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First report of frequencies of Y chromosome microdeletions at a reproductive medicine center in Peru

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A B S T R A C T

Objective: Y chromosome Microdeletions are the second genetic cause of infertility in men. Despite its importance for infertility treatment, there is no previous research in Peru. The aim of this study was to determine the frequencies and characteristics of Y chromosome microdeletions in a group of men who sought infertility consultation at a specialized reproductive medicine center in Peru.
Methods: In this study, 201 semen samples were analyzed. The samples were obtained from Niu Vida's fertility program. Each seminal sample was analyzed according to the recommendations of the Laboratory Manual of the World Health Organization (WHO) 2010. A buccal swab and a 500 μL aliquot of seminal sample were used for the molecular study of Y chromosome microdeletions in each patient. The frequencies and the type of Y chromosome microdeletion in the AZFa, AZFb and AZFc regions were evaluated.
Results: The prevalence of Y chromosome microdeletions in the AZF region was 6.45% in oligozoospermic and azoospermic patients, and a prevalence of 20% was observed specifically in azoospermic patients. No microdeletions of AZFb type were detected. A partial region microdeletion of AZFa was detected in a teratozoospermic patient with a normal sperm count.
Conclusions: The study represents the first report on the incidence of Y chromosome microdeletions in Peru. Our results indicate a high prevalence of microdeletions in azoospermic patients compared to similar studies. It is suggested to assess the presence of AZFa microdeletions and to evaluate additional genetic markers in this region to identify specific mutations that may cause impaired sperm production and male infertility in the Peruvian male population.

1. Introduction

Infertility is a disease characterized by the failure to establish a clinical pregnancy after 12 months of regular, unprotected sexual intercourse or due to an impairment of a person's capacity to reproduce either as an individual or with his/her partner [1]. Male infertility is a global health issue that affects 7% of the male population where the cause remains unexplained [2]. On a global scale, the causes of male infertility are wide ranging and poorly understood in most cases. Although various diagnostic tests are available, their interpretation is imprecise and often subjective [3]. Despite that, about 20% of infertile males have undetermined infertility, includes two categories: idiopathic male infertility that are infertile males with abnormal semen analyses with an unknown cause for that abnormality, and unexplained male infertility-males, with "normal" semen analysis who are unable to impregnate due to unknown causes [4]. In addition, genetic factors are also responsible for male infertility and are commonly due to chromosomal abnormalities such as Klinefelter syndrome 47XXY [5], congenital absence of vas deferens, microdeletions, and cystic fibrosis [6,7]. Because the Yq locus contains a large number of genes that are transcribed at the testicular level, the Y chromosome plays a vital role during sexual differentiation a spermatogenesis in male development. Thus, as occurs in the Azoospermia Factor (AZF) c region, due to the presence of many amplicons and palindromic sequences, these are prone to self-recombination during spermatogenesis and therefore

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susceptible to intrachromosomal deletions [8].

Microdeletions of the Y chromosome are commonly considered the second leading cause of male infertility. It is estimated that the frequency of microdeletions in infertile men is approximately 7% [9]. However, studies show that there is variability of microdeletion frequencies by ethnic parameters throughout [10]the world [11]. Y chromosome Microdeletions are more frequently present in the AZFc region (60%), less frequent in the AZFb region (16%), and rarely present in the AZFa region (5%). There are Y chromosome microdeletions that involve two or three AZF factors simultaneously and are diagnosed in 14% of cases [12].

In recent times, assisted reproduction centers allow couples, with a previous diagnosis of male infertility linked to chromosomal aberrations, to procreate using the assisted reproductive technique: Intracytoplasmic sperm injection [13,14]. Therefore, it is important to perform genomic screening in male patients for the detection of genetic etiologies linked to male infertility.

The aim of this study is to determine the frequencies and characteristics of the microdeletions of the Y chromosome in the AZFa, AZFb and AZFc subregions through the use of the polymerase chain reaction (PCR) in patients who attended a Peruvian center for consultation of assisted reproduction.

2. Materials and methodology

2.1. Patient selection and experimental design

This was a prospective experimental study carried out at Niu Vida assisted reproduction center. We received the clinic's ethics committee approval for this study. Two hundred and one patients from the assisted reproduction program were recruited for the study during the years 2018 and 2019. These patients were informed and they agreed to sign their consent for the use of the corresponding samples. Likewise, it is specified that the study has not carried out a karyotype analysis due to its high cost and the associated ethical issues that are not the objective of the research.

Each seminal sample was analyzed by a spermiogram under the recommendations of the WHO Laboratory Manual of 2010. Likewise, inclusion and exclusion criteria were applied to the samples evaluated in this study as follows:

- Inclusion criteria:
  1. Men with no previously known history of infertility.
  2. Men of legal age. In Peru, from the age of 18 a man is considered of legal age.
  3. Men who agreed to participate in the study by signing the informed consent.
- Exclusion criteria:
  1. Men older than 65 years (retired men in Peru) because it could influence the results due to pre-existing comorbidities and the use of medications that could affect seminal quality. The present study aimed to obtain a more homogeneous and controlled sample.
  2. Men with previous surgical treatments in the reproductive area.
  3. Foreign patients

2.2. Semen analysis

Each seminal sample was received in a sterile container duly labeled; samples were kept for 20–30 min for liquefaction to take place. The macroscopic analysis was carried out and the following parameters were evaluated: Volume, color, pH, appearance, viscosity and liquefaction.

Table 1
Sequences of primers used for PCR. Two internal amplification controls (ZFX/Y and SRY) and two Multiple systems (A and B) were evaluated.

ocus	Primers	Sequences	Product size (bp)
Amplifications controls			
ZFX/Y	ZFX/Y–F	5′-ACC RCT GTA CTG ACT GTG ATT ACA C-3′	495
	ZFX/Y–R	5′-GCA CYT CTT TGG TAT CYG AGA AAG T-3′	
SRY	sY14-F	5′-GAA TAT TCC CGC TCT CCG GA-3′	472
	sY14-R	5′-GCT GGT GCT CCA TTC TTG AG-3′	
MμLtiplex A			
AZFa	sY86-F	5′-GTG ACA CAC AGA CTA TGC TTC-3′	318
	sY86-R	5′ - ACA CAC AGA GGG ACA ACC CT - 3′	
AZFb	sY127-F	5′-GGC TCA CAA ACG AAA AGA AA-3′	274
	sY127-R	5′-CTG CAG GCA GTA ATA AGG GA-3′	
AZFc	sY254-F	5′-GGG TGT TAC CAG AAG GCA AA-3′	380
	sY254-R	5′-GAA CCG TAT CTA CCA AAG CAG C-3′	
MμLtiplex B			
AZFa	sY84-F	5′-AGA AGG GTC CTG AAA GCA GGT-3′	326
	sY84-R	5′-GCC TAC TAC CTG GAG GCT TC-3′	
AZFb	sY134-F	5′-GTC TGC CTC ACC ATA AAA CG-3′	301
	sY134-R	5′-ACC ACT GCC AAA ACT TTC AA-3′	
AZFc	sY255-F	5′-GTT ACA GGA TTC GGC GTG AT-3′	123
	sY255-R	5′-CTC GTC ATG TGC AGC CAC-3′	

Microscopic evaluation was carried out in two phases: in the first step we evaluate concentration and sperm motility using the Mackler chamber, in a second step we smear 20 μL of the sample on a slide to assess the sperm morphology and to determine the presence of other cells, agglutinations and other elements.

2.3. DNA extraction

To improve the sample collection experience for patients a non-invasive method was chosen: an oral swab [10] and an aliquot of 500 μL of seminal sample. This study does not evaluate blood samples to maintain non-invasive methods. DNA was extracted from both samples using the QIAamp DNA Investigator Kit - Cat. No./ID: 56,504 - USA (QIAGEN).

The concentration and purity of the DNA were evaluated by spectrophotometry using the NanoDrop 2000 of Thermofisher. The DNA concentration was adjusted to a range between 5 and 30 ng/μl and maintained to −20 °C.

2.4. Polymerase chain reaction (PCR)

6 STS regions (Sequence-Tagged Sites) of the Azoospermic Factor AZF were evaluated according to what was described by Krausz et al. (2013). The AZF regions and the primers used are described in Table 1. The amplification mix was carried out in a volume of 15 μL of PCR reaction, using 1 μL of DNA for each amplification reaction. The amplification protocols were as follows: denaturation cycle at 95 °C for 5 min; 35 cycles of hybridization at 94 °C for 30 s, annealing at 57 °C for 90 s, extension at 72 °C for 60 s; and a final extension at 72 °C for 10 min; preservation at 4 °C. Two internal controls were used: the sex determining region in the Y chromosome SRY gene as well as the zinc finger expression factor gene: ZFX/Y.

2.5. Detection of amplification products with vertical electrophoresis

The PCR products were processed in Polyacrylamide gels using the Silver Sequence Kit (PROMEGA), and vertical electrophoresis was carried out at 80 V/cm for 45 min. Subsequently, the gel was photo documented for analysis. The analysis of fragments was evaluated with a GeneRuler allelic ladder 100 bp.

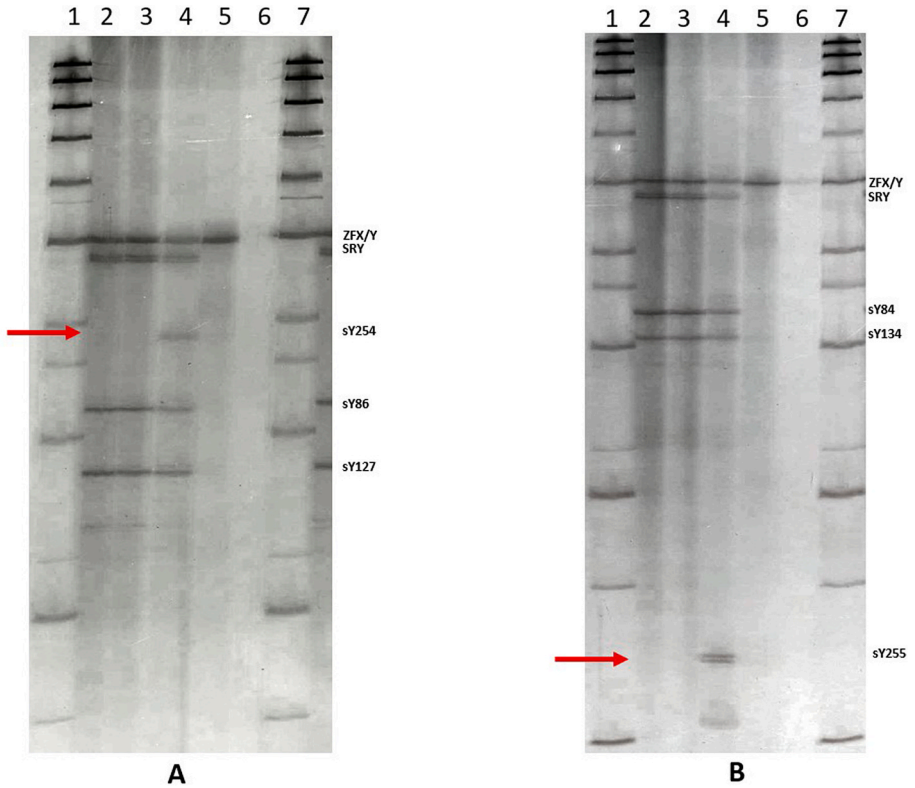

Fig. 1. Vertical Electrophoresis. Ladder 100 bp (Lane 1 and 7). Gel A: Microdeletion in the sY254 region in seminal and oral swab samples (Lane 2 and 3 respectively). Gel B: microdeletion in the sY255 region in seminal and oral swab samples (Lane 2 and 3, respectively). Line 4: DNA of normal male, line 5: DNA female, line 6 Negative control. The red arrow indicates the presence of the AZFc microdeletion.

2.6. Statistical analysis

The experimental data were processed using SPSS 19.0 software. Comparisons were made using the chi square test. The statistic $P < 0.05$ was used as the significance value. Each assay was performed using a normal male DNA control with biological children and a female DNA control.

3. Results

Out of the 201 patients who were evaluated, 167 exhibited alterations in at least one seminal parameter. The distribution of these patients according to their alterations was as follows: 21 patients (10.4%) had oligozoospermia, 50 patients (25%) had asthenozoospermia, 108 patients (54%) had teratozoospermia, and 10 patients (5%) had azoospermia. Furthermore, 33.5% of the patients had two or more seminal parameters that were altered.

We report microdeletions in the Y chromosome AZFc region, the absence of markers in the sY254 bands for seminal and oral swab samples in lane 2 and 3 (See Fig. 1A) and sY255 in lane 2 and 3 (See Fig. 1B), respectively.

Likewise, Fig. 2 shows the microdeletion in the Y chromosome partial region AZFa due to the absence of the sY86 band for seminal and oral swab samples in lane 2 and 3 (Fig. 2).

A total of three microdeletions of the Y chromosome (1.5%) were detected out of a total of 201 samples evaluated in the study, these microdeletions are distributed between the AZFa microdeletion (0.5%) and the AZFc (1%). Microdeletions were not detected in the AZFb region (See Table 3).

Two Y chromosome microdeletions (20%) were detected from a total of 10 samples from Azoospermic patients in the study population. Likewise, the finding of a teratozoospermic patient with a normal sperm count and the presence of a partial microdeletion in the AZFa region is highlighted, which represents 0.6% of the samples with a normal sperm count (See Table 4).

4. Discussion

The report presented by Simoni et al. evaluates 91 studies on Y microdeletions carried out around the world corresponding to 41 countries and 16,316 individuals evaluated [15]. The study points out that the frequencies of microdeletions are distributed

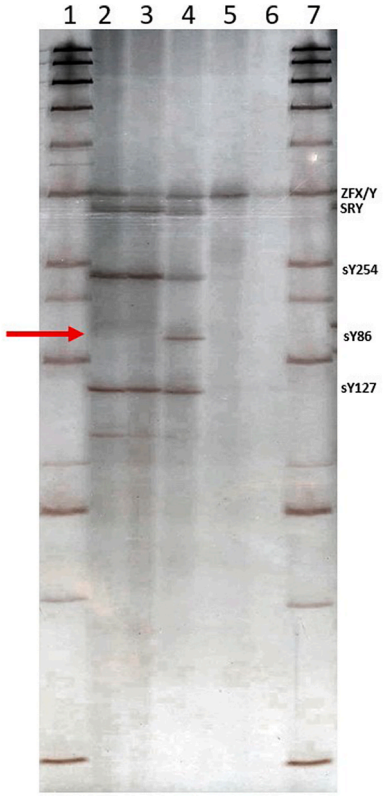

Fig. 2. Vertical Electrophoresis. Ladder 100 bp (Lane 1 and 7). Microdeletion in the sY86 region in seminal and oral swab samples (Lane 2 and 3, respectively). Lane 4: DNA of normal male, line 5: DNA female, line 6 Negative control. The red arrow indicates the presence of the AZFa microdeletion.

Table 2
Distribution of sperm abnormalities referred to seminal parameters according to WHO in the study population.

Anomaly	N° samples	%
Oligozoospermic	21	10.4
Azoospermic	10	5.0
Astenozoospermic	50	25
Teratozoospermic	108	54

Table 3
The AZF microdeletion patterns are shown: AZFa (1) and AZFc (2). AZFb microdeletions were not observed.

n, (%)	Microdeletion patterns					
	AZFa	AZFb	AZFc	AZF (b + c)	AZF (a+b + c)	Total
n	1	0	2	0	0	3
(%)	(0.5)	(0.0)	(1.0)	(0.0)	(0.0)	1.5

AZF: Azoospermic Factor

Table 4
Microdeletions detected in relation to sperm concentration.

Sperm Concentration	N° Microdeletions (%)				
	Absence	%	Presence	%	Total
Azoospermic	8	80	2	20	10
<15 mill/mL	21	100	0	0	21
>15 mill/mL	169	100	1	0.6	170
Total	198		3		201

approximately as follows: AZFc: 80%, AZFa: 0.5–4%, AZFb: 1–5% and AZFbc: 1–3% [9]. This report indicates that AZFb microdeletions are the second highest incidence reported worldwide, this type of microdeletion was not detected in our study group (Table 2). This could be related to the necessity to evaluate a larger population group because the frequencies of microdeletions have a wide range of incidence that goes from 1% [16] to 55% [17] around the world. Likewise, microdeletions studies must be evaluated in different geographic areas in a given region to estimate the variations in microdeletions frequencies in a population [18].

Our study demonstrated a 20% prevalence of microdeletions in Azoospermic patients (2/10) (Table 3), resulting a greater frequency than those reported by other studies on azoospermic patients such as those of Akinsal et al. in Turkey (4.7%) [19], Kim et al. in Korea (14%) [20], Aknin-Seifer et al. in France (8%) [21], Sen et al. in India (3.4%) [22], Al-Ouqaili et al. in Iraq (72.5%) [23,24]and Al-Qaisi et al. in Iraq (62.5%) [24], among others. This fact must be carefully evaluated, since it can be affected by factors of sample size, type of evaluated population and/or criteria for patient selection.

Regarding Latin America, the percentage of Y chromosome microdeletions detected in oligospermic and azoospermic patients in our study was 6.45% (Fig. 2), a similar value compared to other studies in the region reported by Pina-Neto et al. in Brazil 7.5% (12/160) [25], Sánchez et al. in Chile 9.8% (10/102) [26], Torres et al. in Ecuador 8.57% (3/35) [27] and Fernández et al. in Venezuela 3.45% (1/29) [28]. With respect to other continents Peterlin et al. reported information on microdeletions on Azoospermic and Oligospermic patients in Germany 3.2% (12/370), 1.3% (19/1470), Sweden 2% (4/192), Netherlands and Belgium 2.3% (37/1627), Ireland 3.6% (2/56) and Slovenia 4.4% (9/226) [29]. Our study did not detect microdeletions in Oligozoospermic patients.

The partial microdeletion in the AZFa region on STS s86 is unusual due to the low incidence of this type of deletion and it is configured as the first finding reported in our region on a sample from a donor with a normal count and a teratozoospermia diagnosis. This finding is like a case reported by Luddi et al. in their publication three patients with a family link with a diagnosis of normozoospermia and the deletion of the AZFa region on the USPY9 gene located in this region [30], which requires from our study a more in-depth analysis about the impact of the deletion on the expression of proteins on the genetic sequence involved.

In reference to the study by Elfateh et al. who describe the presence of AZFb and AZFc microdeletions on teratozoospermic samples in an Iranian population [31], this study did not report microdeletions from teratozoospermic samples in these regions. On the other hand, Hellani et al. describes the presence of microdeletions in teratozoospermic samples of seminal and blood cells, suggesting that germinal mosaicism could be responsible for teratozoospermia [32], however the present study does not evidence such a hypothesis because the microdeletion is observed in buccal and seminal cells. The study by Sevinç et al. also report the presence of microdeletions on teratozoospermic samples but with a diagnosis of Oligospermia [33].

It is important to mention that there may be other parameters to take into account regarding the association between the presence of Y microdeletions and patient conditions such as anti-sperm antibodies as evaluated in the study by Al-Qaisi et al., whose study found no correlation between both pathologies, considering that the study population had a high prevalence of microdeletions [34].

At present, the application of the testicular sperm extraction (TESE) technique used to recover spermatozoa from the testis has

improved the fertility prognosis in patients diagnosed with infertility, however the success of the testicular sperm extraction will depend to a large extent on the type of microdeletion evaluated in each patient [35].

The microdeletions of the AZFa and AZFb region show that there is a very low success rate on the application of the testicular sperm extraction technique for sperm recovery, as demonstrated by the study by Golcalves et al. and Arshad et al. [36,37]. In contrast, AZFc microdeletions show a better clinical prognosis for sperm retrieval by testicular sperm extraction of approximately 55% [36,38].

In this study, two AZFc microdeletions were detected in samples from azoospermic patients, so that the application of sperm recovery techniques would have a good probability of success for Intra Cytoplasmic Sperm Injection (ICSI) treatment [13]. However, it is important to note that TESE is an invasive procedure that carries certain risks, and that the success rate of testicular sperm retrieval may also depend on other factors such as the age of the patient and the severity of azoospermia and that in addition, microdeletions can be transmitted to sons. Therefore, it is essential that patients with azoospermia diagnosed with microdeletions in AZF regions receive a detailed evaluation by a specialist in genetics and andrology to determine the best treatment option.

5. Conclusions

Studies of microdeletions and based on the PCR should be used with great importance prior to assisted reproduction technology (ART) in patients with a diagnosis of severe oligospermia or non-obstructive azoospermia. Our study reports the presence of AZFc microdeletions in Azoospermic patients without the presence of AZFb microdeletions, which suggests carrying out larger studies with a larger sample size. The first finding of an AZFa microdeletion in our population is highlighted, belonging to a donor with a normal sperm count and a diagnosis of teratozoospermia. For this reason, it is suggested to expand the study to evaluate sperm morphology due to the correlation with the presence of AZF microdeletions and to evaluate additional markers of the AZFa region to identify specific mutations.

Author contribution statement

Juan Martín Gavilan: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Carlos Vivar; Cecilia Choque; Mayra Guzmán: Performed the experiments.

Víctor Núñez: Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Carlos Duarte: Conceived and designed the experiments; Wrote the paper

Data availability statement

Data included in article/supplementary material/referenced in article.

Additional information

No additional information is available for this paper.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e20221.

References

[1] H. World Health Organization, in: WHO Laboratory Manual for the Examination and Processing of Human Semen Sixth Edition, sixth ed., 2021. Geneva.

[2] M.S. Oud, R.M. Smits, H.E. Smith, F.K. Mastrorosa, G.S. Holt, B.J. Houston, P.F. de Vries, B.K.S. Alobaidi, L.E. Batty, H. Ismail, J. Greenwood, H. Sheth, A. Mikulasova, G.D.N. Astuti, C. Gilissen, K. McEleny, H. Turner, J. Coxhead, S. Cockell, D.D.M. Braat, K. Fleischer, K.W.M. D'Hauwers, E. Schaafsma, D. F. Conrad, L. Nagirnaja, K.I. Aston, D.T. Carrell, J.M. Hotaling, T.G. Jenkins, R. McLachlan, M.K. O'Bryan, P.N. Schlegel, M.L. Eisenberg, J.I. Sandlow, E. S. Jungheim, K.R. Omurtag, A.M. Lopes, S. Seixas, F. Carvalho, S. Fernandes, A. Barros, J. Gonçalves, I. Caetano, G. Pinto, S. Correia, M. Laan, M. Punab, E.R.-D. Meyts, N. Jørgensen, K. Almstrup, C.G. Krausz, K.A. Jarvi, L. Nagirnaja, D.F. Conrad, C. Friedrich, S. Kliesch, K.I. Aston, A. Riera-Escamilla, C. Krausz, C. Gonzaga-Jauregui, M. Santibanez-Koref, D.J. Elliott, L.E.L.M. Vissers, F. Tüttelmann, M.K. O'Bryan, L. Ramos, M.J. Xavier, G.W. van der Heijden, J. A. Veltman, A de novo paradigm for male infertility, Nat. Commun. 13 (2022) 154, https://doi.org/10.1038/s41467-021-27132-8.

[3] A. Agarwal, S. Baskaran, N. Parekh, C.L. Cho, R. Henkel, S. Vij, M. Arafa, M.K. Panner Selvam, R. Shah, Male infertility, Lancet 397 (2021) 319–333, https://doi.org/10.1016/S0140-6736(20)32667-2.

[4] S. Pandruvada, R. Royfman, T.A. Shah, P. Sindhwani, J.M. Dupree, S. Schon, T. Avidor-Reiss, Lack of trusted diagnostic tools for undetermined male infertility, J. Assist. Reprod. Genet. 38 (2021) 265–276, https://doi.org/10.1007/s10815-020-02037-5.

[5] M. Bielanska, S. Lin Tan, A. Asangla, Fluorescence in-situ hybridization of sex chromosomes in spermatozoa and spare preimplantation embryos of a Klinefelter 46,XY/47,XXY male, Hum. Reprod. 15 (2000) 440–444, https://doi.org/10.1093/humrep/15.2.440.

[6] J. Sertić, P. Cvitković, A. Myers, R.K. Saiki, A. Stavljenić Rukavina, Genetic markers of male infertility: Y chromosome microdeletions and cystic fibrosis transmembrane conductance gene mutations, Croat. Med. J. 42 (2001) 416–420.

[7] T. Huynh, R. Mollard, A. Trounson, Selected genetic factors associated with male infertility, Hum. Reprod. Update 8 (2002) 183–198, https://doi.org/10.1093/humupd/8.2.183.

[8] T. Kuroda-Kawaguchi, H. Skaletsky, L.G. Brown, P.J. Minx, H.S. Cordum, R.H. Waterston, R.K. Wilson, S. Silber, R. Oates, S. Rozen, D.C. Page, The AZFc region of the Y chromosome features massive palindromes and uniform recurrent deletions in infertile men, Nat. Genet. 29 (2001) 279–286, https://doi.org/10.1038/ng757.

[9] C. Krausz, L. Hoefsloot, M. Simoni, F. Tüttelmann, EAA/EMQN best practice guidelines for molecular diagnosis of Y-chromosomal microdeletions: state-of-the-art 2013, Andrology 2 (2014) 5–19, https://doi.org/10.1111/j.2047-2927.2013.00173.x.

[10] C.R. Quilter, E.C. Svennevik, P. Serhal, D. Ralph, G. Bahadur, R. Stanhope, M. Sütterlin, J.D.A. Delhanty, K.E. Taylor, Cytogenetic and Y chromosome microdeletion screening of a random group of infertile males, Fertil. Steril. 79 (2003) 301–307, https://doi.org/10.1016/S0015-0282(02)04692-7.

[11] M.J. Rabinowitz, P.J. Huffman, N.M. Haney, T.P. Kohn, Y-chromosome microdeletions: a review of prevalence, screening, and clinical considerations, Appl. Clin. Genet. 14 (2021) 51–59, https://doi.org/10.2147/TACG.S267421.

[12] D.K. Choi, I.H. Gong, J.H. Hwang, J.J. Oh, J.Y. Hong, Detection of Y chromosome microdeletion is valuable in the treatment of patients with nonobstructive azoospermia and oligoasthenoteratozoospermia: sperm retrieval rate and birth rate, Korean J Urol 54 (2013) 111–116, https://doi.org/10.4111/kju.2013.54.2.111.

[13] J. Testart, E. Gautier, C. Brami, F. Rolet, E. Sedbon, A. Thebault, Intracytoplasmic sperm injection in infertile patients with structural chromosome abnormalities, Hum. Reprod. 11 (1996) 2609–2612, https://doi.org/10.1093/oxfordjournals.humrep.a019179.

[14] M. De Braekeleer, T.-N. Dao, REVIEW: cytogenetic studies in male infertifity, Hum. Reprod. 6 (1991) 245–250, https://doi.org/10.1093/oxfordjournals.humrep.a137315.

[15] M. Simoni, F. Tüttelmann, J. Gromoll, E. Nieschlag, Clinical consequences of microdeletions of the Y chromosome: the extended Münster experience, Reprod. Biomed. Online 16 (2008) 289–303, https://doi.org/10.1016/S1472-6483(10)60588-3.

[16] K. Van Der Ven, M. Montag, B. Peschka, J. Leygraaf, G. Schwanitz, G. Haidl, D. Krebs, H. Van Der Ven, Combined Cytogenetic and Y Chromosome Microdeletion Screening in Males Undergoing Intracytoplasmic Sperm Injection, 1997.

[17] C. Foresta, A. Ferlin, A. Garolla, E. Moro, M. Pistorello, S. Barbaux, M. Rossato, High frequency of well-defined Y-chromosome deletions in idiopathic Sertoli cell-only syndrome, Hum. Reprod. 13 (1998) 302–307, https://doi.org/10.1093/humrep/13.2.302.

[18] G.C. dos, S. Godoy, B.B. Galera, C. Araujo, J.S. Barbosa, M.F. De Pinho, M.F. Galera, S.F. de Medeiros, The low prevalence of Y chromosomal microdeletions is observed in the oligozoospermic men in the area of mato grosso state and amazonian region of Brazilian patients, Clin. Med. Insights Reprod. Health 8 (2014), https://doi.org/10.4137/cmrh.s15475. CMRH.S15475.

[19] E.C. Akınsal, N. Baydilli, M. Dündar, O. Ekmekçioğlu, The frequencies of y chromosome microdeletions in infertile males, Turk J Urol 44 (2018) 389–392, https://doi.org/10.5152/tud.2018.73669.

[20] J.R.I., Y. Kim, S.Y. Kim, H.J. Lee, B.Y. Park, S.Y. Lee, H.S. Seo, Y Chromosome Microdeletions in Infertile Men with Non-obstructive Azoospermia and Severe Oligozoospermia, 2017.

[21] I.E. Aknin-Seifer, H. Lejeune, R.L. Touraine, R. Levy, Y chromosome microdeletion screening in infertile men in France: a survey of French practice based on 88 IVF centres, Hum. Reprod. 19 (2004) 788–793, https://doi.org/10.1093/humrep/deh157.

[22] S. Sen, A.R. Pasi, R. Dada, M.B. Shamsi, D. Modi, Y chromosome microdeletions in infertile men: prevalence, phenotypes and screening markers for the Indian population, J. Assist. Reprod. Genet. 30 (2013) 413–422, https://doi.org/10.1007/s10815-013-9933-0.

[23] M.T. Al-Ouqaili, S.K. Al-Ani, R. Alaany, M.N. Al-Qaisi, Detection of partial and/or complete Y chromosome microdeletions of azoospermia factor a (AZFa) sub-region in infertile Iraqi patients with azoospermia and severe oligozoospermia, J. Clin. Lab. Anal. 36 (2022), https://doi.org/10.1002/jcla.24272.

[24] M.N. Al-Qaisi, M.T. Al-Ouqaili, D.T. Al-Hadithi, Molecular Analysis for Azoospermia Factor Microdeletions in the Y Chromosome for Azoospermic and Severe Oligospermic Infertile Iraqi Patients, 2020.

[25] J.M. Pina-Neto, R.C.V. Carrara, R. Bisinella, L.F. Mazzucatto, M.D. Martins, E. Sartoratto, R. Yamasaki, Somatic cytogenetic and azoospermia factor gene microdeletion studies in infertile men, Braz. J. Med. Biol. Res. 39 (2006) 555–561, https://doi.org/10.1590/S0100-879X2006000400017.

[26] C. Sánchez, J.I. Vinay, R. Valdevenito, C. Palma, Prevalencia de microdeleciones del cromosoma Y en hombres chilenos infértiles, Rev. Int. Androl. 14 (2016) 80–85, https://doi.org/10.1016/j.androl.2015.11.002.

[27] T.P. Torres, X.B. Rojas, M.B. Narváez, E.L. Montanero, A.N. Sarasti, Triplex real-time polymerase chain reaction optimization for azf y-chromosome microdeletion analysis, J. Bras. Reprod. Assist. 19 (2015) 37–43, https://doi.org/10.5935/1518-0557.20150011.

[28] E. Fernández-Salgado, F. Alvarez-Nava, L. Borjas-Fajardo, J. Osuna, R. Gómez, W. Zabala, M. Zambrano, M.G. Portillo-M, [Molecular analysis of microdeletions of the Y chromosome in Venezuelan males with idiopathic infertility], Invest. Clin. 47 (2006) 395–403.

[29] B. Peterlin, T. Kunej, D. Hristovski, Diagnostic test for Y chromosome microdeletion screening in male infertility, Genet. Test. 8 (2004) 45–49, https://doi.org/10.1089/109065704323016021.

[30] A. Luddi, M. Margollicci, L. Gambera, F. Serafini, M. Cioni, V. De Leo, P. Balestri, P. Piomboni, Spermatogenesis in a man with complete deletion of USP9Y, N. Engl. J. Med. 360 (2009) 881–885, https://doi.org/10.1056/NEJMoa0806218.

[31] F. Elfateh, R. Wang, Z. Zhang, Y. Jiang, S. Chen, R. Liu, Influence of Genetic Abnormalities on Semen Quality and Male Fertility: A Four-Year Prospective Study, 2014.

[32] A. Hellani, S. Al-Hassan, A. Al-Duraihim, S. Coskun, Y chromosome microdeletions, Are they implicated in teratozoospermia? Hum. Reprod. 20 (2005) 3505–3509, https://doi.org/10.1093/humrep/dei254.

[33] D. Sevinç, A. Zehir, A. Biricik, S. Sertyel, S. Kahraman, I. Güney, The relation between human Y chromosome microdeletions and sperm morphology, Marmara Medical Journal 15 (2002) 227|–232.

[34] M.N. Al-Qaisi, M.T. Al-Ouqaili, D.T. Al Hadithi, The correlation between azoospermia factor microdeletion in the Y chromosome and anti-sperm antibody generation in infertile Iraqi patients, Journal of Emergency Medicine, Trauma and Acute Care (2022) 2022, https://doi.org/10.5339/jemtac.2022.aimco.4.

[35] S.H. Park, H.S. Lee, J.H. Choe, J.S. Lee, J.T. Seo, Success rate of microsurgical Multiple testicular sperm extraction and sperm presence in the ejaculate in Korean men with Y chromosome microdeletions, Korean J Urol 54 (2013) 536, https://doi.org/10.4111/kju.2013.54.8.536.

[36] C. Gonçalves, M. Cunha, E. Rocha, S. Fernandes, J. Silva, L. Ferraz, C. Oliveira, A. Barros, M. Sousa, Y-chromosome microdeletions in nonobstructive azoospermia and severe oligozoospermia, Asian J. Androl. 19 (2017) 338–345, https://doi.org/10.4103/1008-682X.172827.

[37] M.A. Arshad, A. Majzoub, S.C. Esteves, Predictors of surgical sperm retrieval in non-obstructive azoospermia: summary of current literature, Int. Urol. Nephrol. 52 (2020) 2015–2038, https://doi.org/10.1007/s11255-020-02529-4.

[38] P.J. Stahl, P. Masson, A. Mielnik, M.B. Marean, P.N. Schlegel, D.A. Paduch, A decade of experience emphasizes that testing for y microdeletions is essential in American men with azoospermia and severe oligozoospermia, Fertil. Steril. 94 (2010) 1753–1756, https://doi.org/10.1016/j.fertnstert.2009.09.006.