LECTURE / LAB 5

Male Reproduction Technologies

1. Semen collection methods
   A) Artificial Vagina; The AV consists of a hard tubular case with a rubber liner, that acts as a water jacket, and is referred to as an outer liner. There is also an inner liner of latex rubber or a non-toxic plastic film that acts as the cone that directs the ejaculate into an attached test tube or bottle. The water jacket is filled with hot water, typically 115^{o} F. The water volume should be adequate to provide firm pressure on the penis.
      1) Typically used in all species of ruminants, stallions, toms, and occasionally dogs and boars.
      2) Can be used in any species were the male animal is tractable and trainable to its use. Teaser animals, mount animals and/or mount dummies are used.
         a. Steers that have been treated with several estrogen implants (Synovex S) are used as teaser and mount animals for bulls.
         b. A mare in heat can be used as a teaser for the stallion. The stallion is preferably mounted on a phantom or mount dummy. A mare in heat can be used as a mount although this can present danger to the stallion and handler if the mare is not gentle or is not completely receptive. It is preferable to train stallions to use a mount dummy without a teaser mare present.
      3) Latex inner liners (cones) should be washed, disinfected, rinsed in deionized water and pure alcohol and air dried to assure chemical cleanliness.
         a. Rubber liners may be toxic to sperm, especially when new
         b. Disposable polyethylene liners may be preferable for cleanliness and reduction of sperm toxicity
      4) Minimal lubricant with a gel (methylcellulose) that contains no preservatives (HR Lube) is applied to the inner liner.
      5) Temperature of the water jacket should be 41 to 50 degrees C.
         a. Elevated temperature required for ejaculation
         b. The length of the AV should allow intromission of the penis through the entire length of the AV so that there is minimal exposure of the semen to the elevated temperature of the water jacket.
      6) Semen is collected through the inner liner into an attached collection vessel
         a. 250 ml Nalgene bottle for equine, include a milk line filter in the neck of the bottle to retain the sperm poor gel fraction of the ejaculate
         b. Boars require a 250 or 500 ml bottle, including a filter
         c. 10 ml or 15 ml centrifuge tube for ruminants and dogs
      7) Place jacket on end of AV to protect collection vessel from temperature fluctuation

B) Digital manipulation in canines, boars, and debilitated stallions.

1) Canine
   a. As erection starts it is necessary to retract the prepuce behind the bulbus glandis before it begins to engorge
   b. The penis is directed posteriorly as full erection occurs
   c. Pressure is applied behind the bulbous glandis to stimulate ejaculation
   d. A 20 ml syringe case (Monoject) or a 50 ml centrifuge tube can be used to collect semen
   c. A bitch in heat works well as a teaser for shy or untrained stud dogs.
2) Boar
   a. A teaser animal is used to initiate erection
   b. Pressure is placed on the glans penis to stimulate ejaculation
   c. A 250 ml or 500 ml Nalgene bottle or clean wide mouth thermos can be used to collect semen
   d. The gel fraction of the ejaculate is filtered out using gauze (thermos) or a milk line filter (Nalgene bottles)

C) Electroejaculation, low voltage (1 to 20V) AC current applied intra-rectally to the area of the pelvic plexus, accessory sex glands and root of the penis. The current is turned on for 1 to 5 seconds and turned off for 2 to 3 seconds. The current is increased until erection and ejaculation occur. It is always best to remove feces from the rectum, manually or using an enema, prior to insertion of the probe.

1) Electroejaculation can be used in conscious bulls, rams, and bucks
   a. Commercially available equipment
   b. Various sized probes for the different species and sizes of animals
   c. Tranquilization rarely needed, Acepromazine
   d. Rams and bucks can be placed in lateral recumbency if additional restraint is required
2) Boar
   a. Requires anesthesia: Thiamylal and/or inhalation anesthesia
   b. Erection is not usually achieved, only ejaculation
   c. Commercial equipment can be used but a custom rectal probe must be used
3) Feline
   a. Requires anesthesia: Ketamine HCL
   b. Custom made equipment
   c. See: Lang CM, A technique for the collection of semen from squirrel monkeys by electroejaculation. Lab Animal Care, 17:218, 1967
   Platz CC, et.al., Semen collection by electroejaculation in the domestic cat. J Am Vet Med Assoc 173:1353, 1978
4) Equine: no published reports of use, but EE should be effective in a properly restrained or anesthetized stallion. Use in endangered wild equid species has been successfully attempted while the stallions were conscious (personnel communication; Bob Green, Lane Manufacturing Company) but should not be necessary in domestic horses.

5) Canine: no reports of use, but EE should be effective in a properly anesthetized dog. Should not be necessary.
6) Primates: Anesthesia required

D) Collection of Epididymal sperm cells, used in laboratory animals.

E) Pharmacologically assisted erection and ejaculation, primarily researched in stallions and humans. These same methods should work in other species.
1) 50 micrograms of GnRH 2 to 3 hours before breeding will increase libido by causing a LH and testosterone surge.
2) Imipramine HCL (an antidepressant drug used in humans) can be used to lower ejaculatory threshold.
a. Can be used in difficult to collect stallions. Administer .5 to 1mg/lb orally 2 to 4 hours before breeding or collection.
b. Used in debilitated stallions for manual collection. Administer .5 to 1mg/lb IV and observe for erection and ejaculation. Manual manipulation is used if needed after 30 minutes.
3) Xylazine can be used by itself or in combination with imipramine for purely pharmacological collection of semen. Used in debilitated or otherwise unable to be collected stallions. Some stallions are fearful or timid at breeding due to previous negative experience and may only be able to be collected by these methods until further training and psychological adaptation is done. Collection may or may not benefit from external sexual stimuli (estrual mare, or estrual mare's bedding).
a. Imipramine at .5 to 1 mg/lb. IV is administered first and if ejaculation is not achieved within 60 minutes administer xylazine at .1 to .3mg/lb IV. Ejaculation should occur in a few minutes after xylazine administration.
b. Xylazine by itself will cause ejaculation in some stallions within 1 to 20 minutes after administration. .3mg/lb. IV.
4) Prostaglandin F2 alpha has been reported to cause ejaculation in some stallions at .005 to .01mg/kg IM in 5 to 90 minutes.

Success with pharmacological ejaculation protocols is unpredictable and will probably not exceed 50%. Some stallions will adapt better than others to one of these protocols and individualized adjustments of the drugs and dosage will be required.

2. Breeding soundness examinations and semen evaluation,

A) Motility; Gross motility or estimate of the percentage of progressively motile sperm cells.
1) Gross motility, used in the field as a quick motility estimate on bull or ram semen. Can also be used on stallion, canine or boar semen but the wave motion in the sample is not observed due to the more dilute nature of the sample. Very dilute bull or ram semen will not show wave motion in spite of good motility percentage.

Score	Class	Description

5	Very good	rapid moving waves in the sample, correlates with 90% motility
4	Good	vigorous movement with moderate wave motion, correlates with 70% motility
3	Fair	slow weak waves and individual sperm cells are easily observed, correlates with 50% motility
2	Poor	no wave motion in the sample and few individual cells are progressively motile, 10% motility
1	Dead	all cells are motionless

2) Percentage of motile sperm cells estimate
   a. A more accurate method to determine motility
   b. Requires a very thin preparation of "neet" semen or a diluted sample.
   c. Sophisticated motion analysis systems exist to assist in this evaluation but with experience the human eye is very accurate.
   d. If individual cells are hard to observe due the sample being too dense, dilution is required.
   e. Diluents include any applicable skim milk extender, egg yolk extender, phosphate buffered saline or 2.9% sodium citrate. Cream or high fat milk extenders are not good for motility estimates since the fat micelles obscure the sperm cells,
   f. Observe several fields and average the estimates of motility of all fields observed.
   g. Motility in most species should exceed 70% for highly fertile semen samples. Chemical contamination, water contamination and temperature fluctuations during semen collection and evaluation can adversely affect motility. These factors should be accounted for in making motility estimates.

B) Concentration; Quantitative or qualitative estimate of the number of sperm cells per ml of neet semen
   1) Hemocytometer count
      a. Dilution: Bull, Ram & Buck Semen, 1:100
         Stallion and Boar, 1:20 or 1:40
         Canine 1:100
      b. A WBC Unopette can be used to achieve a 1:100 dilution, using a 20 microliter pipette and a 2 ml chamber
      c. Saline with a few drops of formalin is used to make manual dilutions of the semen for concentration analysis
      d. For the 1:20 dilution add 1 ml neet semen to 19 ml diluent.
      e. Count the center 1 mm^2 square of the hemocytometer.
      f. The volume of this count is .1 mm^3. Multiply count by 10 to get cells per mm^3, multiply result by 1000 to get cells per cm^3 (ml), multiply the result by the dilution factor to get sperm cells per ml of neet semen.
         Simplified:

Multiply the hemocytometer count x 10,000 x dilution factor = sperm cells per ml of undiluted semen.

g. If the cells are too concentrated on the hemocytometer use a higher dilution. If less than 100 cells are counted use a lower dilution. Many other dilutions and other methods of counting on the hemocytometer can be used, with appropriate adjustments to the calculations.

h. Frozen semen can frequently be counted on a hemocytometer with minimal dilution to estimate the cells per straw of semen.

2) Spectrophotometers can also be used to measure sperm cell concentration. The B&L Spec 20 is the most common model used. Percent absorbence is directly proportional to the semen concentration

3) Visual estimate of concentration are not very accurate but may be used in some situations

Very thick, creamy;	$>1{,}200 \times 10^6$ cells/ml
Moderately thick, milky	800 to 1,200 $\times 10^6$ cells/ml
Moderate, skim milk	500 to 800×10^6 cells/ml
Gray, opaque	200to 500×10^6 cells/ml
Gray, translucent	$<200 \times 10^6$ cells/ml

C) Morphology; Quantitative count of normal sperm cells expressed as a percentage and analysis of sperm cell abnormalities

1) Eosin nigrosin stain is the most common stain used
   a. One drop of semen on a slide
   b. One drop of Eosin-Nigrosin stain placed next to the semen
   c. Use a second slide to mix and spread the two drops
   d. Dry on a heating plate at 37 degrees C or use a flame to gently heat the smear until dry

2) Normal and abnormal cell types; there are two usual ways to classify sperm cell defects
   a. Classification based on origin location of the defect.
      1. **Primary defects**; fault in spermatogenesis, primarily head and midpiece defects
      2. **Secondary defects**; fault in spermatozoa maturation in the duct system, primarily droplets and tail defects
      3. **Tertiary defects**; defects that occur after ejaculation due to temperature, osmotic, chemical or other shock
   b. Classification based on severity of the sperm cell defect
      1. **Major defects**; defects that will render the cell non-functional and dramatically lower fertility
      2. **Minor defects**; sperm cell is still apparently functional and trials reveal that these defect (if not excessive) do not effect fertility

D) Other analysis of semen, see attached article “Ancillary Tests of Bull Semen Quality” Duane L. Garner