Published in final edited form as:

Theriogenology. 2011 July 15; 76(2): 374–380. doi:10.1016/j.theriogenology.2011.02.017.

Trisomy-X with estrous cycle anomalies in two female dogs

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Abstract

Two female dogs were presented with a history of abnormal estrous cycles and infertility, despite multiple breedings. Medical therapy to correct the cycle anomalies did not result in pregnancy. Cytogenetic analysis of blood lymphocyte cultures in each dog revealed three copies of the X chromosome in each cell, constituting a 79,XXX karyotype (trisomy-X). Both dogs were eventually ovariohysterectomized and histological evaluation revealed hypoplastic ovaries and an absence of normal follicular structures. However, partial or immature follicles were noted, which may have been sufficient to cause both females to initiate cycling. The history and clinical characteristics found in these dogs were compared to those described in three other dogs reported with trisomy-X, as well as those reported in other species. These findings highlighted the importance of cytogenetic studies in fertility evaluation and achieving a definitive diagnosis for infertility in the bitch.

Keywords

Dog; Infertility; Karyotype; Aneuploidy; XXX; Abnormal estrous cycle

1. Introduction

Chromosomal aneuploidy is one of the most commonly recognized genetic anomalies in humans, and of those, trisomy-X occurs at a rate of 1 in 1000 human births [1]. Aneuploidy is the result of non-disjunction during meiosis and can be associated with clinical signs in the offspring, depending on the chromosomes that are either absent or in excess. First

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described as a “super female”, trisomy-X is characterized by a wide variety of clinical signs, mainly primary amenorrhea and gonadal failure, in approximately 20% of affected women [2]. However, based on incidence studies, it is suspected that the majority of trisomy-X cases may escape diagnosis, because accompanying symptoms of reproductive disturbance, skeletal malformations, and behavioral or psychiatric disorders can be so mild that karyotyping is not performed [3]. In dogs, the few reports of trisomy-X in the current literature had presenting complaints of either primary anestrus or infertility, despite the occurrence of estrous cycles [4–6]. The incidence of trisomy in the dog would likely be difficult to assess, as most dogs are neutered or spayed. This population would not be presented for infertility and thus, there would be no reason to perform karyotyping.

In dogs, reported estrous cycle abnormalities included persistent anestrus, anovulation, a slow rise in serum progesterone concentrations, “split” heats, insufficient luteal phase, and persistent estrus [7]. The underlying cause of these abnormalities was often investigated using a combination of diagnostics including reproductive hormone profiling, vaginal cytology, vaginoscopy, reproductive ultrasonography, hormone therapy trials, and occasionally gonadal biopsy or ultrasound-guided fine needle aspiration of ovarian cystic structures for both cytology and more importantly hormone analysis. However, underlying cytogenetic abnormalities are rarely investigated, and ovariohysterectomy is pursued when treatment fails to result in pregnancy [7]. This report describes two dogs that presented with estrous cycle anomalies, compares their clinical findings to those of trisomy-X in several species, and emphasizes the importance of cytogenetic analysis in the diagnostic plan for infertility in the bitch.

2. Case histories, methods, and results

2.1. History and physical examination

Dog 1, a 2-y-old Silky Terrier bitch, presented for reproductive evaluation with a history of infertility and shortened interestrous intervals of 4 mo duration. At the time of presentation, she was reported to have had four previous estrous cycles, which were characterized by bloody vulvar discharge and acceptance to mating. She was bred during two of these cycles, with no litters resulting. Dog 2, a 2-y-old Labrador retriever bitch was presented with a history of infertility. She was reported to have had three previous estrous cycles beginning at 6 mo of age, with interestrous intervals approximating 6 mo. During her estrous cycle, she was attractive to males, but not receptive. No previous ovulation timing or assisted reproductive techniques had been attempted in either case prior to presentation.

2.2. Reproductive examination

Both dogs presented for a fertility examination when the owners noted external signs of proestrus, including vulvar enlargement and serosanguinous vaginal discharge. Phenotypically, both dogs had unambiguously female external genitalia and were normal on the remainder of their physical examinations. The only other observation was shy and timid behavior noted in Dog 1. Vaginal speculum examination of Dog 1 revealed minimal crenulation and a pale pink, slightly mottled and slightly edematous vaginal mucosa. She had moderate vulvar enlargement without appreciable vaginal turgor. Vaginal cytology revealed 30% parabasal cells, 50% intermediate cells, 10% nucleated superficial cells, and 10% anucleated superficial cells. Dog 2 had moderate vulvar enlargement, and vaginal cytology identified 100% superficial, cornified epithelial cells consistent with estrus.

Transabdominal ultrasound examination was performed in both dogs. Both dogs were shaved for the ultrasonographic examination and the examinations were performed by board-certified specialists. In Dog 1, both ovaries were inactive, but normal in size and shape for a bitch of her size and breed [9]. In Dog 2, the left ovary was mildly enlarged with

multiple, 1 cm diameter, thin walled (<2 mm), fluid-filled cavities. The right uterine horn had a corrugated wall and contained a small amount of intraluminal fluid and small endometrial cysts. Ultrasonographic examination of the right ovary did not reveal any prominent structures such as cystic structures. The left uterine horn was normal and the cranial vaginal wall appeared thicker than during hormonal quiescence.

Laboratory analyses included complete blood counts and serum biochemistry panels to rule out an underlying systemic disease. The results were within normal limits in both dogs. Urinalysis performed in Dog 2 was normal. Serum progesterone concentrations were measured by radioimmunoassay in both cases and were 0.4 and <0.2 ng/mL, respectively, indicating neither dog's follicles had responded to an LH surge, if it had been present, and neither had progressed into the luteal phase [3].

2.3. Treatment

The owners of Dog 1 elected to pursue ovariohysterectomy. The owners of Dog 2 chose medical treatment as part of a diagnostic procedure prior to consideration of ovariohysterectomy. Based on history, physical examination findings, and diagnostic results, differential diagnoses in Dog 2 included persistent estrus, ovarian cysts, ovarian tumor, endometrial hyperplasia, mucometra, hydrometra, and uterine infection. Medical therapy was directed at treating potential functional ovarian follicular cysts and early cystic endometrial hyperplasia. Pending results of culture and sensitivity of the cranial vagina, enrofloxacin was prescribed. A GnRH agonist, gonadorelin (Factrel®, Fort Dodge Laboratories, Fort Dodge, IA, USA) was given at a dose of 2.2 µg/kg im, once daily for 3 d to induce progression in the cycle [10]. A follow up transabdominal ultrasound examination revealed that the left ovarian cystic structures were smaller than at presentation. However, endometrial cystic lesions were still present in the right uterine horn. The left uterine horn was within normal limits, and the vaginal wall was no longer thickened. Microbial cultures revealed mixed flora of normal vaginal bacteria that were sensitive to almost all antibiotics including enrofloxacin, which was discontinued after 3 d, upon receiving the laboratory results.

Dog 2 was stable and afebrile after GnRH treatment during 4 wk of monitoring, but serum progesterone concentrations failed to rise above 0.2 ng/mL. Daily mibolerone was prescribed according to manufacturer's instructions (120 µg orally every 24 h; Cheque Drops®, Upjohn, Kalamazoo, MI, USA) to prevent cycling and allow time for uterine repair [11]. After 4 mo, treatment with mibolerone was discontinued, external signs of proestrus and estrus followed, and the bitch was bred three times by the owner without waiting for a rise in progesterone. Serum progesterone concentrations remained below 0.5 ng/mL on the days she was bred. When presented approximately 1 mo after breeding, the bitch was in estrus, not pregnant, and serum progesterone concentrations were <0.5 ng/mL. Medical treatment with enrofloxacin was resumed and mibolerone was added upon cessation of cytologic signs of estrus. After 4 mo, the owner discontinued mibolerone, and the bitch exhibited proestrus 1 mo later. Once the bitch progressed to estrus as determined by vaginal cytology, hCG (22 U/kg IM, given three times, 12 h apart; Chorionic Gonadotropin®, Bristol Myers Squibb, New Brunswick, NJ, USA) was used in an effort to induce ovulation [10]. The owner reported that a natural breeding took place 1 d after hCG administration, and two intravaginal AI with fresh semen were performed 4 and 6 d after hCG administration, based on the assumption that ovulation was induced 2 d after hCG treatment.

Transabdominal ultrasound examination after hCG treatment revealed cystic structures on both ovaries, with multiple endometrial cystic lesions persisting in the right uterine horn. Behavioral and cytologic signs of estrus persisted in this bitch for 7 d after hCG treatment, without evidence of ovulation or luteinization. Serum progesterone concentrations never

exceeded 0.5 ng/mL, indicating failure of ovulation and/or failure of progression to the luteal phase. As a primary ovarian abnormality was suspected, with a poor prognosis for future fertility, the owner consented to ovariohysterectomy and histopathologic evaluation of the reproductive tract.

2.4. Ovarian pathology

Ovariohysterectomy was performed in both cases and the ovaries and uterus were submitted for histopathology. The ovarian lesions were similar in both bitches. In Dog 1, both gonads were hypoplastic ovaries (Fig. 1 and 2). The cortex was thin, but subepithelial structures were present (Fig. 1B). Normal follicles were absent. Numerous prominent structures, interpreted as immature sex cords, were present (Fig. 1B and 1C). Some had differentiation towards seminiferous tubules, whereas others resembled follicles.

As in the first case, both ovaries of Dog 2 were hypoplastic with poorly differentiated sex cords and no evidence of primordial or mature follicles (Fig. 2). However, in one ovary there were some immature sex cords lined by granulosa cells, confirming partial follicular development. In addition, prominent hyperplasia of the rete epithelium and ectopic adrenal tissue were identified in the contralateral ovary.

2.5. Uterine pathology

In Dog 1, uterine glands were dilated and filled with inspissated proteinaceous debris. The glandular epithelium was tall and columnar with basal nuclei and long, apical cytoplasmic profiles. Small numbers of lymphocytes and plasma cells were scattered throughout the endometrial stroma, indicating minimal lymphocytic endometritis. In Dog 2, the uterine endometrium was thick and convoluted. On histopathology, endometrial glands were present in some areas of the myometrium. Inflammatory cells infiltrated the stratum compactum and the uterine lumen contained cellular debris and inflammatory cells, indicating subacute cystic endometrial hyperplasia, mild suppurative endometritis, and focal adenomyosis.

2.6. Karyotype

Heparinized blood was collected from both dogs for cytogenetic evaluation of short-term peripheral lymphocyte cultures, as previously described [12]. Fifty Giemsa-banded metaphase spreads from each dog were evaluated and all revealed an extra X chromosome constituting a 79,XXX karyotype (Fig. 3). A PCR assay of genomic DNA from Dog 2, according to the methods of Meyers-Wallen et al [13], was negative for the presence of the *SRY* gene (data not shown).

3. Discussion

Chromosomal aneuploidy results from chromosomal non-disjunction during meiosis or mitosis. Meiotic nondisjunction can result in an additional chromosome or loss of a chromosome in the oocyte or spermatocyte, resulting in trisomy or monosomy in the zygote, respectively [14].

Sex chromosome aneuploidy is one of the most commonly recognized chromosomal anomalies in humans. Of those, trisomy-X is the most common sex chromosome anomaly reported in women, and has been associated with a wide variety of phenotypic abnormalities, including reproductive anomalies, facial dysmorphism, earlier growth with longer legs, behavioral disorders, and mental retardation [1]. There are also reports of increased incidence of cardiovascular, skeletal, and nervous abnormalities, including kyphosis, facial asymmetry, and pulmonic artery stenosis [2,15]. Even though X-inactivation occurs in somatic cells, two active X chromosomes remain, which is a potential

cause of somatic abnormalities reported [31]. In contrast, for normal oogenesis to occur, both X chromosomes must be active during meiosis in oocytes [32]. The additional X chromosome in oocytes of trisomy-X patients likely interferes with meiosis, leading to early oocyte death, follicular degeneration, and failure to cycle [14]. These ovarian defects are the likely cause of reported reproductive anomalies, including primary amenorrhea, delayed puberty, premature menopause, and irregular menstrual cycles. However, some women with trisomy-X have been able to reproduce, especially with assisted reproductive techniques, and the majority of their children have had a normal karyotype [1]. It is possible that such patients are mosaic in the gonad (79,XXX/78,XX). Primary oocytes having trisomy-X and their surrounding follicles would undergo atresia. Other primary oocytes having a normal female chromosome constitution could undergo normal meiosis and their surrounding follicles could function to some extent. This could also explain the occurrence of estrous cycles in the two cases of this report.

Other species in which trisomy-X has been reported include the mouse [16], Murrah buffalo [17], mare [18–20], river buffalo [21,22], bovine heifer [23–27], macaque [28], and sheep [29]. The majority of these animals had cytogenetic investigations prompted by a history of infertility, failure to cycle, or infantile vulva [5]. Associated non-reproductive anomalies are reportedly rare in species others than humans. There is a report of two heifers with kyphosis and underdevelopment [5] and a dog with congenital absence of teeth [6].

In dogs, sex chromosome aneuploidy is rarely reported, as there are only two previous reports of trisomy-X presenting with infertility due to primary anestrus and one presenting with failure to conceive [4–6]. Most clinicians pursue cytogenetic analysis based on phenotypic anomalies or complaints of primary anestrus [30]. The two cases presented here had evidence of estrus cycles with infertility, revealing the value of karyotyping for fertility evaluation in this species. Furthermore, it is currently unknown whether dogs mirror humans in the frequency of reproductive anomalies associated with trisomy-X. In one case report of a mixed breed dog with trisomy-X, normal ovarian histology was identified [4]. Because exogenous hormone therapy was not attempted, it is not known whether this dog could have responded to assisted reproductive techniques. It is likely that there are dogs with this disorder that are capable of reproduction, as in women and other animals. One heifer diagnosed with trisomy-X was reported to deliver two phenotypically normal calves with normal karyotypes [23]. In addition, a Murrah buffalo with trisomy-X was reported to have two calves prior to diagnosis [17]. However, this hypothesis needs further investigation, especially considering the poor response to hormone therapy in Dog 2 of this report, and the possibility that reproductive success in other reports might be attributed to gonadal mosaicism (79,XXX/78,XX), as reported in two cats [30]. Further investigation will help to establish the prognosis for fertility based on cytogenetic analysis of dogs presenting for infertility.

Also noted, Dog 1 was shy and timid. Such behavior was also noted in a previous report of a mixed breed dog with trisomy-X [6]. Psychiatric and behavioral disorders are not uncommon in humans with trisomy-X. Auditory processing, language development, and impaired social skills have all been described [15]. In addition, behavioral anomalies, such as learning deficiencies, have been reported in macaques [28] and a cat [30]. It is difficult to determine whether behavioral anomalies noted in those reports are attributable to trisomy-X. However, since trisomy-X is often undetected in women, the relationship between chromosomal and behavioral anomalies in dogs may warrant further investigation.

In summary, the few cases of trisomy-X in dogs reported in the literature had a poor prognosis for future fertility and highlighted the importance of cytogenetic analysis in definitive diagnosis of fertility disorders.

Acknowledgments

These studies were supported in part by the National Institutes of Health grant #RR02512 and by the Baker Institute for Animal Health. The authors thank Anita Hesser for manuscript preparation.

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Fig. 1.

(Case 1) A. Photomicrographs of ovary and cross sections of the uterine tube (arrows) within the wall of the ovarian bursa. Both ovaries were small with relatively thin cortices (bilateral ovarian hypoplasia). B) Higher magnification of the cortex in section of ovary from 1A. Normal appearing subepithelial structures (SES; arrow) were present, but there is an absence of normal follicular structures. Poorly differentiated sex cords (arrowheads) were present deeper in the parenchyma. C) Higher magnification of immature sex cords, which varied from poorly differentiated follicle-like structures (large arrows) that did not contain oogonia or oocytes, to those resembling poorly differentiated seminiferous tubules (arrowhead).

Figure 2.

(Case 2) Photomicrograph of the cortex and superficial medulla of one ovary, with subepithelial structures and prominent cross sections of immature sex cords. The latter ranged in morphology from poorly differentiated follicles without oocytes (large arrow) to smaller structures that resembled poorly differentiated seminiferous tubules (arrowheads). Histologic features of the contralateral ovary were similar.

Fig. 3. Metaphase spread from peripheral blood lymphocyte culture (Dog 1) demonstrating a 79,XXX (trisomy X) karyotype. The X chromosomes were indicated (arrow).