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## INVITED OPINION

Male Infertility

# Azoospermia: vasal agenesis

J Sam Fisher, Edward D Kim

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### SPECIFIC GENETIC TESTS (CFTR AND INTRON 8 SPLICE VARIANT TESTING) AND THEIR SIGNIFICANCE

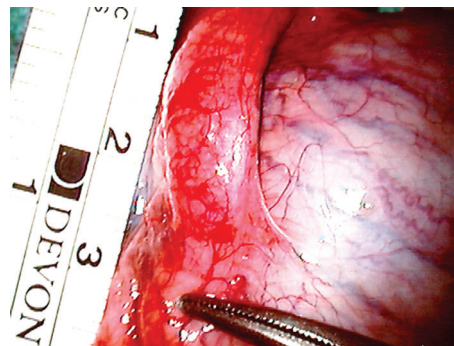
Congenital bilateral absence of the vas deferens (CBAVD) and cystic fibrosis (CF) began as separate entities with an apparent clinical correlation. CBAVD occurs in 1%–2% of men presenting with infertility at urological departments and has an incidence of 1:1000–1:2000 in Caucasian males. An example of this anatomic variant is shown in **Figure 1**. The rates in other ethnicities drop to 1:14 000–1:17 000 in African descendants and 1:90 000 in individuals of Asian descent. As early as 1971, researchers suspected an unusually mild form of CF existed in some males with CBAVD. Since that time, the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene has been determined as the causative defect in CF. Many men with no clinical signs of CF with CBAVD on examination exhibited silent mutations in the *CFTR* gene. Nearly 99% of patients carry at least one CF-associated *CFTR* mutation in the setting of CBAVD.<sup>1</sup> Clinical laboratories generally test for the most common 30–50 mutations found in cystic fibrosis.<sup>2</sup> A broader panel identifies up to 100 mutations; however, there are over 1800 total mutations, suggesting that a negative test may only rule out common abnormalities. Beyond screening tests, direct sequence analysis may provide further information on atypical mutations, but at a greater financial burden to patients.

Intron 8 variations in the *CFTR* protein also contribute to abnormal phenotypes through repeat sequences acting as a rheostat and affecting *CFTR* protein expression. If the intron 8 splice variant was 5 polythymidine bases long (5T allele) compared to 7 or 9, exon 9 skipping could reduce

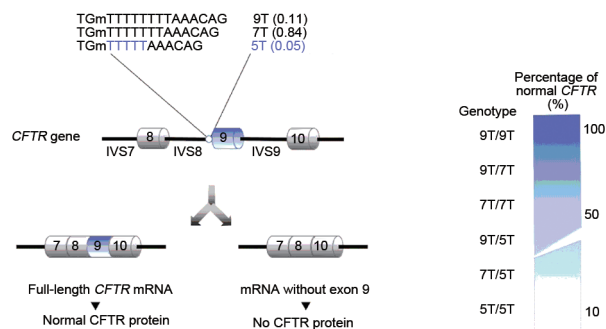
production of functional *CFTR* protein.<sup>3,4</sup> Sequencing also showed that the number of thymidine–guanine (TG) repeats adjacent to the *CFTR* expression site inversely affected *CFTR* expression and presence of CBAVD. This relationship only holds true in the presence of 5T variant patients. **Figure 2** demonstrates the utilization of gene sequencing for these introns.

In this case, the  $\Delta F508$  deletion (deletion of phenylalanine at amino acid position 508) can be considered a severe mutation. This deletion results in decreased efficiency of protein folding with premature *CFTR* degradation in the endoplasmic reticulum.<sup>5</sup> The individual in this case should ideally

be offered, if available, whole *CFTR* gene analysis using Sanger or next-generation sequencing (NGS) including intronic poly T and poly TG tract analysis if he planned to undergo treatment for fertility with assisted reproductive technologies (ART). These tests detect over 98% of *CFTR* mutations, but genetic sequencing can cost \$500–\$1500 compared to \$100–\$300 for carrier testing.<sup>6</sup> Each couple must weigh the risks and benefits in the settings of this test as the risk of transmission remains low at around 1/100 if the female carries the mutation and 1/1000 if her screening is negative. Each couple must independently weigh this risk with the cost of testing. The benefits of full diagnostic workup



**Figure 1:** Intraoperative photo of the CBAVD testicular-epididymal unit with missing 2/3 of epididymis. CBAVD: congenital bilateral absence of the vas deferens.



**Figure 2:** *CFTR* gene testing-genetic sequencing. Net effect: normal *CFTR* channel but reduced amount at the cell membrane. *CFTR*: cystic fibrosis transmembrane conductance regulator.

Department of Urology, University of Tennessee, Knoxville, TN 37920, USA.

Correspondence: Dr. JS Fisher (jfisher@utmck.edu)

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allow for an accurate risk assessment for the couple's offspring.

### PARTNER TESTING RECOMMENDATIONS

Testing guidelines utilized in the setting of *CFTR* include a number of parameters to justify both carrier and diagnostic testing. A male factor is solely responsible in about 20% of infertile couples and contributory in another 30%–40%.<sup>7</sup> In general, partners should undergo carrier testing in the setting of a male with CBAVD. It is worth noting that guidelines would suggest the general population of reproductive couples should undergo carrier testing as well.<sup>8</sup> If the wife is also a carrier for the same mutation, the couple should be counseled that there is a one in four chance that their child would be affected. The inheritance of a CF trait occurs in an autosomal recessive fashion, which explains the 25% chance to pass on the disease for two heterozygous carriers. The clinical severity would vary as a function of specific mutations present. Roughly 5% of Caucasian females are found to be heterozygote carriers. If identified as a carrier, preimplantation genetic diagnosis could be used to reduce offspring risk of both infertility and a diagnosis of CBAVD.<sup>9</sup> In general, all partners of patients with potential *CFTR* mutations should undergo standard cystic fibrosis screening, which covers the 32 most common mutations. These various blood assays provide appropriate evaluation to determine if a familial mutation exists and might prompt the need for a formal meeting with a genetic counselor.

### THE ROLE OF ULTRASONOGRAPHY

In patients with CBAVD, associated abnormalities of the seminal vesicles and ejaculatory ducts almost always exist. Renal ultrasonography may identify the concurrent finding of renal agenesis corresponding with unilateral agenesis of the vas deferens. Development of the genital and renal systems occurs through a series of signals with close correlation and dysfunction can affect both systems. In men with unilateral vasal agenesis, the presence of genitourinary anomalies affecting the contralateral testis approaches 80%. Unilateral renal agenesis occurred in 26% of those with unilateral and 11% of those with bilateral vasal agenesis.<sup>10</sup> Of those with renal agenesis in the CBAVD group, almost all men had no *CFTR* mutation present in the setting of renal anomalies. The AUA guidelines recommend imaging for renal abnormalities in all men with unilateral or congenital bilateral absence of the vasa deferentia with

no evidence of *CFTR* abnormalities. This known mutation in *CFTR* makes the risk of renal abnormality extremely rare, but a renal ultrasound provides a relatively inexpensive test that may provide clinical information for the patient going forward. While the risk does not compare to a patient with unilateral vasal agenesis, a patient with CBAVD should undergo renal ultrasound at some point during their diagnostic evaluation.

### SPERM RETRIEVAL METHODS (PERCUTANEOUS VS MICROSURGICAL; EPIDIDYMAL SOURCE VS TESTICULAR SOURCE)

In the setting of CBAVD, multiple options of sperm retrieval remain available in these patients. Due to high efficacy of egg vitrification, collection may be performed before, after, or at the time of cryopreservation of sperm if clinically indicated. Percutaneous epididymal sperm aspiration (PESA) and microsurgical epididymal sperm aspiration (MESA) are the preferred retrieval methods because of the high rate of sperm retrieval. PESA may be favored due to a lower cost. Vasovasostomy would be excluded on account of absent anatomical components. MESA offers high clinical rates of fertility along with a large number of sperm retrieved. This technique provides adequate samples for cryopreservation. The disadvantages include the expense, anesthesia, and the need for an incision along with the associated discomfort. Testicular sperm extraction (TESE) offers a simplified and repeatable technique that can be offered without microsurgical expertise and at a fraction of the cost.

Similar limitations apply to percutaneous approaches such as PESA or testicular biopsy. Testicular aspiration, for example, can provide excellent success rate of providing spermatozoa acceptable for ICSI, but may require multiple passes of the needle in order to yield adequate sperm. Even so, the ability to perform this procedure in an office setting with local anesthesia and minor postoperative discomfort makes it an appealing option in some patients. Rates of harvest are quoted from 90% to 100%, and the procedure can be performed without microsurgical training under local anesthesia. PESA obtains appropriate sperm more frequently in CBAVD patients compared to postinfectious or vasectomy patients, which by contrast more often require TESE due to difficulty. The need to expand to TESE in the latter two scenarios occurs in almost 1/3 of patients.<sup>11</sup>

Results of some studies show that PESA and TESE provide comparable reproductive

potential independent of extraction site. Epididymal spermatozoa are optimal for technicians in *in vitro* fertilization (IVF) labs compared to testicular sperm due to improved motility. While MESA achieves more robust extracted specimens under the care of a fellowship-trained andrologist, PESA remains an excellent alternative due to the more manageable cost to the patient.

### CLINICAL OUTCOMES (FERTILIZATION, PREGNANCY, AND LIVE BIRTH RATES) ASSOCIATED WITH EPIDIDYMAL VERSUS TESTICULAR SPERM

Men can achieve comparable clinical outcomes with epididymal compared to testicular sperm. As reflected below, the rates for fertilization, pregnancy, and live birth are 34%–81%, 31%, and 27%, respectively, in testicular extraction. By comparison, a fertilization rate of 45%–72% was seen with no significant difference in pregnancy and birth rate from epididymal sperm. **Table 1** shows these rates as reflected in a recent meta-analysis below.<sup>12</sup>

In a study by Chen *et al.*,<sup>13</sup> patients underwent epididymal aspiration procedures in the setting of obstructive azoospermia along with ART. The fertilization rate for spermatozoa harvested from the caput was lower than other areas of the epididymis. Compared to alternative causes of acquired obstructive azoospermia (prior vasectomy, infectious, or traumatic causes), patients with CBAVD attained a higher pregnancy rate versus the acquired group (20% vs 5.9%,  $P > 0.05$ ).<sup>13</sup> The dysfunctional epididymis likely contributes to some degree of reduced sperm quality in the cases of acquired patients. Outcomes of ICSI based on epididymal or testicular sperm were comparable in early series of patients.<sup>14</sup> Equivalent success rates were confirmed later with a larger cohort of 1121 patients with obstructive azoospermia, and it was suggested that sperm source should be left to surgeon preference.<sup>15</sup>

Alternatively, another study reported higher fertility rates using epididymal spermatozoa compared with testis in patients with obstructive azoospermia yielding 77.2% versus 67.5%. The testicular sperm patients in this series had a significantly higher implantation rate and higher pregnancy rate with lower miscarriage rate. Prolonged transit in the epididymis causing structural chromosomal aberrations may contribute to these outcomes, while testicular sperms are less motile or immotile, but still viable for use in ART.<sup>16,17</sup>

**Table 1: Comparison in success rates with testicular versus epididymal sperm in patients with obstructive azoospermia**

Study	ICSI cycles (n)	FR, n (%)	CPR, n (%)	OPR, n (%)	Implantation, n (%)
Dohle <i>et al.</i> <sup>20</sup> 1998					
E	22	101/186 (54)	9/21 (43)	6/21 (29)	–
T	11	32/90 (36)	4/9 (44)	4/9 (45)	–
Fahmy <i>et al.</i> <sup>21</sup> 1997					
E	36	242/401 (60)	9/36 (25)	–	–
T	58	329/568 (58)	20/58 (34)	–	–
Hovatta <i>et al.</i> <sup>22</sup> 1995					
E	9	64/108 (59)	1/6 (17)	–	–
T	21	64/190 (34)	21 (4/19)	–	–
Mansour <i>et al.</i> <sup>23</sup> 1997					
E	44	280/471 (60)	12/44 (27)	–	–
T	91	519/955 (54)	29/91 (32)	–	–
Nagy <i>et al.</i> <sup>24</sup> 1995					
E	43	306/547 (56)	12/40 (30)	–	–
T	17	116/242 (48)	5/13 (38)	–	–
Nicopoullous <i>et al.</i> <sup>25</sup> 2003					
E	113	587/1202 (49)	28/100 (28)	20/100 (20)	36/239 (15)
T	14	72/140 (51)	4/14 (29)	3/14 (21)	5/32 (16)
Palermo <i>et al.</i> <sup>26</sup> 1999					
E	129	1027/1429 (72)	83/129 (64)	73/129 (57)	–
T	14	95/118 (81)	8/14 (57)	7/14 (50)	–
Rosenlund <i>et al.</i> <sup>27</sup> 1997					
E	7	47/69 (68)	–	–	3/15 (20)
T	12	94/139 (68)	–	–	3/27 (11)
Silber <i>et al.</i> <sup>28</sup> 1995					
E	33	201/431 (47)	20/31 (65)	12/31 (39)	–
T	32	215/436 (49)	14/27 (52)	10/27 (37)	–

E: epididymal aspiration; T: testicular aspiration; CPR: clinical pregnancy rate; OPR: ongoing pregnancy rate, –: data unavailable from the study; FR: fertilization rate

In general, TESA seems to provide adequate sperm for ICSI, particularly when multiple aspirations are performed. In some cases, six repeated retrievals may be performed on the same patient. In one study, 97% of patients had adequate spermatozoa for ICSI. No correlation existed between the number of aspirations and fertilization rates. The pregnancy rate of 57% along with a 52% live birth rate reflected similar results to epididymal aspiration.<sup>18</sup>

### THE ROLE OF PREIMPLANTATION GENETIC DIAGNOSIS (PGD)

PGD offers an opportunity to avoid the difficult decision to have therapeutic abortions with known risk factors for CF pregnancies. PGD remains a good option if both parents are CF mutation carriers and want an unaffected child but would not undergo a termination of pregnancy if prenatal abnormalities were identified. IVF would be required which could be analyzed for any *CFTR* mutations with selective implantation of mutation-negative embryos. One or two single blastomeres undergo genetic analysis and three intragenic and four extragenic polymorphic markers can be screened for

mutations. Nearly 15% of PGD tests for monogenic disorders are for CF, which is the most common indication. Geneticists use PCR to identify these markers and exclude these embryos from implantation. The markers associated with CBAVD have incomplete penetrance making counseling difficult as the phenotypic outcomes vary. A center in Montpellier (France) reported outcomes of PGD in 130 couples with *CFTR* mutations of which 76 enrolled in testing.<sup>19</sup> In patients with CBAVD, 6 couples completed 19 cycles with 6 healthy children born. Of couples with comparable risk of CF-affected children, a mutation was present in both partners in 47.7% and one in 36.7%.<sup>19</sup> PGD provides additional information prior to fertilization, but may not be utilized in every case. Instead, the presentation of data in the clinical setting provides shared decision-making in this population. It should be noted that some couples opposed the idea of abortion and couples need to discuss this prior to performing PGD.

### AUTHOR CONTRIBUTIONS

JSF contributed to literature review, data collection, and creation of manuscript.

EDK collaborated in creation and edit of manuscript. Both authors read and approved the final manuscript.

### COMPETING INTERESTS

Both authors declared no competing interests.

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