

Male pseudohermaphroditism in dogs: three case reports

M.R. ALAM¹, Y.G. CHO², S.J. CHO³, J.I. LEE¹, H.B. LEE¹, H.J. TAE¹, I.S. KIM¹, N.S. KIM¹

¹College of Veterinary Medicine, ²Medical School, Chonbuk National University, Jeonju, Republic of Korea

³Family Animal Medical Clinic, Gunsan, Republic of Korea

ABSTRACT: Three Cocker Spaniel dogs, 2–3 months old, weighing 3–4 kg, were presented to the Chonbuk Animal Medical Centre, Chonbuk National University, with intersex anomalies. Physical, radiological, gross, histological, hormonal and cytogenetic studies were performed. Physical examination of the external genitalia revealed that the dogs possessed the vulva with an enlarged clitoris protruding from the vulvar juncture and the scrotum with an undescended testis in Case 1 and 2, and both testes remained undescended in Case 3. Hyperoestrogenaemia and low testosterone serum concentrations were found. Laparotomy revealed persistent Mullerian ducts (PMD) in Case 1 and 2, and abdominally located testicle(s) in all the cases. Histological examinations of the gonads revealed inactive seminiferous tubules. Cytogenetic analysis showed 78XY male karyotype in Case 1 and 2, whereas Case 3 showed 79XX female karyotype. The congenital defects were diagnosed as male pseudohermaphroditism (MPH) and PMD in Case 1 and 2, and XX sex reversal MPH in Case 3.

Keywords: male pseudohermaphroditism; XX sex reversal; persistent Mullerian duct; dog

Disorders of genital development occur in all species of mammals (Cribiu and Chaffaux, 1990; Passello-Legrand and Mowat, 2004; Weng et al., 2005). The intersex animal is an animal possessing the characteristics of both sexes. Intersex animals are also called pseudohermaphrodites or hermaphrodites depending on their gonads (Howard and Bjorling, 1989). Hermaphroditism is a condition when the subject has ambiguous genitalia with a part or all of the genital organs of both sexes present (Passello-Legrand and Mowat, 2004). True hermaphrodites have the gonadal tissue of both sexes whereas pseudohermaphrodites possess the gonads of one sex (according to which they are classified as male or female pseudohermaphrodites), but the secondary sex characteristics and external genitalia of the opposite sex. Male pseudohermaph-

rodites possess testes while having mixed or female external genitalia. Female pseudohermaphrodites have ovaries but phenotypically they have a masculine appearance (Howard and Bjorling, 1989; Del Amo et al., 2001). In true hermaphroditism, both testicular and ovarian tissues are present in various combinations. A testis may be found on one side in combination with an ovary on the contralateral side, an ovotestis only may be present, or an ovotestis may be paired with a testis or ovary. Problems associated with intersex animals are not unique to intersexes but can be found in animals with normal chromosomal karyotypes (Svendsen et al., 1985; Howard and Bjorling, 1989). These developmental disorders are caused by abnormalities of genetic or chromosomal origin, or inappropriate hormonal or chemical exposure (Passello-Legrand

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and Mowat, 2004). This study presents 3 cases of male pseudohermaphroditism in dogs that were investigated by clinical, histological, hormonal and cytogenetical methods.

Case presentation

Three American Cocker Spaniel dogs, 2–3 months old, weighing 4–5 kg, were presented (Case 1 on 16 January 2003, Case 2 on 4 January 2005 and Case 3 on 5 September 2005) to the Chonbuk Animal Medical Centre, Chonbuk National University, with intersex anomalies. Physical examination of the external genitalia revealed the presence of the vulva with an enlarged clitoris protruding out from the vulvar juncture (Figures 1, 2 and 3). Palpation of the scrotum revealed an undescended testis in Case 1 and 2, and both testes remained undescended in Case 3. In addition, physical examination in Case 1 showed generalized seborrhoea, alopecia and hyperkeratotic lesions on the ventral neck, thorax, abdomen, flanks, vulva, axilla and caudal thighs (Figure 1). A skin scraping test showed the presence of mange mites. Male pseudohermaphroditism was a tentative diagnosis.

Blood samples were collected for cytogenetic and sex hormone analysis. Exploratory laparotomy was performed as a diagnostic and therapeutic procedure. Before laparotomy, Case 1 was treated with ivermectin (Ivomec Inj[®], MSD Agvet, Holland) 0.2 mg/kg, *s.c.*, with a repeated dose after 1 week for mange. After premedication with atropine sul-

phate (Atropine Sulfate Inj[®], Dai Han Pharm. Co. Ltd., Korea) 0.05 mg/kg, *s.c.*, the anaesthesia was induced using thiopentone sodium (Thionyl Inj[®], Dai Han Pharm. Co. Ltd., Korea) 25 mg/kg, *i.v.* and maintained with enflurane and oxygen delivered through a cuffed endotracheal tube. Supportive fluid therapy was maintained throughout the procedure and cephalexin (Methilexin Inj[®], Union Korea Pharm. Co. Ltd., Korea) 25 mg/kg, *i.v.* was administered at the time of induction. The dogs were then placed on dorsal recumbency and the caudal abdominal and inguinal regions were surgically prepared with 7.5% povidone-iodine surgical scrub. Through a pre-scrotal paramedian laparotomic incision the gonadal and genital tract tissues were removed. Castration of the intrascrotal testis was performed using a standard open orchidectomy technique. The postoperative treatment was given with cephalexin (Methilexin Inj[®], Union Korea Pharm. Co. Ltd., Korea) 25 mg/kg, *i.v.*, every 8 hours, for 5 days.

The internal genitalia somewhat resembled those of the female with testicle(s) instead of ovary. A persistent Mullerian duct was identified (PMD) in Case 1 and 2, and abdominally located testicle(s) in all the cases. After a gross examination the gonadal tissues were fixed for a histopathological study in 10% buffered formalin and embedded in paraffin. Five μm sections were made and stained with haematoxylin and eosin and the slides were examined under a light microscope. The gonads revealed inactive seminiferous tubules and epididymides (Figure 4). The gonads were confirmed as testes. Serum oestra-



Figure 1. Enlarged clitoris protruding out from the vulva and generalized seborrhoea, alopecia and hyperkeratotic lesions in Case 1



Figure 2. Enlarged clitoris protruding out from the vulva in Case 2

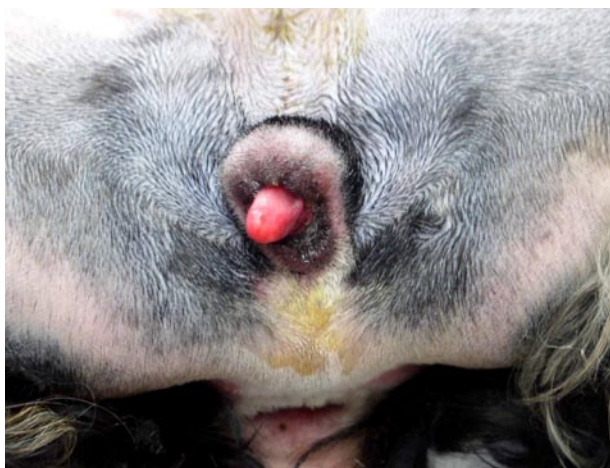


Figure 3. Enlarged clitoris protruding out from the vulva in Case 3

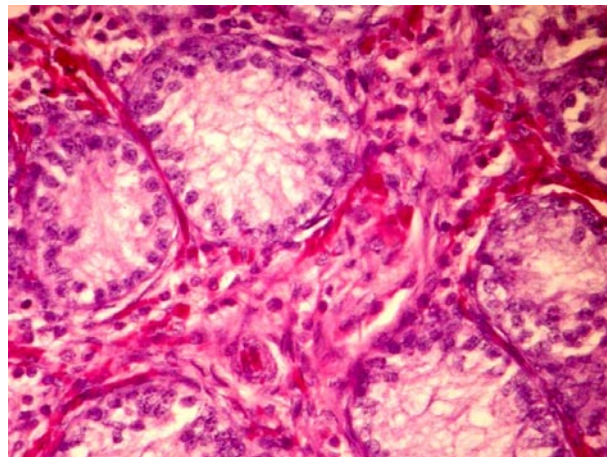


Figure 4. Seminiferous tubules revealed inactive spermatogonia, relatively extensive interstitium and loosely arranged Sertoli cells in the interstitium, H&E 40×

diol 17 β and testosterone concentrations were assayed using a commercial radioimmunoassay kit (Coat-A-Count, DPC[®], Los Angeles, USA), which revealed high oestradiol 17 β and low testosterone levels in all 3 cases. Cytogenetic analysis was carried out on blood lymphocyte cultures. Heparinized blood samples were collected for 72 hours in Ham's F12 culture medium, supplemented with pokeweed and 10% foetal calf serum. Slides were made and stained with 5% Giemsa. Thirty metaphase samples were scored and a karyotype was performed according to the recommendation of the Committee for the Standard Karyotype for the Dog (Switonski et al., 1994). Cytogenetic analysis showed 78XY male karyotype in Case 1 and 2, whereas Case 3 showed 79XX female karyotype. The congenital defects were diagnosed as male pseudohermaphroditism (MPH) with PMD in Case 1 and 2, and XX sex reversal MPH in Case 3.

DISCUSSION

In mammals, genital developmental disorders have been described in numerous species including humans, pigs, goats, horses, dogs, mice, marsupials and moles (Cribru and Chaffaux, 1990; Pailhoux et al., 2001; Kim and Kim, 2006). The term hermaphrodite is used independently of the chromosomal constitution. The individuals may have an ovary on one side and a testis on the other or they may have combined ovotestes (Kai et al., 2003). They may be classified as bilateral (testis and ovary or ovotestis

on each side), unilateral (ovotestis on one side and testis or ovary on the other) or lateral (testis on one side and ovary on the other). A pseudohermaphrodite shows a disagreement between phenotypic and gonadal sex (Howard and Bjorling, 1989; Del Amo et al., 2001). These individuals have a single type of germinal tissue, according to which they are male or female pseudohermaphrodites (Kennedy and Miller, 1993). A female pseudohermaphrodite has ovaries but male external genitalia, and for a male pseudohermaphrodite the reverse is true. These were in full agreement with our cases.

The genital developmental disorders are caused by abnormalities of genetic or chromosomal origin, or inappropriate hormonal or chemical exposure. During the early embryonic life the gonad is bipotential and capable of developing into either testis or ovary. 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 (Passello-Legrand and Mowat, 2004). 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) (Sinclair et al., 1990). The development of the testis is initiated by the SRY gene and in its absence the ovarian differentiation normally occurs (Sinclair et al., 1990; Kennedy and Miller, 1993; Hubler et al., 1999).

The phenotypic sex is regulated by the gonads after their differentiation (Kennedy and Miller, 1993; Hubler et al., 1999). If the gonad develops along a testicular pathway, two hormones responsible for phenotypic masculinization are produced, Mullerian inhibiting factor (MIF) and testosterone. The Sertoli cells secrete MIF (Hubler et al., 1999) and in the absence of this substance passive development of the Mullerian ductal system takes place, no stimulatory factor being required. Testosterone is also responsible for masculinization of the external genitalia. For this action to occur, testosterone must be converted into dihydrotestosterone (DHT). This transformation is effected in the target tissue itself, the cells of the external genitalia. Thus, DHT promotes the formation of the prostate, urethra, penis and scrotum (Kennedy and Miller, 1993; Hubler et al., 1999). In the absence of DHT, the external genitalia will develop along female lines. In the present cases, hyperoestrogenism and low testosterone levels are thought to contribute to the dysgenesis of the male external genitalia and unilateral or bilateral cryptorchidism (Brown et al., 1976).

Androgen resistance or disorders of androgen biosynthesis are also thought to cause male pseudohermaphroditism. Sometimes male hormones cannot exert their full effects on the tissues due to various congenital abnormalities. 5α -reductase deficiency is a form of androgen resistance, in which the enzyme responsible for the formation of DHT, the active form of testosterone, is decreased (Miller, 2002). There are two 5α -reductase isoenzymes, type 1 and type 2, in humans and animals. In humans, this deficiency is a rare autosomal recessive trait, i.e. the result of any of several mutations in the gene encodes the steroid 5α -reductase-2 gene. The type 2 gene is expressed in the foetal genital skin and in the normal prostate (Miller, 2002). The mutation in type 2 isoenzymes causes male pseudohermaphroditism (Imperato-McGinley et al., 1985; Passello-Legrand and Mowat, 2004).

The XX sex reversal, a form of the intersex in 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 most often in American Cocker Spaniels, the animals reported to be MPH having XX genotype and sxr gene located on an autosome (Selden et al., 1984; Kuiper and Distl, 2004). These findings are in agreement with Case 3 in our report.

Failures in the establishment of chromosomal, gonadal and phenotypic sex can cause intersexuality in dogs. Thus, the diagnosis of sex reversal syndrome, male or female pseudohermaphroditism in intersex individuals has to be based on the inspection of chromosomes, gonads and the phenotypic appearance of the reproductive organs (Svendsen et al., 1985; Kuiper and Distl, 2004). As these animals did not receive any hormonal treatment during their lives, and based on the histological characteristics of their gonads, they were considered to be spontaneous male pseudohermaphrodites, probably caused by a genetic disorder. The present cases were classified as male pseudohermaphrodites (MPH) because of the presence of testes and ambiguity of external genitalia. Cryptorchidism and failure of androgen dependent masculinization of the external genitalia might be due to the dysfunction of embryonic testes.

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Corresponding Author:

Dr. Nam-Soo Kim, Assoc. Prof., Chonbuk National University, College of Veterinary Medicine, Department of Surgery, and Vice Dean, Academic Affairs, Jeonju 561-756, Republic of Korea
Tel. +82 63 270 2800, fax +82 63 270 3778, e-mail: namsoo@chonbuk.ac.kr
