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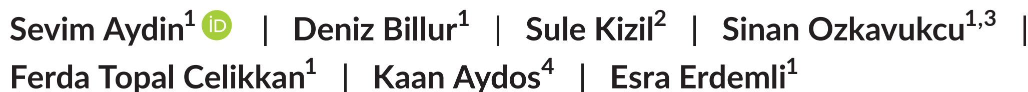
Evaluation of blood–testis barrier integrity in terms of adhesion molecules in nonobstructive azoospermia



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Abstract

Blood–testis barrier (BTB) is critical for maintaining fertility. The integrity of tight junctions (TJs) provides restricted permeability of BTB. The aim of this study was to evaluate the relationship between BTB and Sertoli cells. Testicular sperm extraction (TESE) obtained from nonobstructive azoospermia (NOA) patients was examined: Group I (spermatozoa+) and Group II (spermatozoa−). The tissues were stained with haematoxylin eosin, periodic acid–Schiff and Masson's trichrome for Johnsen's score evaluation. Apoptosis and adhesion molecules such as claudin-11, occludin and ZO-1 were assessed. In Group I, the integrity of the seminiferous tubules was intact. In Group II, some seminiferous tubule walls were lined only with Sertoli cells, had a thickening of the basement membrane, and oedema in interstitial spaces. In Group I, the seminiferous tubule consisted of a stratified columnar epithelium, claudin-11 expressions were observed as linear staining in the basal zone of the tubule, while seminiferous tubules, with low epithelium, displayed a punctate type of staining. Immunohistochemical observations were consistent with the ultrastructural findings. In Group II, high apoptosis and unstained/irregular TJ formation in claudin-11, occludin and ZO-1 were observed. In conclusion, disruption of relation between BTB and TJs may reveal inadequate spermatogenesis, which is one of the mechanisms behind azoospermia.

KEYWORDS
blood–testis barrier (BTB), claudin-11, occludin, tight junctions (TJs), ZO-1

1 | INTRODUCTION

The blood–testis barrier (BTB) structured by specific connections between adjoining Sertoli cells, to preserve newly formed male gametes from interstitial microvasculature, provides a selective passage through the basal portion of the seminiferous epithelium (Cheng & Mruk, 2012). The BTB concretely separates the seminiferous tubule epithelium into two parts and several different co-existing intersections such as tight junctions, basal ectoplasmic units and desmosome–gap junctions (Vogl, Vaid, & Guttman, 2008). These intersections have been described as the 'blood–testis barrier', which is thought to be involved in the maintenance of an exclusive biological location for sperm maturation and in the protection of spermatogenic cells, particularly in the post-meiotic state from the immune response (Moroi et al., 1998).

The principal functions of tight junctions (TJs) are to preclude the free passage of ions, solutes and water across the intercellular area and to act as a physical barrier to generate and maintain cell polarity. The main structures of the BTB are TJ proteins, that is zonula occludens (ZOs), claudins and occludin (Chihara, Otsuka, Ichii, Hashimoto, & Kon, 2010; Erkanli Senturk, Ersoy Canillioglu, Umay, Demiralp-Eksioglu, & Ercan, 2012).

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The membrane-associated guanylate kinase proteins include ZO-1, ZO-2 and ZO-3 (Gumbiner, Lowenkopf, & Apatira, 1991; Haskins, Gu, Wittchen, Hibbard, & Stevenson, 1998; Stevenson, Siliciano, Mooseker, & Goodenough, 1986). Concerning the TJ proteins of the human BTB, solely ZO-1 has been recognised (Fink et al., 2006). Integral membrane proteins, which are claudins and occludin, are restricted to the TJ (Chihara et al., 2010). The discovery of a second family of transmembrane TJ proteins and claudins displays the complexity of TJs. Claudin-11 is critical for developing BTB in mice. The absence of claudin-11 in mouse testes inclines to low organ volume, abnormally narrow seminiferous lumens and lack of BTB (Gow et al., 1999).

Disruption of the BTB is found to be related to male infertility (Park et al., 2011). Lack of spermatogenesis and obstruction of excretory ducts lead to azoospermia and the aetiology determines the treatment method. Azoospermia is a condition identified as the lack of spermatozoa in ejaculate, after evaluation of semen. Such analysis should be confirmed for an individual at least twice to name the diagnosis. In the general population, male infertility frequency is nearly 10% to 15% and azoospermia is found in 1% of these infertile men (Berookhim & Schlegel, 2014). Testicular sperm extraction (TESE) is performed in the majority of azospermic patients to recover viable sperm for intracytoplasmic sperm injection (ICSI) (Yu et al., 2018). The aim of this study was to investigate the relationship between the integrity of BTB and viable sperm recovery from testicular tissue biopsies in patients with nonobstructive azoospermia (NOA).

2 | MATERIALS AND METHODS

2.1 | Patient selection and evaluation

The study was performed at the Center for Assisted Reproduction and Department of Histology and Embryology, Faculty of Medicine, Ankara University. Informed and written consent was obtained from all patients, the study was performed according to the Declaration of Helsinki, and procedures were approved by the Ankara University Local Ethics Committee (Number: 39-837). All patients included in the study were diagnosed with azoospermia after two successive semen analyses, which were handled and processed according to the World Health Organization (WHO) protocols (WHO, 2010). After getting written voluntary consent forms, testicular biopsy specimens were taken from infertile men (n = 38, age = 24–45), who underwent microdissection TESE for ICSI. Before the TESE procedure, a complete andrologic assessment was made in each man to define the aetiology of azoospermia (routine physical examination of vasa deferentia, epididymides and testes; testicular volume calculation by ultrasound; levels of serum FSH; and total testosterone).

After the diagnostic biopsy or at the time of TESE, patients were excluded with lack of ductal obstruction (Sacca et al., 2016). Biopsied materials were transferred to the embryology laboratory, dissected and evaluated for sperm recovery, as per the methodology used by Ozkavukcu, Ibis, Kizil, Isbacar, and Aydos (2014). Testicular tissue pieces of 1x2 mm were included in one of the study groups according to the presence of spermatozoon, that is Group I with recovered spermatozoon (n = 12) and Group II with no sperm recovery (n = 26) and fixed for different methods, explained below. Patients with normal semen analysis during the ICSI procedure could not be included in the study as a control group for ethical reasons, since the TESE procedure was not required. Patients with obstructive azoospermia, Klinefelter syndrome, bilateral agenesis of the vas deferens or globozoospermia were excluded.

2.2 | Brightfield Microscopy

Testicular biopsy specimens were separated into two pieces for light and transmission electron microscopy. One part was fixed with 10% buffered formalin and dehydrated with a series of ethanols after washing with water. Following incubation in xylene, tissues were embedded into a paraffin mixture. Transverse serial sections (4–6 μm) were cut and stained with haematoxylin–eosin (H-E), using standard methods to evaluate for tissue integrity and to provide an overall assessment of any morphological changes before analysis by immunohistochemistry. Masson's trichrome staining was performed for changes in testis' connective tissue, and periodic acid–Schiff (PAS) staining was performed to define basement membrane changes. Microscopic evaluation and photography of sections were done with a Zeiss Axio Scope A1 light microscope (Zeiss, Oberkochen, Germany).

2.3 | Testicular biopsy scoring

Haematoxylin–eosin-stained sections were evaluated according to Johnsen's testicular biopsy scoring (Johnsen, 1970) and briefly depicted in Table 1. In each patient, 100 seminiferous tubule sections were examined histologically under 400× magnification and scored between 1 and 10. A mean score was calculated for each patient as the average testicular biopsy score.

2.4 | Apoptosis evaluation with the TUNEL Assay

To detect testicular apoptotic cells, DNA fragmentation was shown in tissue sections via the TUNEL technique using an in situ apoptosis detection kit (Cat# S7100; Apoptag®, Merck, Germany). Briefly, testicular tissue slides were deparaffinised in xylene (5 min), rehydrated with an ethanol series (100%, 100%, 96% and 75%) (2 min) and treated with citrate buffer (pH = 6, 0.01 M) for antigen retrieval. Afterwards, rTdT enzyme was added for the end-labelling reaction in a humidified chamber (1 hr, at 37℃). Slides were washed with PBS (5 min), and then, 3% hydrogen peroxide blocked endogenous peroxidase (30 min). Tissue sections were incubated with anti-digoxigenin peroxidase solution in a humidified chamber (30 min, at room temperature). After washing in PBS,

TABLE 1 Johnsen's scoring system for testicular biopsies

Score	Histological criteria
10	Complete spermatogenesis with many spermatozoa. Germinal epithelium organised in a regular thickness leaving an open lumen.
9	Many spermatozoa present but germinal epithelium disorganised with marked sloughing or obliteration of lumen.
8	Only few spermatozoa (<5–10) present in section.
7	No spermatozoa but many spermatids present.
6	No spermatozoa and only few spermatids (<5–10) present.
5	No spermatozoa, no spermatids but several or many spermatocytes present.
4	Only few spermatocytes (<5) and no spermatids or spermatozoa present.
3	Spermatogonia are only germ cells present.
2	No germ cells but Sertoli cells present
1	No cells in tubular section.

diaminobenzidine (DAB) solution was used for visualisation until a light brown background appeared. Counter staining was done by haematoxylin. All slides were dehydrated via an ethanol series, cleared in xylene (5 min) and covered with entellan. Staining was observed under an Axio Scope A1 light microscope (Zeiss, Oberkochen, Germany). All photographs were taken with a Zeiss AxioCam MRc5 camera.

2.5 | Immunohistochemistry

To detect claudin-11, immunohistochemistry was used via a streptavidin–biotin technique. Paraffin-embedded tissues were cut in 4–5 μm thickness and laid on poly-lysine-covered slides. The slides were dewaxed and hydrated in an ethanol series. For antigen retrieval, the sections were incubated in a microwave (5 min in 0.01 M citrate buffer, pH = 6). 3% hydrogen peroxide blocked endogenous peroxidase activity (GBI Labs). The slides were incubated for one hour at 37˚C with the primary antibody against claudin-11 (HPA013166, Sigma-Aldrich Co.) at a dilution of 1:100. The secondary antibody (GBI Labs) was incubated for 30 min. The streptavidin–peroxidase complex was applied for 30 min, and the sections were incubated with 3,3'-diaminobenzidine (DAB) (GBI Labs) until a brown colour appeared (prepared as 1:20). Then, the tissue sections were washed in water; Mayer's haematoxylin was applied for counterstaining after dehydration, and covered with entellan. In the negative controls, only the secondary rabbit IgG antibody was added without using the primary antibody (DakoCytomation, Denmark).

2.6 | Immunofluorescence microscopy

Immunofluorescent staining was performed for mouse monoclonal antibody generated against zonula occludens (ZO-1) (33-9100, Invitrogen) and rabbit polyclonal antibody generated against occludin (C-Term GST) (42–2400, Invitrogen). 0.01% trypsin was applied for 8 min for permeabilisation in deparaffinised and rehydrated 4-μm-thick sections. A microwave was used to heat the slides, which were in citrate buffer (pH 6, 0.01 M) for 15 min and blocked with blocking solution (GBI Labs) for 60 min at room temperature. Then, sections were incubated with primary antibodies for 1 hr at 37˚C, washed in Tris buffer solution (TBS), and a secondary antibody incubation was performed in a humidified chamber for 1 hr using fluorescein isothiocyanate (FITC)-conjugated anti-rabbit immunoglobulin G (1:200) (F1262, Sigma). The sections were covered in mounting medium with Hoechst. The negative sections were treated with 1.5% BSA in Tris–HCl buffer without the primary antibody. The samples were observed with fluorescent microscope (Zeiss Axio Scope A1, Oberkohen, Germany).

2.7 | Transmission electron microscopic (TEM) evaluation

For each patient in both groups, a volume of 0.5–1.0 mm^3 of testicular biopsy was treated in 2% phosphate-buffered glutaraldehyde and 10% paraformaldehyde solutions at pH 7.2, fixed for 2–4 hr at 4°C and followed by post-fixation in phosphate-buffered 1% osmium tetroxide. After dehydration in an ethanol gradient, the samples were transferred to propylene oxide and embedded in Araldite 6005. Ultra-thin sections (60–80 nm) were cut, by using an ultramicrotome (Leica Ultracut R) and stained with uranyl acetate and lead citrate. Ultra-thin sections were examined by transmission electron microscope (LEO 906E, Zeiss, Oberkochen, Germany).

2.8 | Statistical analysis

Statistical differences for age of patients, and the average testicular biopsy scores were evaluated using Student's *t* test. Mann–Whitney *U* test was performed to compare hormone profiles and testes volumes. All data analyses were performed using SPSS, version 11.5

TABLE 2 Statistical comparison of the groups

	Group I (Spermatozoa+)		Group II (Spermatozoa-)		
	Mean ± SS	Median (Min_Max)	Mean ± SS	Median (Min_Max)	*p*
Age	35.83 ± 7.69	36.00 (26.00–51.00)	33.26 ± 5.11	32.50 (24.00–44.00)	.23
FSH (mIU/mL)	22.29 ± 13.52	21.50 (5.00–52.00)	22.92 ± 15.16	17.00 (4.80–61.00)	.98
Testosterone (ng/ml)	3.09 ± 1.87	2.75 (0.50–7.40)	3.53 ± 1.96	3.00 (1.20–9.00)	.58
Right testis volume (cm^3)	10.25 ± 5.94	9.00 (3.00–18.00)	9.61 ± 5.53	11.00 (2.00–18.00)	.54
Left testis volume (cm^3)	11.08 ± 6.51	11.00 (3.00–22.00)	9.88 ± 5.47	12.00 (2.00–18.00)	.53
Johnsen's score	4.03 ± 1.77	4.77 (1.00–6.40)	4.98 ± 1.41	4.96 (1.96–7.00)	.09

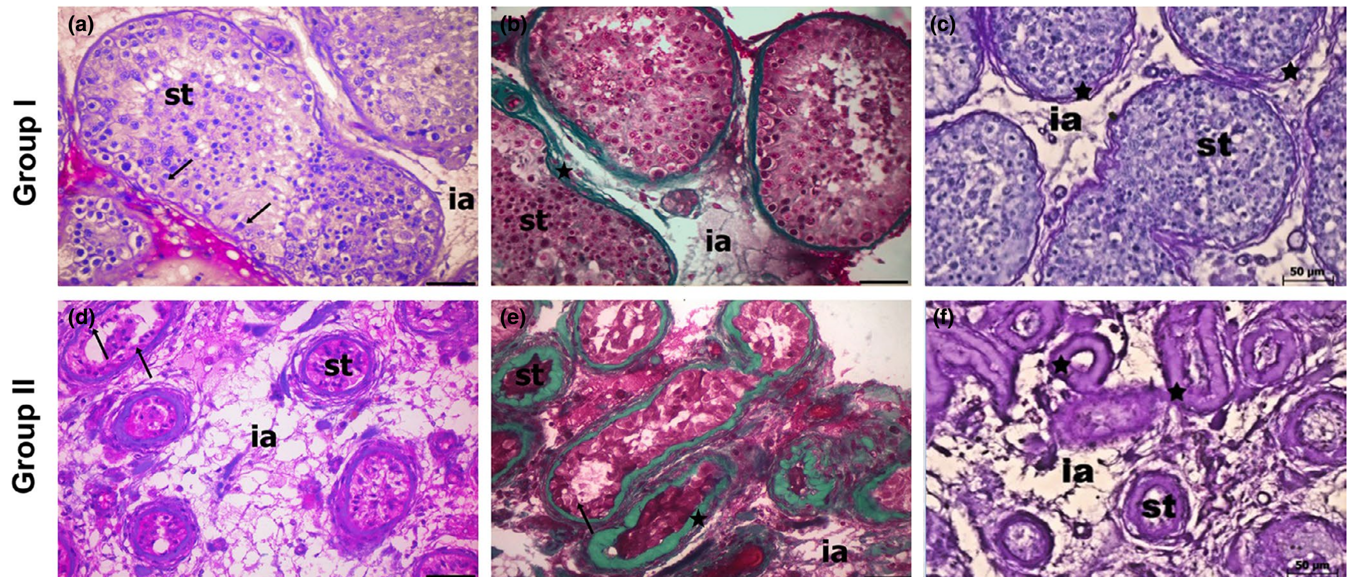

FIGURE 1 Testicular tissue sections of groups I (a–c) and II (d–f). a, d; H&E stained. b, e; Masson's trichrome stained. c, f; PAS stained. st: seminiferous tubules, arrow: Sertoli cells, ia: interstitial area, star: collagen fibres of the connective tissue, which is under the basement membrane. Scale bars indicate 50 μm

(SPSS Inc., IBM). Statistical significance was determined when $p < .05$.

3 | RESULTS

3.1 | Patient andrologic evaluation

The mean age of the patients was 35.83 ± 7.69 in Group I and 33.26 ± 5 in Group II. Mean FSH and testosterone levels, testicular volumes and Johnsen's scores for each group are depicted in Table 2. No statistically significant difference was observed in any of these parameters between the two groups.

3.2 | Histological observations

Under light microscopic examination, in Group I, more spermatogenetic cells (SC) in the seminiferous tubules were observed, compared to Group II (Figure 1a,d). The loose connective tissue appeared to have mild oedema with Leydig cells in the interstitial area. The collagen fibres, which exist in interstitial connective tissue under the basement membrane, were thin and regularly arranged (Figure 1b). Basement membrane integrity was preserved on observation of PAS staining (Figure 1c). In the examination of Group II, an excessive thickness of basement membrane, a significant reduction in the thickness of the tubule epithelium, an irregular structure in the majority of spermatogenic cells consisting of spermatogonia and spermatids, a shortening in the columnar height of Sertoli cells and an existence of relatively pale cytoplasm of Sertoli cells were observed. In some tubules, almost all parts of the walls were lined with Sertoli cells. Enlargement and oedema in the interstitial area were noticed (Figure 1d–f).

3.3 | Assessment of apoptosis (TUNEL staining)

TUNEL staining, in Group I, indicated that a few apoptotic cells were present in the seminiferous tubule epithelium (Figure 2a), whereas in Group II, an increase in apoptosis was detected with spermatogenic cells detached from the epithelium into the tubule lumen (Figure 2b).

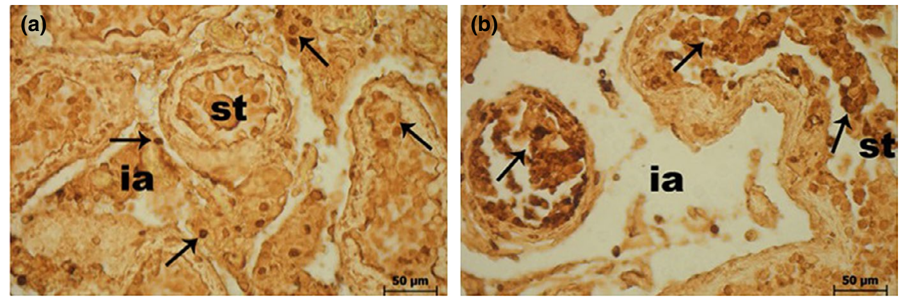

FIGURE 2 TUNEL staining. Immunoreactivity in apoptotic cell nuclei (arrows). (a) Testicular tissue samples from Group I. (b) Testicular tissue samples from Group II, st: seminiferous tubule, ia: interstitial area. Scale bars indicate 50 μm

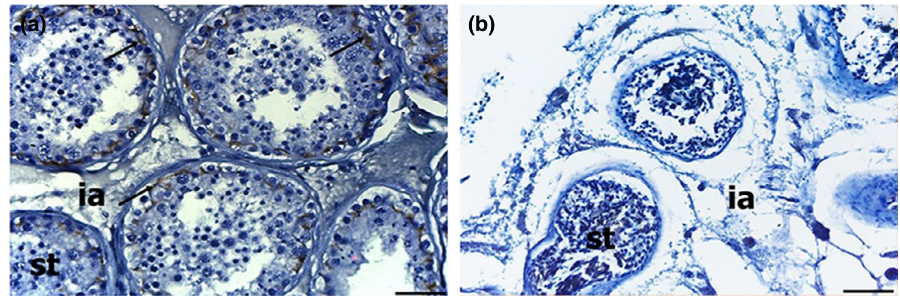

FIGURE 3 Immunohistochemical staining of claudin-11. (a) A testicular tissue sample from Group I. (b) A testicular tissue sample from Group II. st: seminiferous tubules, ia: interstitial area, arrow: filamentous expression against claudin-11 antibody. Scale bars indicate 50 μm

Apoptotic cells seen in the interstitial region were also observed more in Group II.

3.4 | Immunohistochemistry

Immunohistochemical staining for claudin-11 revealed a strong expression pattern in the basal sections of the epithelial wall in Group I (Figure 3a); however, weak staining was observed in Group II (Figure 3b). In Group I, tubules with a high epithelium demonstrated a filamentous expression pattern, whereas in Group II, the expression pattern was punctuated and scattered.

3.5 | Immunofluorescence

Linear and strong immunofluorescent staining (arrow) was observed against the occludin (occ) and zonule occludens-1 (ZO-1) antibodies in Group I. Negative immunofluorescent staining was observed against the occludin (occ) and zonule ocludens-1 (ZO-1) antibodies in Group II (Figure 4).

3.6 | Transmission electron microscopy (TEM)

Ultrastructural examination of Group I revealed that the basement membrane was conserved and that spermatogenic cells were present above the basement membrane with their relative stage morphology. In some of the spermatogenic cells' cytoplasm, vacuoles and lipid droplets were observed. The boundaries between the Sertoli and spermatogenic cells were intact and connections between cells showed continuity (Figure 5a–d). In the TEM examination of Group II, basement membranes were observed intact, and conversely, the boundaries of the Sertoli and spermatogenic cells were found to be diminished and connections between Sertoli cells were not observed. Shrinkage in spermatogenic cells, cytoplasmic vacuolisation, lipid droplets, smooth endoplasmic reticulum (SER) residues and severe degeneration were detected. Deep nuclear grooves were also noticed in some cell nuclei (Figure 5f,g).

4 | DISCUSSION

The probability of retrieving spermatozoa in TESE is related directly to testicular histopathology. Evaluating the histopathology is useful for patients' further prognosis in assisted reproduction treatment. In the current study, the aim was to investigate the status of testicular tissues by means of evaluating the blood–testis barrier and overall appearance of the seminiferous tubules in azoospermic patients, using light, immunofluorescent microscopy and transmission electron microscopy.

Light microscopic evaluation revealed that Group I had better histologic findings than Group II. Basal membrane thickness was significantly higher in Group II. The presence of spermatogenetic cells was clearly observed in Group I. As noted by Dabaja et al., testicular histopathology may differ in patients with NOA (Dabaja & Schlegel, 2013) and this study's findings between the groups are consistent with literature. It is possible to see different testicular pathological patterns obtained in different time intervals or from anatomic regions for the same patient (Ezeh, Moore, & Cooke, 1998), and the probability of successful testicular sperm retrieval was found to be related only to the presence of spermatids and high Johnson scoring (Ezeh, Taub, Moore, & Cooke, 1999). Taking these observations into account, a high rate of apoptosis and pathologic changes were detected in the Sertoli cells' cytoplasm in Group II, which can be investigated further in controlled studies in order to introduce additional

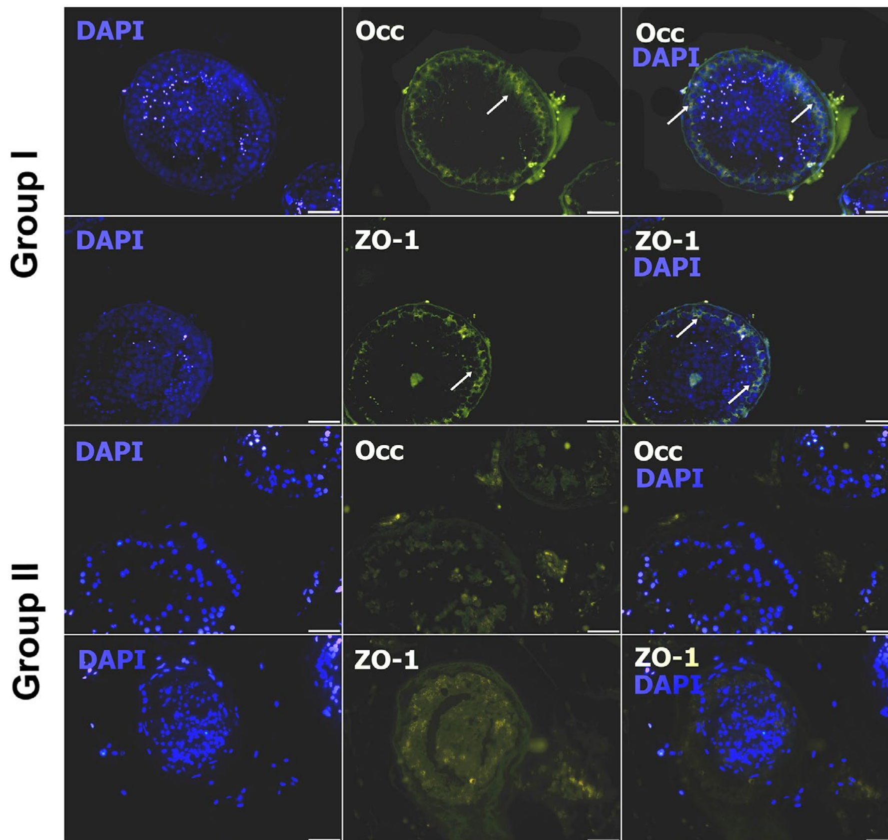

FIGURE 4 Immunofluorescence staining of Occ and ZO-1 on testis sections. Immunofluorescent staining (arrow) against occludin (Occ) and ZO-1 antibodies (green). DAPI: nucleus (blue). Scale bars indicate 50 μm

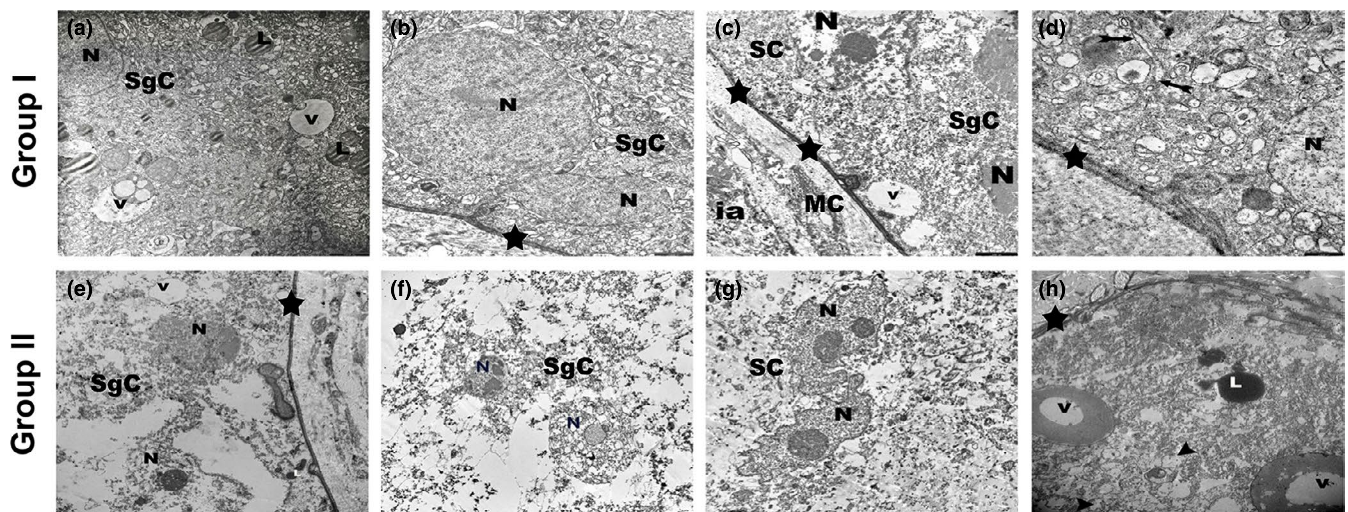

FIGURE 5 TEM images of groups I (a–d) and II (e–h). Spermatogenic cells (SgC), spermatogenic cell nucleus (N), vacuoles (v), lipid droplets (L), basement membrane (star), Sertoli cells (SC), myoid cells (MC), cell–cell connection (arrow) interstitial area (ia) and SER residues (arrowheads)

predictive factors for sperm retrieval probability in azoospermic men.

In this study's TEM observation, the evidence of cell degeneration, including vacuole formation and lipid droplets in the spermatogonial cells, was detected. Additionally, an apoptotic appearance was distinct in Group II. As Erkanli Senturk et al. mentioned, disrupted TJs cause disconnection in cell-to-cell interactions and damage BTB (Erkanli Senturk et al., 2012). In this study, comparatively less observation of apoptosis in Group I was related to intact cellular connection and cell integrity.

In the seminiferous epithelium, BTB, which is a selectively permeable barrier, is formed between neighboring Sertoli cell by TJs and separates the seminiferous epithelium into basal and adluminal compartments (Fink et al., 2006). Spermatogenic cells are surrounded by complex processes of Sertoli cells that participate in the formation of the BTB, which maintains a homeostatic environment for developing

spermatogenic cells (Aydos, Yukselten, Ozkavukcu, Sunguroglu, & Aydos, 2019; Tian et al., 2017). Sertoli cells are bound to each other by junctional complexes. The TJ structure, which constitutes the main essential piece of junctional complexes, consists of a paracellular region that involves small molecules, which provide adhesiveness to adjacent cells by binding to the uniform molecule (occludin, claudin, and JAM). The molecules of the cytoplasmic region of the tight junction act as a bridge between the anchor transmembrane proteins and actin-based cytoskeleton (Perez et al., 2012). This study focused on the differential expression patterns among ZO-1, occludin and claudin-11 proteins in NOA patients.

Fink et al. reported that the epithelium of the seminiferous tubule, ZO-1 and ZO-2 formed continuous rings of robust staining on the level of the BTB. Occludin and claudins directly connect to the ZO-1, and its attachment to occludin is crucial in fixing in the extracellular imprint (Fink et al., 2006). In this study, expressions of ZO-1 and occludin protein were detected as linear and intense in group I, but were absent in Group II. Despite the presence of NOA, it was thought that positive sperm retrieval is associated with strong expression of ZO-1 and occludin, which can be related to the maintenance of the delicate physiology of the seminiferous epithelium. Inversely, the absence of ZO-1 and occludin indicates a loss of integrity in the design of the BTB and disruption of spermatogenesis.

Claudins are proteins that bind to cytoskeletal proteins such as actin filaments and are crucial constituents of tight junctions (Pan et al., 2018). Expression patterns and localisations of claudin-11, ZO-1 and occludin can be variable in different seminiferous epithelial cycles (Chihara et al., 2010). In the testis, expression of claudin-11 shows to be restricted to Sertoli cells and has an essential role in tight junctions; for example, claudin-11 deficiency in mice causes sterility. In men with testicular biopsies showing defective spermatogenesis, the cause of increased expression of claudin-11 is unexplained (Ilani et al., 2012). Yang et al. reported that in NOA patients, the development and progression of spermatogenic disruption might arise from the upregulated claudin-11 expression in Sertoli cells (Yang et al., 2018). In this study, the expression of claudin-11 was stronger in Group I than Group II, whereas claudin-11 expression in Group II showed dislocated patterns, which were punctuated and scattered. Stammler et al. expressed that in impaired spermatogenesis there is an increase in Sertoli–Sertoli junctions related to claudin-11 expression (Stammler et al., 2016). It can be concluded that both over expression and absence of claudin-11 may result in impairment in spermatogenesis but according to this study's findings, the latter decreases the probability of sperm retrieval after TESE in azoospermic patients. Chiba et al. also mentioned that a dislocated expression pattern of claudin-11 causes decreased sperm discovery after TESE (Chiba, Yamaguchi, Ando, Miyake, & Fujisawa, 2012) and different expression patterns of claudin-11, such as linear and punctate pattern, are identified in impaired spermatogenesis (Haverfield, Meachem, O'Bryan, McLachlan, & Stanton, 2013). In this study, claudin-11 expressions were observed in the form of strong and dislocated expression patterns in accordance with literature and it was associated with the presence of spermatozoa in patients with NOA.

The authors of this study concluded that the disruption of BTB in NOA patients is related to the disorganisation or dysfunction of ZO-1, occludin and claudin-11. The impaired maturation status of Sertoli cells and their faulty interaction with sperm cells are related to the distribution of ZO-1, occludin and claudin-11, and all together may cause the breakdown of the BTB. Determination of the distribution pattern or absence of the transmembrane TJ proteins together with apoptosis in the seminiferous epithelium, status of basement membrane and morphology of Sertoli cells need to be investigated in prospective controlled trials for the assignment of their novel predictive values for positive sperm retrieval in TESE in azoospermic patients.

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REFERENCES

Aydos, O. S., Yukselten, Y., Ozkavukcu, S., Sunguroglu, A., & Aydos, K. (2019). ADAMTS1 and ADAMTS5 metalloproteases produced by Sertoli cells: A potential diagnostic marker in azoospermia. *Systems Biology in Reproductive Medicine*, *65*(1), 29–38. https://doi.org/10.1080/19396368.2018.1467512

Berookhim, B. M., & Schlegel, P. N. (2014). Azoospermia due to spermatogenic failure. *Urologic Clinics of North America*, *41*(1), 97–113. https://doi.org/10.1016/j.ucl.2013.08.004

Cheng, C. Y., & Mruk, D. D. (2012). The blood-testis barrier and its implications for male contraception. *Pharmacological Reviews*, *64*(1), 16–64. https://doi.org/10.1124/pr.110.002790

Chiba, K., Yamaguchi, K., Ando, M., Miyake, H., & Fujisawa, M. (2012). Expression pattern of testicular claudin-11 in infertile men. *Urology*, *80*(5), 1161.e13–1161.e17. https://doi.org/10.1016/j.urology.2012.06.036.

Chihara, M., Otsuka, S., Ichii, O., Hashimoto, Y., & Kon, Y. (2010). Molecular dynamics of the blood-testis barrier components during murine spermatogenesis. *Molecular Reproduction and Development*, *77*(7), 630–639. https://doi.org/10.1002/mrd.21200

Dabaja, A. A., & Schlegel, P. N. (2013). Microdissection testicular sperm extraction: An update. *Asian Journal of Andrology*, *15*(1), 35–39. https://doi.org/10.1038/aja.2012.141

Erkanli Senturk, G., Ersoy Canillioglu, Y., Umay, C., Demiralp-Eksioglu, E., & Ercan, F. (2012). Distribution of Zonula Occludens-1 and Occludin and alterations of testicular morphology after in utero radiation and postnatal hyperthermia in rats. *International Journal of Experimental Pathology*, *93*(6), 438–449. https://doi.org/10.1111/j.1365-2613.2012.00844.x

Ezeh, U. I., Moore, H. D., & Cooke, I. D. (1998). Correlation of testicular sperm extraction with morphological, biophysical and endocrine profiles in men with azoospermia due to primary gonadal failure. *Human Reproduction*, *13*(11), 3066–3074. https://doi.org/10.1093/humrep/13.11.3066

Ezeh, U. I., Taub, N. A., Moore, H. D., & Cooke, I. D. (1999). Establishment of predictive variables associated with testicular sperm retrieval in men with non-obstructive azoospermia. *Human Reproduction*, *14*(4), 1005–1012. https://doi.org/10.1093/humrep/14.4.1005

Fink, C., Weigel, R., Hembes, T., Lauke-Wettwer, H., Kliesch, S., Bergmann, M., & Brehm, R. H. (2006). Altered expression of ZO-1

and ZO-2 in Sertoli cells and loss of blood-testis barrier integrity in testicular carcinoma in situ. *Neoplasia*, *8*(12), 1019–1027. https://doi.org/10.1593/neo.06559

Gow, A., Southwood, C. M., Li, J. S., Pariali, M., Riordan, G. P., Brodie, S. E., … Lazzarini, R. A. (1999). CNS myelin and sertoli cell tight junction strands are absent in Osp/claudin-11 null mice. *Cell*, *99*(6), 649–659. https://doi.org/10.1016/S0092-8674(00)81553-6

Gumbiner, B., Lowenkopf, T., & Apatira, D. (1991). Identification of a 160-kDa polypeptide that binds to the tight junction protein ZO-1. *Proceedings of the National Academy of Sciences of the United States of America*, *88*(8), 3460–3464. https://doi.org/10.1073/pnas.88.8.3460

Haskins, J., Gu, L., Wittchen, E. S., Hibbard, J., & Stevenson, B. R. (1998). ZO-3, a novel member of the MAGUK protein family found at the tight junction, interacts with ZO-1 and occludin. *Journal of Cell Biology*, *141*(1), 199–208. https://doi.org/10.1083/jcb.141.1.199

Haverfield, J. T., Meachem, S. J., O'Bryan, M. K., McLachlan, R. I., & Stanton, P. G. (2013). Claudin-11 and connexin-43 display altered spatial patterns of organization in men with primary seminiferous tubule failure compared with controls. *Fertility and Sterility*, *100*(3), 658–666. https://doi.org/10.1016/j.fertnstert.2013.04.034

Ilani, N., Armanious, N., Lue, Y.-H., Swerdloff, R. S., Baravarian, S., Adler, A., … Wang, C. (2012). Integrity of the blood-testis barrier in healthy men after suppression of spermatogenesis with testosterone and levonorgestrel. *Human Reproduction*, *27*(12), 3403–3411. https://doi.org/10.1093/humrep/des340

Johnsen, S. G. (1970). Testicular biopsy score count–a method for registration of spermatogenesis in human testes: Normal values and results in 335 hypogonadal males. *Hormones*, *1*(1), 2–25.

Moroi, S., Saitou, M., Fujimoto, K., Sakakibara, A., Furuse, M., Yoshida, O., & Tsukita, S. (1998). Occludin is concentrated at tight junctions of mouse/rat but not human/guinea pig Sertoli cells in testes. *American Journal of Physiology*, *274*(6), C1708–1717. https://doi.org/10.1152/ajpcell.1998.274.6.C1708

Ozkavukcu, S., Ibis, E., Kizil, S., Isbacar, S., & Aydos, K. (2014). A laboratory modification to testicular sperm preparation technique improves spermatogenic cell yield. *Asian Journal of Andrology*, *16*(6), 852–857. https://doi.org/10.4103/1008-682X.132468

Pan, J., Zhu, Z., Xu, G., Niu, L., Yu, L., Luo, Z., & Yan, J. (2018). Expression of claudin11 in a rat model of varicocele and its effects on the blood-testis barrier. *Molecular Medicine Reports*, *18*(6), 5647–5651.

Park, C. J., Lee, J. E., Oh, Y. S., Shim, S., Kim, D. M., Park, N. C., … Gye, M. C. (2011). Postnatal changes in the expression of claudin-11 in the testes and excurrent ducts of the domestic rabbit (Oryctolagus cuniculus domesticus). *Journal of Andrology*, *32*(3), 295–306. https://doi.org/10.2164/jandrol.110.010611

Perez, C. V., Sobarzo, C. M., Jacobo, P. V., Pellizzari, E. H., Cigorraga, S. B., Denduchis, B., & Lustig, L. (2012). Loss of occludin expression and impairment of blood-testis barrier permeability in rats with autoimmune orchitis: Effect of interleukin 6 on Sertoli cell tight junctions. *Biology of Reproduction*, *87*(5), 122.

Saccà, A., Pastore, A. L., Roscigno, M., Naspro, R., Pellucchi, F., Fuschi, A., … Da Pozzo, L. F. (2016). Conventional testicular sperm extraction (TESE) and non-obstructive azoospermia: Is there still a chance in the era of microdissection TESE? Results from a single non-academic community hospital. *Andrology*, *4*(3), 425–429. https://doi.org/10.1111/andr.12159

Stammler, A., Luftner, B. U., Kliesch, S., Weidner, W., Bergmann, M., Middendorff, R., & Konrad, L. (2016). Highly conserved testicular localization of claudin-11 in normal and impaired spermatogenesis. *PLoS ONE*, *11*(8), e0160349. https://doi.org/10.1371/journal.pone.0160349

Stevenson, B. R., Siliciano, J. D., Mooseker, M. S., & Goodenough, D. A. (1986). Identification of ZO-1: A high molecular weight polypeptide associated with the tight junction (zonula occludens) in a variety of epithelia. *Journal of Cell Biology*, *103*(3), 755–766. https://doi.org/10.1083/jcb.103.3.755

Tian, J., Ding, Y., She, R., Ma, L., Du, F., Xia, K., & Chen, L. (2017). Histologic study of testis injury after bisphenol A exposure in mice. *Toxicology and Industrial Health*, *33*(1), 36–45. https://doi.org/10.1177/0748233716658579

Vogl, A. W., Vaid, K. S., & Guttman, J. A. (2008). The sertoli cell cytoskeleton. *Advances in Experimental Medicine and Biology*, *636*, 186–211.

WHO (2010). *WHO laboratory manual for the examination and processing of human semen* (5th ed). Switzerland: World Health Organization Press.

Yang, X. F., Fu, C., Liu, Y. Q., Liu, R. S., Feng, H., Huang, R. Y., … Pan, Z. H. (2018). Expression of CLAUDIN-11 in the testicular tissue of the patient with non-obstructive azoospermia and its clinical significance. *Zhonghua Nan Ke Xue*, *24*(3), 221–225.

Yu, Y., Xi, Q., Pan, Y., Jiang, Y., Zhang, H., Li, L., & Liu, R. (2018). Pregnancy and neonatal outcomes in azoospermic men after intracytoplasmic sperm injection using testicular sperm and donor sperm. *Medical Science Monitor*, *24*, 6968–6974. https://doi.org/10.12659/MSM.912613

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