Selection of quality breeding bulls: Use of testicular needle aspiration biopsy and recent semen evaluation techniques

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Challenges in front of us

- Expanding the AI services to 50% of breedable population.
- Increasing frozen semen production from 81 millions to 140 millions.
- Two fold increase in the number of breeding bulls.
- Optimizing the frozen semen out put in semen stations and quality improvement.
- Tools to forecast the future fertility of bulls at young age

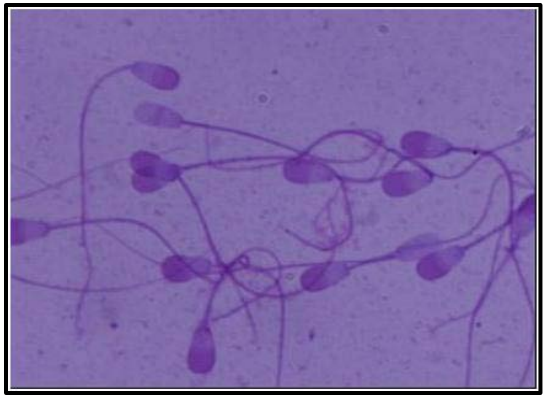
How we select breeding bulls?

Superior genetics

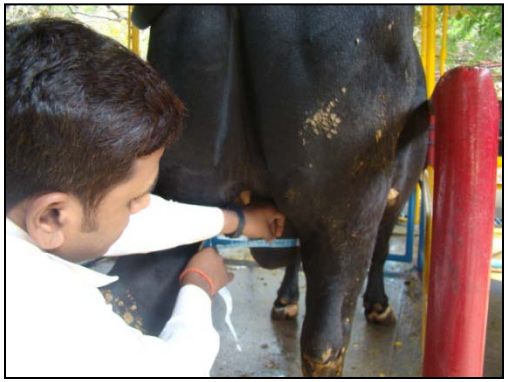
Anatomical evaluation

Special feeding

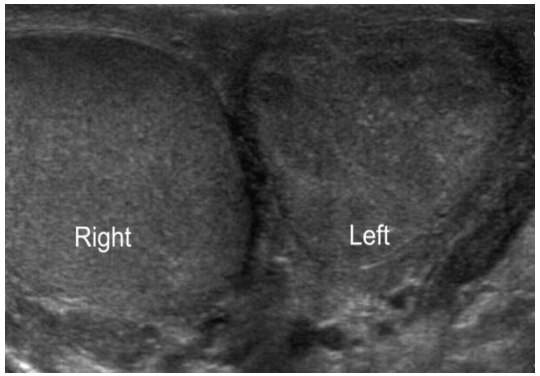
Disease testing



Semen evaluation



Scrotal circumference



Testicular ultrasound

Induction into semen collection program

Culling due to sub-fertility

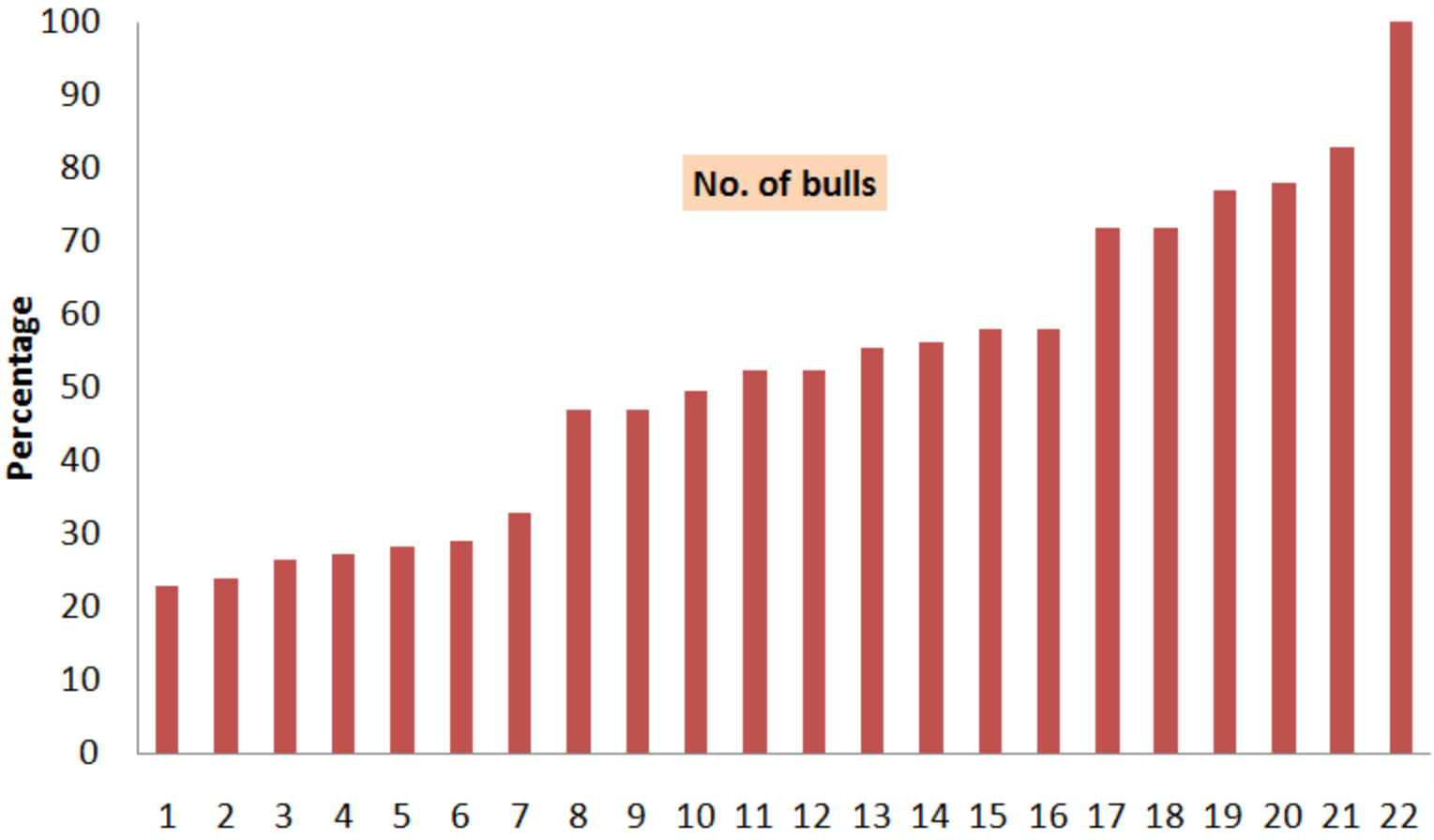
Particulars	Karan Fries	Frieswal	Sunandini	Sahiwal
Poor Libido	26.92	NA	36.46	76.67
Poor Semen Quality	55.77	54.91	45.42	13.32
Poor freezability	17.31	45.09	10.87	10.00
Sub-fertile	42.98	50	-	29.41
Reference	Mukhopadyay, 2010	Mathur, 2002	Sudheer, 2000;	Mukhopadyay, 2010

Which breed is more affected?

10 – 25%

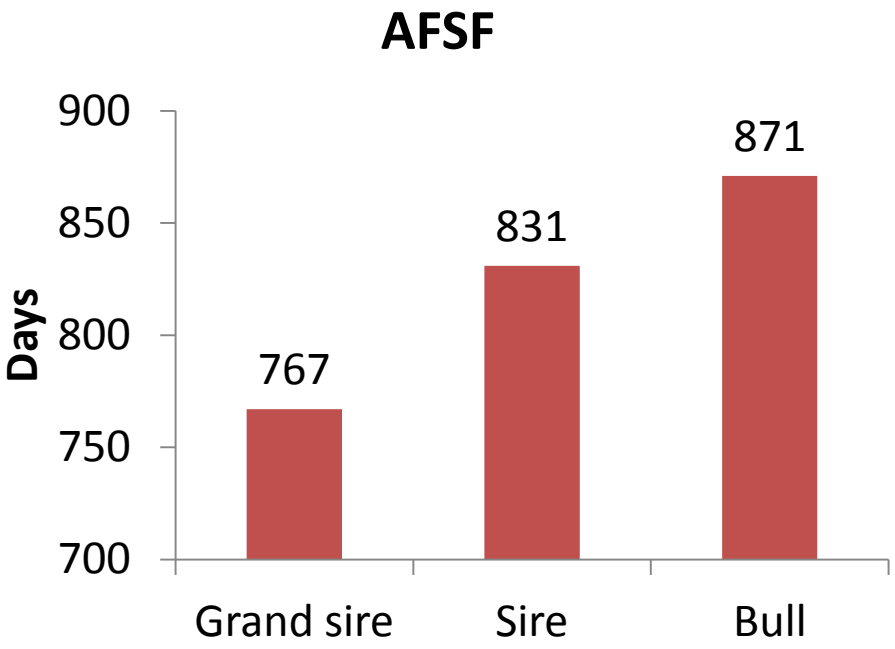
45 – 55%

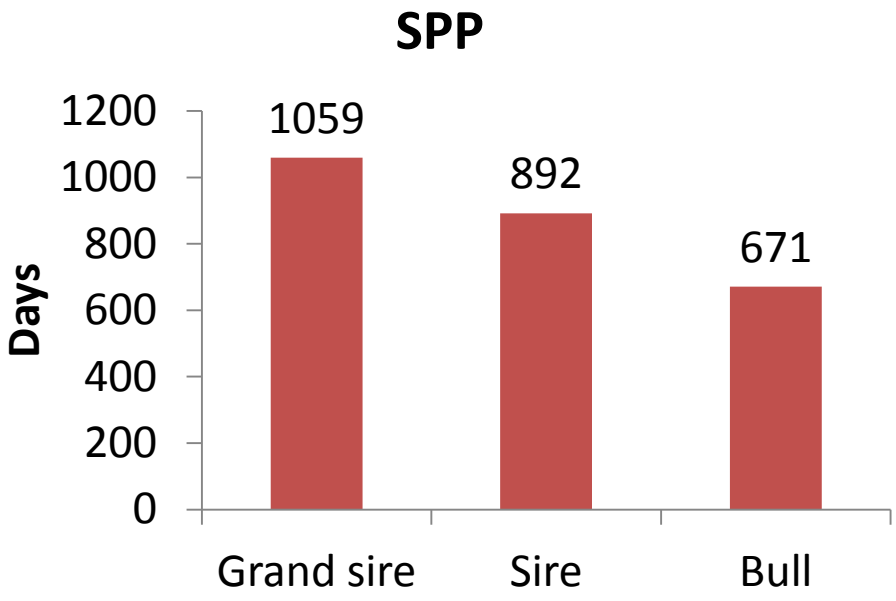
Ejaculate rejection rate in crossbred bulls



Vijetha et al, 2014

Decreasing trend of semen production ability





AFSF - Age at First Semen Freezing
SPP – Semen Production Period

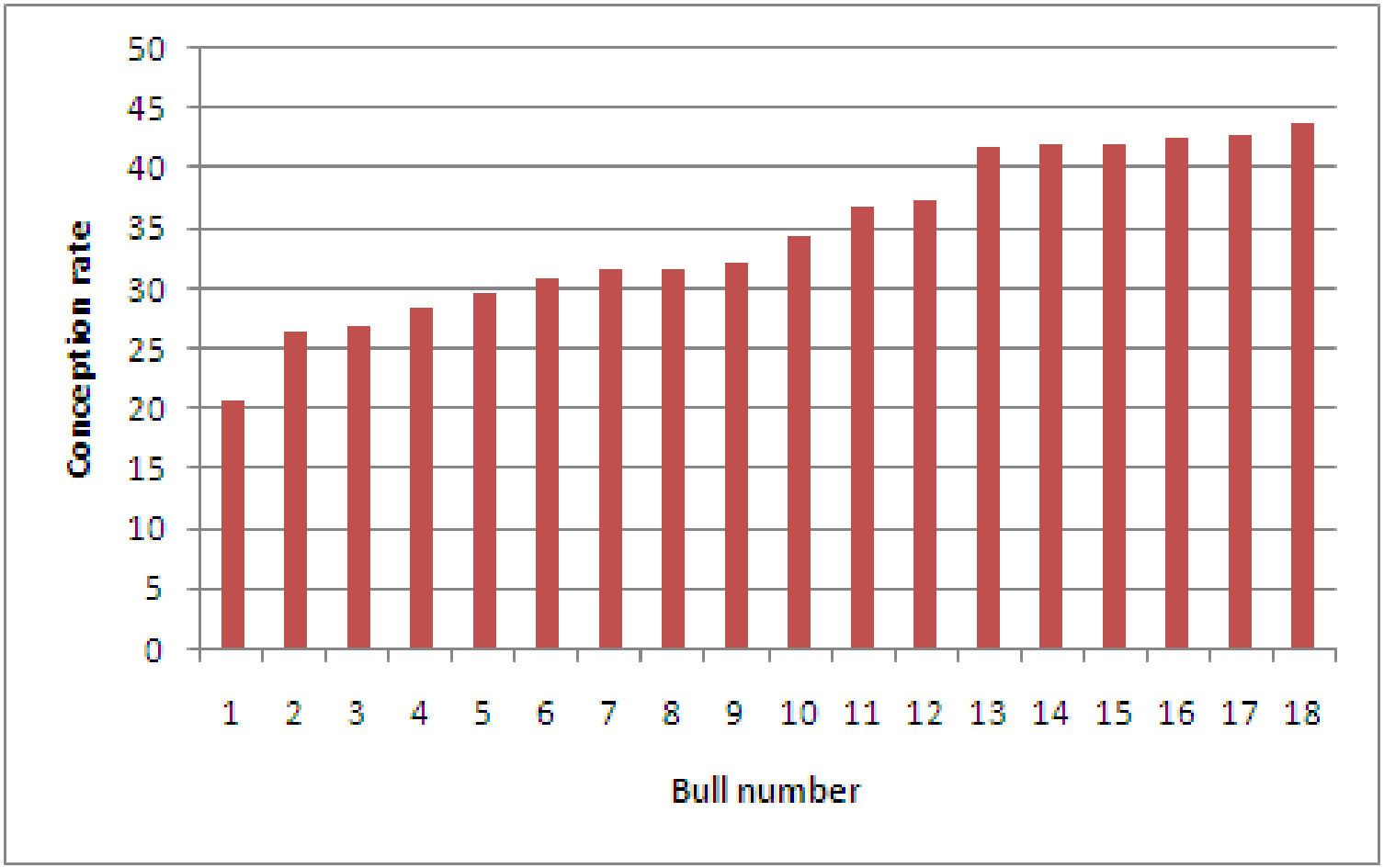
Vijetha et al, 2014

Increasing trend of poor semen quality

ERR – Ejaculate Rejection Rate

Vijetha et al, 2014

Fertility differences among the bulls that passed all BSE tests (20-25%)



CR based on minimum of 100 inseminations

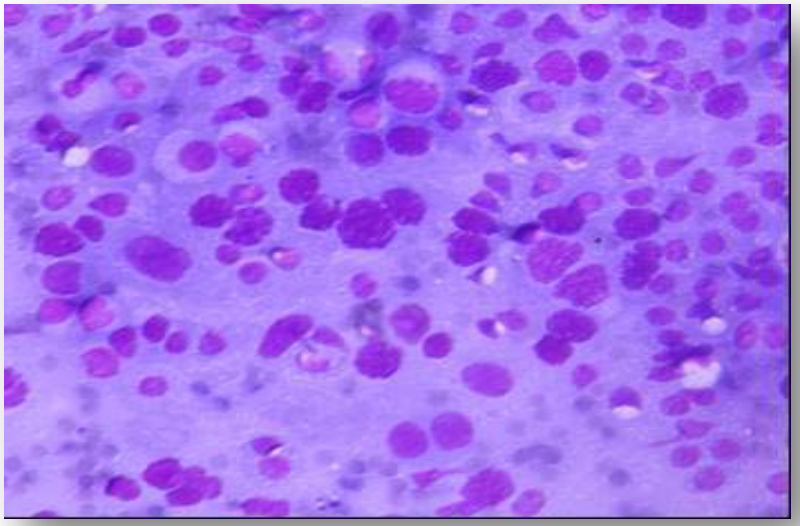
Why fertility of the bulls cannot be predicted accurately?

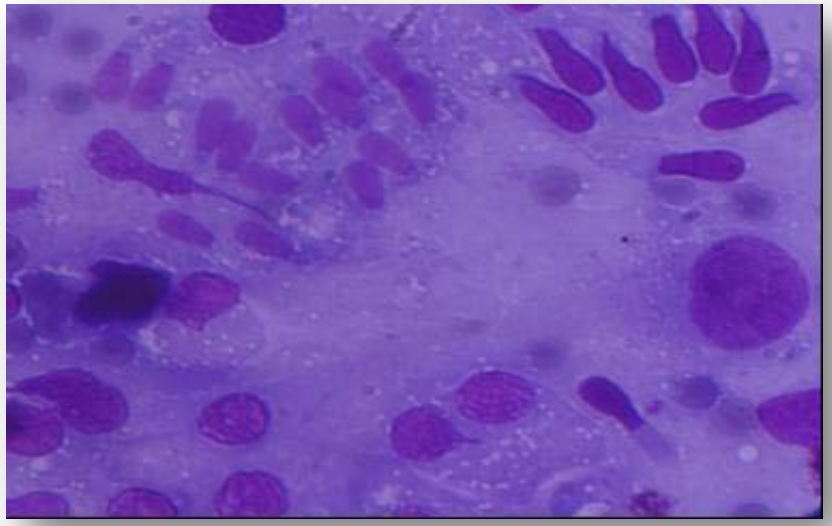
- Scrotal circumference indicate only the quantity of testicular parenchyma and not quality.
- The appearance of the testicular parenchyma of the in ultrasonography do not correlate with breeding ability of bulls.
- Lack of relationship between scrotal circumference, testicular echotexture and breeding soundness evaluation and fertility.

So search for fertility markers in bulls remains a continuing endeavor…..

Percutaneous needle aspiration method

Testicular cytology smears cells at 40 x and 100 x magnification





PNAB process did not alter SC and semen profile…

Parameters	Pre-PNAB	Post-PNAB
Scrotal circumference (cm)	38.07± 0.81	38.50 ± 0.70
Volume (ml)	4.63 ± 0.33	4.59 ± 0.26
Mass activity (0-5)	2.32 ± 0.33	2.44 ±0.30
Individual motility (%)	53.15±4.29	58.07±4.42
Sperm Concentration (10^6/ml)	761.40 ± 52.62	767.18± 47.61

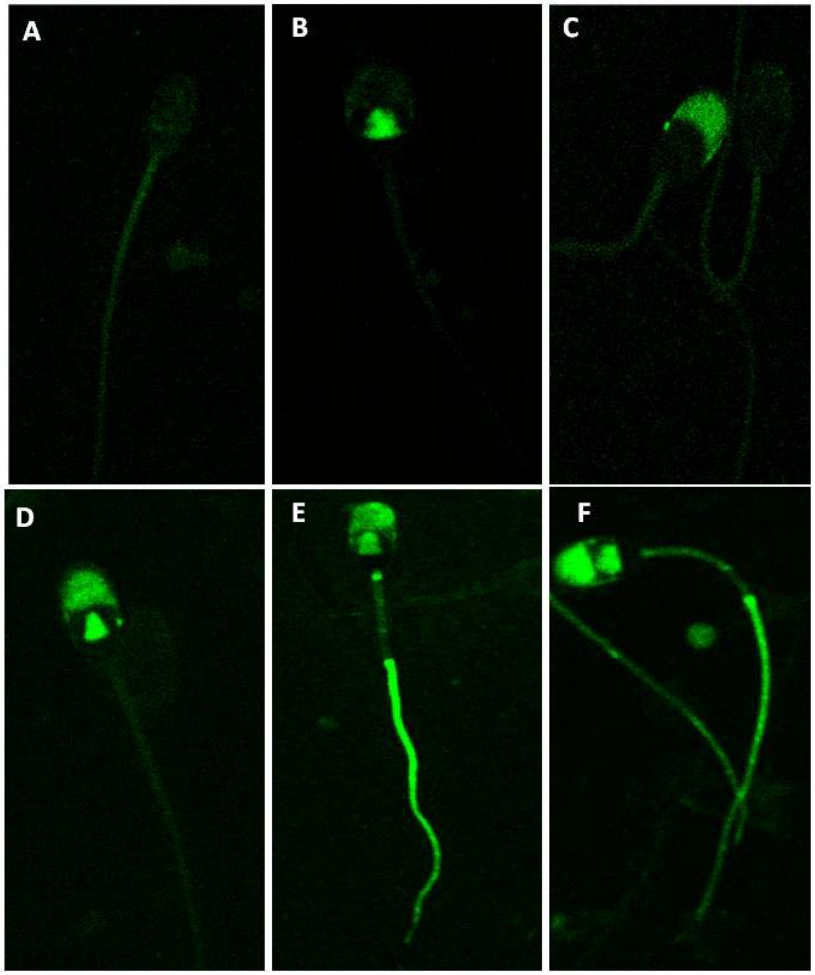
Rajak, 2012

Results indicate..

- Percutaneous needle aspiration can be employed safely in bulls with out affecting their reproductive health.

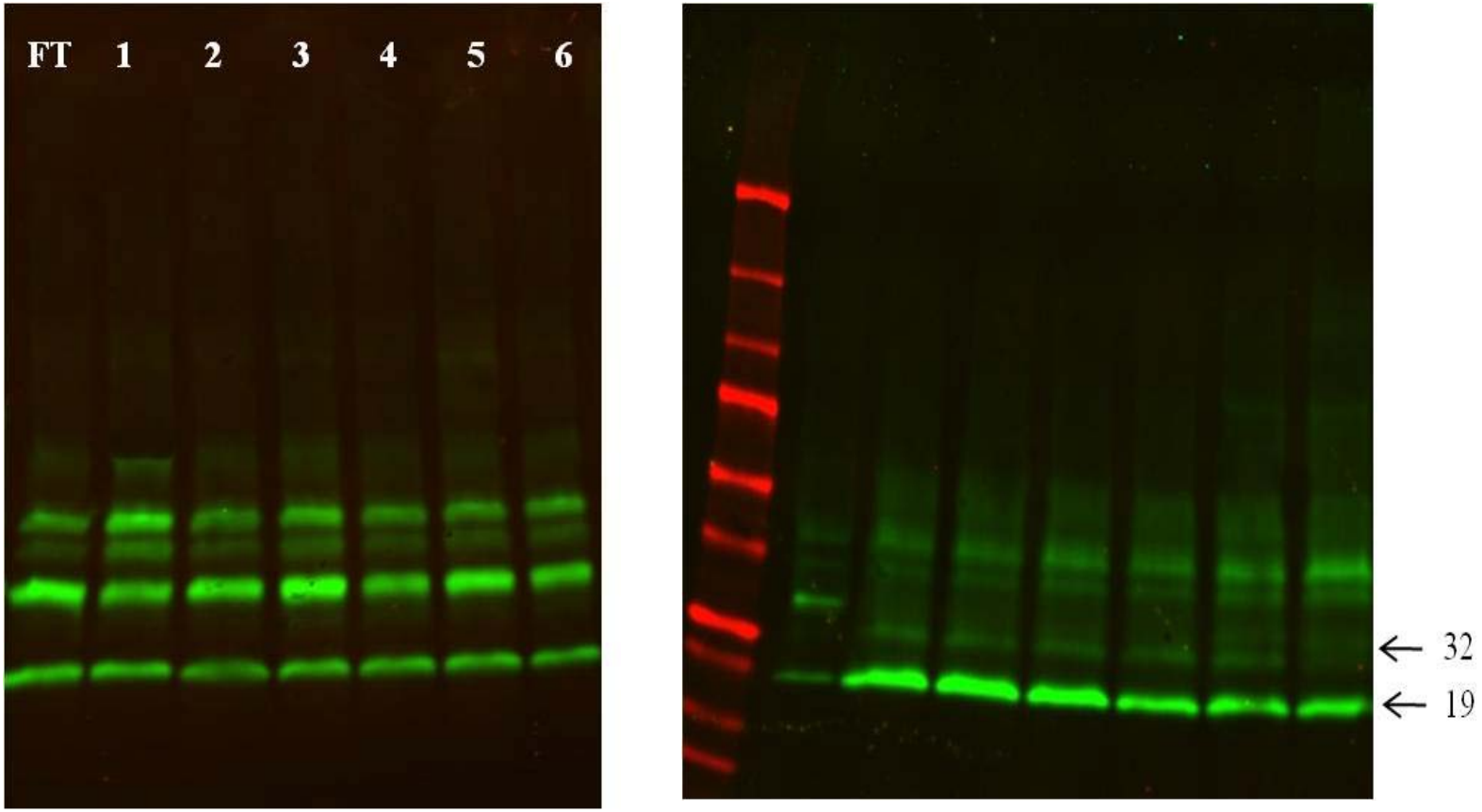
- Testicular cytology can be used for evaluating semen production ability in crossbred bulls.

Protein tyrosine phosphorylation pattern in seprmatozoa



Kumaresan et al, 2012

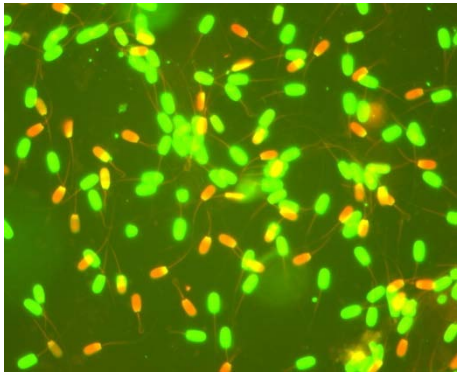
Differential expression of specific phosphoproteins is related to fertility

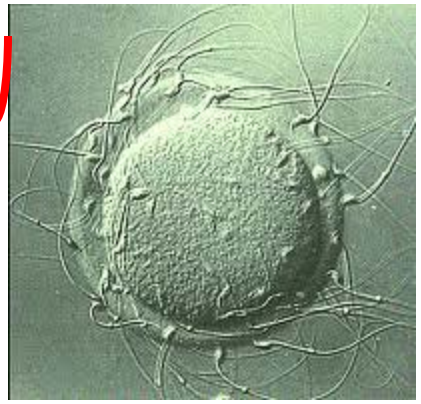


Kumaresan et al, 2012

Sperm function tests

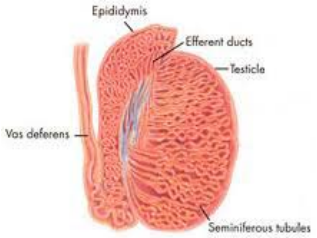
Reproduction cycle





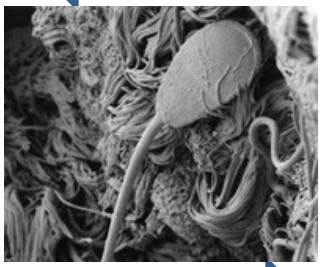
Changes in the sperm environment

- Low pH (epididymis)

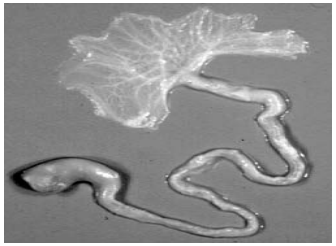


- High pH (in ejaculate)

- Low pH (uterus)



- Low pH (UTJ)
- High pH (in AIJ)

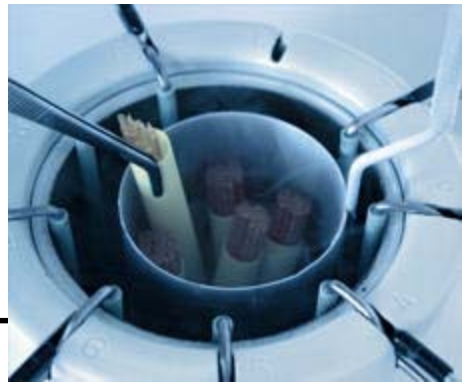


- Further High pH (in Ampulla)

Human made additional change

Low pH (epididymis)

High pH (in ejaculate)



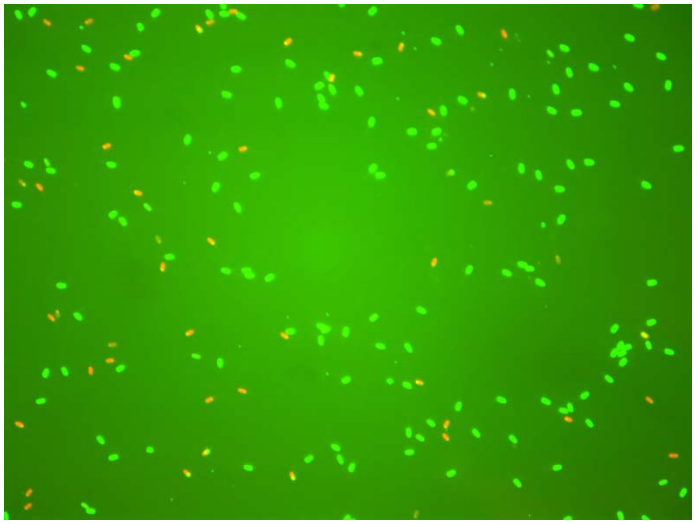
Cryopreservation

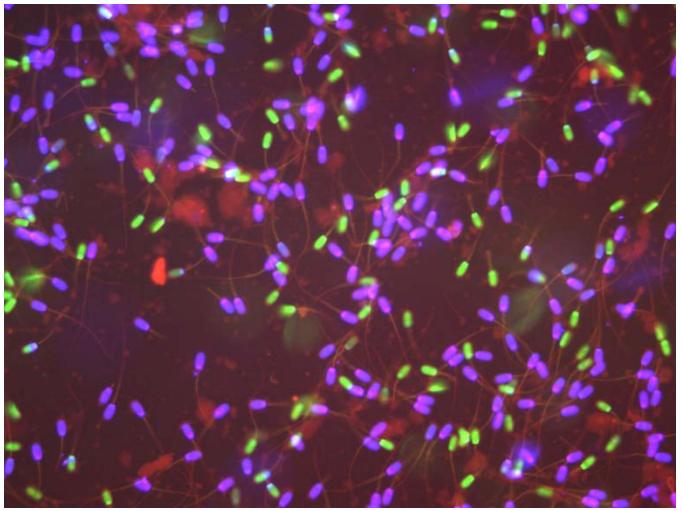
Low pH (UTJ)

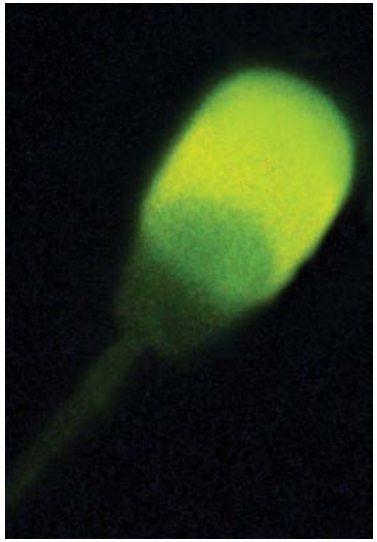
High pH (in AIJ)

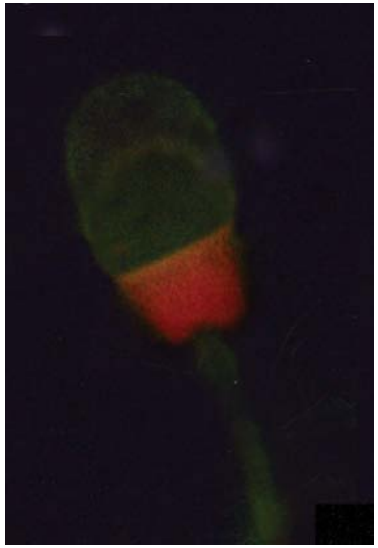
Further High pH (in Ampulla)

What happens to spermatozoa during cryopreservation??

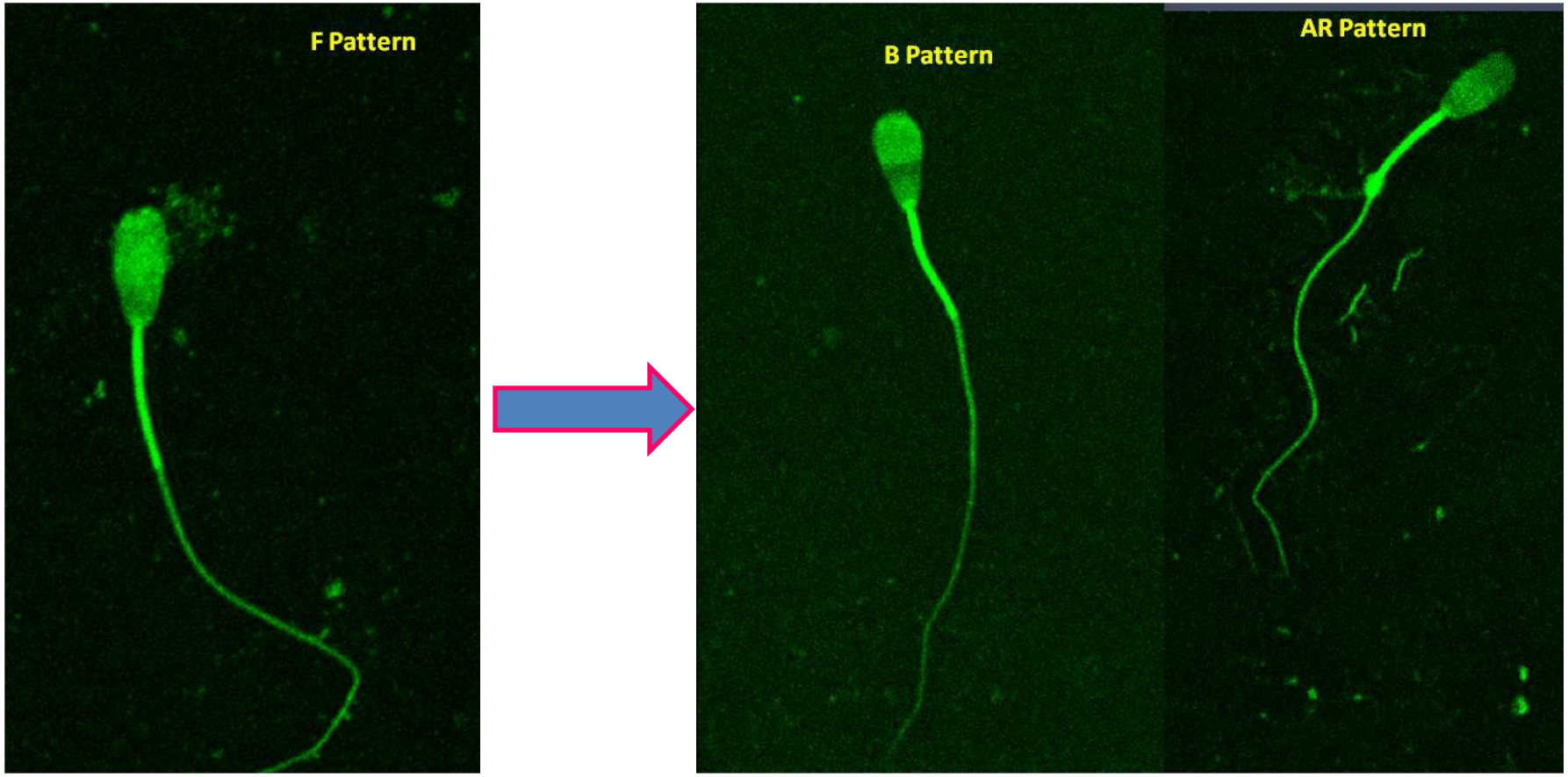




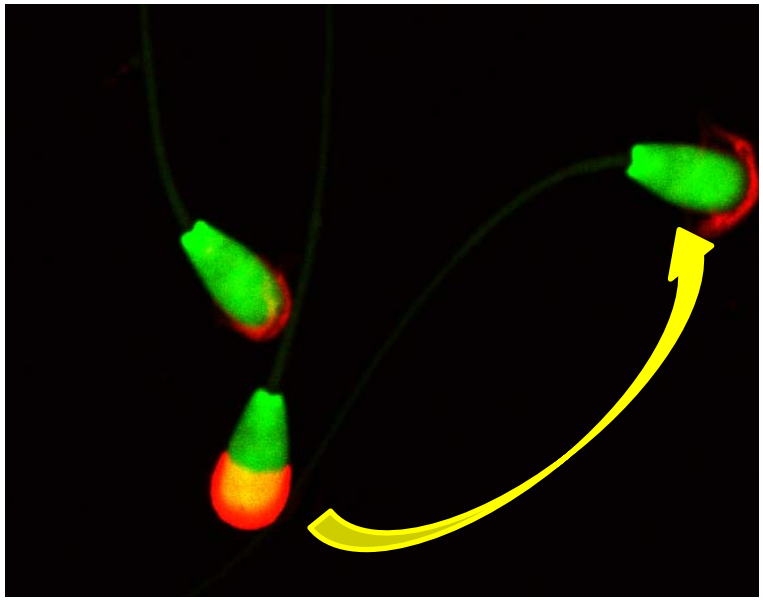


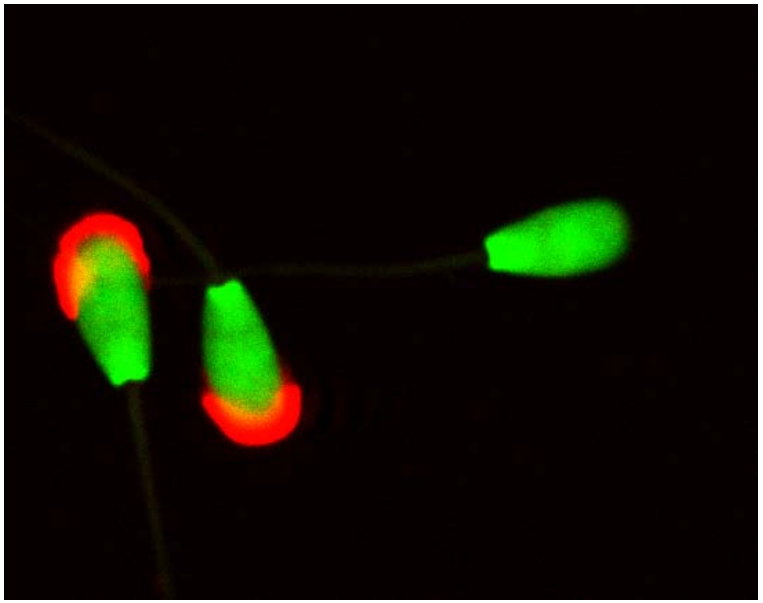


Pre-mature capacitation



Progressive loss in acrosome





Why the fertility of cryopreserved sperm is compromised?

- Changes in membrane components
- Sensitivity to extenders and additivies
- Thermotolerance
- **Capacitation-like changes**
- **Reactivity of sperm to the changing environments**

Do we estimate all these changes in frozen semen with current evaluation methods??

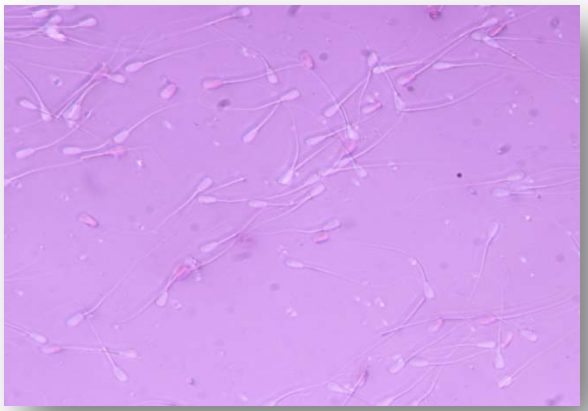
Current procedure - Routine semen analysis

Post-thaw

- Sperm motility
- Sperm viability
- Acrosomal Integrity

At some places

- HOSST
- BCMPT



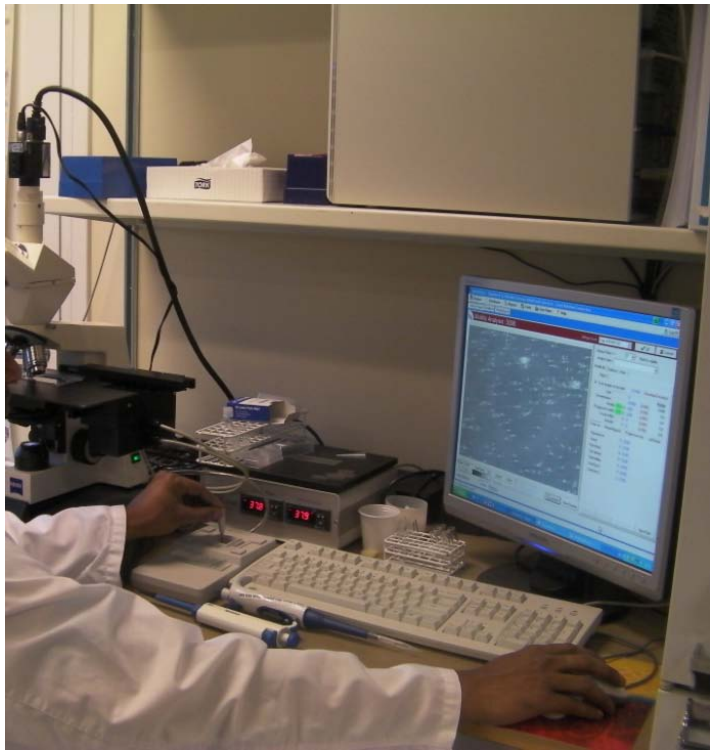
With these methods can we differentiate between the potential one and the others ?????

What are the sperm function tests related to fertility

- Sperm motility
- Intracellular calcium
- Protamine deficiency
- Membrane integrity
- Capacitation status
- Acrosomal integrity/reaction ststus
- Mitochondrial membrane potential
- Apoptosis
- Early membrane scrambling
- Lipid peroxidation

Motility estimation using CASA

- Estimate not only the progressive motility but also give information about sperm kinematic parameters
  - Curvilinear velocity
  - Straight line velocity
  - Average path velocity
  - Linearity
  - Straightness
  - Beat cross frequency
  - Lateral head displacement



Sperm kinematic parameter and fertility

CASA Motility parameter	High fertile bulls	Low fertile bulls
Average path velocity	87.22±1.6 µm/s	79.02±2.4 µm/s
Straight linear velocity	68.93±1.9 µm/s	63.42±1.2 µm/s
Curvilinear velocity	156.52±4.3 µm/s	142.37±2.8 µm/s
Amplitude of lateral head displacement	6.8±0.07 µm	6.5±0.1 µm

(Kumar *et al.*, 2012)

Average path velocity, Curvilinear velocity, Straight linear velocity and ALH are related to bull fertility

Sperm intracellular calcium

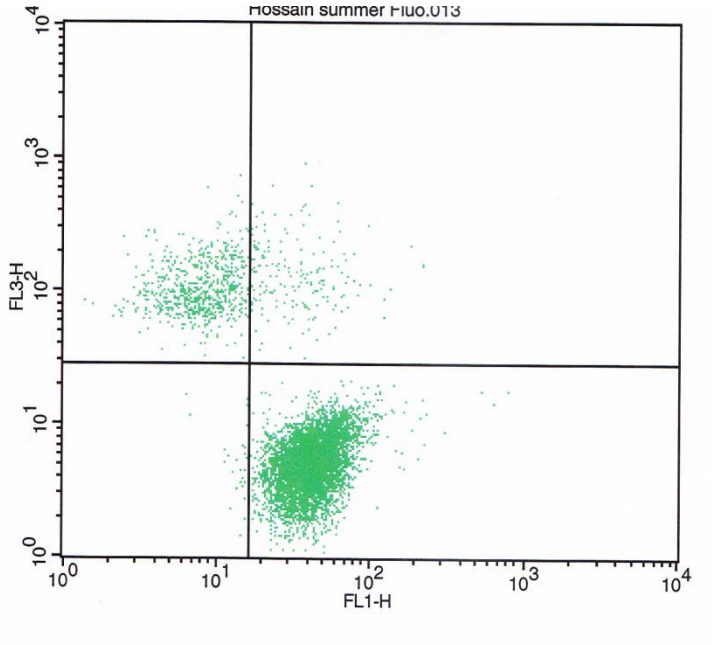
- The levels of [Ca_2+]i are higher in cryopreserved than in fresh bovine spermatozoa

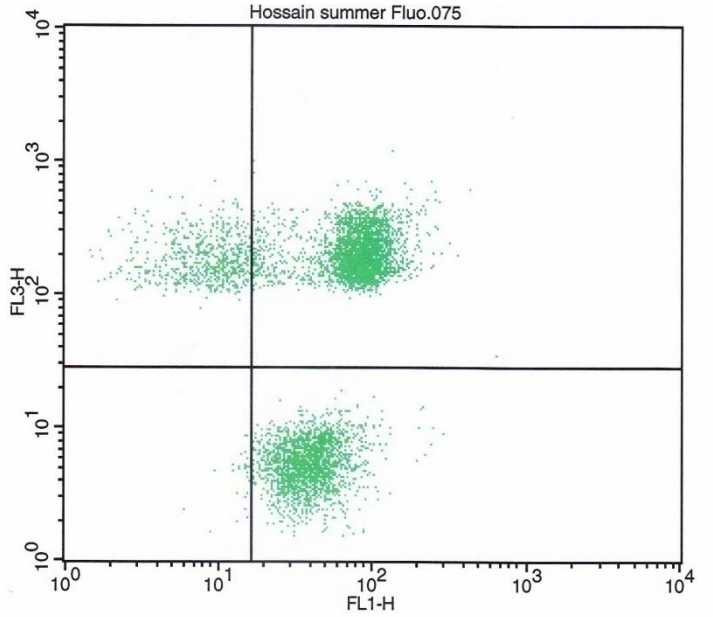
- Cryopreservation is known to disrupt the sperm plasma membrane and induces premature capacitation of a sperm subpopulation, which may be a result of the increased internal calcium levels after thawing

- Increased levels of [Ca_2+]i induces capacitation

- Quantification of sperm population with high intracellular calcium would help in assessing fertilizing potential

Subpopulations with high intracellular calcium





The intracellular calcium level is negatively correlated with bull fertility immediately after thawing

Kumaresan et al, 2011

(Collin *et al.*, 2000)

Protamine deficiency

- Protamine is a nuclear protein which is necessary for sperm head chromatin condensation and DNA stabilization.

- During spermatogenesis the histones are replaced with protamines.

- The higher histone are related with infertile individuals and sperm anomalies like immature sperm, abnormal structure, abnormal motility etc

Sperm Membrane Integrity

- Sperm plasma membrane covers the entire cell and also plays role in cell to cell interaction

- The proportion of moribund sperm is higher in sperm from low- vs high-fertility bulls, and the proportion of moribund sperm were correlated with non-return rates.

- Conversion of viable sperm to a moribund state is higher in low-fertility bulls, and that viability of sperm from low-fertility bulls was inherently compromised

(Saadi *et al.*, 2011)

Sperm capacitation Status

- Capacitation of spermatozoa takes place in female reproductive tract (especially at oviduct).

- The proportion of viable, uncapacitated spermatozoa present in semen was positively correlated ($r=0.5; p=0.03$) to fertility in cattle.

(Thundathil *et al.*, 1999)

- However, premature capacitation or presence of high proportion of capacitated spermatozoa in freshly ejaculated or immediately after thawing of cryopreserved semen is associated with low fertility

Sperm Acrosome Reaction

- Acrosome is a cap like structure covering the anterior portion of spermatozoon nucleus and having enzymes useful in acrosomal reaction

- Acrosome reaction is the pre-requisite event for the spermatozoa before acquiring fertilizing capacity.

- Acrosomal reaction had a high correlation with fertility. i.e. 0.91 in cattle and 0.77 in buffalo.

(Goswami, 2006)

- However, premature acrosome reaction is related to low fertility

Sperm Apoptosis

- Apoptosis is a programmed cell death; phosphotidylserine is present in the inner cytoplasmic leaflet of the plasma membrane and translocates to the outer leaflet during early apoptosis.

- The correlation coefficient between frozen thawed semen and fertility for apoptosis, early necrosis and necrosis were -0.48, -0.32 and -0.16 respectively.

- High proportion of apoptotic sperm is associated with low fertility.

Lipid peroxidation status

- Sperm mitochondria produce byproduct of oxygen metabolism like ROS. A certain level of ROS is essential for sperm oocyte fusion, zona pellucida binding and sperm capacitation.

- When ROS level exceeds the required level it causes ATP depletion, reduced motility, DNA damage and block sperm egg fusion, ultimately all lead to fertility reduction

- A negative correlation existed between MDA and sperm motility (-0.97) in buffaloes. (Kumaresan *et al.*, 2009)

- Higher the lipid peroxidation lower the chances of fertilizing potential.

Epilogue

- The current system of bull evaluation and selection needs require a reviewing and remodeling - it is high time to implement a uniform BSE protocol across the semen stations.

- The PNAB method and testicular cytology indices offer a scope to improve the efficiency of bull selection.

Epilogue

- Routine semen analysis (motility, viability, acrosomal integrity etc.) do not reflect the fertilizing capacity of spermatozoa.

- The sperm function tests assess minute details of spermatozoa essential for fertilizing ability - Easy to carry out and avoid human error in interpretation of the results.

- Semen stations can start with at least three tests FITC-PNA, CFDA-PI, CTC to improve the quality control of frozen semen.

Our lab continues to..

- Work on standardization of a "***Battery of sperm function tests***" that could be used to fairly judge the superiority of a bull over the others in terms of fertility.

- Train manpower in the area of sperm function tests.

- Provide technical help in establishing facilities for sperm function tests.

The Theriogenology Team at NDRI


thank you