Evaluating Boar Semen Quality[1]

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Introduction

Determining the initial quality of a boar ejaculate is the first step in semen processing and should ensure that prior to further processing, a high quality artificial insemination dose of semen will be produced. Effective screening methods for ejaculates prior to processing are necessary for improving on farm reproductive performances. Ideally, ejaculates that are thoroughly evaluated prior to processing help identify poor quality semen. Daily evaluations of gross motility and morphology of stored semen sample will helps ensure that due to some unforeseen reason, deteriorated semen doses are not used at the farm level.
With this in mind, the objectives of this fact sheet are to describe boar semen evaluation methods and (Table 1) and outline specific guidelines for acceptance and rejection of boar ejaculates upon laboratory entry.

Concentration

Generally, there are four basic parameters that are measured to evaluate boar semen quality: Concentration, motility, morphology and acrosome integrity. Of these, concentration and motility are perhaps most routinely used for sorting ejaculates prior to processing since they require the least amount of time and are required to calculate semen doses/ejaculate. Measuring semen concentration or total numbers of spermatozoa is not a component of semen quality evaluation, but more so, as a tool to monitor the health and productive output of the boar and as the primary feature in processing boar ejaculates for optimizing the genetic potential of a single individual. Accurate assessment of sperm numbers is not the only factor for increasing semen doses per ejaculate and boar stud efficiency in terms of semen output. Semen quality estimates, as discussed in the proceeding text, must be integrated into processing as a means to ensure that an sufficient number of viable sperm are used for insemination.

Gross Motility

Gross ejaculate motility appears to an important aspect of semen evaluation. A recent study that evaluated and inseminated split ejaculates shortly (<24h) after collection suggests that farrowing rates and litter sizes will decrease when initial semen motility is recorded and used at levels below 62.5 %. However, it is important to considering that semen from commercial studs, unless hand delivered after processing, is seldom used within this time period. Because semen motility decreases during storage, the minimum motility rates during initial evaluation of semen at the boar stud should be higher than

[1] Reviewed by W.L. Flowers, Department of Animal Science, North Carolina St. University and W. Singleton, Department of Animal Science, Purdue Univerisity.

Table 1. Minimum procedures and equipment for semen quality evaluation of boar ejaculates following collection and prior to processing.

Evaluation Procedures	Equipment Needed[a]
1. Visual and olfactory assessment of ejaculate	None
2. Determine semen volume and sperm concentration	Balance and a hemacytometer or photospectometer
2. Motility	
a. Prepare a 1:10 dilution of semen with semen extender	
b. Gently rotate the semen	
c. Remove a small sample (5 to 10 ml) and place in a clean glass test tube.	
d. If, necessary, warm it to 36 to 37 degrees centigrade (body temperature)	Small Water Bath
e. Place a small drop on a pre-warmed slide and gently place a cover slip over the drop.	Slide Warmer
f. Immediately examine the sample at 100x and then at 400x	Self illuminating microscope Capable of 100x, 400x, magnification and glass slides with coverslip
g. Estimate the percentage of sperm in field that are progressively	
h. Examine several fields and establish an average.	
i. Record your estimate to the nearest 5 or 10 percentage units.	Small, disposable plastic pipette
3. **Morphology**	
a. After the motility estimate is complete, allow the slide to cool. Motility will slow or stop and individual sperm cells can be observed	Self-illuminating microscope capable of 100x and 400x and 1000x (oil) magnification; glass slides and immersion oil.
or	
Prepare a stained semen sample-using step 4a, with a mixture (1:1) of morphology stain and formal saline.	Eosin-nigrosin stain or Williams stain
b. Switch to the 400x objective and observe individual cells in several fields.	
c. Estimate, in several fields, the percentage of cells that are "normal". (see example pictures)	
4. **Acrosome Integrity**	Self illuminating phase contrast microscope capable of 100x, 400x, 1000x (oil) magnification;
a. From the same semen sample in step 1a, prepare a 1:1 dilution of semen and a mixture (1:1) of formal saline and Acrosome stain on a glass slide.	Formal saline: 6.19g $Na_2HPO_32H_2O$: 2.54g KH_2PO_4: 4.41g NaCL: 125 ml 38% formaldehyde: 1000 ml distilled water. naphthol yellow or erythrocin stain
b. Place one or two drops of semen and 1-2 drops of the stain mixture on a glass slide and mix gently with the tip of the pipette. Use the edge of a second slide to draw the mixture across the flat slide to produce a thin layer. Allow the slide to air dry.	
c. Place a drop of microscope immersion oil under the slide and view first at 10x to focus, and then switch to either 40x or 100x and view individual cells. (Be sure that you don't get oil on non-oil lens)	
d. Estimate, in several fields, the percentage of cells that are "normal". (see example pictures for normal vs. abnormal)	

[a] Available commercially from boar semen equipment suppliers. Contact our extension office for a complete list.

60 %, and many studs have established a motility cutoff level between 70-80%. The minimum motility rate for processing a semen collection at each facility needs to be based on the projected storage length before use and expected motility rate decline over this period of storage time. Boar studs must also recognize that semen storage conditions and handling are perceived to be poorer on the farm than in the stud. Therefore, retained semen samples in the stud for daily quality monitoring will most likely have better motility rates than the homogeneous samples that were sent to the farm. Communication between the sow farm and boar stud in regard to this discrepancy, will enable the stud to select an initial motility rate acceptance level that help ensure that when shipped semen is actually used, motility rates are above 60 %.

Visual estimates of the percentage of motile spermatozoa by light microscopy are the most widely used and acceptable method. Technician skill and experience greatly influences the relative accuracy of this procedure. Briefly, a very small drop of diluted spermatozoa (dilution rate must be standard for all evaluations) is placed on a warmed microscopic slide and overlaid with a cover slip. The sample should be dilute enough to view individual sperm cells at 400 X power. Although gross estimates can be derived from viewing groups of sperms, technicians should be trained by first giving a gross estimate and then counting 10 cells in 5 different fields and averaging the % of motile cells (only those with forward motility) from the count for determining overall gross motility.

Morphology

Sperm morphology and acrosome integrity are also effect tools to estimate semen viability and can also provide more information about the ejaculate in terms of its quality than is possible with just a motility evaluation. Both of these criteria are important to use, along with motility, as a determinant for keeping or discarding ejaculates.

Because motile sperm may be morphologically abnormal, poorly motile sperm can fertilize eggs, and sperm without intact acrosomes cannot fertilize eggs, boar studs which do not evaluate all three of these semen quality components likely underestimate the true fertility potential and quality of an ejaculate.

Like motility, it appears that a certain percentage normal morphological spermatozoa are needed in an AI dose to optimize fertility rates. Research data supports routine evaluation of spermatozoa for morphological normality. Semen collections with less than 70 % normal morphological sperm can be identified as inferior collections if the semen is used at or below this level. Since the rate of morphological deterioration during storage is probably highly variable between boars, the initial processing level for normal spermatozoa is probable higher than 70 % normal morphology when semen is used after extended storage lengths (>24 h).

A rough morphological examination can be easy performed at the same time as semen motility, however ideal morphological examinations are conducted with phase contrast

microscopy that allows for a greater distinction of sperm membranes and parts. A precise evaluation will be obtained by performing one separate count for sperm head morphology, droplets and tail morphology. Morphology counts should be immediately conducted under phase contrast microscopy (400x or 1000x) using 1-2 drops of semen diluted 1:10 with semen extender. If semen cannot be immediately analyzed (<30 min), fix or preserve the semen drops on the slide with 0.5-1 ml of formal saline. In addition to preserving the sample, sperm will be immobilized, and thus, much easier to view. Samples can be viewed wet or dry mounted and viewed under oil immersion after fixing. Deformities in head shape, tail formation and cytoplasmic droplets (proximal-near the head; distal-middle of tail) should be counted as abnormal spermatozoa (Fig. 1).

Although the incidence of proximal droplets is quite low, fertility as measured by farrowing rates and litter size, gradually decreases as the prevalence of proximal (near the head) droplets increase. The same affect appears to be true, if not more severe for distal droplets (mid-piece) and unfortunately, distal droplets are more commonly found in semen collections than proximal droplets. Although there are limited scientific references regarding the impact of cytoplasmic droplets in boar ejaculates, it has been suggested that the incidence of plasmas droplets should not exceed 15 % when semen is stored for extended periods of time (at least 2 days).

Figure 1. Common Sperm Head, Mid Piece, Tail and Acrosome Abnormalities

Acrosome Integrity

The acrosome membrane covers the top 2/3rds of the sperm head and contains the necessary enzymes that are for oocyte penetration. Although acrosome evaluations are time consuming and require a more advanced type of microscope (phase contrast) and technique to operate the scope, some research suggests that acrosome integrity may be a better indication of sperm quality than motility. Acrosomal integrity can be

examined using the same methodology as morphological examination, however, examination of individual sperm cells should be performed under oil immersion with a phase contrast microscope to see the distinct "acrosome layer". Ejaculates with less than 51 % intact acrosomes can be identified as inferior collections if semen is inseminated at this level. The same principal as motility and morphology can be applied to acrosomes such that this 51% normal acrosomes is likely to low for initial evaluation when semen will be stored for extended periods of time.

Estimates of motility, morphology, or acrosome integrity do not appear to be an estimate of fertility but instead are basic evidences of sperm viability. These described measurements are more likely to influence fertility through elimination of poor quality ejaculates as contrast to a predictor of the ejaculates ability to sire more piglets or impregnate a female.

Using Stains to Evaluate morphology

Rough morphology evaluations of individual boars ejaculates are necessary, however periodic detailed exams may provide a measure of quality control to ensure that rough estimates are not grossly under or over estimating the true morphological quality of an ejaculate. To conduct a thorough morphology evaluation, stains are sometimes used to accentuate the outline of the sperm cell under a light microscope. This type of evaluation, in contrast to a gross morphological examinations, should be conducted under a higher-powered lens (1000x: oil Emerson) focused on individual sperm cells. A detailed morphology examination is performed using three separate counts; one each for headpieces, mid pieces, and tails. Glass slide smears made from a 1:1 dilution of semen in eosin-nigrosin (commercially available as a "morphology stain") stain is sufficient for differential counting of sperm head morphology. One hundred total sperm cells are counted and the percent of normal sperm cells is calculated.

Other stains useful for morphology examinations are Williams stain for sperm head morphology and naphthol yellow and erythrocin stain for acrosome morphology.

Appearance, Color and Odor

Even though microscopic evaluations are the standard for acceptancing or rejecting ejaculates, it is important not to forget obvious visual and olfactory characteristics of semen. The normal whole boar ejaculated must be of considerable volume (>150 ml) and depending of the concentration, should be a milky white color. It’s color can be somewhat yellowish, but is normally has an similar appearance to that of skim milk. Occasionally small amounts of blood, usually originating from the urethra, may be present in the ejaculate, which gives the semen a pinkish hue. This normally does not reduce the fertility or the viability of the ejaculate, but a darker red color associated with a pungent odor should be cause for discarding the collection.

Collected semen must not have a noticeable odor, an if so, poor sanitary procedures during collection probably occurred. The emitting odor is most likely reflective of prepuce fluids

(urine) which are generally heavily laden with bacterial and foreign contaminants. Antibiotics in semen extenders are designed to control pathogen growth during storage, not to reduce or combat large bacterial contamination in semen collections. Ejaculates must be evaluated for deviating odors, and discarded if detected. Furthermore, routine periodic semen culturing is becoming standard operating procedure in most boar studs and should be conducted in on farm studs to monitor facility sanitation and hygiene processing procedures. Bacteriology cultures are fairly inexpensive, and are performed at all diagnostic laboratories. Even though contamination of semen with bacteria is almost inevitable, there is very little scientific information regarding interactions between type and level of bacteria and fertility. However, since bacterial contamination has been associated with decreased storage life, clumping, and persistent uterine infections, it is necessary to periodically test ejaculates for bacterial contamination, identify the source of contamination, and take proactive measures to reduce contamination. Table 3 list some of the more common bacteria found in boar semen and possible sources of origin.

Temperature Shock and Clumping

It is important to consider the impact temperature fluctuations on sperm cells when semen is not directed into the lab right after collected (i.e. transported between barn and lab or to the house). A rapid drop in temperature results in cold shocked. This is evident when sperm are initially viewed under the microscope. Sperm tails will be curled around the head or tightly spiraled beneath the sperm head. Small numbers of sperm cells exhibiting this general appearance may normally be present in an ejaculate, however, more than 10% curled tails in association with a temperature of less than 32° C is a good indication of cold shock. It may not be necessary to discard this sample, but consider immediate dilution with extender (at the same temperature to prevent further damage.

Table 2. Partial list of bacterial flora isolated from the boar ejaculates (Adapted from Althouse, 2000)

Bacteria	Potential Sources
Bacillus sp. Actinobacillus sp. Staphylococcus spp. Flavobacterium sp. Klebsiella sp. Pseudomonas spp. Micrococcus sp. E. coli Citrobacter sp. Proteus sp. Actinomyces sp. Serratia sp. Enterobacter sp. Bacillus sp. Streptococcus sp.	Laboratory equipment, disposable and non-disposable supplies, Powdered/reconstituted extenders, boars and the boar stud environment

There is very data to suggest that sperm clumping has a significant effect on fertility. However, these cells are often non-motile spermatozoa that may have been either damage during transit through the testicle or traumatized following ejaculation. Other possible causes for semen clumping can include wide temperature fluctuations during transport (± 5º C), and bacterial contamination.

It appears is important to consider the degree of clumping when calculating sperm concentration even though there is very little evidence to suggest that clumping in semen affects fertility. However, clumped sperm often dissociate when diluted for spectrophotometer or hemacytometer counting and viable sperm numbers for semen doses may be grossly underestimated if the prevalence of sperm clumping is not initially accounted for. Occasionally you may see spermatozoa movement within these clumps of cell, however, there is not enough evidence that these cells dissociate after insemination. Therefore, regardless of the apparent motility in clumps, adjustments to whole sperm counts should be based on the occurrence of clumping in these ejaculates. Clumping may be associated with individual boars, boar and semen extender interactions, and management. The prevalence and impact that sperm cell clustering has on semen viability and fertility needs further investigation.

Summary

Table three shows the normal and minimal values for the semen quality parameters that have been outlined in the preceding paragraphs. It is important to remember that some of these measurements are difficult to determine accurately and morphological and acrosome quality should not be approached as absolute values. Recognize that sperm orientation and slide preparations can influence the counted values significantly. For example, acrosomes can only be view if the sperm cell is lying flat on the microscopic slide, since spermatozoa that are not lying flat could appear to have no acrosome when if fact they do. The techniques described here cumulatively provide us with the best estimate of fertilizing potential of boar ejaculate. Exclusion of one or more of these measurements for evaluating boar semen quality will fail to fully insure that a quality product is shipped, and thus creating the potential for lower reproductive performances on the sow farm that will eventually be attributed to the semen source.

Table 3. Initial Semen Quality Standards and suggested limit value for semen used within 24 h after collection.

Ejaculate Characteristic	Normal Value	Limit Value
Ejaculate Volume[a]	100-500 ml	50 ml
Total Sperm per Ejaculate (x 10^9) [a]	10-100	10
Progressive Motility[c]	70-95%	62 %
Clumping (% coverage of microscopic field)	0-10 %	25%
Curled Tails	1-2 %	10 %
Morphological Abnormalities [c]	5-10 %	30 %
Acrosome Abnormalities [c]	5-10 %	49 %
Cytoplasmic Droplets	<5 %	15 %

[a] Data from Larsson K: Current Therapy in Theriogenology, 2nd ed. P.972.

[b] Pork Industry Handbook no. 136. Semen collection, evaluation, and processing in the boar.

[c] Flowers, 1998.

Suggested Readings

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The fresh semen evaluation is highly important because. it reflects the seminal quality of the boars that are being. used for roofing. This evaluation can be performed on. both, the animals for natural mating and the ejaculate to be. used for the preparation of AI doses in order to determine. its quality. In the case of AI, since the ejaculate is divided. into several doses, it is necessary to evaluate the quality of. the insemination dose, because problems of infertility and. subfertility may have a direct influence on increasing the.Â efficient method for evaluating the sperm morphology of boar semen, and thats pecial care should be taken when preparing the smear to avoid. affecting the results, mainly regarding tail alterations. Evaluating Boar Semen Quality. Kevin J. Rozeboom, Ph.D. Extension Swine Specialist.Â Determining the initial quality of a boar ejaculate is the first step in semen processing and should ensure that prior to further processing, a high quality artificial insemination dose of semen will be produced. Effective screening methods for ejaculates prior to processing are necessary for improving on farm reproductive performances. Ideally, ejaculates that are thoroughly evaluated prior to processing help identify poor quality semen. Daily evaluations of gross motility and morphology of stored semen sample will helps ensure that due to some unforeseen reason, deteriorated semen doses are