

Analysis of benign neoplasms of the rete testis formerly termed “Sertoliform cystadenomas” demonstrates that they are not Sertoli cell tumours with intra-rete growth

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Analysis of benign neoplasms of the rete testis formerly termed “Sertoliform cystadenomas” demonstrates that they are not Sertoli cell tumours with intra-rete growth

Aims: Benign tumours of the rete testis include mostly cystadenomas and adenomas. A subset with tubular or tubulopapillary architecture shows morphological similarities to Sertoli cell tumours; these neoplasms were previously termed “Sertoliform cystadenomas of the rete testis”. In the most recent WHO classification, they have been interpreted as Sertoli cell tumours, not otherwise specified (NOS), with pure intra-rete growth, and therefore excluded as an entity. The remaining cystadenomas of the rete testis vaguely resemble tumours of Mullerian origin arising in the ovaries. In this study we analyse benign tumours of the rete testis, including a subset with Sertoliform features.

Methods and Results: Benign neoplasms of the rete testis were identified through query of consultation

and institutional files. Clinicopathologic data were collected, and available slides were reviewed. Cases were assessed using IHC and three separate DNA sequencing panels. Eleven tumours from patients 32–78 years old were evaluated. Four were classified as Sertoliform adenomas/cystadenomas, displaying tubulo-papillary or tubular/trabecular architecture; all of them were PAX8-positive and lacked nuclear beta-catenin expression. The remaining seven tumours were benign cystadenomas NOS. Genomic analysis was performed successfully in 10/11 tumours (including all Sertoliform adenomas/cystadenomas) and revealed no pathogenic variants in *CTNNB1*, *KRAS*, or *BRAF*.

Conclusion: Sertoliform cystadenomas of the rete testis differ from Sertoli cell tumours NOS, as evidenced

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Abbreviations: IHC, immunohistochemistry; NOS, Not otherwise specified; WHO, World Health Organization.

by the absence of molecular markers characteristic of Sertoli cell tumours. The remaining benign

cystadenomas lack molecular alterations seen in Mullerian tumors of the ovaries.

Keywords: adenomatous hyperplasia rete, orchiectomy, paratestis, rete testis, Sertoliform cystadenoma, testicular neoplasm

Introduction

Benign tumours of the rete testis are rare and include mostly cystadenomas and adenomas with papillary or tubulopapillary architecture. A subset of the latter was previously termed Sertoliform cystadenoma of the rete testis, given their partial morphologic resemblance to Sertoli cell tumours.^{1,2} However, unlike Sertoli cell tumours not otherwise specified (NOS), Sertoliform cystadenomas of the rete testis demonstrate pure intra-rete growth patterns.^{1,3,4} In the latest edition of the World Health Organization (WHO) classification of urinary and male genital tumours, Sertoliform cystadenomas were considered Sertoli cell tumours NOS growing within the rete testis, and therefore excluded as an entity.⁵

Although most benign neoplasms of the rete testis are readily recognized as such, some adenomas/Sertoliform cystadenomas may require exclusion of malignant tumours that are significantly more frequent, including metastatic carcinomas from different primary sites. However, the histopathologic, immunophenotypic, and molecular characteristics of these benign neoplasms of the rete testis remain largely undescribed. In this study we provide an overview of our experience with 11 benign tumours of the rete testis, including morphologic, immunophenotypic, and molecular data.

Materials and methods

This study was performed with approval of the Institutional Review Board (IRB) of Indiana University (protocol #18697, 2023) and the remaining participating institutions (when applicable).

CLINICOPATHOLOGIC FEATURES AND IHC OF THE STUDY CASES

Institutional and personal consultation files were queried to identify benign neoplasms of the rete testis. Relevant clinicopathologic findings of all the cases were collected from electronic medical records and pathology reports, including patient's age,

clinical presentation, procedure, and tumour size. Available haematoxylin and eosin-stained slides were reviewed in all cases. Immunohistochemistry (IHC) studies were performed on a representative section per case using the antibodies listed in Table 1. In some cases, the contributing pathologist provided results of IHC stains performed during the original diagnostic work-up.

GENOMIC ANALYSIS

Tumour tissue was manually dissected from unstained formalin-fixed paraffin-embedded tissue sections. DNA was extracted following standard laboratory protocols; sequencing was performed at the Solid Tumour Molecular Pathology Laboratory at the University of Bologna Medical Center using three different laboratory-developed panels that analyse the genomic regions reported in Table S1 (human reference sequence hg19/GRCh37). Briefly, ~30 ng of DNA was used per each panel to amplify sequencing libraries using the AmpliSeq Plus Library Kit 2.0, according to the manufacturer's recommendations (Thermo Fisher Scientific, Waltham, MA, USA). Sequencing was performed on an Ion S5 Prime machine (Thermo Fisher Scientific) and the results were analysed with the Ion Reporter tool (v5.20—Thermo Fisher Scientific). As described in the validation study,⁶ single nucleotide variants / Indels present in at least 5% of the generated reads and observed in both strands were considered for variant calling. Sequencing results were further assessed for clinical and biologic relevance by one

Table 1. Immunohistochemistry antibody

Antibody	Clone	Dilution	Vendor
PAX-8	MRQ-50	RTU	Cell Marque
WT1	6F-H2	RTU	Dako
CDX2	DAK-CDX2	RTU	Dako
Beta-catenin	14	RTU	Cell Marque

RTU, ready to use.

of the authors with expertise in molecular pathology (D.dB.).

Results

CLINICOPATHOLOGIC FEATURES OF THE CASES

Eleven tumours from 11 patients 32–78 years old (mean, 56.7 years; median 57.5 years) were included in the study (Table 2). Of the five patients with available clinical information, four (80%) presented with testicular or scrotal masses, all treated by orchiectomy; one tumour was found incidentally in a paratesticular resection specimen for spermatocele repair. Past medical history was significant for: suspected/possible Klinefelter syndrome, bilateral undescended testes, and hypogonadism (case #6), with

preoperative serum markers within normal limits. Tumour sizes ranged from 1.2 to 7.0 cm (mean, 3.0 cm; median 3.1 cm).

Four cases showed morphologic features compatible with so-called “Sertoliform adenoma/cystadenoma” of the rete testis. More specifically, these tumours were solid (one case) or solid and cystic (three cases), and well-circumscribed. Confinement within expanded profiles of the rete testis was clearly identifiable in three tumours. Architecture was tubulo-papillary (three tumours, Figure 1A–C), or tubular and trabecular (one tumour, Figure 1D,E), reminiscent of a Sertoli cell lesion. Tubules were elongated and branching, often showing continuity with papillary structures. Papillae were slender and showed occasional branching; in one case, the fibrovascular cores showed prominent hyalinization consistent with

Table 2. Summary of clinical and pathologic features of the study cases

Case no.	Age	Clinical presentation	Diagnosis	Tumour size (cm)	IHC*	
					Positive	Negative
1	53	NA	Benign cystadenoma	NA	PAX8, WT1	CDX2
2	37	NA	Benign cystadenoma	NA	CK20, CDX2 (focal)	PAX8, WT1, AR
3	48	NA	Benign cystadenoma	1.5	CDX2	PAX8, WT1
4	32	NA	Benign cystadenoma with mucinous epithelium	1.2	CDX2	PAX8, WT1
5	60	NA	Benign cystadenoma with focal papillary structures	3.3	PAX8 (rare), WT1	CDX2
6	59	Testicular mass	Benign cystadenoma with ciliated cells	4.3		PAX8, WT1, CDX2
7	67	Scrotal mass	Benign cystadenoma with mucinous epithelium	7.0	AE1/AE3	PAX8, WT1, CDX2, calretinin
8	78	Incidental (found during spermatocele repair)	Adenoma of rete testis (Sertoliform)	3.0	Pancytokeratin, CK7, EMA, vimentin, AR, SOX9 (patchy)	PAX8, WT1, CDX2, SALL4, inhibin, calretinin, WT1, D2-40, Melan-A, S100, ER, PR, SYN, CG, GATA3, CK5, CK20, TTF1, PSA, PRAP, PSMA, SF1
9	56	Testicular mass	Adenoma of rete testis (Sertoliform)	1.6	PAX8 (diffuse) CK7 (patchy)	Beta-catenin (membranous), p63, inhibin
10	77	Testicular mass	Adenoma of rete testis (Sertoliform)	NA	PAX8 (diffuse), CK7 (multifocal), CA9	CDX2, PSA, NKX3.1, TTF1, GATA3, CDX2, RCC, calretinin
11	NA	NA	Adenoma of rete testis (Sertoliform)	NA	NA	NA

NA, not available; RO, radical orchiectomy.

*Immunohistochemistry performed as part of the initial diagnostic workup.

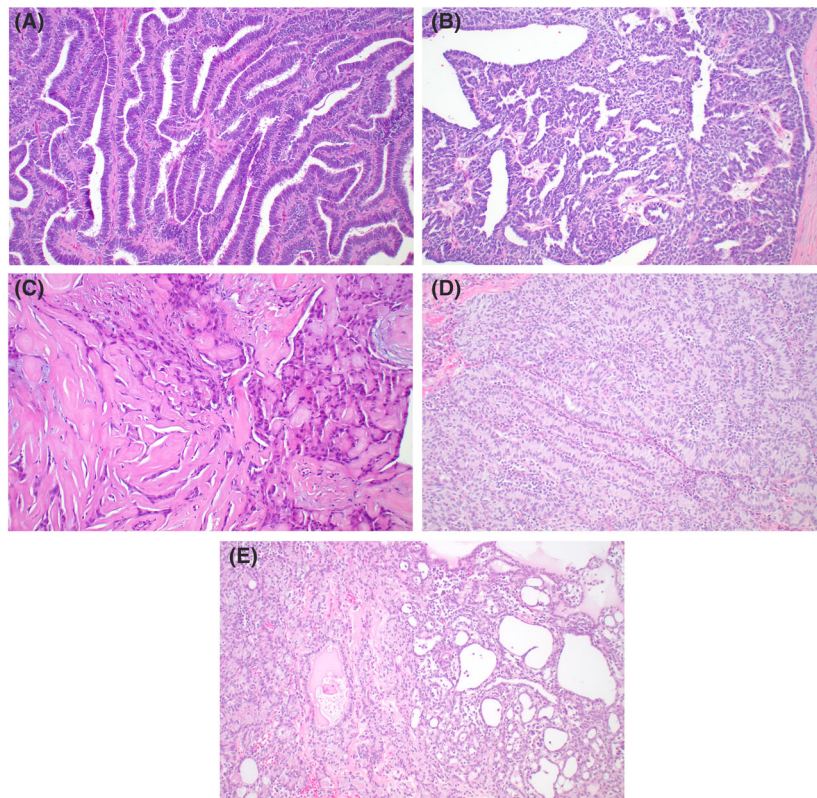


Figure 1. “Sertoliform” adenoma/cystadenoma of the rete testis. A,B: The tumours displayed elongated, branching tubules, often continuous with slender papillary structures that occasionally branched. C: Fibrovascular cores showed marked hyalinization, suggestive of abundant basement membrane material from one tumour. D,E: Solid areas displaying tubular and trabecular growth patterns. The trabeculae exhibited a parallel arrangement of epithelial cells, reminiscent of a “train track” in certain regions, along with a prominent intratumoral lymphocytic infiltrate. Some tubules contained hyaline globules.

deposits of abundant basement membrane material. One tumour was solid, with tubular and trabecular growth patterns. In this lesion, trabeculae showed a parallel arrangement of epithelial cells, focally resembling a “tram track”, with prominent intratumoral lymphocytes and occasional hyaline globules. Tumour cells were eosinophilic, cuboidal to columnar with minimal to mild atypia; nuclei were oval, elongated, or slightly irregular containing fine chromatin. Nucleoli were inconspicuous, and mitotic activity was consistently low (<1 mitotic figure per 10 high-power fields). Necrosis, invasive growth, or angioinvasion were not identified. In three of these tumours, areas of transition between the flat nonneoplastic epithelium of the rete and the neoplastic epithelium of the adenoma were clearly identifiable (Figure 2A–C), arguing against a Sertoli cell origin of these lesions. Immunohistochemistry demonstrated that all of them expressed PAX8 diffusely (Figure 3A). Expression of beta catenin was restricted to the cytoplasmic membrane in all cases (4/4; Figure 3B), with no tumour

showing nuclear expression of the marker. Additional IHC results are described in Table 3.

The remaining seven tumours were entirely cystic and classified as benign cystadenomas. The lining epithelium contained mucinous cells in two tumours (Figure 4A), a mixture of mucinous and ciliated columnar cells in one tumour (Figure 4B); and cuboidal to short columnar cells in the remaining four tumours. Based on their microscopic appearance, the cysts seemed unilocular/simple (i.e. they lacked internal septations) and were lined by a single layer of epithelial cells. Focal stubby, branching papillae were seen in one neoplasm that did not contain mucinous cells (Figure 5A,B). Some lesions showed inflammatory cells (within the mucin) and/or evidence of reaction to extravasated mucin. True stratification was not seen, although pseudostratification occurred focally, including in the tumour with stubby papillae. Of note, in the latter, the epithelium lining the papillae showed more significant nuclear atypia, with conspicuous nucleoli, than the

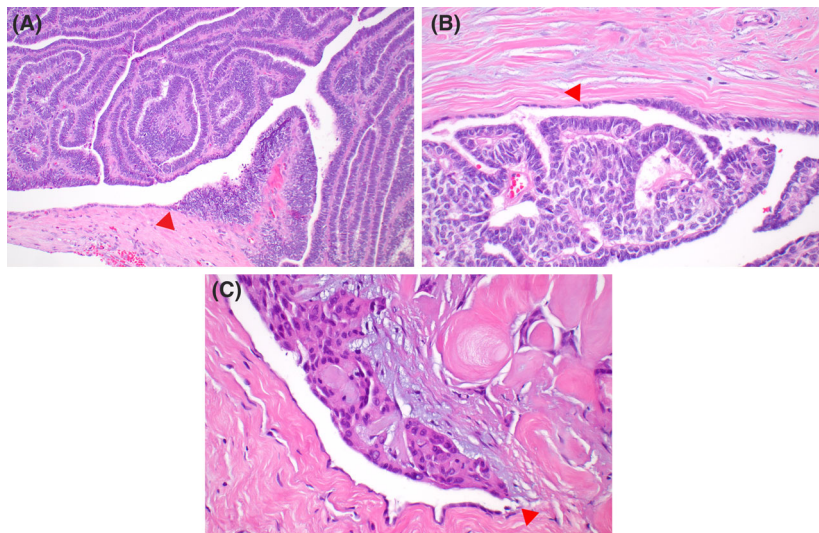


Figure 2. “Sertoliform” adenoma/cystadenoma of the rete testis. A–C: Transition areas between the flat, nonneoplastic epithelium of the rete and the neoplastic epithelium.

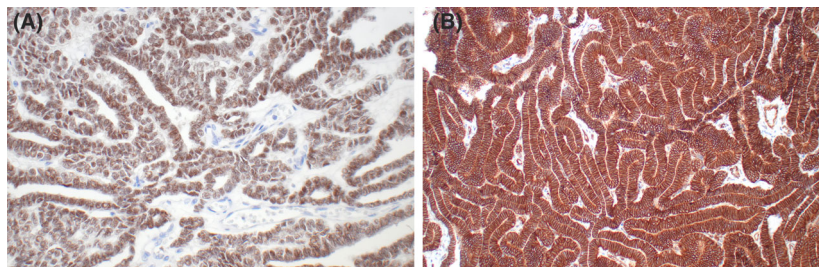


Figure 3. “Sertoliform” adenoma/cystadenoma of the rete testis. A: PAX8 showed diffuse nuclear expression. B: Beta catenin expression restricted to the cytoplasmic membrane.

remaining neoplasms in this group (Figure 5B). Very focal areas with ovarian-like stroma were seen in only two tumours (Figures 5 and 6). Mitotic activity was consistently low (below one mitotic figure per 10 high-power fields), and necrosis, invasive growth, or lymphovascular invasion were not seen. All lesions were tested *de-novo* for PAX8, WT1, and CDX2. Immunohistochemistry demonstrated that PAX8 was expressed in scattered cells of 1/7 tumours, WT1 in 2/7 tumours, and CDX2 in 2/7 tumours (Table 2).

GENOMIC ANALYSIS

Genomic analysis was successful with at least one of the three panels in 10/11 tumours (including all Sertoliform cystadenomas) (Table 3). Pathogenic variants were not identified in this series; notably, no pathogenic *CTNNB1*, *KRAS*, and *BRAF* variants were identified in any of the

Sertoliform adenomas/cystadenomas and benign cystadenomas, respectively. Given their morphologic features, lesional cellularity of most mucinous tumours was low (the presence of variants was checked manually—D.dB.).

Discussion

Benign neoplasms of the rete testis comprise a spectrum of predominantly cystic or solid and cystic lesions that have been classified as different entities. A subset of them was formerly called “Sertoliform cystadenomas” of the rete testis due to their partial resemblance to Sertoli cell lesions. The latest WHO classification of urinary and male genital tumours has excluded this entity under the assumption that it comprises examples of Sertoli cell tumour with intra-rete growth,⁵ but evidence to support this decision was lacking. Most of the remaining benign neoplasms of the rete testis are benign cystadenomas and

Table 3. Ancillary testing results of the study cases

Case no.	Immunohistochemistry				NGS findings		
	PAX8	WT1	CDX2	Beta-catenin	Panel 1	Panel 2	Panel 3
1	POS	POS	NEG	NP	WT	WT	WT
2	NEG	NEG	NEG	NP	WT	WT	WT
3	NEG	NEG	POS	NP	WT	NE	WT
4	NEG	NEG	POS	NP	NE	NE	NE
5	POS	POS	NEG	NEG	WT	WT	WT
6	NEG	NEG	NEG	NP	WT	WT	WT
7	NEG	NEG	NEG	NP	WT	WT	WT
8	NEG	NEG	NEG	NEG	WT	WT	WT
9	POS	NP	NP	NEG	WT	WT	WT
10	POS	NP	NEG	NEG	RET p.Arg677Trp (45%—VUS)	WT	WT
11	POS	NA	NA	NEG	WT	WT	WT

Immunohistochemistry considered positive if $\geq 5\%$ of tumour cells showed expression of the marker (regardless of intensity). NE, not evaluable due to low DNA quality.

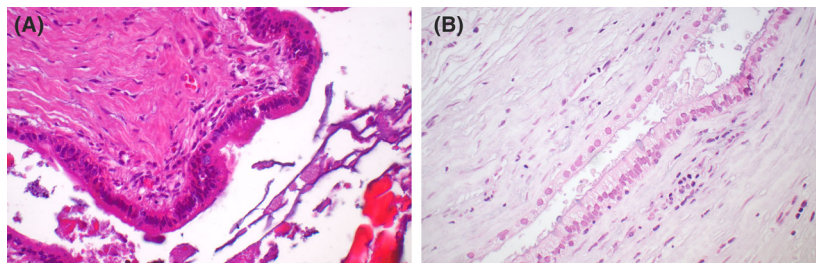


Figure 4. Cystadenoma of the rete testis. A: Mucinous subtype. B: Seromucinous subtype, lined by cuboidal to columnar epithelial cells, including a population of ciliated cells (fallopian tube-like epithelium).

have been historically thought to represent testicular counterparts of Mullerian tumours with similar morphology to those of the ovary.⁷ Data for these tumours were also limited and, therefore, the hypothesis that they may represent counterparts of Mullerian neoplasms of the ovaries was largely based on assumptions.

The current WHO classification of urinary and male genital tumours does include a category for adenomas of the rete testis, but there is no explanation of how these tumours differ from those previously described as “Sertoliform” cystadenomas in the literature.⁸ Hence, the interpretation of what constitutes a Sertoli cell tumour NOS with growth confined to the rete testis and a true adenoma of the rete testis seems entirely arbitrary. Moreover, to the best of our knowledge, there is no convincing documentation of

a Sertoli cell tumour with pure intra-rete growth. The present study demonstrates that four lesions within the spectrum of Sertoliform cystadenoma of the rete testis (and originally classified as such) were negative for pathogenic *CTNNB1* variants, lacked nuclear expression of beta-catenin, and were positive for PAX8. Additionally, three tumours showed a clear transition between the nonneoplastic rete epithelium and the neoplastic epithelium. These findings argue against the possibility of a Sertoli cell tumour NOS growing into the rete testis, especially considering that over 90% of indolent Sertoli cell tumours show *CTNNB1* or *APC* variants and nuclear beta-catenin expression^{9,10} and are negative for PAX8 nuclear expression.

Unlike Sertoliform cystadenomas, which are solid and cystic or solid, most of the remaining benign

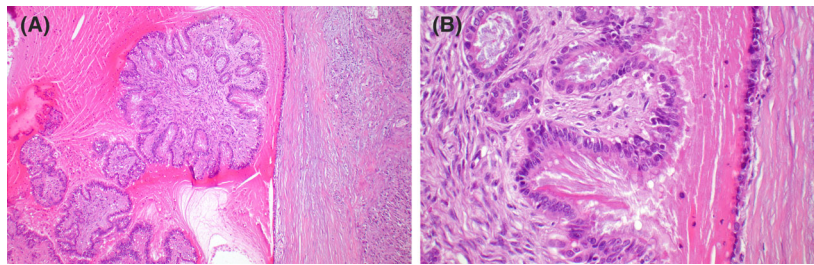


Figure 5. Cystadenoma of the rete testis. **A,B:** Serous subtype, characterized by unilocular structure with no internal septations, lined by a single layer of epithelial cells. **B:** One serous neoplasm exhibited focal epithelial proliferation with stubby, branching papillae. The epithelium lining the papillae exhibited mild nuclear atypia, featuring conspicuous nucleoli.

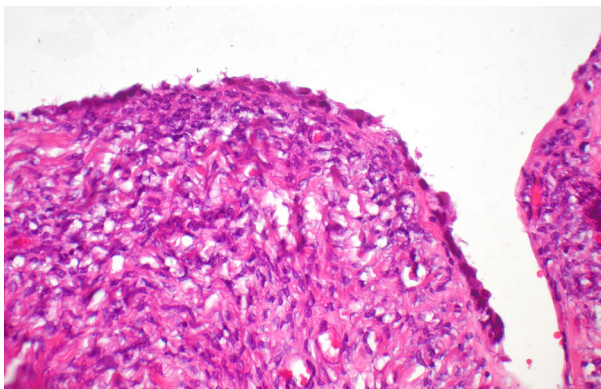


Figure 6. Cystadenoma of the rete testis. Focal areas with ovarian-like stroma.

neoplasms of the rete testis are entirely cystic. These benign cystadenomas are generally well-circumscribed, discovered incidentally, and characterized by cystic spaces lined by a single layer of cuboidal to columnar epithelial cells.¹¹ A subset may contain mucinous epithelium with intraluminal mucin, and some tumours include a subpopulation of ciliated cells. Based on morphologic findings, these tumours have often been interpreted as testicular counterparts of similar-appearing Mullerian tumours of the ovaries, but the immunophenotypic and molecular features of these lesions had not been previously studied in detail.^{1,12} Mucinous or serous ovarian neoplasms may harbour *KRAS* or *BRAF* mutations, which were not identified in benign cystadenomas of the rete testis (including a case with focal epithelial proliferation consisting of stubby papillae lined by short columnar cells).^{7,13,14} An important caveat is that, due to their nature, tumour cellularity is often low in these neoplasms, making the molecular analysis challenging.

In light of the latest changes in classification endorsed by the WHO, neoplasms of the rete testis that fall within the spectrum of “Sertoliform

cystadenoma” need to be distinguished from Sertoli cell tumour, NOS. In prior studies, IHC performed in a small subset of these tumours demonstrated that they may variably express SF-1, inhibin, and calretinin, suggesting sex cord differentiation.⁸ However, these markers are not entirely specific and show overlap between lesions of sex cord-stromal and purported rete testis origin (for instance, adenocarcinomas of the rete testis are commonly positive for calretinin, whereas SF-1 has not been systematically assessed, to the best of our knowledge).¹⁵ Additionally, it is possible that neoplasms with different phenotypes may arise from the different portions of the rete testis (septal versus mediastinal versus extratesticular), with those arising in the septal portion (tubuli recti) showing overlapping features with Sertoli cell tumours NOS. In fact, recent work in animals¹⁶ suggests that the rete testis derives from SF-1-expressing cords of cells that have some Sertoli-like features but are distinct from them, a situation that may explain the partially overlapping immunophenotypes of Sertoliform cystadenomas and Sertoli cell tumour, NOS. In support of the idea that distinct tumours may arise from different portions of the rete testis, we have recently encountered incidentally a hyperplastic proliferation of efferent ducts (extratesticular rete testis) in an orchiectomy specimen (Figure 7).

From a practical perspective, the distinction between these tumours of the rete testis and metastases, germ cell tumours, or other malignant neoplasms, is seldom problematic, especially when they are entirely cystic. However, tumours with solid components may show significant overlap with sex cord stromal tumours and may occasionally raise the possibility of metastases to the testis. In particular, neoplasms within the spectrum of Sertoliform cystadenoma, given their morphologic and immunophenotypic features, may need to be distinguished from Sertoli cell tumor NOS, metastatic ductal

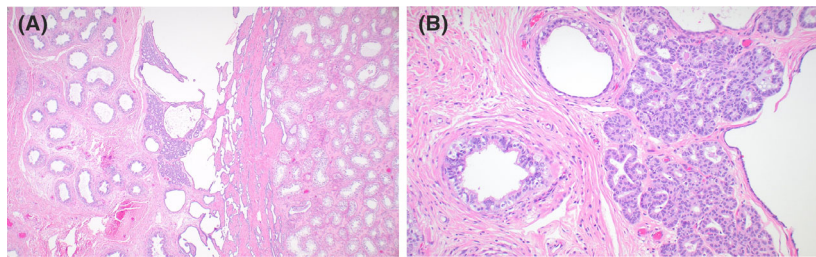


Figure 7. Hyperplasia of the extratesticular portion of the rete testis. A: The rete testis consists of three parts: the septal rete testis containing tubuli recti (right towards the testicular parenchyma), the hilar/mediastinal rete testis containing wide anastomosing channels lined by flat epithelium (centre), and the extratesticular rete testis containing efferent ducts (left, towards the epididymis). B: This case shows hyperplasia of efferent ducts confined to the extratesticular portion of the rete testis. We hypothesize that lesions arising in different portions of the rete may show phenotypic differences, which could explain the variable immunoprofile reported in adenomas of the rete testis.

adenocarcinoma of the prostate, and papillary renal cell carcinoma, among others. As shown in this study, the presence of areas of transition between non-neoplastic and neoplastic epithelium within the rete testis can be helpful to support a diagnosis of Sertoliform cystadenoma of the rete testis. Additionally, positivity for PAX8 and absence of nuclear beta-catenin expression are helpful to distinguish between these neoplasms and Sertoli cell tumor NOS. In our opinion, the presence of nuclear beta-catenin expression is so consistent among indolent Sertoli cell tumour NOS that, in its absence, this diagnosis should be restricted to cases with prototypical features.^{9,10,17} Given that Sertoli cell tumours NOS typically occur within the testis proper, the combination of purely intra-rete growth pattern and the absence of nuclear beta-catenin expression argue against it, even in the presence of SF-1 expression.

Despite the multiple limitations of the current study, including the use of sequencing panels (rather than genome-wide or exon-wide assays) and the limited number of cases, two relevant conclusions can be drawn from its results. First, except for the presence of tubular and/or tubulopapillary architecture, benign tumours previously considered Sertoliform cystadenomas of the rete testis and now regarded as Sertoli cell tumours, NOS with intra-rete growth by the WHO classification of urinary and male genital tumours lack the hallmark findings of Sertoli cell tumours NOS. Hence, this assumption is likely incorrect. Second, cystadenomas of the rete testis lack molecular alterations seen in most mucinous tumours of Mullerian origin arising in the ovary and may therefore not represent a true counterpart. Unfortunately, the use of sequencing panels precluded identification of novel variants; additional studies are needed to determine the molecular alterations that underlie oncogenesis in these neoplasms.

Author contributions

Concept: AMA. Design and coordination: KC, CR, DdB, AMA. Compilation and analysis of clinicopathologic and clinical data: KC, CR, DdB, AMA. Article draft and figures: KC, AMA. Cases and/or intellectual contributions (including article editing): All authors.

Conflict of interest

The authors declare that they have no conflicts of interest pertaining to the content of this article.

Data availability statement

The data generated in this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Next generation sequencing custom-designed multi-gene panel.