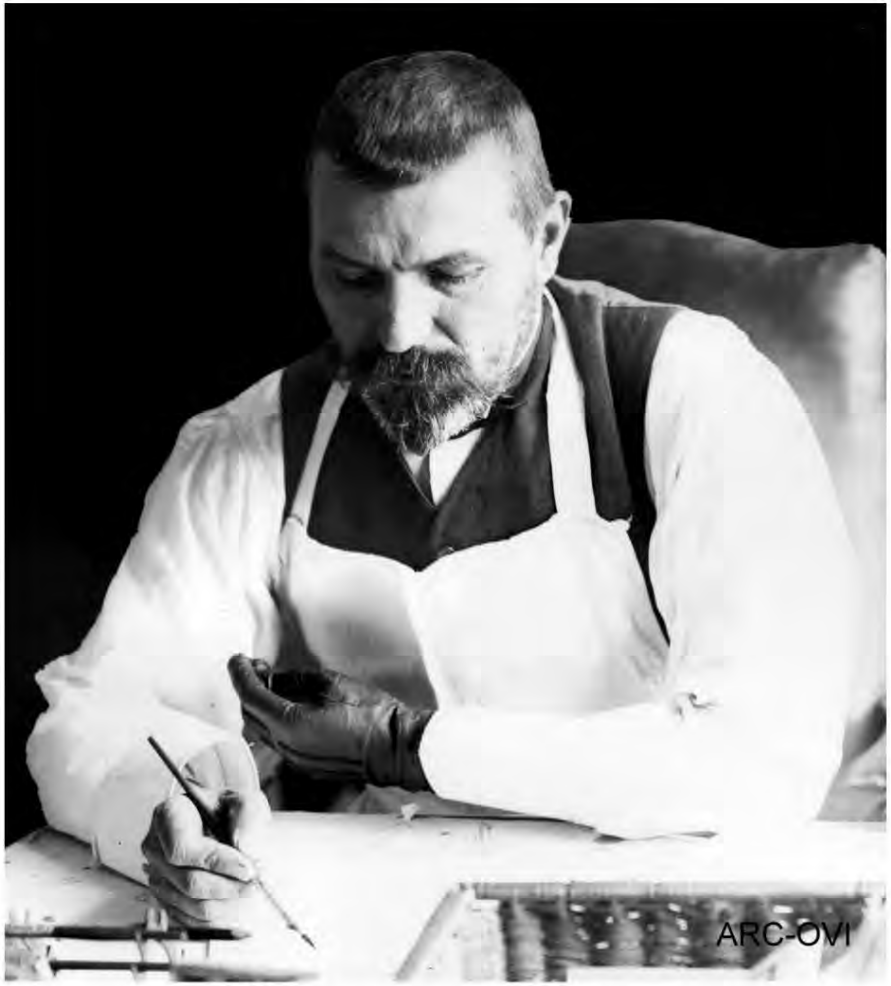
Thank you

- Prof. Vinny Naidoo
- Prof. Marinda Oosthuizen
- Department of Anatomy
- Prof. Joseph Panashe Chamunorwa
- Dr. Lizette Du Plessis
- Prof. John Soley
- All Faculty, Staff & Students

Storyline: Sir Arnold Theiler



Arnold Theiler was born on 26 March 1867 to Franz and Maria Theiler in the town Frick in Switzerland. His father, son of a farmer, taught natural history and mathematics at the local three-man school, of which he was the headmaster. Although he had no academic qualification, Franz Theiler excelled in his vocation, developing a passion for natural history that he shared with his young son, taking him on long hikes in the mountains and green fields. Arnold was thus exposed to all the marvels of natural history in his environment including rocks, plants, trees, animals, birds, insects and mushrooms as well as his father's beekeeping hobby.
Arnold was initially inclined to follow in his father's footsteps, studying to become a teacher, but then opted for veterinary surgery at the Veterinary School of the University of Zürich. Despite partaking wholeheartedly in typical exuberant student activities, he completed his course successfully, obtaining a Veterinary Diploma in August 1889 at the age of 22.

National Museum of Natural History Pretoria 2019

Male Infertility & Semen Evaluation

Andrology in The Age of Precision Medicine and Agriculture

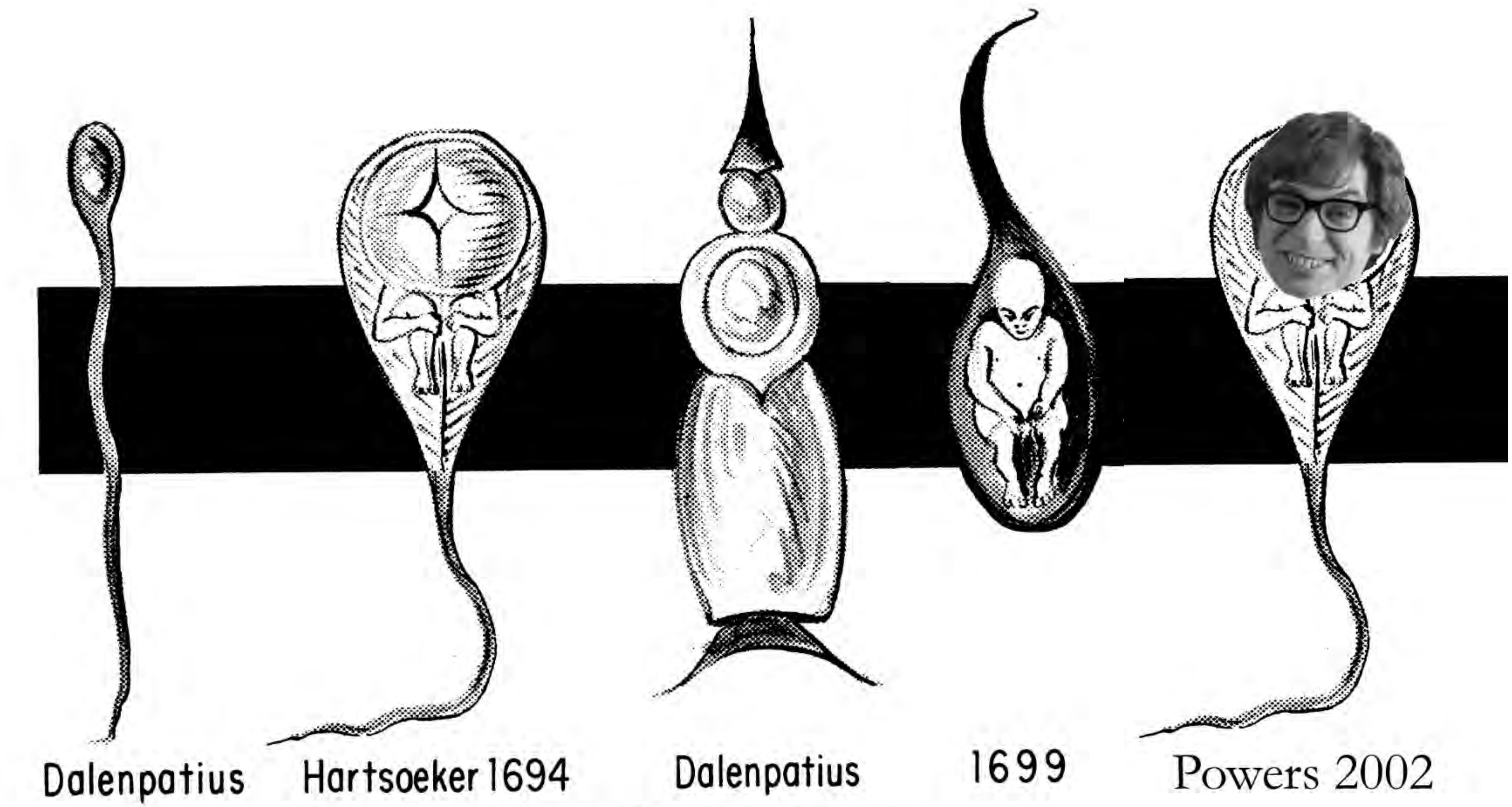


Fig. 15-74. Types of Homunculi – As imagined by their creators.

Peter Sutovsky

Andrology

Andrology (from Ancient Greek: ἀνήρ, anēr, genitive ἀνδρός, andros, "man"; and -λογία, -logia) is the medical specialty that deals with male health, particularly relating to the problems of the male reproductive system and urological problems that are unique to men.

Fertility

- The state or quality of being fertile.
- *Biology:* the ability to produce offspring; power of reproduction.
- The birthrate of a population.

- Clinical definition: Ability of a couple to achieve a clinical pregnancy after 12 months of regular unprotected sexual intercourse.

Factors Affecting Livestock Fertility/Reproductive Performance

- Health/disease, body condition & age
- Herd size
- Reproductive management & technology
- Nutrition/feed (balance, toxicants)
- Genome/genomic selection
- Climate (heat stress)
- Photoperiod
- Environment

Market Need/Opportunity

Total cattle in the world in 2021 was 1.468 billion head

Annual semen sales:

- $1.5 billion (dairy)
- $250 million (beef)
- $600 million (swine)

Trend & Hotspots:

- Genomics
- Sexed semen
- Bulls: Pubertal sire collection (no BSE)
- Boars: Single sire AI (no semen pooling)

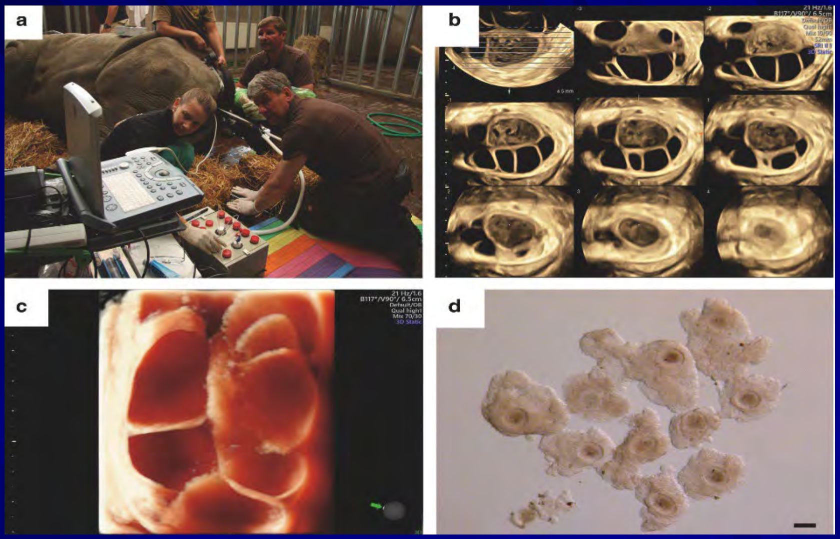
One extra piglet/litter would add $135 million/year to US swine industry

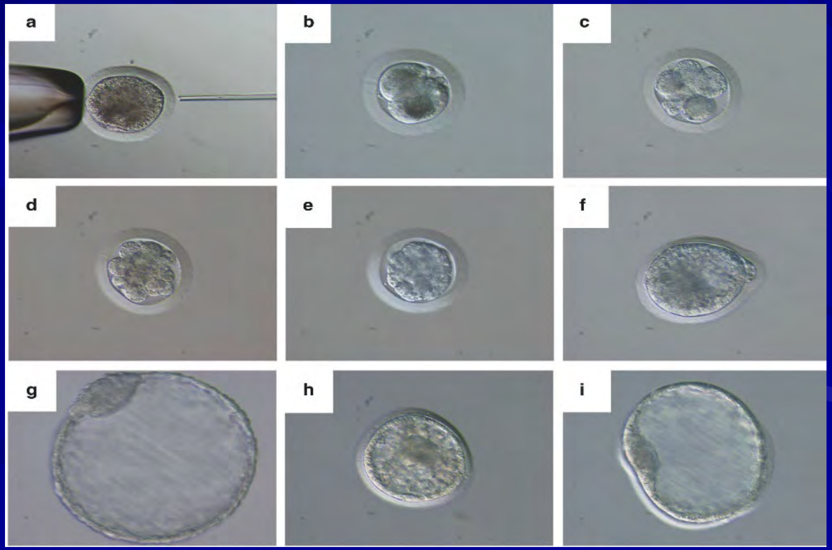

INVENTIONS

A Bull Market

Peter Sutovsky Finds Fertile Opportunities In Research by SANDY SELBY photos by L.G. PATTERSON

ART in Wildlife Management, Fertility Preservation & Nature Conservation





nature COMMUNICATIONS
ARTICLE
DOI: 10.1038/s41467-018-04959-2 OPEN

Embryos and embryonic stem cells from the white rhinoceros

Thomas B. Hildebrandt[1,2], Robert Hermes[1], Silvia Colleoni[3], Sebastian Diecke[4,5], Susanne Holtze[1], Marilyn B. Renfree[6], Jan Stejskal[7], Katsuhiko Hayashi[8], Micha Drukker[9], Pasqualino Loi[10], Frank Göritz[1], Giovanna Lazzari[3,11] & Cesare Galli[3,11]

Dual Purpose With Dual Benefit

Large animal models for biomedical research:

- Anatomical and genetic similarity to humans
- Genetic modification becoming routine
- Cost efficient and ethically more acceptable compared to non-human primates
- Ample fertility records from AI (reproductive research)

Cover by Dalen Zuidema & Peter Sutovsky
University of Missouri

One health, one medicine

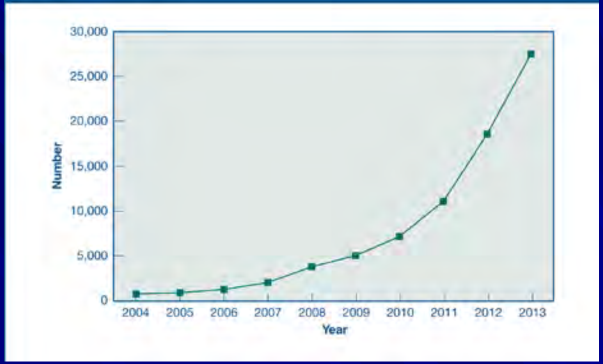
Human Infertility

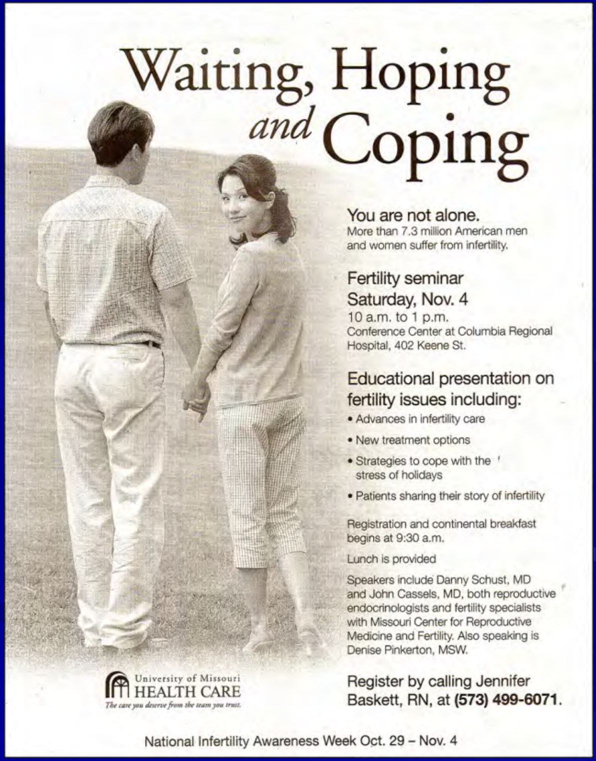
Est. 20% of US population are affected by infertility

~300,000 infertility treatment-cycles/year realized in USA (2018); >500,000/year in Europe; *annual expenditure on fertility services in US: >$2.2 billion*

Steadily increasing cycles/year but stagnant success rate (~30%)

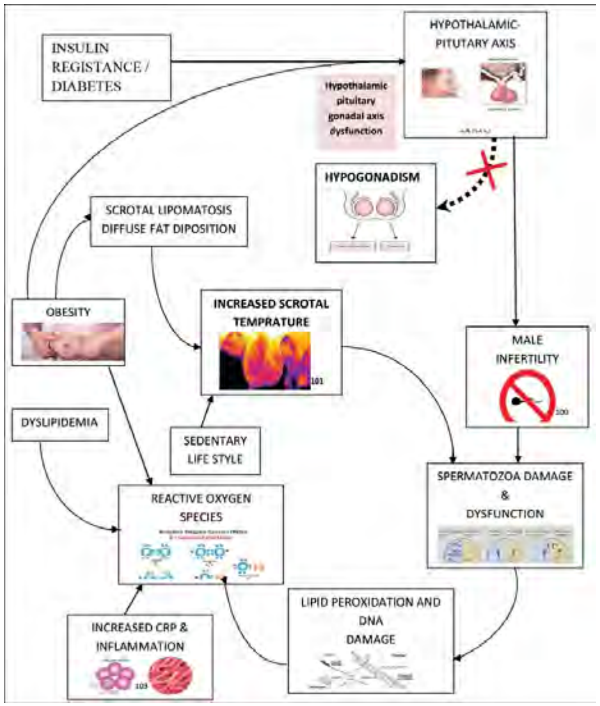
Figure 44
Numbers of ART Cycles Performed for Banking All Fresh Nondonor Eggs or Embryos, 2004–2013





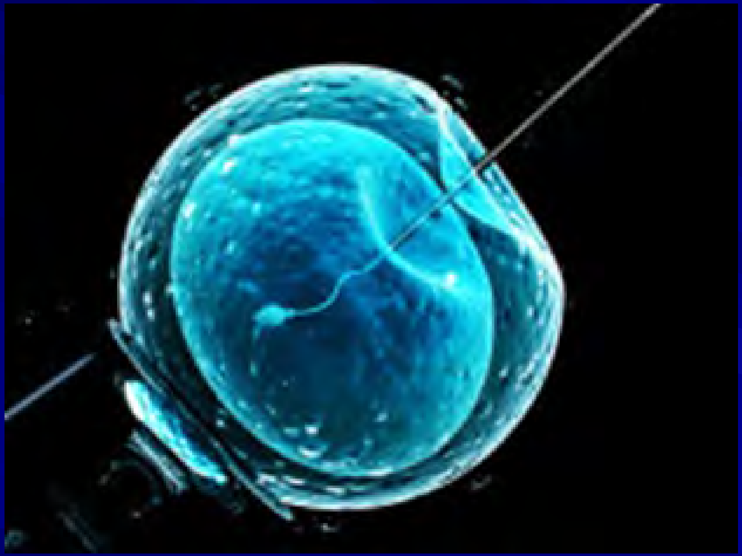
Fertility Status as a Marker for Overall Health*

- *Program supported by US National Institutes of Health
- Chronic conditions such as cancer, diabetes, cardiovascular disease and obesity can impair fertility (somatic-reproductive comorbidities)
- Less is known about the extent to which fertility status can impact or act as a marker for overall health
- *Andrology:* spermogram could serve as a sentinel of somatic disease



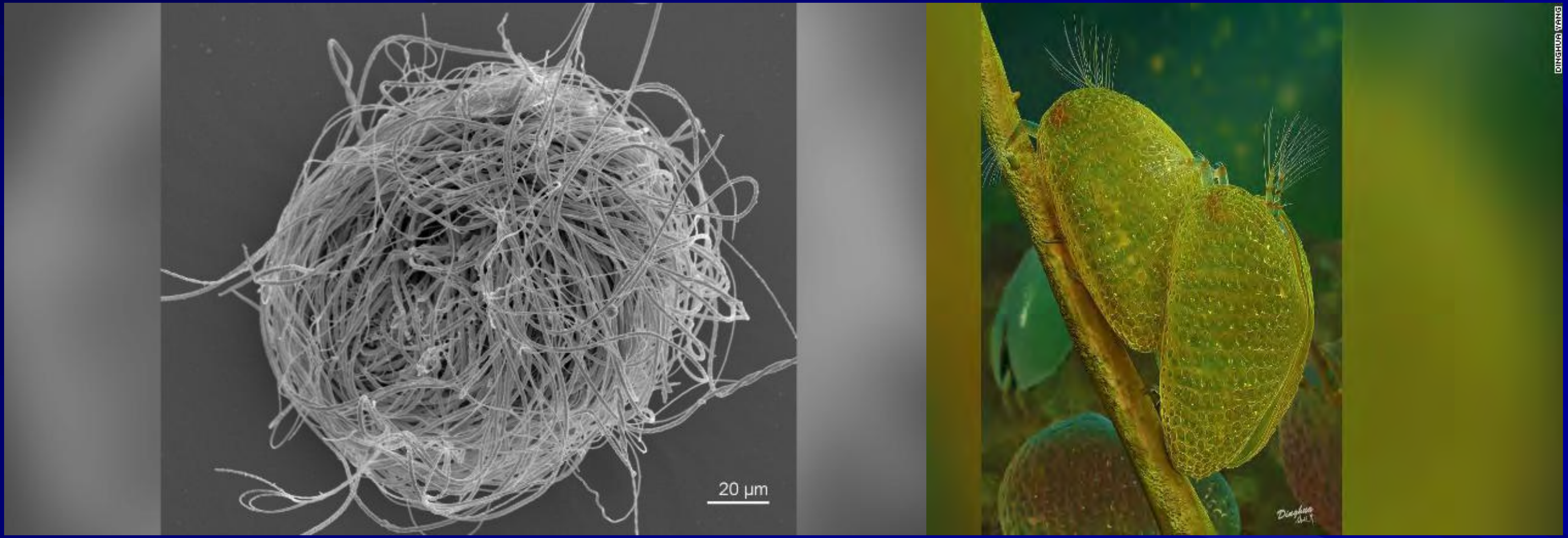
Eunice Kennedy Shriver National Institute of Child Health and Human Development

Livestock animal reproductive efficiency, and the success rate and safety of human ART* can be improved by new technologies for semen analysis, handling and purification



*Assisted Reproductive Therapy

Oldest animal sperm discovered in 100-million-year-old amber



- *Paleontologists discovered the sample in the reproductive tract of an ancient female crustacean encased in resin -- one of several samples of ostracods from Myanmar.*
- *The previously unknown species of crustacean, now named Myanmarcypris hui, resembles a modern day mussel.*

First (Human) Sperm Observations Van Leeuvenhook & Hamm 1677-79

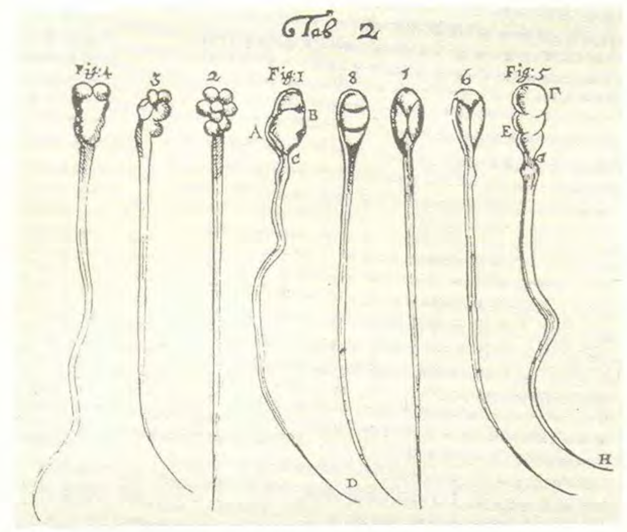
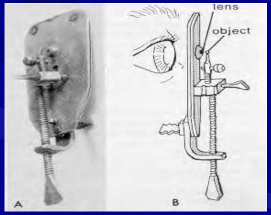


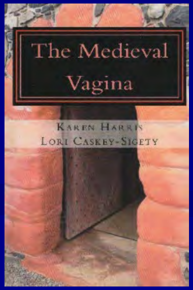
Fig. 19. Leeuwenhoek's drawings of different types of sperm cells. (From "The Observations of Mr. Antoni Leeuwenhoek, on animalcules engendered in the semen," *Philosophical Transactions of the Royal Society of London,* 1679; as reprinted by Meyer in *The Rise of Embryology,* 1939.)



First Fertility Test

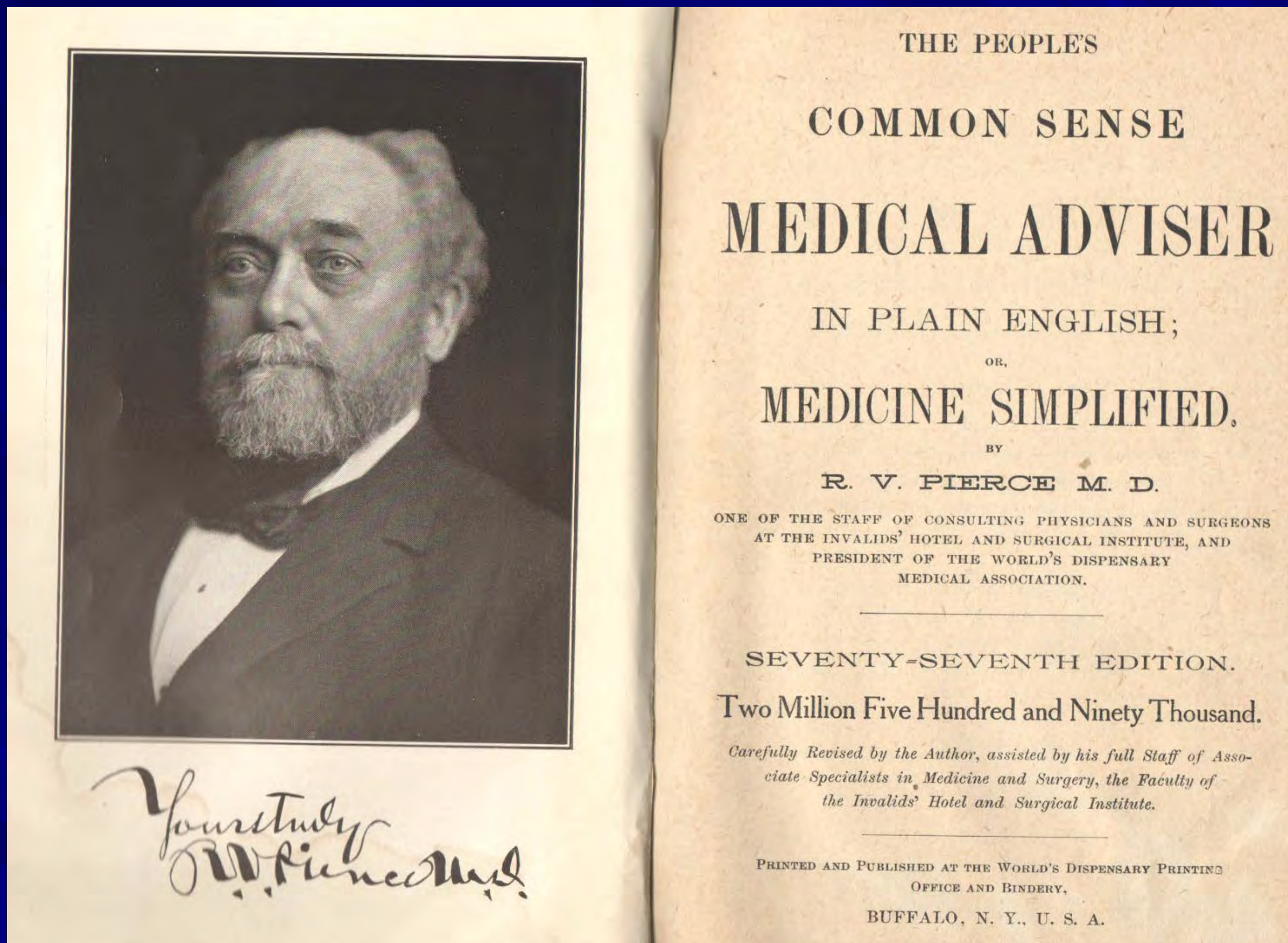
Take two new earthen pots, each by itself; and let the woman make water in the one, and the man in the other; and put in each of them a quantity of wheat bran, and not too much, that it be not thick, but be liquid or running; and mark well the pots for identification, and let them stand for ten days and ten nights, and thou shalt see in the water that it is in default small live worms; and if there appear no worms in either water, then they be likely to have children in process of time when God will.

~Common medieval fertility test, often attributed to the female physician, Trota of Salerno (Trocta/Trotula; 1050-1097)



De curis mulierum (On Treatments for Women)
Practica secundum Trotam (Practical Medicine According to Trota)

Conventional Semen Analysis 1909



THE PEOPLE'S

COMMON SENSE

MEDICAL ADVISER

IN PLAIN ENGLISH;

OR,

MEDICINE SIMPLIFIED.

BY

R. V. PIERCE M. D.

ONE OF THE STAFF OF CONSULTING PHYSICIANS AND SURGEONS AT THE INVALIDS' HOTEL AND SURGICAL INSTITUTE, AND PRESIDENT OF THE WORLD'S DISPENSARY MEDICAL ASSOCIATION.

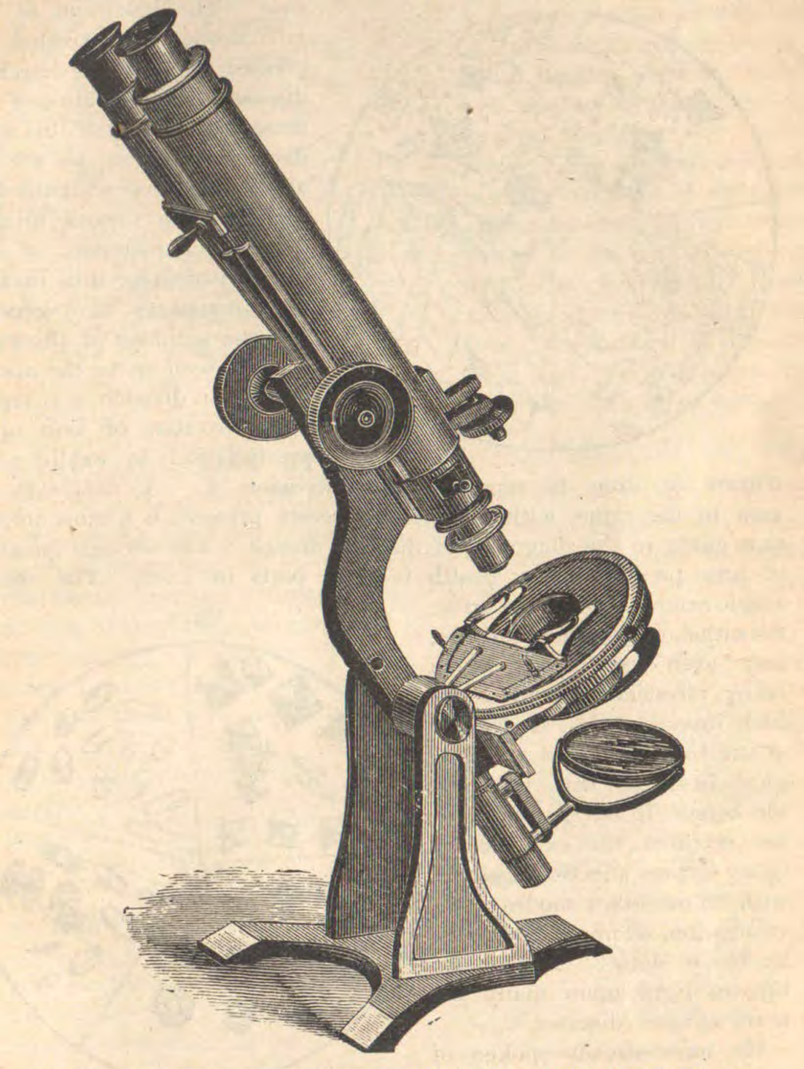
SEVENTY-SEVENTH EDITION.

Two Million Five Hundred and Ninety Thousand.

Carefully Revised by the Author, assisted by his full Staff of Associate Specialists in Medicine and Surgery, the Faculty of the Invalids' Hotel and Surgical Institute.

PRINTED AND PUBLISHED AT THE WORLD'S DISPENSARY PRINTING OFFICE AND BINDERY,

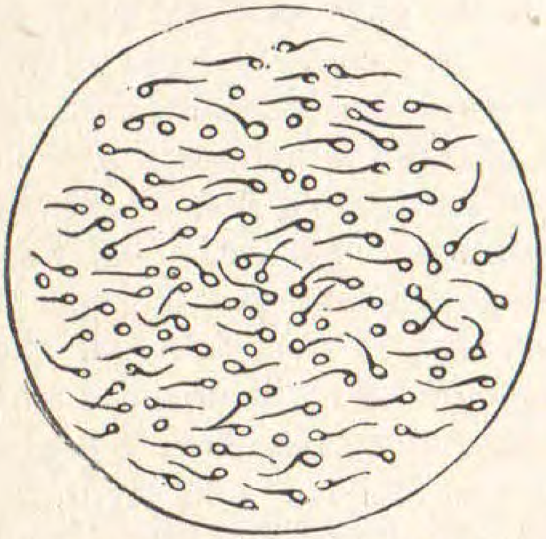
BUFFALO, N. Y., U. S. A.


Binocular Microscope used at the Invalids' Hotel and Surgical Institute.

A careful microscopic examination is a valuable aid in determining the nature of chronical diseases of generative organs

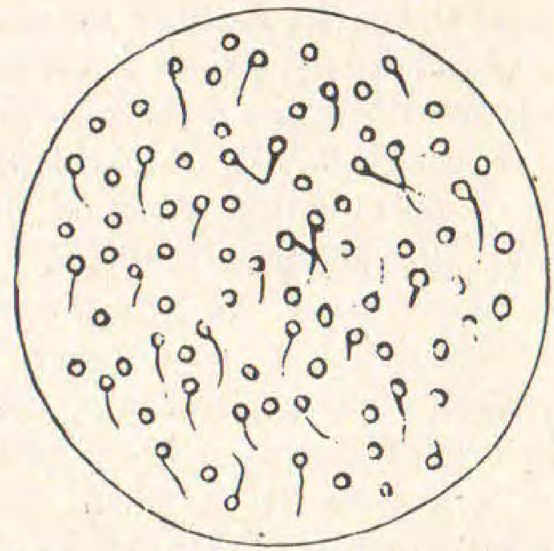
Spermatorrhea – Seminal Weakness

Fig. 3.



Microscopic appearance of healthy semen.

Fig. 4.



Microscopic appearance of semen which will not fecundate.

"May be a result of marital excess"

Conventional Semen Analysis 2015

- Front line semen assessment (volume, density, color, swirl)
- Sperm Count
- Motility
- Appearance/Morphology
- Contaminants (Leukocytes, spermatids, epithelial cells, residual bodies, cellular debris).

Semen Parameters - Livestock

	Bull (desired)	Boar (desired)
Volume mL	5 mL (range 1-15 mL)	100-500 mL
Concentration/mL	>500 million (range 300-2500 million)	100-200 million
% Motile	>60% (>30% BSE)	Min. 80% (prog. mot.)
% Normal	>70%	80-90%
Single AI dose	20 million	1-3 billion

- ❑ Past: Sires with poor semen quality eliminated during breeding soundness evaluation
- ❑ Present: Emphasis on genetic value, not fertility

Do ConventionalSemen Parameters Reflect Fertility?

- Yes, but to a limited extent…
- Why? Because…

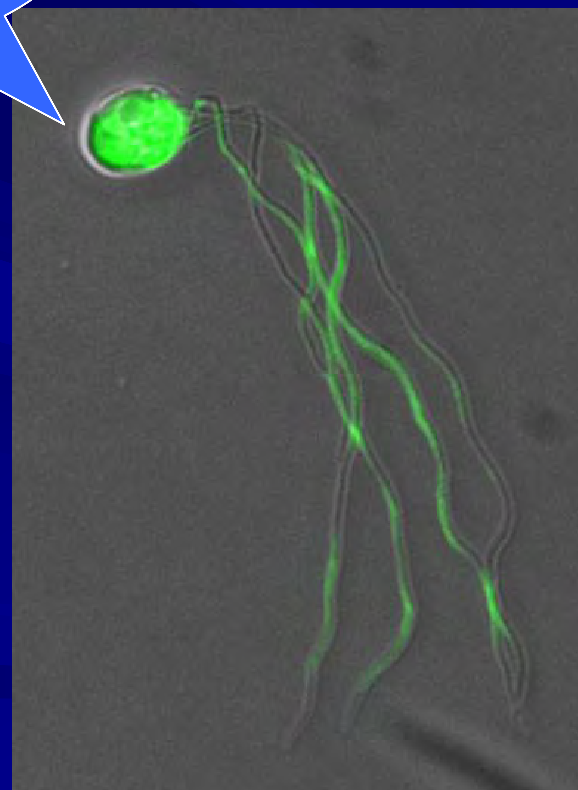
SPERMATOZOA- THE GOOD, THE BAD AND THE UGLY

STARRING PETER SUTOVSKY, PHD
AS
BLONDIE "THE GOOD" (SPERM GUY)

PRODUCTION: DIVISION OF ANIMAL SCIENCES AND THE DEPARTMENTS OF OBSTETRICS, GYNECOLOGY AND WOMEN'S HEALTH, UNIVERSITY OF MISSOURI, COLUMBIA, MO, USA

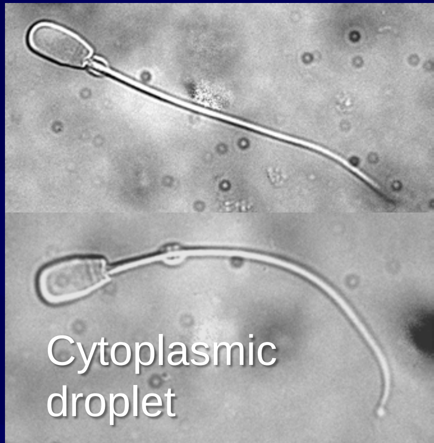
Bad & Ugly

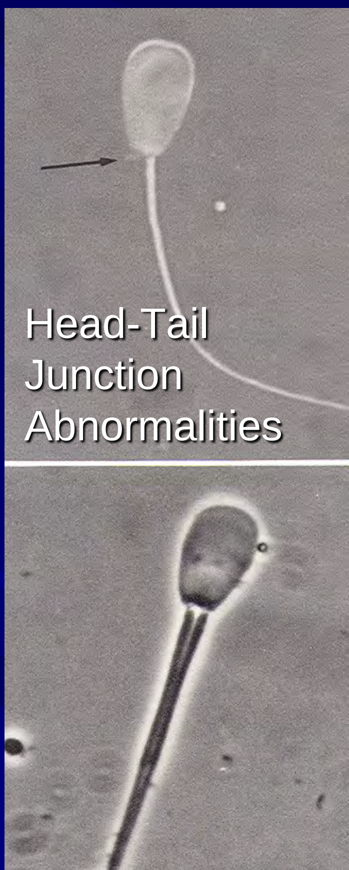
Do You Feel Lucky, Punk?

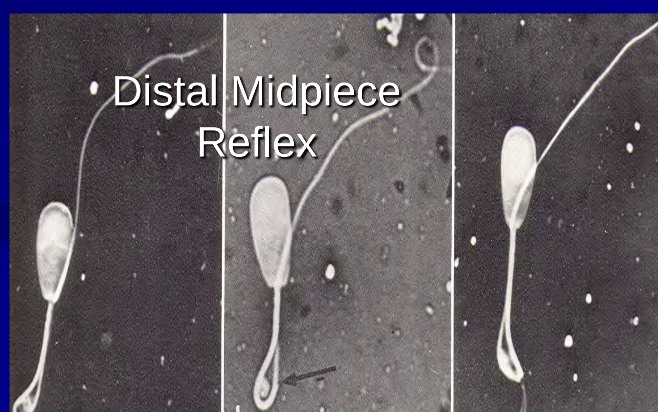


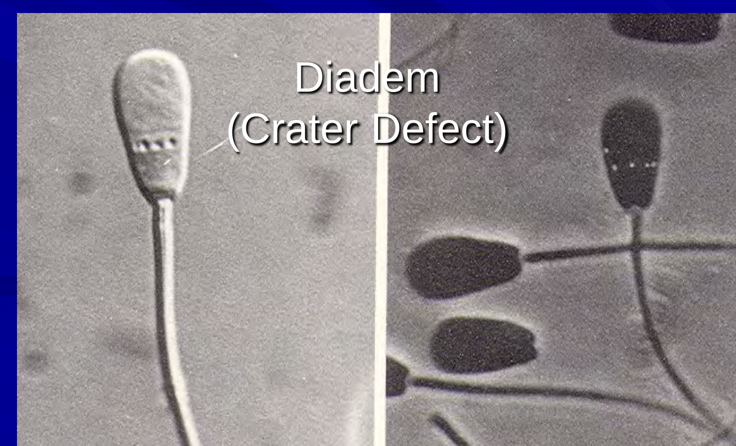
Multinuclear – multiflagellar sperm defect in bull

Livestock Animal Sperm Defects

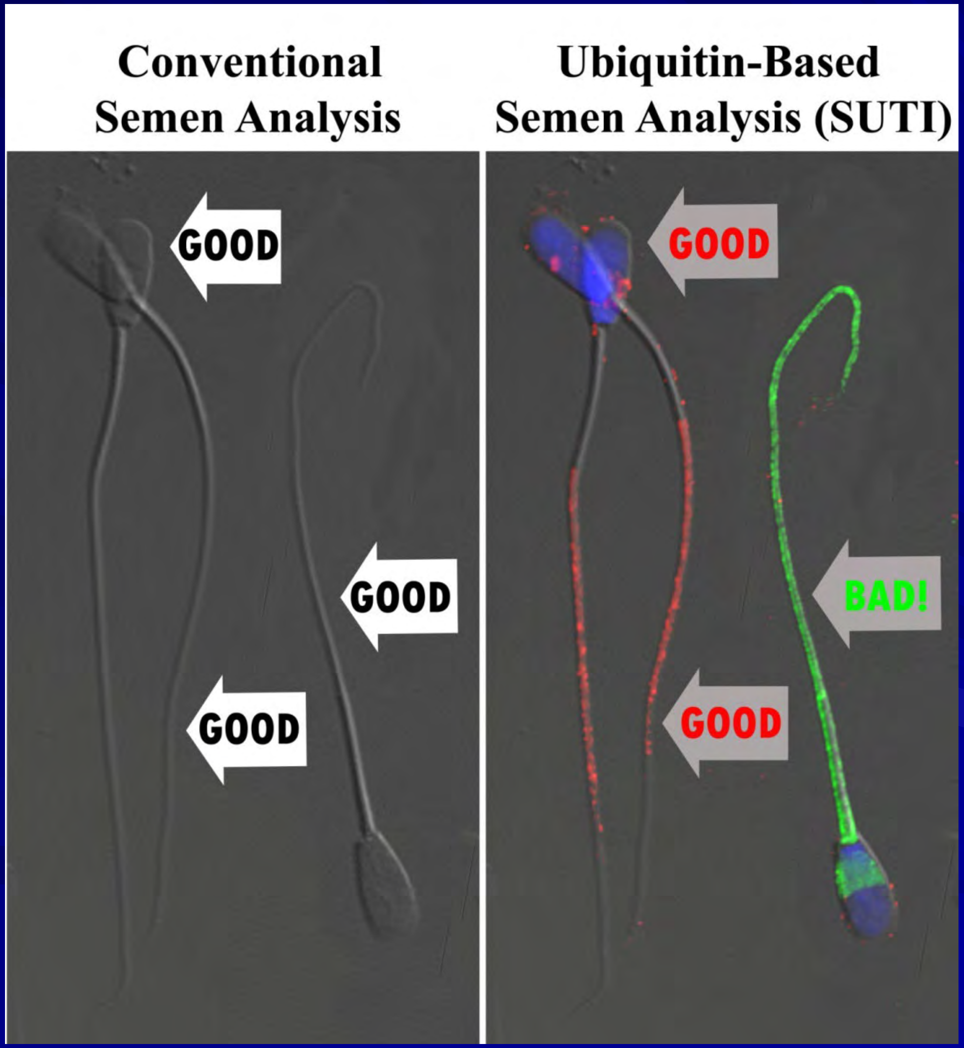








All Good?


Conventional
Semen Analysis
Ubiquitin-Based
Semen Analysis (SUTI)
GOOD
GOOD
GOOD
GOOD
BAD!
GOOD

Goal:

Provide new tools and fertility markers for farm animal semen evaluation and diagnostics of human male infertility

Improved Semen Quality Assays: What to Consider:

- Objective: evaluation is not based on subjective judgment
- Universal; recognizes multiple types of semen abnormalities
- Detects hidden sperm abnormalities
- Correlates with fertility
- High throughput 50-500 samples /day per technician
- Representative of a sperm sample: measures 10-20,000 cells in each semen sample as opposed to 100-200 cells/sample capacity of microscopic evaluation
- Measurements are not distorted by sperm damage during collection, storage and thawing
- Low cost: ready to use technology; probes & reagents are commercially available or easy to produce on a large scale
- Combined/multiplex tests with other related assays possible

Objective Semen Evaluation Methods

1. Automated morphometry & motility (CASA, IVOS)
2. Viability tests (HOS, Eosin-nigrosin, trypan blue, live/dead kit, vital mitochondrial stains*)
3. Acrosomal Integrity
4. HOS
5. DNA/chromatin structure tests (SCSA, TUNEL*, Comet, FISH, Hallo, Sperm Protamination)
6. Sperm capacitation assays (Ca-influx)
7. Fertility-associated sperm proteins
8. Sperm-defect associated proteins
9. **NEW: Postranslational (sperm) protein modifications (oxidation, acetylation, methylation, protamine index)**

*ANALYZED BY FLOW CYTOMETRY

Integrated Computer Assisted Semen Analysis (CASA)

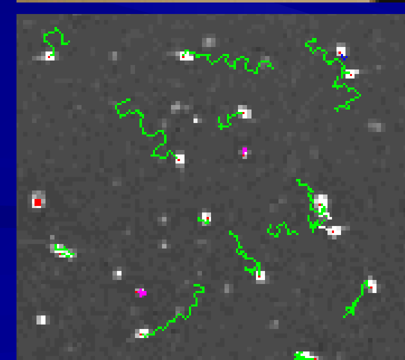
- **<u>IVOS/CEROS systems:</u>**

- **Counts:**
  - Total, Motile, Progressive
  - % Motile, % Progressively Motile
  - Rapid, Medium, Slow and Static Cells

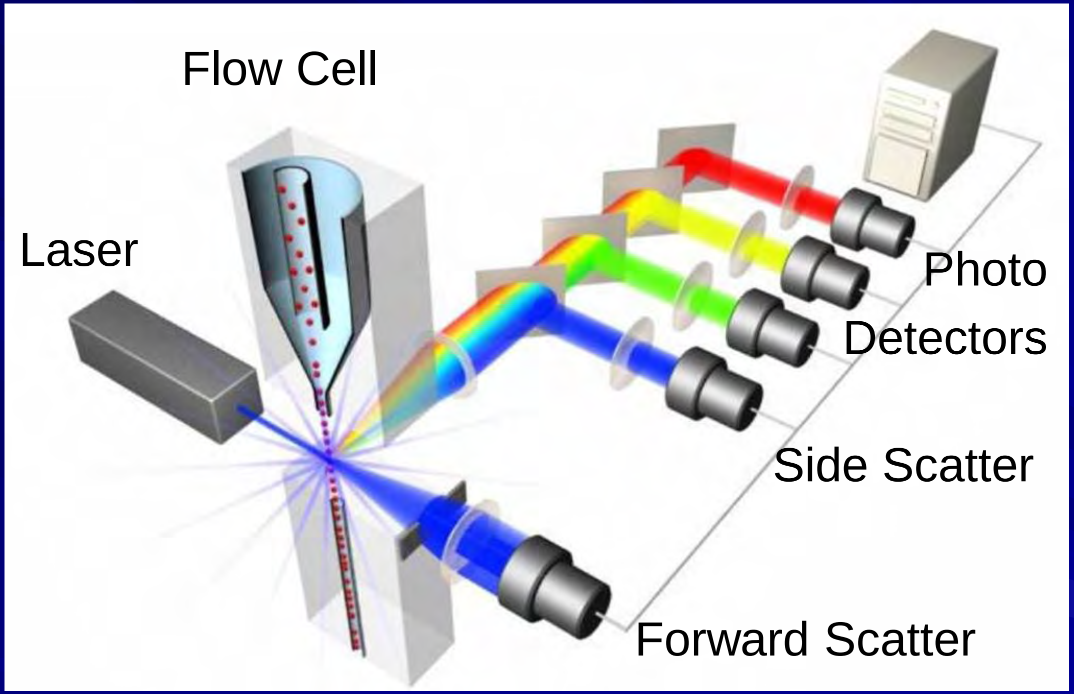
- **Concentrations**
  - Total, Motile, Progressive (millions/ml)
  - Rapid, Medium, Slow and Static Cells (millions/ml)

- **Mean Values** and Morphometry
  - VAP: Smoothed Path Velocity (microns/sec)
  - VCL: Track Velocity (microns/sec)
  - VSL: Straight Line Velocity (microns/sec)
  - ALH: Amplitude of Lateral Head Displacement (microns)
  - BCF: Beat Cross Frequency (hertz)
  - LIN: Linearity (ratio of VSL/VCL)
  - STR: Straightness (ratio of VSL/VAP)
  - Elongation: head shape (ratio of minor to major axis of sperm head)
  - Area: head size (square microns)

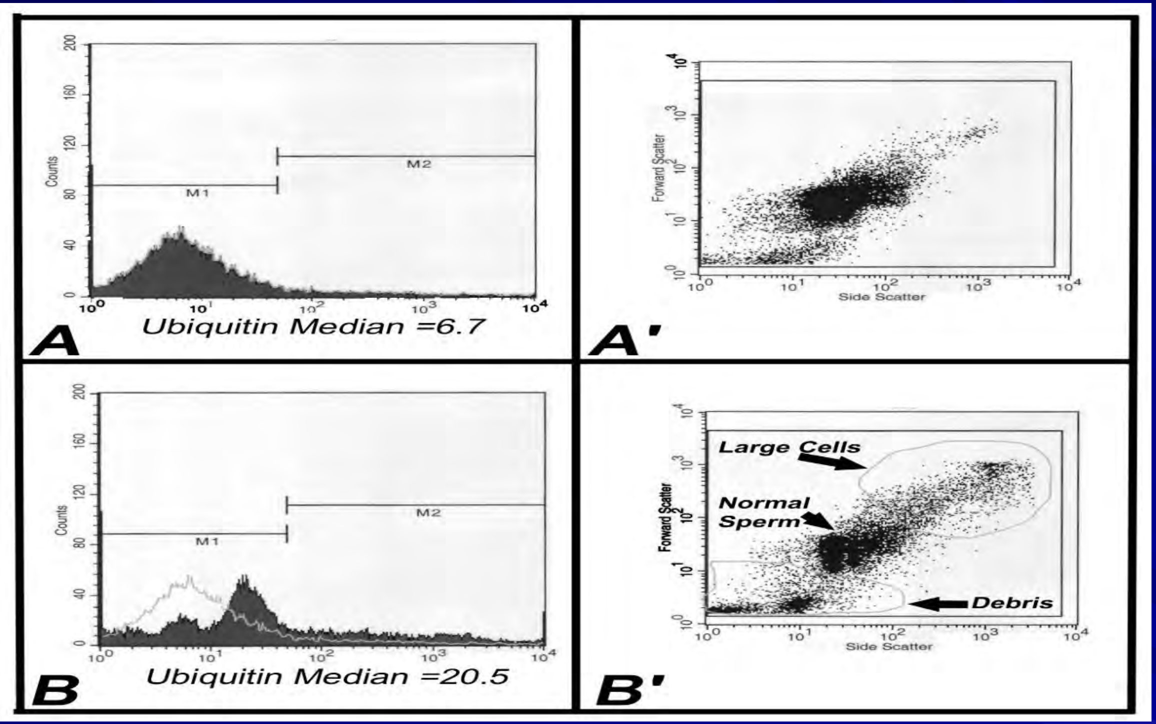
- **Bar Chart Distributions**
  - VAP, VCL, VSL, Elongation, ALH, BCF, LIN, STR

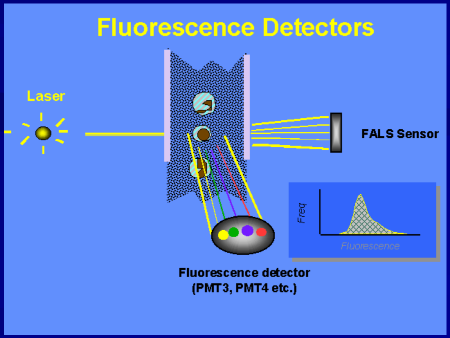


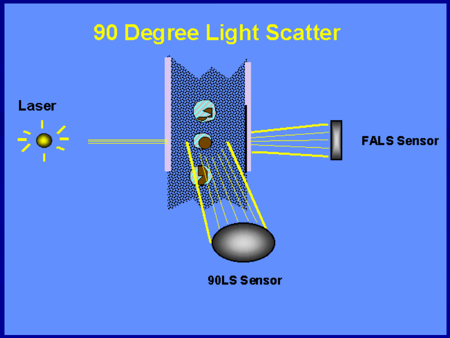
Flow Cytometry



Histogram & Scatter Diagram

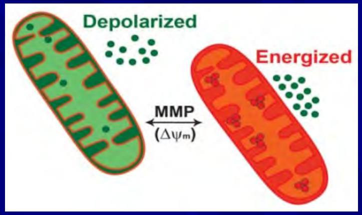




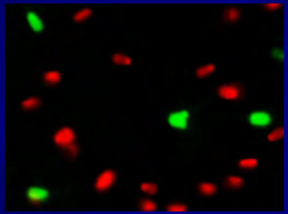


Vital Stains and DNA Stains

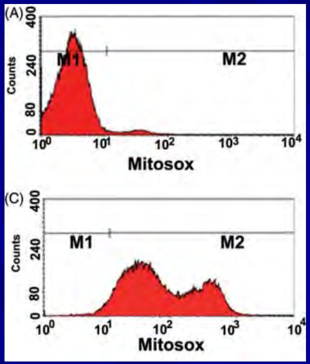
Mito Potential



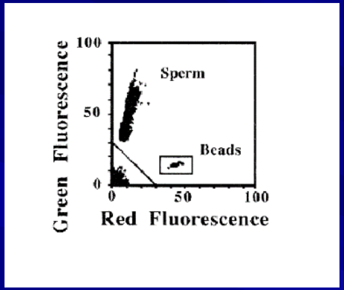
Viability - Live/Dead



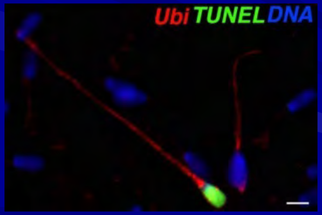
ROS



SCSA – Chromatin Structure

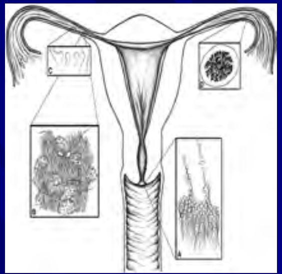


TUNEL – DNA Damage

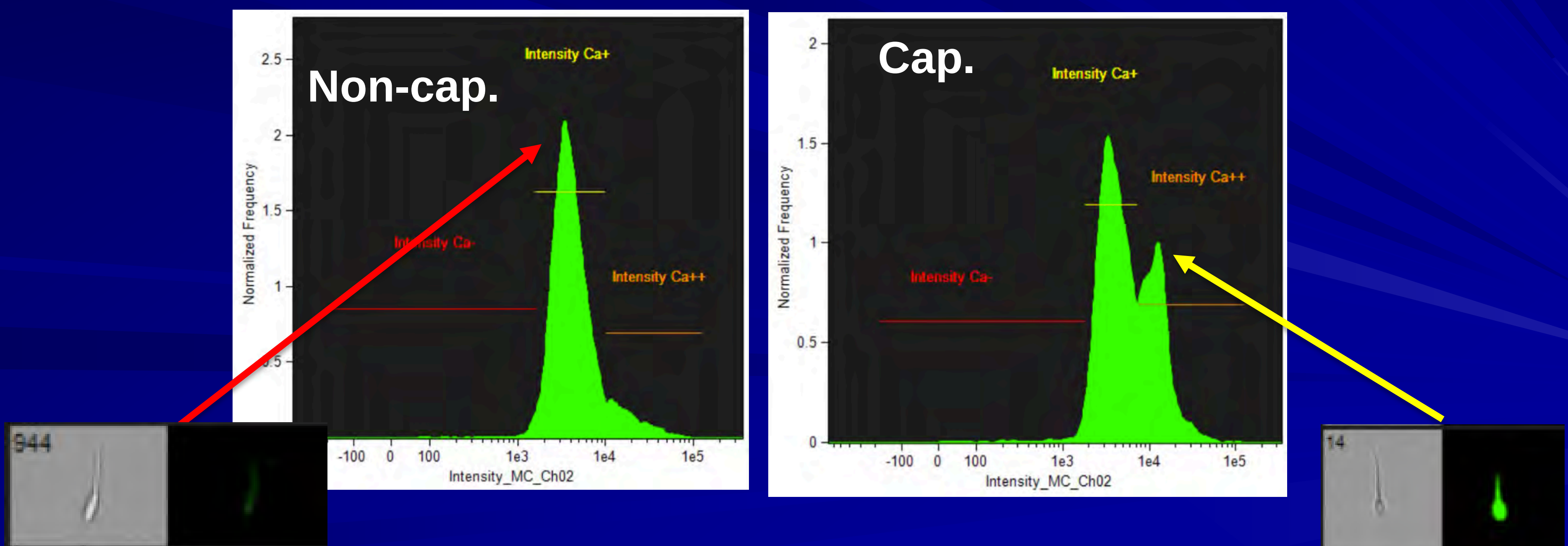


Acrosomal Integrity – PNA lectin

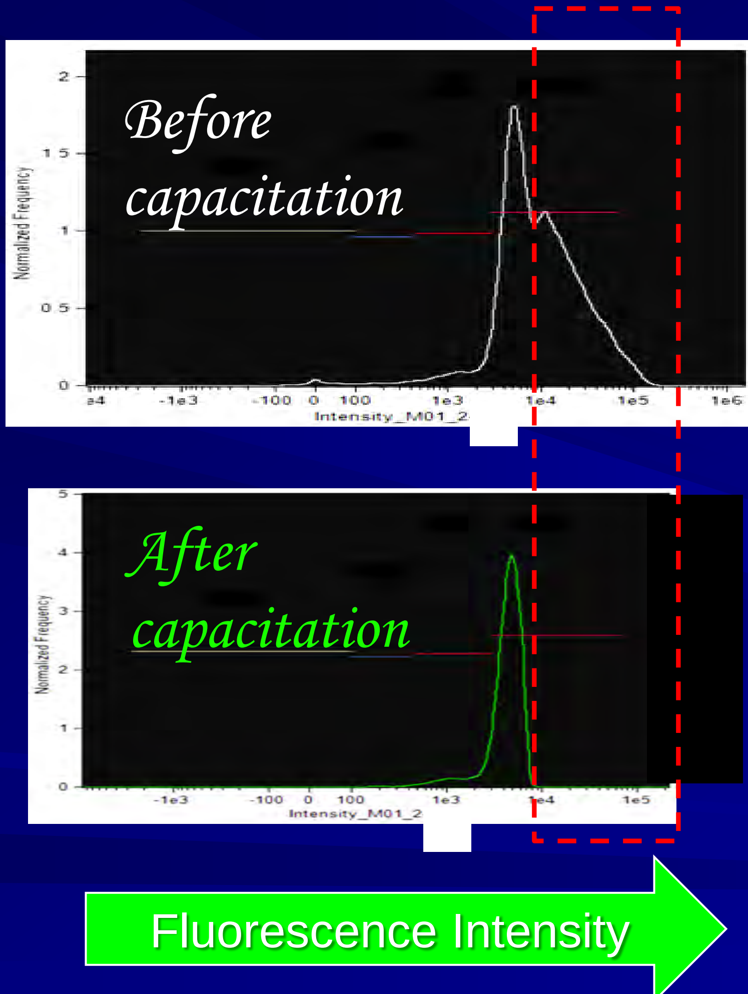
Sperm Capacitation Status



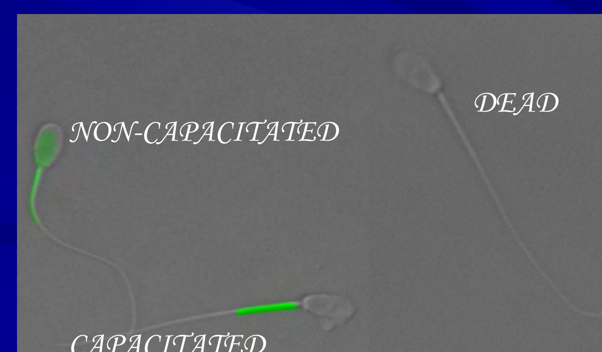
- Endows spermatozoa with FERTILIZING ABILITY
- Spermatozoa do not capacitate naturally until they bind to oviductal sperm reservoir epithelium
- Premature capacitation kills spermatozoa
- Associated with Ca-influx in sperm
- Measured by fluorescent Ca-dyes Fluo-3/Fluo-4 (flow cytometry) or by chlorotetracycline (epifluorescence microscopy)



Zinc Signature of Capacitation

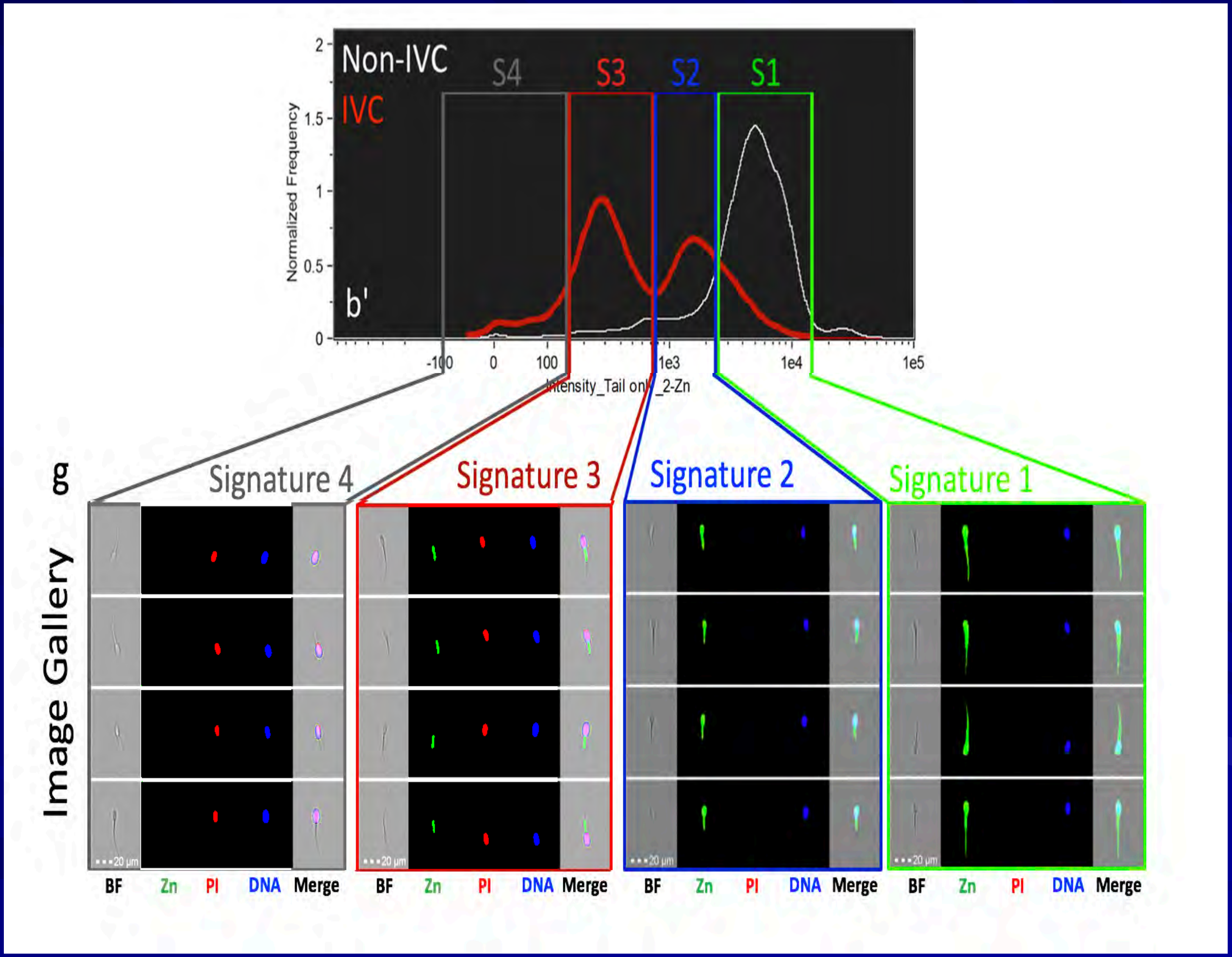


- Quick one step live sperm staining, detects & quantifies premature capacitation
- Also detects & quantifies death spermatozoa
- Indicates sperm ability or readiness to undergo timely capacitation



Kerns E et al 2018 Nature Communications

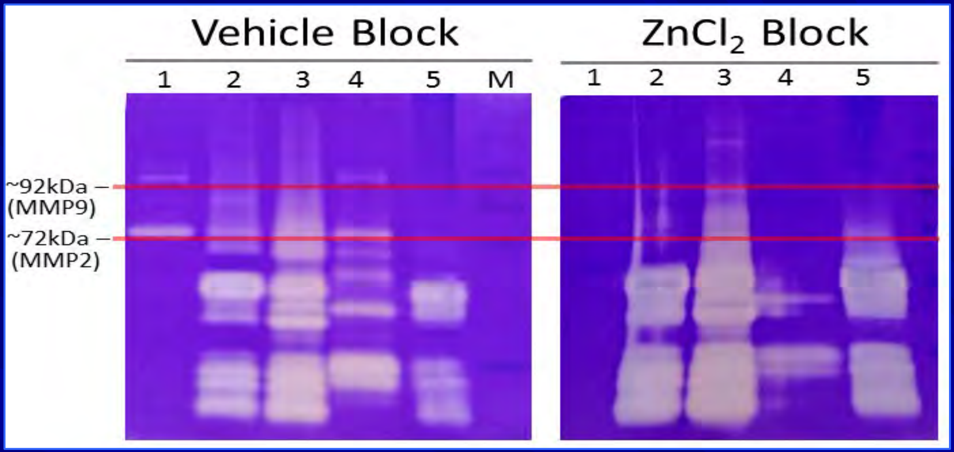
Zinc Signature Changes During *In Vitro* Capacitation (IVC)



Kerns et al., 2018, Nat Comm

Zinc Efflux Alters Zincoprotein Activities

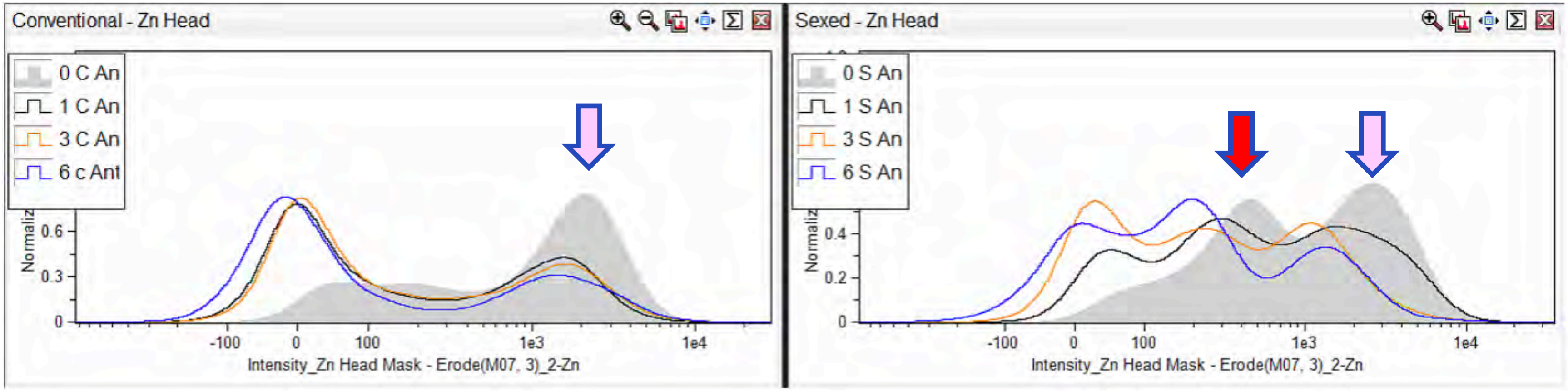
- Zinc release can inactivate or activate a zincoproteins, and change their affinity for zinc binding matrices



Matrix metalloproteinase MMP2 and MMP9 zymography. Inhibition of MMP activity is indicated by the absence of bands at 92 and 72 kDa. Lanes: 1) Trophoblast cell line control, 2) Boar sperm SDS after NP40 extraction, 3) Boar Sperm NP40 extr., 4) Bull sperm NP40 extr., 5) Boar sperm SDS extraction.

Does Semen Sexing Affect Zinc Signature?

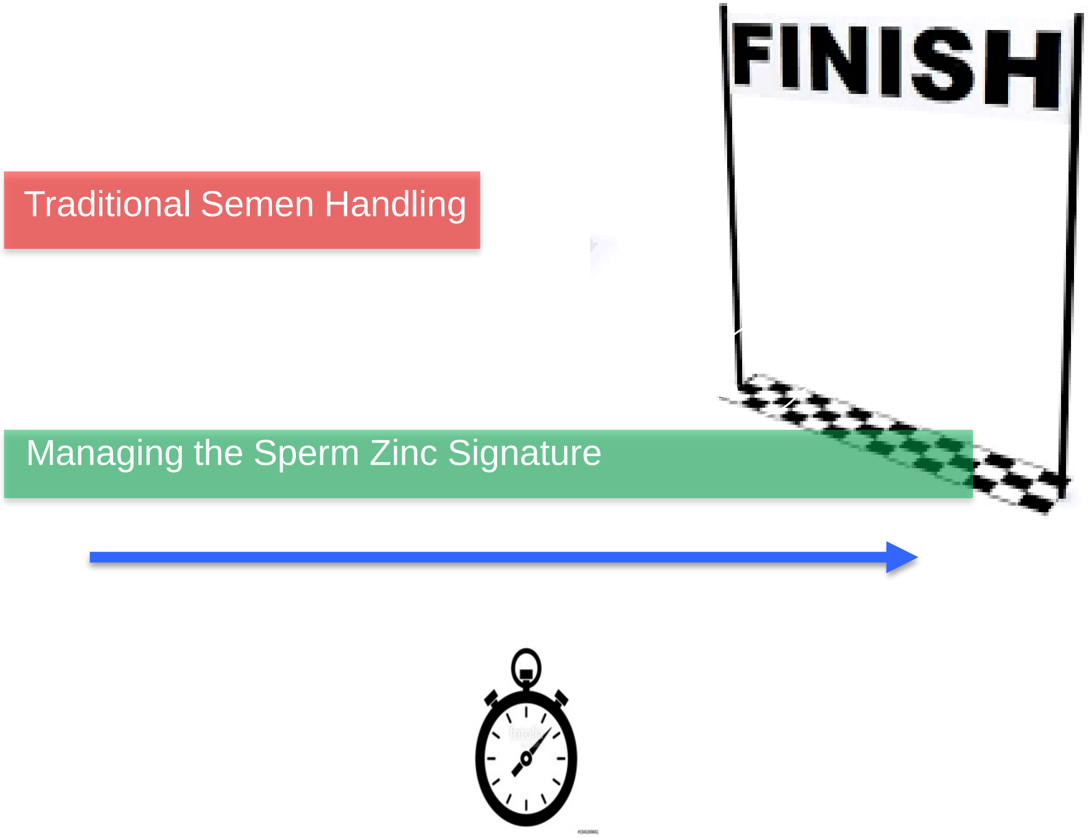
- Same bull, same ejaculate
- **½ neat, ½ XY-sorted**



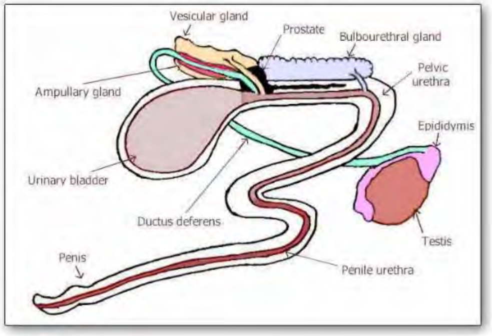
Control Sexed

(Zn^{2+} fluorescence in sperm head)

Co-Management of Sperm Mitochondrial Health and Zinc Signature



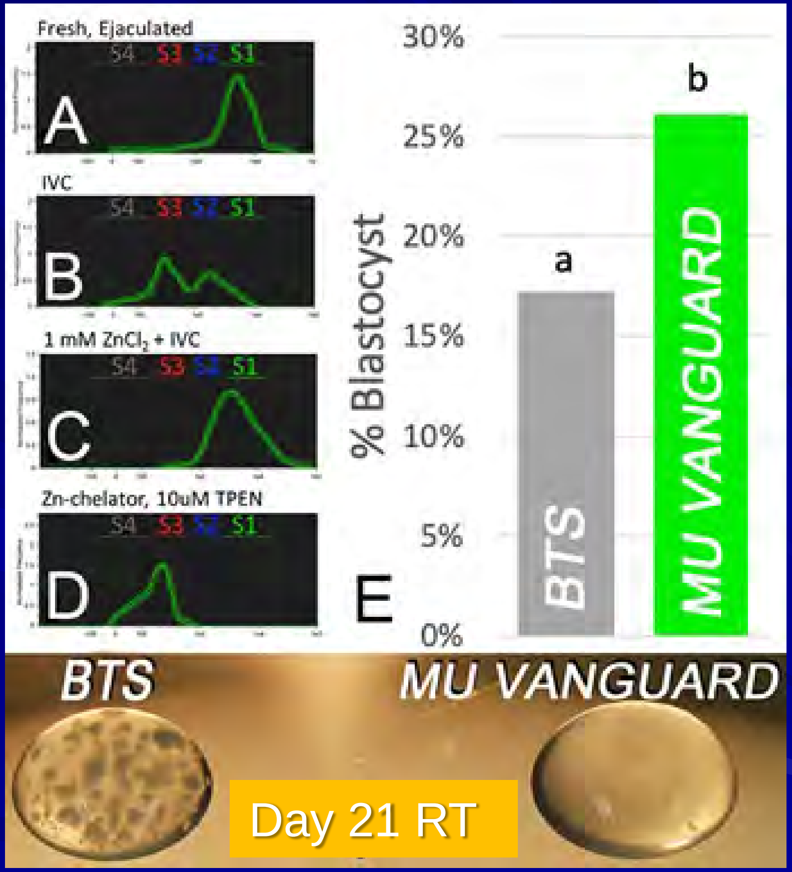
Seminal Plasma:



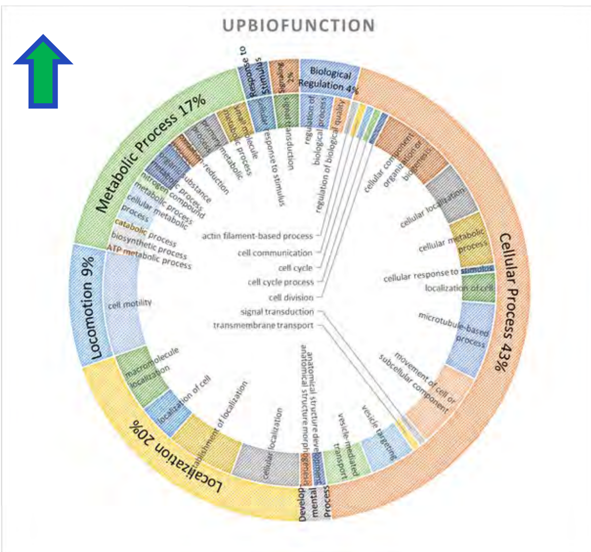
- Produced by male accessory sex glands, rich in Zn^{2+}
- Semen extension dilutes Zn^{2+} ions in seminal plasma
- FUNCTIONS:
  - Alkaline pH for neutralization of vaginal environment
  - Induction of sperm progressive motility (activates sperm soluble adenylate cyclase/SACE, elevates cAMP)
  - Formation of vaginal /copulatory plug (rodents)
  - Protease inhibitors, prostaglandins, growth factors, immuno-suppressors are present in seminal plasma
  - Protective coating of sperm surface (spermadhesins/binder of sperm proteins)
  - Induction of CD removal (boar)
  - Induction of ovulation (alpaca, beta-nerve growth factor)
  - Sperm surface-binding proteins required for sperm binding to oviductal sperm reservoir (e.g. BSPs in bull, spermadhesins in boar)
  - Seminal plasma may influence gene expression in uterine epithelia directly and indirectly regulate embryo development through uterine secretion of embryotrophic growth factors (Bromfield *et al.,* 2014, PNAS 111:2200-5.)

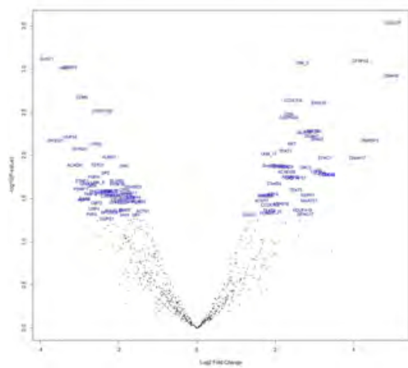
Zinc Reloading

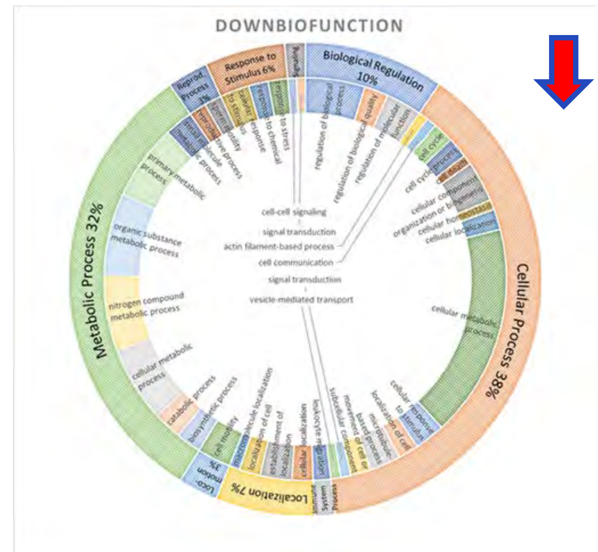
- Prevents spontaneous capacitation of extended livestock semen
- Extend shelf life and fertility of liquid semen, refrigerated or stored at RT
- Cold be also useful for sperm prep in human ART



Zincoproteome of Mammalian Sperm Capacitation





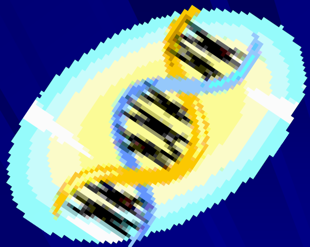


Increased

Decreased

Affinity of zinc-containing/interacting proteins to zinc-binding protein purification matrices before vs. after sperm capacitation

OMICS



- Sperm transcriptome/mRNA & miRNA analysis in semen: Ejaculated spermatozoa contain a complex repertoire of mRNAs that could be used as a non-invasive proxy for investigations of testis-specific infertility. (Ostermeyer et al., Lancet. 2002; 360:772-7) Also includes sperm epigenome.

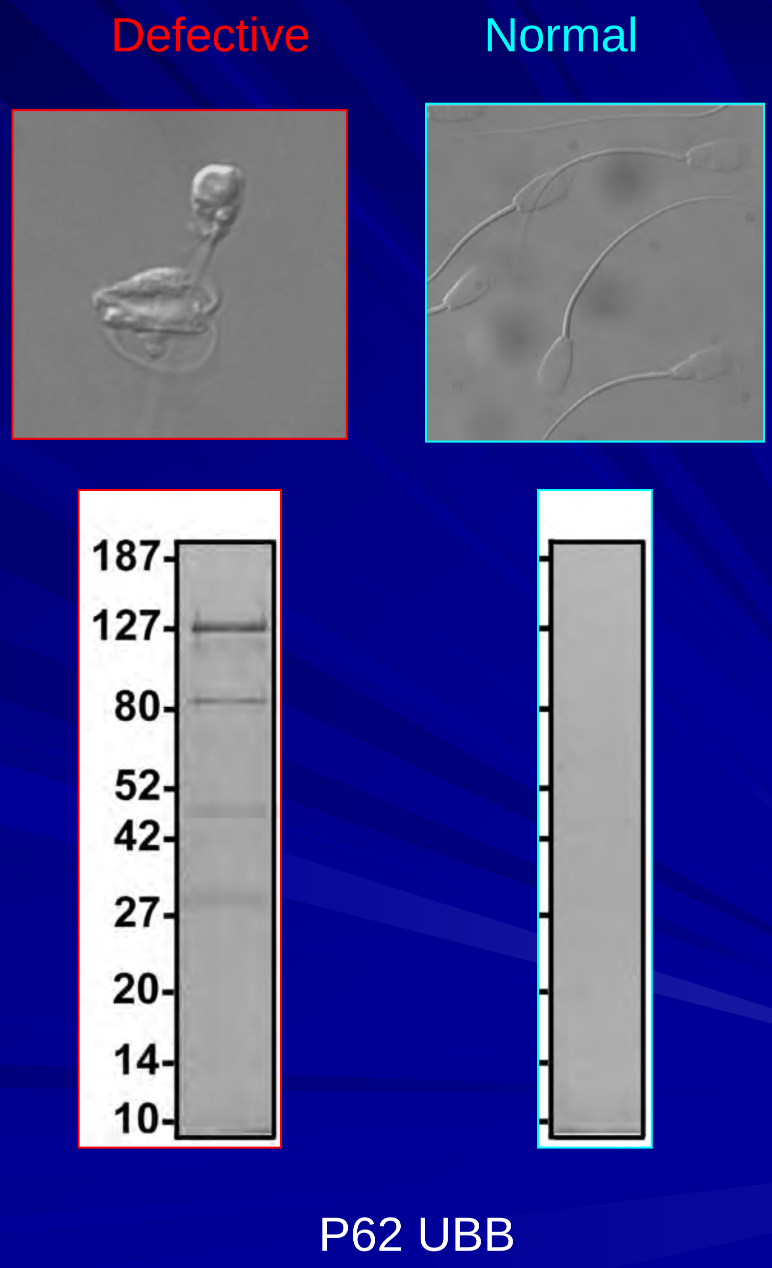
Spermatozoa carry small non-coding RNAs that could affect female reproductive system & zygote

- Proteomics: Sperm proteomes differ between fertile and infertile males

- Metabolomics: Sperm and seminal plasma metabolite profile reflects fertility

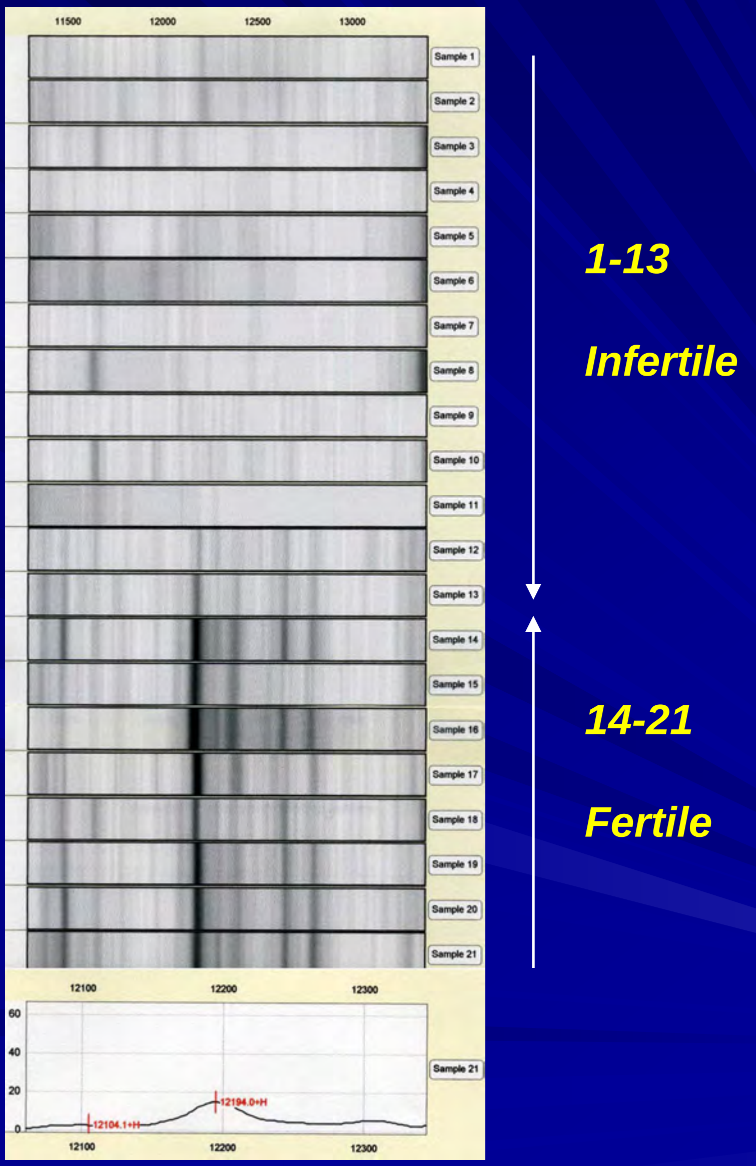
Protein Biomarker Identification

- Comparing Normal vs. Defective Sperm Fractions After Gradient Separation



Sperm Quality Biomarkers:

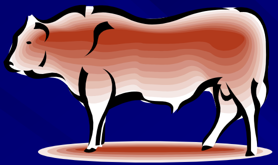
Identification by Comparing Fertile vs. Infertile Sperm Sample



Surface-Enhanced Laser Desorption/Ionization (SELDI)

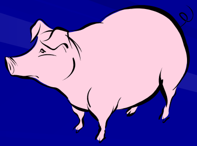
Negative Markers of Male Fertility

- Ubiquitin (UBB)



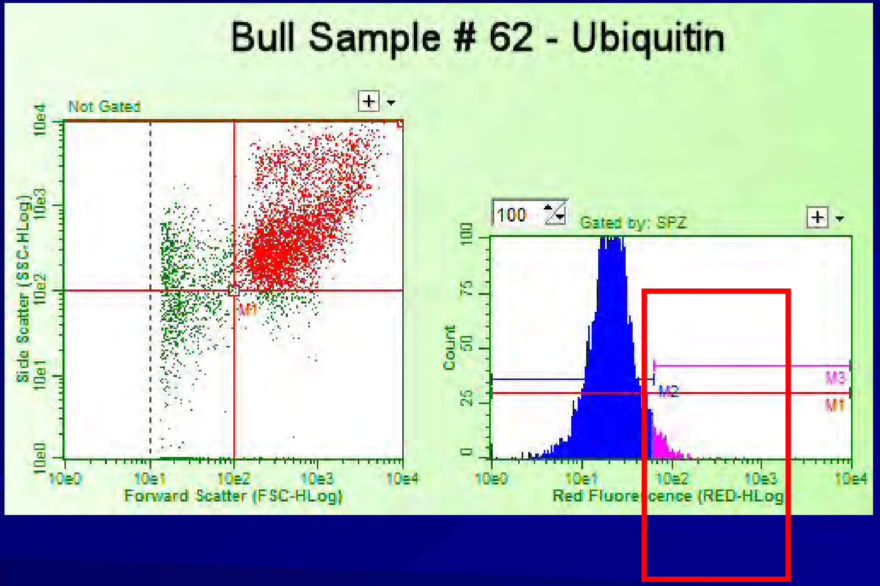
- 15-lipoxygenase (15LOX)
- Spermatid-Specific Thioredoxin 3 (SPTRX3)

- *Lens culinaris* agglutinin (LCA) and PNA-lectin-binding sperm glycans
- PAWP, Aggresome (AGG)

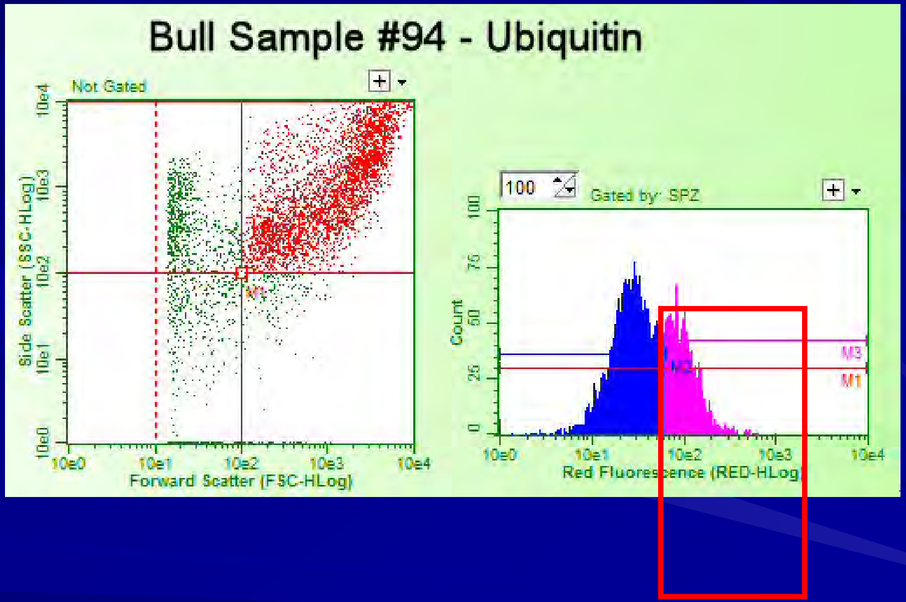


- Nuclear protamines, acetylated histones

Ubiquitin – Too Much Bad Protein



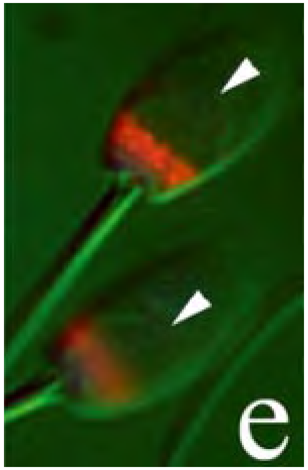
Normal Histogram



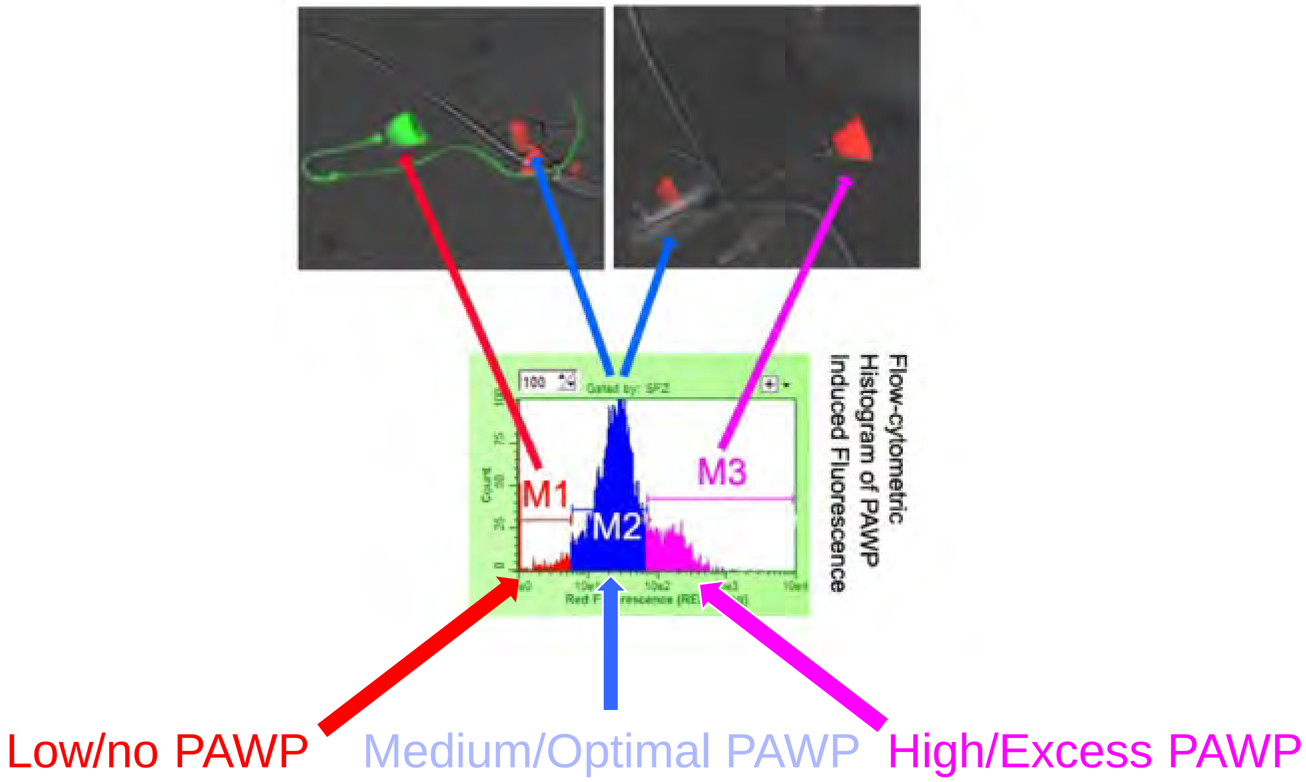
Abnormal Histogram

Postacrosomal Sheath WW-Domain-Binding Protein (WBP2NL/PAWP)

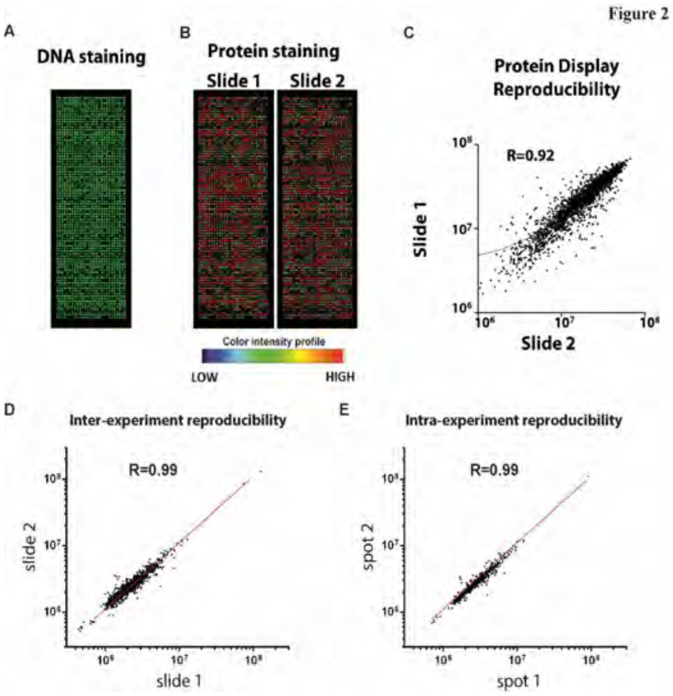
- Resides in the post-acrosomal sheath of the sperm head perinuclear theca (PT)
- Promotes oocyte activation and pronuclear development during fertilization
- Present in normal sperm *but a*bnormal sperm may have elevated levels of PAWP



Gating of Low, Medium and High PAWP in Bull Spermatozoa



PAWP-Candidate Breast Cancer Marker



Wang et al., 2015

Nourashrafeddin et al., Biomark Cancer 2015;7:19-24

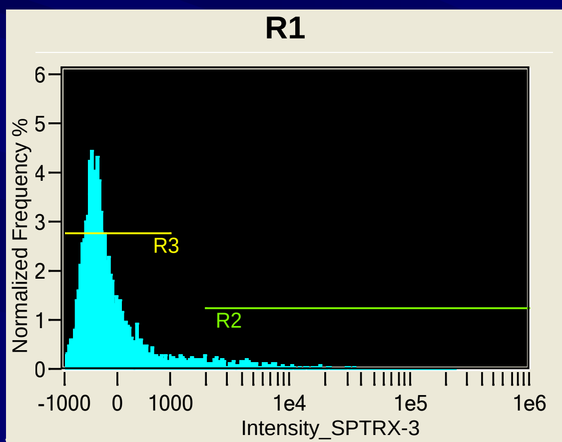
Nourashrafeddin et al., Pathol Oncol Res. 2015; 21(2):293-300

Wang et al., Cancer Epidemiol Biomarkers Prev. 2015 PMID: 26070530

^↑⇨ ⇆▶◀▶△⇨ ↓▽
⤷⇕▷
(The Future is Now)

Beginning by Sherstin Schwartz

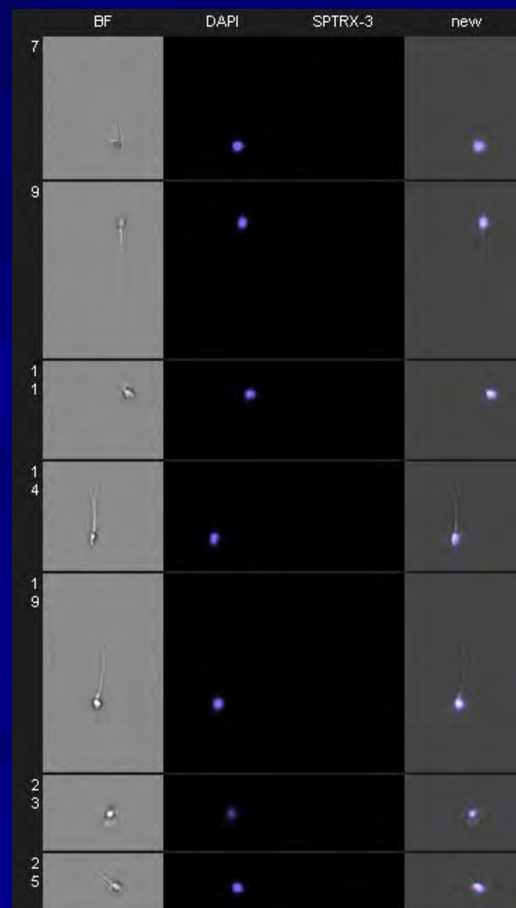
ImageStream-Flow Cytometer & Microscope in One Box



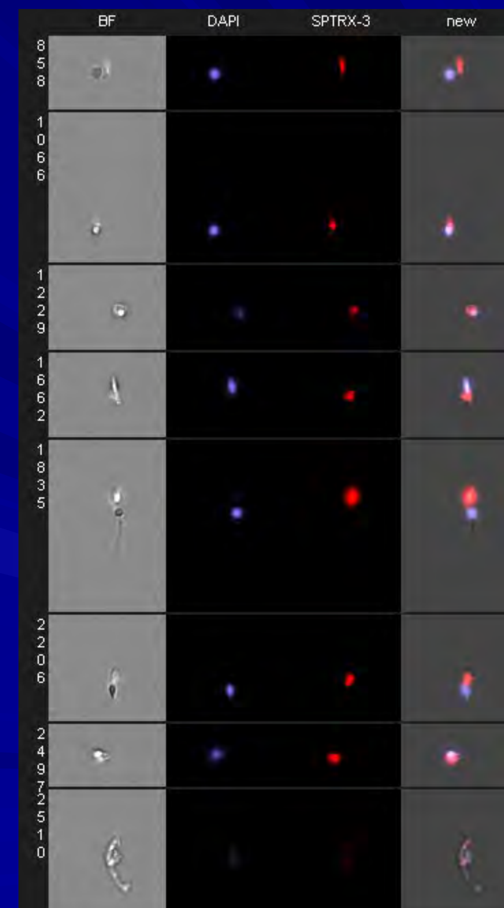
Population Statistics

Population	Count	%Gated
R1	5099	100
R3 & R1	4567	89.6
R2 & R1	319	6.26

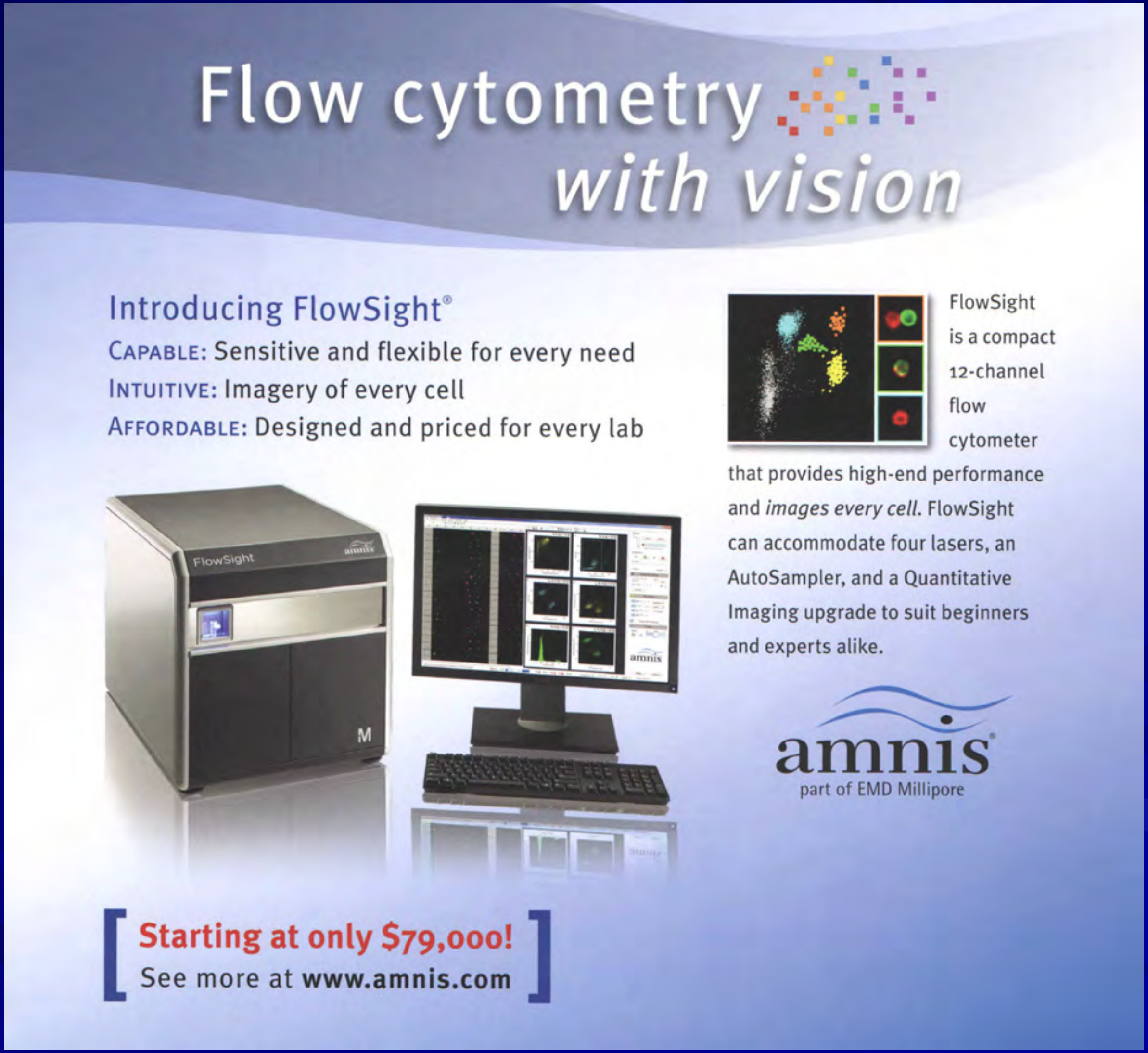
Normal Sperm (R3)



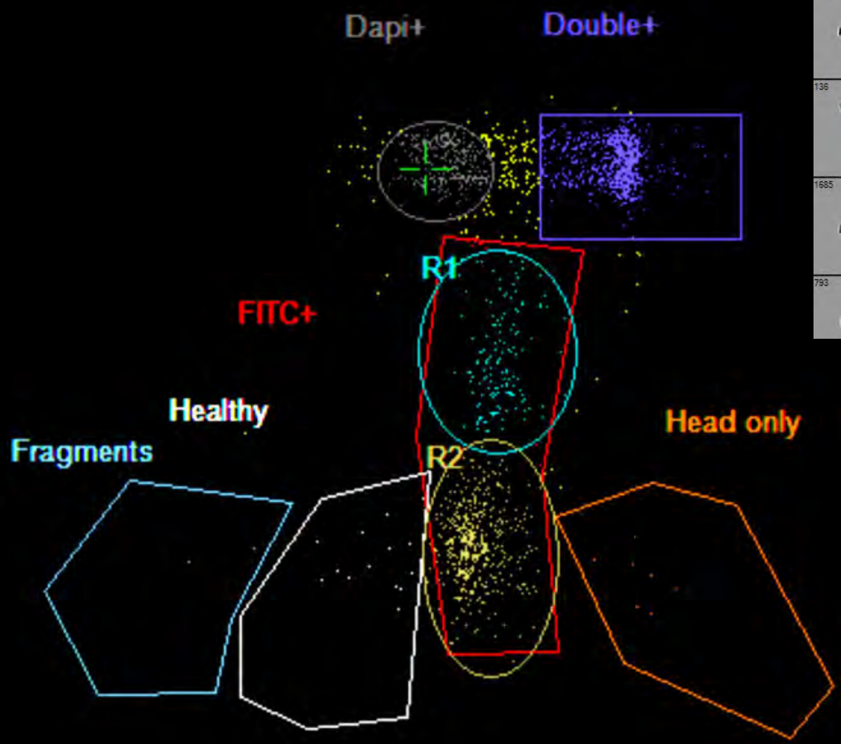
Defective Sperm (R2)

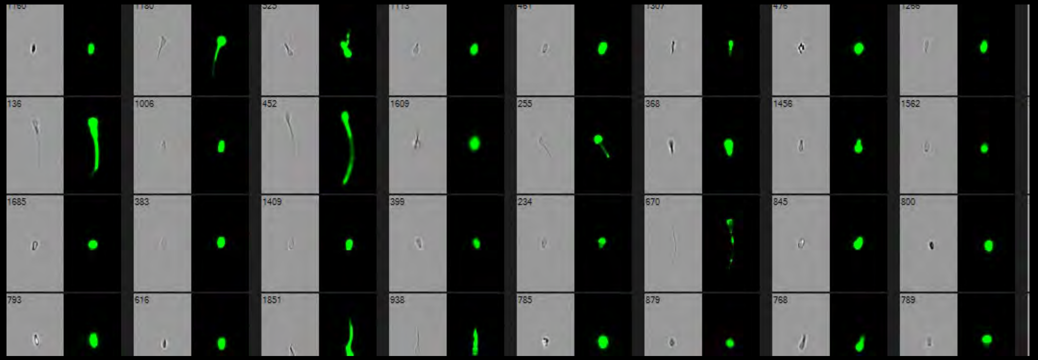


- Amnis Corp., Seattle WA [Buckman *et al.*, 2009, *Systems Biol. Reprod. Med.*, 55(5-6):244-251]


Flow cytometry
with vision
Introducing FlowSight®
CAPABLE: Sensitive and flexible for every need
INTUITIVE: Imagery of every cell
AFFORDABLE: Designed and priced for every lab
FlowSight is a compact 12-channel flow cytometer that provides high-end performance and images every cell. FlowSight can accommodate four lasers, an AutoSampler, and a Quantitative Imaging upgrade to suit beginners and experts alike.
FlowSight
amnis
part of EMD Millipore
Starting at only $79,000!
See more at www.amnis.com

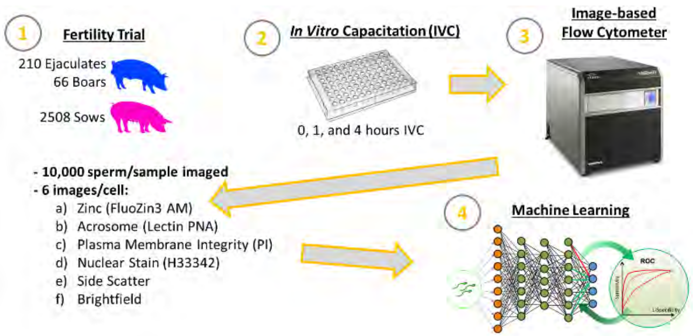
FlowSight DAPI+ LCA

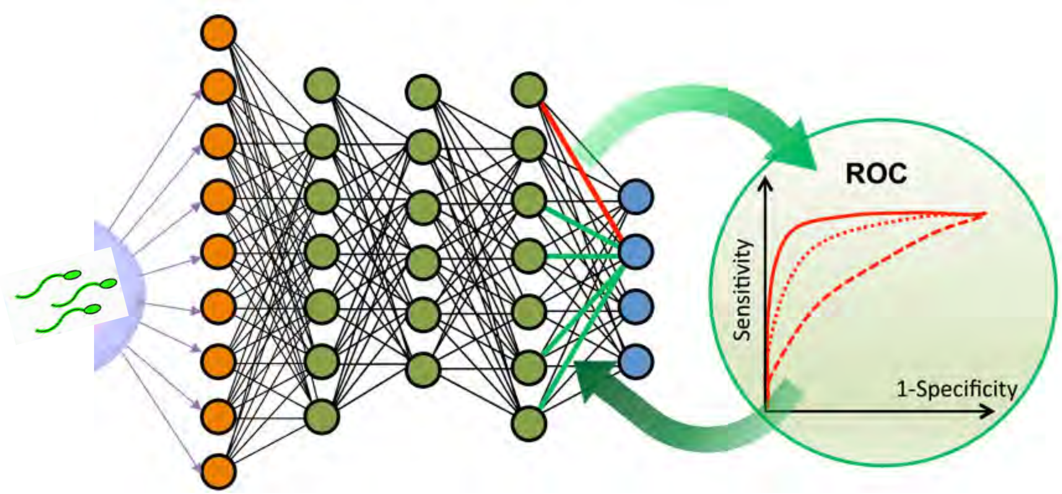




AI & Machine Learning

- “Face ID” for spermatozoa





Artificial Intelligence Analysis of the Mammalian Sperm Zinc Signature Predicts Male-factor Subfertility

Karl Kerns[1], Skyler Kramer[2], Michal Zigo[1], Amanda Minton[3], Dong Xu[4], Susanta Behura[1], Peter Sutovsky[1,5]

Biomarker Development Workflow

Step 1 - BIOMARKER DISCOVERY

Cell biology, proteomics, genomics, polymorphism identification, infertility screening, fertility analysis in animal models propagated by artificial insemination, knock-out animals.

Step 2 - BIOMARKER VALIDATION

Cell biology, immunocytochemistry, biochemistry, CASA, flow cytometry, infertile couple screening, low-fertile sire screening in livestock models. Antibodies & fluorescent probes are required.

Step 3 - MACHINE LEARNING

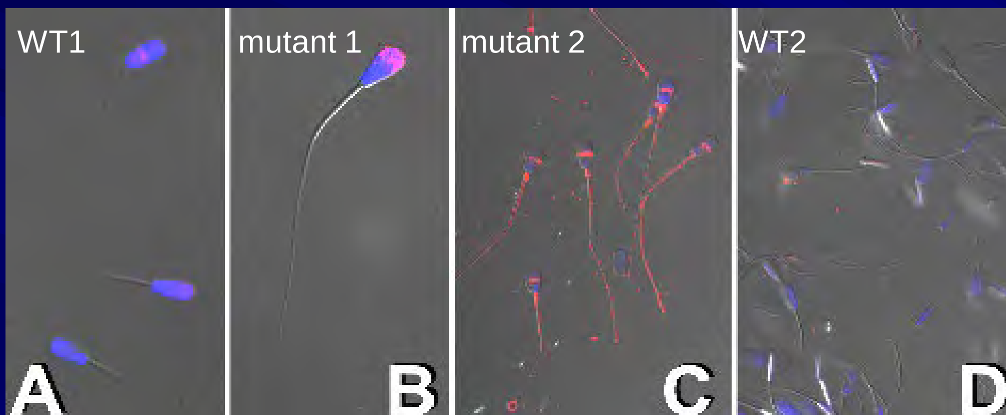
IBFC – association of infertile biomarker phenotypes with advanced multifactorial sperm morphometry patterns.

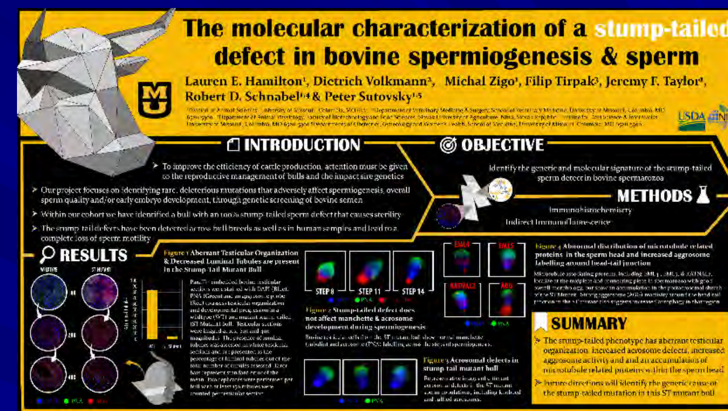
Step 4 - APPLICATION

Limited/No probes required (“label-free”)

Male infertility diagnostics	Single sperm selection for ICSI

Sperm Genomics – SNPs, INDELS Affecting Spermatogenesis, Sperm Structure and Sperm Function





Unique sperm phenotypes associated with predicted loss of function (LOF) affecting sperm quality/male fertility

Haplotypes Associated With Bull Fertility

- 255 AI sires with varied semen quality *Cooperative Dairy DNA Repository* aligned to NCBI sequences over 2,500 bull genomes
- Variants were called and the variant data were submitted to the *1000 Bull Genomes Project* for inclusion in the global variant dataset which will be derived from approximately 4,000 genomes
- Master list of candidate single nucleotide polymorphisms (SNPs) affecting bull fertility will be used for construction of genotyping microarrays.
- Focus on rare homozygous recessive mutations

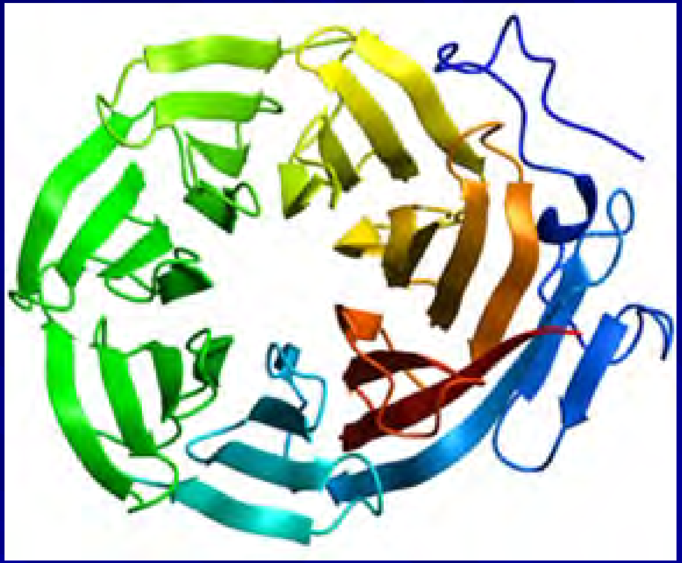
Table 1 *Haplotypes or mutations responsible for embryonic lethality discovered by genome-scanning for haplotype or genotype homozygote insufficiency*

Breed	Haplotype	OMIA[1] 9 913 ID	Gene(s)[2]	Frequency (%)	BTA[3]	Region (bp)
Ayrshire[4]	AH1	1934	*UBE3B*	13.00	17	65 921 497
	AH2	2134	*RPAP2*	9.80	3	51 267 548
Brown Swiss[4]	BH1	1825	–	6.67	7	42 811 272 to 47 002 161
	BH2	1939	*TUBD1*	7.78	19	11 063 520
Holstein – United States[4]	HH0	151	*FANCI*	2.76	21	21 184 869 to 21 188 198
	HH1	1	*APAF1*	1.92	5	63 150 400
	HH2	1823	–	1.66	1	94 860 836 to 96 553 339
	HH3	1824	*SMC2*	2.95	8	95 410 507
	HH4	1826	*GART*	0.37	1	1 277 227
	HH5	1941	*TFB1M*	2.22	9	93 223 651 to 93 370 998
	HHC	1340	*SLC35A3*	1.37	3	43 412 427
	HCD	1965	*APOB*	2.50	11	77 958 995
Holstein – France[5]	BY	151	*FANCI*	3.60	21	20 200 000 to 22 300 000
	HH1	1	*APAF1*	2.60	5	61 400 000 to 66 200 000
	HH2	1823	–	1.70	1	93 000 000 to 97 400 000
	HH3	1824	*SMC2*	2.50	8	94 000 000 to 96 500 000
	HH4	1826	*GART*	3.60	1	1 900 000 to 3 300 000
	HH5/HH6	1340	*SLC35A3*	3.90-4.60	3	45 800 000 to 52 600 000
	HH13	1836	–	3.70	18	56 400 000 to 58 400 000
Holstein – Nordic[6]	05-1351/05-1476	1907	–	1.60-2.02	5	106 713 645 to 114 405 063
	07-501	1909	–	1.92	7	34 633 456 to 36 127 497
	08-1276/08-1301/08-1326/08-1351	–	–	1.48-1.54	8	83 888 935 to 89 859 523
	11-926/11-976/11-1001/11-1026	1910	–	1.35-1.37	11	55 345 639 to 63 759 322
	19-151	1911	–	1.95	19	13 154 786 to 14 478 389
	21-276/21-301/21-326	–	–	1.94-2.05	21	20 477 690 to 24 844 501
Holstein – New Zealand[7]	–	2036	*TTF1*	3.52	11	102 485 897 to 102 515 271
	–	2037	*RABGGTB*	2.13	3	69 316 067 to 69 322 906
	–	2038	*RNF20*	1.82	8	92 911 255 to 92 935 750
Jersey – United States[4]	JH1	1697	*CWC15*	12.10	15	15 707 169
	JH2	1942	–	1.30	26	8 812 759 to 9 414 082
Jersey – New Zealand[7]	–	2035	*OBFC1*	6.59	26	24 700 354 to 24 737 868
Montbeliarde[5]	MH1	1827	*SHBG*	9.00	19	27 600 000 to 29 400 000
	MH2	1828	*SLC37A2*	7.00	29	27 900 000 to 29 100 000
	MH3	1842	–	5.10	2	31 500 000 to 32 800 000
	MH5	1844	–	7.10	6	73 300 000 to 74 400 000
	MH6	1845	–	2.60	7	80 100 000 to 81 700 000
	MH8	1847	–	3.50	13	76 400 000 to 77 600 000
Normande[5]	NH1	1851	–	1.80	24	38 100 000 to 39 200 000
	NH2	1852	–	3.80	1	145 700 000 to 146 800 000
	NH5	1829	–	1.90	7	3 600 000 to 4 600 000
	NH6	1855	–	1.90	15	59 800 000 to 61 100 000
Danish Swedish and Finnish Red Cattle[8]	A27	1901	*RNASEH2B*	6.50-16.00	12	20 101 696 to 20 755 193
Nordic Red Cattle[9]	–		*BTBD9, GLO1, DNAH8*		23	12 291 761 to 12 817 087
Angus[10]	ANH1	–	–	2.30	1	27 786 985 to 29 095 768
	ANH2	–	–	7.60	4	82 467 969 to 83 996 686
	ANH3	–	–	2.30	8	62 040 920 to 63 000 189
	ANH4	–	–	3.20	12	59 989 293 to 61 258 655
	ANH5	–	–	3.80	15	82 317 986 to 83 144 172
	ANH6	–	–	4.50	17	46 514 063 to 47 462 424
	ANH7	–	–	4.40	29	43 043 207 to 44 243 444
Fleckvieh[11]	FH1	1957	–	2.90	1	1 668 494 to 6 187 555
	FH2	1958	*SLC2A2*	4.10	1	97 239 973
	FH3	1959	–	3.30	10	26 929 817 to 35 479 280
	FH4	1960	*SUGT1*	3.30	12	11 131 497
Belgian Blue[7]	–	2042	*EXOSC4*	1.33	14	1 947 198 to 1 949 074
	–	2043	*MED22*	1.15	11	104 305 076 to 104 311 650
	–	2039	*MYH6*	4.99	10	21 325 414 to 21 344 965
	–	2041	*RPIA*	1.89	11	47 220 160 to 47 254 704
	–	2040	*SNAPC4*	5.13	11	103 884 749 to 103 905 548

[1]Online Mendelian inheritance in animals. Taxon ID 9913 represent cattle.
[2]Multiple listed genes represent a deletion.
[3]*Bos taurus* chromosome.
[4]Cole *et al.* (2017).
[5]Fritz *et al.* (2013).
[6]Sahana *et al.* (2013).
[7]Charlier *et al.* (2016).
[8]Kadri *et al.* (2014).
[9]Sahana *et al.* (2016).
[10]Hoff *et al.* (2017). Haplotypes not validated as fertility associated.
[11]Pausch *et al.* (2015).

Identification of a Rare, Fertility Affecting Mutation in Bovine *Eml5* Gene

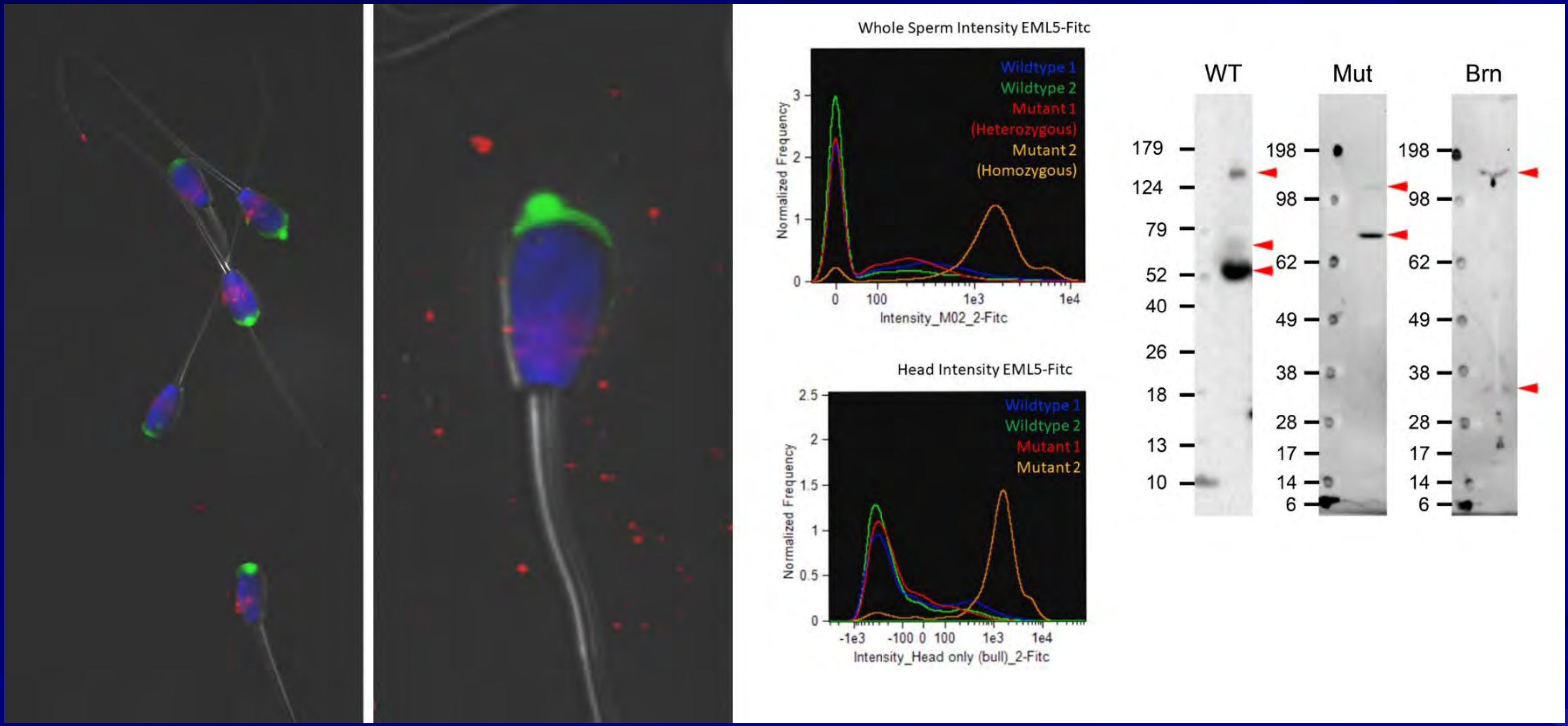
- Echinoderm microtubule-associated protein-like 5 isoform X5
- Rare mutation in WD40 domain
- Repetitive, circular solenoid protein domain for multi-protein complex assembly



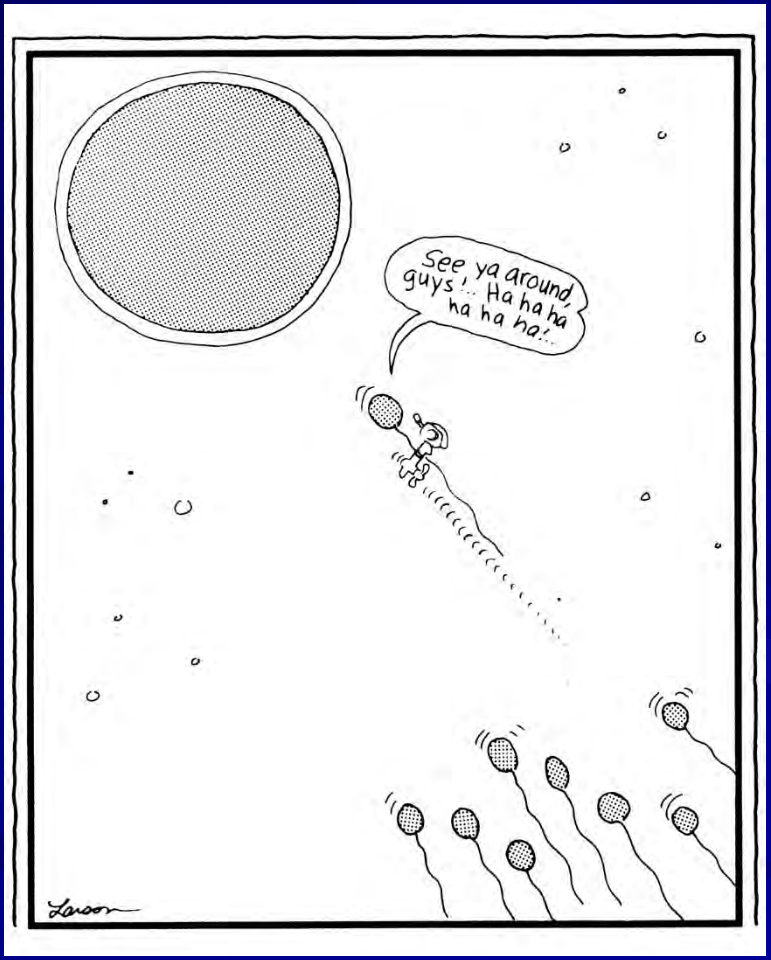
Michal Zigo[1], Eriklis Nogueira[2,3], Karl Kerns[1], Miriam Sutovsky[1], JaeWoo Kim[1], Filip Tirpak[4], Thomas E. Spencer[1,5], Jeremy F. Taylor[1], Robert D. Schnabel[1,6], Peter Sutovsky[1,5]

Embrapa

Protruding Knobbed Acrosome Phenotype in Homozygous *Eml5*$^{wd40+/+}$ Mutant Bull



Selecting the fittest spermatozoa…

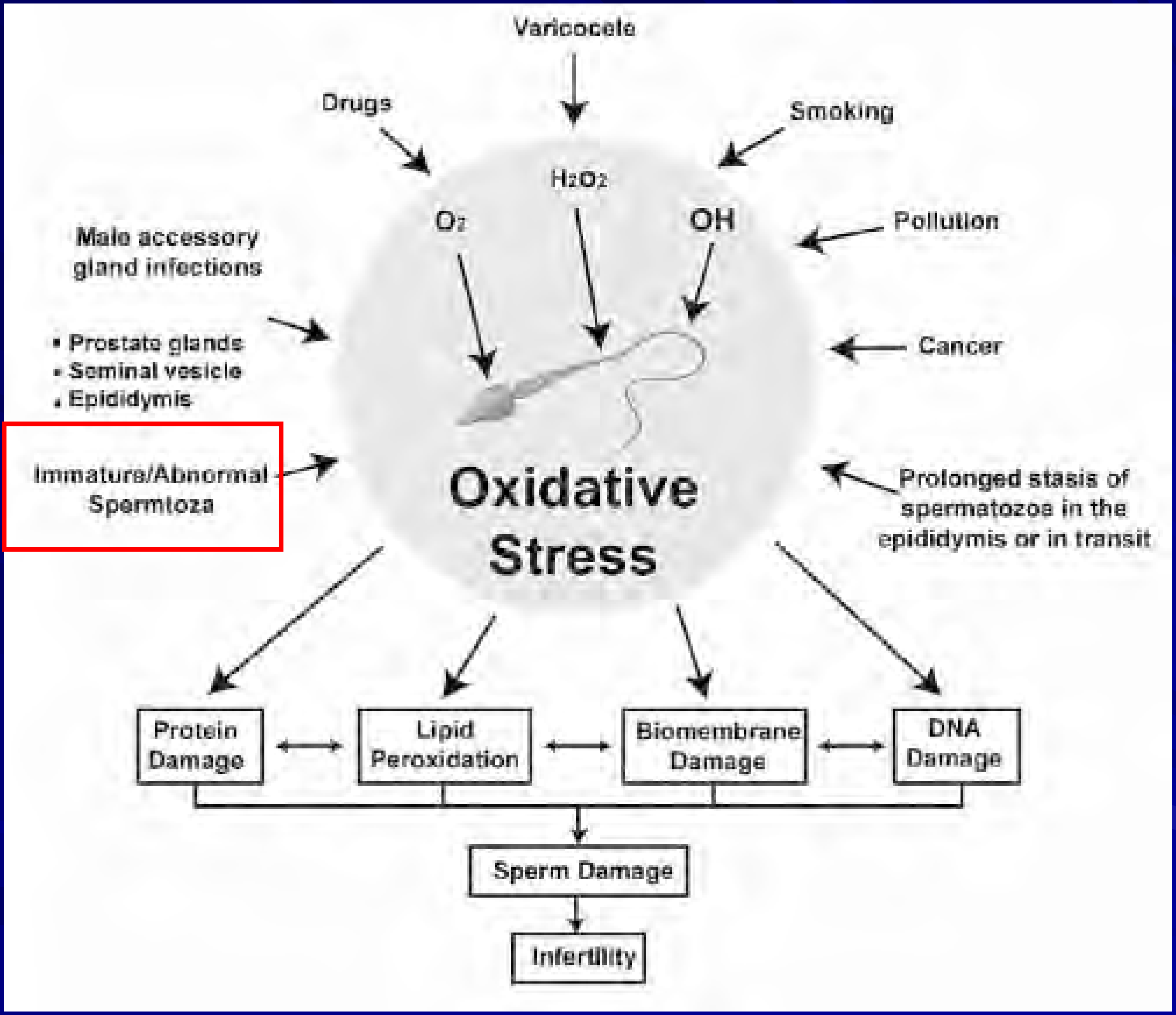


…AND GETTING THE RID OF THE BAD AND THE UGLY ONES

Semen Purification

Goal: Remove defective spermatozoa from semen used or artificial insemination (AI)

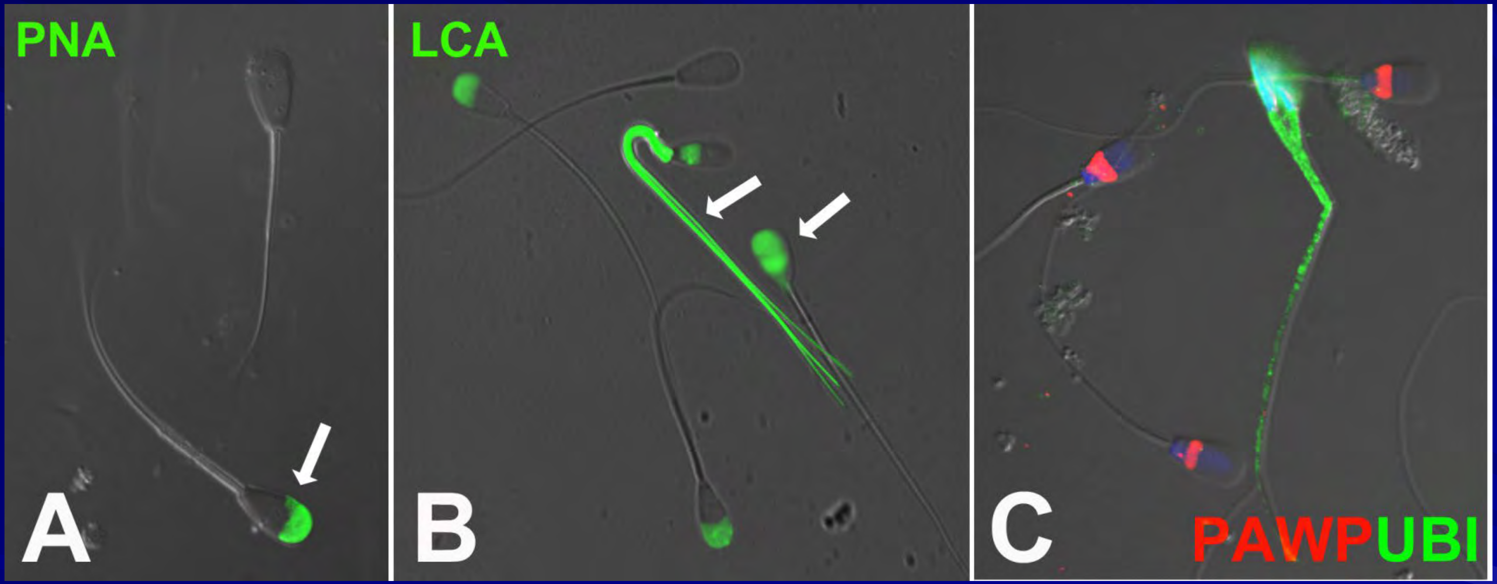
Benefits: Increased fertility/pregnancy rate, lower dose of spermatozoa per insemination, efficient use of genetically valuable sires, possibly eliminating the need for semen pooling

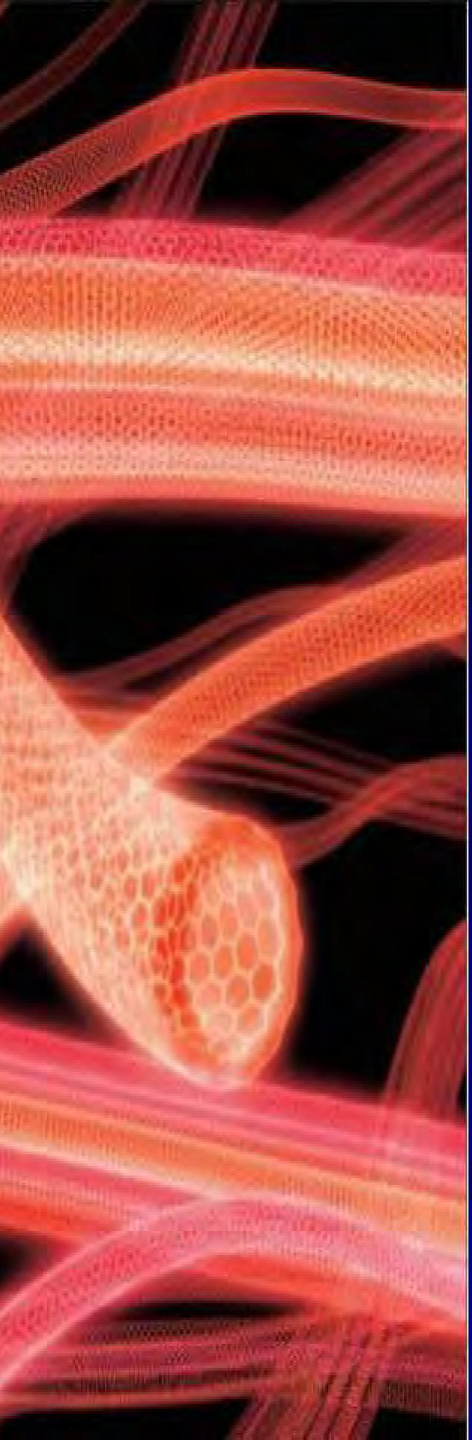

Varicocele
Drugs
Smoking
H_2O_2
O_2
OH
Pollution
Male accessory gland infections
• Prostate glands
• Seminal vesicle
• Epididymis
Cancer
Immature/Abnormal Spermtoza
Oxidative Stress
Prolonged stasis of spermatozoa in the epididymis or in transit
Protein Damage
Lipid Peroxidation
Biomembrane Damage
DNA Damage
Sperm Damage
Infertility

Current Methods:

- Swim Up
- Gradient (e.g. PureSperm, OptiPrep)
- Glass wool filtration
- Magnetic activated cell sorting (Annexin 5 beads)
- Hyaluronan based methods (HA-coated dishes)

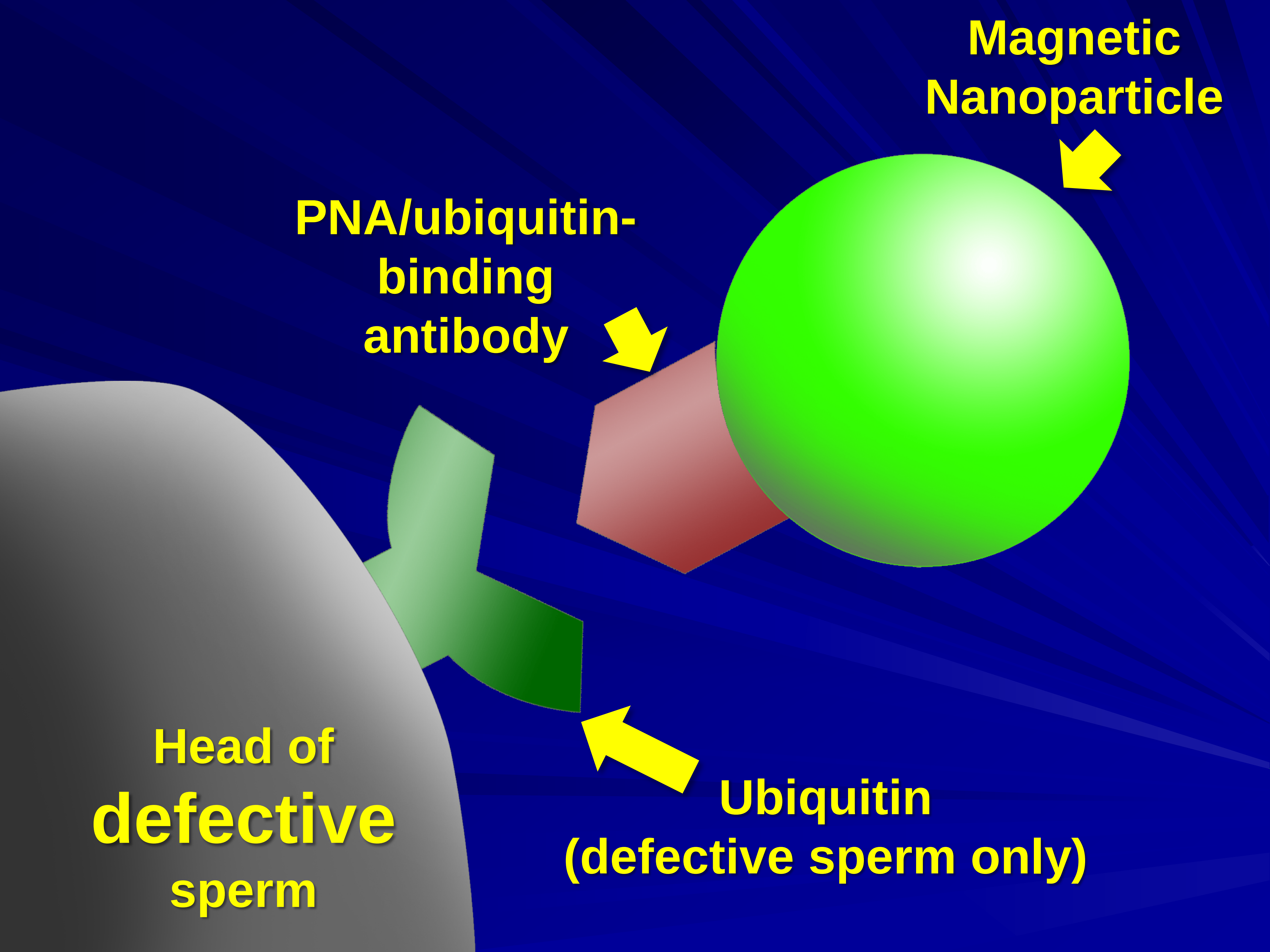
Surface Ligands in Defective Spermatozoa

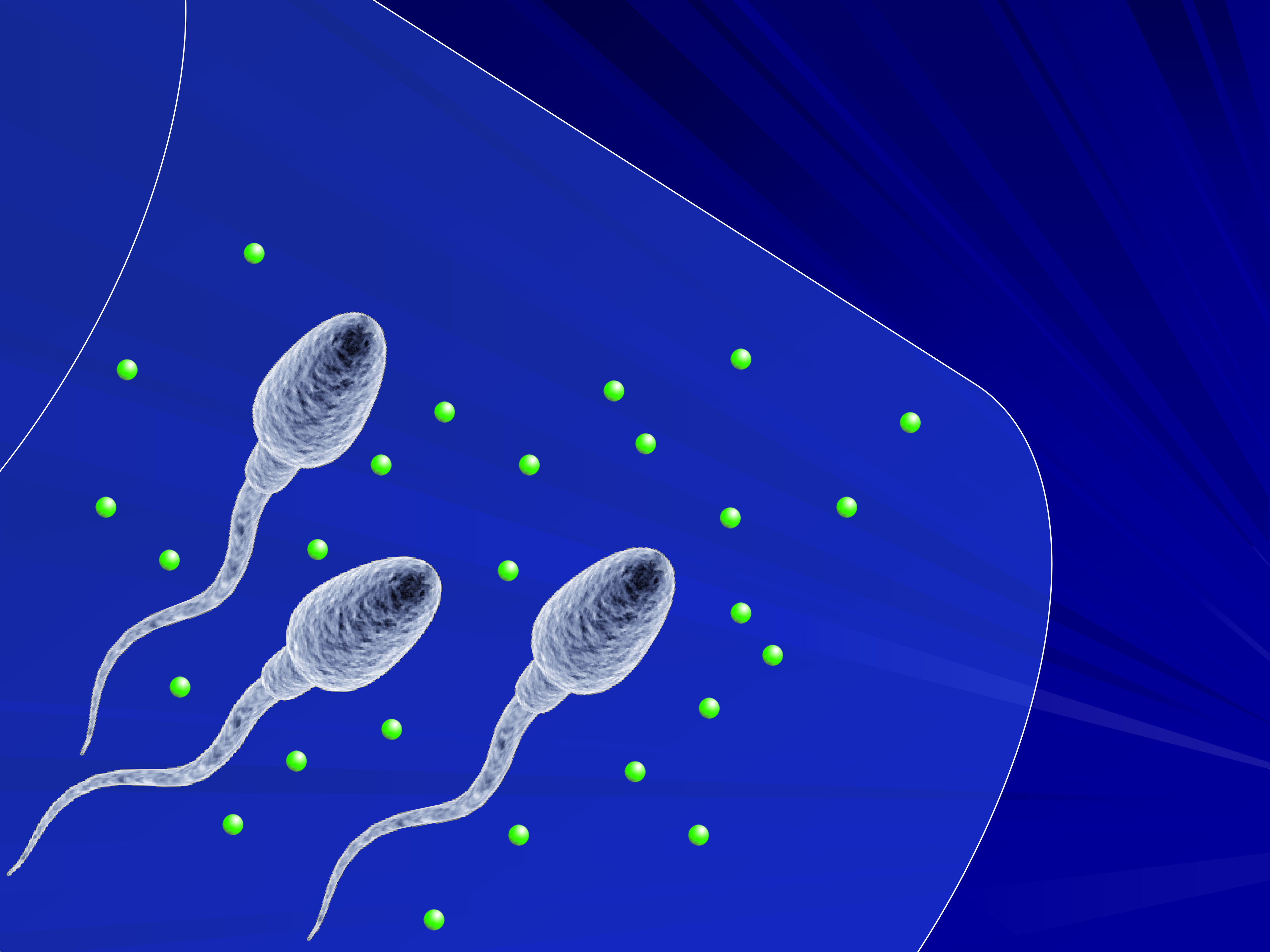


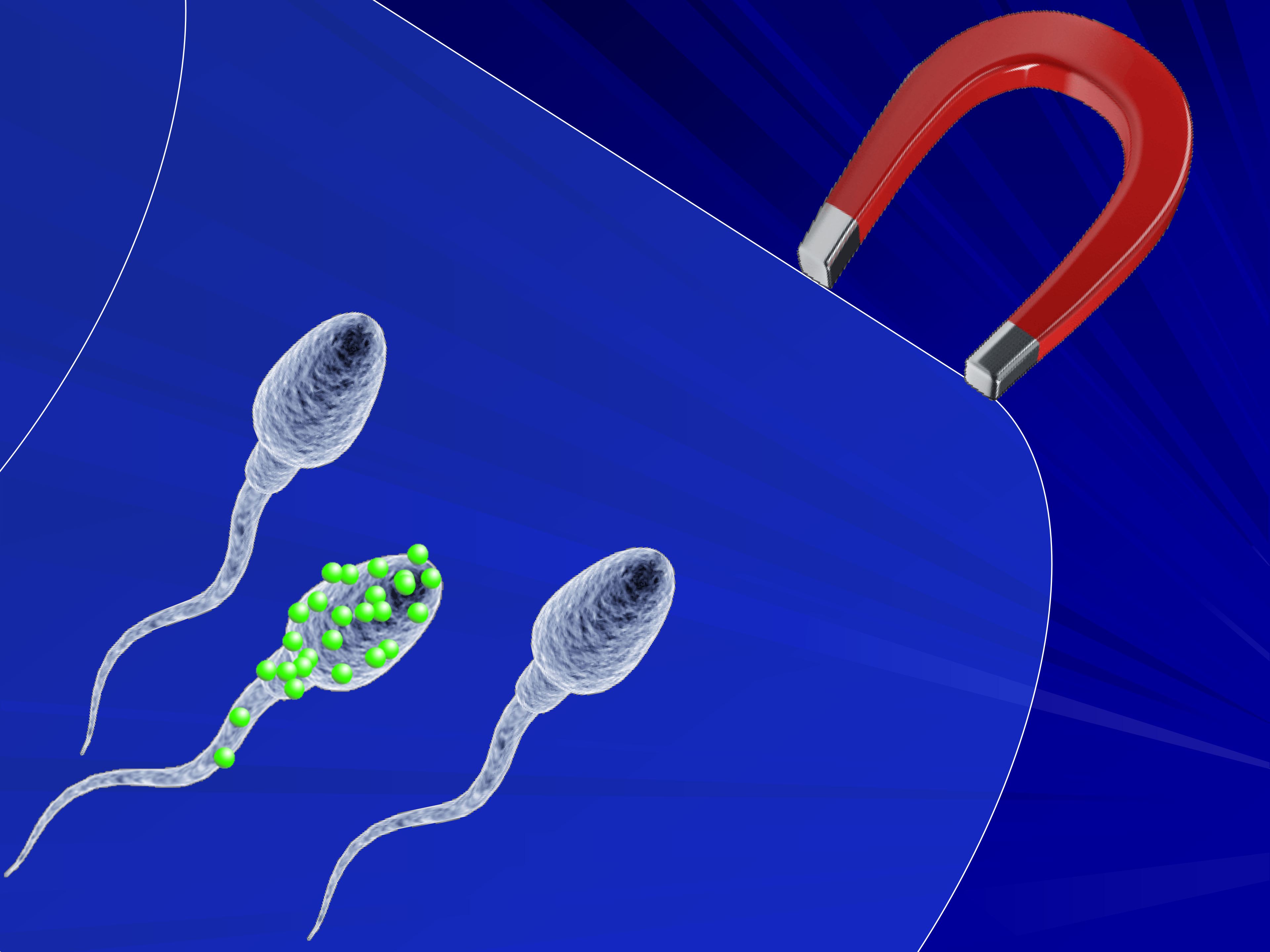


νᾶνος (nanos) Greek for ‘dwarf’

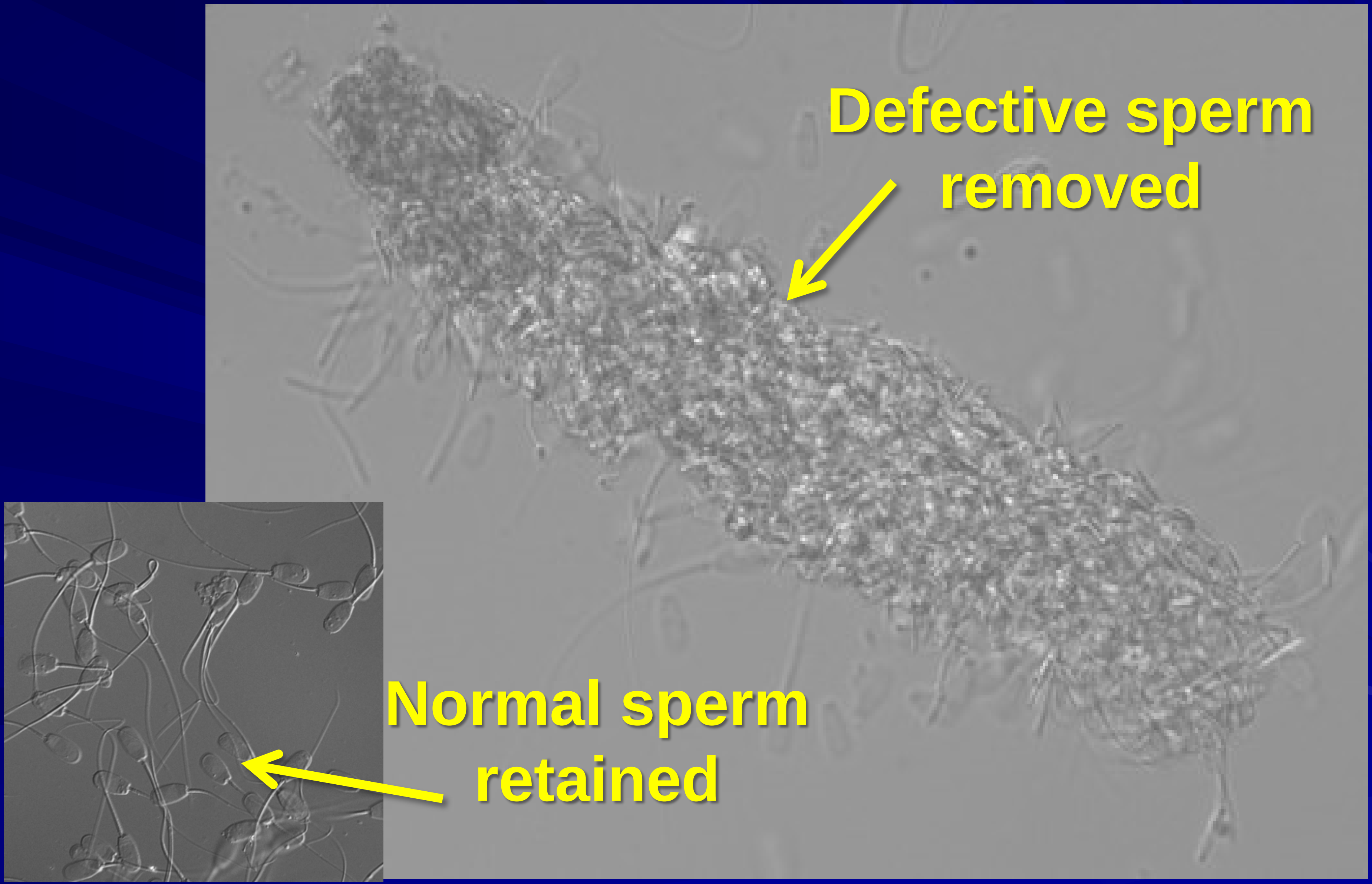
- Nanotechnology: manipulation of a matter on an atomic or molecular scale
- Nanotechnology may refer to a material or to a manufacturing process
- Applications in medicine, electronics, energy production and agriculture
- Concerns about toxicity and environmental impact


Magnetic
Nanoparticle
PNA/ubiquitin-
binding
antibody
Head of
defective
sperm
Ubiquitin
(defective sperm only)

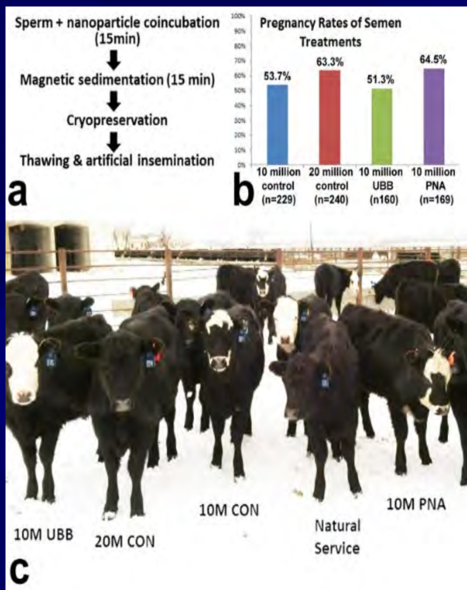




Following depletion . . .

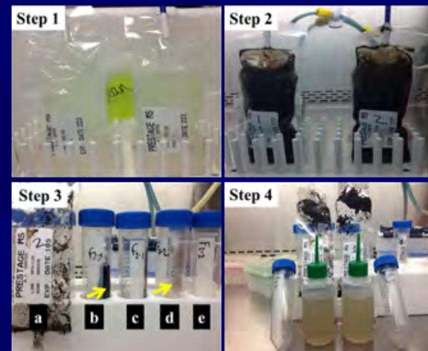


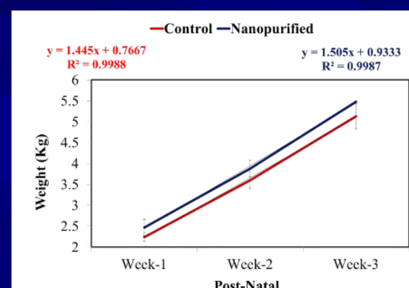
Validation In Livestock Models



798 AI Services, 466 Healthy Calves Born

Odhiambo *et al.*, 2014, *Biol. Reprod.*,





Boar Sperm Nanopurification

Feugang *et al.*, 2015, JFIV Reprod. Med. Genet. 3:145

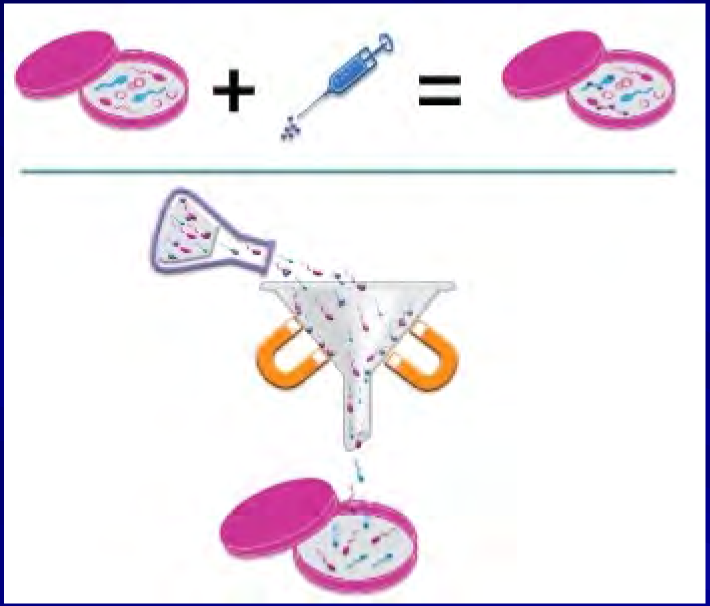
Nano-depletion of acrosome-damaged donkey sperm by using lectin peanut agglutinin (PNA)-magnetic nanoparticles.

Yousef MS, et al. Theriogenology. 2020

Purification of cryopreserved camel spermatozoa following protease-based semen liquefaction by lectin-functionalized DNA-defrag magnetic nanoparticles

Rateb SA, Reprod. Dom. Anim. 2021

MCRM FERTILITY



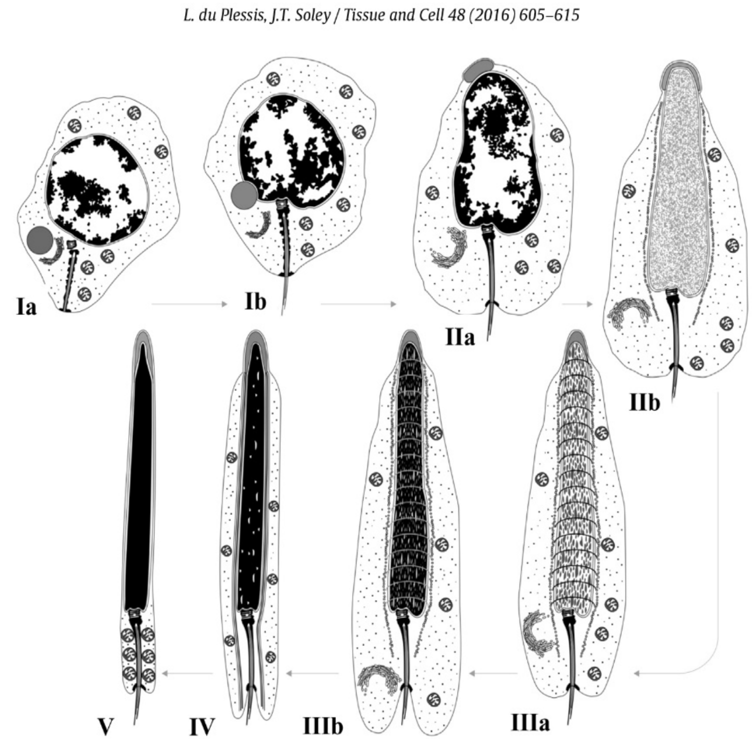
https://www.youtube.com/watch?v=ZsFsyFQTljs

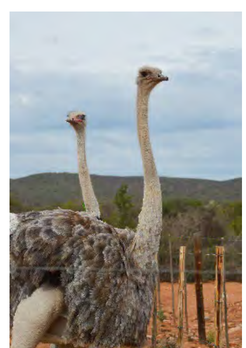
http://www.mcrmfertility.com/treatment-options/in-vitro-fertilization-ivf-/selecting-the-perfect-sperm/nanobead-sperm-selection-process.aspx

SUMMARY

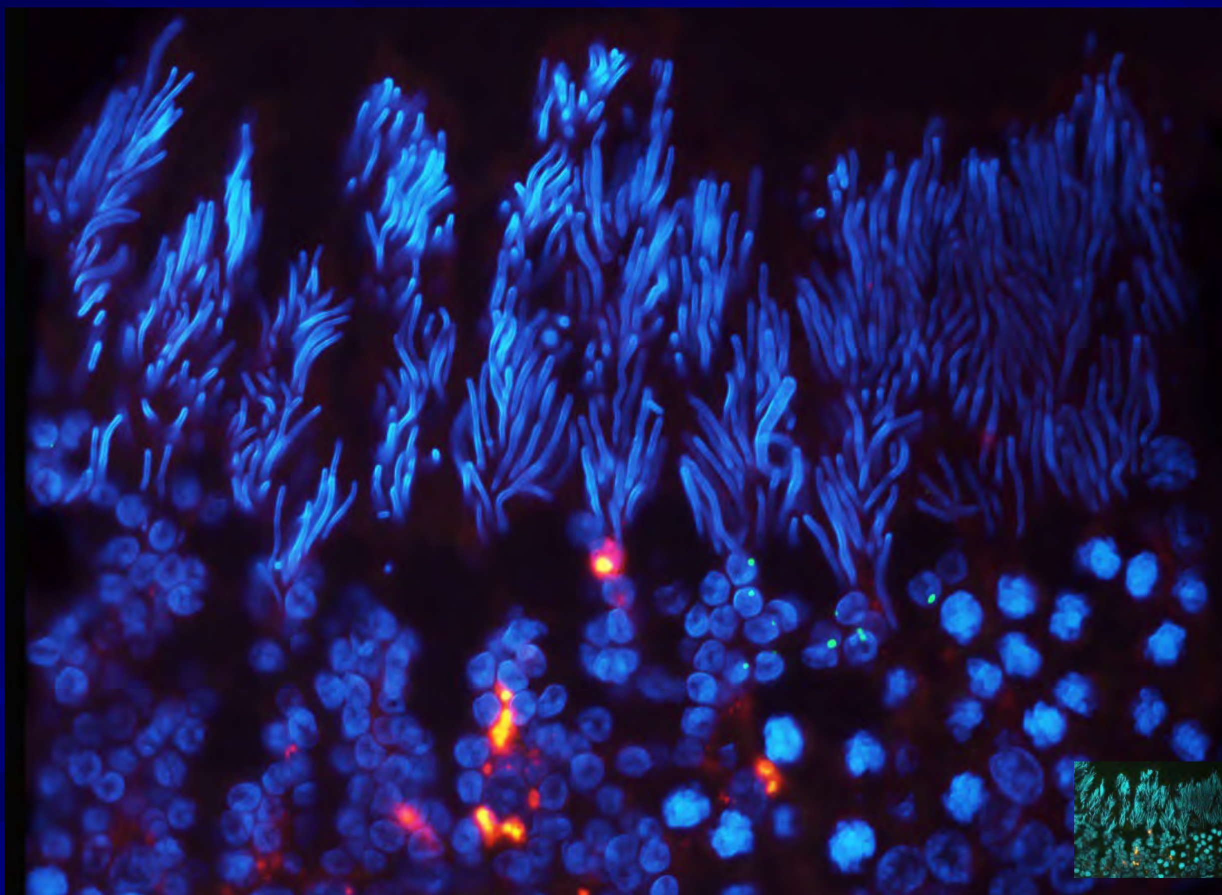
- Biomarker based sperm quality assays reveal molecular sperm defects undetectable by conventional semen analysis
- Livestock models propagated by artificial insemination are ideal for biomarker development
- Omics and biomarker based computer training and machine learning will enable label-free, fully automated andrological analysis, *both in men and male livestock*
- Pre-selection of fittest spermatozoa is possible through a variety of approaches, including nanotechnology

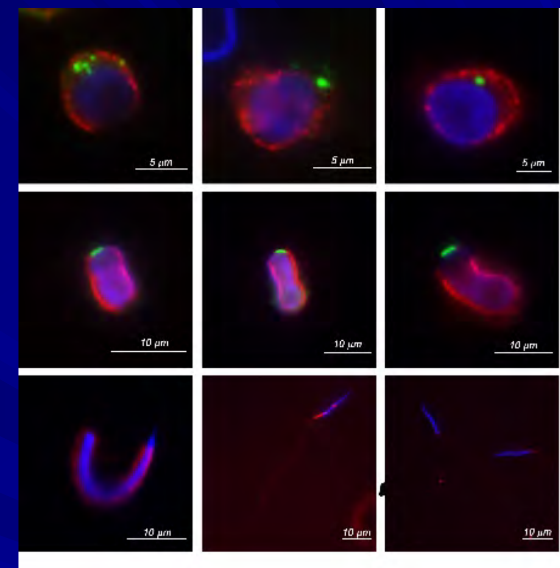
The Oudtshoorn Project





Ostrich Testis





Lab-Present

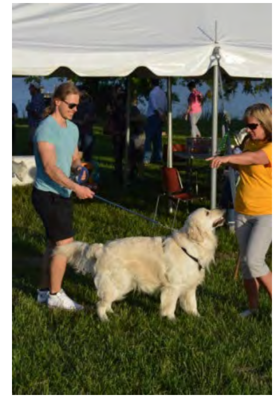
Miriam Sutovsky
Michal Zigo
Lauren Hamilton
Dalen Zuidema
Alexis Jones
Edgar Miranda
Betsy Pascoe

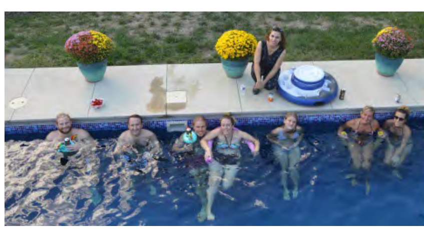
Lab-Past

Karl Kerns (MU Adjunct Faculty)
Kathy Craighead
Eriklis Nogueira
Filip Tirpak
Seda Ocakli
Lucie Tumova
Miriama Stiavnicka
Clio Maicas
Jiude Mao
Jenny Jankovitz
Wonhee Song
Linda Yan
Jan Nevoral
Peter Petruska
Veronika Benesova
Young-Joo Yi
TJ Miles
Peter Vargovic
Shawn Zimmerman
Chelsey Kennedy
Gauri Manandhar
Fred Odhiambo
T.J. Myles
Katie Fischer
Kathleen Baska
Kyle Lovercamp
Kelly Moore
Dawn Feng
Jen Antelman
Heinz Leigh
Nicole Leitman
Clayton Buckman

MU Collaborators

Randy Prather MU ANSCI
Kevin Wells MU ANSCI
Jerry Taylor & Bob Schnabel MU ANSCI & GENOMICS
Tim Safranski MU ANSCI
Gavin Conant MU ANSCI
Jamal Ibdah – MU School of Medicine
Kathy Sharpe-Timms MU-OBGYN
Gary Clark MU-OBGYN
Laura Schultz MU OBGYN
Yuksel Agca MU VETMED
Heide Schatten MU VETMED
Dietrich Volkmann MU VETMED





External Research Collaborators

John Soley & Lizettte DuPlessis U. of Pretoria
Richard Oko-Queen's U. Kingston, ON
Tom Geary USDA-ARS
David Miler, U. of Illinois
Wansheng Liu, Penn State U.
Bill Ballard, Sydney, AUSTRALIA
Justin St John, Hudson Institute, AUSTRALIA
Mark Baker & John Aitken, Newcastle, AU
Stuart Meyers, UC Davis
Markéta Sedmíková, Lucie Tumová, CZU (CULS) Prague
Věra Jonáková , Jana Pěknicová ,
Pavla Postlerová, Inst. Biotech., Prague
Jan Nevoral, Miriam Štiavnická, Charles University in Pilsen CZ
Katerina Dvoraková-Hortová, Charles U., Prague
Eva Nagyová, Andrej Šušor, IAPG, CAS, Liběchov, CZ
Keith Latham, Temple U.
Erdogan Memili, U. Mississippi
Namdori Mtango, Temple/Minitube
Susan Suarez, Cornell U.
Satish Gupta, Natl. Inst. Immunol., New Delhi
Antonio Miranda-Vizuete, UPdO, Sevilla
Klaus van Leyen, Harvard
Winston Thompson, Morehouse School of Medicine
Fred van Leeuwen, University of Maastricht

Current Funding

USDA-NIFA
NIH-USDA Dual Purpose – Dual Benefit
NIH-NICHD
Seed Funding UM

Industry Collaborators

Patrick Blondin, Semex Canada
Mel DeJarenette-Select Sires
Kari Beth Krieger – Genex
Abdullah Kaya -Alta
Tom Geary, USDA
Eric Schmitt, IMV Technologies
Peter Ahlering, MD, MCRM, St. Louis

National Institutes of Health
The Nation's Medical Research Agency

IRHG
Integrated Reproduction and Health Group

USDA NIFA
United States Department of Agriculture
National Institute of Food and Agriculture

Thank you for keeping the flame lit

SutovskyP@missouri.edu

Sutovsky Lab // University of Missouri

Photo: Histological section of a rat seminiferous tubule