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Post-Natal Changes In The Morphology Of Sertoli Cells In The Scrotal And Abdominal Testes Of Unilaterally Cryptorchid Goats.

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ABSTRACT

Sertoli cells of scrotal and intra-abdominal testes from 25 unilaterally cryptorchid West African dwarf goats between the ages of 1-30 months were examined by light and electron microscopy. In the scrotal testes, the major developmental changes included differentiation of Sertoli-to-Sertoli cell junctional specializations, alteration of cell shape due to proliferation of the spermatogenic cells, development of nucleolar vesicles, convolution of nuclear profiles and the profusion of SER. In the intra-abdominal testes, the first alteration was noted in the Sertoli cells of 3-4-month-old goats, which contained dense masses shown ultrastructurally to be clumped SER with narrow cisternae. In 6-8-month-old animals, further changes included atrophy of Golgi complex, dilatation of cisternae of RER, accumulation of lipid droplets and lipofuscin granules. Many of these organelles and inclusions were no longer obvious in the Sertoli cells of 12-15-month-old goats; rather intracellular vacuoles and dilated intercellular spaces had become common. In the 24-30-month-old goats, the Sertoli cells contained mostly microfilaments and basally located mitochondria with circular cristae in dense matrices. The alterations in the architecture of Sertoli cells in the intra-abdominal testes imply Sertoli cell dysfunction. This, in turn, would contribute largely to the failure of spermatogenesis that characterizes cryptorchidism.

Key words: Sertoli cell morphology. unilateral cryptorchidism.testis. goats.

The multi-functional Sertoli cell has been recognized as the central regulatory unit of the seminiferous epithelium (de Kretser & Kerr, 1988; Bardin et al., 1988). Their specialized tight junctions constitute the blood/testis barrier that creates the microenvironment of the intra-tubular compartment in which the germ cells undergo specific developmental changes. Setchell & Waites (1975) have demonstrated that the composition of the fluid in this special compartment varies from that of blood plasma and lymph, a variation most probably mediated by Sertoli cells. Sertoli cells are the target cells of FSH and testosterone (Grootogoed et al., 1977; Fritz, 1978), hormones that have been shown by Steinberger (1971) and Fritz (1978) to be responsible for initiation and maintenance of spermatogenesis.

In cryptorchidism, Sertoli cell function is impaired and spermatogenesis fails (Bergh and Damber, 1984; Kerr et al., 1979; Ezeasor and Singh, 1989; Ezeasor and Singh, 1987; Ezeasor, 1985). The nature of the morphologic alteration accompanying this impairment has not been elucidated. Related morphologic data from domestic animals refer to certain aspects in cryptorchid pigs (Toyama, 1975; Chevalier, 1978) and to light microscopical observations of experimentally induced cryptorchidism in rams and bulls (Blackshaw & Samsoni, 1967; Skinner & Rowson, 1967).

The paucity of literature on the ultrastructure of testis from cryptorchid domestic animals and the availability of naturally cryptorchid animals, prompted the author to investigate the ultrastructure of Sertoli cells in cryptorchid goats. What follows is an account of morphological events in the scrotal and abdominal testes of 1-30 month old unilaterally cryptorchid West African dwarf goats.

MATERIALS AND METHODS

25 healthy West African dwarf goats were obtained from local stock and maintained at the facilities of the Veterinary

Farm, University of Nigeria, Nsukka. The goats were divided into 5 age groups each consisting of five animals aged 1, 3-4, 6-8, 12-15 or 24-30 months. Animals in the last three groups were considered mature.

Animals were euthanized by administration of an overdose of pentobarbitone sodium, their abdominal cavities opened and the testes were fixed by perfusion using a modification of the procedure described by Forssman et al. (1977). The abdominal aorta was ligated cranially below the origin of the left renal artery and caudally at its bifurcation to give origin to the external iliac arteries. Also ligated were the posterior mesenteric artery and dorsal lumbar arteries. This procedure assured exclusive access into the testicular arteries from which both took origin. Perfusion was carried out through a tubing by gravity from a reservoir suspended at the height of about 90cm. The caudal vena cava was incised in the same region for the drainage of the perfusates.

The rinsing solution comprising mammalian Ringer's solution containing 0.4% procaine hydrochloride was first allowed to flow and as soon as both testes blanched, it was stopped and perfusion with the fixative resumed and continued for 30 minutes. The fixative was cold 6.25% glutaraldehyde in 0.1M phosphate buffer, pH 7.3. The hardened testes were diced into 1mm cubes which were fixed for additional 2h by immersion in fixative of the same composition. The cubes were post-fixed in 1% phosphate buffered osmium tetroxide, dehydrated in graded acetone series and embedded in Epon812. One-micrometer-thick sections were cut, stained with 1% toluidine blue in 1% sodium tetraborate, then examined and photographed. Appropriate areas for electron microscopic were selected and ultrathin sections were cut with a diamond knife and stained with uranyl acetate in 70% ethanol and with lead citrate. The sections were examined by use of H-7000 Hitachi electron microscope at 80kV.

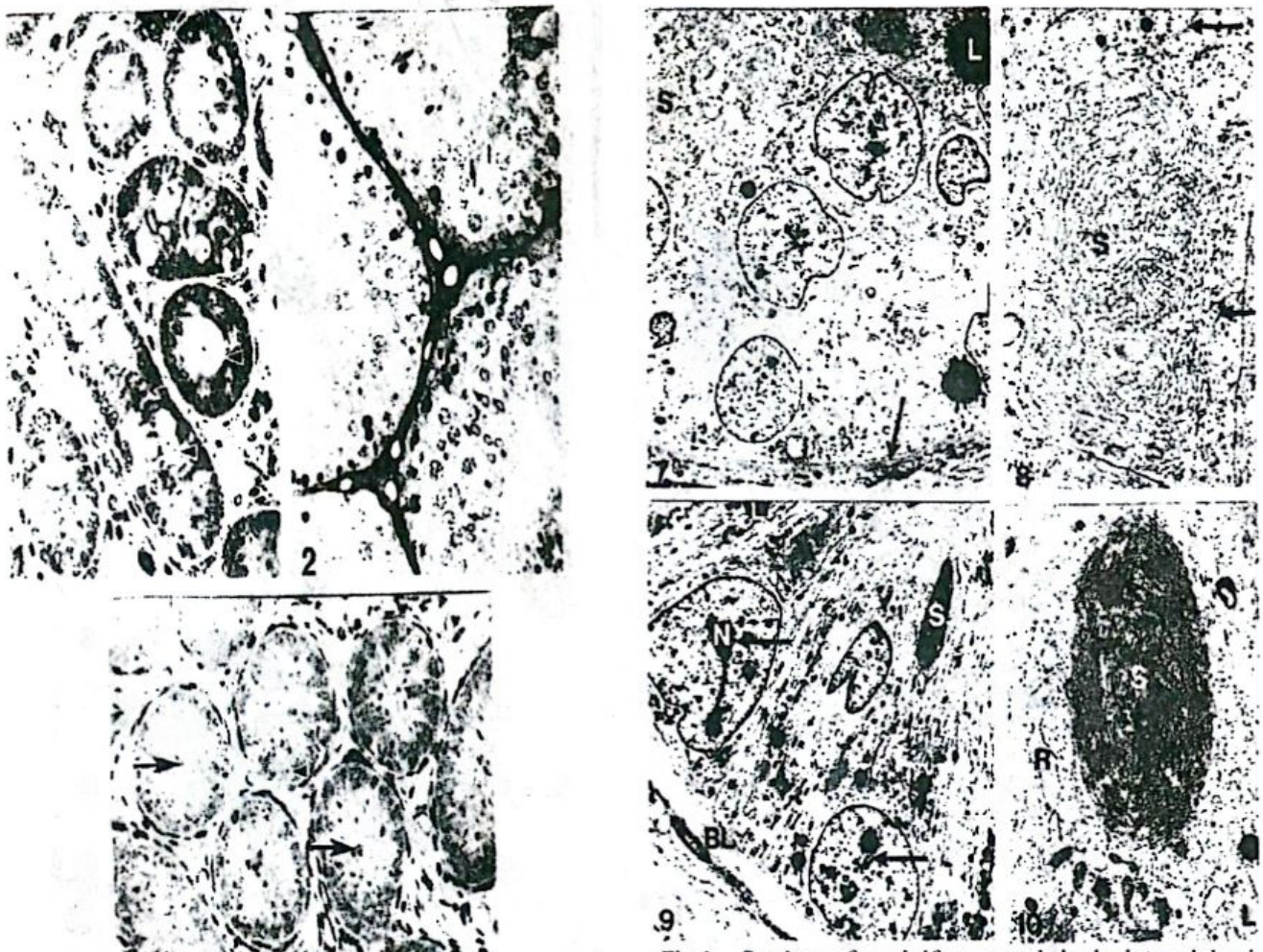
RESULTS

Light Microscopy

Both scrotal and intra-abdominal testes of 1-month-old goats appeared similar histologically. Their epithelium comprised columnar Sertoli cells interspersed with large round pale-staining prespermatogonia (Fig. 1). In 3-month-old goats, Sertoli cells of the scrotal testes were uniformly stained and intermingled with numerous primary spermatocytes; a few cords developed lumina. Thereafter, the seminiferous epithelium in all pubertal and adult goats assumed mature characteristics ie., development of all the diverse germ-cell modifications associated with Sertoli cells (Fig. 2). The basal and supranuclear cytoplasm contained osmiophilic (lipid) droplets. The seminiferous epithelium of the intra-abdominal testes, in contrast, was markedly dissimilar as a result of lack of germinal cells and degeneration of Sertoli cells. In 3-month-old goats, Sertoli cells contained dense-staining elliptical masses in the apical cytoplasm and a few spermatogonia were observed at the base of the epithelium (Fig. 3). The masses were intermingled with osmiophilic bodies of various sizes in the 6-8 month-old-goats. The Sertoli cells of 12-15 month-old goats contained many clear vacuoles and a few osmiophilic bodies (Fig. 4). In 24-30 month-old bucks, the seminiferous tubules varied widely in their overall and luminal diameters and comprised only pale-staining Sertoli cells of varying heights (Fig. 5).

Electron Microscopy

The columnar configuration of the immature Sertoli cells was apparent in the scrotal and intra-abdominal testes of 1-month-old goats (Fig.6). The smooth contoured nuclei were mostly oval and basally located. The apical cytoplasm had extensive arrays of rough endoplasmic reticulum (RER), well-developed Golgi complexes and mitochondria. Several alterations were apparent in both testis types in 3-4 month-old goats. All nuclei developed indented outlines, and their nucleolonemas became associated with vesicles. Profiles of smooth endoplasmic reticulum (SER) became apparent in the cytoplasm but in the scrotal testis, they occupied large areas and comprised wide cisternae (Fig. 7, 8). In the intra-abdominal testis, the dark cytoplasmic masses seen in light microscopy proved to be discrete masses of SER with narrow, whorled, closely packed cisternae (Fig. 9, 10). Closely associated with these were cisternae of RER that were narrow and regular in scrotal testis but wide, irregular



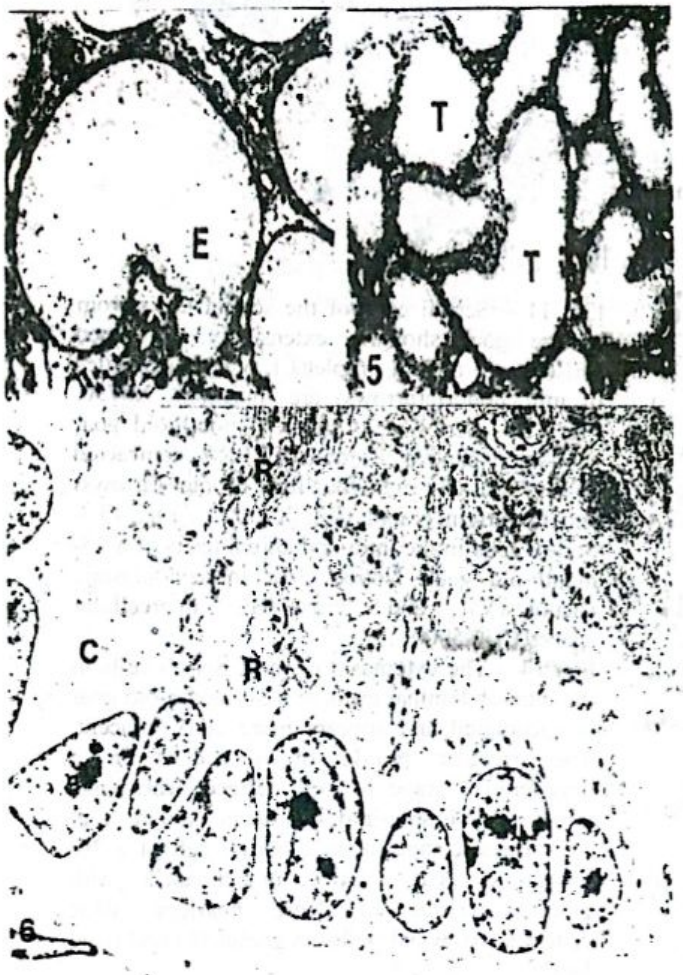
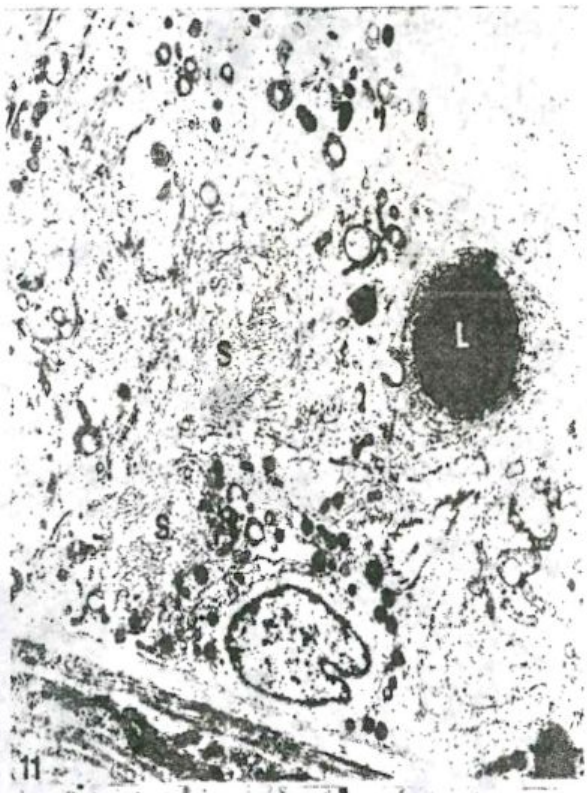
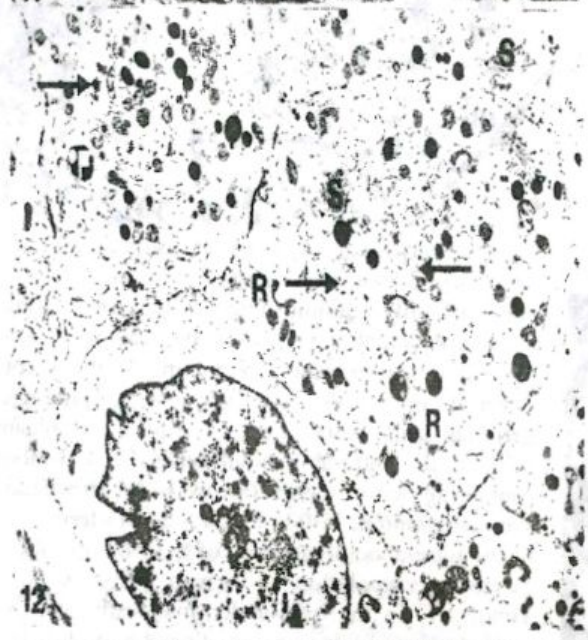
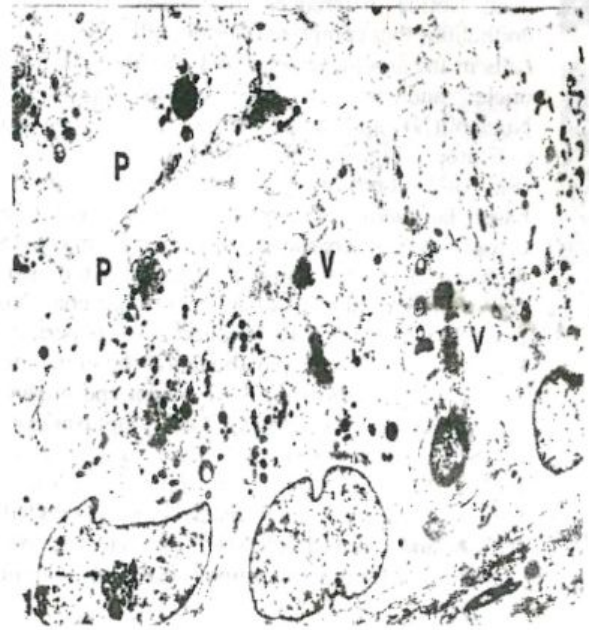


Fig.1 – Sections of seminiferous cords in the intra-abdominal testis of a 1-month-old goat. Sertoli cells have basal nuclei and uniformly staining apical cytoplasm among which are many pale-staining prespermatogonia (arrowhead) X 300. Fig. 2 – Seminiferous epithelium in the scrotal testis of pubertal goats. Observe various morphologic types of germinal cells. X300. Fig. 3 – Apical cytoplasm of Sertoli cells in the intra-abdominal testis of a 3-month-old goat containing dense masses (arrows). An rrowhead indicates a spermatogonium. X300. Fig. 4 – Seminiferous epithelium(E) in the intra-abdominal testis of a 15-month-old goat. Notice extensive vacuolation of Sertoli cells. X300. Fig. 5 – Tubules(T) in the intra-abdominal testis of a 30-month-old goat. Notice differences in diameter and height of lining epithelium that comprises Sertoli cells. X300. Fig. 6- Sertoli cells in the scrotal testis of a 1-month-old goat. Notice basal nuclei and profuse RER in the apical cytoplasm(R). Nucleoli(N) and a portion of a germinal cell(C) are indicated. Fig. 7 – Seminiferous epithelium in the scrotal testis of 3-month-old goat characterized by an intact and thin basal lamina(arrow), nuclei with irregular outlines and cytoplasm that contains profuse SER masses(S) and lipid droplets(L). X3000. Fig. 8 – Apical cytoplasm of a Sertoli cell of the scrotal testis of a 3-month-old goat. Notice an SER mass(S) with normal RER cisternae at its periphery. X12000. Fig. 9 – Sertoli cells in the intra-abdominal testis of a 3-month-old goat showing basal nuclei and nucleoli associated with small vesicles(arrows). The cytoplasm contains masses of SER cisternae(S) and lipid droplets(L). The basal lamina(BL) is split into many thin layers. X4000. Fig. 10 – Clumped masses of SER cisternae(S), expanded cisternae of RER® and lipid droplets(L) characterize the Sertoli cell cytoplasm of intra-abdominal testes of 3-month-old goats. X12000.







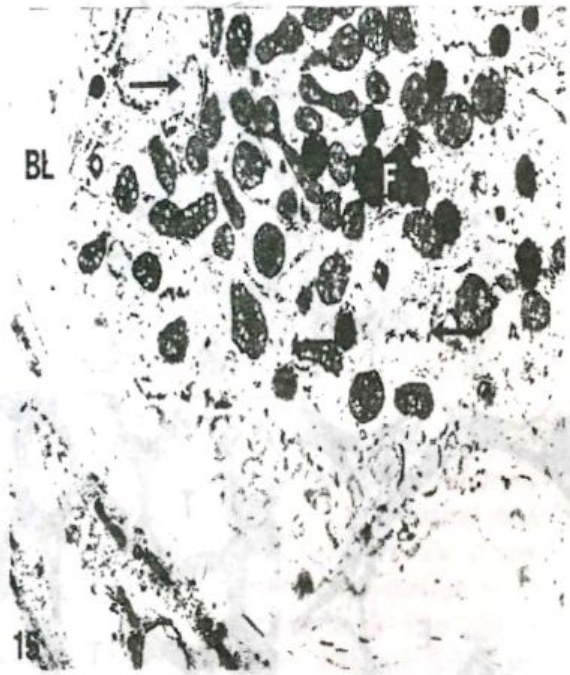


Fig. 11 – Sertoli cells of the scrotal testis from mature goat showing extensively developed SER(S) and a lipid droplet(L). Varying profiles of mitochondria(arrows) are indicated. X8750. Fig. 12 – Sertoli cells of an 8-month-old goat showing expanded cisternae of RER®, contracted SER masses (S), numerous lipid droplets(arrows) and lipofuscin granules(F). X7000. Fig. 13 – Sertoli cells in the intra-abdominal testis of a 15-month-old goat. Notice clear intracytoplasmic vacuoles(V) and expanded intercellular spaces(P). X5000

Fig. 14 – The cytoplasm of most Sertoli cells in the intra-abdominal testis of a 30-month-old goat is vacuolated and appears more electronlucent. The irregular profile of a cell(A) in a degenerative stage is well defined. The basal lamina (BL) is split into thin layers. X5000. Fig. 15 – Basal cytoplasm in a Sertoli cell of a 30-month-old goat. Notice mitochondria with globular cristae in dense matrices, RER cisternae(arrows), lipofuscin granule(F) and basal lamina(BL) X20,000.

and contained pale flocculent material in the intra-abdominal testes. Lipid droplets were also evident in Sertoli cells of both testis types, but the globules were smaller and more abundant in the intra-abdominal testes.

At this stage, the typical Sertoli-to-Sertoli cell junctional specializations were observed at the lateral plasmalemma. The basal lamina in the intra-abdominal testes was split into supernumerary thin layers while it was thinner and intact in the scrotal testes.

In the pubertal and older goats, alterations in the Sertoli cells of the scrotal testes included:

(1) a change from columnar to irregular configuration to accommodate the proliferating germinal cells;
(2) increased number and size of the vacuolar components of the nucleoli;
(3) abundance of microtubules and microfilaments that often aggregated into bundles in the apical cytoplasm;
(4) an augmented SER and
(5) increased complexity of the Sertoli-to-Sertoil cell junctional specializations. Lipid droplets were observed as large globules associated with SER profiles (Fig. 11).

In sharp contrast, Sertoli cells of the contralateral intra-abdominal testis of corresponding age groups had evidence of further degeneration. In 6-8-month-old goats, Sertoli cell cytoplasm presented irregular instead of elongated RER profiles with an increase in the number of lipid droplets and lipofuscin granules (Fig. 12). In 12-15 month-old animals, the cells depicted focal intercellular expansions, irregular nuclear profiles and vacuolated cytoplasm. There was progressive shrinking of SER masses (Fig. 13). In 24-30 month-old goats, Sertoli cells were in advanced stage of degeneration (Fig. 14, 15). The cell outline was extremely irregular; the cytoplasm had numerous vacuoles of varying size and paucity of organelles. The Golgi complexes were inconspicuous in the cells of all age groups examined. Sertoli-Sertoli cell junctions in most instances remained intact.

DISCUSSION

This study compared the sequence of structural events undergone by Sertoli cells in the scrotal and intra-abdominal testes of unilaterally cryptorchid West African dwarf goats, between the ages of 1 and 30 months. The results indicated that up to the age of 1 month, the morphologic features of Sertoli cells in both testis types appeared similar and conformed generally to the features described for the normal postnatal development of the mammalian testes (Ramos & Dym, 1979; Hatier & Grignon, 1980; van Vorstentbosch et al, 1984).

From the 3rd month onward, nuclear and cytoplasmic features typical of normal mature Sertoli cells differentiated progressively in the scrotal testis, but Sertoli cells of the contralateral intra-abdominal testis degenerated, perhaps because of sensitivity of the cytoplasmic organelles to the unfavourable intra-abdominal environment.

The altered structure of RER and the atrophy of the Golgi apparatus that were evident in the intra-abdominal testis of 6-8 month-old goats constitute likely correlates of disruption in the secretory process. The involvement of RER in the elaboration of androgen-binding protein and inhibin has been reported (Tran & Josso, 1982). Thus this finding may explain the impairment in the secretion of these products reported consistently in experimentally induced cryptorchidism (Hagenas & Titzen 1976; Kerr et al, 1979).

The SER proliferated initially but progressively shriveled so that in goats between the ages of 24 and 30 months, only traces of these structures were visible. This observation represents a process of atrophy leading to altered metabolism that, in turn, might result in spermatogenic failure characteristic of cryptorchidism.

The accumulation of lipid droplets in combination with cytoplasmic vacuoles and focal intercellular dilations are well-known features of Sertoli cells in cryptorchidism of natural or experimental origin (Amat et al, 1985; Clegg, 1963). These alterations may

represent a non-specific reaction of the cell to insults.

Sertoli cells and peritubular myoid cells seem to act in partnership to synthesize various components of the extracellular matrix of the basal lamina (Skinner et al, 1985). The splitting of the basal lamina of the seminiferous epithelium reported here may, therefore, be a reflection of the interruption of this process.

The pattern of Sertoli cell degeneration reported here appears to be lacking in human cryptorchidism, in which impaired differentiation and even hyperplasia of the cell have been documented (Amat et al, 1985; Schulze et al, 1976).

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