

Seminar on Boar Semen Application for Pork Quality Improvement

SCREENING ON SPERM CHROMOSOMAL BREAKAGE OF YOUNG BREEDING BOARS



Mr. Kuo Ting-Yung
Division of Breeding and Genetics, TLRI
2017/11/09

Outline

- ✓ ELITE BREEDING SWINE OF TAIWAN
- ✓ INTRODUCTION
- ✓ EXAM SPERM QUALITY ?
- ✓ METHODOLOGY OF SEMEN EVALUATION
- ✓ FLOW CYTOMETRY SPERM INTEGRITY ANALYSIS
- ✓ SCREENING ON SPERM CHROMOSOMAL BREAKAGE OF YOUNG BREEDING BOARS
- ✓ SCREENING ON SPERM CHROMOSOMAL BREAKAGE OF NATIVE MINIATURE PIG
- ✓ CORRELATION COEFFICIENTS (R) BETWEEN NEW SPERM PARAMETERS QUALITY TRAITS AND FERTILITY PARAMETERS
- ✓ CONCLUSION

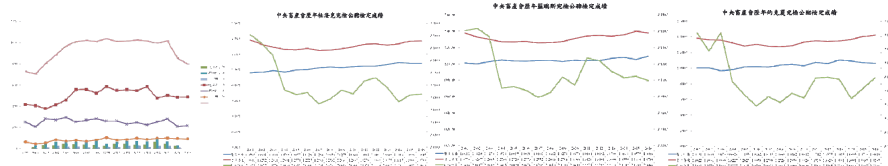
A-47

Elite breeding swine of Taiwan

- Performance test station/Growing boars from 40-110kg of body weight/AVE. FE/BF/ADG/2016

Traits	Duroc	Landrace	Yorkshire
Ave. ADG	1.08	1.09	1.06
Ave. BF	1.37	1.37	1.42
Ave. FE	2.06	2.09	2.11

➤ ADG/Average daily gain, BF/Back fat, FE/Feed efficiency (Feed/Gain)

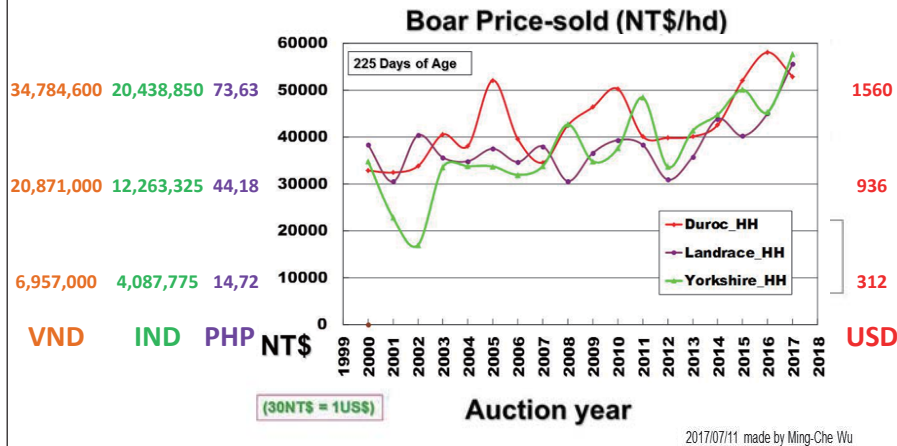


Elite breeding swine of Taiwan Total sperm count per collection in boars within 300 days of age

	Number of boars In Auction	Ave. Total sperm number (billion)	Top 1 Total sperm number (billion)
Duroc	1186	71.3	155.3
Landrace	296	75.6	232.2
Yorkshire	69	66.9	114.0
Black	42	63.5	102.4

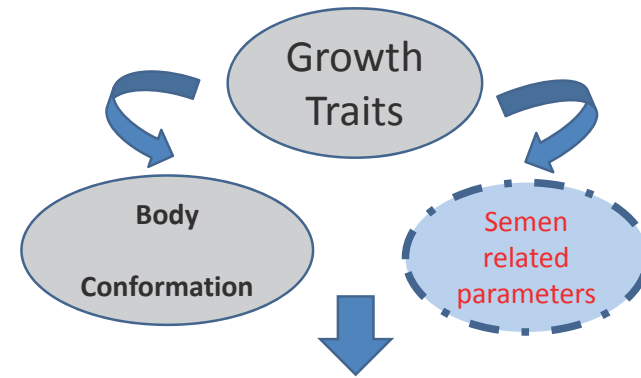
Year from 2011~2017

Elite breeding swine of Taiwan



The Best Boar Selection Index

Focus on semen related parameters



Future: New boar selection Index combine with Growth Traits- Body Conformation- **Semen parameters**

A-48

Introduction

- The integrity of mammalian sperm is of importance for the male genetic contribution (ex: meat, litter size) to normal offspring.

semen doses/ boar/year	mean fertility	Dose/ inseminated sow	Litters/ year	piglets / litter	piglets obtained / boar/ year
1,800	85%	3	510	10	5,100

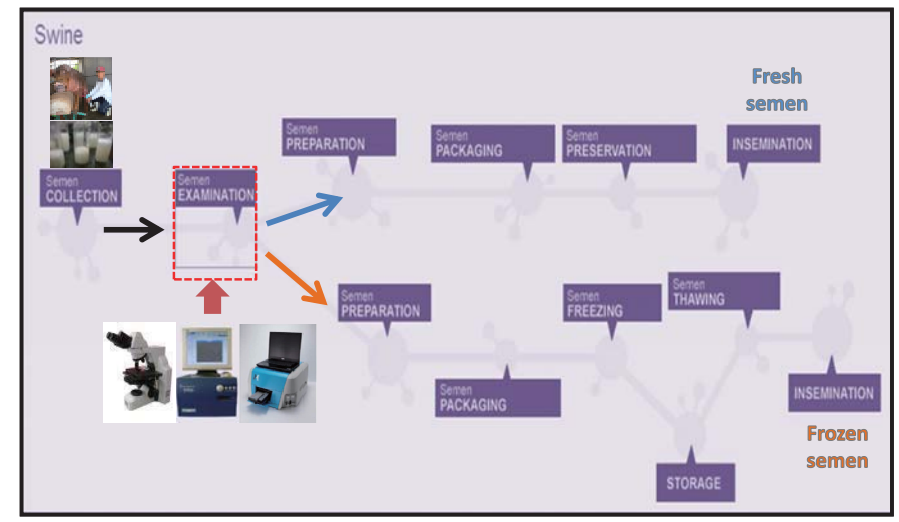
Mating times/ sow/year	Litters/ year	piglets / litter	piglets obtained / sow/ year
2	2	12	24

Field fertility of a boar

Male genetic contribution to normal offspring

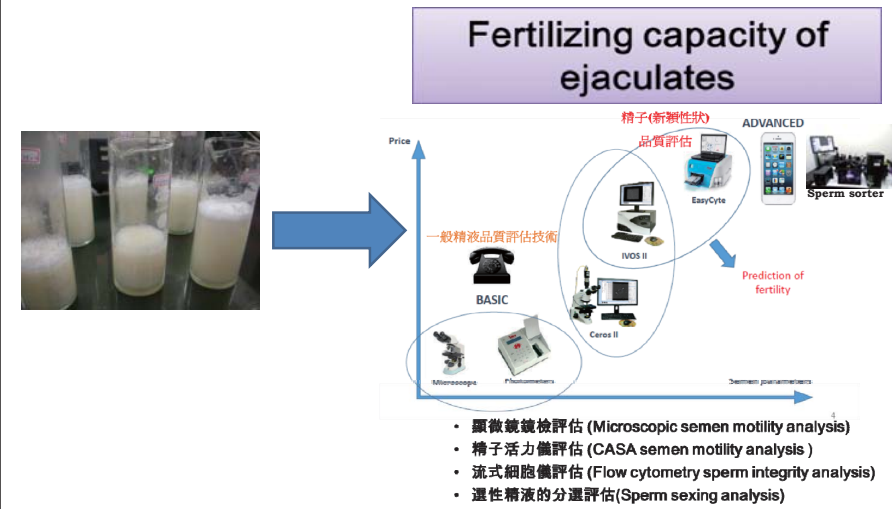
Farrowing rate (FR) and Total number of piglets born (TNB)

The importance of semen examination



- 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 **new 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.

Exam sperm quality ? Sperm selection



Evaluation method used for relating boar semen quality with field fertility?

A. Conventional assay
一般分析

- Breed/品種
- Birth Date/出生月份
- Month of ejaculate /採精月份(ME)
- Ejaculate age/採精日齡(DE)
- desires to mount/屬乘意願W
- Dummy/屬上假母台(秒)DS
- penis appearance/陰莖外觀PA
- penis length/鞭長(公分)PL
- semen volume/精液量(毫升)MSC
- semen color/精液顏色SC
- ✓ sperm motility/精子活力(90+++強)SM
- ✓ /精子數等級(+++濃厚)SG
- ✓ semen concentration/精子濃度(億/毫升)SBMI
- ✓ Total sperm number/總精子數(億)TSN
- ✓ hind legs strength/後肢強度FS
- ✓ Sperm Morphology/型態不正常率

B. Computer assisted semen analysis
精子運動力分析

- > **Velocities and Parameters**
 - Total Motile
 - Progressive
 - Slow
 - Static
- > **Motion Parameters**
 - DAP: Distance Average Path
 - DCL: Distance Curvilinear
 - DSL: Distance Straight line
 - VAP: Smoothed Path Velocity (microns/sec)
 - VCL: Track Velocity (microns/sec)
 - VSL: Straight Line Velocity (microns/sec)
 - ALH: Amplitude of Lateral Head Displacement (microns)
 - BCF: Beat Cross Frequency (hertz)
 - LIN: Linearity (ratio of VSL/VCL)
 - STR: Straightness (ratio of VSL/VAP)
- > **Morph Categorization**
 - Proximal Droplet
 - Distal Droplet
 - Bent Tail
 - Coiled Tail
 - Distal Midpiece Reflex (DMR)

C. Flow cytometry assay
精子新穎性狀分析

- ◆ Viability
- ◆ Viability Acrosome
- ◆ Mitopotential
- ◆ High Calcium Level
- ◆ Average Calcium Level
- ◆ Chromatin Structure
- ◆ Oxidation
- ◆ Bacterial Count
- ◆ Ubiqui
- ◆ Phospholipids disorder
- ◆ Apoptosis
- ◆ hyposmotic swelling test

D. Gene selection
基因型分析

- porcine stress syndrome/PSS/AGENE
- Estrogen Receptor /ESR/MGENE
- Heart-type fatty acid binding protein /HFABP/HGENE
- Insulin Like Growth Factor /IGF27/FGENE
- Insulin Like Growth Factor /IGF23/QGENE
- prolactin receptor /PRLR/PGENE

FLOW CYTOMETRY SPERM INTEGRITY ANALYSIS)

EasyKit 5 Viability & Acrosome Integrity - Indicator of sperm membrane and acrosome integrity

EasyKit 2: Mitochondrial activity - Indicator of mitochondria

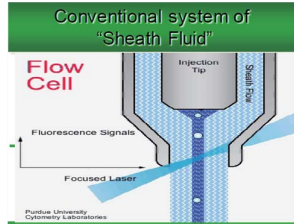
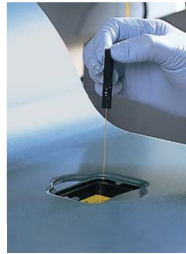
EasyKit 1: concentration - total number of sperm cells

EasyKit 1: Viability - measure of sperm membrane integrity

EasyKit 6: Bacterial concentration - Measure the total bacteria in an ejaculate

EasyKit 3: Oxidation molecule D - Detection of free radicals in spermatozoa

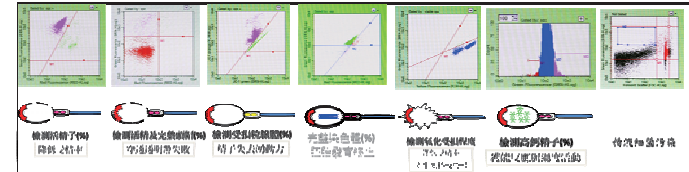
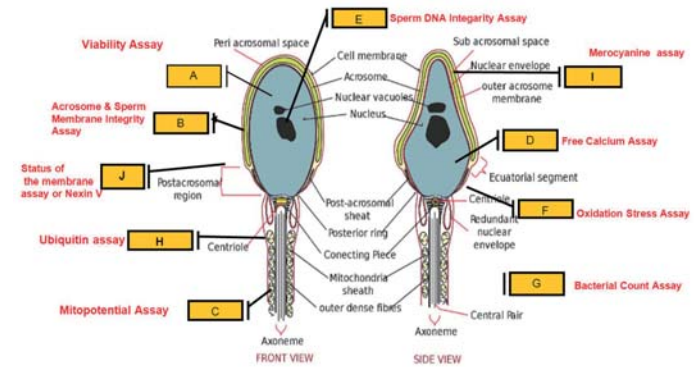
Analyze by Flow Cytometer



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

A-50

New Sperm parameters



Result of 7 new sperm parameters of young breeding boar 2012

9月龄种公猪精子参数分析
(检测日期2011/11/03至2012/09/03)

参数等级	V750 活精子(%) (≥74%为优)	A400 活精子且 膜完整性(%) (≥39%为优)	M30B 活精子中 粒球膜完整性(%) (≥31%为优)	C800B 活精子中 高尔基体完整性(%) (≥91%为优)	DC950 活精子且 膜完整性(%) (≥94%为优)	O300B 活精子中 氧化应激(%) (≤91%为优)	BO 精子膜完整性 (≥0.50为优)
0	0	24	0	0	0	0	0.00
5	5	31	5	5	5	5	0.05
10	10	37	10	10	10	10	0.10
15	15	40	15	15	15	15	0.15
20	20	44	20	20	20	20	0.20
25	25	45	25	25	25	25	0.25
30	30	43	30	30	30	30	0.30
35	35	35	35	35	35	35	0.35
40	40	43	40	40	40	40	0.40
45	45	39	45	45	45	45	0.45
50	50	36	50	50	50	50	0.50
55	55	35	55	55	55	55	0.55
60	60	26	60	60	60	60	0.60
65	65	39	65	65	65	65	0.65
70	70	24	70	70	70	70	0.70
75	75	22	75	75	75	75	0.75
80	80	31	80	80	80	80	0.80
85	85	14	85	85	85	85	0.85
90	90	2	90	90	90	90	0.90
95	95	0	95	95	95	95	0.95
100	100	0	100	100	100	100	1.00



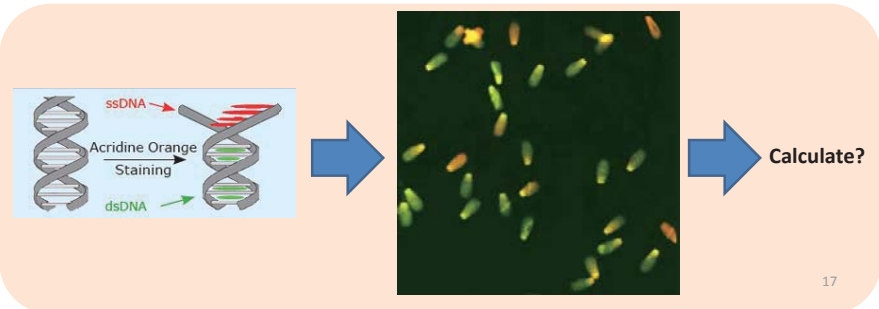
New Sperm parameters- DNA Breakage



- ▶ Sperm Chromosome DNA
- ▶ Screening in pig industry
- A. In auction (young boar)
- B. In reservation farm (Native pig)

The sperm chromatin structure assay

To estimate the structure stability of the sperm nucleus chromatin after an acid attack. The acridine orange (AO) has the capacity to change from red fluorescence when it is linked to fragmented DNA green fluorescence to green when it is linked to intact DNA.



17

compensable and noncompensable semen quality trait

Semen Used for AI		
Trait	compensable semen quality trait	Noncompensable semen quality trait
	sperm numbers	sperm DNA fragmentation
	i.e., increased sperm numbers can be added to produce a higher pregnancy rate.	it is the percent of sperm with fragmented DNA being considered and no matter how many sperm are added, the percent sperm with fragmented DNA remains the same; thus, the probability is the same for decreased pregnancy outcome due to this factor alone.

One of the main causes of sperm DNA damage is the exposure to reactive oxygen species (ROS) that are highly reactive and damaging to nucleic acids (Ochsendorf, 1999).

Sperm cell defense against DNA damage relies on two factors: the tight packaging of chromatin, based on condensation and substitution of histones with protamines, and the antioxidant agents present in seminal plasma. These defenses are extremely important as mature sperm is unable to repair DNA damage and even if a successful fertilization occurs, embryo undergoes apoptosis at the time of genomic activation. (De Ambrogi et al., 2006) (Italy)

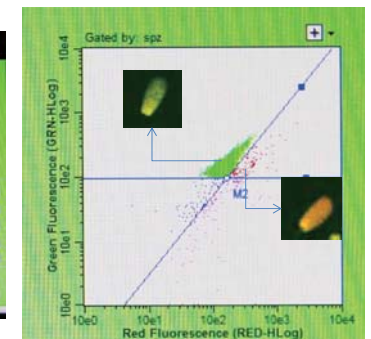
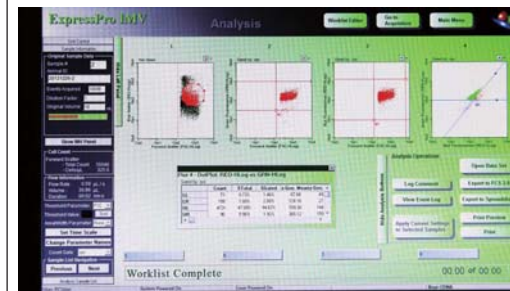
DNA damaged sperm presented normal zona-pellucida binding characteristics and even the fertilization and cleavage rates of fertilized oocytes remained normal. However, in their experiments about all four to eight cell embryos initiated apoptosis. Thus, reproductive failure, caused by DNA aberrations, does not seem to occur at the level of fertilization [14] but at the onset of embryonic DNA expression. (Spinaci M, De Ambrogi M, Volpe S, Galeati G, Tamanici C, Seren E. Effect of staining and sorting on boar sperm membrane integrity, mitochondrial activity and in vitro blastocyst development. Theriogenology 2005;64(1):191-201.)

Sperm chromatin damage was quantified by percentages of spermatozoa with detectable DNA Fragmentation Index – DFI divided into moderate (m-DFI) and high (h-DFI) DFI. Percentage of immature cells (HDS; cells with High DNA Stainability) was also evaluated. (Rybar et al., 2004)

The threshold level for SCSA parameters DFI%

- Results from a recently published meta-analysis indicate that human couples with no known infertility problems were 7.0 times (confidence interval [CI] 3.17, 17.7) more likely to achieve a natural pregnancy/delivery if the DFI was <30% (n =362, P= .0001) (Evenson and Wixon, 2006). (USA)
- Results from a 18 boars study suggests that a >6% DFI places certain commercial boars into a statistical group that produces a reduced FR and ANB. (Ddion et al., 2009).(USA)
- Boe-Hansen et al (2008) reported on a study of ejaculates from 145 boars used in 3276 experimental inseminations in Danish breeding herds. The total number of piglets born (litter size) for Hampshire, Landrace, and Danish Large White boars was, respectively, 0.5, 0.7, and 0.9 piglets smaller per litter when the SCSA-defined %DFI values were above 2.1% as opposed to below this value. (Denmark)
- Increased percentages of spermatozoa with abnormal chromatin were found in bulls with lower fertility (Bochenek et al., 2001). (Poland)
- Boars had significantly higher percentages of spermatozoa with h-DFI and HDS (P < 0.0001) in comparison to bulls. (Rybar et al., 2004) (Czech Republic)
- Six hundred ninety two (692) ejaculates from 79 Piétrain boars in an AI center were analyzed for motility, morphology and DFI over a period of 24 weeks. 95.5% of the semen samples had a DFI 5% with low distribution of variation for DFI due to boar and ejaculate (5%). 61.3% of ejaculates with DFI 5% showed values below thresholds for sperm motility or morphology. Waberski et al.,2011 (Germany)
- The threshold for considering human sperm quality low or unsuitable for assisted reproduction is 30% of DFI using SCSA. On the basis of the threshold established in humans, Rybar et al. [19], proposed that 15% in boar sperm could be considered high.

流式細胞儀對染色體DNA染色後精子的分析



影片播放

20

SCSA Method for Boar sperm DFI Assay in LRI

Status	A	B	C	D	E
Flow Cyto SCSA (Dot Plot)					
Result					
DFI%	0-1%	>1-5%	>5-10%	10-20%	>20%
Quality	Very Good	Good	Acceptable	Not Good	Bad

Fig. Examples of data generated by flow cyto with boar semen.

DFI: Sperm DNA Fragmentation Index

21

Frequency distribution of DFI in boars/2013

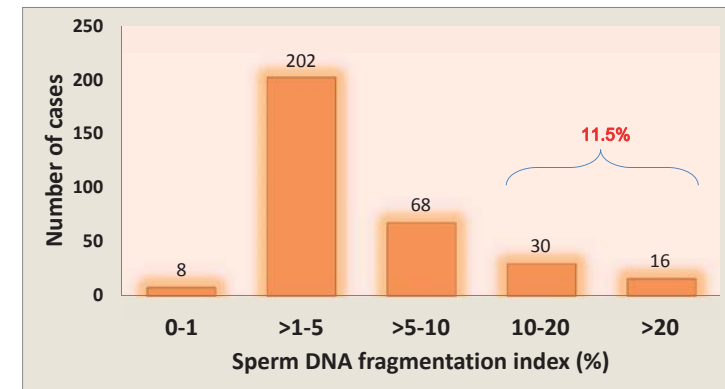


Fig. Frequency distribution of data of sperm DNA fragmentation index in a sample of 398 boar ejaculates in 2013

Simon Kuo, 2013
Unpublished Data

A-52

Frequency distribution of DFI in boars/2014

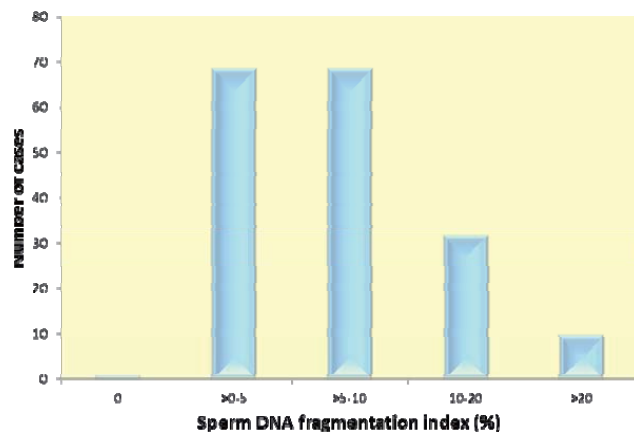
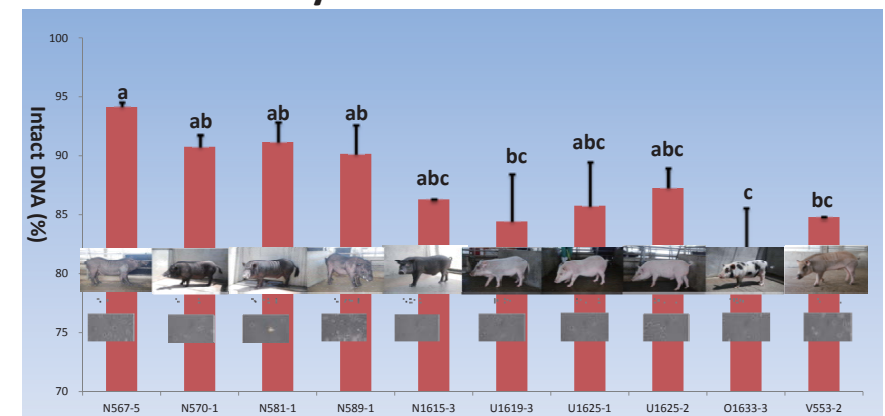


Fig. Frequency distribution of data of sperm DNA fragmentation index in a sample of 180 boar ejaculates in 2014

Native miniature pig sperm DNA breakage was screened by FC

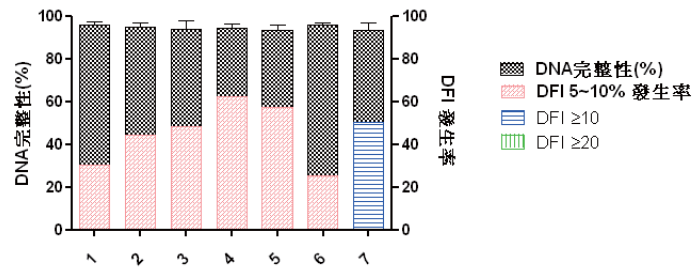


a, b, c values with different subscripts were significantly different ($P < 0.05$)

Simon Kuo, 2014

Investigation of sperm DNA breakage in various ages of breeding pigs

年齡	< 1歲 (N=13)	1 - 2歲 (N=39)	2 - 3歲 (N=33)	3 - 4歲 (N=26)	4 - 5歲 (N=7)	5 - 6歲 (N=4)	6 - 7歲 (N=2)	All ages (N=124)
染色體完整率 (Mean±SD)	95.7±1.4	94.5±2.1	93.8±4.1	94.3±2.2	93.4±2.1	95.8±0.8	93.3±3.6	94.4±2.8
最大值~最小值	(97.7~93)	(97.5~88.3)	(97.9~74.7)	(98.2~90.2)	(96.3~90.1)	(96.7~94.6)	(96.9~89.7)	(98.2~74.7)
DFI 5%~10% (隻)	4 (30%)	17 (44%)	16 (48%)	16 (62%)	4 (57%)	1 (25%)	0	58(46.7%)
DFI 10%~20% (隻)	0	1(2.6%)	1(3%)	0	0	0	1(50%)	3(2.4%)
DFI>20% (隻)	0	0	1(3%)	0	0	0	0	1(0.8%)

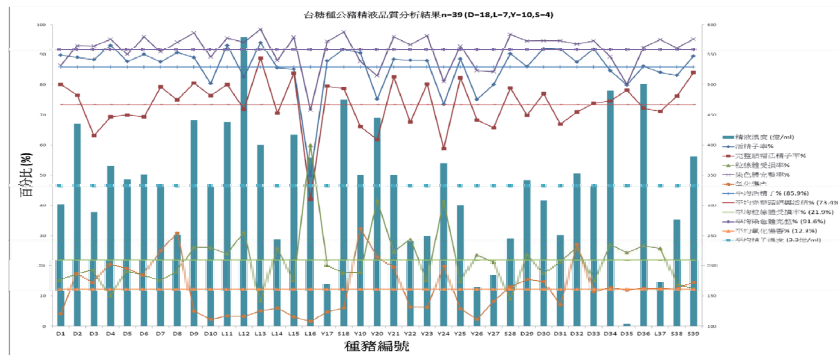


correlation coefficients (r) between new sperm parameters quality traits and fertility parameters

	AVE. Fertility rate	AVE. farrowing rate	AVE. No. Piglets born
Intact sperm membrane (%)	0.518*** (SD=0.152)	0.531* (SD=0.075)	-0.282 (SD=0.328)
Un-intact sperm mitochondria(%)	-0.715** (SD=0.003)	-0.592*** (SD=0.019)	0.025 (SD=0.934)
Intact sperm DNA(%)	0.637* (SD=0.019)	0.514 (SD=0.127)	0.699** (SD=0.0054)

A-53

How my boar is preparing for 2015



Conclusion

- In a sample of 398 random boar ejaculates in 2013, where 70-88% of them are of acceptable quality to use in AI, an incidence of 11.5% of ejaculates with a DFI higher than 20% has been observed.
- The SCSA technique appears to be able to identify individuals with higher DNA damage, and could in the future be implemented by the pig industry.
- 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.

Mr. Mai Van Sanh
Mr. Pham Van Tiem
Mr. Luong Anh Dung
Mr. Pham Vu Tuan
Mr. Phan Van Hai



Seminar on Boar Semen Application for Pork Quality Improvement Hanoi, Vietnam

Thank you !

