Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Colchicine induces epithelial disruption associated with transient MAPK/ERK suppression in the mouse proximal epididymis

Ye-Seol Park
Kongju National University
Sung Woo Kim
sungwoo@korea.kr
National Institute of Animal Science
Bongki Kim
Kongju National University

Research Article

Keywords: colchicine, epididymis, ERK signaling, apoptosis, aquaporin-9

Posted Date: November 12th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11560107/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Abstract

Although colchicine is a widely used therapeutic drug, its effects on the epididymis remain unclear. Because the epididymis is essential for sperm maturation and male fertility, maintaining its structural and functional integrity is critical. This study investigated the effects of colchicine on the epididymal epithelium, focusing on ERK signaling, apoptosis, and epithelial cell-specific alterations in the initial segment (IS). Male mice were administered colchicine, and epididymal tissues were analyzed immediately after treatment and following a recovery period. Immunofluorescence staining was performed to assess ERK phosphorylation, apoptosis, and epithelial markers: keratin 5 (KRT5) for basal cells, aquaporin-9 (AQP9) for principal cells, and B1-V-ATPase for clear cells. Colchicine treatment substantially reduced phosphorylated ERK (pERK) levels, increased apoptosis, and induced epithelial atrophy. Basal cells lost their luminal-reaching projections, and principal cells showed a striking reduction in AQP9 expression. In contrast, the number of B1-V-ATPase-positive clear cells remained unchanged. After recovery, pERK expression and basal cell projections returned to control levels, and epithelial height was partially restored. However, AQP9 expression remained suppressed, indicating incomplete recovery of principal cell-associated function. These findings demonstrate that colchicine induces both transient and persistent alterations in the epididymal epithelium. Although pERK expression and basal cell projections recovered during the recovery period following colchicine treatment, AQP9 expression remained markedly reduced. Given the widespread therapeutic use of colchicine, these findings provide preclinical evidence of potential epididymal effects and support further investigation into their functional consequences for sperm maturation and male reproductive health.

1. Introduction

Male infertility is primarily caused by impaired sperm motility and failed sperm-oocyte interactions. An appropriate epididymal environment is essential for sperm to acquire normal functionality. The epididymis is a highly convoluted duct that connects the testis to the vas deferens through the efferent ducts and is divided into three major regions: the caput, corpus, and cauda. Each region provides a unique microenvironment through the synthesis and secretion of region-specific proteins that are crucial for sperm maturation, protection, storage, and transport [1–3]. In mice, the caput epididymis is further subdivided into the initial segment and the caput proper. The initial segment acts as a primary barrier against external influences entering the epididymis, and its absence prevents normal sperm maturation [4]. Due to its pivotal role in male fertility, recent studies have increasingly focused on the morphological and functional characteristics of the mouse initial segment.

The epididymal duct comprises multiple epithelial cell types that regulate the internal microenvironment by secreting and transporting ions and nutrients. Of these, three major epithelial cell types (principal, clear, and basal cells) play critical roles in epididymal function. Principal cells, the predominant epithelial cell type, contribute to luminal homeostasis by synthesizing and secreting essential proteins required for sperm maturation and storage. These cells express cystic fibrosis transmembrane conductance regulator (CFTR) and aquaporin-9 (AQP9) on their apical membrane, which contribute to ion aand water

transport and luminal fluid homeostasis [5–8]. Clear cells maintain an acidic luminal environment through apically localized vacuolar ATPases (V-ATPases) [9] and are also involved in endocytosis, removing excess cytoplasmic droplets and residual materials from the sperm [10]. Although less studied, basal cells have been suggested to play a role in immune responses [11]. However, dendritic cells function independently of basal cells in the epididymis [12]. Additionally, basal cells extend cytoplasmic projections through tight junctions, allowing them to monitor the luminal composition [12, 13].

The acidic luminal environment of the epididymis is essential for maintaining the sperm in a quiescent state and ensuring proper maturation. This acidification is regulated by coordinated interactions between the principal, clear, and basal cells. CFTR-mediated bicarbonate release from principal cells promotes V-ATPase accumulation in clear cells, which secrete protons into the lumen to lower pH [7, 9]. Basal cells further modulate this process by releasing nitric oxide, which enhances the V-ATPase activity in neighboring clear cells [14]. These coordinated functions establish the optimal microenvironment for sperm storage and function.

Of the molecular pathways that regulate epididymal function, the mitogen-activated protein kinase (MAPK) signaling pathway plays a fundamental role in cellular metabolism, growth, differentiation, and apoptosis. This pathway follows a hierarchical cascade, with MAP4K activating MAP3K, which in turn phosphorylates MAPKK, leading to MAPK activation and subsequent MAPKAPK signaling. A key component of this pathway is the extracellular signal-regulated kinase (ERK) signaling cascade, in which ERK is activated through MEK (MAPK/ERK kinase)-mediated phosphorylation. The activated form of pERK is essential for the regulation of cell proliferation, survival, and mobility [15]. Dysregulation of the MAPK/ERK pathway has been implicated in various diseases including cancer, diabetes, and rheumatoid arthritis [16]. ERK overexpression has been detected in lung cancer [17], breast cancer [18], and gastric adenocarcinoma [19], which highlights its biological significance. In the epididymis, ERK expression is particularly high in the initial segment, where it plays a crucial role in cell proliferation, differentiation, and survival [20, 21]. Furthermore, ERK has been implicated in the regulation of tight and gap junctions between epithelial cells [22]. Tight junctions are essential for maintaining the blood-epididymis barrier (BEB), which protects the sperm from autoimmune responses and cytotoxic molecules [23–25]. These findings suggest that ERK signaling contributes to the maintenance of epididymal homeostasis and male fertility.

Colchicine is an alkaloid derived from *Colchicum* spp. and is widely used to treat acute gout, Behçet's disease, familial Mediterranean fever, chondrosarcoma, systemic sclerosis, and amyloidosis [26]. It binds to tubulin, disrupting microtubule assembly and leading to protein aggregation, inhibition of endocytosis/exocytosis, cytoskeletal reorganization, reduced cell motility, and mitotic arrest [27, 28]. Previous studies have reported colchicine-induced reproductive toxicity. Allard et al. [29] observed reduced testicular weight and structural abnormalities in rats after colchicine administration, whereas Correa et al. [30] demonstrated dose-dependent testicular toxicity. Additionally, colchicine exposure resulted in abnormal sperm heads and acrosome defects in mice, with increased sperm abnormalities

persisting for up to five days after administration [31]. Although the potential of colchicine as an anti-tubulin agent in cancer therapy has been investigated [32], its effects on the epididymis are unclear.

This study investigated the effects of colchicine on the structure and cellular characteristics of the epididymal epithelium in mice. By examining changes in pERK expression, epithelial morphology, apoptosis, and epithelial cell markers in the initial segment, this study aimed to characterize epididymal alterations associated with colchicine treatment. Given the widespread clinical use of colchicine, understanding its potential effects on the epididymis may provide relevant preclinical information regarding male reproductive health.

2. Materials and methods

2.1 Animals and Ethics Statement

This study was conducted using C57BL/6 male mice aged 8–10 weeks. All mice were housed under controlled conditions at 22–24°C, humidity of 50–60%, and a 12-hour light/dark cycle, with *ad libitum* access to food and water. All animal experiments were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Kongju National University (Approval number: KNU_2019-06) and conducted in accordance with ethical guidelines for animal research.

2.2 Colchicine Administration

Colchicine (Sigma-Aldrich, St. Louis, MO, USA) was administered via intraperitoneal (i.p.) injection at a dosage of 1 mg/kg. Mice in the colchicine-treated group received two injections 24-h apart. [33, 34]. Epididymal tissues were collected 24 h after the second injection (48 h after the first injection). In the recovery experiments, one epididymis was surgically removed at this same time point, and the contralateral epididymis was collected after an additional 72-h colchicine-free recovery period to evaluate epithelial recovery. This experimental design was adapted from previous studies investigating epididymal epithelial recovery and enabled paired assessment of treatment and recovery responses within the same animal [22]. A minimum of six mice per experimental condition were used for analysis.

2.3 Tissue Preparation

Epididymal tissue was fixed in 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS) at 4°C for 24 h. Following fixation, tissue samples were washed three times with PBS, then cryoprotected in 30% sucrose solution (in PBS) at 4°C for 24 h. Cryoprotected tissues were embedded in Optimal Cutting Temperature (OCT) compound (Tissue-Tek; Sakura Finetek, Torrance, CA, USA) and frozen for cryosectioning. Cryosections were prepared using a Leica 3050S cryostat (Leica Microsystems, Bannockburn, IL, USA) at thicknesses of 6 μm and 10 μm and mounted on Fisher Scientific slides (Pittsburgh, PA, USA). Prepared slides were stored at − 20°C until further analysis.

2.4 Immunofluorescence Staining

The cryosections were washed twice with PBS (5 min each) and subjected to antigen retrieval by incubating in 10 mM Tris/EDTA buffer (pH 9.0), followed by heat treatment in a microwave oven. Heat treatment was conducted in four cycles (2 min – 1 min – 1 min – 1 min), with a 10-min cooling interval between cycles. After cooling, the slides were incubated in 1% bovine serum albumin (in PBS) at room temperature for 1 h to block nonspecific binding.

To detect phospho-ERK expression, rabbit monoclonal Phospho-p44/42 MAPK (Erk1/2) antibody (Cell Signaling Technology, Boston, MA, USA) was used. To analyze apoptosis, rabbit monoclonal Cleaved Caspase-3 antibody (Cell Signaling Technology, Boston, MA, USA) was used. Epididymal macrophages were identified using rat monoclonal F4/80 antibody (Thermo Fisher Scientific, Rockford, IL, USA), and epithelial structure was visualized using rat monoclonal E-cadherin antibody (Thermo Fisher Scientific, Rockford, IL, USA). Basal cells were labeled with a rabbit monoclonal keratin 5 (KRT5) antibody (Thermo Fisher Scientific, Rockford, IL, USA). The water channel protein, AQP9, was detected using a rabbit polyclonal anti-AQP9 antibody (a gift from Dr. Breton, MGH, Boston, USA), and clear cells were identified based on B1-V-ATPase expression using rabbit polyclonal anti-B1-V-ATPase antibody (a gift from Dr. Breton, MGH, Boston, USA). After incubation with the primary antibody, the sections were treated with secondary antibodies (Jackson ImmunoResearch Laboratories, West Grove, PA, USA). The secondary antibodies used were Cy3-conjugated donkey anti-rabbit IgG, Cy3-conjugated donkey anti-rat IgG, and Alexa Fluor 488-conjugated donkey anti-rabbit IgG. The specificity of all the primary antibodies used in this study was validated by positive and negative control staining (Online Resource 1). All antibodies were diluted in 1% BSA, and slides were mounted using Vectashield (Vector Labs, Burlingame, CA, USA). Imaging was performed using a Zeiss confocal microscope (LSM800), and data analysis was conducted using Zen Blue Software, Adobe Photoshop, and ImageJ.

2.5 Hematoxylin and Eosin (H&E) Staining

For histological analysis, cryosections were subjected to hematoxylin and eosin (H&E) staining. The slides were sequentially immersed in a graded ethanol series (100%, 95%, 70%, and 50%) for rehydration, followed by washing with tap water. The sections were then stained in hematoxylin solution for 7 min, after which excess hematoxylin was removed by rinsing with tap water. To differentiate the nuclei, the slides were briefly immersed in 0.1% hydrochloric acid (HCl) for 3 s, followed by an additional tap water rinse. Sections were then counterstained with eosin for 2 min, dehydrated in 95% and 100% ethanol, and cleared in xylene. Finally, the coverslips were mounted using a toluene-based mounting medium.

2.6 Statistical Analysis

To quantitatively analyze the cellular changes induced by colchicine treatment, basal cell projections and clear cell populations were assessed using immunofluorescence staining. The proportion of KRT5-positive basal cells with luminal-reaching projections was quantified following KRT5 staining, and clear cells were quantified based on B1-V-ATPase-positive staining. Additionally, changes in AQP9 expression were evaluated by measuring fluorescence intensity in principal cells. To quantify epithelial cell apoptosis, at least six tubules from each section were analyzed. The epididymides of at least six mice

were examined per experimental group, and three cryostat sections were analyzed for each epididymis. All experiments were conducted using a minimum of six independent biological samples. Statistical analyses were conducted using one-way ANOVA followed by Tukey's HSD post hoc test. Data are presented as mean ± SEM. Statistical significance was indicated by asterisks in the graphs: **p < 0.01 and ***p < 0.001. ns indicates no significant difference.

3. Results

3.1 Decreased pERK Expression in the Initial Segment of the Epididymis Following Colchicine Treatment

To examine the effect of colchicine treatment on MAPK signaling, adult male mice were treated with colchicine (1 mg/kg/day, i.p.) for two consecutive days. This treatment led to a marked reduction in ERK1/2 phosphorylation in the initial segment (IS) of the epididymis compared to that in the controls (Fig. 1a, b). Immunofluorescence staining showed that phosphorylated ERK1/2 (pERK) was highly expressed in the IS of control mice, but was markedly reduced following colchicine treatment. In the recovery group, in which mice underwent a 72-h colchicine-free period after the final colchicine treatment, pERK1/2 expression in the IS returned to levels comparable to those in controls (Fig. 1c). Quantitative analysis showed that pERK1/2 expression was significantly reduced in the colchicine-treated group compared with the control group and significantly increased after the 72-h recovery period ($p < 0.01$), with recovery values returning to levels comparable to those in controls (Fig. 1d).

3.2 Morphological Changes in the Epididymal Epithelium Following Colchicine Treatment

Hematoxylin and eosin (H&E) staining revealed distinct histological differences in the initial segment (IS) of the epididymis between control and colchicine-treated mice. In colchicine-treated mice, the epithelial height of the IS was substantially reduced compared to that in the control group, and exfoliated cells and debris were prominently observed in the lumen (Fig. 2a, b, d). Additionally, the luminal diameter was increased following colchicine treatment (Fig. 2a, b, e). After a 72-h recovery period, the IS exhibited partial restoration of its morphology; epithelial height and luminal diameter showed improvement compared to the colchicine-treated group, but did not fully return to control levels (Fig. 2c, d, e). However, exfoliated cells and debris, which were evident in colchicine-treated mice, were no longer present after recovery (Fig. 2a–c). Statistical analysis confirmed significant differences in epithelial height and luminal diameter among all three groups ($p < 0.01$) (Fig. 2d, e).

3.3 Increased Apoptosis in the Epididymal Epithelium Following Colchicine Treatment

Immunofluorescence staining for caspase-3 (left panels), F4/80 (middle panels), and their merged images (right panels) indicated distinct changes in apoptosis and macrophage distribution in the initial segment (IS) of the epididymis across experimental groups (Fig. 3a–c). In the control mice, caspase-3-positive apoptotic cells were rarely detected (Fig. 3a, left panel). Macrophages labeled with F4/80 were present and exhibited luminally directed projections (white arrowheads) (Fig. 3a, middle panel), as observed in the merged image (Fig. 3a, right panel).

In the colchicine-treated mice, caspase-3-positive apoptotic cells were abundant, indicating increased epithelial apoptosis (Fig. 3b, left panel). Macrophages were frequently observed surrounding caspase-3-positive apoptotic cells (white arrows), indicating a close spatial association between macrophages and apoptotic epithelial cells following colchicine treatment (Fig. 3b, middle and right panels). Quantitative analysis showed a significant increase in caspase-3-positive apoptotic cells following colchicine treatment and a significant reduction after the 72-h recovery period ($p < 0.001$) (Fig. 3d).

After a 72-h recovery period, caspase-3-positive apoptotic cells were markedly reduced in the IS (Fig. 3c, left panel). Macrophages in the recovery group exhibited a morphology similar to that of the control group, with the reappearance of luminal-directed projections (white arrowheads) (Fig. 3c, middle and right panels). Additionally, the total number of epithelial cells per tubule decreased substantially in the colchicine-treated epididymis (Fig. 3e). However, after the 72-h recovery period, the epithelial cell count fully recovered to control levels, with no statistical difference between the recovery and control groups (Fig. 3e).

3.4 Morphological Changes in Basal Cells Following Colchicine Treatment

To assess the effects of colchicine on basal cells (BCs) in the initial segment (IS) of the epididymis, immunofluorescence staining was performed for E-cadherin (E-cad, left panels), keratin 5 (KRT5, middle panels), and their merged images (right panels) (Fig. 4a–c). E-cadherin staining was used to mark cell-cell junctions and delineate epithelial cell boundaries, whereas KRT5 staining was used to identify basal cells.

In the control mice (Fig. 4a), a marked proportion of BCs extended luminal-reaching projections (white arrows). Other BCs exhibited either short projections or non-projecting morphologies (white arrowheads). In colchicine-treated mice (Fig. 4b), the number of BCs with luminal-reaching projections was markedly reduced, and most BCs exhibited a retracted morphology with no cytoplasmic extensions (white arrowheads). However, a very small number of BCs that retained luminal-reaching projections were still present (white arrow), although much less frequently than in controls.

After a 72-h recovery period (Fig. 4c), the proportion of BCs with luminal-reaching projections increased and exceeded that observed in control mice. The proportion of basal cells with luminal-reaching projections differed significantly among all three groups ($p < 0.01$) (Fig. 4d).

3.5 Changes in Aquaporin-9 Expression and B1-V-ATPase-Positive Clear Cell Numbers Following Colchicine Treatment

To examine the effects of colchicine on principal cells, immunofluorescence staining for aquaporin-9 (AQP9) was performed (Fig. 5a). In control mice (left panels), AQP9 expression was prominently detected in the apical region of principal cells. However, in colchicine-treated mice (middle panels), AQP9 expression was substantially reduced, and this reduction persisted after the 72-h recovery period (right panels) (Fig. 5a). Quantitative analysis confirmed that AQP9 intensity was significantly reduced in both the colchicine-treated and recovery groups compared with controls ($p < 0.01$), whereas no significant difference was detected between the colchicine-treated and recovery groups (Fig. 5c). To assess the potential changes in clear cells, immunofluorescence staining for B1-V-ATPase, a clear cell-specific marker, was conducted (Fig. 5b). B1-V-ATPase-positive clear cells were observed in all groups without noticeable differences in their distribution. Quantitative analysis showed no significant difference in the number of B1-V-ATPase-positive clear cells among the control, colchicine-treated, and recovery groups (Fig. 5d).

4. Discussion

Colchicine treatment was associated with marked alterations in the morphology and cellular characteristics of the initial segment (IS) epithelium, including reduced pERK expression, increased epithelial apoptosis, loss of luminal-reaching basal cell projections, and decreased AQP9 expression in principal cells. Although some of these changes were reversible during the recovery period following colchicine treatment, others persisted throughout the recovery period, suggesting differential recovery responses among epididymal epithelial cell populations. Colchicine treatment resulted in reduced epithelial height, increased apoptosis with luminal cell debris, loss of luminal-reaching projections in basal cells, and a pronounced reduction in apical AQP9 expression in principal cells (Fig. 6). In contrast, pERK expression, epithelial cell number, and basal cell projections showed substantial recovery during the observation period.

4.1 Colchicine-Induced ERK Suppression and Its Reversibility

One of the key findings of this study was a marked reduction in phosphorylated ERK (pERK) expression in the IS following colchicine treatment. Given that ERK signaling is essential for epithelial cell proliferation, survival, and differentiation [15,20,21], the reduction in pERK observed following colchicine treatment may be associated with the epithelial apoptosis and morphological alterations observed in the present study. However, the current findings do not establish a direct causal relationship between ERK suppression and the observed epithelial phenotype. Efferent duct ligation (EDL) led to sustained ERK suppression and increased apoptosis in the epididymis [20, 35, 36]. However, the underlying

mechanisms of ERK suppression differ between EDL and colchicine treatment. In EDL, blockage of luminal fluid flow prevents luminal signaling factors from reaching the initial segment (IS), leading to sustained ERK suppression and progressive epithelial degeneration. In contrast, colchicine exerts its effects directly on microtubules and intracellular dynamics, rather than through luminal factor deprivation. Unlike EDL, pERK expression returned to control levels during the 72-h recovery period after colchicine treatment, indicating that ERK suppression under the present experimental conditions was transient. However, the mechanisms underlying the recovery of ERK signaling were not examined in the present study.

4.2 Epithelial Cell Loss, Structural Changes, and Recovery

Colchicine treatment induced marked structural alterations in the initial segment (IS) epithelium, characterized by a reduction in the epithelial height and an increase in the luminal diameter. These changes suggest epithelial cell loss and structural disruption, which could alter the luminal microenvironment necessary for sperm maturation. Although epithelial height recovered partially after 72 h, control levels were not restored, indicating that epithelial regeneration requires a longer timeframe for complete structural restoration.

The presence of exfoliated apoptotic cells in the lumen of colchicine-treated mice further indicates colchicine-associated epithelial cell detachment, which may alter the luminal environment. Macrophages were frequently observed adjacent to or surrounding the apoptotic epithelial cells, suggesting their potential involvement in apoptotic cell clearance. Mononuclear phagocytes in the proximal epididymis undergo morphological and phenotypic changes following efferent duct ligation, facilitating rapid clearance of apoptotic epithelial cells [37]. Although the macrophage distribution observed in the present study is consistent with a role in epithelial homeostasis, direct phagocytic activity was not assessed. After the 72-h recovery period, apoptotic cells were rarely observed, and the total number of epithelial cells returned to control levels. These findings indicate substantial recovery of the epididymal epithelium during the recovery period following colchicine treatment. However, the incomplete restoration of epithelial height raises the question of whether the restored epithelial cell population fully recovers its structural and functional characteristics.

These findings align with a previous study demonstrating the ability of the epididymal epithelium to regenerate following an injury. The epididymal epithelium can rapidly regenerate after efferent duct ligation to maintain epithelial integrity through dramatic proliferation of principal cells (PCs) and basal cells (BCs) [38]. BCs within the epididymal epithelium are considered adult stem cells with the potential to differentiate and regenerate the epithelium, thereby playing a crucial role in maintaining structural integrity [39]. Previous studies have demonstrated regenerative capacity of the epididymal epithelium involving principal and basal cells. However, cell proliferation and lineage relationships were not directly assessed in the present study; therefore, the cellular mechanisms underlying the observed recovery remain unclear.

In summary, colchicine-induced structural changes in the IS epithelium involved epithelial cell loss and structural disruption, with partial recovery observed after 72 h. The reduction in apoptotic cells and the return of epithelial cell numbers to baseline indicate substantial epithelial recovery during the recovery period. However, incomplete restoration of epithelial height suggests that full functional recovery may require extended periods or additional regulatory mechanisms.

4.3 Recovery of Basal Cell Projections Following Colchicine Treatment

Basal cells (BCs) in the IS extend luminal projections, allowing them to monitor and regulate luminal composition [12, 14, 35]. In the current study, colchicine treatment led to a marked reduction in BC projections, indicating potential impairment of BC function. However, following the 72-h recovery period, the proportion of BCs with luminal-reaching projections exceeded control levels. The biological significance of this increase remains unclear and warrants further investigation. ERK signaling is required for the formation and maintenance of BC projections [13, 35]. Future studies should determine whether this increase is transient and whether it has functional consequences for epididymal homeostasis.

4.4 Persistent Downregulation of AQP9 in Principal Cells

Colchicine treatment led to a marked reduction in AQP9 expression in principal cells, which persisted after the recovery period. Principal cells constitute approximately 60–80% of the epididymal epithelium [40] and play a major role in establishing the luminal environment required for sperm maturation and storage. Previous studies have also shown that principal cells are particularly susceptible to apoptosis under certain conditions of epididymal injury or androgen deprivation [41]. However, because apoptotic cells were not co-localized with cell-type-specific markers in the present study, it was not possible to determine whether principal cells were preferentially affected by colchicine treatment. Principal cells contribute to luminal homeostasis through the secretion and absorption of various substances [42], and AQP9 is involved in water transport and fluid regulation within the epididymis [5]. Therefore, the persistent reduction in AQP9 expression observed after the recovery period may reflect incomplete recovery of principal cell-associated functions and may contribute to prolonged alterations in luminal fluid homeostasis. Although pERK expression returned to control levels while AQP9 remained suppressed, this differential recovery does not establish that AQP9 regulation is independent of ERK signaling. Future studies using cell-type-specific approaches and extended recovery periods will be required to determine the mechanisms underlying sustained AQP9 suppression and its functional consequences for sperm maturation.

4.5 Preservation of B1-V-ATPase-Positive Clear Cell Numbers

In contrast to the marked reduction in AQP9 expression, the number of B1-V-ATPase-positive clear cells remained unchanged following colchicine treatment and during recovery. This finding suggests

preservation of the clear-cell population under the present experimental conditions; however, clear-cell function was not directly assessed.

Several limitations should be considered when interpreting the present findings. First, although colchicine treatment was associated with reduced pERK expression and epithelial disruption, the present study was not designed to establish a direct causal relationship between ERK suppression and the observed epithelial phenotype. Second, the identity of apoptotic epithelial cells was not determined using cell-type-specific co-localization analyses. Finally, recovery was evaluated using the contralateral epididymis following unilateral epididymectomy; therefore, potential systemic effects associated with the surgical procedure cannot be completely excluded. Additional studies will be required to address these limitations and further define the mechanisms underlying colchicine-induced epididymal alterations.

In summary, colchicine treatment was associated with transient reduction of pERK expression, increased epithelial apoptosis, loss of basal cell projections, and persistent reduction of AQP9 expression, whereas the number of B1-V-ATPase-positive clear cells remained unchanged. During the 72-h recovery period, pERK expression, epithelial cell number, and basal cell projections substantially recovered, whereas epithelial height remained partially reduced and AQP9 expression remained suppressed. These findings indicate differential recovery of colchicine-induced epithelial alterations and support an association between ERK signaling and epithelial homeostasis. Given the widespread therapeutic use of colchicine, the persistent reduction of AQP9 observed in this study warrants further investigation to determine whether colchicine-related epididymal alterations have functional consequences for sperm maturation and male reproductive health.

Declarations

Competing interests

The authors declare that they have no competing interests.

Ethics approval

All animal experiments were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Kongju National University (Approval number: KNU_2019-06).

Funding

This work was supported by a research grant from Kongju National University Industry-University Cooperation Foundation in 2024 and carried out with the support of "Cooperative Research Program for Agriculture Science and Technology Development (Project No. RS-2024-00351672).

Author Contribution

Kim B. conceptualized and supervised the study. Park YS performed data curation, formal analysis, and investigation. Methodology and validation were conducted by Park YS, Kim SW, and Kim B. Park YS performed image and data analysis using Zen Blue, Adobe Photoshop, and ImageJ. Park YS drafted the manuscript, and all authors contributed to reviewing and editing the manuscript.

5.4 Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

1. 1. Cornwall GA. New insights into epididymal biology and function. Hum Reprod Update. 2009;15:213 – 27. https://doi.org/10.1093/humupd/dmn055
2. 2. Shum WW, Da Silva N, Brown D, Breton S. Regulation of luminal acidification in the male reproductive tract via cell-cell crosstalk. J Exp Biol. 2009;212:1753-61. https://doi.org/10.1242/jeb.027284
3. 3. Belleannee C, Thimon V, Sullivan R. Region-specific gene expression in the epididymis. Cell Tissue Res. 2012;349:717 – 31. https://doi.org/10.1007/s00441-012-1381-0
4. 4. Xu B, Washington AM, Hinton BT. PTEN signaling through RAF1 proto-oncogene serine/threonine kinase (RAF1)/ERK in the epididymis is essential for male fertility. Proceedings of the National Academy of Sciences. 2014;111:18643-8. https://doi.org/10.1073/pnas.1413186112
5. 5. Badran HH, Hermo LS. Expression and regulation of aquaporins 1, 8, and 9 in the testis, efferent ducts, and epididymis of adult rats and during postnatal development. Journal of andrology. 2002;23:358 – 73. https://doi.org/10.1002/j.1939-4640.2002.tb02243.x
6. 6. Cheung K, Leung C, Leung G, Wong P. Synergistic effects of cystic fibrosis transmembrane conductance regulator and aquaporin-9 in the rat epididymis. Biology of reproduction. 2003;68:1505-10. https://doi.org/10.1095/biolreprod.102.010017
7. 7. Ruz R, Andonian S, Hermo L. Immunolocalization and regulation of cystic fibrosis transmembrane conductance regulator in the adult rat epididymis. Journal of andrology. 2004;25:265 – 73. https://doi.org/10.1002/j.1939-4640.2004.tb02787.x
8. 8. Pietrement C, Da Silva N, Silberstein C, James M, Marsolais M, Van Hoek A, et al. Role of NHERF1, cystic fibrosis transmembrane conductance regulator, and cAMP in the regulation of aquaporin 9. Journal of biological chemistry. 2008;283:2986-96. https://doi.org/10.1074/jbc.M704678200
9. 9. Da Silva N, Shum WW, Breton S. Regulation of vacuolar proton pumping ATPase-dependent luminal acidification in the epididymis. Asian J Androl. 2007;9:476 – 82. https://doi.org/10.1111/j.1745-7262.2007.00299.x

10. 10. Hermo L, Dworkin J, Oko R. Role of epithelial clear cells of the rat epididymis in the disposal of the contents of cytoplasmic droplets detached from spermatozoa. American Journal of Anatomy. 1988;183:107 – 24. https://doi.org/10.1002/aja.1001830202
11. 11. Yeung C, Nashan D, Sorg C, Oberpenning F, Schulze H, Nieschlag E, et al. Basal cells of the human epididymis—antigenic and ultrastructural similarities to tissue-fixed macrophages. Biology of reproduction. 1994;50:917 – 26. https://doi.org/10.1095/biolreprod50.4.917
12. 12. Shum WW, Smith TB, Cortez-Retamozo V, Grigoryeva LS, Roy JW, Hill E, et al. Epithelial basal cells are distinct from dendritic cells and macrophages in the mouse epididymis. Biol Reprod. 2014;90:90. https://doi.org/10.1095/biolreprod.113.116681
13. 13. Roy J, Kim B, Hill E, Visconti P, Krapf D, Vinegoni C, et al. Tyrosine kinase-mediated axial motility of basal cells revealed by intravital imaging. Nat Commun. 2016;7:10666. https://doi.org/10.1038/ncomms10666
14. 14. Shum WW, Da Silva N, McKee M, Smith PJ, Brown D, Breton S. Transepithelial projections from basal cells are luminal sensors in pseudostratified epithelia. Cell. 2008;135:1108-17. https://doi.org/10.1016/j.cell.2008.10.020
15. 15. Guo Y-J, Pan W-W, Liu S-B, Shen Z-F, Xu Y, Hu L-L. ERK/MAPK signalling pathway and tumorigenesis. Experimental and therapeutic medicine. 2020;19:1997–2007. https://doi.org/10.3892/etm.2020.8454
16. 16. Kyriakis JM, Avruch J. Mammalian MAPK signal transduction pathways activated by stress and inflammation: a 10-year update. Physiological reviews. 2012;92:689–737. https://doi.org/10.1152/physrev.00028.2011
17. 17. Tang Q, Wu J, Zheng F, Hann SS, Chen Y. Emodin increases expression of insulin-like growth factor binding protein 1 through activation of MEK/ERK/AMPKα and interaction of PPARγ and Sp1 in lung cancer. Cellular Physiology and Biochemistry. 2017;41:339 – 57. https://doi.org/10.1159/000456279
18. 18. Rao A, Herr DR. G protein-coupled receptor GPR19 regulates E-cadherin expression and invasion of breast cancer cells. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research. 2017;1864:1318-27. https://doi.org/10.1016/j.bbamcr.2017.05.001
19. 19. Bang Y-J, Kwon JH, Kang SH, Kim JW, Yang YC. Increased MAPK activity and MKP-1 overexpression in human gastric adenocarcinoma. Biochemical and biophysical research communications. 1998;250:43 – 7. https://doi.org/10.1006/bbrc.1998.9256
20. 20. Xu B, Abdel-Fattah R, Yang L, Crenshaw SA, Black MB, Hinton BT. Testicular lumicrine factors regulate ERK, STAT, and NFKB pathways in the initial segment of the rat epididymis to prevent apoptosis. Biology of reproduction. 2011;84:1282-91. https://doi.org/10.1095/biolreprod.110.090324
21. 21. Xu B, Yang L, Lye RJ, Hinton BT. p-MAPK1/3 and DUSP6 regulate epididymal cell proliferation and survival in a region-specific manner in mice. Biology of reproduction. 2010;83:807 – 17. https://doi.org/10.1095/biolreprod.110.085613

22. 22. Kim B, Breton S. The MAPK/ERK-signaling pathway regulates the expression and distribution of tight junction proteins in the mouse proximal epididymis. Biology of reproduction. 2016;94:22, 1–12. https://doi.org/10.1095/biolreprod.115.134445
23. 23. Setchell B. The functional significance of the blood-testis barrier. Journal of andrology. 1980;1:3–10. https://doi.org/10.1002/j.1939-4640.1980.tb00003.x
24. 24. Cyr DG, Gregory M, Dube E, Dufresne J, Chan PT, Hermo L. Orchestration of occludins, claudins, catenins and cadherins as players involved in maintenance of the blood-epididymal barrier in animals and humans. Asian J Androl. 2007;9:463 – 75. https://doi.org/10.1111/j.1745-7262.2007.00308.x
25. 25. Mital P, Hinton BT, Dufour JM. The blood-testis and blood-epididymis barriers are more than just their tight junctions. Biology of reproduction. 2011;84:851-8. https://doi.org/10.1095/biolreprod.110.087452
26. 26. Slobodnick A, Shah B, Krasnokutsky S, Pillinger MH. Update on colchicine, 2017. Rheumatology. 2018;57:i4-i11. https://doi.org/10.1093/rheumatology/kex453
27. 27. Andreu JM, Timasheff SN. Tubulin bound to colchicine forms polymers different from microtubules. Proceedings of the National Academy of Sciences. 1982;79:6753-6. https://doi.org/10.1073/pnas.79.22.6753
28. 28. Finkelstein Y, Aks SE, Hutson JR, Juurlink DN, Nguyen P, Dubnov-Raz G, et al. Colchicine poisoning: the dark side of an ancient drug. Clinical toxicology. 2010;48:407 – 14. https://doi.org/10.3109/15563650.2010.495348
29. 29. Allard EK, Johnson KJ, Boekelheide K. Colchicine disrupts the cytoskeleton of rat testis seminiferous epithelium in a stage-dependent manner. Biology of reproduction. 1993;48:143 – 53. https://doi.org/10.1095/biolreprod48.1.143
30. 30. Correa LM, Nakai M, Strandgaard CS, Hess RA, Miller MG. Microtubules of the mouse testis exhibit differential sensitivity to the microtubule disruptors Carbendazim and colchicine. Toxicological Sciences. 2002;69:175 – 82. https://doi.org/10.1093/toxsci/69.1.175
31. 31. Handel MA. Effects of colchicine on spermiogenesis in the mouse. Development. 1979;51:73–83. https://doi.org/10.1242/dev.51.1.73
32. 32. Naaz F, Haider MR, Shafi S, Yar MS. Anti-tubulin agents of natural origin: Targeting taxol, vinca, and colchicine binding domains. European journal of medicinal chemistry. 2019;171:310 – 31. https://doi.org/10.1016/j.ejmech.2019.03.025
33. 33. Ramos-Ibeas P, Pericuesta E, Fernandez-Gonzalez R, Ramirez M, Gutierrez-Adan A. Most regions of mouse epididymis are able to phagocytose immature germ cells. Reproduction. 2013;146:481-9. https://doi.org/10.1530/REP-13-0145
34. 34. Nagy F, Edmonds RH. Cellular proliferation and renewal in the various zones of the hamster epididymis after colchicine administration. Fertility and sterility. 1975;26:460-8. https://doi.org/10.1016/S0015-0282(16)41118-0

35. 35. Kim B, Roy J, Shum WW, Da Silva N, Breton S. Role of testicular luminal factors on Basal cell elongation and proliferation in the mouse epididymis. Biol Reprod. 2015;92:9. https://doi.org/10.1095/biolreprod.114.123943
36. 36. Turner TT, Riley TA. p53 independent, region-specific epithelial apoptosis is induced in the rat epididymis by deprivation of luminal factors. Mol Reprod Dev. 1999;53:188 – 97. https://doi.org/10.1002/(sici)1098-2795(199906)53:2
37. 37. Smith TB, Cortez-Retamozo V, Grigoryeva LS, Hill E, Pittet MJ, Da Silva N. Mononuclear phagocytes rapidly clear apoptotic epithelial cells in the proximal epididymis. Andrology. 2014;2:755 – 62. https://doi.org/10.1111/j.2047-2927.2014.00251.x
38. 38. Kim B, Breton S. Androgens are essential for epithelial cell recovery after efferent duct ligation in the initial segment of the mouse epididymis. Biology of reproduction. 2020;102:76–83. https://doi.org/10.1093/biolre/ioz152
39. 39. Pinel L, Mandon M, Cyr DG. Tissue regeneration and the epididymal stem cell. Andrology. 2019;7:618 – 30. https://doi.org/10.1111/andr.12635
40. 40. Akbarsha MA, Faisal K, Radha A. The Epididymis: Structure and Function. In: Singh SK, editor. Mammalian Endocrinology and Male Reproductive Biology. Boca Raton, FL: CRC Press; 2015. p. 115 – 66. https://doi.org/10.1201/b18900-7
41. 41. Fan X, Robaire B. Orchidectomy induces a wave of apoptotic cell death in the epididymis. Endocrinology. 1998;139:2128-36. https://doi.org/10.1210/endo.139.4.5888
42. 42. Zhou W, De Iuliis GN, Dun MD, Nixon B. Characteristics of the Epididymal Luminal Environment Responsible for Sperm Maturation and Storage Maturation and Storage. Front Endocrinol (Lausanne). 2018;9:59. https://doi.org/10.3389/fendo.2018.00059

Figures

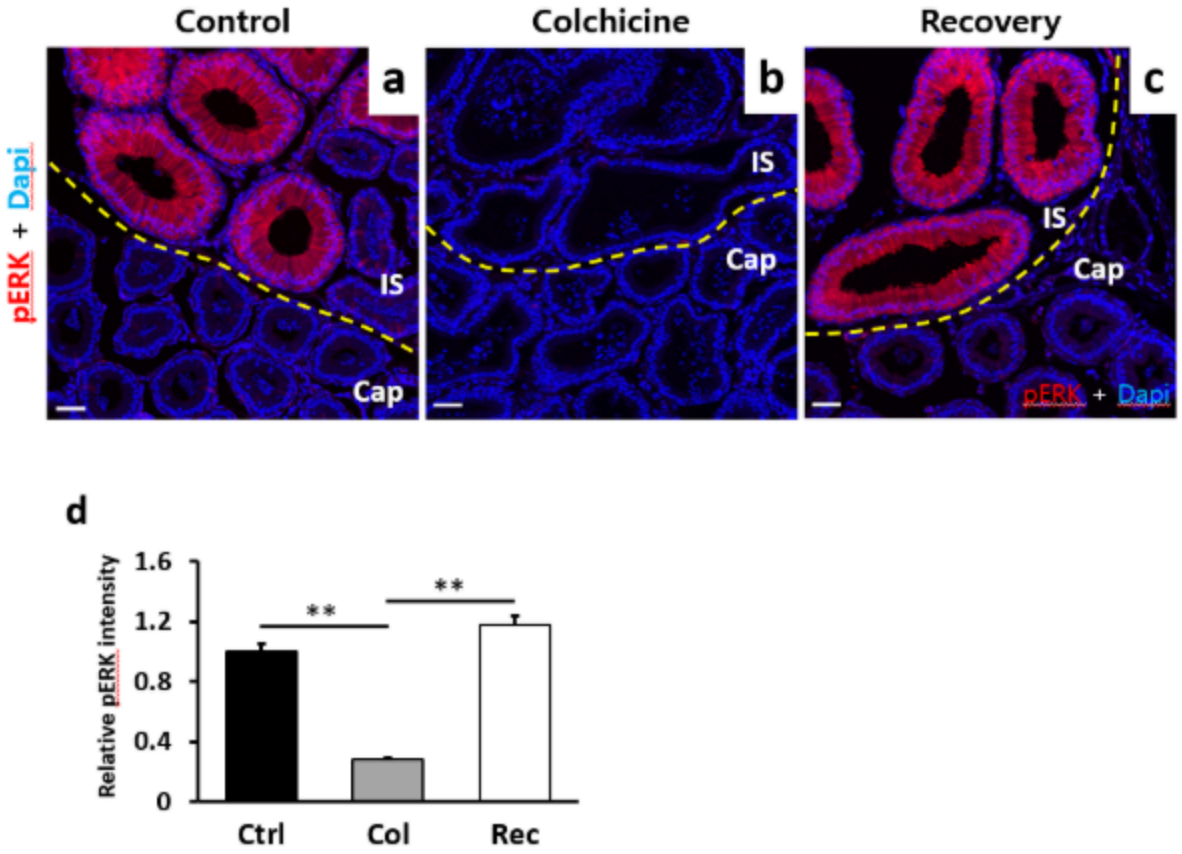


Figure 1

Colchicine treatment reduces pERK expression in the initial segment of the epididymis. (a–c) Immunofluorescence staining of phosphorylated ERK1/2 (pERK) in the initial segment (IS) of mouse epididymis. Control mice (a) exhibited strong pERK immunoreactivity in the IS. Colchicine-treated mice (b) showed a marked reduction in pERK expression. After a 72-h recovery period (c), pERK expression returned to levels comparable to those in controls. Nuclei were stained with DAPI (blue). Scale bars = 50 μm. (d) Quantification of relative pERK intensity in the IS. Data are presented as mean ± SEM. **p < 0.01.

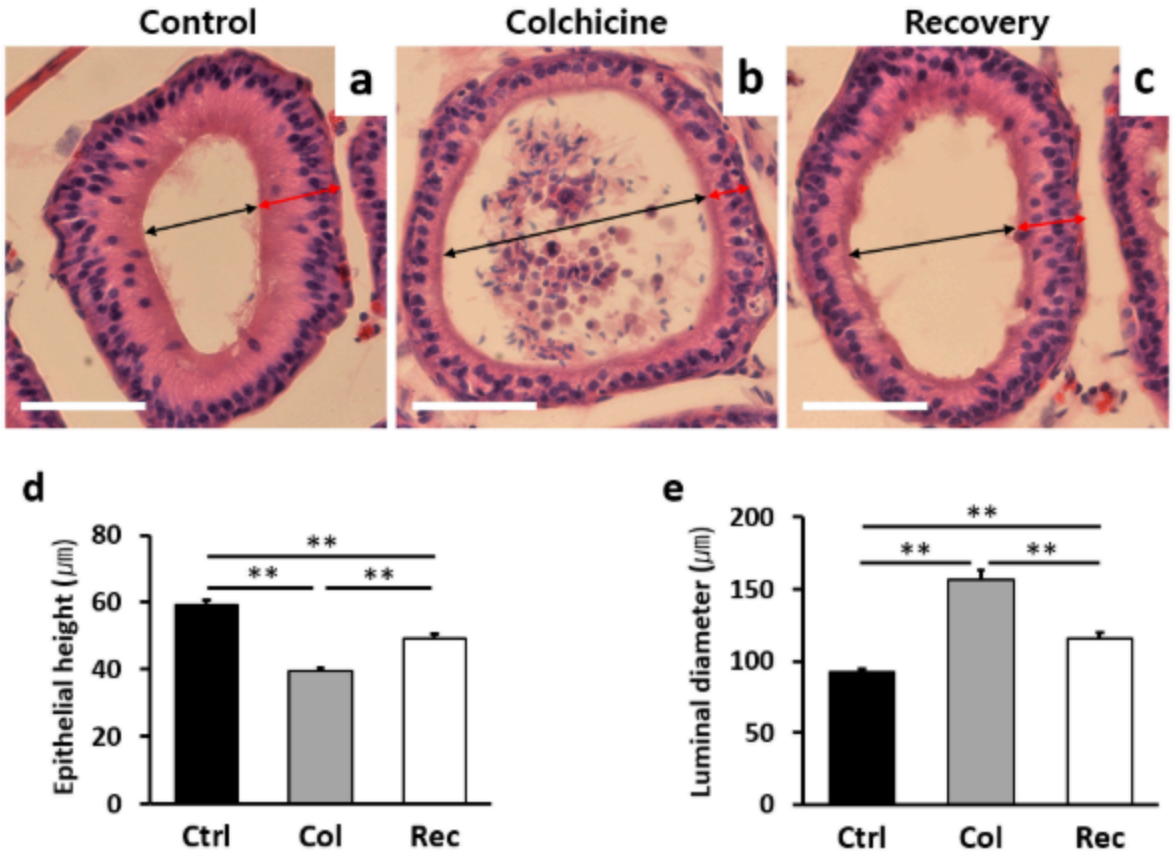


Figure 2

Morphological alterations in the epididymal epithelium after colchicine treatment. (a-c) Hematoxylin and eosin (H&E) staining of the initial segment (IS) of the epididymis from control (a), colchicine-treated (b), and recovery (c) mice. Colchicine treatment resulted in reduced epithelial height and increased luminal diameter compared with controls. After a 72-h recovery period, epithelial height increased and luminal diameter decreased compared with the colchicine-treated group but did not fully return to control levels. Black double-headed arrows indicate luminal diameter and red double-headed arrows indicate epithelial height. Scale bars = 100 μm. (d) Quantification of epithelial height in the IS. (e) Quantification of luminal diameter in the IS. Data are presented as mean ± SEM. **p < 0.01.

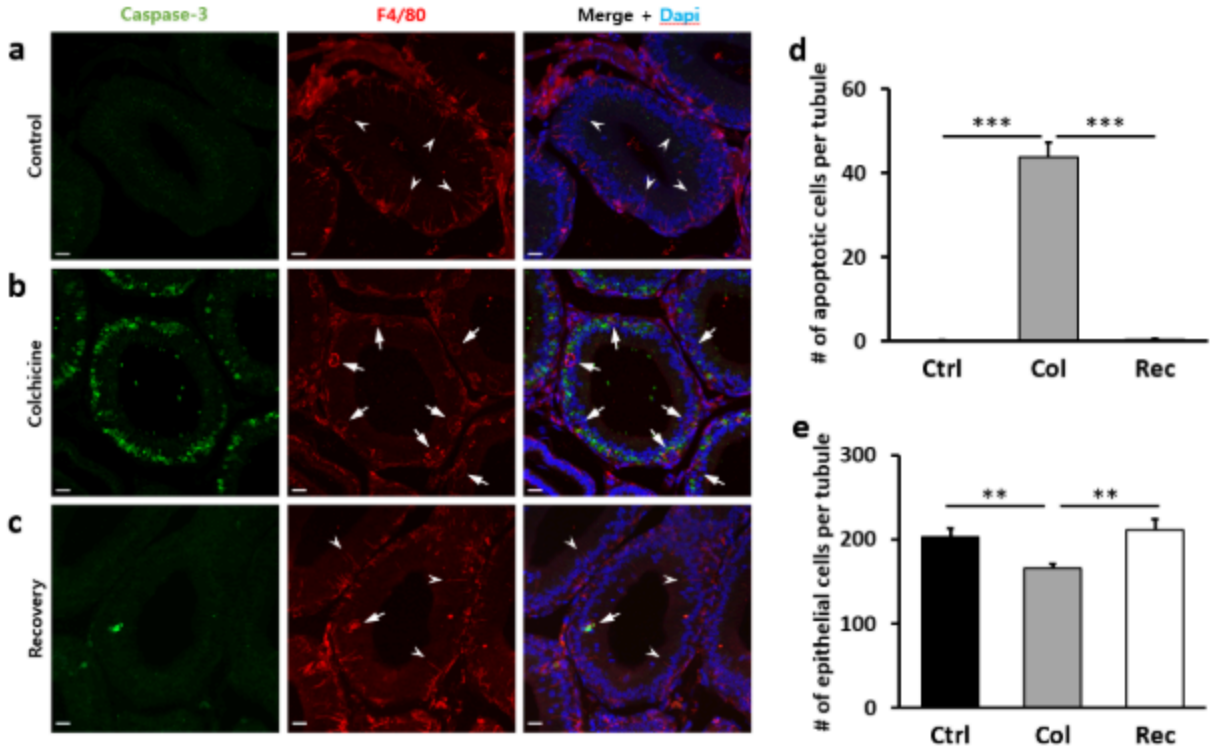


Figure 3

Colchicine treatment increases apoptosis in the epididymal epithelium. (a-c) Immunofluorescence staining for cleaved caspase-3 (green, apoptosis marker) and F4/80 (red, macrophage marker) in the initial segment (IS) of the mouse epididymis. Merged images with DAPI-stained nuclei (blue) are shown in the right panels. Control mice (a) exhibited few caspase-3-positive apoptotic cells, and macrophages displayed luminal-directed projections (white arrowheads). In colchicine-treated mice (b), the number of caspase-3-positive apoptotic cells markedly increased, and macrophages were frequently observed adjacent to or surrounding apoptotic cells (white arrows). After a 72-h recovery period (c), caspase-3-positive apoptotic cells were markedly reduced, and macrophages again displayed luminal-directed projections (white arrowheads). Scale bars = 20 μm. (d) Quantification of caspase-3-positive apoptotic cells per tubule in the IS. (e) Quantification of the total number of epithelial cells per tubule in the IS. Data are presented as mean ± SEM. **p < 0.01; ***p < 0.001.

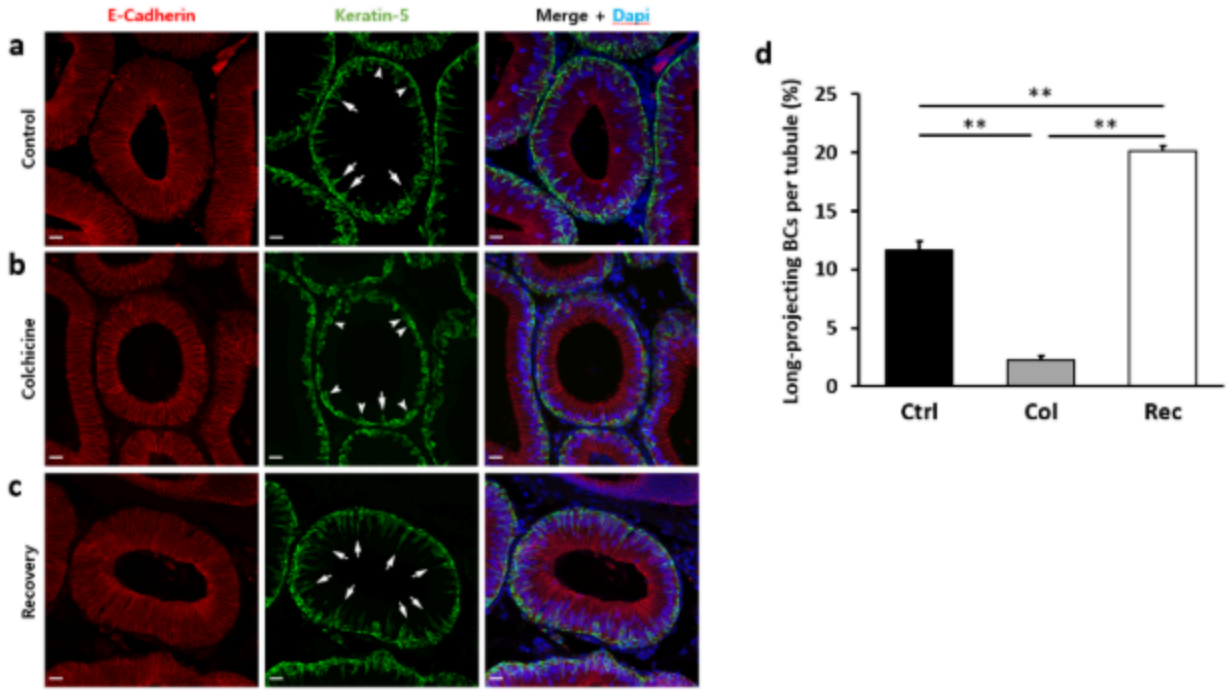


Figure 4

Colchicine-induced changes in basal cell projections. (a-c) Immunofluorescence staining for keratin-5 (KRT5; green, basal cell marker) and E-cadherin (E-cad; red, cell adhesion molecule) in the initial segment (IS) of the mouse epididymis. Merged images with DAPI-stained nuclei (blue) are shown in the right panels. In control mice (a), basal cells with luminal-reaching projections (white arrows) were frequently observed, whereas some basal cells exhibited a non-projecting morphology (white arrowheads). Colchicine-treated mice (b) showed a marked reduction in luminal-reaching projections, with most basal cells exhibiting a retracted morphology (white arrowheads), although a few projecting cells remained (white arrows). After a 72-h recovery period (c), luminal-reaching projections were restored and exceeded the level observed in controls. Scale bars = 20 μm. (d) Quantification of basal cells with luminal-reaching projections in the IS. Data are presented as mean ± SEM. **p < 0.01.

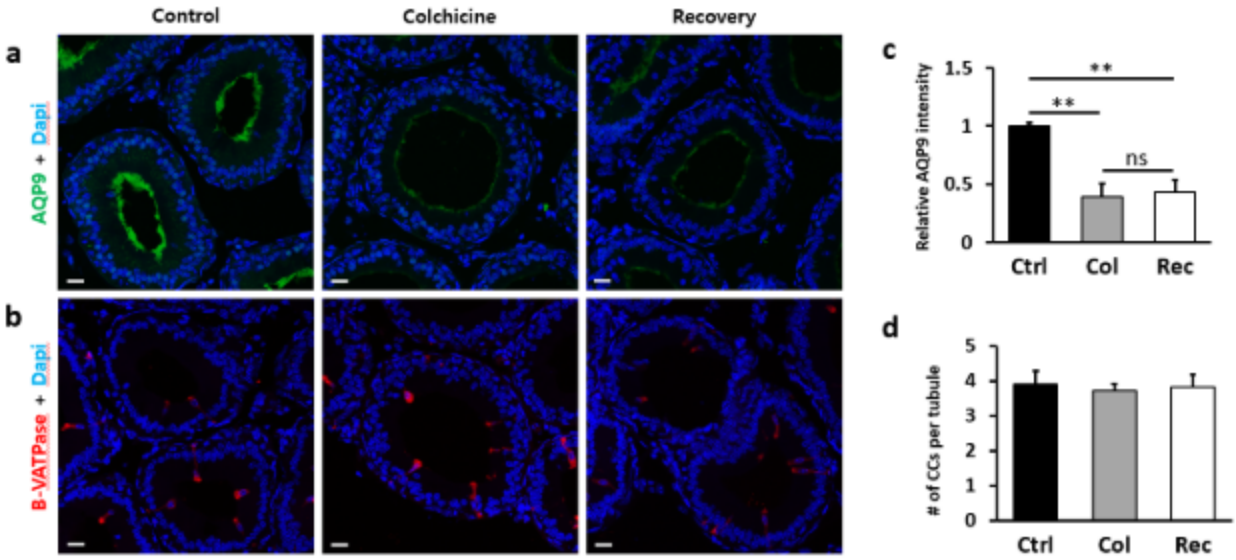


Figure 5

Altered AQP9 expression and B1-V-ATPase-positive clear cell counts in the epididymis after colchicine treatment. (a) Immunofluorescence staining for aquaporin-9 (AQP9; green) in principal cells of the initial segment (IS). AQP9 was prominently expressed in the apical region of principal cells in control mice, whereas its expression was markedly reduced in colchicine-treated and recovery groups. (b) Immunofluorescence staining for B1-V-ATPase (red), a marker for clear cells. B1-V-ATPase-positive clear cells were observed in all groups without noticeable differences in their distribution. Nuclei were stained with DAPI (blue). Scale bars = 20 μm. (c) Quantification of relative AQP9 intensity in the IS. AQP9 intensity was significantly reduced in both the colchicine-treated and recovery groups compared with controls, with no significant difference between the colchicine-treated and recovery groups. (d) Quantification of B1-V-ATPase-positive clear cells per tubule in the IS. No significant differences were detected among the three groups. Data are presented as mean ± SEM. **p < 0.01; ns, not significant.

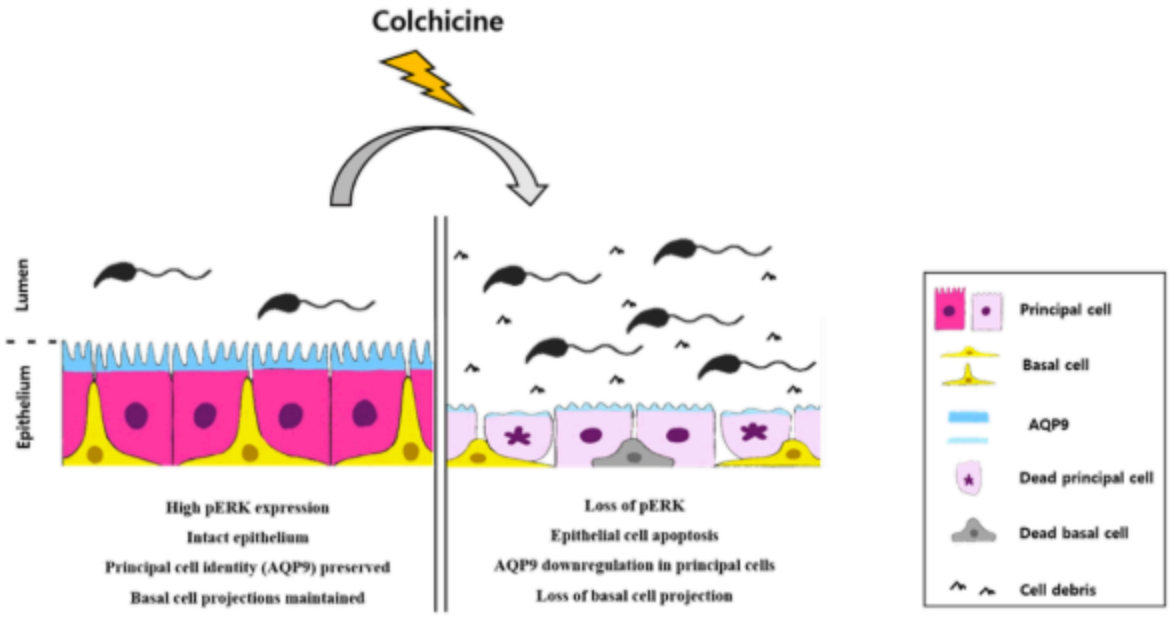


Figure 6

Summary of colchicine-induced alterations in the initial segment (IS) epithelium of the mouse epididymis. Colchicine treatment decreased the epithelial height and induced marked apoptosis, resulting in luminal cell debris. Basal cells showed loss of luminal-reaching projections, whereas principal cells exhibited a pronounced reduction in apical aquaporin-9 (AQP9) expression.