

# Oestrogen in fluid transport in efferent ducts of the male reproductive tract

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This review focuses on the importance of oestrogen and oestrogen receptors in the male reproductive system, with a special interest in the newly discovered role of oestrogen in the regulation of fluid reabsorption in the efferent ductules of the testis. Early work on oestrogen synthesis indicated that Leydig and Sertoli cells were the only important cells in the production of this steroid in the adult testis. However, more recent work has shown that germ cells and spermatozoa also contain aromatase and produce oestrogen. The observation that germ cells synthesize oestrogen contributed to a new hypothesis that oestrogen in the lumen of the male reproductive tract targets the epithelial lining of efferent ductules and the epididymis. The location of nuclear oestrogen receptors in the male reproductive tract has also been investigated and it has been found that oestrogen receptor  $\alpha$  is more abundant in the efferent ductules of the testis than in any other tissue of the male or female. In all species examined to date, oestrogen receptor  $\alpha$  has been found to be abundant in the efferent ductules. The structure and function of the efferent ductules are taken into account as these tubules are responsible for the reabsorption of almost 90% of the luminal rete testis fluid. Thus, it was logical to hypothesize that oestrogen receptors play a role in the regulation of fluid reabsorption in efferent ductules. The oestrogen receptor  $\alpha$  knockout mouse was used to help define this role of the receptor in males. In this animal model, the efferent ductules are altered markedly from a reabsorptive epithelium to a squamous epithelium devoid of lysosomes and endocytotic organelles. Although the separate roles for oestrogens and androgens in the regulation of fluid reabsorption are controversial and remain to be resolved, it is now established that loss of oestrogen receptor function in males interferes with the resorptive function of efferent ductules, a function that is essential for fertility. Future studies will focus on the biochemical and physiological mechanisms involved in the regulation of water and ion movement by oestrogen in the male reproductive tract.

It has been known for many years that oestrogen is synthesized in males. Many studies have been made of oestrogen synthesis by cytochrome P450 aromatase (P450arom) in Leydig and Sertoli cells of the testis (van der Molen *et al.*, 1981; Papadopoulos *et al.*, 1986; Carreau *et al.*, 1988). P450arom is also recognized in the brain, where oestrogen is important for imprinting male behaviour (Resko *et al.*, 1996; Beyer, 1999). However, until recently, there has been little evidence that oestrogens function as important steroid hormones in the male reproductive tract. The major discovery that unlocked this topic was the development of an oestrogen receptor  $\alpha$  knockout mouse (ERKO), which showed that a functional oestrogen receptor (ER)- $\alpha$  is necessary for fertility in both males and females (Lubahn *et al.*, 1993). Although only the first steps toward understanding these functions in the male have been taken, there is clear evidence that the role of ER in males is associated with the maintenance of fluid reabsorption in the head of the epididymis (Hess *et al.*, 1997a). In this review, the structure and function of the efferent ductules of the testis will be discussed along with a historical perspective of studies in male reproduction that have led to the current hypotheses for the sources of oestrogen and its function in fluid dynamics in the excurrent ducts of the testis.

## Oestrogen source and concentration

Testosterone is recognized as the primary sex steroid in men. However, oestrogen is produced in recognizable quantities in the testis, as well as in the brain (Roselli *et al.*, 1997) and is found in the semen of a number of species (Ganjam and Amann, 1976; Free and Jaffe, 1979; Claus *et al.*, 1992, 1987). The concentration of oestrogens in peripheral blood is typically very low in males (Table 1), but ranges from 2 to 180 pg ml<sup>-1</sup>, depending upon the species. An exception to this is the peripheral blood of horses, which contains concentrations of oestrone sulfate of up to 2447 pg ml<sup>-1</sup> (Setchell and Cox, 1982). Oestrogen concentrations are typically higher in the testicular vein than in the general circulation. In the male reproductive tract, oestrogen concentrations are normally higher than in blood (Table 1). In rats, the concentration of oestradiol in rete testis fluid is approximately 250 pg ml<sup>-1</sup> (Free and Jaffe, 1979), which is considerably higher than the average serum concentrations of oestradiol in female rats (Overpeck *et al.*, 1978; Robaire and Hermo, 1988). Oestrogens have also been found to be abundant in semen (Table 1). Boar semen contains the highest concentrations of oestradiol, at almost 430 pg ml<sup>-1</sup> (Claus *et al.*, 1985),

Table 1. Oestrogen concentrations in males

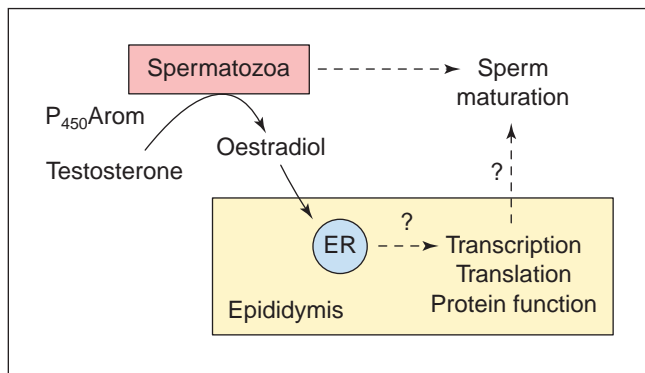
Source	Concentration	Species	Reference
Peripheral blood	19–46 pg ml <sup>-1</sup>	Man	Overpeck <i>et al.</i> , 1978; Bujan <i>et al.</i> , 1993
	40–145 pg ml <sup>-1</sup>	Monkey	Waites and Einer-Jensen, 1974
	2–25 pg ml <sup>-1</sup>	Rat	de Jong <i>et al.</i> , 1973; Dohler and Wuttke, 1975
	73.4 pg ml <sup>-1</sup> (oestradiol)	Horse	Setchell and Cox, 1982; Claus <i>et al.</i> , 1992
	2447 pg ml <sup>-1</sup> (oestrone sulphate)		
	250 ng ml <sup>-1</sup> (oestrone sulphate)		
	9–180 pg ml <sup>-1</sup>	Bull	Ganjam and Amann, 1976; Eiler and Graves, 1977
	6.3 pg ml <sup>-1</sup>	Ram	Melnyk <i>et al.</i> , 1992
	~180 pg ml <sup>-1</sup>	Boar	Setchell <i>et al.</i> , 1983; Claus <i>et al.</i> , 1985
	0.18 nmol l <sup>-1</sup> (total oestrogens)		
21.5 nmol l <sup>-1</sup> (oestrone sulphate)			
Testicular vein	104–200 pg ml <sup>-1</sup>	Monkey	Waites and Einer-Jensen, 1974
	17.5 pg ml <sup>-1</sup>	Rat	de Jong <i>et al.</i> , 1973
	450 ng ml <sup>-1</sup> (oestrone sulphate)	Horse	Setchell and Cox, 1982
	1.09 nmol l <sup>-1</sup> (total oestrogens)	Boar	Setchell <i>et al.</i> , 1983
52.4 nmol l <sup>-1</sup> (oestrone sulphate)			
Testicular lymph	900 ng ml <sup>-1</sup> (oestrone sulphate)	Horse	Setchell and Cox, 1982
Rete testis	14–195 pg ml <sup>-1</sup>	Monkey	Waites and Einer-Jensen, 1974
	249 pg ml <sup>-1</sup>	Rat	Free and Jaffe, 1979
	11.5 pg ml <sup>-1</sup>	Bull	Ganjam and Amann, 1976
	0.38 nmol l <sup>-1</sup> (total oestrogens)	Boar	Setchell <i>et al.</i> , 1983
8.60 nmol l <sup>-1</sup> (oestrone sulphate)			
Semen	162 pg ml <sup>-1</sup>	Man	Bujan <i>et al.</i> , 1993
	73 pg ml <sup>-1</sup> (oestradiol)	Horse	Claus <i>et al.</i> , 1992
	4116 pg ml <sup>-1</sup> (oestrone sulphate)		
	50–890 pg ml <sup>-1</sup>	Bull	Ganjam and Amann, 1976; Eiler and Graves, 1977
	430 pg ml <sup>-1</sup> (oestradiol)	Boar	Claus <i>et al.</i> , 1985
860 pg ml <sup>-1</sup> (oestrone)			

but horse semen also contains high concentrations of total oestrogens, primarily oestrogen sulphate (Claus *et al.*, 1992).

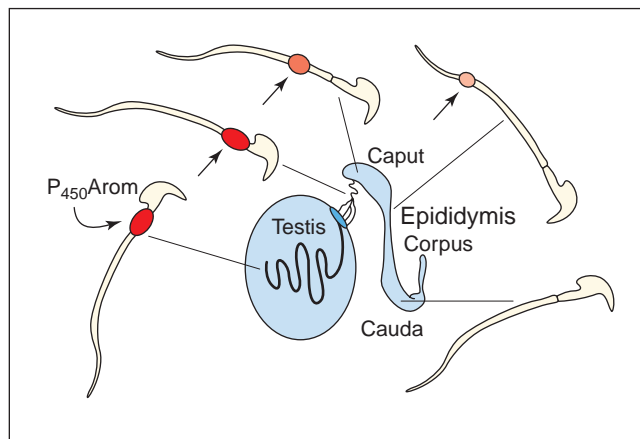
Early studies of oestrogen in the male reproductive tract reported that the primary source of oestrogen in immature males was the Sertoli cells (van der Molen *et al.*, 1981). This finding was based primarily on data from rats. Further studies of rats and other species have shown both Leydig and Sertoli cells to be sources of oestrogen at all ages (Carreau *et al.*, 1999). However, in the adult testis, Leydig cells express P450arom and actively synthesize oestradiol at a rate much greater than that seen in adult Sertoli cells (Payne *et al.*, 1976; Levallet and Carreau, 1997; Levallet *et al.*, 1998; Carreau *et al.*, 1999). For nearly 20 years the Leydig cell was considered the only cell type in the adult rodent testis to actively produce oestrogen. However, there were suggestions that seminiferous tubules were capable of oestrogen synthesis and a growing body of evidence indicates that germ cells, at least in some species, also participate in oestrogen synthesis and possibly serve as the major source of oestrogen in the male reproductive tract. Nitta *et al.* (1993) first reported that P450arom is present in testicular germ cells of the adult mouse testis. The protein was localized by immunocytochemistry,

using an antiserum generated against purified human placental cytochrome P450arom. In the germinal epithelium, P450arom was located primarily in the Golgi of round spermatids, throughout the cytoplasm of elongating spermatids, and along the flagella of late spermatids. The protein 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 <sup>3</sup>H<sub>2</sub>O water assay and was found to equal or exceed that found in isolated interstitial cells. Thus, it appeared from this early work that spermatozoa could serve as mobile endocrine units, capable of producing oestrogen that would target ER downstream from the testis and possibly regulate the epididymal functions necessary for normal sperm maturation (Fig. 1).

The presence of P450arom in male germ cells has been demonstrated in several species, including mice, rats, brown bears and roosters (Nitta *et al.*, 1993; Tsubota *et al.*, 1993; Hess *et al.*, 1995; Kwon *et al.*, 1995; Janulis *et al.*, 1998). The presence of P450arom in germ cells and spermatozoa has been confirmed and shown to represent approximately 62% of the total testicular



**Fig. 1.** Spermatozoa as mobile endocrine units. Spermatozoa carry P450 aromatase ( $P_{450}Arom$ ), which is capable of synthesizing oestradiol from testosterone in the lumen of the male reproductive tract. Oestradiol synthesized by the germ cells and spermatozoa will target oestrogen receptors (ER) 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 spermatozoa 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 spermatozoa traverse the epididymis, until the cytoplasmic droplet is lost in the cauda epididymidis.

aromatase (Levallet *et al.*, 1998; Carreau *et al.*, 1999). However, it remains unclear whether oestrogen is synthesized by germ cells of the human testis. Most reports have noted that Leydig cells or interstitial tissue are the only source of oestrogen (Payne *et al.*, 1976). However, cultures of Sertoli cells from the human testis show aromatase activity (Foucault *et al.*, 1994). 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, where ER is known to be present (Schleicher *et al.*, 1984). P450arom has been immunolocalized in the cytoplasmic droplet of the sperm tail and shows its most intense staining in the seminiferous tubule lumen, efferent ductules and proximal caput epididymis (Janulis *et al.*, 1996) (Fig. 2). Staining was less intense as spermatozoa traversed the epididymis, until all labelling was lost in the cauda region. On the basis of these observations, oestrogen appears to have a major function in the efferent ductules and head of the epididymis. Therefore, considerable effort has been made to localize ER in these regions in several species.

### Oestrogen receptors

Oestrogen receptors have been detected in the male reproductive tract of numerous species (Schleicher *et al.*, 1984; Brenner *et al.*, 1990; West and Brenner, 1990; Cooke *et al.*, 1991b; Greco *et al.*, 1993; Danzo, 1997; Fisher *et al.*, 1997; Goyal *et al.*, 1997; Hess *et al.*, 1997b; Iguchi *et al.*, 1991; Prins *et al.*, 1998; Saunders *et al.*, 1997, 1998; van Pelt *et al.*, 1999). In these reports, the distribution of ER varies widely within the male reproductive tract, depending upon the species and the method used to localize the ER: binding assay, autoradiography or immunohistochemistry. Danzo *et al.* (1975) were the first to suggest that oestrogen is capable of binding to receptors in the epididymal epithelium. However, McLachlan (1975) and coworkers reported that exposure to the potent oestrogen, diethyl stilboestrol (DES) *in utero* induced epididymal granulomas in

mice. These early studies were the stimulus that began the search for the role of oestrogen in male reproduction.

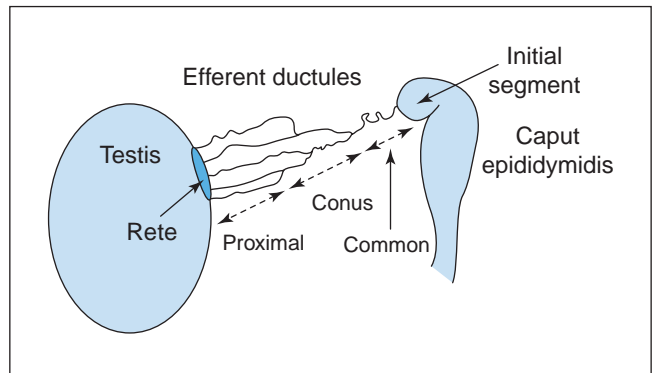
In immature rabbits, cytosol-specific oestradiol binding was highest in the cauda epididymidis (Toney and Danzo, 1988). In adult rams, oestradiol binding was also highest in the cauda epididymidis (Tekpetey and Amann, 1988). In mice, autoradiographic localization of [ $^3H$ ]oestradiol was greatest in the epithelium and connective tissues of the ductuli efferentes and the initial segment epididymis, with less binding in the distal tract (Schleicher *et al.*, 1984). Immunocytochemistry studies have shown that ER is localized primarily in the epithelium of the efferent duct (West and Brenner, 1990; Iguchi *et al.*, 1991; Fisher *et al.*, 1997; Goyal *et al.*, 1997, 1998; Hess *et al.*, 1997b; Kwon *et al.*, 1997). However, in goats and monkeys, only the non-ciliated cells of the efferent ductal epithelium stained ER-positive (West and Brenner, 1990; Goyal *et al.*, 1997). ER $\alpha$  localization in the epididymis has been less clear, with some autoradiographic (Schleicher *et al.*, 1984) and some immunocytochemical evidence (Hess *et al.*, 1997b; Kwon *et al.*, 1997), but some reports showing no evidence for ER $\alpha$  in the epididymis (West and Brenner, 1990; Fisher *et al.*, 1997; Goyal *et al.*, 1997). In both rats and mice, the epithelium of the vas deferens was negative, but the surrounding stromal cells were positive for ER $\alpha$  (Iguchi *et al.*, 1991; Hess *et al.*, 1997b). Connective tissue in the rat efferent ductules and caput epididymidis was positive for ER $\alpha$  (Hess *et al.*, 1997b), whereas these regions were negative in mice (Iguchi *et al.*, 1991). ER $\alpha$  is also found in the prostate, but is specific for the periductal smooth muscle cells from the proximal regions out to the distal tips of the developing prostate lobes (Prins and Birch, 1997). In the prostate, it appears that ER $\alpha$  is regulated by oestrogen (Prins and Birch, 1997). Little is known about ER regulation in the efferent ductules and epididymis, although Goyal (1998) showed by immunocytochemistry an upregulation of ER $\alpha$  by testosterone in goat efferent ductules after castration. In addition, exposure to DES at birth causes an upregulation of ER in the male reproductive tract stroma and epithelium (Sato *et al.*, 1994).

The discovery of a second form of ER (ER $\beta$ ) has further complicated the interpretation of earlier data. Autoradiography, which detects ER by localizing oestradiol binding, cannot distinguish between ER $\alpha$  and ER $\beta$ . ER $\beta$  differs from ER $\alpha$  primarily in the N-terminus domain (Kuiper *et al.*, 1996). Therefore, some antibodies against regions highly conserved in ER $\alpha$  and ER $\beta$  may recognize both forms of ER, while other antibodies recognize only one form. ER $\beta$  has now been found in the testis, efferent ductules, epididymis and prostate (Kuiper *et al.*, 1996, 1997; Hess *et al.*, 1997b; Saunders *et al.*, 1997, 1998; Krege *et al.*, 1998; Prins *et al.*, 1998; Rosenfeld *et al.*, 1998; van Pelt *et al.*, 1999) and appears to be much more extensive in the male tract, and is found in both epithelium and connective tissue cells. ER $\beta$  is located in prostate epithelial cells, but not in stroma, while ER $\alpha$  is found in the stromal cells but not in prostate epithelium (Prins and Birch, 1997; Prins *et al.*, 1998). ER $\beta$  is expressed extensively in the testis and is found in Sertoli cells, spermatogonia and pachytene spermatocytes (Kuiper *et al.*, 1996; Saunders *et al.*, 1997, 1998; Shughrue *et al.*, 1998; van Pelt *et al.*, 1999). A function for ER $\beta$  in the male reproductive tract awaits further investigation. However, the ER $\beta$  knockout mouse has been shown to be fertile and appears to have a normal testis and epididymis (Krege *et al.*, 1998). Preliminary data from the author's laboratory indicates that some epithelial cells of the epididymis have abnormal morphology, but that their function is not disrupted enough to inhibit fertility (Krege *et al.*, 1998).

### Structure of efferent ductules (*ductuli efferentes*)

The efferent ductules are small tubules between the rete testis and epididymis that arise separately from rete testis near the tunica albuginea (Fig. 3). There are between 2 and 33 efferent ductules, depending on species (for review, see Ilio and Hess, 1994). Near the rete testis, the ductules have a wider lumen and are embedded in the superior epididymal ligament and a thick layer of fat. Near the epididymis in rats, a conus vasculosa is formed into a series of highly tortuous tubules. Within the conus, the ductules form a common duct by anastomosis and are invested with a connective tissue capsule. As the common duct enters the head of the epididymis, it becomes smaller in diameter and remains highly coiled. In mammals, there are two basic designs of efferent ductules (Ilio and Hess, 1994). The first pattern is that of a funnel in which the ductules form a single tubule by anastomosis that changes abruptly into the initial segment of the epididymis; this pattern is typical for rats, mice and some guinea-pigs (Jones and Jurd, 1987; Ilio and Hess, 1994). The second pattern of organization involves parallel coils of ductules that form multiple entries into the head of the epididymis; this pattern is observed in most guinea-pigs and large mammals, including men. The caput epididymis in men, unlike other mammals, is occupied mostly by the efferent ductules, which leave the testis as parallel straight tubules and become coiled into lobules that fold over one another before emptying into the epididymis (Yeung *et al.*, 1991).

Columnar, nonciliated principal cells and ciliated cells with kinocilia (Fig. 4) line the efferent ductule epithelium (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 concentrated. Non-ciliated cells



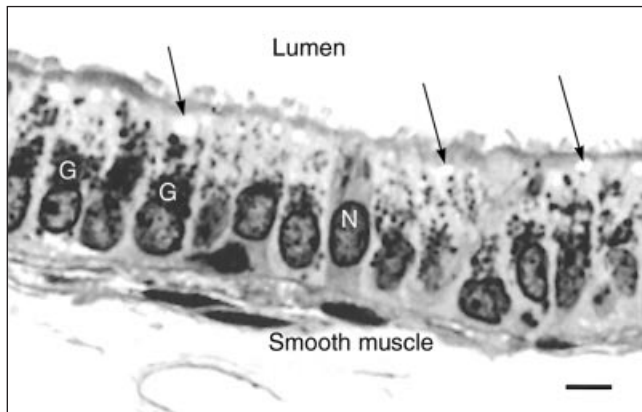
**Fig. 3.** The rodent testis, efferent ducts and head of the epididymis. Efferent ductules consist of parallel coiled ducts arising from the rete testis as proximal straight ductules, which merge in the conus region into a common duct that enters the initial segment epididymidis.

appear to have a common function in all species examined. These cells have a well developed endocytotic system with features specialized for fluid reabsorption (Fig. 4). 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 (Herms *et al.*, 1988; Robaire and Herms, 1988; Ilio and Hess, 1994). Markers for electron microscopy have demonstrated that coated pits, apical tubules, endosomes, multivesicular bodies and lysosomes are components of an elaborate endocytotic apparatus capable of fluid-phase, adsorptive and receptor-mediated endocytosis (Veeramachaneni and Amann, 1990, 1991; Veeramachaneni *et al.*, 1990; Morales and Herms, 1983; Herms and Morales, 1984; Herms *et al.*, 1985; Ilio and Hess, 1994).

The lateral plasma membranes in the basal and supranuclear regions of the efferent ductules form a localized 'tubular network' (Robaire and Herms, 1988; Ilio and Hess, 1994). The intercellular spaces become dilated when absorption is active and the occurrence of these dilated intercellular channels indicates that fluid movement in this part of the tract is coupled to active solute transport (Suzuki and Nagano, 1978). However, these tubular networks show considerably less amplification than is found normally in resorptive epithelium such as the proximal convoluted tubules of the kidney (Ilio and Hess, 1992).

### Efferent ductules and fluid reabsorption

The efferent ductule epithelium is recognized as a fluid-transporting epithelium (Clulow *et al.*, 1998). The efferent ductules function to transport spermatozoa, reabsorb water, ions and proteins and carry out spermophagy under certain conditions (for review, see Ilio and Hess, 1994). The time required for spermatozoa to travel the length of the ductuli efferentes is approximately 45 min in rats (English and Dym, 1981), but little is known for other species. In rats, the rate of fluid flow from the testis is between 30 and 50  $\mu\text{l h}^{-1}$  (Hinton and Setchell, 1993). Many physiological and micropuncture studies on the



**Fig. 4.** Light micrograph of the efferent ductules of the rat. There are a large number of dark, periodic acid–Schiff's (PAS)<sup>+</sup> granules (G) in the apical cytoplasm and large clear vacuoles that are part of the extensive endocytotic apparatus (arrows) that underlie the microvillus border of the lumen. Smooth muscle layers support the base of the epithelium. N: nucleus. Scale bar represents 10  $\mu$ m.

proximal segments of the excurrent ducts in different species have confirmed the original findings of Crabo (1965) that > 90% of the fluids secreted by the seminiferous epithelium is reabsorbed in the efferent ductules. The reported values vary from 74 to 96% reabsorption (Jones, 1980; Djakiew and Jones, 1983; Jones and Clulow, 1987; Clulow *et al.*, 1994; Man *et al.*, 1997). Although the efferent ductules are recognized as the major site for rete testis fluid absorption, the underlying mechanisms for absorption remain unsettled. However, the work of several laboratories indicates that the primary mechanism of fluid (that is, water) resorption in the ductules involves the coupling of water and active ion transport (Jones and Clulow, 1987; Jones and Jurd, 1987; Ilio and Hess, 1992; Clulow *et al.*, 1994, 1996, 1998; Chan *et al.*, 1995; Man *et al.*, 1997; Hansen *et al.*, 1999).

For many years, the 'standing osmotic gradient' model was used to explain water and ion movement in efferent ductules (Diamond and Bossert, 1967; Hamilton, 1975). This model was modified by Ilio and Hess (1992) to include the presence of Na<sup>+</sup>,K<sup>+</sup>-ATPase along the basolateral membranes and not in the apical regions of the epithelium. This finding was consistent with a generalized model of Na<sup>+</sup> transport in the kidney. Na<sup>+</sup> transport across the basal membranes was coupled with the movement of other ions such as Cl<sup>-</sup> and HCO<sup>3-</sup>. The movement of water from the lumen to interstitium was predicted to include both paracellular and transcellular pathways. However, this model was based upon the limited data available at the time.

Clulow *et al.* (1998) presented an updated model of fluid transport by the efferent ductule epithelium which attempted to account for several new types of data. For example, a Na<sup>+</sup>-H<sup>+</sup> exchanger was demonstrated in the apical membrane by the addition of amiloride to the luminal perfusate, which decreased fluid reabsorption by nearly 70% (Hansen *et al.*, 1999). Other data showed that the luminal contents of efferent ductules rapidly approach osmotic equilibrium with the blood (Clulow *et al.*, 1996). Thus, rapid permeability of both ions and water were noted in the model (Clulow *et al.*, 1998), including

the incorporation of a leaky tight junction between epithelial cells (Suzuki and Nagano, 1978), which was supported by the low transepithelial resistance found in cultures (Chan *et al.*, 1995). The movement of water in this model involves several pathways, as does the previous model (Ilio and Hess, 1992), including the free movement of water through apical and basal aquaporin (AQP) water channels (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 the situation in kidney proximal tubules, which contain AQP1 in both apical and basolateral plasma membranes (Verkman, 1998; Verkman, 1999).

This 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. If this is found to be the case, it would be a major discovery, considering the emphasis that has been placed on the homology of efferent ductules to the proximal tubules of kidney (Clulow *et al.*, 1996; Hinton and Turner, 1988). Alternatively, if other AQP isoforms are not identified in the efferent ductules, this would mean that the leaky cell junctional complex provides a rapid route for the equilibration of water across this epithelium. The model proposed by Clulow *et al.* (1998) requires further experimentation, if it is to be claimed that the efferent ductules function in a different manner from the simplified 'three-compartment model system' that is being promoted currently to explain fluid absorption and secretion by other epithelia (Spring, 1998). In the kidney, it appears that AQP1 channels that are located in both the 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 AQP1 knockout mice, water movement is reduced by nearly 80% (Verkman, 1999), indicating that the transcellular pathway is the dominant mechanism for water absorption in the proximal tubules. The paracellular pathway may prove more important in the efferent ductules, as 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 efferent ductules or the initial segment (Koskimies and Korman, 1975; Olson and Hinton, 1985; Jones, 1987). It has been calculated that 50–90% of the total protein leaving the testis is absorbed in the efferent ductules (Jones and Jurd, 1987; Veeramachaneni *et al.*, 1990; Veeramachaneni and Amann, 1991; Clulow *et al.*, 1994). The capacity of the efferent ductal epithelium to reabsorb molecules, 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). In addition, it has been shown that up to 30% of inulin is reabsorbed in microperfused rat efferent ductules (Clulow *et al.*, 1998), emphasizing the role of endocytosis in the transcellular movement of water, ions and proteins. Endocytosis may also provide an alternative route for water in AQP1 knockout mice and thus help to prevent fluid

accumulation in the lumen and subsequent infertility, in comparison with the permanent infertility found in the ERKO mouse (Eddy *et al.*, 1996; Hess *et al.*, 1997a).

### Regulation of efferent ductules

Efferent ductules reabsorb nearly 90% of the rete testis fluid, thereby concentrating spermatozoa as they enter the epididymis, which ensures that a large number of spermatozoa are released upon ejaculation (Robaire and Hermo, 1988; Clulow *et al.*, 1994; Ilio and Hess, 1994; Yeung *et al.*, 1994; Chan *et al.*, 1995). On the basis of these data and the observation that efferent ductules contain the highest concentration of ER in males, it was hypothesized that oestrogen participates in the regulation of fluid reabsorption in the male reproductive tract (Hess *et al.*, 1997a). This hypothesis was tested using the ER $\alpha$  gene knockout mouse (ERKO; Lubahn *et al.*, 1993) to evaluate 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, after which they begin to degenerate as early as 20–40 days of age (Eddy *et al.*, 1996). Fluid secretion by the testis only begins at puberty (Hinton and Setchell, 1993), which may account for the delay in effects on spermatogenesis. ERKO mouse testes are atrophic by 150 days of age. Spermatozoa from the ERKO male are abnormal and sperm concentrations are significantly reduced in the epididymis (Eddy *et al.*, 1996). The 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, with luminal areas more than double those of wild-type males at 90 days of age (Hess *et al.*, 1997a, 2000). From these observations, it appeared that luminal fluid was not being removed by the efferent ductules, causing fluids to accumulate in the lumen of rete testis and seminiferous tubules. As predicted from these observations, a transient increase in testis mass in ERKO males was noted between 32 and 81 days of age, followed by a continuous decrease in weight until 185 days of age, indicating that the long-term atrophy of testes in the knockout mice was caused by back pressure of the accumulating fluids (Hess *et al.*, 1997a). The initial segment epididymis was surgically cauterized to occlude the terminal end of the efferent ductules in the adult male to test this hypothesis. At 48 h after surgery, testis mass was increased 30% more in ERKO than in wild-type males (Hess *et al.*, 1997a).

The efferent ductal epithelium in ERKO tissue was reduced in height by nearly 50%, with a loss of cellular organelles, a flattening of the nucleus and the loss or shortening of the microvillus border (Hess *et al.*, 2000). All of these changes are consistent with a decrease in fluid reabsorption in these ductules in ERKO males (Hess *et al.*, 1997a). The endocytotic apparatus, including apical vesicles and periodic acid–Schiff's (PAS)<sup>+</sup> lysosomal granules, which are prominent in nonciliated cells of normal efferent ductules (Morales and Hermo, 1983; Hermo and Morales, 1984; Ilio and Hess, 1994), was non-existent in the ERKO efferent ductule epithelium. Thus, when ER $\alpha$  was lacking, the apical surface of this absorbing epithelium was transformed into what appeared to be a non-absorbing structure. This hypothesis was 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 24 h. Efferent ductules from wild-type males were able to reabsorb the luminal fluid rapidly, resulting in a collapse of the ductule walls; however, the luminal area of ERKO ductules did not collapse, but rather showed a marked increase in area (Hess *et al.*, 1997a). Thus, ERKO mice provide evidence that oestrogens or, more specifically, a functional ER $\alpha$ , is involved in the regulation of fluid transport in the male reproductive tract, and is responsible for increasing the concentration of spermatozoa as they enter the epididymis.

These data from the ERKO mouse appear unequivocal. However, efferent ductules also contain androgen receptors (Cooke *et al.*, 1991a; Goyal *et al.*, 1997), and the question of the potential androgen regulation of these ducts remains unanswered. Few studies have attempted to examine this question from a physiological perspective (Ilio and Hess, 1994; Hansen *et al.*, 1997). In one study, male rats were administered daily doses of testosterone propionate, flutamide (anti-androgen), oestradiol 3-benzoate or tamoxifen (anti-oestrogen) for 7 days. The results of this study appear to contradict the findings in the ERKO mouse (Hess *et al.*, 1997a), as testosterone caused a small increase in fluid reabsorption and oestrogen caused a large increase in the rate of fluid flow (Hansen *et al.*, 1997). These data are confusing because the anti-androgen, flutamide, actually caused a greater reduction in fluid reabsorption than did testosterone. Further complication was seen with the tamoxifen treatment, which showed the greatest stimulation of fluid reabsorption, and in the finding that the only treatment that appeared to decrease reabsorption was oestradiol. Clulow *et al.* (1998) attempted to explain these conflicting data by equating oestrogen and tamoxifen effects to an anti-androgen action and suggesting that flutamide would cause an increase in systemic androgen concentrations. In another study, Ilio and Hess (1994) found that castration reduced and testosterone partially restored the activity of Na<sup>+</sup>,K<sup>+</sup>-ATPase in the efferent ductules, and that oestrogen treatment inhibited the activity and partially blocked the restoration of activity by testosterone. Meistrich *et al.* (1975) found that oestrogen treatment reduced the time required for spermatozoa to transit the epididymis, an effect that has been interpreted as indicating that oestrogen inhibits fluid reabsorption in the efferent ductules (Hansen *et al.*, 1997). Such an effect would increase the amount of fluid in the ductules, which would then cause an increased flow rate through the reproductive tract. The potential effects of treatment on smooth muscle contraction and luminal diameter were not discussed.

A first impression of these studies would lead us to conclude that androgens, not oestrogens, stimulate the reabsorption of efferent ductal fluids. However, the overwhelming data from the ERKO mouse do not support this conclusion. In ERKO males, testosterone is increased (Eddy *et al.*, 1996), not decreased, and fluid reabsorption is suppressed (Hess *et al.*, 1997a). Therefore, there must be another explanation of the results from the systemic administration of oestrogen and androgens (Hansen *et al.*, 1997). One consideration is the potential effects of treatment on the physiological feedback of hormones on the hypothalamus–pituitary–testis axis (Barton and Andersen, 1998). Systemic treatment with hormones and anti-hormones either decreases or increases testosterone and thus it is possible that spermatogenesis is altered. Indeed, flutamide and oestradiol treatments showed decreases in daily sperm production (Hansen *et al.*, 1997). The flow of rete testis fluid

was not affected by gonadotrophins nor by hypophysectomy until after 24 h (Setchell, 1970).

The injection of oestradiol would be likely to induce a rapid decrease in LH, which would result in a decrease in testosterone and spermatogenesis (Huhtaniemi *et al.*, 1983) and thus an indirect decrease in luminal oestradiol synthesized by the germ cells. The only study to address the question of hormonal regulation of ER in the male reproductive tract found that androgens were sufficient to maintain the presence of ER in the efferent ductules (Goyal *et al.*, 1998). Therefore, on the basis of the ERKO model (Hess *et al.*, 1997a), any decrease in testosterone resulting from oestrogen treatment would be likely to downregulate ER in the efferent ductal epithelium, which would further decrease fluid reabsorption.

In conclusion, there is clear evidence that steroids are important in the regulation of the function of the efferent ducts, particularly in fluid reabsorption. However, aldosterone and other mineralocorticosteroids are not involved (Man *et al.*, 1997). Instead, oestrogens and androgens appear to be the primary regulators (Hansen *et al.*, 1997; Hess *et al.*, 1997a; Clulow *et al.*, 1998). The mechanisms of steroid action in this resorptive epithelium have not yet been agreed upon and it is not clear how best to test the hypothesis that oestrogen stimulates fluid reabsorption under normal physiological conditions. All treatments with a steroid hormone, with or without castration or rete testis ligation, create complications due to interference with the feedback regulation of gonadotrophin release. Castration and rete testis ligation experiments remove luminal contents and thus eliminate a major pathway of entry along the apical surface of the ductal epithelium by regulatory factors. Clarification of these steroid action mechanisms is likely to come from the use of *in vitro* methods or *in vivo* treatments with pure anti-oestrogens that target the epithelium from the luminal side. However, it is already well established that the loss of oestrogen receptor activity in males interferes with the resorptive function of the efferent ductule epithelium, a function that is essential for fertility.

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