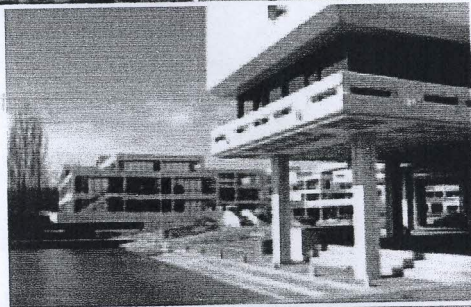


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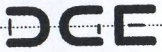
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The Morphological Evaluation in Azoospermia Cases of Testicular Tubular Tight Junctions

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Testis possesses intrinsic mechanisms to modulate and regulate different stages of germ cell development under the intimate and indirect cooperation with Sertoli and Leydig cells [1]. In the seminiferous tubules the epithelium is physically divided into the adluminal compartment by the blood-testis barrier (BTB) which is constituted by adjacent Sertoli cells near the basement membrane. BTB is the crucial role to spermatogenesis. Spermatogenesis is a highly complicated process in which functional spermatozoa are generated from primitive mitotic spermatogonia. A compromise in this process can lead to infertility [2]. Tight junctions (TJs) at the site of the BTB function in the testis are maintained by intricate regulatory mechanisms. Several TJ-related proteins, such as claudins, occludin, junctional adhesion molecules and zonula occludens-1 (ZO-1), -2 and -3 have been identified [3]. Of these, ZO-1 is the main constituents. In this study we aim to compare the ZO-1 in human testicular tissue with azoospermia, either with spermatozoa or without by using immunohistochemistry and electron microscopy.

For this study, we obtained samples from ages between 22-55 year old man presented infertile laboratory. Tissue samples were obtained from azoospermic patients whose semen fluids did not contain spermatozoa. These azoospermic patients were divided into two groups which do have spermatozoa in testicular sperm extraction (TESE) samples (n=20) and which don't have spermatozoa in their TESE samples. The localization of ZO-1 protein was analyzed by immunohistochemistry and these groups were also examined by transmission electron microscopy.

It was observed that ZO-1 expression in blood testis barrier. BTB was normal in localization in spermatozoa-detected group "Figure 1". Sertoli cell-only syndrome we refer to as the spermatozoa-absent group was examined and ZO-1 in the structure of TJs was not only in the basal compartment of the seminiferous epithelium but also observed on the lateral surface covering the adluminal compartment "Figure 1". The ultrastructural results were also supported with light microscopy findings "Figure 3-4".

These findings suggested that disturbance of ZO-1 reaction in Sertoli cells might be one of the factors involved in the impairment of spermatogenesis. Especially spermatozoa absent group we assumed that maturation of Sertoli cells is not completed. Therefore, TJs among Sertoli cells are regarded as having crucial roles in spermatogenesis.

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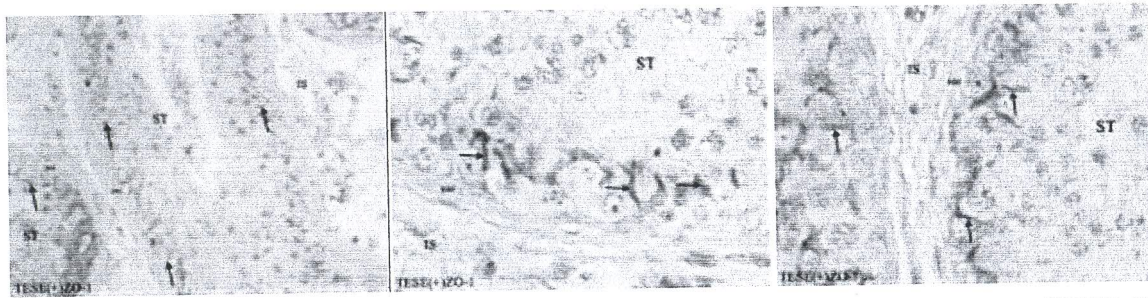


Figure 1. Photomicrographs of azoospermic cases' testicular tissue with spermatozoa detected group [TESE(+)] after testicular sperm extraction. ZO-1 immunostaining (arrows) is present between Sertoli cells (S) in normal localization. The diameter of the seminiferous tubules (ST) were normal and were lined by Sertoli cells and spermatogenic cells. Seminiferous tubules were outlined by basement membrane (BM). In the interstitium (IS) where the Leydig cells appear normal. Magnifications: x40 and x100.

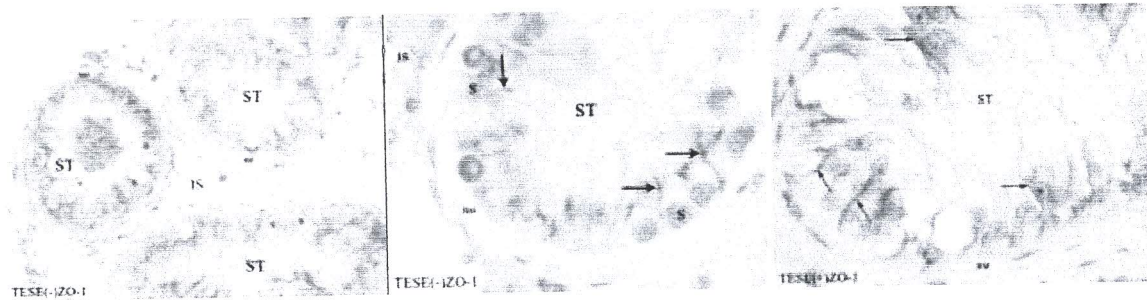


Figure 2. Photomicrographs of azoospermic cases' testicular tissue with spermatozoa absent group [TESE(-)] after testicular sperm extraction. Sertoli cell sendrome was determined in the tubules. ZO-1 immunostaining (arrows) was examined not only in the basal compartment of the seminiferous epithelium (ST) but also observed on the lateral surface covering the adluminal compartment. In the interstitium area (IS) Leydig cells were not seen. Sertoli cells (S) and basement membrane (BM). Magnifications: x40 and x100.

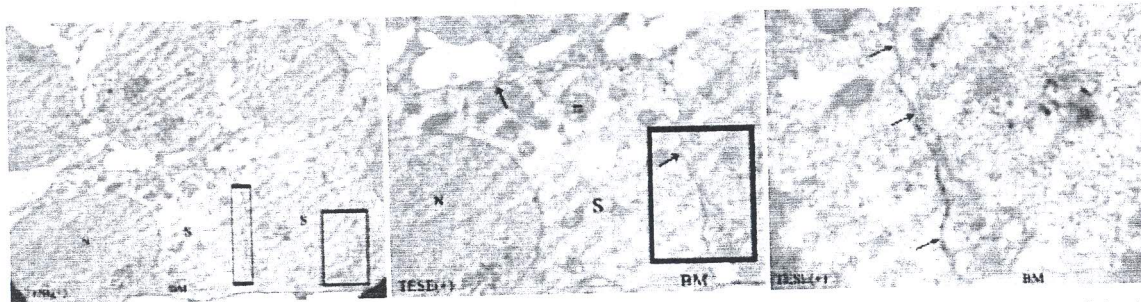


Figure 3. Electron micrographs showing the cross section of a seminiferous tubule from the spermatozoa detected group [TESE(+)]. Tight junctions (arrows, □) were seen between Sertoli cells. Sertoli cells (S), Sertoli cells' nucleus (N), basement membrane (BM) and mitochondria (m). Magnifications: x5000, x7500, x20000.



Figure 4. Electron micrographs showing the cross section of a seminiferous tubule from the spermatozoa absent group [TESE(-)]. Tight junctions (arrows) were seen adluminal compartment. Sertoli cells (S), Sertoli cells' nucleus (N) and mitochondria (m). Magnifications: x5000, x7500, x20000.