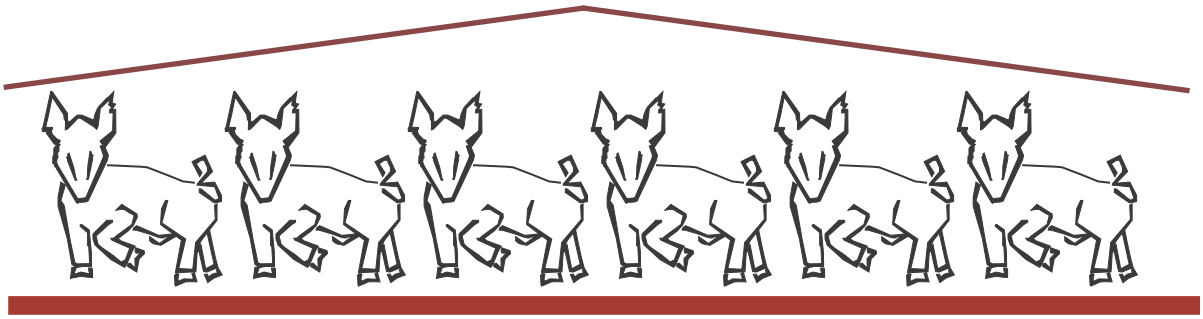

Midwest Boar Stud Managers Conference

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Midwest Boar Stud Managers Conference III

2007 North American Boar Stud Survey Summary

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Introduction

The development of AI technology for the swine industry has facilitated great improvements in the genetic characteristics of the modern swine. This has resulted from increased rates of genetic transfer and greater selection differentials for traits that translate into growth and feed conversion advantages, highly valued pork production traits, and in traits such as liter size, milk production, and overall herd fertility as measured by pigs weaned /sow/year. Many additional dramatic industry changes have also occurred as a result of the conversion from natural service to AI. These changes have included size and layout of breeding units, labor specialization and requirements, and animal flow within the breeding herd. In parallel, new support industries have arisen to provide commercial access to genetics and semen, equipment and supplies that are labor efficient, low cost, and provide sanitary and safe AI.

Boar Studs and Operations

The boar studs developed in the 1990's out of an industry need to manage highly valued genetic resources for frequent distribution to farms at numerous remote locations without risk of spread of disease from the semen supplier. The boar studs themselves became a separate industry segment that would maintain reservoirs for the genetic resources to populate the swine industry. This segment would protect, maintain ensure the health of the boars from specific pathogens, and provide a reliable method to ensure and test boar fertility. This segment would be responsible for distributing the genetic resources using regulated procedures and guidelines for reliable health and fertility.

The development and separation of boar studs and sow centers reduced risk of disease to sow farms entry through frequent boar entry. Today, the studs operate as isolated units that supply only semen to the sow centers to meet optimal fertility needs of the breeding farms. High biosecurity and routine health monitoring is the norm of the boar stud units. Boars are managed for longevity, fertility and maximized semen dose production. The housing, feeding, and health management is controlled to maximize boar fertility. Semen collection, processing, and evaluation are all highly regulated procedures that minimize contamination and maximize fertility and doses produced. The semen evaluation processes at studs involves the basic assessments of the ejaculate ensuring that each dose will meet the requirements for sperm numbers, motility, contamination. Semen processing is performed using semen extenders that have been optimized for sperm fertility in storage for 3 to 10 days following collection. The extenders maintain sperm viability, motility, and minimize bacterial growth during the time in storage. Semen is shipped within a day from collection and is targeted to reach the farm the next day. Semen shipping and storage is regulated to maintain temperatures at 16-18 °C that reduce sperm cell bioactivity and microbe growth and aids in preserving the semen dose until use a few days later. The

controlled storage of semen allows the semen to be stored for use on the farm for between 3-7 days depending upon the type and label the extender used.

The studs have also implemented a system for monitoring and assessing quality control and product safety. The boar studs usually retain samples of semen sent to farms to monitor and alert farms for risk of lowered fertility and potential disease risk. This system allows studs to contact sow centers if problems arise in quality control. Alternatively, the sow centers can contact the stud to alert them to reproductive failures or problems that may be related to semen shipments. While these cannot be corrected in previous breeding groups, this type of feedback system allows the studs to identify boars or problems in the semen production system for future groups.

The boar stud industry itself continues to evolve with the further consolidation of the ownership of the swine industry and the continued isolation of the production segments. With these changes comes larger farms, a more isolated and distant industry, and to some extent, with little chance for industry interaction or sharing of production information. The boar stud industry is the key to the future success of the swine breeding herd. The past and current success rate of the swine breeding segment is unparalleled in livestock production, as the reproductive rates for breeding herds as measured by farrowing rate and litter sizes, pigs produced per sow per year. This rate of production would not be possible without the present semen production system that relies on the boar stud. The boar studs are the foundation for providing semen and genetic renewal for the nearly 6 million sows bred annually in the USA and 7.8 million sows in the USA and Canada combined. Maintaining the health of fertile boars and supplying high fertility semen is the sole responsibility of the boar studs to ensure and protect the North American breeding herds.

The purpose of this survey

As the industry continues to evolve and face new challenges, competition and challenges to the North America swine industry will force the industry to change and evolve. The major issues of high feed and energy costs, risks of new of old diseases, a dwindling labor force, and distance between industry members, and outside market forces make maintaining competitive edges, and sharing information to bolster our industry ever more important. Sharing information that can assist the studs and sow farms to make improvements in business efficiency, and aid in management decisions that aid in product quality and production efficiency will help keep the operations a viable and integral part of the North American swine industry.

The survey

A online survey was conducted in April to June 2007 was help capture some of the size, structure, and management practices of the North American Boar Stud industry. A parallel survey was also conducted for sow farms but is not included in the present proceedings. Briefly, the survey was created using the Zoomerang® survey program. The survey contained ~65 questions with predetermined answers and with write in capability for some answers. The surveys were designed to capture individual boar stud size and management procedures. The survey was electronic and was sent out via email extension and

industry contacts, and also used open invitations via the popular press electronic newsletters. The Survey was open to all respondents classified as managers of a boar stud. There was only 1 survey allowed for each computer address. The survey took ~20 minutes.

Results

The following results are summarized responses from the survey. The full and original manuscript will appear as "An update on North American boar stud practices" in the scientific journal, Theriogenology from the 6th International Conference on Boar Semen Preservation . The article in press: Knox DTDDIFFDR, et al., An update on North American boar stud practices, Theriogenology (2008), doi:10.1016/j.theriogenology.2008.06.036.

Stud demographics and characteristics

There were 44 boar studs that responded to the survey. These studs were located in 5 provinces of Canada and 15 states within the USA. The total number of boars represented from the studs was ~10,000, based on median values for the category of boars in inventory. From table 1, the studs with 51-500 boars were most numerous and accounted for 84% of responses.

Table 1.

Total number of boars in inventory for a single site for the stud		
1-10	2	5%
11-25	1	2%
26-50	2	5%
51-100	5	11%
101-250	18	41%
251-500	14	32%
501-1000	2	5%
1001-2000	0	0%
Total	44	100%

The genetics of the boars in studs were mixed, and many studs had multiple types of genetics (Table 2). The responses are sorted in descending order for frequency of responses. It was also clear that all genetics were not easily classified and represented in the survey questionnaire as the "other" category was selected in 23% of the responses.

Table 2.

Genetics for the boars in Stud		
PIC lines	24	55%
Duroc or crosses	15	34%
Landrace or crosses	14	32%
Large White or crosses	12	27%
Other	10	23%
Monsanto / Dekalb lines	8	18%
Pietran or crosses	7	16%
Newsham lines	5	11%
Hampshire or crosses	5	11%
Danbred lines	4	9%
Genetiporc	4	9%
Berkshire or crosses	2	5%
Meishan or crosses	2	5%
Chester White or crosses	1	2%
Hypor	1	2%

The average boar culling rate is shown in table 3 and supports previous reports that boar turnover is very high and 88% of the studs replaced between 21-70% of boars annually.

Table 3.

The average annual boar culling rate :		
1-10%	0	0%
11-20%	3	7%
21-40%	10	23%
41-50%	9	21%
51-60%	10	23%
61-70%	9	21%
>70%	2	5%

The reasons for the high replacement rates are shown in Table 4. The responses list reasons that studs cull boars. The top reasons for this procedure involve genetic improvement, followed

by failure for boars to meet semen quality standards, boar soundness failure, and failure of boars to allow semen collection.

Table 4.

The primary reasons boars are removed from the stud include:		
genetic improvement	39	91%
poor semen quality	38	88%
feet and legs	35	81%
failure to collect	23	53%
disease or health	16	37%
poor ejaculate characteristics	15	35%
Aggressiveness	10	23%

Sperm production statistics

The responses listed in table 5 show responses for how studs produce doses of semen. The days of collection were typically on multiple days of the week for each stud, with Monday through Friday the most frequent days and with Sunday a significant collection day in 25% of studs. For production of semen, 71% of studs reported 51-150 billion sperm produced per boar per week with 70% of studs reporting 21-40 doses produced per week from this range of sperm cells. Mid range values for each category of sperm (100 billion sperm) and doses (30 doses / week) translates into 3.3 billion sperm per dose. Over 85% of studs reported that boars received 3-7 days of rest between collections.

Table 5. Semen collection and dose production.

	Response Categories						
Day of collection	Monday 89%	Tuesday 64%	Wednesday 48%	Thursday 93%	Friday 64%	Sat 2%	Sunday 25%
Sperm (billions)/boar/week	<50 7%	51-100 29%	101-150 32%	>150 5%	Unsure 27%		
Doses produced /boar/week	<15 5%	15-20 14%	21-30 37%	31-40 33%	41-50 9%	>51 2%	
Days of rest between collections	1-2 days 2%	3-4 days 36%	5-7 days 61%	>7 days 0%			

Most boars in stud were between 1-2 years of age. Training of boars started between 6-9 months of age in 96% of studs with training occurring in isolation and in the stud and lasting 1-3 weeks only in 93% of studs (table 6).

Table 6. Boar training.

Age of boars at start of training	*< 7 mo* *32%*	*8-9 mo.* *64%*	*>10 mo* *4%.*	
Location of training	*Isolation* *45%*	*Stud* *45%*	*Other* *9%*	
Duration of training	1 week 52%	2-3 weeks 41%	1 month 7%	

Semen collection procedures used double gloves in most studs to limit contamination and prewarming of collection containers to thermally protect semen (Table 7).

Table 7. Semen collection.

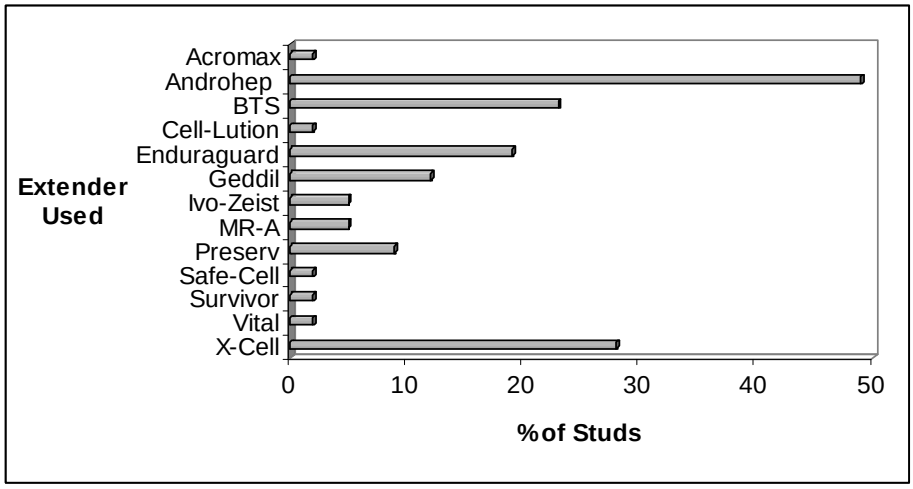
	Response category			
Glove requirement	Double pair 86%	Single pair 11%	None 2%	
Pre-Warming collection devices	Yes 75%	Sometimes 2%	No 23%	

Semen processing occurred almost exclusively by indirect volume assessment using weight (table 8). In addition, almost 81% of studs indicated that semen was pooled using from 2-6 ejaculates. The extenders sued by studs in the survey are shown in Figure 2.

Table 8. Semen processing.

	Response categories			
Volume assessment	Weight 98%	Volumetric 2%		
Semen Pooling	4-6 ejaculates 49%	2-3 ejaculates 16%	No pooling 19%	Line specific pooling 16%

Figure 2. Extender used at studs.



The ejaculates were evaluated using the basics of motility and concentration. The procedures used for motility were highly variable and included warming times for microscopic semen evaluation ranging from 0-30 minutes. Most studs used extender as a diluent while others did not dilute the raw sample (Table 9). The microscope magnification used was also highly variable and ranged from low magnification (100X) to high magnification at 400X. Semen concentration was estimated predominantly by photometer in 60% of studs and by CASA in 28% of studs.

Table 9. Proportion of studs indicating method of semen evaluation.

	Response Categories			
Warming time	0 min. 35%	1-5 min. 28%	5-15 min. 21%	20-30 min. 5%
Diluent used for fresh semen motility	None 23%	Saline 2%	Extender 72%	
Microscope magnification level for motility	100X 21%	200X 35%	400X 26%	Unsure 5%
Concentration estimation method	Photometer 60%	CASA 28%	Microscope Counting 7%	DNA staining 2%

Quality control procedures were evident as studs reported discard reasons and rates in table 10. Discard rates were most common in>80% of studs at 1-10% levels. The predominant reasons included poor motility and abnormalities almost all studs, followed by discard for bacteria, low sperm numbers and low volume of ejaculate. Other factors related to the reason for the discard included the boar itself in most cases, season of the year, disease, and genetic line (table 9). Most studs retained samples 3-7 days as a check for quality control.

Table 10. Proportion of studs indicating quality control practices.

	Response Categories					
Discard Reason	Poor motility 100%	Abnormal Sperm 98%	Bacteria 63%	Low sperm 56%	Low volume 26%	Contaminants 9%
Discard rate (%)	0 0%	1-5 47%	5-10 35%	11-15 9%	16-20 9%	>20 0%
Factors most related to ejaculate discard	Individual boar 77%	Season 58%	Disease or health 23%	Genetic line 19%	Collector 12%	
Ejaculate sample retention	Yes 5-7 days 77%	Yes 3-4 days 19%	Yes 1-2 days 2%	No 2%		

The studs indicated that doses of semen were packaged predominantly with 2-4 billion sperm cells. Over 70% of studs indicated they adjusted the dose of sperm cells for fertile sperm and packaged the dose in bottles and bags in 60-80 mL (table 11).

Table 11. Proportion of studs indicating semen packaging methodology.

	Response Categories		
Sperm numbers (billions)	2-3 billion 47%	3-4 billion 44%	4-6 billion 9%
Adjustment for fertile sperm	Yes 72%	No 28%	
Packaged volume	90-100 mL 7%	60-80 mL 93%	

Boars were essentially housed in stalls using fully or partially slatted floors (table 12). The boars were thermally regulated using evaporative and mechanical cooling systems for most studs. The animals were fed by both drop and hand feeding methods and received water by nipples or trough delivery systems. Feed amount was determined primarily by body condition or size of boar.

Table 12. Proportion of studs indicating housing and feeding systems used.

	Response Categories			
Housing	Stalls 91%	Pens 14%		
Flooring	Fully slatted 70%	Partial slats 26%	Solid 4%	
Cooling	Evaporative 74%	Mechanical 44%	AC 7%	None 5%
Feeding	Drop feeders 60%	Hand feed 40%		
Water delivery	Nipples 84%	Troughs 33%	Cups 5%	
Feed Amount / day	Body condition 94%	Boar size or weight 44%	Standard for all 12%	Adjusted for sperm production 9%

The labor in the stud and lab was related to the size of the stud within a day. Studs with 251-500 boars had between 3-4 collectors in 71% of studs and more persons in the remaining studs. For studs with 50 boars or less, 1-2 people were needed within a day. In larger studs more time was spent on collection. Of all studs, 36% indicated their people spent >25 h/week collecting of a 40 hour work week, while 36% reported their employees spent 16-25 hours/week collecting boars. Over 79% indicated they sometimes observed an effect while 19% indicated they always observe an employee effect on doses produced.

For all size of studs, 77% expected to hire a new employee with the year, with 27% indicating they would hire 1 employee, 16% needing 2, and another 16% needing 3 or more people. Not surprisingly, the larger the stud, the greater the frequency and higher need for new employees and this was most evident in the studs with more than 50 boars. The percentage of the stud labor force that speaks and understands predominantly Spanish was low and 80% of studs indicated there were no employees with this classification. Questions pertaining to employee training asked whether education would be beneficial for existing personnel and 64% indicated a positive response, 9% a negative and 23% unsure. Selection of employees with degrees beyond high school was considered beneficial by 45% of respondents, not beneficial by 32% and 23% were unsure.

The individual labor within a stud allocated to perform general tasks is reported by proportion of studs indicating the average employee hours spent on certain tasks within a 40 hour work week (Table 13). It would appear that there was great similarity by all respondents on the labor used for animal health, equipment repair and maintenance, and record keeping, but more variation in time spent feeding, moving boars and cleaning. For moving boars, there was more variation in the larger studs but little variation in the smaller sizes. For the most part the data on labor activities was somewhat related to size of the stud with more variation in the mid-range and larger sized studs.

Semen processing and evaluation lab labor revealed 1-2 people were employed for studs with >50, and 51-250 boars. Studs with 251-500 boars had an equal need for 1-2 or 3-4 people in the lab and the larger studs with >500 boars, used 3-6 people. In studs with more than 100 boars, more hours were devoted to processing within a work week with 36% of studs reporting employees spending more than 25 hours each week processing, and 36% of studs reporting 16-25 hours spent each week processing.

Table 13. Percentage of respondents indicating hours of stud employee labor hours spent during a 40 hour work week on job related tasks.

	Hours each week						
	0 hours	**1-5 hours**	**6-10 hours**	**11-15 hours**	**16-25 hours**	**>25 hours**	**unsure**
Animal health	0%	82%	11%	5%	0%	0%	2%
Repairing equipment	5%	81%	7%	0%	2%	0%	5%
Record keeping	7%	77%	9%	5%	0%	0%	2%
Feeding	2%	64%	30%	5%	0%	0%	0%
Moving boars	2%	52%	25%	11%	5%	2%	2%
Collecting	0%	7%	7%	14%	36%	36%	0%
Cleaning	0%	43%	32%	16%	7%	0%	2%

To see the full survey questions and responses go to The Illinois Livestock Trail swine repronet site and click on: http://www.livestocktrail.uiuc.edu/uploads/swinerepronet/papers/ZoomBoarSurveyIII.pdf

PRRS monitoring techniques and success stories

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Introduction

It is well documented that PRRS virus can be transmitted through semen and infect sows or gilts[1,2]. The best way to prevent downstream infection is for the boar stud to remain negative to PRRS. However, if infected, early detection is critical so shipment of semen can be stopped to minimize the risk of downstream infection.

How should boar studs be sampled

Most boar studs should sample blood rather than semen. It is clear the virus can be detected sooner and more readily in blood than semen in the early stages of infection in a boar[3,4,5,6,7,8]. The ideal sample is serum because the concentration of virus would be higher than in blood. Boars whose semen will be distributed downstream should be sampled at the time of collection to minimize the risk of distributing semen containing PRRS virus. An alternative is the blood swab method[8]. While ejaculating and without restraint, a needle prick is made in an ear vein. Using a polyester tipped swab, the blood is collected and the swab placed into a tube containing saline or PBS. Most of the universities would need a volume of 0.5 ml to be able to do an individual PCR and repeat the test (in the event of a positive or suspect result). Some sensitivity is lost with this technique due to the dilution effect in the saline or PBS and also the dilution effect caused by the red blood cells. Pooling of samples can be done in pools of 3 or 5, understanding that some sensitivity is lost and the chances of missing a positive are greater. This is particularly true during early infection, when the odds are higher that only one positive would exist within the pool.

The blood swab method can be done on the same boars at each collection. In the field, the flinch rate (% of boars responding negatively to the needle prick) has matched the research setting, at around 10%[8]. Due to the short time frame needed to do the needle prick, the blood swab method is easier for staff when compared with drawing blood for a serum sample on an unrestrained animal.

How often should boar studs be tested

The simple answer is that every boar should be blood sampled at every collection and tested individually for all strains of PRRS by PCR. Of course, this is not economically feasible due to the high cost of PCR testing. Most boar studs can afford to do statistical sampling as a means of risk management downstream. I recommend to further manage the risk by selectively sampling boars whose impact of infecting farms downstream is greater. For example:

1. All boars whose semen will be distributed to nucleus or multiplication herds should be sampled each day they are collected.
2. A statistical sample of the population collected each day should be sampled, as determined by the risk downstream farms or the stud are willing to take. The studs I work with do either a 95/10 or 95/5 sample on each collection day. For a large stud the numbers approach 30 or 60 per day, respectively.
3. Any boar who is off feed, feverish, or showing any clinical signs should be sampled immediately. Although clinical signs or fever by themselves are poor indicators of PRRS PCR status, a boar who has been infected with PRRS[3,8] virus would be more likely to be showing clinical signs or fever than a boar who has not been infected with PRRS virus.
4. The following table shows the number of boars that need to be tested to be 95% confident of detecting a positive boar given 5% prevalence rate

	95/5
# boars collected	# to sample by blood/serum (pools of 5)
<=30	Test all
31-35	30
36-50	35
51-70	40
71-100	45
101-175	50
176 or more	55

Withholding of semen

Semen should always be withheld until negative results are obtained. There is little value in testing a stud if semen could have already been used when results are obtained. If it is impossible to do this, it may be more appropriate to test weekly using ELISA and PCR tests, understanding that downstream infection is much more likely to have occurred when positives are found.

Economics of PCR testing for boar studs

I have developed an economic impact analysis to compare testing costs related to the costs of a 200 boar stud breaking with PRRS and the boar stud supplying semen to 35,000 sows. The costs to downstream production are based on a recent publication which quantified the costs of a farm breaking with PRRS[9]. A summary of the costs are as follows:

- $74.16 per litter loss with PRRS break
- Median 4 months loss
- $6.01 nursery loss per pig
- $7.67 finishing loss per pig

Based on these numbers, a 2500 sow unit would have an estimated total loss of $321,726 loss in 4 months. The following assumptions were made to determine this calculation:

- 17 weeks x 106 farrowings per week x $74.16 loss per farrowing
- 17 weeks x 106 x 7.63 pigs/litter (extrapolated from same paper) x $13.68 combined nursery and finisher loss per pig.

The losses for the boar stud were estimated to be $812 per boar. I estimated this number based on multiple stud breaks that I have been involved with. An assumption was made that boars could be replaced at cost of production, which is how most boars are sold today. An assumption was also made that 25% of downstream farms would be infected if the boar stud was only doing monthly monitoring rather than daily PCR monitoring and that stud closure would happen when clinical signs were significant enough to warrant diagnostics.

Summary of losses

Number of boars	200
Estimated loss per boar	$812
Total loss for boar stud	$162,400
Estimated loss per infected 2500 sow unit	$133,636
Estimated loss for nursery and finishing pigs	$188,090
Number of sows served by boar stud	35,000
Projected number of farms infected	25%

Estimated TOTAL loss	$1,288,441

Costs of PCR testing include the following

- $22 per PCR test
- Blood swabs or serum tested in pools of 3
- 42 boars tested per day on average using a 95/5 statistical sampling chart based on the number of boars collected each day
- 3 collection days per week
- Consumables (swabs, needles, microcentrifuge tubes) of $63/week
- Extra labor of 10 hours at $12 per hour
- Estimate of 200 miles each way to Diagnostic Lab at $.50 per mile
- Total cost of $1707 per week
- Total cost of $88,764 per year

If we use the estimated total loss numbers ($1,288,441) and divide by the total cost of testing per year ($88,764), we can estimate that the stud would have to operate for 14.5 years without a PRRS break to justify NOT PCR testing a statistical sample on a daily basis.

Realistic Expectations of Testing

Unless testing 100% of the boars every day, which in most cases is economically prohibitive, there needs to be a realistic expectation of the result. The following table shows the probability of finding virus in the stud within the first week after a single animal was infected. Of course, we don't know if one animal gets infected or if more than one get infected at the initial disease introduction. So if more than one animal gets infected when the virus initially enters the farm, you could expect better results than shown in this example.

The following table shows the percent of boars tested to be 90% confidence of finding the stud positive within two weeks after virus infected a single boar.[10]

Confidence Level (%)	**Serum or blood swab (individual)**	**Serum or blood swab (pooled)**	**Semen (individual)**
90	37%	44%	74%

Techniques for testing boar studs

Semen

Semen sampling should be done on raw semen only. Because of the poor of sensitivity, semen samples should not be pooled for testing. They should be tested individually.

Blood Swab

Blood samples have only a slightly reduced sensitivity from serum testing. This is due to the fact that the sample needs to be diluted in saline or in phosphate buffered saline (PBS). For most of the diagnostic labs, about 0.5 ml of sample is needed to run the necessary PCR tests to confirm an initial positive sample. As a result, we normally put the blood swab into 0.6 ml of saline or PBS. It is extremely important that the swab is 100% saturated with blood. Otherwise, the swab will soak up the saline and you will be short on sample and have potentially diluted out virus to the point a positive sample is missed. Use a swab that holds a lot of blood. The standard swab that should be used is a Quickvue® in-line strep A swabs (REF. 25-806 1PRLB QUICKVIEW, Puritan Medical products Company LLC). It is important to use this swab because it holds about 2.5 times the amount of blood as a standard Rayon or Dacron swab.

Serum

Serum is the ideal sample to use. PRRS virus is in much higher levels in the serum than in semen and at least 1-2 days sooner. Serum samples can be taken from the ear vein (auricular vein), leg vein (saphenous vein), or tail vein. Sampling is much easier if done while the boar is ejaculating. Another option is to retrain the boar and bleed from the neck. I have included a slide show on antemortem collection techniques for swine with the proceedings paper.

PRRS Elisa Testing

Even with extensive PCR testing, there is the chance that the PCR test will not detect the strain of virus that has infected a herd. This has happened, and in one case a new, undetectable (by PCR) strain was introduced into a boar stud. For this reason, it is still important to do PRRS ELISA testing. The ELISA test detects antibodies about 2 weeks after an animal has been exposed to PRRS virus, but it is extremely sensitive to all PRRS virus strains. I recommend that this be done on a weekly basis. Most studs do about 30 boars throughout a monthly period.

Case Reports

Seven case reports will be summarized. In each case, a boar stud was infected with PRRS virus. Also, all were sampled daily using the blood swab method. Intervention and results will be reviewed. Six of seven avoided depopulation of the whole stud site. No known downstream infections occurred in any of the cases.

Summary

Boars are positive in serum/blood before semen by PCR and in much higher quantity in the early stages of infection, thus blood sampling is the preferred method for monitoring of negative studs. Monitoring should occur daily. Choosing how many boars to test and which boars to test should be determined on an individual system basis. This is dependent on the estimated risk of being infected and the potential negative costs of downstream infection. Semen should always be withheld prior to when PCR negative results are obtained.

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Hands off boar semen collection

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Introduction

Artificial insemination (AI) revolutionized the commercial swine industry in the late 1980's and early 1990's to become an established component of pork production in the US. As a result, boar studs have become an integral part of pork production. At the time, little was known about managing large numbers of boars and volume semen production in a dedicated facility. However, rapid integration of AI into the swine industry has provided much opportunity to improve boar stud efficiency.

Boar stud labor represents approximately one half of the cost of an AI dose. However, ergonomic injury, competitive labor supplies and employee retention have added to labor costs. Boar stud labor costs have forced questions about time allotment per task, how many employees are needed and how can employee retention be improved? As a result, time spent using the industry standard glove-hand method to collect a single boar was targeted as an area to improve labor efficiency by decreasing employee time dedicated to each collection. Modifying boar collection also had the potential to improve safety and work environment, reduce ergonomic injury and reduce bacterial contamination of ejaculates.

Therefore, the need to improve the efficiency of boar semen collection in the swine industry was addressed by Minitube Today the AutoMate® is the most accepted and used alternative to the industry standard gloved-hand method of boar semen collection. The Automate has significantly reduced the labor requirement for boar semen collection, minimized or eliminated ergonomic injury, reduced bacterial contamination and improved employee safety and work environment.

Comparison of the AutoMate® and Gloved-Hand Method

The functional components of the AutoMate® are an artificial cervix (AC), which provides a firm texture and grip for the boar's penis and a hygienic double sheath plastic liner to facilitate separate collection of the pre-sperm fluid and the ejaculate. When the penis is exposed, the collector places an AC (Figure 4) on a gloved hand and grips the glans end of the penis to initiate erection and ejaculation. The pre-sperm fraction is collected in the tear-away inner sheath and discarded (Figures 4&5). The AC is then inserted into the AC clamp and the outer sheath and plastic ring are inserted into the cup to create a closed system of semen flow from the boar to the filtered collection bag (Figure 6). The collection apparatus rides on a mechanism under the dummy to allow thrusting while the boar continues ejaculation unassisted (Figure 7). The employee is now free to begin collecting another boar. When the boar has finished, reduction in penis diameter allows automatic release from the AC for a safe dismount from the dummy.

A trial was conducted at Minitube's International Center for Biotechnology to compare the AutoMate® and gloved-hand boar collection systems for the variables of collection efficiency, semen production and sperm quality.

Materials and Methods

Nine boars representing three lines and three age groups (9, 18 and 30 months) were used in this study. All boars were trained for gloved-hand and AutoMate® semen collection. A switchback design was used so that each boar served as its own control. Boars were collected on Monday and Thursday alternating between AutoMate® and gloved-hand at each collection. Boars were moved directly to the collection chute from housing pens. Time was recorded when a boar entered the collection chute. Time was subsequently recorded when the boar began thrusting and when ejaculation began and ended. Ejaculation was defined as complete when the boar began dismounting from the collection dummy. The pre-sperm fraction was discarded from both methods of collection followed by collection of the full ejaculate. Ejaculates were weighed to record volume. SpermVision® was used to determine concentration and motility values for each

ejaculate. Bacterial swabs of the gloved-hand, artificial cervix (AC) and neat semen were streaked on blood agar plates immediately after semen collection. Colonies were counted 24 hours after incubation at 37°C. Data were analyzed using SAS (Cary, NC) Proc GLM for the main effects of boar, collection system and boar x collection system.

Results

Differences between AutoMate® and gloved-hand collection of boar semen were not observed for any of the variables measured (Table 1; P>0.05) in this study. Boars approached collection from AutoMate® and gloved-hand collection systems similarly. Time from chute entry to beginning of ejaculation (2.7 ± 0.13 vs. 2.6 ± 0.13 minutes; P=0.3653) and ejaculation time (7.1 ± 0.23 vs. 7.2 ± 0.23 minutes; P=0.8031) were not different. Boars collected on the AutoMate® compared to the gloved-hand system produced a similar volume (230 ± 5.7 vs. 223 ± 5.7 ml; P=0.369) and concentration of semen ($0.28x10^9$/ml ± 0.02 vs. 0.26 $x10^9$/ml ± 0.02; P=0.4281). Semen motility from AutoMate® (88.2%) collections was not different from gloved hand (88.3%) collections (P=0.880). Similar results were recorded for progressive motility (74.7% vs. 74.8%; P=0.9611). Bacterial contamination was not influenced by collection method (Table 2) as measured by colonies formed in 24 hours after sampling the AC (94.1 ± 2.0), or the glove (97.4 ± 2.0). Semen from AutoMate® and gloved-hand collection systems formed similar numbers of colonies (19.6 ± 4.4 vs. 28.5 ± 4.5; P=0.1582). Although there was no collection system effect, a boar effect was observed for all variables measured in Table 1. Examples of these typical boar effects include ejaculation time (Figure 1; P<0.05), total sperm cells (Figure 2; P<0.05) and motility (Figure 3; P<0.05) as measures of efficiency, quantity and quality. However, despite boar effects no differences were observed within a boar for these variables when comparing the AutoMate® and gloved-hand systems of collection (Figures 1-3; P>0.05).

Discussion

Improvements in pork production systems must maintain or increase quantity and quality while maintaining or decreasing cost. Hands-off boar semen collection (HOC) methods have accomplished these measures of improvement and have become a viable alternative to the gloved-hand method of semen collection.

In this study, boars occupied the collection chute for approximately 10 minutes regardless of collection system. Boars occupied the chute for 2.7 minutes before starting ejaculation and ejaculated for 7.2 minutes (Table 1). Employees used the 2.7 minutes for cleaning the boar and preparing for collection in both the AutoMate® and gloved-hand systems. Warm-up boar pens were not used in this study. Warm-up or staging pens could reduce total chute time by up to 2.5 minutes to approximately 7-8 minutes, substantially increasing chute throughput.

Both collection systems require gloved-hand gripping of the penis to initiate ejaculation. However, in contrast to the gloved-hand system, the AutoMate® AC maintains ejaculation hands free for approximately 7 minutes. Seventy two percent of collection time was saved using AutoMate®. This difference provides employees approximately 7 minutes to finish and process a previous collection, begin the next collection, record data, obtain a blood sample or do other tasks. Since boars release themselves from the AutoMate® without assistance after completing ejaculation, boars can be efficiently moved in and out of the collection chute. Depending on the location of boars in reference to the collection chute it is feasible for one person to collect 6-8 boars per hour. At a base of 4-5 collections/hour and 25 doses/collection, doses/man/hour has increased by 50-75 doses or approximately 33%.

Gloved-hand collection requires employees to grip the penis and maintain moderate pressure for more than seven minutes (Table 1) to initiate, maintain and complete ejaculation. Daily repetitive gripping has resulted in ergonomic injury to some employees. In contrast, the AutoMate® system requires minimal gripping for extension of the penis, initiation of ejaculation and positioning in the AC which takes approximately 30-45 seconds. Gripping and pressure are then transferred to the AC for maintenance of ejaculation. AutoMate® collection effectively eliminates more than seven minutes of prolonged gripping, likely eliminating ergonomic injury.

Most bacteria were surface contaminants found on the glove or AC and lesser amounts of bacteria were found in neat semen. While no statistical difference in bacterial contamination was observed between the collection systems (Table 2), neat semen from AutoMate® collection tended to have a lower number of colonies compared to the gloved-hand system. Reduction of contamination is attributed to the closed system of semen collection (Figure 6). Methods that prevent preputial fluids and foreign matter from entering the semen collection vessel are beneficial to semen quality. Assembly of the closed system eliminates preputial and pre-sperm fluid from contaminating the ejaculate. Similarly the closed system also prevents bacteria from the boar and environment from entering the collection cup during the 7 minute hands free ejaculation period. In contrast, semen is routinely exposed to the glove during gloved-hand collection and the open-top collection vessel is open to the barn environment. The magnitude of contamination difference between the gloved-hand technique and AutoMate® could vary with individual stud collection conditions.

Tables 1-3 demonstrate that AutoMate® collection of boar semen is an efficacious system of boar collection for a variety of boar ages and lines. Boars of all ages generally adapt well to the AutoMate® after installation. In this study, boars responded equally to alternate gloved-hand and AutoMate® collection. There are occasions where a small number of older boars do not adapt and still require gloved hand collection. Young boars train well to HOC systems. Most boars that do not train to the AutoMate® do not train to the gloved-hand method either.

The unique designs of HOC have facilitated modification of alley ways and penning to more effectively separate the employee from the boar before, during and after collection. These designs have not only increased efficiency of boar handling but have also eliminated the uncomfortable and dangerous postures required for the gloved-hand technique. By improving safety, the quality of the work environment has been improved. This improvement has a positive impact on employee morale and retention rates.

In conclusion, HOC systems reduced the labor requirement by approximately 70% for a single collection. Time during the ejaculation phase of one boar can be used to complete the collection process of a previous collection and begin the next collection. Producers can expect boars to perform efficiently and produce normal quantity and quality of semen when using the AutoMate®. Furthermore, the closed system of sperm cell harvest may reduce or eliminate environmental bacterial contamination of the semen and therefore, enhance semen quality.

Table 1: Comparison of Automate® and gloved-hand systems of boar semen collection (lsmeans ± SE).

Variable	AutoMate®	Gloved-Hand	P value
Number of boars	9	9	
Number of collections	54	63	
Entry to begin ejaculation (min.)	2.7 ± 0.13	2.6 ± 0.13	0.3653
Ejaculation time (min.)	7.1 ± 0.23	7.2 ± 0.23	0.8031
Ejaculate volume (ml)	230 ± 5.7	223 ± 5.7	0.369
Concentration (10^9sperm cells/ml)	0.28 ± 0.02	0.26 ± 0.02	0.4281
Total sperm cells (10^9)	57.3 ± 2.7	53.3 ± 2.8	0.3017
Motile cells (%)	88.2 ± 0.81	88.3 ± 0.81	0.8808
Progressive cells (%)	74.7 ± 1.3	74.8 ± 1.3	0.9611

Table 2: Number of colony forming units after 24 hours of incubation at 37°F from swabs taken from the AC, collection glove or samples from neat semen immediately after semen collection from the AutoMate® and gloved-hand systems (lsmeans ± SE).

	AutoMate® AC	Gloved-Hand glove	P value
Swab	94.1 ± 2.0	97.4 ± 2.0	0.2644
Neat semen	19.6 ± 4.4	28.5 ± 4.5	0.1582

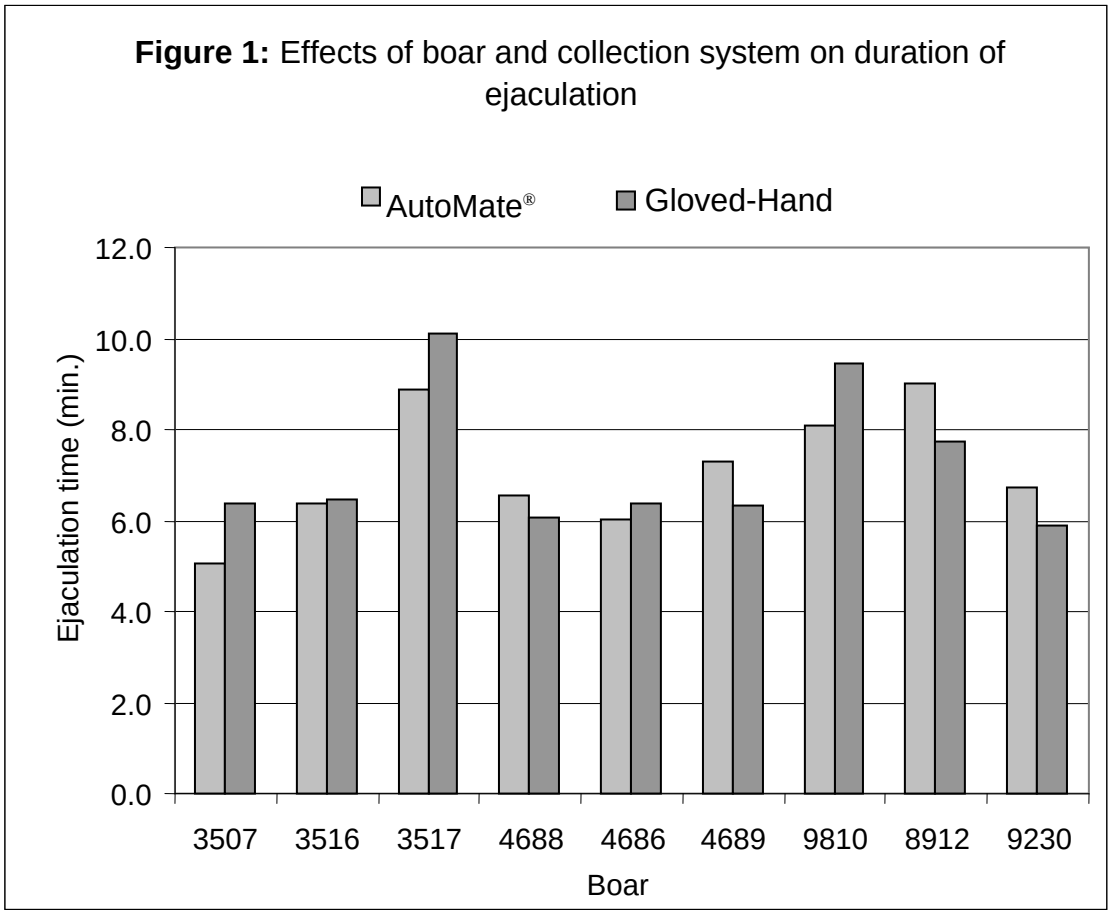

Figure 2: Effects of boar and collection system on total sperm cells per ejaculate

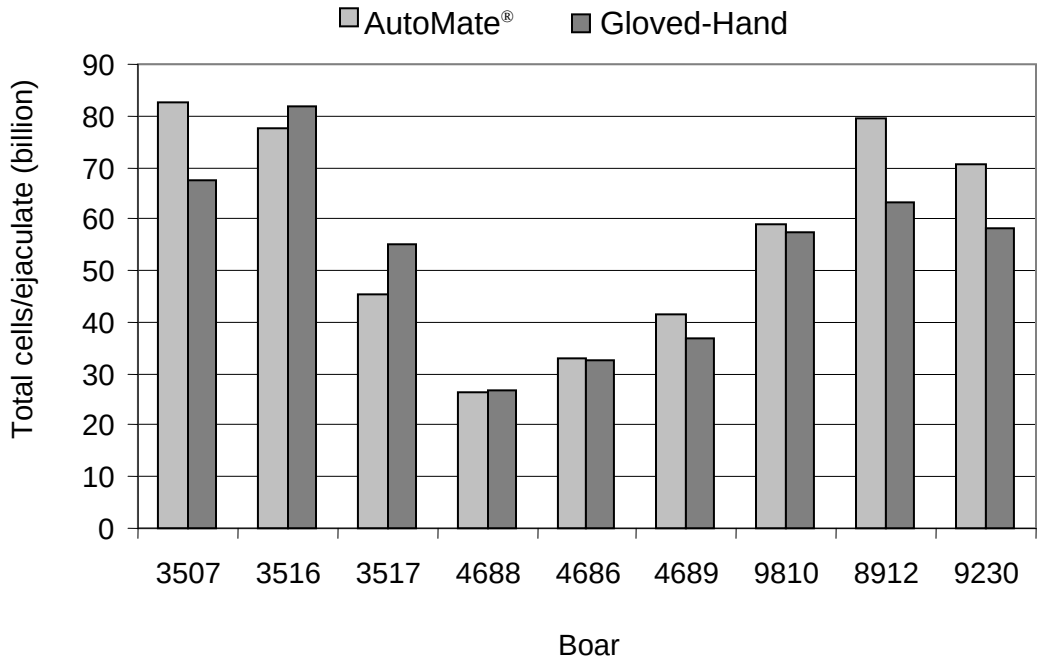

Table 3: Effects of boar and collection system on sperm motility of neat semen immediately after collection

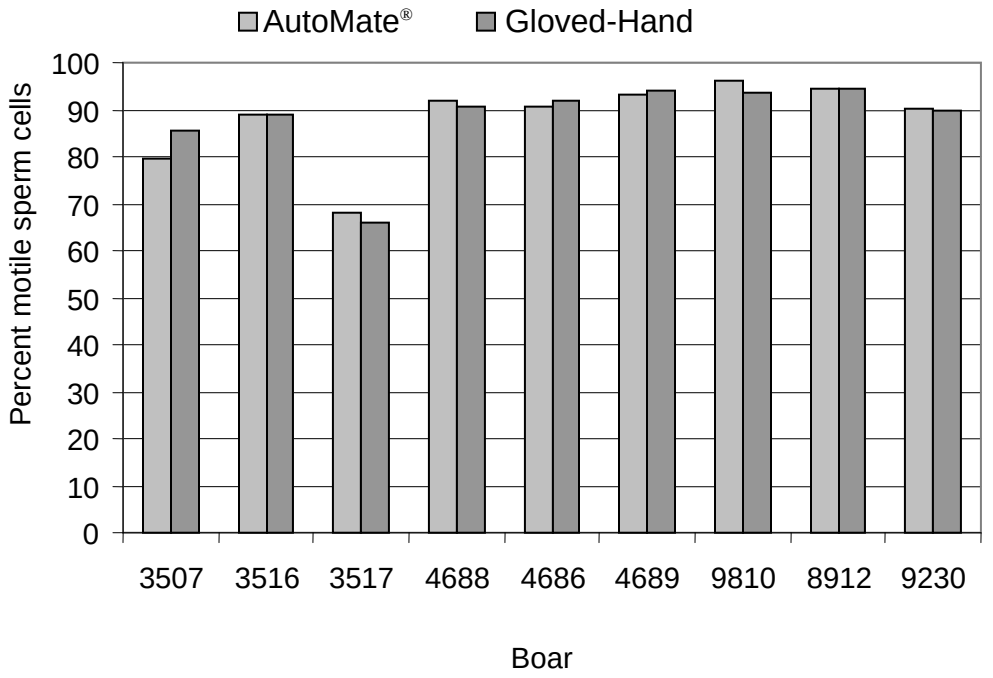

Figure 4. AC with double-sheathed plastic liner.

Figure 5. The inner bag with the pre-sperm fluid is torn away and discarded.

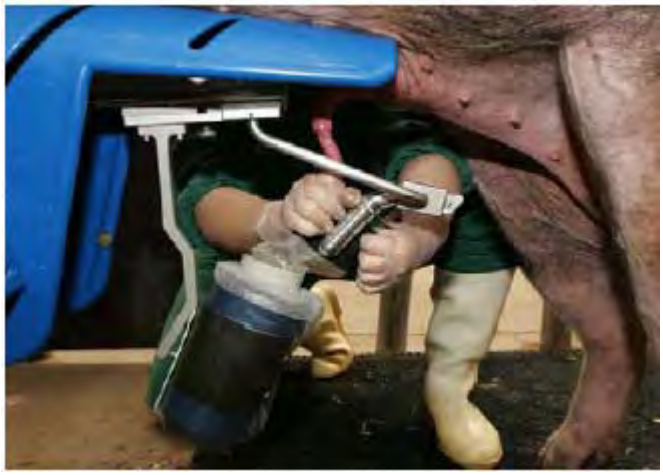

Figure 6. The AC is inserted into the AC clamp; the boar can finish ejaculation unattended.

Figure 7. Closed system of sperm cell harvest.

Sperm Quality Tests: What Are Other Species Using?

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INTRODUCTION

This paper outlines various semen quality tests that are used in a variety of mammalian species, with emphasis on the human, bovine, and porcine. The objectives are to explain why semen testing is useful, to explain how basic semen evaluation is conducted, and to describe more sophisticated assays of sperm function. Boar stud managers should consider this information from other species as it pertains to their own units.

WHY SEMEN TESTING IS (OR IS NOT) USEFUL

Accurately predicting semen fertilizing capacity would be extremely beneficial to boar studs since semen from one male is subdivided and used to inseminate many females. To the best of my knowledge, however, there exists no "quick and dirty" assay that can predict semen fertility in typical commercial artificial insemination (AI) conditions (Tardif et al., 1999).

Semen testing can provide useful information. In the human andrology clinic, simple tests and more complicated assays of semen quality can give insight into confirming and, sometimes, diagnosing infertile or subfertile men. Appropriate therapies or assisted reproductive techniques can then be recommended to counter any apparent male-mediated problem. Alternatively, semen analysis should always be conducted to verify that an intentional sterilization procedure or contraceptive approach has been effective.

In a zoo setting, sperm quality may be assessed if there is concern that the male animal of interest has fertility problems or to ensure reasonable reproductive potential prior to his being committed to a cross-zoo breeding effort. Wild animals may also be temporarily restrained and subjected to semen analysis if the semen is to be processed and used for captive breeding programs. In addition, because there is concern that environment toxicants interfere with reproductive development and function, wild animals may also be screened for semen quality as an index of reproductive health. Semen analysis is conducted in zoo species and companion animals such as dogs, cats and horses used for controlled reproduction in a similar manner to agriculturally-important species that are to be bred. In these cases, relatively simple assays are carried out in the hope of ensuring good fertility rates following hand mating or AI with fresh or cryopreserved semen.

Unfortunately, semen analysis is not a test of male fertility. The goals of semen analysis to predict fertility are quite generalized, such as to determine the minimum conditions (e.g. sperm numbers) to ensure fertilization (or contraception) in an individual, to evaluate the probability of reduced fertilizing potential in a population, or to determine which males in a particular group (e.g. with a desirable pedigree) might be the most fertile. Predicting the fertility of an individual or group of individuals, which is desirable in human medicine, dairy cattle AI, or the zoo setting, is not the same as predicting the fertility of a semen sample, which would be useful for the boar stud.

Successfully predicting male fertility is limited by the characteristics of the mammalian ejaculate, in that the total number of sperm/ejaculate far exceeds the numbers necessary for fertility. Moreover, the sperm population in an ejaculate is tremendously heterogeneous and we have no way of knowing which sperm will be the one that will fertilize the oocyte. Similarly, sperm fertilizing capacity is often confounded by the female, since fertilization is internal and the female reproductive tract influences sperm function. Fertility prediction based on semen analysis is also limited by the methods that exist in the laboratory to evaluate sperm quality, as there are few techniques that can evaluate a representative number of sperm in the ejaculate, it is difficult for one assay to adequately assess the full functionality of sperm, and it is not always possible to evaluate an adequate number of samples per male.

Whether the semen to be used is fresh or cryopreserved also has an influence on the predictability of semen fertility. Similarly, predicting male fertility also depends on the type of reproduction to be conducted, for example natural mating, AI, *in vitro* fertilization (IVF), or intracytoplasmic sperm injection (ICSI). These methods vary in terms of the numbers and functional quality of sperm required for fertilization and embryo development.

BASIC SEMEN EVALUATION

For most species, including human and bovine, quality is generally assessed at body temperature on undiluted semen that has been collected within minutes. For samples that have been collected far from the laboratory or cooled, an aliquot should be re-warmed for 5-10 minutes prior to microscopic analysis.

Standard microscopic semen evaluation is principally focused on sperm motility and morphology. At its crudest, “mass motility” can be estimated by observing the characteristics of a droplet of undiluted semen on a slide without a coverslip. My laboratory will use this technique in the field if we are quickly verifying that a particular sample is motile or not, such as immediately post-collect or prior to insemination. A scale from 0 (immotile), 1, 1+… to 4 (very rapid) or 1 to 10 can be used; the scale used can vary, but should be repeatable within a species and unit to facilitate comparisons.

Sperm motility can also rapidly be estimated using a wet preparation under phase contrast microscopy. Semen is diluted and examined on a coverslipped slide after waiting about a minute until the “flow” stops and sperm motility can be observed. At least 200 sperm on about 5 different fields should be observed in duplicate (400 sperm total) and reported as the percentage of motile sperm. Progressive motility could also be calculated by categorizing sperm patterns into four categories that are then expressed as percentages (rapidly progressive - WHO class a, slowly progressive - WHO class b, slow and non-progressive – WHO class c, and immotile – WHO class d; WHO, 1999). Handheld counters greatly facilitate sperm motility assessment. As for mass motility, wet mount motility should be assessed rapidly to avoid artifacts caused by either a temperature decrease or dehydration of the preparation, or before sperm metabolism decreases and motility falls. It should be pointed out that many semen extenders are intentionally designed to decrease sperm metabolism during storage, therefore, extended or thawed sperm may appear sluggish. Full motility typically resumes after dilution and warming in physiological

media; this approach is rarely carried out in human clinics or in animal studs, although I feel it provides a better assessment of sperm quality.

Wet mounts also permit evaluation for sperm agglutination and the presence of other cell types or debris in the sample. The percentage of agglutinated sperm is determined from 10 fields and estimated to the nearest 5%). Non specific agglutination occurs when the sperm aggregate with particles (debris, dead sperm, other cells) present in the seminal plasma and is frequently observed with boar and stallion sperm if gelatinous bits remain in the sample. Human infertility clinics often perform tests (commercially available) for specific agglutination (head to head or tail to tail) caused by the presence of anti-sperm antibodies; this is not considered to be problematic in domestic animals, other than, perhaps, horses. Head to head agglutination, however, is common as sperm become ready for fertilization (capacitation), and is often observed in frozen-thawed bovine sperm and even in fresh boar sperm.

SPERM FUNCTION ASSAYS

Even following the complicated processes of spermatogenesis and epididymal maturation, the ejaculated spermatozoon must undergo a daunting series of modifications (summarized in Table 1) to achieve fertilization and produce a competent embryo.

Table 1. Biological events involving ejaculated sperm required for reproduction.

Vigorous sperm motility
Sperm-oviduct binding and release
Capacitation
Transit to the site of fertilization
Binding to the oocyte zona pellucida
Acrosome reaction
Penetration of the zona pellucida
Sperm-oolemma fusion
Penetration of the oocyte
Sperm chromatin decondensation
Syngamy
Embryo development
Implantation
Fetal survival

Furthermore, sperm must demonstrate numerous biological attributes that enable them to carry out these events (Table 2). More sophisticated assays can provide more information on the reproductive capacity of the sample than for basic semen evaluation alone. As mentioned earlier, although these functional assays may not correlate with field fertility, the results could identify a problem should fertility rates be suboptimal.

Objective Sperm Motility Tests – Although it is generally accepted that precisely determining the proportion of motile sperm in a sample is an important assay of semen quality, the basic techniques of motility analysis described above are subjective.

Computer-Assisted Sperm Analysis (CASA) was developed to objectively measure sperm motion characteristics. The basic components of a CASA system include a microscope with heated stage, a video recorder, and a computer that digitalizes sperm images with software to measure

and analyze the motion parameters. This technology has been commercially available for 20 years, however, the cost and apparent inability to easily predict semen fertilizing ability limited CASA use to the human andrology lab or for research. In addition, proper CASA use requires considerable technical attention, for example to properly identify sperm (as opposed to debris) and slow or immotile sperm, to validate the settings for different diluents, etc. More recently, however, less costly CASA systems are available and are designed specifically for the AI industry. Properly conducted, CASA is very objective, enabling standardized evaluations among different technicians or labs.

Table 2. A partial list of sperm characteristics necessary for fertility (modified from Amann & Hammerstedt, 1993).

Appropriate morphology
Sufficient energy production
Progressive motility
Capacity for hyperactivated motility
Membrane lipids
- To stabilize plasma and acrosomal membranes
- Flippase enzyme activity
- Facilitate membrane fusion in a timely manner
Membrane proteins
- Immunosuppressive factors
- Attachment ligands available but masked until required
- Acrosome reaction inhibiting factor(s)
Enzymes associated with fertilization
- To modify membrane glycoproteins
- Acrosomal enzymes
Intact and functional genome

The sperm-cervical mucus interaction test is used in the human lab to objectively determine the sperm's ability to penetrate the man's partner's cervix (WHO, 1999). Either the man's partner's cervical mucus, bovine cervical mucus, or a synthetic medium may be used. Mucus is aspired into a capillary tube, which is then placed vertically in an aliquot of sperm. After a certain time period, the numbers of sperm present along the capillary. Samples that have the most sperm furthest along the capillary are then considered to be the most motile in these physiologically relevant conditions.

Sperm Vitality - For human semen, the WHO (1999) recommends that if less than 50% sperm are motile, the vital staining should be also conducted to differentiate between dead sperm and live, immotile sperm. For human and bovine sperm, eosin Y and nigrosin staining coupled with phase contrast microscopy is often used as it is rapid, inexpensive, and the slides can be assessed at a later time (Barth and Oko, 1989). We use this stain for porcine (Tardif et al., 1999), ovine (Morrier et al., 2000), caprine and rooster sperm. The principle of the staining is that a cell with an intact plasma membrane does not incorporate the eosin, while a dead cell (i.e. one with damaged membrane) takes up the purple stain. Nigrosin is used as a background stain to provide contrast for the unstained (clear), live cells. Other similar stains can be used based on the same principle of dye exclusion, such as trypan blue and eosin-analine blue.

In recent years, flow cytometry has been used to assess sperm viability using fluorescent probes such as ethidium bromide, which colors the plasma membrane and propidium iodine, which penetrates damaged plasma membranes to stain the DNA. Similar commercial kits designed for use with sperm are commercially available through Molecular Probes (Eugene, OR). Flow cytometry is a very powerful technique as tens of thousands of sperm can be evaluated in minutes, which is far more representative than a couple hundred sperm on a microscope slide! Flow cytometers, however, are costly (>$100,000) and require a certain technical expertise. Nonetheless, many assays other than vitality can be performed by flow cytometry (e.g. sperm intracellular calcium level, internal pH, membrane fluidity, etc.), so it is more frequently being used in human andrology and bovine sperm laboratories.

Morphology - Sperm morphology is generally very good in domestic animal semen samples (>70% normal), whereas as few as 30% morphologically normal sperm is considered acceptable in men (WHO, 1999). Certain species of large felines (in the wild and in captivity) are also reported to show extreme teratospermia, possibly as a function of inbreeding (Pukazhenthi et al., 2006). Morphologically abnormal sperm may be motile, but rarely penetrate the uterus. Indeed, human sperm demonstrate large variations in morphology, and observations on sperm obtained from post-coital cervical mucus have helped to define the morphology of an "ideal" human spermatozoon, and it is presumed that the fertilizing spermatozoon is selected among these ideal cells (WHO, 1999). To the best of my knowledge, such investigations have not been thoroughly conducted on domestic animal species. Moreover, the percentage of morphologically normal sperm is positively correlated with *in vivo* fertilization and IVF outcome in humans (Coetzee et al., 1998). Human andrology laboratories, therefore, consider sperm morphology to be an important factor in male fertility, whereas it is much less emphasized in AI studs. In human clinics, sperm are fixed and stained (e.g. Giemsa, Bryan-Leishman, or Papanicolaou) to thoroughly evaluate morphology, whereas wet mounts or simple fixation might be used in animal units. Our laboratory usually uses eosin-nigrosin (Tardif et al., 1999) to evaluate morphology as well as vitality. Sperm morphology is typically described according to the anatomic site of the defect (head, midpiece, tail, or presence of cytoplasmic droplets). Some abnormal sperm may have defects in more than one site. Understanding the nature of the abnormality is also important. Primary defects are the more severe and are thought to originate while the sperm was still within the semeniferous epithelium of the testis. Secondary defects are clinically considered to be less serious and thought to arise during passage thought the epididymis or by mishandling after ejaculation. Some of these defects, therefore, could be avoided.

Assays of Fertilization-Related Events – The ability to bind sufficiently to the oocyte's zona pellucida is essential to fertilization *in vivo*. At least two approaches can be used to assess sperm-zona binding ability. The Hemizona Assay involves laser bisection of oocytes to divide the zona in two equal fragments. Sperm from a known fertile male (control) are added to one hemizona and sperm from the male undergoing testing are incubated with the second hemizona. After a period of time to encourage capacitation and binding, the numbers of tightly bound sperm are assessed and compared between the two individuals. Competition assays can also be conducted on intact zonae where the sperm from the control and test males are differentially stained using fluorochromes. After the heterospermic insemination and incubation, the numbers of sperm from each male are clearly discernable by fluorescent microscopy. These zona binding assays are most common in the human infertility clinic, but may also occur on a zoo setting with a specialized team in reproductive biology.

After binding the zona, the capacitated sperm must undergo the acrosome reaction. Sperm that do not acrosome react in response to appropriate stimuli are considered to be non-responsive and may not be fertile. In contrast, sperm that spontaneously acrosome react in the absence of stimuli are highly fragile and poorly fertile *in vivo*. In most mammals, the acrosome is very small and requires staining to be visible with a microscope. Non-fluorescent stains include Coomasie blue, erythrosine B-flavanic acid among many others. Often lectins or antibodies coupled to fluorochromes are used that bind either the outer or inner acrosomal membrane so that the intact or acrosome reacted sperm, respectively, can be visualized. To fully assess the ability of a sperm sample to appropriately acrosome react, capacitation is first induced, the level of spontaneously acrosome reactions should be noted, and then a stimulator of the acrosome reaction is added to the sperm aliquot. Homologous zonae pellucidae is the universal inducer of the acrosome reaction in mammals, although preparing sufficient quantities of zonae can be difficult and/or expensive. Common alternative acrosome reaction inducers include calcium ionophore, progesterone and lysophosphatidylcholine (a fusigenic lipid).

The acrosome reacted sperm penetrates the zona to fuse with the oocyte membrane and the gamete interactions necessary fertilization are essentially complete. The classic Hamster Oocyte Penetration Assay tests the ability of human sperm to penetrate zonae-free hamster oocytes. Heterologous systems have also been very useful in the zoo setting where salt-stored oocytes from domestic cats have been used to test the fertilizing ability of sperm from wild felines (Donaghue et al., 1992). Homologous *in vitro* fertilization (IVF) can also be used as an indicator of sperm fertilizing capacity, usually with cattle as bovine IVF works well. For most other mammals, however, homologous IVF is technically challenging, time consuming, costly, impractical (e.g. unethical in humans, poor results in most other species). It should also be clearly noted that successful IVF does not ensure that the sperm will be fertile *in vivo*.

Sperm Chromatin Tests – Assays of sperm DNA packaging are becoming more popular in human clinics and in animal studs. Sperm can be perfectly functional and fertile, however, DNA defects can lead to embryonic lethality or developmental abnormality. The Analine Blue Assay is used primarily for human sperm (WHO, 1999). Aniline blue or blue toluidine blue stains are used to mark lysine-rich sperm nucleoproteins (histones), which are visible by microscopy, thereby indicating nuclear maturity, since sperm histones should be mostly replaced by protamines.

Terminal Uridine Deoxynucleotidyl Transferase dUTP Nick End Labelling (TUNEL) is a method for detecting DNA fragmentation. TUNEL is based on the principle that damaged genomic DNA may have breaks (“nicks”) that can be identified by labelling that free 3’-OH terminal with fluorescent nucleotide markers in an enzymatic reaction. This assay can be conducted using fluorescent microscopy or flow cytometry.

The Comet Assay is based on single cell microgelelectrophoresis. Individual sperm are embedded in a thin agarose gel on a microscope slide. All sperm proteins are then removed from the cells by lysing and the DNA unwinds. Following the unwinding, the DNA undergoes electrophoresis, allowing the broken DNA fragments or damaged DNA to migrate away from the nucleus. After staining with a DNA-specific fluorescent dye such as ethidium bromide or propidium iodide, the gel is read for amount of fluorescence in head and tail and length of tail. The damaged cell looks

like a comet, hence the name of the assay. The extent of DNA liberated from the head of the comet is directly proportional to the amount of DNA damage.

The Sperm Chromatin Structure Assay is based on the principle that the fluorescent marker, acridine orange, interacts with DNA and RNA. When bound to DNA, acridine orange fluoresces green, but when it associates with RNA or broken, single-stranded DNA, it fluoresces red. Therefore, a ratio of the red/green fluorescence is an indication of sperm DNA damage, which has been correlated to pregnancy rates in humans. We have used this assay extensively in sheep (Peris et al., 2004), bovine, chickens, and in pigs.

CONCLUSION

There are numerous assays to assess for sperm function, most of which are performed in human clinics. Nonetheless, zoos and the bovine AI industry, which relies heavily on the use of specific bulls, also tend to analyze semen quality more intensively than do boar studs. A number of these assays could easily be incorporated into a porcine AI laboratory to aide in quality control, assess the semen of new or questionable boars, or test the suitability of new extenders or storage protocols.

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Cytoplasmic droplets: the most frequently observed abnormality

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Introduction

One of the most widespread and poorly understood sperm abnormalities within the boar ejaculate is commonly referred to as the sperm cytoplasmic droplet (CD). The purpose of this review is to provide a basic understanding of the CD including its formation on the sperm in the testis, movement on the sperm in the epididymis, and shedding from the sperm at ejaculation. Special emphasis will be placed on addressing the potential effect of the sperm-attached CD on the fertilizing ability of spermatozoa in the ejaculate, as well as identifying detrimental biological and environmental factors which may increase CD retention on spermatozoa in the ejaculate.

Definition, shape, size and location of the CD

The CD is a membrane-enclosed vesicle of cellular cytoplasm that remains attached to the sperm tail midpiece after the completion of spermatogenesis in the testis. Studies using specialized microscopy have determined the average shape and size of the mammalian CD to be generally spherical and 2-3μm in diameter (Kaplan et al., 1984). During microscopic evaluation the CDs can be observed either attached or unattached to the spermatozoa. When attached to the spermatozoon, the CD is associated with the sperm tail midpiece in one of two locations: 1) at the proximal end of the midpiece, i.e. next to the sperm head (proximal CD; Figure 1A) or 2) located at the distal end of the midpiece (distal CD; Figure 1B) at the junction of the midpiece and principle piece of the sperm tail. The distal CD can also be associated with the sperm tail abnormality known as the distal midpiece reflex, which is characterized as a 180° bend of the sperm tail around the distal CD at the midpiece/principal piece junction (DMR CD; Figure 1C). The CD can also be observed detached from the sperm and free-floating in the ejaculate (unattached CD). Unattached CDs can be observed individually or as clusters present in boar semen (Figure 1D).

Formation of the CD during spermatogenesis

Formation of the CD occurs during spermatogenesis when the haploid round-shaped sperm cell, a round spermatid, matures into the elongated sperm cell, an elongated spermatid, and eventually into a fully differentiated sperm cell, a spermatozoon. A hallmark of this morphological change is the removal of the residual cellular cytoplasm from the elongated spermatid. The residual cellular cytoplasm is removed from the elongated spermatid in order for the sperm cell to become an efficient motile transporter of the male DNA (Barth and Oko, 1989). Formation of the CD begins when the residual cellular cytoplasm is removed from the elongated spermatid and is retained and destroyed by the Sertoli cell, the nurturing cells of the seminiferous epithelium. When the spermatid stalk, which connects the residual cellular cytoplasm to the presumptive CD is severed, a small amount of cytoplasm remains attached to the sperm tail midpiece (Figure 2).

This small amount of cytoplasm is the CD. The residual cellular cytoplasm is resorbed and destroyed by the Sertoli cell. It is important to understand that all newly formed sperm cells in the testis possess a proximal CD following spermatogenesis (Russell, 1984).

Movement of the CD on the sperm tail midpiece during epididymal transit and at ejaculation

After spermatogenesis, spermatozoa pass from the testis into the epididymis, which is a tubular gland located on the outside of the testis. The epididymis consists of three contiguous areas: the head (caput), the body (corpus) and the tail (cauda). Spermatozoa travel through the epididymis for a period of 7 to 14 days, depending on the species. It is during epididymal transit that the spermatozoa mature and become capable of fertilization. When released in the testis, all spermatozoa possess a proximal CD (Barth and Oko, 1989). As the spermatozoa pass from the caput epididymis into the corpus epididymis, the CD migrates along the midpiece from the proximal to the distal position. It is not yet understood why or by what mechanisms movement of the CD occurs in the epididymis. However, due to other sperm maturational events occurring in the caput, one could speculate that an unidentified product secreted by the caput epididymis could induce CD movement from the proximal position to the distal position (Bassols et al., 2005). Following migration to the distal position, the CD can be released in the cauda region of the epididymis in some species. However, in the boar, the majority of spermatozoa in the cauda epididymis still possess a CD in the distal position (Kato et al., 1996), and some spermatozoa possess a CD in the proximal position. During ejaculation or quickly thereafter, the CDs can be released from the sperm and found free-floating within the seminal plasma of the ejaculate, although most unattached CDs disintegrate within one minute of ejaculation (Kato et al., 1996). It is likely that the majority of the free floating CDs were of attached to spermatozoa as distal CDs, however it is possible that some may have been proximal CDs. Not all boar spermatozoa lose the CD at ejaculation. Ejaculated spermatozoa can have a CD in the proximal or distal position, or as part of the distal midpiece reflex. Sperm with an attached CD are sometimes referred to as immature, due to the CD being attached to the sperm tail midpiece (Sancho et al., 2004).

Extensive research has analyzed the migration of the CD in swine and other farm animal species during epididymal transit. Table 1 summarizes the results of studies characterizing the movement of the CD from the proximal position to the distal position for each region of the epididymis. In the boar, the percent of spermatozoa with a proximal CD in the caput epididymis ranges from 40-90%. This figure drops to around 5-15% in the cauda epididymis. Conversely, the percentage of spermatozoa with a distal CD ranges from 0-20% in the caput epididymis, rising to 11-97% in the cauda epididymis. Non-farm animal species do not differ greatly from swine in CD migration patterns during epididymal passage, though the retention of the CD in ejaculated spermatozoa may be less common (Temple-Smith, 1984; Hermo et al, 1988; Axnér et al., 2002).

Few studies have evaluated the frequency of the DMR CD (Bonet, 1990; Briz et al., 1995). It is not currently understood if formation of the DMR CD occurs in the epididymis, or is present only following ejaculation. Briz et al. (1995) found sperm with the DMR CD in the caput region of the epididymis of the boar. Though not confirmed, it has been hypothesized by Koefoed-Johnsen and Pedersen (1971; reviewed Barth and Oko, 1989) the DMR CD is caused by lysosomal enzymes released from the distal CD. These enzymes could mediate break down of

the sperm tail structural components, thus allowing the bending and breaking of the flagellum at the midpiece/principle piece junction. The DMR CD is also a common defect in mutant mice lacking the gene for tyrosine kinase c-Ros (Yeung et al., 1999). Spermatozoa with the DMR CD defect may have compromised motility due to the abnormal tail structure (Figure 1C). It is difficult to evaluate the effect of sperm with the DMR CD because studies will often group the DMR CD sperm with other sperm tail abnormalities or with other general sperm abnormalities instead of identifying it as a unique defect related to the CD.

Purpose of the CD

It is not known if the CD is formed on the spermatozoa to serve a specific purpose during sperm maturation or fertilization, or if the CD is simply a rudiment remaining after spermatogenesis is completed. Early hypotheses proposed the droplet as an endogenous source of energy following ejaculation (Guraya, 1963). However, others (Fawcett and Ito, 1965) have concluded that the CD is nothing more than a functionless remnant of cytoplasm. Current hypotheses suggest the CD may be responsible for proper maturation of the mitochondrial sheath (Briz et al., 1995), maintenance of correct osmotic balance to prevent sperm tail angulation in the female reproductive tract (Cooper and Yeung, 2003), or antioxidant protection in the epididymis (Nichi et al., 2006)

Structural contents of the CD

Research on the structural contents of the CD has found very similar results between different species (bull, ram, rat, rabbit, boar, sheep, hamster, opossum and bat). It is widely accepted that the CD is surrounded by a continuous plasma membrane (Bloom and Nicander, 1961). Many studies characterize the CD as containing small vesicles, curved saccular elements (horse-shoe shaped or circular) and smooth surface tubules, identified as lamellae. The composition and distribution of contents within the CD are modified during migration of the CD from the proximal to the distal position, which typically coincides with sperm transit from the caput to the corpus epididymis.

Proteins, enzymes and molecules associated with the CD

Proteins, enzymes and molecules required for cellular homeostasis prior to terminal differentiation of the spermatozoon (i.e. during spermiogenesis) have been identified within the CD. As previously described, the CD is a small mass of cellular cytoplasm not removed from the elongated sperm cell with the residual cellular cytoplasm during spermatogenesis. Therefore, it can be assumed that proteins, enzymes and other molecules required for formation of the elongated sperm cell during spermiogenesis will be present in the CD. It is not yet known if this is a deliberate mechanism of the sperm cell to retain certain molecules for maturation and maintenance during epididymal transit, or if it is simply a by-product of spermatogenesis. Most researchers currently agree that enzymes associated with the CD provide no benefit to the mature sperm cell or its surrounding environment.

Table 2 provides a summary of the proteins, enzymes and molecules that have been identified as components of the CD. Though research is lacking, the possible actions of these components of the CD include reactive oxygen species generation (Aitken and Sawyer, 2003), formation of the mitochondrial sheath on the midpiece (Westhoff and Kamp, 1997), CD removal and degradation

of organelle membranes within the CD (Janulis et al., 1996; Sutovsky et al., 2001; Rago et al., 2003; Fischer et al., 2005) and maintenance of the lipid membrane (James et al., 1999).

Possible factors causing removal of the CD

Removal in the epididymis
Removal and destruction of the CD within the epididymis has been documented in the rat (Hermo et al., 1988) and the brush-tailed opossum (Temple-Smith, 1984). However, a similar mechanism for removal and destruction of the CD in the epididymis has not been clearly identified in the bull or boar. The idea that the epididymis can act as a quality control system has been gaining more favor with the recent work of Sutovsky et al. (2001) and Baska et al. (2008).

Removal at ejaculation
As previously discussed, the majority of boar sperm CDs are removed upon sperm mixing with seminal plasma at ejaculation (Kato et al., 1996). It is thought that components present in the seminal vesicle fluid cause CD removal at ejaculation. Harayama et al. (1996) collected sperm from the cauda epididymis of mature boars. In all of the samples, >90% of the sperm had a distal CD. The authors found that mixing the epididymal spermatozoa with a seminal vesicle fluid component, D-fructose, resulted in shedding of the CDs from sperm tails. It is apparent that seminal vesicle fluid is required for CD removal in ejaculated sperm since a vesiculectomized boar still had most of the ejaculated spermatozoa retain a CD (Harayama et al., 1996). Inadequate seminal vesicle gland secretions could explain why some boars have a high number of sperm with an attached CD following ejaculation.

Percentages of attached CDs in the boar ejaculate
From a review of the literature it is difficult to conclude what might be considered a ‘normal’ percentage of sperm-attached CDs in the boar ejaculate. This is due to different nomenclature of the attached CD in regards to its position on the sperm (Harayama et al. 1992; Pruneda et al. 2005) or the reporting of only one category of the attached CD (generally only the PCD; Malmgren and Larsson 1984). The ‘normal’ range for spermatozoa possessing a CD appears to be between 10 to 15% in the boar ejaculate (Kaplan et al., 1984; Waberski et al., 1994; Lovercamp et al., 2007b) although higher percentages have been reported (Kuster et al., 2004). Such discrepancies could be caused by varied semen quality in the sampled boars or a difference in the stringency of light microscopic semen evaluation. Table 3 shows the mean percentages for the CD categories of proximal CD, distal CD, DMR CD and total number of spermatozoa with an attached CD. Only studies reporting at least two of the CD categories are evaluated. The CD percentages reported are not from the boars that were exposed to conditions that have been shown to increase the CD. In other words, the percentages reported are from ‘control’ boars.

Possible factors for retention of the CD

Temperature of semen
A study by Zou and Yang (2000) determined that temperature has an effect on CD retention of ejaculated boar spermatozoa. Four different temperatures, 39°C (102°F), 20°C (68°F), 15°C (59°F) and 4°C (39°F) were used to evaluate the effect on droplet shedding in a single collection of freshly ejaculated spermatozoa for 48 hours. The semen was not extended prior to initiation of

the experiment. At time 0 hr the percentage of sperm exhibiting CDs was 18.7% in all treatment groups. Shedding of the droplet was significantly faster in the lower temperature groups than the higher temperature groups; however, the authors did not propose an explanation for this observation. This experiment provides interesting results; however, caution should be used when considering application to AI since the ejaculates were not extended prior to the initiation of the experiment.

Environmental temperature of the male

High temperatures have been shown to increase the percentage of sperm with a CD. Boars housed in controlled environmental conditions (18ºC, 64°F) and then exposed to heat stress conditions (35°C, 95°F) for 100 hours showed a significant increase in the percentage of sperm with a proximal CD for up to six weeks after the heat stress. Seven to nine weeks following the heat stress, the percentage of proximal CDs returned to pre-heat stress levels (Malmgren and Larsson, 1984). These results suggest that male germ cells in the early stages of development were damaged since the percentage of spermatozoa with a proximal CD persisted for six weeks following the heat stress. Spermatogenesis in the boar takes approximately 35 days, and epididymal transit takes approximately 12 days (six to seven weeks total). The percentage of spermatozoa with a distal CD was not evaluated in this study. Huang et al. (2000) looked at sperm quality traits in three western breeds (Duroc, Landrace and Yorkshire) during a cool weather season and a hot weather season. The cool season average temperature range was 15-22°C (59-71°F) while the hot season average temperature range was 23-32°C (73-89°F). There was a significant increase in the number of proximal CDs (Duroc and Landrace) and distal CDs (all breeds) from cool season to hot season.

Chemical exposure

Exposure to chemicals can have an effect on CD retention in rats as reported by Akbarsha et al. (2000). In this study, 3-month old Wistar rats were either injected or orally treated with malathion, dichlorvos (organophosphate insecticides), andrographolide or ursolic acid (phytothreapeutics). Control rats displayed normal CD removal in the cauda epididymis whereas treated rats showed CDs retained on 60-95% of the sperm residing in the lumen of the cauda epididymis.

Sexual maturity

Retention of the droplet in the ejaculate can also be affected by sexual maturity of the animal. Several analyses have identified the pubertal bull as a classic example of this age-CD relationship. Typically, beef bulls attain sexual maturity between 8 and 12 months of age at which time the scrotal circumference measurement reaches 28 cm (reviewed by Arteaga et al., 2001). Ejaculates of immature bulls have more sperm abnormalities than mature bulls, with the proximal CD being one of the most common. A study performed by Johnson et al. (1998) evaluated breeding soundness examinations (BSE) on 2,497 beef bulls. Spermatozoa with proximal CDs were found most commonly in bulls 10 to 12 months old, but prevalence decreased significantly in bulls 13 to 18 months old and greater than 24 months old. Distal CDs were more prevalent in bulls 10 to 18 months old, and prevalence decreased significantly in bulls greater than 19 months old. Arteaga et al. (2001) evaluated the relationship between semen quality traits, age, and scrotal circumference in bulls between 11 and 15 months of age. The mean percentage of morphologically normal spermatozoa increased from 41% (11 months) to

69.6% (14 months). The corresponding percentage of spermatozoa with proximal CDs decreased from 17.8% (11 months) to 5.8% (14 months). The authors noted that proximal CDs were common in young bulls at the time of puberty. In this study, proximal CDs represented 37% of the main sperm defects contributing to an immature classification.

Diet
Retention of the proximal CD after feeding gossypol (8 mg/kg/day for 56d) to yearling cross-bred Angus x Hereford bulls was analyzed by Hassan et al. (2004). Gossypol is a natural component of cotton seed meal that has been found to impair spermatogenesis, increase the number of sperm morphological abnormalities and possibly reduce sperm motility (reviewed by Hassan et al., 2004). The treatment group receiving gossypol showed significant increases for proximal CDs and other abnormalities. This study shows that although gossypol does have a detrimental effect on the quality of the ejaculate, removal of gossypol from the diet will allow a return to sperm quality comparable to control levels.

Disease
The percentages of sperm cells with proximal and distal CDs were shown to increase following controlled infections of boars with *Pseudorabies* virus (PRV, Larsen et al., 1980). Similarly, boars infected with Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) demonstrated increases for distal CDs (Prieto et al., 1996) and proximal CDs (Shin et al., 1997). The authors speculated that the increased body temperature from the viral infection may have resulted in abnormal sperm development in the testis or sperm maturation in the epididymis, thereby increasing the frequency of sperm-attached CDs in the ejaculate.

Photoperiod
Studies show conflicting results when evaluating the effect of an increasing or decreasing photoperiod on the percentage of spermatozoa with a CD. A trial using natural photoperiods found that the percentage of spermatozoa with a proximal CD was highest during the decreasing photoperiod, while the percentage of spermatozoa with a distal CD was highest during the increasing photoperiod (Sancho et al., 2004). Conversely, an experiment using artificial photoperiods found that the percentage of spermatozoa with a proximal CD was highest during the increasing photoperiod (Andersson et al., 1998). The distal CD was not evaluated in this study.

Collection Frequency
A high semen collection frequency (two collections per day, taken every 12 hours for four days) increased the percentage of ejaculated spermatozoa with a proximal CD compared to a collection frequency of one collection every two days for the same four day period (Pruneda et al., 2005). The authors speculate that the percentage of spermatozoa with a proximal CD was increased in response to an increased rate of sperm passage through the epididymis, which hastened the sperm maturation process. The number of distal CDs in the ejaculate was similar for the two collection frequencies.

Relationships between attached CDs and fertility
Ejaculated spermatozoa that possess an attached CD can have a negative relationship with fertility in farm animal species, although the exact mechanisms by which this occurs have not

been fully elucidated. Waberski et al. (1994) found that sperm-bound CDs in extended boar semen have detrimental relationships with pregnancy rates and litter size. The authors noted that CDs represented the most frequent morphological abnormality in the spermatozoa of AI boars. In 24 ejaculates from 10 Pietrain boars, the average percentage of spermatozoa with CDs varied from 5.5 to 39.9%. Analysis showed a significant negative correlation between the percentage of spermatozoa with CDs and pregnancy rates or litter size when using semen stored for two or four days. Specifically, the percentage of distal CDs was negatively correlated with pregnancy rates in day two ($r=-0.87$) and day four ($r=-0.92$; $P<0.001$) extended semen and with litter size ($r=-0.80$) in day two extended semen ($P<0.01$). Proximal CDs showed a negative correlation with litter size ($r=-0.87$; $P<0.001$) and pregnancy rate ($r=-0.79$; $P<0.01$) using semen extended for four days ($P<0.001$). These results do not determine whether proximal CDs or distal CDs are more detrimental to boar fertility. It is difficult to speculate why proximal CDs did not have a negative correlation with pregnancy rates and litter size until the extended semen was stored for four days. Possibly, proximal CDs are not detrimental in extended semen until a certain amount of time has passed, or the removal of proximal CDs during extended semen storage is less likely than removal of the distal CDs. Conversely, distal CDs reduced fertility in both day 2 and day 4, extended semen. This suggests that distal CDs have a greater detrimental effect on fertility than proximal CDs.

An *in vitro* study performed by Petrunkina et al. (2001) analyzed sperm binding to explants of the pig oviductal epithelium. It is thought that spermatozoa must bind transiently to the oviductal epithelium of the oviductal sperm reservoir in order for successful fertilization of the oocyte to occur *in vivo.* The results of the sperm binding were used to calculate a binding index. There was a significant negative correlation of the percentage of sperm with attached CDs ($r= -0.29$) and the binding index. Furthermore, there was a negative correlation between motility of the sperm sample ($r=-0.47$) and the percentages of spermatozoa with attached CDs. These results suggest that boars with a high percentage of attached CDs had a lower binding index, illustrating either the selectivity of the oviductal epithelium to bind spermatozoa without an attached CD, or the lowered ability of spermatozoa with an attached CD to bind to the oviductal epithelium.

Lovercamp et al. (2007a; 2007b) examined relationships between sperm morphology parameters in boar ejaculates and resulting fertility data (farrowing rate and total number born). In this study 13 boars accounted for 71 ejaculates that were extended and used for 1,754 services in commercial system. The results showed significant negative linear correlations for the distal CD ($r=-0.37$; $P<0.01$) and DMR CD ($r=-0.25$; $P<0.05$) with farrowing rate. In addition, there was a significant negative linear relationship ($r=-0.46$; $P<0.01$) between farrrowing rate and accrued attached CDs (proximal CD + distal CD + DMR CD). Regression analysis showed that the sperm-attached CDs accounted for 30% of the variability seen in the farrowing rate. Intriguingly, of this 30%, the distal CD was responsible for 20%, whereas the proximal and DMR CD were responsible for 6% and 4%, respectively (K.W. Lovercamp, unpublished results). These results suggest that the distal CD may have the most profound effect on farrowing rate compared to the proximal CD and DMR CD. No relationships were found with total number born. Further analysis by Lovercamp et al. (2007b) demonstrated that boars with a farrowing rate below the average for the study ($74.01 \pm 1.43\%$) had a higher number of distal CDs and total attached CDs compared to the boars above the average farrowing rate (5.49 vs. 3.18% and 17.14 vs. 8.55%, respectively). The boars in this study were ranked based on their percentage of total attached

CDs, farrowing rate and total number born (Table 4). All boars were considered fertile and suitable for AI service. The existence of a relationship between farrowing rate and the total attached CDs suggests that boars with the highest percentage of total attached CDs will produce the lowest farrowing rates and vice versa. Therefore, ranking the boars from highest to lowest based on the percentage of total attached CDs should, in theory, be comparable to ranking boars from lowest to highest based on farrowing rate. This relationship is supported by the published data. For example, there was an agreement between total attached CDs and farrowing rate for two boars (#6 and 5) ranked in the bottom third, as well as for three boars (#10, 14 and 17) ranked in the top third. These results suggest that ranking boars based on the percentage of total attached CDs can be used as a diagnostic tool to identify those boars that may have the highest farrowing rate and total number born.

Possible mechanism(s) by which the CD influences fertility

The mechanism(s) by which the attached CD may adversely influence fertility are not known. An important point to remember is that all the spermatozoa in a given ejaculate underwent spermatogenesis and epididymal transit at approximately the same time. An ejaculate that has a high number of spermatozoa with an attached CD may be indicative of abnormal spermatogenesis and (or) abnormal epididymal sperm maturation. As a result, all the sperm cells in ejaculates with a high percentage of attached CDs may be subfertile, including morphologically 'normal' spermatozoa that may actually be deficient on the molecular level (Althouse, 1998; Thundathil et al., 2001). The molecular interactions of spermatozoa with and without an attached CD are not understood.

It may be that the sperm-attached CD interferes primarily with those sperm properties that are required for interaction of the spermatozoon with the oocyte during the initial stages of fertilization prior to sperm-egg fusion (i.e. binding to the oviductal epithelium, capacitation, hyperactivation, sperm-egg recognition and binding, and acrosomal exocytosis). The lack of a relationship between spermatozoa with an attached CD and litter size indicates that if the oocytes are successfully fertilized (most likely by spermatozoa not possessing a CD; Amann et al. 2000) then the attached CD does not appear to negatively affect total number born by such means as disturbing embryonic development or contributing to embryonic mortality. The above considerations warrant further investigation of the attached CD and its specific mechanisms of action on the initial stages of fertilization.

Conclusions

Spermatozoa with an attached CD are a frequently observed abnormality in the boar ejaculate. Following spermatogenesis in the tesis, all sperm cells possess a CD in the proximal position. During epididymal transit, the CD moves from the proximal position to the distal position. At ejaculation, the CD is typically removed from the sperm cell. However, many ejaculated spermatozoa can retain a CD in the proximal or distal position. The effect of attached CDs on fertility is not well understood. To date, research has only indicated negative relationships between the attached CD and fertility; however, no conclusive evidence exists for the CD to cause a reduction in fertility. Furthermore, it is not fully understood if attached CDs and (or) unattached CDs are detrimental to the normal, motile spermatozoa that do not possess a CD in the ejaculate. Additionally, it is not known whether the proximal CD or distal CD is the most detrimental to fertility. Therefore, any negative relationships for attached CDs with fertility

should be considered a cumulative effect of all attached CDs in the ejaculate. The mechanisms through which attached CDs reduce fertility have not been identified, although researchers speculate that spermatozoa with an attached CD are restricted in their ability to interact with the oviductal epithelium and (or) oocyte during fertilization.

Recommendations

Acceptable levels of sperm with an attached CD for artificial insemination
Currently, it is recommended that ejaculates with greater than 15% of the sperm possessing an attached CD should not be used for artificial insemination (Waberski et al., 1994; Althouse, 1998).

Monitoring boars that produce unacceptable levels of spermatozoa with an attached CD
Boars that produce ejaculates containing a high percentage of spermatozoa with an attached CD should be evaluated for approximately a three month period using a once-per-week collection frequency. If the percentage of attached CDs does not drop to acceptable levels, then the boar should be culled (Althouse, 1998). At present, there are no known treatments for boars that produce a high percentage of sperm with attached CDs. Furthermore, there are not any practical methods to promote the shedding of CDs in the ejaculate. Until more information is established about the effects of CDs on fertility, the safest course of action is to reject ejaculates with more than 15% attached CDs.

Management practices to prevent ejaculates with high levels of spermatozoa with an attached CD
The following management practices are recommended to prevent the production of ejaculates containing a high percentage of spermatozoa with an attached CD. Following these practices should increase the likelihood that the ejaculates produced will contain sperm with attached CDs in the normal range (Lovercamp, 2005).

1.) Keep boars on a regular collection schedule of one to two collections per week, with at least two to three days rest between collections
2.) Maintain an environment with an ambient temperature range of 15 to 24ºC (60 to 75°F) and daily light exposure of 8 to 10 hours
3.) Prevent disease outbreaks and be aware that vaccinations could affect the level of retained CDs

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Figures

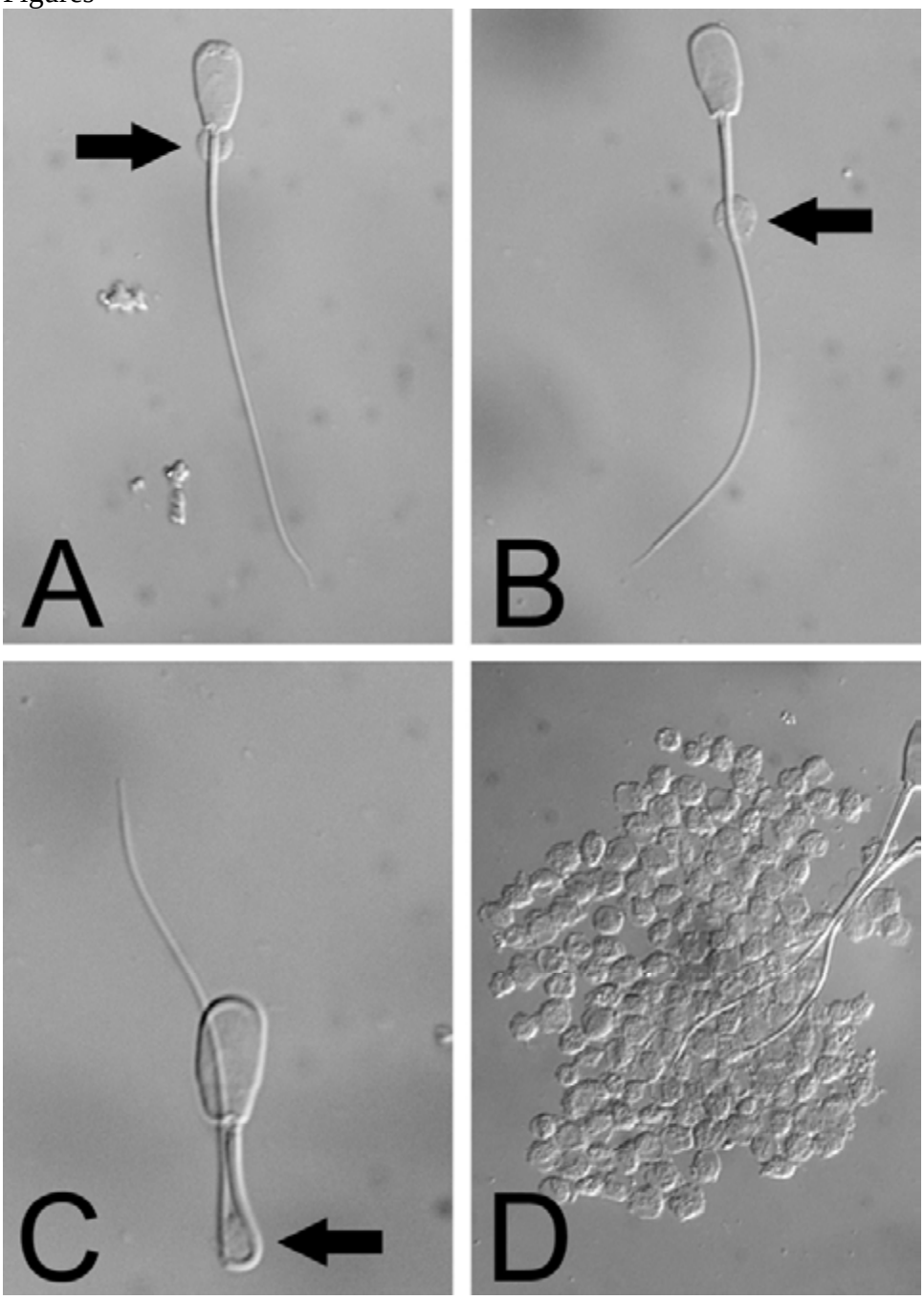

Figure 1. Images of attached and unattached CDs in the boar ejaculate visualized by high power differential interference contrast microscopy (primary magnification x 100x infinity). (A) Sperm with a proximal CD; (B) Sperm with a distal CD; (C) Sperm with the distal midpiece reflex possessing a distal CD (DMR CD); (D) cluster of unattached CDs.

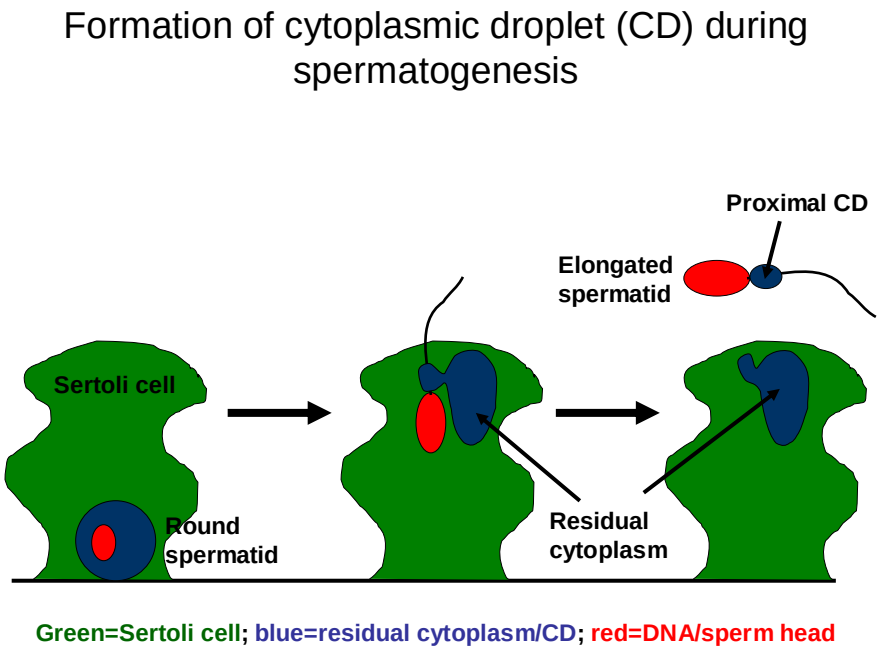

Figure 2. Formation of the CD occurs when the residual cellular cytoplasm is removed from the elongated sperm cell. The small amount of cytoplasm remaining on the elongated sperm cell is the CD.

Tables

Table 1. Percentages of proximal CDs and distal CDs in the different regions of the epididymis and the ejaculate. Overall, the percentage of proximal CDs is highest in the caput epididymis and decreases in the cauda epididymis while the reverse is true for the distal CD. This transition demonstrates the movement of the attached CD from the proximal position to the distal position as the sperm transits the epididymis.

Species	Epididymis region/Ejaculate	Proximal CD	Distal CD	Source
Landrace boars	Caput	~49%	Not evaluated	Briz et al., 1995
	Corpus	Not evaluated	~54%	
	Cauda	~5%	~11%	
	Ejaculate	Not evaluated		
Miniature boars	Caput	80-90%	Not evaluated	Crabo et al., 1979
	Corpus	5-10%	Not evaluated	
	Cauda	≤5%	Not evaluated	
	Ejaculate	Not evaluated		
Domestic boars	Caput	Proximal and distal CDs: 93%		Kaplan et al., 1984
	Corpus	Not evaluated		
	Cauda	Proximal and distal CDs: 72%		
	Ejaculate	Proximal and distal CDs: 14%		
Domestic boars	Caput	48%	34%	Fischer et al., 2005
	Corpus	16%	64%	
	Cauda	14%	72%	
	Ejaculate	Not evaluated		
Meishan boars	Caput	Not evaluated	Not evaluated	Harayama et al., 1992
	Corpus	Not evaluated	Not evaluated	
	Cauda	1-3%	95-97%	
	Ejaculate	Not evaluated	Not evaluated	
Meishan and Jinhua boars	Caput	Not evaluated	Not evaluated	Kato et al., 1996
	Corpus	Not evaluated	Not evaluated	
	Cauda	2%	97%	
	Ejaculate	Proximal and distal CDs: 24%		
Domestic boars	Caput	90%	0%	Pruneda et al., 2005[1]
	Corpus	15%	75%	
	Cauda	10%	80%	
	Ejaculate	5%	5%	

[1]Values estimated from graph for control boars in proximal compartment of the respective epididymal region

Table 2. Identified components of the mammalian CD

Species	Component	Classification	Putative action	Source
Boar, stallion, rat, bull and human	hexokinase	Enzyme	Glycolytic capacity for biogenesis of mitochondrial sheath	reviewed in Westhoff and Kamp, 1997
	glucose phosphate isomerase	Enzyme		
	lactate dehydrogenase	Enzyme		
	glyceraldehyde 3-phosphate dehydrogenase	Enzyme		
Boar and ram	Cholesterol	Lipid	Maintenance of the lipid membrane	James et al., 1999
Boar, human, mouse and stallion	15-Lipoxygenase	Enzyme	CD removal	Fischer et al., 2005
Boar and bull	Ubiquitin	Protein	CD removal	Sutovsky et al., 2001
Boar	β-hexosaminidase	Enzyme	?	de Vries and Colenbrander, 1990
Human	NADPH oxidase	Enzyme	ROS synthesis	Aitken and Sawyer, 2003
	superoxide dismutase	Enzyme	ROS synthesis	
	glucose-6-phosphate dehydrogenase	Enzyme	ROS synthesis	
Bull and ram	hydrolase	Enzyme	?	Dott and Dingle, 1968; Garbers et al., 1970
	lysosomal	Enzyme	?	
	acid phosphatase	Enzyme	?	
	acid protease	Enzyme	?	
	β-glucuronidase	Enzyme	?	
	aryl sulphatase	Enzyme	?	
	RNase	Enzyme	?	
	DNase	Enzyme	?	

Table 3. Mean percentages for the proximal CD, distal CD, DMR CD and total sperm with an attached CD. Only studies reporting at least two of the CD categories are evaluated. The CD percentages reported are not from the boars that were exposed to conditions that have been shown to increase the CD.

	CD category			
Study	Proximal CD, %	Distal CD, %	DMR CD, %	Total sperm with attached CD, %
Waberski et al., 1994[1]	7.4	6.5	Not reported	Not reported
Huang et al., 2000[2]	4.5	7.3	Not reported	Not reported
Gadea et al., 2004[3]	3.1	4.1	Not reported	Not reported
Sancho et al., 2004[4]	5.3	4.2	Not reported	Not reported
Kuster et al., 2004[5]	Not reported	29	Not reported	52
Pruneda et al., 2005[6]	~5	~5	Not reported	Not reported
Lovercamp et al., 2007b	6.2	4.6	2.7	13.5

[1]Values estimated from graph
[2]Calculated by averaging all reported values for the cool season
[3]Calculated by averaging the low fertility and high fertility groups
[4]Calculated by averaging all reported values
[5]Total sperm with attached CD includes proximal, distal and DMR CDs
[6]Values estimated from graph for control boars in proximal compartment of the respective epididymal region

Table 4. Boars ranked by total attached CDs (TACDs), farrowing rate (FR) and total number born (TNB). Total attached CDs and farrowing rate are reported as a percentage. Boars #7, 11, 15, 16, and 18 were not used for any matings in the study. Values are means (± SEM) and were calculated by weighting the number of services for each boar. Correlation coefficients for the averages shown are: $r^{TACD-FR}$=-0.44, $r^{TACD-TNB}$=-0.30

	Boar ID	TACD[1]	Boar ID	FR[1]	Boar ID	TNB[1]
	6	41.50 ± 3.50	13	51.22 ± 12.99	6	9.29 ± 4.96
Bottom	1	26.36 ± 3.99	6	58.33 ± 8.33	13	9.76 ± 0.96
	3	24.46 ± 3.05	5	61.26 ± 4.21	1	10.39 ± 0.90
	5	20.52 ± 4.34	9	63.41 ± 9.32	9	10.64 ± 0.83
	4	15.68 ± 2.41	12	66.67 ± 3.10	8	11.13 ± 0.26
	2	13.25 ± 1.88	2	67.25 ± 3.75	2	11.37 ± 0.57
Middle	12	12.80 ± 0.80	3	67.72 ± 3.38	4	11.60 ± 0.39
	13	12.02 ± 0.45	1	67.80 ± 5.60	17	12.08 ± 0.29
	8	10.33 ± 1.12	4	72.98 ± 3.45	12	12.26 ± 0.23
	17	9.51 ± 1.41	8	77.78 ± 5.03	14	12.30 ± 0.29
	10	9.38 ± 1.28	14	79.26 ± 2.18	3	13.45 ± 0.46
Top	14	7.06 ± 0.89	17	80.82 ± 3.82	5	13.50 ± 0.92
	9	5.32 ± 1.58	10	87.84 ± 2.78	10	13.55 ± 0.11

[1]Column abbreviations: TACD = PCD + DCD + DMR; FR = farrowing rate; TNB = total number born

Perspectives on cryopreservation, quality control, and artificial insemination of frozen-thawed boar sperm in a national repository

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Introduction

The National Animal Germplasm Program (NAGP) is a repository for animal germplasm (sperm, eggs, embryos, blood, tissue, DNA) that has been collected, cryopreserved, and cataloged for the purpose of creating a living compilation of genetic resources. The repository is multipurpose and can be used to expand a breeds' genetic base, repopulate/recreate breeds or lines, for gene discovery, or for other research programs. What is essential in the creation of a collection of this type is to have an understanding of the post-thaw quality and fertility of the preserved samples. This enables management to make projections about the amount of material, by type, species, and breed needed to meet the aforementioned uses. Consequently, quality control throughout the cryopreservation process is extremely important to ensure populations can be reconstituted with predetermined fertility levels.

During a typical year NAGP routinely freezes 30,000 straws (0.5 mL) of boar semen. Accordingly, quality control issues are important in our day to day operation. By analyzing the collection, handling, cryopreservation, and insemination processes we have adapted an approach that is beneficial for the repository and swine industry. We have therefore sought to answer the following questions: 1) What are the optimal conditions prior to freezing for transporting cooled, liquid samples so that they are in an optimal condition for cryopreservation?; and 2) Can we create better tools, or a better understanding of the tools we have, to more thoroughly evaluate the pre-freeze and post-thaw quality of an ejaculate? By addressing these issues we endeavor to improve the cryopreservation and artificial insemination processes. The information presented in this manuscript is a compilation of numerous experiments that have been performed by NAGP personnel.

Materials, methods and experimental design

Collection and processing boar semen

All samples used for analysis were collected at commercial studs and processed according to the NAGP shipping protocol. The samples were collected using the gloved hand technique and the gel-free fraction was separated using sterile gauze. The samples were then diluted (1:5) with 37 ºC Androhep Plus (Minitube of America, Verona, WI), cooled to 23 ºC (60 min), cooled to 15 ºC (90 min) and then packed to maintain the 15 ºC temperature and shipped to arrive 24 h later at the NAGP laboratory.

Experiment 1 - Extenders

In Experiment 1 the lactose-egg yolk (LEY; Almlid and Johnson, 1988) and the Beltsville freezing extender 5 (BF5; Pursel and Johnson, 1975) cryopreservation diluents were compared to determine which medium would work best for freezing purebred and composite breeds of boars (Blackburn et al., 2003). Semen samples from both Yorkshire and Composite breeds were shipped to the NAGP as previously described. Once at the laboratory the samples were centrifuged (800 x g; 10 min) and the supernatant was removed. Sperm pellets were combined for each ejaculate and the concentration and motility were determined using spectrophotometry and computer assisted sperm analysis (CASA), respectively. For CASA analysis a minimum of 1000 sperm were analyzed, before and after freezing, in at least seven fields using the default settings for boar sperm (Purdy, 2008). The samples were then diluted to 300 x 10^6 sperm/mL in either the LEY or BF5 diluents and cooled to 5 ºC over two h. The samples were then diluted to 200 x 10^6 sperm/mL with the LEY and BF5 freezing extenders (diluents containing glycerol and Equex/Orvis paste; Minitube), loaded into 0.5 mL CBS straws (IMV, Minneapolis, MN), placed on a rack 6.4 cm above liquid nitrogen and frozen over 10 min in the liquid nitrogen vapor. Samples were then plunged into the liquid nitrogen for storage. Samples were thawed by placing the straw into a 50 ºC water bath for 20 s. Data of the motility characteristics were recorded for each sample at 15 min increments from 0 (immediately after thawing) to 105 min post-thaw.

Experiment 2 – Genetic and stud differences

This research was an investigation of the variation in sperm quality that could be attributed to individual boars, genetic lines, and the commercial studs where the boars were housed (Stewart et al., 2006). Semen samples from 163 boars, 2 ejaculates (collections) per boar, from two studs and representing four closed composite lines were collected, processed, and cryopreserved using the BF5 cryopreservation diluent and protocol described in Experiment 1. In this and the following experiments a programmable freezer (Minidigitcool UJ400, IMV) was used and the following freeze curve: 5 ºC to -8ºC at 20 ºC/min; -8 ºC to -120 ºC at 69 ºC/min; -120 ºC to -140 ºC at 20 ºC/min. After thawing the samples the sperm motility was analyzed as previously described.

Experiment 3 – Shipping fresh semen

This experiment investigated the influence of temperature and pH during shipping to determine if these affected pre-freeze and post-thaw motility characteristics. Again, boar semen samples (N = 199) were collected at four commercial studs and shipped to the NAGP laboratory. Upon arrival the temperature and pH of the liquid samples was determined using a pH/Temperature Plus meter (Corning Inc., Corning, NY). The samples were then processed, cryopreserved and the motility (pre- and post-thaw) was analyzed using CASA.

Experiment 4 – Fertility trials

Finally, in this experiment the fertility of frozen-thawed boar sperm was investigated, using gilts and sows, to assess the quality of samples in the repository. Semen for these experiments was processed and cryopreserved as described previously. Seven sows were artificially inseminated at Purdue University using 1 x 10^9 motile

frozen-thawed sperm per dose diluted to 80 mL, either one or two insemination doses, and transcervical catheters. In the single insemination group (n = 3) sows were inseminated 24 hours after the first observed estrus (weaning heat was used in conjunction with twice daily observation using a boar and fence line contact) whereas the double insemination group (n = 4) was inseminated at the same time, as well as 11 hours later. Sows were taken to term and the number of piglets born per farrowing sow (prolificacy) was determined.

Gilt estrous was synchronized using a Matrix feeding regimen and ovulation was induced using an injection of PG600 (Spencer et al., 2008). Estrus detection was performed twice daily and inseminations were performed either 32 hours post-estrus detection (single insemination) or 24 and 32 hours post-estrus detection (double insemination). Insemination doses contained 1, 2 or 4 x 10^9 motile frozen-thawed sperm which was diluted to 80 mL and inseminated either once or twice depending on the insemination treatment. The fertility and the prolificacy (number of fetuses per pregnant gilt) were determined by analysis of the dissected reproductive tracts determined at slaughter 30 days post insemination.

Results

Comparison of cryopreservation diluents in Experiment 1 demonstrated a significant effect of extender and the extender x breed interaction (Figure 1). At time 0 breed differences (Yorkshire or Composite) in the percentage of motile or progressively motile sperm were not observed when the samples were frozen using the LEY diluent but differences in total and progressive motility were observed for the breeds when the BF5 diluent was used (Figure 1). Furthermore, when the motility was analyzed using regression analysis over the 105 min period it became evident that the BF5 diluent yielded superior post-thaw motility/survivability compared with the LEY diluent ($P < 0.05$; Figure 2). Because of these results BF5 was selected for use by the NAGP and consequently the remaining experiments were performed using this diluent.

It has been suggested that breed or genetic effects are important contributors to sperm freezability. Experiment 2 clearly illustrated that genetic line had no significant effect on motion parameters (Table 1). Another interesting result was that collection was a non-significant source of variation (Table 1). In this experiment the importance of stud and individual boar were clearly identified for motility characteristics (Table 1).

In Experiment 3 our goal was to understand the influence of the temperature and pH of the samples which were determined upon arrival at the NAGP. Significant differences in temperature, pH and post-thaw motility characteristics were observed by commercial stud ($P < 0.05$; Table 2). It was theorized that temperature and pH may have non-linear effects and there may be interactions of the two. Therefore, a regression model was constructed that included temperature, pH, their interactions, and the quadratic effect of each of the variables. This model revealed that temperature and pH acted in an obviously non-linear manner, as demonstrated in Figures 3 and 4 (post-thaw results), where the variables were regressed on progressive and total fresh and post-thaw motility ($P < 0.0001$).

From a repository perspective it is extremely important to demonstrate that the stored germplasm is viable. In addition, this information can help refine our collection process in regards to the number of straws needed for reconstitution. In the fertility trial

using sows, all of the animals became pregnant and the prolificacy for the single and double insemination groups was 8.70 and 6.75 live piglets, respectively (7.57 live piglets across treatments). The pregnancy and prolificacy results for the gilt inseminations are presented in Table 3. No differences in pregnancy or prolificacy rate due to number of sperm inseminated or number of inseminations performed were detected ($P > 0.05$). Individual boars did not have a significant effect on pregnancy rates ($P > 0.05$) but did have a significant effect on the prolificacy rate within a treatment ($P < 0.002$).

Discussion

The experiments described are meant to address a vast number of characteristics of cryopreserving, storing boar sperm samples, and to ascertain their practical utility. The first experiment was performed to determine the best cryopreservation diluent available for freezing boar semen regardless of its genotype. It was apparent that the superior diluent was BF5 due to its ability to maintain the viability of the samples over the 105 min evaluation time. Consequently, this diluent has been used at the NAGP as the standard for preserving boar semen samples.

In an effort to further identify sources of variation which may negatively influence post-thaw quality, a larger study was performed (Experiment 2) to determine differences in post-thaw sperm quality due to boar, line, or commercial stud assessed using CASA. There were differences in post-thaw quality attributed to individual boars, the commercial stud, and only in a few instances to lines (beat cross frequency and elongation) but the boar (line*stud) variable was responsible for a significant source of variation in all motility characteristics measured. These results reveal two issues of importance to the NAGP. First, cryopreservation protocols do not need to be tailored to specific lines to optimize post-thaw quality because few differences in motility attributed to lines were detected in the frozen-thawed samples. This suggests that additional efforts to freeze pig lines differently is not necessary, thus greatly simplifying the collection process. Second, this information also underscores the importance of how semen is processed at the stud before shipment and calls for greater standardization of industry semen handling protocols. Standardization of methodologies will guarantee samples of higher quality, regardless of line, which is of interest to NAGP and commercial swine breeders.

Our results clearly illustrate the importance of shipping temperature and pH. It was obvious that the pH fluctuated minimally during shipping. This is not surprising because the Androhep Plus shipping diluent is designed, and is well proven, to maintain pH over multiple days. This would also account for the narrow range of pH observed in this study (5.91 to 7.14; average 6.74); thus the Androhep Plus is fulfilling its purpose. Analysis of the effects of the shipping temperature and diluted semen pH revealed a number of intriguing issues. Most importantly, an optimal temperature and pH has been determined (~ 17 ºC and 6.5 pH). Shipping samples in conditions even slightly above or below this result in decreased percentages of fresh and post-thaw progressive and total motility (Figures 3 and 4); thus diminishing the overall quality of the sample regardless of whether it will be used in a fresh or frozen-thawed manner. For example, even if a pH of 6.5 was maintained, a drop in sample temperature from 17 ºC to 14ºC could result in a decrease in the percentage of progressively motile sperm from 20% to 16%; a 25 % decline. This is an example of when only one factor (temperature) changes but the

effects become exacerbated when both temperature and pH are altered. The same decline in temperature coupled with an increase in pH from 6.5 to 6.7 results in a 50 % decrease in progressive motility which exemplifies the precarious interaction of temperature and pH; a balance necessary for maintaining sample quality.

The fertility trials performed provide an important measure of cryopreserved semen quality and increase confidence in the ability of the repository to reconstitute populations. Although the number of sows used was small the results were substantial due to the 100% fertility, while using only 1 x 10^9 motile sperm per AI dose. The prolificacy results were lower than what is normally reported for fresh semen but were within proximity of that reported by Johnson et al. (2000; 7-10 live piglets per litter) for frozen-thawed boar sperm. The fertility and prolificacy rates from the fertility trial with gilts were further validation of reconstitution capacity and the prospects for utilizing cryopreserved semen. Considering that the inseminations were performed with frozen-thawed semen, overall the pregnancy and prolificacy rates were quite similar to those reported with fresh semen (pregnancy: 73 to 98%, prolificacy: 8.8-12.0; Ruiz-Sánchez et al., 2006) when gilts were inseminated.

Conclusions and future directions

This series of experiments performed by NAGP have covered the range of boar semen handling, cryopreservation, evaluation, and utilization. Careful monitoring of the product through these steps provides assurance that samples in the collection will be of utility when used.

There is additional research needed which is mutually beneficial to ourselves and the swine industry. For example, an artificial insemination trial is planned for the near future using sows, single inseminations, and four semen doses; 0.25, 0.5, 0.75 and 1.0 x 10^9 motile frozen-thawed sperm. This is beneficial to the NAGP because the fertility and prolificacy data will enable us to better estimate the amount of samples needed for line and breed recreations. This will also be beneficial to producers because the techniques will demonstrate what can be achieved with minimal cells and single inseminations; a methodology which will decrease the costs and increase the profitability of frozen-thawed boar semen.

Further exploration of the use of CASA for estimating sample quality and determining infertility is also underway. Recent research performed at the NAGP has resulted in the creation of a model using fertility records and CASA analysis of semen samples from 135 dairy bulls. We are currently adapting this model to swine so that a similar product can be created and implemented at a commercial stud. From our perspective, cryopreservation of boar semen is entering an extremely interesting era. With this technology we have greater ability to combine useful genetic resources within the U.S. and in the growing swine industries of developing countries.

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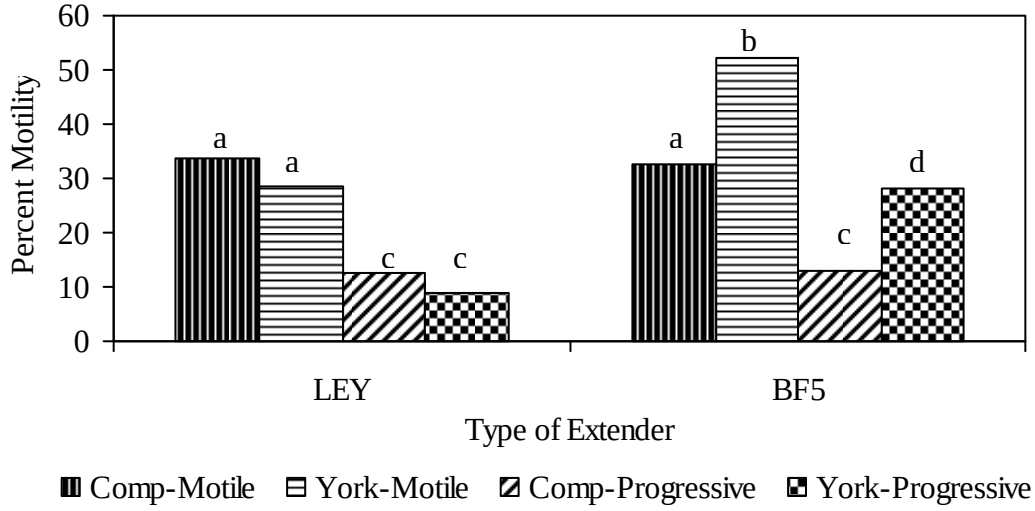

Figure 1. The post-thaw motility and progressive motility of boar sperm that was cryopreserved in either lactose egg yolk (LEY) or BF5 cryopreservation diluents.

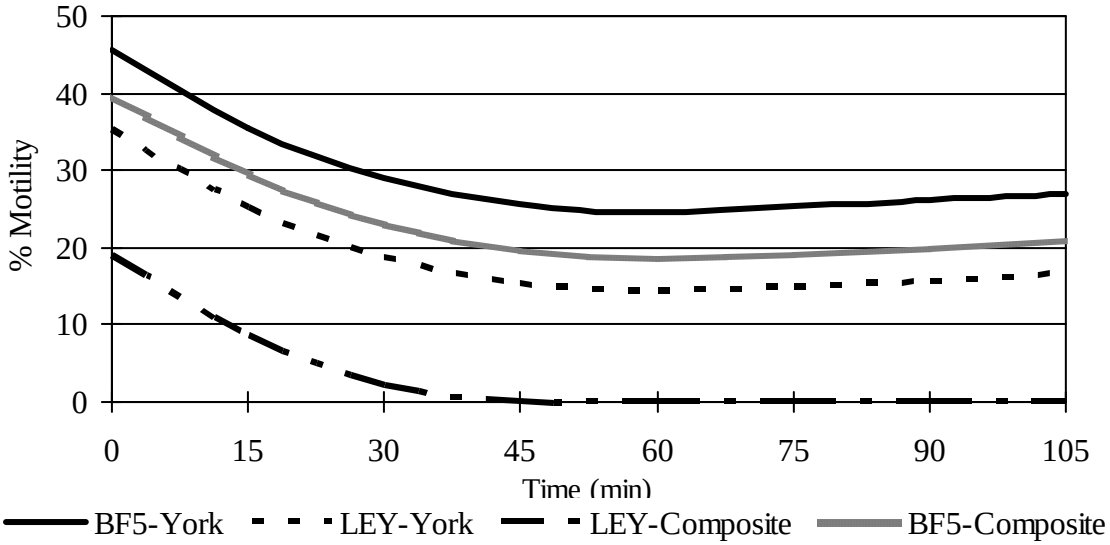

Figure 2. Regression analysis of frozen-thawed boar sperm total motility on time. The samples were analyzed for motility using CASA every 15 min from time 0, immediately after thawing, up to 105 min.

Table 1. Levels of significance for sources of variation for motility parameters.

Trait*	Stud (S)	Line (L)	Collection	Boar (L*S)
VAP	.01	.62	.73	.01
VSL	.01	.90	.15	.01
VCL	.01	.39	.82	.01
ALH	.01	.73	.70	.01
BCF	.15	.01	.83	.01
STR	.32	.14	.02	.01
LIN	.38	.31	.07	.01
ELONG	.02	.01	.53	.01
AREA	.01	.52	.62	.01
Motility	.01	.23	.85	.01
Progressive motility	.01	.45	.44	.01

*VAP = path velocity; VSL = progressive velocity; VCL = track velocity; ALH = lateral head amplitude; BCF = beat cross frequency; STR = straightness; LIN = linearity; ELONG = elongation; AREA = area of sperm head

Table 2. Differences in semen sample characteristics by commercial stud after 24 hours of liquid storage.

Stud	Temp.	pH	Motility	Prog. Motility	ALH	ELONG	SIZE
1	$16.2^{c} \pm .4$	$6.85^{a} \pm .02$	$46^{ac} \pm 5.9$	$20^{bc} \pm 3.61$	$7.7^{a} \pm .1$	$50.7^{b} \pm .4$	$9.2^{b} \pm .1$
2	$17.2^{b} \pm .2$	$6.69^{c} \pm .01$	$62^{a} \pm 3.0$	$32^{a} \pm 1.81$	$7.5^{a} \pm .1$	$52.4^{a} \pm .3$	$9.8^{a} \pm .07$
3	$19.8^{a} \pm .4$	$6.69^{c} \pm .01$	$33^{c} \pm 5.5$	$14^{c} \pm 3.35$	$6.9^{b} \pm .1$	$50.3^{b} \pm .3$	$9.1^{b} \pm .08$
4	$15.2^{d} \pm .2$	$6.76^{b} \pm .02$	$53^{a} \pm 3.5$	$24^{b} \pm 2.16$	$7.4^{a} \pm .2$	$50.4^{b} \pm .5$	$9.4^{b} \pm .1$
P	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

Temp = Temperature in ºC; Motility, Prog. (Progressive) Motility, and ELONG (elongation) values are presented as percentages; ALH (lateral head amplitude) and SIZE are presented as μm and μm^2, respectively.

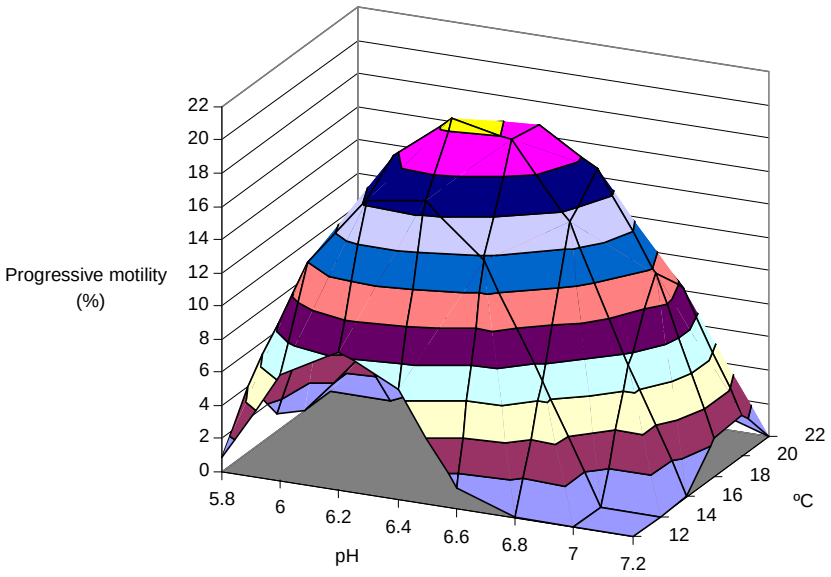

Figure 3. Results of a regression analysis demonstrating the effects of the temperature and pH of diluted, cooled boar semen which was held for 24 hours prior to cryopreservation on the post-thaw progressive motility ($P < 0.0001$).

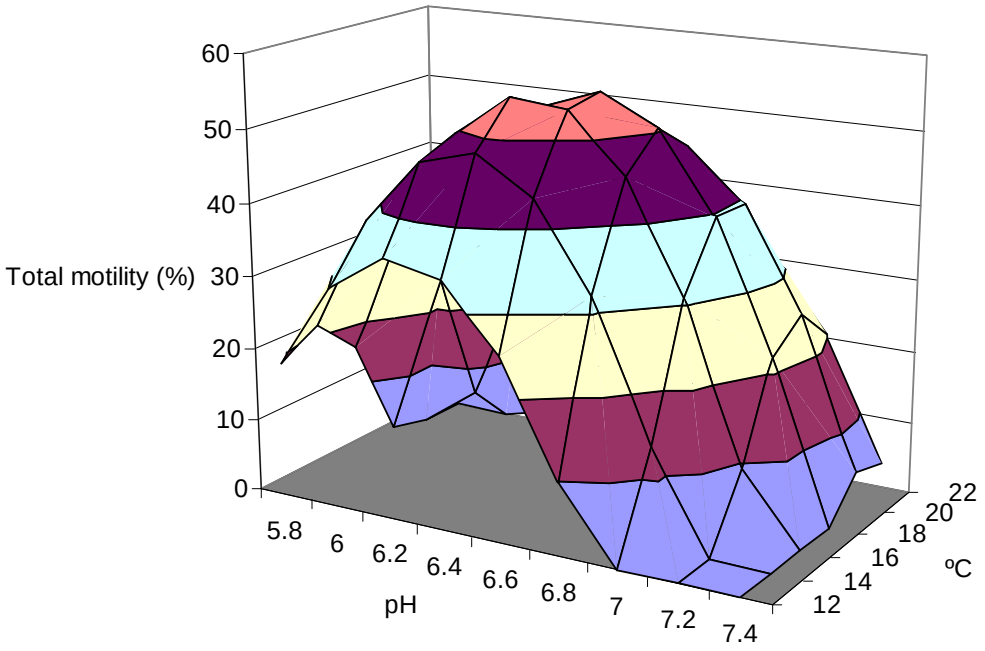

Figure 4. Results of a regression analysis demonstrating the effects of the temperature and pH of diluted, cooled boar semen which was held for 24 hours prior to cryopreservation on the post-thaw total motility ($P < 0.0001$).

Table 3. The mean pregnancy rate and litter size of gilts inseminated with frozen-thawed sperm.

Treatment	Motile sperm x 10^9	AI number	N	Pregnancy (%)	Litter Size
1	1	1x	18	77 ± 10.0	9.9 ± 1.2
2	1	2x	19	68 ± 10.9	11.2 ± 1.4
3	2	1x	19	68 ± 10.9	10.7 ± 1.2
4	2	2x	17	82 ± 9.5	12.8 ± .66
5	4	1x	18	67 ± 11.0	10.9 ± 1.9
6	4	2x	18	83 ± 9.0	11.0 ± 1.5

Genetic variation in testis size and testicular development

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Clay Center, NE 68933

Breed differences in sperm production have been described in a number of studies (Figure 1)with these differences reflectingvariation in testicular size (Ford et al., 2006; Smital, 2008).

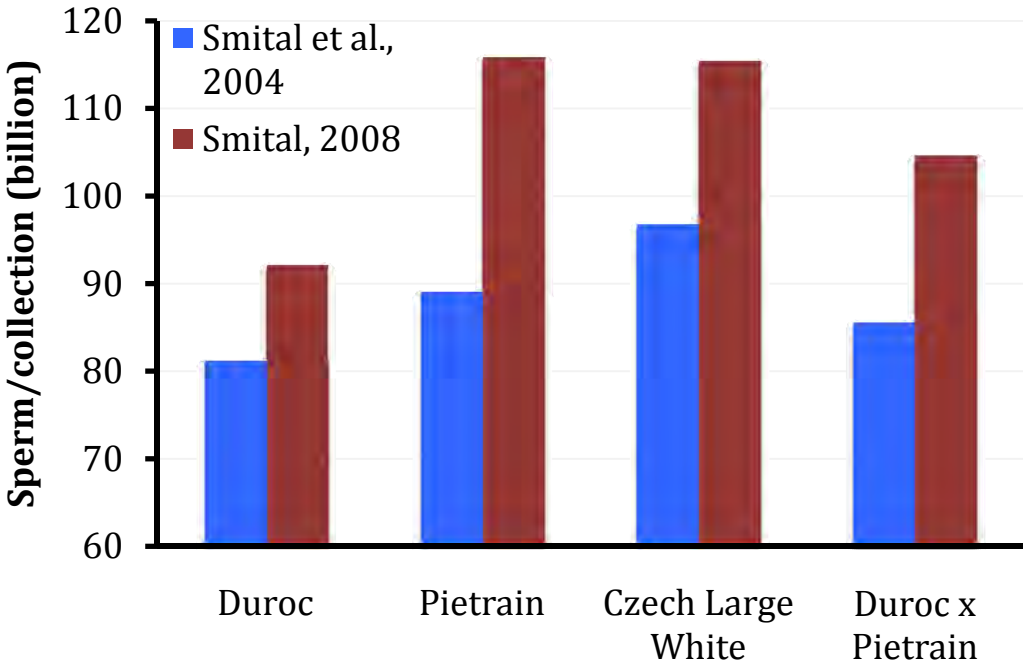

Figure 1. Differences in sperm production of selected breeds were evaluated in successive reports from the Czech Republic. The number of boars ranged from 186 to 980 per breed; the number of ejaculates ranged from 13,619 to 69,281 per breed. Duroc boars produced significantly less sperm than Pietrain or Czech Large White boars in both reports.

Within a given breed or genetic line of boars, sperm production increases as testicular size increases.Furthermore, the number of Sertoli cells within the testes at puberty establishes adult testicular size (Lunstra et al., 2003). Sertoli cells provide physical and nutritional support for developing germ cells in their early development toward sperm cells (Figure 2). Sertoli cells first become apparent in the testes on day 26 of gestation. Thereafter, these cells continue to increase in number until boars reach puberty after which they undergo final maturation. This maturation process is associated with accumulation of fluids within

the tubules, enlargement of tubule diameter and formation of a lumen that subsequently allows transport of sperm through the tubules followed by their eventual exit from the testes into the epididymis. The potential for sperm production and testicular size is established at puberty when Sertoli cells cease to proliferate.

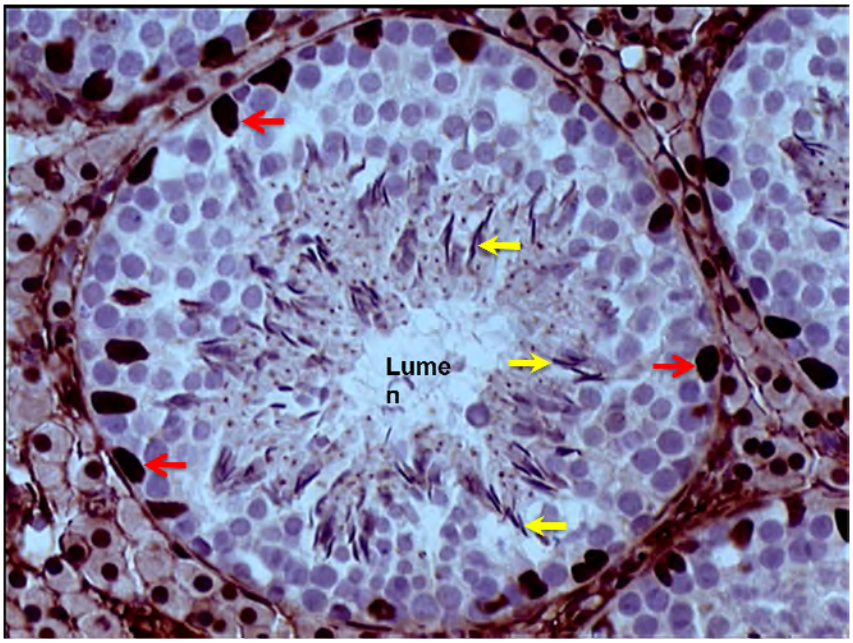

Figure 2. Seminiferous tubule of a boar testis with Sertoli cell nuclei indicated by red arrows and spermatids by yellowarrows. Germ cells arelight violet, oval bodies within the seminiferous tubule.

Figure 3 illustrates patterns of testicular growth in breeds or crossbred lines of boars that diverge greatly in mature testicular size. Meishan and Piau are breeds with somewhat smaller mature body weight and distinctly smaller testicular weight than today's crossbred boars; however, these two breeds differ vastly in their age at puberty. Meishan boars attain expanding seminiferous tubules (> 100 µm in diameter) by 50 days of age while Piau do not achieve this degree of testicular development until 140 days of age. Furthermore, testes of adult Piau boars contain a much greater proportion of seminiferous tubules than Meishan boars. For the three crossbred lines of pigs, diameter of seminiferous tubules advance to 100 µm by 90 days of age in BX boars, by 105 days in LR x D boars and by 120 daysin H x D x Y boars. These different studies were conducted under well-managed conditions, but management may have had subtle effects on age for the initiation of pubertal development. Also, the number of boars in each age group is small in most of these studies. Nonetheless, these data emphasize that age of puberty and genetic potential for testicular size are not correlated traits.

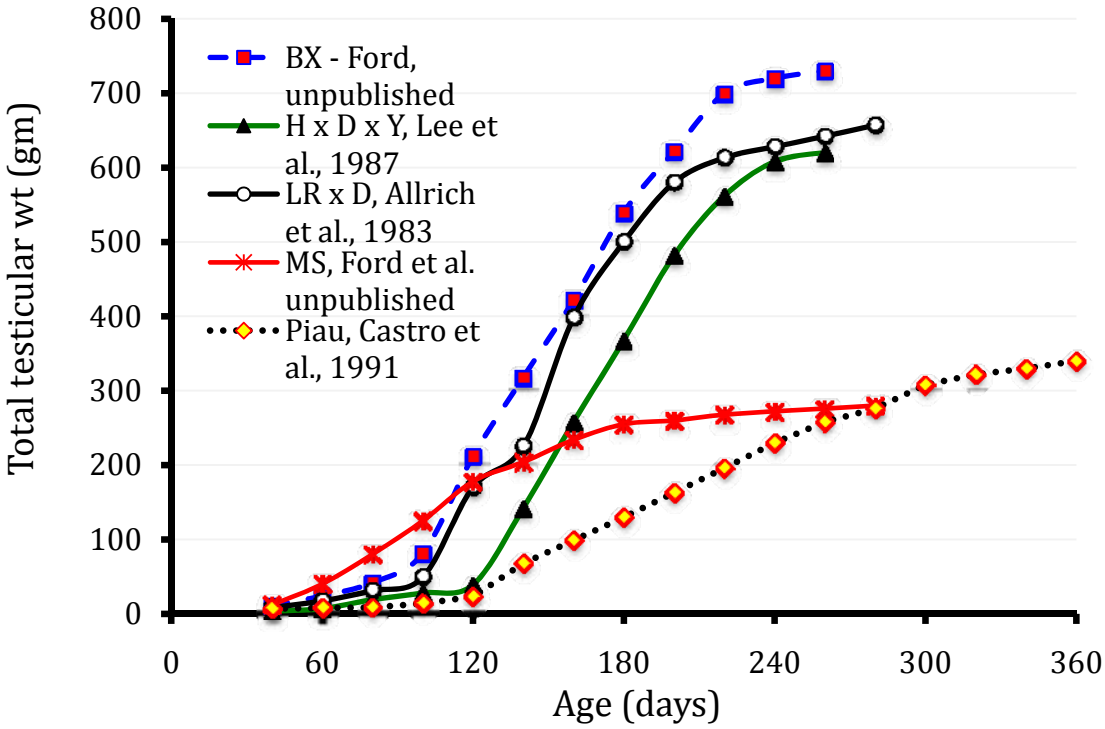

Figure 3. Differences in rate and magnitude of testicular growth in boars of divergent lines; BX – a four-breed composite of Duroc, Yorkshire, maternal Landrace and terminal Landrace; H x D x Y – Hampshire, Duroc and Yorkshire; LR x D – maternal Landrace and Duroc; MS – Chinese Meishan; Piau – an unimproved breed from Brazil.

Heritability for testicular size was employed in a study conducted at the University of Nebraska to improved sperm production significantly by 18% in association with a 24% increase in testicular size (Huang and Johnson, 1996). These boars were selected for 11 generations for increased testicular size at 150 days of age. A correlated response was earlier age of puberty in boars of the selected line that was substantiated by earlier enlargement of seminiferous tubules and earlier onset of sperm production (Harder et al., 1995; Rathje et al., 1995). In contrast with this, Meishan crossbred boars that reach puberty early have smaller testes and lower sperm production as adults than boars with later pubertal development (Lunstra et al., 2003). Collectively, these studies emphasize that the relationship of age at puberty and mature testicular size varies with the genotype of the boars that are under investigation.

Informative genetic markers for testicular size would provide a means to select boars early in life with the best potential for greater sperm production as adults. A stimulus for this area of research was the observation that plasma concentrations of follicle-stimulating hormone (FSH) were negatively correlated with testicular size (Ford et al., 1997; Ford et al., 2001). Initial efforts to identify geneticmarkers utilized Meishan crossbred boars (Rohrer et al., 2001; Sato et al., 2003). Evidence to date indicates the presence of quantitative trait loci (QTL) on swine chromosomes X, 3, and 8 that harbor genes for

testicular weight and/or plasma FSH concentrations.Additional information relating to chromosomes 3 and 8 has not advanced since these initial reports. On chromosome X, the thyroxine-binding globulin (TBG) gene resides in the QTL for plasma FSH (Figure 4), and Nonneman et al. (2005) determined that alleles forTBGpredicted testicular size. Boars with the TBG allele inherited from the Meishan breed had smaller testes than boars with the TBG allele from a white composite line. However, to date, the Meishan allele for TBG has not been found in breeds of pigs used for commercial swine production. Studies are in progress to identify QTL for testicular size and sperm production in crossbred boars that do not contain the Meishan breed.

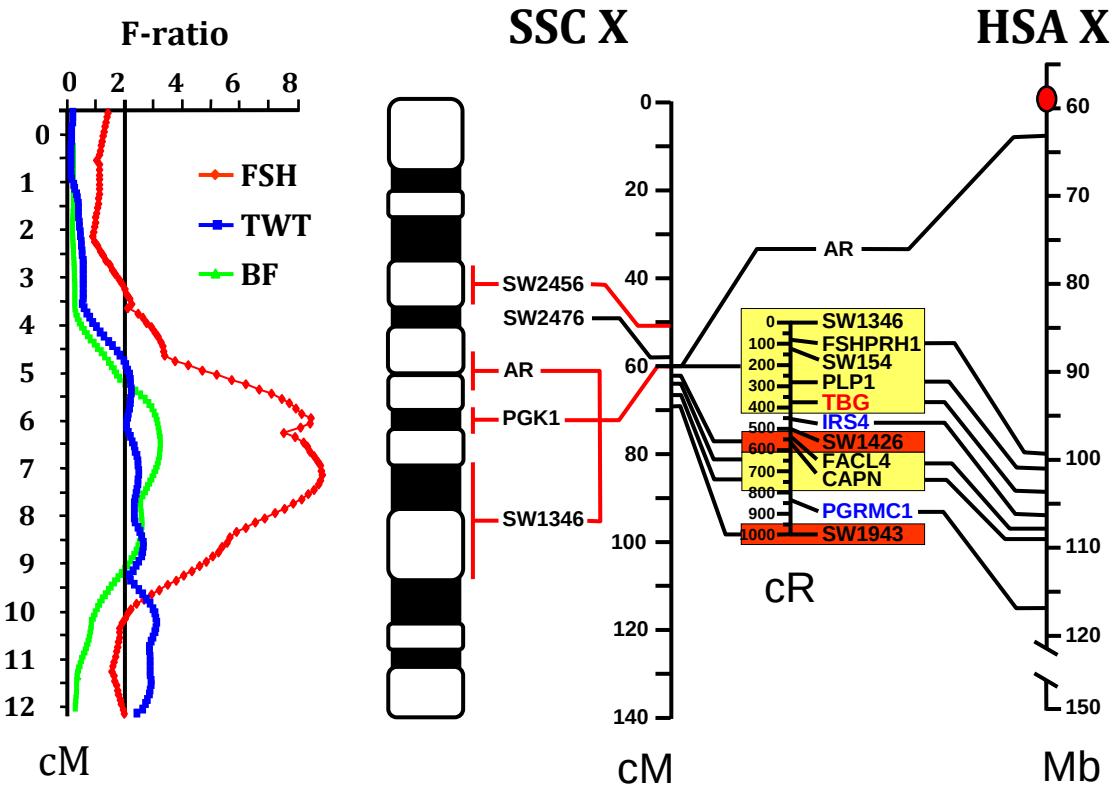

Figure 4. F-ratio profiles on swine chromosome X indicating evidence of QTL for plasma follicle-stimulating hormone (FSH), testicular weight (TWT), and backfat thickness (BF);reproduced from Nonneman et al. (2005). Genetic markers are aligned in their relative position on the porcine cytogenetic, genetic, and physical maps and compared with the human physical sequence map. Units are in centimorgans (cM), centirays (cR), and megabases (Mb).

Worth of mentioning is that the Meishan TBG allele is also predictive of greater backfat thickness (Figure 4; Ponsuksili et al., 2005). Data in Figure 2 draw attention to how easily testicular weight increased as pigs were selected for faster growth rate and leaner

Harder, R. R., D. D. Lunstra, and R. K. Johnson. 1995. Growth of testes and testicular morphology after eight generation of selection for increased predicted weight of testes at 150 days of age in boars. J. Anim. Sci. 73:186-219.

Harris, J. 1883. In: Harris on the Pig. Breeding, Rearing, Management, and Improvement. Orange Judd Company, New York.

Huang, Y. T., and R. K. Johnson. 1996. Effect of selection for size of testis traits. J. Anim. Sci. 74:750-760.

Lee, K. -H., M. A. Diekman, G. E. Moss, and R. D. Allrich. 1987. Pituitary gonadotropins, hypothalamic gonadotropin-releasing hormone, and testicular traits of boars exposed to natural or supplemental lighting during pubertal development. Biol. Reprod. 36:1164-1169.

Lunstra, D. D., T. H. Wise, and J. J. Ford. 2003. Sertoli cells in the boar testis: changes during development and compensatory hypertrophy after hemicastration at different ages. Biol. Reprod. 68:140-150.

Nonneman, D., G.A. Rohrer, T.H. Wise, D.D. Lunstra, and J. J. Ford. 2005. A variant of porcine thyroxine-binding globulin has reduced affinity for thyroxine and is associated with testis size. Biol. Reprod. 72:214-220.

Ponsuksili, S., E. Murani, K. Schellander, M. Schwerin, and K. Wimmers. 2005. Identification of functional candidate genes for body composition by expression analyses and evidencing impact by association analysis and mapping. Biochim. Biophys. Acta 1730:31-40.

Rathje, T. A., R. K. Johnson, and D. D. Lunstra. 1995. Sperm production in boars after nine generations of selection for increased weight of testis. J. Anim. Sci. 73:2177-2185.

Rohrer G. A., T. H. Wise, D. D. Lunstra, and J. J. Ford. 2001. Identification of genomic regions controlling plasma FSH concentrations in Meishan-White Composite boars. Physiol. Genomics 6:145-51.

Sato, S., Y. Oyamada, K. Atsuji, T. Nade, S. -I. Sato, E. Kobayashi, T. Mitsuhashi, K. Nirasawa, A. Komatsuda, Y. Sato, S. Terai, T. Hayashi, and Y. Sugimoto. 2003. Quantitative trait loci analysis for growth and carcass traits in a Meishan x Duroc F2 resource population. J. Anim. Sci. 81:2938-2949.

Smital, J., L. L. De Sousa, and A. Mohsen. 2004. Differences among breeds and manifestation of heterosis in AI boar sperm out. Anim. Reprod. Sci. 80:121-130.

Smital, J. 2008. Effects influencing boar semen. Anim. Reprod. Sci. (Epub ahead of print).

carcasses. This supports the observation by Harris (1883) that testicular size changed rapidly when boars were selected for greater body mass and greater fat content; "The truth of the matter is, that good breeders increase the development of the choice parts of the pig at the expense of the offal; and the ham of a well-bred and well-formed boar has been enlarged at the expense of some portion of the contiguous parts. We have known this carried to such an extreme, that causal observers would suppose they were looking at a barrow-pig. Any one who will contrast a coarse Chester County boar with a refined Essex will understand our meaning." Although data are unavailable, association of fatness with small testicular size is supported by photographs of older breeds of pigs such as the Iberian and Mangalitza breeds that have not been selected intensively for leanness. This ability to modify testicular size rapidly as preference for specific carcass characteristics underwent change substantiates a moderate to high heritability for testicular size.

The BX line of pigs at the U.S. Meat Animal Research Center is a composite of one-quarter Duroc, Yorkshire, maternal Landrace and terminal Landrace. Considerable variation exists for testicular size of post-pubertal boars in this line creating a resource to investigate non-invasive procedures to use early in life as a means to predict post-pubertal testicular size and sperm production. However, estimation in pubertal boars of testicular size by ultrasound has proved less informative than desired thereby leaving identification of informative QTL as the most promising selection tool to develop for improving sperm production of boars used in commercial swine production.

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Ford, J. J., T. H. Wise, D. D. Lunstra, and G. A. Rohrer. 2001. Interrelationships of porcine X and Y chromosomes with pituitary gonadotropins and testicular size. Biol. Reprod. 65:906-912.

Ford, J. J., S. A. McCoard, T. H. Wise, D. D. Lunstra, and G. A. Rohrer. 2006. Genetic variation in sperm production. Soc. Reprod. Fertil. Suppl. 62:99-112.

Anatomy and Physiology of the Boar

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Introduction

The boar has a tremendous impact on the reproductive efficiency of the swine breeding herd. Depending on the collection frequency and insemination dose, semen from a single boar can be used to breed between 750 and 1000 sows per year. As a result, reproductive failure of a single male influences a large number of sows. Consequently, a thorough understanding of the basic aspects of male reproductive physiology is important in managing boars for optimal fertility. This paper will review the anatomy, physiology and sexual development of boars placing particular emphasis on spermatogenesis and the ejaculatory process.

Anatomy of the Boar

The male reproductive system is composed of a variety of different structures including the testes; the urogenital duct system; the secondary sex glands; the pituitary gland; and the hypothalamus. These communicate via the endocrine and nervous system to coordinate normal reproductive activity in boars. Abnormal activity in one or more of these areas can result in reproductive problems.

Hypothalamus and Pituitary Gland - The brain is the component of the male reproductive system that gathers internal signals from within the body and external cues from the environment; integrates them; and regulates physiological and behavioral functions associated with reproduction. The hypothalamic portion of the brain secretes gonadotropin releasing hormone (GnRH) which controls the production and secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary gland (Hafez, 1993). These two hormones are responsible for regulating testicular function (Figure 1).

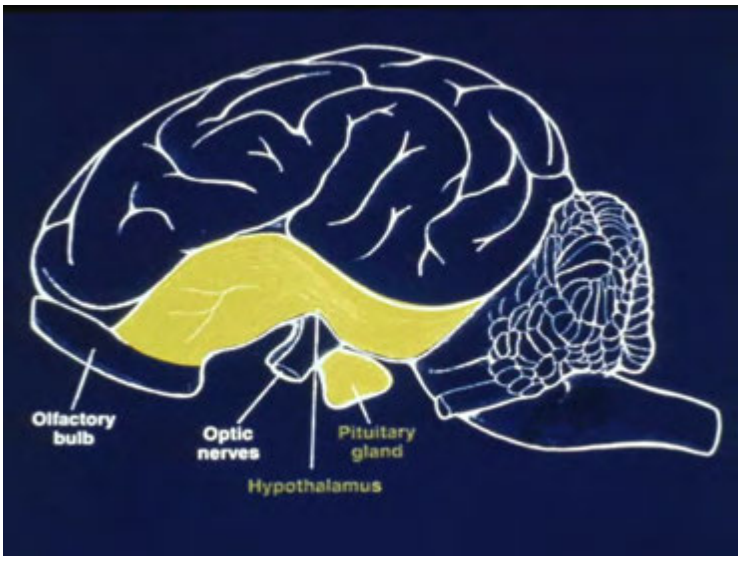

Figure 1. Diagram of the hypothalamus and pituitary gland. Notice the close anatomical association between the optic and olfactory nerves and the hypothalamus. Sights and sounds perceived by the boar, such as a female in estrus travel to the brain via these nerves and stimulate the hypothalamus to secrete GnRH. Secretion of GnRH stimulates the release of LH and FSH from the pituitary, which, in turn, stimulate the production of testosterone from the testes.

Testes - The primary functions of the testes (Figure 2) are to produce spermatozoa and hormones. The majority of each testes is seminiferous tubules. The seminiferous tubules are a convoluted network of ducts in which spermatozoa are produced. Sertoli cells are specialized cells involved in the maturation of spermatozoa and hormone production and line the lumen of the seminiferous tubules. Interstitial cells of Leydig, blood and lymph vessels and nerves are located in between the seminiferous tubules. Important interactions between the Sertoli and Leydig cells regulate virtually every aspect of male reproductive function. A series of tubules leave the seminiferous tubules and connect to form collecting duct located in the center of each testis called the rete testis. During spermatogenesis, spermatozoa leave the seminiferous tubules and enter the rete testis during their passage into the epididymis (Setchell et al., 1993). Because the testes are located externally, special anatomical systems are needed for thermoregulation. The most important of which is a complex vascular arrangement of testicular arteries and veins in the spermatic cord called the pampiniform plexus (Garner and Hafez, 1993). The testicular artery forms a convoluted structure in the shape of a cone in which arterial coils are enmeshed with testicular veins. From a functional perspective, this countercurrent mechanism enables arterial blood entering the testis to be cooled by venous blood exiting the testis. In most species, the temperature of arterial blood drops between 2 and 4^{o}C prior to its entry into the testes. In addition, two groups of muscles, the tunica dartos and cremaster, play an important role in thermoregulation. The tunica dartos lines the inside of the scrotum and controls its proximity to the testis. It contracts during cold weather pulling the scrotal sac closer to the testis for added insulation and relaxes during warm weather allowing the scrotum to recoil into a distal position. The cremaster muscle is located in the spermatic cord and is attached to the thick membranous sac surrounding the testis. It contracts during cold weather pulling the

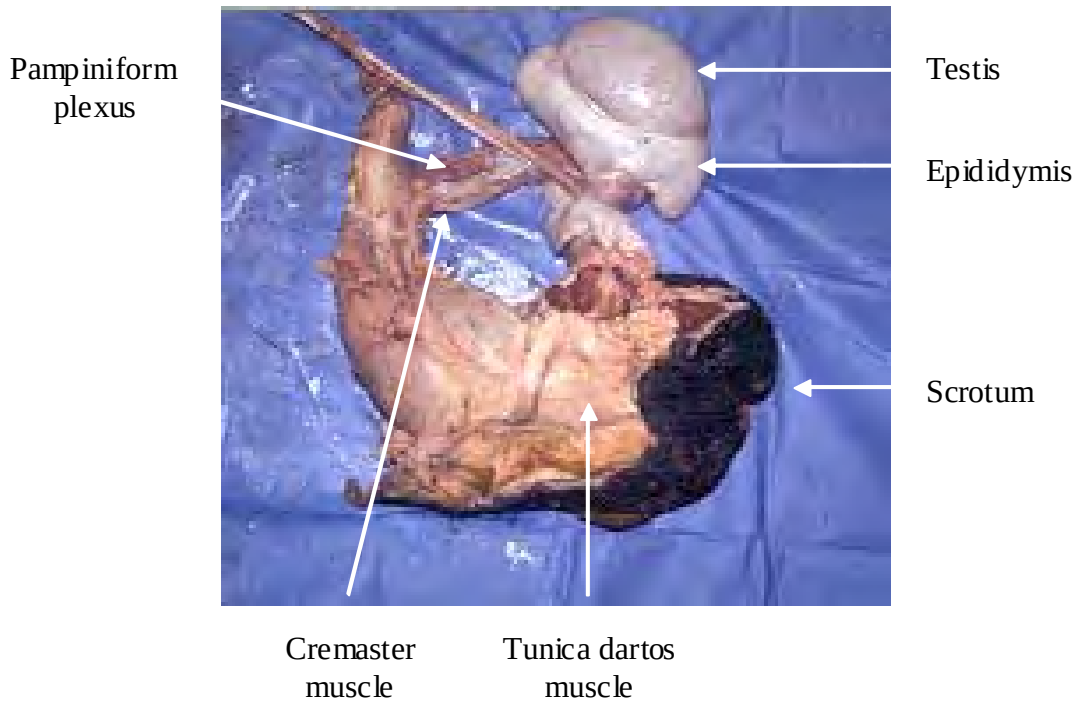

Figure 2. Testes and associated structures from a boar. The testis and epididymis in the upper right hand corner of the picture have been separated from the connective tissue, tunica dartos, and scrotum that normally surround them. The other testis and epididymis, which still is surrounded by the connective tissue layers, are located in the lower left hand corner of the picture.

scrotal sac and testis closer to the body core and relaxes during warm conditions allowing the testis to return to its normal position. Both muscles have an abundant supply of nerve fibers that respond to temperature sensors located in the central nervous system. Because boars do not have pendulous testicles like bulls, the tunica dartos is more important than the cremaster muscle in the regulation of testicular temperature.

Epididymis - The rete testis enters the efferent ducts, which eventually form a single coiled duct called the epididymis. The epididymis is similar to the seminiferous tubules in that in coils back upon itself many times and forms three distinct sections - the caput (head), corpus (body) and cauda (tail) epididymi. The convoluted duct of the epididymis is surrounded by a prominent layer of circular muscle fibers and contains pseudostratified columnar, stereociliated epithelium. Masses of spermatozoa are commonly found along the entire lumen of the epididymis (Setchell et al., 1993).

The primary function of the epididymis is sperm maturation, transport and storage. Spermatozoa entering the epididymis are neither motile nor fertile. It takes spermatozoa between 9 and 14 days to migrate from the head to the tail of the epididymis, the primary storage site. It has been estimated that the tail of the epidiyimis contains about 75% of the total epididymal spermatozoa. Spermatozoa become motile and acquire fertilizational competence in the body of the epididymi due to the secretion of factors by the cells located in this region. Movement of spermatozoa through the epididymi is thought to be due to the flow of rete fluid, the action of the sterociliated epithelium and contractions of the circular muscle layer. Unejaculated spermatozoa are gradually eliminated by excretion into the urine. Spermatozoa that are not excreted in the urine

undergo a gradual aging process. During the aging process, fertilizational competence is lost first and is followed by a decrease in motility (Garner and Hafez, 1993). Eventually, dying spermatozoa disintegrate. Ejaculates with dying spermatozoa often appear "clumpy", ie. - have large groups of spermatozoa bound together by their heads.

Vas Deferens, Accessory Sex Glands and Penis[1,3] - The vas deferens (Figure 3) is a thick, heavily muscled tube through which sperm are transported from the tail of the epididymis to the pelvic urethra, at which point the paired genital systems of the boar meet and converge with the urinary tract just before the bladder. Adjacent to the pelvic urethra are three secondary sex glands: the vesicular glands or seminal vesicles; the prostate gland; and the bulbo-urethral glands (Hafez, 1993).

The seminal vesicles lie lateral to the terminal portion of each vas deferens. In the boar, they are large, lobulated and relatively diffuse. They often appear to have an orange color. They are responsible for the majority of the fluid volume of boar semen. In addition, they secrete high levels of fructose and citric acid as well as inositol, ergothioneine, several amino acids and glycerylphosphoroylcholine. Most of these compounds are used as energy substrates by ejaculated spermatozoa (Garner and Hafez, 1993).

The prostate gland is located next to the vesicular glands with the majority of its body being embedded in the muscle layer surrounding the pelvic urethra. Secretions from the prostate gland during ejaculation are primarily alkaline and contain calcium, acid phosphatase and fibrinolysin. The primary function of the fluid from the prostate gland is to neutralize the acidic vaginal secretions (Setchell et al., 1993). Secretions from the prostate gland also are believed to give semen its characteristic odor.

The bulbourethral glands are long cylindrical glands in the boar located on either side of the pelvic urethra near the ischial arch of the pelvis. The bulbourethral glands secrete the gel fraction of the semen characteristic of porcine ejaculates. Many functions for the gel component of semen have been postulated, but few have been proven.

The terminal portion of the boars urogenital system is the penile urethra, which is the central tube within the penis. The penile urethra opens into the glans penis. In the boar, the glans penis has a counter

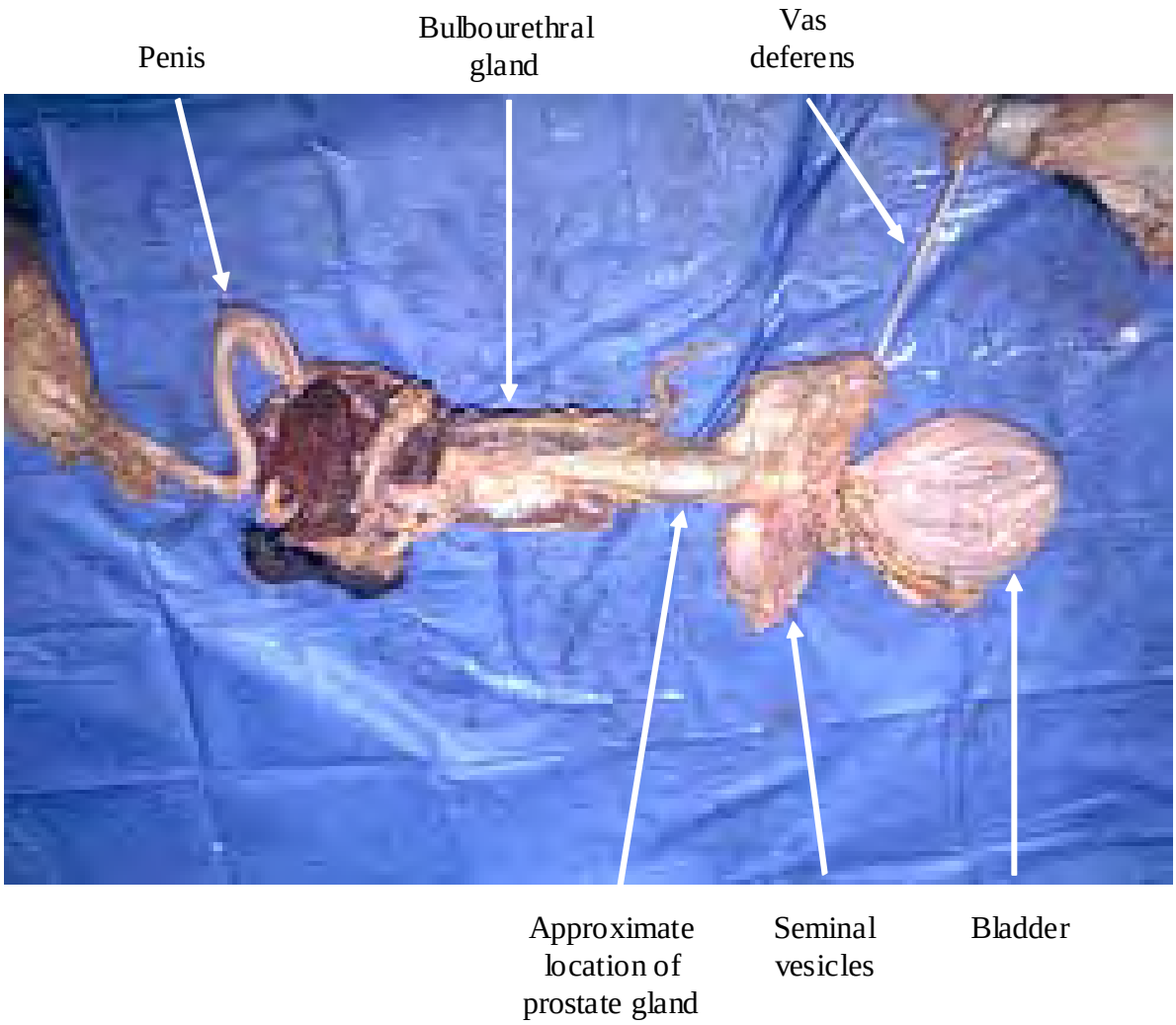

Figure 3. Vas deferens, secondary sex glands, bladder, and penis (urogenital tract) from a boar. The seminal vesicles and bulbourethral glands are paired glands. The prostate gland is embedded in muscle and cannot be seen without additional dissection. The vas deferens originates from the tail of the epididymis.

clock-wise spiral. The glans penis is highly innervated and must be stimulated properly for normal ejaculation to occur. The porcine penis also contains three cavernous bodies or sinuses that surround the penile urethra. During erection blood in pumped into and retained in these areas. In the resting state, the porcine penis is retracted and forms a characteristic "S" fold called the sigmoid flexture. The free end of the penis in the retracted state resides in the prepuce or sheath (Figure 4). In young prepubertal boars, the glans penis cannot be extended fully because it is fused with the lining of the prepuce. As a boar matures, androgens produced by the testis initiate keratinization of the inner preputial lining and the penis is eventually freed from its connection with prepuce. Persistent frenulum is a condition in which strands of tissue did not keratinize fully and are still attached to the

penis (Garner and Hafez, 1993). When this occurs, the end of the penis curves back toward to the prepuce during erection and ejaculation. Removal of these strands of tissue with a pair of sterile scissors corrects this situation. Near the end of the prepuce is a diverticulum called the preputial sac. Urine, semen and secreted fluid collect in this sac and are broken down via bacterial action. Contents of the preputial sac are often expelled during detection of estrus or natural matings and often believed to be the source of the odor associated with mature boars.

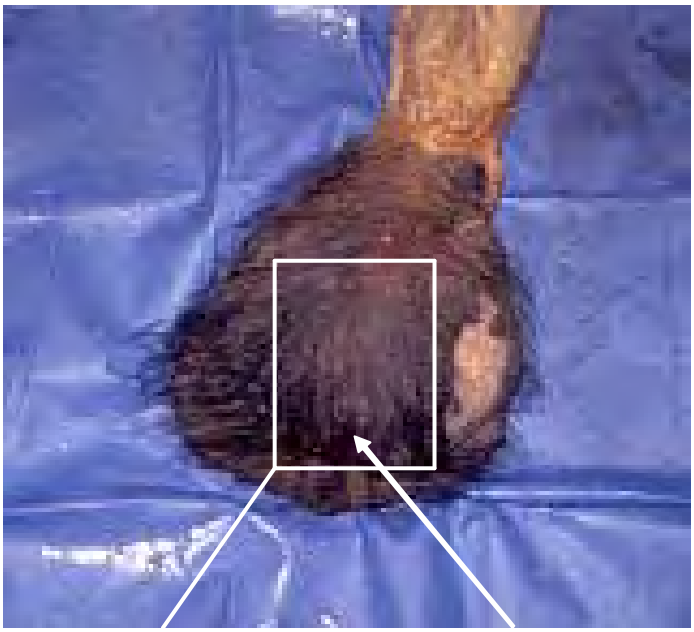

Figure 4. Sheath and preputial sac. The general area of the preputial sac is outlined by the white box.

Physiology of the Boar

Endocrine Activity within the Testes - Leydig cells and sertoli cells are the two primary endocrine producing cells in the testes. Luteinizing hormone (LH) released from the anterior pituitary gland stimulates production of androgens from the Leydig cells. The primary androgen produced is testosterone. Testosterone has a variety of important functions in spermatogenesis and male sexual behavior. Follicle-stimulating hormone (FSH) stimulates the Sertoli cells to produce androgen-binding proteins; convert testosterone to dihydrotestosterone and estrogen; and secrete inhibin (Bartke et al., 1978). Androgen-binding protein forms a complex with androgen and is carried along with the spermatozoa to the epididymis. High local levels of androgen are necessary for the normal function of the epididymal epithelium. Inhibin diffuses out of the seminiferous tubules; enters the vascular system; and transported to the brain where it has a negative effect on the secretion of FSH. Inhibin production by the testes is an important component of gonadotropin regulation in the male. In the boar, high quantities of estrogen are found in semen. The source of these estrogens is the sertoli cells, which convert testosterone to estrogen via the aromatase enzyme (Setchell et al., 1993). It appears that the primary role of seminal estrogens is to stimulate important reproductive events in

the female reproductive tract during breeding.

Recent investigations have demonstrated that both Sertoli and Leydig cells have receptors for a variety of growth factors including IGF-I, EGF and TGF. It has been proposed that growth factors may be produced in response to gonadotropin or growth hormone action on testicular tissue and mediate many of the actions of these hormones (Hafez, 1993). In addition, growth factors are believed to be the primary mode in which Sertoli cells and developing spermatozoa regulate each other's secretion of proteins along the length of the seminiferous tubule (Setchell et al., 1993).

Erection and Ejaculation - Sexual stimulation causes dilation of the arteries supplying the sinuses in the penis. In has been suggested that parasympathetic fibers originating from the pelvic nerve are responsible for providing the neural signal for dilation and thus the initiation of erection. At the same time vasodilation begins, the ischiocavernosus muscle begins to contract repeatedly which causes blood to be pumped into the sinuses in the penis. In the boar, no veins drain the distal portion of these spaces, which facilitates the increase in pressure within the penis and ultimately, erection. As pressure increases from blood being trapped in the sinuses, the retractor penis muscle relaxes allowing the sigmoid flexure to straighten and the penis to protrude from the sheath. Several studies demonstrate that erection failures in boars are caused primarily by structural defects rather than psychological problems (Benson, 1993).

Ejaculation is primarily under neural control and involves contractions of smooth muscles. The process is initiated by rhythmic contractions of smooth muscles lining the tail of the epididymis and the ductus deferens. These contractions are controlled by sympathetic nerves that arise from the pelvic plexis of nerves, which is a branch of the hypogastric nerve. During ejaculation, the bulbospongiosus muscle compresses the penile bulb and forces blood into the remainder of the cavernosus tissue resulting in a slight enlargement of the glans penis in boars.

Spermatogenesis - Spermatogenesis is divided into two basic processes: spermatocytogenesis and spermiogenesis (Figure 6). In a general sense, spermatocytogenesis is the process involved with the mitotic and meiotic divisions of sperm cells, while spermiogenesis refers to the maturational phase of development. Although both hormones are important, it is believed that LH plays a more active role than FSH in spermatocytogenesis, while FSH is the main hormone involved with spermiogenesis.

Spermatocytogenesis - Just prior to puberty in boars, undifferentiated germ cells called gonocytes differentiate to form type AO spermatogonia. These are the precursor sperm cells from which all other sperm cells originate. There is some evidence that the number of AO spermatogonia is directly related to the sperm production capacity of adult males. In adult boars, AO spermatogonia differentiate into A1 spermatogonia which divide progressively to form various types of immature sperm cells. The final mitotic division during spermatocytogenesis occurs in primary spermatocytes. Although the average number of mitotic divisions cells would undergo between the A1 and primary spermatocyte stages is a subject of some controversy, a figure of 6 to 8 is commonly used (Garner and Hafez, 1993). This means that between 32 and 124 primary spermatocytes are formed from a single spermatogonia. After the formation of primary spermatocytes, no new DNA synthesis occurs and the resulting secondary spermatocytes divide to form haploid cells known as spermatids. The entire divisional process of spermatocytogenesis occurs in the testis. It is interesting to note that many of the divisions are actually incomplete in that small cytoplasmic bridges that are retained between most cells originating from a common type A1 spermatogonia. Some researchers speculate

that these bridges are important in coordination of development of sperm cells as a group (Setchell et al., 1993).

Spermiogenesis and Spermiation - The round spermatids are transformed into spermatozoa by a series of morphological changes referred to as spermiogenesis. Maturational changes that spermatozoa undergo during spermiogenesis include condensation of nuclear material, formation of the sperm tail and

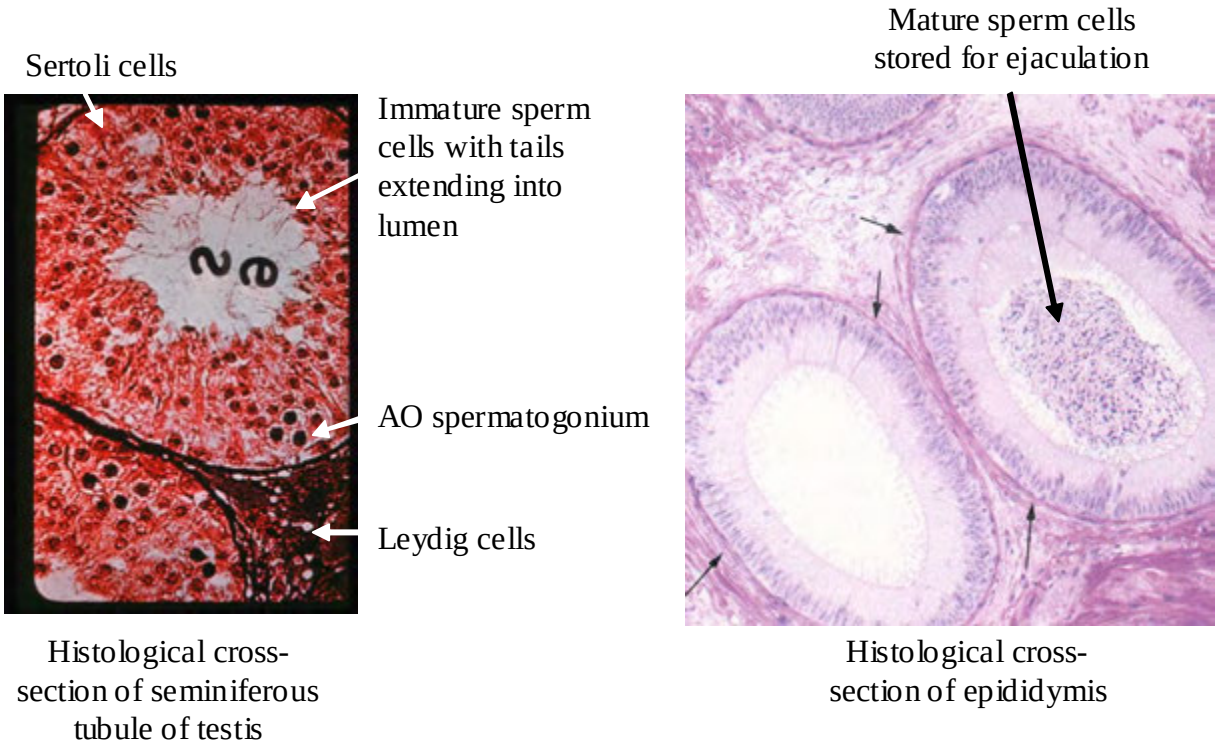

Figure 6. Histological cross-sections of seminiferous tubules (left) in the testes and the tail of the epididymis (right).

development of the acrosomal cap and its contents (Garner and Hafez, 1993). During most of spermiogenesis the sperm cells appear to have their heads imbedded in Sertoli cells. In reality, the membrane of the Sertoli cell actually is wrapped around the sperm head. Communication and exchange of materials between the Sertoli and developing sperm cells occurs via intercellular bridges. The actual release of spermatozoa into the lumen of the seminiferous tubule is called spermiation. The elongated spermatids are gradually extruded or pushed out of the Sertoli cell into the lumen of the seminiferous tubule until only a small cytoplasmic stalk connects the head of the sperm to the residual body in the Sertoli cell. Breakage of the stalk results in the formation of a cytoplasmic droplet in the neck region of the sperm. These commonly are referred to as proximal cytoplasmic droplets.

Epididymal Maturation - Spermatozoa enter the head of the epididymis incapable of fertilization, however, acquire this ability at some point during their transit to the cauda epididymis. It is believed

that epididymal secretions contain maturation factors than stimulate biochemical changes within the sperm cells necessary for fertilization (Garner and Hafez, 1993; Setchell et al., 1993). These changes include development of the potential for progressive forward motility; alteration of metabolic mechanisms; loss of the cytoplasmic droplet; and changes in the plasma membrane, acrosomal cap and nuclear material. It is interesting to note that during storage in the caudal portion of the epididymis, the metabolic activity of mature sperm is actually suppressed by secretion of a "quiescence factor". The entire process of spermatogenesis requires 45 to 55 days in the boar. The majority of this time is spent in the testicle and involves changes associated with both spermatocytogenesis and spermiogenesis. Maturation in the epididymis is thought to require only 10 to 14 days.

Sexual Behavior

Certain aspects of sexual behavior begin as early as 1 month of age in boars. Mounting activity of penmates is observed more frequently for males than females. Consistent mounting activity accompanied by erection occurs around 4 months of age (Hemsworth, 1982). However, most boars are not capable of producing ejaculates with normal quantities of fertile spermatozoa until 6 to 8 months of age (Figure). In general, testosterone is the male hormone that is the most closely linked with sex drive or libido. It is true that castrated males or boars with extremely low testosterone levels exhibit virtually no sexual interest. However, there have been a number of documented cases in which boars with normal levels of testosterone have low libido. Consequently, determining the relative importance of the endocrine system and prior sexual experience in male reproductive behavior is extremely difficult.

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Impact of uterine environment on life time sperm production

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Early Gestation:

Genetic sex is established at conception; however, the female phenotype inherently develops unless specific signals dictate the male phenotype. These signals must occur during defined periods of embryonic, fetal and neonatal development. The timing of this sequence of events in boars is well characterized by a number of studies (reviewed by Ford et al., 2001).

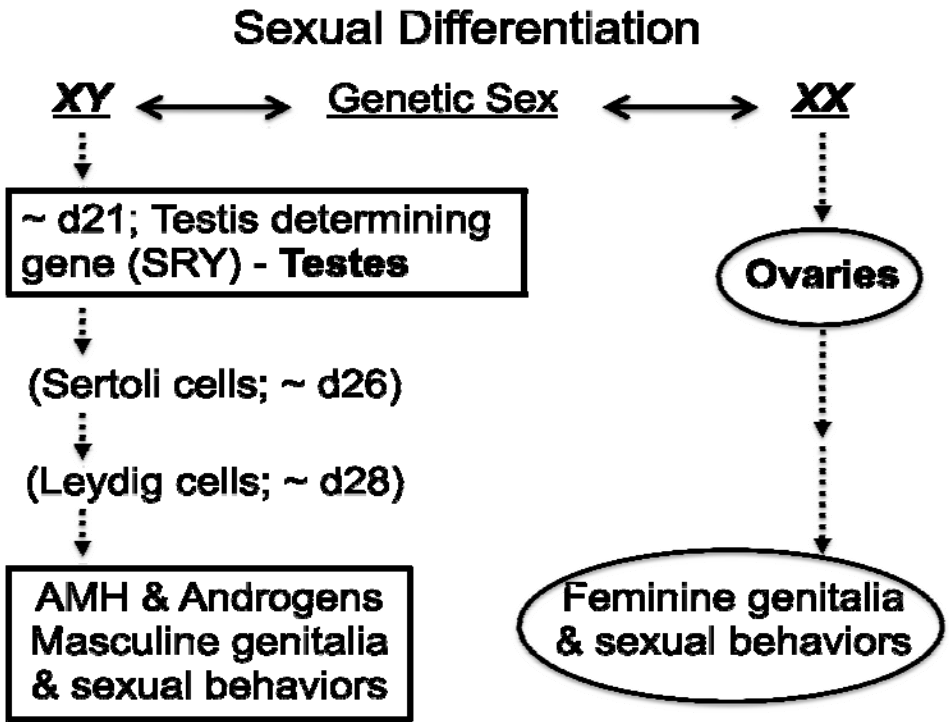

Figure 1. The sequence of events required for formation of testes and development of a masculine phenotype in boars. Embryos are essentially programmed to acquire the female phenotype.

During normal, porcine embryos development, the gonads are undifferentiated at day 19 of gestation; histological appearance of gonad in males and females is indistinguishable from one another. On approximately day 21 of gestation, testis formation is signaled by activation of the gene for sex determination, SRY, followed

by the appearance of Sertoli cells by day 26 (Figure 2; McCord et al., 2001) and Leydig cells on day 28 (Figure 3). Sertoli cells secrete anti-Mullerian hormone (AMH, formerly named MIS), which causes regression of the Mullerian ducts in males. In female embryos these ducts would develop into the oviducts, uterus and upper vagina.

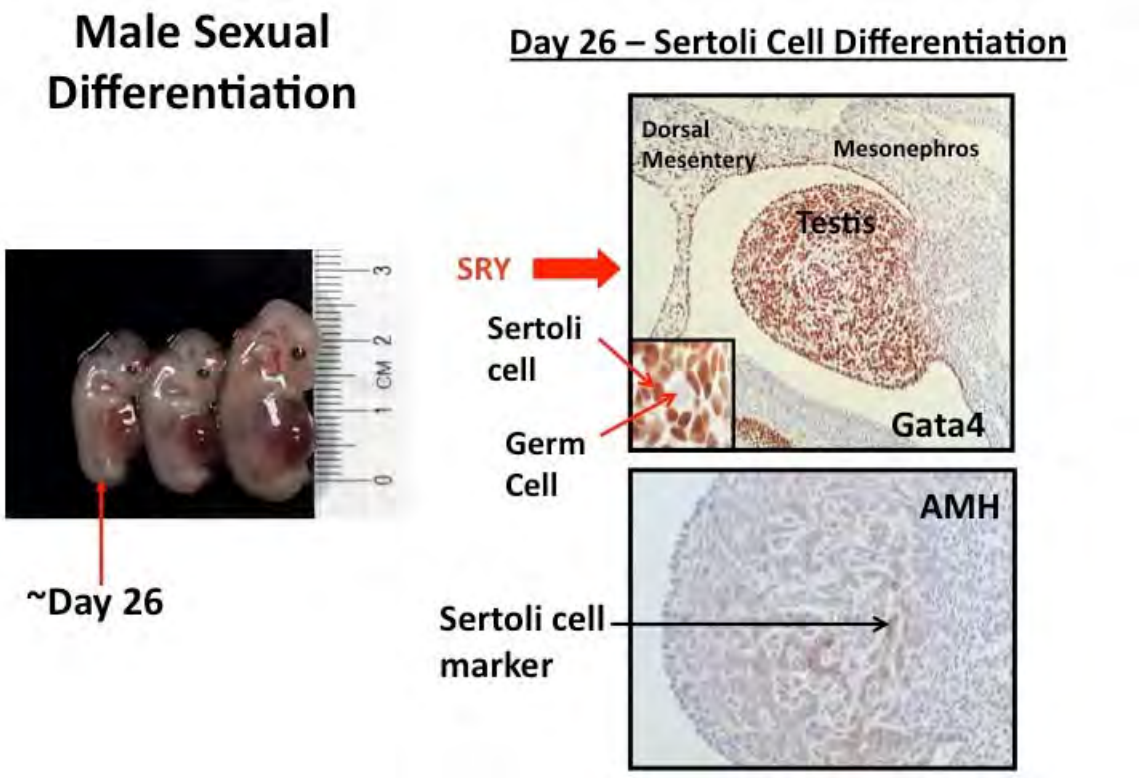

Figure 2. Male embryo on day 26 of gestation. Somatic cells within developing testes are distinguished from germ cells by their immunoreactivity for a zinc finger transcription factor, Gata4 (brown staining cells). Sertoli cells that align to form testicular cords secrete anti-Mullerian hormone (AMH) identified by their light brown appearance.

Leydig cells, apparent by day 28 of gestation, are the only cells within the testis that contain 17α-hydroxylase/17, 20-lyase cytochrome P450 (P450c17), an essential enzyme for synthesis of androgens. Production and secretion of testosterone stimulates the Wollfian ducts to develop into the vas deferens and epididymis and achieves maximal concentrations in the blood of male fetuses on day 35 of gestation (Ford et al., 1980). Dihydrotestosterone, a potent metabolite of testosterone, stimulates development of the scrotum and migration of the genital tubercle in association with formation of the penis. This permits visual differentiation of male from female embryos by day 40 of gestation. Numerous physiological processes

throughout fetal and postnatal development subsequently undergo sexual differentiation as a consequence of testicular steroid secretions. Among these, sexual behavior has a distinct postnatal component (Ford, 1990).

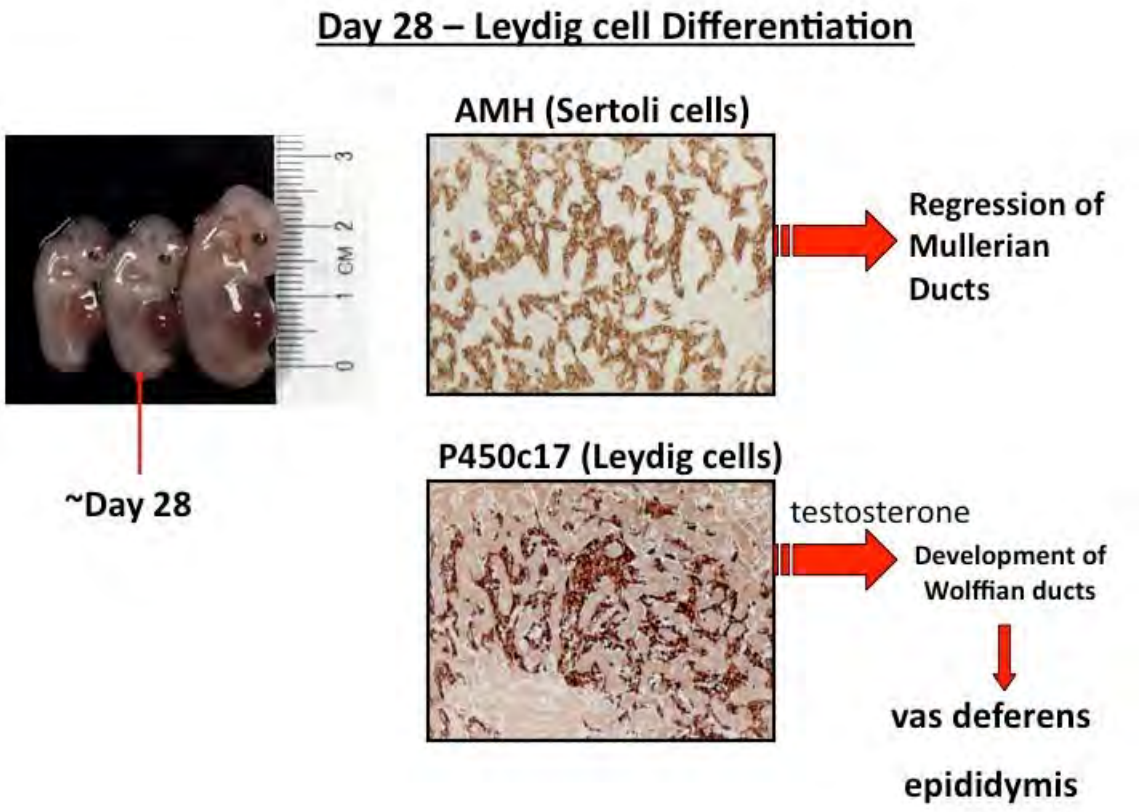

Figure 3. Male embryo on day 28 of gestation. Sertoli cells produce AMH throughout gestation and into early prepubertal development. Leydig cells, identified by immunoreactivity for P450c17, secrete testosterone.

Chinese Meishans boars provide an opportunity to address the timing of sexual differentiation in a unique breed that reaches puberty at a younger age and has smaller testes as adults than commercial crossbred boars (Ford et al., 1997; Lunstra et al., 1997). In spite of this distinct diversity in reproductive traits, sexual differentiation of embryonic and early fetal Meishan boars occurred at ages indistinguishable from Yorkshire x Landrace (YxLR) boars (McCoard et al., 2002). Fetal boars of these two breeds had similar crown-rump length through the first 50 days of gestations, but by day 50 YxLR boars achieved greater body weight than Meishan boars. As a result of these findings, gonadal differentiation in embryonic pigs emerged as fixed in its timing and seemingly not subject to environmental modification. However, this conclusion should be tempered in light of the observation that growth retardation can occur as early as day 30 of gestation (Ashworth et al., 2001; Foxcroft et al., 2006). Such retardation impacts muscle

development (myogenesis), but it remains to be established if components of testicular maturation align with embryonic muscle development.

Late gestation:

In contrast to the similarity of development during early gestation, YxLR boars had heavier body and testicular weights during the last one-half of gestation than Meishan fetuses (McCoard et al., 2003). Composition of testes also differed between these two breeds with Meishan boars having a greater proportion of their testes occupied by seminiferous tubules. Difficulties arise with interpreting the basis of these differences because the genotype of the fetus is confounded with the uterine environment in which it develops. When Yorkshire fetuses matured in a Meishan uterus, they weighted 25 percent less than when they matured in a Yorkshire uterus (Biensen et al., 1998; Wilson et al., 1998). Likewise, these investigators determined that Meishan fetuses undergoing gestation in Yorkshire uteri weighed 30 percent more than Meishan fetuses developing in Meishan uteri. The impact of these reciprocal embryo transfers upon testicular development and testicular weight of mature boars has not been evaluated.

In an effort to estimate the impact of intrauterine development upon sperm production of mature boars, data from nine studies totaling 500 crossbred boars were examined (Ford, unpublished data). The influence of birth weight upon mature testicular weight was inconclusive. Factors examined in addition to birth weight included total number of pigs born in the litter and parity of the sow, first or second. In the studies in which birth weight accounted for significant variation in testicular weight of postpubertal boars, the magnitude of the relationship was no greater than in those studies in which this relationship was not significant. Presently, no data in boars implicate prenatal influences upon postpubertal testicular size and sperm production other than genotype of the boar (Smital et al., 2004; Ford et al., 2006; Smital, 2008).

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Anatomy and Physiology of Boar Spermatozoa

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Introduction

The only purpose of a boar stud is to produce the largest quantity of the highest quality sperm cells (doses of semen) in an efficient period of time. Several factors can have negative affects on the production of high quantity and quality of semen, such as: high ambient temperature, inadequate nutrition, bacterial or viral infection, and collecting boars too frequently. Because the production of sperm cells is the sole purpose of a boar stud, it is advantageous for people working in a boar stud to have a better understanding about the anatomy and physiology of sperm cells. A person has a better chance of solving a semen quality problem due to morphologically abnormal sperm cells when they have an understanding about the anatomy, "manufacturing" and "maturing" of sperm cells.

Formation of spermatozoon

The morphological development of a spermatozoon (one sperm cell) is a complex process. Spermatozoa (plural of spermatozoon) are developed from a process called spermatogenesis. Figures 1 and 2 are schematics of the spermatogenesis process.[1] Spermatogenesis occurs in the seminiferous tubules of the testis. Although the process of spermatogenesis is very complex, it can be partitioned into two basic aspects.

Spermatocytogenesis. The first aspect is called spermatocytogenesis. Spermatocytogenesis is the transformation of spermatogonia (sperm mother cell) into spermatids (spermatogonia transform into primary spermatocyte; primary spematocyte transforms into secondary spermatocyte; secondary spermatocyte transforms into spermatids). Spermatogonia increase in number by mitosis. Mitosis is a type of cell division in which the daughter cells are nearly identical with the mother cell. It takes about 8.6 days for a spermatogonium to reach the primary spermatocyte stage. It takes 12.3 days for a primary spermatocyte to become a secondary spermatocyte. It takes 7.8 days for a secondary spermatocyte to become a spermatid.

Spermiogenesis. The second aspect is called spermiogenesis. Spermiogenesis is the morphological transformation of spermatids into spermatozoa. It takes 6.2 days for a spermatid to become a spermatozoon.

Take Home Message: The total time required to produce a sperm cell in the testis is approximately 34.4 days.[2] Sperm production occurs 24 hours per day and 7 days per week.

Therefore, all boars have to be correctly managed everyday to make sure they do not encounter factors that will cause problems with spermatogenesis. Heat-stress and sickness are two examples that will cause semen quality problems.

Sperm transport through epididymis

After the sperm cells are formed in the testis, they move through the caput (head), corpus (body) and cauda (tail) portion of the epididymis (Figure 3). It is estimated that the transport time from the caput through the cauda epididymis is approximately 9 to 14 days in the boar (caput – 3 days; corpus – 2 days; cauda – 4 to 9 days).[2,3] During the passage of the spermatozoa through the caput and corpus epididymis, the cytoplasmic droplet near the neck of the spermatozoa moves further down the middle-piece of the tail. The sperm cells are stored in the cauda epididymis where the concentration of spermatozoa is high and the lumen of the duct is larger. By the time spermatozoa are ejaculated most of the cytoplasmic droplets have become completely detached from the spermatozoa.[4] Some researchers have indicated that the majority of spermatozoa lose their distal droplets during the ejaculation process.[21] Researchers have also suggested the droplet releasing is induced by fructose coming from the seminal vesicle fluid during ejaculation.[22]

Take Home Message: Research has indicated that heat-stress and sickness can have detrimental effects on the epididymis. Heat-stress and sickness does cause an increase in cytoplasmic droplets. Therefore, all boars need to be correctly managed everyday. An insult to the epididymis can cause an increase in morphologically abnormal sperm cells for 14 days.

Anatomy of a boar spermatozoon

A boar spermatozoon consists of a head, neck, middle-piece of tail, main-piece of tail and end-piece (terminal portion) of tail (Figure 4). The total length of a boar spermatozoon is 47.2 ± 1.5 micrometers (µm).[5] One micrometer equals one-thousandth part of a millimeter (1 µm = .001 mm). One millimeter equals .039 inch.

Head. The head is oval and very thin (7.4 ± .5 µm long; 3.72 ± .4 µm wide and .35 ± .6 µm thick). A cap-like structure (acrosome) covers about 66% of the head. The remaining 33% of the nucleus is covered with a post-acrosomal sheath. The head contains the nucleus (heredity material: four nucleic acids [DNA] that encodes for every characteristic of the pig. This DNA is contained in the cell's chromosomes).

Neck. The region where the neck fibers of the middle-piece of the tail attach to the head is called the neck. The neck consists of a number of fibers gathered into three bundles. These bundles are not generally visible with a light microscope.

Middle-piece of tail. The middle-piece (10.7 ± .2 µm long and .75 ± .15 µm in diameter) is the thickened region of the tail between the head and main-piece of the tail. Individual mitochondria are wrapped spirally around the outer nine fibrils of the tail to form the mitochondrial sheath. The mitochondrial sheath is the "power" house (engine) for supplying energy to the spermatozoon. The middle-piece is more rigid than the main-piece and more

resistant to twisting. The middle-piece is more distinguishable when viewed with a phase contrast microscope instead of a bright-light microscope.

Main-piece of tail. The main-piece (26.6 ± 1.4 µm long and .65 ± .15 µm in diameter) is the longest part of the tail and very flexible. The main-piece contains fibrils that allow the spermatozoon to be motile. The coarse nine fibrils of the outer ring diminish in thickness and disappear leaving only the 11 inner fibrils (9 double inner fibrils + 2 central fibrils) in the axial core of much of the length of the main-piece. The main-piece fibrils are surrounded by a fibrous tail sheath.

End-piece of tail. The end-piece (2.5 ± .2 µm long) is not surrounded by a sheath and the outer nine fibrils are absent.

Take Home Message. Because sperm cells are very small, they need to be evaluated at a magnification of 400x.

Morphologically abnormal spermatozoa

Malformations in spermatozoa can originate in the testis or epididymis. Abnormalities in the shape and size of sperm heads can be: (1) of testicular origin as a result of alteration in the pattern of chromatin condensation, or (2) epididymal orign due to anomales in the maturation of the nucleus and the acrosome. Spermatozoa with abnormal tail shapes develop in the epididymal duct.[6] Abnormalities of sperm cells are generally classified into three categories, namely: head, middle-piece of tail, and main-piece of tail.

Abnormal Head. Some people refer to head abnormalities as cephalic (of or relating to the head) abnormalities. Spermatozoa head abnormalities include microcephalic (small) head; macrocephalic (large) head; short, broad, flattish head; elongated narrow head; rounded shape head; pyriform (pear-shaped) head; multiple heads; head detached from tail; and acrosome damage (Figure 5).[4,7-10] Acrosome damage has been classified as knobbed[9], loosened, swollen, crater defects, or lost (Figure 5). Generally, a boar stud does not evaluate acrosome damage while processing raw ejaculates.

Abnormal Middle-Piece of tail. Abnormalities of the middle-piece include extended middle-piece attachment to the head; swollen (broadened) middle-piece; narrow (filiform) middle-piece; double middle-piece; middle-piece bent in middle; middle-piece sharply bent at the neck region; curved middle-piece; broken middle-piece; middle-piece without a tail; and middle-piece containing cytoplasmic droplets (Figure 6).[5,11] Cytoplasmic droplets are classified as either proximal (droplet next to head) or distal (droplet at end of middle-piece or on main portion of tail). It is not uncommon to observe the middle-piece attached to the head at an off-center (abaxial or eccentric) location. The off-center attachment does not influence the ability of the sperm cell to fertilize eggs.

Abnormal Main-piece of Tail. Abnormalities of the main portion of the tail include sharply bent tail at distal end of middle-piece; multiple-coiling of tail under the head; coiling of tail around the head; single coil of tail under the head; "hairpin" tail with distal cytoplasmic

droplet; bent tail with distal cytoplasmic droplet, curved tail, broken tail, headless tail, multiple tails, and two tails fused together (Figure 7).[4,5,11,12]

Artifacts. Numerous artifacts (problem caused by technician error) can be the result of improper handling and processing of semen. Head without tail artifacts can be the result of excessive agitation and improper mounting on microscope slide. Coiled-tails artifacts can be caused by too rapid cooling, presence of water, urine in semen, and letting a wet mount dry without a fixative.

Agglutination or "Clumping". The head-to-head agglutinability of boar spermatozoa increases during their passage through the caput and proximal corpus epididymides, followed by a decline by the time of their arrival at the cauda epididymidis (Figure 8).[13] The cause of agglutination or "clumping" (Figure 9) observed at the time of processing an ejaculate is not well understood. If the number of cells in the clump are dead or cannot get loose from of the clump, the number of viable cells in the dose of semen will be reduced. Agglutination has been associated with bacterial contamination[14] incompatibility between two or more ejaculates (pooled semen), high ambient temperature, and failure to rotate doses during storage. It has been shown that boar semen contaminated with gentamicin-resistant bacteria will cause sperm agglutination.[15] Gentamicin-resistant bacteria associated with reduced sperm longevity in extended semen are *Acinetobacter spp., Aeromonas schubertii, Alcaligenes spp, Enterobacter cloacae, Escherichia coli, and Serratia marcescens*.

"Normal" rate of abnormalities. The percentage of morphologically normal sperm cells of most boars will be greater than 85%. It has been suggested that the following sperm abnormalities may be expected in boars of high fertility: abnormal acrosome - less than 5%; abnormal heads - less than 5%; proximal and distal cytoplasmic droplets - less than 5%; abnormal middle-piece of tail - less than 5%; head without tail - less than 2%; bent-tails - less than 5%, and tightly coiled tails - less than 5%.

Take Home Message. Boar semen needs to be evaluated for abnormalities of sperm cells. The evaluation should be at a magnification of 400x.

Physiology of a boar spermatozoon

Definition of Physiology: *A branch of biology that deals with the functions and activities of life or of living matter (as organs, tissues, or cells) and of the physical and chemical phenomena involved.* As this definition implies, the physiology involved with the development of a viable boar spermatozoon is highly complex. The development of a viable spermatozoon involves numerous morphological, biochemical and physiological changes as the sperm cell is "manufactured" in the testis and "undergoes maturation" while moving through the epididymis. This paper briefly discusses the initiation of motility in sperm cells and the use of boar semen extenders to keep the sperm cells viable.

Initiation of Motility. The corpus and cauda epididymis is a critical region for the development of sperm motility.[13] A French study removed spermatozoa from 10 regions of the epididymis and estimated percentage of mobile spermatozoa after a 10-minute incubation at

37 degrees C (98.6 degrees F). The pattern of spermatozoa motility showed qualitative changes between the epididymal regions (Figure 8). Spermatozoa taken from the posterior portion of caput only had their tails oscillated and less than 1% showed progressive movement. Spermatozoa taken from the corpus had an increase in the vibration of the tails and the sperm cells moved in the medium with a circular or irregular pattern of progression (head of sperm cell exhibited a rotary movement out of phase with the tail beat). Forward progression and straight-line movement of sperm cells appeared in the cauda epididymis (regions 8, 9, and 10). Spermatozoa are immotile during the time of storage in the cauda epididymis. Upon ejaculation, these sperm cells undergo a transition from the quiescent to the actively motile state. The initiation of motility of ejaculated spermatozoa involves complex biochemical factors that will not be presented.

Purpose of boar semen extender to keep sperm cells alive. When observing the powder of extender to be dissolved in distilled water, it appears that the composition of the extender is very simple. However, the chemical composition has to be carefully formulated to keep the sperm cells alive and viable. The basic role of semen extenders has not changed since their inception. Extenders are still used to increase the total volume of diluted sperm cells, provide an adequate supply of nutrients for sperm cell metabolism, provide an environment to protect the sperm cells against rapid cooling, provide buffers to protect sperm cells against extreme shifts in pH, provide electrolytes for proper osmotic pressure, and provide antibiotics to inhibit bacterial growth. Storage of sperm cells in seminal plasma by itself does not permit a long preservation of spermatozoa. Therefore, a suitable medium has to be added to the sperm in order to prolong their survival and to maintain their ability to fertilize ova.

Energy source. Spermatozoa primarily produce energy through glycolytic pathways occurring in the mitochondrial sheath of the midpiece of the sperm's tail. Thus, the midpiece is the power source for sperm motility. The primary source of energy in boar semen extenders is glucose. Although other energy sources (galactose, fructose, ribose, and trehalose) have been included in boar semen extenders, they have not shown any benefit over glucose.

Buffers. The hydrogen ion [H^+] concentration of a solution is known as pH. Many biochemical reactions must be carried out in a fairly narrow range of pH values. The pH of the sperm-rich fraction is 6.8 to 7.4 and 7.0 to 7.6 for post-sperm fraction.[16] Sperm cells and bacteria produce metabolic products such as lactic acid. As the concentration of lactic acid increases during storage of semen the pH value of the semen becomes lower over time. Thus, buffer ingredients are used to minimize fluctuations in pH due to metabolic products.

Electrolytes. Electrolytes (a nonmetallic substance which in solution becomes an ionic conductor) are used to regulate osmotic pressure. Bulk inorganic ions, potassium chloride and sodium chloride, are sometimes added to extenders to balance osmotic pressure. Osmotic flow is the passage of a solvent through a semipermeable membrane from a dilute solution into a more concentrated solution. Osmotic pressure is the force required to oppose the osmotic flow. The osmotic pressure of a solution is a function of the number of particles (ions or molecules) and is independent of the nature of the solutes. A hypertonic solution, one that has a greater osmotic pressure than the cellular (sperm cell) contents, will cause a loss of water from sperm cell and a corresponding shrinkage of the cell. Conversely, hypotonic solutions will produce a

flow of water into sperm cells and may cause them to burst. Isotonic solutions maintain the same osmotic pressure on both sides of the sperm cell membrane. The osmotic pressure of neat (raw) semen is 290 to 300 mOsm. Sperm motility appears to be similar when osmolality ranges from 210 to 290 mOsm.[17]

Antibiotics. It is almost impossible to collect boar semen ejaculates free of bacterial contamination. In addition, the temperature at which boar semen is normally stored (16 to 18 C) does not inhibit bacterial growth. Some of the metabolic products produced from bacteria in boar semen have detrimental effects on survival of spermatozoa.[18] There is not a single antibiotic that will control all bacterial growth in boar semen.

Membrane stabilizers. The sperm cell is enclosed in a plasma membrane. The plasma membrane is essentially a thin film of lipid and protein molecules assembled during spermatogenesis and modified during epididymal transit, storage, and ejaculation (7, 8). Special components have been added to boar semen extenders in an effort to prevent or retard unwanted alterations in the structure and function of plasma membranes.

Boar spermatozoa are sensitive to temperatures below room temperature. One of the purposes of a boar semen extender is to protect the spermatozoa from cold shock; thus, the current practice is to store extended liquid boar semen between 15 to 18 C.

Take Home Message. A suitable extender has to be added to the sperm in order to prolong their survival and to maintain their ability to fertilize ova. The longer semen is stored, the better the extenders should be!

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Figure 1. Drawing of a transverse section through a seminiferous tubule

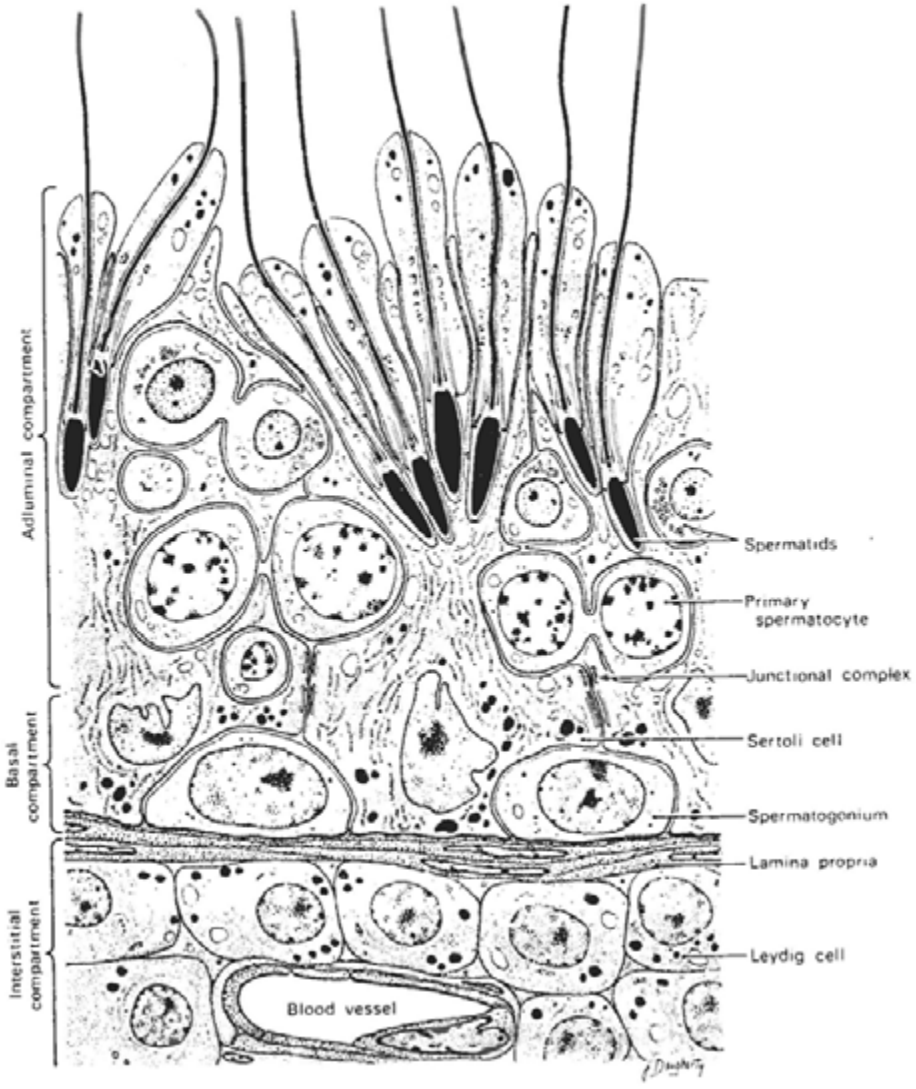


Figure 2. Schematic drawing of spermatogenesis in the boar

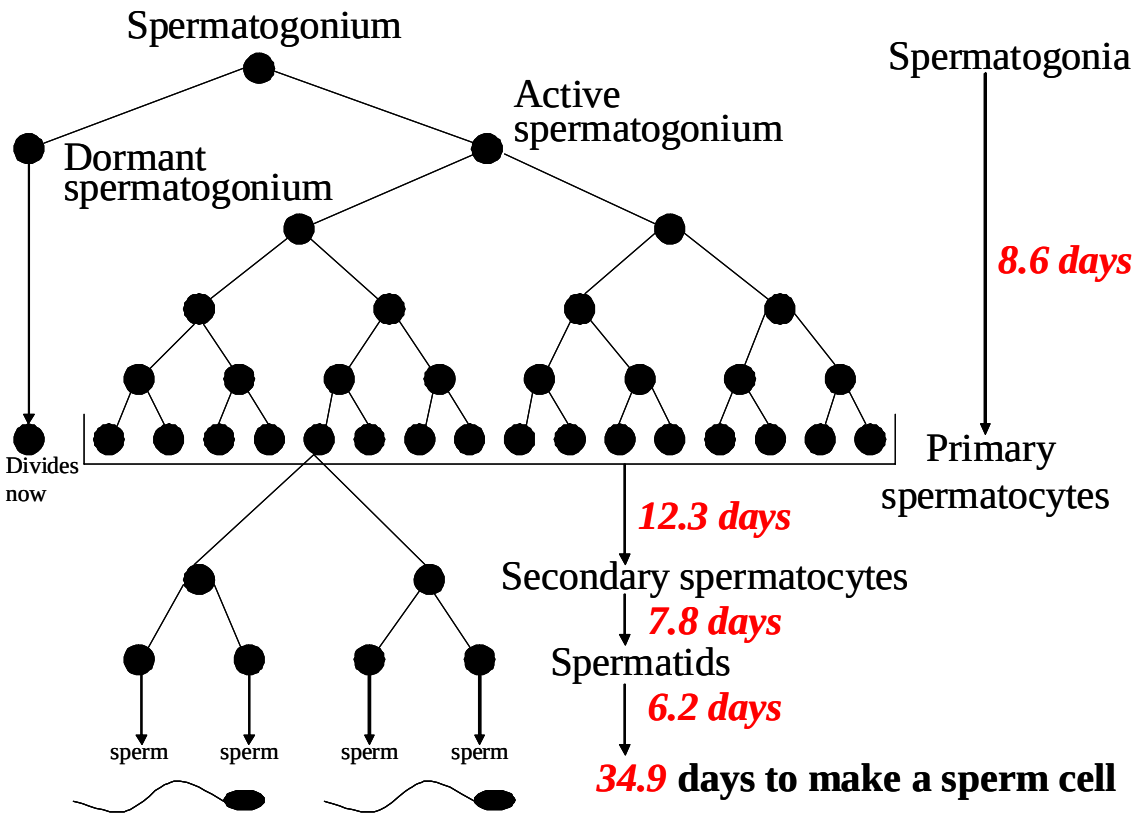


Figure 3. Photograph of testis and epididymis of a boar

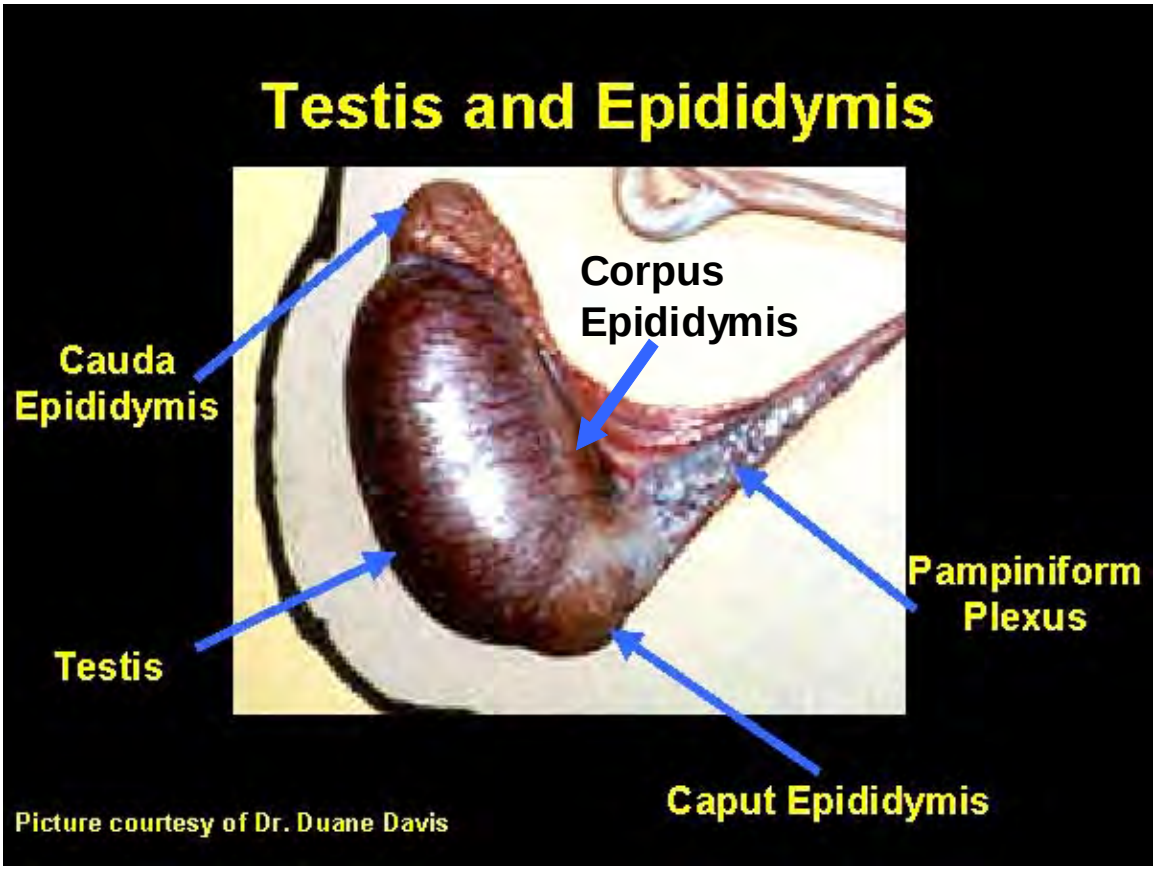


Figure 4. Schematic drawing of a boar's sperm cell

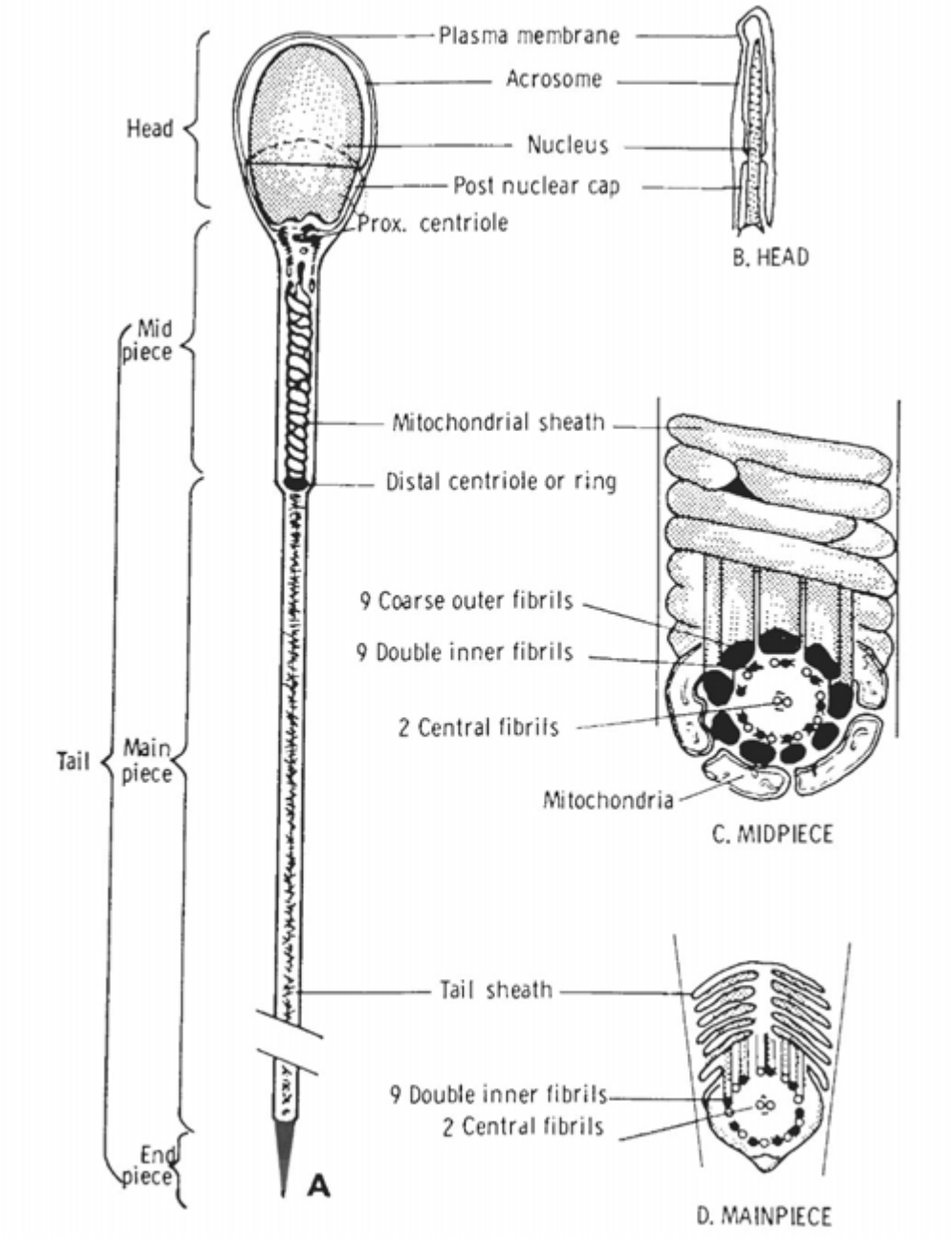


Figure 5. Abnormal heads

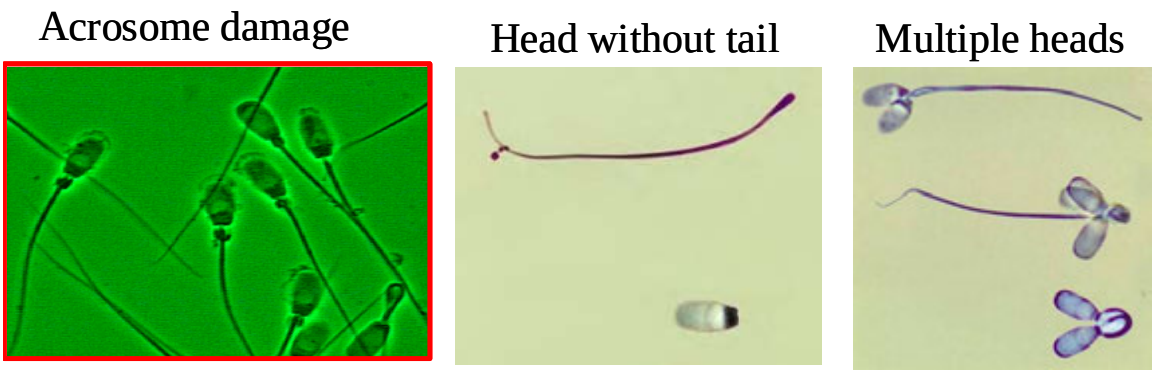


Figure 6. Sperm cells with cytoplasmic droplets

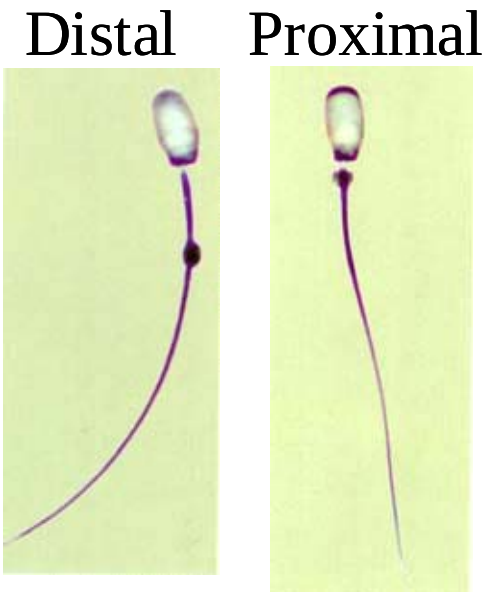
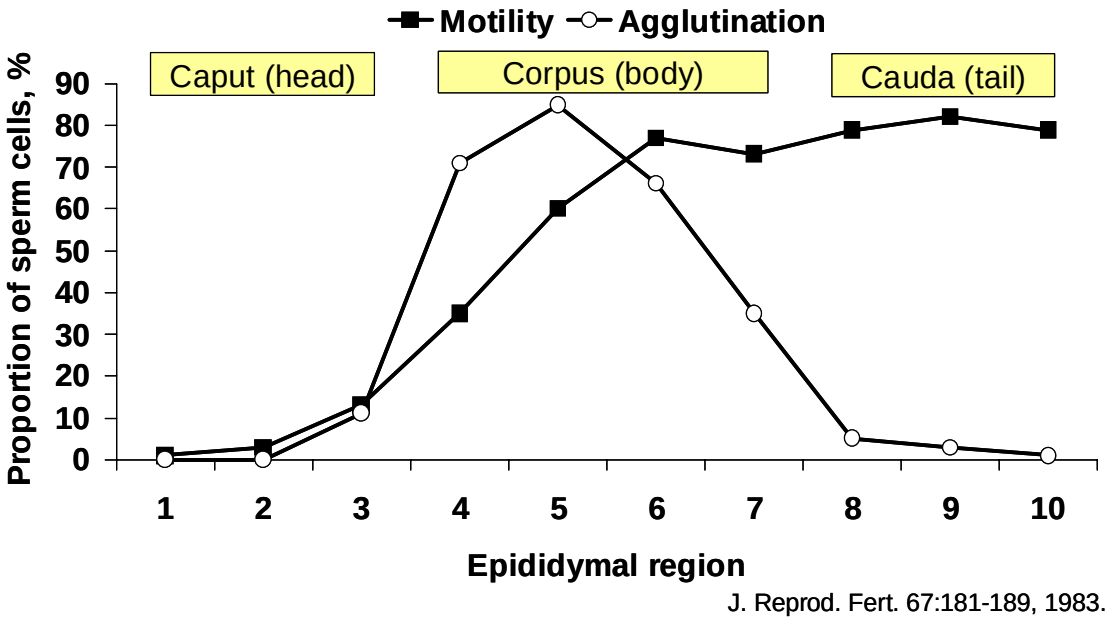


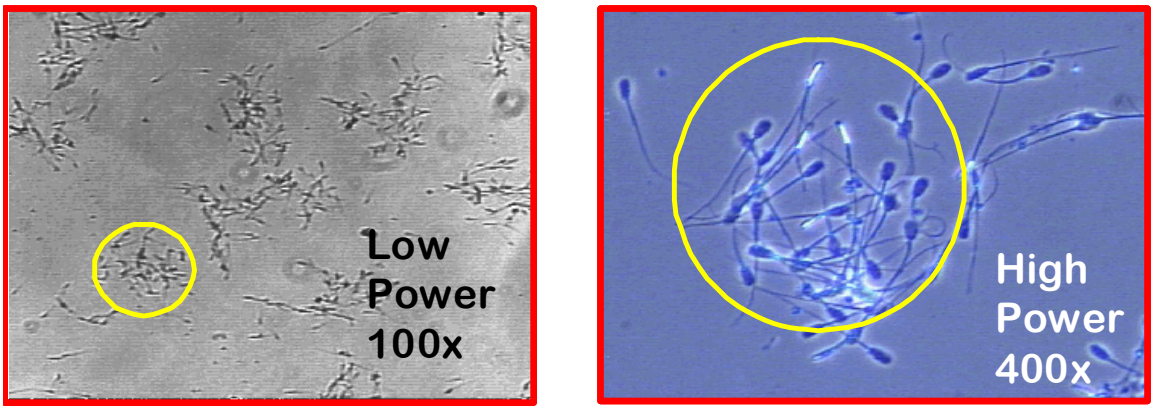
Figure 7. Abnormal tails (Photographs by Dr. Wayne Singleton, Purdue University)

Figure 8. Changes in sperm agglutination and motility by region of epididymis



J. Reprod. Fert. 67:181-189, 1983.

Figure 9. Agglutinated sperm cells



Hygiene and Contamination in the Lab

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Boar Stud Biosecurity

It seems prudent to begin a discussion about hygiene and laboratory contamination with a few words about biosecurity. When considering hygiene and external contamination of semen doses, we generally think of bacterial contamination, while biosecurity generally conjures thoughts of viral contamination of the boars themselves, which may result in subsequent contamination of semen doses. Only in recent years have sow farms begun to incorporate biosecurity protocols to protect themselves from potential contamination from outside sources. At the boar stud level, however, biosecurity has been second nature for many years. Boar stud managers should strive to create a culture within all employees that embraces biosecurity in such a way that it is taken into consideration for all processes and activities within and outside of the stud. With an average ratio of 150 boars on stud per sow in inventory, the potential impact of disease in a boar stud contaminating the sow units it supplies semen to is enormous, and the liability to the stud immense.

Biosecurity encompasses a large number of processes from rodent control and bird-proofing to traffic control and showering and changing clothing. Feed sources should be assessed to insure that no animal products are incorporated which could potentially contaminate the boars as well. Contaminants may enter the boar stud from outside sources via a number of routes, including laboratory supplies, personnel, boars, air, water, etc. Periodic audits of biosecurity practices and monitoring of feed should be routine in the management of the boar stud.

The National Pork Board has published a series of documents on biosecurity that can be accessed either directly from them at P.O. Box 9114, Des Moines, IA 50306, (515) 223-2600 or at www.porkboard.org (http://www.pork.org/Producers/Security.aspx?c=Security). Every boar stud manager should be familiar with the questions (and their answers) that are outlined for producers to ask their semen suppliers regarding the stud's biosecurity practices. These documents include a guide for general principals of biosecurity as well as a guide for pork producers in general.

The American Association of Swine Veterinarians (AASV) has published a document titled Health, Hygiene, and Sanitation Guidelines for Boar Studs Providing Semen to the Domestic Market.[7] This document contains a section on Hygiene and Sanitation Requirements for Semen Collection, Processing, and Storage and is available to all AASV members. Every boar stud's veterinarian should provide this document to the staff so they can be familiar with its principals.

Once biosecurity has become a culture with the employees of a stud, the risk of disease introduction to the stud should be reduced dramatically. It is critical to the success of the stud that this culture be cultivated in every new employee as quickly as possible. Having the entire team on board will expedite this process with new hires, provided the team works together to educate each new employee. The best biosecurity protocols are useless without monitoring and meaningful punishment for breaches. The manager's actions and attitude towards biosecurity will set the tone for the entire team.

Hygiene and Bacterial Contamination

Bacterial contamination of boar semen ejaculates begins with the boar and is often considered "normal." Neat semen can regularly be used as a positive control for bacteria cultures and bacterial contamination of extended, stored semen may reduce fertility in sows.[3,17] Indications of bacterial contamination of extended semen range from sperm cell agglutination and decreased storage life to reduced fertility and vaginal discharges[2]. In extreme cases with high levels of contamination, endometritis may occur in mated sows, which could result in sow

death. Other consequences of bacterial contamination include acrosome damage, which cannot be corrected once it has occurred, and pH change, which can be adjusted with the addition of fresh extender in most cases. Factors other than bacterial contamination, such as heat stress or fever, antibiotics and contamination from other sources such as residue on equipment or supplies, may also be responsible for these problems, so confirmation by culturing and microscopic examination is warranted to correct the problem.

By design, semen extenders contain ingredients that make them well suited as culture media for the bacteria that may be present in neat semen. Antibiotics are added to semen extenders to control bacteria growth and allow for longer storage times.[16] Cooling of semen to storage temperatures 16°C to 18°C also aids in suppression of bacterial growth. Breakthroughs in semen extender formulations that would allow storage closer to 0°C, or improvements in semen freezing technology could further prolong storage times. Microscopic examination of samples throughout the useful storage life of the semen will often reveal problems with bacterial contamination and should be carried out at least to the recommended maximum shelf life (or longer if a problem is suspected). Visual analysis of sperm cells, both fresh and stained, can be coupled with culturing of samples on agar plates with incubation for 48 hours to confirm contamination. Keep in mind that problems will often only be revealed after the semen in question has been used at the farm. When a problem is found in the lab, follow-up of fertility results with that pool or ejaculate should be done at all farms where that semen was sent.

Important considerations for choosing antibiotic(s) for a semen extender are spermicidal activity, cost, and effectiveness against (sensitivity test) and resistance of the target bacteria. The first step is to determine which bacteria are normally present and what antibiotics provide control. Table 1 lists antibiotic combinations and levels that are available from some of the semen extender suppliers in the U.S.A.

Table 1. Antibiotic combinations available in semen extenders in the U.S.A. by supplier.

Supplier	Antibiotic – Level per Liter
A	Ampicillin – (level not given)
A	Ceftiofur – (level not given)
A	Gentamycin – (level not given)
A	Neomycin – (level not given)
B	Apramycin – 250mg + Ampicillin – 250mg
B	Gentamycin – 250mg or 400mg
B	Gentamycin – 400mg + Ampicillin – 200mg
B	Gentamycin – 400mg + Ampicillin – 200mg + Cefoperazone – 50mg
B	Gentamycin – 400mg + Neomycin – 600mg
B	Gentamycin – 400mg + Spectinomycin – 270mg + Lincomycin – 120mg
B	Neomycin – 1,000mg
C	Ampicillin – 100 to 200mg
C	Ceftiofur – 50mg
C	Gentamycin – 250 to 500mg
C	Neomycin – 250 to 600mg
D	Amikacin – per customer request
D	Gentamycin – 250 to 400mg
D	Neomycin – 0.95g
D	Penicillin – 100,000 IU
E	Gentamycin – 200mg
E	Gentamycin + Amoxicillin + Tylosin
F	Colistine – 30mg + Neomycin – 80mg
F	Colistine – 30mg + Enrofloxacine – 30mg + Gentamycin – 60mg

F	Colistine – 30mg + Gentamycin – 60mg + Lincospectin – 30mg
F	Colistine – 33mg + Enrofloxacine – 33mg + Gentamycin – 83mg
F	Colistine – 30mg + Gentamycin – 60mg + Lincospectin – 30mg
F	Colistine – 60mg + Gentamycin – 60mg + Enrofloxacine – 30mg

Samples of extended semen should be routinely submitted for bacterial culture to an independent laboratory. This service is provided by many veterinary clinics, diagnostic laboratories and even artificial insemination (AI) supply companies. It is best to store samples at the boar stud in its controlled environment until the test is desired. Information desired would include types and relative levels of bacteria found and a list of antibiotics they are both sensitive and resistant to. This is an on-going process as the micro-flora of the boars can change over time. These processes are extremely important to provide a high quality and fertile product for the consumer as well as maintain their confidence that the boar stud is monitoring their own production. Customers should rely on the boar stud to provide the quality control (QC) for the semen they provide. Sow farms rarely have the equipment or expertise to properly evaluate semen they have received, so it is best to keep this process in the hands of the boar stud personnel. Placing an expiration date on semen does not necessarily result in proper semen usage and storage times and can result in shortages if doses are discarded prior to the next shipment arriving at the farm, particularly in the event of an unforeseen delay. Semen does not die on its appointed expiration date, but farm employees will perceive that to be the case if such a date exists. Teaching sow farm employees proper semen handling, storage and timeliness of use should be considered the responsibility of the boar stud that is supplying it.

Barn Hygiene

Routine cleaning of the boar barn should include daily scraping of manure from alleys and power washing and disinfecting of the semen collection areas. With the exception of the very largest boar studs that have multiple rooms, it is unlikely that most studs would replace boars on an all-in, all-out basis, making regular cleaning and disinfecting of an empty barn or room difficult. Power washing of the entire barn should be done on a routine basis, taking ambient temperatures and boar comfort into consideration. When boars are present during washing, supplemental heat and increased air movement may be required to dry them and the barn quickly, and care must be taken to not spray boars directly with the power washer. Considerable variation exists in the ability to remove contaminants with a power washer, depending on the type of surface being cleaned.[4] Surfaces that are rough will give the boars more traction and potentially result in fewer injuries due to slipping, but may result in more residual bacteria following cleaning.[12] Therefore, a compromise must be reached between abrasiveness for traction and cleaning ability. Slat quality in the boar stalls and pens and in warm-up pens and alleys is particularly important to insure injury is not caused in normal day to day housing and moving within the boar stud. A thick rubber mat (1 cm or more) with large (2.5 to 3 cm diameter) holes is always recommended for the semen collection pen. The mat must be removable to facilitate complete washing and disinfecting on a daily basis. Rotation of disinfectants is recommended only after thorough testing to insure that resistant bacteria are not present.

There is limited information regarding the presence of bacteria in showers at swine barns, but evidence reported to date suggests that the risk of such contamination is low, despite the fact that most farm showers are not visibly kept clean.[5] Simple hand washing has been demonstrated to be linked with a reduction in *Salmonella* prevalence on swine farms.[18] This suggests the minimum requirements of showering into the barn and wearing unit clothing should prevent most contamination problems that could be potentially introduced by employees. Other

biosecurity practices that should be followed are observing minimum "down" times between exposure with other swine and entry into the boar stud, off-site isolation with daily chores performed by non-stud personnel, washing of vehicles before returning to the boar stud after a visit to another swine operation (particularly for semen delivery vehicles) and fogging of packages with disinfectant prior to their entry in to the unit. As a practical matter, personnel with active influenza infections should be discouraged from reporting for work, both to protect the boars from possible cross-contamination (low risk) and to prevent infection of other workers at the unit (high risk).

Due to the nature of semen collection by the gloved hand technique and the source from which we obtain it, we should not expect an ejaculate to arrive in the laboratory completely free of bacteria. Under normal circumstances, and provided good hygiene practices are followed, the limited numbers of bacteria that are typically present should not pose a significant problem with semen storage and fertility. If, however, the bacteria level is significant and/or there are bacteria present that are resistant to the antibiotic(s) in the semen extender used, semen quality can deteriorate rapidly in storage and/or fertility may be compromised. Often, this is manifested by a discharge in the sows approximately three weeks post mating.

There are a number of ways to minimize the bacterial load in semen collections. The first is to maintain a high level of hygiene in the barn, including daily power washing and disinfection of the warm-up pens, semen collection area and dummy sows. With the proper type of flooring and ventilation, the warm-up and semen collection areas should dry between periods of use. The dummy sow should not be covered with any type of carpet or material which can easily become contaminated with bacteria, which can be present on the skin, hair and fecal material of the boar. Plain metal or plastic coated dummy sows are preferred for ease of cleaning and disinfecting. Periodic cleaning between collections can also help reduce the environmental load of bacteria present in the semen collection area.

The second step is to keep all semen collection related materials as clean as possible prior to use. Reusable equipment should be avoided when possible. Single use collection bags or insulated containers that can be prepared ahead of time and kept in clean plastic bags until immediately prior to use are preferred. The new automated semen collection systems that take the place of the technician in the actual semen collection process use disposable products that can be kept clean and sterile prior to use.

The third step is to use clean collection gloves for each boar. A second plastic glove over the collection glove should be worn until the penis is actually exposed and the boar is ready to fully extend. Many boars will attempt several mounts and extensions (full or partial) prior to their final extension and ejaculation. This may necessitate the wearing of two or more collection gloves, plus a plastic glove over these. Keeping additional gloves in a pocket to put on if the collection glove gets contaminated should be encouraged. However, you must keep in mind that the pockets of the technician's clothes are not sterile, or even clean in some cases, but these extra gloves are a good alternative to using a glove contaminated by contact with the boar. Some technicians also have a habit of placing a hand on the back of the boar as he mounts and extends to help balance or support himself and/or the boar. This may result in direct contamination of the collection glove and should be avoided unless the plastic glove worn over the collection glove is kept on during this process.

The fourth step involves the semen collection process itself. Two of the four ejaculate fractions have been shown to have a higher level of bacterial contamination than the other two, and also contain very few sperm cells. The first fraction, or pre-sperm portion, and the fourth, or gel plug, fraction may contain higher levels of bacteria and do not normally contain any appreciable number of sperm cells and, therefore, should not be collected into the semen collection container.[10] It should be relatively easy for the technician to distinguish the pre-sperm fraction, as it is clear and ends with the first jets of the darker, milky sperm-rich fraction (the first

fraction to save). The fourth or gel fraction is normally accompanied by pellets of sticky "gel" that make it easily distinguishable from the sperm rich and seminal fluid fractions that are to be saved. One disadvantage of the automated semen collection equipment is that this gel fraction cannot be diverted from the semen collection container, although it will be caught in the filter. Preputial fluids have been shown to be a major contributor to bacterial contamination of ejaculates in the boar.[17] Surgical removal of the preputial diverticulum is an effective method of eliminating this source of contamination.[1] While this intervention is not practical in most studs, there may be a limited number of applications where it could be useful.

The final step in the barn is to remove the collection filter prior to transport of the ejaculate into the lab. This step reduces the bacterial load in the lab, not the barn, but is no less important than the previous steps listed above in overall hygiene of the stud.

Having sterile, disposable products available does not guarantee they will be sterile at the time of use. If the bag of collection supplies is left open or a technician uses a dirty hand to remove the items from the bag, bacterial contamination can compromise the entire bag's contents. Placing a sterile collection container on the dirty floor, or even placing it in a pocket or under an arm can also result in contamination. An open box of collection gloves kept in the barn will become contaminated with dust and bacteria circulating in the air and should therefore be kept in a closed plastic bag. The goal is not to make the process so cumbersome that the technician cannot perform his or her duties, but to provide the best practices possible that result in the least amount of bacterial exposure during these processes.

Lab Hygiene

Everything that enters the laboratory, from water and air to people and ejaculates, has the potential to introduce bacterial contamination. The ideal semen processing laboratory is physically separated from the barn with independent showers, break rooms, etc. If it is not possible to provide showers into the lab, a minimum of a change of clothing and shoes and washing of hands should be required. In smaller units in which barn personnel may also help in the lab, they should change clothing and wash hands prior to entering the lab if it is not possible for them to shower between duties. Unfortunately, having showers does not guarantee every employee will take a complete shower every time they enter the unit. Developing a "culture" among all employees where taking a shower or changing clothing and washing hands (depending on the facilities) is considered "normal" should be the goal of every boar stud manager. For all hand washing in the unit, the type of soap used is not as important as the washing of hands itself. Antibacterial soap does not remove more bacteria than ordinary soap when hands are washed for 20 seconds, although E2 rated soaps (those containing 50 parts per million chlorine) are significantly more effective in removing bacteria than either antibacterial or ordinary soap. Instant hand sanitizers are not as effective in removing bacteria as washing with soap and water.[14]

For cleaning laboratory surfaces, just as with washing hands, cleaning appears to be more important than "disinfecting". In 1995, the disinfection protocols from a hospital were compared to cleaning (without disinfection) with a commercial cleaning solution at a large boar stud. The hospital protocol incorporated wiping all surfaces with a solution of 10% bleach (5.25% sodium hypochlorite) in water (final solution of 0.525% sodium hypochlorite), which was compared to using only a commercially available detergent cleaning solution and disposable towels before and after daily work. In all tests run at the boar stud, there were fewer bacterial colonies grown from swabs of counters using the commercially available detergent compared to those wiped with the bleach solution (Gall, T.J., unpublished data). Chlorine bleach solutions are routinely used in other process for disinfection, such as for disinfecting carcasses and killing mold, but are not effective as cleaning agents for removal of organic matter or dirt.[8,15] When selecting cleaning and disinfecting agents for the boar stud, first you must decide whether you want a

cleaning agent alone, a combination cleaning and disinfecting solution, or a disinfecting solution alone. The label of each agent should clearly state which type it is and all disinfectants must be registered with the Environmental Protection Agency (EPA) and are required to submit to the EPA proof that they kill the organisms that they claim to control. The label should also state which types of surfaces the product is safe to use on. Ten per cent bleach solution was once considered the "Gold Standard" for disinfection in hospitals, but many hospitals, as well as food service and medical care facilities have switched from bleach to quaternary ammonia chloride solutions (Quats) for disinfection of surfaces. Quats provide broad spectrum bacteria, virus and mold control, are less corrosive than bleach and have some cleaning properties that bleach solutions do not provide.[13] The active ingredient in Quats is benzalkonum chloride which can be formulated with a variety of ingredients to provide a neutral pH disinfectant. Since Quats provide both cleaning and disinfectant properties, they provide an economical one-step process in the lab. There are several other types of disinfectants that may be considered for use in the lab. One is phenols, which are flammable and corrosive, can leave a residue that eventually has to be removed and must be mixed up fresh every day. Another type is iodine solution (iodophors) which are slow to kill organisms, making exposure time critical. They are also corrosive to metal and rubber and stain many types of surfaces. Iodophors must be mixed fresh daily and may be inactivated by hard water. Ethyl alcohol diluted to 70% (with water) is effective against most organisms, but is flammable and requires that exposure time be monitored[9]. Ethanol is very effective as a disinfectant for skin and can be used on hands prior to handling clean supplies and equipment. The importance of cleaning organic material to achieve disinfection has been shown for similar surfaces in the swine industry, such as rubber boots.[6] For washing lab surfaces, disposable cleaning cloths are ideal, but reusable clothes that are washed daily are an acceptable alternative. Sponges should be avoided for cleaning, unless they are replaced regularly (daily, or at least weekly). Ultraviolet light (253.7nm wavelength) is effective against airborne and surface organisms but will not penetrate solid materials. Bulbs must be checked for intensity and kept clean to be effective and should not be turned on while people are present in the lab.

Protocols for minimizing contamination within the lab begin with thorough cleaning on a regular basis. Surfaces that are used routinely should be cleaned and disinfected frequently throughout the processing period. Technicians should wash hands frequently and use disposable towels as much as possible. All surfaces that are touched need frequent cleaning, including telephones, the computer keyboard and mouse, doorknobs, drawer pulls, etc. A plastic cover can be ordered for the most popular computer keyboards which allows for effective cleaning as well as unimpeded operation. Plastic kitchen food wrap makes an ideal cover for the mouse and telephone, and can be replaced easily and frequently at low cost. Cell phones should not be allowed past the dirty side of the showers at any time, since they cannot be disinfected.

Water is the single largest component of semen extender and is used for routine cleaning and rinsing of lab equipment. Regardless of the source, water should be periodically tested for contaminants. Low cost in-line ultraviolet light systems are available that will kill bacteria present in water used in the lab.

As in the barn, use of disposables in the lab is preferred to using equipment that must be washed and sterilized. Just as with supplies in the barn, you cannot assume that disposable items taken from an open bag are sterile, so following best hygiene practices will help minimize contamination. Thoroughly washing hands before handling supplies is the best way to decrease cross contamination. Once containers of bulk supplies are opened, their contents should be placed in re-sealable bags or boxes. Bulk semen extender should be repackaged in individual aliquots for use when the bulk package is initially opened to prevent repeated opening and possible contamination of the extender. Semen extenders should only be purchased from

reputable suppliers who can verify QC processes and routinely perform them on their ingredients.

Dr. Chris Kuster has defined three discrete levels of risk associated with bacterial contamination of extended boar semen.[11] Risk Level I is the lowest and most desired level and is characterized by no significant growth of bacteria from extended semen samples after 48 hours of aerobic incubation on agar. Level II is defined as medium risk with culture of non-spermicidal bacteria that do not affect shelf life of the sperm cells. Level II indicates a breakdown in hygiene in the lab and/or barn which needs to be addressed immediately. Level III is the highest risk level and is characterized by the presence of spermicidal bacteria at a significant level. Intervention should involve the unit's veterinarian to determine the source and most expedient means of correcting the problem. Level III may also require a temporary change in extender antibiotic until the problem is corrected.

Conclusion

Biosecurity and hygiene in the boar stud include processes and mindsets that are best served by developing a culture within the employees that support them. Providing written protocols will enhance training of new employees and serve as a guide in the event of a contamination problem for addressing it. Having the boar stud certified by the International Organization of Standards (ISO) will require the documentation of every process in the unit, resulting in detailed protocols, and offer an avenue for tracking down problems that have occurred. Although ISO certification can be an expensive process, going though the steps to be certified without actually completing the certification itself can be a great place to start. Finally, boar studs that rely on the regular use of outside consultants, veterinarians and laboratories for input on QC and processes will likely be more successful in their endeavors than those that do not.

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NUTRITIONAL REQUIREMENTS AND RESPONSES OF THE BREEDING BOAR

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Breeding herd efficiency is influenced by the performance of both the sow and the boar. The sow needs to produce an adequate number of fertile ova and maintain the correct uterine environment for both embryonic and foetal development, whereas the boar must provide a sufficient number of fertile spermatozoa with a high fertilisation capacity. Maintaining the fertility of the boar is therefore of major consequence for the efficiency of reproduction on many farms. This paper considers the major nutritional factors influencing boar fertility.

THE REARING PERIOD

If boars are to be reared for breeding purposes, their physical soundness and future reproductive performance are as important as good growth rate. Young boars are normally selected according to an index that includes characteristics such as growth rate, appetite, feed conversion efficiency, lean tissue growth rate, carcass quality and breeding potential. They are normally fed to appetite and it is assumed that this does not prejudice subsequent reproductive capacity. It may however affect their physical ability to perform, since the tendency to leg weakness may be exacerbated by high rates of growth and by feeding to appetite (Grondalen, 1974; Hassen and Grondalen, 1979; Kesel *et al.*, 1983). Penny and Guise (1989) reported that the annual culling rate of boars in commercial herds is 40 to 60%, with the primary reason being excessive weight gain and animals becoming too large and too lethargic.

Evidence suggests that nutrition during the rearing period may influence both the age at puberty and the rate of sexual development in young boars. However, other than the obvious effect on the growth and mature body size of the animal and unless severely undernourished, this does not appear to impose any lasting damaging effect upon reproductive capacity (Close and Roberts, 1991). In practice, most young boars are offered feed close to their appetite potential during rearing and at this level any effects upon sexual development or subsequent reproductive capacity are unlikely.

NUTRITION OF THE BREEDING BOAR

Energy and amino acid requirements

The feeding of the young boar after arrival on farm is often a contentious issue, but consideration has to be given to the animal's body weight, its rate of growth, level of use, body condition, as well as the environmental circumstances under which it is kept. For example, excessive growth and over-fatness may affect its physical ability to perform and may exacerbate the tendency to leg weakness in later life. Underfeeding, on the other hand, not only affects growth rate and body condition, but also the quality and quantity of the sperm produced.

It seems prudent to allow boars to make some positive growth during their working life. However, this should be controlled, with young boars having higher growth rates than older animals. Modern boars have been bred for high rates of lean tissue growth and hence they may have less backfat cover. Thus, their temperature requirements may be higher than those of pregnant sows of similar body weight.

The energy requirements of breeding boars can be calculated according to the factorial procedure which takes account of the body weight of the animal, that is its maintenance energy needs, the rate of growth required, the energy expended in the activity associated with mating *per se* and the energy cost of semen production. Consideration must also be given to the environmental conditions when animals are kept below their lower critical temperature (LCT) of 20°C.

The energy requirements of working boars of different body weights and rates of gain calculated according to the above procedure are presented in Table 1. The requirements increase from 6.6 Mcal ME/day for a boar of 100 kg body weight, gaining 0.5 kg/day to 9.4 Mcal ME/day for a boar at its mature body weight of 350 kg. These values compare with those calculated by Kemp (1989): 8.2 and 8.8 Mcal ME/day for animals of 150 and 350 kg body weight, respectively,

Table 1. Factorial estimates of the daily energy requirements of breeding boars (Close and Roberts, 1991)

Body weight (kg)	100	150	200	250	300	350
Growth rate (kg/d)	0.50	0.40	0.30	0.20	0.10	-
Protein gain (g/d)	80	64	48	32	16	-
Fat gain (g/d)	125	100	75	50	25	-
	Requirements (Mcal ME/day)					
Maintenance[a]	3.87	5.07	6.14	7.12	8.03	8.90
Protein deposition[b]	0.84	0.66	0.51	0.34	0.17	-
Fat deposition[c]	1.60	1.28	0.96	0.64	0.32	-
Mating activity[d]	0.14	0.18	0.23	0.27	0.31	0.35
Semen production[e]	0.10	0.10	0.10	0.10	0.10	0.10
Total ME (Mcal/day)	6.6	7.3	7.9	8.5	8.9	9.4
Total feed[f] requirements (kg/d)	2.2	2.45	2.65	2.85	3.0	3.1

[a] $0.181\ W^{0.665}$, where W is the body weight (kg)
[b] (Protein gain x 0.0057)÷0.54
[c] (Fat gain x 0.0095)÷0.74
[d] $0.0043W^{0.75}$, where W is the body weight (kg)
[e] 0.62÷0.6
[f] Based on diet containing 3.0 Mcal ME/kg

The major energy cost is that associated with the maintenance of the animal, representing between 60 and 95% of the total intake, whereas that associated with mating activity and semen production is small and represents no more than 5% of the total. If a diet containing 3.0 Mcal ME/kg is fed, then these calculations suggest that the feeding level of the breeding boar should be increased from 2.2 kg/day at 100 kg body weight to 3.1 kg/day at 350 kg body weight. These compare with estimates of between 2.7 and 3.1 kg/day determined by Kemp (1989).

There have been few experiments to assess the requirements for protein or for specific amino acids in the growth and development of the breeding boar. Most studies have been designed to investigate aspects of semen characteristics and reproductive performance. Indeed, sexually active boars do not seem to have any increased amino acid needs. From a review of the literature, Close and Roberts (1991) concluded that dietary supplementation with additional lysine or methionine had little effect on semen production, unless boars were severely over-used.

These results suggest that the protein and amino acid needs of the developing and working boar are small in relation to the growing animal. A protein intake of ≈260 g/day and with an amino acid pattern similar to that of the pregnant sow (AFRC, 1990; NRC, 1998) will meet the daily requirements of the working boar. A diet containing 140-160 g CP/kg and 6-8 g lysine/kg should suffice. If boar usage is extreme, that is 3-4 times per week, then these values need to be increased. Dietary lysine levels below 5 g lysine/kg reduce growth rate and delay sexual maturity. Similarly, feed levels below 2.0 kg/day must be avoided, as they have been shown to reduce the number of sperm cells.

Plane of nutrition, and hence variations in both energy and amino acid supply, affects semen quality, especially in young boars. Kemp *et al.* (1989) fed boars at three different feed levels and noted that changes in semen production were not evident before 6 weeks of treatment. However, significant changes were noted between 8 and 12 weeks, with semen production decreasing as feeding level changed from high (17.7 Mcal ME/day) to medium (11.2 Mcal ME/day) and from medium to low (6.0 Mcal ME/day).

Louis *et al.* (1994 a,b) investigated the effects of different intakes of energy, protein and amino acids on growth, libido and semen characteristics of young boars (Table 2). At similar levels of protein or lysine intake, a reduction in energy intake resulted in reduced volumes of ejaculate and overall sperm output. However, when protein or lysine intake was also reduced, the reduction in the volume of ejaculate and sperm output was even more marked. There was also a significant reduction in the gelatinous fraction of the ejaculate.

Table 2. The effect of energy and protein intake on the growth, libido and semen characteristics of boars (weeks 8-27 of trial). From Louis *et al.*, 1994b)

Energy intake (Mcal ME/day)	8.4	6.1	6.1
Protein intake (g/day)	363	356	188
Lysine intake (g/day)	18.1	18.1	7.7
Growth			
Rate of gain (g/day)	373	169	92
Change in P_2 (mm) (0-27 weeks)	4.8	-0.8	-0.6
Boars' libido			
Time to ejaculation (sec)	145	114	149
Duration of ejaculation (sec)	417	396	306
Semen characteristics			
Sperm motility (%)	79.9	79.2	82.7
Volume of ejaculate (ml)	331	295	184
Gelatinous fraction (ml/ejaculate)	63.9	59.6	37.0
Sperm concentration (sperm no x 10^6/ml)	286	284	380
Sperm output (sperm no x 10^6/ml)	73	66	53

Fats and fatty acids

Although fats are major providers of energy to the boar, specific lipid components, especially long-chain polyunsaturated fatty acids (PUFA), are involved in the process of spermatogenesis and other functions. Indeed, their levels in the lipids of the spermatozoa are extremely high and show distinct characteristics. Boar spermatozoa contain high levels of 22:6 n-3 (docosahexaenoic acid, DHA) and 22.5 n-6 (docosapentaenoic acid, DPA).

The proportion of 22:6 n-3 and 22:5 n-6 is inversely related and a reduction in the proportion of DHA and increase in that of DPA within the sperm are correlated with a reduction in fertilisation capacity (Penny *et al.*, 2000). Supplementation with tuna oil, which is rich in DHA, has resulted in positive effects on sperm quality and boar fertility (Penny *et al.*, 2000; Rooke *et al.*, 2001) (Table 3). The concentration of viable sperm per ml ejaculate was increased, as was the motility and proportion of sperm with normal acrosomes. In the study reported by Penny *et al.* (2000) this was reflected in an increase in the number of piglets born alive per 100 matings.

Adding high levels of polyunsaturated fatty acids will result in higher rates of lipid peroxidation in boar semen (Surai, 2002). Thus, antioxidants need to be provided and, as indicated in Table 3, additional vitamin E and selenium need to be added to the diet (Table 3).

Table 3. Response of working boars to dietary supplementation with tuna orbital oil

Study	Penny *et al.* (2000)		Rooke *et al.* (2001)	
Diet	**Control**	**Tuna oil[1]**	**Control**	**Tuna oil[2]**
Sperm concentration (10^8/ml)	5.0	5.8***	2.0	4.1^{NS}
Sperm viability (% live)	78	88*	71	80**
Motile sperm (%)			72	77**
Normal acrosome			45	54***
Piglets born alive per 100 matings	846	954		

1) Diet supplement with tuna orbital oil, vitamin E and selenium
2) Diet supplemented with 30 g tuna orbital oil/kg
Significance: * $p<0.05$ ** $p<0.01$ *** $p<0.001$

Dietary fibre

During rearing, boars are fed at a level of 0.75 to 1.00 of appetite, but this is reduced later in life, so that feed intake may be no higher than that required to maintain the animal in energy equilibrium. However, restriction in feed intake may result in hunger and reduction in feeding behaviour and this may enhance frustrated feeding motivation and stereotypic behaviour (Lawrence *et al.*, 1988). Increasing the fibre content or bulkiness of the ration has been shown to improve welfare, decrease stereotypic behaviour and reduce hunger on restricted feeding regimens (Appleby and Lawrence, 1987; Brouns *et al.*, 1997; Meunier-Salaün, 1999) and may therefore have application in the feeding of boars.

There may also be a positive health and welfare advantages associated with the feeding of fibre. Working boars are kept for a longer period of time than animals reared for meat production and they are more likely to develop health problems associated with chronic nutritional inadequacies. For example, lesions of the *pars oesophagae* are common in cereal-fed animals, resulting in reduced feed intake and poorer overall performance. Indeed, about 5% of boars are culled because of ulceration of the stomach (Penny and Guise, 1989). Increasing the fibre content of the diet has been shown to reduce the severity and incidence of such lesions (Potkins *et al.*, 1984). Adding fibre to boar diets may also improve their ability to thermoregulate under cold conditions because of the high heat increment associated with fermentation in the large intestine (Close, 1987). There is also evidence to suggest that fibre may play a role in steroidogenesis of the boar (Ruoff and Dzuik, 1994).

MINERAL AND VITAMIN REQUIREMENTS

Mineral and vitamin allowances for the breeding boar are presented in Table 4. These are broadly similar to those of the sow, but there are several that are of specific relevance to the boar and hence require comment.

Table 4. Suggested mineral and vitamin allowances for the breeding boar (per kg diet) (from Close and Cole, 2000)

Minerals:		
Calcium	7.5 - 9.0	g
Phosphorus (total)	6.0 – 7.5	g
Phosphorus (available)	3.5 – 4.5	g
Sodium	1.5	g
Chloride	1.5	g
Salt	3 – 4	g
Potassium	2.5	g
Magnesium	400	mg
Copper	5	mg
Iodine	0.5	mg
Iron	100	mg
Manganese	20	mg
Zinc	100	mg
Cobalt	0.1	mg
Selenium	0.3-0.5	mg
Chromium	200	ppb
Vitamins:		
Vitamin A	6,000	IU
Vitamin D3	500	IU
Vitamin E	50-100	IU
Vitamin K	1	mg
Thiamine (B1)	1.5	mg
Riboflavin (B2)	4	mg
Niacin	15	mg
Pantothenic acid	15	mg
Pyridoxine (B6)	2.0	mg
Cyanocobalamin (B12)	0.020	mg
Biotin *	0.3 - 1.0	mg
Folic Acid	1.3	mg
Choline	1.5	g
Ascorbic Acid (C)	500	mg/day
Essential fatty acids:		
Linoleic acid	7	g
Arachidonic acid	5	g

** Higher level with persistent leg problems*

Calcium and Phosphorus

Since leg problems are a common affliction of too many boars, it is recommended that dietary allowances for calcium and phosphorus should be 9.5 and 7.5 g/kg for young developing boars and 7.0 and 6.0 g/kg for mature boars, respectively. The addition of a phytase enzyme to the diet allows the phosphorus levels to be reduced.

Zinc

Zinc is essential for spermatogenesis. The Leydig cells in the testis are responsible for testosterone production. In zinc deficiency, the Leydig cells are abnormal, with a concomitant loss of epithelial tissue in the seminiferous tubules in the testes.

Zinc is also important in tissue involving keratin and it is essential for adequate leg and foot health. Foot and leg problems are a frequent cause of premature culling of boars. A dietary requirement of 100 mg/kg is recommended. Chelated or proteinated sources appear more effective than inorganic sources at meeting the animal's needs because of their higher bio-availability (Close, 2002).

Selenium (Se) and vitamin E

Both act as anti-oxidants and have a direct effect on sperm quality by protecting them from oxidative damage.

Vitamin E deficiency causes testicular degeneration that affects the sperm cell within the testicular parenchyma. Sperm structural damage occurs when diets do not contain sufficient vitamin E. The benefits of vitamin E are within or on the surface of the spermatozoa and not in the seminal plasma. Supplemental vitamin E has been reported to increase the concentration of sperm in the boar ejaculate. However, vitamin E has no effect on sperm cell structural abnormalities, but serves as an anti-oxidant on the sperm cell.

Se deficiency, on the other hand, affects the integrity and morphology of the sperm tail, and hence motility, as well as the number of maturing spermatids and testicular sperm reserves. Thus, both selenium and vitamin E affect sperm quality and hence fertilisation rate. However, selenium appears to play a greater role than vitamin E in that it is essential for sperm development and maturity.

Several studies have demonstrated the importance of dietary selenium for boar fertility. Marin-Guzman *et al*., (1997) fed boars a basal diet supplemented with either 0.5 ppm Se or 220 IU vitamin E/kg. Semen from adult boars fed the Se-supplemented diet produced greater volumes and concentrations of sperm which had higher motility than from boars fed the vitamin E supplemented diet. There was no decline in sperm motility in the 16-week period of the trial and there were fewer abnormal sperm. In addition, sperm from the boars fed the Se-supplemented diet had higher fertilisation capacity which resulted in the fertilisation rate of inseminated gilts also being higher (Table 5).

In subsequent studies (Marin-Guzman *et al.*, 2000a,b) evaluated boars at different ages and body weights, when fed diets containing 0 or 0.5 ppm Se and 0 or 220 IU vitamin E/kg (Table 6). At 18 months of age, the Se-fed boars had higher numbers of sperm reserves, whereas vitamin E had no effect on testicular sperm reserves. The plasma membrane connection to the tail piece was not as tightly bound in those boars fed diets without added selenium than in those that were fed Se-supplemented diets. This affects motility.

The concentration of ATP in the spermatozoa of the boars fed 0.5 ppm Se was 25% higher than that of boars fed 0 ppm Se, and indeed, higher than when vitamin E was provided (Table 6). The higher metabolic activity of the sperm in the ejaculate increased motility and enhanced fertilisation rate (Table 5).

Table 5. The effect of dietary Se and vitamin E on boar fertility *(Marin-Guzman et al., 1997)*

	Selenium (ppm)		***Vitamin E (IU/kg)***	
	0	***0.5***	***0***	***220***
Semen				
Volume (ml)	158	213	175	195
Concentration (no. x 10^6/ml)	807	946	965	788
Sperm motility (%)	60	88	72	76
Normal sperm (%)	24	62	41	45
Fertilisation				
Fertilisation rate (%)	73	98	89	83
Accessory sperm	14	60	36	38

Table 6. The effect of age and dietary Se and vitamin E on testicular reserves of boars *(Marin-Guzman et al., 2000a,b)*

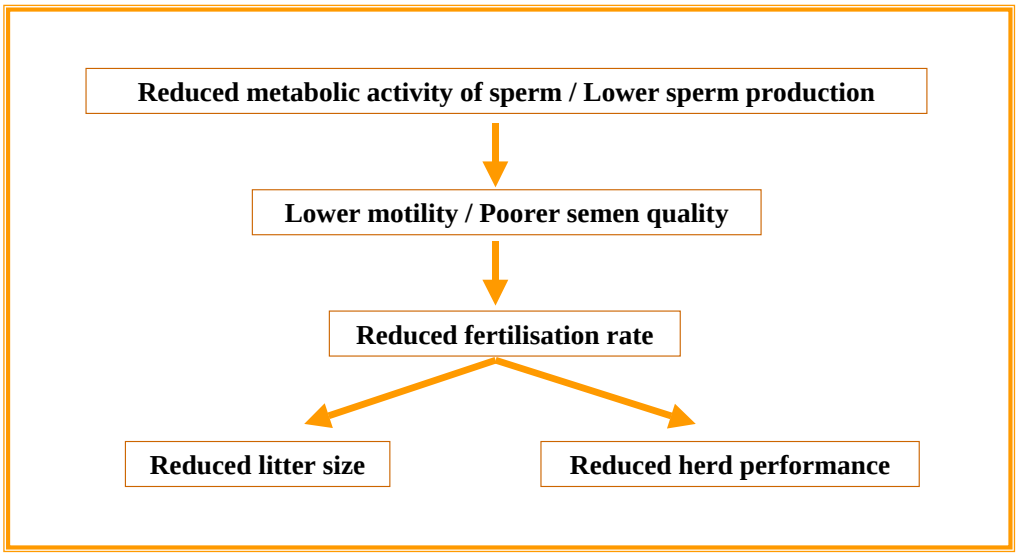
	Selenium (ppm)		***Vitamin E (IU/kg)***		***SEM***
	0	***0.5***	***0***	***220***	
Age (months)	*Sperm produced per g testis (no. x 10^6)*				
5.4	39.4	50.7	48.2	41.9	8.6
6.2	65.9	73.0	64.2	74.7	7.3[a]
9.0	64.0	89.8	73.8	79.9	8.1[b]
18.0	92.4	163.8	134.0	122.2	8.7[b]
	Average spermatozoal ATP concentration (nanomol/10^6 sperm)				
	1.15	1.55	1.30	1.37	0.10[c]

[a, b, c] Dietary Se response ($p<0.05$)

More recently, Jacyno *et al.*, (2002) measured the reproductive performance of young boars receiving inorganic or organic Se and vitamin E in their diet during both winter and summer. There was no effect of treatment on the volume of the ejaculate or the percentage of motile sperm, but the concentration of spermatozoa and total spermatozoa produced were higher in boars fed the diets containing the organic Se. There was also a higher proportion of sperm with normal acrosomes and a lower percentage of sperm with minor or major morphological abnormalities. These data show that supplementation of diets with organic Se is superior to supplementation with inorganic Se, but in this study the source of Se was confounded with the dietary level of vitamin E.

The mechanism by which low selenium status influences reproductive performance is suggested in Figure 1.

Figure 1. How low dietary selenium influences boar reproduction and herd fertility



It is therefore suggested that boar diets be supplemented with 50-100 IU vitamin E and 0.3-0.5 ppm Se from the most available sources. Selenium yeast, such as Sel-Plex™, with its active component seleno-methionine is more effective than inorganic sodium selenite at meeting the requirements of the boar.

Chromium

Chromium is known to influence male fertility and reduce stress. It should be included in the diet where allowed (Close and Cole, 2000).

Biotin

Biotin reduces lameness and the incidence of claw lesions. Because of the vulnerability of the boar, the dietary inclusion should be 0.3 mg/kg, but if foot problem exist, then this should be increased up to 1.0 mg/kg (AFRC, 1990).

Vitamin C

The physiological functions of vitamin C are as an antioxidant in the body and in the synthesis of steroid hormones. Both of these processes are important for spermatogenesis in boars. Indeed, the requirement for vitamin C increases under conditions of stress, and especially under hot, stressful summer conditions. Providing some additional vitamin C under these conditions may be of benefit. For example, Henman *et al.* (2001) examined the effect of additional vitamin C on the sperm quality of young boars. The addition of 2 kg of vitamin C per ton of feed significantly increased the number of semen doses obtained from each ejaculate from 15.2 to 21.6. This was the result of a significant improvement in sperm quality and a tendency to increased total sperm count per ejaculate.

Vitamin supplementation

Audet *et al.* (2004) fed young breeding boars diets containing additional vitamin C, fat soluble and water soluble vitamins and noted that sperm morphology was not affected

by treatment. However, when boars were intensely worked, that is, every day, additional vitamin supplements tended to benefit sperm production, but it was less pronounced for the fat-soluble vitamins than for the water-soluble ones. They concluded that dietary supplementation of water- and fat-soluble vitamins appeared to attenuate the depressed sperm production during a period of intensive collection, or when under heat stress.

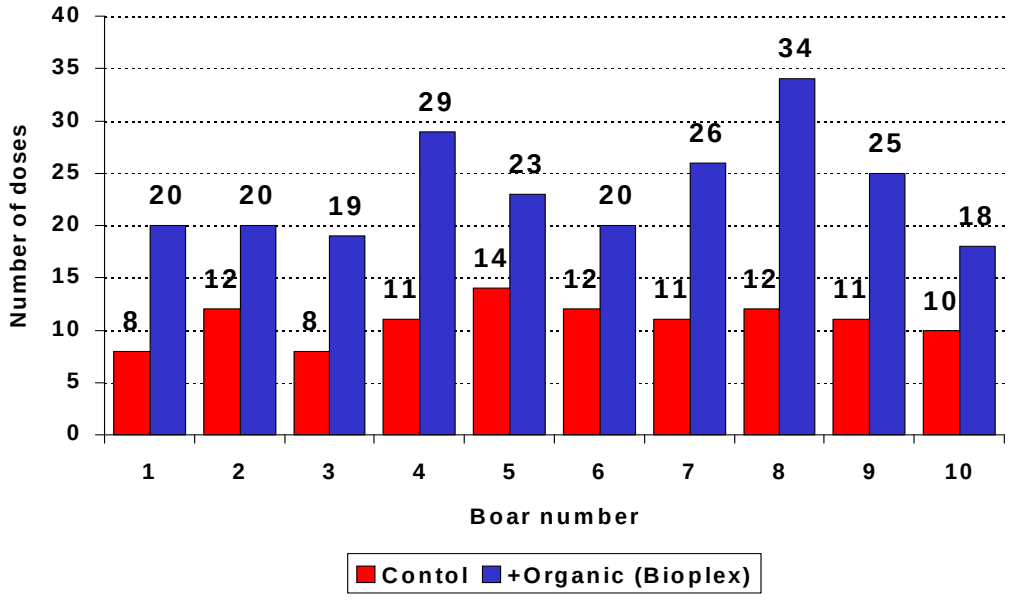
Source of minerals

High levels of some minerals in inorganic form are known to react with others in the intestinal tract and this reduces their availability for absorption. Typical examples are the reaction /combination of calcium and zinc or of iron - copper - zinc. It is for this reason that there has been an increase in the use of organic minerals, with their inherent production advantages.

A study conducted in a commercial boar stud evaluated several organic (Bioplex™) minerals and their effect on semen quality in boars (Mahan *et al.*, 2002). The organic minerals added to the boar diet for a 1-year period were: copper, zinc, manganese, selenium and chromium. The results showed that feeding the organic mineral complex to 10 boars increased average number of semen doses per ejaculate from 10.9 to 23.4 (Figure 2). These result suggest that supplementation of the diet with organic (Bioplex™) minerals dramatically improved semen production and quality.

More recently, Groenewegen *et al.* (2006) have demonstrated that the supplementation of trace minerals from organic (Bioplex™) sources (Zn, Cu, Mn, and Fe) and Se from Sel-Plex™ to diets of boars resulted in a 11.1% (from 774 to 860 cells/ml) increase in semen concentration and a 9.7% (30.0 *vs* 32.9) increase in the number of tubes produced per ejaculate, compared with control-fed boars.

Figure 2. Effect of added organic minerals on the number of boar semen doses per ejaculate (Mahan *et al*., 2002)



FEED QUALITY

Often the boar is seen as the 'dustbin' of the farm and is fed diets and ingredients left over or found not suitable for other classes of pigs. However, the quality of the feed does have a major effect on boar fertility. Weather-damaged or mouldy feeds are likely to contain mycotoxins and these have a major influence on fertility (Table 7). If weather-damaged grain is used in the diet it is imperative that an effective mycotoxin binder is added to the feed to limit any ill-effects.

It is recognised that mycotoxins seldom occur alone and there are often additional and synergistic interactions between them that reduce even further the threshold at which symptoms occur. The inclusion of an effective mycotoxin adsorbent at low levels is therefore to be recommended.

Table 7. Effects of mycotoxins on boar fertility (various sources)

Zearalenone	Delayed puberty Reduced testes size Diminished libido Poor sperm quality
Aflatoxin	Oedema of the prepuce - loss of hair Poor semen quality Low sperm concentration Increased morphological abnormalities Reduced fertilisation capacity
Ochratoxin	Off feed Gastric ulcers Poor sperm quality
Trichothecenes (T2, DON, DAS)	Off feed Vomiting

WATER REQUIREMENT

There is a scarcity of information on the water requirements of boars and there are no reported studies on the effects of dehydration on boar fertility. The water requirements of boars may be between 15 and 40 l/day, depending upon their body weight and the climatic conditions. Boars should always have continuous access to a clean and fresh supply of water.

Where nipple drinkers and drip-type drinkers are used, these must be large and freely accessible because of the physical size of the boar's head and mouth. If nipple or bite drinkers are used, then the water delivery rate should be at least 1.5 l/minute.

EFFECTS OF ENVIRONMENTAL TEMPERATURE

The boar is extremely sensitive to both cold and hot environments. Kemp *et al.* (1989) have established that the LCT of a boar of 250 kg body weight at its maintenance energy intake is approximately 20°C in a draught-fee environment and on a well insulated floor. Generally, for each 1°C decrease in temperature below 20°C the feed intake needs to be increased by 4% to compensate for the increased heat production. Thus, if the temperature falls from 20 to 15°C, then the feed requirement of a 200 kg

boar increases from 2.75 kg/day to 3.2 kg/day. However, temperature is not the sole component of the environment to influence LCT and hence feed requirement. Thermal components, such as air movement or draughts, radiation, floor time and bedding all influence heat exchange (Close, 1981) and these must also be taken into account in the assessment of the thermal environment.

Kemp *et al.* (1989) have shown that for each 1°C decrease in temperature below LCT, protein deposition was reduced by 10 g/day and fat deposition by about 20 g/day. Thus, at lower ambient temperatures fat deposition is more suppressed than that of protein. This underlines the importance of adequate and proper nutrition of the breeding boar - particularly when kept in poor housing conditions - in order to maintain body condition and to ensure good reproductive performance. A body condition score of 3 (scale 1-5) is a good target. Cold conditions *per se* do not influence sperm production, provided extra feed is given to compensate for the increased requirement.

Under hot conditions, on the other hand, both sperm motility and sperm production are decreased. Indeed, boars seem to be more sensitive to higher environmental temperatures than the males of other species and this is probably related to their limited ability to sweat and lose heat by evaporation. Exposure of boars to high environmental temperatures results in rapid increases in respiration rate and rectal temperature and reduced reproductive performance. However, the effects of heat stress on semen quality take 4-6 weeks to appear and a period of at least 5 weeks is required for the quality and quantity of semen to return to normal levels.

The fertility of sows mated with heat-stressed boars is therefore reduced (Wettemann *et al.*, 1976; 1979) The conception rate of gilts either naturally-mated or artificially inseminated with semen from heat-stressed boars was reduced (Table 8). However, embryo survival was only influenced when gilts were artificially inseminated. This may suggest that the spermatozoa from heat-stressed boars may be less suitable for preservation.

To have boars in good breeding condition, it is therefore essential that they are kept cool - ideally below 25°C - and not overworked. Measures, such as those as indicated in Table 9, will help to achieve this.

Table 8. Fertility of gilts artificially inseminated or naturally mated with control or heat stressed boars (Wettemann *et al*, 1976, 1979)

	No. of boars	*No. of gilts*	*Gilts pregnant at 30±3 d after mating* *%*	*Embryo survival* *%*
Artificially inseminated				
Control	6	88	41	71 ±4
Heat-stressed	6	77	29	48 ±5
Naturally mated				
Control	6	37	82	82 ±2
Heat-stressed	6	60	88	78 ±5

Table 9. Ways to overcome heat stress and seasonal infertility in boars

Housing and Management

- Provide adequate pen and floor space
- Reduce environmental demands:
  - Artificial cooling
  - Use of shades
  - Water sprinklers
  - Evaporative cooling
  - Wallows
- Provide adequate ventilation: air movement over animals
- Optimise mating management
  - Reduce frequency of mating / collection
  - Mate / collect semen at cooler parts of day
  - Increase herd size to compensate
- Continuous supply of good quality, fresh water is absolutely essential
- Good stockmanship and people management is important

Nutrition

- Provide special quality diet of correct composition
- Feed boars according to body weight and body condition
  - Selenium is very important for semen quality:
    0.3 – 0.5 ppm Se from an organic source
  - Zinc is essential for male reproduction; especially organic Zn
  - Consider Boar-Pak of organic minerals
  - Adequate levels of Vitamin E
  - Extra Vitamin C may help
  - Biotin and L-carnitine may also be useful
  - Fish oil can enhance semen quality
  - Consider adding a mycotoxin binder

Conclusions

It is often said that the boar is 'half the breeding herd', but the potential of the boar in terms of piglets produced per lifetime is much greater than that of the sow. Maintaining the fertility of the boar is therefore of major significance for the efficiency of reproduction on many farms, regardless of whether natural or artificial mating is practised.

Nutrition, management, housing, welfare and health status all influence the reproductive potential of the breeding boar. In terms of nutrition, a prolonged period of restriction of both energy, protein and amino acids adversely affects libido and semen quality in boars. The nutrient requirements of the boar and dietary specification are known and a separate boar diet is essential. Several vitamins and minerals influence boar reproduction and recent results support the addition of organic Se, which has resulted in improved semen quality and fertilisation rate. Similarly, supplementing the diet with a combination of proteinated minerals resulted in more doses of semen per ejaculate because of higher semen quality. Recent evidence also suggests that dietary supplementation with omega-3 fatty acids improves semen quality. It is also important to keep boars in the correct body condition.

Boars should never be exposed to environmental temperatures that cause heat stress, as this reduces semen quality, libido and results in lower fertilisation rates and subsequent litter size. Indeed, it takes 3 months to correct any adverse effects on semen quality. Control of the environment is therefore essential.

The technical competence of the staff can have a major effect on reproductive performance. It is important to monitor the reproductive performance of sows in order to assess the technical competence and practical skills of those working in the breeding unit. For this, a good recording system is essential.

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Safe Sex at -196°C - Is Cryopreservation of Boar Semen Possible?

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INTRODUCTION

Artificial insemination (AI) is arguably the most important management practice in modern animal reproduction. Indeed, AI dramatically accelerates herd/flock genetic improvement by increasing the distribution of genes from superior males. This benefit of AI has been long recognized and is reflected in dramatic tales of 14th century Arab chieftains stealing semen from their rival's stallions to breed their own mares. Artificial insemination is also an important tool in disease control, which is particularly significant to intensive pig productions.

The benefits of AI are intensified if the semen can be cryopreserved. An especially valuable sire can remain in use even long after his death if his semen had been frozen and stored. Cryopreservation greatly facilitates the transport of semen over long distances or to remote areas. In particular, international trade regulations stipulate that semen be frozen prior to export/import, therefore, semen cryopreservation greatly enhances market expansion. Protocols to successfully cryopreserve bovine semen have been used for over a half century (Polge, 1952), however, boar sperm are notoriously sensitive to cooling damage and AI with cryopreserved semen is not widely used in swine production.

My laboratory is interested in characterizing the nature of sperm damage caused by cryopreservation and developing strategies to improve the fertility of thawed semen. The objectives of this paper, therefore, are to describe the cryopreservation procedure, to explain why boar sperm are so temperature sensitive, and propose novel approaches to improve the quality of frozen-thawed boar semen.

SPERM FUNCTION

Sperm are highly specialized cells that are well-designed to carry out their role of delivering the paternal genome to the oocyte. They are small, streamlined, and contain only the organelles necessary to achieve their task. Most obvious, the mitochondria-rich flagellum permits the mobility essential to penetrate the oocyte vestments and the nucleus contains the highly condensed genetic material required for embryo formation. Sperm have little cytoplasm and do not have the cellular machinery to synthesize or repair proteins and lipids. The sperm head demonstrates an astonishing level of compartmentalization. Like all animal cells, the entire sperm is enveloped by the outer head plasma membrane, which consists of both lipids and proteins. The basic structure of the plasma membrane is a phospholipid bilayer, which forms a stable barrier between the inside and the outside of the sperm. Proteins embedded within the phospholipid bilayer carry out the specific functions of the plasma membrane; for example, the sperm head plasma membrane is particularly important as it contains the protein receptors that interact with the female tissues. The acrosome is a large, enzyme-filled vesicle situated over the nucleus and beneath the head plasma membrane.

Ejaculated sperm are unable to fertilize an oocyte. They must first undergo "capacitation", which can be loosely defined as a series of biochemical modifications to the sperm that permits them to bind the zona pellucida of the oocyte and undergo the acrosome reaction. Capacitation normally occurs in the oviduct as the sperm transits the female reproductive tract. Capacitation can also be reproduced *in vitro*, which has been important in helping researchers understand the mechanisms leading to this phenomenon. A loss of cholesterol from the head plasma membrane is considered to be a major trigger of capacitation, and many sterol acceptors are present in the female reproductive tract. As a consequence of the cholesterol loss, the sperm head plasma membrane becomes much more fluid and molecular reorganization of the membrane architecture occurs, presumably to facilitate sperm-egg interactions.

The acrosome reaction is an exocytotic event initiated by an influx of calcium into the capacitated sperm head. The calcium permits the formation of fusion points between the head plasma membrane and the outer acrosomal membrane, which liberates the acrosomal enzymes and exposes the inner acrosomal membrane. The acrosomal enzymes are thought to be important in helping the sperm to penetrate the zona pellucida and reach the oocyte itself. The newly exposed inner acrosomal membrane contains the receptors for the oocyte plasma membrane so fusion between the sperm and the oocyte can occur.

Clearly, both capacitation and the acrosome reaction are essential for fertilization. Any abnormalities of the sperm head plasma membrane would compromise the ability of the sperm to undergo capacitation. Moreover, if either capacitation or the acrosome reaction fails to occur or do so prematurely, fertilization cannot happen.

THE CRYOPRESERVATION PROCESS

Semen cryopreservation involves several general steps, each of which affects sperm structure and function (Hammerstedt et al., 1990): dilution, cryoprotection, cooling and freezing, storage, and thawing. For boar semen, the ejaculated sperm-rich fraction of the semen is first centrifigued to remove the excess seminal plasma and the sperm are then diluted with a cooling medium. Traditionally, egg yolk-based solutions have been used, although there is a clear need for media devoid of biological materials. The diluted semen is then slowly cooled to 5°C at which point it is further diluted with medium containing glycerol, a membrane-permeable cryoprotectant. The glycerolated, cooled semen is then packaged into straws and frozen in a programmable freezer or over liquid nitrogen vapours. Once frozen, the semen is stored indefinitely at -196°C in liquid nitrogen. To thaw, the sealed straws are quickly plunged into a water bath for a few seconds, the semen is re-diluted, and then used for AI.

COLD TEMPERATURE DAMAGE TO SPERM

The primary site of cooling and freezing damage to the sperm is the outer plasma membrane. The two principal causes of membrane stress are the cold temperature itself and the osmotic effects generated during cryopreservation.

During cooling, membrane lipids undergo "phase transitions" at specific temperatures. These phase transitions change the lipid physical state from a disordered liquid crystalline phase, where the lipid's hydrocarbon chains are randomly oriented and fluid, to the ordered gel phase, where the chains are fully extended and closely packed. Upon re-warming, the membrane lipids should revert to their more fluid phase. Because of its rigid ring structure, cholesterol plays a distinct

role in membrane structure and phase transitions. At high temperatures, cholesterol interferes with the movement of the phospholipid fatty acid chains, thereby stabilizing the membrane. At low temperatures, however, cholesterol has the opposite effect and interferes with interactions between fatty acid chains, lower the phase transition temperature and better maintaining membrane fluidity.

During the cryopreservation process, the sperm are also subjected to extreme shifts in osmolality. During the addition of glycerol there is a major transitory drop in sperm volume, during freezing, cell volume drops again as ice crystals form in the extender, and there is a major transitory increase in volume at thawing. Such dramatic changes in cell volume induce considerable mechanical membrane stress due to shrinking and expanding.

It is quite remarkable that many cells withstand cryopreservation. But why are boar sperm so sensitive to even moderate cooling? Boar sperm membranes do not fully recover from cooling and rewarming as do bovine sperm (de Leeuw et al., 1993). It has been suggested that because boar sperm membranes contain a relatively low proportion of cholesterol, they are more susceptible to cooling damage than are sperm with higher cholesterol levels in their membranes. Moreover, the irreversible ultrastructural changes to the boar sperm membranes destabilize the membrane and make it "leaky" to calcium (White, 1993). Moreover, boar sperm membranes contain high levels of unsaturated fatty acids, which are particularly vulnerable to peroxidation, which is a major problem since cryopreservation is known to reduce endogenous antioxidant protection in a number of species (reviewed in Bailey et al., 2000).

What are the implications for such membrane damage to boar sperm? For those cells that survive, they have damaged lipids and proteins, resulting in modified enzyme activity, altered receptor structure and poor selective permeability as manifested by calcium influx. As a consequence, sperm function would be highly compromised, having problems with oviduct binding, capacitation, the acrosome reaction and oocyte interactions. In fact, while studying the bovine system, we observed that despite the reportedly high fertility of frozen-thawed bull sperm cooling and cryopreservation facilitate capacitation and spontaneous acrosome reactions (reviewed in Bailey et al., 2003). Specifically, cooled and frozen-thawed bull sperm were able to fertilise without capacitating them *in vitro*. Boar semen that has been subjected to cryopreservation or even cooling below 15°C also shows many signs of precocious capacitation, such as specific fluorescent patterns associated with capacitation according to the choletracycline (CTC) assay, calcium influx and protein changes (Green and Watson, 2001; Bailey et al., 2008). Moreover, the sperm undergo acrosome reaction in the absence of normal physiological stimuli as if they have become capacitated due to the temperature changes. Table 1 compares the changes in boar sperm that are observed during normal capacitation and cold temperature exposure. Although true capacitation and the capacitation-like changes during cooling are not identical, there are clearly many molecular similarities.

Ultimately, mammalian sperm are on a "do or die mission". If they undergo capacitation but do not fertilize, they cannot really repair themselves or become quiescent and await the next oocyte. Once capacitated, sperm are highly fragile and rapidly undergo necrosis. Similarly, the ultimate consequence of these cooling or cryopreservation capacitation-like modifications is that, survival in the female reproductive tract is reduced, thereby decreasing the fertility of the semen.

Table 1. Summary of boar sperm modifications associated with capacitation and cold temperatures.

CAPACITATION	COOLING
CTC fluorescent pattern B	CTC fluorescent pattern B
Ability to undergo the acrosome reaction	Spontaneous acrosome reactions
Cholesterol loss	Cholesterol loss
Fluidisation of the plasma membrane	Disorganisation of the plasma membrane
Increased cytosolic calcium	Increased cytosolic calcium
Appearance of p32 proteins	Appearance of p32 proteins

NOVEL STRATEGIES TO PROTECT THE SPERM PLASMA MEMBRANE

We have proposed that reinforcing the sperm plasma membrane to prevent capacitation-like changes during cooling and freezing will improve the fertility of cryopreserved semen. We suggest that incorporating exogenous cholesterol to stabilise the plasma membrane will help boar sperm improve their tolerance to cooling.

Cyclodextrins are oligomers of glucose that form water-soluble complexes with other organic molecules. Cyclodextrins are very popular for delivery of hydrophobic pharmaceutics and are, therefore, very common molecules. Methyl-β-cyclodextrin has been used to induce capacitation of boar sperm *in vitro* by removing cholesterol, thereby activating the capacitation process (Shadan et al., 2004). Conversely, we hypothesize that cyclodextrins can be used to deliver supplemental cholesterol to boar sperm membranes, thereby increasing their resistance to cold temperatures. Indeed, we and others have shown that cyclodextrin + cholesterol treatment improves the quality of bovine and ovine sperm following freezing-thawing (Purdy and Graham, 2004; Bailey et al., 2003).

For our initial study, methyl-β-cyclodextrin loaded with cholesterol was added to fresh boar sperm during cold shock. The semen was incubated at ~2°C for up to 1 h, and then re-heated to 39°C. Cyclodextrin + cholesterol effectively maintained the percentage of motile sperm at around 50% as compared to < 10% for untreated sperm. Interestingly, cyclodextrin alone or cholesterol alone did not protect the sperm, indicating that the cyclodextrin is important in delivering the exogenous cholesterol to the cell. Moreover, our colleagues showed that cyclodextrin + cholesterol treatment reduces the protein changes observed with cooling (see Table 1; Galantino-Homer et al., 2006; Bailey et al., 2008) and decreases the rate of spontaneous acrosome reactions. Our preliminary experiments with freezing boar sperm have also yielded promising data. Although motility did not significantly improve in sperm pre-treated with methyl-β-cyclodextrin + cholesterol, sperm survival was higher and the rate of spontaneous acrosome reactions decreased. Together, these observations confirm that reinforcing the plasma membrane with extra cholesterol does prevent some of the capacitation-like changes seen in boar sperm subjected to cold temperatures. We are in the process of continuing this line of research and fine-tuning our protocols.

CONCLUSION

In summary, it is now established that sperm from a number of mammals undergo capacitation-like changes during cryopreservation, thus reducing the efficacy of frozen semen. Boar sperm, however, are particularly susceptible to cooling damage, even at temperatures well above

freezing, possibly due to the low proportion of cholesterol and high level of polyunsaturated fatty acids in their plasma membranes. It is also anticipated that stabilising the plasma membrane prior to freezing using novel molecules such as cyclodextrin coupled to exogenous cholesterol appears to improve the tolerance of boar sperm to cooling and may make cryopreservation of pig semen a commercial reality.

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Boar Stud Employee Management

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1) Introduction:

The successful operation of the large boar stud involves the interaction of people, processes and pathways, not all of which are within the manager's control. The most important of these is the staff that the manager puts together to perform the duties at the unit. An effective manager must be intimately familiar with all of the processes that occur within the unit and should be able to fill in at any position, should a temporary staffing shortage occur. Only in the very smallest stud can one person perform all tasks required to successfully collect, process and distribute semen daily. Therefore, selection and training of a competent, dedicated staff should be a top priority of the manager. Staffing is determined by a variety of parameters, including size of the unit, layout or design, equipment, and semen collection, processing and delivery schedules. Boar studs can be divided into three general categories: those that sell semen to the commercial market, those dedicated to a closed, integrated system and those that are a combination of both. Staffing needs will vary according to the type of stud and semen delivery schedule. Units dedicated to supplying semen to an integrated system may have a more even production schedule spread over the entire week, allowing a higher ratio of boars per employee compared to studs selling semen on the commercial market that may have two or three heavy production days and two or three very light production days each week. These latter types of units may require more part-time labor, which provides its own set of challenges.

The semen delivery schedule determines the semen collection schedule, which can influence salaries and the workforce available to fill the positions in the stud. For example, in an integrated system's stud, semen delivery schedules may be set up such that semen is produced during the day and delivered at the end of the day for the sow farms to use the following day. In this case, staffing for a "normal" daytime shift can be relatively easy. Also, if a commercial stud provides semen mainly to customers who receive it by commercial carrier, such as UPS or FedEx, a daytime shift may provide the best schedule. If, however, the stud must provide semen for dedicated delivery routes that are scheduled to leave the stud in the early morning to provide same day delivery over a wide area, production would be required to be completed in the early morning, necessitating an overnight or third shift. Third shift staffing can provide many challenges, particularly with part-time employees, and may require higher salaries than comparable positions for daytime shifts.

2) The Manager:

Management is as much a philosophy as a procedure. Management does involve overseeing people as well as very specific processes that determine how the work at the unit is accomplished. The successful operation of a boar stud involves strict attention to details, while an effective manager must also be able to see the big picture at all times. A balance between these two characteristics creates an effective manager. Keeping an open mind to new or different ideas and constantly seeking knowledge are two additional key traits of a good manager. The true test of a manager, or any employee, is not how they perform when things are operating smoothly, but how they perform under pressure or in a crisis situation. Management requires responsibility, which denotes the ability to respond to situations and make decisions.

Numerous books and articles have been written about management techniques and philosophies. This paper is not intended to be a review of those transcripts, but will incorporate ideas from several different authors. One of those authors is Stephen Covey who, in his book

Seven Habits of Highly Effective People[2], has given us some insight into the habits of effective leaders. The effective boar stud manager would incorporate these behaviors into his or her management philosophy. From the employer's perspective, the most effective and desirable manager is one who is constantly developing their replacement with aspirations of moving up within (or outside of) the organization. When you ask an employee where they would like their career to be in five or ten years, you will seldom hear them say they would like to be doing the same job they are performing today. If their goals do not involve advancement, they may well not be the best candidate for the manager's position.

3) Prioritize:

Procrastination is detrimental to being a good manager, regardless of the type of business you are involved in. To be successful, every manager must set his or her priorities and do the most important things first, regardless of how difficult they may be. Some managers can keep these tasks prioritized mentally, while a majority do a better job if they make lists. A list can make it much easier to prioritize tasks, as we can assign dates and times to the list and update it as we complete each task and add new ones as they come up. If these tasks can be kept on a computer program tied to a calendar with automatic reminders, it can be much easier to keep current with what needs to be done. We often find ourselves performing activities which may seem important, instead of the more critical tasks, even to the point of putting off making the call to an overdue customer or addressing an employee problem until the next day, or the day after that, or not at all. We may put some tasks off because we dread completing them, but once they are accomplished, we feel a tremendous sense relief, as in achieving a private victory, and are able to more effectively perform the other tasks of the day, and even sleep better at night.

4) Recruit and Hire:

Making a concerted effort to hire the right people will result in fewer employee problems with lower turnover which can provide a greater number of possible successors for the manager's position. Often, we look at the cost of hiring and training new and replacement employees when we should focus on the value of finding and recruiting the best candidate. Unless a local industry has recently closed, we should not expect the best candidates to be waiting for us to offer them a job. The best workers already have a job, so we must be creative in finding them and enticing them to join our team. This will require offering competitive salaries and benefits and possibly enticing them with signing bonuses. When interviewing prospective employees, keep in mind that resumes can be written to make the employee look better than they may be. Some people know how to manipulate the interview process to make themselves appear more capable and qualified than they are. The best thing to do is follow up with references and confirm as much of each applicant's background as possible. Find out why they left their last job, how long they worked there and how much their pay scale changed over that period of time. The most important question to ask is, "Would you re-hire this person if they were to apply at your company again today?" Look at the number of positions the person has held over the past ten years and with how many different employers. Applicants that have had a number of different positions with different companies in a short period of time should be thoroughly researched to minimize turnover. Search out each applicant's short and long-term goals and make sure their goals fit with the position you are hiring them for and the culture of your company. Take into consideration potential advancement within the company for that employee. Ask yourself, "Will this person who is interviewing for an entry level lab technician position be the right person to take over as lab manager in the future?" An applicant is typically hired for a specific position, but may be a better fit in a different area of your company than you originally planned. In the words of Jim Collins in his book *Good to Great*[1], first you must get the right people on the bus, get the wrong people off the bus, and get the right people in the right seats on the bus. Often we

have good people working for us, but they either are not given proper direction or we have them working in the wrong position.

The processes of the boar stud are singularly unique and rare as an industry, making it difficult to explain to an applicant exactly what the various positions at a boar stud entail. Watching a video of semen collection and processing or allowing an applicant to spend part of a day observing production are excellent ways for them to see ahead of time what will be expected of them if they are hired. Either method of previewing the job could prevent an applicant from accepting a position that they would not remain at once they learn the details of what their duties entail. Do not be too quick to judge a person on how they may perform at a specific job based on appearance or experience. Be willing to give them a chance to prove themselves.

Provide a somewhat detailed job description to make it clear to the prospective employee what will be expected of them. Job descriptions should be both general and specific. It is unlikely that every activity of a position can be listed on a job description, and yet it should not be assumed that the applicant will understand your expectations without your explication. Some tasks may be so engrained in the manager's mind as expected to be part of a job that they become second nature and are inadvertently omitted from the job description. For example, because biosecurity is such a high priority at a boar stud and becomes part of the culture of the employees, most maintenance and repairs are performed by the employees instead of risking the entry of an outside contractor. The manager cannot assume that an applicant who has welding skills will automatically be expected to make welding repairs when semen collection duties are completed. This misunderstanding can be avoided by simply stating on the job description that repairs and maintenance, and even remodeling, are the responsibility of all unit employees without listing specific tasks. The same holds true for cleaning. Both the lab and barn cleaning chores should be part of the respective technician's job, so cleaning should be included on the job description, without details, as the processes themselves will be expected to change over time. The unit manager, along with the lab and barn managers, must spend some time periodically evaluating the processes of the stud to understand their needs when hiring new employees. Duties at the various positions in the unit also change over time, so job descriptions should be reviewed and updated every time a new employee is to be hired.

A company handbook should be provided to each new employee that spells out company policies. This can serve as a basic guide for both the manager and the employee in knowing what behavior is acceptable and what is not, what benefits are provided and when they are available to the employee and what actions or inactions can result in disciplinary action and/or termination. Most companies hire employees without a contract, which allows them to terminate employment at any time without reason, but also allows the employee to resign at any time. The rules for employment vary by state and should be checked for the state you work in prior to hiring new employees.

5) Engage, Challenge and Trust:

Prior to the new hire's first day on the job, equipment that the person will be using should be in place and either ready to set up or use. They should be engaged from the first day to perform their assigned tasks. New hires should be provided with a set of specific goals to be reached within a designated period of time. For example, a newly hired lab technician may be expected to be able to clean the laboratory on their second day without supervision. They may also be expected to be able to extend ejaculates by the end of the second week and to perform any duty in the laboratory without supervision by the end of eight weeks of employment. If these expectations are spelled out in a document that the manager, the employee and their direct supervisor sign at the time of hire, questions regarding the employee's abilities to meet your expectations will be minimized. These expectations can only be considered if you provide the tools with which the employee can accomplish the goals, particularly education and training.

This agreement will also provide you with the tools to make decisions regarding retaining employees, instead of relying on a subjective interpretation of the employee's performance.

All employees should be challenged to do more. Most people have the ability to perform beyond our expectations, so as managers, we must provide them with opportunities to do so. If we tell an employee they cannot do a job, they will be convinced that they cannot, and if a situation arises that requires them to perform that task, they likely will refuse to try or simply fail to do it. If, however, we challenge them to perform beyond their current boundaries, they will likely do so with ease. As managers, we have a tendency to underutilize and underestimate the abilities of our employees.

We must trust our employees in order to challenge them. Mistakes will be made, as we are all human, and we must be able to accept them. We must also be ready to accept that, when challenged to go beyond expectations, employees may do things differently than we would do them, and it may turn out that they have found a better way to do the task.

6) Develop, Coach and Lead:

We must spend time developing our new hires and employees promoted to new positions through education and training. This process will include coaching and leading them through decisions and issues. We cannot expect new hires and promotions to find their way in the dark without guidance, and it is the responsibility of the manager to provide that guidance. Resources are available to aid in this process for managers who have not been formally exposed to these processes. Companies such as Development Dimensions International, Inc.[3] (DDI) have programs that can help in leading and coaching of employees and make training a more complete process.

7) Praise, Review and Evaluate:

Giving praise and recognition for a job well done is as important as dispensing disciplinary action. Praise and recognition should be done for specific actions and not given out every day when the normal expected job is done[7]. When someone takes initiative to do something that is beyond the routine, solves an unexpected problem that comes up, or goes the extra mile to complete a difficult task, praise should be given in a timely manner. Most people are proud of the extra things they do and recognition is a great stimulus for people to put forth extra effort and foster working as a team. Small rewards can also be effective in fostering a sense of belonging and pride.

Most people do not like performing employee evaluations, and yet feedback from their manager is one of the things that employees desire most. The manager can make this process much easier on him or herself with a little preparation. If job descriptions are clear and performance expectations are spelled out at the time of hire, with specific goals stated clearly, evaluations will be made considerably easier. DDI can provide guidelines for evaluating performance of employees, making the review process much easier and more equitable for both parties.

Employee reviews should include a specific review of the person's ability to perform the various activities in that person's area of work, as well as their attitude towards the company, their position, coworkers, livestock and customers, if applicable. A schedule for reviews should be spelled out at the time of hiring and must be followed. Consideration should also be given to absences and tardiness. Termination of an employee who fails to show up to do chores on a weekend may be justified, as it can put the life and health of the boars at risk. An employee who is tardy twice in their first month of employment may only need a disciplinary warning to correct the problem. It is the responsibility of the manager to impress upon every employee the importance of his or her job to the successful operation of the unit. Employees that are made to feel important will usually be more loyal and hard working than those that are not.

8) Promote:

When making decisions about promotions, it is expected that seniority will be the primary criteria for moving up. However, you should consider other traits when deciding whom to promote. Promotion from within the unit is almost always better than hiring a new person from outside, unless there is simply no suitable candidate on your payroll as a replacement. If you develop your employees and promote from within, you will set a precedent that will build confidence in employees taking on new tasks and trust in you to reward them for doing an exemplary job. It will also make it easier to hire good employees who have the desire to improve their status within the company. Make note of employees who are always willing to go the extra mile, who volunteer to stay late to finish an important task or come in on their day off when the other employees are unwilling to do so.

9) Be Trustworthy:

Just as the manager must trust their employees, he or she must also be trustworthy. Being trustworthy is the first step in building and gaining trust with employees. One important way to gain trust with your employees is to always keep your word. If you say you will do something for your employees, whether for an individual or group, it is paramount that you follow through and do it in a timely manner. If you are unable to keep your commitment, you must inform them of the situation and give them a satisfactory reason why you cannot do so. If you build the level of trust with your employees to the point of being able to leave them in charge for a period of time, such as being gone for a week of vacation, you will have started the process of developing a suitable replacement – a trait of a good manager! Procrastination is a potent enemy of trust and should always be avoided.

Admitting mistakes will also build trust with your employees. Everyone makes mistakes from time to time. Admitting your mistakes as soon as you realize them will help you gain the respect of your employees and encourage them to do the same.

10) Communicate and Listen:

Many problems that occur are the result of poor or inadequate communication. While it is impractical and often inappropriate to notify your staff of all the things that are going on that impact the unit, it is important to keep them informed of things that directly affect them and production. Ultimately, management must decide what is important enough and appropriate to tell employees, and what is insignificant. Always address concerns as soon as you recognize that a problem exists.

Good communication involves not only speaking, but effective listening as well, and it is difficult to hear when you are talking! A large part of employee evaluations and dealing with employee problems involves listening. In order for the manager to understand how an employee feels about his or her duties, co-workers or to get ideas for improving the system, he or she must listen effectively.

11) Dealing with Employee Problems:

In the case of disputes between two co-workers, the people involved will often have very different versions of the situation. In the event of a dispute, it is important to interview both parties separately and write or record (with their knowledge and permission) their responses and comments. It is just as important to write or record information from any witnesses. Recording as much information as possible regarding the dispute enables the manager to make unbiased decisions and determine if disciplinary action is warranted.

If an incident occurs in which termination of an employee is necessary, the decision to end their employment should be delayed until the next day. Once all the information that the

employee has to offer is recorded, they should turn in their keys and any other company equipment and be sent home. The manager should then assemble all the information that is available from other employees and assess the situation carefully before confronting the employee the next day. If a human resources director or advisor is available, the manager should consult with them to insure the proper steps are being followed for terminating an employee. If termination is warranted, it is best not to delay in carrying it out. When confronting an employee, it must always be done in private, never in front of other employees, and the manager must maintain self control and leave personal emotions and bias out of the situation. The manager must remain professional under all circumstances. Providing the employee with any type of compensation beyond termination is a decision that must be made based on the situation, company policy and the employment agreement, if applicable. If there is fear of retaliation by a disgruntled employee, local law enforcement may be consulted for advice. In the most severe case, a restraining order may be necessary to protect employees and the business.

Problems that employees experience at work at times may be related to situations taking place outside of the workplace. For example, if an employee is going through a divorce, the employee's attitude at work may be influenced, which can affect their performance and/or interaction with other employees. It is unreasonable to expect an employee in this type of situation to forget all of their anxieties when they come to work, but it is not unreasonable to expect them to treat coworkers with respect and do the best they can at their duties. The performance of breeding technicians undergoing this type of stress at home has been shown to have a dramatic effect on the reproductive performance of the sows they are mating with AI[4]. Employees at the boar stud in comparable circumstances could be affected in a similar way. While the manager should not act as a counselor, he or she should listen to the employee if they wish to discuss the problem and suggest professional counseling if it seems prudent.

12) Refresh:

It is essential that every employee, including the manager, take time off to relax and allow the mind to unwind from the pressures of day to day work. All employees are given vacation time each year and it is important that the manager see that each uses his or her allotted time. In some cases, it may be necessary to put restrictions on vacations so the unit is not short handed at a very busy time. For example, if several employees are avid deer hunters and would all like to take opening week off, you may have to have a lottery to determine who can be off first, etc.

13) Educate:

New information from research, customers, suppliers, media, etc., must constantly be integrated into the stud and passed on to employees in a practical way and on a continual basis. Employees should be encouraged to read and learn new things related to the industry. Provide them with appropriate educational materials and send them to related training schools or workshops. When given an opportunity to interact with other people in similar positions from divergent systems, employees will more readily consider implementing new techniques to improve productivity or the quality of the product they are producing.

14) Standard Operating Procedures (SOPs):

All procedures at the boar stud and isolation, including biosecurity, should have written protocols or SOPs. By putting these SOPs in writing and presenting a copy to every new employee at the time of the interview or of hiring, no employee will have an excuse for not knowing the protocols. The exercise of writing the SOPs also helps the manager become more familiar with all of the process at the unit.

One may argue that making detailed, specific protocols of all the processes performed at a boar stud does not encourage employees to think creatively and make decisions when a unique

situation arises that is not covered by an SOP. It is prudent, however, to spell out the details of the processes as they are expected to be performed so they will be done correctly every time. Over time, changes will occur that will require updating of the SOPs, whether due to new equipment or technology or new information on the processes that are being performed. The intent is not to write the SOPs and place them in a binder until something goes wrong. SOPs should be reviewed and updated on a regular basis with input from all employees. Without periodic review, procedures can unintentionally change over time, resulting in them being performed incorrectly. Periodic review on a regular basis will help prevent procedures from changing and insure that the protocols are current any time a new employee is hired. These reviews also keep SOPs current with changes in processing methods and equipment as new technology is adapted by the boar stud.

Appendix A contains a list of topics for which SOPs might be written for a boar stud. This list is by no means intended to be complete and some of these suggestions may not apply to every type of stud.

15) Biosecurity:

It is important for all employees to have a general understanding of biosecurity and its implications in operation and management of the boar stud. The National Pork Board has published a guide for pork producers on *Security and Biosecurity*[6]. Biosecurity entails the implementation of procedures and practices that minimize or eliminate the risk of disease entering the unit. The manager's job is to make certain these protocols are followed without exception. These protocols should be written and/or critiqued by the herd veterinarian to insure they meet guidelines for specific diseases that are a concern to the unit. Disease issues will change over time, so it is important to review these protocols on a regular basis, and educate all employees of these changes when they occur.

By having SOPs for biosecurity, employees will understand the commitment of management to biosecurity and it will help every employee to think "*biosecurity*" every day. Employees tend to emulate the practices and habits of the manager, particularly with regard to biosecurity. Managers are leading by example at all times, so it is paramount that they follow the SOPs of the unit. Managers must also remember that if a biosecurity SOP is violated without corrective action it will be perceived by employees as not being important and treated as such by them. This can quickly escalate to a total breakdown in biosecurity protocols, which could put the boar stud at risk of disease contamination and shut down. A document should be drafted outlining the expectations of management with regard to biosecurity and each employee should sign and date his or her receipt of the document.

Semen delivery drivers pose a significant biosecurity risk to the boar stud because they could be delivering to sow farms that are in the midst of a disease outbreak before returning to the boar stud. They also have the potential of spreading disease from one farm to another on the delivery route. A separate biosecurity protocol should be established for delivery drivers, just as for the other unit employees. Delivery drivers should never be allowed to enter the boar stud.

16) Future Technologies:

New technologies will change how we manage the boar stud and its employees. Changing from processing fresh semen to freezing semen would require a new set of skills and training of employees. It may also have a dramatic effect on work schedules. Semen sexing would also require a change to more complexes processing, should it become commercially feasible in swine[5]. The implementation of trans-cervical insemination could result in a significant reduction in the number of boars required in the industry and would impact our boar and employee needs[8]. These technologies have the potential to greatly impact the operation of boar studs by both

reducing the number of boars required to service the sows in the industry and changing current semen processing to more sophisticated technologies that utilize expensive equipment and may impact the number of insemination doses attained per boar. These more complex processes will require a higher level of management and attention to detail to be implemented successfully.

17) Conclusion:

There are many aspects to the successful management of employees in the boar stud which require attention to detail, focus on the big picture, proper selection, training and supervision of employees, etc. Prioritizing time effectively will allow the manager to accomplish the most important tasks each day. Build trust and be trustworthy to build a team spirit among all employees. Deal with employee problems swiftly and give praise where it is warranted. Provide the tools your employees need to do their job, including additional education. Provide SOPs for all processes and monitor biosecurity at all times. As with any swine operation, the care and welfare of the animals should be a constant concern for the manager and their employees.

Appendix A

Possible SOPs for the Boar Stud:

Biosecurity policies
Monitoring health status of source herds and pre-delivery disease screening and vaccinations
General isolation procedures and time-lines
Delivery and acceptance of boars
Vaccination, deworming, treating and health monitoring of boars in both isolation and the stud
Training and evaluation of boars for semen collection
Transfer of boars to and from isolation and to and from the stud
Necropsy and disposal of dead boars
Trailer and chute cleaning, disinfecting and drying
Daily monitoring of boar health status
Culling of boars
Feed deliveries and feed spill management
Shower-in and shower-out procedures
Veterinarian interpretation of health status and tissue testing
Safety policy and regulations
Employee hiring, training and evaluations
Barn and laboratory cleaning and disinfecting
Semen collection, evaluation, processing, packaging, cooling, storage and shipping protocols
Laboratory equipment cleaning and monitoring protocols
Water quality monitoring
Semen quality and contamination monitoring
Back-up plan in the event of stud closure
Visitor policy

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Management Factors that Affect Boars Before They Arrive at the Stud

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Introduction

Sperm production in adult boars is influenced by a number of management factors. Most of the critical ones such as nutrition, housing, ambient temperature and collection frequency are associated with or occur after boars are mature and have already entered production. There is no question that these are critical aspects of managing A.I. boars and influence both the quantity and quality of spermatozoa. Therefore, it is appropriate and wise for boar stud managers to pay close attention to them.

In contrast, considerably less attention is devoted to management conditions of future replacement boars during their pubertal development. In fact, the management program for most commercial A.I. boars before they enter the stud probably is similar in many aspects to those used for market pigs. This is an oversight. Boars are unique in that much of the anatomical framework that they need to produce spermatozoa as adults develops within the first 30 days after they are born (Franca et al., 2000). For example, it is believed that the number of Sertoli cells is already established by the time replacement boars are weaned (McCoard et al., 2001). This is an important observation because the number of Sertoli cells is directly related to the number of spermatozoa boars can produce as adults. Those with high numbers have the potential to produce large numbers of spermatozoa. Those with low numbers have a limited capacity for sperm production. Consequently, the management conditions to which boars are exposed before they arrive at the stud probably impact adult sperm production as much as those to which they are exposed as adults. The main purpose of this paper is to discuss various aspects of the boar management during sexual maturation and their potential effects on the production of spermatozoa as adults.

Critical Periods Associated with Reproductive Function in Adult Boars

Adult sperm production in boars occurs in two phases. The first phase can be thought of as a "building" phase, while the second is, in essence, a "functional" or "active" phase. As alluded to earlier, the active phase of sperm production is the period of time that adult boars are housed in studs and are actively producing insemination doses. In contrast, the building phase takes place during the sexual maturation of adolescent boars and probably is mainly associated with the proliferation of Sertoli cells. In swine, as is the case with most mammalian species, sertoli cells can only support the development of a finite number of germ cells during spermatogenesis (Sharpe et al., 2003). Consequently, the number of sertoli cells typically is thought to be the "rate-limiting" factor in terms of sperm production levels in adults (Amann and Schanbacher, 1983). Sertoli cell proliferation in pigs begins during the prenatal period (McCoard et al., 2002) and continues after birth (Swanlund et al., 1995; Franca et al., 2000). There is some debate over when mitotic activity ends postnatally; however, it is generally accepted that a very active and, probably, critical period occurs during the first 3 weeks after birth (McCoard et al., 2003). Consequently, it

is possible that a boar's potential for sperm production as an adult might be established by the time it is weaned from its mother. Thus, the 3-week period of time immediately after birth and associated with lactation fits the definition of a "critical period" associated with spermatogenesis. As a result, sperm production could either be positively or negatively affected in adult boars depending on how they are managed when nursing their mother.

Another important reproductive function in adult boars is the desire to breed or be collected in the case of A.I. boars. Development of this behavioral trait also begins before a boar is sexually mature. Libido primarily is under the control of testosterone. Testosterone is produced by the interstitial cells of Leydig, which are located between the seminiferous tubules in the testicles. While these cells have not been studied in as much detail as their sperm-producing counterparts, it is currently accepted that they also undergo an active period of proliferation which mirrors that of the Sertoli cells. If this is, in fact, true, then the amount of testosterone and, thus, libido that boars potentially can display as adults might also already be determined at weaning.

Neonatal Environment of Boars and Adult Reproductive Function

It is difficult to make definitive recommendations with regards to management programs for future replacement boars, because there is still quite a bit of basic information that is unknown concerning the puberty in boars. Therefore, the approach that will be taken in this paper is to review studies that have been conducted in these areas and then present some educated guesses or speculations in terms of management conditions for adolescent boars.

Recently, a retrospective study was conducted with a limited number of boars (n=20) in order to begin to look for possible reasons why some boars consistently produce more spermatozoa than others (Flowers, unpublished). It is of interest to note that there were several pairs of littermates in this data set and in several instances they were on opposite ends of the spectrum in terms of number of spermatozoa produced per ejaculate as adults. One of the factors that was highly correlated ($r = 0.79$) with adult sperm production was a boar's weaning weight, which, in turn, exhibited a strong inverse relationship ($r = -0.85$) with the size of the litter from which the boar was weaned. These relationships are logical since boars weaned in small litters should experience less competition and have the opportunity to consume larger quantities of milk than their counterparts in large litters. The additional nutrition that they received during lactation would coincide with the active period of Sertoli cell mitosis and key developmental periods of other male reproductive organs. Thus, it seems physiologically plausible that manipulation of the litter size in which boars nurse may be a way to enhance their sperm production and other aspects of adult reproductive function.

Based on this observation, a prospective study was conducted in order to examine the influence of the neonatal environment of boars on their adult reproductive performance (Griffith et al., 2006). In this study, 40 terminal-line, crossbred boars were crossfostered at one day of age in such a way that littermates were raised in litters of 6 (n=20) or in litters of 9 or more pigs (n = 20). Boars were selected from birth litters that had equal numbers of gilts and boars and crossfostering was done in such as way that potential milk production difference among sows were minimized. After weaning, boars were raised under conditions that simulate normal

management practices within the swine industry. Boars were trained to collect off a dummy sow beginning around 5 months of age. Total number of spermatozoa and semen quality estimates were evaluated weekly until the boars were 2 years of age. In addition, when the boars were 20 months, their fertility was evaluated via heterospermic inseminations and paternity testing.

There were no differences in the number of boars successfully being collected by the end of the 30-day training period (Figure 1). However, the overall training period was significantly reduced for boars from small than large litters. One interpretation of these data is that boars allowed to nurse in litters of 6 pigs had greater libido than boars nursing in litters of 9 or more pigs. Boars raised in small litters also had increased testicular size at relatively young ages compared with boars raised in large litters (data not shown). Based on these observations, it appeared that testicular maturation and, thus, testosterone production began earlier in the boars reared in small litters during lactation. This, in turn, should result in attainment of puberty at a younger age as measured by their desire to mount a dummy sow and be collected. It was particularly impressive that the majority of boars that nursed in small litters mounted and were collected during the first 5 days of the training period. In contrast, only 5 of the 20 boars that nursed in large litters were trained for semen collection during the first 5 days of training.

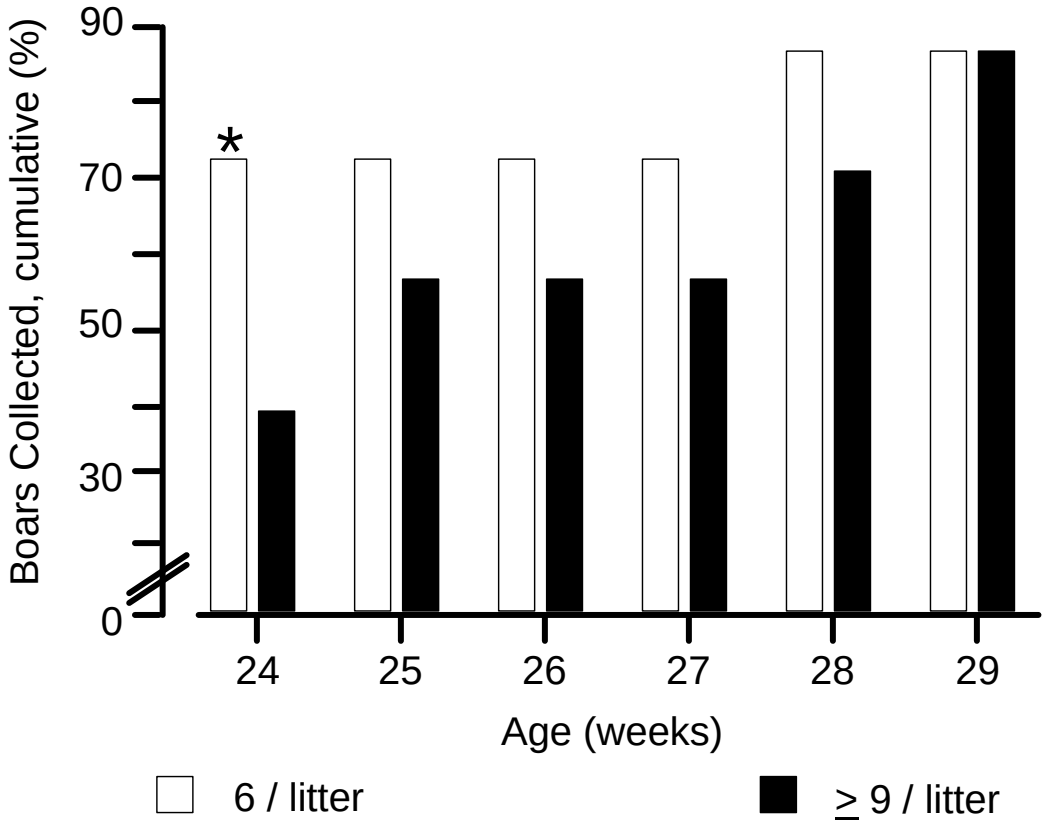

Figure 1. Effect of neonatal litter size on boars trained for semen collection on a dummy sow. *More boars raised in litters of 6 were trained to collect from a dummy sow compared with boars raised in litters of $\geq$ 9 ($P < 0.05$).

Table 1. Semen Quality and Fertility Estimates from Boars raised in Small or Large Litters during Lactation (mean $\pm$ s.e.).

Variable	6 pigs / litter	$\geq$ 9 pigs / litter
Total number of spermatozoa per ejaculate (x 10^9)	108.7 $\pm$ 6.3*	84.2 $\pm$ 5.8
Motile spermatozoa (%)	87.6 $\pm$ 5.2	84.4 $\pm$ 4.8
Normal morphology (%)	86.3 $\pm$ 3.4	86. 8 $\pm$ 5.1
Normal acrosome morphology (%)	85.4 $\pm$ 4.2	83.6 $\pm$ 6.4
Acrosin activity (%)	95.3 $\pm$ 4.5	92.8 $\pm$ 4.1
Normal capacitation (%)	80.2 $\pm$ 7.8	79.7 $\pm$ 4.2
Proportion of piglets sired in heterospermic matings (%)	65.3 $\pm$ 5.7*	34.7 $\pm$ 4.3

* Boars raised in litters of 6 are greater than boars raised in litters of $\geq$ 9 ($P \leq 0.05$)

Numbers of spermatozoa per ejaculate were consistently around 20 billion sperm cells greater in boars raised in small versus large litters (Table 1). From a practical perspective, the collective advantage of being raised in a small litter was an additional 468 insemination doses per year assuming boars are collected 1.5 times per week and insemination doses consist of 3 billion motile spermatozoa. No significant differences among treatments in motility, morphology, acrosome morphology, acrosin activity, or capacitation status were observed (Table 1). The observed increase in sperm production in the boars raised in small litters is consistent with the speculation that reduced competition resulted in an increased proliferation of Sertoli cells during this early, critical period of testicular development.

Finally, boars raised in small litters sired, on average, around 65% of the piglets resulting from heterospermic inseminations (Table 1). Consequently, they appeared to be more fertile than boars raised in large litters. It is difficult to translate this relative advantage into differences in farrowing rate and numbers of pigs born alive at the present time. This is due to the fact that use of heterospermic inseminations and paternity testing of the resulting offspring is a relative

assessment of fertility. In other words, it can be used to rank boars from most to least fertile. However, this technique cannot really establish whether the most fertile boar produces farrowing rates of 95% or 85%. Nevertheless, these data do indicate that regardless of what the actual fertility level, boars raised in small litters would be higher than those reared in large litters.

Implications for Management Factors for Adolescent Boars

There are two important messages that have practical implications from the study discussed previously. First, it is clear that changes in management during periods of testicular development in maturing boars can influence their adult reproductive function. This is evident from the increases in training success, sperm production, and relative fertility of the boars reared in litters of 6 compared with their counterparts that nursed in litters of 9 or more. In this particular study, a management change was applied only during the first 21 days of their life, yet it had fairly dramatic results. Thus, it is clear management programs for replacement boars need to begin at birth.

Second, it appears that reducing competition and facilitating animal growth early in a boar's life have positive long-term benefits. In the case of the previous study, litter size during lactation was deliberately reduced to a level which probably would not be feasible in current commercial production systems. It was done this way to prove a concept and not to provide recommendations for application within the industry. Nevertheless, it is not unreasonable to expect to see the same general trend for any reduction in litter size. For example, if a herd normally averages 11 pigs born alive, then reducing the litters in which potential replacement boars are raised to 8 pigs should result in some benefit in their reproductive function as adults.

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Markers of inferior boar performance

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Introduction

The overall reproductive efficiency of the herd is highly correlated to the reproductive capacity (fertility) of the males. Poor quality boars, because of the polygamous structure of swine production, will affect the reproductive outcome of numerous females. In the case of artificial insemination (AI) this could be thousands of females. We inherently know that not all boars and not all ejaculates from those boars are created equal. Yet ejaculates collected for use in AI are subjected to a minimum standard of semen analysis. Using sub-fertile boars and low quality ejaculates reduces production efficiency and lowers profit margins for the producer.

The ultimate measures of boar fertility in production records are pregnancy rate and litter size born. However, these are retrospective measures of boar fertility and can be highly influenced by breeding management and the quality of the females (Colenbrander et al., 2003). Livestock production managers have come to accept that a combination of a thorough physical examinations of the boar and conventional semen evaluation (concentration, morphology, motility) can provide an alternative to actual fertility data (Gibson, 1989) While these evaluations can establish that an animal is either sub-fertile or infertile, they cannot identify the relative fertility of boars males that meet accepted industry standards for sperm and ejaculate quality (Ruiz-Sanchez, 2006). However, the "predictors of useable semen" currently used in most commercial AI centers provide a very conservative estimate of the relative fertility of individual boars. Furthermore, the relatively high sperm numbers used in commercial AI practice (usually more than 3 billion total sperm per dose of extended semen), and the pooling of semen from boars that may have inherently different fertility, likely masks the reduced fertility that can be demonstrated in some of these boars when lower numbers of sperm are used for AI, or if boars are used on an individual basis.

If the full economic impact of the genetically highest indexed boars is to be realized at all levels of the breeding pyramid, then the number of gilts and sows bred per boar must be maximized. A number of innovations in insemination technology, including post-cervical (Watson and Behan, 2002) and deep-uterine (Vazquez et al., 2005) insemination are conducive to the use of lower sperm numbers per insemination. The further possibility of using controlled ovulation techniques to achieve single fixed-time insemination protocols (Baer and Bilkei, 2004; Cassar et al., 2005) would also substantially increase the utilization of genetically superior boars. Therefore, effective predictors of relative boar fertility would be essential for excluding less fertile boars from commercial boar studs and thus optimizing the use of proven high fertility, and genetically high indexed, boars with lower sperm numbers per AI dose. At the nucleus

level this will allow for increased selection pressure by increasing the number of offspring bred per collection from high ranking boars. At the level of terminal line production, this would allow considerable improvements in production efficiency to be realized, by capitalizing on boars with a high index for traits such as growth rate, feed conversion efficiency and the carcass characteristics of their progeny. Even if the same production costs were paid in genetic royalties, by purchasing less total doses of semen but from genetically superior boars, the cost benefits realized by producers in the grow-finish performance of the progeny produced and the quality of the carcass sold, would nevertheless be positive.

However, if these changes in production strategy are to be realized, it is critical to be able identify boars of relatively low fertility when used in the more challenging situation of reduced sperm numbers per AI dose or per insemination. Thus, the very definition of "useable semen" changes in this new context. Existing information and recent research directed to achieving these more demanding criteria of useable semen objectives is presented below.

General approaches to boar semen evaluation

Researchers have searched for decades to find a single or combination of tests that can accurately predict male fertility from a seminal sample (Amann, 1989). Unfortunately, there appears to be no simple answer to this very complex question (Rodriguez-Martinez, 2003). Often laboratory assays examine all of the sperm present in a sample for fertility, yet only one to 30 or so sperm are necessary to fertilize all available oocytes. Braundmeier and Miller (2001) suggested that the sperm that fertilizes the oocytes *in vivo* may be a small (even a single), highly selected, sub-population that is not representative of the average sperm evaluated in the sample. They also suggest that, because sperm must meet many requirements for successful fertilization, testing a single attribute is unlikely to be a true measure of ultimate fertility. Further, Rodriguez-Martinez (2003) suggested that to accurately predict semen fertility, it is necessary to test all sperm attributes relevant for fertilization and embryo development within large sperm populations, and to develop *in vitro* techniques that will predict the fertility of low sperm doses used for AI. This author also suggested that these techniques must accommodate, and accurately predict, in vivo fertility.

Braundmeier and Miller (2001) reviewed a number of functional and molecular tests used to assess male fertility. In this review they describe two different sperm traits that affect fertility.

- *Compensable* traits are those that can be overcome by introducing large numbers of sperm during insemination. Problems with motility and morphology will reduce the number of sperm that are able to reach the oocyte, but by introducing large numbers of sperm the reduction in fertility can be minimized.
- *Uncompensable* traits are those that cannot be overcome by introducing larger numbers of sperm. These defects affect fertilization and embryo development and include nuclear vacuoles, sperm chromatin structure and morphological problems that do not inhibit movement.

Therefore, to effectively predict fertility, it is therefore essential to discriminate between compensable and uncompensable traits in an ejaculate.

Conventional semen evaluation generally includes a measure of seminal volume, sperm concentration, and the percentage of sperm progressively motile and morphologically normal (Amann et al., 1995). Although some of these parameters are correlated with fertility in the boar (Flowers, 1997; Xu et al., 1998), several authors suggest that this information, while important, does not accurately predict whether a male is truly fertile (Brahmkshtri et al., 1999; Correa et al., 1997; Rawls et al., 1998). As shown in Table 1, existing analyses are also usually inadequate for predicting relative fertility in healthy boars with ejaculate quality that meets normal industry standards (>70 % motility and <30% abnormal sperm) (Flowers, 1997; Alm et al., 2006), even though the reproductive efficiency of these boars may still be substantially different (Flowers, 1997; Tardif et al., 1999;Popwell and Flowers, 2004; Ruiz-Sanchez, 2006). As illustrated in Tables 2, differences in relative fertility become increasingly evident when low sperm doses (<2.5 billion sperm) are used for AI (Tardif et al., 1999; Watson and Behan, 2002; Ardon et al., 2003; Ruiz-Sanchez, 2006). This approach likely avoids the compensatory effect of using excessive sperm numbers per AI dose (Saacke et al., 2000 ; Alm et al., 2006), allowing the detection of fertility differences between relatively fertile boars.

Options for semen evaluation

Semen has several characteristics that can be evaluated by macroscopic, microscopic and functional analysis. Routine evaluations used in commercial boar studs may include color, odor, sperm numbers and ejaculate volume, ejaculate pH, and sperm motility, morphology and viability. These traditional measures of ejaculate quality verify the suitability of the ejaculate for extension and the number of doses per ejaculate that can be produced. On an ongoing basis, these characteristics also provide a measure of sperm production and the health of the boar. However, if more discriminatory tests of relative boar fertility are to be developed, those characteristics of the ejaculate that might contribute to such discriminatory analyses are of particular interest. These characteristics are summarized below and discussed in the context of potential discriminatory tests of relative boar fertility. Where relevant, evaluations that provide additional information on sperm that might be optimal for in vitro fertilization procedures are also discussed.

Sperm motility.

Flowers (1997) concluded that the percentage of motile sperm only gives a qualitative estimate of fertility and is only useful for estimating the reproductive performance of boar semen with less of 60 % motile spermatozoa (Table 1). Xu et al. (1998) found no correlation between sperm motility and litter size, using 3 and 2 billion sperm per AI dose to breed weaned sows with an early return to estrus. However, Tardif et al. (1999) reported that when 0.3×10^9 sperm were used per AI dose, the percent of sperm with normal motility was positively correlated with farrowing rate (r=0.783,

P=0.01). This suggests that the use of large numbers of sperm per AI dose might mask actual boar fertility potential, and therefore, that sperm motility is a compensable trait.

In contrast to motility estimates in raw or extended semen on the day of collection, sperm motility in extended semen at day 7 has been correlated with *in vitro* fertility estimates (do Lago et al., 2005), whereas reported correlations with *in vivo* fertility are variable (Juonala et al., 1998; Xu et al., 1998; Sutkeviciene et al., 2005). Subsequently, Ruiz-Sanchez et al. (2006) established significant correlations between the motility of extended semen at day 7 and 10, and farrowing rate and total litter size born in groups of gilts bred with 1.5 x 10^9 sperm per AI dose (Table2). Therefore, the motility of extended semen at different days of storage may offer a practical and inexpensive approach to identifying relative boar fertility. However, more studies are needed to confirm this relationship and should involve more precise methods of measuring sperm motility and motility characteristics.

Table 1. Relationships among sperm motility, sperm penetration rates, farrowing rates and number of pigs born alive for boar semen (from Flowers, 1997).

Motility[a] (%)	**Sperm penetration rate [b] (%)**	**Farrowing rate [c] (%)**	**Number born alive**
94.7	89.5[v] (58)	86.9[v] (460)	10.6[v]
82.3	81.7[v w] (55)	87.1[v] (330)	10.5[v]
76.1	84.3[v w] (50)	84.5[v] (300)	10.5[v]
66.2	74.7[w] (44)	86.1[v] (264)	10.1[v]
52.4	55.5[x] (40)	72.4[w] (201)	9.2[w]
44.2	34.7[y] (28)	72.3[w] (168)	9.2[w]
32.6	21.3[z] (17)	51.7[x] (85)	7.8[x]
SEM	4.8	5.8	0.3

*Motility is expressed as the average number of motile spermatozoa within the following classes: >90; 80-89; 70-79; 60-69; 50-59; 40-49; and 30-39.

[b]: Sperm penetration rate is defined as the percentage of eggs that were fertilized. The numbers in parentheses represent the number of ejaculates within a motility category.

[c:] Number in parentheses represent the number of sows inseminated within a motility category.

SEM: Standard error of the mean.

[v,w,x,y,z:] Means within the same column with different superscripts are significantly different (P<0.05).

Sperm viability.

Several studies, have demonstrated that sperm viability results, obtained with vital stains or fluorescent vital stains, have no significant correlation with fertility *in vitro* (Berger et al., 1996; Brito et al., 2003) or *in vivo* (Juonala et al., 1999). Nevertheless, there is evidence for positive correlations between the HOST, and zona pellucida penetration and *in vitro* fertilization rates (Gadea, 2005; Brito et al., 2003). Unfortunately, HOST results have a low or no correlation with *in vivo* fertility in boars (Pérez-Llano et al., 2001).

Sperm morphology.

Berger and Parker (1989) did not find any correlation between sperm morphology and boar fertility in a competitive mating situation. However, Xu et al. (1998) found that morphological characteristics are a useful tool for assessing semen quality in boars, in that differences in normal sperm morphology contributed to the variance in litter size born when 2 billion normal and motile sperm per AI dose was used for AI in weaned sows.

Table 2. Semen characteristics of ejaculates collected from the nine boars during the period of breeding and evaluation for which fertility data are shown in Table 2. (From (Ruiz-Sanchez, 2006)

	Fertility of doses used for AI			Sperm Motility (%)		
Boar	Farrowing Rate (%)	Total born	Fertility index	Motility day 3	Motility day 7	Motility day 10
R-2	98 ^{x}	11.7±0.4 ^{ab}	11.4±0.6^{a}	79 ± 1.3 ^{a}	71 ± 1.9 ^{a}	60 ± 2.0 ^{a}
Y-2	89 ^{xy}	12.0±0.5 ^{a}	10.9±0.6^{ab}	77 ± 1.3 ^{ab}	70 ± 1.9 ^{a}	61 ± 2.0 ^{a}
Pu-3	91 ^{xy}	11.2±0.5 ^{abc}	10.2±0.6^{ab}	72 ± 1.3 ^{bc}	64 ± 1.9 ^{abc}	55 ± 2.1 ^{ab}
B-1	94 ^{xy}	10.7±0.5 ^{abcd}	10.2±0.6^{ab}	71 ± 1.3 ^{bc}	61 ± 1.9 ^{bc}	52 ± 2.3 ^{abc}
R-3	95 ^{xy}	10.9±0.4 ^{abcd}	10.1±0.6^{ab}	76 ± 1.3 ^{ab}	67 ± 1.8 ^{ab}	60 ± 2.1 ^{a}
G-2	91 ^{xy}	10.1±0.5 ^{abcd}	9.5±0.6^{ab}	76 ± 1.3 ^{ab}	67 ± 1.9 ^{ab}	55 ± 2.0 ^{ab}
B-3	93 ^{xy}	9.6±0.5 ^{cd}	8.8±0.6^{abc}	73 ± 1.3 ^{abc}	66 ± 1.9 ^{ab}	59 ± 2.1 ^{a}
R-1	84 ^{yz}	10.0±0.4 ^{bcd}	8.4±0.6^{bc}	62 ± 1.5 ^{d}	55 ± 2.0 ^{c}	47 ± 2.5 ^{bc}
G-1	71 ^{z}	8.4±0.6 ^{d}	6.0±0.6^{c}	69 ± 1.3 ^{c}	59 ± 1.9 ^{bc}	42 ± 2.3 ^{c}
	P =0.0003	P < 0.001	P <0.0001	P <0.0001	P <0.0001	P <0.0001

P: probability of main effect of boar
^{abcd}: LSM with different superscripts within each column differ (P <0.05).
Values in the table are least means (LSM) ± standard errors (SE) of LSM.

Sperm DNA evaluation.

Strong positive correlations have been found between DNA fragmentation index (DFI) in boar semen and routine semen evaluation parameters such as motility (%), normal acrosomes (%), abnormal sperm (%) and cells positive to HOST, (Perez-Llano et al., 2006). Differences in sperm DNA Fragmentation Index have been found between different boar breeds (Boe-Hansen et al., 2005) and between individuals of the same breed (Perez-Llano et al., 2006); therefore, DNA evaluation could be used as an additional test for assessing semen quality and boar fertility.

Integrated evaluation of sperm function

Several investigators have developed tests to evaluate sperm functions that are more directly related to the fertilization process, such as the ability to undergo capacitation, hyperactivation, the acrosome reaction, sperm membrane fusion, zona pellucida penetration and fertilization of the oocyte:

Sperm-zona pellucida binding test.

Sperm binding to the zona pellucida has been assessed using hemizona (HZA) and zona pellucida binding assays which measure the sperm's ability to approach and attach to the homologous zona pellucida of immature eggs (species specific). Low sperm binding to the zona pellucida may indicate issues related to the capacitation process (Burkman et al., 1990) but associations with male fertility have been variable. For example, a sperm-zona binding assay was used with cryopreserved ram and fresh boar semen (Berger et al., 1996) and in neither species was zona binding correlated with *in vivo* fertility. Interestingly, Fazeli et al. (1997)) used the zona pellucida binding test to show that a high percentage of the sperm that initiate binding to the zona pellucida have an intact acrosome. This may be a clear indicator of why low fertility rates are obtained with cryopreserved semen. This information seems to indicate that the sperm zona pellucida binding test could be used to predict *in vitro* fertilization capacity and as a complementary test for assessing *in vivo* fertility.

Oocyte penetration assay.

The classic sperm penetration test uses zona-free hamster eggs as an *in vitro* assessment of sperm functional competence to fuse with the egg membrane, and to undergo decondensation. Several studies have reported a high correlation between penetration rate (zona-free hamster ova assay) and male fertility. For example, sperm from fertile boars had a high penetration rate compared with sperm of infertile boars (Berger and Horton, 1988; Berger and Parker, 1989; Ivannova and Mollova, 1993). Subsequently, Martinez et al. (Martinez et al., 1993) used zona-free pig oocytes (a homologous *in vitro* penetration assay (hIVP)) to study the relationship between the hIVP and boar fertility, and concluded that this assay effectively identified fertile from subfertile boars. In all these studies, an ability to identify differences in relative fertility among fertile boars seems limited.

In vitro fertilization assay.

Considering the IVF data obtained, *in vitro* characteristics that are not affected over time but are different among boars, are potentially useful as predictors of fertility *in vivo*. In the studies of Ruiz-Sanchez et al. (2006), male pronuclear formation rate was the only IVF variable that explained some of the variation in fertility *in vivo* (from 12 to 17%). Overall, other IVF characteristics lacked strong correlations with *in vivo* fertility, suggesting that thresholds for sperm quality were being met when relatively fertile boars are compared. However, sperm from the lower fertility boars in this study produced lower oocyte penetration and MPN-formation rates and critical thresholds for such traits (e.g. >50 % oocyte penetration rate) can still possibly be used to identify subfertile boars.

The processes involved in IVF compared to *in vivo* fertilization may also contribute to the low correlations between *in vivo* and IVF data. There is a need to improve IVM/IVF techniques for the specific purpose of sperm evaluation.

- The use of standardized total sperm numbers per oocyte for IVF, without any adjustments for motility after sperm capacitation, *in vitro*, would probably help to identify the variation in sperm quality between the boars that affect the efficiency of fertilization process.
- Another approach could be to use much lower numbers of sperm per oocyte for IVF, thus placing the sperm in similar challenging situations *in vitro* and *in vivo*.
- Likewise, the use of the same ejaculate fractions for both *in vivo* and *in vitro* fertility evaluations could confirm the relationship between them.

Recent studies by Rodríguez-Martínez et al. (2005) demonstrate that the sperm from the sperm peak (SP) fraction of the ejaculate are superior (sperm membrane integrity, percentage of live cells, etc) than sperm from the bulk ejaculate (including subsequent Sperm-Rich fractions and Sperm-Free fractions of the ejaculate). These results could provide a better understanding of why the SP fraction presents the least variability when used for *in vitro* fertilization (do Lago et al., 2005), and represents the best sperm subpopulation to test in order to obtain a predictor of fertility. The difference between these fractions is likely produced by interactions with seminal plasma components that may not influence the sub-population of sperm that rapidly establish themselves in the oviductal reservoir in vivo (Zhu et al., 2000;Rodriguez-Martinez et al., 2005). Therefore, further investigation needs to be done in this area to get a better understanding of the effects of sperm and seminal plasma interactions *in vivo*.

From a practical perspective, even if certain IVF parameters are correlated with *in vivo* boar fertility, such as oocyte penetration rates and number of sperm attached per oocyte, male pronuclear formation rate, and potential embryo production rate in the study of Xu et al. (1998), this technique is expensive and time consuming. It is, therefore, unlikely to be practical for routine semen evaluation at commercial level. However, if the results of such tests are predictive of relative lifetime fertility, and can be carried out during the initial quarantine and training period of potential stud boars, then these tests may still make an important contribution to boar fertility assessment.

Sperm-oviduct binding assay.

Differences in the binding index between boars of different fertility indicate that this test could be useful as a complementary test to assess boar fertility gives potentially valuable information on any changes in sperm binding function during storage (Waberski et al., 2006). It is also important to note that the sperm population in the oviductal reservoir depends on the initial sperm quality (ejaculate and/or boar); the site of sperm deposition, number of sperm inseminated, and may, therefore, reflect differences in ejaculate and male fertility.

Seminal plasma proteins and boar fertility

Roles of seminal plasma in vivo

Important physiological functions of seminal plasma have been described in mammals. However, from the perspective of using analysis of seminal plasma to characterize relative boar fertility, it is important to recognize that seminal plasma composition and volume differ between species, individuals, ejaculates from the same individual, and even between different fractions of individual ejaculates. An understanding of the roles of seminal plasma in the complex peri-fertilization events in mammals helps to assess the potential for using seminal plasma components as indicators of relative fertility. The functions of seminal plasma proteins are usually classified as: 1) spermadhesins, 2) sperm motility inhibitory proteins and 3) decapacitation factors. In a more general sense, seminal plasma proteins have multiple roles in promoting fertility.

- Transport of spermatozoa from the male tract into the female genital tract.
- They acts as a buffer solution and a source of nutrients, providing the energy substrates (glucose, fructose, etc) that increase the life span of the spermatozoa.
- Binding of specific seminal plasma proteins to the sperm plasma membrane is involved in sperm-oviductal binding, prevention of early capacitation, maintenance of sperm viability, sperm-zona pellucida interactions and oocyte-sperm binding.
- At the uterine level, specific seminal plasma proteins seem to control the neutrophil response.
- There is also evidence that seminal plasma components play an active role in the female reproductive tract, enhancing uterine contractions, advancing the time of ovulation and eliminating non-viable spermatozoa.

Evidence of functional roles for boar seminal plasma proteins

As seminal plasma proteins play an important role in the fertilization process, several studies have investigated the action of those proteins before and during fertilization. Flowers (1997) demonstrated that mixing seminal plasma of high fertility ("dominant") boars with the sperm of low fertility ("non-dominant") boars improves the fertility of the sperm used for *in vivo* fertilization. Furthermore, a reduction in fertility

was observed when sperm of dominant boars was exposed to seminal plasma of non-dominant boars. Likewise, Zhu et al. (2000) found differences in oocyte penetration rates *in vitro* when spermatozoa from the SP fraction were pre-incubated with different fractions of seminal plasma from the same ejaculate. Subsequently, Caballero et al. (2004) reported negative effects of seminal plasma from specific boars on viability of sperm after extension. Finally, beneficial effects of including seminal plasma during different stages of the sperm cryopreservation process (Perez-Pe et al., 2002; Petrunkina et al., 2005; Suzuki et al., 2002; Vadnais et al., 2005a; Vadnais et al., 2005b) and when cryopreserved sperm are thawed and used for AI (Crabo, 1991) have been demonstrated.

Collectively, this information demonstrates the important role of seminal plasma, and particularly seminal proteins, during the fertilization process. Therefore, it would still likely be beneficial to identify fertility markers present in seminal plasma and include these as part of the assessment of ejaculate quality and boar fertility.

Seminal plasma proteins as markers of boar fertility and semen quality

Specific seminal plasma proteins have been identified as potential markers of male fertility in the bull (Killian et al., 1993), stallion (Brandon et al., 1999) and the boar (Flowers, 2001). Flowers (2001) was able to identify two seminal plasma proteins (55 kDa, pI 4.5 and 26 kDa, pI 6.2) that were positively associated with *in vitro* and *in vivo* fertility. Ruiz-Sanchez (2006) reported that fertility *in vivo* was negatively correlated with relative abundance of PSP-I, and with 20 kDa, pI 6.0 and 60 kDa, pI 6.5 proteins, and positively correlated with a 25 kDa, pI 5.9 protein which could be homologous to a fertility-associated seminal plasma protein in reported in the boar (Flowers, 1995), bull (Killian et al., 1993; Gerena et al., 1998) and stallion (Killian et al., 1993). A 70 kDa protein was also identified as osteopontin, which has also been associated with differences in bull (Cancel et al., 1997, 1999) and stallion fertility (Brandon et al., 1999). Results on the further elucidation of these boar seminal plasma proteins are shown in Figure 1.

The 42 spots analyzed by the Imagemaster software are numbered 1 to 42 in Figure 1. Proteins 7, 17, 22 and 24 were different between boars ($P<0.05$): However none of these proteins were related to in vivo fertility nor in vitro semen characteristics. Protein 4 was negatively correlated ($r = -0.66$, $P = 0.04$) to Farrowing rate and Fertility Index and showed a negative correlation ($r = -0.70$, $P = 0.05$) with sperm motility in day 7 extended semen (Figure 2). Similarly, protein 27 showed a strong negative correlation with total litter size born ($r = -0.76$, $P = 0.01$) and a negative correlation ($r = -0.74$, $P = 0.04$) with sperm motility in day 7 extended semen. Protein 26 showed a strong tendency to be positively correlated with Fertility Index ($r = 0.48$, $P = 0.07$), Pregnancy Rate ($r = 0.45$, $P = 0.09$) and Farrowing Rate ($r = 0.45$, $P = 0.09$) and may be the lipocalin-type prostaglandin D synthase that has been previously identified in the stallion (Flowers, 1995), bull(Killian et al., 1993), and boar (Flowers, 1995).

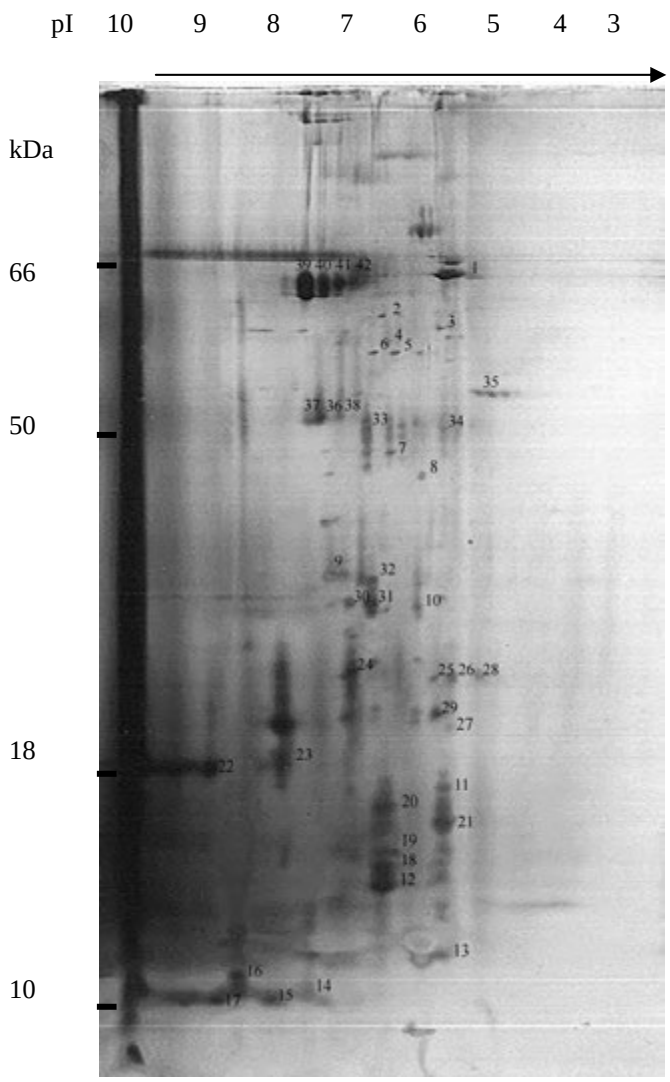

Figure 1. A representative 12 % 2-D SDS-PAGE gel showing seminal plasma proteins identified from the sperm peak fraction of boar R-1 for which data are shown in Tables 2 from the study of Ruiz-Sanchez et al.(2006).

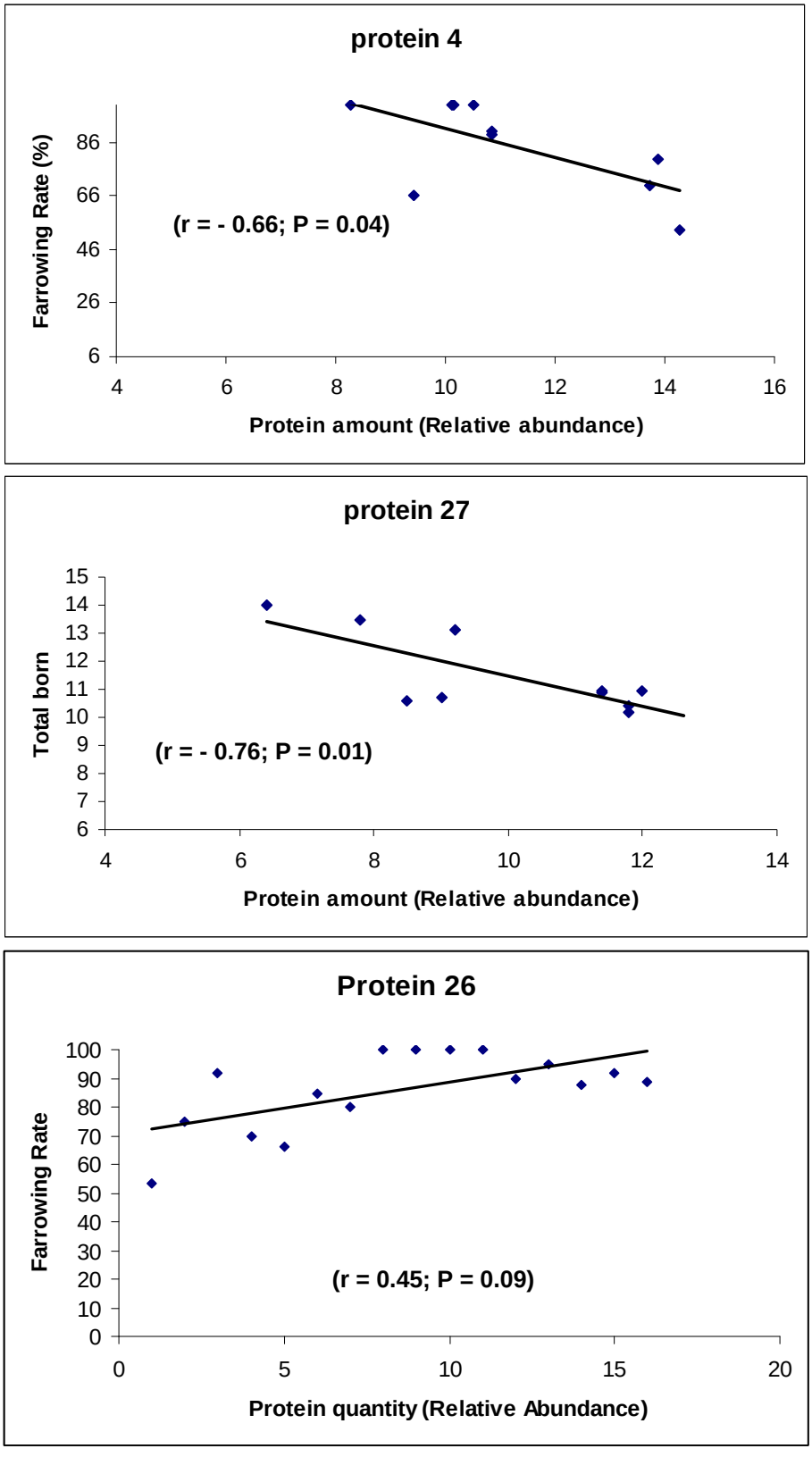

Figure 2. Correlation analyses linking protein abundance in seminal plasma to differences in relatively fertility traits in boars from the study of Ruiz-Sanchez et al. (2006). For these proteomic analyses, replicate 2-D gels (as shown in Figure 1) were run on the seminal plasma samples from two of the more fertile (R-2, Y-2) and the two lowest fertility (G-1, R-1) boars for which fertility data are shown in Table 2.

Additionally, continued research in this area is illustrated in Figure 3, comparing seminal plasma protein profiles from two boars with dramatically different fertility (Novak et al., 2008). The fertilizing capacity of these boars (Boar A and B), who had similar semen parameters, was initially tested with homospermic matings (i.e. single boar AI). Following 31 and 27 single boar matings with semen from Boars A and B respectively, Boar A inseminations resulted in a 45% farrowing rate with an average total born of 9.22, while Boar B gave a farrowing rate of 100% and average total born 12.04 piglets. The semen from these boars was then pooled with 3 other boars with similar semen parameters and used for insemination (i.e. heterospermic semen from 5 boars in total, with equal numbers of sperm from each boar within an AI dose). These inseminations resulted in a 90% farrowing rate (9 of 10 inseminations) and 104 piglets. Paternity testing of the resulting piglets, using a panel of up to 84 single nucleotide polymorphisms markers, identified Boar B as the sire of 29.8% (31) of the piglets, while Boar A was identified as the sire for only a single piglet.

Collectively, these results confirm that specific seminal plasma proteins are associated with boar fertility, providing the basis for their use as a complementary tool to identify sires with high and low potential fertility.

Molecular markers of inferior semen quality

Preliminary data from research targeted at developing more accurate semen evaluation techniques, based on molecular markers present in defective spermatozoa, have recently been presented by Sutovsky (2008). Tagging with ubiquitin occurs on the surface of defective spermatozoa during sperm maturation in the epididymis. Ubiquitin is a small chaperone protein that binds to defective protein molecules (protein ubiquitination). Ubiquitin associates with lipoxygenase (15-LOX), a lipid peroxidating enzyme with high affinity to organelle membranes which also participates in organelle degradation. In boar sperm, 15-LOX is present exclusively in the cytoplasmic droplet and labeling of sperm samples with fluorescently-conjugated anti-ubiquitin and anti-15-LOX antibodies can be used to distinguish defective spermatozoa by measuring the presence of ubiquitin and 15-LOX by epifluorescnce microscopy. Preliminary trials in boars showed significant, negative correlations between sperm ubiquitin and 15-LOX with farrowing rates and litter sizes. In contrast to other available semen evaluation techniques, this marker-based method is not skewed by damage caused during semen collection, processing and cryopreservation.

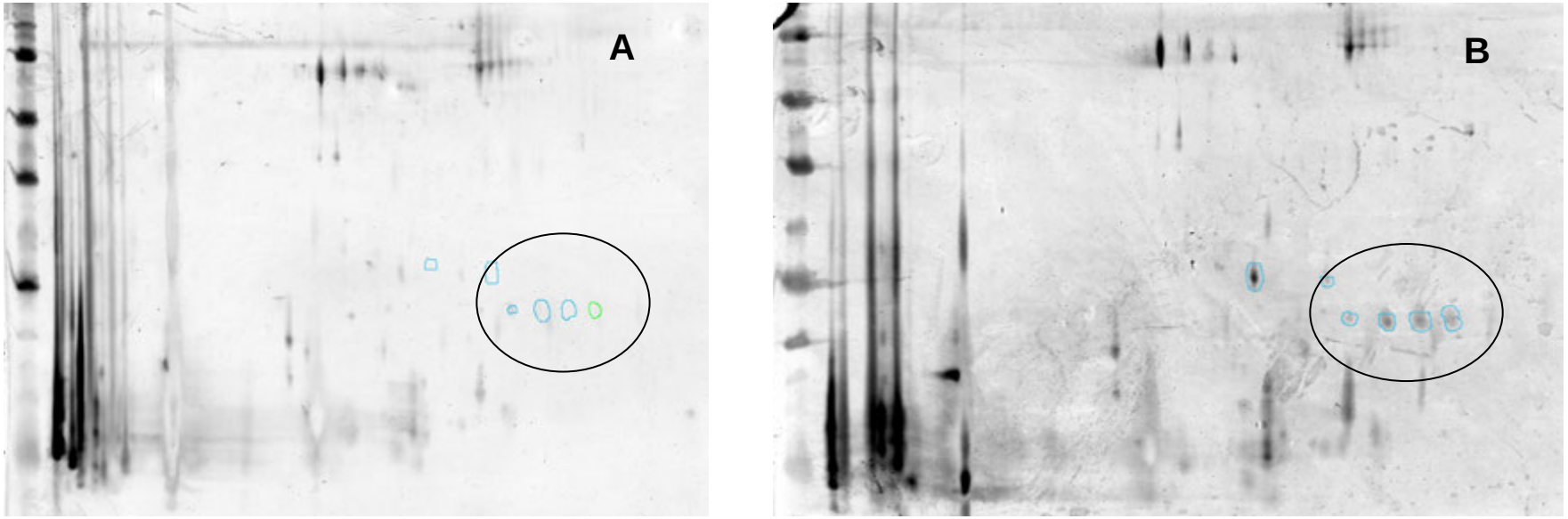

Figure 3: Representative 12% 2-D SDS-PAGE gels showing seminal plasma proteins from boars with demonstrated differences in fertility: A) boar with 45% farrowing rate B) boar with farrowing rate of 100%. The encircled region on the gels demonstrates the four proteins that are more highly expressed in Boar B (Novak et al. 2008).

Conclusions

The ability to differentiate relative fertility amongst boars would have a significant economic impact on the swine industry, by eliminating or optimizing the use of less fertile boars. It would also allow for the use of lower numbers of sperm from the most fertile and genetically high-indexed boars per AI dose, without any loss in productivity. Evaluation of the motility of extended stored semen appears to provide a practical and inexpensive approach to identifying less fertile boars at an early stage. However, evaluation of this technique is required at a commercial level to confirm these relationships.

In contrast to more conservative evaluations of semen quality, the use of IVM/IVF assays demonstrates that this technique is able to discriminate between relative boar fertility and their likely productivity under more stringent (lower sperm numbers per dose) or demanding (use of ageing stored semen) conditions of AI use. However, further studies are again needed to optimize the sensitivity and repeatability of this and other in vitro techniques. In any event, the complexity of these tests requires that they provide meaningful estimates of expected life-time performance of boars at an early stage of the boar evaluation process. Alternatively, the use of low sperm numbers per AI dose to breed gilts at their pubertal estrus, combined with the use of breed and abort protocols to determine the number of conceptuses around day 30 of gestation, can also provide meaningful information on relative boar fertility at a reasonable cost. This program would have little impact on breeding herd productivity, but could produce invaluable data about the potential fertility of boars being considered for use in AI studs, and requires virtually no laboratory facilities.

Finally, evidence for a role of specific boar seminal plasma proteins in the fertilization process suggests that they may contribute to observed differences in semen quality and boar fertility. Further assessment of these potential fertility markers may provide the basis for using seminal plasma proteins to identify sires with high and low fertility.

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Ventilation and Cooling Systems for Boar Studs

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Buildings are used for housing boars within the boar stud system. The building creates the environment for the boars. Several conditions must be addressed when defining the environmental needs of the boar. Facility issues affecting the environmental needs of the boar include 1) air temperature; 2) humidity level; 3) air velocity or air movement; 4) indoor air quality; 5) space; 6) manure removal or handling; 7) feed and water access; 8) building type and construction. These issues affect the boar's environment individually as well as interactively. A ventilation system directly impacts or influences five of the eight issues [1) air temperature; 2) humidity level; 3) air velocity or air movement; 4) indoor air quality; and 8) building type and construction]. So, providing an effective ventilation system is a critical aspect of facilities for boar studs.

Why Ventilate and How Much

The ventilation system is the primary system that regulates the thermal and gaseous environment of the pig. The failure of a ventilation system to provide the necessary conditions for swine production can and often does result in numerous swine health problems and lead to significant decrease in production performance and efficiency. A properly operating ventilation system will remove heat and moisture generated by the housed boars as well as remove carbon dioxide and odors produced by the boars.

The amount of sensible heat and moisture produced by boars at various temperatures can be seen in Table 1 (MWPS-32, 1990). The sensible heat production from the boars adds heat to the air inside the building. The moisture production or latent heat production from the boars adds moisture to the air inside the building. A properly operating ventilation system will remove the moisture produced from the building. The ventilation system is also removing heat from the building as it removes the moisture. If the sensible heat produced from the boars is not sufficient to maintain inside temperature during winter conditions, a supplemental heater, such as a propane heater, adds additional heat to the building to maintain the desired minimum temperature. When the sensible heat produced from the boars causes an inside air temperature rise, additional ventilation fans will begin operating to remove excess produced heat from the building.

Table 1. Heat and Moisture Produced by Boars at Various Temperatures

Boar Weight	Inside Temperature (°F)	Moisture Production (lb water / hour – head)	Sensible Heat Production (BTU/hr – head)
300 lbs	40	0.24	850
	50	0.24	710
	60	0.25	570
	68	0.28	475
	77	0.34	365
	86	0.40	255
400 lbs	40	0.25	1040
	50	0.25	860
	60	0.26	740
	68	0.28	600
	77	0.32	490
	86	0.38	390

Source: MWPS-32

A minimum amount of ventilation is required to maintain adequate indoor air quality conditions and remove moisture from the building during cold/winter conditions. Table 2 (MWPS-43, 2001) provides recommended ventilation rates for boars during cold/winter weather. The ventilation rates presented in Table 2 are rates required to maintain reasonable indoor air quality conditions while also controlling the moisture level in the building. The ventilation rate increases slightly as boars get large because larger boars produce more moisture per head and therefore require a greater minimum ventilation rate.

The target minimum ventilation rates, shown in Table 1, should be provided on a continuous basis. The inlet air for winter ventilation should be provided in a distributed and uniform manner. The minimum ventilation rate should be provided at a constant rate. The cycling of a large fan on and off to give an equivalent required minimum ventilation rate over time within a building results in documented inside air temperature variation that is usually unacceptable and generally results in increases in ammonia and odor levels within the building regardless of the manure management system.

Table 2. Recommended Ventilation Rates for Boars during Cold Weather

Weight (lbs)	Moisture Control[a] (cfm/hd)	Odor Control (cfm/hd)	Add for Unvented Heaters (cfm/hd)
250-350	12	20	1.0
350-450	14	24	2.8
450-550	16	28	4.6
[a] Ventilation rate for partially slotted floors. Multiply values by 0.6 for fully slotted floor or 1.2 for total solid floor to get adjusted ventilation rates for floor type.			

Source: MWPS-43

The delivered ventilation rate needs adjust to maintain desired inside conditions depending upon the outside temperature or season. Ventilation curves can be developed to show how the

ventilation rates should adjust depending upon outside temperature. Basically, the required ventilation rate increases as outside temperature increases. As the outside temperature approaches the desired inside temperature, the required ventilation rate quickly approaches impractical rates. So, a maximum ventilation rate is recommended to be provides so that a hot weather ventilation system can be designed. From ventilation curves and design experience, recommended ventilation rates are presented in Table 3 (MWPS-43, 2001) for various outside weather conditions. These ventilation rates are normally staged so that more fans will operate as the outside air temperature increases. As the outside air temperature increases, an increasing amount of air required to remove the heat generated by the boars.

Table 3. Recommended Mechanical Ventilation Rates for Boars Based on Weather Conditions

Weight (lbs)	Cold (cfm/hd)	Mild (cfm/hd)	Warm (cfm/hd)	Hot[a] (cfm/hd)
250-350	From Table 2	40	85	150
350-450	From Table 2	50	115	300
450-550	From Table 2	65	155	500
[a] For climates with extreme heat and humidity, increase hot weather ventilation rates by 25 to 50%.				

Source: MWPS-43

For hot weather conditions, another reasonable upper limit for the maximum ventilation rate would be for a ventilation system to deliver between 1 and 1.5 air changes per minute (acm) to the entire pig space being ventilated. If tunnel ventilation is used during summer/hot weather conditions, the ventilation rate is selected to provide a 3 to 5 mph air speed through the cross section of the building. However, the recommended ventilation rate for hot weather given in Table 3 needs to be confirmed for long buildings. In some cases a slow tunnel ventilation air speed does not provide the needed ventilation rate per animal in some long buildings.

Since the required ventilation rates significant changes with outside temperature, a ventilation system needs properly designed to allow for numerous delivery rates between required minimum and maximum rates and include a control system to allow the system to adjust ventilation rates automatically as the outside temperature changes.

The Ventilation System

The ventilation system is the primary system that regulates the thermal and gaseous environment inside the building for the boars. Mechanical ventilation systems have some common basic functional components regardless of specific inlet layout and fans selected. The ventilation system must be capable of delivering the required recommended ventilation rates previously discussed for the various outside weather conditions. The remainder of this section discusses the basics of ventilation systems and the different types that may be used in particular boar stud.

System Functional Components

All ventilation systems have five functional components. These functional components are an inlet, an outlet, a driving force, distribution and a path.

1. The **inlet** provides a location or locations for air to enter the pig space.
2. The **outlet** provides the location for air to leave the pig space.

3. The **driving force** provides the means to move air into, through, and out of the pig space.
4. **Distribution** defines how air moves through the pig space.
5. The **path** must exist so that air can enter the facility, go through the inlet, pass through the pig space, leave through the outlet, and finally exit the facility.

If all five functional components exist and are operating properly, the ventilation system probably is working for the given weather and animal stocking conditions. Solutions to most ventilation problems are found by identifying which functional component is missing or improperly operating.

Two other aspects of ventilation that relate to these functional components include the ventilation control system and operational targets. The control system adjusts the operation of the ventilation system in response to inside temperature as impacted by changing outside temperature conditions. The operational targets include the desired inside set point temperatures and acceptable relative humidity levels. Both of these topics are addressed later in this paper.

Negative Pressure Exhaust Systems

Negative pressure exhaust ventilation systems are the most common mechanical systems used for boar facilities. The exhaust fan serves as the outlet and creates the driving force for the system. Exhaust fans create a slightly lower pressure inside the building as compared to the outside of the building. Since the inside pressure is lower than the outside pressure, air will enter the animal space through any opening in the building shell. The goal is to have only the inlets, which serve as the inlet component, allow air to enter the boar space and minimize any other openings. By properly locating and sizing inlets, the distribution of air within the boar space can be quite uniform. Proper air distribution contributes to good pig performance.

Negative pressure exhaust systems can be and are utilized during all seasons of the year. The actual configuration of the ventilation system varies and depends upon the actual, overall construction of the boar facility.

Tunnel Ventilation Systems

Tunnel ventilation is a particular type of negative pressure mechanical ventilation system that is used for hot weather ventilation. In a tunnel ventilation system, the fans serve as the outlets and create the driving force as with other negative pressure systems but are located in one end of the building. The opening at the other end of the building serves as the inlet and usually has an evaporative cooling pad incorporated into the inlet opening. All ventilation air passes through the evaporative cooling pad and then travels from one end of the facility to the other. Tunnel ventilation with evaporative cooling pads incorporated does provide an effective hot weather ventilation system to minimize heat stress regardless of building location and orientation. However, tunnel ventilation is a summer time or hot weather only system. If tunnel ventilation is to be used during cold weather, distribution of the cold air is an unsolvable problem within the context of a tunnel ventilation system. Some other inlet system must be used for cold weather if tunnel ventilation is incorporated in the facility design.

Positive Pressure

Positive pressure exhaust ventilation systems are mechanical systems sometimes used for boar facilities. The fan in this case serves as the inlet and distribution as well as creating the static pressure, which serves as the driving force for the system. The fans create a slightly higher pressure inside the building as compared to the outside of the building. Since the inside pressure is higher than the outside pressure, air will exit the animal space through any opening in the building shell. Problems can arise from warm, moist air leaving the boar space through an undesired opening. For example, an open feed supply line with pelleted feed can serve as an outlet. When warm, moist air leaves the building through a feed line in cold weather, the moisture in the air will condense in the feed line causing moisture problems in the feed system.

Neutral Pressure

Neutral pressure exhaust ventilation systems are mechanical systems sometimes used for boar facilities and use double the number of fans as are required for either negative or positive pressure systems. One fan set serves as the inlet and distribution and a second fan set serves as the outlet. Both fans create static pressure to move air through the system. However, the fan set pairs are operated such that the pressure inside the building is kept equal to the pressure outside of the building. Since the inside pressure is similar to the outside pressure, air tends not to enter or exit the animal space through any undesired opening in the building shell unless the wind creates pressures to exchange air.

Operational Targets

The operational targets for a ventilation system include the desired air temperature and indoor relative humidity. Also, the required minimum ventilation rate needs to be established based on recommendations in Table 2. These operational targets should be met as the first step to ensure a ventilation system is operating efficiently and controls are properly set. If these targets are compromised, boar health and performance may be adversely affected.

The target air temperature will vary depending upon management preferences. The typical optimum air temperature for boars is 60 °F with a range 50 °F to 70 °F as published by MWPS-1 (1987). A minimum air temperature of 55 °F is recommended for individually housed boars with no bedding (MWPS-43, 2001). Setting the air temperature at lower end of the temperature range for the boars will tend to optimize utility type energy usage by minimizing heating costs. However, boar health and condition must be diligently monitored. Slightly more feed may be required for boars housed at the lower end of the air temperature comfort range.

The indoor relative humidity should range between 40% and 70% for boars at all temperatures when cold or mild weather fans are operating. This humidity range tends to be the healthiest range. If the indoor humidity level is consistently below 40% or above 70%, the operation of the ventilation system should be examined. Low levels of humidity tend to increase dust problems and may indicate excessive energy use. Constant high humidity levels may lead to boar health problems. High humidity levels will also tend to reduce the life of crates and penning because corrosion and rust occur more readily at high humidity levels.

The required minimum ventilation rate is needed to provide for adequate indoor air quality removing moisture and other contaminants. The required minimum ventilation rate for boars is

provided in Table 2. However, one needs to be sure that the ventilation rate being delivered during winter conditions for a given boar stud is adequate. One method to evaluate the delivered minimum ventilation rate is to monitor the relative humidity level. If the humidity level is low, the minimum ventilation rate is probably excessive. However, the minimum ventilation rate should be reduced only if other indoor air quality parameters such as odor remain acceptable. If the relative humidity levels are consistently high, the minimum ventilation rate should be increased.

For warm and hot weather conditions, the ventilation system should deliver ventilation rates similar to ones provided in Table 3. During hot weather (when outside temperature is higher than 5 8F below inside desired temperature), the inside temperature should be within 2 to 5 8F of the outside temperature unless evaporative cooling pads or fogger systems are used. If the inside temperature consistently exceeds 5 8F above outside temperature, the hot weather ventilation rate delivered to the pig space should be increased.

Cooling Options

Supplemental cooling is required for boars during hot weather. Boars have been shown to have reduced semen quality at temperatures in excess of 84 8F (MWPS-43). Some boars have shown signs of heat stress beginning at 81.5 8F (Levis and Reicks, 2005). So, cooling systems should be function when the inside air temperature reaches 80 8F to minimize any heat stress on the boars. The two most common cooling options for boars, drippers and evaporative cooling pads, are presented below. Air conditioning is being used in some boar stud to address heat stress issues for boars. A section below will discuss some aspects of incorporating air conditioning into boar housing facilities.

Dripper Systems

Dripper systems apply water directly onto the skin of the boar. The water droplets should be large enough to quickly fall and wet the skin. This water is then allowed to evaporate from the skin surface. As the water evaporates, the boar is cooled because latent heat transfer occurred. To increase the potential of latent heat transfer, ventilation must be effective to exchange the air from around the boar. Also, air speed over the skin of the boar should be at least 75 to 100 feet per minute to enhance convective heat transfer. Each pound of water that evaporates from the surface of the boar's skin will dissipate about 1,000 BTU of heat energy from the boar. So evaporating water off the surface of the boar's skin can provide an effective method to minimize heat stress for boars.

Dripper systems can function well by providing effective cooling for pigs located in both humid and dry climates. Drippers systems operate at low pressures and should emit between 0.5 and 1 gallon per hour per dripper. Drippers should be placed out of reach and above the boar and located at both the shoulder and rear of the boar. The shoulder location provides a large surface from which water can evaporate directly from the boar. The rear location should wet the testicles and help cool them by evaporating water from them as well.

Evaporative Cooling Pads

An evaporative cooling pad evaporates water into the air to reduce the dry bulb temperature of inlet air. This reduced temperature reduces the heat load that pigs will experience inside a

building. The decrease in air temperature from using an evaporative pad system will depend upon the initial climatic conditions. If the outside dewpoint temperatures are relatively low indicating a dry climate, a significant temperature decrease will result from using a pad system. However, if the outside dewpoint temperatures are relatively high indicating a humid climate, a small temperature decrease will usually result. Sometimes in humid climates, the temperature cooling resulting from using evaporative cooling pad systems is not large enough to sufficiently relieve the pigs of the heat stress conditions. Therefore, evaporative cooling pad systems by themselves may not be the most effective choice when trying to reduce heat stress conditions for boars located in very humid climates.

Evaporative Cooling Equipment Maintenance Hint

Water quality can have impacts on maintenance requirements of equipment. An evaporative cooling pad system will have significantly different maintenance requirements as well as life expectancy due to water quality. An evaporative cooling pad or sprinkler nozzle will have water evaporate from its surface. Since the water evaporates off the pad or nozzle, all salts and minerals will be deposited on and in the evaporative cooling pad or on the surface of the nozzle orifice. These salt and mineral build-ups need to be removed to maintain the pad or nozzle. Evaporative pad systems, which use water with lower hardness levels, will have less maintenance requirements and a long life expectancy than pads using harder water. A water softener either will change the kind of salts that are in the water to ones that should be easier to clean from the cooling pads or may even remove salts from the water to minimize the amount of cleaning effort required. However, if a water source without the initial hardness, such as a surface water source, is used, cleaning requirements for evaporative cooling pads will usually be reduced.

Incorporating Air Conditioning

Air conditioning is another method to reduce the effects heat stress on boars. An air conditioning system has the potential to provide a cool facility for boars regardless of outside temperatures. An air conditioning system must be sized properly to accomplish a cool facility.

The sensible heat and moisture (latent heat) loads an air conditioning system must be capable of handling include the heat and moisture produced by the boars, the dehumidification and cooling of ventilation air and the heat gain from building conduction. The amount of heat and moisture produced by the boars can be estimated from values presented in Table 1. The boar stud must be ventilated at minimum recommended ventilation rates as indicated in Table 2. If an adequate ventilation rate is not provided, odor levels will tend to be higher than desired. When outside conditions are warmer and moister than inside conditions, the air conditioning system must be capable of dehumidifying (removing the moisture from) the inlet air as well as cooling the air to a lower temperature than outside. A heat load will also result from conducting from the outside to the inside through the building structure itself via conduction. This building heat load exists because the inside temperature of the building is lower than the outside temperature. An air conditioning system must be sized based on the sensible and latent loads and not just the total load. If the latent load is not properly accounted for in the design and operation of an air conditioning system, the inside conditions will tend to be cool and clammy because enough dehumidification is not incorporated into the system design.

Ventilation System Control

An important segment of a ventilation system is the control system or scheme to coordinate the five ventilation system components. The control segment provides the logic and control necessary for the ventilation system equipment to adjust the ventilation rate depending upon outside weather conditions.

Integrated controls can automatically operate the ventilation equipment to adjust the ventilation rate. Inside temperature is the primary parameter to control ventilation equipment. Control for a ventilation system can be provided watching inside temperature and manually adjusting the equipment to vary the ventilation rate. However, manual control is not recommended because the operator must be available to adjust the system when weather conditions change and must completely understand the entire operation of the ventilation system itself. If the operator does not understand the operation of the system to know which of the various fans system need to operate to provide the various ventilation rates, the ventilation system will not be operated properly. An improperly functioning ventilation system can not only increase operating costs but also reduce boar performance due to inadequate environmental control.

The control system and the various ventilation system components operate in harmony when a system is functioning properly. Once a system is operating properly, all system settings should be left alone. If caretakers require and/or desire the need to adjust the inside temperature for boar comfort, integrated controllers should be used, and allow only the overall set point temperature to be changed. The set point temperature for an integrated control will allow the inside temperature to be increased or decreased without upsetting the overall operational harmony of the ventilation system.

To better understand the operation and settings of a controller for a boar stud, the following example settings are presented in Table 4. Some management decisions need to be made in order to present the example. First, the desired inside target temperature is selected to be 60 8F. When the inside building temperature is 60 8F, only the minimum ventilation will be operating and no supplemental heat will be provided. Four stages of ventilation rates are incorporated into the ventilation system design as outlined in Table 3. Both evaporative cooling pads and drippers will be used to address heat stress for the boars. Since some boars can experience heat stress at 81.5 8F, the maximum ventilation rate will be operating as well as the evaporative pads and drippers when or before the inside temperature reaches 80 8F. Table 4 shows an example set of controller “on” settings for the various ventilation system components. A discussion about these setting follows.

Table 4. Example Ventilation Controller Settings for Boar Stud

System Component	“On” Temperature
Heater	58 8F
Target (Set point)	60 8F
Stage 2 Fan (Mild)	65 8F
Stage 3 Fan(s) (Warm)	70 8F
Stage 4 Fan(s) (Hot)	75 8F
Stage 5 Evaporative Pad	78 8F
Stage 6 Drippers	80 8F

Some separation between the different stages is required for the ventilation system to function properly. A two degree difference is shown between the heater “on” temperature and the target

set point of 60 8F. This difference will allow the room to cool slightly before the heater is activated. The stage 2 fan (mild weather) is set to come on when the room temperature reaches 65 8F. When a heater operates, the room temperature tends to rise above the set point a few degrees before the heater shuts off and continues to circulate air as it cools. Setting the stage 2 fan five degrees above the set point helps ensure that the stage 2 fan should not operate when the heater is cycling on and off. If the heater on temperature is set too close to the stage 2 on setting, the heater may be operating at the same time as the stage 2 ventilation fan. When the heater is operating at the same time the stage 2 fan is running, high heating costs will occur. The five degrees between the fan stages reduces the likely hood that more than one fan stage will be cycling on and off at the same time. Without the separation of the on settings for the fan stages, many fans will cycle on and off with only a minor change in the inside room temperature. Remember that boars will easily tolerate a fairly wide temperature range as indicated in Operating Targets section. However, the variation of temperature within a short period of time (like 15 minutes or less) should be minimized. Separating the on temperature settings for the various stages of a ventilation system will help reduce the temperature variation within a short period of time.

If a boar stud is located in a fairly dry climate, stages 4 and 5 can be switched if the evaporative pad and hot weather fan stage cycle on and off within a short period when the weather is not real hot. Sometimes when an evaporative pad system begins to wet, the inlet temperature will drop by more than 10 to 15 degrees when all the fans are operating. Starting the hot weather fans after the evaporative pad is operating will reduce the on-off cycling of the pad and hot weather fans.

Inlet Air Filter Challenges

Filtering inlet air has become a desired option on some boar stud facilities. Care needs to be taken when considering and incorporating inlet air filters. The design of the entire ventilation system needs to be considered when adding inlet air filters.

When air filters are desired to filter inlet air, the filters will be part of the “path” air must flow through when ventilation occurs. The filters will often create a significant amount of static pressure “resistance” that must be delivered by the “driving force” of the ventilation system. For negative pressure ventilation systems, this driving force is created by the total static pressure created by the exhaust fan. The static pressure driving force created by the fan is the total driving force available to move air through the entire path air must pass when a swine facility is ventilated.

The amount of static pressure resistance created by air flowing through a common filter considered for swine ventilation inlet air filters can be seen in Figure 1. The Durafil 4V MV16 has been used in PRRS research to filter inlet air. The resistance to airflow for this filter (top line in Figure 1) is fairly significant. If the air speed is reduced to low speeds, the resistance to air movement is significantly reduced as seen in Figure 2. More information can be found on the web at www.camfilfarr.com for these filters.

The amount of static pressure driving force available for exhaust fans typically used in swine facilities can be obtained from the fan curves shown in Figure 3. The data for the fan curves was obtained from the BESS lab fan performance information.

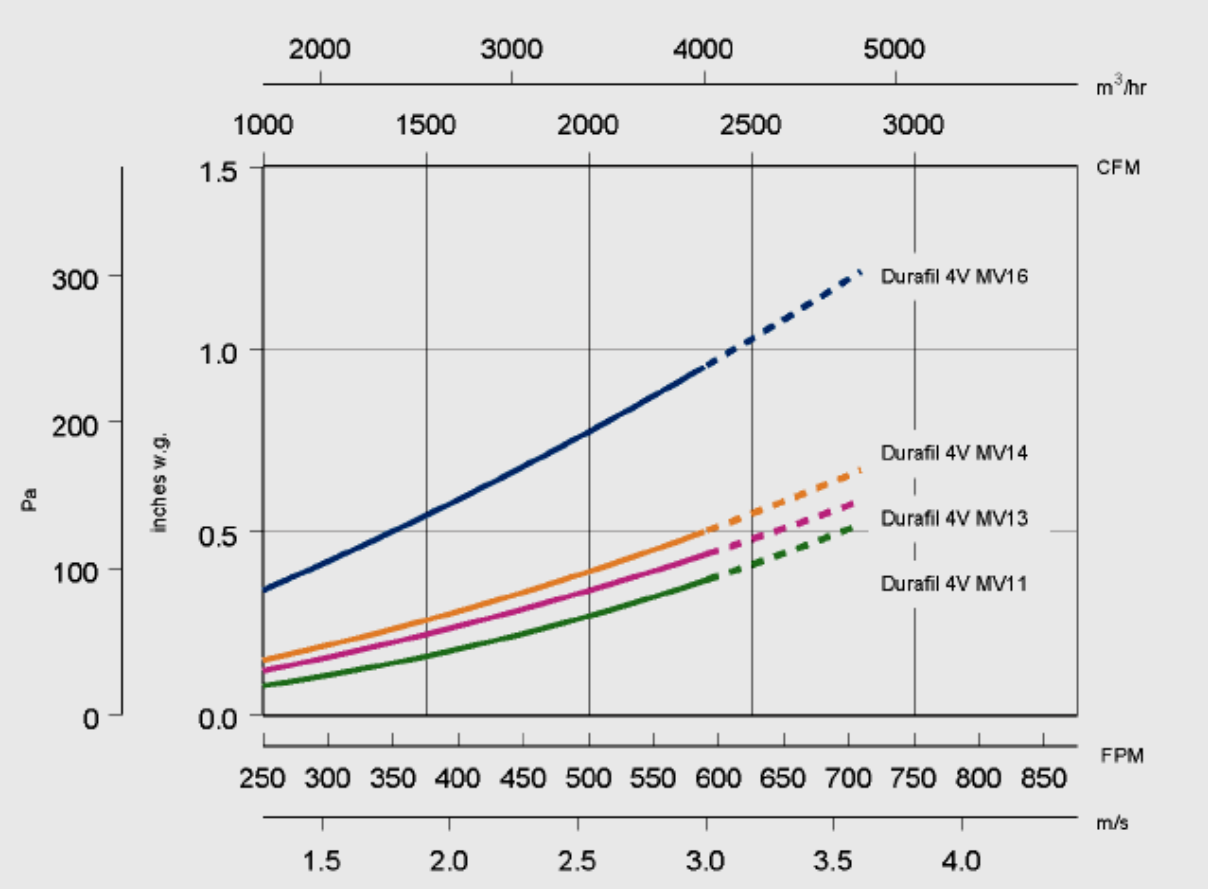

Figure 1. Initial resistance to air flow for various Durafil air filters as presented by Camfil Farr Technical Data.

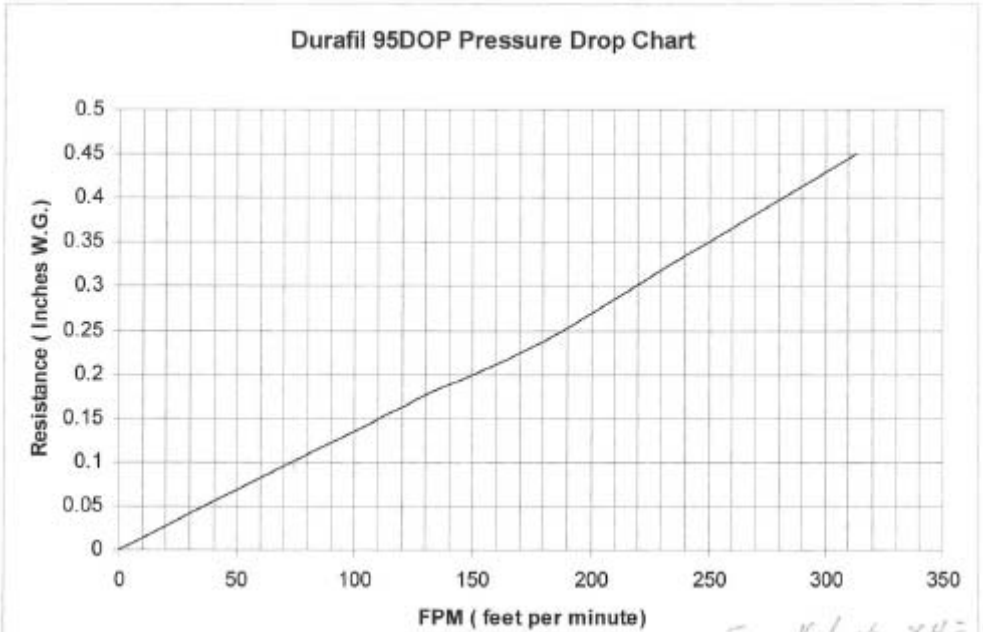

Figure 2. Initial resistance to air flow for Durafil 4V MV 16 filter at low flow rates as presented by Camfil Farr Technical Data.

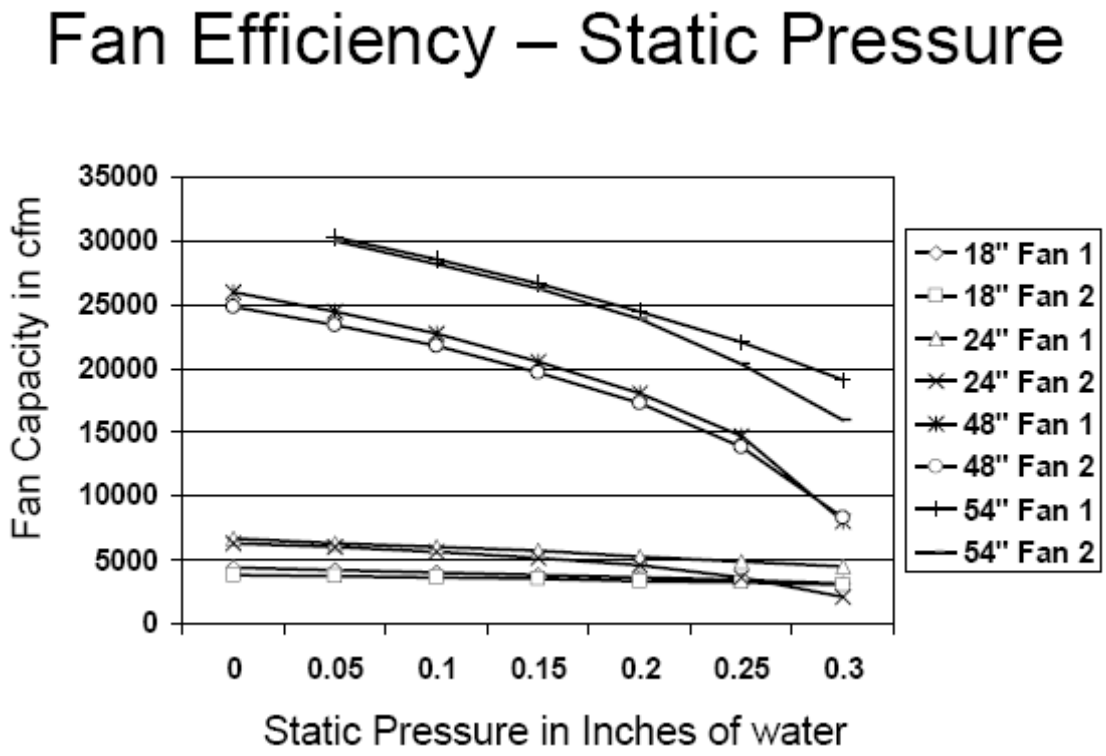

Figure 3. Fan curves from BESS lab data for various agricultural ventilation fans.

The challenge now becomes how to "match" the available static pressure driving force of the exhaust fans with the air flow resistance created by the "path" air must flow through when a swine facility is ventilated. Most swine facility ventilation systems are designed to operate so that the total resistance created by the "path" is between 0.05 and 0.10 inches of water. If air filters are added to the system, the "path resistance" will be increased based on the data provide in Figures 1 and 2. This increased "path resistance" must be provided by the fans whose performance is given in Figure 3. So, if agricultural ventilation fans will be providing the total driving force for air to move through the system, any filter added into the path can not add much more than 0.10 inches of water resistance without significantly affecting fan capability to deliver the necessary ventilation. Using the filter resistance data provided in Figure 2, a flow rate of about 75 cfm per square foot of filter will add about 0.10 inches of water resistance. So, a 4 ft^2 filter will filter approximately 300 cfm of inlet air. The total number of filters required to filter inlet air can be significant if the ventilation rate is high. To reduce the total number of filters required to filter inlet air, the static pressure resistance of the filter will increase and additional fans or blowers need to be incorporated into the design of the airflow path. These additional fans or blowers will increase the available total static pressure driving force but will not increase the delivered ventilation rate.

Building Effectiveness

How well a boar stud facility performs can be determined by its effectiveness. The effectiveness of a boar stud building is defined as whether a building can provide conditions needed for good boar performance and how well these conditions are maintained throughout the year. The conditions that need to be provided include temperature; humidity level; air velocity or

movement; indoor air quality; space; waste removal or handling; and feed and water access. For a boar stud building to be effective, the building needs to have had all five of the following criteria addressed:

1. Properly **sited** to minimize any off site impacts;
2. **Designed** to incorporate any site limitations and management preferences;
3. **Constructed** to meet design specifications;
4. **Operated** to incorporate selected management preferences, and;
5. **Maintained** to meet design requirements and equipment recommendations.

All five of these criteria and activities must be integrated for the building to provide an optimal environment for profitable semen production.

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Air Filtration Systems for Boar Studs

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Introduction

The goal of all boar studs is to be PRRS negative, either through initial stocking or some type of eradication program. The industry has demanded that boar studs be negative due to the potential of spreading PRRS virus through the semen to downstream farms. Thus the percentage of boar studs that are PRRS negative, in relation to other types of the production pyramid, is much higher than in any other segment of the industry.

One question is whether boar studs are more vulnerable than other farms. For most boar studs, all of the biosecurity research of the past five years has been implemented. However, some still have been vulnerable to PRRS breaks. In several cases, no other explanation could be determined other than aerosol infection from neighboring herds. Thus, air filtration is the next logical step, aside from relocation, to protect the boar stud population and the downstream production system from PRRS virus introduction.

In the field, air filtration has been adopted to a number of farms[1,2]. Dr. Scott Dee and Andrea Pitkin have shown us that aerosol transmission of PRRS virus can be repeated experimentally and it is becoming widely accepted to be a common way that negative farms become re-infected[3,4] One challenge for implementation of air filtration is the number of filters needed get adequate air flow through a barn during summer time ventilation.

How does it work?

The true HEPA filters are normally rated at 99.99% efficient down to 0.3 micron particle size.[5] This means that when they are new, they will filter 99.99% of particulate that is 0.3 micron in diameter. The efficiency percentage increases for larger particles and decreases for smaller particles, but as the particle size becomes extremely small, the efficiency actually increases again. As the filter becomes "used" it actually becomes more efficient, as trapped particles help to filter an even higher percentage of small particles.

There are three basic ways filtration works to stop particulate (including viruses and bacteria):

1. Impaction: larger particles are unable to avoid the filter fibers and imbed in them directly. This is how larger particles (>0.4 um) are caught up in the filter
2. Interception: smaller particles(<0.4 um) come within 1 radius of a fiber and adhere to it. The particles are held there with van der Waals forces.
3. Diffusion: Brownian motion (turbulence) increases the likelihood that small particles will be stopped by Interception or Impaction

For the primary agents of concern with aerosol spread in swine, the diameter of each is as follows:

- Swine Influenza Virus – 80-120 nm (.080 - .120 micron)[6]
- PRRSV – 50-65 nm (.050 - .065 micron)[7]
- PCV2 – 17-22 nm (.0017 - .0022 micron)[8]
- Mycoplasma – 0.3 – 0.9 micron[8]

As you can see, SIV, PRRSV, and PCV2 should be able to get through the HEPA filters. However, it is what they are carried on which is important, as bioaerosols are generally 0.4 – 0.7 micron. Also, tiny particulate is likely to stick to the filter fibers due to interception and diffusion processes.

Filter options

There have been many inquiries from producers about cheaper filters. I have found that not all filters are equal, and that the current rating systems are not necessarily applicable to the applications we are doing. For example, we are running the barns typically at .05-.20 (inches W.G.) for static pressure. When these types of filters are tested, they are done so at much higher pressures. As a result, quality of materials, frame integrity, etc. become much more important to make sure no air can bypass the filter material itself.

There has been some redesign of the frame and also a new filter which has better air flow, but may have a breaking point and allow virus to pass through at very high concentrations. Our strategy right now is that we will use the same filter as in the past (now called L9 filter since the new frame design) for farms that have a poor history with PRRS, have farms within about two miles, or have a large number of pigs within three or four miles. We will use the higher air flow filter for lower risk farms.

Partial filtration continues to be utilized due to cost. The problem is with just putting filters on the ceiling inlets and having no filtration for cool cell pads, most of those farms are unfiltered for about 4 months of the year. We've had one farm switch from partial filtration to 100% filtration just by switching the filter bank to the new higher airflow L6 filter.

For the Camfil Farr Merv 16 filter, now revamped to the PB L9 filter, we are figuring 600 cfm of air will go through the filter at 0.2 inches W.G. For the PB L6 filter, we are figuring 1000 cfm of air go through the filter at 0.2 inches W.G.[5] Most of the sites have been able to run the static pressure to 0.2 without any trouble, although it is important to know what fans you have and how they perform at these higher loads.

Partial filtration

Many of these sites simply put a filter above each of the ceiling inlets. If one filter was placed above the inlet, the air flow is adequate until the outside temperatures generally reach 65°-70° F. After that point, there is too much restriction and they must either remove the filters or utilize air coming through a cool cell in tunnel mode ventilation. One of the challenges with this system is that during the late spring and early fall in the Midwest, the temperature often gets hot enough during the day where tunnel mode is necessary. Then at night the temperature drops down into the 50's or 60's F when survivability of the PRRS virus is favorable.[9]

Bail out

Utilizing the survivability curve of the PRRS virus, it may be possible to bail out of the filtration system at a certain temperature while only taking on minimal risk of aerosol introduction of PRRS virus. In order to handle the large volume of air needed through a cool cell during summertime ventilation, a filter bank needs to be about three times the size of the cool cell pad or utilize squirrel cage type fans to pressurize the air going through the filter bank. Both of these can be quite costly options. An alternative to either of these systems is to bail out once the outside temperature gets hot, above 80° F for example. With this system, a filter bank can still be utilized to allow air to go through the evaporative cool cell pad but the high end ventilation needs can still be met without the additional cost of fans or large building structures containing the larger filter banks.

Another application of the bail out option is in farms where all of the air comes through ceiling inlets year-round. An extra row of inlets can be put in at a fairly minimal cost. If these inlets are actuated, they can also be tied to the ventilation system to provide a bail out option during the higher temperatures. These unfiltered inlets would open up when the outside temperature rises into the 80's F for example, and then are pulled shut as the temperature would drop again.

The bail out option can be a feasible low cost way to handle the majority of the risk throughout the year. It probably is not going to be a good application in high risk areas.

100% Filtration

100% filtration for a barn that goes to tunnel mode in the summer basically involves the construction of a filter bank in front of the cool cell. This does add construction cost. In addition, these facilities normally pull air through ceiling inlets in the winter so filters need to be mounted on top of each inlet box. The cost of implementation of a filtration system for these facilities, including cost of construction for the filter banks, has been $180-$200 per animal.

Some facilities pull air through inlets year round so just need filters mounted in front of each inlet. The cost of implementing filtration with this type of building design has generally cost $80-90 per boar.

Air conditioning Systems

Air conditioning systems greatly reduce the number of filters needed (because summer time ventilation needs don't exist anymore). Because the air comes in cool year round, the ventilation needs are similar to winter time year round.

Types of air conditioning systems

We have implemented five boar studs with air conditioning and 100% filtration using the MERV 16, 95% DOP filters over the last three years. In all cases, we use ceiling inlets and negative pressure ventilation for the cooler weather seasons, same as a normal swine facility would. All of these have filters above each inlet. As we transition into late spring, where winter time ventilation rates become inadequate to meet the moisture and heat removal needs of the barn, the air conditioning system starts to operate. There is some variation in systems on how this transition happens.

Recirulated air system

We have one farm that recirculates the air in the room to air condition the air. As the temperature outside rises into the upper 60's to low 70's Fahrenheit, the air conditioner turns on and recirculates and cools the air that is in the room. Fresh air continues to come in through the ceiling inlets and filters.

Fresh air systems

The other sites all use fresh air rather than recirculated air. There are three slight variations on how this is accomplished:

- One of the sites has air handlers in the attic and the air conditioned air comes in through the same ceiling inlets as before. In this site, the air is pulled through the air conditioning duct work year round. As the temperature warms up first the blowers come on to push outside air through the ceiling inlets. As the temperature in the barn continues to rise, the condensing units come on and air condition the air. The air handlers are in the attic but the condensing units sit outside so that the heat is released into the outdoors.
- Two of the sites have a similar system to above, except that the air conditioning unit and air handlers all sit outside and push the air in through the side wall rather than through ceiling inlets. This seems to be a simpler system because it avoids having all the air handling equipment and duct work in the attic. However, getting the air evenly distributed within the room is more of a challenge.
- Another system also has air coming in through the side walls, but inserts an evaporative cooling pad in front of the air conditioner and air handler. With this system, as the temperature rises, the blower comes on to help push air through the filters as above. However, as the temperature rises, water circulates through the evaporative cooling pad to cool the air as a stage prior to the condensing units of the air conditioner coming on. The purpose of this is to delay the condensing

units coming on until the upper 70's Fahrenheit rather than coming on with temperature in the upper 60's. This should save considerably on electrical costs.

Filter and air conditioning needs

Because the air conditioned facilities have control of the temperature of the incoming air, the ventilation rates can be winter time rates year round. All of the facilities operate in the range of 6 to 20 cfm per animal year round. This means a minimal number of filters is needed and greatly reduces the cost of filters. Normally a 24" x 24" filter would supply only 2 adult swine under maximum summer ventilation. With air conditioning operating under 20 cfm/animal year round, the same filter can supply 30 adult swine.

A rule of thumb is air conditioning needs is 5.5-7.0 animals per ton of air conditioning. The number of animals that can be supplied per ton of air conditioning is higher for a unit that recirculates the air. This air can be hard on equipment however. Farms using fresh air are in the 5.5 – 6.25 animals per ton range.

Costs

The costs of implementing an air conditioning system with filtration have varied widely. We have seen a range of $300-$600 per animal[10]. The reasons for this wide range have primarily depended on:

- Whether 3-phase electricity is already on the site or needs to be either brought in or a converter installed.
- The existing capability of the generator on site.
- Whether using recirculated air or fresh air. The recirculated air system is cheaper but will have more maintenance.
- New construction or retrofit. The new construction will be cheaper because one can avoid all the fans, cool cells, inlets, etc. that would normally be needed for summer ventilation.
- The type of air conditioning system. We've seen a range of using household type systems to large commercial systems.

Operating costs have run around $20-30 per animal more for the warm weather season.

Challenges with air filtration

Some of the challenges with the air filtration have been as follows:

- How can you get everything sealed up? Because the system relies on negative pressure, there is always the challenge of minimizing air entry into the building other than through the filter. Positive pressure systems address this but there has been concern about driving warm, moist barn air into cracks and rotting out the barn due to this air condensing in the walls in the winter time.
- Summer filtration. To cover the ventilation needs of a non air conditioned barn, you need about three times the number of filters in the summer as in the winter. Current research is suggesting that night time in the summer is still a significant risk, especially with gentle winds coming from an infected facility. Cooler night temperatures also are favorable for virus survival. 100% filtration or making sure the facility drives all the air through the filters at night are ways to control this risk.
- All doors have to be secured. We have implemented a rule that no external doors can be opened. Deads are taken out through isolation or a hallway where doors to the barn can be closed. Culls are taken out in a similar fashion. Entering animals also go through a double door system to minimize any air re-routing around the cool cell.
- Back-drafting through inactive fans is a challenge as fan louvers can stick open and don't provide a great seal when closed. We have put fan covers inside and outside during the winter and try to delay opening up fans as long as possible. Also, it is important that fans are working and should be checked at least once per week.
- Installing filters without damaging them. It is important that filters are not damaged during installation. Often, those installing filters only have experience with changing their furnace filter

at home. It important that those installing the filters understand that the filter material cannot be torn or damaged in any way during installation.

Results

The first boar studs in our practice were filtered in August 2005. Since implementation of air filtration, we have had three PRRS breaks on farms using partial filtration. All three were infected when the air was not being filtered. We have had two Swine Influenza breaks on 100% filtered farms. We have not had any Mycoplasma hyopneumoniae breaks on any of the filtered farms, although most are vaccinated.

Summary

Air filtration systems have been in place in our practice for three years, in a wide variety of farms and in large and small farms. For boar studs, filtration has become the standard, except for studs with no history of PRRS and located five miles or more from other pigs.

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Health, Hygiene, and Sanitation Guidelines for Boar Studs Providing Semen to the Domestic Market

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Article 1. Domestic (USA) Requirements

Section 1.1 Pre-entry (Herd of Origin) Health Requirements of Semen Donor Boars

1.1.1 All pre-entry qualifying procedures performed on the farm of origin are to be performed by or under the supervision of a United States Department of Agriculture (USDA) accredited veterinarian or, if the farm of origin is located in Canada, by or under the supervision of a Canadian Food Inspection Agency (CFIA) accredited veterinarian.

1.1.2 The herd of origin must be inspected by a USDA accredited veterinarian (or, when appropriate, by a CFIA accredited veterinarian) and found free from clinical evidence of infectious or communicable diseases and insofar as can be determined, from any history of infectious or communicable diseases during the preceding 30 days.

1.1.3 The herd of origin must be free from clinical evidence of infectious or communicable diseases of swine, and be considered a negative herd for brucellosis and pseudorabies (Aujeszky's) within 30 days prior to animal dispatch from the herd of origin to isolation at the designated AI stud facility.

1.1.4 All potential semen donor boars must be examined individually by a USDA accredited veterinarian (or, when appropriate, by a CFIA accredited veterinarian) within 30 days of farm-of-origin dispatch and any evidence of heritable physical defects is to be documented. Boars exhibiting any heritable physical defects should not be used as donor semen boars.

1.1.5 A Certificate of Veterinary Inspection is to be completed by a USDA accredited veterinarian (or, when appropriate, by a CFIA accredited veterinarian) and a copy of this certificate must accompany the animal(s) to the AI stud center isolation facility.

1.1.6 The entry of visitors to the pre-entry site should be controlled. Personnel allowed access to the pre-entry site should be technically competent and observe high standards of personal hygiene to preclude the introduction of pathogenic organisms. Protective clothing and footwear for use only on the pre-entry site should be provided.

1.1.7 Animals shall not have been fed garbage, food byproducts, or meat/bone meal products in diet.

Section 1.2 Isolation Health Requirements of Semen Donor Boars

1.2.1 All procedures associated with the assessment of the isolation health status are to be performed by or under the supervision of a USDA accredited veterinarian.

1.2.2 Only animals which have a completed Certificate of Veterinary Inspection and have followed the pre-entry requirements outlined in Section 1.1 can enter into an AI stud center's isolation site.

1.2.3 Isolation in this section is defined as a self-contained facility physically separated from swine and other animals. The isolation facility is maintained exclusively for the purpose of isolating incoming boars for observation. Movement out of isolation will be all-out, with the start of isolation commencing after introduction of the last boar into the self-contained facility.

1.2.4 All boars presented for entry as additions to the resident stud of a semen production center must undergo a minimum 15-day isolation to allow completion of the necessary tests as outlined in Section 1.2.6.

1.2.5 All animals in isolation will be observed for clinical signs of disease on a daily basis. If clinical signs such as excessive coughing, sneezing, changes in consistency or amount of manure, decreased appetite or water consumption, skin lesions, lameness, or lethargy are observed, the attending veterinarian must be contacted to determine if the animal(s) should be removed from the group for further diagnosis and/or therapeutic management. An examination and necropsy shall be performed by a veterinarian on any animal which succumbs to an unexplained death.

1.2.6 All animals in isolation shall be serologically tested through an accredited diagnostic laboratory with negative results for brucellosis and pseudorabies (Aujesky's). At the attending veterinarian's discretion, tests for the following pathogens and disease conditions may be performed: influenza, leptospirosis, mycoplasmosis, *Actinobacillus pleuropneumoniae*, porcine reproductive and respiratory syndrome virus (PRRSV), tuberculosis, and others as deemed necessary.

1.2.7 Removal or release of animals from isolation must be done only with the permission of the attending veterinarian.

1.2.8 The entry of personnel to the center's isolation facility should be controlled. Personnel allowed access to the isolation facility should be technically competent and observe high standards of personal hygiene to preclude the introduction of pathogenic organisms. Protective clothing, footwear, and all husbandry equipment must be provided for use only in the isolation facility.

1.2.9 Animals shall not be fed garbage, food byproducts, or meat/bone meal products in diet.

Section 1.3 Health Requirements for the Resident AI Stud Herd

1.3.1 Resident AI Stud facility requirements include:

1.3.1.1 Protective clothing and footwear specific for stud.

1.3.1.2 Constructed as a bird-proof facility.

1.3.1.3 Rodent control in place.

1.3.1.4 Insect control in place.

1.3.1.5 Physically separated from other swine and preclude direct contact with other livestock.

1.3.1.6 Entry of visitors to the resident AI stud should be controlled. Personnel allowed access to the resident AI stud should be technically competent and observe high standards of personal hygiene to preclude the introduction of pathogenic organisms. Protective clothing, footwear, and all husbandry equipment must be provided for use only on the resident stud site.

1.3.1.7 Feed and other supplies must originate from a premise free of livestock and be delivered directly to the stud from the source.

1.3.1.8 Loading and unloading areas for boars and supplies must be kept clean and free of organic material.

1.3.1.9 Animals shall not be fed garbage, food byproducts, or meat/bone meal products in diet.

1.3.2 Once a boar has completed the pre-entry and isolation requirements, and is officially released by the attending veterinarian as outlined in Section 1.1 and 1.2, he may enter the resident AI stud where he shall continue to be tested in accordance with the testing procedures listed below so long as he remains in the stud.

1.3.3 The resident AI stud should be maintained as a Validated Brucellosis-free and Qualified Pseudorabies (Aujesky's) Negative Herd.

1.3.4 At the attending veterinarian's discretion, tests for the following pathogens may be performed: influenza, leptospirosis, mycoplasmosis, *A. pleuropneumonia*, PRRSV, tuberculosis, and others as deemed necessary.

1.3.5 If on any given day greater than four percent (>4%) of the boars standing at the resident AI stud facility exhibit similar clinical signs which could be associated with an infectious disease, a USDA accredited veterinarian must immediately assess the resident AI herd, and will be required to determine if sufficient risk warrants closure of the herd to further shipments of donor semen. Closed herds can be released by the USDA accredited veterinarian after he/she determines there is minimal risk in the transmission of disease via semen.

Section 1.4 Hygiene and Sanitation Requirements for Semen Collection, Processing, and Storage

1.4.1 General Requirements

1.4.1.1 Semen may only be collected, processed, and stored from boars that fulfill the requirements set forth in Sections 1.1, 1.2, and 1.3 of this document.

1.4.1.2 Only semen originating from resident boars may be analyzed, processed, and stored at the resident stud.

1.4.1.3 Semen collection, processing, and storage takes place only on the premises set aside for this purpose and under conditions of the strictest hygiene.

1.4.1.4 All implements which come into contact with the semen or the donor animal during semen collection and processing are single-use, disposable materials or, if re-usable, are properly disinfected or sterilized between uses.

1.4.2 Semen Collection

1.4.2.1 Semen may only be collected from boars which show no clinical signs of infectious disease on the day the semen is collected.

1.4.2.2 Each collection of semen, whether or not it is separated into individual doses, is clearly marked in such a way that the identification of the donor animal(s) is evident.

1.4.2.3 Each collection of semen is obtained using prudent minimum contamination protocol practices, which include:

1.4.2.3.1 Use of a collection pen which is cleaned after each daily use following proper sanitary techniques.

1.4.2.3.2 Use of double gloves of a non-spermicidal nature, with the outer glove discarded after preparation and stimulation of the boar, allowing for a clean gloved hand for direct grasping of the penis.

1.4.2.3.3 Clipping of preputial hair surrounding preputial opening.

1.4.2.3.4 Cleaning of the preputial opening and surrounding area (if needed) with a single-use disposable wipe.

1.4.2.3.5 Evacuation of preputial fluids prior to grasping of the penis for semen collection.

1.4.2.3.6 Holding of the penis perpendicular to the boar to minimize the contamination of the semen with preputial fluids.

1.4.3 Semen Processing

1.4.3.1 The entry of personnel to the semen processing site should be controlled. Personnel allowed access to the semen processing site should be technically competent and observe high standards of personal hygiene to preclude the introduction of pathogenic organisms. Protective clothing and footwear for use only at the semen processing site should be provided.

1.4.3.2 Only single-use disposable products or sterilized re-usable products should come into contact with semen in order to prevent cross-contamination of ejaculates or pooled semen during processing.

1.4.3.3 Semen extenders or diluents:

1.4.3.3.1 Whenever any animal protein is used as part of a semen diluent, the product must be free of pathogens and sterilized.

1.4.3.3.2 An effective preservative antibiotic or antibiotic combination using chemicals of U.S.P. grade must be present in the extender or diluent which is to be used to expand the volume of boar semen. Preservative antibiotic or antibiotic combination choices with minimal active concentrations at final dilution are as follows:

1.4.3.3.2.1 500 IU penicillin/500 mg streptomycin per mL final dilution.

1.4.3.3.2.2 150 µg lincomycin/300 µg spectinomycin per mL final dilution.

1.4.3.3.2.3 250 µg gentamicin sulfate/250 µg neomycin sulfate per mL final dilution.

1.4.3.3.2.4 200 µg gentamicin sulfate per mL final dilution.

1.4.3.3.2.5 50 µg ceftiofur sodium per mL final dilution.

1.4.3.4 Each dose of diluted semen must be clearly marked in such a way that, at a minimum, the date of semen collection and appropriate identification of the donor animal(s) are evident. If donor identification is coded, the semen processing center must keep on file for no less than 4 months a record of donor animal(s) which contributed to the coded doses.

1.4.3.5 Each extended/diluted semen dose shall have a unique origin/laboratory identity clearly marked on it. Semen originating from any other laboratory shall not have the same identity.

1.4.4 Extended/Diluted Semen Storage

1.4.4.1 Only extended/diluted semen doses that originate from the resident AI boars which have fulfilled the requirements set forth in Sections 1.1, 1.2, and 1.3, and have been collected and processed as set forth in Section 1.4, may be stored in individual semen containers and storage areas at the stud.

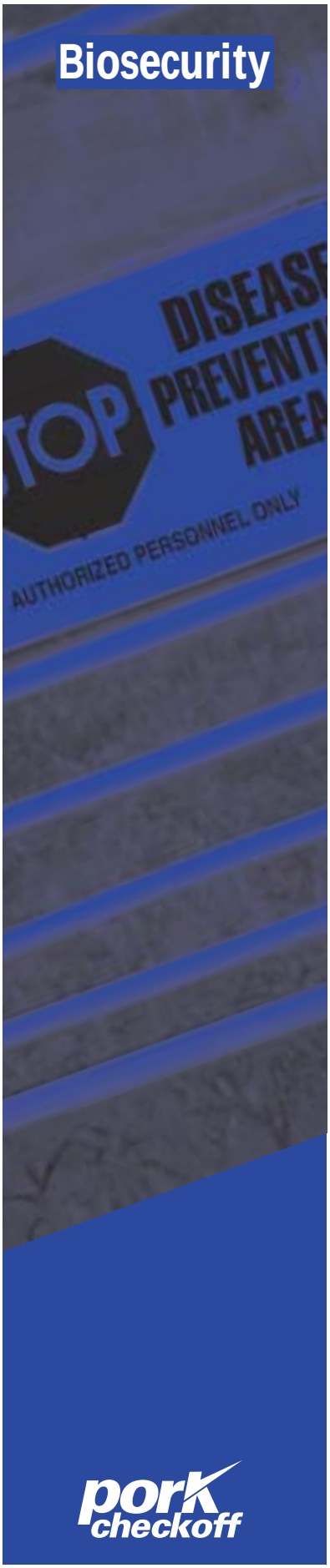
1.4.4.2 Extended/diluted bulk and packaged semen is to be stored only in individual semen containers and storage areas which are capable of being disinfected.

1.4.5 Disease Control of Extended/Diluted Semen

1.4.5.1 Monthly aerobic bacteriological culturing is to be performed on randomly selected individual or pooled semen lots which are at least 48 hours of age post-processing, with the number sampled representing 1% of total monthly collections or four (4) samples/week, whichever is greater. Identification of samples positive for significant bacterial contamination will be followed up with a review of stud hygiene and sanitation by a veterinarian.

1.4.5.2 An established monitoring program which minimizes the risk of PRRSV transmission in the extended semen product is to be in place.

Biosecurity



Biosecurity and Health Assurance at a Boar Stud

An Outline of Questions to Ask Your Semen Supplier

Introduction

Preventing the introduction of disease agents is a continuous challenge for pork producers and veterinarians. When a farm or site is affected by disease, the impact can be devastating to the health of the swine and the producer's bottom line. If a foreign animal disease were to overcome the biosecurity safeguards in place on our farms and our country, it would have a devastating effect on all pork producers.

One route of disease entry to a farm is through introduction of genetic material. Introduction of live animals offers the greatest risk of disease transmission. Artificial insemination can lessen this risk; however, biosecurity still is very important because bacteria and viruses can spread from infected boars to females through semen. Consequently, it is recommended that producers and veterinarians develop farm-specific biosecurity protocols for purchased or delivered semen.

Bacteria Found in Boar Semen*

Common	Infrequent
Staphylococcus spp.	Corynebacterium spp.
Pseudomonas spp.	Streptococcus spp.
Escherichia spp.	Proteus spp.
Klebsiella spp.	Bacillus spp.
Citrobacter spp.	Enterobacter spp.
Micrococcus spp.	Aerobacter spp.
Eubacterium spp.	Bordetella spp.
	Mycoplasma spp.

Viruses Found in Boar Semen*

Common	Infrequent
Adenovirus	Pseudorabies virus**
African swine fever**	Porcine parvovirus**
Classical swine fever virus**	Porcine reproductive respiratory syndrome virus**
Cytomegalovirus	Reovirus
Enteroviruses	Swine vesicular disease virus
Foot-and-mouth disease virus	Transmissible genital papilloma virus
Japanese encephalitis virus	

**Known to be transmissible through semen

* Source: The Swine AI Book: Second Edition, Chapter 3; Publisher: Dr. Morgan Morrow, NCSU

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Detection of bacteria and viruses in semen does not necessarily correlate with transmission of those agents through semen. In fact, most bacterial and at least some viruses present in semen can be the result of contamination during collection and processing and not actual shedding by the boars.

Biosecurity Considerations for the Stud Facility

The following questions can be used as a framework to assist pork producers and their veterinarians in assessing the biosecurity risk associated with a potential new semen supplier for their herd or to evaluate the biosecurity of their current semen supplier. More detailed, farm-specific questions may evolve from these questions through active participation by your veterinarian. As you work through this exercise, keep the following questions in mind:

1. **Has the semen supplier been able to answer your questions?**
2. **Are you satisfied with the answers you received?**

Figure 1 is designed to assist readers in understanding some of the terminology used in the suggested questions that follow.

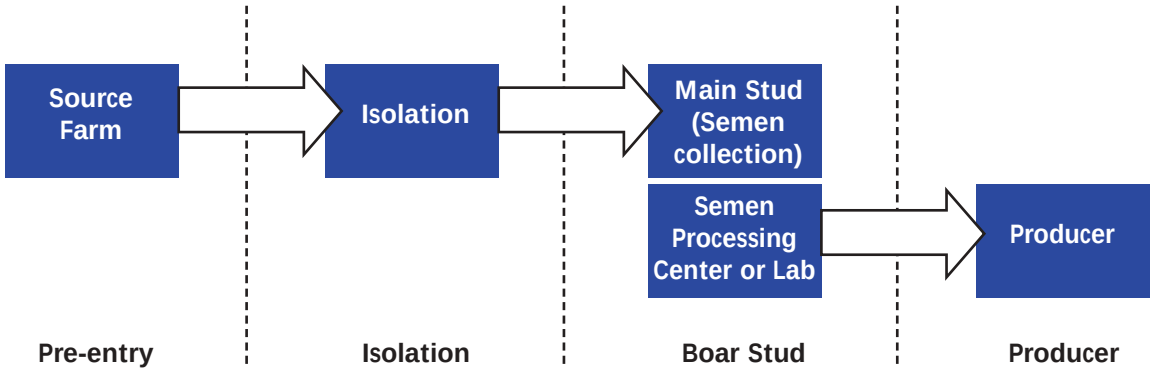

Figure 1. Schematic Production Flow
Fresh semen cannot be "isolated" as you would live animals. Good biosecurity at the stud is your only means for minimizing the disease risks that come with using semen from an outside supplier. However, even when your semen supplier does everything correctly, the biosecurity risk is never zero.

General Inquiries for Boar Stud:

This section is designed to establish the general health status and biosecurity practices of the boar stud.

What is the number of source herd(s) from which the current boar population at the stud originated?

What is the number of source herd(s) that have contributed to the population at the boar stud since its original stocking?

Does the boar stud utilize an isolation facility for replacement boars?

How far is the boar stud facility from other swine? From a major highway?

Are biosecurity procedures for the boar stud in written format and available for review?

Do you have a written visitor policy that is available for review?

Is the boar stud a shower-in, shower-out facility?

Is downtime from pig contact required before people can enter the facility? If yes, what is the length of the downtime required?

Are employees allowed to raise pigs on their home farm?

The National Pork Board developed this questionnaire. Content was reviewed and revised by the National Pork Board (NPB) Swine Health Committee, the NPB/American Association of Swine Veterinarians Biosecurity Working Group, and Dr. Sandy Amass, Director of the National Biosecurity Center at Purdue University.

Please provide documentation to indicate the status of the boar stud for the following diseases: porcine reproductive and respiratory syndrome (PRRSV), pseudorabies (PRV), brucellosis, and leptospirosis.

Have there been any clinical signs of disease within the boar stud in the last 12 months? If yes, please provide diagnostic information and the actions taken.

Is semen ever collected from boars that have been allowed to serve naturally?

Has the boar stud ever been depopulated? If yes, for what reason?

Does semen shipped from the processing center originate from a single stud or multiple studs?

Where is feed for the boar stud produced? Are biosecurity protocols in place at the mill?

Are animal by-products allowed as a feed ingredient?

Is the feed delivered to the stud in meal form or is it pelleted?

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Pre-entry or Source Herd Inquiries:

Are there any specific health procedures and requirements of the source farm(s) prior to shipment of individual animals to the boar stud?

Does the source herd(s) have a written herd health assurance program(s) that is available for review?

Does the source herd(s) have a designated herd veterinarian? Does the herd veterinarian make regular visits to observe the animals?

How frequent are the visits? Please provide the contact information in the event my veterinarian wishes to initiate a vet-to-vet health communication.

Describe the current health status of each source herd(s).

Are biosecurity procedures to prevent disease introduction, including isolation protocols, available from the source herd(s) in a written format?

Please identify any vaccines used in the past 24 months. What is the current vaccination protocol at the source herd(s)?

Is any diagnostic testing performed on a routine basis? If yes, what tests are performed?

Does a veterinarian interpret all diagnostic test results?

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How frequently does communication occur between the source herd veterinarian and the boar stud veterinarian?

_____

_____

What transportation biosecurity protocols are used when delivering boars to the boar stud?

_____

_____

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Isolation Procedures:

Isolation allows time to observe new boars for signs of disease before entry to the stud. Isolation also provides the opportunity to test animals for exposure to certain diseases and to acclimate or vaccinate animals. Continuous pig flow through an isolation facility cannot be considered proper isolation. Failure to isolate new boars offers the greatest risk of disease introduction into the boar stud, and subsequently into your herd.

Does the boar stud utilize isolation procedures?

Is pig flow through isolation managed in an all-in, all-out manner?

Is the isolation building cleaned and disinfected between groups of boars?

Are boars in the isolation unit exposed to the outdoors or totally enclosed?

Is the isolation facility on-site or off-site?

How far is the isolation facility from other swine, including the main stud?

What is the length of the isolation period?

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Does the isolation facility serve more than one boar stud or swine farm?

What vaccination and parasite control protocols are used during isolation?

Are the boars in isolation routinely monitored for signs of clinical disease? If yes, how often and by whom?

Are boars in isolation routinely monitored for seroconversion to specific pathogens?

If yes, which pathogens?

Please describe the timing and frequency of diagnostic testing in isolation as it relates to boar entry dates and vaccinations.

Serological testing at the time of entry may provide a source herd baseline for interpreting results and can indicate exposure to diseases in the past. Testing two to three weeks after entry into isolation gives the boar's immune system time to produce the antibodies that are detected by the test. Contamination during transportation may not become serologically evident until two to three weeks into isolation. It requires ten days to three weeks after exposure before a boar will test serologically positive to most diseases.

If routine serological testing is performed in isolation, is this testing performed on the entire population or on a subset of the entire population?

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If a subset (percentage) of the boars is tested, how is the number of boars to be tested determined?

Does a veterinarian review and interpret all test results?

Are any boars ever moved to the main stud before diagnostic results are received and interpreted?
What is the policy if a boar in isolation tests positive for a pathogen on a diagnostic test?

- Is the sample rerun utilizing the same test?
- Are other tests for the same pathogen run?
- Is the boar retested?
- Are any of the other boars in isolation retested? If yes, how many and how long after the positive result?
- If the positive boar continues to test positive, then what is the protocol?
  - Is the boar removed from isolation?
  - Does a veterinarian perform a post-mortem on the boar?
  - Is a complete diagnostic work-up performed with samples submitted to an accredited diagnostic lab?
  - What health assurance measures are taken on the remaining boars?

What is the protocol if a boar dies in isolation?

- Does a veterinarian perform a post-mortem on the boar?
- Is a complete diagnostic work-up performed with samples submitted to an accredited diagnostic lab?

What transportation biosecurity protocols are used when delivering boars from isolation to the boar stud?

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Health Assurance of the Main Stud:

Disease monitoring is often a routine part of health maintenance at a boar stud. Testing of boars and semen can be useful in the detection of disease. In addition to concern about new diseases entering the stud, attention must be paid to the overall well-being of individual boars and their freedom from common diseases and injuries. These questions will define the normal standard of care for boars once they have entered the main stud.

How often are boars entered into the stud?

Does a formal and written health assurance plan exist for the main stud?

Are boars routinely monitored for specific pathogens through serological testing or other diagnostic procedures? If yes, describe which pathogens and the method, timing, and frequency of the diagnostic testing.

If serological testing is used, is it performed on the entire population or on a subset of the entire population?

If a subset (percentage) of boars is tested, how is the number of boars to be tested determined?

What percentage of the boar stud population is tested...
- on a monthly basis?
- on an annual basis?
- other?

Are diagnostic tests performed on semen? If yes, which tests and how often?

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Has the boar stud ever been determined to be positive for Porcine Reproductive and Respiratory (PRRS) virus? If yes, please describe the status today.

Does a veterinarian review and interpret all test results?

Please identify any vaccines used in the past 24 months. What is the current vaccination protocol for the boar stud?

Is a new needle used for each boar that is vaccinated or treated?

What is the response protocol if a boar in the main stud tests positive for a pathogen on a diagnostic test?

- Is the sample rerun utilizing the same test? Are other tests for the same pathogen run?
- Is the boar retested?
- Are any of the other boars in the main stud retested? If yes, how many and how long after the positive result?
- If the positive boar continues to test positive, then what is the protocol?
  - Is the boar removed from the main stud?
  - Does a veterinarian perform a post-mortem on the boar?
  - Is a complete diagnostic work-up performed with samples submitted to an accredited diagnostic lab?
  - What health assurance measures are taken on the remaining boars?

What is the protocol if a semen sample tests positive for a pathogen on a diagnostic test?

What is the protocol if a boar dies in the main stud?

- Does a veterinarian perform a post-mortem on the boar?
- Is a complete diagnostic work-up performed with samples submitted to an accredited diagnostic lab?

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Herd Closure:

Herd closure occurs when a confirmed or suspected disease situation occurs at a boar stud that requires the termination of all semen deliveries from the stud. Customers should be aware of the criteria that would initiate a herd closure event, understand how and when they would be contacted by the semen supplier, and have plans for alternative sources of semen before they enter into any arrangement with a single boar stud.

What constitutes closure of the boar stud for semen shipments? Is this protocol formal and written? If so, please provide a copy of this protocol.

Who decides the stud should close for shipments…
- herd veterinarian?
- manager?
- genetic supplier?
- other?

Is there a written communication plan to quickly notify customers in the event of a closure? Please explain the procedure.

Is there a back-up plan to supply semen from an alternative source in the event that the boar stud is closed for health or any other reason? Is this plan formal and written? If so, please provide a copy of that plan.

If the back-up plan involves another stud or semen supplier, is that alternative source compatible in the areas of health status and quality assurance?

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Semen Processing Center or Lab:

The semen laboratory processes are critical to successful implementation of an artificial insemination program. Semen quality from even the healthiest boars can be compromised if proper technique is not followed in the laboratory.

Please describe the minimum standards for a dose of semen in regards to concentration (number of sperm per dose), motility, and morphology.

Is there a designated clean area and clean sterile equipment for semen, collection, processing, and storage?

Is there a written protocol available to determine if a boar is eligible or ineligible for collection?

Does the stud have written procedures available for semen collection, processing and storage?

Does the semen processing area have written sanitation protocols available?

Is there a written protocol for monitoring the quality and bacterial contamination levels of semen samples? If yes, please provide.

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Notes:

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