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ORIGINAL ARTICLE

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# Expression of vimentin, cytokeratin, and desmin in Sertoli cells of human fetal, cryptorchid, and tumour-adjacent testicular tissue

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**Abstract** The intermediate filament of mature human Sertoli cells is vimentin. A co-expression of vimentin together with cytokeratin has been demonstrated in Sertoli cells during embryonal development and under pathologic conditions in adult testes. We analysed the presence of vimentin, cytokeratin, and desmin in Sertoli cells of fetal testes ($n$=20), in seminiferous tubules of cryptorchid testes ($n$=10) and adjacent to testicular germ cell tumours ($n$=47) using specific monoclonal antibodies and single and double-labelling immunohistochemistry. During embryonal development prominent cytokeratin expression disappears after the 20th week of gestation. Interestingly, we also found desmin in immature intratubular Sertoli cells between weeks 11 and 14. In adult cryptorchid testes and in peritumour tubules, desmin was also prominently present in Sertoli cells in the vast majority of the cases investigated, as well as vimentin and cytokeratin co-expression. This first description of desmin immunoreactivity may shed some light on the ontogeny of human Sertoli cells and demonstrates that this cell type is able to express three types of intermediate filaments in a complex manner.

**Key words** Human Sertoli cells · Intermediate filaments · Cytokeratin · Vimentin · Desmin

## Introduction

Numerous studies have established intermediate filaments (IF) as markers for cell differentiation and identification of the origin and type of various cells and tissues [9, 15 ,20]. It has generally been accepted that vimentin is found in cells of mesenchymal origin, desmin in muscle, and cytokeratins in epithelial cells [14, 26]. However, an increasing number of reports indicate the expression of two or more different IF within the same cell. This coexpression phenomenon has been found physiologically in various fetal tissues during embryonal development, but also under certain pathologic conditions in reactive, degenerative, and proliferative lesions, as in malignant tumours (for reviews, see [7, 17, 18, 28]).

Sertoli cells (SC) are involved in genital ridge differentiation as well as in the regulation of spermatogenesis and spermiogenesis, and are an important component of the blood-testes barrier (for reviews, see [13, 24]). Morphologically, distinct nuclear features distinguish between prepubertal SC – also designated as pre-Sertoli or immature SC – and postpubertal or mature SC [19]. At the intermediate filament level vimentin has been demonstrated in normal mature SC exclusively [8]. However, during the development of the human testes and SC maturation cytokeratins and vimentin are transiently coexpressed [4, 27]. Their simultaneous occurrence has also been described under certain pathologic conditions, including cryptorchidism, severe disturbances of spermatogenesis [1, 2, 27] and in tubules adjacent to testicular germ cell tumours [1, 16, 25, 27]. The presence of the intermediate filament desmin reported in SC precursors in fetal rats, which might shed some light on their ontogeny [10], has yet not been described in fetal or adult human SC.

We have investigated the expression of the intermediate filaments cytokeratin, vimentin, and desmin in human SC in both fetal and adult testes, including cryptorchid gonads and parenchyma adjacent to germ cell tumours either harbouring atypical spermatogonia or exhibiting SC only. Our findings that human SC can express all three intermediate filaments under certain conditions indicate that this cell type reacts to tissue alterations with complex changes of its cytoskeletal proteins.

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**Table 1** Specimens analysed

| Specimen data | *n* |
|---|---|
| Fetal testes | 20 |
| Cryptorchid testes | 10 |
| *Testicular tumours* | 47 |
| I Seminoma | 17 |
| II Differentiated teratoma | 2 |
| III Malignant teratoma | |
| Intermediate malignant teratoma | 8 |
| Malignant undifferentiated teratoma | 6 |
| Malignant trophoblastic teratoma | 3 |
| IV Seminoma and teratoma | 11 |

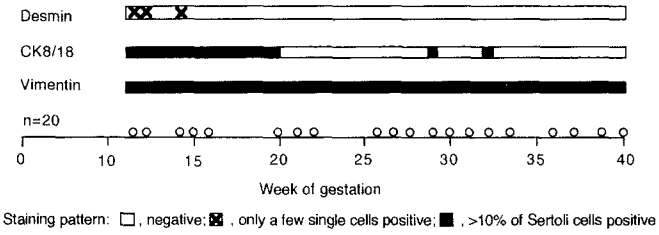


**Fig. 1** Intermediate filament expression of Sertoli cells during embryonal development

## Materials and methods

### Tissues

Fetal testes tissue for this study came from 20 late abortions and preterm deliveries; the gestational age, ranging from week 11 to week 40, was determined on the basis of crown–rump length or the respective clinical information. Furthermore, 10 orchiectomy specimens from patients operated on for unilateral postpubertal cryptorchidism and 47 testicular germ cell tumours were available. The testicular germ cell tumours were diagnosed according to the British Testicular Tumour Panel and Registry [22]. Tumour-adjacent parenchyma was studied in selected tissue blocks each containing more than 50 cross-sectioned seminiferous tubules affected by intratubular carcinoma in situ or SC-only syndrome. The specimens analysed are specified in Table 1.

### Immunohistochemistry

Immunohistochemistry was performed either on consecutive sections of the selected blocks or by double labelling techniques. Briefly, the slides were deparaffinized and rehydrated. After blocking of endogenous peroxidase activity [12] and microwave antigen retrieval [23] the following monoclonal antibodies were applied in appropriate dilutions: anti-desmin (D33, DE-U 10), anti-vimentin (V9), anti-cytokeratin (CK)8 (35ßH11), and anti-CK18 (DC10). The streptavidin-biotin-peroxidase complex and diaminobenzidin were used as detection system. With the exception of anti-desmin, clone DE-U 10 (Sigma Biochemicals; Munich, Germany), all antibodies and secondary reagents were purchased from Dakopatts, Glostrup, Denmark. Sections were counterstained with haemalaun and mounted with coverslips.

To demonstrate the co-localization of different intermediate filaments in the same cell, sequential double-labelling immunohistochemistry was applied in selected cases. Briefly, antigen detection by the streptavidin-biotin-peroxidase method described above was followed by incubation with another primary antibody to the respective IF and visualized using the alkaline phosphatase/anti-alkaline phosphatase system (APAAP; Dakopatts) [3]. The APAAP reaction was developed with Fast Red BB salt and naphthol AS-B1 phosphate containing Levamisole (all reagents from Sigma, Munich, Germany). Both the biotynilated antibody (final dilution 1:500) and the streptavidin-peroxidase complex (final dilution 1:800) as well as the APAAP complex (final dilution 1:50) were soluted in TRIS-buffered saline and pooled in human serum mixed 1:1(vol/vol); incubation time was 30 min for all steps [6]. Finally, the slides were counterstained with haemalaun and mounted in Kayser's glycerine gelatin. Immunostaining was evaluated by light microscopy; in fetal testes and in cryptorchid testis and in tubules adjacent to germ cell tumours the staining results of SC were scored semiquantitatively (Fig. 1, Table 2).

Immunocytochemical staining of peritubular myoid cells and blood vessels served as positive internal controls for desmin and vimentin, rete testis, mesothelium and epithelial tumour components for cytokeratins. For negative controls the respective primary antibodies were omitted.

**Table 2** Intermediate filament profile of Sertoli cells in cryptorchid testis and parenchyma adjacent to testicular germ cell tumours (++ positive Sertoli cells in >50% of altered tubuli seminiferi, + positive Sertoli cells in <50% of affected tubuli evaluated, - negative)

| | *n* | Vimentin (++/+/-) | CK 8/18 (++/+/-) | Desmin (++/+/-) |
|---|---|---|---|---|
| Cryptorchid testes | 10 | 10/0/0 | 0/ 4/6 | 2/ 7/1 |
| Testicular tumours | | | | |
| Seminoma | 17 | 17/0/0 | 1/11/5 | 3/14/0 |
| Differentiated teratoma | 2 | 2/0/0 | 0/ 0/2 | 0/ 0/2 |
| Malignant teratoma | 17 | 17/0/0 | 12/ 5/0 | 7/10/0 |
| Seminoma and teratoma | 11 | 11/0/0 | 4/ 7/0 | 2/ 9/0 |

## Results

### Fetal testes

The results of IF expression in fetal testes are summarized in Fig. 1. A prominent vimentin expression of tubular SC was seen throughout the specimens covering the period from week 11 to week 40. A co-expression of vimentin with low-molecular-weight cytokeratins was primarily detected through weeks 11–20 and was only very occasionally found in later gestational stages (Fig. 2a). The presence of desmin was restricted to single SC in the three cases analysed within weeks 11–14 (Fig. 2b). Immunoreactivity for all three IF was also detectable in the coelomic epithelial cells.

### Cryptorchid testis

Postpubertal cryptorchid testes showed characteristic histopathologic changes of the seminiferous tubules, mostly exhibiting a "Sertoli cell only syndrome" with few intermingled tubuli and with heavily disturbed spermatogenesis. In general, SC had an immature appearance, with elongated or round, regularly outlined nuclei in a pseudostratified disposition. The intermediate filament expression is summarized in Table 2. Vimentin was most prominently and homogeneously expressed in all

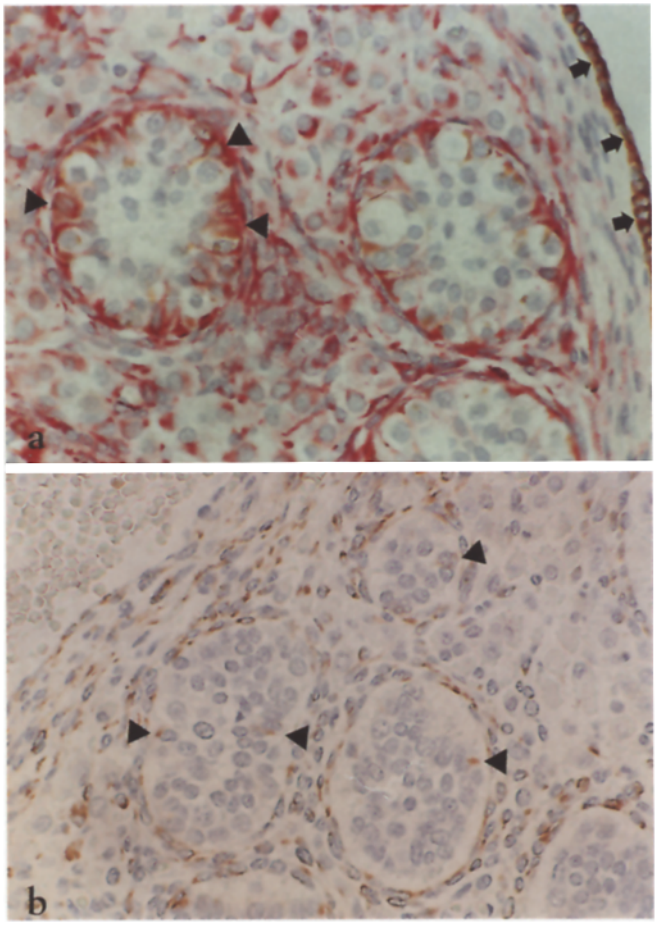


**Fig. 2a, b** IF expression in human fetal testis. **a** Co-expression of vimentin (APAAP, *red*) and cytokeratin 8 (peroxidase, *brown*) in human fetal testes of gestational week 14 (*triangles*); in addition, mesothelial cells (*arrows*) exhibited a prominent cytokeratin 8 immunoreactivity (*brown*) as well as a slight vimentin expression (*red*). ×400. **b** Perinuclear desmin immunoreactivity in few intratubular Sertoli cells in week 11 (*triangles*). ×600

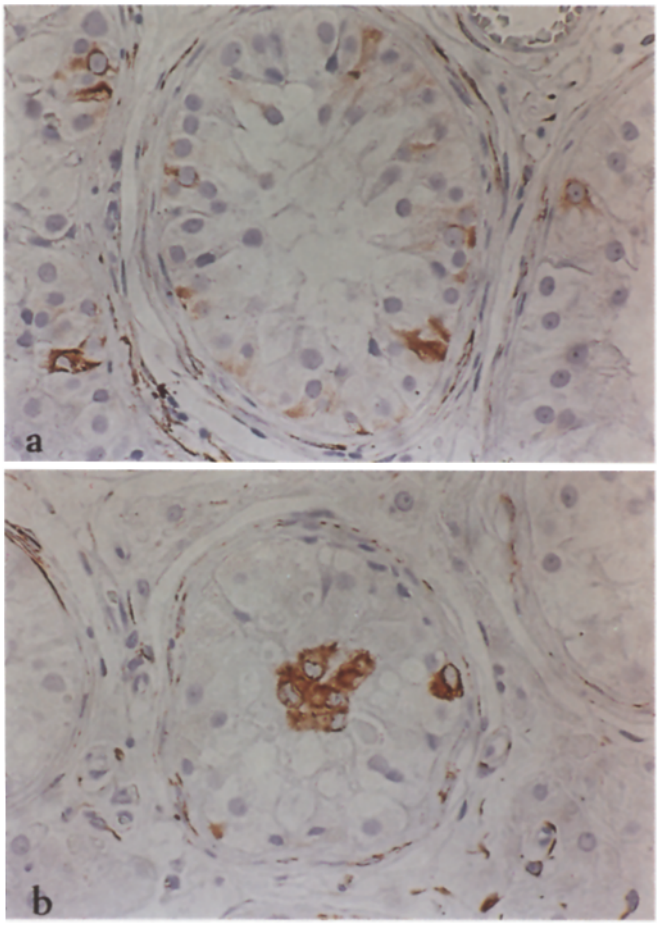


**Fig. 3** Desmin-positive Sertoli cells in a cryptorchid testes **a** located close to the basement membrane, and **b** shed into the tubular lumen. ×400

cases. The expression of cytokeratin was generally weak, restricted to single cells in few tubules, and detectable in SC of 4/10 cases. Desmin was present in SC dispersed throughout the testicular parenchyma in nine of ten cryptorchid testes. This desmin immunoreactivity was found predominantly in SC adjacent to the basement membrane (Fig. 3a), but also in clustered SC shed into the tubular lumen (Fig. 3b). Double-labelling immunohistochemistry revealed co-expression of IF for vimentin and desmin (Fig. 4a) as well as for vimentin and cytokeratin (Fig. 4b). In addition, a cellular co-localization for desmin and cytokeratin was seen (Fig. 4c).

## Parenchyma adjacent to testicular germ cell tumours

With the exception of two mature teratomas the testicular parenchyma adjacent to the tumours displayed severe pathologic changes, including intratubular germ cell neoplasia and/or exclusively SC with an immature morphologic appearance. As in cryptorchid testes, a complex pattern of intermediate filament expression of SC was also observed in seminiferous tubules, as summarized in Table 2. However, SC with strong cytokeratin expression were much more frequently seen than in cryptorchid testis. Desmin immunoreactivity was found in a similar pattern (close to the basement membrane), but also prominently in SC *clusters* within the tubular lumen. Double-labelling IHC again demonstrated the co-expression in SC of all three intermediate filaments (Fig. 5a, b).

## Common findings

In all specimens investigated from fetal, cryptorchid or tumour-adjacent parenchyma, the immunoreactivity for CK 8 and 18 was identical, albeit generally weaker for CK18 than for CK8. Furthermore, the immunoreativity for desmin was essentially the same with two different clones of monoclonal antibodies when tested in selected cases (not shown).

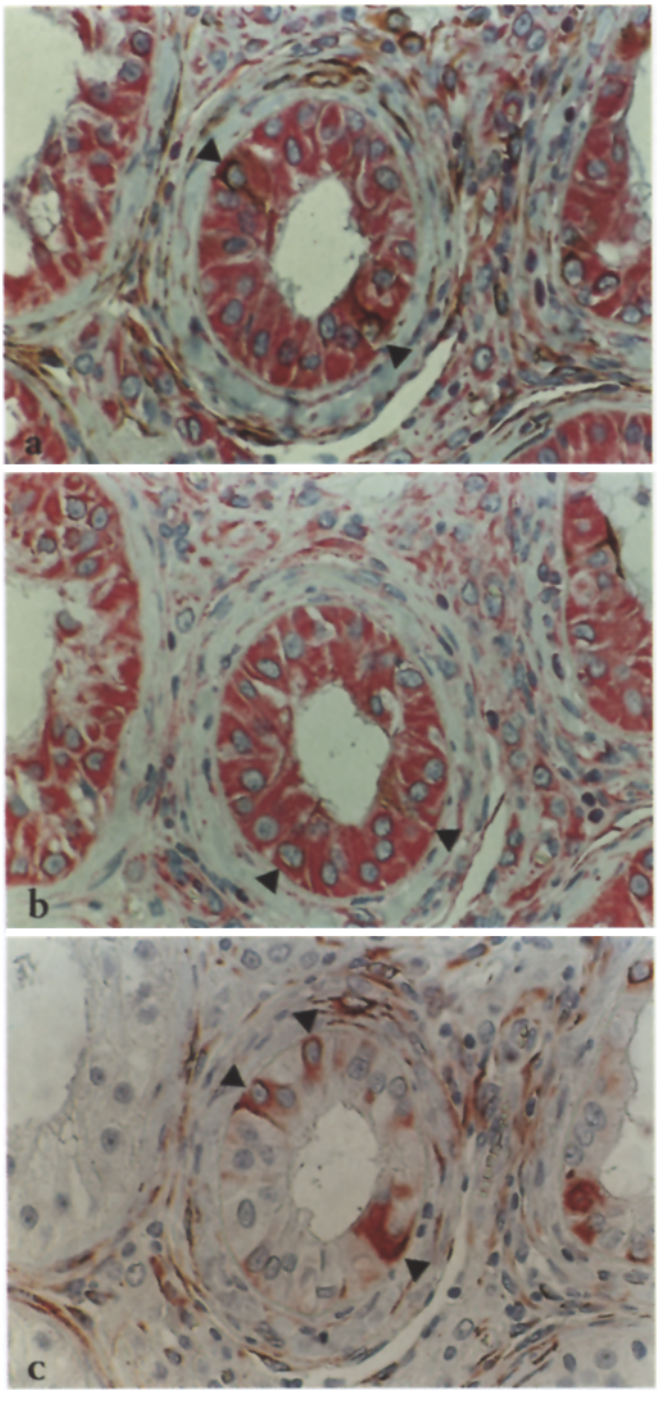


**Fig 4** Double-labelling immunohistochemistry on consecutive sections in a cryptorchid testes for **a** vimentin (APAAP, *red*) and desmin (peroxidase, *brown*), **b** vimentin (APAAP, *red*) and cytokeratin 8 (peroxidase, *brown*), and **c** cytokeratin 8 (APAAP, *red*) and desmin (peroxidase, *brown*); **a–c** Sertoli cells with co-expression of IFs are marked by *triangles*. ×400

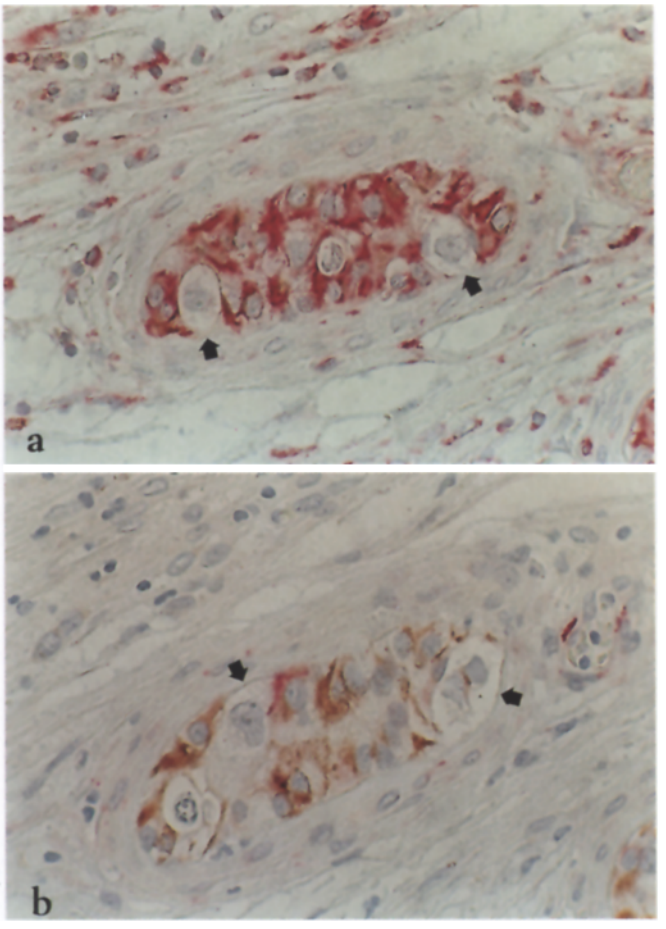


**Fig 5** Double-labelling immunohistochemistry on consecutive sections in a tubule adjacent to a testicular tumour exhibiting atypical spermatogonia (*arrows*): **a** vimentin (APAAP, *red*) and cytokeratin 8 (peroxidase, *brown*); **b** desmin (APAAP, *red*) and cytokeratin 8 (peroxidase, *brown*). ×400

## Discussion

The present study demonstrates a complex pattern of intermediate filament expression in SC during embryonal development and under various pathologic conditions. In agreement with previous findings, co-expression of vimentin and cytokeratins was observed in fetal and cryptorchid testes as well as in tubules adjacent to germ cell tumours. Furthermore, we describe for the first time the expression of desmin in human SC during testicular development and in testicular disorders.

During the fetal period we observed a prominent co-expression of vimentin and cytokeratin in SC up to a gestational age of 20 weeks. Furthermore, our data indicate a dramatic drop in the expression of CK in SC at a gestational age of about 20 weeks, while only a few weakly stained SC can be detected at later gestational ages. This is in accordance with two previous studies reporting transient co-expression of cytokeratins 8 and 18 together with vimentin in functionally not yet differentiated, immature SC [4, 27]. An important additional finding was the presence of desmin-positive intratubular SC in fetal testes of weeks 11–14. This is notable, because with the exception of a single observation on extratubular SC precursors positive for desmin in an embryo of 8 weeks' ge-

stational age [4], such a finding has only been reported in animals. Thus, in male rats a transient co-expression of vimentin and desmin and a later switch from desmin to cytokeratin as being concomitant with early SC differentiation has been described [10].

Two main theories on the origin of SC have been propagated. Some authors favour their derivation from coelomic epithelium, whereas others argue that SC originate from mesonephric blastema or both [21, 29]. The *desmin* and *cytokeratin reactivity* detected in immature SC during early embryonic development would fit both hypotheses – the derivation of SC precursors from coelomic epithelium and their dual origin.

In general, co-expression phenomena of all three intermediate filaments have been found in several fetal tissues, including mesothelium and intestinal, lingual, and myocardial muscle cells before stabilization of the final cell-type-specific IF, which for SC is vimentin [8, 28]. We also observed an expression of vimentin, cytokeratin, and desmin in the mesothelial lining of the fetal testes in line with such previous findings. In pathologic conditions such as cryptorchidism, severely disturbed spermatogenesis, and *tubular changes* adjacent to germ cell tumours, our data – like those recorded in previous studies – have confirmed a shift of the intermediate filament expression in SC, resulting in a reappearance of CK8 and CK18 [2, 16, 25, 27]. Whether this reflects a dedifferentiation phenomen with re-expression of CK or a persistence of immature SC in altered testes is still not known. Our observations of an additional co-expression of desmin together with cytokeratin within the same SC (as shown by double-labelling immunohistochemistry in cryptorchid testes and also in peritumour tubules) are more suggestive of a progressive re-acquisition of a more undifferentiated phenotype. It was also interesting to note that an expression of all three IF was present in SC clusters shed into the tubular lumen. The reasons for these complex focal changes of SC are not understood, however. Changes in the microenvironment are likely to be involved. These could include alterations of the basement membrane composition [5, 11] and perturbations of peritubular myoid cells secreting paracrine factors [24], both of which are necessary for the induction and maintenance of SC differentiation. Finally, an impaired interaction of SC with abnormal germ cells or a complete lack of spermiogenesis could also induce the observed modulation of SC intermediate filament expression.

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