

Ultrastructural Analysis of Sertoli and Myoid cells in Non-Obstructive Azoospermia Men Admitted to TESE-ICSI

Mojtaba Karimipour, Ph.D.[☆], Ahmad Hosseini, Ph.D.[☆], Mojtaba Rezazadeh Valoojerdi, Ph.D.^{☆ †}, Jalil Hosseini, Ph.D.[☆]

[☆] Anatomy Department, Urmia University & Medical Sciences

[☆] Anatomy Department, Shaheed Beheshti Medical Sciences University

[☆] Embryology Department, Royan Institute

[†] P.O.Box 19395-4644, Royan Institute, Tehran, Iran

Abstract

Introduction: Morphology and ultrastructure of myoid and sertoli cells were studied in patients with non-obstructive azoospermia who were admitted to testicular sperm extraction (TESE) and intracytoplasmic sperm injection (ICSI), by transmission electron microscopy (TEM).

Materials and Methods: 12 biopsies of testicular tissues from patients with non-obstructive azoospermia referred to Royan institute were used as a patient group, another 5 biopsies from person with prostate cancer were used as a control group. The tissues were fixed and processed for TEM, and Sertoli and myoid cells were examined under TEM.

Results: Myoid cells of the patient group had lost their slender shape and orientation in relation to the wall of seminiferous tubules. These cells were separated by increased amount of collagen fibers, furthermore, their polarity was different from normal and the nuclear shape changed. Sertoli cells were round to ovoid in shape, and the nuclei were more regular in shape than that of the normal with peripherally located nucleoli.

Conclusion: Sertoli cells in the patient group were immature or undifferentiated. This may have resulted in other changes in the testicular tissues such as the alteration in myoid cells and basement membrane, moreover, the immaturity of Sertoli cells may be the cause for the failure in initiating or triggering normal process of spermatogenesis.

Key words: Testicular tissue, Electron microscopy, Sertoli cell, Myoid cell





Introduction

Intracytoplasmic sperm injection (ICSI) with fresh spermatozoa obtained by testicular sperm extraction (TESE) is a well established treatment for patients with obstructive azospermia (1, 2, 3). Human seminiferous epithelium consists of somatic cells such as Sertoli and myoid cells; as well as germ cells, which are at different stages of differentiation from early stem cells to spermatozoa (4). Sertoli cells are believed to play a key role in triggering and regulation of spermatogenesis (5). Human Sertoli cells undergo morphological and functional changes from birth to puberty. The most dramatic changes occur at puberty, where the nucleus acquires more irregularity in shape, and the nucleolus changes into a large tripartite structure (6, 7). Ultrastructural studies of testis in different pathological conditions such as Klinefelter's syndrome, cryptorchidism, estrogen treatment and Sertoli-cell-only syndrome have revealed that Sertoli cell morphology differs from the normal mature Sertoli cell (6, 20). Peritubular myoid cell (PMC) is myoepithelial type, which resides within the boundary tissue of seminiferous tubules. It is generally assumed that PMC contraction is responsible for peristaltic-like movements of the seminiferous tubules (8). In vitro studies, have demonstrated cooperation between Sertoli cells and PMC in synthesis of extracellular matrix components of the lamina propria (9, 10). Light microscopic studies of the testis in men with oligospermia or azospermia have shown that the peritubular tissue is disorganized either by excessive collagen accumulation or by clumps of eosinophilic materials (11).

The aim of this study was to investigate the changes in PMC and Sertoli cells in men with obstructive azospermia using TEM, which was compared with ultrastructure of testis of normal individuals using qualitative features.

Materials and Methods

Testicular biopsies were obtained from 12 patients (aged 24-41 years) with non-obstructive azospermia who were referred to Royan institute. These patients were admitted to TESE to obtain spermatozoa for ICSI.

All patients were thoroughly evaluated by a clinical questionnaire, physical examination and, hormonal and semen analyses. In addition, five testicular biopsies from men with prostatic cancer, who had underwent orchidectomy, were used as controls. After fixation in 5.5% glutaraldehyde solution (in 0.1 M phosphate buffer, pH 7.2) for 2-4 hours at 4°C the tissues were rinsed several times in phosphate buffer, then post-fixed in 1% osmium tetroxide for 3 hours. Dehydration was performed in ethanol at room temperature and the samples were embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate, and then examined and photographed with an electron microscope (Zeiss EM 900) at 80 kv.

Results

The lamina propria in normal samples consisted of three to four thin laminae which lie parallel to each other and are arranged concentrically around the tubular wall, contained myoid cells with spindle-shaped nuclei and filled with dark heterochromatin (Fig 1).

The extracellular space contains few collagen fibers. Also, the seminiferous tubule showed normal spermatogenesis. In patients with non-obstructive azospermia, the lamina propria distended and thickened, the laminae were separate from each other. The extracellular space between the basal lamina of the tubule and the subjacent lamina of myoid cells was largely broader than normal and was filled with an irregular meshwork of collagen fibers. In most cases, the myoid cells lost their slender shapes, and orientation in relation to the wall of the tubule (Fig 2).

They were separated by increased amounts of collagen fibers. Not only the shapes, but also the polarity of the myoid cells was completely different from that of the normal. Their nuclei also strikingly altered, the nuclei deeply indented, and occasionally contained vacuoles. Most of the myoid cells usually had an oval-to-round distended shape (Fig 3), in some cases, their number increased.

The chromatin and was uniformly distributed throughout the nucleus (Fig 4).

The nucleolus was prominent which exhibited a tripartite structure consisted of a round fibrillar center, a compact granular, and a reticular portions (Fig 5).

150



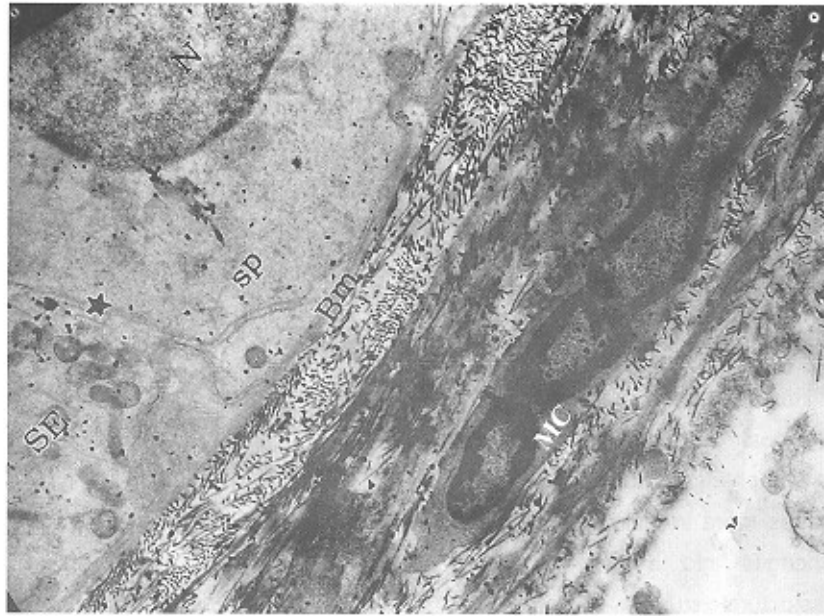


Fig 1: An electron micrograph of a part of normal seminiferous tubule showing a peritubular tissue that contains a myoid cell and a basement membrane. MC: Myoid cell, BM: Basement membrane, SP: Spermatogonia, SE: Sertoli cell (X 4,400).

181

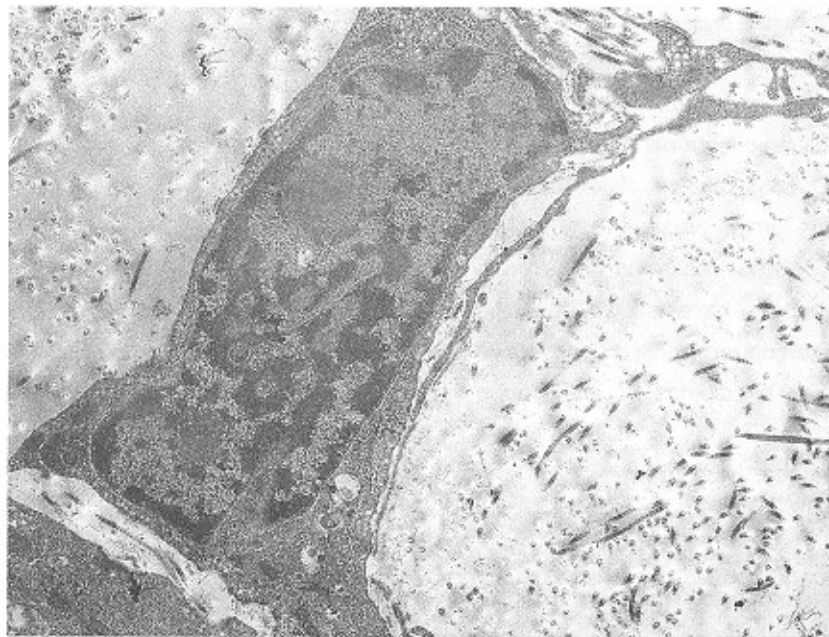


Fig 2: An electron micrograph shows a myoid cell from a patient, the cell has lost slender shape and orientation, while the nucleus is deeply indented (X 5,000).

Sertoli cells in the testes of men with non-obstructive azoospermia, had round to ovoid regularly outlined nucleus, with homogeneously dark or

pale stained chromatin. Each nucleus had peripheral nucleolus which did not show the tripartite structure (Fig 6).

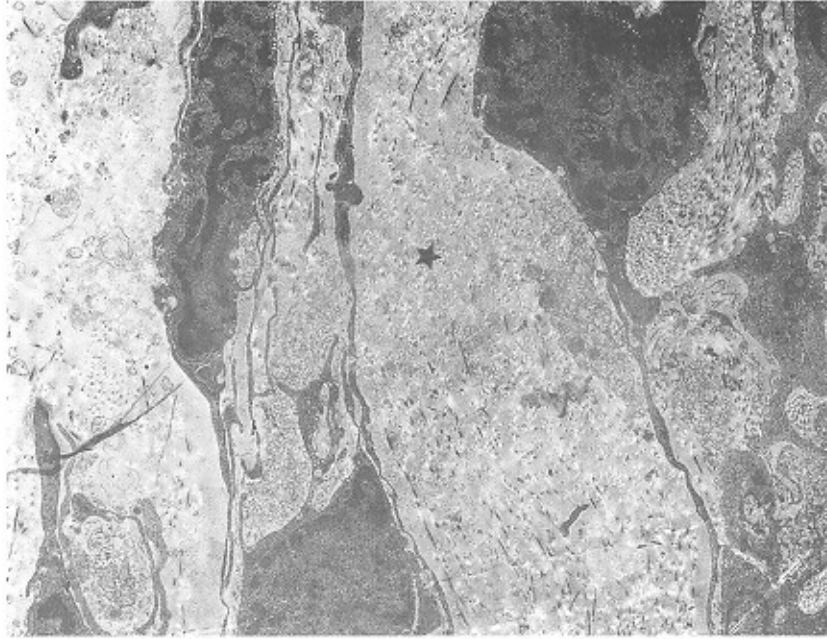


Fig 3: An electron micrograph shows the lamina propria of a seminiferous tubule with a myoid cell showing with an irregularly outline nucleus. The lamina is distended and thickened. The number of myoid cells increased and the polarity of the cells is completely different from the normal condition (X 4,000).

182

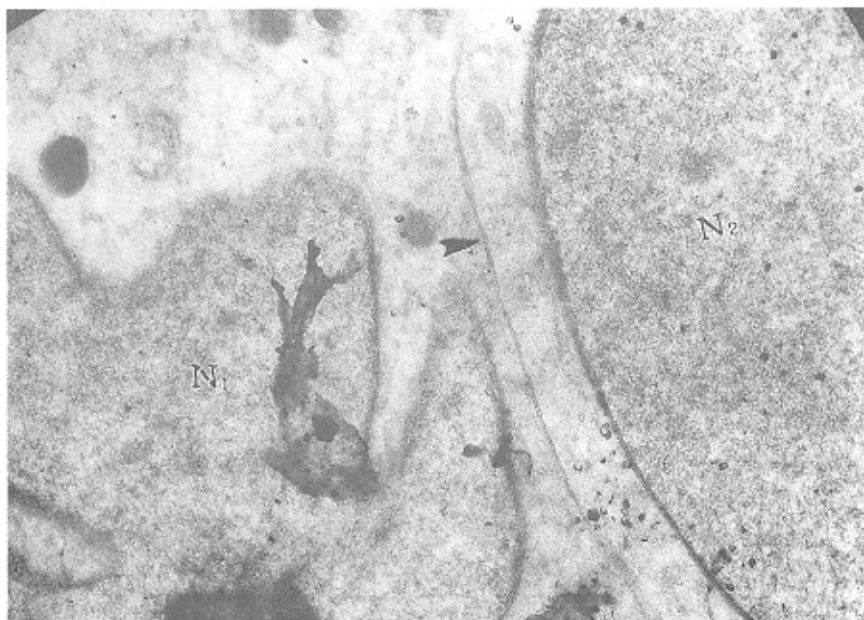


Fig 4: An electron micrograph shows a nucleus in normal and mature Sertoli cell which shows irregular and deep folds. The chromatin appears uniform and distributed throughout the nucleus. The junction between a Sertoli cell with a spermatogonia is also seen N1: nucleus of Sertoli cell, N2: nucleus of spermatogonia (X 4,400).

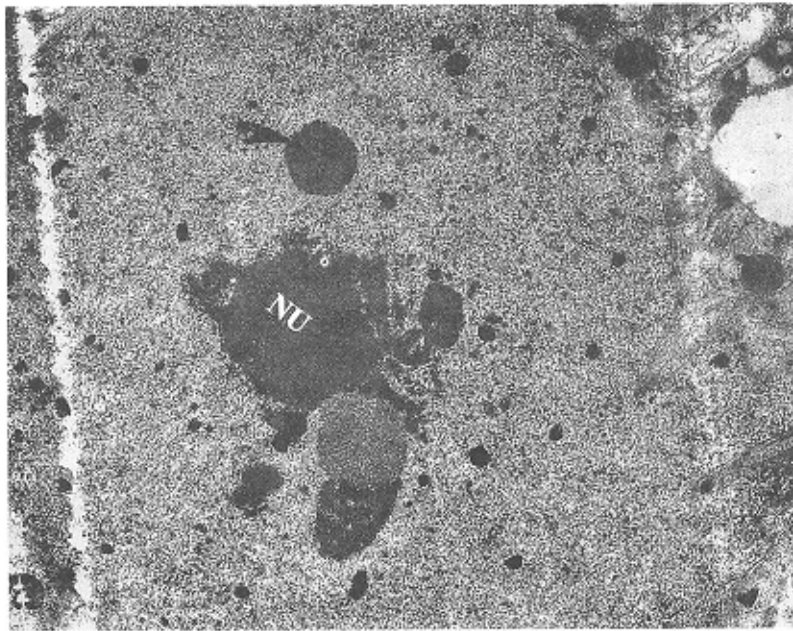


Fig 5: An electron micrograph shows a nucleolus of a normal Sertoli cell that exhibits a tripartite structure (X 5,000).

183

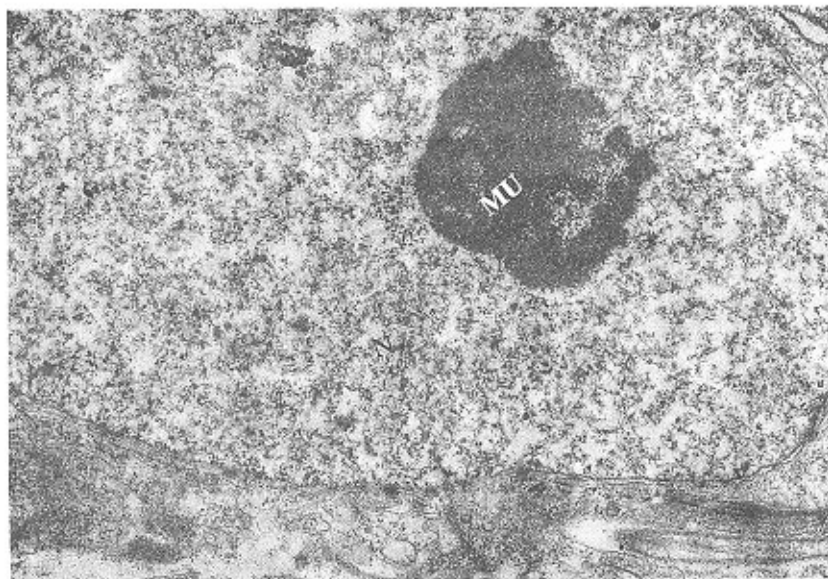


Fig 6: An electron micrograph shows a nucleus and nucleolus of an immature Sertoli cell in a patient with non-obstructive azoospermia. The cell shows a round to ovoid regularly outline nucleus with homogeneously dark stained chromatin. The nucleolus is peripherally located and does not display the tripartite structure (X 10,000).

Discussion

This preliminary study investigated the ultrastructure of Sertoli and myoid cell in limited number of testicular biopsies of non-obstructive azoospermia patients who admitted to TESE and ICSI. The cooperation between

the peritubular myoid and Sertoli cells has been demonstrated by many investigators (12, 13). There are reports emphasizing the paracrine regulation of Sertoli cells by peritubular myoid cells (12, 14). This



suggests that the myoid cell alteration observed here, might be involved in alterations in Sertoli cell function, which resulted in seminiferous tubule lesions. On the other hand, Sertoli cells modulate several morphological and functional aspects of myoid cells, for example, some investigators reported the role of Sertoli cells in myoid cell differentiation using in vitro techniques (14). Tong and Fritz experiments showed that when peritubular myoid cells were cultured together with Sertoli cells, they developed their characteristic myoid pattern, whereas if these cells are cultured without Sertoli cells, they would differentiate into fibroblasts (8, 15). We have observed alterations in myoid cells that were resemble a fibroblast. Furthermore, in another study (unpublished) using immunohistochemistry for vimentin, we observed that immunostaining with anti-vimentin antibodies was more intense in samples from patients than that of the controls, which was consistent with electron microscopy findings. These results suggested differentiation of peritubular myoid cells into fibroblastic characteristics. Furthermore, the myoid cell alterations reported here might be caused by Sertoli cells alterations. In any case, the alteration in both cell types seemed to be associated, although it is difficult to determine which one causes the changes in the other (16). Our ultrastructural study also revealed that the morphological alterations in myoid cells nucleus might be related to the development of vimentin filaments, because these filaments were possibly involved in determining the nuclear shape and the location (16).

In some cases, we observed that the number of myoid cells. The increased in myoid cells during testicular development of rat was documented, they form a continuous and quiescent monolayer surrounding the seminiferous tubule wall, and manifest low rate of mitosis in situ (8). On the other hand, in monoculture of a medium containing serum, however, myoid cells had high rate of proliferations, and cells having proliferative phenotype which is were characterized by rapid growth and accompanied by

increase in the production of extracellular matrix, while myoid cells in direct contact with Sertoli cells in co-culture had a low mitotic rate, and they retain their characteristic contractile phenotype. The mechanisms regulate the phenotype of myoid cells are virtually unknown (8). However, the morphological, functional and biochemical characteristics of myoid cells are influenced greatly by interactions with neighboring Sertoli cells in co-culture (8, 17). The change in myoid cells phenotype in the present study might be due to Sertoli cells alteration that was not able to modulate the myoid cells. One characteristic of myoid cells, in our study, was lack of polarity, which led to the malfunction of myoid cells peristaltic movement of seminiferous tubules. This is necessary for propelling the tubules content (18). The Sertoli cells were morphologically undifferentiated with relatively smooth round nucleus laid adjacent to the basal lamina, and the nucleoli were small and located peripherally.

These features are not only typical of Sertoli cells in hypoplastic zones and after estrogen therapy (19), but also of Sertoli cells before puberty. Moreover, Sertoli cells in cases of hypogonadotropic hypogonadism, testicular feminization and cryptorchid evidently also exhibit these characteristics (5, 9, 20, 21).

Lack of normal spermatogenesis in patients with non-obstructive azoospermia is characterized by other testicular tissue alterations; such as myoid cells, basement membrane and extracellular matrix. This can be explained on the basis of association of these cells with a population of immature Sertoli cells which are unable to initiate or trigger the normal process of spermatogonial proliferation and differentiation.

Acknowledgments

This study was performed in the Department of Anatomy, Shaheed Beheshti university of Medical Sciences and was supported financially by Royan institute, Tehran, Iran. We greatly appreciate Dr J. Hosseini and Dr MH. Haidiri for their helps.

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