Inherited diseases of the reproductive tract in dogs and cats

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Introduction

Many veterinarians encounter an abnormality of sexual development during the course of routine practice, particularly during surgery for neutering dogs and cats. Theriogenologists are also likely to encounter these through their evaluations of infertile dogs and cats, and we are motivated to reach a definitive diagnosis. In addition to helping the owner and breeder, this careful "phenotyping" is also needed to further research, with the goal of determining the genetic basis of these disorders. Such research has led to the development of practical genetic tests for other inherited disorders and is proving successful in reducing production of affected cats and dogs.

To simplify the diagnostic plan, we can categorize affected animals according to the first step in sexual development that is identified as abnormal. These were identified as either errors in chromosomal sex, gonadal sex, or phenotypic sex. In that scheme phenotypic sex includes the internal and external genitalia, but excludes the gonad. This is still a practical plan, but requires incorporation of the new nomenclature for disorders of sexual development that was first developed for human patients is now used for animals. In addition to incorporating molecular diagnosis, the new nomenclature replaces older terms that are outdated and/or confusing. For example, the general term intersex has been replaced with disorder of sexual development (DSD), and the terms hermaphrodite and pseudohermaphrodite have been eliminated. Disorders of sexual development are now divided into three main categories: sex chromosome DSD, XX DSD, and XY DSD. The examples below illustrate how the diagnostic plan and the new nomenclature can be integrated to reach a definitive diagnosis. Previous terminology is included for reference purposes. Other canine and feline examples are also reviewed.

Keywords: Disorder of sexual development, inherited disease, abnormal karyotype

Basic steps in the diagnostic plan

1. Obtain a karyotype. If indicated, obtain molecular tests to confirm the presence of genes located on the sex chromosomes.
2. Obtain gonadal histology to confirm the gonadal sex.
3. Identify all internal genital organs present. If indicated, confirm with histology.
4. Identify the external genitalia.
5. To reach a tentative diagnosis, identify the first step that is abnormal. Is it at the level of the sex chromosomes, the gonads, the internal genitalia or the external genitalia?
6. Confirm the diagnosis by DNA testing, or other molecular and/or functional tests.

Sex chromosome DSD

Abnormal karyotype

The normal cat karyotype is 38,XX or 38,XY, while that of dogs is 78,XX or 78,XY. If the karyotype is abnormal, the diagnosis lies in the category of sex chromosome DSD. These are caused by abnormalities in the number or structure of the sex chromosomes. The clinical diagnostic plan should include a description of the internal and external genitalia, and gonadal histology.

Well-known examples of sex chromosome DSD include the XXY syndrome and X monosomy (XO). XXY cats and dogs are sterile, phenotypic males. However, many cats reported have actually been mosaic variants such as XY/XXY and XY/XYY. Most had either a tortoiseshell or calico coat color. Since the orange coat color locus is X-linked in the cat, a coat color containing both orange and black occurs in cats that have at least two X chromosomes, and not in XY males. Therefore these coat colors in a cat with male external genitalia should prompt investigation for XXY or variants. There is no coat color association in dogs, and only four 79,XXY dogs have been reported.
Monosomy X and mosaic variants have been reported in cats and dogs. They present as phenotypic females with primary anestrus. Although short stature may be associated, signs have been variable in mosaic variants.

Trisomy X (79,XXX) has been reported in dogs. Most presented as phenotypic females with primary anestrus. However, two reported had abnormal estrous cycles, including shortened interestrus intervals, persistent estrus, and anovulation, but failed to become pregnant when bred. Both ovaries in each were hypoplastic, suggesting sex chromosome mosaicism in the gonads (78,XX/79,XXX). Only one feline mosaic variant (37,X/39,XXX) has been reported, which was pregnant at presentation.

Normal karyotype

If the karyotype is that of a normal female (38,XX or 78,XX) or a normal male (38,XY or 78,XY), this places the diagnosis in the category of XX DSD or XY DSD, respectively, and not sex chromosome DSD. The next step is to evaluate gonadal histology to determine if there is an error in gonadal sex.

XX DSD

**The karyotype is 78,XX, but the gonads are ovotestes or testes.** If the karyotype is 78,XX but the gonads contain seminiferous tubules characteristic of the testis, then the first step that is abnormal is in the determination of gonadal sex. The tentative diagnosis is XX DSD, ovotesticular or testicular, which replaces the terms XX sex reversal, XX true hermaphrodite and XX male. After obtaining histologic confirmation of ovotestes or testes, the next step is to test for the presence or absence of *SRY* in the genomic DNA. Finally, the internal and external genital organs, and location of the urethral orifice should be identified. This type of XX DSD has been reported in dogs, but not in cats.

Detailed studies on ovotesticular and testicular XX DSD in dogs from a research pedigree derived from the American cocker spaniel (ACS) confirmed that all affected dogs were 78,XX. Affected siblings had ovotestes or testes, and variable external phenotypes. Subsequent studies confirmed that affected dogs were *SRY* negative, ruling out *SRY* translocation as the cause. The previous name for this disorder was *SRY*-negative XX sex reversal. Breeding experiments conducted in the ACS model indicated the mode of inheritance was compatible with sex-limited autosomal recessive inheritance. To date, ovotesticular or testicular XX DSD has been reported in at least 27 canine breeds and one mixed breed. Although dogs in early reports were not tested for *SRY*, because a test was not available until 1995, all cases tested since have been *SRY*-negative. The causative mutation is likely to be identical by descent in closely related breeds, such as English and American cocker spaniels (genetic homogeneity), but may be different in other breeds (genetic heterogeneity).

The phenotype of affected dogs varies widely. In the ACS model, approximately 10% of affected dogs had bilateral testes, which were often cryptorchid. These dogs were previously called XX males. Epididymides and a complete uterus were also present. In histologic sections, the vas deferens was often identified running parallel to the uterine horn. The penis and prepuce were caudally displaced and exhibited mild hypospadias. The remaining 90% of affected dogs in the ACS model usually had bilateral ovotestes, but occasionally had an ovary and ovotestis, and rarely a testis paired with an ovotestis. These dogs were previously called XX true hermaphrodites. Internal genitalia in these dogs included an epididymis or an oviduct, or both, adjacent to the ovotestes, and a complete uterus. Externally, 15% of dogs with ovotestes had a prepuce-like vulva and 15% had an enlarged clitoris containing a bone. The remaining 70% of dogs with ovotestes had an apparently normal vulva. Subsequently, a narrowed caudal vagina has been identified in dogs with this phenotype. Corrective surgery may be needed to alleviate clinical signs related to hypospadias or clitoral enlargement.

Most affected dogs from the ACS model pedigree have been sterile. However, some have exhibited estrous cycles, and some produced offspring even in the presence of a narrowed vagina or an enlarged clitoris containing a bone. Nevertheless, to prevent production of affected dogs and reduce the carrier frequency in any breed, affected dogs should be removed from the breeding population. Male (78,XY) carriers of the trait and obligate heterozygote carrier females have been fertile. However, further
breeding of the parents of affected dogs should be discouraged to prevent production of affected dogs and reduce the frequency of the mutation within the breed.

The karyotype is 38,XX or 78,XX and the gonads are ovaries, but the internal or external genitalia are fully or partially male. If histology confirms that the gonads are normal ovaries, tentative diagnosis is reached by identifying the first step thereafter that is abnormal. If male genitalia are present, this suggests a tentative diagnosis of XX DSD, androgen excess. As a developing fetus, these animals have been exposed to androgens, which were either produced by the developing fetus itself, or crossed the placenta from the maternal into the fetal circulation.

XX DSD, androgen excess, maternal origin. These disorders have not been reported in the cat. However there are several research and clinical reports in dogs where females were masculinized by steroids administered to their pregnant dam. These preparations included mibolerone, testosterone, and progestagens. As adult responses to steroids are not reliably predictive of fetal response, it is inadvisable to administer steroid preparations to pregnant dogs or cats, and particularly during the period of internal and external genital development.

The karyotype is 38,XX or 78,XX, the gonads are ovaries and the genitalia are unambiguously female, but abnormal. The first step that is abnormal is in the female genitalia. These abnormalities are included under XX DSD, other: Mullerian agenesis/hypoplasia. The phenotype is clearly female, as there are no male genitalia present, but the vagina or uterus has failed to develop properly. In humans, such defects are highly associated with renal agenesis and/or ectopy, and cervicothoracic somite dysplasia. This syndrome is termed MURCS for Mullerian Duct Aplasia, Unilateral Renal Agenesis and Cervicothoracic Somite Anomalies (Online Mendelian Inheritance in Man, number %601076). If one component of MURCS is identified in a human patient, this prompts evaluation for the other anomalies.
A syndrome similar to MURCS may occur in cats and dogs. In a survey of 53,258 cats and 32,660 dogs undergoing elective ovariohysterectomy, congenital uterine abnormalities were identified in 0.09% of female cats and 0.05% of female dogs. These included unicorneatus uterus, segmental aplasia of one uterine horn and uterine horn hypoplasia. In 29.4% of cats and 50% of dogs with uterine abnormalities in which the kidneys were also evaluated, ipsilateral renal agenesis was present. Although cervicothoracic abnormalities were not described in this report, careful evaluation of such cases could establish the prevalence of Mullerian agenesis and hypoplasia as isolated defects, the prevalence of their association with ipsilateral renal agenesis, and whether the full MURCS syndrome occurs in cats and dogs.

**XY DSD**

The karyotype is 38,XY or 78,XY but at least one gonad is an ovotestis. In this case, the first step that is abnormal is in the determination of gonadal sex. The tentative diagnosis is XY DSD, ovotesticular, which replaces the terms XY sex reversal and XY true hermaphrodite. The next diagnostic step is to confirm the presence or absence of SRY in the genomic DNA. Finally, the internal and external genital organs, and location of the urethral orifice should be identified.

One case of feline XY DSD, ovotesticular has been confirmed. The year old mixed breed cat presented as a phenotypic male with bilateral cryptorchidism. The SRY gene sequence was the same as in a normal male control. Bilateral ovotestes were located at the caudal pole of the kidneys. Epididymides were adjacent to each gonad and partially developed vasa deferentia, along with a complete bicornuate uterus and oviducts.

The karyotype is 38,XY or 78,XY, the gonads are testes, but female genitalia are present in regions dependent upon androgens for masculinization. The first step that is abnormal is androgen dependent masculinization, indicating a tentative diagnosis of XY DSD, disorder in androgen synthesis or action. The most commonly reported disorders of this type are androgen receptor defects.

1) When the androgen receptor is nonfunctional, there is complete failure of masculinization in the internal and external genitalia (complete androgen insensitivity syndrome, or CAIS).

One case of feline CAIS has been reported. The external genitalia were unambiguously female at six months of age when the cat was presented for ovariohysterectomy. Testes were located just caudal to the kidneys, and there were no Mullerian or Wolffian duct derivatives present. The testes contained seminiferous tubules and abundant Leydig cells. Inability of the androgen receptor to bind androgens was demonstrated in vitro.

2) When the androgen receptor is functional to some degree, there is variable masculinization of the internal or external genitalia (partial androgen insensitivity syndrome, or PAIS).

One case of canine PAIS has been reported in a mixed breed that was phenotypically female at six months of age. Later, scrotal-like swellings containing testes were identified on each side of the vulva. Spermatogenesis was absent in both hypoplastic testes. A well-developed epididymis and partially developed deferent duct were adjacent to each testis. Abnormal androgen binding to the androgen receptor was demonstrated in vitro.

The karyotype is 38,XY or 78,XY, the gonads are testes, the external genitalia are male, but a uterus is present. The first step that is abnormal here is in Mullerian duct regression. Although classified under the category of XY DSD, other, these are disorders in the synthesis or action of Mullerian inhibiting substance (MIS), also known as anti-Mullerian hormone (AMh). The example below has the specific diagnosis of persistent Mullerian duct syndrome (PMDS). Mutations in MIS or its Type II receptor (MISRII/AmhR2) cause the same PMDS phenotype in human patients.

Persistent Mullerian duct syndrome in the miniature schnauzer has been reported frequently and in several continents. Externally, PMDS dogs are unambiguously male, except that approximately 50% are unilaterally or bilaterally cryptorchid. In addition to having complete male internal and external genitalia, these dogs also develop all Mullerian duct derivatives. Bilateral oviducts and epididymides are adjacent to the testes. The deferent ducts are included in the lateral walls of the uterus, and the cranial end of each uterine horn is attached to the caudal pole of the ipsilateral testis. The cervix is present, and
the cranial vagina terminates within the craniodorsal aspect of the prostate gland. Radiographic contrast studies in some PMDS dogs confirmed a patent connection between the cranial vagina and the prostatic urethra. Pyometra and neoplasia in cryptorchid testes are common sequelae.

Cryptorchidism and infertility are not consistently associated with PMDS in the miniature schnauzer. Fifty percent of PMDS dogs had bilateral scrotal testes and externally appeared to be normal males. Such dogs could easily escape clinical recognition. The remaining 50% of PMDS dogs were cryptorchid. The close attachment of the testis to the uterine horn likely interferes with testis descent. PMDS dogs with at least one scrotal testis were fertile.

Persistent Mullerian duct syndrome in the miniature schnauzer is inherited as a sex-limited, simple autosomal recessive trait. The causative mutation in the MIS type II receptor has been identified and a DNA test is available to detect affected, carrier and noncarrier miniature schnauzers. Persistent Mullerian duct syndrome has also been reported in the basset hound in Europe and a mixed breed dog, but in those cases, the causative mutations are unknown.

**The karyotype is 38,XY or 78,XY, the gonads are testes, the external genitalia are male, but there is abnormal development of the male urethra.** The first step that is abnormal occurs in only one portion of the external genitalia. Although classified under the category of XY DSD, other, the specific diagnosis is isolated hypospadias. This disorder has been rarely reported in cats and dogs, and no molecular etiology has yet been identified. Two reports in Himalayan cats described the severe phenotype. The scrotum is bifurcated by a urethral canal that is open along the entire dorsal aspect, and the penis and prepuce are diverted dorsally. Canine isolated hypospadias of varying severity has been reported, where the urethral orifice can be located in the glans penis, the penile shaft, or the perineum. The Boston terrier had the highest prevalence of isolated hypospadias in a survey of veterinary hospitals.

**The karyotype is 38,XY or 78,XY, the gonads are testes, the external genitalia are male, but one or both testes have failed to descend into the scrotum.** The first step that is abnormal occurs in only one portion of the external genitalia. Although classified under the category of XY DSD, other, the specific diagnosis is isolated cryptorchidism, which is different from cryptorchidism that is associated with other DSD. In breeds where cryptorchidism has been associated with other DSD, such as PMDS in the miniature schnauzer (above), affected dogs can be screened for those mutations to obtain a definitive diagnosis.

The following discussion is limited to isolated cryptorchidism, wherein XY males are phenotypically male in all respects except that one or both testes are undescended. The undescended testis may be located anywhere from the caudal pole of the kidney to the inguinal canal, or external to the canal but cranial to the scrotum. Thus, cryptorchidism is a term encompassing several phenotypic categories, likely reflecting a genetically complex control of testis descent.

In cats, scrotal testes are not easily palpable in young kittens, so cryptorchidism is often identified in males presented for neutering before one year of age. Prevalence has been described as 1.7% and 1.3% of cats presented for neutering, with most being unilaterally cryptorchid. Prevalence in Persian cats was reported to be significantly greater than in other breeds. In dogs, canine cryptorchidism is prevalent, ranging from 6.8% of males presented for neutering to 1.4% of dogs at six to twelve months of age. It is also more prevalent in some breeds. In one study, inguinal cryptorchid testes were most common.

As in humans, late testis descent has been identified in dogs. In one study of cryptorchid dogs examined regularly until one year of age, late descent occurred in 24.6% of cryptorchid testes, with 63.3% of those being unilaterally cryptorchid. However, of the 24.6% that descended, most did so by 14 weeks of age and none descended after six months of age. An increased risk of neoplasia in undescended testes
Conclusions

By following the basic steps in the diagnostic plan and identifying the first abnormal step in sexual development in a clinical case, we can narrow the tentative diagnosis to an error in chromosomal sex, gonadal sex or phenotypic sex. This allows us to categorize the abnormality as a sex chromosome DSD, XX DSD or XY DSD, which narrows the list of diagnostic tests needed to reach a definitive diagnosis. Definitive diagnoses are important to the owners and breeders, who can benefit from genetic counseling for their breeds. A definitive diagnosis also provides the accurate phenotype information that is needed for further research to identify causative mutations, create DNA tests, and ultimately reduce production of affected animals. Incorporation of the new DSD nomenclature into our communications should lead to increased collaboration between veterinarians, researchers, and physicians, to the benefit of animals and humans.

References