EXPERIMENTAL ACTINOBACILLUS SEMINIS INFECTION OF RAMS: A SCANNING ELECTRON MICROSCOPY STUDY OF SEMINAL VESICLES

by

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Major Professor

DEDICATION

Dedicated to my mother,
Elisabeth Stockinger, for
her continual encouragement
and understanding.

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INTRODUCTION

The significance of Actinobacillus seminis infection in sheep, apart from being a diagnostic and control problem for ovine brucellosis, and its various manifestations are undetermined. It is reported to be primarily a genital disease of rams with lesions varying from acute to chronic uni- or bilateral epididymitis or epididymoorchitis. Scattered reports from sheep raising countries indicate that A. seminis infection has broader pathological manifestations: purulent polyarthritis, posthitis, placentitis and abortion. The pathology of genital actinobacillosis in sheep has had little study in contrast to that of brucellosis. In spite of the incidence of ovine infertility and positive serological titers or presence of pathogens in semen in absence of palpable lesions in the scrotal contents, the pathology of the accessory sex glands in rams has received little attention. The accessory sex structures consist of the vas deferens, ampullae, seminal vesicles, prostate, bulbourethral glands, urethra, penis and prepuce. The main function of the accessory sex glands is secretion of seminal plasma that is added to spermatozoa during emission. The most frequently affected and studied accessory sex gland in bulls is the seminal vesicles and the bulbourethral gland the least.

The objective of this study was to examine the ultrastructure of seminal vesicles following intraepididymal injection of A. seminis.

REVIEW OF LITERATURE

1. Ovine Actinobacillosis

Ovine epididymitis due to Actinobacillus seminis was first described in Australia (Baynes and Simmons 1960). Since then it has been reported from the USA (Livingston and Hardy 1964), South Africa (Worthington and Bosman 1968; Van Tonder and Bolton 1968), and New Zealand (Bruere et al. 1977). A. seminis epididymitis was reported to be clinically and pathologically indistinguishable from that due to Brucella ovis, the major cause of ovine epididymitis (Baynes and Simmons 1960, 1968; Worthington and Bosman 1968; Van Tonder 1973).

Genital actinobacillosis is a disease of rams primarily manifested by acute to chronic epididymitis or epididymo-orchitis (Baynes and Simmons 1960, 1968; Van Tonder 1973). A. seminis was isolated from semen of clinical cases for long periods (Simmons et al. 1966; Worthington and Bosman 1968). Subclinical cases were reported (Simmons et al. 1966; Van Tonder 1973) and a high incidence of clinical and subclinical A. seminis infection in South African rams was recorded by Van Tonder (1973). Reports of involvement of the accessory sex organs are virtually nonexistent.

Baynes and Simmons (1960) reported isolating A. seminis from the right seminal vesicle and left bulbourethral gland of the right seminal vesicle and left bulbourethral gland of

a ram with epididymitis. A. seminis was isolated from the ampullae, seminal vesicles and bulbourethral gland of a ram slaughtered nine months after epididymitis was observed (Worthington and Bosman 1968). Baynes and Simmons (1960) reported isolating A. seminis from the vas deferens and the epididymis, 59 days after intraepididymal inoculation. No description of any lesions were given apart from the seminal vesicles being enlarged.

Al-Katib (1980) concluded from his studies that A. seminis may infect part or all of the reproductive tract of rams and that infection may result from extragenital routes of inoculation such as intravenous, intramuscular and oral.

2. Seminal vesiculitis due to A. seminis

Al-Katib (1980) described histopathologic findings in seminal vesicles following intraepididymal inoculation of A. seminis. Within 24 hours postinoculation (PI) the epithelium had hydropic degeneration and was infiltrated by neutrophils. Serous exudation with neutrophilic and eosinophilic infiltration was present in the interstitium. By 48 hours PI the lumen of alveoli contained neutrophils, proteinaceous material, degenerated sperms and the interstitium was infiltrated by lymphocytes and plasma cells.

Epithelial changes at 72 hours PI included desquamation, swelling, hyperplasia and neutrophils.

The lumen contained cellular debris, proteinaceous material and degenerated sperms. At 96 hours PI, hydropic degeneration of the epithelium, cellular debris within the lumen, and serous exudate in the interstitium were observed.

Day 5 PI revealed desquamation and swelling of the epithelium with desquamated epithelium within the lumen. At day 6 PI, the epithelial changes included swelling, hyperplasia, infiltration by neutrophils, and polypoid formations, and the alveoli contained cellular debris and neutrophils together with proteinaceous material. Serofibrinous exudate, lymphocytes, plasma cells and macrophages were present in the interstitium. At day 7 PI, no changes were observed. By day 8 PI only hydropic degeneration of the epithelium was observed.

3. Gross comparative anatomy of seminal vesices

Seminal vesicles, a paired vesicular gland, are the largest of the accessory genital glands in ruminants. Grossly it is distinctly lobulated, firm and compact with an uneven surface. In the bull, an irregular, elongated form, often bent on itself or S-shaped is not uncommon, and in small ruminants, the gland is shorter and more rounded.

In the bull, the gland is 10-12 cm long and 1.5-3.5 cm thick, and in small ruminants, 2.5-4 cm long, 2-2,5 cm wide, and 1-1.5 cm thick (Nickel et al 1973).

Each gland may be regarded as consisting of a very thick-walled, sacculated tube, bent on itself in a tortuous manner. They are commonly asymmetrical in size and shape (Sisson and Grossman 1975). The vesicular gland lies dorso-lateral to the neck of the bladder, and between the right and the left sides the terminal segments of the ureters and deferent ducts are located. The excretory ducts, one for each gland, joins the terminal part of the ductus deferens to form the short ejaculatory duct that opens on the colliculus seminalis in the dorsal wall of the pelvic urethra (Nickel et al 1973). The structure of the vesicular gland is masked by a thick capsule of fibrous tissue and smooth muscle that maintains the bent shape and sends trabeculae between the alveoli (Sisson and Grossman 1975).

4. Histology

According to Dodd (1979), the seminal vesicles in man have a nodular surface because the walls form bulging, irregular pouches, recesses, and chambers, all of which open onto a common lumen.

In ruminants there is a central canal into which the secretion from alveoli passes (Sisson and Grossman 1975). As an extraepithelial gland (subepithelial) it develops from the lining epithelium by invagination into the underlying connective tissue (Dellmann 1971).

None of the above authors described the form of secretion. Nickel et al (1973) described the vesicular gland of the ruminants as a branched, tubulo-alveolar gland, the secretion of which is stored in large intralobular collecting spaces. However, Kroelling and Grau (1960) and Dellmann and Wrobel (1976) classified it as a compound tubular or tubuloalveolar gland with intralobular secretory ducts that drain the slightly coiled tubular portions and in turn being drained by the main secretory duct.

Under influence of testosterone, seminal vesicles produce a large amount of gelatinous, white or yellowish-white secretion, rich in fructose that serves as an energy source for ejaculated spermatozoa and amounts to 8% of the total ejaculate of the ram (Dodd 1979; Dellmann and Wrobel 1976).

The tunica adventitia is a thin, fibrous layer of loose connective tissue. The tunica muscularis consists of an inner circular and an outer longitudinal layer that surrounds the entire gland (Dellmann 1971). The interlobular septa are predominantly muscular and are derived from the thick tunica muscularis (Dellmann and Wrobel 1976). The tunica

submucosa is continuous with the lamina propria (Dellmann 1971). The highly vascularized loose connective tissue of the propria-submucosa is continuous with the dense connective tissue trabeculae that may subdivide the organ into lobes and lobules (Dellmann and Wrobel 1976).

Dodd (1979) described the mucosa of human seminal vesicles as being formed into many tall, thin primary folds that have secondary and tertiary anastomotic folds that extend well into the lumen of the gland, giving it the appearance of a network of irregular spaces and compartments.

A distinct lamina muscularis mucosae is absent in ruminants, but a few single smooth muscle fibers are present around the end pieces (Dellmann 1971). The glandular epithelium is stratified columnar or simple columnar (Dellmann 1971). Dellmann and Wrobel (1976) described the glandular epithelium as pseudostratified columnar with tall columnar cells and small, spherical, often sparse basal cells. The intralobular and main secretory ducts are lined by a similar cuboidal epithelium. Some secretory columnar cells have light, bleb-like apical projections. In man, the epithelium may be simple columnar or pseudostratified columnar, but the latter is common in active, healthy seminal vesicles (Dodd 1979). In man the epithelial lining consists essentially of a layer of tall columnar cells, but small cells may be irregularly distributed between these and the lamina propria (Ham and Cormack 1979).

5. Ultrastructure

Scanning electron microscopy of the human seminal vesicle was described by Kessel and Kardon (1979). The interior of the distal end of the seminal vesicle revealed large outpocketings formed by invaginations of the mucous membrane. The topography of the interior surface in this region appeared very folded. The folding produced long, tube-like structures that often branched and projected into the lumen. These tube-like structures contained secretory material identical to that found in the lumen proper. Much of the extensive folding of the mucosa appeared to originate as epithelial invaginations such that the lumen of the tube-like structure is continuous with the lumen of the seminal vesicle. The tube-like structures are made up of extensively folded epithelium that forms two layers of cells, one facing the lumen of the seminal vesicle, and the other facing the interior of the tube. The latter produces a secretory product that fills the interior of the tube.

The cells of the epithelial surface appeared to become rounded at their apical border as secretory products accumulates within them, and there is a decrease in the number of surface microvilli. The cells that have released their secretory product appear collapsed. Some of the secretory product appears in the form of filamentous material, whereas

the majority of the secretion has been removed by washing. The secretory material is released by exocytosis.

a. Scanning electron microscopy of human and canine prostate

Because of lack of reports concerning scanning electron microscopy of the male ruminant reproductive system and especially its epithelium, the detailed description of the SEM appearance of normal human prostatic epithelium by Yates and Gordon (1977), and the SEM appearance of normal prostatic epithelium of the dog by Nowell and Faulkin (1974) were used for comparison.

Depending on the functional state of the human prostate, the epithelium may be simple squamous, cuboidal, columnar, or pseudostratified columnar (Dodd 1979). The epithelium of the prostate gland in the dog is simple cuboidal or columnar with occasional basal cells. The simple epithelium changes from stratified columnar to transitional toward the terminal portions of the ducts (Dellman and Wrobel 1976).

The hemispherical apices of the columnar luminal cells exhibited consistent size and hexagonal shape in cross section, characteristic of epithelial secretory cells. Microvilli of constant length and diameter, 0.5 μ X 0.1 μ, are distributed uniformly over the cell surfaces. Prominent secretory blebs, 0.8-4.5 μ diameter, extend from the surfaces of many cells and distribution of these structures is

apparently randomized within an acinus. The blebs are generally smooth-surfaced, but some possess microvilli and small pits.

Also present in the apex of some cells are large circular craters (0.5-4.0 μ diameter), presumably related to secretory processes. The floor of the craters are granular and pitted, and do not give the appearance of being covered with plasma membrane; rather, the granular material may represent prostatic secretion or exposed cell cytoplasm. Small caveolae dot the cell surfaces, and represent exocytotic pits produced by merocrine secretion. Merocrine secretion is the dominant form and was found in all prostatic cells examined.

Apocrine secretion in the prostate, in which part of the cell apex becomes distended in the form of a large bleb or saccule, is less common, but nevertheless, is demonstrated in normal as well as malignant tissue. The blebs release their secretory product by dissolution or rupture, and there is evidence from serial studies that blebs detach from the apical surface in true apocrine fashion. The craters in the cell apices may represent areas from which blebs have detached or ruptured. The columnar epithelium rests partially or completely upon a polygonal layer of basal (reserve) cells of unknown function, that in turn, rest upon a delicate basement membrane that completely inverts the acinus and separates it from the underlying fibromuscular stroma (Yates and Gordon 1977).

In the dog, the alveoli are lined with cuboidal secretory cells, the majority of which are covered with short microvilli (Nowell and Faulkin 1974).

b. Specimen Preparatory Techniques

Humphrey et al (1973) dehydrated 4 x 1 x 1 mm sections of fixed tissue through graded ethanol to absolute. The tissue was sealed in parafilm cylinders, frozen in liquid nitrogen and fractured, warmed to room temperature in fresh absolute ethanol and critical point dried with carbon dioxide. Nowell and Faulkin (1974) used 10 x 1 x 1 mm sections of tissue with wedge-shaped ends to aid in identifying the fractured surface, and replaced the absolute ethanol with amyl acetate prior to critical point drying. Since water in the tissue was replaced with absolute ethanol, there was no ice crystal formation during freezing and no discernible freeze-fracture artifacts were revealed on the surface. No separation of epithelium from the lamina propria was observed by SEM of critical point dried samples, either freeze-fractured or not (Nowell and Faulkin 1974).

The extracellular product is removed by sectioning and subsequent washing of the tissue in 0.9% sodium chloride before fixation. Specimens are then preserved by immersion fixation (Kessel and Kandon 1979).

MATERIALS AND METHODS

Experimental Animals

Twenty yearling Suffolk and Suffolk cross rams were randomly divided in 16 test and 4 controls. The sixteen test rams were housed in two isolation rooms and the control rams in a third room. The tail of the left epididymis of each test ram was injected with 1 ml of a 24 hour brain hear infusion broth (BHI)[a] culture of A. seminis via a 26 gauge 1/2" needle. The concentration was 2.3×10^9 CFU/ml. The control rams were injected similarly with 1 ml of sterile BHI broth with 10% inactivated calf serum. The uninoculated right side of both test and control rams served as additional controls (Al-Katib 1980).

Two test rams were randomly selected every 24 hours post inoculation (PI) and euthanatized by intravenously administrated T61.[b] One control ram was euthanatized at 24, 48, 96 and 144 hours. Each ram was subjected to a standardized necropsy as described in Part II. The reproductive tract was carefully examined, cultured, lesions photographed, and duplicate thin sections fixed in both Bouin's solution and in buffered formaldehyde-glutaraldehyde solution (McDowell and Trump 1976).

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[a]Difco Laboratories, Detroit, Michigan.

[b]National Laboratories Corporation, Somerville, New Jersey.

Preparation of Tissues

The whole seminal vesicles remained unprepared in buffered formaldehyde-glutaraldehyde solution (BFG). They were stored at 72 F (22 C). Freeze-fractured specimens were prepared using the procedures described below. Tissue 79-2294 was freeze-fractured according to the previously described technique of Humphrey et al (1973). 6 x 4 x 6 mm pieces of fixed tissue were washed with phosphate buffered saline in a test tube to flush out the lumens. The test tube was placed on a Vortex mixer,[c] a setting of 7 for 2 minutes. The tissues were then dehydrated through graded ethanol, 40% to absolute. After changing the absolute ethanol twice, the specimens were sealed in parafilm cylinders[d] and placed into a container with methylbutane which was cooled with liquid nitrogen. After being fractured with a cooled razor blade, specimens were gently warmed to room temperature in absolute ethanol and critical point dried (CPD) with carbon dioxide; coated with gold in a sputter coater[e] with a working distance of 15 mm for 180 sec. with a high tension setting of 8.

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[c]Vortex genie mixer, S8223, Scientific Products, Evanston, Illinois.

[d]Parafilm "M", American Can Company, Neenah, Wisconsin.

[e]Edwards S150A, Edwards High Vacuum, Manor Royal, Crawley, West Sussex, England.

The 6 x 4 x 6 mm pieces of tissue (79-2231) were washed with 0.9% (normal saline) in a test tube, Vortex mixer at a setting of 5 for 5 x 1 min. duration with saline changes between mixing. The dehydrated specimens, held with forceps, were directly placed into liquid nitrogen and fractured open with a razor blade. The fractured surface was given a wedge-shaped edge for identification. CPD and coating were the same for all specimens.

The 6 x 4 x 2 mm pieces of the other tissues were washed with 0.9% sodium chloride in a test tube, at a setting of 4 for 6 x 1 min. duration. The dehydrated specimens were not freeze-fractured. The seminal vesicles were cut into four cross sectioned pieces. Two nonadjactant pieces were taken for further processing while the other two remained in 10% BFG. The prepared tissues were examined by an ETEC-Scanning Electron Microscope and photographed.[f]

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[f]ETEC-Scanning Electron Microscope U1, ETEC Corp., Hayward, Calif.

RESULTS

Control rams

Ultrastructure of the epithelium and the propria-submucosa

The epithelium of the seminal vesicles of the control rams were variable (Table 1, Fig. 1-8). The size of the minispherical apices of the columnar luminal cells varied from 7.5 µ (79-2287) to 5.0 µ (79-2273 & 79-2239) in diameter; 79-2260 was in between with 7.0 µ diameter.

The cellular shape was mostly hexagonal (79-2239, 79-2273, 79-2287), and the epithelial membrane generally had an even surface. In 79-2260 single cells rose above others and their hemispherical apices were rounded. The surface microvilli were distributed uniformly over the cell surface in 79-2287 and 79-2273. In 79-2239 and 79-2260, however, some epithelial cells had only a few microvilli, especially the taller cells. The length of microvilli was approximately 1.0 µ, diameter 0.1 µ.

Secretory blebs in form of spherical droplets of 0.3-1.3 µ diameter extended randomly from many cells. A few blebs were observed in all epithelial cells of the control rams (3,000 x). Some epithelial cells in 79-2260 and 79-2239 appeared collapsed. Specimen 79-2239 and 79-2273 had large craters in the apex of some epithelial cells that were approximately 3.0 µ in diameter. The shape varied

from irregular to circumscribed. A network of granular and pitted material was visible inside most craters. Small caveolae dotted the cell surfaces, especially those of partially collapsed cells. The number and distribution of caveolae appeared to be coincidental. Alveoli in 79-2239 revealed epithelium resting upon a continuous basement membrane that separated it from the lamina propria (500 x). The term "basement membrane" was chosen bearing in mind that only EM studies in the range of 16,000 x can reveal the difference between a single layer basal lamina and a two-layered basement membrane (Ham and Cormack 1979).

The mucosal folds with a propria-submucosa thickness of about 200-100 μ consisted of dense connective tissue. Mucosal folds of less than 60 μ (79-2260) thickness of the propria-submucosa were composed of loose connective tissue with a spongy appearance.

Table I: Sequential pathological changes in ovine seminal vesicles infected by A. seminis

Findings	Controls	1	2	3	4	5	6	7	8
Epithelial Cells									
Membrane tension	0	1	0	0	0	0	0	0	1
Collapsed	3	0	3	0	4	0	3	3	2
Craters in apex	1	1	1	1	1	1	1	1	0
Surface caveolae	4	2	4	2	4	4	2	2	2
Hemispherical apices									
Size variation in μ	5.0-7.5	<4	1.7-8.3	1.7-10.0	3.3-10.0	1.7-7.0	5.0-10.0	2.0-5.0	2.3-5.0
Shape	(a)& (b)	(b)	(b)& (c)	(b)& (c)& (d)	(b)& (c)	(a)& (b)	(a)& (b)	(d)	(b)& (c)
Epithelial Membranes									
Surface	Even	Humpy	Even	Even	Even	Even	Even	Humpy	Even
Secretory blebs	3	3	3	3	3	2	3	4	3
Defects	0	0	0	0	0	1	0	0	0

Table I: Contd

Findings	Controls	1	2	3	4	5	6	7	8
Basement Membrane Continuity	1	1	1	1	1	1	1	0	0
Microvilli									
Population	4	2	4	4	4	4	3	4	4
Clumping	0	0	1	1	0	1	0	0	0
Morphology change	0	0	0	0	0	0	0	0	0
Alveoli - contents	2	3	3	3	3	2	3	2	2
Propria - submucosa between alveoli									
Normal	1	0	0	1	1	1	1	0	1
Irregular and spongy	0	1	1	0	0	0	0	1	0
Intercellular lacunae	1	larger	larger	larger	larger	1	1	larger	larger

0 = No
2 = Yes
3 = Few
4 = Some
5 = Many

Hexagonal = a
Round = b
Oval = c
Polyhedral = d

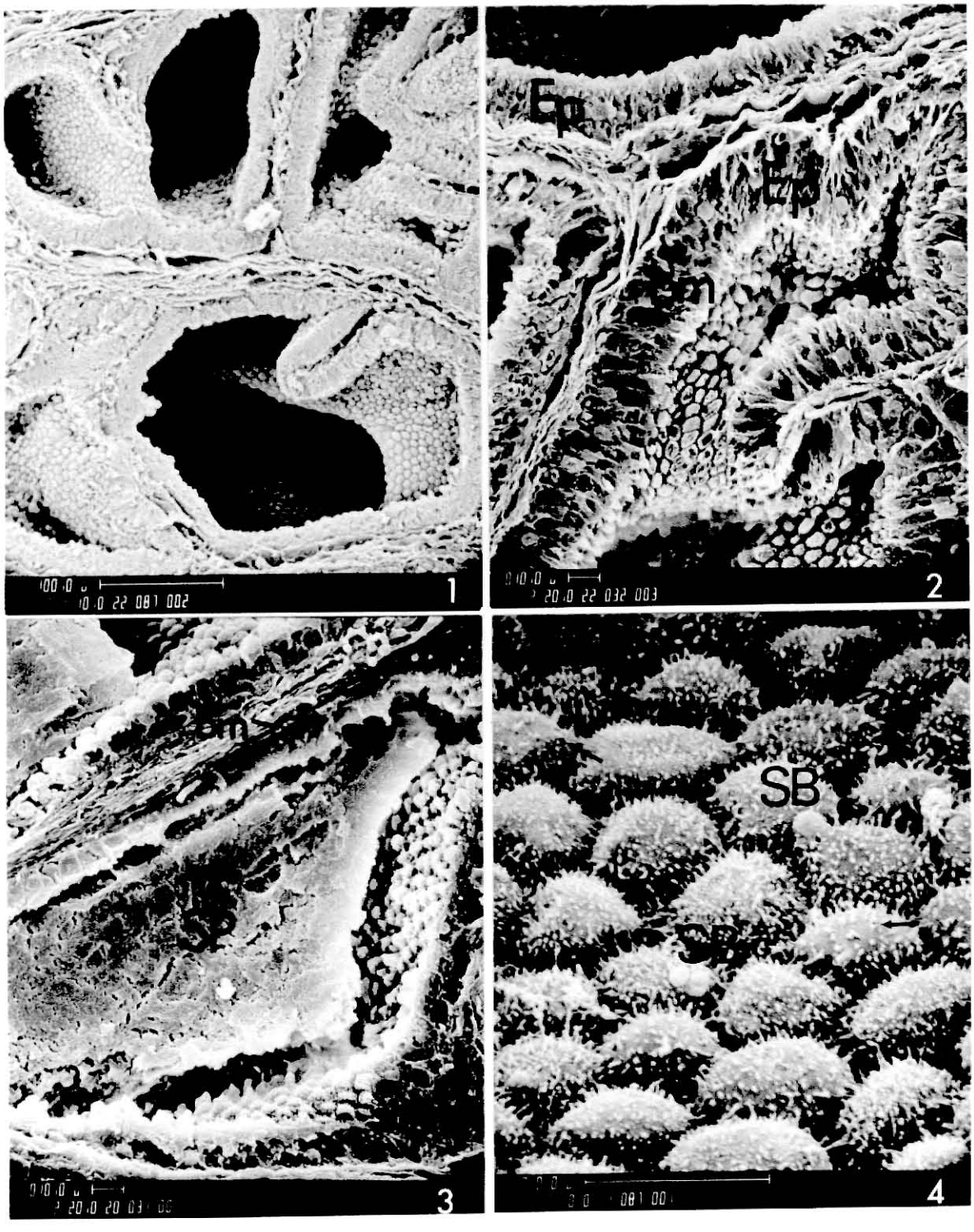
Scanning electron photomicrographs of normal ovine seminal vesicles.

Fig. 1 Working distance (WD) 22 mm
Accelerating voltage of the primary electron beam (AV) 10 KV; x 200

Fig. 2 Epithelium with 2 layers of cells separated by the propria-submucosa and the basement membrane.
WD 22 mm, AV 20 KV, x 500
Legend: Ep = epithelium
bm = basement membrane
SP = secretory product

Fig. 3 Secretory product filling the interior of the alveolus
WD 20 mm, AV 20 KV, x 500

Fig. 4 Epithelial cells with secretory product within them. Secretory blebs (SB) in form of spherical droplets. Small caveolae dot the cell surfaces (arrows).
WD 11 mm, AV 10 KV, x 3,000


Ep
SB
1
2
3
4

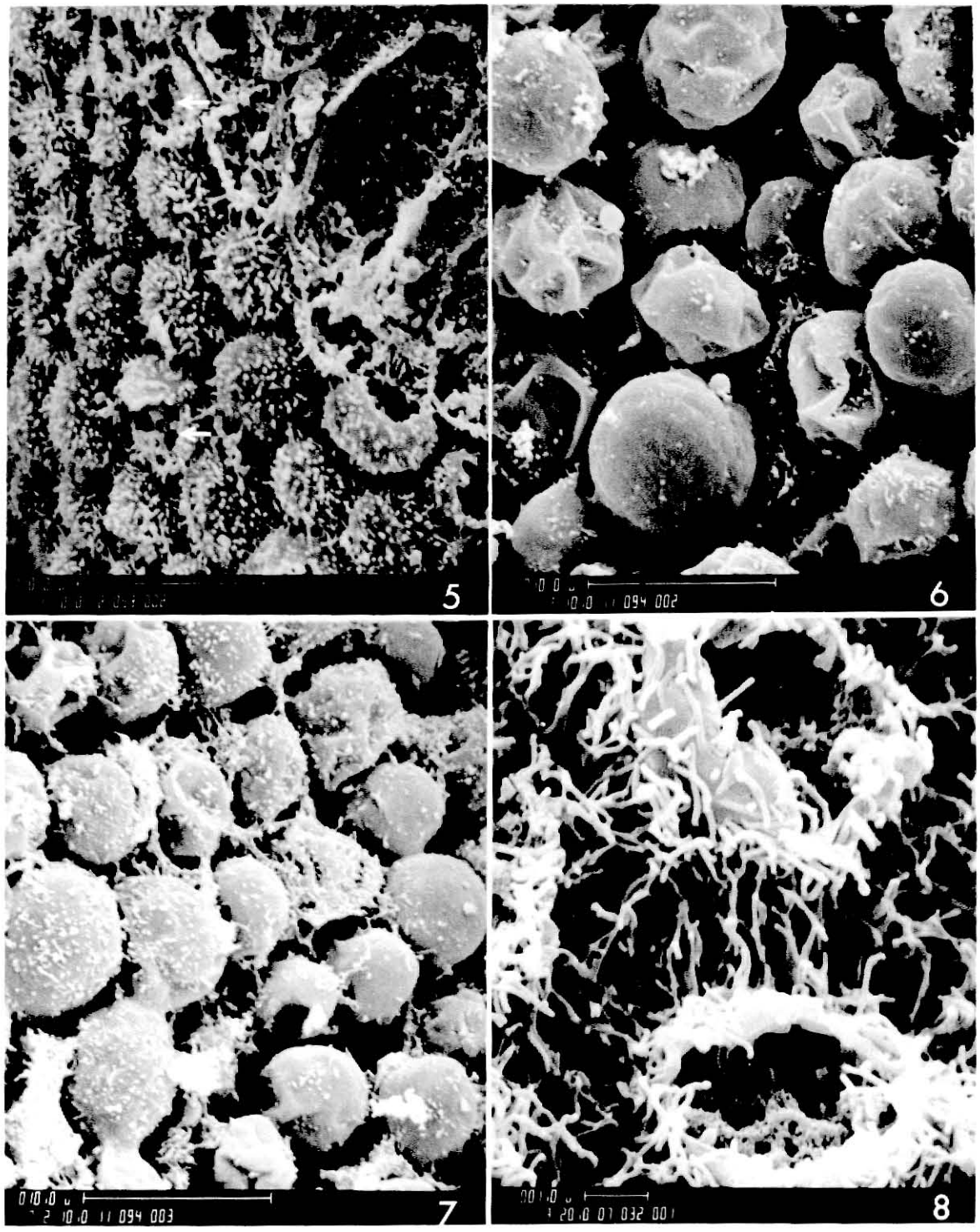
Scanning electron photomicrographs of normal ovine seminal vesicles.

Fig. 5 Note large craters in the apex of some epithelial cells and the network of granular and pitted material inside (arrows).
WD 12 mm, AV 10 KV, x 3000

Fig. 6 Epithelial cells rising above others that have only a few microvilli. Note that some epithelial cells are partially collapsed.
WD 11 mm, AV 10 KV, x 3000

Fig. 7 Note that some epithelial cells are rounded at their apical border.
WD 11 mm, AV 10 KV, x 3000

Fig. 8 High magnification illustrating craters in the apex of epithelial cells. Surface microvilli can be seen at the wall of the epithelial cells and between them--which is probably the apical surface of smaller epithelial cells.
WD 7 mm, AV 20 KV, x 10000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Test rams, 24 hours PI

The epithelial cells had rounded hemispherical apices, 4 μ and smaller. The surface of the epithelial membrane had a hump-backed appearance. Many epithelial cells rose above others with larger intercellular lacunae between apposing epithelial cell membranes than in control rams. Most epithelial cells had only a few microvilli, and cell membranes were tense. Some small caveolae dotted the cell surfaces (Table 1; Fig. 9-11).

There were large craters in the apex of some epithelial cells; however, no collapsed epithelial cells were found. Secretory blebs extended from some cells. The thin propria-submucosa between the alveoli had more irregular and spongy appearance than that of the controls. The basement membrane was continuous. Some alveolar contents were not removed by washing.

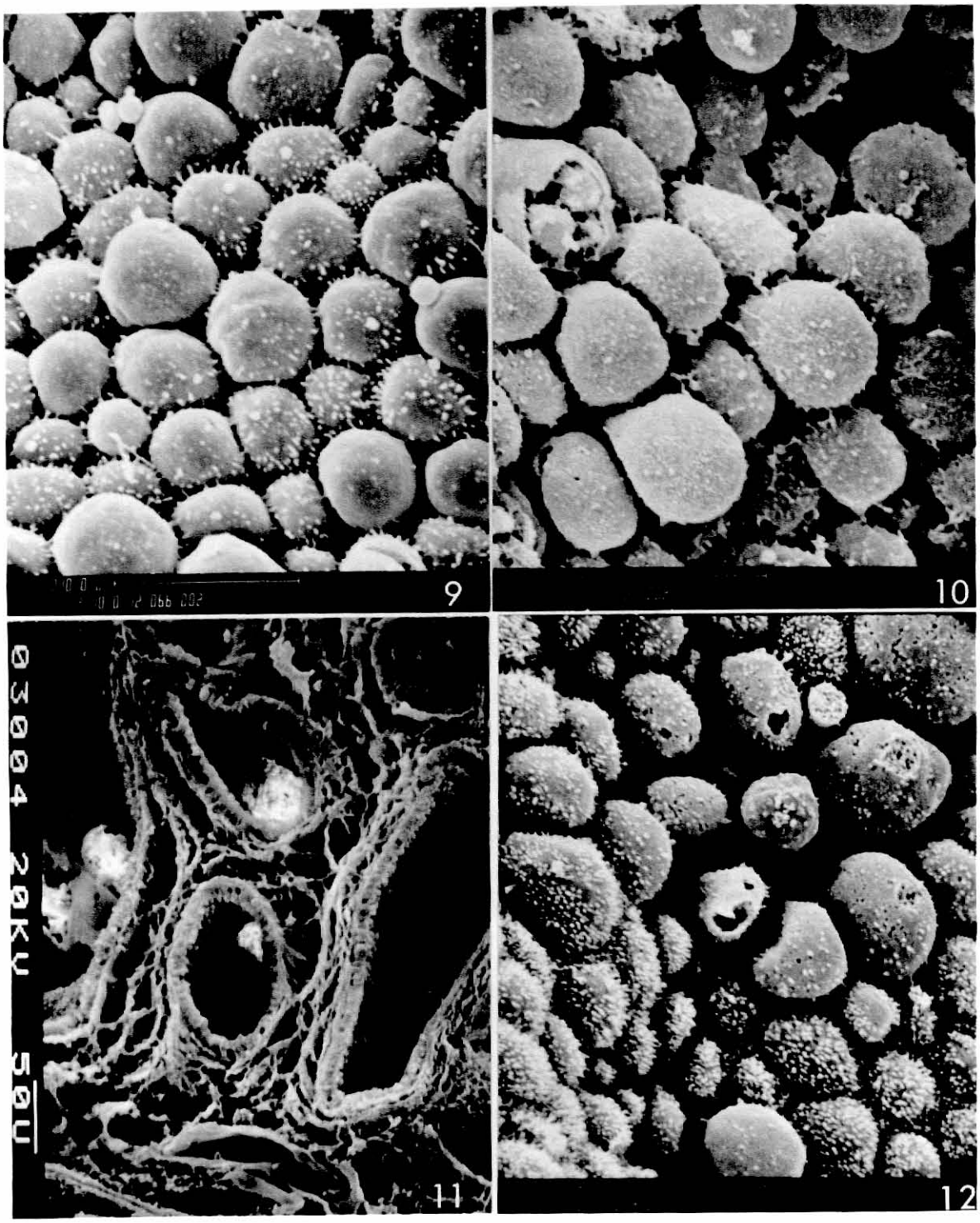
Scanning electron photomicrographs of seminal vesicles of rams infected with A. seminis.

Fig. 9 24 hours PI. Note decrease in size of hemispherical apices of the alveolar columnar luminal cells. The cell membranes are extremely tense.
WD 12 mm, AV 10 KV, x 3000

Fig. 10 24 hours PI. Note few microvilli and no epithelial cell collapse.
WD 11 mm, AV 10 KV, x 3000

Fig. 11 24 hours PI. Note the spongy and irregular propria-submucosa between the alveoli.
AV 20 KV, x 200

Fig. 12 48 hours PI. Note the difference in number of microvilli (compare with 24 hours PI--Fig. 10).
WD 11 mm, AV 10 KV, x 3000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10
030004 20KV 50U
11
12

Test rams, 48 hours PI

Hemispherical apices of epithelial cells varied from 1.7 μ to 8.3 μ (Table 1). The apices of epithelial cells rose about the same height, and gave an even appearance to the epithelial membrane. Intercellular lacunae between apposing epithelial cell membranes were larger than in the controls. The cells were studded with microvilli, small caveolae on cell surfaces and varied craters in the apex. There was slight clumping of some groups of microvilli, with some cells appearing collapsed. Secretory blebs extended from some cells. The thin propria-submucosa between the alveoli had a more irregular and spongy appearance in contrast to the controls. The basement membrane was continuous. Some contents remained in the alveoli in spite of the washing (Fig. 12-15).

Test rams, 72 hours PI

The hemispherical apices of columnar luminal cells varied from 10 μ to 1.7 μ (Table 1). The variety of the apices ranged from oval to polyhedral to round. In general, the intercellular lacunae present between apposing epithelial cell membranes were larger than in the controls. The cells were well equipped with microvilli, with a slight to moderate clumping of microvilli. Secretory blebs extended

from some cells. Craters in the apex of some cells could be seen and some small caveolae dotted the cell surfaces. No cells appeared collapsed and no changes were observed in the propria-submucosa. The basement membrane was continuous. Much of the contents of alveoli was still present (Fig. 16-18).

Test rams, 96 hours PI

Size of the epithelial cells' hemispherical apices varied from 3.3 μ to 10.0 μ, with a round or oval shape. Intercellular lacunae between apposing epithelial cell membranes were enlarged in one photomicrograph (Fig. 21). Epithelial cells had many surface microvilli, and no villary clumping. Many epithelial cells appeared collapsed. Small vacuolae dotted the cell surfaces, secretory blebs extended from some cells and craters in the apex of some epithelial cells were seen. No changes of the propria-submucosa were visible. Some contents of alveoli was not removed by washing.

Test rams, 5 days PI

Size of the epithelial cells' hemispherical apices varied from 1.7 μ to 7.0 μ (Table 1). The shape was hexagonal or rounded as in the control rams. Size of intercellular

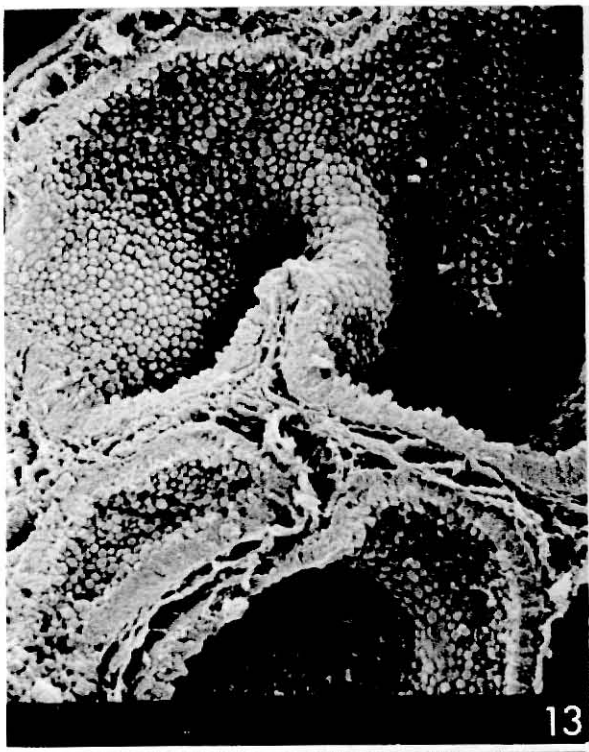
Scanning electron photomicrographs of seminal vesicles of rams infected with A. seminis.

Fig. 13 48 hours PI. Note the spongy and irregular propria-submucosa between the alveoli.
WD 21 mm, AV 10 KV, x 200

Fig. 14 48 hours PI. Note clumping of groups of microvilli (arrow).
WD 11 mm, AV 10 KV, x 3000

Fig. 15 48 hours PI. Note large intercellular lacunae between apposing epithelial cell membranes.
WD 11 mm, AV 10 KV, x 3000

Fig. 16 72 hours PI. Note amount of content in the alveoli.
WD 21 mm, AV 10 KV, x 200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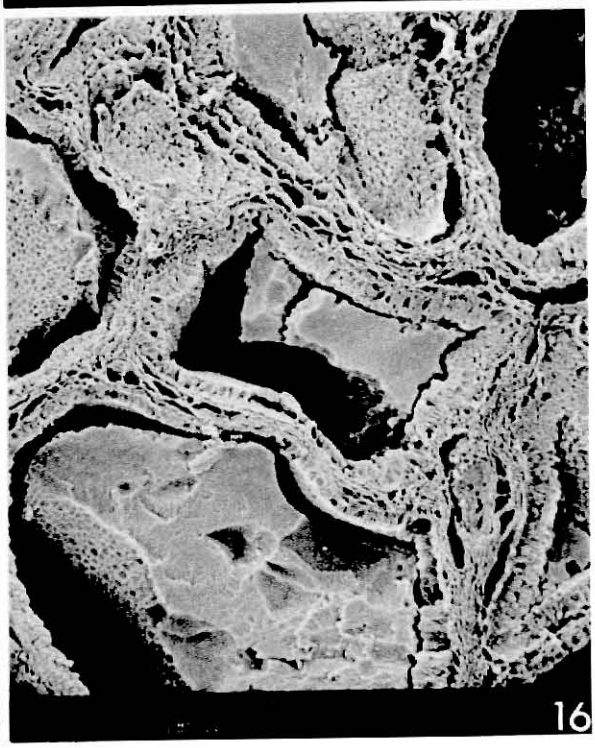
16

Scanning electron photomicrographs of seminal vesicles of rams infected with A. seminis.

Fig. 17 72 hours PI. Note the clumping of microvilli (arrows) and craters.
WD 11 mm, AV 10 KV, x 3000

Fig. 18 72 hours PI. Note variation in size of hemispherical apices of columnar luminal cells.
WD 12 mm, AV 10 KV, x 3000

Fig. 19 96 hours PI. Note the normal propria-submucosa between the alveoli (arrows).
WD 22 mm, AV 10 KV, x 200

Fig. 20 96 hours PI. Note collapsed epithelial cells.
WD 10 mm, AV 10 KV, x 3000


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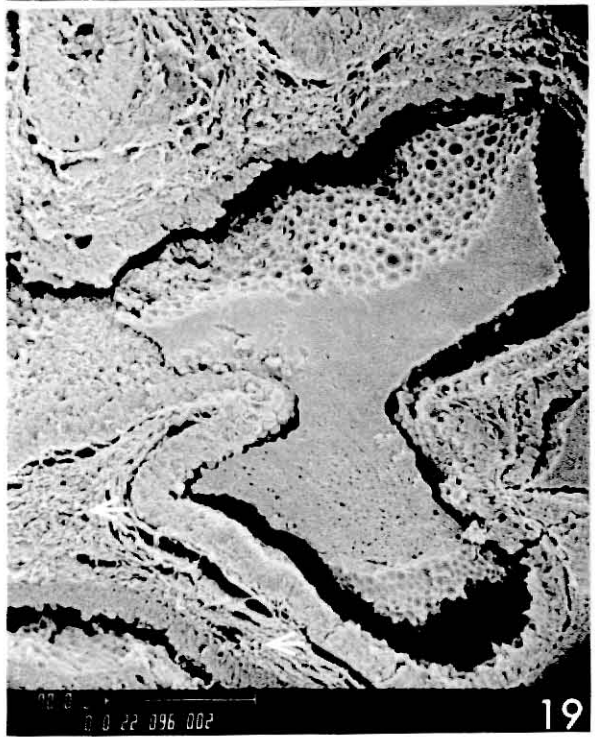
18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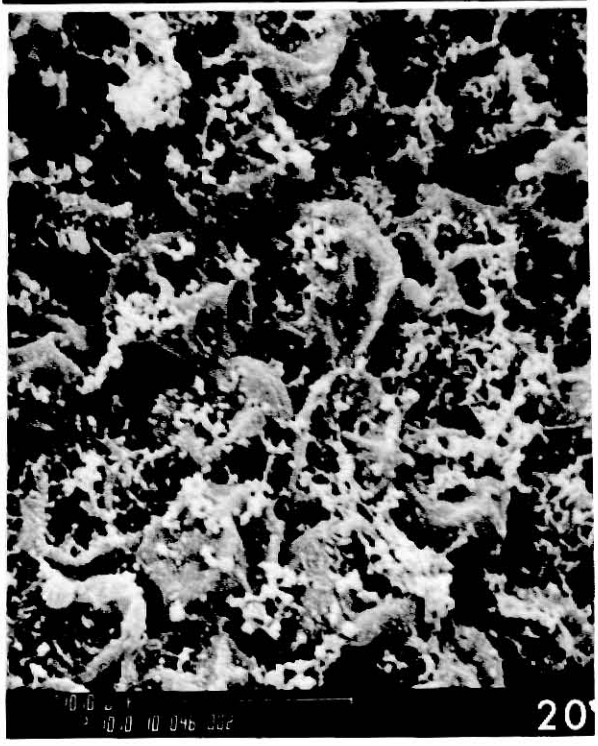
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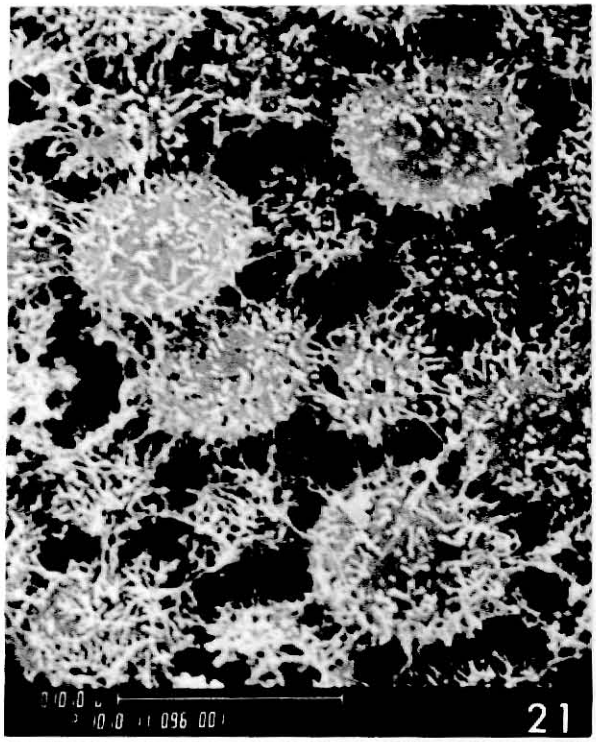
Scanning electron photomicrographs of seminal vesicles of rams infected with A. seminis.

Fig. 21 96 hours PI. Numerous small caveolae dot the cell surfaces.
WD 11 mm, AV 10 KV, x 3000

Fig. 22 5 days PI. Note number of microvilli.
WD 11 mm, AV 10 KV, x 3000

Fig. 23 5 days PI. Note hexagonal and rounded hemispherical apices.
WD 11 mm, AV 10 KV, x 3000

Fig. 24 5 days PI, Note defects in the epithelial membranes (arrows).
WD 21 mm, AV 10 KV, x 200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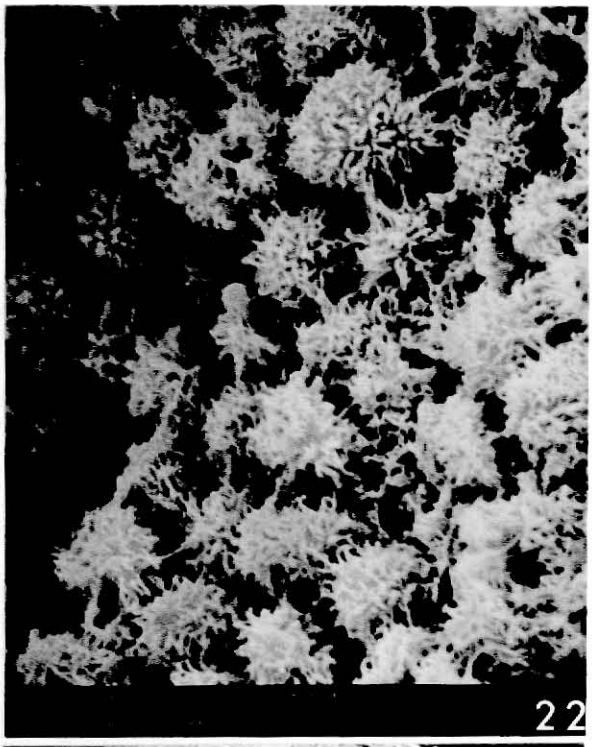
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lacunae between apposing epithelial cell membranes was similar to that of the control rams. Many surface microvilli but no collapsed epithelial cells were seen. There was slight clumping of groups of microvilli. Small vacuolae dotted cell surfaces, craters in the apex of some epithelial cells were present, but there were few secretory blebs (Fig. 22-24). The propria-submucosa appeared normal. Round defects of 25 μ in diameter in the epithelial membranes appeared in x 200 photomicrographs (Fig. 24). Little contents remained in the alveoli. The basement membrane was continuous.

Test rams, 6 days PI

Size of the epithelial cells' hemispherical apices varied from 5.0 μ to 10.0 μ (Table 1). Shape and size of intercellular lacunae were similar to unaffected tissues. A photomicrograph of specimen No. 79-2279 revealed a more hexagonal to polyhedral shape of hemispherical apices (Fig. 25). Surface microvilli and some collapsed epithelial cells were visible. Few small caveolae dotted the cell surfaces, craters in the apex of some epithelial cells and some secretory blebs were seen. The propria-submucosa was normal. Much of the contents in the alveoli was not removed by washing (Fig. 26). The basement membrane appeared unaffected.

Test rams, 7 days PI

Epithelial cells' hemispherical apices were small and varied from 2.0 μ to 5.0 μ in diameter (Table 1). The shape was polyhedral and the intercellular lacunae between apposing epithelial cell membranes were larger than in the control rams. Photomicrographs revealed many surface microvilli, many secretory blebs and some collapsed epithelial cells (Fig. 27). Craters in the apex of epithelial cells were abundant. Few small caveolae dotted the cell surfaces. The propria-submucosa had a spongy and irregular appearance (Fig. 28) and the surface of the epithelial membrane revealed a hump-backed appearance as many of the epithelial cells rose above others. Little content remained in the alveoli. The basement membrane was interrupted at several points.

Test rams, 8 days PI

The round to oval epithelial cells' hemispherical apices measured from 2.3 μ to 5.0 μ (Table 1). Intercellular lacunae between apposing epithelial cell membranes were large and some cell membranes appeared extremely tense (Fig. 29). However, other photomicrographs revealed many surface microvilli, some secretory blebs and a few collapsed epithelial cells There were few small caveolae on the cell

surfaces and absence of craters in the apex of epithelial cells. The continuity of the basement membrane was interrupted (Fig. 30).

Artifacts

Various artifacts due to defective procedures and other causes are illustrated: freeze fracturing (Fig. 31), mechanical damage (Fig. 32), and defective dehydration (Fig. 33-36).

Scanning electron photomicrographs of seminal vesicles of rams infected with A. seminis.

Fig. 25 6 days PI. Note hexagonal shape of hemispherical apices.
WD 12 mm, AV 10 KV, x 3000

Fig. 26 6 days PI. Note contents in the alveoli and normal propria-submucosa (arrow).
WD 21 mm, AV 10 KV, x 200

Fig. 27 7 days PI. Note amount of secretory blebs and example of true apocrine secretion (arrows).
WD 13 mm, AV 10 KV, x 3000

Fig. 28 7 days PI. Note irregular and spongy propria-submucosa and discontinuity of basement membrane (arrows).
WD 22 mm, AV 10 KV, x 200


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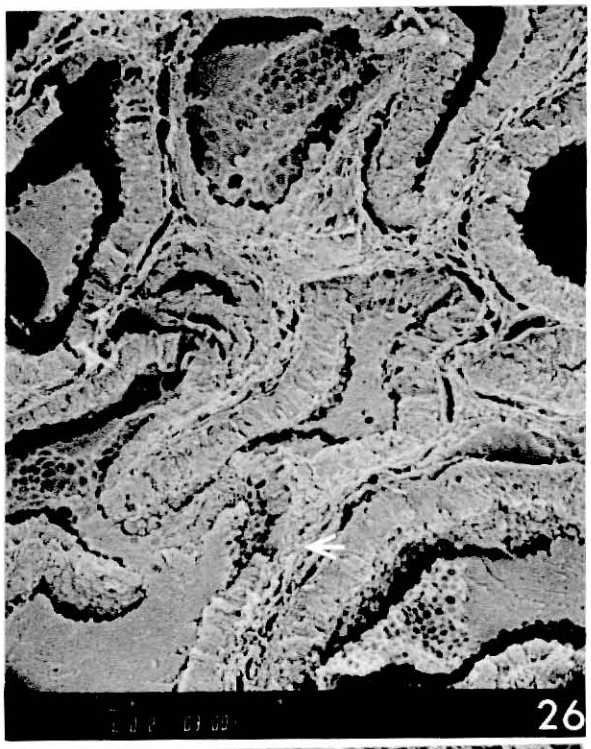
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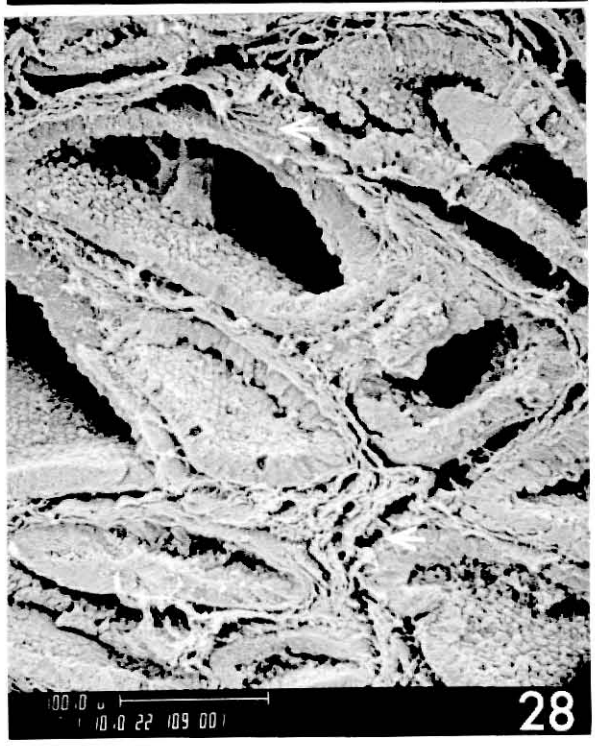
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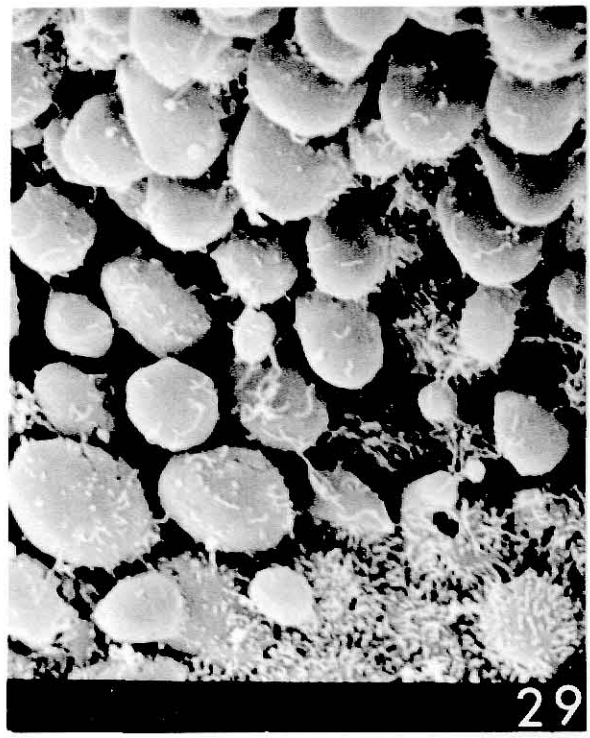
Scanning electron photomicrographs of seminal vesicles of rams infected with A. seminis.

Fig. 29 8 days PI. Most cell membranes are extremely tense.
WD 12 mm, AV 10 KV, x 3000

Fig. 30 8 days PI. Note interruption of basement membrane and relatively normal propria-submucosa (arrow).
WD 22 mm, AV 10 KV, x 200

Fig. 31 8 days PI. Freeze fractured specimen. Note the inferior quality of the surface.
WD 21 mm, AV 10 KV, x 200

Fig. 32 Scanning electron photomicrograph of normal seminal vesicle of ram with artifacts caused by mechanical damage. Note fissures in the epithelial membranes (arrows).
WD 21 mm, AV 10 KV, x 200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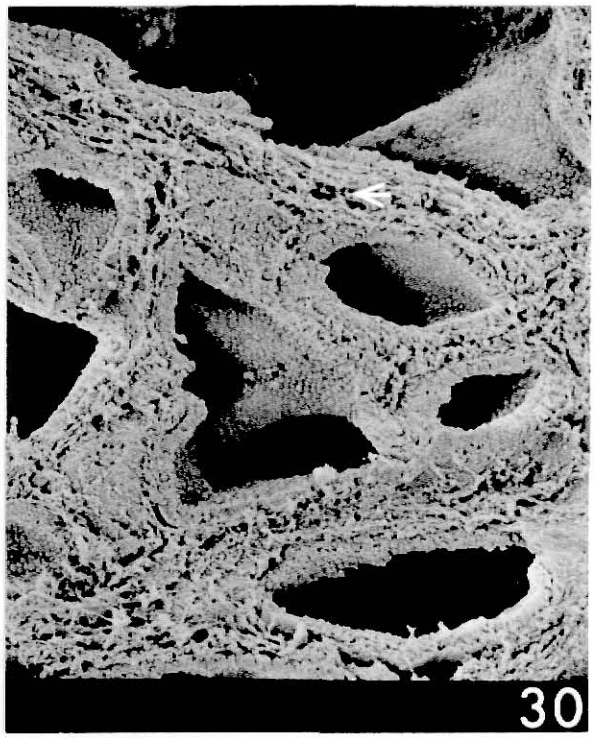
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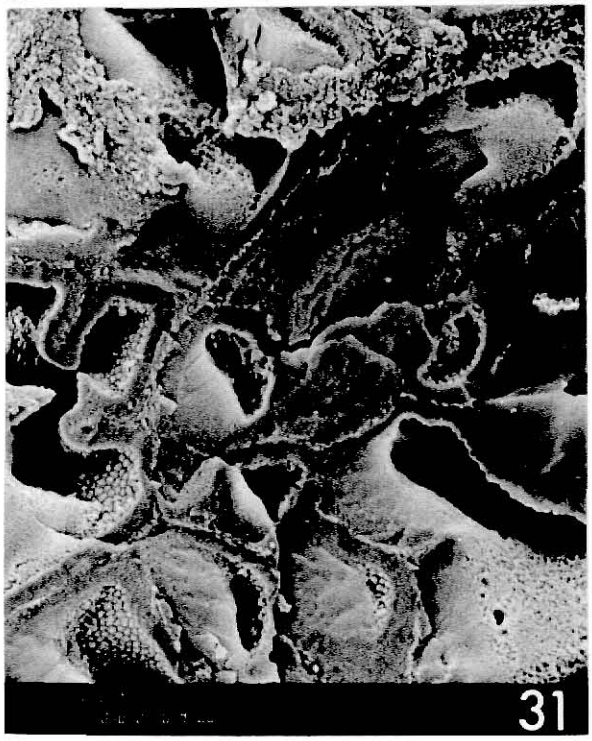
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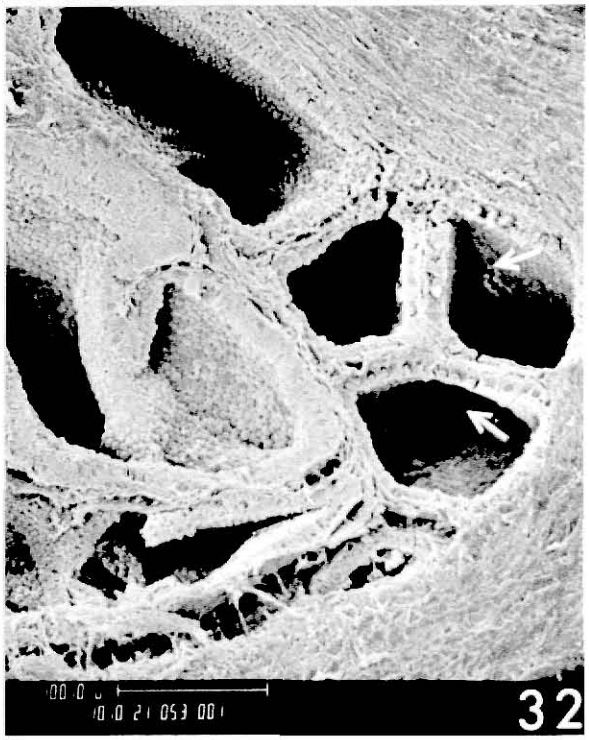
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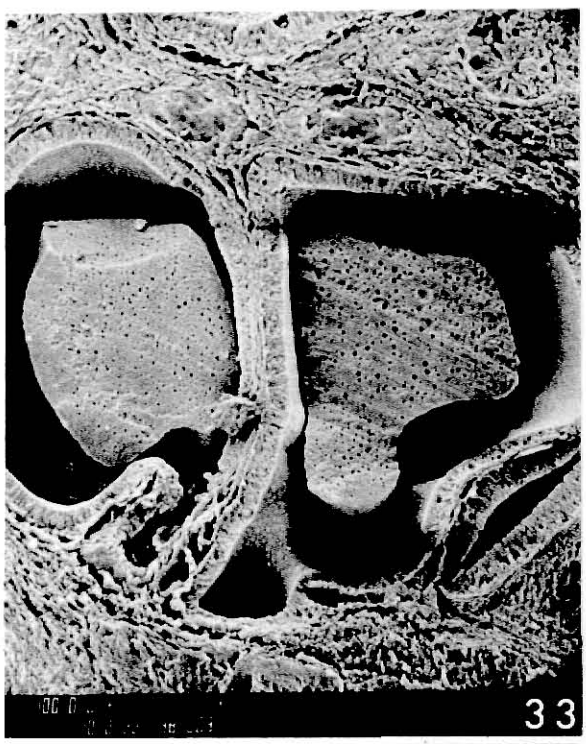
Scanning electron photomicrographs of seminal vesicles of rams infected with A. seminis.

Fig. 33 48 hours PI. Artifacts caused by defective dehydration procedures. Note the spongy, shrunken secretory product within the alveoli.
WD 22 mm, AV 10 KV, x 150

Fig. 34 48 hours PI. Artifacts caused by defective dehydration procedures. Note ruptures in the surface of the epithelial membrane (arrows).
WD 21 mm, AV 10 KV, x 130

Fig. 35 48 hours PI. Artifacts caused by defective dehydration procedures. Note distinctive clumping of all microvilli and the filamentous material between apposing epithelial cell membranes.
WD 10 mm, AV 10 KV, x 3000

Fig. 36 48 hours PI. Artifacts caused by defective dehydration procedures. Note separation of upper layers of the propria-submucosa (arrows).
WD 19 mm, AV 10 KV, x 60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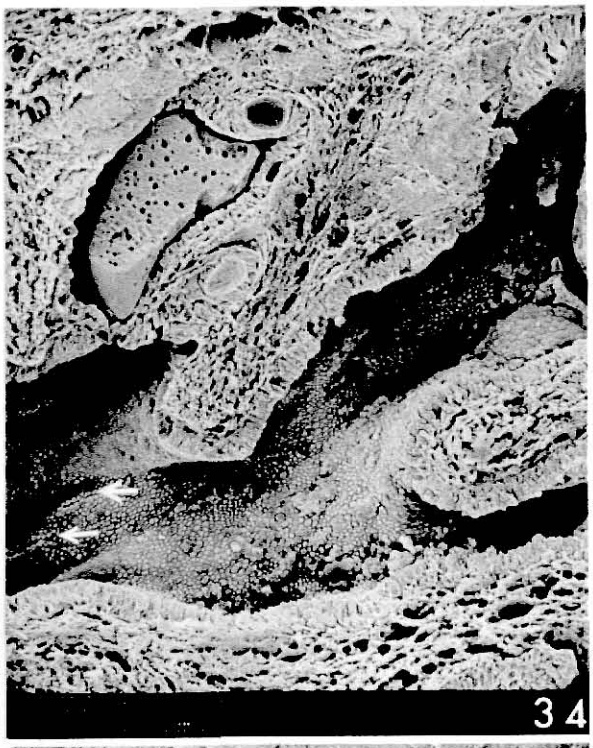
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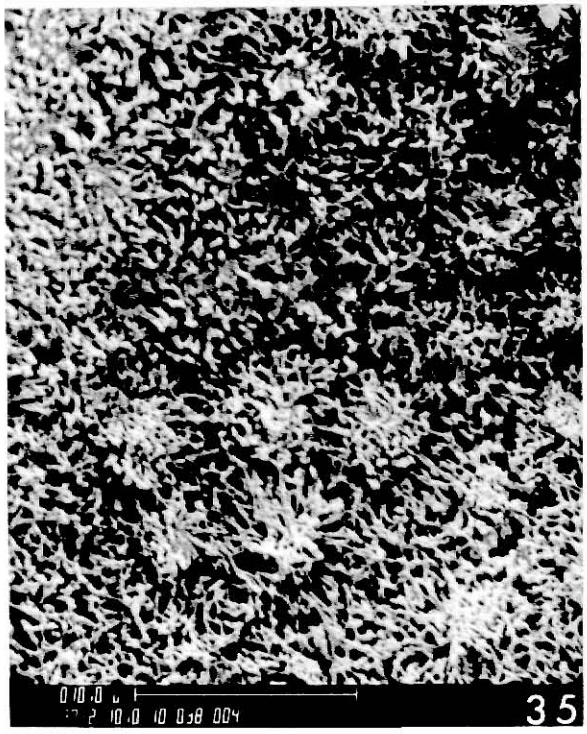
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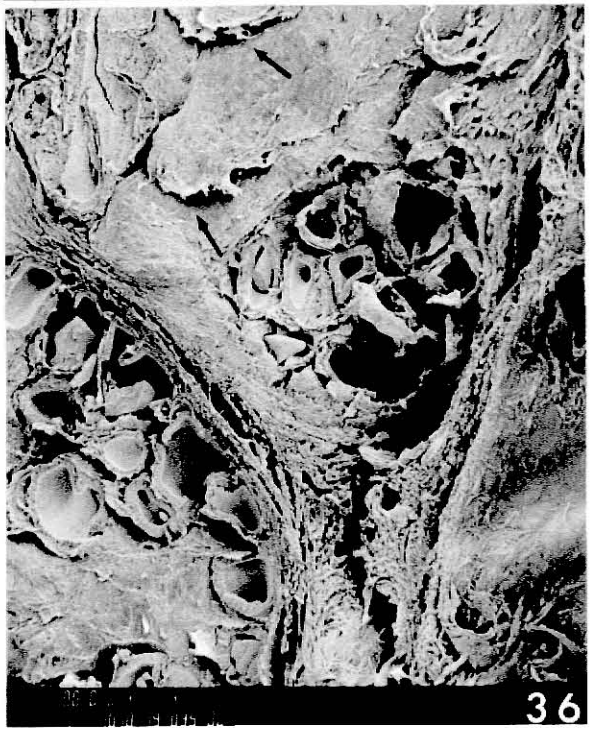
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DISCUSSION

Scanning electron microscopy (SEM) provides a 3-dimensional view of the surface texture of ovine seminal vesicles (SV). SEM-pictures do not include interior details such as changes in the luminal contents and infiltration of lymphocytes, eosinophils and plasma cells in the septa between alveoli. Therefore, the emphasis of this study was to supplement and to add to the changes seen by light microscopy rather than to "compete" with the light microscopic findings.

Changes in size of hemispherical apices of the columnar luminal cells were striking. The control tissues varied in size from 5.0-7.5 μ with an average of 7.0 μ in diameter. The light microscopic changes of the epithelium within 24 hours PI expressed by SEM-pictures revealed a marked decrease in size of the hemispherical apices of the columnar luminal cells; all were less than 4 μ in diameter and the cell membranes were extremely tense. The decrease in size was accompanied by marked enlargement of the intercellular lacunae between apposing epithelial cell membranes. Epithelial cells were not collapsed and few small caveolae dotted the cell surfaces indicating a change in the secretory process as there were still craters in the epithelial cell apices. Merocrine secretion was reduced and the cells released their secretory product by apocrine secretion.

According to Yates and Gordon (1977), the appearance of microvilli and secretory activity are closely related in a number of epithelia. Epithelial cells with only a few microvilli--as with most of the SV epithelial cells within 24 hours PI--should therefore have decreased secretory activity.

A remarkable variation in size of hemispherical apices of the SV columnar luminal cells occurred between day 2 to 6 PI. Smaller cells as well as larger cells were observed in the test rams from day 2 to 4 PI. Variation in size decreased again at day 5 and 6 PI. Day 7 and 8 PI revealed smaller apices in general compared with those found in the control rams.

The number of superficial epithelial cells was the same while some were smaller in diameter than those in control rams with intercellular lacunae between apposing epithelial cell membranes becoming larger except during day 5 and 6 PI.

The shape of hemispherical apices varied considerably from day to day, while at day 5 and 6 PI the shape was similar to that of the control rams. The variation in shape may be cyclical and related to some extent to function. Epithelial changes such as hyperplasia at day 3 and 6 and desquamation at day 3 and 5 by light microscopy (Al-Katib 1980), were observed by SEM as defects (25 μ in diameter)

in the epithelial membranes at day 5 PI. Hyperplasia was barely detected with SEM, because most of the low epithelial cells were overshadowed by the superficial epithelial cells.

Changes in the lumen of the seminal vesicles, for example cellular debris, leukocytes and proteinaceous substance, were mainly undetected by SEM. However, more contents were found in affected alveoli compared to those in the control tissues. Since the procedures employed were the same for all non freeze-fractured tissues, the increase in alveolar contents at day 1-4 and 6 was striking. The decrease in secretory activity at 24 hours PI changed to hyperactivity by day 2 PI. Many small caveolae dotted the cell surfaces, cell membranes lost their extreme tension and in addition, formed craters in the apex of epithelial cells where some collapsed epithelial cells were found.

Larger magnifications (3000 x) revealed a change in microvilli distribution. A slight clumping of some groups of microvilli occurred by day 2 PI that increased at day 3 PI and found again at day 5 PI. Clumping of groups of microvilli was not with unaffected epithelium and served as an indicator for disturbed cellular activity. At day 3 and 5 PI no collapsed epithelial cells could be found whereas at day 4 PI, the distribution of microvilli was normal, but the amount of collapsed epithelial cells was even greater than in the control rams. Discrepancies between the amount

of secretory blebs found in the 3,000 x photomicrographs and the number of small caveolae that dotted the cell surfaces were obvious at day 2, 5 and 7 PI. The remark by Kessel and Kardon (1979) that some secretory product appears in the form of filamentous material could explain many caveolae in the cell surfaces with only a few secretory blebs visible.

The 3,000 x photomicrographs of day 7 PI revealed that large blebs as well as spherical droplets were being released by apocrine secretion and thus accounted for the many secretory blebs observed at that time. Craters in the apex of epithelial cells, a sign of apocrine secretion, appeared on all days PI except day 8 when the cell membranes were extremely tense.

Al-Katib (1980) found a serofibrinous exudate present in the interstitium on day 1, 4 and 6 PI. A more irregular and spongy propria-submucosa between alveoli was found in the SV of test rams at day 1, 2 and 7 PI (200 x). The condition of the basement membrane, however, was hard to evaluate. The continuity of the basement membrane appeared unaffected till day 6 PI and interrupted at day 7 and 8 PI. The functions of the basement membrane and/or basal laminae are elastic support and a filtration or diffusion barrier (Ham and Cormack 1979). Discontinuity has serious consequences for metabolism of the gland and its architecture

and leads to swelling of epithelial cells as observed at day 8 PI.

Elemental leaching and shrinkage of biological specimens during preparation for SEM observations are well-documented serious problems (Postek et al 1980). Accurate critical point drying procedures result in a dry specimen that has not been subjected to damage by the surface tension forces of a gradually evaporating pool of liquid (Postek et al 1980). A transitional fluid, carbon dioxide (CO_2), has to pass through its critical point, resulting in a gaseous phase. The critical pressure of CO_2 is 1072 psi and the critical temperature 31 C.

The following changes occurred in a specimen that underwent incomplete critical point drying procedure because of CO_2 shortage. The CO_2 pressure reached only 900 psi and the temperature was 42 C. The secretory product in the alveoli is shrunken and has a spongy appearance. The surface of the epithelial membranes reveals several ruptures of about 60 u length. A distinctive clumping of all microvilli could be seen with 3,000 x photomicrographs with the space between apposing epithelial cell membranes being stuffed with filamentous material. The upper layers of the propria-submucosa separate at several points.

Fissures in the epithelial membranes of about 50-100 μ length were occasionally found in specimens that underwent

complete dehydration procedures. Those areas represented mechanical damage from mounting and coating and could be reduced by careful handling of the delicate dehydrated specimens and adjusting the working distance in sputter coater. Some damage to the edges of the epithelial membranes was usually caused by cutting the fixed specimens.

Since the unprepared specimens had been fixed for months in BFG, the size of the cuts as well as duration and vigor of the washing process had to be estimated in order to achieve a rather complete removal of the extracellular product without destroying the delicate structure of the seminal vesicle's interior. Vigor and duration of the washing procedure depend on the thickness of the specimen. If the specimen is more than 2-3 mm thick it has to be treated more vigorously in order to clean it. Freeze fracturing requires a specimen thickness of at least 6 mm to provide adequate surface size for fracturing with the cooled razor blade.

A disadvantage of freeze fracturing is difficulty in marking the fractured surface. Marking the freeze fractured surface by cutting an edge off proved impractical because all sides of the specimen looked similar after critical point drying with CO_2. Freeze fractured specimens had an inferior surface quality compared to specimens cut with a sharp razor blade.

It is concluded from this study that SEM serves as an additional method for explaining changes seen by light microscopy and in detecting early changes that are not recognized by light microscopy. The former includes changes in the basement membranes, microvilli and hemispherical apices of columnar luminal cells; the latter includes changes in the secretory process.

In doing this research one obvious deficiency was in trying to determine which epithelial cell changes were due to the experimental infection and which were normal cyclic cell changes. It is believed that the changes in the basement membranes and propria submucosal changes were critical evaluating the epithelial changes observed.

The light microscope findings of swelling and hydropic degeneration of the epithelial cells at day 1, 3, 4-6 and 8 PI were explained by SEM as changes in secretory process 24 hours PI and cell swelling at day 8 PI. Serous exudation with leukocytic infiltration in the interstitium by light microscopy at day 1, 2, 4 and 6 PI were observed by SEM as irregular and spongy propria-submucosa between alveoli at day 1, 2 and 7 PI.

Light microscopic detectable changes in the lumen of the seminal vesicles, for example proteinaceous substance, cellular debris and leukocytes at day 2-6 PI were expressed by an increase in alveolar contents at day 1-4 and 6 PI in

SEM photomicrographs. Desquamation of the epithelium at day 5 PI was detected by both light microscopy and SEM.

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EXPERIMENTAL ACTINOBACILLUS SEMINIS INFECTION OF RAMS: A SCANNING ELECTRON MICROSCOPY STUDY OF SEMINAL VESICLES

by

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DVM, Justus Liebig-University Giessen, West-Germany, 1979

AN ABSTRACT OF A THESIS

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requirements for the degree

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Department of Pathology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1980

This scanning electron microscopy study examined the ultrastructure of epithelial cells, membranes and the propria-submucosa of four normal and 16 ovine seminal vesicles infected with Actinobacillus seminis.

The tail of the left epididymis of each test ram was injected with 1 ml of a 24 hour broth culture of A. seminis. Two test rams were euthanatized every 24 hours post inoculation (PI) and one control ram was euthanatized at 24, 48, 96 and 144 hours.

Findings in test rams at 24 hours PI included changes in size of the hemispherical apices of the columnar luminal cells, tense cell membranes and apparent changes in secretory activity. Findings on the remaining days PI included variation in size and shape of the hemispherical apices of the columnar luminal cells, enlargement of the intercellular lacunae between apposing epithelial cell membranes, defects in the epithelial membranes and changes in the distribution of microvilli. Artifacts caused by defective dehydration procedures and mechanical damage from cutting, mounting and coating were explained. Different specimen preparation methods were evaluated.

The light microscope findings of swelling and hydropic degeneration of the epithelial cells at day 1, 3, 4-6 and 8 PI were explained by SEM as changes in secretory process 24 hours PI and cell swelling at day 8 PI. Serous exudation

with leukocytic infiltration in the interstitium by light microscopy at day 1, 2, 4 and 6 PI were observed by SEM as irregular and spongy propria-submucosa between alveoli at day 1, 2 and 7 PI. Light microscopic detectable changes in the lumen of the seminal vesicles, for example proteinaceous substance, cellular debris and leukocytes at day 2-6 PI were expressed by an increase in alveolar contents at day 1-4 and 6 PI in SEM photomicrographs. Desquamation of the epithelium at day 5 PI was detected by both light microscopy and SEM.