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TITLE

Canine urethral sphincter pressure profile under incremental inflation of an artificial cuff: a cadaver study.

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SUMMARY

Objectives: This preliminary study aimed to determine if artificial urethral sphincter filling volume is proportional to peak pressure exerted on the urethra.

Methods: Urethral pressure profilometry was performed in five female, medium sized, mixed-breed canine cadavers following artificial urethral sphincter placement. Maximum urethral pressure was recorded following sequential incremental inflation of 0.15mL and compared to baseline pressure and between dogs using a two-way ANOVA.

Results: Artificial urethral sphincter placement in cadavers was associated with an increase in urethral pressure, which was significantly correlated with artificial urethral sphincter volume. The correlation was non-linear and demonstrated considerable individual variation. Maximum urethral pressures after artificial urethral sphincter placement exceeded those reported in conscious continent dogs within a narrow volume range, in which a 0.15mL infusion more than doubled maximal urethral pressures.

Clinical implications: Rapid increases in urethral pressure from the artificial urethral sphincter over a small range of filling volumes (0.15mL increments) might explain why some clinical cases can become suddenly dysuric following incremental inflations. We suggest that smaller increments of filling (0.05-0.1mL) may achieve finer pressure control.

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INTRODUCTION

Urinary incontinence may be defined as involuntary loss of urine during bladder filling or storage (Reichler and Hubler, 2014). Urethral sphincter mechanism incompetence (USMI) is the most common cause of urinary incontinence in dogs (Holt, 1990a) with a prevalence of 20% in neutered bitches (Arnold *et al.* 1989). Neutering is a confirmed risk factor (Holt and Thrusfield, 1993). The pathophysiology of USMI in neutered dogs is incompletely understood, although reduced urethral pressure is implicated, since significant reduction in urethral pressures have been recorded in incontinent compared to continent bitches (Arnold, 1997). Salomon and colleagues (2006) reported that the mean urethral closing pressure significantly reduces post-spaying with a lag period of at least 6 months. Structural urethral changes, including reduced smooth muscle mass and increased collagen content, have also been described (Noel *et al.*, 2010).

USMI is treated medically with the α-adrenergic agonist phenylpropanolamine and estriol with success varying from 75 to 95% (Mandigers and Nell, 2001, Bacon *et al.*, 2002, Noel *et al.*, 2010). Surgical treatments for USMI are usually pursued when medical treatment fails (Claeys *et al.*, 2010a). Described techniques include colposuspension (Holt, 1990b), urethropexy (White, 2001, Martinoli *et al.*, 2014), sphincter bulking (Byron *et al.*, 2011), trans-obturator vaginal tape (Claeys *et al.*, 2010b) and artificial urethral sphincters (AUS). Colposuspension is the surgery most commonly performed for treatment of USMI, and its long-term outcome is successful in 53% cases (Holt, 1990b).

The use of AUS for management of urinary incontinence in dogs by manipulating urethral pressure profiles was first reported in cadavers (Adin *et al.*, 2004). A significant increase in both urethral closing pressure and cystourethral leak point pressure was recorded at 50% occlusion compared to baseline. Adin and co-workers (2004) measured 'percentage occlusion' of the AUS device *ex vivo* by measuring digital images of the AUS lumen at various increments of filling. This was then correlated with urethral closing pressures achieved after AUS placement and inflation to the predetermined volumes of filling equivalent to 0%, 25% and 50% occlusion. However, it is difficult to interpret these results when an AUS is already *in situ* in a patient and the level of functional urethral occlusion cannot easily be determined. A more useful parameter would be the occluder

filling volume correlated with pressure exerted on the urethra, which can be measured clinically by profilometry, as this may help to direct clinicians on how much AUS inflation is required after placement.

Outcomes following AUS placement have been reported in prospective (Rose *et al.*, 2009) and retrospective (Reeves *et al.*, 2012, Delisser *et al.*, 2012, Currao span style="font-family:Verdana; font-style:italic">et al., 2013, Gomes *et al*., 2018, Morgan *et al*., 2018) studies.   Overall, AUS placement has a good outcome with up to 80% of cases reported ascompletely continent, without the need for additional medical treatment at 12 months post-operatively (Gomez *et al*., 2018). Minor complications were seen in 30 to 81.8% of cases at varying periods post-operatively and resolved with either no, or minimally-invasive, treatment. Major complications such as urethral obstruction, requiring surgical intervention, were recorded in 7 to 27% of cases and these were managed by AUS deflation or removal (Delisser *et al.*, 2012, Reeves *et al.*, 2012, Currao *et al.*, 2013, Gomes *et al*., 2018). Port-dislodgement, and peri-AUS fibrosis requiring surgical correction have also been reported (Morgan *et al*., 2018).

Study Aims

AUSs are typically placed uninflated and repeated inflations of 0.1 to 0.5mL of saline are carried out on persistently incontinent dogs over prolonged follow-up periods (Delisser *et al.* 2012).   Clinical experience of using AUSs has shown that it can be challenging to manage persistent incontinence via sequential cuff inflation without inducing dysuria in dogs (Gomes *et al*., 2018). The aim of this study was to perform a preliminary investigation to determine if the volume of AUS filling is proportional to the peak pressure exerted on the urethra, and investigate whether the relationship between AUS filling and pressure exerted is linear. In order to derive clinically useful data, small increments of infusion volume were used to reflect the range of volumes used clinically (0.1mL to 0.5mL).

MATERIALS AND METHODS

Cadavers

Five female, medium-sized (15-22kg), mixed breed (four Staffordshire bull terrier crosses, one collie cross) dogs were euthanised for reasons not related to this study. Cadavers were obtained frozen and defrosted for 48 hours before testing took place. A ventral midline coeliotomy was performed from umbilicus to the pubic brim, with the cadaver supported in dorsal recumbency. The bladder was retracted cranio-ventrally to expose the urethra and vagina. An episiotomy was performed and a urinary catheter was placedto identify the urethra. The peri-vaginal fat was reflected cranially, and blunt dissection was performed around the proximal urethra approximately 2 cm caudal to the bladder neck.   This zone of dissection was extended sufficiently along the urethral axis that the full width of the AUS could be passed around the urethra without the cuff becoming pinched by tissues. The circumference of the urethra at this site was measured in each cadaver using a piece of free tape and loosely placing iaround the urethra. The corresponding length was measured against a ruler. A 10mm (internal diameter) x 14mm (width) AUS (DOCXS Biomedical Products, California, USA) was inflated maximally with Hartmann's solution to displace any air, test for leakage and to unstick any areas of adhesion between the silicone leaves of the cuff. The fluid was removed to leave onlythe priming volume before placement. The uninflated AUS was placed around the proximal urethra and sutured in place with 4-0 Nylon. A stab incision was made in the lateral body wall adjacent to the occluder, and the AUS connector tubing was tunnelled into the subcutaneous space lateral to the body wall where it was connected to an injection port.

Urethral pressure profilometry

Urethral pressure profiles (UPP) were measured as previously described (Life-Tech, 2008, Goldstein and Westropp, 2005). Briefly, a 7F double-lumen UPP catheter (Life-Tech, Vermont USA) was used to generate a urethral pressure profile whist being withdrawn from a start point in the bladder neck into the urethra and past the site of the AUS. An automated syringe driver (Alaris GH, CareFusion,

Basingstoke, UK), connected to the UPP catheter under tension, was set up with a primed

10mL syringe discharging at a rate of300mL/h to withdraw the UPP catheter at a constant rate of

0.5mm/s (Goldstein and Westropp, 2005). The UPP catheter was attached to a 3-way

tap and connected to a fluid infusion pump and to a pressure monitor (SurgiVet Advisor Vital Signs

Monitor, Smiths Medical, Massachusetts USA) via a fluid column. The pressure sensor was zeroed to

atmospheric pressure at the level of the urethra. The UPP catheter was inserted into the urethra,

palpated and positioned with the pressure transducer cranial to the bladder neck, the approximate

distance of the transducer from the cranial edge of the AUS was recorded. The catheter was infused

with isotonic fluids (Hartmann's) at a rate of 2mL/min (Goldstein and Westropp, 2005). In

each cadaver, profilometry was performed before placement of the AUS, following placement of

the AUS with a priming volume, and with incremental doses of 0.15mL reflecting a clinical approach

to AUS inflation (Delisser *et al.*, 2012). Pressure readings were recorded at 10 second intervals as the

catheter was withdrawn from the urethra; each interval represented a 5mm

distance. Maximal observed urethral pressures were recorded for each increment of occluder volume.

Each volume was tested in triplicate. Separate occluders and UPP catheters were used in each

cadaver, and UPP was recorded with the cadaver in dorsal recumbency.

Statistics

Statistics were carried out using GraphPad Prism 5. The effect of incremental inflation of the AUS by

0.15mL inflations was measured using a two-way ANOVA. Changes in urethral pressure measured at

each level of inflation were compared to the background urethral pressure with the AUS placed, but

uninflated. A/span>P value of 0.05 was used to indicate a significant difference. Urethral pressure

values achieved following AUS inflation were described as higher or lower than those pressures

recorded in clinically normal dogs, as reported by Fischer and co-workers (2003).

RESULTS

The effect of AUS placement on UPP

Urethral size at the site of AUS placement ranged from 15-25 mm (median 20 mm; Table 1). Dogs 2, 3 and 5 showed no change in urethral pressure after placement of an uninflated AUS. In Dog 1, a reduction in urethral pressure from 15.7 to 11.3 cm H_2O was detected after AUS placement but this difference in pressure profile did not coincide with the position of the AUS. Dog 4 demonstrated a doubling of urethral pressure from 13.3 before AUS placement to 27.0 cm H_2O after placement of an uninflated AUS. This increase in urethral pressure was at the approximate position of the AUS, indicating that the uninflated AUS may have been exerting pressure at this point.

Effect of AUS inflation on maximal urethral pressure

An increase in maximal urethral pressure (above background levels) was observed in each cadaver following incremental inflation of the AUS (P <0.0001) (Table 1). When represented graphically, the increase in maximal urethral pressure followed a sigmoid curve (Figure 1): (i) a first portion of the curve representing the infused volume needed to obtain a significant change in maximal urethral pressure above the uninflated pressure; (ii) an exponential rise in pressure with incremental inflation of liquid; (iii) a plateau phase.

During the first phase of the inflation, the AUS significantly increases maximum urethral pressure above baseline when the cuff is uninflated but the magnitude of this effect was variable between dogs: Dog 1 required 0.9mL, Dog 2 required 0.3mL, Dog 3 required 0.6mL, Dog 4 required 0.75mL and Dog 5 required 0.6mL.

The clinical implications of the pressure changes recorded are best understood when compared to normal urethral pressure; 146.5+/- 41.9 cm H_2O (Fischer *et al.*, 2003). According to this data, the minimum urethral pressure in normal, conscious, continent dogs was 104.6 cm H_2O. This pressure could be achieved after AUS placement andincremental inflation in each cadaver (Figure 1), although the target volume of inflation varied between dogs. From a clinical stand point, the variations observed were large because in some dogs, a 0.15mL infusion led to more than a doubling of the maximal urethral pressure: (i) in Dog 1 the maximum urethral pressure was 63.7 cm H_2O at

0.9mL of filling and increased to 159.3 cm H_2O when the AUS was filled to 1.05mL; (ii) in Dog 2 an increase in urethral pressure from 84.3 cm H_2O to 187.7 cm H_2O occurred when the AUS was filled from 0.3mL to 0.45mL; (iii) in Dog 3 the maximum urethral pressure at 0.75 mL of inflation was recorded as 62.0 cm H_2O and increased to 132.7 cm H_2O after the addition of another 0.15mL to the AUS. In other dogs, the relative change in pressure was smaller but did reach values above normal urethral pressure for a single 0.15mL increment: (i) in Dog the maximum urethral pressure at 0.75 mL of inflation was 98.7 cm H_2O, increasing to 193.3 cm H_2O after another 0.15mL inflation to 0.9 mL; (ii) Dog 5, which demonstrated the smallest increase in urethral pressure to reach the normal range, recorded a maximum urethral pressure of 56.0 cm H_2O at 0.6mL of

filling increasing to 108.3 cm H_2O when the AUS was filled to 0.75mL. Following incremental filling of the AUS, the urethral pressure profiles indicated that increases in pressure exerted on the urethra are localised to the section of the urethra surrounded by the AUS (data not shown).

DISCUSSION

While this is not the first study to assess the use of AUS in canine cadavers, no previous study has correlated incremental AUS inflation with the maximal pressure exerted on the urethra. Normal urethral pressure in conscious, continent dogs has been reported as 146.5+/- 41.9 cm H_2O (Fischer *et al.*, 2003). As shown by Adin and colleagues (2004), the urethral pressure observed in a cadaver model is much lower than that in conscious dogs. Despite this, the effect of hydraulic AUSs in the treatment of USMI is independent of muscle tone, and cadavers may still be used to demonstrate relevant patterns of response, even if the recorded pressures are not within physiologically normal limits (Adin *et al.*, 2004). Following this it was considered more acceptable, in the initial instance, to perform preliminary investigations using cadavers rather than studying live companion dogs.

The results of this study demonstrate that the placement of an AUS around the proximal urethra is associated with incremental increases in urethral pressure capable of exceeding 300 cm H_2O. This is above normal resting urethral pressure (Fischer *et al.*, 2003). However, the relationship observed is both non-linear and considerably variablebetween cadavers. This finding supports clinical observations that some USMI cases treated with AUSs will require greater cuff inflation volumes than others to reach continence, with some cases becoming continent following AUS placement without any additional inflation, while others necessitate repeated deflations or removal (Reeves *et al.*, 2012, Delisser *et al.*, 2012, Gomes *et al.*, 2018, Morgan *et al.*, 2018). This important patient variation in addition to the intensive follow-up management and potential need to resolve complications makes the placement of AUS devices undesirable for most primary care practices.

The clinical relevance of a 'significant increase' in urethral pressure following inflation of the AUS from the uninflated position is questionable, because it does not necessarily represent an effect seen clinically. Incontinence occurs when resting bladder pressure exceeds urethral pressure, therefore a resting urethral pressure exceeding bladder pressure is required to prevent urinary incontinence in the nomal dog. This pressure level (146.5+/- 41.9 cm H_2O) would appear to be an appropriate therapeutic aim when inflating AUSs to treat USMI (assuming there is no underlying detrusor instability). Thus, a better approach to answer the clinical question (of whether a specific AUS volume can be recommended for treatment of USMI) would be to identify the volume of AUS inflation required to generate a urethral pressure exceeding that of resting urethral pressure in normal dogs. Figure 1 shows the correlation of AUS volume on maximal urethral pressure compared to the above stated range of 'normal resting urethral pressures' as described by Fischer and colleagues (2003). In each cadaver, this urethral pressure was achieved by incremental AUS inflation. This result indicates that AUS is an effective means to treat incontinence by focally and artificially restoring urethral pressure. In addition to achieving 'normal urethral pressure', the graphs also demonstrate that this pressure is exceeded over a small increase of AUS volumes. An increase in urethral pressure of 74- 97 cm H_2O could be achieved by a single

incremental increase of 0.15mL in the cadavers to achieve a maximum pressure exceeding 100 cm H_2O. The pattern of a rapid increase in urethral pressure exerted by the AUS over a small range of filling volumes might explain why some clinical cases can become suddenly dysuric following incremental AUS inflations despite previous incontinence, and smaller increments of filling over this range of urethral pressures may achieve finer pressure control.

There are many limitations to this study that restrict the drawing of inferences regarding the clinical significance of AUS inflation volume on urethral pressure in live dogs. Foremost, from a study size of five cadavers it is difficult to relate the results to a general population. There is, arguably, limited value in comparing UPP recorded in cadavers to those recorded in conscious dogs as they are known to be different (Adin *et al.*, 2004). In a conscious dog muscle

tone, abdominal and pelvic pressures may influence UPP but cannot be assessed in a cadaver

model. Despite this, correlations between AUS volume and maximal urethral pressure, and patterns of responses of individual dogs are still relevant, and are a useful preliminary

investigation before conducting clinical studies. In this study, all cadavers demonstrate a significant increase in urethral pressure from baseline (uninflated cuff in position) at 0.9mL. Clinically, a mean volume of 0.4mL (0.1-0.7mL) is required to achieve maximal continence (Reeves *et al.*, 2012). This is a smaller volume than seen in this cadaver study, although in three of five cadavers the normal urethral pressure range was reached with 0.75mL inflation, and one dog responded adequately to an inflation of 0.3mL. This difference in volume requirement between cadavers and clinical cases is most likely due to thertificially low basal urethral pressures in the cadavers. It could be expected, therefore, that higher resting urethral pressures, even in an incontinent dog, will require a smaller pressure exerted by an AUS to achieve continence. In addition, irritation and tissue disruption due to surgical placement of the AUS in clinical cases may induce a degree of muscular spasm in the early postoperative period whilst periurethral fibrosis may develop at a later stage (Morgan et al., 2018), also contributing to increasing urethral tone post-operatively.

The magnitude of effect of AUS inflation was variable between cadavers. In the present study, a standard sized (10x14mm) AUS was placed on each cadaver irrespective of urethral

circumference (range 15-25mm). AUS size selection in clinical cases has previously been based on approximate measurement of the circumference of the urethra and placement of an AUS with equal or larger circumference (Reeves *et al.*, 2012). Whereas Currao and co-workers (2012) reported a surgical technique whereby AUS luminal diameter size was calculated as 50% of the urethral circumference at the site of placement, although there is no consensus for selection of, and little evidence to recommend,specific AUS sizes in dogs. The use of a single size of AUS on different sized urethras may account for some of the variation in the individual responses measured, although a larger sample size would be required to demonstrate the importance of matching AUS and urethral size. In humans, there was no effect in short-term incontinence following AUS placement with implants 4mm smaller or 4mm larger than the urethral circumference (Rothschild *et al.*, 2014), suggesting that matching to exact urethral measurement may not be of clinical importance. Use of the relatively larger cuff size was associated with improved long-term outcome (Rothschild *et al.*,2014). While Adin and co-workers (2004) showed no significant variation in inflation between different occluders, the use of a different AUS in each cadaver may contribute to some individual variation between cadavers. However, this method appeared preferable risking damage associated with repeated fixation and replacement that might have altered AUS performance if the same device was used repeatedly. The study may have been improved by repeating the AUS placement and measurements of urethral pressures a further two more times on each cadaver to establish repeatability. While a pattern has been observed in this dataset, it is not possible to confirm that the variability between dogs was due to difference in urethral size, or an intrinsic property of the AUS inflating around a soft tissue structure. Collection of these additional data was limited due to time and resources, and therefore the results remain preliminary.

The effect of incremental AUS inflation on maximum urethral pressure was not linear. The manner of placement of the AUS around the urethra means that the occlusive effect during inflation is not concentric; instead, the leaves of the AUS hinge asymmetrically. Depending on the size or relative position of the urethra within the AUS, the shape of the AUS will differentially affect urethral pressure. Adin and co-workers (2004) demonstrated strong correlation between percentage

occlusion of the AUS when not positioned around the urethra and incremental AUS filling, as determined by using digital images to calculate the resultant luminal area. The use of percentage occlusion *ex vivo* is less useful clinically, as when the AUS is placed *in situ*, the "luminal area" can no longer be observed and entrapped soft tissues will alter filling profiles. When placed around the urethra of cadavers and the AUS inflated with pre-determined volumes corresponding to reach 25% and 50% occlusion, a non-linear relationship between filling volume and mean cysto-urethral leak point pressure was observed, with significant difference from baseline cysto-urethral leak point pressure apparent at 50% occlusion (Adin *et al*., 2004). The effect of incremental inflation on urethral pressure profilometry was not directly assessed.

It is beyond the scope of this study to determine whether a specific AUS volume can achieve continence in all cases of USMI but we provide here preliminary evidence of arelationship between AUS volume and maximal urethral pressure. To fully determine the clinical significance of such a correlation, a further study evaluating urethral profilometry in clinical cases undergoing AUS placement filling is required. We have been able to show that use of small volume increments (0.15mL) is a valid approach and our preliminary data can be used as a benchmark to design the next study in live companion dogs. In fact, it can now be considered that researching the effect of incremental filling with use of aliquots ranging from 0.05mL to 0.1mL would be valuable The results of a clinical study would be able to direct clinicians as to whether incremental filling, use of urethral pressure profilometry, or use of clinical effect should be considered the recommended standard of care.

CONCLUSION

The placement of AUSs in cadavers was associated with a focal, non-linear increase in urethral pressure significantly correlated with AUS volume. Although the correlation between maximal urethral pressure and AUS volume was significant in all cadavers, the magnitude of the response was different between individuals. This observation is also reflected in clinical experience of treating

USMI with AUS placement. These preliminary results suggest that incremental AUS inflation is

suitable for the treatment of USMI butfurther investigation of UPP in live companion

animals undergoing AUS placement is indicated for interpretation of the true impact of this data.

No conflicts of interest have been declared

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TABLES

Cadaver	Urethral circumference (mm)	AUS inflation volume generating significant urethral pressure change after placement (mL)	t value
Dog 1	15	0.9 [*]	6.091
		1.05 [†]	20.56
Dog 2	20	0.15[*]	3.712
		0.3 [†]	10.09
		0.45 [†]	24.84
		0.6 [†]	36.98
		0.75 [†]	40.88
		0.9 [†]	40.88
		1.05 [†]	40.88
Dog 3	25	0.6 [†]	3.665
		0.75 [†]	6.045
		0.9 [†]	16.13
		1.05 [†]	29.56
Dog 4	20	0.75 [†]	9.995
		0.9 [†]	23.51
		1.05[†]	37.27
Dog 5	25	0.6 [*]	6.140
		0.75 [†]	13.61
		0.9 [†]	24.27
		1.05 [†]	31.36

Table 1: This table displays, for each cadaver, the size of the urethra and the volumes of AUS inflation (after the priming volume) at which the urethral pressure change was increased significantly from that measured with a placed, but uninflated, AUS. $P < 0.05$ was used as the significance level, [*] denotes $P < 0.01$, [†] denotes $P < 0.001$. The corresponding t values for each inflation are displayed. In all cadavers, the maximum urethral pressure was recorded within the section of the urethra surrounded by the AUS.

FIGURES

Figure 1 : The line graphs (a) to (e) display the correlation between maximum recorded urethral pressure and incremental increases in AUS volume for each cadaver Dog 1 to 5 respectively. Each of the three repeats for each recording is displayed along with a line joining the mean/span> recorded pressure. The dotted line highlights the range of normal urethral pressures of 104.6-188.4 cm H_2O measured in conscious, continent dogs (Fischer *et al.*, 2003), which can be focally exceeded in cadavers by the pressure exerted by filling of the AUS. * denotes a significant increase in maximal urethral pressure compared to an uninflated cuff; $P<0.05$.

(a)

(b)

(c)

(d)

(e)