Journal of Andrology, Vol. 22, No. 2, March/April 2001

Epididymal Growth and Differentiation Are Altered in Human Cryptorchidism

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ABSTRACT: Despite the knowledge and histological classification of testicular lesions, epididymal lesions associated with cryptorchidism are not well defined and only macroscopic alterations have been reported. We have evaluated the alterations in the growth of both the epithelium and muscular wall of efferent ducts and epididymis in human patients with cryptorchidism from infancy to adulthood. In addition, by cytokeratin immunostaining we have also evaluated the stage of differentiation of each segment along the human postnatal life in these patients. A decrease is shown in the size of efferent and epididymal ducts in cryptorchid children compared with normal, age-matched controls. The height of the epithelium, muscular wall, and lumen of the cryptorchid epididymis were reduced at every age studied. This decrease in all regions was seen even in the testicular quiescent period (1 to 4 years of age). In addition, the cryptorchid epididymis grows more slowly during the transition to the pubertal period. The smaller size of the cryptorchid epididymis in pubertal and adult men compared with that of normal men is due primarily to underdevelopment of the muscular wall and a reduction in epithelial height. The pattern of growth of cryptorchid efferent ducts and ductus epididymides parallels that in normal men, except that development of the lumen and muscular layer in the cauda epididymis region are delayed. Epithelial differentiation, monitored by cytokeratin expression, is minimal in efferent ducts and throughout the epididymis of the cryptorchid male, and this is already seen in children. In conclusion, our immunohistochemical and morphometric results show a reduced development of the human cryptorchid epididymis that is already evident in childhood. They indicate that cryptorchidism is a primary congenital illness of the testis and spermatic ducts, with evident lesions from the first years of life, and suggest that surgical descent would probably not be able to completely reverse these alterations.

Key words: Efferent ducts, cytokeratins, immunohistochemistry, morphometry.

J Androl 2001;22:212–225

Cryptorchidism is an anomaly in which normal descent of the human testis into the scrotum is inhibited, leaving the undescended testis in the abdominal cavity, inguinal canal, or in the neck of the scrotum. The histopathology of the human cryptorchid testis is well established. In the infant cryptorchid testis, three types of tubules have been distinguished: 1) seminiferous tubules with minimal lesions; 2) moderately hypoplasic seminiferous tubules; and 3) immature seminiferous tubules, exclusively constituted by dysgenetic Sertoli cells. After puberty these alterations can give rise to 1) Sertoli cell only (SCO) pattern, 2) arrested spermatogonial maturation, 3) tubule-interstitial sclerosis, or a combination of these (for a review see Nistal and Paniagua, 1996).

By contrast with the testis, the epididymal lesions associated with cryptorchidism are not well defined, and only macroscopic alterations have been reported (Koff and Scaletscky, 1990; Elder, 1992; D'Agostino et al, 1994). Epididymal abnormalities are classified anatomically according to Scorer and Farrington (1971), with a few modifications, such as: 1) elongated loop-like epididymis (from 2 to more than 4 times the length of the testis), 2) separation of the testis from the epididymis (tail only, head and tail but the epididymis near the testis, and large distance between the epididymis and testis), 3) angulation (pure or associated to narrowing), 4) atresia or loss of continuity between regions of the epididymis or vas deferens, and 5) long mesorchium. The most frequent epididymal abnormalities are elongated loop-like epididymis and separation of the testis and epididymis. In contrast, angulation and atresia of the epididymis are rare and long mesorchium is exceptional in human cryptorchidism (Koff and Scaletscky, 1990).

Although developmental alterations of the human cryptorchid epididymis have not been studied in depth, inconclusive data indicate that infants usually do not show important epididymal anomalies, and that in chil-

Supported by grant 99/0459 from FISS Spain.

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Received for publication February 28, 2000; accepted for publication September 11, 2000.

dren 7–10 years of age with testicular atrophy the epididymis can display a remarkable immaturity (Zondek and Zondek, 1980).

Cytoskeletal intermediate filaments, among them keratin filaments, the intermediate filament proteins that are characteristic of epithelial cells, are known to be mechanical integrators of the cytoplasm, play a central role in structural integrity (Fuchs and Cleveland, 1998). Their pattern of expression is tissue specific and is highly controlled during embryonic development (Cadrin and Martinolli, 1995). Epithelial cells express between 2 and 10 different keratin types, thus reflecting the epithelial type and their differentiation status. Numerous pathologies are known to involve alterations in keratin expression. For instance, in urothelial transitional cell carcinoma, keratins 8 and 18 are overexpressed (Southgate et al, 1999). We and others have previously defined cytokeratin immunoexpression in healthy human epididymis (Achtstatter et al, 1985; Kasper and Stosiek, 1989; Palacios et al, 1993; Martínez-García et al, 1995) and also in epididymal pathologies associated with aging and spermatic tract obstruction (Regadera et al, 1998).

In the present study only epididymides from the first classification group (elongated epididymis) were used because they are the most frequent, because the different portions of the epididymis can be easily separated, and because these patients have the best prognosis for surgical testicular descent. We have identified alterations in the growth of both the epithelium and muscular wall of efferent ducts and epididymal regions in human cryptorchid patients from infancy to adulthood. In addition, by differential cytokeratin immunostaining we have also evaluated the stage of epithelial differentiation of each segment along the human postnatal life in these patients. Finally, the observed alterations are systematized and the significance of surgically induced testicular descent on the evolution of epididymal hypoplasia is discussed.

Materials and Methods

Materials

Right testes and their epididymides from 42 children and men were used in this study. In 21 of these cases testes were located in the abdominal cavity or inguinal canal, and were obtained at the Departments of Pediatric Surgery and Pathology of La Paz Hospital of Madrid. The other 21 cases of normal scrotal testes and epididymides, used as controls, were obtained at autopsy (6–15 hours after death) in the Department of Pathology of La Paz Hospital, and presented no testicular or related pathology. Both the normal and cryptorchid specimens were classified into 3 age groups: 1) 1–4 years of age, 2) 5–14 years of age, and 3) 15–60 years of age. Each normal or cryptorchid group consisted of 7 individuals. Tissues were fixed in 10% buffered formaldehyde for 48 hours, embedded in paraffin, and cut serially into 6-μm-thick sections.

Immunohistochemistry

The antibodies and dilutions used in this study were 1) mouse monoclonal antibody against broad-range human cytokeratins (Pancytokeratin CK22; Biomeda, Foster City, Calif) recognizing keratins 1–19, prediluted; 2) mouse monoclonal antibody against human low-molecular-weight keratins (AE1; Biomeda), recognizing keratins 10, 14, 15, 16, and 19 at 1:200 dilution; 3) mouse monoclonal antibody against human keratin 18 (K18; Biomeda) prediluted; and 4) mouse monoclonal antibody against human keratin 19 (4.62; Bio-Makor, Rehovast, Israel) at 1:40 dilution. Paraffin sections were processed by the phosphatase-anti-phosphatase method. Briefly, sections were deparaffinized, hydrated, and washed in immunoassay buffer (Biomeda). Sections were blocked in goat serum (Zymed, San Francisco, Calif) and then incubated with the primary antibodies at the indicated dilutions for 1.5 hours at room temperature. The sections were then washed and incubated in goat anti-mouse immunoglobulins (Biocell, Cardiff, United Kingdom), washed and incubated with the phosphatase-anti-phosphatase (Dako, Glostrup, Denmark), developed with Fast Red (Sigma, St. Louis, Mo), weakly counterstained in Harris hematoxylin, and mounted in CrystalMount. (Biomeda).

Negative controls performed at the same time omitted the first antibody. For positive controls, human skin containing sweat glands and human mammary gland tissues were used. Care was always taken to incubate all sections with the developing Fast Red solution for exactly the same time.

The relative immunostaining intensity in the epithelial cell cytoplasm was performed using an image analyzer (Videoplan-Kontron, Oberkochen, Germany). Before any measurements were made in the epididymal sections calibration of the optical density of the immunohistochemical procedure was performed. For calibration, the above-described positive and negative control sections were used and 100 cells were measured in each case for each antibody. The average in optical density per unit surface area in the positive and negative sections were given the values 100% and 0% of relative intensity, respectively. For the epididymal measurements, a representative section of each specimen analyzed and stained by each antibody was measured. In each section the optical density per unit surface area of 100 cells was measured and then adjusted to percentage with respect to the calibration.

Morphometry

Morphometric studies were performed on a representative transverse section of each region of the epididymis (caput, corpus, and cauda) and efferent ducts of each patient. In each section, 15 cross-sectioned ducts were measured. In 2 of the cryptorchid cases, epididymal atrophy was so extensive that only 6 to 9 ducts could be used for measurements. In each duct the following parameters were measured with an image analyzer (Videoplan-Kontron): 1) total tubular surface area (area occupied by epithelium plus muscular layer plus lumen), 2) luminal surface (area occupied by the lumen), 3) muscular layer surface (area occupied by the muscular layer), 4) total tubular diameter (diameter of the

*Immunohistochemical detection of cytokeratins in the human epididymis**

		Children								Adults							
		Normal				Cryptorchid				Normal				Cryptorchid			
		CK22	AE1	18	19	CK22	AE1	18	19	CK22	AE1	18	19	CK22	AE1	18	19
Efferent	PC	+	+	+	+/−	+/−	+/−	−	−	++	++	+	+/−	+	+	−	+/−
Caput	PC	++	+	+	+	+/−	+/−	−	−	+	+	+/−	+/−	+/−	+/−	+/−	+/−
	BC	++	++	+	++	+/−	+/−	−	−	++	++	+	+	+/−	+/−	−	+/−
Corpus	PC	+	++	+	+	+/−	+/−	−	−	+	++	+	++	+	+	+/−	+/−
	BC	++	++	++	++	+/−	+/−	−	−	++	++	+	++	+	+/−	−	+/−
Cauda	PC	++	++	+	++	+/−	+/−	−	−	++	++	++	++	+	+	+/−	+/−
	BC	+	++	+	++	+/−	+/−	−	−	++	++	++	++	+	+	+/−	+/−

*CK22 indicates anti-broad-range-keratin antibody; AE1, anti-low-molecular-weight keratins antibody; 18, anti-keratin 18 antibody; 19, anti-keratin 19 antibody; ++, optical density per unit surface 51% to 100%; +, optical density per unit surface 11% to 50%; +/−, optical density per unit surface 2% to 10%; −, optical density per unit surface less than 2% (similar to background); PC, principal cells; BC, basal cells. The average in optical density per unit surface area in 100 cells of the positive and negative sections were given the values 100% and 0% of relative intensity, respectively. For the epididymal measurements, the optical density per unit surface area of 100 cells was measured and then adjusted to percentage with respect to the calibration.

duct, including epithelium plus muscular layer plus lumen), 5) luminal diameter, and 6) height of the epithelium. Calibration was performed with a graduated slide prior to epididymal measurement using the same magnification. Data are expressed in μm^2 for the areas and in μm for the diameters, as mean ± SEM. Intergroup comparisons (paired comparisons between 1 age group and the preceding age group, and those between the normal and cryptorchid measurements at the same age) for each parameter at each ductal segment were assessed by the Tukey test (5% error, Hahn and Hendrickson, 1971).

Results

Keratin Immunohistochemistry

A summary of the differential keratin immunoreactiveness in the distinct regions of the epididymis for the different ages and conditions studied is shown in the table.

Children—Efferent ducts of normal children are positively stained with pancytokeratin CK22 antibody, which recognizes keratins 1–19; the same result is obtained with the AE1 antibody, which recognizes low-molecular-weight keratins (Figure 1A). The positive reaction obtained with these antibodies is probably mostly due to keratin 18, as efferent ducts show a particularly strong reaction with the anti-keratin 18 antibody. In addition, a weak labeling is also seen for keratin 19 (see Table). In cryptorchid children, epithelial cells of the efferent ducts were immature, which made it difficult to distinguish between ciliated and principal cells. However, principal cells still reveal weak immunoexpression of low-molecular-weight keratins (as shown by AE1 antibody immunohistochemistry, Figure 1B) and very low keratin 18 expression (see Table). In cryptorchid children, immunolabeling with every antibody used was generally weaker than that of age-matched controls. This difference is best seen in the corpus and cauda regions due to the strong reaction to keratin antibodies of the same regions of the healthy specimens (see Table and below).

In the normal caput epididymidis, basal cells showed stronger reactivity than principal cells with almost all antibodies used. However, keratin 18 showed a moderate intensity in this region with no differences in intensity between cell types (Figure 1C). The cryptorchid caput epididymidis shows weak immunostaining both with the anti-broad-range-keratin antibody and the anti-low-molecular-weight keratin antibody, with keratins 18 and 19 almost undetectable (compare Figure 1, C and D). The cryptorchid ductus epididymidis showed hypoplasic regions, with undifferentiated cubical cells, and other regions with flat epithelium.

The corpus epididymidis showed in general a stronger keratin expression than the upper regions in the normal conditions (see Table). In the normal corpus epididymidis, reactions with anti-broad-range-keratin antibody and low-molecular-weight keratins antibody (Figure 1E) were very strong, due in part to reactions with keratin 18 and keratin 19 (Figure 2A). In the cryptorchid corpus epididymidis, the level of low-molecular-weight keratins in general (Figure 1F) and keratins 18 and 19 (Figure 2B) in particular, is lower than that of the age-matched controls (compare Figure 1, E with F, and Figure 2, A with B).

In the cauda epididymidis, reaction to anti-low-molecular-weight keratins is very strong in healthy specimens (Figure 2C), whereas it is almost undetectable in cryptorchid patients (Figure 2D). The same lower reaction in cryptorchid patients with respect to controls is seen for keratin 18 (compare Figure 2, E and F). In addition, in both corpus and cauda of the cryptorchid specimens, there is an increase of clear cells that lack low-molecular-weight keratins with respect to normal ones (compare Figure 2, A with B and E with F).

Adults—In general, the pattern of keratin immunoex-

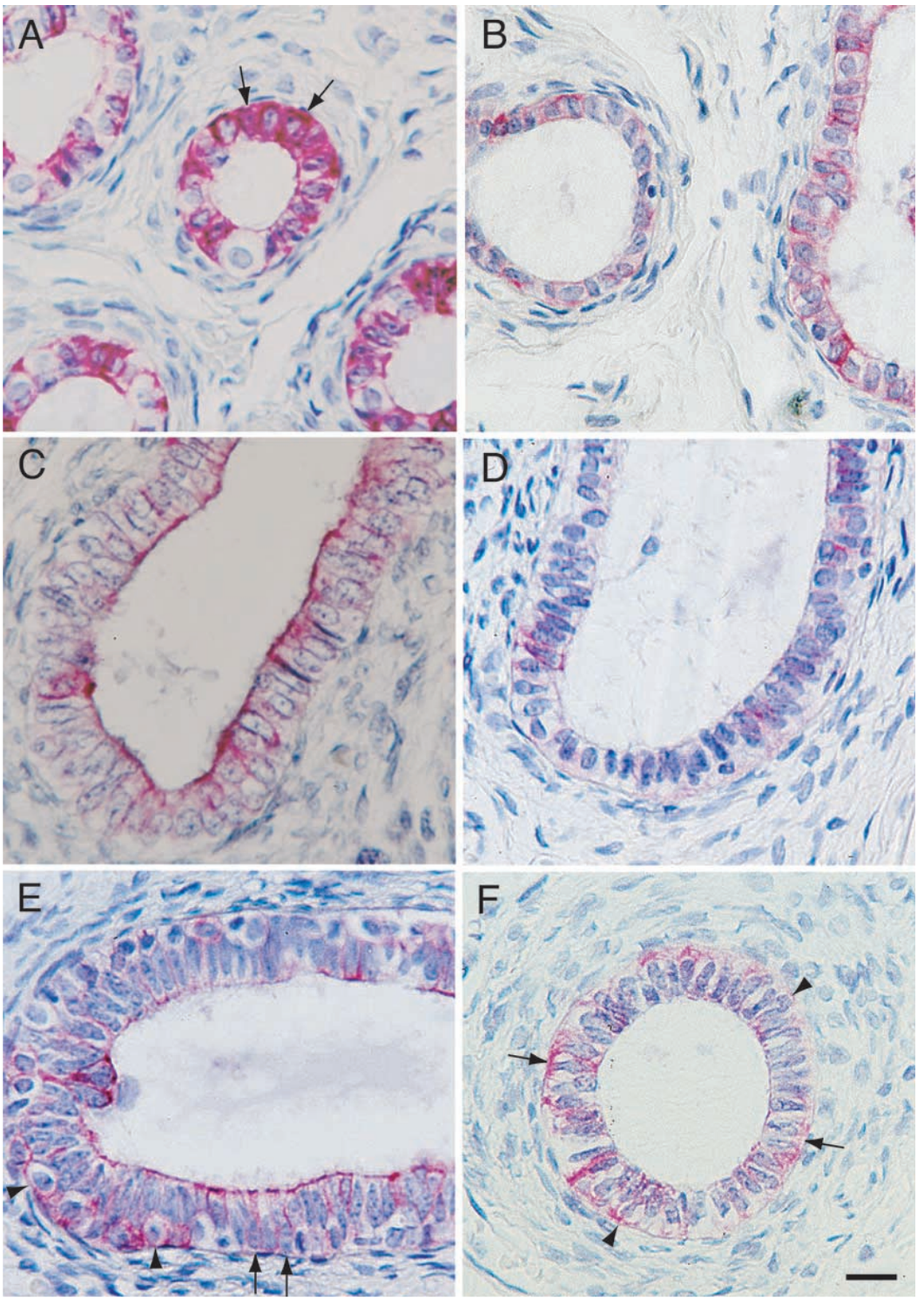

Figure 1. Keratins immunoexpression in the efferent ducts and epididymis of children. **(A)** Efferent ducts of a 2-year-old child immunostained with AE1 antibody showing strong immunoexpression of low-molecular-weight keratins in the principal cells (arrows). Hematoxylin counterstain. **(B)** Efferent ducts of a 3-year-old cryptorchid child immunostained with AE1 antibody showing weak immunoexpression of low-molecular-weight keratins. Note the scarce development of the muscular layer. Hematoxylin counterstain. **(C)** Caput epididymidis of a 5-year-old child showing keratin 18 immunoexpression. Hematoxylin counterstain. **(D)** Caput epididymidis of a 5-year-old cryptorchid child showing very weak keratin 18 immunoexpression in the epithelium. Hematoxylin counterstain. **(E)** Corpus epididymidis of a 3-year-old child immunostained with AE1 antibody. Both basal cells (arrowheads) and principal cells (arrows) show the same strong intensity of immunoreaction. Hematoxylin counterstain. **(F)** Corpus epididymidis of a 3-year-old cryptorchid child immunostained with AE1 antibody showing weaker immunoreaction to low-molecular-weight keratins than the age-matched control. Both basal cells (arrowheads) and principal cells (arrows) show the same intensity of immunoreaction. Hematoxylin counterstain. Bar = 17 μm.

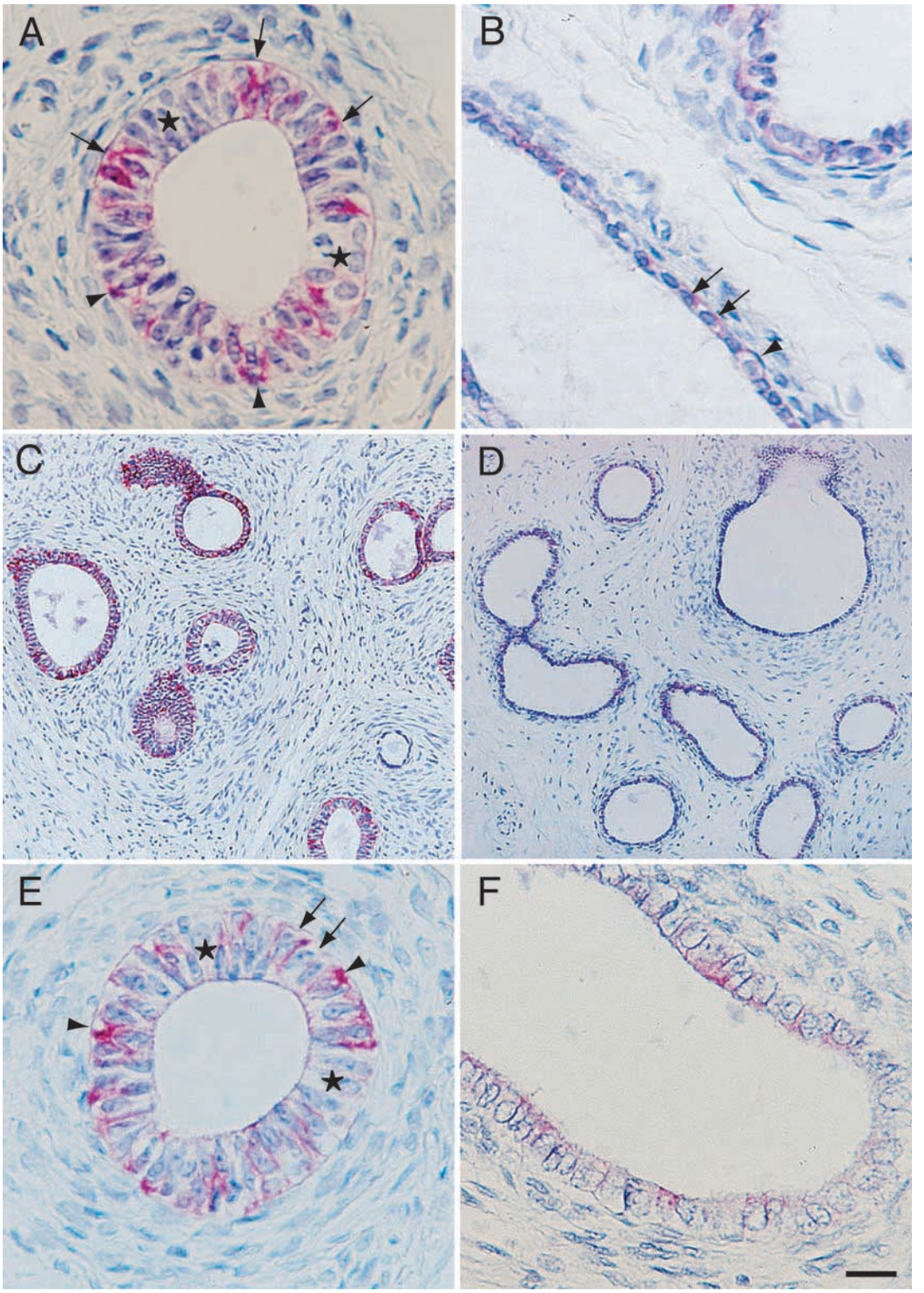

Figure 2. Keratin immunoexpression in the epididymidis of children. **(A)** Corpus epididymidis of a 3-year-old child showing stronger keratin 19 immunoexpression in the basal cells (arrowheads) than in the principal cells (arrows). Note that clear cells (stars) are not immunoreactive. Hematoxylin counterstain. **(B)** Corpus epididymidis of a cryptorchid 4-year-old child. The epithelium is not differentiated and shows very scarce keratin 19 expression both in the basal cells (arrowhead) and in the principal cells (arrows). Note the scarce development of the muscular wall. Hematoxylin counterstain. **(C)** Cauda epididymidis of a 2-year-old child immunostained with AE1 antibody. All epithelial cells show strong labeling whereas the muscular wall is not immunoreactive. Hematoxylin counterstain. **(D)** Cauda epididymidis of a cryptorchid 5-year-old child. The epithelium is lower in height and shows very scarce keratin expression (compare with the age-matched control in **(C)**. Anti-low-molecular-weight-keratins AE1 and hematoxylin counterstain. **(E)** Cauda epididymidis of a 10-year-old child showing moderate keratin 18 immunoexpression in the basal cells (arrowheads) and in the principal cells (arrows) of the epithelium. Note the irregular distribution in the clear cells (stars) due to lack of keratin 18 immunoexpression. Hematoxylin counterstain. **(F)** Cauda epididymidis of a cryptorchid 10-year-old child with moderate atrophy of the epithelium, labeled with anti-keratin 18 antibody. Most of the cells are not immunoreactive and the antibody reaction is scarce in the rest. Hematoxylin counterstain. Bar = 17 μm in A, B, E, and F; 100 μm in C and D.

pression is maintained from childhood to adulthood in normal men; that is, the intensity of immunolabeling with every antibody tested being progressively stronger from caput to cauda (see Table). However, the intensity of keratins is usually higher in adults than in children. This was seen most remarkably in the efferent ducts, which showed a weaker reaction in the children studied (see Table).

In the normal adult epididymis, principal cells of the efferent ducts showed low-molecular-weight keratins, whereas ciliated cells lacked them (Figure 3A). In adult cryptorchid specimens, the efferent duct epithelium was formed almost exclusively by principal cells that showed moderate keratin immunoexpression (Figure 3B).

In all regions of the normal adult epididymis, low-molecular-weight keratin antibody showed a strong reaction (see, for example, in the caput, Figure 3C). The epithelium of adults with cryptorchidism at the level of the caput was atrophic, and showed little keratin immunoexpression, its intensity being weaker than that of age-matched controls (compare Figure 3, C with D). In contrast to the reaction to the low-molecular-weight keratin antibody, keratins 18 and 19 were only weakly expressed in the normal caput, were restricted almost exclusively to basal cells (Figure 3E), and were progressively more abundant in the corpus and cauda (Table). In patients with cryptorchidism, keratin 18 was almost undetectable in this region (compare Figure 3, E with F).

As in children, with every antibody adult cryptorchid specimens generally showed a weaker immunoreactiveness than that of healthy age-matched controls (see Table). Again this difference was most remarkable in the corpus and cauda regions due to their strong reaction to keratin antibodies in healthy specimens. In the corpus epididymidis of normal specimens, the reaction to anti-low-molecular-weight keratins antibody was very strong (Figure 3G). In contrast, the epithelium at the level of the corpus in patients with cryptorchidism was also atrophic and showed lower keratin immunoexpression than that of age-matched controls (compare Figure 3, G with H). The same immunocytological characteristics apply to the cauda region (see Table).

Morphometry

Efferent Ducts—The total surface area of the efferent ducts was smaller in cryptorchid specimens than in age-matched controls at all ages studied (Figure 4A; and compare Figure 3, A and B). Luminal surface was smaller only in cryptorchid adults (Figure 4B; and compare Figure 3, A and B), so the decrease in total surface was mostly due to a reduced development of the muscular layer surface (Figure 4C; and compare Figure 1, A and B) seen at all ages in cryptorchid specimens. In parallel, the total tubular diameter was also reduced in cryptorchid efferent ducts at every age (Figure 5A). Luminal diameter of the efferent ducts did not decrease significantly in the cryptorchid versus normal specimens at any given age (Figure 5B). However, at 5–14 years, the efferent duct lumen was dilated in cryptorchid specimens (Figures 4B and 5B), but was smaller in adults (Figure 4B; and compare Figure 3A and B). The epithelial height was greatly reduced in cryptorchid efferent ducts of both infants and children (Figure 5C; and compare Figure 1A and B). This difference is not significant in adults, however, due to the higher standard deviation in the measurements at that age (but compare Figure 3A and B).

Despite the smaller size of the efferent ducts of the cryptorchid specimens, their pattern of growth paralleled that of normal specimens; that is, every significant increase in size from infancy to childhood or from childhood to adulthood in normal men was also evident in the cryptorchid ones (see # in Figures 4 and 5). In general, there is a progressive increase in the efferent ducts in all parameters measured from infancy to childhood and then from childhood to adulthood, both in healthy and in cryptorchid specimens.

Caput Epididymidis—The total surface area was smaller in cryptorchidism than in controls at every age studied (Figure 4A). The same was seen for total diameter (Figure 5A). However, the caput lumen was not significantly different in patients with cryptorchidism versus normal patients at any age (Figures 4B and 5B; and compare Figure 1, C with D, and Figure 3, C with D). The decrease in total surface area seen in cryptorchid specimens is mostly due to a reduction in the muscular layer surface at every age (Figure 4C), together with a reduced epithelial development in infancy and childhood (Figure 5C). The epithelium was also smaller during adulthood (Figure 5C), but as has been described for the efferent ducts, there was a high standard deviation in this regard (but compare epithelial height in Figure 3, C and D, and also in E and F).

As happened in efferent ducts, in most parameters the growth of the normal caput epididymidis from infancy to adulthood was paralleled by the cryptorchid caput (see Figures 4 and 5). In particular, in the caput, there is no increase in size from infancy to childhood, either in normal specimens or in the cryptorchid ones. The development of the caput takes place between childhood and adulthood. The most striking difference is seen in the increase in epithelial height in the cryptorchid specimens from infancy to childhood, which is not seen in healthy ones (Figure 5C), however, this growth of the epithelium is not enough to significantly reach normal parameters.

Corpus Epididymidis—Total surface (Figure 4A) and total diameter (Figure 5A) were in general smaller in cryptorchid than in normal specimens. However, this difference was not as evident for efferent ducts and caput. Lumen surface (Figure 4B) and diameter (Figure 5B)

were significantly smaller in cryptorchid infants (compare Figure 1, E and F), but this difference disappeared at later ages (see Figures 4B and 5B). In parallel, the muscular layer was underdeveloped in cryptorchid infants (Figure 4C; and compare Figure 2, A and B), but not in cryptorchid children and adults (Figure 4C). In this region, the epithelium tended to be lower in cryptorchid specimens from all ages (Figure 5C; and compare Figures 1, E with F; 2, A with B; and 3, G with H), although again, differences were not significant in adulthood because of a large variation between specimens (Figure 5C).

As for the efferent ducts and caput, the pattern of growth of the cryptorchid corpus epididymidis parallels that of normal ones. As in the caput, in the corpus there is no significant increase in size from infancy to childhood, either in normal specimens or in the cryptorchid ones. The only exception is seen in the increase in muscular layer surface area in the cryptorchid specimens from infancy to childhood, which is not seen in the healthy ones (Figure 4C). This development is sufficient to reach normal parameters.

Cauda Epididymidis—The cryptorchid cauda epididymidis was smaller than that of controls at every age studied (Figures 4A and 5A). The lumen was generally smaller in cryptorchid specimens, but the difference was significant only in the period of 5–14 years of age (Figures 4B and 5B). The muscular layer was significantly smaller in cryptorchid infants and children (Figure 4C). The height of the epithelium of the cauda epididymidis of the cryptorchid specimens did not show significant differences from that of the controls although the epithelial height of the cryptorchid cauda was smaller (Figure 5C; and compare Figure 2, E and F).

In general, as in more proximal regions, the pattern of growth of the cryptorchid cauda epididymidis paralleled that of normal ones, but there are several exceptions. The lumen of the normal cauda epididymidis grows significantly from infancy to childhood, whereas that of the cryptorchid does not (Figures 4B and 5B). This is a delay of growth because this difference is no longer seen in adulthood anymore (Figures 4B and 5B). The same is true for the muscular layer surface, where there is also a delay in the development of this layer in the cauda of cryptorchid specimens, which does not take place before but after puberty (Figure 4C). The differences in these two parameters, lumen and muscular layer, account for the differences in general growth, as seen in the total tubular diameter and area (Figures 4A and 5A).

Discussion

Cytokeratins, the epithelial cell intermediate filaments, are markers of epithelial cell differentiation. The keratin patterns of most epithelia are established early during embryonic development and remain constant during fetal and postnatal development (Moll et al, 1982; O'Guin et al, 1990). During embryogenesis, cytokeratins that are specific to a stage of differentiation always become detectable before corresponding morphologic changes. Among potential diagnostic applications, analysis of keratin patterns of epidermal cells may provide earlier information than morphological studies (for a review see Sawaf et al, 1992). In neoplastic cells, the expression of intermediate filaments reflects their morphologic and functional differentiation. For example, sarcomas of muscle origin contain desmin; the carcinomas contain keratin. Identification of individual keratin components may allow further subclassification (Bernal and Stahel, 1985). In fact, oncogenes that activate Ras signal transduction pathways stimulate expression of the keratin 18 gene (Oshima et al, 1996).

During human embryo development the epithelia of the different excretory ducts, including the primitive efferent ducts and epididymis, are formed by a single layer of undifferentiated epithelial cells. In many of these ductal systems the first keratin expressed is keratin 8, and from the 14th week of gestation keratin 18 is coexpressed (Jackson et al, 1980; Moll et al, 1982). Keratin 19 is also found very early in the human epididymis (Achtstatter et al, 1985; Kasper and Stosiek, 1989) and appears to be a major component in most simple epithelia and a minor one in several stratified epithelia. The immunohistochemical pattern of expression of the different keratins in the human epididymis is in general similar in newborns and in adults (Kasper and Stosiek, 1989; Dinges et al, 1991). It includes, in addition to keratins 8 and 18, keratins 7 and 19 in the principal cells of the efferent ducts, and keratins 5 and 17 in the basal cells of the ductus epididymidis (Achtstatter et al, 1985; Kasper and Stosiek, 1989; Dinges et al, 1991). This keratin pattern is preset at 24–27 weeks in the human fetus (Regadera et al, 1993). However, the intensity of immunostaining by different anti-keratin antibodies changes with maturation of the different regions of the normal human epididymis.

According to our results, there is a decrease in low-molecular-weight keratin expression in human cryptorchid children compared with their age-matched controls. In cases of massive epithelial atrophy keratin 18 expression is very weak or even absent, suggesting an alteration in the epithelial differentiation, because the keratin pair 8/18 is the first to be expressed in embryonal differentiation of the epithelial tissues (Moll et al, 1982). This lack of differentiation becomes more evident with time. In adults, the principal and ciliated cells of the efferent ducts of cryptorchid patients are atrophied, with a significantly lower cytokeratin immunoexpression than in normal men (Regadera et al, 1993). This atrophy of the ciliated cells

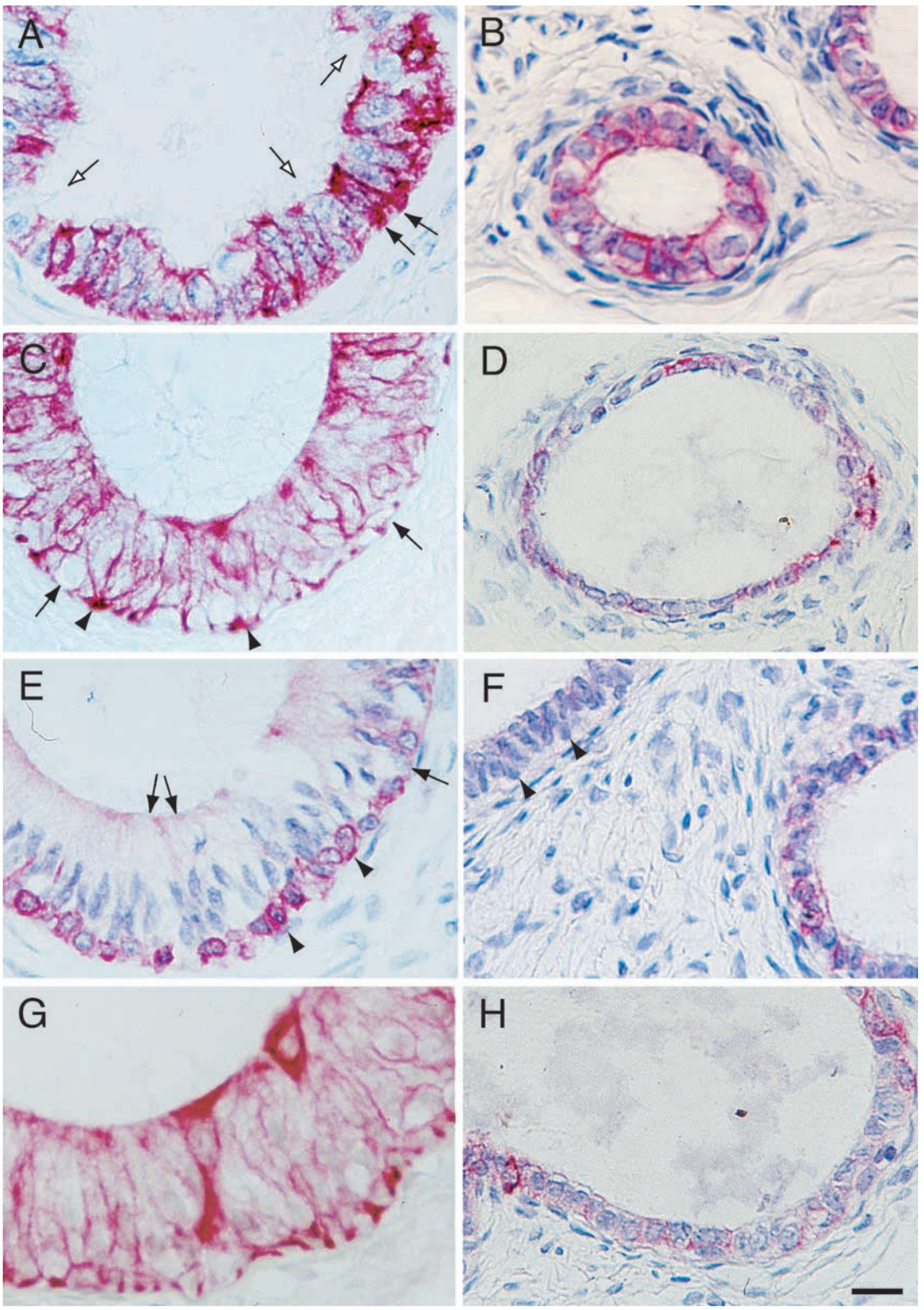

Figure 3. Keratin immunoexpression in the adult efferent ducts and epididymis. **(A)** Efferent ducts of a 41-year-old man showing very intense expression of low-molecular-weight keratins in the principal cells (arrows). Ciliated cells (open arrows) are negative. Anti-cytokeratin AE1 and hematoxylin counterstain. **(B)** Efferent ducts of a cryptorchid 42-year-old man showing atrophy of the epithelium, reduction in tubule and lumen diameters, and moderate expression of low-molecular-weight keratins. Anti-cytokeratin AE1 and hematoxylin counterstain. **(C)** Caput epididymidis of a 41-year-old man showing stronger expression of low-molecular-weight keratins in the basal cells (arrowheads) than in the principal cells (arrows). Anti-cytokeratin AE1. **(D)** Caput epididymidis of a cryptorchid 34-year-old man showing deficient epithelial development and weak expression of low-molecular-weight keratins.

in the efferent ducts could be associated with impaired epididymal function, as normal mammalian efferent ducts are involved in the absorption of testicular fluid and electrolytes (Clulow et al, 1998) and various proteins (Andonian and Hermo, 1999) including androgen-binding-protein (ABP; Pelliniemi et al, 1981; Hermo et al, 1998), and may also contribute to the transport of testicular fluid and spermatozoa from the rete testis to the epididymis (Hermo and Morales, 1984). Keratins are very stable proteins and, once formed, it is difficult for them to disappear from the expressing cells. The lower keratin expression in the epithelial cells of the cryptorchid epididymis is most likely due to a lack in their formation than to a dedifferentiation process. In transgenic mice lacking a specific keratin, lethality at midgestation is normally observed (i.e., keratin 8; Baribault et al, 1993). Cellular phenotypes include lack of maturation and basal hyperplasia (keratin 4; Ness et al, 1998), providing evidence that keratins are not only involved in cell differentiation but also in cell proliferation. In this regard, keratin 18 expression has been spatiotemporally related to the pattern of cell proliferation at least in the rabbit bladder (Pampinella et al, 1996).

In the human cryptorchid epididymis, abnormalities have been reported in association with 36% to 79% of testes that are undescended (Mininberg and Schlossberg, 1983; Koff and Scaletscky, 1990; Elder, 1992). In the present study a decrease in the size of efferent and epididymal ducts in cryptorchid children is shown. This decrease is already seen at the testicular quiescent period (1 to 4 years of age), and cryptorchid epididymis then grows only slowly during the transition to the pubertal period. During puberty and in adulthood the smaller tubular surface area of the cryptorchid epididymis is due primarily to a minimal development of the muscular wall and a decrease in epithelial height. From infancy to childhood the efferent duct is the only region that grows significantly. However, the main development of all the regions of the epididymis, including the efferent ducts, takes place between childhood and adulthood. The pattern of growth of the cryptorchid efferent ducts and epididymis parallels in general that of normal ones, with the exception of retarded development of the lumen and muscular layer in the cauda epididymidis.

These findings point to a hypoplasia of the cryptorchid epididymis that could be due to one of the following mechanisms: 1) postnatal temporary hypogonadotrophic hypogonadism, which has been observed in some patients; 2) a negative effect of increased temperature, which could act directly on the epididymis or indirectly cause hormonal alterations due to damage of the testicular Leydig cells; and 3) a primary congenital anomaly in the epithelium and muscular wall development throughout the epididymis.

With respect to the first possible mechanism, it is known that androgens are responsible for development of the epididymis and accessory glands (Ganjam and Amann, 1976; Wilson et al, 1995). High local testosterone concentrations in childhood in the epididymis are important for epididymal differentiation (Hadziselimovic and Kruslin, 1979). It has been shown that androgens are required to achieve tissue organization (Vazquez et al, 1989) and epithelial differentiation (Tezon and Blaquier, 1981) in epididymal cultures. The testosterone content of epididymal tissue in children is 10-fold larger than that of the plasma (Bidlingmaier et al, 1983). The epididymis can bind testosterone even during the period when androgens are not yet required for sperm maturation (Bidlingmaier et al, 1983). In the androgen-deficient epididymis, atrophic epithelial changes and impaired secretion have been observed (Cooper et al, 1988). This is in accord with androgen insensitivity syndrome, in which epididymal development is impaired (Regadera et al, 1999a). In addition, the level of androgen receptors is also decreased in cryptorchid testes (Regadera et al, 1999b) compared with the normal level (Ungefroren et al, 1997; Suarez-Quian et al, 1999). Moreover, Leydig cell numbers are decreased in cryptorchid adult patients, with only 5% showing normal testosterone immunoexpression (Regadera et al, 1991).

However, not all the epididymal regions respond equally to androgens. The efferent ducts and the initial portion of the caput epididymidis do not express androgen receptors (Ungefroren et al, 1997), indicating that these portions are independent of androgens. In fact, these regions do not respond to androgens following castration (for a review see Hutson and Donahoe, 1986), so the differences in growth and differentiation seen in the efferent ducts and the initial portion of the caput epididymis in the cryptorchid specimens of this study can not be explained by hypogonadotrophic hypogonadism.

Our morphometric results show a decrease in the size

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Anti-cytokeratin AE1 and hematoxylin counterstain. **(E)** Caput epididymidis of a 41-year-old man. Keratin 18 expression is strong in the basal cells (arrowheads) and weak in the principal cells (arrows). Hematoxylin counterstain. **(F)** Caput epididymidis of a cryptorchid adult man. The epithelial height is lower than the corresponding age-matched control. Principal cells show very weak keratin 18 immunostaining and basal cells (arrowheads) are negative. Hematoxylin counterstain. **(G)** Corpus epididymidis of a 37-year-old man showing very strong expression of low-molecular-weight keratins in all epithelial cells. Anti-cytokeratin AE1. **(H)** Corpus epididymidis of a cryptorchid 47-year-old man showing evident atrophy of the epithelium together with a weak expression of low-molecular-weight keratins. Anti-cytokeratin AE1 and Hematoxylin counterstain. Bar = 17 μm.

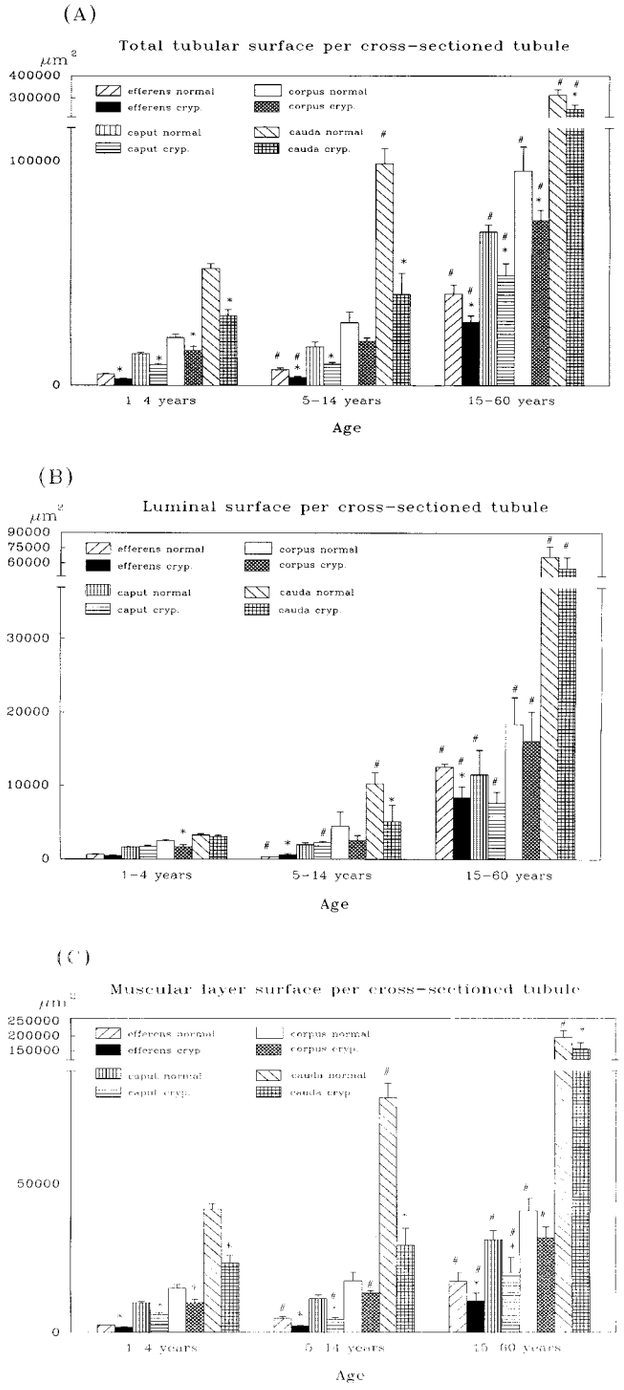

Figure 4. Changes in surface areas (per cross-sectioned tubular section) in the efferent ducts and different segments of the ductus epididymidis (caput, corpus and cauda) in the normal versus cryptorchid specimens during development. Data are expressed as mean ± SEM in μm². *Difference between cryptorchid and the normal specimens at this age is significant ($P = .05$) for this parameter. #Difference between this age group and the preceding age group is significant ($P = .05$) for this parameter. **(A)** Total surface (area occupied by epithelium + muscular layer + lumen). **(B)** Area occupied by the lumen. **(C)** Area occupied by the muscular layer.

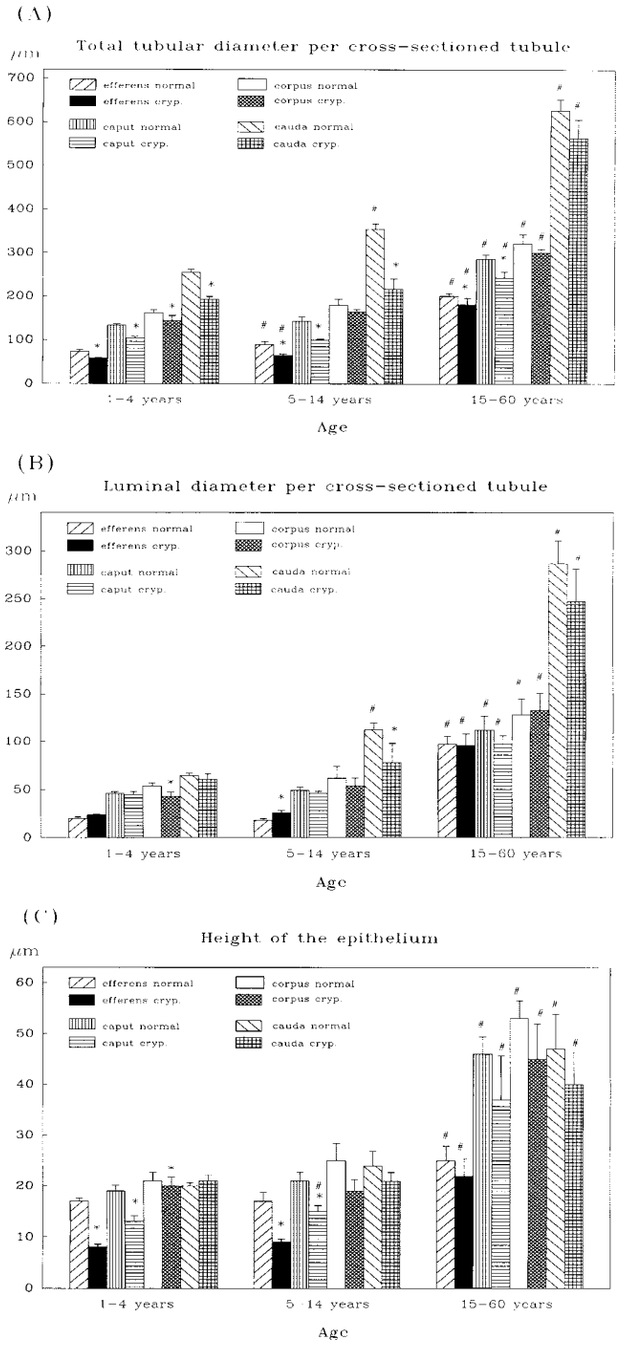

Figure 5. Changes in **(A)** total tubular diameter, **(B)** lumen diameter, and **(C)** height of the epithelium, in the efferent ducts, and the different segments of the ductus epididymidis (caput, corpus, and cauda) in the normal versus cryptorchid specimens during development. Data are expressed as mean ± SEM in μm. *Difference between cryptorchid and normal specimens at this age is significant ($P = .05$) for this parameter. #Difference between this age group and the preceding age group is significant ($P = .05$) for this parameter.

of the cryptorchid epididymis compared with the normal epididymis (Oshima et al, 1984; Rubinstein and Mandarim-de-laCerda, 1995; De Miguel et al, 1998). This alteration, together with the poor cytokeratin cytoskeletal differentiation of the epithelium begin during fetal development, as we see these variations already in children of 1–4 years of age. In most testes that are undescended at birth, spontaneous descent occurs during the first months of life (Scorer and Farmington, 1971), probably due to the testosterone surge that occurs soon after birth (Forest and Cathiard, 1975). Epididymal maldevelopment in these children could result from deficient testosterone production by the embryonic testis (Elder, 1992). However, cryptorchid epididymal hypoplasia could also be due to alterations in the hypothalamic-hypophysis-testicular axis, as the increased luteinizing hormone (LH) levels in cryptorchidism suggest (Schanbacher and Ford, 1977). In addition, LH receptors are diminished in cryptorchidism (Huhtaniemi et al, 1984). This altered hormonal regulation could start in embryonic life when inadequate levels of fetal testicular androgens would result in inadequate stabilization of the wolffian ducts, giving rise to alterations in epididymal lumen development (Husmann and Levy, 1995).

It has been suggested that androgen deficiency could also induce anomalies in the genito-femoral nerve by producing a disruption in gubernaculum migration (Hutson et al, 1995). In the rat, the testicular gubernaculum possesses high levels of androgen receptors (Husmann and Levy, 1995), suggesting that androgens favor testicular descent by acting on the gubernaculum. In this regard, recent findings also involve estrogens in gubernaculum migration. Mice having a disruptive mutation in the estrogen receptor gene displayed a smaller cremaster sac and a larger cremaster muscle (both structures derived from the gubernaculum; Donaldson et al, 1996). Fetal exposure to the endocrine disruptor, dioxin, causes increased expression of estrogen receptor in the testis but decreased expression in the gubernaculum and the epididymis, resulting in cryptorchidism (Barthold et al, 1999). However, gubernaculum development is inhibited in estrogen-treated fetal mice (Shono et al, 1996), suggesting that specific levels of androgens and estrogens during fetal life are necessary for adequate gubernaculum and epididymal development.

Because the epididymis is attached to the gubernaculum, epididymal abnormality may interfere with normal testicular descent (Hadziselimovic and Kruslin, 1979; Mininberg and Schlossberg, 1983). Moreover, it has been suggested that some advantage for the cauda epididymidis has been the prime influence on the evolution of the scrotal state and that, in terms of functional significance, testis descent was of secondary importance (Bedford, 1978). The poor development of the cryptorchid epididymis could not be enough to exert the adequate pressure that the normal epididymis does, which literally pushes the testicle into the scrotum (Hadziselimovic, 1984; Heyns and Hutson, 1995). However, a series of men with an absent vas deferens and epididymis with a descended testis have been reported (Donohue and Fauver, 1989).

The second possible mechanism that may be affecting several aspects of epididymal function in an adverse manner is temperature elevation. A rise in temperature would especially affect the storage and viability functions of spermatozoa, and thereby the quality of the ejaculate (Bedford, 1994). In fact, in rabbits if the cauda epididymis is elevated to the abdomen with the testis, there is a suppression of its ability to maintain sperm in a viable state (Glover, 1960). In the rat, exposure of the cauda epididymis to deep body temperature provokes changes in cauda fluid protein concentration (Esponda and Bedford, 1986; Regalado et al, 1993), which is associated with oligozoospermia (Foldesy and Bedford, 1982) and reduction of both the diameter and apparent length of the caudal epididymal region (Bedford, 1991). In another study, experimental cryptoepididymides in prepubertal rats exhibited a significantly lower weight, a true growth retardation and reduced length of the epididymis, but the histology and diameter of epididymal ducts are unchanged (Johansen et al, 1989). The same growth retardation of the cryptorchid cauda epididymidis has been shown in this study, yet with lower epithelial height and differentiation. These studies suggest that epididymal hypoplasia could be a direct consequence of temperature increase, as it is for the testis in these animals (Steinberger, 1991). However, we see a smaller size and poor differentiation of the cryptorchid efferent ducts and epididymis at infancy, suggesting that a rise temperature is not the trigger of the retarded development but may possibly contribute only to its maintenance.

As stated above, in regard to a third possible mechanism these alterations in cryptorchid epididymal development could be primary and inherited, in accordance with classic anatomic and surgical studies showing that the principal epididymal abnormalities include ductal fusion and elongation and separation of the testis and epididymis (Gill et al, 1989). These and our observations are in accordance with the hypothesis that human cryptorchidism has multiple factors involving inherited lesions of testicular germ cells and Sertoli cells as primary determinants of the fertility alterations (Nistal and Paniagua, 1996). In human cryptorchidism there is frequently an inherited decrease in testicular germ cell numbers, which progressively diminish and disappear by puberty (Nistal and Paniagua, 1996). During fetal development and infant and pubertal testicular maturation (for a review see Pelliniemi et al, 1993; Gondos and Berndston, 1993), follicle-stimulating hormone (FSH) acts directly on the Sertoli

cells (Sharpe, 1994; Huhtaniemi and Toppari, 1998), increasing the number of FSH receptors (Tsutsui, 1991). The alterations in Sertoli cells associated with cryptorchidism can be related to an increase in FSH levels, a decrease in androgen binding protein (ABP) (De Kretser et al, 1979), and a decrease in the secretion or storage of testicular inhibin (Demura et al, 1987).

In conclusion, our immunohistochemical and morphometric examination reveals a suppression of development in the human cryptorchid epididymis that is evident from infancy. This suggests that cryptorchidism is a congenital and primary illness of the testis and spermatic ducts, with lesions evident from the first years of life, and leads to the speculation that surgically induced descent would probably not be able to entirely reverse these alterations. Anomalies in the epididymis have already been suggested to contribute, at least in part, to reduced fertility in patients with cryptorchidism (D'Agostino et al, 1994). A significant proportion of boys undergoing orchiopexy ultimately are infertile (Elder, 1992), with about 20% of adults presenting azoospermia (D'Agostino et al, 1993) and fertility is reduced by about 20% in unilateral and by about 50% in bilateral cryptorchidism (Kogan, 1987; Cendron et al, 1989).

Acknowledgments

The authors thank Ms Carmen Sanchez-Palomo for technical help in histological and immunohistochemical procedures and Mr David Dollinger-Few for photographic figure processing.

References

Achtstatter T, Moll R, Moore B, Franke WW. Cytokeratin polypeptide patterns of different epithelia of the human male urogenital tract: immunofluorescence and gel electrophoretic studies. *J Histochem Cytochem.* 1985;33:415–426.

Andonian S, Hermo L. Cell- and region-specific localization of lysosomal and secretory proteins and endocytic receptors in epithelial cells of the cauda epididymidis and vas deferens of the adult rat. *J Androl.* 1999;20:415–429.

Baribault H, Price J, Miyai K, Oshima RG. Mid-gestational lethality in mice lacking keratin 8. *Genes Dev.* 1993;7:1191–1202.

Barthold JS, Kryger JV, Derusha AM, Duel BP, Jednak R, Skafar DF. Effects of an environmental endocrine disruptor on fetal development, estrogen receptor (alpha) and epidermal growth factor receptor expression in the porcine male genital tract. *J Urol.* 1999;162:864–871.

Bedford JM. Anatomical evidence for the epididymis as the prime mover in the evolution of the scrotum. *Am J Anat.* 1978;152:483–507.

Bedford JM. Effects of elevated temperature on the epididymis and testis: experimental studies. *Adv Exp Med Biol.* 1991;286:19–32.

Bedford JM. The status and the state of the human epididymis. *Hum Reprod Update.* 1994;9:2187–2199.

Bernal SD, Stahel RA. Cytoskeleton-associated proteins: their role as cellular integrators in the neoplastic process. *Crit Rev Oncol-Hematol.* 1985;3:191–204.

Bidlingmaier F, Dorr HG, Eisenmenger W, Kuhnle U, Knorr D. Testosterone and androstenedione concentrations in human testis and epididymis during the first two years of life. *J Clin Endocr Metab.* 1983; 57:311–315.

Cadrin M, Martinolli MG. Alterations of intermediate filaments in various histopathological conditions. *Biochem Cell Biol.* 1995;73:627–634.

Cendron M, Keating MA, Huff DS, Koop CE, Snyder HM III, Duckett JW. Cryptorchidism, orchiopexy and infertility: a critical long-term retrospective analysis. *J Urol.* 1989;142:559–562.

Clulow J, Jones RC, Hansen LA, Man SY. Fluid and electrolyte reabsorption in the ductuli efferentes testis. *J Reprod Fertil (Suppl).* 1998; 53:1–14.

Cooper TG, Yeung CH, Nashan D, Nieschlag E. Epididymal markers in human infertility. *J Androl.* 1988;9:91–101.

D'Agostino S, Campobasso P, Spata F, Belloli G. Il criptorchidismo: anomalie dei dotti escretori ed azoospermia. *Ped Med Chir.* 1994;16: 509–512.

D'Agostino S, Zen F, Ioverno E, Pesce C, Belloli G. Criptorchidismo e fertilita: valutazione in eta adulta. *Ped Med Chir.* 1993;15:275–278.

De Kretser DM, Sharpe RM, Swanston IA. Alterations on steroidogenesis and human chorionic gonadotropin binding in the cryptorchid rat testis. *Endocrinology.* 1979;105:135–138.

De Miguel MP, Mariño JM, Martínez-García F, Nistal M, Paniagua R, Regadera J. Pre- and post-natal growth of the human ductus epididymidis. A morphometric study. *Reprod Fertil Dev.* 1998;10:271–277.

Demura R, Suzuki T, Nakamura S, Komatsu H, Jibiki K, Odagiri E, Demura H, Shizume K. Effect of uni and bilateral cryptorchidism on testicular inhibin and testosterone secretion in rats. *Endocrinol Jpn.* 1987;34:911–917.

Dinges HP, Zatloukal K, Schmid C, Mair S, Wiensberger G. Co-expression of cytokeratin and vimentin filaments in rete testis and epididymis. *Virchow Arch A Pathol Anat.* 1991;418:119–127.

Donaldson KM, Tong SY, Washburn T, Lubahn DB, Eddy EM, Hutson JM, Korach KS. Morphometric study of the gubernaculum in male estrogen receptor mutant mice. *J Androl.* 1996;17:91–95.

Donohue RE, Fauver HE. Unilateral absence of the vas deferens. A useful clinical sign. *JAMA.* 1989;261:1180–1182.

Elder JS. Epididymal anomalies associated with hydrocele/hernia and cryptorchidism: implications regarding testicular descent. *J Urol.* 1992;148:624–626.

Esponda P, Bedford JM. The influence of body temperature and castration on the protein composition of fluid in the rat cauda epididymis. *J Reprod Fertil.* 1986;78:505–514.

Foldesy RG, Bedford JM. Biology of the scrotum. I. Temperature and androgen as determinants of the sperm storage capacity of the rat cauda epididymis. *Biol Reprod.* 1982;26:673–682.

Forest MG, Cathiard AM. Pattern of plasma testosterone and delta 4-androstenedione in normal newborns: evidence for testicular activity at birth. *J Clin Endocr Metab.* 1975;41:977–980.

Fuchs E, Cleveland D. A structural scaffolding of intermediate filaments in health and disease. *Science.* 1998;279:514–519.

Ganjam VK, Amann RP. Steroids in fluids and sperm entering and leaving the bovine epididymis, epididymal tissue, and accessory sex gland secretions. *Endocrinology.* 1976;99:1618–1630.

Gill B, Kogan S, Starr S, Reda E, Levitt S. Significance of epididymal and ductal anomalies associated with testicular maldescent. *J Urol.* 1989;142:556–558.

Glover TD. Spermatozoa from the isolated cauda epididymidis of rabbits and some effects of artificial cryptorchidism. *J Reprod Fertil.* 1960; 1:121–128.

Gondos B, Berndston WE. Postnatal and pubertal development. In: Russell LD, Griswold MD, eds. *The Sertoli Cell.* Clearwater, Fla: Cache River Press, 1993:115–153.

Hadziselimovic F. Mechanism of testicular decent. *Urol Res.* 1984;12: 155–157.

Hadziselimovic F, Kruslin E. The role of the epididymis in descensus testis and the topographical relationship between the testis and epididymis from the sixth month of pregnancy until immediately after birth. *Anat Embryol.* 1979;155:191–198.

Hahn GJ, Hendrickson RW. A table of percentage points of the distribution of the largest absolute value of k Student t varieties and its applications. *Biometrika.* 1971;58:323–332.

Hermo L, Barin K, Oko R. Androgen binding protein secretion and endocytosis by principal cells in the adult rat epididymis and during postnatal development. *J Androl.* 1998;19:527–541.

Hermo L, Morales C. Endocytosis in nonciliated epithelial cells of the ductuli efferentes in the rat. *Am J Anat.* 1984;171:59–74.

Heyns CF, Hutson JM. Historical review of theories on testicular descent. *J Urol.* 1995;153:754–767.

Huhtaniemi IT, Bergh A, Nikula H, Damber JE. Differences in the regulation of steroidogenesis and topic hormone receptors between the scrotal and abdominal testes of unilaterally cryptorchid adult rats. *Endocrinology.* 1984;115:550–555.

Huhtaniemi I, Toppari J. Hormonal regulation of the testis. In: Martínez-García F, Regadera J, eds. *Male Reproduction. A Multidisciplinary Overview*. Madrid, Spain: Churchill Communications Europe España, 1998:67–80.

Husmann DA, Levy JB. Current concepts in the pathophysiology of testicular descent. *Urology.* 1995;46:267–276.

Hutson JM, Donahoe PK. The hormonal control of testicular descent. *Endocr Rev.* 1986;7:270–283.

Hutson JM, Terada M, Zhou B, Williams MPL. Normal testicular descent and the aetiology of cryptorchidism. *Adv Anat Embryol Cell Biol.* 1995;132:1–56.

Jackson BW, Grund C, Schmid E, Burki K, Franke WW, Illmensee K. Formation of cytoskeletal elements during mouse embryogenesis. Intermediate filaments of the cytokeratin type and desmosomes in preimplantation embryos. *Differentiation.* 1980;17:161–179.

Johansen TE, Clausen OP, Nesland JM. The effect of non-union of testis and epididymis and of cryptorchidism on the development of epididymis and ductus deferens in the rat. *Andrologia.* 1989;21:441–448.

Kasper M, Stosiek P. Immunohistochemical investigation of different cytokeratins and vimentin in the human epididymis from the fetal period up to adulthood. *Cell Tissue Res.* 1989;257:661–664.

Koff WJ, Scaletscky R. Malformation of the epididymis in undescended testis. *J Urol.* 1990;143:340–343.

Kogan SJ. Fertility in cryptorchidism. *Eur J Pediatr.* 1987;146:21–24.

Martínez-García F, Regadera J, Cobo P, Palacios J, Paniagua R, Nistal M. The apical mitochondria-rich cells of the mammalian epididymis. *Andrologia.* 1995;27:195–206.

Mininberg DT, Schlossberg S. The role of the epididymis in testicular descent. *J Urol.* 1983;129:1207–1208.

Moll R, Franke WW, Schiller DL, Geiger B, Klepler R. The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cell. *Cell.* 1982;31:11–24.

Ness SL, Edelmann W, Jenkins TD, Liedtke W, Rusgi AK, Kucherlapati R. Mouse keratin 4 is necessary for internal epithelial integrity. *J Biol Chem.* 1998;273:23904–23911.

Nistal M, Paniagua R. Non-neoplastic diseases of the testis. In: Bostwick DG, Eble JN, eds. *Urologic Surgical Pathology.* St Louis, Mo: Mosby-Year Book; 1996:457–565.

O'Guin WM, Schermer A, Lynch H, Sun T. Differentiation-specific expression of keratin pairs. In: Goldman RD, Steiner PM, eds. *Cellular and Molecular Biology of Intermediate Filaments.* New York: Plenum; 1990:301–333.

Oshima RG, Baribault H, Caulin C. Oncogenic regulation and function of keratins 8 and 18. *Cancer Metastasis Rev.* 1996;15:445–471.

Oshima S, Okayasu I, Uchima H, Hatakeyama S. Histopathological and morphometrical study of the human epididymis and testis. *Acta Pathol Jpn.* 1984;34:1327–1342.

Palacios J, Regadera J, Paniagua R, Gamallo C, Nistal M. Immunohistochemistry of the human ductus epididymis. *Anat Rec.* 1993;235:560–566.

Pampinella F, Roelofs M, Castellucci E, Chiavegato A, Guidolin D, Passerini-Glazel G, Pagano F, Sartore S. Proliferation of submesothelial mesenchymal cells during early phase of serosal thickening in the rabbit bladder is accompanied by transient keratin 18 expression. *Exp Cell Res.* 1996;223:327–339.

Pelliniemi LJ, Dym M, Fawcett DW, Gunsalus GL, Musto NA, Bardin CW. The fate of the androgen binding protein in the reproductive tract of male rats. *Int J Androl (Suppl).*1981;3:49–55.

Pelliniemi LJ, Fröjdman K, Paranko J. Embryological and prenatal development and function of Sertoli cells. In: Russell LD, Griswold MD, eds. *The Sertoli Cell.* Clearwater, Fla: Cache River Press; 1993:87–113.

Regadera J, Cobo P, Palacios J. Epithelial cell pathology of the epididymis secondary to spermatic tract obstruction and senescence. In: Martínez-García F, Regadera J, eds. *Male Reproduction. A Multidisciplinary Overview*. Madrid, Spain: Churchill Communications Europe España, 1998:293–304.

Regadera J, Cobo P, Paniagua R, Martínez-García, F, Palacios J, Nistal M. Immunohistochemical and semiquantitative study of the apical mitochondria-rich cells of the human prepubertal and adult epididymis. *J Anat.* 1993;183:507–514.

Regadera J, Codesal J, Paniagua R, Gonzalez-Peramato P, Nistal M. Immunohistochemical and quantitative study of interstitial and intratubular Leydig cells in normal men, cryptorchidism and Klinefelter's syndrome. *J Pathol.* 1991;164:299–306.

Regadera J, Martinez-Garcia F, Paniagua R, Nistal M. Androgen insensitivity syndrome: an immuno-histochemical, ultrastructural and morphometric study. *Arch Pathol Lab Med.* 1999a;123:225–234.

Regadera J, Martínez-García F, González-Peramato P, Serrano A, Nistal M, Suarez-Quian CA. Androgen receptor (AR) in hypoplastic Sertoli cells of human cryptorchidism. *J Androl (Suppl).* 1999b;20:54.

Regalado F, Esponda P, Nieto A. Temperature and androgens regulate the biosynthesis of secretory proteins from rabbit cauda epididymis. *Mol Reprod Dev.* 1993;36:448–453.

Rubinstein I, Madarim-de-laCerda CA. Stereologie de l'epididyme humain en differents groupes d'age: foetus, enfants, adolescents, adultes et personnes agees. *J d'Urologie.* 1995;101:153–158.

Sawaf MH, Goffaux JC, Forest N, Ouhayoun JP. Expression of cytokeratins during embryogenesis and in pathologic epithelia. *Pathologie Biologie.* 1992;40:667–672.

Schanbacher BD, Ford JJ. Gonadotrophin secretion in cryptorchid and castrate rams and the acute effects of exogenous steroid treatment. *Endocrinology.* 1977;100:387–393.

Scorer CG, Farrington GH, eds. *Congenital Deformities of the Testis and Epididymis.* London, United Kingdom: Butterworths; 1971:136–146.

Sharpe RM. Regulation of spermatogenesis. In: Knobil E, Neill JD, eds. *The Physiology of Reproduction.* 3rd ed. New York: Raven Press; 1994:1363–1434.

Shono T, Hutson JM, Watts L, Goh DW, Momose Y, Middlesworth B, Zhou B, Ramm-Anderson S. Scanning electron microscopy shows inhibited gubernacular development in relation to undescended testes in oestrogen-treated mice. *Int J Androl.* 1996;19:263–270.

Southgate J, Harnden P, Trejdosiewicz LK. Cytokeratin expression in normal and malignant urothelium: a review of the biological and diagnostic applications. *Histol Histopathol.* 1999;14:657–664.

Steinberger A. Effects of temperature on the biochemistry of the testis. In: Zorgniotti AW, ed. *Temperature and Environmental Effects on the Testis.* New York: Plenum Press; 1991:33–47.

Suarez-Quian CA, Martinez-Garcia F, Nistal M, Regadera J. Androgen

receptor distribution in adult human testis. *J Clin Endocrinol Metab.* 1999;84:350–358.

Tezon JG, Blaquier JA. The organ culture of human epididymal tubules and their response to androgens. *Mol Cell Endocrinol.* 1981;21:233–242.

Tsutsui K. Pituitary and gonadal hormone-dependent and -independent induction of follicle-stimulating hormone receptors in the developing testis. *Endocrinology.* 1991;128:477–487.

Ungefroren H, Ivell R, Ergun S. Region-specific expression of the androgen receptor in the human epididymis. *Mol Hum Reprod.* 1997;3:933–940.

Vazquez MH, De Larminat MA, Scorticai C, Blaquier JA. Effect of in vivo estrogen administration. *J Steroid Biochem.* 1989;25:239–244.

Wilson JD, George FW, Renfree MB. The endocrine role in mammalian sexual differentiation. *Recent Prog Horm Res.* 1995;50:349–364.

Zondek LH, Zondek T. Normal and abnormal development of the epididymis of the fetus and infant. *Eur J Pediatr.* 1980;134:39–44.