PRACTICAL STAG SEMEN COLLECTION & PROCESSING

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Introduction

Practical demonstrations of semen collection have been presented at past Deer Branch NZVA Conferences. This presentation will deal with aspects of semen handling immediately after collection.

As AI becomes utilized on a national basis, demand for on-farm semen collection increases Good semen handling is vital to subsequent semen quality. Any shortcuts will certainly be evident in post-freeze evaluation results. One of the most difficult problems to combat is temperature control.

On-Farm Collection and Processing

We have assembled a simple "hot box" to ensure all equipment used is kept warm and will eliminate "cold shock" (manifest by coiled tails). The box is heated by a 40W light bulb. A list of necessary equipment for semen collection and processing would include:-

Collection funnels	Diluent
10ml graduated tubes (stoppered)	Chilly Bin and slika pads
Insulated tube covers	Microscope and accessories
Identification labels	Electro Stimulator and lubricant
0.05ml glass pipettes	Towel and lengths of bandage
Spectro sample tubes	Tea Towels and rubber bands

A Step by Step procedure is suggested as follows:-

1. On arrival at site plug "hot box" into mains and place diluent near light source.
2. Place collection funnels, tubes and covers close to heat also. (Rubberwear not too close).
3. Set up microscope and place spectro sample tube close at hand.
4. 0.05 pipette should also be warmed as well as tea towels used to wrap diluted sample in for transport.

Warming of equipment will take approximately 15-20 mins. Diluent will be the most difficult to warm and can be placed close to the operator's body, inside clothing, to hasten the process.

5. The stag should be anaesthetized and left in a clean well lit pen. Noise and activity should be eliminated at this stage
6. Once the stag is fully "under" a towel should be wrapped around the head and eyes to

avoid overstimulation. The penis should then be exteriorised and bandaged firmly (not too tight). A towel should be placed over the flank to keep all contaminants off the penis.

7. Insert the probe and begin stimulation, ensuring that 2-3 funnels and tubes are available and warm. After 3-4 emissions are collected the tube should be changed to avoid the chances of spoiling the sample with urine or excess seminal fluid. Urination may occur without warning and the operator must be prepared to remove the collecting device at any time.

8. Normal volume will vary from 1ml to 3mls dependent on density. Normal density will be 75×10^9 to 2.0×10^9 spermatozoa/ml. The collected sample should be measured for volume (and recorded) and .05ml should be withdrawn and placed in the spectro sample tube. Dependent on estimated density, dilution should be:-

   Dense samples normally .5ml - 1.5mls - dilute 3:1
   Moderate samples normally 1.5mls - 2.5mls - dilute 2:1
   Thin samples normally over 2.5mls - dilute 1:1.

   The diluted sample should be identified and wrapped in a towel and secured with a rubber band.

9. Place in chilly bin with relevant collection details and transport to laboratory as quickly as possible.

Laboratory Processing

On arrival at the laboratory the sample will be examined for motility, have a concentration count evaluated from the accompanying sample and placed in the cold room to adjust temperature. Final concentration is decided by assessing motility - good samples can be diluted at a higher rate than poorer samples. The range is normally between 20×10^6 and 40×10^6 sperm/ml. After final dilution with the two part diluent the semen is filled and sealed in 0.25cc straws identified by stags name, number and batch date. Freezing is completed after a minimum of 4.5 hours equilibration period (after collection) and stored in liquid nitrogen. The maximum time that semen should be held between collection and freezing should not exceed 12 hours. Further field work is required to test this claim and the period may well be extended after data for conception rates are available. This, however, is our recommendation at this stage.

Quality Control

Semen evaluation post freeze is undertaken a day or so after processing using photomicrographs which differentiate motile from non-motile sperm. This photographic method also allows us the opportunity to examine both progressively forward motility and morphological aspects. A minimum of 8 million live sperm per straw is our recommended minimum to achieve satisfactory results.

We are currently also incubating samples for 3 hours in conjunction with the above method. Our objective is to identify the samples which have a rapid "drop off" in motility after thawing. Correlations between these factors and conception rates will be available after this years calving details are finalized.

Health Status

When contemplating on-farm semen collection some form of health testing should be undertaken. As a company (Ambreed) we have set our own criteria for projects of this nature. Inevitably stag owners are placed under pressure to sell semen at some stage, no matter how determined they may be at the time of collection not to sell. Our screening includes Brucella, Johne's disease and Leptospirosis tests, plus Tb if no current test is available e.g. accredited herd or whole herd test negative in the last twelve months. To protect our status as a licensed centre, we will insist in the future that all semen processed and stored by our laboratories will require a similar protocol.