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Therapeutic Effects of Intracavernosal Stromal Vascular Fraction in an Ischemic Cavernosal Injury Model of Erectile Dysfunction

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Title page

Therapeutic Effects of Intracavernosal Stromal Vascular Fraction in an Ischemic Cavernosal Injury Model of Erectile Dysfunction

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Ethics Statement

Institutional Animal Care and Use Committee (IACUC) approval was obtained from the Bagcilar Training and Research Hospital Local Ethics Committee for Animal Experiments (HADYEK) on February 23, 2024 (No. 2023-29). The study was conducted by the physicians involved in the research at the Bagcilar Experimental Research and Skills Development Center (BADABEM).

Conflict of Interest

The authors have nothing to disclose.

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AUTHOR CONTRIBUTION

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Abstract and Keywords

Abstract

Purpose
To evaluate the effects of intracavernosal stromal vascular fraction (SVF) on erectile function and tissue-level changes in an ischemic priapism model.

Methods
Male Wistar rats (n=24) were randomized to Sham, Priapism, or Priapism+SVF (n=8 each). Priapism was induced with a penile base constrictor for 60 min. Autologous SVF from peritesticular fat was injected at band removal. At 4 weeks, cavernous nerve–stimulated intracavernosal pressure (ICP) and mean arterial pressure (MAP) were recorded; penile tissue underwent histology, immunohistochemistry, and qRT-PCR.

Results
Two rats died before model creation; analyses included Sham (n=8), Priapism (n=7), and Priapism+SVF (n=7). Baseline ICP and MAP were similar ($p=0.105$; $p=0.911$). Priapism lowered ICPmax, ICPmean, and ICP/MAP versus Sham (all $p<0.001$). SVF improved ICPmax and ICP/MAP versus Priapism ($p=0.002$; $p=0.013$) but did not reach Sham. ICPmean difference was not significant between Priapism and SVF groups ($p=0.079$). qRT-PCR showed no differences in NGF, TGF-β1, VEGF, or BDNF (all $p>0.05$). Histology showed more inflammation, fibrosis, cellular injury, hypoplasia, and loss of elastin in Priapism; with SVF these changes shifted to milder grades and elastin partially recovered (all $p=0.001$). Immunohistochemistry: VEGF unchanged; Bcl-2 higher in Priapism and intermediate after SVF (extent $p=0.035$; intensity $p=0.001$). eNOS reduced in Priapism and partially restored with SVF (extent $p=0.006$). nNOS elevated in Priapism and attenuated with SVF (both $p=0.001$). iNOS remained higher in both Priapism arms compared to Sham (both $p=0.003$). The muscle-to-fibrosis ratio did not differ ($p=0.561$), whereas the type I/type III collagen ratio did ($p=0.003$).

Conclusions
Intracavernosal SVF conferred partial protection in ischemic priapism. Its minimally processed, point-of-care profile supports feasibility after detumescence, and its endothelial, antifibrotic, and neurotrophic actions make it promising for broader erectile dysfunction phenotypes. Larger preclinical and controlled clinical studies should define optimal dose/timing and establish long-term efficacy and safety.

Keywords: Erectile dysfunction; Fibrosis; Mesenchymal stem cells; Priapism; Regeneration; Stromal Vascular Fraction

Introduction

Priapism is characterized by prolonged penile erection lasting over four hours in the absence of sexual stimulation (1). It predominantly affects men over 40 and is categorized primarily into ischemic (low-flow) and non-ischemic (high-flow) types (2). Ischemic priapism, the most common subtype, arises due to impaired venous drainage, causing painful erections that necessitate immediate treatment (3). Without prompt intervention, ischemic priapism significantly increases the risk of irreversible erectile dysfunction (ED) (4).

Pathophysiologically, ischemic priapism represents a compartment syndrome induced by venous blood stasis within the corpora cavernosa, potentially triggered by hematologic conditions, pharmacological treatments, trauma, malignancies, neurological disorders, and infections (5). Early-stage intervention (within 12 hours) is critical to preserve erectile function, as prolonged ischemia leads to smooth muscle destruction and fibrotic changes, ultimately resulting in permanent ED (6).

Recent advances in regenerative medicine suggest mesenchymal stem cells (MSCs) have significant therapeutic potential for tissue repair and fibrosis prevention in various diseases, including those affecting penile tissue (7–9). However, clinical translation of MSC therapies is limited by cell culture requirements and practical constraints in acute conditions (10). Stromal vascular fraction (SVF), derived directly from adipose tissue, contains MSCs and other beneficial cell populations, and is rapidly obtainable without culture (11). Previous research has shown SVF's effectiveness in several ischemic and fibrotic conditions, yet its potential in ischemic priapism remains unexplored (12–14).

This study evaluated whether intracavernosal administration of SVF can reduce cavernosal tissue injury and preserve erectile function after ischemic insult, supporting its potential as a broadly applicable early intervention strategy for erectile dysfunction across diverse etiologies.

Materials and Methods

Institutional Animal Care and Use Committee (IACUC) approval was obtained from the Bagcilar Training and Research Hospital Local Ethics Committee for Animal Experiments (HADYEK) on February 23, 2024 (No. 2023-29). All procedures complied with institutional and national guidelines for the care and use of laboratory animals.

Animals and study design

Twenty-four adult male Wistar Albino rats (350–400 g) were housed under controlled conditions (20 ± 2 °C; 12-h light/12-h dark cycle) with ad libitum access to standard chow and water. Animals were randomly assigned (computer-generated) to three groups (n=8/group): Sham, Priapism (ischemic control), and Priapism+SVF (treatment).

Anaesthesia

Surgical procedures were performed under intraperitoneal xylazine hydrochloride (10 mg/kg; 2% Rompun, Bayer, Türkiye) and ketamine hydrochloride (50–60 mg/kg; 10% Alfamine, Ege Vet, Türkiye).

Ischemic priapism model

Ischemic priapism was induced for 60 min using a previously described method based on a vacuum-assisted erection and a constrictor band at the penile base (15). Briefly, erection was achieved by gentle negative pressure; then a 2-mm band (cut from a 16Fr Foley catheter) was positioned at the penile root and removed after 60 min. Sham animals received intracavernosal (IC) 0.2 mL saline without sustained ischemia.

SVF isolation and characterization

Adipose tissue (perirenal/peritesticular) harvested from donor rats was processed under sterile conditions to obtain stromal vascular fraction (SVF). Tissue was mechanically fragmented and microprocessed to nanofat using a closed tissue-processing system (GCELL® Istanbul, Turkiye), followed by centrifugation (400 RCF, 10 min) and 41-µm filtration, yielding a cell suspension for IC use. Cell viability was assessed by propidium iodide staining; immunophenotyping (CD105, CD90, CD73, CD45) was performed using FITC-conjugated monoclonal antibodies and analysed by flow cytometry (NovoCyte 3005, Agilent, Santa Clara, USA) with NovoExpress 6.0 (Agilent). Flow staining buffer was from Tonbo Biosciences (USA).

Intervention

Immediately after removal of the constrictor band, the treatment group received IC SVF (0.2 mL); Priapism controls received IC saline (0.2 mL). Dosing volume and route were adapted from prior penile fibrosis models (10).

Electrophysiological assessment of erectile function

At 4 weeks, erectile responses were recorded under anaesthesia. Mean arterial pressure (MAP) was measured via carotid cannulation (PE-50) connected to a pressure transducer and amplifier (COMMAT Pharmacology & Physiology Instruments, Ankara, Türkiye) and a data acquisition module (MP35, Biopac). Intracavernosal pressure (ICP) was recorded through a 25-gauge needle placed in the proximal corpus cavernosum. The cavernous nerve was identified near the major pelvic ganglion and stimulated using a bipolar electrode connected to a stimulator (STPT02, COMMAT). Stimulation parameters were 1.5 mA, 15 Hz, 5 ms pulse width, 35 ms delay, applied for 60 s. Erectile function was quantified as the ICPmean/MAP ratio to normalize for systemic hemodynamics (16).

Tissue collection and histology

After functional testing, penectomy was performed at the crura level. Each penis was hemisected: one half fixed in 10% neutral buffered formalin for histology, the other snap-frozen (−80 °C) for molecular analyses. Paraffin sections were stained with hematoxylin-eosin (H&E) (Figure 1), Masson's Trichrome (Figure 2), Periodic Acid–Schiff (PAS), and Picro-Sirius Red (all reagents: Bioptica, Italy) (Figure 3). Blinded histological scoring assessed inflammation and fibrosis (0: none; 1: mild; 2: moderate; 3: severe) and endothelial injury, according to predefined criteria.

We quantified the smooth muscle-to-fibrosis area ratio (muscle/fibrosis, not muscle/collagen) with Masson's Trichrome using Fiji (ImageJ) colour deconvolution to separate red (smooth muscle) and blue (fibrotic collagen)

channels, followed by thresholding and area measurement; the ratio was computed as red area / blue area. For **Picro-Sirius Red**, polarized-light images were split into red (Type I) and green (Type III) channels; after thresholding (Otsu/Huang as appropriate), we measured areas and calculated the **Type I/Type III collagen area ratio**.

Immunohistochemistry
Sections were processed on an automated platform (BenchMark ULTRA, Ventana Medical Systems, Tucson, AZ, USA) using primary antibodies against TGF-β1, Elastin (Figure 4), VEGF (Thermo Scientific, USA), Bcl-2 (Bioss, USA) (Figure 5), eNOS (Boster, USA) (Figure 6), nNOS (Biorbyt, UK), and iNOS (Thermo Scientific, USA). Staining intensity/extent were semi-quantitatively scored (0–3) as per standard practice.

RNA isolation and RT-qPCR
Frozen tissue was homogenized (TissueLyser LT, Qiagen, USA). Total RNA was extracted using TRIzol™ Reagent followed by column purification (PureLink™ RNA Mini Kit; Thermo Scientific, USA). cDNA was synthesized with the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, USA). Quantitative PCR was performed on a Rotor-Gene Q (Qiagen) using Power SYBR™ Green Master Mix (Thermo Scientific). Target genes included TGF-β1, VEGF, NGF, and BDNF; G6PDH served as the reference gene.

Statistics
Statistical analyses were performed using IBM SPSS Statistics, version 26.0 (IBM Corp., Chicago, IL, USA). Descriptive statistics are presented as mean ± standard deviation, median (interquartile range [IQR]), and percentages.

Normality was assessed with the Shapiro–Wilk test, histograms, and probability (Q–Q) plots; based on these assessments, the numerical variables were determined to follow a normal distribution. Given the normality assumption, between-group differences for continuous measures—across the Sham, Priapism, and P+SVF groups—were examined using one-way analysis of variance (ANOVA). The analyzed variables included electrophysiological parameters (baseline intracavernosal pressure [ICP], maximal ICP, mean arterial pressure [MAP], ICP/MAP ratio) and elastin, as well as PCR-derived markers (NGF, TGF-β, VEGF, BDNF). In addition, the Masson's trichrome muscle-to-collagen ratio and the Picrosirius red type I/type III collagen ratio were compared using ANOVA.

To identify pairwise group differences, Tukey's honestly significant difference (HSD) post hoc test was applied. This procedure was chosen because it yields reliable results when group sample sizes are similar and variances are homogeneous, thereby clarifying the sources of any observed differences among the three groups.

For categorical variables, associations between groups were analyzed using the chi-square test. In this step, histopathological variables (inflammation, fibrosis, cellular injury, granulation, hypoplasia, elastin; categories: none–mild–moderate) and immunohistochemical markers (VEGF, Bcl-2, eNOS, nNOS, iNOS; categories: 0–25%, 25–75%, 75–100%) were evaluated. The eta coefficient (η) was also calculated to quantify effect size.

Finally, Bonferroni correction was applied in the chi-square analyses to control the family-wise error rate in multiple comparisons. All statistical analyses were considered significant at a two-sided p value <0.05.

Results

A total of 24 rats were randomized. Two animals (one each in the Priapism and Priapism+SVF groups) died during induction of anesthesia before model creation; thus, analyses included Sham (n=8), Priapism (n=7), and Priapism+SVF (n=7). All remaining procedures were completed per protocol.

Electrophysiology

Group comparisons are summarized in Table 1 and Figures 7–8. ANOVA showed no between-group difference for baseline ICP or MAP ($p=0.105$ and $p=0.911$, respectively). In contrast, there were significant group effects for ICPmax, ICPmean, and ICP/MAP (all $p<0.001$). Pairwise testing (Tukey HSD) demonstrated lower ICPmax and ICPmean in the Priapism group versus Sham (both $p<0.001$); the Priapism+SVF group improved versus Priapism for ICPmax ($p=0.002$) and ICP/MAP ($p=0.013$) but did not reach Sham levels. The ICP mean difference between Priapism and Priapism+SVF did not reach significance ($p=0.079$).

qRT-PCR

Relative mRNA expression of NGF, TGF-β1, VEGF, and BDNF did not differ significantly among groups (Tables 2–3; all $p>0.05$). No pairwise contrast reached significance.

Histopathology

Table 4 summarizes group-wise distributions. Sham specimens showed no inflammation, fibrosis, cellular injury, or hypoplasia, with high elastin in 7/8 (87.5%). Priapism specimens exhibited predominantly moderate–severe fibrosis (5 and 2 cases, respectively), severe cellular injury (5), increased hypoplasia (6 moderate), loss of high elastin (0), and inflammation (5 mild/2 moderate). In contrast, Priapism+SVF shifted toward milder grades—fibrosis mostly mild–moderate (2 and 5), no severe injury (0) with more mild–moderate injury (4 and 3), milder hypoplasia (4 mild/3 moderate), partial restoration of elastin (2 moderate), and inflammation mainly mild (7). Granulation did not differ among groups. Chi-square test tests showed significant group effects for inflammation, fibrosis, cellular injury, hypoplasia, and elastin (all $p = 0.001$), but not for granulation.

Immunohistochemistry

VEGF staining (extent and intensity) did not differ significantly among groups (Table 5). Bcl-2 showed significant group differences for both extent and intensity ($p=0.035$ and $p=0.001$, respectively), with increased expression in Priapism and intermediate levels in Priapism+SVF relative to Sham. eNOS showed a significant difference in extent ($p=0.006$) but not intensity ($p=0.890$); distribution was highest in Sham, reduced in Priapism, and partially restored in Priapism+SVF (post-hoc difference between Priapism and Priapism+SVF significant). nNOS differed for both extent and intensity (both $p=0.001$), being elevated in Priapism versus Sham and attenuated toward Sham in Priapism+SVF. iNOS differed for both extent and intensity (both $p=0.003$), with low expression in Sham and higher levels in both Priapism and Priapism+SVF; attenuation in the treatment group was limited.

Collagen ratios

Quantitative image analysis of Masson's trichrome (muscle/fibrosis) and Picro-Sirius Red (type I/type III collagen) is presented in Table 6 and Figures 9-10. The muscle/collagen ratio did not differ among groups ($p=0.561$). The type I/type III collagen ratio showed a significant group effect ($p=0.003$), indicating remodeling differences among Sham, Priapism, and Priapism+SVF groups.

Discussion

This study evaluated whether intracavernosal stromal vascular fraction (SVF) mitigates ischemia–reperfusion (I/R) injury and preserves erectile function after ischemic priapism. Three findings were consistent across modalities. First, cavernosal hemodynamics improved with SVF: ICPmax**,** ICPmean, and particularly ICP/MAP increased versus untreated priapism, although values remained below Sham (Table 1 and Figures 7–8). Second, histopathology shifted from moderate–severe injury in Priapism toward milder inflammation and fibrosis with SVF, together with partial restoration of endothelial features (Table 4). Third, immunohistochemistry suggested plausible mechanisms—greater eNOS distribution and modulation of Bcl-2—while iNOS persisted, indicating ongoing inflammatory drive (Table 5). Collectively, these data support incomplete but meaningful protection with a single SVF dose within the 4-week window.

These observations fit the paracrine/immunomodulatory model of SVF action: a heterogeneous mixture (mesenchymal stromal cells, endothelial/progenitors, pericytes, immune cells) secreting trophic, angiogenic, and anti-inflammatory factors that support NO-dependent smooth muscle relaxation and limit matrix accumulation (17–19). Our functional rescue parallels prior cell-based reports in erectile injury models (7) and aligns conceptually with Kılıç et al. who, using cultured MSC therapy in 1-h and 12-h priapism models, showed improved ICP metrics and favorable tissue remodeling (10). Unlike culture-expanded MSCs that require days to prepare, SVF is bedside-obtainable, which could be clinically advantageous immediately after detumescence/aspiration in ischemic priapism.

We quantified Masson's trichrome as the smooth muscle/fibrosis area ratio (red/blue after colour deconvolution), rather than a generic muscle/collagen metric. At 4 weeks, this muscle/fibrosis ratio did not differ among groups, despite qualitative downgrading of fibrosis severity with SVF. In contrast, Picro-Sirius Red (Type I/Type III collagen) showed a significant group effect, highest with SVF. Because Type III often predominates in immature/early repair whereas Type I increases with organized remodeling, a higher Type I/III in the setting of overall milder histologic fibrosis may reflect matrix maturation rather than exuberant scar formation (18, 19).Two technical factors may explain the Masson–Sirius divergence: (i) what is measured (Masson targets "fibrosis-positive" blue signal; Sirius partitions fibrillar subtypes), and (ii) timing/power—smooth muscle re-expansion may lag behind collagen subtype re-equilibration at 4 weeks, and variance in blue-channel thresholds can dilute small between-group effects in modest samples. Persistently low elastin across both priapism groups further argues that elastic fiber restoration lags collagen remodeling, plausibly limiting full normalization of compliance within this timeframe.

eNOS distribution improved with SVF versus Priapism, consistent with the ICP/MAP recovery and SVF-supported endothelial tone. In contrast, iNOS remained elevated in both Priapism and Priapism+SVF, implying that a single IC dose did not extinguish I/R-triggered inflammatory transcription; this lingering iNOS likely contributes to the incomplete functional rescue. Our nNOS signal differed by extent and intensity at group level, trending toward Sham with SVF but not normalizing—this partially diverges from Kılıç et al. who reported a selective eNOS increase without significant nNOS/iNOS change (10). Differences in cell product (SVF vs cultured MSCs), dose, injury duration, immunostaining quantification, and sampling time likely account for the discrepancy.

Gene-expression analyses (NGF, BDNF, VEGF, TGF-β1) did not differ significantly among groups at 4 weeks, in contrast to some reports showing transcriptional shifts in earlier windows (20, 21) and to signals discussed by Kılıç et al. (10). Several factors could underlie this: the small sample size, inter-animal variability, and the inherently dynamic, time-dependent expression of these mediators. Transient early peaks may have been missed, and paracrine signaling at the protein level may outweigh steady-state transcriptional changes detectable in bulk tissue (19–21). Importantly, the lack of significant mRNA differences does not negate the functional and histological benefits observed; rather, it frames future studies to include earlier and serial time points, protein quantification, and cell-tracking to dissect mechanisms.

Our results are broadly concordant with prior reports that mesenchymal cell–based therapies can ameliorate cavernosal injury in various ED models, while extending those observations by using a culture-free, rapidly obtainable cell source (7, 10). Animal studies have explored antifibrotic strategies after ischemic priapism—including PDE5 inhibitors, anti-TGF-β agents, melatonin, pentoxifylline, dipyridamole, , oxytocin, pirfenidon with encouraging preclinical signals (15, 22–27), yet no approved therapy exists for preventing post-ischemic penile

fibrosis in humans (3). In the clinical setting, SVF could be deployed immediately after detumescence/aspiration in acute ischemic priapism as an adjunct aimed at limiting downstream fibrosis and ED. Its bedside feasibility distinguishes it from culture-dependent cell products that require days to prepare.

Strengths of this work include random allocation, a validated ischemic model with objective neurostimulation-based ICP measurements, blinded histopathological scoring, and triangulation of outcomes across function, histology, immunohistochemistry, and qPCR. Limitations should temper interpretation: modest group sizes with two peri-anesthetic losses; a single SVF dose and single harvest time point; use of allogeneic donor SVF without direct comparison to culture-expanded MSCs or cell-free products; semi-quantitative staining assessments; and absence of oxidative-stress biomarkers or behavioral erectile testing. These factors, together with persistent iNOS, likely contributed to only partial functional rescue.

Beyond priapism, the same pathobiology underpinning common ED—endothelial dysfunction, smooth-muscle loss with maladaptive matrix remodeling, oxidative/inflammatory stress, and neural injury—maps to the domains improved with SVF in our model (eNOS support, fibrosis downgrading, ICP/MAP gains). Because SVF is point-of-care, autologous, and minimally processed, it can be prepared from small-volume lipoaspirate and delivered intracavernosally in the same session under local anesthesia/ultrasound guidance, making it operationally attractive for early adjunctive use in vasculogenic ED and peri-rehabilitation after prostatectomy. Crucially, this bypasses the major practical handicap of stem-cell therapies—the need for cell sourcing and ex vivo expansion under GMP conditions with days–weeks of lead time, high cost, and complex logistics—potentially enabling broader, real-world deployment. Pragmatic early-phase trials could randomize recent-onset vasculogenic or early post-prostatectomy ED to SVF vs control (± secretome) atop standardized rehab (PDE5, vacuum/traction), with IIEF-EF/EHS and penile Doppler (PSV/EDV/RI) as primary endpoints and safety monitoring (pain, nodularity, de novo fibrosis, priapic events). Given persistent iNOS in our dataset, combination strategies (selective iNOS inhibition and antioxidants) and serial dosing may be required to achieve fuller functional recovery.

Future work should map early and late molecular responses to SVF (hours to months), test dose, timing, and repeat-dosing paradigms, compare SVF with enriched subfractions or secretome/vesicle preparations, evaluate co-therapies that target complementary pathways (e.g., antioxidants, selective iNOS inhibitors, PDE5 modulation), and extend to long-term function and safety in larger animals. Such studies will clarify whether SVF primarily acts via endothelial support, anti-inflammatory signaling, antifibrotic remodeling, or a combination thereof.

Conclusions

In a neurostimulation-validated rat model of ischemic priapism, intracavernosal stromal vascular fraction (SVF) conferred incomplete but meaningful protection across functional and tissue domains. SVF improved erectile hemodynamics versus untreated priapism—most notably ICPmax and ICP/MAP—while histopathology shifted from moderate–severe to milder injury and inflammation. Immunohistochemistry supported endothelial recovery (greater eNOS distribution) alongside ongoing inflammatory drive (persistent iNOS). Quantitative matrix readouts diverged by modality: Picro-Sirius Red showed a significant shift in type I/type III collagen consistent with organized remodeling, whereas Masson's trichrome (quantified as smooth muscle/fibrosis ratio) was unchanged at 4 weeks, likely reflecting timing and power constraints. Bulk qRT-PCR for NGF, BDNF, VEGF, and TGF-β1 was non-significant, underscoring the need for earlier, serial, and protein-level mechanistic sampling.

Operationally, SVF's point-of-care, minimally processed, autologous nature bypasses key barriers that have limited clinical adoption of culture-expanded cell therapies, suggesting near-term translational feasibility as an adjunct after detumescence/aspiration to limit downstream fibrosis and erectile dysfunction. The same mechanistic axes—endothelial support, antifibrotic remodeling, neurotrophic paracrine signaling—provide a biological rationale to test SVF in selected ED populations (e.g., vasculogenic or early post-prostatectomy), potentially within combination strategies (selective iNOS inhibition, antioxidants, PDE5 modulation) and serial-dosing paradigms. Future studies should optimize dose/timing and product class (SVF vs enriched fractions vs secretome/EVs), standardize collagen/elastin metrics, and extend to long-term efficacy and safety in larger animals and controlled human trials. Collectively, these data position SVF as a pragmatic, mechanism-aligned candidate for penile tissue preservation and erectile function rescue after ischemic injury.

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Figure Legends

Figure 1. Hematoxylin–eosin (H&E), ×20. Overview of rat penile tissue. Corpora cavernosa (arrows) and urethra (asterisk).

Figure 2. Masson's trichrome, ×40, section from the corpus cavernosum. Blue-stained areas indicated by the **orange arrow** represent fibrotic tissue; pink-stained areas indicated by the **green arrow** represent smooth muscle.

Figure 3. Picro-Sirius Red, ×40, corpus cavernosum. **Green arrow:** red birefringent areas consistent with type I collagen; **blue arrow:** green birefringent areas consistent with type III collagen.

Figure 4. Elastin immunohistochemistry. (A,B) ×20, 1+ intensity and extent; (C) ×10, 3+ intensity and extent. **Blue arrows** highlight darker regions where staining is most intense.

Figure 5. Bcl-2 immunohistochemistry. (A) ×40, 1+ intensity and extent; (B) ×40, 3+ intensity and extent. **Blue arrows** indicate brown chromogenic signal marking higher Bcl-2 expression.

Figure 6. eNOS immunohistochemistry. (A) ×20, 1+ intensity and extent; (B) ×20, 3+ intensity and extent. **Blue arrows** indicate brown staining corresponding to higher eNOS expression.

Figure 7. Group comparisons of intracavernosal pressure (ICP) metrics—baseline, maximum, and mean—at 4 weeks. Lines show group means. Sham exhibited the highest values; Priapism showed marked reductions; Priapism+SVF showed partial recovery.

Figure 8. ICP/MAP ratio by group at 4 weeks. Values represent group means. The ratio was lowest in Priapism and partially improved in Priapism+SVF, remaining below Sham.

Figure 9. Masson's trichrome–derived muscle/fibrosis ratio by group (Sham n=8; Priapism n=7; Priapism+SVF n=7). Quantification was performed in Fiji (ImageJ) using color deconvolution. One-way ANOVA found no significant between-group difference ($p=0.561$).

Figure 10. Picro-Sirius Red–derived type I/type III collagen ratio by group. Channel-splitting and thresholding (Otsu/Huang) in Fiji (ImageJ) were used for quantification. ANOVA indicated a significant group effect ($p=0.003$).

Figures

Figure 1.



Figure 2.

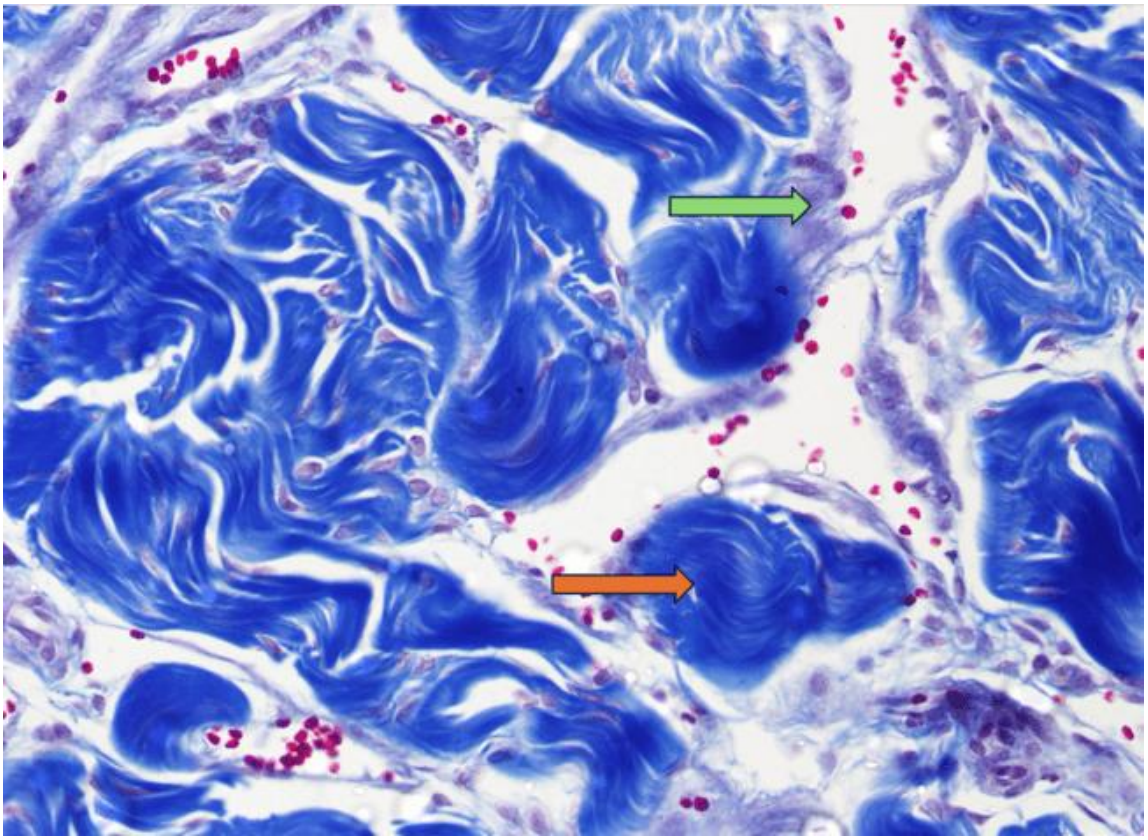


Figure 3.

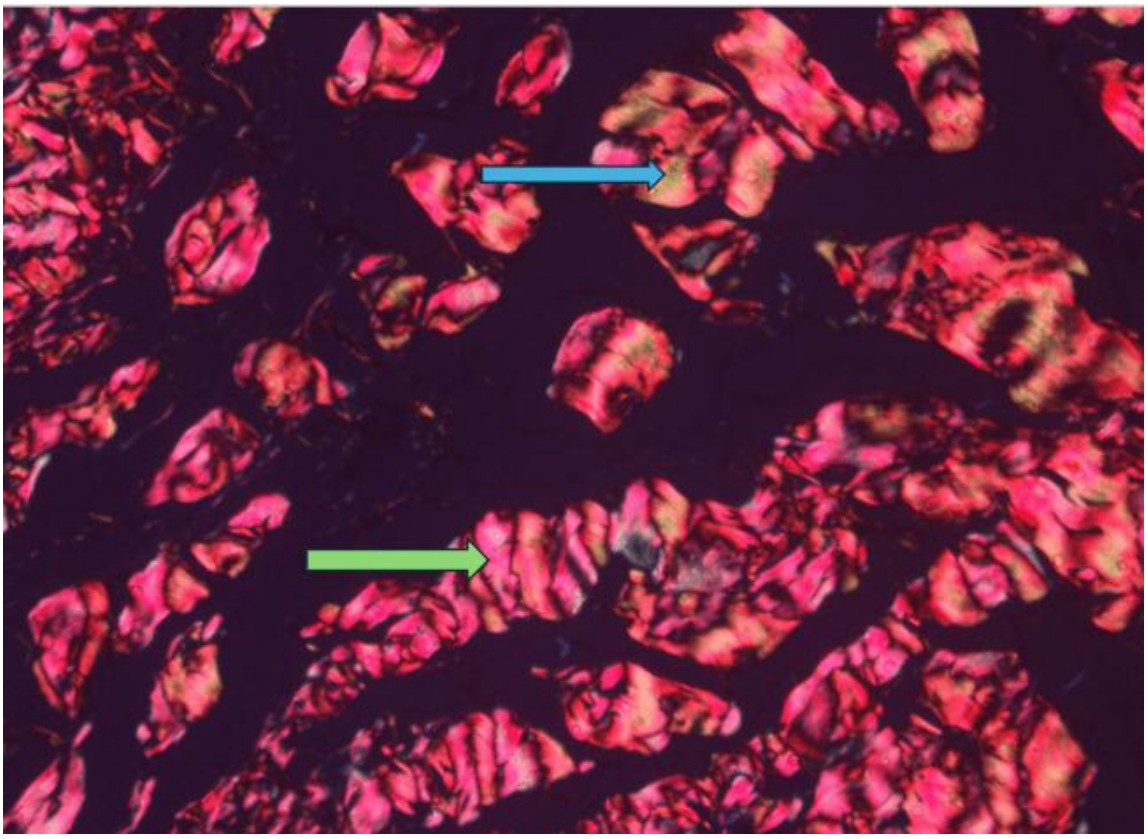


Figure 4.

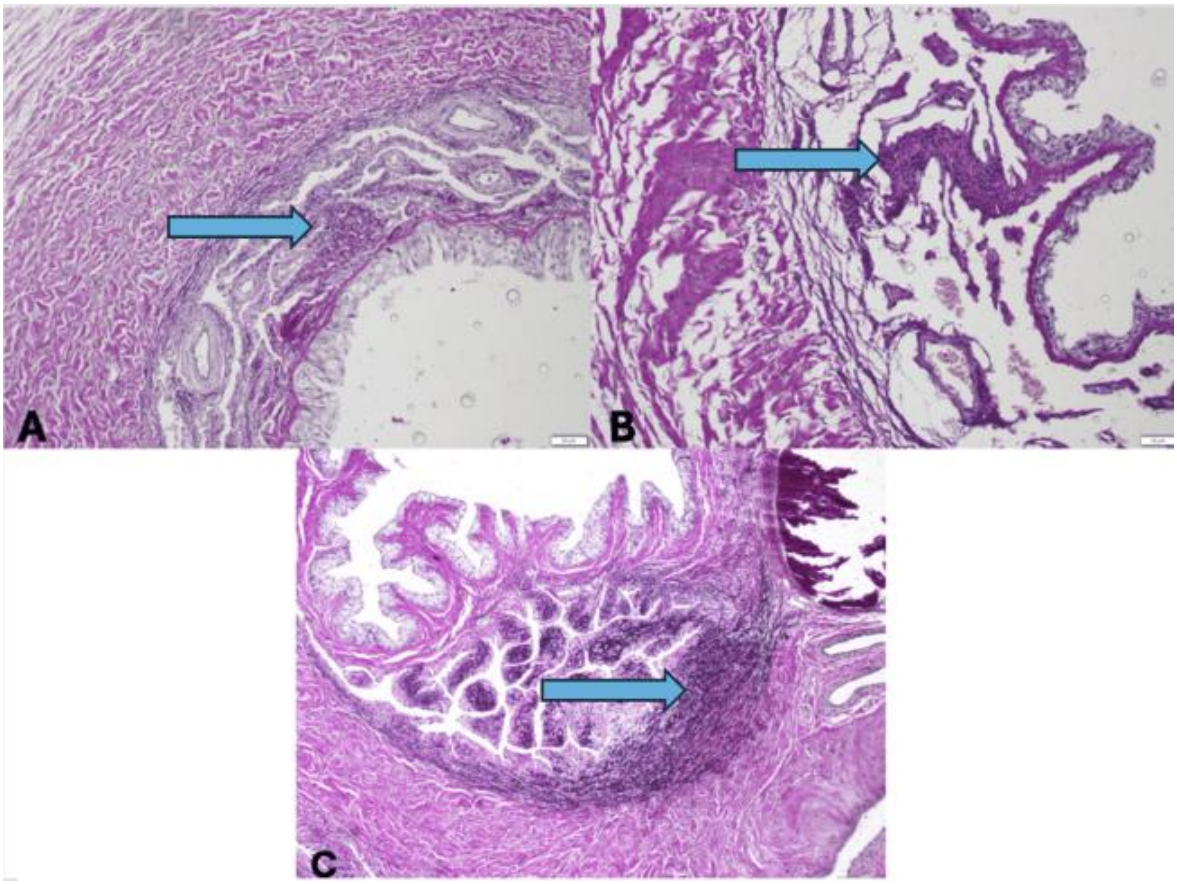


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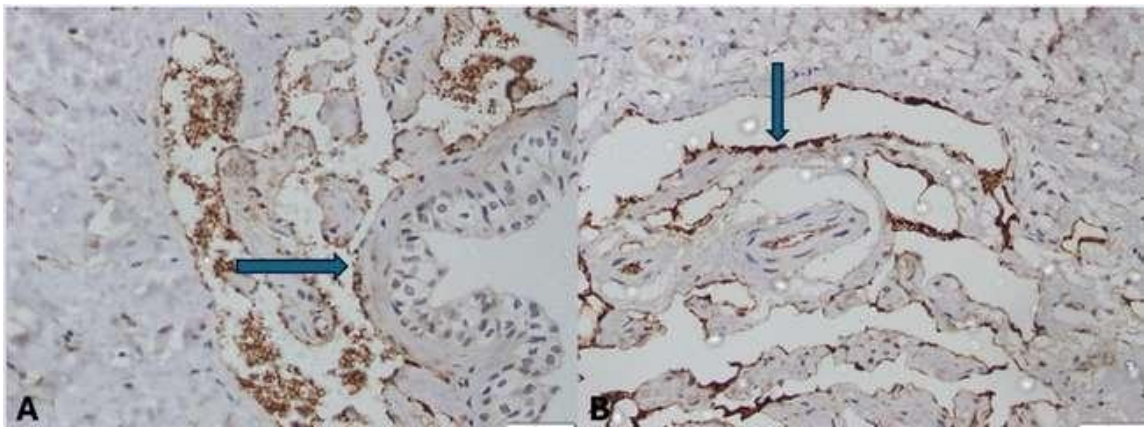


Figure 6.

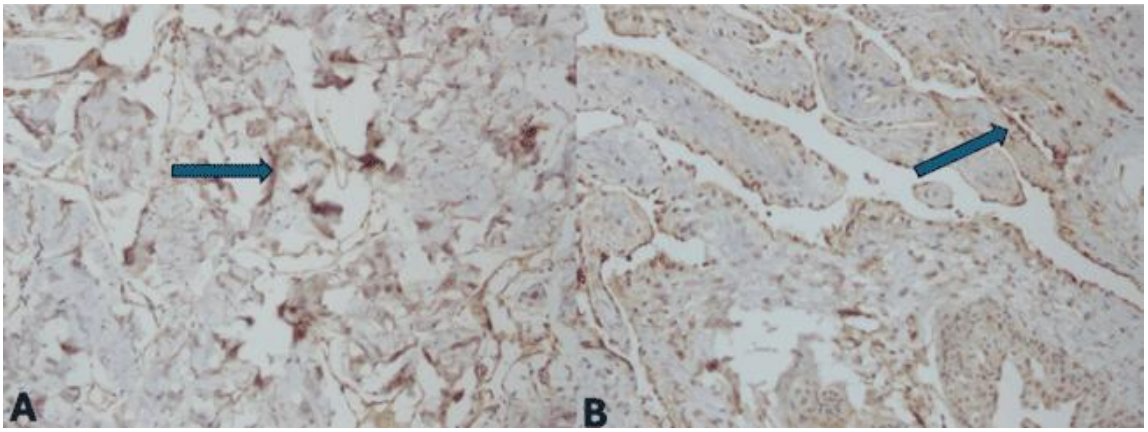


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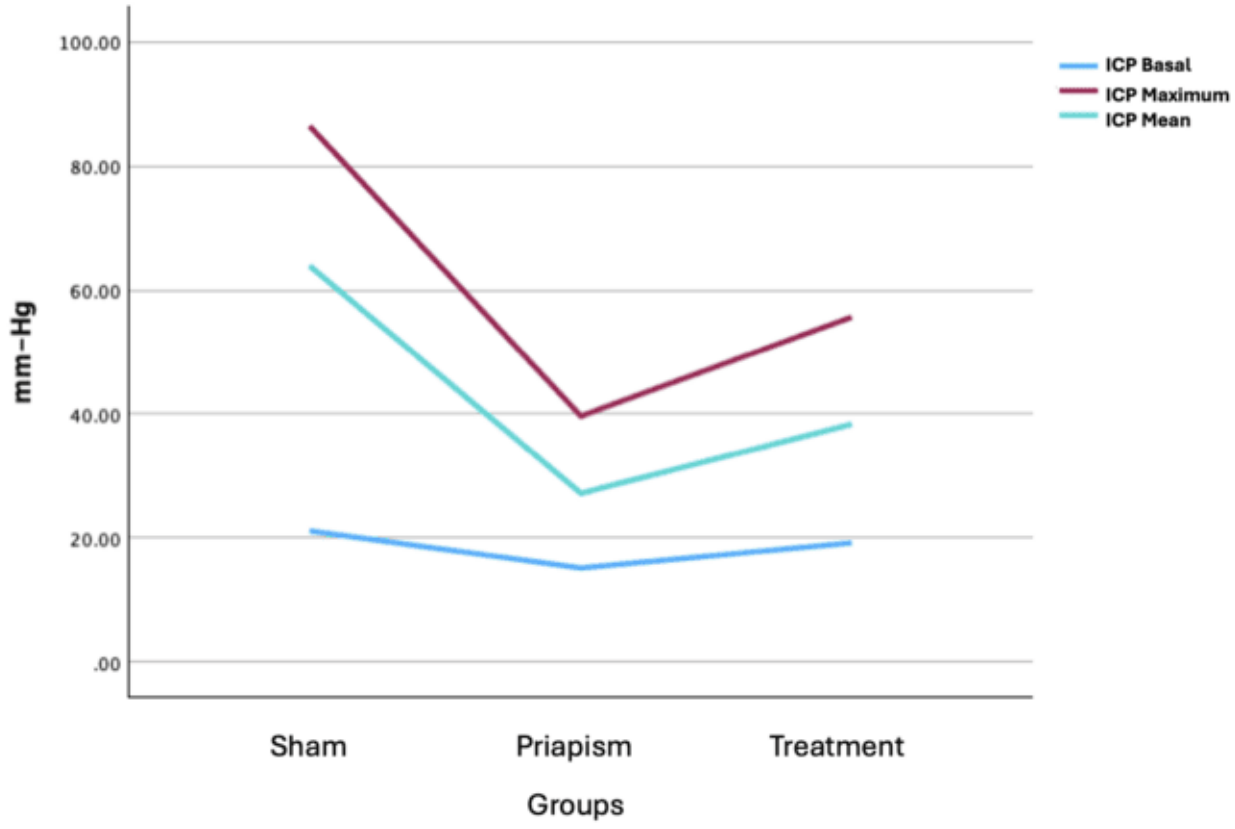

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mm-Hg
Sham
Priapism
Treatment
Groups
ICP Basal
ICP Maximum
ICP Mean

Figure 8.

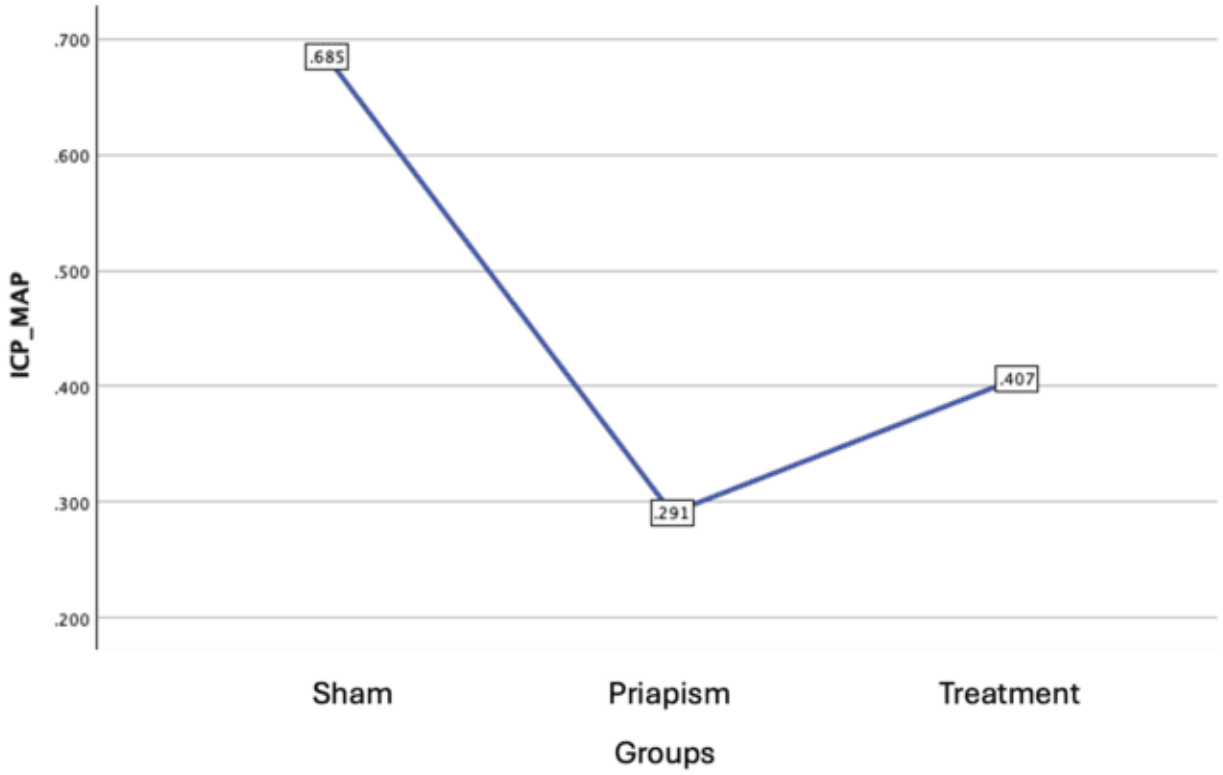

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Sham
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Treatment
Groups

Figure 9.

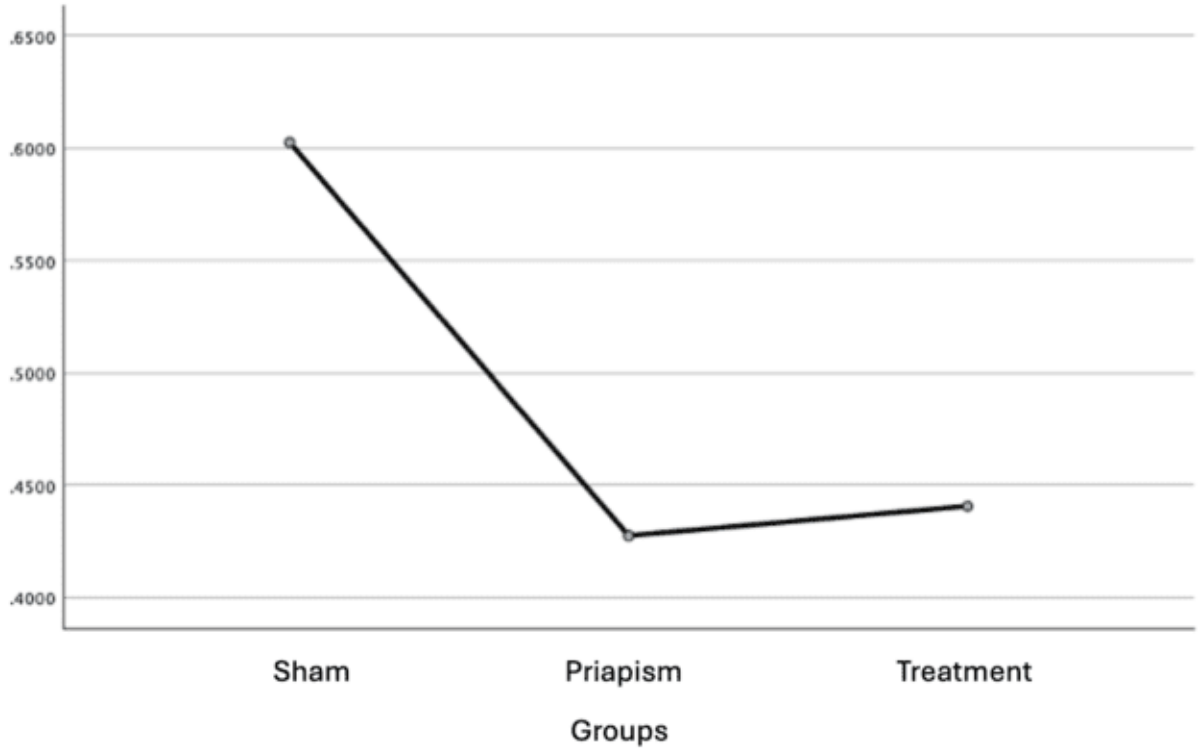
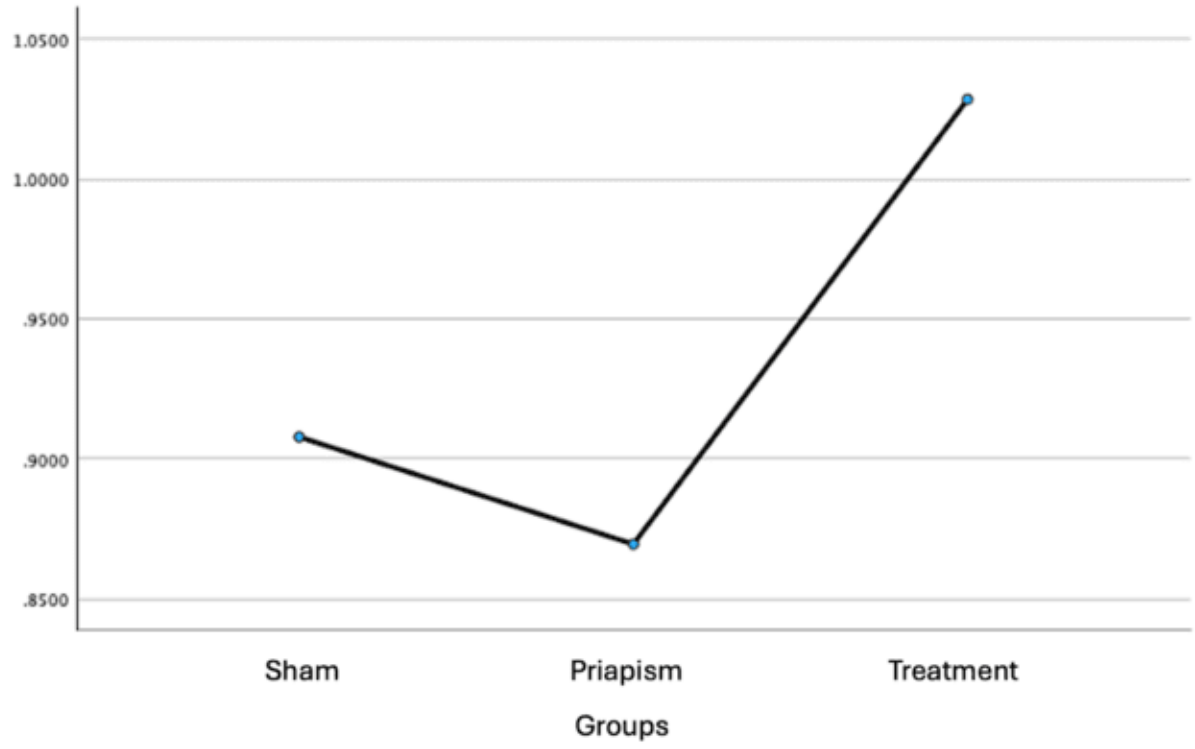

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Sham
Priapism
Treatment
Groups

Figure 10.



Tables

Table 1. ANOVA results for electrophysiological parameters

Explanatory note: This table summarizes group-wise erectile function at 4 weeks. Priapism markedly reduced ICP metrics relative to Sham, whereas SVF treatment partially improved ICPmax and ICP/MAP without fully restoring Sham levels.

Electrophysiological parameter	Sham (n=8), mean ± SD	Priapism (n=7), mean ± SD	Priapism+SVF (n=7), mean ± SD	p-value
ICP basal (mmHg)	21.12 ± 5.59	15.31 ± 5.02	19.21 ± 4.90	0.105
ICP max (mmHg)	86.44 ± 9.59	39.67 ± 6.16	55.66 ± 5.52	0.001
ICP mean (mmHg)	63.92 ± 13.10	27.18 ± 5.55	38.35 ± 5.40	0.001
MAP (mmHg)	93.73 ± 5.80	94.11 ± 5.39	95.31 ± 9.93	0.911
ICP/MAP (ratio)	0.68 ± 0.14	0.29 ± 0.06	0.40 ± 0.07	0.001

(a) One-way ANOVA across groups; two-sided $p<0.05$ considered statistically significant.
(b) Abbreviations: ICP, intracavernosal pressure; MAP, mean arterial pressure; max, maximum; mean, average.

Table 2. Group means (±SD) for qRT-PCR gene expression (2^−ΔΔCt)

Explanatory note: Relative mRNA expression normalized to G6PDH; values are presented as mean ± standard deviation.

Gene	Sham (n=8), mean ± SD	Priapism (n=7), mean ± SD	Priapism+SVF (n=7), mean ± SD
NGF	1.16 ± 0.71	0.70 ± 0.37	2.93 ± 3.43
TGF-β1	1.04 ± 0.32	0.90 ± 0.23	0.96 ± 0.16
VEGF	1.03 ± 0.25	1.04 ± 0.33	0.88 ± 0.36
BDNF	1.10 ± 0.11	0.27 ± 0.36	1.19 ± 0.47

(a) Data expressed as relative expression (2^−ΔΔCt) normalized to G6PDH.
(b) Abbreviations: NGF, nerve growth factor; TGF-β1, transforming growth factor-β1; VEGF, vascular endothelial growth factor; BDNF, brain-derived neurotrophic factor.

Table 3. Tukey HSD multiple comparisons for qRT-PCR gene expression

Explanatory note: Pairwise mean differences (first group minus second) following one-way ANOVA; two-sided p-values. No comparison reached statistical significance at $p<0.05$.

Dependent variable	Comparison	Mean difference	p-value	95% CI
NGF	Sham − Priapism	0.45	0.907	−2.31 to 3.23
	Sham − Priapism+SVF	−1.77	0.259	−4.55 to 1.00
	Priapism − Priapism+SVF	−2.23	0.128	−5.01 to 0.54
TGF-β1	Sham − Priapism	0.13	0.566	−0.20 to 0.48
	Sham − Priapism+SVF	0.08	0.821	−0.26 to 0.42
	Priapism − Priapism+SVF	−0.05	0.903	−0.39 to 0.28
VEGF	Sham − Priapism	−0.01	0.997	−0.45 to 0.42
	Sham − Priapism+SVF	0.15	0.664	−0.29 to 0.59
	Priapism − Priapism+SVF	0.16	0.622	−0.27 to 0.60
BDNF	Sham − Priapism	−0.16	0.650	−0.64 to 0.31
	Sham − Priapism+SVF	−0.09	0.872	−0.57 to 0.38
	Priapism − Priapism+SVF	0.07	0.918	−0.40 to 0.55

(a) Abbreviations: CI, confidence interval; NGF, nerve growth factor; TGF-β1, transforming growth factor-β1; VEGF, vascular endothelial growth factor; BDNF, brain-derived neurotrophic factor.

Table 4. Comparison of histopathological parameters among groups (inflammation, fibrosis, cellular injury, granulation, hypoplasia, elastin)

Explanatory note: Data are presented as n (%). p-values are from chi-square tests across groups. Lettered superscripts indicate post-hoc Bonferroni results—cells not sharing a letter differ at $p<0.05$. η denotes effect size.

Parameter	Category	Sham (n, %)	Priapism (n, %)	Priapism+SVF (n, %)	p-value	η
Inflammation	None	(8, 100%)a	(0, 0.0%)b	(0, 0.0%)b	0.001*	0.911
	Mild	(0, 0.0%)a	(5, 41.7%)b	(7, 58.3%)b		
	Moderate	(0, 0.0%)a	(2, 100%)a	(0, 0.0%)a		
Fibrosis	None	(8, 36.4%)a	(0, 0.0%)b	(0, 0.0%)b	0.001*	0.940
	Mild	(0, 0.0%)a	(0, 0.0%)a	(2, 100%)a		
	Moderate	(0, 0.0%)a	(5, 100%)b	(5, 100%)b		
	Severe	(0, 0.0%)a	(2, 100%)a	(0, 0.0%)a		
Cellular injury	None	(8, 100%)a	(0, 0.0%)b	(0, 0.0%)b	0.001*	0.948
	Mild	(0, 0.0%)a	(0, 0.0%)a,b	(4, 100%)b		
	Moderate	(0, 0.0%)a	(2, 40.0%)a	(3, 60.0%)a		
	Severe	(0, 0.0%)a	(5, 100%)b	(0, 0.0%)a		
Granulation	None	(8, 36.4%)	(7, 31.8%)	(7, 31.8%)	—	—
Hypoplasia	None	(8, 100%)a	(0, 0.0%)b	(0, 0.0%)b	0.001*	0.921
	Mild	(0, 0.0%)a	(1, 20%)a,b	(4, 80%)b		
	Moderate	(0, 0.0%)a	(6, 66.7%)b	(3, 33.3%)a,b		
Elastin	Low	(0, 0.0%)a	(5, 71.4%)b	(5, 71.4%)b	0.001*	0.880
	Moderate	(1, 12.5%)a	(2, 28.6%)a	(2, 28.6%)a		
	High	(7, 87.5%)a	(0, 0.0%)b	(0, 0.0%)b		

(a) Post-hoc Bonferroni: cells not sharing a letter differ at $p<0.05$.
(b) η, effect size.

Table 5. Chi-square analysis of immunohistochemistry (VEGF, Bcl-2, eNOS, nNOS, iNOS)

Explanatory note: Data are presented as n (%). p-values are from chi-square tests across groups. Lettered superscripts indicate post-hoc Bonferroni results—cells not sharing a letter differ at $p<0.05$. η denotes effect size (e.g., Cramér's V).

Parameter	Subcategory	Sham (n, %)	Priapism (n, %)	Priapism+SVF (n, %)	p-value	η
VEGF – Extent	0–25%	(5, 31.3%)	(6, 37.5%)	(5, 31.3%)	0.668	0.268
	25–75%	(2, 40.0%)	(1, 20.0%)	(2, 40.0%)		
	75–100%	(1, 100%)	(0, 0.0%)	(0, 0.0%)		
VEGF – Intensity	None	—	—	—	0.163	0.406
	Mild	(5, 27.8%)	(7, 38.9%)	(6, 33.3%)		
	Moderate	(3, 75.0%)	(0, 0.0%)	(1, 25.0%)		
Bcl-2 – Extent	0–25%	(6, 50.0%)	(1, 8.3%)	(5, 41.7%)	0.035*	0.553
	25–75%	(2, 20.0%)	(6, 60.0%)	(2, 20.0%)		
Bcl-2 – Intensity	None	(7, 87.5%)a	(0, 0.0%)b	(1, 12.5%)b	0.001*	0.866
	Mild	(1, 14.3%)a	(1, 14.3%)a	(5, 71.4%)a		
	Moderate	(0, 0.0%)a	(6, 85.7%)b	(1, 14.3%)a		
eNOS – Extent	0–25%	(0, 0.0%)a	(4, 66.7%)b	(2, 33.3%)a,b	0.006*	0.485
	25–75%	(8, 61.5%)a	(3, 23.1%)b	(2, 15.4%)b		
	75–100%	(0, 0.0%)a	(0, 0.0%)a	(3, 100.0%)a		
eNOS – Intensity	None	(3, 33.3%)	(3, 33.3%)	(3, 33.3%)	0.890	0.122
	Mild	(4, 36.4%)	(4, 36.4%)	(3, 27.3%)		
	Moderate	(1, 50.0%)	(0, 0.0%)	(1, 50.0%)		
nNOS – Extent	0–25%	(8, 80.0%)a	(0, 0.0%)b	(2, 20.0%)b	0.001*	0.842
	25–75%	(0, 0.0%)a	(4, 44.4%)b	(5, 55.6%)b		
	75–100%	(0, 0.0%)a	(3, 100%)a	(0, 0.0%)a		
nNOS – Intensity	None	—	—	—	0.001*	0.855
	Mild	(8, 61.5%)a	(0, 0.0%)b	(5, 38.5%)a		
	Moderate	(0, 0.0%)a	(7, 77.8%)b	(2, 22.2%)a		
iNOS – Extent	0–25%	(5, 100.0%)a	(0, 0.0%)b	(0, 0.0%)b	0.003*	0.717
	25–75%	(3, 17.6%)a	(7, 41.2%)b	(7, 41.2%)b		
iNOS – Intensity	None	—	—	—	0.003*	0.717
	Mild	(5, 100%)	(0, 0.0%)	(0, 0.0%)		
	Moderate	(3, 17.6%)	(7, 41.2%)	(7, 41.2%)		

(a) Post-hoc Bonferroni: cells not sharing a letter differ at $p<0.05$.
(b) Abbreviations: VEGF, vascular endothelial growth factor; Bcl-2, B-cell lymphoma 2; eNOS, endothelial nitric oxide synthase; nNOS, neuronal nitric oxide synthase; iNOS, inducible nitric oxide synthase.

Table 6. ANOVA of Masson's trichrome muscle/fibrosis and Picro-Sirius Red type I/type III collagen ratios by group

Explanatory note: Quantitative histomorphometry of penile tissue. Ratios were computed from area measurements in Fiji (ImageJ); values are presented as mean ± standard deviation.

Parameter	Sham (n=8), mean ± SD	Priapism (n=7), mean ± SD	Priapism+SVF (n=7), mean ± SD	p-value
Masson's trichrome (muscle/fibrosis) ratio	0.60 ± 0.44	0.42 ± 0.25	0.44 ± 0.30	0.561
Picro-Sirius Red (type I/type III collagen) ratio	0.90 ± 0.06	0.86 ± 0.06	1.02 ± 0.20	0.003*

(a) One-way ANOVA across groups; two-sided $p<0.05$ considered statistically significant.
(b) SD, standard deviation.