Commercialization of Novel Biomarkers of Male Fertility in Humans and Farm Animals

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Goals

- Provide new tools and fertility markers for farm animal semen evaluation and diagnostics of human male infertility
- **Improve Assisted Reproductive Technologies (ART)** in humans and animals
- Identify new contraceptive targets
Need & Opportunity

Est. 20% of US population are affected by infertility

135,000 infertility treatment-cycles/year realized in USA; 350,000/year in Europe; annual expenditure on fertility services in US: >$2 billion

Farm animal industry: 16,289,681 dairy bull semen units sold in US and 8,313,572 units exported in 2003; est. market $500 million/yr

Animal reproductive performance: One extra piglet/litter would add $135 million/year to US swine industry
Technologies & Customers

Doctor’s Office/Home Test Kit
- Infertility Clinics
- Veterinary Clinics
- Over the counter fertility test for men

Reference Lab Test
- Forensic lab test kit
- Human Infertility Clinics
- Farm Animal Producers
- Artificial Insemination Companies
- Pharmaceutical Companies
- Government (Environmental Monitoring)
Conventional Male Fertility Evaluation/Infertility Diagnostics: Subjective & Outdated!

- Sperm Count
- Movement (Motility)
- Appearance (Morphology)
- Contaminants (White blood cells, immature sperm cell forms, dead epithelial cells, cellular debris)
Solution: Biomarkers of Male Fertility/Sperm Quality

- Ubiquitin (UBI)
- 15-lipoxygenase (15LOX)
- Spermatid-Specific Thioredoxin 3 (SPTRX3)
- Lectin-binding glycans
Biomarker Validation

- Correlate with sperm/semen quality in humans and male farm animals
- Correlate with fertility (human ART, animal AI)
- Predictive of spontaneous pregnancy loss
- Predictive of multiple births after ART
Fertility Kit Development

- Chute-side/home use kit (dipstick)
- Andrology lab (light microscope)
- Reference lab (flow cytometry)
- Forensic: Sperm detection
So simple, a caveman could do it...

- Add 1 µL semen in 500 µL 100% methanol (10 min)
- Spread on a slide (let it dry for 5 min)
- Add 200 µL tagged UBI probe for 20 min
- Wash slide in water
- Observe & Count
The Future is Now: Guava EasyCyte Plus

Advantages:

• High throughput
• No clogging
• Easy to operate
• Low maintenance
• Low acquisition cost
• Fully networked

Current Sperm Assays:

• Viability
• Calcium concentration
• Acrosomal integrity
• Motility
• Mito Potential
• Sperm Count
• Bacterial Count
NanoTime: Magnetic Depletion of Bad Sperm Cells

- Anti-UBI antibody conjugated to metallic nanoparticles
- Semen is enriched with particles during cooling
- Particles bind to ubiquitin on defective sperm surface
- Magnet applied to tube for 5 min
- Semen slowly decanted, leaving defective sperm in the vessel

Missouri Life Sciences Trust Fund Grant #13324
Magnetic Cell Separator
Clemente Associates, Madison, CT
Sperm Depletion Protocol

15 min incubation

15 min. magnetic particle pull down

Evaluation by flow cytometry

Forward & side scatter - cell size

CYBR-14 & PI Viability Test
Nanodepletion Improves Bull Sperm Viability

![Chart showing sperm viability comparison between MK, MK/PNA, and Control groups. The MK/PNA group has the highest viability at 27.5, followed by the MK group at 23.9, and the Control group at 23.6.]
SPARC Quarterly Networking Event

Bringing science and business together to facilitate technology transfer and economic development
# Off-hours crime fighter

*By day, he studies reproductive efficiency of farm animals, by night, he invents ways to link felons with their victims*

## Paternity Testing Corporation

*Columbia, Missouri*

- **Christian Carson, Ph.D.**
  - Principal Investigator for Research and Development
- **Kim Gorman**
  - President, DNA Supervisor, Technical Leader
- **Michelle Beckwith**
  - Vice President
- **Joe Gorman**
  - General Counsel
- **John Beckwith**
  - Business Manager

## University of Missouri-Columbia

- **Peter Sutovsky, Ph.D.**
  - Associate Professor Animal Sciences and Clinical OBGYN
- **Gaurishankar Manandhar, Ph.D.**
  - Research Assistant Professor
  - Animal Reproductive Biology Group

PS co-discovered the SPTRX3 gene with **Antonio Miranda-Vizuete et al.**, Andalusian Center for Developmental Biology, Seville, Spain

**Special thanks to ALL of the staff at PTC**

**National Institute of Justice SBIR Grant #2008-IJ-CX-K007**
QuantAssure

A Microscope-Free Method of Semen & Sperm Cell Detection and Quantification

Prototype development funded by SBIR grant from the National Institute of Justice
Sexual Assault

coital interval

Medical Examination

Forensic evidence
dried human secretions
swab, tissue, cloth, etc.

Serology
identify semen,
identify sperm cells

Differential extraction
separate sperm cells/DNA
and from epithelial cells/DNA

DNA testing
male fraction,
epithelial fraction
Lateral Flow Cassette

1. Sample with dissolved sperm cells deposited in the well
2. Sperm cell-specific proteins labeled by colloidal gold-tagged antibody
3&4. Gold particles with bound sperm protein, if present are captured on test lines
Summary

- Commercialization opportunities are substantial for reproductive biology applications

- Clients include infertility clinics, farm animal AI companies, forensic labs, men who care about their reproductive health

- Commercialization is being pursued through industry collaborators/licensees (IMV, PTC) and through our own start-up effort (AndroLogika LLC)

- Efforts described here were made possible, and in some cases initiated by University tech transfer and funding from the State of MO
Lab-Present
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Vera Jonakova, Inst. Mol. Biol., Prague
Fred van Leeuwen, University of Maastricht
Mark Tangowski, Phizer & VirtualScopics Inc.
Mike Powell, Morehouse School of Medicine
Gustavo Doncel, CONRAD

Current Funding
USDA-CREES
F21C-UM
MO-Life Sci. Trust Fund
NIH-NICHD
NIJ-SBIR
IMV Technologies (in kind)

Industry Collaborators
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Peter Ahlering, MD, SIRM, St. Louis
Christophe Staub & Eric Schmitt, IMV Technologies
Cliff Marshal & Mel DeJarenette-Select Sires
Henry Clemente & William Fry, Clemente Associates
Phil Morrissey & Tad George, AMNIS Corp.

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Kelly Moore
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Heinz Leigh
Nicole Leitman

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Go Tigers!

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