

Infertility and Testicular Atrophy in the Antiestrogen-Treated Adult Male Rat¹

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ABSTRACT

The estrogen receptor- α (ER α) knockout mouse (α ERKO) lacks ER α throughout development; therefore, an adult model for the study of estrogen effects in male mice was recently developed using the antiestrogen ICI 182,780. However, differences between species have been noted during immunostaining for ER α in the male tract as well as in response to treatments with antiestrogens. Therefore, we developed the antiestrogen model in the adult male rat to test, in another species, the hypothesis that estrogen regulates fluid reabsorption in efferent ductules. Estrogen receptor in the rat was blocked using ICI 182,780 for 100–150 days. Male Sprague-Dawley rats were treated weekly with s.c. injections of ICI 182,780 (10 mg) or castor oil (as control). The effects of ICI included testicular atrophy and infertility, similar to terminal effects in the α ERKO male. Additionally, ICI induced dilations of the rete testis and efferent ductules and a reduction in the height of the ductule epithelium, which are changes similar to those in both α ERKO and ICI-treated mice. One difference between species was a large variation in effects on the rat efferent ductule epithelium, including a transient increase in the number of periodic acid-Schiff-positive, lysosomal-like granules. These data confirm that estrogen is required for normal function of the efferent ductules and is essential for long-term fertility in the male rodent.

epididymis, estradiol receptor, male reproductive tract, mechanisms of hormone action, testis

INTRODUCTION

Estrogen receptor (ER)- α and ER β are present with overlapping and/or distinct cellular distributions in all organs of the male reproductive system [1–5]. The male mouse lacking a functional ER α (α ERKO) is infertile [6, 7]. The absence of ER β has no apparent effect on male reproduction, and these mice (β ERKO) are fertile [8, 9]. In agreement with the separate phenotypes for α ERKO and β ERKO, the male double knockout ($\alpha\beta$ ERKO) is also infertile [9, 10], indicating that ER α has greater importance in the male reproductive system. The α ERKO male has abnormal rete testes and efferent ductules [11, 12]. The lumen of these tubules is dilated, and fluid reabsorption is inhibited [11], which appears to be involved in causing the infertility.

Because the α ERKO mouse lacks ER α throughout development, an adult model for the study of estrogen effects in male mice was recently developed using the antiestrogen ICI 182,780. After 35 days of treatment, the ICI-treated

mouse reproduced most of the phenotype seen in the α -ERKO reproductive tract, except for testicular atrophy and infertility [13], which raises questions regarding length of the exposure and whether antiestrogen action is the same in all species. The mouse has been the species most resistant to the action of antiestrogens having the triphenylethylene formulation, such as tamoxifen [14, 15]. Other species differences in antiestrogen action have also been reported [14, 16, 17]. Additionally, differences have been noted between species during immunostaining for ER α in the male reproductive tract [1, 18]. Therefore, interspecies differences in the function of estrogen on male reproduction and responses to antiestrogen treatment need to be compared.

A comparative analysis of estrogen in rete testis fluid revealed relatively high concentrations (249 pg ml⁻¹) compared to blood levels in the rat [18]. This high concentration of estrogen in rete testis fluid is consistent with an abundant expression of ER α protein and mRNA in efferent ductules, which is 3.5-fold greater than in uterus [2]. Although the concentration of estradiol and ER are relatively high in the male rat, we are unaware of any studies examining the effects of ER disruption on the histology of efferent ductules in this species. One study looked at the effects of estrogen and tamoxifen on efferent ductule fluid reabsorption [19], but those authors did not account for the potential feedback effects of systemic treatment on the hypothalamus-pituitary-gonadal axis.

The pubertal male rat was treated with the ICI 182,780 (AstraZeneca, Macclesfield, UK), a pure steroidal antiestrogen that inhibits both ER α and ER β [20, 21]. Treatment was given over a long period of time (100–150 days), which was chosen to investigate whether ER disruption in the adult rat would result in infertility, as seen in α ERKO mice [6, 7] but not in mice treated with ICI for only 35 days [13]. Because ICI down-regulates the ER and causes complete abrogation of the ER effects [21–23], the use of this antiestrogen provides a functional inactivation of ER similar to that obtained by the genetic knockout of ER expression [24].

Our results show that ICI treatment promotes adult dysfunctional changes in rat efferent ductules similar to those of α ERKO mice, including the induction of testicular atrophy and infertility. The results demonstrate that a functional ER is essential for efferent ductule physiology in the rat, similar to the mouse. However, in the rat, the efferent ductule epithelium shows greater variation in morphological response than is seen in the α ERKO and ICI-treated mouse models [13].

MATERIALS AND METHODS

Animals

This experiment used 16 outbred, 30-day-old, male Sprague-Dawley rats purchased from Harlan Bioproducts (Indianapolis, IN). The rats were maintained under controlled temperature (22°C) and lighting (12L:12D). They were allowed free access to commercial diet (Teklad Chow; Harlan Teklad, Madison, WI) and tap water. All animal experiments were ap-

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TABLE 1. Effects of ICI 182,780 on body, testis, prostate, and seminal vesicle and coagulating gland weights.^a

Day	Treatment	Weight (g)			
		Body	Testis	Ventral prostate	Seminal vesicles + coagulating gland
100	Control	431.63 ± 36.69	1.88 ± 0.15	0.67 ± 0.18	1.56 ± 0.28
	ICI 182,780	394.50 ± 17.22	2.54 ± 0.52 ^b	0.69 ± 0.18	1.69 ± 0.20
150	Control	448.25 ± 6.73	2.07 ± 0.06	0.88 ± 0.28	1.86 ± 0.06
	ICI 182,780	455.48 ± 32.88	1.71 ± 0.77	1.04 ± 0.34	1.73 ± 0.53

^a Values are mean ± SEM.

^b $P < 0.05$ vs. control.

proved by the Laboratory Animal Care Advisory Committee and conducted in accordance with the *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (<http://www.olar.uiuc.edu>).

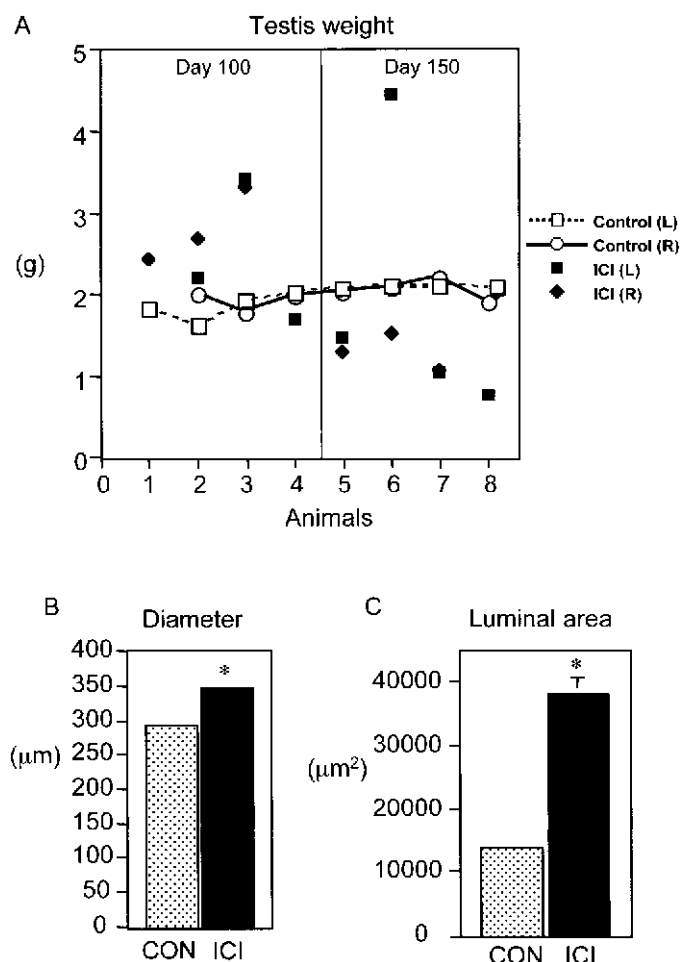


FIG. 1. Effects of long-term ICI 182,780 treatment on rat testes. **A**) This graph represents individual testis weights for each animal from the ICI-treated (◆) and control (○) groups on the two different days of evaluation. One testis each from the ICI and control groups is missing on Day 100. On Day 100, 75% of the ICI-treated testes weighed more than controls, whereas on Day 150, 75% of the ICI-treated weights were substantially less than controls, showing that atrophy occurred after Day 100. L, Left testis; R, right testis. **B**) The seminiferous tubule diameter was increased significantly (*) on Day 100 in the ICI-treated testes ($P < 0.001$). Values represent mean ± SEM (4.58 and 5.69, control and ICI SEM values, respectively; $n = 3$ in each group, from glutaraldehyde-fixed tissues). **C**) The luminal area per seminiferous tubule was also increased significantly (*) on Day 100 after ICI treatment. Values represent mean ± SEM; ($n = 3$ in each group, from glutaraldehyde-fixed tissues).

Treatment

The rats were treated once per week with s.c. injections of ICI 182,780 (AstraZeneca) at a dosage of 10 mg in a volume of 0.2 ml. The control group received the same volume of castor oil (vehicle). The dose of ICI 182,780 was selected based on the results of a previous dose-response study, which showed maximum effects on efferent ductules. One group of matched control and ICI-treated rats (four animals per group) were killed at 100 days after the first injection. However, based on testis weight (the mean was higher in treated rats than in controls) and fertility results (100% fertile), it was decided to extend the experiment to 150 days with the remaining four animals per group.

Breeding

During the treatment period, mating studies were performed to compare the fertility of the ICI-treated and control animals on Days 45, 73, 100, 125, and 150. Males were housed individually with two adult female Sprague-Dawley, proven breeder rats (Harlan Bioproducts) for a period of 15 days. The females were permitted to adapt to the conditions of the vivarium for at least 7 days before starting the experiment. They were kept under the same conditions of light and temperature as the males, and they received food and tap water ad libitum. At the end of the breeding period (15 days), the females were killed by an overdose of sodium pentobarbital (i.p.), and the uterus was examined for pups or implantation sites.

Tissue Preparation

After each treatment period, the rats were anesthetized (i.p. sodium pentobarbital, 0.1 ml per 100 g body weight), weighed, and perfused intracardially with 4% (w/v) glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) or neutral buffer formalin. Following perfusion, the testis, reproductive tract, and accessory glands were removed, immersed in the same fixative, and stored at 4°C. The weights of testes as well as the ventral prostate and combined seminal vesicles and coagulating gland were recorded after fixation. The efferent ductules were dissected away from the testis and epididymis, and the testis was cross-sectioned at the mid-rete testis. The testis and efferent ductules were embedded in glycol methacrylate resin, sectioned at 2.5-μm thickness, and stained with 1% toluidine blue or periodic acid-Schiff (PAS) with hematoxylin counterstaining.

Morphometry

Using NIH image software (developed at the U.S. National Institutes of Health, Springfield, VA; <http://rsb.info.nih.gov/nih-image>), quantitative estimations were performed on histological sections obtained from glutaraldehyde-fixed tissue. Images were taken using a Spot-2 digital camera (Diagnostic Instruments, Sterling Heights, MI) mounted on an Olympus Vanox-T Microscope (Olympus America Inc., Melville, NY) and captured with a Mac-G3 computer (Apple Computer, Cupertino, CA) using Adobe Photoshop 5.5 (Adobe Systems, Inc., San Jose, CA). The following parameters were analyzed: on Day 100 of treatment, seminiferous tubule diameter and luminal area in 10 randomly selected cross-sections of stages VII–VIII (measurements were not performed on Day 150, because most seminiferous tubules were atrophic); luminal diameter of proximal efferent ductules at the widest region in five tubule sections; and height of efferent ductule epithelium from the basement membrane to the microvillus tip, in areas of straight sections, from 25 cells with evident nuclei.

Statistics

Data from the body and reproductive organ weights (except for testis weight) as well as the morphometric study were compared using the t -

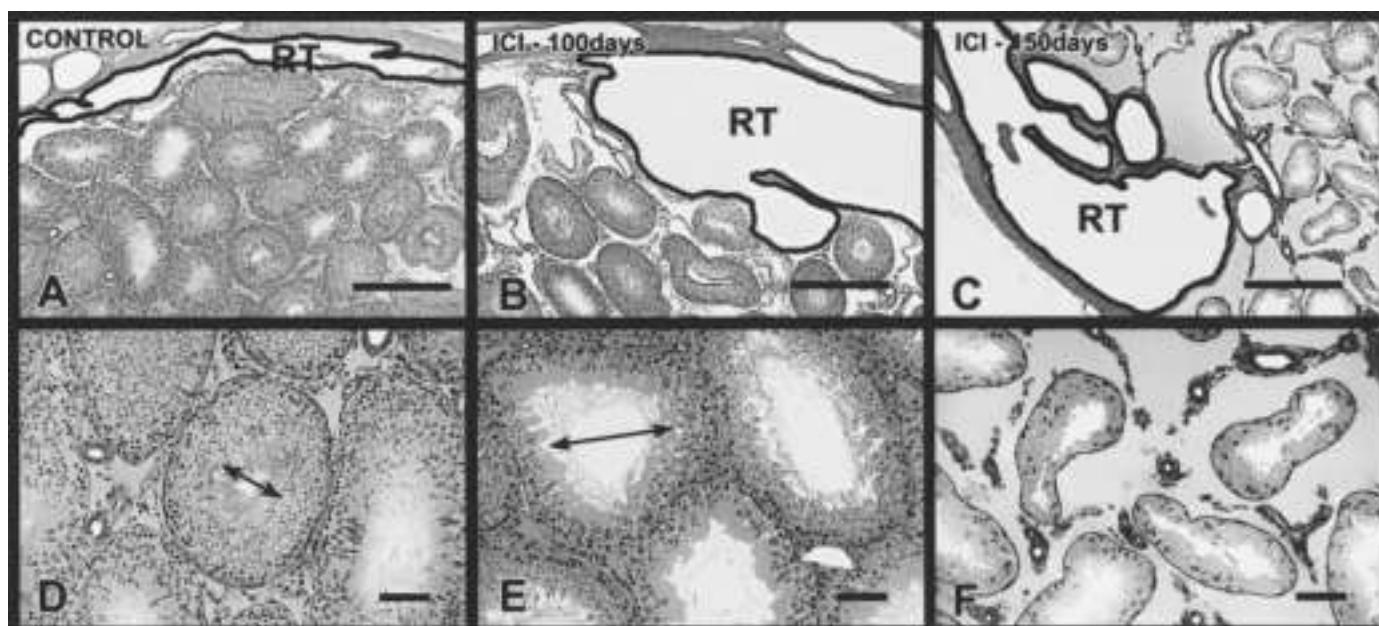


FIG. 2. Morphology of the rat testis after ICI 182,780 treatment. **A)** Control rat testis and rete testis (RT; outlined with black line). The rete is thin, lying along the edge of the seminiferous tubules. Bar = 500 μm . **B)** ICI-treated testis and rete testis on Day 100. The rete testis (RT; outlined with black line) is markedly dilated. Bar = 500 μm . **C)** ICI-treated testis and rete testis on Day 150. The rete testis (RT; outlined with black line) is markedly dilated, and the interstitium between seminiferous tubules appear edematous. Bar = 500 μm . **D)** Control rat testis. The lumens of the seminiferous tubules are thin (double arrow). Bar = 100 μm . **E)** ICI-treated testis on Day 100. The lumens of the seminiferous tubules are dilated (double arrow). Bar = 100 μm . **F)** ICI-treated testis on Day 150. The lumens of the seminiferous tubules are collapsed, and the tubules are atrophic and surrounded by edematous fluid. Bar = 100 μm .

test. Testis weights lacked normality of error variances, and the data were first transformed to the Log (10) for analysis by the Welch alternate *t*-test. Results are presented as means \pm SEM.

RESULTS

The ICI treatment caused a large variation in testis weight compared to that of controls, but no significant difference in the weight of the body and secondary sex glands was found (Table 1). A significant increase in testis weight occurred on Day 100 of ICI treatment ($P < 0.05$). Treated rat testes were 35% heavier than those in controls, and they also showed a significant increase in diameter and luminal areas of seminiferous tubules (Figs. 1 and 2). The luminal dilation was evident in seminiferous tubules independent of the cycle stage of the seminiferous epithelium [25]. Spermatogenesis appeared to be normal, and degeneration or sloughing of germ cells was not evident.

On the other hand, on Day 150, a decrease of 33% was noted in the mean testis weight ($P = 0.065$) of the ICI-treated rats compared to that on Day 100. This decrease in mean testis weight was seen in all animals, but the effect was variable, with unilateral and bilateral changes. Testes weighed as much as 4.46 g and as little as 0.76 g after ICI treatment, which resulted in a mean difference that was not statistically significant compared to that of controls, even when the means were transformed. However, 75% of the testes weighed less than the mean control value (1.71 g). Histologically, after 150 days of ICI treatment, the seminiferous tubules showed atrophy in all testes except for one. This exception was the testis with the greatest weight (4.46 g), which was due to dilation in seminiferous tubules and the rete testis, indicating the accumulation of fluid. The atrophy observed in the remaining testes resulted in reduced diameters of seminiferous tubules, with tubules being dominated by Sertoli cells in most areas (Fig. 2). Occa-

sionally, some tubules exhibited sparse spermatogenic cells, but normal spermatogenesis was not observed in the atrophic testes.

The rete testis (Fig. 2) and efferent ductule lumens in all treated rats were greatly dilated compared to those in controls. In contrast to α ERKO and ICI-treated mice, epithelial cells filled with glycogen were not observed at the rete

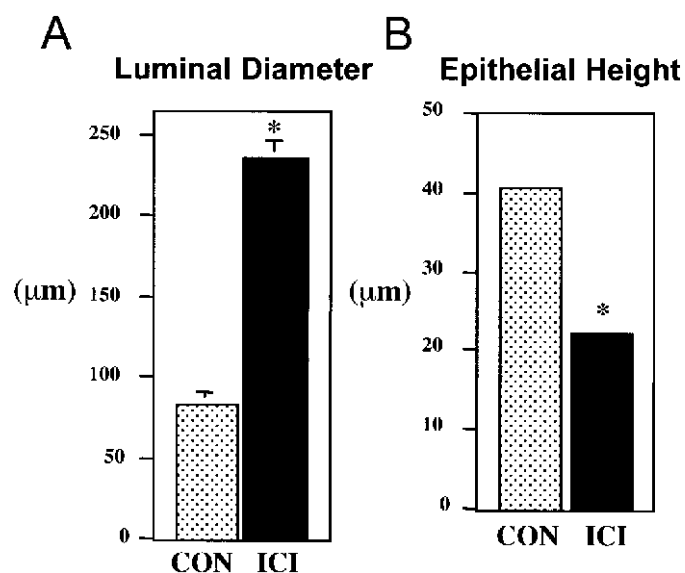


FIG. 3. Effects of ICI 182,780 treatment on the rat proximal efferent ductules (combined period of 100–150 days). **A)** Luminal diameter of the efferent ductules was increased 183% in ICI-treated rats compared to controls. Values represent mean \pm SEM ($n = 5$, from glutaraldehyde-fixed tissues). $*P < 0.001$. **B)** Efferent ductule epithelial height was decreased significantly after treatment with ICI compared to controls. Values represent mean \pm SEM ($n = 5$, from glutaraldehyde-fixed tissues). $*P < 0.001$.

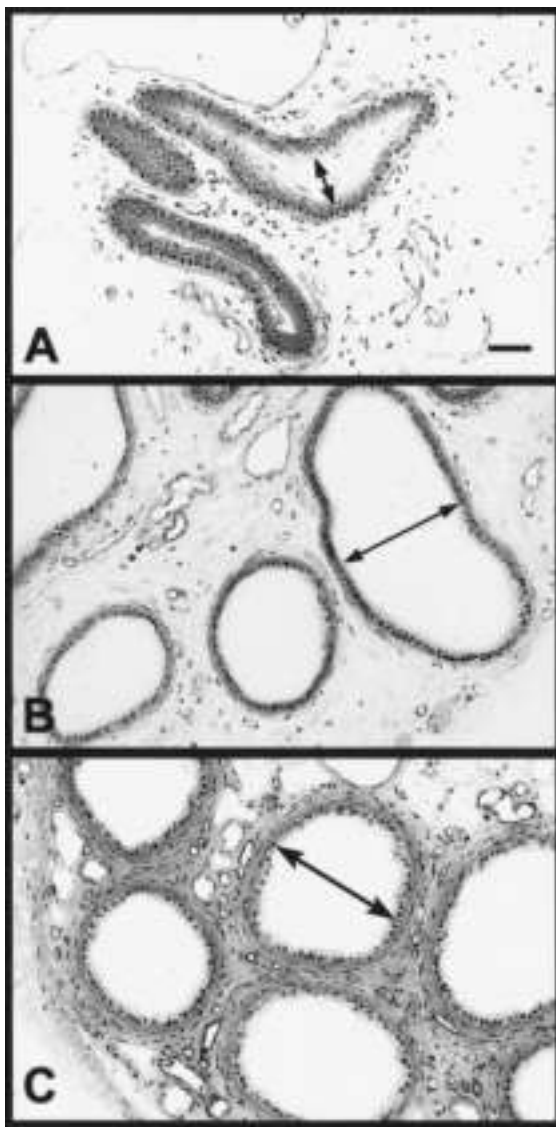


FIG. 4. Morphology of the rat efferent ductules following ICI 182,780 treatment. **A**) Control efferent ductules on Day 100. The lumen is narrow, and the epithelium is tall. Surrounding the ductules is one thin layer of smooth muscle and large spaces of loose connective tissue. **B**) ICI-treated efferent ductules on Day 100. The lumen is dilated (double arrow), and the epithelium is shorter in height, compared to control (**A**). The peritubular smooth muscle surrounding the ductules appears thicker than that in the control. **C**) ICI-treated efferent ductules on Day 150. The lumen is dilated (double arrow), and the epithelium is shorter in height compared to control (**A**). The peritubular smooth muscle surrounding the ductules appears thicker than on Day 100 (**B**). Bar = 50 μm .

testis-efferent ductule junction. The efferent ductule dilation was more pronounced in the proximal region, where an increase of 183% was observed (Figs. 3 and 4). Treatment with ICI also caused effects in the efferent ductule epithelium, the height of which was decreased significantly by 46% (Fig. 3). However, morphology of the epithelial cells was highly variable (Fig. 5), even in the same efferent ductule section. On Day 100, some ICI-treated epithelial cells were columnar, comparable to those in control animals, whereas other cells were cuboidal in appearance. Variations between these extremes of height were observed (compare Fig. 5B to 5E). The same variation was found in the microvillus border and in the presence of endocytotic vesicles, which were apparently normal in some cells but

shorter, fewer in number, or sometimes missing in others. On Day 150, variations in the height of epithelium and microvilli were still present. However, most areas of the epithelium had decreased height, and microvilli were also shorter compared to those in controls (compare Fig. 5F to 5I). It was difficult to make a correlation between epithelium and microvillus height, because some taller cells had reduced microvillus borders and some short cells still retained taller microvilli. At all time periods, lipid droplets (as identified by large histological vacuoles) were absent in the basal cytoplasm of the ICI-treated rats.

The number of PAS-positive, lysosomal-like granules present in the cytoplasm of nonciliated cells of the efferent ductules appeared to be increased on Day 100 of ICI treatment (Fig. 5). Also, the position of these granules changed, from a supranuclear position to a more basal location and surrounding the nucleus (Fig. 5, B and C). In contrast, on Day 150, the presence of PAS-positive granules was decreased compared with either the control group or with the Day 100 ICI-treated rats. In ICI-treated animals, some cells contained large PAS-positive granules, often filling the entire cytoplasm. The granules appeared to be coalesced, forming large globules. These cells were mostly basal in position and had cytoplasmic extensions. Control animals never displayed these types of cells (Fig. 5A).

Abnormalities in the peritubular basement membrane area were evident mainly on Day 150 of ICI treatment. The basement membrane appeared to be thick and irregular compared to control epithelium. Also, the supporting peritubular cell layer of the efferent ductules was thicker, with an increased number of smooth muscle cells (Figs. 4 and 5).

The reproductive breeding performance of the ICI-treated rats was no different from that of the control males through 100 days of treatment. However, after Day 100, a significant decrease was noted in fertility, with only 50% fertility on Day 125 and 25% at the end of treatment on Day 150 (Fig. 6A). The number of pups per male was also significantly decreased on Days 123–150 (Fig. 6B), revealing subfertility in those ICI-treated males that did breed successfully.

DISCUSSION

In this study, long-term treatment with ICI 182,780 in the adult male rat resulted in testicular atrophy and infertility similar to that observed in the αERKO mice [6, 7]. Additionally, morphological characteristics of the efferent ductules were similar to those observed in the αERKO and ICI-treated mice [13], including luminal dilation suggestive of inhibited fluid reabsorption in all models. However, the differences noted between the ICI-treated mice and rats may be due to both species and treatment-time differences.

To our knowledge, this is the first report of an antiestrogen treatment protocol in the male that reproduces the terminal reproductive effects on the testis that were seen in the αERKO mouse [7, 11, 13]. This study demonstrated that chemical blockage of ER function in the rat using the antiestrogen ICI 182,780 resulted in alterations in structure and function of the male reproductive tract, including: 1) a transient increase in testis weight; 2) luminal dilation of seminiferous tubules, rete testis, and efferent ductules; 3) testicular atrophy; and 4) infertility. These changes reinforce the importance of a functional ER in the efferent ductules to ensure male fertility. The ICI-rat model also revealed features that were distinct from both the αERKO and the ICI-mouse models, including: 1) the absence of

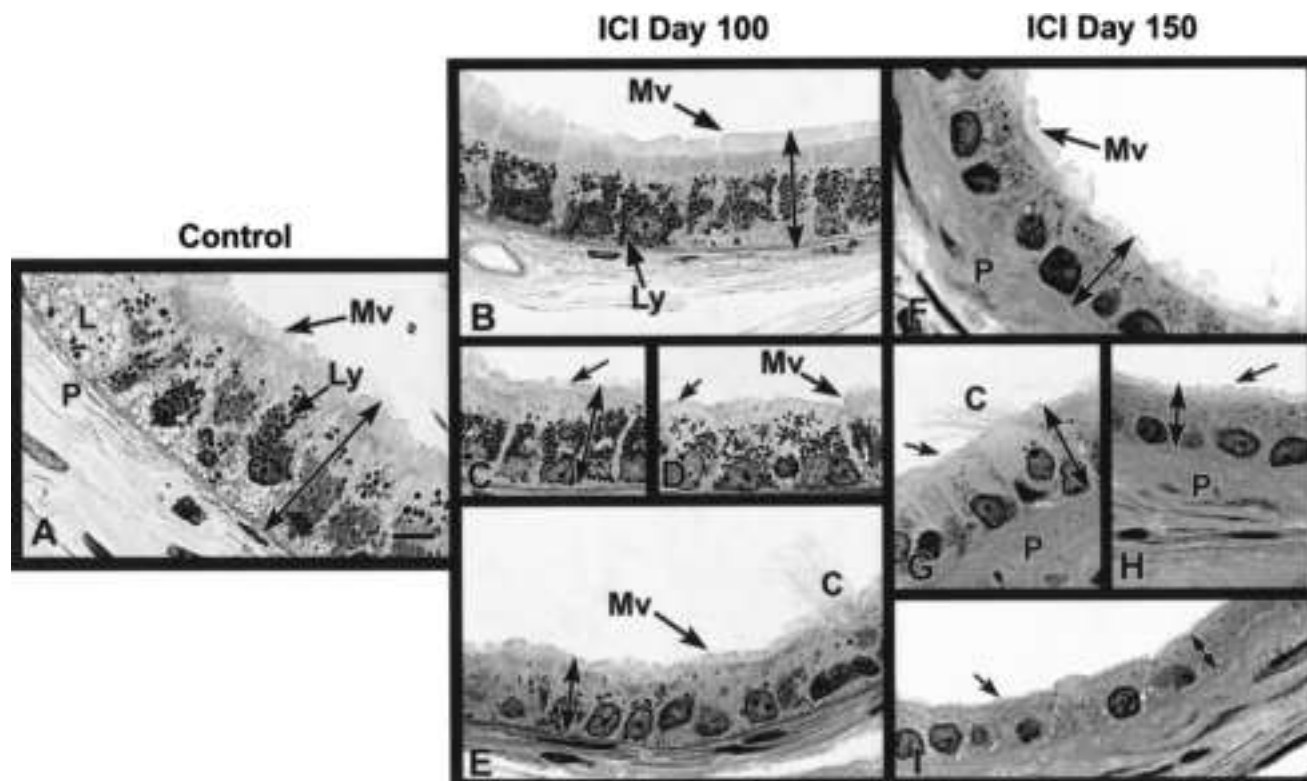


FIG. 5. Morphology of the rat efferent ductules epithelium after ICI 182,780 treatment. Bar = 10 μ m. **A**) Control epithelium on Day 100. Nonciliated cells are columnar in height (double arrow), with tall microvilli (Mv) protruding into the lumen. The PAS-positive, lysosomal-like granules (Ly) are found in the apical cytoplasm, and lipid droplets (L) are seen in the basal cytoplasm. The peritubular region (P) contains one to two layers of thin smooth muscle cells. **B**) ICI-treated rat efferent ductule epithelium on Day 100. Epithelial height is slightly reduced (double arrow) but still columnar and showing a thick microvillus brush border (Mv). The PAS-positive, lysosomal-like granules (Ly) have moved into the basal cytoplasm around the nucleus. **C**) ICI-treated rat efferent ductule epithelium on Day 100. Microvilli are shorter in length, with further reduction in epithelial height (double arrow). **D**) ICI-treated rat efferent ductule epithelium on Day 100. The shortened epithelium contains nonciliated cells with longer microvilli (Mv), whereas other cells have barely detectable microvilli (small arrow). The PAS-positive, lysosomal-like granules are also decreased in number. **E**) ICI-treated rat efferent ductule epithelium on Day 100. The epithelial height is very short (double arrow), but some cells still support a small but clear brush border (Mv). The PAS-positive, lysosomal-like granules are reduced. **C**, Cilia. **F**) ICI-treated rat efferent ductule epithelium on Day 150. The epithelium is shorter (double arrow) than normal but still contains some normal microvilli (Mv). The peritubular area (P) of connective tissue stains dark and is thicker than in the control (**A**). **G**) ICI-treated rat efferent ductule epithelium on Day 150. This epithelium is intermediate in height (double arrow) but has very short microvilli (small arrow). The peritubular area (P) is amorphous and thick. **C**, Cilia. **H**) ICI-treated rat efferent ductule epithelium on Day 150. This represents a very short epithelium (double arrow). However, these cells sometimes showed normal but short microvilli (small arrow). The peritubular area (P) stains dark and is thick. **I**) ICI-treated rat efferent ductule epithelium on Day 150. This is the thinnest (double arrow) epithelium observed, being approximately 11 μ m in height. Nonciliated cells have only a few very short microvilli (small arrow).

cells rich in glycogen at the rete testis-efferent ductule junction; 2) a transient increase in PAS-positive, lysosomal-like granules in the nonciliated cells; and 3) greater variation in efferent ductules epithelium and height of the microvillus border. Thus, species differences in male reproductive tract responses to antiestrogen exposure can be detected.

In the present study, a longer treatment period in the rat resulted in testicular atrophy and infertility, whereas in the 35-day ICI-mouse model, these endpoints were not observed [13]. The most likely reason for this difference is the length of treatment time. In the mouse, 35 days may not have been enough time to fully reproduce the α ERKO phenotype. It was fortunate in the present study that treatment was extended beyond 100 days (the original experimental design), because testicular atrophy was not observed until Day 150. Abnormalities in spermatogenesis were also delayed. Thus, even the extended period of normal spermatogenesis that was found before testicular atrophy in the α ERKO male was replicated in the ICI-treated rat. After 150 days of ICI treatment, the rat testis was decreased in weight, and seminiferous tubular atrophy was observed, which correlated directly with the decline in fertility. An

intermediate period of subfertility was seen in the breeding study at 125 days of treatment. Also, the differences in testes weights observed within individual ICI-treated animals on Day 150 were indicative of a gradual atrophy of the testes. Similar changes were seen in α ERKO mice, in which a transient increase in testis weight at approximately 60–80 days of age preceded the testicular atrophy that occurred during the next 100 days [11]. In both the α ERKO mouse and ICI-rat model, testicular atrophy was a long-term effect, requiring 150–180 days. Over such a long period of time, disturbances in other regions of the male reproductive tract, such as efferent ductule dilation and loss of epithelial height, had already occurred. Such ductule changes were also seen as early as 35 days after treatment with ICI in the mouse [13].

An increase in testis weight followed by atrophy has been reported after exposure to several toxicants that affect the efferent ductules [26–28] as well as after the ligation of efferent ductules [29]. At first, it was thought that the α ERKO male was infertile due to abnormal spermatogenesis, and that the epididymis was basically normal [7]. However, a recent study showed that when α ERKO germ

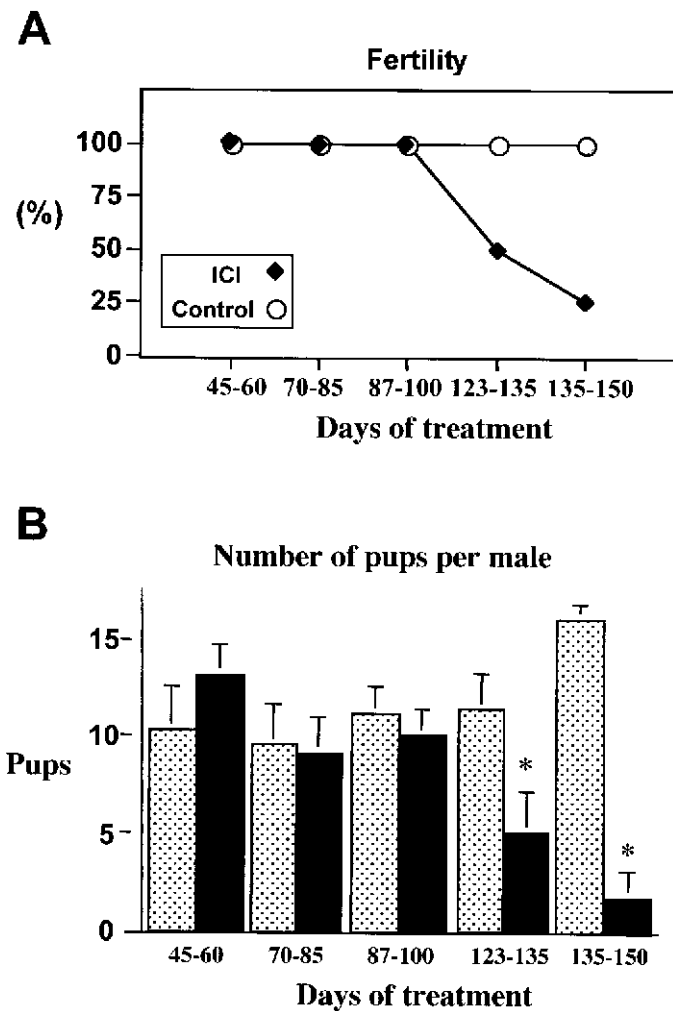


FIG. 6. Effects of ICI 182,780 treatment on reproduction in the male rat. **A**) In the control group (○), the males showed 100% fertility at all time points. A decrease in fertility was seen in the ICI-treated group (◆) after Day 100, resulting in only 50% and 25% fertility on Days 123–135 and 135–150, respectively. **B**) The number of pups per male was also decreased after 100 days of ICI treatment, revealing subfertility as well as infertility. Values represent mean \pm SEM ($n = 4$ per group). * $P < 0.05$.

cells were transplanted into a wild-type testis after depleting the resident germ cells, the α ERKO sperm were capable of fertilization [30]. These observations in α ERKO and ICI-treated rats suggest that the effects on spermatogenesis, and the resulting infertility, are not caused by direct effects on the testis but, rather, by direct effects on the efferent ductule or epididymis. However, because the Leydig cells are ER α -positive [1], the possibility remains that some of the adverse testicular effects are due to changes in Leydig cell function.

Dilation of the proximal efferent ductule lumen in the ICI-rat model (183% increase) was greater than that in the α ERKO mouse (130%) [12], a response that was also found in ICI-treated mice compared to α ERKO mice of the same age [13]. This difference has been attributed to developmental overgrowth of the α ERKO rete testis, which could accommodate more fluid and reduce pressure on the efferent ductules [13]. Alternatively, it may be due to less fluid secretion by the α ERKO testis [11]. The effect of ICI treatment on testicular secretions has not been determined as yet.

Although the efferent ductule epithelium showed an

overall decrease of 46% in height, the ICI-treated rat displayed considerable variation in height in both the epithelium and microvillus border. A comparison of efferent ductules from 100 and 150 days in ICI-treated rats showed important differences in epithelial disruption between the two time periods. The epithelial variation was more pronounced on Day 100, when a large number of cells were still normal in appearance. Although a precise sequence of events leading to epithelial disruption cannot be described from static photos, the data suggest that decreases in height of the epithelium and microvillus border, as well as decreases in lipid droplets, endocytotic vesicles, and the number of PAS-positive granules, may be the terminal pathophysiological events associated with blockage of ER in efferent ductules of the rat.

Large lipid droplets have been associated with the non-ciliated cells in the rat efferent ductules [31–33], and they disappear following ductectomy or ligation [32–34]. Although the significance of lipids in these cells is not understood, several hypotheses have been proposed, including breakdown products of material taken up by the nonciliated cells [33]. The disappearance of the lipid in the data presented here indicates that estrogen is involved in the cellular process that is responsible for the formation of lipid droplets.

The transient increase and redistribution of the PAS-positive, lysosomal-like granules in the nonciliated cells of efferent ductules after ICI treatment was unexpected. In α ERKO and mice treated with ICI for 35 days, these organelles are decreased to nearly undetectable levels [11, 13]. These granules have been recognized as lysosomes and appear to be more numerous in rats than in mice [31]. Thus, this intriguing interspecies difference in lysosomal response to the antiestrogen should be investigated during future studies.

The male β ERKO mice have no reproductive pathological phenotype, and these mice are fertile [8, 9]. However, similar to α ERKO mice, the $\alpha\beta$ ERKO mice are infertile [9, 10]. Even though the $\alpha\beta$ ERKO male has a grossly normal reproductive tract [10], these mice exhibited dilation of the rete testis, loss of germ cells in the seminiferous tubules [9], and reduced epididymal sperm counts [10]. Considered together, these results suggest that ER α is the predominant receptor isoform regulating the physiology of male reproduction. Whether ER β has a specific function in the male reproductive tract remains to be determined. The differences observed between rats and mice following ICI treatment raise the possibility of differences in the expression of ER isoforms in the two species. Such a difference could be important in the study of antiestrogens such as ICI because of its effects on both receptors [23]. It has been proposed that in some cells, ER β may be a modulator of ER α action [35].

To elucidate the role of estrogen in the male, the aromatase gene knockout (ARKO) mouse has been proposed as an alternative model [36–38]. Lacking the enzyme that converts androgens to estrogens, these mice also experienced a progressive infertility, but impairment of the efferent ductule structure and function was not shown [38, 39]. Even though seminiferous tubule dilation or atrophy have not been observed in ARKO males, a significant decrease in seminiferous tubule thickness at stage VII has been described [38], suggesting that some rete testis fluid might have been accumulating. On the other hand, rats treated with an aromatase inhibitor partially resembled the α ERKO mouse and the ICI-treated rat by showing an increase in

testis weight. However, the efferent ductules did not show a consistent response, suggesting that the aromatase inhibitor may not have completely inhibited the enzyme in testicular cells [40]. In any case, ERs are present in ARKO mice and aromatase inhibitor-treated rats, so the possibility of nonligand ER transactivation cannot be ignored [41, 42]. With these considerations, several benefits to using the ICI rather than the inhibiting-aromatase model become apparent, including: 1) as a steroid [21], ICI can probably reach the cells in the testis and efferent ductules more readily than a nonsteroidal compound; 2) ICI causes down-regulation of the ER, avoiding complications induced by other hormones and other factors [21–23]; 3) ICI does not cross the blood-brain barrier [43], so it does not interfere with mating behavior; and 4) in addition to its antiestrogenic action, ICI has aromatase-inhibiting effects as well [44]. Overall, the ICI 182,780 model, in both the mouse and rat, has numerous advantages for the study of estrogen effects on male reproduction.

In conclusion, as previously shown in mice and now confirmed in the rat, efferent ductule dysfunction is induced by the disruption of ER function, which leads to infertility through mechanisms related to the inhibition of fluid reabsorption. These data add support to the general hypothesis that estrogen and a functional ER α may be required for normal fertility in males of all mammalian species.

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