Testicular hypoplasia and azoospermia in a crossbred boar

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Abstract

An 8 month old boar was examined for absence of sperm in semen at initial semen collection in a boar stud. Clinical examination revealed a clinically healthy boar with marginally acceptable testicular size and normal libido. Ejaculation was confirmed by measuring seminal plasma alkaline phosphatase concentrations. Histologic examination of biopsies from both testes revealed testicular hypoplasia. Historical semen production characteristics of close relatives implied a possible genetic cause, and boar was removed from breeding herd.

Keywords: Azoospermia, crossbred boar

Background

Azoospermia is an extreme example of testicular dysfunction. Causes are genetic hypoplasia, congenital or acquired obstructions to sperm outflow, and testicular degeneration secondary to disease, injury and temperature extremes. Reason for azoospermia is diagnosed by concentrations of semen alkaline phosphatase (ejaculation failure) and by testicular biopsy findings (seminiferous tubule dysfunction). Testicular hypoplasia and segmental aplasia of epididymis are associated with genetic defects in pigs.[1] In these instances, cytogenetic analysis of animals and their close relatives are performed to identify carriers of genetic defects for decreased fertility. Critical evaluation of semen parameters of related boars is also recommended to identify congenital causes of subfertile or infertile animals.

Case presentation

An 8 month old, crossbred boar, was presented to University of Illinois Veterinary Teaching Hospital (VTH) when attempts to collect semen on boar resulted in samples with no sperm. Boar was housed in a well-managed breeding facility that sold semen from crossbred boars used primarily for exhibition. Owners reported that members of this boar's family line tended to have small testes and low semen production. Expected sperm number is 10 - 100 billion per collection when semen collection is performed twice per week.[2] Boar's sire, also a crossbred boar, produced good motile sperm with low proportion of morphologic defects; however, sperm output was poor (~ 30 billion sperm in once a week collection). Collecting more than once weekly resulted in considerable reduction in sperm number. Boar's sire sired 6 other boars from 6 different sows, of which 3 boars had small testes and 2 of these had adequate sperm motility with low number (~ 30 billion sperm per collection even if semen collection was once per week) and other 3 boars were normal. A daughter of boar's sire bred to him, produced 2 boars (1 had normal semen production, and other had oligospermia and asthenospermia) from different litters. Boar's grandsire had normal semen parameters; however, a hemicastration was performed when he was 6 years old. Details of medical condition requiring hemicastration were not known. Boar's dam produced 12 litters and mating to boars other than her sire yielded boars with normal semen parameters. Boar's sire was also seen at VTH on 8 occasions over 2 years for idiopathic peritoneal effusion. Inflammatory, infectious, neoplastic, cardiac and hepatic etiologies were ruled out. Approximately 20 liters of peritoneal fluid was drained from abdomen at each visit. Peritoneal effusion stopped without specific treatment.

At presentation, boar had a normal demeanor and gait. He weighed 150 kg and rectal temperature was 37.8°C (reference range 38.6 - 40°C). No abnormalities were detected on physical examination. Boar displayed an acceptable libido and mounted a dummy readily. Penis was examined during semen collection and appeared anatomically normal. Semen was collected using gloved-hand technique into an insulated collection bottle with a filter to separate gel from semen. Approximately 15 ml of clear, gel-free

ejaculate was obtained. Multiple aliquots of ejaculate were examined using a phase contrast microscope (Olympus BX50, Olympus Scientific Solutions Americas Corporation, Waltham, MA) at 100 x and 400 x magnification, and no sperm were observed.

Lack of sperm in an ejaculate can be due to ejaculation failure, hypogonadotropic hypogonadism, defective spermatogenesis, obstruction of sperm outflow, and testicular degeneration. In this case, ejaculation failure was suspected due to low volume of seminal plasma collected. To test for ejaculation failure, an aliquot of gel-free fraction was submitted to clinical pathology laboratory of VTH for measurement of seminal alkaline phosphatase (ALP) concentration. Sample contained 32,500 U/L ALP (reference range: 24,276 - 55,678 U/L), consistent with a successful ejaculation.[3]

Hypogonadotropic hypogonadism is very rare in domestic animals. In a Finnish study reporting results of semen collection on 2,048 boars, 1 animal was diagnosed with hypogonadotropic hypogonadism and had a testicular weight of only 26 g.[1] This condition was not suspected in this case given its rarity and the boar's subjectively near-normal size testes. Due to low probability of this condition and budget constraints, diagnostic efforts were focused on structural and functional problems of testes, including developmental defects. Boar was sedated with tiletamine and zolazepam (Telazol, Zoetis, Parsippany, NJ) with 250 mg of ketamine (Zetamine, Vet One, Boise, ID) and 250 mg of xylazine (AnaSed LA, Vet One, Boise, ID) to yield a solution containing 50 mg/ml of each drug (Telazol, Zetamine, and AnaSed LA) given at a dose of 1.6 mg/kg IM of each drug in combination. Palpation of scrotum and its contents revealed both testes and epididymides free from adhesions, with no palpable abnormalities and normal testicular tone. Ultrasonographic examination was performed with a curved array 5.0 - 2.0 MHz transducer (Sonoscape S8Exp, Universal Imaging Inc., Bedford Hills, NY). Both testes had normal echogenic pattern, and left testis measured 8.2 (length) x 5.3 (width) x 4.8 (height) cm and right testis measured 8.8 (length) x 6.0 (width) x 4.9 (height) cm (Figure 1).

Segmental aplasia of ductus deferens was suspected; however, surgery or necropsy was necessary for visual inspection to confirm. Therefore, testicular biopsy was elected. Biopsies of each testis were performed using a 14 gauge biopsy instrument (Tru-Cut, Merit Medical, South Jordan, UT). Biopsy samples were fixed in 10% buffered formalin and submitted to University of Illinois Veterinary Diagnostic Laboratory for histologic examination. Biopsy samples were embedded in paraffin and stained with hematoxylin-eosin. Testicular parenchyma of both testes was predominantly composed of interstitial cells with rare seminiferous tubules. Seminiferous tubules appeared atrophied and contained few Sertoli cells and rare sperm, consistent with congenital testicular hypoplasia (Figure 2).

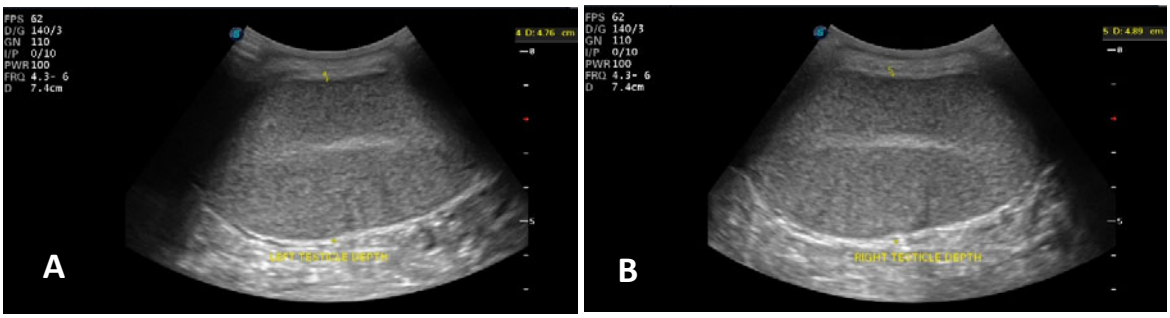

Figure 1. Ultrasonographic images of left (A) and right testis (B)

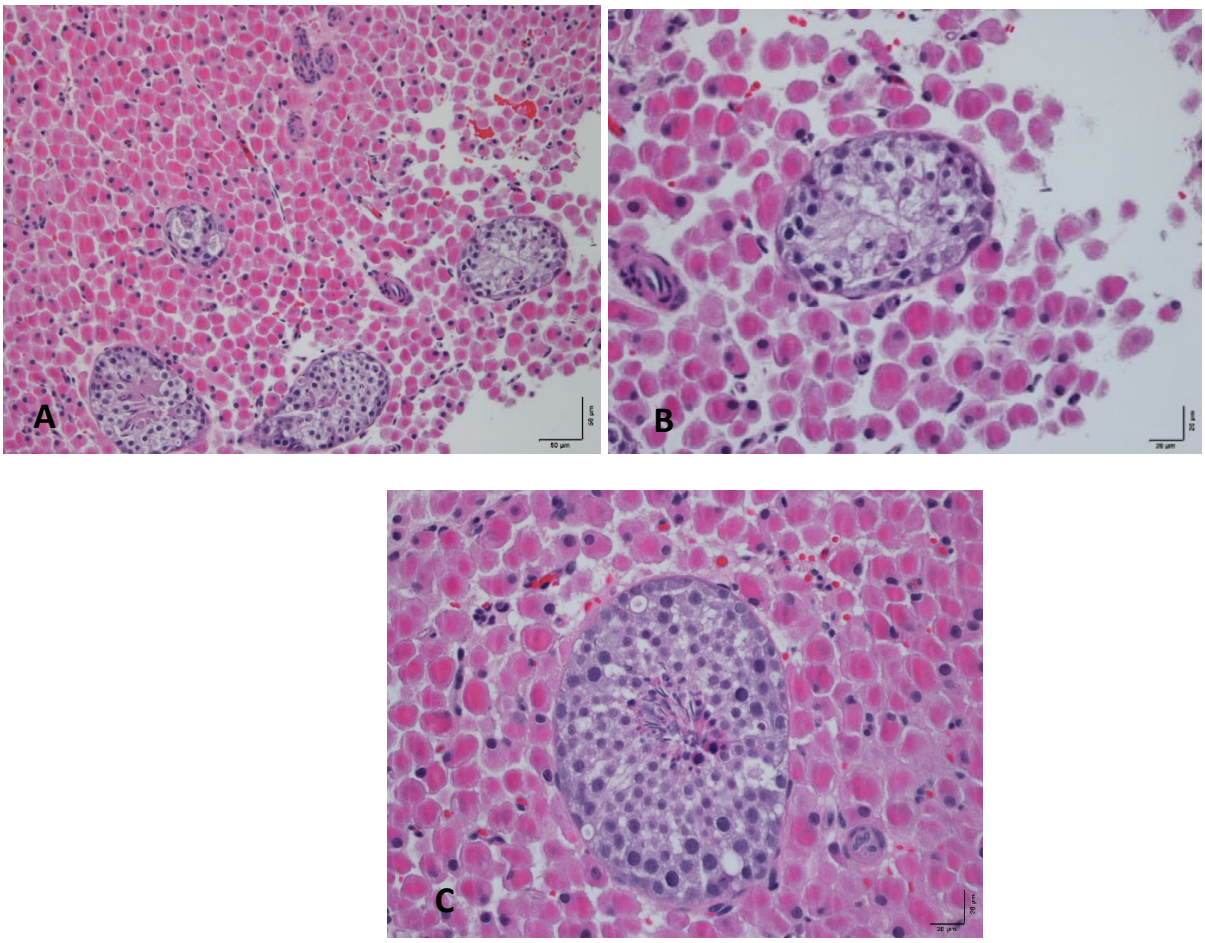

Figure 2. Representative histologic appearance of testicular biopsy samples fixed in formalin and stained with hematoxylin and eosin. (A) A predominance of interstitial cells with rare seminiferous tubules (200 x). (B) Seminiferous tubule with cellular vacuolization (400 x). (C) Seminiferous tubule with rare sperm (400 x).

Outcome

Cause of azoospermia was determined to be due to testicular hypoplasia. Based on our findings, removal of boar from breeding facility was recommended and no further followup was possible.

Discussion

Azoospermia in a young boar could be due to delayed puberty, ejaculation failure, obstruction of sperm outflow, testicular hypoplasia and testicular degeneration. Puberty is loosely defined in males as onset of sexual behavior. Throughout puberty, there is an increase in penis size that results in sigmoid flexure formation. This increase in size, coupled with onset of mounting activity, helps to break prepuce attachment (frenulum) to penis. Time to onset of puberty in boars varies considerably by breed but sufficient pubertal development for breeding activity is not expected until after 5 months of age.[4] Pubertal development could be delayed due to poor nutrition, chronic disease, primary hypogonadism (e.g. Klinefelter's syndrome) or secondary hypogonadism (e.g. pituitary disorder). In this case, boar exhibited appropriate sexual response, exteriorized his penis and ejaculated completely, indicating attainment of puberty by an acceptable age.

Postpubertal, young boars are expected to have an ejaculate volume of 100 - 300 ml.[5] In this case, low volume of seminal plasma was possibly due to ejaculation failure or bilateral obstruction of mesonephric ducts' derivatives. Various congenital defects in epididymis and ductus deferens reported in boars are believed to be of genetic origin.[1,6] High ALP concentrations in ejaculate in this boar confirmed

epididymis and ductus deferens patency. This diagnostic method developed in dogs and stallions (where major sources of ALP include epididymis and testes) has been used in boars.[3,6] Findings in this boar are consistent with those of other species, demonstrating that semen ALP is a useful parameter for ruling out ejaculation failure or outflow obstruction as a cause of azoospermia in swine.

Testicular hypoplasia in domestic animals is due to genetic, endocrine, developmental defects. In boars, hypogonadotropic hypogonadism, chromosomal defects, and arrested spermatogenesis are causes of testicular hypoplasia.[1,7] In testicular hypoplasia, testes do not grow to a normal size because of reduced mass of seminiferous epithelium, but otherwise appear normal. Microscopic abnormalities seen with testicular hypoplasia may include a total lack of germ cells, uniformly arrested spermatogenesis or spermatogenic arrest that varies between tubules.[7]

In boars, there is a proliferation of Sertoli cells between birth and 1 month of age and then another significant proliferation between 3 - 4 months of age. Proliferation of Sertoli cells coincides with significant increase in seminiferous tubule length.[8] Boars are recognized to have rapid testicular growth at 4 - 6 months of age, followed by slow growth to 8 months of age. Testicular diameter only increases by 2 cm from 8 months of age to 2 years of age.[4] Thus, testicular dimensions at 8 months of age are a good representation of a boar's mature testicular size. While generally accepted standards for boar breeding soundness evaluation are lacking, similar to current case, suggested minimum acceptable testicular dimensions for a boar at 8 months of age are 8 x 5 cm.[5] A survey of 431 boars stratified by breed reported a range of average testicular lengths of 9.6 - 11.9 cm and average testicular widths of 5.0 - 5.7 cm in boars 6 - 11 months of age.[9] Measurements in this current case were ~ 1 cm shorter than reported average.

Given low ejaculate volume and marginal testicular size, spermatogenic arrest was considered. Azoospermia associated with spermatogenic arrest was reported in pigs, with testicular weight ~ half of that in normal boars.[1] Several reports linked this condition to genetics, which appears likely in this case, given familial history. In Finnish Yorkshire pigs, a defect in TEX14 gene was linked to spermatogenic arrest.[10] Another report described translocation of a segment of chromosome 13 to Y chromosome resulting in spermatogenic arrest and azoospermia.[11] Although these defects are considered rare in general population, spermatogenic arrest accounted from 9 of 16 cases of azoospermia in a large population of Finnish Yorkshire and Landrace boars.[1] Patient was discharged while awaiting testicular biopsy results and owner was not willing to pursue further. Limitation of this report was lack of genetic analysis.

Similar to testicular hypoplasia, testicular degeneration results in small testicular size, decreased semen production, and histologic abnormalities in seminiferous tubules. Testicular degeneration can be caused by extreme heat or cold, systemic disease, nutritional deficiencies, and trauma.[7] A boar suffering from testicular degeneration would likely have had evidence of reproductive success followed by a decrease in fertility leading to a clinical examination, whereas an animal with testicular hypoplasia will never achieve normal fertility. In this case, problem was recognized at first attempt of semen collection. Boar was presented in November, making exposure to extremes of heat or cold in preceding 3 months unlikely. In some species, testicular degeneration causes changes in testes that appear as hyperechogenic areas in testicular parenchyma,[12] which presumably is due to fibrosis and mineralization.[7] Although ultrasonographic changes in boars with testicular degeneration have not been described, echogenicity of testicular parenchyma in this boar was subjectively normal during ultrasound examination based on comparison to published images.[13] Given boar's age, good management at breeding facility, and a lack of prior disease or trauma based on history, testicular degeneration was unlikely. Given familial history of reproductive problems, testicular hypoplasia was most plausible explanation for clinical findings.

In this case, azoospermia was due to testicular hypoplasia. Familial history was highly suggestive of a genetic defect. Histologic appearance of testes in this case with Sertoli cells only populating some seminiferous tubules and only a few sperm in other tubules was consistent with hypoplasia; however, predominance of interstitial cells and near lack of seminiferous tubules makes histologic appearance in this case distinctly different than other cases of hypoplasia.

Learning points

- Measuring alkaline phosphatase concentrations in semen is a logical first step to rule out ejaculation failure or aplasia of epididymis or ductus deferens in cases of azoospermia.
- Testicular biopsy is next logical step in azoospermic boars with normal seminal plasma concentration of ALP to determine if testicular hypoplasia or degeneration is present.
- Familial history should be gathered in detail for boars with poor sperm production to identify related animals for semen evaluation and genetic testing to identify genetic defects that could affect fertility of future generations.

Conflict of interest

None to report.

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