MARMARA
MEDICAL JOURNAL

Review Article

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The alterations of blood-testis barrier in experimental testicular injury models

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Submitted: 21.12.2021 **Accepted:** 17.03.2022

ABSTRACT

The blood-testis barrier is found between the Sertoli cells and divides the seminiferous tubule epithelium into basal and adluminal compartments. The germinal cell renewal, differentiation and cell cycle progression up to the preleptotene spermatocytes stage take place in the basal compartment, however, meiosis, spermiogenesis and spermiation take place in the adluminal compertment. The blood-testis barrier consists of tight junctions as well as ectoplasmic specialisations, desmosomes and gap junctions to create specific microenvironment for the completion of spermatogenesis to form spermatozoa. The blood-testis barrier is not a static ultrastructure, it undergoes extensive restructuring during the seminiferous tubule epithelial cycle of spermatogenesis to allow the transit of preleptotene spermotocytes at the blood-testis barrier from basal compartment towards the adluminal compartment. The functions of the blood-testis barrier include preventing the transport of biomolecules into the paracellular space, forming an immunological barrier, separating cellular processes during the spermatogenic epithelial cycle, and establishing the cellular polarity of the seminiferous tubule. However, various environmental conditions, chemotherapeutic agents, toxic substances and lifestyle have degenerative effects on blood-testis barrier, resulting in testicular damage, altered sperm parameters and ultimately male infertility. The alterations in morphological and molecular organization of blood-testis barrier in different experimentally induced testis injury models are reviewed in this article.

Keywords: Blood-testis barrier, Sertoli cells, Tight junction, Testicular injury models

1. INTRODUCTION

Spermatogenesis occurs within the seminiferous tubules in testis and begins after the puberty under the control of follicle-stimulating hormone (FSH), luteinizing hormone (LH) and testosterone. Seminiferous tubule epithelium contains germ cells and Sertoli cells. Spermatozoa are formed at the end of the spermatogenesis. The blood-testis barrier is found between the Sertoli cells and regulates the spermatogenesis. Leydig cells, located in the interstitium, secrete testosterone under stimulation of LH. Testosterone is essential for the maintenance of the blood-testis barrier and spermatogenesis, and assists in both the formation and breakdown of the Sertoli-germ cell association [1]. The blood-testis barrier is not a static ultrastructure, it undergoes extensive restructuring during the seminiferous tubule epithelial cycle of spermatogenesis to allow the transit of germinal cells from basal region towards the apical region of seminiferous tubules. When the blood-testis barrier function is disrupted it directly causes male infertility because the germ cells are more vulnerable to the microenvironment. However, the regulation and restructuring of the blood-testis barrier without causing "leakage" in healthy adults is not fully understood, nor is it known how disturbances in blood-testis barrier regulation contribute to testicular damage and infertility. Tight junctions between Sertoli cells are important for the integrity of this barrier. Intracellular communication between Sertoli and germ cells plays a critical role in the adult spermatogenic process. Loss of this blood-testis barrier function causes infertility [2].

This article focused on the alteration of the blood-testis barrier in different experimental testicular injury models. First, the structure and function of the blood-testis barrier and its role in spermatogenesis were discussed. Then, the alteration of the blood-testis barrier in different experimentally induced testicular injury models was reviewed.

How to cite this article: Ercan F. Acikel Elmas M. The alterations of blood-testis barrier in experimental testicular ınjury models. Marmara Med J 2022; 2; 35(2):249-253. doi: 10.5472/marumj.1114971

ISSN: 1309-9469

http://doi.org/10.5472/marumj.1114971
Marmara Med J 2022;35(2): 249-253

Formation of Blood-Testis Barrier

Sertoli cells were first described by Enrico Sertoli and first called 'Sertoli cells' by Von Ebner. The blood-testis barrier was first defined physiologically, later ultrastructurally, and basal and adluminal compartments were described [3]. Sertoli cells are polarized cells lying on the basal lamina and reaching to the tubule lumen. Germinal epithelial cells are found between the adjacent Sertoli cells (Figure 1). Spermatogenesis begins with the division of spermatogonia A to give spermatogonia B. These cells give rise to preleptotene spermatocytes, which form round spermatids after completion of meiosis. After spermiogenesis, spermatozoa are formed and released into the lumen of the seminiferous tubules. Leydig cells localising in the interstitium secrete testosterone under the stimulation of LH. Testosterone regulates the maintenance of the blood-testis barrier, spermatogenesis and fertility. Monocytes, macrophages, dentritic cells, natural killer cells and mast cells which are also present in the interstitium have roles for the maintenence of spermatogenesis [1].

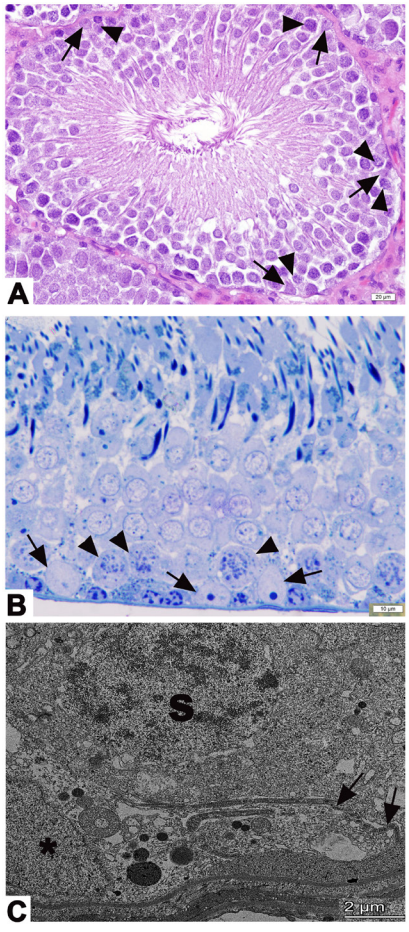

Figure 1. *Primary spermatocytes (arrowheads) and the other germ cells between the Sertoli cells (arrow) are seen in seminiferous tubule epithelium of rat testis (A and B). Tight junctions (arrow), between the Sertoli cells (*) and primary spermatocyte (s) are seen in seminiferous tubule epithelium of rat testis (C). A: Hematoxylin and eosin staining, B: Toluidine blue staining. C: Electron micrograph. Scale bar: A: 20µm, B: 10µm, C: 2µm*

The blood-testis barrier is formed by Sertoli cells close to the base of the seminiferous tubules and divides the germinal epithelium into basal and adluminal compartments. Spermatogonia and preleptotene spermatocytes are located in the basal compartment, while other primary and secondary spermatocytes, round spermatids and elongated spermatids are found in the adluminal compartment [4].

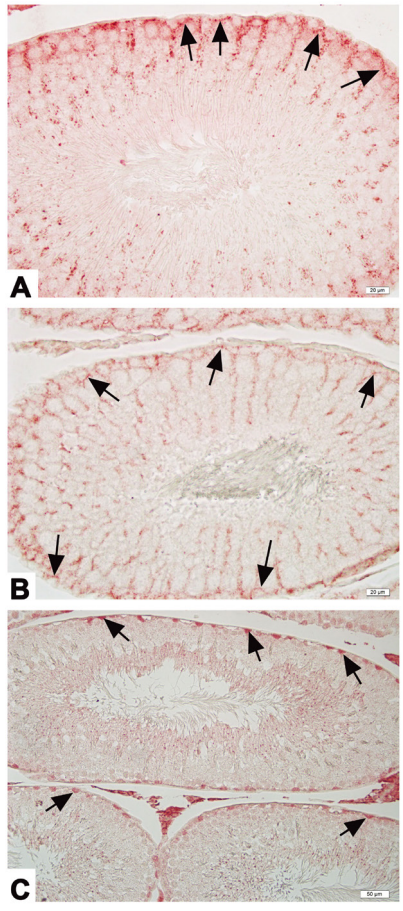

Figure 2: *The localization of ZO-1 (A), occludin (B) and connexin-43 (C) are seen in basolateral cytoplasm (arrow) of the Sertoli cells in rat testis. ZO-1 (A), occludin (B) and CX-43 (C) immunostaining. Scale bar: A and B: 20µm, C: 50µm*

Blood-testis barrier is formed by tight junctions, ectoplasmic specializations, desmosomes and gap junctions. Tight junctions are the most important component of the blood-testis barrier, they have preventive function for the passage of water, solutes and large molecules between the paracellular space, and restrictive function for the movement of proteins and lipids between the apical and basolateral cytoplasm. Ectoplasmic specializations coexist and cofunction with tight junctions. Desmosomes are cell-cell junctions that mediate vigorous adhesion. Gap junctions are cell-cell channels that permit diffusion of metabolites, ions and molecules smaller than 1 kDa. Tight junctions consist of

http://doi.org/10.5472/marumj.1114971
Marmara Med J 2022;35(2): 249-253

integral proteins such as occludin, claudin family (claudin-1, – 3, – 5, – 11, – 12 and – 13), junctional adhesion molecule family, tricellulin, coxsackievirus and adenovirus receptor, and scaffolding proteins such as zonula occludens (ZO) [2]. ZO-1, ZO-2 and ZO-3 are well-studied adaptor proteins and are used by integral membrane tight junction proteins such as occludin and claudin for the attachment to the actin cytoskeleton. ZO-1 colocalizes with gap junction protein connexin – 43 (CX-43) and regulates gap junction communication [1, 4, 5]. Basolateral localization of ZO – 1, occludin, and CX-43 proteins using immunohistochemical technique is shown in Figure 2.

Alteration of Blood-Testis Barrier in Various Testicular Injury Models

Inflammation because of infection [6], sperm extraction procedure such as, testicular sperm extraction (TESE) [7], vasectomy operation [8], reactive oxygen/nitrogene species [9, 10], radiation and hyperthermia [11], chronic unpredictable stress [12] and various environmental contaminants, such as arsenic [13], perfluorooctane sulphonate [14] and cadmium chloride [15] affect spermatogenesis, steroidogenesis, Sertoli cells, blood-testis barrier structure and function and sperm parameters [16]. Impaired blood-testis barrier might cause disruption of spermatogenesis and finally might lead to subfertility or infertility.

It is a well-known fact that the different environmental agents negatively affect the functional development of germ cells, Sertoli cells and Leydig cells during the prenatal period. Prenatal administration of ethanol is shown to cause dilatation between tight junctions, a decrease in ZO-1 and occludin distribution in the blood-testis barrier region, an increase in athrophic tubules with a decrease in germ cells, and an increase in apoptotic cells in the rat testis [17, 18]. These results show that ethanol administration negatively affects the blood-testis barrier during the testicular development. In another study, it is shown that prenatal administration of radiation and postnatal administration of hyperthermia to the rats caused severe increase of atrophic tubules, degeneration of tight junctions with absence of ZO-1 and occludin proteins distribution. This double hit model was represented as Sertoli cell only model [11]. In another prenatal study, electromagnetic waves emitted from cell phones were shown to damage the blood-testis barrier and cause a decrease in the amount of ZO-1 protein distribution, loss of germ cells, an increase in apoptotic cells, a decrease in serum testosterone level, and an increase in oxidative stress in the testes [19, 20]. In addition, electromagnetic waves emitted by mobile phones have been shown to cause tight junction degeneration starting from intrauterine life, with a decrease in ZO-1 intensity in the urothelium, an increase in degranulated mast cells, and an increase in oxidative stress in the urinary bladder [21] and neuronal injury with oxidative stress increase in the brain [22]. It is impossible to avoid mobile phone technology, especially with the current COVID-19 pandemic where children, adults, and even pregnant women are using them for many purposes such as communication, education, etc. However, all these studies have shown that environmental factors in the prenatal period have a negative impact on the development of germ cells and the function of the blood-testis barrier, resulting in spermatogenesis disorder.

Obesity is considered as a global health problem of the 21st century. It is associated with the development of many health disorders, including cardiovascular and hepatorenal failures, type 2 diabetes, respiratory and musculoskelatal disorders as well as infertility [23]. Obesity is usually associated with excessive intake of foods high in fat and sugar, a sedentary lifestyle, genetic predisposition, or a combination of these factors [24]. Increased visceral obesity affects the reproductive function such as steroidogenesis and spermatogenesis as well as peripheral and testicular oxidative stress [25]. It has been shown that high-fat diet induced obesity causes hiperlipidemia, hiperleptinemia, alteration of hormone levels (including FSH, LH, estrogen, and testosterone) in serum, degeneration of seminiferous tubule morphology with increase in apoptosis, degeneration of blood-testis barrier structure with decrease in ZO-1, occludin, and decrease of gap junction protein CX-43, alteration of sperm parameters, increase in testicular inflammation, and oxidative stress [26-29]. However, moderate swimming exercise has been shown to improve all serum and testicular parameters including the blood-testis barrier integrity evaluated in high-fat diet induced obese rats [27, 28]. In addition, the administration of Myrtus communis L. an extract, which is rich in antioxidant components has been shown to ameliorate testicular damage and sperm parameters [26]. In experimental studies of obesity-induced male infertility, impaired blood-testis barrier has also been observed. In addition, the increasing sedentary lifestyle and consumption of high calorie foods are associated with the COVID-19 pandemic that has occurred worldwide in the last two years may cause a rapid increase in male infertility. Therefore, experimental studies are valuable in demonstrating the therapeutic role of lifestyle changes and consumption of foods high in antioxidants in improving male infertility caused by obesity.

Various methods of contraception are used in male sterilization. The most commonly used method is vasectomy. A disrupted blood-testis barrier with a decrease in occludin density and degenerated seminiferous tubules with an increase in apoptotic cells have been observed 6 months later in bilaterally vasectomized rats [8]. This has also been mentioned in the non-surgical reversible and irreversible male infertility models as male contraceptives [30]. In the study by Wong et al., synthetic occludin peptides and testosterone/estrogen implants have been shown to cause disruption of Sertoli cell junction permeability and spermatogenesis, which is reversible [31]. However, administration of the environmental toxin cadmium chloride causes irreversible degeneration of the blood-testis barrier [30] and impaired expression of gap junction protein CX-43 [32]. Moreover, the degenerative effects of thereupeutic agent cisplatin on ultrastructure of blood-testis barrier have been shown in different studies [33, 34]. These studies have indicated that reproductive surgery and various chemical agents degenerate germ cells, integrity of Sertoli cells, reduce sperm production, and thereby cause subfertility or infertility.

In these experimental models, the degeneration of germ cells and the blood-testis barrier have been shown to be reversible or irreversible, which also give an information for the development of male contraceptives.

Conclusion

In conclusion, Sertoli cells have crucial role in the maintenance of spermatogenesis. Various experimental studies show the deleterious effects of environmental factors such as hiperthermia, radiation and electromagnetic waves, toxic substances (cadmium chloride, arsenic, etc.), chemotherapeutic agents (cisplatin, metothrexade, etc.), vasectomy operation, high calorie diet on the tight junction as well as the gap junction and ectoplasmic specialisations. Damage to the junctional complexes may also play a role in the development of male infertility. Demonstrating how the blood testicular barrier as well as other junctional complexes are affected in various experimental testicular injury models shows that it may give an information in both the prevention of male infertility and the development of male contraceptive methods.

Financial support: The authors have no relevant financial information to disclose.

Conflict of interest: The authors have no potential conflicts to declare.

Authors' contributions: FE: Conception and design, MAE and FE: Formal analysis and investigation, FE and MAE: Writing-review and editing. Both authors approved the final version of the article.

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