

Native tissue repair of cardinal/uterosacral ligaments cures overactive bladder and prolapse, but only in pre-menopausal women

Dmitry Shkarupa¹, Anastasiya Zaytseva¹, Nikita Kubin¹, Gleb Kovalev¹, Ekaterina Shapovalova²

¹Saint Petersburg State University Hospital, Department of Urology, Saint-Petersburg, Russian Federation

²Saint Petersburg State University Hospital, Department of Gynecology, Saint-Petersburg, Russian Federation

Citation: Shkarupa D, Zaytseva A, Kubin N, Kovalev G, Shapovalova E. Native tissue repair of cardinal/uterosacral ligaments cures overactive bladder and prolapse, but only in pre-menopausal women. Cent European J Urol. 2021; 74: 372-378.

Article history

Submitted: Oct. 5, 2020

Accepted: May 27, 2021

Published online: June 30, 2021

Corresponding author

Gleb Kovalev
Saint Petersburg
State University Hospital
Department of Urology
36 Universitetskaya
199034 Sankt-Peterburg,
Russian Federation
Kovalev2207@gmail.com

Introduction The aim of this article was to study the effect of native tissue cardinal/uterosacral ligament repair on overactive bladder (OAB) and pelvic organ prolapse (POP).

Material and methods Inclusion criteria included decrease of urge symptoms following insertion of a gauze tampon in the posterior fornix of vagina ('simulated operation'). Exclusion criteria included SUI, POP grades 3–4. The surgery consisted of plication of cardinal/uterosacral ligaments. Post-operative assessment was performed at 3, 6, 12 and 18 months after surgery and included evaluation by stage of prolapse, Urinary Distress Inventory Short Form 6 (UDI-6), Overactive Bladder Questionnaire (OAB-q), Pelvic Floor Impact Questionnaire- Short Form 7 (PFIQ-7), and International Consultation on Incontinence Questionnaire – Urinary Incontinence Short Form (ICIQ-SF) questionnaires and voiding diary.

Results At 3 months, cure rates for frequency, urgency, nocturia and prolapse were comparable. By the 6-month review, catastrophic failure commenced in the postmenopausal group, parallel for all parameters, starkly contrasting with premenopausal group. At 18 months, % cure rates for pre-menopausal (post-menopausal in brackets) were 79.6 (15.4) for POP, 67.3 (20.5) for urgency, 87.7 (20.5) for nocturia and 59.2 (15.4) for frequency.

Conclusions We hypothesize the stepwise parallel recurrence of POP and symptoms in the menopausal group was a consequence of collagen deficiency in the plicated ligaments. Nevertheless, plication of uterosacral-cardinal ligament complex is simple, inexpensive, effective, especially applicable pre-menopausally for POP and as an alternative treatment option in that difficult group of pre-menopausal women who have major OAB/nocturia symptoms but only minimal prolapse.

Key Words: overactive bladder ↔ pelvic organ prolapse ↔ surgical reconstruction of the pelvic floor ↔ ligamentoplasty ↔ uterosacral-cardinal ligament complex ↔ Integral Theory

INTRODUCTION

The female population is ageing, and relatedly, incidence of prolapse and overactive bladder (OAB)/nocturia is increasing. According to the International Continence Society (ICS), prolapse and bladder symptoms are related, but the exact relationship is unknown [1]. Traditionally, anterior and posterior repair of the vagina either with or without mesh placed behind it, have been the most recent methods

applied to repair prolapse. Both methods have been placed in serious doubt by the PROSPECT study [2] which reported up to 83% failure rate for both at 12 months after surgery, with severe incontinence falling from 17% to 8% (native tissue) and vaginal mesh from 21% to 8%.

According to the ICS definition, 'overactive bladder' is characterized by symptoms of urgency (with or without urge incontinence), frequency and nocturia [1]. More than 100 million people worldwide suffer

from this problem [3]. Up to the present day there is no consensus in the treatment for OAB. There are many different concepts regarding OAB causation. The most popular are the neurogenic and myogenic theories, autonomous and urothelial hypotheses [4–8]. Available treatment options for OAB are weak. They are often not effective and may have significant side effects [9, 10]. The absence of a ‘gold standard’ in the treatment for OAB demands a search for new treatment options. These were searched for and summarized by Peyronnet et al. and described as ‘phenotypes’ each with a future potential treatment [11]. No treatment data was presented for these phenotypes. Not mentioned by Peyronnet was a special group of women who have minimal pelvic organ prolapse (POP stage I–II) and major OAB symptoms [12–15]. An important cause of OAB according to the Integral Theory of P. Petros and U. Ulmsten, is the inability of the pelvic muscles to provide tension for the vaginal fascia supporting the urothelial stretch receptors ‘N’ (Figure 1), because the ligaments against which the muscles contract are loose. This can lead to increased afferent signals and activation of the micturition reflex which is perceived by the cortex as urge, frequency, and at night, nocturia [16] (Figure 1). There are a number of studies reporting a decrease of OAB symptoms after cure of POP [17, 18]. It is achieved not only by surgical reconstruction of the pelvic floor, but also with the use of vaginal devices (pessaries) [15] and in our study, temporarily by a gauze roll used as a pessary to support the weakened uterosacral-ligaments ‘L’, (Figure 1). The uterosacral ligaments (USL) lengthen ‘L’ and are unable to suspend the vagina adequately. The downward/backward muscle forces (arrows)* lengthen by ‘L’ and weaken. The vagina cannot be stretched sufficiently to support the stretch receptors ‘N’. ‘N’ fire off increased afferent impulses at a low bladder volume and this is perceived by the cortex as urgency. If the quantum of afferents is sufficient to activate the micturition reflex, the efferent from the micturition centre are activated; the forward muscles relax; the backward muscles open out the urethra; bladder contracts; the patient may uncontrollably lose urine (‘urge incontinence’). The prospect of significant OAB cure with a minimally invasive ligament repair operation is an appealing low-cost option for any nation which has deprived areas with limited financial resources. Currently, there is no clear understanding by learned bodies of the role of surgery in the treatment of the patients suffering from OAB.

* the wavy form and pink colour of the arrows denote weakened muscle contractile force.

The aim of this article was to analyze the functional and anatomical outcomes of the reconstructive surgery for the uterosacral-cardinal ligament complex in patients who had OAB associated with low-stage POP.

MATERIAL AND METHODS

Bearing in mind the many different possible ‘phenotypes’, we performed a screening test to ensure the etiology was consistent with inability of the ligament/muscle complex to control the premature micturition concept (Figure 1). The patient was examined with a bladder sufficiently full for the patients to have urgency symptoms. A gauze roll was gently inserted into the posterior fornix of the vagina such as to support the uterosacral ligaments where they insert into the cervical ring. The gauze roll insertion was carried out by a doctor and consisted of a sterile gauze tampon insertion for a period from 24 to 36 hours. The result was evaluated by patients subjectively and objectively in the voiding diary for a period of gauze inserting. Only patients who reported a decrease of frequency throughout the day and at night, as well as a decrease or elimination of urgency were qualified for the surgery.

Inclusion criteria were women with symptomatic POP stage I–II [Baden-Walker (BW) classification] with concomitant OAB and positive gauze roll test.

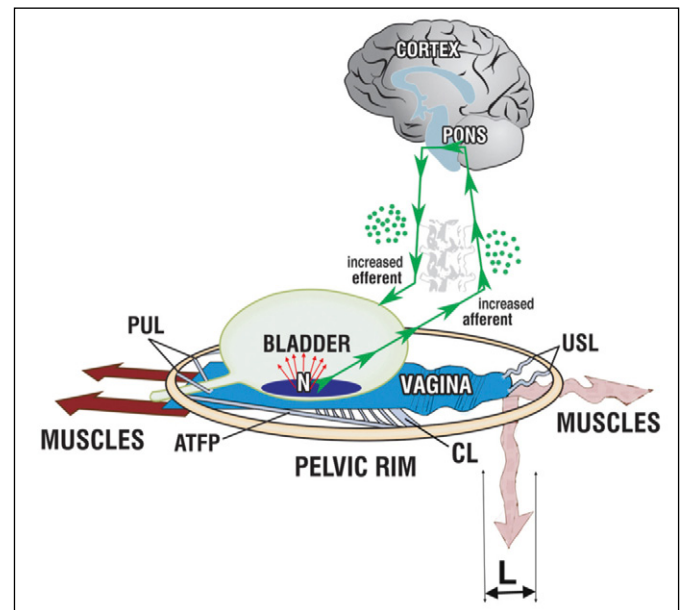


Figure 1. Urge incontinence as interpreted by the Integral Theory.

Reproduced with permission from: Petros P. Pathogenesis of OAB and surgical treatment according to the Integral Theory Paradigm. *Gynecol Obst Clin Med.* 2021; 1: 56.

OAB was defined as the presence of frequency (more than 8 times a day), nocturia (more than 2 or more times during the night's sleep), and urgency with or without urinary incontinence.

Exclusion criteria for the study were: POP stage III–IV, stress urinary incontinence (SUI), any reconstructive operations for pelvic floor in the history, age <18 years, cancer in a different location, psychiatric diseases.

The preoperative examination of the patients included review of the presenting complaints, history of the disease, gynecologic examination of the patient and definition of POP stage and type, cough test (to exclude concomitant SUI), as well review of the results from the completed validated questionnaires [Urinary Distress Inventory Short Form 6 (UDI-6), Overactive Bladder Questionnaire (OAB-q), Pelvic Floor Impact Questionnaire – Short Form 7 (PFIQ-7), and International Consultation on Incontinence Questionnaire – Urinary Incontinence Short Form (ICIQ-SF)] and voiding diary (frequency throughout the day and night, and number of urgency episodes throughout the day were assessed).

All patients were provided with information on the procedure technique, risks and possible complications. Prior to surgery, informed written consent was taken from all patients.

Surgery

For the period from May 2016 to December 2016, 88 women underwent surgery for pelvic floor reconstruction by the cardinal-uterosacral ligament (CL/USL) complex plication.

The surgery (Figure 2) consisted of the plication of uterosacral-cardinal ligament complex along the midline of the anterior semicircle of the cervix. Therefore, the access was performed through an incision in the anterior vaginal wall, the blunt dissection towards the ligaments was carried out. Two-three non-absorbable sutures (Ftorex 1 USP, braided, coated polyester suture) approximated the right and left uterosacral-cardinal ligament complexes. The vaginal wall was sutured with PGA 2-0 USP.

The control examinations were carried out at 3, 6, 12 and 18 months postoperatively. The examination included the same assessment tools as before the surgery.

Statistical analysis

The obtained clinical results in the course of the work were analyzed using the STATISTICA for Windows software system (version 10 License BXXR310F-964808FA-V). The Shapiro-Wilk normality test was

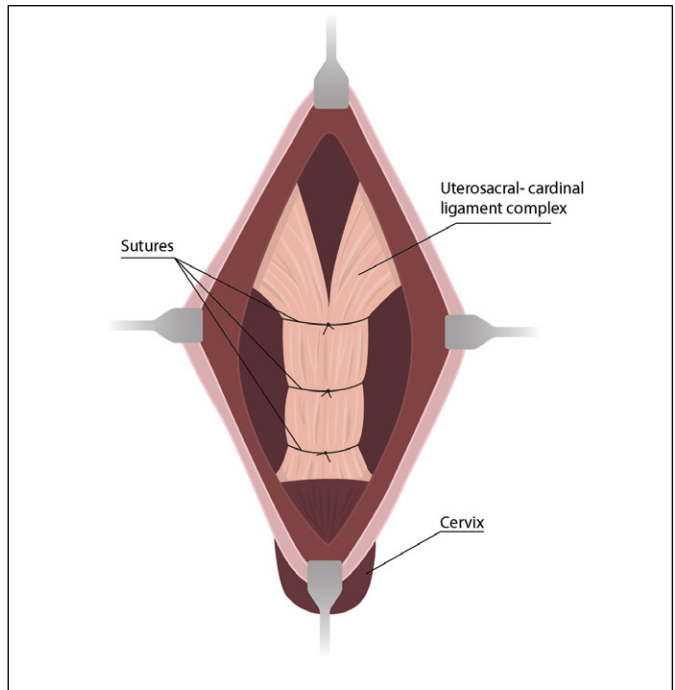


Figure 2. Step of the surgery – approximation of right and left ligament us complexes using Ftorex 1 suture.

performed for quantitative data. The quantitative variables are described using the mean and standard error of the mean, as well as by the median 25 percent and 75 percent quantiles. The t-test criterion was used to compare the effects of treatment options (in groups) over normally distributed data. The Mann-Whitney test was used for data which distribution was different from normal. The dynamics for normally distributed data between several time points was assessed using the repeated measures ANOVA; for data which distribution differs from the normal the nonparametric Wilcoxon test was used (in the case of 2 time points) or the Friedman test. The dynamics was studied in each group separately.

RESULTS

The study included 88 women. The patients were divided into two groups according to the reproductive age: the pre-menopausal (25–55 years old) and the post-menopausal (56–82 years old) groups. The characteristics of patients from each group are shown in Table 1.

During the preoperative phase, all patients underwent the gauze rolltest, whose purpose was to determine the indications for surgical treatment and predict the intended outcome. The patients were qualified for surgery only in case of positive effect which was confirmed by the decrease in OAB symptoms

Table 1. Characteristics of common parameters of patients from Group 1 and 2 (n = 88)*

Parameter	Group 1 n = 49	Group 2 n = 39
Average age	42.3 ±1.1 43.0 (37.0; 48.0)	63.7 ±0.9 64.0 (59.0; 66.0)
Height	165.4 ±0.9 165.0 (162.0; 170.0)	162.0 ±1.0 164.0 (159.0; 167.0)
Weight	71.4 ±1.62 71.0 (61.0; 79.0)	71.3 ±1.8 68.0 (66.0; 80.0)
BMI	26.1 ±0.56 25.5 (22.8; 28.0)	27.0 ±0.74 26 (24.0; 30.0)

*Data are presented as median (M) ± standard deviation (s.d.); Me (Q1;Q3)
n – number; BMI – body mass index

Table 2. The outcomes of the test with gauze roll

Indicator	Group 1		Group 2	
	Before surgery	Test with gauze tampon	Before surgery	Test with gauze tampon
Frequency*	13.71	12.1	14.77	12.18
Nocturia*	3.6	2.45	3.97	2.7
Urgency	2.4*	1.4**	2.2*	1.9**

*p < 0.05; **p < 0.001 – statistically significant differences

based upon the voiding diary. The outcomes of the preoperative test with gauze roll for both groups are shown in Table 2.

The follow-up period of all 88 patients was 18 months with monitoring at 3, 6, 12 and 18 months. The efficiency of surgical treatment was assessed with analyzing of the results of the voiding diary completion for the period of 48 hours. The prolapse stage was assessed via the BW classification. The cure rate during the study is shown in the Table 3. The cure

Table 3. Comparative cure rate (%) of POP and overactive bladder symptoms in different points of follow-up

POP/OAB symptoms	Pre-menopausal group (n = 49)	Post-menopausal group (n = 39)
3 months		
Frequency	71.5	64.1
Urgency	85.7	82
Nocturia	96	64.1
POP	98	89.7
6 months		
Frequency	77.5	48.7
Urgency	85.7	64.1
Nocturia	98	59
POP	85.7	48.7
12 months		
Frequency	63.3	38.5
Urgency	81.6	33.3
Nocturia	71.5	25.6
POP	85.7	20.5
18 months		
Frequency	59.2	15.4
Urgency	67.3	17.9
Nocturia	87.7	20.5
POP	79.6	15.4

POP – pelvic organ prolapse; OAB – overactive bladder; n – number

was defined by the decreasing of OAB symptoms: frequency less than 8 times per day, nocturia less than 2 times per night, absence of urgency.

The influence of the surgery on patient's quality of life was assessed with the questionnaires UDI-6, PFIQ-7, OAB-q, ICIQ-SF. The dynamics of efficiency in different periods of follow-up is shown in Table 4.

Table 4. Dynamics of the subjective efficiency of surgical treatment for both groups depending on the follow-up period according to standardized urological questionnaires on the quality of life. Statistical indicator, M ± 6 Me (Q1; Q3)

Questionnaire	Group 1 (n = 49)					Group 2 (n = 39)				
	Before surgery	3 months	6 months	12 months	18 months	Before surgery	3 months	6 months	12 months	18 months
UDI-6	7.5 ±0.6 8.3 (4.2; 8.3)	1.2 ±0.3 0 (0; 1)	0.41 ±0.3 1 (0; 1)	2.2 ±0.31 1 (1; 4)	3.2 ±0.4 3 (1; 5)	8.23 ±0.59 9 (6; 11)	1.79 ±0.39 1 (1; 5)	2.46 ±0.39 1 (0; 5)	4.7 ±0.5 5 (1; 8)	6.15 ±0.4 6 (5; 8)
OAB-q	21.9 ±6.5 21 (17; 28)	7.63 ±0.3 7 (6;)	8.06 ±0.27 8 (6.9)	9.63 ±0.5 9 (8; 11)	10.8 ±0.6 9 (9; 11)	20.0 5±1.1 18 (14; 27)	8.44 ±0.48 8 (7; 9)	9.41 ±0.4 9 (8; 11)	12 ±4.8 11 (9; 12)	13.6 ±5.5 11 (10; 15)
PFIQ-7	32.2 ±12.64 32 (32; 35)	4.9 ±0.28 4 (4; 6)	5.4 ±0.3 5 (4; 7)	7.2 ±0.5 6 (5; 9)	7.4 ±0.5 7 (5; 9)	38.4 ±2 34 (31; 45)	6.31 ±0.59 8 (3.9)	7.33 ±0.43 8 (6; 9)	9.6 ±3 9 (8; 12)	15.2 ±6 14 (11; 17)
ICIQ-SF	5.08 ±0.44 6 (3; 7)	1.1 ±0.51 1 (1; 2)	1.35 ±0.1 1 (1; 2)	1.9 ±0.2 1 (1; 3)	4 ±0.2 4 (2; 4)	5.87 ±0.42 6 (4; 8)	1.59 ±0.15 1 (1; 2)	2 ±0.2 2 (1; 3)	3.4 ±2.3 3 (2; 5)	6.4 ±2.4 6 (5; 8)

n – number; UDI-6 – Urinary Distress Inventory 6, Short Form; OAB-q – Overactive Bladder Questionnaire; PFIQ-7 – Pelvic Floor Impact Questionnaire – Short Form 7; ICIQ-SF – International Consultation on Incontinence Questionnaire – Urinary Incontinence Short Form

No cases of intraoperative injury to the bladder and rectum, as well as bleedings that required blood transfusion were detected during the study. In the postoperative period, pelvic pain syndrome developed only in one patient (6 months after surgery). The pain was eliminated with twice conducted medicine blockade with local anesthetics.

While analyzing the research data, the improvement of subjective and objective indicators was found in both groups for the follow-up periods of 3 and 6 months. It was objectively noted that the cure of POP was directly associated with the cure of OAB symptoms and symptom failure with the recurrence of POP (Table 3).

DISCUSSION

The recent banning of mesh kits by the Food and Drug Administration (FDA) and other regulatory bodies added to the PROSPECT study's poor results for any type of surgery involving the vagina, and have left pelvic floor surgeons with few options to help women suffering from POP. The vagina is essentially a weak elastic membrane involved in urethral closure (continence) and evacuation (micturition) [16]; it is not structural. It is the ligaments which are structural [16]. In this regard, it is worth noting that our methodology, plication of ligaments without excision of vagina, is really a less invasive evolution of the 100-year-old Manchester/Fothergill operation [19]. Our data shows it is a much more effective option than classical vaginal repair [2], at least in pre-menopausal women.

In our study, we revealed that gauze roll test and subsequent surgical treatment (CL/USL plication) were initially effective in 88 patients with OAB in both pre- and post-menopausal women (Figure 2). This fact evidences that existing myogenic, neurogenic and autonomous hypotheses are insufficient as explanations of all OAB causes. However, both interventions, gauze insertion which supports USLs and surgical plication, appear to confirm the predictions of the Integral Theory [16] which states that opposite muscle forces contract against the suspensory ligaments to provide tension of the vagina in the manner of a trampoline to support the bladder base stretch receptors (Figure 1). Looseness in these ligaments will weaken the muscle forces, weakening underlying support of the stretch receptors; the se-fire off at a lower bladder volume to stimulate the micturition reflex, perceived by the cortex as urgency, frequency during the day and at night, nocturia. This scenario explains the pathway for the 'urothelial hypothesis' [11] which states that urothelium constitutes the 'stretch receptors' (Figure 1).

The effectiveness of POP surgery for decreasing OAB symptoms was published in several studies [15, 17, 18].

Also, no more than 5–6% patients with de novo OAB followed reconstructive POP surgery [20]. Importantly, our study demonstrated that cure of OAB also holds for patients with minor POP. This validates the ICS statement that prolapse and OAB are related [1].

Short-term follow-up (3 months) revealed high efficacy of the surgery in both groups. However, by 6 months, a massive simultaneous failure in both POP, OAB and nocturia had commenced, much more manifest by 18 months (Table 3). This result focused our attention on the question: "What was the pathogenesis of the steady linear decline in cure rate for both symptoms and anatomical cure to the catastrophic levels at 18 months in the post-menopausal group?"

According to the Integral Theory [16], ligament weakness is mainly a consequence of collagen loss. It is known that estrogen level decline in post-menopausal women leads to leaching out of collagen from the body, leading to osteoporosis and many other structural problems related to bone and connective tissue. As collagen is also the main structural component of ligaments and vaginal connective tissue, it is our hypothesis that the loss of estrogen after the menopause leads to leaching out of collagen from the vagina and its supporting ligaments to cause both POP and symptoms such as urge and nocturia (Table 3). Supporting our hypothesis is the finding that hydroxyproline, a key breakdown product of collagen doubles after menopause and continues to excrete at that rate afterwards [21]. This process brings irreversible changes to the anatomical structures which provide organ support. The ligaments lose the necessary collagen, elastin and glycoproteins [22]. We have demonstrated that the efficiency of ligamentoplasty is far higher in women of reproductive age, 80% cure (pre-menopausal) vs 16.7% cure (post-menopausal) at 18 months. These data confirm other studies that show surgical reconstruction with native techniques (including ligamentoplasty) in conditions of estrogen deficiency does not provide a long-term effect [23, 24].

Our data demonstrates very clearly that USL plication in older women cures neither POP nor OAB significantly beyond 3 months, which raises two questions: 1. Will the pre-menopausal group suffer the same fate after menopause as the post-menopausal group due to collagen leaching out of the repaired ligaments? 2. What can be done about the post-menopausal group who have the highest incidence of POP and OAB in the population?

We view the whole problem as a collagen issue. Our protocol which we regularly apply in our university is that older women need a posterior sling tape to create new collagen[25]. This collagen-creation technique was developed in 1990 on experimental animals [26]. It is the basis of the mid urethral sling operation (MUS). It explains cure of POP and symptoms by laparoscopic or robotic sacrocolpopexy: the mesh acting as a firm anchoring point for the posterior vectors (arrows, Figure 1), much in the way of a vaginally inserted posterior sling [25].

CONCLUSIONS

The plication of uterosacral-cardinal ligament complex in patients with POP stage I–II associated with OAB symptoms provides high anatomical and functional efficiency, but only in pre-menopausal patients.

DISCLOSURE OF INTERESTS

The authors declare a lack of financial, personal, political, intellectual or religious interests.

FUNDING

The study was initiated and supported by the Saint Petersburg State University Hospital. Financial assistance from other centers is not provided.

DETAILS OF ETHICS APPROVAL

This study was approved by the local research ethics committee of Saint Petersburg State University Hospital meeting dated 14.5.2019.

AUTHORS' CONTRIBUTIONS

Research concept and design: Shkarupa D., Kubin N.

Collection and processing of material: Kovalev G., Zaytseva A.

Data analysis and interpretation: Shapovalova E., Shkarupa D.

Writing and editing text: Zaytseva A., Kovalev G.

References

- Abrams P, Cardozo L, Fall M, et al. The standardisation of terminology in lower urinary tract function: report from the standardisation subcommittee of the International Continence Society. *Urology* 2003; 61: 37-49.
- Glazener CM, Breeman S, Elders A, et al. Mesh, graft, or standard repair for women having primary transvaginal anterior or posterior compartment prolapse surgery: two parallel-group, multicentre, randomised, controlled trials (PROSPECT). *Lancet*. 2017; 389: 381-392.
- Milsom I, Abrams P, Cardozo L, Roberts RG, Thüroff J, Wein AJ. How widespread are the symptoms of an overactive bladder and how are they managed? A population-based prevalence study. *BJU Int*. 2001; 87: 760-766.
- De Groat WC. A neurologic basis for the overactive bladder. *Urology*. 1997; 50:36-52.
- Brading AF. A myogenic basis for the overactive bladder. *Urology*. 1997; 50: 57-67.
- Drake MJ, Mills IW, Gillespie JI. Model of peripheral autonomous modules and a myovesical plexus in normal and overactive bladder function. *Lancet*. 2001; 358: 401-403.
- Gillespie JI: The autonomous bladder: A view of the origin of bladder overactivity and sensory urge. *BJU Int*. 2004; 93: 478-483.
- Kanai A, Fry C, Ikeda Y, Kullmann FA, Parsons B, Birder L. Implications for bidirectional signaling between afferent nerves and urothelial cells-ICI-RS 2014. *Neurourol Urodyn*. 2016; 35: 273-277.
- Wein AJ. Association between anticholinergic medication use and cognition, brain metabolism, and brain atrophy in cognitively normal older adults. *J Urol*. 2016; 196: 1500.
- Wein AJ. Costs of urinary incontinence and overactive bladder in the United States: a comparative study. *J Urol*. 2004; 172: 1216.
- Peyronnet B, Mironska E, Chapple C, et al. A Comprehensive Review of Overactive Bladder Pathophysiology: On the Way to Tailored Treatment. *Eur Urol*. 2019; 75: 988-1000.
- Cardozo L. *Urogynaecology*. Churchill Livingstone, London, 1997.
- Beck R.P, McCormick S, Nordstrom L. A 25-year experience with 519 anterior colporrhaphy procedures. *Obstet Gynecol*. 1991; 78: 1011-1018.
- Handa VL, Garrett E, Hendrix S, Gold E, Robbins J. Progression and remission of pelvic organ prolapse: a longitudinal study of menopausal women. *Am J Obstet Gynecol*. 2004; 190: 27-32.
- de Boer TA, Salvatore S, Cardozo L, et al. Pelvic organ prolapse and overactive bladder. *Neurourol Urodyn*. 2010; 29: 30-39.
- Petros PE, Ulmsten U. An integral theory of female urinary incontinence. *Acta Obstet Gynecol Scand* 1990; 69: 1-79.
- Fletcher SG, Haverkorn RM, Yan J, Lee JJ, Zimmern PE, Lemack GE. Demographic and urodynamic factors associated with persistent OAB after anterior compartment prolapse repair. *Neurourol Urodyn*. 2010; 29: 1414-1418.
- Miranne JM, Lopes V, Carberry CL, Sung VW. The effect of pelvic organ prolapse severity on improvement in overactive bladder symptoms after pelvic reconstructive surgery. *Int Urogynecol J*. 2013; 24: 1303-1308.
- Fothergill WE. Pathology & the operative treatment of displacements of the pelvic viscera. *Journal of Obstets Gynaecol of the British Empire*. 1907; 13: 410-419.
- de Boer TA, Kluivers KB, Withagen MIJ, Milani AL, Vierhout ME. Predictive factors for overactive bladder symptoms after pelvic organ prolapse surgery. *Int Urogynecol J*. 2010; 21: 1143-1149.
- Sone T, Miyake M, Takeda N, Fukunaga M. Urinary excretion of type I collagen crosslinked N-telopeptides in Healthy Japanese adults: Age- and sex-related changes and reference limits. *Bone*. 1995; 17: 335-339.

22. Goepel C. Differential elastin and tenascin immunolabeling in the uterosacral ligaments in postmenopausal women with and without pelvic organ prolapse. *Acta Histochem.* 2008; 110: 204-209.
23. Tinelli A, Malvasi A, Rahimi S, Negro R, Vergara D, et al. Age-related pelvic floor modifications and prolapse risk factors in postmenopausal women. *Menopause.* 2010; 17: 204-212.
24. Kökçü A, Yanik F, Çetinkaya M, Alper T, Kandemir B, Malatyalioglu E. Histopathological evaluation of the connective tissue of the vaginal fascia and the uterine ligaments in women with and without pelvic relaxation. *Arch Gynecol Obstet.* 2002; 266: 75-78.
25. Shkarupa D Kubin N Pisarev A Zaytseva A Shapovalova E. The hybrid technique of pelvic or-gan prolapse treatment: apical sling and subfascialcolporrhaphy. *Int Urogynecol J.* 2017; 28: 1407-1413.
26. Petros P.E & Ulmsten U, Papadimitriou J. The Autogenic Neoligament procedure: A technique for planned formation of an artificial neo-ligament. *Acta Obstet Gynecol Scand Suppl.* 1990; 153, 69: 43-51. ■