

De Graaf's Thread: the human epididymis

by

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Running Head: The Human Epididymis

Key Words: human, epididymis, efferent ducts, sperm maturation

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Sources of Support: NIH P50-DK45179

Introduction

In 1668 Regnier De Graaf reported his dissection of the human epididymis, which included a partial unraveling of the epididymal tubule (Figs. 1A and 1B). He gave the following description:

“The duct of the epididymis, thus unraveled, becomes thicker the further it proceeds from its origin as six or seven branches at the top of the testicle. Where the branches run together into one duct it can be compared with a rather thin thread which gradually enlarges until it attains the thickness of a piece of string and constitutes the vas deferens.”

[De Graaf (1668) translated by Jocelyn and Setchell (1972)]

De Graaf described the epididymal tubule’s origin as arising from a number of “branches” we now know as the efferent ducts, which comprise much of the proximal epididymis (Figs. 1C-1E). Fig. 1C illustrates the proximal epididymis, dorsal view, of a 53 year old man with a thickened tunica albuginea covering the organ. The arrow indicates the proximal-to-distal direction of the underlying epididymal tubule. The decapsulated tissue (Fig 1D) reveals an amorphous bundle of flaccid tubule that, when dissected and with the core epididymal tubule straightened (indicated by straightened arrow), show themselves to be efferent ducts (Fig. 1E). While the anatomist said these ducts numbered six or seven, they typically vary in number between 11 and 15 (Fig. 1E; Jonte and Holstein, 1978; Yeung et al., 1991). The efferent ducts, which communicate proximally with the rete testis, anastomose distally at multiple points along the single, caput epididymidal tubule (Fig 1E). Even though De Graaf likened the tubule to a thread, he knew it contained “seminal matter” and he stated that the epididymis existed so that matter “might be better elaborated by a long delay in transit” (Jocelyn and Setchell, 1972).

Interestingly, De Graaf’s description of the epididymis predated Leewenhoek’s discovery of spermatozoa (Leewenhoek, 1678), so De Graaf’s term, seminal matter, in his description of

the epididymis only referred to the idea that something in the ejaculate (generally accepted to be of a testicular source) was necessary for the generation of new life. Since De Graaf had no concept of spermatozoa, he could have had no concept of sperm maturation; so he postulated that the long, thin thread he had uncoiled was necessary for the seminal matter to be “elaborated,” i.e. made complete, over time. In stating that opinion, De Graaf was perhaps the first to make a published comment about epididymal function and thereby initiated a discussion that is still active over three hundred years later.

A major difficulty in developing information about the human epididymis has been the lack of human epididymal tissue suitable for research. The epididymis consists of a highly coiled, single tubule (Fig. 1A); thus, the organ may not be biopsied and epididymectomy is extremely rare, especially in men within their reproductive years. This has meant that most studies of the human epididymis have used tissues from older men who have undergone therapeutic orchidectomy for prostate cancer. These men are typically beyond their reproductive years and the results gained from those tissues are unlikely to reflect the biology of the normally functioning epididymis. Tissues extirpated from elderly patients having died from a variety of diseases and having been exposed to a variety of treatments are unlikely to have been receiving the endocrine, paracrine, and lumicrine signaling of the “normal” epididymis. In this regard, Fig 1C showing the proximal epididymis of a 53 year old male after cancer treatments, with its thick tunica and flaccid, indistinct tubules, is in great contrast to a healthy epididymis from a 23 year old with its thin tunica and distinct tubules (Fig. 1F).

Another occasional source of human epididymal tissue is from younger men with testicular cancer, but these testes are often heavily involved with tumor and their ipsilateral epididymides cannot be considered to be normal. Tissues from younger men obtained after lethal

misfortune do not have that same disadvantage, but often suffer from the lack of preservation proper for morphological, biochemical, or molecular studies. Tissues being fixed, frozen, or analyzed unpreserved after several hours of anoxia will be no more appropriate for study than laboratory animal tissues treated in that same way. Protein and RNA degradation can begin within minutes of loss of blood flow, so unless some indication of good preservation is given, even results ostensibly obtained from healthy human tissues must often be regarded with caution.

These limitations have meant that most of what is known about the biology of the human epididymis results from studies performed in laboratory animals with a more occasional use of human tissue to determine where similarities or dissimilarities exist. While this present review will focus on understanding the human epididymis, common reference will be made to selected studies in laboratory animals; however, this is not intended to be an extensive review of mammalian epididymal biology. Those reviews are already available (Robaire and Hinton, 2002; Robaire et al., 2006). Rather, this review will consider some features of epididymal anatomy and histology that have practical relevance to both basic and clinical investigators, but which are rarely, if ever, discussed in context. Following that, the key functions of the organ will be discussed, again with an eye toward issues of practical relevance to both basic and clinical investigators.

Anatomy of the human epididymis.

The epididymis is typically adherent to the testis with the attachment to the cranial pole of the testis being via the efferent ducts and the connective tissue tunica of the caput. Medially, the epididymis is attached to the testis by the epididymo-testicular connective tissue, and distally by both the caudal connective tissue and the epididymal fat pad (Fig. 2A). Classical gross anat-

omy uses the terms *globus major* for the proximal epididymis and *globus minor* for the distal epididymis with the *globus minor* disappearing in the epididymal fat pad (Fig. 2A).

The nomenclature more commonly used in reproductive biology and medicine divides the epididymis into three broad regions, the caput (head), corpus (body), and cauda (tail) epididymidis (Fig. 2B.). As already described (Fig 1), the caput epididymidis in the human largely consists of efferent ducts. This fact has clinical relevance in cases of microsurgical epididymal sperm extraction (MESA) and percutaneous sperm aspiration (PESA) because the tubules of the caput epididymidis are multiple (Fig. 1E), while more distally there is only a single epididymal tubule arranged in multiple coils (Fig. 2B, inset). This means that MESA or PESA-induced occlusion or damage of an efferent duct in the caput epididymidis will not obstruct total luminal flow through the organ whereas such an occlusion of the more distal tubule will cause a complete epididymal obstruction. In practice, MESA and PESA can occur distal to the efferent ducts without causing obstruction, but the possibility of such an injury should be considered when deciding on types and location of aspiration procedure.

The total length of the typical human epididymis-- the organ, not the tubule, itself-- is between 10-12 cm before the cauda tubule evolves to become the convoluted vas deferens (rising on the left side of Fig. 2B). The epididymides often available from elderly patients undergoing orchidectomy can be as small as 7mm in length and can have approximately half the mass of the healthy epididymis. Anatomy texts commonly report the uncoiled epididymal tubule to be 6-7 m in length, though the source of this estimate is unknown. The value may be an underestimation given that the much smaller rat epididymis contains a single tubule that has been uncoiled and measured to be 3.2 m in length (Turner et al., 1990).

Histology of the Epididymal Tubule

The epithelium of the epididymis varies, proximally to distally (Fig. 3), and maintenance of the tissue with regard to both form and function, requires lumicrine secretions of the testis (Hinton et al., 1998; Turner et al., 2007). The epididymis in Fig. 1C- 1E, for example, is from a terminally ill cancer patient well beyond his reproductive years. Further, he had likely received at least one of a variety of treatments that could also affect testicular production of androgens, seminiferous tubule secretions, and spermatogenesis, e.g. radiotherapy, chemotherapy, antiandrogens. As a consequence, that epididymis is clearly dysfunctional as judged by: 1) The epididymal tunica is thickened causing neither the efferent ducts nor the epididymal tubule to be visible beneath the tunica. 2) In the detunicated organ (Fig. 1D), the tubules remain flaccid, indistinct, and with no apparent sperm content. This is a very different appearance from the healthy epididymis wherein the tunica is thin and the tubules are distinct being filled with sperm and luminal fluid (Figs. 1F and 2B, inset). 3) The epididymis illustrated in Fig. 1C-E is much reduced in size relative to epididymides from healthy males (Fig. 1F). The alert scientists should be aware of how inappropriate such epididymides are for investigations aimed at understanding the biology, not the pathology, of the organ.

As noted, an appropriately functioning testis (Fig. 3A.) is key to the regulation and support of the epididymis (Fig 3B-3D). Leydig cells of the testicular interstitium produce androgens, which are required for the support of many epididymal features from morphology to individual gene expressions (Robaire et al.,2006). Sertoli cells support the development of spermatozoa, but also produce secretions like androgen binding protein (Danzo et al, 1977) and basic fibroblast growth factor (Lan et al., 1998) that appear necessary for the regulation of the epididymal epithelium, especially in the proximal regions of the duct. For this reason, a histologically

normal seminiferous epithelium in the ipsilateral testis (Fig. 3A) provides a signal that the epididymis has been appropriately supported.

The human epididymis has no “initial segment” as popularly thought of from studies primarily of the rodent epididymis (Reid and Cleland, 1957; Jelinsky et al., 2007). The efferent ducts make up the majority of the human caput epididymidis (Fig. 3B), and these have as many as seven different types of epithelia, depending on location within the ducts (Yeung et al., 1991). The epithelia of these tubules are commonly irregular in height and the tubule diameters are small relative to true epididymal tubule (Yeung et al., 1991; Fig. 3 B and 3C). The epithelium of the efferent ducts varies from low cuboidal to tall columnar and true cilia (9+2 microtubules; ability to beat) appear throughout the duct (Yeung et al., 1991). The physiology and cell biology of the efferent ducts has been studied largely in laboratory animals, has been well reviewed elsewhere (Hess, 2002; Hess, 2003), and will not be detailed here.

It is common to see histological, cross-sectional profiles of efferent ducts with no or few sperm in the lumen (Fig. 3B). This is not necessarily due to the absence of sperm production in the testis; it can be due to the naturally dilute sperm concentrations coming over in the rete testis fluid. Alternatively, sperm could be released from the rete testis in pulses, and if the inter-pulse intervals are long, most tubule profiles in a random section through the efferent ducts would be expected to have no or few sperm.

Healthy epididymides may have these limited numbers of sperm evident in the efferent ducts (Fig. 3B) yet have many sperm in the lumen of the corpus tubule (Fig. 3C) and even more in the cauda (Fig. 3D). This is because sperm are concentrated during their transit of the epididymis (see below).

In the case of an obstructed epididymis, the normal distribution of sperm in the epididymis changes depending on the level of the obstruction. Typically, there will be no sperm in the lumen below the site of obstruction, but above the obstruction sperm will have accumulated in the lumen potentially backing up all the way to the efferent ducts or even to the rete testis. Near the site of obstruction, the dense sperm pack in the tubule lumen will consist of senescent and degenerating sperm, and in cases of sperm granuloma these will be mixed with macrophages and neutrophils, which can be interstitial, intraepithelial or even intraluminal (Wang and Holstein, 1983; Pollanen and Cooper, 1994).

The true epididymal tubule in the distal caput and corpus has a columnar epithelium with microvilli (no 9+2 microtubules, no capacity to beat) projecting into the tubule lumen. Microvilli at all levels in the epididymis (Fig. 3) provide a huge increase in luminal membrane surface area that may be important in providing area for cell surface receptors, transport channels, and even membrane for endocytic events. At the level of the corpus epididymidis, the lumen should contain readily evident concentrations of spermatozoa (Fig 3C.). At the electron microscope level (not shown) the apical borders of epididymal epithelial cells exhibit cell-cell tight junctions (Friend and Gilula, 1972) composed of a number of cell adhesion molecules (Cyr et al., 2007; Dubé et al., 2007) which impose a blood-epididymal barrier similar in effect to the blood-testis barrier (Howards et al., 1976); that is, the blood-epididymal barrier provides a specialized, immune-privileged microenvironment in which sperm remain isolated from other body compartments (Hinton, 1985).

The lumen of a healthy cauda epididymidal tubule is greatly expanded in diameter, has a short, more cuboidal epithelium with short stereocilia and contains a dense pack of spermatozoa (Fig. 3D). The epididymal epithelium of most species has at least six different cell types: princi-

pal cells, clear cells, basal cells, narrow cells, apical cells, and halo cells, all of which differ in relative abundance depending on the epididymal region and species studies. In all cases, however, including the human, the predominant cell type is the principal cell. Detailed descriptions of these cell types are largely derived from other species and have recently been reviewed elsewhere (Robaire et al., 2006).

Dysgenesis/atresia of the epididymis

Anatomical abnormalities of the epididymis, usually associated with undescended testes, occur in only a small minority of fertile men. On the other hand, in almost 300 cases of obstructive azoospermia, Girgis et al. (1969) found that a dysgenesis or atresia of the cauda or vas deferens had occurred in 39% of the cases and between the caput epididymidis and the testis in 21% of the cases. Kroovand and Perlmutter (1981) described eight different types of developmental anomalies of the epididymis including complete absence of the excurrent ducts, absence of a connection between the testis and caput epididymidis, agenesis of different regions or segments of the epididymis, and extensive disconnection of the corpus and/or cauda of the epididymis from the testis allowing, respectively, a “looping” epididymis or one that can angulate sharply away from the testis

While these conditions can exist in the adult, the most common examinations of the epididymis occur in young boys at the time of repair of cryptorchidism. Estimates of the summed frequency of anomalies like those listed above range from 35% (Mollaeian et al, 1994) to almost 90% (Koff and Scaletsky, 1990) in cryptorchid boys. In boys with epididymides being examined for reasons other than cryptorchidism the summed incidence of similar types of anomalies was less than 5% excluding the “looped” epididymis (Turek et al., 1994). Interest-

ingly, the looped epididymis (attachment only at the caput and cauda thus allowing the corpus to loop away from the testis) occurred in 84% of noncryptorchid epididymides. This implies that the looped condition found with cryptorchidism (Koff and Scaletscky, 1990; Kucukaydin et al., 1998) is not really an anomaly but rather the usual condition of the young epididymis. Since the looped epididymis is not usually seen in the non-cryptorchid adult, the data of Turek et al. (1994) imply that the connective tissue connection between testis and epididymis is still condensing during child- and, potentially, adolescent development.

The histologic appearance of the cryptorchid epididymis also reflects a failure of normal development of the epididymal epithelium and the peritubular musculature of the cauda fails to develop properly (Migel et al., 2001). This developmental failure is already evident in epididymides from boys in the first years of life, so whether orchidopexy will allow sufficient stimulation of the epididymis to produce “catch-up” growth even in a patent system is an open question.

The main clinical point regarding the cryptorchid epididymis relates to the potential for the orchidopexed, formerly cryptorchid testis to eventually contribute to fertility. No amount of spermatogenesis will overcome a dysmorphic epididymis. While long-term studies of epididymides known to have been dysplastic at the time of orchidopexy have not been done, it seems likely that epididymal anomalies will persist over time, especially in the unusual cases of segmental or regional atresia; thus, attention to the condition of the epididymis at the time of orchidopexy can help inform any prognosis being considered, especially in bilateral cases.

While the cause(s) for the different types of epididymal dysgenesis associated with cryptorchidism remains unknown, the molecular biology underlying these lesions is proving to be interesting. Boys with cryptorchidism (thus, with a high rate of epididymal malformation) have

an increased incidence of mutation of the *HOXA-10* gene (Kolon et al., 1999), and *Hoxa-10* and *Hoxa-11* expressions are known to be necessary for the proper development of the epididymis and vas deferens in mice (Hsieh-Li, et al., 1995; Benson et al., 1996). Further, the *Hoxa-10* and *Hoxa-11* knock-out mice have a high rate of cryptorchidism (Branford et al., 2000) and *HOXA-13* mutations in the human have been associated with Hand-Foot-Genital syndrome in which, among other symptoms, the distal genital tract is abnormal (Goodman et al., 2000).

Hox genes (there are *HOXA*, *HOXB*, *HOXC*, and *HOXD* clusters on different chromosomes) produce transcription factors important for anterior-to-posterior, segmental development in the embryo (Carroll, 1995). The numbered genes are arrayed 3' to 5' within their clusters, and the more 5' genes (*HOXA-8* through *HOXA-13*, for example) are typically expressed more posteriorly in the embryo and later in development than their more 3' orthologs (Bomgardner et al., 2001). The connection between the rete testis and the efferent ducts is typically made in the third month of embryogenesis; thus, to the degree that Hox genes are involved, epididymal malformations that include absence of a connection to the testis, perhaps due to agenesis of the efferent ducts, may involve the more 3' genes in the hox clusters. Malformations of the more distal tract, e.g. the cauda epididymidis and vas deferens, may be influenced by misexpression of the more 5' hox genes, as has been shown with *hoxa-10* and *hoax-11* expression in the mouse. Evidence in the mouse suggests that the Notch signaling pathway may also be important in establishing the efferent duct-rete testis connection (Luperin et al., 2006).

Hedgehog proteins also play a role in tissue orientation in the embryo and one of these, sonic hedgehog, has been known for some time to be important for the development of the prostate from the urogenital sinus (Podlasek et al., 1999). More recently, sonic hedgehog gene and protein expressions have been detected in the epididymis of the adult mouse (Turner et al, 2004)

and inhibition of the hedgehog pathway inhibits epididymal sperm maturation (Turner et al., 2006). Current evidence also suggests that sonic hedgehog protein is present in the epithelium of the adult human epididymis, including specific parts of the efferent ducts, but not the testis (Fig. 4). Interestingly, the epithelial cells of the efferent ducts that stain for sonic hedgehog protein (Fig. 4F) appear to be selected principal cell and apical cells while all epithelial cells of true epididymal epithelium stain for the protein (Figs. 4H, J, and L). While sonic hedgehog is typically secreted basolaterally toward mesenchymal cells, in the epididymis the protein is also secreted lumenally where it associates with luminal spermatozoa and cell debris (Figs. 4H, J, and L). It has been speculated previously that disruption of the sonic hedgehog pathway during development could play a role in epididymal malformation, but preliminary evidence here suggests that the pathway continues to play a role in the adult epididymis, a possibility that requires further exploration.

Another cause of epididymal dysgenesis is mutation of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene that occurs in cystic fibrosis. Over 500 different mutations of *CFTR* have been identified, explaining the wide spectrum of the cystic fibrosis phenotype (Wong, 1998). One constant in the disease is that approximately 95% of men with clinical cystic fibrosis have congenital absence of the vas deferens (Wong, 1998), which is commonly accompanied by absence of the cauda and corpus epididymidis, as well. This points to a morphogenic failure of the mesonephric derivatives as early as the 13th wk of gestation. At that time the Wolffian system is differentiating into the epididymis and scrotal vas deferens (Kroovand and Perlmutter, 1981). *CFTR* is expressed in the human embryonic, post-natal, and adult epididymis and the highest expression at all of these times is in the caput epithelium (Tizzano et al., 1993; Tizzano et al. 1994; Patrizio and Salameh, 1998). *CFTR* is also expressed in the human vas def-

erens (Tizanno et al., 1994; Patrizio and Salameh, 1998) and in cultured vas deferens epithelium (Harris et al., 1991). Since the CFTR protein is a regulated chloride channel and mutations disrupt its transport function, the evidence suggests that the *CFTR* mutation causes dysfunction of the early mesonephric tubule and possible lumicrine secretions necessary for sustained development of the embryonic vas deferens and distal epididymis. It is conceivable that upstream secretions altered through *CFTR* misexpression cause downstream effects on the transcription of the 5' Hox genes mentioned earlier or on the activity of their gene products. In this way, mutations of CFTR expressed in the proximal epididymis can lead to agenesis of the distal epididymis and vas deferens.

Finally, epididymal dysgenesis can be induced by mutation of the von Hippel-Lindau (VHL) gene, a condition most strongly associated with renal cell carcinoma (Glassberg, 2002). *VHL* mutation in the male can cause epididymal cystadenomas (Glasker et al, 2006) and, more rarely, obstruction of the epididymis (Ayden et al., 2005; Pozza et al., 1994). This can lead to infertility if bilateral.

Epididymal Functions

The adult epididymis is important for fertility not only as a conduit for sperm between the testis to the vas deferens, but as an active contributor to the formation of a fertile ejaculate. The epididymis transports, concentrates, matures, and stores spermatozoa. Each of these classic functions is discussed briefly, below.

Sperm Transport :

Sperm are moved through the epididymis in part by hydrostatic pressures originating from fluids secreted in the seminiferous tubules (Setchell, 1974) and by peristaltic-like contractions of the tubules (Hinton and Setchell, 1978). Contractions of the tunica albuginea of the testis (Banks et al., 2006) also potentially play a role in the generation of positive fluid pressure in the head of the epididymis. Peristaltic-like contractions of the peritubular myoid surrounding the epididymal tubule (Jaakola, 1983; Turner et al., 1990) plus the positive hydrostatic pressure from the caput (Johnson and Howards, 1975, 1976), aid in moving the luminal content down the more distal duct. Impirical observations during *in vivo* micropuncture experiments in the rat indicate that the contractile activity and the subsequent back and forth agitation of the luminal contents increases with increasing intraluminal pressure. The regulation of such activity and the role of intraluminal pressure on it remains an open question.

Net transport rates are estimated to be most rapid in the efferent ducts and proximal epididymis where fluid is nonviscous and water is being rapidly absorbed from the lumen, but transport rates decrease in the more distal tubule where the lumen content becomes more viscous (Turner et al,1990) . The time required for sperm to transit the epididymis has been assessed in a variety of ways in laboratory animals and is notable for its relative consistency (10-13 days) between species of vastly different sizes. In the human, however, sperm transport time has been estimated to be only 2-6 days (Robaire et al., 2006) meaning that the time the human sperm spends in the epididymis is relatively short. Johnson and Varner (1988) demonstrated that men with high testicular sperm output have shorter epididymal transit times than men with low testicular sperm output (~2 d vs. ~6 d, respectively). This may be because testes that produce more sperm also produce more fluid, thus moving the epididymal content along more rapidly.

The rate of sperm movement through the epididymis might be more important than is generally recognized since the concentration of secreted or absorbed molecules in any luminal fluid is a function of pro- and antiluminal epithelial transport activities and the time the intraluminal fluid is exposed to those activities. In the case of the epididymal lumen fluid, the concentrations of ions, small organic molecules, and specific proteins secreted by the epithelium are likely important for sperm maturation or for the regulation of downstream activities of the epididymal epithelium. Radically altered rates of movement of luminal content down the epididymal tubule could alter the amount of fluid reabsorbed at any one point or the amount of a molecule secreted in a specific volume. Such changes could affect not only the concentration of intraluminal spermatozoa, but the concentration of molecules important to the sperm's maturation environment.

Sperm Concentration:

The sperm concentrating ability of the mammalian epididymis has been established for many years (Crabo, 1965; Levine and Marsh, 1971). The increase in sperm concentrations between the efferent ducts and cauda epididymidis is due to fluid reabsorption subsequent to antiluminal electrolyte transport (Wong et al., 1978; Wong et al., 2002). In a number of laboratory species, 75-95% of fluid leaving the rete testis has been reabsorbed by the time the transported spermatozoa reach the mid-caput epididymidis (Turner, 2002; Robaire et al., 2006). In fact, much of this fluid reabsorption occurs in the efferent ducts where estrogen and estrogen receptors play an important role in its regulation (Hess, 2003).

While direct measurements of fluid reabsorption have not been made in the human epididymis, histological evidence substantiates that the human efferent ducts and proximal epididymis

also concentrate spermatozoa in the tubule lumen (Fig. 3). Catecholamines (Wong and Yeung, 1977), aldosterone (Turner and Cesarini, 1983; Hinton and Keefer, 1985), the renin-angiotensin system (Leung et al., 2000), and estrogen (Hess, 2003), among others, have all been hypothesized to play a regulatory role in this fluid reabsorption. While the issue requires further investigation, it is clear that the mechanism of *in vivo* lumen volume regulation is very complex. Ion transport channels like CFTR and the antiluminal sodium transporters cause osmotic shifts that draw water from the epididymal lumen through aquaporin channels in the epithelial cell membranes (Cheung et al., 2003; Da Silva et al., 2006). This reabsorption of water results in a gradual increase in intraluminal sperm concentrations and, ultimately, to a dense sperm pack filling the lumen of the cauda epididymidis (Fig. 3A). The regulatory molecules mentioned earlier (catecholamines, aldosterone, estrogen, etc) can influence these transporters and either stimulate or repress net water movement out of the lumen.

Since only 5%-10% of the final human ejaculate volume is contributed by the cauda epididymidis (Weiske, 1994; Wetterauer, 1986), it is essential that intraluminal cauda sperm density be high to insure adequate sperm concentrations in the final ejaculate. For this reason, the sperm concentrating function of the epididymis and the cellular mechanisms supporting it are important for the development of the fertile ejaculate.

Sperm Maturation:

More than three hundred years after De Graaf opined that the function of the epididymis was to simply hold its “seminal matter” until it matured, a debate continued as to whether or not this is true (Silber, 1988, 1989; Cooper, 1990). Does the epididymis simply act as a reservoir for

sperm until they mature intrinsically or does it provide extrinsic factors that are required for sperm maturation?

In the early part of the twentieth century, Young (1929a, 1929b, 1931) had used the guinea pig as a model to come to the conclusion that time alone was sufficient for sperm maturation, but later evidence in a number of species showed that this was wrong. In fact, an accumulation of later data demonstrated that sperm do need extrinsic factors from the epididymal microenvironment to become fully mature (Cooper, 1990; Turner, 1995; Toshimori, 2003).

Gene expressions and protein secretions vary in distinct patterns along the human epididymal duct (Dacheux et al., 2006; Dubé et al., 2007; Kirchhoff, 2007) as they do in other species (Cornwall, et al., 2002; Dacheux et al, 2003). Complex associations of proteins in membranous vesicles called epididymosomes are also secreted along the epididymal duct in some species, and those vesicles have been shown to transfer specific proteins directly to luminal spermatozoa (Sullivan et al, 2003). Similar vesicles have also been reported in the human epididymis (Frenette et al., 2006), but their role remains unresolved. Electrolytes and small organic molecules change in characteristic patterns along the epididymis, as well (Turner, 2002), and it is the exposure of sperm to this ever-changing microenvironment that is necessary for their full maturation (Turner, 1995; Robaire et al., 2006). In the mammalian species given as examples in Table 1, sperm attributes like progressive sperm motility and the ability to bind to the ovum increase from essentially zero in the caput epididymidis to much higher values in the cauda.

In obstructed epididymides, it is true that sperm in the caput tubule (Qui et al., 2003) or even in the seminiferous tubules (Belker et al, 1998) can exhibit an increased capacity for motility, but this is likely due to events ranging from remodeling of the existing ductal epithelium subsequent to the obstruction (Turner et al., 2000) to the accomplishment of some steps in the

maturation process that can occur due to time alone. It is also true that human pregnancies through natural mating can occur even though the ejaculated sperm have bypassed most of the epididymis, as when a vasoepididymostomy (VE) has been performed in the caput to bypass an obstruction in the corpus or cauda (Schoysman and Bedford, 1986; Silber, 1989). Even more rare is the natural pregnancy established after vasoeffereostomy (VEF; Silber, 1988); still, it is a legitimate question how such pregnancies occur if it is true that exposure to the epididymal microenvironment is required for complete sperm maturation.

First of all, conventional fertility trials are for one year, yet pregnancies reported by some investigators after VE or VEF have occurred after much longer times. Silber (1989), for example, reported that some of his fertile patients after VE required up to 4 yrs to achieve a pregnancy. Such extended periods of study allow reporting of the rare pregnancy made even rarer by the consideration of the number of attempts made to achieve the pregnancy. Fertility trials extended beyond the norm introduce potential paternity concerns, as well. Putting those issues aside, the fact remains that caput VE's or VEF's do lead to an occasional pregnancy after either brief or even no exposure of sperm to the epididymal microenvironment. Possibilities that allow for sperm maturation in these situations are: 1) long-term obstruction causes remodeling of the caput or efferent duct epithelium which makes the proximal microenvironment more conducive to sperm maturation. 2) With both VE and VEF, sperm cells are exposed to the microenvironment of the vas deferens. That rarely-studied microenvironment may be more useful for sperm maturation than is commonly appreciated.

There are other factors that likely play a role in any pregnancy established after VE or VEF. For example, sperm cell maturation presumably follows a Gaussian distribution; i.e. a few cells will mature much more easily than the average and a few cells will mature much less easily

than the average. The small proportion of sperm cells with a tendency toward early maturation are likely the ones finding sufficient stimulation in the post-VE microenvironment of only the proximal caput plus the vas deferens to reach functional maturity. Those few sperm could be sufficient to produce the occasional pregnancy. Nevertheless, it is quite clear that the chances for developing a fertile ejaculate after VE increases with the length of the epididymis the sperm were able to transit (Silber, 1988; Schlegel and Goldstein, 1993; Belker, 2001).

Since some sperm that have been exposed to only a short portion of the epididymis can fertilize an egg *in vivo*, it is not surprising that sperm in epididymal aspirates from the obstructed proximal epididymis can fertilize an egg *in vitro*. When used for intracytoplasmic sperm injection (ICSI) or even conventional in vitro fertilization (IVF) sperm collected from the proximal regions of an obstructed epididymis have a relatively high fertility potential. The use of aspirated sperm in ICSI totally bypasses the requirement that sperm be motile and be able to acrosome react, penetrate the zona, bind to the egg plasma membrane, fuse with the membrane, and complete fertilization on their own. Even in conventional IVF there is no requirement for sperm survival in seminal plasma then cervical mucus followed by the uterine environment and the oviductal fluids before encountering the egg at the site of fertilization. Neither is there a need for the kind of motility capable of propelling the sperm through the cervical mucus into the uterus then through the utero-tubal junction into the oviduct; thus, MESA and PESA in the very proximal epididymal tubules or efferent ducts can collect sperm useful for both IVF and ICSI.

Interestingly, it has been reported that sperm collected more proximally in the obstructed epididymis are more useful for assisted reproductive techniques (ART) than sperm collected more distally near the site of obstruction (Marmar et al, 1993; Schlegel et al.,1994; Silber, 1990). The reason for this likely relates to at least two factors: 1) the movement of intraluminal content

down the epididymal duct is positive but pendular, with sperm being pushed forward then backward (Jakkola, 1983; Turner et al., 1990) by peristaltic-like contractions of the epididymal tubule (Jakkola and Talo, 1982). In the acutely obstructed epididymides of laboratory animals, our unpublished observations are that the vigor of the back-and-forth surges of luminal content increases as intraluminal pressure increases after obstruction. In the chronically obstructed human epididymis, it appears likely that the movement of luminal content over long periods of time could result in sperm from a more proximal part of the duct being mixed with sperm from the more distal part of the duct; thus some sperm collected from the more proximal duct could have been exposed to a more distal microenvironment than usually imagined. 2) Starting at the efferent ducts, the more distal one goes along the obstructed epididymis, the nearer one approaches the point of obstruction where the epididymal lumen contains nothing but dead cells and cell debris. In such cases, moving proximally from the obstruction gets away from the area containing a high proportion of degraded spermatozoa. Taking samples in this area may be why some investigators have found human testicular sperm more useful in ICSI procedures than sperm from obstructed epididymides (Dozortsev et al., 2006). Moving as far distally along the epididymis as possible while still remaining proximal to the region of degraded sperm allows the highest possibility of success in obtaining sperm useful for ART.

It is important to note in Table 1 that the human data are all from sperm obtained from unobstructed epididymides, not the obstructed epididymides encountered clinically in MESA or PESA procedures. Thus, regardless of the species studied, sperm from these unobstructed epididymides are essentially immotile and infertile in the caput and they gain the capacity for these characteristics during epididymal transit (Moore et al., 1983; Buffat et al., 2006). Also in Table 1, the increasing sperm maturation parameters between proximal and distal epididymis of the

human appear to be less dramatic than in the other species. This is a case where the function of mature sperm are likely diminished by the fact they were acquired from elderly cancer patients.

Sperm Storage:

Approximately 55-65% of total epididymal sperm in the human are stored in the cauda epididymidis (Amann, 1981). Interestingly, while as a proportion of testicular output this compares favorably with other species (Bedford, 1994), it is only the equivalent of 3 average ejaculates (Johnson and Varner;1988) while in some species the cauda contains sperm sufficient for many more ejaculates (Curtis and Amann, 1981). While sperm can pass through the human cauda within a couple of days, fertile sperm can be stored for several weeks in both man (Bedford, 1994) and other mammals (Jones and Murdoch, 1996). How long effective storage may be in the human is uncertain, but sperm motility in the ejaculate of young men can be preserved out to 7-8 weeks after the last ejaculation (Bedford, 1994). In older men, sperm motility in the cauda epididymidis can actually be less than in the distal corpus epididymidis (Yeung et al, 1993).

That reduced motility may have been due to the already mentioned problem of epididymides being obtained from sexually inactive, older men where months of sperm storage leads to senescence of sperm in the cauda epididymidis and vas deferens. Such an explanation makes intuitive sense, but how true it is remains uncertain because we still know so little about the biology of sperm storage in the epididymis. It is an area of epididymal function that has been largely ignored for many years. While the critical features of the storage environment remain unknown, it has been shown that specific proteins secreted into the lumen by the more proximal epithelium, intraluminal ionic concentrations controlled by the epithelium, the subsequent reduction of luminal pH, and the high osmolality of the lumen fluid have all been shown to play a role in main-

taining quiescent cauda sperm in several species (Jones and Murdoch, 1996; Robaire et al., 2006). The cauda microenvironment must also protect stored sperm against microbes, xenobiotics, and oxidative stress, protections that are important in the more proximal epididymis, as well (Hinton et al., 1995).

Antimicrobial defenses are provided in part by epididymal β -defensins or defensin-related proteins, many of which are highly expressed in the epididymis and differentially regulated between the epididymal regions of both humans (Hall et al., 2007; Kirchhoff, 2007) and other mammals (Jelinsky et al, 2006). A variety of protease inhibitors, lipocalins, and metal chelating compounds participate in the epididymal host-defense, as well (Hall et al., 2002; Lundwall, 2007). Antioxidant strategies involving the glutathione system (glutathione peroxidase, glutathione-S-transferase, γ -glutamyl transpeptidase, etc) and the superoxide dismutase/catalase system have been studied in laboratory species (Hinton, et al., 1995; Robaire, et al., 2006) and are known to exist in the human epididymis (Potts et al., 1999). The relative importance of the two antioxidant systems for the protection of sperm is not known, but the presence of at least two major systems in the epididymis suggests a redundancy that is likely important for prolonged sperm survival. Inflammation and oxidative stress are important clinical entities in the epididymis since epididymitis is the fifth most common urological diagnosis in men within their reproductive years (Collins et al.,1998) and has historically been a major cause of lost man-hours in the American military (Moore et al., 1971). While many cases of epididymitis are bacterial in origin, the largest studies available show those cases to be in the minority (Tracy and Steers, 2007). This means that sterile epididymitis is a significant but poorly understood epididymal pathology inviting further investigation.

Conclusion

Much remains unknown about the mammalian epididymis, generally, and the human epididymis, specifically. We know that the epididymis is important for the development of a fertile ejaculate and that a functioning organ depends on both endocrine and lumicrine secretions from the testis; but beyond that, what lumicrine factors are important for the maintenance of epididymal function? Which specific secretions of the epididymis are vital for development of fertile sperm? These things remain unknown. At the same time, approximately 25% of male infertility is idiopathic (Lipshultz et al., 1987) and a significant proportion of that infertility may arise from features of epididymal biology we do not presently understand. From this standpoint, more basic insights about the epididymis could eventually aid the infertile male. On the other hand, it has been recognized for many years that the epididymis might be a vector for male contraception (Hamilton, 1972; Hinton, 1980). Ideally, an epididymal approach to contraception would not involve manipulation of steroid hormones and would not require a cessation of spermatogenesis. Rather, it would inhibit an epididymal function required for the development of a fertile ejaculate. Contraception through an epididymal vector has recently received increased attention (Habenicht, 2003; Cooper and Yeung, 2003; Turner et al., 2006; Gottwald et al., 2006) and remains a possible product of our advancing knowledge about the human epididymis.

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Table 1: Progressive motility and ability to bind to or fertilize the egg, depending on the study, of sperm from the caput, corpus, and cauda epididymides of four mammalian species, including the human. Immature sperm in the caput can do neither, mature sperm can the cauda can do both.

	HUMAN ^{1,A}			BOAR ^B			RAT ^C			MOUSE ^D		
	CPT	COR	CDA	CPT	COR	CDA	CPT	COR	CDA	CPT	COR	CDA
% Prog. Motility ²	3	30	60	1	56	83	0	15	98	0	65	93
% Sperm Bind/Fert.	0	11	43	-	54	83	0	10	75	0	89	95

¹Epididymal sperm from humans were from collected from elderly men undergoing therapeutic orchidectomy; thus their sperm motility and fertilizing capacities are reduced relative to the values from healthy laboratory animals within their normal breeding ages.

²Percent progressive motility.

³Percent of ova binding sperm or having been fertilized *in vitro*, depending on the study.

^Adata from Moore et al. (1983) Int. J. Androl.6:310

^Bdata from Hunter et al. (1976) J. Reprod. Fertil. 46:463 and Dacheux and Paquignon (1980) Reprod. Nutr. Develop. 20:1085.

^CBlandau and Rumer (1964) Fertil. Steril. 15:561 and Turner (1995) J. Androl. 16:292

^DLacham and Trounson (1991) Mol. Reprod. Develop. 29:85

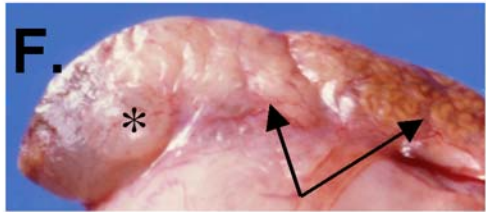
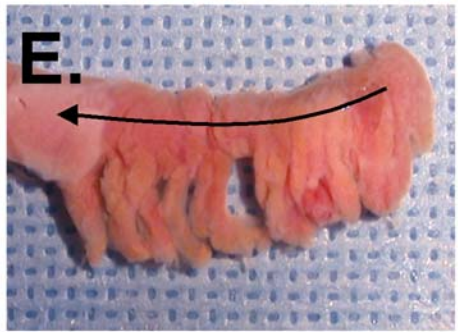
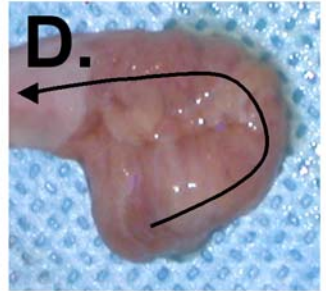
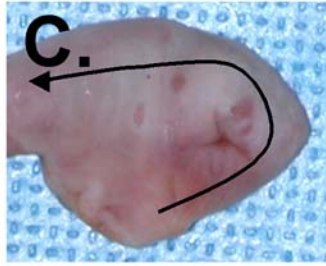
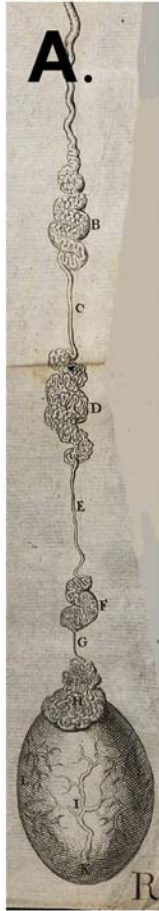
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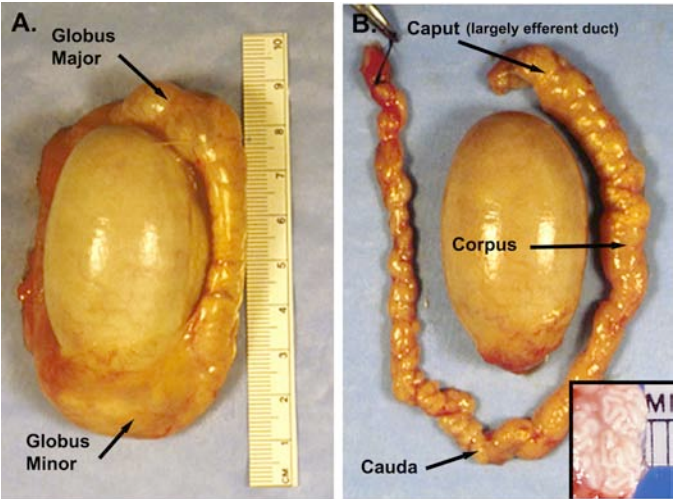
Figure 1. Dissections of the human epididymis. A. Regnier De Graaf's dissection of the human epididymis (1668). The tunica albuginea of the epididymis has been removed exposing the efferent ducts still attached to the proximal pole of the testis. B. Enlargement of panel A showing De Graaf's microdissection to reveal the single epididymal tubule or "thread" arising from a mass of tubules still attached to its origin on the testis. C. The human caput epididymis from a 53 year old male. Tissues were collected 10 hrs post mortem. Appearance of the tissue (thick tunica albuginea, no luminal filling of the tubules) indicates the poor status of this tissue collected from a male who had died with colon cancer. Contrast this with the tissue in Fig. 1F. The arrow indicates the general proximal-to-distal direction of the underlying epididymal tubule. D. Decapsulated human caput epididymidis revealing a mass of indistinct tubules similar to that shown by De Graaf at the bottom of panel B. E. The same epididymis as in panels C and D but with the caput tubules uncoiled revealing 13-15 efferent ducts leading toward the true epididymal tubule. The arrow indicates how the tissue was straightened for display to demonstrate how the efferent ducts make up a majority of the tissue of the human caput epididymis. F. Healthy caput epididymis from 23 yr old male. Note the thin tunica and filled, distinct tubes (arrows) and an epididymal cyst (asterisk) common in the caput region. Here and elsewhere, all human tissues were collected under a protocol approved by the University of Virginia Health Sciences IRB.

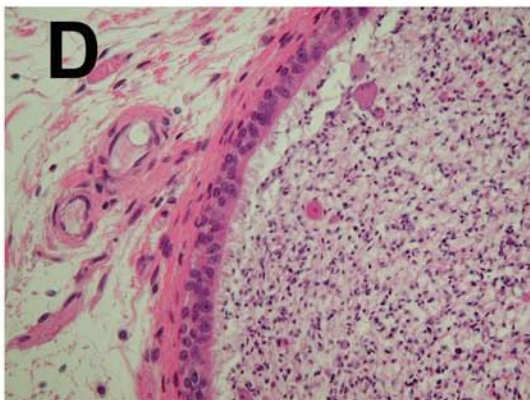
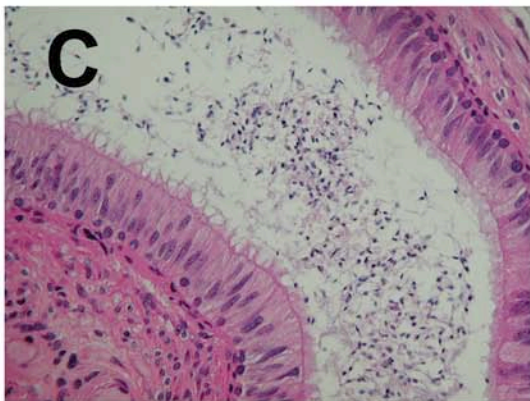
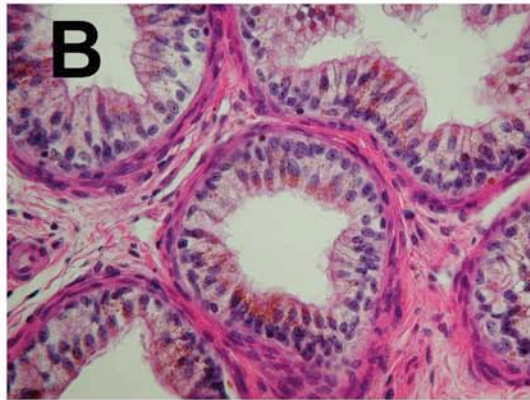
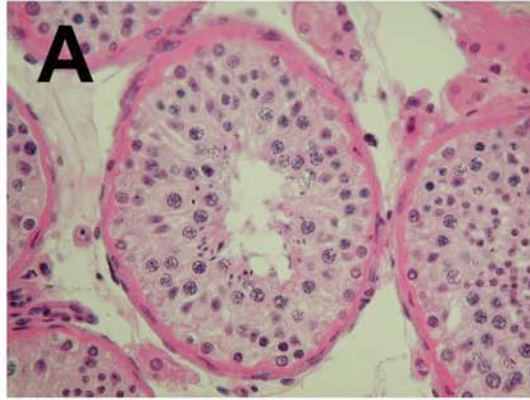
Figure. 2. The human testis and epididymis as they appear removed intact from the scrotum. The *globus major* at the cranial pole of the testis is the proximal part of the organ and receiving the sperm and intraluminal fluids from the testis. The *globus minor* is the distal part of the organ and is shrouded by the epididymal fat pad. B. The partially dissected testis and epididymis with epididymal fat removed. The caput, corpus, and cauda epididymis are indicated with the cauda tubule evolving into the convoluted vas deferens rising vertically on the left. Inset: a magnified view of decapsulated corpus tubules. The white appearance indicates a healthy tubule filled with spermatozoa. The two vertical lines on the right indicate 1 mm.

Figure 3. Histology of human epididymis. Maintenance of the epididymis requires the secretions of a normal seminiferous epithelium in the testis (A). The caput epididymidis (B) is comprised largely of efferent ducts (illustrated) with small tubule diameters and an epithelium of irregular height but with true cilia. The true epididymal tubule of the corpus (C) is larger in diameter, has a tall, columnar epithelium of regular height. Microvilli line the lumen and spermatozoa are more dense than in the caput. The cauda tube (D) has a much larger lumen diameter, a short, columnar epithelium with short microvilli and a lumen with a dense pack of spermatozoa. Examples of cilia (c) of the efferent duct epithelium and microvilli (m) of the epididymal epithelium are indicated. All panels 250X magnification.

Figure 4. Immunohistochemical localization of sonic hedgehog protein (SHH) in the adult human epididymis. SHH did not immunolocalize to the active seminiferous epithelium (A, B) or to the Type 1 efferent duct (caput) tubules (C, D). SHH was detected in the epithelium of Type 3 efferent ducts (E, F) as well as the corpus (G, H), proximal cauda (I, J) and distal cauda (K, L). Importantly, the seminiferous tubules showed complete spermatogenesis and delivered sperm and, presumably, other testicular products to the epididymal lumen (G-L). Sections illustrated in panels A, C, E, G, I, and K received no primary (negative controls). Panels B, D, F, H, J, and L illustrate sections that were serial to the controls and were processed with primary antibody. All panels 62X magnification.







- Primary

+ Primary

