Topics in Companion An Med 45 (2021) 100554

Case of Anorchia in a Mixed-Breed Dog

Vincenzo Cicirelli[a,*], Giulio G Aiudi[b], Stefano Carbonara[b], Michele Caira[a], Giovani M Lacalandra[b]

Keyword:
anorchia
cryptorchidism
dog
testosterone
monorchidism

[a]*Department of Veterinary Medicine, University of Bari "Aldo Moro", Bari, Italy*

[b]*IAMB Mediterranean Agronomic Institute of Bari, Bari, Italy*

A B S T R A C T

Anorchia is a rare and often poorly understood disorder of sexual development. In the present case report, we used a multidisciplinary approach to diagnose a case of anorchia in a 30-month old dog. The diagnostic process began with gathering the dog medical history followed by a clinical visit with the patient, which included a general wellness examination as well as an examination of the genital system. As suggested in the relevant literature, the dog underwent an ultrasound and then computed tomography (CT) of the genital system, which confirmed the diagnosis of testicular agenesis. Genetic testing confirmed the male XY karyotype. Hormone testing also supported the diagnosis: testosterone and anti-Müllerian hormone levels were below their reference ranges, and luteinizing hormone (LH) was above 1 ng/mL. Following the diagnostic procedures suggested in the relevant literature, the present study confirms anorchia in the dog and describes a case of testicular agenesis in the canine species.

Introduction

Of the various pathologies affecting sexual development in mammals, cryptorchidism is the most widespread.[1-3] Cryptorchidism is characterized by the failure of one (unilateral) or both (bilateral) testes to descend from the abdominal cavity into the scrotum.[4] In veterinary medicine, anomalies of the reproductive system can be the result of numeros genetic alterations. Consequently, an accurate diagnosis is not at all simple or rapid, and often requires very complex investigations, because the phenomenon of sexual ambiguity has only been thoroughly investigated in the last three decades.[5] This pathology affects several mammals, including swine[6] equine,[7] feline,[8] and canine species.[9] In dogs, the prevalence of cryptorchidism has been reported to be 1%-2%, and dog breeds such as Chihuahua, German Spitz, Miniature Schnauzer, Pekinese, Maltese, Shetland Shepherd, and Cairn terrier appear to be the breeds most adversely affected.[8] There are numeros significant repercussions of cryptorchidism on reproduction, which may involve a series of notable spermatic anomalies[10] and alterations of the testicle in the abdomen, which can degenerate into neoplasia.[11] Monorchidism, the congenital absence of a single testicle in the scrotum, appears to be less common than cryptorchidism.[1] Congenital anorchia, the complete absence of testicular tissue, has rarely been described in veterinary literature.[7,12] The absence of both gonads is often related to other sexual abnormalities,[13] such as hypospadias[14] and micropenis.[15] The association between testicular dysgenesis and hypospadias is known as testicular dysgenesis syndrome.[16] Previous reports have described a diagnostic methodology for gonad developmental disorders in dogs,[13] but to the best of the authors' knowledge, this is the first study to provide a complete and appropriate diagnosis of anorchia in dogs. To implement an accurate diagnostic procedure, the authors referred to relevant literature from human medicine[17-19] to reach an accurate correct diagnosis based on both clinical criteria and instrumental tests.

Case History

After the veterinary team had recorded the patient's medical history, a general wellness examination and a targeted examination of the genital organs were performed.[20] For clinical completeness, sterile blood collection (5 mL) was performed, which required the use of sodium heparin as an anticoagulant. The sample was shipped to the laboratory (at a temperature of 4°C) within 4 hours, in order to maintain viability of the specimen. General blood tests (blood count, biochemistry, and electrophoresis of plasma proteins) were performed. Hormonal analyses were also performed, including: testosterone (Kit ELISA Demeditec), follicle stimulating hormone (FSH), luteinizing hormone (LH) (Kit ELISA ABNOVA), anti-Müllerian hormone (AMH) (IDEXX Laboratories), thyroid hormones (IDEXX Laboratories), basal cortisol concentrations (IDEXX laboratories), karyotypes X and Y (IDEXX Laboratories), and basic urinalysis (IDEXX Laboratories).[28] Additionally, imaging tests were performed using an Esaote MyLab Alpha device and computed tomography (CT). This advanced diagnostic examination was carried out on a multi-slice CT scanner (Sensation 16) with iodine contrast medium (370 mg/mL) at a dosage of 800 mg iodine/kg. The following anaesthesia protocol was used: premedication with butorphanol (Dolorex, MSD Animal Health, 0.1 mg/kg), induction with propofol (Propovet, Ecuphar Italia, 0.33 mL/kg), and maintenance with isoflurane (Isoflo, Zoetis Italia, 2.5%-3%).

Results

The patient, an obese (37 kg), large mixed-breed male dog, was approximately 30 months old at the time of examination (Fig 1). The owner, who had received the dog as a puppy (at about 2 months of age), stated that the animal had never undergone an orchiectomy or any other type of surgery. This was supported by the fact that the animal had no scars in the genital area which might be attributable to any type of castration. The subject's most important symptoms were a reluctance to move, ataxia, claudicated gait, painless hypotrophic hind limbs, and poor resistance to physical stress. His state of hydration was normal, as were his body temperature and heart rate. His dulled sensory state was ascertainable in his poor sensory system and orientation functions. The subject's skin showed no alterations

*Corresponding author:
E-mail address: vin.cicirelli@libero.it (V. Cicirelli).

http://dx.doi.org/10.1016/j.tcam.2021.100554
1938-9736/

Fig. 1. The patient with the physical malformations described. Color version of figure is available online.

attributable to pathologies of the integumentary system; in fact, his coat was in good condition. The patient also presented with a large neoplasia in the neck region, identified immediately by cytological examination as a lipoma, and therefore unimportant from a diagnostic and scientific standpoint. The patient's penis was very small for his size and age, although it showed a normal preputial sheath. There were no signs of trauma, foreign bodies, or adhesion phenomena attributable to inflammatory events. The mucous membrane of the penis was painless on palpation, smooth, and of a suitable pinkish-white colour. The scrotal sac was absent, and the testicles were also not detected on palpation, either in the inguinal or abdominal areas. The prostate gland was also not detected on palpation.

The veterinary team decided to proceed with biochemical blood (from the cephalic vein), urine, and ultrasound examinations. Ultrasound failed to detect the presence of testicles, either in the abdominal cavity or in the inguinal canal. Additionally, the prostate appeared to be significantly smaller than expected, with a diameter of 3.4 cm (Fig 2). The ultrasound image showed a much smaller than expected penis, at 2.8 cm long (Fig 3). Ultrasonography of the abdominal organs showed hyperechoic parenchyma and the presence of a cystic formation in the left kidney (Fig 4). The ultrasound images of the right kidney showed a hyperechoic parenchyma with two cystic formations located on the right and left sides in an almost specular fashion, with the largest measuring approximately 11 cm (Fig 5). Both kidneys showed an uneven appearance. The blood count showed mild neutrophilia and slight hypochromic macrocytic anaemia. The biochemical profile values were normal, except for the following: creatinine phosphokinase (CPK) of 240 IU/L (normal range, 30-160); amylase of 1783 IU/L (normal range, 487-1181); creatinine of 1.51 IU/L (normal range, 0.70-1.40); and total iron (Fe) of 66 μg/dL (normal range 90-230). These abnormal values are not regarded as significant. Urinalysis performed from spontaneous urination revealed no significant abnormalities (specific gravity dog urine was 1033). The patient's testosterone was $<$ 20 ng/dL, much lower than the range of basal values (80-2560 ng/mL). A testosterone stimulation test was carried out with an intramuscular (IM) injection of 250 IU of human chorionic gonadotropin (hCG), and the testosterone level was unchanged ($<$ 20 ng/dL) 1 hour after the stimulation test. The patient's LH level was 1.2 ng/mL, and FSH was 3 IU/mL. Additionally, further hormone tests revealed low AMH levels (0.3). The endocrine profile of free thyroxine (fT4) was 7.2 pmol/L (normal range, 16-45.5) and thyroid stimulating hormone (TSH) was 0.45 ng/mL (normal range, 0.03-0.40). Haematological tests (basal cortisol concentrations were 3.6 μg/dL) and imaging tests excluded other hormonal diseases, such as hypoadrenocorticism.

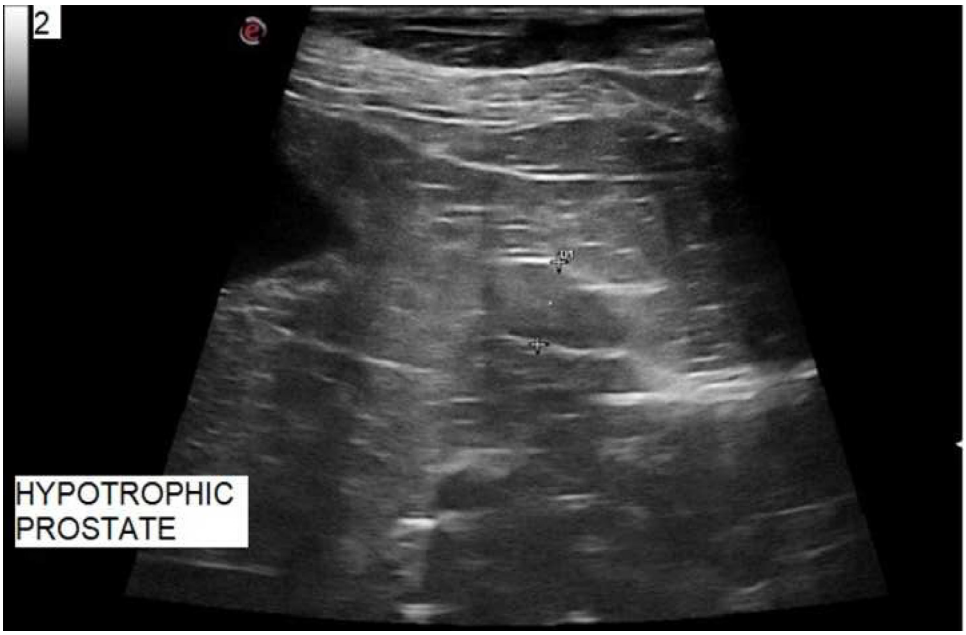

Fig. 2. Ultrasound image of hypotrophic prostate (diameter of 3, 4 cm). Color version of figure is available online.

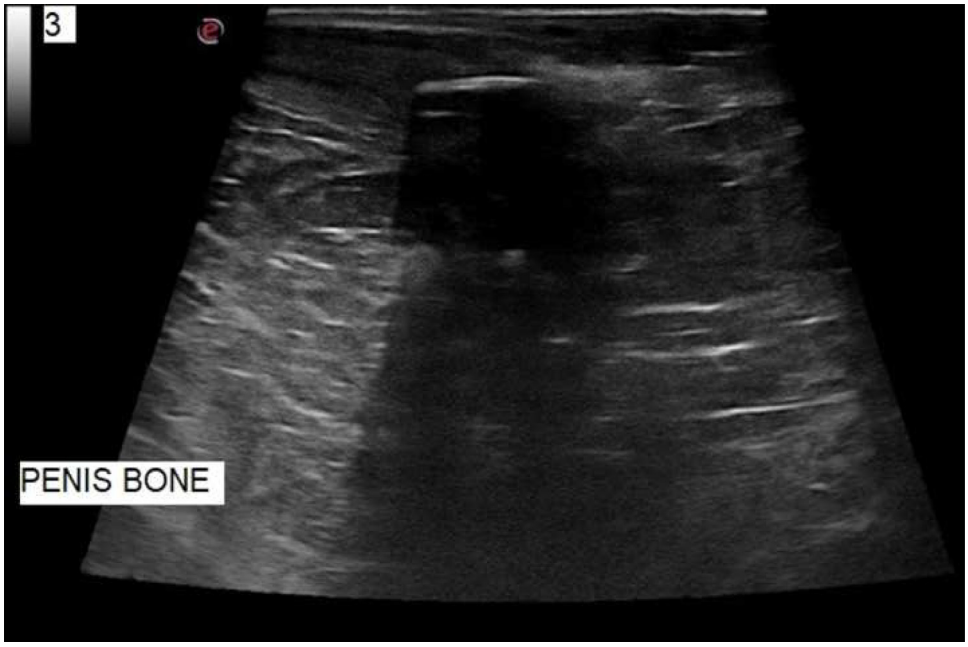

Fig. 3. Ultrasound image of the penis bone (2.8 cm). Color version of figure is available online.

Fig. 4. Ultrasound image of left kidney characterized by hyperechoic parenchymatous structure and the presence of an evident cystic formation. Color version of figure is available online.

Considering the results of the various investigations carried out by the team, which suggested the suspicion of hypoplasia or testicular agenesis, an in agreement with the owner, both blood samples were sent to the laboratory for genetic examination. The owner also agreed to allow the dog to undergo a CT scan, which was performed with the help of a team of anaesthetists to exclude the risk of problems under anaesthesia. The CT scan showed a slightly undersized liver with normal intra- and extra-hepatic circulation and the absence of focal or diffuse lesions seen with contrast medium. Gastroduodenal enlargement was evident due to the presence of food material. Furthermore, the images show splenomegaly, although the structure of the spleen was within normal limits and no focal or diffuse parenchymal lesions were present. Both kidneys were slightly reduced in size, with the loss of normal cortico-medullary differentiation and irregular profiles due to the presence of multiple cortical subcapsular lesions (the largest measuring approximately 11.2 cm) protruding through the margins, hypodense (9 UH) and non-captive to the contrast medium. The abnormalities visualised were compatible with renal dysplasia. There was moderate dilation of the renal pelvis and both ureters. The images of the reproductive system highlighted significant prostatic hypoplasia, with identification of the organ based on the anatomical location in the absence of differentiation of the lobes. With the exception of the prostate and penis, there was no parenchyma compatible with the testicles, either in the abdominal cavity or in the extra-abdominal space. Extremely small in size, more evident on the left than

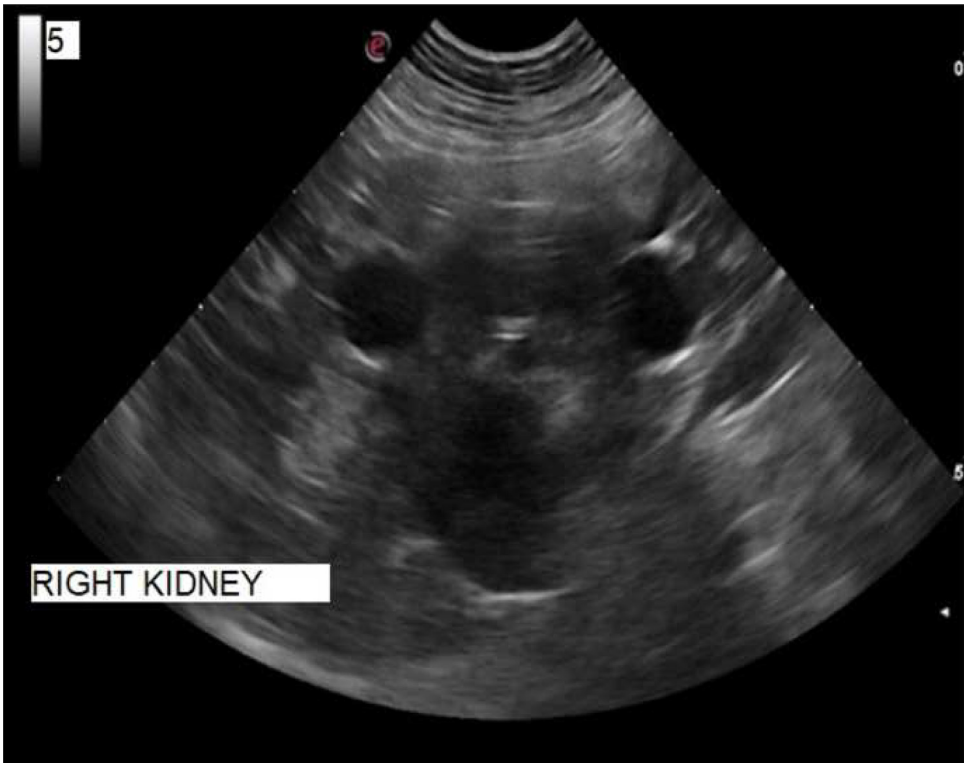

Fig. 5. Ultrasound image of the right kidney shows a hyperechoic parenchymatous structure with two evident cystic formations arranged on the right and left in an almost. Color version of figure is available online.

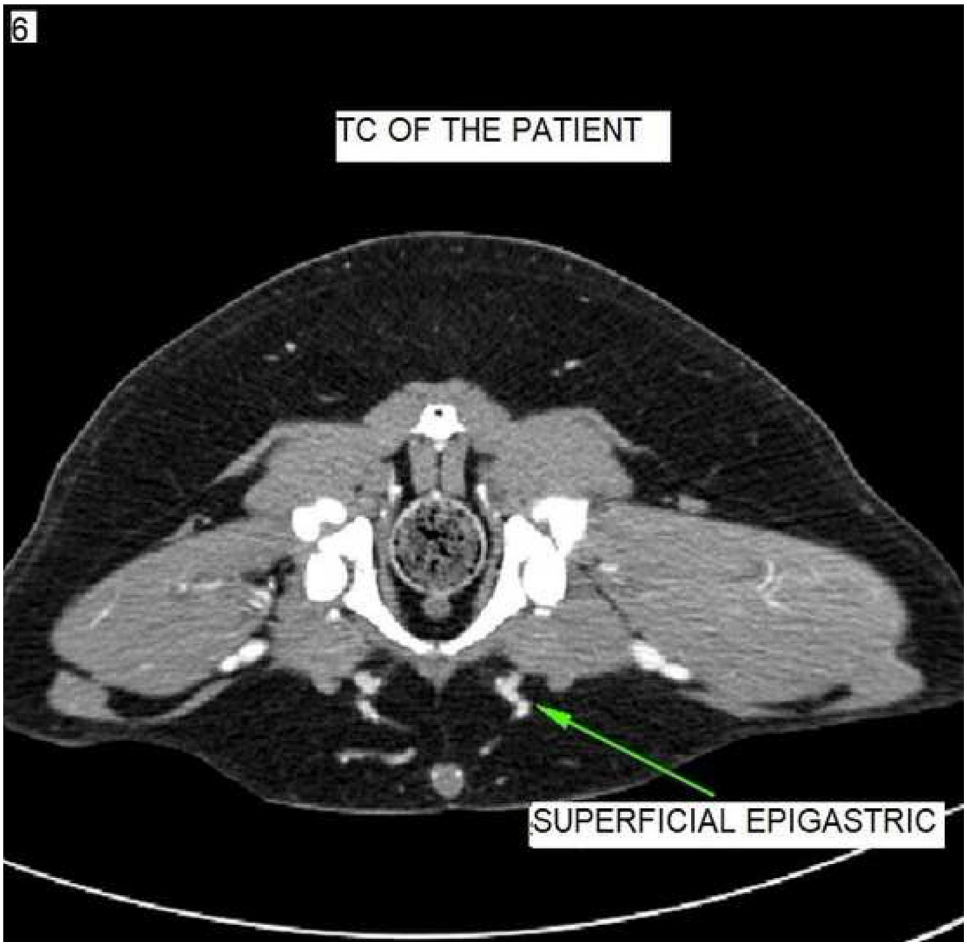

Fig. 6. No testicular compatible structures are visible in the abdominal cavity. Near the superficial epigastric, two small vessels are visible on each side, sketches of spermatic. Color version of figure is available online.

the right, were two vessels likely referable to the spermatic arteries, which did not leave the ventral part of the abdomen. Additionally, the spermatic veins were not visible. By comparing the CT images of the patient (Fig 6) with those of a healthy individual (Fig 7), an understanding of the patient's pathology is evident.

Discussion

The patient in the present study had an XY karyotype and was therefore genetically male,[21,22] and also had a hypotrophic prostate, as confirmed by both ultrasound and computed tomography. The penis had a penis length of 2.8 cm, qualifying it as a micropenis,[23] and both ultrasound and CT imaging showed abnormal kidney morphology. Both kidneys present irregular border and had hyperechoic parenchymal texture, with the left kidney showing the presence of an apparent cystic formation, while the right kidney had two, located in a roughly specular fashion, the largest measuring approximately 11 cm. Most authors agree that polycystic kidney disease (PKD) is a congenital hereditary disease,[24] characterized by multiple cysts of various sizes present in both kidneys.[25] As of yet there have been no studies on the association of PKD with sexual disorders. The diagnostic process in the present study involved hormonal investigations subsequent to diagnostic imaging. The patient's testosterone was very low (12 ng/dL), even after a testosterone stimulation test with hCG, therefore confirming testicular agenesis in the patient. Testosterone produced by the testicles is responsible for the descent of the testicles into the scrotal cavity, although non-descent has unclear and doubtless multifactorial origins.[4] Even though there is no data on the patient's siblings or mother, the patient most likely underwent processes prior to birth which caused the anorchia.

The patient's LH, the pituitary gonadotropin that induces testosterone production in male Leydig cells and is useful in determining the presence of gonads in the patient, was 1.2 ng/mL. In an animal without gonads, as there is no feedback to the hypothalamus, gonadotropins are constantly released. This condition is detectable in dogs by a constant secretion of LH $>$ 1 ng/mL.[26] Hormonal analysis showed that the patient's AMH, which is an important regulator of gonadal function and is a specific gonadal product that can be determined in circulation, was low. An AMH test was performed on the dog's blood as a diagnostic tool to determine the presence of functional canine gonadal tissue,[27] and the extremely low levels confirmed the absence of functioning testicular tissue. This hormone has several diagnostic uses in males, as it is used to distinguish cases of anorchia from cases in which the testicles fail to descend into the scrotum by the age of 6 months[28] and are therefore located either in the abdomen or in the inguinal canal. In the latter, cryptorchidism predisposes the animal to testicular neoplasm; therefore, the undescended testicles must be surgically removed in order to preserve the animal's health.[28] In this regard, the evaluation of AMH is useful when diagnosing animals that have no testicles in the scrotal pouch and to diagnose incomplete castration with a remaining intra-abdominal testis. AMH is also useful for distinguishing cryptorchids and anorchids from previously castrated patients.[28,29]

The haematological tests performed in the present study also included a thyroid profile, due to the patient's clinical and physical symptoms, which are typical with hypothyroidism. The results of these tests showed lower fT4 and slightly higher TSH levels than

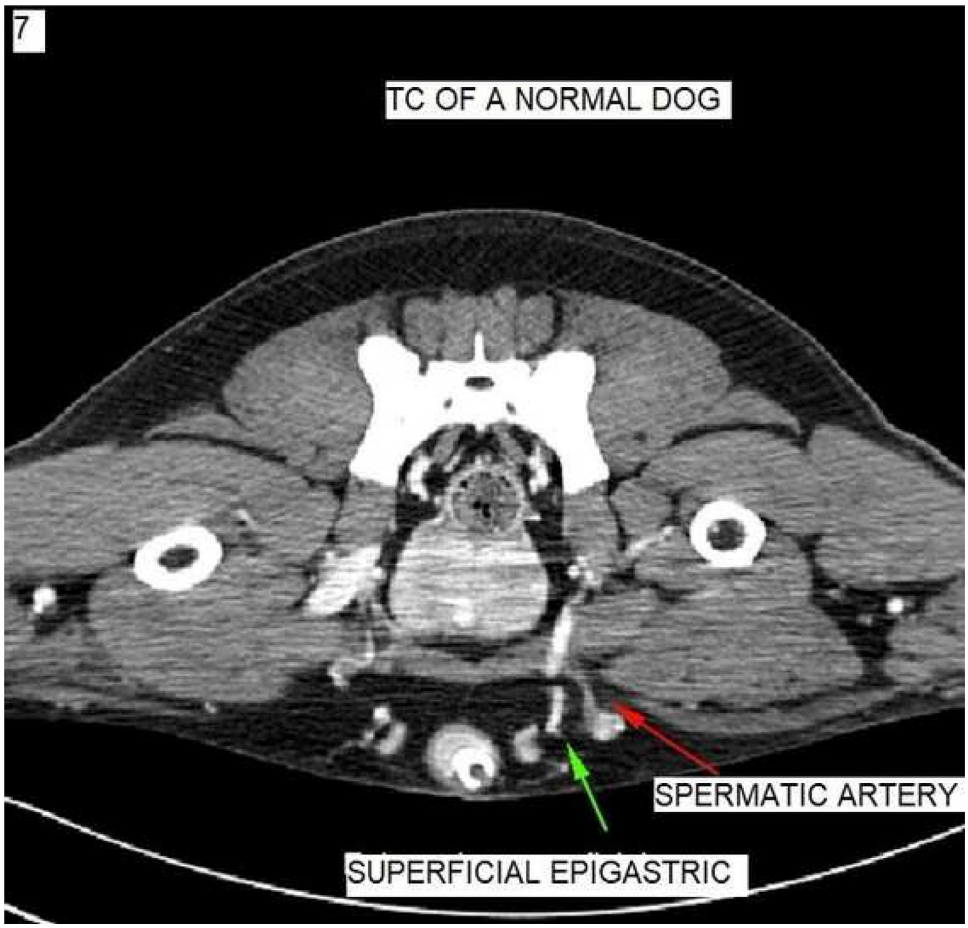

Fig. 7. Tc of a normal dog with clearly visible epigastric, and spermatic artery that descends into the abdominal cavity and irrigates the corresponding testicle. Color version of figure is available online.

expected, suggesting the diagnosis of hypothyroidism. Spayed and neutered animals have a higher relative risk of developing hypothyroidism than those that are sexually intact,[29] and a sterilized dog has a hormonal profile very similar to that of an anorchic animal, indicating an increased risk of hypothyroidism. The most significant clinical symptoms that indicate hypothyroidism are obesity (41%), seborrhoea (39%), weakness (21%), and lethargy (20%).[30] The dog in the present case had many of the clinical symptoms reported by Panciera,[29] especially those related to weakness and lethargy. The thyroid is closely connected to the reproductive system in animals and although thyroid and reproductive pathologies are often related,[31] resolution of one is not always beneficial to the other.[28]

Author Contributions

Data curation, G. M. L., S. C.; Investigation, V.C.; Methodology, G. G. A.; Software, M.C.; Supervision, G. M. L.; Writing – original draft, V. C.

Funding

This research received no external funding.

Data Availability Statement

The data presented in this study are available on request from the corresponding author.

Declaration of Competing Interest

The authors declare no conflict of interest.

References

1. Lyle SK. Disorders of sexual development in the dog and cat. *Theriogenology.* **68:**338–343, 2007
2. Burke TJ. *Small animal reproduction and infertility*. Causes of infertility. Lea & Febiger; 1986. p. 233–235
3. Mickelsen DW, Memon MA. In: Stephen J. Ettinguer, Edward Feldman. Small Animal Reproduction and Infertility: A Clinical Approach to Diagnosis and Treatment. *Philadelphia*. 1689p.
4. Khan AF, Gartley CJ, Khanam A. Canine cryptorchidism: An update. *Reprod Domest Anim* **53:**1263–1270, 2018
5. Meyers-Wallen DF. Patterson Disorders of sexual development in the dog. In: Morrow DA, editor. *Current therapy in theriogenology*, 2nd ed., Philadelphia: W.B. Saunders Co.; 1986. p. 567–574
6. Scollo A, Martelli P, Borri E, Mazzoni C. Pig surgery: cryptorchidectomy using an inguinal approach. *Vet Rec* **178:**609, 2016
7. Searle D, Dart AJ, Dart CM, Hodgson DR. Equine castration: review of anatomy, approaches, techniques and complications in normal, cryptorchid and monorchid horses. *Aust Vet J* **77:**428–434, 1999
8. Wolff A. Castration, cryptorchidism, and cryptorchidectomy in dogs and cats. *Vet Med Small Anim Clin* **76:**1739–1741, 1981
9. Kirby FD. A technique for castrating the cryptorchid dog or cat. *Vet Med Small Anim Clin* **75:**632, 1980
10. Pinart E, Camps R, Briz MD, Bonet S, Egoscue J. Unilateral spontaneous abdominal cryptorchidism: structural and ultrastructural study of sperm morphology. *Anim Reprod Sci* **49:**247–267, 1997
11. Amann RP, Veeramachaneni DNR. Testicular dysgenesis in animals: Cryptorchidism. *Anim Reprod* **3:**108–120, 2006
12. Lyle SK. Disorders of sexual development in the dog and cat. *Theriogenology* **68:**338–343, 2007
13. Rezaei M, Azizi S, Akhtardanesh B, Azari O, Hassibi H, Bojd T. Hypospadias and testicular agenesis in two German Shepherd puppies. *Iranian Journal of Veterinary Surgery* **11:**, 2016. Serial No:24
14. Cassata R, Iannuzzi A, Parma P, et al. Clinical, cytogenetic and molecular evaluationin a dog with bilateral cryptorchidism and hypospadias. *Cytogen Genome Res* **120:**140–143, 2008
15. Hatipoğlu N, Kurtoğlu S. Micropenis: Etiology, diagnosis and treatment approaches. *J Clin Res Pediatr Endocrinol* **5:**217–223, 2013
16. Toppari J, Virtanen HE, Main KM, Skakkebaek NE. Cryptorchidism and hypospadias as a sign of testicular dysgenesis syndrome (TDS): Environmental Connection. *Birth Defects Research (Part A)* **88:**910–919, 2010
17. Morgentaler A, Zitzmann M, Traish AM, et al. Fundamental concepts regarding testosterone deficiency and treatment: International Expert Consensus Resolutions. *Mayo Clin Proc* **91:**881–896, 2016
18. Harrington J, Palmert MR. Clinical review: Distinguishing constitutional delay of growth and puberty from isolated hypogonadotropic hypogonadism: Critical appraisal of available diagnostic tests. *J Clin Endocrinol Metab.* **97:**3056–3067, 2012
19. Aversa A, Morgentaler A. The practical management of testosterone deficiency in men. *Nat Rev Urol.* **12:**641–650, 2015
20. Cicirelli V, Debidda P, Maggio N, Caira M, Lacalandra GM, Aiudi GG. Ultrasound-guided funicular block: Ropivacaine injection into the tissue around the spermatic cord to improve analgesia during orchiectomy in dogs. *Animals* **11:**1275, 2021
21. Switonski M, Payan-Carreira R, Bartz M, et al. Hypospadias in a Male (78,XY; SRY-Positive) dog and sex reversal female (78,XX; SRY-Negative) Dogs: Clinical, histological and genetic studies. *Sex Dev* **6:**128–134, 2012. 8
22. King RC, Stansfield WD, Mulligan PK. *A dictionary of genetics*. 7th ed. Oxford University Press; 2006
23. Blyth H, Ockenden BG. Polycystic diseases of kidney and liver presenting in childhood. *In J Med Genet* **8:**257–284, 1971
24. Appleman E, Bernet A. Polycystic kidney disease. *Emerg Crit Care Med* **8:**7–10, 2006
25. Bay K, Main KM, Toppari J, Skakkebæk NE. Testicular descent: INSL3, testosterone, genes and the intrauterine milieu. *Nat Rev Urol* **8:**187–196, 2011
26. Rohlertz M, Strom Holst B, Axnèr E. Comparison of the GnRH-stimulation test and a semiquantitative quick test for LH to diagnose presence of ovaries in the female domestic cat. *Theriogenology* **78:**1901–1906, 2012
27. Themmen APL, Kalra B, Visser JA, et al. The use of anti-Müllerian hormone as diagnostic for gonadectomy status in dogs. *Theriogenology* **86:**1467–1474, 2016. Pages
28. Thomas R, Reid RL. Thyroid disease and reproductive dysfunction: A review. *Obstet Gynecol* **70**–**75:**789–797, 1987
29. England GC, Allen WE, Porter DJ. Evaluation of the testosterone response to hCG and the identification of a presumed anorchid dog. *J Small Anim Pract* **30:**441–443, 1989
30. Panciera DL. Hypothyroidism in dogs: 66 cases (1987-1992). *J Am Vet Med Assoc* **204:**761–767, 1994. 1
31. Johnson C, Olivier NB, Nachreiner R, Mullaney T. Effect of 131 I-Induced Hypothyroidism on Indices of Reproductive Function in Adult Male Dogs. *J Veter Inter Med* **13:**104–110, 1999