Session 29

The Past, Present, and Future of the Breeding Stallion

The Forces of Darkness and the Gathering of Light

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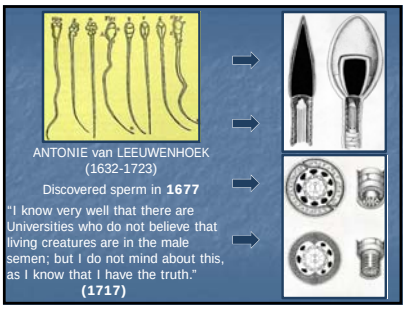
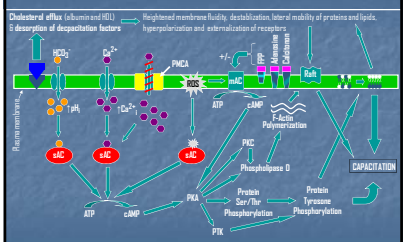


ANTONIE van LEEUWENHOEK
(1632-1723)

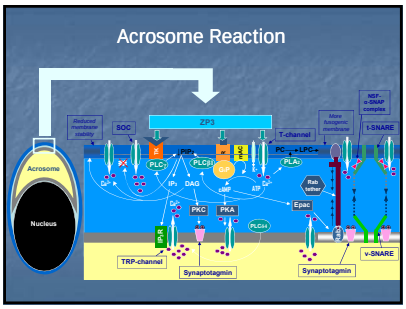
Discovered sperm in **1677**

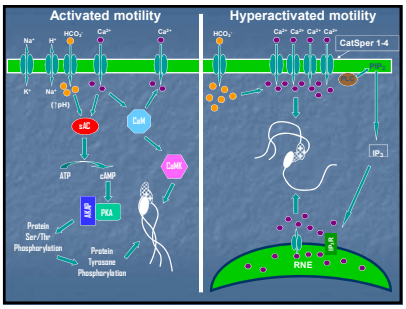
"I know very well that there are Universities who do not believe that living creatures are in the male semen; but I do not mind about this, as I know that I have the truth."
(1717)

Spermatozoal Capacitation



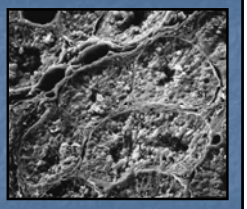
Acrosome Reaction



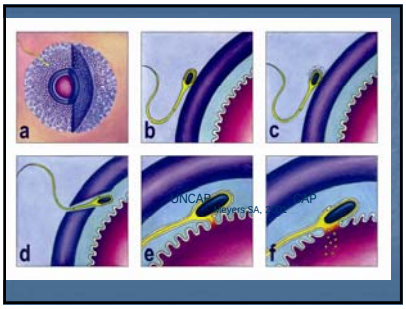


Control of Spermatogenesis??

Classical
- GnRH
- FSH
- LH
- Estrogens
- Testosterone
- Inhibin



Additional pathways: Paracrine, juxtacrine, autocrine, cryptocrine, lumicrine



Clinical Considerations
Breeding Soundness Examination

Traditional tests	Additional tests
Sperm number	Sperm chromatin quality
Concentration x volume	Acrosomal integrity and function
Daily sperm output	Plasma membrane integrity
Sperm morphology	Mito. membrane potential
Sperm motility	Capacitation ability
Testicular size	Oxidative injury
Semen contaminants	Apoptosis-like changes

Chromatin Integrity

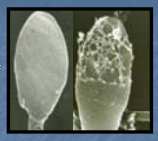
- Sperm Chromatin Structure Assay (SCSA)
  - Flow cytometer

- TUNEL (TdT-mediated-dUTP neck end labeling) Assay
  - Fluorescence microscopy

- Protamine Assay
  - Immunofluorescence

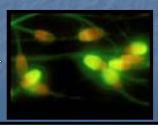
Acrosomal Integrity and Function

SEM →



←TEM

PSA-PI →



Membrane Impermeant Dyes

Eosin Y
Propidium iodide
Hoechst 33258
YO-PRO-1
Ethidium homodimer

Bind with DNA

Cytoplasm

Membrane Permeable Dyes

SYBR®-14 / PI

Carboxyfluorescein diacetate → carboxyfluorescein

Calcein AM → calcein

SYBR®-14

Cytoplasm

Spermatozoa with inability to acrosome react

- Determine differences in surface proteins and solubilized proteins of spermatozoa.
- DIGE (Differential in Gel Electrophoresis)

In collaboration with:

Dr. Nuch Tanphaichitr
Ottawa Health Research Institute
and
Dr. Peter Yau
University of Illinois

Other clinical assays on the horizon

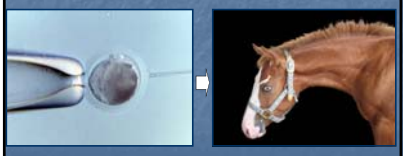
- Capacitation
  - Heparin-binding proteins
  - Protein phosphotyrosine activity
  - Annexin V
  - SP-20 (hyaluronidase)
- Oxidative injury
  - C_{11}-BODIPY (581/591)
  - Reactive oxygen species
- Sperm-cumulus interaction
  - SP20 (hyaluronidase)
- Sperm-zona binding
  - AWN spermadhesion protein
  - P34H protein
  - Zonadhesin
- Sperm-oocyte interaction
  - CRISP proteins
  - PLC zeta

Advancements in Semen Technology Processing and Preservation

Procedures

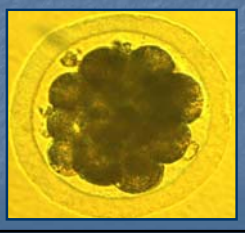
- Cushioned centrifugation
  - Iodixanol
- Gradient centrifugation
  - Silica-particle solution
- Low-dose insemination
  - Trans-rectally guided technique

Intracytoplasmic Sperm Injection (ICSI)



Preservation of Spermatozoa
Lyophilization

Preservation of Spermatozoa Cloning (Nuclear Transfer)



The Evolution of Sperm Science

What can we expect?

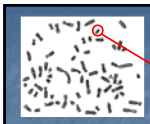
- An "omics" era
  - Genomics
  - Toxicogenomics
  - Transcriptomics
  - Metabonomics
  - Proteomics

Male Fertility & Molecular Genetics

Bhanu Chowdhary & Terje Raudsepp

- Critical male fertility genes on Y chromosome
- Females don't have them
- Critical for testicular development and spermatogenesis
- Detailed Y chromosome map essential!

ECAY



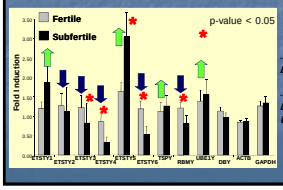
~2% of the genome
~15 Mb euchromatin

35 genes in MSY

- 11 novel genes – no counterpart in other species
- 12 genes with multiple copies on ECAY
- 9 genes with testis specific expression

Y genes & stallion fertility

- qRT-PCR to compare expression levels in testis from:
  - 10 fertile stallions
  - 14 stallions with various fertility problems
  - 9 testis-specific genes + *DBY, ACTB, GAPDH*



Subfertile males:

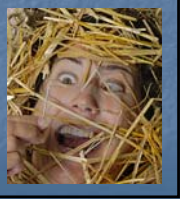
-5 genes ↓-regulated: *ETSTY2-4, 6 & RBMY*

-4 genes ↑-regulated: *ETSTY1, 5, UBE1Y and TSPY*

The rest of the genome?

- Genome size: 2.47 Gb (expected 2.7 Gb)
- Genes: ~20,322 (~16,617 human orthologs)

Genome-wide approaches used to find fertility genes of interest

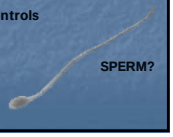


Equine WG expression oligoarray

Texas A & M, Bhanu Chowdhary

- 21,500 genes and ESTs
- 70-mer oligos
- Genome coverage ~ 96%
- Transcriptional profiles
  - between different tissues
  - between clinical cases and controls
- RNA - which tissues?

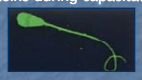
TESTIS –procurement invasive
-Surgery
-Biopsy



SPERM?

Messenger RNA in Sperm

- Active genes produce sperm mRNAs in the testis... so sperm mRNAs may provide a window through which we can view testicular function.
- Sperm mRNA can be translated into new proteins during capacitation.

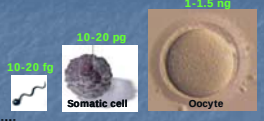


Gur Y & Breitbart H, *Genes & Development*, 2006

- Sperm mRNA may also play a role in early embryonic development

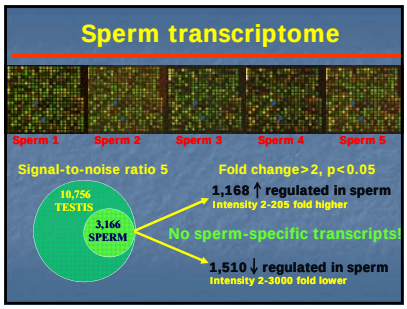
Therefore, it may be possible to develop a spermatozoal mRNA fingerprint as a diagnostic and prognostic tool for stallion fertility evaluation.

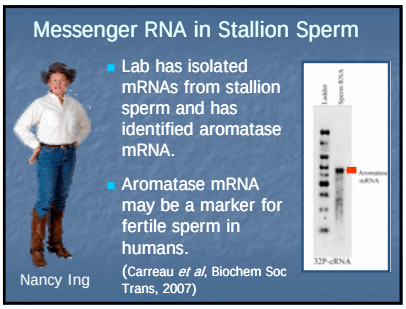
Sperm RNA quantity



Million sperm
↓
10-20 ng total RNA
↓
RNA linear amplification
↓
20-60 µg sense RNA

→ 300 ng-5 µg mRNA per microarray hybridization


Sperm transcriptome
Sperm 1
Sperm 2
Sperm 3
Sperm 4
Sperm 5
Signal-to-noise ratio 5
Fold change>2, p<0.05
10,756
TESTIS
3,166
SPERM
1,168 ↑ regulated in sperm
Intensity 2-205 fold higher
No sperm-specific transcripts!
1,510 ↓ regulated in sperm
Intensity 2-3000 fold lower


Messenger RNA in Stallion Sperm
Lab has isolated mRNAs from stallion sperm and has identified aromatase mRNA.
Aromatase mRNA may be a marker for fertile sperm in humans.
(Carreau et al, Biochem Soc Trans, 2007)
Nancy Ing
32P-cRNA


We need to learn a new language
Nucleofection
Gene silencing
Primers
Gene expression
Gene transfer
mRNA
Single nucleotide polymorphism
Transgenics
Real time RT-PCR
Comparative maps
Amplification cycles
Gene knockout
Annealing temperature
Oligonucleotide
Bioinformatics
Reverse transcription
Differential gene expression
cDNA microarray
Molecular subtyping