INDIAN COUNCIL OF MEDICAL RESEARCH | NATIONAL INSTITUTE FOR RESEARCH IN REPRODUCTIVE HEALTH

Policy Brief

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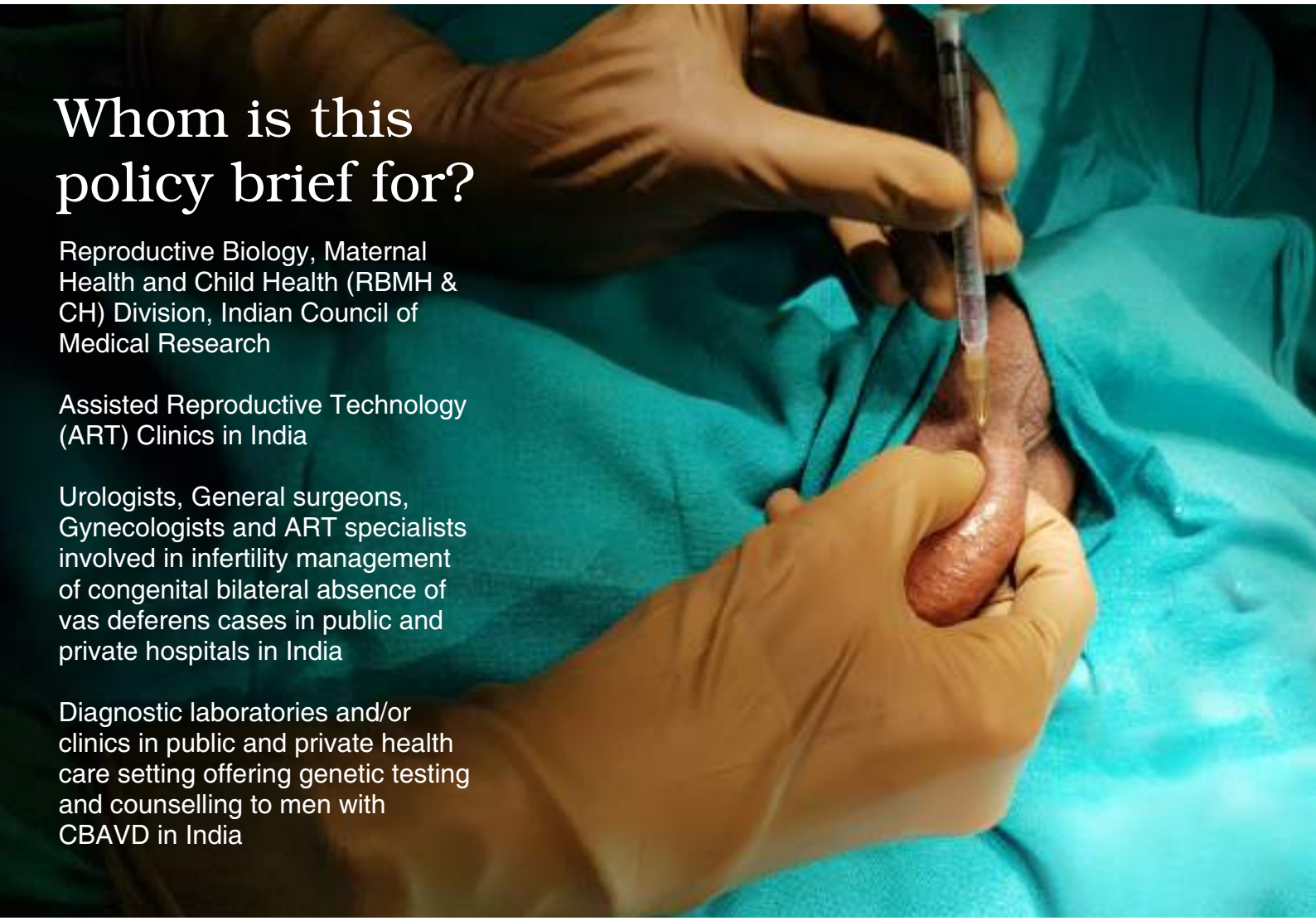
Genetic testing (CFTR-mutations) in Indian men with congenital bilateral absence of vas deferens (CBAVD) before undergoing Intracytoplasmic Sperm Injection (ICSI)



Whom is this policy brief for?

Reproductive Biology, Maternal Health and Child Health (RBMH & CH) Division, Indian Council of Medical Research

Assisted Reproductive Technology (ART) Clinics in India

Urologists, General surgeons, Gynecologists and ART specialists involved in infertility management of congenital bilateral absence of vas deferens cases in public and private hospitals in India

Diagnostic laboratories and/or clinics in public and private health care setting offering genetic testing and counselling to men with CBAVD in India

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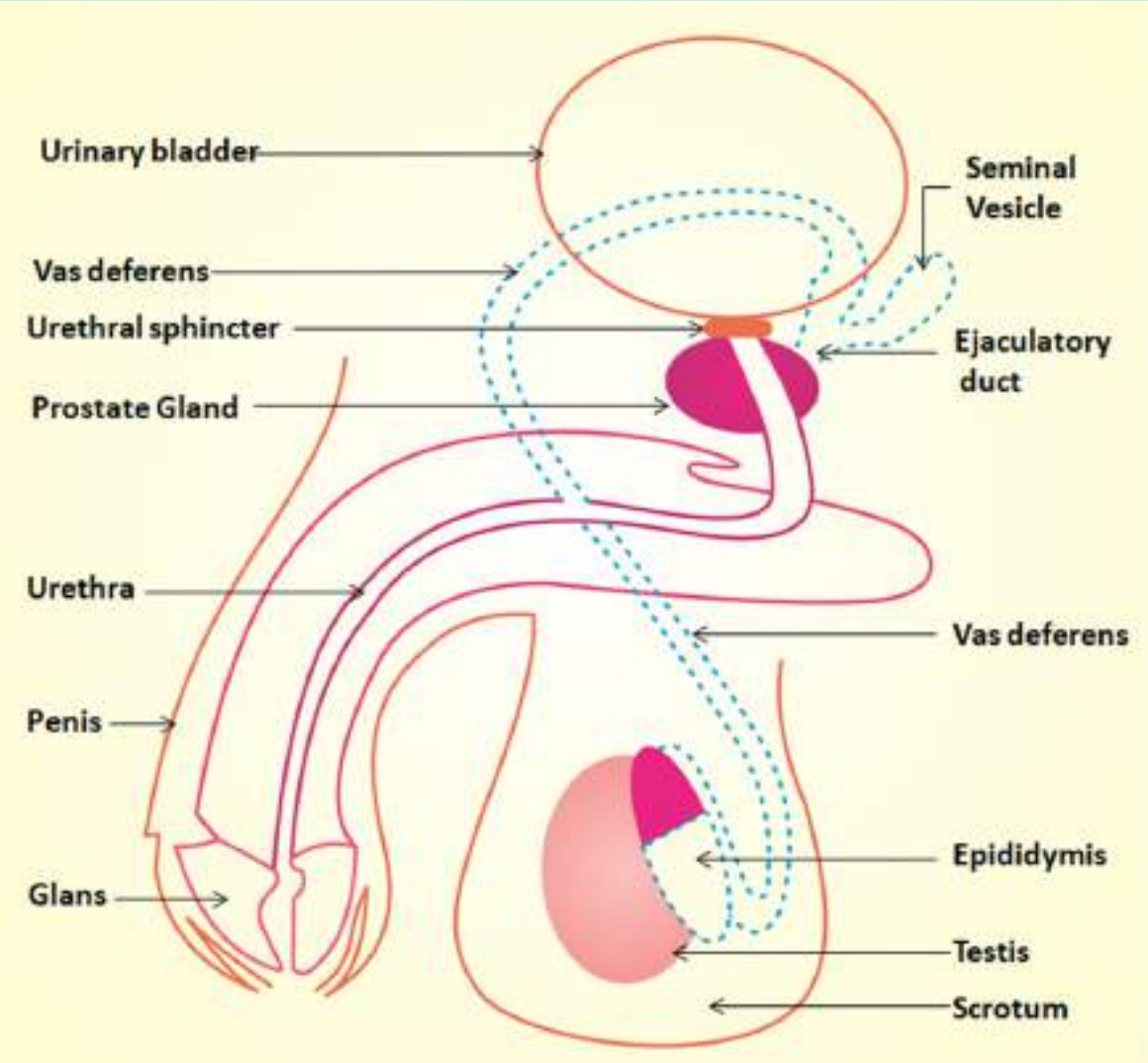

Figure 1: Male reproductive system showing absence of vas deferens (CBAVD)

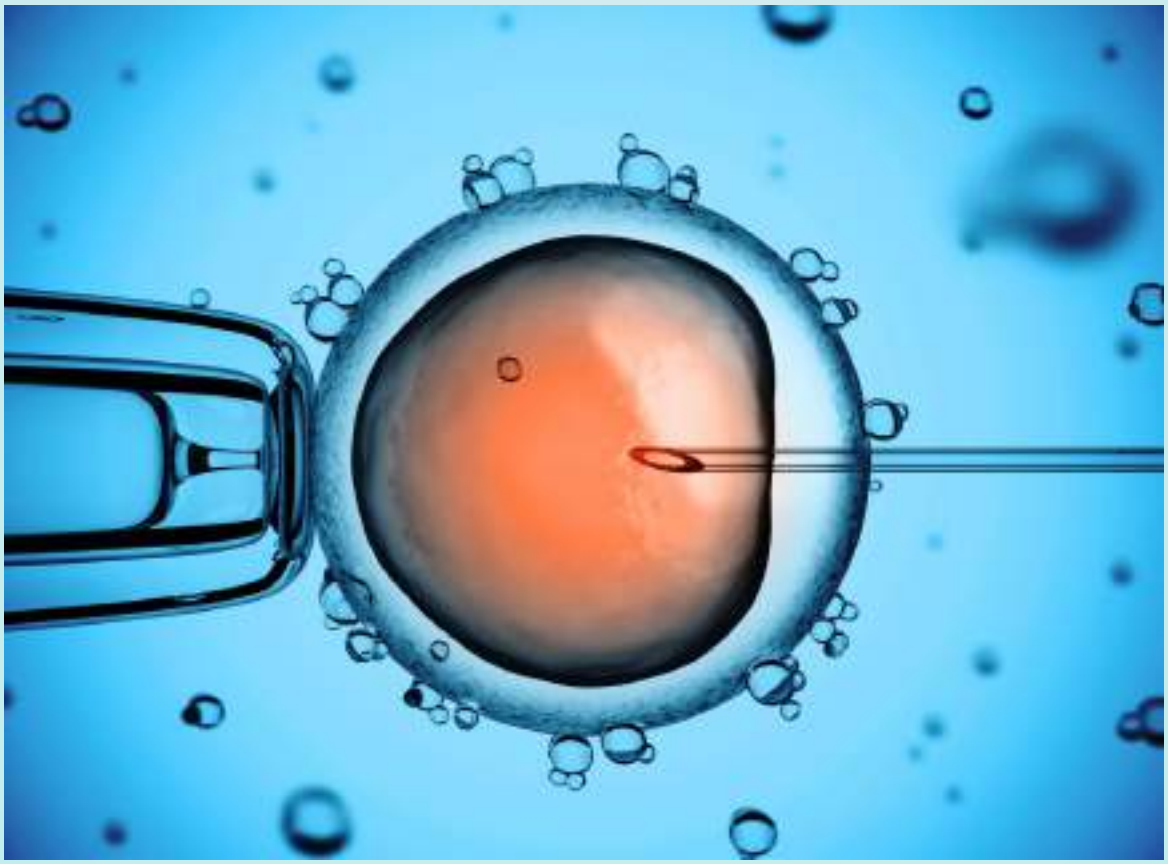

Figure 2: Pictorial presentation of Intracytoplasmic sperm injection (ICSI)

Executive Summary

Congenital bilateral absence of vas deferens (CBAVD) is responsible for 2-6 % of male infertility cases[1] and use of Assisted Reproductive Techniques (ART) especially testicular or epididymal sperm aspiration and Intracytoplasmic Sperm Injection (ICSI) is the treatment of choice to achieve biological fatherhood in men having CBAVD[2]. Currently, there are 490 ART Clinics enrolled under National Registry of ART Clinics and Banks in India[3] ; however, there are around 2000 ART clinics identified across the country[4]. There is a risk of transmission of genetic abnormalities to the progeny if sperm are used from a man with CBAVD for ICSI[3,4]. The most important findings of our recent study at ICMR-NIRRH suggest that the type and incidence of CFTR mutations in Indian population is different from Western populations ; hence limited western panels will not detect many of the mutations, and entire *CFTR* gene needs to be studied [5,6,7]. The aim of the policy brief is to call attention of urologists, general surgeons, gynecologists, ART specialists and reproductive health policy makers in India to sensitize them on extensive screening of *CFTR* gene and importance of genetic counselling for men having CBAVD and their female partners. There is a need to provide instructions and guidelines for *CFTR* gene screening in the National Guidelines for Accreditation, Supervision & Regulation of ART Clinics in India.

Introduction

Male infertility associated with congenital bilateral absence of vas deferens (CBAVD), an autosomal recessive disorder was once considered a rare disorder in non-Europeans[1]. Hence, the diagnosis and management of male infertility due to CBAVD was neglected in Asian population particularly in India. The incidence of CBAVD was reported to be 2-6 % in male infertility[1]. The abnormalities in cystic fibrosis transmembrane conductance regulator *(CFTR)* gene are known to be associated with CBAVD. There are more than 2000 mutations identified in *CFTR* gene (Cystic Fibrosis Mutation Database, CFMD, 2017) which are classified as severe (Classes 1-3) and mild (Classes 4 or 5) based on their functional and phenotypic effect[1]. The distribution of *CFTR* gene mutations and genotypes is different in CBAVD than cystic fibrosis (CF). Two severe mutations lead to CF; whereas two mild mutations or one severe and one mild mutation lead to CBAVD[1]. The frequency of p.F508del is higher in European men with CBAVD and p.F508del in trans with IVS8-5T (28%) and p.F508del in trans with p.R11 7H (6%) are two most common compound heterozygous genotypes in European men with CBAVD[1]. Spectrum and frequency of *CFTR* gene mutations in Indian men with CBAVD was rarely investigated thereby leading to non-availability of population specific *CFTR* gene mutation panel.

Using recent advancement in assisted reproductive technologies such as Intracytoplasmic sperm injection (ICSI), men with CBAVD can become biological fathers[1,2]. If the female partner of a CBAVD male is a CF carrier, they possess a risk of transmitting the CFTR abnormalities to the progeny resulting in a child born with a full blown CF or CF related disorders such as CBAVD[1,2] highlighting the significance of screening female partners. Therefore, it is mandatory to screen for *CFTR* gene mutations in western population[1]. CBAVD and association of *CFTR* gene has been studied extensively in European population. Currently European population specific mutation panels are used for screening even in non-European populations including India.

Gap Analysis

Currently available CFTR mutation panels are based on Western population and have limitation in detection of mutation relevant to Indian population.

Methodology

The data was collected on socio-demographic, clinical, hormonal, semen parameters in Indian men with CBAVD. Ultrasonography (USG) of the abdomen & pelvis along with Transrectal USG (TRUS) was carried out. Extensive screening of *CFTR* gene abnormalities was carried out in 93 CBAVD men, their female partners and 50 proven fertile normal men from general population.

Results and Conclusion

Azoospermia with low semen volume (<1 ml), acidic pH (< 6.8), absence/low fructose in semen, normal testicular volume, normal levels of FSH, LH, testosterone and absence of the bilateral vas deferens on scrotal examination clearly suggest an obstructive azoospermia due to CBAVD[6,9]. The classical phenotype shows presence of part of the caput of epididymis while body and tail are absent in Indian men with CBAVD[6] . However, many variants were observed with some men having larger segments of the epididymis, and sometimes even a part of the vas. Unilateral renal anomalies were detected in 9% of Indian men with CBAVD. Following sub-phenotypes of vas aplasia were detected in Indian population[5,6,7].

1. CBAVD without renal anomalies
2. CBAVD having unilateral renal anomalies (CBAVD-URA)
3. CUAVD (congenital unilateral absence of the vas deferens)
4. CUAVD- URA

Significant association was observed for *CFTR* gene variants in Indian CBAVD men versus controls[6]. A heterogeneous spectrum of *CFTR* gene variants was observed with 66.3% . frequency in Indian men with CBAVD which is lower than Caucasian population. CBAVD men were classified as patients with: i) one variant (51.25%) ii) two variants (15%) and iii) no mutations (33.7%). Twenty *CFTR* gene abnormalities were detected in 53 CBAVD men (66.3%)[6]. Eight novel missense *CFTR* gene variants (L214V, A238P, E379V, L578I, F587L, L926W, R1325K and R1453Q); two novel splice site gene variants (c.1-30C>G and IVS1+2T>G) and ten previously reported mutations (R75Q, c.1210-12[5], F508del, A309G, R334W, I444T, R668C, R709X, A1285V and Q1352H) were detected in Indian CBAVD men[6]. Out of the ten previously known *CFTR* gene mutations, 3 were severe mutations F508del (8.75%), R709X (2.5%) and R334W (1.25%) and majority were mild mutations IVS8-5T (42.5%), R75Q (3.75%), Q1352H (2.5%), A309G (1.25%), I444T (1.25%), R668C (1.25%), A1285V (1.25%).

Of the ten novel variants, seven were heterozygous missense [L214V, A238P, E379V, L578I, L926W, R1325K, and R1453Q]. A homozygous missense novel *CFTR* gene variant F587L was detected. Two of the novel splice site variants detected were c.1-30C>G in the essential promoter region and IVS1+2T>G. The most frequently detected novel *CFTR* gene variants were L926W (2.5%) and R1453Q (2.5%)[6].

Of the eighty female partners of CBAVD men screened, *CFTR* gene variant was detected in thirteen (16.2%) females. Twelve females harbored a c.1210-12[5] allele while one was a carrier of A1285V heterozygous missense *CFTR* gene variant[6]. The mutations identified spanned the different domains of the CFTR protein and are represented in Figure 1.

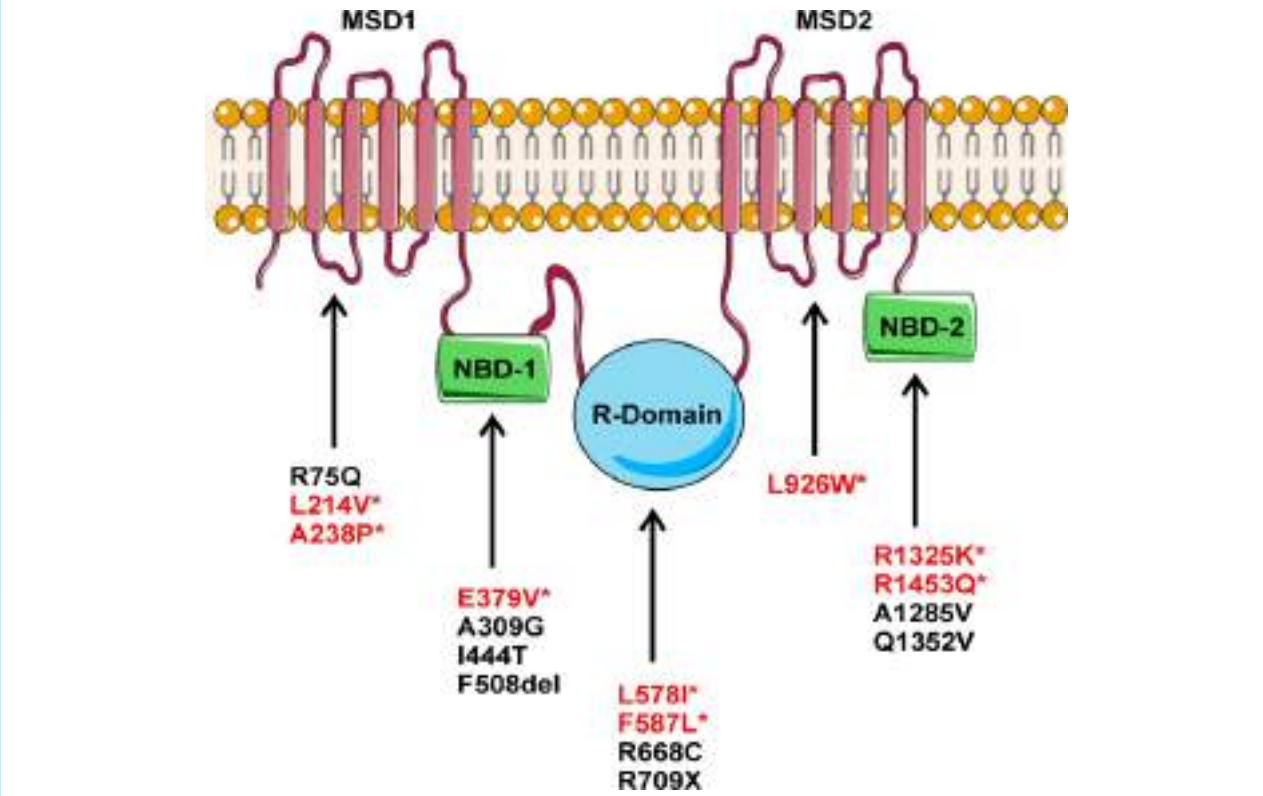

Figure 3: Schematic diagram representing putative domain-type structure of the CFTR protein and identified *CFTR* gene variants. Novel *CFTR* gene variants are indicated in red and *. Previously known *CFTR* gene mutations are indicated in black. MSD, membrane spanning domain; NBD, nucleotide binding domain and R-domain, regulatory domain.

Policy Recommendations and Implications

1. Screening of CFTR gene in Indian men with CBAVD: In India, screening for selected *CFTR* gene mutations based on European population is being practiced. Screening for selected *CFTR* gene mutations in Indian men will miss out clinically important mutations. F508del mutation (most commonly reported in European CBAVD men) was detected in only 8% of Indian CBAVD men. Majority of *CFTR* gene mutations detected in Indian CBAVD men were mild mutations. Only 3 severe mutations (F508del, R709X and R334W) were reported. Severe mutations were detected in 10 (12.5%) and mild mutations were detected in 43 (53.75%) Indian men with CBAVD. Ten novel mutations were also reported in Indian CBAVD men. Detection and understanding of severe and mild mutation is extremely important from the point of view of counselling as it may lead to offspring with milder phenotype such as CBAVD or full blown CF which is a lethal condition. Therefore, it is very important to screen for complete *CFTR* gene in Indian men with CBAVD to calculate accurate genetic risk. Therefore, we recommend extensive screening of *CFTR* gene (all 27 exons, exon-intron boundaries and essential promoter region of *CFTR* gene) in Indian CBAVD men.

2. Female CF Carrier screening in India: Twelve females harbored a mild mutation c.1210-12[5] while one was a carrier of mild mutation A1285V. None of the female partners were carrier of severe mutations. Nine couples had a risk of transmitting mutant CFTR allele to the offspring. Three CBAVD men had compound heterozygous *CFTR* gene mutations (L926W / c.1210-12[5] and c.1521_1523delCTT (F508del) /L578I, and their female partner harbored c.1210-12[5] or a *CFTR* gene mutation, increasing the risk by 50% for the offspring to inherit two *CFTR* gene variants. Six CBAVD men had one *CFTR* gene variant and their female partner harbored c.1210-12[5], thereby increasing risk by 25% for the offspring to inherit two *CFTR* gene variants. Therefore, It is important to screen female partners.

3. Genetic counselling: There is a need to sensitise urologists, general surgeons, gynaecologists dealing with infertility cases to counsel and refer the CBAVD male and his female partner for *CFTR* gene screening before performing Intracytoplasmic sperm injection (ICSI). Genetic counselling shall be provided to the couples based on the *CFTR* gene screening reports to prevent transmission of genetic risks to the offspring.

4. *CFTR* gene screening Policy for ART Clinics and Diagnostic Laboratories: National Guidelines for Accreditation, Supervision & Regulation of ART Clinics in India shall include *CFTR* gene screening in Indian men with CBAVD and their female partners before undergoing ICSI. The diagnostic laboratories in India shall provide screening of extensive *CFTR* gene (all 27 exons, exon-intron boundaries and essential promoter region of *CFTR* gene.

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