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Quality assurance of semen

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Introduction

In pork production systems, throughput—or pounds of pork produced—is an important economic indicator of profit. It depends upon the number of sows farrowed and litter sizes of the farrowed animals. Increased output—up to the point of capacity—has a significant effect on lowering the impact of fixed costs of an operation, therefore improving the return on investment.

To effectively and efficiently operate a boar stud or sow farm, managers must first set production goals and then implement the operating procedures necessary to achieve the standards required to accomplish these goals on a regular and consistent basis. Any variation in procedure that causes production to fall short of these goals has a detrimental impact on profitability of the operation. Therefore, it is important for producers to understand and control the factors of reproduction that result in a reduction of reproductive performance and lost profit.

Reproductive performance is a complex issue that demands diligent management of both boar and sow operations.

For boar studs, consistent reproductive performance requires:

- biosecurity,
- healthy boar environment,
- trained personnel, and
- quality semen dose production according to standards:
    - viable sperm cells,
    - no bacterial contamination,
    - proper semen handling, and
    - controlled semen temperature during shipping and storage.

In sow operations it requires:

- biosecurity,
- controlled semen temperature during storage,
- accurate heat detection,
- accurate timing of insemination,
- good breeding technique,
- gilt pool management,
- gestation management,
- nutritional feed in lactation, and
- trained personnel.

The objective presented here focuses on the standards for boar stud operation and semen storage that directly contribute to consistent reproductive performance.

Set standards and implement SOPs

Standards to minimize variation and optimize semen dose output are fundamental to efficient boar stud operation. This includes standards for the boars' environment as well as semen collection techniques, evaluation, processing, shipping, and storage. To consistently meet these standards, it is necessary to develop and implement standard operating procedures (SOP). These procedures are detailed, written instructions for carrying out the routine operations necessary to achieve the standards. Each employee should be well trained to perform the SOPs in his/her respective area of responsibility. Follow-up is important to periodically check and verify that the SOPs are properly followed and equipment is maintained and operating according to set standards.

Environment impacts semen output

Boars respond to an unfavorable environment where the temperature exceeds 80°F (27°C) and the humidity is above 50% or the temperature drops below 60°F (15°C) (Flowers, 2002); diets are nutritionally deficient; vaccination, relocation, or bleeding cause chronic or acute stress; and/or inadequate biosecurity leads to disease. These stresses, even for short periods of time, cause a reduction in sperm cell output as well as sperm cell quality. If the stress is short-term, the semen output will usually recover within 6–7 weeks. During the recovery period, immature sperm cells continue to develop within the testicle and epididymis. If exposure to the stress conditions occurs sporadically or chronically over a 2-month period or during the recovery time, the immature and more

sensitive sperm cells will be further stressed, and recovery time can be significantly increased. The resulting decreases in boar semen output and semen quality cause variation in the consistency of semen dose production and are contributing factors to inconsistent reproductive performance.

Key standards for environment

- Maintain the boar stud temperature below a heat index (see table) of 80°F (27°C). Heat index is a combination of ambient temperature and humidity. Therefore, if the thermometer reads 80°F (27°C) and the relative humidity is greater than 45%, the heat index is more than 80°F (27°C) and cooling measures should be used.
- Provide a nutritional diet that has been evaluated and recommended for working boars. During summer months, take necessary precautions to prevent feeding rations that contain mycotoxins.
- Perform all vaccinations and veterinary treatments in isolation.
- Minimize stressful procedures such as movement, handling, and injections of boars in production to only 25% of the boar population every 6–8 weeks.
- Impose strict biosecurity measures and reduce health bleeds to 10% monthly population sampling.
- Provide boars with sanitary housing facilities. Sweep floors daily and wash crates at least once each week.

Collect semen to optimize boar's output

Depending upon age, a typical boar on a 5–7 day collection frequency should give 150–400ml ejaculates containing 50–85 billion cells. On average, a mature healthy boar will produce 10–12 billion sperm cells each day (Crabo, 1983). This is not to say that some boars will produce much more or less than average. Volume and total sperm cells will change due to collection frequency and individual ablities, particularly in younger boars (under 12 months of age). Young boars may give less than 50ml and produce fewer sperm cells each day.

Semen collection requires planning and coordination in order to make the best use of a boar's semen production. Collection schedules should be organized to allow sufficient time for proper cleaning of the boar and collection of the ejaculate, including all seminal plasma (modified full ejaculate). This generally means 5–6 boars per hour. Boars should not be collected too often. Collecting three times over a two-week period takes advantage of most

Table 1. Air temperature and relative humidity combine for heat index

Air Temperature (°F) \ Relative Humidity (%)	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
120	107	111	116	123	130	139	148														
115	103	107	111	115	120	127	135	143	151												
110	99	102	105	108	112	117	123	130	137	143	150										
105	95	97	100	102	105	109	113	118	123	129	135	142	149								
100	81	93	95	97	99	101	104	107	110	115	120	126	132	138	144						
95	87	88	90	91	93	94	96	98	101	104	107	110	114	119	124	130	136				
90	83	84	85	86	87	88	90	91	93	95	96	98	106	102	106	109	113	117	122		
85	78	79	80	81	82	93	84	85	86	87	88	89	90	91	93	95	97	99	102	105	108
80	73	74	75	76	77	77	78	79	79	80	81	81	82	83	85	86	86	87	88	89	91
75	69	69	70	71	72	72	73	73	74	74	75	75	76	76	77	77	78	78	79	79	80
70	64	64	65	65	66	66	67	67	68	68	69	69	70	70	70	71	71	71	71	71	72

Shaded area indicates the combination of temperature and relative humidity with a heat index of no more than 80°F.

boars' normal sperm cell production to produce ejaculates with acceptable sperm cell concentration.

There are two critical elements in maintaining sperm cell viability during collection. First, maintain semen temperature and prevent any cold shock due to sudden drop in temperature by prewarming collection vessels in a warming cabinet located in the collection area. Second, eliminate bacterial contamination of the ejaculate by following the "basic practices."

Use these basic practices in *combination* to minimize bacterial contamination during semen collection:

- Divert initial jets of the ejaculate (pre-sperm fraction containing urethral flushings/urine) from the semen collection vessel onto the floor.
- House boars in a facility separated from the collection area.
- Wash and sanitize collection pens and dummies daily and a minimum of once each week with hot water.
- Do not cover collection dummies with carpet.
- Create an airflow pattern that moves air from the collection area to the boar barn.
- Periodically trim hair from around the preputial opening.
- Wash the boar sheath and underside with a weak Nolvasan(r) solution (dry thoroughly) prior to boar mount.
- Wear disposable vinyl gloves.
- Use a double-glove technique to prevent contamination of the collection glove.
- Evacuate preputial fluids prior to collection. This is a *must*. Evacuate in a prep pen (if possible) before entering the collection area.
- Clean the preputial opening and surrounding area with a clean disposable paper towel.
- Clean and dry the glans penis.
- Hold the penis with the tip (glans) slightly elevated to minimize any chance of preputial fluid running down shaft of the penis into the semen collection vessel. An alternate method may be used to block any flow of fluid down the shaft by wrapping a folded strip of paper towel or clean gauze around the penis.
- Do not touch the collection vessel with the collection hand during the collection process.
- Allow 1–2cm of the penis to extend beyond the gloved hand when grasping the penis or open the last finger to allow a free flow of semen into the vessel.
- Remove the collection vessel just before the last few jets of semen and gel plug are ejaculated.
- Use a US BAG(tm) for collection. Always dispose of the top portion before passing the ejaculate to the processing laboratory. The remaining portion with the ejaculate remains free from contact with the boar and collection area.

Key standards for collection

- Wash collection area daily.
- Collect a maximum of 5–6 boars per collector per hour.
- Keep boars clean and dry.
- Follow the "basic practices" outlined above.
- Collect boars no more than three times within a 2-week period (ideal situation).

Evaluate semen according to standards

The physical evaluation immediately follows collection and screens an ejaculate for obvious poor semen quality. This is a visual and olfactory assessment of the semen for volume, color, and odor. Ejaculates that do not meet any one of the required standards fail the physical evaluation. They should be rejected and not transferred to the laboratory for evaluation and processing.

Ejaculates that pass the physical evaluation should be very quickly transferred to the lab in an insulated container that maintains the semen temperature.

Key standards for physical evaluation

Complete the physical evaluation at the time of collection as quickly as possible:

- Volume: minimum of 50ml.
- Color: range from milky white to pale yellow. Individual boars may differ; a dark red or brown color may indicate the presence of blood.
- Odor: none. Any odor indicates some form of contamination such as urine or preputial fluid.

Laboratory operation requires careful planning and staffing to accommodate collection schedules, extender preparation, maintenance and use of proper lab equipment, continual monitoring and inspection of the facility for bacterial contamination and hygiene, and prompt corrective actions as required.

The laboratory evaluation is conducted to ensure that ejaculates meet the standards for semen processing and dose preparation. Standard laboratory operating procedures should quickly and accurately evaluate the acceptable ejaculates as they arrive at the lab. Maintaining semen temperature throughout the evaluation process is

critically important to maintaining sperm cell viability and its reproductive performance.

Volume is determined by weight (1ml = 1g). Concentration is measured by one of three methods: hemacytometer, Sperm Vision(tm) (CASA), or a spectrophotometer. The hemacytometer method is a microscopic manual count of sperm cells using a calibrated slide. Sperm Vision(tm) links digital images to the computer to create a computerized semen analysis system for measuring concentration and motility of a semen sample. The spectrophotometer measures sperm cell concentration by passing light through the sample and reading the optical density of the semen. Generally, the range in variation of multiple sample readings from a hemacytometer or Sperm Vision(tm) is less than that of the spectrophotomer. The spectrophotometer's inherent wider range in variation is due to the technology and procedures used.

Minimum ejaculate standards

- temperature: 93°F (34°C)
- volume: >50ml
- sperm cell concentration: >100 million/ml
- total sperm = days rest × 10–12 billion sperm produced/day
    - example: 7 days rest × 10 billion sperm/day = 70 billion total sperm; 7 days rest × 12 billion sperm per day = 84 billion total sperm
- gross motility (as measured by Sperm Vision(tm))
    - >90% (long-term use)
    - >85% (short-term use)
- gross morphology
    - head defects: <10%
    - tail defects: <20%
    - proximal droplets: <10%
    - total normal: >70%

Data from Larsson, K: *Current Therapy in Theriogenology*, 2nd ed. P. 972. Pork *Industry Handbook no. 136*. Semen collection, evaluation, and processing in the boar and Flowers, 1998.

Semen ejaculates that fail to meet the ejaculate standards, but go on to become processed doses, may cause inconsistency in reproductive performance.

Key standards for laboratory evaluation

- Check temperature of the ejaculate when it arrives in the lab. Discard if the temperature is less than 93°F (34°C).
- Complete the laboratory evaluation within 3 minutes of semen arrival in the lab.
- Discard the ejaculate if the temperature is below 93°F (34°C) at the time of extension.
- Proceed to semen processing as quickly as possible; avoid maintaining semen temperature in a water bath.
- Prevent bacterial contamination of the ejaculate.

Process semen for reproductive performance

Semen processing is extending and packaging semen into artificial insemination (AI) doses that meet minimum standards for volume and viable cells on day of insemination. Preparation for processing begins by selecting and preparing the extender. Selection of extender type ("short": 3 days; "medium": 5 days; and "long term": 10 days) should take into account delivery time to the customer, health testing (i.e., PRRSV), customer order frequency (time until AI), as well as other features offered by different extenders. BTS (Beltsville Thawing Solution; Johnson *et al.,* 1988) is a relatively inexpensive semen extender. When used within 48 hours after collection and under good storage conditions, the reproductive performance associated with its use is quite good. However, the question is often asked, "Is there an advantage to using a long-term extender even when semen is used within two to three days?" The answer to this question can be summed up in four words: fertility, storage, handling, and temperature.

There are numerous factors that can influence reproductive success at the farm level. A decrease in fertility over time is apparent in all extenders. However, long-term extenders contain a more extensive buffering capacity and additives such as, among others, BSA, which—although this effect is unexplained—contributes to improvement in fertilization rates, even in fresh semen. Beyond the common attributes of long-term extenders, the newer generation extenders also contain additional components that help protect sperm from normal temperature fluctuations, enhance fertility, and reduce the effects of poor handling.

Once selected, the extender should be prepared and maintained at 96°F (35°C) in a water bath or extender vat during the processing day. Throughout processing, the semen must not only be carefully handled, but the processing procedures must be accurately performed in a manner that prevents bacterial contamination. Extender must be added to the semen as soon as possible, and semen temperature must be maintained.

In most situations, the relationship between motility and fertility of an ejaculate may be dependent on motile sperm numbers rather than on the absolute percent value of sperm motility. For example, accumulating data from recent studies with varying sperm numbers strongly suggest that the sperm number threshold for achieving satisfactory reproductive performance on U.S. farms with conventional AI

methods is between 2 and 5 billion motile sperm per dose (Baker, *et al.* 1968). As new technologies, such as intrauterine insemination (IUI), develop by which a dose with low sperm number (<1 billion sperm) is inseminated, fertility may be more dependent on percent motility because an abundance of spermatozoa is not present during breeding (Rozeboom *et al*., 2003).

Evaluation of gross morphology and especially acrosome integrity appear to be more effective in pro-actively assessing the potential fertility of an ejaculate. However, these evaluations should not be considered as absolute values. Estimates of motility, gross morphology, or acrosome integrity do not appear to be an estimate of fertility but are instead basic evidences of sperm viability. These measurements are more likely to influence fertility through elimination of poor quality ejaculates rather than predictors of an ejaculate's ability to sire more piglets or impregnate a female.

The major disadvantage of using visual estimates for motility is the subjectivity of the evaluation. The main disadvantage to stained morphology evaluation is lack of knowledge regarding the potential harm to the cell from the stain. It would seem logical, therefore, that to improve the efficiency of a boar ejaculate (produce more doses/collection), an accurate, nonsubjective, and noninvasive method to determine the number of viable sperm in a dose is of greater assistance to the semen processor. This technology is know available in SpermVision'.

To ensure successful reproductive performance, the final AI doses should contain sufficient sperm cells so that 2.5 billion viable cells are available at the time of AI. In general, achieving a concentration of 2.5 billion viable cells with good quality semen at the time of AI (regardless of extender type) usually requires a total sperm cell count per dose within a range of 3.2–3.5 billion cells at processing. These concentrations are recommended when sperm cell concentration is determined by Sperm Vision(tm) or a hemacytometer. Because of the inherent variation due to the technology and procedures when a spectrophotometer is used for sperm cell counts, AI doses should be prepared to contain 0.5 billion total sperm cells more per dose than when using Sperm Vision(tm) or a hemacytometer. Increasing the minimum number of viable sperm cells per dose to at least 3 billion at time of insemination is recommended to help offset some of the effects of seasonal infertility during the summer months. These guidelines are generally acceptable for most all extenders.

As the doses are packaged, they should be placed into a cool room or semen storage unit at 63°F (17°C) as soon as possible after collection to begin the gentle and rapid cooling process.

Minimum semen dose standards

- Volume: >70 ml (most studs average 75–85ml/dose).
- Viable sperm: >2.5 billion. At the time of insemination, AI doses should have >2.5 billion (in summer, >3 billion) viable cells. Sperm cells are considered viable when they appear normal under the conditions observed (this is based upon field experience, and individual boars and situations may vary). Dr. Billy Flowers reported at the Midwest Animal Science Meeting in 2001 that 25% of the boar population may need >5 billion viable sperm cells to optimize fertility.
- Semen to extender ratio: 1:4. A minimum of a 1:4 semen to extender ratio is necessary to optimize storage length and ensure sufficient antibiotic concentrations.
- Seminal plasma: 7%. A minimum of 7% seminal plasma per dose is necessary to optimize reproductive performance of that dose.

Below are the calculations used to process AI doses that meet the standards for reproductive performance. For example: The laboratory received an ejaculate with a volume of 147ml. According to the laboratory evaluation using Sperm Vision(tm), the ejaculate had a total of 70 billion sperm cells. The % gross motility was 90%, and the % gross morphology was 72% normal (there were 5% head defects, 15% tail defects, and 8% proximal droplets; no cell was counted more than once). A long-term extender was used, and the final AI doses were to contain 3 billion viable sperm cells in each 80ml AI dose volume.

The following calculations for viable sperm, potential AI doses, and volume of extender to add were used to process this ejaculate.

Viable sperm per ejaculate

- ((% gross motility) × (% gross morphology) × (total sperm cells)) = viable sperm
    - example: ((0.9) × (0.72) x (70 billion sperm)) = 45.4 billion viable sperm cells

Potential AI doses

Calculation method #1:

- ((viable sperm cells) / (viable cells/dose)) = potential AI doses
    - example: ((45.4 billion sperm) / (3 billion)) = 15.1 potential AI doses

Calculation method #2 (doses may also be calculated using only % gross motility):

- (((total sperm cells) × (% gross motility)) / (viable cells/dose)) = potential AI doses
    - example: (((70 billion sperm) × (0.90)) / (3 bil-

lion)) = 21 potential AI doses

Volume (ml) of extender to add

Using the potential AI doses calculated by method #1:

- ((potential AI doses) × (ml/AI dose)) = ml of extended semen (total vol.)
    - example: ((15.1) × (80ml)) = 1208ml of extended semen
- ((vol. (ml) of extended semen) – (ml raw ejaculate)) = ml extender to add
    - example: ((1208ml extended semen) – (147ml raw ejaculate)) = 1061ml extender to add

Semen doses containing a minimum of 2.5 billion viable sperm at the time of insemination generally have a sufficient number of cells to produce consistent reproductive performance on most sow farms with acceptable to excellent techniques. Remember, additional viable cells at the time of processing are needed if semen is going to be stored for an extended period of time. However, quality semen doses containing 2.5 billion viable sperm cells at the time of insemination will rarely compensate for subpar techniques on the sow farm.

Key standards for semen processing of individual ejaculates

- Fully extend the ejaculate within 3–5 minutes after arrival in the lab.
- Adjust extender (if needed) to within 1.8°F (1°C) of the semen by cooling it or warming it under running water.
- Always add the extender to the semen.
- Never warm semen to match extender temperature.
- Never maintain the temperature of neat or partially or fully extended semen in a water bath or incubator.
- Proceed to semen packaging as quickly as possible.
- Begin the cooling process of packaged semen doses to 63°F (17°C) as soon as possible.
- Design the processing system to avoid partial dilution of ejaculate as partial dilution adds processing steps and reduces efficiency.
- Prevent bacterial contamination.

Key standards for semen pooling

- Extend each ejaculate to its final volume. Refer to the first 6 points in key standards for individual ejaculates.
- Place each extended semen ejaculate in a Styrofoam(r) container until all ejaculates are processed and their individual temperatures are within 1.8°F (1°C) of each other.
- Slowly combine 3–5 extended ejaculates into a large pooling bag supported by a clean cylinder container.
- Mix well (gently) before dispensing.
- Begin the cooling process of packaged semen doses to 63°F (17°C) as soon as possible.
- Prevent bacterial contamination.

Temperature fluctuation reduces viability

Throughout the evaluation and processing procedures, it is important to maintain the semen temperature without fluctuation. However, once semen is extended and packaged, it is ready for cooling to 63°F (17°C), the optimal storage temperature for fresh boar semen.

The cooling process causes a gradual and constant reduction of metabolic activity to conserve sperm cell energy and viability. Cooling reduces the risk of depleting the buffer and nutrients provided by the semen extender. Once semen is cooled, it should be maintained at 63°F (17°C) until it is used for insemination. For each temperature fluctuation up or down 3.6–5.4°F (2–3°C), sperm cell viability and shelf life may be decreased for as much as one day. In short, when properly cooled and stored at a consistent 63°F (17°C), sperm cells will remain viable for a longer period of time.

Key standards for maintaining temperature and preventing fluctuation during storage

- Use semen storage units with forced-air circulation that both heat and cool to maintain cabinet temperature at 63°F (17°C). Temperature should not vary more than 1°C.
- Do not re-warm semen once it has cooled and equilibrated to 63°F (17°C).
- Do not overload storage units with warm semen.
- Avoid opening the unit during the cooling process.
- Record the daily high and low temperatures of semen storage units (use a high-low thermometer).
- Store AI doses away from UV light.
- Regularly clean and disinfect the semen storage unit using a disinfectant cleaner such as Conflict(r) or a bleach solution.
- Keep the semen storage unit in a temperature-controlled room such as an office with even air flow on all sides (avoid hot corners).
- Plug the storage unit into its own electrical circuit to avoid power surges and/or use a UPS surge protector.

Quality control confirms standards

Quality control (QC) testing ensures that semen released to the farm has the potential for consistent reproductive performance. It is also a monitor of the laboratory's analytical and hygiene procedures. Each semen batch is QC tested after semen is extended. The batch is again QC tested after the semen is packaged (Day 1).

The quality control evaluation of semen dose samples is a repeat of the Ejaculate Evaluation procedures. Results of the procedures are used to calculate the viable sperm cells per dose. Acceptable results confirm that the initial evaluation of the ejaculate and the processing procedures were correct and the semen was properly handled. They also ensure that the semen doses have sufficient volume and viable sperm cells (>2.5 billion) to meet the minimum standards for consistent reproductive performance on the farm at the time of insemination. Failure to meet these minimum standards indicates a deficiency and a potential to cause an inconsistency in reproductive performance. The semen batch should be discarded.

Quality control of semen doses is an on-going evaluation of retained semen doses from each semen batch on days 3, 5, 7, and 10 depending upon the maximum-specified use time based on the performance claim for the extender. Once again, AI doses should contain at least 2.5 billion viable sperm cells at the time of insemination. Ongoing QC also includes microscopic evaluation for agglutination or sperm cell clumping. If more than 25% of the cells show clumping, the semen should be discarded.

Key standards for quality control

- Check AI doses for consistent volume (>70 ml).
- Test semen for sperm cell concentration, total sperm, gross motility, and gross morphology to calculate and confirm that AI doses have at least 2.5 billion viable sperm cells at the time of insemination.
- Photometric assessment of cell concentration should be periodically validated with a hemacytometer or Sperm Vision(tm).
- Evaluate sperm cell agglutination (<25%) coverage of microscopic field.
- Conduct QC testing on days 1, 3, 5, 7, and 10 (depending upon extender type) to ensure sufficient viable sperm cells.

Laboratory hygiene is a must

Routinely, bacterial contamination in boar semen is observed when semen is collected by the gloved-hand technique. Studies show that 62.5% of raw ejaculates and 79% of extended semen doses contain bacterial contamination. In one ten-year study, bacterial counts per milliliter (ml) of freshly collected boar semen normally ranged between 5,500 to 48,000 and averaged 27,000.[2] Therefore, when low conception rates and reproductive problems occur with AI, bacterial contamination of semen is an important subject to consider.

Key standards for laboratory hygiene

- Daily clean and disinfect the laboratory including countertops, floors, pneumatic semen delivery shuttles, and pass-through windows.
- Daily clean and sterilize all equipment in direct contact with semen.
- Use disposable supplies as often as possible.
- Prohibit food in the laboratory.
- Prohibit barn clothes in the laboratory.
- Periodically swab counters and equipment and prepare bacterial test culture plates.
- Incubate and dispose of bacterial culture plates outside of the laboratory if possible.
- Immerse culture plates in strong bleach before disposal.

Test semen for bacteria

Key standards for monitoring bacteria

- The minimum standard is no bacterial growth after 24 hours of incubation.
- The goal is no bacterial growth after 48 hours of incubation.
- Bacterial testing is recommended when:
    - semen batches appear to have increased agglutination,
    - there is an abnormal decrease in motilities, or
    - bacteria are suspected when semen is visually inspected.
- Use 5% blood agar plates to check suspected doses.
- Touch sterile swabs to extended semen and streak plate. Incubate at 37°C.
- Count colony forming units (CFU) that grow on the streak after 24 hours incubation.
- Repeat incubation for another 24 hours if no CFUs are present.
- If colonies grow, send aged samples to a diagnostic lab to identify the bacteria and culture for sensitivity.
- Incubate culture plates outside of the laboratory.
- Before disposal, autoclave or immerse culture plates in strong bleach.

Shipping delivers reproductive performance

No matter how well a semen dose is prepared, if it is not carefully transported either in-house or commercially, the semen's reproductive performance will be reduced. The basic goals for semen shipment are (1) gentle semen handling, (2) stable semen temperature, and (3) minimized cost. Because semen handling and temperature can adversely affect the semen, they must be controlled to maintain consistent reproductive performance. Cost may be minimized, but not at the expense of semen handling and stable temperature.

Shipping containers, transit time, and delivery medium are factors that affect the thermal and physical environment of sperm cells during shipment. Transport containers for both in-house and commercial shipment should protect against temperature fluctuation and physical stress with shock absorbent material such as Styrofoam(r) peanuts.

In-house semen delivery time is generally short and made by an employee on the same day as collection. Semen doses are packaged in a Styrofoam(r) container or large insulated cooler along with pre-cooled thermal packs (59°F or 15°C) to maintain temperature and prevent fluctuation. The portable 12-volt semen storage unit is another option.

Commercial delivery is usually overnight for arrival on the farm the next day. Styrofoam(r) shipping containers are most often used because they are lightweight, reduce shipping cost, and the sample can be double-boxed. Containers that are double-boxed offer more insulation to maintain temperature during the increased delivery time. A small box within the container is surrounded by thermal-packs and contains the semen doses. Air cavities within the boxes are filled with Styrofoam(r) peanuts. This double-packing insulates the semen doses from temperature fluctuations and protects them from rough handling.

The number and temperature of the thermal-packs vary depending upon the season. During summer months, frozen packs are placed in the space between the inner and outer containers. Their number depends upon the distance and shipping time, past experience with the shipping container, and volume of semen shipped. When Flowers (1996) examined the influence of shipping containers on semen quality, he determined that using thermal-packs improved the viability of shipped semen in either Styrofoam(r) or plastic containers. The effect, however, was much greater in Styrofoam(r) containers.

In spite of the insulated container, there is still a possibility for temperature variation. Therefore, shipping containers should be labeled "Live Semen Shipment," and the shipping company should receive a handling protocol request for minimal thermal effect during transit.

Temperature fluctuation during semen transport is monitored by two methods: High-low thermometers or digital data loggers. The high-low thermometer shows the highest and lowest temperatures of the semen during transit. It does not show multiple temperature fluctuations. This is the simpler and lower cost of the two methods.

Digital data loggers, such as the TinyTalk(r) II, monitor and record the temperature in shipping containers and semen storage units at preset time intervals over time. The data loggers are returned to the boar stud where they are connected to a computer with software that recovers and prints a temperature fluctuation graph. This is an excellent method, especially in troubleshooting situations, where temperature fluctuation during delivery is suspected as a potential cause of reduction in reproductive performance.

Key standards for shipping

- Ship semen after cooling to 63°F (17°C).
- Use insulated shipping containers that can counteract ambient temperatures and minimize temperature fluctuation to no more than 1.8°F (1°C).
- Use double-boxed containers when environmental shipping conditions are more stressful to maintaining a constant 63°F (17°C).
- Use high-low thermometers or data loggers to monitor temperature fluctuation.
- Label shipping containers with "Live Semen Shipment."

On-farm storage impacts reproductive performance

When semen reaches its destination, the farm accepts responsibility for maintaining the potential for consistent reproductive performance of semen doses. Upon arrival, semen doses should be immediately placed in a semen storage unit at 63°F (17°C). As the doses are transferred from the shipping container to the semen storage unit, delivery date, batch number, number of doses, and expiration date should be recorded on a data sheet kept next to the semen storage unit. When doses are removed for AI, the date, batch number, expiration date, and number removed should also be recorded on the data sheet. This record keeping prevents use of semen doses past the expiration date.

Daily monitoring of the semen storage unit temperature should be standard practice. This is easily done by recording the temperature of a thermometer inserted through a stopper into a bottle filled with 70 ml of water and placed near the center of the semen storage unit. Thermometer readings confirm the accurate operation of unit's temperature controller and digital readout or alert the technician to a possible temperature maintenance problem. When

temperature readings are taken, it is also a good time to rotate the semen doses.

If AI doses are temporarily removed from the semen storage unit, their temperature outside the unit should not fluctuate more than ±5.4°F (±3°C) from the storage unit temperature. If temperature fluctuation does exceed ±5.4°F (±3°C), then reproductive performance may be adversely affected and doses should be discarded and not returned to the storage unit.

Key standards for on-farm storage

- Maintain semen storage units at 61–64°F (16–18°C).
- Record the arrival of all semen batches.
- Record the removal of all semen batches from the semen storage unit.
- Record the daily temperature of semen storage units.
- Store AI doses at 63°F (17°C) away from UV light.
- Do not return AI doses to the semen storage unit if their temperature has fluctuated more than ±5.4°F (±3°C).
- Regularly clean semen storage unit.
- Keep semen storage unit in a temperature-controlled room such as an office with even air flow on all sides (avoid hot corners).
- Plug storage unit into its own electrical circuit to avoid power surges and/or use a UPS surge protector.

The bottom line for peak reproductive performance and profitability is setting and achieving high standards. Any compromise of these standards affects semen quality and causes reduction in reproductive performance.

Suggested readings

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