Purification of *Trypanosoma equiperdum* spiked equine semen by single layer centrifugation

Presentation for The NTTAT

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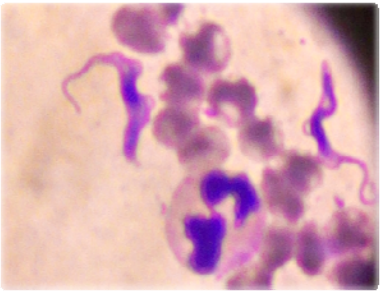
Introduction

Trypanosoma equiperdum

- flagellated protozoan parasite
- tissue parasite
- cause of dourine in equine
- transmitted sexually

- the parasite isolated from
  - ✓ the urethral tract

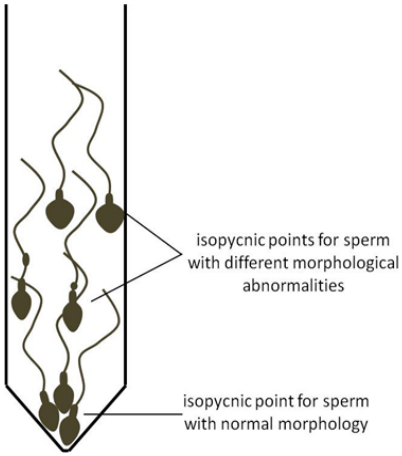
- purification of semen?



Suganuma et al., 2016

Hypothesis

- AI – also transmit the disease
- SLC Principle – density gradient sperm selection
- successful in reducing venereal transmission on other pathogens
- **objective** - to evaluate the purification of *T. equiperdum* spiked semen by SLC



Levy et al., 2002
Blomqvist et al., 2011
Morrell and Wallgren, 2011
Morrell et al., 2013

Materials and Methods

- semen spiked with cryostabilates T. *equiperdum* (Dodola 943)
- spike concentrations : 1+ to 6+
- parasitaemia scoring system

Hagos et al., 2010

Paris et al. 1982

Score	Trypanosome/field*	Estimated parasitaemia trypanosomes/ml
6+	Swarming >100	$>5 \times 10^6$
5+	>10	$>5 \times 10^5$
4+	1–10	10^4–5×10^5
3+	1 per 2 fields to 1 per 10 fields	5×10^3–5×10^4
2+	1–10 per preparation	10^3–10^4
1+	1 per preparation	10^2–10^3

- viability of the parasite tested in spiked semen (wet smear examination)

Materials and Methods cont'd …

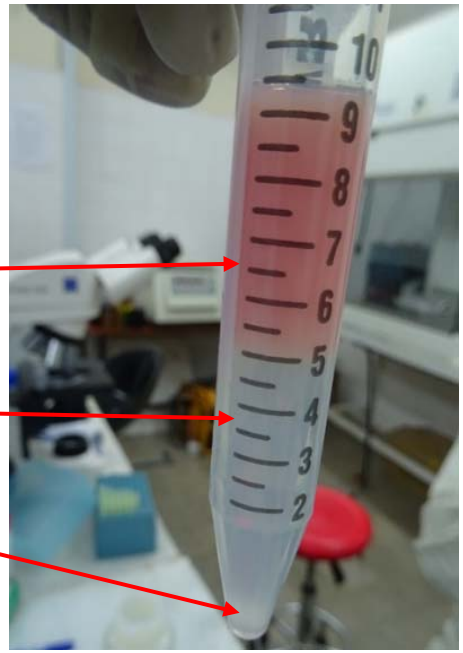
- SLC - standard procedures

Morrell et al., 2013
Hoogewijs et al., 2011

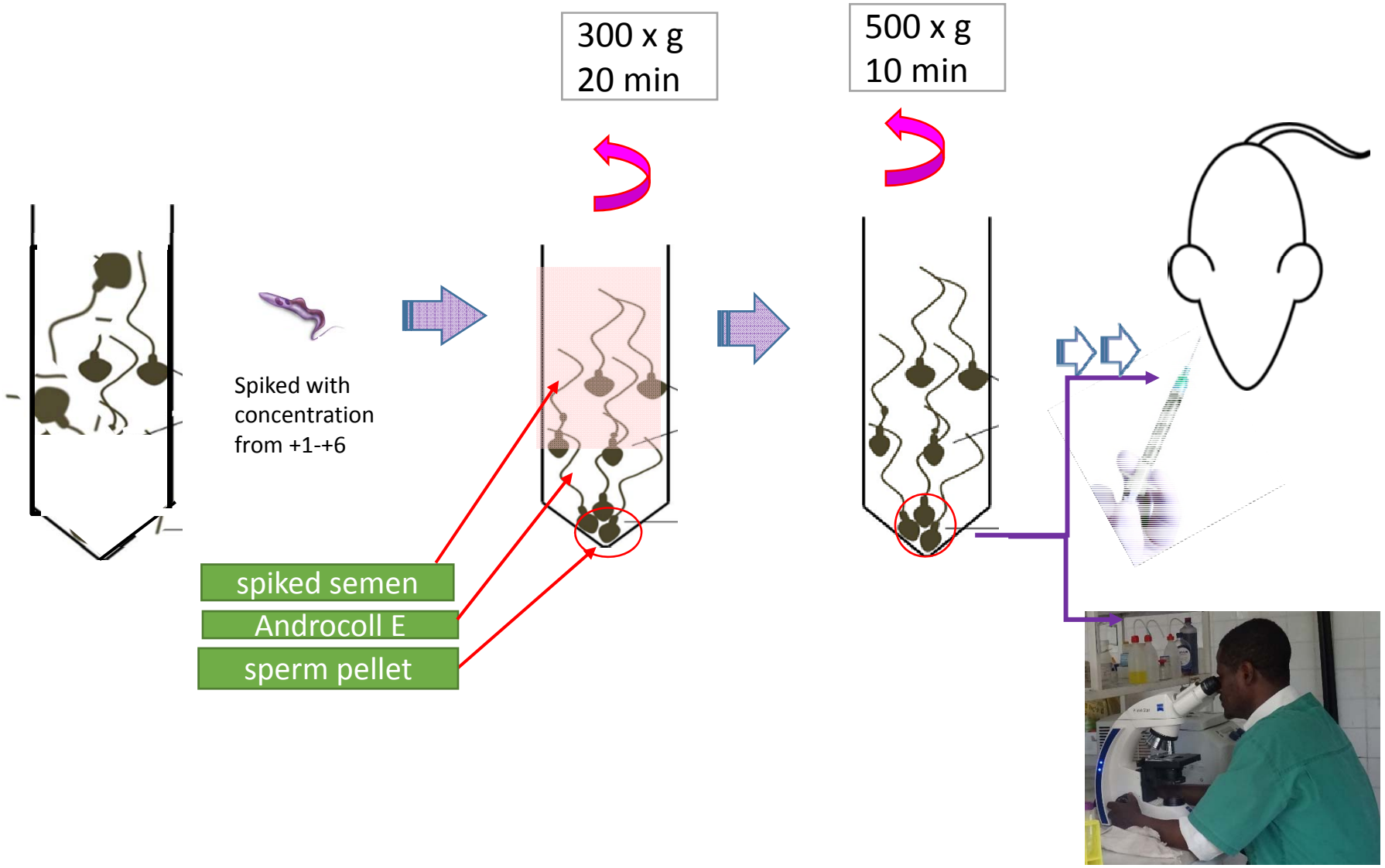
spiked semen

Androcoll E

sperm pellet



Materials and Methods cont'd



Materials and Methods cont'd

spiked+1
SLC- purified

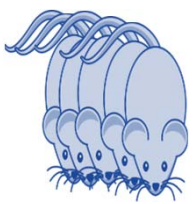
Spiked+2
SLC- purified

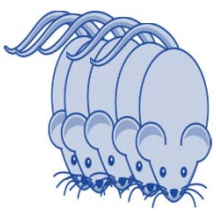
Spiked+3
SLC- purified

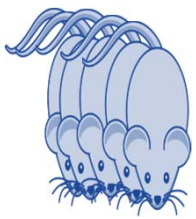
Spiked+4
SLC- purified

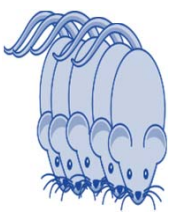
Spiked+5
SLC- purified

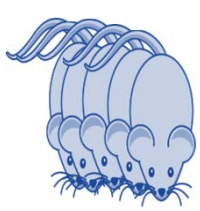
spiked+6
SLC- purified

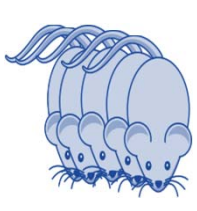






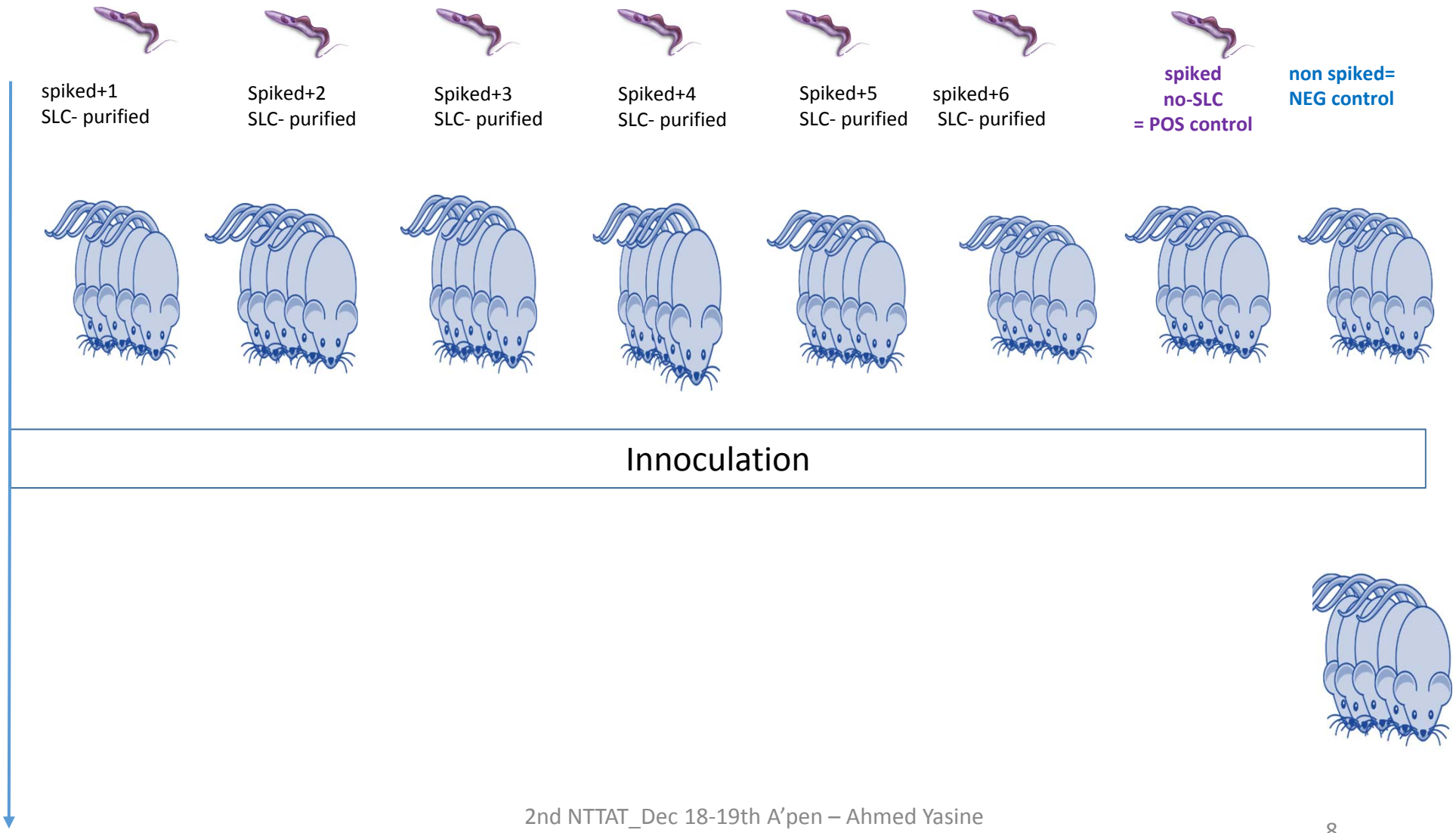






M&M -> Results

- wet smear microscopic examination - Negative



Discussion

- no infection at lower parasite concentration

- previous findings SLC of semen reduced

  - ✓porcine circovirus
  - ✓equine arteritis virus
  - ✓hepatitis C virus
  - ✓bacteria from boar semen

(Blomqvist et al. 2011

(Morrell et al. 2013)

(Levy et al. 2002)

(Morrell and Wallgren, 2011)

- number of parasites in the semen *vs* blood??

- no parasite in the pellet by wet smear examination

- DNA samples for PCR – still to do

Conclusion

- This experiment showed that low levels of parasites can be cleared off the semen

- Further study should be done on the pH, temperature and osmomolar properties of semen processing fluids in relation to the requirements for the Trypanosomes

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