Review

Estrogen in the adult male reproductive tract: A review
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Abstract

Testosterone and estrogen are no longer considered male only and female only hormones. Both hormones are important in both sexes. It was known as early as the 1930's that developmental exposure to a high dose of estrogen causes malformation of the male reproductive tract, but the early formative years of reproductive biology as a discipline did not recognize the importance of estrogen in regulating the normal function of the adult male reproductive tract. In the adult testis, estrogen is synthesized by Leydig cells and the germ cells, producing a relatively high concentration in rete testis fluid. Estrogen receptors are present in the testis, efferent ductules and epididymis of most species. However, estrogen receptor-α is reported absent in the testis of a few species, including man. Estrogen receptors are abundant in the efferent ductule epithelium, where their primary function is to regulate the expression of proteins involved in fluid reabsorption. Disruption of the α-receptor, either in the knockout (αERKO) 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. In addition to this primary regulation of luminal fluid and ion transport, estrogen is also responsible for maintaining a differentiated epithelial morphology. Thus, we conclude that estrogen or its α-receptor is an absolute necessity for fertility in the male.

Introduction

It was known as early as the 1930's that the developing testis was responsive to the "female" hormone [[1], also reviewed by [2]]. It was also known in the 1930's and 40's that developmental exposure to high doses of estrogens could induce malformation of the male reproductive tract [3–6]. Thus, during the formative years of reproductive biology as a discipline it was suggested that estrogen might be important in the male; however, even in the early 1990's many scientists considered estrogen receptor presence in the adult male reproductive tract to be a remnant from the indifferent sex stage of embryological differentiation [7].
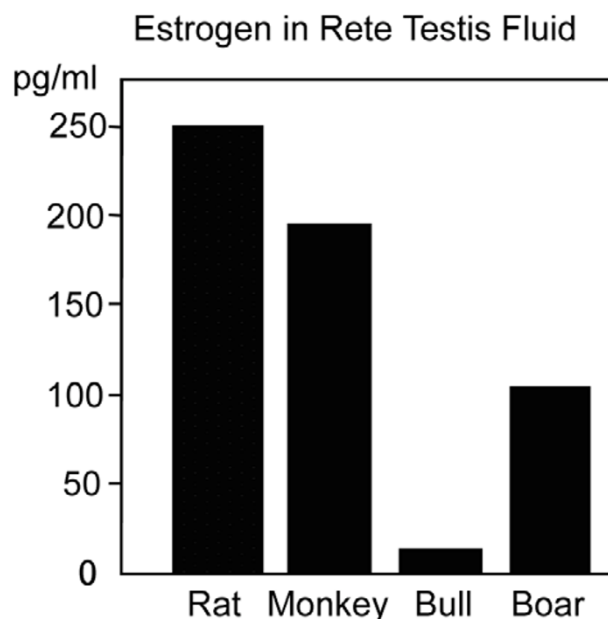
Reference to estrogen production by the testis was more of a curiosity at first, as efforts were made to determine the various metabolites of testosterone being produced [8–11]. During the 1970's, the prediction of an estrogen receptor in testis and epididymis became a reality as estradiol binding was discovered [12–15]. However, it was clear from subsequent publications that most scientists did not consider estrogen to be a major steroid hormone in the male reproductive tract, in the adult [16–19]. The potential importance of estrogen during development of the male reproductive system was made popular by the report that diethylstilbestrol (DES) treatment during pregnancy induced cryptorchidism and epididymal cysts in male mice [20]. This discovery opened the door to numerous investigations into the long-term effects of

developmental exposure to estrogenic compounds on male reproduction, an inquiry that continues today [21,22]. Although estrogen effects in the developing male are important, such studies have not actually proven that estrogen has a role in the adult male reproductive organs. At best, it was thought that an estrogen binding ability was left over from developmental processes and that estrogen played only a small role in the adult male [7,23,24].

Most interesting was the discovery that cytochrome P450 aromatase, which is capable of converting androgens into estrogens, is present in the testis [25–39]. During this same period of discovery, others were using the radioimmunoassay to identify steroids present in body fluids and estrogen concentrations were found to be relatively high in seminal and rete testis plasma [40–48]. Thus, up to the 1990's it appears that most scientific inquiry into estrogen's presence in the male remained a curiosity, as well as a worry that estrogen exposure during development was harmful. Then, in the decade of the 90's new discoveries in the male led to the hypothesis that estrogen not only has important functions in the adult male reproductive tract, but that estrogen and its α-receptor are "essential" for normal fertility. This new paradigm for estrogen's role in the male began with the discovery that testicular germ cells and epididymal sperm contain aromatase and synthesize estrogen [49]. This discovery explained the presence of a high concentration of estradiol in rete testis of the rat [41] and provided a source of estrogen for the high concentration of receptors that were subsequently found to populate the head of the male reproductive tract [50–55]. However, an estrogen function was not uncovered until the ERα knockout (αERKO) was produced. The αERKO mouse, originally generated by Dennis Lubahn and colleagues [56], showed for the first time that ERα is essential for fertility in the male [56–58]. This animal model was further developed to show that estrogen provides a physiological function in regulating fluid dynamics in the male reproductive tract, a function that is "essential" for normal reproductive performance [59–66].

Estrogen in the male tract
Estrogen is produced in sizable quantities in the testis, as well as the brain [67]. It is also present in very high concentrations in the semen of several species [40–48]. Table 1 shows the reported locations for estrogen synthesis in the adult male reproductive system from several species. Early studies reported that the primary source of estrogen in the immature male was the Sertoli cell [68]. In the adult testis, Leydig cells express aromatase (P450arom) and actively synthesize estradiol at a rate much greater than that seen in the adult Sertoli cell [31,32,38,69–72]. Currently, a growing body of evidence indicates that germ cells also synthesize estrogen, and possibly serve as the major source of this steroid in the male reproductive tract

Figure 1
Estrogen in rete testis fluid. Mean concentrations (pg/ml) for estradiol or total estrogens in four species, rat [41], monkey [44], bull [42] and boar [46].

[see review by [72]]. In 1993, in collaboration with the laboratories of Bahr and Bunick [49], we reported for the first time that P450arom is present in testicular germ cells of the adult male mouse. The enzyme was localized in the Golgi of round spermatids and throughout the cytoplasm of elongating and late spermatids. Its presence was confirmed by Western and Northern blot analysis of isolated germ cells. Its activity in germ cells was equal to or exceeded the activity found in the interstitial cells. More recently, Carreau and others [72,73] have shown aromatase expression and activity in the human sperm.

The presence of P450arom in male germ cells has now been demonstrated in several species, including mouse, rat, brown bear, the bank vole, rooster, and man [49,52,73–80]. The enzyme is located in cytoplasmic droplets of the sperm tail, but the staining becomes less intense as sperm traverse the epididymis [73,75]. Its presence in germ cells and spermatozoa was recently confirmed and shown to represent approximately 62% of the total testicular aromatase [69,70,81]. Testicular germ cells in the boar, ram and stallion have not been shown to be aromatase-positive. It is unclear whether this is due to differences in antibodies used or if some species simply do not generate estradiol by the germ cell pathway. It would be interesting to determine if aromatase is expressed in the

Table 1: P450 Aromatase in the adult male reproductive system.

Species	Leydig cells	Sertoli cells	Germ cells	Spermatozoa	References
Mouse[1]	+		+	+	[49,74]
Rat[1]	+		+	+	[31,32,34,36,38,69,75,77,81,151–154]
Rooster			+	+	[76]
Bear[2]	+	+	+		[155]
Boar	+				[156]
Ram	+				[157]
Stallion	+	+	+		[158–160]
Bank vole	+	+	+		[79,161,162]
Rainbow trout fish	+				[163]
Dogfish shark			+		[164,165]
Marmoset			+		[151]
Rhesus	+		+		[166]
Human			+	+	[72,73,80,151]

[1] Early work showed only Leydig cells being positive for Aromatase in the adult testis [2] Location depended upon the season[155].

epididymal tract of those species lacking germ cell expression. Others have shown the absence of aromatase in the mouse epididymis [82]; thus, the conversion of androgens to estrogens by sperm remains the primary source of estrogen in the lumen of the reproductive tract of this species. This observation raises new and exciting hypotheses regarding the potential for estrogen to regulate functions in the efferent ductules, epididymis and vas deferens.
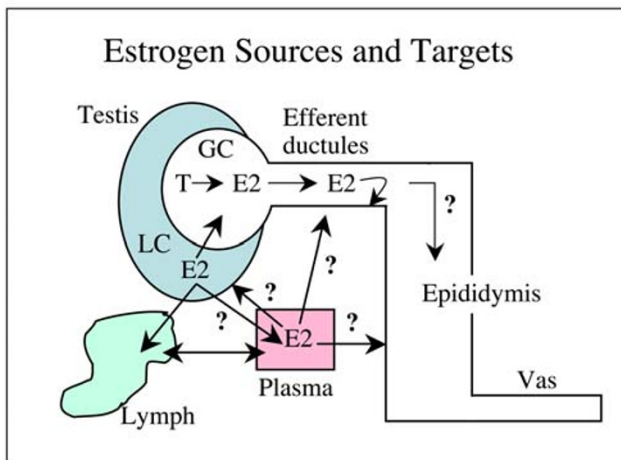
The concentration of estrogens in peripheral blood is typically low in the male, but ranges from 2–180 pg/ml depending upon the species [40,42–47,83–88]. The horse is an exception, where estrone sulfate is found as high as 2,447 pg/ml [40,88]. Estrone-sulfate concentration is 900 ng/ml in testicular lymph in the horse, suggesting that intra-testicular estrogens can be rather high [89]. Estrogen concentrations are typically higher in the testicular vein and lymph than in the general circulation. Also, in the reproductive tract, estrogen can reach relatively high concentrations (Fig. 1). In one report, estrogen concentration in rete testis fluid of the rat was approximately 250 pg/ml [41], which is higher than the average serum concentration of estradiol in the female [83,90]. Estrogens are also abundant in semen and depending upon the species, their concentrations can range from 14 to nearly 900 pg/ml [42–46]. Estrone-sulfate is found as high as 4,000 pg/ml in the horse [40].

The potential sources of estrogen in the male reproductive tract are illustrated in Fig. 2. Although the concentration of estradiol is known for various compartments of the male tract (Fig. 1), the relative amounts of estradiol that are derived from the different sources are not known. For many years it was assumed that most of the testicular estrogen was derived from Leydig cells (Table 1). How-

ever, with the discovery that germ cells also synthesize estrogen, the Leydig cell is no longer required as a source for estrogen in the reproductive tract lumen. Actually, it is more likely that Leydig cell derived estradiol would move toward the lymphatics, because the cells lie adjacent to endothelial cells of the lymphatic system and estrogens are reported to be in very high concentration within testicular lymphatics [47,88]. Because blood estrogens are in low concentrations in the male, we would assume that this source would provide limited endocrine activity in the reproductive tract. In the efferent ductules, the blood source would likely have even less effect than in the remainder of the reproductive tract, as these ductules are responsible for reabsorption of over 90% of the luminal fluids [91] 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 [92].

Estrogen receptors in the male tract
It has been known for at least 25 years that an estrogen receptor-like protein exists in epididymal tissues [12]. However, those early studies lead to the conclusion that estrogen was more important during development of the epididymis than in adult function [17]. Estrogen binding in epididymal tissues has been noted in many species, including the dog [93,94], human [95], turtle [96], monkey [97,98], ram [99], guinea pig [100], and the rat [101]. Autoradiography was also used to show estrogen binding throughout the male reproductive system [55,102]. Schleicher and coworkers [102] found very strong labeling of the efferent ductules and initial segment epididymis, with lesser binding in the distal tract. However, binding assays

Figure 2
Estrogen sources and targets in the male reproductive tract. Estradiol 17β (E2) is produced in peripheral tissues and delivered via the plasma, but is also synthesized by Leydig cells (LC) in the testicular interstitium. The contribution of E2 from testis to plasma and from the vasculature to the testis is unknown, but it is assumed that most of the lymphatic E2 would be derived from LC. LC and germ cells (GC) contain p450 aromatase in the adult testis. LC may also contribute to the E2 concentrations in the rete testis fluid, but it is more likely that germ cell production of E2 provides the estrogen that will target the efferent ductule epithelium, the region that contains the highest concentration of ER. Less is known of E2 function and targets in the epididymis 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, except for the elongated spermatids [108–121]. In contrast, ERα is found only in the interstitium of the testis in most species examined [51,53,109,110,122,123]. In some species both Leydig and peritubular myoid cells are ERα positive but the testis of the goat, monkey and human are reportedly devoid of ERα [98,104,108]. The ERβ knockout mouse [124,125] shows no testicular phenotype and the αERKO and double ERαβ knockout mice [56–58,125,126] show no testicular phenotype during early development, suggesting that these receptors are not essential for normal development of sperm in the testis.

Transplantation of germ cells from the αERKO mouse testis into normal testis (made devoid of sperm) results in normal spermatozoa capable of fertilization and the production of offspring [126], suggesting that testicular ERα has no influence on spermatogenesis. However, loss of estrogen synthesis in the aromatase knockout mouse [127,128] results in decreased fertility with aging. Another study in the mouse also suggests that estrogen may have a testicular function, acting through the Leydig cells. It has been suggested that testosterone concentrations are elevated in the αERKO male [57], but it was generally concluded that this increase was due to the disruption in feedback regulation at the hypothalamus. However, a more recent study found that Leydig cells isolated from the αERKO testis had increased production of testosterone and that normal Leydig cells when treated with the pure ER inhibitor ICI 182,780 also showed increased steroidogenesis [129]. Therefore, ER in the testis, although not necessarily essential for spermatogenesis, does appear to have a subtle function in the Leydig cells.

In the rat, ERα localization has been more controversial. In one study, using a mouse monoclonal antibody (6F11) against the A/B region of the human ERα, positive staining was found only in epithelial cells of the efferent ductules [53]. The epididymal tissues were negative. Our laboratory repeated this study using the 6F11 antibody (Novocastra, UK) and the data are in complete agreement with the Fisher study, showing staining only in epithelia of the efferent ductules [130]. In another study using frozen sections and the ER21 antibody, which is made against a peptide containing the first 21 amino acids of the rat and human ERα (does not cross-react with ERβ), we also found predominant staining in efferent ductules [59], as shown for all species examined to date. However, the initial segment epididymis was also strongly positive and the remaining regions of the epididymis were moderately positive. This study was repeated, but using antigen retrieval methods instead of frozen sections, and the results differed only slightly [130]. The major difference

do not differentiate between ERα and β. Therefore, other methods, such as immunocytochemistry (ICC), *in situ* hybridization and Northern blot analysis, have been used to separate the two ER subtypes. However, these techniques do not always provide identical results, and there are disagreements between laboratories and between species. Using ICC, ER has been localized primarily in the epithelium of efferent ductules [53,55,98,103–108]. However, in the goat and monkey, only nonciliated cells of the efferent ductal epithelium stained ER positive [54,98]. After the discovery of ER subtypes and the production of specific antibodies, ERα localization in the epididymis has also given confusing results [53,59,103–105,109,110]. In the mouse at 90 days of age, the efferent ductule epithelium was strongly positive for ERα immunostaining, using the H222 antibody [51]. Other epithelia along the epididymis were only slightly positive. Using a different antibody, the mouse epididymis showed strong ERα staining in principal cells and other cell types, but in a region specific manner [110]. This immunostaining is somewhat similar to the autoradiography data previously shown by Schleicher [102].

Figure 3
Estrogen receptor-α immunohistochemistry in the efferent ductules. ERα is abundant in the ductules of most species examined. Represented here are ductules from the rat, mouse, dog and cat [109,110,130]. Ciliated (C) and nonciliated (N) cells are strongly positive in all these species, except the cat, where ciliated cells show weak staining. Bar = 25 μm.
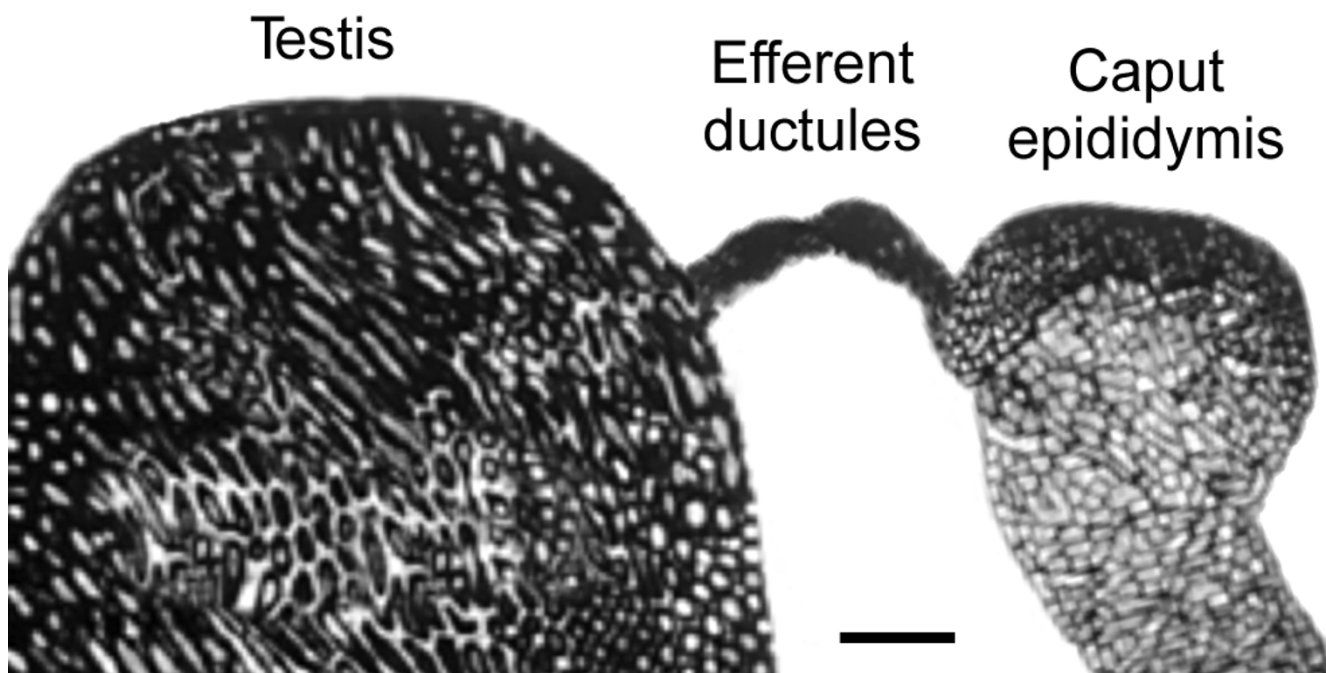
was in staining that was observed in the epithelium of the vas deferens, which was negative using frozen sections. This difference in staining in the rat between the two antibodies, 6F11 and ER21, raises serious questions regarding the literature's description of ER localization in the male reproductive tract using ICC alone. Autoradiography and estradiol binding assays indicate that ER is present in the rat epididymis. RT-PCR data also show that ERα is present in epididymal tissues [59,108]. Therefore, future studies should focus on *in situ* hybridization methods for localizing the mRNA in specific regions and cell types of the epididymis.

Although there are reported differences in ERα localization in the epididymis of various species, its presence in efferent ductule epithelium has remained constant across species (Fig. 3). ERα protein is abundant in epithelial cells of the efferent ductule, with intense immunohistochemical staining of the nonciliated cell nucleus and the ciliated cells showing considerable variability in staining. The presence of an abundance of ERα protein in efferent ductule tissue is supported by an elevated expression of its mRNA. A previous study by our laboratory reported that ERα mRNA expression in efferent ductules of the rat is 3.5 fold greater than in the uterus [55]. Thus, in comparison to the well-recognized estrogen-responsive female tissue, the efferent ductules of the male reproductive tract are also a major target for estrogen action. Several laboratories [95,108,131,132] have reported evidence for ER in the human efferent ductules and epididymis. However, in some cases the principal cells were negative, while the basal cells and stromal cells were positive. The epididymis in nonhuman primates is also ER positive by RT-PCR, but there was no distinction between the α and β subtypes [133].

The discovery of a second form of ER (ERβ) further complicates the interpretation of earlier data from estrogen binding studies. ERβ has now been found in testis, efferent ductules, epididymis and prostate [55,101,108,119,124,134–137]. 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 [124]. ERβ is more widely distributed in the male tract than ERα [130]. ERβ has strong reactivity in efferent ductules, similar to ERα. In the remainder of the tract, ERβ appears to be weaker in initial segment epididymis but stronger in the corpus, cauda and vas deferens. The stromal tissue cells also stain strongly positive for ERβ throughout the male reproductive tract. Thus, there is a large potential for estrogen binding in the epididymis and vas deferens through ERβ.

Estrogen function in testis
There is limited direct evidence that estrogen has a major role in adult testicular function [see review by [127]], other than the recent paper by Hardy and colleagues [129], in which the antiestrogen ICI 182,780 inhibited *in vitro* Leydig cell production of testosterone. Estradiol alone was unable to stimulate Leydig cell steroidogenesis. In the developing testis, estrogen has significant activity in establishing Sertoli cell function [127] and potentially even in establishing Sertoli-germ cell adhesion [138,139]. However, in the total absence of estrogen synthesis, the ArKO male shows normal spermatogenesis at the beginning of puberty and only with aging does the testis begin to develop lesions associated with the round spermatids [127,140]. This is not entirely surprising in light of the fact that ERα is not present within the seminiferous epithelium [109,110] and although ERβ is found in Sertoli cells and nearly all germ cells [108–110,141,142], the ERβ

Figure 4
Testis, efferent ductules and epididymis. The surrounding fat pad was dissected away to show the efferent ductules that lie between the testis and caput epididymis. Bar = 2 mm.
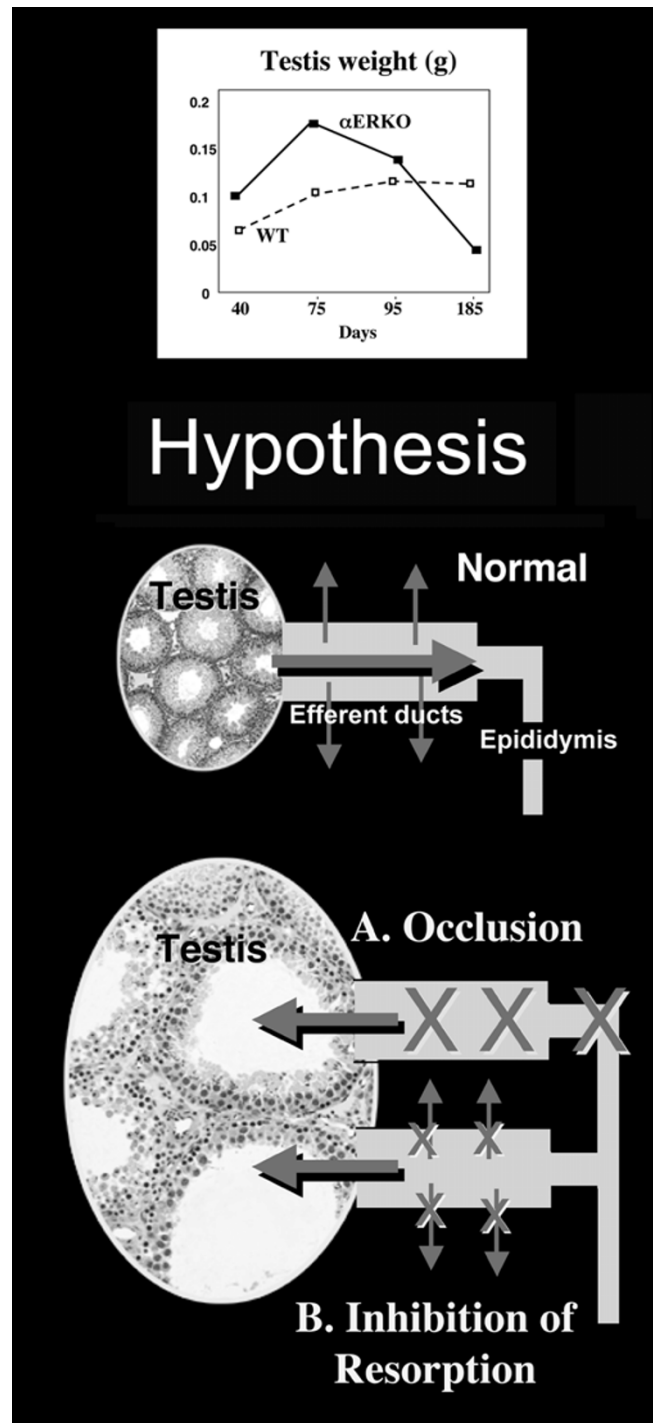
knockout (β ERKO) male testis appears normal and the males are fertile [58,124,125].

Indirect evidence of estrogen's influence on spermatogenesis comes from animal models such as the *hpg* mouse, which is deficient in gonadotropin releasing hormone (GnRH). Ebling and colleagues [143] found that estradiol implants in the *hpg* mouse 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. The ArKO testis is normal at first, but with aging shows decreases in testis weight, seminiferous epithelium, and germ cell numbers [144]. When the ArKO male is maintained on a soy-free diet, these effects are accelerated and enhanced [127,140]. 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 effects due to FSH or LH. This role of estrogen in the testis will most likely be found in the germ cells, as they express ERβ abundantly [108–110,142] and genistein has a higher

affinity for ERβ than for ERα [145]. Finally, although the Sertoli cell does not express ERα, it is interesting that in the αERKO testis there is significantly less seminiferous tubular secretion than in the wild-type testis [59]. The same effect was suggested for the ArKO testis, as seminiferous tubule luminal volume and tubular length was decreased [140]. Thus overall, estrogen does appear to have subtle functions in the testis, not only at the Leydig cell but also possibly targeting the seminiferous epithelium, too.
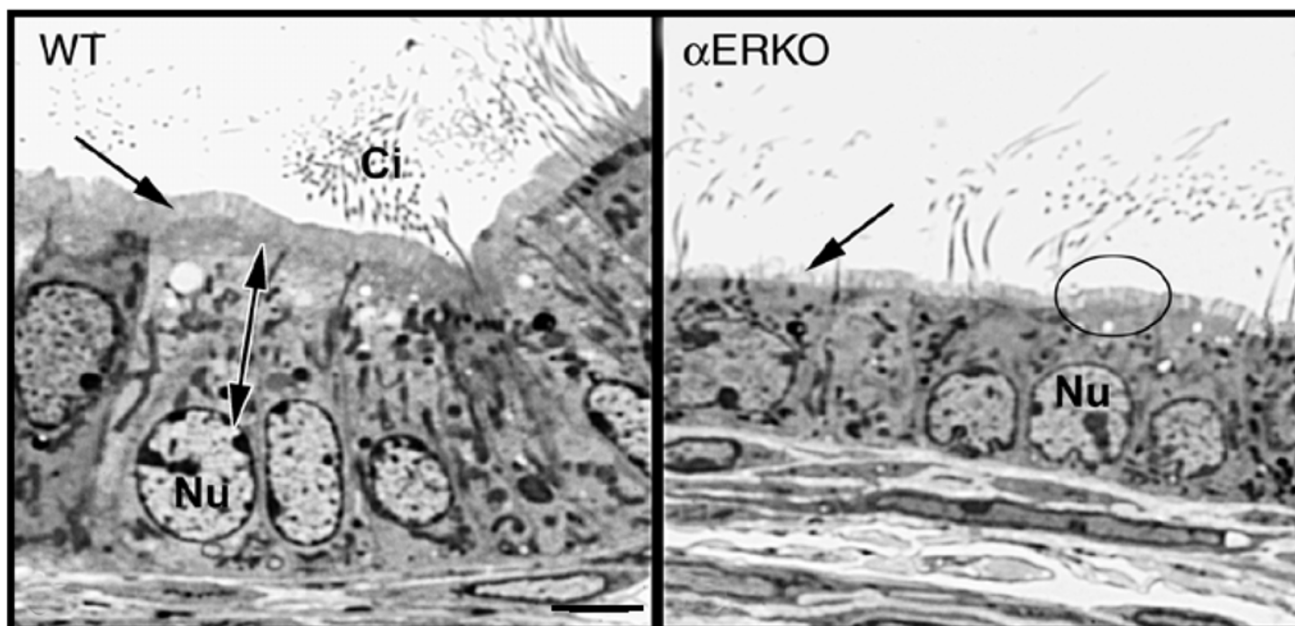
Estrogen function in efferent ductules
Efferent ductules are a major site for estrogen function in the male reproductive tract, across numerous species. These ductules are a series of tubules that connect rete testis to the epididymis (Fig. 4). One-third or more of the head of the epididymis in man and 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 [91]. Nonciliated cells of the epithelium are reabsorptive, similar to proximal tubules of the kidney, having a brush border of microvilli

Figure 5
Hypothesis to account for testicular weight increase in the αERKO mouse. The αERKO mouse testis was shown to increase in weight from day 40 to 75 days of age, and then the weight declined until the testis was atrophied by day 185 [59]. Two hypotheses were proposed to account for mechanisms that could explain the transient increase in testis weight prior to regression. In the normal testis, efferent ductules receive low concentrations of sperm from the rete testis. Approximately 95% of this fluid is reabsorbed by the efferent ductule epithelium, which increases the concentration of sperm that enter the epididymis. Disruption of ERα causes testicular swelling through one of two possible mechanisms: A. the efferent ductules become occluded, or B. the fluid reabsorption pathways are inhibited. Both mechanisms will result in fluid accumulation in the seminiferous tubules and backing up of fluids into the testis. Atrophy occurs by an unknown mechanism that inhibits spermatogenesis.

Figure 6
Histology of the efferent ductule epithelium in αERKO mouse. The wild-type (WT) ductule epithelium is columnar in shape with nonciliated cells that contain large spherical to oblong shaped nuclei (Nu) and extensive apical cytoplasm (double arrow). The nonciliated cell has a tall microvillus brush border (arrow) and extensive endocytotic apparatus. The ciliated cells have motile cilia (Ci) that extend into the lumen. The αERKO efferent ductule epithelium has a low cuboidal shape, with the apical cytoplasm reduced in size and the nucleus (Nu) also smaller. Microvilli are sparse on some cells (arrow) and reduced in height in other cells (circle). Bar = 10 μm.

connecting in the apical cytoplasm to a profusion of apical canaliculi, vesicles, tubules and membrane-bound bodies, which constitutes an elaborate endocytotic/lysosomal system [146]. In the basal region, rough endoplasmic reticulum, mitochondria and lipid droplets are common [147]. The efferent ductules express an abundance of both androgens and estrogen receptors [109,110,130].
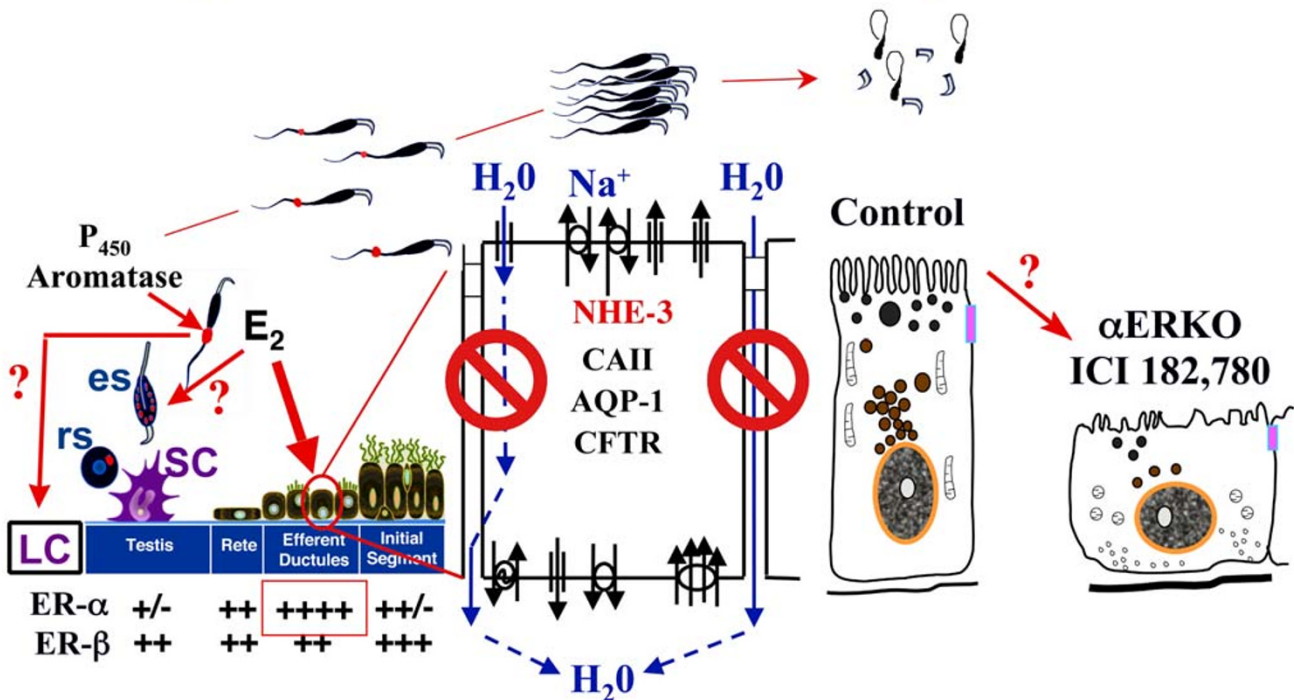
Much of what we know about estrogen's function in efferent ductules has been derived from the study of the αERKO mouse and the use of antiestrogen treatment models. The male αERKO mouse was found to be infertile [56], raising the possibility that ERα is required for normal function of the male reproductive system. Although the αERKO testis appeared normal before puberty, after the onset of spermatogenesis, the testis began to degenerate and eventually became atrophic [57]. By 150 days, cauda sperm from the αERKO male were abnormal and sperm concentrations were significantly reduced [57], suggesting that the reproductive tract was also abnormal. A later study by Eddy's lab showed that αERKO germ cells transplanted into a normal testis (treated with busulphan

to remove native germ cells) were capable of fertilization [148]. That study clearly pointed to extra-testicular regions, such as the efferent ductules and epididymis, being the major source of pathological alterations in αERKO males [57,59].

The rete testis in αERKO males is dilated and protrudes into the testis [57,59]. Based upon this 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 (Fig. 5). After careful examination, we found the second hypothesis to be true, as the efferent ductule lumen was dilated markedly [59]. There appeared to be an inhibition of fluid reabsorption and possibly a net inward flux of water into the ductal lumen. Thus, the excessive accumulation of fluid in the lumen was overloading the funnel-like ductal system found in the rodent. As predicted, the accumulation of fluid caused a transient increase in testis weight in αERKO 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. These data suggested that long-term atrophy of testes in the knockout mouse was caused by backpressure

Figure 7
Estrogen and its inhibition in the male reproductive tract: a summary. In adult males, germ cells, as well as Leydig cells (LC) contain P450 aromatase and actively synthesize estrogen (E2), which produces a relatively high concentration in rete testis fluid. This luminal estrogen targets estrogen receptors that are abundant throughout the male reproductive tract, but particularly ERα that is localized in the efferent ductule epithelium, where its expression is more abundant than even the female reproductive tract. In the testis, E2 may also feedback to influence the function of LC and spermatids, either round spermatids (rs) or elongated spermatids (es). 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, either in the knockout (αERKO) or by treatment with a pure antiestrogen ICI 182,780, results in a decrease in Na+ transport from lumen to interstitium and thus a decrease in water (H_2O) 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 protein and mRNA, which adds to the NHE3 effect by secreting Cl- into the lumen by the cystic fibrosis transmembrane conductance regulator (CFTR) [64]. This inhibition 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 of luminal fluids and ions, estrogen is also responsible for maintaining a differentiated epithelial morphology through an unknown mechanism.

of the accumulating luminal fluids, a well-recognized pathogenesis found after exposure to various toxicants [59,149]. However, atrophy was not induced by antiestrogen treatment in adult mice (unpublished data), suggesting that in the αERKO mouse, this pathological event is due to a developmental anomaly.

In the αERKO efferent ductule epithelium (Fig. 6), the endocytotic apparatus was nearly lost and other cytoplas-mic organelles appeared reduced and scattered randomly [59,60,62,63,149]. The endocytotic pathway includes apical vesicles and PAS+ lysosomal granules, which are prominent in nonciliated cells of normal efferent ductules [91,147,150]. The αERKO epithelium was also flattened and the microvillus border was shortened and even absent in some cells. All of these changes are consistent with a decrease in fluid reabsorption, which was observed in the αERKO male [59]. Thus, in the absence of a functional

ERα, the apical surface of this reabsorbing epithelium appeared to be transformed into a non-absorbing structure.

The αERKO 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 epithelium (NHE3) is down regulated in the αERKO male reproductive tract. Both the mRNA and NHE3 protein were decreased substantially in αERKO tissue, and Na+ uptake by the epithelial cell *in vitro* was negligible [63]. However, the αERKO mouse lacks a functional ERα throughout development. Therefore, the morphological and physiological abnormalities observed could represent developmental defects, rather than adult dysfunction. To test this hypothesis, adult mice were treated with a pure antiestrogen, ICI 182,780 (Astra-Zeneca, Macclesfield, Cheshire, UK). This collaborative study with David Bunick and Janice Bahr showed conclusively that ERα is important for adult function of the efferent ductules, as ICI induced pathological changes that were nearly identical to those seen in the αERKO mouse [60]. A second species, the adult male rat, also responds in a similar manner to ICI treatment over a 125-day period [65,66]. The two major response variables, dilation of efferent ductule lumen and decreased expression of NHE3, show identical responses in rats and mice [63,65]. Although the rats became infertile, they did show greater variation in response overall than was seen in the ICI-treated mice. Long-term treatment in the rat resulted in a transient increase in testicular weight, eventual testicular atrophy at the time of infertility, whereas in the ICI-treated mouse there was no change in testicular weight. After ICI treatment, the rat efferent ductule epithelium also showed a transient increase and redistribution of PAS-positive lysosomal granules in the nonciliated cells [65,66]. However, with continued treatment the rat epithelium showed a decrease in the number of lysosomes to nearly undetectable levels [59], similar to αERKO and mice treated with ICI. Lysosomes are more numerous in the rat than in the mouse efferent ductules [147]; therefore, this intriguing interspecies difference in response to the antiestrogen must be examined in future studies involving other species. Overall, it was shown that ICI promotes adult dysfunctional changes in rat efferent ductules similar to those of αERKO and ICI treated mice, with luminal dilation, decreases in epithelial height, loss of cytoplasmic organelles and decreases in the expression of NHE3 protein and mRNA [65,66].

Summary and Conclusions

Estrogen is important in the regulation of the male reproductive tract, with clear evidence pointing to a direct effect on the function of Leydig cells and the efferent ductule epithelium, but potential effects also on germ cells (Fig. 7). Estrogen is synthesized by the germ cells, producing a relatively high concentration in rete testis fluid. Estrogen receptors are abundant throughout the male reproductive tract, but ERα is primarily localized in the efferent ductule epithelium, where its expression is more abundant than even the female reproductive tract. Estrogen's primary function in the male tract appears to be the regulation of fluid reabsorption in the efferent ductules via the ERα. Disruption of the receptor, either in the knockout (αERKO) 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. In addition to this primary regulation of luminal fluids and ions, estrogen is also responsible for maintaining a differentiated epithelial morphology. Thus, we conclude that estrogen or its receptor is an absolute necessity for fertility in the male.

Acknowledgments

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