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Lesson 31 Volume 39

Congenital Absence of the Vas Deferens: Bilateral and Unilateral*

Learning Objective: At the conclusion of this continuing medical education activity, the participant will be able to describe the epidemiology, genetic basis, presentation, associated features and treatment options for the various syndromes in which one or both vasa are palpably absent.

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INTRODUCTION

Congenital absence of the vas deferens was documented as a cadaveric anatomical anomaly by British surgeon John Hunter in 1786.[1] Congenital bilateral absence of the vas deferens is the proximate cause of infertility in 1%–2% of the male population.[2] Kaplan et al established in 1968 that bilateral vasal absence occurs in nearly all men with clinically diagnosed cystic fibrosis, a common autosomal recessive (biallelic) disease that affects pulmonary and pancreatic function, and can shorten life expectancy.[3] The actual genetic basis of cystic fibrosis was reported in 1989 with discovery of the cystic fibrosis gene and the description of several mutations within it.[4, 5] The protein product of the cystic fibrosis gene is known as the cystic fibrosis transmembrane conductance regulator (*CFTR*), a chloride ion transporter expressed in various epithelial cells. Shortly thereafter in 1992 Anguiano et al linked the seemingly different condition of CBAVD (normal pulmonary and pancreatic function) to mutations in *CFTR* as well, visualizing CBAVD as a "primarily genital phenotype of cystic fibrosis."[6] Further studies have revealed as many as 60%–90% of men with CBAVD harbor at least 1 identifiable *CFTR* mutation, although the majority (but not all) are expected to be compound heterozygotes with abnormalities present on both maternally inherited and paternally inherited alleles.[7] Recently 2 more genetic etiologies underlying CBAVD have been discovered. However, there are many variations on the theme, genetically and phenotypically, that can be confusing: unilateral vasal agenesis in the setting of normospermia, unilateral vasal agenesis coupled with unilateral renal agenesis, bilaterally palpable vasa but low volume, acidic azoospermia etc.

In this Update we will first delve into the anatomy, embryology and histology of the male reproductive ductal system. The importance of the assessment of semen volume and pH of an azoospermic sample will be detailed. This basic knowledge will allow for an easy conceptualization and understanding of the various anatomical and phenotypic scenarios that may present themselves in the clinic setting. It is on this foundation that the genetic underpinnings of a patient's specific condition can be overlaid and appreciated.

ANATOMY, EMBRYOLOGY AND HISTOLOGY OF MALE REPRODUCTIVE DUCTAL SYSTEM

A sperm's journey begins where it was born in the seminiferous tubules of the testis, each of which empties its contents into the rete testis (reviewed by Costabile[8]). The rete is the first stop on the voyage outward, and is an intratesticular meshwork of tubules in the mediastinum testis. From here up to 12 efferent ducts emerge through the tunica albuginea to form the proximal caput epididymis where they, in turn, will join, fuse and finally coalesce into a single tubule.[9] This epididymal tubule is long (3–4 meters), highly coiled and surrounded by supporting cells and the epididymal tunical layers, becoming first the corpus and then the cauda epididymis.[10] There are numerous functions of the epididymal tubule, the most important of which is assistance with the maturation of sperm and the achievement of motility and fertilizing ability.[11] The epididymis then transitions into the convoluted vas deferens, which straightens out to become the scrotal, inguinal and pelvic vas as it courses up and out of the scrotum. The distal end of the vas deferens dilates (the ampullary region) and the seminal vesicle buds off from the lateral side. The confluence of the vas and seminal vesicle is the ejaculatory duct, which pierces the prostate and terminates on the verumontanum.[12, 13] The vas itself measures 30–40 cm and the scrotal portion is easily palpable in the posterolateral aspect of the spermatic cord. If necessary, depending on the clinical scenario, the seminal vesicles, ejaculatory ducts and kidneys can be imaged by a variety of techniques.[14]

In the earliest stages of embryogenesis each mesonephric duct is anticipating giving origin to 2 offshoots, sprouting both ureteral/renal and male reproductive ductal branches (fig. 1).[15, 16] One is the ureteral bud, which induces the nearby metanephric blastema; together they will form the ureteral orifice (mesonephric duct), the ureter (mesonephric duct), the intrarenal collecting system (mesonephric duct) and the kidney (metanephric blastema). At around week 7–10 of gestation the other reproductive ductal portion splits off to morph into the ejaculatory duct, the entire vas deferens, the seminal vesicle and the distal two-thirds of the epididymis (corpus and cauda).[17] This is an androgen dependent process, requiring androgen secretion and receptor function within the mesenchyme, not the epithelium.[16, 18] The prostate gland and caput epididymis (essentially the efferent ducts) originate from different embryological precursors. The caput epididymis derives from the remnants of the degenerating mesonephros (the *mesonephric **tubules***—not the *mesonephric **duct***).[15, 19, 20]

Given the different embryological precursors involved in formation of the male reproductive ductal system, a logical question would be whether there is variance in luminal histology that might correlate with clinical phenotypic/genetic findings. The 12 or so efferent ducts in the human arise from the mesonephric ***tubules*** and make connection on the one end with the rete testis tubules and on the other with the epididymal tubule proper (arising from the mesonephric ***duct***; reviewed elegantly by de Mello Santos and Hinton[21]).

The epithelium is a single layer of interspersed ciliated tall columnar cells and shorter, non-ciliated cells (fig. 2).[22] The dynamics of fluid resorption in this area and the importance of estrogen to the process are nicely described by Hess.[23] It has long been believed that the purpose of the efferent duct cilia was, as it is in most other ciliated epithelia-lined tubular structures throughout the body, to move fluid in a continuous, directional manner into the epididymal tubule. Recent evidence has brought this into question, as demonstrated by Yuan et al, who conclude, "We show that motile cilia lining the efferent ductules do not directly propel sperm, but rather serve as agitators, generating vigorous fluidic turbulence, to maintain suspension of sperm within the intraluminal fluid, thus ensuring an equilibrium in fluid resorption by the non-ciliated cells."[24] **The efferent**

ABBREVIATIONS: CBAVD (congenital bilateral absence of the vas deferens), CFTR (cystic fibrosis transmembrane conductance regulator), ICSI (intracytoplasmic sperm injection), MESA (microsurgical extraction of sperm)

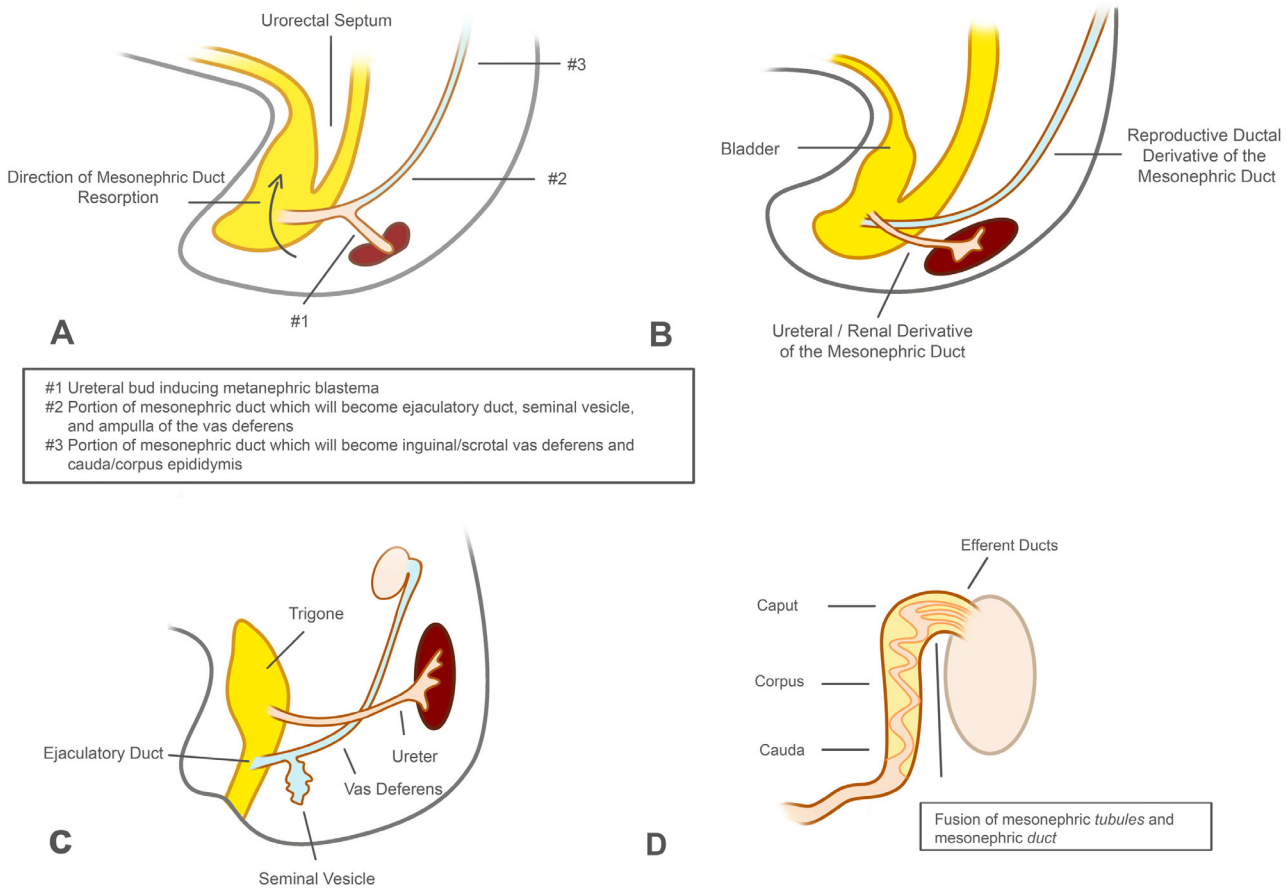

Figure 1. Embryology diagram

ducts intermingle, merge and fuse with the single, epididymal tubule proper. From this point sperm are transported passively down the length of the epididymis.

The epithelium is pseudostratified, and the majority of cells are tall and columnar with stereocilia (fig. 3).[10] The stereocilia are actually cytoplasmic extensions with an actin filament backbone, are non-motile and both absorb and secrete.[11] The

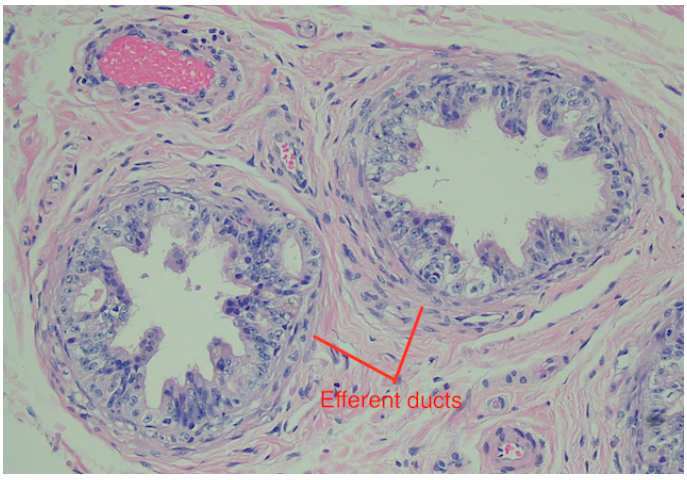

Figure 2. Efferent ducts (derived embryologically from mesonephric *tubules*) with irregular saw-toothed pattern of tall columnar cells (ciliated) alternating with shorter cuboidal cells (non-ciliated) forming crypts and depressions. H&E, reduced from ×40.

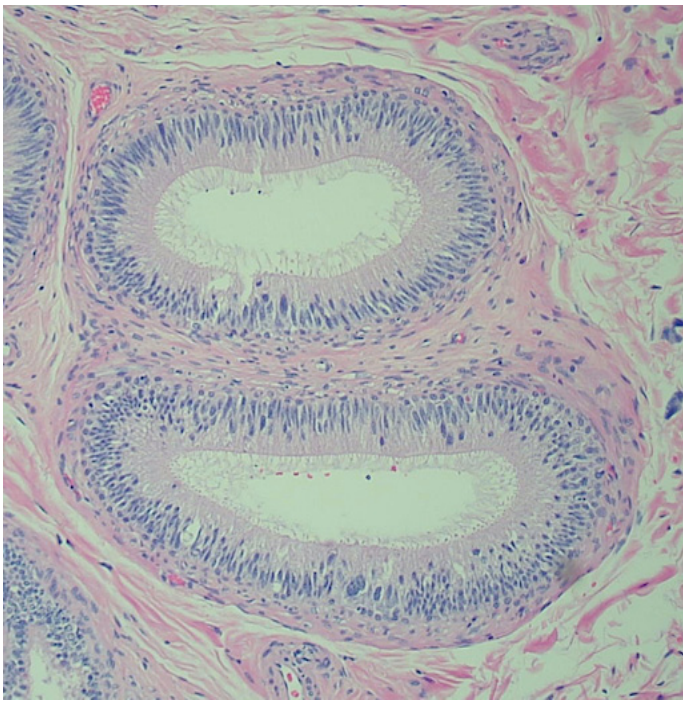

Figure 3. Epididymal tubule proper (derived embryologically from mesonephric *duct*) with its pseudostratified epithelium, majority of which is comprised of tall columnar cells with stereocilia. Stereocilia are actually cytoplasmic extensions with actin filament backbone, are non-motile and both absorb and secrete. H&E, reduced from ×40.

mucosa of the vas deferens is, as would be expected since the embryological origin is the same, very similar to the epididymal tubule showing minor alterations to the pseudostratified columnar epithelium. The seminal vesicle also arises from the mesonephric duct and has an epithelium that is still pseudostratified but is folded and much more irregular and contains cells with secretory function.[19] The time course of growth and development of the efferent ducts and the epididymis follows a biphasic pattern, with progression from the fetal period into the second to fourth month of infancy, after which regression in that development takes place until reinvigorated at puberty.[25]

SEMINAL FLUID CONSTITUENTS AND IMPORTANCE OF VOLUME AND PH

When deciphering an azoospermic semen analysis, it is extremely helpful to know the seminal fluid volume and the pH of the sample.[26, 27] As will be seen, these 2 parameters in combination spearhead an algorithmic split in the differential diagnosis pathway. The majority of the ejaculate (approximately 70%) derives from the paired seminal vesicles. Roughly 20% is secreted by the prostate (circa 0.6 cc) and the remainder is sperm laden fluid from the vasa deferentia. As importantly, all of the alkalinity arises from the seminal vesicles, while the prostatic fluid is acidic (pH 6.5 or so). The typical ejaculate, as a consequence, has a pH >7.2 as the larger, alkaline seminal vesicle contribution overwhelms the smaller, acidic prostatic output.

As described in the preceding section, the vasa and seminal vesicles empty their contents through the ejaculatory ducts into the prostatic urethra. Therefore, when the semen volume is normal, eg 2.5 cc, and the pH is alkaline, eg 8.1, seminal vesicle fluid must be present, proving that at least 1 of the 2 seminal vesicles is functional and its ejaculatory duct is patent. **As a result, the differential diagnosis of an azoospermic, normal volume, alkaline pH semen analysis will NOT include "bilateral ejaculatory duct obstruction" (the blockage to sperm flow as well as seminal vesicle fluid is at the level of the ejaculatory ducts), nor will it be "congenital bilateral absence of the vas deferens" (as will be discussed below, the seminal vesicles are absent/atrophic and do not contribute to the ejaculate in CBAVD).[28, 29]** The reason for the azoospermia lies elsewhere—not within the pelvis, but at the level of the testis (spermatogenic failure) or the more proximal reproductive ductal system (eg from prior vasectomy or idiopathic epididymal occlusion).

Transrectal ultrasonography should not be a reflex next test as the normal volume and alkaline pH are direct evidence arguing against ejaculatory duct obstruction or CBAVD, and thus transrectal ultrasound is not helpful or indicated. However, let's consider the opposite circumstance, when the azoospermic specimen's volume is low, eg 0.6 cc, and the pH is likewise low, eg 6.5. **This is the typical ejaculate consisting only of prostatic fluid, establishing that the seminal vesicles are not present or functional (eg in CBAVD) or are present and functional but bilaterally blocked (eg in ejaculatory duct obstruction).[28–30]** Clinical examination demonstrates no palpable vasa in the former and firm, distended vasa in the latter—an easy determination to make via physical examination. In summary, a relatively clear-cut distinction can be made between the low volume, acidic pH azoospermic semen analysis differential diagnosis (one of the forms of CBAVD or bilateral ejaculatory duct obstruction) and the normal volume, alkaline pH azoospermic semen sample differential diagnosis (NOT one of the forms of CBAVD or bilateral ejaculatory duct obstruction). Attention to these details of the azoospermic semen analysis is extremely helpful in directing one down the proper diagnostic pathway by cognitively focusing and pruning what otherwise is a seemingly confusing array of possible etiological conditions (fig. 4).[28]

The measurement of fructose in a semen sample has long been a test that is performed when the sample is azoospermic. Is it necessary? Fructose is secreted by the seminal vesicle. If the semen volume is low and the pH of an azoospermic ejaculate is acidic, there is no contribution from the seminal vesicles (either bilaterally blocked or absent) and there will be no fructose present. If the semen volume is normal and/or the pH is alkaline, there must be seminal vesicle fluid present and there will be measurable fructose. Semen volume and pH are, therefore, surrogates for the presence or absence of fructose and a fructose assay is neither necessary nor helpful.

CLINICAL/GENETIC SCENARIOS BASED ON PALPATION AND SEMEN ANALYSIS

Bilateral absence of the scrotal vas deferens and low volume, acidic azoospermia. This combination of physical examination and semen analysis findings represents the classic scenario of CBAVD (fig. 4). The seminal vesicles are either atrophic or absent and so do not contribute alkaline contents to the ejaculate, leading to a low volume and acidic sample consisting of just prostatic fluid. Testis size is normal as there is usually no concomitant problem with spermatogenesis. The caput of the epididymis is always present—it is interesting, as described above, that the efferent ducts (which comprise the majority of the human proximal "epididymis") descend from an embryological ancestor different from the remainder of the epididymis and have a very dissimilar epithelium from that of the epididymal tubule proper. On occasion the epididymal remnant may include parts of the corpus or cauda and each side may be distinctive in their anatomy relative to the other. Remember that the original mesonephric duct will "split" into 2 derivatives around week 7 of gestation (renal/ureteral and reproductive ductal). After this time the fate of each is independent of the other. That is if an anomaly in development occurs to the reproductive portion after this division, then renal/ureteral anatomy will be fine but there may be problems with the vas deferens, the seminal vesicle and the distal two-thirds of the epididymis (corpus and cauda). If an insult or genetic aberration occurs prior to that critical time point, then both ureteral/renal and reproductive ductal anomalies may coexist and unilateral renal agenesis or ectopy may be seen along with bilateral vasal absence.

CBAVD and Normal Renal Anatomy: **In this clinical circumstance it is likely that the glitch in reproductive ductal anatomical development transpired after that separation described above.** The putative etiology must **not** have affected the primary mesonephric duct; otherwise coexistent renal abnormalities would be seen. In this clinical circumstance, which is the most common, 3 known genetic etiologies may be present.

As stated in the Introduction, 16 of 25 patients with CBAVD, clinically characterized and studied by Anguiano et al in 1992 were shown to possess mutations in their cystic fibrosis genes (*CFTR*) in the first peer-reviewed publication providing evidence of this phenotype-genotype link.[6] Cystic fibrosis

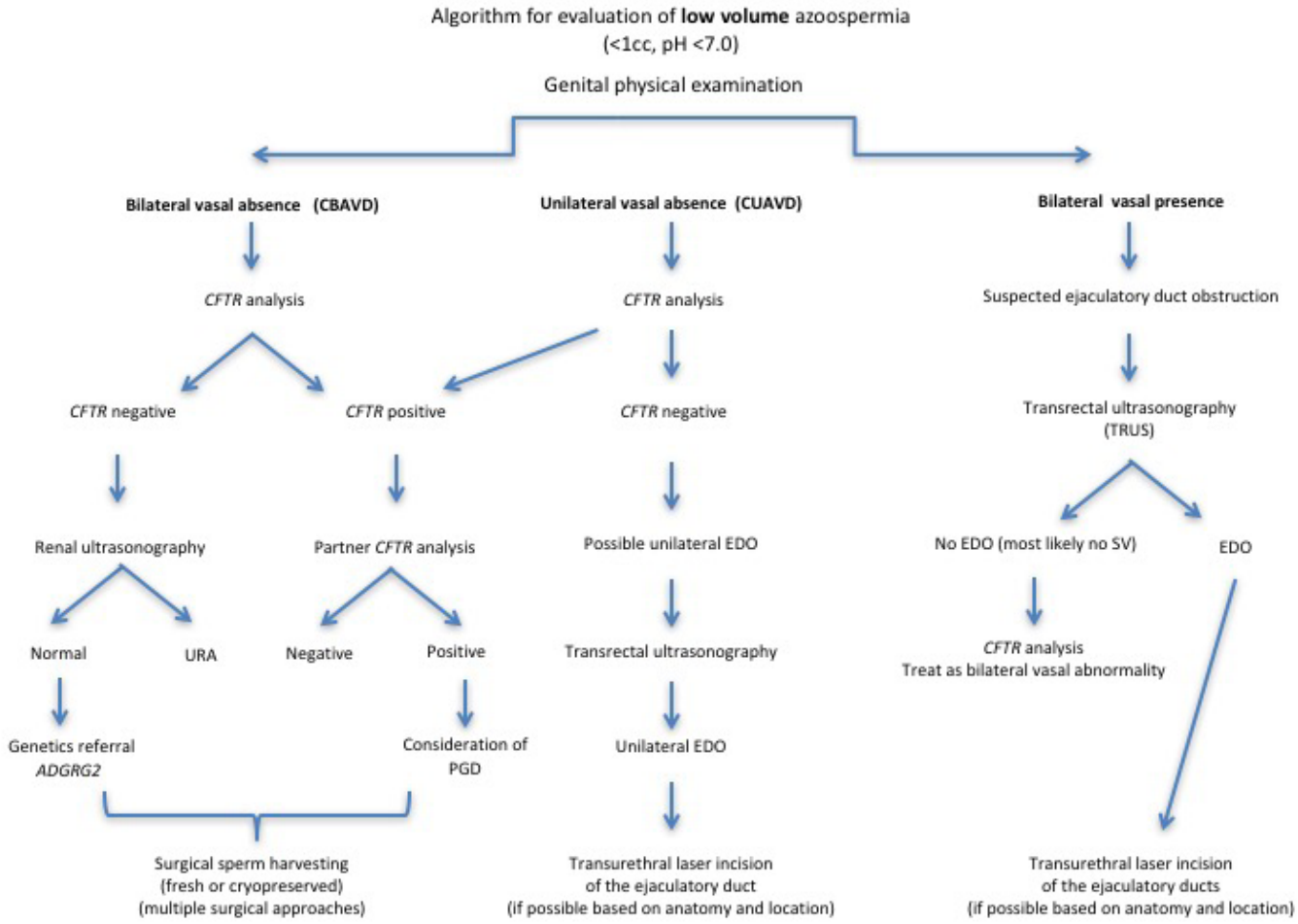

Figure 4. Algorithm for evaluation of male with low volume, acidic, azoospermic semen specimen. Each major branch is determined by physical examination findings: bilateral vasal absence to palpation, unilateral vasal absence to palpation, bilateral vasal presence to palpation. Rarely each vas will be palpable but will be non-patent and seminal vesicles will not exist. *CFTR* mutation analysis is warranted. *EDO*, ejaculatory duct obstruction. *PGD*, preimplantation genetic diagnosis. *URA*, unilateral renal agenesis. *SV*, seminal vesicles.

is a common disorder in Caucasians of Northern European lineage, detected in 1:2500 neonates born in the United States. The prevalence is less in African Americans (1 in 17,000) and even less in Asian Americans (1 in 31,000).[31] More than 2000 mutations/aberrations in the cystic fibrosis gene (*CFTR*) have been described and variant strategies for testing have been recommended, depending on the population base and the clinical condition being assessed. The most common mutation worldwide is a 3 base-pair, in-frame deletion in codon 508 with consequent loss of a phenylalanine from the *CFTR* protein and is known as p.508del.

CFTR functions as a transmembrane chloride ion channel whose ultimate purpose is to keep the intraluminal fluids in certain epithelial lined tubular organs, eg the respiratory tree and the pancreatic ductal system, thin and watery and not thick and viscous. When the total pool of *CFTR* is normal, these structures are just fine. When the total *CFTR* pool is only 50% normal, as occurs in a "carrier" or simple heterozygote, function in these structures is not affected. However, when both *CFTR* genes carry mutations (a compound heterozygote [each mutation is different] or a homozygote [each mutation is the same]), the total pool of *CFTR* may be quantitatively or qualitatively so reduced that the secretions in both respiratory and pancreatic tubular tracts become thick and tenacious and result in the pathological problems of clinically recognizable cystic fibrosis. This is 1 end of the phenotypic spectrum. **If, however, there is a reduction in functional *CFTR* in a compound heterozygote/homozygote that is not as "severe," then the only recognizable manifestation may be bilateral vasal absence (CBAVD) with normal pulmonary and pancreatic function—the opposite end of the spectrum.**

Of course, the effect on a given individual male may be anywhere along the continuum between the extremes. Lucarelli et al suggest a genotype oriented view (as opposed to allele oriented) of *CFTR* genetics, which will allow for a better understanding or prediction of clinical phenotype.[32] Nevertheless, *CFTR* testing for the male with either obvious cystic fibrosis or CBAVD is necessary. The reasons that *CFTR* deficiency of a significant enough degree results in the disappearance of the male reproductive anatomy that derives from the mesonephric duct (distal two-thirds of the epididymis, the entire vas deferens and the seminal vesicle) but not that which originates from the

mesonephric tubules (the efferent ducts) is murky but interesting to contemplate.

Cystic fibrosis mutation disease spans a wide spectrum from devastating clinical cystic fibrosis on the one end to healthy CBAVD on the other. However, some men have conditions such as chronic sinusitis or bronchitis that, in light of the revelation of their *CFTR* mutation based CBAVD, may be seen to have a cystic fibrosis mutation based genetic etiology as well, perhaps altering thoughts and decisions regarding treatment strategies. In addition, many young men will have siblings of reproductive age, and the patient may be the first to be recognized to carry *CFTR* mutations in his family unit, which should precipitate family counseling and screening of appropriate individuals. Defining the genetic basis of CBAVD is, therefore, important for the individual, his partner and his family members.

Due to the high prevalence of *CFTR* mutations in the general population, *CFTR* mutation analysis should be offered to the female partner as well before infertility treatments are carried out. It will be important to know her cystic fibrosis mutation carrier status to determine the different potential genetic outcomes in their progeny, which can easily be surmised due to the Mendelian inheritance pattern of *CFTR* mutations (fig. 5). If she is found to be a carrier, preimplantation genetic diagnosis can be carried out so that only embryos without 2 mutations can be transferred.

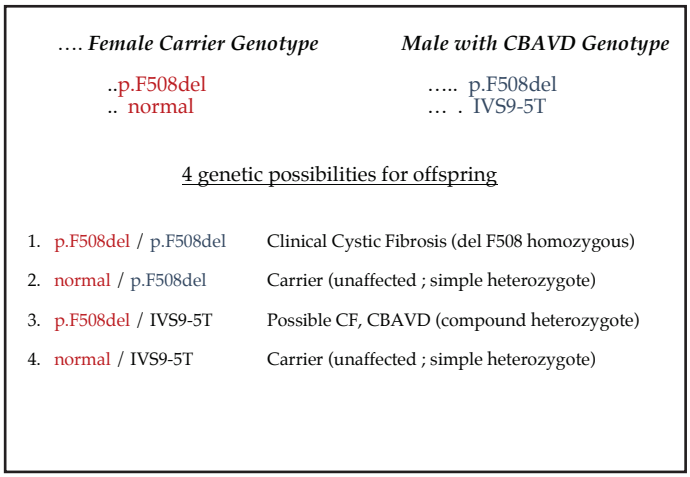

Figure 5. Possible outcomes for offspring if male has CBAVD (p.F508del/IVS9-5T) and his female partner is carrier (p.F508del). It is necessary to define her *CFTR* status prior to surgical sperm aspiration and ICSI so that, if she is carrier, preimplantation genetic diagnosis may be carried out if desired by couple to avoid transfer of embryo destined to have clinical cystic fibrosis (*CF*).

As Yu et al have shown in their review and meta-analysis, at least 78% of patients with CBAVD will have at least 1 mutation identified with p.F508del on 1 allele and the IVS9-5T variant on the other as the most common combination.[33] This is in keeping with most studies but the allele frequency is influenced by ethnic differences.[34, 35] A small number of cases do not have mutations, per se, of *CFTR*, but an allele may have a large rearrangement or deletion within it, altering function. Next generation sequencing methods may prove to be ideal for detection of all possible anomalies.[36, 37]

It is interesting as to why the vas deferens is so extraordinarily "sensitive" to aberrations in *CFTR* function, more so than the respiratory epithelium, for example. Mak et al demonstrated that *CFTR* mRNA splicing is not as efficient in the vasal epithelium as it is in the respiratory epithelium, leading to a more severe pathogenic outcome and at least a possible explanation for this observation.[38] In addition, it has been speculated that the deterioration of the reproductive ductal structures (vas deferens, distal segments of the epididymis and seminal vesicles) due to the altered viscosity of the intraductal fluid milieu occurs in utero, after 12–18 weeks.[39, 40] This time frame is well after the separation of the urinary and reproductive divisions of the primary, antecedent mesonephric duct, a temporal explanation, in part, for why renal anatomy is normal in CBAVD caused by alterations in *CFTR*.

The reproductive ductal system is unique in that it must maintain itself in a quiescent state with little if any passage of contents through it from its inception until the time of puberty, many years later. Just about every other "tube" in the body has air, blood, urine, gut secretions, cerebrospinal fluid etc moving along and interacting with the luminal epithelium from, at the earliest, its moment of creation or, at the very latest, birth. Finally, as regards the type of *CFTR* testing required in the male with CBAVD, full gene sequencing, or next generation technologies, should be employed because, as de Souza et al succinctly state, "Although there are still factors that remain unexplained in the etiology of cystic fibrosis-CBAVD, the main difference between typical cystic fibrosis and cystic fibrosis-CBAVD is the identification of different and rare *CFTR* mutations and variants in high frequency in individuals with cystic fibrosis-CBAVD as compared to typical cystic fibrosis forms...."[34] Next generation technologies is a catchall term for a variety of new techniques that sequence DNA and RNA (and can identify gene mutations or chromosomal abnormalities) at a much more rapid pace and at less cost than prior methodologies.

A second genetic etiology of CBAVD has recently come to light with the finding that hemizygous loss of function mutations in *ADGRG2* (adhesion G protein-coupled receptor G2) are found in 26% (5 of 19 unrelated patients) of French men with CBAVD, normal renal anatomy and no causative *CFTR* aberrations.[41] It has also been noted in a large Pakistani family and in a Chinese man.[42, 43]

ADGRG2 localizes to the X chromosome, which means that it is "inherited" maternally. *ADGRG2* encodes for an orphan G protein-coupled receptor, thought to be involved in fluid resorption and is expressed in the efferent ducts. In their series Pagin et al describe twin brothers, born prematurely at 32.5 weeks, who were discovered to have missing vasa during inguinal hernia repair surgery performed at 6 weeks of life.[41] This observation implies that the vasal atresia occurred in utero and most likely after the divergence of the urinary and reproductive ductal offshoots of the nascent mesonephric duct. Equally as important is that this genomic alteration will be transmitted to 100% of female offspring via sperm aspiration and intracytoplasmic sperm injection. These daughters will be unaffected themselves but will be "carriers" and potentially transmit this deleterious mutation to 50% of their sons (the index patient's grandsons). Preimplantation embryo sexing may be an option for the couple, if they choose, to allow for primary transfer of male embryos.

A third genetic basis for CBAVD and normal renal anatomy has recently been described in Taiwanese patients. Wu et al

elegantly outline the function and location of *SLC9A3* in the reproductive tract of mice and men.[44] *SLC9A3* (solute carrier family 9 sodium/hydrogen exchanger isoform 3) encodes an ion channel regulated by *CFTR*, possibly explaining the interaction of the 2. They showed that *SLC9A3* localized to the stereocilia in the pseudostratified epithelium of the human vas deferens, the apical borders of epithelial and ciliated epididymal cells and the glandular epithelium of the seminal vesicles. Wu et al conclude that "Taiwanese CBAVD is likely due to the cumulative effects of *CFTR* and *SLC9A3* variants."

What is common to all 3 of these etiologies is that they are ion channel proteins, very much involved in the fluidity, pH, osmolality and viscosity of the intraluminal fluids within the duct where they are expressed. Many more will, most likely, be implicated as efferent duct and epididymal transcriptome analysis will provide future clarity.[11, 45]

CBAVD and Unilateral Renal Agenesis: **In this clinical circumstance it is likely that the glitch in reproductive ductal anatomical development transpired before that separation described above**. The putative etiology must have affected ***both*** primary mesonephric ducts, explaining the coexistent renal abnormalities seen. The seminal vesicles are absent or atretic, so that once again the semen analysis demonstrates low volume, acidic azoospermia. As described by McCallum et al, a small percentage of men with CBAVD do not harbor cystic fibrosis mutations and a subset of these men have been found to exhibit unilateral renal agenesis.[46] Of the 4 derivatives of the 2 mesonephric ducts 3 are absent (1 renal and 2 reproductive ductal) and 1 remains (1 renal). It is speculated that this represents a completely distinct etiology involving a genetic mishap or insult derailing proper morphogenesis and differentiation of the mesonephric duct prior to week 7 of gestation, before its division into ureteral and reproductive ductal derivatives. This anomaly thereby alters development of both renal and vasal offshoots but, in a slightly less phenotypic form than bilateral renal agenesis, at least 1 kidney must be present for the patient to survive and present later in life with infertility. McCallum et al found no statistically significant differences between the group of men with *CFTR* mutation based CBAVD and those with unilateral renal agenesis and CBAVD (with no *CFTR* mutations) in terms of physical examination findings, semen analysis parameters, hormonal profiles and anatomical findings of the seminal vesicles on transrectal ultrasonography.

Hence, any man with CBAVD on physical examination and a low volume, acidic azoospermic ejaculate and an absence of *CFTR* mutations on assay should have renal ultrasonography.[47, 48] Although no definite genetic signature has been discovered, transmission to a fetus in the form of bilateral renal and vasal agenesis has been documented in 1 patient, although no family members screened had renal anomalies. It is unclear then if the transmission pattern is autosomal recessive or dominant with a low penetrance.

At this time the genetic evaluation of a male with CBAVD begins with *CFTR* analysis. If he is homozygous or compound heterozygous, the genetic basis is secure. If *CFTR* testing is negative, the next step is renal ultrasonography to identify if 1 kidney is absent. If there is unilateral renal agenesis, the genetic basis is secure. However, if both kidneys are present and since *ADGRG2* mutation analysis is not yet standard in the typical laboratory, a referral to a geneticist may be warranted.

Unilateral absence of the scrotal vas deferens. **Congenital unilateral absence of the vas deferens, as identified by palpation, may have 2 distinct etiologies, as detailed initially and succinctly by Augarten[49] and Mickle[50] et al.** The key to understanding the anatomy, the embryological defect and the clinical phenotype lies in the semen analysis findings as they pertain to presence or absence of sperm as well as volume and pH.

Let us consider the first scenario in which the contralateral epididymis, vas deferens, seminal vesicle and ejaculatory duct are normal and patent as evidenced by sperm in an alkaline ejaculate of decent volume. This proves that the reproductive tract from testis to ejaculatory duct—on the side with the only palpable vas—must be normal in anatomy and function. This is a purely unilateral issue—the problem lies in the development of the ipsilateral (absent) mesonephric duct, often at an early stage prior to its separation into its urinary and reproductive derivatives, and renal agenesis or ectopy may be found on that side in up to 90% of cases.[51] This is a circumstance that may present during a vasectomy in a man who has had children previously—1 vas is not found. Renal ultrasound is necessary to "image" the likely absence of a renal unit on that side. It is not a bilateral problem and *CFTR* mutations are not etiological. Whatever insult occurred to the nascent mesonephric duct affected both urinary and reproductive derivatives and only on 1 side.[52] Zinner syndrome is a rare manifestation and variant of unilateral mesonephric duct malformation, presenting as a triad including unilateral renal agenesis, seminal vesicle cysts/dysplasia and ejaculatory duct obstruction on that same side. Clinical and radiographic diagnosis is typically made in a male in his second or third decade of life with lower urinary tract irritative symptoms.[53]

Let us consider the second scenario in which the semen analysis shows low volume, acidic pH and azoospermia, implying that **both** seminal vesicles are absent or are blocked bilaterally (even on the side of the evident vas; fig. 4). Although 1 vas is palpable in the scrotum, this does not mean it is complete or is patent—it is just demonstrable on physical examination. In this circumstance the reproductive ductal pathology is truly bilateral and the palpable scrotal vas will be found to simply end in the inguinal or pelvic portion, and there is no functional seminal vesicle on that side.[50, 54] This is effectively the same circumstance as CBAVD, just a little less phenotypically severe (1 remnant vasal structure can be felt), and will be found to be *CFTR* mutation based as documented in the majority of cases. The *CFTR* mutation based insult to the reproductive portion of the mesonephric duct occurs after the fission of the mesonephric duct into its ureteral and reproductive ductal derivatives. Therefore, as in cases of CBAVD secondary to *CFTR* mutations, renal anatomy and morphogenesis are unaffected.

Therefore, in cases where a vas deferens can be palpated on 1 side but not the other, the key to understanding the overall anatomy and genetic etiology is the semen analysis. If it shows low volume, acidic azoospermia, both seminal vesicles are absent/atretic and the palpable vas will be found to be nonpatent or will end in the inguinal or pelvic region. Mutations in *CFTR* will most commonly be found. If, however, the semen analysis shows a normal volume, is alkaline and has sperm within it, the seminal vesicle on the side of the evident vas must be present and its ejaculatory duct patent—this is not a bilateral problem and renal ultrasound is necessary to demonstrate the likely renal agenesis on that side.[47, 55] A special circumstance may exist in patients with defects in mesonephric duct differ-

entiation—unilateral vasal absence and contralateral ductal blockage at the ejaculatory duct or vasal/epididymal level as described by Hall and Oates.[56]

TREATMENT OPTIONS AND RESULTS: SPERM HARVESTING AND INTRACYTOPLASMIC SPERM INJECTION

Microsurgical extraction of sperm for use with in vitro fertilization was first successfully performed in 1985, resulting in pregnancy in a patient with obstructive azoospermia secondary to vasectomy and after failed reconstruction.[57] Shortly thereafter in 1987 Silber et al described its effective use in patients with CBAVD.[58,59] However, despite the ability to obtain motile sperm through MESA, conventional in vitro fertilization was unpredictable and produced poor results. The advent of ICSI revolutionized the treatment of infertility of men with CBAVD and irreparable acquired obstructive azoospermia. ICSI allowed for fertilization to occur with the use of a single sperm rather than the millions of motile sperm previously required to fertilize each egg.

Oates et al introduced the concept that not only could spermatozoa be cryopreserved after MESA with similar outcomes to fresh MESA cycles, but this also provided the benefit of separating sperm retrieval from ICSI, allowing for the evaluation of retrieved sperm and division of the harvested sample into multiple vials, which can be used at the convenience of the couple, potentially relieving significant stress for the couple.[60] There are many different techniques to acquire spermatozoa in cases of CBAVD as outlined in illustrative form by Coward and Mills.[61] These include percutaneous epididymal sperm aspiration, testicular sperm aspiration, testicular sperm extraction, microsurgical epididymal sperm aspiration and their own variation of MESA.

Pregnancy rates are excellent, whichever source of sperm is used in conjunction with ICSI. van Wely et al believe that epididymal sperm may have some small clinical benefit over testicular sperm in these types of cases where both types are generally available.[62] They document a live birth rate of 39% after MESA and 24% after testicular sperm extraction. They note no difference in whether the sperm (epididymal or testicular) was fresh or frozen. However, Beauvillard et al report no significant difference, and maybe even a slight benefit, from the use of testis sperm in their study involving 3 different centers.[63] Whichever technique is ultimately chosen for the patient with CBAVD will also be guided by cost and practice patterns but, coupled with ICSI, the sperm should not end up being a limiting factor to the realization of term pregnancy.

SUMMARY

Congenital absence of the vas may come in 2 flavors: bilateral and unilateral. Understanding the embryology of the reproductive ductal and ureteral/renal system is critical in conceptualizing the anatomy and pathology in these cases. Knowing the semen volume and pH is also necessary to formulate a working differential diagnosis after history and physical examination. CBAVD most commonly occurs without ureteral/renal abnormalities and is a consequence of mutations with *CFTR*. Less commonly, unilateral renal agenesis is present and the genetic basis, while unknown, is not related to abnormalities in *CFTR* but to early defects in mesonephric duct morphogenesis. Congenital unilateral absence of the vas deferens, on the other hand, also comes in 2 broad subtypes. The first is secondary to mutations in CFTR and, as in CBAVD, the semen volume will be low and the ejaculate acidic. It is a bilateral process; it is simply that 1 scrotal vasal remnant can be detected on physical examination—it is not patent or it ends blindly in the inguinal or pelvic area. The second involves a defect in nascent mesonephric duct growth and differentiation affecting both ureteral/renal and reproductive offshoots— this is generally a unilateral process and the seminal vesicle, vas and epididymis are normal and functional on the contralateral side. There are a variety of ways to harvest sperm from men with CBAVD but, when combined with ICSI, fertilization, embryo development and pregnancy rates are excellent.

DID YOU KNOW?

- CBAVD is often the result of mutations within *CFTR*; both partners require testing.
- CBAVD may be associated with unilateral renal agenesis.
- Congenital unilateral absence of the vas deferens may be associated with mutations in *CFTR*; both partners require testing.
- MESA, percutaneous epididymal sperm aspiration, and testicular sperm extraction in conjunction with ICSI can help these men achieve biological paternity.

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Study Questions Volume 39 Lesson 31

1. Two derivatives of the mesonephric duct are the
   a. renal parenchyma and rete testis
   b. renal collecting system and efferent ducts
   c. renal collecting system and vas deferens
   d. fallopian tubes and vas deferens

2. During the investigation of azoospermia in an infertile male it is most helpful to know seminal fluid
   a. clarity
   b. volume and pH
   c. liquefaction time
   d. number of leukocytes

3. A 32-year-old man with CBAVD and his 26-year-old partner are planning to have a sperm aspiration with cryopreservation followed by ICSI. He is a CFTR compound heterozygote. Prior to undergoing ICSI the most important test for her to have is
   a. day 3 follicle-stimulating hormone
   b. day 3 anti-müllerian hormone
   c. luteal phase progesterone
   d. cystic fibrosis mutation analysis

4. The most common genetic finding in men with CBAVD are mutations within
   a. VHL
   b. MET
   c. CFTR
   d. PKD1

5. A 30-year-old man with 2 children is undergoing a vasectomy and there is no palpable vas on the left side. Following a unilateral, right vasectomy the next step is
   a. operative exploration to find and ligate the left vas
   b. transrectal ultrasonography
   c. renal ultrasonography
   d. semen analysis in 8 weeks

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