Peer Review File

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Reviewer A

Comment 1: You describe your rationale for methods well in the discussion section. Consider putting the references in the methods section as well.
Reply 1: References have been added throughout the methods section citing drawn influence.

Comment 2: Does placing clamps on the prepuce translate to similar force on the corpora itself rather than penile skin alone? Can this method be related to traction in humans? Consider discussing this limitation/difference in animal vs human.

Reply 2: One difficulty in using a rat model for penile traction is finding a way to apply a traction force to the copora. Lin et al. achieved this by applying a clamp to the prepuce and putting it on stretch. We modeled this approach and found that the corpora were pulled along with the skin overlying them. When done at high forces, we noticed that the copora were the limiting factor in how far the penis could be stretched. This indicates that the force applied to the prepuce was adequately applied to the corpora.
Changes in the text: We have added this explanation to our methods, as well as added a section to our limtations

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Comment 3: For measurement of stretched penile length, has this method been used elsewhere? This should have a reference if so.

Reply 3: This method of measurement was designed by our researchers. Stretching to 0.5N of force has the penis to be on stretch and the glans visible, allowing us to measure from the glans to the pubic symphysis. Taking three measurements and averaging minimizes errors when taking fine measurements. Using the same researcher for each measurement prevents variations due to different measurement styles.
Changes in the text: No references added as this is our technique.

Comment 4: The subtitles are somewhat misleading as western blot results are described under "Trichrome staining/HIF1alpha" category. Consider simplifying to Western blot and IHC categories or eNOS and HIF1-apha categories.

Reply 4: Thank you for catching this. We agree this subtitle is misleading. The reviewer's comment has been implemented.
Changes in the text: Separated HIF 1α and trichrome into separate results sections

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Comment 5: Trichrome images and corresponding bar graphs should be shown whether in the

figures or supplemental files, especially considering unexpected results.

Reply 5: We agree with the reviewer, results from our Trichrome smooth muscle /collegen ratio bar graph has been included.
Changes in the text: Please see added figure that includes samples images from each group along side a bar graph representing the averaged smooth muscle/collagen ratios.

Comment 6: In the results you state trichrome is significant in traction compared to sham, but in discussion state traction is higher compared to crush. Please include figures and clarify.

Reply 6: Thank you for pointing out this discrepancy. We re-reviewed our trichrome slides and processed them using ImageJ quantification to measure the amount of smooth muscle to collagen. After re-analyzing, the Sham and Traction group had more smooth muscle than the Crush group but these differences were not significant. There was a lot of variation in amount of smooth muscle in the Crush group which likely contributed to lack of significant findings. We have included our raw analysis as a supplemental file for full transparency.
Changes in the text: Methodology, results, and discussion involving trichrome stain have been updated.

Comment 7: Mentions HIF1-apha results as being surprising, but mechanism and significance how HIF-1alpha is involved in post-prostatectomy ED/fibrosis is missing. Consider briefly expanding for the reader.

Reply 7: HIF-1α is being used as a surrogate measure for tissue hypoxia. We discuss our theory that traction increases eNOS expression which should increase blood flow and reduce tissue hypoxia. Our traction group has increase eNOS expression, but also increased HIF-1α which is unexpected. We go on to explain that we think this discrepancy may be due to the excessive use of force which had the negative effect of tissue damage.
Changes in the text: included an explanation of the significance of HIF-1α in the discussion section.

Comment 8: Could the increased fibrosis be time dependent and part of the healing/further remodeling that the traction group would have over the crush group?

Reply 8: Thank you for the comment. We agree with the reviewer, fibrosis as a byproduct of the healing processes has shown to be time dependent in several other tissue types. We have no data to prove that remodeling of the penile corpora is any different. However, time was controlled for as all rats were sacrificed at 28 days.
Changes in the text: no changes made

Comment 9: Please discuss limitations of excluding a "traction only" group, especially in the context of 1N of force having potential injurious/fibrotic effects.

Reply 9: A traction-only group would allow us to see the effects of traction on tissue hypoxia and scarring. In our current model, it is unclear how much hypoxia and scarring is caused by traction vs the nerve crush.
Changes in the text: Added to the limitation section

Reviewer B

1. There's no **Abstract** in your manuscript, please add it. You should structure it as: **Background, Methods, Results,** and **Conclusions** (Words limits: 200~350). And please ensure all abbreviated terms are defined the first time they appear in the Abstract.

Abstract added

2. As for this you mean "P" value? Please check the figure legends. Please also check through your whole text and revise if applicable.

Fig. 1. Graph showing average stretched penile length (SPL) of the Sham, Crush, and Traction group preoperatively and at the end of traction. The Traction group had significantly greater SPL than the Sham group. The Traction group had significantly greater SPL than the Crush group.
*α< 0.05. **α< 0.01.

Fig. 2. Graph of erectile function as determined by change in intracavernosal pressure/mean arterial pressure (ΔICP/MAP). The Sham group was significantly greater than the Crush group at 2.5V, 5V, and 7.5V.
*α< 0.05.

Fig. 3. Endothelial nitric oxide synthase (eNOS) and hypoxia-inducible factor 1-α (HIF1-α) expression in corporal tissue on western blot. **A)** eNOS western blot sample from each of the 3 groups. **B)** HIF1-α western blot samples from each of the 3 groups. **C)** Bar graph representation of eNOS expression from all samples, n = 8 per group. The Traction group had significantly greater eNOS than the Crush group (1.6 vs 0.5). All amounts are normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH).
*α< 0.05

α is used for ANOVA w/ Tukey-Kramer post-hoc analysis. ANOVA generates a q-value. Significance of that q-value is dependent on the degrees of freedom in comparison. A q-table was used to determine significance (α < 0.05) based on degrees of freedom.

P is used for Student's t-tests.
The text was reviewed to ensure accuracy of the use of α and p.

3. Figure 3 is a bit vague, please resend us a higher resolution version as separate file (JPG/TIFF format).

Before resubmission, please re-edit those words, the words should not be set vertically.

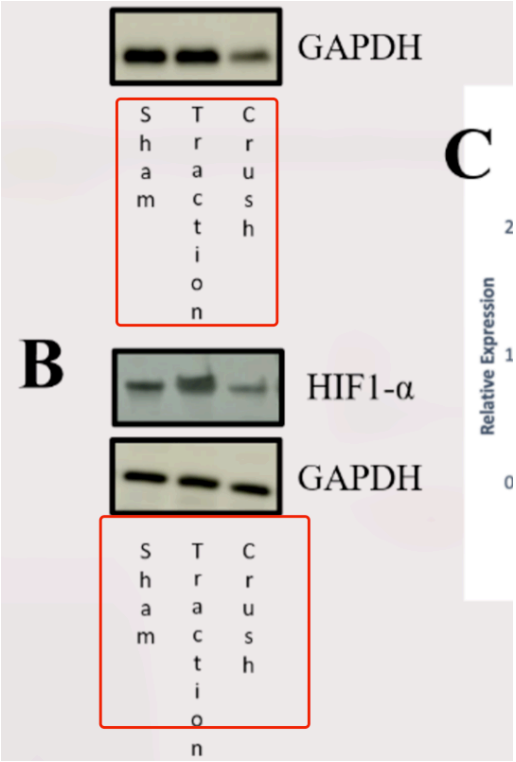

Figure 3 has been edited so the words run horizontally. We have also included jpg images of our eNOS blots, GAPDH blots, and HIF 1α blots as supplementary images. An image of the HIF 1α blot containing animals 1-4 from each group is also included for transparency; this blot was not used as it appeared contaminated. Final HIF1α results were based on n=4. This information has been included in the text.

The selected lanes are intended to show the average amount of protein expressed for each group. We believe that this provides the clearest information for the reader. If you would like us to include all lanes in the figure, please let us know.

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4. Figure 4: Please indicate the scale bar/magnification of Figure 4A-4D in figure/figure legends.

We have indicated that the IHC images are 20x in Figure 4. We have also indicated that our Trichrome images are 4x in Figure 5A.

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5. Figure 5

a. Please provide a summarized legend for figure 5.

Fig. 5.
A) Trichrome staining of the rat penis. RGB images were broken down into separate color components using image J, allowing us to quantify the amount of smooth muscle and collagen. **B)** A graph showing smooth muscle-to-collagen ratios for each group, with standard error shown.

We added information to the figure legend to make it clearer. Please let us know if there is anything else the reviewer recommends adding.

b. Please define RGB in figure legends.

Red-Green-Blue (RGB) is now defined in the figure legend

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6. Figures should be cited consecutively in text. Therefore, please cite Figure 3B between Figure 3A and 3C. Please check and revise.

There was no significant difference in eNOS expression between the Sham and Traction groups (Fig. 3 A and C).

Revised.