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POST-PROSTATECTOMY AND ACQUIRED VOIDING DYSFUNCTION (V TSE, SECTION EDITOR)

Sling Procedures for Post Prostatectomy Incontinence: What Devices Are Out There? and What Are Their Results?

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Abstract

Purpose of Review With increasing rates of radical prostatectomy, post prostatectomy incontinence represents a growing challenge for urologists. The artificial urinary sphincter is a highly effective treatment but is invasive, expensive and associated with mechanical failure. Over the past two decades, a plethora of minimally invasive sling devices to treat post prostatectomy incontinence have become available. This review aims to describe the various male slings available and the evidence supporting their use.

Recent Findings Various sling devices are available, each with limited generally low quality evidence reporting their efficacy and safety. Few comparative studies are available.

Summary Evolving mid-term data suggest that male slings are an effective alternative to artificial urinary sphincter for well-selected patients. Further study is needed to provide higher quality particularly comparative evidence to support the continued use of male sling devices.

Keywords Male sling · Advance · Virtue · ATOMS · Argus · REMEEX

Introduction

Radical prostatectomy (RP) is an effective treatment for localised prostate cancer but has significant implications for lower urinary tract function. Post prostatectomy incontinence (PPI) represents the most common aetiological factor for stress urinary incontinence (SUI) in men and results in significant negative impact on quality of life [1]. The prevalence of post prostatectomy incontinence varies significantly depending on the how it is defined and the time point following surgery that it is assessed. While improvements in surgical technique and understanding of the pathophysiology of PPI has led to improved rates of continence, most men undergoing RP will experience urinary incontinence in the early stages following surgery. Reported rates of incontinence at 1 month range from 45 to 87% [2, 3]. The majority of these men will experience improvement with time and the application of conservative measures including pelvic floor exercises with rates reaching plateau after around 2 years [4]. Prevalence of persistent PPI is reported at between 1 and 40% depending on the precise definition used [5]. Importantly, PPI rates elicited from patient-reported symptoms are significantly higher than those from physician's observations, and similarly, higher rates are seen when self-reported questionnaires are used compared to pad testing [6, 7]. While not all patients with ongoing PPI will seek surgical intervention, an estimated 6–9% of men undergoing RP subsequently undergo surgery for SUI [8].

Options for surgical treatment of PPI include urethral bulking, implantation of an artificial urinary sphincter (AUS), non-circumferential compression device (ProAct®), or a male sling. Urethral bulking injections which offer only limited efficacy and durability are not recommended as a curative option [9, 10]. AUS implantation, although highly effective [10], is more invasive and expensive and poses a significant risk of requiring revision surgery due to infection, erosion or mechanical failure. In addition, the requirement

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for manipulation of the scrotal control pump to permit voiding can exclude some patients from undergoing this procedure. Literature supporting the use of the ProAct device is limited and the reported explantation rate is high [9]. Given the above, over the last two decades, male sling procedures for PPI have become increasingly popular representing an effective yet minimally invasive alternative to the AUS, which avoids the complications associated with a mechanical device.

The idea of a sling type device for creating urethral compression to treat male SUI was first described in the early 1960s by Berry [11] and further developed by Kaufman and Kishev in the 1970s [12, 13]. However, following the development of the artificial urinary sphincter in the same decade, interest in sling devices declined until 1998 when Schaeffer et al. described the use of Dacron bolsters placed under the bulbar urethra and suspended from the rectus fascia. In the initial series of 64 patients, 56% were described as dry and 8% 'significantly improved' at median follow-up of 18 months [14]. However, the procedure was associated with a high need for revision and, over the following two decades, was superseded by a number of different sling designs including initially bone-anchored devices and subsequently trans obturator slings and more recently hybrid and adjustable devices.

Mechanism of Action of Male Slings

The mechanism by which a male sling provides improvement in SUI is not completely understood and varies between different devices. All sling types provide some degree of static urethral compression with the aim of increasing the outlet resistance during storage without significantly compromising voiding function. In addition, some trans obturator devices cause proximal relocation of the bulbar urethra, which has been hypothesised to increase the functional membranous urethral length and augment residual sphincter function [15]. In addition, imaging studies using transperineal ultrasound have demonstrated that appropriately positioned male slings can provide dynamic compression of the urethra during straining similar to the female mid urethral sling [16].

Contemporary male slings can be classified as either fixed or adjustable devices. In this review, we aim to present an overview of the devices available and the literature supporting their use.

Fixed Slings

Bone Anchored

The development of bone-anchored male slings obviated the need for suprapubic incisions of the previously utilised bulbourethral sling and hence became an attractive minimally invasive treatment for PPI in the early 2000s. The bone-anchored sling works by providing broad-based compression of the urethra, imparting outlet resistance through a sling that is stably fixed to the urethra. Advantages of this approach include a single perineal incision, stable fixation to the bony pelvis and virtually no risk of injury to the bladder. In 2001, Madjar et al. first described in the literature the use of a bone-anchored sling, looking at 16 men with PPI and found at 1-year follow-up (mean 12.2 months) that 75% were using less than one pad per day and the remaining 25% improved [17]. A similar study in 2002 by Comiter et al. looked at 21 men with SUI and showed that 76% were continent at 12 months whereas at 24-month post sling, the cure rate had decreased to 67% [18].

The InVance (American Medical Systems Minnetonka, Minnesota, USA) uses a multiperforated polyester sling coated with silicone positioned under the bulbar urethra via a vertical midline perineal incision. The sling is attached to both ischiopubic rami by three titanium screws. Fassi-Fahri et al. in 2007 looked at 50 men who had SUI and showed at 6-month follow-up: 50% were dry, 26% improved and 24% suffered treatment failure [19]. Reports of significant complications related to the bone screws including chronic pain, infection and osteitis pubis and the advent of alternative slings not requiring bony fixation have led to the decline in the use of bone-anchored slings, and currently, no bone-anchored sling is commercially available.

Transobturator Fixed Slings

AdVance

What would become the AdVance (Boston Scientific formerly American Medical Systems, Massachusetts, USA) sling was originally described by Rehder and Gozzi in 2007 [15]. Their initial paper reported results from cadaveric placement and a small clinical series of 20 men. They reported increase in retrograde leak point pressures > 60 cm H20 following appropriate tensioning of the sling along with a mean increase in membranous urethral length of 14 mm and improvement in urethral closure pressure from 13.2 [8–22] to 86.4 (70–100) cm H2O without any significant change in Qmax. Overall, at 6 weeks, 40% were reported as cured (no pads) whilst 30% were improved.

Placement of the AdVance involves a midline perineal incision with dissection continued to expose the underlying bulbospongiosus muscle, which is then opened. The corpus spongiosum is mobilised from the perineal body by dividing the central tendon allowing anterior and cranial relocation of the bulb by around 3 cm when the sling is appropriately tensioned. The trochars are passed in an out to in fashion through the obturator foramen from a point, just below the adductor

longus tendon to a point as high as possible in the triangle formed by the inferior pubic ramus and urethra. The sling arms can then be pulled back to the groin wounds; the central portion of the mesh is secured to the bulb, and the sling then tensioned prior to wound closure.

Over the past decade, the AdVance has become the most commonly implanted male sling device with the largest body of literature supporting its use. However, this is still largely based on single-centre series with a minority of multicentre series and few comparative studies. Rehder reported results from a multicentre European series with a cure rate (dry) of 53.8% and improved (using 1–2 pads with 50% reduction in pad use from baseline) of 23.1% at 12 months which was maintained at 3-year-follow up with cure and improved rates of 53% and 23.8% respectively [20]. Using a similar definition of success (dry and 50% reduction in pad use), Zuckerman et al. were unable to show the same durability of efficacy with success rates declining from 74% at 12 months to 63% at 2 years and 62% at final follow-up (mean 36 months) [21]. Similarly, Li et al. reported progressive decrease in efficacy from initial follow-up to 2 years with mean pad use rising from 0.8 to 1.7/day and the number of patients using two or fewer pads/day falling from 87 to 62.5% [22]. Risk of poor durability of success with AdVance placement has been reported as particularly high in those patients with a history of radiotherapy or storage dysfunction on urodynamics with a recent series reporting a return to baseline levels of incontinence at 3 years of follow-up [23•]. Nevertheless, in well-selected patients, the AdVance represents an efficacious and safe treatment for PPI. Reported adverse events are generally mild with transient urinary retention and groin pain being the most frequently encountered, and the need for explantation is exceedingly low [20, 21].

AdVance XP

In an attempt to improve durability of results, the AdVance XP (Boston Scientific Massachusetts, USA), a second generation transobturator sling, was released in 2010 which incorporated tensioning fibres, chevron anchors to limit sling slippage and altered trochars to aid placement in larger patients. No major change to the surgical technique was required. Thirty-six-month data from a multicentre series was published by Bauer in 2017 with 66% of patients cured (no pads) and 23.4% improved [24•]. Direct comparison of mid-term results between AdVance and AdVance XP has been reported by three authors [25, 26, 27•]; no significant differences in outcomes were seen although in one study, a higher rate of urinary retention was observed with AdVance XP [27•].

I-STOP TOMS

The I-STOP TOMS (CL Medical, France) is a monofilament polypropylene 4-arm sling (2 arms on each side) measuring 4.5 cm × 1.4 cm, with a 2.8-cm central part placed over urethra. It is placed via a transobturator approach in the lithotomy position with a vertical perineal incision [28]. The perineal aponeurosis and superficial fascia are then incised but no muscle dissection is undertaken, and the central tendon is not divided. The sling allows for either an outside-in or inside-out approach with the helical needle used along with blunt dissection to guide the passage through the transobturator space. The two arms on each side are passed through the space simultaneously then repeated on the opposite side. Once in position, the sling is tensioned symmetrically after being sutured to the bulbospongiosum muscle [28].

The I-STOP TOMS developed from the female I-STOP transobturator sling and is the market leading male sling in France. Grise et al. described it initially as a two-arm sling but subsequently, the larger four-arm was released [28, 29]. It was proposed the larger surface area in addition to the fact that the sling is designed to be placed more distally, over the bulbar and post-bulbar urethra, which has bulbospongiosum coverage, would reduce the risk of erosion [28]. Early publication by Grise et al. (2009) looked at 50 men with mild to moderate SUI undergoing TOMS (2 arm) implantation [28]. Twelve months postoperatively, they found 30% were dry with 32% using one pad per day with global improvements of QoL (SF36 and ICIQ) scores [28]. In a later study, Grise et al analysed 103 men with the I-STOP TOMS (4 arm) and showed improved continence outcomes, 59% dry and 20% using one pad per day at 12-month follow-up [29]. They documented nil significant complications within 12 months [29]. Three other studies have looked at 12-month outcomes for the I-STOP TOMS [30–32] and have demonstrated in smaller patient numbers ($n = 26$–40) that 50–58% of patients were cured at 12 months. Two-year outcomes reported by Drai et al. and Ej-Jennane, which have shown reduced dry rates of 47% and 17% respectively with no reporting of any significant complications [30, 33]. The longest study to date is Malval et al. who analysed 100 patients with a median follow-up of 58 months demonstrated 40% dry rate with 77% using one pad or less per day at 1 year decreasing to 15% and 22% respectively at 5 years post insertion [34•]. Eighteen patients required further surgical procedures for recurrence of incontinence, with the majority opting for AUS (12/18) [34•]. Apart from device failure, the only other reported complication was persistent pain in one patient that lasted for more than 2 years [34•].

Hybrid Fixed Slings

Virtue

The Virtue male sling (Coloplast, Denmark) is a four-arm polypropylene mesh hybrid sling with two transobturator (TO) arms and two prepubic arms. This sling was designed to provide both urethral relocation and prepubic compression [35]. The surgical technique is similar to other transobturator sling surgeries. With the patient in lithotomy position a vertical perineal incision is made, dissection then proceeds to the bulbospongiousus which is retracted superiorly to allow division of the perineal body attachments and permit mobilisation. Then, the J-hook passer is used to pass through the transobturator space, and the transobturator sling arms are passed through the transobturator space with an inside-out technique. This is repeated on the opposite side. Next, requires the prepubic sling arms to be passed, so two small suprapubic stab incisions are made, two fingerbreadths lateral to midline. Then, the J-hook passer is moved through the prepubic space to the perineal surgical area just lateral of the urethra. The sling is then attached and pulled through the prepubic space with the J-hook and repeated on the opposite side. The sling is then tensioned via the transobturator arms, then prepubic arms by using the retrograde leak point pressure, with a target pressure of 60 cm H20 [35–37].

Comiter published results of the first Virtue series in 2012 [35] focusing on improvement in retrograde leak point pressures. This was followed in 2014, by a multicentre trial which is the largest study to date ($n = 129$) [38]. The study had two phases, one with the traditional sling fixation as described above and a second where the prepubic and transobturator sling arms were fixed to their opposite side in an effort to improve outcomes. Twelve-month data demonstrated only 42% of the traditional fixation group achieved objective or subjective success (defined has reduction > 50% pad weight or "much" or "very much" better on the Patient Global Impression of Improvement respectively). In the group undergoing the additional fixation steps, subjective and objective success was 70.9% and 79.2% respectively. Neither group developed any complications greater than Clavien grade 1 in the 12-month follow-up period [38]. Three smaller studies have looked at longer-term outcomes with 22–55-month follow-up periods [39, 40•, 41]. Sourial et al. followed 48 men over 22 months and demonstrated that using cystoscopic-guided sling tensioning compared to retrograde leak point testing gave significantly worse continence outcomes 39% vs 70% respectively with 14 patients undergoing repeat procedures due to ongoing incontinence [41]. McCall et al. also identified high failure rates when they analysed 32 patients with a median follow-up of 55 months with 21 (68%) of patients deemed to have had unsuccessful slings with 6 (20%) requiring subsequent AUS implantation [40•]. This is in contrast to 11 (32%) who were improved. They identified prior radiotherapy was strongly related to treatment failure. Additionally, 2 (7%) of the patients reported significant persistent pain but no other long-term complications were mentioned [40•]. Contrasting these results, Ferro et al. looked at 29 men over 3 years and found overall patients were globally satisfied and used no more than one pad per day although 58% (17/29) of the patients experienced complications but all were mild (Clavien grade 1) and transient [39].

Adjustable Slings

The increasing popularity of the male sling has led to the design of adjustable slings to allow for alteration in the compression offered by the device post insertion in response to either persistent incontinence or voiding dysfunction. Three commercially available, adjustable slings have been released since 2006—Argus, REMEEX and ATOMS.

Argus

The Argus male sling (Promedon, Argentina) was first described in the literature by Romano et al. [42]. Promedon has released the Argus adjustable sling system as a retropubic sling and also a transobturator model named the Argus T. The Argus system is composed of a silicone cushion attached to two silicone cone columns that are passed with needles through appropriately depending on the approach, and the sling is then tensioned via assessment of retrograde urethral pressure [42]. Romano published three-year outcomes in 2009 [43]. They followed 47 patients of which 31 (66%) were dry, 6 improved (one pad per day) and 10 failed (2 or more pads per day). ICIQ-SF decreased from 19.5 to 6. Of the ten patients who failed, nine had their sling removed; six because of erosion and three due to infection [43]. In 2011, three other studies analysed 2–3 year outcomes of the Argus male sling [44–46]. These studies showed similar explantation rates with 16% (37 of 225 patients across three studies), with the majority due to erosion (19/37) followed by infection (11/37) [44–46]. Cure rates were varied considerably depending on exact definition but 17–79% of patients were reported dry at follow-up. There were a number of complications reported but the vast majorities were transient and minor (Clavien grade I–II) [44–46].

More recently, results of a multicentre series in 182 patients using the Argus T have been published by Siracusano et al. [47]. At a median follow-up of 22 months, overall success was 86.2%, of which 33% were dry and 53.2% were improved [47]. Success decreased with worsening baseline incontinence from 95% in mild incontinence, 78% in moderate incontinence and 70% in severe incontinence. The explantation rate was 9.3% with the majority due to infection and 30% of

patients required at least one adjustment [47]. Cornel compared inguinal-perineal incision to single-perineal incision for the Argus T and showed that at 12 months postoperatively, there were more infective complications in the inguinal approach compared to the single incision approach, and four patients required explantation, three inguinal vs one single incision suggesting that operative approach can affect outcomes for this device [48].

Chung et al. compared the Argus to Advance male sling in 44 patients [49•]. They found that 25/44 patients preferenced the Argus over the Advance. 6/25 required adjustment procedures, and 23/25 Argus patients were socially continent vs 16/19 with Advance at 2 years postoperatively with similar improvements in QoL domains [49•]. Lima et al. compared the Argus T to the Advance male sling in 22 patients in an RCT over with 18-month follow-up [50]. This small trial found slightly superior results for the Argus T with < 1 pad per day in seven vs five patients. However, this was at the expense of increased rates of explantation of Argus T in four patients (3 erosion, 1 pain) compared to none from the Advance group [50]. Finally, Lim et al. compared the Argus to AUS (AMS 800) in 33 patients with moderate PPI [51]. At 2-year follow-up, they demonstrated success rate (one safety pad or less per day) of 85% compared to 73% for AUS [51].

REMEEX

REMEEX® (Neomedic, Spain) stands for Readjustable Mechanical External device [52]. It was originally based on designs for a suburethral sling for female SUI but was subsequently modified for men. The REMEEX system involves a 1.5 × 3 cm polypropylene suburethral sling connected to a mechanical regulator via traction threads [52, 53]. The mechanical regulator is a subcutaneous permanent implant, (the varitensor), which is implanted over the rectus fascia above the pubis and permits adjustment of the sling externally. The sling is inserted via a retropubic approach and a transverse suprapubic incision, and a combination of blunt and sharp dissection is used to allow a modified Stamey needle to be passed. The traction threads are then drawn through the passage created with the needle until the polypropylene sling mesh is in full contact with the bulbocavernous muscle without pressure. The threads are attached to the varitensor device and the wounds closed. The morning after the surgery, the sling is tensioned appropriately with the external manipulator [52, 53].

There are few studies reporting outcomes for this device in the literature. Sousa-Escandon et al. first published in 2004 with a six-patient case series, and at 18 months post operation, 5/6 patients were not using any pads and later led a multicentre trial in Europe that was published in 2007 analysing 51 men with a mean follow-up of 32 months [52, 53]. 64.7% were considered cured; 19.6% improved and 15.7% failed [53]. Three patients had their sling removed; one due to erosion and two due to infection. Four other smaller studies have looked at outcomes with this sling with mean follow-up between 12 and 40 months. Thirty-six to 54% of patients were using no pads. [54–57]. The majority of patients required multiple sling adjustments (mean adjustments 2.4–3.71 per patient) with two patients requesting to have the sling removed due to the ongoing adjustment requirements [54]. Across all studies where complications were discussed, the most common reason for sling removal was infection.

ATOMS

The ATOMS (AMI, Austria) is a transobturator sling with an adjustable silicone cushion that can be inflated/deflated via a titanium port [58]. Three models have been released with different port arrangements. The original design utilised an inguinal port and subsequent designs have used a scrotal port. The surgical technique is similar for any outside in transobturator sling with the port being tunelled subcutaneously in scrotum. The cushion is left deflated for the first postoperative weeks wherein an outpatient setting saline can be injected into the port to inflate the cushion appropriately, often initially at 4–6 weeks post implantation and adjusted every 6 weeks until the pressure is optimal [58].

Published data on outcomes of ATOMS has been building since Seweryn and Hoda published their findings in 2012–2013 respectively. Seweryn et al. followed 38 patients for 17 months and showed 60.5% of patients were using one pad or less per day [59]. Hoda et al. reported 63% of their 99 patients to be dry at 18-month follow-up with an additional 29% of patients improved. The mean adjustments per patient was 3.8. Four patients required removal due to port infection [58]. Krause et al. reported less success in 36 patients of whom only 38.9% of men achieved "social continence" and 11 (30.6%) underwent device removal, the majority due to infection [60]. More recent publications in 2017 and 2018 have looked at longer-term outcomes with two studies reporting on 3-year outcomes in larger populations (see Table 1) [61•, 62–64, 65].

The largest study to date is Mühlstädt et al. 2018, who analysed 363 men with an average follow-up of more than 3 years (40 ± 27 months) [61•]. They found overall at final follow-up that 50% of men were dry, and 32% improved by at least 50% with an average adjustment rate of 3.4 ± 2.1 per person. Interestingly, they analysed the different models separately and showed similar continence and QoL outcomes but significantly higher complications requiring explantation in the inguinal port group compared to the two scrotal port options [61•]. The predominant indication for device removal was late infection/erosion in 30/37 cases with 24/30 from the inguinal port group alone. The preconnected scrotal port had the lowest complication rate overall [61•]. Friedl et al.,

Table 1 Key published results by device

Author (ref)	Year of publication	Device	Follow up	*N*	Continence results
Rehder et al [20]	2012	AdVance	12 months	156	53% dry and 23% improved at 1 and 3 years
Zuckerman et al [21]	2014	AdVance	36 months	102	74% dry/improved at 12 months 62% at final follow-up
Cornu et al [26]	2014	AdVance vs XP	21 months AdVance 16 months XP	231	AdVance–dry/improved 79% AdVance XP–dry/improved 76%
Bauer et al [25]	2015	AdVance vs XP	24 months AdVance 12 months XP	80	AdVance–dry/improved 69% AdVance XP–dry/improved 90%
Husch et al [27]	2018	AdVance vs AdVance XP	55 months AdVance 31 months XP	128	Advance–PGI-I better 82.6% AdVance XP–PGI-I better 80.8%
Bauer et al [24]	2017	AdVance XP	36months	115	66% of patients dry and 23.4% improved
Grise et al [29]	2012	I-STOP TOMS	12 months	103	59% dry, 20% 1 pad/day
Galiano et al [31]	2016	I-Stop TOMS	12months	34	58% dry, 23.5% 1 pad/day
Malval et al [34]	2017	I-STOP TOMS	12 months	100	40% dry, 37% 1 pad/day
Comiter et al [38]	2014	Virtue	12 months	129	Without fixation—15% dry, with fixation - 46% dry
Ferro et al [39]	2017	Virtue	36 months	29	59% dry, 38% 1 pad/day
McCall et al [40]	2016	Virtue	55months	32	31% less than 2 pads/day
Bochove-Overgaauw [44]	2011	Argus	27 months	100	54% (51) dry
Hubner et al [46]	2011	Argus	25months	101	79% (80) dry
Chung et al [49]	2016	Argus vs AdVance	36 months	44	92% < 1 pad/day Argus vs 84% AdVance < 1 pad/day
Siracusano et al [47]	2017	Argus T	22 months	182	33% < 1 pad/day
Lima et al [50]	2016	Argus T vs AdVance	18 months	22	78% Argus T < 1 pad/day vs 45% AdVance < 1pad/d
Sousa-Escandon et al [53]	2007	REMEEX	32 months	51	49% dry, 16% < 1 pad/day
Leizour et al [55]	2017	REMEEX	31 months	25	36% < 1 pad/day
Muhlstadt et al [61]	2018	ATOMS	40 months	383	50.1% < 1 pad/day
Friedl et al [62]	2017	ATOMS	12 months	287	64% < 1 pad/day

another large multicentre study of 287 men, with 31-month follow-up showed similar continence rates of 64% of men dry and 26% improved but had 20% ($n = 56$) explantation rate [62]. Of note, the majority of the removals were described as being due to titanium intolerance (41%, $n = 23$), device leakage (21%, $n = 12$) at the port or cushion and infection (21% $n = 6$ early, $n = 6$ late). Additionally, to leakage, device failure through dysfunction and dislocation leads to a further eight devices being removed but they did not discuss potential for such high numbers [62].

Conclusion

Stress urinary incontinence post radical prostatectomy is common and significantly impairs quality of life. At present, the artificial urinary sphincter is considered the gold standard surgical treatment for post prostatectomy incontinence; however, the male sling offers a less invasive, non-mechanical and cheaper alternative. There are various different sling designs available which can be broadly categorised into either fixed or adjustable devices. The literature supporting the use of male slings in general is limited and of low quality. In particular, adjustable devices appear to be associated with higher complication and explantation rates relative to fixed slings, and there is currently no evidence of superior outcomes, and as such, only fixed slings are supported as a treatment choice in current European Association of Urology guidelines [9]. Although high level comparative evidence is lacking, it is generally accepted that sling procedures show good efficacy in mild to moderate incontinence that may be comparable to AUS but that results to slings in in severe incontinence, and in irradiated cases are likely to be poor and, here, the sling represents a less favourable alternative to AUS placement. As data matures from each of the sling designs, the ability to select the optimal sling for a particular patient will hopefully

improve. The MASTER trial is a UK multicentre randomised, non inferiority trial comparing male sling surgery to AUS in men with any degree of SUI who are suitable for surgery which will go some way to providing higher level evidence relating to the comparative effectiveness of male sling surgery [66] and the results of which will be keenly awaited.

Compliance with Ethical Standards

Human and Animal Rights All reported studies with human subjects performed by the authors have been previously published and complied with applicable ethical standards (including the Helsinki declaration and its amendments, institutional/ national research committee standards and international/national/institutional guidelines.

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