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Male pseudohermaphroditism in a dog: case report

Pseudohermafroditismo masculino em cão: relato de caso

Joselma da Silva Cavalcanti Bertoldo[1], Apolônio Gomes Ribeiro*[2], Liana Mesquita Vilela[1], Paola Teles Soares[1], Maria Juliana Coelho Dias da Silva Teixeira[1], Júlio Cézar dos Santos Nascimento[1]

ABSTRACT: Genital development disorders or anomalies have been described in several species of domestic animals. Sexual differentiation in mammals occurs sequentially and chronologically with the following distinct events in embryonic and faetal development: the determination of chromosomal sex at fertilisation, the development of gonadal sex and, finally, the development of the phenotypic sex in the foetus. Any errors in these processes lead to some form of intersexuality. In the literature, three types of intersexuality in companion animals are described: true hermaphroditism and male and female pseudohermaphroditism. The diagnosis is based on physical and imaging examinations, with confirmation by histopathological analysis and karyotyping. As there are few reports of this sexual anomaly, this case report addresses a case of male pseudohermaphroditism in a Shiba Inu canine. Clinical examination revealed the presence of a hypertrophied clitoris in the vaginal canal, without obstruction of the urethral canal. An abdominal ultrasound examination revealed splenomegaly, signs suggestive of chronic cystitis associated with bladder and urethral microuroliths, as well as a small tubular structure without cranial continuity. A blood count, serum biochemistry and the determination of the hormonal levels were performed pre- and post-surgery. Surgical treatment was instituted through exploratory laparotomy, revealing the presence of testicles in the abdominal cavity, a uterus, a cervix and uterine horns. The findings were sent for histopathological analysis, where diffuse testicular hypoplasia and endometrial hyperplasia, associated with neutrophilic and lymphoplasmacytic metritis, were evaluated. As these findings are associated with anamnesis, it is concluded that this case is a male pseudohermaphrodite.

KEYWORDS: Sexual Anomaly, intersex, gene mutation, ovotestis, sexual reversion.

RESUMO: Distúrbios ou anomalias do desenvolvimento genital foram descritos em diversas espécies de animais domésticos. A diferenciação sexual em mamíferos ocorre sequencial e cronologicamente com os seguintes eventos distintos no desenvolvimento embrionário e fetal: a determinação do sexo cromossômico na fertilização, o desenvolvimento do sexo gonadal e, finalmente, o desenvolvimento do sexo fenotípico no feto. Quaisquer erros nesses processos levam a alguma forma de intersexualidade. Na literatura são descritos três tipos de intersexualidade em animais de companhia: hermafroditismo verdadeiro e pseudo-hermafroditismo masculino e feminino. O diagnóstico é baseado em exames físicos e de imagem, com confirmação por análise histopatológica e cariótipo. Como existem poucos relatos desta anomalia sexual, este relato de caso aborda um caso de pseudo-hermafroditismo masculino em um canino Shiba Inu. O exame clínico revelou presença de clitóris hipertrofiado no canal vaginal, sem obstrução do canal uretral. A ultrassonografia abdominal revelou esplenomegalia, sinais sugestivos de cistite crônica associada a microurólitos vesicais e uretrais, além de pequena estrutura tubular sem continuidade craniana. Foram realizados hemograma, bioquímica sérica e determinação dos níveis hormonais pré e pós-operatório. O tratamento cirúrgico foi instituído através de laparotomia exploradora, revelando a presença de testículos na cavidade abdominal, útero, colo uterino e cornos uterinos. Os achados foram enviados para análise histopatológica, onde foram avaliadas hipoplasia testicular difusa e hiperplasia endometrial, associadas à metrite neutrofílica e linfoplasmocitária. Como esses achados estão associados à anamnese, conclui-se que este caso é um pseudo-hermafrodita masculino.

PALAVRAS-CHAVE: Anomalia Sexual, intersexo, mutação genética, ovotestis, reversão sexual.

INTRODUCTION

Hermaphroditism or intersex is a general term used to define congenital anomalies of reproductive development, where the individual presents the genital and gonadal system with ambiguous characteristics. Under the term intersex, hermaphrodites can be classified into true and pseudohermaphrodites, both male and female (Mendes *et al.*, 2020).

The chronology of sexual differentiation occurs after the establishment of the chromosomal sex, followed by gonadal development and, finally, the phenotypic sex (Zardo *et al.*, 2021).

[1] Universidade Federal Rural de Pernambuco, Department of Animal Science, Recife-PE, Brasil.
[2] Universidade Federal da Paraíba, Department of Animal Science, Areia-PB, Brasil.
*Corresponding author: apoloniogomes962@gmail.com
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In dogs, the migration and colonisation of germ cells to structure the gonadal sex is organised at approximately 35 days of gestation in male embryos and 45 days for female embryos (Lyle, 2007).

In true hermaphroditism, the male and female sexual organs present simultaneity, combining testicular and ovarian tissue in the same gonad (ovotestis) or in separate gonads, one developing as a testis and the other as an ovary (Rosa *et al*., 2022). Pseudohermaphrodites, on the other hand, have only one type of gonadal tissue and genitalia with characteristics of the opposite sex, being classified as male or female pseudohermaphrodites according to the gonad present. The animal is classified as a male pseudohermaphrodite when the gonad is a testis and a female pseudohermaphrodite when it is an ovary (Bueno *et al*., 2017).

In veterinary medicine, such anomalies are rare, and their etiopathogenesis has not yet been clarified in domestic animals (Prestes *et al*., 2005; Basrur; Stranzinger, 2006). Generally, initial clinical diagnosis is carried out by identifying anomalies in the external sexual organs and the clinical signs that are related to the reproductive system. Ultrasound imaging exams and vaginoscopy are performed; however, the definitive diagnosis is made through the histopathological evaluation of the gonadal tissue and karyotyping examination (Lyle, 2007), techniques that characterise normal and abnormal karyotypes.

Treatment in animals with this anomaly consists of the surgical removal of the gonads, the correction of an anomaly present in the external genitalia as well as prophylaxis for possible testicular and uterine neoplasms, urinary tract infections and dermatitis (Lyle, 2007; Christensen, 2012).

Because pseudohermaphroditism is uncommon in dogs, and data regarding this anomaly are scarce, the objective of this work was to report and discuss a case of male pseudohermaphroditism in a Shiba Inu dog treated at a private veterinary hospital in the City of Recife- PE.

CASE REPORT

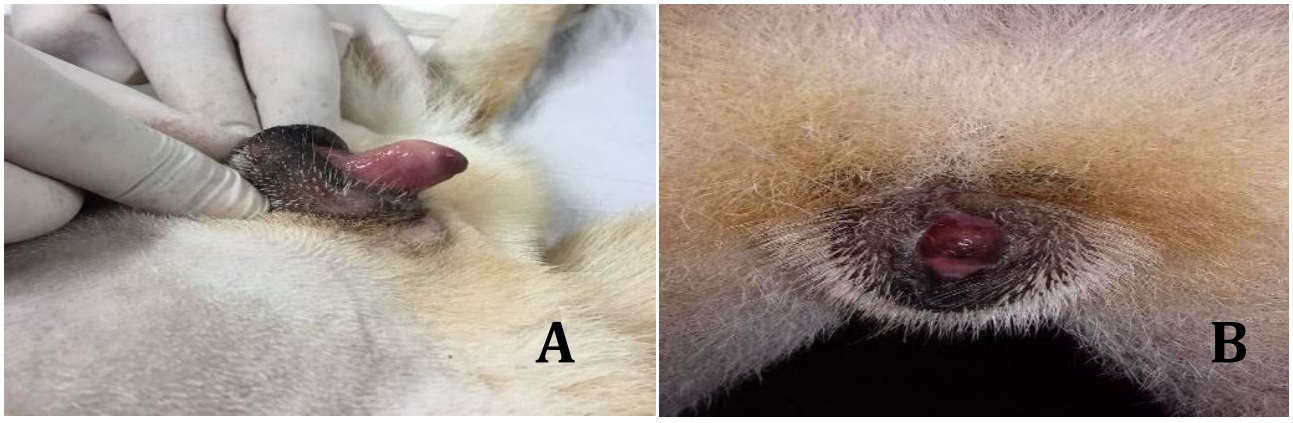
A Shiba Inu dog, 3 years old, weighing 10.3 kg, was treated at the Harmonia Veterinary Hospital – HVH, Recife-PE, with the main complaint of morphological abnormality of the external genitalia and the absence of an oestrous cycle.

On clinical examination, the animal was found to be hydrated, with normal mucous membranes, a heart rate of 95 bpm (reference: 60–160 bpm), a respiratory rate of 28 mpm (reference: 18–36 mpm), a rectal temperature of 38.3°C (reference for adult dogs: 38.1–39.2°C) and a capillary refill time (TPC) of less than 2 seconds. The owner reported delayed puberty and masculine behaviour, sometimes aggressiveness towards other dogs. Upon inspection of the external genitalia, the presence of a hypertrophied clitoris measuring approximately 2.5 cm in length was observed, with a structure similar to that of a penis (Fig. 1).

The clinical suspicion consisted of gonadal dysgenesis (hermaphroditism/pseudohermaphroditism), requiring additional tests. A complete blood count, serum biochemistry (enzymes albumin, pyruvic transaminase, creatinine, gamma glutamyl transferase, alkaline phosphatase, urea) and hormonal levels (oestrogen, progesterone, testosterone) were requested.

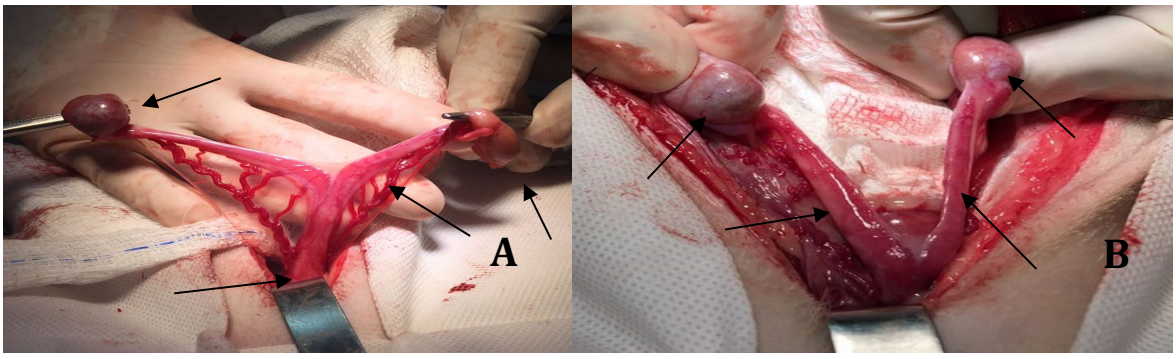
An abdominal ultrasound examination was performed (Suppl. Figs. 1–5), where ultrasound signs were visualised in the urinary bladder suggestive of chronic cystitis, associated with bladder and urethral microuroliths, in addition to splenomegaly and enlarged abdominal lymph nodes (right medial iliac lymph node measuring 0.72 cm in thickness).

In the topography of the cervix/uterine body, a tubular structure without cranial continuity was visualised, measuring 0.59 cm in average diameter and with the absence of intraluminal content (uterine body). In the topography of the left ovary, an oval structure (1.27 x 0.68 cm) was visualised, homogeneous and with a regular contour, without internal vascularisation on Collor Doppler and the presence of a cystic area, suggesting that it was a left ovary. The right ovary was not characterised at the time of examination (Suppl. Fig. 5). In addition to these, the other abdominal organs showed no noteworthy changes.



Source: Case report - Personal Archive

Figure 1. Hypertrophied clitoris, side (A) and frontal (B) views.



Source: case report, personal file, 2018

Figure 2. Macroscopic images of the genital tract with testicle and uterus (surgical procedure); uterine horns and testicles (black arrows).

Figure. intersex, canine, 2 years old, shiba inu. A) Testis, epididymis and spermatic cord, without macroscopic changes. B) longitudinal section of the testis and epididymis without macroscopic changes. C) Uterus with horns without macroscopic changes.

Source: BIOPSIE Veterinary Pathology – Bruno Paiva

Figure 3. Macroscopic images of the testicles and the uterus (removed surgically).

Urine was collected by cystocentesis for analysis, where bacteria, red blood cells and triple phosphate crystals were observed in the sediment. The results of the haematological evaluation revealed thrombocytopenia (platelets 138 million/mm3; reference value 200–500 million/mm^3) and hyperproteinaemia (total proteins 9.3 g/dL; reference value 6–8 g/dL) in the blood count, along with changes in the alkaline phosphatase levels (185 IU/dL; reference values 20–156 IU/dL). Hormonal measurement (oestrogen, testosterone and progesterone) was carried out before the surgical procedure and 15 days after. The first and second dosages of hormones obtained the following values: < 5.00, 20.00 and 0.20 ng/mL for oestrogen,

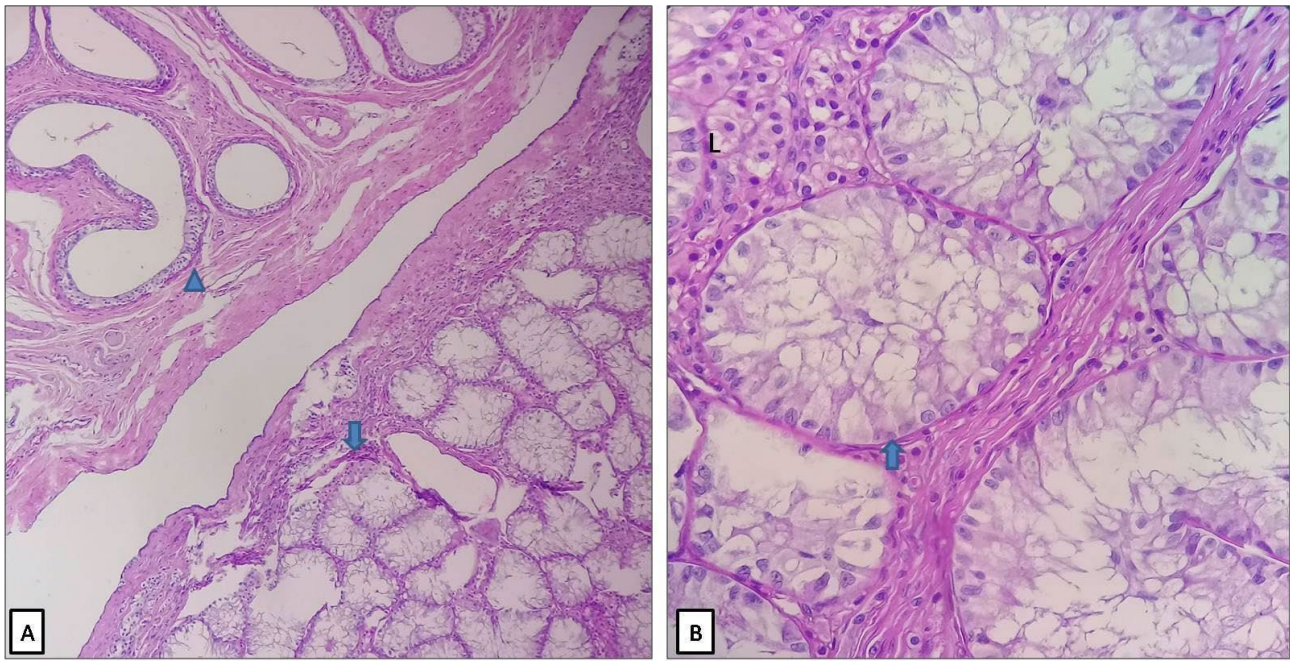


Figure A. Hypoplastic testicular parenchyma, with tubules devoid of germinal epithelium (arrow). Epididymal ducts, lined by moderately hyperplastic epithelium. Haematoxylin and eosin (HE) staining, 4X objective.

B. Seminiferous tubules lined solely by morphologically normal sertoli cells (arrow). In the interstitium, there are some Leydig cells (L). No sperm were seen within the duct lumens. Haematoxylin and eosin (HE) staining, 40X objective.

Source: case report, personal file, 2018

Figure 4. Histopathological examination.

testosterone and progesterone, respectively. The reference values for males are oestrogen up to 20 pg/mL, testosterone 100.0 to 700.0 ng/dL and progesterone 0ng/dL; for females, the reference values are oestrogen 20 to 50 pg/mL, testosterone < 20 ng/dL, progesterone < 2.0 ng/dL at ovulation and > 10.0 ng/dL at gestation.

Given the clinical findings and the results of the complementary exams, the patient was referred for surgical treatment, where castration was performed. The aesthetic protocol used was as follows: pre-anaesthesia (acepromazine, 0.02 mL, propofol, 4 mg/kg; intubation performed with an endotracheal tube number 6.0, where the patient received 100% oxygen and isofluorane, administered diluted in oxygen at 100.00%, with a flow of 30 mL/kg). The animal was monitored using a multiparametric monitor with pulse oximetry, electrocardiography and non-invasive blood pressure. When there was an increase in the monitored values, indicating the perception of pain, a bolus of 5 mcg/kg of fentanyl hydrochloride was administered.

Exploratory laparotomy of the abdominal cavity was performed, obtaining access through a median incision, where upon inspection, the presence of structures similar to testicles, adhered to the uterine horns, was observed, and all internal genitalia were excised. All tissue removed was preserved in 10% formalin for histological analysis (Fig. 2).

The prescribed post-surgical medication was as follows: tramadol hydrochloride (3 mg/kg/bid, for 4 days), enrofloxacin (5 mg/kg/bid, for 15 days), gaviz (1 mg/kg/bid, for 15 days, fasting) The surgical wound was cleaned daily with saline solution and chlorhexidine digluconate, protected with porous tape and surgical clothing, and the patient returned for re-evaluation and the removal of stitches after 15 days.

The macroscopic findings were as follows: a gonadal fragment (Fig. 3 A), measuring 2.5 x 1.2 x 0.4 cm, firm, greyish; when cut, it was homogeneous, firm and grey; a uterus fragment (2), measuring 4.0 x 0.5 x 0.1 cm, firm, greyish in color, with millimetric nodulations in one of the uterine horns; when cut, it was firm, homogeneous, with a white centre and grey periphery; a fragment (Fig. 3 B) of a contralateral gonad measuring 1.8 x 1.4 x 0.8 cm, firm, greyish in colour, with a brown peripheral area and an irregular surface; when cut, it was firm, homogeneous, grey, with a white peripheral area and another brown peripheral area, with numerous multifocal brown spots.

In the histopathological examination (Fig. 4 A and B), the findings were compatible with those of the testicles, presenting (Fig. 3 A and B) normal testicular parenchyma, arranged in numerous seminiferous tubules, composed only of Sertoli cells and interspersed with interstitial tissue with numerous cells Leydig. The uterus (Fig. 3 C) was also evaluated and showed mucosal glands with some foci of superficial cystic dilations. Lamina propria with inflammatory infiltrate composed predominantly of neutrophils, lymphocytes and plasma cells could be observed in some areas in the lumen of both

the glands and the organ. In conclusion, testicles with diffuse hypoplasia and a uterus with endometrial hyperplasia, associated with neutrophilic and lymphoplasmic metritis (Suppl. Figs. 6–9), were observed.

RESULTS AND DISCUSSION

The correct the categorisation of a patient with suspected sexual development disorder, it is important to evaluate a physical examination investigating the external genitalia, measure the sex hormones, histopathologically evaluate the gonads (Campbell, 2004) and perform cytogenetic analysis to verify the reason for this anomaly. These are methods that provide effective information, favoring a more accurate diagnosis. However, cytogenetic examination is little used in veterinary medicine (Romagnoli *et al.*, 2006) and was not possible in this case as it is not available in local veterinary laboratories.

The canine in the present report was characterised as a male pseudohermaphrodite due to its genital tract organs consisting of a uterus, cervix, left and right uterine horns, vulva, hypertrophied clitoris and cryptorchid testicles (Suppl. Figs. 7 and 8), corroborating the clinical and histopathological findings of Vannucci (2008) and Breshears & Peters (2011).

The appearance of structures related to the mechanism of action of the anti-Müllerian hormone confirms the failure in the function of cellular receptors, inhibiting the successful development of internal and external genitalia (Johnston *et al.*, 2001), supporting the hypothesis that this failure occurred in the animal reported. Clinical examination revealed the presence of a vulva with normal morphology and location and a hypertrophied clitoris in addition to the vaginal canal. Ultrasound examination revealed the presence of a uterine body and gonad compatible with the left ovary, and it was not possible to visualise the right gonad.

Although on ultrasound examination, the gonadal structure resembled an ovary, during the surgical procedure, the presence of bilateral male gonads located in the uterine horns, in the anatomical position of the ovaries, was noted, later confirmed through histopathology. Testicles with seminiferous tubules, absent of sperm, were diagnosed; according to Prestes *et al.* (2005) and Vannucci *et al.* (2008), animals with failure in the androgen-dependent masculinisation process have two complete uterine horns and show cryptorchidism.

Regarding the urinalysis result, highlighting the presence of triple phosphate crystals, it is not mentioned in the literature as a finding resulting from this anomaly. However, Lyle (2007) and Christensen (2012) state that some illnesses may arise due to anatomical dysfunction, probably in association with atrophy of the urinary canals, which may explain the reported patient's cystitis.

Among the haematological and serum biochemistry results, the findings revealed thrombocytopenia, hyperproteinaemia and alkaline phosphatase, with slight changes, presumed to a result of the stress suffered by the animal due to handling and not related to the anomaly described.

In the first hormonal measurement carried out prior to surgery, the concentrations of oestradiol, testosterone and progesterone presented values below the physiological basal levels of animals without this anomaly (Table 4). Despite the presence of male gonads, / gonadal hormones are not produced because they are non-functional, as reported by Delfini *et al.* (2007); based on measurements taken 15 days after surgical removal of the gonads, there was no change in the concentration of these hormones.

The histopathological evaluation carried out using the material excised during the surgical procedure confirmed male pseudohermaphroditism since the microscopic analysis revealed the presence of right and left testicular tissue (Prestes *et al.*, 2005).

It is worth noting that, although karyotyping was not performed, the findings of the physical examination as well as the hormonal and histopathological analysis of the gonads determined the phenotypic sex and gonadal constitution, characterising the patient as a male pseudohermaphrodite (Lyle *et al.*, 2007).

After surgical removal of the internal genitalia, the patient's recovery was satisfactory as it was a prophylactic treatment for infections and eliminated the risk of developing changes and neoplasms related to intersex (Romagnoli *et al.*, 2006). Finally, it is worth clarifying that the animal, after surgical treatment, appeared healthy.

FINAL CONSIDERATIONS

This report is the description of a case of male pseudohermaphroditism, providing clarification on a scarce topic with few reports in the literature. Based on the findings, the importance of excluding the parents and siblings of the carrier animal from reproduction is highlighted as this is an anomaly of genetic reproductive development.

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