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Composition of fluids obtained from human epididymal cysts*

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Summary. The fluid composition of five epididymal spermatocoeles, one epididymal cyst and a hydrocoele was examined. The fluid obtained from the spermatocoeles was a dilute suspension of mainly immotile spermatozoa. The sperm-free fluid contained less protein, phosphate, glucose, triglyceride and cholesterol than serum but more testosterone and chloride than peripheral blood. It contained no epididymal secretion products. Proteins in the fluid differed from those in serum. From the fluid composition these cysts appeared to be continuous with the rete testis, either dilatations of efferent ducts or Haller's superior aberrant duct (vas aberrans of the rete testis). Fluid from an epididymal cyst containing no spermatozoa was mainly of similar composition. In contrast, hydrocoele fluid resembles blood serum.

Key words: Human epididymis – Spermatocoele – Cyst fluid – Hydrocoele – Fluid composition

Clarification of the recent debate about the rôle of human epididymis in sperm maturation [6] requires more information about the composition of fluids in different regions of the human male reproductive tract. Epididymal secretions in particular may be important for influencing the maturation and storage of spermatozoa. Some components of fluid from the human vas deferens are known [16, 17] but there are only a few reports of human epididymal fluid obtained by micropuncture [11, 19, 20]. Although access to human testicular fluids is rare, some so-called epididymal cysts containing spermatozoa are thought to be in continuity with the testis itself [23, 31], so study of these cysts may provide information on the composition of testicular fluid in man.

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Materials and methods

Epididymal cysts

A large fluid-filled spermatocoele, located between the testis and epididymis, became available for study after castration for prostatic carcinoma and was analyzed for many components of serum and semen. This cyst was the first large one to be seen after examining the scrotal contents of 54 men over 3 years. A smaller spermatocoele from another patient and three spermatocoeles (Table 1, no. 5 from the right side and nos. 3 and 4 from the left side) obtained from a third patient pre-treated with anti-androgens were also analysed. Spermatocoeles 3 and 4 were apparently unconnected since withdrawal of fluid from one did not lead to the collapse of the other. A sperm-free epididymal cyst from a 93-year-old patient was examined. A pedunculated cyst from a 54-year-old, a pedunculated spermatocoele from a 75-year-old and a larger spermatocoele (ruptured on excision) from a 26-year-old man were too small for fluid collection, but were fixed for observation by light and electron microscopy. Hydrocoele fluid was obtained from a 72-year old patient operated on for prostatic carcinoma.

The first cyst was transported to the laboratory (60 min) in Ham's F-12 medium on ice; the others took 15 min to reach the laboratory from the local hospital. The fluids were aspirated through 23 or 22-gauge needles, examined microscopically, and cells were counted in a Fuchs-Rosenthal haemocytometer and smears were stained (Papanicolou stain [33]). After centrifugation at 1000 *g* for 10 min the fluid was frozen at −20°C and where necessary the cellular pellet was stained by technicians familiar with morphology of ejaculated spermatozoa.

Seminal plasma

A pool of seminal plasma from men attending our infertility clinic was used for comparison of protein profiles.

Biochemical analysis

Clinical chemical analyses were performed on the fluid by an autoanalyser and measurements of testosterone [4] and oestradiol (without chromatography) [1] were made as described previously. Semen markers were assayed as described before [7]. Proteins were separated by sodium dodecyl sulphate – polyacrylamide gel electro-

Table 1. Source and characteristics of spermatocoeles from prostatic carcinoma patients

	Spermatocoele				
	1	2	3	4	5
Age (years)	70	36	81	81	81
Volume (ml)	40	8	1.5	1	4
Sperm concentration (10^6/ml)	0.3	15.8	18.9	16.3	2.7
Normal heads (%)	35	31	11	13	14
Mid-piece defects (%)	21	4	5	20	7
Tail defects (%)	8	14	3	2	0
Motile (%)	30	40	7	9	23

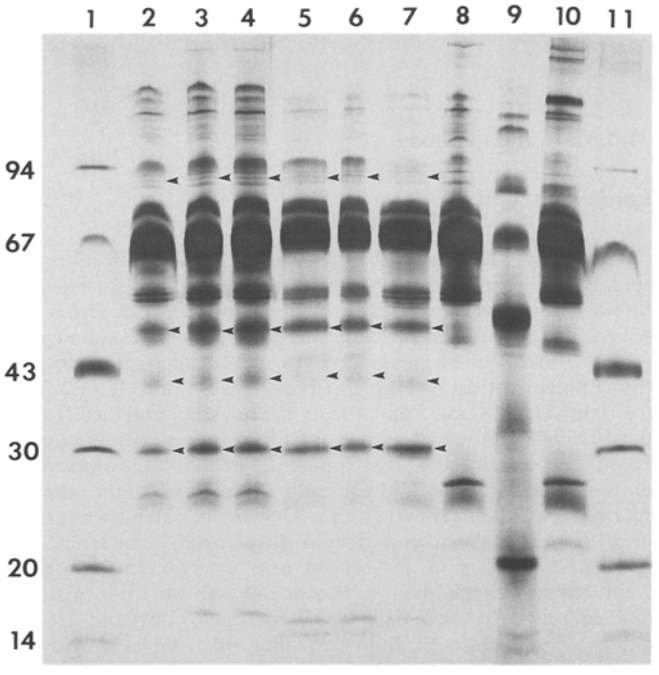


Fig. 1. Proteins (3 μg) separated by sodium dodecyl sulphate gel electrophoresis (12.5% gels) and stained with silver nitrate. Lanes: 1, molecular weight standards; 2, spermatocoele 5;3, spermatocoele 4;4, spermatocoele 3; 5, cyst; 6, spermatocoele 2; 7, spermatocoele 1; 8, hydrocoele; 9, seminal plasma pool; 10, serum; 11, molecular weight markers. Numbers on the left refer to molecular weight standards (kDa); *small arrowheads* indicate protein bands in cyst fluids that are not present in blood

phoresis (SDS-PAGE) in 12.5% gels [22] and stained with a modified [14] silver nitrate method [2, 15]. Cyst fluids were compared with pools of serum and semen from normozoospermic patients.

Microscopy

A piece of cyst wall from spermatocoele 1 was fixed in Bouin's fluid and embedded in paraffin. Portions of spermatocoele 2 and the epididymal cyst from which fluid was collected, together with the three other cystic structures, were fixed in 5% glutaraldehyde, embedded in Epon and prepared for electron microscopy [34].

Results

Composition of fluids

Epididymal spermatocoeles. A few sperm cells displayed forward progression in spermatocoeles 1 and 2. The cell-free fluid was clear and colourless and its composition is given in Table 2. Protein concentration was low in comparison with serum, and electrophoresis revealed the presence of albumin as a major component as judged from densitometric tracings (Table 2, Fig. 1). Several protein bands in the fluid were absent from serum and other blood proteins were absent from the fluid (Fig. 1). Glutamate-oxaloacetate transaminase (GOT), glutamate-pyruvate transaminase (GPT) and γ-glutamyl transferase (GGT) were of similar activity to blood. The activities of alkaline phosphatase and lactate dehydrogenase (LDH) were variable with mean values lower than serum.

Na^+ concentrations were in the range found in blood. K^+, and especially Cl^-, were above the normal range, and Ca^{2+} was below it, with inorganic phosphate especially so. Total androgens were present at about five times the mean peripheral serum concentrations for normal men but no oestradiol-17β was detected in one sample assayed. Little triglyceride, cholesterol or glucose was present in spermatocoele fluid, and uric acid and bilirubin were marginally below serum values. Urea concentrations fell within normal serum limits, whereas creatinine exceeded them. Secretions of the seminal vesicles (fructose), prostate (citrate) and epididymis (neutral α-glucosidase, glycerophosphocholine and L-carnitine) were absent from all samples.

Epididymal cyst. About 1 ml of a straw-coloured fluid containing no spermatozoa was obtained. There was more total protein than in the sperm-containing cysts but the protein profile was similar to that of the sperm-containing cysts (28% albumin; Fig. 1). The activities of LDH and GOT were much lower than in the spermatocoeles, and those of GPT and alkaline phosphatase non-detectable; conversely, GGT was much higher than in blood. Sodium, K^+ and Cl^- concentrations were higher than blood, and calcium lower, but the phosphate concentration was higher than in spermatocoele fluid although still lower than that in blood. Triglyceride, cholesterol and glucose

Table 2. Fluid composition of epididymal cysts in comparison with hydrocoele fluid and serum

Constituent	Unit	Fluids				
		Spermatocoeles		Cyst	Hydrocoele	Serum (range)
		Mean ± SEM	*(n)*			
Protein	mg/ml	2.3 ± 0.2	(5)	7	37	(66–87)[a]
Albumin	%	29.3 ± 1.0	(5)	28.1	22.3	20.6
Glutamate-oxaloacetate transaminase	U/l	8.6 ± 4.5	(5)	1	4	(2–18)[a]
Glutamate-pyruvate transaminase	U/l	14.8 ± 5.4	(5)	ND	6	(2–22)[a]
γ-Glutamyltranspeptidase	U/l	12.6 ± 4.8	(5)	96	16	(6–28)[c]
Alkaline phosphatase	U/l	62.4 ±24.8	(5)	ND	9	(70–175)[c]
Lactate dehydrogenase	U/l	55.2 ±33.1	(5)	2	137	(120–240)[c]
Acid phosphatase (AP)	U/l	1	(1)	NM	NM	(4.8–13.5)[c]
Prostatic AP	U/l	1	(1)	NM	NM	(0–3.7)[c]
Chloride	mM	139.6 ± 3.9	(5)	142	114	(97–107)[a]
Sodium	mM	148.4 ± 4.2	(5)	168	151	(132–155)[a]
Potassium	mM	5.9 ± 0.4	(5)	5.8	4.8	(3.6–5.4)[a]
Calcium	mM	1.87 ± 0.06	(5)	1.2	2.0	(2.1–2.9)[a]
Phosphate	μM	27.3 ±10.8	(5)	31.5	357.8	(263–474)[a]
Androgen	nM	66.0 ±13.2	(5)	19.9	3.2	(10–30)[a]
Oestradiol	pM	nd		NM	NM	(55–165)[b]
Triglyceride	mg/dl	1.2 ± 0.8	(5)	4	23	(74–172)[c]
Cholesterol	mM	0.01 ± 0.01	(5)	0.38	1.24	(6.7–7.7)[c]
Glucose	mM	0.15 ± 0.14	(5)	ND	5.49	(3.9–6.1)[a]
Urea	mM	3.52 ± 0.45	(5)	8.32	3.83	(1.7–8.3)[c]
Uric acid	mM	0.18 ± 0.03	(5)	0.34	0.3	(0.28–1.37)[c]
Total bilirubin	mg/dl	0.02 ± 0.02	(5)	ND	0.5	(0.1–1)[a]
Creatinine	μM	150.5 ±11.5	(5)	NM	115	(60–120)[c]

[a] Normal serum range from clinical laboratory controls
[b] Individual values from a patient pool
[c] Cited by Thomas [27]
NM, Not measured; ND, not detectable

concentrations were still low compared to blood but the urea concentration was at the higher end of the serum range.

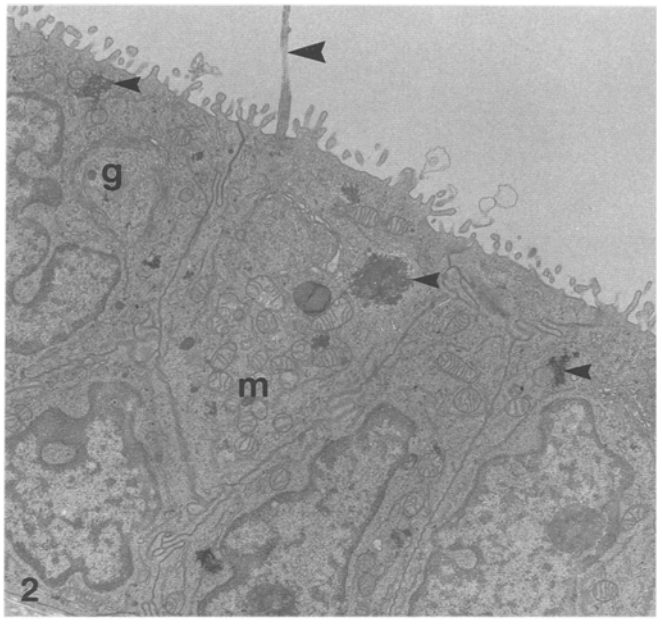
Hydrocoele fluid. About 45 ml of deep yellow fluid was obtained from within the tunica vaginalis. The protein, triglyceride and cholesterol concentrations of this fluid were much higher than in the other fluids, but still lower than in serum. The protein profile was similar to that in serum (22% albumin: Fig. 1). Glucose, urea, uric acid, creatinine, total bilirubin and phosphate concentrations were all in the serum range. The activities of the enzymes LDH, GOT, GPT and GGT were in the serum range but the activity of alkaline phosphatase was low. Of the electrolytes Cl^- was at a marginally higher concentration than in serum.

Seminal fluid. Only the protein composition of seminal fluid was analyzed by SDS-PAGE. All fluids differed markedly from seminal plasma with regard to protein profile (13.6% albumin; Fig. 1).

Epithelial structure

In the light microscope the epithelium lining spermatocoele 1 (the large spermatocoele) was extremely attenuated, resembling an endothelium. The epithelium lining the second spermatocoele was variable in height, in places cuboidal, in places a low columnar epithelium with some regions of low, attenuated epithelium. The sperm-free cyst was lined by cuboidal or columnar epithelium with no evidence of attenuated cells. Epithelial structure in the three cysts from which no fluid was aspirated was similar, in that the sperm-containing cysts had epithelia of variable height with areas with low epithelium, and the sperm-free cyst had a more regular epithelium with no attenuated cells.

With the electron microscope, it was possible to discern within the epithelium non-ciliated cells and cells bearing a single cilium (Fig. 2). Both cell types contained what appeared to be glycogen. True ciliated cells (Fig. 3) were also seen.



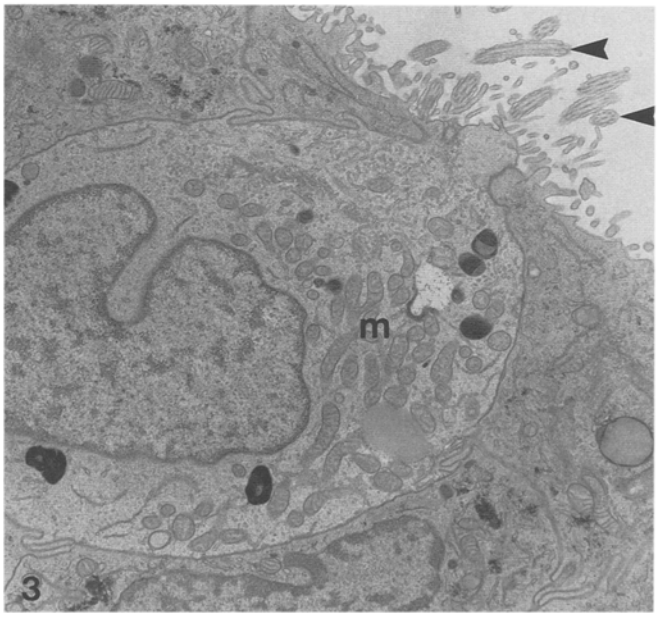


Fig. 2. Electron micrograph of cells lining a pedunculated epididymal cyst from a 54-year-old patient having received 100 mg cyproterone acetate for 10 days before castration. A cell bearing a single flagellum *(large arrowhead)* is evident and this, and the other cells, contain dark deposits *(small arrowheads)* resembling glycogen. Mitochondria *(m)* and Golgi apparatus *(g)* are present in the cells with a highly lobulated nucleus. ×5720

Fig. 3. Several cilia *(arrowheads)* of a true ciliated cell from the same cyst as in Fig. 2. This more pale-staining cell has darker mitochondria *(m)* than the non-ciliated cells. ×7725

Discussion

Congenital anomalies of the human testis and eipididymis include the appendix testis (sessile hydatid of Morgagni), comprising a blind remnant of the cephalic portion of the müllerian (paramesonephric) duct; the appendix epididymis (or pedunculated hydatid of Morgagni), a blind remnant of the cephalic portion of the mesonephric (wolffian) duct; the aberrant ducts (Haller's organs), of which the superior (the vas aberrans of the rete testis) is a remnant of the most cranial mesonephric duct and lies in the caput epididymidis and the inferior lies in the corpus or cauda epididymidis; and paradidymis (Giraldè's organ or Henle's paraepididymis), derived from the most caudal portion of the mesonephric ducts [23, 31]. In addition to these structures, failed congenital connections between individual efferent ducts and the epididymis also lead to "simple cysts" in the caput epididymidis, which, when containing spermatozoa in the adult, are termed spermatocoeles [23].

As the appendix testis, appendix epididymis and paradidymis are blind-ending structures, unconnected to the excurrent duct system, they should not contain spermatozoa. By contrast, the aberrant ducts and dilatations of the ductuli efferentes are continuous with the excurrent duct system and should contain spermatozoa and luminal fluid. The presence of spermatozoa can thus help to establish the nature of the cystic structure, and the fluid composition can provide information about intraluminal fluids in the human genital tract.

In a detailed study of the classification and location of aberrant ducts in man Wollin et al. [32] described them mainly in association with failed testicular descent, although rare cases occur in normal descent [24], as found here. Of sperm-containing cysts, Griffiths [10] distinguished between multiple, small cysts on the coni vasculosi and the larger, single cysts originating as dilatations of the efferent ducts leaving the testis. Wakeley [31] referred to the latter as the "vas aberrans of the rete testis", and they are lined by a simple cuboidal epithelium [25].

From the low protein concentration and absence of established epididymal markers in the fluid obtained in this study, it can be argued that the large cyst examined was not continuous with the corpus epididymal lumen, where these products accumulate [11, 12, 28, 35], and thus is unlikely to represent the "inferior aberrant duct of Haller". The electrophoretic pattern of protein also differed from that reported for human epididymal fluid collected by micropuncture [19, 20]. On the other hand, similarity with reports in the literature supports the view that this fluid was of testicular origin: a colourless dilute suspension of weakly motile spermatozoa is recovered from the rete of many species and the fluid composition is characterized by low concentrations of protein, phosphate, glucose and cholesterol, and by relatively high concentrations of chloride and testosterone [29]. The protein spectrum of the fluid resembled that found in human testicular fluid obtained at autopsy [19] and low activities per unit volume of serum enzymes have also been reported in this fluid from other species [26].

The non-sperm-containing cyst was similar in many respects to the fluid in the spermatocoeles. However, the much lower activity of LDH, GOT and GPT in the fluid suggests that these enzymes originate from within the testis and cannot enter the cystic structure owing to the lack of direct continuity with the rete. The source of the

high activity of GGT in this sample is unclear and needs to be confirmed in other samples of cyst fluid.

The hydrocoele fluid was more similar to serum than the other fluids and had markedly higher concentrations of bilirubin, glucose, cholesterol, triglyceride, phosphate and protein than the luminal fluids. Nevertheless, protein, cholesterol and triglyceride concentrations were still appreciably lower than equivalent serum levels. Lower protein concentration and electrophoretic profiles similar to serum (45% albumin) have also been described for "tunica vaginalis fluid", an intrascrotal fluid bathing the epididymis [9]. However, androgen concentrations reported for "tunica vaginalis fluid" by both Karpe et al. [21] and Gerris and Schoysman [9], which exceeded serum levels 26- to 58-fold, were far greater than the concentration in our samples. We do not know if the low concentration we found reflects loss by diffusion during transport of the material or the testicular function of these particular patients.

The present results extend the observations on epididymal cysts of similar size by Griffiths [10] and Wakeley [31], both of whom noted, but did not quantify, the low albumin content of the fluid. More information is available on the composition of fluid from smaller epididymal cysts. In these, Huggins and Johnson [18] reported little protein and phosphate, traces of glucose and relatively high chloride and calcium levels. There is no other information regarding the composition of rete testis fluid in man, but in macaque monkeys concentrations of Na^+ and K^+ are similar to those found here [30]. The testosterone concentration in the spermatocoeles was 26% of that reported to be present in human spermatic venous blood of men with prostatic cancer [13], and thus higher than the equivalent percentage (10%) in the monkey [30].

The epithelium lining these cystic structures also provides evidence for their being associated with the testis or extratesticular ducts. Firstly, the presence of glycogen deposits and a single cilium is a common feature of cells from the rete testis of a number of species [8]; secondly, the nuclei of some of the epithelial cells resemble that of the extratesticular rete in man [3, 36]; thirdly the presence of ciliated cells is characteristic of epithelia in the efferent ducts, not epididymis proper [36]. Together, these data suggest that the cystic swellings arise as evaginations of rete or efferent duct cavities, at the junctions of these structures or, most likely, from the fine, blind-ending tubuli that branch from the main ductuli efferentes emanating from the testis [36]. When continuity with the testis is maintained (as in a spermatocoele) spermatozoa are present and the continued accumulation of fluid may extend and compress the epithelium. Dilatations of the duct system bearing no connection with the testis contain fluid and no spermatozoa and do not display the distension seen in the spermatocoeles, as reflected by the regularity of the epithelial height.

Testicular fluid in man thus differs markedly from luminal fluid present in more distal parts of the human male tract (the vas deferens [16, 17]), where concentrations of K^+, phosphate and carnitine are much higher and Na^+ and Cl^- lower. Similar changes in concentrations of these substances occur along he length of the human epididymis as in other species [5].

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References

1. Belkien L, Bordt J, Moeller P, Hano R, Nieschlag E (1985) Estradiol in saliva for monitoring follicular stimulation in an in vitro fertilization program. Fertil Steril 44:322
2. Blum H, Beier H, Gross H (1987) Improved silver staining of plant proteins, RNA and DNA on polyacrylamide gels. Electrophoresis 8:93
3. Bustos-Obregón E, Holstein A (1976) The rete testis in man: ultrastructural aspects. Cell Tissue Res 175:1
4. Chandolia RK, Weinbauer GF, Behre HM, Simoni M, Nieschlag E (1991) Comparative effects of chronic administration of the non-steroidal antiandrogens Flutamide and Casodex on the reproductive system of the adult rat. Acta Endocrinol (Copenh) 105:547
5. Cooper T (1986) The epididymis, sperm maturation and fertilisation. Springer, Berlin Heidelberg New York
6. Cooper T (1990) In defense of a function for the human epididymis. Fertil Steril 54:965
7. Cooper T, Jockenhövel F, Nieschlag E (1991) Variation in seminal parameters from fathers. Hum Reprod 6:859
8. Dym M (1976) The mammalian rete testis – a morphological study. Anat Rec 186:493
9. Gerris J, Schoysman R (1984) Tunica vaginalis fluid (TVF): hormonal composition. Assessment of testosterone, 5α-dihydrotestosterone, δ4-androstenedione, dehydroepiandrosteronesulphate, estradiol, LH, FSH and PRL. Acta Eur Fertil 15:205
10. Griffiths J (1893) Observations on the appendix of the testicle, and on the cysts of the epididymis, the vasa efferentia, and the rete testis. J Anat Physiol 28:107
11. Guérin J, Czyba J (1987) Hydrolytic enzymes detected in fluid and in spermatozoa from different portions of human epididymis. In: Spera G, De Kretser D (eds) Morphological basis of human reproductive function. Plenum Press, London, p 197
12. Guérin J, Rollet J, Perrin P, Menezo Y, Orgiazzi A, Czyba J (1981) Enzymes in the seminal plasma from azoospermic men: correlation with the origin of their azoospermia. Fertil Steril 36:368
13. Hammond G, Ruokinen A, Kontturi M, Koskela E, Vihko R (1977) The simultaneous radioimmunoassay of seven steroids in human spermatic and peripheral venous blood. J Clin Endocrinol Metab 45:16
14. Henkel R (1990) Untersuchungen zur Proteinsekretion der Blastocyste des Kaninchens (*Orytologaus cuniculus* L.) während der frühen Gravidität. Inaugrural Dissertation, Philipps-University, Marburg/Lahn
15. Heukeshoeven J, Dernick R (1986) Neue Ergebnisse zum Mechanismus der Silberfärbung. In: Radola B (ed) Elektrophorese Forum. Technical University, Munich p 22
16. Hinton B, Setchell B (1981) Micropuncture and microanalytical studies of rhesus monkey and baboon epididymis and the human ductus deferens. AmJ Primatol 1:251
17. Hinton B, Pryor J, Hirsch A, Setchell B (1981) The concentration of some inorganic ions and organic compounds in the luminal fluid of the human ductus deferens. Int J Androl 4:457
18. Huggins C, Johnson A (1933) Chemical observations on fluids of the seminal tract. I. Inorganic phosphorus, calcium, non-protein nitrogen and glucose content of semen and of seminal vesicle, prostate and spermatocoele fluids in man. Am J Physiol 103:574
19. Jennings M, Temple-Smith P, Smithwick G, Nauau P, Baker H (1987) Patterns of human epididymal fluid proteins on polyacrylamide gel electrophoresis. Int J Androl 10:441

20. Junera H, Alfonsi M,Fain-Maurel M, Dadoune J (1988) Characterization of regional proteins in tissues and fluids in the human epididymis. Reprod Nutr Dev 28:1267
21. Karpe B, Fredricsson B, Svensson J, Ritzén E (1982) Testosterone concentration within the tunica vaginalis of boys and adult men. Int J Androl 5:549
22. Laemmli U (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage. Nature 227:680
23. Nistal M, Paniagua R (1984) Congenital anomalies of the testis and the epididymis. In: Testicular and epididymal pathology. Thieme-Stratton, New York, p 72
24. Rolnick D, Kawanoue S, Szanto P, Buch I (1968) Anatomical incidence of testicular appendages. J Urol 100:755
25. Saitoh K, Terada T, Hatakeyama S (1990) A morphological study of the efferent ducts of the human epididymis. Int J Androl 13:369
26. Suominen J, Setchell B (1972) Enzymes and trypsin inhibitors in the rete testis fluid of rams and boars. J Reprod Fertil 30:235
27. Thomas L (ed) (1984) Labor and diagnose, appendix 2. Medizinische Verlagsgesellschaft, Marburg/Lahn
28. Tomamichel G, Bandhauer K (1986) Seminal carnitine content in obstructive azoospermia. Correlation with the anatomic level of obstruction. J Androl 7:324
29. Waites G (1977) Fluid secretion: In: Johnson A, Gomes W (eds) The testis. Academic Press, New York, p 91
30. Waites G, Einer-Jensen N (1974) Collection and analysis of rete testis fluid from macaque monkeys. J Reprod Fertil 41:505
31. Wakeley C (1943) Cysts of the epididymis, the so-called spermatocoele. Br J Surg 31:165
32. Wollin M, Marshall F, Fink M, Malhotra R, Diamond D (198) Aberrant epididymal tissue: a significant clinical entity. J Urol 138:1247
33. World Health Organization (1987) WHO laboratory manual for the examination of human semen and semen-cervical mucus interaction. Cambridge University Press, Cambridge
34. Yeung C, Cooper T, Meyer R (1987) Immature rat epididymal epithelial cells grown in static primary monolayer culture on permeable supports. II. Histochemistry and ultrastructure. Cell Tissue Res 256:573
35. Yeung C, Cooper T, Senge T (1990) Histochemical localization and quantification of α-glucosidase in the epididymis of men and laboratory animals. Biol Reprod 42:669
36. Yeung C, Cooper T, Bergmann M (1991) The organization of tubules in the human caput epididymidis and the ultrastructure of their epithelia. Am J Anat 191:261

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