Canine Semen: Collection, Frozen, Fresh Chilled, Insemination.
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Osgoode Veterinary Services

Topics

• Bit about me...
• Semen collection
• Semen evaluation
• Fresh Chilled
• Frozen
• Bit about breeding/timing
• Questions
# Semen Evaluation

## OSGOODE VETERINARY SERVICES

### Canine Breeding Soundness Evaluation

<table>
<thead>
<tr>
<th>Date:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Owners Name:</td>
<td></td>
</tr>
<tr>
<td>Dogs Nickname:</td>
<td></td>
</tr>
<tr>
<td>Dogs Registered Name:</td>
<td></td>
</tr>
<tr>
<td>Tattoo:</td>
<td></td>
</tr>
<tr>
<td>Microchip:</td>
<td></td>
</tr>
<tr>
<td>Registration number:</td>
<td></td>
</tr>
<tr>
<td>Collection by:</td>
<td></td>
</tr>
<tr>
<td>Teaser used:</td>
<td>Y  N</td>
</tr>
</tbody>
</table>

### General Physical Examination

<table>
<thead>
<tr>
<th>Prepuce:</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penis:</td>
<td>Normal</td>
</tr>
<tr>
<td>Scrotum:</td>
<td>Normal</td>
</tr>
<tr>
<td>Prostate:</td>
<td>Normal</td>
</tr>
<tr>
<td>Testes:</td>
<td>Normal</td>
</tr>
<tr>
<td>Epididymides:</td>
<td>Normal</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Brucella canis:</th>
<th>Date:</th>
<th>Results:</th>
</tr>
</thead>
</table>

### Semen Analysis

<table>
<thead>
<tr>
<th>Ref. Values</th>
<th>1st collection</th>
<th>2nd collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st Fraction</td>
<td>0.25-3</td>
<td></td>
</tr>
<tr>
<td>2nd Fraction</td>
<td>0.4-8</td>
<td></td>
</tr>
<tr>
<td>Gross appearance (2nd)</td>
<td>milky white</td>
<td></td>
</tr>
<tr>
<td>Sperm Concentration/ml (x10^6/ml)</td>
<td>&gt;200x10^6</td>
<td></td>
</tr>
<tr>
<td>Total Sperm/Ejaculate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Motility (total progressive)</td>
<td>60% to 90%</td>
<td></td>
</tr>
<tr>
<td>Motile Sperm/Ejaculate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diluent if used</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Speed: Slow, Moderate, Fast</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Morphology

<table>
<thead>
<tr>
<th>Ref. Values</th>
<th>1st collection</th>
<th>2nd collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Normal Sperm</td>
<td>75%</td>
<td></td>
</tr>
<tr>
<td>% Abnormal Heads</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Abnormal Midpieces</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Abnormal Tails</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Loose Heads</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Proximal Droplets</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Distal Droplets</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Number Sperm/Ejaculate</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

## OVS - Canine Breeding Soundness Evaluation (page 2)

### Semen Cytology

<table>
<thead>
<tr>
<th>1st Fraction</th>
<th>2nd Fraction</th>
<th>3rd Fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epithelial cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White Blood cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red Blood cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 2nd Fraction Microbiology

<table>
<thead>
<tr>
<th>Culture and Sensitivity</th>
<th>Mycoplasma</th>
<th>Ureaplasmia</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Prostatic Fraction

<table>
<thead>
<tr>
<th>Appearance</th>
<th>(clear)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>(6.0-7.4)</td>
</tr>
<tr>
<td>Cytology</td>
<td></td>
</tr>
<tr>
<td>Culture and Sensitivity</td>
<td>Mycoplasma</td>
</tr>
<tr>
<td>Ureaplasmia</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td></td>
</tr>
</tbody>
</table>

To the best of my knowledge, the results of this examination would indicate that the potential breeding capacity of this animal would be:

- Satisfactory
- Questionable
- Unsatisfactory

### Comments:

- Fresh Chilled
- Extender
- Shipping address

### Frozen

- Extender
- #straws
- Post Thaw Motility
- Sperm/straw
- Storage Location
- Breeding unit
- Storage start date
Semen Collection

Breeding soundness evaluation

Artificial Vagina vs. Cones vs. other

Lubricant and Gloves

Quiet

Training

Female in estrus vs. swabs vs. pheromones
Semen Collection

Moving the Prepuce

Placing the AV

Engorged Bulbous glandis
Semen Evaluation
Semen Evaluation

Motility

Morphology
Semen Evaluation

- Acrosomes - P
- Head Shapes - P
- Normal
  - P - Primary
  - S - Secondary
- Bent, Kinked Tails - S
- Droplets - S
- *P*
- Midpiece - P
- Other cells - Germ Cells
- Detached Heads - S
- Coiled Tails - P
Semen Evaluation
Semen Evaluation
Semen Evaluation

• By the end of the analysis and calculations:
  – Assessed sperm motility
  – Assessed sperm morphology
  – Calculated the number of motile sperm in the ejaculate and per ml of ejaculate = the concentration.
  – In cases of infertility have also looked for specific problems with the semen, assessed concentration, looked for extra cells and started further tests.
What do you do with it?

- **Artificial Insemination**
  - Fresh – 4 to 6 days survival in utero
  - Fresh chilled – 24 to 72hrs survival in utero
  - Frozen – 12 to 24hrs survival in utero

**TIMING IS EVERYTHING!!!**

- Store frozen in liquid nitrogen
  - Lasts indefinitely
Fresh Chilled Semen
- cooking 101

• Why consider Fresh Chilled Semen?

  – Hard to ship female or male
  – Male on show circuit
  – Hard to take time off work when female is in estrus
  – Increased genetic options by shipping semen
Fresh Chilled Semen

Extenders

Using the biochemistry knowledge we have about semen, an extender is added to allow for sperm survival during the chilling process; buffers the solution and adds nutrients for the sperm during the time it is chilled prior to warming and insemination.
Fresh Chilled Semen

Minitube Chill 10

Mix with Egg yolk
Mix volume to volume
Chill and test
Package
Ship
Frozen Semen

Why?

1. for shipping distances (7 days grace)

2. to save genetic potential for the breed

3. insure the breeding potential of a stud dog against loss, death or infertility
Frozen Semen

• Extender
Frozen Semen

• An ideal extender should have nutrients as an energy source, substances that buffer against harmful changes of pH, it provides a physiological osmotic pressure and concentration of electrolytes, that prevents bacterial growth and protects the cells from cold shock during the freezing and thawing processes.
Frozen Semen

• Add 20% egg yolk to both Part A and Part B
Frozen Semen

- Part B and egg yolk to chill to 4°C.
- If Sperm rich fraction is contaminated with Prostatic fraction – wash sperm with part A and egg yolk mix.
- Add Part A to semen in equal volumes – chill for 2hrs
Frozen Semen

• Over 3 min. add equal amounts of Part B with yolk to the original volume of semen
• Fill straws already pre-cooled and labeled
Frozen Semen
Frozen Semen
Frozen Semen

Liquid Nitrogen
Frozen Semen
 Frozen Semen

• Warm part A and B of extender
• Add % egg yolk
• Cool part B of extender to 4°C
• Mix volume of sperm rich fraction with volume part A and egg yolk
• Cool this to 4°C for 2hrs
• Add volume of part B with egg yolk
• Fill straws that have been marked and pre-cooled to 4°C as well – seal and cool for 20-60min.
• Put above liquid nitrogen (4-5cm) for 20min then plunge into liquid nitrogen
• Test one straw for post thaw motility and store rest
Frozen Semen
Frozen Semen
Insemination/Timing

• Most important component of breeding, especially if using fresh chilled or frozen semen
• Knowing the female
• Vaginal cytology
• Progesterone (qualitative and quantitative testing)
• Luteanizing Hormone surge
Insemination/Timing

• Signs of Estrus starts
• Vaginal changes start (cytology) as estrogen increases
• Estrogen starts to drop and LH surge occurs
• Approx. 2-3 days later Ovulation occurs
• Approx 2-4 days later Oocyte maturation occurs
• Approx 2-5 days later Diestrus occurs.
To confuse:

Ovulation does not always coincide with onset of sexual behaviour. Behavioural estrus in some bitches occurs 2-3 days before the LH surge, some 4-5 days after the LH surge and in some extreme cases males have been allowed by the female to breed in pro-estrus 4-5 days prior to the LH surge. Some have even refused a male until 6 days post the LH surge.
Insemination/Timing

Therefore:

Behavioural estrus vs. hormonal cycle and true ovulation etc.
Insemination/Timing
Insemination/Timing
<table>
<thead>
<tr>
<th>Cycle Stage</th>
<th>Length</th>
<th>Hormonal changes</th>
<th>Predominant Cell Types</th>
<th>Erythrocytes</th>
<th>Neutrophils</th>
<th>Behavior of the Bitch</th>
<th>Clinical Signs</th>
</tr>
</thead>
</table>
| Proestrus   | ~9 days| Estrogen         | Early: mixed population of cells  
Late: large intermediate and superficial cells | Early: present  
Late: ± | Early: present  
Late: decreased | Attractive to the male, but won’t stand for mating | Vulvar edema and swelling, bloody discharge |
| Estrus      | ~9 days| LH (surge)       | 90% superficial cells  
Bacteria usually present | ± | Absent | Accepts male and will stand | Less edema, discharge becomes clearer |
| Diestrus    | ~60 days| Progesterone | Abrupt change in relative # of epithelial cells. Superficial cells decrease by 20% | ± | Few to none | Ceases to accept male | Little discharge, edema decreased |
| Anestrus    | Depends on whether pregnant or not. 70-80 days for CL to regress and 130 days to repair endometrium. | Progesterone | Parabasal and Intermediate cells | Few if any | Few if present | No outward signs | Scanty, tenacious secretions |
Proestrus –
early

Proestrus -
late

Estrus

Diestrus

Chart and pictures from University of Georgia, College of Veterinary Medicine web site: http://www.vet.uga.edu/vpp/clerk/beimborn/
Insemination/Timing

Natural coverage or fresh AI
Whenever female will accept the male; breed two days apart for at least 2-3 breedings. Semen lasts average 4-6 days.
Insemination/Timing

- AI rods
- Mavic Catheter
Insemination/Timing

Chilled Extended Semen

Knowing when ovulation has taken place helps and breeding once or twice 2-4 days post ovulation. Semen survival 24-72hrs.

Norwegian Catheter – picture from Louisiana State University School of Veterinary Medicine web site: http://www.vetmed.lsu.edu/eiltslotus/theriogenology-5361/k9_breeding_management.htm
Insemination/Timing

Frozen Semen

Timing critical as semen survival 12-24hrs only.

Thaw instructions very specific

Norwegian Catheter – picture from Louisiana State University School of Veterinary Medicine web site: http://www.vetmed.lsu.edu/eiltslotus/theriogenology-5361/k9_breeding_management.html
Insemination/Timing

Surgical Insemination vs. Transcervical Insemination