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The Influence of Epididymal Agenesis on the Development and Maturation of the Testis: Experimental Model and Clinical Correlations

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Summary. The August X Copenhagen (ACI) rat provides an excellent model to study the effect of congenital epididymal anomalies on testicular function. Despite epididymal absense, testicular maturation and function are unchanged until puberty. At puberty, with increased testicular fluid and spermatozoa output, involution of germinal epithelium occurs. This involution is similar to the changes seen with ligation of the efferent ductules of the epididymis. These findings suggest that in this mammalian model the testis functions normally until the pubertal period. At that time, because of proximal epididymal agenesis and failure to reabsorb testicular secretions, intratesticular hydrostatic pressure increases with rapid germinal epithelial atrophy. Clinical correlations are made in patients with epididymal abnormalities, especially in association with undescended testis.

Key words: Epididymal agenesis, Testicular atrophy, ACI rat.

Introduction

Clinically, epididymal abnormalities have been reported to be more prevalent than previously recognized [12, 18]. The epididymis was once considered primarily a conduit and reservoir for spermatozoa but is now thought to be a highly complex organ with many functions. Classically, these functions are thought to be transport, concentration, and storage of spermatozoa [21, 23, 24]. Integral to these functions are the highly specialized absorptive and secretory functions of the epididymis as demonstrated experimentally by micropuncture techniques [6, 7, 20]. In addition, proximal epididymal patency is important in the maintenance of testicular function. Ligation of the ductuli efferentes or proximal caput epididymidis results in dramatic testicular atrophy with the involution of germinal epithelium [8, 9]. The August X Copenhagen (ACI) rat provides a mammalian model of spontaneous congenital unilateral epididymal anomalies [11]. An early embryological mesonephric ductal arrest is responsible for agenesis of the epididymis. The ductuli efferentes and a minimal portion of the caput epididymidis may remain as they are derivatives of the mesonephric tubules. The complete defect occurs in 10% to 12% of adult ACI rats and is accompanied by the absence of all mesonephric ductal derivatives including the ureter (and kidney), ductus deferens and seminal vesicle [2, 10]. In addition, 1% to 5% of animals demonstrate a partial defect manifested by a dysplastic or hydronephrotic kidney and occasionally an atrophic undescended testis. The testes are descended in rats with the complete defect.

In the adult ACI rat significant testicular atrophy occurs on the affected side. In vitro testicular perfusion studies have also demonstrated diminished ability of the testis to produce testosterone under maximum LH stimulation [13]. The etiology of the testicular atrophy remains uncertain. This study was undertaken to determine the precise time of testicular damage which might also implicate an etiology.

Materials and Methods

The rats employed in this study represented the 103rd generation of ACI rats derived from a pedigreed litter of AXC/Norway rats. The rats were maintained on a 12 h light and 12 h dark schedule and given food and water ad libitum. The ages of the animals ranged from 17 to over 300 days. Thirty-two rats were examined in the prepubertal period (0–40 days), 96 rats in the pubertal period (41–80 days), and 18 rats in adulthood (80–300 days plus). The largest number or rats was examined in the pubertal period because this period is the time of initiation of spermatogenesis and testicular function.

Tissues. Total body, testicular, and renal weights were determined for all rats. The testes of 70 rats were bivalved and half the tissue was preserved in formalin while the other half was immediately processed for spermatid counts.

Determination of Testicular Spermatid Counts. Tissue content of spermatids was determined using a modification of the methods of

Table 1. Table of incidence of complete defects seen in the 146 rats studied

	Right side		Left side	
	No.	%	No.	%
Normal	129	88.5	138	94.5
Partial defect	3	2	5	3.5
Complete defect	14	9.5	3	2
Totals	146	100	146	100

Robb et al. [16]. The weighed testicular specimen was immediately homogenized for one minute with a Potter-Elvehjem teflon-glass homogenizer at 2,500 RPM in 10 ml of a solution containing 0.9% NaCl, 0.1% Thymersol® and 0.5% Triton-X-100. Spermatid heads were then counted in a hemocytometer chamber after appropriate dilution of the homogenates.

Morphometric Studies

Hardware. Morphometric analysis was carried out using a Zeiss microscope equipped with a drawing tube and a Hewlett Packard HP9874 digitizer interfaced to a Hewlett Packard 9825 computer. The digitizer was provided with a cursor on which a light-emitting diode (LED) was mounted. This diode was visible as a bright green light in the microscopic image. The resolution of the Hewlett Packard HP7245A plotter printer was 0.001 inch.

Software. The digitizer was used for digitizing contours of seminiferous tubules under optical manual control. A computer program was developed in this study for calculating the seminiferous tubular cross sectional area, the seminiferous tubular lumenal cross sectional area, the germinal epithelial area, and the tubular roundness factor. The program was linked to a statistical subroutine for the calculation of mean, standard deviation and histograms of the frequency distribution of a series of measured values.

Morphometric Methods. The formalin-fixed testicular tissue was stained with hematoxylin and eosin (H&E). Twenty-six specimens were analyzed. Affected rats and randomly selected normal cohorts were digitized. Random cross sections at 8 μ were used. The seminiferous tubular perimeter and lumen were circumscribed using the digitizing cursor and the results were stored and analyzed. To obtain measurements at the 95% confidence level with a standard error of the mean of less than 4%, 25 tubules had to be digitized. The digitized tubules were selected randomly by the observer and were thought to be orthogonal sections. The tubular roundness factor that was computed for each series of digitizations gave an estimate of the standardization of the sampling. The tubular roundness factor was not found to be statistically different between cross sections.

Statistical Methods. Statistical analysis of linear regressions was performed using the T test as described by Rao [15].

Results

One hundred and forty-six consecutive rats were examined (Table 1). A total of 25 rats was found to be affected (17%). The right side was affected in 17 of the animals and

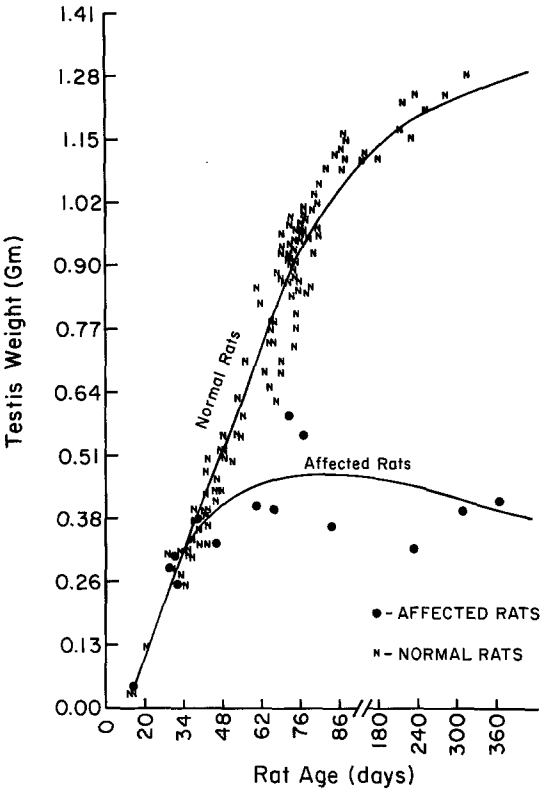

Fig. 1. Plot of testis weight vs. rat age. Affected testes demonstrated an exponential decrease in their relative growth rates after day 50

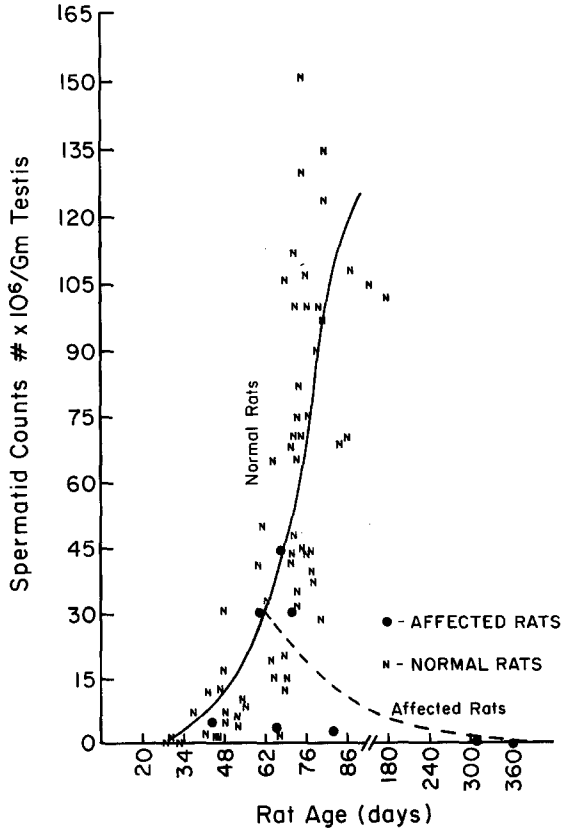

Fig. 2. Plot of spermatid count vs. rat age. Spermatogenesis is initiated but abruptly decreases when the animals are more than 50 days of age (weight 100–125 g)

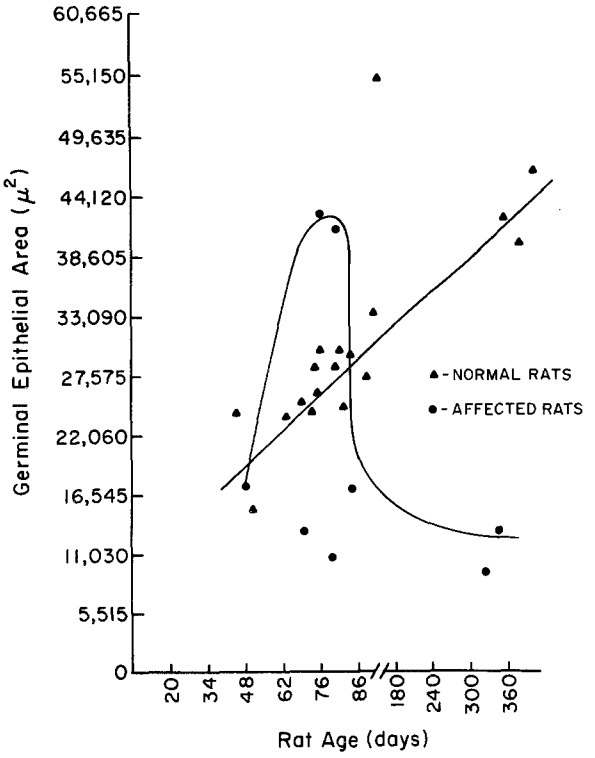

Fig. 3. Plot of germinal epithelial area vs. rat age. As the animals age there is an abrupt decrease in germinal epithelial area in affected rats

the left side in 8. The anomaly was complete in 17 (12%). The partial mesonephric ductal defect was demonstrated in 8 rats (5%). The partially affected rats demonstrated undescended testes and ectopic ureters with dysplastic or hydronephrotic kidneys. The rats with complete renal and epididymal agenesis all had descended testes. These findings are consistent with the previously reported series of ACI rats [1, 2, 4, 10]. The rats with a partial defect of the mesonephric duct were not investigated. Only animals with right-sided agenesis were plotted because of the small number of animals with a left-sided defect.

Gravimetric Study. Subsequent to weaning the increase in body weight was linear from day 20 to day 80; the animals gained 19 g per week. After this rapid growth phase there was a mean weight gain of only 2.7 g/wk. There was no statistically significant difference between the rate of growth of the affected vs. the non-affected rats.

The right and left renal growth rates in unaffected rats demonstrated no statistically significant difference. There was marked compensatory renal hypertrophy in affected rats with renal growth rates nearly double that of normal control kidneys. The weight of hypertrophied kidneys in the affected animals was approximately twice that of normal control kidneys. This difference was manifested as early as 23 days (approx. rat wgt. 25 g).

Left and right testicular growth rates in normal rats were not statistically different. The testicular growth rate of affected rats was no different from that of control rats until approximately 50 days of age (approx. body weight 95 g) when a sharp, and significant decrease occurred (Fig. 1). No affected testis grew after day 70 (approx. body weight 135 g).

Spermatid Counts. Spermatid heads were not found in animals younger than 40 days (approx. weight 60–70 g). After 40 days there was an exponential increase in the spermatid counts. Corresponding with the decrease in testicular weight in affected animals, there was a decrease in spermatid counts to virtually undetectable levels in the adult affected rats (Fig. 2). The initiation and cessation of spermatogenesis occurred very rapidly at the time of puberty in affected animals.

Morphometric Studies. Morphometric analysis was performed on 26 rats (8 affected, 18 normal). There appeared to be an increase in the germinal epithelial area at the time of puberty but again there was an abrupt decrease in germinal epithelial area in the older animals as in the gravimetric and spermatid count studies (Fig. 3). At puberty the microscopic appearance of a normal rat testis was virtually identical to an affected rat testis (Figs. 4a and 4b). Over one month later, profound changes were seen in the affected testis with loss of germinal cell epithelium associated with striking testicular atrophy (Figs. 4c and 4d).

Figure 5 summarizes the changes that occurred in affected rat testes between puberty and early adulthood. During that brief period of time there was a marked reduction in the weight of the testis, spermatid counts and germinal epithelial area.

Discussion

The proximal epididymis is vital for normal testicular function [8, 19]. The ACI rat is an ideal model to study the effect of epididymal absence on testicular function and maturation.

Other experimental studies have demonstrated previously the effects of ligation of the ductuli efferentes and proximal caput epididymidis on testicular function [8]. Histological examination of the tubules revealed transient dilation of the seminiferous tubules at 48 h followed by atrophy of the germinal epithelium at 5 days. As early as 14 days post ligation, some seminiferous tubules contained only Sertoli cells. No compromise was seen in Leydig cell function as measured by the ability of the testes to maintain sex accessory tissue in bilaterally treated animals.

Scheer and Robaire [17] have characterized the developmental, functional, and hormonal changes that occur in the normal rat epididymis and testis. Serum levels of androgens do not begin to increase until age 35 days. Pubertal sex accessory tissues demonstrated a dramatic increase in their growth rate until approximately day 65. This growth is accompanied by the concomitant appearance of spermatids in the testis at day 49. There is a progressive increase in

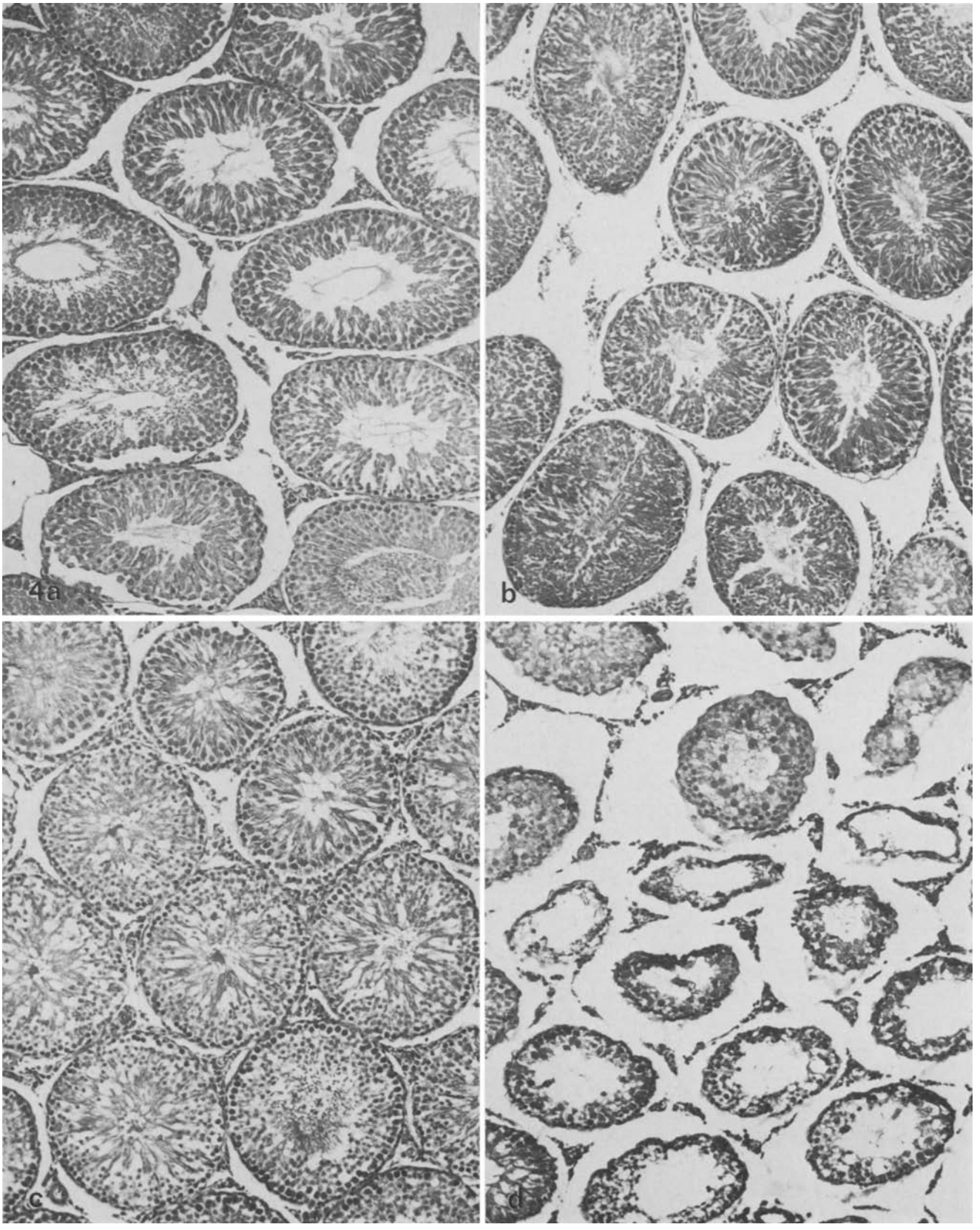

Fig. 4a–d. Photomicrograph (125 x) of a normal rat testis at puberty (52 days) (**a**) and of a pubertal affected rat testis (52 days) (**b**). There is no difference microscopically or in morphometric analysis in these testes. Photomicrograph (125 x) of young adult normal rat (**c**) and affected testis (**d**). Pronounced atrophic changes are already present in the affected testis. Hematoxylin and Eosin

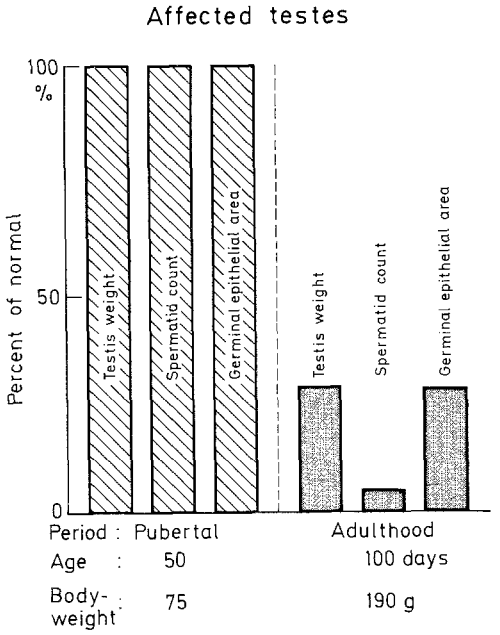

Fig. 5. Relative changes in testicular weights, testicular spermatid counts, and germinal epithelium between affected and control rats. Expressed as percent of normal controls

spermatid counts distally in the epididymis until day 91 at which time a plateau is reached.

Similar initial development of spermatogenesis was seen in affected ACI rats. Spermatogenesis was clearly initiated in affected animals which indicates that testicular atrophy was not a primary testicular process but rather a secondary manifestation of epididymal absence. During a brief period at puberty there was marked diminution in testicular weight, spermatid count, and germinal epithelial area (Fig. 5). The rapidity of atrophic testicular changes is consistent with the atrophic changes described by Smith [19] and Neaves [14] in the ligation of the efferent ductules in normal rats. The abrupt decrease in testicular growth corresponds to the period during which there is appearance of spermatozoa in the testes associated with an increased flow of the rete testis fluid towards the proximal epididymis [17, 21]. Normally this fluid would undergo absorption in the ductuli efferentes and proximal epididymis [21]. This absorptive capability does not exist in the affected ACI rat because the majority of these structures never form. Because of the development of an effective blood testis barrier at puberty [3], this lack of absorption may contribute to the increased seminiferous tubular lumenal pressure with subsequent rapid damage to the germinal cell epithelium. The Sertoli cells and Leydig cells are more resistant to this injury as they are to most noxious insults.

The few affected ACI testes that did not appear to undergo rapid atrophy may represent either physiological variability in the onset of pubertal changes or variability in the completeness of epididymal agenesis.

Clinical Correlates. It has been recognized that congenital anomalies may occur in as many as 36% of patients with undescended testis [12]. It has been noted that the infertility rates are greater in patients with unilateral or bilaterally undescended testes [9]. Houissa [5] has described atrophic testicular changes in patients undergoing orchidopexy. As many as 50% of the undescended testes demonstrated atrophic changes of increased peritubular fibrosis and germinal epithelial atrophy. These studies suggest that a subset of patients with undescended testis may have proximal congenital epididymal abnormalities with potential testicular changes. Although congenital epididymal abnormalities may be far more common than previously realized, the epididymis must be carefully inspected at the time of orchidopexy to recognize them. The effects of this congenital anomaly may not be obvious in infancy or childhood, and atrophy of the testis may not become obvious clinically until the time of puberty, many years following an orchidopexy. The present ACI rat model clearly demonstrates the potential for severe testicular atrophy after puberty in a testis with a proximal epididymal abnormality. With the advent of improved microsurgical techniques and with increased understanding of spermatozoal maturation, some patients may benefit in the future from early epididymal bypass surgery before puberty in order to prevent subsequent testicular dysfunction.

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