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THE EFFECT OF PENILE TOURNIQUET AND CONTINUOUS ARTIFICIAL ERECTION ON PENILE ERECTILE TISSUES: AN EXPERIMENTAL STUDY

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THE EFFECT OF PENILE TOURNIQUET AND CONTINUOUS ARTIFICIAL ERECTION ON PENILE ERECTILE TISSUES: AN EXPERIMENTAL STUDY

Abstract

Introduction

Penile tourniquet (PT) is known to cause ischemic injury, which worsens with prolonged application. Artificial erection (AE), formed by intracorporal saline injection mostly under PT, has been practiced for decades to evaluate penile curvature, yet its effect on erectile tissues has never been investigated. In this study, we examined a modified approach, continuous artificial erection (CAE), and investigated its effects on erectile tissues.

Objective

This study aims to investigate the histopathological and immunohistochemical effects of CAE on penile erectile tissues.

Study Design

Thirty-five rats were randomized into five groups. Four experiment groups received 20 or 40 minutes of isolated PT (20T and 40T) or PT with CAE (20T&E and 40T&E). CAE was achieved through continuous intracavernosal saline injection. Penectomy was performed three weeks post-procedure in the experiment groups and directly in the control group. Erectile tissue samples were evaluated using light microscopy for histopathological parameters including inflammation, neovascularization and fibrosis, and by immunohistochemistry. Endothelial function was assessed by eNOS and e-selectin staining, while ICAM-1 staining was used to assess chronic inflammation. Tissue samples were also spared for further investigation involving ultrastructural analysis.

Results

40T showed the highest levels of inflammation, fibrosis, and endothelial dysfunction. 20T had significantly less inflammation than 40T, with a non-significant increase in fibrosis and alteration of endothelial markers. 40T&E displayed the second-highest fibrosis rate (adjusted $p>0.05$), while 20T&E showed complete absence of fibrosis. Both 40T&E and 20T&E preserved strong eNOS and e-selectin expression, identical to controls. ICAM-1 expression in 20T&E was also consistent with the control group. The most significant difference in erectile tissue damage was noted between 40T and 20T&E.

Conclusion

This is the first study to evaluate the effects of AE on erectile tissues. Findings of this experimental model support that, CAE does not increase the tissue damage that is already caused by PT, but rather reduces it, likely through the washout of blood elements contributing to reperfusion injury. CAE possibly provides a protective effect on erectile tissues by preserving endothelial function, reducing inflammation and fibrosis, especially under 20 minutes of duration. These findings may support that AE maneuvers such as "artificial erection test" and CAE are potentially safe, while further studies involving ultrastructural analysis are needed to assess the detailed effects of CAE.

Keywords: penile erection, penile tourniquet, artificial erection, ischemia reperfusion injury

Introduction

Penile tourniquet (PT) is the most commonly used method for hemostasis during pediatric penile surgery [1]. Artificial erection (AE) is also commonly used to evaluate penile curvature and is formed by intracavernosal injection of saline solution, mostly under PT [2]. Although AE was first described in 1974 and has been widely used ever since, its effects on erectile tissues have not yet been investigated.

Experimental studies on PT and hypospadias repair models have indicated that PT can cause ischemia–reperfusion injury (IRI), even after short durations, and that the severity increases with prolonged application [3-6].

Recently, in our clinical practice, we hypothesized that maintaining artificial erection continuously during penile surgery would ensure that erectile tissues are filled with saline rather than blood, thereby minimizing bleeding and potentially facilitating dissection. In addition, curvature correction can be reassessed intraoperatively, allowing the procedure to be completed with a single PT, without repeated ischemia-reperfusion cycles. As a preliminary step to explore this potential surgical relevance, we designed an experimental model.

Our current study was therefore conducted to investigate whether continuous saline injection into the corpus cavernosum would exacerbate the tissue damage that is already caused by PT, due to increased intracavernosal hydrostatic pressure, or conversely, whether it would alleviate tissue injury by continuously washing out harmful blood products known to contribute to reperfusion injury.

This study aims to evaluate the histopathological and immunohistochemical effects of "continuous artificial erection" (CAE) under PT on penile erectile tissues.

Material and methods

Thirty-five male Sprague Dawley rats aged 16 to 18 weeks, weighing 320 to 450 grams, were used for the study groups. All animals were housed in individual cages under standardized conditions of temperature, moisture, light, and were fed ad libitum.

Experimental model design

For this study, we firstly designed and standardized a method for achieving CAE on the rat penis. The most feasible and reproducible system was gained by using double folded 1.3 mm silicone band, 25-gauge butterfly needle and a pressure control system formed by an infuser pump with 4cc/minute infusion rate that is attached to a two-way venous valve and a 10cc syringe for controlled manual support (Figure 1-2). This system was tested by connecting an empty fluid line to the venous valve during full erectile state and was seen to not exceed the physiological erectile intracavernosal pressure for the adult rat penis [7]. Saline solutions were used at room temperature.

Penil degloving (PD) was found to be necessary. Since the preputium of rat penis is multilayered and very elastic, secure needle placement was challenging with trans-dermal injections. Even with precise needle placement, secondary saline leakage resulted in severe mucosal edema and subcutaneous tissue damage, which was foreseen to influence the protein expression and histopathological findings. Trans-glanular injections did not provide adequate erection. Most preferable place for needle placement was the proximal corpora, with sufficient width for stabilization and distance from the neurovascular bundles posterolaterally and the urethra anteriorly. The needle was advanced until the end of its open tip entered the corpora and was checked to be in position with the infusion onset, then was manually stabilized using tactile feedback throughout the procedure.

Study groups

Thirty-five rats were randomly allocated into 5 study groups, one control group and 4 experiment groups, each containing 7 rats. PD and penectomy were done in the control group (C). Experiment groups were as follows: Group 20T: PD and 20 minutes of isolated PT, Group 20T&E: PD and 20 minutes of CAE under PT, Group 40T: PD and 40 minutes of isolated PT, Group 40T&E: PD and 40 minutes of CAE under PT.

Rats were anesthetized by intraperitoneal injection of ketamine hydrochloride (80-100mg/kg, Ketalar, Eczacıbası ®) and midazolam (4-5 mg/kg, Dormicum, Deva Holding ®). The naturally buried rat penis was exposed, and manual traction was applied. The penile area was cleaned with 10% povidone-iodine solution and prepared with sterile drapes. After total PD, PT was placed to the base of the penis and kept for 20 or 40 minutes for the isolated PT groups. CAE was added for the T&E groups. At the end, the needle was taken out and PT was removed. After ensuring there was no bleeding from the puncture site, degloving line was repaired with separate 7/0 Maxon sutures and the penis was buried back. Postoperatively, enteral paracetamol (1-2mg/mL) was added to the rats' drinking water. Three weeks following the procedures, rats were anesthetized in the same manner and penectomies were done following PD, as done in the control group.

Histopathological evaluation

The specimens were fixed with 10% formalin and embedded in paraffin blocks. Tissues were cut into 5 μm sections using a microtome, followed by staining with hematoxylin-eosin and Masson's trichrome protocols. Samples were investigated under light microscope by a pathologist with twenty years of experience who was blind to the study groups.

All samples were evaluated and scored (0–3) for inflammation, neovascularization, and fibrosis, as described in the literature [3]. Inflammation was graded as: none (0), mild (1), moderate (2), or severe (3), based on the density of polymorphonuclear and mononuclear cell infiltration. Neovascularization was graded as: none (0), mild (1), moderate (2), or severe (3) vascular proliferation. Fibrosis was graded as: none (0), mild (1), moderate (2), or severe (3, with hyalinization), based on the degree of collagen deposition and fibroblast proliferation.

Immunohistochemical evaluation

Two μm sections were prepared, deparaffinized and rehydrated. Automatic specimen staining equipment (Ventana Medical Systems, Tucson, AZ, USA) and indirect "biotin-free" system was used. eNos (endothelial nitric-oxide synthase) antibody (ABCAM/ab185698, 1:100), e-selectin (CD62E) antibody (ABCAM/ab5589, 1:100) and ICAM-1 (intracellular adhesion molecule-1) antibodies (ABCAM/ab282575-10U1, 1:100) were stained for 1 hour. Amplification Kit was used for signal augmentation. ROCHE/Cell Conditioning 1 (CC1) (EDTA) 60-minute standard protocol was used for antigen retrieval. Positive control tests with appropriate tissues and 0.1% hematoxylin counterstaining were conducted. Following dehydration and coverage, specimens were investigated under light microscope. Cytoplasmic staining was interpreted as positive staining. Density was scored as: negative (0), weak (1), moderate (2) and strong (3), in accordance with the literature [3].

Statistical analysis

SPSS 15.0 for Windows program was used. Descriptive data were presented as numbers and percentages. Scorings were evaluated as ordinal variables (scale: 0 to 3). Group

differences were assessed using the Kruskal–Wallis test. When significant differences were detected ($p < 0.05$), post-hoc pairwise comparisons were conducted using the Bonferroni correction to adjust for multiple comparisons. An adjusted p-value < 0.005 was considered statistically significant.

Results

Table 1A-B demonstrate the scoring results.

1) Histopathology

a) Inflammation

Group C showed no inflammation. All cases in 40T showed inflammation with lymphoplasmacytic cells (LPC) (Figure 3a). Among all animals, only one in 40T showed marked inflammation (score 2), remaining responses were mild (score 1). The second highest rate was in 40T&E, which exhibited hemosiderin loaded macrophages along with LPC (Figure 3b). Group 20T&E showed LPC's in 2 animals. Group 20T showed hemorrhagic congestion in one animal. With adjusted analysis, differences between 40T and C, 20T, and 20T&E were statistically significant ($p < 0.05$). In the initial analysis, 40T vs. 40T&E also showed a significant difference; however, this was lost after adjustment. Group 40T&E showed no significant difference compared to C and either of the 20T or 20T&E groups, and 20T&E did not show a significant increase in inflammation compared to C or 20T.

b) Neovascularization

All animals in 40T, 40T&E, and 20T exhibited mild neovascularization (score 1) compared to C (adjusted $p = 0.000$). The corresponding ratio was 57.1% for 20T&E, which did not show a significant difference from the control group after post-hoc analysis (adjusted $p = 0.197$), despite a raw p-value of 0.02.

c) Fibrosis

Group C showed no fibrosis. The highest rate of fibrosis was present in 40T, followed by 40T&E and 20T respectively, and all were of mild density (score 1) (Figure 3c). Compared to the control group, only 40T showed a significant increase in fibrosis (adjusted $p < 0.005$). No fibrosis was present in any of the cases in 20T&E. In pairwise comparisons, 20T&E vs. 40T&E showed a significant difference in the raw analysis (raw $p = 0.007$) but did not retain significance after correction (adjusted $p = 0.72$). In contrast, 20T&E vs. 40T remained significant after adjustment (adjusted $p = 0.013$). Other pairwise comparisons did not show statistical significance ($p>0.05$).

2) **Immunohistochemistry**

eNOS

Groups C, 20T&E, and 40T&E showed strong density staining (score 3) for eNOS, in all of their cases (Figure 3d-e). The lowest levels of eNOS positivity were detected in 40T, where all cases revealed moderate density staining (score 2) (Figure 3f). Group 20T had 71.4% strong and 28% moderate density staining. Differences between 40T and C, 20T&E and 40T&E remained significant after adjustment (adjusted $p < 0.05$). In contrast, the difference between 40T and 20T was significant in the raw analysis (raw $p < 0.05$) but lost significance after adjustment. Group 20T did not show significant differences from C or T&E groups ($p>0.05$).

a) e-selectine (CD62E)

Groups C, 20T&E, and 40T&E showed strong density staining (score 3) for e-selectine, in all their cases (Figure 3g, i). Groups 20T and 40T revealed equal rates of strong (71.4%) and moderate (28.6%) density staining (Figure 3h). Differences between isolated PT groups and the others were not significant ($p>0.05$).

b) ICAM-1

Groups C and 20T&E did not reveal any strong density staining (score 3), while they both showed 85.7% moderate (score 2) and 14.3% weak (score 1) positive staining (Figue 3j-k). Group 40T&E showed the same rate of moderate staining along with 14.3% strong positivity. Group 20T also showed 14.3% strong positivity, with 71.4% moderate and 14.3% weak positive staining. Group 40T revealed the highest rate of strong (42.9%) density staining, with no cases of weak positivity (Figure 3l). In pairwise comparisons, C vs. 40T (raw $p = 0.005$) and 20T&E vs. 40T (raw $p = 0.04$) were statistically significant in the unadjusted analysis; however, neither retained significance after correction (adjusted $p > 0.05$).There was no statistical significance among other group comparisons ($p>0.05$).

Discussion

IRI secondary to PT has been investigated by various studies [3-6]. However, a consensus on the ideal PT duration is not present. Even 10 minutes of PT was shown to cause a rise in the malondialdehyde levels and affect tissue growth factors [4,5]. In a rabbit model, Bozkurt et al. studied cavernosal endothelial cholinergic relaxation responses under PT and showed they were not affected at 20 minutes but were irreversibly affected at 40 and 60 minutes of PT [6].

There are few studies on PT use in experimental hypospadias models. In a rat model, Boybeyi et al. showed that 10 minutes of PT increased the risk of bacterial adhesion, resulted in significant endothelial damage and limited endothelial cell proliferation [3]. Kajbafzadeh et al. studied the uroepithelial ultrastructural alterations in different hemostasis methods and demonstrated that while PT causes damage to the urothelium, it is safer than other methods [8]. Gulburun et al. demonstrated that intraperitoneally

administered hydrogen-rich saline solution exerted a protective effect against acute IRI in a rat Mathieu flap model, at both 10 and 30 minutes of PT duration [9]. Our model directly infused regular saline intracavernosally during PT, to look for a localized protective approach while also achieving a prolonged erection.

Artificial erection test (AET) has been widely used in pediatric urology for decades. AET has been criticized for the potential of causing supra or sub-physiological intracorporal pressure to alter surgical findings [10]. It is also not known if the uncontrolled pressure may result in erectile tissue damage. To our knowledge, this is the first study to investigate the effects of AE on penile tissues.

AET may represent an intermittent form of PT when repeated to check for curvature correction. Although the duration of each AET is minimal, it still exposes tissues to repeated ischemia–reperfusion cycles. If proven safe and incorporated into practice, CAE would allow dissection and curvature control to be performed under a single continuous erection, thus avoiding repeated tourniquet applications.

eNOS is involved in the synthesis of nitric oxide (NO), a potent vasodilator in penile erection mechanism [11]. İmmunohistochemical decrease of eNOS secondary to chronic cavernosal ischemia was shown to be present in a rabbit model of chronic atherosclerosis [12] and a rat model of age-related ischemia [13].

ICAM-1 is a transmembrane glycoprotein that is continuously expressed on endothelial cells which increases under oxidative stress, with a critical role in inflammation [14,15].There is a known inverse relation between NO and ICAM-1. With IRI, NO decreases while ICAM-1 increases [16]. In our study, the decrease of eNOS was taken as

a pointer of endothelial dysfunction or loss of endothelial integrity, while the increase of ICAM-1 was considered a sign of chronic inflammation.

e-selectin (CD62E) is an adhesion molecule that is increased by inflammation and hypoxia, with a major role in endothelial repair following ischemia [17-19]. It is characterized by a rapid peak of expression followed by a faster return to baseline than ICAM-1 [20]. In a study investigating late findings of endothelial inflammation, e-selectin was detected to return to its basic levels in less than 24 hours, while maximum levels of ICAM-1 were preserved for prolonged periods [21]. ICAM-1 also has a "recycling" mechanism that aids in its continuance [22].

e-selectine requires endothelial cells to be "activated" for its synthesis in most organs, while it is continuously present in regions like skin and bone marrow [23].To our knowledge, the expression pattern of e-selectin in the corpus cavernosum has not been previously described. Our study revealed dense positive staining for all animals in group C, which may be an indicator for e-selectin being continuously expressed in cavernous sinusoidal endothelium and provide an insight for future studies. In addition, we observed a non-significant trend towards reduced e-selectin expression in the isolated PT groups, which may suggest a potential role in signaling endothelial integrity, but requires further investigation.

According to our findings, the addition of CAE to isolated PT resulted in favorable outcomes. Among all groups, the most severe injury occurred with prolonged isolated PT (40T), while the most prominent protection was achieved with the addition of CAE at 20 minutes (20T&E). Therefore, in pairwise comparisons, the most consistent statistical differences were observed between groups 40T and 20T&E.

The presence of LPC across all study groups is compatible with penectomy timing and chronic inflammation. Hemosiderin loaded macrophages observed in 40T&E were considered to represent microscopic foci of hemorrhage and were unique to this group. Animals in 40T&E also exhibited more pronounced painful posture during follow-up, which may be a representation of this finding. However, when prolonged PT settings were compared (40T vs. 40T&E), CAE was associated with overall more beneficial trends, including considerably lower inflammation, less fibrosis, reduced ICAM-1, and preserved e-selectin expression. While these changes did not retain significance after adjustment, eNOS showed a clear 100% difference that remained significant. These findings suggest that in a setting of significant cellular damage induced by prolonged PT, the potential benefit of CAE is most sensitively captured by eNOS expression.

When comparing 20 minutes of isolated PT with the addition of CAE (20T vs. 20T&E), inflammation represented the only parameter where 20T&E performed slightly worse ($p>0.05$). In contrast, neovascularization was substantially reduced, and fibrosis was completely resolved in 20T&E. Although these differences did not reach statistical significance, likely due to the limited sample size and variability within groups, they can support the protective nature of CAE. Likewise, in terms of immunohistochemistry, 20T&E maintained a profile consistent with controls, whereas 20T demonstrated small deviations.

When comparing isolated PT at 20 and 40 minutes, inflammation was the only parameter that showed a statistically significant difference. Although fibrosis, ICAM-1 and eNOS showed notable but non-significant variation, neovascularization and e-selectin remained unchanged. Collectively, the lack of consistent differences between the two PT durations likely reflects the fact that both caused a comparable degree of tissue damage.

In the comparison of CAE groups, fibrosis was the only parameter that differed significantly in the raw analysis, and although this was not retained after correction, it likely reflects the marked reduction of fibrosis observed in the 20T&E group. ICAM-1 showed minor variation, in parallel with the histopathological inflammation findings, while eNOS and E-selectin were identical across both groups. The relative similarity between the two CAE groups, and their immunohistochemical resemblance both to each other and the control group, may support the idea that CAE exerts a protective effect even in prolonged durations of PT, and that immunohistochemistry is a more sensitive tool to detect it.

The beneficial effect of CAE may be explained by the washout of harmful blood products, similar to organ preservation models [24]. CAE may also resemble the concept of shear preconditioning, whereby continuous fluid exposure reduces endothelial activation and inflammation [25,26]. These potential mechanisms remain hypothetical and require further experimental validation.

Limitations of this study include the lack of intracorporal pressure transducer usage, where instead we designed a safe, low-cost system that could be applied in clinical practice, if necessary. The small and delicate nature of the rat erectile tissues may make punction and continuity of erection difficult, possibly hindering standardization. We believe this risk was minimized by detailed testing and observation.

In conclusion: CAE, especially when kept under 20 minutes, does not increase the erectile tissue damage that is already caused by PT, but rather reduces it by alleviating inflammation and fibrosis, while exerting a protective effect on endothelial function. Based on the current experimental results, AE maneuvers such as AET and CAE may be

considered safe to apply in pediatric urology. Our ongoing studies involving transmission electron microscopy aim to provide further insight on the cellular impact of PT and, in particular, CAE, which if proven to be non-detrimental, may contribute to improved tissue healing and surgical outcomes during pediatric penile surgery.

Ethical statement

This study was approved by the Animal Research Local Ethics Committee (22.09.2021-2021/01), and the procedures were performed in compliance with relevant laws and institutional guidelines.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this manuscript, generative AI tools (ChatGPT) were used solely for language refinement. All scientific content was produced, reviewed, and approved by the authors.

Declaration of interest

None.

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Figure Legends

Figure 1: A) dorsal view **B)** lateral view after PD +PT application. **C)** injection into proximal corpus cavernosum with 24-gauge butterfly needle to form AE.

Figure 2: A-B) continuous injection and pressure control system involving an infuser pump, two-way venous valve and 10cc syringe.

Figure 3: A) Mild lymphoplasmacytic cell infiltration (arrow) and neovascularization in G20T&E (H&E ×400). **B)** Hemosiderin loaded macrophages (arrow) in G40T&E (H&E ×400). **C)** Mild fibrosis in G40T (MTC ×200). **D)** High-density immunopositivity (score 3) in G20T&E (ENOS ×200). **E)** High-density immunopositivity (score 3) in G40T&E (ENOS ×200). **F)** Moderate-density immunopositivity (score 2) in G40T (ENOS ×200). **G)** High-density immunopositivity (score 3) in group C (e-selectine ×200) **H)** Moderate-

density immunopositivity (score 2) in 20T (e-selectine ×200) **I)** High-density immunopositivity (score 3) in 40T&E (e-selectine ×200) **J)** Moderate-density immunopositivity (score 2) in group C (ICAM-1 ×200) **K)** Moderate-density immunopositivity (score 2) in 20T&E (ICAM-1 ×200) **L)** High-density immunopositivity (score 3) in 40T (ICAM-1 ×200)

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		G20T	G20T&E	G40T	G40T&E	GC
	Score	n (%)	n (%)	n (%)	n (%)	n (%)
Inflammation	**0**	6 (85.7%)	5 (71.4%)	0 (0.0%)	4 (57.1%)	7 (100%)
	1	1 (14.3%)	2 (28.6%)	6 (85.7%)	3 (42.9%)	0 (0.0%)
	2	0 (0.0%)	0 (0.0%)	1 (14.3%)	0 (0.0%)	0 (0.0%)
Neovascularization	**0**	0 (0.0%)	3 (42.9%)	0 (0.0%)	0 (0.0%)	7 (100%)
	1	7 (100%)	4 (57.1%)	7 (100%)	7 (100%)	0 (0.0%)
Fibrosis	**0**	4 (57.1%)	7 (100%)	1 (14.3%)	2 (28.6%)	7 (100%)
	1	3 (42.9%)	0 (0.0%)	6 (85.7%)	5 (71.4%)	0 (0.0%)
e-NOS	**2**	2 (28.6%)	0 (0.0%)	7 (100%)	0 (0.0%)	0 (0.0%)
	3	5 (71.4%)	7 (100%)	0 (0.0%)	7 (100%)	7 (100%)
e-selectine	**2**	2 (28.6%)	0 (0.0%)	2 (28.6%)	0 (0.0%)	0 (0.0%)
	3	5 (71.4%)	7 (100%)	5 (71.4%)	7 (100%)	7 (100%)
ICAM-1	**1**	1 (14.3%)	1 (14.3%)	0 (0.0%)	0 (0.0%)	1 (14.3%)
	2	5 (71.4%)	6 (85.7%)	4 (57.1%)	6 (85.7%)	6 (85.7%)
	3	1 (14.3%)	0 (0.0%)	3 (42.9%)	1 (14.3%)	0 (0.0%)

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414 **Table 1A:** Histopathological and immunohistochemical results. *Scores that have zero*

415 *percentage in all groups are not added.*

416 ***Abbreviations: C**, control; **20T / 40T**, 20/40-minute penile tourniquet; **20T&E /40T&E**,*

417 *20/40-minute penile tourniquet with continuous artificial erection*

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	Inflammation	Neovascularisation	Fibrosis	e-NOS	e-selectine	ICAM-1
	Raw p/Adj p	Raw p/Adj p	Raw p/Adj p	Raw p/Adj p	Raw p/Adj p	Raw p/Adj p
GC vs. G20T	0.599/1	<0.001/0.000*	0.107/1	0.228/1	0.098/0.977	0.569/1
GC vs. G20T&E	0.293/1	0.020/0.197	1/1	1/1	1/1	1/1
GC vs. G40T	<0.001/0.001*	<0.001/0.000*	0.001/0.013*	<0.001/0.000*	0.098/0.977	0.025/0.251
GC vs. G40T&E	0.115/1	<0.001/0.000*	0.007/0.72	1/1	1/1	0.270/1
G20T vs. G20T&E	0.599/1	0.080/0.802	0.107/1	0.228/1	0.098/0.977	0.569/1
G20T vs. G40T	<0.001/0.08*	1/1	0.107/1	0.003/0.26	1/1	0.095/946
G20T vs. G40T&E	0.293/1	1/1	0.282/1	0.228/1	0.098/0.977	0.594/1
G20T&E vs. G40T	0,005/0.047*	0.080/0.802	0.001/0.013*	<0.001/0.000*	0.098/0.977	0.025/0.251
G20T&E vs. G40T&E	0.599/1	0.080/0.802	0.007/0.72	1/1	1/1	0.270/1
G40T vs. G40T&E	0.021/0.21	1/1	0.591/1	<0.001/0.000*	0.098/0.977	0.255/1

Table 1B: Pairwise comparison of histopathological and immunohistochemical scores between study groups. Raw p-values and Bonferroni-adjusted p-values (Raw p/Adj p) are presented. Statistically significant differences (adjusted $p < 0.05$) are indicated with an asterisk (*). ***Abbreviations: C****, control;* ***20T / 40T****, 20/40-minute penile tourniquet;* ***20T&E / 40T&E****, 20/40-minute penile tourniquet with continuous artificial erection.*

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		G20T	G20T&E	G40T	G40T&E	GC
	Score	n (%)	n (%)	n (%)	n (%)	n (%)
Inflammation	**0**	6 (85.7%)	5 (71.4%)	0 (0.0%)	4 (57.1%)	7 (100%)
	1	1 (14.3%)	2 (28.6%)	6 (85.7%)	3 (42.9%)	0 (0.0%)
	2	0 (0.0%)	0 (0.0%)	1 (14.3%)	0 (0.0%)	0 (0.0%)
Neovascularization	**0**	0 (0.0%)	3 (42.9%)	0 (0.0%)	0 (0.0%)	7 (100%)
	1	7 (100%)	4 (57.1%)	7 (100%)	7 (100%)	0 (0.0%)
Fibrosis	**0**	4 (57.1%)	7 (100%)	1 (14.3%)	2 (28.6%)	7 (100%)
	1	3 (42.9%)	0 (0.0%)	6 (85.7%)	5 (71.4%)	0 (0.0%)
e-NOS	**2**	2 (28.6%)	0 (0.0%)	7 (100%)	0 (0.0%)	0 (0.0%)
	3	5 (71.4%)	7 (100%)	0 (0.0%)	7 (100%)	7 (100%)
e-selectine	**2**	2 (28.6%)	0 (0.0%)	2 (28.6%)	0 (0.0%)	0 (0.0%)
	3	5 (71.4%)	7 (100%)	5 (71.4%)	7 (100%)	7 (100%)
ICAM-1	**1**	1 (14.3%)	1 (14.3%)	0 (0.0%)	0 (0.0%)	1 (14.3%)
	2	5 (71.4%)	6 (85.7%)	4 (57.1%)	6 (85.7%)	6 (85.7%)
	3	1 (14.3%)	0 (0.0%)	3 (42.9%)	1 (14.3%)	0 (0.0%)

Table 1A: Histopathological and immunohistochemical results. *Scores that have zero percentage in all groups are not added.*

Abbreviations*: C**, *control;* ***20T / 40T, *20/40-minute penile tourniquet;* ***20T&E / 40T&E***, *20/40-minute penile tourniquet with continuous artificial erection*

	Inflammation	Neovascularisation	Fibrosis	e-NOS	e-selectine	ICAM-1
	Raw p/Adj p	Raw p/Adj p	Raw p/Adj p	Raw p/Adj p	Raw p/Adj p	Raw p/Adj p
GC vs. G20T	0.599/1	<0.001/0.000*	0.107/1	0.228/1	0.098/0.977	0.569/1
GC vs. G20T&E	0.293/1	0.020/0.197	1/1	1/1	1/1	1/1
GC vs. G40T	<0.001/0.001*	<0.001/0.000*	0.001/0.013*	<0.001/0.000*	0.098/0.977	0.025/0.251
GC vs. G40T&E	0.115/1	<0.001/0.000*	0.007/0.72	1/1	1/1	0.270/1
G20T vs. G20T&E	0.599/1	0.080/0.802	0.107/1	0.228/1	0.098/0.977	0.569/1
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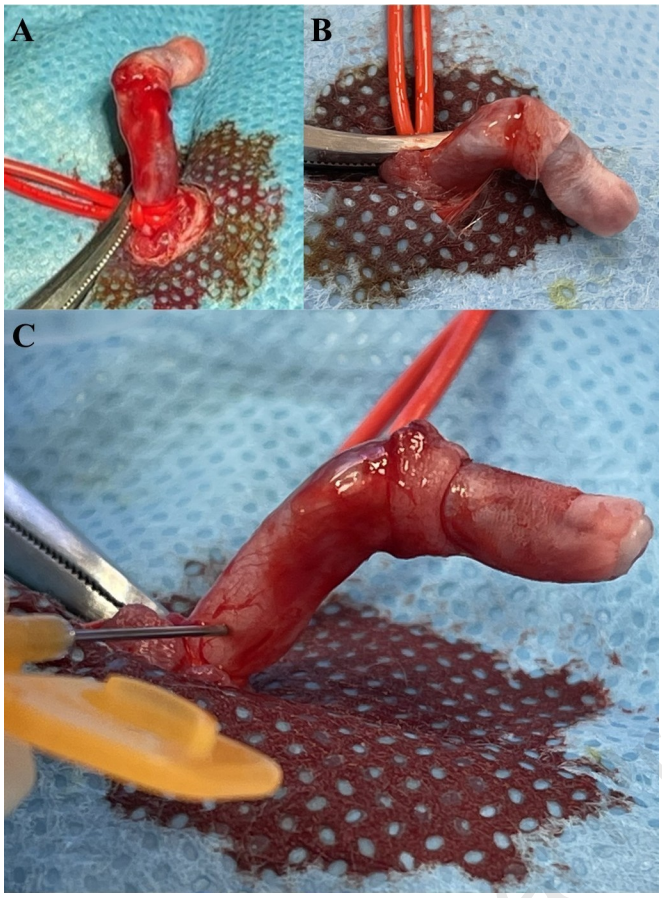
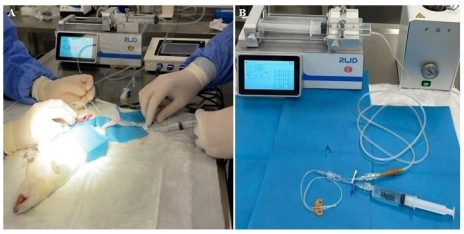
G20T vs. G40T&E	0.293/1	1/1	0.282/1	0.228/1	0.098/0.977	0.594/1
G20T&E vs. G40T	0,005/0.047*	0.080/0.802	0.001/0.013*	<0.001/0.000*	0.098/0.977	0.025/0.251
G20T&E vs. G40T&E	0.599/1	0.080/0.802	0.007/0.72	1/1	1/1	0.270/1
G40T vs. G40T&E	0.021/0.21	1/1	0.591/1	<0.001/0.000*	0.098/0.977	0.255/1

Table 1B: Pairwise comparison of histopathological and immunohistochemical scores between study groups. Raw p-values and Bonferroni-adjusted p-values (Raw p/Adj p) are presented. Statistically significant differences (adjusted $p < 0.05$) are indicated with an asterisk (*). ***Abbreviations: C**, control; **20T / 40T**, 20/40-minute penile tourniquet; **20T&E / 40T&E**, 20/40-minute penile tourniquet with continuous artificial erection*





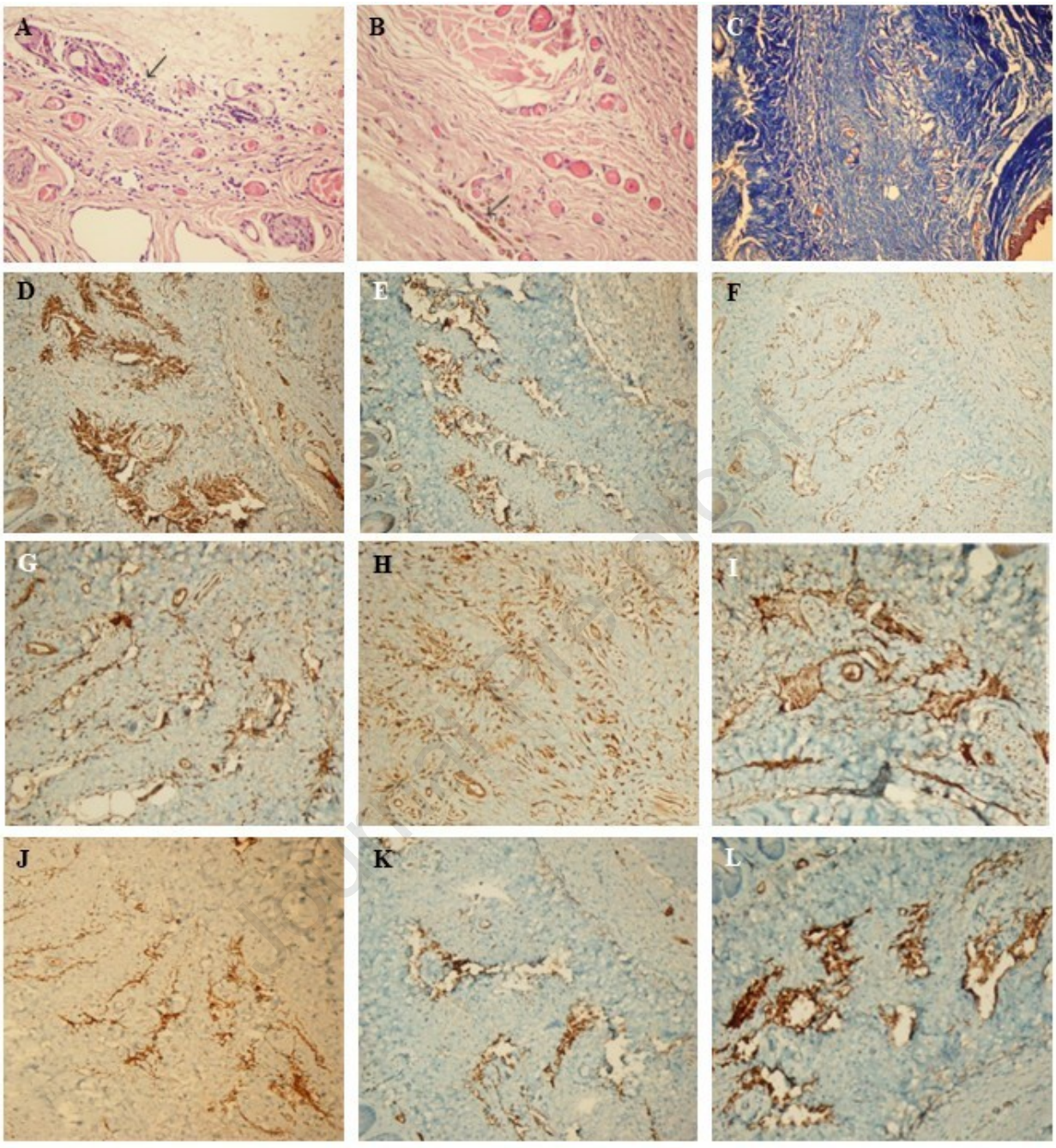


Figure Legends

Figure 1: A) dorsal view **B)** lateral view after PD +PT application. **C)** injection into proximal corpus cavernosum with 24-gauge butterfly needle to form AE.

Figure 2: A-B) continuous injection and pressure control system involving an infuser pump, two-way venous valve and 10cc syringe.

Figure 3: A) Mild lymphoplasmacytic cell infiltration (arrow) and neovascularization in G20T&E (H&E ×400). **B)** Hemosiderin loaded macrophages (arrow) in G40T&E (H&E ×400). **C)** Mild fibrosis in G40T (MTC ×200). **D)** High-density immunopositivity (score 3) in G20T&E (ENOS ×200). **E)** High-density immunopositivity (score 3) in G40T&E (ENOS ×200). **F)** Moderate-density immunopositivity (score 2) in G40T (ENOS ×200). **G)** High-density immunopositivity (score 3) in group C (e-selectine ×200) **H)** Moderate-density immunopositivity (score 2) in 20T (e-selectine ×200) **I)** High-density immunopositivity (score 3) in 40T&E (e-selectine ×200) **J)** Moderate-density immunopositivity (score 2) in group C (ICAM-1 ×200) **K)** Moderate-density immunopositivity (score 2) in 20T&E (ICAM-1 ×200) **L)** High-density immunopositivity (score 3) in 40T (ICAM-1 ×200)