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CASE REPORT
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Disorder of Sexual Development in a French Bulldog (78, MALE XX)

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ABSTRACT

***Background*:** Differentiation of the genital system occurs through a chronological sequence of events involving the determination of chromosomal, gonadal, and phenotypic sex. Disorders of sexual development (DSDs) include chromosomal sex abnormalities, gonadal abnormalities, and phenotypic variations. The purpose of this case report was to demonstrate the clinical signs and diagnosis by histopathology, immunohistochemistry, and karyotyping of a DSD in a French Bulldog (XX, male).

***Case*:** A 7-month-old phenotypically female French Bulldog with a history of clitoral hypertrophy and no signs of puberty is reported. The animal presented with a 3 cm micropenis protruding from the vulvar labia, causing discomfort and constant licking. Pelvic radiography confirmed the presence of a penile bone, supporting the differential for a disorder of sexual development (DSD). There were no abnormalities of the vaginal canal or urethral orifice. The animal underwent exploratory celiotomy to remove 2 uterine horns and their respective gonads, followed by clitoridectomy via episiotomy. Sex reversal was confirmed by histology, as seminiferous tubules and bilateral hypoplastic epididymides were identified on ovarian topography. Immunohistochemistry of gonadal tissues was positive with antibodies to progesterone and estrogen receptors, but negative with polyclonal androgen receptors and prostate-specific antigen. Karyotyping revealed XX sex chromosomes.

***Discussion*:** This dog was diagnosed as an XX DSD male (DSD 78, testicular XX) due to the presence of abdominal testes, uterus, predominantly female external genitalia with vulva and micropenis, and female sex chromosomes. Six cases of DSD have recently been reported in phenotypically female French Bulldogs due to the presence of clitoral hypertrophy or a micropenis with an atypical location. The increase in cases in this breed may be due to its popularity and increased consanguinity. Animals with clinical suspicion of DSD show signs during puberty and most of these animals tend to have a history of externalized structure in the vulva without signs of urethral and vaginal canal obstruction. In animals with ambiguous genitalia and DSDs, the estrous cycle may be normal, absent, or irregular. Because of the discomfort in the clitoral region, clitoridectomy is the 1st line of treatment to prevent self-mutilation and future cases of vaginitis, and gonadectomy is also performed to prevent future uterine disorders and gonadal and mammary carcinogenesis. It is not uncommon for XX male animals to be unilateral or bilateral cryptorchids, in this case, both testes were in the abdominal cavity, and the animal had a normal uterus. Several alternative procedures can be performed for a better understanding of DSDs, namely: histopathology; immunohistochemistry or hormone dosage; karyotyping; and molecular analyses. These tests are important to determine the genetic sex of an animal and to confirm its concordance with the phenotypic sex. There is no evidence of interference of specific genes in the phenotypic expression of XX males, suggesting that the factors involved in their occurrence are still unknown. The hereditary nature of this disorder has not been ruled out and there are currently no laboratory tests available to identify carriers. It is recommended that cases of DSD be thoroughly investigated using complementary examinations to ensure accurate categorization of each disorder.

Keywords: clitoral hypertrophy, ambiguous genitalia, immunohistochemistry, cytogenetics, sexual dimorphism.

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INTRODUCTION

Disorders of sexual development (DSDs) include chromosomal sex abnormalities (such as defects in the number or structure of sex chromosomes), gonadal mismatch (when the gonads do not correspond to the chromosomal sex), and phenotypic variations (when the internal or external genitalia do not match the gonads or sex chromosomes) [7].

Although DSDs are rare genetic conditions in dogs, testicular disorder (DSD XX testicular/male XX) and ovotesticular disorder in chromosomal females are the most reported [14]. Typically, these animals are diagnosed during puberty when their owners seek care for an externalized structure in the vulvar region or abnormalities of the external genitalia, usually without urinary tract changes [8-10].

Surgical treatment is recommended for animals with DSDs to correct external genitalia and prevent urinary tract infections, pain, and vaginitis, and to perform gonadectomy to prevent uterine disorders and gonadal and mammary carcinogenesis [6,8,16]. In addition, histopathology and cytogenetics can help clinicians understand the effects and causes of chromosomal abnormalities at different stages of embryonic development and improve the diagnosis of dogs with DSDs [4].

This paper aimed to report a case of DSD in a French Bulldog (XX male) and to demonstrate the clinical signs and diagnosis by histopathology, immunohistochemistry, and karyotyping.

CASE

A 7-month-old French Bulldog, phenotypically female, weighing 8.6 kg, presented with a history of progressive swelling in the clitoral region for approximately 2 months. The main complaint was discomfort and constant licking, which caused inflammation. During the gynecological examination, a 3 cm firm structure was found in the clitoral fossa which protruded outside the vulvar labia (Figure 1A-B). Pelvic radiography confirmed the presence of a bone structure in the hypertrophic clitoris (Figure 1C). Vaginoscopy revealed a normal vaginal canal and urethral orifice, and vaginal cytology was compatible with the anestrus phase of the estrous cycle.

The animal under study was born from eutocic delivery as a single offspring and has not exhibited any signs of puberty. The mother did not receive any hormone treatments before or during pregnancy.

After identifying reproductive structures on abdominal ultrasound, the animal underwent exploratory celiotomy surgery. During the surgery, the gonads and uterus were removed, and a clitoridectomy was performed through an episiotomy (Figure 1D-F). Postoperative complications did not occur, and the symptoms were resolved.

Macroscopically (Figure 1E), the structure observed in the topography of the ovary presented a morphology compatible with a testicle. Filiform tubular structures were observed in the ipsilateral region to the uterine horns, which were connected to the uterine horns and were compatible with the vas deferens.

The organs were fixed in 10% buffered formalin for histopathology and hematoxylin-eosin staining. Microscopically, the gonads presented seminiferous tubules, and bilateral hypoplastic epididymis was observed (Figure 2A). Notably, the seminiferous tubules lacked germ cells and sperm and were lined with Sertoli cells. The epididymis comprised a few duct segments of cubic pseudostratified epithelium with a scarce amount of stereocilia intermingled with loose connective tissue. Additionally, the morphology of the uterine horn and body tissue was within normal standards, as was the presence of the penile bone.

Immunohistochemistry was performed on samples from both gonads using the following antibodies: AR (Androgen receptor, N-20, 1:100, polyclonal antibody, sc-816)[1], PSA (Prostate-Specific Antigens, 1:2000, polyclonal antibody, bs-4812R)[2], ER (Estrogen receptor, monoclonal antibody, Anti-Human Estrogen Receptor α Clone EP1)[3], and PR (Progesterone receptor, monoclonal antibody, 1:50, Ab-2-clone hPRa2)[4]. Primary antibody was omitted as a negative control. Positive reactions were observed for progesterone and estrogen receptors and negative reactions for androgen receptors and prostate-specific antigen, as shown in Figure 2B-E.

Cytogenetic analysis was performed on a peripheral blood sample collected aseptically. Mitotic chromosomes were obtained using standard procedures and the modified technique of Moorhead *et al*. [11]. All analyzed metaphases showed a normal female karyotype (78, XX) with standard size and morphology of the sex chromosomes (Figure 2F).

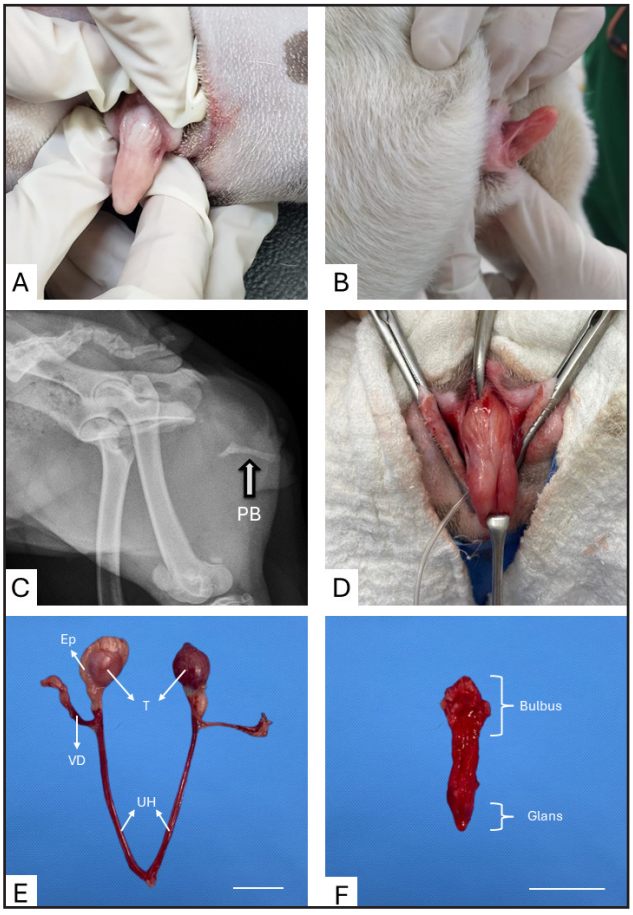

Figure 1. A&B- Photographs of the hypertrophic clitoris protruding from vulvar labia. C- Pelvis radiographic image. Penile bone is observed in the topography of the clitoris (Arrow). D- Exposure of the clitoris through episiotomy in preparation for excision. Dorsal recumbent. E- Internal reproductive tract. From upper region: Epididimides (Ep), Testes (T), Vas deferens (VS), Uterine Horn (UH). F- Clitoris. [Bar= 1 cm].

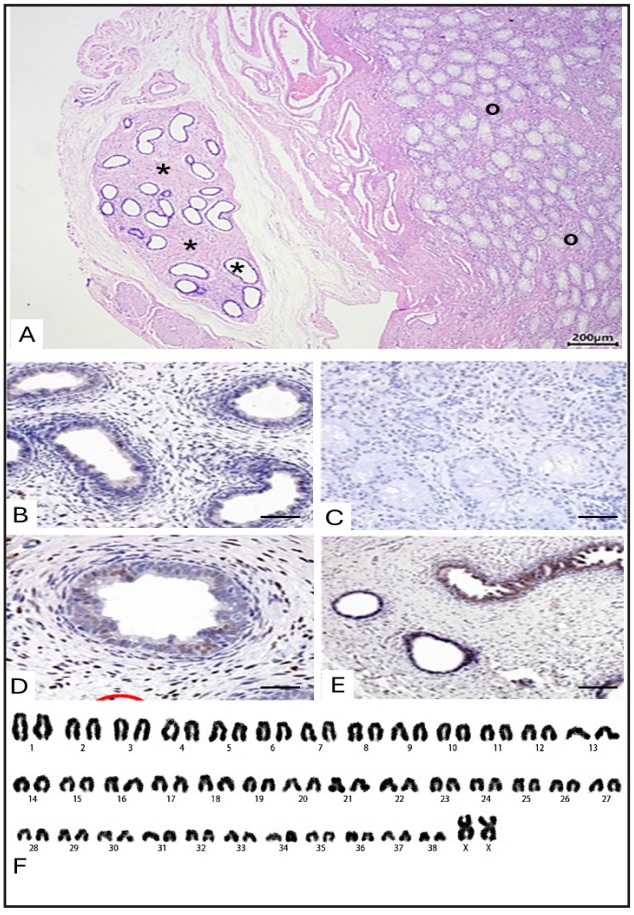

Figure 2. A- Photomicrographs of testicular tissue are presented. The left image shows a few duct segments of epididymis (*), while the right image shows hypoplastic seminiferous tubules (O). [Bar= 200 µm]. B - E: Photomicrographs of immunohistochemistry staining of PSA (B), AR (C), ER (D), and PR (E) in the testicular tissue. Note the absence of reaction on images B and C and the presence of immunostaining areas (brown) on images D and E showing positivity for those receptors (ER and PR, respectively). [Bar= 50 µm (B, D)]. [Bar= 100 µm (C, E)]. F- A normal female karyotype (78, XX).

DISCUSSION

The case study animal presented a discrepancy between chromosomal and gonadal sex, resulting in a testicular disorder in a genetic female. The animal was classified as an XX male, also known as DSD XX testicular [17]. A recent study reported 6 new cases of DSDs in phenotypically female French Bulldogs due to clitoral hypertrophy or a penis-like structure with an atypical location [18]. The rise in the number of cases in this breed may be attributed to its increased popularity and the rise in consanguinity [3,15,18,19]. Typically, animals with clinical suspicion of DSDs seek care during puberty, between the ages of 4 and 12 months. These animals tend to have a history of exteriorized vulva structure without signs of urethral and vaginal canal obstruction [8-10,15,19].

In animals with ambiguous genitalia and DSDs, the estrous cycle can be normal, absent, or irregular. As commonly described in the literature, the animal in this case had not yet started the estrous cycle. However, there is a case report of a true hermaphrodite showing signs of possible estrus after 2 years of age, indicating that most XX true hermaphrodites may not be sterile [13]. In contrast, most XX males are sterile [14].

Notably, the dog in this case had clitoral hypertrophy with a penile bone, which was also observed in most studies in which there was suspicion of DSD with

ambiguous external genitalia. Clitoral hypertrophy can occur due to exogenous exposure to androgens [7], which can be problematic during pregnancy as it can lead to the masculinization of female offspring. However, medication was not administered to the mother of the animal in the present report, ruling out this situation.

Due to the discomfort in the clitoral region, clitoridectomy was chosen to prevent the animal from self-mutilation and future cases of vaginitis. However, some researchers opted not to perform clitoridectomy, particularly when the patients did not demonstrate discomfort or complications in the affected region [10].

During the celiotomy, it was observed that the gonads had a testicular morphological appearance, consistent with reports by other authors [10,19]. A gonadectomy was performed to prevent future uterine disorders, and gonadal and mammary carcinogenesis, and to enable future exploratory studies for a better understanding and diagnosis of these congenital DSDs.

To gain a better understanding of DSDs, several alternative procedures can be performed, including histopathology, immunohistochemistry, hormone dosage, karyotyping, and molecular analyses.

Histopathological examination confirmed sex reversal in the 2 structures observed in the ovary topography. In addition, the horn tissue, uterine body, and penile bone were within physiological standards, consistent with other reports in the literature [9,18]. Immunohistochemistry results showed positivity only for female and non-male hormone markers, which contradicts the presence of a testicular structure in the animal. Notably, other cases in the literature indicate that hormone production has always been positively correlated with gonadal sex [5]. Unfortunately, due to financial constraints, hormone measurements were not possible in this case.

To the authors' knowledge, this is the 1st report in which immunohistochemistry was used as a complementary test in an XX male dog. There is a scarcity of data in veterinary studies to support a discussion regarding the expression of female hormone receptors in the testicles of animals with DSDs. However, studies conducted in humans have demonstrated the presence of progesterone markers in the testes [1]. Progesterone may play a role in regulating spermatogenesis, and altered expression of progesterone may be associated with infertility.

Cytogenetic testing enables the determination of an animal's genetic sex, which is crucial for confirming its concordance with the phenotypic sex. There is a report with 5 cases identified with a female karyotype (78, XX) and the absence of Y-linked genes (SRY and ZFY), but with testicular tissue classified as DSD XX (SRY-negative) [18]. This pattern is frequently observed in the literature [3,8,19]. However, 1 case presented ovotesticular tissue and was classified as a chimera due to the karyotype 78, XX/78, XY [18].

Certain genes have been proposed as responsible for the phenotypic expression of XX males, but their hypotheses have been refuted [2,12]. The exclusion of the interference of specific genes in the phenotypic expression of XX males suggests that unknown factors still contribute to its occurrence. The hereditary nature of this disorder has not been excluded, and there are currently no laboratory tests available to identify carrier animals. Therefore, it is recommended that guardians do not use the parents and siblings of affected individuals for breeding [3]. This recommendation was followed and communicated to the guardian of the animal in this case report.

When selecting animals for breeding, it is important to consider the potential harm that DSDs can cause to small animal reproduction. This can help minimize the risk of producing offspring with sexual and reproductive anomalies. It is particularly important to pay attention to French Bulldogs due to the increasing number of cases and the likelihood of genetic inheritance. To ensure accurate categorization of each disorder, it is recommended to thoroughly investigate all cases of DSDs using complementary exams and analyses, such as immunohistochemistry and karyotyping.

MANUFACTURERS

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