Prerequisites for Quality Semen

Purchase or obtain semen from a source that has an established "quality control" program that:

- Identifies and discards low quality ejaculates prior to processing
- Provides a high level of biosecurity and hygiene
- Uses high quality extenders and water
- Retains a reference sample of each ejaculate or "pool"
- Provides proper control of temperature during processing & transport
- Provides a label on each unit of semen, which includes information such as collection date, sire identity and other information
- Has an established 3rd party verification program.

Note:

- There is no absolute laboratory test or combinations of tests that will accurately predict the subsequent fertility of an ejaculate or a dose of semen.
  - In other words we can not look at 2 samples of semen and accurately predict, for example, that sample A will result in a 92% farrowing rate and that sample B will result in an 85% farrowing rate.
  - However, we should be able to identify samples of obvious low quality, which if used for insemination, would result in very low or zero conceptions.

On-Farm Semen Evaluation

- Ask the semen supplier for a protocol designed specifically for their own product.
- Note: Protocols may vary, depending upon the specific extenders used, as well as other factors.

What should be evaluated?

- Sperm motility or viability
- Gross morphological abnormalities
- Number of normal and viable cells per dose
- Agglutination or "clumping"
- Other contaminants
  - In addition, you may want to monitor temperature of semen upon arrival (especially during extreme weather conditions)
Motility

• An estimate of motility provides an indication of sperm viability.
• Within certain ranges, motility is not highly related to subsequent fertility (farrowing rate & litter size).
• Sperm can be viable without being highly motile. Many factors can influence motility. Temperature is the most important factor when evaluating semen on the sow farm.
• Sperm can be motile, but not fertile.
• In general, expect viability to decrease as the length of storage time increases.
• Sperm motility is not important in sperm transport in the female reproductive tract. Dead cells will be moved up from the site of deposition in the cervix to the oviduct just as rapidly as live cells.
• Sperm motility is important in penetration of the ova.

Motility

• Sperm have a definitive life span, in other words at warm temperatures, they can live for only a given number of hours.
• The degree of motility and livability is related to temperature.
• Once semen is collected and extended, it is cooled to about 17 to 18 degrees C, this slows down the metabolic activity and motility of the cells and extends their life span. Some extenders contain certain ingredients that inhibit motility as well.
• During storage at lowered temperatures, sperm cells more or less "go to sleep" (anabiosis), they require both time and a warmer temperature in order to "wake up" and regain their motility.

Motility

• Within the range of 70 to 95%, the relationship between motility and subsequent reproductive performance is not high (see next slide).
• A motility estimate may be of most benefit when evaluating the shipping and storage conditions, especially during extreme weather conditions.

Relationship Between Estimated Sperm Motility and Fertility

<table>
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<tr>
<th>Motility (%)</th>
<th>No. Sows</th>
<th>In vitro penetration Rate (%)</th>
<th>Farrowing Rate (%)</th>
<th>No. Born Alive</th>
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<td>85.5</td>
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<td>17.3</td>
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</table>
Guidelines for Estimating Motility

- Insure that the sample of semen has been properly prepared.
  - Gently rotate the semen storage package
  - Remove a small sample (5 to 10 ml) and place in a clean glass test tube.
  - Warm it for the appropriate time (according to the supplier’s recommendations). It will usually range from 5 to 20 minutes at 36 to 37 degrees centigrade (body temperature)
  - Place a small drop on a pre-warmed slide and gently place a cover slip over the drop.
  - Immediately examine the sample at 100x and then at 400x

Equipment Required

- “Medium quality” microscope
  - Self illuminated
  - Capable of 100x and 400x magnification
  - Keep it clean and well maintained
- A method for consistently warming the:
  - Semen
  - Slides and cover slips

Emergency, Low Cost Methods for Warming Semen and Slides

- Sample tube with semen
- 37 degree C gel pack
- Microscope slides

Observe at 100x and 400x

- Stain instead of semen is being used for illustration
- Pre-warmed semen
- Small drop of semen on slide
- Placing the cover slip over the drop
- Prepared slide

Motility

- Estimate the percentage of sperm in field that are progressively mobile (moving from one point to another as compared to just undulating).
- Progressive Motility
- Undulating Motility
- Examine several fields and establish an average.
- Record your estimate to the nearest 5 or 10 percentage units.
- Note: In some instances, undulating motility may be an indication that the sample droplet of semen is too small and the sperm are being held in place by the slides and cover slip or that the proper temperature was not achieved.
Motility

• Prepare a second slide if:
  – There is a delay in achieving a focus or in the evaluation of the sample. Remember that motility decreases as the sample cools
  – Motility is less than 60%

• If it is established that the percent motile sperm is less than 60%, contact your source stud and ask them to evaluate the reference sample before you dispose of the lot # in question

Motility

• What about using caffeine coated slides for evaluating motility?
  – Caffeine coated slides are often used for motility evaluations instead of using a proper warming technique. Caffeine stimulates motility of sperm cells and is thought to be correlated with the degree of motility once the cells enter the female reproductive tract.
  – Note: There is some evidence that caffeine may over-stimulate cells of some ejaculates and, therefore, a false estimate would be recorded
  – Consult with your semen supplier about this practice.

Morphology

• Sperm morphology has been correlated with subsequent fertility. Certain abnormalities appear to be more important than others.

• The semen supplier should discard ejaculates with > 20 to 30% abnormal sperm

Morphology

• After the motility estimate is complete, allow the slide to cool. Motility will slow or stop and individual sperm cells can be observed.

• Switch to the 40x objective and observe individual cells in several fields at 400x.

• Note: Mishandling of the sample during preparation could create morphological abnormalities that were not present in the original sample. Cooling or warming the semen too rapidly or physically grinding the cover slip and slide together are examples.
Morphology

• Estimate, in several fields, the percentage of cells which are “normal”.
• If you suspect that < 80% of the cells are normal, contact the source stud and ask them to evaluate the reference sample.

Sperm concentration

• Requires special training and equipment

Clumps and Debris

Clumping

Agglutination or “clumping”
The reasons for agglutination or “clumping” are not well understood. They include:
- Clumping has been associated with bacterial contamination.
- Incompatibility between ejaculates of 2 or more boars which comprise the pooled dose may result in agglutination.
Agglutination or “clumping”

- Some studs report an increased incidence of agglutination during hot weather conditions.
- In some instances, especially with semen prior to extension, cells will clump when they contact the glass surface of the slide.
- Also, in some instances, failure to rotate the doses on a daily basis will result in clumping.
Microscope Maintenance

- Keep the microscope covered when not in use.
- Even with the best of care, dust or debris will accumulate on the eyepiece(s), condenser, light source or the objectives. Use a damp cotton swab to clean the eye piece and other glass parts.

Summary

- Obtain semen from a source which has high quality control standards.
- If on-farm evaluations of processed semen are to be conducted, assure that proper equipment and employee training are provided.
- Do not over-look the importance of proper management of the semen inventory on the farm.
  - Follow the guidelines provided by the semen supplier.