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PIC®
Boar Stud Management

PIC®

INTRODUCTION

Welcome to the latest version of the PIC Boar Stud Management manual. We hope you find it useful to your operation. Now, more than ever before, boar studs must be on the forefront of incorporating new technologies that aid in producing semen doses of the greatest quality through consistent and accurate evaluation, while ensuring the health and well being of the boars. What we do at the boar stud is vital to achieving challenging production targets and maintaining our global competitive advantage in the future.

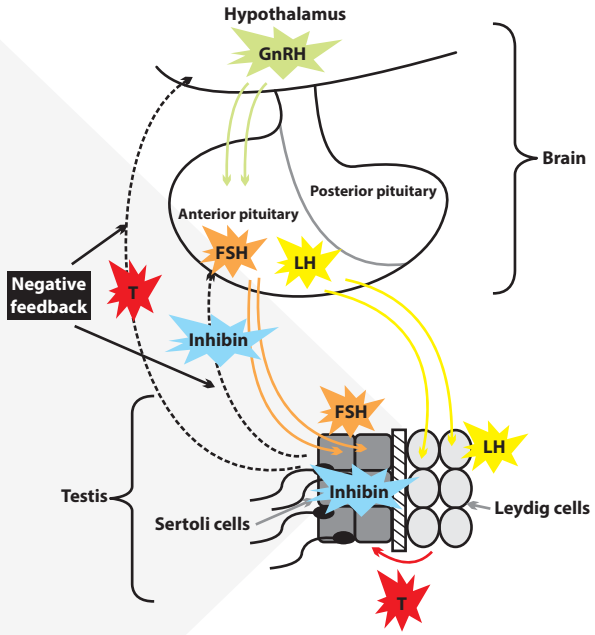
We believe the information included in the manual will be helpful in educating new employees as well as challenge experienced personnel to reevaluate barn and lab processes. Boar sperm production is reviewed as well as management topics in the barn and laboratory in order to improve semen quality and production in your boar stud.

PIC is committed to making improvements to boar stud processes already in place and eagerly seeking out and incorporating new knowledge that will ensure producers remain globally competitive.

CONTENTS

The brain produces the hormones GnRH (gonadotropin releasing hormone), LH (luteinizing hormone) and FSH (follicle stimulating hormone) which work together to promote and regulate testosterone (T) production and ultimately sperm cell development and male behavior (Figure 1). These processes have to occur for reproduction to be possible.

Figure 1: Schematic of hormone production and regulation in sperm production



The process of boar sperm cell and testicular development starts early during the fetal stage with reproductive behavior beginning as early as 1 month and an increase in semen production at 6 months (Table 1).

The testes are responsible for the production of sperm and testosterone. Sperm are produced and move towards the center of each testis or the mediastinum (white area) and then continue on to the head of the epididymis (Picture 1). A sexually mature boar is capable of producing 16 x 109 sperm per day from both testes (Senger, 2005).

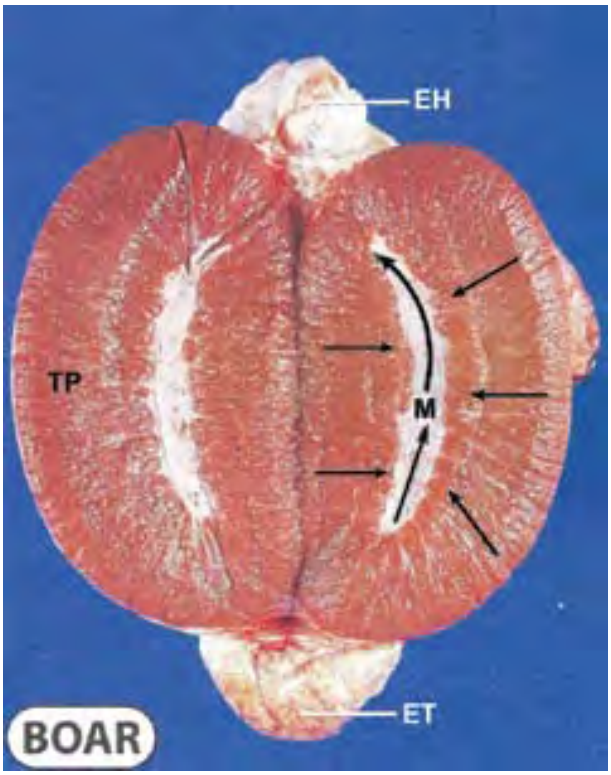
- Sperm production, or spermatogenesis, is a highly complex process that occurs within specialized compartments in the testes called seminiferous tubules. At any given time, there are sperm cells at different stages of development to allow for continual sperm production. The spermatogenesis process takes 39 days on average in a boar (Senger, 2005).

Table 1: Process of Boar Maturation

Age	Maturation Process
Fetus (d 20 – 40 gestation)	Germ cell division and differentiation
Fetus (d 60 gestation)	Testicular dissension from the abdomen into the scrotum
1 – 2 months	Mounting behavior displayed
3 months	2nd germ cell division and increase in testes to body weight ratio
4 months	Sperm appear in seminiferous tubules and erections can occur
5 ½ months	Puberty begins and sperm appear in ejaculate
6 – 18 months	Testes size, semen concentration and ejaculate volume increases

(Knox, 2003)

Picture 1: Flow of sperm production



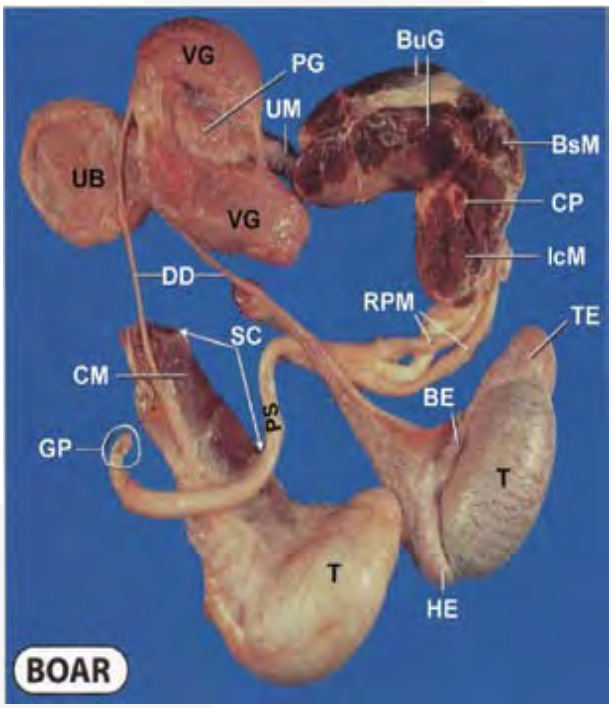
TP = testicular parenchyma, EH = epididymal head, ET = epididymal tail, M = mediastinum (Senger, 2005)

The epididymis is comprised of three sections including the head, body and tail. Each section plays a part in sperm storage, sustenance and the completion of maturation (9-14 days). Sperm cells must transit through the epididymis in order to acquire the potential for fertilization. Without the epididymis, reproduction in the boar would not be possible.

The accessory sex glands include the prostate gland, vesicular glands, and bulbourethral glands and are important for adding seminal plasma to the sperm cells. The prostate gland's function is to remove urine and bacteria from the reproductive tract before sperm enter the urethra. The vesicular glands produce secretions that are viscous and milky in appearance and comprise the majority of the volume of the ejaculate. Lastly, the bulbourethral glands produce the gel fraction of the ejaculate and are large and dense in the boar (Knox, 2003; Senger, 2005).

The penis is divided into three sections including the base, shaft and glans penis. The glans penis contains sensory nerves and is responsible for the commencement of ejaculation. The shape of the glans penis is similar to a cork screw which is unique to the boar (Senger, 2005).

Picture 2. Reproductive organs in the boar



BE	=	Body of Epididymis	PS	=	Penile Shaft
BsM	=	Bulbospongiosus Muscle	RPM	=	Retractor Penis Muscle
BuG	=	Bulbourethral Gland	SC	=	Spermatic Cord
CM	=	Cromaster Muscle	T	=	Testis (left T-parietal vaginal tunic intact; right T-parietal vaginal tunic removed)
CP	=	Crus Penis			
DD	=	Ductus Deferens			
GP	=	Glans Penis	TE	=	Tail of Epididymis
HE	=	Head of Epididymis	UB	=	Urinary Bladder
IcM	=	Ischiocavernosus Muscle	UM	=	Urethralis Muscle
PG	=	Prostate Gland	VG	=	Vesicular Gland

(Senger, 2005)

ISOLATION AND ACCLIMATION

Boars typically enter isolation at 6 months of age and remain there between 4 and 8 weeks. This time period should be used to test for important diseases and establish vaccination protocols. Depending on the number of vaccinations, these should be spaced throughout the isolation period in order to spread out the stress associated with vaccinations.

The isolation facility and its location depend on the regional pig density. Normally a distance of 2.4 - 3.2 km from the stud is preferred but in a pig dense area it is better to have it closer to the main stud and even attached by a covered walkway. The doorway into the stud should be locked during the isolation period until testing releases the boars into the stud. With the development of barn filtration, steps can be taken to reduce the possibility of spreading a disease to the main stud. If the main stud is filtered to prevent entry of disease, the isolation facility should be filtered as well. If the main stud is not filtered due to location in a low pig dense area, the isolation exhaust air can be filtered to prevent possible infection of the stud. The exhaust filters can be opened up after testing, indicating the group is negative for the diseases of concern.

- Do:
  - Have a separate shower-in facility.
  - Stud workers may take care of the isolation unit at the end of the day with an overnight downtime back into the main stud.
  - Perform an initial statistical test on the boars within seven days of arrival and then a 100% testing of the population at the end of the isolation period.
  - For a facility that is located away from the main barn be sure to wash, disinfect and dry the trailer prior to moving the boars to the stud.
  - Move the boars into the stud as soon as possible after receiving negative test results.

- Do Not:
  - Locate the isolation facility too close the main stud (less than 400 meters) if it does not have exhaust filters and the main stud is not filtered.

Managers may choose to train boars in isolation or in the stud. Either option will work as long as the proper training protocol is in place (see Part 8: Training).

Boars should be housed individually and not mixed during transfer to the main stud.

Record daily high and low ambient temperatures in the barn.

Boars should be isolated upon delivery from the source farm per the PIC sales agreement (Conditions of Sale).

The isolation facility must be washed, disinfected, and dried between groups. Downtime of 1 day after appropriate cleaning, disinfecting, and drying is recommended.

The minimum isolation period is 30 days after the last entry of boars into the isolation unit.

All boars in isolation should be clinically monitored each day. Data should be recorded on individuals exhibiting clinical signs or requiring treatment. Any boars that are off-feed or are clinically ill should have their temperature recorded and be monitored and managed on an individual animal basis. Increasing incidence of off-feed or feverish boars day to day is indicative of a disease introduction. It is recommended that the boar stud manager notify their veterinarian if clinical disease and/or deaths occur.

Testing for release of boars into the stud includes the following:

- Test a statistical sample of the boars within 7d of arrival
- Ensure the diagnostic laboratory runs the PCR prior to the ELISA to avoid contamination. The population must be determined to be negative by the veterinarian prior to entry into the main stud.
- Specific disease testing requirements will vary by country and region/state. Consult your

veterinarian.

- See Part 10. Welfare and Health for additional details on testing in isolation.

Do not move boars from the isolation into the main stud if PIC notifies you of a health concern in the source herd or if the isolation is experiencing a clinical outbreak of any disease.

Prior to release of isolation animals to the stud, communicate with your herd veterinarian or PIC Health Assurance to verify the current health status of the source herd.

Biosecurity of the isolation facility should be maintained by requiring all staff and service personnel to shower-in and showerout of the isolation facility.

- Service personnel entering the isolation facility should follow the same restrictions used at the stud and adhere to the same downtime rules.
- Production staff may visit and work in the isolation unit after working in the stud, but must observe one night of downtime prior to returning to the stud.

PIC will inform the stud of any significant change in the health status of the PIC source herd. The stud or its veterinarian will be provided with the results of blood tests performed on the source farm for the boars that are destined to be placed into the quarantine facility on request.

GENERAL MANAGEMENT OF BOARS

Barns should be walked daily by the manager or an assigned technician. Look for boars that did not clean up their feed, get boars up every day at feeding to observe for lameness and also observe for coughing or a respiratory problem. Consult with your veterinarian for suggested treatment protocols

Records should be kept for any boars that are treated or off-feed.

When placed in the barn boars should be placed by line and then by age. Keep young boars grouped together and avoid mixing them in between older boars.

BARN MANAGEMENT

Management recommendations pertain to both the isolation facility and the main stud.

Boars are typically quiet animals but stud staff needs to prevent any unnecessary risks when training, sampling, treating, walking, and collecting them. When taking boars to/from the collection area, walk behind the boars and use a sorting board (see Part 10: Welfare and Health for more information).

Optimal temperature for sperm production is 18-20°C. Misters, atomizers, evaporative cooling and air conditioning are used to control barn temperatures but care must be taken to avoid creating a wet environment.

Barns should have adequate ventilation and air movement to reduce ammonia and odor levels, while maintaining acceptable ambient temperatures for both the boars and barn personnel (See Part 5. Ventilation and Air Flow). The backs of crates should not be solid, but open bars, to allow for adequate air movement and optimum temperature around the testicles.

The minimum water flow rate is 2 L/min and boars consume 5.6-7.8 L per day on average. Flow rate should be measured once per quarter (once a week during the summer months) to make sure the boars have sufficient access to water to prevent tissue water depletion and dehydration. Chemical testing of the water should also be performed to detect impurity, mineral and bacteria levels twice per year. Local municipalities may require more frequent testing.

Foot baths with a copper sulfate solution should be used upon entry to and exit from the isolation facility. In the stud, boars should be walked through the foot bath after collection on the way back to the stall. This will help harden the hooves and prevent lameness problems in the stud.

Mats should be placed under boars with leg or hoof problems to ensure comfort and promote recovery. Enough mats should be on hand for 10% of stud capacity.

Check to ensure that NO stray voltage is flowing through the water lines and equipment.

Many different management factors in the barn can impact semen quality (Table 2).

Each day after collections are finished, the collection area should be power washed with hot water and high pressure. Areas to clean include the warm-up area, collection pens or crates, dummy and mats. After washing, the area should be clean of organic material (i.e. manure, semen). Once a week, disinfect the collection area after washing with a product made specifically for animal facilities. Be sure to include all surfaces (i.e walls, bars on the crates).

Table 2: Management Factors that Impact Semen Quality

Situation	Description	Effect on Boars	Reference	Recovery
High ambient temperatures	>29°C for 3 days or more	Sharp increase in abnormal sperm per ejaculate	McNitt and First, 1970 Wetteman et al., 1976	8 weeks
Moderate ambient temperatures plus high humidity	26 - 29°C + 75% humidity or greater for 4 wks or more	Gradual increase in abnormal sperm per ejaculate	Suriyasomboon, 2005	6-8 weeks
Fever (caused by vaccination or disease)	Body temperature >39°C for 2 or more days	Sharp increase in abnormal sperm per ejaculate	McNitt and First, 1970	8 weeks
Increased and erratic collection regimens	>3 times per week	Gradual decrease in number of normal sperm per ejaculate	Kennedy and Wilkins, 1984	After 2 weeks of rest
Reduced nutrient intake	>15% reduction in energy or protein intake for more than 8 weeks	Reduced libido and gradual decrease in normal sperm per ejaculate	Louis et al., 1994	Variable, depends on severity of the restriction
Suboptimal photoperiods	>16 hours of light or <8 hours of dark	Gradual decrease in libido and no consistent changes in sperm output	Sancho, 2004	NA
Immature boars	<6 to 7 months depending on genotype	Low volume of semen; low numbers of normal spermatozoa and presence of cytoplasmic droplets	Kennedy and Wilkins, 1984	Maturation

VENTILATION AND AIR FLOW

Optimizing environmental conditions for boars are critical for several specific reasons:

- Optimized sperm cell and quality semen production.
- Regulation of daily maintenance feed requirements.
- Control of bacterial growth within the environment.
- Promoting health and minimizing lameness.

The goal of a ventilation program is to achieve desired room temperature (DRT) and humidity through removal of heat humidity to create comfort.

DRT refers to the optimal temperature for boar comfort within a given environment. Adjustments must be made to DRTs to account for different environments such as flooring and building type.

- Different DRTs have an associated set point (the point at which variable stage fans increase speed) considering variable environments in order to achieve maximum boar comfort (Table 3; excerpt from PIC's Ventilation Modeling Tool available upon request).

Table 3: Building Environment Variables and Recommended Optimized Conditions

	Example 1	Example 2	Example 3	Example 4
Flooring Type	Slats	Slats	Solid	Solid
Barn type	Solid Sided	Curtain	Solid Sided	Curtain
Desired room temperature	19°C	20°C	17°C	18°C
Winter set point	21°C	22°C	19°C	20°C
Summer set point	18°C	19°C	16°C	18°C

Relative humidity in a boar stud should be between 40 and 65%.

Humidity and DRT are controlled by managing and manipulating inside and outside air exchange rates measured by cubic feet per minute (m^3/hour).

- During normal respiration the boar produces both heat and water vapor causing an elevation of barn temperature and humidity unless properly exhausted.
- The recommended minimum m^3/hour is 14 and is a calculated estimation of the required air exchange to maintain humidity and temperature.
- When humidity and temperature are outside of the optimal range, changing the m^3/hour is required to properly exhaust the excess heat and replace with cooler, dryer air.
  - Cooler air holds less water vapor allowing an effective drop in the barn's relative humidity.
  - Increasing ventilation rates to improve humidity when outside temperatures are above desired room temperatures will not improve humidity.

Air speed is important to effectively mix cooler air sourced from inlets in order to eliminate drafts and areas of condensation and is measured in meter per second (m/s).

- An air speed of 4.06 m/s is optimal for elevated fan stages, while 2.03 m/s is much more practical in minimum ventilation stages.
- Routinely evaluate air speed from inlets to assure proper mixing of air within the barn.

Supplemental heaters are required to control lower critical temperatures and are essential in emergency situations.

- Each facility requires the addition of supplemental heaters to assure the control of lower critical temperatures and heaters for use in emergency situations.
- If the heaters are set too close to the set point excessive liquid propane or natural gas will be used. Use a minimum of 2 degrees heater off-set below increasing variable fan speeds at set point, i.e. if the set point is 21°C then heaters turn on at 8°C.

Fan staging is designed to keep the building as close to the DRT as possible without causing major temperature variations by progressively removing heat and humidity as the barn warms through increasing m^3/hour.

- Fan speed does not equal m^3/hour (i.e. 50% fan speed does not equal 50% m^3/hour), therefore it's important to understand the relationship between variable fan performance and fan

exhaustion rate (Figure 2).

- Different sizes of fans, along with the presence of a cone, influence m^3/hour output (Table 4).

Figure 2: Viable Fan Performance

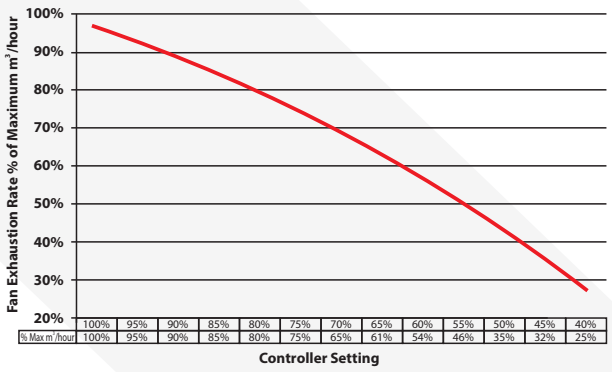


Table 4: Estimations of CFM by Fan Size

Fan Size cm	m^3/hour Output	m^3/hour Output with Cone
20	765	850
25	1869	2038
30	2549	2718
46	5947	6116
61	9684	10194
91	16480	16990
122	28883	30582
127	37378	39077
140	39077	40776

- Motor curves correspond to different fan sizes and are defined as the relationship between the voltages supplied to the motor and the resulting RPM.
  - Incorrect matching of motor curve and fan size may either burn fans up or cause inaccurate fan speeds, i.e. a 60% fan speed setting results in 90% fan speed.
- Instances in which air exchange rates increase include rising outside temperatures, a change in season, and increased heat production due to boar activity,
- Moderate changes in ventilation should be made, while increases of 2x more m^3/hour should be avoided.

Providing an optimal environment for boars requires multiple aspects to operate together. As the total number of m^3/hour increases the following must be considered.

- Each cm^2 of ceiling inlet provides 0.0011692 m^3/hour.
- Each cm^2 of eave inlet provides 0.00065 m^3/hour.
- If the system does not have the proper number of inlets open and the proper amount of attic inlet, it does not matter how many fans are on, the air will not come into the barn effectively.

Water can be used for cooling in the form of dripper systems or evaporative cooling.

- The purpose of dripper systems is to cool the testes to optimize the temperature for sperm production.
  - The production of extra water in the air and on the floor creates risks such as elevated barn humidity, lameness and an environment ideal for bacterial growth.
  - Minimum ventilation rates must be raised to effectively dry the floor above normal rates.
- Evaporative cooling combined with air speed effectively cools the barn but also adds humidity to the air.
  - The addition of evaporative cooling is most effective when inside humidity is less than 70% or outside temperature is lower than inside temperatures.
  - Specific rules should be followed to maximize the effect of this cooling method.
- A soaking cycle should be utilized that allows the pads to partially dry between applications of water.
- Routinely replace the water in reservoir as the evaporative process causes a concentration of salts and minerals potentially decreasing equipment useable life.
- Allow pads to completely dry at least once per day.
- Use evaporative pads at only 10 degrees above DRT.

Several factors should be considered when

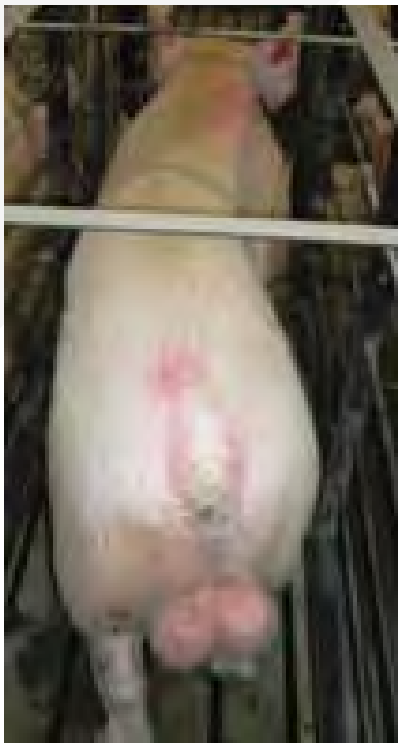
troubleshooting ventilation or air quality issues

- Fans output can be influenced by the following.
  - Dirty louvers and blades may decrease fan efficiency by 30%.
  - Leaking pit pump covers drastically affect air exhaustion.
  - Adding fan cones improves the fan's output by 10-20% m^3/hour
  - Excessive static pressure (>1000 FPM air speed or 0.1 in of water) severely affect a fan's exhaustive m^3/hour rating.
- Wet floors are a major factor in overall boar comfort and can make a boar feel 9 degrees cooler with the same air temperature but can be fixed by the following suggestions.
  - Increase minimum ventilation rates.
  - Assure proper airspeed from inlets.
  - Increase barn temperature until the floors are properly dried.
- A decrease in RPM and exhaustive output as a result of slipping fans can be detected by the following.
  - Measure the temp of the pulley with an infrared thermometer.
  - A thermometer temp of 7 degrees warmer than room temp indicates a slipping belt.

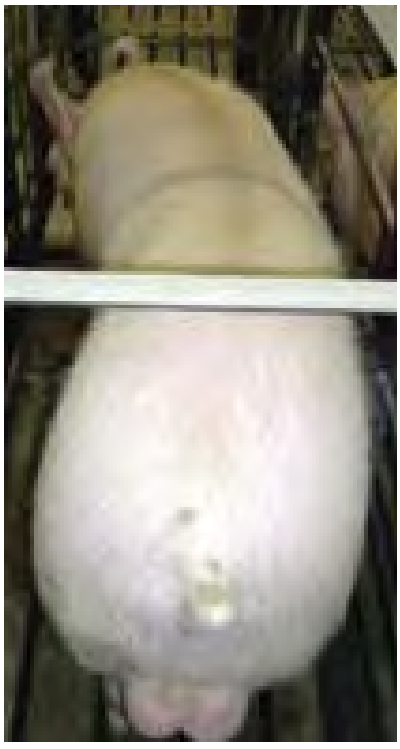
BODY CONDITION

Body condition is important for optimal libido maintenance, semen production and ability to jump on the collection dummy. The target body condition for 90% of boars in stud is 'normal' (See Pictures 3, 4, 5).

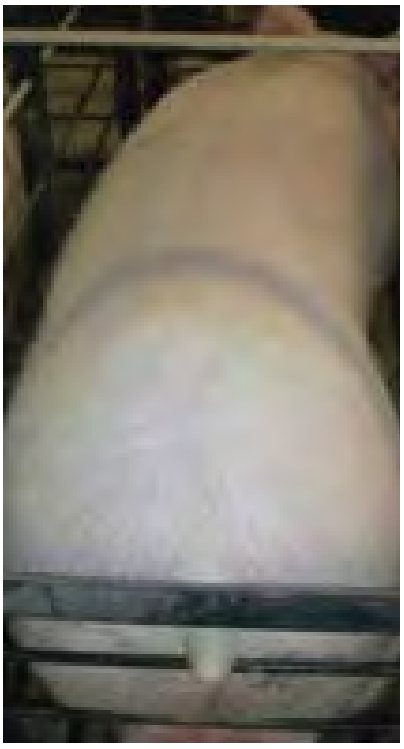
Picture 3: Thin Body Condition



Picture 4: Normal Body Condition



Picture 5: Fat Body Condition



FEEDING AND NUTRITION

Boars should be on full feed upon arrival in isolation to help with the transition into the new facility. This feeding rate should be maintained for 2 weeks and then dropped to 5 lb per day for the remaining isolation period.

Feed boars in normal body condition 2.3-2.7 kg once per day. Adjust appropriately to meet the target body condition (see Part 6: Body Condition). A fat boar should be restricted to 1.6-1.8 kg per day, while a thin boar should receive 73.2- 3.6 kg per day.

If drop feeders are used, weigh samples once per quarter to ensure accuracy. Measurements should be taken with any ingredient adjustment to account for bulk density changes.

Feeders should be adjusted every 2 weeks to maintain proper body condition and semen output. Proper maintenance of body condition will aid in libido and working ability of boars.

Proper feed intake levels and nutrient fortification should be provided to optimize semen production (see Appendices A and B).

Mycotoxins can have several detrimental consequences on boar performance including problems in maintaining high quality semen (Table 5). Avoid the use of by-products or co-products where mycotoxins can be concentrated. Select high quality ingredients and monitor mycotoxin levels on a regular basis. Work with your nutritionist to add a binder to the boar diet.

Table 5: Impact of Mycotoxins in Feed on Boar Performance

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

(P. Matzat, summarized from various sources)

TRAINING

Training for Manual Collection

Before training begins, adjust the height of the dummy to match the size of the young boars being trained. The collection area should be draft free and have good flooring.

Never start training a boar before 150 days of age.

Identify people willing to devote time and patience to train young boars and begin training 3 to 5 days after arrival. Ensure that a recording system is in place to track the progress of each boar. All boars should be trained within 4 weeks once training has started. The protocol below should be followed.

- Remove any source of distraction in the collection area.
- Ensure the boar is comfortable with human contact and ensure personnel safety.
- Squeeze the preputial diverticulum to stimulate the boar and make every effort to get the boar to pay attention to the dummy.
- Once the boar jumps the dummy, lock the penis and collect him.
- Observe possible anatomical problems with the boars (i.e. limp penis, persistent frenulum) at this time.
- The next boar should be placed in the warm-up area to prepare for training while the first boar is being collected.
- If a boar does not show interest in jumping the dummy in 10 minutes, move him to the warm-up area and administer a natural prostaglandin.
- Bring him into the collection area after 5-10 minutes and try to collect him again
- Once the boar is trained, repeat the process for 3 days in a row to enforce the learning experience.
- After boars are trained they must be collected once per week.

Training Using an Automatic Collection System

An automatic collection system includes an artificial cervix (AC), slide arm, AC holder and dummy. The AC mimics a sow's cervix and provides pressure to stimulate the boar. The slide arm allows free back and forth movement during collection.

- Follow the above steps for the first day of collection.
- On day 2, collect the first portion of the ejaculate manually for approximately 1 minute with the left hand.
- After 1 minute attach the penis to the automatic collection system and allow the boar to finish the collection.
- Repeat the process on day 3 of training.
- The time to acclimate each boar to the system will be dependent on the individual boar.

See Appendix C for instructions with photos.

BOAR COLLECTION

Boars should always be brought into the warm-up area first before collection. This allows boars to prepare to be collected. The boar sheath should be cleaned in the warm-up area and make sure the preputial diverticulum is emptied of its contents. The hair around the sheath should be trimmed periodically.

Hygiene must be maintained during collecting in order to limit bacterial contamination. The double gloved method is preferred.

Collections vessels should be prepared the day before collection and stored in a clean, sealed, hygienic, warmed (37°C) area until use.

The boar ejaculate has 4 fractions:

- Pre-sperm
- Sperm rich
- Post-sperm
- Gelatinous (boar plug)

Semen should be collected into a clean disposable container, including polyethylene bags, Styrofoam cups, etc. All methods need to use a filter to remove the gelatin material.

Once mounted on the dummy the boar will make attempts to unsheathe the penis and with a clean gloved hand the collector will catch and hold the glans penis (corkscrew) and follow the movement of the boar until he is locked.

Avoid collecting the pre-sperm fraction into the cup. This is typically a clear emission that contains urine and bacteria.

The sperm rich fraction follows the pre-sperm fraction. Collect the boar until he completes the ejaculation. Typically this process takes 8-10 minutes with some individual boars taking longer.

After collection, the filter should be removed from the bag in the barn and must not enter the lab.

Accurate and clear identification of the boar ID, genetics and technician or collector need to be recorded and attached to the bag or cup that contains the ejaculate.

All boars, regardless of semen demand should be collected on a regular basis. A guideline for collection intervals for sire line boars is provided (Table 6). Understand that individual boars and/or lines may perform better at a different interval than suggested; assuming collections are done regularly. Generally, maternal lines should be collected once per week regardless of age.

Table 6: Collection Interval by Boar Age for Sire Lines

Age	Interval
<12 months	1 x per week
≥12 months	3 x every 2 weeks

Body Temperature and Appetite
Rectal temperatures should to be taken for boars off-feed or with clinical signs of illness. If the boar's temperature remains >40°C, the boar should not be collected that day and the herd veterinarian should be notified immediately. Consideration should be given to a diagnostic work-up including PRRSv PCR testing on serum or blood swabs. If the number of off-feed and/or feverish boars increases from one day to the next day, the stud should be closed and a full diagnostic work-up initiated.

Diagnostic Testing
Weekly PRRSv PCR testing of blood or serum should be conducted at a frequency and number to achieve a minimum statistical sample of 95% confidence level at 5% prevalence based on sample type and the sensitivity/specificity of the PCR test. More rigorous sampling is at the discretion of the stud. Pooling samples up to 5 per pool is permitted.

Samples for PCR should be collected and submitted according to diagnostic laboratory protocol.

Monthly PRRSv ELISA (30 individual samples) screening or weekly sampling of a similar number is recommended.

Consider immediate PRRSv PCR testing from blood samples of any boars with a fever, off-feed or showing other clinical signs. These samples should be PCR tested individually, rather than as a member of a pool.

The blood swab technique has been used over the last several years and is an effective way to collect blood for PRRS PCR testing on a weekly basis. Another method using the tarsal vein on the back leg of the boar is a good technique for collecting blood for PRRS PCR and ELISA. Contact you herd veterinarian or PIC for instructions on blood collection.

Criteria for Stud Closure
The decision to suspend shipment of semen from a boar stud relies heavily on the professional judgment of the manager and herd veterinarian. Semen must not be collected for shipment from individual boars if there is any question of health status on collection day. Temperatures should be recorded on any boars suspected of having a health problem. Suspension of semen sales must be considered when clinical disease (i.e. cough, scours, offfeed) is evident or elevated temperatures (>40°C) are present in more than 5% of the boars in the stud. If the number of off-feed and/or feverish boars is less than 5% but is increasing daily the stud should be closed for a diagnostic work-up. Additional grounds for potential closure can be raised if the manager or herd veterinarian has other disease risk concerns (i.e. biosecurity breach). Suspicion of clinical or sub-clinical disease is to be reported to the herd veterinarian to determine whether distribution of semen can continue.

Confirm positive diagnostic results for diseases transmitted in semen such as PRRS.

Handling and Euthanasia
Mature boars are large and powerful and may cause injury to caretakers during normal handling. Special care should be taken when moving, treating or taking samples from boars. If detailed examination or treatment is required, the boar should be safely, effectively and humanely restrained. Proper techniques for euthanasia are found in the document On-farm Euthanasia for Swine: Recommendations for the Producer (AASV and NPB, 2009).

Transport of Boars

Drivers employed to transport boars should be TQA® certified. Stocking density should be based on weight, temperature and distance traveled (Table 7).

Table 7: Stocking Densities for Boars Based on Weight, Temperature and Distance

Weight	m^2 Requirements per Boar			
Kg	27°C	27-32°C	32°C	32°C >400 kilometers
109-117	0.3	0.4	0.4	0.5
118-138	0.4	0.4	0.5	0.6
139-165	0.5	0.5	0.5	0.7
166-181	0.5	0.6	0.6	0.7
181-203	0.6	0.7	0.7	0.8
204-226	0.6	0.7	0.8	0.9
227	0.7	0.8	0.8	1.0

Barn personnel SHOULD NOT be allowed in the laboratory at any time, unless they have showered and changed clothing.

Lab personnel should wear different attire than that worn in the barn. These items should be washed separately from barn clothes.

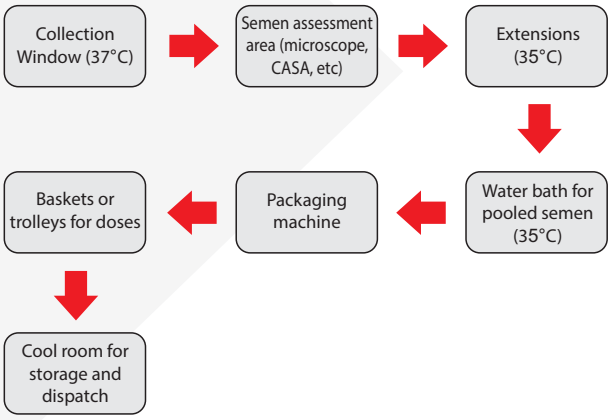
Lab coats and hair nets should be available for lab personnel.

Eating or smoking is not permitted in the lab. The countertops should be cleaned daily with a bleach solution after production is finished.

Hoses should be rinsed with deionized (DEI) water, soaked in alcohol and then re-rinsed and hung to dry before the next production day.

The lab should be set-up to promote efficiency in processing semen (Figure 3).

Figure 3: Flow of the Laboratory



Water Quality

Purified water is the largest component of a dose of semen and therefore water quality and monitoring is paramount.

The options are to buy purified water (best option for fewer than 100 boars) or fitting a water purification system into the stud. The cost can be variable, depending upon the quality and the origin of the water source.

Daily monitoring needs to take place to ensure consistent quality. Many studs in the US use a Myron 'L' 250 II device to monitor Megaohms (MΩ) and have this mounted in the lab for an immediate visual indicator.

Reagent water grades and specifications, micro-biological contamination levels and water quality specifications should be on hand for in-lab use (Tables 8 – 10; ASTM, 1991). The aim of the pure water systems installed in boar studs is to produce water between Type I and Type III grades.

Table 8: Parameters for Water Grades and Specifications

Parameter	Type I	Type II	Type III	Type IV
Electrical conductivity, max, µS/cm at 298 K (25°C)	0.056	1.0	0.25	5.0
Electrical resistivity, min, Ω-cm at 298 K (25°C)	18.0	1.0	4.0	0.2
pH at 298 K (25°C)	A	A	A	5.0 to 8.0
Total organic carbon, max, µg/L	50	50	200	No Limit
Sodium, max, µg/L	1	5	10	50
Chlorides, max, µg/L	1	5	10	50
Total silica, max, µg/L	3	3	500	No Limit

A =The measurement of pH in Type I, II, and III reagent waters has been eliminated from this specification because these grades of water do not contain constituents in sufficient quantity to significantly alter the pH.

Table 9: Types of Microbiological Contamination

Parameter	Type A	Type B	Type C
Max heterotrophic bacteria count	10/1000 mL	10/100 mL	100/10 mL
Cfu[a] /mL	0.01	0.1	10
Endotoxin, EU[b] /ml	0.03	0.25	NA

[a]Cfu = colony forming units; [b]EU = endotoxin units

Table 10: Specifications for in-Lab Water Quality

Parameter	Target
Bacteriology	<1 cfu[a] /mL
Purity inorganics	=18 MΩ @77°F (25°C)
Organics	>0.001 AU@254 nm
Total Organic Carbon	>50 ppb[b]
pH	6.8 to 7.2

[a]cfu = colony forming units; [b]ppb = parts per billion

If the water samples do not meet these specifications, an extensive analysis should be performed to correct the problem.

Semen Arrival
A stud using a pass-through window from the barn into the lab should have a warming cabinet to pre-warm the collection cups prior to collection. It should be set at 37°C.

A stud using a pneumatic tube delivery system should also have a warming cabinet close to the collection area.

The ejaculate of the boars needs to be clearly identified by boar ID and genetics. The barn technicians need to avoid bringing dirty containers into the pass-through window.

The lab technicians need to be aware of when an ejaculate enters the pass-through window. The ejaculate should be properly evaluated and extended within 10 minutes of arrival.

Semen Assessment
Upon entering the lab, semen should be observed for off-color and odor to determine if blood or urine is present.

The total weight of the ejaculate in grams needs to be measured by using a calibrated scale.

Prepare a sample for evaluation by diluting the raw semen with extender or a sodium citrate solution in a 1:20 dilution. If the ejaculate appears watery, a dilution of 1:10 should be used or if it appears very creamy or concentrated use a dilution of 1:40. This is important as is can impact the accuracy of semen concentration.

A visual assessment of semen motility and morphology should be performed on arrival into the lab's semen evaluation area and should meet predetermined quality standards (Table 11).

Table 11: Criteria for Acceptable Semen Quality

Characteristic	Threshold
Gross motility	≥80%
Normal sperm	≥70%
Cytoplasmic droplets, proximal and distal	<15%
Agglutination	<30%

A microscope can be used to assess gross motility using a 37°C warmed slide and cover or a computer-assisted semen analysis (CASA) system.

CASA systems can assess the morphology in the same sample used for motility. If no CASA is used a killed sample should be prepared and 100 cells should be counted in order to get the % normal cells in an ejaculate.

The presence of cellular debris and sperm cell clumping or agglutination should be recorded.

Less than 10% of ejaculates should be trashed for semen quality. If this number is higher, a detailed analysis of trash reasons should be completed to understand where the problem exists.

Concentration Assessment
Options to measure ejaculate concentration include hemacytometers, photometers, spectrophotometers or CASA systems.

Proper mixing of raw semen and pipetting techniques are important to ensuring a representative diluted sample of the ejaculate is used to assess the total sperm concentration. Depending on the equipment available this will provide a measurement expressed as total sperm cells x millions per mL of raw semen.

Semen Extension
Lab personnel need to know how many doses they are targeting ahead of time so they can prepare sufficient extender for the regular collection day. A general rule of thumb is to multiply the target number of doses times the total volume per dose and add 5 to 10% more to have enough extender for semen production and consequential uses like raw semen dilution, spills, pre-extension, or last minute orders.

Follow the extender manufacturer's instructions exactly. Accurate weighing of purified water and extender is vital. Deviations from this can alter the osmolarity of the mix. The extender needs to be continuously mixed for 1 hour to

permit the extender components to stabilize prior to adding it to semen.
The temperature of extender should be maintained at 35°C. At collection semen has a temperature of 37-38°C and there is a 2-3 degrees temperature drop of the ejaculate during the valuation process. Consequently the extender needs to be kept at 35°C.

After the ejaculate and extender is mixed view a sample in the microscope prior to filling the doses.

Extenders may contain one or multiple antibiotics. The antibiotics in the extender can be modified and tailored to your situation. Open communication with your supplier is necessary.

Dispensing Semen Doses
After extension, semen should either be put into a water bath 35°C for pooling or immediately dispensed for doses.

Prior to dispensing, semen should be gently mixed since sperm may have settled.

The entire process from the time the semen arrives in the window to dispensing doses should take 20 minutes.

Semen Cooling and Packaging of Cooled Doses
Cool rooms are used for storing and cooling the semen prior to dispatch. The temperature should be maintained at 15 - 17°C. A stir fan should be used to ensure air circulation. Record daily high and low temperatures in the cool room.

Wire shelves or bakers trolleys are used to move, store and cool the semen. This allows the flow of cool air and a more uniform cooling of the semen doses. The doses need to drop from the extension temperature (35°C) to the preservation temperature (15 - 17°C). With the use of modern extenders, doses can be immediately moved to the cool room.

Semen should be cooled for 4 hours prior to dispatch. This is especially important for semen being shipped in a double-boxed Styrofoam™ cooler combination, as these coolers maintain a constant temperature in shipping. Temperature loggers can be used to monitor temperatures in transit. The key is to fully cool the semen prior to packaging.

Semen Shipping and Transport
Semen shipped via an external courier, such as UPS or FedEx should be packaged into a double-boxed Styrofoam™ cooler and be delivered Next Day Air™. Semen for external shipping should be packaged and sealed inside a controlled (17°C) environment.

For large orders, no more than 200 doses should be placed in a Styrofoam™ cooler. For example, a customer ordering 1200 doses would receive 6 x 200 dose coolers.

When temperatures are 26°C or <4°C, doses should be packaged using double Styrofoam™ coolers with gel packs in the dead air space, otherwise a single cooler may be used. In the winter (<4°C) use 2 warmed gel packs and in the summer (>26°C) use 1 frozen (or 2 refrigerated) gel packs in between the coolers. One to 2 room temperature gel packs should be placed inside the inner cooler or the single cooler for all shipments. See Appendix D for further packaging instructions.

For semen that is sent via an internal courier, the temperature should be noted at the dropoff location.

LABORATORY QUALITY CONTROL

Post-production motility evaluations

- Ensuring the quality of the dose of semen produced is of the utmost importance. The best indicator the stud has to assess the viability of the extended dose is to perform a post-production motility check on all batches and single sire collections.
- A sample of each batch or single sire collection should be saved in a 5 ml glass tube along with a sample in the tube or bag used for packaging. Samples should be prepared for evaluation according to the directions provided by the manufacturer of the extender (see Table 12).
- Studs should perform post-production motility checks on day 1, 3 and 5 at minimum, where the day of collection is d 0.
- If doses are <70%, perform the post-production motility check a second time to confirm results. If results are confirmed, a call should be made to customers who received the semen instructing them to discard the semen.

Extender preparation and traceability

- Post a printed reference guide in the extender preparation and extension area so the technician can quickly reference the pure water to extender ratios recommended by the manufacturer. A guide can be prepared for every type of extender available in the particular stud (see Appendix E).
- Create a recording system to keep track of the amount of extender used. This record should include extender type, name and manufacturer lot number (Table 13).

Disposable materials records

- Record when suppliers change and when products that come into direct contact with semen change. All these entries need to include dates and lot numbers.
- Perform an in-lab trial to monitor for potentially detrimental effects (i.e. decreased motility).
  - For example, when a new lot of tubes are received dispense a semen sample into a tube from the new lot number and another sample into a tube with the current lot number using the same boar or pool of boars.
  - Evaluate both tube samples on d1, 3 and 5 for motility and morphology.
  - For other consumables like gloves, cut a piece from a new glove and a piece from a glove from the current lot number and immerse them into semen samples from the same pools and evaluate the semen as stated above.

Supplier quality control specifications

- Request that your supplier provide all quality control regulations they have in place for consumable production. For example, the extender supplier should provide what protocol is in place for the biological testing of plastic materials. Also ask suppliers about ISO 9000 certification.

Equipment calibration

- Calibration should be done for scales weekly,

Table 12: Manufacturer Guidelines for Sample Preparation for Motility Rechecks

Threshold	Threshold	Threshold	Threshold	Threshold	Threshold
IMV	Gedil	1-5 ml cooled, extended	37°C	10 minutes	Motility
Magapor	Vitasem	3 ml cooled, extended	37°C	5 minutes	Motility
Minitube	Androhep Enduraguard	5 ml cooled, extended	38°C	20 minutes	Motility

*For example purposes only. PIC does not endorse specific extender manufacturers.

Table 13: Example Extender Recording System

Extender: xxx		Extension Rate: xx g/kg			
Date	Water, kg	Extender, g	Lot #	Initials	Notes
11/01/2012	1 kg	50 g	1234	XX	

pipetting techniques monthly and infrared laser thermometers yearly. In the lab a set of master weights need to be available, a sensitive scale (readability to 0.001 g) for pipette volumes (for single channel air displacement pipetters) and an infrared laser thermometer calibration kit.

Pure water analysis

- Studs that have a pure water system (Pictures 6-10) must establish a verification process to ensure all components are operating properly. The frequency of water analysis is dependent on the starting quality of the water and the source (i.e. well, WEB water).
  - Carbon and sand filters should be used to capture gross particles. This equipment is functional for >500 K gallons of water. The filters should be checked every quarter.
  - Monitor the salt levels in the water softener (Picture 6) to ensure the proper ratio of water used per gallon of soft water produced.

Picture 6: Water Softener example

  - The reverse osmosis RO machine needs to be serviced once or twice a year, replacing cartridges and filters. The use of RO meters and test strips can be used to locally monitor water produced by the pure water system (example manufactured by Myron L Company, Picture 7).

Picture 7: Reverse Osmosis Water Meter

  - DEI tanks must work in pairs and need to be replaced twice a year (Picture 8). If the system has indicator lights that change from green to red there is a 1 week window of time to replace the depleted tank. The pair of tanks operate in the 'working and polishing' positions. The tank that is depleted is the one in the working position and when the replacement tank arrives the existing good tank goes from the polishing to the working position and the new tank goes to the polishing position.

Picture 8: Deionizing Tanks for Water System

- The 0.25 micron system and UV lamp should be changed every year (Pictures 9-10).

Picture 9: Micron System

Picture 10: Example of a UV lamp

- Water lines from the UV lamp to the water outlets in the lab should be sanitized every month to control parafilm bacteria. Sanitize the water lines and faucet outlets in the lab using a laundry bleach solution. Let them soak overnight and then thoroughly rinse.
- Review the whole system for possible water leaks weekly.

There are many quality control measures in the lab. A list of the measurements (Table 14) should be available to lab technicians with the name of the person responsible for each task.

Third party analysis assessment

› To ensure the extended dose of semen meets minimum quality standards a rigid monthly assessment of the diluted extender, water and extended semen doses needs to be performed. Establish a program to periodically monitor the overall quality of the semen doses produced in the stud. This consists of sending a random set of extended semen doses for quality control checks including sperm cell concentration, gross motility, morphology, and semen dose volume. At the same time send pure water samples, diluted extender samples and extended semen samples for bacteriology. The protocol for water and extender sample preparation is provided (see Appendix F). After the results of the third party evaluation are provided compare them with your target concentrations.

- The number of semen samples sent should equal 1% of a day's production or a minimum of 10 doses randomly selected among batches.
- The frequency of submission is routinely scheduled monthly and samples randomly selected from all batches.
- Targets should be established for every parameter measured and the accepted variation ranges.
- When consumable source or lot # changes, samples from the same batch should be sent using both sources/lots and the third party made aware of the change.
- The third party doing the semen evaluation service needs to be a truly independent entity. See a PIC representative for recommendations.

Table 14: Quality Control Measure and Frequency of Measurement

QC Measure	Frequency
Motility rechecks	d1, 3 and 5
Disposable materials	New lot numbers or products
Scale calibration	Weekly
Pipette calibration	Monthly
Infrared thermometer calibration	Yearly
RO machine	Replace filter 2x per year
DEI tanks	Replace 2x per year
Water lines and faucets	Sanitize monthly
.25 micron system	Replace 1x per year
UV lamp	Replace 1x per year

PERSONNEL MANAGEMENT AND TRAINING

Boar studs should maintain a boar to employee ratio of 1:60 with 60% of the employees working in the barn and 40% in the lab.

Employees should be PQA Plus® certified.

Stud personnel that work directly with the boars should be trained on animal movement and handling as well as safety.

Lab personnel should be trained by another employee with multiple years of experience or through a 3rd party training program.

Employee standardization should be done 1x per quarter with intense cross referencing of lab employees in terms of slide prep, semen assessment, and other lab functions.

KEY PERFORMANCE INDICATORS

As in sow farms, boar studs should also track parameters that are indicative of boar performance and semen quality (Table 15).

Table 15: Key Performance Indicators for Boar Studs

KPI	Target
Total sperm per ejaculate	>30 billion
Collections per boar per week	1.2
Untrainable boars	<3.0%
Prostaglandin use	<1.5%
Trashed collections	<6%
Unused doses	<5%
Boar mortality	<5%

Few studies have investigated the timeline for when the semen quality of an AI boar begins to deteriorate. Research (Wolf and Smital, 2009) suggests that semen volume, total sperm number and functional sperm reach their maximum by the time a boar is 2 years old. Sperm concentration increases until 11 months followed by a decrease in concentration until boars are 3 years old. The percentage of abnormal sperm increases with time from 8 to 48 months of age. Motility, however, steadily decreases with time but only by a 1% decrease.

Estimates for sperm cell and dose production for PIC lines can be made based on data from owned, affiliate, user group and customer boar studs (Figures 4-6; Tables 16, 17).

Figure 4: Viable (live, normal) Cells per Ejaculate per Genetic Line per Week of Production

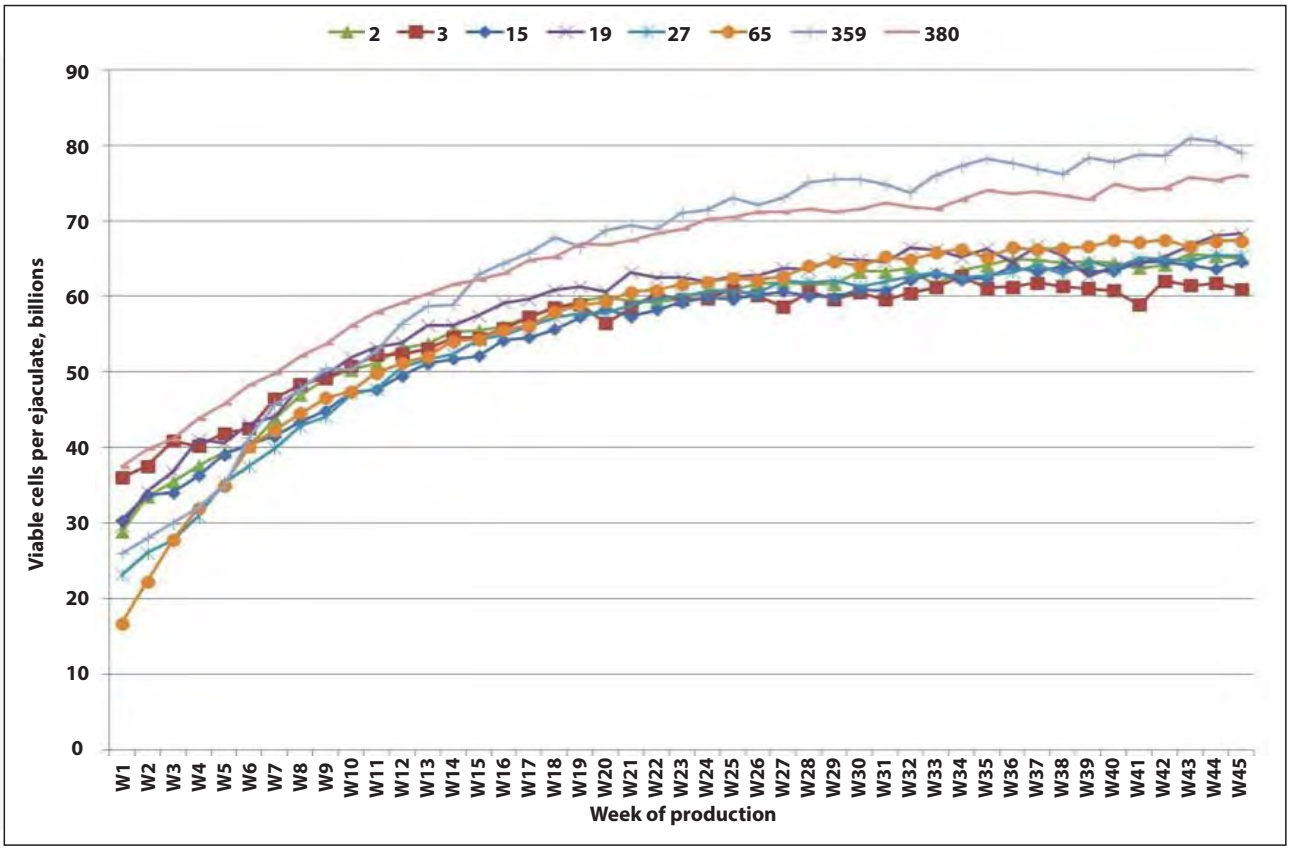


Table 16: Viable Cells per Ejaculate by Genetic Line Averaged for 5 Production Weeks

Line	W1-5	W6-10	W11-15	W16-20	W21-25	W26-30	W31-35	W36-40	W41-45
L02	35.0	46.1	53.8	58.2	60.0	62.0	63.2	64.6	64.8
L03	39.3	47.5	53.3	57.4	59.8	59.9	61.0	61.2	61.0
PIC280/L15	34.7	43.5	50.4	56.0	58.8	60.3	62.1	63.6	64.3
L19	36.5	47.4	55.4	60.3	62.5	64.0	65.7	64.6	66.5
PIC327/L27	28.6	42.3	51.3	56.7	60.0	61.6	62.6	63.7	65.1
PIC337/L65	26.8	44.2	52.3	57.5	61.4	63.5	65.5	66.6	67.2
PIC359	30.2	47.1	57.9	66.6	70.8	74.3	76.0	77.3	79.5
PIC380	41.6	52.0	60.2	65.4	69.1	71.3	72.5	73.7	75.1

Figure 5: Average Number of Doses per Collection by Age and Genetic Line Assuming Doses of 2.5 Billion Viable sperm Cells and an Average of 1.2 Collections per Week Over the Boar's Lifetime

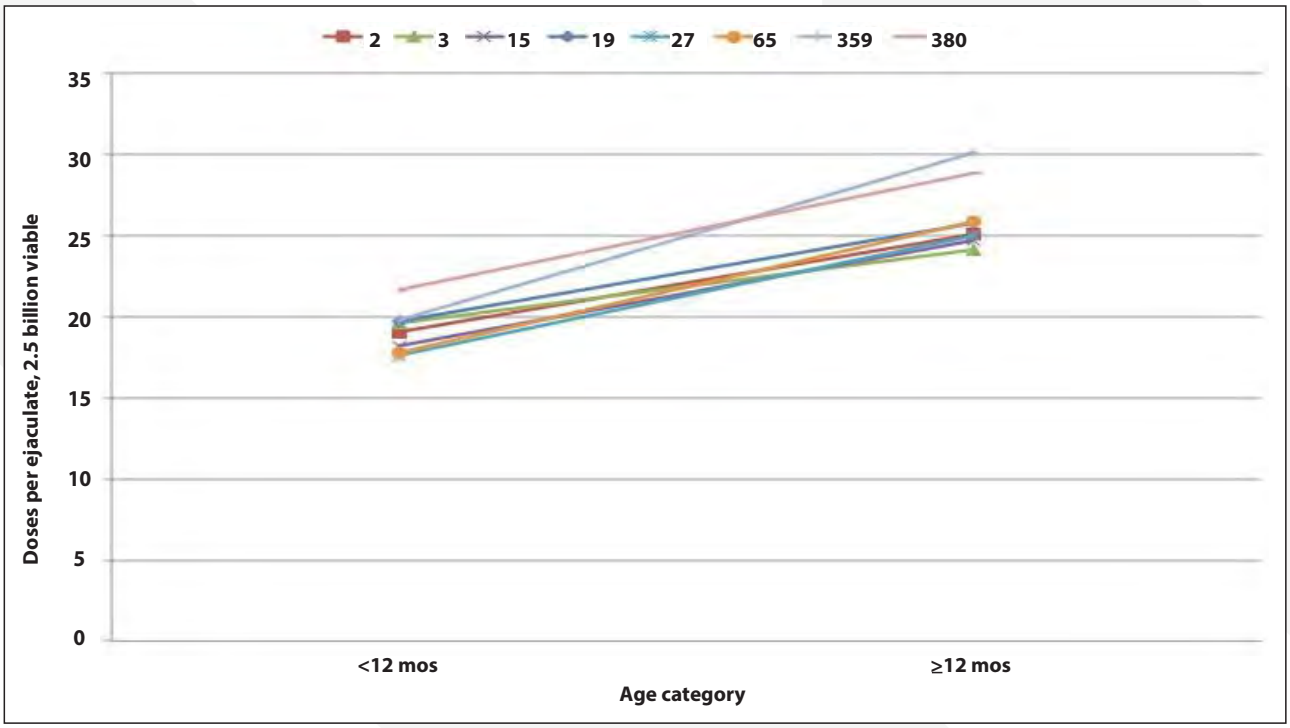
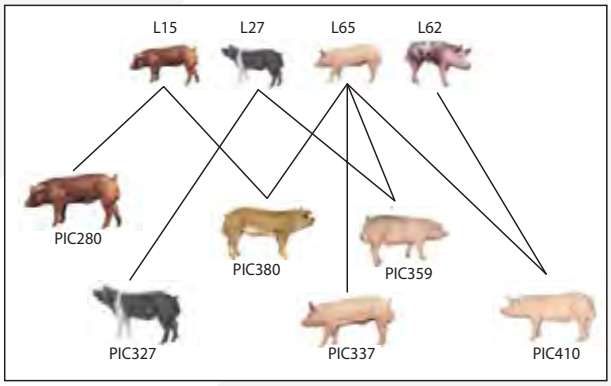


Table 17:Summary of the Average Number of Doses per Collection by Age and Genetic Line

	PIC Doses	
Line	<12 mos	≥12 mos
L02	19.1	25.1
L03	19.6	24.2
PIC280/L15	18.2	24.7
L19	19.7	25.8
PIC327/L27	17.6	25.0
PIC337/L65	17.8	25.8
PIC359	19.8	30.1
PIC380	21.7	28.8

Figure 6: Sireline Makeup



BOAR LIFE AND REPLACEMENT RATE

Over the past few years, PIC, in association with university economists, has developed an economic model to determine the optimum time to cull a boar in a boar stud. Optimum Boar Life (OBL) utilizes customized cost inputs from studs (housing, feeding, purchase price, isolation costs, royalties, etc.) and projected revenues (the value of the doses of semen produced by the boar and the genetic value of the boar compared to its potential replacement) to objectively determine the optimum time a boar should remain in stud. There are two models of OBL that accommodate integrated customers that own both a boar stud(s) and breeding sows and also a gene transfer center model that is specifically for customers who own a boar stud and sell semen. This replaces providing 'target' replacement rates and provides objective metrics based on accurate, real-time information. Please contact Genetic Services at PIC for more information on OBL and its use in your system.

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APPENDIX A

Suggested specifications: Amount per kg. of complete diet.

		Nursery		Grow-Finish		Boar
Nutrient	**Unit**	**< 6 kg**	**6 - 27 kg**	**27 - 68 kg**	**68 - Market**	**Stud**
Vitamin A	IU/kg	11000	9900	6600	4840	11000
Vitamin D	IU/kg	1760	1650	1210	990	1760
Vitamin E	IU/kg	83.6	77	33	22	110
Vitamin K	mg/kg	5.5	4.4	3.3	2.2	4.4
Choline	mg/kg	440	330	110	0	660
Niacin	mg/kg	70.4	44	26	22	44
Riboflavin	mg/kg	13.2	9.9	5.72	4.4	9.9
d-Pantothenate	mg/kg	39.6	33	19.8	14.3	33
Vitamin B12	mcg/kg	55	44	26.4	22	37.4
Folic Acid	mcg/kg	1045	770	0	0	1650
d-Biotin	mcg/kg	275	154	0	0	550
Thiamine	mg/kg	3.52	3.3	0	0	2.2
Pyridoxine	mg/kg	7.04	4.4	0	0	3.3
Vitamin C (stable)	mg/kg	132	0	0	0	132
Zinc	PPM	150[c]	130[c]	120	70	125
Iron	PPM	200[d]	175[d]	80	65	100
Manganese	PPM	50	45	30	25	50
Copper	PPM	18[c]	15[c]	12	10	15
Iodine	PPM	0.65	0.55	0.40	0.35	0.65
Selenium	PPM	0.30	0.30	0.30	0.30	0.30

[a] B-Vitamins supplemented at approximately 3.5 x NRC (1998) for < 5.5 kg pigs. Multiples for other groups approx.as follows:
6 - 27 kg, 3 x NRC.
27 - 68 kg, 2.5 x NRC.
68 kg- Market, 1.5 x NRC.
Sows tend to be 2.5 x NRC for Vitamins in general.
Boars are similar to sows with extra margins set for several micro-nutrients.
Add 2.3 IU of Vitamin E/lb of complete diet for each 1% fat above 3% total dietary fat.

[b] Pelleting and(or) expanding decreases Vitamin stability by 10-12% and 15-20% respectively. Consult Vitaminmanufacturer to verify the extent by vitamin so additional fortification can be made as required.

[c] Nutritional levels are shown for Zinc and Copper. Chemotherapeutic levels of Zinc as follows: < 6 kg 2600PPM; 6 - 27 kg., 2200 PPM; 27 - 68 kg, 1600 PPM. Chemotherapeutic levels of Copper is 220 PPM foreach phase. Inorganic forms assumed.

[d] Supplemental iron are near to NRC levels because of the substantial iron content of di-calcium phosphate andbecause high iron intake encourages E.Coli proliferation in the young pig

APPENDIX B

PIC minimum diet specifications[a]

Nutrient	
NRC ME, Kcal/kg	3086
Protein, %	16
Fiber, %	4.5 to 6.0
SID lysine[b] , %	0.62
Calcium, %	0.80
aPhosphorus[c] , %	0.40
Added salt, %	0.45
Linoleic acid, %	1.90

[a]Amount / kg of complete diet
[b]SID = Standardized ileal digestible
[c]a = available

Example Boar diet

Ingredient	Percent
Corn	69.32
Soybean meal (2.62% SID Lysine)	13.75
Soybean Oil	1.00
Monocalcium Phosphate, 21% P	1.10
Limestone	1.20
Salt	0.45
Lysine HCl	0.11
DL-Methionine	0.02
L-Threonine	0.05
Soy Hulls	12.50
PIC Boar Stud VTM + Phytase	0.50
	100.00

APPENDIX C

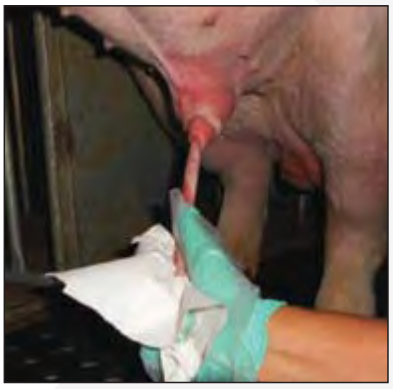
Automated collection systems step-by-step instructions for use.

1. 

Prepare the artificial cervix (AC) by placing it through the ring and wrapping the outer lining around it.

2. 

Squeeze the preputial diverticulum to empty the contents.

3. 

Once extended clean the penis with a single use disposable paper towel.

4. 

Attach the AC to the glove in the palm of the hand by exposing the tape. When the boar starts to thrust, grab and extend the penis.

5.

Place the ends of AC into the holder and press down on the trigger. The tip of the penis should extend slightly beyond the end of the AC. After collecting the pre-sperm fraction remove and discard the inner bag from the AC.

6.

Place the outer bag inside the collection cup and use the ring and mouth of the cup to create a seal.

7.

Attach the collection cup to the dummy but do not apply excessive force to prevent bending. Release the sliding arm lock to allow free movement during the collection process. During collection semen goes through the outer bag of the AC toward the collection bag filter located within the collection cup. Once the ejaculation is completed, the boar will withdraw his penis from the AC and dismount. Release the tension on the trigger to remove the AC and collection cup.

8. Remove the AC from the ring and discard. Then remove the top part from the collection bag that contains the filter and discard. The ejaculate is now in the collection bag and can be delivered for processing to the lab.

APPENDIX D

Packaging semen doses for shipment using double coolers.

1. 

Prepare liners and coolers.

2. 

Layer doses inside Thermalast® bag within inner cooler.

3. 

Add a room temperature gel pack.

4. 

Put on the lid and seal with tape.

5. 

Wrap inner cooler with Thermalast® bag.

6. 

Put inner cooler inside outer cooler and add gel packs (warm or cool depending on season).

7. 

Put on the lid and seal with tape.

8. 

Put in box for shipping.

APPENDIX E

Extender Preparation Guide

Extender: XXX

Manufacturer's Ratio: 50 g/kg of water Tub: 5kg

Extender Volume (L or kg)	Water to add (kg)	Extender to add (g)	Extender Volume (L or kg)	Water to add (kg)	Extender to add (g)	Extender Volume (L or kg)	Water to add (kg)	Extender to add (g)	Extender Volume (L or kg)	Water to add (kg)	Extender to add (g)
1	1	50	26	26	1300	51	51	2550	76	76	3800
2	2	100	27	27	1350	52	52	2600	77	77	3850
3	3	150	28	28	1400	53	53	2650	78	78	3900
4	4	200	29	29	1450	54	54	2700	79	79	3950
5	5	250	30	30	1500	55	55	2750	80	80	4000
6	6	300	31	31	1550	56	56	2800	81	81	4050
7	7	350	32	32	1600	57	57	2850	82	82	4100
8	8	400	33	33	1650	58	58	2900	83	83	4150
9	9	450	34	34	1700	59	59	2950	84	84	4200
10	10	500	35	35	1750	60	60	3000	85	85	4250
11	11	550	36	36	1800	61	61	3050	86	86	4300
12	12	600	37	37	1850	62	62	3100	87	87	4350
13	13	650	38	38	1900	63	63	3150	88	88	4400
14	14	700	39	39	1950	64	64	3200	89	89	4450
15	15	750	40	40	2000	65	65	3250	90	90	4500
16	16	800	41	41	2050	66	66	3300	91	91	4550
17	17	850	42	42	2100	67	67	3350	92	92	4600
18	18	900	43	43	2150	68	68	3400	93	93	4650
19	19	950	44	44	2200	69	69	3450	94	94	4700
20	20	1000	45	45	2250	70	70	3500	95	95	4750
21	21	1050	46	46	2300	71	71	3550	96	96	4800
22	22	1100	47	47	2350	72	72	3600	97	97	4850
23	23	1150	48	48	2400	73	73	3650	98	98	4900
24	24	1200	49	49	2450	74	74	3700	99	99	4950
25	25	1250	50	50	2500	75	75	3750	100	100	5000

APPENDIX F

Water sample preparation for third party analysis (developed by G. Althouse, J. Morales and B. Thompson).

- Water samples should be collected into a sterile Whirl-Pak® bag and sealed immediately after collection.
  - Put on disposable gloves before sampling.
  - Wipe the outer surface of the faucet with lint free tissue lightly sprayed with 70% alcohol; be sure it is not saturated. Physically wipe off the faucet and lightly insert the tissue into the end of the faucet. Wait for 30 seconds for the alcohol to evaporate.
  - Allow the RO water to run for 3 minutes to completely flush the lines.
  - Open the Whirl-Pak® and collect the sample mid-stream from the flow.
  - Sample all faucets with the same technique.

- Diluted extender sampling
  - The employee that weighs and adds the extender to the water should wear a N95 mask and use disposable gloves when working with the extender powder.
  - Use a similar technique with an alcohol wipe for the tubing from the peristaltic pump. Wipe off the outer surface of the tubing and swab the inside of the tubing and allow time for the alcohol to dry.
  - Collect approximately 1 liter of diluted extender into a container. Slow the pump down and free catch a sample into a Whirl-Pak® and seal immediately.

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