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Intermediate filaments in the Sertoli cells of the ageing human testis

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Abstract The presence and distribution of intermediate filaments (vimentin, keratin, desmin) was studied in the Sertoli cells of elderly men by means of quantitative immunohistochemical methods. Sertoli cells from young men showed moderate immunogold labelling to vimentin throughout the entire cytoplasm between the cell organelles in tubules showing complete spermatogenesis. Immunogold particles were more numerous in the perinuclear cytoplasm and beneath the plasma membrane in all its faces. The testes from elderly men showed different tubule types; some showed complete spermatogenesis and a normal lamina propria, while others had spermatogenic arrest at different levels (spermatids, spermatocytes, spermatogonia). The immunohistochemical reaction to vimentin in the Sertoli cells of tubules with complete spermatogenesis (type a) was similar to that in the cells of young men. In the Sertoli cells of severely damaged tubules (type b) the immunohistochemical reaction was more intense and immunogold particles extended in similar proportions throughout the whole cytoplasm. When immunolabelling intensity was compared between the three groups of tubules, by counting the number of immunogold particles per square micrometre of cytoplasm, it was found to be significantly higher ($P \leq 0.05$) in type b tubules of elderly men than either in tubules of young men or in type a tubules of elderly men. Since the average cell surface of Sertoli cells was similar in all tubule types, these data suggest that an actual vimentin increase occurs in Sertoli cells of germ-cell-depleted tubules. Sertoli cell immunogold labelling to keratin was found neither in young men nor in type a tubules of ageing men, whereas a positive immunohistochemical reaction was observed in the Sertoli cells of type b tubules of elderly men. Immunogold particles were localized mainly in the perinuclear cytoplasm, and beneath the lateral and basal cell surfaces. The observation of vimentin increase and keratin re-expression in ageing Sertoli cells only in germ-cell-depleted tubules suggests that the changes in intermediate filaments are related to the local factors associated with completion of spermatogenesis, causing functional changes in Sertoli cells.

Key words Ageing men · Ageing testes · Intermediate filaments · Vimentin · Keratin · Sertoli cel

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Introduction

The Sertoli cell cytoskeleton is responsible for producing many of the organizational features characterizing the seminiferous epithelium. It is involved in determining and maintaining cell shape, transporting and positioning organelles in the cytoplasm, forming plasma membrane domains at sites of cell–cell and cell–extracellular matrix interaction, and also in translocating, anchoring and positioning germ cells and releasing sperm from the seminiferous epithelium [38]. The principal cytoskeletal components of the mammalian Sertoli cells are microtubules [30], microfilaments (actin filaments) [23], and intermediate filaments which, in normal adult Sertoli cells, are mainly of the vimentin type [2, 5, 10, 26, 38].

Vimentin filaments are abundant around the nucleus, resulting in a clear zone free of organelles surrounding the nucleus [5, 6]. From this location, vimentin filaments radiate outwards to the cell periphery. Some vimentin filaments extend towards the lateral plasma membrane, and they are seen intermingled with actin filament bundles [5] that concentrate beneath the inter-Sertoli specialized junctions [23, 38]. Other vimentin filaments extend to the basal region of the cell, where other cytoskeletal components are scanty [38]. In the apical region of Sertoli cells, individual bundles of vimentin filaments run in parallel with microtubules [2, 14, 18]. These are prominent and aligned parallel to the long axis of apical crypts containing spermatid heads; they terminate in association

with the cisternae of the ectoplasmic specializations [6]. In several mammalian species, including the rat [11, 26], the rabbit [6], and man [27, 33], Sertoli cells co-express vimentin and keratins nos. 8 and 18 during fetal and early neonatal life, although Miettinen et al. [20] found isolated occasionally keratin-positive cells in seminiferous tubules of young men with active spermatogenesis. Keratins are also re-expressed in Sertoli cell cultures from adult rats [15]. Recently, an immunohistochemical reaction to desmin has been observed in human fetal testes [27].

In the aging testis, the most frequent histological pattern is a mixture of different seminiferous tubule patterns, varying from tubules with complete spermatogenesis and tubules with spermatogenetic arrest at the level of spermatogonia or spermatocytes to fully sclerosed tubules. In these testes, the Sertoli cells undergo morphological alterations, including elaborate interdigitations between adjacent Sertoli cells, an excessive lipid content forming large lipid droplets or inside vacuoles, and decreased amounts of smooth endoplasmic reticulum [24, 25]. These changes could possibly be accompanied by changes in Sertoli cell cytoskeleton, principally in the intermediate filaments, as occurs in several testicular disorders associated with seminiferous tubule involution.

An increase in Sertoli cell vimentin immunolabelling, although without quantitative demonstration, has also been reported in Klinefelter's syndrome [19], varicocele [29], postpubertal cryptorchidism after antiandrogen administration, and androgen insensitivity syndrome [4, 5], in the vicinity of testicular tumours [6] and in seminiferous tubule atrophy [5]. Re-expression of keratins in Sertoli cells has been observed in several types of testicular tumours, including germ cell tumours [20] and malignant Sertoli cell tumour [21], in the vicinity of testicular tumours [1, 27], in cryptorchid testes [27], in the testes of infertile men [7], and in atrophic human testes [33]. In addition, immunoexpression of desmin has been reported in Sertoli cells of cryptorchid, and tumour-adjacent testicular tissue [27].

In common with age changes in the testis, all these disorders involve seminiferous tubule involution accompanied by germ cell loss and thickening of the lamina propria. The present report analyses the changes in the intermediate filament pattern in the Sertoli cells of elderly men by means of a quantitative immunoelectron microscopic study. The aim was to ascertain whether the increase in vimentin and the re-expression of keratin, and perhaps desmin, also occur in testes from elderly men without any previous history of testicular pathology and whether these alterations are limited exclusively to germ-cell-depleted tubules. By using quantitative methods, we wished to determine whether the increased immunohistochemical reaction to vimentin in atrophic tubules is real, and not apparent as a result of the reduction of seminiferous tubule volume and Sertoli cell grouping caused by germ cell loss.

Materials and methods

Therapeutic orchidectomy specimens were obtained with informed consent from 21 elderly men (aged 70–80 years) with prostatic carcinoma. They had received no previous hormone or drug treatments and had no testicular, endocrine or related disorders. Each had fathered at least one child, and none had any previous history of exposure to toxic agents. For comparison purposes, testicular biopsies obtained from nine young adult men (28–34 years of age) consulting for infertility and showing normal testicular histology (the cause of infertility was at post-testicular levels) were used.

Testicular specimens were processed for light and electron microscopy immunolabelling and Western blot analysis. The primary monoclonal antibodies used were: antibody to bovine vimentin (Boehringer, Mannheim, Germany); antibody to human pan-keratin, which stains a common epitope present in keratins nos. 1–6, 8, 10, 14–16, and 19 (AE1/AE3, Biomeda, Foster City, Calif.); and antibody to human desmin (BioGenex, San Ramon, Calif.). All antibodies were mouse derived and were selected after performance of Western blot analyses with a number of antibodies from different sources provided by several manufacturers.

The specificity of primary antibodies was tested by Western blot analysis as described by Towbin et al. [35]. The testes were homogenized in 0.5 M Tris-HCL buffer (pH 7.4) containing 1 mM EDTA 12 mM 2-mercapto-ethanol, and 1 mM phenylmethylsulphonyl fluoride (PMSF). The homogenates were centrifuged at 10000 *g* for 30 min. After boiling for 2 min at 98° C, aliquots of 25 µg of protein were separated in SDS-polyacrylamide (12%, W/v) slab minigels, according to the procedure of Laemmli [17]. Separated proteins were transferred for 4 h at 0.25 A to nitrocellulose paper, and the nitrocellulose sheets were then soaked in blocking solution [1 M glucose 1% bovine serum albumin (BSA) 0.5% Tween-20 10% glycerol in PBS, pH 7.3] overnight at 37°C and then incubated with the primary antibodies, all at 1:400 dilution in blocking solution for 3 h. After extensive washing with phosphate-buffered saline (PBS)-Tween 20 the sheets were incubated with a peroxidase-labelled second antibody (goat antimouse immunoglobulin; Sigma, Barcelona, Spain), at 1:100 dilution in blocking solution. The filters were developed with an enhanced chemiluminescence (ECL) Western blotting analysis, following the procedure described by the manufacturer (Amersham, Aulesbury, Bucks., UK).

For light microscopy immunohistochemistry, the testes were fixed for 24 h in a 0.1 M phosphate-buffered 10% formaldehyde mixture, dehydrated and embedded in paraffin. Sections 6 µm thick were processed following the avidin–biotin–peroxidase complex (ABC) method [16]. Following deparaffinization, sections were hydrated and incubated for 20 min in 0.3% H_2O_2 in PBS to reduce endogenous peroxidase activity. Sections were then digested with 0.1% trypsin (Merck, Darmstadt, Germany) in 1% $CaCl_2$ (pH 7.6) for 15 min at 37° C to enhance antigenic exposure, and incubated overnight at 4° C with the primary antibody diluted in PBS containing 1% BSA. The primary antibody dilutions found to be optimal for this study were: vimentin 1:50; pan-keratin 1:40; and desmin 1:50. Afterwards, the sections were washed twice in PBS and then incubated with goat antimouse biotinylated immunoglobulin (Sigma) at 1:100 dilution in 20% goat serum-PBS buffer (pH 7.6). After 1 h of incubation at 37° C with the second antibody, the sections were incubated with the avidin-biotin-peroxidase complex (Vector, Burlingame, Calif.) for 30 min at room temperature and developed with diaminobenzidine enhanced with $NiCl_2$ or glucose-oxidase (Sigma). Thereafter, the sections were dehydrated in ethanol, and mounted in DePex (Probus, Badalona, Spain).

For electron microscopy immunohistochemistry, the testes were fixed for 90 min in 0.1 M phosphate-buffered mixture of 2.5% paraformaldehyde and 0.5% glutaraldehyde, pH 7.4. Afterwards, the pieces were washed, dehydrated and embedded in Lowicryl K4M resin. Ultrathin sections were placed on drops of 0.2 M Tris buffer containing 0.1% glycine and 1% BSA. Then they were incubated for 2 h at room temperature with the different primary antibodies at 1:50 dilutions. After washing in Tris buffer,

the sections were incubated with goat antimouse gold-labelled (15 nm gold particles) immunoglobulins (Biocell, Cardiff, UK), at 1:150 dilution in 20% goat serum-Tris buffer (pH 7.8) for 2 h at room temperature. Then the sections were again washed with Tris buffer and distilled water and counterstained with uranyl acetate for 20 min at room temperature.

None of the reactivity patterns was observed either when the primary antibodies were omitted or when the sections were incubated with preimmune serum instead of the primary antibody as negative controls. As positive controls, sections from biopsies of human skin, small intestine, and mammary gland were stained simultaneously with the testicular sections.

To evaluate the differences in immunolabelling between young control testes and ageing testes at the electron microscopy level, the immunohistochemical reaction intensity in Sertoli cells was determined in each subject by measuring the area occupied by the cytoplasm, nucleus, and lipid/vacuoles of the Sertoli cells with an image analyser (Microm, Barcelona, Spain) and counting the number of immunogold particles in their cytoplasm in micrographs (at a final magnification of ×45,000) of non-consecutive ultrathin sections, to give the density expressed as gold particles per square micrometre. The number of micrographs that should be used for calculations in each section was determined by successive approaches to obtain the minimum number of micrographs required to reach the lowest standard deviation (SD). A higher number of micrographs did not lead to a smaller SD. This number varied from one section to another between five and nine micrographs. The same procedure was applied to determine the number of sections from each testis. This number varied from testis to testis between 5 and 12 sections. In elderly men, calculations were made in two well-defined types of tubules: (a) those showing complete spermatogenesis; and (b) severely damaged tubules in which Sertoli cells could be recognized (tubules with spermatogenetic arrest at the levels of spermatogonia or spermatocytes). The means±SD for group (young men, type a tubules of elderly men, and type b tubules of elderly men) were calculated from the average values in each testis. The number of immunogold particles in extracellular locations was also counted in each micrograph used for quantitative studies to evaluate background immunohistochemical reaction. The intensity of background was scored according to the following criteria: intense (>10 particles per μm^2); medium (2–10 particles), and low (<2 particles).

Small fragments of some testes were processed for conventional electron microscopy. Tissues were fixed for 6 h in 3% glutaraldehyde in 0.1 M cacodylate, buffered at pH 7.2 with 0.1 M sodium cacodylate, rinsed in the cacodylate buffer, postfixed for 4 h in 1% osmium tetroxide in the cacodylate buffer, rinsed again in cacodylate, dehydrated in ethanol, and embedded in Epon. Ultrathin sections were double-stained with uranyl acetate and lead citrate.

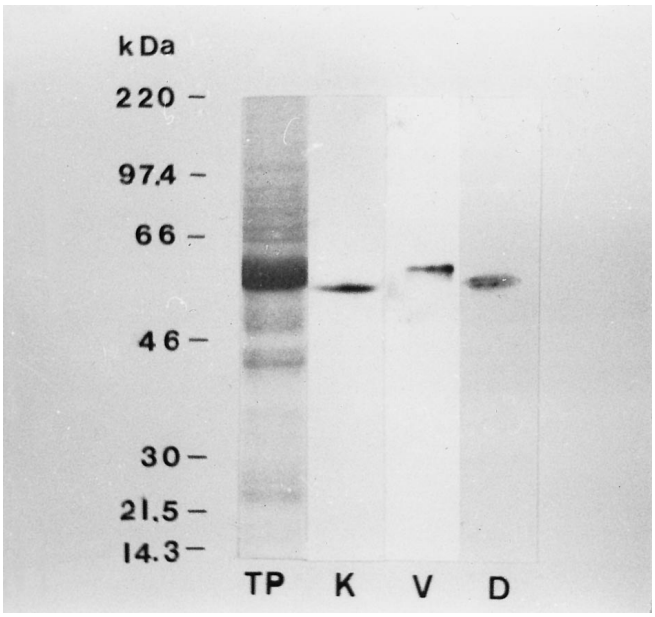


Fig. 1 Western blotting in testicular extracts from a 71-year-old man after 12% SDS-polyacrylamide gel electrophoresis (*TP* total protein markers stained with Coomassie blue, *K* Western blot stained with anti-keratin antibodies, *V* Western blot stained with anti-vimentin antibodies, *D* Western blot stained with anti-desmin antibodies). Molecular weight scale is shown at the left

Results

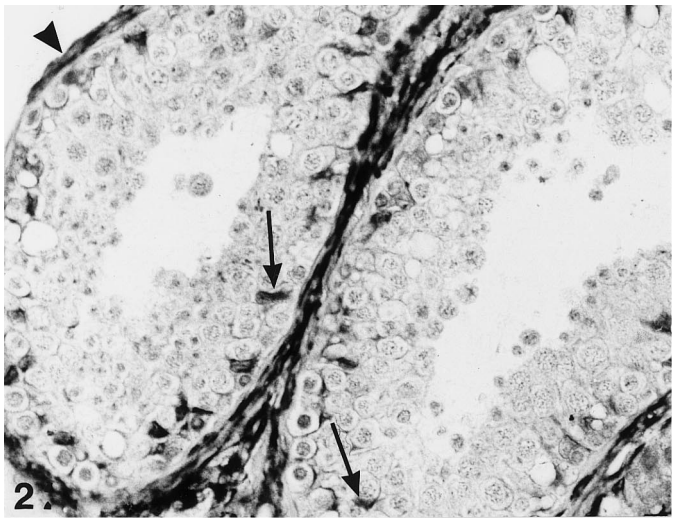
The results of Western blot studies showed a single band, at the corresponding molecular weight, for each of the antibodies used (Fig. 1).

All the control testes selected (young men) showed complete spermatogenesis in most seminiferous tubules (Fig. 2). The testes from elderly men showed different tubule types that varied from those showing complete spermatogenesis and a normal lamina propria to others with spermatogenic arrest at different levels (spermatids, spermatocytes or spermatogonia) and a thickened lamina propria (Figs. 3, 4). Areas of fully sclerosed tubules (with no recognizable seminiferous epithelium) were occasionally seen.

Light microscopy shows a vimentin immunohistochemical reaction throughout the Sertoli cell cytoplasm in both young (Fig. 2) and elderly men (Fig. 3). The immunohistochemical reaction was apparently more abundant in the germ-cell-depleted tubules of elderly men than in the tubules with complete spermatogenesis whether from elderly men or young men, although this might be due to the absence of germ cells. An immunohistochemical reaction to keratins was only observed in the Sertoli cells of the germ-cell-depleted tubules from ageing testes (Fig. 4). An immunohistochemical reaction to desmin was observed only in the lamina propria cells and some interstitial cells in all the testes studied.

The Sertoli cells of young men exhibited a characteristic ultrastructural pattern. They had an indented nucleus with a centrally located prominent nucleolus, and their cytoplasm contained some rough endoplasmic reticulum cisternae, areas of smooth endoplasmic reticulum, dark mitochondria, and some lysosomes and lipid droplets. Specialized junctions between Sertoli cells or between these and germ cells were also observed. Immunogold labelling in response to vimentin was found throughout the entire cytoplasm between the cell organelles. Immunogold particles seemed to be more numerous in the perinuclear cytoplasm (Fig. 5) and beneath the plasma membrane in all its faces; under the lateral surface this is associated with inter-Sertoli junctional specializations (Fig. 6), under the apical surface, it is associated with spermatid crypts, and under the basal surface it faces the basal lamina.

The Sertoli cells of elderly men varied according to the tubule type. The Sertoli cells of tubules with complete spermatogenesis (type a) were similar to those of young men, whereas the Sertoli cells of severely dam-



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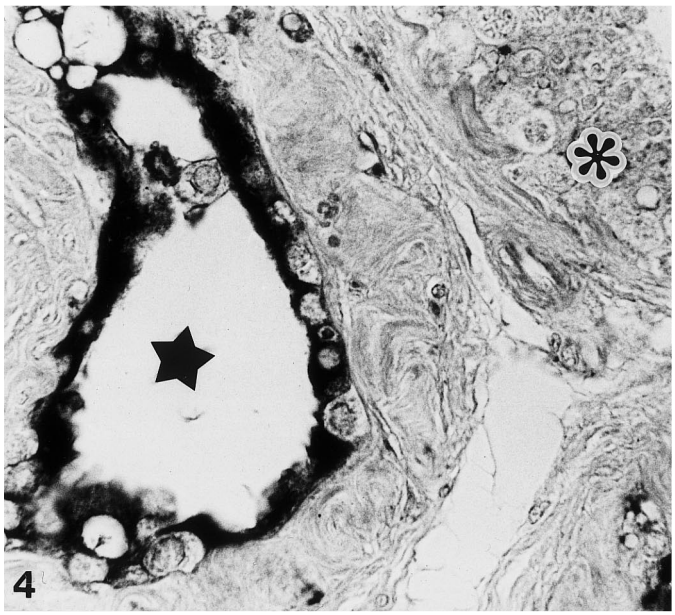

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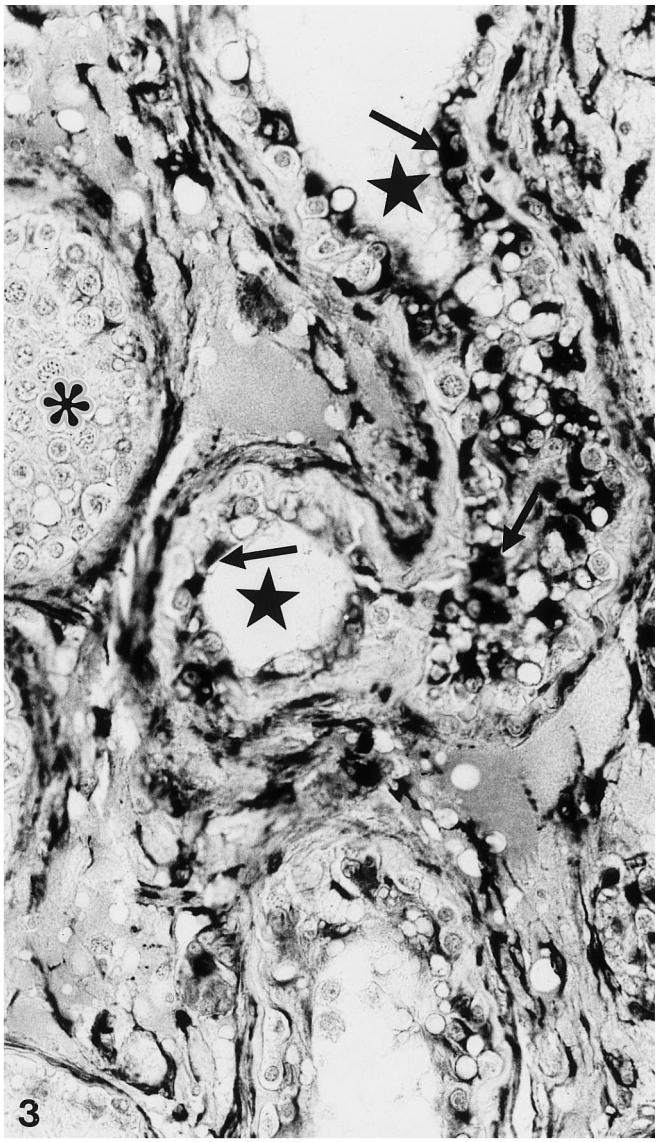

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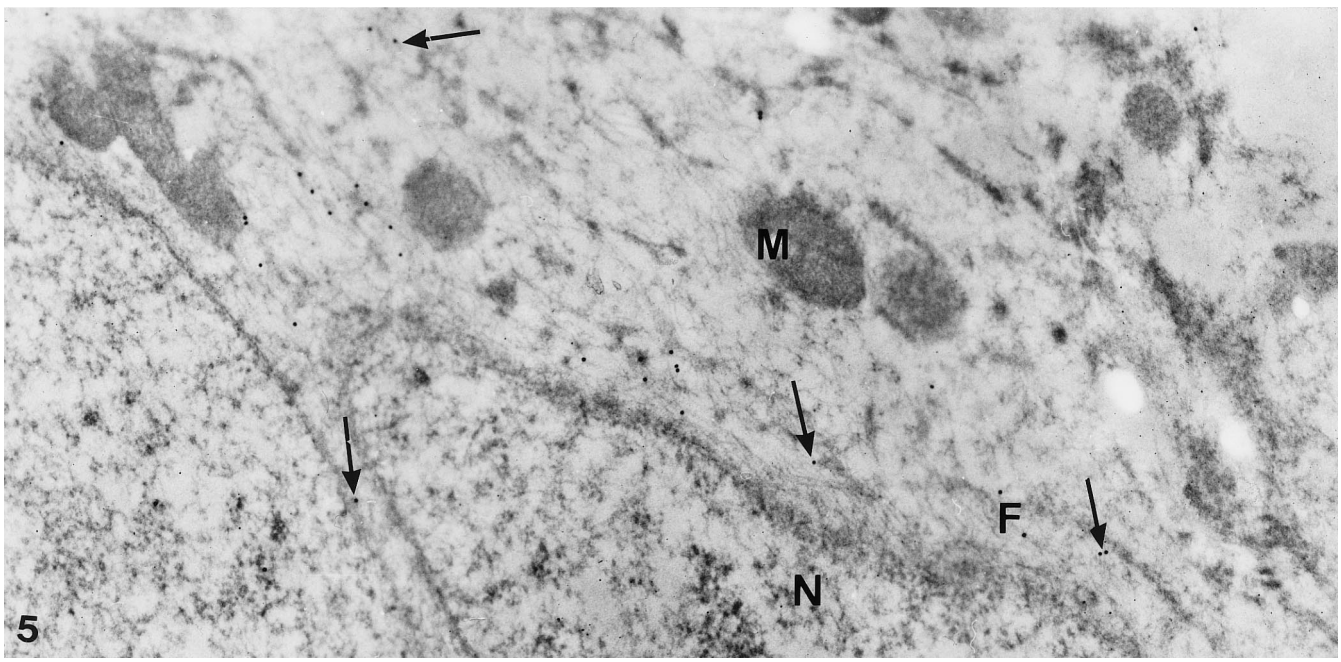

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Table 1 Comparison of Sertoli cell immunostaining intensity to vimentin and keratins between young control testes and two seminiferous tubule types of ageing men (*type a* seminiferous tubules showing complete spermatogenesis, *type b* seminiferous tubules showing maturation arrest at the level of spermatogonia or primary spermatocytes)

Seminiferous tubules	Average values per Sertoli cell (means±SD) in ultrastructural sections					
	Total cell surface (μm²)	Nuclear surface (μm²)	Surface occupied by lipids and vacuoles (μm²)	Surface occupied by lipid- and vacuole-free cytoplasm (μm²)	Vimentin immunolabelling (number of gold particles per total cell surface)	Keratin immunolabelling (number of gold particles per total cell surface)
Young men	128.81±38	31.52±4	6.93±3	90.36±28	905±280	–
Ageing testis type a	130.88±35	30.96±5	8.57±5	91.35±29	897±311	–
Ageing testis type b	169.73±45*	31.70±5	40.84±34*	97.20±44	1555±408*	869±211*

* $P \leq 0.05$ (differences between this value and the other values in the same column)

aged tubules (type b) presented a less elongated shape than those of young men, with extensive interdigitations between adjacent Sertoli cells, diminution of both rough and smooth endoplasmic reticulum, and a vacuolated appearance caused by a marked increase in the number and size of lysosomes and lipids. Electron microscopy immunolabelling to vimentin revealed that the immunohistochemical reaction was not concentrated in the perinuclear and subplasmalemmal cytoplasm but extended in similar proportions throughout the entire cytoplasm (Figs. 7, 8). When the intensity of immunolabelling was compared between the three groups of tubules, by counting the number of immunogold particles per square micrometre of cytoplasm (excluding nucleus and lipid droplets and vacuoles), it was found to be significantly higher ($P \leq 0.05$) in type b tubules of elderly men than either in tubules of young men or in type a tubules of elderly men. Background reaction and average Sertoli cell surface area were similar in the three tubule types (Table 1).

Sertoli cell immunogold labelling to keratin was found neither in young men (Fig. 9) nor in type a tubules of ageing men (Fig. 10). A positive immunohistochemical reaction to these intermediate filaments was observed in the Sertoli cells of type b tubules in elderly men, in which immunogold particles were localized throughout the cytoplasm (Figs. 10, 11). The number of immunogold particles per square micrometre of cytoplasm and per cell surface are shown in Table 1.

Fig. 2 Vimentin immunostaining in seminiferous tubules of a 31-year-old normal man. Immunohistochemical reaction is observed in the lamina propria (*arrowhead*) and in the Sertoli cell cytoplasm (*arrows*). ×215

Fig. 3 Vimentin immunostaining in seminiferous tubules of a 74-year-old normal man. Immunohistochemical reaction in Sertoli cells (*arrows*) is apparently more abundant in tubules with atrophic seminiferous epithelium defective in germ cells (*stars*) than in those showing germ cell development up to spermatids (*asterisk*). ×250

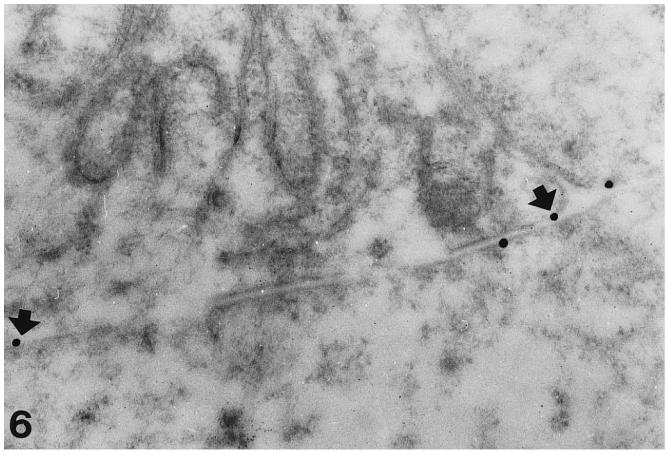
Fig. 4 Keratin immunostaining in the testis of a 77-year-old man. Immunohistochemical reaction is observed in the tubule showing only Sertoli cells and spermatogonia (*star*), but not in the tubule with complete spermatogenesis (*asterisk*). ×450

Fig. 5 Vimentin immunolabelling in a Sertoli cell of a 30-year-old man. Immunogold particles (*arrows*) are mainly concentrated around the nucleus (*N*) and bound to cytoplasmic intermediate filaments (*F*). (*M* mitochondria). ×33,000

Discussion

Vimentin immunolabelling was found to be increased in the Sertoli cells of elderly men in tubules with severe germ cell depletion. It might be suspected that the increase in vimentin filaments suggested by light microscopy immunostaining is only apparent, resulting from the increase in lipid inclusions and vacuoles that constrain the remaining cytoplasm and concentrate the filaments. However, the quantitative ultrastructural study reveals that the lipid- and vacuole-free cytoplasm is not reduced by germ cell loss, and thus, it may be accepted as a real increase in vimentin.

The possible causes and significance of such a vimentin increase are uncertain. It has been suggested that this increase might be related to an alteration in the cell morphology [12]. However, it has recently been shown that excessive amounts of human vimentin, up to fivefold normal, did not appear to alter epithelial cell morphology dramatically [3]. Therefore, the changes in Sertoli cell morphology in severely damaged tubules seem to be related to germ cell loss rather than to vimentin increase. This increase might also be caused by hormonal changes involving the testes. In most testicular disorders with a vimentin increase in the Sertoli cells, follicle-stimulating hormone (FSH) levels are elevated and testosterone levels are decreased [22]. In cultured rat Sertoli cells, vimentin is phosphorylated in an FSH-dependent manner and, during FSH stimulation, the vimentin distribution changes from a diffuse fibrillar network to a flat stellate appearance [32]. However, these hormonal changes are probably not the main factor responsible for vimentin increase in ageing Sertoli cells, because this is only observed in germ-cell-depleted tubules and not in adjacent tubules with complete spermatogenesis. The cause of vimentin increase is possibly related to local factors associated with the completion of spermatogenesis, that is, to a paracrine or autocrine response [31] rather than a change in the endocrine function. Among these factors might be the inhibin secreted by the Sertoli cell to control pituitary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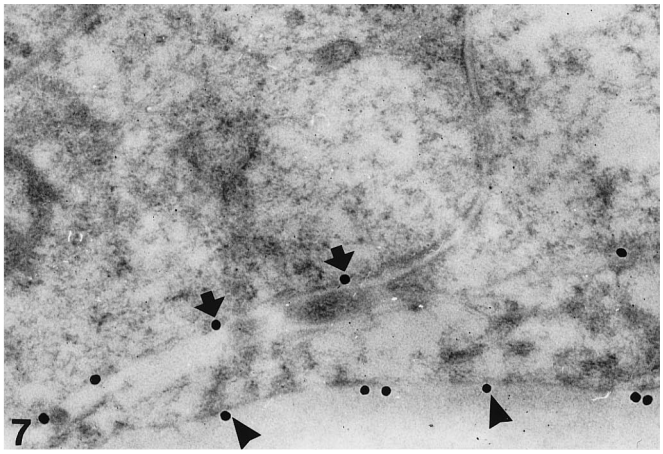
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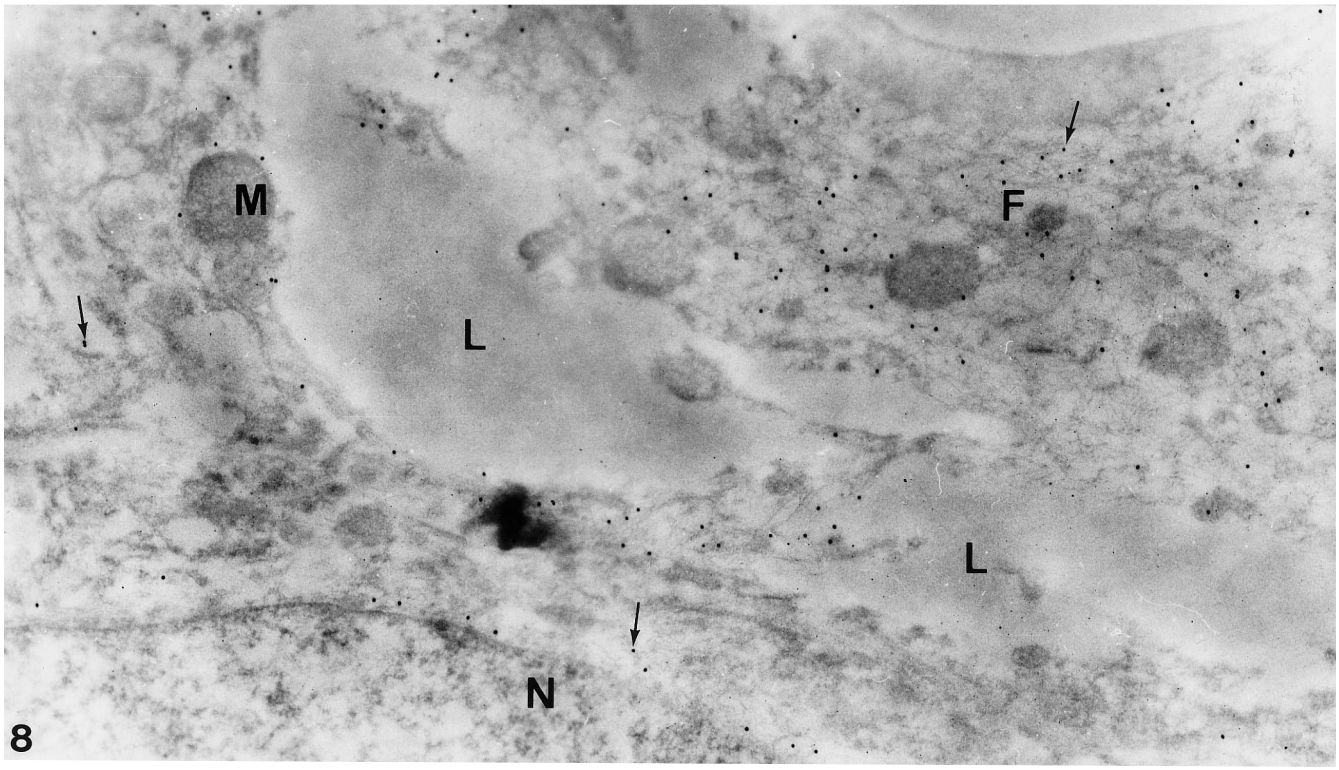

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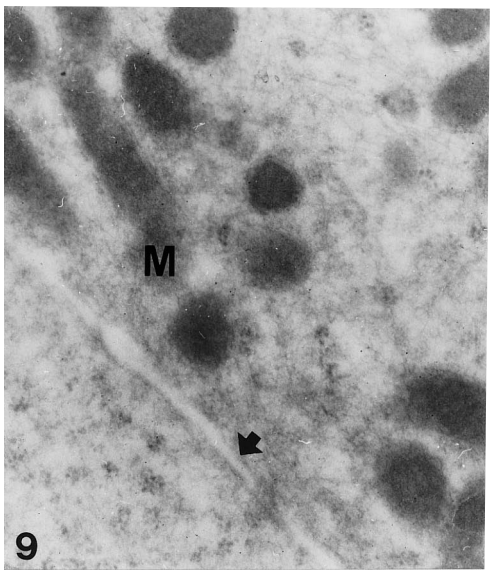

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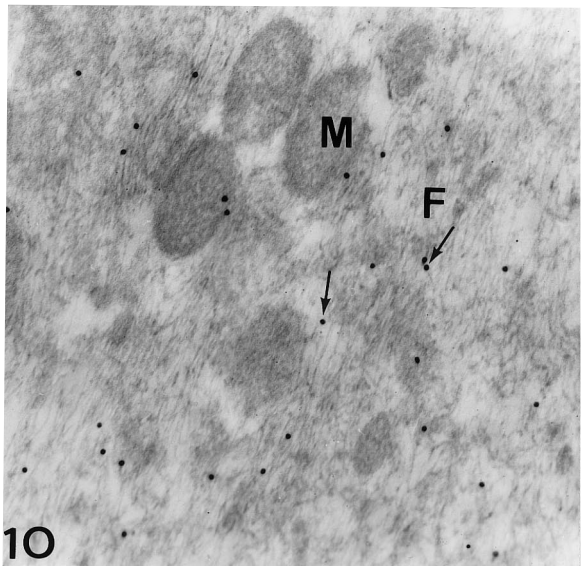

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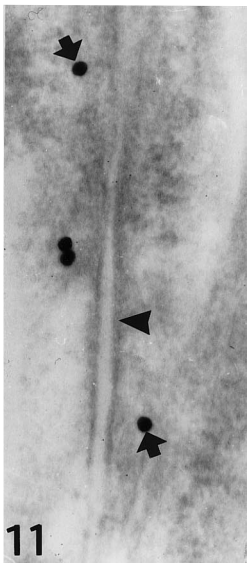
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FSH secretion, which is decreased in elderly men [34], and local factors secreted by pachytene spermatocytes, spermatids, and late spermatid residual bodies, causing functional changes in Sertoli cells [13, 28].

In the present study, keratin has been found in the Sertoli cells of elderly men's tubules with spermatogenetic arrest. In the Western blot study, the molecular weight of the single keratin band coincided with that of keratin no. 8 (52.5 kDa); this, together with keratin no. 18, are the only keratins reported in Sertoli cells.

The biological significance of keratin re-expression in the Sertoli cells is difficult to ascertain. The expression of keratins has been interpreted as an indicator of epithelial origin [26], and Aumüller et al. [6] assumed that, by expressing keratin filaments, Sertoli cells regain undifferentiated features. One of the reported alterations in ageing human Sertoli cells is a dedifferentiated pattern, with the appearance of immature (prepubertal) ultrastructural features [24]. It has been suggested that the presence of keratin immunoexpression in the basal and lateral cytoplasm of the Sertoli cells in infertile men might be related to the observation of multiple desmosome-like structures in these cells [7]. It has also been reported that Sertoli cell desmosome-like junctions are particularly well developed in the vicinity of testicular tumours [6]. Although the Sertoli cells of the ageing testes also showed keratin immunoexpression in these locations, we have not observed the development of desmosomes in these cells, and nor have we found any reference to it in the literature. The dedifferentiated Sertoli cells in the ageing testes maintain their specialized junctions, including the ectoplasmic specializations [24].

In mouse fibroblasts transfected with cDNA-encoding keratins, the coexistence of vimentin with keratins appears to enhance cell migration and invasion [8]. This would agree with the presence of keratins in testicular tumours [20]. However, ageing Sertoli cell are not prone to malignancy; testicular tumours usually occur in young men [36].

The disappearance of keratins in mammalian Sertoli cells after birth, when important developmental modifications take place, has suggested the existence of a relationship between testicular maturation and keratin expression [26]. In human fetuses, the observation of the appearance (12th week of fetal life) and involution (from the 20 week onwards) of Sertoli cell keratins [27] coincides with the reported rise and fall in the levels of gonadotropins and testosterone [9]. The possible influence of gonadotropins in keratin re-expression is in keeping with the elevated gonadotropin levels in ageing men but not with testosterone levels, which are usually decreased. However, as with vimentin increase, keratin re-expression in ageing testes only takes place in germ-cell-depleted tubules, and thus, it is more likely that such a re-expression is related to local factors.

An interesting observation is that Sertoli cells of adult patients with the Sertoli-cell-only syndrome did not stain for keratin [7]. This suggests that keratin expression in Sertoli cells requires the presence of spermatogonia and disappears when spermatogenesis is developed. We failed to corroborate this observation in the ageing testes, since at least isolated spermatogonia were present in all tubule types in which Sertoli cells were recognizable. It would be interesting to investigate the presence of keratins in fetal testes showing Sertoli-cell-only tubules.

◀ **Fig. 6** Parts of two adjacent Sertoli cells of a 32-year-old normal man that were immunolabelled for vimentin. Immunogold particles are concentrated along the intercellular junction (*arrows*) ×58,500

Fig. 7 Vimentin immunolabelling in Sertoli cells of a seminiferous tubule with germ cell development up to spermatogonia (74-year-old man). Numerous immunogold particles are seen in the cytoplasm beneath the intercellular junctions (*arrows*) and in the cytoplasm adjacent to the basal lamina (*arrowheads*). ×58,500

Fig. 8 Vimentin immunolabelling in a Sertoli cell of an atrophic tubule with germ cell development up to spermatogonia (77-year-old man). Immunogold particles (*arrows*) are more numerous and more homogeneously distributed throughout the cytoplasm than in Sertoli cells of young men (cf. Fig. 5; *N* nucleus, *M* mitochondria, *L* lipids, *F* intermediate filaments) ×33,000

Fig. 9 Sertoli cells of a 32-year-old man, showing no immunohistochemical reaction to keratin (*arrow* intercellular junctions, *M* mitochondria). ×44,000

Fig. 10 Keratin immunolabelling in a Sertoli cell of a seminiferous tubule with germ cell development up to spermatogonia (77-year-old man). Immunolabelling (*arrows*) is observed in the filaments (*F*) among mitochondria (*M*) ×44,000

Fig. 11 Keratin immunolabelling (*arrows*) near the plasma membranes of an intercellular junction (*arrowhead*) between Sertoli cells in the same testis. ×150,000

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