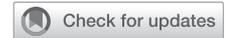


BASIC SCIENCE

Hydro-Jet Dissection of the Cavernous Nerves Preserves Erection Function in a Radical Prostatectomy Animal Model



Jeffrey D. Campbell, MD,^{1,*} Husain Alenezi, MD,^{1,*} Ling X. DeYoung, MD,¹ Gerald B. Brock, MD,¹ and Stephen E. Pautler, MD^{1,2}

ABSTRACT

Background: Postoperative erectile dysfunction (ED) remains a prevalent consequence of radical prostatectomy (RP) that significantly impacts patient quality of life. Water-jet technology is widely used for dissection in neurosurgical procedures but novel to urologic surgery.

Aim: To establish the impact of hydro-jet dissection (HJD) of the cavernous nerves (CN) on postoperative erectile function in an animal model of RP-induced ED.

Methods: 32 male Sprague-Dawley rats were randomized to 4 groups: Sham surgery (n = 8), bilateral HJD of CN (n = 8), blunt CN injury (n = 8), or stretch CN injury (n = 8). After 4 weeks, erectile function was assessed by measuring intracavernous pressure (ICP), and penile tissues were harvested for immunohistologic studies.

Main Outcome Measure: The peak ICP and the area under the curve were calculated for each group. Immunohistologic studies were performed for α -smooth muscle actin and neuronal nitric oxide synthase on cross-sections of penile tissue.

Results: Rats in the HJD group demonstrate a significantly higher mean peak ICP and area under the curve compared with both CN injury groups ($P = .001$). Postoperative erectile function in the HJD group returned to baseline function. Preservation of α -smooth muscle actin and neuronal nitric oxide synthase was observed in the HJD group compared with the other surgical trauma groups.

Clinical Implications: Hydro-jet dissection used in an RP animal model maintains erectile function and offers a potential benefit that warrants further human studies.

Strengths & Limitations: This is a novel animal study comparing a new technology to established CN dissection techniques. This study uses an animal model, which may not completely translate to post-RP ED in humans.

Conclusion: Hydro-jet dissection of the CN during RP in an animal model is associated with significantly better postoperative erectile function when compared with other CN injury. Clinical studies are needed to further investigate the putative benefit of HJD on erectile function in patients undergoing RP. **Campbell JD, Alenezi H, DeYoung LX, et al. Hydrojet Dissection of the Cavernous Nerves Preserves Erection Function in a Radical Prostatectomy Animal Model. Sex Med 2019;7:104–110.**

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Key Words: Hydro-jet; Erectile dysfunction; Cavernous nerves; Radical prostatectomy; Intracavernous pressure

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INTRODUCTION

Radical prostatectomy (RP) is the gold standard surgical procedure for the management of clinically localized prostate cancer in appropriate candidates.^{1,2} In the 1980s, Walsh and Donker³ first described the neurovascular bundle and periprostatic structures, which led to a more comprehensive understanding of male pelvic anatomy. This surgical procedure has evolved over time to achieve the goals of cancer control with satisfactory functional outcomes for patients.⁴ More recently, the application of minimally-invasive approaches including laparoscopic and robot-assisted surgery have

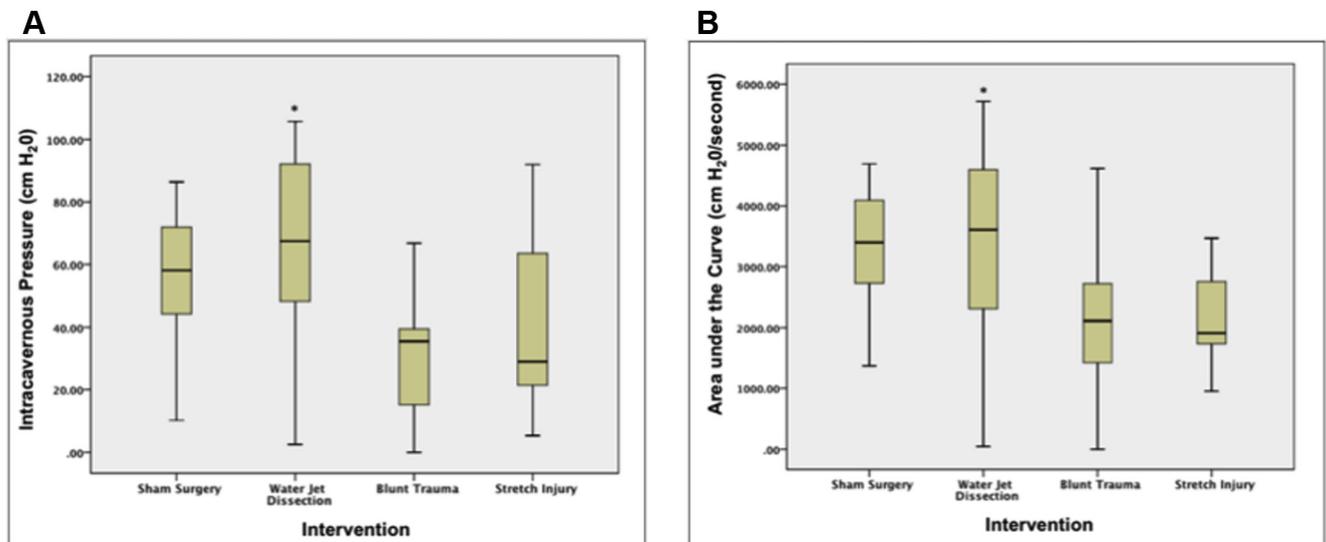


Figure 1. Box plots comparing the (panel A) peak intracavernous pressure (cm H₂O) and (panel B) area under the curve (cm H₂O/sec) for all 4 intervention groups. *Statistically significant (Kruskal-Wallis test). cm H₂O = centimeters of water.

continued the evolution of RP.^{5–7} Minimally-invasive approaches to the standard RP claim to offer fewer perioperative complications and quicker patient recovery, although its proclamation as the current standard of care is still under debate.^{8,9}

One of the major functional outcomes that impact the quality of life for men who undergo RP is the recovery of erectile function.¹⁰ Many factors affect erectile function after surgery, including preoperative potency, age, the degree of nerve-sparing (NS) during surgery, and the need for postoperative adjuvant cancer treatment, such as radiation or androgen deprivation therapy.^{11,12} Specific surgical maneuvers used during NS-RP can have a negative impact on erectile function, including electrothermal injury, excessive traction, and transection or devascularization of the cavernous nerves (CN).¹³

Hydro-jet dissection (HJD) technology involves the application of a thin high-pressure fluid stream to establish and expand surgical planes. The first reported surgical application of HJD was in 1982, using a modified agricultural sprayer during hepatic resection.¹⁴ Commercially-available pressurized hydrodissection equipment has since been used for numerous surgical procedures, including NS-RP and NS-retroperitoneal lymph node dissection.^{15,16} Early clinical studies exploiting hydrodissection and HJD during RP are promising but are limited by small numbers, poor outcome measures, inappropriate patient selection, and inconsistent application of the technology.^{16–19}

Before supporting HJD as an acceptable alternative to current nerve-sparing approaches for RP, preclinical studies must elucidate a mechanistic and functional benefit. We hypothesize that HJD will have less traumatic effects on the CN than blunt dissection, which will ultimately lead to an improvement in postoperative erectile function. To test our hypothesis, we will use a post-RP rat model to compare the effects of HJD to blunt and stretch CN injury (CNI) and evaluate the functional and histologic impact of this novel technology.

METHODS

Animal ethics approval for this study was obtained from the Institutional Review Board at Western University (London, Ontario, Canada; REB 2014-052). 32 Sprague-Dawley rats, male and 9 months of age, were randomly divided into 4 groups: group 1 (n = 8) had sham surgery (SHAM); group 2 (n = 8) had HJD of the CN; group 3 (n = 8) had bilateral CNI (BCNI) by blunt trauma (BT); and group 4 (n = 8) had BCNI by stretch trauma (ST). Each rat was used for both erection studies and immunohistologic staining.

Bilateral Cavernous Nerve Injury

Preoperative and postoperative animal care was carried out in accordance with the Western University animal ethics board. Under isoflurane anesthesia, all groups underwent an inferior midline incision using sterile techniques. Prostate, CN, and major pelvic ganglia were identified in each group. The SHAM group had no further manipulation after CN dissection and then underwent routine skin closure.

The HJD group used the ERBEJET 2 unit (Erbe Elektromedizin GmbH, Tuebingen, Germany) with short, straight, flexible-tip applicators (6-mm outer diameter and 65-mm length). Cavernous nerves were completely dissected and isolated using HJD technology. The water used for the dissection was kept at room temperature.

The comparison groups had gentle, sharp dissection of the CN rather than either blunt or stretch trauma applied to induce moderate nerve injury. Blunt trauma applied to the CN was generated by directly dropping a 34.1-gauge steel cylinder (0.7 × 8.5 cm) through a 28-cm-long guided tube 3 times onto the sharply dissected CN, which were placed on top of a steel plate as previously described.²⁰ The ST group had standard sharp dissection of the CN, followed by a bilateral 10-second stretch of CN using a micro forceps for a total of 3 repeats.²¹

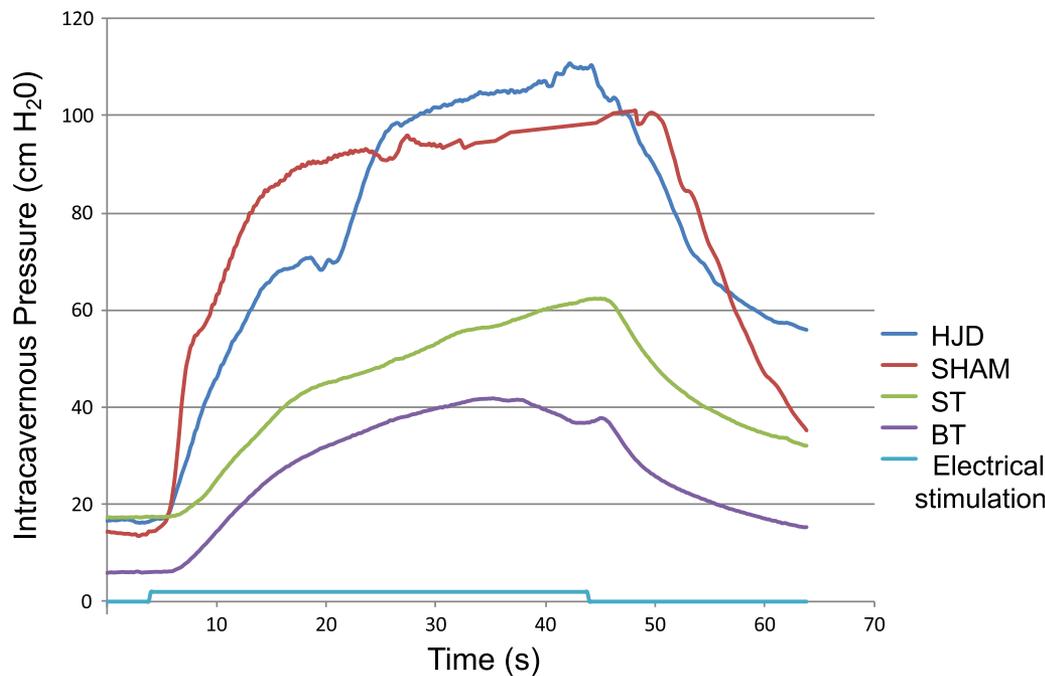


Figure 2. Average intracavernous pressure (cm H₂O) curves for each of the study groups after electrical stimulation of the cavernous nerves. BT = blunt trauma; cm H₂O = centimeters of water; HJD = hydro-jet dissection; SHAM = sham surgery; ST = stretch trauma.

Intracavernous Pressure Measurement

4 weeks after surgery, erectile function was assessed by measurement of intracavernous pressure (ICP), with general anesthesia administered using ketamine 100 mg/kg and xylazine 5 mg/kg as previously described by our laboratory.²² In brief, using our previous lower midline incision, CN were identified, isolated, and hooked with a stainless steel bipolar electrode. The penile crux was exposed through a transverse incision, and a 23-gauge needle was inserted and subsequently connected to a transducer. ICP was evoked with 0.2-msec pulses of 2 mA at 20 Hz for 40 seconds' duration and recorded using LabVIEW 7 software (National Instruments, Austin, Texas, USA). 3 electrostimulations were replicated at intervals of 10 minutes. The peak ICP was noted, and the area under the curve was calculated for each stimulation. After completion of erectile function assessment, the animals were euthanized, and their penile tissue was harvested for further analysis.²²

Immunohistochemistry

After ICP measurements, penile tissue from each rat was fixed in 4% formalin and embedded in paraffin. 5- μ m sections were immunostained with primary antibodies neuronal nitric oxide synthase (nNOS) mouse anti-nNOS (Transduction Lab Mississauga, Mississauga, Ontario, Canada), and mouse anti- α -actin (Sigma, St. Louis, MO, USA), as previously described.²³

The histologic examination was performed using a Zeiss Axioskop microscope with a computerized imaging system (Northern Eclipse; Empix, Mississauga, Ontario, Canada). The

reviewer was blinded to the groups, and the counts were repeated for consistency. The sum of nNOS-positive stained cells was calculated as the total from 3 regions: the right, left posterior, and middle of the corpus cavernosum in the field of view at magnification \times 200. The area of positive staining of α -smooth muscle actin (α -SMA) was calculated as the ratio of total sectional area under magnification \times 25 using Image J computer software.²³

Statistical Analysis

Statistical analysis was performed using STATA10 (StataCorp, College Station, TX, USA) software. Values are expressed as mean \pm standard error. Data were compared using 2-tailed *t*-tests, with $\alpha = 0.05$ and Kruskal-Wallis test.

RESULTS

The preoperative weights in each of the 4 animal groups did vary strictly based on the randomization; however, they were not significantly different (means 629.8 g, 609.6 g, 570.3 g, and 637.6 g; $P = .317$). 8 rats underwent the procedure in each group, with 1 rat death in the SHAM, BT, and ST groups during the postoperative recovery period.

Intracavernous Pressure Measurements

HJD rats demonstrate a significantly higher mean peak ICP of 65.74 ± 32.29 cm H₂O in comparison to a mean peak ICP of 33.43 ± 23.52 cm H₂O and 40.89 ± 26.87 cm H₂O in the BT

	% α SMA	Number of nerve fibers (nNOS)
SHAM	12.9 \pm 1.156	93.88 \pm 25.69
HJD	11.63 \pm 1.685	94.88 \pm 23.65
BT	9.64 \pm 2.495*	54.86 \pm 5.398 **
ST	10.21 \pm 2.099*	51.57 \pm 9.897 **

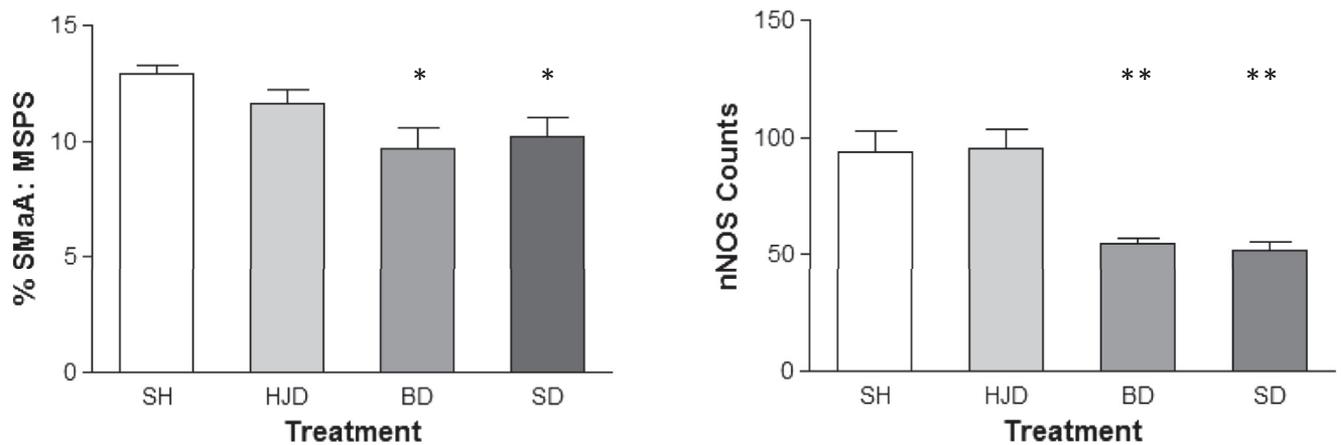


Figure 3. Graphic depiction of the percentage of α -SMA–stained tissue in a representative MSPS and the number of positively stained nerve fibers for nNOS in the dorsal nerve bundle for each of the treatment groups. α SMA = α -smooth muscle actin; BT = blunt trauma; HJD = Hydro-jet dissection; MSPS = mid-shaft penile section; nNOS = neuronal nitric oxide synthase; SHAM = sham surgery; ST = stretch trauma. *Statistically significant decrease in % α -SMA compared with SHAM (Student’s *t*-test). **Statistically significant decrease in nNOS fibers compared with SHAM (Student’s *t*-test)

and ST groups, respectively ($P < .0001$). The mean peak ICP for the SHAM control group is 55.25 ± 22.68 cm H₂O, which is not statistically different from the HJD group. Figure 1A illustrates these findings graphically. HJD was associated with a more sustainable rise in the ICP in comparison to BT and ST because the mean area under the curve was $3,425.7 \pm 1,671$ cm H₂O/sec, $2,163.2 \pm 1,551$ cm H₂O/sec, and $2,120 \pm 768.2$ cm H₂O/sec, respectively ($P = 0.012$). Figure 1B demonstrates the ICP response curves during and after electrical stimulation. A representative ICP curve for HJD and BT groups are shown in Figure 2.

Histologic Analysis

All penile tissue was successfully antibody stained for analysis. There was a higher percent of α -SMA–positive staining in both the SHAM and HJD treatment groups, compared with both BCNI groups ($P < .05$) (Figure 3). In addition, both SHAM and HJD groups had a significantly higher number of nNOS cells ($P < .01$) compared with the other CN dissection techniques (Figure 4). Both α -SMA and nNOS were preserved in penile tissue of rats that had HJD when compared with SHAM.

DISCUSSION

The use of pressurized fluid to perform tasks such as cutting has been used in industrial processes for decades.²⁴ In medicine, Papachristou and Barbers¹⁴ are credited with the first application for solid organ transection using a modified agricultural sprayer to resect liver tissue. Since its initial appearance in medical literature, surgeons have used this technique for a variety of applications. In some instances, the use of fluid for dissection of natural tissue planes was achieved using a simple syringe and needle technique and termed “hydrodissection.” In comparison, the use of water or normal saline solution in a thin (120- μ m) fluid stream under high pressure is termed “hydro-jet dissection.”²⁵ The reported advantages of HJD include precise dissection and selective preservation of critical structures containing increased collagen including nerves and blood vessels larger than 20 μ m in diameter.^{15,25,26} Using HJD at pressures ranging from 15–30 atm has negligible functional and morphologic effects on peripheral nerves, as shown by human and animal studies.^{26,27}

There have been limited studies exploring the use of hydro-dissection of the NVB during RP.^{17–19} The early use of this

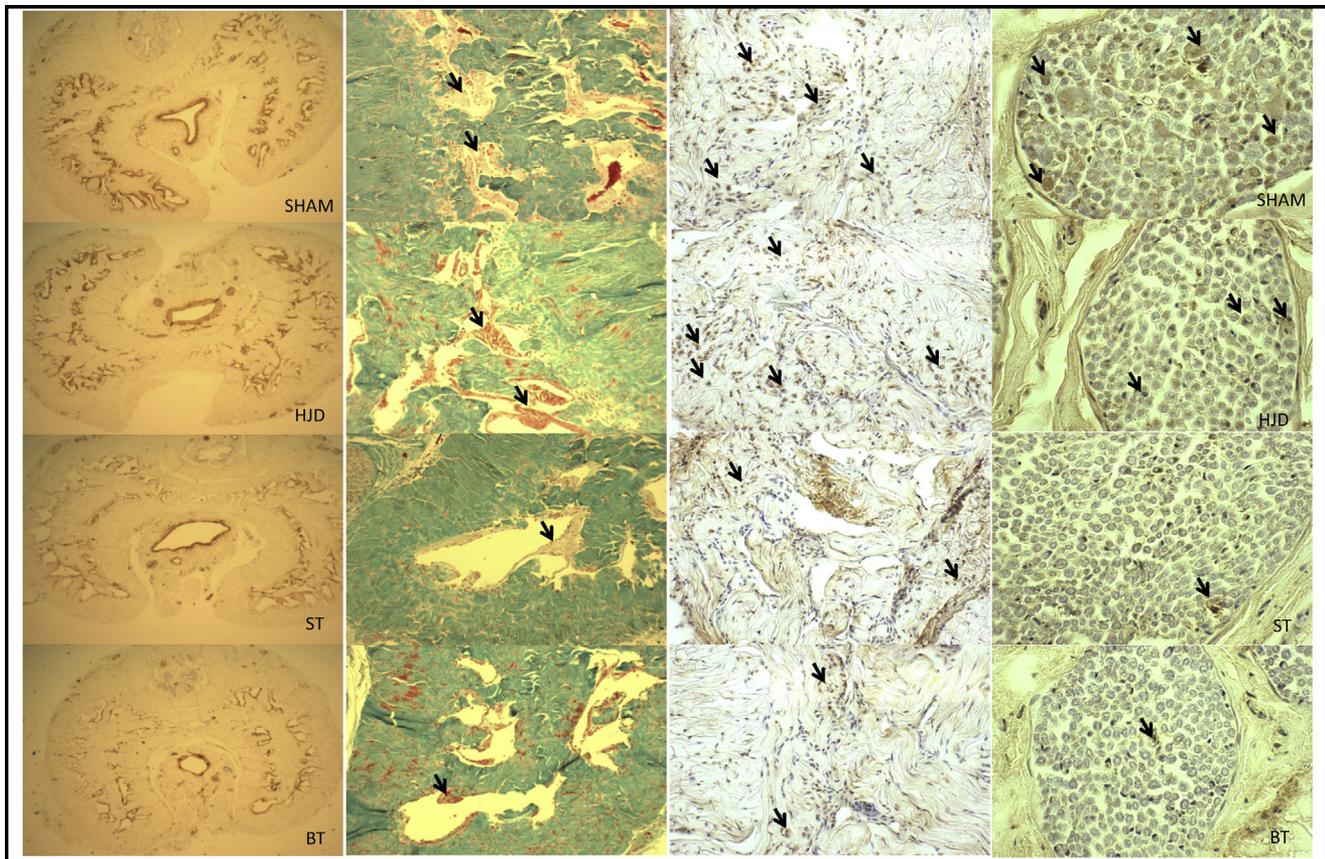


Figure 4. From left to right, representative cross-sectional slides stained for (1) α -SMA (magnification $\times 25$), (2) Masson's trichrome (magnification $\times 100$) and nNOS of (3) penile tissue (magnification $\times 200$); and (4) dorsal nerve bundle (magnification $\times 400$ magnification). Top to bottom: SHAM, HJD, ST, and BT groups. Noticeably more positive staining of both α -SMA (brown; panel 1) and nNOS (brown; panel 3 & 4) are seen in the SHAM and HJD groups compared with the BT and ST groups. Black arrows highlight positive stains in each representation. α -SMA = α -smooth muscle actin; BT = blunt trauma; HJD = hydro-jet dissection; nNOS = neuronal nitric oxide synthase; SHAM = sham surgery; ST = stretch trauma.

technology involved a simple syringe and needle to inject diluted epinephrine into the neurovascular bundle to facilitate dissection of the CN off of the prostate. The safety of this technique was confirmed by Guru et al¹⁷ in 2008; however, they did not report sexual function outcomes. Patel et al¹⁸ did report functional outcomes from their non-randomized consecutive series of patients who underwent open RP. Hydrodissection was associated with an improvement in the postoperative sexual health inventory for men score by 3.5 at 6 months.¹⁸ The most recent use of hydrodissection for nerve-sparing, robot-assisted RP did show improvement in postoperative erectile function; however, there was a high positive surgical margin rate, and therefore this technique needs to be modified to optimize oncologic outcomes before clinical use.¹⁹

In contrast to hydrodissection, HJD during open NS-RP was first reported by a German group in 2002.¹⁶ This early clinical study did confirm safety of this technology, but due to small patient populations and early follow-up regimes, they could not demonstrate statistically improved potency or continence.¹⁶ Over a decade later, this technology has recently been reused for NS-RP. A group from Russia enrolled 116 patients and

randomized them to either HJD or a standard NS-RP approach. They did demonstrate a functional improvement in post-operative erectile function when using HJD strategies. This study does have patient-selection, reporting, and surgeon bias, but it does optimistically present HJD as a safe tool that may preserve erectile function.²⁸ Our animal models support the clinical findings observed in these small, poorly conducted studies; that is, HJD of the CN during RP offers an approach to reduce postoperative neuropraxia. As opposed to stretching or bluntly manipulating the CN off of the prostate during RP, this novel technology could free up these delicate nerves and reduce the intraoperative injury. The pressure elicited during HJD is less than that associated with other dissection techniques and does not appear to cause enough trauma to result in nerve injury. Long-term data from animal studies and well-executed randomized controlled trials are warranted to further develop this novel technology.

The HJD technique was also safely applied during NS-retroperitoneal lymph node dissection. The authors report their pilot study involving 3 pigs and subsequently 5 patients with testicular cancer. Their narrative experience concluded that nodal

tissue could be separated from the nerve and vascular tissue safely and accurately, leading to successful ejaculation in these patients.¹⁵

Our animal model has been used to investigate erectile dysfunction (ED) in multiple prior studies.²¹ To understand whether HJD dissection leads to better functional outcomes compared with other surgical dissection techniques, as well as the mechanism underlying this perceived benefit, we compared this surgical technology to standard with blunt or stretch injury of the CN. After 1 month of recovery, the HJD group had a higher mean peak and more sustainable rise in ICP, which equates to better erectile function than the other dissection groups. Erectile function in the HJD group returned to baseline function, as compared with our SHAM control group, suggesting that this technique has minimal impact on the CN. Our experimental groups both provoke neuropraxia, and thus we unsurprisingly observed a significant preservation of erectile function with the more precise dissection with hydro-jet. Importantly, HJD did not induce ED and, instead, maintained erectile function that was statistically equivalent to no CNJ.

Preservation of both α -SMA and nNOS after HJD of the CN suggests that this is a neuroprotective technology that reduces neuropraxia and strongly supports this as a potential surgical maneuver. Studies exploring penile tissue earlier after HJD would allow us to determine the true impact of this technology as it relates to short-term injury.

To our knowledge, this is the first animal study exploring the functional outcomes of HJD on CN. We compared multiple different animal models of RP to establish a clinical benefit to this modern technology. We recognize that there are discrepancies between human and animal models of ED recovery, but this study in an animal model is an important indicator that this novel technology may offer a benefit on a broader scale. The surgical techniques applied to the animal mimic stretch and blunt injury that occur during RP and compare this to a less-aggressive dissection. Presently, limited human studies have been attempted, and this is the first study that objectively demonstrates improvement in erectile function after HJD of CN compared with other periprostatic dissection techniques. Our findings strengthen the current literature promoting HJD as a surgical strategy to preserve CN during RP.

CONCLUSIONS

This study demonstrates that HJD of the CN in an animal model of RP-induced ED is associated with significantly improved postoperative erectile function when compared with standard dissection techniques. HJD technology has been demonstrated to be safe in humans in other systems, and our animal studies suggest that this technique may prevent CNI during RP and improve the current nerve-sparing surgical strategies. Further clinical studies are needed to investigate the effect of HJD on erectile function in humans undergoing RP.

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