The drive to reduce sperm per female inseminated and deliver sexed semen to the swine industry: Are we there yet?
Outline

• History
  • Swine breeding and AI evolution
  • Sex chromosomes and sperm sorting
  • Reaching the conventional AI benchmark
  • How did we make sorted sperm perform better?

• Fertilization Factors
  • 3-way interaction of the S’s (Sperm*Site*Synchrony)

• Sex Biases and Benefits
  • Gilts vs Boars vs Barrows

• Transition from Billions to Millions
  • Sow reproductive performance at low sperm dose

• When Will We Deliver the Male (Sorted)?
  • Best way to predict the future is to (get busy and) create it
Pros of Genetic Distribution via AI

- **Economics** – Disseminate the genes of a high genetic merit boar over multiple gilts and sows to reduce genetic lag

  100 billion sperm/week; 2.5 billion/dose

  \[ 1\sigma \times 20\varphi (40 \text{ doses})/\text{week} \times 52 \text{ weeks} = 1,040\varphi/\text{year} \times 90\% \text{ FR} = 936\varphi \times 12 \text{ pigs weaned/litter} = 11,232 \text{ pigs} \]

- **Fertility** – Block distribution of low semen quality boars and ejaculates
- **Flexibility** – Rapidly change genetic direction
- **Logistics** – Distribution over geographic distance
- **Health** – Less effective vector of disease than transfer of live animals
- **Labor** – Specialization and economy of scale
## Swine Breeding: Historical Perspective

<table>
<thead>
<tr>
<th></th>
<th>Pen &amp; Hand Mating</th>
<th>On-Farm Al to Al Studs</th>
<th>Specialized Larger Studs</th>
<th>Fewer Boars &amp; Fewer Studs</th>
<th>Making the Future Now</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Circa</strong></td>
<td>&lt; 1985</td>
<td>1990</td>
<td>2000</td>
<td>2010</td>
<td>Today</td>
</tr>
<tr>
<td><strong>Semen Evaluation</strong></td>
<td>None</td>
<td>Manual, Gross, Subjective</td>
<td>Automated, Detailed, Objective</td>
<td>Automated, Detailed, Objective</td>
<td>Better Screening of Sub-Fertile Boars &amp; Ejaculates</td>
</tr>
<tr>
<td><strong>Rejection Rate</strong></td>
<td>None</td>
<td>1-4%</td>
<td>5-9%</td>
<td>10-15%</td>
<td>More? Or Earlier?</td>
</tr>
<tr>
<td><strong>AI Technique</strong></td>
<td>None</td>
<td>Conv Al + Natural Serv</td>
<td>Conv Al 100%, tried &quot;Deep&quot; Al</td>
<td>Uterine Insem, aka &quot;Post-Cervical Al&quot;</td>
<td>Deep Uterine Insem at Utero Tubal Junction (UTJ), Both Horns?</td>
</tr>
<tr>
<td><strong>Semen Deposition</strong></td>
<td>Cervix</td>
<td>Cervix</td>
<td>Cervix</td>
<td>Uterine Body</td>
<td>UTJ, Oviduct, Further?</td>
</tr>
<tr>
<td><strong>Matings, Als/Estrus</strong></td>
<td>3.5</td>
<td>3.0</td>
<td>2.3</td>
<td>2.0</td>
<td>1.0 Fixed-Time, Synch</td>
</tr>
<tr>
<td><strong>Sperm/Dose</strong></td>
<td>90-70×10⁹</td>
<td>5×10⁹</td>
<td>3×10⁹</td>
<td>2×10⁹</td>
<td>0.5×10⁹ and less</td>
</tr>
<tr>
<td><strong>Volume/Dose</strong></td>
<td>450-150 mL</td>
<td>100 mL</td>
<td>75 mL</td>
<td>45 mL</td>
<td>20 mL and less, Bulk?</td>
</tr>
<tr>
<td><strong>Sperm/Female</strong></td>
<td>280×10⁹</td>
<td>15×10⁹</td>
<td>7×10⁹</td>
<td>4×10⁹</td>
<td>0.5×10⁹ and less</td>
</tr>
<tr>
<td><strong>Females/Ejac/Week</strong></td>
<td>6</td>
<td>13</td>
<td>22</td>
<td>180</td>
<td></td>
</tr>
<tr>
<td><strong>Boar:Sow Ratio</strong></td>
<td>1:25</td>
<td>1:100</td>
<td>1:200</td>
<td>1:300</td>
<td>1:500 and more</td>
</tr>
</tbody>
</table>

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**Fast genetics™**
Swine have 38 chromosomes:
- 36 autosomes
- +2 allosomes
- XY/XX

Photo: Alain Pinton, INRA, Toulouse, France
Sex Sorting Concept Diagram

Today

≈ 40,000 events/sec
> 90% purity
≈ 7,000 X- sperm/sec
≈ 25 million X- sperm/hr

Johnson and Pinkel, 1986
X- and Y-Bearing Sperm DNA Content Differences

Garner and Seidel, 2003; creeping vole 9.1 to 12.5% diff

Johnson, 2000; adapted from Johnson and Welch, 1999
Figure 1 Plot 1 is forward 0° (FAF) and side 90° (SAF) fluorescence images. Plot 1 is used to identify live/dead sperm populations and to gate only the cells within the oriented region to plot 2 and plot 3. This removes all dead sperm from the sorting process. These resulting flow cytometry histograms are used to analyze and sort on the relative fluorescence of X- and Y-sperm populations. The plot 2 allows for the gating of the required sex (X or Y or both), while plot 3 is monitoring resolution by means of peak to valley ratio (PVR). Sort speeds of >9000 cells/s can be achieved of each sex. Parallelism can triple productivity through the development of multiple head sorters such as Genesis III.

Vishwanath and Moreno, 2018
Genesis III – 3 Heads, Automated, Digital, Smaller Footprint
USDA ➤ XY®, Inc ➤ Sexing Technologies®

1960’s
• Pioneering work on sex chromosomes by Gledhill, Pinkel, and others

1970’s

1980’s
• Larry Johnson and others at USDA BARC East publish studies that demonstrate live births and skewed sex ratio litters of rabbits and pigs from flow sorted sperm
• US patents filed around 1991 and XY, Inc formed, CO State Univ Research Foundation and Cytomation, Inc (MoFlo®) in 1996

1990’s
• Sexing Technologies (ST) issued license for commercial sorting in 2003; sets up sorting lab in 2004 at Navasota, TX site
• Inguran, LLC (dba ST) acquires XY, Inc and all its licenses in 2007

2000
• XY Legacy Technology upgraded by global introduction of SexedULTRA™ in 2013
• Move from 2 to 4 million SSS/straw with fertility nearly matching 15 million conventional sperm with SexedULTRA-4M™ in 2015
• Inguran, LLC purchases Fast Genetics from HyLife in 2015 to couple SSS with genetic/genomic selection of swine

2010

Lenz et al., 2016
Select Sires study, 8 bulls 6,930 heifers

German Genetics International study, 5 bulls 7,855 heifers 62,398 heifers
How Are Sorted Sperm Different?

• More homogenous population
  • Dead, abnormal sperm removed (clean-up effect)
  • Aneuploid sperm removed (missing or extra chromosomes)

• Primary sorting “stressors”
  • Dilution, media changes
  • Pressure, shear forces, mechanical damage
  • Centrifugation

• How to help sorted sperm compete?
  • Sorted sperm are in a pseudo-capacitated state
  • Sorted boar sperm do not bind to oviductal epithelial cells as well as non-sorted sperm (Winters et al., 2017)
  • Insemination timing closer to ovulation yields better results
  • Site of deposition, boar effects (fertility before/after sorting)
3-Way Interaction of the S’s for Fertilization Success

Other♀Factors?
- Immuno Aspects
- Gilts vs Sows
- ♀Fertility
- Ov Rate

Gonadotropins
GnRH Agonists
1 Fixed-Time AI
AI to Ov Interval

Sperm

Synch

Site

- Number
- Functional Status; Age
- Boar Fertility Effect
- Boar Sort Tolerance Effect
- Uterine Body
- UTJs, Isthmus
- Oviducts
- Polyspermy
- Other?
## Multiplication – Gilt Value (Theoretical)

<table>
<thead>
<tr>
<th>X-/Time</th>
<th>X-Purity</th>
<th>♀/Litter</th>
<th>+Gilts</th>
<th>+Selects</th>
<th>+Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>90%</td>
<td>10.8</td>
<td>4.8</td>
<td>4</td>
<td>$140</td>
</tr>
<tr>
<td>↑</td>
<td>80%</td>
<td>9.6</td>
<td>3.6</td>
<td>3</td>
<td>$105</td>
</tr>
<tr>
<td>↑↑</td>
<td>70%</td>
<td>8.4</td>
<td>2.4</td>
<td>2</td>
<td>$70</td>
</tr>
<tr>
<td>↑↑↑</td>
<td>60%</td>
<td>7.2</td>
<td>1.2</td>
<td>1</td>
<td>$35</td>
</tr>
<tr>
<td>No sort</td>
<td>50%</td>
<td>6.0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

12 pig litter, 6 gilts, $35 value of replacement gilt over market barrow

Overall value is more complex depending on system impact/changes

- Reduce number of sows dedicated to multiplication
  - Convert less efficient sites to commercial; focus on best ones; cut costs
- Maintain number of sows dedicated to multiplication
  - Increase gilt selection criteria
  - Sell surplus gilts and be ready for health contingencies
  - Reduce gilt deficit; achieve genetic replacement targets
Potential Benefits of Gilt Skewed Litters

- **Litter sex ratio effects (androgen exposure)**
  - Gilts from litters of ≥ 2/3 males had ↓ CR (Drickamer et al, 1997)

- **Sex bias on prenatal and postnatal growth and survival**
  - Gilts smaller placentae & occupy less space than boars (Chen & Dzuik, 1993)
  - Uterine crowding affects muscle fiber & postnatal growth (Tse et al., 2008)
  - Sow gestational stress effects larger on boars than gilts (Mack et al, 2014)
  - Boar birthweights tend to be greater than gilts (production data)
  - Pre-weaning mortality greater for boars (w/o castration, Baxter et al, 2012)

- **Gilt/gonadal advantage for finishing efficiency**
  - Barrows tend to lose growth efficiency at high bodyweights
  - Castration may become a consumer issue as it has in Europe
Sex Sorted Sperm (SSS) Use

\[ \Delta G = I \times R \times \sigma / GI \]

- \( \Delta G \): genetic change in deviations per year
- \( I \): selection intensity (sexed semen)
- \( R \): selection accuracy (genomics)
- \( \sigma \): genetic variation in population being selected
- \( GI \): generation interval

<table>
<thead>
<tr>
<th>Technology</th>
<th>Maternal Lines</th>
<th>Terminal Lines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extra FIRE Feeders and Optimization of Data</td>
<td>19%</td>
<td>29%</td>
</tr>
<tr>
<td>Genotyping Selection Candidates</td>
<td>30%</td>
<td>15%</td>
</tr>
<tr>
<td>Sex Sorted semen</td>
<td>16%</td>
<td>Variable</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>65%</strong></td>
<td><strong>44%</strong></td>
</tr>
</tbody>
</table>

Leach, Grossi, Duggan (Fast Geneticists), AASV, 2018
## Sperm, Synch, and Site

<table>
<thead>
<tr>
<th>Technique and Sperm</th>
<th>n</th>
<th>FR, %</th>
<th>LS TB</th>
<th>FR*TB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conv – 3x, 3.0 bill, nonsorted</td>
<td>65</td>
<td>84.6</td>
<td>11.3</td>
<td>9.6</td>
</tr>
<tr>
<td>DUI – 3x, 0.6 bill, nonsorted</td>
<td>68</td>
<td>86.7</td>
<td>11.0</td>
<td>9.5</td>
</tr>
<tr>
<td>Lap Al – 1x, 0.006 bill, sex sorted</td>
<td>45</td>
<td>80.0</td>
<td>11.0</td>
<td>8.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- 4.6</td>
<td>- 0.3</td>
<td>-8.3%</td>
</tr>
<tr>
<td>Conv – 2x, 3.0 bill, nonsorted</td>
<td>45</td>
<td>86.7 ± 6.0</td>
<td>16.5 ± 0.8</td>
<td>14.3</td>
</tr>
<tr>
<td>DUI – 1x, 0.6 bill, nonsorted</td>
<td>17</td>
<td>82.4 ± 9.1</td>
<td>15.4 ± 1.3</td>
<td>12.7</td>
</tr>
<tr>
<td>Lap Al – 1x, 0.010 bill, sex sorted</td>
<td>18</td>
<td>77.8 ± 8.8</td>
<td>15.0 ± 1.3</td>
<td>11.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- 8.9</td>
<td>- 1.5</td>
<td>-18.2%</td>
</tr>
</tbody>
</table>

Univ of Murcia, Spain

ST, Inc field trial data

- Fast herds in SK, ≈ 30 to 35 SSS sows/week
- Working with 1 fixed-time DUI containing 0.4 to 0.5 bill SSS
- Results average ≈ -25% lower than conventional AI in same herds
LAP AI (Surgical) Deposition of SSS at UTJs & Oviducts

Table 2
Reproductive performance of sows inseminted using double laparoscopic insemination (oviducts and the uterine horns) with different numbers of X-sorted sperm per insemination.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Number of sex-sorted sperm per insemination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$3 \times 10^6$</td>
</tr>
<tr>
<td>Sows, N</td>
<td>57</td>
</tr>
<tr>
<td>Pregnancy rate, N (%)</td>
<td>52/57 (91.2)</td>
</tr>
<tr>
<td>Farrowing rate, N (%)</td>
<td>45/57 (78.9)</td>
</tr>
<tr>
<td>Litter size, mean ± SEM</td>
<td>9.2 ± 0.6</td>
</tr>
<tr>
<td>Born alive, mean ± SEM</td>
<td>9.0 ± 0.5</td>
</tr>
<tr>
<td>Birth weight, mean ± SEM</td>
<td>1.8 ± 0.1</td>
</tr>
</tbody>
</table>

The sows were inseminated with a total of $3 \times 10^6$ ($0.5 \times 10^6$ in each oviduct and $1 \times 10^6$ in each uterine horn) or $6 \times 10^6$ ($1 \times 10^6$ in each oviduct and $2 \times 10^6$ in each uterine horn) sex-sorted sperm.

Values in the same row with different superscripts are different ($P < 0.05$).

del Olmo et al, 2014
Sperm/Dose and Boar Fertility

Flowers, 2014
Synchronization of Ovulation with OvuGel®

![Graph showing synchronization of ovulation with OvuGel®. The graph displays the percentage of sows across different time intervals (10 to 26 h, 30 to 34 h, 42 h, 46 to 50 h, 58 to 66 h, 70 to 74 h) for different treatment groups: NO, OG, and PG+OG. The highest percentage is observed at 42 h for OG treatment.]
Site of Deposition with DUI Bicornuate Catheter

Ability to deflect innermost flexible catheter material at an angle near the bifurcation to achieve deep insemination of both uterine horns (one after the other) as close to the UTJs as possible.
Sperm/Dose Effects on Conception Rate (CR), Viable Embryos (VE), and their Product (CR*VE)
One Non-Surgical 10 Million Sperm AI Dose, 7 Embryos

That’s 1/250th of a 2.5 billion ≈ industry average dose
Published Data on Sperm/Dose 2001 to 2017
Take-Home

• Sex sorting sperm works
  • Sorting speeds are a limitation but sperm dose is the greater issue
  • Must continue to progress from billions to millions
  • Will require advances in several areas
  • May require revolutionary innovation
• Initial application – multiplication gilts
  • Dairy industry SSS 4 vs 15 million/dose conventional; 1/4 dose and generating similar results on heifers (≈ 95% of conventional)
  • Fast herds SSS 0.5 vs 2.5 billion/dose conventional, 1/5 dose and generating reduced results on sows (≈ 75% of conventional)
  • Some of the same issues and some porcine-specific ones can be resolved
  • Make better sorted sperm and give them every advantage
Sow Reproductive Tract

R. Nickel, Viscera of Domestic Animals
Why Are so Many Sperm Required?

1) Attrition – within 8 h, \( \approx 0.01\% \) of sperm inseminated remain
2) Timing – Ovulated oocytes viable \( \approx 8 \) h, then degenerate
3) Quality – Some types of “defective” sperm don’t get to compete

Sperm Reservoir

Within 8 h, 300,000 sperm (\( \approx 0.01\% \)) make it to UTJs. Sperm seem to “hang out” here, bind to epithelium, finish capacitation, & “wait” for ovulation? Sperm with abnormalities don’t tend to make it here or cannot bind.

3 billion sperm cervical AI

Within 2.5 h post-AI
70% of volume
25% of sperm ejected
(Steverink et al., 1998)

Within 4 h, most sperm (\( \approx 60 \) to 75%) not ejected are phagocytized by uterine immune response; seminal plasma seems to suppress & improve transport.

Within 8 h, 30,000 sperm (\( \approx 0.001\% \)) reach AIJs (site of fertilization). Reportedly not influenced by number of sperm/dose.

Uterine Diagram: Marrable, 1971 (the embryonic pig)
Sperm at Diff Uterine Sites: Willenburg et al., 2003
Business Overview

- Fast Genetics (“Fast”) offers maternal and terminal swine breeding livestock, producing over 80,000 hogs annually
- Genotypes all animals raised in the nucleus, using proprietary genomic evaluations in order to select for the most economically important traits
- Acquired by Sexing Technologies in 2015

History and Timeline

- Founded in 1982 by the Fast family in Saskatchewan, Canada
- Original stocking of animals was done by placing caesarean derived piglets into the nucleus farm
- In 2006, Fast became a wholly-owned subsidiary of HyLife, one of Canada’s largest pig producers

Breeding Pyramid

- Total genetic effect on 12 million slaughtered pigs per year

Sample Genetic Lines

- Fast 275 is a durable female with exceptional prolificacy, growth, and feed efficiency
- Fast Duroc has been selected for growth, feed efficiency, yield, loin size, and backfat thickness