# Male pseudohermaphrodite in a Shetland cross sheep- a case report

Yehonatan Berkowic,[a] Lisa A. Edwards,[a] Roselle Busch,[b] Marcos Pérez-Nogués,[a] Sarah E. Stevens,[a] Mary Booth,[c] Bret R. McNabb[d]

[a]William R. Pritchard Veterinary Medical Teaching Hospital, School of Veterinary Medicine, University of California-Davis, Davis, CA; [b]Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California-Davis, Davis, CA; [c]School of Veterinary Medicine, University of California-Davis, Davis, CA; [d]Department of Population Health and Reproduction, School of Veterinary Medicine, University of California-Davis, Davis, CA

## Summary

A 1.5 year old Shetland cross sheep was presented to the University of California-Davis (UCD), Veterinary Medical Teaching Hospital (VMTH) for reproductive evaluation and sterilization surgery. On physical examination, female external genitalia were present including an enlarged, erect clitoris, as well as two subcutaneous inguinal masses. On exploratory abdominal laparoscopy, spermatic cords could be identified bilaterally, however no female internal genitalia or gonads were observed. The inguinal masses were surgically removed and confirmed histologically to be testes. Hormonal assay results were consistent with cryptorchidism (testosterone= 658.1 pg/ml; AMH= 4.1 ng/ml; progesterone= 0.1 ng/ml). The sheep was genotypically an XX/XY blood chimera, with male gonads and female external genitalia, and was therefore identified as male pseudohermaphrodite. Of the known etiologies for this condition 5 α reductase deficiency is the most probable cause.

**Keywords**: Sexual differentiation, pseudohermaphrodite, intersex, sheep, 5 α reductase, 17α-hydroxyprogesterone

## Background

The sex of an animal can be described on three levels: the chromosomal sex (determined by parental spermatozoa, which deliver either an X or Y chromosome), gonadal sex (determined mainly by SRY factor encoded by the sex determining region on the Y chromosome), and phenotypic sex. The last can be subdivide to internal genitalia-sex (determined by presence or absence of testosterone and antiMullerian hormone (AMH), secreted by the fetal testes),[1,2] and external genitalia-sex. The development of male external genitalia, penis and scrotum, requires conversion of testosterone into dihydrotestosterone (DHT) by 5 α reductase, an enzyme expressed locally in the embryonic genital tissue.

Disorders of sexual development have been diagnosed in all domestic species but are relatively uncommon. In general, they can be caused by chromosomal/genetic disorders or by abnormal hormonal exposure within critical periods of sexual development.[3] Different definitions are used when describing disorders of sexual development. "Intersex disorders" is a broad term that includes different levels of sexual ambiguity. Hermaphroditism describes the existence of both ovarian and testicular tissue within the same individual. This may be observed as two discrete types of gonads or as part of "ovotestes", which are gonads consisting of both ovarian tissue (typically in the cortex) and testicular tissue (typically in the medulla). Pseudohermaphrodism is a disagreement between the gonadal and phenotypic sex when only one type of gonads exist. "Male- pseudohermaphroditism" is characterized by the presence of testes, while "female- pseudohermaphroditism", is characterized by the presence of ovaries. This should not be confused with "sex- reversal", a term used in the literature describing "disagreement" between the chromosomal sex and gonadal sex.

In this report we describe a case of ovine male pseudohermaphroditism and attempt to determine the etiology by considering possible known causes for this condition reported in veterinary and human medicine literature.

## Case presentation

A 1.5 year old Shetland cross sheep was presented to the UCD VMTH for reproductive evaluation and sterilization surgery. Initially, the sheep was thought to be a female by the owner based on

the appearance of the external genitalia. As the animal aged, male characteristics (masculine appearance, ram behavior and ram like-odor) developed. The sheep was born as part of a triplet litter of which the remaining two lambs were female, one born alive and the other one dead. On physical examination female external genitalia, including a vulva and an enlarged, erect clitoris was present (Fig. 1). No prepuce, penis, or scrotum were present, however inguinal masses, suspected to be gonads, were palpated subcutaneously in the right and left inguinal regions (Fig. 2). The tissue architecture visualized on ultrasound examination was supportive of testes. Complete blood count (CBC) and serum biochemistry values were all within normal limits. On abdominal ultrasonography, no gonads or internal genitalia were observed. A vaginal speculum was inserted into the vagina, which was very narrow and the speculum could not pass further than approximately 5 centimeters. Serum hormone concentrations were consistent with cryptorchidism (testosterone 658.1 pg/ml; AMH 4.1 ng/ml; progesterone 0.1 ng/ml) at the time of presentation. Whole blood used for genetic analysis revealed a blood chimera, with a mixture of male and female genotypes (XX/XY) and the presence of the *sry* gene. For comparison, steroid hormones and their metabolites were measured (using mass spectrometry) from three apparently normal breeding rams, approximately 2 years of age and unrelated to this individual. Results are presented in the table below.

**Treatment and outcome**

Food and water were withheld for 24 hours prior to surgery. Oxytetracycline (10 mg/kg body weight) and flunixin meglumine (1.1 mg/kg body weight) were administered intraveneously preoperatively. The sheep was sedated using midazolam (0.2 mg/kg body weight) and morphine (0.1 mg/kg body weight), and anesthesia was induced with ketamine (4.4 mg/kg body weight) then maintained with isoflurane. The sheep was placed in dorsal recumbency, in Trendelenburg position reaching approximately 45º table angle, which allowed exploration of the caudal abdomen. The abdominal and inguinal area were clipped and prepared for surgery in a standard fashion. A 1.5cm incision was made through the skin and muscle layers 5 cm lateral to the umbilicus on the right side. The peritoneum was entered with scissors using caution to avoid inadvertent bowel damage. A 10mm cannula with a blunt trocar was inserted into the abdominal cavity in a caudal direction with a 30º angle from the abdominal wall. Insufflation using carbon dioxide at 8mm Hg of pressure was maintained during the procedure. A Backhaus clamp was placed in the caudal portion of the portal incision to prevent leakage of insufflation gas. A 10mm 0º laparoscope 33cm in length was inserted (Karl Storz Endoscopy-America, El Segudo, CA), and the omental sac was visualized, which impaired visualization of the viscera. A second portal was made 5cm to the left of the umbilicus. Another 10cm laparoscopic cannula was inserted and a fan retractor (AutoSuture Endo Retract II: 10mm, Medtronic, Minneapolis, MN) was used to pull the omental sac cranially and to allow complete visualization of the caudal peritoneal space by elevating the urinary bladder. Spermatic cords could be identified bilaterally traveling through the internal inguinal ring (Fig. 3). No female internal genitalia or gonads were observed. The laparoscopic portals were closed in two layers, external abdominal fascia was closed using 0 polyglactin 910 (Vycril, Ethicon, Somerville, NJ) and intradermal using 2-0 poliglecaprone (Monocryl, Ethicon). Both inguinal masses (presumptive testicles) were removed via parainguinal approach. Both cords were ligated using 0 polyglactin 910 and the skin closed using an intradermal pattern with a 2-0 poliglecaprone suture. The inguinal masses were submitted for histopathological examination.

Histologically, the submitted samples were consistent with testicular architecture. The samples exhibited severe, generalized seminiferous tubular atrophy and diffusely lacked active spermatogenesis (Figs. 4-6). Atrophied seminiferous tubules were separated by bands of fibrosis that occasionally included mild to moderate Sertoli cell hyperplasia. These are features commonly observed in retained testes due to the effect of body temperature on spermatogenesis, with chronic degeneration manifested by varying degrees of interstitial fibrosis.[3]

Recovery from surgery was uneventful, and the sheep was discharged from the hospital. Based on personal communication with the owner approximately one month after the surgery, the sheep recovered well and the ram behavior and ram like odor resolved.

**Discussion**

Based on genetic analysis and phenotypic appearance this sheep was determined to be a blood-chimera, comprising an XX/XY genotype, with male gonads, male internal genitalia, and female external genitalia. Although this sheep was phenotypically female, some level of masculinization could be observed including an enlarged clitoris, ram-like odor, ram behavior, and a masculine head. Since only testicular tissue could be observed in the gonads, the patient was defined as male pseudohermaphrodite.

Laparoscopy was first described in sheep by Phillippo et al. in 1971 for the diagnosis of pregnancy and fetal counting.[4] Since then it has become a routinely used technique for intrauterine artificial insemination, embryo transfer, oocyte collection, and ovulation rate determination.[5-7] This technique has also been reported to be useful in sheep for direct visualization of ovarian and uterine abnormalities, evaluation of other abdominal organs, inguinal hernioplasty in rams, treatment of urolithiasis and toggle abomasopexy.[8-11] In the present case, the use of laparoscopy provided excellent visualization with low morbidity, no post-operative complications, and a shorter anesthetic and hospitalization time than a laparotomy would have been allowed. The laparoscopic procedure was performed under inhalation general anesthesia due to our assumption of a possible need for gonadal extirpation, however, if laparoscopy is elected just as an exploratory tool, sedation and analgesia have been reported to be successful and minimal adverse consequences and contraindications have been reported.[12-14]

There are three known pathogenesis for a male pseudohermaphrodite: persistent Mullerian duct syndrome, androgen insensitivity (also known as testicular feminization), and 5 α reductase deficiency.[15] Persistent Mullerian duct syndrome is a rare condition described in human patients, with case reports in miniature schnauzer dogs and a goat. The condition is characterized by an XY genotype, male external genitalia, and testes that are attached to the proximal ends of the uterine horns. This syndrome is a result of a mutation in the AMH or AMH-receptor gene. Androgen insensitivity is a condition in which individuals have an XY genotype with hormonally active retained testes. A mutation identified as *Tfm* results in a deficiency of intracellular androgen receptors. As other receptors for steroid hormones these receptors are nuclear and trigger transcription of DNA and eventually translation of mRNA into protein when activated by androgens. The mutation makes all cells insensitive to androgen.[3] Individuals with androgen insensitivity usually have female external genitalia and the internal genitalia is rudimentary due to the action of AMH and the deficiency of intracellular androgen receptors. This condition has been described in humans,[16] observed in cattle, sheep and swine, and is probably inherited.[17] Five α reductase is an enzyme that reduces testosterone to dihydrotestosterone, a potent androgen that is required in utero for masculinization of external genitalia. Deficiency of 5 α reductase in humans has been found to be an autosomal recessive trait, primarily due to mutations of the 5-alpha reductase 2 gene.[18] In animals, no causative mutation has been reported and it has not been reported to be hereditary, however this does not rule out an unknown heritable mutation. The clinical manifestation is very similar to androgen insensitivity syndrome, including an XY genotype, retained testes and predominantly female external genitalia. Testosterone is required for internal ductus deferens and external male genitalia development; thus, androgen insensitive animals will lack both. Conversely, 5-alpha reductase is required only for masculinization of future external genitalia and the genital tubercle. As result, deficient animals will possess internal ductus deferens (unlike androgen insensitive animals) and have predominantly female external genitalia. Humans with 5-alpha reductase deficiency have varying degrees of external genitalia ambiguity, even amongst those with identical 5-alpha reductase 2 mutations.[19] Thus, it could be extrapolated that animals with 5-alpha reductase deficiency may also vary slightly in the appearance of the external genitalia, while appearing predominantly female.

In the present case, persistent Mullerian duct syndrome can be ruled out since no female internal genitalia were found. Testosterone insensitivity is unlikely, since a well-developed spermatic cord could be observed via laparoscopy (Fig. 3). Therefore, 5α reductase deficiency seems to be the most likely cause in this case. Nevertheless, one should take into consideration other possible unknown or unreported etiologies for male pseudohermaphroditism.

Freematinism is one of the most common congenital causes of infertility in cattle. It occurs in cases of heterosexual twins, which share common blood supply and become XX/XY blood chimeras. They display a variety of phenotypes, but usually display masculinized ovaries, small genital tract and short vagina. In one report by Brace et al, blood chimerism had a prevalence of 4.35% in Rideau Arcott sheep, with no effect on litter size or sex ratio.[20] In sheep and goats, although litters containing males and females are common, freemartinism is rare because fusion of placental vasculature occurs after sexual differentiation. In the present case it does not seem likely that sex chimerism can explain the clinical presentation, therefore this was assumed to be an incidental finding.

Testosterone value references for ram lambs are available and ranged from 240- 680pg/ml in lambs from 1-9 weeks of age.[21] In a more recent study testosterone levels ranged between 2.2 ± 0.47ng/mL for lambs 12 weeks of age and 8.92 ± 2.35ng/mL for lambs 21 weeks of age.[22] Cazorla et al reported AMH values in ram lambs to be approximately 15ng/ml at 4-6 weeks of age.[23] They were not detectable in castrated lambs. In the present case, testosterone levels exceed those reported for ram lambs. Anti-Mullerian hormone levels, however were lower than the reported reference. Comparison of mass spectrometry results from the patient and 3 apparently normal breeding rams of approximately the same age did not reveal any differences in circulating DHT. This however, cannot rule out 5 α reductase deficiency, since the expression of the enzyme and its product (DHT) is expected to be local in the external genitalia tissue, sertoli cells and accessory sex glands. Testing directly for 5 α reductase would require biopsy of the genitalia and demonstration of its expression in the tissue, however such diagnostics are unavailable. The steroid analysis reveals detectable 17α-hydroxyprogesterone and 20α-hydroxyprogesterone in our patient, compared to none in the control group of rams. Five α reductase is important for what described as the "backdoor pathway" for synthesis of androgens. In this pathway 17α-hydroxyprogesterone can be metabolized to DHT via androstenedione.[24] As 5 α reductase is a key factor in this cascade, its absence could result in accumulation of 17α-hydroxyprogesterone. This result provides another support to our hypothesis that 5 α reductase insufficiency is the most probable cause of the intersex phenotype in the present case.

**Learning points**

- Blood chimerism in sheep can be an incidental finding, and not necessarily the cause sexual ambiguity.
- Dihydrotestosterone could not be detected in the serum. This is probably because the expression of 5 α reductase and its product (DHT) is local in the external genitalia tissue.
- Elevated serum 17α-hydroxyprogesterone could be a supportive evidence for 5 α reductase insufficiency
- Transabdominal laparoscopy can be utilized for exploration of the caudal abdomen in cases of sexual ambiguity, with the potential advantage of using light sedation and low post-operative morbidity.
- This case demonstrates again the complexity of sexual differentiation and the challenge of detecting the exact etiology.

**Acknowledgements**

Alan J. Conley BVSc, MS, PhD, FRCVS, Erin Legacki PhD, Lee V. Millon

**References**

1. Wilhelm D, Palmer S, Koopman P: Sex determination and gonadal development in mammals. Physiol Rev 2007;87:1-28.
2. Eggers S, Ohnesorg T, Sinclair A: Genetic regulation of mammalian gonad development. Nat Rev Endocrinol 2014;10:673-683.
3. Schlafer DH, Miller RB: Female genital system. In: Maxie MG, editor. Jubb, Kennedy, and Palmer's pathology of domestic animals. 5th ed. Philadelphia: Elsevier, 2007. volume 3: p. 433-440.
4. Phillippo M, Swapp GH, Robinson JJ, et al: The diagnosis of pregnancy and estimation of foetal numbers in sheep by laparoscopy. J Reprod Fertil 1971;27:129-132.

5. Killeen I. CG, Holt N: Fertility of ewes following intrauterine insemination with the aid of a laparoscope. Ann Conf Aust Sot Reprod Biol 1982;104.
6. McKelvey WA, Robinson JJ: Collection of embryos from ewes by laparoscopy. Vet Rec 1984;115:158.
7. Snyder DA, Dukelow WR: Laparoscopic studies of ovulation, pregnancy diagnosis, and follicle aspiration in sheep. Theriogenology 1974;2:143-148.
8. Khan M, Sajjad Nazir S, Ahangar S, et al: Retroperitoneal laparoscopy for the management of renal hydatid cyst. Int J Surg 2010;8:266-268.
9. Daniel AJ, Easley JT, Holt TN, et al: Laparoscopic inguinal hernioplasty in a ram. J Am Vet Med Assoc 2015;246:1118-1121.
10. Franz S, Dadak AM, Schoffmann G, et al: Laparoscopic-assisted implantation of a urinary catheter in male sheep. J Am Vet Med Assoc 2008;232:1857-1862.
11. Thomas KL, Wilson DG, Bracamonte JL, et al: Quality of adhesions after sutured paramedian vs. laparoscopic toggle abomasopexy in an ovine model. Vet Surg 2016;45:488-493.
12. Franz SD, Schöffmann G, Coppens P, et al: Laparoscopic surgery in small ruminants. Proc XXVI World Buiatrics Cong 2010;14-18.
13. Stafford KJ, Chambers JP, Sylvester SP, et al: The stress caused by laparoscopy in sheep and its alleviation. N Z Vet J 2006;54:109-113.
14. Curet MJ, Vogt DA, Schob O, et al: Effects of CO2 pneumoperitoneum in pregnant ewes. J Surg Res 1996;63:339-344.
15. Acland HM: Reproductive system: male: McGavin MD, Carlton WW, Zachary JF, editors. Thomson's special veterinary pathology. 3rd ed. Philadelphia: Mosby; 2001. p. 635-637
16. Mongan NP, Tadokoro-Cuccaro R, Bunch T, et al: Androgen insensitivity syndrome. Best Pract Res Clin Endocrinol Metab 2015;29:569-580.
17. Ladds PW: Congenital abnormalities of the genitalia of cattle, sheep, goats, and pigs. Vet Clin North Am Food Anim Pract 1993;9:127-144.
18. Okeigwe I, Kuohung W: 5-Alpha reductase deficiency: a 40-year retrospective review. Curr Opin Endocrinol Diabetes Obes 2014;21:483-487.
19. Shabir I, Khurana ML, Joseph AA, et al: Phenotype, genotype and gender identity in a large cohort of patients from India with 5alpha-reductase 2 deficiency. Andrology 2015;3:1132-1139.
20. Brace MD, Peters O, Menzies P, et al: Sex chromosome chimerism and the freemartin syndrome in Rideau Arcott sheep. Cytogenet Genome Res 2008;120:132-139.
21. Lee VWK, Cumming IA, de Kretser DM, et al: Regulation of gonadotrophin secretion in rams from birth to sexual maturity. I. Plasma LH, FSH and testosterone levels. J Reprod Fertil 1976; 46:1–6.
22. J. Herrera-Alarco´n, E. Villago´mez-Amezcua, E. Gonza´lez-Padilla, et al: Stereological study of postnatal testicular development in Blackbelly sheep. Theriogenology 2007; 68: 582–591.
23. Cazorla O, Seck M, Pisselet C, et al: Anti-Mullerian hormone (AMH) secretion in prepubertal and adult rams Blackbelly sheep. J Reprod Fertil 1998;112: 259-266.
24. Kamrath C, Hartmann MF, Remer T, et al: The activities of 5α-reductase and 17,20-lyase determine the direction through androgen synthesis pathways in patients with 21-hydroxylase deficiency. Steroids 2012;77:1391-1397.

Figure 1. Female external genitalia were present with an enlarged, erect clitoris. The sheep is in right lateral recumbency.

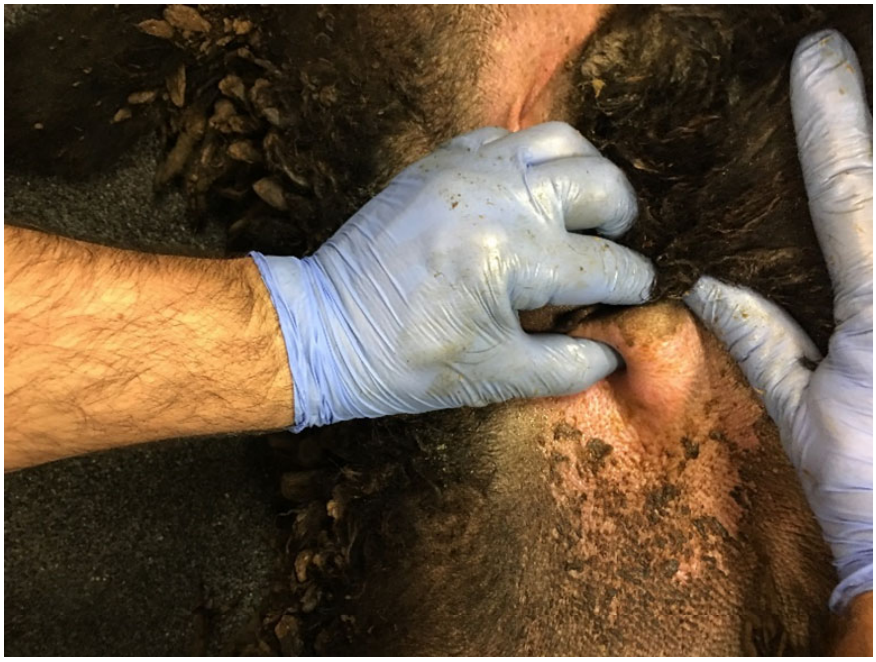

Figure 2. A mass, suspected to be a gonad, in the left inguinal region. The sheep is in dorsal recumbency with the cranium directed to the upper side of the picture.

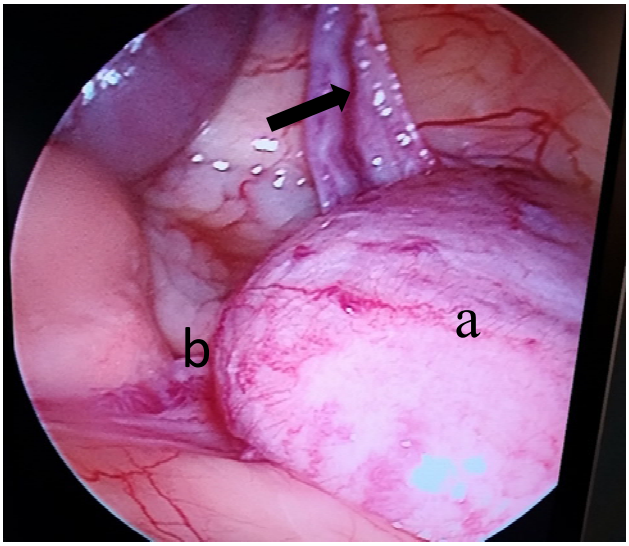


Figure 3. Spermatic cord (black arrow) traveling toward the inguinal canal. In addition, urinary bladder (a), and left ureter (b) observed via abdominal exploratory laparoscopy.

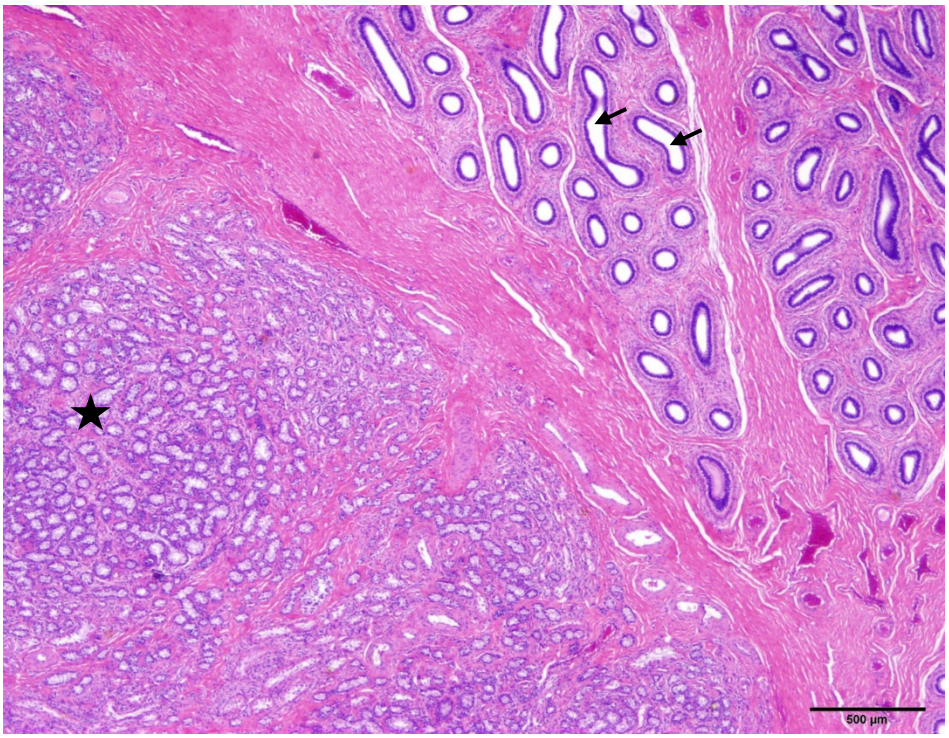


Figure 4. Inguinal testis. Seminiferous tubules (lower left) are severely atrophied and separated by fibrosis (area note by star). Lumina of the epididymis (upper right) are void of contents (examples for cross sections of empty epididymis note with black arrows).

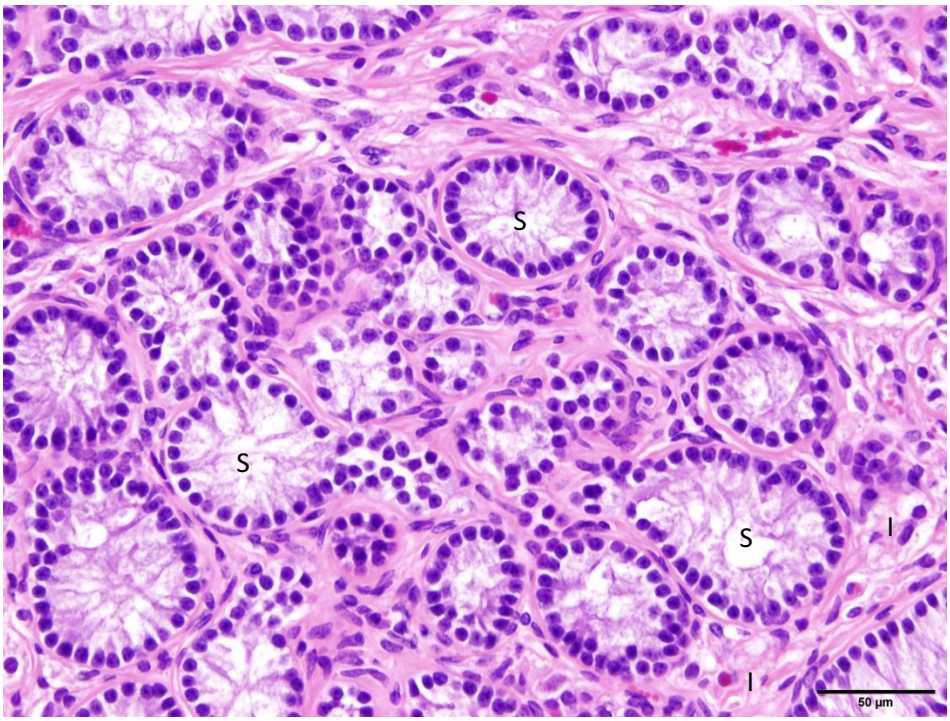

Figure 5. Seminiferous tubules (S). Seminiferous tubules are severely atrophied with decreased germ cells, decreased mature Leydig (interstitial=I) cells and there is absent spermatogenesis.

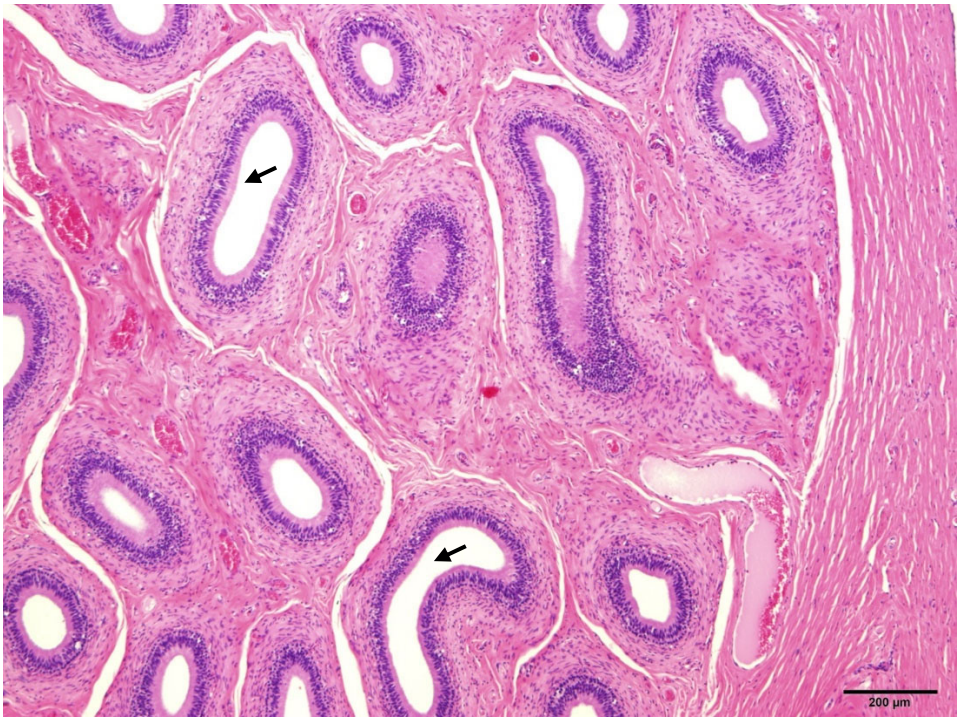

Figure 6: Epididymis of inguinal testis. Lumina are void of contents consistent with absent spermatogenesis (empty epididymal lumen noted by arrow).

Table. Summary of steroid analysis using mass spectrometry for comparison of steroid hormones between the hermaphrodite patient to 3 apparently normal breeding rams of the same age. The main differences were in progestagens (17-OHP and 20a-OHP). None of the animals had detectable levels of dihydrotestosterone (DHT). 17-OHP=17α-hydroxyprogesterone, 20a-OHP=20α-hydroxyprogesterone, A4=androstenedione, P4=progesterone.

| | 17-OHP | 20a - OHP | A4 | P4 | Testosterone |
|---|---|---|---|---|---|
| Ram 1 | 0.00 | 0.00 | 0.10 | 0.00 | 11.51 |
| Ram 2 | 0.00 | 0.00 | 0.00 | 0.00 | 1.74 |
| Ram 3 | 0.00 | 0.00 | 0.00 | 0.00 | 0.99 |
| Intersex | 0.42 | 0.20 | 0.00 | 0.00 | 0.94 |

(Editor's Note: Photographs in this paper are available in color in the online edition of Clinical Theriogenology,)