Canine Disorder of Sex Differentiation

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INTRODUCTION

Disorder of sexual differentiation (DSD) is caused by chromosomal abnormalities, mainly DAX-1 duplication gene on the X chromosome. During normal embryogenesis, DSD can occur, which results in genital tract malformations that can be mild or severe, including pseudo-hermaphroditism (PHP) (García-Acro et al., 2020). Most frequently reported PHP is found in the male, and is due to the failure of paramesonephric duct to regress (Persistent Mullerian Duct Syndrome, PMDS) or due to the failure of masculinization because of hormonal disorders such as androgen insensitivity syndrome (Mullen and Behringer, 2014). PMDS, which is a hereditary autosomal recession in the miniature Schnauzer, is most common in the dog.

After the diagnosis of sexual disorder using different methods and identifying the gender of the animal, surgical intervention seems to be the only available approach to treat the condition (Torad and Hassan, 2016). The objective of this study was to report the history and clinical signs, identify the sexual identity and surgical correction of the deformed parts in a dog.

The disorder of the X chromosome causes defects in sex differentiation in the female phenotype. This work reports a six-month-old, Cocker Spaniel dog with intersex disorder diagnosed as pseudo-hermaphroditism. The dog was brought to the clinic with a swollen reddish penis-like protrusion, which was partially covered by the sheath. Clinical examination revealed the presence of raised nipple-like structures, resembling the nipples and extending from chest to groin region. However, the testes could not be detected. PCR analysis revealed the absence of the Y chromosome. A corrective surgery, including clitidectomy, and removal of uterus and testicles-like structures, was performed. Histological examination of the removed organs showed underdeveloped testes and well-recognized endometrium. This report suggests that surgical correction may be used to overcome any possible complications in a six-month-old dog with pseudo-hermaphroditism.

ABSTRACT


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Primers of canine sex-determining region Y (SRY) genomic sequence were designed according to a previously published study, forward CTCGGATCAAAG GCGCAAGAT and reverse TTTCGGTTCTGTAAGCAT TTTC (Prugnard et al., 2016). PCR kit (Promega Co., Madison, WI, USA) was used for the chromosomal analysis. The PCR was carried out in a thermal cycler (Takara Bio. Inc., Japan) and the product was run in the gel with a DNA ladder marker. The bands were detected using SYBR Green I (Sigma, St. Louis, MO, USA) staining and a blue light trans-illuminator (Piazza et al., 2017). PCR electrophoresis revealed the absence of Y chromosome in the dog (Fig. 3), which suggested that sex identity of the dog was a female. Therefore, the surgical removal of the male gonads like structures was performed, as described below:

**Surgical intervention:** Surgical removal of the male gonads like structures was performed through celiotomy, gonadectomy, and clitoridectomy, as described by Sumner et al. (2018). Briefly, after induction of anesthesia, a ventral midline incision (15 cm) was made. A testicle-like structure connected to epididymis, identified in the right caudal abdomen, was ligated using 3-0 synthetic polymer (Go, Medical Korea) and removed. Then, a 3-cm incision was made over the subcutaneous mass, the left testicle was isolated and removed. After cleaning, a 3-0 synthetic polymer was used to close the skin incision. For clitoridectomy, urethral opening was located using urinary catheter (size 16). Surgical incision was made over the dorsal aspect of the os-clitoris, which was removed using blunt dissection. The prostate gland couldn’t be found. The anomalous gonads and the uterus were removed and a routine closure was made. Simple continues suturing was used to close the clitoral incision. The abdomen was closed, as previously prescribed. To ensure resolving of swelling in the operation site, the urinary catheter was kept for 24 h. Urination was followed up after removal of the urinary catheter to encounter any possible complications. The position of internal genitalia after surgical removal is shown in Fig. 4. Post-operative care included administration of ceftriaxone HCL 500 mg (Daewoong pharm. co. Ltd, Korea) for five days, as well as vaginal wash using povidone-iodine (Daewoong pharm. co. Ltd, Korea). The dog showed complete recovery without any complication.

**Differential diagnosis:** Immediately after removal, samples of testes-like and uterine tube-like structures were taken. These samples were processed for the routine hematoxylin and eosin (H&E) staining for histological examination (Webster et al., 2017).

Histologically, interstitial tissue in the testis was more abundant compared to the healthy adult dog (Fig. 5A), with round Leydig cells were detected in interstitial tissue (Fig. 5B&C). Meanwhile, only spermatogonia were identified in seminiferous tubules (Fig. 5B&C), without any indication of active spermatogenesis. Uterine tissue with endometrial glands could also be detected in the tubule (Fig. 5D-F). These histological findings suggested the distorted testes with aspermato genesis.
DISCUSSION

The chromosomal study of the dog suggested the absence of Y chromosome. Therefore, the possibility of the dog to be a male was excluded. A previous study also revealed that numerous species including, humans and dogs experienced urogenital defects during development (Dietz et al. 2018). Szczerbal et al. (2017) also observed that disorder of sex development in a cat with chromosome mosaicism showed external genitalia ambiguity, such as undeveloped penis and scrotum.

The urethra was abnormally widely separated from the enlarged protruded os-clitoris. Hence, the removal of the clitoris with its entire os-clitoris was done carefully. Urinary tract infection, which is common in such a surgical operation (Park et al., 2016), was successfully avoided.

Removal of testes, ovaries, and uterine horn-like structure is the central part of corrective surgery of FPH to minimize the risk of ovaries and uterine diseases or abnormal growth (Gregory and Trower, 1997; Sacks and Béraud, 2012). In the present study, the externally protruded part in the dog appeared to be an os-clitoris or enlarged clitoris. Corpora cavernosa of male penis resembles the roots of the clitoris in dogs (Gregory and Trower, 1997), ossification and hyperfibroplasia of the corpora cavernosa leads to canine os penis. Os-clitoris could be the outcome of the similar ossification process.

Because of difficulties in performing usual skin preparation for clitoridectomy, antibiotics were used postoperatively. Instead of resembling the normal anatomical position of either a male (distal end of the pars longa glands) or female (approximately 3 cm cranial to the clitoris), the urethral opening was located along the caudo-ventral surface of the clitoris at the caudal end of the hypospadia, which is abnormally located urinary orifice in the male due to incomplete masculinization of urogenital sinus (Marei et al., 2016). Initially, the embryo has the undifferentiated ducts and structures, from which both internal and external genitalia develop (Marei et al., 2016; Araujo et al., 2018). The possible cause of this condition seems to be the genetic disorder during embryogenesis.

Conclusions: The case study has shown that a sex disorder of external genitalia can be confirmed by chromosomal analysis. A surgical management of the external genitalia in case of feminine sexual disorder is recommended in dogs with similar clinical signs.

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