ELSEVIER

Molecular and Cellular Endocrinology 178 (2001) 29–38

MCE
Molecular and Cellular Endocrinology

www.elsevier.com/locate/mce

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

Rex A. Hess [a,*], David Bunick [a], Janice Bahr [b]

[a] *Department of Veterinary Biosciences, University of Illinois, 2001 S. Lincoln, Urbana, IL 61802, USA*
[b] *Department of Animal Sciences, University of Illinois, 2001 S. Lincoln, Urbana, IL 61802, USA*

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.

Keywords: Oestrogen receptor; Testis; Efferent ductules; Epididymis; Fluid reabsorption

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 ^3H_2O 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 shown to represent approximately 62% of the total testicular aromatase (Carreau et al., 1999; Levallet et

* Corresponding author. Fax: +1-217-2441652.
E-mail address: r-hess@uiuc.edu (R.A. Hess).

0303-7207/01/$ - see front matter
PII: S0303-7207(01)00412-9

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 ^{3}H-E_2 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). ERα 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 ERα (Hess et al., 1997b), whereas these regions were negative in the mouse (Iguchi et al., 1991).

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

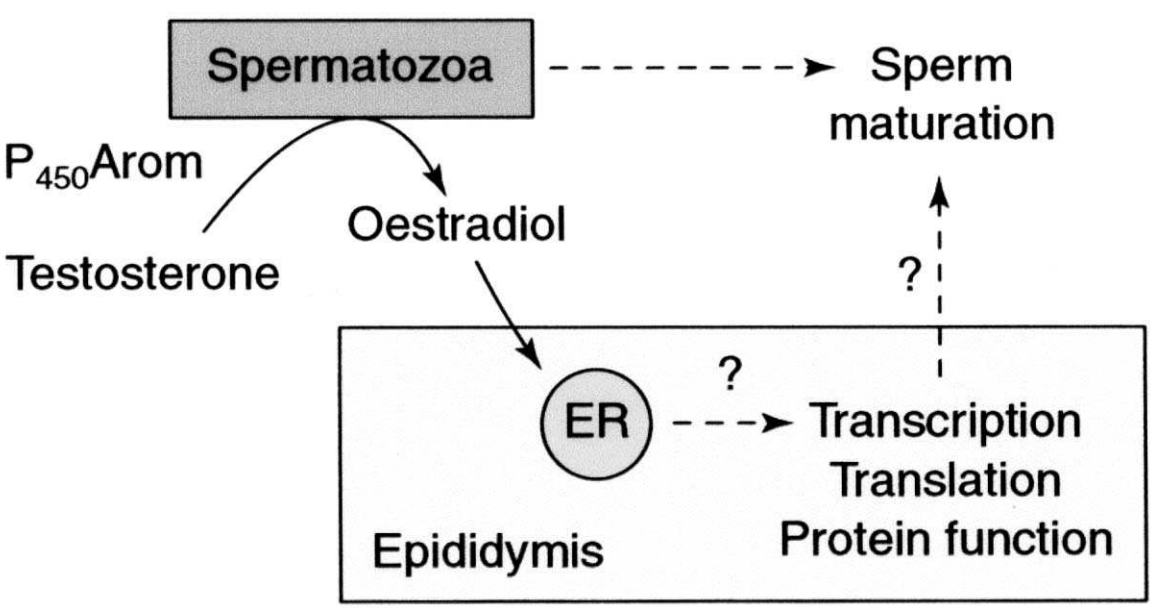

Fig. 1. This illustrates that germ cells and sperm carry P450 aromatase, which is capable of synthesizing estradiol from testosterone in the lumen of the male reproductive tract. Estradiol synthesized by the germ cells and sperm will target oestrogen receptors in the male tract, where as yet unidentified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

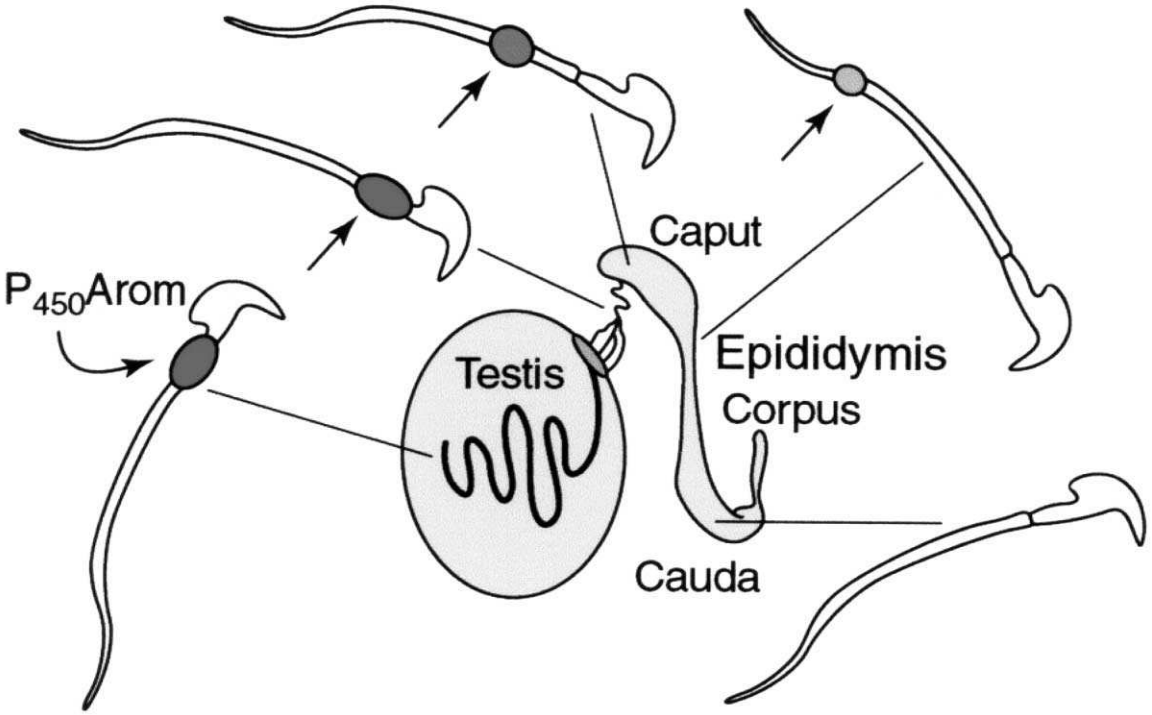

Fig. 2. P450 aromatase (P_{450}Arom) in sperm is noted in the cytoplasmic droplet of the rodent sperm tail (arrow). Immunostaining is stronger in testis and efferent ductules, and is reduced in size and intensity as sperm traverse the epididymis, until the cytoplasmic droplet is lost in the cauda region.

ERβ 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 ERβ in the male reproductive tract awaits further investigation, as the ERβ 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 coni 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; Robaire and Hermo, 1988). Markers for electron microscopy have demonstrated that coated pits, apical tubules, endosomes, multivesicular bodies and lysosomes 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 transcelluar 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

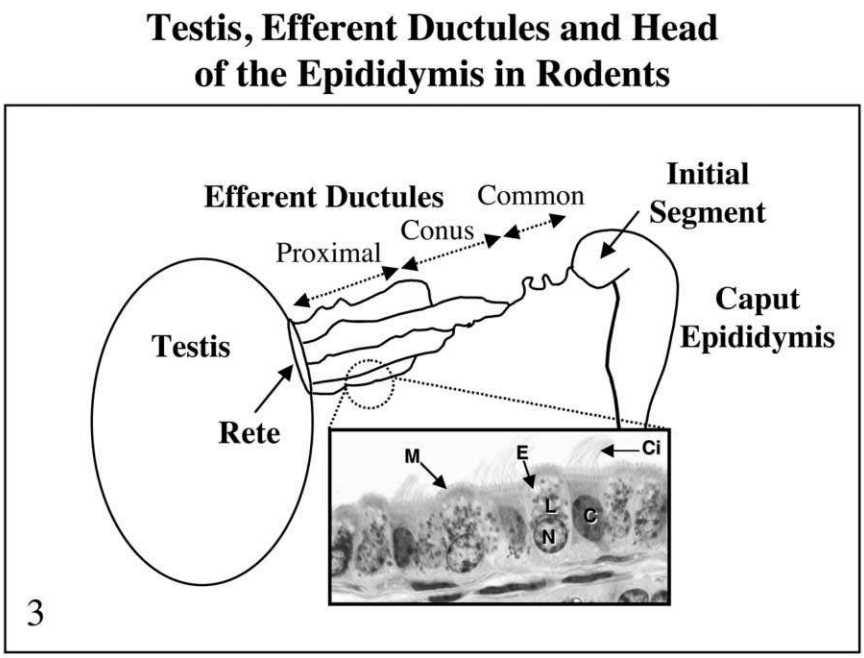

Fig. 3. A drawing of the excurrent ducts of the head of the epididymis with a photomicroscopic inset of the efferent ductule epithelium stained with PAS/hematoxylin. The efferent ductules consist of parallel coiled tubules arising from the rete testis as proximal straight ductules that merge in the conus 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.

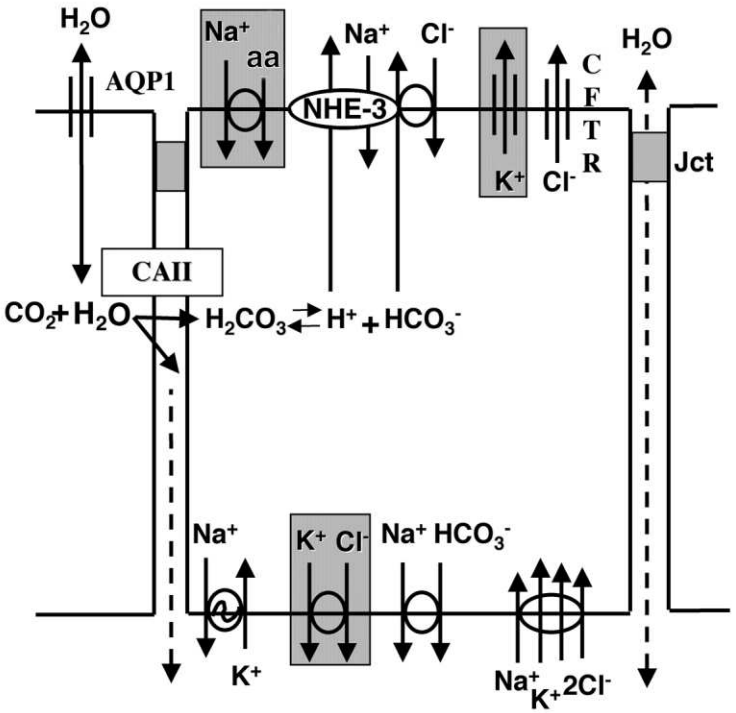

Fig. 4. A physiological model representing potential sites for oestrogen regulation in the efferent ductule epithelium. Aquaporin-1 (AQP1) is a water channel that is known to exist along the apical border. Water also may move paracelluarly through the apical junctions (Jct). Carbonic anhydrase II (CAII) 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 HCO_3^-/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 Na^+/HCO_3^- and $Na^+/K^+/2Cl^-$ 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 transcelluar 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 epididymis, owing to their absorption in the ductuli and/or the initial segment (Jones and Jurd, 1987; Koskimies and Kormano, 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 ERα 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 ERα 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

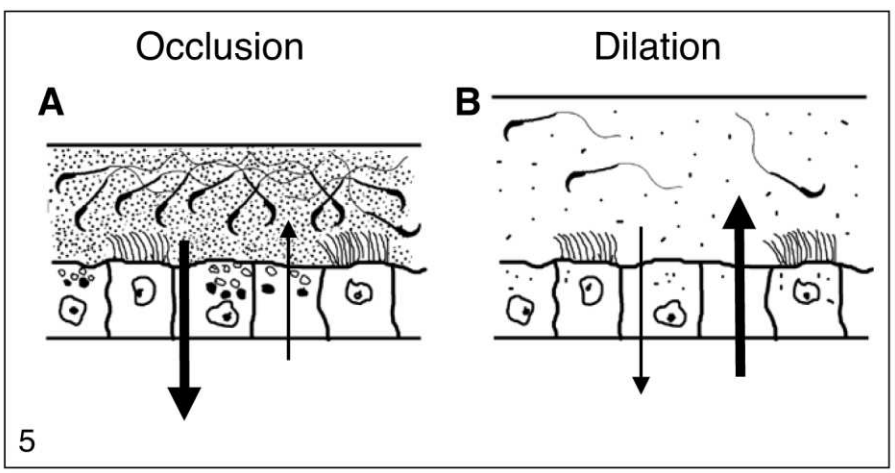

Fig. 5. Hypotheses to explain the effects of ERα disruption on the ERKO male testis, which begin to increase in weight as the males age and finally exhibit atrophy. (A) Hypothesis that excessive fluid is reabsorbed from the lumen (large arrow), causing an increased concentration of sperm in the efferent 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.

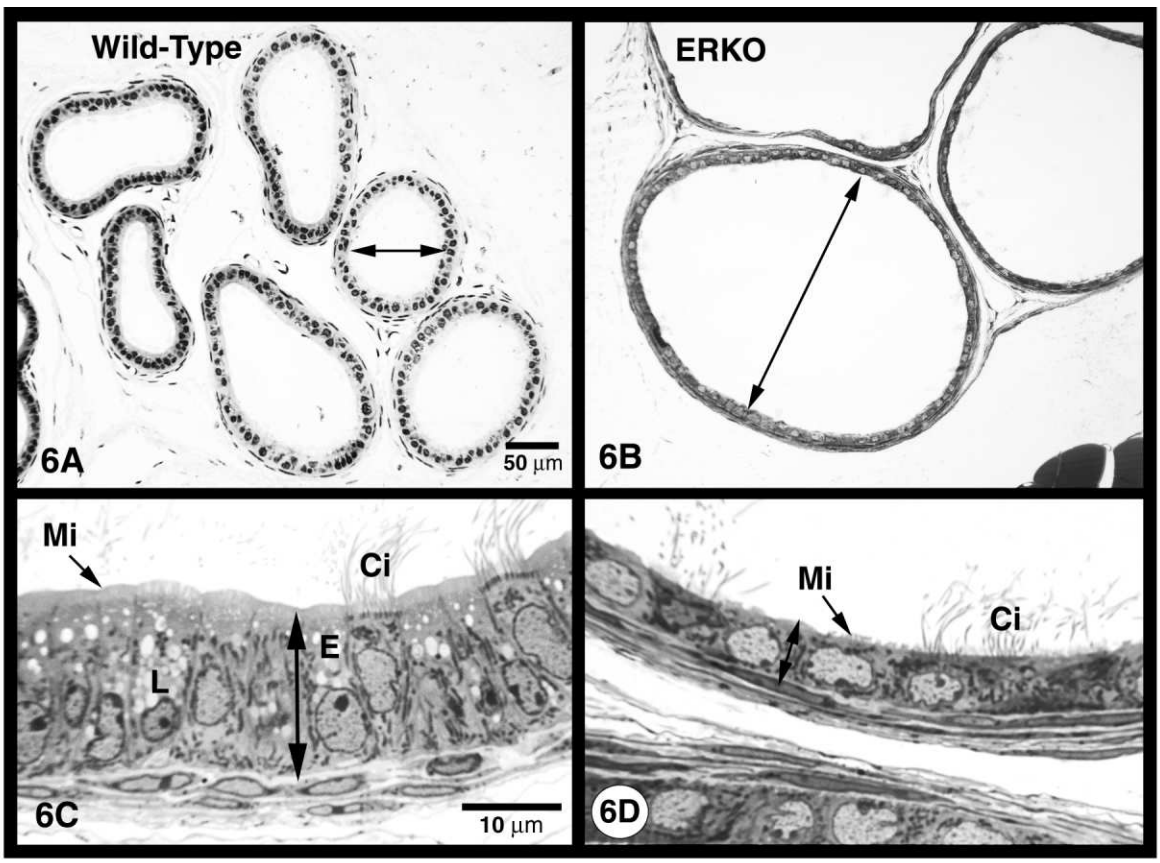

Fig. 6. (A) Wild-type efferent ductules at low magnification exhibit have narrow lumens (arrow). (B) ERKO efferent ductules have greatly dilated lumens (arrow). (C) Wild-type efferent ductule epithelium is tall, contains well-developed endocytotic vesicles (E) and numerous lysosomes (L), and has a prominent 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 μm (A, B); 10 μm (C, D).

ERKO efferent ductule epithelium. Thus, the apical surface of this absorbing epithelium appeared to be transformed into a non-absorbing lining, when ERα 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 ERα 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).

Acknowledgements

We would like to acknowledge several former and current students, including Hiro Nitta, Soga Kwon, Yu-Chyu Chen, Lynn Janulis, Sarah Janssen, Ki-Ho Lee, Qing Zhou, Rong Nie, Eman Jassim and Cleida Oliveira; visiting scientists, Masaaki Nakai and Hyun Cho; the fruitful collaboration of colleagues Dennis Lubahn, Ken Korach, Lane Clark, and Dan Gist; and the excellent technical support of Kay Carnes in the study of oestrogens in the male. Supported by NIH Grant # HD35126.

References

Brown, D., Verbavatz, J.M., Valenti, G., Lui, B., Sabolic, I., 1993. Localization of the Chip28 water channel in reabsorptive segments of the rat male reproductive tract. Eur. J. Cell. Biol. 61, 264–273.

Bujan, L., Mieusset, R., Audran, F., Lumbroso, S., Sultan, C., 1993. Increased oestradiol level in seminal plasma in infertile men. Hum. Reprod. 8, 74–77.

Byers, S.W., Musto, N.A., Dym, M., 1985. Culture of ciliated and nonciliated cells from rat ductuli efferentes. J. Androl. 6, 271–278.

Carreau, S., Genissel, C., Bilinska, B., Levallet, J., 1999. Sources of oestrogen in the testis and reproductive tract of the male. Int. J. Androl. 22, 211–223.

Carter, S.D., Hess, R.A., Laskey, J.W., 1987. The fungicide methyl 2-benzimidazole carbamate causes infertility in male Sprague–Dawley rats. Biol. Reprod. 37, 709–717.

Chan, H.C., Lai, K.B., Fu, W.O., Chung, Y.W., Chan, P.S., Wong, P.Y., 1995. Regional differences in bioelectrical properties and anion secretion in cultured epithelia from rat and human male excurrent ducts. Biol. Reprod. 52, 192–198.

Claus, R., Dimmick, M.A., Gimenez, T., Hudson, L.W., 1992. Estrogens and prostaglandin F2a in the semen and blood plasma of stallions. Theriogenology 38, 687–693.

Claus, R., Hoang-Vu, C., Ellendorff, F., Meyer, H.D., Schopper, D., Weiler, U., 1987. Seminal oestrogens in the boar: origin and functions in the sow. J. Steroid. Biochem. 27, 331–335.

Claus, R., Schopper, D., Hoang-Vu, C., 1985. Contribution of individual compartments of the genital tract to oestrogen and testosterone concentrations in ejaculates of the boar. Acta. Endocrinol. 109, 281–288.

Clulow, J., Hansen, L.A., Jones, R.C., 1996. In vivo microperfusion of the ductuli efferentes testis of the rat: flow dependence of fluid reabsorption. Exp. Physiol. 81, 633–644.

Clulow, J., Jones, R.C., Hansen, L.A., 1994. Micropuncture and cannulation studies of fluid composition and transport in the ductuli efferentes testis of the rat: comparisons with the homologous metanephric proximal tubule. Exp. Physiol. 79, 915–928.

Clulow, J., Jones, R.C., Hansen, L.A., Man, S.Y., 1998. Fluid and electrolyte reabsorption in the ductuli efferentes testis. J. Reprod. Fertil. Suppl. 53, 1–14.

Cooper, E.R., Jackson, H., 1973. Chemically induced sperm retention cysts in the rat. J. Reprod. Fertil. 34, 445–449.

Crabo, B., 1965. Studies on the composition of epididymal content in bulls and boars. Acta Vet. Scand. 6, 8–94.

de Jong, F., Hey, A., van der Molen, H., 1973. Effect of gonadotrophins on the secretion of oestradiol-17b and testosterone by the rat testis. J. Endocrinol. 57, 277–284.

Djakiew, D., Jones, R.C., 1983. Sperm maturation, fluid transport, and secretion and absorption of protein in the epididymis of the echidna, *Tachyglossus aculeatus*. J. Reprod. Fertil. 68, 445–456.

Eddy, E.M., Washburn, T.F., Bunch, D.O., Goulding, E.H., Gladen, B.C., Lubahn, D.B., et al., 1996. Targeted disruption of the oestrogen receptor gene in male mice causes alteration of spermatogenesis and infertility. Endocrinology 137, 4796–4805.

Eiler, H., Graves, C., 1977. Oestrogen content of semen and the effect of exogenous oestradiol-17a on the oestrogen and androgen concentration in semen and blood plasma of bulls. J. Reprod. Fertil. 50, 17–21.

English, H.F., Dym, M., 1981. The time required for materials injected into the rete testis to reach points in the caput epididymis of the rat and observations on the absorption of cationic ferritin. Ann. NY Acad. Sci. 383, 445–446.

Ergun, S., Ungefroren, H., Holstein, A.F., Davidoff, M.S., 1997. Estrogen and progesterone receptors and estrogen receptor-related antigen (ER-D5) in human epididymis. Mol. Reprod. Dev. 47, 448–455.

Fisher, J.S., Millar, M.R., Majdic, G., Saunders, P.T., Fraser, H.M., Sharpe, R.M., 1997. Immunolocalisation of oestrogen receptor-alpha within the testis and excurrent ducts of the rat and marmoset monkey from perinatal life to adulthood. J. Endocrinol. 153, 485–495.

Fisher, J.S., Turner, K.J., Fraser, H.M., Saunders, P.T., Brown, D., Sharpe, R.M., 1998. Immunoexpression of aquaporin-1 in the efferent ducts of the rat and marmoset monkey during development, its modulation by estrogens, and its possible role in fluid resorption. Endocrinology 139, 3935–3945.

Foley, G.L., Bassily, N., Hess, R.A., 1995. Intratubular spermatic granulomas of the canine efferent ductules. Toxicol. Pathol. 23, 731–734.

Free, M.J., Jaffe, R.A., 1979. Collection of rete testis fluid from rats without previous efferent duct ligation. Biol. Reprod. 20, 269–278.

Ganjam, V.K., Amann, R.P., 1976. Steroids in fluids and sperm entering and leaving the bovine epididymis, epididymal tissue, and accessory sex gland secretions. Endocrinology 99, 1618–1630.

Goyal, H.O., Bartol, F.F., Wiley, A.A., Khalil, M.K., Chiu, J., Vig, M.M., 1997. Immunolocalization of androgen receptor and estrogen receptor in the developing testis and excurrent ducts of goats. Anat. Rec. 249, 54–62.

Goyal, H.O., Bartol, F.F., Wiley, A.A., Khalil, M.K., Williams, C.S., Vig, M.M., 1998. Regulation of androgen and estrogen receptors in male excurrent ducts of the goat: an immunohistochemical study. Anat. Rec. 250, 164–171.

Hansen, L.A., Clulow, J., Jones, R.C., 1999. The role of Na^+–H^+ exchange in fluid and solute transport in the rat efferent ducts. Exp. Physiol. 84, 521–527.

Hermo, L., Clermont, Y., Morales, C., 1985. Fluid-phase and adsorptive endocytosis in ciliated epithelial cells of the rat ductuli efferentes. Anat. Rec. 211, 285–294.

Hermo, L., Morales, C., 1984. Endocytosis in nonciliated epithelial cells of the ductuli efferentes in the rat. Am. J. Anat. 171, 59–74.

Hermo, L., Spier, N., Nadler, N.J., 1988. Role of apical tubules in endocytosis in nonciliated cells of the ductuli efferentes of the rat: a kinetic analysis. Am. J. Anat. 182, 107–119.

Hermo, L., Wright, J., Oko, R., Morales, C., 1991. Role of epithelial cells of the male excurrent duct system of the rat in the endocytosis or secretion of sulfated glycoprotein-2 (clusterin). Biol. Reprod. 44, 1113–1131.

Hess, R.A., Bunick, D., Bahr, J.M., 1995. Sperm, a source of estrogen. Environ. Health. Perspect. 103 (Suppl. 7), 59–62.

Hess, R.A., Bunick, D., Lee, K.H., Bahr, J., Taylor, J.A., Korach, K.S., et al., 1997a. A role for oestrogens in the male reproductive system. Nature 390, 509–512.

Hess, R.A., Bunick, D., Lubahn, D.B., Zhou, Q., Bouma, J., 2000. Morphologic changes in efferent ductules and epididymis in estrogen receptor-alpha knockout mice. J. Androl. 21, 107–121.

Hess, R.A., Gist, D.H., Bunick, D., Lubahn, D.B., Farrell, A., Bahr, J., et al., 1997b. Estrogen receptor (α & β) expression in the excurrent ducts of the adult male rat reproductive tract. J. Androl. 18, 602–611.

Hess, R.A., Moore, B.J., Forrer, J., Linder, R.E., Abuel-Atta, A.A., 1991. The fungicide benomyl (methyl 1-(butylcarbamoyl)-2- benzimidazolecarbamate) causes testicular dysfunction by inducing the sloughing of germ cells and occlusion of efferent ductules. Fundam. Appl. Toxicol. 17, 733–745.

Iguchi, T., Uesugi, Y., Sato, T., Ohta, Y., Takasugi, N., 1991. Developmental pattern of estrogen receptor expression in male mouse genital organs. Mol. Androl. 6, 109–119.

Ilio, K.Y., Hess, R.A., 1992. Localization and activity of Na+, K(+)-ATPase in the ductuli efferentes of the rat. Anat. Rec. 234, 190–200.

Ilio, K.Y., Hess, R.A., 1994. Structure and function of the ductuli efferentes: a review. Microsc. Res. Technol. 29, 432–467.

Janulis, L., Bahr, J.M., Hess, R.A., Janssen, S., Osawa, Y., Bunick, D., 1998. Rat testicular germ cells and epididymal sperm contain active P450 aromatase. J. Androl. 19, 65–71.

Janulis, L., Hess, R.A., Bunick, D., Nitta, H., Janssen, S., Asawa, Y., et al., 1996. Mouse epididymal sperm contain active P450 aromatase which decreases as sperm traverse the epididymis. J. Androl. 17, 111–116.

Jones, R.C., 1980. Luminal composition and maturation of spermatozoa in the genital ducts of the African elephant (*Loxodonta africana*). J. Reprod. Fertil. 60, 87–93.

Jones, R.C., Clulow, J., 1987. Regulation of the elemental composition of the epididymal fluids in the tammar, *Macropus eugenii*. J. Reprod. Fertil. 81, 583–590.

Jones, R.C., Jurd, K.M., 1987. Structural differentiation and fluid reabsorption in the ductuli efferentes testis of the rat. Aust. J. Biol. Sci. 40, 79–90.

Koskimies, A.I., Kormano, M., 1975. Proteins in fluids from different segments of the rat epididymis. J. Reprod. Fertil. 43, 345–348.

Krege, J.H., Hodgin, J.B., Couse, J.F., Enmark, E., Warner, M., Mahler, J.F., et al., 1998. Generation and reproductive phenotypes of mice lacking estrogen receptor beta. Proc. Natl. Acad. Sci. USA 95, 15677–15682.

Kuiper, G.G., Carlsson, B., Grandien, K., Enmark, E., Haggblad, J., Nilsson, S., et al., 1997. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. Endocrinology 138, 863–870.

Kuiper, G.G., Enmark, E., Pelto-Huikko, M., Nilsson, S., Gustafsson, J.A., 1996. Cloning of a novel receptor expressed in rat prostate and ovary. Proc. Natl. Acad. Sci. USA 93, 5925–5930.

Kwon, S., Hess, R.A., Bunick, D., Kirby, J.D., Bahr, J.M., 1997. Estrogen receptors are present in the epididymis of the rooster. J. Androl. 18, 378–384.

Kwon, S., Hess, R.A., Bunick, D., Nitta, H., Janulis, L., Osawa, Y., et al., 1995. Rooster testicular germ cells and epididymal sperm contain P450 aromatase. Biol. Reprod. 53, 1259–1264.

Levallet, J., Bilinska, B., Mittre, H., Genissel, C., Fresnel, J., Carreau, S., 1998. Expression and immunolocalization of functional cytochrome P450 aromatase in mature rat testicular cells. Biol. Reprod. 58, 919–926.

Levallet, J., Carreau, S., 1997. In vitro gene expression of aromatase in rat testicular cells. CR Acad. Sci. III 320, 123–129.

Lubahn, D.B., Moyer, J.S., Golding, T.S., Couse, J.F., Korach, K.S., Smithies, O., 1993. Alteration of reproductive function but not prenatal sexual development after insertional disruption of the mouse estrogen receptor gene. Proc. Natl. Acad. Sci. USA 90, 11162–11166.

Man, S.Y., Clulow, J., Hansen, L.A., Jones, R.C., 1997. Adrenal independence of fluid and electrolyte reabsorption in the ductuli efferentes testis of the rat. Exp. Physiol. 82, 283–290.

Morales, C., Hermo, L., 1983. Demonstration of fluid-phase endocytosis in epithelial cells of the male reproductive system by means of horseradish peroxidase-colloidal gold complex. Cell. Tissue Res. 230, 503–510.

Nakai, M., Hess, R.A., Moore, B.J., Guttroff, R.F., Strader, L.F., Linder, R.E., 1992. Acute and long-term effects of a single dose of the fungicide carbendazim (methyl 2-benzimidazole carbamate) on the male reproductive system in the rat. J. Androl. 13, 507–518.

Nitta, H., Bunick, D., Hess, R.A., Janulis, L., Newton, S.C., Millette, C.F., et al., 1993. Germ cells of the mouse testis express P450 aromatase. Endocrinology 132, 1396–1401.

Olson, G.E., Hinton, B.T., 1985. Regional differences in luminal fluid polypeptides of the rat testis and epididymis revealed by two-dimensional gel electrophoresis. J. Androl. 6, 20–34.

Overpeck, J.G., Colson, S.H., Hohmann, J.R., Applestine, M.S., Reilly, J.F., 1978. Concentrations of circulating steroids in normal prepubertal and adult male and female humans, chimpanzees, rhesus monkeys, rats, mice, and hamsters: a literature survey. J. Toxicol. Environ. Health 4, 785–803.

Payne, A., Kelch, R., Musich, S., Halpern, M., 1976. Intratesticular site of aromatization in the human. J. Clin. Endocrinol. Metab. 42, 1081–1087.

Pelliniemi, L.J., Dym, M., Gunsalus, G.L., Musto, N.A., Bardin, C.W., Fawcett, D.W., 1981. Immunocytochemical localization of androgen-binding protein in the male rat reproductive tract. Endocrinology 108, 925–931.

Prins, G.S., Marmer, M., Woodham, C., Chang, W., Kuiper, G., Gustafsson, J.A., et al., 1998. Estrogen receptor-beta messenger ribonucleic acid ontogeny in the prostate of normal and neonatally estrogenized rats. Endocrinology 139, 874–883.

Pudney, J., Fawcett, D.W., 1984. Seasonal changes in fine structure of the ductuli efferentes of the ground squirrel *Citellus lateralis* Say. Anat. Rec. 208, 383–399.

Ramos, A.S. Jr., Dym, M., 1977. Ultrastructure of the ductuli efferentes in monkeys. Biol. Reprod. 17, 339–349.

Robaire, B., Hermo, L., 1988. Efferent ducts, epididymis, and vas deferens: structure, functions, and their regulation. In: Knobil, E., Neill, J. (Eds.), The Physiology of Reproduction. Raven Press, New York, pp. 999–1080.

Roselli, C.E., Abdelgadir, S.E., Resko, J.A., 1997. Regulation of aromatase gene expression in the adult rat brain. Brain. Res. Bull. 44, 351–357.

Rosenfeld, C.S., Ganjam, V.K., Taylor, J.A., Yuan, X., Stiehr, J.R., Hardy, M.P., et al., 1998. Transcription and translation of estrogen receptor-beta in the male reproductive tract of estrogen receptor-alpha knock-out and wild-type mice. Endocrinology 139, 2982–2987.

Sato, T., Chiba, A., Hayashi, S., Okamura, H., Ohta, Y., Takasugi, N., et al., 1994. Induction of estrogen receptor and cell division in genital tracts of male mice by neonatal exposure to diethylstilbestrol. Reprod. Toxicol. 8, 145–153.

Saunders, P.T., Fisher, J.S., Sharpe, R.M., Millar, M.R., 1998. Expression of oestrogen receptor beta (ER beta) occurs in multiple cell types, including some germ cells, in the rat testis. J. Endocrinol. 156, R13–17.

Saunders, P.T.K., Maguire, S.M., Gaughan, J., Millar, M.R., 1997. Expression of oestrogen receptor beta (ER-beta) in multiple rat tissues visualised by immunohistochemistry. J. Endocrinol. 154, R.13–R.16.

Schleicher, G., Drews, U., Stumpf, W.E., Sar, M., 1984. Differential distribution of dihydrotestosterone and estradiol binding sites in the epididymis of the mouse. An autoradiographic study. Histochemistry 81, 139–147.

Schnermann, J., Chou, C.L., Ma, T., Traynor, T., Knepper, M.A., Verkman, A.S., 1998. Defective proximal tubular fluid reabsorption in transgenic aquaporin-1 null mice. Proc. Natl. Acad. Sci. USA 95, 9660–9664.

Setchell, B.P., Cox, J.E., 1982. Secretion of free and conjugated steroids by the horse testis into lymph and venous blood. J. Reprod. Fertil. Suppl. 32, 123–127.

Setchell, B.P., Laurie, M.S., Flint, A.P., Heap, R.B., 1983. Transport of free and conjugated steroids from the boar testis in lymph, venous blood and rete testis fluid. J. Endocrinol. 96, 127–136.

Suzuki, F., Nagano, T., 1978. Regional differentiation of cell junctions in the excurrent duct epithelium of the rat testis as revealed by freeze-fracture. Anat. Rec. 191, 503–520.

Talo, A., 1981. In-vitro spontaneous electrical activity of rat efferent ductules. J. Reprod. Fertil. 63, 17–20.

Toney, T.W., Danzo, B.J., 1988. Developmental changes in and hormonal regulation of estrogen and androgen receptors present in the rabbit epididymis. Biol. Reprod. 39, 818–828.

Tsubota, T., Nitta, H., Osawa, Y., Mason, I., Kita, I., Tiba, T., Bahr, J., 1993. Immunolocalization of steroidogenic enzymes, P450scc, 3β-HSD, P450c17, and P450arom in the Hokkaido brown bear. Gen. Comp. Endo. 92, 439–444.

van der Molen, H.J., Brinkmann, A.O., de Jong, F.H., Rommerts, F.F. 1981. Testicular oestrogens. J. Endocrinol. 89 (Suppl) 33–46.

van Pelt, A.M., de Rooij, D.G., van der Burg, B., van der Saag, P.T., Gustafsson, J.A., Kuiper, G.G., 1999. Ontogeny of estrogen receptor-beta expression in rat testis. Endocrinology 140, 478–483.

Veeramachaneni, D.N., Amann, R.P., 1990. Oxytocin in the ovine ductuli efferentes and caput epididymidis: immunolocalization and endocytosis from the luminal fluid. Endocrinology 126, 1156–1164.

Veeramachaneni, D.N., Amann, R.P., 1991. Endocytosis of androgen-binding protein, clusterin, and transferrin in the efferent ducts and epididymis of the ram. J. Androl. 12, 288–294.

Veeramachaneni, D.N., Amann, R.P., Palmer, J.S., Hinton, B.T., 1990. Proteins in luminal fluid of the ram excurrent ducts: changes in composition and evidence for differential endocytosis. J. Androl. 11, 140–154.

Verkman, A.S., 1998. Role of aquaporin water channels in kidney and lung. Am. J. Med. Sci. 316, 310–320.

Verkman, A.S., 1999. Lessons on renal physiology from transgenic mice lacking aquaporin water channels. J. Am. Soc. Nephrol. 10, 1126–1135.

Waites, G.M., Einer-Jensen, N., 1974. Collection and analysis of rete testis fluid from macaque monkeys. J. Reprod. Fertil. 41, 505–508.

West, N.B., Brenner, R.M., 1990. Estrogen receptor in the ductuli efferentes, epididymis, and testis of rhesus and cynomolgus macaques. Biol. Reprod. 42, 533–538.

Winet, H., 1980. On the mechanism for flow in the efferent ducts. J. Androl. 1, 303–311.

Yeung, C.H., Cooper, T.G., Bergmann, M., Schulze, H., 1991. Organization of tubules in the human caput epididymidis and the ultrastructure of their epithelia. Am. J. Anat. 191, 261–279.