ORIGINAL STUDY

VIA MEDICA

DHT deficiency perturbs the integrity of the rat seminiferous epithelium by disrupting tight and adherens junctions

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Abstract: In rats with a DHT deficiency induced by finasteride, morphological changes in the seminiferous epithelium were observed. The structural alterations were manifested by the premature germ cells sloughing into the lumen of seminiferous tubules. The etiology of this disorder could be connected with intercellular junctions disintegration. We showed in the immunohistochemical study the changes in expression of some proteins building tight and adherens junctions. The depression of N-cadherin, β-catenin and occludin immunoexpressions could be the reason for the release of immature germ cells from the seminiferous epithelium. However, the observed increase of the immunohistochemical reaction intensity of vinculin, one of the cadherin/catenin complex regulators, could be insufficient to maintain the proper function of adherens junctions. The hormonal imbalance appears to influence the pattern of expression of junctional proteins in the seminiferous epithelium. It could lead to untimely germ cells sloughing, and ultimately could impair fertility. *(Folia Histochemica et Cytobiologica 2011, Vol. 49, No. 1, 62–71)*

Key words: DHT-deficiency, intercellular junctions, testis, rat

Introduction

Spermatogenesis is defined as a process whereby germ cells divide, and differentiate into maturing spermatozoa in the company of Sertoli cells [1]. In the epithelium, Sertoli cells support nutrition and proper hormones concentration for germ cell development. Between the lateral processes of neighboring Sertoli cells, tight junctions (TJs) exist that divide the seminiferous epithelium into a basal compartment containing diploid (2n) spermatogonia, preleptotene/leptotene spermatocytes and an adluminal compartment containing more mature, haploid (1n) spermatocytes and spermatids [2]. TJs are constituted by several TJ-integral membrane proteins such as occludin, claudin and junctional adhesion molecules (JAMs). The Sertoli-Sertoli cell TJs constitute an important element of the blood-testis barrier (BTB). However, within the BTB complex of various intercellular adherens junctions (AJs) are also present [3–5].

The physical division of the SE into basal and adluminal compartments is based on the localization of the BTB; therefore the intercellular junctions between adjacent cells are classified according to their localization and the type of attachment cytoskeleton molecules [6]. In both basal and adluminal compartments of the seminiferous epithelium, adherens junctions (AJs) exist. Adherens junctions are actin-based junctions (ectoplasmic specialization, tubulobulbar complex) or intermediate filaments-based junctions (desmosome-like junction) [6]. The ectoplasmic specializations (ESs) are the most studied actin-based testis-specific type of AJs, and are regarded as very important for proper spermatogenesis. The basal ESs support the thigh junctions of the BTB structure between adjacent Sertoli cells [7], whereas the apical ESs between Sertoli cell and spermatids (round/elongating/elongated) participate in intercellular adhesion and movement of germ cells through the SE, as well as contributing to the orientation, positioning and correct shape of

the forming spermatozoa head [8]. The complex of various molecules among the integral membrane junctional components of ESs is present [9–13]. The best recognized is the cadherin/catenin complex that is found in both apical and basal ESs [6]. In the basal ESs, β-catenin co-localizes with N-cadherin [14] and at the ESs site, vinculin, actin-binding protein is located. Vinculin, as a putative substrate for protein kinase, is involved in junction assembly, stabilization and regulation [6, 15]; therefore vinculin is mentioned as a crucial regulator of the cadherin/ /catenin complex.

TJs and AJs strictly interact together to form a very firm, tight and non-leaking barrier that provides a specific microenvironment for developing germ cells by sequestering proteins from the interstitial systemic circulation before they enter the seminiferous epithelium [6, 16, 17]. The barrier participates in isolation of the post-meiotic germ cells from the self-immune system and avoids autoimmune response development [18]. Consequently, the testis is known as an immunologically privileged organ [19]. The main function of intercellular junctions between Sertoli and germ cells is giving the immature germ cells (preleptotene and leptotene spermatocytes) permission to migrate progressively through the height of the SE, from the basal to the adluminal compartment, traversing the BTB [6, 20]. The structure of intercellular junctions, participating in the passage of germ cells throughout the epithelium, is changing during the SE cycle and depends on hormonal balance [18, 20–22].

The male gonad is an androgen-dependent organ. In androgen target tissues, testosterone (T) can be irreversibly reduced into more active dihydrotestosterone (DHT). The process is catalyzed by 5α-reductase (5α-red) that exists in two isoforms: 5α-reductase type 1 (5α-red1) and type 2 (5α-red2) [23]. Some authors have estimated by northern blot analysis that immature rat testes have higher levels of 5α-red1 then those of the adult [24]. The expression of 5α-red2 in the male gonad was detectable at any age [25]. Other studies have strongly suggested that the major isoenzyme present in testicular tissues is 5α-reductase type 2 [26–29]. Therefore, in our study (carried out with mature rats), we inhibited the 5α-red2 activity by finasteride, which is used in basic and clinical studies as the one of the best inhibitors of the enzyme [23, 30]. In our previous studies, we had found that 28 and 56 day DHT-deficiency altered the androgen receptor (AR) [31], estrogen α and β receptors (ERα, ERβ) in epididymis [32], antioxidant enzymes (GPX5, E-SOD, Cu/Zn SOD) [33] and inducible nitric oxide synthase (iNOS) [34] expression patterns. The changes in the morphology of testes were noticed after a long period (56 days) of androgen imbalance [35].

Because of that, the aim of this current study was to examine the immunolocalization of AJ components: β-catenin, N-cadherin, their regulator — vinculin and TJ occludin in the seminiferous epithelium of rat testis during DHT deficiency.

Materials and methods

Animals

Sexually mature male Wistar rats (three months old) were housed singly, kept on a 12 hours of light, 12 hours of dark cycle and given food and water *ad libitum*. The study was approved by the Local Ethics Committee for Scientific Experiments on Animals in Szczecin (Poland). The animals were randomly divided into a control group (ten animals) and two experimental (finasteride-treated rats) groups (five animals per group). Finasteride (Proscar®, MSD Sweden) was administered *per os* at a dose of 5 mg/kg b.w./day for 28 days — two cycles of the seminiferous epithelium (the first experimental group — Fin28) and for 56 days — the total time of spermatogenesis (the other experimental group — Fin56). After the experiment, the rats were anesthetized with thiopental (Biochemie GmbH, Austria), administered in a dose of 120 mg/kg b.w. intraperitoneally. The testes were fixed in 4% buffered formalin and processed for embedding in paraffin for immunohistochemistry and histological staining.

PAS method

The paraffin-embedded tissue samples were stained with PAS (Periodic Schiff Acid) technique according to McManus described by Totty [36] assess the seminiferous tubules morphology and to visualize the acrosome that helps establish the stages of seminiferous cycles.

Immunohistochemistry (IHC)

The paraffin-embedded sections (3–5 μm) of rat testes were immunostained for visualization of chosen components of intercellular junctions. The IHC was performed using specific primary antibodies: for adherens junction: anti-vinculin, anti-N-cadherin, anti--beta-catenin; for tight junction: anti-occludin (Table 1). The deparaffinized sections were microwave irradiated in citrate buffer (pH 6.0) to heat induced epitope retrieval. After slow cooling to room temperature, slides were washed in PBS twice for five minutes each and then incubated for 30 minutes with primary antibodies. Then, sections were stained with

Table 1. Antibody specifications

Antibody/clone	Antibody	Dilution	Producer/cat. no.
Vinculin/h-Vin1	Mouse monoclonal	1:200	Sigma Chemical Corporation/V-9131
N-cadherin/CDH2	Rabbit polyclonal	1:100	Santa Cruz Biotechnology/sc-7939
Beta-catenin/CATNB	Rabbit polyclonal	1:100	Santa Cruz Biotechnology/sc-7199
Occludin/H-279	Rabbit polyclonal	1:100	Santa Cruz Biotechnology/sc-5562

an avidin-biotin-peroxidase system with diaminobenzidine as the chromogen (DAKO (HRP; rabbit/mouse/goat (DAB+) code no. K0679; DakoCytomation) in conformity with the staining procedure instruction included in Dako LSAB+ System-HRP. Sections were washed in distilled water and counterstained with hematoxylin. Our investigation made comparisons between stages I or II, VII or VIII and X–XII of the SE cycle of control and two experimental groups were made. For negative controls, specimens were processed in the absence of a primary antibody. Positive staining was defined microscopically by visual identification of brown pigmentation.

Results

PAS method

After 28 days of finasteride treatment, the seminiferous tubules (Figure 1B) showed normal morphology, similar to that observed in the control rats (Figure 1A). There were all generations of germ cells, characteristic of the appropriate stage of the seminiferous epithelium cycle (Figure 1A, B). After 56 days of finasteride treatment, the cross-sections of the seminiferous tubules showed the presence of sloughed immature germ cells in the lumen (Figure 1C), mainly spermatids in different steps of development (Figure 2A–D), rarely pachytene spermatocytes (Figure 2B). Empty areas within the SE were noticed (Figures 1C, 2A–C).

Immunohistochemistry

In all studied groups (control, Fin28, Fin56) the junctional proteins, within the cells of the SE, were expressed with different intensity (Figures 3–6).

N-cadherin

The most intensive N-cadherin expression was visible in the basal part (white asterisks) of the SE, (spermatogonia A1, intermediate spermatogonia, pachytene spermatocytes) in stages II (Figure 3A)

and VIII (Figure 3D) of the SE cycle in the testes of control rats. The immunoexpression of N-cadherin was quite low in round spermatids steps 2nd and 8th (Figures 3A, D; black arrows). Much more intensive immunoreaction was noticeable in the steps 16th and 19th elongated spermatids (Figures 3A, D; blue arrows). Intensive N-cadherin expression was visible mainly between zygotene spermatocytes (ZS) (white arrows) and steps 12th–13th elongating spermatids (blue arrows) in stage XII of the SE cycle of control rats (Figure 3G). The intensity of IHC reaction was lower in areas of epithelium in which PS were present (Figure 3G; black arrow). The product of IHC-reaction was weakly visible in both finasteride-treated groups (Figures 3B, C, E, F, H, I) in comparison to the control group. It was observed generally in the cells near the basal membrane (white asterisk) and in the elongating spermatids of the 16th (Figures 3B, C; blue arrows), 19th (Figures 3E, F; blue arrows) and 10th–12th (Figures 3H, I; blue arrows) steps. Moreover, the sloughed immature germ cells into the seminiferous tubules lumen of 56Fin rats were also immunopositive (Figures 3C, F; green asterisks).

β-catenin

The β-catenin expression in the testes of the control rats, in all examined stages of the SE (Figure 4A, D, G), was evident at the basal part of epithelium (Figure 4A, D, G; white asterisks). Brown color, indicating the presence of β-catenin, encircled round spermatids at the II (Figure 4A; black arrows) and VIII (Figure 4D; black arrows) stages of the SE cycle. The elongating spermatids of 11th–12th steps (Figure 4G; blue arrows) and elongated spermatids (Figures 4A, D; blue arrows) showed a very weak positive reaction.

The pattern of β-catenin immunoexpression within the SE of Fin28 (Figures 4B, E, H) and Fin56 (Figures 4C, F, I) rats was similar to the control, but intensity of β-catenin immunoexpression was significantly decreased. However, the step 19th spermatids (just prior to spermiation) of the Fin56 group appear immunopositive (Figure 4F; blue arrows).
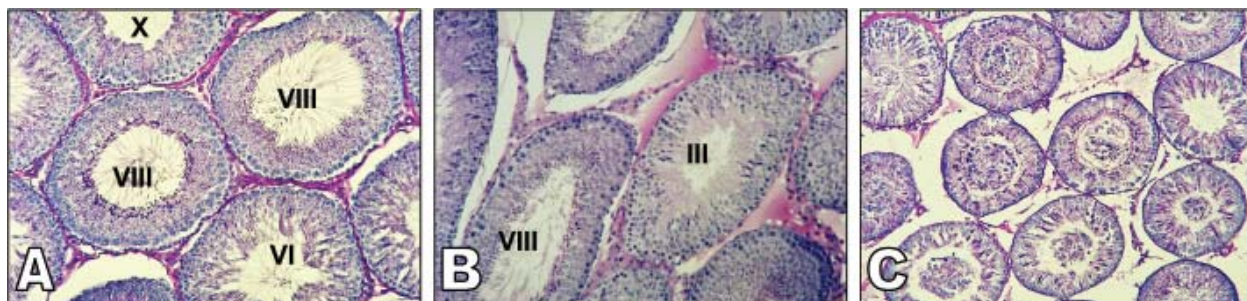
Figure 1. Cross-sections of a control rat testis. **A.** Stages VI, VIII and X of the seminiferous epithelium. Within the tubules, all germ cells generations appropriate for particular stages of the seminiferous epithelium cycle are present; **B.** Testis of a Fin28 rat. Unchanged structure of the seminiferous tubules. Stages III, VIII of the seminiferous epithelium cycle; **C.** The prematurely sloughed germinal cells visible in the lumen of seminiferous tubules of a Fin56 rat. Objectives magnification: A, B × 20; C × 10
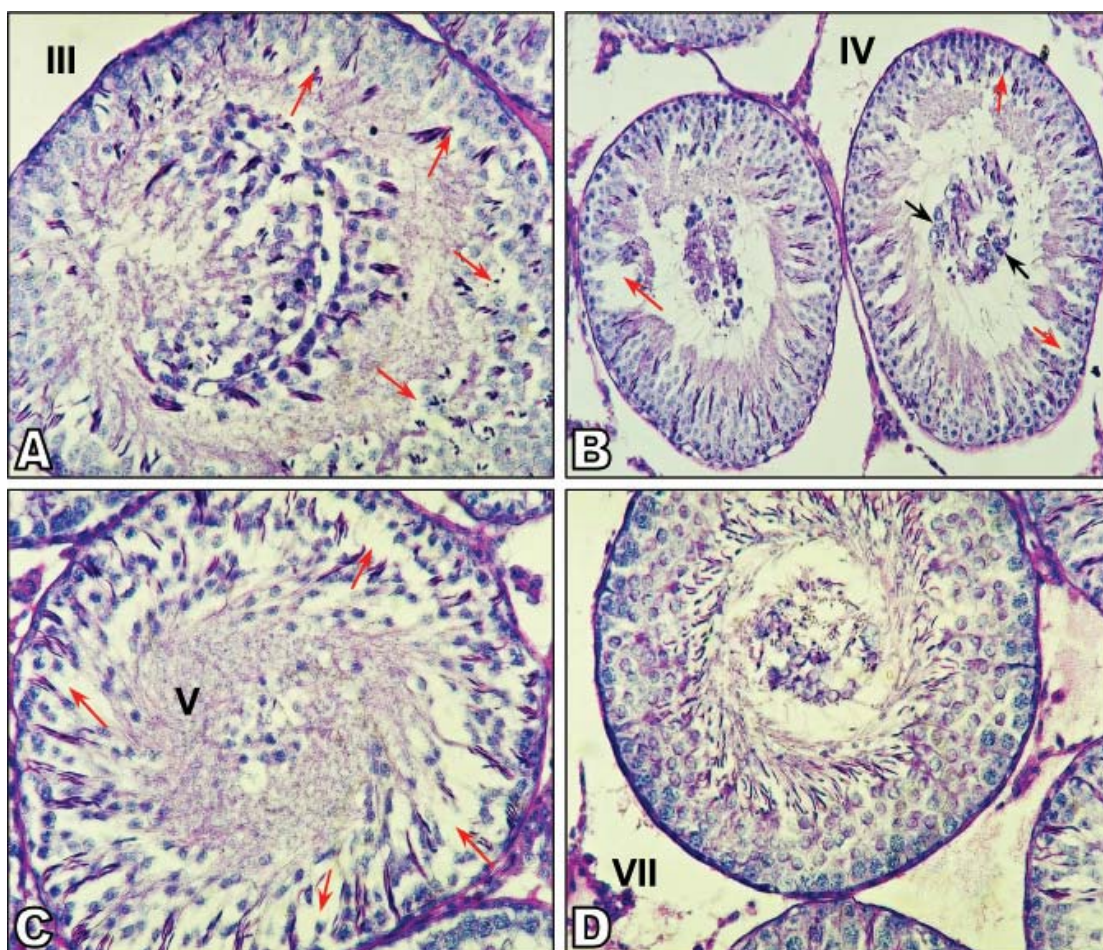

Figure 2. Cross-sections of the seminiferous tubules of a rat which has received finasteride for 56 days. **A.** Stage III of the SE cycle. In the lumen of tubules sloughed spermatids step 3rd (red arrows) are visible; **B.** Stage IV of the SE cycle. In the lumen of tubules, sloughed step 4th spermatids (left tubule) and pachytene spermatocytes (black arrows), step 17th spermatids (right tubule) are present. Within the SE empty spaces (red arrows) are visible; **C.** Stage V of the SE cycle with empty spaces (red arrows) and sloughed into the lumen step 5th spermatids; **D.** Stage VII of the SE cycle with sloughed step 7th spermatids in the lumen of tubules. Objectives magnification: B × 20; A. C, D × 40
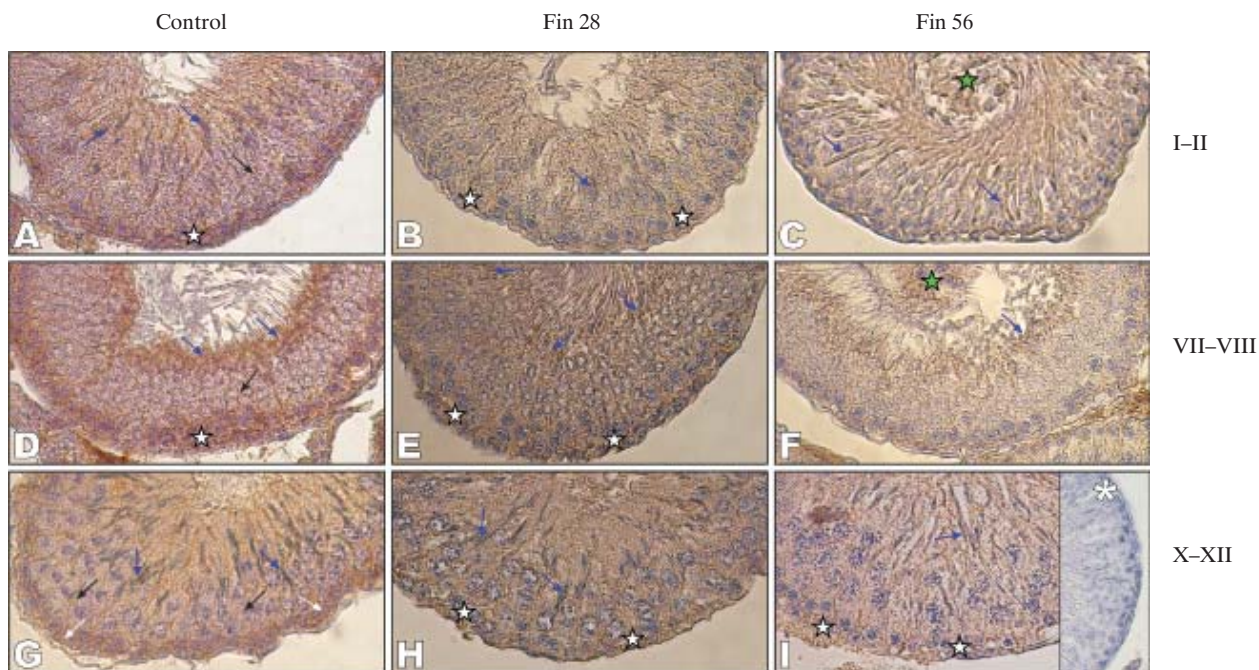
Figure 3. The immunoexpression of N-cadherin in the testes of control (**A, D, G**), Fin28 (**B, E, H**) and Fin56 (**C, F, I**) rats. The seminiferous tubules in stages I or II (**A, B, C**), VII or VIII (**D, E, F**) and X–XII (**G, H, I**) of the SE cycles. No immunostaining in seminiferous epithelium in negative control of IHC reaction with omitting of primary antibody (I — insert *). Objective magnification: A–I × 40. The arrows and asterisks legends: blue arrows — steps 10th–12th elongating SD (**H, I**), 16th (**A–C**) and 19th (**D–F**) elongated SD; black arrows — steps 2nd (**A**), 8th (**D**) round SD and 12th (**G**) elongating SD; white arrows — ZS (**G**); white asterisks — basal part of seminiferous epithelium (**A–F, H, I**); green asterisk — sloughed immature germ cells (**C, F**)
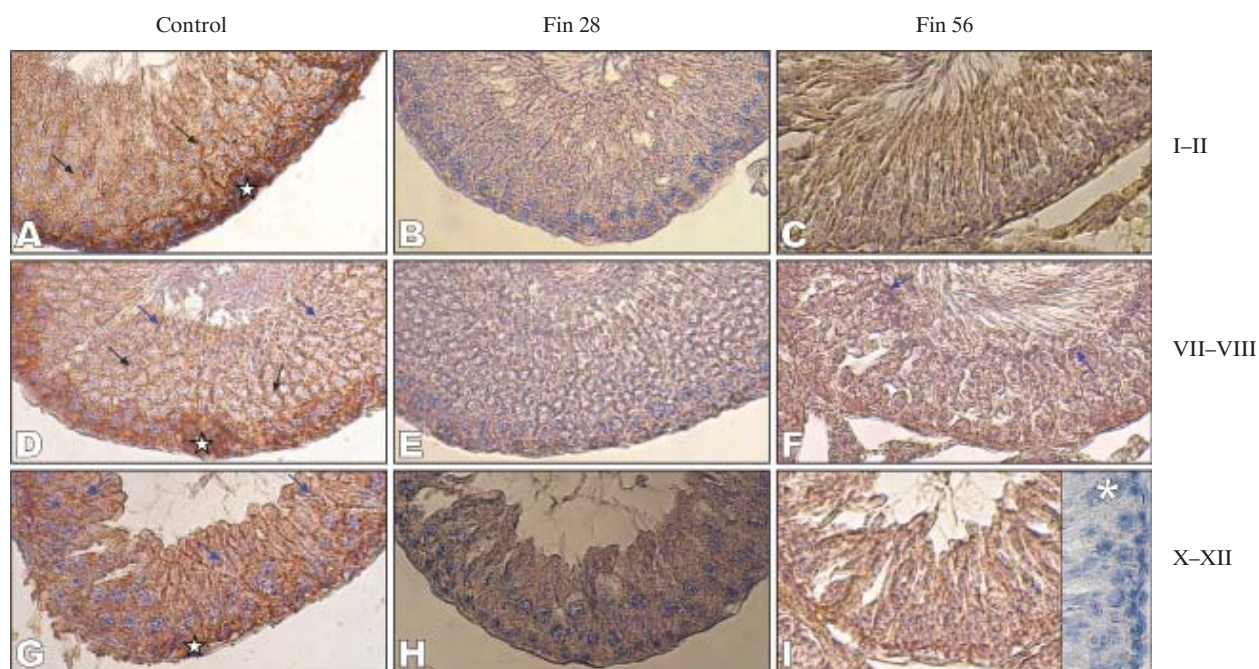
Figure 4. The immunoexpression of β-catenin in the testes of control (**A, D, G**), Fin28 (**B, E, H**) and Fin56 (**C, F, I**) rats. The seminiferous tubules in stages I or II (**A, B, C**), VII or VIII (**D, E, F**) and X–XII (**G, H, I**) of the SE cycles. No immunostaining in seminiferous epithelium in negative control of IHC reaction with omitting of primary antibody (I — insert *). Objective magnification: A–I × 40. The arrows and asterisks legends: blue arrows — steps 10th–12th (**G**) elongating SD, 16th (**A**) and 19th (**D, F**) elongated SD; black arrows — steps 2nd (**A**), 8th (**D**) round SD; white asterisks — basal part of SE (**A, D, G**)

Vinculin

The immunoexpression of vinculin in the testes of control rats was weakly marked (Figures 5A, D, G). Brown pigmentation, indicating the positive statement of immunoreactions, has the strandline pattern extending throughout the SE height. The IHC reaction was visible in the Sertoli — spermatids attachment site in stages I–II (Figure 5A; red arrows) and X–XII (Figure 5G; red arrows) of the SE cycle.

The vinculin immunoexpression increased in finasteride-treated rats' testes (Figures 5B, C, E, F, H, I). Therefore, the radial motif was better evident in the seminiferous tubules cross-sections, particularly in the Fin56 group (Figures 5C, F, I; red arrows), mainly in stages VII–VIII (Figure 5F) of the SE cycle. Additionally, the basal compartment of the SE (spermatogonia, preleptotene spermatocytes) also shows a strong positive product of IHC reaction (Figures 5B, C, E, F, H, I; white asterisks).

Occludin

The immunoexpression of occludin was prominent in the basal part of control rats SE (Figures 6A, D, G; white asterisks). The brown pigmentation, which confirms the positive score of immunoreaction, was located in the area enveloping the primary spermatocytes (preLS, ZS, PS). Both round (Figures 6A, D; black arrows) and elongating/elongated spermatids (Figures 6A, D, G; blue arrows) demonstrate rather low occludin expression.

The level of occludin declined in the testes of rats in the finasteride-treated groups (Figures 6B, C, E, F, H, I) independently of SE cycle stages, much more within long-term DHT deficiency group (Figures 6C, F, I). In the Fin28 group, the positive score of immunoreactions was visible only in a few primary spermatocytes (Figures 6E, H; red arrows).

Discussion

After the first meiotic division of primary spermatocytes, the haploid cells are produced. Consequently, secondary spermatocytes, differentiating spermatids and spermatozoa could be recognized by the immune system as a foreign body. It means that, at the same time, germ cells have to divide and traverse the seminiferous epithelium according to the periodic changes of junctions disassembly and assembly without altering the immunological barrier function [37]. Therefore, the integrity of the blood-testis barrier is extremely essential for separation of the haploid reproductive cells from the immunocompetent cells, and the proper function of the other junctions is required for appropriate arrangement of germ cells within the lateral processes of Sertoli cells and their passage through the epithelium. The germ cells movement across the SE is associated with remodeling of adherens and thigh junctions [6, 38], and in the rat takes place at late stage VIII through early stage IX of the seminiferous epithelium cycle [6, 20].

Very early investigations showed that round spermatids (pre-step 8th spermatids in rat), and less-differentiated germ cells types are loosely attached to Sertoli cells by desmosome-like junctions [22, 39–42]. It was also shown that at stage VIII of the SE cycle in rats, the step 8th spermatids are attached to the Sertoli cell by unique, highly specialized, actin-based cytoskeletal structures — ectoplasmic specializations [41, 42]. Elongating spermatids (post-step 8th spermatids in the rat) are associated with Sertoli cells by the extremely tight ectoplasmic specialization [21, 22]. An *in vitro* study indicated that the cytoskeleton of junction-related structures of Sertoli cells that join pre-step 8th spermatids are different than those observed between the Sertoli cell and post-step 8th spermatids [43]. The ectoplasmic specializations also exert an important role in remodeling BTB [38]. At stage VIII of the rat's SE cycle, the active laminin fragments of apical ESs induce blood-testis barrier disruption directly, by reducing the steady-state levels of integral membrane proteins at the BTB [38].

Similarly, cytokines (TNFα, TGF-β2, TGF-β3) and testosterone induce protein endocytosis at BTB. The factors mentioned above 'de-stabilize' the BTB, facilitating the transit of preleptotene spermatocytes. Testosterone promotes BTB integrity, but also can enhance *de novo* synthesis of TJ-proteins, as well as promote transcytosis of endocytosed proteins from 'old' disrupted TJ-fibrils of above the primary preleptotene spermatocytes to 'new' assembly TJ-fibrils below the pachytene spermatocytes [38].

The results of our study indicate that dihydrotestosterone, a more active metabolite of testosterone, could play an important role in the physiology of junctions complex of BTB. Although we did not notice an alteration in the morphology of SE of rats with 28-day-long DHT deficiency, the changes of immunoexpression of occludin and N-cadherin/β-catenin/vinculin were perceptible and similar to those in Fin56 rats. Moreover, a long period of finasteride treatment caused changes in the morphology of the SE. This concords with the observations of O'Donnell et al. [44–46] who proved suppressed spermatogenesis mainly at the spermatids conversion from step 7th to step 8th during deficit of DHT. In our study, we also detected that finasteride-induced DHT deficiency lasting the period of one spermatogenesis (56 days) resulted in sloughing immature germ cells in 7th step, but also an earlier (3rd–5th) and later (17th) steps of spermatids differentiation. Occasionally, we noticed
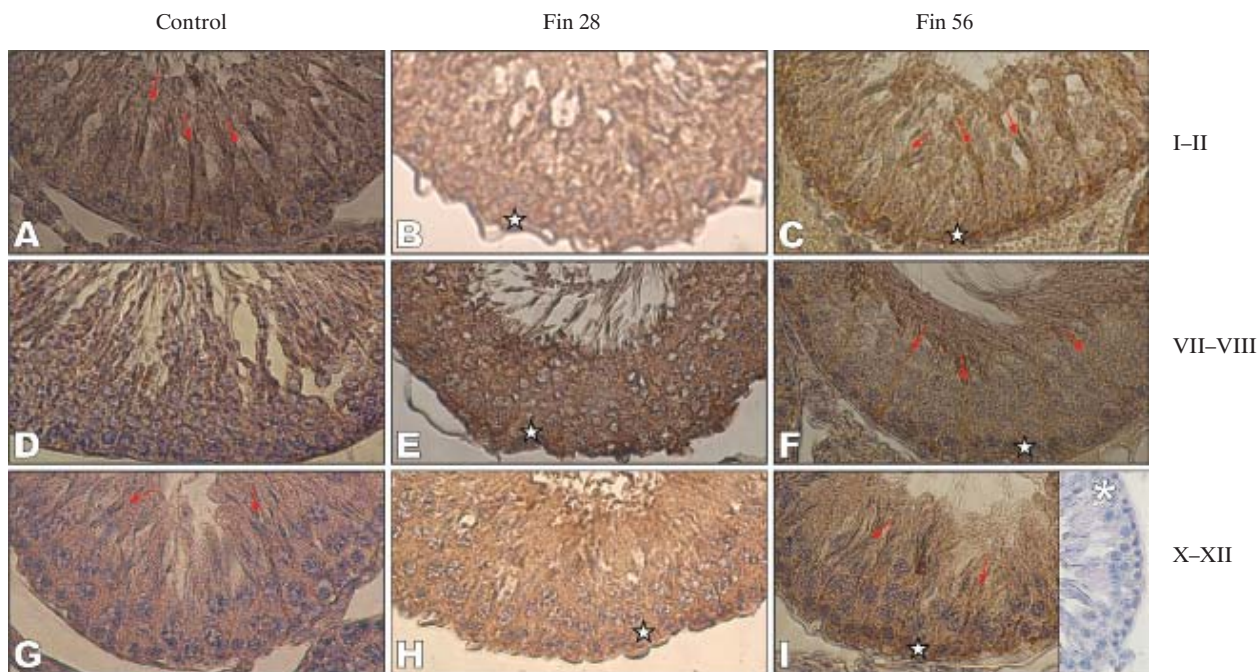
Figure 5. The immunoexpression of vinculin in the testes of control (**A**, **D**, **G**), Fin28 (**B**, **E**, **H**) and Fin56 (**C**, **F**, **I**) rats. The seminiferous tubules in stages I or II (**A**, **B**, **C**), VII or VIII (**D**, **E**, **F**) and X–XII (**G**, **H**, **I**) of the SE cycles. No immunostaining in seminiferous epithelium in negative control of IHC reaction with omitting of primary antibody (I — insert *). Objective magnification: A–I × 40. The arrows and asterisks legends: red arrows — radial motif of vinculin immunoexpression (**A**, **C**, **F**, **G**, **I**); white asterisks — basal compartment of SE with SG and pre-meiotic spermatocytes (**B**, **C**, **E**, **F**, **H**, **I**)
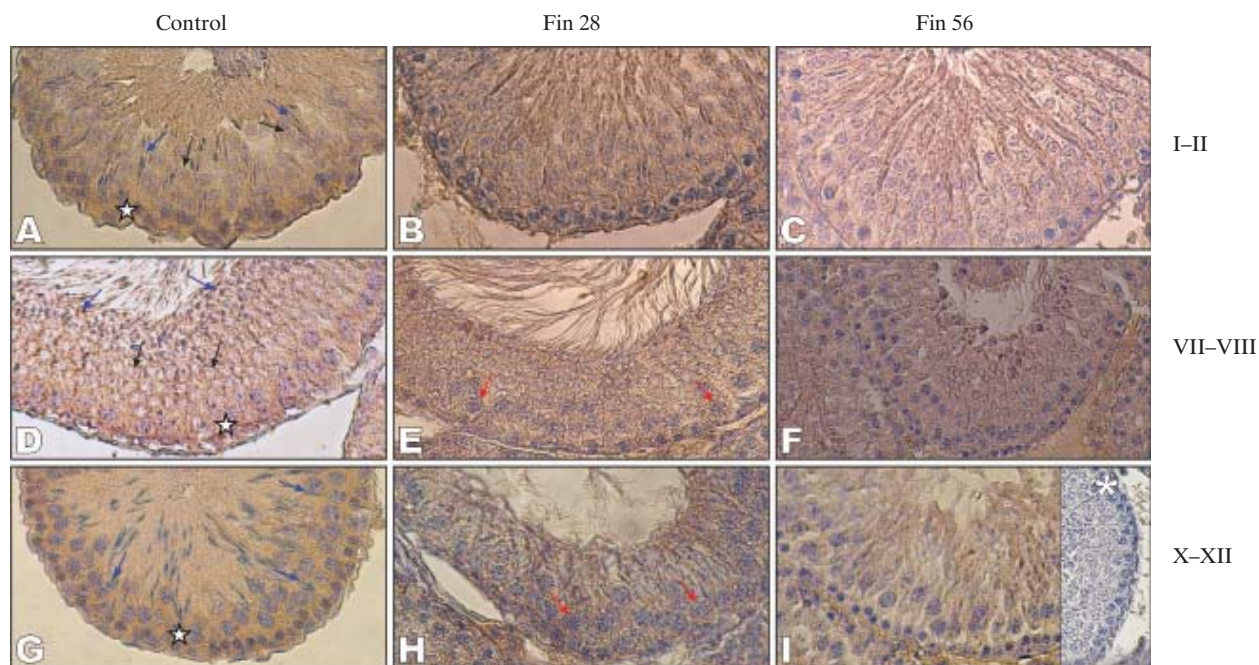
Figure 6. The immunoexpression of occludin in the testes of control (**A**, **D**, **G**), Fin28 (**B**, **E**, **H**) and Fin56 (**C**, **F**, **I**) rats. The seminiferous tubules in stages I or II (**A**, **B**, **C**), VII or VIII (**D**, **E**, **F**) and X–XII (**G**, **H**, **I**) of the SE cycles. No immunostaining in seminiferous epithelium in negative control of IHC reaction with omitting of primary antibody (I — insert *). Objective magnification: A–I × 40. The arrows and asterisks legends: black arrows — steps 2nd (**A**), 8th (**D**) round SD; red arrows — primary spermatocytes; blue arrows — steps 10th–12th (**G**) elongating SD, 16th (**A**) and 19th (**D**) elongated SD; white asterisks — basal part of SE (**A**, **D**, **G**)

the presence of pachytene spermatocytes in the lumen of tubules. A proper level of DHT is important, because it is involved in maintaining the spermatogenesis during very low androgen concentrations (10–20% of the norm) in gonads [47]. The ultrastructural study by O'Donnell et al. [48] showed that ectoplasmic specializations are normal in the SE of adult rats with testosterone suppressed by low-dose T and E2 treatment, and the detachment of the premature step 8th spermatids from Sertoli cell [49] was caused probably by defects in adhesion molecules such as epsin, actin and vinculin between round spermatids and Sertoli cells [48]. We observed an increase of vinculin immunoexpression that was much more visible in the SE of rats treated with finasteride for 56 days. However, the other proteins of adherens junctions studied by us display a somewhat contrary tendency. Because vinculin is considered as the regulator of the cadherin/catenin complex [6, 15], we hypothesised that during the decline in cadherin and catenin expression, the increase of vinculin could be one of the protective mechanisms for preserving as well as possible the function of the SE in hormonal imbalance condition. However, this hypothesis needs more detailed examination.

Lee et al. [50] suggested that interactions between Sertoli cells and germ cell are crucial for expression of β-catenin. So, the reduction of its immunoexpression in our study could be correlated with a decrease of the germ cell adhesive capacity.

The main function of ectoplasmic specializations is to facilitate germ cell movement, but additionally the ESs have an anchoring function to retain germ cells, particularly spermatids in the epithelium until spermiation [7, 16, 42]. Therefore, some hormonal disruption, like DHT deficiency, leads to change in the immunoexpression of structural (cadherin/catenin) and regulatory (vinculin) junctional proteins and finally affects the integrity of ESs and perturbs its functions mentioned above, as well as possibly influencing remodeling of the blood-testis barrier [38].

Our study also found changes in occludin immunoexpression. The occludin decline could be the next reason for the immature germ cell sloughing into the lumen of seminiferous tubules of finasteride-treated rats. Literature data confirming regulation of occludin expression by androgen is scarce. It has been documented that TJ dynamics in the testis is regulated by nitric oxide synthase (NOS) [51]. During testes maturation, the mRNA levels of iNOS and eNOS in both Sertoli and germ cells are changing. In the maturating Sertoli cells, the iNOS expression dramatically increased compared to eNOS and in germ cells the iNOS mRNA plunged, whereas the eNOS mRNA level rose during maturation. Taken together, these findings illustrate the different responses of NOS isoforms in Sertoli and germ cells during testes maturation, and suggest the important biological function of NO to maintain testis function [50].

Our previous experiment indicated that finasteride-induced DHT deficiency intensified expression of iNOS in most rat testicular cells [34]. It can be assumed that DHT deficiency, through the elevated NO production, causes the decline of occludin immunoexpression, and thereby germ cell sloughing. Moreover, the tight junctions secure the isolation of spermatozoa from the immune system, so the increase of BTB leaking could induce local inflammation in the male gonad. We observed that during finasteride-induced hormonal disturbance, the immunoexpression of IL-6, a cytokine considered as a pro-inflammatory factor, increased (data not shown). Therefore, the DHT deficiency could cause the local inflammatory process with IL6 participation and might negatively affect spermatogenesis in the testis.

In conclusion, our data indicates that finasteride-induced DHT deficiency changes the expression of junctional proteins involved in the building of tight and adherens junctions in the seminiferous epithelium of rats. Therefore, it can be expected that the structure and function of the blood-testis barrier can be regulated by DHT. Imbalance in androgens homeostasis can lead to untimely release of immature germ cells from the SE into seminiferous tubules lumen, and consequently can decrease the number of spermatozoa produced by the testis and thus decrease male fertility.

Abbreviations

AJ — adherens junction; 5α-red — 5α-reductase; 5α-red1 — 5α--reductase type 1; 5α-red2 — 5α-reductase type 2; AR — androgen receptor; BTB — blood-testis barrier; Cu/Zn SOD — copper//zinc superoxide dismutase; DHT — dihydrotestosterone; E2 — estradiol; E-SOD — extracellular superoxide dismutase; ERα — estrogen receptor α; ERβ — estrogen receptor β; ES— ectoplasmic specializations; GPX5 — epididymal specific glutathione peroxidase 5; IHC — immunohistochemistry; LC — Leydig cell; NO — nitric oxide; NOS — nitric oxide synthase; iNOS — inducible nitric oxide synthase; eNOS — endothelial nitric oxide synthase; PS — pachytene spermatocyte; preLS — preleptotene spermatocyte; SC — Sertoli cell; SD — spermatid; SE — seminiferous epithelium; SG — spermatogonium; T — testosterone; TJ — tight junction; ZO — zonula occludens; ZS — zygotene spermatocyte

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