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Characterization of Y Chromosome Microdeletions Among Infertile Men in Peru

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ABSTRACT

Y chromosome microdeletions represent the second most common genetic cause of male infertility. Despite their significance in guiding infertility treatment, no studies have previously investigated this in Peru. This study aimed to assess the prevalence and patterns of Y chromosome microdeletions among men seeking infertility evaluation at a specialized reproductive medicine center in Peru. In this study, 201 men enrolled in Niu Vida's fertility program provided semen samples for analysis. Seminal parameters were assessed according to the 2010 World Health Organization (WHO) Laboratory Manual guidelines. For molecular testing, both a buccal swab and a 500 µL portion of each semen sample were collected to investigate Y chromosome microdeletions. The study focused on identifying the presence and distribution of deletions in the AZFa, AZFb, and AZFc regions. Y chromosome microdeletions within the AZF regions were detected in 6.45% of patients with oligozoospermia or azoospermia, with a higher prevalence of 20% observed specifically among azoospermic individuals. No AZFb deletions were identified in the cohort. Additionally, a partial AZFa microdeletion was found in a teratozoospermic patient who exhibited a normal sperm concentration. This study provides the first evidence of Y chromosome microdeletions in the Peruvian male population. The data show a notably higher frequency of microdeletions among azoospermic men compared to previously reported cohorts. These findings highlight the importance of further screening for AZFa deletions and examining additional genetic markers in this region to pinpoint mutations that may underlie defective sperm production and male infertility in Peru.

Keywords: Y chromosome microdeletions, Infertile men, Peru, Sperm

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Introduction

Infertility is recognized as a medical condition in which a clinical pregnancy does not occur after one year of regular unprotected intercourse, or when a person is unable to reproduce independently or with a partner [1]. Male-factor infertility accounts for a considerable global burden, affecting roughly 7% of men, and the underlying 原因 often remains elusive [2]. Although many diagnostic approaches exist, the interpretation of these tests is frequently subjective and imprecise [3]. Notably, approximately one-fifth of infertile men fall into categories in which no clear cause is identified: idiopathic male infertility, where semen parameters are abnormal without an identifiable reason, and unexplained male infertility, in which men present normal semen analyses but still fail to achieve conception [4].

Genetic factors contribute substantially to male infertility. Chromosomal anomalies such as Klinefelter syndrome (47,XXY) [5], congenital bilateral absence of the vas deferens, Y-chromosome microdeletions, and mutations associated with cystic fibrosis are well-documented causes [6, 7]. The Y chromosome is particularly important because the Yq region contains genes essential for testicular function and spermatogenesis. The Azoospermia Factor (AZF) regions, especially AZFc, contain multiple palindromic and repeated sequences that are prone to recombination errors, making them vulnerable to intrachromosomal deletions during sperm production [8].

Microdeletions in the Y chromosome are considered the second most prevalent genetic cause of male infertility, with an estimated frequency of around 7% among infertile males [9]. Their prevalence varies considerably across

different ethnic and geographic populations [10, 11]. Most deletions occur in the AZFc domain (about 60%), followed by AZFb (16%) and AZFa (5%), while combined deletions involving two or all three AZF regions represent approximately 14% of diagnosed cases [12].

Advances in reproductive technology, particularly intracytoplasmic sperm injection (ICSI), have enabled couples affected by chromosomal abnormalities to conceive despite male-factor infertility [13, 14]. Because these genetic defects can be transmitted to future offspring, genomic screening has become an essential component in the evaluation of infertile men.

This study therefore aimed to determine the frequency and specific characteristics of Y-chromosome microdeletions within the AZFa, AZFb, and AZFc subregions using polymerase chain reaction (PCR) in male patients evaluated at a Peruvian assisted reproduction center.

Materials and Methods

Patient selection and design

This prospective experimental study was conducted at the Niu Vida assisted reproduction center following approval from the institutional ethics committee. A total of 201 men who participated in the center's assisted reproduction program during 2018–2019 were enrolled after providing informed consent. Karyotyping was not performed because of cost limitations and ethical considerations that extended beyond the scope of this investigation.

Semen samples were assessed according to the 2010 WHO Laboratory Manual guidelines. Inclusion criteria required participants to be legally adult males (18 years and older in Peru) without known prior infertility and willing to participate in the study. Exclusion criteria removed men over 65 years of age—due to comorbidities and medications that could influence semen quality—as well as individuals with previous reproductive surgeries and non-Peruvian patients, ensuring a more controlled and homogeneous sample group.

Semen analysis

Samples were collected in sterile, labeled containers and allowed to liquefy for 20–30 minutes. Macroscopic evaluation included volume, appearance, viscosity, color, pH, and liquefaction time. Microscopic analysis was performed in two stages: concentration and motility were determined using a Mackler chamber, followed by slide smears of 20 µL for morphological evaluation and inspection for additional cells, agglutinations, or atypical elements.

DNA extraction

To maintain a non-invasive sampling protocol, DNA was extracted from oral swabs [10] and from 500 µL aliquots of semen, without the use of blood samples. Extraction was performed using the QIAamp DNA Investigator Kit (QIAGEN, Cat. No./ID: 56504). DNA concentration and purity were assessed via a ThermoFisher NanoDrop 2000, adjusted to 5–30 ng/µL, and stored at −20 °C.

Polymerase chain reaction (PCR)

Six sequence-tagged sites (STS) corresponding to the AZF regions were amplified according to the methodology of Krausz *et al*. [8]. Primer information for each AZF locus is provided in **Table 1**. PCR reactions were prepared in 15 µL volumes using 1 µL of genomic DNA. Thermal cycling consisted of an initial denaturation at 95 °C for 5 minutes; 35 cycles of 94 °C for 30 seconds, 57 °C for 90 seconds, and 72 °C for 60 seconds; a final extension at 72 °C for 10 minutes; and storage at 4 °C. Two internal controls were included: the SRY gene (sex-determining region of the Y chromosome) and the ZFX/Y zinc-finger gene pair.

Table 1. Primer sequences applied in the PCR assays. The analysis incorporated two internal control genes (ZFX/Y and SRY) and utilized two separate multiplex panels, labeled A and B.

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′	

Detection of amplification products with vertical electrophoresis

PCR amplicons were separated on polyacrylamide gels using the Silver Sequence Kit (PROMEGA). Vertical electrophoresis was performed at 80 V/cm for 45 minutes. Following electrophoresis, gels were photographed for documentation, and fragment sizes were assessed using a 100 bp GeneRuler allelic ladder as a reference.

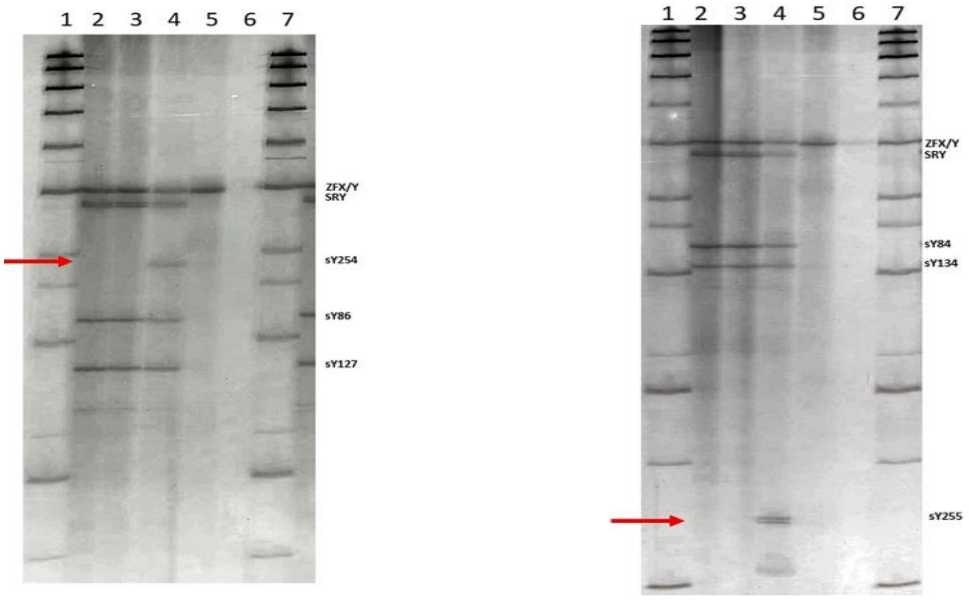
Statistical analysis

Data analysis was conducted using SPSS version 19.0. Differences between groups were evaluated with the chi-square test, and a p-value of less than 0.05 was considered statistically significant. Each experiment included controls consisting of DNA from a fertile male with biological children and from a female.

Results and Discussion

Among the 201 men evaluated, 167 (approximately 83%) presented with abnormalities in at least one semen parameter. The distribution of these alterations was as follows: 21 patients (10.4%) exhibited oligozoospermia, 50 (25%) had asthenozoospermia, 108 (54%) showed teratozoospermia, and 10 (5%) were azoospermic. Additionally, 33.5% of the participants displayed abnormalities in two or more semen parameters.

Y chromosome microdeletions were detected in the AZFc region. Specifically, the sY254 marker was absent in both seminal and oral swab samples in lanes 2 and 3 **(Figure 1a)**, and the sY255 marker was missing in lanes 2 and 3 **(Figure 1b).**



a) b)

Figure 1. Vertical electrophoresis of PCR products**.** Lanes 1 and 7 contain a 100 bp DNA ladder. **Gel A** shows the absence of the sY254 marker, indicating an AZFc microdeletion in both the seminal (lane 2) and oral swab (lane 3) samples. **Gel B** demonstrates the absence of the sY255 marker in the same samples (lanes

2 and 3). Lane 4 represents DNA from a fertile male, lane 5 from a female, and lane 6 is the negative control. The red arrow highlights the AZFc microdeletion.

Figure 2. Partial AZFa microdeletion in the Y chromosome. The absence of the sY86 band in both seminal (lane 2) and oral swab (lane 3) samples indicates a deletion in the AZFa region.

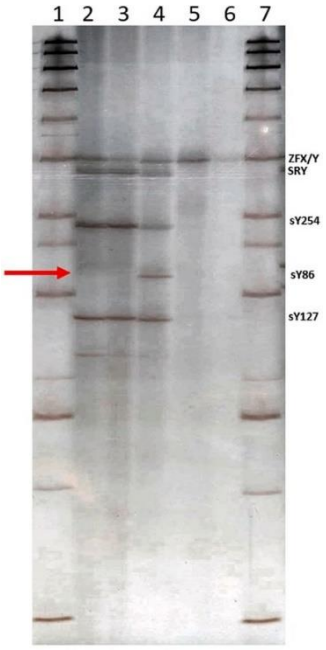


Figure 2. Vertical electrophoresis of PCR products. Lanes 1 and 7 contain a 100 bp DNA ladder. The absence of the sY86 band in both seminal (lane 2) and oral swab (lane 3) samples indicates a partial AZFa microdeletion. Lane 4 corresponds to DNA from a fertile male, lane 5 to DNA from a female, and lane 6 is the negative control. The red arrow highlights the AZFa microdeletion.

Across the 201 samples analyzed, three Y chromosome microdeletions (1.5%) were identified, comprising 0.5% in the AZFa region and 1% in the AZFc region. No microdeletions were detected in the AZFb region **(Table 3).**

Table 2. Distribution of semen abnormalities in the study cohort, classified according to WHO seminal parameters.

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

Table 3. Observed Y chromosome microdeletion patterns in the study cohort. One deletion was detected in the AZFa region and two deletions in the AZFc region. No microdeletions were identified in the AZFb region.

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

Among the 10 azoospermic patients analyzed, two Y chromosome microdeletions (20%) were identified. Additionally, a partial AZFa microdeletion was detected in a teratozoospermic patient with a normal sperm count, representing 0.6% of patients with normozoospermic profiles **(Table 4).**

Table 4. Distribution of detected Y chromosome microdeletions according 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

Simoni *et al*. compiled data from 91 investigations conducted in 41 countries and involving 16,316 men, providing one of the most comprehensive overviews of Y-chromosome microdeletions to date [15]. Their analysis indicates that the distribution of deletions is heavily skewed toward the AZFc region (≈80%), while AZFa, AZFb, and AZFbc deletions remain relatively rare, occurring in roughly 0.5–4%, 1–5%, and 1–3% of reported cases, respectively [9]. In light of these global patterns, the absence of AZFb deletions in our cohort **(Table 2)** is not unexpected, especially considering that published frequencies range dramatically—from as low as 1% [16] to as high as 55% [17]. Such variability underscores the need for studies covering broader sample sizes and diverse geographic subpopulations to establish reliable regional estimates of deletion prevalence [18].

In our analysis, 20% of azoospermic men (2/10) carried Y-chromosome microdeletions **(Table 3)**, a proportion that exceeds many previously reported values. Lower frequencies have been documented in cohorts from Turkey (4.7%) [19], Korea (14%) [20], France (8%) [21], India (3.4%) [22], and several regions in Iraq—although some Iraqi studies report unusually high rates of 62.5–72.5% [23, 24]. These discrepancies likely reflect differences in recruitment strategy, diagnostic criteria, and population genetics, all of which can markedly influence the detection rate.

Focusing on Latin America, our overall microdeletion frequency among azoospermic and oligozoospermic men was 6.45% **(Figure 2)**. This aligns closely with regional reports: 7.5% in Brazil (12/160) [25], 9.8% in Chile (10/102) [26], 8.57% in Ecuador (3/35) [27], and 3.45% in Venezuela (1/29) [28]. Compared with Europe, where Peterlin *et al*. documented rates of 3.2% in Germany, 1.3% in Sweden, 2.3% in the Netherlands/Belgium, 3.6% in Ireland, and 4.4% in Slovenia [29], our findings fall within a similar range. No microdeletions were detected among our oligozoospermic participants.

One noteworthy observation from our study is the partial deletion in the AZFa region (STS s86) detected in a donor with normal sperm concentration but diagnosed with teratozoospermia. Given the rarity of AZFa deletions worldwide, this represents an important first report for our region. A comparable scenario was described by Luddi *et al*., who identified AZFa-associated USP9Y deletions in several normozoospermic individuals with familial links [30]. This parallel suggests that certain AZFa defects may exert subtle effects on spermatogenesis or sperm morphology, meriting further exploration of protein expression pathways in this region.

Studies from other regions provide mixed insights regarding teratozoospermia and Y-chromosome deletions. Elfateh *et al*. reported AZFb and AZFc deletions among teratozoospermic men in Iran [31], whereas such alterations were not observed in our teratozoospermic samples. Hellani *et al*. proposed germline mosaicism as a potential contributor to teratozoospermia after detecting microdeletions in both seminal and blood cells [32]; our findings, however, reveal identical deletions in buccal and seminal samples, arguing against mosaicism in the case we observed. Sevinç *et al*. likewise found microdeletions among teratozoospermic men, though in individuals who also exhibited oligospermia [33].

The interplay between Y-microdeletions and other infertility-related parameters remains an open question. For instance, Al-Qaisi *et al*. found no association between microdeletions and anti-sperm antibodies despite a high frequency of deletions in their population [34], suggesting that additional immunological or physiological factors might modulate these interactions.

Advances in testicular sperm extraction (TESE) have improved reproductive outcomes for men with severe infertility, yet the success of TESE is strongly influenced by the specific AZF region affected. Multiple studies agree that AZFa and AZFb deletions are associated with very poor sperm retrieval rates [35, 36], whereas AZFc deletions offer a far more optimistic prognosis, with retrieval successful in roughly 55% of cases [35, 37]. Both microdeletions identified in our azoospermic cohort involve the AZFc region, indicating a favorable likelihood for sperm recovery and potential intracytoplasmic sperm injection (ICSI) treatment [13]. Nevertheless, TESE remains an invasive technique with risks, and outcomes depend not only on the microdeletion profile but also on patient-specific factors such as age, hormonal status, and the degree of testicular impairment. Moreover, because

AZF deletions can be transmitted to male offspring, comprehensive genetic counseling is essential before pursuing ART.

Conclusion

PCR-based screening for Y-chromosome microdeletions should be considered an essential diagnostic step prior to initiating assisted reproductive technologies (ART) in men with severe oligospermia or non-obstructive azoospermia. In our cohort, AZFc deletions were identified exclusively in azoospermic patients, whereas AZFb deletions were absent, highlighting the need for studies with larger sample sizes to capture less frequent variants. The detection of an AZFa deletion in a donor with normal sperm count but teratozoospermia represents the first such report in our population and emphasizes the importance of evaluating sperm morphology when assessing potential AZF-associated abnormalities. Expanding molecular screening within the AZFa region and incorporating additional markers may help clarify the functional significance of these rare deletions and their impact on male fertility.

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