The role of estrogen in testis and the male reproductive tract: a review and species comparison

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Abstract

Testosterone and estrogen are hormones important to both sexes. In the adult testis, estrogen is synthesized by Leydig cells and germ cells, producing a relatively high concentration in rete testis fluid and in semen of several species. Estrogen receptors (ER) are present in the testis, efferent ductules and epididymis of most species; however, ERα is reported absent in the testis of a few, including man. ERα is abundant in the efferent ductule epithelium of every species examined to date. Its primary function is the regulated expression of proteins involved in fluid reabsorption. Disruption of ERα, either in the knockout (ERαKO) or by treatment with a pure antiestrogen, results in dilution of cauda epididymal sperm, disruption of sperm morphology, inhibition of sodium transport and subsequent water reabsorption, increased secretion of Cl^-, and eventual decreased fertility. Loss of aromatase activity in the ArKO mouse does not result in an ERαKO or antiestrogen phenotype, suggesting that epithelial ERα in the efferent ductules may exhibit ligand-independent activity. In addition to the primary regulation of luminal fluid and ion transport, estrogen is also responsible for maintaining a differentiated epithelial morphology through a mechanism remaining to be discovered. Thus, estrogen or its receptor is important for male reproductive tract function in numerous species.

Keywords: estrogen, aromatase, estrogen receptor, testis, efferent ductules, epididymis, prostate, sperm, fertility

Introduction

Estrogen has been found in the semen and fluids of the male reproductive tract of many species (Waites and Einer-Jensen, 1974; Ganjam and Amann, 1976; Eiler and Graves, 1977; Free and Jaffe, 1979; Setchell *et al.*, 1983; Adamopoulos *et al.*, 1984; Claus *et al.*, 1985;Claus *et al.*, 1992; Bujan *et al.*, 1993.). At first it was thought that this male source of estrogen was produced primarily by the accessory sex glands and that estrogen's function should be relegated to influencing the female reproductive tract after ejaculation, a role that it may indeed play to some degree (Willenburg *et al.*, 2003). In the 1930's it was reported that developing testes were responsive to the "female" hormone (also reviewed by Wolff and Ginglinger, 1935; Weniger, 1990). It was also known in the 1930's and 40's that developmental exposure to high doses of estrogens could induce malformations in the male reproductive tract (Burrows, 1935; Greene *et al.*, 1940; McLachlan, 1979; Arai *et al.*, 1983). However, as late as the early 1990's, many scientists still considered estrogen receptor presence in the adult male reproductive tract to be only a residual of embryological differentiation (Greco *et al.*, 1993). Previous reviews have already covered important aspects of estrogen's influence on male reproductive development (Sharpe, 1998; Hess *et al.*, 2001b; Iguchi *et al.*, 2001; O'Donnell *et al.*, 2001; Hess, 2003; Sharpe, 2003); therefore, here we will focus on a comparison of estrogen synthesis, receptor localization and potential function in a variety of adult male species.

Estrogen synthesis and inactivation

In several species, estrogen levels are remarkably high in the semen (Waites and Einer-Jensen, 1974; Ganjam and Amann, 1976; Eiler and Graves, 1977; Free and Jaffe, 1979; Setchell *et al.*, 1983; Adamopoulos *et al.*, 1984; Claus et al., 1985; Claus *et al.,* 1992; Bujan *et al.*, 1993). Estrogen concentrations within the testis and semen can reach levels that exceed even the female vasculature (Table 1). Of particular note, concentrations of estradiol in testis venous blood

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and lymph are relatively high in all species. Rete testis fluid concentrations vary considerably between species, with the rat showing the highest, at 249 pg/ml (Free and Jaffe, 1979). In semen, conjugated estrogens are often found at extreme levels in the horse, bull and boar (Ganjam and Amann, 1976; Eiler and Graves, 1977; Claus et al., 1985; Claus et al., 1992; Lemazurier *et al.*, 2002).

Table 1. Estrogen concentrations in the male.

Source	Concentration	Species	References
Testis venous blood	104-200 pg/ml	Monkey	(Waites and Einer-Jensen, 1974)
	17.5 pg/ml	Rat	(de Jong *et al.*, 1973)
	450 ng/ml (estrone-sulfate)	Horse	(Setchell, 1982)
	1.09 nmol/L (total estrogens) 52.4 nmol/L (estrone-sulfate)	Boar	(Setchell *et al.*, 1983)
	926 pg/ml	Man	(Adamopoulos *et al.*, 1984)
Testis lymph	900 ng/ml (estrone-sulfate)	Horse	(Setchell and Cox, 1982)
	1.86 nmol/L (total estrogens) 705 nmol/L (estrone sulfate)	Boar	(Setchell *et al.*, 1983)
Rete testis fluid	14-195 pg/ml	Monkey	(Waites and Einer-Jensen, 1974)
	249 pg/ml	Rat	(Free and Jaffe, 1979)
	11.5 pg/ml	Bull	(Ganjam and Amann, 1976)
	0.38 nmol/L (total estrogens) 8.60 nmol/L (estrone-sulfate)	Boar	(Setchell *et al.*, 1983)
Semen	6.7-162 pg/ml	Man	(Purvis *et al.*, 1975; Adamopoulos et al., 1984; Bujan et al., 1993; Luboshitzky *et al.*, 2002a,b; Naderi and Safarinejad, 2003)
	73- 144 pg/ml (estradiol) 385 pg/ml (conjugated estradiol) 739 pg/ml estrone 4116-9612 pg/ml (estrone-sulfate)	Horse	(Claus *et al.*, 1992; Lemazurier et al., 2002)
	50-890 pg/ml	Bull	(Ganjam and Amann, 1976; Eiler and Graves, 1977)
	430 pg/ml (estradiol) 860 pg/ml (estrone)	Boar	(Claus *et al.,* 1985)

Estrogen synthesis in the male reproductive tract was first thought to occur in Sertoli cells during development, but then only in Leydig cells of the adult testis in most species (Rommerts and Brinkman, 1981; van der Molen *et al.*, 1981; Rommerts *et al.*, 1982; Payne *et al.*, 1987; O'Donnell et al., 2001; Carreau *et al.*, 2003; Sharpe *et al.*, 2003). Table 2 shows the reported locations for estrogen synthesis in the adult male reproductive system. There is a consistent presence of aromatase in Leydig cells, but several species also reportedly show activity in Sertoli cells of the adult testis. In the dog, aromatase activity is a marker for Sertoli cell tumors (Peters *et al.*, 2003). In general, aromatase has not been found in rete testis, efferent ductules, epididymis or vas deferens. However, scattered reports are found for epididymal presence of aromatase ([human efferent ductules and proximal epididymis; Carpino *et al.*, 2004]; cultured rat cells; Wiszniewska, 2002). Currently, a growing body of evidence indicates that germ cells also synthesize estrogen, and possibly serve as the major source of estrogen in the male reproductive tract (see review by Carreau *et al.*, 2003).

Table 2. Aromatase presence in adult male reproductive tissues.

Species	Tissues	References
Mouse[1]	Leydig cell Immature germ cell Spermatozoa	(Nitta et al., 1993; Janulis et al., 1996b; Wang *et al.*, 2001b; Bilinska *et al.*, 2003; Catalano *et al.*, 2003; Golovine *et al.*, 2003)
Rat[1]	Leydig cell Immature germ cell Spermatozoa Epididymal epithelium[3]	(Rommerts and Brinkman, 1981; Rommerts et al., 1982; Tsai-Morris *et al.*, 1984; Papadopoulos *et al.*, 1986; Payne et al., 1987; Janulis et al., 1996a; Levallet and Carreau, 1997; Janulis et al., 1998; Carpino *et al.*, 2001; Genissel *et al.*, 2001; Lanzino *et al.*, 2001; Levallet et al., 1998a, b; Turner *et al.*, 2002; Wiszniewska, 2002; Bourguiba *et al.*, 2003a,b; Tirado *et al.*, 2004)
Rooster	Leydig cell Immature germ cell Spermatozoa	(Kwon et al., 1995; Vaillant *et al.*, 2001)
Fish	Total testis analysis Leydig cell Immature germ cell	(Callard *et al.*, 1985; Betka and Callard, 1998; Kobayashi *et al.*, 1998; Freking *et al.*, 2000; Lee *et al.*, 2001b; Agate *et al.*, 2002; Dalla Valle *et al.*, 2002; Gonzalez and Piferrer, 2003; Kobayashi *et al.*, 2003; Blazquez and Piferrer, 2004)
Amphibian	Total testis analysis	(Ohtani *et al.*, 2003; Kuntz *et al.*, 2004)
Turtle	Total testis analysis	(Place *et al.*, 2001)
Bear[2]	Leydig cell Sertoli cell Immature germ cell	(Tsubota *et al.*, 1997; Okano *et al.*, 2003)
Boar	Leydig cell	(Conley *et al.*, 1996)
Cattle	Total testis analysis	(Vanselow *et al.*, 2001)
Ram	Total testis analysis[4] Leydig cell	(Schmalz and Bilinska, 1998; Quirke *et al.*, 2001; Vanselow et al., 2001)
Stallion	Leydig cell Sertoli cell Immature germ cell	(Eisenhauer et al., 1994; Lemazurier and Seralini, 2002; Lemazurier et al., 2002; Sipahutar et al., 2003; Hess and Roser, 2004)
Dog	Leydig cell Sertoli cell (tumors)	(Peters et al., 2003)
Raccoon	Leydig cell Sertoli cell Immature germ cell (elongate spermatid)	(Qiang *et al.*, 2003)
Bank vole	Leydig cell Sertoli cell Immature germ cell	(Bilinska *et al.*, 2001; Fraczek *et al.*, 2001; Kotula-Balak *et al.*, 2003)
Marmoset	Immature germ cell	(Turner et al., 2002)
Rhesus	Leydig cell Immature germ cell	(Pereyra-Martinez *et al.*, 2001)
Human	Immature germ cell Spermatozoa Epithelium of efferent ductule Epithelium of proximal epididymis Prostate stromal cell	(Brodie *et al.*, 2001; Carreau et al., 2002b; Turner et al., 2002; Aquila et al., 2003; Carreau et al., 2003; Lambard et al., 2003; Rago et al., 2003; Simpson, 2003; Carpino et al., 2004; Ellem *et al.*, 2004)

[1] Early work showed only Leydig cells being positive for Aromatase in the adult testis.
[2] Location depended upon the season (Tsubota et al., 1997).
[3] Only in primary culture cells (Wiszniewska, 2002).
[4] One study found no expression of aromatase in the developing and adult sheep testis (Quirke et al., 2001).

The first reports to demonstrate aromatase in testicular germ cells and sperm (Fig. 1) were published through a collaborative effort at the University of Illinois (Nitta *et al.*, 1993; Kwon *et al.*, 1995; Janulis *et al.*, 1996a, b; Janulis *et al.*, 1998). Its presence in germ cells was found in diverse species ranging from mice to chicken testes (Fig. 1) and was localized in the Golgi of round spermatids and throughout the cytoplasm of elongating and late spermatids. The enzyme is also found in the cytoplasmic droplet of epididymal sperm (Fig. 2), but its presence and activity are higher in sperm isolated from the efferent ductules and head of the epididymis than from the cauda region (Janulis et al., 1996a; Rago *et al.*, 2003). Aromatase in germ cells and spermatozoa represent approximately 62% of the total testicular amount (Levallet and Carreau, 1997; Levallet *et al.*, 1998b; Carreau *et al.*, 1999). Its biological activity in developing germ cells has been found to equal or exceeded that found in interstitial cells. More recently, Carreau and others have confirmed aromatase presence in testicular germ cells and sperm and have demonstrated aromatase expression and activity in human sperm (Carreau and Levallet, 1997; Carreau *et al.*, 1998; Carreau et al., 1999; Carreau, 2000; Carreau, 2001; Carreau *et al.*, 2001; Aquila *et al.*, 2002; Carani *et al.*, 2002; Carreau, 2002; Carreau *et al.*, 2002a, b; Aquila *et al.*, 2003; Carreau, 2003; Carreau et al., 2003; Lambard *et al.*, 2003; Rago et al., 2003; Carreau *et al.*, 2004; Lambard *et al.*, 2004). Only a few species, such as the horse (Eisenhauer et al., 1994; Hess and Roser, 2004; Lemazurier et al., 2002; Lemazurier and Seralini, 2002; Sipahutar et al., 2003), have not shown testicular germ cells to be aromatase-positive (Table 2). It is unknown if the lack of staining was due to differences in antibodies or if species simply differ in the sources of estrogen found in the reproductive tract.

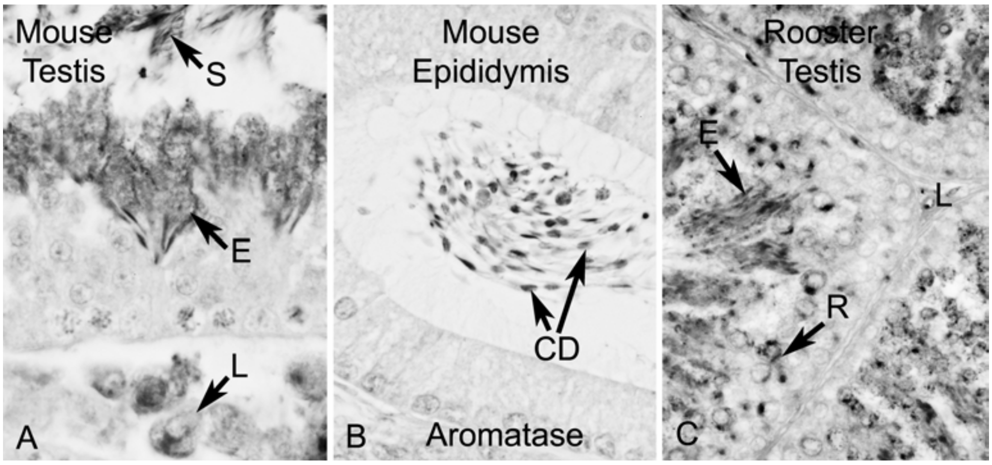

Figure. 1A. Aromatase in the mouse testis show immunohistochemical staining of Leydig cells (L), elongated spermatids (E), and released sperm (S). 1B. Aromatase in the mouse epididymis showing staining of the cytoplasmic droplet on sperm tails (CD). 1C. Rooster testis showing aromatase in Leydig cells (L), round spermatids, and elongated spermatids (E).

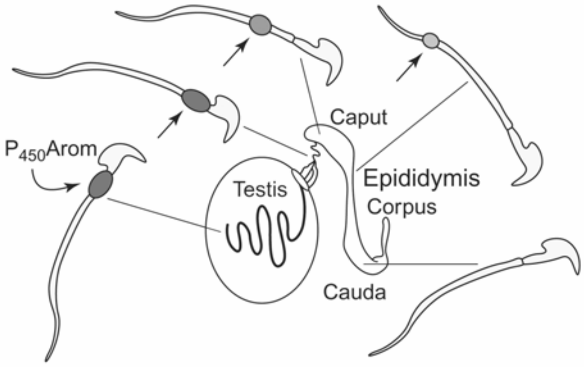

Figure 2. A drawing showing how aromatase (P_{450} Arom) found in sperm cytoplasmic droplets decreases as the sperm traverse the epididymis.

These recent discoveries of germ cell production of estrogen in the male reproductive tract led to new hypotheses regarding estrogen receptor presence in the tract and its potential function. The Leydig cell is no longer considered the only source of estrogen for the reproductive tract and it appears that Leydig cell derived estradiol would more likely target the lymphatics and peripheral circulation, rather than the lumens of rete testis and epididymis. Leydig cells lie adjacent to endothelial cells of the lymphatic system, a region reported to have very high concentrations of estrogens (Setchell, 1982; Setchell et al., 1983). However, blood estrogen concentrations are low in the male, therefore, we presume that estrogens from Leydig cell synthesis would provide limited endocrine activity in the reproductive tract. In the efferent ductules, blood-borne estrogens would likely have even less effect, as these ductules are responsible for reabsorption of over 90% of the luminal fluids (Clulow *et al.*, 1998) and thus display an overwhelming luminal to basal orientation, which could limit the movement of substances from basement membrane into the cell cytoplasm. Although this hypothesis has not been tested directly, there are studies suggesting that this region of the male tract does not respond to exogenous androgens following castration (Fawcett and Hoffer, 1979). More recent studies, however, suggest that after castration the efferent ductules do respond to estrogens and androgens (Oliveira *et al.*, 2004). Nevertheless, current data demonstrate that in most species luminal estrogen, produced by testicular germ cells and luminal sperm, is more than sufficient to target estrogen receptors found in epithelial cells lining the male reproductive tract (Hess, 2002; Hess *et al.*, 2002; Hess, 2003).

Estrogens are inactivated through sulfoconjugation, catalyzed by the enzyme estrogen sulfotransferase, which is abundantly expressed in liver (Song and Melner, 2000; Song, 2001). Interestingly in the male, estrogen sulfotransferase has been found to show the highest concentration and specific organ activity in the testis (Hobkirk and Glasier, 1992; Song *et al.*, 1995; Song, 2001). This enzyme has been studied in the male of only a few species, but was found in testis of pigs, mice, rat, guinea pig and man (Hobkirk *et al.*, 1989; Hobkirk and Glasier, 1992; Song et al., 1995; Song, 2001; Miki *et al.*, 2002). In the testis, its presence is exclusive to the Leydig cell, but along the tract it is found in the epididymal epithelium and the epithelium and smooth muscle of the vas deferens of the mouse (Tong and Song, 2002). It has not been found in prostate or seminal vesicles. The reproductive tracts of other species have not been investigated. Estrogen sulfotransferase is regulated in the testis and epididymis through pituitary gonadotrophins (LH) and androgens (Tong and Song, 2002). The CD-1 mouse testis was shown in 1995 to have the highest organ specific activity (Song et al., 1995) and then in 2001 the testis of this mouse strain was shown to be 16 fold less sensitive to estrogen than the B6 strain of mice (Spearow *et al.*, 2001). Spearow further showed that the CD-1 testis expresses 3.5 times more estrogen sulfotransferase than the B6 mouse testis (Spearow et al., 2001). Testes of the estrogen sulfotransferase knockout mice are reported to be damaged, with Leydig cell hyperplasia and hypertrophy and decreases in the weights of testis and epididymis (Qian *et al.*, 2001). Sperm motility is also reduced, as well as fertility. Exogenous estrogen treatment of the estrogen sulfotransferase knockout mice induces further decline in sperm quality (Tong and Song, 2002).

Estrogen receptors in the male reproductive tract

Estrogen receptor-like proteins were found in epididymal tissues over 30 years ago (Danzo *et al.*, 1975). However, early investigations into estrogen receptor presence and function in the male reproductive tract lead to the conclusion that estrogen was more important during development than in the adult (Danzo, 1986). Estrogen binding in epididymal tissues has been noted in numerous species, including the dog (Younes *et al.*, 1979; Younes and Pierrepoint, 1981), human (Murphy *et al.*, 1980), turtle (Dufaure *et al.*, 1983), monkey (Kamal *et al.*, 1985; West and Brenner, 1990), ram (Tekpetey and Amann, 1988), guinea pig (Danzo *et al.*, 1981), and the rat (Kuiper *et al.*, 1997). In the mouse, estrogen binding was found throughout the testis and epididymis (Schleicher *et al.*, 1984; Hess *et al.*, 1997b). The strongest binding was found in the efferent ductule epithelium and initial segment epididymis, with lesser binding in the distal tract (Schleicher et al., 1984). However, binding assays do not differentiate between ERα and ERβ; therefore, other methods, such as immunocytochemistry, *in situ* hybridization and Northern blot analysis, have been used to separate the two ER subtypes. Unfortunately, these techniques do not provide identical results and disagreements are found in ER presence in the male (Hess et al., 2002).

Using immunocytochemistry, ER has consistently been localized in the epithelium of efferent ductules (West and Brenner, 1990; Sato *et al.*, 1994; Ergun *et al.*, 1997; Fisher *et al.*, 1997; Goyal *et al.*, 1997a; Hess et al., 1997b; Kwon *et al.*, 1997; Goyal *et al.*, 1998; Saunders *et al.*, 2001). However, in the goat and monkey, only nonciliated cells of the efferent ductal epithelium stained ER positive (West and Brenner, 1990; Goyal *et al.*, 1997b). With the discovery of ER subtypes α and β, more precise localization of ERs has been reported, but even the new antibodies can result in confusing data (Fisher et al., 1997; Goyal et al., 1997a; Hess *et al.*, 1997a; Kwon et al., 1997; Goyal et al., 1998; Hess et al., 2002; Nie *et al.*, 2002; Zhou *et al.*, 2002). One of the best examples is the mouse, which shows weak epididymal staining for ERα using the H222 antibody (Iguchi *et al.*, 1991), but strong staining using another antibody, 6F11 (Zhou et al., 2002). ERα has now been localized in the male reproductive tract of at least nine species (Table 3).

Table 3. Localization of ERα, ERβ and estrogen binding (E) in the testis and male reproductive tract epithelium: a species comparison.

Organ	Rat	Mouse *	Dog	Cat	Goat	Rabbit **	Ram	Boar	Bird	Fish ***	Monkey	Man
Testis		αβ			-α					α	-/+α	+/-α
Leydig	αβ	αβ E	α -β	αβ						α		+/-α
Peritubular	αβ	+/-α +/-β	αβ	-α β								-α
Sertoli	-α β	-/+α β	-αβ	-α -β						α		-α
Germ cells	-/+α β	-/+α β	-α	-α β						α		+/-α
Sperm	-/+α	-α								α		+/-α
Rete testis												
Epithelium	-α β	αβ	α β	α β								
Efferent ductules	α E				α E				α			-α
Nonciliated	αβ	αβ E	αβ	αβ							α	α
Ciliated	αβ	αβ E	αβ	-/+α β								-α
Epididymis	E		E		-/+α	E	E	E		E	E	-/+α E
Cell line		αβ	α								-/+α	-/+α
Initial Segment	α											
Principal cell	-α β	-α β	-α β	-α β								
Narrow/apical	-α β	αβ E	-α β	-α β								
Basal cell	-α β	αβ	-α β	-α β								
Caput						E						
Principal cell	-α β	αβ	-α β	αβ								
Apical cell	-α	αβ	-α	αβ								

	β	E	β									
Basal cell	-α β	αβ	-α β	αβ								
<u>Corpus</u>												
Principal cell	-α β	-/+α β	-α β	αβ								
Clear cell	-α β	αβ E	-α β	αβ								
<u>Cauda</u>												
Principal cell	-α β	-α β	-α β	αβ								
Clear cell	-α β	αβ E	-α β	αβ								
<u>Vas deferens</u>												
Principal cell	-α β	-α β	-α β	αβ								
Basal cell	-α β	-α β	-α β	αβ								
Prostate												
Principal cell	-α β	-α β										-α
References	1	2	3	4	5	6	7	8	9	10	11	12

1. Rat: <u>ERα</u>, (Saunders et al., 1998; Shughrue *et al.*, 1998; Pelletier et al., 2000; Sar and Welsch, 2000; Mowa and Iwanaga, 2001; Saberwal *et al.*, 2002; Oliveira et al., 2003; Oliveira et al., 2004). <u>ERβ</u>, (Prins *et al.*, 1998; Saunders, 1998; Shughrue et al., 1998; van Pelt et al., 1999; Makela *et al.*, 2000; Pelletier et al., 2000; Sar and Welsch, 2000; Atanassova *et al.*, 2001; Weihua *et al.*, 2001; Asano *et al.*, 2003; Oliveira et al., 2003; Oliveira et al., 2004; Tirado et al., 2004). <u>Estrogen binding</u>, (Hess et al., 1997b); (Kuiper et al., 1997).
2. Mouse and vole[*]: <u>ERα</u>, (Atanassova et al., 2001; Bilinska et al., 2001; Prins *et al.*, 2001; Risbridger *et al.*, 2001; Shibayama *et al.*, 2001; Zhou et al., 2002; Takao *et al.*, 2003; Sipila *et al.*, 2004). <u>ERβ</u>, (Atanassova et al., 2001; Bilinska et al., 2001; Prins et al., 2001; Risbridger et al., 2001; Shibayama et al., 2001; Zhou et al., 2002; Takao et al., 2003; Sipila et al., 2004). <u>Estrogen binding</u>, (Schleicher et al., 1984; Hess et al., 1997b).
3. Dog: <u>ERα</u>, (Telgmann *et al.*, 2001; Nie et al., 2002). <u>ERβ</u>, (Telgmann et al., 2001; Nie et al., 2002)
4. Cat: <u>ERα</u>, (Telgmann et al., 2001; Nie et al., 2002). <u>ERβ</u>, (Telgmann et al., 2001; Nie et al., 2002)
5. Goat: <u>ERα</u>, (Mansour *et al.*, 2001). <u>Estrogen binding (nonspecific antibodies)</u>, (Goyal *et al.*, 1997a; Goyal *et al.*, 1998;)
6. Rabbit and guinea pig[**]: <u>Estrogen binding</u>, (Danzo *et al.*, 1975; 1977; 1978; Danzo and Eller, 1979; Danzo et al., 1981; Danzo *et al.*, 1982; Hendry and Danzo, 1985; Danzo, 1986; Hendry and Danzo, 1986; Hendry *et al.*, 1987)
7. Ram: <u>Estrogen binding</u>, (Linde *et al.*, 1975; Raeside *et al.*, 1999).
8. Boar: <u>ERβ</u>, (human efferent ductules and proximal epididymis Carpino et al., 2004; cultured rat cells Wiszniewska, 2002). <u>Estrogen binding</u>, (Tekpetey and Amann, 1988).
9. Bird: <u>ERα</u>, (Janssen *et al.*, 1998).
10. Fish, newt[***], amphioxus[***]and turtle[***]: <u>ERα</u>, (Socorro *et al.*, 2000; Arenas *et al.*, 2001; Bouma and Nagler, 2001; ; Wu et al., 2001; Fang *et al.*, 2003; He *et al.*, 2003). <u>ERβ</u>, (Socorro et al., 2000; Arenas et al., 2001; Bouma and Nagler, 2001; Wu et al., 2001; Fang et al., 2003; He et al., 2003). <u>Estrogen binding</u>, (Dufaure et al., 1983).
11. Monkey: <u>ERα</u>, (Heikinheimo *et al.*, 1995; Pelletier, 2000; Saunders et al., 2001). <u>ERβ</u>, (Heikinheimo et al., 1995; Pelletier, 2000; Saunders et al., 2001). <u>Estrogen binding</u>, (Kamal et al., 1985; West and Brenner, 1990).
12. Man: <u>ERα</u>, (Pelletier, 2000; Pelletier and El-Alfy, 2000; Denger *et al.*, 2001; Makinen et al., 2001; Saunders et al., 2001;

Brand *et al.*, 2002; Gonzalez-Unzaga *et al.*, 2003; Aquila et al., 2004; Lambard et al., 2004). ERβ, (Mosselman *et al.*, 1996; Enmark *et al.*, 1997; Moore *et al.*, 1998; Pelletier, 2000; Pelletier and El-Alfy, 2000; Denger et al., 2001; Makinen et al., 2001; Saunders et al., 2001; Brand et al., 2002; Shoda *et al.*, 2002; Gonzalez-Unzaga et al., 2003; Lambard et al., 2004; Aquila et al., 2004).

The most consistent data across species has been ERα presence in the Leydig or Interstitial cells (Fig. 3), even in the fish testis. There are conflicting reports of ERα in germ cells and sperm (Wu *et al.*, 2001; Nie et al., 2002; Zhou et al., 2002; Aquila *et al.*, 2004; Lambard et al., 2004). Efferent ductules are positive for ERα in all species examined (Fig.4), although one study showed no immunostaining in man (Pelletier and El-Alfy, 2000). Analysis of mRNA from the efferent ductules has indicated that the receptor is expressed 3.5 fold greater than in female tissue (Hess et al., 1997b). The epididymis has generally been found to be ERα negative, although select species, such as the cat and mouse, have shown strong staining for this receptor in specific regions and select cell types (Nie et al., 2002; Zhou et al., 2002). Narrow, apical and clear cells of the rodent epididymis show intense binding affinity for estrogens (Schleicher et al., 1984) and also show intense staining by immunohistochemistry for ERα (Saunders *et al.*, 1998; Pelletier *et al.*, 2000; Zhou et al., 2002; Oliveira *et al.*, 2003; Oliveira et al., 2004). The prostate epithelium always appears ERα negative, while stromal cells are positive.

The discovery of a second form of ER (ERβ) complicates the interpretation of earlier data from estrogen binding studies, as it is unknown in those studies to which ER binding has occurred. ERβ was originally discovered because of it high expression in prostate (Kuiper *et al.*, 1996), but it has now been found in all tissues of the male reproductive tract, in both epithelium and stromal tissues (Table 3). 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). ERβ is more widely distributed in the male tract than ERα (Hess et al., 2002) and shows strong reactivity in efferent ductules, similar to ERα. The male tract is an example where both receptors are expressed in high concentrations within the same cell (Nie et al., 2002; Zhou et al., 2002). ERβ appears to be weaker in initial segment epididymis but stronger in the corpus, cauda and vas deferens.

In the testis, ERβ is the more abundant receptor and is typically found in nearly every cell type of the interstitium and the seminiferous tubule (Fig. 3), except for the elongated spermatids (Saunders *et al.* 1997; Rosenfeld *et al.*, 1998; Saunders et al., 1998; van Pelt *et al.*, 1999; Bilinska *et al.*, 2000; Jefferson *et al.*, 2000; Pelletier, 2000; Taylor and Al-Azzawi, 2000; Makinen *et al.*, 2001; McKinnell *et al.*, 2001; Saunders et al., 2001; Takeyama *et al.*, 2001; Nie et al., 2002; Zhou et al., 2002).

In contrast, ERα is found only in the interstitium of the testis in most species examined (Table 3). The ERβ knockout mouse (Krege et al., 1998; Couse *et al.*, 1999) shows no testicular phenotype and double ERαβ knockout mice appear identical to the ERα knockout mice (Lubahn *et al.*, 1993; Eddy *et al.*, 1996; Couse et al., 1999; Dupont *et al.*, 2000; Mahato *et al.*, 2001).

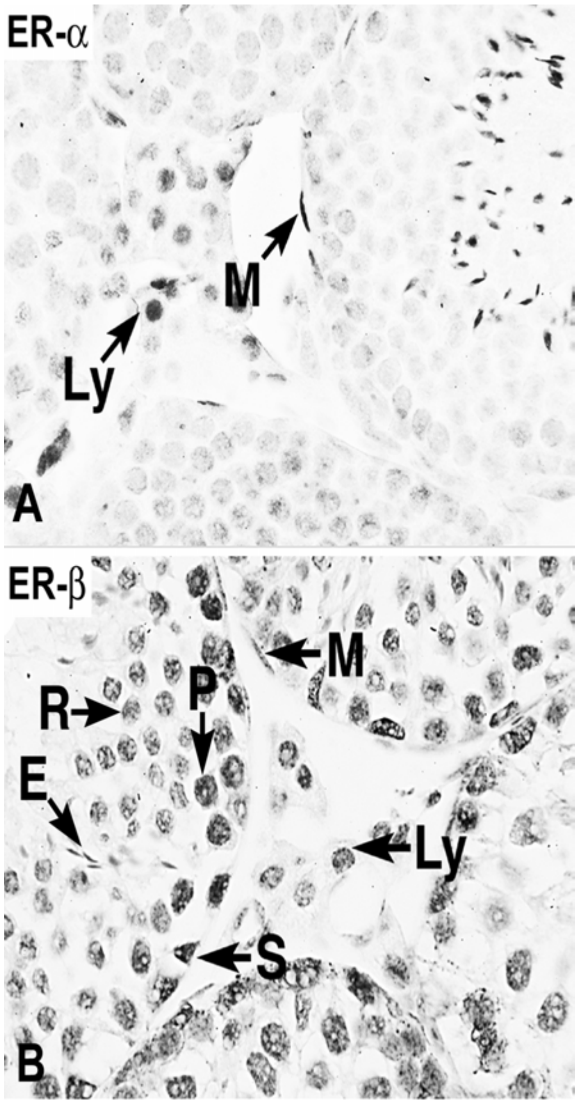

Figure 3A. ERα in the mouse testis. Leydig cells (Ly) and peritubular myoid cells (M) are strongly positive. 3B. ERβ in the mouse testis. Nearly all cell types are positive except for the elongate spermatids (E). Leydig cell (Ly); peritubular myoid cell (M); pachytene spermatocytes (P); round spermatid (R).

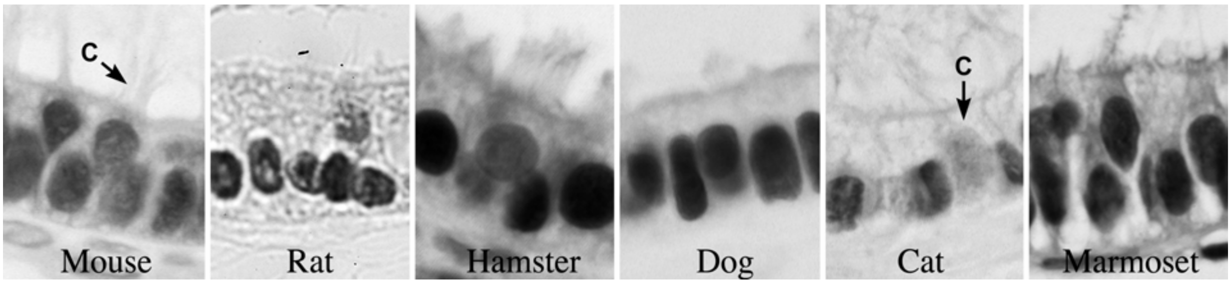

Figure 4. ERα in the efferent ductule epithelium of several species: mouse, rat, hamster, dog, cat and marmoset monkey. Non-ciliated principal cells are strongly positive in all species, but ciliated cells (C) are less positive in some.

Future studies must attempt to resolve conflicting reports found in the literature regarding the presence or absence of ERs in the male reproductive tract of different species. It is difficult to reconcile, for example, the generally accepted lack of ERα expression in germ cells with new reports of ERα expression in human sperm. It will also be important to determine why the cat and mouse express ERα in epididymal tissue, while other species generally show no immunostaining in this region. How could such a divergence in expression evolve? On the other hand, ERβ is nearly ubiquitous in its presence, both in the epithelium and stroma throughout the male reproductive tract. It is possible that in some species ERβ compensates for the lack of ERα, while in the cat and mouse, the duel presence of both receptors may be necessary for balancing unique epididymal functions of fluid reabsorption and sperm maturation.

Estrogen Function in Testis

Estrogen appears to have only a minor role in adult testicular function (see review by O'Donnell et al., 2001). However, Hardy and colleagues (Akingbemi *et al.*, 2003) have demonstrated in mouse cells that anti-estrogen treatment inhibits Leydig cell activity *in vitro*, but estradiol alone was unable to stimulate Leydig cell steroidogenesis. In the developing testis, estrogen has significant activity in establishing Sertoli cell function (O'Donnell et al., 2001) and potentially even in establishing Sertoli-germ cell adhesion (MacCalman and Blaschuk, 1994; MacCalman *et al.*, 1997). However, in the total absence of estrogen synthesis, the aromatase knockout (ArKO) male shows normal spermatogenesis at the beginning of puberty and only with aging does the testis begin to develop lesions associated with round spermatids (O'Donnell et al., 2001; Robertson *et al.*, 2002). This is not entirely surprising in light of the fact that ERα is not present in the seminiferous epithelium of the mouse (Nie et al., 2002; Zhou et al., 2002) and although ERβ is found in Sertoli cells and nearly all germ cells (Saunders et al., 2001; Nie et al., 2002; Saunders *et al.*, 2002; Scobie *et al.*, 2002; Zhou et al., 2002), the ERβ knockout (ERβKO) male testis appears normal and the males are fertile (Krege et al., 1998Couse et al., 1999; Dupont et al., 2000).

There are no data showing that ERα is important in initiating or maintaining spermatogenesis. Transplantation of germ cells from the ERαKO mouse testis into a normal testis (made devoid of germ cells) produces normal spermatozoa capable of fertilization and results in live offspring (Mahato et al., 2001), suggesting that testicular ERα has no influence on spermatogenesis. However, loss of estrogen synthesis in the ArKO mouse (O'Donnell et al., 2001; Robertson *et al.*, 2001) results in decreased fertility with aging. Another study in the mouse also suggests that estrogen may have testicular function, acting through the Leydig cell (Akingbemi et al., 2003). It was suggested that testosterone concentrations are elevated in the ERαKO male (Eddy et al., 1996), due to the disruption in feedback regulation at the hypothalamus, and the more recent study indeed shows that Leydig cells isolated from the ERαKO testis have increased production of testosterone and when treated with the pure ER inhibitor ICI 182,780 show increased steroidogenesis (Akingbemi et al., 2003). Therefore, ER in the testis, although not necessarily essential for spermatogenesis, appear to have a subtle function in Leydig cells.

Although estrogen may not be essential for spermatogenesis, there is indirect evidence of estrogen's influence on spermatogenesis. Ebling and colleagues (Ebling *et al.*, 2000) found that estradiol implants in the *hpg* mouse, which is deficient in gonadotropin releasing hormone (GnRH), stimulated a 4-5-fold increase in seminiferous tubular volume, in the absence of measurable levels of androgens. Although it is possible that this effect was due to the slightly elevated levels of FSH, an alternative hypothesis put forward was direct effects of estrogen on cells of the testis. This hypothesis appears plausible when the ArKO mouse data are taken into consideration, as ArKO testes are normal at first, but with aging show decreases in weight, seminiferous epithelium, and germ cell numbers (Robertson *et al.*, 1999). When the ArKO male is maintained on a soy-free diet, these effects are accelerated and enhanced (O'Donnell et al., 2001; Robertson et al., 2002). Thus, soy based phytoestrogens likely protected the testis somewhat in the ArKO mouse, suggesting that small amounts of estrogen do have testicular effects independent of FSH or LH.

This potential role for estrogen in the testis will most likely be found in the germ cells, as they express ERβ abundantly (Saunders et al., 2001; Nie et al., 2002; Saunders et al., 2002; Zhou et al., 2002) and genistein has a higher affinity for ERβ than for ERα (Kuiper *et al.*, 1998). Finally, although the Sertoli cell does not express ERα, it is interesting that in the ERαKO testis there is significantly less seminiferous tubular secretion than in the wild-type testis (Hess et al., 1997a). The same effect was suggested for the ArKO testis, as seminiferous tubule luminal volume and tubular length was decreased (Robertson et al., 2002).

Another compelling study that would suggest ERβ having a role in spermatogenesis comes from long-term treatment of the rat and mouse with ICI 182,780 (Cho *et al.*, 2003; Oliveira *et al.*, 2002). Similar to the results seen in the ArKO mouse (O'Donnell et al., 2001; Robertson et al., 2002), at first there was no effect on the testis, as spermatogenesis progressed normally. But with time, the testis shows severe atrophy in the rat (Oliveira et al., 2002) and hypospermatogenesis and abnormal germ cell development in the mouse (Cho et al., 2003). In the rat, seminiferous tubular atrophy was caused by back-pressure induced by fluid accumulation within the rete testis, similar to the reported effects seen in the ERαKO mouse (Hess et al., 1997a). However, in the mouse there was no seminiferous tubular dilation or increase in testis weight (Cho-et al., 2003); therefore, the effects on spermatogenesis could not have been induced by fluid accumulation, but were more likely due to direct effects of the antiestrogen on ERβ found in the germ cells (Zhou et al., 2002). It is also possible that indirect effects due to increases in testosterone concentration or alterations in paracrine factors associated with Leydig cell effects (Akingbemi et al., 2003). Thus overall, estrogen appears to have a function in the adult testis, not only in the Leydig cell but also possibly within the germinal epithelium. However, disruption of this function appears to require an extended period of inhibition.

Estrogen Function In Efferent Ductules

In all species studied to date, efferent ductules are a major site for estrogen function in the male reproductive tract. The ductules connect rete testis to epididymis (Hess, 2002). One-third or more of the head of the epididymis in man and other mammals contains these ducts and it was once thought that they simply transported sperm from testis to the epididymis. However, it is now known that efferent ductules have an important function in the reabsorption of over 90% of the rete testis fluid and thereby concentrate sperm prior to entering the epididymal lumen (Clulow et al., 1998). Nonciliated cells of the epithelium are reabsorptive, similar to proximal tubules of the kidney, having a brush border of microvilli connecting in the apical cytoplasm to a profusion of apical canaliculi, vesicles, tubules and membrane-bound bodies, which constitute an elaborate endocytotic/lysosomal system (Hermo *et al.*, 1994). In the basal region, rough endoplasmic reticulum, mitochondria and lipid droplets are common (Ilio and Hess, 1994).

The efferent ductules express an abundance of both androgen and estrogen receptors (Hess et al., 2002; Nie et al., 2002; Zhou et al., 2002; Oliveira et al., 2003; Oliveira et al., 2004). Therefore it was not surprising to discover that the ERαKO mouse and the antiestrogen-treated rodents are infertile or show greatly reduced fertility (Lubahn et al., 1993; Eddy et al., 1996; Oliveira et al., 2002; Cho et al., 2003). Numerous prior reviews have covered this transgenic mouse (Couse and Korach, 1999a, b; Hess, 2000a, b; Hess *et al.*, 2001a, b; Couse and Korach, 2001; Couse *et al.*, 2001; O'Donnell et al., 2001; Carani et al., 2002; Hess et al., 2002; Hess, 2003). Although the ERαKO testis appeared normal before puberty, after the onset of spermatogenesis, the testis began to degenerate and eventually became atrophic (Eddy et al., 1996). By 150 days, cauda sperm from the ERαKO male were abnormal and sperm concentrations were significantly reduced (Eddy et al., 1996), suggesting that the reproductive tract was also abnormal. A later study by the Eddy's lab showed that ERαKO germ cells transplanted into a normal testis (treated with busulphan to remove native germ cells) were capable of fertilization (Mahato *et al.*, 2000). That study clearly pointed to extra-testicular regions, such as the efferent ductules and epididymis, being the major source of pathological alterations in ERαKO males (Eddy et al., 1996; Hess et al., 1997a).

The rete testes in the ERαKO mouse and the antiestrogen ICI 182,780 treated male mouse and rat are dilated and protrude into the testis (Eddy et al., 1996; Hess et al., 1997a; Lee *et al.*, 2000; Oliveira *et al.*, 2001). Based upon these data, we hypothesized that the efferent ductules were either a) occluded due to excessive reabsorption, or b) dilated due to an inhibition of fluid reabsorption. After careful examination, we found the second hypothesis to be true (Fig. 5), as the efferent ductule lumen was dilated markedly when ERα was inhibited (Hess et al., 1997a; Hess *et al.*, 2000; Lee et al., 2000; Nakai *et al.*, 2001; Oliveira et al., 2001; Zhou *et al.*, 2001; Cho et al., 2003). There appeared to be an inhibition of fluid reabsorption and

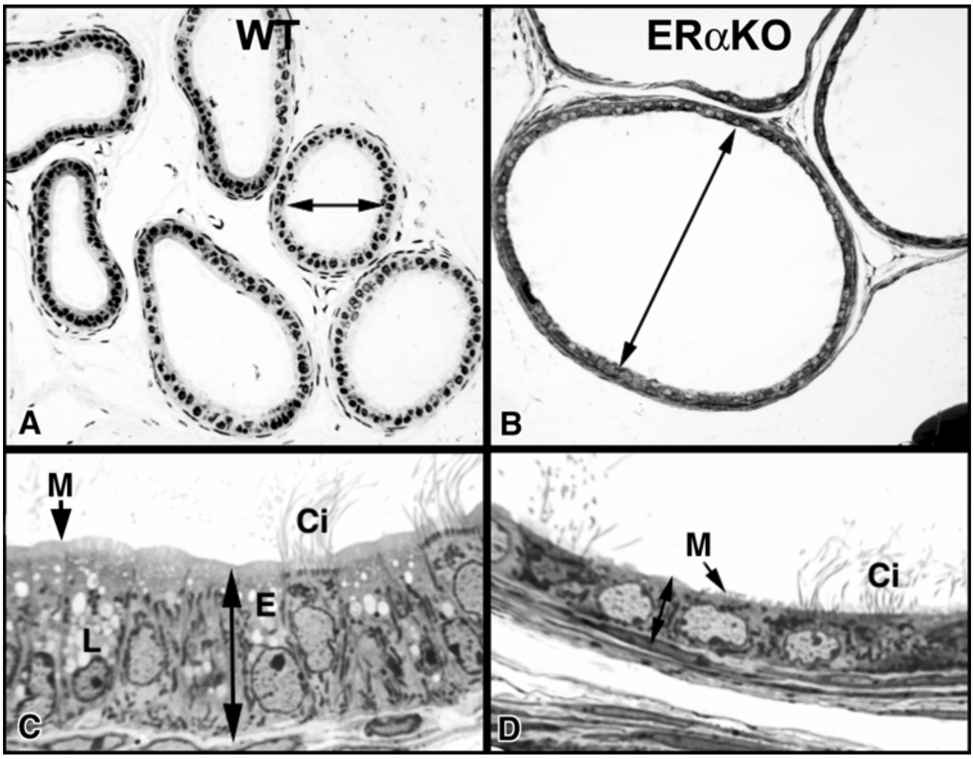

Figure 5A. Wild type mouse (WT) showing normally dilated proximal efferent ductules. 5B. In the ERαKO mouse, the proximal efferent ductule lumen is extremely dilated compared to WT. 5C. WT efferent ductule epithelium by light microscopy showing normal columnar height. Nonciliated cells contain lysosomes (L) and endosomes (E) and have a prominent microvillus border (M) lining the lumen. Cilia (Ci) protrude into the lumen from the ciliated cell. 5D. ERαKO efferent ductule epithelium by light microscopy showing decreased epithelial height. Nonciliated cells contain few cytoplasmic organelles and the microvillus border (M) lining the lumen is greatly reduced. Cilia (Ci) protrude into the lumen from the ciliated cell.

possibly a net inward flux of water into the ductal lumen (Hess et al., 1997a). Thus, excessive accumulation of fluid in the lumen overloaded the funnel-like ductal system that is found in the rodent. As predicted, the accumulation of fluid caused a transient increase in testis weight in ERαKO males between 32-81 days of age and then a steady decrease in weight out to 185 days of age, when total atrophy was observed (Hess et al., 1997a). These data suggested that long-term atrophy of testes in the knockout mouse was caused by backpressure of the accumulating luminal fluids, a well-recognized pathogenesis found after exposure to various toxicants (Hess et al., 1997a; Hess et al., 2000). However, atrophy was only partially induced by the antiestrogen treatment in the adult mice (Cho et al., 2003), but was induced by long-term treatment with ICI 182,780 in the rat (Oliveira et al., 2001; Oliveira et al., 2002). These data have led us to hypothesize that the ERβ that is present within the seminiferous epithelium, which would be blocked in the ICI 182,780 treated males, does have a role in normal spermatogenesis, but is disrupted only after inhibition for an extended period of time.

In the ERαKO and ICI 182,780 treated rodents, the endocytotic apparatus was nearly lost and other cytoplasmic organelles of the nonciliated epithelial cells were greatly reduced and scattered randomly in the efferent ductules (Hess et al., 1997a; Hess et al., 2000; Lee et al., 2000; Nakai et al., 2001; Zhou et al., 2001). The endocytotic pathway includes apical vesicles and PAS+ lysosomal granules, which are prominent in nonciliated cells of normal efferent ductules (Hermo and de Melo, 1987; Ilio and Hess, 1994; Clulow et al., 1998). With ERα inhibition, efferent ductule epithelium was also flattened and the microvillus border was shortened and even absent in some cells (Figs. 5, 6). All of these changes are consistent with a decrease in fluid reabsorption, which was observed in the ERαKO male (Hess et al., 1997a). Thus, in the absence of a functional ERα, the apical surface of this reabsorbing epithelium is transformed into a non-absorbing structure that appears to have lost its terminal differentiation (Al-Awqati *et al.*, 2003).

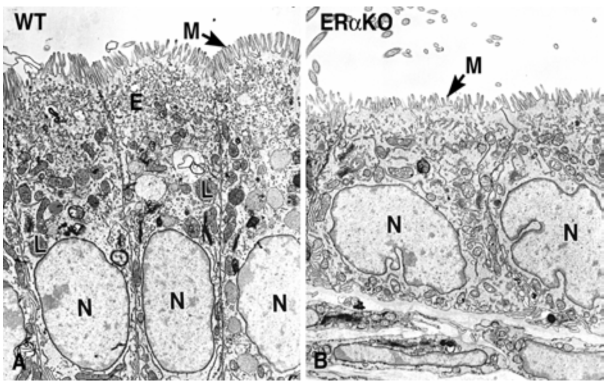

Figure. 6A. Wild type mouse (WT) efferent ductule epithelium at higher magnification by electron microscopy. The nonciliated principal cells are columnar and the apical cytoplasm is filled with lysosomes (L) and the endocytotic apparatus (E). The microvillus brush border (M) shows extensive individual protrusions. N, nucleus. 6B. ERαKO efferent ductule epithelium at higher magnification by electron microscopy. The nonciliated principal cells are short and the apical cytoplasm lacks the typical lysosomes and endocytotic apparatus. The microvillus brush border (M) consists of short irregular protrusions. The nuclei (N) are somewhat distorted and flattened.

The ERαKO mouse provided the first strong evidence that estrogen, 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. Subsequent studies have shown that the major Na+ transporter in the efferent ductule epithetlium (NHE3) is down regulated in the ERαKO male reproductive tract). Both the mRNA and NHE3 protein are decreased substantially in ERαKO and ICI 182,780 treated efferent ductule tissue (Zhou et al., 2001; Oliveira et al., 2002). Because the ERαKO mouse lacks a functional ERα throughout development, the antiestrogen treatment studies are the only ones that effectively demonstrate that ERα is essential for adult function of the efferent ductule epithelium (Lee et al., 2000; Lee *et al.*, 2001a; Zhou et al., 2001; Oliveira et al., 2002; Cho et al., 2003; Oliveira et al., 2003).

ICI 182,780 treatment of the adult male rat (Oliveira et al., 2001; Oliveira et al., 2002) demonstrated that there were species differences in response, with the rat showing greater variability than the mouse (Cho et al., 2003). It is interesting that the rat testes became totally atrophic (Table 4), similar to the ERαKO mouse, while the ICI treated mice testes showed only limited atrophic seminiferous tubules and partial disruption of spermatogenesis. Other species are currently under investigation and it will be interesting to determine whether different species and even strains of rodents show varying sensitivity to the pure antiestrogen. As new ER inhibitors are developed it will be possible to determine the separate contributions of the two receptors in male reproduction. Because both receptors are present in the same cell types of the male reproductive tract, it is possible that ERβ functions to dampen ERα in a manner similar to that found in other tissues (Gustafsson, 2003; Lindberg *et al.*, 2003; Strom *et al.*, 2004).

The aromatase knockout mouse (ArKO) does not exhibit the ERαKO and ICI 182,780 (Table 4) treatment phenotypes (Fisher *et al.*, 1998; Robertson et al., 2001; Robertson et al., 2002). This raises several questions regarding the physiology of estrogen in the testis and efferent ductules, but the most likely answer lies in the fact that ERα is constitutively expressed in the rodent species (Oliveira et al., 2004), although regulated by testosterone (it is not clear that the receptor in this study was ERα) in the goat (Goyal et al., 1998). The ArKO mouse, which lacks estrogen, most likely still expresses ERα abundantly in the efferent ductules. If so, this will be an excellent example of ligand-independent activity of ERα, which could maintain NHE3 expression and subsequent ion transport and fluid reabsorption. Evidence has been accumulating that ERα can be activated in the absence of ligand by several mechanisms; the most well established being EGF induced tyrosine phosphorylation of ERα (Coleman and Smith, 2001; Marquez *et al.*, 2001). Activation of MAP kinase induces ERα translocation to the nucleus (Osborne *et al.*, 2001; Lu *et al.*, 2002) and recently it was shown that acetylation of ERα by p300 cofactors also provides a ligand-independent mechanism for ERα signaling (Wang *et al.*, 2001a). It is possible that fluid reabsorption in the efferent ductules commands extreme important factors? for maintenance of fertility such that down regulation of ion transporter expression in this epithelium requires the loss of more than one receptor to cause a reduction in fluid and ion transport. Thus, it appears that estrogen 'receptor' action in this epithelium is more important than the presence of hormone itself.

Estrogen function in epididymis and vas deferens

The epididymis and vas deferens in most species contain only ERβ and not ERα within the epithelium (Hess et al., 2002; Hess, 2003). However, binding studies suggest that estrogen could have an influence in this region either during development or possibly in the adult. In the first experiment to suggest that estrogen could influence epididymal function in the intact adult mouse, estradiol benzoate plus testosterone propionate decreased sperm transit times through the tract (Meistrich *et al.*, 1975). Estradiol alone was even more effective and resulted in the passage of immature sperm

Table 4. Comparison of animal models: the role of estrogen in male reproduction.

	a	b	c	d	e	f	g	h	i	j	k	l	m
Experimental Model													
ERαKO[1]	+	+	+	+	+	+	+	+	+	+	+	-	+
ERβKO[2]	-	-	-	-	-	-	-	-	-	-	-	+	-
ERαβKO[3]	+	+	+	+	+	+	+	+	+	+	+	+	+
ArKO[4]	+	+	-	+	+	-	-	-	-	-	+	-	+
EsulfotransKO[5]	-	Nd	+	+	+	Nd	Nd	Nd	Nd	Nd	+	+	Nd
ICI 182,780[6]	+	+/-	+/-	+/-	+	+	+	+	+	+	+	+	-
Tamoxifen[7]	-/+	+	+	+/-	+	Nd	Nd	Nd	Nd	Nd	+	+/-	+
Raloxifene[8]	-	-	-	-	-	Nd	Nd	Nd	Nd	Nd	-	+	Nd
Arom Overexpression[9]	Nd	+	-	Nd	+	Nd	Nd	Nd	Nd	Nd	Nd	+	Nd
Aromatase Inhibitor[10]	+	+	+	+	Nd	Nd	Nd	Nd	Nd	Nd	+	-	+/-
Isoflavones (Soy)[11]	-	-	-	-	-	Nd	Nd	Nd	Nd	Nd	-	+	-

a- Infertility or decreased fertility or delayed infertility;
b- Increased or decreased LH and/or testosterone;
c- Change in testis weight or testicular atrophy
d- Seminiferous tubular disruption
e- Leydig cell effects
f- Efferent ductule luminal dilation
g- Decreased efferent ductule epithelial height
h- Decreased efferent ductule endocytosis and/or microvilli
i- Decreased expression of sodium/hydrogen exchanger 3 and carbonic anhydrase II
j- Increased expression of efferent ductule ion transporters
k- Effects on sperm, including cauda sperm counts and/or motility
l- Effects on prostate or prostate cancer cells
m- Effects on sexual behavior
n- Nd- Not determined

[1] ERαKO: (Lubahn *et al.*, 1989; Lubahn et al., 1993; Eddy et al., 1996; Hess et al., 1997a; Dupont et al., 2000; Hess et al., 2000; Lee et al., 2000; Mahato et al., 2000; Lee et al., 2001a; Ogawa *et al.*, 2000; Mahato et al., 2001; Nakai et al., 2001; Prins et al., 2001; Zhou et al., 2001; Akingbemi et al., 2003).

[2] ERβKO: (Krege et al., 1998; Dupont et al., 2000; Gustafsson and Warner, 2000; Risbridger et al., 2001; Weihua et al., 2001).

[3] ERαβKO: (Couse et al., 1999; Dupont et al., 2000)

[4] ArKO: (Fisher et al., 1998; Robertson et al., 2001; Robertson et al., 2002)

[5] Estrogen sulfotransferase knockout: (Qian et al., 2001)

[6] ICI 182,780: Mouse; (Hess et al., 1997a; Lee et al., 2000; Cho et al., 2003); Rat; (Oliveira et al., 2001; Oliveira et al., 2002); Prostate; (Huynh *et al.*, 2001; Turner *et al.*, 2001; Ho, 2004); Human Sperm; (Aquila et al., 2004)

[7] Tamoxifen: (Schill and Landthaler, 1981; Buvat *et al.*, 1983; Danner *et al.*, 1983; Brigante *et al.*, 1985; Dony *et al.*, 1985; Noci *et al.*, 1985; Rozenboim *et al.*, 1986; 1989; Robinzon *et al.*, 1990; Minucci *et al.*, 1997; Li, 1991; Chou *et al.*, 1992; Gill-Sharma *et al.*, 1993; Kotoulas *et al.*, 1994; Adamopoulos *et al.*, 1997; Belmonte *et al.*, 1998; Gopalkrishnan *et al.*, 1998; Parte *et al.*, 2000; Du Mond *et al.*, 2001; Gill-Sharma *et al.*, 2001; Padmalatha Rai and Vijayalaxmi, 2001; Saberwal et al., 2002; Gill-Sharma *et al.*, 2003; Nam *et al.*, 2003; Sethi-Saberwal *et al.*, 2003; Corrada *et al.*, 2004)

[8] Raloxifene: (Neubauer *et al.*, 1993; Neubauer *et al.*, 1995; Hoyt *et al.*, 1998;)

[9] Arom Overexpression: (Hiramatsu *et al.*, 1997; Fowler *et al.*, 2000; Gill *et al.*, 2001; Luthra *et al.*, 2003; Simpson, 2003)

[10] Aromatase Inhibitor: (Trunet *et al.*, 1993; Ulisse *et al.*, 1994; Panno *et al.*, 1995; Shetty *et al.*, 1998; Hayes *et al.*, 2001; Hayes *et al.*, 2000; Mauras *et al.*, 2000; Turner *et al.*, 2000; Omura *et al.*, 2001; Smith *et al.*, 2002; Luthra et al., 2003; Leder *et al.*, 2004;)

[11] Isoflavones (soy): (Mitchell *et al.*, 2001; Robertson et al., 2002; Morrissey and Watson, 2003; Faqi *et al.*, 2004)

into the cauda epididymis, resulting in total sterility. The study did not determine effects on serum hormone concentrations, which leaves open the possibility that estrogen was not acting directly, but instead interfering with gonadotropin secretions and the production of endogenous testosterone. A more recent study has shown that reducing serum testosterone or blocking androgen receptor function will also decrease sperm transit time through the proximal segment of the epididymis (Klinefelter and Suarez, 1997). Other studies have shown that estrogen can influence contractions of the reproductive tract (Markus *et al.*, 1980; Elmallah *et al.*, 1995; Velasco *et al.*, 1997). This potential mechanism for estrogen action in the epididymis should be further studied, as environmental estrogens, when given developmentally, also inhibit sperm transit time in the adult male reproductive tract (Gray *et al.*, 1995).

Other studies have shown that estrogen, even in the presence of maintenance levels of testosterone, produces harmful effects on the epididymis and reduces fertilizing ability of epididymal sperm (Lubicz-Nawrocki, 1974). Although other specific effects have been noted after estrogen treatment, it is not clear whether or not the effects on the epididymis were direct or indirect. In general, the effects of castration on the epididymis are reversible by testosterone administration and estrogen is antagonistic (Jones *et al.*, 1980; Ma *et al.*, 1998). Therefore, the question of estrogen's importance in regulation of the epididymis and vas deferens remains unanswered.

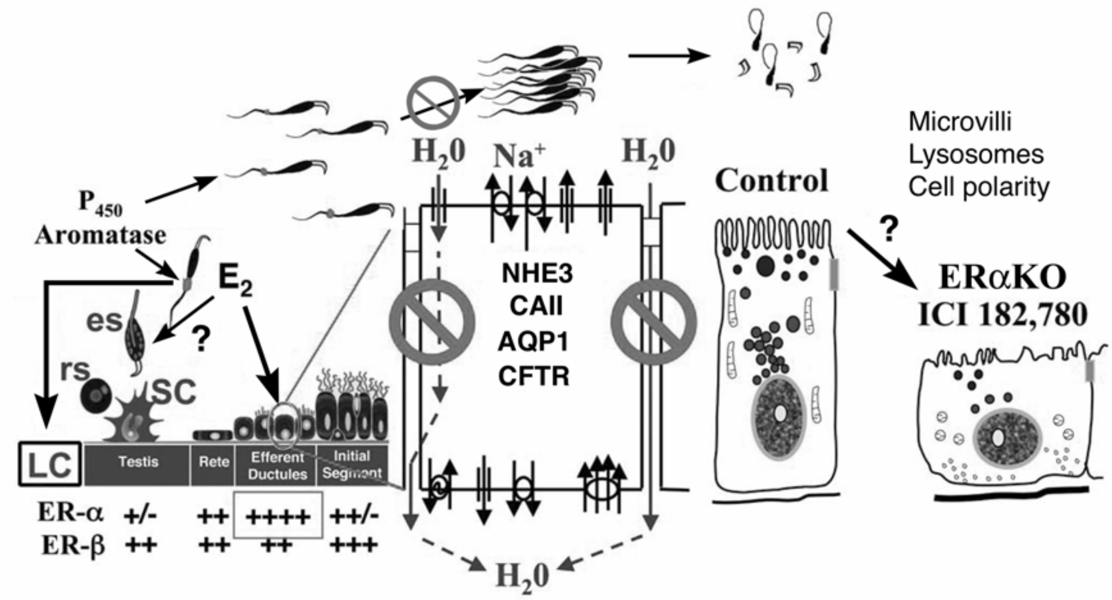

Figure. 7. This summarizes the presence of P450aromatase, estrogen receptors (ER) and targets for estrogen function and dysfunction in the male reproductive tract. In the adult testis of many species, Leydig (LC) and germ cells (round spermatids-rs; elongated spermatids-es) and sperm express aromatase. Sertoli cells (SC) in the adult do not synthesize estrogen to any great extent. Estrogen (E2) synthesized by these sources target the abundance of ERα and ERβ found down stream in the efferent ductules. Estrogen does influence Leydig cell function but questions remain regarding its effect on the germ cells. In the mouse there are many epithelial cell types that contain ERα along the reproductive tract, but in other species only the efferent ductules express this receptor, while ERβ is nearly ubiquitous in epithelial cells of testis and epididymis of all species examined. Estrogen's primary function in the male tract is the regulation of fluid reabsorption in the efferent ductules via ERα, which increases the concentration of sperm prior to entering the epididymis. Disruption of ERα results in decreased Na+ transport and thus decreased water (H2O) and fluid reabsorption. This inhibition is mediated by a decrease in the expression of NHE3 mRNA and protein and also decreases in carbonic anhydrase II (CAII) and aquaporin I (AQP-1) proteins. There is also an increase in cystic fibrosis transmembrane conductance regulator (CFTR) protein and mRNA, which adds to the NHE3 effect by secreting Cl- into the lumen (Lee et al., 2001a). This inhibition (indicated by ⃠) of fluid reabsorption results in the dilution of cauda epididymal sperm, disruption of sperm morphology, and eventual decreased fertility. In addition to this primary regulation, estrogen is also responsible for maintaining a differentiated epithelial morphology, which includes the expression of microvilli, lysosomes through an unknown mechanism that is apparently associated with cell polarity.

Summary and Conclusions

Estrogen is found in abundance in the testis, rete testis fluid and semen of many species. Its importance in the regulation of the male reproductive tract is now evident (Fig. 7), with convincing data showing direct effects on the function of Leydig cells and the efferent ductule epithelium. Potential effects on germ cells remain questionable. Estrogen is synthesized by the germ cells, producing a relatively high concentration in rete testis fluid, which then targets estrogen receptors that are abundant in efferent ductule epithelium in all species examined. In some species, ERα is present even in the epididymis, but in most species only ERβ is expressed in epididymis and vas deferens. Estrogen's primary function in the male tract appears to be the regulation of fluid reabsorption in the efferent ductules via ERα. Disruption of the receptor results in dilution of cauda epididymal sperm, disruption of sperm morphology, inhibition of sodium transport and subsequent water reabsorption, increased secretion of Cl^-, and eventual decreased fertility. The mechanism by which estrogen regulates epithelial morphology, such as microvillus growth and expression of endosomes and lysosomes, remains to be determined. Based upon the data reviewed, we must conclude that estrogen or its receptor is important for male reproductive tract function in numerous species.

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