Small Ruminant Reproductive Management Workshop

Animal Nutrition and Physiology Center, North Dakota State University

Sponsors: American Sheep and Goat Center, North Dakota State University, University of Wisconsin-River Falls
## Workshop Schedule

<table>
<thead>
<tr>
<th>Date</th>
<th>Activities</th>
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</table>
| Wednesday, Nov. 10 | - Reproduction Review  
                     - Semen Collection Evaluation & Storage                                      |
| Thursday, Nov. 11  | - Embryo Collection and Transfer  
                     - Laparoscopic AI                                                           |
| Friday, Nov. 12    | - Pregnancy Diagnosis & Fetal Aging, Sexing and Counting  
                     - Development of Teaser Rams                                              |
Specific Objectives

1. Reproductive anatomy

2. Hormonal control of reproduction

3. Hormonal products for managing reproduction

4. Reproductive management techniques
Male Reproductive Anatomy

Testes

• Male gonad
• Site of sperm production
• Site of testosterone production

Male Reproductive Tract

- Testicle
- Epididymis
- Vas Deferens
- Sigmoid Flexure
- Retractor Penis Muscle
- Accessory Sex Glands
- Penis
- Scrotum
- Testicle
Male Reproductive Anatomy

**Epididymis**

- Connects the testicle to the vas deferens
- Consists of:
  - Head
  - Body
  - Tail
Male Reproductive Anatomy

Epididymis

- Connects the testicle to the vas deferens
- Responsible for sperm cell:
  - Storage
  - Maturation
  - Increase in concentration
Scrotum

- Protective covering of the testicle
- Aids in temperature regulation

Male Reproductive Tract

- Accessory Sex Glands
- Sigmoid Flexure
- Retractor
- Penis Muscle
- Penis
- Scrotum
- Testicle
Vas Deferens

- Connects epididymis to the accessory sex glands
- Portion is removed during a vasectomy
Male Reproductive Anatomy

Sex Glands

- Secrete buffers, energy sources, and contribute to semen volume
- Accessory sex glands:
  - Ampulla
  - Prostate
  - Seminal vesicles
  - Bulbourethral glands

Male Reproductive Tract

- Accessory Sex Glands
- Sigmoid Flexure
- Retractor
- Penis Muscle
- Penis
- Scrotum
- Testicle
- Epididymis
Male Reproductive Anatomy

**Sigmoid Flexure**

- Muscle
- Straightens during an erection

**Male Reproductive Tract**

- Accessory Sex Glands
- Sigmoid Flexure
- Retractor Penis Muscle
- Penis
- Scrotum
- Testicle
- Epididymis
Male Reproductive Anatomy

- Retractor Penis Muscle
  - Muscle
  - Relaxes during an erection

Diagram:
- Testicle
- Scrotum
- Penis
- Epididymis
- Sigmoid Flexure
- Accessory Sex Glands
- Retractor Penis Muscle
Male Reproductive Anatomy

Penis

- Copulatory organ

Fibroelastic

Male Reproductive Tract

- Accessory Sex Glands
- Sigmoid Flexure
- Retractor
- Penis Muscle
- Scrotum
- Testicle
- Penis
Ejaculate Characteristics

Semen Standards

- Motility 50-70%
- Morphology 90%
- Volume 0.5-1.5 ml
- Concentration 2 to 4 billion per ml
- White blood cells <5%
Semen Collection & Evaluation

Collection Methods

Semen characteristics in bucks collected by artificial vagina (AV) or electro ejaculator (EE)

<table>
<thead>
<tr>
<th></th>
<th>AV</th>
<th>EE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>volume, ml</td>
<td>0.6</td>
<td>1.3</td>
<td>.007</td>
</tr>
<tr>
<td>motile sperm, %</td>
<td>62.0</td>
<td>70.3</td>
<td>.109</td>
</tr>
<tr>
<td>sperm/ml, x10⁹</td>
<td>3.06</td>
<td>1.51</td>
<td>.001</td>
</tr>
<tr>
<td>sperm/ejaculate, x10⁹</td>
<td>1.87</td>
<td>2.11</td>
<td>.612</td>
</tr>
<tr>
<td>live sperm, %</td>
<td>71.9</td>
<td>78.3</td>
<td>.296</td>
</tr>
<tr>
<td>normal sperm, %</td>
<td>80.1</td>
<td>89.2</td>
<td>.043</td>
</tr>
<tr>
<td>abnormal heads, %</td>
<td>0.45</td>
<td>0.65</td>
<td>.259</td>
</tr>
<tr>
<td>abnormal tails, %</td>
<td>13.0</td>
<td>7.8</td>
<td>.150</td>
</tr>
<tr>
<td>protoplasmic droplets, %</td>
<td>6.5</td>
<td>2.8</td>
<td>.043</td>
</tr>
</tbody>
</table>
Semen Collection & Evaluation

Techniques for determining sperm cell concentration in the semen

• Visual appraisal
  – Thick, chalky appearance indicates high concentration

• Spectrophotometer
  – Calibrated using a standard curve and comparing sample

• Hemocytometer
  – Glass slide with precision grid etched within
Semen Collection & Evaluation

Techniques for determining sperm cell concentration in the semen

• Hemocytometer

1. Sub-Sample Dilution (1:200)
2. Place sample on hemocytometer
3. Count sperm cells in 5 squares
4. Multiply Step 3 by 5 = cells/0.1 mm³
5. Use the following equation to determine concentration (cells/mL)

\[
\text{Sperm cells/mL} = \frac{\text{cells/0.1 mm}^3 \times 10 \times \text{Dil Rate (200)} \times 1000}{\text{Dil Rate (200)} \times 1000}
\]
Semen Collection & Evaluation

Techniques for determining sperm cell concentration in the semen

- Hemocytometer Example
  - 112 cells/5 squares
  - 560 cells/0.1mm$^3$ (i.e. 112 X 5)

1.12 billion cells/mL = 560 cells/0.1mm$^3$ X 10 X Dil Rate (200) X 1000
Determining the number of units from a collection:

- 2 mL ejaculate collected from a ram
  - 70% Progressive Motility
  - 1.12 x 10^9 Cells/mL

1. Adjust for motility
   
   \[1.12 \times 10^9 \text{ Cells/mL} \times 0.70 = 0.784 \times 10^9 \text{ Motile Cells/mL}\]

2. Determine total number of motile cells in ejaculate
   
   \[0.784 \times 10^9 \text{ Motile Cells/mL} \times 2 = 1.57 \times 10^9 \text{ Motile Cells}\]

3. Determine number of units (if we need 40 x 10^6 Motile Cells/Unit)
   
   \[1.57 \times 10^9 \text{ Motile Cells} / 40 \times 10^6 = 39.2 \text{ Units}\]
Reproductive Anatomy

Vulva

- External genitalia
- Provides opening to the vagina
Reproductive Anatomy

Vagina

- Between vulva and cervix
- Site of semen deposition during natural mating
Reproductive Anatomy

Cervix

- Between vagina and uterine body
- Consists of dense connective tissue and several folds
Reproductive Anatomy

**Uterus**

- Between cervix and oviducts
- Body and two horns

**Female Reproductive Tract**

- Rectum
- Vulva
- Vagina
- Pelvis
- Bladder
- Cervix
- Broad Ligament
- Ovary
- Oviduct
- Caruncles
Reproductive Anatomy

Oviduct

- Between uterine horns and ovary
- Where sperm meets the egg(s)
- Site of fertilization
Reproductive Anatomy

**Ovary**

- **Primary sex organs**
  1. Egg(s)
  2. Hormones
    - Progesterone

**Female Reproductive Tract**

- Rectum
- Uterine Body
- Uterine Horns
- Vulva
- Vagina
- Pelvis
- Bladder
- Cervix
- Broad Ligament
- Oviduct
- Ovary
Reproductive Anatomy

Ovary

- Primary sex organs
  1. Egg(s)
  2. Hormones
     - Progesterone
     - Estrogen

Female Reproductive Tract

- Ovary
- Follicles
Reproductive Anatomy

Ovary

- Rectum
- Vulva
- Vagina
- Pelvis
- Bladder
- Cervix
- Broad Ligament
- Uterine Body
- Uterine Horns
- Oviduct
- Ovary

Brain

- Hypothalamus
- Pituitary

COMMUNICATE VIA HORMONES
Reproductive Anatomy

**Ovary**

- Rectum
- Vulva
- Vagina
- Pelvis
- Bladder
- Cervix
- Broad Ligament
- Uterine Body
- Uterine Horns
- Oviduct
- Ovary

**Brain**

- Hypothalamus
- Pituitary

**COMMUNICATE VIA HORMONES**

Brain produces two hormones:
- Follicle Stimulating Hormone
- Luteinizing Hormone
Specific Objectives

1. Reproductive anatomy

2. Hormonal control of reproduction

3. Hormonal products for managing reproduction

4. Artificial insemination techniques
Reproductive Anatomy

Ovary

Brain

Follicles

Estrogen

Follicle Stimulating Hormone
Reproductive Anatomy

Ovary

Follicles

Estrogen

Luteinizing Hormone

Brain

Ovulation of Eggs for Fertilization
Reproductive Anatomy

Ovary

Brain

Corpus Luteum

Progesterone

Fertilization of Eggs Occurs

Progesterone stays until kids/lambs are born!
Reproductive Anatomy

Ovary

Prostaglandin removes Corpus Luteum

Brain

Progestosterone

Fertilization of Eggs Does Not Occur

Females uterus will release prostaglandin
Reproductive Anatomy

- Ovary
- Brain

Follicles

This process is responsible for controlling the estrous cycle.
Estrous Cycle

- Repetitious cycle of events that occurs in healthy, non-pregnant females during the normal breeding season
- Interval from one estrus or heat to another

### Table: Characteristics of the goat estrous cycle

<table>
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<tr>
<td>Cycle length (d)</td>
<td>21</td>
<td>17-24</td>
</tr>
<tr>
<td>Duration of estrus (hrs)</td>
<td>30</td>
<td>16-50</td>
</tr>
<tr>
<td>Ovulation</td>
<td>shortly after estrus</td>
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Estrous Cycle

• Repetitious cycle of events that occurs in healthy, non-pregnant females during the normal breeding season
• Interval from one estrus or heat to another

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Estrous Cycle in the Doe

Day of the estrous cycle

Estrus & Ovulation

Estrus & Ovulation

Prostaglandin

No Embryo

Estrogen

FSH

LH

Progesterone

0  4  8  12  16  20/0
Estrous Cycle in the Doe

Day of the estrous cycle

- Estrus & Ovulation
- Progesterone
- Prostaglandin
- No Embryo
- Estrogen

FSH

LH
Estrous Cycle in the Doe

Day of the estrous cycle

- Estrus & Ovulation
- Progesterone
- Prostaglandin
- No Embryo
- Estrogen

FSH

LH

20/0

4 8 12 16 20/0
Specific Objectives

1. Reproductive anatomy

2. Hormonal control of reproduction

3. Hormonal products for managing reproduction

4. Artificial insemination techniques
Estrous Synchronization

- Basis for artificial insemination
- Brings does into estrus at a single time for insemination
• We use the same products that are naturally present during the estrous cycle in the doe.

Day of the estrous cycle
Estrous Synchronization

What products can we use?

1. Progestagens
   - Actions of progesterone

2. Gonadotropins
   - Actions of FSH

3. Prostaglandin
   - Regress the CL and decrease progesterone
Estrous Synchronization

What products can we use?

1. Progestagens
   - Actions of progesterone

2. Gonadotropins
   - Actions of FSH

3. Prostaglandin
   - Regress the CL and decrease progesterone

Controlled Internal Drug Release (CIDR) Device

Goat CIDR

Inserter
Estrous Synchronization

What products can we use?

1. Progestagens
   - Actions of progesterone

2. Gonadotropins
   - Actions of FSH

3. Prostaglandin
   - Regress the CL and decrease progesterone

Sponges and Other Progestagen Devices

Chronogest Sponge

Device made from cattle CIDRs
Estrous Synchronization

What products can we use?

1. **Progestagens**
   - Actions of progesterone

2. **Gonadotropins**
   - Actions of FSH

3. **Prostaglandin**
   - Regress the CL and decrease progesterone

**Progestagen Protocol**

- **Day of Treatment**
  - Insert CIDR: -12
  - Pull CIDR: 0
  - Doe in Estrus: 2

**Cost:** $5/female
Estrous Synchronization

What products can we use?

1. Progestagens
   - Actions of progesterone

2. Gonadotropins
   - Actions of FSH

3. Prostaglandin
   - Regress the CL and decrease progesterone
Estrous Synchronization

What products can we use?

1. Progestagens
   - Actions of progesterone

2. Gonadotropins
   - Actions of FSH

3. Prostaglandin
   - Regress the CL and decrease progesterone

P.G. 600 or PMSG
5 ml dose:
- 400 IU PMSG
- 200 IU hCG

Commercially Available

Not Available in the U.S.
**Estrous Synchronization**

**What products can we use?**

1. **Progestagens**
   - Actions of progesterone

2. **Gonadotropins**
   - Actions of FSH

3. **Prostaglandin**
   - Regress the CL and decrease progesterone

**Progestagen & P.G. 600 Protocol**

- **Day of Treatment**
  - Cost: $10

- **Insert CIDR**
  - -12

- **Pull CIDR & Inject PG600**
  - 0

- **Doe in Estrus**
  - 2
Estrous Synchronization

What products can we use?

1. Progestagens
   - Actions of progesterone

2. Gonadotropins
   - Actions of FSH

3. Prostaglandin
   - Regress the CL and decrease progesterone

![Graph showing comparison between PMSG and PG600]
Estrous Synchronization

What products can we use?

1. Progestagens
   - Actions of progesterone

2. Gonadotropins
   - Actions of FSH

3. Prostaglandin
   - Regress the CL and decrease progesterone

Prostaglandin Products

Inject 2 ml (im)
Effective when corpus luteum is present
Two injections are necessary
**Estrous Synchronization**

**What products can we use?**

1. **Progestagens**
   - Actions of progesterone

2. **Gonadotropins**
   - Actions of FSH

3. **Prostaglandin**
   - Regress the CL and decrease progesterone

---

**Prostaglandin Protocol**

- **Day of Treatment**
  - Cost: $3.00
  - **-7**
  - **0**
  - **2**
  - **4**

- **Inject PGF2**
- **Inject PGF2**
- **Doe in Estrus**

---
Specific Objectives

1. Reproductive anatomy
2. Hormonal control of reproduction
3. Hormonal products for managing reproduction
4. Artificial insemination techniques
Artificial Insemination

What methods can we use?

Laparoscopic AI  
Cervical AI
Artificial Insemination

What methods can we use?

Laparoscopic AI
Artificial Insemination

When do we perform Laparoscopic AI?

- Insert CIDR
- Pull CIDR & Inject PG600
- Perform Laparoscopic AI

Day of Treatment
Artificial Insemination

Superovulation Protocol for LAI

**Insert CIDR**
-12

5 mg FSH (a.m. & p.m.)
-2

4 mg FSH (a.m. & p.m.)
-1

3 mg FSH (a.m. & p.m.)
0

**Pull CIDR a.m.**

LAI

60 h

Day of Treatment
Artificial Insemination

How do we perform cervical AI?

AI Gun
Speculum

Rectum
Uterine Body
Uterine Horns

Vagina
Ovary

Pelvis
Oviduct

Bladder
Cervix
Broad Ligament
Artificial Insemination

What factors influence success?

Degree of Cervical Penetration in 53 Does:

- Intra-uterine: 10
- Intra-cervical: 7
- Cervical os: 31
- Vagina: 5

Pregnancy Rate to AI with Fresh Semen:

- Intra-uterine: 60%
- Intra-cervical: 40%
- Cervical os: 20%
- Vagina: 0%

Wildeus & Collins, 2003
Artificial Insemination

What factors influence success?

Pregnancy Rate to AI with Fresh versus Frozen Thawed Semen

- Fresh
- Frozen
- Thawed

- No Cervical Penetration
- Cervical Penetration

Does pregnant, %
Artificial Insemination

What factors influence success?

Other Factors

- Technical Experience
- Seasonality
- Age
- Nutritional Management
- Stress