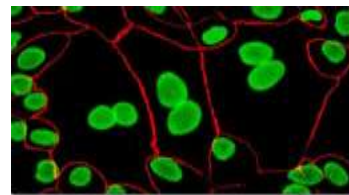


# Application of Pressure Cycling Technology (PCT) in Differential Extraction



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- Pressure Cycling Technology (PCT)
- Differential Extraction
- Objective
- Experimental design
- Results
- Conclusions



# Pressure Cycling Technology

- Barocycler<sup>®</sup> NEP2320  
(Pressure BioSciences Inc.)
- User controlled variables:
  - a) Pressure (5-45k psi)
  - b) Holding time at ambient pressure (T1)
  - c) Holding time at target pressure (T2)
  - d) No. of cycles (1-99)
  - e) Temperature



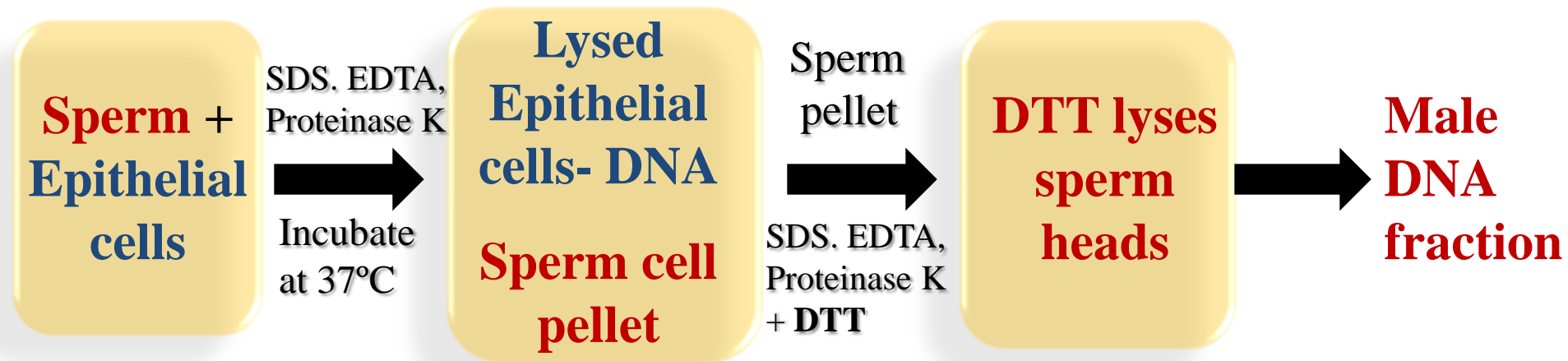
PULSE™ tubes  
(up to 300  $\mu$ l)

MicroTubes  
(50-150  $\mu$ l)



# Differential Extraction

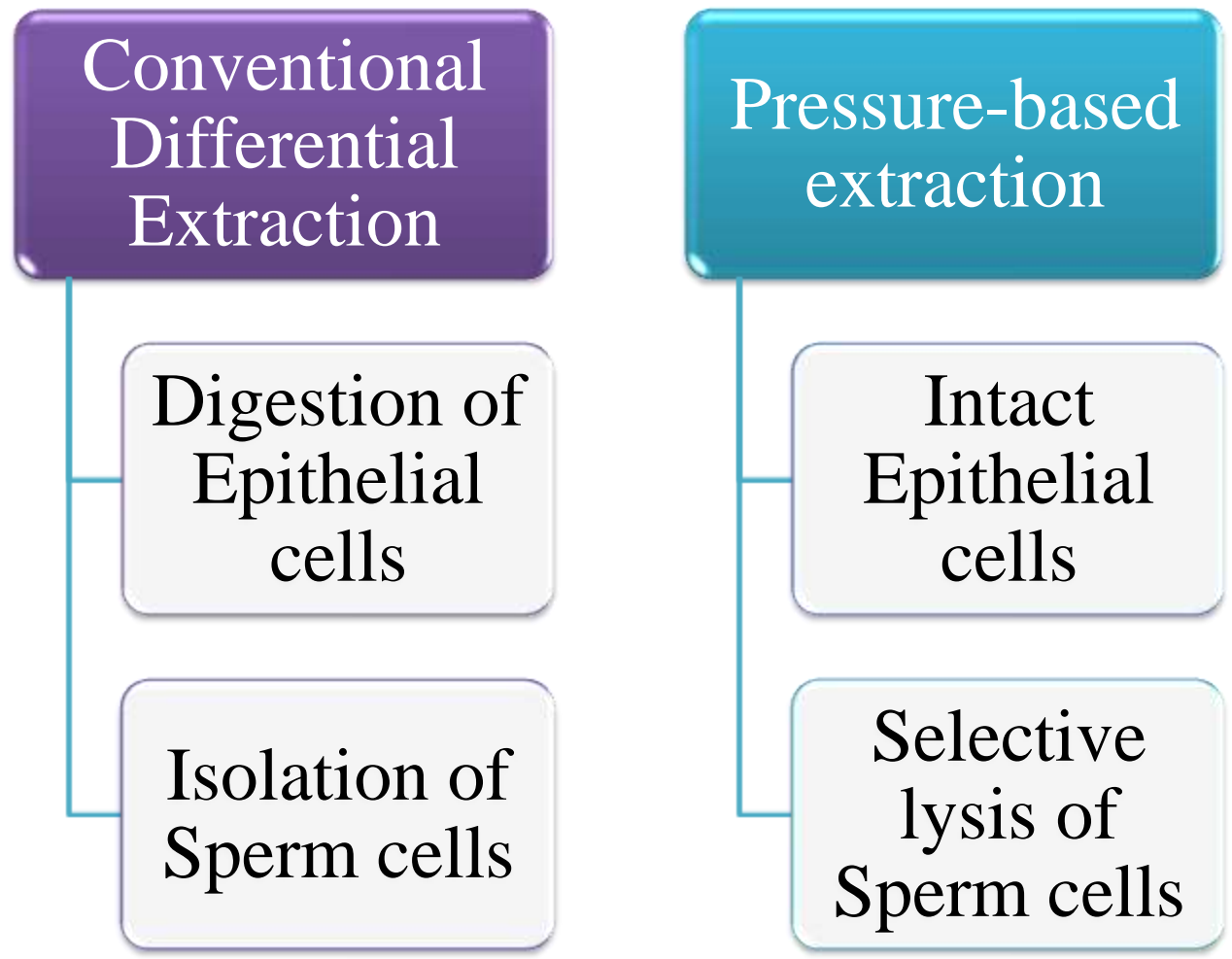
- Sexual assault casework
- Separation of female-derived sperm cells and epithelial cells



- Male DNA profile

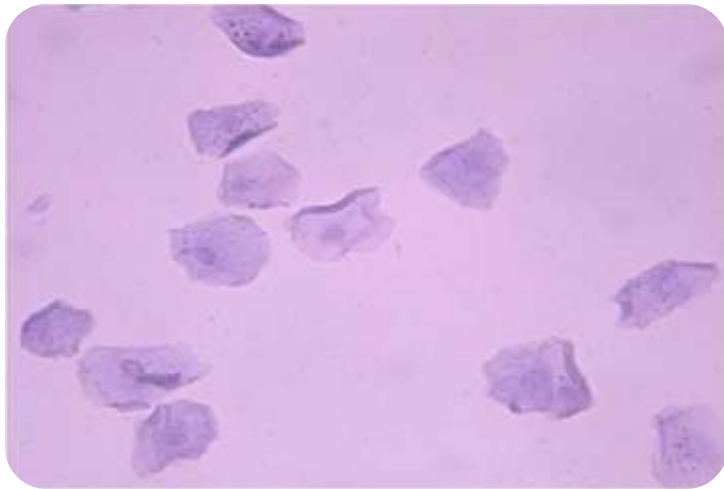


# Objective

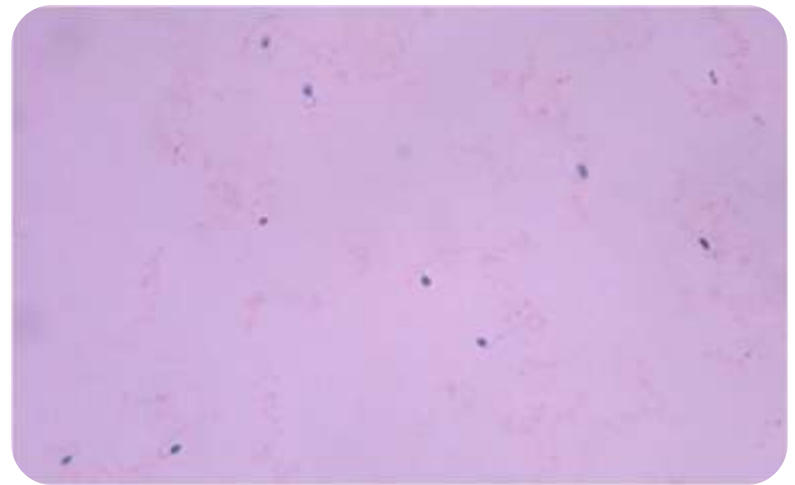


# PCT- Microscopic studies

- Microscopic visualization of PCT-treated cells stained with 0.4% Trypan blue solution
- Trypan blue staining - dye exclusion method
- Cells with a compromised membrane integrity are stained dark blue



Vaginal epithelial cells



Sperm cells



# Experimental design

## SAMPLES

Sperm cells and  
Epithelial cells  
suspended in 1X PBS  
buffer (pH 7.4)



## CELL LYSIS

Pressure cycling using  
Barocycler<sup>®</sup> NEP 2320



## QUANTITATION

Real-time PCR analysis  
using Plexor<sup>®</sup> HY system  
(Promega Corporation)



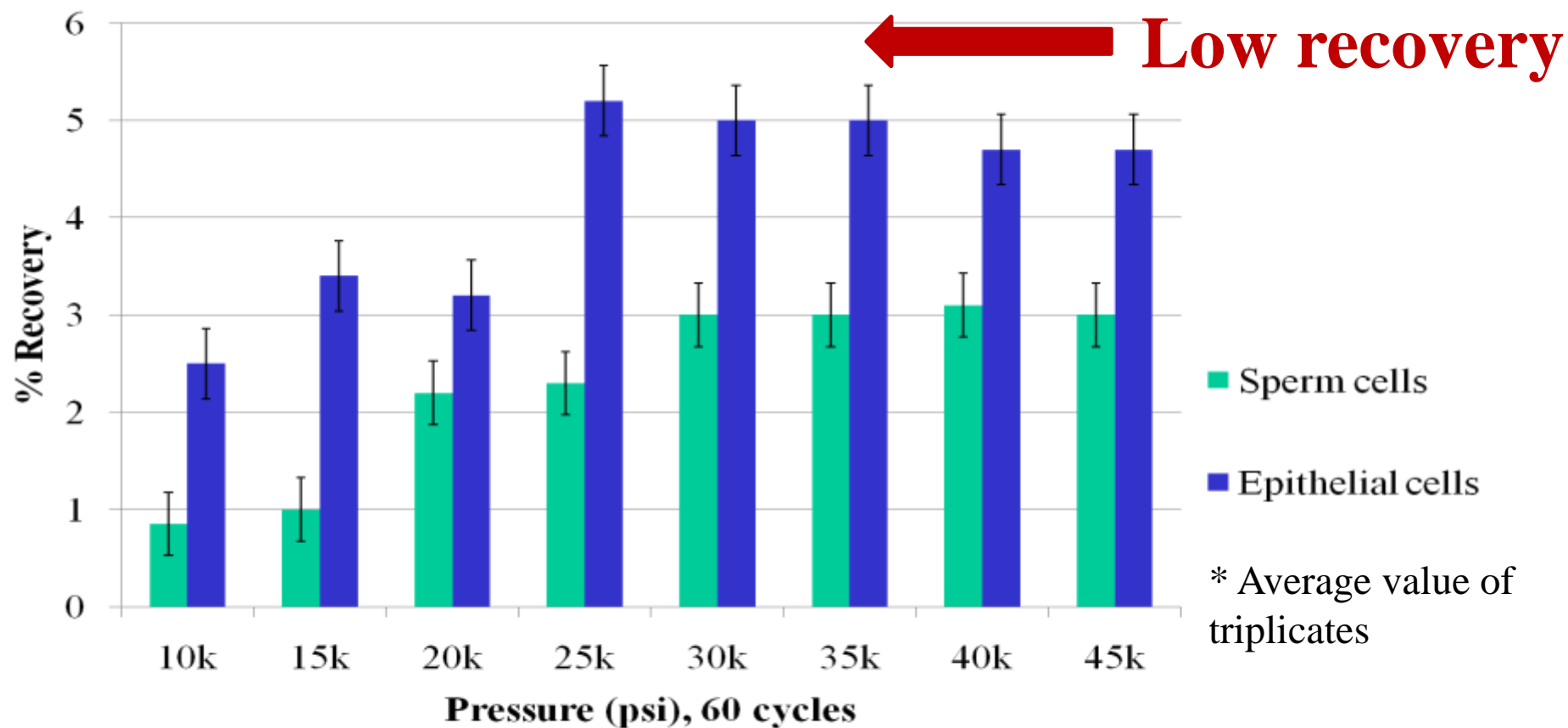
## PURIFICATION

Phenol-Chloroform-  
Isoamyl alcohol extraction



# Dose Response curve

Total DNA in sample- Standard Phenol/Chloroform and Proteinase K extraction





# Maximize DNA recovery

Individual samples

**45000 psi**  
**60 cycles**

Increase in temperature

- 40° C
- 50° C
- 60° C

Addition of detergent

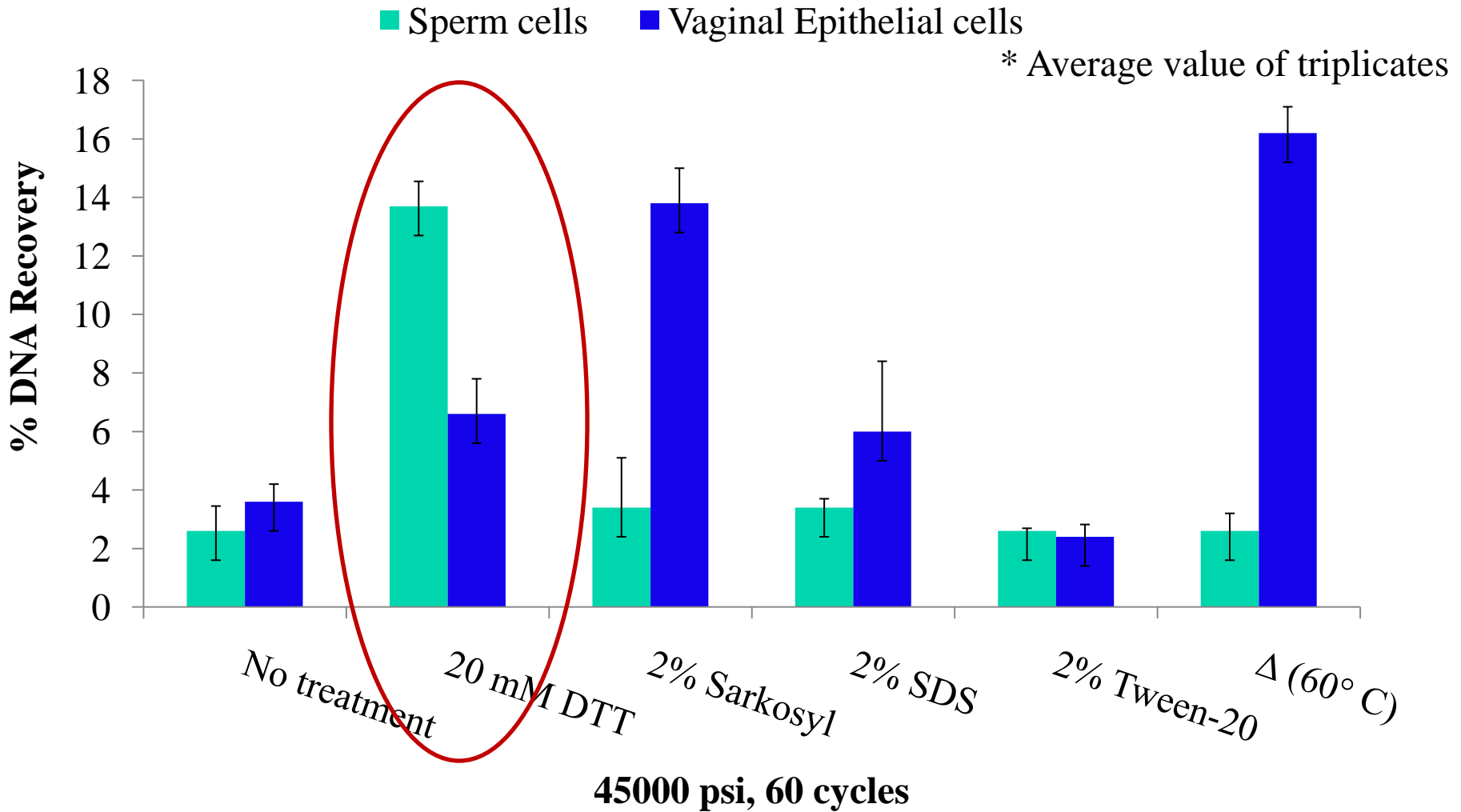
- 2% Sarkosyl
- 2% Sodium dodecyl sulfate (SDS)
- 2% Tween-20

Addition of reducing agent

Dithiothreitol (DTT)

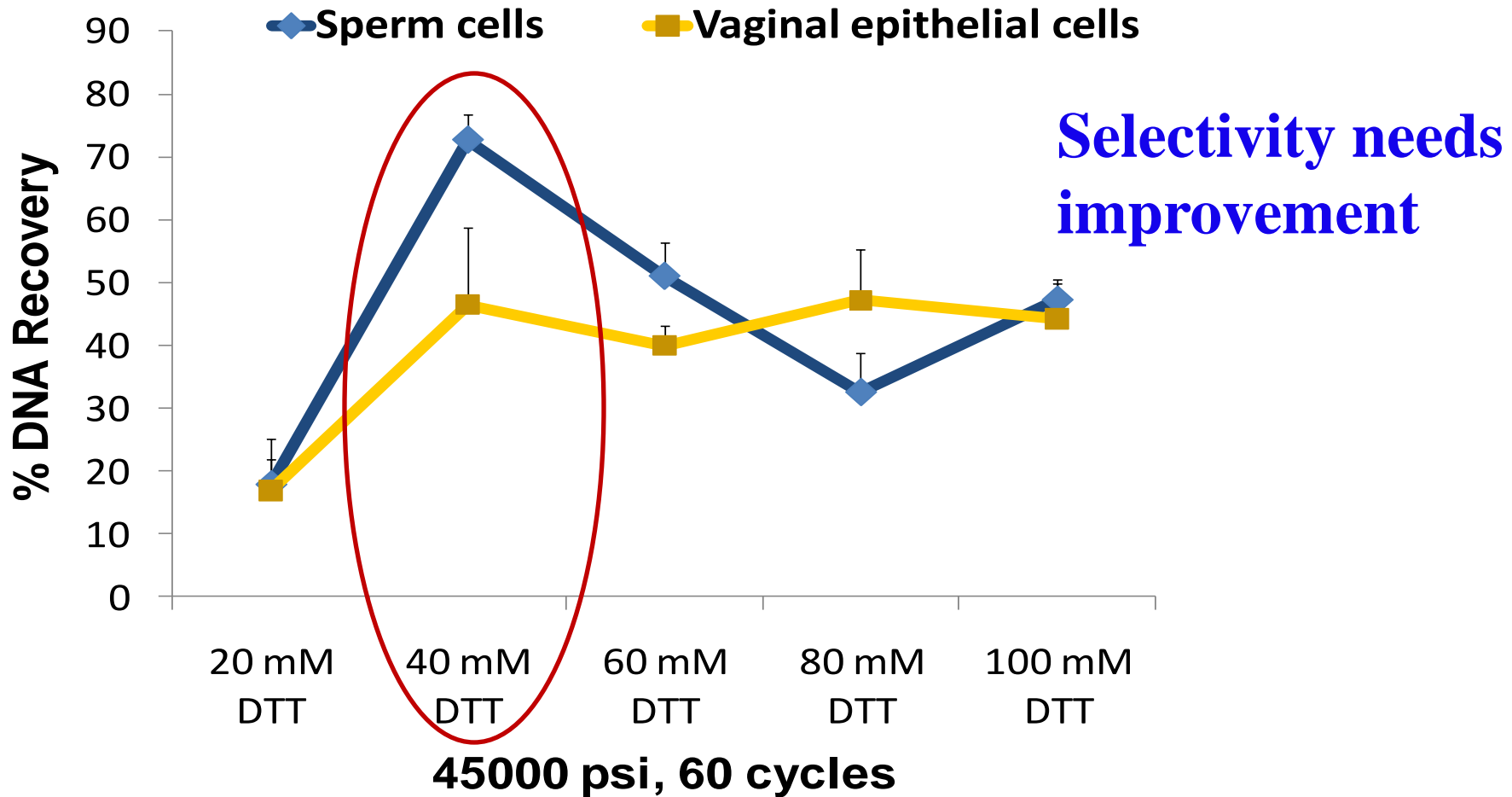


# Effect of PCT on treated cells





# Dithiothreitol (DTT) studies

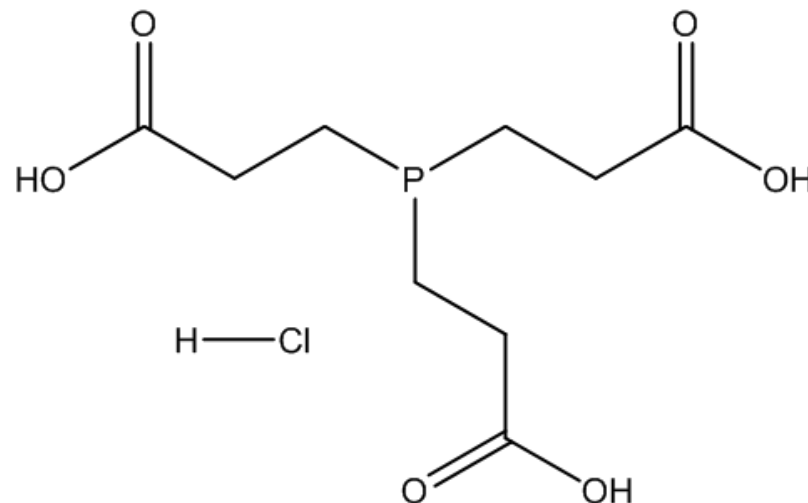


\* Average value of triplicates



# Tris(2-carboxyethyl)phosphine (TCEP)

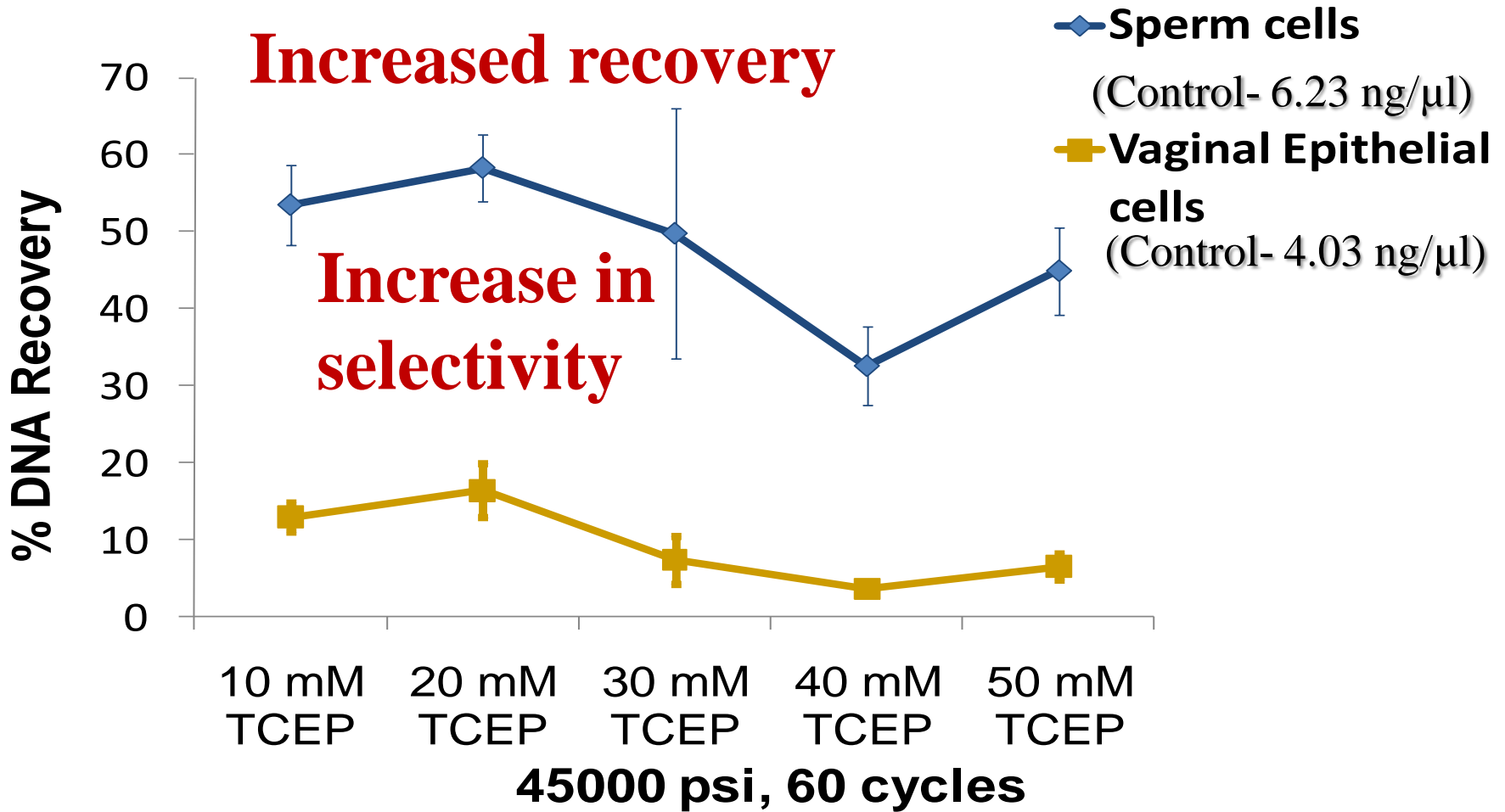
- Soluble in water
- More effective than DTT
- Odorless
- Selectively reduces disulfide bonds over a broad pH range
- Stable- resistant to air oxidation
- TCEP is not particularly stable in phosphate buffers



Chemical Formula:	$C_9H_{16}O_6PCl$
Molecular Weight:	286.6 g/mol



# TCEP studies



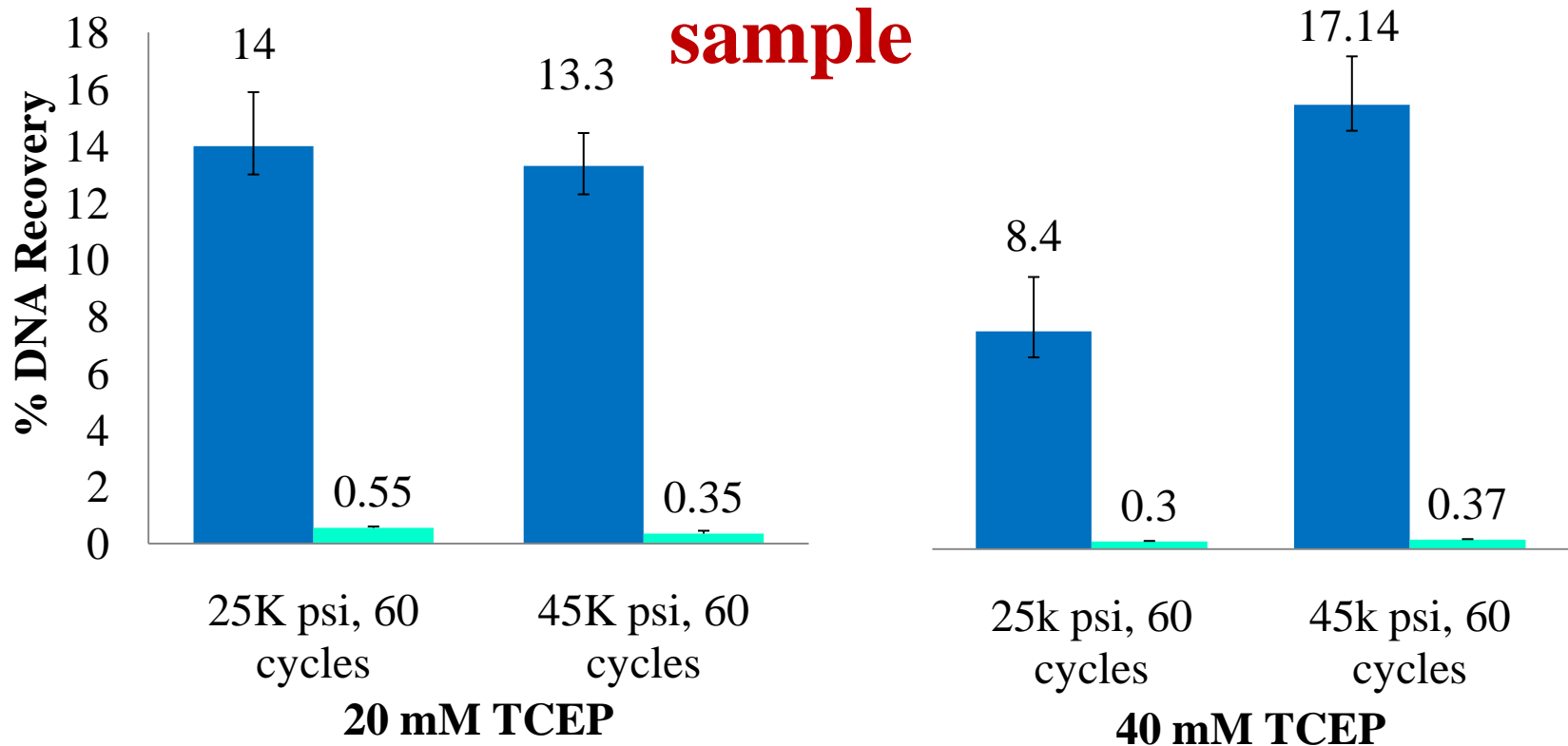
\* Average value of triplicates



# TCEP studies

- Sperm cells (Control- 32.7 ng/ $\mu$ l)
- Vaginal Epithelial cells (Control- 40.3 ng/ $\mu$ l)

**Selectivity reproducible at higher concentrations of sample**

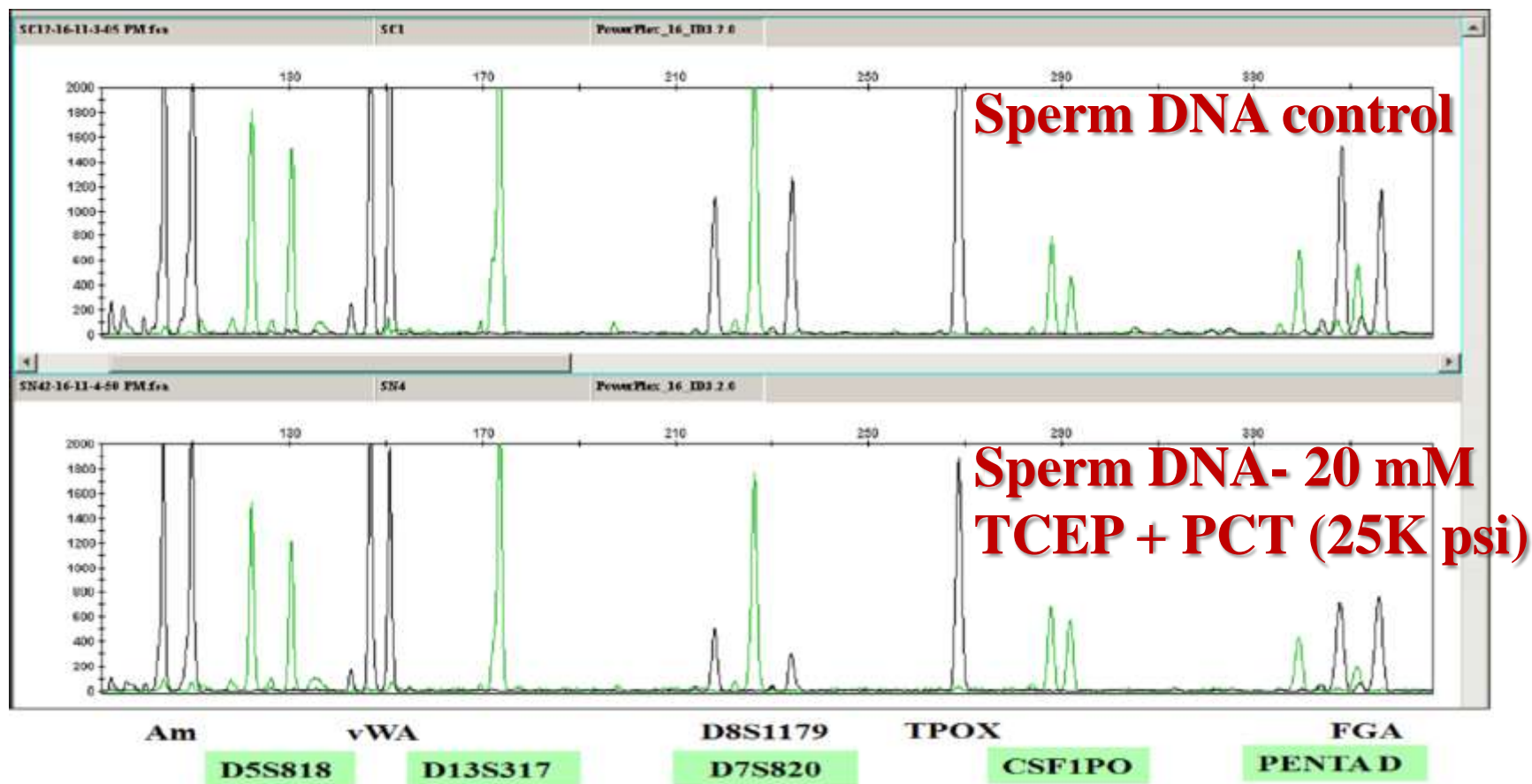


\* Average value of triplicates



# STR profile

PowerPlex<sup>®</sup> 16 HS (Promega Corporation)





# Conclusions

- Pressure Cycling Technology (PCT) - Potential application in differential extractions
- Tris (2-carboxyethyl) phosphine (TCEP)
  - High selectivity
  - Improved recovery
  - Selective detection of sperm cells
- Successful amplification of sperm DNA recovered from pressure lysis
- Future work- Mixtures and dried stains



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Len Goren, Promega Genetic Identity Director

## **Broward Sheriff's Office DNA Laboratory**

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