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Case report

MORPHOLOGICAL AND IMAGING FEATURES OF MALE PSEUDOHERMAPHRODITISM IN A FERAL CAT

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A one-year-old European shorthair feral cat with signs of heat was presented at the Veterinary Teaching Hospital of Las Palmas de Gran Canaria University. After the physical exam, histology, hormonal analysis, ultrasound and computed tomography (CT) studies were performed. Examination of the external genitalia revealed the absence of one of the testes in the scrotal sac and the presence of a structure whose appearance could suggest an enlarged penis-like clitoris with small spines. The ultrasound study showed compatible images with the left ovary and intra-abdominal testis and tubular structures, which closely resembled the uterine horns. Similar structures were confirmed in the CT study. Exploratory celiotomy revealed the presence of the uterus and undescended testis attached to the uterine horn. Histological examination revealed immature testicular tissue in both gonads and the presence of Persistent Müllerian Duct (PMD). Though a karyotype was not performed, it was presumed to be the standard 38 XY found in pseudohermaphrodites. To date, this type of disorder of sexual development (DSD) has been scarcely reported in cats. Further studies are on the way to knowing the genetic mechanism of this disease.

Key words: disorders of sexual development; pseudohermaphroditism; ultrasound; computed tomography; cat.

INTRODUCTION

In mammals, sexual development begins after the establishment of chromosomal sex [1]. During fertilization, the constitution of chromosomal and genetic sex is established [2]. Early XX or XY zygotes are sexually undifferentiated and developed similarly until the beginning of the determination of gonadal sex. A wide variety of genes are involved in sex differentiation, such as the SRY gene (sex determinant region on the Y chromosome) and the Sox9 [3]. SRY is the only gene linked to Y that is necessary and

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sufficient to initiate testicular development [4]. The differentiation of internal ducts, accessory sexual organs, and external genitalia occurs in response to the presence or absence of two testicular hormones, testosterone and anti-Müllerian hormone (AMh), produced by Leydig cells and Sertoli cells, respectively [2]. In the absence of hormones, phenotypic development of the female reproductive tract occurs from the Müllerian ducts. In the presence of testosterone and AMh, the masculinization of the Wolff ducts and the atrophy of the Müllerian ducts occurs, giving rise to male phenotypic sexual development [3]. Nonetheless, alterations may occur during development, which takes place in Disorders of Sexual Development (DSD).

DSD is common and has been reported in numerous species, including the swine, goat, horse, cat, and dog [5-13]. Most DSD are incidental or of a minor nature and do not affect reproductive performance. Some are confused with serious disease during clinical examinations (e.g., detecting cysts in or near the ovary during ultrasound examination), routine surgery, or post-mortem examinations [14]. DSD result from abnormalities of sex chromosome origin, inappropriate hormone production, receptor upregulation, downregulation, or hormone exposure [10]. Therefore, a condition cannot be classified accurately due to the lack of knowledge of many causes that produce these abnormalities [2].

Four criteria are necessary for definitive sex identification: chromosomal composition, gonadal histology, reproductive tract morphology, and external genital appearance [15]. The intersex animal is an animal that possesses the characteristics of both sexes. Depending on their gonads, they are also called pseudohermaphrodites or hermaphrodites [16]. True hermaphrodite animals have gonadal tissue of both sexes, in any possible combination: testis with contralateral ovary, unilateral ovotestes with contralateral ovary or testis, and bilateral ovotestes [12]. In contrast, pseudohermaphrodites possess the gonads of one sex (male pseudohermaphrodites if they have testes, and female pseudohermaphrodites if they have ovaries). In addition, pseudohermaphrodite animals possess secondary sex characteristics and external genitalia of the opposite sex [16,17]. Several DSD, although rare, have been described in cats [18]. Therefore, a complete uterus was identified in a bilateral cryptorchid, phenotypically male cat [19], and a large clitoris, which was penis-like with small penile spines was described in a male pseudohermaphrodite cat [15]. To the author´s knowledge, only a few cases dealing with DSD diagnosis in cats using different diagnostic imaging techniques. Therefore, this study presents a case of male pseudohermaphroditism in a cat that underwent an ultrasound, computed tomography (CT), surgery, and hormonal analysis.

CASE PRESENTATION

A one-year-old male European feral cat coming from a rescue shelter with signs of heat was referred to the Veterinary Teaching Hospital of Las Palmas de Gran Canaria University (Canary Islands, Spain). The animal did not show any physical

abnormalities; however, in the perineal area, there was a structure whose appearance could suggest an enlarged penis-like clitoris with small penile spines. There was one testis in the scrotal sac, and at the level of the inguinal canal, we found another round structure, suggesting the presence of another testis. Hence, to better evaluate the cat, we conducted an ultrasound study prior to other imaging diagnostic techniques. We obtained permission from the caretakers to conduct the different imaging diagnostic techniques.

RESULTS AND DISCUSSION

Ultrasound examination was performed with a GE LOGIQ e Vet ultrasound (USA) using micro convex 8C and 12L linear transducers and this imaging technique allowed assessing different organs within the abdominal cavity. The abdominal hair was clipped, and the cat was placed in dorsal recumbency. This imaging technique allowed assessing different organs within the abdominal cavity. Thus, we could confirm that abdominal organs had a normal appearance, without congenital defects. Therefore, the liver and gallbladder showed size, morphology, and echogenicity within the normal range. The gastrointestinal tract, the stomach and intestine walls maintained their standard thickness and differentiation into layers. The pancreas and the spleen were ultrasonographically normal. The kidneys presented a standard size and morphology, respecting the cortical-medullary differentiation and echogenicity. The urinary bladder showed an average parietal thickness, and urine content, without cellular flocculation or small stones. The uterus was visualized as a cavitary, hypoechoic structure with a slightly hyperechoic lumen and a thin hyperechoic serosal rim. The left uterine horn was associated with a structure consistent with the left ovary or intra-abdominal testis (Fig. 1a and 1b). However, the right uterine horn was identified as coursing to the inguinal canal and attached to a structure that matched the right ovary. This horn showed the presence of a cyst that occluded the lumen.

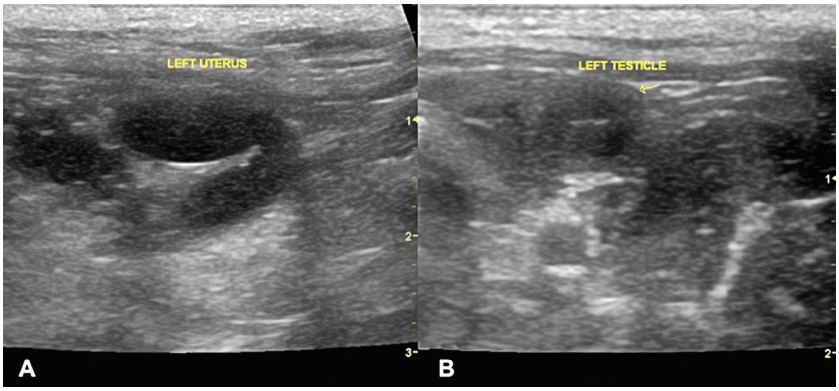

Figure 1. Ultrasound images of the left uterine horn **(A)** associated with a structure consistent with a left ovary or intra-abdominal testis **(B)**

Feline ovaries might not be visualized during a routine ultrasound examination procedure. However, in our case, as this cat was in heat, it became more visible because of a notable increase in size in its anatomic location immediately caudal and slightly lateral to the caudal pole of the kidney [20]. Interestingly, pseudohermaphrodite dogs often have accompanying urogenital tract disorders, such as ectopic ureter [21,22] or hypospadia [23], but the occurrence of this combination of conditions has been poorly described in cats [15,18]. To our knowledge, this is the first description of pseudohermaphroditism in cats by ultrasound technique.

To visualize the above-mentioned findings and associated structures, we conducted a CT study. Thus, the cat received a bolus of the contrast medium, iomeprol (61.24 gr/100ml), at a dose of 2 ml/kg manually injected via a jugular vein (receiving 5.5 ml in total) to better visualize the organs and associated structures. Helical CT was performed using a Toshiba Astelion 16-slice scanner (Japan) with the animal positioned in dorsal recumbency on the scanning table. The scanning parameters were as follows: 100 kVp, 60 mA, 512 X 512 matrix, 283 X 283 field of view, and a spiral pitch factor of 0.94. Transverse images (1 mm thick) were obtained and transferred to the DICOM workstation, where soft tissues window settings were applied to obtain optimal CT images.

Computed tomographic imaging description included soft tissue, bone, and pulmonary algorithm and pre and post contrast study. Two well defined large structures with thin walls and liquid attenuation content located in the left side of the caudal region of the abdomen and right inguinal canal are described. Also, in the transversal plane, one rounded structure with soft tissue attenuation is observed and located cranial to the left tubular structure (left uterine horn). On the other side, the right tubular structure with a caudal and subcutaneous trajectory finish in the cranial aspect of the right gonad located in the scrotum. These findings could also be observed with volume rendering and multiplanar reconstruction (MPR) techniques. (Fig. 2 and Fig. 3). When an elongated tubular structure is identified behind the urinary bladder in a male with normally virilized external genitalia, this rare syndrome can be diagnosed [23].

In human medicine, close imaging features have been observed in similar diseases, such as persistent Müllerian duct syndrome, a rare form of male pseudohermaphroditism [24]. However, pseudohermaphroditism has been poorly described in animals employing computed tomography or other imaging diagnostic techniques.

After the imaging study, an exploratory celiotomy was performed for a better assessment of the organs involved. The anesthesia protocol was the following. The cat was premedicated with dexmedetomidine (Dexdomitor ®, Orion corporation) 10 mcg/kg IM), methadone (Metasedin ®, Esteve Pharmaceuticals S.A.) 0,2 mg/kg IM and ketamine (Ketaset ®, Zoetis Spain, S.L.) 5 mg/kg IM. Anesthesia was induced with 4 mg/kg intravenous propofol (Propovet ®, Zoetis Spain S.L.) to allow intubation and maintained with sevoflurane (Sevoflo ® Zoetis Belgium S.A.) in 100% oxygen through a non-rebreathing circuit. During the anesthesia, we used mechanical

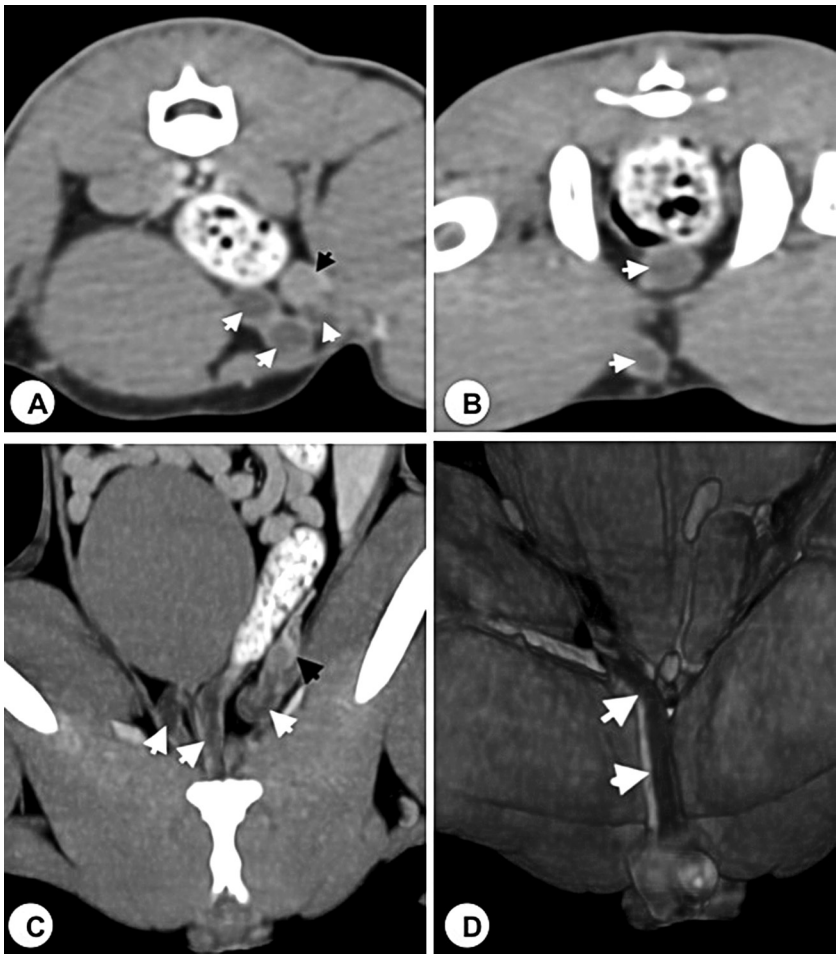

Figure 2 (A). Transversal images with soft tissue postcontrast algorithm in the caudal aspect of the abdomen. Four rounded structures located under the rectum and left to the urine bladder are shown. Three structures with thin walls and liquid attenuation in the center marked with white arrows correspond with the left uterine horns. The black arrow points a round structure with well defined and regular walls with light enhancement and homogeneous soft tissue attenuation. This structure corresponds with the left gonad (left testes). **(B)** Transversal images in soft tissue postcontrast algorithm at the level of the pelvis. Two rounded structures are shown. Both structures have thin and regular walls and liquid attenuation in the center aspect (both marked with white arrows). One located in the pelvis canal and ventral to the rectum corresponding with the uterine body. The second one -ventral white arrow- is located subcutaneously and coursing within the inguinal canal (correspond with the right uterus horn). **(C)** Multiplanar reconstruction (MPR) in dorsal plane with soft tissue postcontrast algorithm. Three tubular structures are visualized with thin and regular walls and liquid attenuation in the center aspect (all marked with white arrows). The tubular structures correspond with both uterine horns and uterine body. The black arrow point a round structure with well define and regular walls with light enhancement and homogeneous soft tissue attenuation. This structure corresponds with the left gonad (left testes). **(D)** MPR-dorsal plane in volume rendering. An enhanced postcontrast tubular structure located in the lateral aspect respect to the uterine horn coursing through the inguinal canal is observed. This structure corresponds with a venous vessel which irrigates the right gonad.

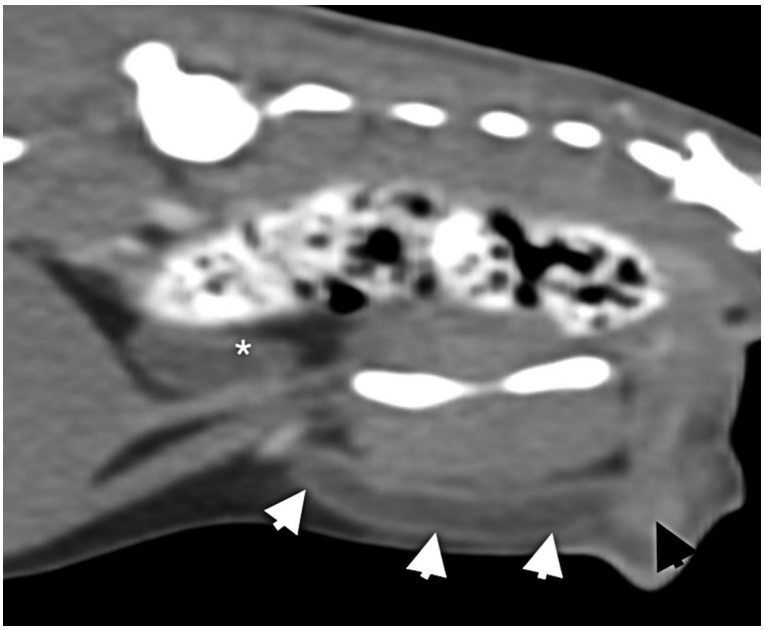

Figure 3. MPR in sagittal plane with soft tissue postcontrast algorithm. An inguinal tubular structure with thin and regular walls and liquid attenuation in the center aspect (all marked with white arrows) is described in the inguinal canal aspect with intrascrotal insertion in the cranial aspect of the right gonad (black arrow). Within the caudal aspect of the abdomen the tubular structure previously described correspond with the left uterine horn (white asterisk).

ventilation to improve intraoperative gas exchange. The cat was continuously monitored using an electrocardiogram, oscillometric non-invasive blood pressure, pulse oximetry, capnography, spirometry, an end-tidal fraction of anesthetic gases, and esophageal temperature. For this purpose, we used a multiparameter monitor (B125 General Electric Medical Systems Information technologies GmbH, Freiburg, Germany). During recovery a dose of meloxicam (Metacam ® Boehringer Ingelheim Vetmedica GMBH) for completing the analgesic protocol. Careful evaluation of the reproductive tract revealed a central tubular form that closely resembled the uterine body and cervix with their corresponding uterine horns (Fig. 4). The right uterine horn progressed until it entered the inguinal canal, ending in a gonadal structure. Nevertheless, the left uterine horn followed its ordinary course and ended equally in the gonadal structure. Therefore, the reproductive tract included uterus horns and body and ovary-like structures that showed some morphological features of testes. Similar findings have been described in other studies performed in other species such as bitch [14] or cat [19,15].

After the celiotomy, samples of the uterus and gonads were collected. All tissues were fixed in 10% buffered formalin, embedded in paraffin, sectioned (4 mm thick), and stained with hematoxylin and eosin for histological examination. Histologically, these gonadal structures did not include the ovarian bursa, being constituted by testicular

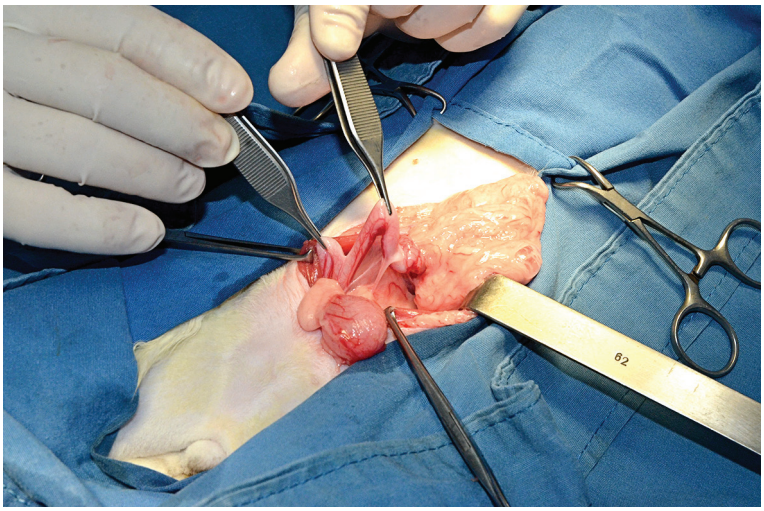

Figure 4. Intrasurgical image of the female reproductive tract. The uterine body and uterine horns are visualized.

parenchyma, with the presence of trabeculae within the organ. The lobules had hypoplastic seminiferous tubules of small diameter, covered by germ cells, mainly by Sertoli´s supporting cells (Fig. 5). Between the seminiferous tubules, there were abundant Leydig interstitial cells with acidophilic or intensely vacuolated cytoplasm. The subcapsular zones contained ducts lined by simple cylindrical-ciliated epithelium and immersed in a connective matrix (epididymis). In addition, the hilum of the organ appeared as tubular structures with planar or cubic epithelium (rete testis). These tubular elements could be derived from the ductal mesonephric and paramesonephric embryonic structures, represented by uterine horns and vas deferens, respectively. The

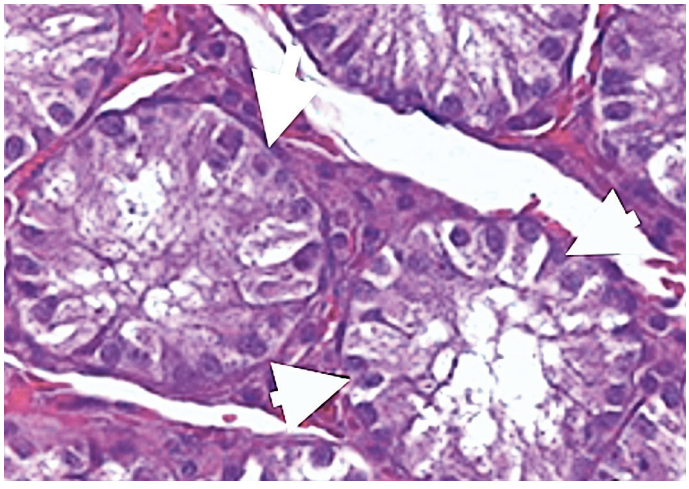

Figure 5. Histology of the abnormal gonads. They were composed by hypoplastic seminiferous tubules of small diameter and covered mainly by Sertoli cells (H&E x 10)

tubular structures were covered by a simple cuboidal epithelium on a basal lamina surrounded by smooth muscle fibers and peripherally, by a ligament (mesometrium) of connective and adipose tissue with large and dilated blood vessels. These findings are similar to those works on pseudohermaphroditism reported in dogs and cats [25] and monkeys [10]. Nevertheless, significant differences were observed in true hermaphroditism where the gonads were identified as ovotestes due to these were composed by testicular portion in the inner and a smaller ovarian portion forming a thin cortical rim [26].

Blood samples were collected for hormonal analysis. In addition, part of the reproductive tract was stored at -80ºC for further genetic studies.

The hormone analysis revealed that serum testosterone concentration was similar to that expected for a cryptorchid male cat (17.9 ng/dl; reference ranges, 10 to 50 ng/dl for cryptorchid males, >100 ng/dl for sexually intact males, and <10 ng/dl for neutered males). Serum estradiol concentration was within reference limits for a spayed female cat (<20 pg/ml; reference range, <20 pg/ml for spayed females, 25 to 30 pg/ml for metaestrous females, 30 to 70 pg/ml for estrous females, and 10 to 20 pg/ml for anoestrous females) [27-29]. The low testosterone levels observed in our cat could contribute to inadequate inhibition of the female reproductive tract [29]. Nonetheless, levels of the anti-Müllerian hormone should be performed to further evaluate the hormone influence.

Although several types of DSD have been reported in the cat and dog, which are often strikingly similar to human DSD, these have been infrequently utilized to contribute to our knowledge of mammalian sexual development.

In conclusion, the features observed in our study suggested the diagnosis of pseudohermaphroditism. Though a karyotype was not performed, it was presumed to be the standard 38 XY commonly found in pseudohermaphrodites. Further studies are on the way in order to better understand the genetic mechanism of the disease.

Authors' contributions

JRJ, ME, AC and FSC designed the study. Imaging study was conducted by JRJ. ME, JAC, AC and FSC. Exploratory celiotomy was performed by MM. FR did the histological study. The manuscript was written by JRJ, and reviewed by JAC and CM. All authors contributed to the improvement of discussion and reviewed the final manuscript for its intellectual content. All authors read and approved the final manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Statement of Informed Consent

The owner understood procedure and agrees that results related to investigation or treatment of their companion animals, could be published in Scientific Journal Acta Veterinaria-Beograd.

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MORFOLOŠKE I IMINDŽING KARAKTERISTIKE PSEUDOHERMAFRODITIZMA MUŽJAKA DIVLJE MAČKE

Francisco SUÁREZ-CABRERA, Mario ENCINOSO, Adrian CARABALLO, Manuel MORALES, Juan Alberto CORBERA, Francisco RODRÍGUEZ-GUISADO, Carlos MELIÁN, Jose Raduan JABER

U ambulantu klinike na fakultetu veterinarske medicine Las Palmas de Gran Canaria Univerziteta, primljena je kratkodlaka divlja mačka sa znacima estrusa. Posle fizičkog pregleda, obavljena su histološka ispitivanja, analiza hormona, ultrazvuk i kompjuterska tomografija (CT). Eksternim pregledom genitalija uočeno je odsustvo jednog testisa u skrotumu kao i prisustvo strukture koja je mogla da ukazuje na postojanje

uvećanog klitorisa nalik na penis sa sitnim bodljama. Ultrazvučnim pregledom uočeni su slični nalazi uz levi ovarijum i intra-abdominalni testis i tubularne strukture koje su podsećale na rogove uterusa. Prisustvo sličnih struktura je potvrđeno i CT testom. Eksplorativna celiotomija ukazala je na prisustvo uterusa i nespuštenog testisa koji je bio prirastao za rog uterusa. Histološkim ispitivanjem ustanovljeno je prisustvo nezrelog testikularnog tkiva u obe gonade kao i prisustvo perzistentnog Müllerian-ovog duktusa. Uprkos tome što nije obavljeno ispitivanje kariograma, predpostavljeno je da se radi o standardnoj dijagnozi nalaza koji je karakterističan za 38 XY pseudohermafrodite. Do danas ovaj tip poremećaja seksualnog razvoja, nije bio često opisivan kod mačaka. Sprovode se dalja istraživanjasa ciljem upoznavanja genetskih mehanizama nastanka ovog poremećaja.