Short Communication

**SRY-Negative XX Sex Reversal in a French Bulldog**

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**Contents**

Here, we describe a 3-month-old XX male French Bulldog. The diagnosis was based on the clinical signs, gonadal histology and cytogenetic analysis. Additionally, the dog was confirmed to be Sry negative by semi-quantitative reverse transcription polymerase chain reaction (sQRT-PCR). Canine Sry-negative XX sex reversal is a disorder of gonadal development where individuals who have a female karyotype develop testes or ovotestes. To our knowledge, this case is the first XX male sex reversal described in a French Bulldog.

**Introduction**

In mammalian sexual development, either an XX or an XY sex chromosome complement is established at the time of fertilization. Zygotes develop in a similar way up to the time of differential gene expression, which determines whether the bipotential gonad becomes an ovary or a testis. Sex determination has been associated with testis induction, which is normally controlled by the Y-linked Sry gene (sex-determining region Y) (Koopman et al. 1990). In the presence of testis secretions, the genitalia are masculinized, and in their absence a female phenotype develops. The only Y-linked gene known to initiate testis development in mammals is the Sry gene; its gonadal expression is the first molecular evidence of testis induction, followed immediately by Sox9 expression. Transgenic Sox9 expression in the absence of Sry can induce a functional testis, indicating that this autosomal gene is also testis determining (Qin and Bishop 2005).

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’. XX sex reversal has been reported in dogs. These dogs have a 78XX karyotype with variable degrees of testicular differentiation of the gonad (Meyers-Wallen et al. 1999). Sex reversal disorder includes XX males with bilateral testes and XX true hermaphrodite with ovotestes. Eighty percent of human XX males are Sry-positive (Sry-positive XX sex reversal) because of autosomal translocation from the Y chromosome (Pereira et al. 1991). The incidence of human XX males is one in 20 000 male births, and approximately 10% of such patients are Sry negative (Ergun-Longmire et al. 2005; Sarafoglou and Ostrer 2000). However, all cases to date of diagnosed canine XX are Sry negative (De Lorenzi et al. 2008; Hubler et al. 1999; Meyers-Wallen et al. 1999). Sry-negative XX sex reversal has been described in goats and pigs as an inherited autosomal recessive syndrome. In addition, it has also been reported in llamas and horses as isolated cases with an unidentified inheritance pattern (Drew et al. 1999; Meyers-Wallen et al. 1997). The genes responsible for testis induction in Sry-negative are unknown for horses, pigs and dogs. It is known that in goats XX sex reversal is linked to the polled trait (Polled Intersex Syndrome) and a functional deletion of two genes, Pisrt1 and FoxL2, which have been proposed as ovary differentiating genes. Loss of these genes leads to testis development in XX homozygous Pisrt1⁻/⁻ mutants (Pailahoux et al. 2005).

Sex reversal is described in at least 18 canine breeds (Meyers-Wallen et al. 1999). Inheritance is likely to be autosomal recessive in the American Cocker spaniel and in the German shorthaired pointer (Meyers-Wallen et al. 1995, 1999). Linkage analysis in the canine model has identified a critical region mapped to a different chromosome than any of the genes previously reported in association with XX males: Pisrt1 (goat), RSPO1 and SOX9 (human). The results of the genome-wide screen suggest that a locus causing sex reversal is likely to be located in the linked region of CFA29 (Pujar et al. 2007). Research continues trying to identify the mutated candidate gene(s) responsible for XX sex reversal in the dog (Meyers-Wallen 2006).

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. The prepuce is usually abnormal in shape and caudally displaced. Most XX males have a hypoplastic penis and hypospadias. Furthermore, abnormal curvature of the penis is common. Occasionally, cryptorchid phenotypic males are not diagnosed until development of signs referable to hyperestrogenism (intraabdominal testis) or leukocytosis (Meyers-Wallen 1999). 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. The amount of testicular tissue present correlates with the degree of masculinization of the internal and external genitalia. A karyotype of 78XX chromosome constitution in conjunction with the presence of testicular tissue (at least one ovotestis or one testis) is needed to verify XX sex reversal (Meyers-Wallen 1999). This is the first report that describes the XX male sex reversion in French Bulldog, the diagnosis includes the
clinical signs, histology of the genital tract, cytogenetic analysis and Sry analysis by semi-quantitative reverse transcription polymerase chain reaction (sqRT-PCR).

Case Report
A 3-month-old female French Bulldog named Tana was presented at the clinic for its first vaccination. During the consultation, the attending veterinary surgeon noticed a reddish mass protruding from the vulva. It was bright and the dog appetite was normal. Clinical examination of Tana revealed a normal-sized vulva in a normal anatomical position with enlarged clitoris, approximately 0.8 cm long, which contained an os clitoris. Vaginal cytology showed high numbers of neutrophiles and typical cells of anoestrus with high nucleus/cytoplasm ratio, round nuclei and basophilic cytoplasm. A contrast agent (Omnigraf-300 DCI; Lab Juste, S.A.Q.F. Madrid, Spain) was used to evaluate internal anatomy by retrograde cystouretrovaginogram. The contrast agent was introduced with a syringe adapted to a foley catheter (Sikolatex®; Teleflex Medical Iberia S.A., Madrid, Spain) through the vaginal vestibule. After the balloon was inflated, the contrast was introduced. It was confirmed that the urethra went directly to the bladder. Structures indicating a cranial vagina were not observed (Fig. 1).

Heparinized blood samples from Tana were collected for cytogenetic analysis and sent to the Department of Genetics of the University of Santiago de Compostela (Lugo, Spain). Chromosome preparations were made following standard procedures, and chromosome spreads were Giemsa stained. The total number of chromosomes and the number of X chromosomes were determined in 20 randomly chosen metaphase spreads of peripheral lymphocytes. A blood sample in an EDTA tube was collected for messenger RNA (mRNA) isolation. Sry gene expression was analysed by semi-quantitative reverse transcription polymerase chain reaction (sqRT-PCR) adapted from Meyers-Wallen et al. (1999). A normal female karyotype (78XX, Fig. 2) was found in metaphase spreads. In sqRT-PCR, the expected Sry product only was obtained from complementary DNA templates of XY male control dogs, but not from XX female dog or the affected French Bulldog (Fig. 3). This indicates that the Y-specific Sry gene was absent in the affected dog.

Two months later, Tana returned with a copious mucopurulent discharge. The owner reported that the dog often licked the vulva. Physical examination of the abdomen revealed inguinal hernias containing testicle-like structures. A gonadectomy and the possible hysterectomy were scheduled, and Enrofloxacine (5 mg/kg SID oral) (Baytril®; Bayer, Barcelona, Spain) was administered daily for a week prior to the surgery. The dog was pre-medicated with Acepromacine (0.05 mg/kg intramuscular) (Calmo Neosan®; Pfizer, Madrid, Spain) as a sedative and Morphine (0.2 mg/kg intravenously) (Morfina 2% B Braun®; B. Braun VetCare, Barcelona, Spain) as analgesic. The induction was performed with Propofol (Propofol lipuro® 1% 10 mg/ml B Braun) and Fentanyl (5 µg/kg intravenously) (Fentanes®; Kern Pharma S.L., Barcelona, Spain) before an endotracheal intubation. General anaesthesia was maintained using isoflurane 2% (Isoba Vet®; Schering-Plough, Madrid, Spain). A clitoridectomy and a ventral laparotomy were performed. The
genital conduct were followed as far as the testicle-like structures inside the hernias. A subcutaneous incision was performed beyond the testicle-like structures, and all the genitalia were removed. After surgery, the dog received post-operative pain treatment including Buprenorphine (6 μg/kg/TID intravenously) (Buprelox®; Schering-Plough) for 1 day and Meloxicam (0.1 mg/kg/ SID subcutaneously) (Metacam®; Boehringer Ingelheim, Barcelona, Spain) for 4 days. Amoxicilin-clavulanic (12.5 mg/kg/BID oral) (Synulox®; Pfizer) was administered as an antibiotic for 1 week. Following surgery, Tana displayed standard behaviour without complications.

Both testicle-like structures were submitted for histopathology (Histolab, Veterinary histopathology centre, Malaga, Spain) and confirmed to be bilateral testes. The testes had seminiferous tubules with small diameter and were covered with degenerated germinal epithelia without visible spermatozoa.

Discussion

To our knowledge, this is the first case of a XX male French Bulldog. XX sex-reversed individuals have been reported in several purebreds (De Lorenzi et al. 2008; Fitzgerald and Murphy 1990; Hubler et al. 1999; Meyerswallen et al. 1995; Meyerswallen and Patterson 1988; Pujar et al. 2006; Selden et al. 1984; Thomas et al. 1986; Wilecken 1987). Different types of sex can be defined: genetic, gonadal and phenotypic sex. Determination of the genetic sex should be made by chromosome analysis of cells from several tissues. For the diagnosis of gonadal sex, histological examination of the gonads is essential, and phenotypic sex is determined both by the internal and by external genitalia (Meyers-Wallen 1999).

The first clinical sign observed in the case reported here was the enlargement of the clitoris. In most reported cases of female pseudo- or true hermaphrodisim, clitoral enlargement is the most common physical finding (Meyers-Wallen 1999). The disturbance of sexual differentiation depends upon the stage of embryonic development. In the present case, the French Bulldog had a mild degree of masculinization, only the clitoris was enlarged and contained a bone, but the vulva was normal in size, shape and position, as described by Hubler in a Basset Hound (Hubler et al. 1999). Therefore, it is likely that there was not enough testosterone present during the critical embryonic period for the androgen-dependent organs to respond normally. On the other hand, the onset of testosterone secretion and testis development could have been delayed. Androgen administration during gestation is known to result in varying degrees of masculinization of the female offspring. The greatest sensitivity to exogenous androgens is shown by the epithelium of the urogenital sinus (De Rooster et al. 2006).

The majority of XX true hermaphrodites and all XX males are sterile. Because this is a heritable trait in breeds that have been closely studied, owners should be advised that both parents of affected individuals be removed from the breeding programme. At least half of the siblings of affected individuals are expected to be carriers, while one quarter may be non-carriers. Since there is no laboratory test available to identify carriers, the best recommendation is not to use any siblings of affected individuals as breeding animals. Gonadectomy and hysterectomy are recommended for affected individuals (Lyle 2007).

The result of the Sry gene assay in our French Bulldog was also negative as described by other authors (Meyers-Wallen et al. 1999). Unfortunately, there is no data on affected dogs of this particular breed to determine the model of inheritance. According to the inheritance pattern found in other breeds, it is highly likely that it is an autosomal recessive inheritance (Meyers-Wallen et al. 1995; Meyerswallen and Patterson 1988). As described by Meyers-Wallen, the identification of these mutant genes will provide insights into the mammalian testis pathway and should lead to practical DNA tests to eliminate carriers from breeding stock (Meyers-Wallen 2003). Information available from genetic research will be useful in improving canine health, as long as veterinary surgeons have the knowledge and skills to use it ethically and responsibly (Meyers-Wallen 2003).

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Conflict of interests

None of the authors have any conflict of interest to declare.

Author contributions

Campos, García-Roselló M and García-Roselló E reported the case and examined the clinical and surgical work. Moreno did the semi-quantitative reverse transcription polymerase chain reaction. All the authors contributed to the writing of the text.

References


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