Poster Exhibit 8: Sexual Dysfunction & Infertility

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UP-143
Finding the cause of the curve: Development of an in vitro 3D Peyronie's disease model

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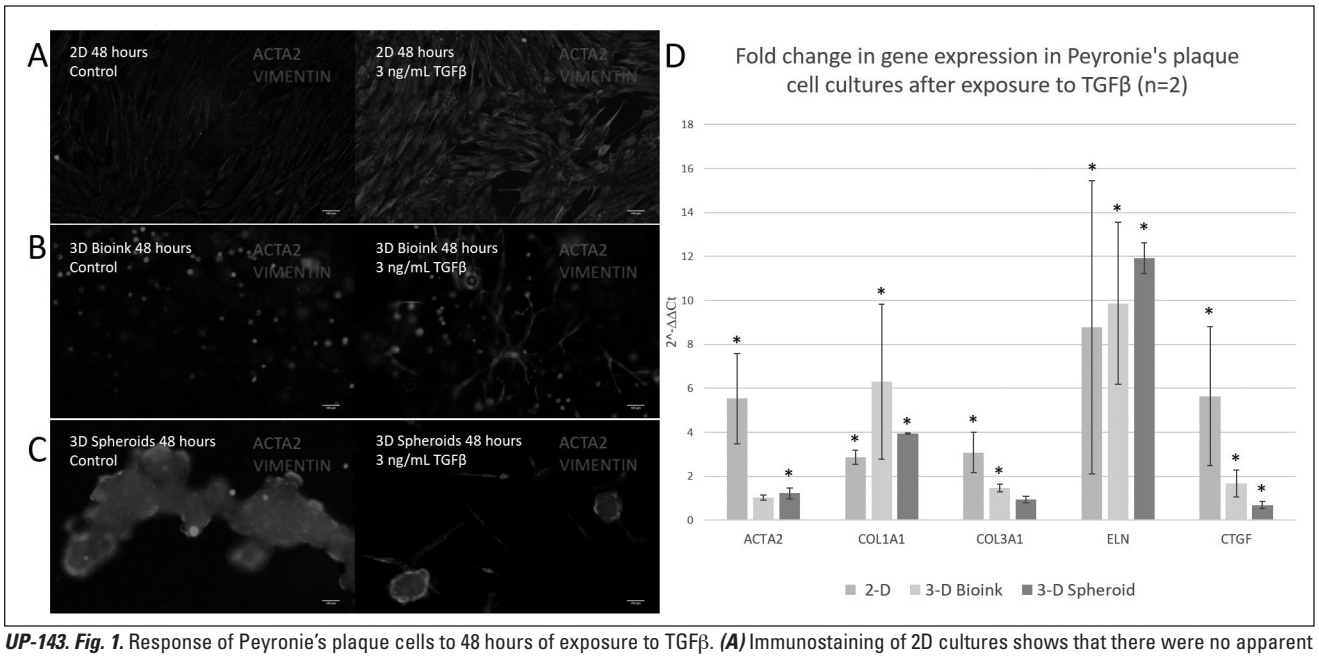
Introduction: Understanding of the pathophysiology of Peyronie's disease is limited. A mechanistic Peyronie's disease model remains elusive, with currently available animal models representing induced penile fibrosis, and thus not replicating the actual disease state. This study set out to develop a three-dimensional (3D) in vitro model to elucidate the pathogenesis of Peyronie's disease.

Methods: Peyronie's plaque tissues were placed in explant culture and expanded. Early passage cells were dissociated and placed either in six-well culture plates for 2D culture, Aggrewell800 plates to form 3D spheroids, or in a collagen-based hydrogel. To induce Peyronie's disease pathogenesis, transforming growth factor beta (TGFβ) was added to cultures at 3 ng/mL for 48 hours. Gene expression was analyzed by real-time polymerase chain reaction (RT-qPCR), and changes in morphology and cell phenotype were assessed by immunocytochemistry.

Results: In 2D plaque cell cultures, exposure to TGFβ for 48 hours resulted in upregulation of genes associated with extracellular matrix protein production, such as collagen I (COL1A1), collagen III (COL3A1), elastin (ELN) and connective tissue growth factor (CTGF) (Fig. 1D). In contrast, 3D cultures displayed a more modest upregulation of COL3A1 and CTGF, but nevertheless were able to attach and spread after 48 hours, indicating increased deposition of matrix proteins (Figs 1A–C). The smooth muscle gene alpha smooth muscle actin 2 (aSMA2/ACTA2), a known marker of myofibroblasts and fibrosis, was upregulated in 2D cultures only, indicating a difference in the reactivity of the plaque cells in 3D vs. 2D cultures.

Conclusions: This study describes two novel 3D culture models of Peyronie's disease and shows that the pro-fibrotic response differs in 3D environments compared to standard 2D cell culture, illustrating the importance of creating in vitro models that closely resemble the in vivo environment.



UP-143. Fig. 1. Response of Peyronie's plaque cells to 48 hours of exposure to TGFβ. ***(A)*** Immunostaining of 2D cultures shows that there were no apparent morphological changes after TGFβ exposure, whereas in 3D cultures ***(B)*** and ***(C),*** the cells are able to attach and spread after TGFβ exposure, indicating an increased cellular deposition of matrix proteins. ***(D)*** Changes in Peyronie's disease marker gene expression in response to TGFβ in the 2D and 3D culture conditions. *Statistical significance compared to controls without TGFβ exposure.

UP-144

Predictors of success after bilateral vasoepididymostomy performed during vasectomy reversal

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Introduction: Vasectomy reversal (VR) represents an excellent option for paternity in men who desire to expand their family following vasectomy. VR using vasovasostomy has a success rate upwards of 90%, but is sometimes not possible, and a vasoepididymostomy (VE) must be performed instead. A VE is successful in 40–60% of cases, and thus factors that can contribute to success are important to understand.

Methods: A multi-institutional, prospectively maintained database with data from the U.S. (Arizona) and Canada (Toronto) was collected from men who underwent bilateral VE at time of VR. Pre-, intra-, and postoperative clinical, surgical, and semen-related data were collected. Possible predictors of success included time since vasectomy, patient age, and intraoperative epididymal fluid characteristics. Success was defined as motile sperm in any postoperative semen analyses. Data were collected from 2008–2020. VE in both centers was performed using two 10-0 sutures in an intussusception technique. Multivariable logistic regression was used to identify predictors of success.

Results: A total of 191 men were included in the analysis. Average age was 45.8 years. Median time elapsed between vasectomy and VE was 14 years (interquartile range [IQR] 10–18). The longest time elapsed for a successful VE was 34 years. Overall success rate was 50%; 77.0% of all men had motile sperm in epidydimal fluid at time of VE. On multiple logistic regression, neither years since vasectomy (odds ratio [OR] 1.01, confidence interval [CI] 0.95–1.06), age (OR 0.96, CI 0.91–1.01), intraoperative presence of motile sperm (OR 0.81, CI 0.41–1.62), or any epidydimal fluid characteristics predicted success.

Conclusions: Bilateral VE at time of vasectomy reversal is successful in 50% of cases in a multi-institutional, North American cohort. Microsurgeons can be reassured that neither time elapsed nor epididymal fluid characteristics negatively impact success rates. Surgeons performing VR >10 years since vasectomy should be comfortable and prepared to perform VE if necessary.

UP-145

Less mortality and less major adverse cardiovascular events (MACE) under long-term testosterone therapy (TTh): 15-year data from a prospective, controlled registry study

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Introduction: We have previously demonstrated improvements in cardiometabolic risk factors in hypogonadal men on long-term testosterone therapy (TTh).

Methods: A cumulative registry study to investigate long-term effectiveness and safety of three-monthly TU to treat hypogonadism was established in 2004 in a urological setting. Of 805 hypogonadal men, 412 received parenteral TU 1000 mg/12 weeks (T-group) for up to 12 years; 393 men opted against TTh and served as controls (CTRL). Ten-year data are reported. Confounders, including age, body mass index (BMI), smoking, alcohol, total and high-density lipoprotein (HDL) cholesterol, systolic blood pressure, and type 2 diabetes, were assessed and considered in statistical modeling.

Results: Baseline age was 57.7±7.4 years in the T-group and 63.7±4.8 years in CTRL ($p<0.001$). The absolute followup time comprised approximately 6.500 patient-years. The mean Framingham risk score at baseline was 15.5 in the T-group and 15.8 in CTRL ($p<0.05$). The mean 10-year risk was 22.7% in the T-group and 23.5% in CTRL ($p=0.11$). Mean and median observation time in the T-group was eight years. There were 16 deaths (3.9%) but no myocardial infarctions (MIs) or strokes. Mean observation time in CTRL was eight years, median nine years. There were 74 deaths (18.8%), 70 MIs (17.8%), and 59 strokes (15%). All classical cardiovascular risk factors, including obesity, glycaemic control, lipid pattern, and C-reactive protein, improved in the T-group and worsened in CTRL. The reduction of cardiovascular events by TTh after applying a linear mixed effect model was 24.7% and 15.5% after applying a random effect longitudinal model.

Conclusions: In hypogonadal men, long-term TTh reduces cardiovascular events and mortality.

UP-147

Percutaneous epididymal sperm aspiration for men with obstructive azoospermia: Predictors of outcomes

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Introduction: We sought to evaluate sperm retrieval and pregnancy outcomes following percutaneous epididymal sperm aspiration (PESA) and intra-cytoplasmic sperm injection (ICSI) in men with obstructive azoospermia (OA).

Methods: Data were collected retrospectively from 533 patients with OA who underwent PESA between March 2007 and June 2019. Sperm retrieval outcomes were reported as motile sperm (>1% motile), rare motile sperm (≤1% motile), non-motile sperm, and no sperm found. We recorded clinical pregnancy per embryo transfer and live birth rates.

Results: Following PESA, motile sperm were detected in 404 patients (75.8%), rare motile sperm in 41 (7.7%), non-motile sperm in 67 (12.6%), and no sperm were found in 21 (3.9%). There was no difference between the groups in terms of paternal age, however, there was a significantly higher testicular volume in men who had motile sperm compared to those who had no sperm or nonmotile sperm ($p=0.015$). The overall clinical pregnancy and live birth rates per embryo transfer were 37.6% and 23.7%, respectively, with a mean of 1.1 (±0.5) embryos transferred. Paternal age, clinical diagnosis, and sperm motility were not associated with clinical pregnancy rates.

Conclusions: The data suggest that PESA yields good motile sperm retrieval rates in patients with OA, with overall good pregnancy rates. Larger testicular volume was predictive of higher motile sperm retrieval rates.

UP-148

Age-stratified potency outcomes of bilateral nerve sparing robotic-assisted radical prostatectomy

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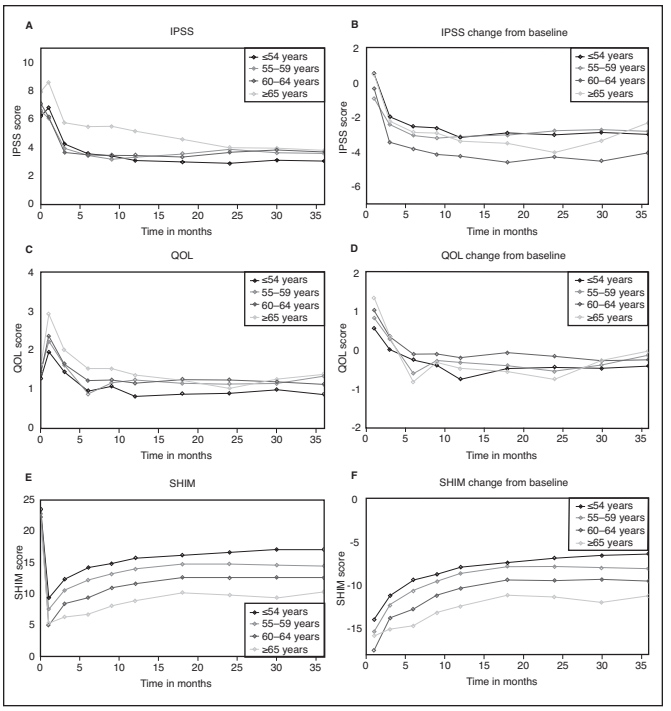
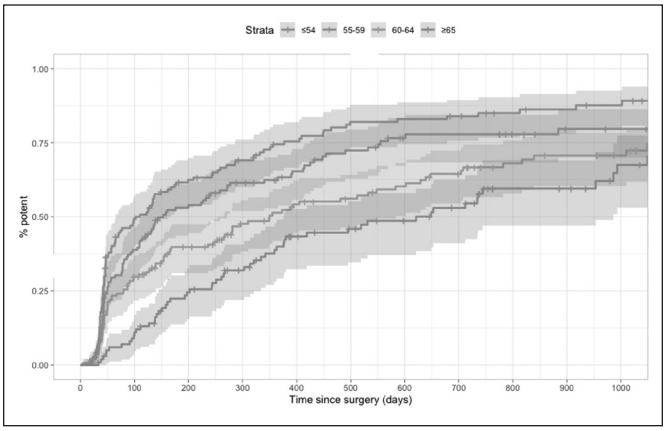
Introduction: This study aimed to report age-stratified potency outcomes in Canadian men undergoing robot-assisted radical prostatectomy (RARP).

Methods: A retrospective review was performed on a database of 1737 patients who underwent RARP for localized prostate cancer between 2007 and 2019. Inclusion criteria consisted of patients undergoing bilateral nerve-sparing RARP. Exclusion criteria were preoperative Sexual Health Inventory for Men (SHIM) score <17 and postoperative androgen deprivation therapy or radiotherapy. Patients were divided into four cohorts based on age: ≤54 years (group 1); 55–59 years (group 2); 60–64 years (group 3) and ≥65 years (group 4). Functional outcomes were measured up to 36 months. Kaplan-Meier analysis was performed to compare the



UP-148. Fig. 1. Age-specific outcomes over time. ***(A)*** Mean IPSS score over time. ***(B)*** Mean IPSS score change from baseline over time. ***(C)*** Mean QOL score over time. ***(D)*** Mean QOL score change from baseline over time. ***(E)*** Mean SHIM score over time. ***(F)*** Mean SHIM score change from baseline over time.

time to recovery of potency stratified by age groups using log-rank testing.
Results: A total of 542 patients met the selection criteria. Potency rates were significantly different between groups. Groups 1 through 4 demonstrated potency recovery rates of 64.2%, 52.3%, 36.6%, and 20.7% at one-year followup, respectively. After three years, groups 1 through 4 had potency rates of 77.9%, 67.0%, 50.5%, and 35.0%, respectively. Recovery of potency was achieved at a median time after surgery of 199, 340, and 853 days for groups 1–3, respectively. The Cox proportional hazard model showed that older age, higher body mass idex, and lower preoperative SHIM score were associated with significantly higher rates of impotence.



UP-148. Fig. 2. Age-specific potency recovery after the surgery. Potency defined as EHS 3–4.

Conclusions: This study shows that RARP has acceptable potency outcomes, regardless of age. However, patient factors, including older age and preoperative SHIM, were significantly associated with poorer functional recovery. This data is valuable in prognostic evaluation and patient counselling.

UP-149

Using artificial intelligence to predict semen upgrading after microsurgical varicocele repair

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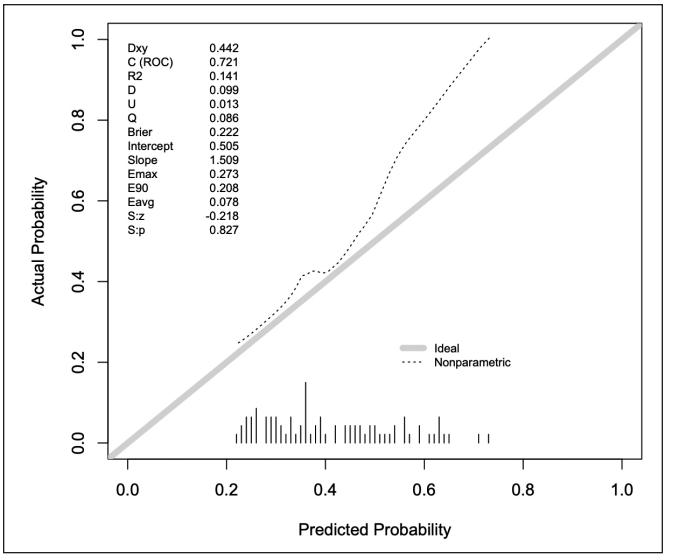
Introduction: Varicocele is one of the most common causes of male infertility. Unfortunately, up to 50% of men who meet criteria for repair will not see meaningful benefit in outcomes despite successful surgery. We developed an artificial intelligence (AI) model to predict which men with varicocele will benefit from surgery.
Methods: We identified men with male infertility, clinical varicocele, and at least one abnormal semen parameter from two large urology centers in North America between 2006 and 2020. Clinical upgrading was defined as an increase in sperm concentration that would allow a couple to access new reproductive technologies. The tiers used for upgrading were 0–1, 1–5, 5–15, and >15 million/cc. AI models were trained and tested using R to predict which patients upgraded after surgery.
Results: Data from 160 men from Miami and 80 men from Toronto were included. Average age was 36 years. Most men had grade 2 left varicocele, and (when present) a grade 1 varicocele on the right; 47% of men experienced an upgrade following surgery, 47% did not change, and 6% downgraded. The data from Miami were used to create a random forest model for predicting upgrade in sperm concentration (SC). The most informative parameters were preoperative follicle-stimulating hormone (FSH), SC, and surgical laterality. The model identified men with unfavorable, intermediate, and favorable features to predict varicocele upgrade. On external validation using Toronto data, the model accurately predicted



UP-149. Fig. 1. Calibration curve for post-repair sperm concentration. The curve measures the correspondence between AI-predicted model and the actual observed values. The grey line represents the ideal scenario when predicted values equal observed values. The dotted line is the calibration curve of the AI model. The model shows good calibration.

upgrade in 87% of men with favorable features, and in 49% and 36% of men with intermediate and unfavorable features, respectively. Overall, the model performed well on external validation, with an area under the curve of 0.72 and good calibration (Fig. 1).
Conclusions: A machine learning model performed well in predicting clinically meaningful post-varicocelectomy semen upgrade using preoperative hormonal, clinical, and semen analysis data. This model can be used by clinicians in preoperative counselling of their patients.

UP-150

Insurance approval rates for collagenase clostridium histolyticum prior to discontinuation: A Canada-wide analysis

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Introduction: Intralesional collagenase clostridium histolyticum (CCH) was the first non-surgical therapy approved for the treatment of Peyronie's disease (PD). CCH has been discontinued in Europe, and more recently, in Canada. The reason cited by the producing company was unsustainably low demand. We sought to examine insurance approval rates for CCH prior this decision to better understand whether this may have contributed to its discontinuation.
Methods: We identified all patients for whom prescriptions for CCH were written, both for the treatment of Dupuytren's contracture (DC) and PD, within Canada from April 2018 to June 2020. Data regarding insurance approval and rejection was obtained. Univariate and multivariate analysis was performed to assess for predictors of insurance approval.
Results: Insurance applications were submitted for 3297 patients during the study period (2535 for DC vs. 748 for PD). On multivariate analysis, insurance applications for patients with PD were more likely to be approved compared to those for DC (92.9% vs. 87.3A%, odds ratio [OR] 2.35, p<0.001). Among approved applications, the median coverage was 90% (interquartile range [IQR] 80–100), and the median out-of-pocket expense paid was $210.40 (IQR 0–283.30). Of all approved patients, 88.8% ultimately filled a prescription, compared to 8.8% whose applications were rejected. The median province in terms of application rate approval was Newfoundland and Labrador (86.6%), with British Columbia, New Brunswick, Nova Scotia, and Ontario more likely to receive insurance coverage approval (range 93.9–98.9%), and Quebec, Manitoba, Saskatchewan, and Alberta less likely to receive coverage approval (range 64.3–79.9%).
Conclusions: Overall insurance application approval rates for CCH were high in Canada for both DC and PD, though approval rates for significantly higher for the treatment of PD. There was substantial inter-provincial variation in approval rates.

UP-151

Possible effects of saffron (Crocus sativus) in treatment of erectile dysfunction: A randomized, double-blind, placebo-controlled trial

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Introduction: A significant portion of erectile dysfunction (ED) patients receiving available treatments fail to respond. This study aimed to evaluate efficacy of saffron in improving erectile function compared with placebo in men with ED.
Methods: Men with ED of at least a mild severity participated in a six-week, parallel-group, double-blind, placebo-controlled trial. Participants were randomized to receive 15 mg saffron or placebo capsules twice daily. The trial continued for six weeks, and participants were evaluated every two weeks. The primary outcome of interest was the change in the erectile function. We used t-test, linear regression, and repeated-measures ANOVA to analyze the results.
Results: Sixty-two participants were equally randomized into two groups, and 29 participants in each group completed the trial. Participants had a mean age of 41years, and the majority suffered from mild ED. Positive changes in erectile function scores reached 6.14 (95% confidence interval [CI] 4.97, 7.30) points in the saffron group, which was superior to the placebo. The CI excluded the minimal clinically important difference of the scale. The adverse events were similar between the two groups and saffron showed a clinically acceptable profile.
Conclusions: Our findings suggest saffron might be an effective and safe option to ameliorate ED, especially for those who decline or are unwilling to use phosphodiesterase type 5 inhibitors.

UP-152

Intracavernous injection of botulinum toxin improves erectile function in an animal model of bilateral cavernous nerve injury

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Introduction: Erectile dysfunction (ED) is common after radical prostatectomy (RP) and can negatively impact patient and partner quality of life. Botulinum toxin (Botox) promotes smooth muscle relaxation and may augment erectile function by reducing basal sympathetic tone in the corpora cavernosa. Botox may promote nerve regeneration after peripheral nerve injury. We investigated Botox as a treatment for ED in a bilateral cavernous nerve injury (BCNI) animal model.
Methods: Sixteen Sprague Dawley rats (4.5 months; 450–550 g) were randomized to four groups: 1) sham + normal saline (NS); 2) BCNI + NS; 3) sham + Botox; and 4) BCNI + Botox. When cavernous nerves were identified during surgery, they were either crushed (BCNI) or left untouched (sham). Next, either 10 units of Botox or 80 umL of NS were injected into the corpora cavernosa. On day 14, erectile function was assessed via cavernous nerve electro-stimulation-induced intracavernous pressure (ICP) changes. Penile tissue was harvested for immunohistochemistry and Western blot for neuronal nitric oxide synthase (nNOS) and smooth muscle α-actin (SMA).
Results: Fourteen animals were included for analysis; one animal in each of groups 2 and 3 were euthanized for weight loss and lethargy. The Botox treatment groups had a net weight loss, whereas the NS groups had weight gain (p<0.05). Erectile function after BCNI recovered with Botox injections and both group 3 and 4 had mean peak ICP and area under the curve values analogous to baseline (group 1) and statistically improved compared to control (group 2). Western blot analysis revealed a recovery of both nNOS and SMA after Botox treatment (p=0.06).
Conclusions: This is the first animal study evaluating intracavernous Botox injections for treating ED after BCNI and compared to placebo; the intervention appears to recover erectile function. Further preclinical studies are needed to support this finding and there may be a future role for using Botox to treat post-prostatectomy ED.

UP-153

Canadian provider perspectives on collagenase clostridium histolyticum for the treatment of Peyronie's disease and the impact of its discontinuation

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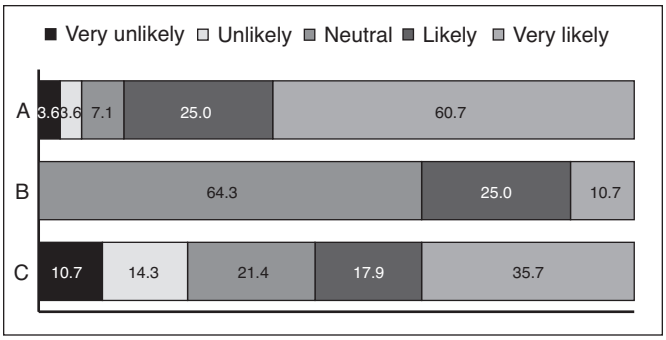
Introduction: Intralesional collagenase clostridium histolyticum (CCh) was the first non-surgical therapy approved for Peyronie's disease (PD). However, CCh's cost and poor market uptake has led to its discontinuation in Europe and Canada. The aim was to better understand Canadian



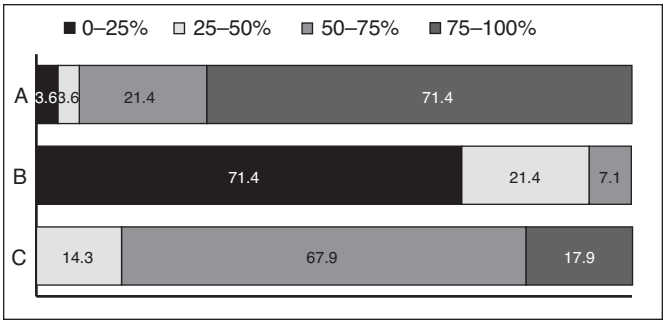
UP-153. Fig. 1. Provider perspectives on insurance approval, clinical outcomes, and whether they would continue treating PD. A=How likely were insurance coverage applications for CCh to get approved? B=How likely were you to see a clinically meaningful response? C=How likely are you to continue to treat PD now that CCh has been discontinued?

providers' perspectives regarding its treatment efficacy and the potential impact of its discontinuation.

Methods: All Xiaflex®-approved Canadian providers were asked to complete an anonymous 20-question survey using an online platform in the summer of 2020. Analysis consisted of descriptive statistics. Outcomes of interest included previous experience with CCh, protocols used, experience with insurance coverage, clinical and patient-reported outcomes, and provider perspectives on the discontinuation of CCh.

Results: Overall response rate was 48.3% (29/60) (Table 1); 93% of respondents felt that CCh was superior to other intralesional therapies for PD and 86% reported a patient satisfaction rate of at least 50%. The majority (75%) saw a clinically meaningful response (Figs. 1, 2). Only 7% expressed difficulty obtaining insurance coverage, with many providers (71%) achieving an insurance approval rate from 75–100%. Only 54% of respondents reported that they would continue treating PD in light of CCh's discontinuation. In light of CCh's discontinuation, few will offer intralesional verapamil (36%) or interferon (7%), and most (79%) are now more likely to offer surgical treatment.

Conclusions: Most CCh providers found CCh to be effective and were dismayed by its discontinuation. The discontinuation of CCh in Canada will lead to a reduction in the number of Canadian urologists offering PD treatment, with increased propensity to offer surgical treatment among those who remain. The survey demonstrated that due to the withdrawal of CCh from Canada, physicians' abilities to offer effective medical therapy may become limited, with more providers offering surgical options for PD.



UP-153. Fig. 2. Insurance approval rates, willingness to pay for CCh without insurance, and patient perspectives on clinical outcomes. A=What was the rate of insurance approval for CCh treatment? B=What percentage of patients were willing to pay for CCh without insurance? C=What percentage of patients were happy with CCh outcomes?

UP-153. Table 1. Baseline demographics

Parameter	Value n (%)
Number of respondents	28
Gender (%)	
Male	25 (89)
Female	3 (11)
Years in practice	
0–5	9 (32)
6–10	8 (29)
11–15	2 (7)
>15	9 (32)
Fellowship-trained	
Yes	13 (46)
No	15 (54)
Province of practice	
British Columbia	10 (36)
Alberta	1 (4)
Manitoba	1 (4)
Ontario	10 (35)
Quebec	6 (21)
Atlantic provinces	0 (0)
Instances of CCh administration	
<10	5 (18)
10–20	6 (21)
>20	17 (61)

UP-154

Getting a straight answer: Survey of collagenase histolyticum treatment practices for treating Peyronie's disease

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Introduction: Peyronie's disease (PD) affects up to 13% of men, although few ultimately seek treatment for this condition. Treatment options are limited, with injectable collagenase clostridium histolyticum (CCh) representing one of the few non-surgical options. We hypothesized that variation exists within practitioners offering this therapy, as clear treatment protocols remain elusive.

Methods: We administered an email survey to members of the Sexual Medicine Society of North America (SMSNA) and CCh providers in Canada. The survey inquired about each urologist's training, workup of patients, treatment protocols, and injection technique.

Results: A total of 35 urologists responded. The respondents had been practicing for a median of 11 years (range 37 years). Sixteen were academic urologists, 17 were community urologists, and two were both. During workup of patients, 13 of 30 urologists identify their point of injection via palpation, while 17 of 30 used palpation and measurement of point of maximal curvature. Only seven of 31 urologists adhere to the FDA protocol of administering four sets of two injections spaced 3–7 days apart. Fanning of the injection was reported in 67% of respondents. During injection, 60% maintain partial penile traction, 33% maintain full traction, and 7% leave the penis flaccid. When asked to estimate the volume of drug delivery 16 of the 30 estimated 50–75% delivery of the CCh drug into the PD plaque. On a scale of 1–5, with 1 being not confident at all and 5 being extremely confident, 85% of urologists reported being less than "very confident" in their injection placement.

Conclusions: Heterogeneity exists within urologists offering CCh treatment for Peyronie's disease. This may stem from a lack of a clear treatment

regime. The variation in injection technique and confidence suggests that improved training, education, and technical assistance may benefit practitioners pursuing these therapies.

UP-155
A novel cell-sorting strategy for the isolation of germ cells transitioning into meiosis

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Support: Vancouver Coastal Health Research Institute (VCHRI). Canadian Urological Association Scholarship Foundation (CUASF). Canadian Institute of Health Research (CIHR). University of British Columbia Department of Urological Sciences.
Introduction: Spermatogenesis is driven by meiotic division, wherein a single cell divides twice to produce four cells containing half the original amount of genetic information. Entry into meiosis is regulated by stimulated by retinoic acid sene 8 (STRA8), and its dysfunction leads to infertility. In this study, we set out to isolate STRA8-expressing germ cells using fluorescent-activated cell sorting (FACs).
Methods: 10X genomics single-cell sequencing of a human testis biopsy identified surface proteins associated with pre-meiotic and early meiotic germ cells, which were used to sort cells via FACS. Sorted cells were analyzed for gene expression using real time polymerase chain reaction (RT-qPCR) assays. Results were validated by immunostaining of tissue sections.
Results: Screening for a surface marker to isolate a pre to early meiotic stage of germ cell resulted in the identification of serine protease 50 (PRSS50/TSP50). TSP50-sorted cells had enhanced expression of pre-meiotic and early meiotic stage germ cells (Fig. 1B). The highest fold change in gene expression was STRA8 (56-fold), followed by the early meiotic marker doublesex- and Mab-3-related transcription factor B1 (DMRTB1); the meiotic transcription factor, deleted in azoospermia like (DAZL); the pre-meiotic/mitotic spermatagonia marker, melanoma-associated antigen 4 (MAGEA4); the meiotic marker, synaptonemal complex protein 3 (SYCP3); and the pre-meiotic marker, mast/stem cell growth factor receptor kit (KIT). Immunostaining confirmed the localization of TSP50 to germ cells, and coexpression of TSP50 and SYCP3 in a subset of germ cells, illustrating a transition between pre-meiotic and early meiotic stages (Fig. 1A).
Conclusions: This study identified the surface marker TSP50 for FACS isolation of STRA8-expressing germ cells in pre-meiotic to early meiotic stages of spermatogenesis. Isolation of these cells provides an avenue for in-depth characterization of STRA8 function in spermatogenesis.

UP-156
A xeno-free-defined culture method for the in vitro expansion of human spermatogonial stem cells

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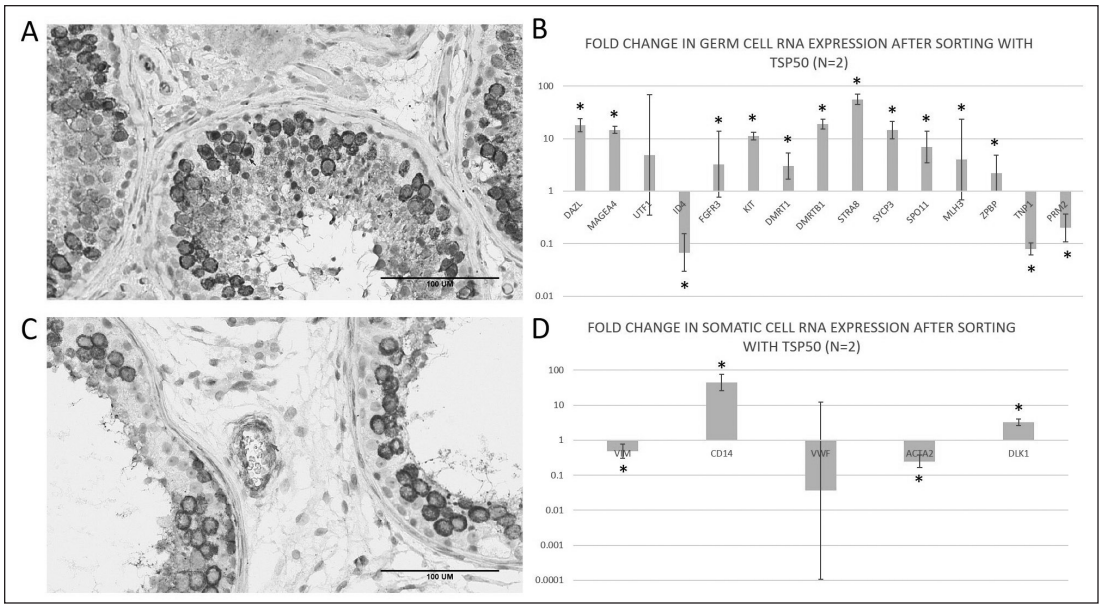
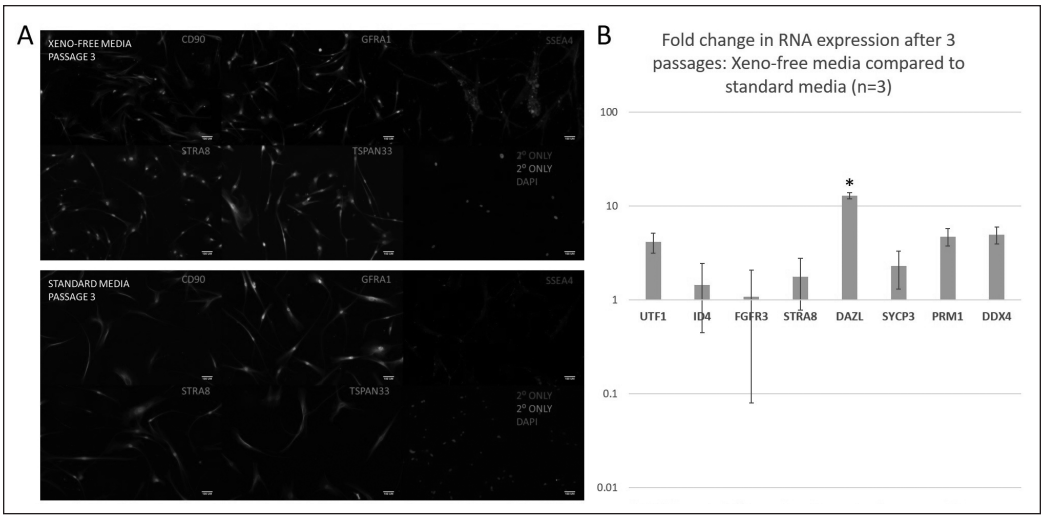
Support: Vancouver Coastal Health Research Institute (VCHRI). Canadian Urological Association Scholarship Foundation (CUASF). Canadian Institute of Health Research (CIHR). University of British Columbia Department of Urological Sciences.
Introduction: In vitro expansion of spermatogonial stem cells (SSCs) has been established using animal-derived fetal bovine serum (FBS), however, animal components introduce the risk of contaminating with pathogens, making them unsuitable for medical use. This study set out to develop xeno-free culture conditions for the expansion of human SSCs.
Methods: SSCs were derived from human induced pluripotent stem cells (hiPSCs) and tested in various xeno-free media conditions in combination with growth factors prostaglandin D2 (PDG2) and insulin-like growth factor 1 (IGF1). Primary SSCs then underwent three passages in the best condition, were cryopreserved and thawed, and then analyzed for changes in gene and protein expression by real-time polymerase chain reaction (RT-qPCR) and immunocytochemistry.



UP-155. Fig. 1. Testis expression of TSP50. ***(A)*** A testis section co-stained with TSP50 antibody and the meiotic germ call marker SYCP3 shows that many TSP50-expressing cells are pre-meiotic, while a subset co-express TSP50 and SYCP3, indicating entry into meiosis. ***(B)*** Fold change of gene expression for spermatogenic markers of TSP50 sorted cell. ***(C)*** A testis section stained with TSP50 showing some interstitial cell immunoreactivity. ***(D)*** fold change of gene expression for interstitial cell markers show an upregulation in the macrophage gene CD14. *Statistical significance.



UP-156. Fig. 1. Human primary spermatogonial stem cell expanded in xeno-free media compared to standard media for 3 passages (216-fold expansion). ***(A)*** Comparison of expression of spermatogonial markers CD90, GFRA1, SSEA4, and TSPAN33 after culture in xeno-free media vs. standard nedia. ***(B)*** Fold change in RNA expression of the spermatogonial stem cells culture in xeno-free media compared to standard media. UTF1, ID4, and FGFR3 are spermatogonial stem cell markers, STRA8 indicates entry into meiosis, DAZL is a regulator of spermatogonial stem cell proliferation and differentiation, SYCP3 is a meiotic germ cell marker, PRM1 is a post-meiotic germ cell marker, and DDX4 (VASA) is a general germ cell marker. N=3. *Statistically significant change compared to controls.

Results: 10 ng/mL PDG2 with 10 ng/mL IGF1 was found to replace FBS and BSA without loss of viability or growth. ROCK inhibitor Y-27632 was determined to be necessary for viability upon thawing. Compared to established conditions, the SSCs shared identical protein expression profiles for the SSC markers, glial cell-derived neurotrophic factor family receptor alpha 1 (GFRA1), G-protein coupled receptor 125 (GPR125), Thy-1 cell surface antigen (CD90), and stage-specific embryonic antigen 4 (SSEA4) (Fig. 1A). RT-qPCR analyses revealed no significant variation in gene expression of undifferentiated germ cell markers undifferentiated embryonic cell transcription factor 1 (UTF1), inhibitor of differentiation 4 (ID4), and fibroblast growth factor receptor 3 (FGFR3). An increase in deleted in azoospermia like (DAZL), a regulator of both SSC proliferation and differentiation, was observed (13-fold) (Fig. 1B).
Conclusions: This study identified a combination of growth factors capable of replacing FBS and BSA components in established SSC expansion cell culture. This xeno-free-defined formulation allows standardized SSC culture and removes the risk of animal pathogens.

UP-157

Vasectomy reversal following percutaneous epididymal sperm aspiration (PESA): Operative implications and postoperative outcomes

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Introduction: Options for fertility following vasectomy include vasectomy reversal (VR) or sperm retrieval with in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI). Percutaneous epididymal sperm aspiration (PESA) poses a theoretical risk of epididymal scarring/obstruction and may have implications on the operative approach and success following VR. In an effort to better inform couples, the current study investigated the intra- and postoperative outcomes of VR following PESA.
Methods: A prospective database of 2023 VRs was reviewed to identify men who had undergone PESA prior to VR. Intraoperative findings, including vasal fluid, sperm quality, and anastomotic technique (vasovasostomy-VV/vasoepididymostomy-VE), were correlated with PESA details. Postoperative semen parameters and patency rates were compared among men presenting with a pre-VR PESA to patients with no history of PESA prior to VR.
Results: Twenty-eight men were identified who underwent unilateral or bilateral PESA prior to VR. Mean age and vasal occlusive interval was 43 and 11.7 years, respectively; 44% and 66% of men reported having a bilateral and unilateral PESA performed, respectively. Bilateral VV was performed in 82% of men, with VE required in 18%. Among men requiring VE, 80% had a PESA performed on the same side. Among all testicular units where a PESA was performed, favorable vasal fluid characteristics (non-pasty) were identified in 81% of men and intraoperative microscopic vasal fluid analysis confirmed the presence of sperm in 82% of men. Compared to an aged-matched sample of patients with no history of PESA prior to VR, postoperative patency rates (motile sperm in ejaculate) were equivalent (94% PESA+VR, 94% VR). Mean total motile sperm counts (TMC) were 18.4 among PESA+VR patients compared to 23.4 among men who underwent VR without PESA.
Conclusions: VR remains an option for couples post-PESA. Favorable vasal fluid characteristics and sperm quality allow for VV >80% of men. Postoperative patency rates and semen parameters compared favorably to patients without PESA prior to VR.

UP-158

Development of a machine learning algorithm for Peyronie's disease curvature assessment

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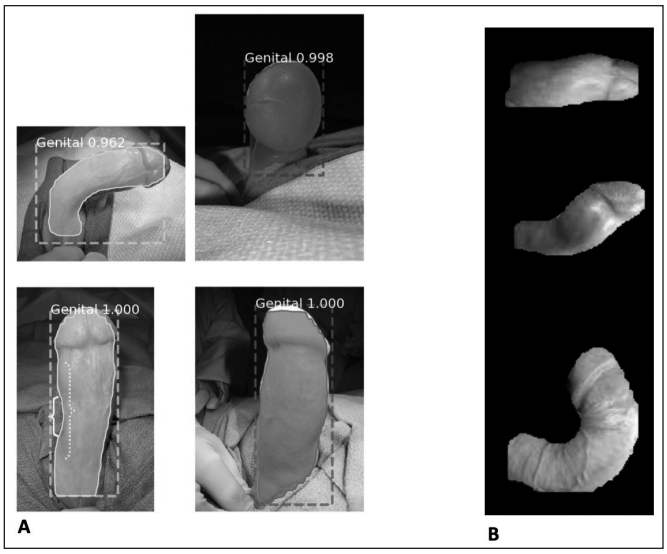
Introduction: Although Peyronie's disease prevalence estimates suggest that 11–15% of men suffer from this disease, only 1% seek evaluation. When patients do seek help, tools for penile curvature assessment are lacking. We present a tool for patient- and clinician-led assessment of Peyronie's disease using a neural network system capable of plaque assessment of photographic images.
Methods: Men underdoing penile curvature assessment in a sexual medicine clinic were recruited to enroll in this study. Images were taken of the



UP-158. Fig. 1. Visual representation of current algorithm for detection of Peyronie's disease and background removal. ***(A)*** Labelled values represent algorithm certainty that a genital (penis) has been identified. ***(B)*** Examples of background subtraction algorithm.

erect penis from the left, right, and top sides using an Apple iPad™. For initial model training 150 images were used from the open access Not Suitable for Work (NSWF) repository (*https://github.com/EBazarov/nsfw_data_source_urls/tree/master/raw_data*) and labelled manually for object detection. Subsequently model training was conducted with a residual neural network (ResNet), a neural network-based framework used for image recognition, to identify male genitals. To assess basic curvature a mathematical formulation was created to calculate the penile curvature after fitting linear lines from genital tip to curvature point and from curvature point to the base of the penis.

Results: Initial training of the image analysis model has allowed for identification of the presence of a curvature of a penis, with ongoing training for curvature quantification (Fig. 1). We have trained the model to remove background images, isolating the genitals (Fig. 1) to aid in patient privacy.

Conclusions: This study provides a proof of concept that machine learning can augment the assessment of patients with Peyronie's disease. With demonstration of curvature assessment via photographic images this will allow patients to perform an initial curvature assessment at home, improving motivation for patients to seek help. Furthermore, this technology can augment clinician-led curvature assessment, providing an objective and easy means for documenting disease severity.

UP-160

Google searches are a poor-quality source of information for erectile dysfunction treatment

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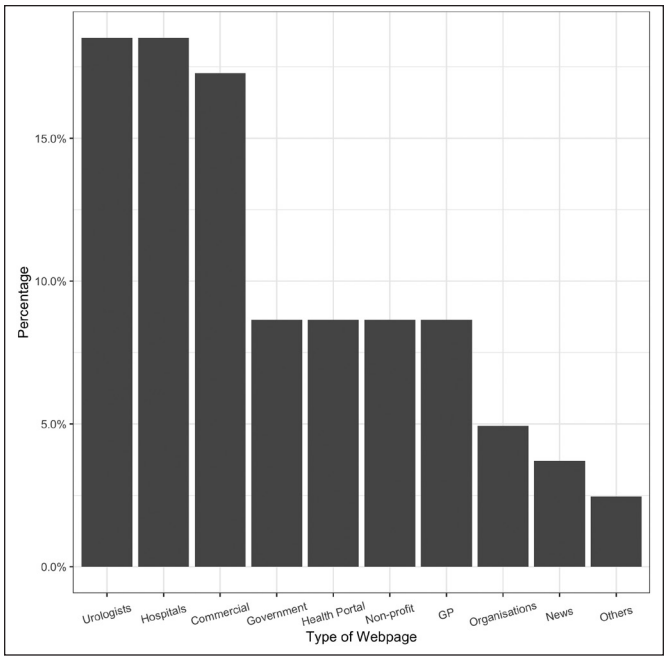
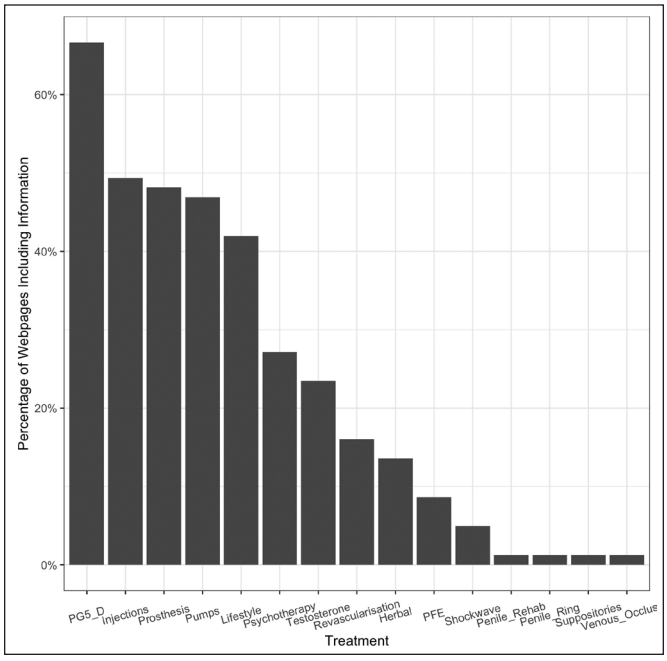
Introduction: Patients often use the internet to find medical information. This study aimed to determine the quality and readability of online information on erectile dysfunction treatments (EDT).

Methods: A Google search for "erectile dysfunction treatment" was performed. Typographic and treatment information within sites was recorded. Readability was measured with four validated items: Fleisch-Kincaid grade, Gunning-Fog, Coleman-Liau, and Simple Measure of Gobbledygook. Quality was measured using three validated items: HON certification, JAMA criteria, and the DISCERN instrument.

Results: Eighty-one websites were included. Most were produced by urologists and hospitals, with 15 (18.5%) each (Fig. 1). Specific information on EDT was found on 74 (91.4%) sites, with phosphodiesterase-5 inhibitor therapy most frequent (n=55, 67.9%) (Fig. 2). The mean readability score was 12.32 (standard deviation [SD] 1.91, 95% confidence interval [CI] 11.90–12.74), equivalent to a year 12 level. Resources were poor-quality, with a median total DISCERN score 35 (interquartile range [IQR] 26–44.5) out of 80. There was a small, significant negative correlation between Google rank and total DISCERN score (τ=-0.160, p=0.036). HON-certified sites had significantly higher total DISCERN scores; median 44 (IQR 25–59.75) vs. 32.5 (IQR 25.13–42.38) (U=832.5, p<0.001). A



UP-160. Fig. 1. Type of webpage included in the study.



UP-160. Fig. 2. Topics covered on each website.

linear regression was used to predict DISCERN score from JAMA criteria, R^2=0.374 (adjusted R^2=0.340), F(4, 76)=11.370, p<0.001. The effects of meeting the attribution and currency criteria were significant (β11.160 and β 9.753).
Conclusions: On average, websites on EDT required a 12th-grade readability level and provided poor-quality information. Health professionals should assess their websites' readability and quality using validated instruments prior to publication, to improve resource quality. HON certification and meeting the JAMA criteria for attribution and currency were significant predictors for higher-quality websites. Educating patients about these markers may improve identification of quality resources.

UP-161

Treatment of Peyronie's disease patients with collagenase clostridium histolyticum: Clinical significance and outcomes

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Introduction: Peyronie's disease (PD) is an inflammatory fibrosis of the penile tunica albuginea that results in penile curvature and may cause sexual dysfunction. In this overview, we evaluated the treatment outcomes in terms of symptoms and complications with the use of collagenase clostridium histolyticum (CCh).
Methods: A prospective, observational, data registry study was recorded for PD patients who underwent CCh treatment. Inclusion criteria were any PD patient with penile curvature >30º and <90º. Exclusion criteria were any ventral curvature and calcified plaque. Data registered through followup to two years using history, physical exam, International Index of Erectile Function (IIEF), and penile ultrasound with induced erection for plaque size and curvature determination pre- and post-treatment. A Pearson Chi-square and t-test (p<0.05) was used to figure out the statistical significance.
Results: A sample size of 99 patients suffering from PD underwent CCh injections. The age of the participants was (mean ± standard deviation) 55.5±9.8 years. Baseline mean curvature was 49º±20.44º. Regarding the curvature direction, 73% were dorsal, 11% lateral, and 16% were in mixed direction. More than 90% of plaques were <2 cm in size (mean size 1.71+6.6 cm). The duration of followup was >6 months after treatment. Forty-nine percent had eight injections in total, while the remaining had 4–6 injections. Our course was one injection per month. The mean change in degree after treatment was 25.77º±11.05º (p<0.001) and almost 87% of the patients had ≥20% reduction in curvature. There was also a mean improvement in IIEF score after treatment of 2.27 points (p<0.001). Twenty-three patients developed complications after taking the injection; the most common were hematoma and transient chest pain, back pain, and lower limb pain (similar to sciatica). All complications were treated conservatively. For the treatment of erectile dysfunction (ED), all patients were given vacuum devices in addition to medical therapy with tadalafil 5 mg daily.
Conclusions: PD treatment with CCh has a good outcome, even with comorbid diseases, with about 87% of our patients showing improvement with a low rate of complications. ED and curvature degree improved significantly. We urged our patient to use vacuum, a phosphodiesterase type 5 inhibitor, and patient stretching daily to improve their ED and strongly recommend this method of combined treatment for ED in PD patients.

UP-162

The hard facts: Patient information and trends in the current use of restorative therapy for the treatment of erectile dysfunction

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Introduction: Erectile dysfunction (ED) is on the rise, with a prevalence of over 50% in men 40–70 years old. Several clinics across Canada offer restorative therapies for ED, including shockwave therapy (SWT) and protein-rich plasma (PRP) injections. This study aims to provide a descriptive analysis of the current restorative therapies offered across Canada and to determine if these clinics supply inquiring patients with necessary information.
Methods: We performed a systematic search of online website directories to identify Canadian clinics that offer restorative therapies for ED. A set of standardized questions were created to simulate what information prospective patients would want to know from clinic websites, including cost, frequency of treatment, patient outcomes, brand/source of product used, and educational background of the provider. The websites were navigated to obtain answers to the pre-formulated questions. Descriptive statistics were used to summarize the information.
Results: A total of 34 clinics were identified as offering rejuvenation therapy; 15 offered SWT and 19 offered PRP. The number of clinics that provided online cost estimates were three (15.8%) and three (20%) for PRP and SWT, respectively. The average cost per treatment was $1191 (standard deviation [SD] 300.4) for PRP and $258 (SD 138.3) for SWT. The percentage of clinics that were supervised by an MD was 68.4% for PRP and 60% for SWT. Of the 22 clinics that listed MD supervision, only four had urology involvement. Only 21% of PRP and 20% of SWT clinics listed any risks associated with their treatments. No clinics listed number of patients treated or their treatment success rates. The most common benefits listed for PRP were stronger erections and improved sensation, and for SWT it was improved circulation.
Conclusions: Overall, there is a lack of consistency in the information provided online for patients inquiring about restorative therapies for ED. Future directions include contacting clinics by phone to obtain more information prospective patients may seek out.

UP-163

Deep learning-based automated sperm identification for non-obstructive azoospermia patients

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Introduction: Over 30 million men worldwide are infertile, and the most severe form of male infertility is non-obstructive azoospermia (NOA). NOA patients require andrologists to find viable sperm to proceed with vitro fertilization (IVF) intracytoplasmic sperm injection (ICSI), which often requires hours seeking rare sperm under a microscope. We evaluate the feasibility of using machine learning methods for the identification of rare sperm in microscopy images taken from a semen sample to improve IVF success rates.
Methods: We prepared samples using density gradient centrifugation to isolate healthy sperm with no debris or non-sperm cells. Sperm are stained using SYBR-14 and propidium iodide nucleic acid to be identified and then imaged using a fluorescent microscope. Images are then combined, binarized, and used as the ground truth to train a U-Net architecture using binary cross-entropy loss to segment sperm pixels. Individual sperm are identified using the watershed algorithm and evaluated through precision-recall metrics and receiver operating characteristic curves.
Results: Unlike previous work, the model is trained on BF images with unwashed and unstained samples to mimic clinical practice. A custom metric was developed in Python to evaluate the model on sperm prediction precision and recall using nearest-neighbour, a k-d tree, and size/distance thresholding. Pilot tests were completed to optimize model performance and speed to determine the use of 10x magnification. Heavily unbalanced datasets were counteracted using weighted losses. At 10x magnification, our model achieves 91% precision and 96% recall in finding sperm in microscopy BF semen images.
Conclusions: Our results indicate it is feasible to use convolutional neural networks to semantically segment sperm to support andrologists for IVF-ICSI. Our custom lab protocol creates training data containing stained sperm and unstained miscellaneous cells, allowing for the first example of a real-world application of artificial intelligence for assisted sperm identification.

UP-164

Generation of peritubular myoid-like cells from human induced pluripotent stem cells

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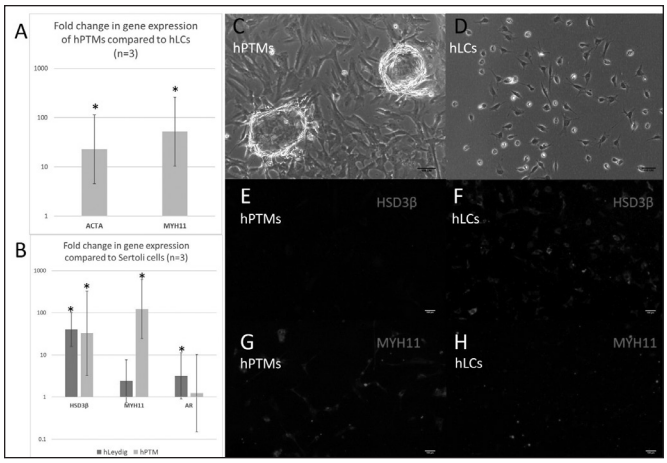
Support: Vancouver Coastal Health Research Institute (VCHRI). Canadian Urological Association Scholarship Foundation (CUASF). Canadian Institute of Health Research (CIHR). University of British Columbia Department of Urological Sciences.

Introduction: Spermatogenesis is a complex process involving cellular interactions between multiple cell types to produce viable sperm. Investigation of spermatogenesis is hampered by the inability to reproduce the unique physiological environment found within the testes. One cell population critical to sperm production are peritubular myoid cells (PTMs), which are smooth muscle cells that line the seminiferous tubules, provide contractility, and contribute to Sertoli and Leydig cell function. PTMs and Leydig cells (LCs) arise from a common progenitor and are dependent upon activation by platelet derived growth factors (PDGFs). This study set forth to generate myoid cells from human induced pluripotent stem cells (hiPSCs).

Methods: To derive PTMs from hiPSCs (hPTMs), hiPSCs were subjected to a LC differentiation protocol, with PDGFbb used in place of the PDGFaa typically used in LC protocols. Gene and protein expression were analysed by real-time polymerase chain reaction (RT-qPCR) assays and immunocytochemistry (ICC).

Results: Our findings show that hPTMs upregulated expression of smooth muscle genes alpha smooth muscle actin (ACTA2) and myosin heavy chain actin 11 (MYH11), 23-fold (18–92) and 52-fold (41–209), compared to the hLCs control condition (Fig. 1A). Compared to Sertoli cells, hPTMs possessed higher expression of MYH11 by 124-fold (99–500), while hLCs possessed higher expression of HSD3β and AR, by 40-fold (24–61) and 3.2-fold (2.2–8.1) (Fig. 1B). The establishment of PTM vs LC fates was further confirmed by morphological examination (Figs, 1C, 1D) and ICC for HSD3β and MYH11 (Figs. 1E–H).

Conclusions: This study describes a method for generating PTMs from hiPSCs. These cells will allow for ongoing understanding of the cellular interactions required for normal spermatogenesis in an in vitro setting.



UP-164. Fig. 1. hPTMs derived by PDGFbb activation. ***(A)*** Fold change in smooth muscle gene ecpression of hPTMs compared to hLCs. ***(B)*** Fold change in smooth muscle (MYH11) and steroidogenic gene expression (HSD3ß and AR) of hPTMs and hLCs compared to Sertoli cells. ***(C)*** Phase contrast image of PTMs morphology. ***(D)*** Phase contrast image of hLCs morphology. ***(E-H)*** Immunocytochemistry images of HSD3ß expression and MYH11 expression in hPTMs and hLCs. *Statistical significance.