Prevalence of Y Chromosome Microdeletion Among Mongolian Infertile Men With Azoospermia and Severe Oligozoospermia

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Research

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Abstract

Backgound: Y chromosome microdeletions are the second most common genetic causes in male infertility. The aim of the present study was to reveal the patterns of Y chromosome microdeletions among Mongolian infertile men.

Method: A descriptive study was performed to 75 infertile men during February 2017 to December 2018. Y chromosome microdeletions were identified by PCR. Semen parameters, hormonal levels, testis biopsy were determined. All collected data were evaluated with Statistical Package for Social Sciences (SPSS, version 22.0).

Results: Among 75 infertile men, 2 cases of Y chromosome microdeletions were determined (2.66%). The first case had AZFa complete deletion and the other one had AZFc partial deletion. The azoospermia patient with AZFa complete deletion had Sertoli cell only syndrome in the testis biopsy, FSH 58.0 mIU/ml and LH 12.0 mIU/ml. The azoospermia patient with AZFc partial deletion showed FSH 23.85 mIU/ml and LH 13.01 mIU/ml. Serum FSH level was significantly higher in the Y chromosome microdeletion patients (p value 0.016).

Conclusion: This study determined Y chromosome microdeletion among Mongolian infertile men to be at 2.66%. Our results showed FSH level is the best predictor of a successful TESE. However, best cut off value for FSH was 9.69 mIU/ml with a sensitivity and specificity 85.6% and 83.3% respectively. There is a possibility that sperm retrieval will be difficult from the TESE since the testicular tissue is severely damaged. The findings can be applied to IVF and Assisted Reproductive Techonology, and our results will help clinicians improve treatment management for Mongolian infertile couples.

Background

Infertility occurs in 10–15% of all couples worldwide, and male infertility consists 40–50% of the infertile cases (1). Infertility frequency in Mongolia was 8.7% in 2003 and 11.6% in 2013 (2). According to findings from Child and Maternity Hospital, male factor constitutes 25.6% of all infertility cases (3). Y chromosome microdeletions are the second most common genetic causes in male infertility after the Klinefelter syndrome. In the general population, Y chromosome microdeletions occur in one among 4000 men, but the frequency is significantly higher among infertile men. The association between Y-chromosome microdeletion and defective spermatogenesis has been studied previously. The incidence of Y chromosome microdeletions is 2–10% or even higher among azoospermic patients with no sperm count or oligospermic patients with sperm count of less than 5 million per milliliter (4). The distal end of the long arm of the Y chromosome includes the azoospermia factor (AZF) locus which contains the genes necessary for spermatogenesis. The AZF locus has been mapped to a region in band q11.23 of the Y chromosome. Microdeletions occur in the AZF region on the long arm of the Y chromosome, which includes AZFa, AZFb and AZFc (5). The diagnosis of Y chromosome microdeletion can establish the cause of the patient's azoospermia and oligozoospermia, and formulate a prognosis.

The purpose of this study was to investigate the frequency of Y chromosome microdeletions among infertile men who visited Mon-CL Fertility Center, Ulaanbaater, Mongolia for evaluation, and to introduce modern infertility diagnosis, contributing to further treatment. Standard method applied by the European Academy of Andrology/European Molecular Genetics Quality Network (EAA/EMQN) was used for the evaluation.

The current data shows that there is a low frequency (2.66%) of Y chromosome microdeletions, in azoospermic and severe oligozoospermic infertile men in the Mongolian population.

Methods

Study population

Having obtained the approval from the local institutional review board, this prospective descriptive study was carried out from February 2017 to December 2018. According to the National Statistics Center of Mongolia, there were about 853018 men (50.6% men of total population) from the age of 15 to 49 in 2019. The confidence interval for the results using the given formular is 95% and the percentile is from 10th to the 95th in this study. Seventy-five infertile men, azoospermic and severe oligozoospermic patients (sperm count of less than 5×10^6) were included in this study. Each patient who agreed to participate in the study was provided with a written informed consent prior to enrollment.

Semen Analysis

In all participants, semen analysis was performed at least twice at one-month intervals, following 3–7 days of sexual abstinence. These samples were collected and examined after 30 min liquefaction. The mean values of different semen analysis results were reported and used as average results. The reference values set by the World Health Organization (WHO) in 2010 were used: a sperm count over 15 million sperms/ml was considered normal, while a sperm count of ≤ 5 million/ml was defined as severe oligozoospermia, and the absence of sperms as azoospermia.

Dna Analysis By Pcr

Blood was taken from all participants for DNA analysis. The DNA was extracted using DNA extraction kits (Chorosh onosh, Mongolia). Then DNA amplification by multiplex PCR was performed using Sequence-tagged sites (STS) primers for the AZFa sub-region (sY84 and sY86), the AZFb sub-region (sY127 and sY134), the AZFc sub-region (sY254, sY255) and the SRY gene (sY14). Samples showing microdeletions on the first screening were verified by subsequent multiplex PCR amplification for another two times. The complete description of primers used for detecting Y-chromosome microdeletion and the amplification sets are shown in Table 1.

Table 1
The STS primers set used in detecting Y-chromosome microdeletions.

STS	Region	Sequence 5'→3'	Size (bp)
sY14	Yp	F: GAATATTCCCGCTCTCCGG	470
		R: GCTGGTGCTCCATTCTTGAG	
AZFa	sY84	F: AGAAGGGTCTGAAAGCAGGT	326
		R: GCCTACTACCTGGAGGCTTC	
	sY86	F: GTGACACACAGACTATGCTTC	318
		R: ACACACAGAGGGACAACCCT	
AZFb	sY127	F: GGCTCACAAACGAAAAGAAA	281
		R: CTGCAGGCAGTAATAAGGGA	
	sY134	F: GTCTGCCTCACCATAAAACG	300
		R: CCACTGCCAAAACTTTCAA	
AZFc	sY254	F: GGGTGTTACCAGAAGGCAAA	380
		R: GAACCGTATCTACCAAAGCAGC	
	sY255	F: GTTACAGGATTCGGCGTGAT	123
		R: CTCGTCATGTGCAGCCAC	

The multiplex PCR amplification condition was optimized as follows: initial denaturation in 95°C for 10 min, followed by 32 cycles of 95°C for 30 s, 60°C for 90 s and 72°C for 60 s; with a final extension at 72°C for 1 min. The PCR products were separated by electrophoresis on a 1.6% agarose gel stained with ethidium bromide. They were then viewed under UV trans-illumination. Negative controls with a DNA template were included with each reaction.

Hormone Assay

The serum samples were obtained by venipuncture from all participants for measurement of follicle-stimulating hormone (FSH), luteinizing hormone (LH) and total testosterone (TT). The serum samples were allowed to clot for 30 minutes and the samples were separated by centrifugation for 10 minutes. All hormone assay was estimated using the Elecsys FSH, Elecsys LH, and Elecsys TT on a cobas e 411 analyzer (Roche Diagnostics, Germany).

Testicular Biopsy

Multiple testicular sperm extraction (TESE) procedures were performed in azoospermic patients for diagnosis and treatment. If sperm non retrieved on TESE, these testicular samples were fixed in 10%

formalin solution, processed, imbedded inparaffin, sectioned, stained with Hematoxylin and Eosin, and then examined for histological anomalies. Based on the most predominant and favourable histopathological pattern, testicular histology was classified into normal spermatogenesis (NS), hypospermatogenesis (HS), maturation arrest (MA), Sertoli cell only syndrome (SCOS), and finally tubular hyalinization (TH).

Data analysis

All collected data were evaluated with Statistical Package for Social Sciences (SPSS, version 22.0). The data was presented as median and range or number and percentage. Receiver operating characteristic (ROC) curve analysis was used to analyse the predictive accuracy and to find the best cut-off level of variables in predicting sperm retrieval in TESE. Student's t test was used for comparison and values of < 0.05 were considered statistically significant.

Result

Patient Characteristics

The patient characteristics are described in Fig. 1. The patients' age ranged from 24 to 46 years (34.51 ± 5.42 in average). Average infertility period was 7.4 ± 5.09 years. The mean body weight was 87.92 ± 14.41 kg and average body mass index (BMI) was 27.85 ± 5.25. A total of 1007 men were analyzed for semen analysis from February 2017 to December 2018. From the total sample, 75 patients who were diagnosed with infertility were analyzed for Y chromosome microdeletion. Six (8.0%) of patients had severe oligozoospermia and 69 (92.0%) patients had azoospermia. Of the group, 39 people underwent TESE. Sperm retrieval rate was 64.1% (25 from 39 patients). Testicular samples of sperm non retrieved 14 patients sent for analysis to the histopathology laboratory.

Semen analysis

The mean age of azoospermic patients was 34.6 ± 5.89 years old, and severe oligozoospermic patients was 33.7 years old. Average pH value of all participants was 7.7. The mean semen volume of all patients was 3.05 ml, 7 ml in maximum and 0.2 ml in minimum. The mean semen volume was 2.9 ± 1.79 ml in azoospermia and 3.9 ± 1.62 ml in severe oligozoospermia. The average sperm count or concentration in 1 ml of semen with severe oligozoospermia was 1.6 ± 1.64 million / ml (Table 2).

Table 2
Result of semen analysis

	Azoospermia n = 69 (92.0%)	Severe oligozoospermia n = 6 (8.0%)	P value
Mean age	34.6 ± 5.39	33.7 ± 5.89	0.684 ns
Mean pH	7.7 ± 0.41	7.6 ± 0.36	0.752 ns
Semen volume (ml)	2.9 ± 1.79	3.9 ± 1.62	0.189 ns
Sperm count (million/ml)	0.0	1.6 ± 1.64	-

Y chromosome microdeletion

A total of 75 patients were analyzed for the Y chromosome microdeletion. The microdeletion in the Y chromosome was detected in the AZFa region (sY84 and sY86) in one patient, and partially detected in the AZFc region (sY254) for the other patient. Deletion of AZFb region was not detected (Fig. 2).

The Y chromosome microdeletion was detected in 2 (2.66%) patients (Table 3).

Table 3
Prevalence of Y chromosomal microdeletion

Deleted loci	Case	Prevalence
AZFa	1	1.33%
AZFb	0	0.0%
AZFc	1	1.33%
Total	2	2.66%

The PCR results of 75 patients are shown in supplementary Fig. 1. The age of the patient with partial deletion of AZFc was 40 years old, diagnosed with azoospermia, did not undergo TESE, FSH level was 23.85 mIU/ml, LH level was 13.01 mIU/ml, and total testosterone level was 4.06 ng/ml. However, the age of the patient with AZFa microdeletion was 31 years old, with azoospermia, sperm was not retrieved from TESE procedure, and the histologic examination result showed Sertoli cell only syndrome. FSH level was 58.0 mIU/ml, LH level was 12.0 mIU/ml, and the total testosterone was 5.0 ng/ml. The hormone levels of the Y chromosome microdeletion group and the non-deletion group were compared. The average FSH level of 2 (2.66%) patients with microdeletion was 40.93 ± 17.07 mIU/ml, the average LH level was 12.5 ± 0.71 mIU/ml, and the average total testosterone was 4.53 ± 0.66 ng/ml, while in average FSH level was 14.86 ± 14.58 mIU/ml, the average LH value was 8.17 ± 5.41 mIU/ml, and the average total testosterone was 3.09 ± 2.16 ng/ml in 73 (97.4%) in patients with no microdeletion (Table 4).

Table 4
Hormone levels of Y chromosome microdeletion and non-deletion group.

Hormone level	Group of Y chromosome microdeletion	Group of Y chromosome non-deletion	P value
FSH (mIU/ml)	40.93 ± 17.07	14.86 ± 14.58	0.016*
LH (mIU/ml)	12.5 ± 0.71	8.17 ± 5.41	0.262
TT (ng/ml)	4.53 ± 0.66	3.09 ± 2.16	0.35

As a result of comparing the hormone levels of the Y-chromosome microdeletion group and the non-deletion group, the FSH levels were significantly different, but the LH and total testosterone levels were not significantly different.

Testicular Sperm Extraction (tese)

Thirty nine of patients with azoospermia underwent TESE procedure for ICSI under regiional anaesthesia. In this surgical procedure, a median raphe incision was made in the scrotum, tunica vaginalis was opened and the testis delivered through the incision. A large piece of testicular tissue was obtained from incision. The specimens were examined by an embryologist and was analyzed for the presence of spermatozoa where all tubules were teased. If no spermatozoa were found, the specimen was taken for histopathological examination. Of the 75 patients, 39 patients received TESE, 25 (64.1%) patients had sperm retrieved, and 14 (35.9%) patients had no sperm retrieved. Average age of sperm retrieval group was 36.7 ± 5.39 years old, and the average age of the unsuccessful group was 31.8 ± 3.37 years old. The FSH hormone level of patients undergoing TESE was 6.31 ± 4.67 mIU/ml in the sperm retrieved group and 21.87 ± 15.08 mIU/ml in the sperm non-retrieved group (p value 0.0001). The LH hormone level of patients undergoing TESE was 5.97 ± 2.8 mIU/ml in the sperm retrieved group and 10.35 ± 5.94 mIU/ml in the sperm non-retrieved group (p value 0.016). Comparison of variables including, age and serum hormone levels with regard to sperm recovery on TESE is shown in Table 5.

Table 5
Comparison of variables with regard to sperm retrieval on testicular sperm extraction (TESE)

Variable	Spermatozoon was retrieved on TESE n = 25 (64.1%)	No spermatozoon was retrieved on TESE n = 14 (35.9%)	P value
Age (years)	36.7 ± 5.39	31.8 ± 3.37	0.006**
FSH (mIU/ml)	6.31 ± 4.67	21.87 ± 15.08	0.0001****
LH (mIU/ml)	5.96 ± 2.81	10.35 ± 5.94	0.016*
TT (ng/ml)	3.42 ± 2.67	2.84 ± 1.37	0.463

Serum FSH and LH levels were significantly lower in the sperm retrieval group on TESE. Age was significantly lower in sperm non-retrieval group. Other variables were found to be comparable between two groups. Histopathological examination showed Sertoli cell only syndrome in 12 patients (85.71%), seminiferous tubule hyalinization 2 patients (14.29%) (Fig. 3).

The ROC curves of FSH and LH hormone levels in the sperm retrieval and non-retrieval group were analyzed. As a result of analyzing the ROC curve to predict the rate of sperm out of the TESE, the FSH level was 9.69 mIU/ml (sensitivity 85.7%, specificity 83.3%), LH was 8.015 mIU/ml (sensitivity 66.7%, specificity 82.4%) (Fig. 4). The sperm was retrieved in 90.9% of patients with FSH levels below 9.69 mIU/ml.

Discussion

The Y chromosome microdeletions are one of the most common causes for male infertility (6). The Y chromosome AZF region contains many genes that are important for spermatogenesis. The region known as azoospermia factor (AZF) includs AZFa, AZFb, and AZFc (7). The AZFa region contains USP9Y, DBY, the AZFb region contains CDY2, EIF1AY, HSFY, PRY, RBMYL1, RPS4YS, SMCY, XKRY, and the AZFc region contains BPY2, CDY1, CSPG4LY, DAZ, and GOLGA2LY (8). Study by L. Tiepolo et al. shows Y chromosome microdeletions to be involved in testicle differentiation and testicle maturation (9). Y chromosome microdeletion plays an important role in predicting sperm extraction from testes. Some studies have shown that Y chromosome microdeletion is associated with testicular cancer and recurrent pregnancy loss (10–12).

Our groups Y chromosome microdeletion study is the first-ever study in Mongolia carried on infertile patients. The study data shows a frequency of Y chromosome microdeletion in the among 75 patients with azoospermia and severe oligozoospermia was 2.66%. The frequency of Y chromosome microdeletion for infertile male patients was 0.7–34.5%, with an average of 8.2% (13, 14). According to the 2008 report, the frequency of AZF microdeletion among infertile men in Sweden, Germany, and

Austria had showed the lowest frequency at less than 2.5% whereas the highest showed more than 10% in Australia, China, and Brazil (4). A comparative study carried throughout Asia among patients with idiopathic azoospermia or severe oligozoospermia showed frequencies of 19.4% in China, 10.6–11.7% in Taiwan, 15.8% in Japan, 9.6–12.0% in India, 3.2% in Saudi Arabia, 3.3% in Turkey, and 2.6% in Kuwait (15–17). We used six different markers for AZFa regions sY84, sY86, AZFb regions sY127, sY134, AZFc regions sY254 and sY255 according to guidelines published by the European Academy of Andrology (EAA) and the European Molecular Genetics Quality Network (EMQN). In a 2012 study of Y chromosome microdeletion with 115 patients in Iran, 1.7% showed deletion in AZFc and AZFbc. They used six markers such as sY84, sY86 (AZFa), sY127, sY134 (AZFb), sY254, sY255 (AZFc) which are the same as what we used in our study (18). In 2011 study, Haluk Akin et al reported Y chromosome microdeletion in 7 patients (3.93%) among 178 infertile men. They were detected in the AZFc and AZFa region (19). From the study of 1738 infertile men by Yong-Sheng Zhang et al in China, the frequency of Y chromosome microdeletion was 8.57%. Of note, the frequency of the AZFa deletion was 2.2%. From the study of 3654 males by Totonchi et al., the frequency of Y chromosome deletion was 5.06%. The deletion in AZFa was 2.16%, which is similar to our results (20, 21). Most patients with AZFa deletion were diagnosed with Sertoli cell only syndrome (22). In the case of complete deletion of AZFa, no sperm was retrieved. However, in partial deletions, it is reported that sperm is retrieved by TESE (22, 23). In our study, the patient with Y chromosome AZFamicrodeletion was 31 years old, with azoospermia, no sperm retrieved from the testis, and histologic examination showed Sertoli cell only syndrome. These results were similar to the results from Kamp et al (22).

Both patients with Y chromosome microdeletion had azoospermia, and FSH and LH hormone levels were higher than normal. FSH was 40.93 ± 17.07 mIU/ml and LH hormone was 12.5 ± 0.71 mIU/ml, but there was a significant difference in FSH hormone level compared to non Y chromosome microdeletion group ($P < 0.05$). These results were similar to the results from Li-Quan Wang and Rajeev Kumar et al (24, 25).

The mean age of the sperm retrieval group was 38.3 ± 5.1 years old. The mean age of the sperm non-retrieval group was 31.5 ± 3.63 years old, showing a significant difference. Studies by Yi-Ru Tsai or Kuo-Chung Lan et al showes that IVF results, pregnancy rates, and miscarriage rates correlate with male age (26). According to the results of a micro TESE study by Noritoshi Enatsu and Hideaki Miyake, the average age of the sperm retrieval group from the testis was 35.0 ± 5.6, and the average age of the sperm not-retrieval group was 33.2 ± 4.9 ($p < 0.05$) (27).

The 14 patients with no sperm retrieved by TESE were diagnosed testicular histopathology. Histopathological examination showed Sertoli cell only in 12 patients (85.71%) and seminiferous tubule hyalinization in 2 patients (14.29%). Of the 25 patients who had sperm retrieved from TESE, 18 (72%) patients had In Vitro Fertilization (IVF) treatment. Of these, two (11.1%) patients were embryo banking and sixteen (88.9%) had embryo transfer. Six patients (37.5%) had successfully clinical pregnancy. Where one patient gave birth to a twin baby and others are successful in ongoing pregnancy.

In conclusion, current data shows that there is a low frequency of Y chromosome microdeletions, in azoospermic and severe oligozoospermic infertile men in the Mongolian population. In the case of non-obstructive azoospermia, AZFa, AZFb and AZFb/c microdeletion occurs in 1–2%, but sperm is not retrieved by TESE. So there is no need for unnecessary TESE procedure. In patients with AZFc microdeletion, sperm formation functions properly. IVF can be performed with sperm of a patient with microdeletion of AZFc, which can result in a successful pregnancy. However, the microdeletion of the AZFc part is inherited to his male child. However, we recommend a larger group of patients and controls to be screened for this microdeletion for confirmation.

Abbreviations

AZF	Azoospermia factor
BMI	Body Mass Index
BPY2	Basic Protein Y 2
CDY	Chromo Domain Y
CSPG4LY	CSPG4 pseudogene 1 Y-linked
DAZ	Deleted in Azoospermia
DBY	DEAD Box Y
DNA	Deoxyribonucleic acid
D.W	Distilled water
EAA	European Academy of Andrology
EIF1AY	Essential Initiation Translation Factor 1A Y
EMQN	European Molecular Genetics Quality Network
FSH	Follicle stimulating hormone
GOLGA2LY	Golgi autoantigen, golgin Subfamiliy a2 Like Y
HSFY	Heat shock transcription factor Y
ICSI	Intracytoplasmic Sperm Injection
IRB	Institutional Review Board
IVF	In Vitro Fertilization
LH	Luteinizing Hormone
PCR	Polymerase chain reaction
PRY	PTP-BL related on the Y chromosome
RBMYL1	RNA Binding Motif Y-linked
ROC	Receiver operating characteristic
RPS4YS	Ribosomal Protein S4 Y linked 2
SMCY	Selected Mouse C DNA Y
SPSS	Statistical Package for Social Sciences
SRY	Sex-determining region Y
STS	Sequence tagged site
TESE	Testicular Sperm Extraction

AZF	Azoospermia factor
TT	Total Testosterone
USP9Y	Ubiquitin specific proteaase 9 Y
UV	Ultraviolet
WHO	World Health Organization
XKRY	X - Kell blood group precursor related Y

Declarations

Ethics approval and consent to participate

This was a descriptive study conducted at the Mon-CL fertility center, Ulaanbaatar, Mongolia. The study was approved by the Institutional Review Board of Mongolian National University of Medical Science (IRB:2017/3-05).

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors do not have any competing interests.

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Author contributions

E.D., P.N. and B.B designed, carried out most of experiments, analyzed data, and wrote the paper, drafted the manuscript. All authors contributed to the interpretation, discussion and editing of the manuscript. All authors approved the last version.

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Figures

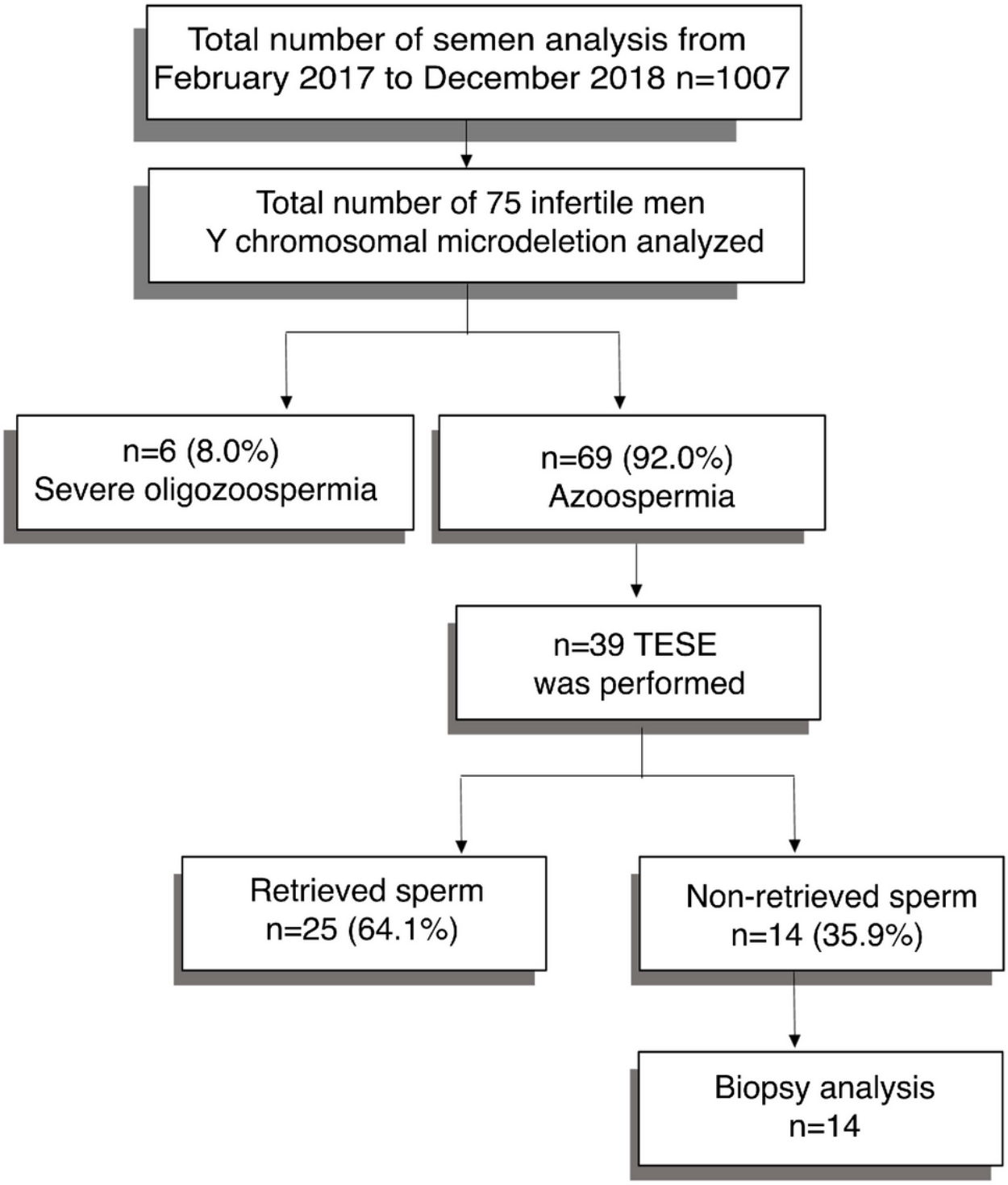

Figure 1

Flow chart of study participants. A total of 1007 men were analyzed for semen analysis and of these, 75 patients who were diagnosed with infertility were analyzed for Y chromosomal microdeletion. 6 (8.0%) patients were with severe oligozoospermia and 69 (92.0%) patients were with azoospermia. Thirty-nine people underwent TESE. In 25 (64.1%) patients there were sperm retrieved, but in 14 (35.9%) patients there were sperm not retrieved and 14 patient's tissue was sent for biopsies.

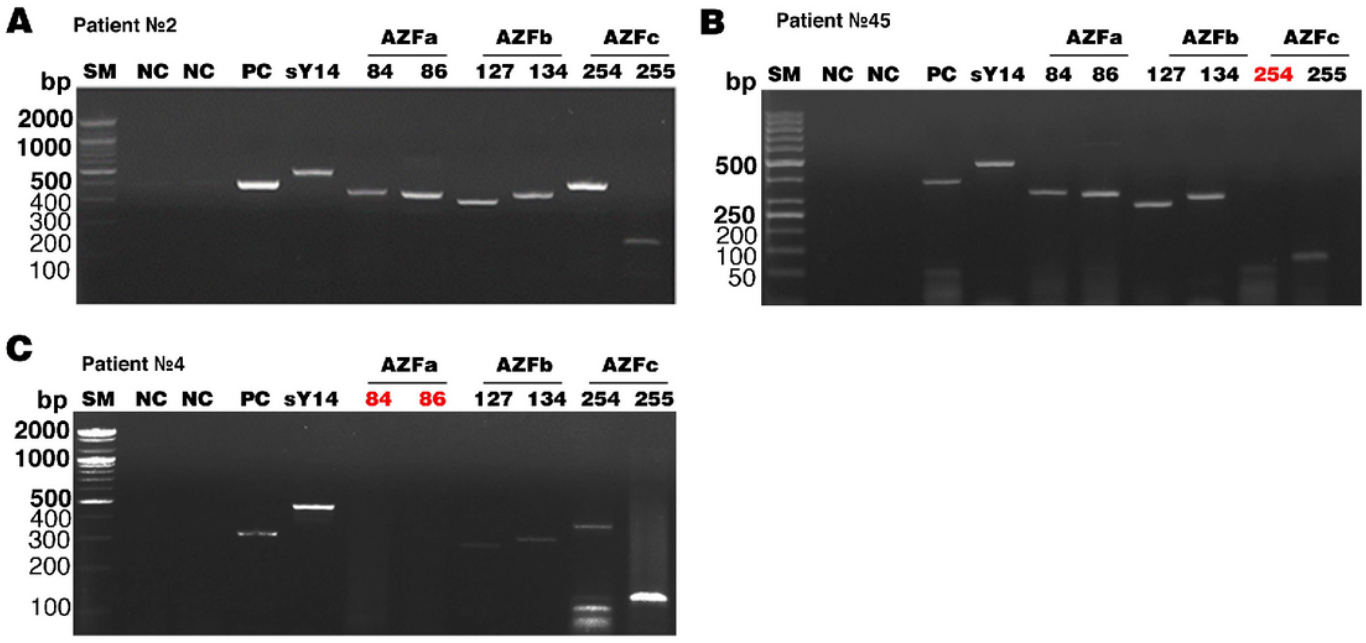

Figure 2

PCR results of Y chromosome microdeletion. (A) A representative picture of Y chromosome non-deletion group. lane 1: Size marker; lane 2: negative control (D.W); lane 3: negative control (Female patient blood); lane 4: positive control (sY127); lane 5: positive control (sY14); lane 6:AZFa- sY84; lane 7:AZFa-sY86; lane 8:AZFb-sY127; lane 9:AZFb-sY134; lane 10:AZFc-sY254; lane 11:AZFc-sY255. (B) A representative picture of AZFc (sY-254) microdeletion. lane 1: Size marker; lane 2: negative control (D.W); lane 3: negative control (Female patient blood); lane 4: positive control (sY127); lane 5: positive control (sY14); lane 6:AZFa- sY84; lane 7:AZFa-sY86; lane 8:AZFb-sY127; lane 9:AZFb-sY134; lane 10:AZFc-sY254; lane 11:AZFc-sY255. (C) A representative picture of AZFa (sY84 and sY86) microdeletion. lane 1: Size marker; lane 2: negative control (D.W); lane 3: negative control (Female patient blood); lane 4: positive control (sY127); lane 5: positive control (sY14); lane 6:AZFa- sY84; lane 7:AZFa-sY86; lane 8:AZFb-sY127; lane 9:AZFb-sY134; lane 10:AZFc-sY254; lane 11:AZFc-sY255.

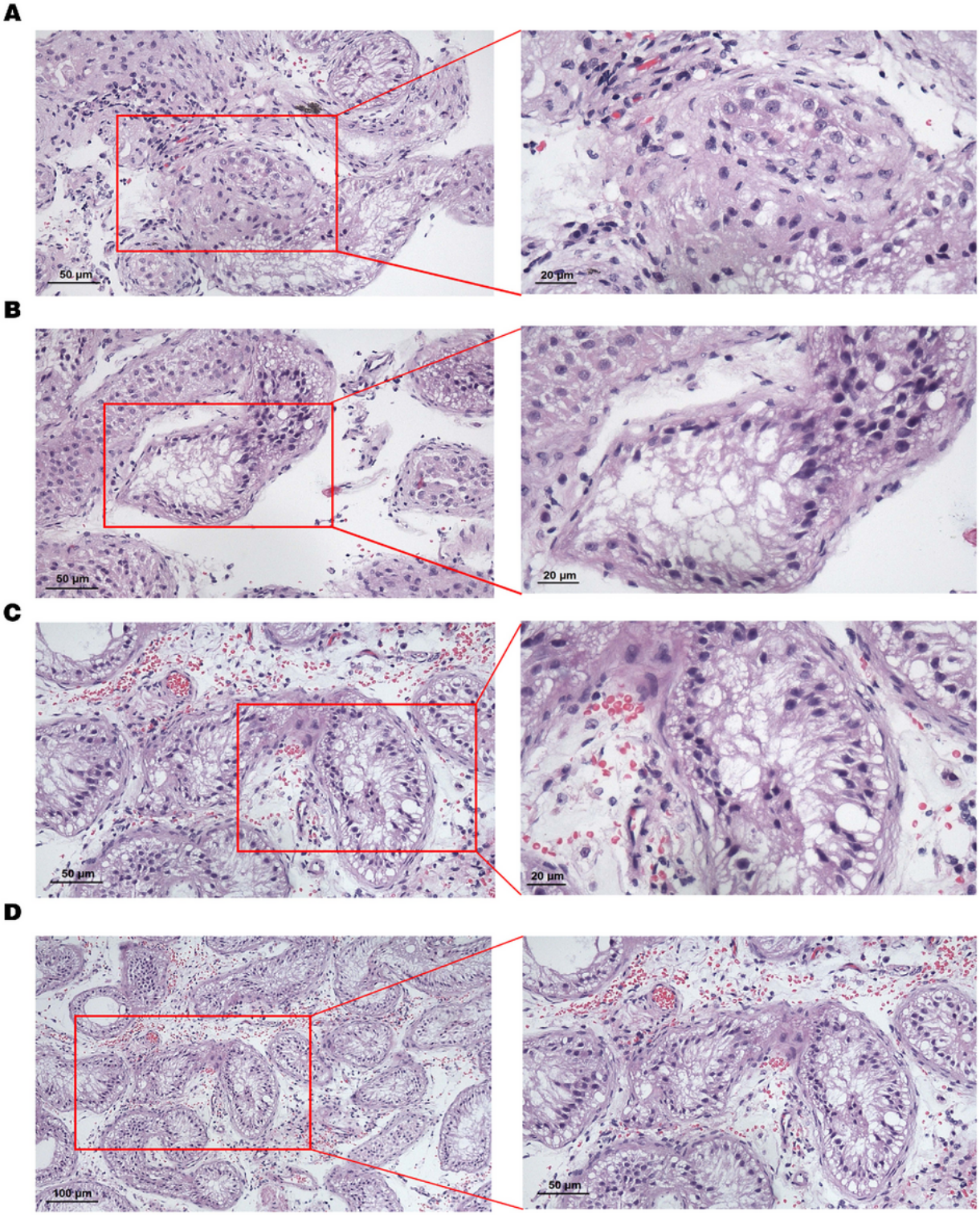

Figure 3

Showing pattern of Sertoli cell only syndrome. (A). Testicular atrophic tubules with hyalinization, HE 10x10, 10x40. (B and C). Tubules with thickened basal membrane are lined by sertoli cells some of which are altered in shape and detached from the basal membrane, and devoid of germ cells, HE 4x10, 10x40. (D). Expansion of interstitial space along with increased connective tissue. Atrophic appearance in the seminiferous tubules. Basement membrane thickening, HE 4x10, 10x10.

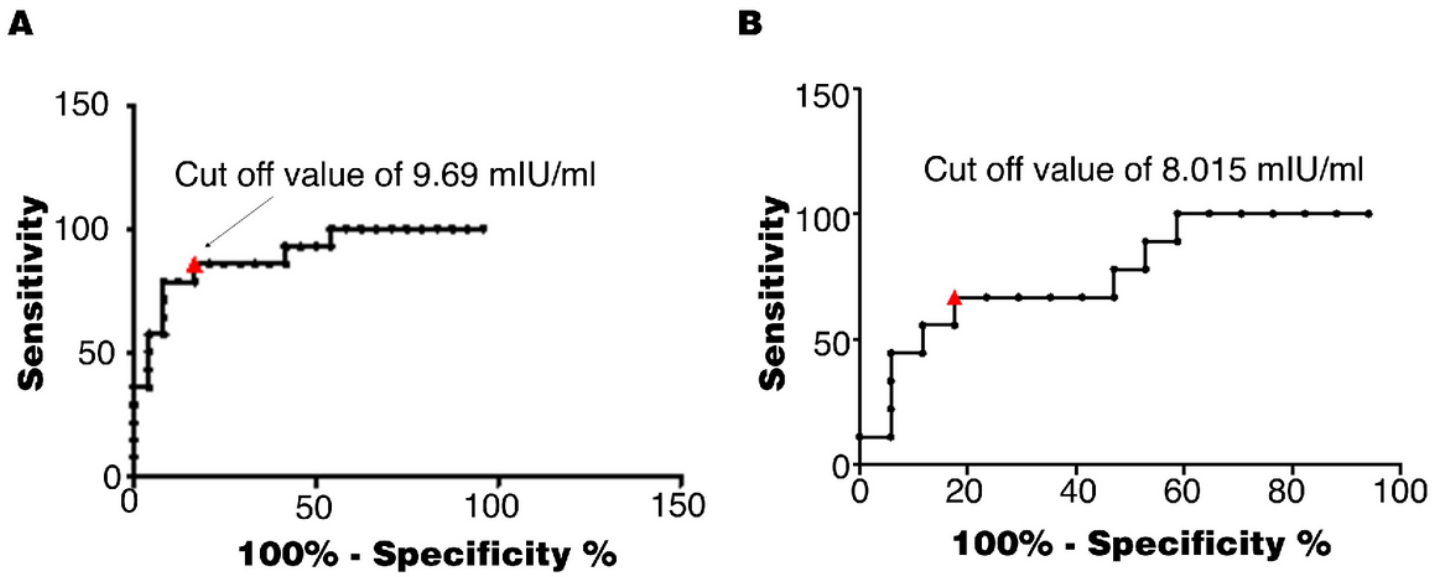

Figure 4

ROC curve of FSH (A) and LH (B).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFigures.pdf