applied cytology

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Infertility in a Dog

A 9-year-old male Labrador retriever presents with a history of possible infertility.

History. The dog is actively being campaigned at field trials. Over the past 7 years, he has been bred to over 50 bitches with conception rates on average over 80% and average litter size of 6 or more puppies. Early last year, the owner noted some decrease in litter size; in the past few months, there have been few pregnancies and the matings that have resulted in pregnancy have been singleton litters.

Physical Examination. The dog was bright, alert, responsive, and in good body condition. Temperature, pulse, respiratory rate, and mucous membrane examination were normal. Auscultation of the thorax and abdominal palpation were within normal limits. There was no lymphadenopathy.

Digital examination of the prostate per rectum revealed a small, smooth, symmetrical gland. Palpation of the scrotum revealed no abnormalities of the scrotal skin. The testicles were less turgid than normal but symmetrical in size and shape. There were no palpable masses. The epididymides and spermatic cords were within normal limits of size, shape, and texture.

Imaging. Ultrasonographic measurement of the testes revealed total scrotal width to be 3.5 cm. There were no masses noted on ultrasonography, but there were multiple pinpoint hyperechoic lesions throughout the testicular parenchyma of both testes.

Laboratory Analysis. Serum biochemistry (including TT₄ and fT₄-ED), complete blood count, and urinalysis were within normal limits. Rare sperm were noted on sediment examination of the urine. Semen collection was performed using manual stimulation in the presence of an estrous teaser bitch. The results of semen evaluation are shown in the Table.

A sample of the ejaculate was stained with eosin-nigrosin and allowed to air dry (Figures 1 through 3). Another sample of the ejaculate was stained with modified Wright's-Giemsa to evaluate round cells (Figure 4).



Eosin-nigrosin stain; original magnification, 40×



Eosin-nigrosin stain; original magnification, 100× (oil immersion)

Results of Semen Evaluation	
5 ml	
30%	
20%	
4/5	
2 to 5	
85 million spermatozoa	
425 million spermatozoa	
500	

 fT_4 -ED = free thyroxine measured by equilibrium dialysis; lpf = lower power field; TT_4 = total thyroxine

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Eosin-nigrosin stain; original magnification, 100× (oil immersion)



Wright's-Giemsa stain; original magnification, 100× (oil immersion)

ASK YOURSELF ...

- What are the primary defects noted in Figures 1 through 3?
- What is the round cell in Figure 4?
- If these sperm are representative of the ejaculate, what is the morphologic assessment of the semen sample?

Orthopedic Bioscaffold

TR MATRIX (IMEX Veterinary,

www.imexvet.com), a new osteopromotive bioscaffold for veterinary orthopedic conditions (eq, fractures, bone defects, delayed unions, osteotomies, nonunions, arthrodeses), is now available. According to the manufacturer, "Host cells bind to [the material] and recognize amino acid sequences resulting in activation of physiologic tissue repair." It is available as a vial of 1gram, shelf-stable, lyophilized powder.—Press release 4/4/08

Leukocyte ID Guide

The American Society for Veterinary Clinical Pathology (www.asvcp.com) and Heska (www.heska.com) have developed a 1-page, 2sided, waterproof visual hematology guide for leukocyte identification. It is being distributed to all North American veterinary schools; individuals can request a copy by calling 800-464-3752 (literature code 240300).—Press release 4/4/08

Animals in Disaster

The 2008 National Conference on Animals in Disaster will be held June 2 to 6 in Sacramento, California. The conference, organized by The Humane Society of the United States, will cover topics such as improving evacuation, rescue, and sheltering policies; connecting with government management agencies; field rescue operations; and planning and response. Visit www.humanesociety.org/ncad2008 for conference information.—Press release 3/6/08

continues

Diagnosis: Primary testicular degeneration

Cytologic Assessment. Morphologic characteristics must be evaluated on fixed, stained semen samples (see Box) under oil immersion $(100\times)$, counting a minimum of 100 cells. Use of low to medium ($10 \times$ to $40 \times$) power wet mounts to assess sperm morphology often results in many missed or misclassified morphologic defects. Use of formol-buffered saline to fix the spermatozoa and subsequent assessment under high power, using phase contrast or differential interference contrast microscopy, is also acceptable but may be unavailable to the general practitioner.

Assessment of the sample should define defects as primary (occurring in the testicle during spermatogenesis) or secondary (occurring during epididymal storage, ejaculation, or semen handling). More than 70% normal sperm is considered acceptable.

Sample Preparation

After semen is collected, it should be maintained at room temperature. All pipettes and slides coming in contact with the semen should be warmed to 37°C.

- A drop of eosin-nigrosin stain is placed on a warm slide, followed by a drop of semen. The slide is gently rocked to mix the stain and semen completely.
- After 30 seconds of incubation with the stain, the drop is pushed gently across the slide using the leading edge of a second microscope slide. This should result in a thick layer of stained semen on the slide.
- The slide is then quickly air-dried.

Rough handling of the sample during preparation can result in artifacts (detached heads, reflexed midpieces, or coiled and bent tails). It is imperative to stain a portion of the sample with Wright's-Giemsa to differentiate white blood cells from germ cells.

DID YOU ANSWER ...

- Distal midpiece reflexes, bent midpieces, detached heads, pyriform heads, knobbed acrosomes
- Germ cell
- Teratozoospermia, asthenozoospermia, and oligozoospermia

Morphologic assessment in this case (Figures 1-3) revealed 15% normal sperm, 34% distal midpiece reflexes, 5% bent midpieces, 16% detached heads, 20% pyriform heads, and 10% knobbed acrosomes. Two large, binucleate germ cells are seen on the slide stained with Wright's-Giemsa (Figure 4).

Discussion. This sample is typical of a dog with primary testicular degeneration. An increase in primary defects is common-specifically,

increased numbers of defects of the sperm head and acrosome, primary defects of the midpiece, and high numbers of detached heads (indicative of abnormalities in midpiece attachment to the head) are noted with primary testicular disease and testicular degeneration. In some cases there may be small to large round cells (germ cells or white blood cells) in the sample. The number of germ cells increases as testicular degeneration progresses.



Spermatozoa. Note the distal midpiece reflexes (arrows), bent midpieces (arrowheads), and detached heads (vellow arrows).



Sperm heads. Note the pyriform heads (arrows); a more subtle pyriform head is noted with the arrowhead.



Sperm head. Note the knobbed acrosome (arrow).

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Large round cells (arrows)

Potential causes of primary testicular degeneration include senescent change, autoimmune orchitis and epididymitis, drugs (anabolic or sex steroids, long-term use of nonsteroidal antiinflammatory drugs or glucocorticoids, environmental toxins), chronic endogenous glucocorticoid release (stress), chronic orchitis/ epididymitis or prostatitis, Brucella canis infection, neoplasia, scrotal overheating, or pyrexia associated with systemic illness.

Further Diagnostics. Fine-needle aspiration or testicular biopsy may provide a definitive diagnosis, and in some cases, the cause of the disorder. Fine-needle biopsies typically have low cell density and spermatogenic cells of varying stages of maturation (large round cells to mature spermatozoa). Sertoli cells are readily aspirated while Leydig cells are not.

Assessment of thyroid function, specifically autoantibodies to T₃, T₄, or thyroglobulin, may be helpful, as there has been a hereditary association between autoimmune thyroid disease and autoimmune orchitis and epididymitis. The semen should be evaluated again in 60 days to determine if the abnormalities are permanent or transient.

See Aids & Resources, back page, for references, contacts, and appendices. Article archived on www.cliniciansbrief.com

Animal Chiropractics

A bill that is awaiting vote by the Minnesota state legislature would allow human chiropractors to practice on animals as long as they had 210 hours of additional training. Critics of the legislation say the training doesn't suffice and that veterinarians should evaluate an animal before it sees a chiropractor. The bill, which would be the broadest in the nation if approved, requires a chiropractor to contact the animal's veterinarian after the first visit.—Minnesota Public Radio 3/25/08

Tamper-Resistant Scripts

SECURE RUB RX



PAPER (Nocopi Technologies, Inc, www.nocopi.com) offers fraud-resistant prescription pads based on new technology. If someone tries to erase a number, the background turns blue, signaling a problem or, if

rubbed hard enough, an entire area will be removed.—Philadelphia Inquirer 4/4/08

Canine Elbow Replacement System

TATE (BioMedtrix, www.biomedtrix.com), total arthroplasty of the elbow, is a new system for canine elbow replacement. It promises minimally invasive resurfacing arthroplasty with milling based on the axis of the center of rotation. TATE was developed with the goal of surgical ease, low morbidity, and a low complication rate. For more information, including training workshops, visit www.biomedtrix.com.—Press release 3/7/08

 T_3 = triiodothyronine; T_4 = thyroxine