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Morphological Features of the Seminiferous and Cauda Epididymides Epithelia of Breeding Zebu Bulls with Normal and Decreased Testicular Consistency

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Abstract. The fine morphology of the testes and epididymes collected from breeding Zebu (*Bos indicus*) bulls with normal or decreased testicular consistency (TC) at clinical palpation was studied. The ultrastructure of testicles with a slight to moderate reduction in consistency showed more obvious, albeit slight, degenerative changes in the seminiferous epithelium, with cellular debris present in Sertoli cells, compared with control animals. Abnormalities in the condensation of the chromatin and acrosomes during spermiogenesis were also common findings in cases of decreased TC. The cauda epididymides of these bulls did not show clear morphological deviations compared with controls, although the macrophagic activity of the epithelium was more pronounced, and cell debris as well as foreign cells were apparent in the lumen. The morphological findings confirm the relationship between a decreased TC at clinical palpation and the lowered normality of the testicular parenchyma, thus supporting the value of palpating scrotal contents as part of the field andrological clinical evaluation of breeding Zebu bulls in the tropics.
Key words: Testicular consistency, *Bos indicus*, Ultrastructure, Testis, Epididymis.
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Beef production in the tropics is based mainly on extensive production systems and natural mating. Under these conditions, it is essential that a complete clinical examination of the breeding bulls be made [1] and that clinical parameters related to the fertility of the bull be used as selection criteria for breeding purposes. One of these clinical parameters is testicular consistency (TC), which is normally evaluated while palpating the scrotal contents. Testicular consistency has been shown to be related to conception rate [2] and semen quality [3] in European breeds under temperate conditions. A decrease in TC is commonly found in connection with acute or sub-acute testicular pathologies, such as testicular degeneration. Attempts to establish a relation between TC and seminal or fertility parameters of *Bos indicus* bulls have been inconclusive thus far [1, 4], although morphological alterations of the seminiferous epithelium were more frequent in Zebu breeding bulls with low TC than in control bulls [5] and were often associated with temperature-induced testicular pathologies [6]. For *Bos indicus*, *Bos taurus* and crossbred breeding bulls extensively reared in Costa Rica, a negative relationship was found between decreased

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TC and the frequencies of abnormal sperm heads in the ejaculate [7]. In addition, bulls with a lower TC were more likely to be classified as unsound for breeding after field andrological evaluation. Despite these indications, it has yet to be determined whether a slight to moderate diminution of TC is associated with clear alterations in the seminiferous epithelium which would have important implications for the clinical diagnosis. This association is of importance since TC has been utilized as a major criterion for disclosal of field breeding soundness evaluations.

Therefore, in the present study we assessed the magnitude and nature of the changes in fine structure (at the light and electron microscopic levels) in the seminiferous and cauda epididymides epithelia of breeding Zebu bulls in Costa Rica with TC ranging from normal to moderately decreased.

Materials and Methods

A total of 11 breeding Zebu (Brahman) bulls, 36 to 42 months old and extensively reared in the North-Dry Pacific region of Costa Rica, were included in the study. The andrological evaluation of the breeding bulls at the farm was always preceded by taking a detailed identification of the bull (breed, tattoo number, and age), followed by the clinical history, including previous illness history and mating system (single or multiple breeding bulls, with restricted or continuous mating, length of breeding season, number of females per bull, mating behaviour and dominance status). Body condition (BC) was scored 1 to 5, according to the categorization used by the International Livestock Centre for Africa [8]. A score ≥ 3 was considered as normal, while scores < 3 were considered as low body condition. After assessment of body condition and gait, each bull was restrained in a chute for clinical examination. The general clinical inspection included the eyes and the muscle-skeletal system (specially feet and legs conformation). The specific andrological examination included inspection of the prepuce and penis (during semen collection), palpation and inspection of the scrotum and its contents, as well as the internal genitalia by rectal palpation. Scrotum length was defined according to the distance between its distal part and the hock joint as follows: *Short*: scrotum clearly close to the body, >15 cm above the hock joint; *Normal*: <15 cm above the hock down to the level of the hock and, *Long*: below the hock joint. Scrotal circumference (SC) was measured using a standard scrotal-metal tape (Nasco, Wisconsin, USA), at the widest mid-scrotal point. Testicular symmetry (TS) was described at inspection, and testicular consistency (TC) was determined subjectively by palpation and scored 1 to 5. A score of 4 was considered normal, while values below or above were considered as softer or higher consistency, respectively [9]. Palpatory findings in the epididymides as well as in the internal genitalia were also registered. The animals, which all were in good body condition and grazing on natural pastures, were finally classified only with regard to testicular consistency and resilience at palpation (TC).

The animals were slaughtered at a local abbatoir where testes and epididymides were collected immediately after slaughter and quickly examined in search of macroscopic morphological abnormalities. The testis was fixed by vascular perfusion with a 3% solution of glutaraldehyde in 0.067 M cacodylate buffer (pH 7.2; 500 mOsm). The testicular artery was cannulated with a blunt needle, and after clearing the vascular bed with isotonic NaCl solution (0.9 %) the tissues were perfused with the fixative. Small (1-mm thick) tissue samples were excised from the perfused testis at the proximal, medial and distal poles and stored at 4 C prior to their analysis. Spermatozoa were pipetted out from the cauda epididymides and, together with pieces of the caudal duct, immersion-fixed in the same fixative. Thereafter, the specimens were rinsed in cacodylate buffer at 4 C, dehydrated by exposure to graded concentrations of ethanol and propylene oxide and embedded in either metacrylate (Historesin®, LKB, Sweden) or, after trimming to form blocks 1-2 mm thick, subjected to a post-treatment with 2% osmium tetroxide in Agar 100^{R} (Agar Aids, Sussex, England) plastic resin. Semithin sections (1–2 μm thick) of metacrylate blocks were cut out with a glass knife on a microtome (Historange®, LKB, Sweden) and stained with hematoxylin and eosin (HE) for light microscopy. Semi-thin sections were also cut out from Agar 100^{R} blocks on a LKB Ultratome^{R} and stained with buffered toluidine blue in order to select the areas from which ultra-thin sections were to be removed for transmission electron microscopy. The ultrathin sections were picked up onto uncoated copper grids, counterstained with uranyl acetate and lead

citrate, and examined in a Philips EM 201 electron microscope at 60-80 kV.

Results

Of the animals examined clinically, three bulls had normal testicular consistency (TC) i.e. controls; while the remaining eight presented a slight to moderate reduction in TC. The scrotal circumference (Mean ± SD) for the controls as well as for the group with reduced TC was 34.4 ± 1.0 and 35.2 ± 1.2 cm, respectively (n.s.). All bulls showed a normal testicular symmetry.

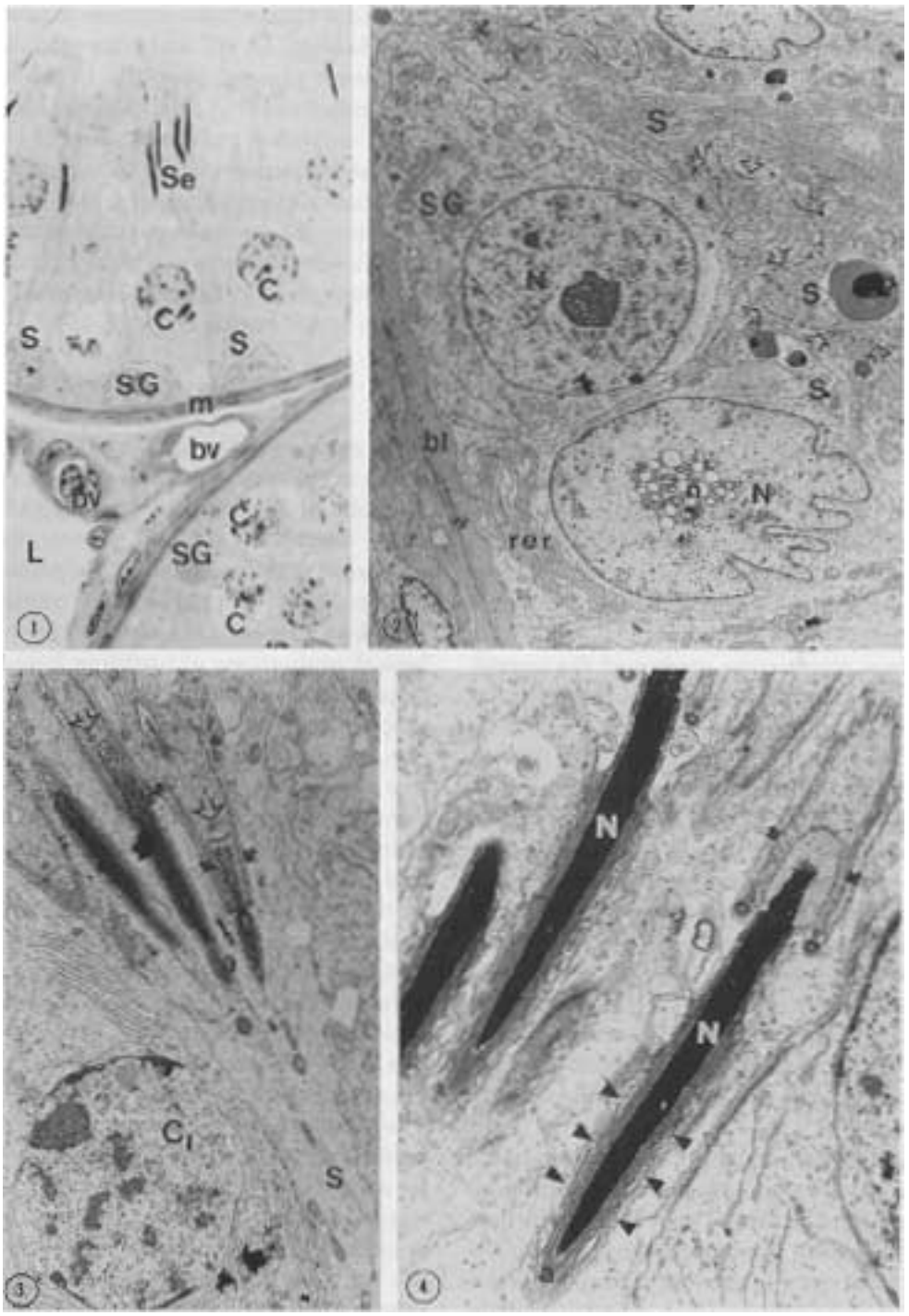
At slaughter, all testes and epididymes appeared macroscopically normal. Light microscopy revealed that testes classified as having normal TC at palpation (controls, n=3) also had a typical histology, with normal spermatogenesis, whereas among those with a mild to moderate reduction in TC (n=8) three showed normal histology and five showed diffuse tubular alterations.

The electron microscopy confirmed the overall histology of the samples. The ultrastructure of the testes and cauda epididymides of Zebu bulls with normal TC (controls) is depicted in Figs. 1 to 8. The ultrastructure of the testes was typical with spermatogenesis under way in the tubuli (Figs. 1–4). Degenerative changes were very uncommon and restricted to the late stages of spermiogenesis (data not shown). The Sertoli cells had lipid inclusions of regular contour and profuse cytoplasmic processes that occupied the spaces among the various types of germ cells (Figs. 1–4). The cauda epididymides of the control Zebu bulls (normal TC) as well as the spermatozoa collected from the caudae appeared to have a normal morphology (Figs. 5 to 8).

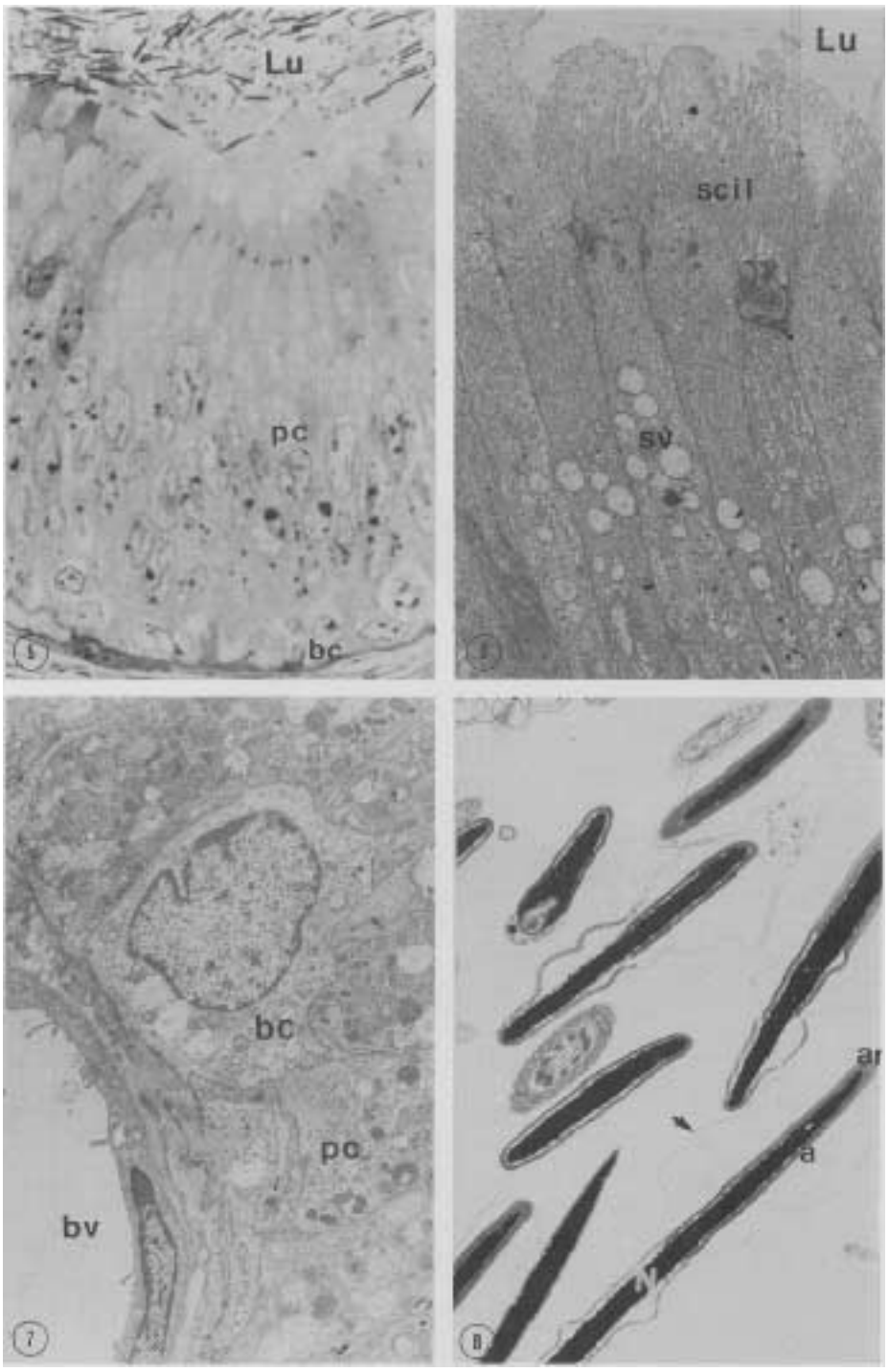
In the animals with a reduced TC, degenerative changes were evident among the tubuli, although they were of minor nature. These changes included an increased view of intercellular spaces at the level of the basal compartment in the seminiferous epithelium in some tubules (Figs. 9 and 10), while in others degenerated primary spermatocytes and spermatids were observed (Figs. 11 and 12). Furthermore, the condensation of the chromatin in the elongating spermatid was disturbed in these animals, which also showed acrosomal defects and increased amounts of cellular debris in the cytoplasm of the Sertoli cells (Figs. 11–13). Also, in these bulls, the cauda epididymides epithelia showed a conspicuous increase in phagosome-like granula (Figs. 15, 16), and intra-epithelial macrophages were present, specially in the basal area of the epithelium (Fig. 16). Although most spermatozoa collected from this ductus segment had normal ultrastructure (Fig. 17), quite a few showed acrosome abnormalities, of which some could also be seen in the seminiferous epithelium, such as the crest defect [10] shown in Figs. 12 and 14. In addition, foreign cells (leukocytes) could be seen in the lumen of the ductus (Figs. 18, 19).

Discussion

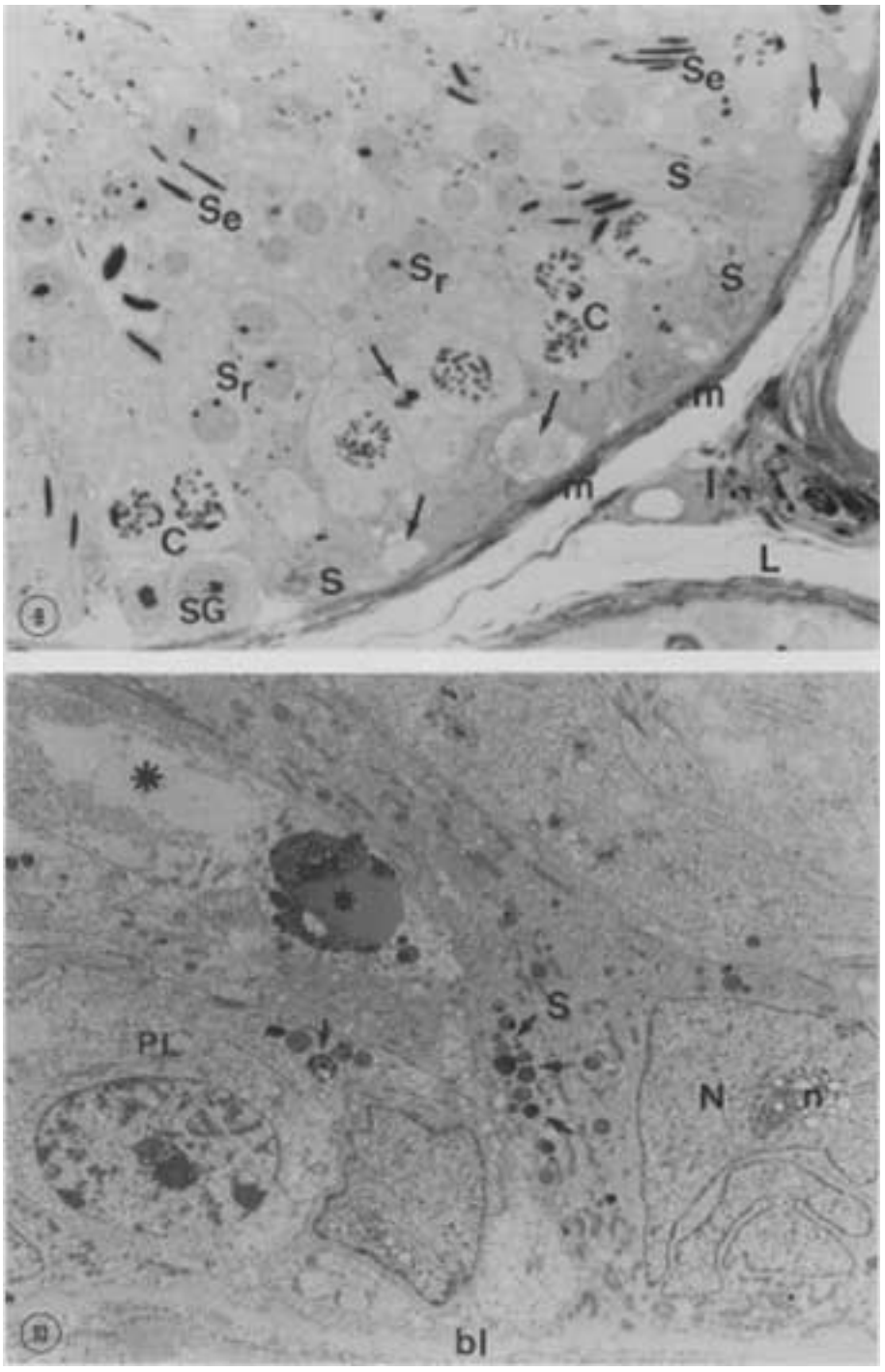
In the present study, minor alterations were found in the seminiferous and cauda epididymides epithelia of Zebu bulls with a slightly to moderately decreased TC at clinical examination, compared with control bulls. Palpation of the scrotal contents is part of the regular clinical examination made in connection with field andrological evaluations of breeding breeding bulls in tropical Costa Rica. Bulls with a decreased TC are suspected of having a diminished testicular function and are usually considered unfit for breeding. In a previous study, a significant negative relation between decreased TC and the prevalence of abnormal sperm heads in the ejaculate of Zebu bulls and crosses was established [5]. This fact suggested that that testicular consistency could be related to the structural integrity of the seminiferous epithelium. The present results confirm these findings, although the intensity of the clinical and morphological findings was minor. A major drawback of this study was the lack of information concerning the status of the breeding bulls spermiogrammes. The ultrastructural changes in the seminiferous epithelium suggest that spermatogenesis was mildly affected [7] as seen in cases of testicular degeneration [1, 6, 7], with degenerated primary spermatocytes and abnormalities in spermiogenesis. In addition, the presence of numerous intraepithelial spaces at the level where spermatocytes are usually located indicated that the these spermatogenetic stages either died and were then phagocytosed by Sertoli cells or escaped into the tubular lumen [11, 12]. The presence of cellular debris in the phago-lysosomes of the Sertoli cells confirmed that some of the germ cells died. Such changes have been reported in bulls with impaired spermatogenesis [12–14].



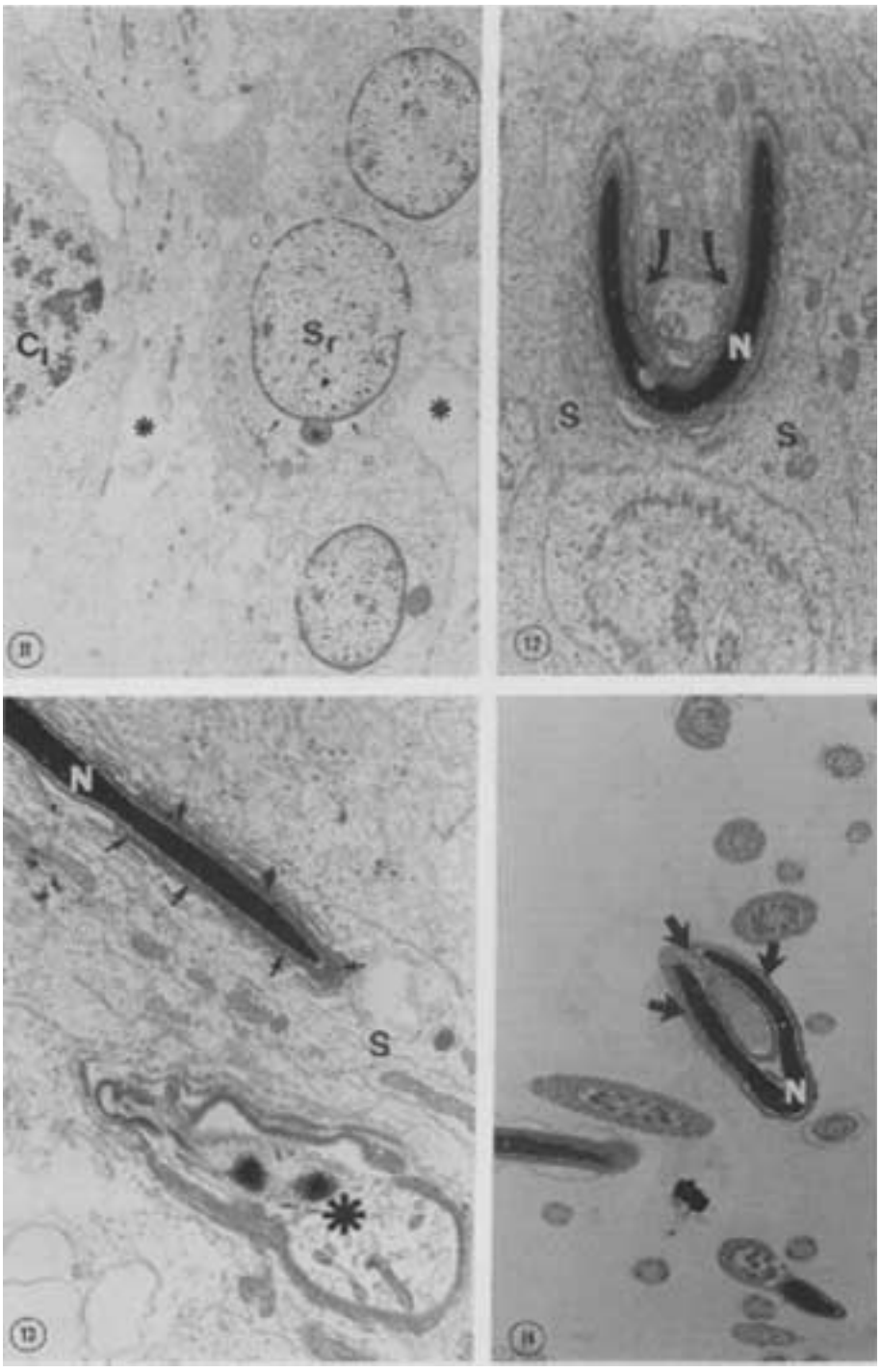
Figs. 1 to 4. Morphology of the testis of Zebu bulls with normal consistency (TC) at clinical palpation (controls). Fig. 1 (HE, 300 ×) presents a partial view of seminiferous tubules and the testicular interstitium (bv: blood vessels; L: lymphatic) depicting the main cell types present (S: Sertoli cells, SG: spermatogonia, C: spermatocytes, Se: elongated spermatids, m: myoid cells). The transmission electron micrograph in Fig. 2 (2800 ×) shows tubular cells apposed to the basal lamina (bl), i.e. a Sertoli cell (S) and spermatogonia (SG) with their typical fine structure. Note the junction complexes forming a part of the hemo-testicular barrier (open arrows), N: nucleus, n: nucleolus, rer: rough endoplasmic reticulum. Figs. 3 (2800 ×) and 4 (8000 ×) show the long cytoplasmic processes of the Sertoli cells (S) where spermatids in the acrosomal phase of spermiogenesis are enclosed. The apical cytoplasm of the Sertoli cell contains accumulations of regularly arranged cisterna of endoplasmic reticulum close to the acrosome region (arrowheads) (C1: primary spermatocyte, arrows: manchette, a: acrosome ridge, open arrows: proximal centriole, N: nucleus).



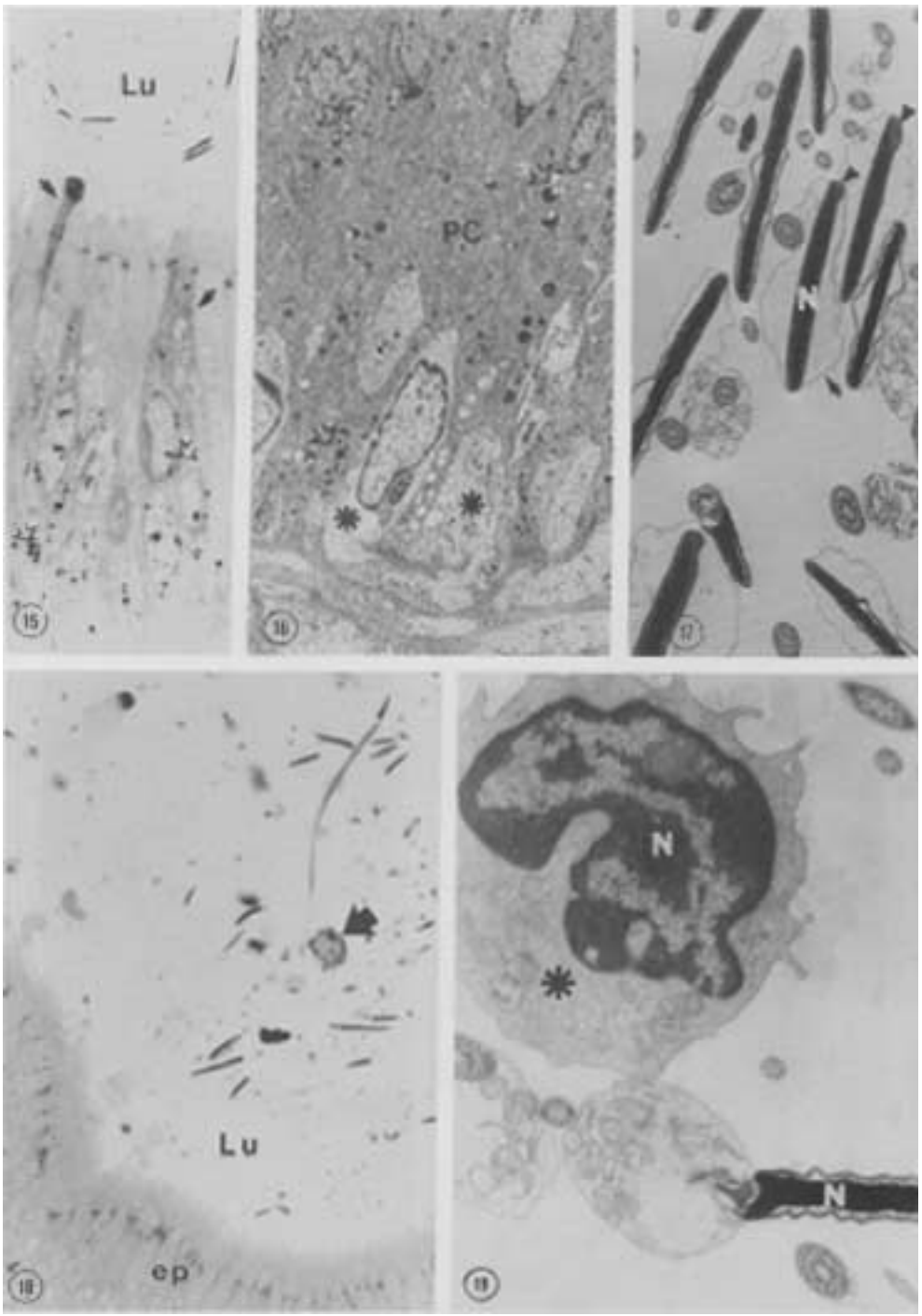
Figs. 5 to 8. Morphology of the cauda epididymides of Zebu bulls with normal TC. Fig. 5 (toluidine blue, 300 ×) shows the general organization of the duct epithelium, with the small basal cells (bc) and the tall principal cells (pc) whose apical region, supplied with numerous stereocilia (scil) and secretory vesicles, is depicted in Fig. 6 (2800 x, Lu: lumen). The basal region of the epithelium is presented in Fig. 7 where both basal and principal cells are apposed to the basal lamina (bv: blood vessel, 4000 ×). The spermatozoa collected from the cauda region are shown in Fig. 8 (8000 ×) (N: nucleus, a: acrosome, ar: acrosome ridge, arrow: distended plasma membrane).



Figs. 9 to 10. Testes of Zebu bulls with a slightly to moderately decreased TC. Minor degenerative changes, depicted as increased amounts of phagocytic activity in the Sertoli cells (compare with Fig. 10) and degenerated germ cells and hollow areas seen in this group of animals are shown in a transversely cut tubules at both the light (arrows in Fig. 9, toluidine blue, 300 ×) and electron microscopic levels (*in Fig. 10, 2800 ×) among the Sertoli cells (S). (In 9: S, Sertoli cell; SG, spermatogonia; C, spermatocyte; Sr, round spermatids; Se, elongated spermatids; m, myoid cells; I, interstitium; L, lymphatic space) (In 10; small arrows: residual bodies, star: lipid contained in a phagolysosome, N: nucleus, n: nucleolus, PL: preleptotene, bl: basal lamina).



Figs. 11 to 14. Testes of Zebu bulls with a slightly to moderately decreased TC. In Fig. 11 (2800 ×) a group of spermatids (Sr) in late cap phase (arrows, the small asterisk is on the acrosome granule) stage is seen close to a primary spermatocyte (C1); note the intercellular spaces (asterisks). Figs. 12 and 13 (8000 ×) show elongated spermatids with their condensed nuclei enclosed in the Sertoli cell cytoplasm (S: Sertoli cell, N: spermatid nucleus, arrows in 13: acrosome, large asterisk in 13: phagosome). Note that the abnormal spermatid in Fig. 12 is also seen in the epididymal cauda contents (Fig. 14, crest defect), the arrows show the invagination of the nucleus and the misplacement of the acrosome.



Figs. 15 to 19. The cauda epididymis of Zebu bulls with a slightly to moderately decreased consistency at palpation. Fig. 15 (HE, 100 ×) presents an apical view of the ductus epithelium showing intact and electron-dense principal cells (filled arrows); note the perinuclear vesicles (open arrows). The basal area is shown in Fig. 16 (1800 ×) shows the presence of vesicles with crescent electron-lucent contents (open arrows) and several pleomorphic cells (macrophage-like) basally located (*). The lumen presented both spermatozoa with normal appearance (17, 6000 ×) and foreign cells (arrow in 18, 100 ×) such as the leukocyte (*) shown in Fig. 19 (N: nucleus, 8000 ×).

The finding that intraepithelial macrophages were more common in cauda epididymides of bulls with reduced TC than in those of normal bulls can probably be explained by the increased spermiophagy along the excurrent ducts. Such a process has been reported in bulls with impaired spermatogenesis [13] where the number of abnormal cells increased above a threshold. However, this finding has been also observed in breeding bulls with normal TC, in which spermiophagy was more conspicuous in the tubuli recta and rete testis than in the corpus and cauda epididymidis [12, 13, 15].

The lack of degenerative changes at the spermatogonial level in bulls with reduced TC corresponds with results indicating that primary spermatocytes and spermatids were the main type of cells affected in this pathology, in contrast to the spermatogonia which are more resistant to these changes in the bull [5, 12, 16–18].

Although changes in TC seemed to be related to the degree of alteration in the testicular parenchyma, as previously determined [5], some of the bulls in this study with slightly reduced TC had a normal histology in most respects. This is not surprising since testicular palpation is a subjective method for determining TC, and variations in scrotum thickness or edema can confound the findings. Further, some of the minor alterations seen might have been present in some individuals without significantly affecting this clinical parameter and would therefore not have been detected, even when using tonometry [1]. Therefore, TC should be considered an important component of the andrological evaluation of breeding bulls, although it should not be used as the only selection criterion.

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