Theriogenology Question of the Month

History

A 6-year-old 45-kg (99-lb) hemicastrated phenotypic male horned Pygmy goat was referred to the Auburn University Veterinary Teaching Hospital because of dysuria and poor appetite of 2 days’ duration. The goat had previously undergone surgeries (hemicastration) to remove 1 testis from the typical scrotal location (owner did not allow exploratory laparotomy for removal of the suspected abdominal testis) and bladder marsupialization to manage urinary obstruction secondary to calculi in the penile urethra.

At the time of admission to the veterinary teaching hospital, the urinary stoma was patent. No obstructions or strictures were detected during digital examination of the urinary stoma. Transabdominal ultrasonography revealed a nondistended urinary bladder, hypoechoic abdominal fluid that contained many fibrin tags, and 2 distinct, 4- to 6-cm-diameter, fluid-filled structures that contained heterogeneous, mixed echogenic fluid.

Abdominocentesis was performed, which yielded yellow and slightly opaque fluid. A Foley catheter was placed through the urinary stoma into the bladder. To assess integrity of the urinary bladder wall, methylene blue was infused into the urinary bladder via the Foley catheter. A second abdominocentesis was then performed, which yielded a foul-smelling, mucopurulent fluid but no evidence of methylene blue; this indicated that the urinary bladder wall was intact. Cytologic examination of the fluid revealed rod-shaped bacteria and degenerate neutrophils. Because of the evidence of sepsis and deteriorating condition of the goat, exploratory laparotomy was selected as the best diagnostic and therapeutic option.

The goat was anesthetized and placed in dorsal recumbency. Celiotomy was performed via a ventral midline incision. Approximately 1 L of clear fluid containing fibrin strands was removed from the abdominal cavity. Exploration of the abdomen revealed a dilated, fluctuant, bilobed soft tissue structure that resembled a uterine body and horns (Figure 1). The right uterine horn was larger than the left, and a gonad-like structure was identified at the proximal end of the left uterine horn. Approximately 1.8 L of mucopurulent fluid was aspirated from the uterus. A soft tissue connection existed between the right uterine horn and apex of the urinary bladder, but no connection was detected between the lumens of the 2 organs. The apex of the urinary bladder was dissected from the right uterine horn, and the broad ligaments were dissected to facilitate removal of the uterus. The uterus and gonad-like structure were excised and submitted for histologic examination. The abdomen was lavaged with isotonic saline (0.9% NaCl) solution and closed in a routine manner.

Question

What are the 3 most common developmental abnormalities that could lead to the gross reproductive anomalies seen in this goat? Please turn the page.

Figure 1—Photograph of a fluctuant, bilobed, soft tissue structure resembling a uterine body and horns and a gonad-like structure at the end of the short left uterine horn found in the abdominal cavity during exploratory laparotomy of a 6-year-old hemicastrated phenotypic male horned Pygmy goat.

ABBREVIATION

PIS Polled intersex syndrome
**Answer**

Polled intersex syndrome, freemartinism, and persistent Mullerian duct syndrome.

**Results**

Histologic examination of the bicornuate structure confirmed that the organ was a uterus, with mucosal folds covered by stratified squamous epithelium and swollen cells resembling epithelium under the influence of progesterone (Figure 2). Mild to moderately dilated endometrial glands bordered an underlying layer of smooth muscle. Extensive submucosal inflammatory infiltrates indicated pyometra. The gonad-like structure consisted entirely of adipose tissue and could not be classified as a testis, ovary, or ovotestis.

The preferred diagnostic tests to determine the cause of the condition for the goat described here included karyotyping of peripheral blood cells (lymphocytes) and somatic cells (fibroblasts) and testing to detect Sry. Somatic tissues collected via punch biopsy of the skin and a blood sample collected from a peripheral vein were submitted for karyotype analysis. In some animals with chromosomal abnormalities, only peripheral blood cells have been analyzed to determine karyotype; however, both lymphocytes and somatic cells (fibroblasts) were analyzed for this goat.

The karyotypes for clinically normal goats are 60,XX (female) and 60,XY (male). Two cell lines were identified in the lymphocytes (n = 165 cells) submitted from the goat. There were 162 (98%) lymphocytes with 60,XX, and the other 3 (2%) lymphocytes were 60,XY (Figure 3). The 60,XY lymphocytes had positive results when tested for male-specific Sry, which confirmed the presence of a Y chromosome. These findings were consistent with 60,XX/60,XY blood chimerism.

Two cell lines were identified in the fibroblasts (n = 185 cells). There were 155 (84%) fibroblasts with 60,XX, and the other 30 (16%) fibroblasts were 90,XXY (Figure 3). The 90,XXY cells had positive results when tested for male-specific Sry, which confirmed the presence of a Y chromosome. These results indicated a diploid-triploid mixoploidy in the somatic cells (fibroblasts).

Additional microsatellite analysis was performed on both lymphocytes and fibroblasts to obtain information on the genotype. Typically, the microsatellites of a subject are compared with results for maternal and paternal samples to determine the origin of the additional chromosomes. In this case, the goat was purchased as a kid, and samples could not be obtained from the maternal or paternal lines for analysis. Instead, microsatellite-derived molecular markers from the goat were compared with markers obtained by the testing facility from 17 clinically normal goats. Given the 90,XXY (triploid) karyotype of 16% of the fibroblasts, the presence of 3 alleles was predicted; however, only 2 could be elicited in all cell lines.

Results of the karyotype analysis indicated that the intersex condition in this goat was not attributable to one of the more common disorders of sexual development. Rather, it was most likely attributable to a 60,XX/90,XXY diploid-triploid mixoploidy.

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**Figure 2**—Photomicrographs of tissue sections obtained from the uterus and gonad-like structure excised from the goat in Figure 1. A—Mucosal folds covered by squamous cell epithelium (black asterisk) are evident. There are many neutrophils, lymphocytes, macrophages, plasma cells, and areas of hemorrhage scattered throughout the superficial submucosal layer (arrow). B—A higher magnification view of the mucosa revealed swollen cells resembling squamous epithelium under the influence of progesterone (arrow). Notice the extensive submucosal inflammatory infiltrates (arrowheads) indicative of a pyometra. C—The presumed gonadal tissue consisted entirely of adipose tissue; thus, the structure could not be classified as a testis, ovary, or ovotestis. H&E stain; bar = 750, 75, and 150 µm in panels A, B, and C, respectively.
Discussion

Sexual differentiation in mammals is categorized at 3 levels: chromosomal, gonadal, and phenotypic. The most likely differential diagnoses for intersex conditions in goats are PIS, freemartinism, and persistent Mullerian duct syndrome. However, none of these potential differential diagnoses accounts for all of the abnormalities in the goat of the present report. For the goat described here, mixoploidy (60,XX/90,XXY) was responsible for the anomalous development, and diagnosis was only possible through the use of karyotyping of fibroblasts and cultured lymphocytes.

One of the most common disorders of sexual development in goats is PIS, a genetic intersex condition that has been linked to the polled gene.1 The most common abnormal condition associated with PIS is a sex-reversed female (60,XX) that does not express Sry (ie, male pseudohermaphrodite with a female genotype). The mutation is the result of an 11.7-kb deletion to the PIS gene; the result is a loss in transcriptional regulation for a factor (Forkhead box L2) that typically inhibits male gonadal differentiation.1 This loss of inhibition for testicular development results in the formation of testis-like gonads, variable development of the tubular reproductive tract, and inconsistent phenotype, secondary sex characteristics, and sexual behavior.1 The presence of Sry in the horned goat of the present report made PIS unlikely, despite variability in potential phenotypic development and the inability to accurately classify the gonads.

Fusion early in gestation of placental blood vessels between fetuses of the opposite sex leads to XX/XY chimeraism, with the female twin sometimes displaying a freemartin phenotype.2 There are numerous reports of XX/XY chimeraism in goats. The presence of both 60,XX and 60,XY lymphocytes in the goat of the present report is consistent with freemartin syndrome; however, there may be blood cell chimeraism in phenotypically normal females, and this is not considered diagnostic for freemartinism in goats. Goats are a polytocous species that routinely give birth to twins and triplets. Therefore, the placenta of goats is prone to fusion and vascular anastomosis during gestation. Freemartinism classically is evident as an animal with female external genitalia, incomplete development of the tubular reproductive tract, and variable gonadal development (commonly testes); animals are genetically female (60,XX) in somatic cells (fibroblasts) but chimeras (60,XX/60,XY) in blood cells. The presence of diploid (60,XX) and triploid (90,XXY) fibroblasts in the goat of the present report indicated a more complex situation, with freemartinism alone likely not accounting for all the developmental abnormalities.
Persistent Müllerian duct syndrome is characterized by a male genotype (60,XY) and physiologically normal testicular development accompanied by variable amounts of a female tubular reproductive tract. Anti-Müllerian hormone, which is a glycoprotein secreted by sustentacular (Sertoli) cells, mediates the regression of the paramesonephric ducts in males; therefore, mutations in the genes encoding anti-Müllerian hormone or its receptors result in the persistence of Müllerian-paramesonephric ducts and development of the female tubular reproductive tract in the presence of testes. This condition has been described in goats. In the goat of the present report, gonadal classification was not available and persistent Müllerian duct syndrome could have accounted for both the male and female phenotypic characteristics found. However, genotypic determination indicated a more complicated situation.

Deviation of karyotypes consisting of abnormal sex chromosomes (eg, aneuploidy) or the coexistence of cells with different sex chromosome constituents (eg, mosaicism or chimerism) is the main cause of anomalies of sex determination and sex differentiation in domestic species. Variation in the clinical syndromes and anatomic anomalies associated with the classic disorders of sexual development makes it essential to perform karyotyping to obtain a definitive diagnosis. Karyotypes for the goat of the present report indicated an interesting genetic anomaly and potentially accounted for the intersex condition. The genotype in the lymphocytes and somatic cells illustrates chimerism, in which an individual has >1 genetically distinct population in a line of cells that originated from >1 zygote. Conversely, the unmatched genotypes between lymphocytes and somatic cells reported here illustrated chromosomal mosaicism, whereby cells within an individual have different genetic makeup and originated from only 1 zygote. The disappearance of the aneuploid (eg, triploid) cells from peripheral blood cells appears to be a common yet unexplained phenomenon reported in humans with mosaicism. In the goat of the present report, Sry was detected in both somatic cells and peripheral lymphocytes, so the presence of a uterus cannot be attributed to lack of Sry. Microsatellite analysis can provide useful information about intersex animals because of its applications in linkage analysis and use in tracing inheritance patterns; however, the detection of 2 alleles did not define the source of the genetic anomalies. Instead, multiple theories may be considered regarding each chromosomal anomaly.

The most likely scenario for the abnormalities in the peripheral lymphocytes is that a somatic diploid-triploid embryo was formed and shared blood through placental anastomosis with a sibling that contributed the 60,XY lymphocytes (as with freemartinsm). A less likely scenario would be the possibility that the goat was the chimeric product of the fusion of 2 conceptuses (a 60,XY conceptus and a diploid-triploid 60,XX/90,XXY conceptus). The most probable explanation for the abnormalities in the somatic cells is delayed secondary fertilization whereby an ovum was initially fertilized by a spermatozoa carrying an X chromosome and subsequently was fertilized (polyspermy) by a spermatozoa carrying a Y chromosome, which thus created a triploid cell line. The detection of only 2 alleles via microsatellite analysis might be explained if the 3 genomes in the triploid cells had at least 2 alleles in common. An alternate possibility could include fertilization of the zygote followed by incorporation of the second polar body, which would result in triploid cells. This is consistent with microsatellite analysis results but is unlikely because the diploid cell line would have been 60,XY, rather than 60,XX. An even less likely but rare possibility is the fusion of diploid and triploid conceptuses. Whole-body chimerism in which a substantial mixture of XX and XY cells is distributed throughout the various organs and tissues of the body of sheep and goats has been described; this is postulated to result from fusion of embryos prior to the development of the placenta. Experimental fusion of sheep embryos at the blastocyst stage results in a chimeric intersex animal, thus confirming that embryo fusion is a possible mechanism leading to chimerism. Unfortunately, the reproductive anatomy of those intersexes was not described in that study.

A final potential explanation is that the ovum failed to complete meiosis II and began to develop parthenogenetically; the zygote was then fertilized, which created triploid cells, and developed as a result of the paternal contribution. However, this possibility is unlikely because once an ovum begins to develop parthenogenetically, it usually does not complete development.

These genetic findings are important for a diagnosis of intersex animals in the future. Mixoploidy is rarely reported in small ruminants, and to the authors’ knowledge, it has not been reported in combination with blood cell chimerism or mosaicism, which illustrates the need for karyotyping of peripheral blood cells as well as somatic cells in animals with suspected genetic anomalies. With the increasing availability of cytokinetics and molecular analyses, reports of mixoploid animals may increase. Also, in vitro fertilization, which is more likely to result in a higher number of mixoploid embryos, is becoming more common in the animal production industry. Therefore, it is important for breeding program personnel to be familiar with the numerous ways to screen for certain genetic developmental anomalies that affect fertility and reproductive capacity.

Outcome

The goat recovered from surgery without complications. After surgery, the goat received systemically administered antimicrobials (ceftriaxone sodium, 3.3 mg/kg [1.5 mg/lb], IV, q 24 h) and an NSAID (flunixin meglumine, 2.2 mg/kg [1 mg/lb], IV q 24 h) for 6 and 3 days, respectively. The goat made an uneventful recovery and was discharged to the owner.

References