

Sexual development disorder in a dog

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Abstract

A 1-year intact, phenotypic female Doberman Pinscher dog, was evaluated for suspected sexual development disorder. Patient had a history of white mucoid vaginal discharge with no estrous signs or behavior. Clitoral hypertrophy with a palpable os clitoris were noted. Transabdominal ultrasonography revealed a right gonad and tubular structures (possibly uterus). Exploratory laparotomy revealed 2 underdeveloped gonads in caudodorsal abdomen and a markedly underdeveloped right uterine horn. Histopathology confirmed bilateral ovotestes. Karyotyping (number and morphology of chromosomes) and fluorescence in situ hybridization (XX and XY cells) results were normal. Samples were PCR positive for sex-determining region Y (SRY) and X-linked androgen receptor gene. We concluded that the patient was either a mosaic or chimera with normal female 78,XX and normal male 78,XY cells and genetically a mix of male and female. Final diagnosis was sex chromosome (78,XX/XY), SRY-positive, ovotesticular, disorder of sexual development with female phenotype.

Keywords: Abnormality of chromosomal sex, chimera, mosaic, os clitoris, ovotestes

Background

Disorders of sexual development (DSDs) include congenital reproductive tract abnormalities.¹ Abnormalities in chromosomal sex, gonadal differentiation, and phenotypic differentiation of the external genitalia result in a variety of phenotypic presentations. Therefore, determining both gonadal sex and chromosomal sex is critical to allow for precise diagnosis and to understand underlying pathophysiology.^{2,3}

In Y chromosome presence, sex-determining region Y (SRY) gene acts on SRY-box transcription factor 9 (SOX9) that is supported by positive feed-forward loops with the fibroblast growth factor 9 (FGF9) gene.⁴ Potentially, steroidogenic factor 1 (SF-1) also acts to initiate activation of SOX9;⁵ SOX9 expression allows Sertoli cell development and inhibits expression of wingless-related MMTV integration site 4 (WNT4) and forkhead box L2 (FOXL2) genes, important for ovarian development.⁶ Sertoli cells continue to express SOX9 throughout life⁷ and also secrete antiMüllerian hormone that regresses Müllerian ducts and stimulates differentiation of Leydig cells.⁶ In contrast, absence of SRY expression results in differentiation of bipotential gonads into ovaries. Expression of R-spondin family member 1 (RSPO1) activates WNT4⁸ upregulating

dosage-sensitive sex reversal-adrenal hypoplasia congenital critical region on X chromosome, gene 1 (DAX1), an X chromosome gene that inhibits Sertoli cell development by suppressing SF-1 activation of SOX9.⁹ RSPO1 also activates the β -catenin signaling pathway that further drives expression of WNT4 along with other genes associated with female differentiation.⁸ Failure in 1 or more of these pathways, led by a multitude of causes, might lead to an ambiguous phenotype.

Chromosomal abnormalities have been reported in dogs, but are considered uncommon disorders.^{10,11} Chimerism originates through the combination of 2 differing zygotes whereas mosaicism originates from a mitotic error in a single zygote, making 2 or more genetically varying type of cells 78,XX and 78,XY. Chromosomal mosaicism may take place at any stage of development.¹² Chimeras that occur in early development are defined as primary chimeras, but if they occur postimplantation are called secondary (e.g. blood chimeras, which originate due to the exchange of blood cells between twins). Freemartinism is a common condition in cattle where there is exchange of blood between twin male and female through placental anastomosis. Affected females may present with differing degrees of reproductive tract masculinization.¹³ Female or male dogs with XX/XY leukocyte chimerism is uncommon,

may present with ambiguous phenotype, and sterility of the individual is common.¹⁴⁻¹⁶ A dog diagnosed with a sex chromosome DSD is presented.

Case presentation

A 1-year intact, phenotypic female Doberman Pinscher dog, was referred for evaluation of a suspected DSD due to an enlarged clitoris. Owner reported that the dog had multiple episodes of white mucoïd vaginal discharge with no evidence of estrus. Dog's dam was not supplemented with progesterone during pregnancy and it was unknown if any of the other littermates were affected.

Physical examination findings were normal, except for slight increase in anogenital distance, clitoral hypertrophy, and palpable os clitoridis (Figure 1A). Urethral groove extended from os clitoridis base to ~ ¾ distance to distal clitoral tip. No other phenotypic abnormalities were noted.

Serum progesterone concentrations (0.25 ng/ml) were consistent with lack of luteal activity and anestrus. Right gonad was identified via transabdominal ultrasonography. It was located craniomedial to right kidney, adjacent to multiple mesenteric lymph nodes, and was 2.7 x 1.6 cm, circumscribed, heteroechoic, with small, round, cyst-like hypoechoic to anechoic structures within it that were interpreted to be ovarian follicles. A hyperechoic, relatively linear structure was observed in gonadal center that was suspected to be rete testis (Figure 1B). Left gonad could not be detected via transabdominal ultrasonography. Additionally, small, tubular, hypoechoic structure with hyperechoic parallel walls were observed in the expected region of uterus; structure appeared to bifurcate right and left at the caudal aspect of urinary bladder into small, tubular, structures that abruptly terminated. Abnormally developed uterus or vas deferens was suspected.

Treatment

Abdominal exploratory surgery identified 2 small, underdeveloped gonads in the caudodorsal abdomen between kidneys' caudal pole and bladder. There was evidence of a markedly underdeveloped right uterine horn. Uterine body and left uterine horn were not palpable.

Reproductive tissue was surgically removed and histopathology of gonads revealed bilateral ovotestes. These ovotestes consisted of a central testicular component and peripheral ovarian component surrounded by bursa (Figure 2A). Adjacent to gonad were numerous closely tortuous arteries and veins (resembling pampiniform plexus) as well as several tortuous mesonephric ducts (resembling epididymis) surrounded by dense fibrous connective tissue (Figure 2B). Central testicular component was composed of large numbers of variably pigmented Leydig cells and irregular seminiferous tubules that consisted solely of Sertoli cells with no evidence of spermatogonia, spermatocytes, spermatids, or sperm (Figure 2C). Peripheral ovarian component lacked overt follicular structures and was merely composed of irregular and tortuous rete ovarii and abundant interstitial cells (Figure 2D).

Outcome

Cytogenetic analysis (Texas A&M University) revealed normal number and morphology of chromosomes. Approximately 60% of the cells had 2 X chromosomes and ~ 40% of the cells were XY. Fluorescent in situ hybridization (FISH) assay was performed using Y chromosome-specific probes. PCR analyses were performed to identify SRY gene presence and an X-linked androgen receptor gene. Results indicated the presence of both XX

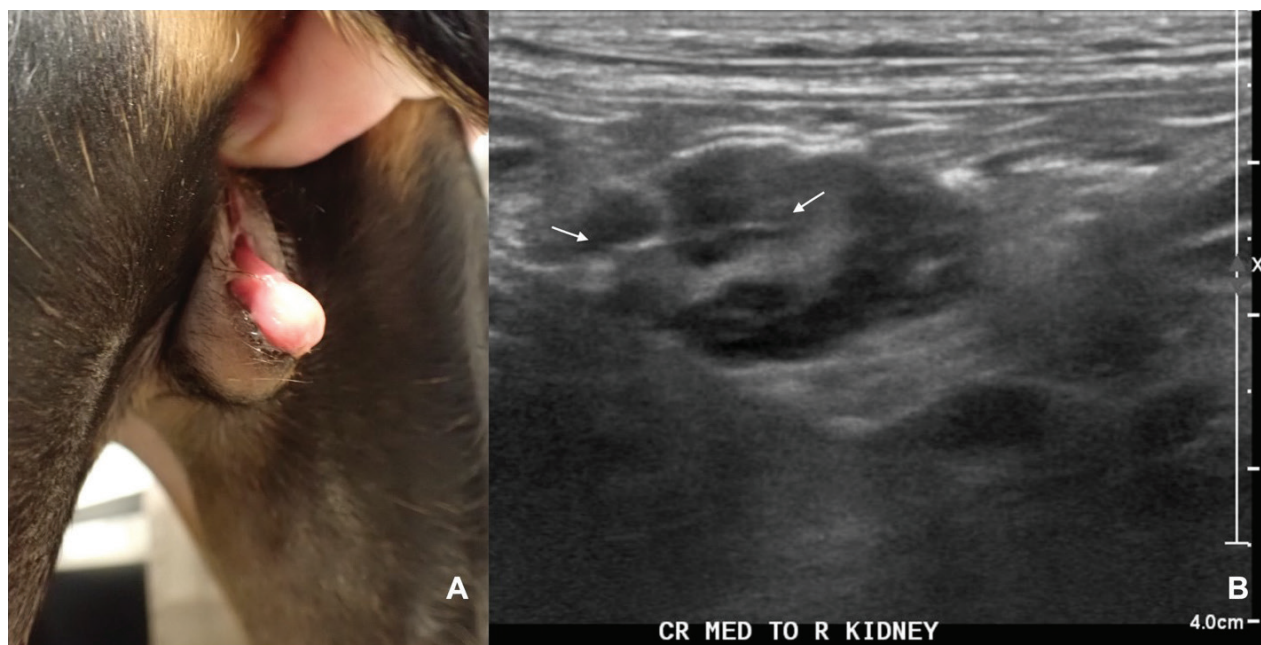


Figure 1. A. Os clitoridis exposed for size visualization; B. Ultrasonographic image (craniocaudal view) of suspected right gonad, note: multifocal hypoechoic follicles and suspected rete testis (hyperechoic band [arrows]).

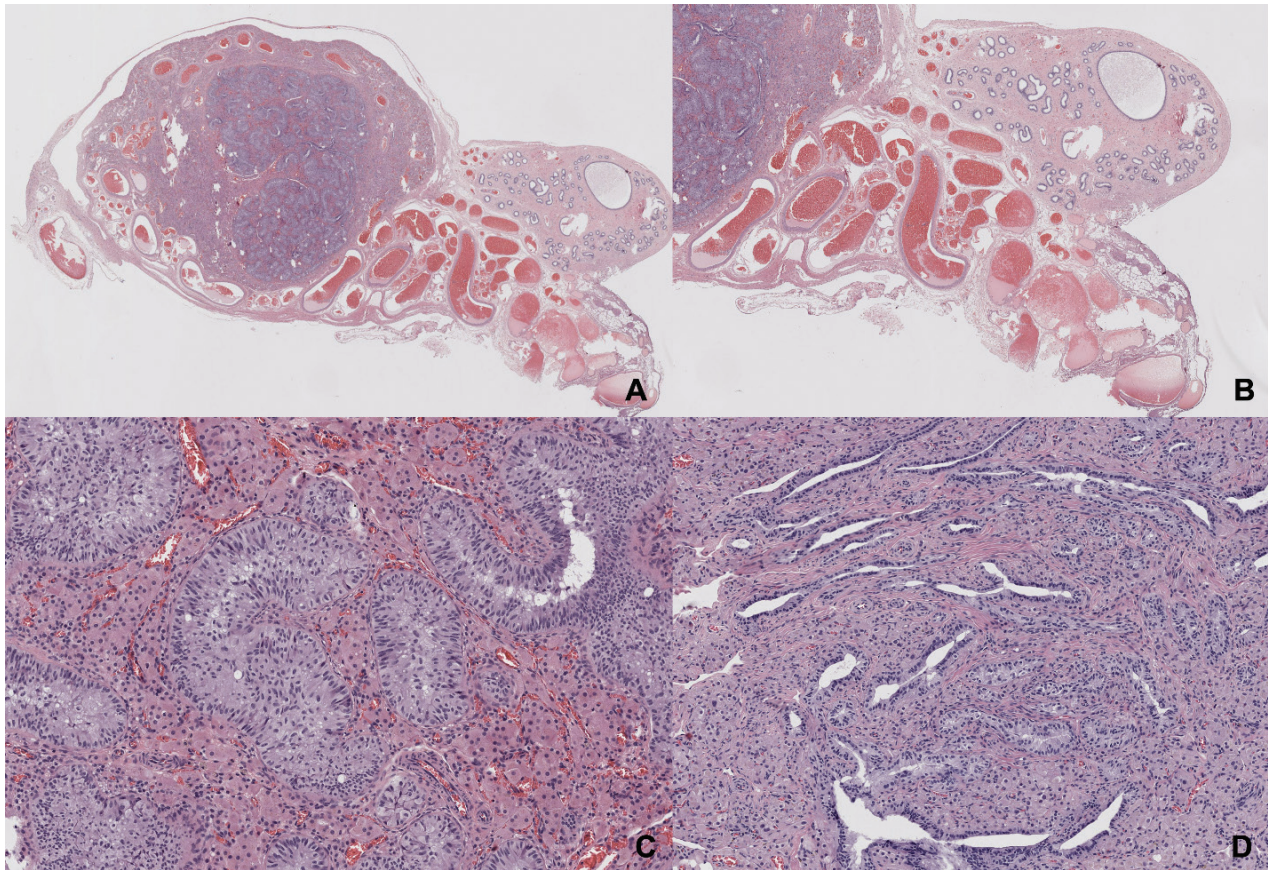


Figure 2. Light microscopic images of gonad stained with hematoxylin and eosin: A. central testicular component and peripheral ovarian component surrounded by bursa (1X); B. tortuous arteries and veins (resembling the pampiniform plexus) and several tortuous mesonephric ducts (resembling the epididymis) surrounded by dense fibrous connective tissue (2X); C. large numbers of variably pigmented Leydig cells and irregular seminiferous tubules that consist solely of Sertoli cells with no evidence of spermatogonia, spermatocytes, spermatids, or sperm (20X), and D. tortuous rete ovarii and abundant interstitial cells (20X).

and XY cells, confirming the final diagnosis of sex chromosome (78,XX/XY), SRY-positive, ovotesticular, disorder of sex development with a female phenotype.

Discussion

Phenotypic abnormality with chromosomal anomaly (XX/XY) and bilateral ovotestis suggested a development disorder due to either 1 or both of them. Abnormalities of chromosomal sex are caused by errors in the number or structure of X and Y chromosomes. Additional sex chromosomes or missing sex chromosome can affect phenotype. Additionally, errors in chromosomes' structures such as insertions, deletions, or translocations can disrupt the expression of genes that reside on altered chromosomes and can result in ambiguous sex phenotype.^{17,18}

This patient was diagnosed with a disorder of sexual development that included clitoral hypertrophy, bilateral ovotestes, underdeveloped uterus, and a chromosome disturbance consistent with chimerism/mosaicism. Chimerism occurs when different zygotes or cells from different zygotes fuse, whereas mosaicism is due to nondisjunction in a single zygote or cells derived from a single zygote.^{3,19} In this case, cytogenetic and FISH analyses were performed only in blood. Various nonhemapoetic tissues along with blood and other genetic diagnostic tests such as DNA

microsatellite, short tandem repeats (STR) and/or single nucleotide polymorphism (SNP) microarray are required to differentiate the underlying mechanism of mosaicism/chimerism.^{20,21}

Amount of testicular gonadal tissue and its corresponding hormonal activity determines the animal's phenotype²² and may include unilateral or bilateral ovotestes as in this patient.^{23–27} Chimerism in a dog was previously reported wherein both blood leukocyte and skin cytogenetic analyses were performed.²⁸ A limitation of the present case report was that the cytogenetic analysis was performed only on blood leukocytes.

Inability to identify left gonad via transabdominal ultrasonography was most likely due to positioning of animal and transducer angle. Additionally, follicle-like structures identified via transabdominal ultrasonography were likely distended mesonephric ducts within the structure resembling epididymis, as follicles were not identified on histopathology. This case is unique because the subset of chimeric and mosaic dogs is rare,² with only a few case reports of abnormalities of chromosomal sex in dogs.^{27,28}

Conflict of interest

Authors have no conflict of interest or funding to declare.

Learning points

- Mosaicism occurs when there is nondisjunction in a single zygote or cells derived from a single zygote.
- Chimerism occurs when different zygotes or cells from differencing zygotes fuse.
- Sex chromosome disorders of sexual development are uncommon in dogs.

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