Breeding Soundness Evaluation of the Stud Dog

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The Stud Dog…

“A near-extinct species that continues to be decimated by humans with sharp knives” - Unknown
Overview

• Review of clinically relevant
  – Reproductive physiology
  – Anatomy

• The breeding soundness evaluation as a clinical and management tool
  – Goals
  – Indications
  – Limitations
  – Procedures
  – Classification

• Reproductive dysfunction
Reproductive Physiology

Descent of the Testes

- Testes are intra-scrotal in **normal** males by 14 days after birth
  - Males in which both testes are not fully descended by this time should NOT be retained as breeding stock.

- Latest likely time of descent is 6 months in any breed
  - This is NOT normal!
Cryptorchidism in the Dog

• Heritable as a sex-limited autosomal recessive trait

• Breeds at increased risk
  – Many toy and miniature breeds
  – Boxer
  – Old English Sheepdog

• Castration to decrease potential complications
  – Neoplasia of testis/es retained in abdomen
  – Spermatic cord torsion
  – Priapism, paraphimosis
Reproductive Physiology

Puberty

- **Puberty**
  - Requirements
    - Behaviors consistent with successful “courtship”, intromission and copulation
    - Ejaculate contains minimum number of spermatozoa required to achieve pregnancy
  - On average, 9 months (5-12 months, range)
  - Age of attainment dependent upon
    - Breed
    - Body condition/plane of nutrition
Reproductive Physiology

Testes

- **Sperm production**
  - Spermatogenic cycle requires 62 days for a sperm to develop and be capable of fertilization

- **Endocrine gland**
  - Testosterone – Leydig cells
  - Estrogen – Sertoli cells

- **Blood-testis barrier**
  - Tight junctions between Sertoli cells
  - Adluminal compartment is “immuno-privileged”
Reproductive Anatomy
Testicular/Epididymal Orientation

Long axis of testis roughly horizontal
Epididymal tail caudal (near tail of animal); body dorsal (on top)
Reproductive Anatomy and Physiology

Epididymis

• A SINGLE coiled duct!!!

• Components
  – Head
  – Body
  – Tail

• Functions
  – Sperm maturation: requires final ~ 14 of 62 days of spermatogenic cycle
    • Acquisition of motility
    • Alterations in plasma membranes
    • Achievement of fertilization competence
  – Storage until ejaculation
Reproductive Anatomy and Physiology

Prostate

- Development and maintenance of function is testosterone-dependent!!!
- Functions
  - Secrete components of seminal plasma, facilitating delivery of sperm
  - Large volume of prostatic fluid emitted during the copulatory tie
Reproductive Anatomy
Musculocavernous Penis

- Blood engorgement of cavernous spaces increases diameter substantially during erection
- Os penis facilitates intromission prior to achievement of complete erection
The Breeding Soundness Evaluation
What is It?

• A complement of examinations and evaluations that allow us to formulate an opinion as to whether or not a male is capable of breeding and successfully impregnating (a given number of females under prevailing industry management conditions)
The Breeding Soundness Evaluation
What is It Not?

• A guarantee of fertility
  – Breeding sound does not guarantee fertility
  – Poor performance on a breeding soundness evaluation does not necessarily mean that a male is sterile or infertile. He is, however, subfertile.
The Breeding Soundness Evaluation

Goals

- Selection of genetically superior individuals to promote continued genetic improvement across a breed
- Discrimination against heritable diseases
The Breeding Soundness Evaluation

General Indications

• Selection of individuals to retain/promote as breeding stock?
• Pre-sale/pre-purchase
• Prior to use
• Subfertility (infertility)
Breeding Soundness Evaluation

Indications

• Bitch owner wants “guarantee” prior to committing
  – Scheduling and transporting dogs for breeding is labor intensive and expensive
  – Often

It’s cheaper to mail my sperm than to buy the bitch a plane ticket!
Breeding Soundness Evaluation

Indications

- Stud dogs generally not presented unless there is a (perceived) problem
  - “He missed his last bitch”
  - “He doesn’t like the bitch” or “he doesn’t like any bitches” or “he doesn’t like the doctor’s hand”
  - “He acts funny when he poops” or “his penis is bleeding”

I’d rather jump off of here!
The Breeding Soundness Evaluation

Limitations of the Evaluation

• Results are valid only for the day of examination and for the conditions under which it was performed

• Fertility is…
  – Multifactorial
  – Difficult to evaluate completely given the limitations of the examinations available to us, particularly microscopy
The Breeding Soundness Evaluation

Components

• History and signalment
  – Primarily as it relates to reproductive performance
  – But also general health, musculoskeletal disease/lameness
• General physical examination
• Examination of the external genitalia
• +/- Direct examination of the prostate
• +/- Evaluation of libido and breeding behavior
• Semen collection and evaluation
• +/- Testing for the presence of infectious diseases
• +/- Testing for the presence of genetic diseases
• Collation of data and pronouncement of classification
Breeding Soundness Evaluation

Signalment

• Age: Pubertal vs. mature vs. geriatric
• Breed
  – Size of gene pool affects fertility
  – Heritable defects
• Use
  – Show
  – Hunting/field trial
  – Performance
  – Working
  – Breeding
Breeding Soundness Evaluation

History

• Breeding
  – Has the dog bred?
  – If so, by what method(s)?
  – What was/were the outcome(s)?
    • Pregnancy rate
    • Litter size

Expected pregnancy rate*:
Natural service >75%
Artificial insemination 50-90%
Breeding Soundness Evaluation

History

• Breeding
  – Has the dog had semen collected and evaluated?
  – Has he been tested for antibodies against *Brucella canis*?
    • Most recent test date?
    • Result?
  – Any other reproductive problems

• General health
  – Routine preventive care
  – Other medications
  – Nutraceuticals
Breeding Soundness Evaluation
General Physical Examination

• General health
  – Body condition
  – Skin lesions
  – Evidence of endocrine disease

• Musculoskeletal defects that would interfere with ability to breed

• Although not part of a BSE, certifications may be relevant
  – OFA hips, elbows
  – CERF
  – Cardiac
Breeding Soundness Evaluation
General Physical Examination

• Heritable defects
  – Eyes and adnexa
  – Dentition
  – Cryptorchidism
  – Hernias
  – Musculoskeletal conformation
  – Many, many others that are not readily apparent and would require detection via genetic testing

www.covemountainkennels.com
Breeding Soundness Evaluation

Heritable Diseases Resources

• Ever expanding identification of heritable diseases and available genetic tests
• Canine Health Information Center (CHIC)
  • Database maintained by OFA
  • Recommended pre-breeding screening evaluations and tests by breed
  • Pedigree analysis available to breeders for proposed matings
  • www.caninehealthinfo.org
• “The Veterinary Medical Guide to Dog and Cat Breeds”
  • Comprehensive guide to genetic disease predispositions by breed
  • Listing of testing centers
Breeding Soundness Evaluation

Examination of Scrotum and Its Contents…
aka, “Checking under the Hood”

- Scrotal integument
  - Smooth and thin
  - Free of lesions (dermatitis)

- Scrotal contents
  - Spermatic cord
  - Epididymides
  - Testes

- Owners should be performing this exam once monthly!
Breeding Soundness Evaluation

Examination of Scrotum and Its Contents…
a.k.a., “Checking under the Hood”

• Epididymides
  – Symmetrical
  – Asymmetrical: epididymitis, segmental aplasia

• Spermatic cords
  – Symmetrical
  – Lesions rare
Breeding Soundness Evaluation
Examination of the Testes

• Location
  – Both must be IN the scrotum
  – One may sit slightly cranial to other
  – Freely movable

• Size
  – Testes size roughly correlated with normal body weight for breed
  – Objective measurement of scrotal width using calipers or ultrasound helpful if serial examinations likely

• Consistency
  – Too soft: hypoplasia, degeneration
  – Too firm: neoplasia, terminal fibrosis
Table 16-3. Influence of Body Weight on the Reproductive Capacity of Adult Dogs*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>10-34</th>
<th>35-39</th>
<th>60-84</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total scrotal testes width (mm)</td>
<td>36 ± 2§</td>
<td>50 ± 1†</td>
<td>56 ± 1**</td>
</tr>
<tr>
<td>Paired testes weight (g)</td>
<td>16 ± 1§</td>
<td>31 ± 1†</td>
<td>44 ± 2**</td>
</tr>
<tr>
<td>DSP/g parenchyma (10⁴)</td>
<td>20 ± 2</td>
<td>17 ± 1</td>
<td>20 ± 3</td>
</tr>
<tr>
<td>DSP/dog (10⁴)</td>
<td>287 ± 33</td>
<td>472 ± 32</td>
<td>750 ± 111</td>
</tr>
<tr>
<td>Exagonadal spermatozoal reserves (10⁴) at sexual rest</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caput epididymidis</td>
<td>0.07 ± 0.01</td>
<td>0.23 ± 0.04</td>
<td>0.23 ± 0.05</td>
</tr>
<tr>
<td>Corpus epididymidis</td>
<td>1.10 ± 0.18</td>
<td>1.85 ± 0.16</td>
<td>2.27 ± 0.24</td>
</tr>
<tr>
<td>Cauda epididymidis</td>
<td>2.06 ± 0.31</td>
<td>3.30 ± 0.36</td>
<td>4.68 ± 0.39</td>
</tr>
<tr>
<td>Ductus deferens†</td>
<td>0.06 ± 0.02</td>
<td>0.21 ± 0.03</td>
<td>0.23 ± 0.04</td>
</tr>
<tr>
<td>Semen ejaculated after sexual rest</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume (ml)‡</td>
<td>2.4 ± 0.3</td>
<td>3.9 ± 0.5</td>
<td>5.4 ± 1.3</td>
</tr>
<tr>
<td>Concentration (10⁶/ml)‡</td>
<td>209 ± 42</td>
<td>359 ± 72</td>
<td>228 ± 58</td>
</tr>
<tr>
<td>Total sperm (10⁹)</td>
<td>0.4 ± 0.11</td>
<td>1.12 ± 0.13</td>
<td>1.43 ± 0.46</td>
</tr>
</tbody>
</table>

* Mean (± SEM) for 30, 53, and 32 dogs in the 10-34, 35-59, and 60-84 lb groups, but data on exagonadal spermatozoal reserves are for 17, 32, and 14 dogs and data for characteristics of a single ejaculate collected after ≥7 days of sexual rest are for 12, 14, and 11 dogs, respectively. DSP = daily spermatozoal production. For a characteristic, means without a superscript symbol (§, †, ‡) or with the same superscript symbol are not different (p > 0.05).
† Not all of the ductus deferens was available for dogs castrated rather than euthanized.
‡ The presperm and sperm-rich fractions were collected together, but ejaculation was terminated when ejaculation of the postsperm prostatic fluid started.

Breeding Soundness Evaluation
Testicular Hypoplasia

- Inadequate number of germinal cells present from birth
- Bilaterally small testes and epididymal tails
- Low number of sperm, many of which are abnormal
- Etiology
  - Sporadic but with familial predisposition
  - Chromosomal abnormalities, e.g., XXY “males”
- No treatment or management method will improve semen quality
Breeding Soundness Evaluation

Testicular Degeneration

• Clinical signs
  – Decreased testicular size
  – Prominent epididymal tails
  – Altered testicular consistency
    • Soft, flabby – acute
    • Very firm, fibrotic - chronic
  – Normal libido
  – Abnormal spermiogram
    • Decreased sperm count (oligospermia) to complete absence of sperm (azoospermia)
    • Abnormalities of sperm structure
      – Usually marked
      – Principally primary defects
Breeding Soundness Evaluation
Testicular Degeneration: Causes

- Thermal-induced
  - Insulation
    - Excessive hair
  - ↑ Temperature
    - Fever
    - ↑ Environmental temperature
- Inflammation
- Infection
Breeding Soundness Evaluation
Testicular Degeneration: Causes

• Autoimmune
  – May be associated with autoimmune thyroiditis

• Toxins
  – Estrogens (Sertoli cell tumor)

• ↑ Corticosteroids → ↓ LH secretion
  – Endogenous secondary to stress (social, physiologic)
  – Exogenous administration

• Age-related/senile**

• Idiopathic**

Sertoli cell tumor in stallion
Breeding Soundness Evaluation
Testicular Degeneration

• Diagnosis
  – Clinical signs and spermiogram highly suggestive
  – Testicular biopsy (rarely performed)
  – Hormone concentration measurements of limited utility

• Prognosis highly dependent upon
  – Etiology
  – Bilateral or unilateral
  – Duration of insult
Breeding Soundness Evaluation

Testicular Hypoplasia vs. Degeneration

How do you know the difference?

Small testes + proportionally small epididymides = testicular hypoplasia

Small testes + relatively large epididymis = testicular degeneration
Breeding Soundness Evaluation
Semen Collection

Now, into the cubicle and give us a sample…
Breeding Soundness Evaluation
Semen Collection...and More

• “Setting the mood”
  - Estrus teaser
    • “Size-matched” is optimal
    • Maximizes total sperm count
  - Non-estrus bitch
  - Secretions from estrus bitch
    • Swab or 4”x4”
    • Designated rug
  - No stimulus at all
Breeding Soundness Evaluation

A Note Regarding “Inherently Poor Libido”

• Over-diagnosed by owners
• RARE!!!
  – Can be associated with
    • Abnormality of sexual development
    • Estrogen-secreting neoplasia (Sertoli cell tumor)
  – Most commonly misdiagnosis of a management problem
Breeding Soundness Evaluation

Immaturity or Inexperience

• Successful copulation requires practice, particularly in dogs
• Physical demands of copulation require muscles, nerves, postures that have not been used before
• “Practice” often involves sorting out social interactions first
• Stud dogs, particularly inexperienced ones, should never be taken to a bitch’s territory for mating, especially if she is very dominant
Breeding Soundness Evaluation

Psychogenic Impotence

- In absence of physical impediment in a mature animal, most cases have management problems at their origin
  - Negative reinforcement for exhibition of sexual behavior
    - Dogs disciplined harshly for interest in other canines in show settings
    - Dogs disciplined for interest in bitches in heat in own home → may require breeding on neutral territory and in absence of owner
Breeding Soundness Evaluation

Unwillingness/Inability to Mount

• Male unable or too painful to mount due to a physical abnormality
  – Musculoskeletal
    • Poor conformation predisposing to development of joint pathology
    • Chronic athletic demands or injuries in performance dogs
  – Neurologic
    • Lumbosacral disease
    • Degenerative myelopathy
  – Inadequate facilities
    • Slippery floors preventing sufficient traction
    • Use throw rugs, yoga mats, etc.
Breeding Soundness Evaluation
Semen Collection…and More

• Tools of the trade
  – Semen collection apparatus
    • Rubber cone with vial at end
    • Funnel with collection vial
    • +/- Warm water bath
  – Lubricant
  – +/- Examination gloves
Breeding Soundness Evaluation
Semen Collection...and More

- Tricks of the trade
  - Stimulate bulbus glandis through the prepuce
  - Retract prepuce to exteriorize bulb prior to full engorgement
  - Clamp proximal non-erectile portion of penis circumferentially
  - Retroflex penis and allow male to “tie”
Breeding Soundness Evaluation
Semen Collection…and More

- Opportunity to examine penis and prepuce
  - Chronic, healed injuries
  - Foreign bodies and/or infection in preputial cavity
  - Mass lesions on preputial integument
  - Urethral prolapse
Breeding Soundness Evaluation
Semen Collection…and More

• Collect 2\textsuperscript{nd} and 3\textsuperscript{rd} fractions into separate tubes
  – 1\textsuperscript{st}: pre-sperm, clear
  – 2\textsuperscript{nd}: sperm-rich, opaque
  – 3\textsuperscript{rd}: prostatic, clear
• Lubricate bulbus glandis
• Remove teaser bitch
• Assure that penis has detumesced and returned to prepuce!!!
Breeding Soundness Evaluation

Semen Evaluation

- Color and opacity
- Volume
  - Fraction #2: 0.5-2.0 mL
  - Fraction #3: up to 30 mL in large breeds
- Progressive motility
- Morphology
- Concentration
Breeding Soundness Evaluation
Semen Evaluation - Motility

• Progressive motility (PM)
  – Instruments and disposables should be warm (~35°C)
  – Canine sperm should swim forward in a straight line
  – Estimate % that are PM, % abnormally motile, % dead
  – Normal: >70-80% PM sperm
  – Dilution may be necessary in order to make accurate determination
    • Physiologic saline
    • Semen extender
Breeding Soundness Evaluation

Semen Evaluation - Morphology

- Morphology
  - Preparation of sample
    - Eosin-nigrosin stain, light microscopy, 1000x oil
    - Buffered formyl saline, phase contrast microscopy, 1000x oil*
  - Count at least 100 sperm
  - Abnormal sperm defects classified
    - <10% primary defects
    - <20% secondary defects
Breeding Soundness Evaluation
Semen Evaluation - Morphology

- Morphology: classification schemes
  - Primary vs. secondary vs. tertiary defects
    - Morphologic abnormalities classified largely as to organ of origin, with a few exceptions
    - Primary: testes; defect in production
    - Secondary: epididymis; abnormality in latter stages of maturation or while in storage
    - Tertiary: post-ejaculatory/iatrogenic (may be difficult to differentiate from secondary)
**Breeding Soundness Evaluation**

**Semen Evaluation - Morphology**

- Normal canine sperm
  - Head
    - Acrosome
    - Equatorial region
    - Post-acrosomal region
  - Midpiece
    - Slightly narrowed neck
    - Axial attachment
  - Tail

Breeding Soundness Evaluation
Semen Evaluation – Primary Defects

Knobbed (lipped) acrosome
Macrocephalic
Pyriform head
Diadem defect

Breeding Soundness Evaluation
Semen Evaluation – Primary Defects

- Double sperm
- Detached abnormal heads
- Dag defect
- Tightly coiled tail

*Breeding Soundness Evaluation*

**Semen Evaluation – Secondary Defects**

- **Terminally coiled tail**
- **Distal midpiece reflex with retained distal cytoplasmic droplet**
- **Bent tail**
- **Detached normal head**

Breeding Soundness Evaluation
Semen Evaluation - Morphology

• Morphology
  – Normal: >70-80% morphologically normal sperm (MNS)
  – Fewer than 60% MNS adversely affects fertility*
  – Increasing age is often negatively correlated % MNS*
  – Retained proximal cytoplasmic droplets have a questionable impact upon fertility in the dog

Breeding Soundness Evaluation
Semen Evaluation – Other Cells

- **Hematospermia**: blood in semen
  - Iatrogenic (rupture of superficial vessels on penis)
  - Benign prostatic hypertrophy
  - Urethral or cavernosal rent

- **Leukospermia/pyospermia**: pus in semen
  - Balanoposthitis
  - Prostatitis
  - Orchitis/epididymitis
Breeding Soundness Evaluation
Semen Evaluation - Concentration

• Concentration
  – Number of sperm (millions/mL) in sample
  – Varies widely with collection of variable volumes of pre-sperm and prostatic fractions

• Methods of measurement
  – Hemacytometer considered to be the gold standard
  – CASA demonstrated to over-estimate sperm concentration in dogs by 1.7 times*

Breeding Soundness Evaluation
Semen Evaluation - Concentration

• Preparation
  – 1:100 dilution of sample in buffered formyl saline
  – Sample may be raw or extended semen
  – Commercially available dilution systems, e.g., BMP LeukoChek™

• Number of sperm in red cell counting chamber equals number of sperm in millions/ml
  – Number of sperm in two counting chambers should not differ by >10% or entire process should be repeated
Breeding Soundness Evaluation

Semen Evaluation - Concentration

Total sperm count = \textbf{concentration} (million sperm/mL) \times \textbf{volume} (mL)

* Minimum across breeds should be 300 million sperm, but that is a very low standard for medium-sized and larger dogs!!!
Breeding Soundness Evaluation

Prostatic Fraction

• Cytologic evaluation
  – Centrifuge prostatic fraction sample
  – Remove supernatant
  – Utilize pellet to prepare smear
  – Stain using Diff Quik

• Interpretation
  – Sperm → normal
  – Neutrophils → prostatitis
  – Erythrocytes → benign prostatic hyperplasia

• Digital prostate evaluation **only** if indicated
Breeding Soundness Evaluation

Ejaculatory “Failure”

- Incomplete ejaculate: Omission of entire sperm-rich fraction from ejaculate during semen collection
- Mixing of fractions: Alternation between sperm-rich and prostatic fractions
- Ejaculation in incorrect location/“outside tie”: During natural mating, dog fails to achieve intromission, but ties with external preputial orifice and ejaculates
- “Slip mating”: Dog achieves, but does not maintain intromission; breeding may not result in pregnancy
Breeding Soundness Evaluation
“Failure to Launch”

• What do you do if you obtain no or few sperm?
  – Oligospermia not uncommon
    • < 200 million sperm in most breeds
    • < 50 million sperm in toy breeds
  – Azoospermia rare
  – In the complete absence of sperm, to determine if the dog actually ejaculated, i.e., emitted fluid from the epididymal tails,
    • Measure [alkaline phosphatase] in sample
    • Alkaline phosphatase concentration very high in epididymal fluid
    • [Alkaline phosphatase] > 5000 U/L consistent with ejaculation of at least some fluid from the epididymis
    • [Alkaline phosphatase] < 5 U/L suggests bilateral blockage of sperm outflow
Breeding Soundness Evaluation

“Failure to Launch”

- What do you do if you obtain no or few sperm?
  - [Alkaline phosphatase] 5-5000 U/L consistent with ejaculatory failure, i.e., an incomplete ejaculate
    - “Sweeten the deal” by collecting and evaluating semen when teaser bitch available
    - Collect urine sample via cystocentesis and examine for the presence of sperm
Breeding Soundness Evaluation

Testing for Infectious Disease

- Genital and semen cultures not routine
- *Brucella canis*
  - Serologic testing: rapid card agglutination test
    - Excellent screening test
    - False positive possible due to cross-reactivity with antibodies to cell wall antigens of commonly encountered canine pathogens (*Bordatella bronchiseptica, Pseudomonas aeruginosa, some Staphylococcus spp*).
    - Add 2-ME to card test to eliminate cross-reactivity (very effective)
  - Positive serologic results confirmed using
    - Agar gel immunodiffusion (AGID) test: Cornell
    - Blood culture: Missouri; requires 5 blood samples!
Breeding Soundness Evaluation

The Bottom Line

• Stud dogs are generally not formally classified as satisfactory or unsatisfactory prospective breeders
  – Ultimately, we don’t often select dogs to breed based solely upon fertility

• Satisfactory Prospective Breeder
  – Two scrotal testes
  – > 300 million total sperm (> 800 million for large and giant breeds)
  – > 70-80% PMS
  – > 70-80% MNS
  – Free of infectious reproductive disease
  – +/- Free of heritable disease
Breeding Soundness Evaluation
Re-Evaluation

- Dogs usually do not generally suffer from “rusty loads”
  - Older sperm are evacuated via “retrograde ejaculation” and are voided in the urine
  - If preponderance of secondary morphologic defects, semen may be collected a second time after 30 minute rest
    - Total sperm count will be $\leq 50\%$ of first ejaculate
    - $\%$ MNS may increase
Breeding Soundness Evaluation

Re-Evaluation

• If re-evaluation recommended, performed two months later at the soonest

• Very few male dogs suffering from severely compromised fertility ever return to normal fertility
  – If no improvement occurs after 6 months, it will never happen!
"Man, I think I'm shooting blanks - I've been on the Morrie show six times and I'm never the father."