Hermaphroditism in two dogs – pathological and cytogenetic studies: a case report

A. Gurel[1], F. Yildirim[1], G. Sennazli[1], K. Ozer[1], M. Karabagli[1], A. Deviren[2], A. Cirakoglu[2]

[1]Faculty of Veterinary Medicine, Istanbul University, Istanbul, Turkey
[2]Cerrahpasa Medical Faculty, Istanbul University, Istanbul, Turkey

ABSTRACT: A Pit-bull and a Beagle, both one-year-old, with complaints of an enlarged clitoris were clinically suspected for hermaphroditism. The enlarged clitoris, the uteri and the gonads were removed surgically from each animal and submitted to our department for histopathological evaluation. Tissue samples were fixed with 10% buffered formalin solution and processed routinely, after which paraffin sections were obtained and stained with H&E. A blood sample was taken from the Beagle dog and a peripheral lymphocyte culture was prepared. While the clitoris and uteri were confirmed histopathologically, the gonads were detected as a testis instead of an ovary for both dogs. Additionally, cytogenetic evaluation revealed a normal female chromosome complement, 78, XX for the Beagle dog. According to the gonadal and phenotypic sexes, both cases were first determined as 'male pseudohermaphroditism', a phenotypic sex disorder. However, after karyotyping analysis, we concluded that the 78, XX Beagle dog should be defined as suffering from XX sex reversal syndrome, a gonadal sex disorder.

Keywords: dog; hermaphroditism; XX sex reversal; cytogenetic

Intersexuality (hermaphroditism) is a sexual development disorder caused by an abnormality during chromosomal, gonadal and phenotypic sex determination or differentiation, leading to defects in the reproductive tract (Kuiper and Disti 2004). Therefore, intersex develops as a consequence of certain pathological disorders like chimaerism, mosaicism, sex reversal syndrome, and male or female pseudohermaphroditism. The diagnosis of these disorders has to be based on the inspection of the chromosomes, gonads and the phenotypic appearance of the reproductive organs (Basrur 2006; Lyle 2007).

Chromosomal sex disorders include several defects in the number or structure of the sex chromosomes such as XXY syndrome (Klinefelter syndrome in humans), XO syndrome (Turner syndrome in humans), XXX syndrome, true hermaphrodite chimeras, XX/XY chimeras with testes or XY/XY chimeras with testes. Individuals with gonadal sex disorders have either an XX or XY sex chromosomal constitution, but the gonadal sex does not match with the chromosomal sex ("sex-reversed") (Basrur 2006; Lyle 2007). The most frequent type of hermaphroditism in dogs is XX sex reversal syndrome. No cases of XY sex reversal have been reported in the dog, and no cases of XX sex reversal have been reported in the cat so far (Lyle 2007). Recently, a paper reporting XY sex reversal in the cat was published (Schlafer et al. 2011). XX sex reversal includes XX true hermaphrodites (have ovotestes) and XX males (have bilateral testes). In individuals with phenotypic sex disorders, chromosomal and gonadal sex match, but the phenotypic sex (internal or external genitalia or both) don't match with the gonadal sex. Phenotypic sex disorders in cats and dogs include female and male pseudohermaphroditism, Persistent Mullerian Duct Syndrome (PMDS) and androgen-dependent masculinisation (Lyle 2007).

The type and frequency of malformations in the genital tract have been studied in different species of domestic animals (McLachlan 2001; Lyle 2007; Schlafer et al. 2011; Silversides et al. 2011). These differ mainly due to differences in their ge-

Supported by the Istanbul University, Scientific Research Projects Department (Project No. UDP-26818).

netic background and the embryological clock that shifts the developmental targets of environmental factors. Breed predisposition for specific birth defects and recurrence in families often point to their genetic aetiology (McLachlan 2001; Basrur 2006). It is important to determine the dog breeds which are susceptible to genital tract malformations in terms of dog breeding (Silversides et al. 2011). Therefore, clinical, pathological and cytogenetic aspects should be evaluated all together in order to describe the intersex cases encountered in small animal practice.

The aim of this study was to pathologically evaluate the inner and outer genital tract organs and to cytogenetically compare the chromosomal sex and phenotype in two dogs clinically diagnosed with hermaphroditism.

Case description

A Pitbull and a Beagle, both one-year old, with complaints of an enlarged clitoris, were clinically diagnosed with hermaphroditism. The enlarged clitoris and radiologically detected uteri and gonads were removed surgically from each animal at the Surgery Department of the Faculty of Veterinary Medicine, Istanbul University and submitted to our department for histopathological evaluation. The tissue samples were fixed with 10% buffered formalin solution, processed routinely and embedded in paraffin wax. Then, paraffin sections were cut at 4–5 µm thickness with a rotary microtome, stained with haematoxylin and eosin (H&E) and examined under light microscopy. For karyotyping analysis, a blood sample was taken from the Beagle dog into a heparinised tube and submitted to the Cerrahpasa Medical Faculty, Department of Medical Biology and Genetics at Istanbul University. The sample was incubated with PB-MAX™ culture media (GIBCO) for 72 h at 37 °C in order to culture peripheral blood lymphocytes. After a 1.5 h incubation with colchicine the lymphocytes were harvested according to routine procedures, the chromosomes were stained using the GTG-banding method and metaphases were photographed using a CCD camera (Reimann et al. 1996).

The gross pathologic examination for both dogs revealed an enlarged clitoris which contained hard ossified tissue resembling an os penis (Figure 1A). Visualisation of the *cervix*, *corpus* and *cornu uteri* revealed a testis together with epididymis on the cranial edge of the *cornu uteri* (Figure 1B). The oviduct was absent in both dogs. Microscopically, the penis-like structure was surrounded by squamous epithelium and was comprised of collagenous fibrous tissue (Figure 2A), and diffuse vascular and cavernous structures. It was diagnosed as a clitoris (Figure 2B). Osseous tissue was detected at the medial part of the mass. While the *cornu* and *corpus uteri* were confirmed histopathologically (Figure 2C), the testes and epididymis tissue were marked by abundant seminiferous tubules characterised by poor lumen formation, evident sertoli and the absence of spermatocytes belonging to testis tissue at the location where the ovary should have been (Figure 2D). There were several interstitial cells (leydig cells) between theseminiferous tubules. All chromosomes in dogs are acrocentric, except for X and Y which are metacentric in structure. In this study, two metacentric X chromosomes were easily distinguished from the others (Figure 3). According to the gonad sex both cases were initially, clinically and pathologically, diagnosed as 'male pseudohermaphroditism'. However, as a rule, individuals with male pseudohermaphroditism have the XY karyotype (Lyle 2007). Therefore, we concluded that the Beagle, which was XX genetically, was in fact suffering from XX sex reversal syndrome which is a gonadal sex disorder. In the case of the Pit-bull, we could not distinguish between male pseudo-

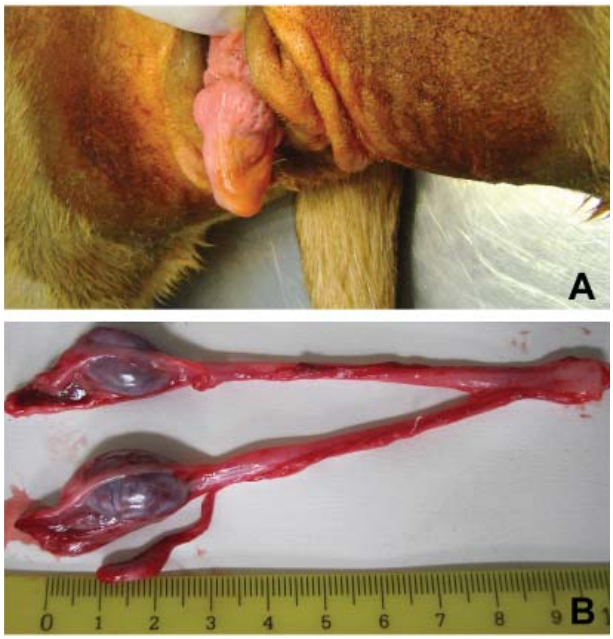

Figure 1. **A**. The enlarged clitoris resembling a penis structure. **B**. The *cervix*, *corpus* and *cornu uteri*; a testis together with an epididymis on the cranial edge of the *cornu uteri*

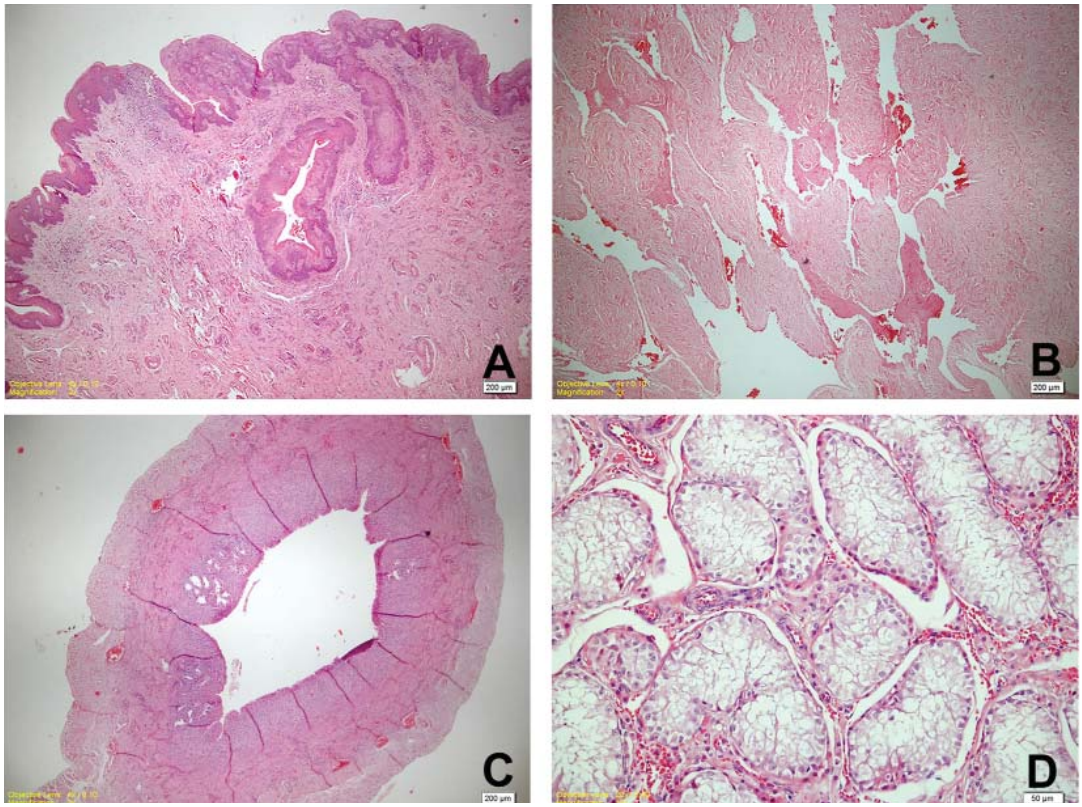

Figure 2. **A**. The enlarged clitoris section, surrounded by squamous epithelium and constituted from collagenous fibrous tissue; H&E, bar = 200 μm. **B**. Enlarged clitoris section, diffuse vascular and cavernous structures; H&E, bar = 200 μm. **C**. *Cornu uteri*; H&E, bar = 200 μm. **D**. Testes and epididymis tissue, abundant seminiferous tubules characterised by poor lumen formation, evident sertoli and seminiferous cells in tubules and absence of spermatocytes; H&E, bar = 50 μm

hermaphroditism and XX sex reversal syndrome because of the lack of a blood sample for karyotyping analysis. Cytogenetic evaluation revealed a normal female chromosome compliment, 78, XX for the Beagle dog.

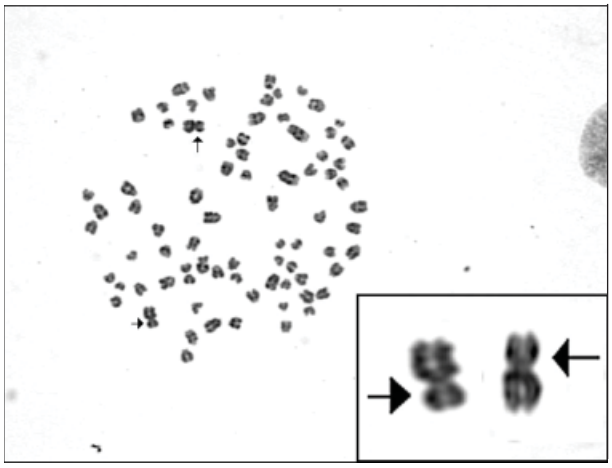

Figure 3. Representative karyotype of the Beagle dog. A normal female chromosome complement, 78, XX. Two metacentic X chromosomes (arrows). GTG-banding staining, 100×

DISCUSSION AND CONCLUSIONS

Male pseudohermaphroditism is a rarely observed disorder in dogs and mainly involves PMDS and androgen insensitivity syndrome (Lyle 2007; Matsuu et al. 2009). In the present study both dogs had female internal and external genital organs, with testes taking the place of the ovary. Because of these findings male pseudohermaphroditism was considered. It has been reported that the most frequent types of intersexuality in dogs are XX sex reversal syndrome and cryptorchidism (Meyers-Wallen et al. 1999; Lyle 2007; Nowacka-Woszuk et al. 2007).

Even though sexual development disorders in the dog can be sporadic, these disorders are a significant problem for dog breeding (Lyle 2007; Meyers-Wallen 2009; Switonski et al. 2012). XX sex reversal syndrome occurs as a result of Y-effective autosomal genes (like *Sxr* gene) or translocation of the *Sry* gene from the Y chromosome (Lyle 2007). XX sex reversal includes XX true hermaphrodites (have ovotestes) and XX males (have bilateral

testes). This syndrome has been reported in at least 15 dog breeds (Nowacka-Woszuk et al. 2007; Meyers-Wallen 2009; Silversides et al. 2011). In this study, at first, because of the gonadal sex diagnoses both cases were diagnosed as 'Male pseudohermaphroditism', which is a phenotypic sex disorder.

The importance of the present cases lie in drawing the attention of clinicians and breeders to sexual disorders observed in dogs with respect to their breed. There have been several reports regarding intersexuality in domestic animals. However, molecular mechanisms and useful markers for diagnosing sex reversal syndromes have not yet been well-described. We conclude that molecular investigations are needed in order to clarify the pathogenesis of sex reversal syndrome. To the best of our knowledge, this study is the first case report in veterinary pathology in our country regarding pathological and cytogenetic aspects of cases of genital abnormalities.

REFERENCES

Basrur PK (2006): Disrupted sex differentiation and feminization of man and domestic animals. Environmental Research 100, 18–38.

Kuiper H, Disti O (2004): Intersexuality in dogs: causes and genetics. Deutsche Tierarztliche Wochenschrift 111, 251–258.

Lyle SK (2007): Disorders of sexual development in the dog and cat. Theriogenology 68, 338–343.

Matsuu A, Hashizume T, Kanda T, Nagano M, Sugiyama A, Okamoto Y, Hikasa Y (2009): A case of persistent Mullerian duct syndrome with sertoli cell tumor and hydrometra in a dog. Journal of Veterinary Medicine Science 71, 379–381.

McLachlan JA (2001): Environmental signalling: what embryos and evolution teach us about endocrine disrupting chemicals. Endocrine Reviews 22, 319–341.

Meyers-Wallen VN (2009): Review and update: genomic and molecular advances in sex determination and differentiation in small animals. Reproduction in Domestic Animals 44, 46–46.

Meyers-Wallen VN, Schlafer D, Barr I, Lovell-Badge R, Keyzner A (1999): Sry-negative XX sex reversal in purebred dogs. Molecular Reproduction and Development 53, 266–273.

Nowacka-Woszuk J, Nizanski W, Klimowicz M, Stiwonski M (2007): Normal male chromosome complement and a lack of the SRY and SOX9 gene mutations in a male pseudohermaphrodite dog. Animal Reproduction Science 98, 371–376.

Reimann N, Bartnitzke S, Bullerdiek J, Schmitz U, Rogalla P, Nolte I, Ronne M (1996): An extended nomenclature of the canine karyotype. Cytogenetics Cell Genetics 73, 140–144.

Schlafer DH, Valentine B, Fahnestock G, Froenicke L, Grahn RA, Lyons LA, Meyers-Wallen VN (2011): A case of SRY-positive 38, XY true hermaphroditism (XY sex reversal) in a cat. Veterinary Pathology 48, 817–822.

Silversides DW, Benoit J-M, Collard F, Gilson C (2011): Disorder of sex development (XX male, SRY negative) in a French bulldog. Canadian Veterinary Journal 52, 670–672.

Switonski M, Payan-Carreira R, Bartz M, Nowacka-Woszuk J, Szczerbal I, Colaco B, Pires MA, Ochota M, Nizanski W (2012): Hypospadias in a male (78, XY; SRY-positive) dog and sex reversal female (78, XX; SRY-negative) dogs: clinical, histological and genetic studies. Sexual Development 6, 128–134.

Received: 2014–01–03
Accepted after corrections: 2014–01–27

Corresponding Author:

Funda Yildirim, Istanbul University, Faculty of Veterinary Medicine, Department of Pathology, 34320 Avcilar, Istanbul, Turkey
E-mail: funda@istanbul.edu.tr