CASE REPORT

Urogenital anomalies and urinary incontinence in an English Cocker Spaniel dog with XX sex reversal

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1 | INTRODUCTION

A 3-year-old English Cocker Spaniel dog weighing 8 kg was referred with a disorder of sexual development and persistent urinary incontinence before and after gonadohysterectomy performed at a local animal hospital. Histopathological examination disclosed hypoplasia of the testes, epididymis, pampiniform plexus, and uterus. On ultrasonography, an anomalous structure containing anechoic fluid was identified in the region dorsal to the urinary bladder. An anomalous communication between the proximal urethra and the remnant uterus and vagina was found on retrograde urethrography under fluoroscopy. Reflux of contrast medium into the anomalous structure, suspected to be the uterus and cranial vagina, from the urethra was detected. Computed tomography identified the anomalous structure between the rectum and urethra. The anomalous structure was removed via laparotomy and the urinary incontinence resolved. The diagnosis of XX sex reversal with a developmental anomaly of the genitourinary tract was made on the basis of laparotomy findings and cytogenetic and SRY gene analyses.

KEYWORDS
canine, clitoral bone, urethral anomaly, urethrovaginal fistula, urogenital anomaly, urogenital sinus

The dog underwent gonadohysterectomy at the local animal hospital. Gonads suspected to be testes were located at the cranial end of the uterine horns. The transformation zone between the uterine body and cervix was not identifiable on palpation. Therefore, the uterus was transected and ligated as caudally as possible.

The uterus and 3 sections of the gonads and attendant structures were histologically evaluated at a laboratory (IDEXX Laboratories Inc, Seoul, South Korea). Hypoplasia of the testes, epididymis, and pampiniform plexus and absence of spermatozoa were identified in hematoxylin and eosin-stained tissues (Figure 1). The uterus had normal morphology with uterine glands and columnar epithelium. After

Abbreviations: CB, clitoral bone; CT, computed tomography; DSD, disorder of sexual development; SRY, sex-determining region Y; UB, urinary bladder

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surgery, a single dose of cefovecin (4 mg/kg) was administered SC. Although the pollakiuria resolved, urinary incontinence persisted, and was particularly severe when pressure was placed on the dog’s abdomen. Treatment with phenylpropanolamine (1 mg/kg PO q12h) for 1 week also was ineffective.

The dog was referred to Konkuk University Veterinary Medical Teaching Hospital, where physical examination disclosed an enlarged vulva, enlarged clitoris with hard bony material, a small external urogenital opening, and the absence of a vestibule. In the sitting position, the external genitalia were in contact with the ground. A suspected urethral opening was abnormally located along the dorsal surface of the enlarged clitoris (Figure 2A).

Abdominal radiography (Titan 2000M, Comed Medical Systems Co, Ltd, Seoul, South Korea) identified the CB in the enlarged clitoris (Figure 2B). Abdominal ultrasonography (Prosound F75, Aloca Co, Ltd, Tokyo, Japan) using 3.6–13.3 MHz linear transducers and 3.75-10 MHz microconvex transducers identified an anomalous structure containing anechoic fluid dorsal to the urinary bladder (UB) suspected to be a remnant uterus and cranial vagina after gonadohysterectomy performed at the local animal hospital (Figure 3A,B). On mild abdominal compression during scanning of the caudal abdomen, urine was observed to flow from the anomalous structure to the exterior of the body, after which the size of the structure decreased. A urine sample was collected using ultrasound-guided cystocentesis. No infectious organisms grew in bacterial and fungal cultures of the urine sample. A urethral catheter was inserted through an opening dorsal to the enlarged clitoris. Interestingly, urine drained through the catheter when it was inadvertently introduced into the anomalous structure. However, on color Doppler examination, bilateral ureteral jets were identified only in the UB, which confirmed that the ureters did not drain into the anomalous structure. Hyperechoic material suspected to be suture material used for ligation during gonadohysterectomy was identified in the cranial region of the anomalous structure (Figure 3A). When the urethral catheter was removed and reintroduced, it correctly located the UB.

Retrograde urethrography was performed under fluoroscopic guidance using 1 mL/kg iohexol (Omnihexol 300, Korea United Pharm Inc, Seoul, South Korea), as shown in Figure 4A and Supporting Information Video S1. The dog was awake during the procedure. The urethral catheter was positioned in the distal urethra and contrast medium was injected. The study showed reflux of contrast media into the remnant uterus and vagina from the proximal urethra.

Computed tomography (CT) was performed using a 4-multidetector-row system (LightSpeed, GE Medical Systems, Milwaukee, Wisconsin). The imaging parameters were as follows: 120 kVp; 200 mAs; matrix size, 512 × 512; rotation time, 0.6 seconds; and, slice thickness, 1.25 mm. Plain CT images were obtained first, after which iohexol (800 mg iodine/kg) was manually injected into the cephalic vein. Then, postcontrast images were acquired with a 60-seconds delay. Images were acquired without elimination of the contrast medium from the lower urinary tract to confirm its structure (Figure 3C,D). All acquired images were uploaded to the GE workstation and reviewed by 2 radiologists (H. Yoon and J. Kim).

The CT images showed a tubular structure with pooling of iohexol between the rectum and urethra. The tubular structure was interpreted
to be the anomalous structure suspected to be the remnant uterus and cranial vagina visualized on ultrasonography and fluoroscopy. A parenchymal connection between this structure and the urethra was suspected. The CB in the enlarged clitoris was identified.

Blood samples were collected from the cephalic vein for karyotyping and SRY gene sequencing. Twenty metaphase cells were obtained from peripheral blood lymphocyte cultures using Giemsa staining, as described in a previous study. A normal female karyotype (2n = 78, XX) was identified in the metaphase of all of these cells. The canine SRY coding region (GenBank AF107021) was amplified using forward (5'-3': ATCACAGCACCAGATCTAG) and reverse (5'-3': GCGTTGGAAACTTGCTTAACA) primers. The polymerase chain reaction products then were compared with those from normal male and female Beagle dogs. The SRY gene with approximately 900 base pairs was identified in a normal male dog, but was not detected in the female Beagle or the subject of this report. The genetic disorder in this dog was determined to be XX sex reversal based on the presence of XX sex chromosomes without the SRY gene, hypoplastic testes without spermatozoa, a uterus, and ambiguous external genitalia.

Although both clitoridectomy and removal of the anomalous structure to prevent urine reflux were required, the dog's owner only wished to resolve the problem of the external genitalia that made contact with the ground when the dog sat down. Accordingly, clitoridectomy was performed and the CB was excised. However, the urinary incontinence persisted, and eventually, laparotomy for removal of the anomalous structure was performed at 3 weeks after gonadohysterectomy. Anesthesia was induced and maintained with IV propofol (Anepol, Hana Pharma, Hwasung, South Korea; 6 mg/kg) and inhaled 1.5% isoflurane (Foran solution, Choongwae Pharma, Seoul, South Korea), respectively. A urethral catheter (All Silicone Foley Catheter, Yushin Medical Co, Ltd, Bucheon, South Korea) was placed in the UB and urethra just before surgery. Laparotomy was performed, and the anomalous structure suspected to a remnant uterus and cranial vagina was identified in the region dorsal to the proximal urethra. It was carefully retracted in the cranial and lateral direction without pubic osteotomy (Figure 4C). The cranial part of the anomalous structure was adhered to the dorsal part of the bladder, whereas the caudal part was connected to the proximal urethra. The structure was ligated and cut at the level immediately cranial to the anomalous communication between the urethra and uterus and vagina, located dorsal to the proximal urethra (Figure 4D). Some of the tissue from the structure adherent to the UB was not completely removed, because complete removal could have resulted in a UB tear or injury.

Minimal reflux of contrast medium into the anomalous structure was observed on retrograde urethrography performed 1 day after surgery (Figure 4B). The urethral catheter was replaced, and continuous drainage was maintained for 7 days. Histopathological examination of the anomalous structure showed stratified columnar epithelium, connective tissue, and smooth muscle (Figure 4E), which would be consistent with a cervix. The dog was closely monitored for remnant pyometra or persistent urinary incontinence and infection, but no such abnormalities were identified. No infectious organisms were identified.
in bacterial and fungal cultures of a urine sample obtained using ultrasound-guided cystocentesis 2 months after surgery. The dog has had no episodes of urinary incontinence in the 7 months since treatment. The final diagnosis was XX sex reversal with developmental anomalies of the genitourinary tract.

2 | DISCUSSION

XX sex reversal is a DSD with discordance between the XX karyotype, the gonad, and genital phenotypes. Six dogs with DSD, urogenital anomalies and urinary incontinence have reported previously. One dog had bilateral uterine horn insertion directly into the bladder, 2 exhibited a vestibule and an abnormal communication between the vagina and urethra, and 3 exhibited a narrow distal vestibule with abnormal communication between the vestibule and perineal region. Five cases were identified as female with ovaries and 1 case was a neutered female. All 6 cases had an ambiguous phenotype. Urinary incontinence has been associated with DSD and congenital adrenal hyperplasia in children, primarily in those with ambiguous female external genitalia. Various urinary tract anomalies, including agenesis of the urethra and unilateral kidney or bladder and vagina, rectovaginal communication, and hydrocolpos, ectopic ureters, and urethral ectopia with anomalous cervix, can accompany anomalies of the urogenital sinus, regardless of karyotype, gonadal type, or phenotype of internal or external genitalia. However, to our knowledge, ours is the first report to describe physical, imaging, surgical, histopathological, and genotypic findings in a dog with XX sex reversal and urinary incontinence caused by anomalous communication between the proximal urethra and the uterus and cranial vagina.

In the normal female embryo, the cranial portion of the paramesonephric (Müllerian) ducts gives rise to the uterine tubes, whereas the caudal portion forms the horns and body of the uterus. The metanephric duct, which is derived from a ureteric bud of the distal mesonephric duct, forms the ureter. The mesonephric duct undergoes degeneration, and the cranial portion of the urogenital sinus gives rise to the primordium of the bladder and vesicourethral canal, whereas the caudal portion forms the urethra and vestibule. The vaginal plate develops from the Müllerian tubercle of the urogenital sinus, and fuses with the ends of the paramesonephric ducts to give rise to the vagina (Figure 5A).
XX sex reversal associated with the SXR gene has been reported most frequently in American Cocker Spaniels. In this case, XX sex reversal resulted in formation of the testes from the urogenital ridge. Androgen secretion by the abnormal testes presumably resulted in masculinization of the external genitalia, with development of an enlarged (phallic) clitoris, a CB, and a long penile urethra with an external urethral orifice in the clitoris. Insulin-like peptide 3 and its receptor, and calcitonin-gene-related peptide regulate intra-abdominal testicular descent. Although the regulatory pathways were not evaluated in this case, defects might exist in the pathways. In addition, because of androgen secretion and deficiency of Müllerian inhibiting substance (MIS), the dog’s anomalous internal genitalia arose from both the mesonephric (epididymis, deferent duct) and paramesonephric (uterus, cranial vagina) ducts.

A urethrovaginal communication also can occur as a sequela of surgery, trauma, or the presence of a foreign body. Differentiation between congenital and acquired urethrovaginal communications requires careful history taking and identification of chronic inflammation, foreign bodies, and the location and morphology of the fistula via imaging modalities such as ultrasonography, retrograde urethrography, CT, vaginoscopy, and urethrocystoscopy. In particular, urethrocystoscopy and vaginoscopy provide excellent visualization for identification of lower urinary tract disease. Although urethrocystoscopy and vaginoscopy were not performed in this dog, the studies performed provided adequate information about urinary incontinence and the congenital anomalies present.

The exposed clitoris, which contained the external urethral orifice and urine pooling in the anomalous structure, presumably, contributed to urinary tract infection and urinary incontinence. Pollakiuria was resolved after antibiotic treatment and clitoridectomy. Urinary incontinence was resolved after the remnant uterus and vagina was excised.

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CONFLICT OF INTEREST DECLARATION
The authors declare that they have no conflict of interest with the contents of article.

OFF-LABEL ANTIMICROBIAL DECLARATION
Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION
Authors declare no IACUC or other approval was needed.
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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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