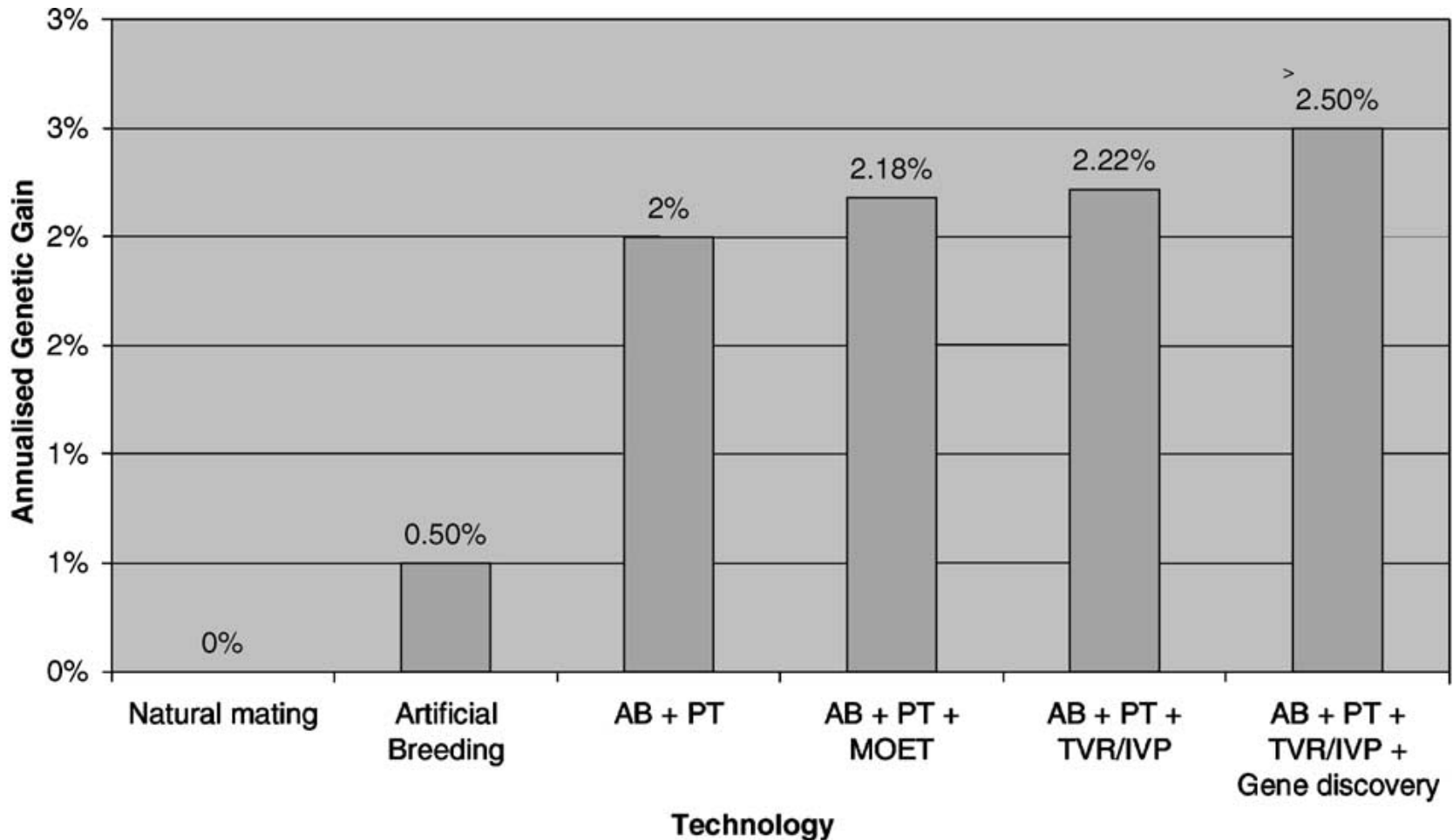


The production and processing of bovine semen and quality control

Dr R (Vish) Vishwanath



The value proposition for artificial insemination



Critical areas in Frozen Semen Production – focus on lab procedures

- The initial evaluation is very important
- Sets benchmark for all future decision making



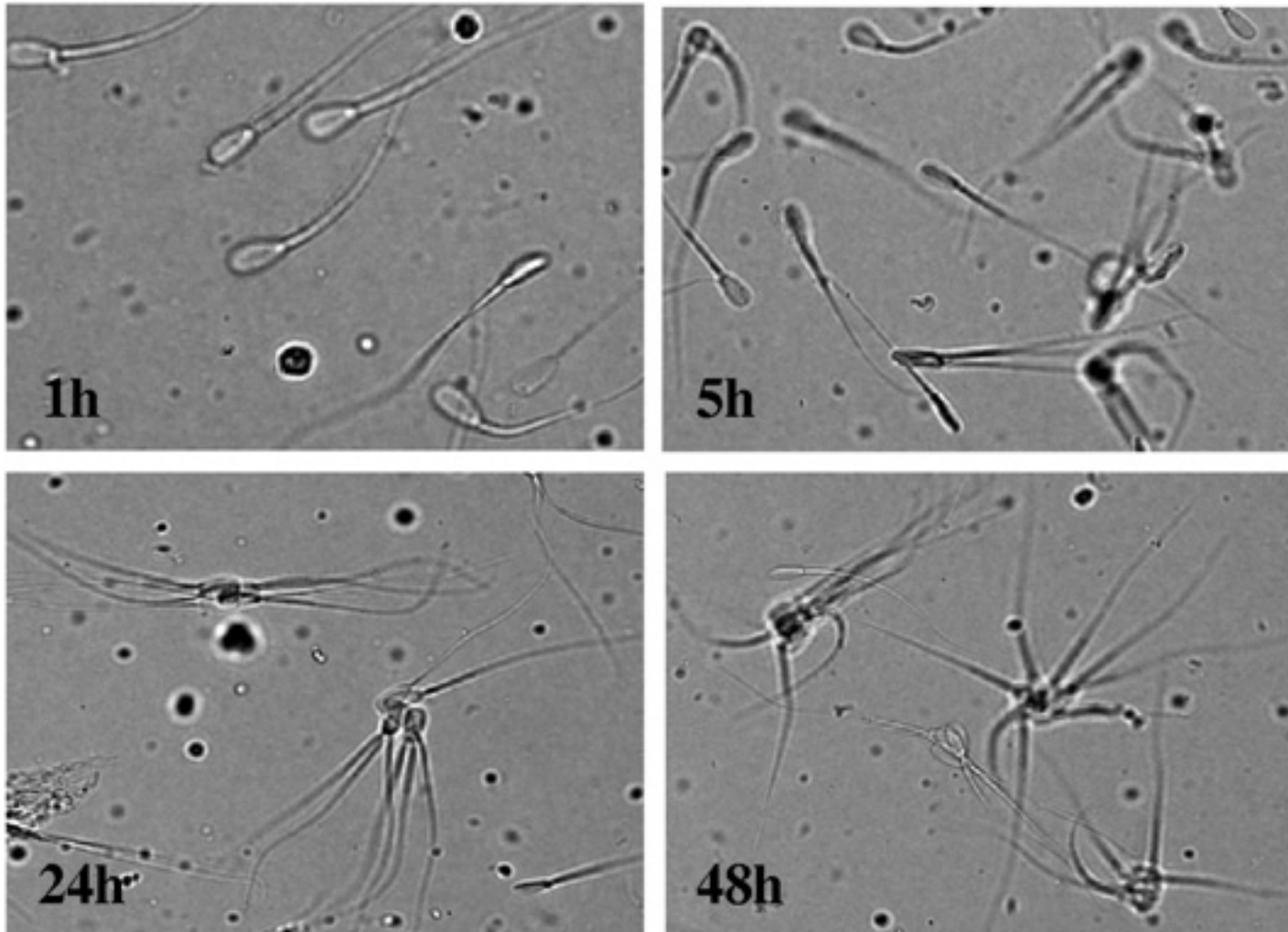
Attention to detail is the key

- Sperm concentration and volume estimation are two of the biggest sources of error
 - Spectrophotometer calibration using haemocytometers has error range of + / - 10%
- Sampling errors due to pipetting are extremely high. Ensure staff are properly trained in techniques.
- Consider using nucleocounters – useful for neat semen as well as processed straws.
- Consider using mass estimations rather than eyeball volume measurements.

Tris- EY medium is perfectly adequate

- A well researched medium and has functioned adequately over many years
- Managing temperatures during dilution is very important. Agglutination is an after effect of pH vs temperature interacting with +ve charged fractions from egg yolk.

Head to head agglutination due to egg yolk effects



Freezing protocols are very good

- Visit to various centres – freezing protocols very good
- Attention to post freeze handling of straws.



- Biggest concern is discrepancy between laboratory post thaw results and post thaw results of straws returned from the field.

Post thaw quality control

- Battery of tests being done
 - How do you categorize which is the most important?
 - Post thaw motility to be the single biggest determinant
 - The other tests are only supplementary

Correlation to fertility:

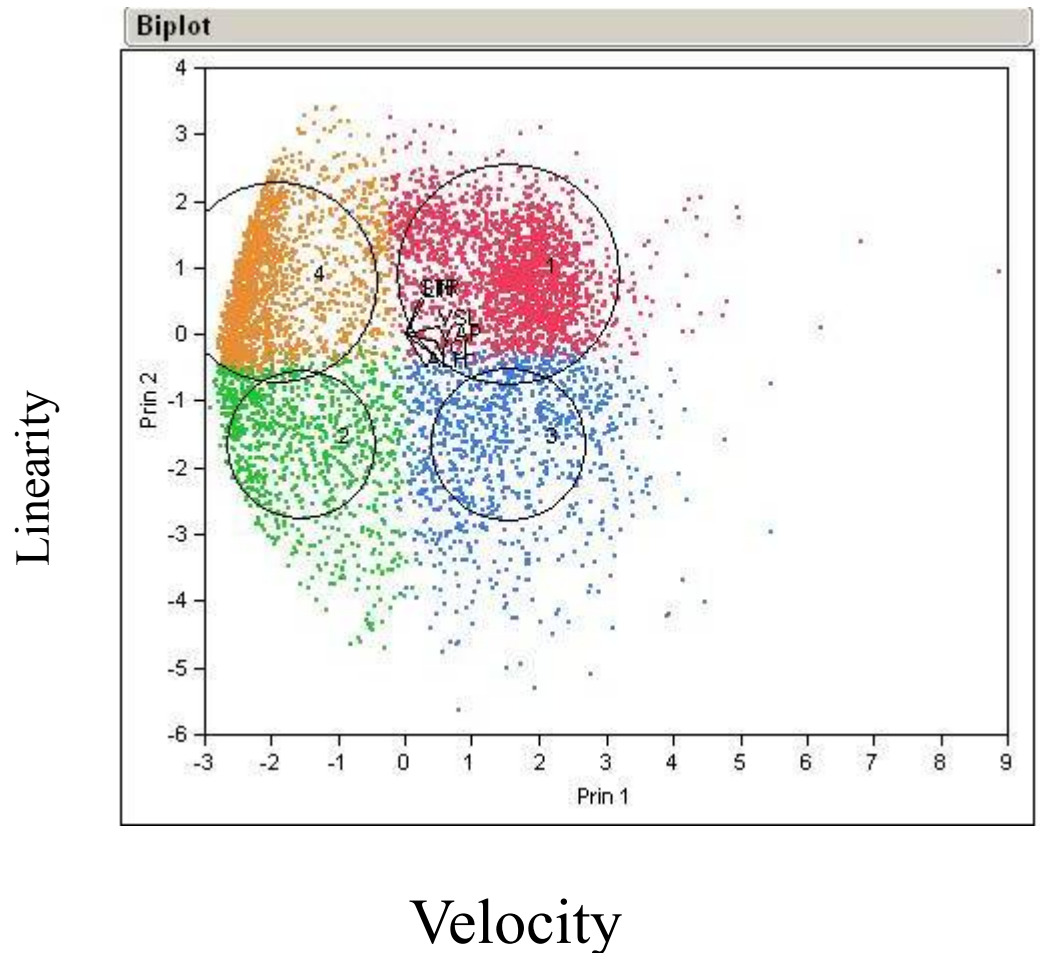
Sellem et al., found that individual assays were uninformative, but combining outcomes could create a model correlation to fertility of **0.69**.

1. CASA: Post thaw motility
2. DIC microscope: sperm morphology
3. Flow cytometer:
 - Acrosome integrity
 - Oxidative damage
 - Mitochondrial activity
 - Chromatin (DNA) integrity

The value of CASA

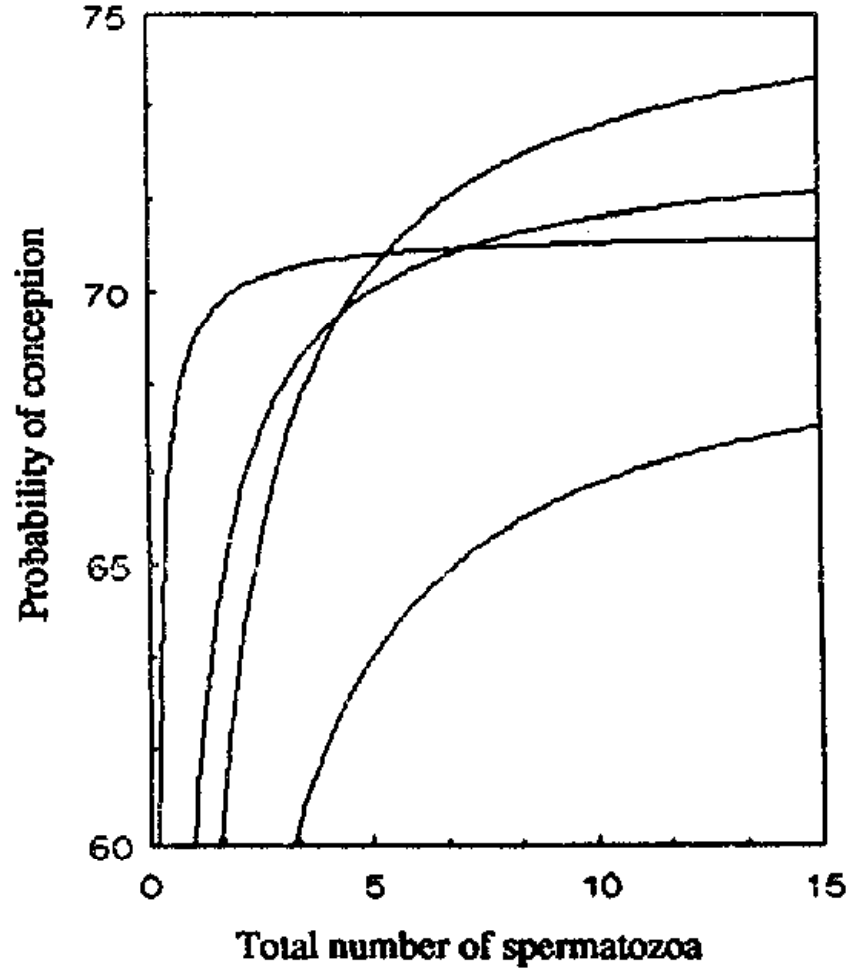
- Objective vs subjective – the often cited reason
- Set appropriate gates and have a strict operating protocol for operation.
- Standardise across all centres

**Semen analysis by CASA...
...but understanding comes from
experience**

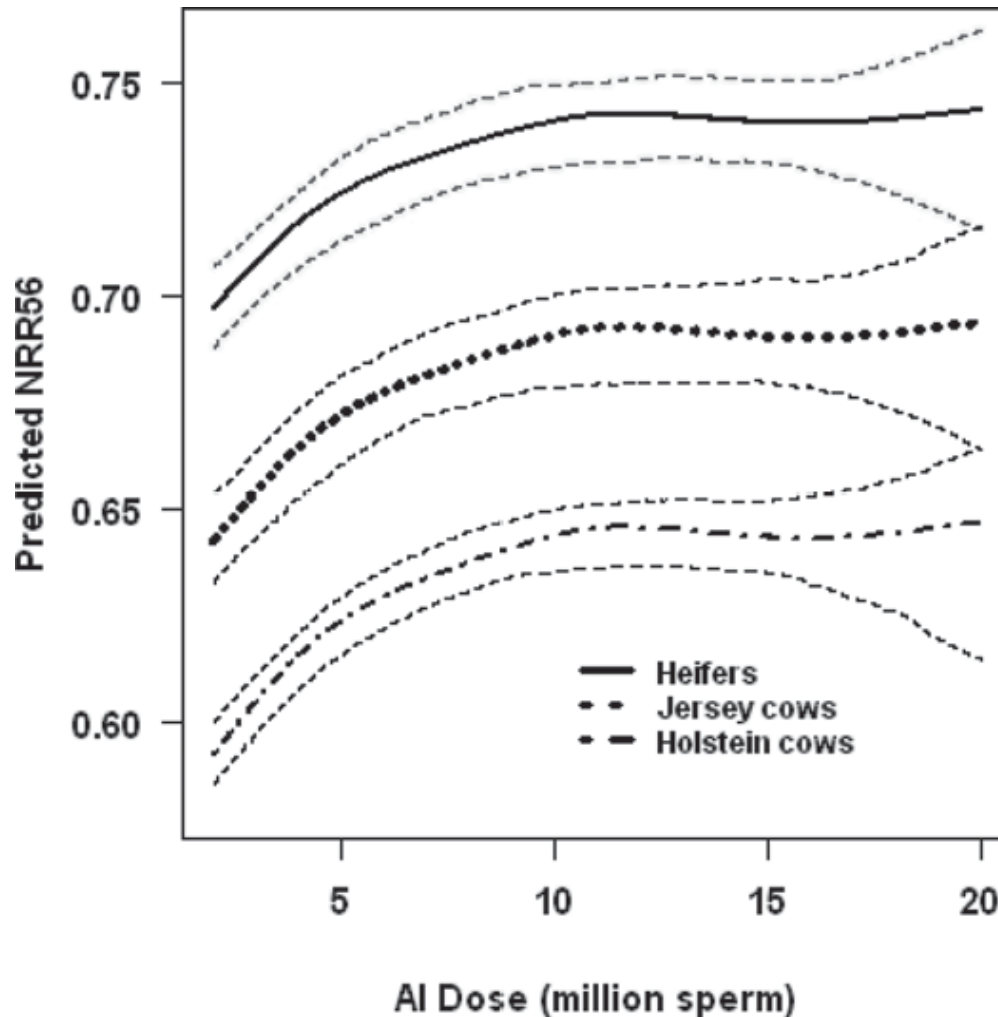


Why is dose rate or absolute sperm concentration in a straw important?

Bulls differ in inherent fertility



In most cases sperm numbers above 10 million sperm per AI dose are not consistently helpful.



(Christensen, et al., 2011)

The Standard Operating Protocol is very important

- What do you do if the genetics of the bull is very valuable but poor semen quality?
- Cross road analysis.



Your choices are

- Increase dose rates - ??
- Find out the reason for the problem??
- Seek alternative processing methods
- Cull the bull

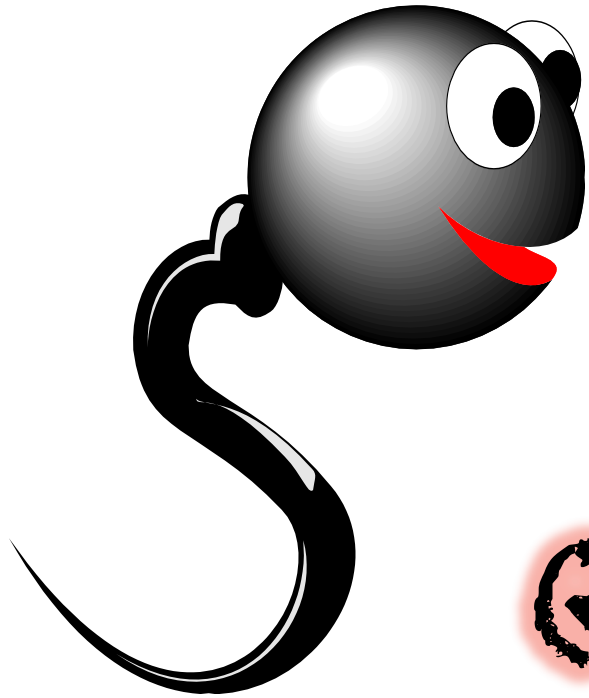
Why do you do what you do?

- Information is the key
- Shared knowledge by senior staff on sperm physiology
- Monitoring data, trends and anomalies
- Peer group mentoring & training



Observations relevant to India

- Semen production
 - World class
 - Missing is feedback loop data on CR and field performance
- R&D relevant to needs
 - Different challenges, different breeds, different environment.



Questions ?