

## An Intersex with Os Clitoris and Ovotestes in Korean Native Jindo Dog

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**Abstract :** Hermaphroditism occurs rarely in human and animal populations. Previously, in Korea, The hermaphrodite dog in American Cocker Spaniel dogs have been reported in six cases. However, in this case, we found a hermaphrodite dog in Korean native Jindo dog. The characteristics were its enlarged os clitoris that was shown a reddish finger-like structure protruding from the vulva. The dog was diagnosed as hermaphroditism with ovotestes. To our knowledge, this report is the first case of hermaphroditism in Korean native dog.

**Key words :** hermaphrodite, hermaphroditism, dog, Korean native dog, Jindo.

### Introduction

The intersex animal is an animal possessing the characteristics of both sexes. Intersex animals are also called pseudohermaphrodites or hermaphrodites depending on their gonads (4). Disorders of genital development occur in all species of mammals (2,14,20). Hermaphroditism is a condition when the subject has ambiguous genitalia with a part or all of the genital organs of both sexes present (14). The canine disorder may be genetically heterogenous, potentially with a different mutation in each breed, and may provide several models of sex determination including SRY (sex-determining region Y gene), especially for human SRY-negative XX Sex Reversal (XXSR) (12). Normal sexual differentiation of the embryo is dependant upon the completion of 3 major events (11,13). It begins with the establishment of the chromosomal constitution of the zygote as XX or XY at fertilization. The chromosomal sex then determines gonadal sexual differentiation. The development of the testis is initiated by the Sry gene, which is normally located on the Y chromosome. Expression of the SRY gene in the testis occurs only during a short period in the embryonic development (9). In the absence of a Y chromosome, ovarian differentiation normally occurs. Hermaphrodite means an animal or a human being in which male and female sex organs are present simultaneously, or in which the sex organs contain both ovarian and testicular tissue (13). Sometimes one gonad becomes a testis while the other becomes an ovary; sometimes the gonads become ovotestes containing a mixture of male and female components (3). Although hermaphroditism is often associated with infertility, that is not always the case. Hermaphroditism occurs rarely in

human and animal populations (12).

We reported previously the case studies of three hermaphrodite American Cocker Spaniel (8,15). Furthermore, we also met other three reported cases in Korea (1). In this study, we found newly a hermaphrodite dog in Korea native Jindo dog and analyzed the characteristics of the submitted case.

### Case

The studied animal (WK28052) was 5-months old Korean Jindo dog with 13 kg body weight and white coat color (Fig 1). It was evaluated with vaginal bleeding, and intersex anomalies. Intersex was identified in the dog with an os clitoris that was buried in the intact vulva. We analyzed the characteristics

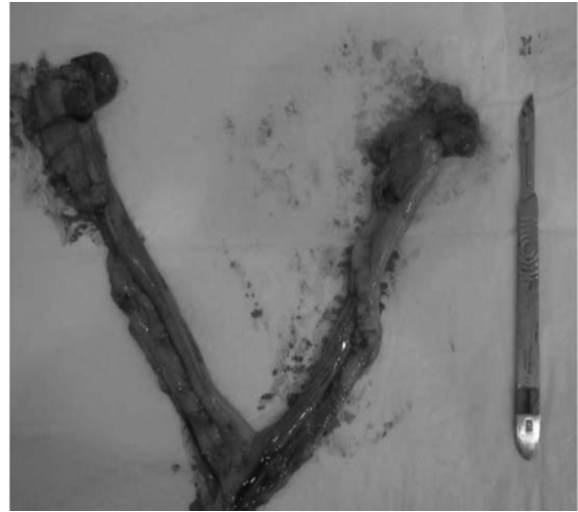


**Fig 1.** The studied animal (WK28052) was 5-months old Korean Jindo dog with 13 kg body weight and white coat color.

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**Fig 2.** The case revealed external female appearance and the intact vulva was normal structure.



**Fig 4.** The gonadectomy of WK28052 revealed bilateral testes-like gonads instead of ovaries.



**Fig 3.** WK28052 had a reddish finger-like structure, os clitoris (arrow), protruding about 4 cm from the vulva.

of the submitted hermaphrodite case. The morphological observations and surgical dissections were performed. The gonadectomy, clinical exam and pathological studies were performed. The trimmed tissues were fixed in 10% neutral buffered formalin, and embedded in paraffin. Four- $\mu$ m sections were made and stained with haematoxylin and eosin for histopathological examination. Also, in order to identify their sexing, DNAs were extracted from the hair roots and amplified by polymerase chain reaction (PCR) with a set of primer described by Takami *et al* (19). SRY primers used in this study were designed according to conserved region of the human pY53.3 DNA sequence (17,19): the upstream primer was 5'-AAGCGACCCATGAATGCATTCATGGTGTGGT-3' and the downstream primer was 5'-GAGGTCGATACTTATAGTTCGGGTATTTCTCTCTGTG-3' (6,19). The amplification profile comprised 32 cycles: at 93°C for 1 min (dissociation),

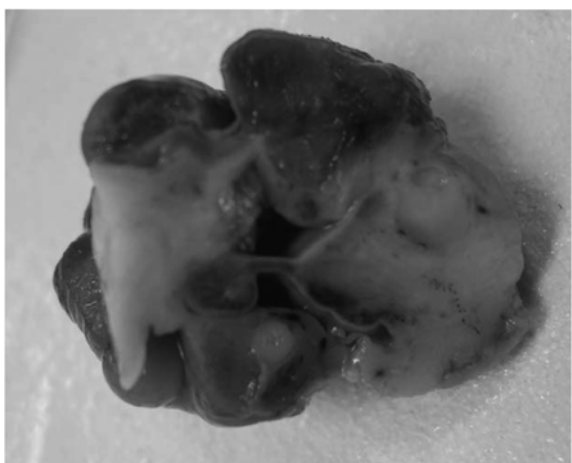
55°C for 2 min (annealing) and 72°C for 3 min (extension). The final cycle included an additional 7 min at 72°C for complete strand extension. The reaction mixture contained 2  $\mu$ l template DNA, 2  $\mu$ l of 50 pmole of upstream primer, 2  $\mu$ l of 50 pmole of downstream primer, 5  $\mu$ l of 0.2 mM each dNTP, 5  $\mu$ l 10xPCR buffer, 1  $\mu$ l of 2.5 unit/ $\mu$ l Tth DNA polymerase and 33  $\mu$ l distilled water (DW) to 50  $\mu$ l (19). After PCR amplification, the PCR products were separated by electrophoresis for 30 min, at 100V in a 3% MetaPhore Agarose gel in 1xTBE stained with ethidium bromide and visualized under UV light.

In this study, the case (WK28052) was a 5-months old Korea native Jindo dog with external female appearance and the intact vulva was normal structure (Fig 2). However, the dog had the os clitoris buried in the vulva. The dog was presented due to bleeding, a reddish finger-like structure, os clitoris, protruding about 4 cm from the vulva (Fig 3). Clinicopathological features of the dog suggested hermaphroditism. The gonadectomy of WK28052 revealed bilateral testes-like gonads instead of ovaries and the uterine horns and uterus were shown normally (Fig 4). The gonads were situated caudal to the kidneys at the cranial tips of the uterine horns. The testes-like gonads showed dark reddish spheres with 2-2.5 cm diameters and revealed lobular shapes (Fig 5). The cut surfaces of testes-like gonads showed dark reddish and revealed lobular shapes (Fig 6). The vulva bleeding symptom and the os clitoris was recovered by surgical correction. The finger-like os clitoris was about 1.3 cm diameters and revealed an enlarged os clitoris including bone.

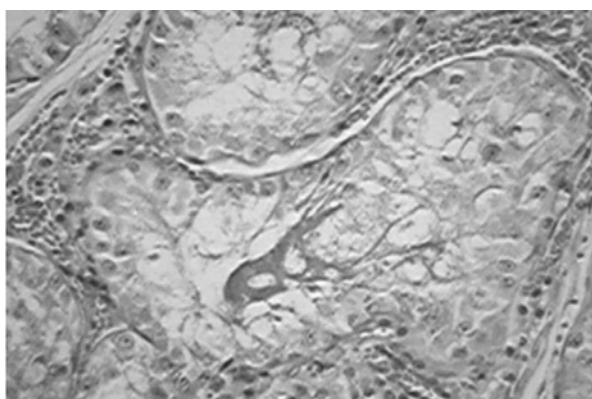
Clinical diagnosis was confirmed by gross and histopathological examination of the dissected tissues revealing hermaphrodite with bilateral ovotestes. The histology of both gonads was similar, being ovotestes. The gonads were composed of seminiferous tubules and interstitial cells (Fig 7). However, in the other area, ovarian follicles were observed (Fig 8). In those ovotestes, the seminiferous tubules may show incomplete



**Fig 5.** The testes-like gonads showed dark reddish spheres with 2-2.5 cm diameters and revealed lobular shapes.

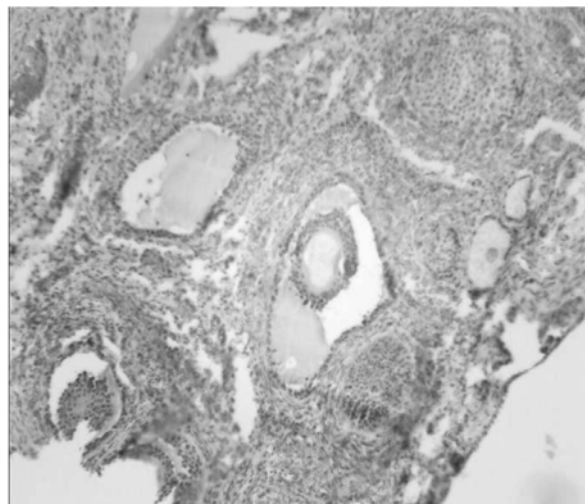


**Fig 6.** The cut surfaces of testes-like gonads showed dark reddish and revealed lobular shapes.

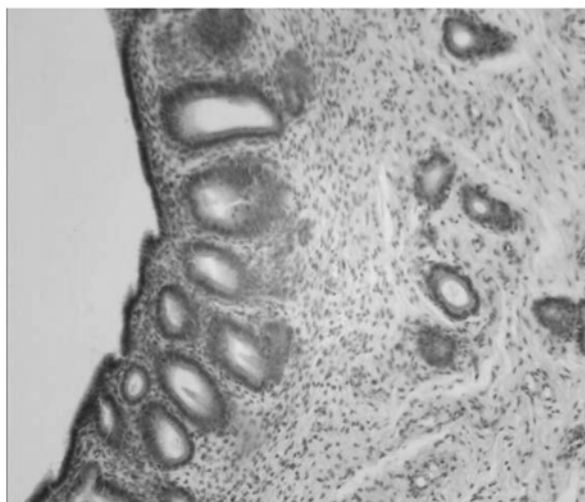


**Fig 7.** The gonads were composed mainly of seminiferous tubules and interstitial cells. H & E,  $\times 200$ .

spermatogenesis. Oviductal tissue and incompletely developed epididymis were found adjacent to each gonad. Each gonad had pampiniform plexus which contained with dilated tubules. The typical uterus appearances were observed from the tissues



**Fig 8.** In the other area of gonads, ovarian follicles were observed. H & E,  $\times 200$ .



**Fig 9.** The typical uterus appearances were observed from the tissues of uterus. H & E,  $\times 200$ .

of uterus (Fig 9).

PCR amplification of sex determining region in Y chromosome and agarose gel electrophoresis were used to determine unknown sex of WK28052 sample. In this study, we used a method based on Y chromosome specific region which was unique to male, so its presence or absence in a sample of genomic DNA was indicative of sex. In male, an amplified Y-specific region made a band in agarose gel whereas there was no band in female. In the present study, male control had one band and female control had no band. These results indicated that WK28052 not showing band in agarose gel was female.

## Discussion

Problems associated with intersex animals are not unique to intersexes but can be found in animals with normal chro-

**Table 1.** The characteristics of hermaphrodite dogs in Korea

No	Breed	Age	Characteristics	Sexing	Reference
1	American Cocker Spaniel	8 months	Bilateral ovotestes, os clitoris	Female	Rho <i>et al.</i> (15)
2	American Cocker Spaniel	3 months	Bilateral ovotestes, os clitoris	Female	Rho <i>et al.</i> (15)
3	American Cocker Spaniel	3 years	Bilateral ovotestes, os clitoris	Not tested	Kim and Kim (8)
4	American Cocker Spaniel	2-3 months	unilateral ovotestes, os clitoris	Male	Alam <i>et al.</i> (1)
5	American Cocker Spaniel	2-3 months	unilateral ovotestes, os clitoris	Male	Alam <i>et al.</i> (1)
6	American Cocker Spaniel	2-3 months	Bilateral ovotestes, os clitoris	Female	Alam <i>et al.</i> (1)
7	Korean native Jindo	5 months	Bilateral ovotestes, os clitoris	Female	Cho <i>et al.</i> (this report)

mosomal karyotypes (4,18). These developmental disorders are caused by abnormalities of genetic or chromosomal origin, or inappropriate hormonal or chemical exposure (14). The control mechanisms of sexual development are sequential and normal sexual differentiation of the embryo depends on three major events. The chromosomal sex determines the gonadal sex differentiation, and the translation of the gonadal sex to phenotypic sex (14). Differentiation along ovary or testis pathways is determined by the sex chromosomal constitution of the embryo, which is established as XX or XY at fertilization. The Y-chromosome carries a dominant inducer of testis development, the SRY gene (sex-determining region of Y) (17). The development of the testis is initiated by the SRY gene and in its absence the ovarian differentiation normally occurs (5,7,17). The phenotypic sex is regulated by the gonads after their differentiation (5,7), which discordance exists between the sex chromosomal composition and the gonadal and external phenotype. It is due to a gene referred to as the sex reversal gene (*sxr*). In dogs sex reversal occurs frequently in American Cocker Spaniels, which reported to be male pseudohermaphroditism (MPH) having XX genotype and *sxr* gene located on an autosome (10,16).

We have previously described the occurrence of a hermaphrodite American Cocker Spaniel (8) and two other cases of hermaphrodite dogs (15). Alam *et al.* (1) reported also three hermaphrodite dogs in Korea. The internal genitalia somewhat resembled those of the female with testicles instead of ovary. A persistent Mullerian duct was identified in two cases, and abdominally located testicles in all the cases. The gonads revealed inactive seminiferous tubules and epididymides (1). The gonads were confirmed as testes (1). Previously, we analyzed that the reported hermaphrodite dogs in Korea were American Cocker Spaniel and American Cocker Spaniel dogs have susceptible to hermaphroditism (15). However, in this case, we found a hermaphrodite dog in Korea native Jindo dog like as Table 1. The characteristics were its enlarged os clitoris that was shown a reddish finger-like structure protruding from the vulva. The dog revealed vulva hypertrophy and clitoris hypertrophy. Polymerase chain reaction amplification of sex determining region in Y chromosome and agarose gel electrophoresis were used to determine unknown sex. In this study, we used a method based on Y

chromosome specific region which is unique to male. As the results, the case WK28052 could be identified as a female sex. The dog was diagnosed as hermaphroditism with ovotestes.

## Conclusion

We found a hermaphrodite dog in Korean native Jindo dog. The characteristics were its enlarged os clitoris that was shown a reddish finger-like structure protruding from the vulva. The dog was diagnosed as hermaphroditism with ovotestes. To our knowledge, hermaphroditism may be a rare case in dog. This report is the first case of hermaphroditism in Korean native dog.

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## 진도개에서 발생한 Os clitoris와 Ovotestes를 특징으로 하는 양성자 사례 연구

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**요 약** : 양성자(Hermaphroditism)는 사람과 동물에서 매우 드물게 발생단계의 이상으로 발생한다. 한국에서 개의 양성자 발생 보고는 6 사례가 있으며 그들 모두가 American Cocker Spaniel 품종이었다. 본 연구자들은 진도개에서 발생한 양성자 사례를 발견하고 그 특징 분석과 병리조직학적 연구 및 분자생물학적 성별감별 연구를 수행하였다. 본 사례 연구를 통하여 os clitoris와 ovotestes를 가지며 암컷 성을 가진 양성자 진도개를 최초로 확인하고 보고하는 바이다.

**주요어** : 양성자, 성별이상, 개, 진도개.