The 6th Fatty Pig International Conference

Identification of nucleus boar semen quality to ensure the sustainable conservation of Taiwan native Lanyu minipigs



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Aim

 The purpose of this study is to ensure optimal fertility of nucleus boars and major advances in the selection of young boar to guarantee their semen fertility before get into nucleus herd.

 To screen and estimate genetic parameters and genetic trend for new sperm parameters such as sperm chromosomal breakage and oxidative damage of on-farm breeding boar.

outline



- **1. Introduction**
- 2. Boar Semen Quality Evaluation
- **3. Nucleus Boar**
- 4. Result of Semen Quality Assay
- 5. Conclusions



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 5,100	
1,800	85%	3	510	10		
Mating times/ sow/year			Litters/ year	piglets / litter	piglets obtained / sow/ year	
2			2	12	24	



Male genetic contribution to normal offspring

Field fertility of a boar



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

Traditional heritability estimates for a number of traits in swine

- T			
	性狀 (Trait)	遺傳率 (h²)	
	精液量 (Semen volume)	0.58]
	精液濃度 (Sperm concentration)	0.49	
	精子前進性 (Progressive motion of spermatozoa)	0.37	
	型態不正常 (Abnormal spermatozoa)	0.34	
	總精子數 (Number of total spermatozoa)	0.42	
	人工授精劑數(Number of insemination doses)	0.40	
	出生活仔數 (Number of piglets born alive)	0.08	
	總出生胎仔數 (Total number of piglets born)	0.05	
	懷孕率 (Conception rate)	0.29]

(Smital *et al.,* 2005)

Oxidative Stress

A major reason for reduced sperm survival seems to be the presence of reactive oxygen species (ROS). Oxygen radicals are known to cause a decrease in motility and induce precapacitation as well as damage to the membrane system by lipid oxidation, especially when the seminal plasma content is reduced after excessive dilution and washing of spermatozoa.



Figure. Association of increasing reactive oxygen species (ROS) production with infertility. Evenson and Wixon (2006)

Aitken et al.,2015

Sperm DNA Damage



Figure. Mechanistic pathway showing sperm DNA damage due to oxidative stress.

Compensable and noncompensable semen quality trait

Semen Used for Al						
Trait	compensable semen quality trait	Noncompensable semen quality trait				
	sperm numbers	sperm DNA fragmentation				
Ex.	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.				

- 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 but at the onset of embryonic DNA expression. (Spinaci M,2005)

Evaluation method used for relating boar semen quality





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/駕乘意願W
- □ Dummy/駕上假母台(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 精子湧動力分析

- 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)



hypoosmotic 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





New Sperm parameters (NSP)

New semen quality assay other than conventional assay



Fig. sperm structural and functional integrity related parameters

Simon Kuo,2012

 Detection and application of new sperm parameters in breeding farm animals by flow cytometer in TLRI



One software for data analysis and interpretation

The SCSA test: concept of sperm DNA fragmentation (sDF) tests NSP: Sperm Chromatin Structure Assay



- 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.
- Sperm chromosomal breakage has been classified as Noncompensable semen quality trait (Spinac *et al.,*2004)

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



Fig. Schematic of fluorescence color shifting from normal DNA (green) to fragmented DA (red)



The Nucleus Boar House









The Nucleus Boar (1)



N584-01



N589-01



N1615-3







N581-01

N567-05

N570-01

Fig. Black Lanyu pigs (Lanyu 200)

The Nucleus Boar (2)





U1625-01



U1619-3

U1625-02









Fig. Lanyu 100 (Spotty pig), Lanyu 400 (Binlang pigs) by their hair coat and Lanyu 50 (Mitase pig) a cross mating with commercial pig breed

Ejaculate collection



Result-General Semen Quality Assay (1)

No.	N567-5	N570-1	N581-1	N589-1	N1615-3	U1619-3	U1625-1	U1625-2	01633-3	V553-2
Date	2013.06.24	2013.06.30	2013.08.31	2013.09.14	2014.01.05	2014.01.12	2014.01.27	2014.01.27	2014.02.19	2013.04.19
Semen volumn	90	90	85	75	85	80	40	60	90	125
Motility A	90+++	90+++	90+++	90+++	90++	80++	70+++	70++	80++	80+++
Motility B	82	80	81	82	90	84	89	89	87	88
Semen con.	4.09	2.5	2.97	3.12	2.66	2.77	3.8	2.96	2.43	3.52



Result-General Semen Quality Assay (2)



Fig 4. Semen quality assay. (a)Semen volume (b)Sperm concentration (c) High free calcium level (d)Normal sperm (e) Morphology/Abnormal sperm (head), Abnormal sperm (tail), Droplets (distal), Droplets (proximal)

Result- Flow Cytometry Assay



Fig 5. From left to right: sperm motility, viability, sperm acrosome damage, mitochondrion integrity, sperm DNA integrity, oxidation

Native miniature pig sperm DNA breakage was screened by FC



^{a, b,c} values with different subscripts were significantly different (P < 0.05)

Fig . An incidence of ejaculates with a sperm DNA fragmentation index (DFI), a damaged sperm DNA can lead to early embryonic or fetal death and can have a dramatic impact on health of the offspring, higher than 20% has been observed in Spotty pig and Binlang pig

Simon Kuo, 2014

Sperm DNA fragmentation (sDF) data related to fertility potential



Fig. Scatter Diagram of intact DNA (%) and field fertility

Simon Kuo,2015

Male genetic contribution to normal offspring



Field fertility of a boar



Farrowing Rate (FR) and Total Number of Piglets Born (TNB)

The threshold level for SCSA parameters DFI% of boar

- 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)
- 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)

Conclusions

- Adding sperm DNA fragmentation index as a new parameter to the routine assessment of every ejaculate may be beneficial to the field.
- The sperm DFI values could be an useful reference in extrusion of subfertility breeding animals.
- In conclusion, by further and routinely identification of nucleus boar semen quality to ensure the sustainable conservation of Taiwan native Lanyu minipigs could be expected.

Future and prospects

- Judging sDF and ROS bring us such questions as what is the added value for the animal industry?
- Can we relate sDF and ROS with field fertility and Which opportunities does it give us?
- □ What are the economic perspectives of SCSA and ROS assessment ?
- □ Can the worldwide population use less number of males (bull, boars,...)?
- Flow cytometric semen quality assessment will be the solution and gives possibilities for further screening and culling animals in an AI center and farm to the development of semen quality assessments, which improves the prediction of male fertility.
- Knowing which semen characteristics, and to what extent, contribute to male fertility and makes the field fertility more predictable.
- The deterioration of genetic ability indicated that new sperm parameters should be taken into account for boar selection at young age and may further to improve and enhance the overall fertility of breeding boars

