LECTURE 6

Artificial Insemination and Semen Processing

A. Artificial insemination; There are two major divisions of AI. 1) fresh semen insemination and 2) frozen semen insemination. Although processing of the semen differs the timing of insemination and the placement of the semen is the same.

1. Advantages (and disadvantages) of artificial insemination
   - A) Increased genetic influence of superior sires or popular sires. A corollary disadvantage is that rapid dissemination of faulty genetic traits is also possible.
   - B) Increased opportunity to obtain production data on offspring and make genetic evaluation of young sire prospects used to breed large numbers of females. In dairy and beef cattle, young sire genetic evaluation programs are used extensively.
   - C) Ability to ship frozen semen or chilled semen anywhere in the world.
   - D) Disease control
     - 1) Testing of sires for contagious and venereal diseases
     - 2) Addition of antibiotics to semen for control of specific diseases and to prevent proliferation of bacterial contamination.
   - E) Financial gain from semen sales or stud fees for owners of top sires.
   - F) Cost of AI is frequently less than owning a service sire for small breeders.
   - G) The most obvious disadvantage is the amount of planning and labor required.

2. Estrus detection and signs of "heat";
   The main sign of heat is receptivity of the female to advances by the male. There are other signs of heat that help in detecting animals for insemination.

   Mare: Winking, urination and a bracing posture are common signs when stimulated by a stallion, other mare or even spontaneously.

   Cow: Standing to be mounted by another cow or calf, excessive walking, swollen vulva and mucous discharge. Cows in heat will also attempt to stimulate sexual activity in other cows by nudging, butting and attempting to mount others.

   Ewe and doe: Same as a cow except that there is less homosexual activity. Teaser rams or bucks are more effective in detecting animals in heat.

   Bitch: Blood spotting that is typical of proestrus decreases or ceases, tail flagging and receptivity in the presence of a male. Once proestrus starts, vaginal cytology can help to identify the onset of estrus. Just prior to estrus the vaginal cytology reveals maximum cornification of the epithelial cells (over 98% cornified cells) reduction in RBCs and no (or very few) WBCs. Vaginal cytology must be done daily to be of any value. Maximum cornification occurs 2 to 3 days prior to the onset of true estrus. LH and progesterone assays can also be used to assist in determining optimum timing of insemination.

Sow: standing to be mounted or typical "sawhorse" stance when pressure is applied on the rump, swollen edematous vulva and mucous discharge

Heat detection aids:

A) Progesterone assays; in most species when progesterone drops below 1ng/ml it can be assumed that cycling animals are in or very near estrus. In the canine a twofold rise in progesterone from below 1ng/ml to over 2ng/ml precedes the LH surge and start of estrus. Requires daily progesterone analysis.

B) Heat Mount Detectors: Several methods of detecting mounting behavior have been developed for use in cattle, where homosexual activity is prevalent, and are extensively utilized as labor saving devices. Heat detection by observation can be very time consuming and occasional cows will not display estrus due to fear or shyness in the presence of humans. These methods will not only save labor but also increase accuracy of heat detection

1) K-Mar detectors; The K-Mar is a patch glued to the tail head which contains a capsule of red dye that breaks when the cow is mounted. The dye then stains the detector indicating that the cow has been mounted. The biggest problem is false positive patches due to rubbing or inappropriate mounting of a cow not in heat.

2) Electronic detection devices;

a. Heat Watch ™; A pressure sensitive telemetry device is glued to the cow's tailhead. The device sends a radio telemetry message to a central computer every time a cow is mounted. . The message contains the date, time and animal ID. This system is quite accurate, but may require some interpretation of results. For instance is a single mount a heat or can it be ignored.

b. A pedometer is a radio telemetry device that will count the number of steps that an animal takes. Increased walking and general activity is highly correlated with estrus.

3) Tail head painting with All Weather Paint Sticks™ or some other paint; The simplest and cheapest method of mount detection is to apply a small amount of paint to the tail head and observe the cow occasionally for roughing up or wiping off the paint.

3. Timing of insemination; Ideally, identification of the LH surge would be the best method to determine the timing of AI. Ovulation occurs 24 to 72 hours after the LH surge (depending on species). In practice it is necessary to estimate the occurrence of the LH surge and impending ovulation from physical signs of estrus and simple diagnostic tests. In all species except the canine the optimum time for insemination is 6 to 12 hours prior to ovulation since matured oocytes, that are ready to be fertilized, are ovulated. The ovulated oocyte may only retain its capacity to develop for 6 to 12 hours after ovulation. In the canine the optimum time for insemination is 24 hours after ovulation since the oocyte requires this time to finish maturation. When using frozen semen it is more important to determine the proper time of insemination due lower total sperm cell numbers and shorter time of viability of frozen semen.

Mare: Duration of estrus is 4 - 7 days.

- The LH surge occurs during the last half of estrus.
- Ovulation occurs 30 hours after the LH surge and 12 to 24 hours prior to the end of heat.
- In mares it is difficult to determine the ideal time for breeding due to variability in the long period of estrus. The end of estrus and receptivity occurs at ovulation or shortly after.
- Typically mares are breed every other day, starting on the second or third day of estrus. Rectal palpation or ultrasonography can be used to help determine the time to commence breeding. Mares usually ovulate when the follicle reaches 40 mm. Breeding can commence when a follicle reaches 35 mm.

Cow: Duration of estrus is 8 to 18 hours.

- LH surge occurs just prior to or at the start of estrus.
- Ovulation occurs at 24 to 30 hours after the LH surge and the start of estrus.
- Breed 12 to 24 hours after the start of estrus.

Ewe and doe: Duration of estrus; 18 to 36 hours

- LH surge occurs just prior to or at the start of estrus.
- Ovulation occurs at 20 to 30 hours after the start of estrus.
- Breed 18 to 24 hours after the start of estrus.

Sow: Duration of estrus; 4 - 7 days

- Breed twice; first insemination at 12 to 18 hours after the start of estrus and a second insemination 24 hours later. If only one insemination is used, breed at 24 hours after onset of estrus.
- Ovulation occurs at 24 to 42 hours after the start of estrus

Bitch: Duration of proestrus; 7 to 9 days, Duration of estrus; 9 to 10 days

- The LH surge occurs immediately prior to the start of true estrus.
- Ovulation is variable, occurring 36 to 72 hours after the LH surge and onset of true estrus.
- Breed every other day starting on the second day of true estrus.
- The canine is unique among the domestic species in that primary oocytes are ovulated and must complete the first meiotic division before they are ready for fertilization. Due to this the optimal time for insemination is 24 hours after ovulation. Thankfully the timing of insemination in the bitch is not as critical as in other species. Primary oocytes are capable of being fertilized although incorporation of the sperm cell chromosomes and formation of the male pronucleus must wait until completion of the oocyte's second meiotic division. Also, oocytes in the bitch are somewhat longer lived after completion of maturation (2 to 3 days compared to 12 hours in other species).
- There are two practical methods for determining the start of true estrus or the occurrence of the LH surge.
    1) The latest technology involves a semi-quantitative progesterone assay for detection of the first significant rise in serum progesterone to over 2 ng/ml. This test can be run in the kennel by the inseminator. When this test is used daily, a progesterone rises above 2 ng/ml indicates that the LH surge and the start of true estrus has occurred 12 to 36 hours prior to

the sample tested. Ovulations can begin to occur at any time after the elevation in progesterone is detected. Breeding can commence in 1 day.

2) Vaginal cytology is the traditional method for determining the start of true estrus. Although not as accurate as the progesterone assay it is still adequate for determining the timing of insemination. Determination of the degree of cornification of vaginal epithelial cells is the basis for the vaginal cytology test. Cornification of the epithelial cells is under the influence of estradiol and reaches a maximum at about 2 to 3 days prior to the start of true estrus. Once maximum cornification is observed (90 to 98% cornified epithelial cells) breeding can commence in 2 to 3 days.

4. Method and location of insemination

Mare: Intrauterine transcervical

Cow: Intrauterine transcervical

Ewe and doe: Intrauterine transcervical,
Oxytocin assisted Intracervical
Intrauterine laparoscopic

Bitch: Intrauterine transcervical (very difficult)
Intravaginal or intracervical
Intrauterine laparoscopic
Intrauterine laparotomy

Sow: Intrauterine transcervical

5. Semen preparation for artificial insemination.

A) Extenders:

Extension of the raw semen with an egg yolk or skim milk extender is typical when semen is not used immediately. Extenders stabilize the cell membrane, buffer against pH, temperature and other fluctuations that can shock the sperm cells.

1) The powdered skim milk, glucose extender (Kenney extender) is very simple and effective for fresh insemination. 2:1 to 10:1 extension

Semen Extender Preparation	for 100ml		for 500ml
R.M. Kenney Extender			
Sanalac (Instant non-fat dry milk w/o preservatives)	2.4 gm	Add last	12.0 gm
Glucose monohydrate	4.9 gm		24.5 gm
Sodium Bicarbonate (2 ml of 7.5% solution)	150.0 mg		750.0 mg
Gentamicin (2 ml of 50mg/ml reagent grade solution)	100.0 mg		500.0 mg
Distilled or deionized water (approx.92 ml/100ml)	QS to 100.0 ml		QS to 500.0 ml

2) An extender composed of 20% Egg yolk, 2.9% sodium citrate and 3 to 5% glycerol (as the cryopreservative) are typically used for frozen semen

3) Antibiotics such as Penicillin/Streptomycin, Gentamicin, Tylosin, Lincocin / Spectinomycin, Ticarcillin or Amikacin are always added to the semen extender to combat bacterial proliferation.

B) Semen Variable and formulas used to calculate dilution factor and inseminating dose. It is usually necessary to dilute the semen at least 4 to 1 to achieve adequate preservation of motility for freezing or chilling for long term storage.

In the case of frozen semen, which is usually frozen in ½ ml straws, the inseminating dose is a given. In the case of fresh or chilled semen the inseminating dose can be a variable in the calculation.

The complete list of variable, constants and formulas for calculation follows:

1) Initial Volume: Vi (ml, of undiluted semen)
2) Initial Motility: Mi (decimal percent motile cells, i.e. 0.75)
3) Initial Concentration: Ci (cells/ml, motile & non-motile cells)
4) Total Cells (in ejaculate): TC (Ci x Vi)
5) Insem. Dose, Motile Cells: Dmc (assigned by technician)
6) Inseminating Dose, ml: Dml extended semen
   Note: Dml can be assigned by the tech. in order to limit volume of the final extended dose of semen to fit a packaging system such as ¼ ml straws or where insemination volume must be limited (as in mares; under 30 ml). Alternatively Dml can be calculated, as when raw non-extended semen is to be inseminated (Vf = Vi), or when semen that has been quickly extended 1:1 with extender for fresh AI (where Vf = Vi x 2).
   Dml = Vf / PND (or Dml = (Vf x Dmc) / TMC)
7) Total Motile Cells: TMC = Ci x Vi x Mi Calculation 1
8) Potential Number of Doses: PND = TMC / Dmc Calculation 2
9) Final Extended Volume: Vf = PND x Dml Calculation 3
10) Extender Volume: Ve = Vf – Vi Calculation 4
11) Concentration of motile cells/ml of extended semen (Cme) = Dmc / Dml

C) Calculation of semen extender dilution and insemination dose, details;

1) Measure the “Initial Volume” (Vi). Vi is the total volume of the sperm rich fraction of the ejaculated neat semen, in ml. Usually a graduated cylinder or a graduated disposable test tube is used to store the semen in after collection. Volume can be read directly off of this container.

2) Determine the “Initial Motility” (Mi). This is the decimal percentage of motile cells estimated on the ejaculate. Can be done on raw semen only if the concentration is not too high (equine & porcine). If the semen is too concentrated to identify individual cells on the slide mount, an approximate 1:20 to 1:100 dilution may be quickly made on a small (50ul) semen sample using buffered saline or extender.

3) Determine the "Initial Concentration" (Ci) using a hemacytometer sperm cell count or a spectrophotometer count. This count includes motile as well as non-motile cells and is expressed as cells/ml.

a. For hemacytometer counts the semen must be diluted in H_2O or formalin saline solution to kill the cells. It is not possible to count motile cells.

b. Use a 1:20 dilution for equine and porcine semen
Use a 1:100 dilution for all other species
The dilution can be varied on an individual animal as needed

c. Place diluted semen in the counting chamber of the hemacytometer and let settle for 3 minutes

d. Count all sperm cells in the center 1mm square area (It is OK to count 5 medium squares inside of the center 1mm square area and multiply that count by 5 to arrive at a count for the entire 1mm square area. At least 100 cells should be counted to assure accuracy). The count is referred to as N and the dilution factor is referred to as D.

e. The calculation is: Ci = N x D x 10,000
Note: 10,000 is the volume factor and is arrived at by dividing 1 ml by the volume of the center 1mm square of the hemacytometer, which is .1mm^3 (1mm x 1mm x .1mm) or .1µl (microliter). There are 1000µl in a ml and we counted the cells in a 0.1µl volume.

4) Calculation 1: Determine "Total Motile Cells" (TMC) per ejaculate. Multiply the Concentration of sperm cells per ml of neat semen by the initial volume of sperm rich fraction of the ejaculated semen by the decimal percentage of motile cells by. TMC = Ci x Vi x Mi

5) Determine the "Inseminating Dose of Motile Cells" (Dmc). The Dmc is a variable that is selected by the technician. This variable is based on knowledge of the number of motile cells required in a dose of semen to achieve optimal fertility. See the chart below to select an Insemination Dose of Motile Cells or Dmc. The Dmc should be based on the number of live cells near to or in excess of the ideal inseminating dose.

Inseminating Dose of Motile Sperm Cells

Species	Motile cells per inseminate Ideal	Motile cells per inseminate Minimum	Volume typically used
Stallion	500,000,000	100,000,000	Fresh, split & extended: 10ml , Frozen 1ml
Bull	20,000,000	10,000,000	Frozen semen, 0.5ml straw
Canine	>500,000,000	100,000,000	Fresh, entire ejaculate, Chilled 5ml, Frozen .5ml
Ovine, Caprine	50,000,000	15,000,000	Fresh or Frozen, 0.25 or 0.5ml straws

If semen is to be frozen or chilled for storage, a decrease in motility of up to 50% should be factored into the Dmc. In other words the Dmc should be increased (or even doubled) above the listed ideal to compensate for the inevitable loss in motility that occurs during storage or freezing.

If the maximum possible numbers of doses are not required then the Dmc can be dramatically increased above the ideal inseminating dose listed in the chart above.

6) Calculation 2: Determine the "Potential Number of Doses" (PND) of semen. The PND is calculated by dividing the TMC by the Dmc. PND = TMC / Dmc

7) Determine the "Inseminating Dose in ml" (Dml) or volume of the inseminating dose of extended semen. As with the Dmc, the Dml is a variable that is selected by the technician. There are many things to consider based on the use of the semen.

a. Chilled, stored and/or shipped semen (porcine, canine, equine)
One of the main criteria for chilled semen is that a minimal dilution ratio be adhered to. In general a 4:1 to 9:1optimum dilution ratio is a good rule of thumb if the semen is to be chilled and stored. If the semen is too dilute to achieve a minimum dilution ratio of 3:1 and not exceed a maximum volume (60ml in the equine) it should be used immediately, as chilling or shipping will give poor results.

b. Fresh incubated semen.
A 1:1 to 3:1dilution with extender is adequate for fresh incubated semen. This type of semen should be minimally diluted so as to limit the volume of insemination. In general a lower volume will result in less irritation to the endometrial lining of the uterus. (High volumes of insemination are one of the factors that contribute to lower pregnancy rate with chilled/shipped semen in the equine.)

c. Frozen semen
Extension rates for frozen semen vary depending on the species and the packaging system. Equine semen must be centrifuged and the packed cells re-suspended in extender due to the low concentration of the ejaculated semen and the relatively high number of cells required for optimal fertility. Most other species require only extension of the semen so that an optimal number of cells are included in a ½ ml dose, for freezing in ½ ml straws.

To assist in determining this amount, a person can make a quick calculation of the raw undiluted semen dose (Vi / PND). In the equine for instance; if the Vi is 60ml and PND is 12 the undiluted semen dose is

5ml. A 4:1 dilution means that 20ml of extender should be added to the semen for a Dml of 25ml.

8) Calculation 3: Determine "Final Volume of Extended Semen" (Vf). Multiply the potential number of doses by the dose in ml.
Vf = PND x Dml

9) Calculation 4: Determine "Volume of Extender" (Ve) to add to the raw semen. Subtract the initial volume from the final volume.
Ve = Vf - Vi

There are many variations on the calculations that can be done to make semen extensions and determine insemination dose. The scheme outlined is one that works well on most species for fresh or chilled/stored/shipped semen. Additional calculations are necessary though in some situations such as when semen must be centrifuged or when extender must be added in more than 1 fraction. These special situations arise when semen is to be frozen using special techniques.

SEMEN PROCESSING LAB

Laboratory session at Aristocrat Reproduction Center

SEMEN COLLECTION AND PROCESSING

Assembly of an AV

Equine AV

Bovine AV

Electro-Ejaculator set up

Set up the collection cone and tube

SEMEN PROCESSING

Preparations:

Semen Extender Preparation	for 100ml		for 500ml
R.M. Kenney Extender			
Sanalac (Instant non-fat dry milk w/o preservatives)	2.4 gm	Add last	12.0 gm
Glucose monohydrate	4.9 gm		24.5 gm
Sodium Bicarbonate (2 ml of 7.5% solution)	150.0 mg		750.0 mg
Gentamicin (2 ml of 50mg/ml reagent grade solution)	100.0 mg		500.0 mg
Distilled or deionized water (approx.92 ml/100ml)	QS to 100.0 ml		QS to 500.0 ml

Place the extender into a 37°C incubator.

Prepare sperm cell counting solutions

(These solutions are lethal to sperm cells. Use caution around semen that will be used for AI)

1) .1% Formalin in .9% sodium chloride
2) H_2O

Prepare dilution vials for sperm cell count:

Use a 10 ml volumetric pipette to place 4.95 ml of formalin saline solution into a 10 ml culture tube. Label tube "Cell Count". Place into TT rack on lab bench.

Prepare dilution vials for motility estimate:

Use a 10 ml volumetric pipette to place approximately 2.45 ml of Kenney extender into a 10 ml culture tube. Label each tube "Motility". Place into test tube rack in 37°C incubator.

Place a box of slides and the cover slips on the slide warmer.

Place several 1 ml volumetric pipettes on the slide warmer.

Place several 50 µl pipettes on the slide warmer.

Place collection tubes in the 37°C incubator.

Place semen collection insulating jacket into a 37°C incubator or over a shielded 50 watt incandescent light bulb or prepare a 37°C water bath to place over collection tube at collection time.

SEMEN COLLECTION

1. Collect semen from the bull using electro-ejaculation
2. Maintain Temperature of collection tube above 32°C. using a water bath or insulated jacket while outdoors.
3. In the laboratory place the ejaculate into the 37°C heater block or in the 37°C incubator.

- Record volume:_____ml (Vi)

SEMEN PROCESSING

The complete list of variable and constants follows:

1) Initial Volume: Vi (ml, of undiluted semen)
2) Initial Motility: Mi (decimal percent motile cells, i.e. 0.75)
3) Initial Concentration: Ci (cells/ml, motile & non-motile cells)
4) Total Cells (in ejaculate): TC (Ci x Vi)
5) Insem. Dose, Motile Cells: Dmc (assigned by technician)
6) Inseminating Dose, ml: Dml extended semen

   Note: Dml can be assigned by the tech. in order to limit volume of the final extended dose of semen to fit a packaging system such as ¼ ml straws or where insemination volume must be limited (as in mares; under 30 ml). Alternatively Dml can be calculated, as when raw non-extended semen is to be inseminated (Vf = Vi), or when semen that has been quickly extended 1:1 with extender for fresh AI (where Vf = Vi x 2).
   Dml = Vf / PND (or Dml = (Vf x Dmc) / TMC)

7) Total Motile Cells: TMC = Ci x Vi x Mi Calculation 1
8) Potential Number of Doses: PND = TMC / Dmc Calculation 2
9) Final Extended Volume: Vf = PND x Dml Calculation 3
10) Extender Volume: Ve = Vf – Vi Calculation 4
11) Concentration of motile cells/ml of extended semen (Cme) = Dmc / Dml

4. Motility estimate. Use a 50 µl micropipette to place a 50 µl semen sample into a 2.45 ml motility dilution tube. Approximate is good enough. This will result in a 1:50 dilution for making our motility estimate. All we need to do is dilute the semen enough to prevent clumping of cells and offer a good view of the individual cells. The volume of semen added to the 2.45 ml of extender can be varied according to the preference of the technician.
   Use the 50 µl pipette or a Pasteur pipette to place a small amount of the extended semen sample on a pre-warmed slide and cover with a 22mm^2 cover slip. Observe for % motile cells.

- Record decimal percent motile:_____ (Mi)

5. Make a 1:100 Dilution of the semen sample for the cell count.
   A. If not already done, use a 10 ml volumetric pipette to dispense 4.95 ml of formalin saline into a 10 ml culture tube.
   B. A 50 µl micropipette will be used to male a 1:100 dilution by adding 50 µl of semen to 4,950 µl (4.95ml) of Formalin saline solution.

6. Cell Count
   A. Ideally it is good to use a spectrophotometer to do the cell count on semen samples. This type of equipment results in a high degree of repeatability in the cell count. In practice a hemacytometer will provide a good working count that is adequate for many applications.
   B. Load a clean dry hemacytometer with diluted semen,
   C. Count sperm cells in the center 1 mm square area
   D. If a 1:100 dilution was used, multiply count by 1,000,000 to get # of cells per ml of semen.

   - Record initial concentration:_____ (Ci)

7. Calculate total number of motile cells
   TMC = Vi _____ x Ci_____ x Mi_____

   - Record TMC:_____

8. Determine # cells per inseminate

Species	Motile cells per inseminate Ideal	Motile cells per inseminate Minimum	Volume typically used
Stallion	500,000,000	100,000,000	Fresh, split & extended: 10ml , Frozen 1ml
Bull	20,000,000	10,000,000	Frozen semen, 0.5ml straw
Canine	>500,000,000	100,000,000	Fresh, entire ejaculate, Chilled 5ml, Frozen .5ml
Ovine, Caprine	50,000,000	15,000,000	Fresh or Frozen, 0.25 or 0.5ml straws

Although we are working with bull semen you are at liberty to experiment with packaging semen at different inseminate concentrations and total motile cell numbers.

- Record inseminate dose in total motile cells:_____ (Dmc)

9. Calculate potential number of doses.
   PND = TMC / Dmc

   - Record potential number of doses:_____ (PND)

10 Decide on an extender : semen dilution ratio

<u>The minimum extender dilution that can be used for semen that is to be chilled and stored or shipped is 3:1 (extender to semen)</u>

The minimum extension ratio must be balanced against logical constraints on the volume to be inseminated. For instance, in mares an extended semen volume over 30 ml may lower conception rate. In cattle the volume should be kept below 2 to 3 ml. In the canine an adequate extension can be made and still keep the volume below 10 ml while inseminating $500x10^6$ cells.

EXAMPLE

Equine: Say that the initial concentration (Ci) of the gel free semen is $200x10^6$ / ml (a fairly normal concentration), initial motility (Mi) is 60% and initial volume (Vi) is 30ml.

TMC = Ci x Vi x Mi = $200x10^6$ x 30 x .60 = <u>$3{,}600x10^6$</u>

For the equine the following are good dose parameters to strive for when using chilled semen:

Dmc = $500x10^6$

Dml <= 30 (<=20ml is even better).

With the above information and goals the following is calculated:

PND=TMC/Dmc = 3,600 $x10^6$/500 $x10^6$ = 7.2, Round(7.2) = 7.0 doses

INITIAL TRIAL CALCULATIONS

Decide on a Dml;

Let use a 4:1 dilution ratio of extender to raw semen (4 parts extender and 1 part raw semen, total of 5 parts = Dilution factor = 5).

Dml (unextended) = Vi/PND = 30/7 = 4.29ml of raw semen per dose

Dml (extended) = 4.29ml x 5 = 21.45ml, Round(21.45) = <u>21.5ml</u>

This volume of semen is acceptable for insemination in a mare and the dilution factor is acceptable for chilled semen. Accepted.

FINAL CALCULATION

Vf = PND x Dml = 7 x 21.5 = 150.5ml, Round(150.5) = 150ml

Ve = Vf –Vi = 150 – 30 = 120ml extender

Check calculations: Random calculations to double check you math and verify that rounding errors do not adversely affect the final dose (i.e. lower Dmc below that which is required).

Dml = Vf/PND = 150 / 7 = 21.429ml

Dmc = TMC / PND = $3{,}600x10^6$ / 7 = $514x10^6$ motile cells per dose.

DOSAGE CALCULATIONS

PND = TMC / Dmc = _____ $x10^6$ / _____ $x10^6$ = _____, Round(___) = ___ doses

INITIAL TRIAL CALCULATIONS

Decide on a Dml;

Select a potential dilution ratio of extender to raw semen

___ parts extender and 1 part raw semen, total of ___ parts = Dilution factor.

Dml (unextended) = Vi / PND = ____ / ____ = ____ml of raw semen per dose

Dml (extended) = Dml (raw) x Dilution factor = _____ x ____ = ___ ml

Round(_____) = _____ ml

FINAL CALCULATION

Vf = PND x Dml = ____ x _____ = _____ml, Round(_____) = _____ml

Ve = Vf – Vi = _____ – ____ = _____ml extender

Check calculations:
Dml = Vf / PND = _____ / ____ = _____ml
Dmc = TMC / PND = _____x10^6 / _____ = _____x10^6 motile cells per dose.

11 Extend semen;

A. If trouble is encountered in calculating a workable extension ratio you should extend the semen 1:1 as soon as possible. With using non-automated methods for semen analysis, it is probably a good idea to extend the semen 1:1 as soon as all samples required for the semen analysis are obtained. You can then go back and make a final extension prior to packaging the semen or performing AI.

B. Once the extension is calculated, slowly pour the warmed extender into the semen. Allow the extender to run down the side of the tube or cylinder containing the semen so that it does not splash into the semen. Bubbles and air are deleterious to semen.

12 Package semen in Whirl Pac bags or baby bottle liners. Exclude <u>all</u> air from bag when sealing.

13 Load semen into Refrigerated Shipping Container (RSC)

SEMEN PROCESSING WORKSHEET

Extend all ejaculates as soon as evaluation samples are obtained 1:1 with extender

	Ejac #1	Ejac #2	Ejac #3
Record volume: (Vi)	_____ml	_____ml	_____ml
Record decimal % motile: (Mi)	_____	_____	_____
Record concentration: (Ci)	_____/ml	_____/ml	_____/ml
Record total cells: (TC)	_____	_____	_____
Calculate total motile cells			
TMC = Vi x Ci x Mi	_____	_____	_____

Combine Useable Ejaculates

Combined Vi = Vi1 + Vi2 + Vi3 = _____

Combined TC = TC1 + TC2 + TC3 = _____

Combined TMC = TMC1 + TMC2 + TMC3 = _____

Record combined Ci = Combined TMC / Combined TC = _____

Record dose in total motile cells:_____ (Dmc)

Calculate PND = TMC / Dmc = _____ doses

Decide on an extender : semen dilution ratio

INITIAL TRIAL CALCULATIONS

Decide on a Dml;

If Dml is known, i.e. semen is to be frozen in a ½ ml straw, go on to final calculations

If Dml is to be determined:

Select a potential dilution ratio of extender to raw semen

___ parts extender and 1 part raw semen, total of ___ parts = <u>Dilution factor</u>.

Dml (unextended) = Vi / PND = ____ / ____ = ____ml of raw semen per dose

Dml (extended) = Dml (raw) x <u>Dilution factor</u> = _____ x ____ = ___ ml

FINAL CALCULATION

Vf = PND x Dml = ____ x _____ = _____ml

Ve = Vf – Vi = _____ – ____ = _____ml extender

Check calculations:

Dml = Vf / PND = _____ / ____ = _____ml

Dmc = TMC / PND = _____x10^6 / _____ = _____x10^6 motile cells per dose.