Granular changes in Sertoli cells in children and pubertal patients

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Objective: To characterize lysosomes and histochemical function of granular Sertoli cells in developmental alterations.
Design: Prospective and retrospective study.
Setting: University hospital and research centers.
Patient(s): Nineteen infantile and pubertal patients undergoing testicular biopsy; four rat testes for lysosomal study.
Intervention(s): CD-68, α-1-antitrypsin, vimentin, inhibin α subunit, and anti-müllerian hormone antibodies were evaluated. Morphometric measures in seminiferous tubules with and without granular Sertoli cells were obtained. Ultrastructural data of lysosomes in human and rat Sertoli cells were compared.
Main Outcome Measure(s): Quantification of mean diameter of seminiferous tubules, tubular fertility index, and germ and Sertoli cell indexes were obtained in human testis.
Result(s): Granular changes in Sertoli cells are due to the accumulation of large amounts of lysosomes. Vimentin immunoexpression in infantile and pubertal granular Sertoli cells was lower than in adjacent nongranular Sertoli cells. Inhibin was negative in granular cells. Anti-müllerian hormone–positive and -negative granular Sertoli cells were present within the same tubules.
Conclusion(s): The presence of early granular changes in Sertoli cells in childhood and pubertal cryptorchidic patients, associated with other developmental alterations, suggests an intense and irreversible dysfunction of phagocytosis in the granular Sertoli cells. These alterations might be considered primary and irreversible anomalies of Sertoli cells, which might be contributing factors in the infertility seen in these patients. (Fertil Steril® 2005;83:1489–99. ©2005 by American Society for Reproductive Medicine.)

Key Words: Testis, Sertoli cell, cryptorchidism, lysosomes, inhibin, anti-müllerian hormone

The Sertoli cell is the principal type of cell participating in the control of spermatogenesis and steroidogenic regulation (for review, see Griswold and McLean) (1). In humans, Sertoli cells might show a significant variety of cytoplasmic alterations in different congenital and acquired testicular conditions (2, 3). On the basis of their nuclear morphology, four types of Sertoli cells have been recognized: [1] normal mature cells, [2] immature cells with round or ovoid nuclei, [3] dysgenetic cells, and [4] involuting cells (4). In pathologic Sertoli cells, the most frequent changes in the cytoplasm are vacuolization by lipid droplets (4), dense filament deposits (5, 6), and granular changes. Granular changes in Sertoli cells were first described in cases of cryptorchidism (7) and retractile testes of adult male patients (8). Only one series of granular changes in Sertoli cells has been reported in the literature (9). This included 18 patients (only 1 of them prepubertal) in whom spermatogenesis was severely defective or even completely absent. The most frequent diseases in the adult testis associated with granular changes in Sertoli cells were cryptorchidism (5 patients) and varicocele (4 patients) (9).

The aim of this study was to examine by light and electron microscopy, morphometric methods, and immunohistochemistry the granular changes in Sertoli cells observed in a series of 19 pediatric patients (8 prepubertal children and 11 pubertal boys) whose testes were biopsied or orchiectomized for diagnosis or treatment of their undescended testis. For functional characterization of Sertoli cells and their granular changes we used various well known markers of Sertoli cell activity, such as α-1-antitrypsin, CD-68, vimentin, inhibin α-subunit, and anti-müllerian hormone (AMH) antibodies. Alpha-1-antitrypsin and CD-68 antibodies were used as lysosomal markers, as previously demonstrated in testicular macrophages of infertile men (10). Vimentin has been shown to be a good Sertoli cell marker in various testicular conditions (6). In addition, AMH and inhibin α-subunit typically

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localize in human Sertoli cells and have functional variations during development (11–13).

Granular changes in Sertoli cell lysosomes were further characterized by high-resolution light microscopy and transmission electron microscopy, and the findings were compared with cyclic changes in Sertoli cell lysosomes, enzymatic activity, and residual body phagocytosis in the testes of control rats.

MATERIALS AND METHODS

Patients

During the 10 years leading up to this study, 730 testicular biopsies and orchiectomy specimens from children and pubertal patients with cryptorchidism or other testicular diseases were examined at the Department of Pathology of La Paz Hospital (Madrid, Spain) and the Pathology Laboratory, Department of Endocrinology, Buenos Aires Children Hospital, Argentina. We found 19 patients (2 with bilateral lesion) with granular transformation in Sertoli cells (Table 1).

Histologic and Immunohistochemical Methods

Testicular biopsies and specimens were fixed in Bouin's fixative and embedded in paraffin wax. Six to ten nonconsecutive sections in each testicular specimen were produced. The sections were stained with hematoxylin-eosin, periodic acid-Schiff (PAS), and Masson's trichrome stain. Immunohistochemistry was performed with anti-CD-68 (Dako, Glostrup, Denmark; 1:400 dilution), anti-α-1-antitrypsin (Dako; 1:400 dilution), anti-vimentin (Dako; 1:400 dilution), anti-inhibin α-subunit (Serotec, Oxford, United Kingdom; 1:10 dilution), and anti-AMH (a rabbit polyclonal antibody raised against recombinant human AMH at 1:1000 dilution) antibodies. The AMH antibody had been extensively used previously (12).

After dewaxing, paraffin sections were processed according to the streptavidin–biotin immunohistochemical method (Dako). Briefly, after treatment with a solution of methanol–hydrogen peroxide to block endogenous peroxidase activity, the sections were successively incubated with the first antibody, according to dilutions previously mentioned, followed by a biotin-labeled secondary antibody and a streptavidin–peroxidase complex. Peroxidase activity was localized with diaminobenzidine as a chromogen.

Morphometric Methods

Different morphometric parameters were determined, depending on the degree of sexual development. Variables measured included [1] mean tubular diameter, [2] tubular fertility index, defined as the percentage of tubules showing at least one germ cell, [3] germ cell number per cross-sectioned tubule, and [4] Sertoli cell index, defined as the number of Sertoli cells per cross-sectioned tubule. All histometric variables were measured in transverse tubular sections. In each case, the presence of granular changes in Sertoli cells was classified as [1] isolated (only one tubule affected), [2] focal (a group of tubules), or [3] multiple (several groups of tubules). These morphometric data were compared with previous control data obtained from unaltered testicular biopsy specimens (3, 14).

Ultrastructural Methods in Human Biopsies

Small blocks (1 mm in diameter) were fixed in Karnovsky's fixative and then in 1% phosphate-buffered osmium tetroxide for 2 hours, dehydrated in ethanol, and embedded in Epon-Araldite. Sections (1 μm) were stained with toluidine blue. Ultrathin sections were double-stained with uranyl acetate and lead citrate and then studied in an electron microscope (Zeiss EM 109; Carl Zeiss, Jena, Germany).

Ultrastructural Methods for Evaluation of Rat Sertoli Cell Lysosomes

Morphology, cyclic variations, and histochemical demonstration of acid phosphatase in Sertoli cell lysosomes were studied by electron microscopy in four control, 90-day-old fertile Sprague-Dawley rats. Animals were anesthetized and, after perfusion fixation with 5% glutaraldehyde, the testes were processed for electron microscopy as previously described (5). For ultrastructural histochemistry of acid phosphatase, 2.5% glutaraldehyde buffered with sodium cacodylate–maleate (pH 7.4) was used. Thick (50-μm) slices were obtained with a vibratome and subsequently incubated at pH 5 in Gomori medium containing sodium β glycerophosphate as substrate (15), followed by postfixation in osmium tetroxide. After Epon-Araldite embedding, thin sections were slightly contrasted and studied with electron microscopy (Zeiss EM 109).

RESULTS

Granular Sertoli Cells in Children and Pubertal Patients

The general histologic alterations of spermatogenesis, Leydig cells, and interstitium are summarized in Table 1.

In children with granular Sertoli cells (GSCs), a severe lesion of seminiferous tubules was found (Fig. 1), as also revealed by morphometric studies. The tubules with GSC changes showed a slightly increased mean tubular diameter compared with the neighboring ones, but no significant differences were observed between them. Three boys presented a Sertoli cell–only pattern, and others showed a pronounced decrease in the tubular fertility index (Table 1). Sertoli cell index was diminished in tubules with granular changes ($P<.05$).

Initial pubertal maturation was determined by the presence of mature Sertoli cells and/or abundant primary spermatocytes associated with focal spermatid differentiation. Interstitial Leydig cells displayed abundant eosinophilic cytoplasm, similar to that found in normal adult men. In the pubertal group, GSCs were similar to those described in