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The mediastinum of the bovine testis

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Summary. The bovine testis has a central mediastinum consisting of longitudinally oriented rete channels and spacious lymph vessels, embedded in the mediastinal stroma. The latter represents a contractile-elastic unit and is composed of myofibroblasts, collagen bundles and accumulations of elastin, connecting the myofibroblasts. The dimension of the mediastinum varies in cross sections at different levels between 3.5 and 31.8 mm^2. In one cross section ~30 rete channels and ~30 openings of straight testicular tubules are encountered. Nearly 25% of the area is occupied by thin-walled, valveless lymph vessels. Arterial convolutes, interpolated between straight centripetal and straight centrifugal branches of the testicular artery flank the rete on all sides. It is concluded that the pulsation within these convolutes together with the contractile-elastic stroma promotes lymph and rete content in a caudo-cranial direction. Chordae retis as described by Roosen-Runge and Holstein (1978) for the human testis are a common feature in the bovine mediastinum testis. The rete channels are lined by a simple cuboidal or columnar epithelium. Short intraepithelial crypts are present and function as epithelial reserve for dilatation and expansion of the rate. The inventory of organelles is rather inconspicuous in the rete epithelium. The apical border bears short microvilli and gives a strong reaction for alkaline phosphatase. The basal cytoplasm contains many small to medium-sized electron-dense bodies and is site of a strong acid phosphatase reaction. The rete epithelium as a whole reacts strongly with leucine aminopeptidase, the marker enzyme of the testicular excurrent duct system. Many free mononuclear cells, mostly macrophages, are observed in the basal half of the rete epithelium.

Key words: Testis – Mediastinum testis – Rete testis – Myofibroblasts – Lymphatics – Bovine

The importance of the topographic unit mediastinum testis and the functional cooperation of its various components – connective tissue, vessels and rete testis – have found little attention to date. Roosen-Runge and Holstein (1978) in their extensive work on the human rete testis included also the significance of the mediastinal stroma in their study. All other investigations concentrated mainly on the rete testis as an important region of the intratesticular excurrent duct system, on the permeability of its epithelial lining and on the presence of free cells in the rete epithelium (see Dym 1976). The ultrastructure of cells lining the rete testis is well documented and seems to be rather similiar in man (Roosen-Runge and Holstein 1978), higher mammals (Dym 1976), marsupials (Rodger 1982) and even in birds (Barker and Kendall 1984). Structure and development of the mediastinum, however, differ among the species, as does the localization of the rete. When a superficial or marginal rete is present, as in man, monkey, stallion, rat, mouse, and hamster (Benoit 1926), the mediastinum is small and restricted to the cranial testicular pole. In ruminants such as bull (Hees et al. 1987), buffalo (Goyal et Dhingra 1973), zebu (Orsi et al. 1983), ram, and goat (Orsi et al. 1984), a relatively voluminous rete is embedded in a well-developed connective tissue complex, situated in the longitudinal testicular axis. We have studied the structural organization of such a central mediastinum testis using the bull as a model.

Materials and methods

Testes of 25 adult bulls were obtained 5–10 min after slaughter (10 for light microscopy, 7 for TEM, 5 for SEM and 3 for histochemical studies). Perfusion fixation of the testes for TEM and light microscopy was performed using a gas-driven pump described by Scheubeck and Wrobel (1984).

Light microscopy

A cannula was inserted into the intratunical portion of the arteria testicularis, which runs parallel to the corpus epididymidis. Rinsing procedure and perfusion with Bouin's solution were performed as described elsewhere (Wrobel et al. 1978). 5–7 µm thick serial sections (longitudinal as well as cross sections) of the mediastinum testis were prepared from Paraplast-embedded material and stained with a modified Masson-Goldner sequence and with resorcin-fuchsin for elastic fibers. Lipid was visualized with Sudan Black B in frozen sections.

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Transmission electron microscopy

Fixation was performed with the formaldehyde-glutaraldehyde fixative as described by Karnovsky (1965). Small pieces of the mediastinal tissue were separated and washed in 0.2 M phosphate buffer. After osmication (1% OsO_4) the blocks were dehydrated in graded ethanol and embedded in ERL 4206 (Spurr 1969). Ultrathin sections were mounted on copper grids, stained with uranyl acetate and lead citrate (Reynolds 1963) and examined with a Zeiss EM 10 A electron microscope.

Scanning electron microscopy

The testes were perfused with Ringer's solution (at 38° C, containing 1 ml/l Liquemine and 0.5% Procaine) and stored in ice water for 3 h to prevent vasoconstriction and premature polymerization of the injection mass. Following a second perfusion with Ringer's solution (at 25° C) the injection compound was instilled: Low viscosity methyl-methacrylate mixtures (Kohler and Leiser 1983; Hees et al. 1984)) were used: (A) Batson Nr. 17 corrosion compound (Polysciences Inc. Warrington, Pennsylvania, USA); 25 ml monomer base solution 4° C; 7.5 ml catalyst, room temperature; 0.5 ml promotor, 4° C, (B) 12 ml Sevriton (available from most dental suppliers), 4° C.

After injection, testes were stored at room temperature for 30 min, then transferred to a water bath at 80° C for 4 h to enable polymerization. Tissue corrosion was performed by alternating submersion in 40% KOH and distilled water (60° C) for a few days. The liquids were changed once a day. Some of the finished corrosion casts were dissected under a stereo microscope to visualize the mediastinal region for a general survey; others were examined with a Philips 500 SEM.

Histochemistry

For the demonstration of enzyme activity at the light-microscopical level small blocks of native tissue were immersed in liquid nitrogen and transferred to a cryostat for sectioning. 7–10 µm thick sections were subjected to procedures demonstrating alkaline phosphatase (Gomori 1952), adenosine triphosphatase (v. Deimling 1964), acid phosphatase (Barka and Anderson 1962), indoxylacetate esterase (Holt and Withers 1958), leucine aminopeptidase (Nachlas et al. 1968), NADH-T-reductase and NADPH-T-reductase (Hess et al. 1958; Nachlas et al. 1958; Lojda et al. 1976).

Results

Topography of the mediastinum testis

The bovine testis has a centrally located mediastinum, which is laterally and caudally surrounded by parenchyma. The functional unit of the mediastinum comprises the rete testis, large blood vessels and spacious lymph vessels, all embedded in the mediastinal stroma, which apart from the tunica albuginea, represents the largest connective tissue complex of the bovine testis (Fig. 1).

Originating in the tunica albuginea large straight arteries (rami parenchymales centripetales) run in a radial course to the mediastinum where they turn into heavily convoluted coils flanking the rete testis. Number and size of the coils vary, thus the length of the convoluted portions changes between 1.5 and 5 cm. The shape of the convolutes also differs considerably between corkscrew-, loop- and glomerulum-like, the latter configuration dominating. When the convolutes are elongated, they are arranged in most cases parallel to longitudinally oriented rete channels (Fig. 1). The convolutes in the caudal and middle thirds of the mediastinum are generally more voluminous than those in the cranial portion. Histological cross sections through the mediastinum reveal that in a given plane the convolutes mostly flank the rete from all sides (Fig. 2a). The distribution of centripetal straight arteries, however, is not uniform within the testes: a reduced number comes from the testicular surface adjacent to the epididymal body. As a result of this uneven distribution convolutes may be lacking at the mediastinal surfaces, pointing to the epididymis (Fig. 2b). However, transversally arranged arterial convolutes, originating from straight centripetal arteries of other regions may also reach the epididymal sector of the mediastinum. The arterial coils often protrude centrally and thus narrow the rete testis channels (Fig. 2c); in some cross sections the coils are situated in the axis of the mediastinum, or they separate small portions of the rete from the remainder. In longitudinal sections, or to better advantage in corrosion casts, the convolutes are seen arranged in subsequent levels with regular intervals (Fig. 3). Anastomoses between adjacent convolutes of different levels are not observed.

The diameter of the mediastinum and its cross-sectional configuration vary in different planes. Evaluation of the dimensions of the mediastinum in 30 evenly spaced planes revealed differences between 3.5 mm^2 and 31.8 mm^2 (arterial convolutes not included). Thus, the mediastinum is a central longitudinal connective tissue complex with frequently and abruptly changing diameter. Maximal dimensions occur with a certain regularity at a distance of 2–3 cm. The mediastinal cross sections may be round but also star-, U- or hourglass-shaped (Fig. 2c), probably due to the local configuration of the arterial convolutes.

Within one cross section of the mediastinum on the average 30 (10–40) rete channels were encountered. There exist no channels that pass through the total length of the mediastinum. The longitudinally oriented channels anastomose frequently, form larger spaces and branch again at higher levels, thus constituting a labyrinthine duct system of high complexity. The cross section of the individual channel is seldom round, but oblong or slit-like lumina prevail. In one cross-sectional plane on the average 30 entering straight testicular tubules are observed.

Mediastinum and rete testis begin at a distance of nearly 3 cm from the caudal pole of the testis. A cross section at this level shows a number of closely adjoining straight testicular tubules, intermingled with narrow rete extensions of the same diameter, both structures embedded in abundant connective tissue (Fig. 5). Only a few millimeters more cranial the now more numerous rete channels develop the first anastomoses and dilatations.

The straight centripetal arteries from the caudal pole run in the central longitudinal axis to the beginning of the mediastinum; the other straight centripetal arteries of the caudolateral testicular portion converge also to the same point. Therefore, the caudal initial region of the rete is intimately surrounded by many arterial convolutes (Figs. 4, 7). In cranial direction, the approaching straight centripetal arteries reduce gradually their angle of ascent when passing from the tunica albuginea to the mediastinum but they are

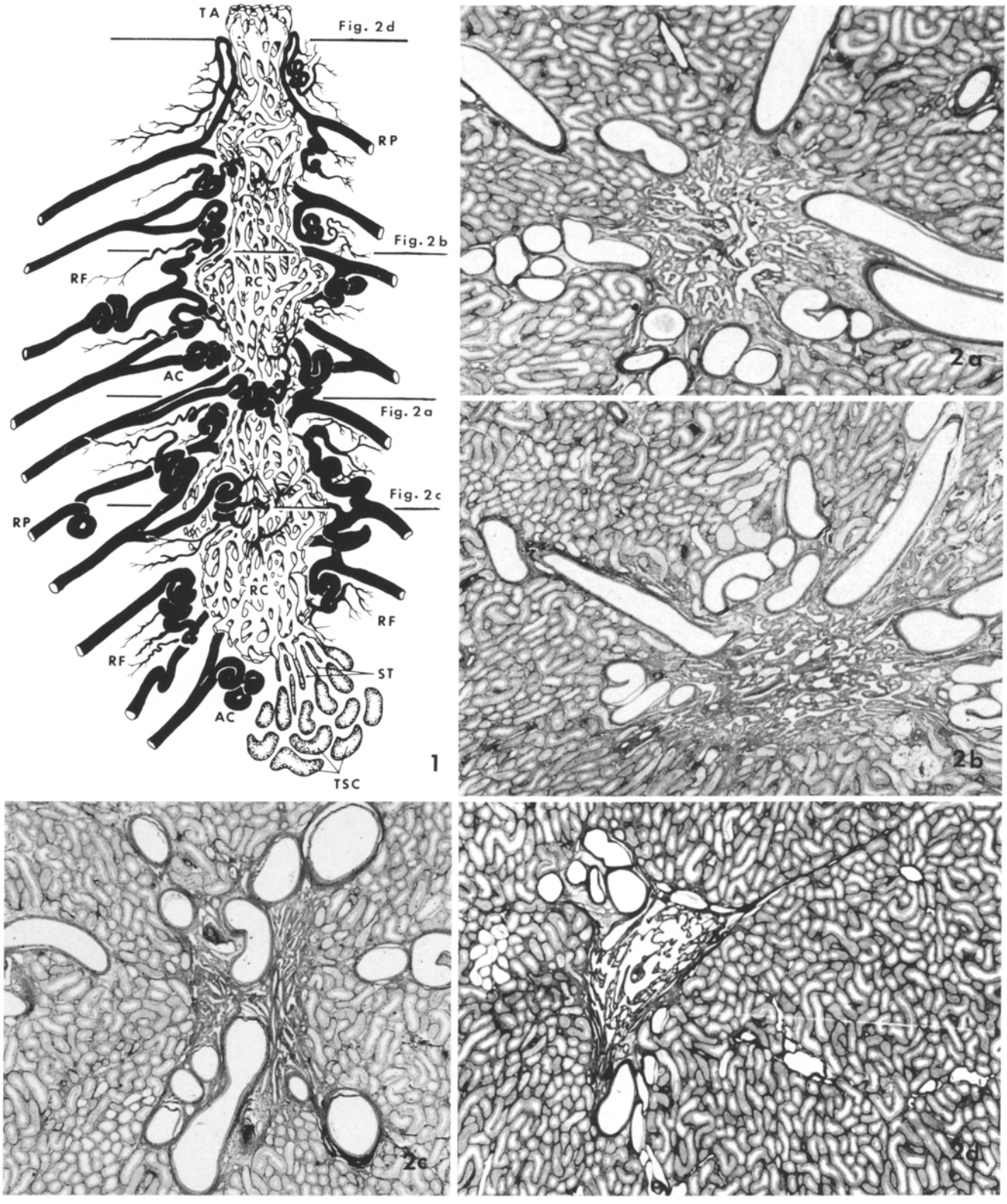


Fig. 1. Schematic drawing of spatial arrangement and topographic relationship of straight rami parenchymales centripetales (*RP*) and straight rami parenchymales centrifugales (*RF*) of the testicular artery, arterial convolutes (*AC*) and rete channels (*RC*) within the mediastinum testis. Tubuli seminiferi contorti (*TSC*) and the openings of straight tubules (*ST*) into the rete and the transitional area (*TA*) between rete and efferent ductules at the cranial testicular pole are also indicated. 1:1

Fig. 2a–d. Cross sections of the mediastinum testis at the levels indicated in Fig. 1, illustrating varying relationship between arterial convolutes and mediastinal dimensions. Masson-Goldner, ×10. **a** The mediastinum is completely surrounded by arterial convolutes. **b** Arterial convolutes are absent at the side pointing to the epididymis. **c** Hourglass-shaped narrowing of the mediastinum caused by centrally protruding arterial convolutes. **d** At the cranial pole of the testis (area confluens retis) convolutes decrease in number and size

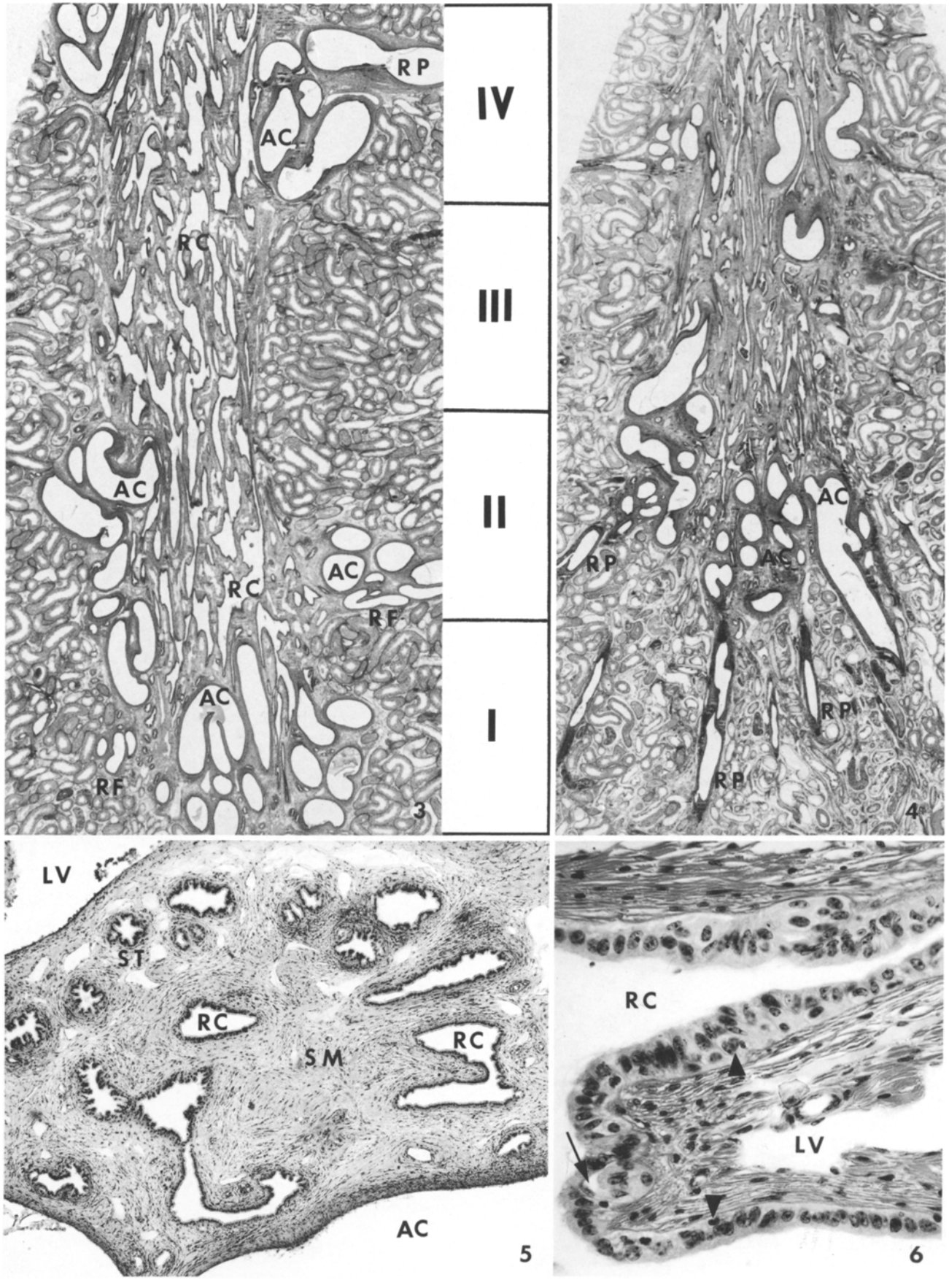
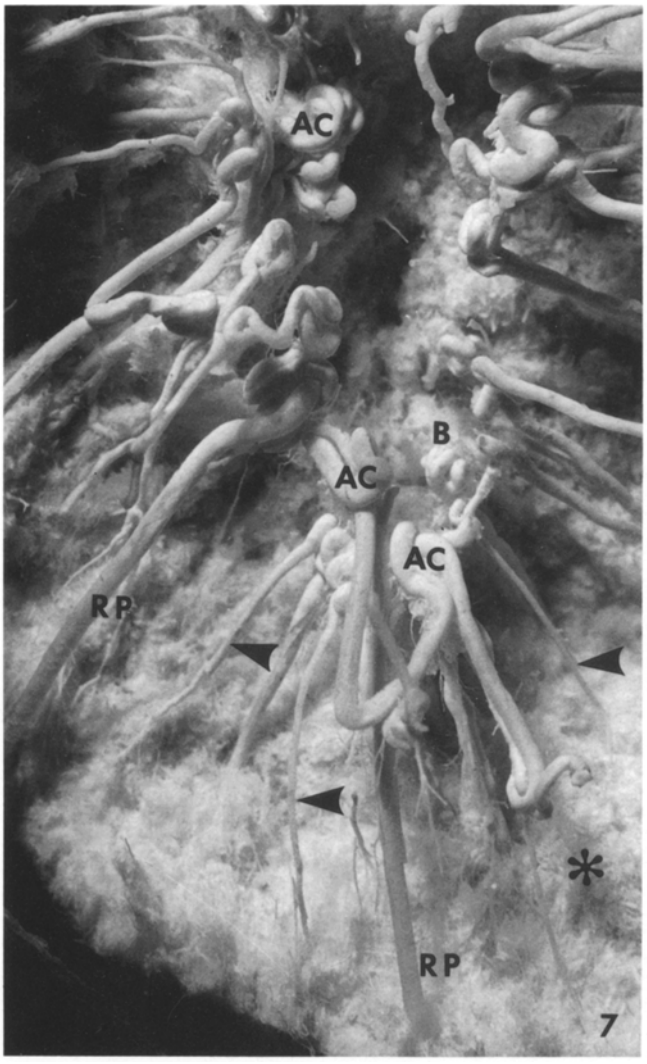


Fig. 3. Longitudinal section through part of the mediastinum testis, representing individual arterial convolutes (*AC*) at subsequent levels (*I–IV*). Masson-Goldner, × 10. Rete channels (*RC*), rami parenchymales centripetales (*RP*) and rami parenchymales centrifugales (*RF*) of the testicular artery. In I, RP run perpendicular to the plane of section, AC is cut tangentially. In II and IV, flanking arteries and convolutes are in the plane of section and cut longitudinally. In III, arteries are not visible because they are situated outside the plane of section



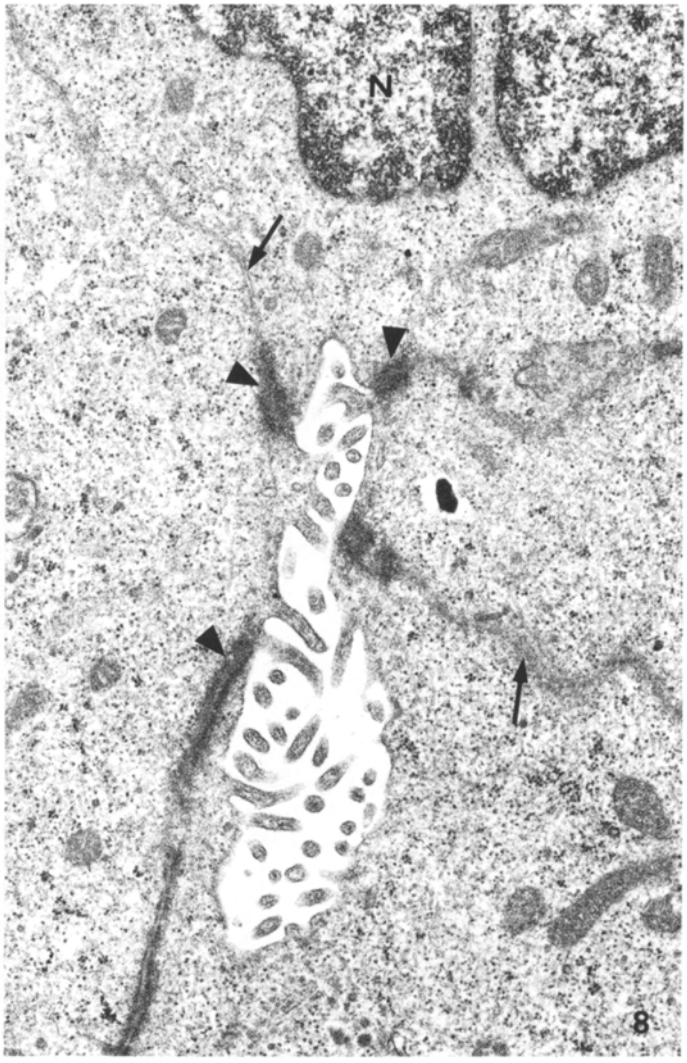


Fig. 7. Arterial corrosion cast from the caudal region of the testis (see description of this area in the text). Arterial convolutes (*AC*), rami parenchymales centripetales (*RP*) and rami parenchymales centrifugales (▶) of the testicular artery; beginning of rete testis (*B*), capillaries in the parenchyma of the testis (*). ×4

Fig. 8. Epithelial crypt with microvilli protruding into the lumen. Nucleus (*N*), junctional complexes (▶), intercellular spaces (→). ×20000

never oriented in a plane perpendicular to the longitudinal axis of the rete (Figs. 1, 4, 7).

At the cranial pole of the testis, mediastinum and rete reach the level of the tunica albuginea, where the rete ends with the area confluens (Fig. 2d). An extratesticular rete is absent in the correctly perfusion-fixed bovine testis (Hees et al. 1987). The cranial termination of the rete is flanked by a small number of arterial convolutes. The individual convolutes, however, are rather long, the coils having a narrow lumen. The uppermost straight centripetal arteries often form recurrent loops pointing to cranial.

The epithelium of the rete testis

The rete channels are lined by a simple cuboidal or columnar epithelium. At certain sites it appears stratified due to the existence of short intraepithelial crypts (Fig. 6). These crypts often form an acute angle with the basal lamina;

Fig. 4. Longitudinal section through the beginning of mediastinum testis and rete at a distance of ~3 cm from the caudal pole. Caudal straight rami parenchymales centripetales (*RP*) converge to this location and their narrow-spaced convolutes (*AC*) form an arterial cup encompassing the mediastinum from caudal. Masson-Goldner, ×10

Fig. 5. The beginning of mediastinum and rete at a distance of ~3 cm from the caudal pole. Rete channels (*RC*), straight tubules (*ST*), lymph vessels (*LV*), arterial convolute (*AC*), stroma of mediastinum testis (*SM*). Masson-Goldner, ×56

Fig. 6. Mediastinum and rete testis. Epithelial lining of the rete testis. Rete channels (*RC*), lymph vessel (*LV*), mononuclear free cells (▶), intraepithelial crypt (→). Masson-Goldner, ×140

their lumina are generally narrow and slit-like, so that they may escape light-microscopical detection. The lining cells of these oblique-running intraepithelial crypts possess a common basal lamina. The cells lining the basal aspect of the crypt are low, those flanking the lateral and apical aspects are columnar, bend around the lumen and establish contacts above the lumen of the crypt. The nuclei of these irregularly shaped columnar cells are situated basally or apically, creating a pseudostratified appearance. The columnar cells of the crypts face two surfaces: the common rete lumen as well as the lumen of the crypts.

Extensive junctional complexes connect adjacent epithelial cells at the luminal border and seal the intracellular spaces against the crypts (Fig. 8). The lateral plasmalemmata display interdigitations at the level of the nuclei or supranuclearly. The basal epithelial border is rather irregular with many projections into the folded basal lamina. Many hemidesmosomes are developed (Fig. 9).

The inventory of organelles is rather inconspicuous. Small mitochondria are distributed at random. The nuclei occupy the middle of the cell and are often indented. Short microvilli project from the apical surface and also into the crypts. The cells bear a single very long cilium, showing the usual 9+2 pattern. Endoplasmic reticulum is scarcely developed, free ribosomes and polyribosomes, however, are abundant. A small Golgi apparatus and some multivesicular bodies are present. A characteristic feature is the occurrence of a multitude of small and medium-sized osmiophilic dense bodies in the basal cytoplasm. Such dense bodies tend to accumulate within the basal processes, which invaginate the basal lamina (Fig. 9).

Many free mononuclear cells, mostly macrophages, to a lesser degree lymphocytes, are localized in the basal half of the bovine rete epithelium. Here they are observed between autochthonous epithelial cells. Contacts between free cells and the basal lamina are rare, because generally narrow plasma strands of the epithelium separate the two elements (Fig. 9). The amount of free cells is subject to local variations but may reach 30% in some areas of the rete.

Histochemical results

In some regions of the rete lipid material is situated in the basal portions of the epithelial cells as well as in macrophages. Mitochondrial enzymes and tetrazolium reductases react moderately and uniformly. A strong alkaline phosphatase reaction concentrates in a narrow apical epithelial zone (Fig. 10a). Adenosine triphosphatase is restricted to the free mononuclear cells (Fig. 10b). Acid phosphatase in low concentrations is observed in a supranuclear position corresponding to the localization of the Golgi apparatus. Furthermore, a rather strong, often granular reaction is obvious at the epithelial bases (Fig. 10c). Granular deposits of indoxylacetate esterase are also present in this basal area. Leucine aminopeptidase is the marker enzyme of the bovine excurrent duct system and displays a strong and diffuse reaction in the rete epithelium (Fig. 10d).

The mediastinal stroma

Rete channels, blood and lymph vessels are surrounded by several layers of elongated stroma cells with slender, extensive processes. Processes of adjoining cells make desmosomal contacts or gap junctions. The stroma cells of the mediastinum are myofibroblasts combining features of (i) fibroblasts (large Golgi apparatus, well-developed RER, many free ribosomes, all structures being located predominantly close to the poles of the elongated nuclei) and (ii) smooth muscle cells (filaments 5 and 10 nm in diameter, oriented in the long axis of the cell body and processes). In the course of the filament bundles, as well as at the inner side of the cell membrane, dense attachment plaques are visible. Many micropinocytotic vesicles are observed in a subplasmalemmal position. A discontinuous basal lamina covers each individual myofibroblast (Fig. 11c).

Those myofibroblasts that lie close to the rete epithelium show a strong reaction for alkaline phosphatase; stroma cells further distant, react weaker (Fig. 10a). The opposite is the case with adenosine triphosphatase: a strong reaction in the peripheral mediastinal stroma cells contrasts with a diminished reaction in subepithelial cells (Fig. 10b).

Individual stroma cells are separated by wide interstices, filled with bundles of collagen fibers, oriented in the long axis of the rete channels. Where two myofibroblasts come in close contact, their cell bodies or processes are connected by accumulations of elastin (Figs. 11a–c). Furthermore, elastin is found between rete epithelium and stroma cells. These elastic fibers play an important role in the coordination of epithelium and stroma, since they are attached to the basal lamina and to the myofibroblasts (Fig. 11b).

Narrow strands of mediastinal tissue covered by typical rete epithelium are found traversing the lumina of the channels (Fig. 12a). Such chordae retis in the definition of Roosen-Runge and Holstein (1978) are noticed as small round or oval islets in cross sections (Fig. 12b) but are best seen in corrosion casts (Fig. 12c). Sometimes two chordae retis are observed in parallel and in close proximity. Within the chordae the myofibroblasts form a loose network; occasionally a small blood or lymph vessel lies in the center.

Vessels of the mediastinum testis

The arteries supplying the mediastinal tissue come either from the convolutes of the straight centripetal rami, or they are recurrent branches of the centrifugal parenchymal rami. The supplying arteries lie mostly in close neighborhood to the many large lymphatics of the mediastinum. The arteries and arterioles often invaginate the wall of the lymph vessel and are situated in the lumen of the latter, drawing out a short fold of a "meso". ~65 blood vessels (arterioles and capillaries) were counted in one cross section of the mediastinum.

Numerous valveless lymph vessels were located within the mediastinum testis, often in close contact to the rete channels. The vessels collect the content of the many intertubular testicular lymphatics. In the mediastinum ~115 lymph vessels were counted in individual cross sections; their lumina represent 25% of the cross-sectional area. The wall of the mediastinal lymph vessels comprises a flattened endothelium and a discontinuous basal lamina, no musculature being present.

Discussion

The bovine mediastinal stroma contains an interconnected network of myofibroblasts surrounding the rete channels, lymph and blood vessels in widely spaced layers. Between the cell layers thick bundles of collagen fibers and connect-

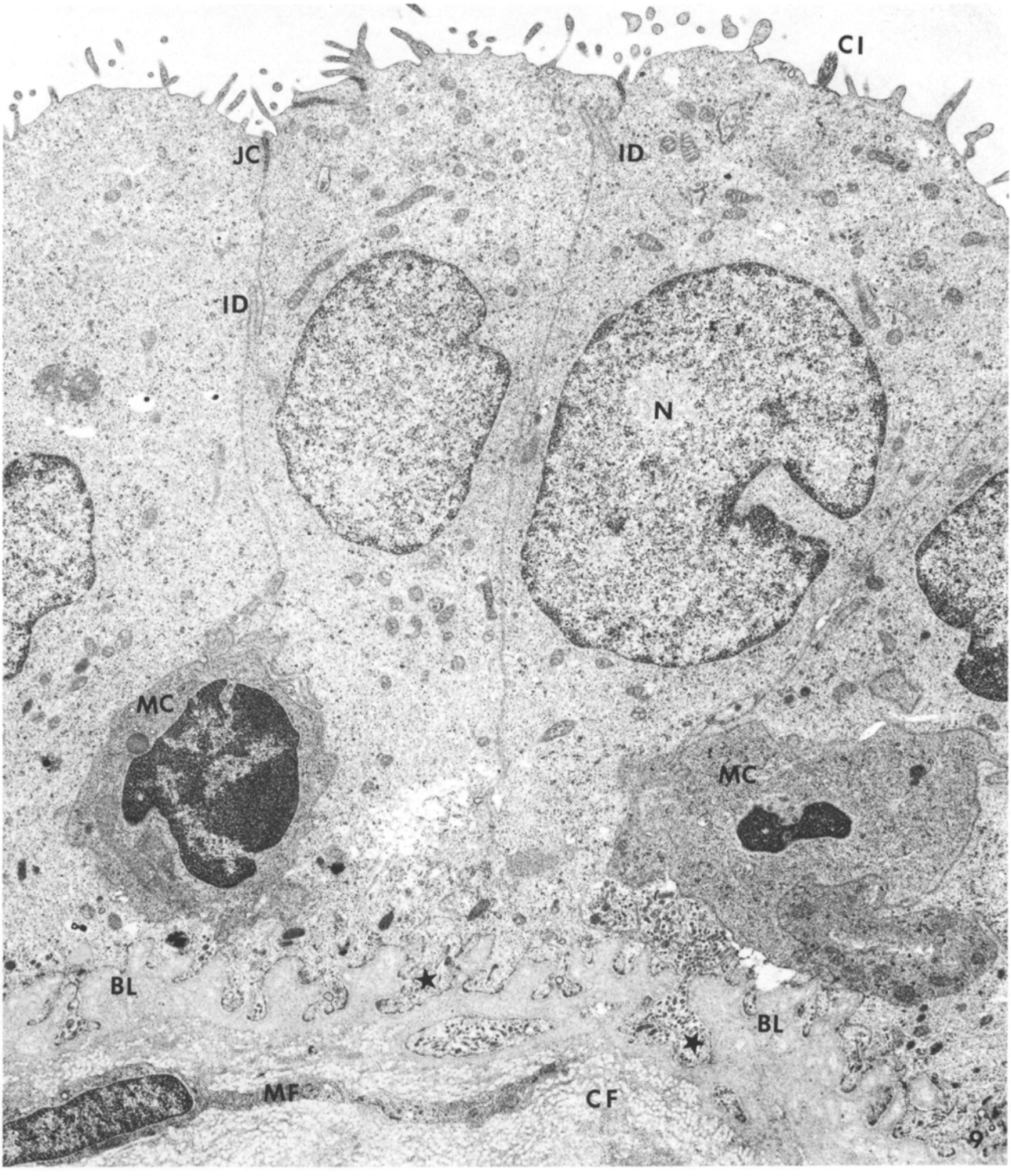


Fig. 9. Epithelium of rete testis. Junctional complexes (*JC*), nucleus (*N*), interdigitations of the lateral plasmalemmata (*ID*), basal lamina (*BL*), collagen fibers (*CF*), myofibroblast (*MF*), mononuclear cells (*MC*), cilium (*CI*), basal processes invaginating the basal lamina (*). ×9900

ing deposits of elastin are observed. The mediastinal stroma as a whole can be regarded as a contractile and elastic unit, acting upon lumen and content of rete channels and lymph vessels.

Cross sections through the mediastinum reveal compressions of the latter at the level of the arterial convolutes. It is well known that in convoluted arteries the arterial pulse causes signficant changes in intravasal volume, depending on the length of the convoluted portion and on number and bending of coils. Thus, it seems very likely, that a lateral compression of the mediastinum by the caudocranially arranged series of arterial convolutes exerts a peristaltic wave on the mediastinal lymph vessels and the interconnected rete channels and thus is a means to promote

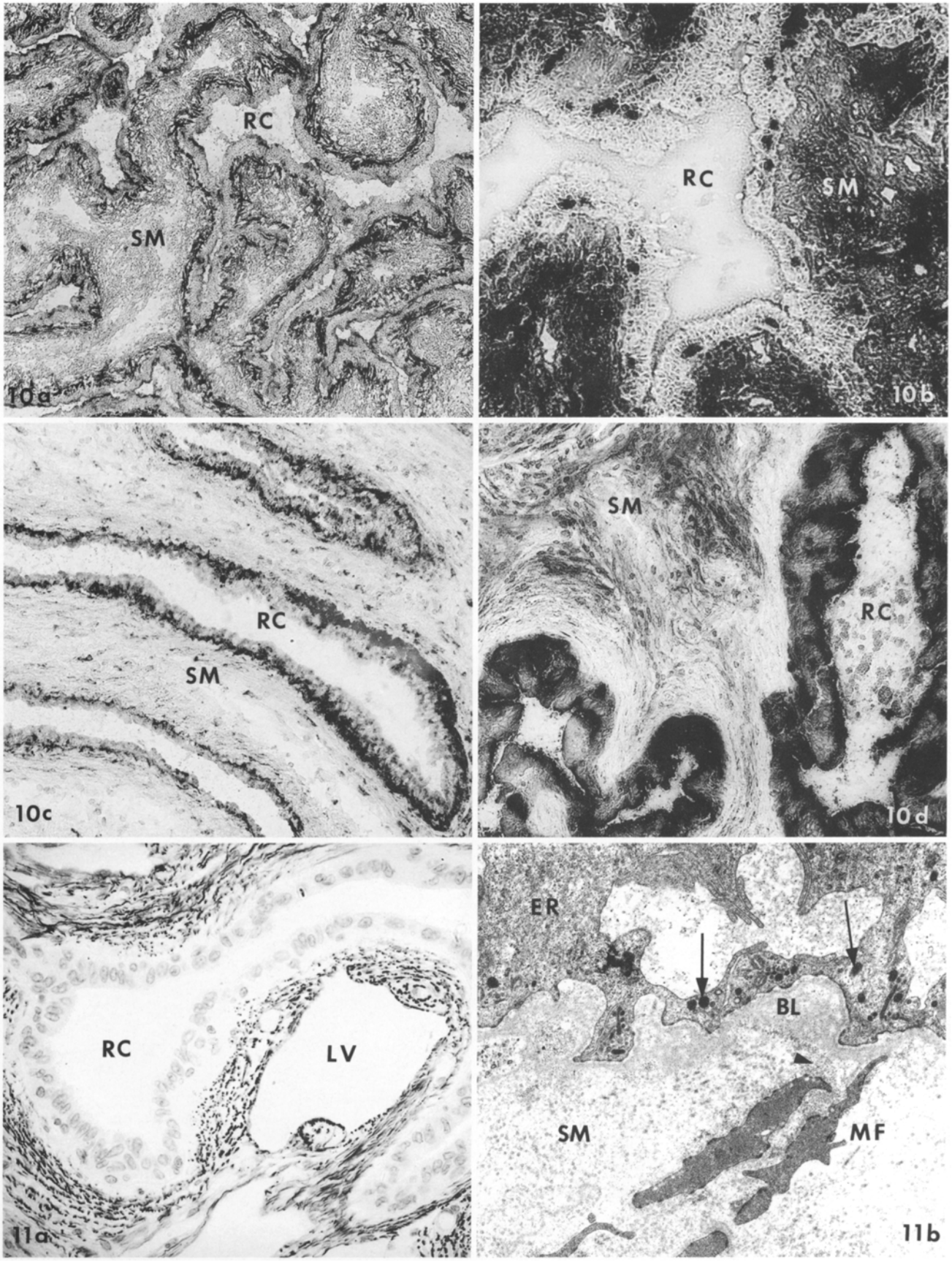


Fig. 10a–d. Histochemical reactions. **a** Alkaline phosphatase. × 56. **b** Adenosine triphosphatase. × 140. **c** Acid phosphatase. × 140. **d** Leucine aminopeptidase. Rete channels (*RC*), stroma of mediastinum testis (*SM*). × 140

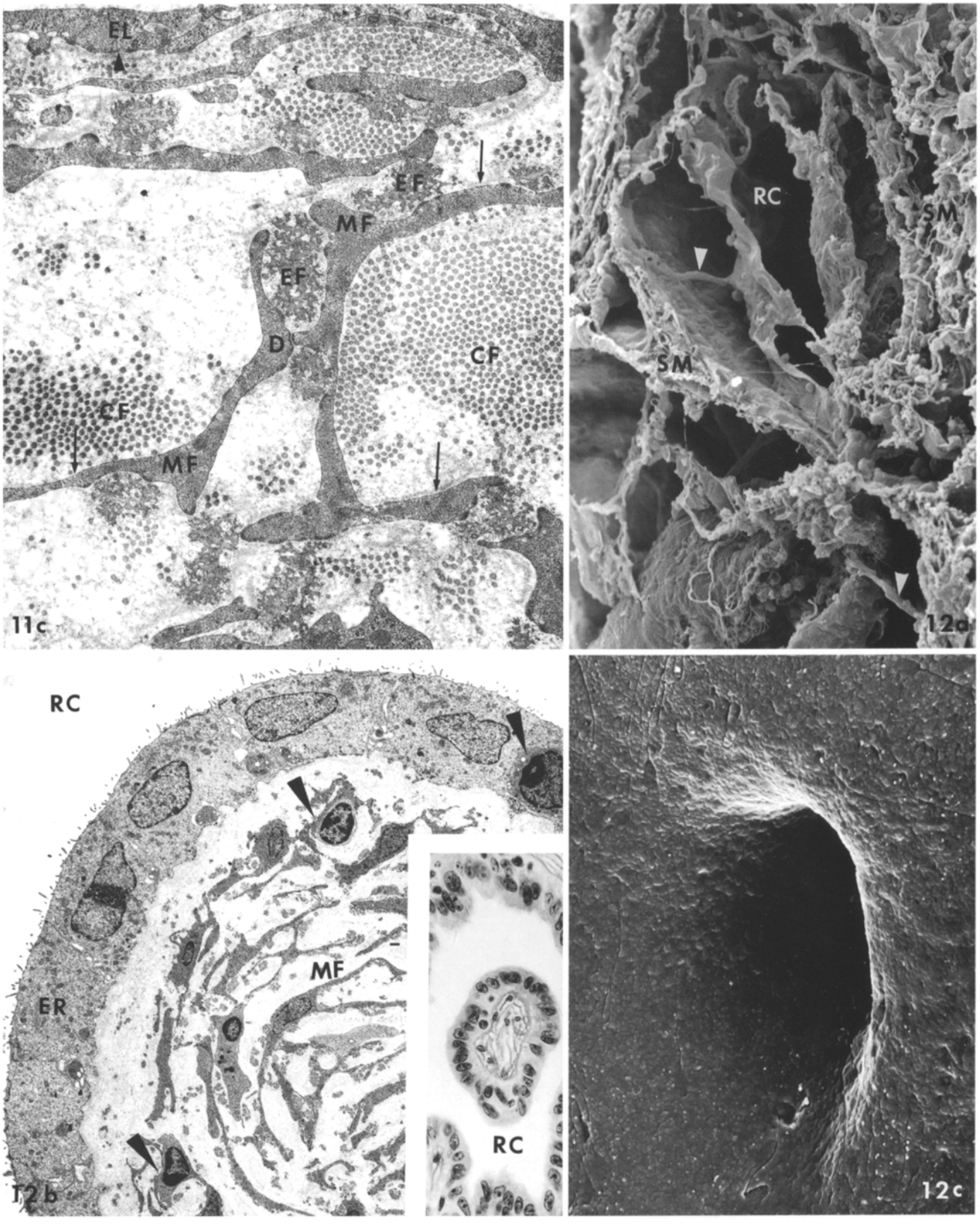


Fig. 11. Stroma of the mediastinum testis. **a** Representation of elastic material by resorcin fuchsin. Rete channels (*RC*), lymph vessel (*LV*). × 350. **b** Myofibroblast (*MF*) in contact with the basal lamina (*BL*) of rete epithelium (*ER*), osmiophilic dense bodies (➙) in the basal cytoplasm, stroma of mediastinum testis (*SM*), elastic fibers (◗). × 9900. **c** Ultrastructure of the mediastinal stroma. Endothelium of a lymph vessel (*EL*), myofibroblasts (*MF*), desmosome (*D*), collagen fibers (*CF*), elastic fibers (*EF*), basal lamina of myofibroblasts (➙), basal lamina of the lymph vessel. (◗). × 14600

Fig. 12. Chordae retis. **a** TEM micrograph showing chordae retis (◗) traversing the lumina of rete channels (*RC*), stroma of mediastinum testis (*SM*). × 40. **b** Cross section of a chorda retis with core of mediastinal stroma. Myofibroblasts (*MF*), mononuclear free cells (◗), epithelium of rete testis (*ER*), rete channels (*RC*), × 2600. *Inset:* Cross section of a chorda within a rete channel (*RC*). Masson-Goldner, × 140. **c** In the injection-compound filling rete channels a chorda retis appears as a smooth-walled empty recess. × 300

lymph flow in the spermatic cord and thereby to enhance transport of rete content to the efferent ductules. Such a combined conveying system consisting of arterial pulsation and contractile-elastic mediastinal stroma elements may have a similar importance for the long central rete of the bull as the smooth muscle cells in the tunica albuginea for the evacuation of the marginal rete in the human testis (Holstein 1968). The well-known high pressure in the bovine seminiferous parenchyma causing tissue protrusion following incision of the tunica albuginea, prevents displacement of the compressed rete channels in areas where no flanking arterial convolutes are present.

Adenosine triphosphatase activity in the mediastinal stroma reflects the contractile potential of the myofibroblasts; alkaline phosphatase is involved in carbohydrate metabolism. The observed differences in the activity of these two enzymes between stroma cells immediately adjacent to the rete epithelium and those further distant, point to functional differences within the morphologically uniform population of stroma cells and underline the mutual relationships between epithelium, basal lamina and subepithelial stromal elements.

In the center of the mediastinum the stroma between rete channels and lymph vessels is scarcely developed. The strand-like chordae retis may here have a special importance as transluminal coupling devices for the contractile system of myofibroblasts. Chordae retis have been described so far for the human (Roosen-Runge and Holstein 1978) and for the monkey (Burgos et al. 1979), but probably represent common and characteristic features of the rete testis in many species.

In all species studied, the epithelium of the rete testis is simple squamous to low columnar (Dym 1976; Bustos-Obregón and Holstein 1976; Osman 1978; Roosen-Runge and Holstein 1978; Aire 1979; Rodger 1982; Barker and Kendall 1984; Suzuki and Racey 1984; Orsi et al. 1984). For human rete Bustos-Obregón and Holstein (1976) report two, Goto (1981) by means of SEM even four different cell types. In the bovine, cuboidal and elongated lighter and darker cells are encountered; histochemistry and ultrastructure, however, reveal that they all belong to the same type. Furthermore, a number of common features has been observed in the rete epithelium of different mammalian species (man, marsupials and birds): extensive tight junctions are situated near the adluminal border (Dym 1976; Bustos-Obregón and Holstein 1976). Tracer studies of Dym (1976) and Osman (1978) have revealed that these junctions represent a potent diffusion barrier between mediastinal stroma and rete lumen. Finger-like interdigitations of the lateral cell border (Dym 1976; Aire and Malmquist 1979; Barker and Kendall 1984) and a highly clefted basal surface (Dym 1976) are generally developed. The apical cell border displays short microvilli and bears one typical cilium per cell (Dym 1976; Goto 1981; Barker and Kendall 1984). The nuclei exhibit irregular contours, indentations and invaginations occur (Dym 1976). Heterochromatin is preferentially located marginally, 1–3 characteristic nucleoli are present. Depending on cell size some species (cat, ram, monkey) have extraordinarily large nuclei. In the bovine, nuclei are regular in size and may be found at all levels inside the low to high columnar cells, even just below the lumen. The existence of narrow intraepithelial crypts is a bovine-specific feature. Similar structures have been reported by Rodger (1982) only for the marsupial rete. The crypts resemble intercellular secretory capillaries of glandular epithelium, and the lining surface possesses microvilli, but secretory activity of the bovine rete epithelium is morphologically not obvious. Another interpretation for the epithelial crypts is, that they represent epithelial reserve, necessary for dilatation and expansion of the rete channels.

Mononuclear free cells (macrophages, lymphocytes) are regular constituents of the rete epithelium (Dym 1976; Osman and Plöen 1978; Aire and Malmquist 1979), but their number may vary among the different species. The mononuclear cells of the bovine rete epithelium are predominantly macrophages (Wrobel et al. 1978), which support the autochthonous epithelial cells in phagocytosis and decomposition of spermatozoa. Macrophages send their filopodia and processes into the enlarged intercellular clefts of the basal half of the epithelium. Contrary to Osman and Plöen (1978), who studied the rete of bulls, boars, rams, goats, and rats, we observed macrophages also in contact with the basal lamina. In the shrew (Suzuki and Racey 1984) a number of rete epithelial cells contains glycogen. In the guinea pig also, Fawcett and Dym (1974) described glycogen in this portion of the rete, which is adjacent to the openings of straight testicular tubules. In the bull, epithelia in the rete as well as in tubuli recti are completely devoid of glycogen.

References

Aire TA (1979) The epididymal region of the Japanese quail (*Coturnix coturnix japonica*). Acta Anat 103:305–312

Aire TA, Malmquist M (1979) Intraepithelial lymphocytes in the excurrent ducts of the testis of the domestic fowl (*Gallus domesticus*). Acta Anat 103:142–149

Barka T, Anderson PJ (1962) Histochemical methods for acid phosphatase using hexazonium parasaniline as coupler. J Histochem Cytochem 10:741–753

Barker SGE, Kendall MD (1984) A study of the rete testis epithelium in several wild birds. J Anat 138:139–152

Benoit M (1926) Recherches anatomiques, cytologiques et histologiques sur les voies excretrices du testicule chez les Mammifères. Arch Anat Microsc Embryol 5:175–412

Burgos MH, Cavicchia JC, Einer-Jensen N (1979) Electron microscopy (SEM and TEM) of the rete testis in the monkey. Int J Androl 2:559–571

Bustos-Obregón E, Holstein AF (1976) The rete testis in man: Ultrastructural aspects. Cell Tissue Res 175:1–15

Deimling OH von (1964) Die Darstellung phosphatfreisetzender Enzyme mittels Schwermetall-Methode. Histochemie 4:48–55

Dym M (1976) The mammalian rete testis – A morphological examination. Anat Rec 186:493–524

Fawcett DW, Dym M (1974) A glycogen-rich segment of the tubuli recti and proximal portion of the rete testis in the guinea pig. J Reprod Fertil 38:401–409

Gomori G (1952) Microscopic histochemistry, principles and practice. University of Chicago Press, Chicago

Goto K (1981) Surface morphology of the epithelium of human seminiferous tubules, rete testis, ductuli efferentes and ductus epididymidis. Biomed Res 2 (Suppl):361–374

Goyal HO, Dhingra LD (1973) Postnatal study on the histology and histochemistry of mediastinum testis in buffalo (*Bubalus bubalis*) from birth to one year. Acta Anat 86:65–70

Hees H, Leiser R, Kohler T, Wrobel KH (1984) Vascular morphology of the bovine spermatic cord and testis. I: Light- and scanning electron-microscopic studies on the testicular artery and pampiniform plexus. Cell Tissue Res 237:31–38

Hees H, Wrobel KH, Kohler T, Leiser R, Rothbächer I (1987) Spatial topography of the excurrent duct system in the bovine testis. Cell Tissue Res 248:143–151

Hess R, Scarpelli DG, Pearse AG (1958) The cytologic demonstration of oxydative enzymes. II: Pyridine nucleotide-linked dehydrogenases. J Biophys Biochem Cytol 4:753–760

Holstein AF (1968) Die glatte Muskulatur in der Tunica albuginea des Hodens und ihr Einfluß auf den Spermientransport in den Nebenhoden. Anat Anz 121 (Erg.-Heft):103–108

Holt SJ, Withers RFJ (1958) Studies in enzyme cytochemistry V: An appraisal of indigogenic reactions for esterase localization. Proc R Soc Lond [Biol] 148:520–532

Karnovsky MJ (1965) A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. J Cell Biol 27:137A–138A

Kohler T, Leiser R (1983) Blood vessels of the bovine chorioidea. Acta Anat 116:55–61

Lojda Z, Gossrau R, Schiebler T (1976) Enzymhistochemische Methoden. Springer, Berlin Heidelberg New York

Nachlas MM, Monis B, Rosenblatt D, Seligman AM (1968) Improvements in the histochemical localization of leucine aminopeptidase with a new substrate, L-leucyl-4-methoxy-2-naphthylamide. J Biophys Biochem Cytol 7:261–264

Nachlas MM, Walker DG, Seligman AM (1958) A histochemical method for the demonstration of diphosphoridine nucleotide diaphorase. J Biophys Biochem Cytol 4:29–38

Orsi AM, Mello Dias S, De Melo VR, Dall Pai V, Vicentini CA (1983) Estrutura da rede testicular no bovino zebu (*Bos indicus*). Ciência e Cultura 36:238–242

Orsi AM, Mombrum de Carvalho I, Moreira JE, Valente MM, Guazzelli Filho J (1984) Morfologia de la red testicular en el caprino doméstico (*Capra hircus*, L). Zbl Vet Med C 13:42–49

Osman DI (1978) The ultrastructure of the rete testis and its permeability barrier before and after efferent ductule ligation. Int J Androl 1:357–370

Osman I, Plöen L (1978) The mammalian tubuli recti: Ultrastructural study. Anat Rec 192:1–18

Reynolds EG (1963) The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J Cell Biol 17:208–212

Rodger JC (1982) The testis and its excurrent ducts in American caenolestid and didelphid marsupials. Am J Anat 163:269–282

Roosen-Runge EC, Holstein AF (1978) The human rete testis. Cell Tissue Res 189:409–433

Scheubeck M, Wrobel KH (1984) Eine einfache, transportable Apparatur zur Durchführung von Perfusionsfixierungen. Mikroskopie 41:108–111

Spurr AR (1969) A low viscosity epoxy resin embedding medium for electron microscopy. J Ultrastruct Res 26:31–43

Suzuki F, Racey PA (1984) Light and electron microscopical observations on the male excurrent duct system of the common shrew (*Sorex araneus*). J Reprod Fertil 70:419–428

Wrobel KH, Sinowatz F, Kugler P (1978) Zur funktionellen Morphologie des Rete testis, der Tubuli recti und der Terminalsegmente der Tubuli seminiferi des geschlechtsreifen Rindes. Zbl Vet Med C: Anat Histol Embryol 7:320–335

Wrobel KH, Sinowatz F, Mademann R (1982) The fine structure of the terminal segment of the bovine seminiferous tubule. Cell Tissue Res 225:29–44

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