AJVR—11-07-0247R—Noël—1fig—3tab—VLS—CRB

Urodynamic and morphometric characteristics of the lower urogenital tracts of female Beagle littermates during the sexually immature period and first and second estrous cycles

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Objective—To compare values of lower urogenital tract urodynamic and morphometric variables determined during the prepubertal (sexually immature) period and first and second estrous cycles in healthy female Beagle littermates to determine functional and anatomic changes of the lower urogenital tract during those periods.

Animals—5 female Beagle littermates.

Procedures—Urethral pressure profilometry, diuresis cystometry, and vaginourethrography were performed when dogs were 3.5, 4.5, 5, 6, 7, 8, 8.5, and 9 months old and during proestrus; estrus; early, middle, and late diestrus; and early and late anestrus of the first and second estrous cycles.

Results—At the end of the prepubertal period, values of urodynamic and morphometric variables increased significantly, compared with values at earlier times. Maximum bladder capacity developed when dogs were 9 months old. In all dogs, the bladder was intermittently located in an intrapelvic position during the prepubertal period; the bladder was intra-abdominal, from the time dogs were 9 months old until the end of the study. Urethral pressure decreased significantly during estrus and early diestrus of the first and second estrous cycles. Bladder capacity increased significantly during diestrus of both estrous cycles. Urethral and vaginal lengths were significantly longer during proestrus and estrus than they were during anestrus.

Conclusions and Clinical Relevance—Values of lower urogenital tract urodynamic and morphometric variables were influenced by age and phases of the estrous cycle of immature and young adult Beagles in this study. Age of dog and phase of estrous cycle should be considered when interpreting urodynamic and vaginourethrography data. (*Am J Vet Res* 2012;73:xxx–xxx)

Abbreviations

FPL	Functional profile length
IP	Integrated pressure
MUCP	Maximum urethral closure pressure
MUP	Maximum urethral pressure
UL	Urethral length
USMI	Urethral sphincter mechanism incompetence
VL	Total vaginal length

Urethral sphincter mechanism incompetence is a micturition disorder of juvenile and adult dogs. The congenital form of USMI is the second most frequent cause of urinary incontinence in juvenile animals,[1,2] and approximately half of affected juvenile bitches become continent following the first or second estrus.[1–3] The acquired form of USMI is the most common cause of urinary incontinence in adult spayed bitches.[1,2] Urinary bladder neck position and UL may have a role in development of USMI. Pelvic bladder, defined as > 5% of the urinary bladder length located inside the pelvis,[4] is a frequent radiographic finding for incontinent immature and adult bitches[1,5] and is typically associated with a short urethra.[6–8] A pelvic position of the bladder neck could decrease transmission of abdominal pressure to the urethra in dogs, leading to urine leakage, especially during recumbency, exercise, or barking.[5,6]

Development of urinary bladder function in healthy humans during neonatal to early childhood pe-

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Received July 18, 2011.
Accepted November 23, 2011.

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This manuscript represents a portion of a thesis submitted by the senior author to the University of Liège Department of Companion Animal Clinical Sciences as partial fulfillment of the requirements for a Doctor of Philosophy degree.

Dr. Noël is a PhD student supported by the Fonds de la Recherche Scientifique—FNRS, Belgium.

The authors thank Laurent Massart for assistance with statistical analysis and Véronique Limpens for technical assistance.

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riods has been investigated.[9–12] Human neonates have neuronal pathways between the bladder and cerebral cortex[9,11]; in human neonates, voiding of the bladder is not voluntary but involves more neural pathways than the automatic reflex to bladder distension. During the first months after birth, humans have a small bladder capacity and high bladder voiding pressure.[9,10] The bladder capacity increases during the first year after birth, and the voiding pressure decreases progressively.[9] Bladder capacity is stable when children are 1 to 2 years old but nearly doubles by the time children are 3 years old.[10] As human infants age, the total number of bladder voiding episodes, volume of residual urine in the bladder after voiding, and number of bladder voiding episodes during the night decrease, and bladder capacity increases.[9,10]

Male children have sex hormone receptors in transitional epithelium of the urinary bladder neck.[13] Adult women have estrogen receptors in the bladder, urethra, vagina, and pubococcygeus muscles.[14,15] Sex hormone receptors are expressed in squamous epithelium of the urethra and bladder neck of women, regardless of circulating estrogen concentrations.[16] However, expression of progesterone receptors in subepithelial tissues of the lower urinary tracts of women is dependent on circulating concentrations of estrogen.[16] Female dogs have estrogen receptors in the proximal urethra but do not have such receptors in the kidneys, ureters, bladder, or distal urethra.[17]

Results of other studies regarding the effects of variations in sex hormone concentrations during the menstrual cycle on the urinary tracts of women are controversial. One group of investigators[18] found that FPL of the urethra increases during the middle of the menstrual cycle in women, whereas other investigators[19–22] identified no significant changes in urodynamic variables during the menstrual cycle. Another group of investigators[23] found that bladder tone increases during the follicular phase of the menstrual cycle in women and that bladder tone is decreased during the luteal phase. Other investigators[24] determined that the lower urogenital tract in adult female Beagles is affected by variations in circulating concentrations of hormones during the estrous cycle. Urethral resistance decreased during estrus and early diestrus in the Beagles in that study,[24] whereas bladder tone increased and values of urinary tract morphometric variables changed during anestrus and proestrus. However, other investigators[25] found that urethral resistance significantly decreases in female dogs after ovariectomy; that finding is not consistent with urodynamic changes that develop in sexually intact female Beagles during anestrus.[24]

Functional and morphometric characteristics of urinary tracts in continent and incontinent adult female dogs have been determined. However, to our knowledge, urodynamic and morphometric characteristics of lower urogenital tracts in sexually immature female dogs have not been investigated. Therefore, the purposes of the study reported here were to compare values of lower urogenital tract urodynamic and morphometric variables determined during the prepubertal (sexually immature) period and first and second estrous cycles and to determine functional and anatomic changes that develop in the lower urogenital tract during those times in healthy female Beagle littermates.

Materials and Methods

Dogs—Five female Beagle littermates were included in the study. The Beagles were 3.5 months old at the start of the study and 20 to 26 months old at the end of the study. All of the dogs were born and housed at the animal facilities of the Research Unit of the Department of Clinical Sciences of the College of Veterinary Medicine, University of Liège. Animal housing, care, and experimental procedures were approved by the Ethical Committee of Animal Use of the University of Liège. Prior to the start of each experiment, each dog was weighed, a complete physical examination was performed, and a urine sample (5 mL) was obtained via cystocentesis. Urinalysis included performance of a dipstick test[a] (for determination of urine specific gravity and pH and urine concentrations of blood, protein, bilirubin, and glucose) and cytologic examination of urine. Urine samples were submitted for bacteriologic culture if urinary tract infection was suspected on the basis of results of cytologic examination. Blood samples (5 mL) were obtained from a jugular vein of each dog, and a CBC and serum biochemical analyses were performed. Dogs included in the study had no signs of lower urinary tract disease or infection.

Study design—Testing of dogs was performed during 3 periods of sexual development. During the prepubertal (ie, sexually immature) phase, urodynamic testing and vaginourethrography were performed when dogs were 3.5, 4.5, 5, 6, 7, 8, 8.5, and 9 months old. At each of those times, anesthesia was induced with an IV bolus of propofol[b] (6 mg/kg) and maintained with a continuous IV infusion of propofol (maximum dosage, 0.3 mg/kg/min). A light and stable depth of anesthesia characterized by a central eye position and presence of a palpebral reflex and jaw tone without movement was maintained during testing of each dog. Dogs were placed in right lateral recumbency after a light and stable plane of anesthesia had been attained. Three successive urethral pressure profilometry measurements were obtained followed by performance of diuresis cystometry (once) as described[26] by use of a medical measurement system.[c] After urodynamic testing, each dog received buprenorphine[d] (15 g/kg, IV) and was maintained under anesthesia in right lateral recumbency for performance of vaginourethrography.[27]

The mean ± SD age of the dogs at the first proestrus was 11.2 ± 1.5 months. Urodynamic testing and vaginourethrography, as described for the prepubertal phase, were performed during each of the following phases of the first and second estrous cycles of each dog: proestrus (4 to 5 days after the beginning of vaginal bleeding), estrus (day 4 after peak circulating luteinizing hormone concentration), early diestrus (day 10 to 12 after peak circulating luteinizing hormone concentration), mid-diestrus (day 30 after peak circulating luteinizing hormone concentration), late diestrus (day 60 after peak circulating luteinizing hormone concentration), early anestrus (day 90 after peak circulating luteinizing hormone concentration), and late anestrus (day 150 after peak circulating luteinizing hormone concentration). Phases of the estrous cycle were identified via visual examination of the vulva, cytologic examination of a vaginal smear,[28,29] and determination of plasma progesterone concentration. The first day of proestrus for each dog was defined as the first day that serosanguinous discharge from the vulva was observed and erythrocytes and multiple types of epithelial cells were identified via cytologic examination of a vaginal smear. The first day of estrus was defined as the day on which the circulating concentration of luteinizing hormone substantially increased; this was indirectly determined on the basis of an increase in plasma progesterone concentration. Progesterone concentrations were measured every 48 hours; the first day of estrus was defined as the day on which progesterone concentrations was ≥ 2.0 ng/mL. Estrus was also confirmed via cytologic examination of a vaginal smear (detection of bacteria and > 90% cornified cells). The first day of diestrus was defined as the first day that cells in vaginal cytology samples comprised > 50% parabasal and intermediate cells (associated with an increase in plasma progesterone concentration). The first day of anestrus was defined as the first day that plasma progesterone concentration was < 1 ng/mL and cells in vaginal cytology samples comprised > 90% parabasal and intermediate cells.[28,29] Plasma progesterone concentrations were determined via a progesterone assay,[e] which was a 1-step immunoassay with chemiluminescent microparticles.[30]

Data interpretation—Variables measured or calculated by use of urethral pressure profilometry measurements included MUP and MUCP (difference between MUP and intravesicular pressure). Definitions of these variables were in accordance with those accepted by the International Continence Society.[31] The IP was calculated by use of urethral pressure profiles and was defined as the area under the urethral functional profile curve.[32] In the present study, FPL was defined as the distance between the point in the urethra at which urethral pressure exceeded intravesicular pressure and the point at which either a pressure plateau was detected or the urethral pressure was less than intravesicular pressure.[26]

The following variables were determined by use of cystometrograms: threshold pressure (intravesicular pressure at the time of micturition reflex), threshold volume (volume of fluid collected from the urinary bladder at the time of micturition reflex), and compliance. Compliance was calculated by use of the following equation: $C = (Vth - V0)/(Pth - P0)$, where C is compliance, Vth is threshold volume, Pth is threshold pressure, and V0 and P0 are the intravesicular volume and pressure, respectively, at the start of cystometry. Definitions of these variables were in accordance with those accepted by the International Continence Society.[31]

The following variables were measured on lateral vaginourethrograms: UL (distance between the bladder neck and the external urethral orifice), vaginal distance A (distance between the cranial aspect of the dorsal cervical cul-de-sac and the caudal aspect of the caudal tubercule of the dorsal median fold of the vagina), vaginal distance B (distance between the dorsal median fold of the vagina and the vestibulovaginal junction), vaginal distance C (distance between the vestibulovaginal junction and the external urethral orifice), and VL (sum of vaginal distances A, B, and C; **Figure 1**). Measurements were corrected for magnification by use of a metal ruler placed near the pelvis of each dog during radiography. Position of the bladder was also determined via evaluation of lateral vaginourethrograms; the bladder was defined as a pelvic bladder when > 5% of the bladder length was located in the pelvis.[5]

Statistical analysis—Statistical analysis was performed with software.[f] Comparisons among values of urodynamic and morphometric variables for each phase of the estrous cycle and for weights of dogs during the study were performed via a mixed procedure. The influences of age and weight of dogs on pelvic position of the bladder were determined via a logistic procedure. Body weight was included as a covariate in the statistical model; time (in days) was included as a classification variable. Repeated measures of data for each dog were correlated via a compound symmetry structure. Differences attributable to time were calculated, and significance was determined by use of analysis of least square means. Values of $P < 0.05$ were considered significant.[33]

Results

Urethral pressures—During the prepubertal period, IP was significantly higher when dogs were 7, 8, 8.5, and 9 months old than it was before those times

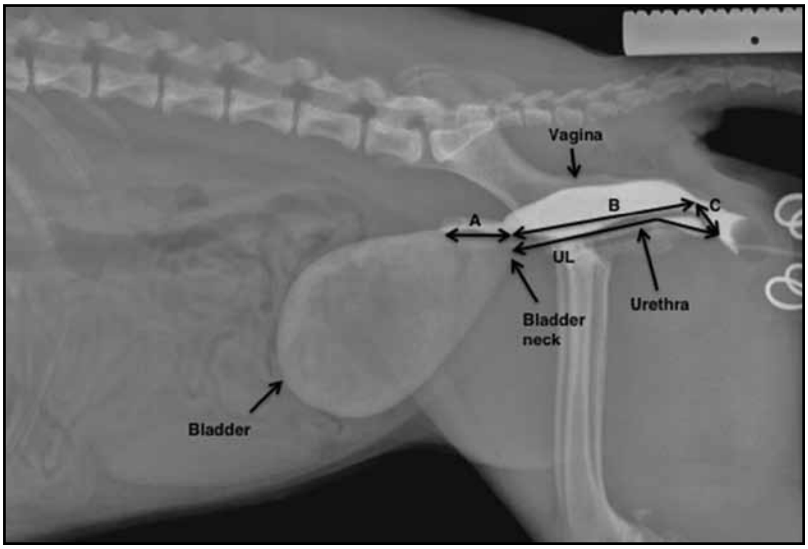

Figure 1—Representative lateral vaginourethrogram of a 6-month-old female Beagle indicating landmarks for determination of urogenital tract morphometric variables. UL = Distance between the bladder neck and the external urethral orifice. A = Vaginal length distance A (distance between the cranial aspect of the dorsal cervical cul-de-sac and the caudal aspect of the caudal tubercule of the dorsal median fold of the vagina). B = Vaginal length distance B (distance between the dorsal median fold of the vagina and the vestibulovaginal junction). C = Vaginal length distance C (distance between the vestibulovaginal junction and the external urethral orifice).

Table 1—Mean ± SD values of lower urogenital tract urodynamic and morphometric variables for five 3.5- to 9-month-old female Beagle littermates during the prepubertal period of development.

Variable	3.5 months	4.5 months	5 months	6 months	7 months	8 months	8.5 months	9 months
MUP (cm H_2O)	51.2 ± 17.1^{a}	49.4 ± 8.3^{a}	$61.7 \pm 9.3^{b,d,e}$	$47.7 \pm 13.0^{a,d}$	61.5 ± 15.6^{b}	$66.6 \pm 12.3^{b,e,f}$	$73.2 \pm 10.4^{c,f}$	65.7 ± 8.0^{b}
MUCP (cm H_2O)	36.7 ± 17.3^{a}	41.1 ± 9.0^{a}	$52.7 \pm 11.1^{b,d,e}$	$43.1 \pm 11.9^{a,d}$	60.8 ± 15.3^{b}	$64.4 \pm 12.2^{b,e,f}$	$71.5 \pm 9.7^{c,f}$	61.8 ± 7.9^{b}
FPL (cm)	$4.8 \pm 0.8^{a,c}$	5.1 ± 0.9^{a}	4.8 ± 0.6^{a}	$5.5 \pm 1.2^{a,c}$	$6.3 \pm 1.5^{b,d}$	$6 \pm 0.9.^{c,d}$	$5.9 \pm 0.5.^{c,d}$	$6.2 \pm 1.8^{b,d}$
IP (cm^2 H_2O)	152.9 ± 32.3^{a}	141.1 ± 17.1^{a}	154.9 ± 17.1^{a}	142.6 ± 31.6^{a}	203.9 ± 56.1^{b}	221.7 ± 66.9^{b}	205.1 ± 38.1^{b}	199.7 ± 47.2^{b}
Bladder threshold volume (mL)	135.6 ± 52.9^{a}	199 ± 55.0^{b}	126.7 ± 33.0^{a}	$255.7 \pm 44.2^{c,d}$	270.3 ± 70.1^{c}	270.2 ± 93.4^{c}	$232.5 \pm 76.3^{b,d}$	247.7 ± 54.4^{c}
Bladder threshold pressure (cm H_2O)	$23.9 \pm 6.9^{a,c}$	20.5 ± 4.9^{a}	$24.4 \pm 12.9^{a,c}$	29.5 ± 8.6^{c}	38.4 ± 10.3^{b}	36.4 ± 5.6^{b}	39.7 ± 9.7^{b}	40.1 ± 10.5^{b}
Bladder compliance (mL/cm H_2O)	$7.8 \pm 2.7^{a,d}$	$9.9 \pm 3.2^{b,c}$	$7.9 \pm 4.0^{a,d}$	$9.9 \pm 2.6^{a,c}$	$7.3 \pm 1.3^{a,d}$	$8.8 \pm 3.4^{a,c}$	6.4 ± 1.9^{d}	$8.5 \pm 1.5^{a,c}$
UL (cm)	$6.4 \pm 0.6^{a,c,d,e,f}$	$6.3 \pm 0.6^{a,d}$	6.1 ± 0.8^{d}	$6.9 \pm 0.8^{a,c}$	$7.4 \pm 0.3^{c,f}$	$7.6 \pm 1.2^{e,f,g}$	$7.7 \pm 0.9^{b,c,f}$	$8.1 \pm 0.6^{b,g}$
VL (cm)	9.7 ± 3.8^{a}	9.8 ± 1.4^{a}	$11.4 \pm 1.6^{b,c,f}$	$10.8 \pm 1.0^{a,c}$	$11.2 \pm 0.7^{a,c}$	$12.8 \pm 1.1^{e,f,d}$	13.2 ± 0.5^{d}	15.1 ± 1.9^{g}

Within each row, values with different superscripts are significantly ($P < 0.05$) different.

Table 2—Mean ± SD values of lower urogenital tract urodynamic and morphometric variables for 5 female Beagle littermates during the first estrous cycle.

Variable	Proestrus	Estrus	Early diestrus	Mid-diestrus	Late diestrus	Early anestrus	Late anestrus
MUP (cm H_2O)	62.6 ± 17.3^{a}	23.2 ± 13.6^{b}	37.5 ± 15.3^{c}	76.7 ± 10.1^{d}	73.7 ± 6.4^{d}	76.3 ± 9.4^{d}	70 ± 8.8^{d}
MUCP (cm H_2O)	59.7 ± 17.9^{a}	18.8 ± 14.2^{b}	31.7 ± 14.4^{c}	73.1 ± 9.3^{d}	70.5 ± 5.2^{d}	75.1 ± 10.4^{d}	70.6 ± 8.8^{d}
FPL (cm)	6.7 ± 1.1^{a}	7.5 ± 1.7^{b}	$6.4 \pm 0.7^{a,c}$	5.6 ± 0.7^{d}	$5.9 \pm 0.8^{c,d}$	$5.8 \pm 0.9^{c,d}$	$5.8 \pm 0.5^{c,d}$
IP (cm^2 H_2O)	215.9 ± 48.9^{a}	93.1 ± 29.1^{b}	137.1 ± 51.4^{c}	217.3 ± 17.9^{a}	218.6 ± 24.2^{a}	221.9 ± 46.0^{a}	185.7 ± 19.4^{d}
Bladder threshold volume (mL)	$258.8 \pm 39.0^{a,d}$	$261 \pm 98.2^{a,d}$	$297.6 \pm 59.8^{b,c}$	$273.9 \pm 61.6^{a,c}$	$305.1 \pm 29.6^{b,c}$	$255.7 \pm 86.1^{a,d}$	232.1 ± 62.9^{d}
Bladder threshold pressure (cm H_2O)	35.4 ± 8.3	36.5 ± 7.1	35.1 ± 9.9	39.7 ± 6.2	41.3 ± 11.5	36.7 ± 6.5	40.5 ± 16.7
Bladder compliance (mL/cm H_2O)	8.6 ± 2.4	7.8 ± 2.4^{a}	10.1 ± 2.3^{b}	$7.9 \pm 3.2^{a,c}$	$8.7 \pm 1.7^{a,b}$	7.7 ± 3.1^{a}	6.8 ± 2.6^{a}
UL (cm)	8.6 ± 0.4^{a}	NA	10.1 ± 0.8^{b}	8.9 ± 2.2^{a}	$10.1 \pm 0.6^{b,c}$	10.1 ± 0.8^{b}	$9.1 \pm 0.7^{a,c}$
VL (cm)	19.3 ± 3.5^{a}	19.3 ± 2.4^{a}	19.7 ± 1.2^{a}	18.8 ± 1.2^{a}	18.6 ± 1.8^{a}	16.8 ± 1.7^{b}	15.5 ± 1.1^{c}

NA = Data not available.
See Table 1 for remainder of key.

Table 3—Mean ± SD values of lower urogenital tract urodynamic and morphometric variables for 5 female Beagle littermates during the second estrous cycle.

Variable	Proestrus	Estrus	Early diestrus	Mid-diestrus	Late diestrus	Early anestrus	Late anestrus
MUP (cm H_2O)	47.8 ± 17.4^{a}	21 ± 12.6^{b}	27 ± 8.6^{b}	$77.2 \pm 6.7^{c,d}$	$78.3 \pm 9.8^{c,d}$	72.7 ± 9.6^{c}	79.9 ± 7.1^{d}
MUCP (cm H_2O)	48.5 ± 18.7^{a}	20.1 ± 11.2^{b}	$26.2 \pm 8.9a^{,b}$	$76.3 \pm 5.3^{c,d}$	$76.9 \pm 11.1^{c,d}$	71.3 ± 8.9^{c}	80.2 ± 8.2^{d}
FPL (cm)	8.3 ± 0.9^{a}	8.1 ± 0.9^{a}	6.5 ± 0.7^{b}	$6.4 \pm 0.6^{b,c}$	6.7 ± 1.1^{b}	5.8 ± 0.8^{c}	$6.2 \pm 0.09^{b,c}$
IP (cm^2 H_2O)	$199.9 \pm 89.5^{a,d}$	99.8 ± 47.6^{b}	98.6 ± 31.0^{b}	$240.2 \pm 25.2^{c,d}$	248.4 ± 43.7^{c}	$216.4 \pm 27.7^{a,d,e}$	$239.8 \pm 29.6^{c,e}$
Bladder threshold volume (mL)	$265.5 \pm 54.1^{a,d}$	249.1 ± 71.5^{a}	304.3 ± 32.5^{c}	$285.5 \pm 28.7^{c,d}$	280.9 ± 53.6	277.4 ± 89.1	251.3 ± 47.2^{a}
Bladder threshold pressure (cm H_2O)	49.6 ± 11.7^{a}	$40.4 \pm 9.4^{b,c}$	$46.4 \pm 8.0^{a,c}$	$37.9 \pm 9.8^{b,e}$	$44.2 \pm 8.5^{a,c,e}$	$46.6 \pm 12.2^{a,c}$	34.1 ± 9.3^{d}
Bladder compliance (mL/cm H_2O)	5.3 ± 1.0^{a}	$6.7 \pm 1.8^{a,c}$	$7.2 \pm 2.1^{b,c}$	8.9 ± 2.3^{d}	$7 \pm 2.4^{b,c,e}$	$6.2 \pm 1.9^{a,c}$	8.9 ± 3.7^{f}
UL (cm)	10.4 ± 0.0	11.5 ± 0.0^{a}	11.1 ± 0.8^{a}	10.7 ± 0.5	10.7 ± 0.5	10.3 ± 0.8^{b}	10.2 ± 1.1^{b}
VL (cm)	21.8 ± 1.6^{a}	20.3 ± 1.4^{b}	18.6 ± 2.0^{c}	18.7 ± 1.5^{c}	18.3 ± 1.4^{c}	15.7 ± 1.3^{d}	15.8 ± 1.0^{d}

See Table 1 for key.

(**Table 1**). During the prepubertal period, MUP and MUCP were significantly higher when dogs were 7, 8, 8.5, and 9 months old than they were when dogs were 3.5 and 4.5 months old. During the first and second estrous cycles, urethral pressures during estrus and early diestrus were significantly lower than they were during any other phase (**Tables 2 and 3**); urethral pressures during early diestrus of the first estrous cycle were significantly higher than they were during estrus of that cycle.

ULs—During the prepubertal period, FPL increased when dogs were 7 months old; FPL was significantly higher when dogs were 7, 8, 8.5, and 9 months old than it was when dogs were 4.5 or 5 months old (Table 1). During the first and second estrous cycles, the highest values of FPL were detected during the follicular phase (ie, proestrus and estrus), although FPL remained high during early diestrus of the first estrous cycle; FPLs during the follicular phase were significantly higher than they were during the luteal phase (ie, mid-diestrus to late diestrus) and anestrus (Tables 2 and 3).

During the prepubertal period, ULs when dogs were 8.5 and 9 months old were significantly higher than they were when dogs were ≤ 6 months old (Table 1). Values of UL could not be determined for estrus of the first estrous cycle because urethras were not identifiable in vaginourethrograms obtained at that time (Table 2). During the second estrous cycle, UL was significantly higher during estrus and early diestrus than it was during anestrus (Table 3).

Vaginal length—Vaginal length increased significantly during the prepubertal period (Table 1). During the first and second estrous cycles, VL during anestrus was significantly shorter than it was during any other phase (Tables 2 and 3).

Cystometrogram variables—During the prepubertal period, urinary bladder threshold volume was significantly higher when dogs were ≥ 6 months old than it was when dogs were < 6 months old (Table 1). Threshold volume was significantly higher when dogs were 4.5 months old versus when dogs were 3.5 (P = 0.001) or 5 ($P < 0.01$) months old. Urinary bladder compliance was significantly higher when dogs were 4.5 months old than it was when dogs were 3.5 (P = 0.03) or 5 (P = 0.04) months old. During the first and second estrous cycles, urinary bladder threshold volume was significantly higher during diestrus than it was during other phases (Tables 2 and 3), except during late diestrus and early anestrus of the second estrous cycle, during which threshold volume was not significantly different.

During the prepubertal period, urinary bladder threshold pressure was significantly higher when dogs were ≥ 7 months old versus when dogs were < 7 months old (Table 1). Threshold pressure did not change significantly during the first estrous cycle (Table 2). During the second estrous cycle, threshold pressure varied and was significantly (P = 0.005) lower during estrus than it was during proestrus. Threshold pressure was significantly lower during late anestrus that it was during any other phase of the second estrous cycle (Table 3).

Values at the end of the prepubertal period versus those during anestrus—The MUCP and UL were significantly higher during anestrus of both estrous cycles than they were when dogs were 9 months old. The IP was significantly (P = 0.007) higher during anestrus of the second estrous cycle and VL was significantly (P = 0.004) higher during anestrus of the first estrous cycle versus values of those variables when dogs were 9 months old.

Bladder position—Bladder position was significantly (P = 0.003) influenced by age of dogs. A pelvic bladder was detected in vaginourethrograms of all dogs at various times during the prepubertal period. All dogs had a pelvic bladder when they were 5 months old. The bladder was in an intraabdominal position in all dogs when they were 9 months old and during the first and second estrous cycles.

Weight—Weight of the dogs was significantly ($P < 0.001$) influenced by age. Significant changes in weight of dogs were not detected after late anestrus of the first estrous cycle.

Discussion

In the present study, significant changes in values of urodynamic and morphometric variables of the lower urogenital tracts of female Beagles were detected during the prepubertal period and first and second estrous cycles. During the prepubertal period, urethral resistance was higher when dogs were ≥ 7 months old, compared with urethral resistance when dogs were < 7 months old, and urethral resistance continued to increase during the first and second anestrus periods. Few studies have been performed to determine urethral resistance in human infants. Urethral pressure profilometry is rarely performed for human infants because withdrawal of the catheter used in the procedure through the urethral lumen induces reflex activity in muscles of the pelvic floor, which causes artifacts in measurements.[34,35] In human infants, urethral sphincter activity is better determined via electromyography of pelvic floor muscles combined with performance of cystometry and determination of leak point pressure.[34–37]

In the present study, the urethral and vaginal lengths measured via vaginourethrography significantly increased during the prepubertal period, which was attributed to growth of the dogs. Adult body weight of Beagles is attained when they are 9 months old.[38] Continued increases in UL and urinary pressures detected during anestrus of dogs in the present study could indicate that complete growth of the dogs was not attained at 9 months, but that dogs continued to grow and urethral resistance continued to increase after this age.

A decrease in urinary bladder volume and compliance was detected when dogs were 5 months old, after which time bladder volume increased and remained high until the end of the prepubertal period. Urinary bladder threshold pressure increased when dogs were between 7 and 9 months old. In human infants, bladder capacity increases with age and body weight,[39] but not linearly,[40] and a stepwise increase in bladder capacity is detected during the first months after birth. This pattern of increasing bladder size in human infants could be similar to that in immature dogs. However, bladder capacity and compliance at the end of the prepubertal period and during the second anestrus period were similar among dogs in the present study. That finding indicated maximum bladder capacity was attained at the end of the prepubertal period in dogs in this study (although bladder capacity varied during subsequent estrous cycles because of variations in circulating concentrations of sex hormones). In human infants, urinary bladder function is characterized by low bladder capacity, high urine voiding pressure, and dyscoordination during voiding of urine (with or without complete voiding).[40] These characteristics change with increasing age and development of bladder control in infants.[10,40]

Interestingly, all of the dogs in the present study intermittently had a pelvic bladder during the prepubertal period. All of the dogs had a pelvic bladder by the time they were 5 months old. The bladder was in an intra-abdominal position in all dogs when they were ≥ 9 months old. Pelvic bladder is a radiographic finding more frequently detected for incontinent bitches than for other dogs and may be a predisposing factor for urinary incontinence.[1,5] When the neck of the bladder is intra-abdominal, abdominal pressure is transmitted equally to the bladder and urethra.[3] When the neck of the bladder is intrapelvic, more abdominal pressure is transmitted to the bladder than is transmitted to the urethra, creating a pressure gradient that causes urine leakage during periods of high intra-abdominal pressure (eg, during recumbency or barking).[3] In dogs in the present study, pelvic bladder was not associated with urinary incontinence. However, findings regarding changes in bladder position detected in dogs in this study (ie, pelvic bladders in dogs were only detected during the prepubertal period) could be reason for spontaneous resolution of congenital USMI in affected juvenile bitches following the first or second estrous cycle.[1–3] To further investigate that finding of the present study and to determine mechanisms of USMI, further studies are warranted in which immature dogs of breeds predisposed to this problem are evaluated.

Urodynamic and lower urogenital tract morphometric characteristics of dogs during the first estrous cycle in the present study were similar to those of middle-aged dogs during the estrous cycle.[24] During the first estrous cycle of dogs in the present study, urethral pressure decreased during estrus and early diestrus. During these phases of the estrous cycle, plasma estrogen concentration decreases and plasma progesterone concentration increases. Estrogens induce an increase in the number of α-adrenergic receptors and responsiveness of these receptors to sympathetic stimulation.[41,42] Estrogens also induce an increase in blood flow to urethral tissues,[43] which causes an increase in urethral sphincter tone.[44] However, progesterone potentiates β-adrenergic receptor activity in urethras of female dogs, leading to urethral relaxation and a decrease in urethral smooth muscle tone.[45,46] Progesterone may exert an effect on the lower urinary tracts of women by inhibiting action of estrogen or via a direct effect on urethral progesterone receptors.[16] Such responses could develop in urethral tissues of female dogs.

The highest FPLs were detected during proestrus and estrus in dogs in the present study. Estrogens increase the maturation index of urethral epithelium[47] and stimulate metabolism and collagen production in connective tissue in women.[48] In women, FPLs increase during phases of the estrous cycle with high serum concentrations of estrogen.[18]

Bladder volume increased during diestrus, and the highest bladder capacity was detected during periods of maximal plasma progesterone concentration[28] in dogs in the present study. Results of other studies regarding the effects of progesterone on bladder capacity are controversial. Results of some studies[49,50] indicate bladder capacity increases during pregnancy in rats[49] and after administration of exogenous progesterone and during the luteal phase of a normal menstrual cycle in women.[50] Results of other studies indicate bladder capacity is not influenced by plasma progesterone concentration in women[21] or female dogs.[24]

An increase in bladder pressure, associated with a decrease in bladder compliance, was detected during proestrus of the second estrous cycle in dogs in the present study; this finding was similar to that of another study[24] conducted by personnel in our laboratory. Results of another study[51] indicate a nonphysiologic increase in plasma estrogen concentration is correlated with an increase in the number of α-adrenergic and muscarinic receptors in rabbits.[51] To the authors' knowledge, it is unknown whether such an increase in receptor number develops after a physiologic increase in plasma estrogen concentration. Results of other studies[52,53] indicate that high circulating concentrations of estrogens are associated with decreased muscarinic receptor density in rabbits. Results of other studies regarding the influence of the estrous cycle on bladder threshold pressure in women are controversial.[21,50,54]

During the second estrous cycle in dogs in the present study, VL decreased from proestrus to anestrus, and values during anestrus were significantly lower than they were during other phases of the first and second estrous cycle. These findings may be attributable to hormonal influences during the estrous cycle. Results of another study[24] conducted by personnel in our laboratory indicate vaginal length in adult dogs increases from late anestrus to proestrus.

The present study had several limitations. A small number of dogs were included in the study. However, we chose to include as homogenous a group of dogs as possible (ie, dogs that were littermates). Despite similarity among dogs in breed, age, weight, and sex, data had high SDs, which was attributed to biological variation among the dogs. Another limitation was the breed of dog used in the study. Although Beagles are not predisposed to USMI, they are frequently used in studies such as this. Other studies may be warranted in which dogs of breeds predisposed to USMI are used; results of such studies may be compared with those of the present study. Another limitation of this study was the technique used to determine urodynamic variables; this technique must be performed for anesthetized dogs to minimize artifacts attributable to movement. Development of telemetry techniques may allow accurate determination of urodynamic variables during physiologic conditions in conscious dogs. In addition, the vaginourethrography technique also had limitations attributable to phases of the estrous cycle; UL could not be determined during estrus of the first estrous cycle. During that period (when cervixes of dogs were open), passage of radiopaque contrast dye into the uterus was observed, and filling of the urethra was incomplete. To determine UL during estrus of the second estrous cycle, urethrography was performed before vaginography by use of foam placed in the vagina to avoid passage of radiopaque contrast dye into the uterus. After the urethra was completely filled with contrast dye, the foam was removed, and the vaginourethrography technique was continued in the typical manner.

Results of this study indicated female Beagles developed urinary continence, characterized by an increase in urethral resistance and bladder capacity, during the prepubertal period. Urodynamic and morphometric variables were influenced by growth of dogs and phases of the estrous cycle (including during the first estrous cycle). Results of this study also suggested that urinary continence continues to improve during the first 2 estrous cycles. An intrapelvic position of the urinary bladder during the prepubertal period may contribute to congenital USMI in sexually immature dogs. Results of this study provided information regarding factors that may predispose female dogs to urinary incontinence.

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a. CombiScreen VET 11 PLUS, Analyticon Biotechnologies AG, Lichtenfels, Germany.
b. Propovet, ECUPHAR, Oostkamp, Belgium.
c. Libra+ Medical Measurement System, Benetec, Retie, Belgium.
d. Vetergesic, ECUPHAR, Oostkamp, Belgium.
e. ARCHITECT progesterone assay, Abbott, Lisnamuck, Longford, Ireland.

f. SAS/STAT software, version 9.1, SAS Institute Inc, Cary, NC.

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