Tips and Tricks of Testicular Tissue In Vitro Culture: Optimizing Progressive Sperm Motility, ICSI Selection and Cryopreservation

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Disclosure

- The speakers have no conflicts of interest to disclose

- Developmental /Applied research studies were performed at previous clinical affiliates

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Learning Objectives

- Understand cellular characteristics of testicular tissue dispersed into suspension;
- Gain insight into the value of pre-freeze in vitro culture (IVC) at 30-32°C to promote progressive sperm motility;
- Apply the KISS principle: minimizing processing aimed to optimize the efficacy of TBx evaluation and usage;
- Discuss best practices to severe male factor evaluations when low numbers of ejaculatory sperm are present; a
- Pro and cons to fresh TBx usage versus frozen tissue/sp

Background

- TESE / ICSI

  (Craft et al, 1993; Devroey et al., 1994, Nagy et al., 1995)

- TBx processing – later 1990's publications

  (Crabbé E, Verheyen G, Tournaye H, Nagy ZP and co-workers) – *UZ Brussel Group*

- Testicular Sperm IVC / IVM

  (Liu et al., 1997; Angelopoulos et al., 1999; Emiliani et al., 2000)

  * Numerous publications on TBx from 1998 to 2004

* The processing of Testicular Biopsies continues to b procedure in which tremendous variability and frustra still experienced in different laboratories worldwide

1st testicular sperm ICSI – Feb 1994

Seminal events:

Feb 1995: Cappy Rothman +24h TBx

July 4, 1995: +4d IVC @RT…….triplet LBR

Fall 1996, Serono Symposia-Rome

Winter 1997:PCRS Whole TBx cryo

2000 : FSAC Lab Sm Lab, 30C RT

*Great TBx pMot

2004 : New Lab more sqft. P… environm… controll…

TMPS re…

Deduced

Introduction

J Assist Reprod Genet
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GAMETE BIOLOGY

Validation-verification of a highly effective, practical human testicular tissue in vitro culture-cryopreservation procedure aimed to optimize pre-freeze and post-thaw motility

M. C. Schiewe[1,2] · C. Rothman[2,4] · A. Spitz[3] · P. E. Werthman[4] · S. I. Zeitlin[5] · R. E. Anderson[6]

- Extended IVC with minimal processing
- Whole TBx tissue cryopreservation

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Experimental Aim

- Study 1- To show that IVM of TBx sperm at an intermediate temperature could effectively enhance progressive motility and promote sperm longevity.

- Study 2 – To prove that the use of a 30°C incubation temperature was superior to RT or 37°C for optimizing TBx sperm motility.

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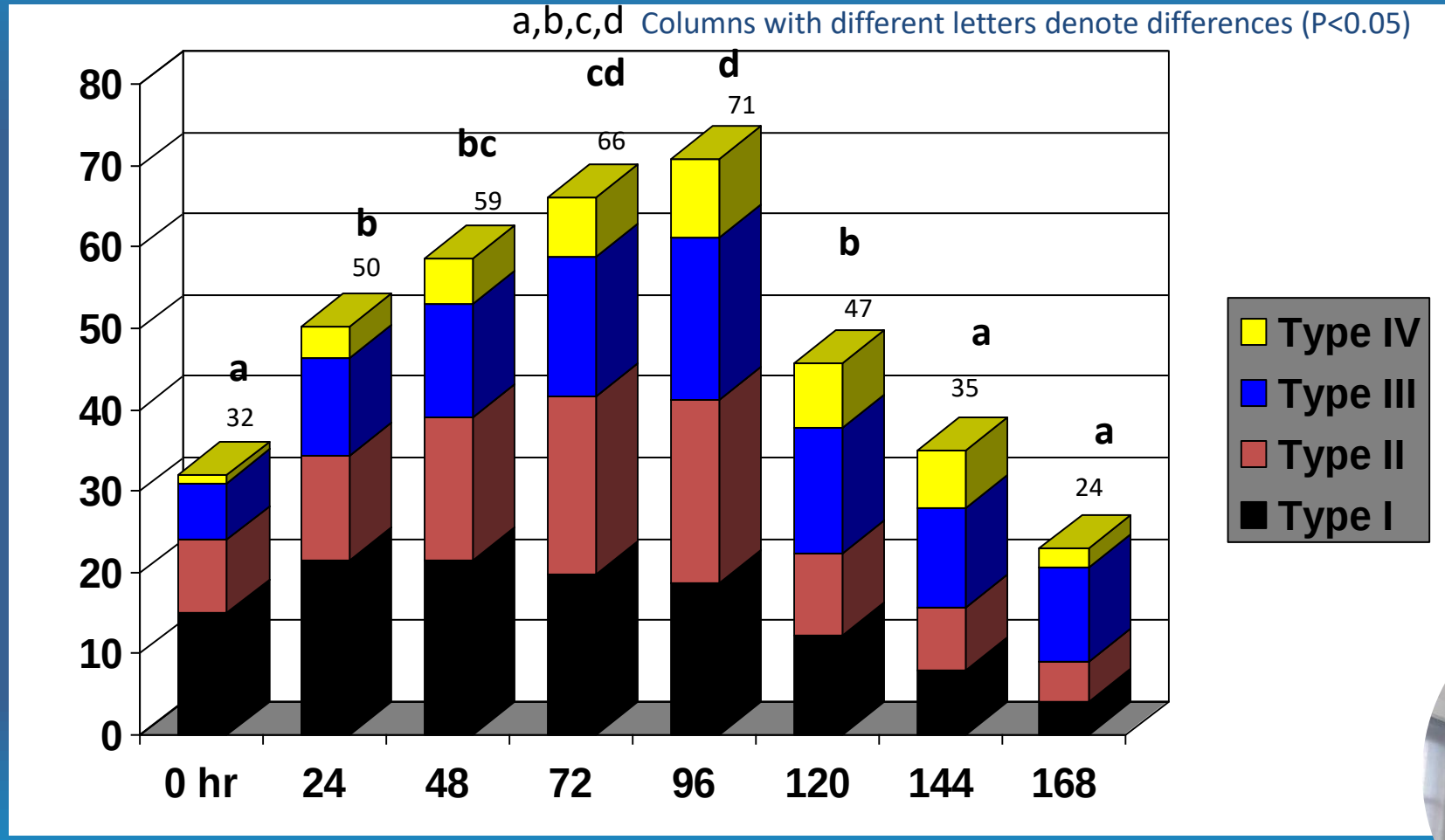
Materials & Methods

- Upon sperm confirmation, TBx were analyzed for the Total % motility and categorized by motility index (Type 1= twitching, 2= undulating motion of the flagellum, 3= progressive motion and 4= rapid movement)
- Sperm longevity was evaluated over a 1 wk interval. TBx were maintained in a styrofoam box on a warmer (internal temp=30-31^{o}C).

Study 1 – Fresh TBx



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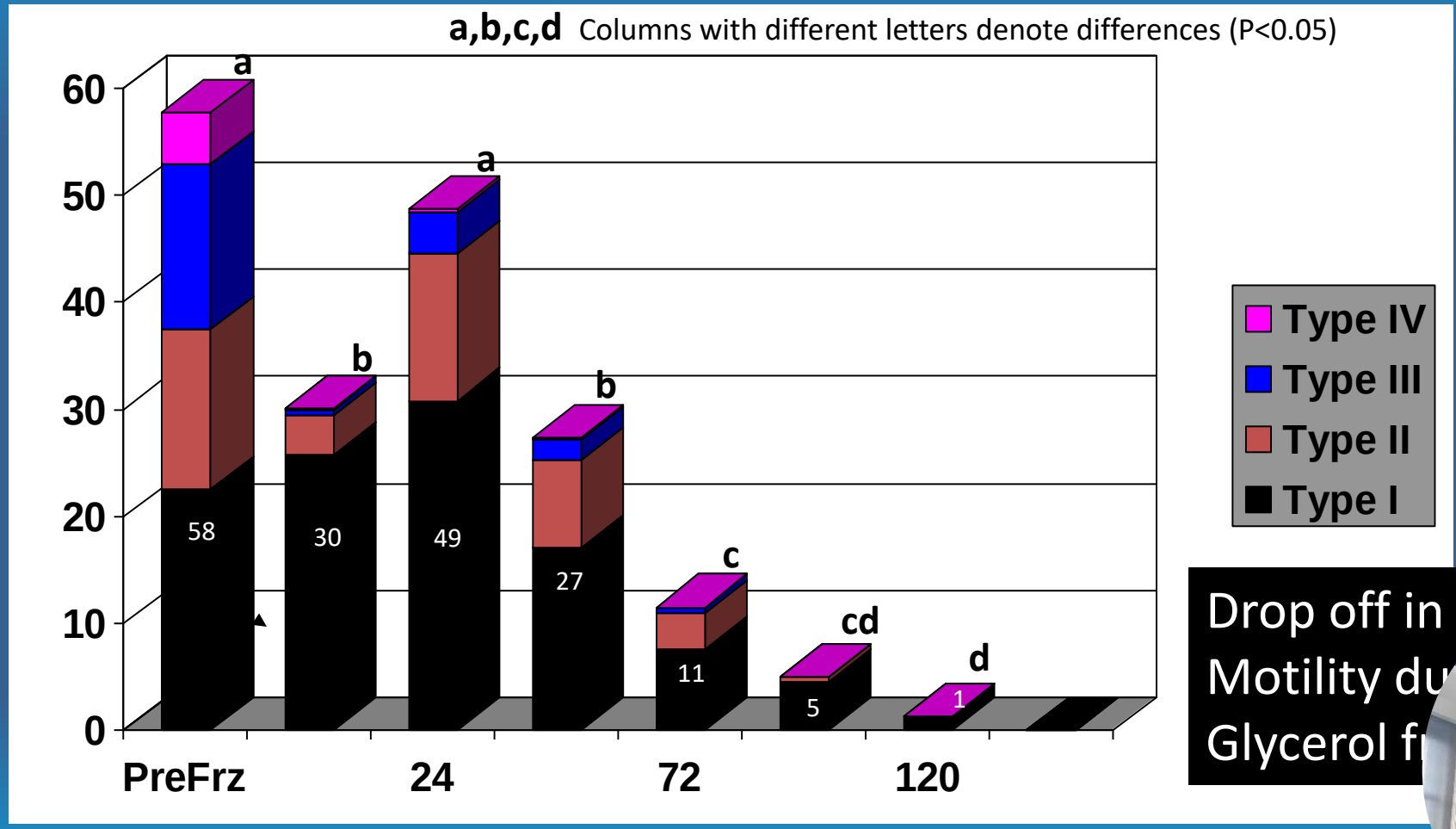
Study 1 – Frozen-thawed TBx



Drop off in po
Motility du
Glycerol f

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Study 2

Design

- Maintain shredded TBx for IVM at: RT (21°C), 30°C or 37°C
- M&M: warm in 0.5M Suc at 37C for 5min , 2x wash
- Measure motility at +24hr, +96hr and +168

RESULTS

- Increased Total Mot and Progressive Mot ($P<0.05$)

96hr: 30°C (66%, 44%) > RT (42%, 14%) > 37°C (18%, 10%)

1wk: 30°C (42%, 23%) > RT (24%, 8%) > 37°C (9%, 5%)

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ROS & DNA Frag. Concerns ?

Relationship of Motility to DNA condition

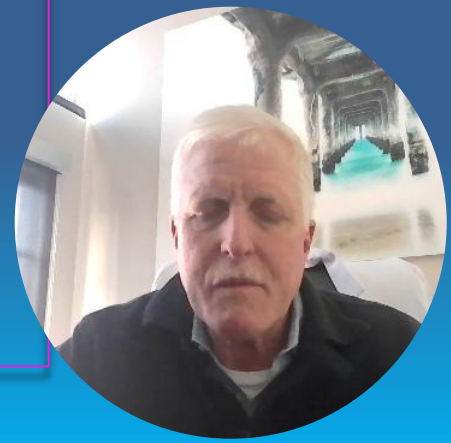
- Wyrobek et al., 2006
  - Paternal age effects: Inc. Mot% - Dec. DFI%

DNA condition of TBx sperm

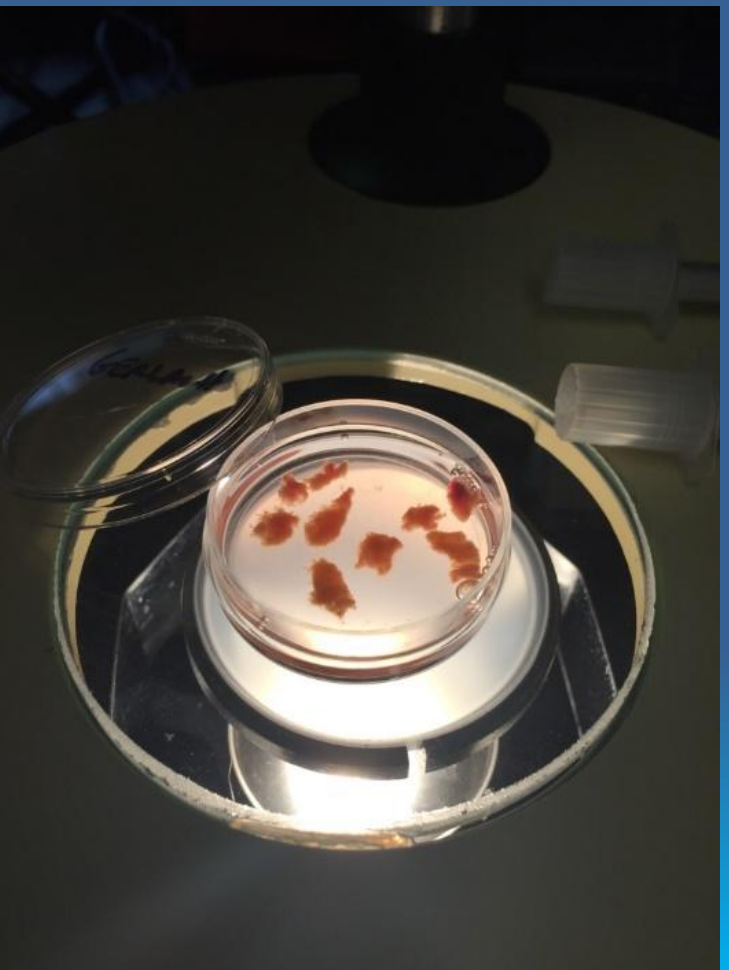
- Emiliani et al., 2001
  - Single stranded (ss)-DNA are more vunerable to oxidative & denaturing stress vs. double sDNA
  - +72hr IVM: Inc. Mot% and reduced portion of ssDNA thus Inc. dsDNA sperm for ICSI

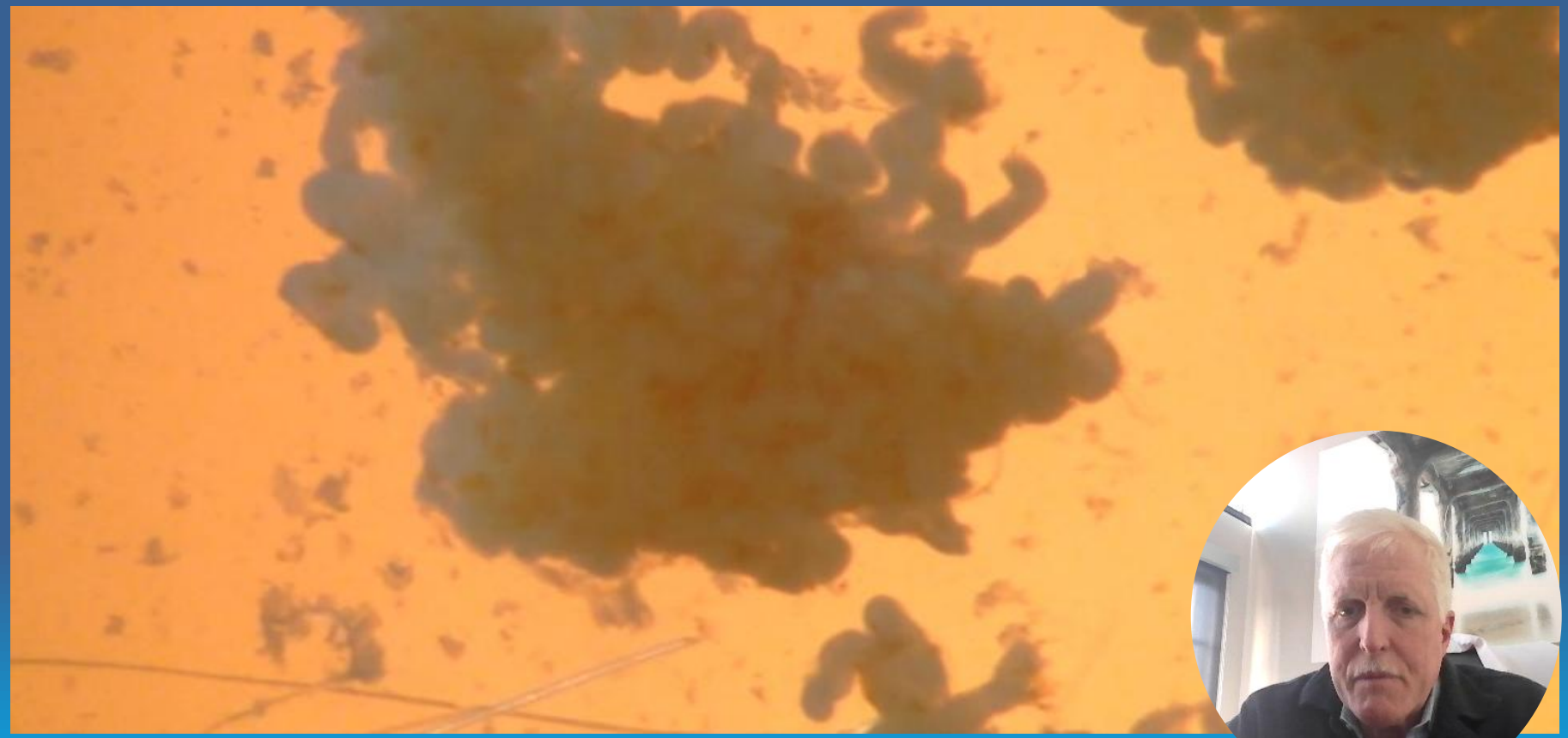
Clinical outcomes of ICSI cases using IVC/frozen-thawed whole testicular biopsy sperm at the SCIRS laboratory between 2011-2014.

Mean Female Patient Age	35.7 ± 4.8 S.D.
# of Oocyte Retrievals	100
# of Mature Oocytes Injected	1356
# of 2 Pronuclear Zygotes	920 (67.9%)
Total # Fertilized	1023 (75%)
# of Embryo Transfers-fresh embryos	60
# of Positive Pregnancies	35 (58.3%)
# of Clinical Pregnancies	32 (53.3%)
# of Live Birth Outcomes	28 (47%)



Testicular Tissue : Processing and Freeze Preservation

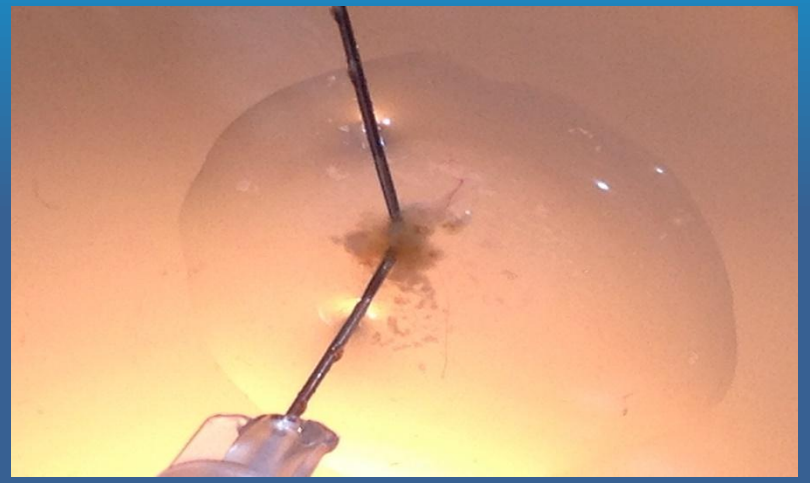


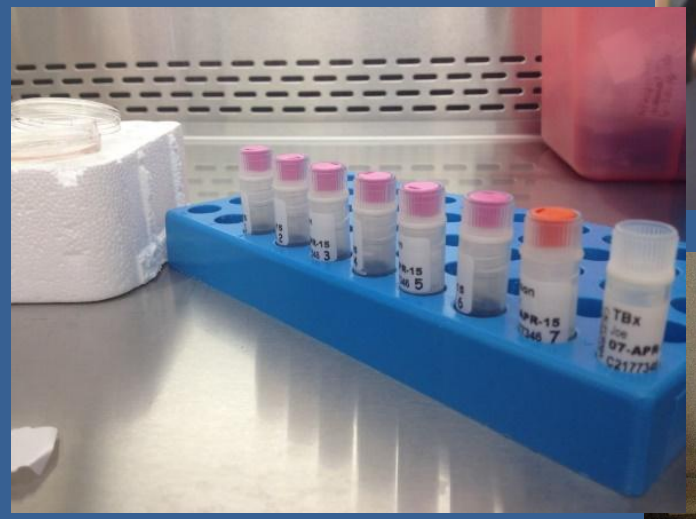


CCB Study Design

- 24 month interval (2014-2015): 40 adult men (24 to 62 years old) were scheduled to have testicular tissue frozen for possible future ICSI use.
- Testicular tissue freeze preservation services: Provided to men scheduled for either a Vasovasostomy surgery (n=32) or to assist NOA/Anejaculatory Cancer patients (n=8).
- Retrospective analysis: Efficacy of whole tissue freeze preservation/IVC methodology for cryobanking purposes.
- Assessed sperm motility patterns for up to 1 week: Compared pre-freeze and post-thaw total and progressive sperm motility, contrasting differences by ANOVA ($p<0.05$).

Processing & Evaluation

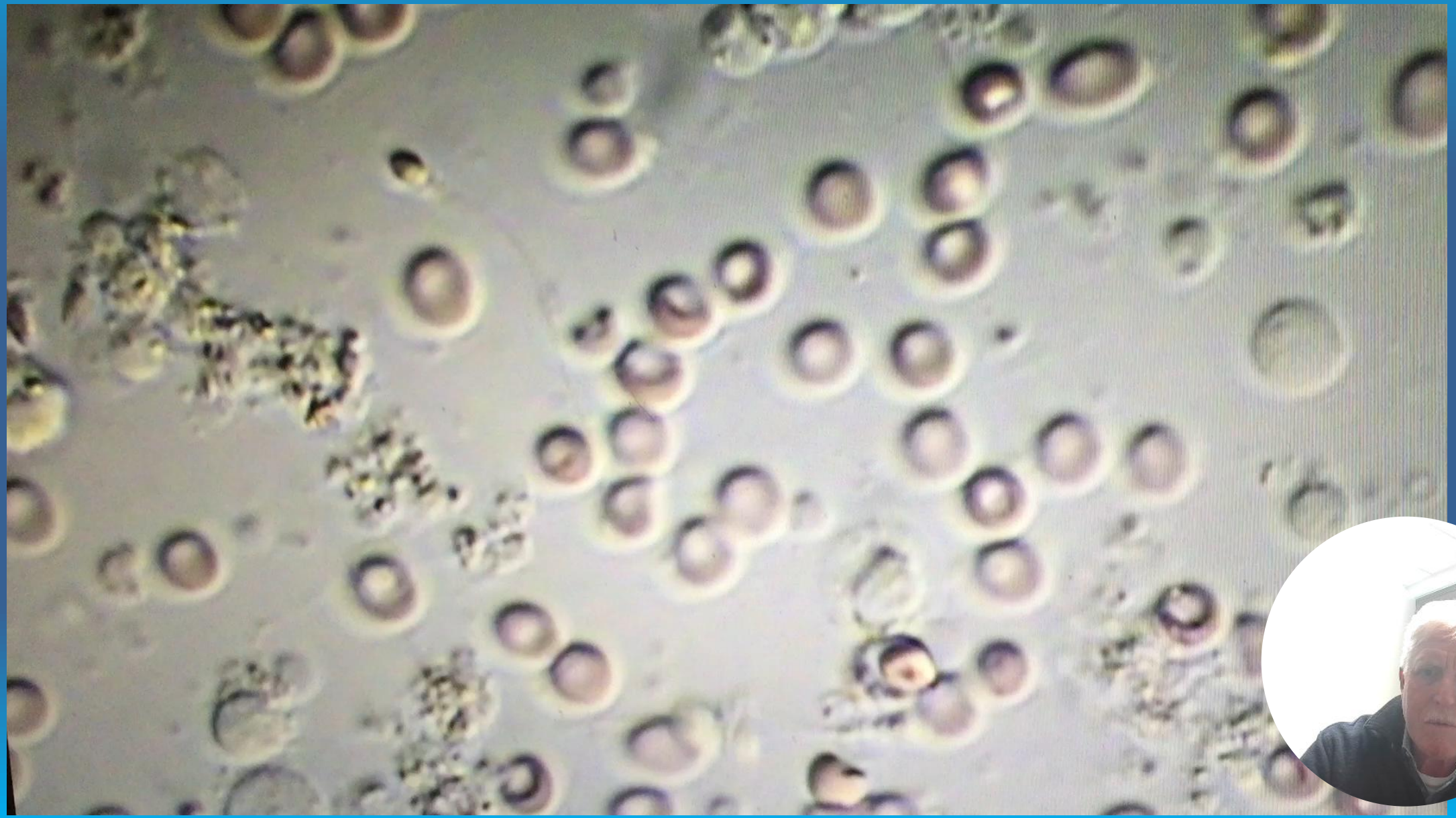




Results : Pre-Freeze

- Only 2 of 8 NOA patients failed to yield sperm, thus 95% of the clients had tissue cryopreserved.
-
- Total and progressive motility significantly elevated by +48hr IVC (46.8% and 12.4%) compared to 26.4% and 5.2% at +3hr and 32.5% and 5% at +24hr, respectively. Total motility peaked at +96hr (52.1%), while progression elevated ($p<0.05$) to 20.3% and 24.5% by +96 and 120hr, respectively, and remained at +168hr (20+%).

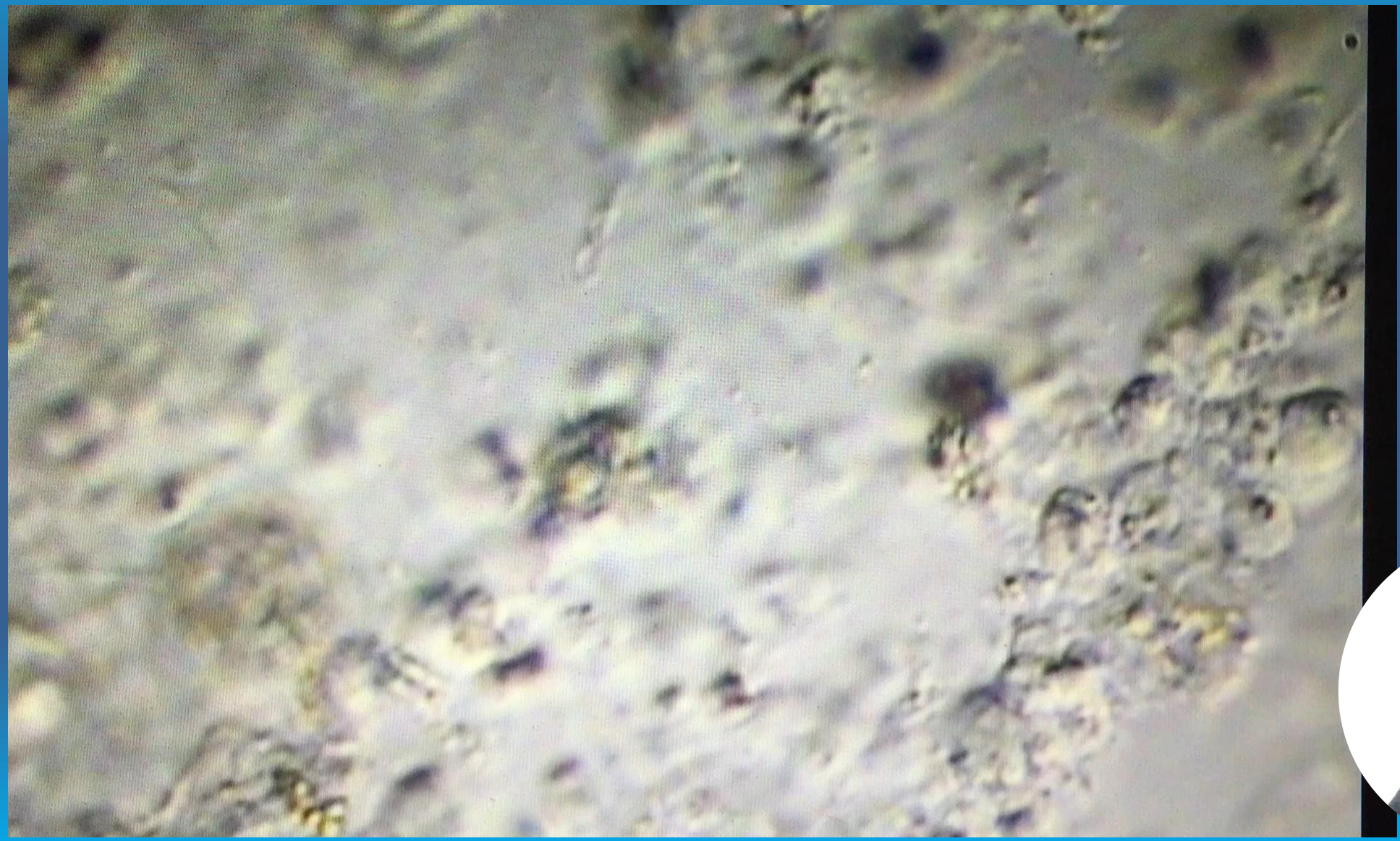
+ 96 hr IVC

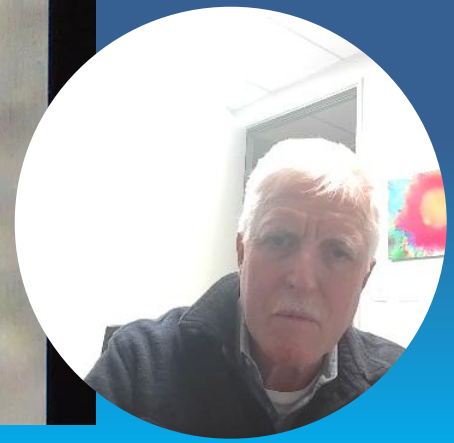


Results : Post-thaw

- Comparing pre-freeze to post-thaw total and progressive sperm motilities : Declined ($p<0.05$) from 44.4%±2.0%SE and 13.3% to 32.1%±2.2 and 6.7%, respectively, post-thaw.
- The reduced motility recovery rate of both total (73%) & progressive (50%) movements, are generally lessened by additional overnight IVC considering the median pre-freeze IVC interval was +72hr.
- The motility patterns of pre-freeze / equilibrated (3hr, 37°C), post-thaw samples was: I=18.7%/17[illegible] II=12.8%/8.4%; 9.6%/5.1%; III=16.7%/9.8% and IV=3.7%/1.6%, respectively.

Post-thaw at 0h





Conclusion - I

- Overall, excellent conservation of sperm viability *minimal pre-freeze processing, by cryopreserving whole testicular tissue pieces in a 13.4% glycerol solution and five equal mixed dilutions.
  - less time / labor expended
  - no chemical treatments required
  - no loss of sperm in processing / excellent recovery
    - Pentoxifylline use on rare occasions (NOA's)

" the KISS pr

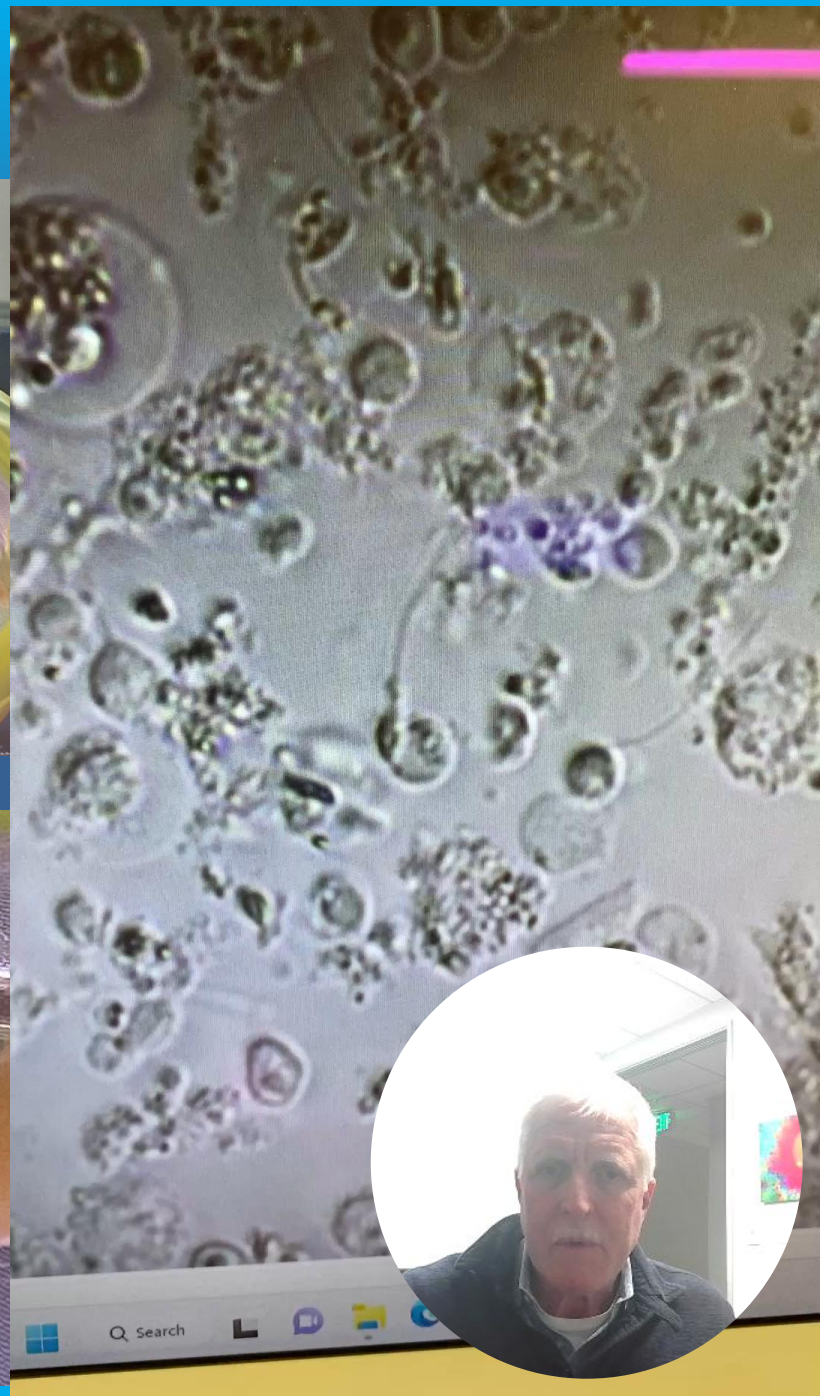
Conclusion - II

- Whole testicular tissue freezing following IVC ..... motility enhancement has proven be a highly effective, while minimizing excessive and laborious processing procedures. The availability of overtly motile sperm, simplifies valuable Embryology time to isolate viable sperm to ICSI oocytes in a timely manner.
  - Excellent post-thaw motility outcomes
  - Simple detection of active sperm post-thaw
  - Less time / labor expended
  - ICSI motile FT sperm ⟶ Optimal Outcome
  - Method used for post-thaw processing ? Choice

“ the KISS principle “

Current Progress in TBx processing: Cell Strainer

Ease of Cell Strainer Processing



Troubleshooting “KISS Approach”

- Old inverted microscope Optiics can last forever

“good cheap investment for Urology work”, but not the light source…….. Costly repair $2K, however a $15 emergency LED light source is an effective alternative

Best practices to severe male factor evaluations

- Severe Sperm Preps following Ejaculatory Clean-out protocol
  - Pelleted cellular suspension into 50-100ul square microdroplets/oil
  - Determine an estimated TMPS count following 1hr 37C swim-out
  - Possible Cryo, repeat to assess reliability of TMPS/ejac + more Cryo b/u
- Pro and cons to fresh TBx usage versus frozen tissue/sperm
  - OA vs NOA
  - IVC allows great flexibility in scheduling TBx around estimated O
  - Goal : easy motile sperm isolation when oocytes are recover delay in acceptable ICSI interval

Thank You

Questions?