RESEARCH UPDATE IN ARTIFICIAL INSEMINATION AND SEMEN PROCESSING

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Anand 2014

Research Overview:

- Research in processing and packaging
- Research in semen analysis
- Cryobiology
- Physiology of breeding animals
- AI practices
- Fate of sperm in the cow
- Embryo quality

Research selected to make specific points:

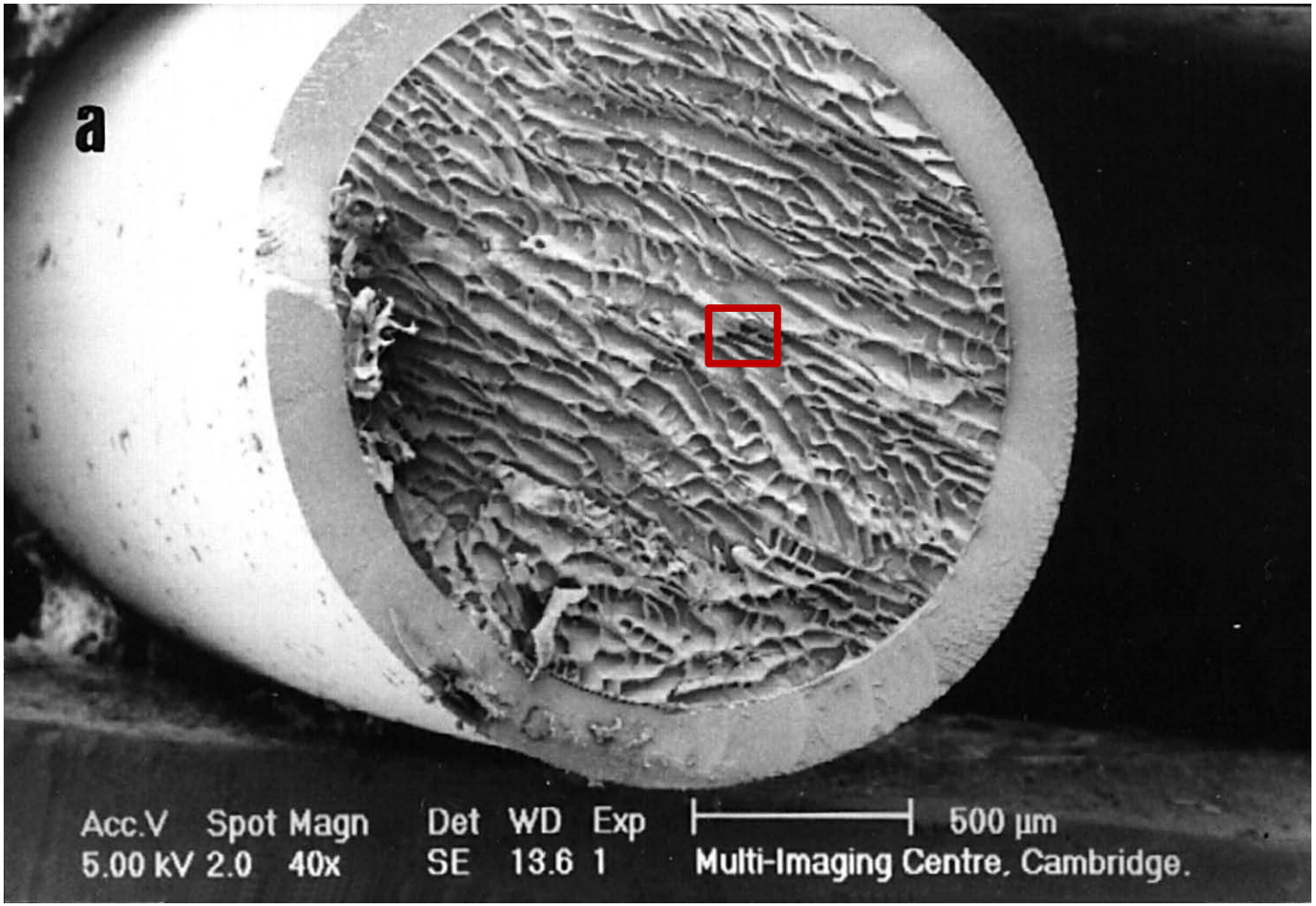
Part one:

Research in processing and packaging

We know that all sperm *are not now* being frozen at optimum rates, due to inexactness of controlling the freezing curves for individual straws and groups of straws, and the physical geometry of the straw.

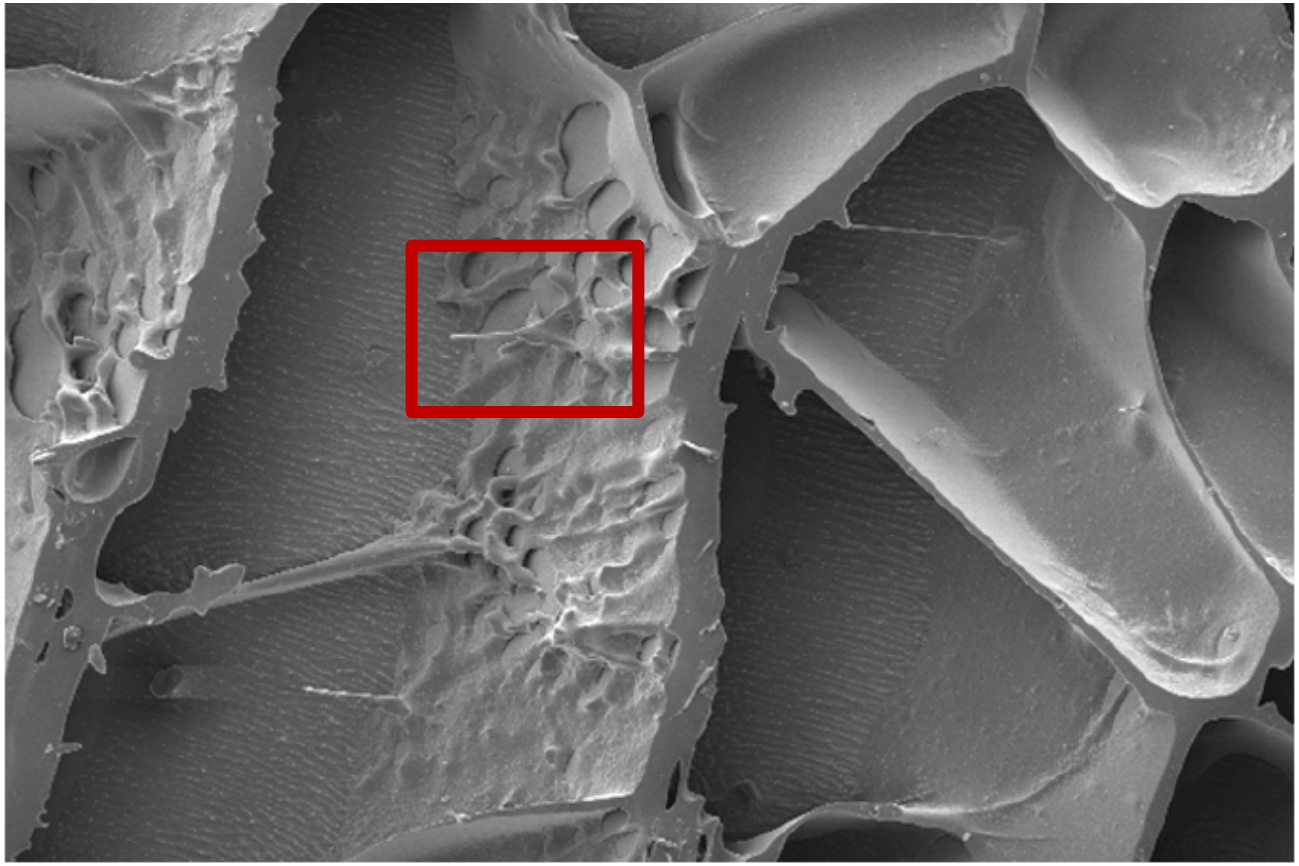
Research?

Sperm frozen in straws:



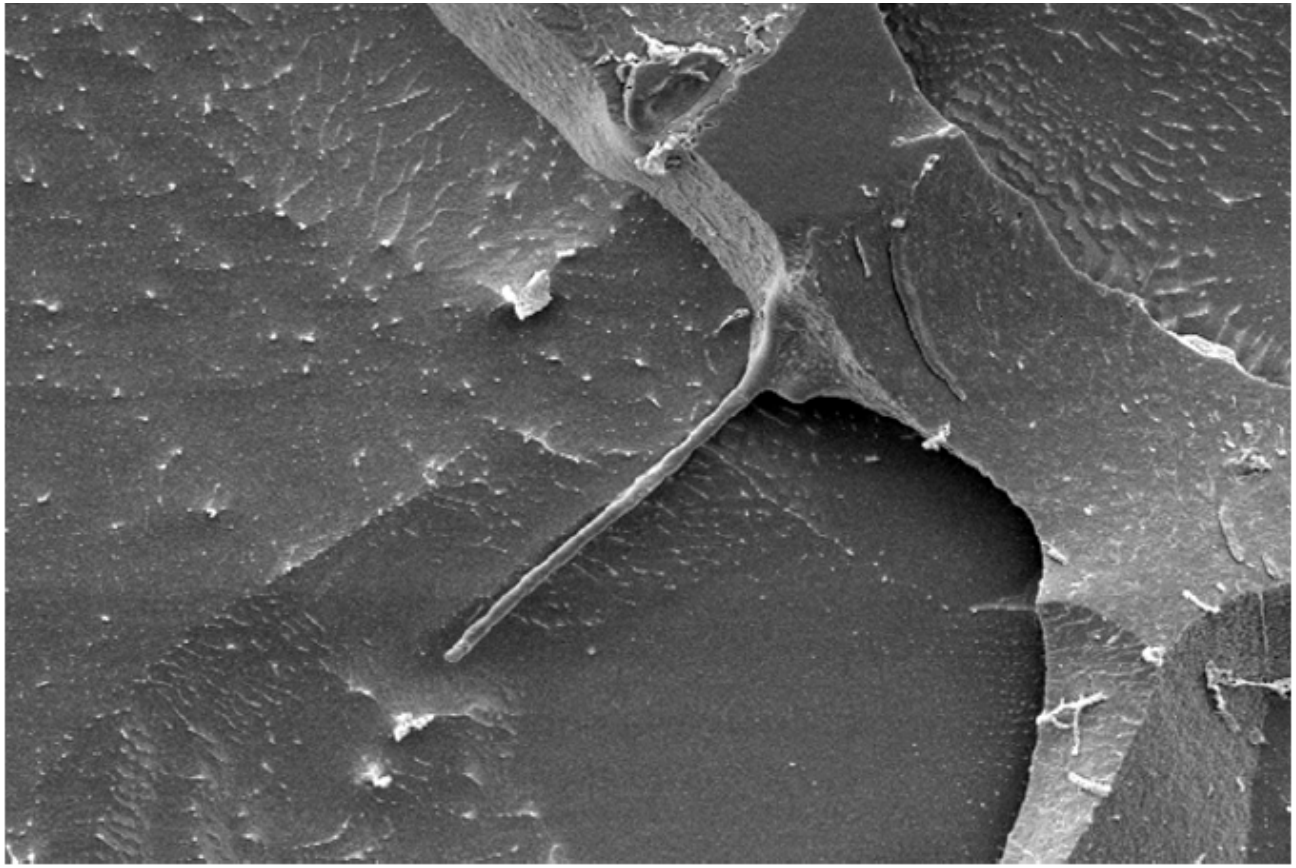
Morris, G. J., E. Acton, and S. Avery. 1999. A new approach to sperm cryopreservation. Human Reprod. 14:1013-1021.

Sperm frozen in straws:



Morris, G. J. 2006. Rapidly cooled human sperm: no evidence of intracellular ice formation. Human Reprod. 21:2075-2083.

Sperm frozen in straws:



Morris, G. J. 2007. Asymptote guide to cryopreservation. Asymptote Ltd. 2:1-42.

Semen Freezing Concepts

- Turbo Freezer
- Rotating Drum Freezer
- Directional Freezer

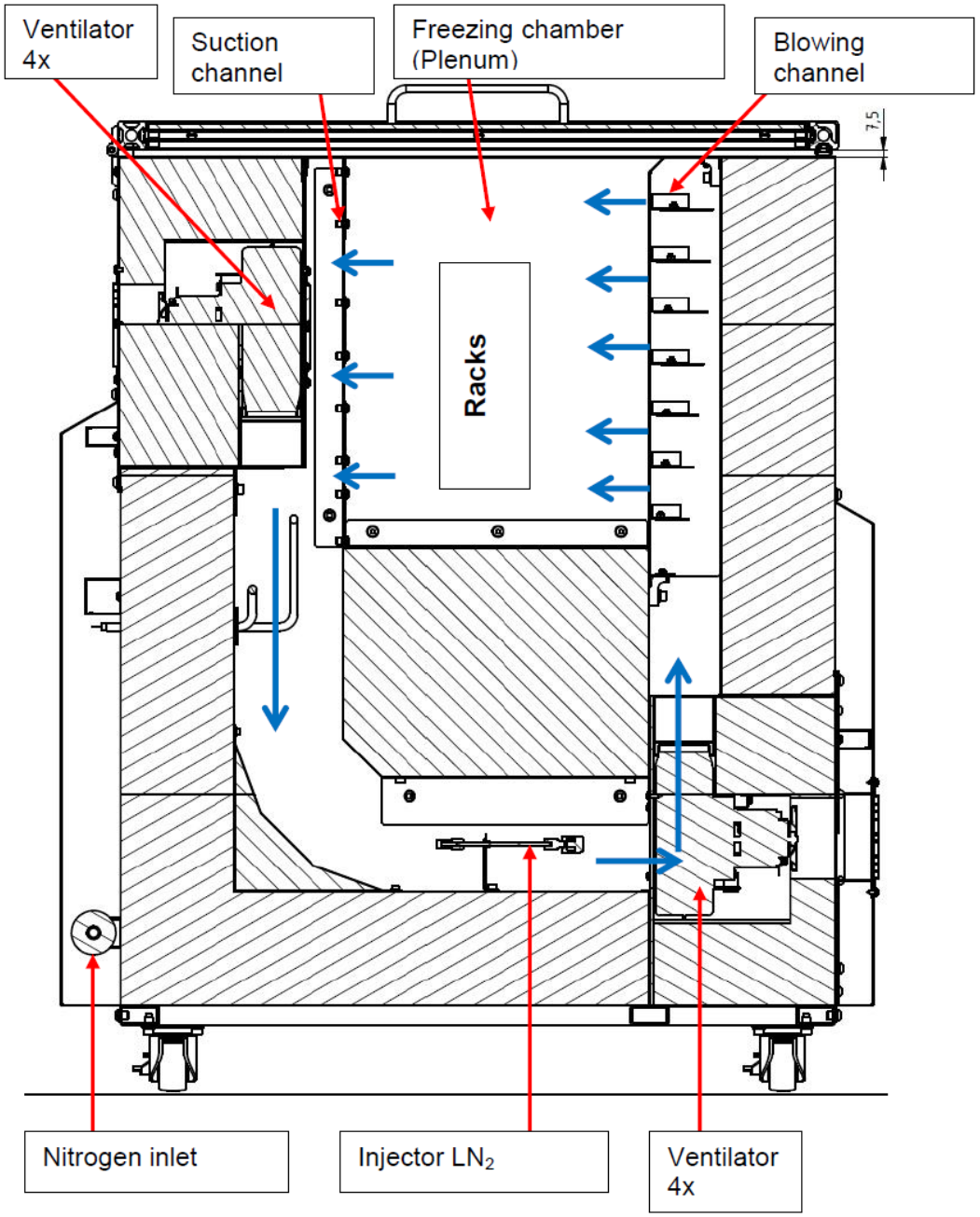
Semen Freezing Concepts

Turbo Freezer

Turbo Freezer

All straws frozen at same rate

Minitube trials

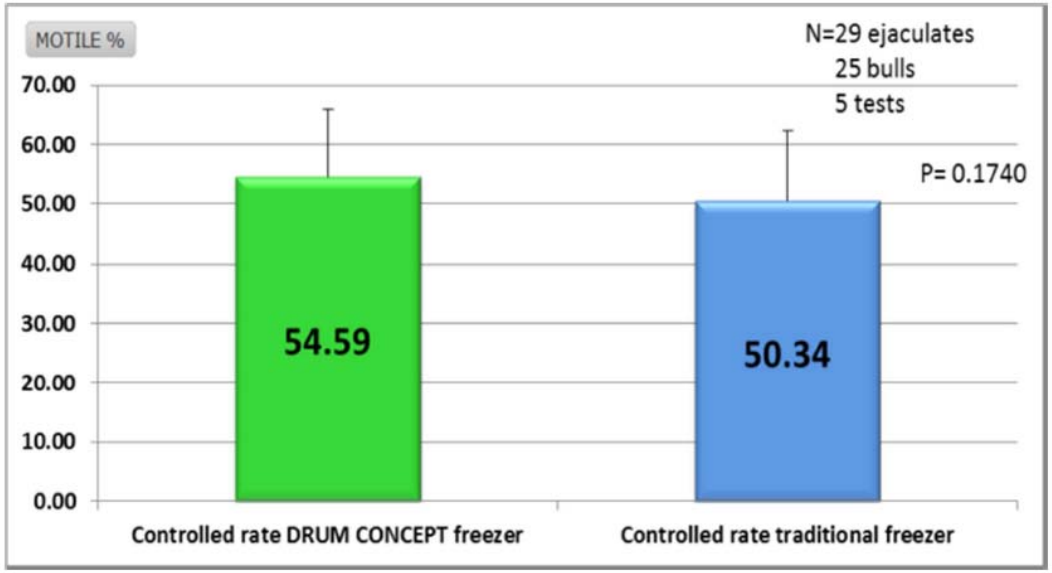


Semen Freezing Concepts

Rotating Drum Freezer

- IMV
- 4.2% increase post thaw motility

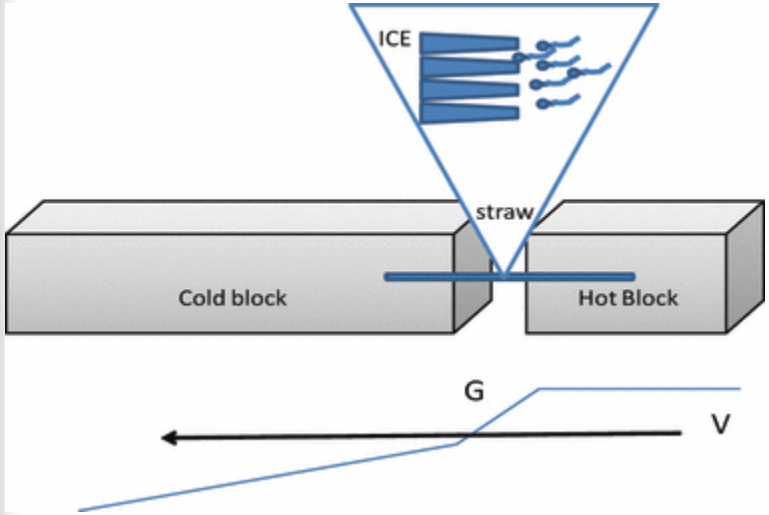
All sperm in a straw frozen at same rate

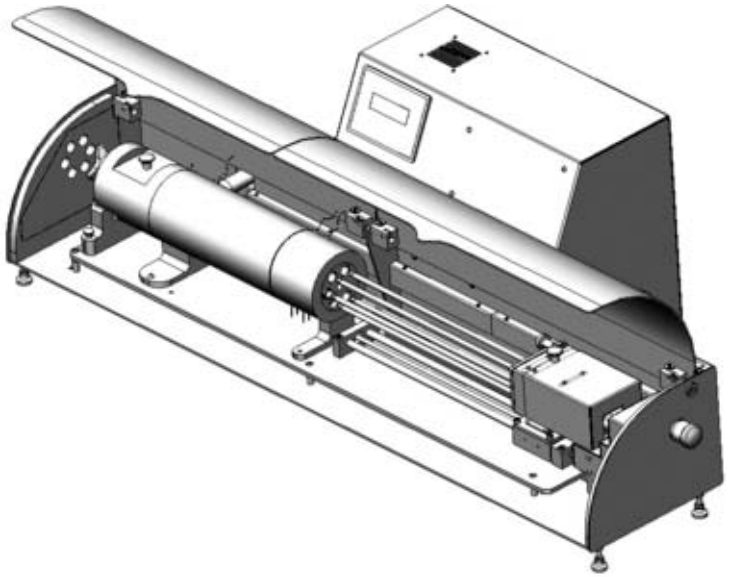


Agnes Camus, Christian Beau, Eric Schmitt. A rotating drum container as the basis for automation of straw freezing in the bovine semen producing laboratory. IMV-Technologies

Semen Freezing Concepts

Directional Freezer



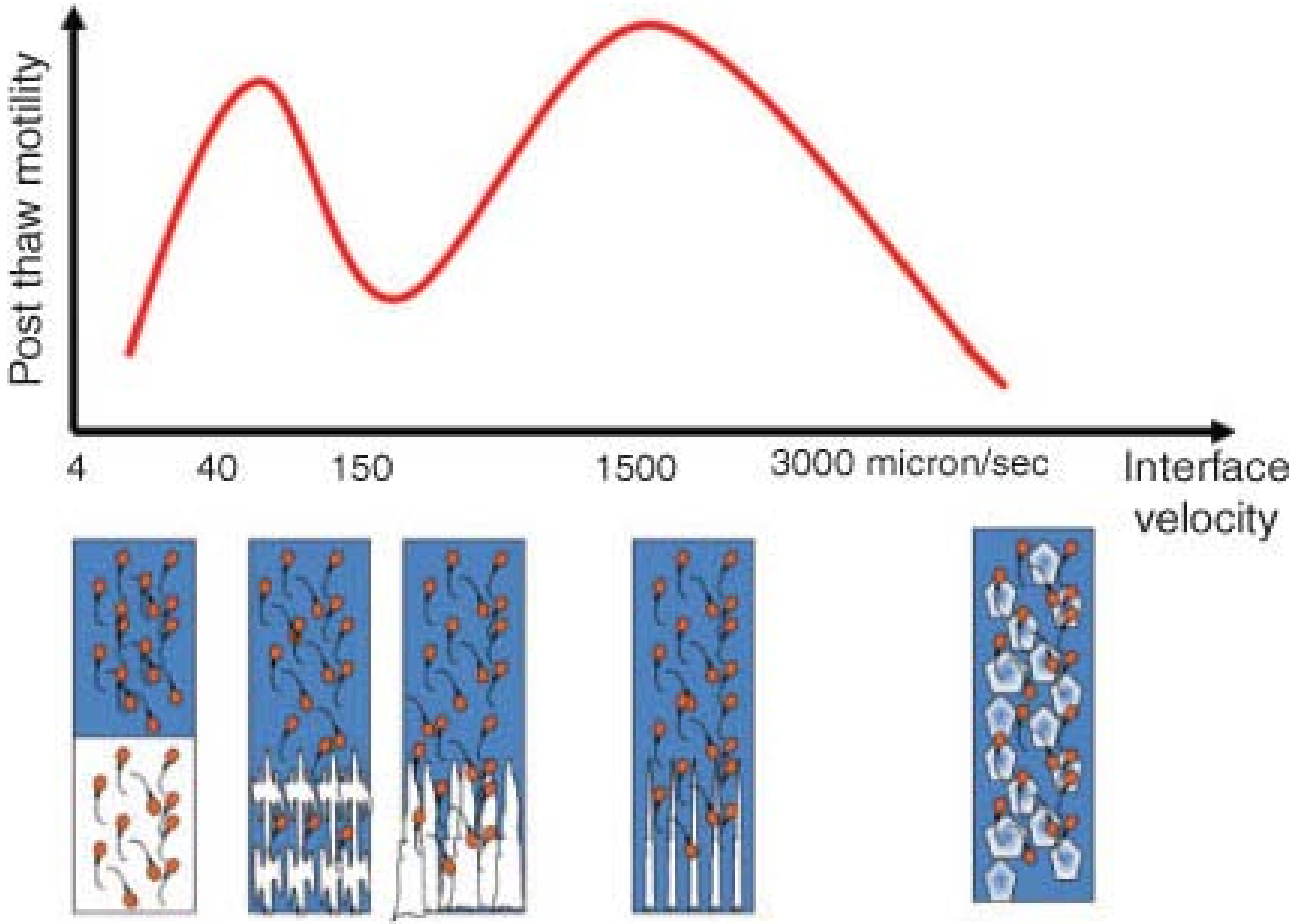


- Uniform wave of ice formation: avoids random ice damage. Trials (Japan): No benefit in total or progressive motility, but higher viability, and acrosome integrity

Arav, A., D. Natan. 2012. Directional freezing of reproductive cells and organs. Reprod. Dom. Anim. Vol 47 s4: 193-196.

Hayakawa, H., T. Yamazaki, M. Oshi, M. Hoshino, O. Dochi, and H. Koyama. 2007. Cryopreservation of conventional and sex-sorted bull sperm using directional freezing method. Reprod. Fert. Devel. 19(1) 176 – 177.

Arav and Saragusty 2014: Determined relationship of freezing velocity and PT sperm motility outcomes.



Encapsulation

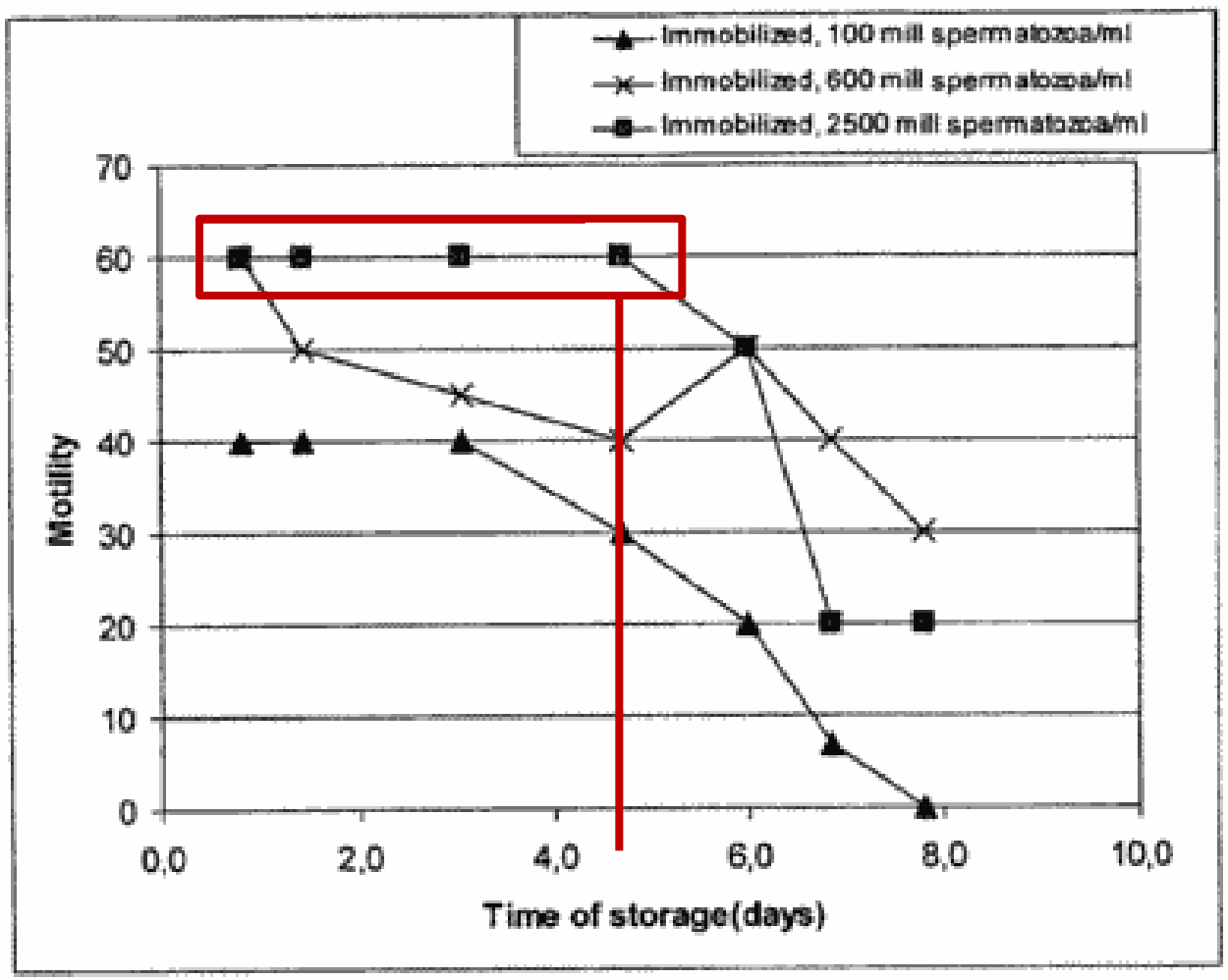
Put extended semen in capsules of cellulose…

or a

Biopolymer like barium or calcium alginate gels.

Do sperm like to be crowded together? Maybe…

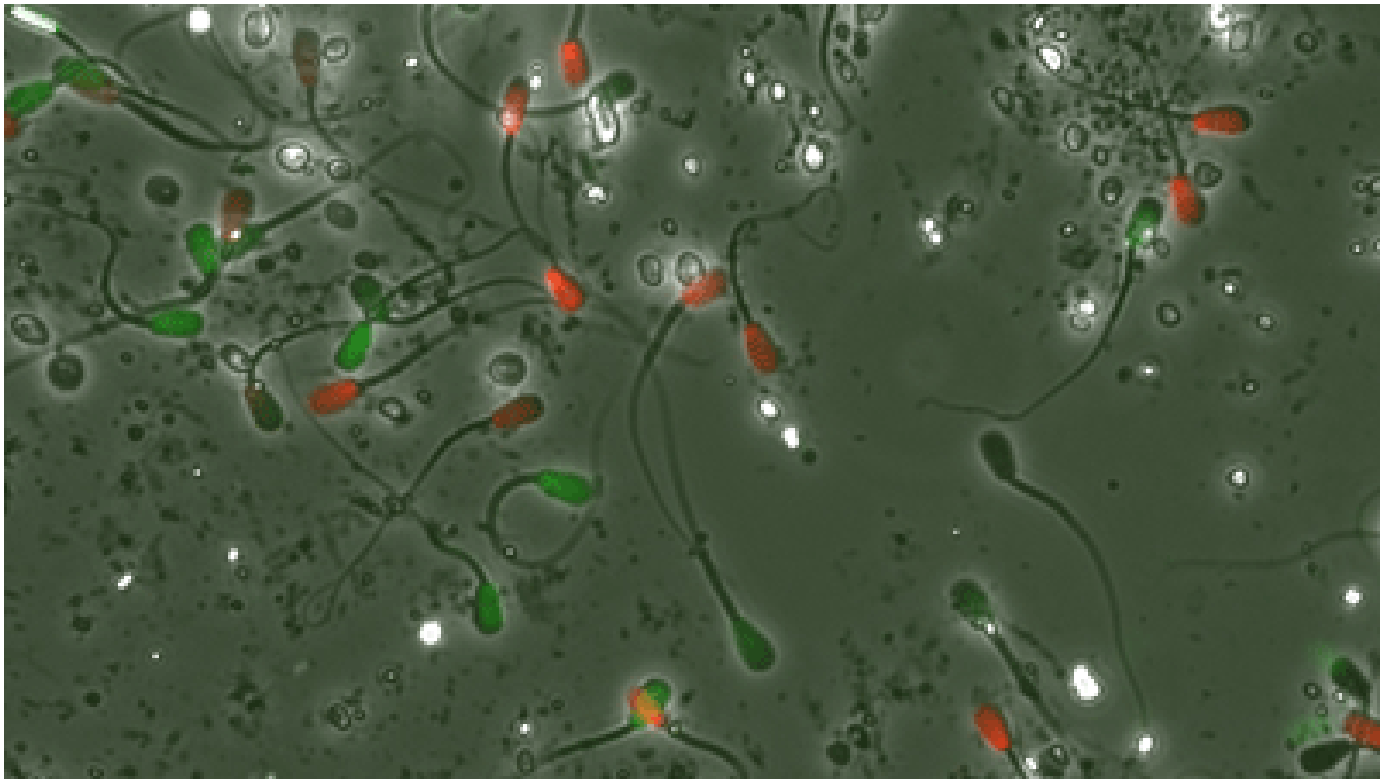
High concentration encapsulation: 2.5 billion sperm/mL for 5 days (swine).



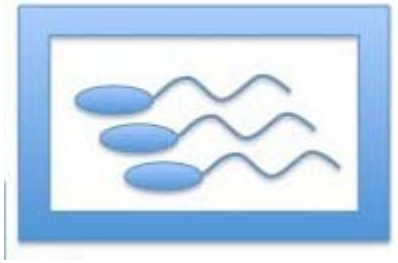
But how should sperm released? … Mechanical, or by controlled release?

US Patent 8178130; May 15 2012

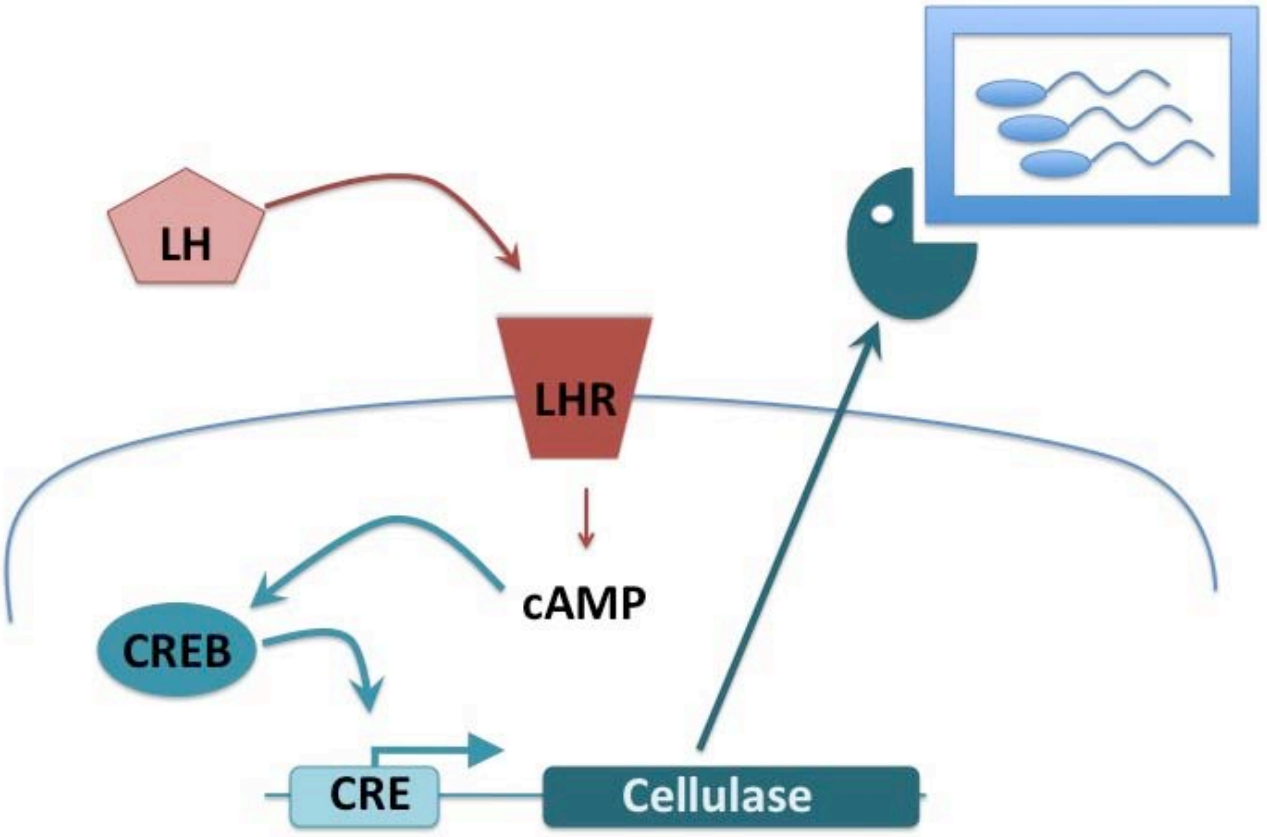
“SpermVital”, Geno, image of 18 hr alginate-stored sperm.



But how are sperm released?



How to release cellulose encapsulated sperm: uses ovulation associated LH surge



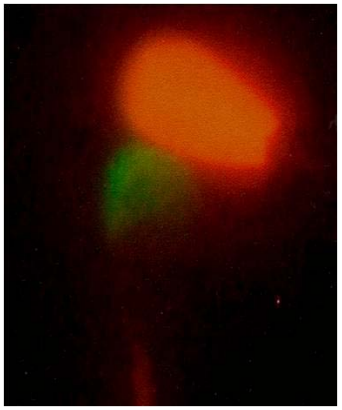
A SwissGenetics idea

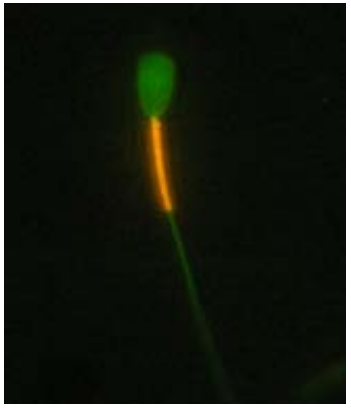
Part two: **Research in semen analysis.**

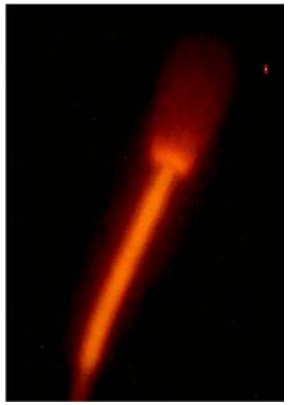
There are several roles for advanced semen analysis.

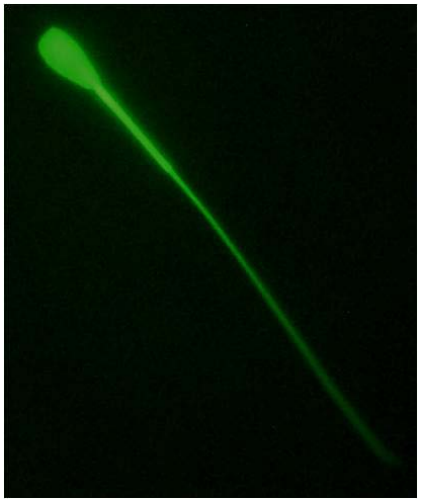
1. Confirm that everyday duties in semen processing and routine QC procedures are in control and functioning as planned.
2. Identify and remove semen batches which should not be used.
3. Identify those bulls who should not be used (to be culled).
4. Identify those bulls who have both superior genetics, and whose semen can safely be more greatly extended.

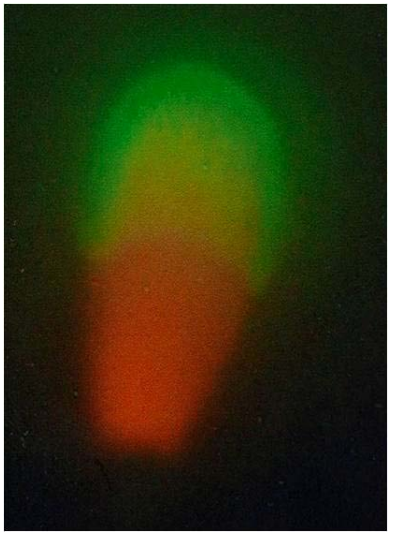
Flow cytometric analysis











Images courtesy IMV EasyCyte Program.

Flowcytometric analysis

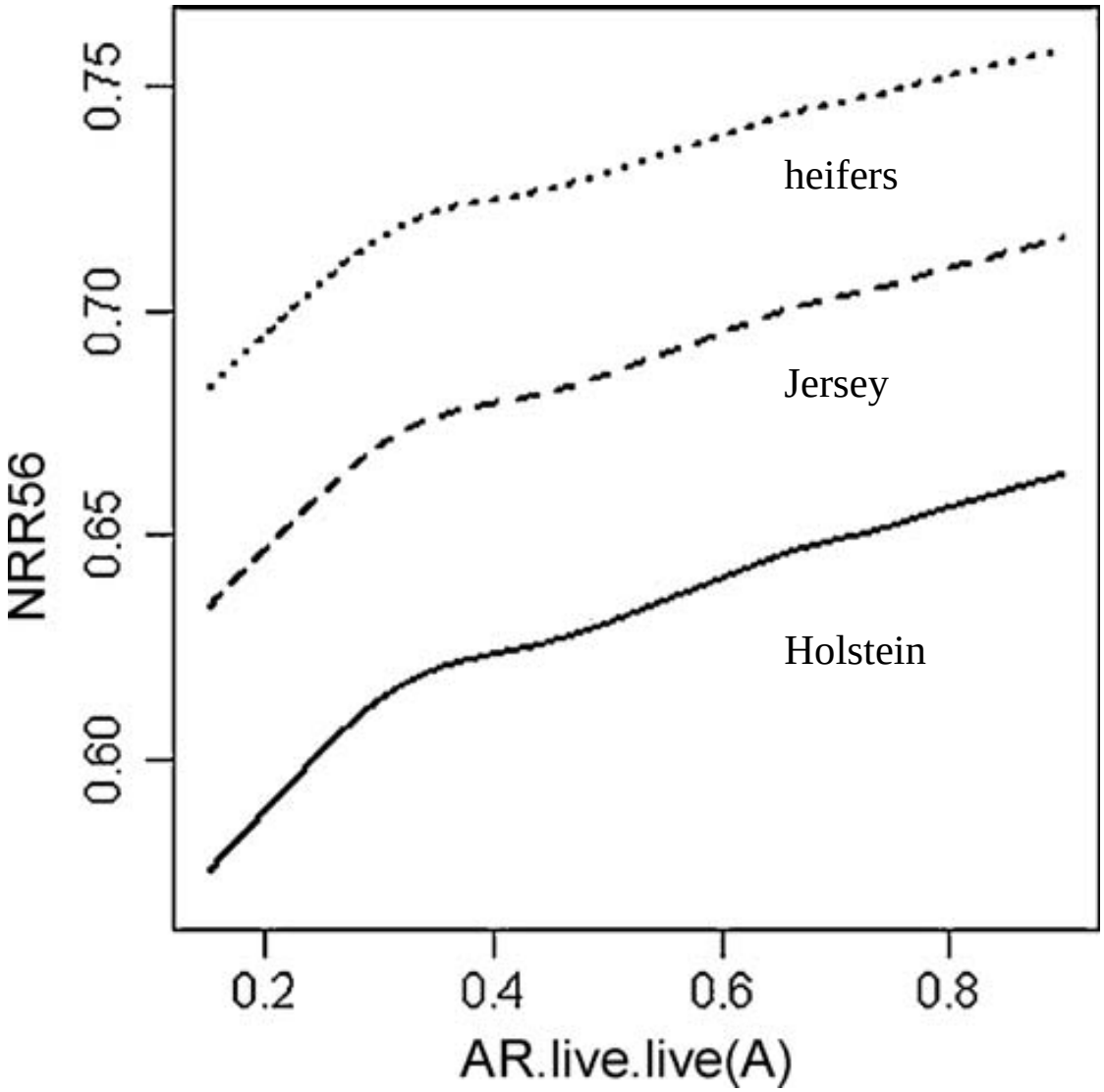
- Viability assay
- Plasma integrity and acrosome membrane integrity
- Cell counting
- Mitochondrial membrane potential
- Lipid peroxidation
- DNA quality by SCSA

  (Sperm Chromatin Structure Assay)
- DNA quality by TUNEL

  (Terminal transferase dUTP Nick End Labeling)

Improving the correlations to fertility …

…Measuring the induced acrosome reaction for young sire AI doses (Denmark)



% of live cells with induced acrosome response.

(Birck, et al., 2010)

In young sire AI doses:

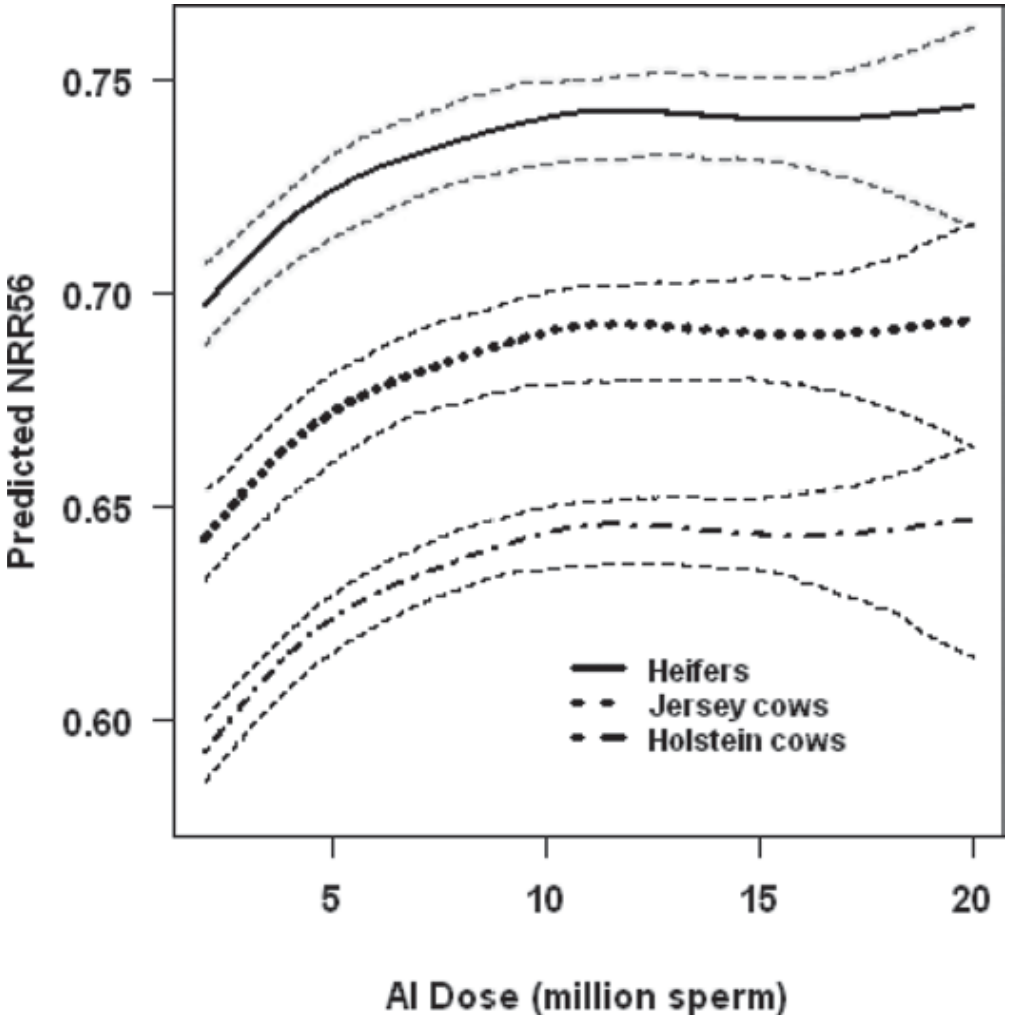
The concentration of sperm in neat semen, and its prefreeze viability (by flowcytometry) were very important predictors in final model predicting fertility. **Surprising information.**

When combined with post thaw viability by flowcytometry, the model became powerful for predicting young sire fertility.

Other measures of semen quality: acrosome response, sperm chromatin (DNA) assay, morphology were individually useful but did not provide additional predictive value past the above measures.

(Christensen, et al., 2011)

Sperm numbers above 10 million sperm per AI dose are not consistently helpful.



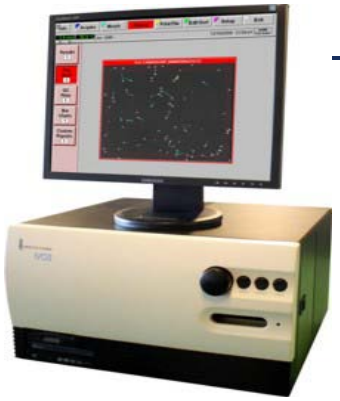
(Christensen, et al., 2011)

Correlation to fertility: Combined 50,000 AI outcomes with:

EasyCyte™

– Flowcytometeric assays

IVOS™
with Ident



– CASA: motility

DIC microscope

– Morphology

Correlation to fertility:

For assays of semen quality with fertility, Sellem et al., found that individual assays were uninformative, but combining outcomes could create a model correlation to fertility of 0.69.

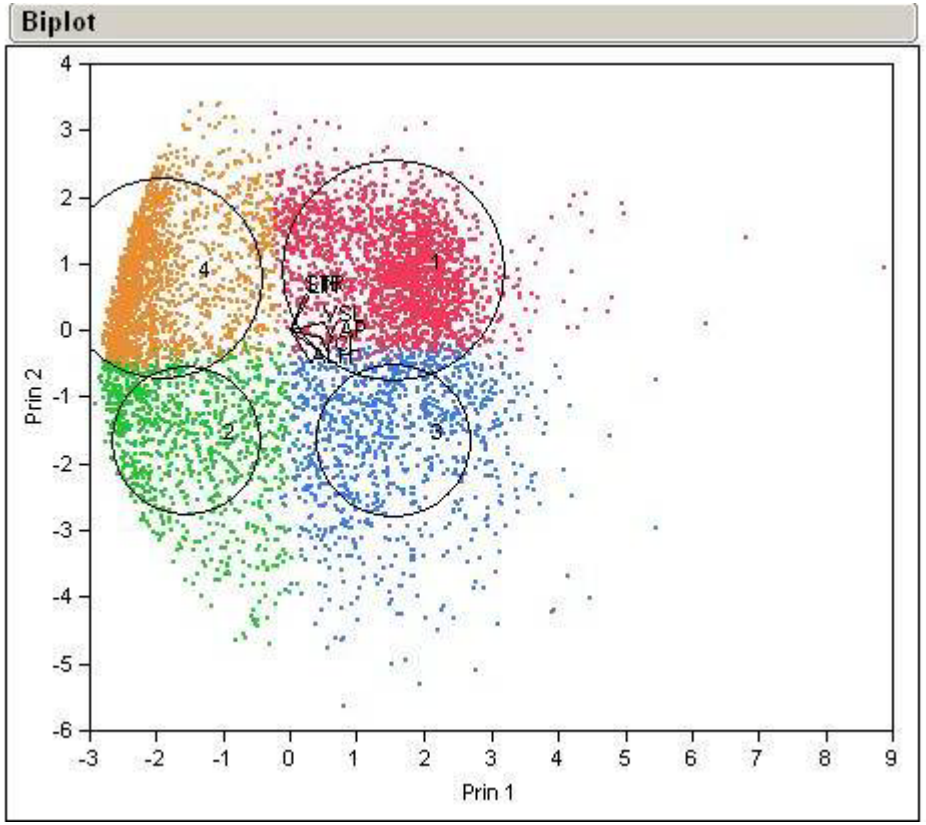
1. CASA: Post thaw motility
2. DIC microscope: sperm morphology
3. Flow cytometer:
    - Acrosome integrity
    - Oxidative damage
    - Mitochondrial activity
    - Chromatin (DNA) integrity

Sellem, E., L. Chevrier, S. Camugli, O. Gérard, E. Schmitt, and C. Ponsart. 2012. Use of in vitro assessed semen quality criteria to predict fertility of bull semen. Reprod. Dom. Anim. Vol 47, s4.

Semen analysis by CASA

- Computer assisted sperm analysis
- Measurement
  - Point curve velocity (VCL)
  - Straight line velocity (VSL)
  - Average path velocity (VAP)
  - Straightness (STR=VSL/VAP)
  - Linearity (LIN=VSL/VCL)
- Subpopulation research is common

Semen analysis by CASA…
…but understanding comes from experience



Factor 1

Factor 2

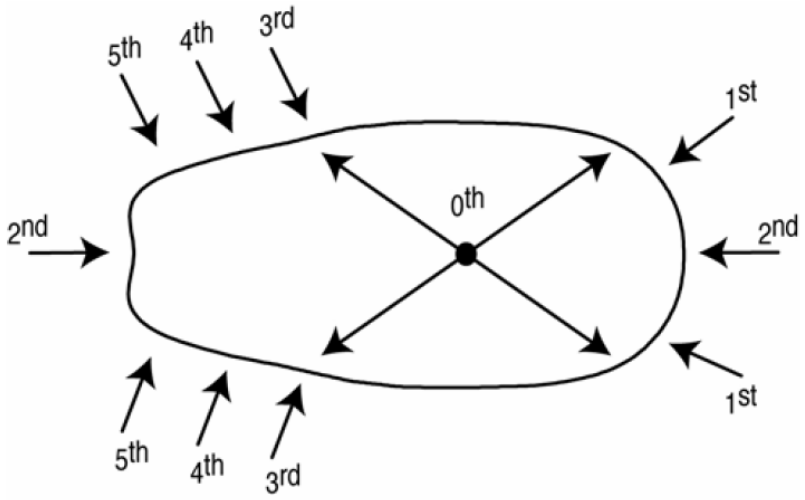
Nucleocounter for verifying sperm numbers

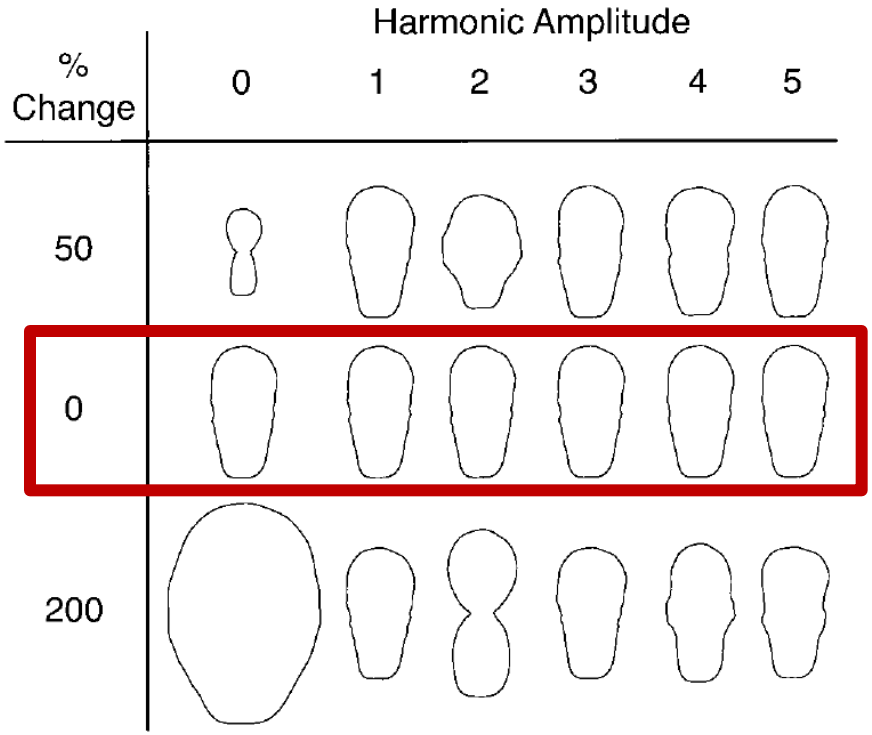
Nucleocounter for verifying sperm numbers

- Excellent for counting Sperm cells/mL in neat semen or from processed straws.
- Sample gets Hoecsht stain, which labels sperm nuclei. Sample travels in microfluidic channel to fluorescence reader. Obtained fluorescence level is proportional to sperm concentration.
- Pay attention to details:
  - Watch for operator error in dilution rates, dilution accuracy
  - For unknown reasons, it lacked hemacytometer agreement at some cell concentrations. (Anzar, et al., 2009)
  - Like all counting chambers, it is sensitive to *Segre-Silberberg* effects and corrections (Kuster and Althouse 2010).

Relationship of sperm head shape to fertility

- Harmonic amplitudes to analyze head shape





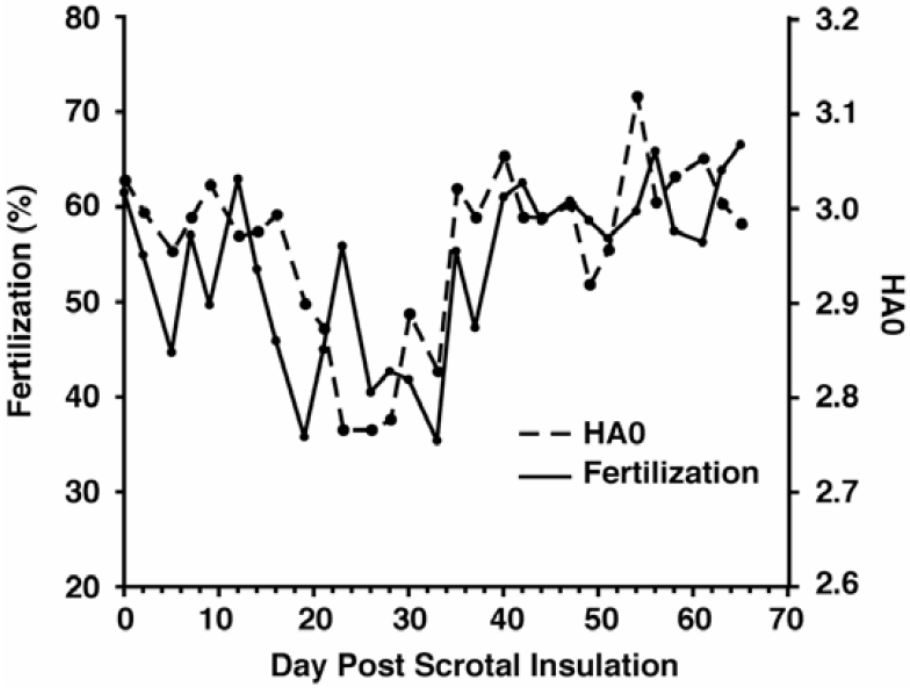
Parrish, J., L. Enwall, A. Kaya, C. Pawshe, A. Siddiqui, and M. Shamsuddin. 2006. Sperm shape research: An update. Proceed. 21st Tech. Conf. Artif. Insem. Reprod. NAAB. 19-26.

Ostermeier, G. C., G. A. Sargeant, B. S. Yandell, J. A. Parrish. 2001. Measurement of bovine sperm nuclear shape using fourier harmonic amplitudes. 22:584-594.

Relationship of sperm head shape to fertility

- Harmonic amplitudes (HA0) assay.
- When combined with other assays, correctly ID'd low fertility bulls: all 32 of 210 NAAB bulls.
- Also finds heat stress damage:

Heat stress: 48 h of scrotal insulation; test span was 3 weeks prior, and following insulation. Confirmed with ability to penetrate oocytes in vitro.



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Part three:

Cryobiology

In both of the studies following, with LDL/cholesterol, and cyclodextrin/cholesterol, cryopreserved semen was produced with altered cholesterol to phospholipid ratios. It is expected that changes in the cholesterol to phospholipid ratios will also alter the in vivo performance of the sperm with respect to timing of AI and its relationship to ovulation.

Low density lipoprotein (LDL) production

The Low Density Lipoprotein (LDL) portion of egg yolk contains most of the cooling protection and seminal plasma protection as used in AI. Bailey reviewed its characteristics and recovery from egg yolk. It is an attractive alternative to egg yolk. Parks reviewed the difficulties of transferring cholesterol from egg yolk to sperm membranes and offered alternative tools to modify sperm membranes.

Coupled with new tools such as CLC (cyclodextrin loading of cholesterol), and other additions of cholesterol to the sperm membrane, we now have new tools for controlling sperm quality and function by adjusting sperm membrane phospholipids and cholesterol.

Bailey, 2010; Parks et al., 2010

Why should we adjust membrane cholesterol?

This study (Ram) similar to bull studies, but Ram sperm is more sensitive:

Cholesterol was added to neat semen with cyclodextrin molecules. Cyclodextrins, essentially hollow, transports cholesterol to the sperm membrane. Cholesterol loads up in the sperm.

CLC significantly improved PT motility: CLC at 62%, control at 43%.

CLC treated sperm maintained greater percentages of motile sperm during a 3 h incubation after thawing.

CLC treated sperm maintained greater percentages of motile sperm through a wide range of osmotic solutions (150 and 425mOsm) while control sperm lost motility in solutions outside a more narrow range (270 to 370 mOsm).

Moce, et al., 2010

Part four:

Physiology of breeding animals

Feeding bulls calves for sperm production

- Can you make up for poor early calfhood nutrition with a high plane of nutrition later?
- Likely not.

Trial: Beef calves weaned at 8weeks.

Two groups: Diet different only during 10th to 30th week

Calves received either:
control normal diet (conventionally fed to beef bulls)
or high nutrition diet (+ 58% crude protein; +11% energy)

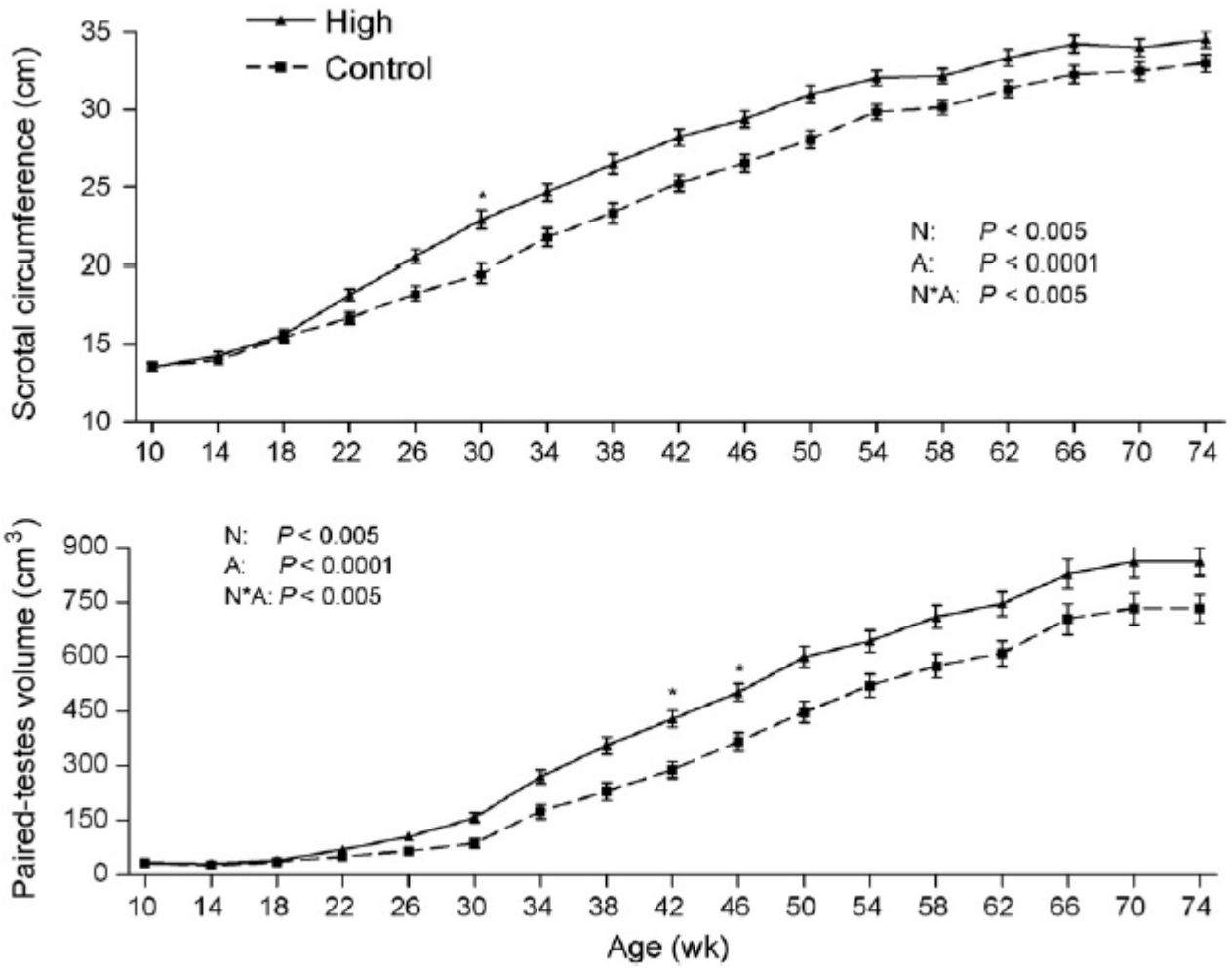
Thereafter received identical diet through 70th week.

Brito et al., also measured gonadotropins, serum hormones throughout.

But: Control bulls never caught up in testis development.

Brito et al., Domestic Animal Endocrinology 33 (2007) 460-469.

From 10-30 weeks calves (weaned 8 weeks) received control normal diet or high diet (+ protein, energy); thereafter received identical diet.



Testis development never caught up.

Heat Shock Protein 70 (Hsp70)

- Chaperone protein that increases during heat stress.
- In reproductive tissues during gametogenesis.
- Trials tested Hsp70 for semen quality and sperm production by season.
- Sires grouped by different Hsp70 SNP haplotypes.
- Certain haplotypes are associated with favorable semen quality values even during summer heat stress.
- Will it be useful to include this haplotype search when considering breeding stock for warm climates?

Patterson, J., G. Gilbert, M. Sales, C. Rosenkrans Jr. 2012. Effect of season of collection and heat shock protein 70 haplotype on semen quality characteristics of Holstein bulls. J. Anim. Sci. 90, Sup 1, p 12.

Part five:

AI practices

Best practices for using multiple straws: …….current practice recommendation

- As reviewed by NAAB:
  - Safe to thaw groups of straws when AI is completed within 10-15 minutes,

    AND…
  - Straws and AI equipment are protected from harmful environmental effects (solar, heat, cold).

New report about multiple straws: Brazil, tropical heat.

Three bulls (3 batches per bull and 1000 total inseminations), thawing one cane, 10 straws, at one time. All straws were deposited in cows over a span of 1-7 minutes, and fertility outcomes were recorded according to the sequence.

Results:
One bull did not maintain his level of fertility for his final (9^{th}, 10^{th}) straws relative to his fertility for earlier straws.

The other 2 bulls maintained unchanged fertility over these 7 minutes.

Tested straws of each batch in lab, mimicking the time sequence.

New report about multiple straws: Brazil, tropical heat.

Combining lab and fertility results?
Bull with depressed fertility: no change in incubated quality.
Bull who dropped in incubated quality: no change in fertility.
Third bull unchanged in both fertility and incubated quality.

What's going on here?

Investigator recommends now that due to high environmental temperatures, overheating of sperm during AI may be occurring.

Message: Protect sperm and AI equipment from solar and high temp conditions. Can't predict from lab measures how individual bull's sperm will react to stressful conditions.

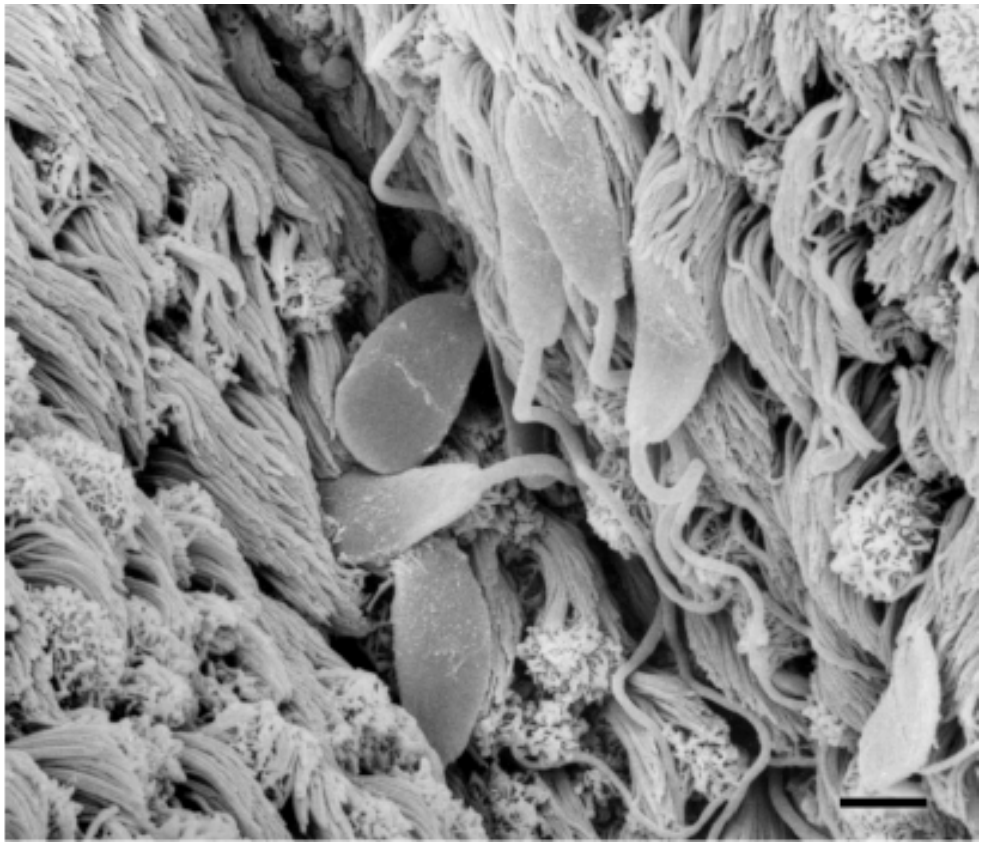
Oliveira, L., et al., 2012. Therio. In press.

Part six:

Fate of semen in the cow

More is now known about the bovine sperm reservoir created in the cow following AI, populated by sperm, and at ovulation becomes the functional sperm population available for fertilization.

Nature's plan for sperm reservoir release



Bovine sperm in oviductal epithelium.

Lefebvre et al., 1995. BOR 53: 1066-1074

Seminal plasma proteins needed for sperm reservoir release

- Inseminated sperm attached to the oviductal epithelium is the bovine sperm reservoir.
- In vivo, it takes more than 6 hours for bull sperm inseminated by natural service to become capacitated.
- Sperm undergo capacitation in order to be able to fertilize eggs.
- When capacitated, bull sperm begin to loosen their binding to oviductal epithelium.
- This loss of binding releases sperm from the storage reservoir and they can resume moving to the site of fertilization.

- Release of sperm due to loss of one of 3 seminal plasma proteins (example this research report: BSP PDC-109) which happens during capacitation.
- Each BSP protein likely responds differently to capacitation. Why?
- The function of differential responses of the three BSP proteins may be to regulate the gradual release of sperm from the oviductal storage reservoir and direct their subsequent movement toward the egg.

Hung, P., S. Suarez. 2010. In: Reproduction in domestic ruminants VII. M. Lucy, J. Pate, M. Smith, T. Spencer, eds. Nottingham University Press. pp 257-266.

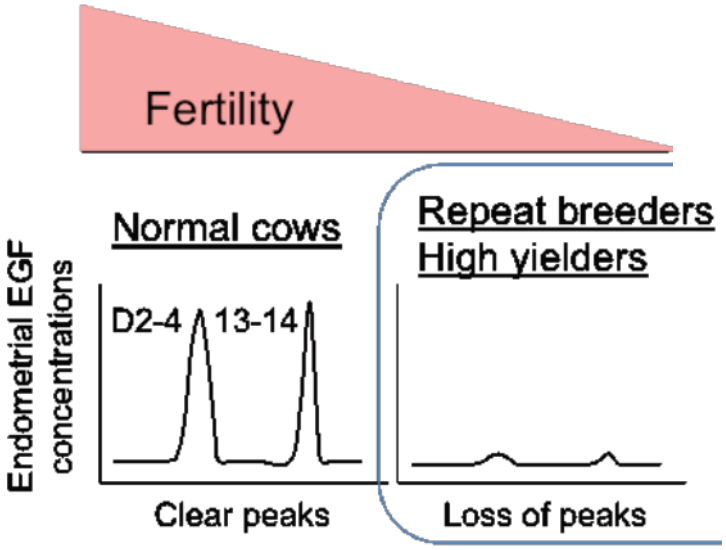
Nature's plan for sperm reservoir release

- This is an elegant picture of how sperm behave following natural service.
- With sperm release triggers at differing times allowing a steady population of sperm to meet the oocyte.
- This group determined that processing and cryopreservation changes the abundance of binding proteins associated with sperm.
- How do we modify AI processing to better mimic nature?

Ardón and Suarez. 2012

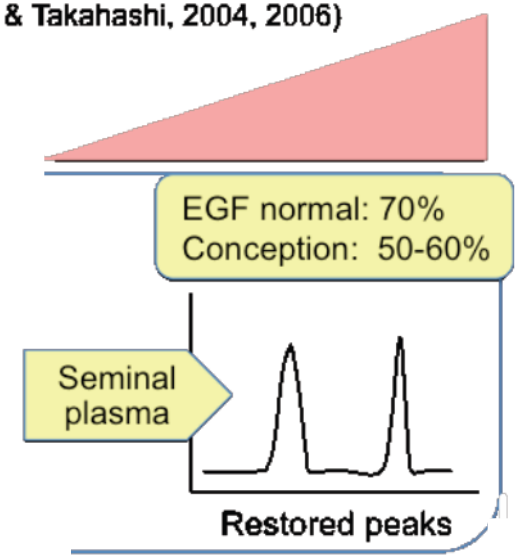
Repeat breeder cows have abnormal cycles of epidermal growth factor (EGF)

- Uterine EGF indicator of fertility problems



(Katagiri & Takahashi, 2004, 2006)

- EGF pattern restoration
  - Infusion of uterus with seminal plasma on Day 0
  - Improved fertility with restored peaks



(Katagiri, 2010)

Katagiri, S., G. Gokarna, and M. Moriyoshi. 2012. Treatment with seminal plasma (SP) modulates the endometrial cytokine network and improves fertility in dairy cows. Reprod. Dom. Anim. Vol 47, s4: 558-559.

Part seven:

Embryo quality

An estimated **30%** of fertilized oocytes do NOT go on to produce a successful pregnancy. Are there *male-specific factors* involved in establishing the quality of the resulting embryo?

Sperm factors introduced at fertilization

- Sperm-borne protein, PLC ζ
  - May stimulate calcium oscillations that are required for oocyte activation.

    (Ito, et al., 2011)
- mRNA transcripts (and possible translation)
  - To synthesize novel proteins that may play a role in storage in sperm reservoir, during capacitation, and post fertilization.

    (Gur and Breitbart, 2008)
- First complete transcriptome of sperm mRNA
  - Found transcripts for genes of all areas of physiology and maturation

    (Card, et al., 2012)

Conclusions

- Research is improving:
  - Post-thaw semen quality
    - Establish functional uniformity between bulls and batches
    - Allow better defined sperm function
- Additional research:
  - Fully utilize genomic opportunities
  - Molecular biology
    - High embryonic mortality rate