FEMALE UROLOGY (L COX, SECTION EDITOR)

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Management of Occult Urinary Incontinence with Prolapse Surgery

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Abstract

Purpose of Review The purpose of this paper is to review (1) the epidemiology and pathophysiology of pelvic organ prolapse (POP) and occult stress urinary incontinence (SUI), (2) examine the data on combined operative management of POP and occult SUI, (3) discuss the approaches to clinical decision making, and (4) present future therapies.

Recent Findings Prospective data on many approaches to concomitant treatment of prolapse and occult stress urinary incontinence, such as minimally invasive sacrocolpopexy and midurethral sling, or older approaches that have regained favor among patients and clinicians wishing to avoid synthetic mesh, such as native tissue prolapse repair and pubovaginal sling, are limited. Safe durable treatments with absorbable graft materials that promote a beneficial host response are intriguing but may be far from clinical implementation. Stem cell therapy for the treatment of stress urinary incontinence has demonstrated benefit in phase I/II trials but has not been studied in the setting of concomitant treatment of occult SUI with POP surgery and remains in the preclinical phase for the treatment of POP.

Summary A personalized approach to concomitant SUI surgery that incorporates individual risk assessment as well as informed patient preferences likely optimizes the risk/benefit ratio and patient satisfaction. Novel therapies, including graft materials and cellular therapies that stimulate a regenerative response, may improve or maintain continence outcomes while mitigating risk and alter the approach to both POP and SUI surgery.

Keywords Pelvic organ prolapse · Stress urinary incontinence · Midurethral sling · Cystocele

Introduction

The consensus definition of pelvic organ prolapse (POP) is the falling, slipping, or downward displacement of one or more pelvic organs, including the uterus and vaginal compartments associated with neighboring bladder, rectum, or bowel [1]. The definition of POP and its components have remained essentially stable since the first joint terminology report by Bump et al. in the early 1990s [2],

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although the concept of POP as a common condition with an unfortunate impact on quality of life dates back to at least 1835 B.C.E, when the condition was described in ancient Egyptian papyri [3]. Historical treatment of prolapse was primitive and in many cases disturbing, based upon a poor understanding of pathophysiology and anatomy that did not begin to reflect our modern understanding of the condition until the sixteenth century.

Urinary symptoms such as stress urinary incontinence (SUI) commonly accompany POP but may not present until after the prolapse is treated. SUI, defined by the International Continence Society (ICS) as the "involuntary loss of urine on effort or physical exertion, or on sneezing or coughing" [4], was increasingly recognized as a condition requiring unique surgical approaches in the late 1800s [5]. Occult SUI is defined as SUI only observed after the reduction of co-existent prolapse [1, 4]. As understanding of the underlying pathophysiology of POP and SUI increased, so was the understanding that both conditions frequently coexisted; however, it was not until 1900 that Howard A. Kelly performed anterior colporrhaphy and bladder neck plication, the first routine

clinical procedure concomitantly addressing both POP and SUI [5, 6].

Unlike the ancient Egyptians or even the urogynecologic pioneers of the late nineteenth and early twentieth centuries, we now have a markedly improved understanding of the mechanisms underlying both POP and SUI, modern surgical techniques, and randomized trials guiding management of both conditions in isolation and concomitantly. However, important questions remain regarding appropriate evaluation for and treatment of occult SUI. Herein, we review the epidemiology of POP and occult SUI, the pathophysiology underlying their coexistence, management options, and novel therapies that may better balance efficacy and morbidity.

Epidemiology of POP and Occult SUI

POP and SUI share several common epidemiologic risks, such as aging, increased parity, and family history, although reduced bother from both conditions relative to comorbid pelvic floor or systemic disorders may decrease care seeking in the elderly [7, 8]. POP may be found in up to three quarters of women, increasing by 40% with each decade of life [9]. However, the prevalence of symptomatic POP is likely closer to 3–6% [10, 11, 12•]. SUI, when the diagnosis of mixed urinary incontinence (MUI) is also considered, has been estimated to be present in up to 50% of adult women [13]. Although the incidence of SUI increases with age, bother from SUI may be masked after the 4th or 5th decade of life due to a rising incidence of concomitant urgency urinary incontinence (UUI) [14, 15]. Obesity has been associated with an approximately 25–50% increased likelihood of symptomatic POP [16] and 3–4 times increased risk of severe SUI [17]. With the population of US women over age 65 years projected to rise from 27 million in 2016 to 40 million in 2030 [18] and rising rates of obesity, which already impacts approximately 40% of US adults [19], the already substantial burden of POP and SUI is likely to continue to increase.

In general, symptoms resulting from anatomic changes of POP may be experienced as vaginal bulge or pressure, and urinary symptoms, although not universally present or associated with prolapse, may include storage symptoms such as urinary urgency and frequency as well as voiding symptoms such as hesitancy, straining, and feeling of incomplete bladder emptying. The pathophysiology of voiding and subsequent storage symptoms may be related to bladder outlet obstruction (BOO) with resultant bladder wall hypertrophy and hypoxia, detrusor irritability, or upregulation of spinal reflexes [20]. Alternatively, bladder stretching such as in the case of a large anterior compartment prolapse may activate sensory neurons or allow urine to enter the proximal urethra with resultant overactivity [20]. Somewhat ironically given significantly overlapping etiology, SUI is frequently masked by advanced POP as a result of urethral kinking and BOO. The unmasking of incontinence with prolapse reduction is known as "occult" SUI.

Bothersome symptoms of SUI are reported by 40–50% of patients with symptomatic POP [21, 22]. Interestingly, in the Colpopexy and Urinary Reduction Efforts (CARE) trial, which attempted to exclude patients with clinical SUI, any SUI was reported by approximately 20% and bothersome SUI by 10%, underscoring the frequency with which these conditions coexist and challenges in diagnosis [23••]. The preoperative diagnosis of occult SUI is especially challenging but important, as this finding is generally thought to merit greater consideration for concomitant SUI surgery due to the increased likelihood of symptomatic SUI after prolapse repair. Occult SUI was present in 36% of women on preoperative urodynamics in the CARE trial but may be present in as many as 70–80% of women with advanced POP [24–27].

Clinical Evaluation

The evaluation of women considering operative management of POP must include a detailed assessment of symptoms potentially related to POP, specific questions on SUI and history of SUI prior to prolapse progression, comorbid conditions, and prior abdominal and pelvic or vaginal surgery. Pelvic examination is directed at objective quantification of POP, identification of urethral pathology including hypermobility and incontinence, pelvic floor strength and pelvic pain, and health of the external genitalia and vaginal epithelium.

Occult SUI may be suggested by a history of incontinence that improved or resolved completely as prolapse symptoms worsened, the need for manual reduction of prolapse in order to void, or the development of SUI with pessary use [10]. Ideally, assessment for occult SUI should be performed with the bladder full and the apex and anterior compartments effectively elevated to reduce the prolapse by any number of methods, including manual reduction, pessary, vaginal packing, cotton swab, ring forceps, or a half-speculum. A negative supine test should prompt testing in the standing position with the prolapse reduced. A secondary analysis of preoperative prolapse reduction methods from the CARE trial identified occult SUI in only 6% with a pessary versus 16–30% with all other methods, suggesting pessary reduction alone may be insufficient [28]. Examination by manual reduction, cotton swab, ring forceps, or a half-speculum was also imperfect, with positive and negative predictive values for postoperative SUI of 50–79% and 51–66%, respectively. Despite limitations in pessary reduction to identify incontinence on physical examination, an ambulatory pessary trial in women who can comfortably tolerate it may identify women with occult SUI not identified by other means [29].

The role of urodynamics (UDS) for the detection of occult SUI is unclear. The results of the Value of Urodynamic Evaluation (ValUE) trial, which demonstrated no difference in treatment success in women with uncomplicated SUI based upon receipt or non-receipt of UDS, have been extrapolated as evidence against the routine use of UDS for the evaluation of SUI alone [30]. However, women with high-grade prolapse were excluded from the ValUE trial, as alterations in anatomy associated with POP disqualified the designation of "uncomplicated." It is probably reasonable to assume that UDS adds little to a high-quality examination for the detection of occult SUI. However, UDS may identify BOO, present in 7–60% [31–33], and/or detrusor overactivity, present in 43% overall and 19% in the absence of demonstrable SUI [34]. Furthermore, UDS may provide another opportunity to reduce the prolapse, unkink the urethra, and assess for leakage with Valsalva and cough in patients in whom the practitioner has a high index of suspicion of occult SUI or in those patients who elect additional testing prior to informed decision making.

It is the authors' preference to selectively perform UDS in women undergoing surgery for symptomatic POP, in whom occult SUI is not elicited but a high index of suspicion remains. This is most relevant to women who would elect a concomitant sling procedure if occult SUI was demonstrated.

Outcomes of Concomitant Treatment

Burch Colposuspension

The CARE trial randomized 322 women with clinically absent SUI based upon report of "never" or "rarely" to the SUI-relevant questions in the Medical, Epidemiological, and Social Aspects of Aging (MESA) questionnaire undergoing abdominal sacrocolpopexy to concomitant performance of Burch colposuspension or no concomitant SUI surgery [23••]. Despite screening criteria, preoperative questionnaires suggested bothersome clinical SUI in 10%, and 36% had SUI with prolapse reduced on UDS.

At 3-month follow-up, SUI was reported by 34% randomized to concomitant Burch procedure versus 57% of controls ($p < 0.001$). Bothersome SUI was reported by 25% of controls versus 6% in the Burch group ($p < 0.001$). No significant differences were observed in storage LUTS or receipt of treatment or adverse events, although a higher MESA urge score was reported by controls (16.8 vs. 11.8, $p = 0.007$). At 2 years, bothersome SUI was reported by 12% in the Burch group and 25% of controls ($p = 0.004$) [35]. 7-year data were somewhat discouraging on multiple fronts. The incidence of recurrent POP symptoms (29% Burch, 24% controls) and de novo or recurrent incontinence of any kind (75% Burch, 81% controls) were substantial, and recurrent or de novo SUI was common.

However, its incidence was 15% lower among women randomized to receive a Burch procedure (Table 1).

Midurethral Sling

The Outcomes following Vaginal Prolapse Repair and Midurethral Sling (OPUS) trial randomized 337 women undergoing vaginal prolapse repair to retropubic midurethral sling (MUS) or sham surgery [38••]. Eligible women reported bothersome vaginal bulge symptoms on questions 4 or 5 of the Pelvic Floor Distress Inventory (PFDI) and had anterior vaginal prolapse defined by POP-Q Aa point ≥ 1 cm. Women with symptoms of SUI, defined by a positive response to any of 3 SUI-related questions on the PFDI, and those undergoing treatment for SUI with a pessary/incontinence device, pelvic floor physical therapy, or medication, were excluded [39].

At 3 months, the rate of urinary incontinence or treatment was 24% in women randomized to MUS and 43% in those randomized to sham ($p < 0.001$) and bothersome incontinence 9.4% vs. 24.8% ($p < 0.001$). Continence remained significantly better among women randomized to MUS at 12 months, even allowing for treatment (27.3% vs. 43.0%, $p < 0.01$). However, improved continence came at the expense of significantly greater risk of several adverse events associated with MUS, including bladder perforation, UTI, incomplete bladder emptying, and need for urethrolysis (Table 1).

Pooled Data

In a recently published meta-analysis, Baessler et al. examined 19 randomized controlled trials across 2717 women assessing the impact of concomitant SUI surgery at the time of prolapse repair [37]. The findings are summarized in Table 1. The authors determined the quality of evidence to be low to moderate overall. Their conclusions regarding the following SUI procedures at the time of prolapse repair were as follows:

- MUS appears to benefit incontinent women undergoing vaginal prolapse repair
- The benefit of Burch colposuspension in incontinent women undergoing abdominal sacrocolpopexy is uncertain
- Midurethral sling appears to be of benefit in women found to have occult SUI on examination
- The benefit of MUS in continent women at the time of vaginal prolapse surgery is uncertain
- The benefit of Burch colposuspension at the time of abdominal sacrocolpopexy in continent women is uncertain based upon widely conflicting results of two studies
- Native tissue prolapse repair may be associated with a lower risk of de novo SUI than vaginal mesh prolapse repair among continent women

Table 1 Summary of selected randomized controlled trials assessing outcomes for concomitant treatment of occult stress urinary incontinence at the time of surgery for pelvic organ prolapse

Trial	Randomization	Symptoms (intervention vs. control)	Bothersome symptoms (intervention vs. control)	Adverse outcomes (intervention vs. control)	Notes
Brubaker et al. [23••], Brubaker et al. [31], Nygaard et al. [36]	Abdominal sacrocolpopexy with Burch colposuspension vs. no Burch	SUI 3 months: 19.0 vs. 39.7% ($p < 0.001$) 24 months: 25.9 vs. 40.6% ($p = 0.016$) 7 years: 62% vs. 77% ($p < 0.05$)	SUI 3 months: 6.1 vs. 24.5% ($p < 0.001$) 24 months: 11.6 vs. 25.2% ($p = 0.004$)	3 months: 14.6 vs. 14.5% ($p = 0.79$) 24 months: 9.8 vs. 13.9%	Similar rates of bothersome urgency, UUI Mesh erosion rate 10.5% at 6.18 years
Wei et al. [32]	Vaginal prolapse repair with midurethral sling vs. sham	Any incontinence 3 months*: 23.6 vs. 49.4% ($p < 0.001$) 12 months**: 27.3 vs. 43.0% ($p < 0.01$)	Any incontinence 3 months*: 9.4 vs. 24.8% ($p < 0.001$) 12 months**: 11.4 vs. 18.8% ($p = 0.07$)	Within 12 months: Bladder perforation 6.7 vs. 0% ($p < 0.01$); UTI 31.0 vs. 18.3% ($p = 0.008$); major bleeding 3.0 vs. 0% ($p = 0.03$); Incomplete bladder emptying at 6 weeks 3.7 vs. 0% ($p = 0.01$); Urethrolysis 2.4 vs. 0% ($p = 0.06$)	No significant difference in storage symptoms at either timepoint Number needed to treat 6.3 to prevent one case of urinary incontinence at 12 months
Baessler et al. [37]	Meta-analysis of 19 RCTs	*When clinical SUI present:* Vaginal repair with vs. without concomitant sling (2 studies), 8–19% vs. 39%, RR 0.30 (95% CI 0.19–0.48) Vaginal repair with concomitant vs. delayed sling (1 study), RR 0.41 (95% CI 0.12–1.37) Abdominal sacrocolpopexy with vs. without Burch (1 study) RR 1.38 (95% CI 0.74–2.60) *When occult SUI present:* Vaginal repair with or without concomitant sling (5 studies), rate of SUI 10–22 vs. 38%, RR 0.38 (95% CI 0.52–1.07) *Continent women:* Vaginal repair with or without concomitant sling (1 study), RR 0.69 (05% CI 0.47–1.00) Abdominal sacrocolpopexy with vs. without Burch (2 studies), RR 1.31 (95% CI 0.19–9.01)			Conclusions: MUS likely reduces postoperative SUI Reasonable to stage MUS Pooled data not as convincing in favor of concomitant Burch

SUI stress urinary incontinence, *UUI* urgency urinary incontinence, *UTI* urinary tract infection, *RCT* randomized controlled trial

*Stress, urge, or mixed urinary incontinence or treatment for incontinence

**Stress, urge, or mixed urinary incontinence regardless of interim treatment

Other Procedures

What the meta-analysis by Baessler et al. indirectly highlights is a lack of randomized trials evaluating commonly performed concomitant procedures, such as midurethral sling at the time of sacrocolpopexy or fascial sling at the time of vaginal prolapse repair. Here, the data are primarily limited to retrospective comparisons and case series.

Barnes et al. reported absence of SUI in 36/38 women with high-grade prolapse and occult SUI undergoing concomitant fascial sling and vaginal prolapse repair [40•]. At 15 months mean follow-up, the rate of de novo UUI was 9.5% and resolution of preoperative UUI 45%. An earlier study evaluating a similar operative approach, although in which nearly half of women had preoperative clinical SUI, reported continence in the absence of further intervention in 85% at a mean follow-up of 20 months [41]. Chung et al. reported cure of SUI in 17/18 women undergoing pubovaginal sling and anterior repair with cadaveric dermis at an average follow-up of 28 months [42].

Retrospective data suggests retropubic tension–free vaginal tape (TVT) is more likely than Burch to result in freedom from SUI after abdominal sacrocolpopexy [43], findings congruent with the extensive body of literature demonstrating superiority of MUS over Burch for the treatment of SUI in non-prolapse clinical settings [44]. The efficacy of both pubovaginal and midurethral slings at the time of sacrocolpopexy mirrors that reported after vaginal prolapse surgery, although transobturator approaches may be less likely to result in freedom from SUI. At a mean follow-up of 3–5 years, Tubre et al. reported rates of freedom from SUI of 83.7%, 82.8%, and 66.7% for pubovaginal, retropubic, and transobturator slings, respectively, performed concomitantly at the time of sacrocolpopexy [45•].

Clinical Decision Making

For women with symptomatic SUI and POP, the decision to undergo concomitant anti-incontinence surgery is based on patient preference after weighing the risks and benefits of the additional surgery. For these patients, it is generally accepted that their incontinence is unlikely to improve and may worsen after prolapse surgery. Similarly, in patients with a history of SUI that improved after prolapse progression and in those with demonstrable occult SUI with prolapse reduction, the decision to undergo concomitant anti-incontinence surgery is based on patient preference after weighing the risks and benefits of the additional surgery. For these patients, it is generally accepted that they are at increased risk of postoperative symptomatic SUI but that it is not universally experienced. In women who do not demonstrate occult SUI and do not have a history of SUI, the consideration for concomitant anti-incontinence surgery is more challenging. Several approaches can be considered.

In a universal approach, a concomitant SUI procedure is recommended at the time of anterior and/or apical repair in all patients with the goal of reducing the burden of postoperative SUI and need for subsequent treatment. In a selective approach, concomitant SUI procedures are recommended only in women in whom a high index of suspicion exists, particularly if subsequent surgery would be expected to be challenging or associated with considerable surgical or medical risk. In a staged approach, performance of an SUI procedure is recommended as a subsequent surgery in women who develop bothersome SUI following prolapse repair.

Twiss et al. argued for a universal approach to concomitant SUI surgery based upon the limited ability of exam findings to predict postoperative incontinence [46]. Data from the CARE and OPUS trials are illustrative. Among controls in the CARE trial, de novo SUI developed postoperatively in 60% with occult SUI and 39% without occult SUI [23••]; in the OPUS trial, the rates of incontinence in women randomized to sham were 72% and 38% when occult SUI was present and absent, respectively [38••], indicative of the significant potential for postoperative incontinence even when not clinically present or identifiable on exam. Furthermore, a universal approach may also be the most cost-effective [47].

A universal approach would seem to be most appropriate when the risks of the concomitant SUI procedure are limited. This may be the case when a Burch colposuspension is added to sacrocolpopexy [35], even though a Burch is not risk free as an isolated procedure [48]. In contrast, a selective approach would be most appropriate when the risks of the SUI procedure are not insignificant, which may not be the case with a midurethral sling. The rate of concomitant SUI procedures increased from 38% to 47% between 2001 and 2009 [49]; however, there are several reasons why it is unlikely this trend continued. Although relatively uncommon, midurethral slings may be associated with unique complications such as mesh erosion or exposure, pain, discharge, decreased bladder sensation, and numbness [50]. Furthermore, despite being excluded from the 2011 FDA Public Health Notification informing of the significant risk of mesh-related complications in transvaginal prolapse repair, midurethral slings were not exempt from the ensuing litigation, resulting in several major manufacturers exiting the market altogether and increasing patient and clinician anxiety regarding this procedure [51]. Illustrative of this anxiety internationally, in July 2018, the National Health Services (NHS) placed a national "pause" on the use of surgical mesh to treat SUI, completely limiting access to synthetic slings in the UK [52]. It would seem, then, that although the midurethral sling remains the gold standard treatment by American urologic and gynecologic societies [53], in the current clinical and medicolegal landscape, its indiscriminate use has become increasingly fraught with risk.

Pubovaginal slings are a mesh-free alternative to the midurethral sling but are also not free from perioperative or long-term morbidity [48].

In an effort to optimize informed patient and clinician-decision making, Jelovsek et al. developed a predictive model for postoperative SUI in the absence of concomitant surgery in the OPUS trial, validated against the CARE trial cohort [54]. This model incorporates 7 clinical variables, including age, parity, body mass index, preoperative stress test, prior continence surgery, urgency urinary incontinence, and diabetes. With an area under the curve of 0.72, it outperformed expert clinicians and preoperative stress testing and may be a useful tool for clinicians pursuing a selective approach to concomitant SUI surgery.

A staged approach avoids unnecessary SUI surgery in all patients but may subject many others to a second operative procedure. In the OPUS trial, despite urinary incontinence in 49% in the sham group, only 25% reported bother and just 5% chose to undergo a sling procedure in the first year [38••]. Borstad et al. randomized women with POP and SUI to TVT at the time of prolapse repair or 3 months later, if required [55]. At 1 year, freedom from SUI was high in both groups, at 95% and 89% in the immediate and delayed TVT groups, respectively. Only 53/94 women in the delayed group underwent TVT, and 27% appeared to be free of symptoms of SUI by prolapse surgery alone.

In general, we prefer a selective approach to the concomitant use of anti-incontinence surgery at the time of POP repair, tailored in each case to the risk of incontinence, risk of the SUI procedure, acceptability of a subsequent surgery, and the patient's aversion to the relative risk of each. Due to greater efficacy, a sling, either midurethral or pubovaginal, is preferred to culposuspension. Patients with clinical or demonstrable occult SUI are presented the option of and offered a concomitant SUI procedure. All women, including those without clinical or occult SUI, are informed of the relative likelihood of SUI, potential need for subsequent surgery, and the risks of undesirable perioperative or long-term functional outcomes associated with anti-incontinence surgery. Such an approach appears to optimally balance outcomes and patient satisfaction, both in retrospective studies and in our clinical experience [56, 57].

Future Therapies

The "holy grail" of pelvic floor reconstruction are effective, durable therapies that incur limited risk to the patient. A treatment that promotes restoration of the patient's natural support mechanisms would presumably be closer to the ideal. Novel graft materials or cellular based therapies may hold such promise. They may also lead to a minimal risk anti-

incontinence procedure that permits a universal treatment approach to urethral management at the time of POP repair.

In a rabbit model, Roman et al. compared the host response to 2 established graft materials, polypropylene and polyvinylidene, to 2 alternative materials, poly-L-lactic acid (PLA) and polyurethane (PU) [58]. At 90 days, all materials maintained similar mechanical properties; however, the established materials were associated with chronic inflammation, whereas PLA and PU were associated with cell infiltration, neovascularization, and organized collagen formation, an encouraging finding. Furthermore, PLA is known to degrade in 1–2 years, a significant presumed benefit given justified aversion to permanent transvaginal mesh implants. Novel treatments for POP via mesenchymal stem/stromal cells derived from human endometrium are also in preclinical development and may hold considerable promise [59].

The use of stem cells to regenerate urethral closure mechanisms has moved beyond the preclinical phase. Peters et al. reported pooled data from 2 phase I/II clinical trials inclusive of 80 women with stress-predominant urinary incontinence who underwent intrasphincteric injection of autologous muscle–derived stem cells at variable doses (10, 50, 100, and 200×10^6 cells) [60]. Across all treatment groups, median stress leaks per 72 h improved significantly, from a baseline of 5–10 to 1–3 at 12 months. Pad weights improved significantly in the highest dose group, from a baseline of 86.4 g to 37.2 g at 12 months and trended towards improvement in all other groups. A randomized placebo–controlled phase III clinical trial across 29 sites is ongoing, with an estimated study completion date of June 2020 (https://clinicaltrials.gov/ct2/show/NCT01893138). Although promising, this therapy is unlikely to be a panacea in its current form. At the present time, the 6th International Consultation on Incontinence recommends against the use of stem cell technologies for SUI outside of the context of clinical trials [61].

Conclusions

POP and SUI are common conditions whose incidence is expected to rise due to an increasingly aging and comorbid patient population. Approximately half of women without clinical SUI may develop de novo SUI after prolapse repair, with an increased risk among women with demonstrable occult SUI or prior history of SUI preoperatively. A clinical approach which treats all women undergoing prolapse repair to a concomitant SUI procedure may subject up to 75% to the risk of these procedures without clear benefit. Therefore, we advocate a selective approach to concomitant SUI surgery that incorporates risk assessment and informed patient preferences. Novel therapies, including graft materials and stem cells that stimulate a regenerative response may improve or

maintain outcomes while mitigating risk and alter the approach to both POP and concomitant SUI surgery.

Compliance with Ethical Standards

Conflict of Interest   Joshua A. Cohn and Ariana L. Smith each declare no potential conflicts of interest.

Human and Animal Rights and Informed Consent   This article does not contain any studies with human or animal subjects performed by any of the authors.

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