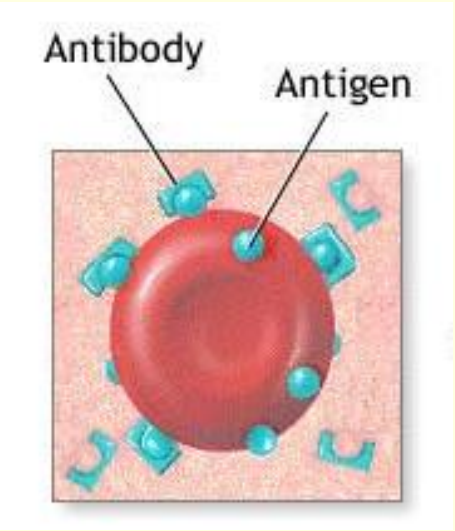
Immune response in obstructive male infertility



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Sperm transport from the testicle

- Seminiferous tubule contractions of the myoid cells (hormone dependent)
- Fluid build up and pressure
- Testicular capsule contractions

Epididymis

- Responsible for sperm maturation and motility.
- Usually two-thirds of the epididymis is needed for sperm transport in the human for normal sperm fertilization capacity.
- Potential markers of epididymal function include carnitine and glycerol phosphocholine.
- Sperm take approximately two weeks to get through the epididymis.
- Sperm are stored near the tail portion and in the vas deferens until they are ejaculated

Stereological analysis of the human testis after vasectomy indicates impairment of spermatogenic efficiency with increasing obstructive interval

- "Vasal obstruction results in significant reductions in germ cells in the later stages of spermatogenesis and increases in testicular fibrosis, both worsening with an increasing obstructive interval. Testicular damage after vasectomy might impact upon the prospects for reversal."

Fertil Steril. 2004 Jun;81(6):1595-603.

Some gross observations of the epididymides following vasectomy

- In 114 vasectomized men, 220 epididymides were grossly examined during vasovasostomy. Fullness, distention, turgidity, thickening, induration, and other gross changes of the epididymides, including the formation of cystic spermatic granuloma, or spermatocele, indicated inadequate removal of spermatozoa and testicular fluid from the sequestrated proximal seminal ducts and the epididymis.

Fertil Steril. 1976 Mar;27(3):267-70

Vasectomy review: sequelae in the human epididymis and ductus deferens

- Following vasectomy, spermatogenesis continues, the human epididymis and ductus deferens may distend and leak, and the extravasated spermatozoa stimulate formation of a sperm granuloma.
- Granulomas may occur at 60% of vasectomy sites and are usually asymptomatic and relieve intraluminal pressure. About 3-5% of patients experience pain. Intraluminal phagocytosis may explain why some reproductive tracts become depleted of spermatozoa.
- Most episodes of painful epididymitis and granulomas resolve with conservative treatment, but < 1% require vasectomy reversal or, if this is ineffective, excision of the epididymis and obstructed ductus deferens.

Clin Anat. 1996;9(5):337-42.

Cellular responses to vasectomy

- Following vasectomy, epididymal distension and sperm granuloma formation result from raised intraluminal pressure. The sperm granuloma is a dynamic structure and a site of much spermatozoal phagocytosis by its macrophage population. In many species, spermatozoa in the obstructed ducts are destroyed by intraluminal macrophages, and degradation products, rather than whole sperm, are absorbed by the epididymal epithelium. In laboratory animals, there is evidence that pressure-mediated damage to the seminiferous epithelium can follow sperm granuloma formation and obstruction in the epididymal head.

Int Rev Cytol. 2000;199:295-339.

The response of the male reproductive tract to vasectomy

- Inflammation of the interstitial tissue of the epididymis in Lewis rats at intervals following bilateral vasectomy, vasectomy followed 1 month later by vasovasostomy, or sham operations.
- In areas of interstitial reaction, numerous macrophages, monocytes, lymphocytes, neutrophils, and plasma cells occupied the connective tissue. Macrophages, containing many lysosomes and vesicles, aggregated and assumed the appearance of epithelioid cells. Many plasma cells with distended rough endoplasmic reticulum appeared in the interstitium. The majority of animals in the vasectomy and vasovasostomy groups exhibited epididymal interstitial changes by 2-3 months; the cauda epididymidis was the region most often affected.
- The ultrastructural features were indicative of chronic granulomatous inflammation and were consistent with an immune response.
- The nearly complete absence of sperm or recognizable parts thereof in the interstitial tissue in the areas of the reactions suggests that these lesions formed in response to soluble antigens leaking from the duct.
- Vasovasostomy was not effective in reversing or retarding epididymal inflammation at the intervals studied.

Anat Rec. 1993 Jan;235(1):61-73

Vasectomy-related changes on sonographic examination of the scrotum

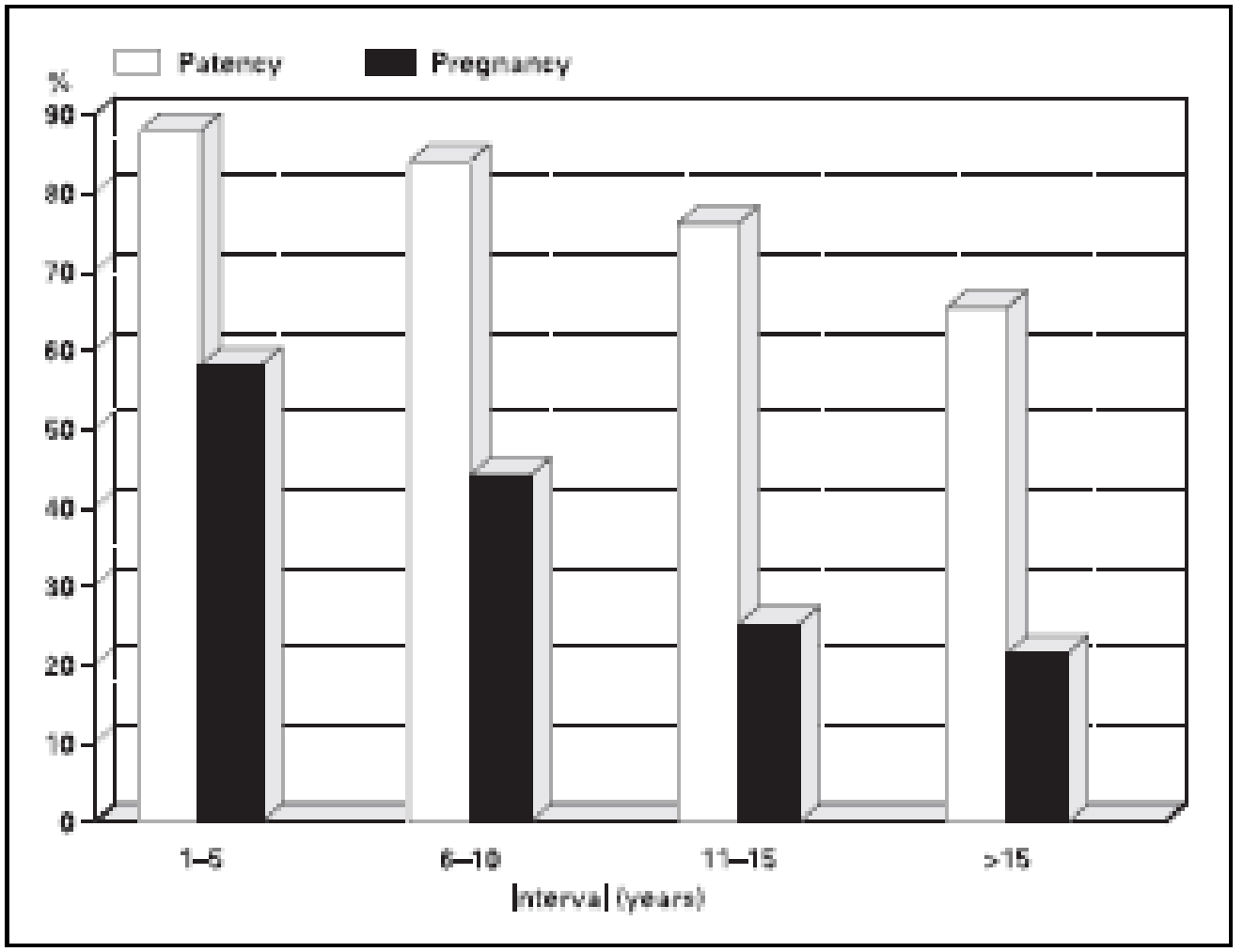
- A retrospective study of 30 patients with a history of vasectomy and 30 patients without who were referred for scrotal sonography for various indications over a 1-year period. The following findings had a statistically higher incidence in the vasectomy group than in the controls:
  - thickened epididymides (53% versus 17%, $p < 0.05$);
  - epididymal tubular ectasia (43% versus 7%, $p < 0.001$);
  - and both of the previous 2 findings simultaneously (37% versus 7%, $p < 0.01$).
  - sperm granulomas were found in 3 patients in the vasectomy group, and none was seen in the control group.
  - other findings (eg, epididymal cysts) showed no statistical difference in incidence.

J Clin Ultrasound. 2004 Oct;32(8):394-8.

Vasovasostomy Study Group

- This largest multicenter group to assess vasovasostomy efficacy has found that if the interval was less than 3 years from the vasectomy, patency was 97% and pregnancy was 76%. In men more than 15 years from their vasectomies, patency and pregnancy rates fell to 71% and 30%, respectively.

Patency and pregnancy rates after vasovasostomy at different intervals between vasectomy and reversal.



Eur Urol 2001;40/4 (Curric Urol 1–10)

Failure following vasovasostomy

- Epididymal obstruction
- Autoantibodies
- Oxidation
- Other lower fertility factors
  - Protein P34H
  - Sperms with abnormal heads

Abnormalities of sperm morphology in cases of persistent infertility after vasectomy reversal

- Persistent infertility after vasectomy reversal by vasovasostomy may be due to irreversible changes in epididymal physiology, producing morphologic abnormalities of the sperm tail.
- The epididymal environment is required for the final maturation of spermatazoa and the acquisition of normal motility and fertilizing ability. The study results suggest that these epididymal functions may be impaired in some men after vasectomy due to a disturbance of epididymal physiology.

Fertil Steril. 1982 Jul;38(1):112-4.

Male reproductive immunology

- Immunologically the testis is a privileged site with the blood-testis barrier morphologically and humorally separating the germ cell population from the interstitial tissue.

- The epididymis is considered to be the most likely source of antibody secretion. It is densely populated with lymphocytes and macrophages.

Infertil Reprod Med Clin North Am 1999; 10:435-470.

Sperm degradation products

- In case of obstructive azoospermia, spermatozoal degradation occurs proximal to the site of obstruction in the epididymis. It is currently believed that soluble spermatozoal fragments are either absorbed or they leak from the basal lamina and the ductuli efferentis for absorption in the interstitium.

Fertil Steril 1979; 32:253-260

Changes following vasectomy

- Gross anatomical changes
  - cystic swelling at the site of operation
  - development of spermatic granuloma.
  - In experimental auto-immune orchitis
  - varying degree of vacuolization of the Sertoli cells; premature exfoliation of immature germ cells; coalescence of cellular units of the germ cell clones from multinucleate giant cells; distortion of spermatid nucleus and acrosome; and retention and degeneration of late spermatids within the epithelium.

- Due to phagocytosis of the spermatozoa the soluble antigens of the sperm leak into the circulation and produce autoantibodies.
  - Sperm agglutinating antibodies in sera have been detected as soon as the 3rd or 4th day after vasectomy, but they appear in the sera more commonly between 6 weeks to 6 months.
  - Circulating sperm immobilizing antibodies are found in 1%-3% of the nonvasectomized individuals. Within 6 months post operation, about 31% of men develop these antibodies.
  - Swollen sperm head antibodies are found in less than 2% of nonvasectomized male populations and in 28%-33% of vasectomized males within a year following the operation.

Arch Androl. 1981 Sep;7(2):193-9.

Histologic changes in the mouse testis after bilateral vasectomy

- The seminiferous epithelium in the tubules was only 2-3 layers thick and showed much depletion of germ cells; in severe cases, the epithelium consisted of only a thin layer of Sertoli cells, spermatogonia and a few spermatocytes.
- Exfoliation of germ cells, occurrence of multinucleated giant cells and vacuolated appearance of the epithelium were of common features in the tubules.
- Furthermore, lumen of the retetestis was greatly dilated and showed accumulation of spermatozoa with immature germ cells; in mice vasectomized for 6-12 months, several macrophages ingesting spermatozoa were often observed in the lumen of the rete testis.
- Spermatic granuloma was also sometimes noticed in corpus or in cauda regions of the epididymis in mice vasectomized for 6-12 months.

Asian J Androl 2000 Jun; 2: 115-120

Immune orchitis

- Clinical and pathologic evidences of immune orchitis in men exhibiting natural autoimmunity to sperm has never been provided.

Frontiers in Bioscience 4 1999; e9-25

Antisperm antibodies

- Following vasectomy the prevalence of ASA is 34% to 74% and they persist in 38% to 60% following successful vasovasostomy.

Frontiers in Bioscience 4 1999; e9-25.

- Production of ASA after obstruction of the epididymis has also been reported in experimental animals.

J Androl 1998; 19:136-144.

Fertil Steril 2000; 73:229-237.

Production of ASA

- The degradation spermatozoal products may also leak at the breached epididymal sites

Fertil Steril 1979; 32:253-260.

- or may be produced in larger quantities than to be overcome by the immunological suppressor barrier

Int Rev Cytol 2000; 199:295-330;

Infertil Reprod Med Clin North Am 1999; 10:435-470;

Br J Urol 1985; 57:769-774

Antisperm antibodies & infertility

- Clinically antisperm antibodies are found in the serum in 3% to 12% of men who undergo evaluation for infertility as compared to 2% in the normal fertile population.

  Br J Urol 1977; 49:757-762.

- Among men from couples with unexplained infertility significant titres have been recorded in the serum in 10%-16%.

  J Reprod Immunol 1985; 8:279-299.

Antisperm antibodies

- May result in the serum, seminal fluid or on sperm surface either as a result of transport of spermatozoal degradation products by the macrophages to the regional lymph nodes, breakdown of the blood-testis / blood-epididymis barrier due to trauma or infection with escape of sperm antigens directly into the microcirculation, or, by production of antibodies against infective agents like Chlamydia which cross react with spermatozoa.

Front Biosci 1999: 4: e9-25.

ASA following vasectomy

- Antisperm antibodies are found in approximately 50%–70% of men after vasectomy and in every mammalian species studied. Percentages of men with antibodies detected after vasectomy vary between 52% and 68% at 6 months, and 52%–60% after 1 year, and antibodies have been found to persist in the circulation for several years.

Fertil Steril 2000; 73(5): 923-936.

ASA & congenital vas obstruction

- The association of ASA with obstructive azoospermia due to congenital causes is not yet well established with conflicting data being reported in literature.

Frontiers in Bioscience 4 1999; e9-25.

Antibody interference with fertility

- There is little evidence that suggests a cause/effect relationship between ASA and abnormality of principal semen parameters (sperm count, motility and morphology) while the impairment of sperm penetration through the cervical mucus represents the best known and established mechanism.
- Analysis of human IVF results also indicate that sperm bound antibodies impair the fertilization process, which, to some extent, is related with the degree of sperm autoimmunization.

Frontiers in Bioscience 4 1999; e9-25

ASA & conception following vasectomy reversal

Studies of the immunologic consequences of vasectomy indicate that the sperm antibodies when they appear in the ejaculate following vasovasostomy, are likely to affect the sperm function, suggesting that they are clinically important.

- Linnet et al. documented a conception rate in the partners of men who had undergone sterilization reversal, with no evidence of antisperm antibodies in their seminal plasma, of 85% versus 14% for the partners of men whose semen contained antibodies.

  Lancet 1981; I:117-119.

- Parslow et al. confirmed this finding but also documented that fertility is impaired only when high titers of ASA are present in the seminal fluid.

  Fertil Steril 1985; 43:621-625.

- Meinhertz et al. studied the ejaculates of 216 men following sterilization reversal. The conception rate was 85% in the partners of a subgroup of men with a pure IgG response, while only 43% of men who had IgA on their sperm fathered children. The conception rate was reduced even further when 100% of sperm were coated with IgA (21.7%). The combination of IgA on all sperm and a high serum titer (more than 1:250) detected by agglutination test was associated with a zero conception rate.

  Fertil Steril 1990; 54:315-321.

Interference with the fertilization process

- The fertilization rate with *in vitro* fertilization and embryo transfer (IVF-ET) was significantly lower in the presence of sperm-bound antibodies than in the case of other indications, the likelihood of fertilization is higher with intracytoplasmatic sperm injection (ICSI), where the reported fertilization rates are similar to those in other indications.

Frontiers in Bioscience 4 1999; e9-25

Qualitative differences in the effects of ASA in the fertility impairment

- Different immunoglobulin isotypes of antibodies occurring on sperm surface can produce different biological effects (i.e., cytotoxic effects due to complement activation can be produced by IgG but not IgA sperm bound antibodies).

- Due to their polyclonal nature, ASA are directed against more than one sperm antigen, which may differ among patients and may be more o less relevant to fertility.

Frontiers in Bioscience 4 1999; e9-25

ICSI in obstructive azoospermia

- In patients of obstructive azoospermia, the pregnancy rate with ICSI using sperms aspirated from the epididymis in terms of pregnancies per cycle has been reported to be 41% - 47%.

Hum. Reprod. 10:148-152

Fertil. Steril 1995; 61:1045-1051

- In 49% of failed cycles of ICSI , fertilization failure was related to a sperm factor like the absence of motile spermatozoa for injection, or the injection of morphologically abnormal spermatozoa.

Human Reprod 1995;. 10:626-629.

Analysis of spermatozoa from the proximal vas deferens of vasectomized men

- Sperm concentration in the proximal vas from vasectomized men was significantly higher than that of fertile men and was maintained at a constant level independent of the duration of vas obstruction.
- The means of sperm motility, sperm viability (and hypo-osmotic swelling test were statistically lower than the respective values for normal fertile men. There was no significant correlation between the duration of vas obstruction and the above semen parameters.
- In 46.4% of vas fluids all spermatozoa were immotile and this condition was more common after 3 years of vasectomy. Immotile spermatozoa in the proximal vas fluids at the time of vasectomy reversal may be an important factor for predicting semen quality and fertilizing ability after vasovasostomy.
- There were not, however, significant differences in the results of sperm-cervical mucus penetration test between spermatozoa from vasectomized and fertile men.
- It is noted that the presence of antisperm bodies on the spermatozoa from the vas of vasectomized men, as determined by the immunobead test, may partially explain the lower pregnancy rate after vasovasostomy, with spermatozoa parameters potentially closely reflecting those in the cauda epididymis after vasectomy.

Int J Androl. 1994 Aug;17(4):181-5

Following vaso-epididymostomy

- By fistula method **patency rate 43.7 %** and ***pregnancy rate 16.8%***

Urol Clin North Am 1987; 14:527-538.

- By microsurgical technique **patency rate 75.3%** and ***pregnancy rate 29%***

Fertil Steril 1982; 34:149-153

HYPOTHESIS

- Normally, epididymal epithelial lining prevents the extravasation of sperms or their degradation fragments into the interstitium, thus blocking a direct interaction between antibody producing cells and sperm proteins. A breach in the epididymal epithelium would lead to a direct contact between sperm antigens and immune effector cells.

Post vasectomy epididymal changes

- Following vasectomy, dilatation of epididymal ducts with sperm extravasation due to obstruction has also been reported.

J Reprod Fertil 1964; 7:1-12.

- Inflammatory cell infiltrate with lymphocytes, plasma cells and macrophages, perivasculitis and fibrosis in epididymal histology have been demonstrated following vasectomy similar to the findings in our study.

Br J Urol 1982; 54:769-773;

Fertil Steril 1979; 32:546-550.

- Histologic changes in the epididymis to be due to a local immune response resulting from contact of sperm proteins with local immune competent cells secondary to obstruction to sperm egress.

Br J Urol 1982; 54:769-773.

Local immune response

- It is also possible that in obstructive infertility breach in the epithelium causes extravasation of sperms into the interstitium and brings them in contact with the immunocompetent cells in insufficient amounts to induce detectable ASA. *However, repeated exposure to the antigenic sperms may induce a local cell mediated immune response which may be responsible for the histological changes reported in the epididymis.*

J Reprod Fertil 1964: 7: 1-12.

Br J Urol 1982: 54: 769-73.

FSH (U/L) and Spermatogenesis

	<8	8-20	>20
Gr. I	27	8	2
Gr. II	3	3	0
Gr. III	2	1	1
Gr. IV	0	2	1

Operative Criteria for Prognosticating Outcome

Vas patency	Absent	Not patent	Patent	
Epididymal distension	Not distended (15)	Mildly distended (4)	Obviously distended (31)	
Epididymal discharge	+ (13)	++ (17)	+++ (11)	- (9)

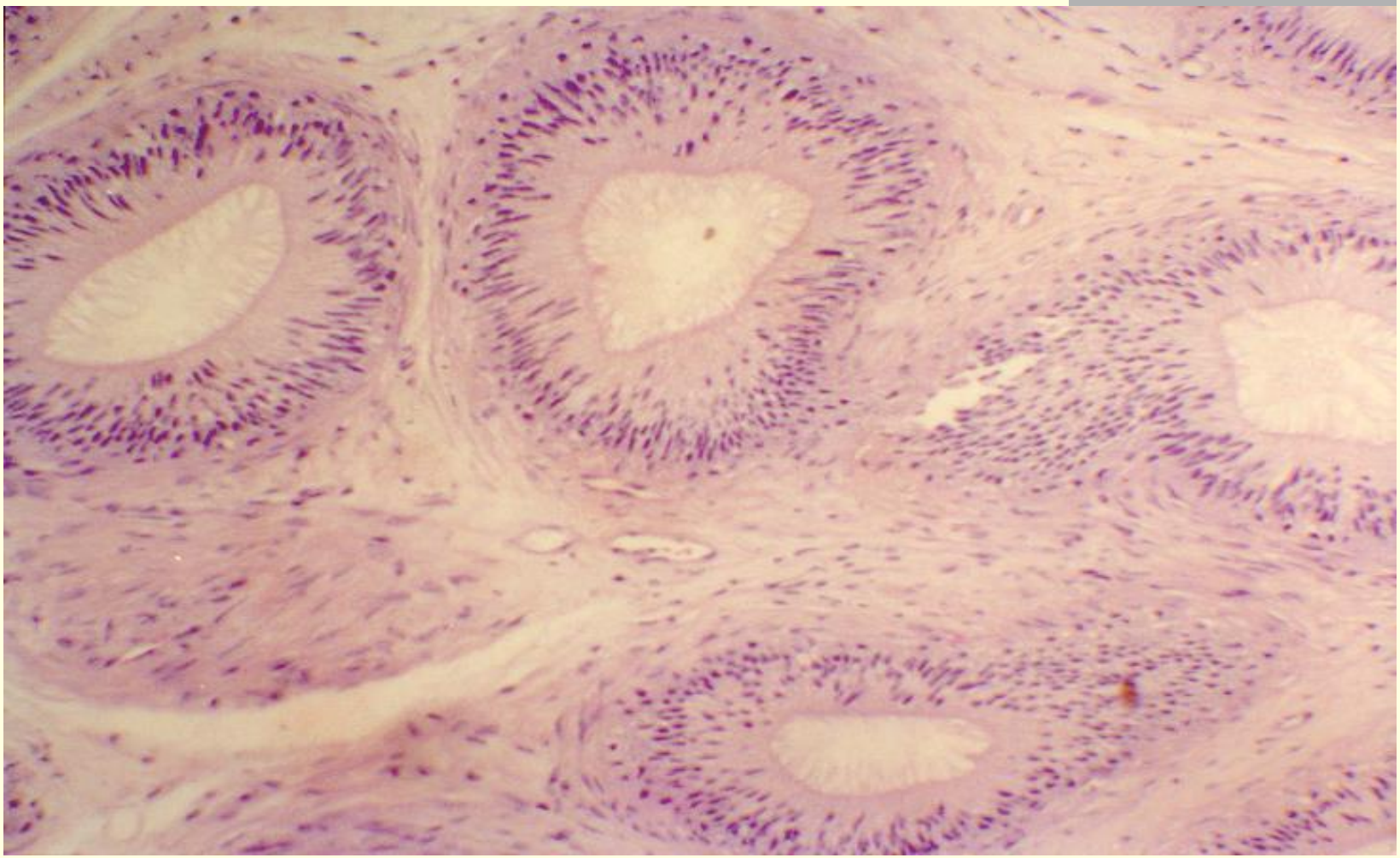
Seminal fluid antibody levels (n=30)

Histological Findings	Patients (n=30)	Epididymal fluid absent	Significant ELISA in epididymal fluid
Dilatation of ducts	19	3	9/16
Loss of cilia	14	4	6/10
Presence of Macrophages in Ducts	18	4	9/14
Sperm Ingestion by Macrophages	10	2	6/8
Sperm Extravasation	8	2	4/6
Epithelial Breach	13	2	8/11
Lymphocytic and Plasma cell Infiltration in Interstitium	22	6	**16/16**
Macrophage Infiltration in Interstitium	4	0	3/4

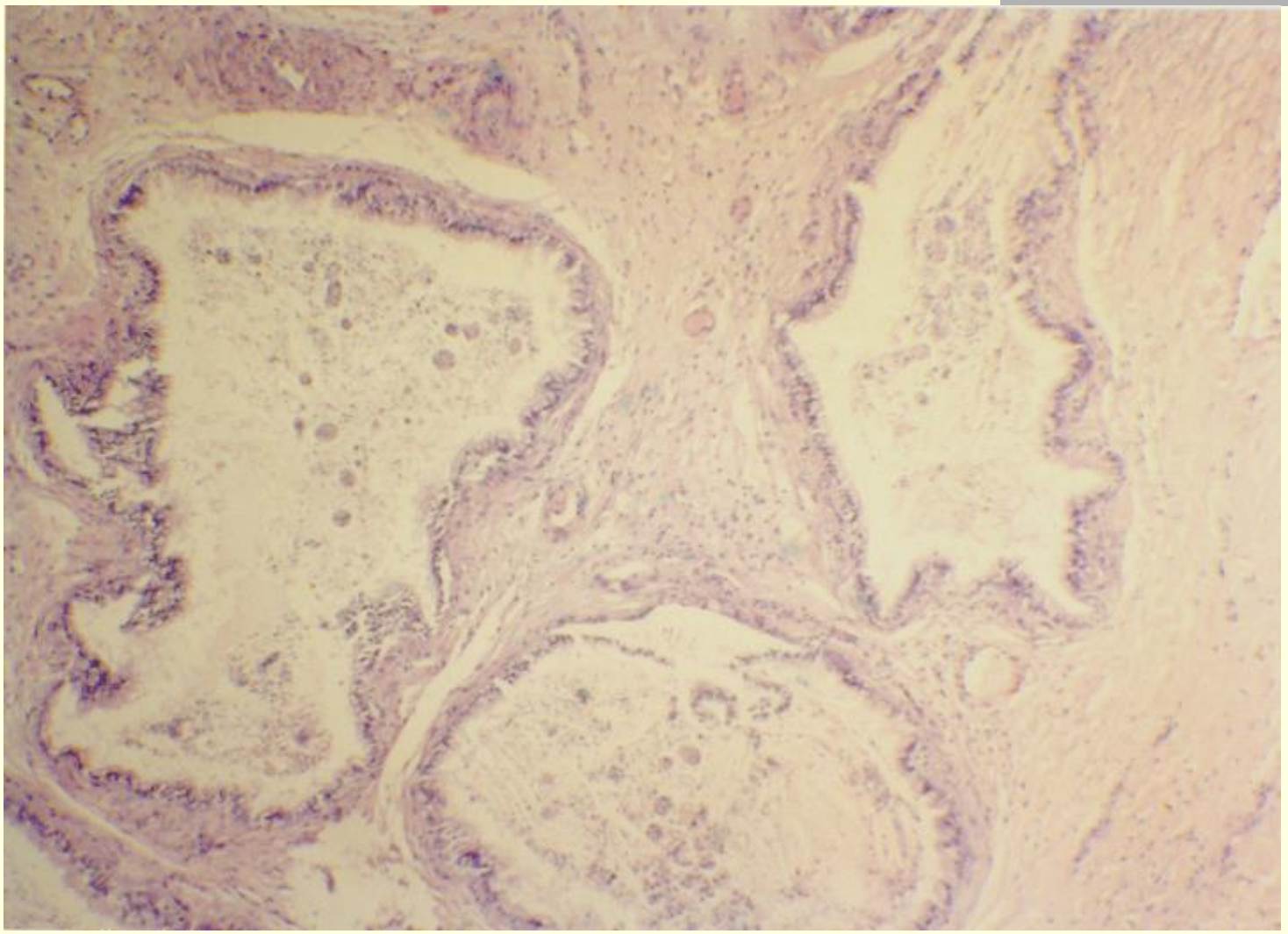
Features in epididymal histology

DUE TO OBSTRUCTION OF SPERM EGRESS	
*** Epithelial flattening with loss of cilia**	**25 (50%)**
*** Pigment in epithelial cells**	**20 (40%)**
*** Ductal dilatation**	**31 (62%)**
*** Ductal sperms**	**33 (66%)**
*** Ductal macrophages** **** Vacoulation** **** Sperm ingestion** **** Pigment**	**29 (58%)** **[25 (86.2%)]** **[18 (62.1%)]** **[09 (31.0%)]**
*** Epithelial breach with sperm extravasation**	**20 (40%)**
DUE TO SPERM EXTRAVASATION IN THE INTERSTITIUM	
*** Interstitial macrophages**	**11 (22%)**
*** Interstitial lymphocytes, plasma cells, etc**	**31 (62%)**

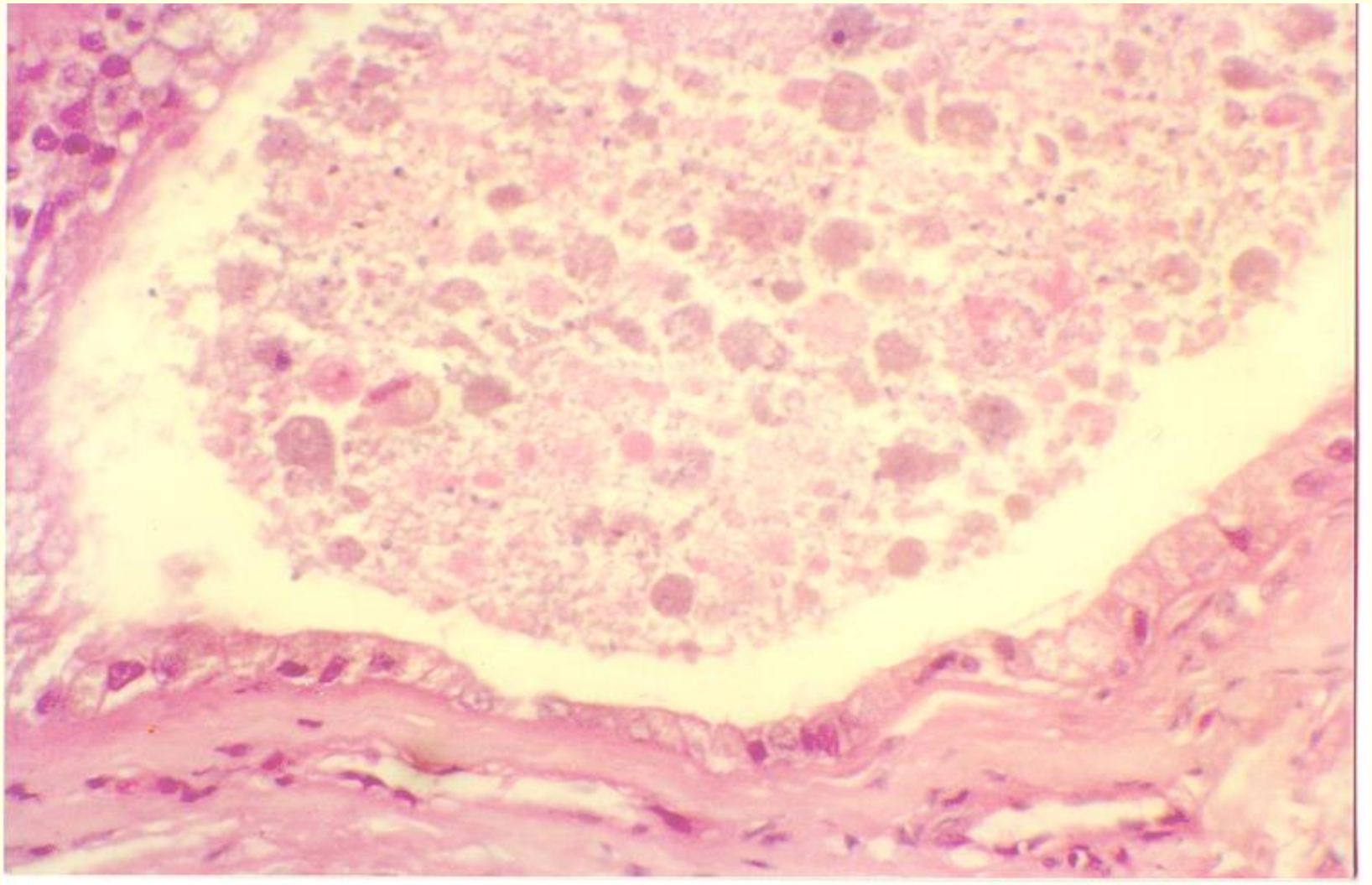
Epididymis showing normal epididymal ducts with orderly layering of nuclei and cilia on the luminal surface (H&E x100)



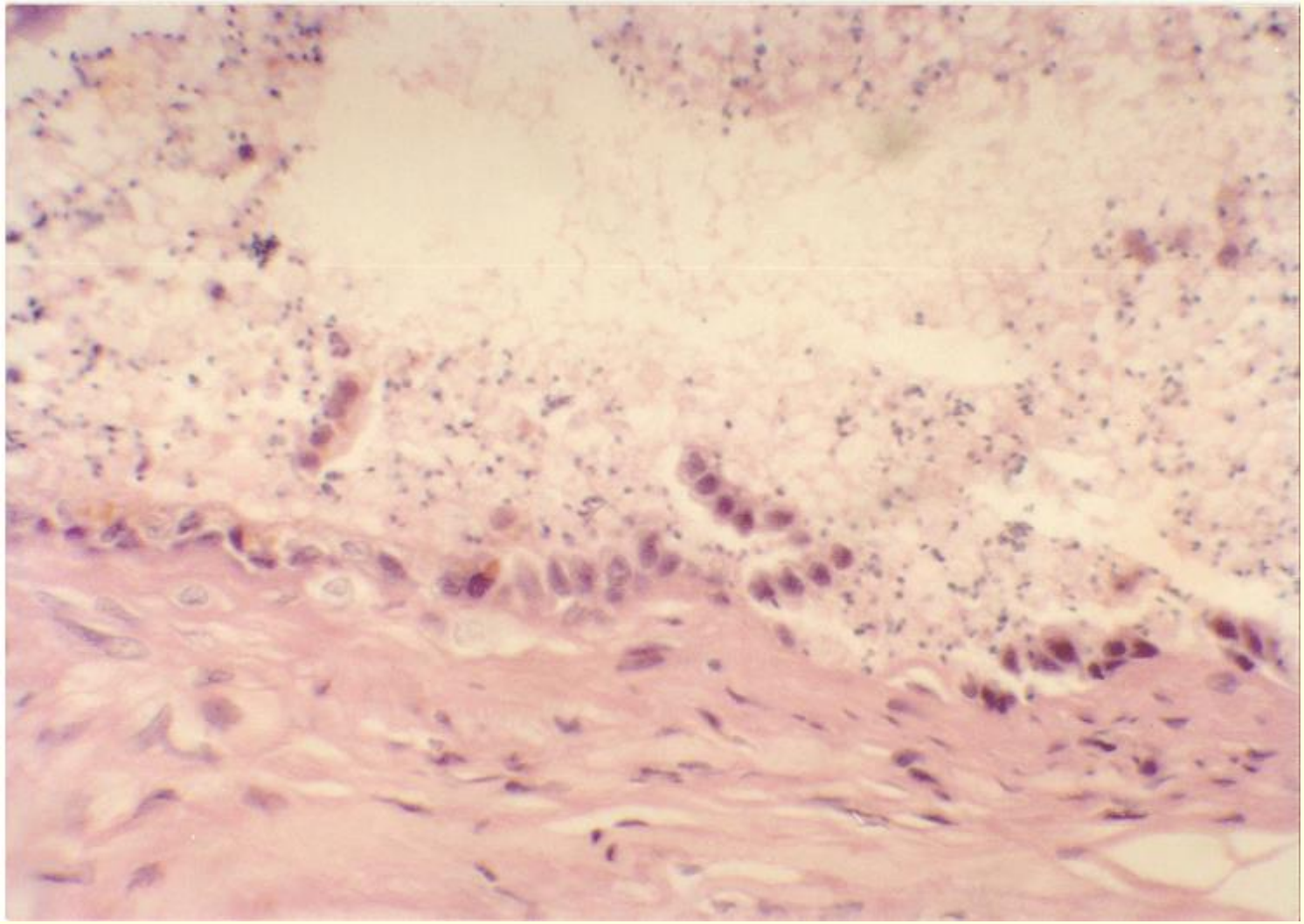
Epididymis showing markedly dilated ducts containing sperms and macrophages (H&E x60)



Epididymis showing dilated ducts with focal flattening of the epithelium (H&E x400)

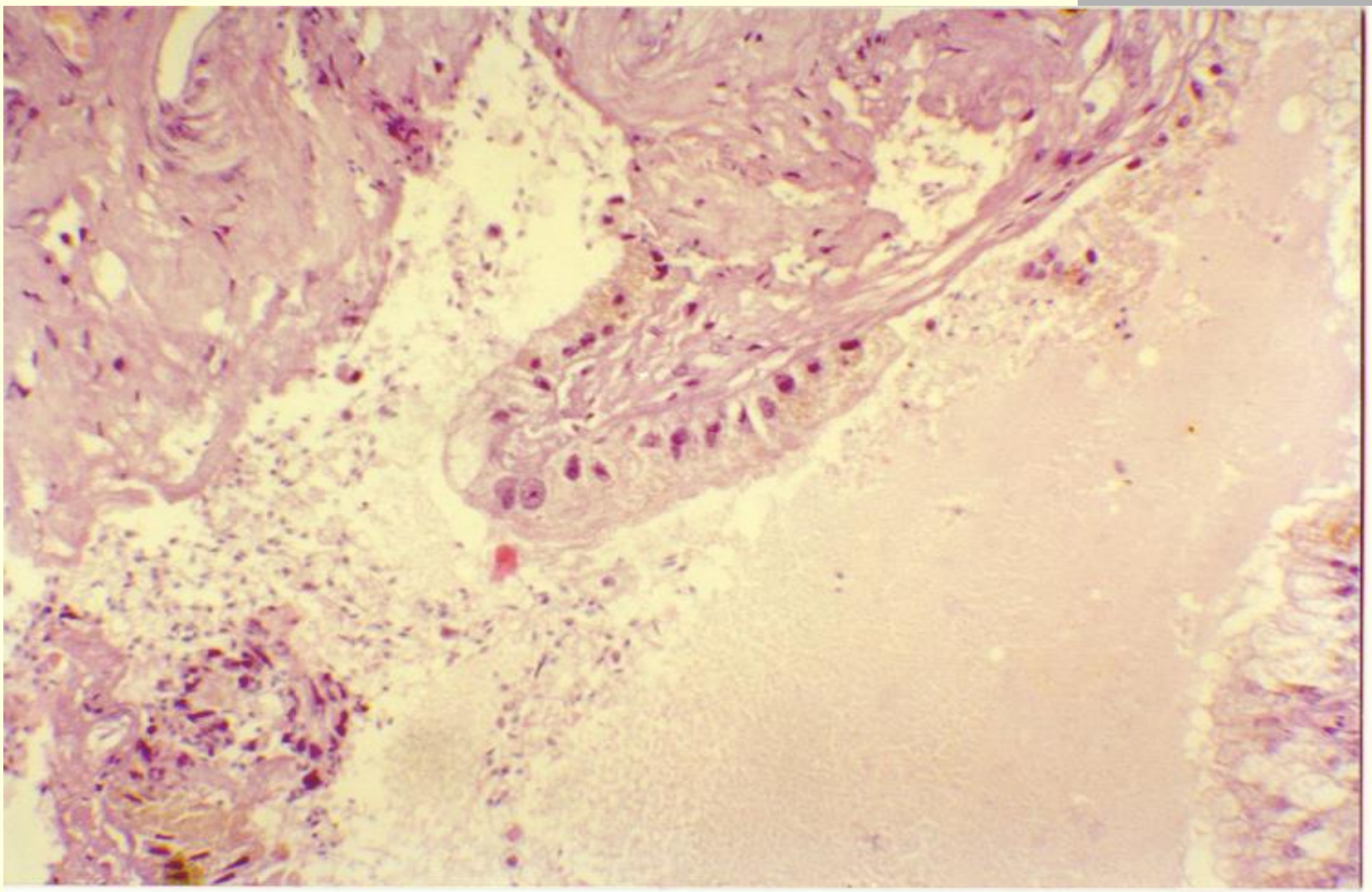


Epididymis of a patient showing sperm extravasation with epithelial breach (H&E x250)

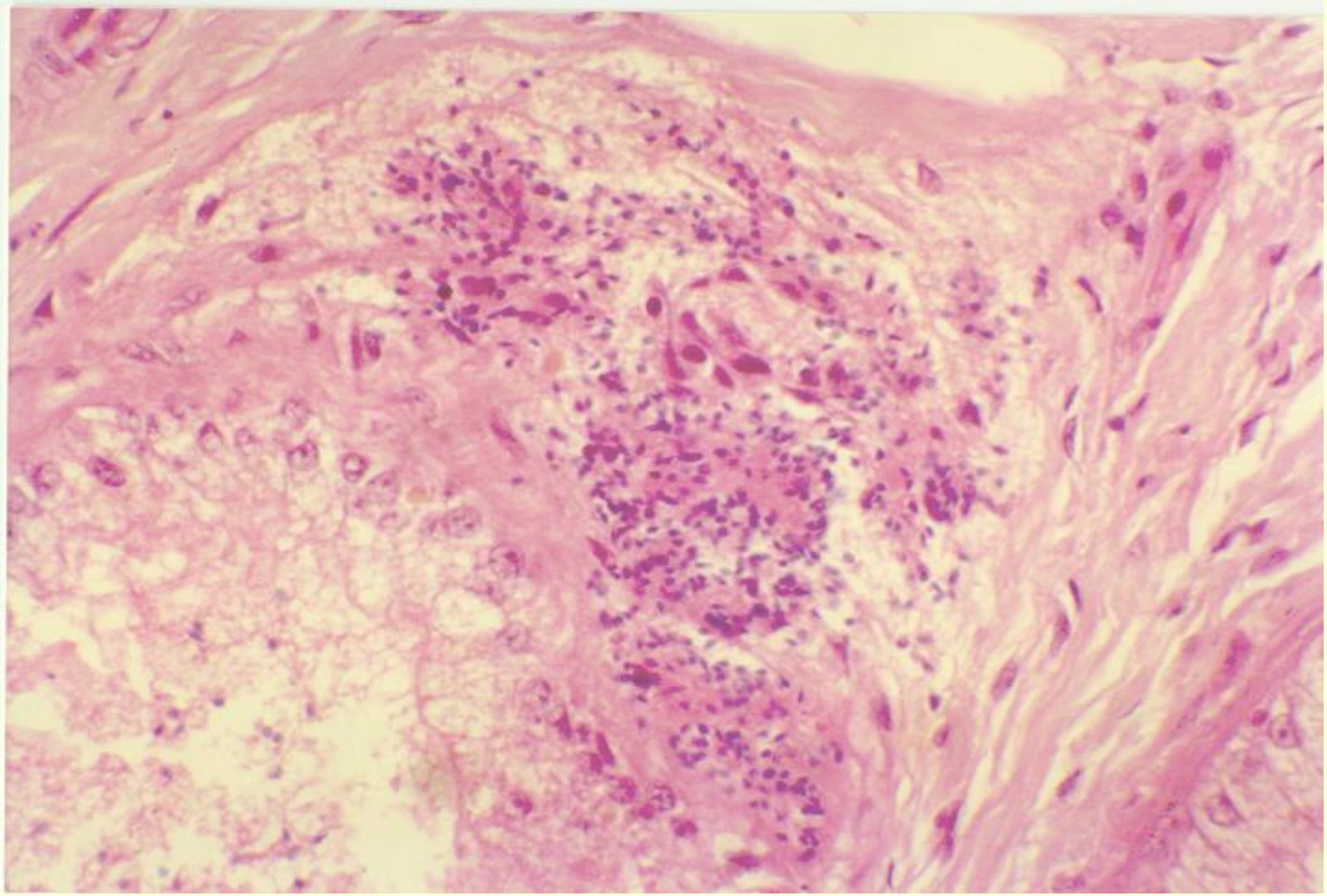


Epididymis showing epithelial flattening and breach, and extravasation of sperms

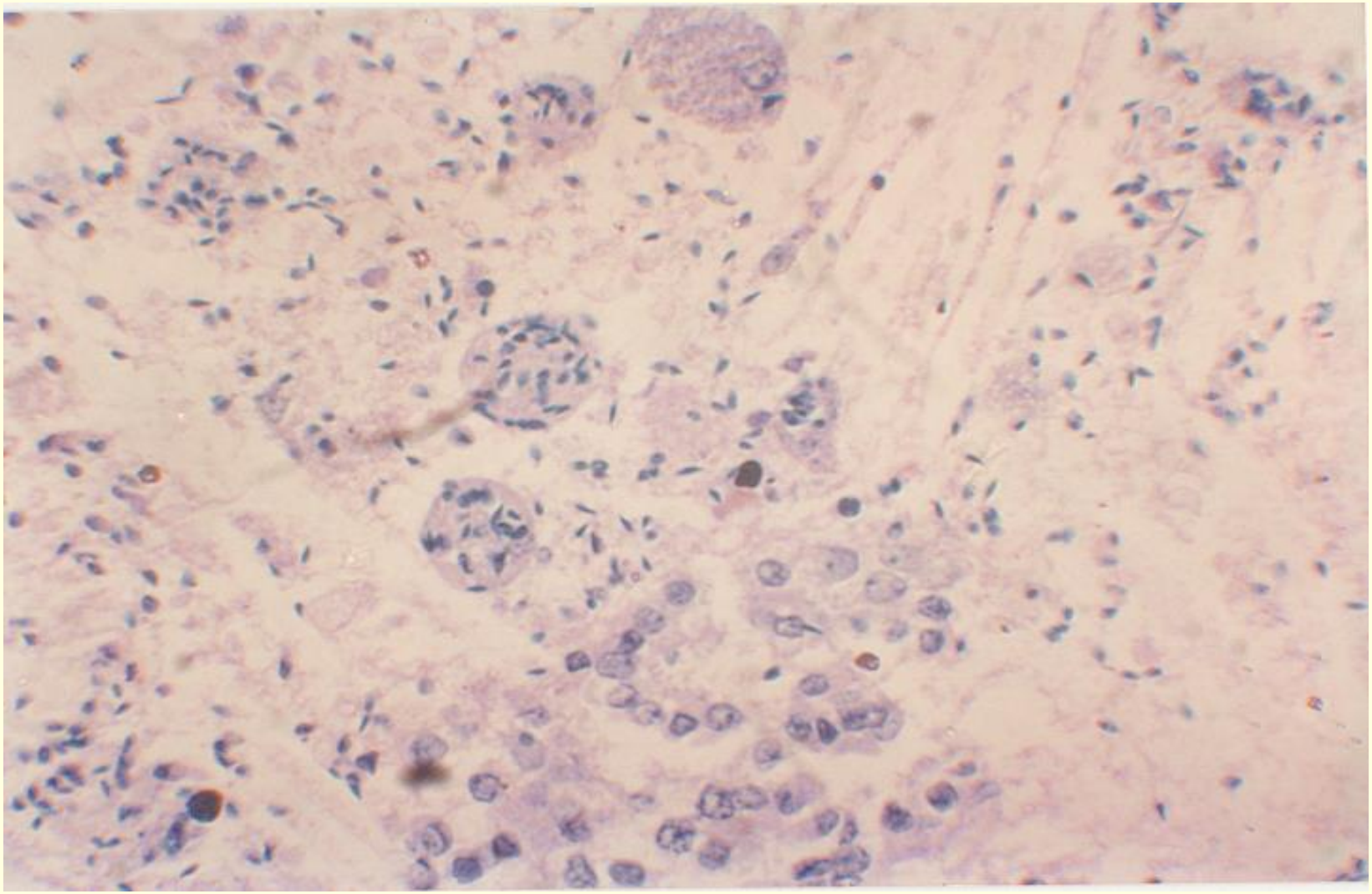
(H&E x400)



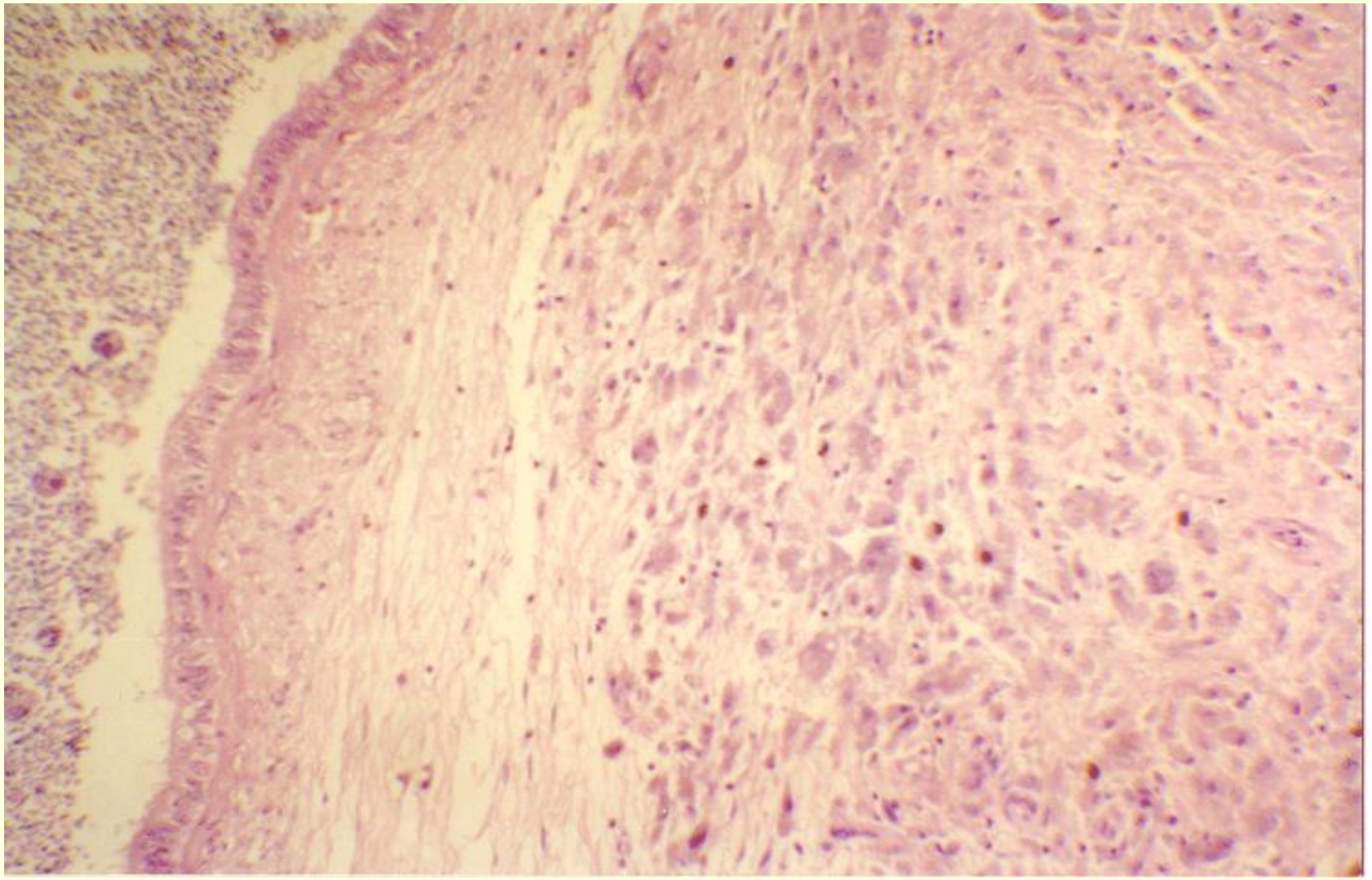
Epididymis showing sperm extravasation with interstitial inflammatory cell infiltration (H&E x400)



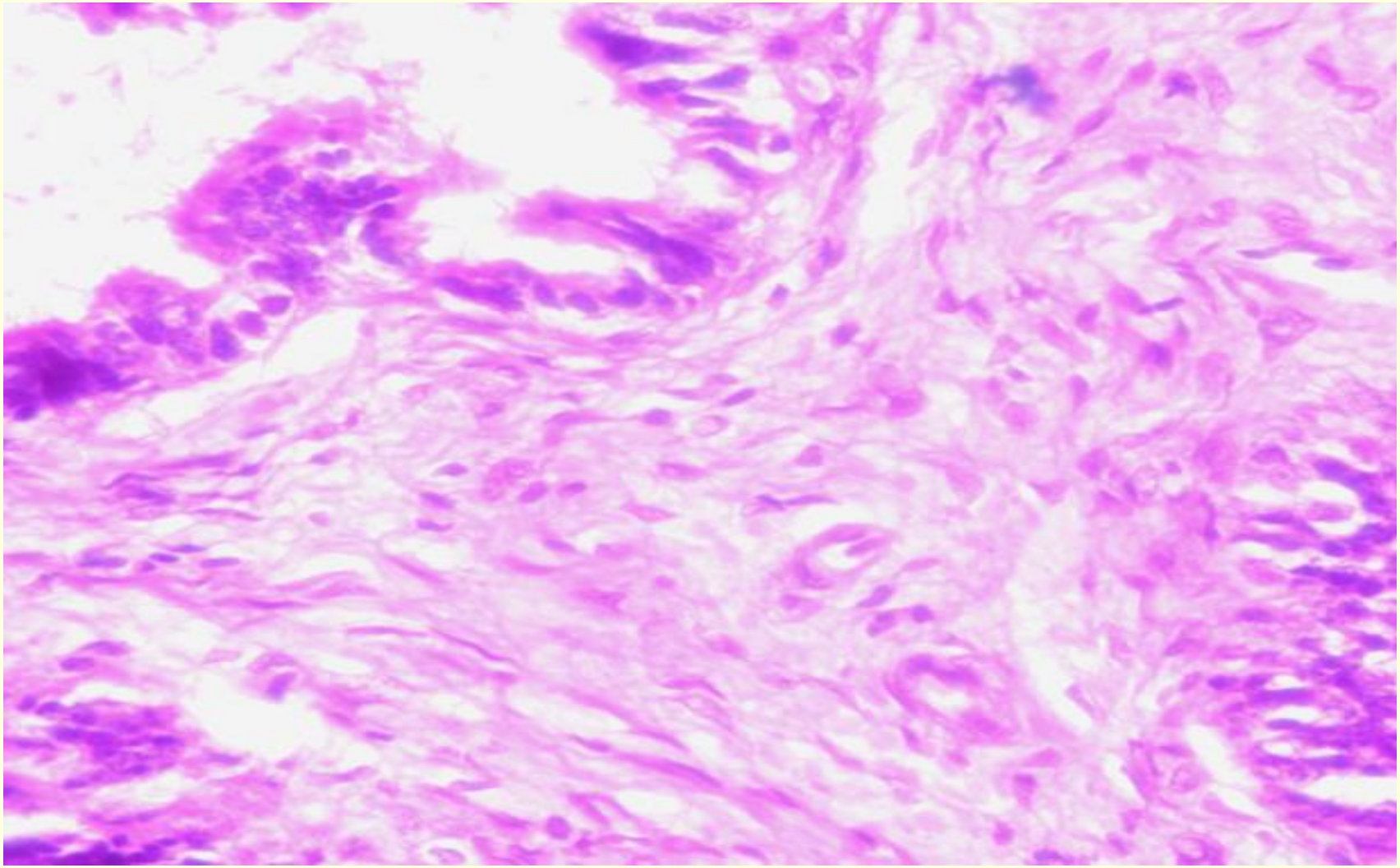
Dilated epididymal ducts contents showing macrophages with phagocytosed sperms and desquamated lining cells (H&E x400)



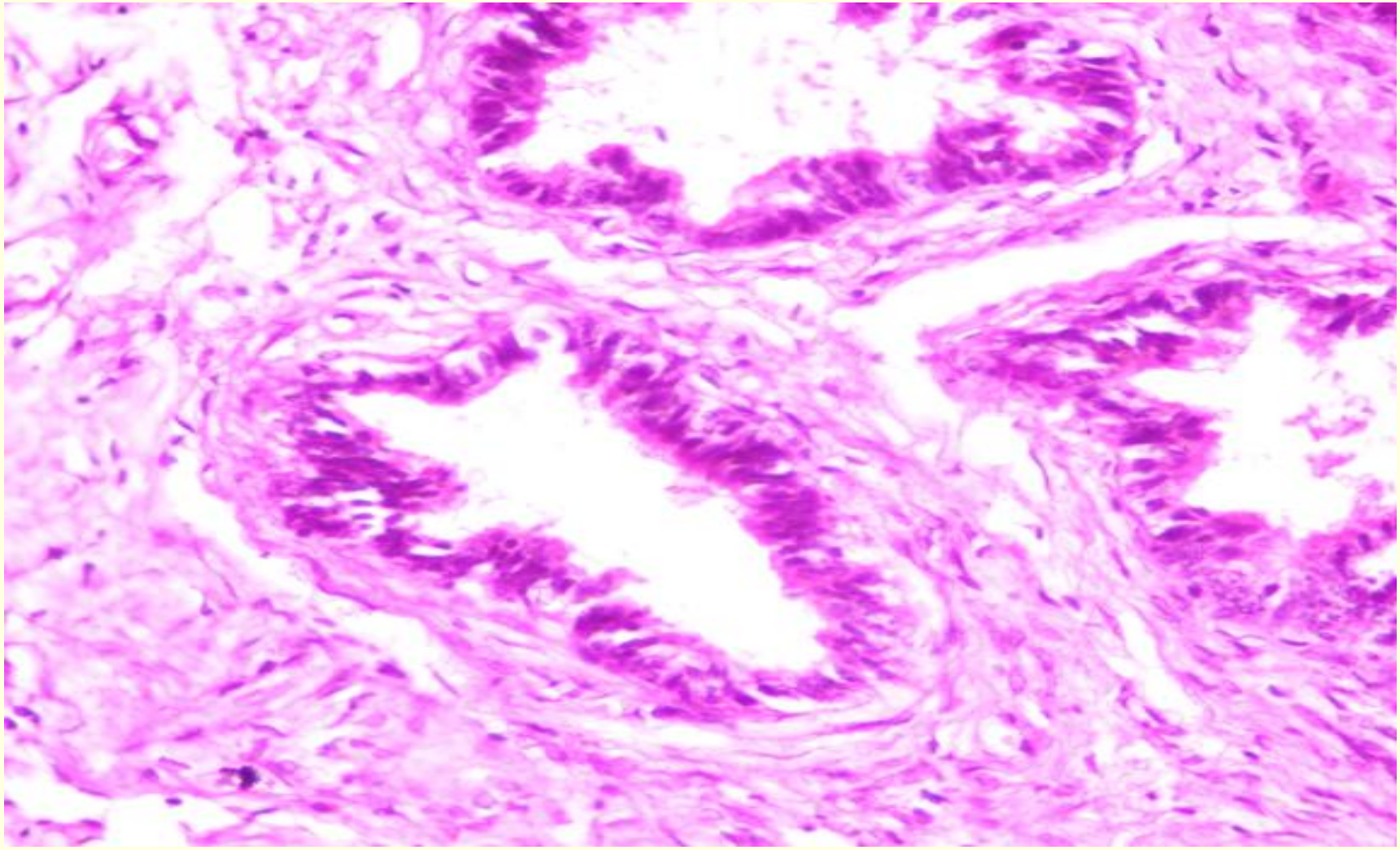
Epididymis showing interstitial collection of macrophages with a dilated epididymal duct to the left (H&E x400)



Epididymis showing peritubular fibrosis (H&E x600)



Interstitial epididymal fibrosis with mild chronic inflammation (H&E x400)



Drawbacks of the study

- Direct correlation between seminal fluid antibodies and local immune response
- Estimation of sperm bound antibodies and their correlation with seminal fluid antibodies
- Reversal of seminal fluid and sperm bound antibodies following reversal
- Effect of local immune response on failure of vasoepididymostomy results
- Similar studies in vasectomized and vasectomy reversal subjects

Differences in immune response in primary & secondary obstruction

- Levels of antisperm antibody production
- Extent of local autoimmune response
- Effect on sperm (count & morphology) proximal to obstruction

Conclusion

- Systemic and local immune response may be an important factor in failure of fertility following successful recanalisation and in assisted reproductive techniques.

Thank you