

Analysis of *SRY*-negative XX True Hermaphroditism in an English Cocker Spaniel

Goo Jang, Ye-Eun Byeon*, Min-Kyu Kim**, Ok-Jae Koo and Byeong-Chun Lee¹

Department of Theriogenology & Biotechnology, College of Veterinary Medicine, Seoul National University, Seoul 151-742, Korea

**Department of Veterinary Surgery, College of Veterinary Medicine, Seoul National University, Seoul 151-742, Korea*

***Department of Animal Science and Biotechnology College of agricultural life Science*

Chungnam National University, Deajeon 305-764, Korea

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Abstract : *SRY* gene is normally responsible for testis induction, yet testis development can occur in the absence of *SRY*. In here, we analyzed the *SRY*-negative sex reversal in cocker spaniel, at 1.5 year-old. The attacked dog was suffered from enlarged clitoris, and resulted in disorder of urination. By surgically approach, enlarged clitoris and one testis, which are apparently seen, are removed. Additionally, thorough the abdomen surgery, uterus and ovary-like mass were removed. The dog had XX, chromosome, showed negative for *SRY*-gene, and the mass had the ovary-testis structure. In other words, based on the macroscopic, cytogenic, and histological study, we can diagnose the cocker spaniel as *SRY*-negative sex reversal.

Key words : cocker spaniel, enlarged clitoris, *SRY*, sex-reversal

Introduction

The XX sex reversal syndrome is known in at least 17 different dog breeds (3,5), and an autosomal recessive mode of inheritance has been proven in the American Cocker and seems most likely in all other dog breeds (5-7,11). The pathogenesis of the XX sex reversal syndrome in dogs and the reason why in some cases one litter mate develops as an XX sex reversed male while another becomes an XX true hermaphrodite is not completely understood.

Normal mammalian sexual development occurs in three steps; 1) establishment of chromosomal sex, 2) development of gonadal sex, and 3) development of phenotypic sex, with each step depending upon successful completion of the previous step. *Sry* (Sex determining Region of the Y chromosome) known as testis-determining factor (TDF) is a gene that located on the short arm of the Y chromosome and has a function of initiate male sex determination (2). But diagnosis of intersexuality in dogs is difficult because, the visible alterations are sometimes small and differentiation between the various forms of intersexuality requires clinical, histological and cytogenetic examinations. Nevertheless, breeding dogs suspected of being intersexes must be diagnosed for type of intersexuality as some forms are inherited.

Materials and Methods

Experimental Animal

A 1.5 year-old female English cocker-spaniel was referred to the Veterinary Medical Teaching Hospital, Seoul National University with primary complaint of polyuria due to exposed penis-like tissue out of female external structure.

Clinical Examination

The affected animal was evaluated with physical examination of reproductive tract, a complete blood count (CBC) and serum biochemistry profiles.

Imaging Diagnosis

Based on physical examination, reproductive and urinary tract radiography were performed. A contrast cystourethrogram was performed for differential diagnosis of polyuria caused by urinary tract anatomical disorder.

Surgical Correction

The patient was anesthetized by premedication atropine sulfate (Atropine, Daehan, 0.1 mg/kg) subcutaneous and acepromazine (Sedaject, Samwoo, 0.1 mg/kg) intramuscularly and then induced with 6 mg/kg of intravenous propofol (Anepol, Hana pham). And general anesthesia was maintained with 2% isoflurane in oxygen. The ventral abdomen and the perineal region were clipped and prepared for aseptic surgery.

To treat the signs of enlarged clitoris tissue, a ventral

¹Corresponding author.
E-mail : bcleee@snu.ac.kr

reconstructive procedure (ventral episioplasty) was performed to lift up the labia to a more vertical position in order to prevent urine accumulation. Therefore, a sufficient area of skin was resected from the ventral surface of the vestibulum. The first incision initiated lateral to the dorsal commissure of the vulva, proceeded ventrally, and continued to the opposite side. The second incision started at the same point as the first, but extended in a wider arc outlining the segment of skin to be removed.

A midline laparotomy was performed, extending from the umbilicus up to the pubic bone. The whole abdomen was thoroughly explored for congenital abnormalities. The internal genitalia somewhat resembled testicles instead of ovaries and both persistent uterus and cystic structure were with mucous fluid. Those tissues were removed by routine surgical procedure.

PCR and Histological analysis

Blood samples were collected in 3 mL EDTA container; control blood samples were collected from a normal male and a female dog. Genomic DNA from blood samples was extracted according to instruction of DNA extraction kit (Quiagen, Germany). Using the PCR primer SRY_F 5'-AAGCGACCCATGAACGCATT-3' and SRY_R 5'-TTCGGGTA-TTTCTCTCTGTG-3'; X_F 5'-CCTGACTTTAAGGCGAT-AGCA-3' and X_R 5'-GGT ACC TTG CTG ACT TTC GC-3', SRY gene and X chromosome specific region of genomic

DNA was amplified. PCR products were analyzed by gel electrophoresis on 2% agarose gels, stained with ethidium bromide and visualized under ultraviolet light.

The removed tissue were fixed in 10% buffered formalin and were taken for routine hematoxylin and eosin (H & E) staining for light microscopic examination.

Karyotyping

For analysis of chromosomes, skin fibroblasts were isolated by general primary culture. Cells were treated with 0.1 $\mu\text{g}/\text{mL}$ colcemid (cat no. 15212-012, Invitrogen, Carlsbad, CA, USA) for 1 hour and harvested with 0.1% trypsin (Invitrogen). Trypsinized cells were incubated in hypotonic solution (0.075 M, KCl, Sigma-Aldrich) buffer for 45 minutes prior to fixation with methanol-acetic acid (3:1). After fixation, metaphase chromosome was spread on the slide and then they were stained with Giemsa solution.

Results and Discussion

The present study, describes a case of XX sex reversal in a 1.5 year-old English Cocker Spaniel with external female appearance. On physical examination, the dog had an enlarged clitoris. CBC and serum biochemistry profiles revealed within reference ranges. Because the enlarged clitoris caused persistent irritation, surgical resection was suggested. Since most dogs affected with this disorder are sterile, the owner



Fig 1. Sry-negative XX sex reversal in Cocker Spaniel. A) Enlarged clitoris (arrow), B) Os penis (arrow) on the X-film.

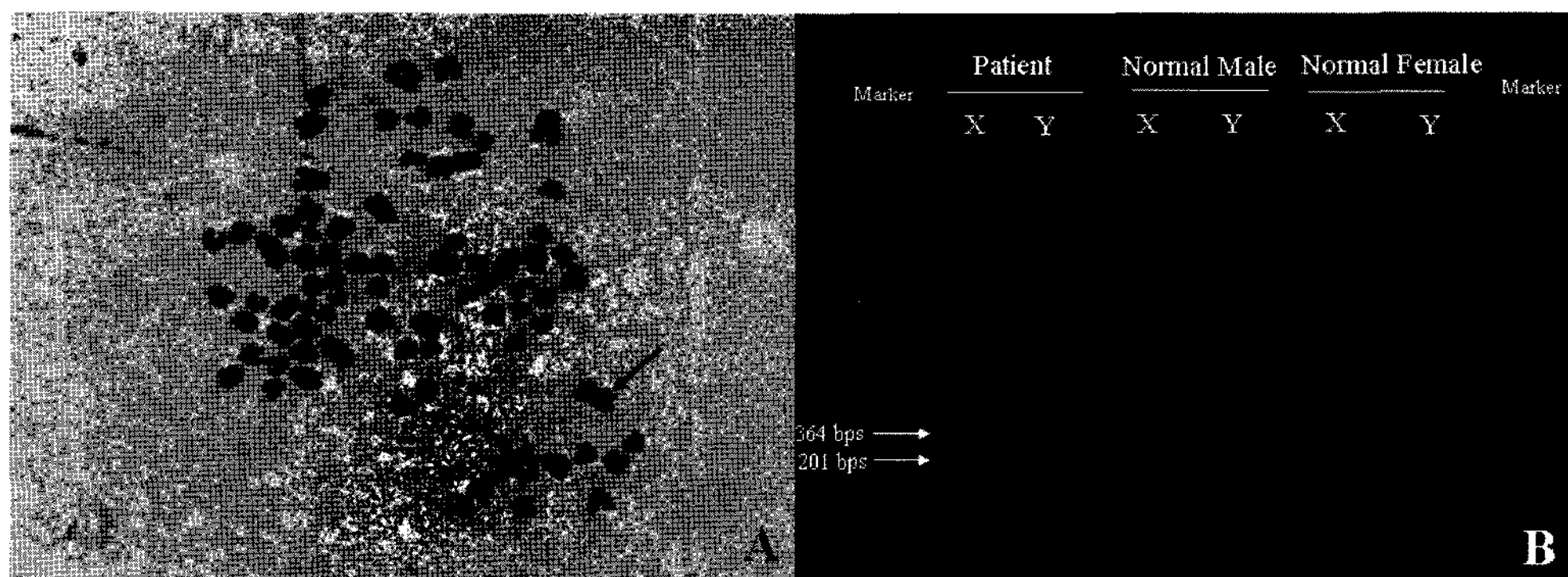


Fig 2. A) XX (arrow) chromosome ($2n = 78, XX$) shown by karyotyping, B) Sry-negative in patient by PCR.

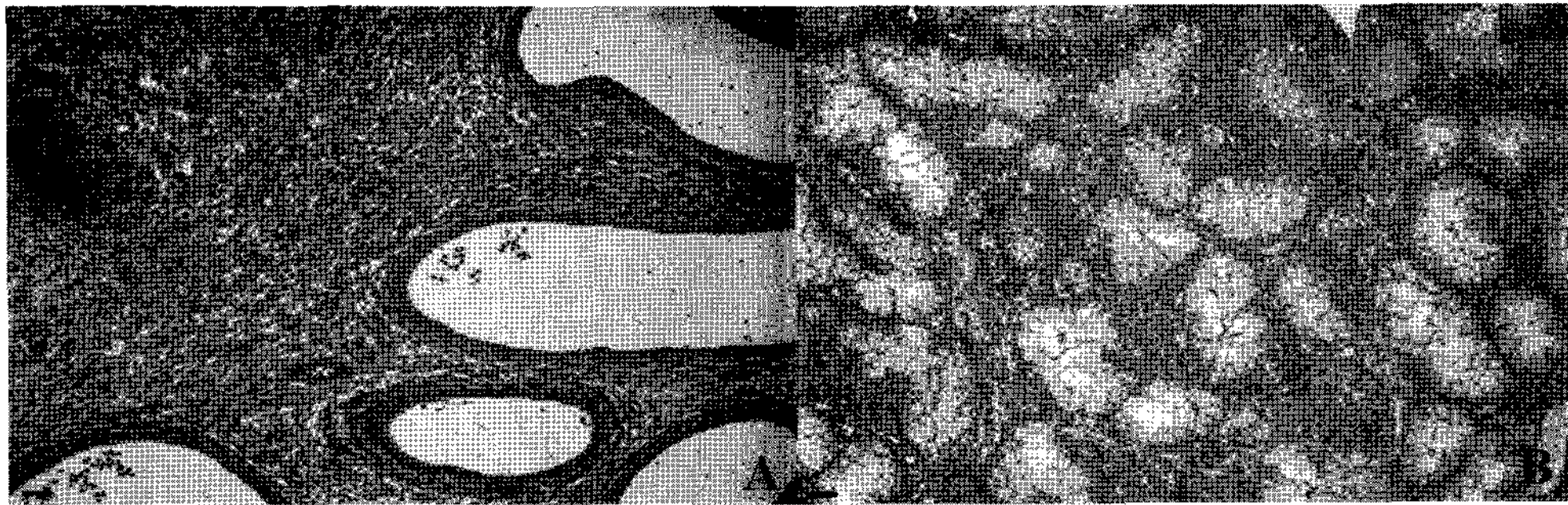


Fig 3. A) and B) histopathologic figure of cystic and seminiferous tubule structure in the ovo-testis. Scale bar = 40 μ m.

agreed to have the dog neutered at the same time. Before surgery, to know more exact structure of exposed tissue, image diagnosis was performed. As results, radiography revealed as os-clitoris (Fig. 1) and testes-like structure were located caudally to the kidney was detected ultrasonographically with a 7.0 MHz transducer in the abdomen scanning. After removal of os-clitoris, in exploratory laparotomy for neutering the patient, we found the normal uterus and unilateral ovary-like mass in macroscopic view of removed reproduction tract.

As further approaches for the patient, molecular and histopathological analysis was performed. Amplified PCR products were the same with that of the normal female dog (Fig. 2B). Analysis of karyotyping from cultured cells using standard cytogenetic techniques revealed a female chromosomal constitutions of $2n=78$, XX (Fig. 2A). Sequently, histopathological examination of the mass which was located in ovary showed the seminiferous tubule without spermatogonia and follicular cystic structure. There was no evidence of spermatogenesis (Fig. 3).

Sex reversal is a congenital abnormality whereby the sex chromosome composition of the individual is normal but it does not agree with gonadal sex. The putative trigger for testis formation is the *SRY* (Sex determining Region of the Y chromosome) gene located on the Y chromosome (1,10). The XX sex reversal may occur due to translocation of the *SRY* gene to an X chromosome or an autosome, resulting in an *SRY*-positive XX individual (9). However, XX sex-reversed individuals that do not have the *SRY* gene have been reported in humans (4), pigs (8), goats (12) and dogs (7).

In this experiment as described above, taking into consideration on these results of cytogenetic, molecular and histopathological examinations, the dog was diagnosed to be a *SRY*-negative XX sex reversed. The authors suggest that removing the genetic disorder of sexual organ development in specific breeds with exact diagnostic tools as mentioned above reduce the transmission of this genetic error to the next generations.

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