

Disorders of sexual development in the dog and cat

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Abstract

Normal sexual differentiation occurs in three sequential steps—establishment of chromosomal (genetic) sex, development of gonadal sex, and development of phenotypic sex. Errors in the establishment of chromosomal, gonadal, or phenotypic sex cause abnormal sexual differentiation. Affected individuals are identified with a wide variety of patterns from ambiguous genitalia, to apparently normal genitalia with sterility or infertility. When a patient is suspected of having a disorder of sexual development, analysis of the chromosomal constitution and complete gross and histopathologic description of the gonads, internal and external genitalia are required to correctly categorize the type of disorder.

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1. Introduction

Normal sexual differentiation occurs in three sequential steps—establishment of chromosomal (genetic) sex, development of gonadal sex and development of phenotypic sex. At fertilization, chromosomal sex is established (XX or XY); this composition is maintained in all cell lines throughout life. Despite their chromosomal sex, all early embryos are sexually indifferent; they possess bipotential genital ridges, wolffian and müllerian ducts, a urogenital sinus, a genital tubercle, and genital swellings. Sex chromosome constitution determines gonadal differentiation: the presence of a Y chromosome results in differentiation of the genital ridge into a testis. In the absence of a Y chromosome, the genital ridge differentiates into an ovary. The gene *Sry*, named for the sex-determining region on the Y chromosome, is necessary for either

activation of genes inducing testis formation or suppression of genes encoding ovarian formation. The exact mechanisms of the pathways controlled by *Sry* are unknown. Genes identified as being involved in testis differentiation in humans, transgenic mice, or cell culture systems include *Sox9* (*Sry*-like HMG box), *Amh*, *Wt1*, *Sf1*, and *Drmt1* [1]. Normal XX individuals, which lack *Sry*, possess two X-linked *Dax1* alleles which are involved with ovarian differentiation. *Dax1* may play more of a role in turning off male-specific genes during gonadal differentiation, rather than turning on ovarian differentiation; it also likely plays a role in adrenal, pituitary, and hypothalamic development [2]. Determination of phenotypic sex (differentiation of the tubular reproductive tract and external genitalia) is dependent on gonadal sex. The default embryonic plan is female; if the genital ridges are removed from XX or XY embryos prior to gonadal differentiation, the female phenotype results. In normal XY individuals, Leydig cells within the testis secrete testosterone (T), which promotes differentiation of the wolffian duct into the epididymis and vas deferens. Sertoli cells in the testis secrete

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müllerian inhibiting substance (MIS), which causes regression of the müllerian ducts. Secretion of these two hormones must occur within a critical time window during embryonic development (different for each species) for normal masculinization to result. In the urogenital sinus, genital tubercle, and genital swellings, testosterone is converted by 5 α -reductase to dihydrotestosterone (DHT), which causes the closure of the urethra, and development of the prostate, penis, and scrotum. Descent of the testes into the scrotum completes phenotypic development in the male. In normal XX individuals the absence of MIS, T, and DHT, allows the müllerian ducts, urogenital sinus, and genital tubercle and swellings to develop into female internal and external genitalia. The müllerian ducts develop into the oviducts, uterus, cervix, and cranial vagina; the urogenital sinus develops into the caudal vagina, and vestibule; the genital tubercle develops into the clitoris; and the genital swellings develop into the vulva. Errors in the establishment of chromosomal, gonadal, or phenotypic sex cause abnormal sexual differentiation. Affected individuals are identified with a wide variety of patterns from ambiguous genitalia, to apparently normal genitalia with sterility or infertility.

2. Disorders of chromosomal sex

Abnormal chromosomal sex results from defects in the number or structure of the sex chromosomes. These derangements result from random events during gamete formation or early embryonic development; therefore, they can be observed in dogs and cats of any breed and generally do not display a familial pattern. Abnormalities of chromosomal sex include XXY syndrome, XO syndrome, XXX syndrome, true hermaphrodite chimeras, XX/XY chimeras with testes, and XY/XY chimeras with testes.

The majority of animals with abnormalities of chromosomal sex have few clinical symptoms. Detection is most likely if the animal's intended use is in a breeding program. The most common historical complaint is primary anestrus for phenotypic females and inability to sire litters for phenotypic males. A karyotype is necessary to define the error in sex chromosome constitution—this is typically performed on heparinized peripheral blood lymphocytes. In all patients with a suspected disorder of sexual development, careful gross descriptions of the external and internal genitalia, as well as histopathology of the gonads and tubular tract are necessary to accurately categorize the disorder. Gonadectomy and hysterectomy are recommended.

2.1. XXY syndrome

XXY syndrome is one of the most common sex chromosome abnormalities observed in man (Klinefelter's syndrome) with a prevalence of 0.1–0.2% in the general population and up to 3.1% of infertile men [3]. Although the true incidence of this disorder in dogs and cats is unknown, it is the most commonly reported sex chromosome abnormality. Affected dogs have a 79,XXY karyotype, hypoplastic testes, epididymides, vas deferentia, male external genitalia (varying from normal to hypoplastic), and are sterile. The completely male phenotype is explained by the presence of testes capable of producing MIS and testosterone. The presence of two X chromosomes prevents normal spermatogenesis, resulting in sterility. Affected cats are 39,XXY with internal and external genitalia and testes similar to that described for the dog (review, [4]). In cats, the genes for orange and non-orange (black or brown) are X-linked alleles at the Orange locus. In normal XX females, the random inactivation of one X chromosome in all somatic cells during embryonic development leads to the formation of the Barr body. Each cell can now only express either an orange or non-orange coat color. Females heterozygous at the Orange locus develop the random patches of the tortoise-shell or calico (T-C) pattern because only one allele is expressed in a given patch of hair. Normal males with only one X chromosome should only be able to express one coat color, orange or non-orange. A review of 25 T-C male cats showed that two-thirds possessed two X chromosomes (XXY, XX/XXY, XY/XXY, or other chimeric or mosaic combinations), and all were sterile except for one (XY/XXY). The remaining T-C male cats were mosaics or chimeras (XY, or XX/XY) [5].

2.2. XO syndrome

Affected dogs are sterile, having a 77,XO karyotype, dysgenetic ovaries (streak gonads), female internal genitalia, and infantile external genitalia. In humans (Turner's syndrome) and horses this syndrome has associated somatic abnormalities, most notably small stature; this is likely the case in the dog as well. There have been two reports of canine X-chromosomal monosomy with stunted growth; a Doberman pincher and an American Eskimo bitch [6,7]. This syndrome has also been reported in a 2.5-year-old Burmese cat with primary anestrus (37,XO) but without somatic abnormalities [8].

2.3. XXX syndrome

A single report of an Airedale bitch with primary anestrus at 4 years of age was associated with a 79,XXX karyotype. The ovaries lacked follicles, the uterus was small, and the remainder of the genitalia was female. Resting concentrations of FSH and LH were elevated, and progesterone was baseline (consistent with anestrus) [9]. Trisomy X occurs in 1 out of 1000 live female human births and affected women typically have normal sexual development and are usually fertile, but there are reports of abnormal reproductive cycles or developmental anomalies [10].

2.4. True hermaphrodite chimera

Chimerism results when two or more cell populations, each from different individuals, are present with the same individual. For example, fusion of two zygotes with different sex chromosome constitutions gives rise to a single zygote that is a XX/XY chimera. Mosaicism results when two or more cell populations with different chromosome constitutions are present. Both of these cell populations arise from within the same individual, and are usually caused by meiotic or mitotic non-disjunctional events. True hermaphrodites have both ovarian and testicular tissue—any combination can be seen (unilateral ovotestis with a contralateral ovary or testis, bilateral ovotestes, or unilateral ovary and unilateral testis). Three canine cases of true hermaphrodite chimeras have been reported. The karyotype of these individuals was either XX/XY or XX/XXY; all were phenotypic females with an enlarged clitoris (review, [4]). One feline case has been reported, which was externally male in phenotype with one scrotal testis and one abdominal ovary [11].

2.5. XX/XY chimera with testes

XX/XY chimerism with testes has been described in the dog and cat. An Old English sheepdog with ambiguous genitalia (cranially displaced vulva containing a hypoplastic penis) possessed a XX/XY karyotype, aspermatogenic testes located at the caudal pole of the kidneys, and a hypoplastic uterus [4]. Several cats with XX/XY chimerism with an external male phenotype have been reported, with variable fertility. It appears that when XY cells are present in higher proportions than XX cells in the testis, the greater the likelihood of fertility. Some tortoise-shell males have this chromosomal anomaly [12].

Chimeric or mosaic individuals may be presented as puppies or kittens with an abnormal vulva or prepuce. Some hermaphrodite chimeras may also present with complaints of chronic vulvar irritation. It would also be possible to see hermaphrodite chimeras or chimeras with testes present with signs of hyperestrogenism secondary to Sertoli cell tumor in abdominal testicular tissue. If the clitoris is enlarged, such as is seen with some chimeras, then amputation is recommended to eliminate continual mucosal irritation.

2.6. XY/XY chimera with testes

Reports of tortoise-shell males, with normal testicular histology and normal fertility are most likely due to XY/XY chimerism.

3. Disorders of gonadal sex

Individuals with disorders of gonadal sex have either an XX or XY sex chromosomal constitution, but the gonadal sex does not agree with the chromosomal sex (“sex-reversed”). Only XX sex reversal has been reported in the dog: affected dogs have a 78,XX karyotype with variable degrees of testicular differentiation of the gonad. No cases of XX sex reversal have been reported in the cat, and no cases of XY reversal have been reported in either the dog or cat. XX sex reversal includes XX true hermaphrodite and XX males—XX true hermaphrodites have ovotestes and XX males have bilateral testes. It is possible to see both of these phenotypes within the same affected familial line. Eighty percent of human XX males are *Sry* positive due to autosomal translocation from the Y chromosome. However, all cases to date of canine XX males that have been tested are *Sry* negative [13]. *Sry*-negative XX sex reversal has been described in goats and pigs as an inherited autosomal recessive syndrome, and has also been reported in the llama and the horse as isolated cases with an unknown inheritance pattern. The genes responsible for testis induction in the absence of *Sry* are unknown for dogs, humans, pigs, and horses. In goats, XX sex reversal (Polled Intersex Syndrome) is linked to the polled trait, and a functional deletion of two genes, *Pisrt1* and *FoxL2* that are proposed ovary-differentiating genes. Loss of these genes leads to testis formation in XX homozygous *PIS*—/— mutants [14]. Functionally active *MIS* is present; however, failure of complete müllerian duct regression suggests insensitivity of the target organ to *MIS*.

In the American Cocker spaniel XX sex reversal is inherited as an autosomal recessive trait [15]. Inheritance

in the German shorthaired pointer is likely to be autosomal recessive as well. It has been described as a familial disorder in the English Cocker spaniel, Beagle, Weimaraner, Kerry blue terrier, and Chinese Pug; the mode of inheritance in these breeds is not known, but is likely autosomal recessive. Cases of XX sex reversal have also been described in the Basset hound, Vizsla, Soft-Coated Wheaten terrier, Pomeranian, Doberman pinscher, American pit bull terrier, Border collie, Walker hound, and Afghan hound [16]. The canine homologue of *Pisrt1* has been shown to not be responsible for canine Sry-negative XX sex reversal [17]. Research continues to identify the mutated candidate gene(s) responsible for XX sex reversal in the dog.

Affected individuals are usually presented as phenotypic females with primary anestrus, phenotypic females with an abnormal vulva, or males with bilateral cryptorchidism and an abnormal prepuce and penis. 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. Most individuals 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), uterus, oviducts, epididymes, and vas deferentia. XX males have testes, the entire wolffian duct system (epididymes and vas deferentia), and a prostate. Both oviducts are usually absent, but a bicornuate uterus is present. The prepuce is usually abnormal in shape and caudally displaced. Most XX males have a hypoplastic penis, and hypospadias or abnormal curvature of the penis is common. Occasionally cryptorchid phenotypic males are not diagnosed until signs referable to hyperestrogenism (intraabdominal testis) or leukocytosis with PU/PD (pyometra) arise. Gonadectomy and hysterectomy are recommended for affected individuals.

A karyotype of 78,XX chromosome constitution in conjunction with the presence of testicular tissue (at least one ovotestis or one testis) is needed to verify XX sex reversal. Elevation of testosterone in response to a gonadotropin releasing hormone (GnRH) or human chorionic gonadotropin (hCG) stimulation test suggests that testicular tissue is present; however, a negative stimulation test does not completely rule out the presence of testicular tissue. A polymerase chain reaction test for the presence of *Sry* accurately describes this disorder.

On rare occasions, XX true hermaphrodites have reproduced; however, it is not recommended to maintain these individuals in a breeding program. The majority of XX true hermaphrodites and all XX males are sterile. Since this is a heritable trait in breeds that have been closely studied and is most likely a heritable trait in those breeds where breeding trials have not yet been conducted, owners should be counseled that both parents of affected individuals should be removed from the breeding program. At least half of the siblings of affected individuals are expected to be carriers, and one quarter may be non-carriers. Since there is no laboratory test that can identify carriers, the best recommendation is to not use any siblings of affected individuals as breeding animals.

4. Disorders of phenotypic sex

In individuals with disorders of phenotypic sex there is agreement of the chromosomal and gonadal sex, but the phenotypic sex (internal or external genitalia or both) disagrees with the gonadal sex. Syndromes that have been described in dogs and cats include: female pseudohermaphroditism, male pseudohermaphroditism, persistent müllerian duct syndrome, and defects in androgen-dependent masculinization. Descent of the testes into the scrotum completes the development of phenotypic sex. The genetic and hormonal control of testicular descent is not completely understood, and the classification of cryptorchidism as a disorder of phenotypic sex is considered by some debatable.

4.1. Female pseudohermaphroditism

A female pseudohermaphrodite has a XX chromosomal constitution, ovaries, and masculinized internal or external genitalia. Female pseudohermaphroditism resulting from the virilizing effects of endogenous in utero androgen exposure (such as X-linked mutations of *Dax1* leading to congenital adrenal hyperplasia in humans [2]) has not been reported in the dog or cat. Rare reports of female pseudohermaphrodites in the dog suggest that iatrogenic exposure of the fetus to exogenous androgens or progestagens during gestation are responsible for this syndrome. There have been no reports of this syndrome in the cat.

4.2. Male pseudohermaphroditism

Male pseudohermaphrodites have a XY chromosomal constitution, testes, and feminization of the internal or external genitalia feminized to some extent. Male

pseudohermaphrodites include XY males in whom the müllerian ducts fail to regress and individuals with defects in androgen-dependent masculinization.

4.3. Persistent müllerian duct syndrome

Persistent müllerian duct syndrome (PMDS) is recognized as a form of male pseudohermaphroditism in the miniature schnauzer in the United States [18], sporadically in other breeds, and possibly in the cat (no cytogenetic evaluation or gonadal histology was performed) [19]. In the miniature schnauzer, affected individuals are XY males, with bilateral testes, external male genitalia, but all müllerian and wolffian duct derivatives are present. PMDS has been shown to be an inherited as an autosomal recessive pattern in the miniature schnauzer; only homozygous individuals will display the abnormal phenotype. Affected individuals secrete bioactive MIS at the critical time period during embryonic development [20], suggesting that the defect in animals affected with PMDS is insensitivity of the müllerian ducts to MIS, possibly due to a defect in the MIS receptor.

4.4. Defects in androgen-dependent masculinization

Animals that possess defects in androgen-dependent masculinization have an XY sex chromosomal constitution, bilateral testes, and no müllerian duct derivatives. However, the internal and external genitalia display variable degrees of feminization. The resulting phenotype can vary from complete (severe) to incomplete (mild). These are grouped according to the primary defect: (1) defects in androgen production and (2) androgen resistance or insensitivity, which includes defects in 5 α -reductase type 2 isoenzyme (failure to convert T to DHT), and defects in the androgen receptor (testicular feminization). Defects in 5 α -reductase type 2 isoenzyme is inherited as an autosomal recessive pattern in man [21,22], but has not yet been described in the dog or cat. Defects in androgen production have also not been reported in the dog or cat.

Hypospadias is the abnormal location of the urinary orifice and occurs when there is incomplete masculinization of the urogenital sinus (closure of the urethra). The location of the orifice can vary from the ventrum of the glans penis, the penile shaft, the penoscrotal junction, the scrotum, or the perineum. The incidence of canine hypospadias is 0.003%, although this surveyed population did not include stillborn, neonatal deaths of severely affected individuals, and probably

undiagnosed mild cases [23]. Although no pedigree analysis has been performed, this condition may have a familial basis in Boston terriers, based on 24% of reported cases being of this breed. The remainder of the external genitalia of these animals is usually not ambiguous, but concurrent cryptorchidism, penile hypoplasia, ventral deviation of the penis, or abnormalities of the ventral prepuce has been described. In man, the presence of hypospadias and cryptorchidism in the same individual was associated with an increased incidence of sex chromosome abnormalities [24], underscoring the need for complete diagnostics for individuals with defects of the external genitalia.

Testicular feminization syndromes (Tfm) result from mutations, qualitative or quantitative, in the X-linked androgen receptor gene. Affected animals are XY males with bilateral testes. Since testes are present which secrete normal amounts of T and MIS, no müllerian duct derivatives are present. However, since there is a defect in the androgen receptor gene, androgen-dependent masculinization is either absent or incomplete, despite normal production of T and DHT. A 38,XY Himalayan cat with testes residing in a bifid scrotum, an enlarged clitoris with hypoplastic spines protruding from a vulvar-like structure, and no müllerian duct derivatives has been described [25].

5. Agenesis and dysgenesis of the reproductive tract

Agenesis is the failure of a structure or organ system to develop due to non-appearance of its primordium during embryonic development. Dysgenesis is a defect in development of a structure or organ. In the reproductive tract of dogs and cats, agenesis or dysgenesis of the gonads, müllerian or wolffian ducts, urogenital sinus, genital tubercle, or genital swellings can be seen. Some examples would include monorchidism and testicular hypoplasia; ovarian agenesis and ovarian hypoplasia; segmental aplasia of the epididymides, vas deferentia, oviducts, uterus, and vagina; and penile hypoplasia. In females, failure of fusion of the caudal müllerian ducts or urogenital sinus can give rise to a wide variety of vaginal anatomical anomalies. In animals whose chromosomal sex, gonadal sex, and phenotypic sex agree it is unknown whether there is some genetic, hormonal, or heritable component to these anomalies.

6. Conclusion

Abnormalities in sexual differentiation arise when errors in the establishment of chromosomal, gonadal, or

phenotypic sex occur. Since the external genitalia of many of these disorders can appear similar, and some disorders have a heritable basis, whereas others do not, it is important to correctly diagnose the type of disorder. Correct categorization of a patient suspected of having a disorder of sexual development requires cytogenetic evaluation, and gross and histopathologic description of the gonads, internal genitalia, and external genitalia. Appropriate counseling on breeding programs is only possible by correctly defining the type of disorder.

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