

Article

# Impact of Local Anesthesia Techniques on Epididymal Morphology: An Experimental Study

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**Abstract:** This experimental study evaluated morphological changes in epididymal tissue following different methods of local anesthesia using lidocaine and novocaine. The effects of intratesticular injection and conduction anesthesia on epididymal tubules, stromal structures, and microvasculature were assessed using histological (hematoxylin–eosin, van Gieson) and morphometric analyses. Intratesticular administration resulted in significantly more pronounced structural damage compared to conduction anesthesia, including epithelial disruption, thickening of intertubular septa, and microcirculatory disturbances ( $p < 0.05$ ). The most severe alterations were observed after intratesticular novocaine injection, whereas conduction anesthesia with lidocaine showed minimal morphological changes. The integrated structural injury index (ISI) confirmed a significant relationship between the method of administration and tissue damage severity. These findings indicate that the anesthesia technique is a key determinant of epididymal tissue response and support the use of less traumatic approaches, particularly in patients of reproductive age.

**Keywords:** Epididymis, local anesthesia, lidocaine, novocaine, intratesticular injection, conduction anesthesia, morphology, morphometry, hematoxylin and eosin, van Gieson staining, integrated injury index (ISI).

## Introduction

In Modern urological and andrological practice is characterized by a steady increase in the number of diagnostic and therapeutic interventions on the scrotum, including testicular and epididymal biopsies, microsurgical sperm retrieval techniques (micro-TESE), and puncture and aspiration procedures for male infertility [1]. Given the increasing prevalence of reproductive disorders in men, the problem of preserving the morphological and functional integrity of testicular and

epididymal tissues is of particular clinical significance[2]. The choice of local anesthesia plays a key role, as it determines not only the adequacy of pain relief but also the degree of local tissue effect [3].

The epididymis is a functionally highly sensitive organ responsible for the maturation, accumulation, and transport of sperm. Its epithelial structures and interstitial tissue are highly susceptible to ischemia, edema, microcirculatory disorders, and drug exposure [4]. Even short-term disruption of the trophic or structural organization of the epithelium can lead to the development of inflammatory-dystrophic changes, fibrosis, and secondary obstructive processes that negatively impact fertility [5].

In clinical practice, lidocaine and novocaine, administered intratesticularly or by conduction, are widely used for local anesthesia of the scrotum [6]. Most studies have assessed the analgesic efficacy and safety of these drugs, while their local morphological effects on epididymal tissue remain poorly understood [7]. Data concerning a comparative analysis of not only the anesthetics themselves but also the routes of administration in terms of structural tissue damage are particularly limited.

Experimental studies indicate that intratesticular administration of drugs can be accompanied by significant microcirculatory disturbances, interstitial edema, epithelial disorganization, and increased vascular permeability [8]. However, the data obtained are fragmentary, and uniform quantitative criteria for assessing morphological damage under various anesthesia options have not yet been developed. The use of morphometric methods and integral damage indices allows for an objective assessment of the degree of tissue changes and increases the reproducibility of results [9].

In the Republic of Uzbekistan, there has been an increase in the number of patients with primary and secondary male infertility, as well as an increase in the volume of invasive diagnostic procedures in andrological practice [10]. However, data from domestic authors on morphological changes in the epididymis under various methods of local anesthesia remain limited, highlighting the need for experimental research in this area.

In connection with the above, a comparative morphological study of the tissues of the epididymis under various methods of local anesthesia is a relevant scientific and practical task aimed at substantiating the least traumatic approach to diagnostic and therapeutic interventions in men of reproductive age[11].

### **The aim of the study**

is a comparative assessment of morphological changes in the tissues of the epididymis with various methods of local anesthesia using lidocaine and novocaine based on histological and morphometric analysis, in order to determine the least traumatic method of anesthesia that ensures the preservation of the structural organization and microcirculation of tissues.

### **Research methods and methodology.**

The experimental study was conducted on 90 albino male laboratory rats weighing 180–220 g, maintained under standard vivarium conditions in compliance with bioethical regulations and laboratory animal care requirements. All procedures were performed in accordance with international guidelines for the use of laboratory animals[12].

Animals were divided into groups based on the anesthetic used and the route of administration: a control group (no anesthetic administration), a group receiving intratesticular and conduction injection of 1% lidocaine, and a group receiving intratesticular and conduction injection of 1% novocaine. The volumes of administered drugs were adjusted for body weight and the anatomical features of the organs.

Epididymal tissue was collected at the standard postoperative time. The obtained material was fixed in 10% neutral formalin, underwent standard histological processing, and then paraffin sections were prepared at a thickness of 4–5  $\mu\text{m}$ . The sections were stained with hematoxylin and eosin and van Gieson staining to assess the overall tissue architecture, epithelial status, interstitial stroma, and vascular bed.

Morphological assessment was performed using light microscopy, followed by morphometric analysis, including measurement of tubule wall thickness, lumen area, edema severity, and the degree

of epithelial destruction. To comprehensively assess the severity of tissue changes, the morphological damage index (ISI) was calculated.

Statistical processing of the data was performed using standard methods of variation statistics. Results are presented as mean values and standard errors ( $M \pm m$ ). The significance of differences was assessed at a  $p < 0.05$  level.

#### Results of the research:

In the control group, epididymal tissue exhibits preserved histoarchitecture. The convoluted tubules are regularly rounded or oval in shape, with clearly defined lumens and uniform diameters. The epithelial layer consists of pseudostratified columnar epithelium, the cells of which are arranged in an orderly fashion, without signs of disorganization, desquamation, or cytoplasmic vacuolization (Fig. 1)[13].

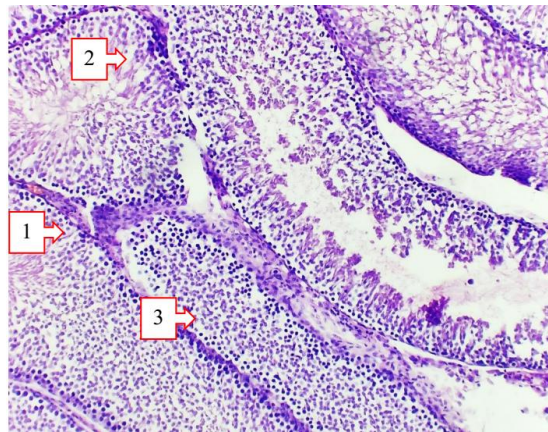


Figure 1. Epididymis.

#### Control group.

##### Hematoxylin and eosin staining, $\times 200$ .

- 1 - convoluted tubules of the epididymis;
- 2 - epithelium of the tubules, uniformly lining their lumen;
- 3 - intertubular interstitial tissue.

The nuclei of epithelial cells have clear contours, are located predominantly basally, and the chromatin is evenly distributed. The basement membrane is clearly visible, without signs of thickening or fragmentation. Intercellular boundaries are preserved, indicating the integrity of the epithelial barrier.

The intertubular interstitial tissue is moderately dense and consists of loose connective tissue without signs of edema or fibrous changes. Blood vessels are of normal caliber, with preserved lumen, their walls are not thickened, and there are no signs of plethora or stasis. Inflammatory cellular infiltration is not detected.

Thus, the morphological picture of the control group reflects the normal structural state of the epididymis and serves as a standard for subsequent comparative analysis of morphological changes that occur with different methods of local anesthesia.

Van Gieson staining in the control group clearly reveals the normal organization of the connective tissue elements of the epididymis. Collagen fibers of the intertubular stroma appear as thin, evenly distributed bundles, stained light red, with no signs of thickening, sclerosis, or fibrous transformation (Fig. 2)[14].



**Figure 2.** Epididymis.

**Control group. Van Gieson staining, ×100.**

- 1 - connective tissue membrane of the epididymis;
- 2 - intertubular interstitial tissue with thin collagen fibers;
- 3 - wall of the epididymal tubules.

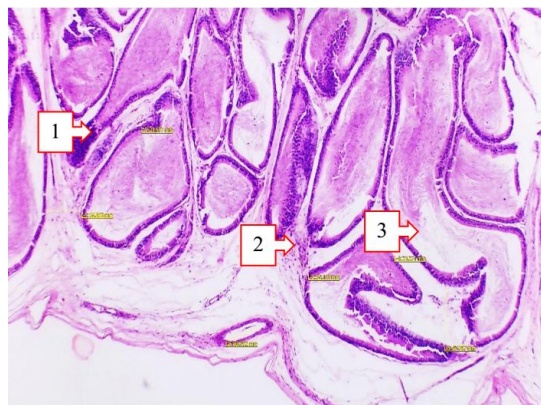
The connective tissue sheath and intertubular septa have clear contours and are of moderate thickness, indicating preserved organ architecture. No signs of edema, loosening, or excessive collagen accumulation are detected. The tubular wall retains its normal structure, with no signs of deformation or lumen compression.

The ratio of parenchymal and stromal components remains physiological.

There are no signs of inflammatory infiltration in the interstitial tissue; the vessels are of normal caliber, without thickening of the walls or signs of congestion.

Thus, the Van Gieson staining data confirm the absence of fibrous and sclerotic changes in the control group and serve as a morphological standard for assessing the degree of connective tissue restructuring under various methods of local anesthesia.

Following intratesticular injection of lidocaine, moderate morphological changes are observed in the epididymal tissue compared to the control group. The convoluted tubules retain their general shape in most fields of view, but in some areas, irregularities in their contours and moderate narrowing of the lumen are noted (Fig. 3).



**Figure 3.** Epididymis after intratesticular injection of lidocaine.

**Hematoxylin and eosin staining, ×200.**

- 1 - convoluted tubules of the epididymis with moderately altered epithelium;
- 2 - epithelial layer of tubules with signs of disorganization;

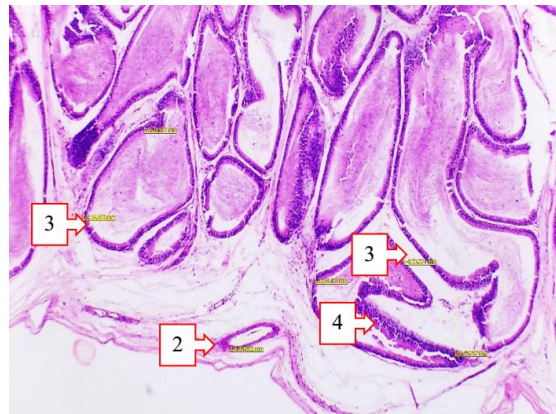
### 3 - intertubular interstitial tissue with moderate edema.

The tubular epithelial layer is partially disorganized: areas of epithelial cell flattening, moderate cytoplasmic vacuolization, and disrupted nuclear arrangement are observed. The basement membrane is generally preserved, but in certain areas, it is visualized as blurred, indicating the beginnings of structural damage.

The intertubular interstitial tissue is moderately dilated due to edema. The vessels are locally congested, with moderate lumen dilation, without significant wall thickening. Signs of massive inflammatory infiltration are absent, but isolated cellular elements are visible in certain areas, indicating a weak inflammatory response.

Overall, intratesticular lidocaine administration is accompanied by the development of moderate degenerative changes in the epithelium and interstitial edema without severe destruction of tissue architecture. The changes observed are less pronounced compared to subsequent groups and reflect the initial level of morphological damage to the epididymal tissue.

After intratesticular administration of a 1% solution of novocaine, more pronounced morphological changes were detected in the epididymis tissue compared to the group of intratesticular administration of lidocaine (Fig. 4).



**Figure 4.** Epididymis after intratesticular injection of novocaine.

#### **Hematoxylin and eosin staining, ×200.**

- 1 - convoluted tubules of the appendage with a deformed lumen;
- 2 - tubular epithelium with foci of dystrophy and desquamation;
- 3 - intertubular interstitial tissue with pronounced edema.

Convoluted tubules in most visual fields have an uneven diameter and a deformed lumen. The average tubule diameter was  $112.4 \pm 6.8 \mu\text{m}$ , which is significantly smaller compared to the control group ( $134.7 \pm 5.9 \mu\text{m}$ ;  $p < 0.01$ ) and the lidocaine group ( $126.1 \pm 6.2 \mu\text{m}$ ;  $p < 0.05$ ).

The tubular epithelial layer is significantly disrupted. Epithelial thickness is reduced to  $18.6 \pm 1.9 \mu\text{m}$  versus  $26.3 \pm 2.1 \mu\text{m}$  in the control ( $p < 0.001$ ). Areas of focal dystrophy, cytoplasmic vacuolization, and partial desquamation of epithelial cells into the tubular lumen are noted. Epithelial cell nuclei are hyperchromatic, with signs of pyknosis in 21.4% of cases.

The intertubular interstitial tissue is significantly dilated due to edema. The area of the interstitial component is increased to  $38.7 \pm 3.4\%$  of the visual field, compared to  $24.1 \pm 2.8\%$  in the control group ( $p < 0.001$ ). The vessels are congested, with local stasis of blood cells and moderate swelling of the endothelium.

The number of cellular elements in the interstitium increased to  $17.3 \pm 2.1$  cells/field of view  $\times 200$ , which exceeds the values of both the control group ( $5.6 \pm 1.2$ ;  $p < 0.001$ ) and the lidocaine group ( $9.8 \pm 1.7$ ;  $p < 0.01$ ), indicating a more pronounced tissue reaction.

Thus, intratesticular administration of novocaine is accompanied by significant disruption of the epididymis's architecture, severe epithelial dystrophy, interstitial edema, and vascular disorders. Based on a combination of morphometric parameters, the degree of tissue damage with novocaine significantly exceeds similar changes with intratesticular lidocaine.

Van Gieson staining after intratesticular administration of novocaine reveals significant changes in the connective tissue component of the epididymis compared to the control group and the lidocaine group (Fig. 5).



**Figure 5.** Epididymis after intratesticular injection of novocaine.

**Van Gieson staining,  $\times 200$ .**

- 1 - intertubular connective tissue with thickened collagen fibers;
- 2 - wall of convoluted tubules with increased collagenization of the basement membrane;
- 3 - perivascular connective tissue with signs of sclerosis.

In the intertubular stroma, significant thickening and compaction of collagen fibers, intensely stained red, are noted. The proportion of the connective tissue component in the structure of the appendage increased to  $41.9 \pm 3.6\%$  of the visual field area, which significantly exceeds the values in the control group ( $23.7 \pm 2.5\%$ ;  $p < 0.001$ ) and the intratesticular lidocaine injection group ( $29.4 \pm 2.9\%$ ;  $p < 0.01$ ).

The tubular basement membrane is thickened and uneven in places. The average basement membrane thickness was  $4.6 \pm 0.5 \mu\text{m}$ , while in the control group this value did not exceed  $2.1 \pm 0.3 \mu\text{m}$  ( $p < 0.001$ ). In some areas, signs of initial collagenization of the tubular walls with a tendency toward rigidity are detected.

Perivascular connective tissue also underwent significant restructuring: a concentric accumulation of collagen fibers around the vessels was observed, accompanied by a narrowing of their lumen. The area of perivascular fibrosis increased by 1.8 times compared to control values ( $p < 0.01$ ).

The severity of connective tissue changes correlates with previously identified dystrophic and vascular disorders when stained with hematoxylin and eosin, which indicates the formation of early fibrous processes in the tissue of the epididymis after intratesticular administration of novocaine.

Thus, the results of Van Gieson staining indicate that intratesticular administration of novocaine is accompanied by activation of collagen formation and the development of connective tissue restructuring, significantly exceeding similar changes when using lidocaine.

Under novocaine conduction anesthesia, the epididymis generally retains its ordered architecture. The convoluted tubules are predominantly regular in shape, with uniform lumens and no significant deformation. The average tubule diameter was  $128.6 \pm 5.7 \mu\text{m}$ , which is not statistically significant compared to control values ( $134.7 \pm 5.9 \mu\text{m}$ ;  $p > 0.05$ ) (Fig. 6).



**Figure 6.** Epididymis after conduction anesthesia with novocaine.

**Hematoxylin and eosin staining,  $\times 200$ .**

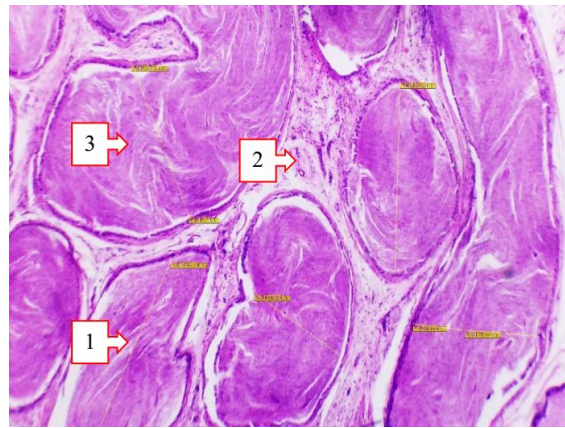
- 1 - convoluted tubules of the epididymis;
- 2 - tubular epithelium with moderate dystrophic changes;
- 3 - intertubular interstitial tissue.

The epithelial layer of the tubules is preserved in most fields of view. The epithelial thickness was  $23.9 \pm 2.2 \mu\text{m}$ , which is significantly higher than the same indicator after intratesticular administration of novocaine ( $18.6 \pm 1.9 \mu\text{m}$ ;  $p < 0.01$ ) and comparable to the control group ( $26.3 \pm 2.1 \mu\text{m}$ ;  $p > 0.05$ ). Foci of vacuolization and disorganization of epithelial cells are isolated and do not lead to a violation of the integrity of the epithelial layer.

The intertubular interstitial tissue is moderately expressed, without signs of significant edema. The area of the interstitial component was  $27.8 \pm 2.6\%$ , which is significantly lower than the values after intratesticular administration of novocaine ( $38.7 \pm 3.4\%$ ;  $p < 0.01$ ) and only slightly exceeds the control values ( $24.1 \pm 2.8\%$ ;  $p > 0.05$ ). The vessels are moderately congested, without signs of stasis and pronounced microcirculatory disorders.

Thus, conduction anesthesia with novocaine is associated with only moderate morphological changes in the epididymal tissue. Compared to intratesticular administration of novocaine, this method provides a significantly gentler effect on the epithelium and interstitial tissue, indicating a reduced degree of local tissue damage.

Van Gieson staining after lidocaine conduction anesthesia reveals minimal changes in the connective tissue component of the epididymis. Collagen fibers of the intertubular stroma are represented by thin, evenly distributed bundles without signs of thickening or excessive accumulation (Fig. 7).



**Figure 7.** Epididymis after conduction anesthesia with lidocaine.

**Van Gieson staining, ×200.**

- 1 - intertubular connective tissue with thin collagen fibers;
- 2 - basement membrane of the tubules without signs of collagenization;
- 3 - perivascular zone without fibrous changes.

The area of the connective tissue component was  $25.6 \pm 2.4\%$ , which does not differ from the control group ( $23.7 \pm 2.5\%$ ;  $p > 0.05$ ) and is significantly lower than the values for both intratesticular ( $41.9 \pm 3.6\%$ ;  $p < 0.001$ ) and conductive administration of novocaine ( $31.2 \pm 2.9\%$ ;  $p < 0.01$ ).

The tubular basement membrane maintains a uniform thickness ( $2.3 \pm 0.4 \mu\text{m}$ ), with no signs of collagenization or fibrous remodeling. The perivascular connective tissue is not thickened, and the vessels maintain a preserved lumen, with no signs of compression or sclerosis.

The obtained data indicate that conduction anesthesia with lidocaine has a minimal effect on the connective tissue framework of the epididymis and is characterized by the most favorable morphological profile among all the studied methods of anesthesia[15].

**Conclusions:**

The prevalence of hepatitis B and C markers among blood donors was characterized by a low rate of active infection and a substantially higher frequency of markers indicating previous exposure to hepatitis B virus, particularly anti-HBc, which demonstrated a clear age-related increase.

Younger donor cohorts were predominantly characterized by vaccination-associated immunity, reflected by a higher prevalence of isolated anti-HBs positivity, whereas older age groups showed a shift toward combined anti-HBc and anti-HBs profiles, consistent with resolved natural infection.

The identified heterogeneity of serological profiles among blood donors highlights the epidemiological significance of age-stratified analysis and supports the need for comprehensive assessment of HBV and HCV markers to optimize donor screening strategies and ensure transfusion safety.

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