

Oestrogen, its receptors and function in the male reproductive tract — a review

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Abstract

Oestrogen is synthesized in the male reproductive system by at least three different cell types; Sertoli, Leydig and germ cells. Although testosterone is recognized as the primary sex steroid in man, oestrogen is produced in sizable quantities in the testis, as well as the brain and is found in extremely high concentrations in the semen of several species. The high concentration of oestrogen in rete testis fluid of the rodent is now thought to be derived from the conversion of testosterone to estradiol by P450 aromatase in germ cells of the testis and spermatozoa traversing the reproductive tract. This new major source of oestrogen would target oestrogen receptors in the male reproductive tract, in particular the efferent ductules, which contain the highest concentration of oestrogen receptor- α . This recent data raises new hypotheses regarding the role of oestrogen in the function of the male reproductive system. The oestrogen receptor- α knockout mouse was used to help define the function of oestrogen in the male. It was found that oestrogen receptor- α is essential for fluid reabsorption in the efferent ductules and in the absence of expression the male is infertile. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

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1. Oestrogen source and concentration

Oestrogen is synthesized in the male reproductive system by at least three different cell types; Sertoli, Leydig and germ cells. Although testosterone is recognized as the primary sex steroid in man, oestrogen is produced in sizable quantities in the testis, as well as the brain (Roselli et al., 1997) and is found in extremely high concentrations in the semen of several species (Claus et al., 1992, 1987; Free and Jaffe, 1979; Ganjam and Amann, 1976). Early studies of oestrogen reported that the primary source of oestrogen in the immature male was the Sertoli cell (van der Molen et al., 1981). In the adult testis, Leydig cells express P450arom and actively synthesize estradiol at a rate much greater than that seen in the adult Sertoli cell (Payne et al., 1976; Carreau et al., 1999; Levallet and Carreau, 1997; Levallet et al., 1998). Currently, a growing body of evidence indicates that germ cells also synthesize oestrogen, and possibly serve as the major source of this steroid in the

male reproductive tract. In 1993, in collaboration with the laboratories of Bahr and Bunick (Nitta et al., 1993) we reported for the first time that P450arom is present in testicular germ cells of the adult male testis. The enzyme was localized in the Golgi of round spermatids, throughout the cytoplasm of elongating spermatids, and along the flagella of late spermatids. Its presence in these cell types was confirmed by Western blot analysis of isolated germ cells and Northern blot analysis demonstrated that its mRNA was present in testicular germ cells. P450arom activity was also measured in germ cells using the $^3\text{H}_2\text{O}$ water assay. Its activity was equal to or exceeded the activity found in isolated interstitial cells. Thus, it appeared from this early work that sperm could serve as mobile endocrine units, capable of producing oestrogen that would target oestrogen receptors (ER) downstream from the testis (Fig. 1).

The presence of P450arom in male germ cells has now been demonstrated in several species, including mouse, rat, brown bear and rooster (Hess et al., 1995; Kwon et al., 1995; Nitta et al., 1993; Janulis et al., 1998; Tsubota et al., 1993). P450arom presence in germ cells and spermatozoa was recently confirmed and

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shown to represent approximately 62% of the total testicular aromatase (Carreau et al., 1999; Levallet et al., 1998). The observation that germ cells of the testis are capable of synthesizing oestrogen raises new and exciting hypotheses regarding the potential for oestrogen to regulate functions along the epididymal tract (Fig. 2). The protein has been immunolocalized in the cytoplasmic droplet of the sperm tail, and the staining becomes less intense as sperm traverse the epididymis (Janulis et al., 1996).

The concentration of oestrogens in peripheral blood is typically very low in the male, but ranges from 2 to 180 pg/ml depending upon the species. The horse is an exception, where estrone sulfate is found as high as 2447 pg/ml (Setchell and Cox, 1982; Claus et al., 1992). Oestrogen concentrations are typically higher in the testicular vein than in general circulation (Waites and Einer-Jensen, 1974; de Jong et al., 1973; Setchell and Cox, 1982; Setchell et al., 1983). In the male rat reproductive tract, oestrogen concentrations are quite high, approximately 250 pg/ml in rete testis fluid (Free and Jaffe, 1979), which is higher than the average serum concentrations of estradiol in the female rat (Overpeck et al., 1978; Robaire and Hermo, 1988). Oestrogens have also been found to be abundant in semen (Bujan et al., 1993; Claus et al., 1992; Eiler and Graves, 1977; Ganjam and Amann, 1976; Claus et al., 1985).

2. Oestrogen receptors in the male tract

In 1975, Danzo suggested that oestrogen might be

capable of binding to receptors in the epididymal epithelium and serve some type of function in the male. He found in the rabbit that cytosol-specific estradiol (E₂) binding was highest in the cauda epididymidis of the immature animal (Toney and Danzo, 1988). During the 1980s, autoradiographic localization of ³H-E₂ was used to identify epithelial-positive tissues in the male reproductive system. Schleicher et al. (1984) found strong labeling of the efferent ductules and initial segment epididymis, with lesser binding in the distal tract. Using immunocytochemistry (ICC), ER have been localized primarily in the epithelium of efferent ductules (Goyal et al., 1998; Fisher et al., 1997; Goyal et al., 1997; Kwon et al., 1997; Ergun et al., 1997; Hess et al., 1997b; Sato et al., 1994; Iguchi et al., 1991; West and Brenner, 1990). However, in the goat and monkey, only non-ciliated cells of the efferent ductal epithelium stained ER positive (Goyal et al., 1997; West and Brenner, 1990). ER α localization in the epididymis has been less clear (Hess et al., 1997b; Kwon et al., 1997; Fisher et al., 1997; Goyal et al., 1997; West and Brenner, 1990). In both rat and mouse, the epithelium of vas deferens was negative, but the surrounding stromal cells were positive (Hess et al., 1997b; Iguchi et al., 1991). In contrast, rat connective tissue in the efferent ductules and caput epididymis was positive for ER α (Hess et al., 1997b), whereas these regions were negative in the mouse (Iguchi et al., 1991).

The discovery of a second form of ER (ER β) has further complicated the interpretation of earlier data.

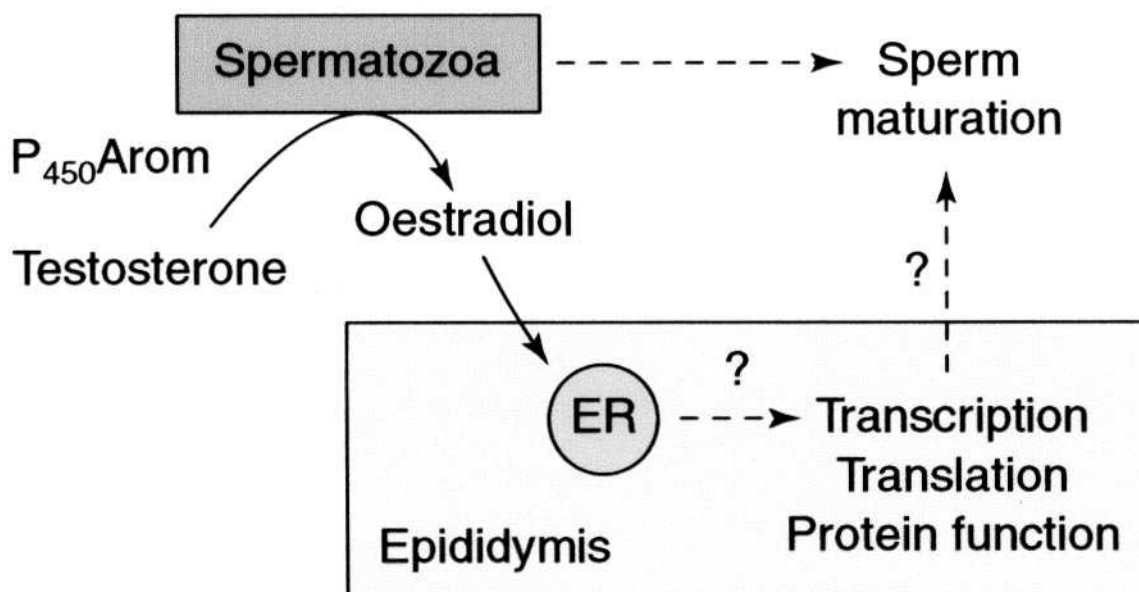


Fig. 1. This illustrates that germ cells and sperm carry P450 aromatase, which is capable of synthesizing estradiol from testosterone in the lumen of the male reproductive tract. Estradiol synthesized by the germ cells and sperm will target oestrogen receptors in the male tract, where as yet unidentified genes (?) are regulated, which have the potential (?) to regulate sperm maturation in the head of the epididymis.

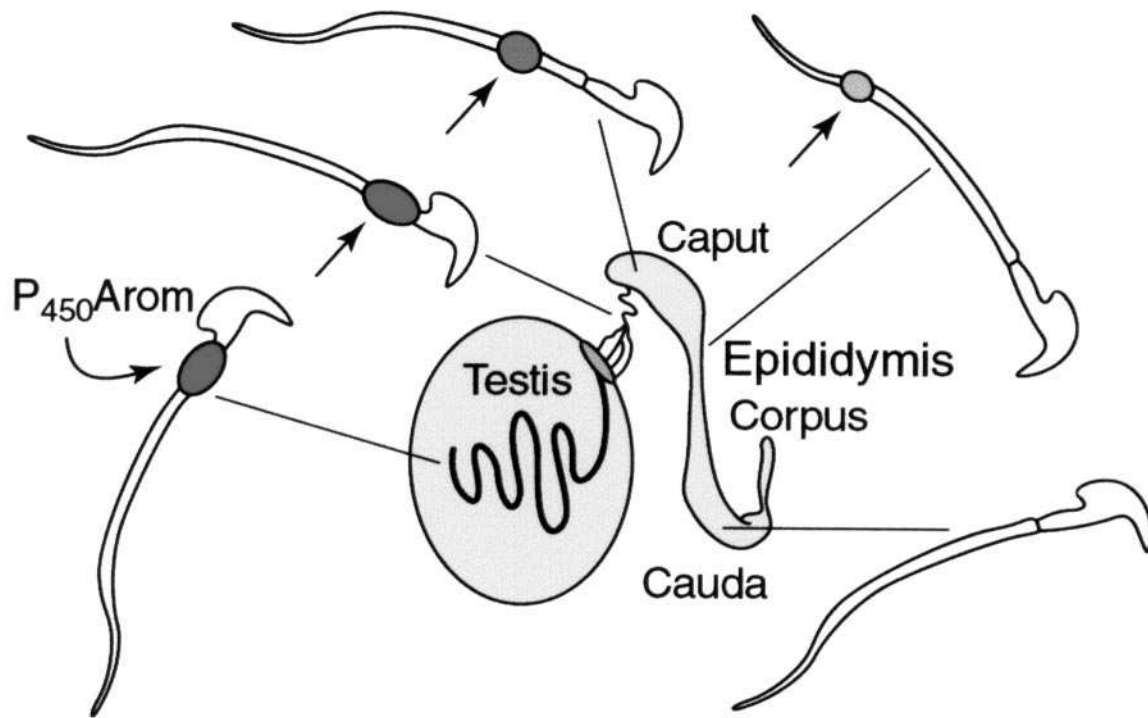


Fig. 2. P450 aromatase ($P_{450}Arom$) in sperm is noted in the cytoplasmic droplet of the rodent sperm tail (arrow). Immunostaining is stronger in testis and efferent ductules, and is reduced in size and intensity as sperm traverse the epididymis, until the cytoplasmic droplet is lost in the cauda region.

ER β has now been found in testis, efferent ductules, epididymis and prostate (Kuiper et al., 1996, 1997; Hess et al., 1997b; Saunders et al., 1997; van Pelt et al., 1999; Krege et al., 1998; Rosenfeld et al., 1998; Saunders et al., 1998; Prins et al., 1998). However, a function for ER β in the male reproductive tract awaits further investigation, as the ER β knockout mouse has been shown to be fertile and appears to have a normal testis and epididymis (Krege et al., 1998).

3. Oestrogen function in the male reproductive tract

Oestrogen receptors are consistently present in abundance in efferent ductules of every species examined, but less so in other regions of the male tract. Therefore, these small ducts have remained a primary focus of oestrogen studies. Efferent ductules are small tubule offshoots of the rete testis, which form a series of coiled tubules between the rete testis and epididymis (Fig. 3). They may number between 2 and 33 depending on species (see Ilio and Hess, 1994 for an extensive review). Near the rete, the ductuli have a wider lumen but near the epididymis, a conus vasculosa forms a series of highly tortuous and narrow tubules. Within the conus, the ductules anastomose into a common duct in the rat and become invested with a connective tissue capsule. In

other species, some ductules merge, but others enter the epididymis as separate tubules. As the common duct enters the head of the epididymis it becomes smaller in diameter and remains highly coiled. The caput epididymidis in man and larger mammals is occupied mostly by the efferent ducts that leave the testis as parallel straight tubules, which become coiled into lobules that fold over one another before emptying into the epididymis (Yeung et al., 1991; Foley et al., 1995; Ilio and Hess, 1994). Columnar, non-ciliated principal cells and ciliated cells line the efferent ductule epithelium (Fig. 3) with kinocilia (Ilio and Hess, 1994). The lumen of the ductule is typically empty or contains few spermatozoa, except in the common duct where spermatozoa become more (Talo, 1981).

Non-ciliated cells in the efferent ductules appear to have a common function in all species examined. These cells have a well-developed endocytotic system that is specialized for fluid reabsorption. Apical cytoplasm is characterized by the presence of a microvillus brush border, a profusion of apical canaliculi, vesicles, and a variety of large vacuoles and membrane-bound bodies of different shapes, sizes and staining intensities (Hermo et al., 1988, 1991; Ilio and Hess, 1994, # 3604; Robaire and Hermo, 1988). Markers for electron microscopy have demonstrated that coated pits, apical tubules, endosomes, multivesicular bodies and lyso-

somes are components of an elaborate system for fluid phase, adsorptive and receptor-mediated endocytosis (Byers et al., 1985; Hermo and Morales, 1984; Hermo et al., 1985; Morales and Hermo, 1983; Veeramachaneni et al., 1990; Veeramachaneni and Amann, 1990, 1991; Ilio and Hess, 1994). Rete testis fluid is taken up by the endocytotic apparatus from coated pits to multivesicular bodies and then to lysosomes for digestion by hydrolytic enzymes (Hermo and Morales, 1984). The lateral plasma membranes in the basal and supranuclear regions of the efferent ductules form a well-localized ‘tubular network’ (Ramos Jr. and Dym, 1977; Jones and Jurd, 1987; Robaire and Hermo, 1988; Ilio and Hess, 1994). The intercellular spaces become dilated when absorption is active (Pudney and Fawcett, 1984). The occurrence of these dilated intercellular channels strongly suggests that fluid movement in this part of the tract may be coupled to active solute transport (Suzuki and Nagano, 1978). However, these tubular networks show considerably less amplification than is normally found in resorptive epithelium such as the proximal convoluted tubules of the kidney (Ilio and Hess, 1992).

It is now well established that efferent ductules function to reabsorb luminal fluids (Clulow et al., 1998; Ilio and Hess, 1994). These ductules function to transport sperm and reabsorb water, ions and proteins (Fig. 4). The time interval required for spermatozoa to travel the length of the ductuli efferentes is approximately 45 min

in the rat (English and Dym, 1981), but little is known for other species. Many physiological and micropuncture studies on the proximal segments of the excurrent ducts in different species have confirmed the original findings of Crabo (1965) that more than 90% of the fluids secreted by the seminiferous epithelium is reabsorbed in the efferent ductules. The reported values, in which the caput epididymidis is excluded, vary between 74 and 96% reabsorption (Clulow et al., 1994; Djakiew and Jones, 1983; Jones, 1980; Jones and Jurd, 1987; Man et al., 1997). Although the efferent ductules are now recognized as the major site for rete testis fluid absorption, the underlying mechanisms for absorption remain unsettled. However, the work of several laboratories suggests that the primary mechanism of fluid (i.e. water) movement in the ductules involves the coupling of water and active ion transport (Clulow et al., 1996; Man et al., 1997; Clulow et al., 1994, 1998; Hansen et al., 1999; Jones and Jurd, 1987; Jones and Clulow, 1987; Ilio and Hess, 1992; Chan et al., 1995).

The movement of water involves several pathways, including paracellular flow and the use of apical and basal aquaporin (AQP) water channels for transcellular movement (Brown et al., 1993; Fisher et al., 1998). However, AQP1 appears to be expressed only on the apical surface of efferent ductules (Fisher et al., 1998), in contrast to that reported for kidney proximal tubules, which contain AQP1 in both apical and basolateral plasma membranes (Verkman, 1998, 1999). This

Testis, Efferent Ductules and Head of the Epididymis in Rodents

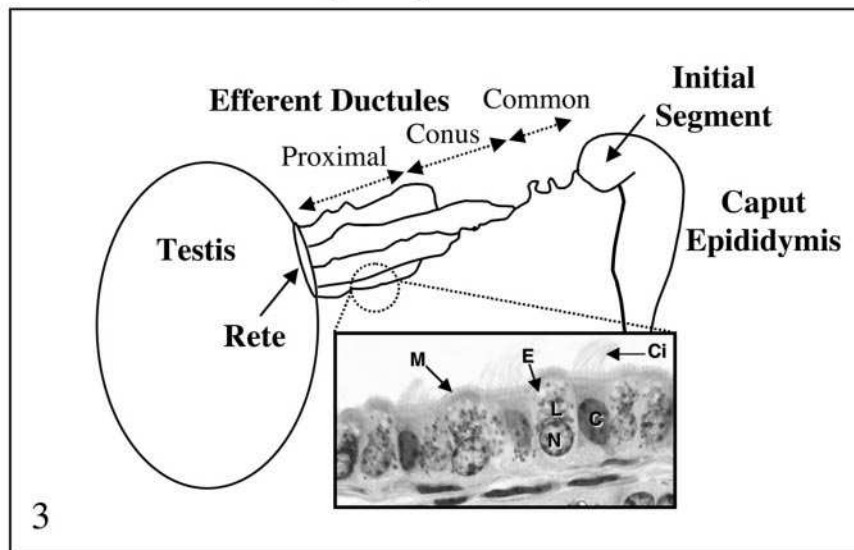


Fig. 3. A drawing of the excurrent ducts of the head of the epididymis with a photomicroscopic inset of the efferent ductule epithelium stained with PAS/hematoxylin. The efferent ductules consist of parallel coiled tubules arising from the rete testis as proximal straight ductules that merge in the conus region into a common duct that enters the initial segment epididymidis. N, non-ciliated cell; C, ciliated cell; Ci, cilia; E, endocytotic vesicle; L, lysosomes; M, microvillus border.

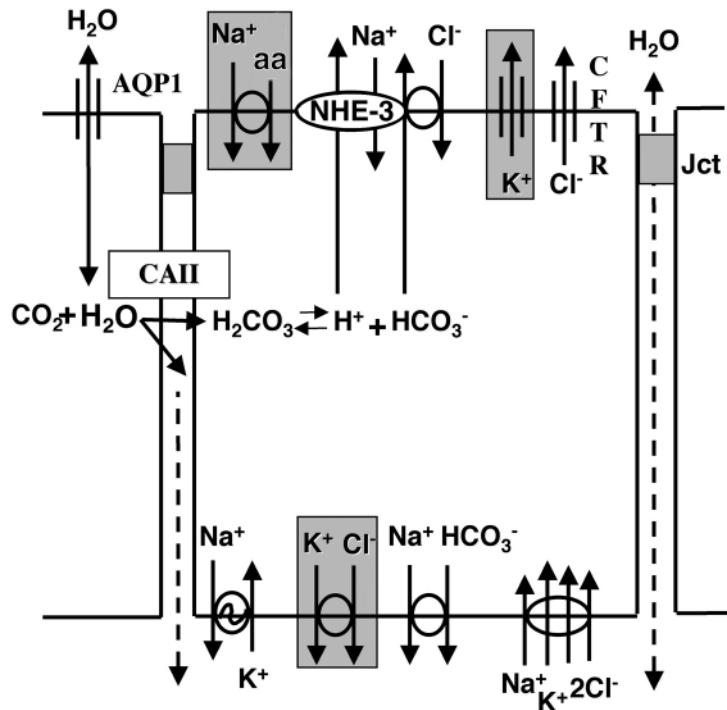


Fig. 4. A physiological model representing potential sites for oestrogen regulation in the efferent ductule epithelium. Aquaporin-1 (AQP1) is a water channel that is known to exist along the apical border. Water also may move paracellularly through the apical junctions (Jct). Carbonic anhydrase II (CAII) is abundant in efferent ductules and catalyzes the formation of carbonic acid. NHE-3 is the Na^+/H^+ exchanger along the apical membrane that apparently co-functions with the $\text{HCO}_3^-/\text{Cl}^-$ exchanger. The Cl^- channel, cystic fibrosis transmembrane regulator (CFTR) is present in the apical membrane. Along the basal membrane are found the Na^+/K^+ , ATPase and the $\text{Na}^+/\text{HCO}_3^-$ and $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ co-transporters. Channels and transporters outlined in gray are yet to be detected.

difference in the location of AQP1 in efferent ductules may explain why males are fertile even in the absence of AQP1 in knockout mice (Schnermann et al., 1998; Verkman, 1999). However, other AQP molecules may be present in the basolateral membranes of efferent ductal cells. Alternatively, if other AQP isoforms are not identified in the efferent ductules, the leaky cell junctional complex that is present could provide a rapid route for the equilibration of water across this epithelium. In the kidney, it appears that AQP1 channels that are located in both apical and basal plasma membranes of proximal tubules (Verkman, 1999) are capable of ensuring rapid movement of water by small differentials in hyperosmolality from epithelial cytoplasm to intercellular and connective tissue spaces. In the kidney of the AQP1 knockout mouse, water movement is reduced by nearly 80% (Verkman, 1999), strongly suggesting that the transcellular pathway is the dominant mechanism for water absorption in the proximal tubules. The paracellular pathway may prove to be more important in the efferent ductules, because the AQP1 knockout mouse is fertile.

The absorption of protein in the efferent ductules has been demonstrated by the disappearance of certain bands of proteins from the rete testis fluid between the ductuli efferentes and the initial segment of the epi-

didymis, owing to their absorption in the ductuli and/or the initial segment (Jones and Jurd, 1987; Koskimies and Korman, 1975; Olson and Hinton, 1985). It has been calculated that approximately 50–90% of the total protein leaving the testis was reabsorbed in the efferent ductules (Jones and Jurd, 1987; Clulow et al., 1994; Veeramachaneni and Amann, 1990, 1991). The capacity of the efferent ductal epithelium to reabsorb molecules, both through fluid-phase, adsorptive endocytosis and receptor-mediated endocytosis has been confirmed by several studies (Pelliniemi et al., 1981; Morales and Hermo, 1983; Hermo and Morales, 1984; Hermo et al., 1985; Veeramachaneni and Amann, 1991). More recently it has been shown that up to 30% of inulin is reabsorbed in the microperfused rat efferent ductules (Clulow et al., 1998), which emphasizes the role of endocytosis in transcellular movement of water, ions and proteins. Endocytosis may also provide an alternative route for water in the AQP1 knockout mouse and thus help to prevent fluid accumulation in the lumen and subsequent infertility, as seen in the ERKO mouse (Eddy et al., 1996; Hess et al., 1997a).

It was hypothesized that oestrogen regulates fluid reabsorption in the male reproductive tract (Hess et al., 1997a), based upon the following: (a) oestrogen is found in high concentrations in rete testis fluids; (b)

efferent ductules contain the highest concentration of oestrogen receptors in any organ examined to date; and (c) efferent ductules function to reabsorb nearly 90% of the luminal fluids. To test this hypothesis, the ER α gene knockout mouse (ERKO Lubahn et al., 1993) was evaluated for histological changes in efferent ductule epithelium, fluid reabsorption and fluid dynamics in the testis over time. The ERKO male is infertile, but its testes appear normal until puberty, when unexpectedly they begin to degenerate as early as 20–40 days of age (Eddy et al., 1996). By 150 days, ERKO testes are atrophic. Sperm from the ERKO male are abnormal and sperm concentrations are significantly reduced in the epididymis (Eddy et al., 1996). Rete testis in ERKO males is dilated and protrudes into the testis (Hess et al., 1997a). Downstream from the rete, the efferent ductules are swollen (Hess et al., 1997a). From these observations, two hypotheses were formed to explain how the disruption of ER α could cause fluid to accumulate in the ERKO testis (Fig. 5). The first hypothesis involved excessive fluid reabsorption from the efferent ductule lumen, which would increase the concentration of sperm and cause luminal contents to become compacted. This rapid response would induce an occlusion of the efferent ducts, which would produce fluid build-up in the testis and subsequent backpressure atrophy of the testis (Cooper and Jackson, 1973; Hess et al., 1991; Carter et al., 1987; Nakai et al., 1992). The second hypothesis suggests that an opposite mechanism would cause an inhibition of reabsorption and possibly a net inward flux of water into the ductal lumen. This excessive accumulation of fluid in the lumen would overload the ductal system, because in the rodent the efferent ductules anastomose until they form a single tubule

that enters the epididymis. This funnel-like effect of the efferent ductules requires that luminal fluids be reabsorbed along the length of the tubules in order to permit the continual movement of seminiferous tubule fluids out of the testis (Winet, 1980). Thus, the inhibition of reabsorption would also cause the accumulation of fluid in the lumen, which would subsequently cause backpressure atrophy of the testis.

In the ERKO male, it appeared that luminal fluid was not being removed by the efferent ductules, causing fluids to accumulate in efferent ductules, rete testis and seminiferous tubules (Fig. 6). As predicted from these observations, a transient increase in testis weight in ERKO males was noted between 32 and 81 days of age and then a continual decrease in weight out to 185 days of age, suggesting that the long-term atrophy of testes in the knockout mouse was caused by back pressure of the accumulating luminal fluids (Hess et al., 1997a). Luminal diameter was increased in ERKO ductules by 130%, 78% and 297% for the proximal, conus and common regions, respectively and epithelial cells of the efferent ductules in ERKO males were reduced in height by approximately 48% (Hess et al., 2000).

The efferent ductal epithelium in ERKO tissue also showed a loss of cellular organelles, a flattening of the nucleus and the loss or shortening of the microvillus border (Hess et al., 1997a, 2000). All of these changes are consistent with a decrease in fluid reabsorption observed in these ductules in the ERKO male (Hess et al., 1997a). The endocytotic apparatus, including apical vesicles and PAS+ lysosomal granules, which are prominent in non-ciliated cells of normal efferent ductules (Ilio and Hess, 1994; Hermo and Morales, 1984; Morales and Hermo, 1983), was greatly reduced in the

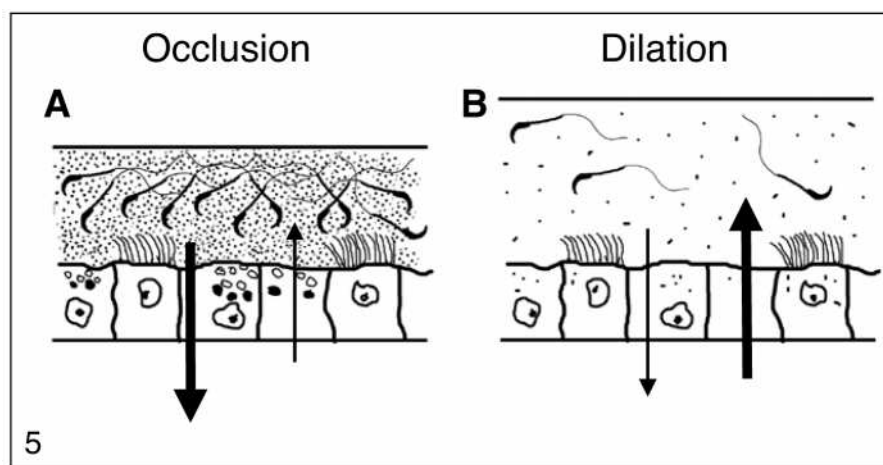


Fig. 5. Hypotheses to explain the effects of ER α disruption on the ERKO male testis, which begin to increase in weight as the males age and finally exhibit atrophy. (A) Hypothesis that excessive fluid is reabsorbed from the lumen (large arrow), causing an increased concentration of sperm in the efferent ductules and subsequent occlusion of the ductules. Occlusion of the ductules would lead to backpressure of luminal fluids in the testis. (B) Hypothesis that fluid reabsorption is inhibited (small arrow) and water is leaking or secreted into the lumen (large arrow), causing dilation of the ductules and dilution of luminal sperm and subsequent the accumulation of luminal fluids. Both mechanisms would result in the backpressure of luminal fluids in the testis and long term atrophy of the seminiferous epithelium.

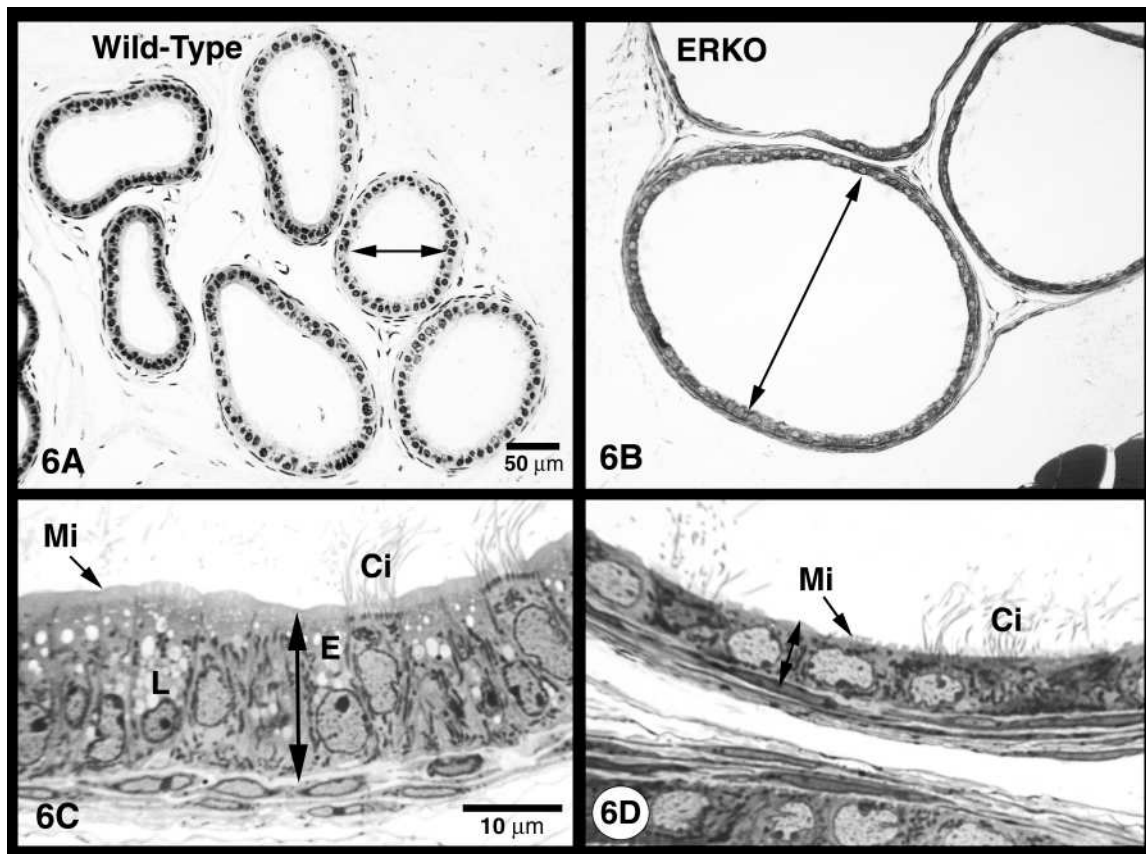


Fig. 6. (A) Wild-type efferent ductules at low magnification exhibit have narrow lumens (arrow). (B) ERKO efferent ductules have greatly dilated lumens (arrow). (C) Wild-type efferent ductule epithelium is tall, contains well-developed endocytotic vesicles (E) and numerous lysosomes (L), and has a prominent microvillus brush border (Mi). Ci, cilia. (D) ERKO efferent ductule epithelium is reduced in height, contain fewer lysosomes and endocytotic vesicles, and microvilli (Mi) are short and often absent. Ci, cilia. Bars = 50 μ m (A, B); 10 μ m (C, D).

ERKO efferent ductule epithelium. Thus, the apical surface of this absorbing epithelium appeared to be transformed into a non-absorbing lining, when ER α was lacking. To test this hypothesis, the initial segment epididymis was surgically cauterized to occlude the terminal end of the efferent ductules in the adult male. Testis weight 48 h post-surgery was increased 30% more in ERKO than in wild-type males (Hess et al., 1997a). The hypothesis was also tested in vitro, using small segments of adult efferent ductules in organ culture. The tubular ends were ligated with fine suture, which prevented the inflow of culture medium, and the lumen was observed over a 24-h period. Efferent ductules from wild-type males were capable of rapidly reabsorbing the luminal fluid, resulting in a collapse of the ductule walls; however, the luminal area of ERKO ductules did not collapse, but instead showed a dramatic increase in area (Hess et al., 1997a).

Thus, the efferent ductules in ERKO mice appear to follow the second hypothesis proposed for inducing fluid accumulation in the male tract. Thus, the ERKO mouse provides strong evidence that oestrogen, or more specifically, a functional ER α is involved in the regulation of fluid transport in the male reproductive tract,

and responsible for increasing the concentration of sperm as they enter the epididymis. Future studies are aimed at uncovering the biochemical and physiological mechanisms underlying these changes in the ERKO male and in a new animal model, in which ER are inhibited in the adult male by treatment with the pure anti-oestrogen, ICI 182,780 (AstraZeneca, Macclesfield, Cheshire).

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