Sperm Chromosome Breakage Screening

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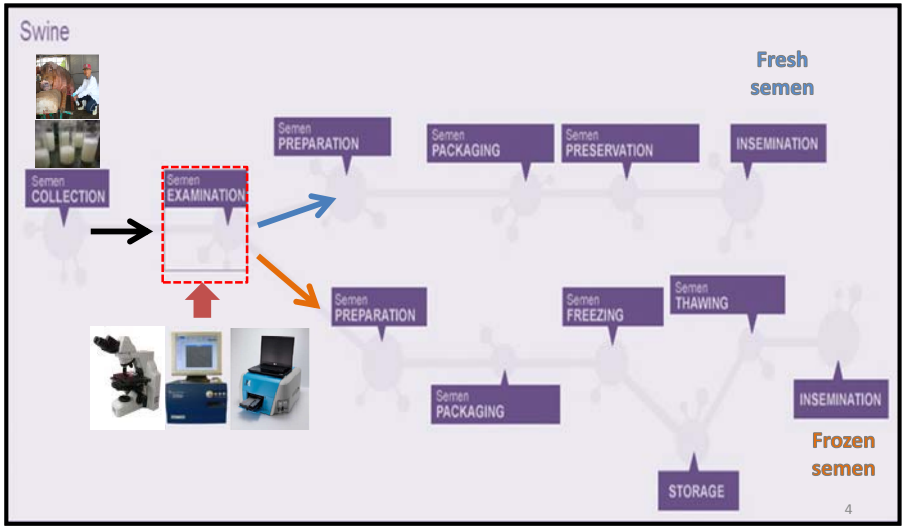
Introduction

- **The integrity of mammalian sperm is of importance for the male genetic contribution to normal offspring.**

Semen doses/ boar/year	Mean fertility	Dose/ sow	Litters/ year	Piglets / litter	Piglets obtained / boar/ year
1,800	85%	3	510	10	5,100

- The **greatest quality semen** to ensure maximum litter sizes and farrowing rates is expected.
- The control of quality in semen used for artificial insemination (AI) must be **as precise as possible**.
- Analysis of **sperm parameters** is very important for predicting the outcome of assisted reproductive techniques and is necessary for determination of fertility of males tested for artificial insemination.
- The number of intact and functional spermatozoa in semen can be assessed with **flow cytometry** and is believed to relate to male fertility.
- The **DNA damaged sperm** have the ability to fertilize the oocytes, but the embryonic development is very much related to the degree of DNA damage.
- Seems to be convenient as **additional method for semen quality detection** in farm animals before their exploitation in breeding.

The importance of semen examination



Sperm parameters

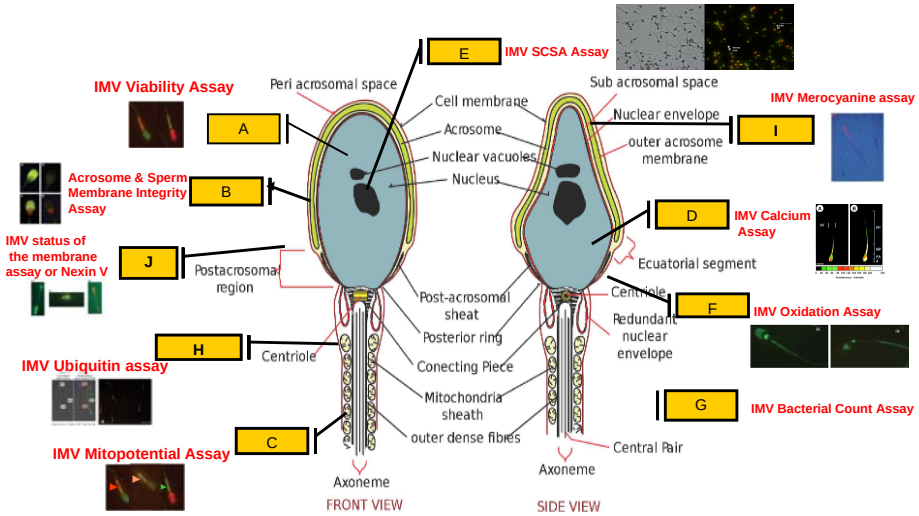
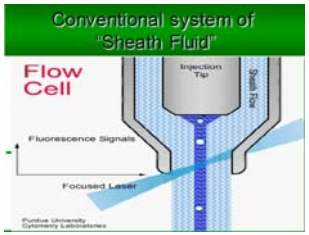


Fig. 10 sperm structure and function related parameters Simon Kuo,2012

Semen Quality Examination
Different systems for different options

ASSAY	Microscope	CASA	Easy Cyte ®
Motility	++	+++	-
Concentration	-	+++	+++
Viability	+	+	+++
Acrosome	+	+	+++
Mitochondria	-	-	+++
Capacitation (Ca)	-	-	+++
DNA fragment	-	-	+++
Bacterial count	-	-	+++
Morpho/physio	+/-	++/-	++/+++
Objectivity	+	++	+++

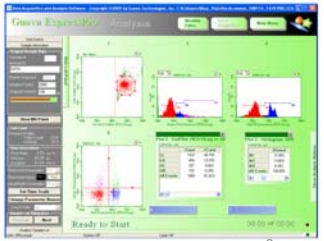
Analyze by Flow Cytometer



- One machine (the flow cytometer)
- One computer (laptop with the machine)
- One software for data analysis and interpretation

Why to use a flow cytometer?

- To improve the semen analysis
- Standardization with high statistic power
- For male management
- For quality control (dose certification & ability of cryoprotectant)
- High value animal



Sperm parameters- DNA Breakage

- **Sperm Chromosome DNA**
- **Screening in pig industry**

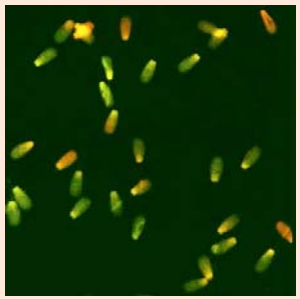
Methods for assessment of sperm DNA fragmentation

- SCD Test (Sperm Chromatin Dispersion)
- Comet Assay
- Tunel Assay (Terminal transferase dUTP Nick End Labeling)
- SCSA (Sperm Chromatin Structure Assay)

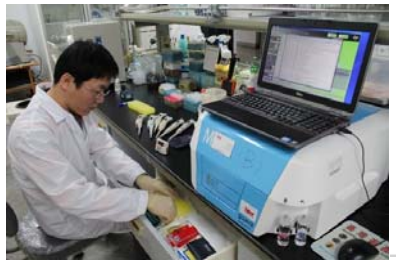
∗ the best experiments to determine what semen quality traits are most important for pregnancy outcome

Sperm Chromatin Structure Assay

Definition : Estimate the structure stability of the sperm nucleus chromatine after an acide attack. The acridine orange (AO) has the capacity to change from red fluorescence when it is linked to fragmentised DNA green fluorescence to green when it is linked to intact DNA.



- **Detection and application of sperm DNA parameter in breeding farm animals by flow cytometer**



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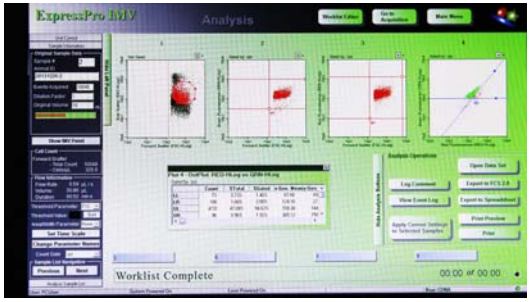
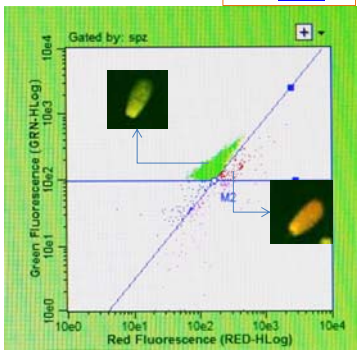
SCSA combined with flow cytometer to access the level of fragmented DNA within a sperm head

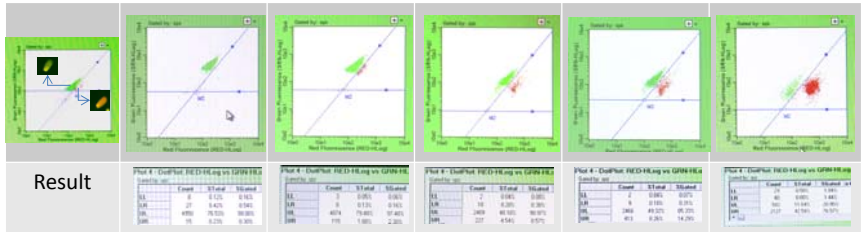
影片播放





Our data indicate the number of intact and functional spermatozoa in semen can be assessed with flow cytometry.

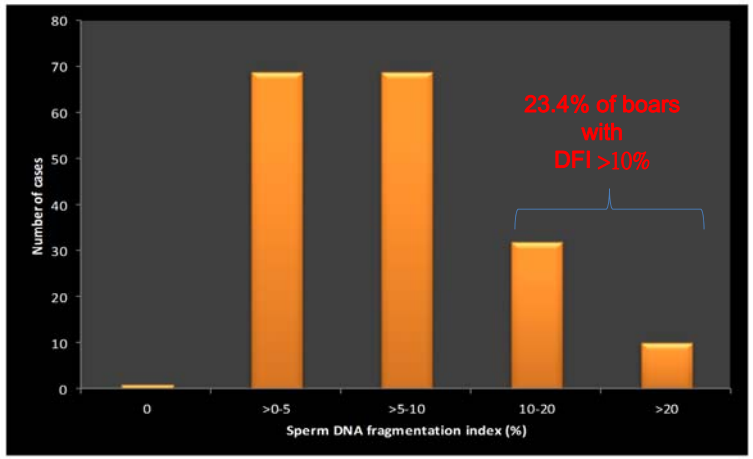
- **To establish and investigate the sperm DNA fragmentation index (DFI) which could be considered normal in pig.**

Status	A	B	C	D	E
Result					
DFI%	0-1%	>1-5%	>5-10%	10-20%	>20%
Quality	Very Good	Good	Acceptable	Not Good	Bad

DNA Fragmentation Index (%DFI; % sperm cells containing damaged DNA)
- **< 10% DFI = excellent fertility potential**
- **> 10 to < 20% DFI = good fertility potential**
- **> 20% DFI = fair to poor fertility potential**

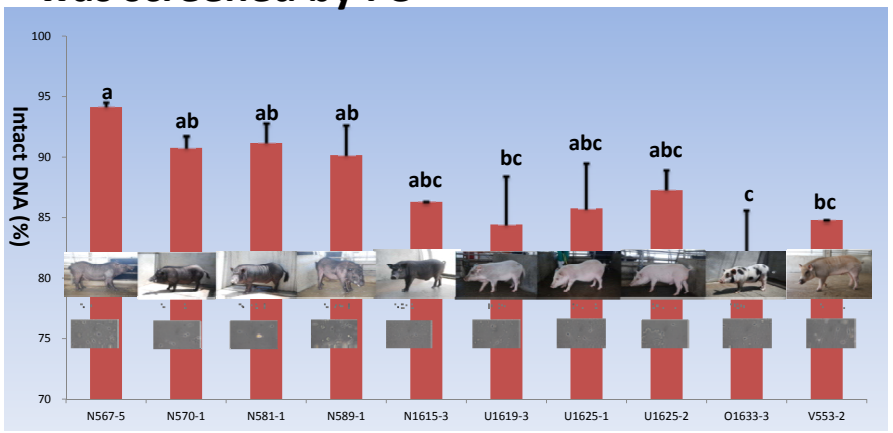
Simon Kuo, 2013

- **Ejaculates of 180 boar were screened and it's DFI values were calculated by FC**



An incidence of ejaculates with a DFI higher than 20% has been observed in boar

- **Native miniature pig sperm DNA breakage was screened by FC**



[a, b,c] values with different subscripts were significantly different (P < 0.05)

Simon Kuo, 2014

Conclusion

- **It is recommended to periodically assess DNA defragmentation, such as at a set number of times over the year, or as criterion for boars entering into an AI center.**

Thank you !