Genetic Screening of Yq Microdeletions Using Multiplex PCR in Infertile Men: A Review Report

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Abstract: Male infertility is a complex condition with multifactorial etiologies, among which genetic abnormalities play a crucial role. Y chromosome microdeletions, particularly in the Azoospermia Factor (AZF) regions located on the long arm (Yq11), are one of the most significant genetic contributors to spermatogenic failure. These deletions affect key genes involved in testicular development, sperm production, and maturation, often resulting in azoospermia or severe oligozoospermia. This review explores the structure and functional relevance of the AZF regions (AZFa, AZFb, AZFc, and AZFd), the mechanisms leading to microdeletions, and their clinical significance in male infertility. Emphasis is placed on the use of multiplex polymerase chain reaction (PCR) as a widely adopted molecular diagnostic tool for detecting microdeletions using sequence-tagged site (STS) markers. The review summarizes key findings from various studies across different populations, highlighting region-specific deletion frequencies and phenotype correlations. Additionally, the importance of integrating genetic screening in routine infertility evaluations and the need for standardized molecular protocols are discussed. Understanding the molecular basis of Y chromosome microdeletions can improve diagnostic accuracy, guide therapeutic interventions, and support informed reproductive counseling.

Keywords: Male infertility, Y-chromosome, Azoospermic factors, Microdeletion, , Multiplex PCR.

Introduction:

According to International Committee for Monitoring Assisted Reproductive Technology, World Health Organization (WHO) and Practice Committee of American Society for Reproductive Medicine (ASRM), infertility is failure to conceive an offspring during unprotected intercourse for 12 months (Zegers-Hochschild et al., 2009). Infertility arising as a major health problem and approximately 15% of men attending infertility clinics (Kamp et al., 2001).

Global Prevalence:

Estimating global infertility prevalence is challenging, as both male and female factors contribute to the condition, and most assessments tend to focus solely on female partners or

outcomes such as pregnancy or live birth. One in every four couples in developing countries had been found to be affected by infertility (WHO, 2021). Largescale studies have shown that about half of all cases of infertility occur due to female factors, 20-30% male factors and 10-20% due to common causes of both gender (WHO, 2021).

Within a year, around 25% of couples fail to conceive pregnancy, 15% seek medical help for infertility, and less than 5% of couples remain childless. Both men and women experience infertility (Dohle et al., 2005). Doctors of All India Institute of Medical Sciences (AIIMS) have reported that over 12–18 million couples in India are diagnosed with infertility every year. It has been reported that the average sperm count in Indian adult males has declined from approximately 60 million/ml three decades ago to around 20 million/ml today

Causes:

There are many factors affecting infertility like Lifestyle factors, Environmental factors, Genetic factors, Hormonal factors, Pathological causes and Unknown causes (Bala et al., 2021). Genetic factors have been claimed to be responsible for about 10% of cases. These genetic disorders affect semen parameters by causing alternation of chromosome materials such as Y chromosome microdeletion (Teka and Alfageih., 2018).

Male factor infertility accounts for approximately half of the cases (Attia et al., 2013) (Mascarenhas et al., 2016). In male infertility various factors affect infertility and one of the major factors is genetic factor where microdeletion in Y-chromosome is the second highest common genetic cause (Krausz et al., 2014).

Male infertility with genetic abnormalities being a leading cause (Tuttelmann and Nieschlag., 2010). Among these, microdeletions in specific regions of the Y chromosome, especially the AZFa, AZFb, AZFc and AZFd loci, have been well-documented in men with non-obstructive azoospermia and severe oligozoospermia (Krausz and Casamonti., 2017). Multiplex PCR has emerged as a rapid, cost-effective method for identifying these microdeletions, significantly improving diagnostic precision.

Prevalence of Y-chromosome microdeletion and male infertility:

The global frequency of Y chromosome microdeletions ranges from 1 percent to 55 percent, with rates of 7.6 percent to 16.5 percent in Japan, 11.0 percent to 19.4 percent in China, 10.6 percent to 11.7 percent in Taiwan, 6.4 percent in Hong Kong, and 2.0 percent to 12.0 percent in India. According to a recent study, the cumulative incidence of Y chromosomal microdeletions in infertile males was 3.5 percent (Kim et al., 2012).

The human Y chromosome contains genes critical for spermatogenesis, particularly within the AZF regions on the long arm (Yq11). A genetic factor located at Yq11 has been established to be important for male germ cell development, and this gene cluster is referred to as the Azoospermia Factor (AZF) (Mitra et al., 2008). Y-chromosome microdeletions represent the absence of DNA segments or gene(s) from the functionally active part of the Y chromosome (Suganthi et al., 2014). Genetic deletions within these regions are known to impair spermatogenesis and hinder sperm maturation (Vogt et al., 1996). Among genetic anomalies, AZF region microdeletions on the Y chromosome represent the most prevalent cause of male infertility

AZF subdivided into four non-overlapping sub-regions: -
1) AZFa (Proximal)
2) AZFb (Middle)
3) AZFc (Distal)
4) AZFd (Between AZFb and AZFc)

At both ends of the Y chromosome are the pseudoautosomal regions (PARs), which pair with the X chromosome during meiosis. The non-recombining region of the Y chromosome is the area outside the PARs that does not recombine (NRY). The SRY (sex-determining region Y) gene, which plays a pivotal role in testicular development, has been identified on the short arm of the Y chromosome, positioned near the pseudoautosomal boundary (Mojtabanezhad et al., 2018). AZFa deletions are associated with Sertoli cell-only syndrome, AZFb with spermatogenic arrest, and AZFc with hypospermatogenesis (Reijo et al., 1995).

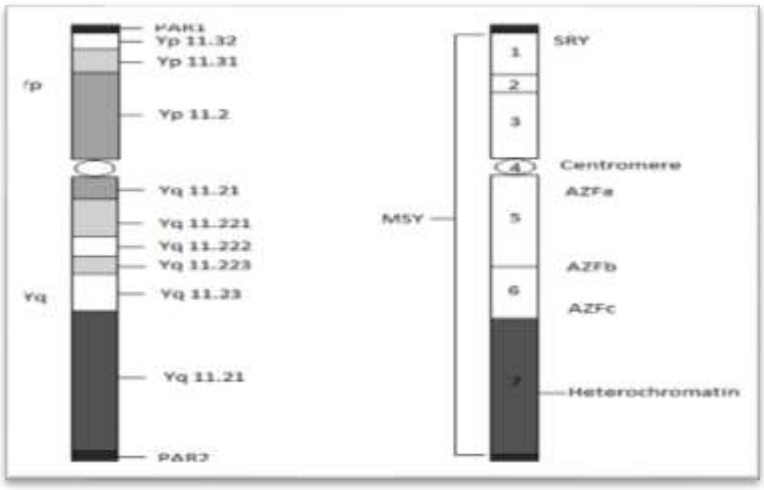

Figure 1: Representation of cytogenetic partitions of Y chromosome and showing pseudoautosomal region- PAR1 and PAR2. Seven interval map of Human Y chromosome and AZF regions- AZFa, AZFb and AZFc (Nailwal and Chauhan, 2017).

Multiplex PCR:

Multiplex PCR enables simultaneous amplification of multiple STS markers in a single reaction. Primers are designed to avoid dimerization and ensure uniform annealing temperatures. Commercially available kits (from Promega, HiMedia) and customized protocols are used depending on lab settings. The European Academy of Andrology (EAA) and EMQN have standardized guidelines for multiplex PCR in detecting Yq microdeletions (Simoni et al., 2004).

Multiplex PCR is widely applied in fertility clinics to screen men with idiopathic infertility. It helps in pre-IVF genetic counseling and avoids unnecessary treatments for non-reversible cases like complete AZFa deletion. The assay is reliable, and results are often confirmed with repeated runs or sequencing. Studies across various populations report differing prevalence rates of Yq microdeletions, ranging from 5–10% in azoospermic men. Indian cohorts show

AZFc as the most frequently deleted region (Thangaraj et al., 2003). Such studies reinforce the role of ethnicity in genetic variation.

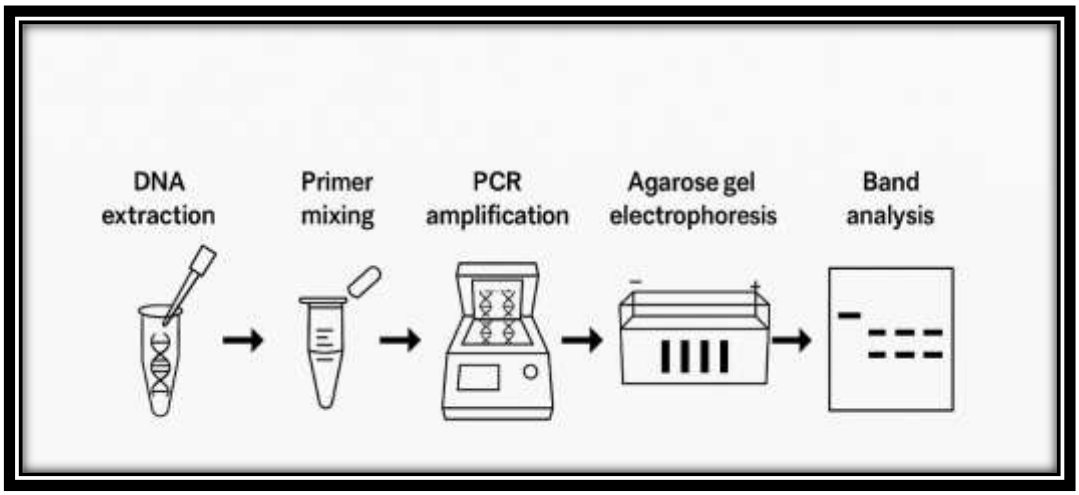

Figure 2: Schematic diagram of multiplex PCR workflow showing: DNA extraction → primer mixing → PCR amplification → agarose gel electrophoresis → band analysis.

Y Chromosome Microdeletions and Multiplex PCR:

Mojtabanezhad et al. analyzed 80 male subjects, including 40 non-obstructive infertile men, 20 with partners experiencing recurrent pregnancy loss (RPL), and 20 fertile controls. Using multiplex PCR to amplify 19 STS markers within the AZF regions, only one infertile individual showed Y chromosome microdeletions (SY254, SY157, SY255). No significant association was found between these deletions and either non-obstructive infertility (P = 0.48) or RPL (Mojtabanezhad et al.,2018).

Elsaid et al. examined 51 infertile men and four fertile couples (controls) to investigate AZF microdeletions using multiplex PCR targeting 12 STS markers. Among the infertile group, AZF microdeletions were found in 16 of 25 azoospermic and 14 of 26 oligozoospermic men. The AZFa region showed the highest frequency of deletions (n = 11), followed by AZFc and AZFd (n = 4 each), and AZFb (n = 3). Notably, AZFa deletions were most common among oligozoospermic individuals (10 out of 14) and showed a significant association with the oligozoospermic phenotype (Elsaid et al.,2021).

Kovacheva et al. conducted a retrospective study on 142 infertile Bulgarian men, including 63 with azoospermia and 79 with severe oligozoospermia. Among 109 subjects tested using multiplex PCR for AZF microdeletions, chromosomal abnormalities were observed in 16.8% overall—20.7% in azoospermic and 13.9% in oligozoospermic patients. The overall frequency of Yq microdeletions (delYq) was 5.5%, rising to 9.5% in azoospermic individuals. Combined, chromosomal abnormalities and Yq deletions accounted for approximately 22% of severe male infertility cases in this population (Kovacheva et al.,2018).

Akbarzadeh et al. investigated chromosomal abnormalities and AZFb, AZFc, and AZFd microdeletions in 100 idiopathic non-obstructive infertile men (78 azoospermic, 22 oligospermic) compared to 100 fertile controls. Y chromosome microdeletions were identified using STS-based multiplex PCR. Microdeletions were detected in 32.05% of azoospermic patients, with no deletions observed in the oligospermic group. Among the azoospermic cases,

AZFc was the most commonly affected region, accounting for 80% of deletions (Akbarzadeh et al., 2020)

Waseem et al. analyzed Y chromosome microdeletions in 379 infertile Indian males (302 azoospermic, 77 oligozoospermic) and 265 fertile controls using PCR and gel electrophoresis. AZF deletions were found in 10.02% of infertile men, with no deletions detected among controls. The AZFc region showed the highest frequency of deletions, followed by AZFa and AZFb. Across Indian populations, the prevalence of AZF deletions ranged from 0.59% to 32.62%, averaging 13.48% (Waseem et al.,2020).

Ambulkar et al. investigated Y chromosome microdeletions in 160 infertile men (90 oligozoospermic, 70 azoospermic) and 50 fertile controls using PCR screening with 18 STS markers spanning AZFa, AZFb, AZFc, and SRY regions. Microdeletions were identified in 10.6% of cases, with AZFc being the most frequently deleted region (58.8%), followed by AZFb and AZFa. Four individuals showed deletions across multiple STS loci (Ambulkar et al., 2017)

Suganthi et al. conducted a PCR-based screening for Y chromosomal microdeletions in 75 men, including 30 non-obstructive azoospermic, 20 severe oligozoospermic, and 25 fertile controls, using 15 STS primer pairs targeting the AZF regions. An optimized multiplex PCR protocol was developed. The overall microdeletion frequency was 36%, with deletions detected in 40% of azoospermic and 30% of oligozoospermic individuals, while no deletions were found in fertile controls (Suganthi et al.,2014).

Mitra et al. analyzed 271 males, including 170 infertile patients (51 oligospermic and 119 azoospermic) and 101 fertile controls, to investigate Y chromosome microdeletions. Deletions were detected in 9 out of 170 (5.29%) infertile men, all of whom were azoospermic. Among them, deletions were distributed across AZFa (2), AZFb (1), AZFc (3), and combined AZFb+c (3). No deletions were observed in severe oligospermic or fertile control subjects (Mitra et al.,2008)

Dutta et al. examined Y chromosome microdeletions in 118 infertile males categorized as azoospermic (N = 63), severe oligozoospermic (N = 38), and oligozoospermic (N = 17), using STS-based PCR targeting the AZF region and SRY. Microdeletions were detected in 16.1% (19/118) of the cases, with a higher frequency among azoospermic individuals (17/19) and none in controls (Dutta et al.,2021)

Nailwal and Chauhan conducted the first analysis of Y chromosome microdeletions in the Gujarati population, screening 100 men (50 infertile and 50 fertile controls) using STS markers for the SRY gene and AZFa/AZFb regions. Microdeletions were observed in 6% (3/50) of the infertile men, specifically in the AZFa sub-region among oligozoospermic individuals, with no deletions found in the AZFb region (Nailwal and Chauhan.,2017).

World-wide comparative study on Y chromosome microdeletion using multiplex PCR:

International					
Multiplex No.	**Kind of multiplex**	**AZF sub-region STS markers**	**Author and Year**	**Place**	**Sample studied**
2	AZF b+c AZF a+b+c	A- sY81(209bp), sY86(318bp), sY182(125bp) B- sY212(190bp), sY124(274bp), sY127(274bp), sY128(228bp), sY133(177bp), sY134(301bp), C- sY157, sY208, sY242, sY254(380bp), sY255(123bp).	(Mojtaban et al., 2017)	Iran	40
5	AZF b+c AZF b+c+d AZF a+b+c AZF b+d AZF a+c	A- sY81(209bp), sY86(318bp), sY84(326bp) B-sY127(274bp), sY128(228bp) C- sY239(201bp), sY254(380bp), sY255(123bp)	(Elsaid et al., 2021)	Sudan	51
1	AZF b+c	A- sY84(326bp), sY86(318bp) B- sY127(272bp), sY134(301bp) C- sY254(380bp), sY255(123bp)	(Kovacheva et al., 2018)	Bulgaria	137
3	AZF b+c AZF c+d AZF b+c+d	B- sY127(274bp), sY133(177bp), sY134(301bp) C- sY254(380bp), sY255(123bp), sY277 D- sY145(125bp), sY152, sY153(135bp)	(Akbarzadeet al., 2020)	Iran	100
2	AZF c+d AZF b+c+d	A- sY84(326bp), sY86(318bp) B- sY127(272bp), sY134(301bp) C- sY254(380bp), sY255(123bp), sY160	(Okutman et al., 2018)	Turkey	374
2	AZF b+c AZF a+b+c	A- sY84(326bp), sY86(318bp) B- sY127(272bp), sY134(301bp) C- sY254(380bp), sY255(123bp)	(Bahmanimehr et al., 2018)	Iran	40
1	AZF a+b+c	A- sY84(326bp), sY86(318bp), sY82(264bp), sY88(123bp), sY1182(274bp) B- sY127(272bp), sY134(301bp), sY105(301bp), sY212(190bp), sY153(139bp), sY143(311bp) C- sY254(380bp), sY255(123bp), sY160(236bp), sY1191(385bp), sY1291(509bp)	(Luong *et al*, 2021)	China	150

National					
Multiplex No.	**Kind of multiplex**	**AZF sub-region STS markers**	**Author and Year**	**Place**	**Sample studied**
3	AZF b+c AZF a+b AZF b+c	A- sY84(326bp), sY86(318bp) B- sY127(272bp), sY134(301bp), sY118(218bp), sY113(304bp) C- sY255(123bp), sY158(231bp), sY153(139bp)	(Ambulkar & Pande, 2017)	India	110
1	AZF a+b+c	A- sY84(326bp), sY83(275bp), sY90(176bp) B- sY134(301bp), sY127(274bp), sY143(311bp), sY117(260bp) C- sY254(380bp),sY158(231bp), sY283(497bp)	(Suganthi et al., 2014)	India	75
1	AZF b+c	A- sY84(326bp), sY86(318bp), sY81(209bp) B- sY127(272bp), sY134(301bp), sY118(218bp), sY113(304bp) C- sY255(123bp), sY158(231bp), sY153(139bp), sY254(380bp)	(Mitra et al., 2008)	India	271
1	AZF b+c	A- sY84(326bp), sY86(318bp) B- sY127(272bp), sY134(301bp) C- sY254(380bp), sY255(123bp)	(Waseem et al., 2020)	India	379
1	AZF a+b+c	A- sY84(326bp), sY86(318bp), sY746(216bp) B- sY127(272bp), sY134(301bp), sY99(350bp) C- sY254(380bp), sY255(123bp), sY156(950bp)	(Poongothai et al., 2021)	India	300
2	AZF a+b+c AZF b+c	A- sY84(326bp), sY86(318bp) B- sY127(272bp), sY134(301bp) C- sY254(380bp), sY255(123bp)	(Rabinowitz et al., 2021)	India	250

Conclusion:

Molecular analysis using STS PCR helps us to identify microdeletions in Y-chromosome specifically AZFa, AZFb and AZFc sub-regions. **Multiplex PCR** help use to simultaneously detect many microdeletions in a single individual (sample) associated with AZFa, AZFb and AZFc sub-regions in males with infertility. Multiplex PCR remains a robust and cost-effective method for detecting Y chromosome microdeletions in infertile men. It plays a vital role in molecular diagnostics, guiding reproductive decision-making. Ongoing advances promise even more accurate and accessible tools for genetic screening in the near future.

Infertility represents a major crisis for most couples, with both partners experiencing loss in ways that affect them as individuals, as family members, and as members of society as a whole. Infertility has significant negative social impacts on the lives of infertile couples and particularly women, who frequently experience violence, divorce, social stigma, emotional stress, depression, anxiety and low self-esteem. Present study helps in the sensitive and specific diagnosis of male infertility which in turn helpful to society and reduces psychological and economic burden.

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