Midwest Boar Stud Managers Conference

August 4-5, 2021

Table of Contents

Bulk vs individual doses: considerations and cautions on choosing
Mr. Dale Hentges, *Acuity Genetics*[3]

Training boars for semen collection
Dr. Mark Estienne, *Virginia Tech University*

What do we really (need to) know about feeding boars for sperm production and quality?
Dr. Mark Wilson, *FeedworksUSA*[3]

Screening new boars for fertility in commercial studs
Mr. Tom Gall, *Gall Consulting Service*
and
Closing the gap between semen QC and boar fertility
Dr. Justin Fix, *Acuity Genetics*[3]

Application of preserved boar semen for artificial insemination: Past, present and future challenges
Dr. Dagmar Waberski, *University of Veterinary Medicine Hannover, Germany*[1, 2]

Cryopreservation of boar semen: where we are (going)
Dr. Rob Knox, *University of Illinois*

Semen gets stressed after collection too
Dr. Martin Schulze, *Institute For Reproduction of Farm Animals Schönow, Germany*[1, 2]

Practical considerations for cooling boars: Why AC? How much is enough?
Dr. Chris Kuster, *Kuster Research and Consulting*

What the survey says.
Dr. Tim Safranski, *University of Missouri*

Secure Pork Supply Plan needed for boar studs too???
Dr. Pam Zaabel, *National Pork Board, USA*[3]

Comparing semen extension in hypothermic vs isothermic conditions
Dr. Darwin Reicks, *Reicks Veterinary Research and Consulting*

Managing feet and legs in the stud
Dr. Mark Wilson, *FeedworksUSA*[3]

Remember when: perspectives on advances in bar studs and swine AI Dr. Wayne Singleton, *Purdue University, retired*

What's going on out there? Strange but true stories from the field Dr. Chris Kuster, *Kuster Research and Consulting*

An eight year history of bacteria in semen

August 2021

Martin Schulze

Biotechnological Research

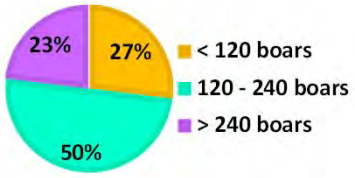
Science-based Quality Control Program

AI Center	26	24
Animals	7.000	1.000
Doses	12 Mio.	10.5 Mio.

- Boar
- Ejaculate
- Evaluation
- Preservation
- Filling
- Transport
- Storage

Characteristics: 26 × Boar Studs

✓ Number of boars/stud	80 to 500
✓ Replacement Rates	40 to 60%
✓ Housing	Straw *vs.* Sawdust
✓ Main production days	Monday, Tuesday & Friday (night)
✓ Technical equipment	CASA eFlow & 95 mL QuickTip Flexitube®
✓ Extender	Short-term
✓ *Sperm Quality Standard*	



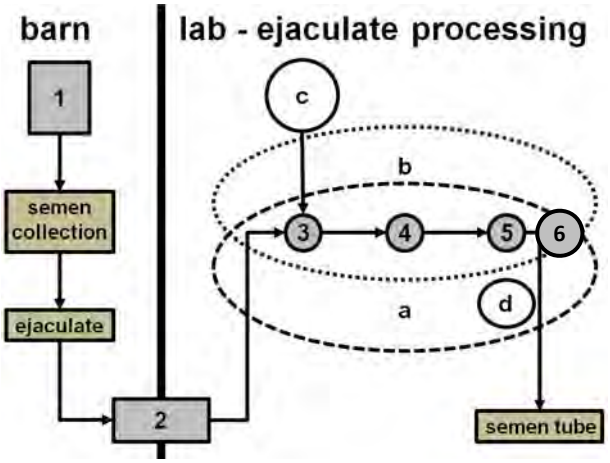
Guideline

a. Which hygienic critical control points are important at all?
b. Which methods are used?
c. Where are limits for contaminated samples?
d. Results
e. Case studies
f. Recommendations

CLSI = Clinical & Laboratory Standards Institute

Methods - Work Flow - HCCPs

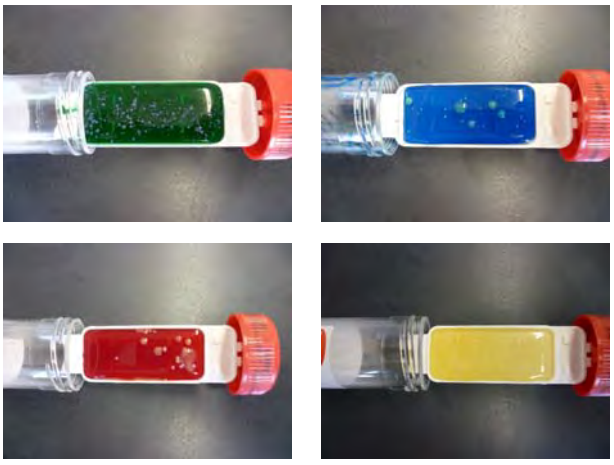
~~1~~	~~Heating Cabinets~~
2	Ejaculate Transfer
3	Extenders
4	Dilution Tank Lids
5	Dyes
6	Filling
a	Manual Operating Elements
b	Lab Surfaces
c	Ultrapure Water
d	Sinks/Drains



HCCPs = Hygienic Critical Control Points

Schulze *et al.* 2015
Theriogenology 146:133-39

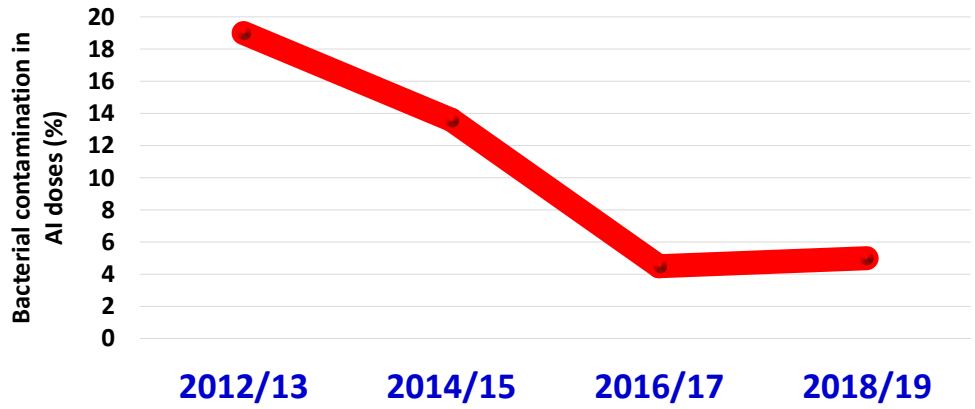
Methods - Dip-Slides



<u>Score 1</u> = 10^1 CFU/mL or 1 CFU/cm²
<u>Score 2</u> = 10^2 CFU/mL or 5 CFU/cm²
<u>Score 3</u> = 10^3 CFU/mL or 45 CFU/cm²
<u>Score 4</u> = 10^4 CFU/mL or 80 CFU/cm²
<u>Score 5</u> = 10^5 CFU/mL or 100 CFU/cm²
<u>Score 6</u> = 10^6 CFU/mL or >100 CFU/cm²

Dip-Slide Plate Count/VRBD, Oxoid-Thermo Fischer, Germany

Results

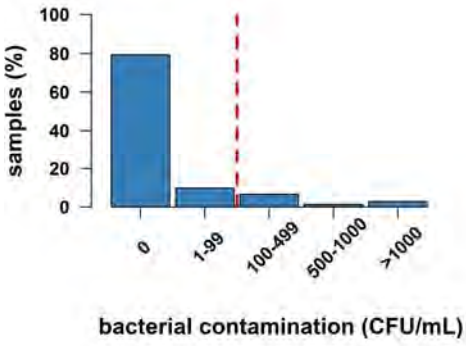


8 years
92 audits
28 boar studs
N=828 HCCPs
N=1,434 ejaculates

Nitsche-Melkus *et al.* 2020
Theriogenology 146:133-39

Results

Fig. 1. Fraction of 1,434 extended ejaculates falling in 5 different bacterial contamination groups (cutoffs) during an eight-year retrospective study (2012 to 2019) in 28 European AI centers.



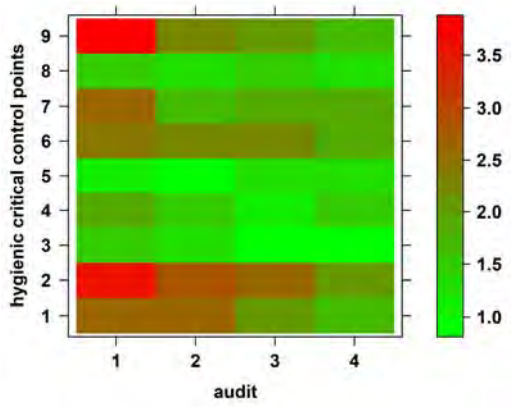
8 years
92 audits
28 boar studs
N=828 HCCPs
N=1,434 ejaculates

Nitsche-Melkus *et al.* 2020
Theriogenology 146:133-39

Results

Fig. 3. Average score per hygienic critical control point (HCCP, n = 828) and audit period (audit 1 to 4) during an eight-year retrospective study.

HCCP1: filling machine
HCCP2: ejaculate transfer
HCCP3: extender
HCCP4: inner face of dilution tank lids
HCCP5: dyes
HCCP6: manual operating elements
HCCP7: lab surfaces
HCCP8: ultrapure water treatment plants
HCCP9: sinks/drains



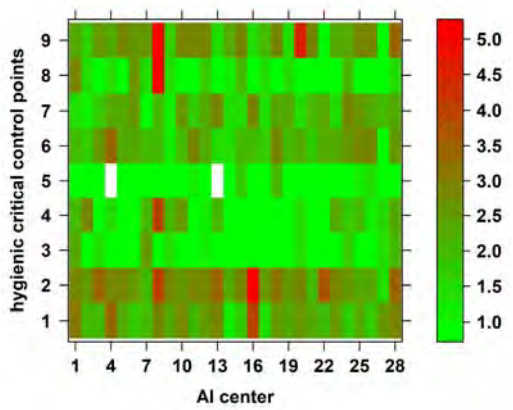
Score 1 = 10^1 CFU/mL or 1 CFU/cm²
Score 2 = 10^2 CFU/mL or 5 CFU/cm²
Score 3 = 10^3 CFU/mL or 45 CFU/cm²
Score 4 = 10^4 CFU/mL or 80 CFU/cm²
Score 5 = 10^5 CFU/mL or 100 CFU/cm²
Score 6 = 10^6 CFU/mL or >100 CFU/cm²

Nitsche-Melkus *et al.* 2020
Theriogenology 146:133-39

Results

Fig. 4. Average score per hygienic critical control point (HCCP, n = 828) in 28 (1 to 28) European AI centers during an eight-year (2012 to 2019) retrospective study.

HCCP1: filling machine
HCCP2: ejaculate transfer
HCCP3: extender
HCCP4: inner face of dilution tank lids
HCCP5: dyes
HCCP6: manual operating elements
HCCP7: lab surfaces
HCCP8: ultrapure water treatment plants
HCCP9: sinks/drains

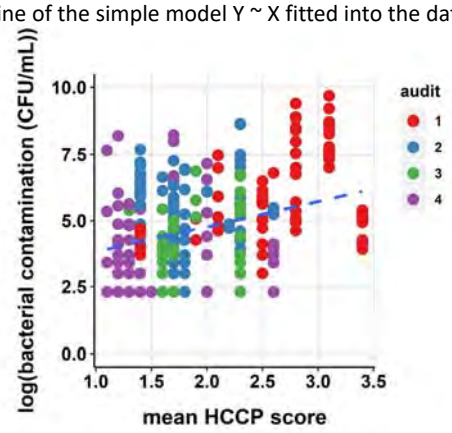


Score 1 = 10^1 CFU/mL or 1 CFU/cm²
Score 2 = 10^2 CFU/mL or 5 CFU/cm²
Score 3 = 10^3 CFU/mL or 45 CFU/cm²
Score 4 = 10^4 CFU/mL or 80 CFU/cm²
Score 5 = 10^5 CFU/mL or 100 CFU/cm²
Score 6 = 10^6 CFU/mL or >100 CFU/cm²

Nitsche-Melkus *et al.* 2020
Theriogenology 146:133-39

Results

Fig. 5. Logarithmized bacterial growth in 1,434 extended semen samples during an eight-year retrospective study as function of hygienic conditions at nine AI center comparable HCCPs evaluated in 28 European AI centers. The blue dashed line shows the regression line of the simple model Y ~ X fitted into the data (adjusted R^2 = 0.11; *P*=3.434 x 10^{-9}).



Multiple linear regression (MLR)

8 years
92 audits
28 boar studs
N=828 HCCPs
N=1,434 ejaculates

Nitsche-Melkus *et al.* 2020
Theriogenology 146:133-39

Results

Tab. 4. Risk of observing a bacterially contaminated semen sample (n = 1,434 ejaculates) depending on hygiene conditions at different hygienic critical control points (HCCP 1 to 9, n = 828 samples) during an eight-year retrospective study (2012 to 2019) in 28 European AI centers (audit 1 to 4, in total 92 quality audits).

Hygienic critical control points	Odds ratio	*P*-value	Lower confidence interval	Upper confidence interval
1. Filling machine	3.02	0.06	0.92	9.92
2. Ejaculate transfer	1.67	0.39	0.52	5.34
3. Extender	8.97	$<10^{-5}$	1.77	45.57
4. Inner face of dilution tank lids	3.14	0.09	0.81	12.2
5. Dyes	NA	NA	NA	NA
6. Manual operating elements	0.67	0.53	0.2	2.32
7. Lab surfaces	1.43	0.56	0.43	4.68
8. Ultrapure water treatment p.	0.62	0.66	0.07	5.32
9. Sinks/Drains	0.89	0.84	0.29	2.74

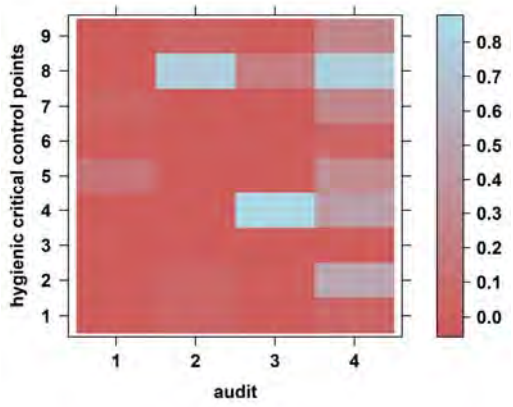
NA: not sufficient observations;
Odds ratio (≥ 100 CFU/mL in extended semen samples) given a contaminated HCCP (HCCP score ≥3). *P*-value: Pearson's chi-squared test

Nitsche-Melkus *et al.* 2020
Theriogenology 146:133-39

Results

Fig. 6. P-values of the Pearson's chi-squared test of independence, showing the effectiveness of hygienic measures along audit periods in 28 European AI centers, quantified by the loss dependency between bacterial contamination in extended semen samples and HCCPs contamination significance.

HCCP1: filling machine
HCCP2: ejaculate transfer
HCCP3: extender
HCCP4: inner face of dilution tank lids
HCCP5: dyes
HCCP6: manual operating elements
HCCP7: lab surfaces
HCCP8: ultrapure water treatment plants
HCCP9: sinks/drains



***P* < 0.05** demonstrates that the occurrence "bacterial contamination of the semen sample" & the occurrence "bacterial contamination of the HCCP (score 1-6)" are dependent occurrences.

Nitsche-Melkus *et al.* 2020
Theriogenology 146:133-39

Take Home Message

1. We could show that hygiene has a positive impact on the reduction of bacterial load.

2. AI centers being evaluated on a regular basis have a good over-all hygiene status.

3. Better education & training reduce the risk of bacterial contamination.

www.ifn-schoenow.de

Metagenomics of bacteria found in semen, prepuce, feces and the barn of boar studs

Darwin L. Reicks, BS, DVM

Introduction

The presence of certain bacteria such as Serratia in the extended semen can cause loss of sperm viability and fertility[1,2]. This can have a significant effect on litter size and fertility[3]. Although the barn is believed to be a primary source of bacteria, the specifics have not been thoroughly defined. Two experiments were conducted to determine the specific source of certain bacteria in the barn.

Materials and Methods

Experiment 1

Fifteen boars were collected in 6 different boar studs. Half of the boar studs had a history of Serratia marcescens within the prior year. On each boar, prepuce fluid was collected mid-stream and raw semen was collected 5 seconds after the start of ejaculation. All samples were cultured on Tryptic Soy Agar with 5% sheep blood. Plates were read at 48 hours and identification of positive isolates was made at the University of Minnesota Veterinary Diagnostic Lab. Quantification of bacteria in each sample was also determined.

Experiment 2

Four of the same six boar studs were sampled, with three having a history of Serratia within 6 months prior to sampling, and one stud having never identified Serratia in extended semen. Prepuce fluid and semen were collected as in experiment 1. In addition, a fecal sample was taken directly from the rectum. An environmental sample (Swiffer) was also obtained, by adding approximately 30 ml of Phosphate Buffered Saline to a Swiffer pad, wiping the pad on the slat at the mid-point of the stall after the boar left, and squeezing the Swiffer pad into a 50 ml tube. 16S rRNA gene sequencing was done on all samples in experiment 2 at the University of Minnesota Veterinary Diagnostic Lab. 16S sequencing utilizes PCR to target and amplify portions of the hypervariable regions of the bacterial 16S rRNA gene. Amplicons from separate samples are then given molecular barcodes, pooled together, and sequenced. After sequencing, raw data is analyzed with a bioinformatics pipeline which includes trimming, error correction, and comparison to a 16S reference database.

Results and Discussion

Experiment 1

The quantity of bacteria in semen ranged from 300 cfu/ml to 12,000 cfu/ml in positive samples. Overall, 73% of the raw ejaculates had no detectable growth (minimum detection level of 150 cfu/ml). For prepuce fluid, 98% of the samples had growth and overall an average of 500,000 cfu/ml. The frequency of the top 6 most frequently isolated bacteria in prepuce compared to raw semen is shown in table 1.

Table 1: data from 90 boars from 6 boar studs showing the top 6 most common aerobic culture isolates from prepuce and raw semen.

Prepuce Bacteria	No. of boars out of 90 - Prepuce	No. of boars out of 90 – Raw Semen
Pseudomonas aeruginosa	45	7
Gram positive cocci	34	5
Corynebacterium sp.	30	7

Staphylococcus sp	21	4
Proteus mirabilis	20	1
Corynebacterium xerosis	18	5

Experiment 2

Porphyromonas endodontalis is the most common bacteria isolated out of the prepuce fluids and is present in the highest concentrations at all four studs. In the feces, Prevotella copri and Faecalibacterium prausnitzii were the dominant bacteria found in all four studs.

Results were more variable for the environmental Swiffer, but in general, Acinetobacter lwoffii and Staphylococcus epidermidis, common skin inhabitants, were the most common isolates. Results were also variable in semen, with no consistent bacteria dominant.

Table 2: Predominant bacteria found by sample type in three boar studs

Sample type	Stud	Isolate	% of isolates
Prepuce fluid	1	Porphyromonas endodontalis	26.4
	1	Staphylococcus aureus	12.5
Prepuce fluid	2	Porphyromonas endodontalis	31.9
	2	Propionibacterium acnes	4.3
Prepuce fluid	3	Porphyromonas endodontalis	31.3
	3	Staphylococcus aureus	7.1
	4	Porphyromonas endodontalis	27.1
	4	Staphylococcus aureus	11.7
Feces	1	Prevotella copri	16.4
	1	Faecalibacterium prausnitzii	13.3
Feces	2	Faecalibacterium prausnitzii	19.9
	2	Prevotella copri	11.0
Feces	3	Prevotella copri	19.0
	3	Faecalibacterium prausnitzii	14.6
	4	Prevotella copri	14.6
	4	Faecalibacterium prausnitzii	12.4
Environmental Swiffer	1	Staphylococcus aureus	11.6
	1	Clostridium perfringens	7.3
Environmental Swiffer	2	Acinetobacter lwoffii	12.9
	2	Staphylococcus epidermidis	5.5
Environmental Swiffer	3	Acinetobacter lwoffii	10.5
	3	Staphylococcus epidermidis	8.2
	4	Staphylococcus epidermidis	13.2
	4	Clostridium perfringens	12.2
Semen	1	Staphylococcus aureus	9.9
	1	Prevotella copri	6.9
Semen	2	Porphyromonas endodontalis	18.1
	2	Staphylococcus epidermidis	9.2
Semen	3	Acinetobacter lwoffii	10.7
	3	Prevotella copri	9.9
	4	Staphylococcus epidermidis	12.3

	4	Propionibacterium acnes	2.7

Serratia marcescens was not a dominant isolate in any of the samples from the four different boar studs, but it was found in lower concentrations in most samples (126 out of 160).

The percentage of isolates in prepuce fluid identified as Serratia marcescens varied from 0.03% in stud 1 to 2.4% in stud 3. For feces, the range was from 0.05% in stud 4 to 1.7% in stud 1. In the semen, the percentage was from 0.2-0.5% in all three studs, and for the environmental Swiffer, between 0.2-0.5%. Although one of the studs has never cultured Serratia from extended semen, Serratia was identified in 36 out of 40 samples from that stud.

The three-glove method was used[4], and an attempt was made to obtain a free-catch collection on all boars. Twenty-four of 40 boars had no detectable Serratia marcescens in the semen. The concentrations were low in most of the boars with 88% of the Serratia found in 7 boars out of 40. It is possible that Serratia in those boars was higher due to cross contamination with feces or prepuce fluid.

The correlations between the 4 different sample types and quantity of Serratia were not particularly strong. The strongest correlations were between prepuce fluid and environment (r=0.24) and between feces and environment (r=0.26), which seems logical.

Table 2: Predominant bacteria found by sample type in four boar studs

Sample type	Stud	Isolate	% of isolates
Prepuce fluid	1	Porphyromonas endodontalis	26.4
	1	Staphylococcus aureus	12.5
Prepuce fluid	2	Porphyromonas endodontalis	31.9
	2	Propionibacterium acnes	4.3
Prepuce fluid	3	Porphyromonas endodontalis	31.3
	3	Staphylococcus aureus	7.1
	4	Porphyromonas endodontalis	27.1
	4	Staphylococcus aureus	11.7
Feces	1	Prevotella copri	16.4
	1	Faecalibacterium prausnitzii	13.3
Feces	2	Faecalibacterium prausnitzii	19.9
	2	Prevotella copri	11.0
Feces	3	Prevotella copri	19.0
	3	Faecalibacterium prausnitzii	14.6
	4	Prevotella copri	14.6
	4	Faecalibacterium prausnitzii	12.4
Environmental Swiffer	1	Staphylococcus aureus	11.6
	1	Clostridium perfringens	7.3
Environmental Swiffer	2	Acinetobacter lwoffii	12.9
	2	Staphylococcus epidermidis	5.5
Environmental Swiffer	3	Acinetobacter lwoffii	10.5
	3	Staphylococcus epidermidis	8.2
	4	Staphylococcus epidermidis	13.2
	4	Clostridium perfringens	12.2
Semen	1	Staphylococcus aureus	9.9
	1	Prevotella copri	6.9
Semen	2	Porphyromonas endodontalis	18.1

	2	Staphylococcus epidermidis	9.2
Semen	3	Acinetobacter lwoffii	10.7
	3	Prevotella copri	9.9
	4	Staphylococcus epidermidis	12.3
	4	Propionibacterium acnes	2.7

Summary

This was a first look at results from multiple boar studs using 16S rRNA gene sequencing to determine what bacteria are prevalent in semen, prepuce fluid, feces, and environmental Swiffer samples from boar studs. Porphyromonas endodontalis, a gram-negative anaerobic rod, was a dominant bacterium of the prepuce fluid. Prevotella copri and Faecalibacterium prausnitzii, also gram-negative anaerobic rods, were dominant in the feces from all three studs. Acinetobacter lwoffii and Staphylococcus epidermidis were the most common bacteria overall on environmental Swiffer samples. Although the three-glove method was used to obtain a free catch collection, bacteria were still found in the semen, with a variety of isolates identified. In experiment 1, most semen samples had no detectable aerobic growth. The 16s rRNA technique appears to be a much more sensitive technique than Tryptic Soy Agar (with 5% sheep blood) plates in identifying all bacteria present and can help us understand what type of normal background bacterial flora are present. Serratia marcescens was not a dominant bacteria present in any of the samples, suggesting that when it presents itself in extended semen, it may be due to a disruption or altering of the normal flora in prepuce fluid, feces, semen, or the environment.

References

1. Althouse GC, Kuster CE, Clark SG, Weisiger RM. Field investigations of bacterial contaminants and their effects on extended porcine semen. Theriogenology 53 (2000) 1167-1176.
2. Ubeda JL et al. Adverse effects of members of the Enterobacteriaceae family on boar sperm quality. Theriogenology 80 (2013) 565-70.
3. Maroto Martín LO et al. Bacterial contamination of boar semen affects the litter size. Animal reproduction science 120 (2010) 95-104
4. Reicks DL. Best collection techniques to avoid bacterial and viral contamination. Boar Stud Managers Conf. Webinar series. September 2018.
5. Reicks DL. Determining the source of Serratia and other bacteria in boar semen. AASV 2021

BSMC VI August 4th-5th, 2021, St. Louis, MO

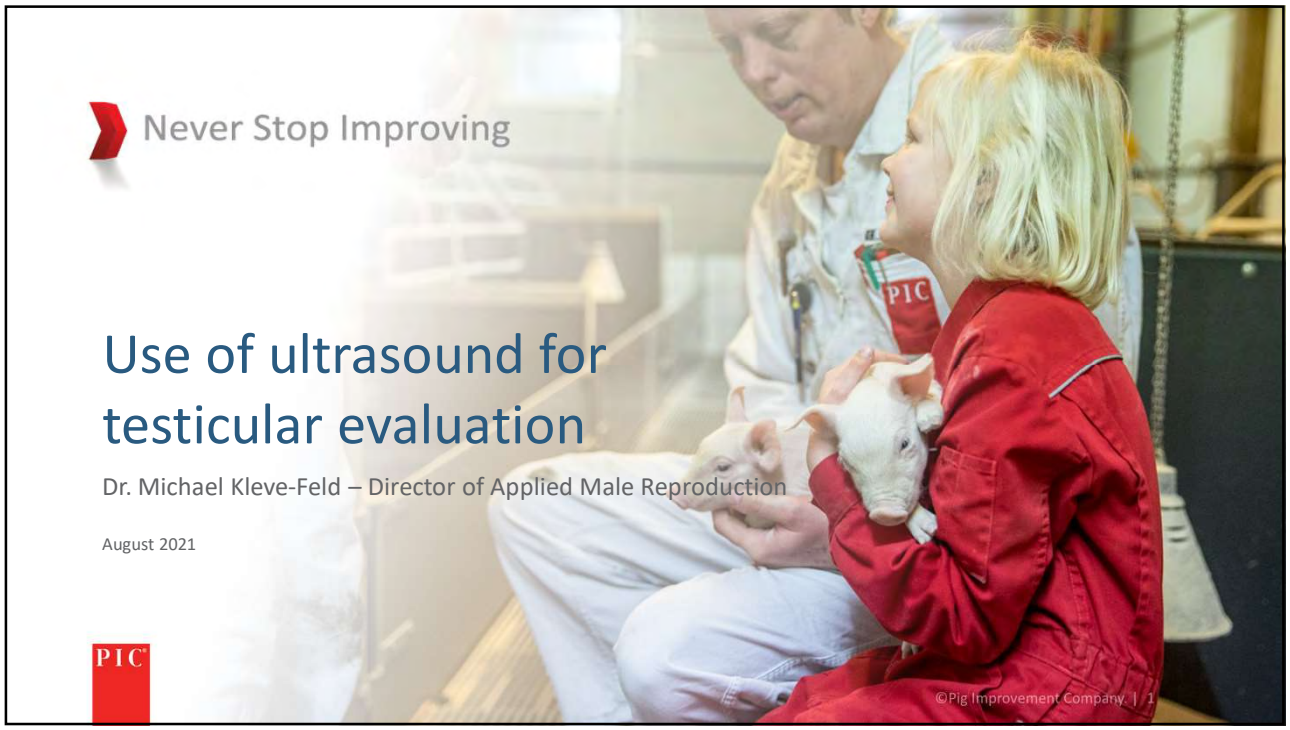
Living without antibiotics in extended semen

Dagmar Waberski, University of Veterinary Medicine Hannover, Germany

Antimicrobial control of bacteria in boar semen doses is required to ensure sperm longevity and to avoid transmission of infectious diseases in the female. Antibiotics, therefore, currently are a standard component of semen extenders. However, with worldwide increased use of AI in swine breeding, the contribution of antibiotics in semen extenders to the global bacterial resistance threat for human health came into focus. Concurrently, multiple bacterial resistance in AI centers is increasing, thus leading to an overuse of antibiotics including those with highest priority for human treatment.

Efforts are being made to reduce and replace antibiotics in boar semen extenders. Typically, the aim is complete eradication of bacteria. Noteworthy, during natural mating the female tract is flooded with male bacteria as part of the natural cellular component of ejaculates. Coevolution of female and male reproduction includes the immunogenic interaction of the ejaculate microbiome with the female reproductive tract, which is now suggested to promote fertility chances. At the same time, an array of studies demonstrated that moderate amounts of bacteria (for most strains < 10^6 CFU/ml) do not harm sow fertility.

Based on these notions, this presentation will critically discuss the intended endpoint of antimicrobial control in extended boar semen and propose strategies for antibiotic-free semen preservation. In any case, reduction of contamination during semen collection and processing by efficient hygiene measures and regular control of boar health and semen for specific pathogens remains mandatory.



1

Never Stop Improving

Background

Boar Multiplier (SLN)

- Boars get selected for sale at ~6 month of age
- Boars are sexually pre-mature at that point
- No option for training and semen collection in SLN

PIC

2

Never Stop Improving

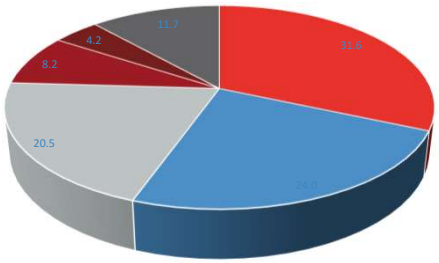
Background

Gene Transfer Centers (GTC)

- GTCs invest in boar quarantine and training
- Quarantine space as limited resource
- Boar Retention relevant for overall efficiency
- Boar retention rate NAM ~75%*
- Semen quality accounts for >30% of all early removals

*Measuring percentage of animals in stud 7month after delivery to quarantine

Non voluntary boar removals LT210 days



Distribution of 2020 NAM boar removals by reason

3

Never Stop Improving

The Challenge

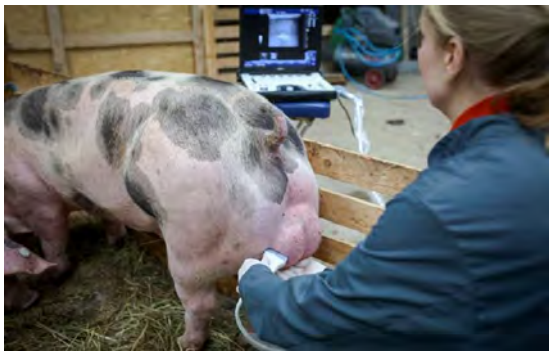
- No possibility to test boar semen quality prior boar sale
- Limited boar introduction capacities of GTC partners
- Poor SQ boars affect GTC efficiency and satisfaction
- Boars failing in SQ unavailable for genetic dissemination

4

Never Stop Improving

Working Hypothesis

"Ultrasound pictures of testicular tissue help to predict later semen quality"



PIC

5

Never Stop Improving

Ultrasound

AKA sonography

- Transmit sound waves in body, record echo
- Waves travel into body until boundary is hit
- Some waves reflect, others travel further
- Speed, direction and distance information from returning soundwaves get analyzed and converted into picture

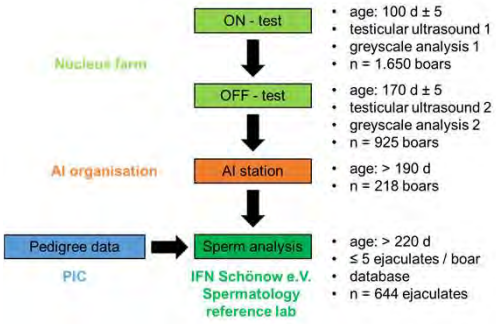
PIC

6

Never Stop Improving

Research Trial

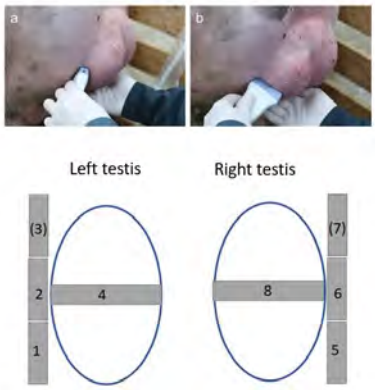
- Collaboration IFN Research Institute
- Germany 2017-2020
- Testicular Ultrasound with Grey Scale Analysis
- Analysis in correlation with semen quality



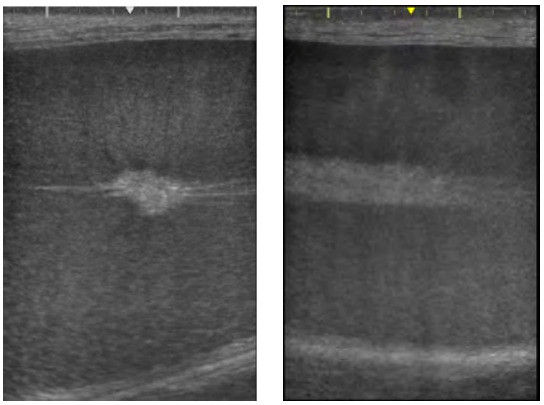
7

Never Stop Improving

Impressions Pictures Capturing and Analysis



Testicular ultrasound evaluation scheme



Horizontal and vertical segment pictures

8

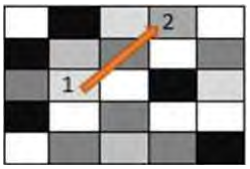
Never Stop Improving

Grey Scale Parameters

Measurement of Grey Scale Parameters

- Min., Max, Mean grey value
- Heterogenity, contrast,…

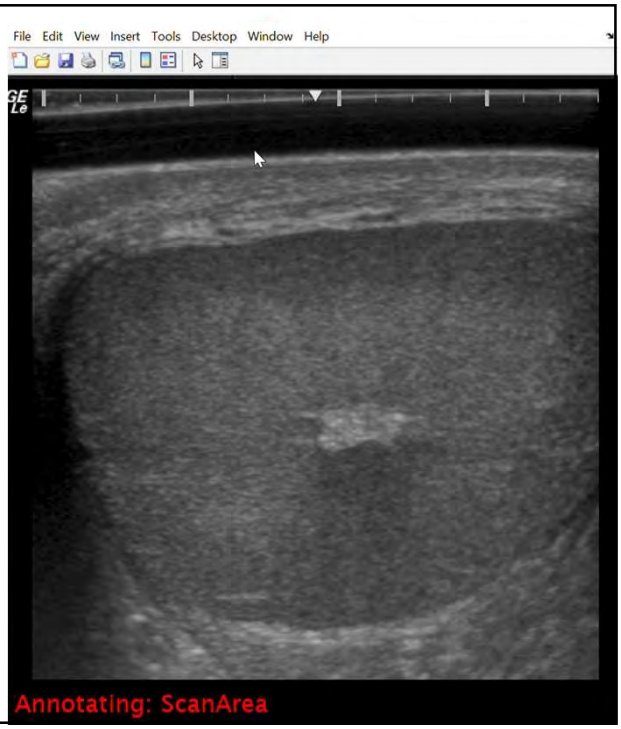
- Grey scale parameters describe tissue composition



9

Get Dataset

Draw boundary of parenchyma followed by boundary of mediastinum.



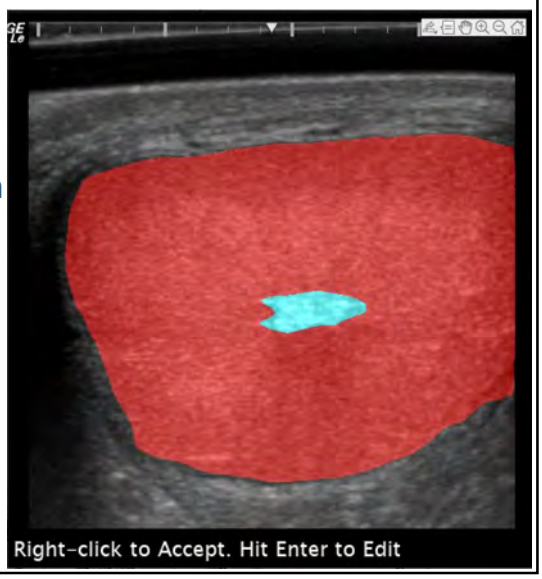
10

Never Stop Improving

Assisted Annotation

Let the network try first pass at annotation

- Correct 80% of the time
- 20% labor required



11

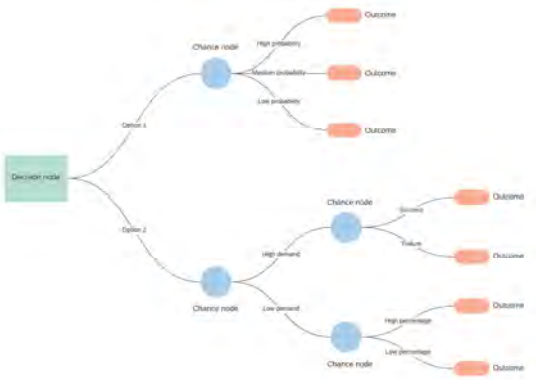
Never Stop Improving

Decision Tree Model

- Supervised learning algorithm
- Groups:

A) 2-5 ejaculates collected to achieve 2 good ones

B) Less than 2 suitable ejaculates after 5 collections



12

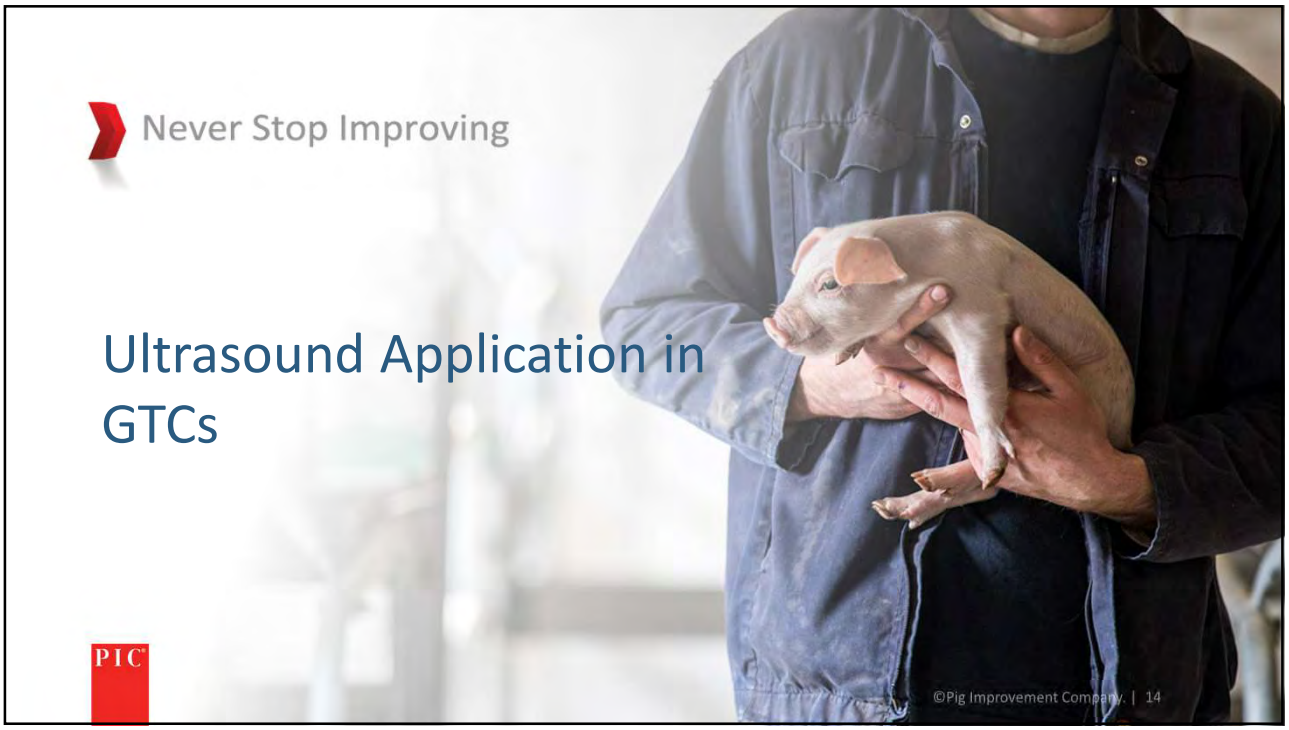
Never Stop Improving

Predictive Power

- Proposes that >90% boars with poor semen quality can be identified correct while 10% of good semen quality boars get categorized wrong as poor semen quality
- Final numbers to be confirmed by further test runs based on new observations

PIC

13



14

Never Stop Improving

GTC Application

Commercial solutions available

- Basic Ultrasound machine
- Software optimized for end-user

User case

- Evaluate boars at poor semen quality – Perspective?

Challenges

- Differentiation permanent vs. temporary damages
- Breed specific parameters

Exago Ultrasound Machine – Picture courtesy of Humeco

Never Stop Improving

GTC Application

- Results suggest correlation between primary sperm abnormalities and measurement parameters
- Correlation with primary abnormalities?
- Clear identification of severe tissue damages

Boars with more than 30% of sperm with primary abnormalities showed significant differences in these 2 echotexture parameters

	TEST 1	TEST 2	TEST 3	% AREA AH	DIAMETER AH (microns)	DENSITY AH/cm2
More tan 30% Spz with primary abnormalities (n=13)	14,38±32,28	186,18±257,8	100,95±14,61	5,69±4,61	90,18±33,71	116,23±30,14
20-30% Spz with primary abnormalities (n=8)	6,41±6,73	65,03±76,37	93,79±8,45	7,05±2,94	92,96±18,31	140,22±32,64
Less tan 20% Spz with primary abnormalities (n=91)	3,46±6,33	57,14±104,97	94,31±8,83	7,02±2,51	93,12±13,77	145,67±18,46
Sig.	0,13	0,05	0,66	0,289	0,850	0,000

Courtesy of Humeco

Never Stop Improving

Craft vs. Technology – Start simple

- Manual evaluation of testis to identify relevant testicular defects
- Test for texture, smooth surface, temperature, pain,…
- Consider testicular size (and changes) as well
- Subjective, comparative approach

Significant odds ratios for boars with abnormal genital organs to develop dysspermia

Group		Semen Analysis		Genital Result		Odds Ratio	Confidence Intervall
Boars (n)		Abnormal Parameter	Boars (n)	Abnormal Organ	Boars (n)		
Adult	145	Morphology*	9	Testis	7	17.0	3.3-87.5
Young and Adult	228	Morphology*	13	Spermatic Cord	40	2.4	1.1-5.0
Young and Adult	219	Membrane** Integrity	38	Spermatic Cord	18	2.0	1.0-4.1

In 17.5 % of the boars, a varicocele testis with significant higher risk (odds ratio) for the occurrence of teratozoospermia and sperm membrane damages was detected. Kleve-Feld et al. 2015

PIC

17

Never Stop Improving

Summary

- Ultrasound capable of identifying testicular tissue damages
- Promising pre-sorting boars for prospective semen quality prior arrival to GTC
- Commercial applications for GTCs available as well
- Start easy – manual evaluation is an easy and cheap first step

PIC

18

Boar Sperm Zinc Ion Flux as a Marker for the Acquisition of Fertilization Competency

Karl Kerns[1,2]*

[1]Iowa State University, Department of Animal Science
[2]University of Missouri, Division of Animal Sciences
*kkerns@iastate.edu

Abstract: Previously, we reported on the changes of sperm zinc ion localization, as detected by zinc reporter FluoZin 3 AM, in boar, bull, and man. These distinct zinc localization patterns were termed collectively the sperm zinc signature. The sperm zinc signature change corresponds with major events on the path to fertilization competency, including major physiologically relevant events during sperm capacitation. Here we briefly describe this zinc signature and elucidate previous findings. The boar sperm zinc signature is indicative of important boar sperm capacitation events, necessary for sperm progression to a fertilization competent state. These findings represent a paradigm shift in the understanding of boar fertilization competency, paving the way for improved boar fertility diagnostics and artificial insemination as well as a diagnostic tool for developing new boar semen preservation methods.

Keywords: biomarker; fertility; sperm capacitation.

Introduction

The highest concentrations of zinc (Zn) in bodily fluids are found in seminal fluid, reportedly containing 1.6-3.6 mM zinc ions (Zn^{2+}) [1]. It is known that male infertility stemming from the accidental Chernobyl radiation in Ukraine was associated with decreased human seminal fluid zinc content [2]. Likewise, it is known that men with low-motile or immotile spermatozoa have decreased zinc ions in their seminal fluid [3] while fertile men have increased seminal fluid zinc levels compared to infertile men [4, 5]. These relationships, among others, led us to inquire about zinc signaling pathways in spermatozoa at the cellular level and whether zinc localization was constant. We previously reported zinc localization in boar, bull, and man [6] using epifluorescent microscopy and image-based flow cytometry (IBFC). In these studies, we described zinc ion fluxes associated with key points leading up to sperm fertilization competency. This includes hyperactivated motility [6], the capability to bind glycans of the porcine sperm oviduct reservoir [7], remodeling of the sperm plasma membrane, remodeling and exocytosis of the acrosome [6], and regulating the enzymatic activity of sperm-borne zona pellucida metalloproteinase MMP2 [7]. For further details regarding sperm zinc-dependent pathways and their necessary involvement in sperm fertilization competency, see our recent review [8].

Methods

Semen processing

Fresh *Sus scrofa* spermatozoa was collected and 1 mL aliquot was immediately placed into a 15 mL conical tube with 6 mL of TL-HEPES as previously reported [8]. The spermatozoa were then centrifuged in a swing hinge rotor centrifuge at 110×g for 5 minutes. The number of washes and g-force used were minimized as these were found to compromise sperm structural integrity. Sperm concentration was then determined using a hemocytometer.

In vitro capacitation

Boar spermatozoa were subjected to an in vitro capacitation (IVC) protocol as previously reported [6]. Briefly, spermatozoa were washed of seminal plasma once with noncapacitating media (NCM), a modified TL- HEPES medium, free of calcium dichloride ($CaCl_2$) and addition of 11 mM D-glucose, with pH adjusted to 7.2. Spermatozoa were then resuspended in 0.5 mL IVC media, TL-HEPES-PVA supplemented with 5 mM sodium pyruvate, 11 mM D-glucose, 2 mM $CaCl_2$, 2 mM sodium bicarbonate, and 2% (m/v) bovine serum albumin, adjusted to 7.40 pH, and incubated in a 37 °C water bath for 4 hours. Microcentrifuge tube

rotation was performed every 60 minutes. This medium hyperactivates spermatozoa, induces protein tyrosine phosphorylation, and renders spermatozoa capable of recognizing and binding to the zona pellucida, undergoing acrosomal exocytosis and penetrating the oocyte zona pellucida [9]. All reagents were purchased from Sigma unless otherwise noted.

Multiplex fluorescence probing

Upon 4 hours of IVC, 100 µL volume (4 million spermatozoa) of spermatozoa were incubated for 30 minutes with 1:500 FZ3 (FluoZin™ 3 AM), 1:2000 lectin PNA Alexa Fluor 647 (PNA-AF647), 1:1000 Hoechst 33342 (H33342), and 1:1000 propidium iodide (PI). Spermatozoa were then washed of probes once and resuspended in NCM to allow complete de-esterification of intracellular AM esters, followed by an additional wash and resuspension in 100 µL PBS for IBFC.

Image-based flow cytometric data acquisition

The IBFC data acquisition was performed as previously described [6]. Specifically, using a FlowSight flow cytometer (FS) fitted with a ×20 microscope objective (numerical aperture of 0.9) with an imaging rate up to 2,000 events/second. The sheath fluid was PBS (without calcium or magnesium). The flow-core diameter was 10 µm set to a speed of 66 mm/second. Raw image data were acquired using INSPIRE® FS data acquisition software. Two bright-field channels were collected (channels 1 and 9), one FZ3 image (channel 2), one PI image (channel 5), one side scatter (SSC; channel 6), one H33342 (channel 7), and one PNA-647 image (channel 11), with a minimum of 10,000 spermatozoa collected. The following lasers and power settings were used: 405 nm (to excite H33342): 10 mW; 488 nm (to excite FZ3): 60 mW; 561 nm (to excite PI): 40 mW, 642 nm (to excite PNA-647): 25 mW; and 785 nM SSC laser: 10 mW.

IBFC data analysis

Data were analyzed using IDEAS® analysis software version 6.2 from AMNIS EMD Millipore. Gating approach used standard focus and single cell gating calculations created by IDEAS software as previously described [6].

Results

Using zinc reporter FluoZin-3 AM (FZ3) paired with image-based flow cytometry (IBFC), we show the four originally discovered sperm zinc signatures [6] in **Figure 1**. Specifically, zinc signature 1 has zinc localization reported by FZ3 fluorescence across the entire sperm head and sperm tail, including the middle, principal and end pieces (**Figure 1a**). Zinc localization restricted to only the sperm head and midpiece, excluding principal and end pieces is signature 2 (**Figure 1b**). Localization of zinc to the sperm midpiece alone is that of signature 3 (**Figure 1c**) and no zinc localization (as reported by FZ3) is that of signature 4 (**Figure 1d**).

A physiological summary of the sperm zinc signature subpopulations is presented in **Figure 2a** along with physiological meaning of the four sperm zinc signatures in **Figure 2b**, as reported in [6].

Figure 1. Sperm zinc signatures. **A.** Signature 1 with zinc localization to the entire sperm head and sperm tail, including the principal and end pieces. **B.** Signature 2 with zinc localization to the sperm head and sperm tail midpiece. **C.** Signature 3 with zinc localization to the sperm midpiece only. **D.** Signature 4, with no zinc localization reported by the FZ3 probe.

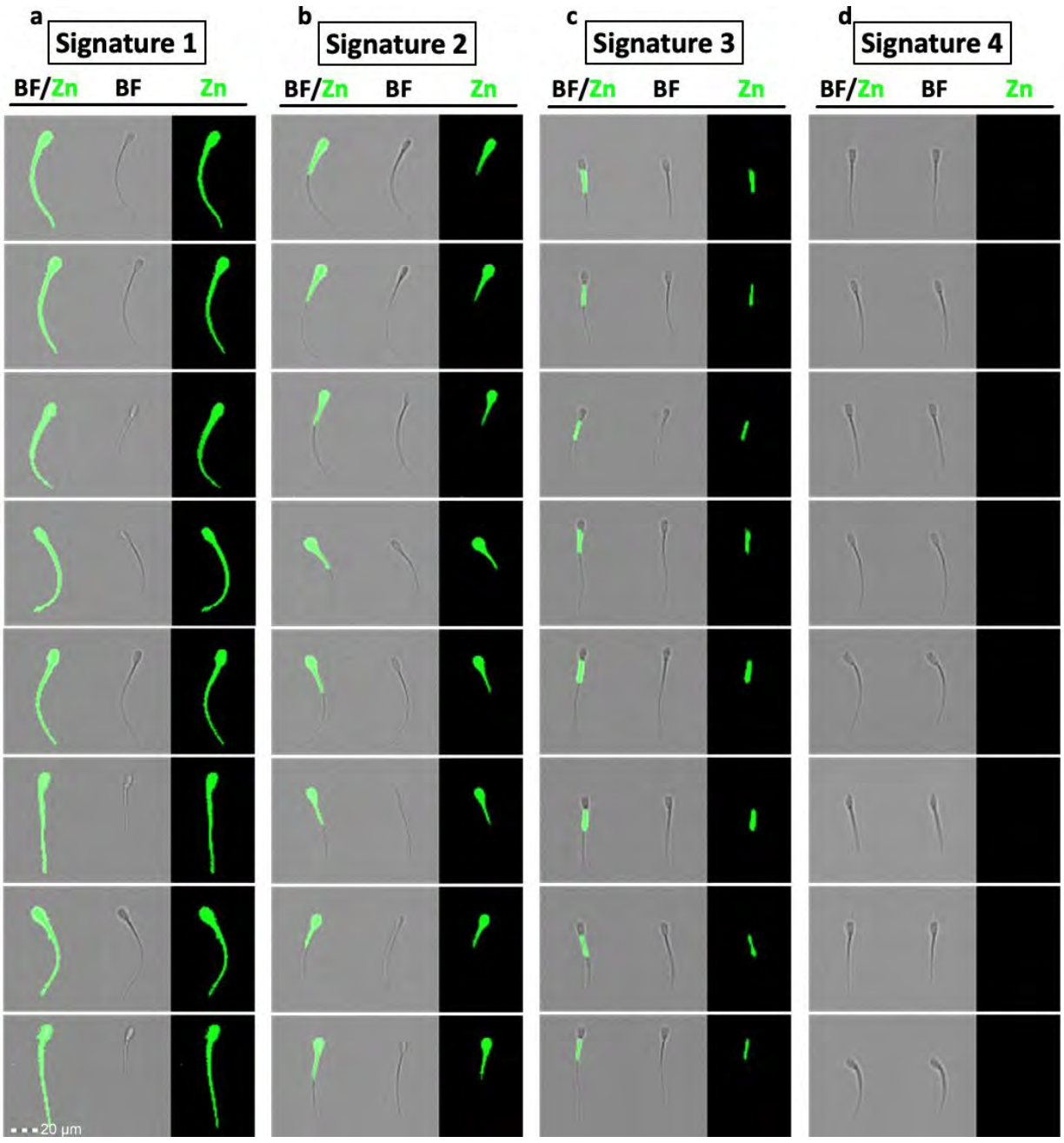
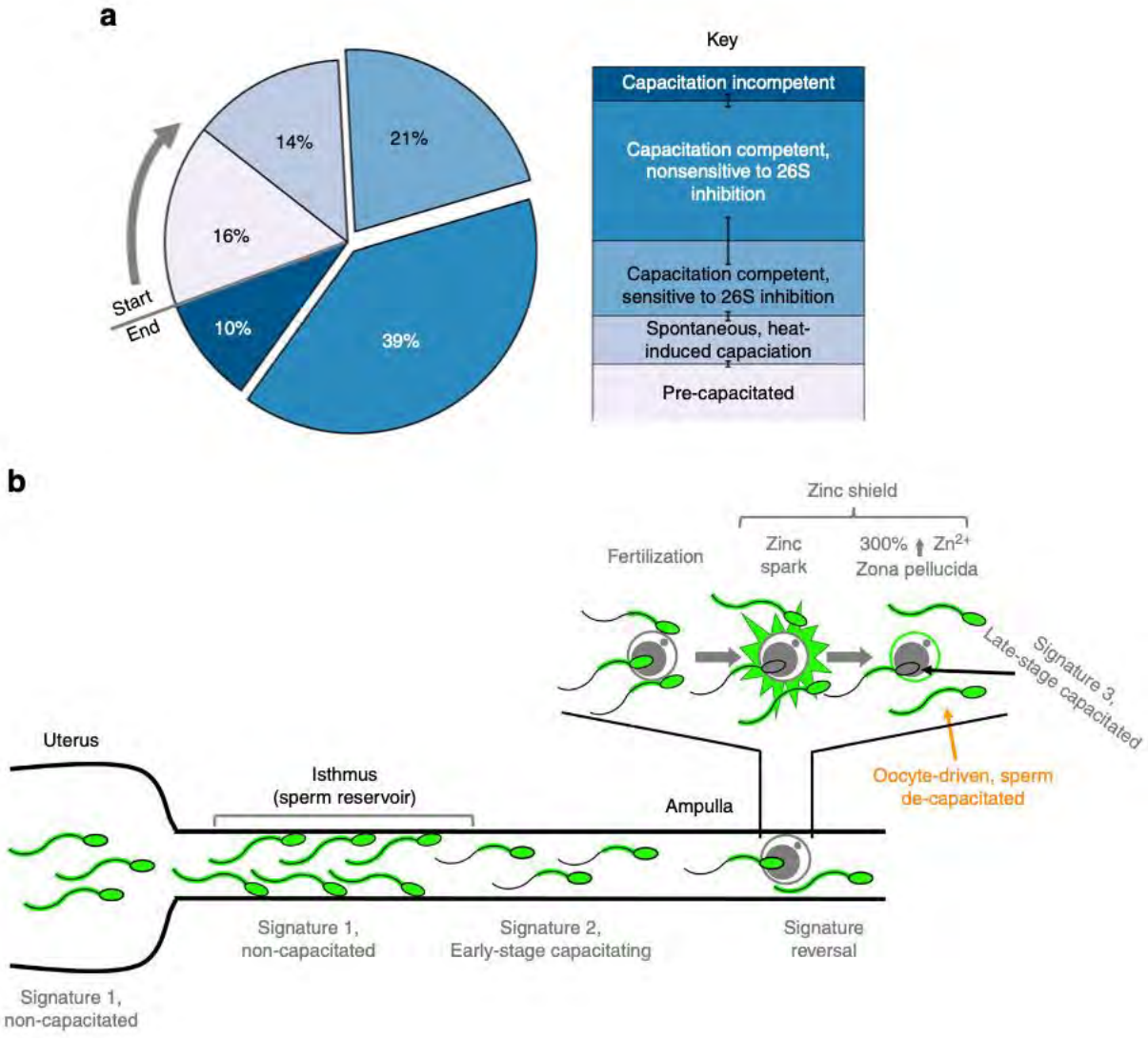


Figure 2. Proposed zinc signature population interpretation. **A.** Example of an individual boar's sperm subpopulations identified by the sperm zinc signature and meaning: 16% of fresh, ejaculated spermatozoa underwent early-stage capacitation upon semen collection (lightest blue working to darkest); 14% of spermatozoa spontaneously underwent early-stage capacitation during incubation without IVC inducers; 60% of spermatozoa remained capacitation competent with IVC inducers, with 21% sensitive to proteasomal inhibition; remaining 10% of sperm were capacitation incompetent under IVC conditions (darkest blue) (s.e. bars included). **B.** Proposed zinc signature changes throughout female reproductive tract and oocyte zinc spark interference with sperm zinc signature as a combined polyspermy defense mechanism, the zinc shield. Summary as reported in [6].



Discussion

We previously reported in detail on the relationship between sperm capacitation state and sperm zinc signatures 1-4 [6] and more recently regarding the zinc ion efflux from the sperm head [7]. Fresh preserved boar spermatozoa have few (as percent of entire population) spermatozoa in a zinc signature 1 state as compared to fresh ejaculated. This is due to chemicals within semen extenders that promote early stages of sperm capacitation.

Figure 2 represents a mechanistic way to help identify sperm capable of undergoing the last maturation events of sperm capacitation, endowing sperm the ability to fertilize the oocyte. It is important to note that the percent of sperm in each cohort is boar dependent and can vary from ejaculate-to-ejaculate. These differences observed might explain boar-to-boar and ejaculate-to-ejaculate fertility differences.

Conclusion

The sperm zinc signature is a new, physiologically relevant biomarker of boar sperm fertilization competency, correlating with key biological events during sperm capacitation. Here we briefly summarize the sperm zinc signature. These findings represent a shift in the understanding of boar fertilization competency, paving the way for improved boar fertility diagnostics and artificial insemination.

Acknowledgments

This work was supported by the National Institute of Food and Agriculture (NIFA), U.S. Department of Agriculture (USDA) award number 2019-67012-34005 (K.K.).

References

[1] Massanyi P, Trandzik J, Nad P, Korenekova B, Skalicka M, Toman R, et al. Concentration of copper, iron, zinc, cadmium, lead, and nickel in boar semen and relation to the spermatozoa quality. Journal of environmental science and health Part A, Toxic/hazardous substances & environmental engineering. 2003;38:2643-51.

[2] Andreychenko SV, Klepko AV, Gorban LV, Motryna OA, Grubska LV, Trofimenko OV. Post-Chornobyl remote radiation effects on human sperm and seminal plasma characteristics. Experimental oncology. 2016;38:245-51.

[3] Nenkova G, Petrov L, Alexandrova A. Role of Trace Elements for Oxidative Status and Quality of Human Sperm. Balkan medical journal. 2017.

[4] Colagar AH, Marzony ET, Chaichi MJ. Zinc levels in seminal plasma are associated with sperm quality in fertile and infertile men. Nutrition research (New York, NY). 2009;29:82-8.

[5] Zhao J, Dong X, Hu X, Long Z, Wang L, Liu Q, et al. Zinc levels in seminal plasma and their correlation with male infertility: A systematic review and meta-analysis. Sci Rep. 2016;6.

[6] Kerns K, Zigo M, Drobnis EZ, Sutovsky M, Sutovsky P. Zinc ion flux during mammalian sperm capacitation. Nature Communications. 2018;9:2061.

[7] Kerns K, Sharif M, Zigo M, Xu W, Hamilton LE, Sutovsky M, et al. Sperm Cohort-Specific Zinc Signature Acquisition and Capacitation-Induced Zinc Flux Regulate Sperm-Oviduct and Sperm-Zona Pellucida Interactions. Int J Mol Sci. 2020;21.

[8] Kerns K, Zigo M, Sutovsky P. Zinc: A Necessary Ion for Mammalian Sperm Fertilization Competency. International Journal of Molecular Sciences. 2018;19:4097.

[9] Zimmerman SW, Manandhar G, Yi YJ, Gupta SK, Sutovsky M, Odhiambo JF, et al. Sperm proteasomes degrade sperm receptor on the egg zona pellucida during mammalian fertilization. PloS one. 2011;6:e17256.

Boar Management Conference

What have we learned from scrotal insulation?

Parrish JJ, Berndtson JL
Department of Animal and Dairy Science, University of Wisconsin-Madison.

Seasonal declines in reproductive efficiency of swine occur during warm months and are globally recognized as sources of significant economic loss for the pork industry. Excessive heat leads to declines in overall boar fertility that manifest as changes to semen quality, such as a reduction of sperm count, volume, motility, morphology, and sperm head. Attempts to reduce this subfertility by controlling environmental factors have been met with limited success.

Spermatogenesis occurs within the testes in the seminiferous tubule. Leydig cells, blood vessels, lymphatics, and nerves reside in the interstitial space outside the seminiferous tubules; Sertoli cells and the germ cells reside within the seminiferous tubules. Sertoli cells support and direct developing germ cells through mitosis, meiosis, spermiogenesis, and spermiation.
Germ cells in the male first undergo a series of mitotic division, followed by meiosis and spermiogenesis, resulting in morphologically recognized spermatozoa. In the boar, type A spermatogonia initiates mitosis and will divide multiple times to eventually form 64 primary spermatocytes. These divide twice through meiosis to yield 256 round spermatids which then form 256 spermatozoa. At least this is the theoretical maximum number of cells produced. In reality, between 70% and 90% of these cells are lost in the boar and is certainly subject to increased losses during heat stress.

Spermatogenesis in the boar lasted 34.4 days. Transit of sperm through the epididymis required 10.2 days. Therefore, approximately 45 days are required from the point that a type A spermatogonium begins to divide until sperm are ejaculated.

The two most important, sperm-production-related somatic cells in the testis are Leydig and Sertoli cells. As puberty approaches, the adult Leydig cells become more sensitive to luteinizing hormone (LH) from the anterior pituitary. Adult Leydig cells, hereafter referred to as “Leydig cells,” respond to Luteinizing hormone by producing testosterone as well as other growth factors that regulate Sertoli cell function. Sertoli cells possess receptors for testosterone and Follicle-stimulating hormone, which comes from the anterior pituitary. Follicle-stimulating hormone (FSH) regulates the proliferation of Sertoli cells prior to puberty and then ability of Sertoli cells to support spermatogenesis. Expansion of the Sertoli cell population is accompanied by increased seminiferous tubules length and testis size. The final number of Sertoli cells at puberty (between 3 and 4.5 months of age in the boar) determines the total number of sperm that can be produced by the testis. All germ cell progeny of a single type A spermatogonium associate with a single Sertoli cell.

The blood testes barrier (BTB) established between adjacent Sertoli cells is critical for spermatogenesis. The mammalian BTB is formed by tight junctions, adherens junctions, gap junctions and desmosomes. This barrier separates the seminiferous epithelium into basal and

adluminal compartments. Mitosis occurs in the basal compartment whereas germ cell meiosis is restricted to the adluminal compartment. The adluminal compartment is also an immunologically privileged site, isolated from the circulatory and lymphatic secretions by the BTB and by the immunosuppressive action of Sertoli cells. The latter immune-related function of Sertoli cells evolved because all germ cells, whether in the basal or adluminal compartment, can trigger an autoimmune response.

Primary spermatocytes in meiosis must cross the BTB, transiting from the basal to adluminal compartment, before meiosis can progress. Primary spermatocytes are connected to their siblings by cytoplasmic bridges, so they must cross the BTB together. This action is not a migration but a reorganization of the BTB tight junctions. This process must occur without allowing communication between the adluminal and basal compartments of the seminiferous tubule.

The boar testis resides outside the body cavity and is kept between 2 and 5°C below core body temperature. Elevated environmental temperatures negatively impact boar sperm production. For example, exposure of boars to temperatures between 33.4–37.7°C for 4–7 days, for as little as 6 hr per day, resulted in more abnormal sperm ejaculated 2–5 weeks after treatment.

An interaction of temperature and length of exposure exists for fertility metrics. Long-term, ambient exposure intensifies the effects of heat stress in boars. There are numerous studies reviewed by Parrish et al. (2017) that show when temperatures rise above the thermo-neutral zone of the pig, heat stress damage to spermatogenesis occurs. Currently the thermo-neutral zone temperature in modern adult lean pigs is believed to have an upper value of 22.7°C (72.9°F; Brown-Brandl et al., 2013). While whole animal heat exposure is relevant to the industry, it complicates the interpretation of experiments since effects on physiological, metabolic, and endocrine parameters beyond the testes may be impacted by heat.

Scrotal insulation provides an alternative approach to study the impact of heat stress on the testes that can be performed year-round. The boar testis is normally maintained below body temperature by heat loss from the scrotum, counter-current exchange of heat between the arterial and venous system in the pampinform plexus, and heat loss from the spematic chord/pampiniform plexus in the inguinal region. The experimental approach of scrotal insulation is to affix insulating material to the scrotum, sometimes including the area adjacent to the spermatic chord that contains the pampinform plexus. We developed a repeatable scrotal insulation model to explore short-term heat stress impacts on spermatogenesis in the boar. We designed the system to produce abnormal sperm in post-insulation ejaculates, but to minimize the impact on sperm density in ejaculates. Longer periods (84–96 hr) and more insulation resulted in sperm numbers crashing to null after 3 weeks and not returning even 60 days following insulation. Insulated sacks were constructed with layers of nylon, cotton batting, mylar (as a vapor and insulation material), cotton batting, and then canvas (ordered from the outside in); when used, sham sacks were constructed similarly, but consisted of only nylon and canvas. These sacks covered the scrotum and under the inguinal region to insulate the pampiniform plexus. A temperature monitor was first glued to the scrotum, midway between the two testes, and then the sacks were glued to the scrotum; this method did not require local anesthesia or alter boar behavior. Temperatures in the scrotal sacks increased from 32.1 ± 0.4 to 34.0 ± 0.3°C at 48 hr of insulation ($p < 0.05$; five control and five insulated boars), with control values at 31.1 ±

0.6°C in some experiments. More recently, smaller temperature monitors applied below the scrotal skin prior to insulation revealed resting temperatures of 34.9 ± 0.3°C (n = 6) that increased to 38.0 ± 0.1°C (n = 3) upon insulation using the same scrotal sack design. The impact of scrotal insulation on post-insulation semen quality was examined by collecting ejaculates three times a week, to avoid the stress of an altered semen collection schedule and to minimize mixing of sperm in the tail of the epididymis. Semen was collected 2 weeks before insulation and 6 weeks post insulation. All semen parameters were compared to average values before the insulation was applied (designated as Day 0). Semen was evaluated for sperm morphology, sperm nucleus shape (by Fourier harmonic analysis), and motility (via computer-assisted sperm analysis). Sham insulation has no effect on sperm motility, morphology, or Fourier harmonic analysis ($p>0.05$). Changes in these sperm metrics occurred over time and in a specific sequence with sperm with cytoplasmic droplets (tertiary abnormalities) appearing first at Day 19, followed by sperm with abnormal tails (secondary abnormalities) and sperm with abnormal heads (primary abnormalities) at Day 21 (all metrics, $p < 0.05$). The percentage of motile sperm did not decline until Day 30 ($p < 0.05$). Sperm nuclear shape was evaluated with Fourier Harmonic analysis (FHA) that produced Harmonic amplitudes (HA) 0, 3, and 5 changes starting at Day 23 ($p < 0.05$). Relative to Day 0, 48 hr scrotal insulation decreased motility on Days 30–35, morphology abnormalities increased between Days 19 and 37, and Fourier harmonic analysis shape parameters changed between Days 23 and 33 (all metrics, $p < 0.05$). Similar to other whole-animal heat stress or scrotal insulation studies, changes to semen quality parameters occur between Weeks 3 and 5 post-insulation or heat exposure; however, our system allowed for more frequent semen collection and tighter control of heat exposure to the testis.

To determine how scrotal insulation or heat impacted specific cell types of spermatogenesis was first assessed based on the days post-scrotal insulation when ejaculated sperm characteristics change. Data from the bull indicated that, although short-term scrotal insulation produces abnormal sperm, it does not alter the length or timing of spermatogenesis. Therefore, calculations based on known cycles and stages of spermatogenesis allowed for the prediction of when a specific cell type will be ejaculated following its maturation. This approach was used to examine how scrotal insulation impacts spermatogenesis in the bull. Accuracy of this approach requires frequent collection of semen following scrotal insulation. In the boar, semen was collected three times per week both before and after scrotal insulation. A 48 hr scrotal insulation period revealed heat sensitivity of spermatogenesis during meiosis, particularly the period from primary spermatocytes through round spermatids, which lead to sperm with abnormal morphology and/or changes to sperm nucleus shape.

Direct histological evaluation of changes in spermatogenesis was performed to understand the source of the sperm defects observed in the ejaculates. Testicular histology following scrotal insulation was measured in two trials, first with two control boars and three boars with full scrotal insulation, followed by five control and five insulated boars. Tissue was fixed with Karnovsky solution, stored in 70% ethanol at 4°C until embedding in Epon, sectioned (2 μm thick), and then stained with 0.5% toluidine blue with 1% sodium borate in water. Of significance in the testis post-insulation versus controls were: (i) a 15–18% decrease in the height of the seminiferous tubule epithelium ($p < 0.05$); (ii) a 20–30% fewer preleptotene/leptotene primary spermatocytes and pachytene primary spermatocytes ($p < 0.05$); (iii) a 15% reduction in the number of round spermatids in the second trial ($p < 0.05$) but not the first trial ($p > 0.05$); and

(iv) an 1.8- to 2.2-fold increase in debris and in the remains of primary, secondary, and round spermatids in the lumen of the seminiferous tubule ($p < 0.05$). Vacuoles were also noted in the seminiferous epithelium following scrotal insulation which are indicative of junctional dysregulation observed between adjacent murine Sertoli cells. A clear loss of germ cells migrating through the BTB and cells in meiosis was also observed, although the germ cells remaining in the seminiferous epithelium will likely produce sperm, as demonstrated by the first study using the same scrotal insulation technique and semen collection. In the bull, sperm produced post-scrotal insulation (Days 19 and 33) are not normal, possessing elevated markers of apoptosis and exhibiting reduced in vitro fertility. These attributes corresponded with changes to Fourier harmonic analysis metrics and suggested that DNA damage occurred in these sperm during their development in the testis.

Clearly scrotal insulation produces changes in spermatogenesis that result in fewer and more abnormal sperm with altered sperm nuclear shape. Scrotal insulation however also provides us the means to measure if mitigation strategies could lessen the impact of heat stress on the testes. While in the boar stud, cool cell technology, air conditioning, scrotal mist or drips are among the measures used to reduce heat stress it still occurs likely due to failure to achieve temperatures below the maximal thermal neutral zone of the pig. We have considered an alternative using pharmaceutical manipulation. Based on the studies we have conducted with scrotal insulation it appears that there is both an impact on Leydig cells and Sertoli cells that leads to increased cell death and production of abnormal sperm. We have chosen therefore to evaluate PG600 which has: 1) Human Chorionic Gonadotropin (HCG) that has LH-like effects and 2) Equine Chorionic Gonadotropin (ECG) that has FSH-like effects. Scrotal insulated (48 hr) boars were treated with saline or PG600 (standard dose for gilts) at 24 hr before and at the start of scrotal insulation. The standard semen collection schedule was followed. Scrotal insulation produced a 3.5°C (5°F) increase in average scrotal temperature for 48 hours ($p<0.0001$). Total sperm output was unchanged ($p>0.05$) following scrotal insulation as our model is designed to do. Increased scrotal temperature without PG600 treatment caused: motility to decrease days 26-30; primary abnormalities to increase days 21-30; secondary abnormalities to increase days 28-30; tertiary abnormalities to increase days 14, 19-26 and 30; perimeter to decrease days 30-35; HA0 to decrease days 21-33; HA1 to increase day 30; HA2 to decrease day 35; HA3 to increase days 21-33; HA4 to increase days 21-26; HA5 to increase days 21-30; and overall sperm nuclei shape change on days 5, 30, and 35 ($p<0.05$). Treatment with PG600 decreased the days when various changes in sperm quality, post scrotal insulation, were seen as well as the magnitude of the response. PG600 thus mitigated the response of boars to scrotal insulation and testicular heat stress. Increasing the dose of PG600 for boars may improve results.

Scrotal insulation is short-term and is different from environmental heat stress traditionally experienced by boars during the summer season. While induced heat stress, like scrotal insulation, is an excellent starting point for the examination of heat stress due to its' repeatability and consistency, it does not cause whole animal heat stress, which induces various systemic physiological responses. For use in a production setting to offset the effects of heat stress on spermatogenesis in boars it is critical to experiment with the use of PG600 during environmental heat stress.

The use of antioxidants has also been suggested to mitigate heat stress in boars. However, results are inconsistent. Certain antioxidants, like zinc, have in fact shown negative impacts on sperm quality. Even in studies where antioxidants are suggested to reduce DNA damage to spermatozoa from heat stress increases in sperm head width, regardless of season, are seen possibly indicating impaired compaction, indicating long-term supplementation of zinc, an antioxidant, is detrimental. No negative changes in sperm quality were noted with PG600 treatment without heat stress.

In a commercial setting PG600 would need to be given multiple times during the summer. A potential limitation to the repeated use of PG600 is the possible production of antibodies leading to declining effectiveness after long-term use. Anti-eCG antibodies, an important component of PG600, have been seen in some but not all species. Anti-hCG antibodies, another component of PG600 have also been seen in some but not all species. If antibodies form it is unclear how long they persist in the body and if these interfere with pharmacological and biological action, as research is controversial. Further research on antibodies against eCG and hCG, which could limit the effectiveness of PG600 as a treatment for heat stress, should be conducted in boars.

Conclusions

Heat stress in boars leads to a negative impact on spermatogenesis most importantly on meiosis. Scrotal insulation clearly demonstrates such impacts on primary, secondary and round spermatids. However, some of these germ cells survive but produce altered spermatozoa of likely reduced fertility. PG600 was shown to mitigate some of the impact of scrotal insulation but requires more research. A more detailed set of references appears in Parrish et al., 2017.

References

Brown-Brandl, T. M., R. A. Eigenberg, and J. L. Purswell. 2013. Using thermal imaging as a method of investigating thermal thresholds in finishing pigs. Biosystems Engineering. 114:327–333.

Parrish, J. J., K. L. Willenburg, K. M. Gibbs, K. B. Yagoda, M. M. Krautkramer, T. M. Loether, and F. C. S. A. Melo. 2017. Scrotal insulation and sperm production in the boar. Molecular Reproduction and Development. 84:969–978.

THE PHYSIOLOGY OF SPERM PRODUCTION AND THE REPRODUCTIVE MANAGEMENT OF BOARS FOR FERTILITY

Rob Knox, Ph.D. and Lidia Sbaraini Arend, DVM
Department of Animal Sciences, University of Illinois, Champaign-Urbana USA

Introduction

In most major swine producing countries, pork production relies almost exclusively upon AI for dissemination of genetics and breeding herd management (Riesenbeck 2011). Adoption of AI began for the pig industry in the early 1990's and rapidly increased in developed countries and on operations with 500 or more pigs (USDA-APHIS 2006). The rapid rate of AI adoption occurred as a result of the advantages of labor efficiency with increased breeding herd size, animal health, faster rates of genetic progress, and improved reproductive efficiency (Flowers and Alhusen 1992). Capturing these critical advantages depended upon the development of a highly regulated quality control system for production and use of the highest quality boar semen. Today, much of the commercial production of boar sperm occurs in boar studs even in developing countries. These are specialized entities separated from other pork production phases that allow for specified management of boars and labor for sperm production. Most boar studs apply the highest levels of biosecurity processes for management of facilities, animals and people. Biosecurity typically involves new boar isolation and acclimation, vaccination and regular health monitoring. Personnel biosecurity involves training, education, and stringent processes to control human movement in order to limit disease entry and risk (AASV 2003, Althouse 2008b, Singleton and Flowers 2006).

In commercial breeding farms that rely exclusively on AI, the consistent flow of pigs to market relies on high quality sperm for breeding herd fertility. Of critical importance to the commercial swine industry is that the semen produced is free of disease and meets high quality semen characteristics. Since AI allows rapid dissemination of genes to multiple farms at great distances in a short period of time, it might also contribute to rapid disease spread through contaminated semen when correct prevention procedures are not followed, which can have devastating consequences for breeding farms for months or years. Furthermore, the distribution of poor fertility semen can result in lower breeding herd performance and reduced flow of pigs for market. As a result, there is great dependence upon the boar studs to provide reliable and frequent delivery of high quality semen with individual boars producing enough doses to breed approximately 650 sows each year. In the USA, this translates to a need of 1 boar in stud for every 250 to 300 sows and would require at least 20,000 sires to cover all sows to breed within a year.

The success of AI and the boar stud industry can be appreciated by examining fertility results from use of AI when examining the changes in farrowing rates, litter sizes and pigs produced per sow per year over time (PigCHAMP 2012, USDA 2012). Current fertility data in North America for herds that maintain more than 1,000 sows, illustrates the production measures on average and in the top 10% of farms (Table 1, (PigCHAMP 2012). These fertility measures can be attributed, in large part to improvements in sperm quality, boar fertility, and AI success. To ensure that semen production needs are met, managers of studs must select boars to be physically capable of semen production and collection, and that the semen meets minimal fertility standards. The studs help accomplish these tasks by evaluating a boar's libido, sperm production efficiency, and fertility performance when possible. To allow expression of a sire's genetic potential for sperm production, boars must be managed properly. This article will provide an overview of the anatomy and physiology of boar reproduction and factors that can influence sperm production and boar fertility.

Table 1. Current data on herd fertility in North American databases (adapted from PigCHAMP Benchmarking, USA 2015 and CANADA 2013).

	US
Number of herds	411
Average herd size	2022
Average farrowing rate (%)	83.4
Average farrowing rate for the top 10% of farms	91.74
Average total born pigs	13.64
Average total born for the top 10% of farms	14.91

Boar Anatomy

The major components of the reproductive system of the boar (Figure 1) include the testicle, epididymis, ductus deferens, blood vessels, nerves, connective tissues, muscles, accessory glands and penis (Ashdown and Hafez 1993, Bearden and Fuquay 1997, Bone 1988, Frandson et al. 2003). The reproductive tract is supported by connective tissue attachments to both the pelvis and the sacral vertebrae. While some of the tissues of the reproductive tract are housed in the abdomen but outside the peritoneum, the testes are located outside the body cavity in order to allow spermatogenesis to occur at temperatures a few degrees below core body temperature. The primary function of the testes is to produce both sperm cells and the male hormone testosterone. In the boar, the testes are paired and inverted with the caudal epididymis and ductus deferens located at the top of the inverted testicle (Figure 2). Spermatogenesis occurs within seminiferous tubules of the testicles which are highly convoluted and densely packed within the testes (Figure 3). Numerous tubules converge into a single collection tube in the center of the testicle (rete testes) which exits the testicle and enters the head (caput) of the sperm maturation tube, called the epididymis. The epididymis is located on the outside of the testicle and contains a highly coiled tubule that may be >50 meters in length. The testicles are contained within a pouch called the scrotum which has multiple tissue layers that serves to protect and thermoregulate the temperature of the testes. Unlike males from other species with a pendulous scrotum (bulls, ram), the boar's is not well defined and remains close to the body wall (Figure 4). The testes are connected to the internal components of the male reproductive system by the spermatic cord which passes through a small opening in the abdominal wall called the inguinal canal. Failure of the canal to close following testicle decent usually allows for the intestines to pass through the opening resulting in scrotal hernia. The spermatic cord contains blood vessels, nerves, muscles, and connective tissue (Figure 2). These are all involved with temperature regulation, sperm production and movement of sperm out of the epididymis. For each testicle, the epididymis connects to a tube called the ductus deferens which leaves the testicle to become part of the spermatic cord and passes through the inguinal canal to enter into the abdomen. In cases where sterile or vasectomized or V-boars are required, the ductus deferens is surgically cut and then sutured to create sterile males. These V-boars maintain high libido since testosterone production is normal and are often used for estrous detection, but are sterile since they do not have sperm in the ejaculate. Each of the ducti deferentia merge into a single tube called the pelvic urethra which forms at the neck of the bladder (Figure 1). The urethra continues and passes through various muscles and accessory glands in the pelvic region until it forms the penile urethra. The function of the urethra is to transport both semen and urine. Urine enters the urethra from the bladder by relaxation of a muscle under voluntary control of the boar, but relaxation of this muscle is prevented during erection and ejaculation by the nervous system to prevent urine from entering into the ejaculate.

Figure 1. Drawing of the boar reproductive (adapted from W. Singleton, Purdue University, IN USA).

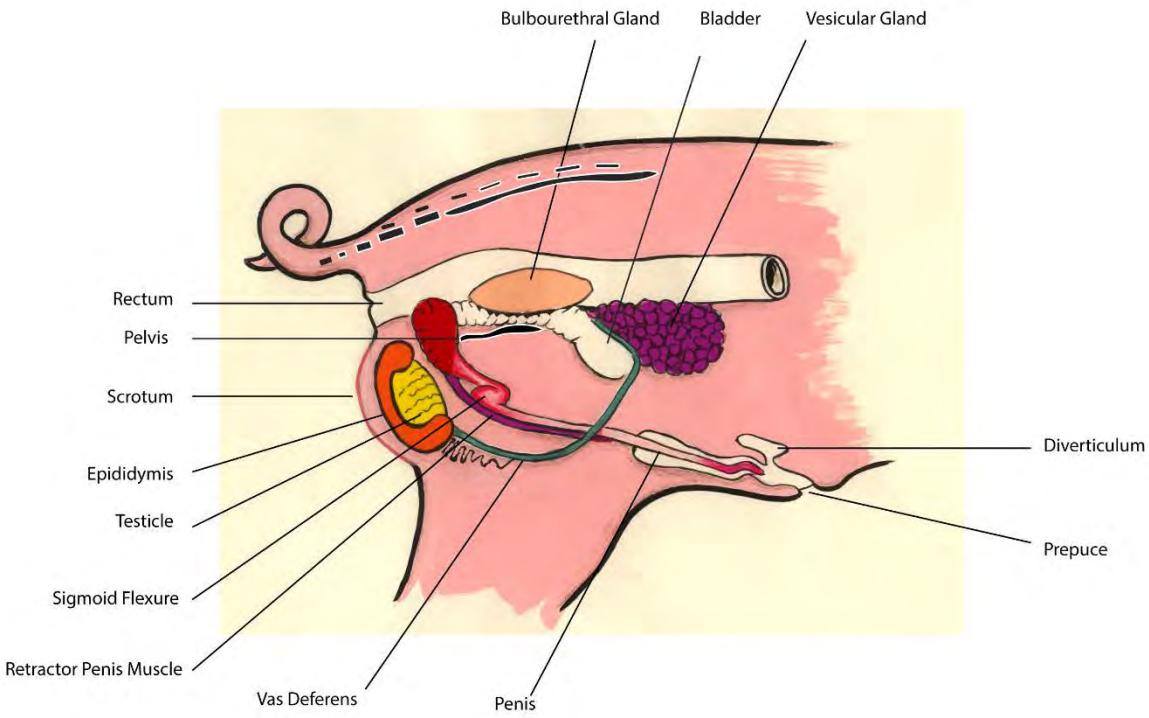


Figure 2. Labeled picture of an inverted boar testicle.

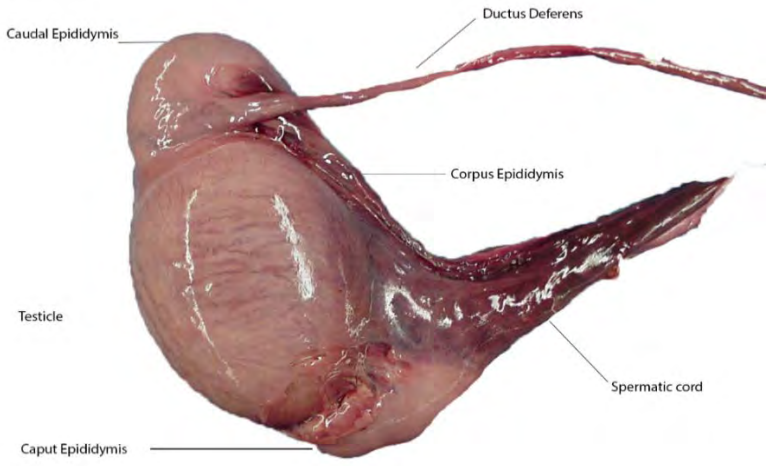


Figure 3. Drawing of a cross section of an inverted boar testicle with an adjacent picture of a dissected boar testicle.

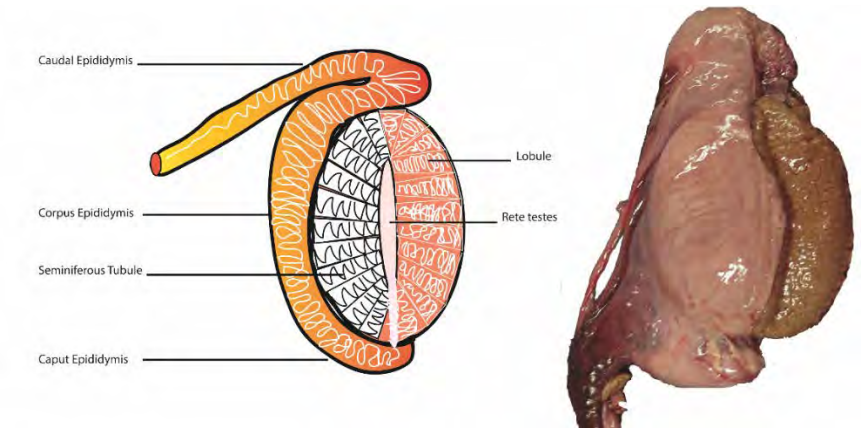
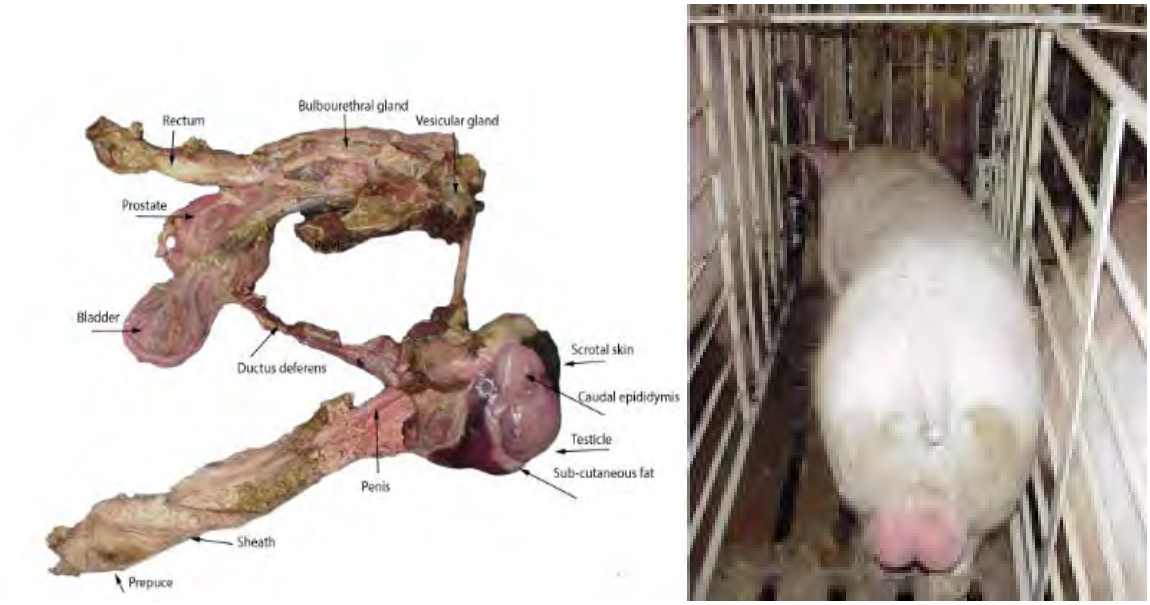


Figure 4. Dissected boar reproductive tract with images of the boar scrotum.



Semen is composed of sperm cells and seminal plasma which contains approximately 95% water with numerous organic and inorganic molecules that maintain a neutral pH of ~7.4. The importance of seminal plasma to fertility has received much attention over the years due to its complex array of components and the fact that when using AI, the seminal plasma is diluted to such a great extent that any physiological effect is greatly diminished (Flowers et al. 2013). Several roles have been attributed to the components of seminal plasma that include sperm transport, immune modulation of the uterus, and altering time of ovulation. Fluid, ionic substances, organic molecules, steroids, and proteins are all added to sperm to aid in fertility. These substances are added in different volumes and concentrations by the different parts of the boar reproductive tract (Setchell and Brooks 1988). Small amounts of fluid originate from the seminiferous tubules and epididymis, while the majority of semen volume originates from the prostate, vesicular, and bulbourethral glands. The secretions from the tubules and glands are important to sperm fertility as they regulate volume, motility, cell metabolism, membrane integrity, osmotic balance, pH buffering, and oxidation. Reviews and evaluation of ejaculate components has been performed for the boar (Setchell and Brooks 1988, Garner and Hafez 1993, Louis et al. 1994, Rodriguez-Martinez et al. 2009, Claus et al. 1983, Borg et al. 1993, Park et al. 2005, Colenbrander and Kemp 1990, Claus 1990, Colenbrander et al. 1993) and a summary is shown in Table 2. Proteins in the ejaculate increase with the

phases of the ejaculate and the major proteins have been identified as porcine seminal plasma proteins PSP-I and II, which belong to the spermadhesin family and AQN-1 and 3, and AWN 1 and 2, which are classified as heparin binding proteins. Collectively, these proteins appear to function in sperm membrane stabilization and may affect reproductive functions in the female reproductive tract as well (Rodriguez-Martinez et al. 2009). Hormone concentrations in the ejaculate have been reported (Table 2) with androgens reported to be approximately 10% of circulating concentrations while estrogens may be at 300% of those in found in circulation (Claus 1990, Setchell and Brooks 1988). Estrogens in semen originate from the epididymis while testosterone is produced by the accessory glands. Other hormones, such as prostaglandin and oxytocin are also detected in semen and are thought to aid in sperm transport in the uterus.

Table 2. Boar seminal plasma components and concentrations (Adapted from Setchell and Brooks, 1988; Garner and Hafez 1993; Rodriguez-Martinez et al., 2009; Claus, 1983; Claus 1990; Louis et al., 1994; Borg et al., 1993; Park and Yi, 2002; Colenbrander and Kemp, 1990)

Boar semen	Measure
Volume (mL)	100-300
Concentration ($x10^{6}$/mL)	150-400
Motility (%)	65-80%
Total sperm ($x10^{9}$/ejaculate)	30-130
Sodium (mM)	20-23
Potassium(mM)	1.50-4.60
Bicarbonate (mM)	2.50-24
Calcium (mM)	85-105
Magnesium (mM)	0.40
Chloride (mM)	0.50
Phosphate (mM)	0.06-0.30
Fructose (mM)	0.40-0.70
Glucose (mM)	28
Sorbitol (mM)	2.20
Inositol (mM)	2.60-10.40
Lactic acid (mM)	2
Citric acid (mM)	4-6.80
Glutamic acid (mM)	0.26
Glycerophoshpcholine (mM)	0.01
Glycerophosphoinositol (mM)	0.03
Arginine (mM)	0.70
Creatinine (mM)	0.03
Ergothionine (mM)	0.70
Protein (mg/mL)	30-39
pH	7.30-7.80
Estrogens (ug/ejaculate)	10-12
Prostaglandins (ng/mL)	0.50
Oxytocin (pg/mL)	1.50
Androgens(ng/mL)	0.20-0.60
Testosterone (ng/mL)	0.20-5.00

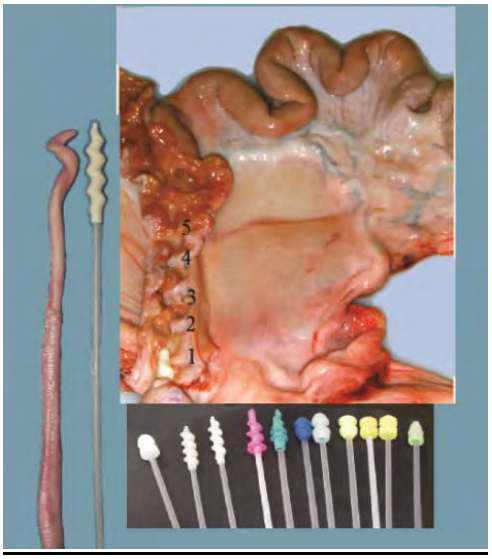
The boar ejaculate is emitted in waves with different fractions of the ejaculate (Figure 5) produced by different glands (Rodriguez-Martinez et al. 2009). The pre-sperm fraction contains limited epididymal fluid and sperm and is composed of fluid from the bulbourethral and prostate glands. The prostate gland is the first gland to add components into the pelvic urethra and is responsible for adding fluids that flush out urine and bacteria before sperm enter the urethra. The sperm rich fraction originates from the caudal epididymis and is diluted by fluids from the prostate and vesicular glands. The sperm poor fraction is composed of fluids from the prostate, vesicular and bulbourethral glands (Rodriguez-Martinez et al. 2009) and comes just after sperm rich fraction. The vesicular gland produces most of the volume, energy sources, buffers and ions. The bulbourethral gland produces a sialomucin which is responsible for the gelation fraction of the semen. The gelatin is added in many fractions but predominantly at the end of the ejaculation process. This sticky fraction serves to seal the cervix to keep the large ejaculate volume in the female uterus following natural service. The plug sets up quickly and may remain intact hours before expulsion. When

semen is collected, the pre-sperm fractions of the ejaculate are commonly not collected, while the remaining sperm rich and sperm poor fractions are collected through a filter to prevent gel contamination.

Figure 5. Picture of the unfiltered semen on sequence of collection fractions from a boar ejaculate.

The penis contains the penile urethra and is a common passageway for both semen and urine. The boar penis is composed of limited erectile tissue and when sexual stimulation occurs, shows limited increase in length and width. When the boar is not sexually stimulated, the retractor muscle is contracted and forms an S-shaped bend to maintain the long boar penis inside the body (Figures 1 and 4). However, once excited, neural stimulation allows retractor penis muscle relaxation and the S-bend unfolds, and the penis extends out of the prepuce. The boar has a preputial pouch which is located just above the prepuce. This diverticulum is a source of mucus, but will also accumulate urine, dead cells, and bacteria which can contaminate the ejaculate. The fluid in this diverticulum is manually massaged out using a gloved hand before collection, and the glove discarded. Upon extension of the penis, tension in the fibroelastic tissue causes twisting of the free end to form a corkscrew shape. The end is formed to match the pattern of pads to allow insertion into the cervix (Figure 6). Upon intromission of the penis into the cervix, the locking of the penis into the cervical pads is associated with pressure and temperature which is most important to induce the ejaculation reflex in the boar.

Figure 6. Image of the intact boar penis and AI catheter with an image of the cervix of a sow.



Neuroendocrine Control of Male Reproduction

The process of reproduction in males is regulated by the hypothalamus which is a neuroendocrine organ located near the base of the brain and in response to different stimuli, releases the hormone GnRH (Gonadotropin Releasing Hormone). This small deca-peptide is responsible for inducing the release of two hormones, FSH (Follicle Stimulating Hormone) and LH (Luteinizing Hormone) from the pituitary gland, which is located just below the hypothalamus. GnRH is either released or inhibited in response to neural perception of many physiological factors associated with maturity, metabolic state, environment, stress, sensory stimulation, and hormone feedback from the testes (Figure 7). Upon release of GnRH, FSH and LH are released from the pituitary and enter the circulatory system where they travel to bind specific receptors in the testicles. FSH binds to the Sertoli cells that line the seminiferous tubules of the testes (Figure 8). FSH stimulates cell metabolism, hormone production, and supplies nutrients and growth factors for the developing sperm. The nurse cells can contain up to 20 sperm cells in various stages of development at a time (Figure 9). Sperm production relies on Sertoli cell production of proteins that allow testosterone accumulation and its conversion to estrogen. The Leydig cells bind LH to activate enzyme pathways to convert cholesterol to testosterone. This cell is located outside of the seminiferous tubules but in close association with the blood supply to allow multi-directional flow of testosterone (Figure 10). Testosterone will cross the basement membrane to enter the seminiferous tubules which is required for Sertoli cell support of sperm production. The basement membrane of the tubule isolates sperm production from the immune system and systemic circulation. The hormone products of the Sertoli (inhibin) and Leydig (testosterone) cells also enter circulation where they feedback at the level of the hypothalamus and pituitary to regulate the release of GnRH, FSH and LH and sperm production.

Figure 7. Schematic illustration of the endocrinology of sperm production in the boar.

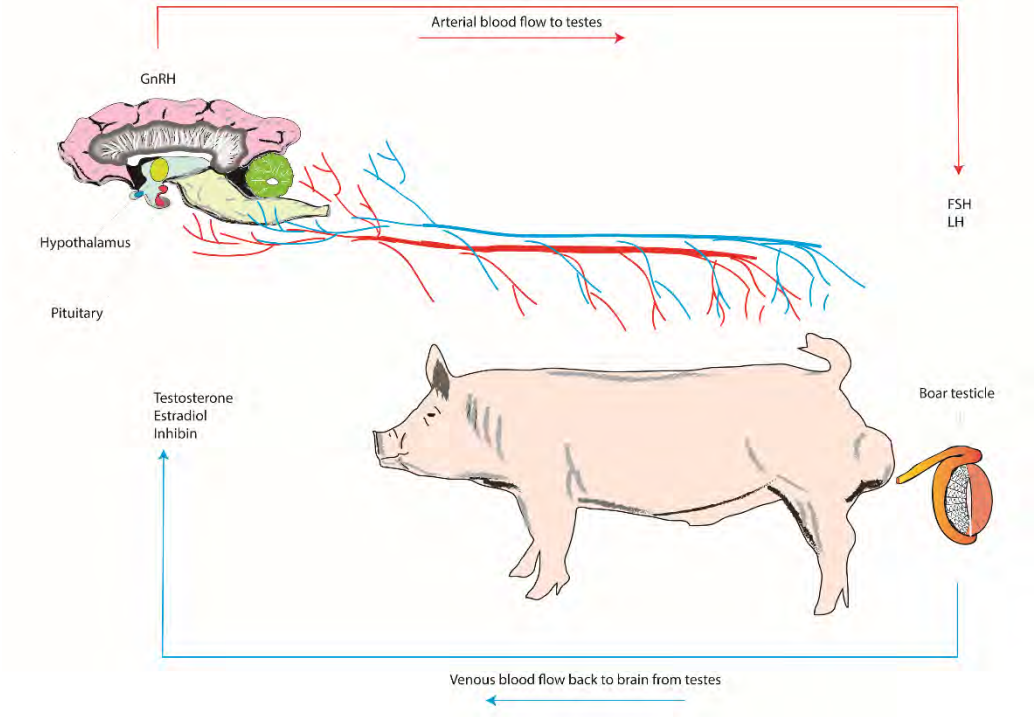


Figure 8. Illustration of gonadotropin binding to the Leydig and Sertoli cells.

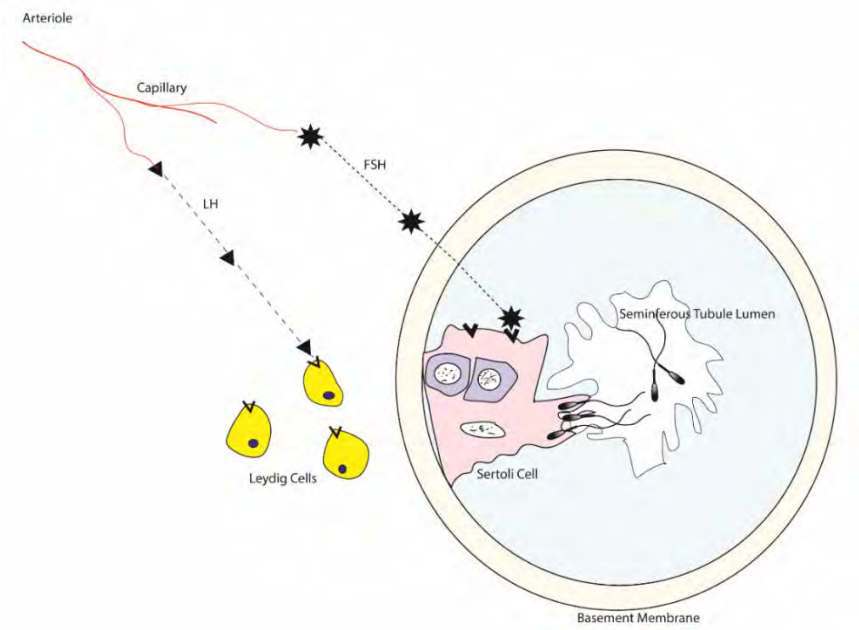


Figure 9. Drawing of the seminiferous tubules (adapted from Bearden and Fuquay, 1997)

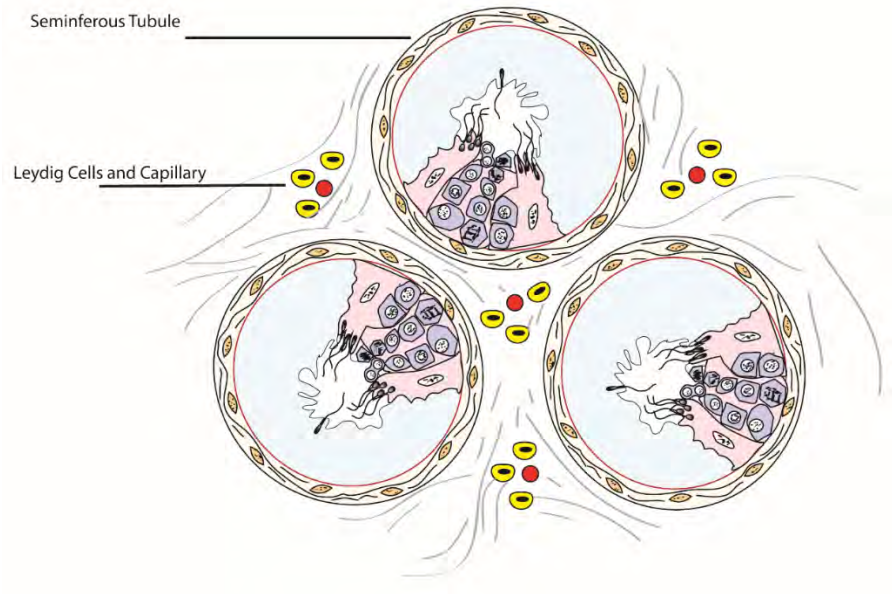
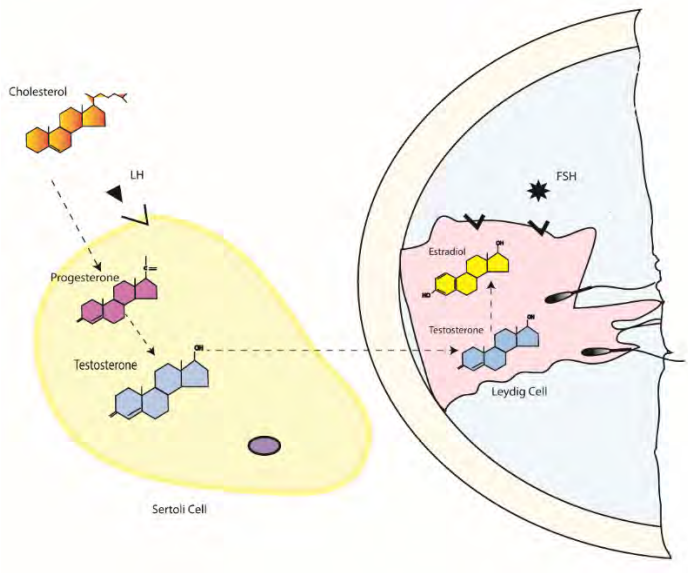


Figure 10. Illustration of steroid hormone production within the Leydig and Sertoli cells.



Testosterone in circulation allows responsive tissues to bind the steroid for male reproductive tract development, sexual behavior, and sperm production. It is produced in the testis of the fetal boar between 20 to 40 days of age when germ cell division rates are high. The levels of this hormone then decline and remain low until puberty. Testosterone production from the fetal testes is important for establishing male hormone patterns of release and behaviors. In the developing male fetus, testosterone is converted to estrogen in the brain. Exposure of either sex to testosterone during fetal or neonate development can alter hormone and behavior patterns. Alpha feto-protein circulates in both sexes and binds free estrogen in circulation to prevent estrogen entry into the brain and masculinization of the neuroendocrine system of female fetuses. Production of testosterone results from conversion of cholesterol to progestagen, androgen, testosterone, and in some cases to estrogen. This pathway to estrogen production in males is also involved in seminal plasma production and estrogen content. Estrogen in seminal plasma is thought to aid uterine contractions for sperm transport, and advance time of ovulation through uterine prostaglandin release (Claus 1990, Waberski 1997). Androgens and forms of testosterone control libido, aggressiveness, muscling, and development of the reproductive tissues such as the penis, testes, and accessory sex glands. Androgens also function as pheromones when produced in the sub-maxillary gland and are converted to the 5-α androgen form which are aerosolized to stimulate estrus in female pigs (Hughes et al. 1990). This hormone has been synthesized and can stimulate standing in some female pigs in estrus when sprayed toward the snout.

Physiology of Boar Sexual Development and Puberty

Sexual development in the boar is determined by the presence of the Y chromosome. The testes begin to form in the embryo at day 30 when germ cells migrate to the genital ridge and colonize the undifferentiated gonad near the kidney. The Y chromosome in Sertoli cells expresses the Sry gene which produces a male factor to stimulate androgen production from the testes (Franca et al. 2005). Androgen production induces male reproductive tract development as certain cells in the fetal reproductive tract are able to bind this steroid. At day 30 of embryo development, Sertoli cells initiate the first stage of cell division, with assembly of Sertoli and Leydig cells to form the seminiferous tubules. As the embryo develops further, the male reproductive tract grows slightly. Division of testes cells at this stage is independent of FSH and LH. At time of birth and to one month of age, there is a second proliferation of Sertoli cells, and then the third at 3 to 4 months of age. At the end of the third proliferation phase, a barrier forms to restrict blood flow into the

seminiferous tubules and to regulate the flow of fluids within the tubules. Sertoli cell division and their numbers prior to puberty will dictate the size of the testes and the number of sperm cells that can be matured and is thought to be the determining factor for lifetime sperm producing capability in mature males. Periods of post-natal increases in Sertoli cells are associated with higher FSH but these cells do not proliferate after puberty and sperm production potential is fixed by this time. Leydig cells are under control of LH and is the essential hormone driving testosterone production. Testosterone production in the Leydig cells depends upon the size of the smooth endoplasmic reticulum and the number of LH receptors, and not on number of Leydig cells. Leydig cells develop during the early fetal and peri-natal periods, but also continue to divide from onset of puberty into adulthood. During fetal development, the testes develop inside the abdomen. Then at ~60 days of gestation, they are gradually pulled out of the abdominal cavity and through the inguinal canal and into the scrotum. Later the canal becomes smaller, allowing only enough room for the spermatic cord. Failure of both testicles to descend can cause sterility. Failure of one testicle to descend is called unilateral cryptorchidism and can lead to a male that is normal in appearance but with reduced spermatogenic capability. The reduction occurs since normal sperm production must occur in the scrotum where temperatures are lower than body temperature. Testosterone levels are not affected in these males and except for reduced sperm production, all behaviors and growth are normal. In the case where both testes fail to descend into the scrotum (bilateral cryptorchidism), these males are sterile even though testosterone levels are normal and they appear to act like fertile males. Another abnormality associated with testicular descent is scrotal hernia where some of the gut is able to pass through the inguinal canal. This problem is thought to be genetically linked, and is most often observed in castrates, where improper pressure or technique alters the supportive structures of the testes during castration.

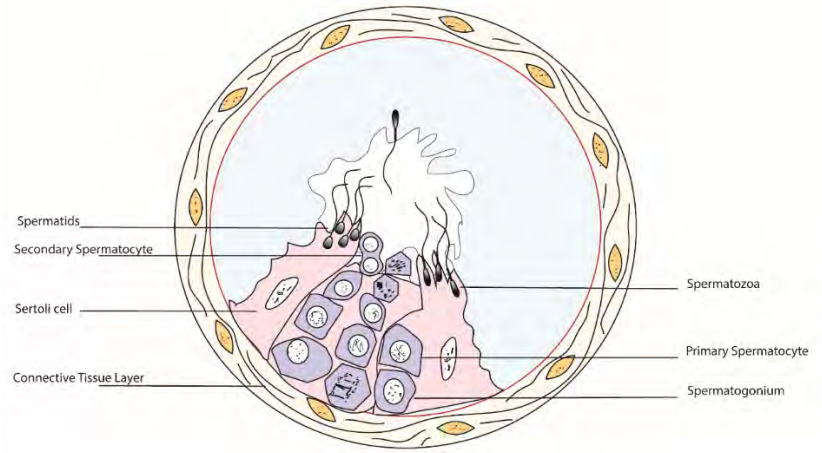
From the time of birth, the testis and epididymis grow continuously. Yet the factor that determines the number of sperm produced in the adult boar are the number of Sertoli cells. The number of cells are determined by periods of mitotic activity prior to birth and then for 3-4 weeks after birth (Franca et al. 2005). Factors which influence these rates of activity and number of cells can affect lifetime sperm production. There is interest in male piglet birth and weaning weights as well as growth rate since heavier weight has been shown to be related to increased testes size and sperm production (Rathje et al. 1995). At 1-2 months of age, behaviors such mounting are observed and at 3-4 months of age, in occurrence with the third period of germ cell division, there is a notable increase in the testes to body weight ratio. By 4 months of age, sperm appear in the tubules and erection can be achieved. At 5½ months of age, puberty begins with sperm first appearing in the ejaculate. Boars less than 9 months of age have lower ejaculate volumes and sperm concentrations compared to boars 18 months of age or older. Up to 18 months, the testes increase in size and both semen concentration and ejaculate volume increases. By 18 months of age no further increases in fertility are observed and the boar is considered fully mature.

The Physiology of Sperm Production

Inside the testicle, immature pre-sperm cells, called spermatogonia, begin to mature near the outside wall of the seminiferous tubes. It will take 34 to 40 days for this cell to change from a rounded cell on the outer wall of the tube to its release into the seminiferous tubule as a sperm cell (Figure 11). It will require another 10 to 12 days to mature the sperm as they move through the epididymis for a total duration of 44 to 52 days to produce a mature sperm. Immature sperm cells start their maturation process near the outer wall of the tube and as they mature they move through the Sertoli cell and closer to the lumen where they will be released as a spermatozoan. During maturation inside a Sertoli cell, the sperm cell changes shape from a nearly round shape to the elongated sperm head with a mid-piece and tail. Within a Sertoli cell, there are many sperm cells in different stages of spermatogenesis. The entire process can be defined into three phases: spermatogonial (mitotic or proliferative), spermatocytary (meiotic) and spermatogenic (differentiation). Spermatogonia are diploid round cells and are found farthest away from the lumen, while spermatocytes, undergoing meiosis, are still round-shaped but are closer to the lumen (Senger 2003). The most advanced spermatids are haploid, are found closest to the lumen, and look like a sperm cell. Development and

production of sperm cells is not synchronized along the tube and occurs in sequential stages that repeat along the entire length of the tube. This system allows substantial numbers of sperm cells to be produced continuously on a daily basis from different segments along the tube. In the boar, the time required for a single cell to advance to the next stage is called a cycle, and requires 9 days. It will take 4.5 cycles or ~41 days to mature a single sperm for release. Not all cells become sperm and cell loss is estimated at 70% and is thought to result from apoptosis or cell death related to stress, toxins, and chromosomal damage during meiotic development. The boar is efficient in daily sperm production with 85% of the testes parenchymal tissue made up of Sertoli cells, a short spermatogenic cycle, and as many as 10 spermatids matured/Sertoli cell at a time (Franca et al. 2005). Collectively, this results in ~25 million sperm produced/gram of testes each day and with a 500 gram paired testes capable of producing >12 billion sperm day in a mature boar.

Figure 11. Illustration of sperm production in the seminiferous tubule (Adapted from Beardon and Fuquay, 1997).



Following the production and release of spermatozoa into the lumen, they leave the testicle and enter the head of the epididymis. Maturation in the epididymis is required for sperm to acquire the ability to fertilize an egg. The epididymis is long in mature boars and the cells along the epididymis have tight junctions to maintain a specific environment and flow within the tubule and to isolate sperm from blood. In the caput section of the epididymis, sperm are concentrated and immotile in response to estrogen (Dacheux et al. 2005). In the epididymis, the sperm incorporate proteins into the membrane which are thought to be essential for fertility. As they are transported down the length of the epididymis by fluid flow and smooth muscle contractions, they enter the mid-piece where they acquire the capability for tail movement, but are still too concentrated to move. As the sperm enter into the tail of the epididymis, additional proteins are added which further modify the cell to enable fertility. Sperm are stored immotile in the tail region in concentrated form. The entire duration of the pathway through the epididymis requires ~12 days. The sperm will acquire full motility and fertilizing capability when diluted with seminal plasma in the ejaculate. During sperm transport the surface and membrane proteins are removed and added when also phospholipids in the membrane are remodeled. Each section functions to mature and transport sperm by secreting or absorbing fluids and proteins for the purposes of immunity, acidification, or cell protection and stabilization. All of the functions appear to be supported by DHT, as epididymal cells contain 5 α reductase for conversion of testosterone to DHT. It is thought that stressors and toxins can alter the function of the epididymis and its environment to reduce sperm fertility by altering epididymal rate of transport, absorptive or secretory function, pH, osmolarity, O_2 tension, and temperature.

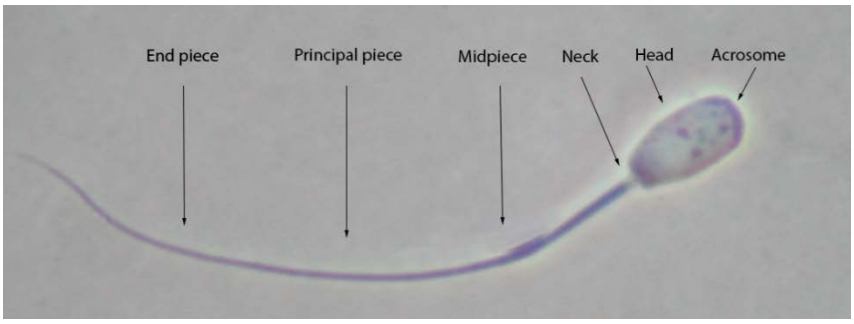
The entire process of spermatogenesis is dependent upon temperature and appears most sensitive to elevated temperatures. The scrotum regulates testes temperature to be ~3.5°F (2°C) lower than body

temperature. The scrotum has structural features and responsive mechanisms to regulate testes temperature which include low amounts of subcutaneous fat to limit heat retention, and a plexus of intertwining arteries and veins that function to cool arterial blood and warm venous blood. This system cools arterial blood to ~98°F (36°C) before it enter the testes. In addition, the scrotum contains thermoreceptors that respond to induce body heat loss through panting, and relaxation of the tunica dartos muscle which can increase scrotal surface area in hot weather. This muscle can also contract and wrinkle the scrotal skin to reduce surface area in cold weather. Another muscle called the cremaster, is located in the spermatic cord and contracts to pull the testes closer to the body in cold weather or relaxes to allow let them drop further away in hot weather to dissipate heat.

Sperm Cell Structure and Physiology

The sperm cell is an elongated, motile cell that is responsible for transferring chromosomes from the male to join with the chromosomes of the female following fertilization of the egg inside the female oviduct. The entire sperm cell is covered with a plasma membrane. The haploid cell is composed of a flattened, oval head containing the nucleus, with highly condensed DNA. The head contains the acrosome which is a double walled structure situated between the plasma membrane and the nucleus. It contains enzymes needed for penetration of the sperm into the egg. The head is connected to the tail by a neck and the tail is divided into the mid, principal and end pieces (Figure 12). The mid-piece contains microtubules for energy production for sperm tail motion, while the principal piece and tail propel the sperm. Sperm do not store energy and rely on substrates such as sugars in the seminal plasma or media that can easily be metabolized. Sperm survive in isotonic media but are most active in alkaline pH. Sperm cells are very sensitive to both low and high temperatures, and even short periods of exposure can shorten their lifespan. Temperatures 5°C above their normal temperature can cause irreversible damage in only 5 minutes. Cooling sperm can be effective for increasing shelf-life if the proper steps and media are used. Sperm produced in the testes and stored in the epididymis cannot fertilize sperm and must undergo capacitation, or a modification of the membrane outside of the epididymis in order to allow fertilization. This appears to be a complex series of events taking as long as 2 hours and results when sperm are mixed with seminal plasma. It would appear that uncapacitated sperm may be the only ones capable of establishing a sperm reservoir in the oviduct to await ovulation of the eggs. Sperm cells bound to the oviductal epithelial cells in the reservoir appear to be released upon some signal or signals and upon their release, are capable of binding to the cells or surface of the egg. Fertilization is typified by numerous sperm binding to the cumulus and corona cells surrounding the eggs. Sperm have enzymatic capability in their acrosome, and upon activation, the acrosome reaction occurs. With the release the enzyme content of the acrosome, sperm can digest through the cells surrounding the egg in order to bind the outer membrane layer of the egg. A single sperm will penetrate the zona pellucida and bind to the vitelline membrane, resulting in a zona reaction in the egg that immediately blocks all other sperm from entry. Subsequently, the sperm and egg membranes fuse and the sperm becomes hyper-activated and pushes its head containing the condensed DNA into the egg cytoplasm.

Figure 12. Image of intact boar sperm cell (1000X).



In order to for sperm to be capable of fertilizing an egg, they must have attributes of normal motility, morphology, and function. As such, semen samples are evaluated following collection, processing, or storage for concentration, motility, viability, morphology, contamination, and clumping. However, problems can occur is the processes of spermatogenesis, sperm maturation, and even during processing and storage, which can lead to dramatic changes in sperm cell attributes and semen fertility. Expected sperm concentration is important for efficient production of AI doses and can be estimated based on the daily production capability of testis and the frequency of semen collection. When these expectations are not met, investigation into causes can be determined. Problems in sperm viability, morphology, and DNA integrity can often be related to stresses before, during, or after semen collection. These measures and others, such as the hyper-osmotic swelling test can provide indicators for problems in sperm sample quality. For example, damaged sperm have heads that swell in hypo-osmotic media and assessment has been correlated to fertility problems. In high quality ejaculates, measures of motility, viability, and morphologically normal sperm may exceed 85%, while concentration is expected at 200 to 400 million sperm/mL. In these samples, clumping from sperm-sperm binding or bacterial contamination is not evident.

Management and Environmental Factors Influencing Fertility of the Boar

Boar pubertal development is an important component for lifetime sperm production. Feeding and nutrition of growing boars must provide adequate protein and energy to support growth and sperm production. Problems most often occur with under feeding growing boars and over feeding mature boars, both of which can result in poor fertility and early culling. Age of boar is related to sperm production, since puberty is a gradual process and not a singular event. Boars <1 year of age are not fully mature and produce fewer sperm than older boars, and should be collected no more than one time each week. Mature boars on the other hand can be collected up to two times per week with adequate numbers of sperm in each collection. Most maternal line boars remain in stud for only 1 to 2 years due to fast rates of genetic advancement, while terminal line sires may remain active in studs for 2 to 4 years before voluntary culling for higher indexing sires. Fertility can be diminished by advancing age in boars, but this has not been well established since modern studs use high rates of boar turnover. However, it has been reported that boars >4 years of age have higher incidences of sperm abnormalities and lower fertility. In mature boars, daily sperm production is not constant and can vary from 10 to 16 billion sperm produced/day. At ejaculation, ~60% of the caudal stores of sperm are emptied and it may take a week to completely replenish these stores. Sperm stored in the caudal epididymis are fertile for several weeks with older and excess sperm eliminated through muscle contractions and voided in the urine. Boars are typically rested between collections and when collected twice a week, ~50 billion sperm can be collected in each ejaculate. With one week rest between collections, ~100 billion sperm can be collected. Collection intervals at 2 weeks or more can increase sperm numbers in the ejaculate, but with longer periods of sexual rest, an increase in the number of degenerating and non-fertile sperm are observed. It has been reported that aged sperm first lose their ability to fertilize an egg before they lose their motility and membrane integrity.

Boar sperm collection efficiency can be improved by the use of increased sexual excitement before collection. This is accomplished by the use of warm up pens for 5-10 minutes, where boars can watch other boars being collected. This can be used to increase the concentration and volume of the boar ejaculate as a result of smooth muscle contractions in the tail of the epididymis and the vas deferens. The use of warm up pens also aids in sanitation by stimulating the boar to urinate and defecate before he enters the collection pen. Manual collection is most common but new auto collection devices are also in use in some larger studs (Aneas et al. 2008) and results suggest these can produce similar results to manual collection. Collection of only the sperm rich or with the sperm poor fraction is commonly performed. The boar usually takes 7-10 minutes to ejaculate a 150-400 mL volume. Semen collection practices for optimal fertility require procedures to minimize temperature changes and contamination. Collection vessels for the ejaculate should be close to 37°C but are reported to range from 24 to 37°C. The boar ejaculates semen in fractions and does not mix all the accessory sex gland fluids with sperm prior to ejaculation. Therefore, the different fractions of the ejaculate have varying numbers of sperm. The first fraction of a boar ejaculate is ~25 mL and is

called the pre-sperm fraction. It contains no sperm and only residual urine, cell debris, and some fluid from the prostate gland. This fraction is often discolored, may contain bacteria, and is discarded. The second fraction is known as the sperm-rich fraction and can range in volume from 50-150 mL depending upon collection frequency and age. It contains 80% of the total sperm and is milky in appearance. The third fraction is known as the sperm-poor fraction and has a large volume of 70-400 mL but only 20% of the total sperm. This is a primary source for seminal plasma but is sometimes discarded to prevent over-dilution of the sperm. The last fraction is the gelatinous phase and is 20-40 mL in volume. Except for the first fraction which is discarded, all phases can be collected, but a filter should be used to remove the gel material produced by the bulbourethral gland. Filtering the ejaculate is important since some of the gel material is ejaculated in all of the fractions and can lead to sperm clumping.

Abnormalities in ejaculates are noted and as such minimal criteria are set for processing ejaculates for production efficiency and fertility. These criteria include a sperm rich fraction >80 mL, >70% sperm motility, >200 million sperm/mL and with abnormalities in <15% of sperm (Colenbrander et al. 1993). Many boar studs also retain samples for quality control checks 2 to 7 days following semen delivery to evaluate motility and bacteria in the processed dose. Problems in the ejaculate can occur during the stages of sperm formation, maturation or may have occurred in the collection, processing, shipping or storage steps. Inside the boar, since sperm are continuously developed and matured, the type of the abnormality in an ejaculate can be indicative of when the problem occurred. In cases where a problem is diagnosed, the issue may result from a single event that is limited to that sample and day. However, in other cases, when ejaculate issues are identified, it is possible that the problem may have occurred weeks or months ago, may have lasted more than a few days, and the problem may be evident for months to come. The type of the abnormality is the key to identifying the source and extent of the problem. Problems in ejaculate volume and concentration can be related to management, genetics, and environment and may be associated with lowered production efficiency but not lowered fertility. Sperm motility can be affected by sperm formation, maturation and post-collection handling conditions. Motility is one of the most common assays used in studs, and does provide a subjective measure of quality, but its correlation to fertility is low. Some have reported effects of motility on pregnancy rates and litter sizes, but these often are not evident until motility declines <70% (Flowers 1997). It has been suggested, that the use of CASA may improve the measure of motility, as sub-populations of motile sperm can be identified (Flowers 1997, Gadea 2005). Sample morphology may be a better indicator for fertility failure or stress. Samples can be evaluated for problems in the sperm head, acrosome, neck and tail. Some of these can be quantitative and related to fertility. Healthy boars show few head defects and are not common. But when they occur can be an indication of problem during sperm formation. The defects identified include micro and macro, and mis-shaped heads. However, in many cases they may represent only a small sub-population of the total sperm in an ejaculate. Membrane integrity for live sperm and acrosome status have been used but little data is available on their fertility effects. Acrosome integrity is viewed using microscopy and problems have been identified such as damaged, missing, or detached acrosomes and when these occur at >30%, have been shown to lower fertility. Tests such as the HOST show promise in identifying sperm membrane problems, and may be more sensitive. Neck or mid-piece problems show up as bent, displaced, or swollen attachments. Tail defects are by far the most common with proximal, mid piece, and distal droplet most frequently observed. Droplets originate during the separation of the sperm from the Sertoli cell with a small remnant of cytoplasm at the neck. This droplet migrates down the tail and falls off at the mid-piece during maturation in the epididymis. Sperm with proximal droplets render sperm immotile and infertile. Mid-piece droplets can prevent motility if the tail forms a loop around the droplet. Distal drops allow motility and may not cause fertility problems unless they occur at >20 % in a sample. The droplet problems result from cases of stress which may alter sperm formation in the Sertoli cell or maturation in the epididymis. Immature and overused boars are known to have a greater frequency of droplets as less developed sperm are moved along the epididymis at faster rates. Some tail defects can indicate problems in maturation or post-ejaculation handling. Curved or bent tails result from sperm that have been exposed to extremes in temperature, pH, osmolarity, pressure, UV radiation, toxic compounds, or bacteria. Samples are also evaluated for clumping. This is an indication of

sperm-sperm binding and is not a problem unless it is evident in >30% of sperm. Sperm clump as a result of dead sperm, cell debris, gel, bacteria, rapid cooling and damaged acrosomes.

Many ejaculate problems may take weeks to appear due to the time required for sperm formation and maturation from the time of the stress or insult. After this delay, the length of time and degree to which abnormalities appear depends upon the severity and length of the stress and which stages of sperm cell development were susceptible to injury. Boar response to heat stress is the best studied, but individual responses are highly variable and depend on the intensity of heat and humidity and the length of exposure. In this case, problems may not appear for 1-2 weeks and then can last 5-7 weeks. Since droplets are the best studied and most prevalent defect, it has been reported that most droplets are distal (8%), with <5% classified as proximal or distal mid-reflex (Lovercamp 2008). Further, heat stress at 95°F (35°C) for 4 days has been shown to increase the percentage of droplets from 5 to 40% for a period lasting 4-6 weeks. Reports also suggest boar vaccination has been associated with problems in almost half of studs immediately after vaccination with a delay in semen problems of 20 days and with recovery at 5.5 weeks afterwards (Althouse 2008a). In cases where poor quality semen is evident, it is suggested that increasing collection frequency from once to twice a week or collection of boars twice daily for 3 consecutive days and with a rest of 3-4 days with subsequent evaluation, can aid in return to fertility and help in determining the extent of the problem. Regardless of the outcome, these boars should be monitored over the next 2 months, since over 61% of involuntary replacement occurs in cases related to ejaculate fertility.

Seasonal effects of boar fertility and semen production have been widely reported (Claus et al. 1983, Colenbrander et al. 1993, Knox et al. 2008, Sonderman and Luebbe 2008) with daily sperm production changing over months (Colenbrander and Kemp 1990). However, the problem varies among boars and between temperate or tropical climates. The decline in fertility is associated with summer heat and long day length, but can also be associated with transition periods in early fall. As a result, attention has focused on the housing and management of boars in stud with emphasis on temperature, housing and lighting. Boars are commonly housed in stalls or pens for controlling, temperature, stress, aggression, feed intake, and aiding structural soundness. The design and housing for boars in studs has been reported (Gall 1999, Levis and Reicks 2004). Managing boars in separate pens from other boars can be important for fertility and longevity but raising boars in isolation should be avoided since libido and ejaculate volumes are lower when raised in physical isolation from other males or females. In most studies, lighting intensity is not a factor in libido or semen quality and supplemental light during short days has had little effect. However, increasing or decreasing light duration in the opposite season can advance age of puberty. This response likely occurs since the wild pig is a short-day, seasonal breeder and still may retain some seasonal responsive genes. There is variation in the breeding season of the wild boar (European) which runs from late autumn to early winter. In the domestic boar, sperm production appears to be greatest between September-February compared to March-August. The differences between the wild and the domesticated boar may be a reflection of the differences in available feedstuffs, environmental housing, and management but could clearly be related to the higher incidence of days above the heat stress level and exposure to daylength. Low temperatures have not been found to cause great problems for boar fertility except when temperatures drop below -10°C (14° F). This low temperature problem is thought to occur as a result of increased feed intake and excessive body heat generation which affects sperm production. On the other hand, elevated environmental temperatures are much more of a concern (Flowers 1997) and both chronic and acute heat stresses can reduce fertility. High temperatures and humidity result in heat stress in boars. This can be observed by measuring increased respiration rates and body temperatures in boars. Interestingly, some boars do not show the same responses to heat stress and may be related to boar anatomy, physiology, or genetics. In the fall, some fertility effects could be related to the extremes in temperature fluctuations between day and night. It has been suggested that day and night temperature changes of 18°F (10°C) may cause problems in sensitive boars. Temperatures >86° F (30°C) can reduce sperm production by 30% and affect motility. Temperatures above 95°F (35°C) with high humidity for 6 hours/day for 3

consecutive days has been shown increase heat stress by altering testes function with production of more sperm with damaged heads, acrosomes, persistent proximal, mid-piece and distal droplets, bent and coiled tails, and lowered total sperm output (Colenbrander and Kemp 1990). These abnormalities begin 2 weeks after the stress and can last for months. Heat stressed boars also have higher levels of basic proteins in their ejaculates and these are associated with a reduced life of extended sperm in storage. Evaporative coolers have been helpful at lowering temperatures in hot weather but have resulted in increased humidity with little effect on improved fertility (Kunavongkrit et al. 2005). As a result, many studs plan for increased rates of ejaculate discards in the summer season and increase boar inventory for collection by 20%.

Boars are chosen for placement in studs and used as sires primarily based on their EBV (Robinson and Buhr 2005) for dam (moderate growth efficiency and prolificacy) or sire (offspring efficient growth) line traits and then for their semen production and fertility potential. Age at puberty is an important trait in sires for improving lifetime sperm production and rate of gene transfer. Both age at puberty and size of the testes have been reported to be moderately heritable and in some cases have been used to increase fertility (Rathje et al. 1995). Other studies indicate that selection for birth weight increases testes size, but results in only a 2% increase in total sperm (Ford 2008). A review of the impact of selection for semen traits in sires reveals that most are in fact lowly heritable (<25%) and negatively related to daily gain, backfat, and muscle depth (Safranski 2008). Others have studied the variation in reproductive traits of AI boars, and noted that libido, breed, and growth rate have no clear genetic component for mature sperm production, while 6-15% of seasonal declines in sperm production are clearly related to genetic line (Flowers 2008). This is supported by data showing many genetic lines and breeds exhibit increased ejaculate discard rates from summer and fall, with purebred lines having the highest rates and crossbred lines showing lower discard rates for ejaculates (Sonderman and Luebbe 2008).

Boar stud management and semen production

In North America there are an estimated 120 boar studs with an inventory of 24,000 boars (Burke, Singleton 1999) with stud sizes of 51 to 500 boars most numerous (Knox et al. 2008). In these studs, the average boar culling rate was between 21-70% with reasons due to genetic improvement, followed by failure for boars to meet semen quality standards, boar soundness, and failure of boars to be collected. Stud production involved multiple collection days of the week, 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 these collections. Mid-range values for sperm produced (100 billion sperm) and doses (30/week) translated into 3.3 billion sperm per dose. Other stud survey data also noted an average of 100 billion sperm/ejaculate when boars were collected once /week with 26 doses produced each week containing ~ 3 billion fertile sperm/dose (Kuster and Althouse 2004). A large CASA data set from the Netherlands reported that doses currently contain 1.5 billion sperm /80 mL and that collection intervals of 4.5 days were used to produce 84 billion sperm/ejaculate for 35 doses/ejaculate (Broekhuijse et al. 2011). In the US, over 85% of studs reported that boars received 3-7 days of rest between collections. Most boars in stud were between 1-2 years of age. Training of boars started between 6-9 months of age in most studs with training occurring in isolation and in the stud and lasting 1-3 weeks. Semen collection procedures used double gloves to limit contamination and pre-warming of collection containers to thermally protect semen. Semen processing occurred almost exclusively by indirect volume assessment using weight. In addition, almost 81% of studs indicated that semen was pooled using 2-6 ejaculates. Most ejaculates are evaluated using 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 for evaluation. 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. Quality control procedures were evident as studs reported discard rates at 1-10% in the majority of studs. Discard rates reported by others in the US indicated 13% occurs in summer and 8% the rest of year (Kuster and Althouse

2004). The predominant reasons for discard include poor motility and abnormalities (droplets and abnormal tails), followed by bacteria, low sperm numbers and low volume of ejaculate. Other factors related to the reason for the discard included the boar, season of the year, disease, and genetic line. Semen was extended in a variety of extenders but the most common ingredients are listed in Table 3 and have been reported (Gadea 2003, Levis 2000, Johnson et al. 2000). The extenders chosen or developed mostly used glucose as the predominant energy source although sodium pyruvate and other sugars are also included in some and others have been tested for use. Buffers varied from the very simple to the more complex with the more complex providing a greater buffering range over temperature, time, and pH. There is limited information on addition of electrolytes, but potassium and sodium chloride appear to be the predominant ones used. The use of membrane stabilizers to protect sperm from cold shock, osmotic change, and oxidation varied greatly as well as use of antibiotics and have been reviewed for the pig (Großfeld et al. 2008). The use of antibiotics is also common in extended semen and their effectiveness reported (Althouse and Lu 2005, Althouse et al. 2008). Most studs retained samples 3-7 days as a check for quality control. The studs indicated that doses of semen were packaged with 2-4 billion sperm cells and most of the studs indicated they adjusted the dose of sperm cells for fertile sperm and packaged the dose in bottles and bags in 60-80 mL volumes. Boars were mostly housed in stalls using fully or partially slatted floors in thermally regulated buildings using evaporative and mechanical cooling systems. The animals were fed by either drop or 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 3. Common ingredients and their concentration in commercial semen extenders (Adapted from Johnson et al., 2000; Levis, 2000).

Components		Conc.
Energy source		
	Glucose (nM)	180.2
Buffers		
	Sodium citrate (mM)	294.10
	TRIS (mM)	121.1
	Sodium bicarbonate (mM)	85
	HEPES (mM)	238.3
	Citric acid (mM)	191.1
	MOPS (mM)	209.3
Electrolytes		
	Potassium chloride (mM)	75.55
Membrane stabilizers, chelators, cold shock protectors, antioxidants		
	EGTA	
	EDTA (mM)	292.24
	BSA (%)	0-5%
	Polyvinyl alcohol (%)	1%
	Powdered skim milk (%)	6.90%
	Egg yolk (%)	2-3%
	Cysteine (mM)	121.2
Antibiotics		
Extender properties		
	Osmolarity (mOsm)	240-380
	pH	6.4-7.2

References

AASV, 2003: Boar stud guidelines: Health, hygiene, and sanitation guidelines for boar studs providing semen to the domestic market. J. Swine Health Prod., *July and August* 204-206.

Althouse G., 2008a: Infectious and noninfectious causes of infertility in boars. In: R. S. Youngquist; W. R. Threlfall, (eds), Current therapy in theriogenology 2. Saunders Elsevier, St. Louis, MO, pp. 722-724.

Althouse G. C., 2008b: Sanitary procedures for the production of extended semen. Reprod Domest Anim, *43 Suppl 2* 374-378.

Althouse G. C.; Lu K. G., 2005: Bacteriospermia in extended porcine semen. Theriogenology, *63* 573-584.

Althouse G. C.; Pierdon M. S.; Lu K. G., 2008: Thermotemporal dynamics of contaminant bacteria and antimicrobials in extended porcine semen. Theriogenology, *70* 1317-1323.

Aneas S. B.; Gary B. G.; Bouvier B. P., 2008: Collectis® automated boar collection technology. Theriogenology, *70* 1368-1373.

Ashdown R. R.; Hafez E. S. E., 1993: Anatomy of male reproduction. In: E. S. E. Hafez, (ed), Reproduction in farm animals 6th ed. Lea and Febiger, Malvern, PA.

Bearden H. J.; Fuquay J. W., 1997: Applied animal reproduction 4th ed. Prentice-Hall Inc., Upper Saddle River, NJ.

Bone J. F., 1988: Animal anatomy and physiology 3rd ed. . 3rd Ed. edn. Prentice Hall, Englewood Cliffs, NJ.

Borg K. E.; Lunstra D. D.; Christenson R. K., 1993: Semen characteristics, testicular size, and reproductive hormone concentrations in mature duroc, meishan, fengjing, and minzhu boars. Biol Reprod, *49* 515-521.

Broekhuijse M.; Feitsma H.; Gadella B. M., 2011: Field data analysis of boar semen quality. Reprod. Dom. Anim., *46* 59-63.

Claus R., 1990: Physiological role of seminal plasma components in the reproductive tract of the female pig J. Reprod. Fertil. Suppl., *40* 117-131.

Claus R.; Schopper D.; Wagner H. G., 1983: Seasonal effect on steroids in blood plasma and seminal plasma of boars. J Steroid Biochem, *19* 725-729.

Colenbrander B.; Feitsma H.; Grooten H. J., 1993: Optimizing semen production for artificial insemination in swine. J Reprod Fertil Suppl, *48* 207-215.

Colenbrander B.; Kemp B., 1990: Factors influencing semen quality in pigs. J Reprod Fertil Suppl, *40* 105-115.

Dacheux J.-L.; Castella S.; Gatti J. L.; Dacheux F. o., 2005: Epididymal cell secretory activities and the role of proteins in boar sperm maturation. Theriogenology, *63* 319-341.

Flowers W. L., 1997: Management of boars for efficient semen production. J Reprod Fertil Suppl, *52* 67-78.

Flowers W. L., 2008: Genetic and phenotypic variation in reproductive traits of ai boars. Theriogenology, *70* 1297-1303.

Flowers W. L.; Alhusen H. D., 1992: Reproductive performance and estimates of labor requirements associated with combinations of artificial insemination and natural service in swine. J. Anim. Sci., *70* 615-621.

Flowers W. L.; Stewart K. R.; Gall T.; Novak S.; Dyck M. K.; Kirkwood R. N., 2013: Boar seminal plasam proteins and thier relevance to reproductive technologies. In: H. Rodriguez-Martinez; N. M. Soede; W. L. Flowers, (eds), Control of pig reproduction ix. Contex Limited, Nottingham, England, U.K., pp. 33-45.

Ford J. J., 2008: Impact of uterine environment on life time sperm production Midwest boar stud managers conference III. St. Louis, MO.

Franca L. R.; Avelar G. F.; Almeida F. F. L., 2005: Spermatogenesis and sperm transit through the epididymis in mammals with emphasis on pigs. Theriogenology, *63* 300-318.

Frandson R.; Wilke W. L.; Fails A. D., 2003: Anatomy and physiology of farm animals. Sixth edn. Lippincott, Williams, and Wilkins, Baltimore, MD.

Gadea J., 2003: Review: Semen extenders used in the artificial insemination of swine Spanish Journal of Agricultural Research, *1* 17-27.

Gadea J., 2005: Sperm factors related to in vitro and in vivo porcine fertility. Theriogenology, *63* 431-444.

Gall T., 1999: Boar stud design as it relates to functionality. In: L. A. J. a. H. D. Guthrie, (ed), Boar Semen Preservation IV. Allen Press Inc. Lawrence, KS, Beltsville, MD.

Garner D. L.; Hafez E. S. E., 1993: Spermatozoa and seminal plasma. In: E. S. E. Hafez, (ed), Reproduction in farm animals, Sixth edn. Lea & Febiger, Philadelphia, pp. 165-187.

Großfeld R.; Sieg B.; Struckmann C.; Frenzel A.; Maxwell W. M. C.; Rath D., 2008: New aspects of boar semen freezing strategies. Theriogenology, *70* 1225-1233.

Hughes P. E.; Pearce G. P.; Patterson A. M., 1990: Mechanisms mediating the stimulatory effects of the boar on gilt reproduction. Control of pig reproduction iii. J. Reprod. Fert. (Suppl.), *40* 323-341.

Johnson L. A.; Weitze K. F.; Fiser P.; Maxwell W. M., 2000: Storage of boar semen. Anim. Reprod. Sci., *62* 143-172.

Knox R.; Levis D.; Safranski T.; Singleton W., 2008: An update on north american boar stud practices. Theriogenology, *70* 1202-1208.
Kunavongkrit A.; Suriyasomboon A.; Lundeheim N.; Heard T. W.; Einarsson S., 2005: Management and sperm production of boars under differing environmental conditions. Theriogenology, *63* 657-667.
Kuster C. E.; Althouse G. C., 2004: Update on current us boar stud practices. Midwest Boar Stud Managers Conference II. St. Louis, MO.
Levis D., 2000: Liquid boar semen production: Current extender technology and where do we go from here? In: L. A. Johnson; H. D. Guthrie, (eds), Boar Semen Preservation IV. Allen Press, Lawrence, KS, pp. 121-128.
Levis D. G.; Reicks D. L., 2004: Boar housing and semen collection pen considerations. Midwest boar stud managers conference II. St. Louis, MO.
Louis G. F.; Lewis A. J.; Weldon W. C.; Miller P. S.; Kittok R. J.; Stroup W. W., 1994: The effect of protein intake on boar libido, semen characteristics, and plasma hormone concentrations. J. Anim. Sci., *72* 2038-2050.
Lovercamp K., 2008: Cytoplasmic droplets: The most frequently observed abnormality. Midwest boar stud managers conference III. St. Louis, MO.
Park J. S.; Kim J.; Ha Y.; Jung K.; Choi C.; Lim J. K.; Kim S. H.; Chae C., 2005: Birth abnormalities in pregnant sows infected intranasally with porcine circovirus 2. J Comp Pathol, *132* 139-144.
PigCHAMP, 2012: Data summary.
Rathje T. A.; Johnson R. K.; Lunstra D. D., 1995: Sperm production in boars after nine generations of selection for increased weight of testis. J. Anim. Sci., *73* 2177-2185.
Riesenbeck A., 2011: Review on international trade with boar semen. Reprod. Dom. Anim., *46* 1-3.
Robinson J. A. B.; Buhr M. M., 2005: Impact of genetic selection on management of boar replacement. Theriogenology, *63* 668-678.
Rodriguez-Martinez H.; Kvist U.; Saravia F.; Wallgren M.; Johannisson A.; Sanz L.; J. P. F.; Martinez E. A.; Roca J.; Vasquez J. M.; Calvete J. J., 2009: The physiological roles of the the boar ejaculate In: H. Rodriguez-Martinez; J. L. Vallet; A. J. Ziecik, (eds), Control of pig reproduction viii. Nottingham University Press, Nottingham, UK, pp. 1-22.
Safranski T. J., 2008: Genetic selection of boars. Theriogenology, *70* 1310-1316.
Senger P. L., 2003: Pathways to pregnanacy and parturition. 2nd edn. Current Conceptions, Inc., Pullman, WA.
Setchell B. P.; Brooks D. E., 1988: Anatomy, vasculature, innervation, and fluids of the male reproductive tract. In: E. Knobil; J. Neill, (eds), The physiology of reproduction, Vol. 1. Raven Press Ltd., New York, pp. 753-836.
Singleton W. L.; Flowers B., 2006: Managing boars in ai centers. Pork Information Gateway, *PIG 08-02-01*.
Sonderman J. P.; Luebbe J. J., 2008: Semen production and fertility issues related to differences in genetic lines of boars. Theriogenology, *70* 1380-1383.
USDA-APHIS, 2006: National animal health monitoring system: Swine. Vol. 2013.
USDA, 2012: National agricultural statistics service: Hogs and pigs. Vol. 2013.
Waberski D., 1997: Effects of semen components on ovulation and fertilization. J Reprod Fertil Suppl, *52* 105-109.

Training Boars for Semen Collection

Mark J. Estienne and Kimberly A. Williams
Virginia Tech-Tidewater Agricultural Research and Extension Center
Suffolk, VA

Introduction

There are approximately 120 boar studs in the U.S. with a total inventory of 20,000 head (Knox *et al.*, 2008). An important component of overall boar stud management is the successful introduction of new sires into the operation. This requires young boars trained for semen collection in a quick and efficient manner, whether training occurs while in isolation before entering the stud, or in the stud itself. Results of a survey of 44 commercial studs in Canada and the U.S. with a total inventory of approximately 10,000 boars revealed that the duration of training required one week in 52% of studs, but 2 to 3 weeks in 41% of studs, and up to one month in the other 7% of operations (Knox *et al.*, 2008). Reasonable goals for boar studs are the successful training of 90% of boars within 3 weeks, and less than 3% of boars characterized as "untrainable".

Factors affecting the efficiency of training boars for semen collection fall into four general categories: 1) boar, 2) facility and environment, 3) management, and 4) people. The objective of this paper is to describe the different factors influencing the training process, as well as strategies for enhancing the efficiency with which the training occurs.

Factors affecting the training for semen collection: Boar

In terms of a practical definition, attainment of puberty (or sexual maturity) is the time at which a boar exhibits normal mounting behavior and an erection, and produces an ejaculate containing a sufficient number of fertile sperm cells to impregnate a sow. Most boars reach puberty by 6 to 8 months of age, however, the trait is affected by breed and heterosis or "hybrid vigor", with crossbred boars achieving sexual maturity approximately 40 days before corresponding purebreds (Levis *et al.*, 1997).

A young, developing boar displays reproductive behavioral traits in advance of the ability to ejaculate sperm cells. Moreover, the number of sperm cells ejaculated generally increases until approximately 15 to 18 months of age. Blood concentrations of testosterone and estradiol increase throughout the pre-pubertal period in boars until achieving threshold levels required to maintain normal sexual behavior (Allrich *et al*., 1982; Estienne *et al*., 2000). Once reaching these thresholds or minimum concentrations, the level of sexual motivation is unrelated to blood concentrations of sex steroids. An inverse relationship exists between blood levels of estradiol and the time required to mount and begin ejaculation once boars are in the presence of a dummy, and estradiol concentrations are greater in boars that readily mount an artificial sow than in boars that refuse to do so (Louis et al., 1994b).

The weight of boars at birth impact the production of sperm and semen quality displayed in adulthood (Almeida et al., 2013; Dysart, 2014). Sexual behavior at maturity, including the readiness to mount a dummy and allow semen collection, is also affected. Estienne (2015) reported that birth weights were significantly greater in boars that were successfully trained for semen collection (n = 29) than for those that were not (n = 8) (1.67 and 1.29 kg, respectively).

Factors affecting the training for semen collection: Facility and Environment

For training, technicians typically move boars to a collection room or pen housing a stationary artificial sow (or dummy) (Althouse, 2007), or take a moveable dummy to the boars' home pen. Research comparing efficiency of the training process when employing either stationary or portable dummies is limited, however in one study, the proportion of boars successfully trained during a two-week test period was greater when a portable dummy was used (Godara *et al.*, 2018).

A suitable collection area typically measures six to eight feet x eight to nine feet, is well lighted and free of distractions (Althouse, 2007). Most importantly, the floor is clean and dry, and provides excellent footing. The dummy is secured to the floor such that one end is against the partition or wall, preventing boars from circling it. Commercially available dummies are adjustable to match the height of the dummy with the size of boar, and have side supports allowing the boar to stabilize his front legs during mounting and thrusting.

Most studs have a "warm-up" pen in close proximity to the semen collection pen. Boars in the warm up pen observe and hear another boar in the collection area, and these stimuli frequently help excite the boar warming up, so that when moved into the semen collection area, he quickly mounts the dummy. Collecting semen from an older, mature boar helps stimulate an inexperienced boar to mount the dummy.

Factors affecting the training for semen collection: Management

Griffin *et al.* (2006) conducted a study during which boars born in litters of 10 or more pigs were cross fostered so as to nurse in litters of six (n = 18) or greater than nine pigs (n = 18). Pigs were weaned after a 21-day nursing period and were then managed similarly. Birth weights were similar between groups; however, weaning weights were approximately 35% greater for boars nursed in the smaller litters. Moreover, by 170 days of age, a significantly greater proportion of boars from small litters compared with large litters, were trained to mount a dummy and allow semen collection (73 versus 39%, respectively). These findings are consistent with the notion that increased access to milk occasioned by the reduction in litter size, increased growth and positively impacted reproductive function in adulthood.

Training for semen collection should commence when boars have reached, or are approaching, sexual maturity. Approximately one third of operations begin training when boars are less than 7 months old, and 64% begin training at 8 to 9 months of age (Knox *et al*., 2008). It is reasonable to hypothesize that compared with younger individuals a greater proportion of older boars will mount a dummy and allow semen collection after just a few exposures.

For training, boars are allowed time to explore the collection area. When the boar focuses on the dummy, the preputial diverticulum is lightly gripped and stroked. It is common for a boar to mount the dummy shortly after the initial exposure. Once the boar has mounted and begun thrusting, the penis is grasped and the ejaculate collected. After the first successful collection, boars are collected two or three times over the following few days to reinforce the learned behavior.

Limit individual training sessions to no more than five to 10 minutes per day and record the training history of individual boars. Increasing the number of five-minute training sessions from two/week to five/week increased the proportion of six-month old boars successfully trained in a three-week period from 37.5 to 88.9% (Stewart and Lovercamp, 2017).

Commercially available prostaglandin-$F_{2\alpha}$ (**$PGF_{2\alpha}$**) products expedite mounting behavior and the rapidity with which boars mount and allow semen collection (Estienne, 2014). For example, in a study conducted in our laboratory (Estienne *et al.*, 2007), 36 Yorkshire x Landrace boars (298 days of age and 155 kg body weight) were moved to a semen collection pen on five consecutive days. Approximately one minute before entering the pen each day, boars received i.m. treatment with saline (controls); dinoprost tromethamine (2 mL; 5 mg/mL), a naturally occurring prostaglandin **(**Lutalyse, Pharmacia and Upjohn; Kalamazoo, MI); or cloprostenol sodium (1 mL; 250 µg/mL), a synthetic analogue of $PGF_{2\alpha}$ (Merck Animal Health, Madison, NJ). On each of day 1, day 2, and day 3, the percentage of boars collected after treatment with Lutalyse, but not cloprostenol, was greater than controls. There were, however, no effects of treatment on the proportion of boars collected on day 4 and day 5.

Variation exists in the effectiveness of $PGF_{2\alpha}$ for expediting the training of boars for semen collection, which perhaps reflects differences in genetics, age, or weight of boars, use of different commercial products or doses of products, or other undetermined management practices. Finally, the use of $PGF_{2\alpha}$ for induced farrowing is the only use of the compound in swine approved by the U.S. Food and Drug Administration. Treating boars with $PGF_{2\alpha}$ to stimulate sexual behavior is an "extra label" use performed only after consultation with a licensed veterinarian.

Although research focusing on the effect of nutrition on sexual behavior in boars is rather limited, some general conclusions can be drawn. A prolonged period of restricted protein, or both energy and protein, adversely affects libido and semen characteristics (Louis *et al.,* 1994a, b). Reduced libido probably reflects decreased estradiol concentrations, and precedes altered semen characteristics in boars that are chronically protein restricted. Although semen characteristics are enhanced, there are no exceptional positive effects of supplementing large levels of Vitamin C, fat soluble vitamins, or water soluble vitamins (Audet *et al.*, 2004) or omega-3 fatty acids (Estienne *et al.*, 2008).

Factors affecting the training for semen collection: People

Effective training of boars for semen collection requires an experienced and patient technician. This point cannot be overemphasized. Boars must be comfortable with human contact and "trust"

built with the technician. Dysart (2014) conducted an experiment during which high (2.1 kg; n = 10) or low (1.1 kg; n = 10) birth weight boars received socialization or no socialization protocols beginning at 5 weeks of age. Socialization consisted of 60 minutes of human contact, 3 days per week, for 5 weeks. During the first 2.5 weeks, the researcher stood in the aisle way and the last 2.5 weeks, actually entered the pen. Boars in the no socialization group received human exposure approximately 7 minutes per day during routine feeder maintenance and health observations. At 173 days of age, training for semen collection began. Socialization improved the rapidity of training low-birth weight boars (by an average of 6 days), but had no effect in high-birth weight boars.

Summary

Successful introduction of new sires into the boar stud requires young boars trained for semen collection in a quick and efficient manner. Most boars reach puberty by 6 to 8 months of age, however, the trait is affected by breed, heterosis, birth weight, and litter size during lactation. Training for semen collection should commence when boars have reached, or are approaching, sexual maturity. For training, boars are allowed time to explore the collection area. When the boar focuses on the dummy, the preputial diverticulum is lightly gripped and stroked. Although it is common for a boar to mount the dummy shortly after the initial exposure and allow semen collection, most boars will require a number of training sessions of five to 10 minutes per day. Increasing the number of five-minute training sessions from two/week to five/week increased the proportion of six-month old boars successfully trained in a three-week period. Commercially available $PGF_{2\alpha}$ products expedite mounting behavior and the rapidity with which boars mount and allow semen collection. A prolonged period of restricted protein, or both energy and protein, adversely affects libido. Reduced libido probably reflects decreased estradiol concentrations, and precedes altered semen characteristics in boars that are chronically protein restricted. There are no exceptional positive effects of supplementing large levels of Vitamin C, fat-soluble vitamins, water-soluble vitamins, or omega-3 fatty acids. Finally, effective training of boars for semen collection requires an experienced and patient technician, who builds trust with the boars. Socialization improved the rapidity of training low-birth weight boars (by an average of 6 days), but had no effect in high-birth weight boars.

References

Allrich, R.D., R.K. Christenson, J.J. Ford, D.R. Zimmerman. 1982. Pubertal development of the boar: testosterone, estradiol-17β, cortisol and LH concentrations before and after castration at various ages. *Journal of Animal Science* 55:1139-1146.

Almeida F.R.C.L., P.A. Auler, G.H.F.A. Moreira, R.B.C. Jardim, F.P. Bortolozzo and H. Chiarini-Garcia. 2013. Birth weight and its impacts on testicular development in boars In: Control of pig reproduction IX. Rodriguez-Martinez, H., N.M. Soede and W.L. Flowers., Context Products Ltd., Leics, UK. pp. 113-114.

Althouse, G.C. 2007. Artificial insemination in swine: Boar Stud Management. In: Youngquist R.S. and W.R. Threlfall (eds.): Current therapy in large animal theriogenology. 2. Elsevier, St. Louis, MO. p. 731-738.

Audet, I., J.-P. Laforest, G.P. Martineau, and J.J. Matte. 2004. Effect of vitamin supplements on some aspects of performance, vitamin status, and semen quality in boars. *Journal of Animal Science* 82:626-633.

Dysart, N.E. 2015. Effect of birth weight and human socialization on reproductive behaviors, sperm production, semen quality, and fertility of AI boars. *M.S. Thesis, North Carolina State University, Raleigh*, 163 pages.

Estienne, M.J. 2014. A review of the effects of prostaglandins on sexual behavior in boars. *Applied Animal Behaviour Science* 154:1-7.

Estienne, M.J., A.F. Harper, W.E. Beal, and R.J. Crawford. 2007. Effects of prostaglandins and prostaglandin synthesis inhibitors on sexual behavior in boars. *Reproductive Biology* 7:163-175.

Estienne, M.J., A.F. Harper, and R.J. Crawford. 2008. Dietary supplementation with a source of omega-3 fatty acids increases sperm number and the duration of ejaculation in boars. *Theriogenology* 70:70-76.

Estienne, M.J. 2015. Boar reproduction as affected by early-in-life experiences. *Reproduction in Domestic Animals* 50(Supplement 2):115-116.

Estienne M.J., D.S. Broughton, and C.R. Barb. 2000. Serum concentrations of luteinizing hormone, growth hormone, testosterone, estradiol, and leptin in boars treated with n-methyl-D, L-aspartate. *Journal of Animal Science* 78:365-370.

Godara, A.S., T. Dutt, G.K. Gaur, G.K. Das, Mukesh Singh, and P.K. Bharti. 2018. Short communication: Training and sexual behavior of boars on portable and fixed dummies for semen collection. *Indian Journal of Animal Sciences* 88:915-917.

Griffin JK, Seal MC, Flowers WL, 2006: Effect of neonatal environment on adult reproductive function in boars. *Journal of Animal Science* 84(Supplement 1), 205.

Knox, R., D. Levis, T. Safranski, and W. Singleton. 2008. An update on North American boar stud practices. *Theriogenology* 70:1202-1208.

Levis, D.G., V.D. Leibbrandt, and D.W. Rozeboom. 1997. Development of gilts and boars for efficient reproduction. Faculty Papers and Publications in Animal Science. 619. https://digitalcommons.unl.edu/animalscifacpub/619

Louis, G.F., A.J. Lewis, W.C. Weldon, P.M. Ermer, P.S. Miller, R.J. Kittok, and W.W. Stroup. 1994a. The effect of energy and protein intakes on boar libido, semen characteristics, and plasma hormone concentrations. *Journal of Animal Science.* 72:2051-2060.

Louis, G.F., A.J. Lewis, W.C. Weldon, P.S. Miller, R.J. Kittok, and W.W. Stroup. 1994b. The effect of protein intake on boar libido, semen characteristics, and plasma hormone concentrations. *Journal of Animal Science* 72:2038-2050.

Stewart, K. and K. Lovercamp. 2017. Increased training frequency improves boar collection. *National Hog Farmer*. Minneapolis, MN (September 26, 2017).

What do we really (need to) know about feeding boars for sperm production and quality?

Mark Wilson, Tom Gall and Mike Parsley
FeedworksUSA, Cincinnati, Ohio

Introduction

Boars represent a small portion of the amount of feed utilized by swine. You can find all ranges of nutrients provided in a boar diet from gestation feed that the sows receive to diets that are over formulated and likely are costing more money with little additional benefit for sperm production or sperm quality. It is important to understand what issues are significant to a boar diet to help with longevity of the top EBV boars. Many products have been tested to see if a greater impact on boar sperm numbers or sperm quality can be improved. Unfortunately, many of the trials on products for boars have indicated a response from a single experiment and often when products are tested multiple times we find minimal value for the addition of those products. Because the boar is 50% of the input for producing piglets, we add additional products to hopefully create a genuine impact on quantity of sperm cells and/or quality of sperm cells. Most attempts from a management and nutrient input strategy have not been very successful at improving sperm production of mature boars (Flowers, 1997).

Young Boar Nutrition May Impact Sperm Production

In male pigs the sertoli cell proliferation begins in the prenatal period and continues after birth (Swanlund et al., 1995) for approximately 3 weeks (McCoard et al., 2003). Sertoli cells can only support the development of a finite number of germ cells in the process of spermatogenesis (Sharpe et al., 2003). Interestingly, a young boar's nutrition during the three weeks after birth may establish a boar's potential for sperm production as an adult.

Dr. Flowers set up an interesting trial to investigate this process. He set up to select 40 terminal line crossbred boars cross fostered on day 1 so littermates were raised in litters of 6 (n=20) or in litters of 9 or > (n=20). Care was taken to select birth litters that had equal numbers of gilts and boars and cross fostered to minimize potential milk production differences in sows and represented 5 different genotypes. The study was conducted with a group of boars in the Fall and in the Spring (n =10 boars/treatment/season). Same sires were used to produce the piglets for each season. Boars from small litters (6 pigs) and from large litters (≥ 9 pigs) were co-mingled at weaning so that all boars were allow the same diet and space through finishing at 5 months, at which time they were placed in individual crates. At 5.5 months boars were trained to a dummy sow and collected once per week until they were at least 2 years of age. Boars body weight and testicular size were measured at birth, weaning and ever three weeks thereafter for the study.

All 20 boars that were from the small litters were trained in 5 days to jump the dummy sow and collect. From the large litter size, only 5 boars out of 20 were trained in the first 5 days. In addition, the amount of sperm cells per ejaculate were much larger for the spring born replicate from small litter, compared to the boars nursed in the large litters by 10 billion sperm cells/ejaculate. The boars from small litters in the fall had 20 billion more sperm cells per ejaculate over the boars from a large litter. Testicular size was larger for the small litter boars than boars from large litters. There were no differences in motility,

morphology, acrosome morphology, acrosin activity or capacitation status between the two groups of boars.

Semen from the two groups of boars was mixed to make heterospermic insemination doses and the litters were analyzed to determine which boars sired the most offspring by DNA fingerprinting techniques. Boars from small litters were highly favored over the boars from large litters, siring 65% of pigs.

Reviews for Feeding Boars

Several reviews on feeding boars and calculating estimates of for energy, protein, amino acid, mineral and vitamin requirement for boars of different weights and ages have been conducted (Kemp, B. 1989). Kemp and Soede (2001) wrote a book chapter on boars in *Swine Nutrition* on the topic as well. Estienne and Harper, (2004) wrote an update in Feeding and Nutrition with the objective of finding practical research to examine boar diets with as many projects and trials as possible. Tokach and Goodband (2007) wanted to go a different direction and focused on issues in the boar stud that may help with nutrition and feed management. A planned nutritive program for boars showed an advantage over trying to maintain a body condition score program. Ning Lu et al. (2021) designed some new parameters to protect against overfeeding and used a factorial approach to energy estimates for boars in isolation and the range of age of boars within a stud.

Most boar stud diets are adequate or over formulated for nutrients. Always avoid feedstuffs that increase the risk for mycotoxins, such as DDGs, wheat middlings, and small grains. Feed stuffs that have a higher risk of mycotoxins must be tested numerous times to make sure they are not contaminated. The age, weight, locomotion soundness, sperm quantity and quality along with estimated breeding values of boars are all important parameters. Boars should be gaining a minimum of 100 grams (1/4 pound) per day. This will benefit sperm production and avoid over conditioning boars. Energy is the first priority followed by amino acids and protein. Protein should be a minimum of 14.5% and lysine should be at 0.68% with a minimum intake of 15 g of lysine/d. There are limited data on the range that should be fed to young boars verses older boars. Kemp (1989) studied 18g lysine/ day verses 32 g lysine/ day and showed there was no difference in performance. Even though boars are a different genotype than 20 years ago, a safe range of 15 to 20 g lysine/d (maximum) for younger boars is recommended until we have results from further testing.

Calcium and Phosphorus levels should be maintained at 0.8 to 0.9 % Ca and 0.7 to 0.8% P in boar diets. Vitamin and trace minerals should be similar to gestating sows without some of the very wild numbers that one might see on some of the diet specifications. The nutrient requirements for young boars may not satisfy the nutrient requirements of older boars weighing 650 lbs. (294 kg) body weight from a tissue mass basis. Additional testing of various products on libido, sperm production, sperm motility and other parameters of sperm quality is needed. The most important issue for the boar stud is number of sperm cells produced per ejaculate or a calculated level of sperm production to account for collection frequency to compare all boars on a similar basis, such as billion sperm produced per day. Ultimately, the desire is to achieve maximum semen production from the boars with the highest estimated breeding value (EBV) to improve performance in grow finish pigs for meat production.

Examples of trials and production impacts of nutrients for boars

Boars that are greater than 14 months of age generally have an advantage over younger boars on sperm production. Therefore, longevity is an important concern to maximize sperm output and maintain boars with the highest EBVs that can have a significant, positive impact on progeny performance.

Biotin levels are the most effective when keratinocyte proliferation is not damaged and is not dealing with inflammation. Most dietary levels of biotin for boars tend to be in the range of 0.200- 0.500 mg/kg while the requirement estimate is 0.200 mg/kg of diet. Discussions around lameness prevention in boars aim at the range of levels of biotin for the most effective responses, while levels far greater than this are seen in diet formulations for boars.

Marin-Guzman et al. (1997) studied the effects of vitamin E and selenium supplementation in boar diets. The addition of higher levels of selenium had a greater response on semen characteristics than the effects of increasing Vitamin E. Legal mill limits for FDA regulations allows up to 0.136 mg of added Se/pound (0.3 ppm) in the diet for all pigs. The levels fed for this project for selenium were at 0.23 mg/pound of diet or 0.5 ppm, which is currently not legal in the US.

Audet et al. (2004) concluded that supplementation of boar diets with high levels of vitamin C, fat soluble vitamins or water soluble vitamins had no appreciable effects on semen or libido characteristics in boars. There were no treatment effects on number of sperm cells per ejaculate during the regular collection period.

Kozink et al. (2004) looked at dietary L-carnitine supplementation on semen characteristics in boars. For the weekly intensive collections in both experiments, there were no positive effects of increasing L-carnitine levels in the boar diet.

Reicks et al. (2012) looked at sixty-seven (7 month old) boars that were in isolation for 6 weeks. Thirty-three treated boars were allowed access to a 20% stock solution of Solutein (product of APC), while the control (34) boars only drank water. Using a data set of only the terminal boars, the number of extended doses favored the Solutein group compared to the control boars with 23.3 doses verses 18.1 doses per ejaculate ($P< 0.05$). This response may have been due to the stress conditions of moving from the boars from isolation to the boar stud.

It is not uncommon to see rations where several nutrient ingredients will exceed NRC estimates at greater than 4 times the suggested amount. This is particularly a problem with levels of micro minerals which may compete and have antagonism with other minerals in the diet. another issue is that some of the exaggerated levels of nutrients can create a burden on dietary cost per dose of semen. Stay with the dietary suggestions of the nutritionists and your boars will be ok!

Why consider using fiber in the diet at all?

Fiber often has is considered as a no impact item. Claims are that there is no evidence that fiber improves reproductive performance. There are several reasons fiber still may be considered for boar diets to help with 1) increase satiety 2) decrease cost 3) improve welfare 4) stabilize microbial population and decrease the impact from reabsorption of hormones from the intestinal tract due to microbial breakdown of the cholesterol bound to sex hormones that have been excreted from the liver.

There is increasing evidence that fiber may play an additional role in hormone regulation. A mechanism for the absorption and recycling of steroid hormones from the intestine via the entrohepatic circulation which passes the steroids back into either bile or peripheral circulation (Ruoff and DZuik 1994 a, b). This mechanism is similar in both sows and boars and can affect steroidogenesis. A similar disruption of this pathway occurs with higher dietary fiber in humans (Goldin et al., 1981). Fiber sources that contain possible levels of mycotoxins such as DDGs, wheat middlings and small grains should be tested prior to feeding these products to boars.

Omega 3 fatty acids

Rooke et al. (2001) conducted an experiment during which boars were fed 5.5 lb of a control diet or the control diet with 13.6g/lb of diet with added tuna oil. Boars in both groups were fed vitamin E (134 mg/lb of diet) to serve as an antioxidant. The number of sperm cells per ejaculate averaged 74.1 billion for controls and 83.4 billion for boars fed Prosperm. These researchers then mated 478 gilts to each set of boars. Conception rate was 90% vs. 83% and number born alive 10.6 vs. 10.2 ($P< 0.05$) for gilts bred to boars that were fed a DHA product compared to the control boars, respectively.

Parsley et al. (2021) studied the comparison of a competitor DHA product (control) verses Salmate® at the exact same levels providing 1.83 g DHA/boar/day for both treatments. In addition, another trial looked at a titration of three levels of Salmate® of 1.83, 2.38 and 2.94 g DHA/boar/day and showed that there were no differences in sperm production or differences in quality of sperm. What the titration trial did show was that if treatment levels were having any issues with rejected ejaculates that the medium level of Salmate (2.38 g/boar/day) showed a reduction in the % rejected ejaculates by 7.5% and 6.4% compared to the lowest and highest inclusion levels, respectively. Comparison of control boars (competing product) to treatment boars (Salmate®), both at 1.83 g DHA/boar/day, resulted in an increase in the number of sperm cells per ejaculate from 69.7×10^9 vs. 82.0×10^9 for the Salmate® fed boars. Salmate® is a highly protected fish oil product, and the method of protection of the DHA component is important to the response of the sertoli cells in the control of spermatogenesis in the testicles.

References

Amann, R. P. and Schanbacher, B. D. 1983. Physiology of male reproduction. J. Anim. Sci. Suppl. 57, 380-403.

Audet, I., J. P. Laforest, G. P. Martineau, and J. J. Matte. 2004. Effect of vitamin supplements on some aspects of performance, vitamin status and semen quality in boars. J. Anim. Sci. 82:626-633.

Flowers, W. L. 1997. Management of boars for efficient semen production. J. Reprod. Fert. Suppl. %2, 67-78.

Kemp, B. (1989) Investigations on breeding boars to contribute to a functional feeding strategy. PhD Thesis, Agricultural University of Wageningen, the Netherlands.

Kemp, B. and Soede, N.M. (2001). Feeding of Developing and Adult Boars. Chapt. 34:771-782. *Swine Nutrition.* CRC Press LLC, Boca Raton, Florida. Eds. Lewis, A. J. and Southern L. L.

Kozink, D. M., M. J. Estienne, A. F. Harper, and J. W. Knight. 2003. Effects of L-carnitine supplementation on semen characteristics in boars. Theriogenology 61:1247-1258.

Lu, N., M. Kleve-Feld, M. Wilson, C. Vier, W. Cast, R. Navales, S. Dritz and U. Orlando. 2021. A Factorial Approach to Estimate Energy Requirements and Feeding Levels for Boars in Studs.

Marin-Guzman, J., D. C. Mahan, Y. K. Chung, J. L. Pate, and W. F. Pope. 1997. Effects of dietary selenium and vitamin E on boar performance and tissue responses, semen quality, and subsequent fertilization rates in mature gilts. J. Anim. Sci. 75:2294-3003.

McCoard, S. A., Wise, T. H. and Ford, J. J. 2002. Expression levels of müllerian-inhibiting substance, GATA-4 and 17α-hydroxylase/17, 20-lyase cytochrome P450 during embryonic gonadal development in two diverse breeds of swine. J. Endocrin. 175, 365-374.

McCoard, S. A., Wise, T. H., Lunstra, D. D., and Ford, J. J. 2003. Stereological evaluation of Sertoli cell ontogeny during fetal and neonatal life in two diverse breeds of swine. J. Endocrin. 187, 395-403.

Parsley, Michael A., Mark E. Wilson, Thomas J. Gall and M. R. Malcolm Ballard. 2021. Effect of Stabilized Fish Oil Source on Sperm Quality and Production of Boars. ojas 11, 197-207. DOI:10.4236/ojas.2021.112015 April 15, 2021.

Reicks, D., B. Allen, J. M. Campbell, J. D. Crenshaw, and J. Polo. 2012. Effect of Solutein given to boars in isolation on discard rate and sperm quality. American Association of Swine Veterinarians yearly meeting.

Rooke, J. A., C. C. Shao, and B. K. Speake. 2001. Effect of feeding tuna oil on the lipid composition of pig spermatozoa and in vivo characteristics of semen. Reproduction 121:315-322.

Ruoff, W. L. and Dzuik, P. J. 1994a. Absorption and metabolism of estrogens from the stomach and duodenum of pigs. Domestic Animal Endocrinology. 11(2):197-208.

Ruoff, W. L. and Dzuik, P. J. 1994b. Circulation of estrogens introduced into the rectum or duodenum of pigs. Domestic Animal Endocrinology. 11.(4):383-391.

Sharpe, R. M., Mckinnel, C., Kirlin, C. and Fisher, J. S. 2003. Proliferation and functional maturation of sertoli cells and their relevance to disorders of the tetis function in adulthood. Reproduction 125, 769-784.

Swanlund, D. J., N'Diaye, M. R., Loseth, K. J., Pryor, J. L. and Crabo, B. G. 1995. Diverse testicular responses to exogenous growth hormone and follicle-stimulating hormone in prepubertal boars. Biol. Reprod. 53, 749-757.

Measuring Boar Fertility

Tom Gall

The purpose of this presentation is to demonstrate one method of assessing boar fertility at the sow farm level while experiencing a minimal amount of fertility loss. Measuring true boar fertility tends to be a very elusive endeavor at best and may seem an impossible task for the boar stud manager who thinks "I measure motility and morphology, isn't that enough? Besides, I don't have any sows to test with!" Boar fertility, when measured as farrowing success and litter size, has a relatively low correlation to the typical motility and morphology measurements made in the laboratory with a microscope. Correlation estimates range from r = 0.36 to 0.46 for motility measured after 10 days of storage, and r = 0.59 for morphology measurements taken at the time of collection (Flowers, 2009).

It has been reported that the semen dose accounts for only 6.7% of the variation in litter size and the number of sperm cells in a dose, when reduced from 2.2×10^9 to 1.7×10^9 sperm, explained only 1.2% of the variation in litter size (Feitsma, 2009). These data were derived from multiple years of fertility records in The Netherlands where sow farms are typically small compared to the USA and sows are checked for oestrous every 12 hours, but inseminated only once every 24 hours that they will stand for the boar, with an average of 1.6 inseminations per sow. Semen doses contained from 2.2×10^9 to 1.5×10^9 motile homospermic cells per 80ml dose. It has been reported that total sperm numbers less than 3×10^9 per insemination dose may result in a reduction in litter size for some boars (Flowers, 2002). So, if less than 7% of the variation in fertility on the sow farm is due to semen, then greater than 93% of the variation in fertility is due to…….what? Be that as it may, as boar stud managers, we still have a responsibility to send the highest quality, most fertile product that we can produce, to our customer every day.

With that as our background, what influences fertility, and can we measure it? While there are many ways to evaluate spermatozoa as well as the accompanying seminal plasma that the boar ejaculates with it, and correlate those findings with fertility, we will only discuss a few of those methods here. The methods available for evaluating boar spermatozoa have been presented numerous times at this conference in the past.

Motility:

Humans have been looking at sperm cells under a microscope since Antoni van Leeuwenhoek first observed them in 1677 in The Netherlands. With the advent of Computer Assisted Semen Analysis (CASA) systems in the 1990's, some, but not all of the error and subjectivity has been removed from this process. CASA systems still rely on the technician to properly mix the sample, make the proper dilution, load the chamber or slide, maintain the proper temperature of all equipment, insure there are no contaminants on any of the surfaces that contact the semen sample, evaluate the sample in a rapid and timely manner, etc. (Feitsma, et al. 2011). With the implementation of CASA systems coupled with proper training and routine evaluation, however, the variation in fertility due to the evaluating technician has been eliminated in The Netherlands (Broekhuijse, et al., 2011). Without CASA, however, it has been determined that fertility differentiation cannot be realized as long as observed motility is greater than 60% to 70% (Flowers, 2009). This author has personally observed frozen-thawed boar sperm with less than

5% motility that achieved fertility. Do we want high motility? Yes, but it does not guarantee a specific level of fertility.

Morphology:

When coupled with motility, we gain some value in fertility assessment by measuring morphology, but again, the two together will not predict fertility accurately in all samples (Flowers, 2009). It has been reported that reducing the threshold for the maximum number of abnormal cells allowed to pass an ejaculate from 30% to 20% will increase farrowing rate 0.07% and total number born by 0.08 piglets (Feitsma, 2009). As with motility, higher quality seems to result in better fertility, but there is no guarantee. It has also been reported that reducing the total sperm per insemination dose increases the correlation between swine sperm cell morphology and litter size variation to $R^2 = 0.59$ (Xu, et al. 1998).

Seminal Plasma Proteins:

The measurement of various seminal plasma proteins has been studied to determine if their levels can be correlated with fertility (Flowers, et. al., 2013). However, these evaluations involve the use of two-dimensional gel electrophoresis, chromatography, western blot testing, or mass spectrometry; tests which are not practical to run at line speed in a boar stud and/or involve expensive equipment.

Combined Evaluations:

Of course, combining several types of evaluations increases the predictability of boar fertility. When per cent motility and acrosome-reacted spermatozoa was evaluated in conjunction with the relative levels of 28 kDa, p/ 6.0 and 55 kDa, p/ 4.5 seminal plasma proteins, variation in boar fertility was predicted with nearly 90% accuracy (Flowers, 2009). Again, these evaluations do not allow for line speed results at the boar stud and may be very cost prohibitive to obtain.

Reciprocal Translocations:

First demonstrated in 1930 in maize, reciprocal translocation (RTL) involves an exchange DNA between two chromosomes in an individual. They occur when a part of one chromosome breaks off and attaches to a different chromosome during meiosis. The result can range from no observable effect to death of the embryo, usually after implantation. In swine, conception rate is typically not impacted, but affected boars can manifest themselves with a reduction in litter size between 10% and 100%, meaning some will have a very low impact and others a very significant impact on piglet production. For boars, RTL carriers nearly always have normal spermiograms, so semen evaluation will not reveal carrier status (Kuster, et al. 2019). RTL is passed from the affected parent to its offspring when the offspring do survive, so eliminating carriers is particularly critical in nucleus and multiplication scenarios. However, a negative test result of parents does not guarantee offspring will be negative, as the estimate for occurrence in each new generation is 0.5%. The prevalence of RTL in unscreened populations has been estimated to be less than 5% with a range of 0.7% to 4% considered typical for swine (Feitsma, 2009). Elimination of carrier boars in The Netherlands had resulted in an increase of 0.05 piglets per litter with an estimated value of €1.4 million to the pig industry at a cost of €200,000 in testing (Feitsma, 2009). In the USA, an analysis in 2011 calculated the cost of a single boar with RTL to be $84,240, based on a loss of 4,212 weaned pigs over the boar's lifetime (Kuster, et al. 2019).

RTL can be detected from a blood sample at any time (birth, weaning, selection, isolation, etc.) by labs such as Kuster Research and Consulting.

Semen age:

It should be noted that semen age can have a significant impact on fertility, outside of all of the other parameters that are measured. Broekhuijse, et al., reported that semen age had no significant effect on farrowing rate or total number born up to 5 days after production. Personal experience of this author has found that fertility, measured as farrowing rate and total number born, was higher for semen used the day following collection (day 1) than when used the same day of collection (day 0), then gradually declined from day 2 until day 5, when it increased again, before declining on day 6 and beyond. While there was no statistical analysis of these matings performed, the number of matings observed add some credence to the overall picture.

Boar age:

The sooner a boar can be evaluated for fertility, the more efficiently he can be removed from the pool of contributing boars in the event he is sub-fertile. However, it has been shown that when boars of 3 age groups (7-10 months, 18-33 months, and 51-61 months) were compared, the youngest group had a significantly ($P < 0.001$) lower overall farrowing rate (65%) compared to the 2 older groups (87.2% and 84.7% respectively) (Tsakmakidis, et al., 2012). Therefore, it is important to keep in mind that some boars may be culled for low fertility who would improve as they age.

THE GOLD STANDARD:

Ultimately, farrowing rate and total number born on the farm are the optimal measure of the fertility of boars. But how can this be achieved by the boar stud, which does not have sows of its own to breed, unless it is part of a large, integrated system? Even in the integrated model, it can be difficult to get sow farm managers to cooperate with the boar stud to do the testing, which will involve some additional labor for the sow farm manager, breeding manager and breeding technician. One must also consider the accuracy of information coming from the breeding barn and farrowing house, as incorrect counting of piglets may have a false negative or false positive impact on the final evaluation of individual boars (Broekhuijse, et al., 2011).

Objective number one: convince the sow farm manager and upper management to do the extra work. This is only realistic by considering the value in real dollars that would be returned to the producer by eliminating boars with low fertility. In order to do so, some assumptions must be made, none the least of which is: this process can be accomplished accurately at both the boar stud and sow farm levels. The questions to be asked would include:

1) What is the value of 1% difference in farrowing rate?
2) What is the value of 1 additional pig born?
3) What is the cost of 1 replacement boar?
4) How many doses of semen does a boar produce per week?
5) What is the cost to produce the test doses, store them separately at the farm, ensure accuracy in semen use, and obtain, compile, and analyze fertility data above the cost of a standard insemination dose?

1) Value of 1% difference in farrowing rate:

The value of 1% difference in farrowing rate is a complex variable to define, but we will simplify it for this example. The value is greatly influenced by the policy of each sow farm regarding recycle sows. If they are given a second opportunity and remated on their next estrous, their value may be much greater than if the open sow is simply culled and replaced with a gilt.
Cost to maintain a non-pregnant sow for 45 days (time from mating until removed from the farm as a cull, assuming found open at the 21-day estrous check):
Feed cost at 5 pounds per day x 45 days x $0.^{12}$ per pound = $27.^{00}$
Housing cost at $0.^{24}$ per day x 45 days = $10.^{80}$
Total cost to maintain an open sow until culled = $37.^{80}$
Cost of replacement gilt = $350.^{00}$
Salvage value of cull sow = $212.^{50}$ (assume 425 lb. average cull sow weight x $0.^{50}$ per pound)
Replacement cost of sow = $350.^{00}$ - $212.^{50}$ = $137.^{50}$
Total cost if sow is replaced with a gilt = $37.^{80}$ + $137.^{50}$ = $175.^{30}$
Cost if sow is remated = $37.^{80}$
For every 10,000 sows in production, assuming 2.45 litters per sow per year and 92% farrowing rate, (10,000 x 2.45) ÷ 0.92 = 26,630 matings per year
26,630 matings x 1% = 266 matings per year difference
266 matings per year x $37.^{80}$ = $10,054.^{80}$ per year cost if sows are remated
266 matings per year x $175.^{30}$ = $46,629.^{80}$ per year cost if sows are replaced
Neither of these 2 scenarios take into account the cost of the additional doses of semen that would be required, which would vary considerably from $2.^{50}$ to $4.^{00}$ in most cases but could be much higher in the case of maternal semen ($7.^{00}$ to $25.^{00}$ per dose). There may be additional genetic fees involved if they are charged through the cost of the semen doses.
Multiply the cost above times the per cent of sows above or below 10,000 in your operation to get your cost per year. These cost figures do not take into account the loss from the delay in having the litter lost in inventory to sell approximately 11 months post mating, which may be positive or negative, depending on the market value at that time.

<u>2) Value of 1 additional piglet born:</u>
The second question relates to the value of an additional pig born. For the boar stud, total born is a better measure to use than born alive as the number of mummies and stillborn piglets are generally considered to not be influenced by sperm quality. Two scenarios must be considered: the sow farm that is paid for acceptable quality weaned pigs out the door and the sow farm that is part of the integrated system, where weaned pigs out the door are a very important metric, but ultimately, the system is paid for pigs on the rail at the plant where they are harvested.

The first scenario is a simple calculation where each piglet weaned has a set value. For this example, we will assign $40.^{00}$ as the value of an acceptable quality weaned piglet. Deducting vaccine, labor and other variable costs, we will reduce the amount to $35.^{00}$ per weaned piglet. No fixed cost is assigned to this piglet as it will be considered a “bonus” piglet, above the minimum number set to cover fixed costs, except possible employee production bonuses. The calculation then looks like:
wpv = weaned piglet value = $35.^{00}$
tb = total number of piglets born (we will use 16.1 for our example)
pba = per cent of piglets born alive (we will use 92.5% for our example)
nba = number of piglets born alive

pw = per cent of acceptable quality piglets weaned of those born alive (we will use 91.5% for our example)
nw = number of acceptable quality piglets weaned that the farm gets paid for
Therefore:
nba = tb x pba (16.1 x 0.925 = 14.8925) vs. (15.1 x 0.925 = 13.9675)
nw = nba x pw (14.8925 x 0.915 = 13.6266) vs. (13.9675 x 0.915 = 12.7803)
value of 1 piglet more in total born = nw x wpv (13.6266 x $35.^{00}$ = $476.^{93}$) vs. (12.7803 x $35.^{00}$ = $447.^{31}$)
Value of 1 pig more total born = $476.^{93}$ - $447.^{31}$ = $29.^{62}$

The second scenario will use the same calculations up to the value of the weaned piglet. From that point, it becomes a function of the value of the carcass at harvest, which can vary considerably. For this example, we will assume a value over all fixed and variable cost of $15.^{00}$ per pig at harvest. We simply now take the number of pigs weaned times the per cent harvested as full market value and we get the following numbers:
mv = value above costs of each pig harvested (we will use $15.^{00}$ per pig for this example)
ph = per cent of weaned pigs harvested at full market value (we will use 94.5% for this example)
nfmv = the number of pigs harvested at full market value
Using the same production numbers as above:
nfmv = (13.6266 x 0.945 = 12.8771) vs. (12.7803 x 0.945 = 12.0774)
mv = (12.8771 x $15.^{00}$ = $193.^{16}$) vs. (12.0774 x $15.^{00}$ = $181.^{16}$)
Value of 1 pig more total born per litter = $193.^{16}$ - $181.^{16}$ = $12.^{00}$
For the 10,000 sow operation, the calculation is 10,000 x 2.45 = 24,500 x $12.^{00}$ = $294,000.^{00}$ per year. Multiply the cost above times the per cent of sows above or below 10,000 in your operation to get your cost per year.

3) Cost of 1 dose of semen:
Every boar stud manager should know exactly what the cost of producing each dose of semen is for his or her stud and differences in maternal vs. terminal doses should be calculated as well. In fact, every boar stud manager should know, or at least see on a regular basis, the cost of all inputs (labor, feed, veterinarian cost, barn supplies, lab supplies, utilities, depreciation, isolation, repairs, taxes, etc.) on a total monthly or period basis and annual basis, and see these values broken down on a per dose basis. He or she should also know the income side of this equation.

4) Cost of 1 replacement boar:
While this would appear to be quite simple to determine on the surface, several considerations must be included. The calculation should include:
1. Boar price delivered to isolation (this may vary by line or source)
2. Cost of maintaining each boar in isolation (feed, housing, labor, vet, depreciation etc.)
3. Cost of diagnostic tests, vaccinations, treatments, etc.
4. Cost of housing the boar in the stud until the boar is producing usable doses of semen.
5. Boar replacement rate, which is an important component in the overall calculation and should be broken down by genetic line where applicable, particularly maternal vs. terminal sires.

The simplest method of determining the per boar cost is to take the annual cost of the items listed above and divide by the number of boars purchased, including boars that never make it into production. Salvage value of these boars can be deducted from the total cost, if applicable.

5) Number of doses produced per boar per week:
Another simple calculation that every boar stud manager should be intimately familiar with for their stud. Keep in mind, however, that you are normally only testing new boars, so doses per collection should be calculated only from boars that have been in the stud for a few months and not include boars that have been there for a year or longer.

6) Additional costs to do the single sire matings:
It may be difficult, or even impossible to calculate these costs, but at least keep in mind that they do exist. It has been determined that single sire doses used to evaluate boar fertility should be made at a lower number of sperm per dose to challenge the system, as some defects that impact fertility are compensable. It has been suggested in the literature that doses should contain between 1×10^9 and 2×10^9 total sperm per dose (Flowers, 2009).

What is the cost of a low fertility boar to a system per 10,000 sows in inventory?
As the examples above clearly demonstrate, the cost to a boar stud's customer or to the integrated system is dependent on numerous variables, but it can be calculated. The following examples will demonstrate the cost in two scenarios. The first will be for a boar that has a ten per cent lower farrowing rate than desired and the second will be a boar that has four fewer total born than desired.

Example 1: 10% reduction in farrowing rate:
When looking at boar effect, conception rate is a more accurate measurement than farrowing rate, as most sows that abort or are removed due to prolapse are not due to semen, but rather a health or environmental effect on the sow itself. These sows, then, should not count against the fertility assessment of the boar. They will not, however, contribute to the litter size component for that boar.

For this exercise, we will consider that the sow farm is part of a system of 10,000 total sows that owns all pigs through finishing and harvest and does not rebreed recycle sows, but rather replaces them with a gilt. We will also use a boar replacement rate in the stud of 70% per year. From our cost estimates above, for 10,000 sows and a 10% reduction in farrowing rate, the cost to the system would be $\$466{,}298.^{00}$ per year times the per cent of sows that this boar would mate. Using 300:1 ratio of sows per boar, approximately 33 boars would be required to produce semen for this farm; this boar would be responsible for 3% of the matings at a cost to the system of $\$13{,}988.^{94}$ on an annualized basis. If this boar were replaced after spending the average length of time in the boar stud with a 70% replacement rate, the total cost to the system would be $\$19{,}984.^{20}$.

Example 2: 4 pig reduction in total born:
Using the same scenario of the 10,000 sow system that finishes all pigs it produces, a boar that produces 4 fewer pigs total born than the best boars in the stud, the cost to the system would be $\$48.^{00}$ per litter produced by this boar ($\$12.^{00}$/pig x 4 pigs), times 2.45 litters per sow per year =

$117.^{60} times 3% of the sows mated to this boar (300 litters) = $35,280.^{00} per year. With the same 70% replacement rate at the stud, the lifetime loss to the system is $50,400.^{00}. Using a much more conservative value of $5.^{00} profit per market animal, this translates to $4.^{00} per piglet lose at birth and the total loss to the system from the boar siring 4 fewer piglets per litter becomes $16,800.^{00}.

In both cases, finding just one boar per year with either a 10% lower farrowing rate or 4 fewer pigs in the litter would easily pay for the cost of testing boars as they enter the stud.

The cost of testing all boars for reciprocal translocations would be a fraction of the cost outlined above, but would not catch every boar, as some fertility problems would not be eliminated by this test. This does point out, however, the value in RTL testing for all boars in isolation.

Single sire testing

If you choose to fertility test boars via single sire matings, a protocol must be developed for both the boar stud and the sow farms that will be performing the matings to insure they are performed properly and everyone knows exactly what their roll will be in the process. It is critical to the success of the program that the boar stud manager communicate with the sow farm or breeding manager to insure everyone is in synch with the process and timing of delivery of the single sire doses. We will assume that adequate sows and/or farms are available to do the testing and the owners and managers are on board with conducting the tests. A minimum of 50 "good" matings are required per boar to give adequate confidence in the results. This is by no means a level that would allow for statistical verification of fertility, but it will be enough to identify boars with low fertility. It is recommended that doses be sent out to make 60 matings (120 doses) in groups of 20 to 30 doses to mate 10 to 15 sows single sire per group. A group may be available once per week, or on large farms, twice per week. The number will depend on the size of the sow farm and the weaning schedule of the farm. Doses must be delivered the sow farm packaged separately from the rest of the semen the farm receives and delivered so that they will be used within 72 hours of collection to avoid the influence of semen age on the process. An important part of the sow farm protocol is that recycle or "opportunity" sows must never be used for single sire test matings, as the general fertility of these sows is not as predictable as weaned sows mated on the first post-weaning oestrous. Also, gilts generally are not used for the same reason. It should be stated in the sow farm's protocol that only sows returning to oestrous within 7 days post-weaning are to be used for the single sire mating tests. This will give the boars the best opportunity to achieve success and keep the sows grouped together to facilitate data analysis.

If the sow farms have been receiving semen with 2.5×10^9 to 3×10^9 sperm per dose, there may be some reluctance to participate in the program with doses at 1.5×10^9 total sperm, particularly on the sows with the highest potential for reproductive capacity. The data generated in this author's experience showed no difference in fertility, over a long period of time, when compared to the overall fertility of the sow farm, when comparing single sire matings to the rest of production.

Invariably, there will be some matings in which a sow receives a single sire dose from the test boar and the second mating is from a pool of the regular production semen. These sows cannot be used as part of the single sire evaluation and must be eliminated from the single sire data set.

Sows which only receive one dose because they will not stand for mating the second day can be included in the data set but should be noted as single matings. Sows that stand for a third mating and receive it can also be included and recorded as triple matings, but only if all three matings are with single sire doses. If they are not, they must be eliminated from the single sire data set. Single sire doses must also be designated in such a way that they are easily distinguishable from regular production doses in the sow record system. For example, if each pool of semen receives a unique identification (ID) number that is entered into the sow record system for the boar or mating ID, the same could be done with the single sire doses with "SS" in front of the number to easily pick them out of the record system database. Keeping the numbering system intact also allows the person looking at results to verify that the pool ID was in fact a pool ID of a single sire batch produced within a few days prior to that mating. Alternatively, if the software at the boar stud allows or automatically assigns the boars individual ID as the ID for the doses of semen, as opposed to a rotating pool ID, then the boar's individual ID should be sufficient to verify the single sire matings as such.

The matings for any given boar should be performed at more than one sow farm to spread out any extremely low fertility boars and to give each boar as unbiased chance as possible to achieve pregnancy. It is not uncommon for sow farm managers to call the boar stud at the 3 week post mating oestrous check to report a high incidence of returns, should they occur. However, it should be noted that this author has experienced more than one case in which an ejaculate was split between 2 farms, one of which reported 100% of the sows returning to oestrus at 3 weeks post mating and the other reported 0% returns at 3 weeks for sows they mated with doses split from the same ejaculate. When a sow farm manager states that a single sire boar, or even a pool of semen, has had a large number or per cent of returns, always verify the numbers with data from the sow database. If the sow farm is not recording pool IDs in the sow record system for both first and second matings, and third matings when they occur, the only option is to go back to the sow farm and look at the individual sow cards and breeding sheets to verify the fertility results.

A sample protocol is shown below in Appendix A

Once data begin to be generated, one or two Excel spreadsheets will be needed, depending on how matings will be tracked. Each time doses are sent to a sow farm, the date, boar ID, sow farm name and number of doses sent must be recorded. This allows the lab to track doses sent so the technicians can stop sending that boar when they have reached 120 doses on each boar, and so the data manager knows when and where to look for the breeding data. It can also be used to track the final fertility results, which can also be done from a separate spreadsheet, if desired. The inclusion of rupture or other abnormality data is separate from the fertility information but may be deemed useful if a problem exists or is perceived to exist. Many problems seem to automatically disappear once the farm is required to track them.

The single most important thing to do is report the results back to all stakeholders (owners, sow farm managers, etc.) for each group of boars tested, both good and bad. The sow farm workers are generally curious to know if any boars are culled due to the work they have contributed to the process, and it helps maintain a healthy relationship between the boar stud and sow farms to share the data.

References:

Broekhuijse MLWJ, Feitsma H, Gadella BM. 2011. Field Data Analysis of Boar Semen Quality. *Reproduction in Domestic Animals*. vol. 46 (Suppl. 2). pp. 59-63.

Feitsma H, 2009. Artificial insemination in pigs, research and developments in The Netherlands, a review. Acta Scientiae Veterinariae. Vol. 37: 61-71.

Feitsma H, Broekhuijse MLW, Gadella, BM. 2011. Do CASA Systems Satisfy Consumer Demands? A Critical Analysis. *Reproduction in Domestic Animals*. vol. 46 (Suppl. 2). pp. 49-51.

Flowers WL. 2002. Increasing fertilization rate in boars: influence of number and quality of spermatozoa inseminated. *Journal of Animal Science*. vol. 80 (E Supplement 1) pp. 47-53.

Flowers WL. 2009. Selection for boar fertility and semen quality – the way ahead. In *Control of Pig Reproduction VIII,* pp 67-78. H Rodriquez-Martinez, JL Vallet & AJ Ziecik editors. University of Nottingham Press.

Flowers WL, Stewart KR, Gall T, Novak S, Dyck MK, Kirkwood RN. 2013. Boar seminal plasma proteins and their relevance to reproductive technologies. In *Control of Pig Reproduction IX*, pp 33-44. H Martinez-Rodriguez, N Soede & WL Flowers editors. University of Nottingham Press.

Kuster C, Robinson J, White E. *Proceedings 50th Annual AASV* 2019:17-19.

Tsakmakidis, IA, Khalifa TAA, Boscos CM. 2012. Age-related changes in quality and fertility of porcine semen. *Biological Research*, 45:381-386.

Xu X, Pommier S, Arbov T, Hutchings B, Sotto W & Foxcroft GR. 1998. In vitro maturation and fertilization techniques for assessment of semen quality and boar fertility. *Journal of Animal Science*, 76:3079-3089.

Appendix A

Single Sire Mating Protocol

1. Objectives of process:
    a. Determine the reproductive performance of terminal genetic sires. Parameters to be measured will include: Conception Rate, Farrowing Rate, Total Born, and Ruptures per Litter.

b. Parameters will be measured from data from XXXX Sow Record system from commercial sow farms.

2. Materials and methods
    a. Semen will be sourced from YYYY boar stud from boars within genetic lines currently in use by the commercial sow farms receiving it.
    b. Semen will be processed in the same semen extender used for all semen processing and with the same processing methods for normal production at YYYY boar stud.
        i. Doses will be diluted to a final concentration of 1.5×10^9 (billion) total sperm per dose in a standard volume of XX milliliters.
        ii. Doses will be sent to the various commercial sow farms in pairs according to the number and on the days determined by the boar stud and sow farm managers. Single sire doses must be packaged separately from other doses. Semen from only one single sire boar may be sent to an individual farm on a given day.
        iii. A minimum of 10 pairs (20 doses) will be sent from each boar to be tested on a given day, to achieve a minimum of 60 matings that can be recorded. The 60 pairs of doses should not be sent to the same farm. All 60 pairs (120 doses) from a given boar should be sent out within the shortest time period possible (i.e., 2 to 3 weeks maximum).
            a. The individual boar's identification number or a unique pool ID number must appear on the label of each dose of semen.
            b. Participating sow farms will be: ZZZZ
    c. Semen delivered to the sow farm will be stored and handled in the same manner as normal production semen.
        i. Sow farms must communicate with the boar stud manager regarding the days of the week and number of single sire doses they prefer to receive per delivery.
        ii. Storage temperature will be 17ºC (±1°C)
        iii. Doses will be rotated gently at least once each day until used up.
        iv. Mating method may be by normal (intercervical) Artificial Insemination (AI) or Post Cervical Artificial Insemination (PCAI). If an individual farm is utilizing multiple mating methods, the mating method used for each insemination must be recorded.
        v. Only sows that are confirmed in normal standing estrous within 7 days after weaning should be mated with a maximum of 2 matings per sow, 20 to 26 hours apart. Sows should be mated per the farm's normal mating protocol, but with never more than 2 matings per sow. Gilts that have not farrowed a litter must not be used for these matings. Parity 7 or higher sows must not be used for these matings. Opportunity sows must never be used for these matings.
        vi. Matings must be recorded in XXXX sow production record system with the four digit boar ID number as the boar, semen or pool ID.
        vii. Farrowing data should include Total Born and number of Ruptures. The number of Ruptured piglets in each litter should be recorded in the sow production record system. Other abnormalities should be recorded on the farrowing card and that information communicated directly to the project and data manager.
        viii. Accurate data recording is critical, as it will determine culling of individual boars, which have a significant cost to the entire production system.

3. Boar culling criteria:

a. Boars with less than 70% conception rate based on returns and 5 week pregnancy exams will be culled.
b. Boars with less than 11 pigs total born will be culled.
c. Additional matings may be required of boars that are within 5% of the target farrowing rate or 0.5 total born.

4. Contacts: (list names here)
    a. Project & Data manager:
    b. Boar stud manager:
    c. Boar stud office manager:
    d. Boar stud lab manager:

Contacts Names and Addresses

List details for each of the persons listed above, including address, email, office phone and cell phone information.

Date	Farm	Pool ID	Boar ID	# Doses	Line	# Mated	# Pregnant	% Pregnant	Total Mated	Total Farr	% Farr by Boar	Total Born	Live Born	# Weaned	Parity	Cull Date	Cull Reason
9/19/2016	YYYY	597	Z8700	30	359	13	12	92.31%	44	39	88.64%	14.10	12.10	8.80	3.15		
11/21/2016	YYYY	242	Z8700	30	359	17	16	94.12%				14.19	12.25	10.13			
11/28/2016	YYYY	314	Z8700	30	359	14	11	78.57%				13.55	12.09	11.27			
11/21/2016	XXXX	243	Z8703	30	359	14	14	100.00%	28	26	92.86%	16.36	14.64	11.64			
10/14/2016	ZZZZ	869	Z8703	30	359	14	12	85.71%				14.08	12.75	11.75	3.64		
9/23/2016	ZZZZ	648	Z8705	30	359	14	12	85.71%	14	12	85.71%	14.00	12.36		4.31	12/6/2016	Unsound
9/23/2016	**AAAA**	**647**	**Z8706**	**30**	**359**	**15**	**8**	**53.33%**	**31**	**19**	**61.29%**	12.25	11.88		3.00	2/10/2017	Unsound
11/22/2016	**AAAA**	**264**	**Z8706**	**30**	**359**	**16**	**11**	**68.75%**				15.27	14.18		3.38		
9/26/2016	YYYY	670	Z8707	30	359	15	13	86.67%	15	13	86.67%	13.92	11.62	9.23	3.20		
11/25/2016	ZZZZ	297	Z8707	30	359	0											
11/29/2016	**AAAA**	**334**	**Z8708**	**30**	**359**	**13**	**12**	**92.31%**	**28**	**21**	**75.00%**	12.42	11.17		4.00		
9/26/2016	**BBBB**	**671**	**Z8708**	**30**	**359**	**15**	**9**	**60.00%**				13.89	10.11	8.67	2.20		
12/2/2016	ZZZZ	364	Z8709	30	359	14	13	92.86%	29	24	82.76%	14.92	13.15	9.69	2.77		
9/19/2016	BBBB	598	Z8709	30	359	15	11	73.33%				15.20	13.30	10.20	1.81		
12/5/2016	YYYY	388	Z8710	30	359	15	12	80.00%	33	28	84.85%	9.67	9.08	9.92	3.00	6/27/2017	Semen Quality
3/9/2017	ZZZZ	314	Z8710	30	359	18	16	88.89%				14.07	12.31	11.25	1.89		
9/30/2016	AAAA	705	Z8711	30	359	6	6	100.00%	20	19	95.00%	15.00	12.83		1.50		
12/6/2016	AAAA	407	Z8711	30	359	14	13	92.86%				14.38	12.15	8.77	2.79		
9/30/2016	ZZZZ	706	Z8712	30	359	15	14	93.33%	30	29	96.67%	12.57	11.79		2.60		
12/9/2016	ZZZZ	431	Z8712	30	359	15	15	100.00%				14.53	13.07	11.00	4.47		
10/3/2016	YYYY	743	Z8713	30	359	13	13	100.00%	13	13	100.00%	13.13	12.13	10.73	4.00		
12/13/2017	AAAA	472	Z8714	30	359	15	14	93.33%	28	24	85.71%	15.82	14.36	11.55	3.20		
10/3/2016	BBBB	744	Z8714	30	359	13	10	76.92%				14.56	13.11	11.67	2.87		
10/7/2016	**ZZZZ**	**789**	**Z8716**	**30**	**359**	**0**			**14**	**8**	**57.14%**						
12/16/2016	**ZZZZ**	**499**	**Z8716**	**30**	**359**	**14**	**8**	**57.14%**				9.88	8.88	10.25	4.13		
12/19/2016	YYYY	510	Z8717			15	13	86.67%	29	23	79.31%	12.08	11.69	7.77	1.38		
10/7/2016	AAAA	787	Z8717	30	359	14	10	71.43%				13.10	11.30	11.80	3.86		
12/19/2016	AAAA	549	Z8718	30	359	13	12	92.31%	28	25	89.29%	15.25	12.25	9.83	4.15		
12/26/2016	AAAA	598	Z8718	30	359	15	13	86.67%				15.38	11.85	10.08	3.20		
10/10/2016	BBBB	812	Z8718	30	359	0											
10/10/2016	YYYY	811	Z8719	30	359	15	11	73.33%	15	11	73.33%	14.55	13.45	10.45			
1/8/2017	YYYY	723	Z8719	30	359	0											
3/12/2017	**YYYY**	**329**	**Z8720**	**30**	**359**	**13**	**8**	**61.54%**	**25**	**9**	**36.00%**	13.25	10.75	9.25	3.14		
10/14/2016	**AAAA**	**864**	**Z8720**	**30**	**359**	**12**	**1**	**8.33%**				10.00	7.00	9.00	2.17		
1/9/2017	YYYY	???	Z8723	???	359	13	9	69.23%				12.88	8.25	4.13			
3/19/2017	YYYY	401	Z8723	30	359	12	6	50.00%				12.50	9.50	6.83	2.08		
10/17/2016	YYYY	886	Z8725	30	359	14	9	64.29%	23	18	78.26%	10.33	7.67	6.89			
1/9/2017	AAAA	750	Z8725	30	359	9	9	100.00%				8.11	7.00	6.89	2.07		
8/18/2016	BBBB	273	Z8725	30	359	0					#DIV/0!						
8/22/2016	YYYY	316	Z8726	30	359	13	10	76.92%	28	10	35.71%	13.67	12.67	9.50	1.31		
1/12/2017	ZZZZ	765	Z8726	30	359	15	0	0.00%							3.40		
1/15/2017	YYYY	783	Z8727	30	359	14	8	57.14%	29	23	79.31%	11.60	6.40	6.60	3.20		
10/21/2016	AAAA	939	Z8727	30	359	15	15	100.00%				16.64	13.21	10.71	3.73		
8/22/2016	BBBB	317	Z8727	30	359	0											
1/16/2017	AAAA	816	Z8728	30	359	15	15	100.00%	30	26	86.67%	13.40	11.53	9.60	2.50		
10/21/2016	ZZZZ	940	Z8728	30	359	15	11	73.33%				12.36	12.00	10.36	4.08		
10/24/2016	**YYYY**	**955**	**Z8729**	**30**	**359**	**15**	**11**	**73.33%**	**40**	**22**	**55.00%**	14.00	11.60	9.20			
8/26/2016	AAAA	356	Z8729	30	359	12	2	16.67%				14.50	14.00	11.50	3.83		
1/20/2017	ZZZZ	834	Z8729	30	359	13	9	69.23%				11.67	8.89	10.33	2.79		

Date	Farm	Pool ID	Boar ID	# Doses	Line	# Mated	# Pregnant	% Pregnant	Total Mated	Total Farr	% Farr by Boar	Total Born	Live Born	# Weaned	Parity	Cull Date	Cull Reason
11/7/2016	YYYY	O94	Z8730	30	359	15	11	73.33%	29	18	62.07%	14.00	11.09	8.45	5.47		
1/23/2017	YYYY	852	Z8730	30	359	14	7	50.00%				12.40	10.20	8.00	3.57		
8/26/2016	ZZZZ	355	Z8730	30	359	0											
8/29/2016	YYYY	379	Z8731	30	359	13	10	76.92%	27	21	77.78%	15.50	14.90	11.10	2.92		
1/24/2017	AAAA	884	Z8731	30	359	14	11	78.57%				12.44	11.56	12.33	3.00		
11/1/2016	AAAA	O41	Z8733	30	359	14	12	85.71%	42	39	92.86%	15.75	12.83	10.33	3.14		
9/2/2016	ZZZZ	437	Z8733	30	359	14	14	100.00%				16.23	14.77	12.08	4.21		
1/28/2017	ZZZZ	??	Z8733	30	359	14	13	92.86%				13.67	10.67	9.67	3.53		
8/29/2016	BBBB	382	Z8733	30	359	0											
9/2/2016	AAAA	428	Z8734	30	359	10	0	0.00%	43	29	67.44%				3.10		
2/7/2017	AAAA	O22	Z8734	30	359	18	15	83.33%				13.40	11.87		2.33		
11/4/2016	ZZZZ	O70	Z8734	30	359	15	14	93.33%				11.62	9.62	8.54	3.61		
11/7/2016	XXXX	O95	Z8735	30	359	0											
1/30/2017	YYYY	933	Z8735	30	359	14	9	64.29%	14	9	64.29%	11.57	9.14	8.14			
9/6/2016	YYYY	466	Z8736	30	359	6	4	66.67%	34	29	85.29%	11.50	7.25	6.75	2.83		
2/5/2017	YYYY		Z8736	30	359	13	10	76.92%				12.80	12.00		2.57		
11/8/2016	AAAA	116	Z8736	30	359	15	15	100.00%				15.53	14.20	11.80	2.67		
1/30/2017	AAAA	953	Z8737		359	14	13	92.86%				14.89	12.89	8.67	4.00		
9/6/2016	BBBB	468	Z8737	30	359	0										4/13/2017	Unsound
9/9/2016	AAAA	502	Z8738	30	359	0											
3/19/2017	XXXX	408	Z8738	30	359	15	13	86.67%				13.23	10.38	8.92	2.87		
2/9/2017	ZZZZ	O40	Z8738		359	14	12	85.71%	14	12	85.71%	14.25	12.92		3.27		
11/14/2016	XXXX	171	Z8739	30	359	17	16	94.12%	34	27	79.41%	13.81	12.44	11.00	3.06	6/27/2017	Semen Quality
9/9/2016	ZZZZ	503	Z8739	30	359	0											
11/11/2016	ZZZZ	136	Z8739	30	359	0											
2/3/2017	ZZZZ	973	Z8739	30	359	17	11	64.71%				7.30	7.10	9.90			
9/12/2016	YYYY	529	Z8740	30	359	14	11	78.57%	27	24	88.89%	10.36	9.18	7.82	2.93		
11/14/2016	YYYY	170	Z8740	30	359	13	13	100.00%				12.31	11.31	9.38			
10/31/2016	YYYY	O26	Z8741	30	359	17	15	88.24%	45	37	82.22%	12.53	11.07	8.87			
2/12/2017	YYYY	??	Z8741			17	15	88.24%				12.53	11.07	8.87	4.35		
9/12/2016	BBBB	530	Z8741	30	359	11	7	63.64%				12.86	10.86	9.14	1.64		
9/16/2016	AAAA	572	Z8742	30	359	0			13	12	92.31%						
11/15/2016	AAAA	189	Z8742	30	359	13	12	92.31%				16.58	14.67	11.58	4.69		
2/13/2017	YYYY	O61	Z8744	30	359	14	12	85.71%	28	22	78.57%	13.00	11.80		1.71		
3/6/2017	YYYY	263	Z8744	30	359	14	10	71.43%				10.80	5.90	6.40	2.86		
2/20/2017	YYYY	126	Z8747	30	359	12	10	83.33%	12	10	83.33%	12.14	10.43				
2/13/2017	XXXX	O63	Z8748	30	359	0											
3/5/2017	XXXX	267	Z8748	30	359	15	12	80.00%	15	12	80.00%	11.08	10.42	12.00	2.44		
4/9/2017	XXXX	617	Z8749	30	359	15	14	93.33%				13.36	12.21	9.71	2.60		
2/14/2017	AAAA	O91	Z8749	30	359	18	15	83.33%				12.40	11.27		2.50		
2/26/2017	YYYY	194	Z8750	30	359	16	11	68.75%				12.00	11.00	11.10	3.19		
4/10/2017	AAAA	645	Z8750	30	359	16	8	50.00%				10.75	10.00	9.25	3.25		
2/17/2017	ZZZZ	110	Z8750	30	359	15	12	80.00%				10.25	9.33		2.67		
4/16/2017	XXXX	684	Z8751	30	359	14	13	92.86%	14	13	92.86%						
2/20/2017	XXXX	130	Z8753	30	359	0											
2/22/2017	AAAA	156	Z8754	30	359	14	13	92.86%	29	27	93.10%	13.62	12.07		4.07		
4/20/2017	ZZZZ	741	Z8754	30	359	15	14	93.33%				14.21	12.43	11.86	3.73		
2/23/2017	ZZZZ	175	Z8755	30	359	14	10	71.43%	14	10	71.43%	11.25	10.88		2.06		
2/26/2017	XXXX	196	Z8756	30	359	14	12	85.71%	28	24	85.71%	14.42	13.17	11.50	3.47		

Date	Farm	Pool ID	Boar ID	# Doses	Line	# Mated	# Pregnant	% Pregnant	Total Mated	Total Farr	% Farr by Boar	Total Born	Live Born	# Weaned	Parity	Cull Date	Cull Reason
4/16/2017	YYYY	683	Z8756	15	359	0											
4/24/2017	AAAA	781	Z8756	30	359	12	11	91.67%				10.27	9.27	7.36	3.08		
2/27/2017	AAAA	226	Z8757	30	359	16	16	100.00%				16.10	13.10	10.70	2.81		
3/13/2017	AAAA	358	Z8757	30	359	20	17	85.00%				13.63	12.88	11.50	2.55		
4/23/2017	YYYY	762	Z8758	30	359	12	9	75.00%				12.11	10.78	7.78	3.38		
3/3/2017	ZZZZ	237	Z8758	30	359	12	11	91.67%				13.55	11.45	10.45	3.83		
3/26/2017	YYYY	485	Z8764	30	359	16	14	87.50%	14	10	71.43%	14.14	10.57	7.43	6.00		
4/16/2017	YYYY	688	Z8764	15	359	0											
3/26/2017	XXXX	489	Z8765	30	359	15	14	93.33%	15	14	93.33%						
3/27/2017	AAAA	502	Z8766	30	359	15	13	86.67%	15	13	86.67%	14.15	10.38	10.00	3.60		
4/2/2017	YYYY	545	Z8767	30	359	14	8	57.14%	14	11	78.57%	14.50	11.88	10.50	2.60		
4/2/2017	XXXX	547	Z8769	30	359	14	12	85.71%	14	12	85.71%	14.42	13.50	10.42	2.21		
3/20/2017	AAAA	426	Z8770	30	359	16	14	87.50%	16	14	87.50%	14.77	13.08	9.62	4.25		
4/3/2017	AAAA	577	Z8771	30	359	15	15	100.00%	15	15	100.00%	13.58	12.17	10.00	3.53		
4/9/2017	YYYY	615	Z8772	30	359	13	7	53.85%	14	10	71.43%	11.00	10.50	7.33	2.57		
4/23/2017	XXXX	763	Z8773	30	359	15	13	86.67%				15.15	13.23	10.38	3.00		
5/8/2017	AAAA	919	Z8773	30	359	16	13	81.25%				14.15	12.62	9.67	2.94		
6/11/2017	YYYY	O23	Z8774	30	359	14	10	71.43%									
5/14/2017	YYYY	963	Z8776	30	359	13	11	84.62%									
4/27/2017	ZZZZ	816	Z8776	30	359	15	15	100.00%				13.13	12.20	9.40	3.94		
4/30/2017	XXXX	822	Z8777	30	359	15	14	93.33%				13.79	12.50	9.36	2.50		
5/15/2017	AAAA	981	Z8777	30	359	16	13	81.25%				13.23	11.85		2.63		
4/30/2017	YYYY	821	Z8778	30	359	13	8	61.54%				15.50	13.38	11.00	4.08		
5/21/2017	YYYY	O27	Z8778	30	359	15	14	93.33%				14.71	12.79		3.67		
5/28/2017	YYYY	O89	Z8779	30	359	16	11	68.75%									
5/1/2017	AAAA	846	Z8779	30	359	13	9	69.23%				14.11	12.00	11.89	3.08		
5/7/2017	YYYY	891	Z8780	30	359	14	11	78.57%									
6/4/2017	YYYY	165	Z8780	30	359			#DIV/0!									
5/7/2017	XXXX	890	Z8781	30	359	15	15	100.00%				15.20	13.13	10.50	2.33		
6/18/2017	XXXX	289	Z8781	30	359	15	15	100.00%									
6/25/2017	XXXX	363	Z8784	30	359	14	14	100.00%			#DIV/0!						
7/2/2017	YYYY	437	Z8785	30	359	15	13	86.67%			#DIV/0!						
3/7/2017	AAAA	290	Z8785	30	359	15	11	73.33%				12.20	11.10	9.10	2.47		
7/9/2017	YYYY	504	Z8786	30	359	15	15	100.00%			#DIV/0!						
6/18/2017	YYYY	288	Z8792	30	359	17	14	82.35%			#DIV/0!						
6/25/2017	YYYY	362	Z8793	30	359	14	13	92.86%			#DIV/0!						

Boar ID	Total # Matings	# Farrowed	Farrow Rate	# Matings	# Farr.	Breed Week	Farm	# Matings	# Farr.	Breed Week	Farm	# Matings	# Farr.	Breed Week	Farm	# Matings	# Farr.	Breed Week
8082	**52**	**36**	**69.2%**	12	10	1138	B	11	8	1140	A	10	6	1142	A	10	7	1144
8083	**50**	**38**	**76.0%**	25	22	1208	B	6	3	1208	A	10	6	1209	A	9	7	1210
8087	**54**	**44**	**81.5%**	16	12	1151	B	8	8	1150	A	15	13	1202	B	15	11	1203
8090	**51**	**48**	**94.1%**	7	7	1148	B	5	5	1148	A	15	15	1149	A	14	13	1150
8091	**47**	**42**	**89.4%**	17	15	1207	B	1	1	1207	C	14	13	1208	A	15	13	1208
8093	**54**	**45**	**83.3%**	7	6	1207	A	10	8	1150	B	15	13	1202	A	12	10	1203
8094	**54**	**37**	**68.5%**	14	11	1204	B	15	9	1203	B	15	11	1206	D	10	6	1207
8101	**53**	**47**	**88.7%**	10	9	1140	B	15	14	1141	A	15	13	1142	B	13	11	1144
8102	**50**	**49**	**98.0%**	9	9	1210	A	9	9	1145	A	17	17	1206	B	15	14	1208
8103	**48**	**34**	**70.8%**	7	5	1152	A	1	1	1133	A	15	11	1149	D	10	7	1138
8104	**52**	**44**	**84.6%**	9	7	1211	A	8	8	1151	A	15	13	1150	E	10	8	1148
8107	**50**	**45**	**90.0%**	19	17	1134	B	1	1	1135	A	15	14	1136	D	15	13	1137
8108	**51**	**45**	**88.2%**	16	14	1139	B	19	17	1140	B	16	14	1141	E			
8112	**50**	**16**	**32.0%**	20	2	1136	B	15	6	1137	E	15	8	1139	C			
8114	**65**	**52**	**80.0%**	3	3	1206	A	51	49	1134	B	5	0	1142	A	6	0	1206
8116	**50**	**46**	**92.0%**	10	10	1146	A	15	14	1147	E	10	9	1148	D	15	13	1149
8120	**50**	**44**	**88.0%**	15	13	1204	B	17	16	1205	B	8	7	1204	A	10	8	1206
8121	**48**	**44**	**91.7%**	18	16	1210	B	15	13	1135	B	15	15	1211				
8127	**55**	**29**	**52.7%**	15	8	1132	B	15	7	1133	D	15	9	1135	A	10	5	1136
8129	**52**	**47**	**90.4%**	17	16	1208	B	15	13	1209	A	10	9	1210	A	10	9	1211
8130	**53**	**41**	**77.4%**	22	17	1212	B	16	13	1210	A	15	11	1211	A			
8131	**51**	**44**	**86.3%**	16	14	1132	B	20	17	1134	B	3	3	1135	A	12	10	1138
8134	**70**	**60**	**85.7%**	19	17	1146	B	16	13	1148	B	15	13	1203	B	20	17	1209
8139	**48**	**46**	**95.8%**	18	18	1210	B	15	15	1211	D	15	13	1212	C			
8141	**53**	**37**	**69.8%**	16	10	1205	B	15	11	1206	D	12	9	1208	C	10	7	1209
8152	**52**	**44**	**84.6%**	4	4	1148	B	11	9	1149	B	7	6	1152	A	15	13	1202
8154	**48**	**35**	**72.9%**	8	0	1202	A	10	10	1203	B	15	13	1205	B	15	12	1206
8155	**47**	**38**	**80.9%**	30	27	1211	B	6	5	1211	A	11	6	1202	B			
8240	**48**	**42**	**87.5%**	19	16	1137	B	15	14	1138	D	14	12	1139	A			
8242	**55**	**49**	**89.1%**	15	14	1136	B	15	13	1137	C	15	14	1138	C	10	8	1139
Ave.	**1,561**	**1,268**	**81.2%**															
Good	**1,098**	**965**	**87.9%**															
Bad	**463**	**303**	**65.4%**															

Farm	Total # Born	# Pigs/ Litter	# Ruptures	# Ruptures/ Litter	% Ruptures/ Total # Born	# Matings	# Farr.	Week	Farm	Cull Reason	Cull Date	10 Day Motility
B	**367**	**10.19**	**1**	0.03	0.27%	9	5	1145	B	**SQ**	3/30/12	
A	**506**	**13.32**	**0**	0.00	0.00%							
A	**468**	**10.64**	**0**	0.00	0.00%					**SQ**	6/2/12	
B	**641**	**13.35**	**0**	0.00	0.00%	10	8	1151	B			
C	**567**	**13.50**	**1**	0.02	0.18%							
C	**566**	**12.58**	**2**	0.04	0.35%	10	8	1205	D			
B	**401**	**10.84**	**0**	0.00	0.00%					**SQ**	7/7/12	
D	**623**	**13.26**	**0**	0.00	0.00%							
D	**691**	**14.10**	**2**	0.04	0.29%							
E	**361**	**10.62**	**1**	0.03	0.28%	15	10	1139	A	**SQ**	6/2/12	
D	**536**	**12.18**	**0**	0.00	0.00%	10	8	1149	C			
A	**631**	**14.02**	**0**	0.00	0.00%					**SQ**	3/30/12	
	583	**12.96**	**4**	0.09	0.69%					**SQ**	3/30/12	
	129	**8.06**	**0**	0.00	0.00%					**Low Fertility**	3/30/12	
A	**679**	**13.06**	**5**	0.10	0.74%							
E	**647**	**14.07**	**2**	0.04	0.31%							
E	**574**	**13.05**	**2**	0.05	0.35%							
	629	**14.30**	**1**	0.02	0.16%							
B	**209**	**7.21**	**2**	0.07	0.96%					**Low Fertility**	3/30/12	
D	**578**	**12.30**	**0**	0.00	0.00%							
	485	**11.83**	**0**	0.00	0.00%							
C	**606**	**13.77**	**2**	0.05	0.33%							
B	**816**	**13.60**	**1**	0.02	0.12%							
	681	**14.80**	**0**	0.00	0.00%							
B	**407**	**11.00**	**0**	0.00	0.00%					**Low Fertility**	7/7/12	
D	**538**	**12.23**	**2**	0.05	0.37%	15	12	1203	A			
C	**438**	**12.51**	**10**	0.29	2.28%					**Ruptures**	7/7/12	
	448	**11.79**	**2**	0.05	0.45%							
	539	**12.83**	**0**	0.00	0.00%							
A	**638**	**13.02**	**1**	0.02	0.16%							
	15,982	**12.60**	**41**	**0.03**								
	12,679	**13.14**										
	3,303	**10.90**										

BSMC VI August 4th-5th, 2021, St. Louis, MO

Application of preserved boar semen for artificial insemination: Past, present and future challenges

Dagmar Waberski, University of Veterinary Medicine Hannover, Germany

Starting from the seventies in the last century, swine AI became highly efficient with increasing knowledge of reproductive physiology, development of sophisticated AI techniques, semen extenders, establishment of transport logistic and lab automation. All together this has resulted in high fertility with less than 2 billion sperm per dose preserved up to seven days in long-term extenders. Doubtless, the tight conjunction between research, AI industry and breeding organization made swine AI a success story. Contrary to earlier expectations, frozen semen has not yet found entry into breeding routine, probably due to a still lower efficiency and higher demands on transportation and storage on farm. A fast and sperm-safe sexing technology, together with an easy performable and reliable insemination technique, would revolutionize swine breeding but is yet to come.

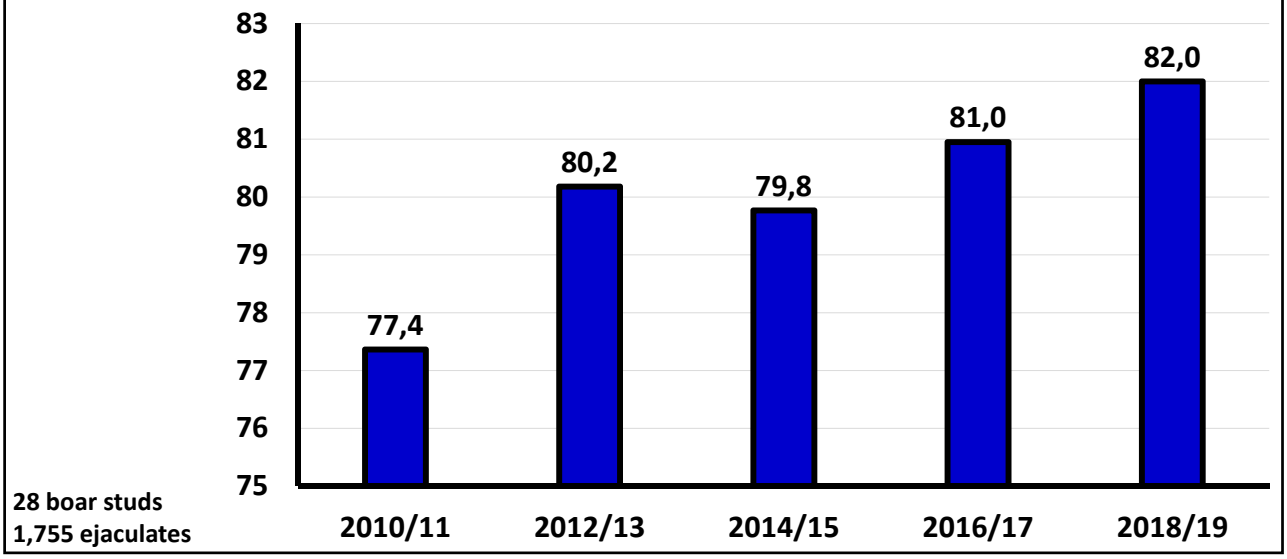
Meanwhile new challenges for swine breeding have arisen alongside with climate changes and a society increasingly demanding consideration of health, welfare and environmental impact of animal production. Additionally, non-animal ("clean") meat production has launched and is foreseen to compete with conventionally produced meat. Under this influence, and with recognition of the breeder's responsibility in their part of the food production chain, the sustainability of swine AI gains increasing importance. Hence, the transition of swine AI towards bioeconomy is needed to maintain competitiveness while meeting environmental and societal challenges.

Semen gets stressed after collection too

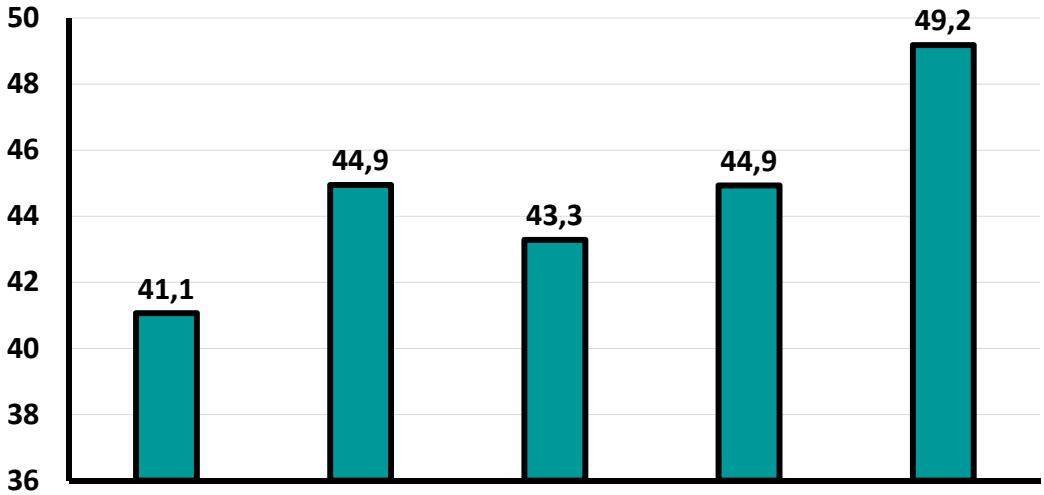
August 2021

Martin Schulze

Day 3 Total Sperm Motility (%)

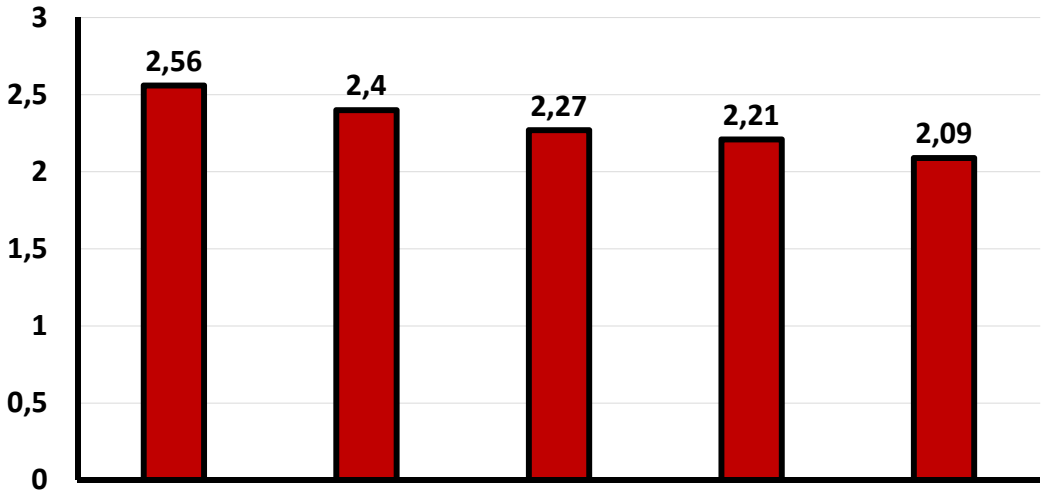


Day 7 Thermo-Resistance (%)



28 boar studs
1,755 ejaculates

Total Sperm Number per Dose (bn)



28 boar studs
1,755 ejaculates

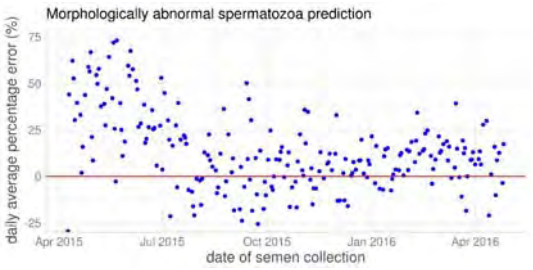
A look back…

Response variable	Input variables	Type III SS	Coefficient	SE	F-value	*P*-value
Thermo-resistance	Intercept		-15.53	14.78	-1.05	0.290
	Boar stud	69157			8.76	<0.001
	Month	12753			4.85	<0.001
	Dilution steps	2595			3.95	0.020
	Bacterial contamination, CFU/mL	1625			4.94	0.027
	Year	6146	2.16	0.50	18.69	<0.001
	Morphologically intact sperm, %	763	-0.11	0.07	2.32	0.128
	Mitochondrially active sperm, %	1959	0.30	0.12	5.96	0.015
	Membrane-intact sperm, %	6578	0.52	0.12	20.01	<0.001
	Arrival temperature at IFN, °C	1692	-1.21	0.54	5.15	0.024
	Electrical conductivity, µS/cm	6510	-0.17	0.04	19.8	<0.001
	Boar age, months	2158	0.12	0.05	6.56	0.011
	Ejaculate volume, mL	1556	-0.03	0.01	4.73	0.030
	Sperm concentration, 10^9/mL	1677	23.58	10.44	5.10	0.024
	Sperm output, 10^9	3877	0.20	0.06	11.79	<0.001
	Dose/ejaculate	4134	-0.29	0.08	12.57	<0.001

Adj. R^2 ≈ 0.4
ANCOVA Analysis

Schulze *et al.* 2019
Theriogenology 127:15-20

A look into the future…

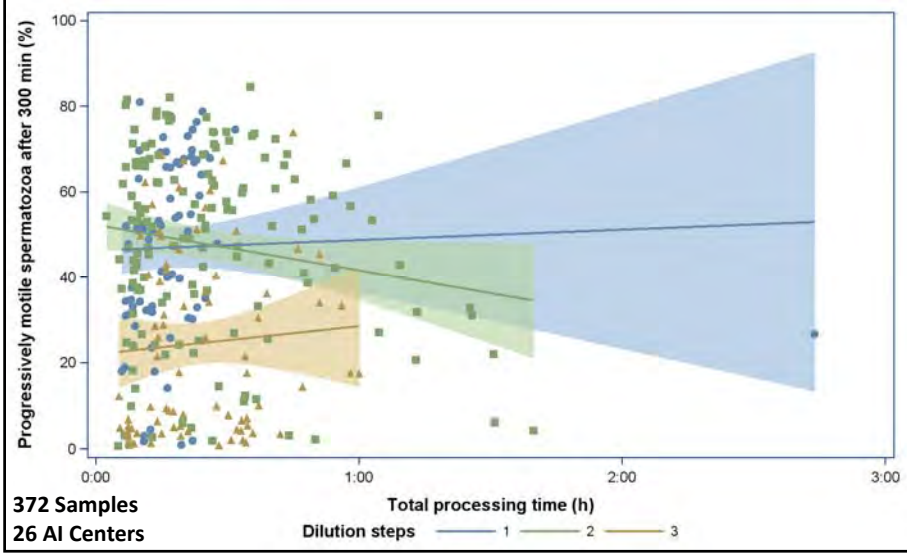


	MAPE (%)
Motility	4.4
Output	23.9
Morphology	44.7

n=7,455 ejaculates
n=637,875 equations

Kuhlgatz *et al.* 2019
Theriogenology 134:129-140

Impact of Total Processing Time - TPT



372 Samples
26 AI Centers

Dilution	TPT (min)
One-step	16.3 ± 14.5
Two-step	26.0 ± 20.4
Three-step	23.9 ± 13.3

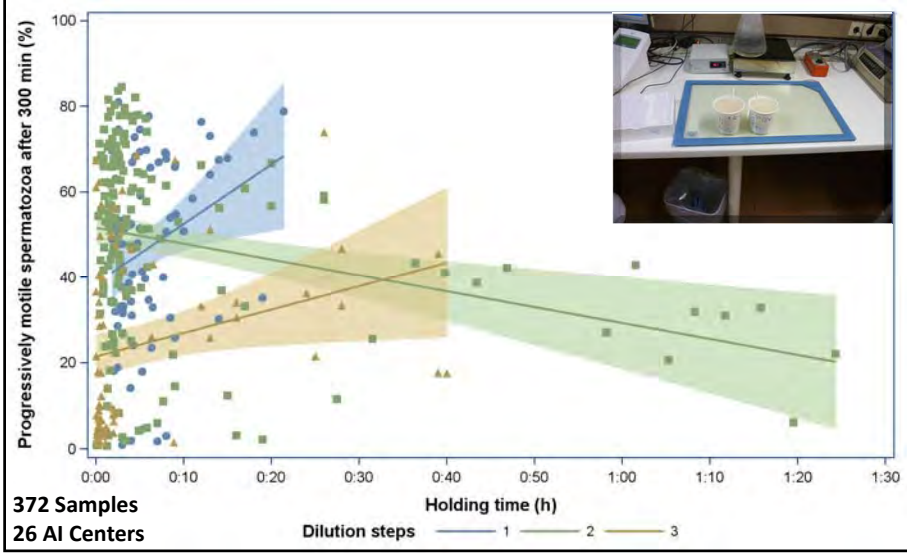
Main dilution

Ø 7 min

Dose filling

Schulze *et al.* 2018
Anim Rep Sci 190: pp 94-101

Impact of Holding Time - HT

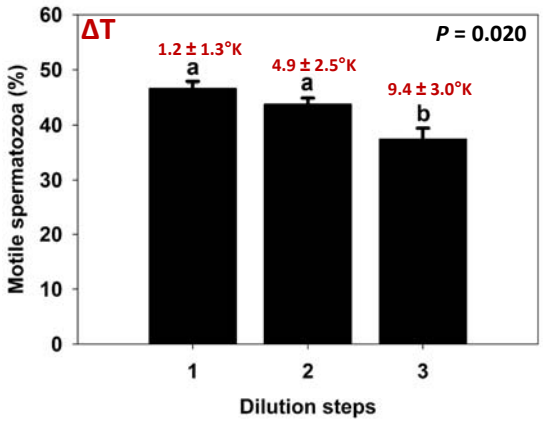


372 Samples
26 AI Centers

Dilution	HT (min)
One-step	5.31 ± 3.53
Two-step	8.03 ± 10.19
Three-step	8.15 ± 6.78

Schulze *et al.* 2018
Anim Rep Sci 190: pp 94-101

Impact of Dilution Management

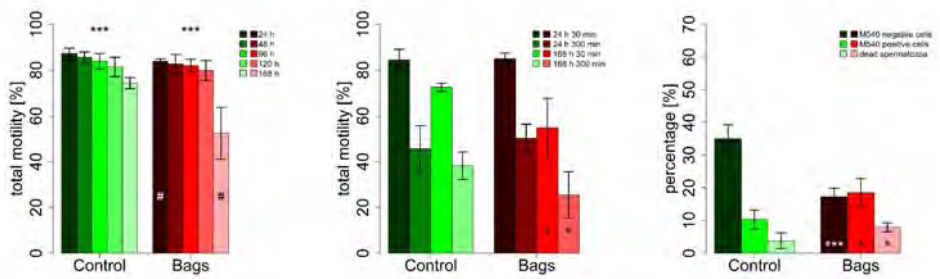


	1. Ejaculate	1. Extender
Boar studs	75%	25%
Time Management	-	+
Foam Formation	- -	+ +
Hygiene	- - -	+ + +
Homogeneity	- -	+ +
Sperm Quality	+	+

Schulze *et al.* 2019
Theriogenology 127:15-20

Schulze *et al.* 2017
Anim Rep Sci 182:138-145

Impact of the Material - BADGE



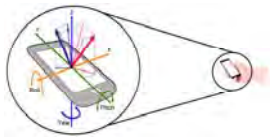
Period	Breeded sows (n)	Farms (n)	Farrowing rate (%)	Total born alive (n)	Total born/sow (n)	Born alive/sow (n)	Abortions (%)
Before	2,412	4	92.6 ± 2.3	32,746	16.3 ± 0.2	14.6 ± 0.2	0.2 ± 0.2
During	2,627	4	63.7 ± 11.1	16,991	10.8 ± 1.9	9.9 ± 1.7	0.04 ± 0.1
After	2,737	4	89.7 ± 0.9	35,934	16.0 ± 0.2	14.6 ± 0.2	0.2 ± 0.3
P-Value	n.a.	n.a.	*<0.0001*	n.a.	*<0.0001*	*<0.0001*	0.44

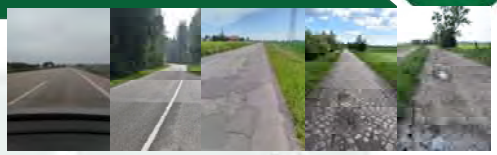
Values are represented as mean ± SD. *P* values: ANOVA/Fisher LSD Test.

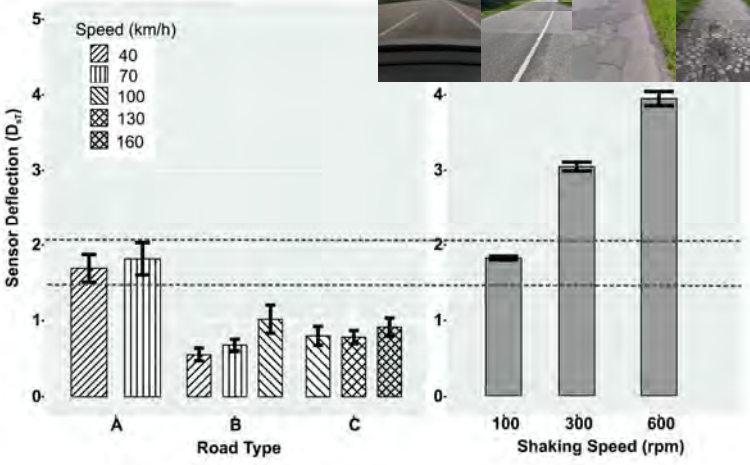
BADGE = 0.37 ± 0.05 mg/L sample
GC-MS & UPLC-QTOF-MS
Institute for Food & Environmental Research, Potsdam, Germany

Schulze *et al.* 2020
Sci Rep 10:22258

Impact of Semen Transport



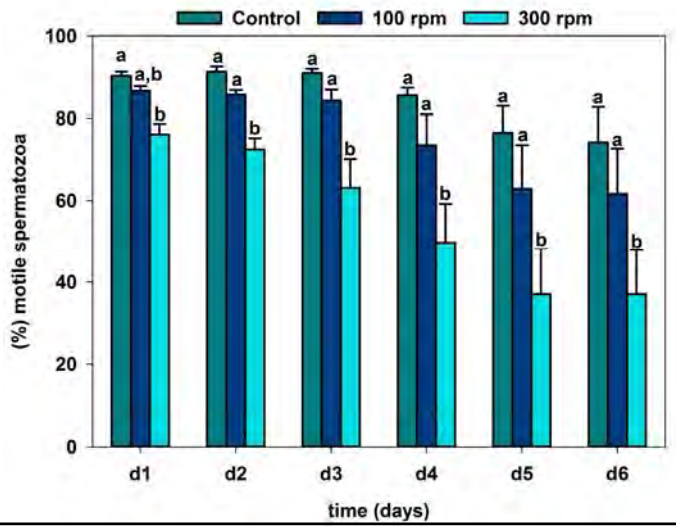




Schulze *et al.* 2018
Anim Rep Sci 190:109-115

Impact of Semen Transport

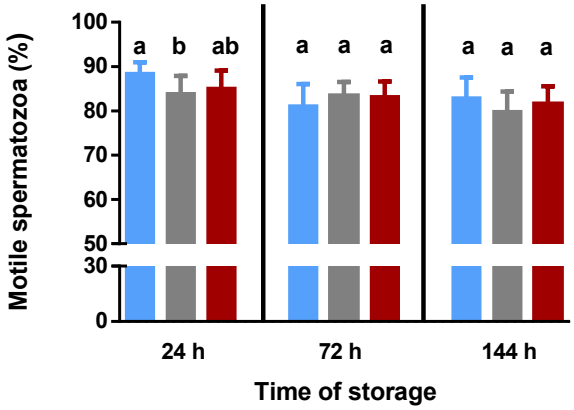
n = 20
ANOVA
$^{ab}P < 0.05$
6 h stress
16°C BTS Minitüb
1.8 × 10^9 spermatozoa/dose
85 ml QuickTip Flexitubes®



Schulze *et al.* 2018
Anim Rep Sci 190:109-115

Impact of Semen Transport

Federal Ministry of Food and Agriculture



n = 11
ANOVA
$^{ab}P > 0.05$
4 h stress 200 rmp
5°C in Androstar® Premium
1.8×10^9 spermatozoa/dose
85 ml QuickTip Flexitubes®

Paschoal *et al.* 2019
Reprod Dom Anim 54 (Suppl. 1): p17

Take Home Message

1. 40% of the total variability in boar sperm quality can be explained by production management factors.

2. We are able to predict boar sperm quality.

3. Vibration emissions during semen transport can affect sperm quality.

www.ifn-schoenow.de

Secure Pork Supply Plan
Needed for boar studs too???

Pamela Zaabel, DVM, and Patrick Webb, DVM
National Pork Board

Introduction

Foot and mouth disease (FMD), classical swine fever (CSF), and African swine fever (ASF) are highly contagious trade-limiting foreign animal diseases (FADs) present in many countries, causing severe animal production losses. Animal agriculture in the U.S. is highly vulnerable to FADs due to a variety of factors which include U.S. production animals have no immunity to FADs; export markets will be lost and prices will drop dramatically; emergency vaccine stocks are far below what would be required to address a livestock dense state or multi-state outbreak; and the size, structure, efficiency and extensive movement inherent in the U.S. livestock industries will present unprecedented challenges in the event of an FAD outbreak. However, FMD, CSF, and ASF are not public health or food safety concerns.

The USDA APHIS Veterinary Services developed Foreign Animal Disease Preparedness and Response Plans. The FMD and ASF Response Plans call for the implementation of an initial 72- hour nationwide standstill order for susceptible animals and germplasm at the beginning of an FAD outbreak, and establishment of disease Control Areas and network-based controls for contact premises. The response goal would be for rapid contact tracing and identification of infected animals, strategic depopulation with proper disposal for confirmed positive sites, and vaccination if available. Total duration of the national standstill may vary depending on the circumstances of the outbreak.

The confirmation of a trade-limiting foreign animal disease in the U.S. swine herd would result in disease control measures that could negatively impact semen distribution. The duration of negative effects could be extensive, and resolution will be dependent on the industry's ability to quickly communicate the right data and information to satisfy a competent veterinary authority's criteria for differentiating disease-free populations by region, state, or production site(s). These criteria may change over time or by state, so industry will need the ability to quickly communicate compliance to authorities if regulations change.

Control Area Effects on Semen Movement

At the state level, after the confirmation of an FAD outbreak, the state animal health officials will work to identify a regulatory Control Area and begin designating premises as infected, contact, suspect and at-risk or monitored. Early in an outbreak if a boar stud is designated as an infected premises then the most likely outcome would be depopulation. If a boar stud is epidemiologically linked to an infected premises it would be designated as a contact premises (regardless of being in the Control Area or not) and would be subject to disease control measures and testing. Studs located in a Control Area that report clinical signs consistent with the FAD would be designated as a suspect premises and tested. Boar studs located in a Control Area that do not have animals with case-compatible signs and are not designated as infected, contact or suspect are designated as at-risk premises and may request permission to move animals or semen. At-risk premises are eligible to become a monitored premises after they can objectively demonstrate they are not infected, contact, or suspect and meet a set of defined criteria in seeking to move susceptible animals or products out of the Control Area by permit.

Early in an outbreak, non-infected boar studs located in Control Areas will be extremely limited in their ability to move semen, which will negatively affect the availability of semen, effectively limiting supply. Duration of this status could be extensive due to the time it takes for authorities to figure out all the

unknowns before they are comfortable with permitting semen shipments. As the outbreak evolves and the stud moves to a monitored status the ability to move semen may improve depending on the scope of the outbreak and the overall demand. Outside of Control Areas there may also be new rules and regulations put in place that restrict or are additive to current regulations for shipping semen that increase costs and affect supply. Unless the industry is very lucky, and the FAD is quickly contained and eradicated there will be negative impacts due to all the unknowns of an FAD outbreak on semen supply. However, there are steps the industry can take today to help provide needed clarity to authorities to facilitate business continuity through the pork chain.

Secure Pork Supply Plan Overview

The goal of the Secure Pork Supply (SPS) Plan is to provide a workable business continuity plan for pork premises with no evidence of the FAD infection located in a regulatory Control Area and allied industries that is credible to Responsible Regulatory Officials. Continuity of business (COB) for the swine industry revolves around the ability to move animals located within a Control Area to market and between production premises. Officials must balance the risk benefit of allowing movement of animals to market and between production premises against the risk benefit of not allowing movement.

Movement restrictions will be put in place for the regulatory Control Area(s) to limit risk of disease spread by animals, animal products, visitors, vehicles, and other equipment. Movement will be by permit only which will be issued based on the risk posed by movement of that item and the site's ability to meet permit requirements. Production sites that follow the guidance in the SPS Plan will be better prepared to request a movement permit when Officials are ready to allow permitted movements.

Participation is voluntary. Development of the SPS Plan was led by the Center for Food Security and Public Health in collaboration with industry, state, federal, and academic representatives. Funding for its development was provided by USDA APHIS and the National Pork Board. The SPS Plan provides guidance only. In an actual outbreak, decisions will need to be made by the Responsible Regulatory Officials and the industry based on the unique characteristics of the outbreak.

SPS Components

Biosecurity

During an FAD outbreak, it is the boar stud's responsibility to keep their animals from becoming infected, focusing on what they can control on their site. To facilitate business continuity (movement), boar studs will need to provide assurances to the animal health officials that they are not contributing to the spread of disease nor putting their own animals at risk of exposure. Some movements carry more risk than others. An enhanced biosecurity plan increases individual preparedness to maintain COB in the face of an FAD outbreak. Resources to enhance biosecurity implemented on a production site are available at www.securepork.org. Existing biosecurity plans for pork production sites may offer protection against endemic diseases but heightened precautions are needed for FADs.

Surveillance

Surveillance includes both observations as well as diagnostic testing. Animal caretakers should be able to recognize abnormal findings (clinical signs and/or changes in production parameters) associated with FMD, CSF, and ASF, and be able to document that there is no evidence of an FAD virus infection. Educational materials are available at www.securepork.org.

Negative surveillance test results are also needed. The samples will need to be collected by a government official, veterinarian, or certified swine sample collector. The program standards and resources for the certified swine sample collection training program are available at www.securepork.org. Having

designated individuals on the site trained and ready to collect and submit samples will enable the premises to start surveillance sampling at the direction of Responsible Regulatory Officials at the onset of an outbreak or if the outbreak outstrips available response resources. Diagnostic tests to be performed and sampling protocols may evolve throughout the outbreak based on new knowledge and technology.

Traceability

Boar studs need to request a National Premises Identification Number (PremID or PIN) from the office of the State Animal Health Official if they do not have one for the site. For sites that have PIN's the company should make sure the State has the most current information regarding the site to keep the State premises repository up to date on the data elements associated with the PIN in their records. Having a PIN facilitates requesting movement permits during an outbreak. A PIN includes a valid 911 address and a set of matching coordinates (latitude and longitude) reflecting the actual location of the animals on the premises.

Premises in a regulatory Control Area will be required to provide epidemiological information at the beginning of an outbreak to identify potential exposure to the disease. Maintaining accurate records of movement of animals, feed, supplies, equipment, personnel, and visitors enables producers to provide accurate trace-back epidemiological information. Maintaining electronic records is preferred.

AgView is one tool provided by NPB to assist with traceability. AgView provides a mechanism for boar studs to share premises attributes that are needed by Responsible Regulatory Officials to facilitate the movement of semen from studs located in regulatory Control Areas. AgView can also serve a similar purpose if there are new health requirements from animal health officials for boar studs located outside of a disease Control Areas. AgView could also serve as a database of record for any industry-driven programs related to verifying site-specific health status or compartments prior to an outbreak of African swine fever or other foreign animal diseases of swine.

Conclusion

The SPS Plan provides tools for boar studs to help prepare prior to an FAD outbreak. While the Plan will always be evolving as new information becomes available; it will be a valuable document to facilitate decision making to maintain business continuity when an FAD outbreak occurs. Resources are available at www.securepork.org, and www.AgView.com.

References

- Information on the Secure Pork Supply Plans is available at www.securepork.org.
- Information on AgView is available at www.AgView.com.
- USDA APHIS ASF Response Plan: The Red Book. Available at https://www.aphis.usda.gov/animal_health/emergency_management/downloads/asf-responseplan.pdf
- USDA APHIS FMD Response Plan: The Red Book. Available at www.aphis.usda.gov/animal_health/emergency_management/downloads/fmd_responseplan.pdf
- USDA APHIS CSF Response Plan: The Red Book. Available at https://www.aphis.usda.gov/animal_health/emergency_management/downloads/csf_responseplan.pdf

Comparing semen extension in hypothermic vs isothermic conditions

Darwin L. Reicks, BS, DVM

Introduction

A focus for boar studs laboratories has been to keep the semen and extender within 1 degree Celsius through out processing and cool the final product after final extension in a controlled manner. Support for these processes was from studies showing that a rapid decrease of temperature could cause a thermotropic phase transition[1], and that separation of lipid phases causes alterations of membrane proteins which impairs viability.[2] Various methods of using a two-stage extension, with the last portion being hypothermic, have showed no significant difference in various laboratory assessments of the sperm [3,4,5,6]. However, one study showed a negative effect on motility and sperm membrane integrity.[7] The negative effect was less with a long-term extender, AndroStar Plus, when compared to a BTS. A recent study evaluated both the laboratory assessments as well as fertility of sows inseminated with semen processed by hypothermic vs. isothermic conditions, showing no differences.[8] In this study, semen was diluted isothermically (32-34 C) at 1:2 and then held for 15 minutes prior to a final extension at 21-25 C.

The purpose of this study was to repeat the work of Soler-Llorens, but to pool the pre-extended ejaculates together, and then extending the entire pool with 22-23 C extender. This process would provide significant advantages in efficiency in that the final extension process would only be needed one time per pool of semen. The other advantages of quicker cooling time and reduced utility costs also would be significant.

Materials and Methods

Boar Stud

Twenty-eight Landrace boars out of a 300-boar stud were utilized for the study. Extender was mixed first thing in the morning, then half of the extender was allowed to cool to 22 C (21-23 C). The other half of the extender was held at 35 C. On 2/3 of the production days, Vitasem was used and Nutrixcell Plus was used on 1/3 of the production days. Each ejaculate was pre-extended at 35 C within 1 minute of entering the lab to achieve a concentration of 0.2-0.4 sperm per ml. Motility and morphology evaluations were done with a minimum of 70% normal acceptable for each individual ejaculate and >70% normal acrosomes. Following the last individual boar designated to a pool, 5-6 boars were pooled together, and the pool held at 35 C for 15 minutes.

After the 15 minutes of equilibration time, the pool was stirred, and then divided in half. Half of the pool was extended with 35 C extender and the other half with 22 C extender. Following full extension, doses were dispensed into IMV cochette bags, either full (80 ml) or PCAI (60 ml) doses, and cooling completed to <18 C prior to shipment.

Sow Farms

2 Sow farms (2500 and 4000 sows) received the semen and were blinded as to treatment group. All sows were purebred Large White genetics. The 35 C extended semen was bred to even numbered sows and the 22 C extended semen was bred to odd numbered sows. Semen was used day 1-4 after collection.

Results and Discussion

Semen Temperatures

The average temperature after pooling was 33.2 (+-1.1). After final extension with 35 C extender, the temperature average was 34.6 (+-1.1). For the pools extended with 22 C, the temperature average was 23.3 (+-0.7). The time to reach a final temperature <18 (at which semen was packaged) was 107 minutes for the 35 C, and 77 minutes for the 22 C. A difference of 30 minutes. This resulted in a cooling rate of 7-14 degrees per hour for the 35 C and 3-7 degrees for the 22 C.

Total Born

There was a significant difference in total born (P<0.01) and born alive (P<0.01) between the groups in favor of the 22 C group. The difference was consistent on both sow farms.

Group	# Farrow	Ave Total Born	Ave Born Live	Sow Farm 1 TB	Sow Farm 2 TB
22 C	390	15.47	13.64	15.18	16.07
35 C	393	14.92	13.19	14.76	15.25
Total	783	15.20	13.82	14.97	15.66

Farrow Rate

There was no difference in farrow rate (0.11) or conception rate between the groups.

Group	# Bred	Farrow Rate	Conception Rate	Sow Farm 1 FR	Sow Farm 2 FR
22 C	463	84.2	87.5	82.3	88.4
35 C	476	82.6	88.0	83.7	80.4
Total	939	83.4	87.7	83.0	84.2

Motility

There was no difference in CASA motility between the groups. P=0.39

	# pools 35 C	# pools 22 C	Motility 35 C	Motility 22 C
Regular	8	8	80.9	84.4
PCAI	9	9	84.2	83.1
Total	17	17	82.6	83.7

Acrosomes

There was no difference in Acrosomal ridge integrity. P=0.38

Acrosomes	35 C extended	22 C extended
Regular	93.7	95.7
PCAI	96.2	96.6
Total	95.2	95.8

Morphology

There was no difference in CASA automated morphology (P=.40), or in microscopic morphology(P=.33) between the groups.

Sperm counts

The total sperm (P=0.45) and normal sperm counts (P=0.42) were the same between the groups.

Bacteria

There was no bacterial growth on aerobic culture media for any of the pools.

Sow Farm variables

There was no difference in parity, gestation length, or matings per service, service periods per parity, or 35-day conception rates between the groups.

Summary

There was no difference in semen analysis parameters between the groups. There was no difference in variables of data from the sow farm, except for a statistical difference in total born and born alive. The differences in total born and born alive were in favor of the hypothermically extended pools and the difference was consistent with both sow farms involved. Under the conditions of this study, hypothermic extension provided higher total born than the traditional isothermic extension method.

References

1. Parks JE, Lynch DV. Lipid composition and thermotropic phase behavior of boar, bull, stallion, and rooster sperm membranes. Cryobiology. (1992). 29(2), 255-25 https://doi.org /10.1016/0011-2240(92)90024-V.
2. Drobnis E Z, Crowe LM, et. Al Cold shock damage is due to lipid phase transitions in cell membranes: A demonstration using sperm as a model. J. Experimental Zoology. (1993). 265(4), 432-437. https//doi.org/10.1002/jez.1402650413.
3. Lopez Rodriguez A, Fresh boar semen: Quality control and production. (2012) Merelbeke, Belgium: Ghent Univer. https://biblio.ugent.be/publication/3006176.
4. Lopez Rodriguez A, Rijsselaere T, et al. Effect of dilution temperature on boar semen quality. Rep. in Domestic Animals. (2012). 47(5), e63-e66. https://doi.org/10.1111/j.1439-0531.2011.01938.x.
5. Petrunkina AM, Volker G, et al. Detection of cooling-induced membrane changes in the response of boar sperm to capacitating conditions. Theriogenology (2005). 63(8), 2278-2299. https://doi.org/10.1016/j.theriogenology.2004.10.008.
6. Schmid S, Henning H, et al. The specific response to capacitating stimuli is a sensitive indicator of chilling injury in hypothermically stored boar spermatozoa. Andrology (2013). 1(3), 376=386. https://doi.org/10.1111/j.2047-2927.2013.00045.x.
7. Schulz M, Henning H, et al. Temperature management during semen processing: Impact on boar sperm quality under laboratory and field conditions. Theriogenology (2013), 80(9), 990=998. https://doi.org/10.1016/j.theriogenology.2013.07.026.
8. Soler-Llorens, Mendoza N, et al. Insemination of sows with seminal doses prepared by a two-step hypothermic dilution does not impair the reproductive performance at farm. Reprod. Dom. Anim. (2020). 00:1-8. DOI:10.1111.rda.13763.

Managing feet and legs in the stud

Mark Wilson, Mike Parsley and Tom Gall

FeedworksUSA, Cincinnati, Ohio 45243

Introduction:

Lameness is associated with aberrant behavior and manifests itself in the animal by a change in gate. This may not only be displayed in the legs, but also includes swinging the head or hips to assist motion and to relieve weight off a sore limb. The willingness, or lack of willingness to stand or walk would classify an animal as lame. An animal that has pain from any mechanical function or shows inhibition to any physical activity would also be classified as lame.

Claw lesions or injury to the claw can cause lameness. From surveys around the globe the per cent of sows or boars with claw lesions might be as high as 80% or greater. A claw lesion does not necessarily cause lameness unless there is bleeding and/or a wound is deep enough through the outer horn tissue and into the corium tissue which may become inflammatory due to bacteria in the injury. A producer must make careful observations each day of behaviors of boars in order to notice small changes towards lameness. Most surveys agree that greater than 80% of the boars have a few small lesions, but only about 30% of the boars are lame due to claw lesions. Lameness is hard to treat because blood flow is quite poor in the claw, except for the heel. The heel will have 10 times as much blood flow and nerve endings compared to the outer horn tissue and horn tissue of the sole. Use of antibiotics or topical sprays to treat a claw lesions often is not very successful. The sooner you can identify a problem, the more likely it will be that you can get the claw lesion healed up. Zinpro's scoring for lesions is a 0 1 2 3 with 0 being no lesions, 1 is a mild lesion, 2 a moderate lesion and 3 lesion score should consider the option of culling the boar. If the boar has gotten to level 3, it will likely be quite painful for the animal. If the lameness persists, semen production will almost always decline, the boar will lose body weight, and the boar becomes a likely candidate to be culled from the boar stud.

It is important to have hospital pens in the boar stud so that when lameness issues are caught early, the boar can be in a space that allows for more comfort and quicker healing with rest and assist in reducing the inflammatory signals. Placing a pad or mat in the pen for the boar to walk and stand on may assist in relieving some pain. Some hospital pens have solid floors with straw or other bedding material to create comfort. Boars often prefer to be standing or resting on sand as opposed to laying on slatted flooring. This scenario can be difficult to create in a typical boar stud. The problem is how to sterilize the sand and get it cleaned out of the building each time the pen is used. Boars that pick their leg up often need to be considered for treatment to prevent further issues with lameness. Picking up the injured claw and setting it back down is often called "toe taping". Foot baths are another option that have been used in boar studs. If you are only collecting 1 time per week, the foot bath treatment may not be as effective as collecting multiple times. If the boar travels to the collection pen more than 1 time per week you have better chances of drying tissues and reducing inflammatory process at the lesion. A boar that lays down very quickly after feeding should be watched closely for any lameness issue.

How to best prevent or reduce the incidence of lameness:

Flooring matters. Flooring that has very course gravel protruding from the concrete must be smoothed out. It is important to have dry alley-ways for boars to move to the dummy sows and to return without having any hazards for the boar. Slick flooring and crates where the flooring stays messy with urine and feces can cause the uptake of ammonia into the claws. These claws can hold about 30% more weight due to absorption of water in the outer horn tissue. The softer that tissue is increases the injury potential of the claw, particularly when constantly exposed to water or urine. Floors with holes where a boar may step and injure a foot must be fixed as soon as possible to prevent further injury.

Claw lesions

There are 7 major lesions that have been utilized in a scoring scheme from 0 to 3 for these lesions:

1. Heel over-growth and erosion, 2. heel sole lesion, 3. white line lesion, 4. vertical wall crack, 5. horizontal wall crack, 6. long toes and uneven toes, and 7. dew claw length and injury There is one of other lesion that should at least be noted that is a lot more common in finishing buildings with moderately wet flooring called heel-abaxial horn lesion, which is at the back portion of claw and can be fairly painful.

How do we help reduce claw lesions and lameness:

Build up bone, joints, ligaments, and muscle by implementing a feeding program of inorganic minerals and vitamins. If there are additional stressors such as disease, heat stress, or mycotoxins, for example, it may be wise to feed a portion of the minerals as an organic mineral such as Availa®Zn. This mineral source is important in maximizing the utilization of mineral attached to a single amino acid because they are not interfered with by bacteria, they don't have to use the mineral transport systems as they can pass through the amino acid transport chain to bring the intact molecule of Zn attached to a single amino acid utilizing essential 17 different amino acids. As boars leave the nursery they will build protection to reduce inflammatory responses and improve horn quality due to the Availa®Zn. It can be important on new concrete to increase the amount of Availa-Zn utilized in the diet to reduce the wear and friction to the heels until the new concrete gets smoothed down.

Issues such as mycotoxins and/or heat stress, even though they are attacking the tight junction between the enterocytes, can result in the inflammatory signals travelling throughout the body of the boar and create issues with the claws. Injury can cause issues in the claw if the dew claw gets caught in between the slatted flooring and either breaks the dew claw or tears it off completely at the joint. Often an injury such as this gets bacteria in the wound and the swelling impacts the leg and the animal is lame and often needs to be culled.

Steps to help reduce lameness in the Boar Stud

Boars do not remain in the herd nearly as long as the sow does. If an injury or lameness is quite severe, the best option is to cull that boar as it will take many weeks to get the boar back into condition and then the stud has lost the output of many doses of semen. Here are suggestions on how to help minimize lameness issues in boars:

1. Walk the barn each day to check on any behavior modifications from what the boar used to do.
2. Signs of not eating or laying down quickly after feed has been dropped may be signals that something is wrong with that boar which could possibly be lameness.
3. During times of stress, such as movement into isolation and then later to the boar stud, it is a good idea to use Availa®Zn at higher levels to minimize inflammatory signals, which will help maintain better keratinocyte proliferation. When the boar has inflammatory issues that are severe, that boar should be placed into a hospital pen. Treatment with antibiotics should be done only with veterinarian advice and testing. If a boar has a lesion score severity of 3, the best advice is to cull or euthanize that boar.
4. If any boars get long dew claws on the rear legs, it is a good idea to trim the dew claw to the proper length. Do not trim the dew claw too short. If you cause bleeding it will likely increase inflammatory responses.
5. If a joint injury becomes obvious give the boar time in the hospital pen to recover. If there is no improvement, it may be best to cull that boar. Osteochondrosis is a joint disease that starts at an early age (10-12 weeks of age) that creates areas where bone does not fill in where it should and results in an area of weakness where cartilage was not replaced by bone. Always use the proper size chute or ramp to get the boars loaded safely into or out of the transport trailer.
6. Do not allow the boars to become over conditioned. Body condition score greater than a 4 score out of 5 is unacceptable.
7. Make sure flooring conditions are not slick or wet and the isles are always clean.
8. Do not get boar diets way out of balance for minerals that compete with each other and/or are antagonistic.

A brief history of the implementation of swine AI in the US

August 4-5, 2021
St Louis, MO

Wayne L Singleton
Professor Emeritus
Department of Animal Sciences
Purdue University
West Lafayette, IN
wsinglet@purdue.edu

Midwest Boar Stud Conference

April 13-14, 2000

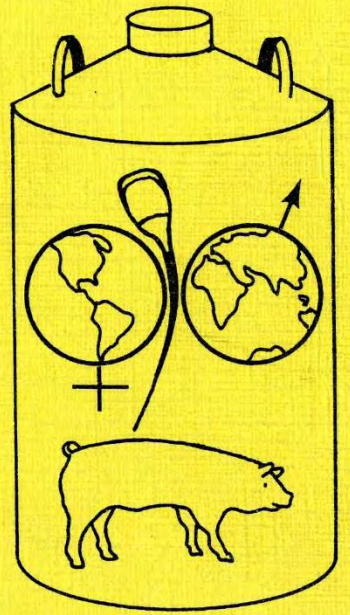
Holiday Inn North
West Lafayette, Indiana

1985

SINGLETON

DEEP FREEZING OF BOAR SEMEN

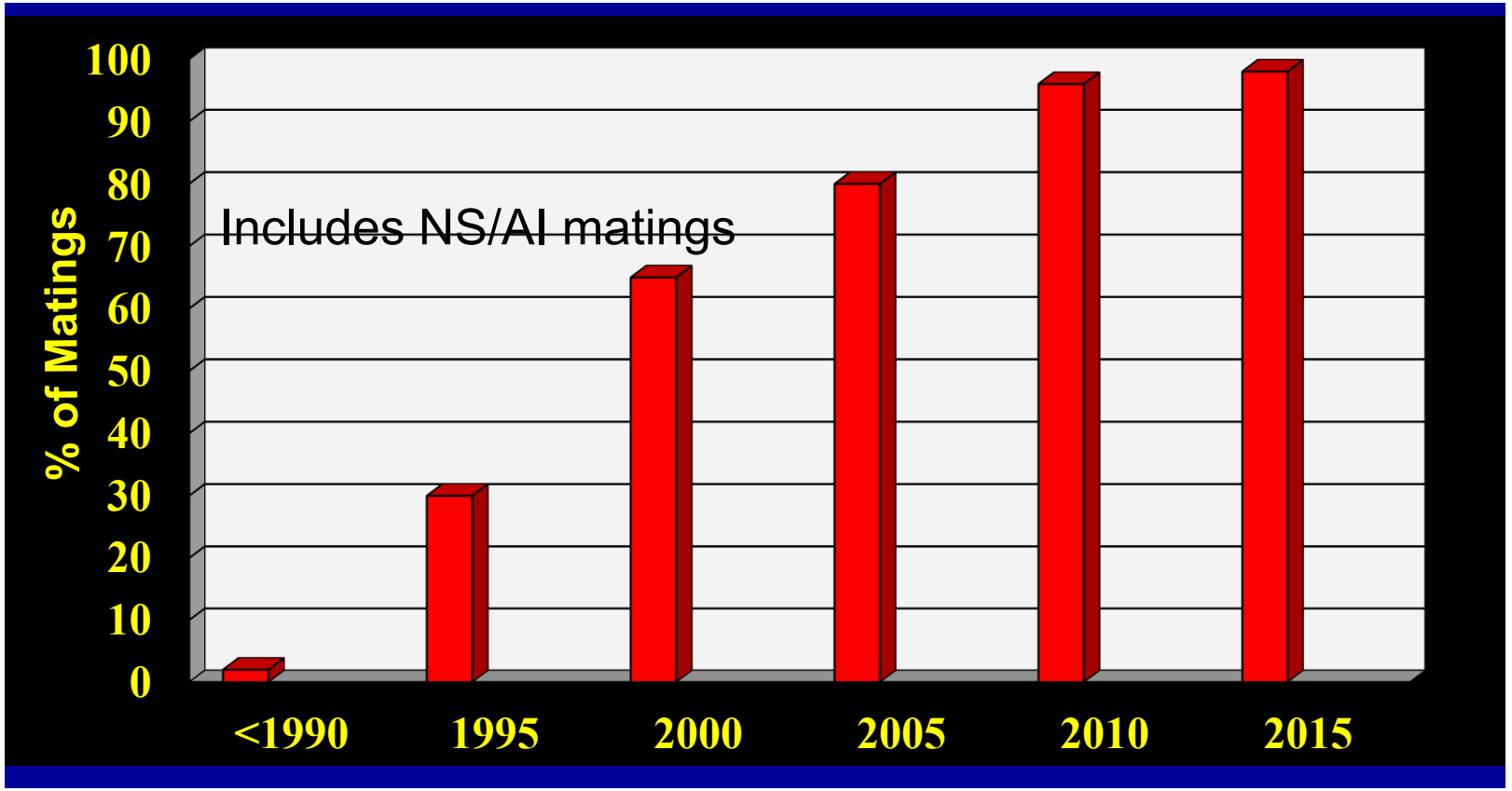
Uppsala, Sweden



SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES

UNITED STATES DEPARTMENT OF AGRICULTURE

Industry Application of AI Technology in the US



Based upon industry estimates

Some Landmark Discoveries and Applications

1677 Anton van Leewenhoek discovered sperm “animalcules”

1780 L. Spallanzani successfully inseminated a dog

1907 Ivanow (Russia) was first to inseminate a pig

1933 Arthur Walton pioneered long distance transport of ram semen from England to Poland

1938 First US bovine cooperative was formed in New Jersey

1946-49 Almquist introduced antibiotics to bull semen (+10% PR)

1949 C. Polge successfully froze bull semen with glycerol at -79 C

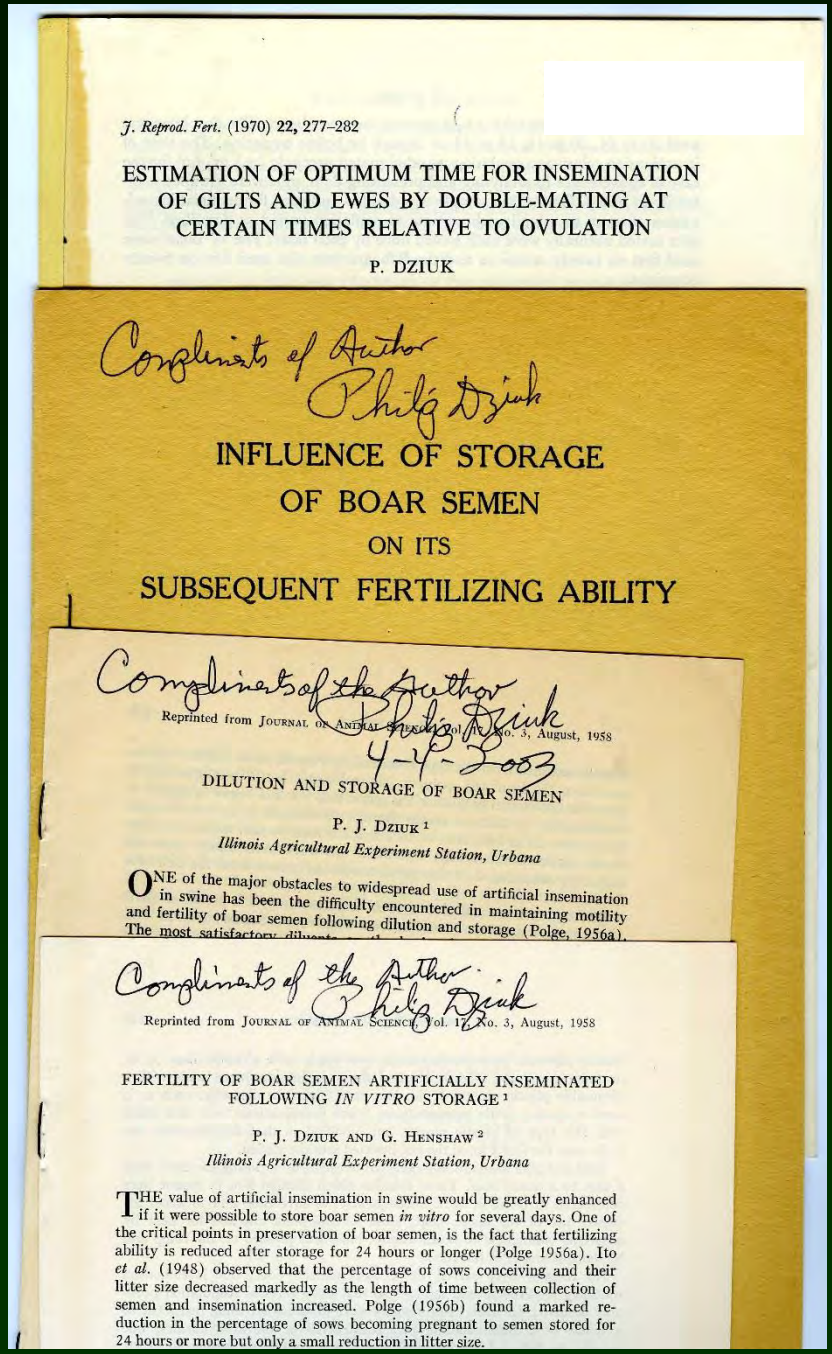
1950’s

- 1950 - Foote and Bratton (Cornell) coined the term “extender”
  - They considered using the word “suspender”
- 1954 – T.Glover and T.Mann, On the composition of boar semen, J. Agricultural Science, 44(3) , pp355-360.

1950-60's

- Series of articles related to dilution and storage of boar semen were published.
  - Dzuik, IL
  - Stratman and Self, WI

  - Herrick and Self published the gloved hand technique for boar semen collection (1962)



J. Reprod. Fert. (1970) **22,** 277–282

ESTIMATION OF OPTIMUM TIME FOR INSEMINATION OF GILTS AND EWES BY DOUBLE-MATING AT CERTAIN TIMES RELATIVE TO OVULATION

P. DZIUK

Compliments of Author Philip Dziuk

INFLUENCE OF STORAGE OF BOAR SEMEN ON ITS SUBSEQUENT FERTILIZING ABILITY

Compliments of the Author Philip Dziuk 4-4-2003

Reprinted from JOURNAL OF ANIMAL SCIENCE, Vol. 17, No. 3, August, 1958

DILUTION AND STORAGE OF BOAR SEMEN

P. J. DZIUK [1]

Illinois Agricultural Experiment Station, Urbana

ONE of the major obstacles to widespread use of artificial insemination in swine has been the difficulty encountered in maintaining motility and fertility of boar semen following dilution and storage (Polge, 1956a). The most satisfactory diluents

Compliments of the Author Philip Dziuk

Reprinted from JOURNAL OF ANIMAL SCIENCE, Vol. 17, No. 3, August, 1958

FERTILITY OF BOAR SEMEN ARTIFICIALLY INSEMINATED FOLLOWING *IN VITRO* STORAGE [1]

P. J. DZIUK AND G. HENSHAW [2]

Illinois Agricultural Experiment Station, Urbana

THE value of artificial insemination in swine would be greatly enhanced if it were possible to store boar semen *in vitro* for several days. One of the critical points in preservation of boar semen, is the fact that fertilizing ability is reduced after storage for 24 hours or longer (Polge 1956a). Ito *et al.* (1948) observed that the percentage of sows conceiving and their litter size decreased markedly as the length of time between collection of semen and insemination increased. Polge (1956b) found a marked reduction in the percentage of sows becoming pregnant to semen stored for 24 hours or more but only a small reduction in litter size.

REPRODUCTIVE EFFICIENCY OF SWINE

Cooperative Extension Service
IOWA STATE UNIVERSITY
Ames, Iowa 50010

ARTIFICIAL INSEMINATION OF SWINE AND ESTRUS SYNCHRONIZATION

Artificial insemination of swine has been undergoing extensive research for the past 20 or 25 years. During that time, techniques for collection of semen, short term storage, and insemination have been developed. Prolonged storage techniques by freezing and accurate and easy estrus detection are two problems that have not been solved.

Several researchers have studied the chemical and physical properties of boar semen. Boars secrete the largest volume of semen of all domestic animals. The average ejaculate is 250 ml in volume and contains over 40 billion sperm. Chemical composition of the semen varies between boars, but these variations are seldom associated with fertility differences between boars.

Boar spermatozoa are sensitive to sudden temperature changes and are easily damaged by cooling too rapidly. Their metabolism is influenced by light, oxygen, carbon dioxide, nitrogen, electrolytes, metabolic substrates, and osmotic pressure of the storage medium. Hence, in handling boar semen extreme caution and attention to detail are important if its fertilizing capacity is to be maintained.

Two of the more commonly used extenders of boar semen are as follows:

I. Glucose	15 grams
Sodium bicarbonate	0.75 grams
Egg yolk	150 milliliters
Distilled water	350 milliliters
Penicillin	500,000 I.U.
Streptomycin	500 mg.

Add one ejaculate of semen to give quantity sufficient for 10 to 15 inseminations.

II. Glucose	13 grams
Sodium citrate	14 grams
Potassium chloride	0.29 grams
Sodium bicarbonate	1.5 grams
Streptomycin	3 grams
Penicillin	3 grams
Distilled water	1000 ml

100 ml egg yolk can be used in place of 100 ml of distilled water.

Add above extender to the semen to give a total volume of 1000 ml. This will be sufficient for 20 inseminations.

A third extender of boar semen is:

Whole homogenized milk	1000 ml
Dihydro-Streptomycin sulfate	1 gram
Penicillin	one million I.U.

Add the above combination to one ejaculate of semen to give a total volume of 1000 ml. This quantity is sufficient for 20 inseminations.

Short term storage of boar semen is permitted with any of the above diluents at a temperature of 7°C. or 45°F. Temperatures below these will not protect the fertilizing capacity. Semen that has been properly processed, gradually cooled and stored for 24 hours will give equally good results as when used fresh. Storage for 48 or 72 hours will reduce the resulting conception rates to about 50 and 30 percent, respectively.

As with natural service, artificial breeding has shown differences in conception rates and litter size between boars. However, conception rates and litter size are not influenced by artificial insemination when insemination is performed at the proper time.

The optimum time for inseminating is as near the middle of the estrus period as possible. This will usually be 12 to 30 hours after first detection of estrus.

The most commonly used volume of extended semen is 50 ml. per insemination, and usually at least 2 billion sperm are used per insemination. The use of smaller volumes and less sperm frequently reduces conception rates.

The biggest detriment to successful artificial breeding of swine is the difficult and time-consuming task involved in accurate estrus detection.

Prepared by Emmett J. Stevermer, extension swine specialist

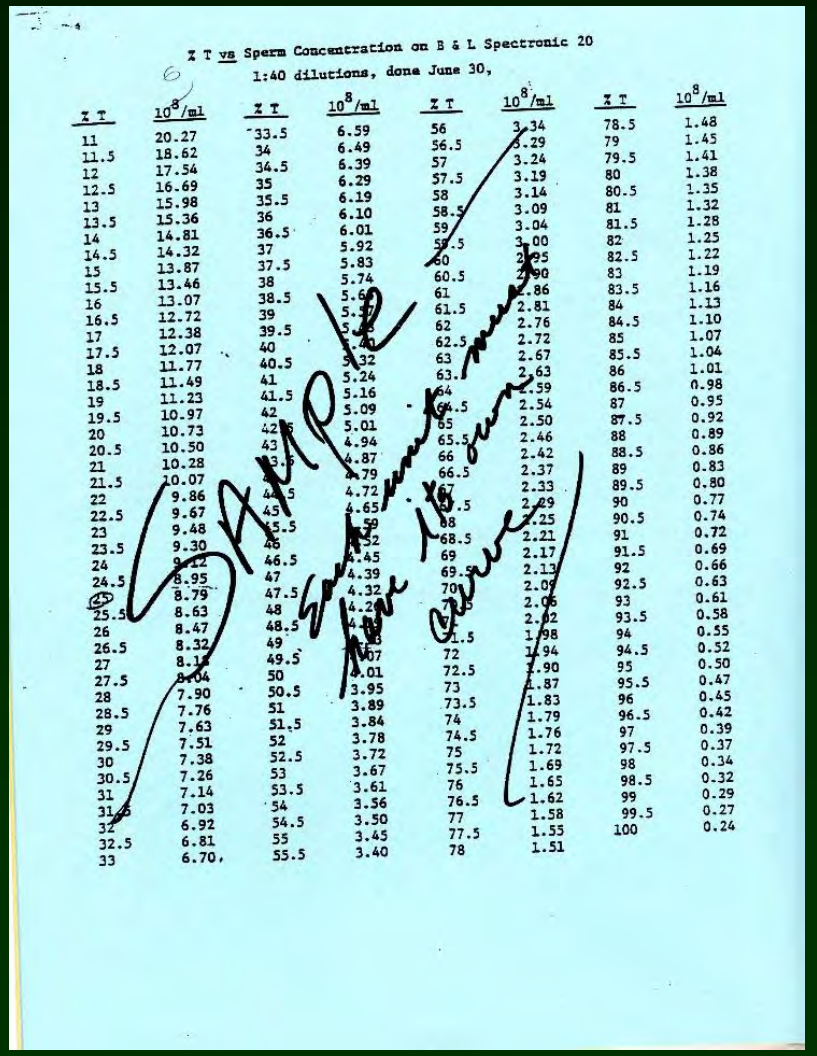
Cooperative Extension Service. . . . . .Ames, Iowa. . . . . .October, 1969. . . . . .Pm-476

1969

Series of fact sheets were published. Sponsored by the National Pork Producers Council

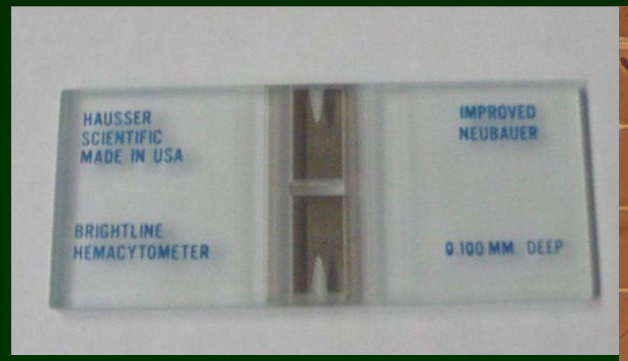
Written by Emmett Stevermer, ISU

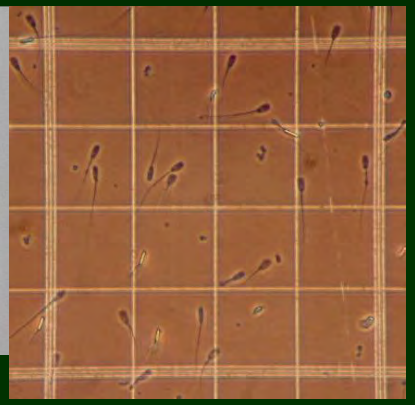
1970
Young, Foote and Turkheimer published photoelectric method for estimating sperm concentration.



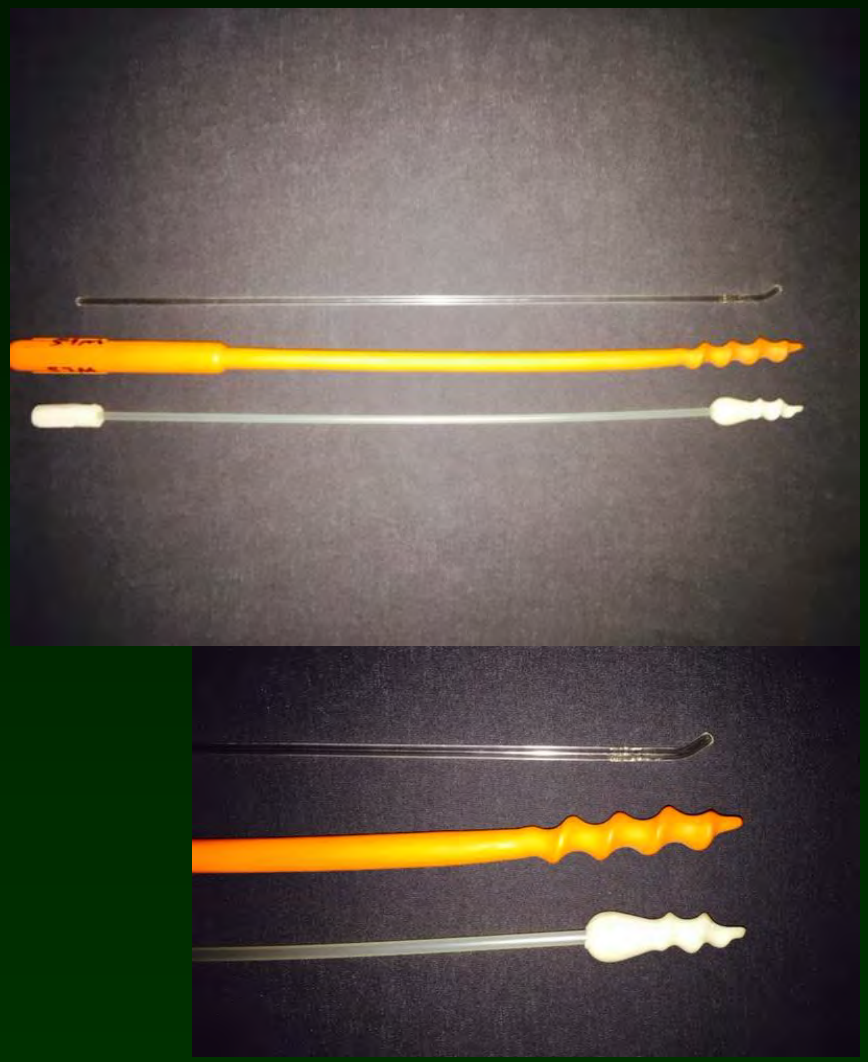
% T vs Sperm Concentration on B & L Spectronic 20
1:40 dilutions, done June 30,

% T	10^8/ml	% T	10^8/ml	% T	10^8/ml	% T	10^8/ml
11	20.27	33.5	6.59	56	3.34	78.5	1.48
11.5	18.62	34	6.49	56.5	3.29	79	1.45
12	17.54	34.5	6.39	57	3.24	79.5	1.41
12.5	16.69	35	6.29	57.5	3.19	80	1.38
13	15.98	35.5	6.19	58	3.14	80.5	1.35
13.5	15.36	36	6.10	58.5	3.09	81	1.32
14	14.81	36.5	6.01	59	3.04	81.5	1.28
14.5	14.32	37	5.92	59.5	3.00	82	1.25
15	13.87	37.5	5.83	60	2.95	82.5	1.22
15.5	13.46	38	5.74	60.5	2.90	83	1.19
16	13.07	38.5	5.66	61	2.86	83.5	1.16
16.5	12.72	39	5.57	61.5	2.81	84	1.13
17	12.38	39.5	5.48	62	2.76	84.5	1.10
17.5	12.07	40	5.40	62.5	2.72	85	1.07
18	11.77	40.5	5.32	63	2.67	85.5	1.04
18.5	11.49	41	5.24	63.5	2.63	86	1.01
19	11.23	41.5	5.16	64	2.59	86.5	0.98
19.5	10.97	42	5.09	64.5	2.54	87	0.95
20	10.73	42.5	5.01	65	2.50	87.5	0.92
20.5	10.50	43	4.94	65.5	2.46	88	0.89
21	10.28	43.5	4.87	66	2.42	88.5	0.86
21.5	10.07	44	4.79	66.5	2.37	89	0.83
22	9.86	44.5	4.72	67	2.33	89.5	0.80
22.5	9.67	45	4.65	67.5	2.29	90	0.77
23	9.48	45.5	4.59	68	2.25	90.5	0.74
23.5	9.30	46	4.52	68.5	2.21	91	0.72
24	9.12	46.5	4.45	69	2.17	91.5	0.69
24.5	8.95	47	4.39	69.5	2.13	92	0.66
25	8.79	47.5	4.32	70	2.09	92.5	0.63
25.5	8.63	48	4.26	70.5	2.05	93	0.61
26	8.47	48.5	4.20	71	2.02	93.5	0.58
26.5	8.32	49	4.13	71.5	1.98	94	0.55
27	8.18	49.5	4.07	72	1.94	94.5	0.52
27.5	8.04	50	4.01	72.5	1.90	95	0.50
28	7.90	50.5	3.95	73	1.87	95.5	0.47
28.5	7.76	51	3.89	73.5	1.83	96	0.45
29	7.63	51.5	3.84	74	1.79	96.5	0.42
29.5	7.51	52	3.78	74.5	1.76	97	0.39
30	7.38	52.5	3.72	75	1.72	97.5	0.37
30.5	7.26	53	3.67	75.5	1.69	98	0.34
31	7.14	53.5	3.61	76	1.65	98.5	0.32
31.5	7.03	54	3.56	76.5	1.62	99	0.29
32	6.92	54.5	3.50	77	1.58	99.5	0.27
32.5	6.81	55	3.45	77.5	1.55	100	0.24
33	6.70,	55.5	3.40	78	1.51		





Bent tipped bovine catheters and the Melrose catheter were used for insemination in the early days.



Professor Christopher Polge, who carried out the initial work on the technique in the United Kingdom, recalled that "I trained some boars to mount a 'dummy' sow and learned how to collect the semen. I first used an artificial vagina designed on lines similar to those suggested by Arthur Walton some years previously, but later switched to using the 'gloved hand' method, which was a lot easier. Initially, the sows were then inseminated using a long plastic tube with an inflatable cuff at the end".

This was superseded by the Melrose spiral catheter, which Dick Melrose, then of the Reading AI centre, 'had made from a mould of a boar's penis', and which, in Polge's opinion, 'was an important advance in AI technique and in promoting its development' Polge's initial work was done in the 1950s, and there were further developments in the 1960s

From: Brassly, Stud. Hist. Phil. Biol. & Biomed. Sci. 38 (2007) 442–461

1972 - First commercial disposable spirette was introduced

1989 - First commercial foam tip(Golden Pig) was introduced

Swine A.I. Co-op to be Formed in Wisconsin

1974

Thirty-five agricultural leaders gathered at Waupun, Wisconsin on Wednesday, December 18 for the purpose of forming a swine A.I. cooperative. The meeting, which was called by East Central Breeders, was held at the Community Room of the State Bank Of Waupun. Included in the group were swine producers and breeders from Wisconsin and Iowa; representatives of East Central Breeders Association Cooperative, Midwest Breeders Cooperative, Select Sires, Inc., Tri-State Breeders Cooperative and Minnesota Valley Breeders Association; Wisconsin Feeder Pig Marketing Cooperative, Equity Cooperative Livestock Sales Association and Midwest Livestock Producers; University of Wisconsin and Purdue University; the St. Paul Bank For Cooperatives; The Wisconsin Department of Agriculture; The United States Dept. of Agricultural; Oscar Mayer, Jones Dairy Farm and Hillshire Farms; Wisconsin Pork Producers and many others.

Clarence Boyke, Fond du Lac, President of East Central Breeders, welcomed the group and explained East Central's involvement in swine A.I. since 1967. Boyke said, "Dr. Ed Graham's successful freezing of boar semen in 1970 and the interest by swine producers and other organizations prompted the East Central Board of Directors to continue swine A.I. as a research program. Now with the success we are having with frozen boar semen, the time to form a swine A.I. cooperative is now here." Boyke further pointed out the East Central Board of Directors approved the formation of a swine A.I. cooperative in April of this year and in September approved leasing land just south of East Central headquarters to the new cooperative with option to eventual purchase by the swine A.I. cooperative. Also East Central would turn over to the swine A.I. cooperative when established, its boars, swine A.I. equipment and its investment in the swine A.I. program as its equity in the swine A.I. cooperative. He explained East Central members have invested nearly $53,000 in the program from 1967 thru April of this year. Boyke said, "Formation of a viable swine A.I. cooperative must be a cooperative effort, one organization cannot do it alone. Today is a history making event and you gentlemen are part of it."

Dr. Wallace Erickson, General Manager and Jack Eichel, Swine A.I. Supervisor for East Central explained the early work of swine A.I. in Wisconsin and at East Central and presented information regarding the success presently obtained with frozen boar semen.

Dr. Roger Gerrits, Staff Scientist, National Program Staff, United States Department of Agriculture, Beltsville, Maryland spoke on the need of a swine A.I. organization from which semen from quality boars would be available to swine producers. He said a national boar testing program would be an eventual reality. Dr. Gerrits said swine A.I. really got its start in Wisconsin with some of the first research work done by the University of Wisconsin and later by the A.I. cooperatives. He praised East Central Breeders for their continuing efforts on swine A.I.

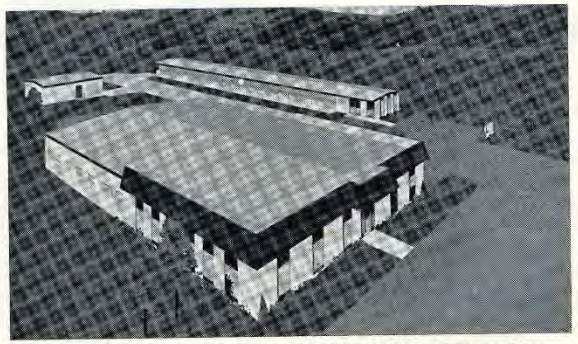
Dr. Robert Grummer, Department of Meat and Animal Science, University of Wisconsin spoke briefly on the swine A.I. research work at the University of Wisconsin. He said Dr. L. E. Cassida conducted the first research on development of boar semen extenders and the number of sperm necessary per insemination. He emphasized the University has continually supported swine A.I. efforts in the past and would do so in the future.

Dennis Braun, Economist of the Bureau of Marketing Service, Wisconsin Dept. of Agriculture, Madison, lead the group in a discussion on a sample articles of incorporation and by-laws. The discussion centered around naming of the new cooperative, definition of its purposes, how the cooperative is to be capitalized, types of membership and the composition of the board of directors.

It was decided that a temporary board of directors would be selected with the first meeting of the board to be held in January. Several advisory committees would also be appointed, namely: research, health, public relations, membership, finance, genetic and building.

An architect's drawing of the new facility which includes a laboratory, semen collection room, frozen semen storage and shipping area, office and housing of 30 boars was displayed.

The goal for the new cooperative to be fully organized is May 1, 1975. Construction of the facility should get underway sometime this spring or summer.



Architect's sketch of proposed new swine A.I. facility at Waupun, Wis.

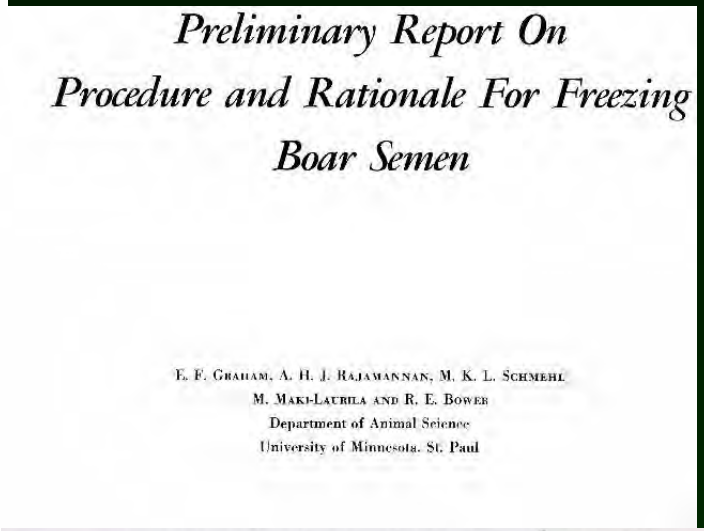
- 1974 Swine AI Coop formed by East Central Breeder's
  - Wally Erickson and Jack Eichel

Early boar studs

1971

- Pursel and Johnson ARS-USDA bulletin 44-227 on Procedure for freezing boar semen

- Article by Ed Grahham and Bo Carbo on boar semen freezing procedure.



Early Extenders

Gadea, 2003

- **Illinois VariableTemperature**
- **Kiev**
- **EDTA**
- **Merck I**
- **Guelph**
- **Zorlesco (1st “long-term)**
- **Modena (1981)**

1988

Johnson, et al., published the paper comparing fecundity of BTS, Modified Modena and MRA

Beltsville Thaw Solution

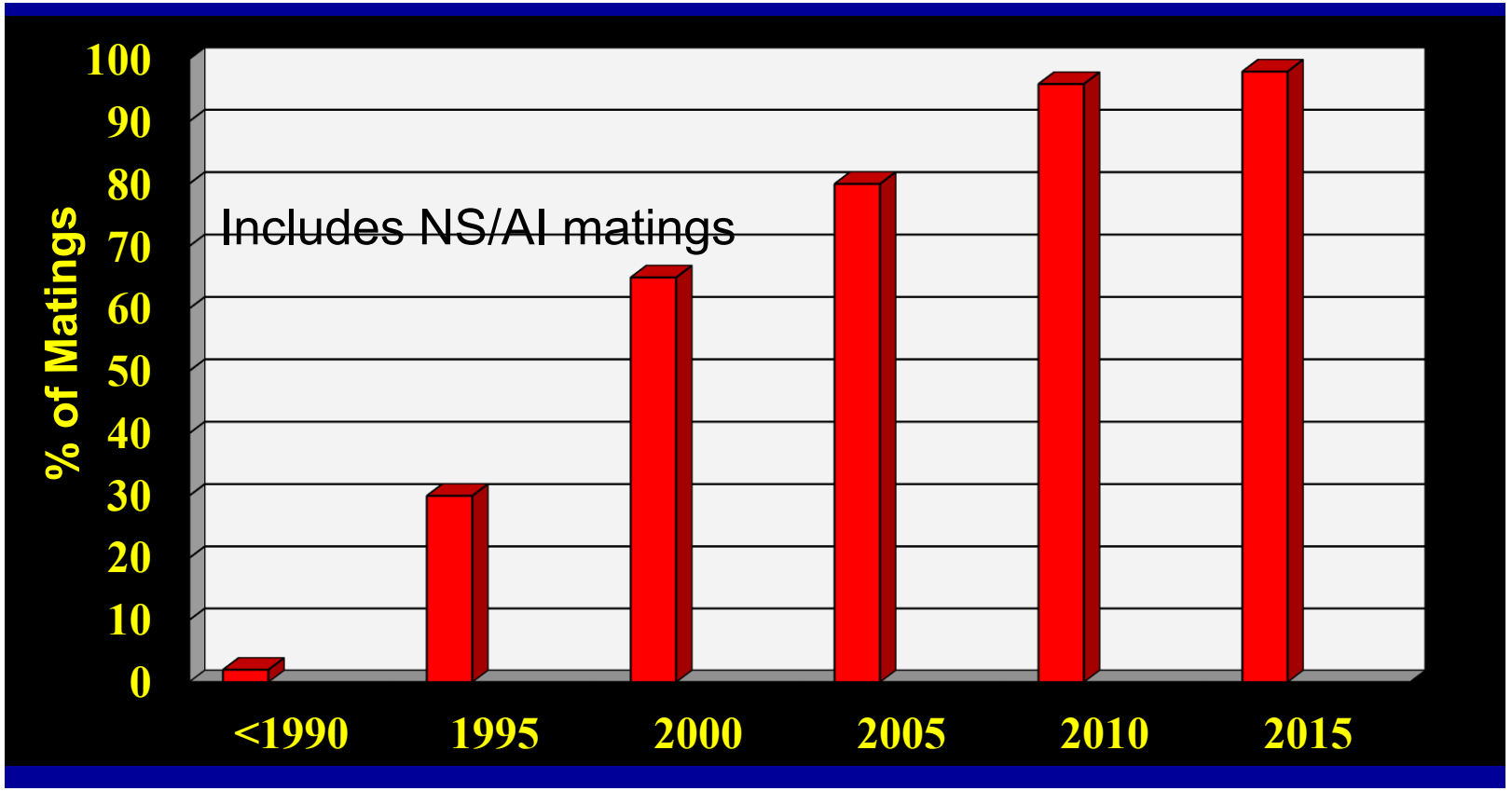
- Glucose
- Sodium Citrate
- Sodium bicarbonate
- Potassium chloride
- Penicillin G
- Dihydrostreptomycin

Early “Long-term” extenders

Gadea, 2003

- Androhep (1990)
- Acromax
- Vital
- SpermAid
- Mulberry III
- X-Cell
- Safe Cell Plus

Industry Application of AI Technology in the US



Based upon industry estimates

General Comments

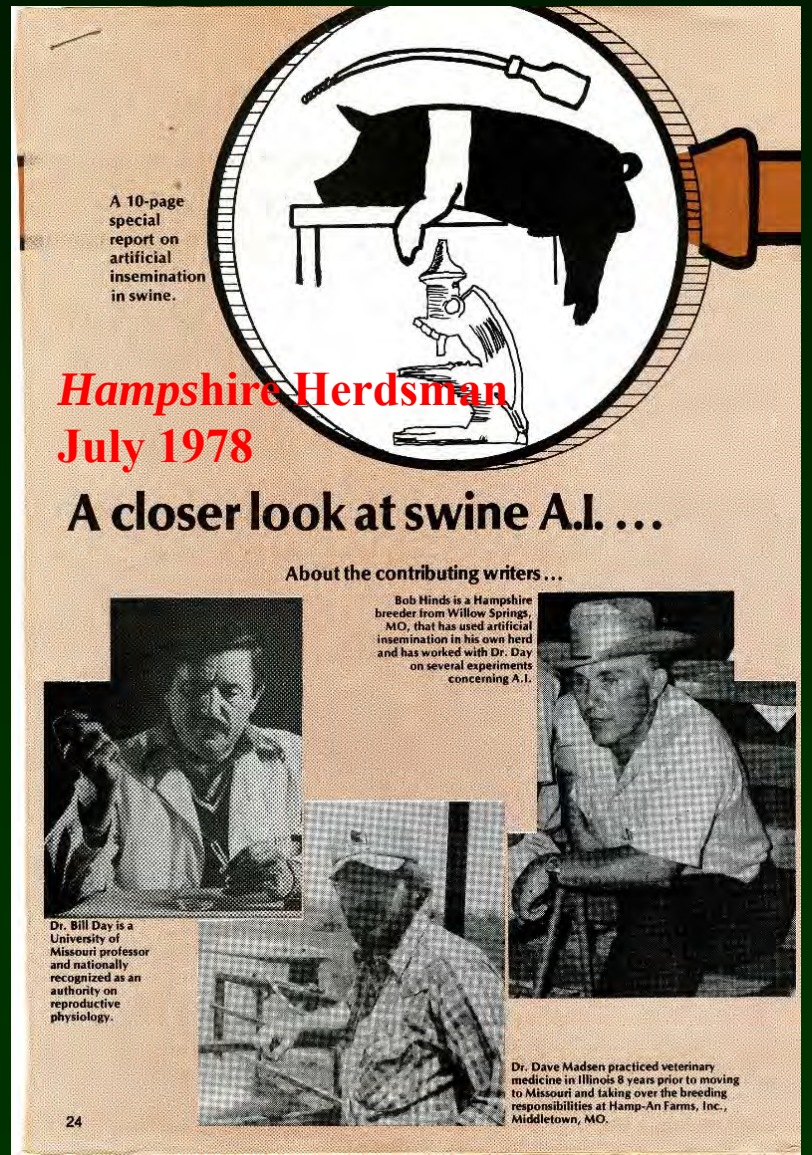
- Prior to the mid 1980's about 300,000 boars were required to service the US sow herd
  - Approximately 22,000 +/- boars are in US studs today (Burke, personal communique)
- In the late 1960's and early 1970's some purebred associations required part ownership of the sire in order to register his offspring
- The seed stock industry underwent major changes in the late 80's and 90's

Prior to the use of AI, a weekly breeding and farrowing schedule with natural service and hand mating, a 1200 sow unit required about 50-60 boars in inventory.

Boar libido and soundness and worker safety were major issues.

Imagine using hand mating in a 5000 to 10,000 sow unit!!

Early use of AI was basically limited to a few seed stock producers



A 10-page special report on artificial insemination in swine.

Hampshire Herdsman
July 1978

A closer look at swine A.I. . . .

About the contributing writers . . .

Bob Hinds is a Hampshire breeder from Willow Springs, MO, that has used artificial insemination in his own herd and has worked with Dr. Day on several experiments concerning A.I.

Dr. Bill Day is a University of Missouri professor and nationally recognized as an authority on reproductive physiology.

Dr. Dave Madsen practiced veterinary medicine in Illinois 8 years prior to moving to Missouri and taking over the breeding responsibilities at Hamp-An Farms, Inc., Middletown, MO.

24

SWINE BREEDING SURVEY

MSU Survey
National Hog Farmer
July 1989

Few Herds Using A.I.

Most value technique as way to produce superior breeding stock.

By Dale Miller
Managing Editor

Artificial insemination has never caught on "big" in the hog industry. But the number of producers willing to tackle A.I. appears to be edging upward. Perhaps even more important, the units using the breeding technique tend to be the larger operations.

Those are some of the findings gleaned from the "Survey of Swine Breeding Systems in the U.S." conducted by Michigan State University and *National Hog Farmer*. Over 90% of respondents indicated they never use A.I. (See Table 3.)

But those that do use it, 8.5%, have a specific purpose in mind. As the pie chart shows, nearly 15% use it to raise their own replacement gilts, just over 5% use it for home-raised boars, nearly 70% use it for both. Just over 10% said they use it for market hog production. Table 4 offers a more detailed analysis of use, based on herd sizes.

On the surface, those percentages may seem relatively high, but keep in mind they are percentages of only those that said they use A.I. at all — again, just 8.5% of the total.

There are several reasons why producers resist A.I., but the one that drew the most discussion focused on the difficulty of breeding and raising boars in a commercial hog unit:

▶ Raising boars requires another whole set of sows. Often producers maintain a set of purebred sows from one of the white breeds which are mated A.I. to another white breed to generate F-1 replacement females.

But, to effectively produce boars in like fashion, another line of sows would be needed to produce either purebred or F-1 boars to maximize heterosis. Those sows claim more space from a breeding herd that's supposed to be producing market hogs.

Of course, the need for boars is far less than the need for gilts, too. Theoretically, if only 10-20 boars are needed per year, 5-6 purebred sows should be able to provide them. But there's a limitation there, too. Small numbers restrict genetic diversity and selection. Then, if one or two sows fail, 20-30% of the boar production capacity is quickly lost.

Wayne Singleton of Purdue and John Mabry of the University of Georgia agreed producers quickly become discouraged when they try to raise their own boars. "I only recommend it if they have a certain size sow herd and have specific health concerns," Mabry noted. "It puts a lot of added stress on labor and management."

▶ Another major management hassle is where to put growing boars in facilities designed for market hog flow. Extra replacement gilts aren't a big problem because they are handled the same as market hogs until they are about 5 months old. If they aren't selected for the gilt pool, they're simply shipped with other market hogs.

Boars are another matter. Separate pens, preferably separate facilities, are needed. Boars must reach sexual maturity before they can be screened. If they don't make the grade, they're culled.

"With gilts, there's no real price risk; no penalty. Not so with boars. You really get hammered when you sell cull boars," observed Michigan State Economist Gerald Schwab. Those lightweight culls are often worth 25-50% less than their littermate market hogs, and chances are they've been fed 4-6 weeks longer.

Those producers who justify the effort most often list minimizing disease and health risks as the key reasons. A few also cite the chance to tap superior genetic lines as an incentive.

Users Use A.I. Sparingly

Singleton observed: "The percentage that use it is quite low, but the percentage that use it to any degree

Table 3. Use of Artificial Insemination (A.I.) to Breed Sows

Extent of Use	Percent
Do not use A.I.	91.5%
Use A.I. for less than 25% of all matings	7.1%
Use A.I. for 25% to 50% of all matings	.9%
Use A.I. for 50% to 75% of all matings	.1%
Use A.I. for more than 75% of all matings	.4%
Total	100.0%

AI requires the same personality traits that make natural service work in a breeding herd: precision, patience, confidence, curiosity and enthusiasm.

1993

Four years later!
National Hog Farmer
June, 1993

AI: This Time It's Here To Stay

Carcass-merit buying, genetic continuity, breeding herd efficiency are the driving forces behind a renewed interest in AI.

First in a series

By Dale Miller
Managing Editor

Interest in artificial insemination (AI) is back on the fast track in the hog industry.

"It's like the 'seven-year itch,'" says swine reproductive physiologist Billy Flowers. "About every seven years, interest in AI peaks."

But this time around, the North Carolina State University specialist believes, the interest is here to stay.

Fellow reproductive specialist Wayne Singleton at Purdue University agrees. "Producers setting up to do artificial insemination are doing it right this time. Ten years ago they tried to do it using a 'kitchen table' mentality."

New incentives are driving the latest interest in this breeding option:

▶ The leading reason appears to be the belief that the packing industry will soon be buying hogs exclusively on carcass merit – with heavy emphasis on muscling.

▶ A related incentive is the push toward more consistency in market hogs. Truckloads of hogs with wide variations in body composition and quality give packers fits. One way to reduce variation is to use fewer genetic lines. The semen collected from one boar can be used to inseminate from five to 20 females (depending on sperm cell count), rather than one natural-service mating.

▶ Spreading one semen collection over more sows reduces the number of boars needed, allowing producers to buy better quality boars with their

A.I.

-A Tool for Profitable Pork Production

July 19-20, 1994

Holiday Inn Lafayette
and On-Site Training Facilities
West Lafayette, Indiana

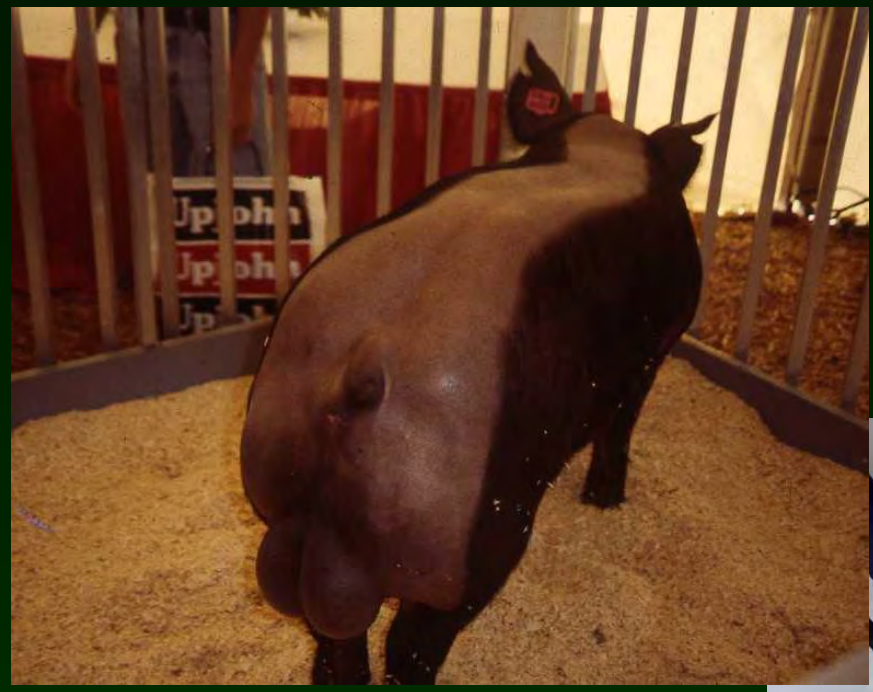
TABLE OF CONTENTS

Increased use of AI during the early 1990's

Major shift from outdoor to environmentally controlled production systems.

Sow herds became larger and specialized management could be applied the breeding phase

Increased use of AI during the early 1990's



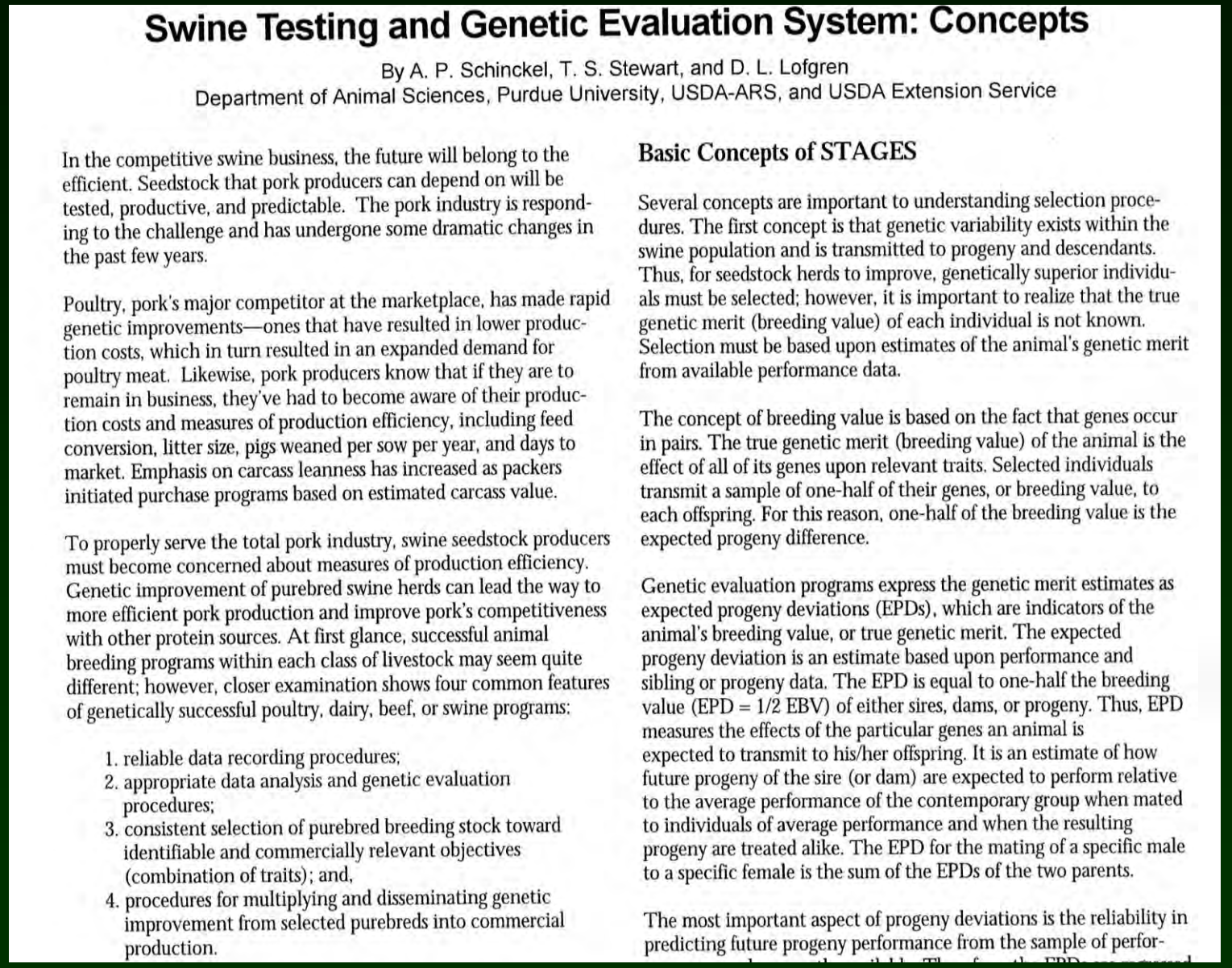
Quest for carcass leanness

- Carcass measurement and payment for lean programs were instituted by packers

Increased use of AI during the early 1990's

STAGES and BLUP analysis became available.

Sires with superior growth and carcass traits could be more accurately identified.



Swine Testing and Genetic Evaluation System: Concepts

By A. P. Schinckel, T. S. Stewart, and D. L. Lofgren
Department of Animal Sciences, Purdue University, USDA-ARS, and USDA Extension Service

In the competitive swine business, the future will belong to the efficient. Seedstock that pork producers can depend on will be tested, productive, and predictable. The pork industry is responding to the challenge and has undergone some dramatic changes in the past few years.

Poultry, pork's major competitor at the marketplace, has made rapid genetic improvements—ones that have resulted in lower production costs, which in turn resulted in an expanded demand for poultry meat. Likewise, pork producers know that if they are to remain in business, they've had to become aware of their production costs and measures of production efficiency, including feed conversion, litter size, pigs weaned per sow per year, and days to market. Emphasis on carcass leanness has increased as packers initiated purchase programs based on estimated carcass value.

To properly serve the total pork industry, swine seedstock producers must become concerned about measures of production efficiency. Genetic improvement of purebred swine herds can lead the way to more efficient pork production and improve pork's competitiveness with other protein sources. At first glance, successful animal breeding programs within each class of livestock may seem quite different; however, closer examination shows four common features of genetically successful poultry, dairy, beef, or swine programs:

1. reliable data recording procedures;
2. appropriate data analysis and genetic evaluation procedures;
3. consistent selection of purebred breeding stock toward identifiable and commercially relevant objectives (combination of traits); and,
4. procedures for multiplying and disseminating genetic improvement from selected purebreds into commercial production.

Basic Concepts of STAGES

Several concepts are important to understanding selection procedures. The first concept is that genetic variability exists within the swine population and is transmitted to progeny and descendants. Thus, for seedstock herds to improve, genetically superior individuals must be selected; however, it is important to realize that the true genetic merit (breeding value) of each individual is not known. Selection must be based upon estimates of the animal's genetic merit from available performance data.

The concept of breeding value is based on the fact that genes occur in pairs. The true genetic merit (breeding value) of the animal is the effect of all of its genes upon relevant traits. Selected individuals transmit a sample of one-half of their genes, or breeding value, to each offspring. For this reason, one-half of the breeding value is the expected progeny difference.

Genetic evaluation programs express the genetic merit estimates as expected progeny deviations (EPDs), which are indicators of the animal's breeding value, or true genetic merit. The expected progeny deviation is an estimate based upon performance and sibling or progeny data. The EPD is equal to one-half the breeding value (EPD = 1/2 EBV) of either sires, dams, or progeny. Thus, EPD measures the effects of the particular genes an animal is expected to transmit to his/her offspring. It is an estimate of how future progeny of the sire (or dam) are expected to perform relative to the average performance of the contemporary group when mated to individuals of average performance and when the resulting progeny are treated alike. The EPD for the mating of a specific male to a specific female is the sum of the EPDs of the two parents.

The most important aspect of progeny deviations is the reliability in predicting future progeny performance from the sample of perfor-

1992 Flowers and Alhusen JAS article on AI vs NS

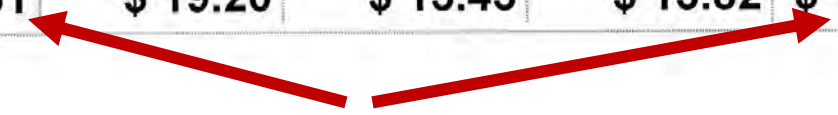
EFFECTS OF COMBINATIONS OF NATURAL SERVICE AND AI ON REPRODUCTIVE PERFORMANCE OF GILTS

TRAIT	NS / NS	NS / AI	NS / NONE	AI / AI
No. GILTS	67	60	61	65
AGE AT MATING	180	185	188	178
FARROWING RATE (%)	71.7	88.9	56.3	82.3
No. BORN ALIVE	9.8	9.5	8.0	9.3
TOTAL BORN	10.9	10.9	9.0	10.5

FLOWERS AND ALHUSEN, 1992, JAS

Table 1. Cost per mated sow using various programs.

Mating Program	Natural Service	AI w/ Purchased Semen	AI w/ On-Farm Collection; 200 Sows	AI w/ On-Farm Collection; 1,000 Sows	AI w/ Small Boar Stud; 50 Boars
Min./Mating	22.6	13.5	15.3	15.3	15.3
Labor Cost ($10/hr)	$ 7.53	$ 4.55	$ 5.10	$ 5.10	$ 5.10
Boar Fixed Costs	$ 7.94	-------	$ 5.56	$ 5.56	$ 5.56
Boar Variable Costs	$ 8.14	-------	$ 1.22	$ 1.22	$ 0.20
Semen Cost	-------	$ 14.00	-------	--------	-------
AI Lab Structure Cost	-------	-------	$ 0.68	$ 0.68	$ 0.14
AI Lab Equipment Cost	-------	-------	$ 2.04	$ 0.41	$ 0.07
AI Disposable Supplies Cost	-------	$ 0.65	$ 0.85	$ 0.85	$ 0.85
Total	**$ 23.61**	**$ 19.20**	**$ 15.45**	**$ 13.82**	**$ 11.92**



See, NC ST ,ANS 96-809s

There was a major shift from on-farm semen production to commercial boar studs during the late 1990's and early 2000's

- "The trend seems clear. More pork producers are dumping on-farm boar collection, opting instead to have semen delivered for use in their AI (artificial insemination) programs. For many just switching to AI, the choice may be one of convenience more than cost. Although substantial cost savings can be gained with on-farm boar collection as unit size increases, more and more producers say they would rather not mess with collecting or the lab work"

- Joe Vansickle National Hog Farmer Article, June 01, 1998

Automation equipment and quality control protocols were developed

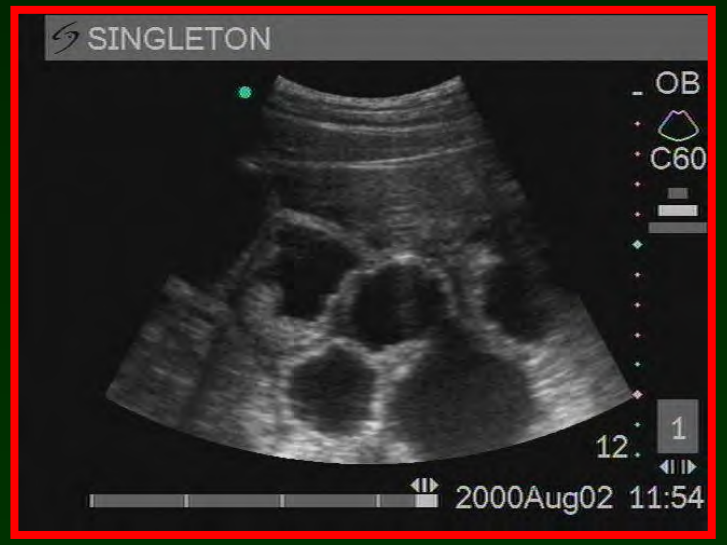
Farrowing results with IUI and reduced sperm numbers (N=3210)

	Golden Pig			Deep Golden Pig		
Dose (billion)	FR	TB	BA	FR	TB	BA
1	65.8	10.3	9.0	86.9	12.1	10.9
2	91.8	12.6	10.9	92.5	12.3	10.8
3	91.1	12.1	10.9	90.5	12.3	11.0

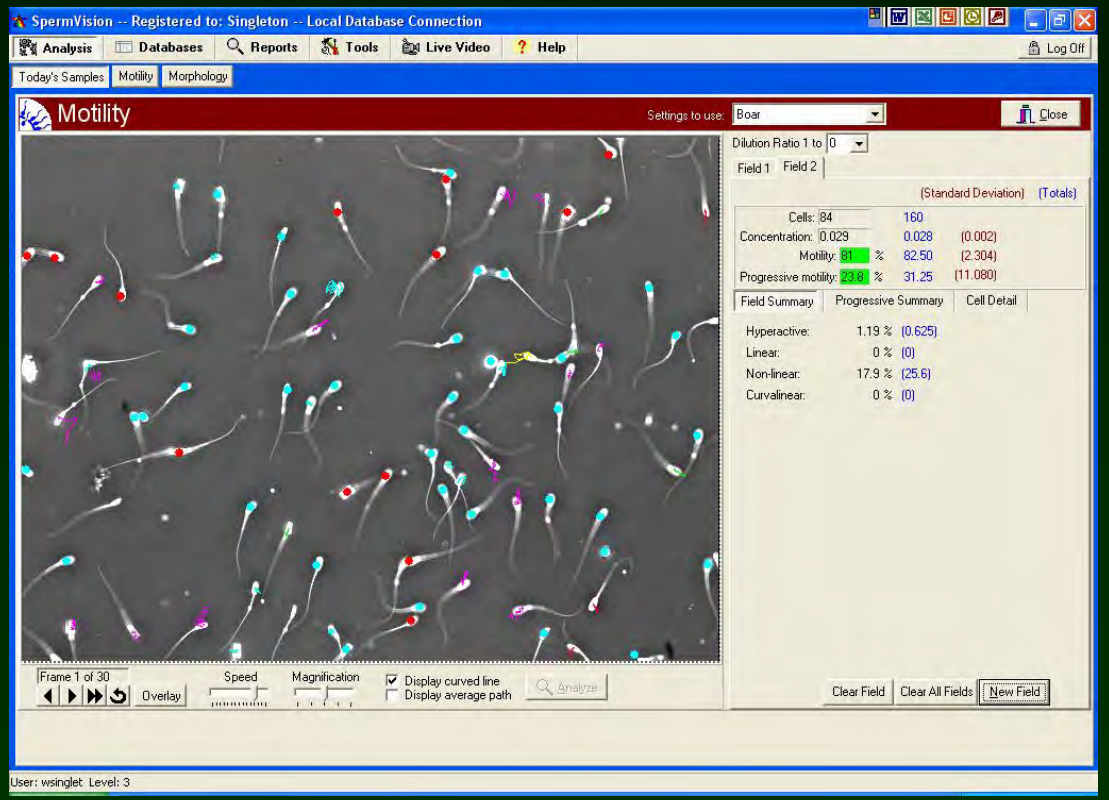
Watson and Behan, Theriogenology,2002

Mid 90’s-Early 2000’s

- RTU’s became more portable and affordable



Computer Assisted Semen Analysis system became widely used

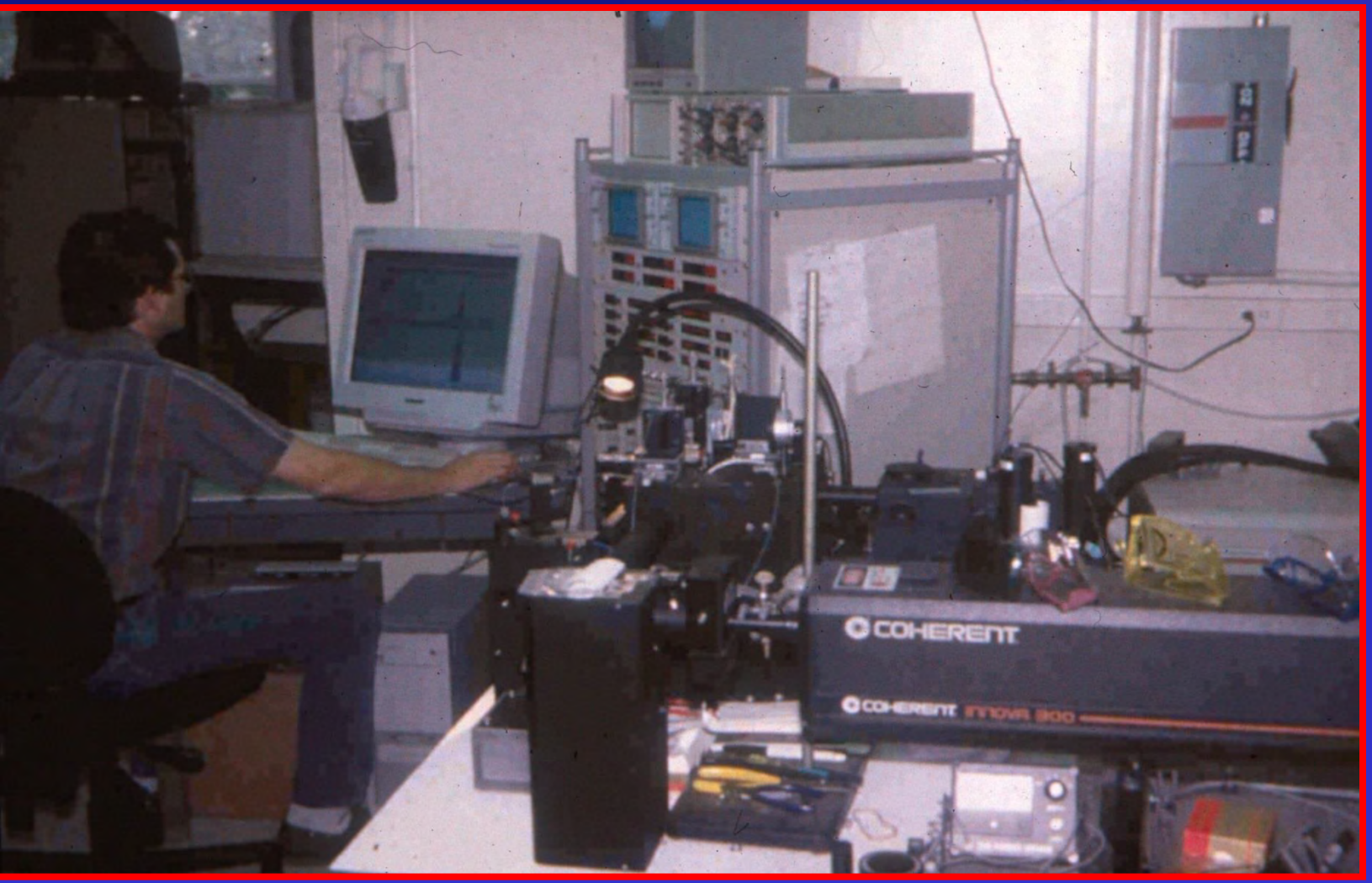


U of I
Semen Evaluation workshop

Beltsville Sperm Sexing Technology

Flow cytometry

1999




110
108
107

For More Information

- Artificial insemination in pigs, research and developments in the Netherlands, Hanneke Feitsma, Acta Scientiae Veterinarian, 37(Supp1) 2009
- Evolution and adoption of A.I. in the US. W.L. Flowers, 2015, Billy Day Symposium
- The history of artificial insemination: Selected notes and notables. R.H. Foote, 2002 J. An Sci.
- Cutting across nature? The history of artificial insemination in pigs in the United Kingdom, P Brassley, 2007