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Invited Review

Basic science and translational research: recommendations from the Fifth International Consultation for Sexual Medicine (ICSM 2024)

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Abstract

Introduction: Sexual function is a critical issue for human health and impacts the quality of life of patients and their partners. In this ICSM report, basic science and translational perspectives have been examined from the past decade of literature since the last ICSM report, and have been integrated to produce a state of the art summary of the physiology and molecular biology of sexual function/dysfunction and development of novel nanotechnology-based vehicles and treatments to aid regeneration and clinical translation in men and women.

Objectives: Examine, critically assess, and curate the most important and impactful basic and translational research findings on male and female sexual dysfunction since 2015.

Methods: Literature reviews were performed by a multidisciplinary committee of sexual medicine experts between June 2023 through May 2024. Findings were presented at the ICSM meeting in Madrid (June 2024), and comments from the consultation were incorporated to develop this consensus report.

Results: Erectile dysfunction (ED), which accompanies prostatectomy, diabetes, aging, and vascular disease in men, develops through both common and distinct mechanisms that involve neural injury, penile remodeling (smooth muscle (SM) apoptosis and increased collagen/fibrosis), dysregulated SM contractility, increased oxidative stress, immune response, and genomic instability. In women, disorders of genital pain, arousal, sexual desire, and orgasm involve multiple, overlapping neurological and endocrine mechanisms. Research on ED has been more extensive and the underlying molecular mechanisms have been better characterized than female sexual dysfunction. Future research directions should focus on pathways that underlie penile tissue remodeling and fibrosis associated with cavernous nerve injury in prostatectomy and diabetes, since this leads to irreversible ED. Particular emphasis should be placed on therapeutic targets to improve/enhance nerve regeneration, neuroprotection, "on demand" sexual function, SM contractility/relaxation, oxidative stress, immune response, and hormone function. In women, despite the existence of approved and off-label treatments for disorders of sexual desire and orgasm, the greater influence of psychosocial factors for these aspects of sexual function demands a multidisciplinary approach, along with predictive animal models. Genome-wide association studies have great potential in advancing the field but require replication and functional validation of findings from bioinformatic analyses. Progress in nanotechnology and regenerative therapies offers an exciting frontier in the targeted delivery of ameliorative/restorative treatments.

Conclusions: Research in sexual medicine has expanded through accelerated rates of discovery and increased breadth and diversity. However, much work remains in translating preclinical findings into biomarkers and clinical therapies that can improve patient outcomes.

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Summary/Recommendations

1) Significant advances in basic and translational research in sexual medicine have occurred over the past decade. A central underlying mechanism in prostatectomy, diabetic, and aging erectile dysfunction (ED) patients is cavernous nerve (CN) injury-induced penile remodeling.
2) Substantial strides have been made developing nanotechnology-based delivery vehicles for corpora cavernosa and CN regeneration that enable site-directed delivery and avoid systemic side effects; these innovative delivery vehicles are critical for the development of neuroprotective and neuroregenerative treatment strategies.
3) Progress in elucidating genomic mechanisms underlying ED (tissue remodeling, smooth muscle contractility, immune response) may lead to future targeted therapies.
4) Progress in understanding the mechanisms associated with vasculogenic, immune and aging-related ED require further investigation but offer potential for early-stage ED interventions.
5) The value of shockwave treatment remains unclear due to a lack of consensus regarding its effectiveness despite it being well utilized in clinical practice.
6) The clinical utility of stem cell therapy has been limited by the inability of stem cells to remain within the tissue at the site of delivery; migration to other organs presents potential risk.
7) In women, disorders of genital pain, sexual arousal and orgasm have multiple, overlapping neurological and endocrine components that are being actively characterized.
8) Several lines of research applied mostly to women may also be of benefit to male sexual dysfunction that go beyond ED.
9) Due to the complexity of interacting systems of male and female sexual dysfunction, it is likely that multiple combined strategies will be needed for prevention and treatment.
10) While established therapies play a significant role in managing sexual dysfunctions, there are currently no clinical therapies that delay or change the progression of any sexual dysfunction. However, many independent lines of research may positively impact disease progression and prevention.
11) While substantial barriers to clinical translation exist, current basic science research efforts will likely provide more opportunities than ever before to develop disease-specific treatments and prevention strategies that have been lacking in the field.

Keywords: erectile dysfunction; nanotechnology; tissue remodeling; immune response; dyspareunia; hypoactive sexual desire disorder.

Introduction

The last International Congress on Sexual Medicine (ICSM) occurred in 2015, and the basic science portion focused primarily on animal models for studying sexual dysfunction. The Basic Science and Translational Research Committee of the 2024 ICSM, was tasked with analyzing and highlighting the most novel and impactful basic and translational research that was published since the last congress. The area of focus is on the molecular mechanisms that underlie erectile physiology, development of normal and abnormal tissue morphology, and sexual dysfunction. Advancements in experimental models and analytical capabilities, identification of novel therapeutic targets, increased interdisciplinary collaboration, and cross-fertilization of investigative and therapeutic approaches have broadened the scope of viable treatments for a variety of sexual dysfunctions in men and women. In this expert consensus review, we have summarized important developments in the past 10 years and critically assessed relevant laboratory and clinical research findings, resulting in recommendations for future directions of research, and development of innovative therapies.

Methods

A multidisciplinary team of sexual medicine experts was convened in June 2023. Committee members were chosen based upon area of expertise, body of work/publications, and contributions to the field of sexual medicine. Each committee member was assigned a topic and was tasked with reviewing and evaluating the basic and translational sexual function and dysfunction research that was published during the last 10 years. Using relevant keyword literature searches in PubMed, Web of Science, Scopus, and Embase, each researcher was asked to highlight and curate the most significant and impactful research within their areas of expertise, which include: post-prostatectomy ED and nanotechnology, ED and diabetes, SM dysfunction and ED, aging and ED, immune response and ED, stem cell and shockwave therapy for ED, female sexual pain, arousal, and desire disorders, and orgasm disorders. A consolidated report of the committee's findings was presented at the ICSM conference in Madrid in June 2024 and comments from the Consultation were incorporated into this summary so that the review represents an expert consensus of the field of sexual medicine. The modified Delphi method was used to achieve consensus on findings and recommendations. [1,2] As this is a review primarily of basic science and translational research, rather than clinical practice, we did not include any graded recommendations in this report.

Results

Male sexual function/dysfunction

Erectile dysfunction (ED), the inability to achieve or maintain an erection, has high impact on the physical and mental health of men and their partners. ED affects ~50% of men between the ages of 40 and 70[3] and 22% of men under 40.[4,5] ED is common as men age, and risk factors include prostate cancer treatment, diabetes, hypertension, cardiovascular disease, smoking, and medication side effects. Phosphodiesterase type 5 inhibitors (PDE5i), vasodilators, vacuum devices and inflatable penile implants are commonly used to treat ED. While PDE5i are useful in some men, they are not very effective in patients with neuropathy of the cavernous nerve (CN, provides innervation to the penis), as occurs with prostatectomy, and in diabetic and aging men. Therefore, novel therapies are needed to help these difficult to treat patients.

A significant contributor to the development of ED is penile remodeling that occurs with loss of innervation. This can be an acute injury such as occurs during prostatectomy, or a slow development of neuropathy, as in diabetic and aging men. The

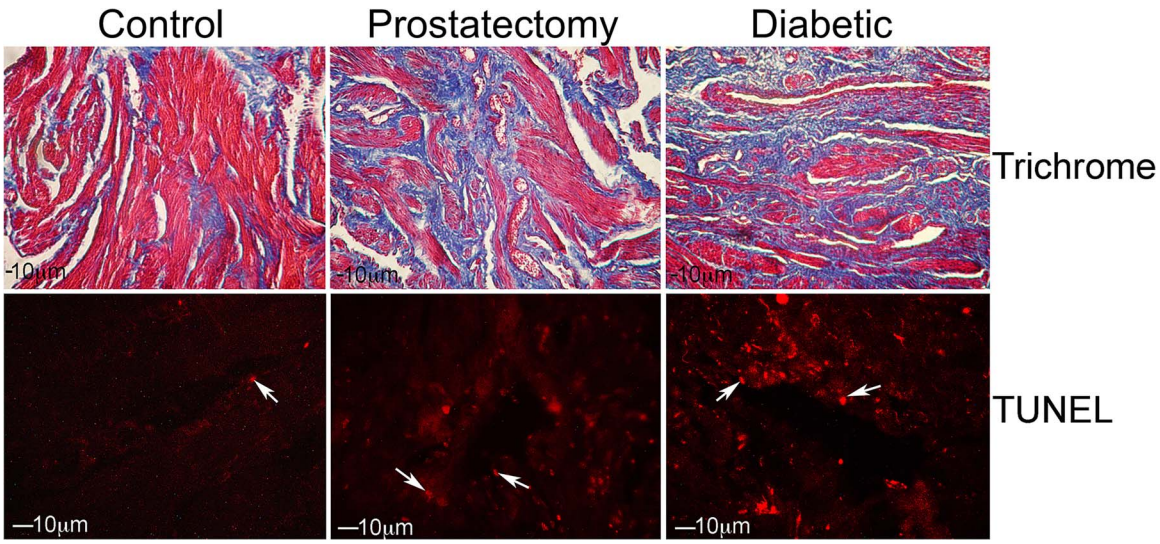

Figure 1. Penile remodeling in corpora cavernosa tissue of prostatectomy and diabetic patients in comparison to Peyronie's (control). Western analysis and trichrome stain show a 50% reduction in corpora cavernosa SM in prostatectomy and diabetic patients in comparison to Peyronie's controls. TUNEL assay shows that apoptosis remains elevated at a low level in prostatectomy and diabetic patients despite it being an average of 6 years (1–17 years) post prostatectomy and 12 years (7-24 years) since onset of diabetes. Reproduced from Angeloni et al.[6] Used with permission.

remodeling process includes apoptosis of corpora cavernosa smooth muscle (SM) and endothelium, followed by increasing collagen[6] (ie, fibrosis; Figure 1). The architectural changes in ED patients are significant with ~50% loss of corpora cavernosa SM in prostatectomy and diabetic patients.[6] The loss of SM and increased collagen make the corpora cavernosa less able to relax in response to neurotransmitter signals, and ED results. Many of the most impactful advances that have happened since 2015 focus on understanding and preventing the penile remodeling process. Priapism and Peyronie's disease are not addressed in this publication, as these are the purview of ICSM Committee 9 and Committee 10, whose reports will be published separately in Sexual Medicine Reviews.

Post-prostatectomy ED and nanotechnology (*Carol Podlasek, Ph.D.*)

Progress in understanding the mechanisms underlying the development of post-prostatectomy ED can be divided into three main avenues of exploration (68 publications since 2015): (1) Materials science/nanotechnology-based delivery vehicles for proteins/cells/genes to the corpora cavernosa to prevent penile remodeling (13 publications). (2) Factors that improve CN regeneration or are neuroprotective, and vehicles for delivery to the pelvic ganglia (MPG)/CN (9 manuscripts). (3) Genomic analysis via RNA sequencing or microarray to identify genes and pathways that change with CN injury in both patients (prostatectomy, Peyronie's) and rodent models (seven publications). Some issues were noted, and some exclusions were based on: (1) Animal models that were not adult (<115–120 days old); Developmental factors are upregulated during postnatal penile development and the results may not reflect signaling and mechanisms that take place in adult or aged rats.[7] (2) Manuscripts where the sympathetic innervation of the dorsal nerve bundle was misinterpreted as parasympathetic innervation from the CN. (3) Manuscripts that utilized poor quantitative methodology for SM, collagen and apoptosis analysis, or did not compare the same region of the penis.[8]

Nanotechnology

How to deliver proteins, inhibitors, and stem cells to the corpora cavernosa of the penis at the time of CN injury, to prevent penile remodeling, is challenging; however, significant advances have been made in animal models. Leading this area are development of peptide amphiphiles (PA; (C_{16})-$V_3A_3E_3$-COOH) that can self-assemble to form macroscopic hydrogels with proteins intercalated within the fibers as the gel forms.[9] PAs are a type of nanoparticle that can be combined as a liquid with the protein of interest, Sonic hedgehog (SHH), in a syringe, before adding $CaCl_2$ and immediately injecting the mixture into the corpora cavernosa of the penis where it forms an extended-release gel *in vivo*, which lines the sinuses of the corpora cavernosa.[9] The PA gel forms within a minute and releases protein as it breaks down over 7–14 days.[9] SHH protein treatment of the penis by PA suppresses apoptosis, preserves SM,[9] and decreases collagen.[10] The delivery methodology was optimized such that longer delivery with a second injection 5 days after CN injury, resulted in 110% more SM preservation than CN injured controls treated with an inert protein (mouse serum albumin).[11] Treatment of both the penis and CN further preserved penile SM 127%.[11] PA was used to deliver bone morphogenetic protein 4 (BMP4) and Gremlin1 (GREM1, BMP4 antagonist) to the penis to identify that part of the mechanism of how SHH regulates the balance of penile SM[12] and collagen[13] involves a switch in BMP4 and GREM1 signaling; BMP4 and GREM1 are responsive to changes in SHH signaling that occur with CN injury, and are downstream targets of SHH.[12] The novelty of the PA derives from its ability to form the hydrogel *in vivo* with extended release of protein directly to the SM target for preservation.

Development of other hydrogel vehicles are in progress. A heparin-pluronic hydrogel/gelatin-poly(ethylene glycol)-tyramine hydrogel has been used to deliver basic fibroblast

growth factor (bFGF) and nerve growth factor (NGF) to the penis of a CN injury rat model.[14] Apoptosis was suppressed synergistically with bFGF/NGF treatment and α-actin and CD31 were increased in the treated group relative to controls.[14] A benzaldehyde-terminated polyethylene glycol/glycol chitosan hydrogel is under development as a stem cell carrier and was applied to eight-week-old Sprague Dawley rats made diabetic with streptozotocin.[15] This type of hydrogel is formed outside the body and then injected into the penis. The corpora cavernosa tissue is likely to be damaged from injection of the already formed gel into the penis due to compression and sheer stress; however, this type of hydrogel has novelty in its capacity to reseal with stem cells within the hydrogel. With this methodology, stem cells were retained up to 14 days and the SM to collagen ratio was improved.[15] This type of hydrogel would potentially be applicable to a CN injury model. In a study by Lin et al., NanoShuttle magnetic nanoparticles were bound to adipose-derived stem cells.[16] When injected into the penis of 56-day old Sprague Dawley rats that underwent CN injury, the particles migrated toward an externally applied magnet and were retained within the corpora cavernosa for 3–5 days, with an increase in SM and endothelial markers noted.[16,17]

A nanoparticle hybrid hydrogel/glass (PEG, TMOS, chitosan, sodium nitrite, glucose, delivered as a suspension in carboxymethylcellulose), which releases encapsulated material with hydration, has made progress in delivering erectogenic agents (tadalafil, sialorphin, and nitric oxide [NO]) to the penis when topically applied, for on demand erectile function.[18] This technology has been further developed to topically deliver NO which when combined with orally administered PDE5i has a synergistic effect in eliciting an erectile response in a rat model of CN injury with significantly more spontaneous erections and faster onset for the first erectile response.[19]

CN regeneration and neuroprotection

Advances have been made in nanoparticle-based delivery vehicles to the CN/MPG of animal models of CN injury. A sprayable adhesive hydrogel, composed of gelatin, adenine, carbon nanotubes and mesaconate, is being developed for repair of the CN. The hydrogel has conductive properties allowing for an electrical bridge to be formed with the damaged nerve.[20] A second delivery vehicle, a linear PA hydrogel ((C_{16})-$V_2A_2E_2$-(NH_2)) with the SHH protein intercalated between the fibers, has been developed which promotes CN regeneration,[21] is neuroprotective,[22] promotes neurite formation in adult[23] and aged[24] rats, and the mechanism of how SHH promotes CN regeneration has been examined.[25] The linear SHH PA is unique in that it is formed by extruding PA and SHH protein onto a glass slide containing $CaCl_2$, which triggers gel formation. The linear gel that is formed is highly flexible and can be moved easily onto the CN and MPG to deliver SHH protein in an extended-release manner over 3–5 days. In addition to the effects of SHH protein in the CN/MPG, the linear shape of the PA, with the PA fibers aligned in one direction, may be advantageous for regeneration of CN fibers. A third approach includes small interfering RNA (siRNA) wafers that have been developed to inhibit fidgetin-like 2 in the CN/MPG to enhance CN recovery after injury.[26] A fourth approach involves injectable thermosensitive hydrogels composed of hydroxyethyl chitosan/sodium β glycerophosphate, which contain adipose derived stem cells derived from exosomes, and are being developed for treatment of CN injury.[27] Oxygen releasing microparticles are also under development to enhance erectile function in a rat CN injury model.[28]

Genomic studies

Significant advances have been made in utilizing microarray and RNA sequencing technology to identify genes that play a role in maintaining erectile function and in the development of ED. Very few genetic studies were performed prior to the last few years because of the large amounts of data acquired, and the difficulty of data analysis. These studies included a microarray analysis of corpora cavernosa from CN resected rats[29] and from diabetic rat penis.[30] The first analysis of corpora cavernosa from prostatectomy and Peyronie's (control) patients, was performed and compared to a rat CN resection model.[31] Ingenuity Pathway Analysis (QIAGEN) identified altered signaling in pathways involving embryonic development, connective tissue development and function, skeletal muscle development and disorders, tissue and cellular development, cell survival and death, neurobiological disease, immune response, cell movement, cell cycle, cell function and maintenance, growth and proliferation, and cell to cell signaling (Figure 2).[31]

A seminal manuscript examined a large scale genome-wide association study of ED in 36 649 men in the multiethnic Kaiser Permanente Northern California Genetic Epidemiology Research in Adult Health and Aging Cohort.[32] A single locus (rs17185536-T) on chromosome 6 near the single-minded family basic helix–loop–helix transcription factor 1 (SIM1) gene was identified that was significantly associated with the risk of ED.[32] The significance of *SIM1* is its established role in body weight homeostasis and sexual function as part of the leptin-melanocortin system.

A plethora of microarray and RNA sequencing manuscripts have examined changes in gene expression in CN injury rat models. Surprisingly, inflammation was not identified at 6 hours after CN injury, but a later inflammatory response was observed at 3 days after injury.[33] A comparison of CN injury and diabetic changes were examined in animal models[34]; however, the rats examined were not adult (84 days old). Cldn4 over expression was identified to promote CN SM cell fibrotic response via the JNK signaling pathway; however, the rat model was only four weeks old.[35] A comparison of biomarkers was performed in two ED models based on Gene Expression Omnibus (GEO) database and NOS-cGMP-PDE5 pathway.[36] Whole-transcriptome analysis of nerve injury related ED pathogenesis was performed on rat cavernosum, and circ RNA-miRNA-mRNA networks were identified.[37] Long non-coding RNA (lncRNA) expression pattern and the lncRNA-miRNA-mRNA network was examined in a rat model of CN injury and ED[38]; however, complete denervation did not occur in the rat model since CN injury was performed less than 5 mm from the MPG. Changes in microRNA expression in the corpora cavernosa of a rat CN injury model were examined.[39]

Conclusions

In summary, significant advancements have been made in preventing corpora cavernosa remodeling that occurs with CN injury, development of delivery vehicles for proteins, inhibitors, and stem cells to the penis to preserve penis morphology, identification of targets important for CN

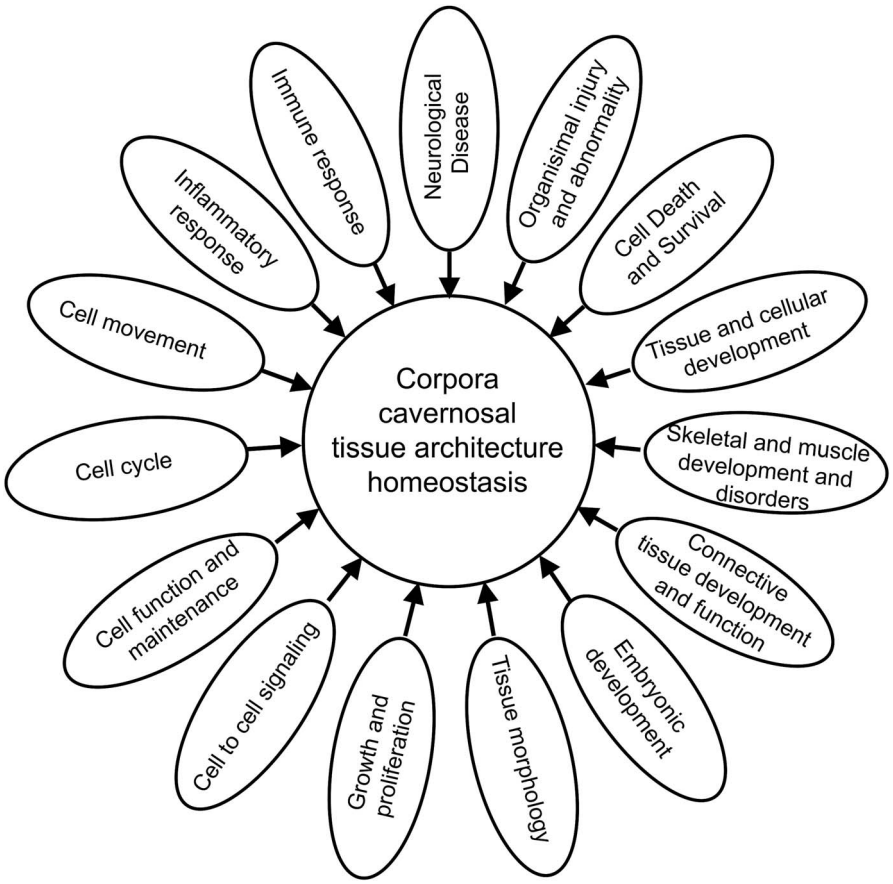

Figure 2. Analysis of altered pathways identified with Ingenuity Pathway Analysis (QIAGEN, Inc.) in corpora cavernosal tissue from patients with a prostatectomy and erectile dysfunction in comparison with Peyronie's disease controls. Ingenuity software was used to examine the Disease and Function databases. Reproduced from Searl et al.[31] Used with permission.

regeneration and neuroprotection and delivery vehicles for the novel targets, and therapies targeted for improvement of on-demand erectile function. These areas of research focus have been most impactful and future research in these directions would have the most significant translational/clinical impact.

ED and diabetes *(Hunter Wessells, M.D.)*

The burden of ED is significant in men with diabetes, who have higher risk and earlier onset compared to other etiologies.[40] Despite great progress in our understanding of the pathophysiology of diabetes mellitus-associated ED (DMED), it remains highly prevalent, resistant to first line therapies, and without interventions that alter the course of its onset or inexorable progression. Precision approaches to the diagnosis, treatment and prognosis of diabetes have emerged as important concepts.[41] A more precise strategy to predict and prevent complications has the potential to greatly reduce disease burden and distress and improve quality of life for all persons with diabetes, including those with ED. Analysis of the German Diabetes Study showed variation in the rate of ED based on type of DM and severity (see Figure 3).[42] ED heritability has also been elucidated. Investigators used genome-wide association studies (GWAS) to identify a risk locus for ED implicating hypothalamic sim1/leptin/melanocortin neurobiology and diabetes in its etiology.[43] These examples along with epidemiological studies in men with longstanding diabetes[44] foreshadow the ability to predict the onset of ED and identify individuals eligible for future intervention and/or prevention trials.

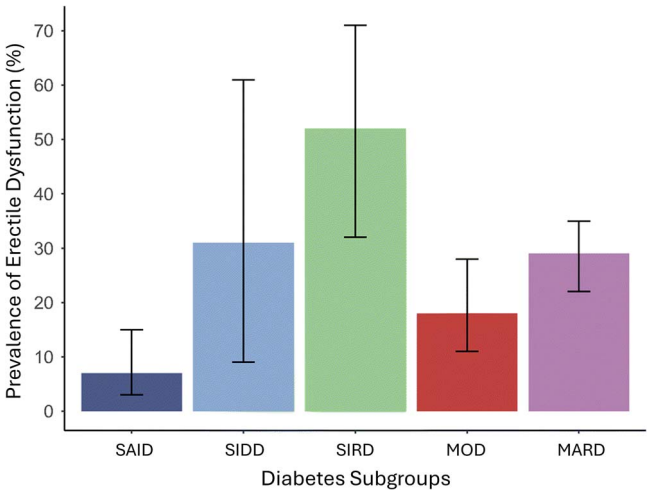

Figure 3. Prevalence of erectile dysfunction across subgroups of recent-onset diabetes from the German Diabetes Study. Data are given as percentages and corresponding 95% confidence intervals. SAID = severe autoimmune diabetes; SIDD = severe insulin-deficient diabetes; SIRD = severe insulin-resistant diabetes; MOD = mild obesity-related diabetes; MARD = mild age-related diabetes. Reproduced from Maalmi et al.[42] Used with permission.

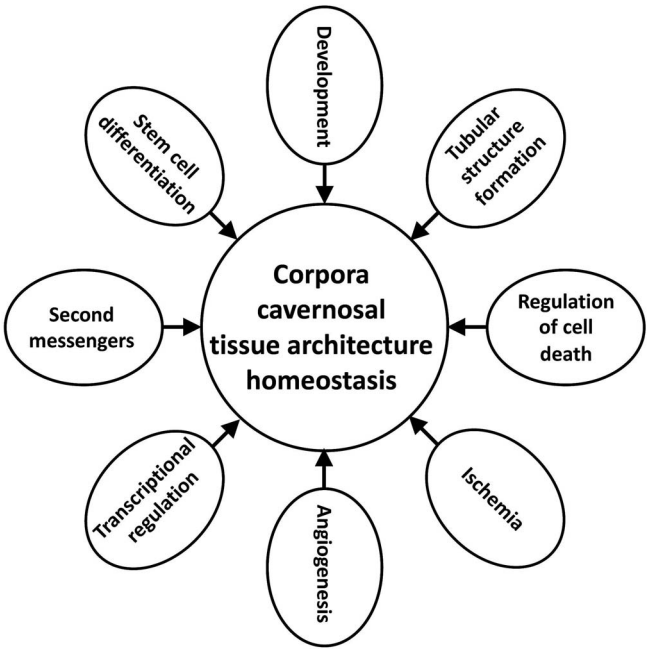

Figure 4. Pathway analysis identified altered signaling in corpora cavernosa tissue from diabetic patients with ED in comparison to Peyronie's (control) patients.

However, an important gap in DMED exists between extensive basic science findings and a lack of actionable targets for human studies to delay the onset, alter the disease progression, or restore normal functioning of the corpora cavernosa. Advances have been made in our understanding of the role of glycosylated hemoglobin, hypogonadism, neuropathy, the role of inflammation and endothelial dysfunction, advanced glycation end products, increased free oxygen radicals, impaired NO synthesis, upregulation of the RhoA/Rho kinase pathway, and impaired cGMP-dependent protein kinase-1 expression in the development of ED. Further study is required for translation of these basic science findings.[45,46]

The complex pathophysiology of ED patients with diabetes has been elucidated recently by RNA expression studies from human subjects. Podlasek and associates found that men with DMED incur significant loss of normal regulatory processes required for repair and replacement of injured corpora cavernosa tissue.[47] Combined with loss of apoptotic regulatory mechanisms, this results in significant architectural remodeling of the corpora cavernosa, and loss of regenerative capacity in the penis (see Figure 4). The promise of stem cells, shockwave therapy, and other treatments with restorative potential are especially relevant to diabetic patients and are covered in more detail in other areas of this report.

Fortunately, technological innovations over the past 10 years have advanced translational ED research. These include better models of type 2 diabetes,[48] use of single cell RNA sequencing to characterize penile cell populations,[49] and cell-specific inducible gain and loss of function mouse models.[50] In our literature review, we sought to identify mechanistic opportunities that were: (1) discovered through in vivo animal models; (2) specific to DMED; and (3) associated with therapeutic targets. We believe these have potential to yield progress towards treatment intervention or prevention strategies relevant to persons with diabetes.

Table 1 summarizes progress since 2015 along multiple lines of *in vivo* animal investigation of DMED, organized by cavernosal cell type. The importance of pericytes (mural cells) and fibroblasts to normal corpus cavernosa homeostasis has recently become recognized.[50,51] Complementary work on the contribution of disrupted pericyte function to ED mechanisms, as well as potential therapeutic effects to ameliorate DMED through Wnt signaling antagonism, hold promise.[51-53] Established endothelial signaling pathways (eg, adenosine) are being targeted by novel agents with anti-inflammatory and regenerative properties such as CF602.[54] Beneficial effects through Ang-1 and Tie-2 have been demonstrated using RNA interference to IGFBP5,[55] cholesterol derivatives,[56] and pericyte derived DKK2.[53] Although another section of this report covers smooth muscle contractile elements in great detail, four signaling pathways listed in the table have been identified as contributing to DMED and/or through inhibition, ameliorating DMED.[48,57-60] Finally, DMED includes a significant component of neural degeneration that may or may not be reversible.[62] Thus, neural protection or regeneration[13,47,61] will ultimately be required for some of the advanced forms of DMED that involve corporal remodeling and structure changes listed above.

Central to several of the above mentioned therapeutic innovations is the impact on oxidative stress, systemic and vascular inflammation, and fibrosis. Because these pathophysiological mechanisms are shared across various etiologies of ED and may be operational early in the pathophysiology of DMED, the potential to apply them as interventional or prevention therapies in high risk or early ED provides a novel prospect that we could shift the curve so that fewer men develop ED or suffer its most severe and irreversible phenotypes. Although no treatments currently have been shown to alter the course of ED, in other chronic diseases, endophenotyping has been proposed to improve precision approaches to conditions such as chronic obstructive pulmonary disease and depression.[63,64]

Conclusions

Diabetes impacts all components of penile erectile physiology, explaining both the severity of DMED and the range of targets for potential translation. Novel cellular and molecular mechanisms of penile health and dysfunction overlap with other pathophysiologies and offer developmental leads for treatment, intervention or prevention strategies. However, complementary work in largescale human cohorts is required to define ED subtypes and a precision approach to DMED.

ED and smooth muscle dysfunction *(Michael DiSanto, Ph.D.)*

Penile erection involves relaxation of the corpus cavernosum SM (CCSM); however, it is the SM contracted, flaccid (non-erect) state in which the penis exists most of the time.[65] Although contraction of bulbocavernosus and ischiocavernosus skeletal muscles can further increase erection rigidity, this section focuses on recent advances in CCSM dysfunction research. Penile CCSM contractility is regulated by SM myosin light chain kinase (MLCK) which phosphorylates the regulatory light chain of SM myosin, increasing CCSM contractility (Figure 5). SM myosin light chain phosphatase

Table 1. Target cells and molecular pathways implicated in experimental in vivo models of diabetes associated ED.

Target Cell	Molecular Pathway in DMED (Specific Target)	Potential Therapeutic Strategies	References
Pericyte			
	Wnt signaling (DKK2)	HGF, DKK2 protein Pericyte derived EVs	51-53
Endothelium			
	Adenosine (CD73)	CF602	54
	Angiogenesis (Ang-1/Tie-2)	shIGFBP5, Sac1004 DKK2 protein	53, 55,56
Smooth Muscle			
	Sphingosine-1-Phosphate (S1PR2)	JTE-013	57
	Renin Angiotensin System (MAS1)	AVE0991 Ang-[1-7]	58, 59
	Calcium Channel (Kca1.1)	—	48
	Toll Like Receptors TLR-4	CLI-095	60
Neuron	Neuronal regeneration (SHH, Gremlin)	Shh nanoparticles	13, 47,61

EV: extracellular vesicles. DKK2: Dickkopf WNT Signaling Pathway Inhibitor 2. CD73: ecto-5'-nucleotidase. shIGFBP5: short hairpin RNA interference Insulin-like growth factor 5. MAS1: Mas receptor. TLR-4 Toll like Receptor 4. S1PR2: Sphingosine-1-Phosphate Receptor 2. SHH: sonic hedgehog.

(MLCP) dephosphorylates the regulatory light chain, decreasing contractility.[66] Knockout mice with reduced expression of myosin phosphatase target subunit 1 have impaired erections, as shown by reduced intracavernous pressure in response to electrical field stimulation.[67] Rho-kinase phosphorylates and inhibits MLCP, which then allows MLCK to be more effective in promoting phosphorylation of MLC, resulting in SM contraction (termed "calcium sensitization").[68] Stress (a known risk factor for ED) has been shown to upregulate Rho-kinase, which would be expected to cause CCSM contraction leading to ED.[69] In addition, transfection of miRNA-200a into CCSM cells significantly increased expression of RhoA and the Rho-kinase isoforms ROCK1 and ROCK2.[70] These studies directly support therapeutic targeting of the enzymes that regulate phosphorylation levels of SM myosin regulatory light chains to improve erectile function.

Sphingosine 1-phosphate (S1P) is a naturally occurring bioactive sphingolipid. Once phosphorylated, S1P is recognized by a family of G-protein coupled receptors (S1PR1 – S1PR5) that regulate downstream effectors.[71] S1PR1, S1PR2 and S1PR3 are predominant in SM including CCSM. S1PR2 and S1PR3 receptors signal downstream via the RhoA/Rho-kinase pathway. The S1PR2 antagonist JTE-013 has been shown to improve ED in rats with streptozotocin (STZ)-induced type I diabetes via inhibition of the rho-kinase pathway, fibrosis, and apoptosis.[57] Hypertension may also impair erectile function by means of downregulating the expression of S1PR1 (eNOS activator) and upregulating the expression of S1PR2 and S1PR3 (RhoA/Rho-kinase activators) in cavernous tissues of spontaneously hypertensive rats (SHR).[72] Further evidence supporting the role of S1PRs is derived from studies that demonstrated improved erectile function of SHRs after delivery of S1PR3 siRNA[73] or S1PR1 gene transfection.[74] Taken together, the S1P/S1PR/RhoA/Rho-kinase signaling pathways appear to provide a wealth of new molecular targets for treatment of ED.

The interplay between CCSM cell death and cell proliferation is crucial for maintaining proper erectile function, and the molecular programs regulating these processes are highly conserved.[75,76] Exogenous caveolin-1 scaffolding domain administered to rats with bilateral CN injury significantly prevented ED at the molecular level, decreasing oxidative

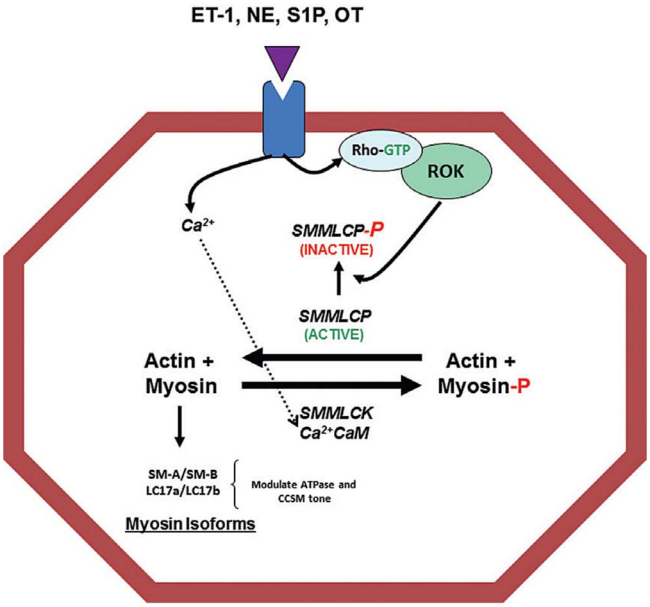

Figure 5. Regulation of corpus cavernosum smooth muscle contractility. In response to the various contractile stimuli (endothelin-1[ET-1], norepinephrine [NE], sphingosine-1-phosphate [S1P] and oxytocin [OT]) working through their respective receptors, an increase in intracellular calcium occurs and/or the RhoA/rho-kinase (ROK) pathway can be activated. Free calcium binds with calmodulin (CaM) and activates smooth muscle myosin light chain kinase (SMMLCK) which phosphorylates the regulatory 20 kDa light chain (MLC20) on smooth muscle myosin to generate force. Activation of the RhoA/rho-kinase pathway involves RhoA moving to the cell membrane and associating with ROK wherein ROK is activated. Activated ROK directly phosphorylates the smooth muscle myosin light chain phosphatase (SMMLCP) which inactivates it and allows the SMMLCK to work with less opposition favoring SM contraction. Reproduced from Zhang et al. J Sex Med 2011;8:1865–1879. Used with permission.

stress, Bax/Bcl-2 ratio, apoptosis, and the levels of caspase3.[77] In addition, the cytokines BMP4 and GREM1 have been identified in rat and human CCSM.[13] CN injury-induced ED is associated with an increase in collagen and BMP4 and GREM1 form part of the mechanism of how SHH suppresses collagen induction following CN injury.[13] Moreover, treatment with BMP_4 increased human CCSM cell growth while GREM1 decreased cell growth.[12] Also, in rats with CN injury, exogenous hydrogen sulfide inhibited the phenotypic modulation of CCSM cells by suppressing RhoA/ROCK1 signaling and affecting a number of its downstream targets[78] while also inhibiting apoptosis of CCSM cells.[79] Subsequently, it was discovered that ED was associated with a defective L-cysteine/hydrogen sulfide pathway in both the corpora cavernosa and penile arteries in patients.[80]

Autophagy, which recycles selective intracellular organelles and molecules, rather than whole cells, has been increasingly examined over the past decade for a role in erectile function.[81,82] Vitamin D_3, nitro-oleic acid (NO_2-OA), tankyrase 1 and human tissue kallikrein 1 all improved erectile function recovery in various *in vivo* rat models of ED by enhancing autophagy.[83-86] In contrast, certain processes/agents (hyperlipidemia and 3-hydroxy-3-methylglutaryl-CoA synthase 2) attenuated erectile function by decreasing autophagy.[87,88] Additionally, *in vitro* culture of CCSM cells with glucose within a certain range of concentration can promote autophagy, but at too high a glucose concentration it can reduce autophagy and with rapamycin further increasing autophagy.[89]

Myostatin is a cytokine that is a negative regulator of myoblast proliferation and differentiation. Although originally thought to be exclusively expressed in skeletal muscle, expression in penile and vascular rat SM was reported in 2017.[90] More recently in 2022, when myostatin was silenced in pigs, the area fraction of SM in the corpora cavernosa was increased from 41.7% to 65.9%, suggesting myostatin as a novel target in treating SM dystrophy-related dysfunction.[91] Guo et al. provided novel data that bilateral CN injury increased the protein levels of GRP78 (a master regulator of endoplasmic reticulum stress (ERS) and decreased the protein levels of α-SM actin.[92] Moreover, 4-phenylbutyric acid (4-PBA), a potent ERS inhibitor, improved EF by inhibiting the level of ERS-related proteins and increasing the protein level of α-SM actin, thereby providing a new target and theoretical basis for the clinical treatment of CN injury-induced ED.[92]

Stem cell therapy has been tried for many years as a restorative method for return of erectile function in animals, but only limitedly in humans.[93] More recently, newer combinational therapies have emerged. Yilmaz-Oral et al. in 2024 employed a dual strategy of adipose-derived stem cells (ADSCs) and L-arginine in a rat radical prostatectomy model and found the decreases in SM mass and NADPH-diaphorase-positive nerve fibers were partially ameliorated by monotherapy, whereas combined therapy led to recovery.[94] Shin et al. reported in 2021 that extracorporeal shockwave therapy combined with engineered mesenchymal stem cells expressing stromal cell-derived factor-1 can improve ED in STZ-induced diabetic rats.[95]

Mesenchymal stem cell-derived exosomal miR-296-5p and miR-337-3p improved ED via the PTEN/PI3K/AKT pathway leading to an improved ratio of SM to collagen.[96] A human study confirmed the feasibility and safety of minimally invasive, same-day delivery of ADSCs with 3/10 men achieving an improvement equal to or greater than the minimal clinically important difference according to their baseline IIEF-EF score[97] while in a separate study intracavernosal injection of stem cells improved sexual function and peak systolic velocity and resistance indices of penile arteries in diabetic patients, however did not reach significance.[98] Clearly, the implementation of stem cell therapy for ED in patients has been slow to date.

Finally, circadian rhythm disorders, also known as sleep–wake cycle disorders, can affect multiple organ systems and even individual cells.[99] Evidence has been growing that the penile erection may be influenced by our circadian clock.[100] Alkan et al. provide evidence that core molecular clock genes are distinctly expressed in penile tissue in patients including both vascular endothelial cells and SM cells.[101] Specifically, BMAL1, CLOCK, PER1, PER2, PER3, CRY1, and CRY2 were expressed. Molecular manipulation of these proteins may provide a new avenue for treatment of ED.

Conclusions

ED remains a multifaceted condition influenced by an intricate interplay of molecular, cellular, and systemic mechanisms. Recent discoveries and advances in our understanding of penile CCSM contractility, the RhoA/Rho-kinase pathway, S1P receptor signaling, autophagy, and stem cell therapy have identified numerous potential therapeutic targets and delivery systems for improving erectile function. Emerging evidence also substantiates a critical role for oxidative stress, apoptosis, cellular pathways like the L-cysteine/hydrogen sulfide pathway, ER stress, and myostatin signaling in the regulation of CCSM structure and function. Finally, novel approaches such as gene manipulation, combinational stem cell therapies, and circadian rhythm modulation provide promising avenues for future treatments. However, while preclinical studies have laid the groundwork for many of these innovations, the translation of these findings to effective, safe, and widely available therapies in patients remains a significant challenge. Continued research and clinical trials are essential to bridging the gap between experimental insights and therapeutic implementation, ultimately improving outcomes for patients suffering from ED.

ED, aging, and fibrosis *(Monica Ferrini, Ph.D.)*

Age-related ED is caused by multiple factors, with veno-occlusive dysfunction being the clinical manifestation.[102] It is believed that oxidative stress, mitochondrial dysfunction, impaired resistance to molecular stressors, genomic instability, and cellular senescence, among other factors, contribute to the pathogenesis of vascular aging and, consequently, age-related ED (Figure 6).[103]

During aging, the corpora cavernosa undergoes morphological changes[102,104] due to a decrease in the number of corpora cavernosa smooth muscle cells (CSMC)[105] and reduced endothelial cell functionality.[106] In addition, proliferation of fibroblasts and myofibroblasts leads to a change in the extracellular matrix composition, ultimately resulting in collagen deposition and fibrosis.[49] The alteration of the extracellular matrix components is associated with the accumulation of senescent cells that express factors linked to a senescence-associated secretory phenotype (SASP) that promotes age-related tissue dysfunction[107] and, hence, fibrosis. A similar mechanism occurs in CN injury and diabetes-associated neuropathy. The lack of innervation impacts RhoA/ROCK,

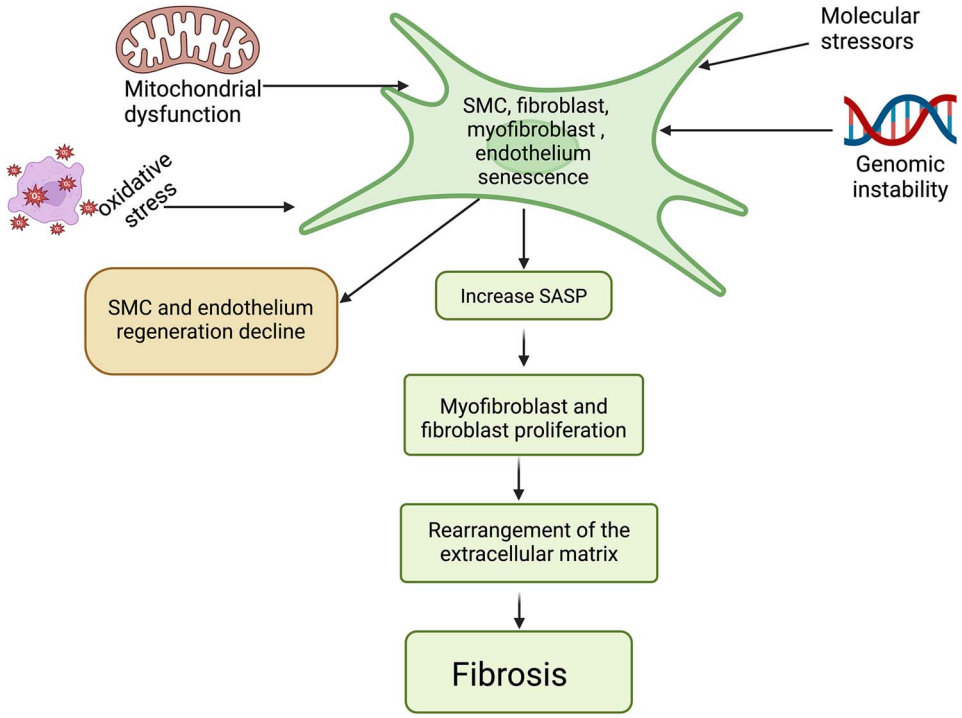

Figure 6. Pathogenesis of fibrosis-age-related erectile dysfunction. Cells in the corpora cavernosa become senescent due to factors like oxidative stress, mitochondrial dysfunction, impaired resistance to molecular stressors, and genomic instability. Senescent cells secrete factors associated with a senescence-related secretory phenotype (SASP), which leads to age-related tissue dysfunction by causing changes in the extracellular matrix and resulting in fibrosis. Created with Biorender.com.

sonic hedgehog,[108] and TGF-β/SMAD/CTGF[109] pathways, promoting CSMC apoptosis and penile tissue fibrosis. Lack of NO release by the CN can also lead to hypoxia-induced oxidative stress.

During senescence and neuropathy, CSMCs exhibit a heterogenous phenotype, switching from a contractile to a synthetic phenotype and expressing a combination of SMC and fibroblast markers such as iNOS,[110] CTGF,[111] TGF-β,[111] osteopontin, and Krüppel-like factor 4 (KLF4),[112] indicating a transition state from CSMCs to fibroblasts. In addition, apoptotic markers[113] and modification of microRNAs and long noncoding RNAs[108,114,115] are accompanied by a decrease in α-SM actin.[116] Fibroblasts exhibit the most heterogeneity among the cellular components of the aging corpora cavernosa. SASP genes such as CDKN2B, IGF1, MMP2, CTSK, CTSH, and IGFBP3 were highly expressed in old rat fibroblasts, revealing that fibroblast heterogeneity plays a role in the development of fibrosis associated with age-related ED.[117] Interestingly, siRNA's knockdown of IGFBP3[112] and KLF4 expression[118] increased CSMC viability and proliferation while decreasing apoptosis, oxidative stress, and fibrosis.

The leading cause of fibrosis in age-related ED is the rearrangement of the extracellular matrix. A study by Sun et al. using qPCR and bioinformatics showed that aged rats had upregulated levels of collagen (COL1A2, COL3A1, COL6A2) and biglycan, a proteoglycan, considered an essential gene regulating fibrosis.[113] Moreover, in aged rats, the content and length of elastin fibers are reduced, which decreases the compliance of the corpora cavernosa.[113] Senescent endothelial cells (ECs) also exhibit functional abnormalities, such as decreased endothelial nitric oxide synthase (eNOS) expression and downregulation of the PI3K/AKT pathway.[108,113] These changes are accompanied by an increased expression of pro-inflammatory molecules, such as interleukins IL-1α, IL-6, IL-8, and IL-17, prothrombotic factors (PAI-1, PAI-2), angiotensin II, and endothelin I. Endothelial dysfunction also contributes to the onset of corpora cavernosa fibrosis.[119]

An approach to mitigating fibrosis-associated age-related ED in animal models is to utilize PDE 5 inhibitors.[116,120] Advances in single-cell RNA sequencing have identified new biomarkers for treating fibrosis and restoring erectile function. Senolytic drugs have emerged as a new alternative for the treatment of fibrosis-associated age-related ED. They selectively induce anti-inflammatory effects and cell death in senescent cells, disrupting their pro-survival pathways. Quercetin has shown a promising impact in increasing NO and antioxidant enzymes while reducing arginase activity in the corpora cavernosa of cyclosporine-treated rats.[121] In a rat model of arterial endothelial dysfunction, quercetin improves erectile function and increases eNOS and iNOS mRNA and protein.[122] In addition, isorhamnetin, a metabolite of quercetin, has a protective effect on erectile function in diabetic rats by reducing inflammation, oxidative stress, corpora cavernosa fibrosis and improving endothelial function.[123] This effect may be linked to the activation of the PI3K/AKT/eNOS pathway. Moreover, a senolytic nutraceutical combination consisting of ginger rhizome, muira puama, *Paullinia cupana*, and L-citrulline (COMP-4) enhances intracellular NO, reduces fibrosis, and increases CSMC content, and has an antioxidant effect on the aging corpora cavernosa and peripheral vasculature.[124,125] In addition to senolytics, regenerative therapies utilizing stem cell therapy and platelet-rich plasma (PRP) show potential for restoring erectile function and reducing fibrosis (Figure 7).[126,127] These therapies are discussed in greater detail in the next section.

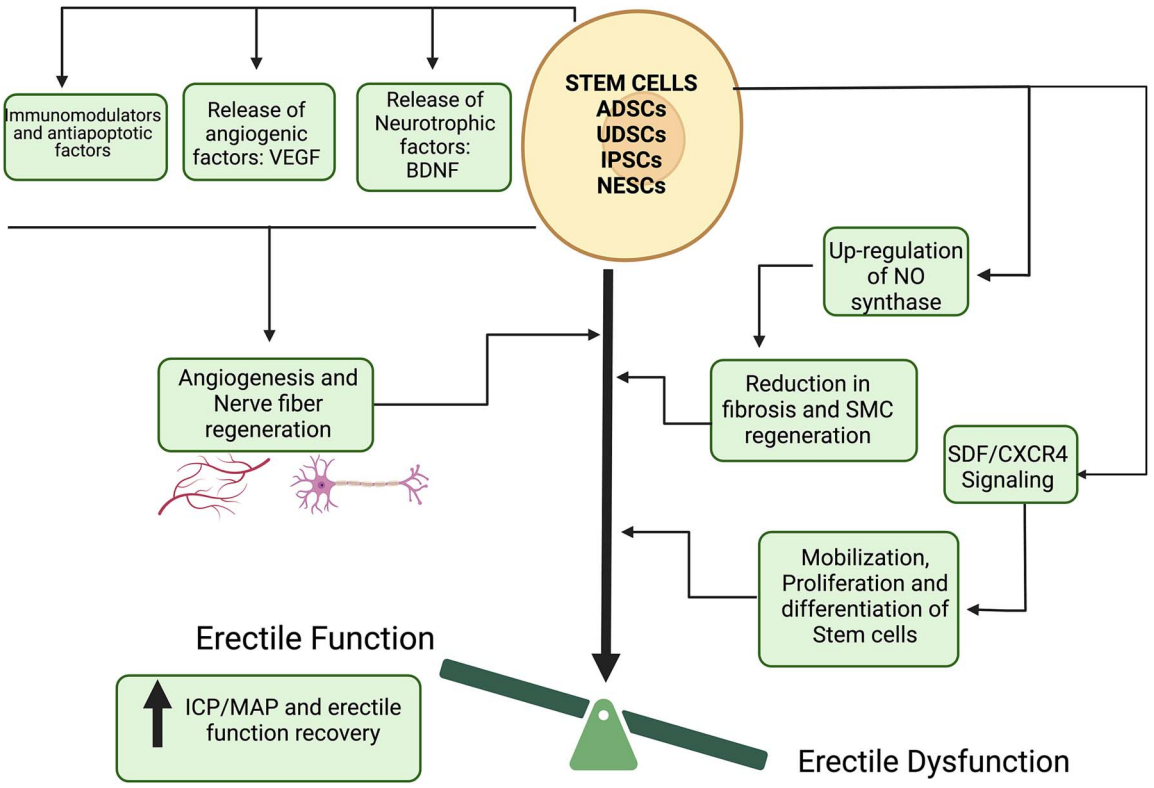

Figure 7. Mechanistic insight into stem cell-based therapy of ED. During repair and regeneration, the stem cells regulate signaling pathways such as SDF/CXCR4 to mobilize, proliferate, and differentiate stem cells into nerve and endothelial cells. Further, these cells also release growth factors such as VEGF and BDNF to promote angiogenesis and nerve fiber regeneration, respectively. Upregulation of NO synthase results in reduced fibrosis and an increase in smooth muscle cell content in corpus cavernosum. Eventually, the immunomodulatory and anti-apoptotic impact of stem cells controls any further injury to penile nerves or muscles. Thus, the cumulative effect of stem cells renders it a potent regenerative therapeutic candidate for ED. MAP = mean arterial pressure. Adapted from Liu et al.[126] Used with permission. Created with Biorender.com

Conclusions

Age-related ED stems from multiple factors. These factors drive structural and functional changes in the corpora cavernosa, including smooth muscle cell loss, fibroblast proliferation, extracellular matrix remodeling, and fibrosis. The accumulation of senescent cells plays a critical role in the development of fibrosis by releasing paracrine senescence-associated secretory phenotypes (SASPs, which drive cavernosal tissue remodeling. While PDE5 inhibitors have historically been the first-line treatment for reducing fibrosis, advancements in single-cell RNA sequencing have uncovered novel biomarkers that pave the way for innovative therapeutic approaches. Among these, senolytic drugs emerge as a promising alternative by reducing inflammation, oxidative stress, and fibrosis while improving nerve and endothelial function and, hence, NO production. The elucidation of the molecular pathways associated with senescence and fibrosis will provide a more comprehensive and targeted approach to managing age-related ED and improving patient outcomes.

ED, stem cell, and shockwave therapy (*Guiting Lin, M.D., Ph.D.*)

The advent of regenerative medicine has heralded a new era in medical care, introducing innovative treatments capable of restoring and revitalizing damaged tissues and organs.[128] Among these, stem cell therapy and shockwave therapy have emerged as potentially promising and rapidly evolving fields.[129,130]

Stem cell therapy

Current research primarily focuses on induced pluripotent stem cells (iPSCs), mesenchymal stem cells, adipose-derived stem cells, and tissue-resident stem cells (TRSCs).[130-132] The activity of stem cells is less dependent on "stemness" and more on the indirect paracrine effects mediated by the secretome of locally injected stem cells.[133-135] The efficacy of stem cell therapy hinges on several key mechanisms. Firstly, through differentiation, stem cells mature into specific cell types required for repairing and replacing damaged tissue. Secondly, stem cells exert their therapeutic effects by secreting growth factors and cytokines, thereby enhancing tissue repair and mitigating inflammation. Thirdly, stem cells can modulate immune system responses, rendering them valuable in scenarios involving compromised or hyperactive immune systems. Thus, stem cell therapy represents an approach to the treatment of ED with the potential to improve erectile function in patients who do not respond to traditional treatments.

In an analysis of nine publications on using stem cells for the treatment of ED in a total of 164 men, Siregar et al. noted various stem cells have been used.[136] They include mesenchymal stem cells, placental matrix-derived stem cells, mesenchymal stem cell-derived exosome, adipose-derived stem cells, bone marrow-derived mononuclear stem cells, and umbilical cord blood stem cells. The authors concluded that stem cell therapy

showed a good efficacy and safety profile, although not enough studies on the protocol, dosage, and mechanism of action have been conducted. In another review, Wang et al. also concluded that further research is needed to optimize the use of stem cells, stem cell products, or combination therapies for the treatment of ED and to determine the long-term safety and efficacy of this approach.[137] A key issue with stem cell treatments is that the stem cells do not stay where they are injected, but rather migrate to other parts of the body, particularly the lung. This is an issue that needs to be resolved prior to thinking about clinical translation.

Shockwave therapy

Since the pioneering application of shockwaves in the treatment of kidney stones in 1987,[138] this field has undergone rapid evolution, progressing from high-energy lithotripsy to its current role in low-intensity shockwave regenerative medicine. In 2010, Vardi et al. reported a pilot study of applying shockwave therapy to improve erectile function in men with vasculogenic ED.[139] Shockwave therapy finds extensive utility across various medical domains, encompassing musculoskeletal disorders, cardiovascular disease, wound healing, neurological applications, and extra-urological applications.[140-142] The safety and efficacy of shockwave therapy has been examined through several clinical studies and meta-analyses; however, further study is needed.[141]

Shockwave therapy capitalizes on the distinctive characteristics of shockwaves, characterized by rapid pressure changes, high peak pressure, small stretching amplitude, and brief duration to elicit biological effects within target tissues. The mechanisms of shockwave therapy have been postulated to involve four stages of action: propagation, physical effect, mechanotransduction (the interaction of shockwaves with biological tissues), and biological effects that include the activation of tissue resident stem cells, release of growth factors and cytokines, and stimulation of angiogenesis, promoting tissue healing and regeneration (Figure 8).[144]

In a recent meta-analysis that evaluated the efficacy of low-intensity extracorporeal shockwave therapy (Li- ESWT) in the treatment of ED in 16 randomized controlled trials including 1064 participants, the authors conclude that Li-ESWT could significantly increase International Index of Erectile Function score and Erection Hardness Score in ED patients, especially in the moderate ED group, but had no significant improvement in positive response rate of SEP2 and SEP3.[145] In general, Li-ESWT has become a popular choice for the treatment of ED because of its low risk, but more clinical experiments, longer duration of follow-up, and more detailed data are still needed to support potential efficacy.

Synergistic potential of combined stem cell and shockwave therapy

Stem cell therapy utilizes the ability of stem cells, particularly mesenchymal stem cells (MSCs), to differentiate into various cell types to achieve therapeutic goals for treating ED. At the molecular level, stem cells can differentiate into specific cell types necessary for tissue repair, such as endothelial cells or SM cells in the case of ED. Additionally, they can secrete paracrine factors, such as cytokines, growth factors (like VEGF and FGF), and anti-inflammatory molecules. However, stem cell therapy has significant limitations. Beyond ethical concerns, a major challenge is targeting and retaining stem cells to the injured or diseased site. Studies have shown that many injected stem cells migrate to non-target tissues, such as the lungs, shortly after administration, even with techniques like magnetic nanoparticles to anchor them.[146]

Many studies have demonstrated that there are overlapping mechanisms for restorative therapies including stem cell therapy, shockwave therapy, and PRP therapy.[147-149] Recent studies indicate that Li-ESWT can significantly increase VEGF levels in tissues, potentially offering therapeutic benefits for ED.[150] Some of the key signaling pathways that are targeted by these therapies include VEGF, transforming growth factor-beta (TGF-β), NO, stromal cell-derived factor-1, and MAPK. Several studies have investigated the combination of shockwave therapy with stem cell therapy for the treatment of ED. For example, a publication reported that combining shockwave therapy with ADSC therapy resulted in a significant improvement in erectile function compared to shockwave therapy alone.[151] Another study investigated the combination of shockwave therapy with bone marrow-derived stem cell therapy.[95] The study found that the combination therapy significantly improved erectile function and penile blood flow and resulted in the regeneration of penile tissue. The combination of shockwave therapy with PRP therapy has also been proposed as a potential treatment for ED.[147,148]

Conclusions

The integration of stem cell and shockwave therapy represents a novel paradigm in regenerative medicine, harnessing the distinct strengths of each modality to amplify treatment efficacy. While stem cell and shockwave therapy, as well as their integration, hold some promise in regenerative medicine, they also present significant challenges that must be addressed to fully realize their clinical potential. Collaboration among researchers, clinicians, and industry stakeholders will be pivotal in overcoming these challenges and realizing the full potential of regenerative medicine. Although combining stem cell therapy with shockwave therapy theoretically enhances tissue repair, the limitations of stem cell therapy remain. Even if shockwave therapy improves stem cell recruitment and angiogenesis, stem cell retention is still a critical challenge. Techniques such as hydrogels, scaffolds, and further optimization with nanoparticles may improve outcomes, but the tendency for injected stem cells to be absorbed by the lungs remains a major clinical obstacle.

ED and the immune response (*Fernanda Priviero, Ph.D.*)

The role of the immune response in the pathogenesis of vasculogenic ED has been increasingly recognized after a significant number of studies has shown that the activation of the immune system and the inflammatory response play a pivotal role in the development of endothelial dysfunction, and therefore, might contribute to ED. In the sexual medicine field, the insight was prompted in the mid-2000s by studies linking ED and sterile (non-infectious) inflammation independent of other comorbidities in patients[152,153] and rodents.[154] However, in the last decade, this idea has been reinforced by studies showing the association between ED and non-sterile (caused by microorganisms) inflammation such as periodontitis.[155-160] The occurrence of ED in patients with chronic periodontitis raises the hypothesis that the pathogenic

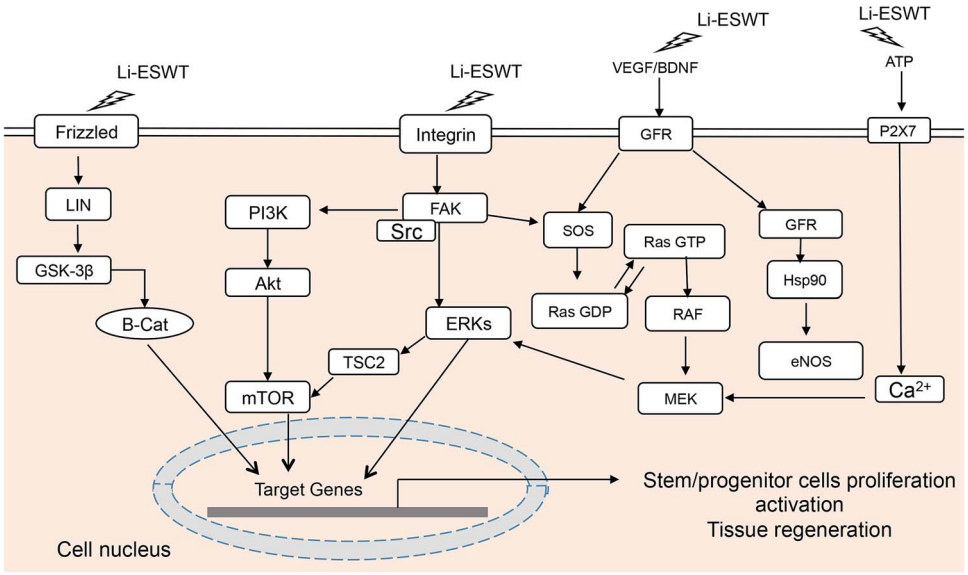

Figure 8. Cellular signaling pathways modulated by Li-ESWT. Li-ESWT = low-intensity extracorporeal shockwave therapy; Frizzled = receptor of WNT; BDNF = brain-derived neurotrophic factor; GSK-3β = glycogen synthase kinase-3 beta; β-cat = beta-catenin; PI3K = phosphoinositide 3-kinase; Akt = protein kinase B (PKB), also known as Akt; Src = sarcoma, a Protooncogene tyrosine-protein kinase; TSC2 = tuberous sclerosis complex 2; GFR = growth factor receptor; RAS = molecules of MAPK/ERK pathway; RAF = molecules of MAPK/ERK pathway; MEK = mitogen-activated protein kinase kinase; mTORC1 = mammalian target of rapamycin complex 1; FAK = focal adhesion kinase; VEGF = vascular endothelial growth factor; SOS = Son of Sevenless. Reproduced from Liu et al.[143] Used with permission.

invasion caused by the oral infection culminates in immune response activation, and the resulting systemic inflammatory state induces endothelial dysfunction, potentially contributing to the onset of ED.[161,162] In fact, this hypothesis is supported by other infectious conditions that either cause or aggravate the severity of ED, such as in diabetic patients with viral infection[163] or patients infected with COVID-19,[164-166] herpes zoster,[167] and human papilloma virus.[168]

The immune system responds not only to pathogen-associated molecular patterns (PAMPs) but also to damage-associated molecular patterns (DAMPs), and therefore evidence has emerged pointing out that the inflammatory response triggered by the cellular damage imposed by the metabolic challenge of conditions such as diabetes, obesity and hypertension might lead to ED. While the inflammatory response participates in signaling and repairing cellular damage, its exacerbation in pathological conditions leads to low-grade chronic inflammation associated with activation of the immune response.

Through the activation of pattern recognition receptors (PRRs), the cells sense PAMPs and DAMPs triggering cell death processes such as apoptosis, necroptosis and pyroptosis. Although inflammation has been demonstrated in patients and animal models of vasculogenic ED, the role of PRRs in this response has been modestly studied in rodents, whereas no studies in patients have been reported. Herein, the most recent advances in the field of innate immunity in the etiology of vasculogenic ED will be highlighted. Of note, a recent study attributed stress-related ED to impaired parasympathetic neurotransmission[169]; however, there is no study reporting the immune response in psychosocial disorder-associated ED.

Toll-like receptors (TLRs) are a family of PRRs (TLR1-9 and TLR11-13) ubiquitously expressed in either cell membrane or cell compartments (such as endosomes).[170] Currently, TLR4 is by far the most studied PRR in endothelial dysfunction. However, the first observation regarding a potential role for TLRs in ED emerged in 2015 from an *in vitro* investigation demonstrating that rat corpus cavernosum expresses TLR2 and the TLR1/2 activator potentiated phenylephrine-induced contraction.[171] At the same time, it was observed that in a rat model of heart failure, there was an increase in the expression of TLR9 in the corpus cavernosum while the deletion of TLR9 was associated with decreased cavernosal contractility.[172] Later, it was demonstrated that TLR9 activation in macrophages contributes to cavernosal dysfunction in a murine model of obesity.[173]

The growing evidence that TLR4 contributes to vascular damage urged investigations on this PRR, revealing its participation in damage to the corpus cavernosum in diabetes and hypertension.[60,174] Increased expression of TLR4 was observed in these pathological conditions and both acute and chronic inhibition of TLR4 improved cavernosal reactivity through mechanisms associated with decreased oxidative stress and inflammation, and improved NO activity. In hypoxia, ED was associated with apoptosis and TLR4 was demonstrated to play a key role in the progression of ED.[175] The downstream mechanisms associated with the activation of TLRs include the activation of transcription factors (such as NFκB) and the release of cytokines leading to upregulation of signaling pathways that favor the flaccid state of the penis, including MAPK and Rho-kinase pathways.[176]

Inflammasomes are another important component of the immune system and mediate pyroptosis (ie, cell death mediated by inflammation). Among the inflammasomes, NLRP1, NLRP3, NLRC4, NLRP10, and AIM2 have been demonstrated to play a role in endothelial dysfunction. In this context, NLRP3 is the most studied inflammasome, and the only one investigated in sexual dysfunction. Interestingly, in mouse corpus cavernosum, both deletion and overactivation of NLRP3 caused an impairment in NO- and endothelium-dependent relaxation, respectively,[177] suggesting that NLRP3

is necessary for normal erectile function but its overactivation negatively impacts the erectile tissue. Indeed, NLRP3 activation was associated with ED induced by endothelin-1, bilateral CN injury and in diabetic ED, since NLRP3 inhibition improved erectile function in these animal models of ED.[178-180] The downstream activation of NLRP3 and the other inflammasomes is the production of two important cytokines, IL-1β and IL-18, and both might be key mediators of ED.[181-185]

Regarding the adaptive immune response, no direct studies evaluated the role of T-cells on sexual (dys)function. However, indirect studies on HIV patients suggest that T-cells may not be a significant contributor for sexual dysfunction. There was no correlation between CD4 and CD8 T-cells and ED,[186] and CD4 and CD8 T-cell count was similar in HIV patients taking ED drugs compared to HIV patients not taking ED drugs.[187] On the other hand, increased levels of cytotoxic T-cells (CD3+ and CD8+) were detected in the tunica albuginea of patients in the chronic stage of Peyronie's disease (no pain and stable curvature)[188] and therefore, the inflammatory environment of this fibrotic disease could contribute to ED.[189] Hence, the contribution of the adaptive immune system to ED development cannot be ruled out, and might vary according to the etiology of ED.

Conclusions

Despite a limited number of studies, the recent findings outlined herein provide clear and growing evidence of the association of ED and the heightened activation of the immune system (Figure 9). Preclinical studies demonstrate that inhibition of the immune system improves erectile function in different animal models of ED, while in the clinical setting, measurement of inflammatory markers associated with immune activation (such as TNF-α, IL-1β, and IL-18) in ED patients could help to identify diagnostic biomarkers. Nonetheless, there remains a significant knowledge gap regarding the impact of immune system inhibition on human erectile function. Some clinical trials carried out to address immunosuppressive drugs like IL-1β or TNF-α inhibitors reported a positive association of these drugs and a reduction in cardiovascular events. Therefore, it would be pertinent to incorporate assessments of sexual function into these trials as a step towards elucidating whether targeting the immune system could represent a novel therapeutic approach or adjuvant treatment for ED.

Female sexual function/dysfunction

Female sexual dysfunctions encompass disorders of pain, desire, arousal, and orgasm.[190] Each type of disorder can be multifaceted in the manifestation of symptoms and the affected individual's perception of their condition, as well as the inter-relational dimension. Although the following sections address each of these disorders in women, the presentations and discussions on hypoactive sexual desire disorder and orgasmic disorder are also pertinent to men. While this report summarizes recent basic science and translational research, the clinical evaluation and treatment of female sexual dysfunctions are presented by other ICSM committees. Lastly, the specific subcategory of genito-pelvic dysesthesias that include persistent genital arousal disorder are not discussed, as these conditions are also the focus of a separate ICSM committee.

Female sexual pain disorders (***Paul Yong, M.D., Ph.D.***)

Pain disorders associated with sexual activity may be broadly categorized into two anatomically-distinct subtypes termed superficial and deep dyspareunia. In this section, the focus of superficial dyspareunia will be on provoked vestibulodynia (PVD), and the focus of deep dyspareunia will be on endometriosis. The vulvar vestibule for PVD, and pelvic regions adjacent to the vaginal fornices for endometriosis (in particular, the posterior pelvis and vaginal fornix), are directly implicated in dyspareunia.[191-193] Mechanisms leading to tenderness at these two anatomic locations include inflammation (with a role for mast cells) that both sensitizes peripheral nerve endings and leads to local neuroproliferation (neurogenesis or hyperinnervation), amplifying signals to the central nervous system (Figure 10). It should be emphasized that for both superficial and deep dyspareunia, the pathophysiology of pain is multifactorial and complex. This includes central nervous system sensitization, concomitant pelvic floor dysfunction, the presence of pain comorbidities such as painful bladder syndrome, and psychological factors.[194]

Provoked vestibulodynia

Tomalty et al. confirmed previous work by demonstrating that cadaveric tissue from individuals with PVD had more PGP9.5 (pan-neuronal marker) immunostaining in the vulvar vestibule compared to tissue from controls.[195] Nerve fibers staining positive for nerve growth factor (NGF) and for the sensory marker calcitonin gene-related peptide (CGRP) were also increased in the PVD cases. Moreover, c-Kit expression (marker of mast cell activation) was also increased and sometimes co-localized with NGF in the PVD cases, providing evidence for a relationship between mast cells and neuroproliferation.

In a rat model of PVD, Awad-Igbaria et al.[196] injected zymosan (a yeast glycan) into the vulva, which resulted in a reduced mechanical and thermal sensitivity threshold at the vulva (ie, greater sensitivity). This model mimics initial inflammatory events (eg, from recurrent yeast infection) that may trigger longer lasting hypersensitivity of the vulvar vestibule, manifesting as an increase in mast cells that release mediators such as histamine, tryptase, chymase, and NGF, resulting in hyperinnervation (neuroproliferation). The authors observed significant increases in the vulvar vestibule of mast cells, mast cell granulation, NGF expression, and PGP9.5 (pan-neuronal) immunostaining density with an increase in number and length of nerve fibers and an increase in expression of transient receptor potential cation channel vanilloid 1 (TRPV1) (involved in heat/burning) in these neurons. Treatment with a mast cell stabilizer (ketotifen fumarate) prior to zymosan administration improved the mechanical/thermal sensitivity thresholds, and also prevented the increase in nerve density, NGF, and TRPV1 expression in the vulva. This study provides animal model evidence for the role of mast cells in neuroproliferation in PVD, and the potential therapeutic use of mast cell stabilizers.

In mice, methylisothiazolinone (MI), a preservative in cleaning products, has been used to model PVD,[197] as household exposures may be associated with risk of vulvar pain.[198] MI exposure, including the labia, was associated with an increase in cytokine expression (such as IL-1β) and mast cells at the

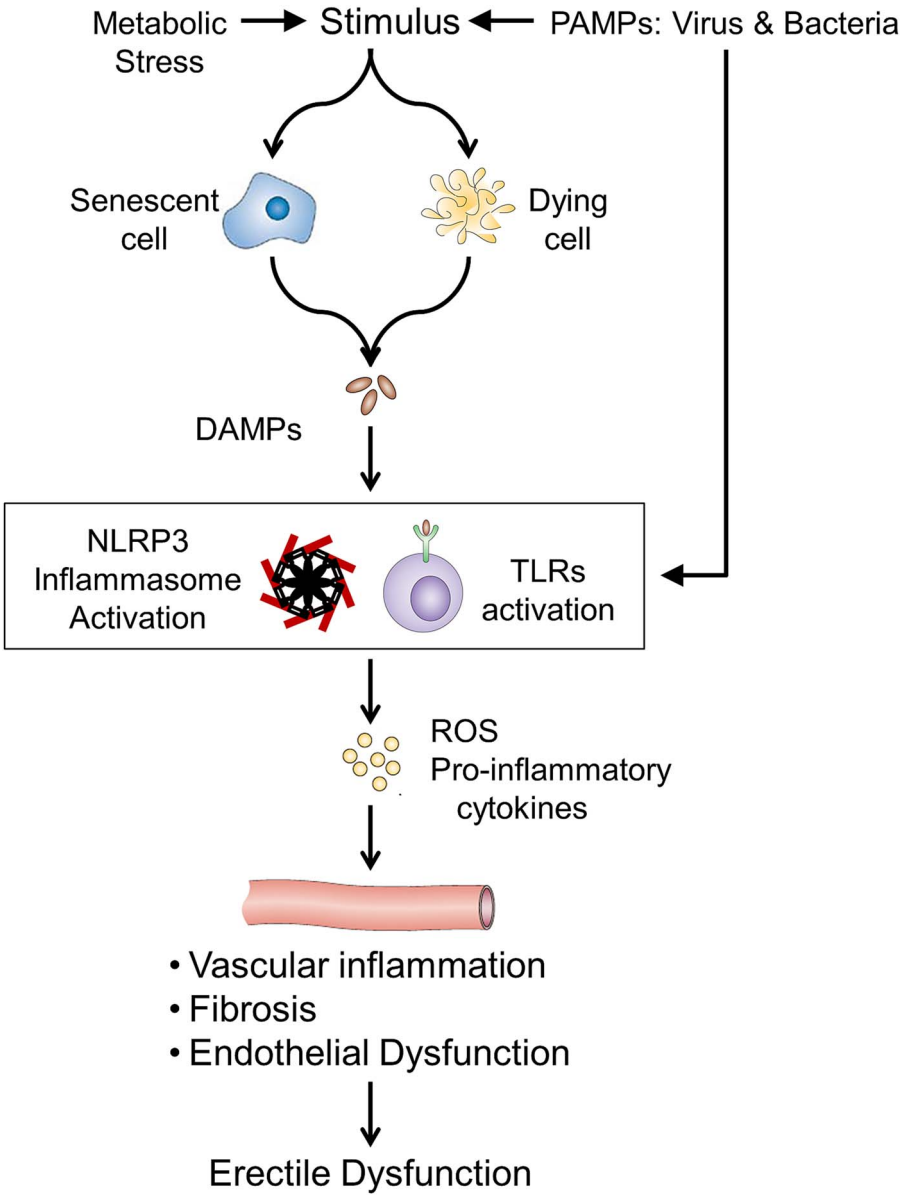

Figure 9. Pathogens or cellular damage induce activation of the immune response through NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasomes and Toll-like receptors (TLRs). The subsequent increase in reactive oxygen species (ROS) and cytokines leads to chronic vascular inflammation, fibrosis and endothelial dysfunction, ultimately contributing to erectile dysfunction. Adapted from Calmasini et al.[176] Used with permission.

labia, as well as increased tactile sensitivity of the labia. The use of imatinib, a c-Kit tyrosine kinase inhibitor known to reduce mast cells, resulted in a reduction in both mast cells and labial tactile sensitivity. This study provides additional evidence for mast cells and the potential role of inflammation-associated neuroproliferation in PVD.

In another mouse model of PVD where complete Freund's adjuvant (CFA) was injected into the vaginal introitus,[199] CFA increased macrophages (both M1 and M2), vaginal hyperinnervation (neuroproliferation) assessed by PGP9.5 immunostaining density, and vaginal hypersensitivity to distention measured by the visceromotor reflex. Interestingly, the administration of clodronate to deplete macrophages did not change hyperinnervation, but did reduce vaginal hypersensitivity, pointing to differential pathways leading to vaginal hypersensitivity beyond neuroproliferation.

Falsetta and colleagues have examined local inflammation (and potential therapeutics) in PVD. In one study, transient receptor potential cation channel subfamily V member (TRPV4) mRNA expression, involved in hyperalgesia and allodynia, was higher in fibroblast cultures derived from tissue biopsied from painful vestibular areas of PVD patients compared to non-painful areas from the same patients and from the vestibule of control subjects.[200] Knockdown of TRPV4 resulted in decreased PGE_2 and IL-6 production in the fibroblasts, while IL-1β increased TRPV4 mRNA expression and PGE_2 production. Given that chemical irritants like MI can increase IL-1β expression in the vulva (as noted above), this elevated IL-1β may then in turn promote prostaglandin levels and TRPV4-mediated pain.

In the second study, Falsetta examined specialized pro-resolving mediators (SPMs) in vulvodynia, which are known

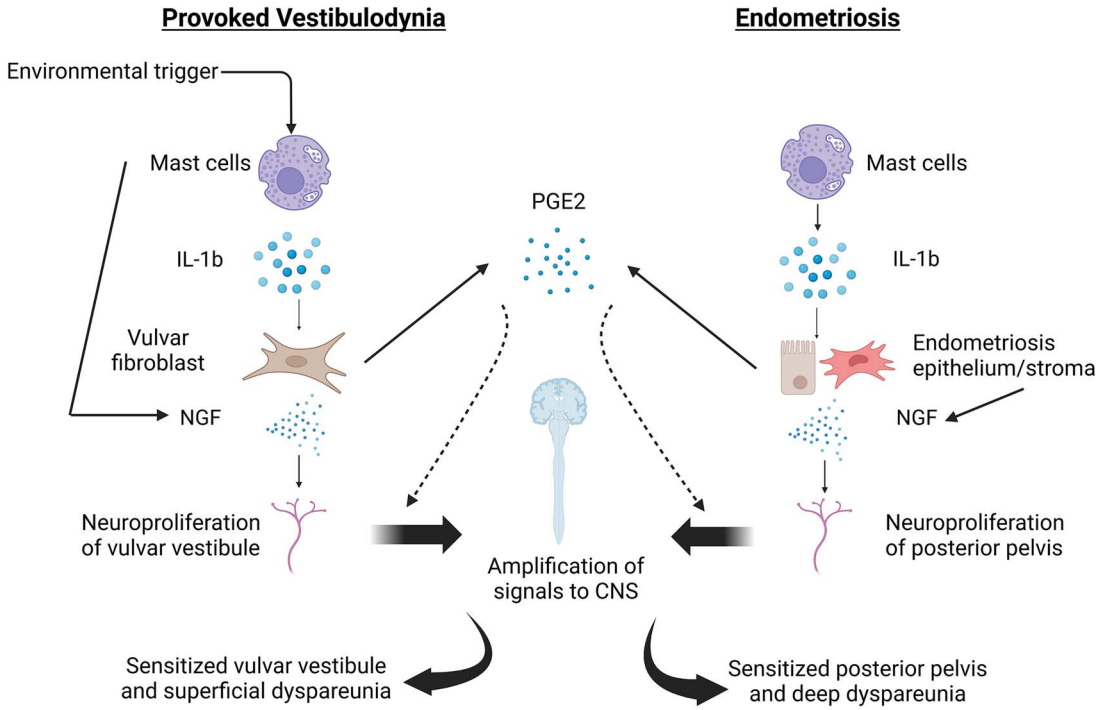

Figure 10. Summary of sexual pain pathways in provoked vestibulodynia and endometriosis. Both conditions are associated with an increase in mast cells and inflammatory cytokines (eg, IL-β), which results in expression of neurotrophins such as nerve growth factor (NGF) that in turn promotes neuroproliferation (neurogenesis or hyperinnervation) at the vulvar vestibule and posterior pelvis, respectively. Local neuroproliferation amplifies signals to the central nervous system (CNS). IL-1β can also lead to an increase in PGE_2, which further sensitizes peripheral nerve endings. The result is a sensitized vulvar vestibule (which upon contact during insertion, results in superficial dyspareunia in provoked vestibulodynia) and a sensitized posterior pelvis (which upon contact of the posterior vaginal fornix during deep insertion, results in deep dyspareunia in endometriosis). Created with Biorender.com.

to have anti-inflammatory effects.[201] SPMs reduced IL-1β-induced effects (IL-5 and PGE_2 expression) in vulvar fibroblasts. The SPM maresin-1 decreased mechanical sensitivity thresholds in a zymosan-induced mouse model of PVD. Polyunsaturated fatty acid (PUFA) precursors to SPMs, in particular DHA, were able to lower PGE_2 and improve mechanical sensitivity thresholds. This study again illustrates the importance of IL-1β in PVD, and point to SPMs (like maresin-1) or PUFA precursors to SPMs, as a means to reduce inflammation and vulvar sensitivity.

Findings from these studies are synthesized in Figure 10. Environmental irritants may induce IL-1β expression and increase mast cells in the vulvar vestibule, which together further increase inflammation (PGE_2), TRPV1 and TRPV4 expression, as well as NGF and associated neuroproliferation, resulting in sensitization of the vulvar vestibule and thus superficial dyspareunia.

Endometriosis

Studies in the last 5 years in endometriosis have shown evidence for similar pathways as in PVD. A recent study described a standardized method for quantifying PGP9.5 nerve bundle density around endometriosis, including a semi-automated protocol, for the study of neuroproliferation.[202] A higher PGP9.5 nerve bundle density was observed in deep (invasive) endometriosis and superficial peritoneal endometriosis, compared to ovarian endometriosis, suggestive of differences in neuroproliferation by anatomic subtype. Peng et al. found that expression of IL-1β and NGF in endometriosis epithelium/stroma correlates with PGP9.5 nerve bundle density around endometriosis, and together they are correlated with more severe deep dyspareunia.[203] IL-1β induced NGF expression by endometriosis stromal cells with NGF promoting PGE_2 expression and also increasing neurite growth and branching in a PC12 cell neurogenesis model.[203]

Similar findings were observed by Velho et al.[204] Building on this group's previous work, they found increased PGP9.5 nerve fiber density in endometriosis compared to control peritoneum, including more substance P (SP)-positive sensory nerve fibers. Among endometriosis cases, PGP9.5 nerve density was lower in those on hormonally suppressive therapies, but with no difference based on surgical staging. Dyspareunia severity correlated with both PGP9.5 nerve fiber density and with SP-positive nerve fibers. Nerve fibers that were positive for NGFp75 and TrkA, NGF family receptors, were higher in endometriosis compared to controls. TRPV1-positive nerve fibers were higher in endometriosis compared to controls using one measure of quantification. This study confirms the correlation between dyspareunia in endometriosis and hyperinnervation around endometriosis lesions. It also implicates TRPV1-positive nerve fibers in endometriosis, similar to PVD.

As in PVD, mast cells are also implicated in endometriosis. Summarizing the literature in endometriosis, a recent review noted that mast cells are increased in endometriosis and are seen in proximity to nerve fibers, that mast cells correlate with NGF levels, and that SP provokes mast cells to degranulate and release inflammatory mediators.[194] Also, mast cells release IL-1β, which in turn promotes NGF expression in endometriosis, as noted above. In a

study that further characterized mast cells in endometriosis, McCallion et al. found evidence of increased expression of mast cell-related genes involved in recruitment, differentiation, and activation in endometriosis compared to uterine endometrium.[205] Immunohistochemistry for mast cell tryptase showed more mast cells in endometriosis compared to uterine endometrium. Higher stem cell factor with ELISA was also seen in endometriosis. In a mouse model of endometriosis, estrogen increased mast cell-related genes compared to control endometrium. Figure 10 summarizes these pathways in endometriosis with comparisons to PVD. Mast cells are elevated in endometriosis and produce IL-1β, which may cause endometriosis epithelium/stroma to produce NGF that leads to neuroproliferation (including TRPV1 positive nerves), sensitization of the posterior pelvis (corresponding to the posterior vaginal fornix), and thus deep dyspareunia.

Conclusions

The commonalities in these pathways has led to the hypothesis of "neuroproliferative dyspareunia" in both PVD and endometriosis.[194,206] Remaining work is needed to confirm and characterize this entity, and to determine its implications for diagnosis and personalized treatment of dyspareunia. Any consideration of neuroproliferative dyspareunia needs to be situated in the context of multifactorial contributors to pain in both conditions, including the role of the pelvic floor, psychological comorbidities, and central nervous system sensitization.

Female sexual arousal disorders (*Noel N. Kim, Ph.D.*)

Arousal disorders in women have been categorized as female genital arousal disorder (FGAD) and female cognitive arousal disorder (FCAD).[207] FGAD is defined as the inability to develop or maintain adequate genital response (vulvovaginal lubrication, engorgement of genitals, and sensitivity of genitalia) for ≥6 months not due to insufficient stimulation or vulvovaginal conditions such as atrophy, infection, or inflammatory disorders.[208] FCAD is described as "distressing difficulty or inability to attain or maintain adequate mental excitement associated with sexual activity as manifested by problems with feeling engaged or mentally turned on or sexually aroused for ≥6 months.

Due to analogous lines of research in male erectile dysfunction, FGAD and the underlying neurovascular pathophysiology has been investigated more robustly than FCAD. While each of the genital organs/tissues (clitoris, vulva, vestibule and associated glands, vagina, and cervix) plays an important role during genital arousal, the clitoris and vagina have almost exclusively been the focus of studies in the realm of genital arousal disorder, while the vulva and vestibule have been investigated within the context of pain conditions. The clitoris and vagina are both highly vascular organs with the capacity to become engorged, primarily due to the corpora cavernosa within the clitoris and the subepithelial plexus of arterioles, capillaries, and merged venous sinusoids in the lamina propria layer of the vagina.[209] Foundational mechanisms of neural and hormonal regulation of the genital sexual arousal response have been summarized previously.[209] This section will focus on more recent research elucidating various aspects of female sexual arousal.

Cardiometabolic risk factors

In both laboratory and clinical studies, cardiometabolic risk factors (atherosclerosis, hypertension, diabetes, obesity) have been associated with FGAD at varying degrees of stringency.[210-213] For example, clitoral pulsatility index, a measure of vascular resistance assessed by color Doppler ultrasound, was significantly and positively correlated with body mass index, waist circumference, insulin, triglycerides, total cholesterol, LDL cholesterol, and insulin resistance (HOMA-IR score) in women (n = 71; mean age = 44.8 y) and these correlations remained statistically significant after adjusting for age, smoking, and years since menopause.[214] Clitoral pulsatility index was also positively correlated with an increasing number of metabolic syndrome criteria and was negatively associated with arousal and satisfaction domain scores of the Female Sexual Function Inventory (FSFI). In a separate study in healthy eumenorrheic women (aged 18– 35 y), pulsatility index of ophthalmic and internal carotid arteries, and vasculature of the labia, clitoris, and uterus was significantly higher in heavy smokers versus non-smokers.[215] While there was a trend for a greater percentage of heavy smokers to have scores below 35 on the McCoy Female Sexuality Questionnaire (indicative of sexual dysfunction) compared to nonsmokers, the difference did not reach statistical significance. However, in a mixed cohort of pre- and post-menopausal women with type 1 diabetes, genital sensation and vaginal pulse amplitude (a parameter correlated with blood flow) in response to visual sexual stimulation was similar to non-diabetic women.[216] Significant decreases in vaginal pulse amplitude and genital sensitivity were noted only in a subgroup of diabetic women who had retinopathy. Thus, in contrast to type 2 diabetics who commonly have insulin resistance, genital arousal does not appear to be compromised in type 1 diabetics unless severe neurological damage has occurred. Unlike penile erection in men, increased genital blood flow in women does not lead to rigidity, potentially conferring a broader window of functionality. In addition, estrogen in premenopausal women may exert greater protective effects on vascular function, moderating the impact of cardiometabolic disease.[217-219]

Effects of sex steroid hormones

In healthy eumenorrheic women, transvaginal Doppler ultrasound analysis indicated that clitoral volume was significantly increased during the periovulatory period when estradiol levels were ~4.5 times higher than during the follicular phase of the menstrual cycle.[220] Clitoral vascular resistance was also significantly decreased during the periovulatory versus the follicular phase.[220,221] The influence of sex steroid hormones on genital arousal is further confirmed by the effects of oral contraceptive pills that have been shown to reduce vaginal blood flow and lubrication in response to visual sexual stimulation.[222] This reduction in vaginal blood flow and lubrication was greatest in women using oral contraceptives containing anti-androgenic progestins (drospirenone, desogestrel, or norgestimate). Conversely, transdermal testosterone (T) therapy (300 μg/day) has been shown to significantly increase clitoral peak systolic velocity after 6 months in women diagnosed with hypoactive sexual desire disorder (HSDD).[223] This improvement in clitoral hemodynamics was associated with significantly increased arousal and lubrication

domain scores on the FSFI. However, it should be noted that flibanserin, a non-hormonal treatment for HSDD, has also been shown to increase FSFI scores for arousal and lubrication domains,[224] demonstrating the interrelated nature of sexual function domains.

Effects of cancer

Sexual dysfunctions in women with cancer are being increasingly investigated as the number of cancer survivors and those living with cancer increases. In a systematic review of longitudinal studies of sexual dysfunction in women diagnosed with breast, cervical, endometrial, ovarian, vulvar, and unspecified gynecological cancers, most women remained sexually active and the incidence of developing sexual dysfunctions (most frequently reduced desire, arousal, and orgasm) ranged from 30– 80%.[225] It remains unclear to what extent sexual function is impacted by a specific cancer versus the sequelae of cancer therapies. In general, with the exception of fertility preservation, there has been minimal investigation into alternative cancer therapy regimens that decrease the impact on sexual function without compromising treatment efficacy. However, investigators are beginning to study the effects of radiotherapy on female erectile tissues to develop evaluation/prevention/mitigation strategies to improve outcomes in patients with pelvic malignancies.[226]

Cognitive arousal disorder

The biopsychosocial factors associated with FCAD may be the least characterized due to the complexity of interactions and the adoption of a combined sexual interest/arousal disorder (SIAD) by psychologists and sex therapists.[227] Nevertheless, cognitive behavioral therapy and mindfulness have been shown to be effective in treating SIAD in women and in couples.[228-230] A limitation of these findings is the use of waitlist control groups that are necessarily not blinded to their treatment status. Future studies using functional brain imaging to assess changes in cognitive arousal could present opportunities to better characterize this response and evaluate the efficacy of psychological interventions.

Conclusions

The high degree of research interest in FGAD has provided basic mechanistic understanding of various pathophysiological mechanisms and insight into similarities and differences between female and male genital arousal. Yet, despite the long-standing availability of oral PDE5 inhibitors for male ED, approved therapies for FGAD are lacking. As with many types of sexual dysfunction, effective treatment of this condition will likely involve both pharmacological and adjunctive non-pharmacological approaches. Regenerative therapeutic approaches have not been actively investigated in the context of FGAD but may offer much potential in providing additional treatment options.

Hypoactive sexual desire disorder (*James G. Pfaus, Ph.D.*)

From a basic science standpoint, hypoactive sexual desire translates to a lack of motivation for sexual interaction in circumstances where sex partners are available and sexual activity with them is normally manifest.[231] This broad definition applies in a translational way to both patients and preclinical animal models that display a lack of appetitive sexual motivation in a sexual context.[231,232] This can present itself as low excitation to sexual cues or stimuli, enhanced inhibition in the presence of those cues, or both.[233-237] A number of causes of hypoactive sexual desire have been identified, including clinically low steroid hormone levels (especially T), effects of drugs or medications that act in the central nervous system (eg, dopamine antagonists, selective serotonin reuptake inhibitors, high doses of sedative-hypnotics, opiates, and opioids), and experience with non-rewarding or otherwise unpleasurable sexual activity.[208,232,233,238-240] When the lack of sexual desire causes distress, it is considered a clinical disorder. This is exemplified in the ICD-11 as HSDD[241] or in the DSM-5-TR as "male HSDD" and "female sexual interest and arousal disorder".[242]

Identification of effective treatments for HSDD have been aided greatly by basic research utilizing well-controlled clinical trials, cognitive measures of attention to sexual stimuli, like eye-tracking,[243,244] and a host of subjective measures, including the FSFI, Decreased Sexual Desire Screener, and the Female Sexual Distress Scale (FSDS).[245-247] In addition to sensate focus[248] and mindfulness therapies,[249] hormonal and pharmacotherapies for HSDD in women also exist. One of the first was the combination of T and estradiol (E_2),[250] given first as an injection to perimenopausal women, and then as a transdermal T patch to surgically menopausal women maintained on E_2.[251] Small enhancements of sexual desire were observed in some studies with the transdermal T patch in premenopausal women, but those effects were inconsistent and potentially related to low circulating androgen levels.[252] However, acute sublingual T was also shown to increase subjective measures of desire in premenopausal women in response to visual sexual stimuli.[253] Although the peak concentration of T in blood occurred within an hour of administration, subjective assessments of desire increased between 4 and 6 hours after administration, suggesting a genomic effect of T on excitatory neurochemical systems involved in sexual attention. An important question is whether T works on androgen receptors to induce this effect, or whether it must be metabolized by aromatase to E_2. Although the combination of E_2 and T increases lordosis and the number of sexual solicitations made by female rats to male rats significantly over either steroid alone,[254] co-administration of the aromatase inhibitor fadrazole produced a dramatic increase in solicitations over the combined effect of E_2 and T.[255] Additionally, the effect occurred 4 hours after T administration, suggesting that T enhanced solicitations by a specific androgen receptor-mediated mechanism. Subsequently, another study reported that the 5α reduced T metabolite, dihydrotestosterone (DHT), a non-aromatizable androgen, also produced a dramatic facilitation of solicitations and lordosis 4 hours after administration in females primed with E_2.[256] These data indicate that T or DHT enhance desire by an androgen receptor-mediated mechanism, but that this mechanism requires effects induced by E_2 to create a specific brain state that enhances sexual excitation.[233,257] To date, however, the mechanisms by which T enhances sexual desire in female patients and rats have not been identified.

Preclinical rat models have aided in the delineation of more specific neuroanatomical and neuropharmacological mechanisms of sexual desire, especially in women. The neural pathways of desire involve the activation of medial preoptic (mPOA), hypothalamic, and limbic circuits involving

dopamine, noradrenaline, melanocortins, and oxytocin, and the inhibition of normally inhibitory circuits involving serotonin, especially those that activate the prefrontal cortex and its outputs that inhibit sexual excitation.[233] Identification of these mechanisms was made possible by studying the effects of selective compounds on homologies of both patient female and male sexual desire, including female solicitations and hyperlocomotion made by males in anticipation of sexual activity. Two compounds known to increase measures of patient female sexual desire on the FSFI, along with decreases in sexual distress on the FSDS, are chronic administration of the mixed 5-HT_{1a} agonist/5-HT_{2a} antagonist drug flibanserin (Addyi), and acute administration of the melanocortin type 4 receptor agonist bremelanotide (Vyleesi). These drugs were approved by the US Food and Drug Administration for the treatment of HSDD in premenopausal women in 2015 and 2019, respectively. Both were shown to increase sexual solicitations and reduce defensive responses in female rats.[233,236,258-260] Notably, bremelanotide alone activated neurons in a subset of regions normally activated in female rats by both conditioned and unconditioned sexual cues when females are sexually receptive.[259,261] This activation was subsequently shown to involve the presynaptic release of dopamine coming from the zona incerta to the mPOA, and its actions on dopamine D1 receptors that send projections to both the ventromedial hypothalamus and the ventral tegmental area in the midbrain,[262] the cell body source of the mesolimbic dopamine pathway. A recent fMRI study in premenopausal women with HSDD showed that bremelanotide induced a pattern of brain activation similar to that induced by an erotic video, and enhanced activation by the erotic video in cerebellum, striatum, dorsal motor cortex, and visual cortex, while reducing activity in secondary somatosensory cortex, compared to placebo.[263] Bremelanotide also enhanced and maintained functional connectivity between the amygdala and the insula during the erotic video. Finally, bremelanotide produced small but significant elevations in plasma luteinizing hormone, follicle-stimulating hormone, and T consonant with its subjective enhancement of sexual desire over a 24-hour period after administration. Using microdialysis of extracellular fluid from the brains of female rats, flibanserin alone was shown to reduce serotonin levels in the prefrontal cortex (PFC), medial preoptic area, and nucleus accumbens, while at the same time increasing both dopamine and noradrenaline levels in the PFC and mPOA.[264]

Tuiten and colleagues took this a step further, showing that women with HSDD had patterns of single nucleotide polymorphisms (SNPs) of genes that encode serotonin, oxytocin, and dopamine receptor subtypes (along with other synthesizing enzymes, reuptake proteins, and peptides) in plasma that predicted either low excitation or high inhibition.[265,266] Those data suggested that the pattern of SNPs could be utilized as a companion diagnostic to better determine whether HSDD therapies should be focused on the treatment of low excitation, high inhibition, or both. It is not known yet to what extent the two SNP patterns correspond to assessments of low excitation or high inhibition on subjective tests (eg, the sexual excitation and inhibition scales developed by Janssen[267,268]). These researchers also conceived of respective treatments for those conditions using a combination of sublingual T paired with sildenafil (Lybrido) for low excitation, or sublingual T paired with buspirone (Lybridos) for high inhibition (Figure 11).[269] These potential treatments are still undergoing clinical trials. Interestingly, the combination of T and the PDE5 inhibitor vardenafil increased hops and darts (proximal sexual solicitations) and lordosis in female rats primed with E_2.[270]

Although understanding HSDD has been a primary focus of studies in women, male HSDD is emerging as a distressing clinical condition.[271,272] It is likely that future research will shed light on sex differences or similarities in its expression in men, and the ability of compounds like bremelanotide,[273] flibanserin, and others (eg, kisspeptin[274]) to provide suitable treatments.

Conclusions

The past decade has seen the approval of two pharmacotherapies, flibanserin, and bremelanotide, for the treatment of HSDD in premenopausal women, although both drugs are prescribed off-label for HSDD in post-menopausal women and men. Two main sources of HSDD, low excitation and arousal to sexual cues or hyper-inhibition in sexual circumstances, have been identified in different populations of women using both subjective tests and SNPs. These tests can help place women with HSDD into more personalized treatment regimens with better outcomes. Two on-demand medications, Lybrido and Lybridos, are slated for Phase 3 trials, and mindfulness therapy continues to be refined. Female rat models of HSDD have identified neural mechanisms of low excitation and high inhibition, and it is likely that these will be examined in male rats in the future as male HSDD comes more into focus.

Orgasmic disorders (*James G. Pfaus, Ph.D.*)

Orgasm is a complex, multimodal reflex induced typically by genital stimulation and coming at the height of the sexual response cycle, prior to the inhibition that characterizes sexual refractoriness.[275] Masters and Johnson originally described a singular orgasm pattern for men, but three distinct patterns for women.[276] This result was replicated by Bohlen et al. using both anal and vaginal probes that detect muscular pressure changes and pelvic floor muscle contractions.[277,278] This can now be done in at-home settings using blue-tooth intravaginal devices that detect pelvic floor muscle pressure.[279] Mechanistically, genital stimulation activates the sensory pudendal and hypogastric nerves (and pelvic nerves in women), along with excitatory neurochemical pathways in the brain and spinal cord that ultimately stimulate sympathetic outflow and the inhibition of parasympathetic spinal circuits in the lumbar cord to induce the feeling of release that characterizes climax. At the same time, the conscious awareness of orgasm occurs as a rush of ecstatic pleasure due to the release of endogenous opioids in the brain, followed by a feeling of satiety and relaxation due to the release of serotonin and other neurochemicals.

In men, orgasm and climax are usually consonant with ejaculation, which is typically achieved by stimulation of the glans and shaft of the penis. In women, climax and orgasm are typically achieved by stimulation of the external glans of the clitoris. Some women can also achieve qualitatively different orgasms by stimulation of the internal bulbs and crura of the clitoris and paraurethral glands (prostate) that are now referred to as the clitoral-urethral-vaginal (CUV) complex,[280] formerly known as the "G-spot". Orgasms can also be achieved by stimulation of the anterior cervix and even by intense stimulation of the nipples and other erogenous

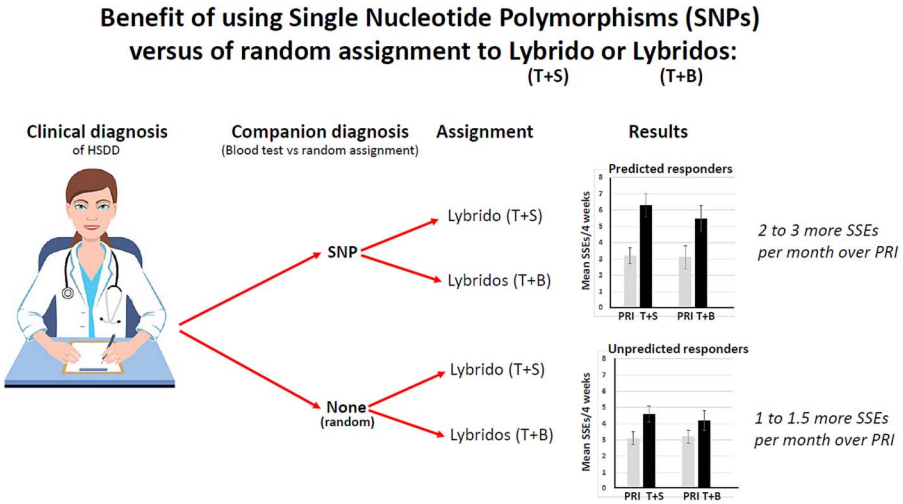

Figure 11. A companion diagnostic approach to the treatment of HSDD with Lybrido and Lybridos. Random assignment of women after clinical diagnosis resulted in a similar increase of 1 to 1.5 sexually satisfying events (SSEs) per month over the placebo run-in (PRI) period for both drug combinations. However, when the analysis of SNPs was added as a companion diagnosis to place women into HSDD subtype categories of low excitation versus high inhibition, treated subsequently with Lybrido or Lybridos, respectively, the treatment efficacy increased to 2 to 3 more SSEs per month over the PRI. Data from Tuiten et al.[265]

zones in sensitized individuals.[281-283] Although the reflex is a product of "bottom-up" genitosensory stimulation, it is also controlled by "top-down" processing of excitation and inhibition that controls both the timing of parasympathetic and sympathetic blood flow, and the subjective ability to "let go" into the orgasm when it is imminent. Indeed, orgasms activate cortical, limbic, hypothalamic, and brainstem structures,[284] and can be rated subjectively in terms of the perceived type and quality of sensory stimulation, affective experience, and the evaluation of pleasure.[285]

Orgasms are accompanied by reflexive activation of pelvic floor muscles along with changes in facial expressions and arm, leg, and toe flexions.[286] This motor activation induces movement artifacts reflected in overall brain activation and patterns of electrical activity in the cortex that are difficult to control for.[287] However, orgasms are also accompanied by neurochemical and endocrine changes that characterize both the euphoric state of pleasure and longer-term inhibition (refractoriness).[233,288] Among these correlates is a consistent, orgasm-induced surge of prolactin released from the anterior pituitary into the peripheral bloodstream.[289,290] Because neuroendocrine cells in the hypothalamus that contain prolactin releasing factor are kept under tonic inhibition by hypothalamic dopamine transmission, the surge in pituitary prolactin release is indicative of the sudden inhibition of both hypothalamic and mesolimbic dopamine transmission at orgasm.[233,291] The prolactin surge and the characteristic pelvic floor movements can be used in basic research as objective markers of orgasm in patients, and this has been used recently in women to validate non-genitally stimulated orgasms (NGSOs) induced by pelvic floor and abdominal movements alone.[292] Subjective aspects of orgasm can be assessed using questionnaires like the Orgasm Rating Scale[293] or the orgasmometer.[294] In preclinical animal models, ejaculations can be studied in males as an orgasm-like response (OLR). OLRs also occur in female rats and can be distinguished on the basis of ultrasonic vocalization patterns[295] in response to copulation or clitoral stimulation.

Among the sexual dysfunctions, orgasm disorders affect 25% to 40% of women and men.[296] These include anorgasmia, delayed ejaculation/orgasm, anhedonic orgasm (feeling of climax or release without pleasure; also known as Pleasure Dissociated Orgasmic Disorder, or PDOD), premature (rapid or early) ejaculation (PE) and post-orgasmic illness syndrome (POIS, including fever, illness, and inflammatory responses likely due to a high sensitivity to histamine released from mast cells at orgasm). Most people with orgasm disorders experience moderate to intense distress, and the incidence of orgasm disorders increases with age. According to both the DSM-5-TR and the ICD-11, orgasm disorders can be lifelong or acquired, generalized or situational, and organic or multifactorial. They can be due to medications that delay or abolish orgasm, like major tranquilizers, antihypertensives, opioid antagonists, anxiolytics, and antidepressants, especially selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine,[297] or to the use of psychomotor stimulants that activate sympathetic outflow and produce more rapid ejaculations. Most of these effects have been modelled in female and male rats.[298-300] For example, bupropion (an antidepressant that blocks the reuptake of noradrenaline and dopamine) facilitates appetitive aspects of sexual behavior in both female and male rats,[301,302] and has been used off-label to counteract the delayed ejaculation/orgasm induced by SSRIs.[303] Similarly, acute administration of oxytocin or caffeine to rats can counteract the effect of the SSRI fluoxetine.[298] The opioid receptor antagonist naloxone, or its longer-acting analogue naltrexone, block the rewarding properties of orgasm in both rats and voles, leading them to avoid sexual contact with partners associated with the state of non-reward.[239,304-306] Naltrexone, in particular, has been used with some success in the treatment of compulsive sexual behavior disorder,[307] presumably by inhibiting the orgasm-induced reward state. Preclinical rat models have also been useful in determining brain regions that are activated at the cellular level by OLRs, including orbitofrontal cortex, anterior cingulate cortex, insula, terminal regions of the mesolimbic dopamine system like the nucleus accumbens, lateral septum,

and basolateral amygdala, medial preoptic area, paraventricular nucleus, subparafascicular nucleus of the thalamus, ventral tegmental nucleus, pedunculopontine tegmental nucleus of the brainstem, and regions of the central grey.[284] This is virtually identical to regions activated in patients by orgasm,[308,309] making rat models useful in determining neuroanatomical and neurochemical pathways that can be disrupted using lesioning techniques, psychopharmacological and molecular techniques, and/or experiences related to sexual non-reward to model processes at various levels of analysis that are involved in orgasmic disorders in humans.

Conclusions

There is a move toward a more refined assessment of orgasm and its disorders. In patients, this includes the use of validated subjective and objective assessments of orgasm, a focus on new orgasmic disorders, such as POIS and PDOD (in addition to naturally-occurring and drug-induced PE and anorgasmia), and the study of NGSOs. Rat models have also been developed to examine the behavioral, hormonal, and neurochemical systems that induce OLRs and that can be perturbed by drugs, stress, or other conditions that produce orgasm disorders in patients.

Strengths and limitations

This expert consensus review summarizes the most up-to-date research on active areas of investigation that have made the greatest strides in elucidating physiological and molecular mechanisms that impact sexual function/dysfunction. Due to considerations of overall length and the overlapping focus of other ICSM committees, we did not engage in performing a comprehensive analysis of all areas of investigation related to sexual medicine. In addition, while the committee was primarily composed of researchers from North America, publications were considered without bias to geographical location of the primary research group. Finally, the psychological aspects of sexual function were not specifically addressed and were considered to be beyond the scope of this committee's charge and expertise.

Conclusions and future directions

There have been significant advances in basic science and translational research in sexual medicine over the past decade. Despite the myriad of distinct physiological processes and molecular signaling pathways being studied in relation to the various dysfunctions and disease states, common pathophysiological mechanisms are being elucidated in the context of ED associated with prostatectomy, diabetes, and aging. A central underlying mechanism includes neuropathy-induced remodeling of the corpora cavernosa including SM apoptosis and fibrosis. With less SM and increased collagen, the penile tissue is less able to relax in response to neurotransmitters (NO) and ED occurs. Therapies that are neuroprotective and promote CN regeneration more quickly so that less overall penile remodeling occurs, are critical. Development of improved delivery mechanisms for proteins, cells and factors that impact CN and corpora cavernosa regeneration are essential to move the field of ED research forward, and substantial strides have been made developing nanotechnology-based delivery vehicles for this purpose with the advantage of avoiding systemic side effects and increasing therapeutic safety. Substantial progress has been made with genomic analyses of penile tissue from patients and animal models, and this will facilitate identification of novel signaling mechanisms involved in penile apoptosis, fibrosis, SM contractility and relaxation, and the immune response, for future therapy development. Progress in understanding the mechanisms underlying vasculogenic ED remains poorly understood and requires further investigation. The potential opportunities related to immune and aging related mechanisms will require substantially more preclinical investigation and offer potential for early stage ED interventions. The utility and benefit of some treatments is ambiguous at this time including shock wave and stem cell therapy. In order for stem cell therapy to be effective, the stem cells need to stay where they are injected and not migrate to other areas of the body where they can be problematic. This is an obstacle that needs to be addressed/overcome in order for effective and safe treatment. The current literature has mixed messages as far as the value of shockwave treatment, despite it being well utilized.

In women, genital pain disorders, and disorders of sexual arousal and orgasm also have multiple and overlapping neurological and endocrinological components that are still being actively characterized. While the knowledge base is significantly greater with regard to aspects of male ED, numerous lines of research that have been applied mostly to women may also be of benefit to studying sexual dysfunction in men that go beyond aspects of penile erectile dysfunction. Due to the complexity of interacting systems for various sexual dysfunctions, different strategies and combined treatment regimens will be required for prevention and treatment.

There are many substantial barriers to clinical translation even with the most promising treatments that are effective in animal models. At present, there are no clinical therapies that definitively change the course of sexual dysfunction. With regard to sexual desire and orgasm disorders, future research will shed light on sex differences or similarities between women and men, and the ability of current and newer approaches to provide suitable treatments. There are many independent lines of research in progress with the potential for positive outcomes. Thus, current basic science research efforts are likely to provide more opportunities than ever before to develop disease-specific treatments or prevention strategies that have been lacking in the field if the hurdle to clinical translation can be overcome.

Conflicts of interest

Medical Society Board/Leadership: Sexual Medicine Society of North America, Society for Pelvic Research (CAP); International Society for the Study of Women's Sexual Health (NNK); World Endometriosis Society, International Society for the Study of Women's Sexual Health, Canadian Society for Advancement of Gynecologic Excellence, Society of Obstetricians and Gynaecologists of Canada (PJY).

Medical Society Honoraria/Consultant/Advisor: International Society for the Study of Women's Sexual Health (NNK); Society for Endometriosis and Uterine Disorders, Society for Obstetricians and Gynaecologists of Canada, Women's Health Research Institute, World Endometriosis Society, Vulvodynia Summit (PJY).

Pharmaceutical/Device Company Grants: Ohnut (PJY).

Pharmaceutical/Device Company Stock: Acoustic Wave Cell Therapy Inc (GL).

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