

Disorder of Sexual Development in a Cocker Spaniel Dog: Sry-negative XX Sex Reversal

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Abstract : A 3-year-old Cocker spaniel was presented for evaluation because of a mass protruding from the vulva. This mass confirmed the presence of an internal bone in clitoris on the radiography. We recommended to the owner of the surgical removal of the gonads and uterus as well as the excision of the enlarged clitoris and os clitoris. The dog had gonads in the region of where the ovaries were located and presented what appeared to be a bicornate uterus and ductus deferens. Histological analysis revealed a morphologically normal uterus and two testes with their epididymis and ductus deferens. Karyotyping revealed a normal female chromosome constitution (78, XX), and polymerase chain reaction (PCR) identified the absence of Sry. Based on these findings, the patient was diagnosed with Sry-negative XX sex reversal.

Key words : XX sex reversal male, hermaphrodites, Sry-negative, dog.

Introduction

The normal chromosome count for dogs is 78 chromosomes including the sex chromosomes. Normal mammalian sex determination begins at fertilization with establishment of chromosomal sex as either XY or XX (2). Some dogs with a normal karyotype will develop inappropriate gonadal tissue, for example, 78, XX with testicular tissue or 78, XY with ovarian tissue (2).

Individuals with disorders of gonadal sex have either an XX or an XY sex chromosomal constitution, but the karyotype does not agree with the respective gonads. This disorder is termed 'sex-reversed' (2,12). Sex reversal disorder includes XX males with bilateral testes and XX true hermaphrodite with ovotestes (14).

Sry is translocated to the X chromosome and as a result testicular tissue is developed consequently. In mice and humans, XX sex reversal can be due to the translocation of the Sry gene from the Y chromosome to the X chromosome during male meiosis (3). The genes responsible for testis induction in Sry-negative are unknown for horses, pigs and dogs.

Here, we present the case of a dog with Sry-negative XX sex reversal male. We obtained this result with the clinical, histopathological and genetic analysis of a Sry-negative XX sex reversal male.

Case

A 3-year-old Cocker spaniel was presented to animal medical center of Gyeongsang National University for evaluation because of a mass protruding from the vulva. The owners associated the mass with the dog's reluctance to maintain a sitting posture and another owner's complaint was urinary incontinence. The dog had never been in estrus before and exhibited male behavior in presence of bitches in estrus. Upon physical examination, a fleshy protuberance extending caudally between the labia of the vulva was detected. The upper skin of vulva was loosed like as scrotum (Fig 2C).

The results of a CBC and serum biochemical analysis were within normal ranges. Radiography confirmed the presence of an internal bone in clitoris (Fig 1). An ultrasonographic examination of the abdomen was then performed using a high frequency (12 MHz) linear transducer. The gonadal structures with the vascular structures were observed at the cranial part of the aortic bifurcation (Fig 1).

We recommended to the owner of the surgical removal of the gonads and uterus as well as the excision of the enlarged clitoris and os clitoris. The dog was premedicated with atropine (0.04 mg/kg, SC), diazepam (0.2 mg/kg, IV) and Butorphanol (0.2 mg/kg, SC). The dog was induced with propofol (6 mg/kg, IV) and maintained with isoflurane.

Laparotomy revealed two gonads resembling testis each with an epididymis and plexus pampiniformis, caudal to normal position of ovary. A complete bicornate uterus was also presented (Fig 2). Both gonads and the uterus were removed and submitted for histological evaluation. Episiotomy was

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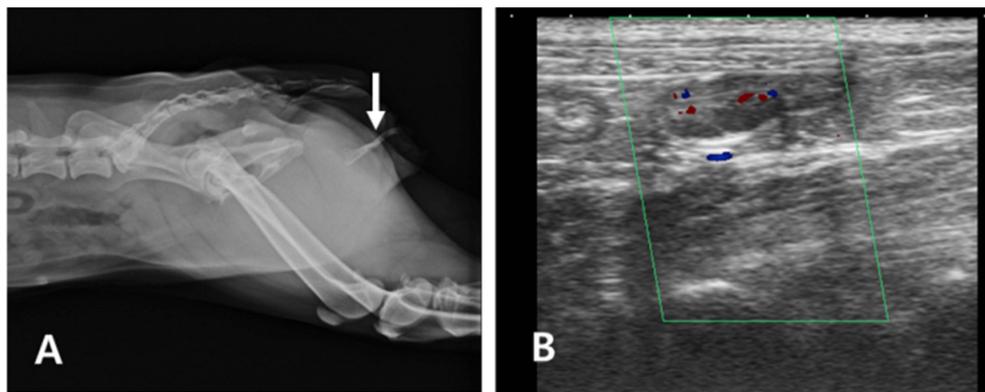


Fig 1. (A) Right lateral radiographic view, the linear mineralized opacity was shown (arrow). (B) The gonadal structures with the vascular structures were observed at the cranial part of the terminal of aorta.

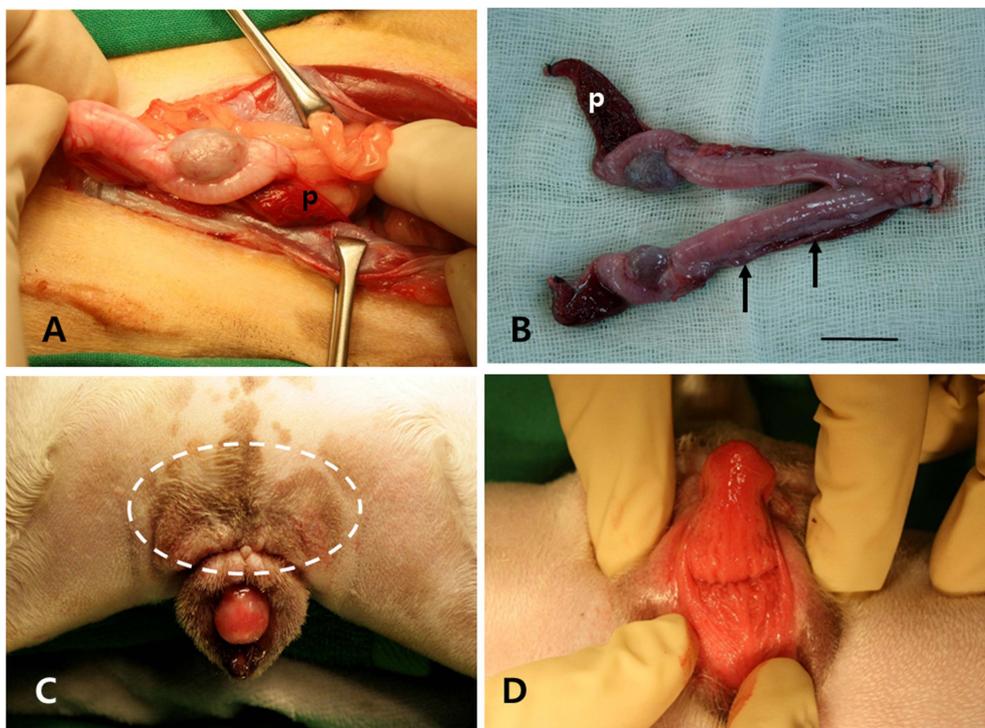


Fig 2. (A) Finding during surgery. The testis is connected to the epididymis and the plexus pampiniformis (p). (B) Gross appearance of the extracted uterus with the structure of ductus deferens (arrow). (C) The reddish penis-like structure protruding from the vulva is (D) an enlarged clitoris with a bone (os clitoridis). Note the scrotum-like skin at the dorsal of vulva (C, dashed circles).

performed to remove the enlarged clitoris tissue. The incision was made around the base of the clitoris. The submucosal incision was closed with absorbable suture.

Histological examination revealed the morphologically normal uterus and two testes with their epididymis and ductus deferens (Fig 3). The uterus was composed of a normal layer (perimetrium, myometrium, and endometrium) and lined by a continuous layer of cuboidal epithelial cells. Both testes were composed of non-spermatogenic convoluted seminiferous tubules. Both epididymes consisted of a loose connective tissue supporting empty tubules lined by a pseudo-stratified non-ciliated columnar epithelium rather than normal efferent

epididymal ductules.

For the analysis of chromosomes, fibroblasts were isolated from uterine tissue by general primary culture. The monolayer cultured cells were arrested in metaphase by adding 0.1 $\mu\text{g/ml}$ of colcemid for 1 hour and harvested using 0.25% trypsin/EDTA solution. The single cell suspension was incubated in hypotonic solution buffer (0.075 M KCl) for 45 minutes and fixed with methanol-acetic acid (3 : 1). After fixation, condensed chromosomes were spread on pre-cleaned slide glasses and stained with Giemsa solution (5). Analysis of karyotyping from cultured cells using standard cytogenetic techniques revealed a female chromosomal constitution of

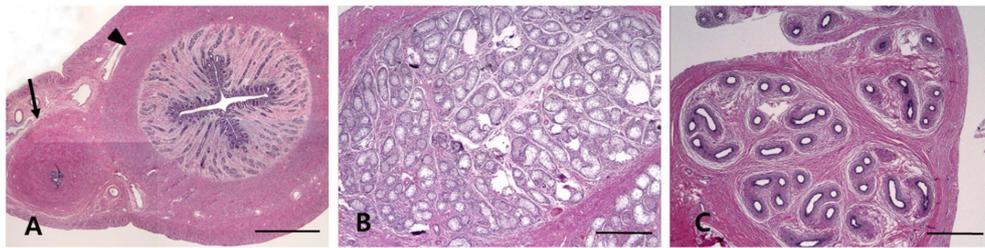


Fig 3. (A) Histological examination shows a ductus deferens (arrow) with epithelium and both inner circular and outer longitudinal smooth muscle layers, and the uterus (arrowhead) with thick mucosa containing glands, surrounded by myometrium and perimetrium (H&E, Bar = 2 mm). (B) A cross-section of testicular tissue, showing abundant seminiferous tubules lined by Serotoli cells and separated by fibrovascular septa and prominent Leydig cells (C) The epididymis structure. No spermatozoa are visible (H&E, Bar = 200 μ m).

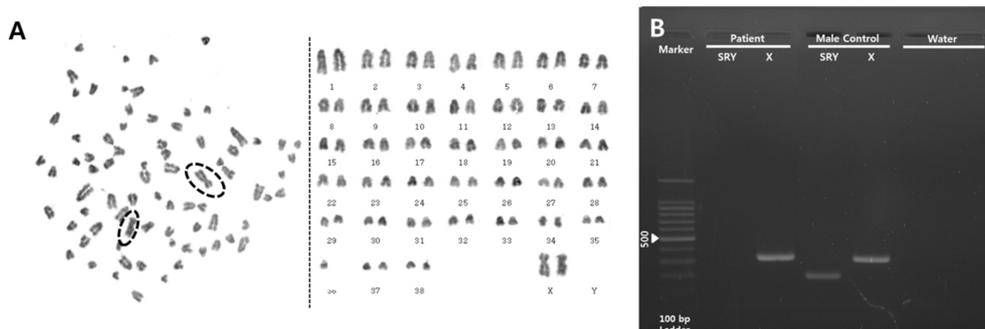


Fig 4. (A) Representative metaphase chromosomal spread (1000 \times) taken from uterine tissue. XX (dashed circle) chromosome ($2n = 78$) shown by karyotyping. (B) Polymerase chain reaction analysis of XX male genomic DNA for the presence of Sry gene and X chromosome. Patient shows Sry-negative. Male control shows Sry gene (Lane 3) and X chromosome (Lane 4).

$2n = 78$, XX (Fig 4A).

Genomic DNA was isolated from the uterine and gonadal tissues using ExgeneTM, Tissue SV kit (GeneAll Biotechnology, Korea). SRY gene and X chromosome specific region of genomic DNA were amplified by PCR. The primer pairs were used according to the previous report (Jang *et al.*, 2008). The PCR products were separated on 1% agarose gels containing ethidium bromide and visualized under ultraviolet light. PCR analysis revealed the presence of the Sry gene in the male control dog (Fig 4B, Lane 3). The patient was Sry negative (Fig 4B, Lane 1).

Discussion

In the XX sex reversals, chromosomal and gonadal sex do not agree and genetically female individuals have gonads with either ovarian and testicular tissue (XX true hermaphrodites) or testicular tissue alone (XX males) (10), and more recently XX sex reversals have simply been included under the broader category of the disorder of sexual development (2). In normal males the development of the testes is initiated by the Sry gene (sex determining region of the Y chromosome) located on the Y chromosome (7,13). Eighty percent of human XX males are Sry-positive (Sry-positive XX sex reversal) because of an autosomal translocation from the Y chromosome (14). The incidence of human XX males is one in 20,000 male births, and approximately 10% of such patients

are Sry negative (4,16). A dog with a chromosome count of 78, XX and the formation of testes or ovotestes have been reported in at least 18 canine breeds (12). XX sex reversal was originally described in the American cocker spaniel and seems most likely in all other dog breeds (1,8,11,12,15). To date, there are no documented cases of true XY sex reversals in dogs (2). However recently, a case of 78, XY ovotesticular disorder of sex development was reported in a cloned wolf (6).

XX true hermaphrodite individuals have both ovaries and testes. Bilateral ovotestes are the most common combination of gonads, followed by one ovotestis and one ovary, with one ovotestis and one testis being the least common combination (12).

Most XX males are phenotypic females or have a partially masculinized female phenotype that varies from a normal to abnormal vulva, normal sized or enlarged clitoris (commonly with an os clitoris). XX males have testes, the entire Wolffian duct system (epididymes and vas deferentia) and a prostate (11,12).

Diagnosis of intersexuality in dogs is difficult because abnormalities in sexual development leading to intersex conditions can occur at different levels, which includes the chromosomal composition, gonadal structures and phenotypic sexual development of the animal (2,10). XX animals with female appearance but with an enlarged clitoris cannot be diagnosed as pseudohermaphrodites or as XX sex reversals. Definitive diagnosis of the disorder of sexual development

minimally requires a karyotype analysis, gonadal histology, and description of genital anatomy.

The persistence of Müllerian structures in the presence of testicular tissue suggests that Müllerian inhibiting substances may be deficient or ineffective in the Cocker spaniel dog (17). For further diagnostic tests (2), hormonal or receptor assays and fluorescence in situ hybridization may be required. For dogs with this reproductive failure, further investigation is necessary to identify the gene-carrier dogs.

If clinical signs accompany the presentation, the removal of gonadal or genital tissue is recommended to treat sex-reversed dogs (2). Animals are generally presented for infertility or evaluation of ambiguous genitalia, but sometimes also for other complications such as pyometra, tumor or gonadal disease (2,9,12). In this report, the dog was uncomfortable in maintaining a sitting posture and predisposed urinary problems such as cystitis and vaginitis. This may ultimately develop into pyometra.

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코커 스파니엘 개에서 발생한 성-발달장애: Sry-negative XX 성전환

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요 약 : 3세령 코커 스파니엘 개가 질의 종괴를 평가하기 위해서 내원하였다. 이 종괴는 방사선 상에서 음경골이 있는 음핵이 확인 되었다. 생식선과 확장된 음핵의 제거를 보호자에게 추천하였다. 수술적으로 제거하는 동안 정관과 자궁 둘 다 관찰 되었으며, 난소가 있어야 할 곳에서 정소형태의 생식선이 관찰 되었다. 조직병리학적인 평가에서 정상적인 자궁과 고환과 부고환조직, 정관이 확인 되었다. 핵형분석에서는 정상적인 암컷의 염색체 (78, XX)를 보였고, PCR에서 Sry 가 나타나지 않았다. 이 결과들을 바탕으로 본 환축은 Sry-negative XX 성전환으로 진단되었다.

주요어 : XX 성전환 수컷, Sry-negative, 반음양, 개