



Pig Genetic Networking - Philippines & Taiwan

第二屆台菲種豬聯合育種研討會



Sperm Selection/產精選拔

Boar Sperm Assay by Flow Cytometry Technology

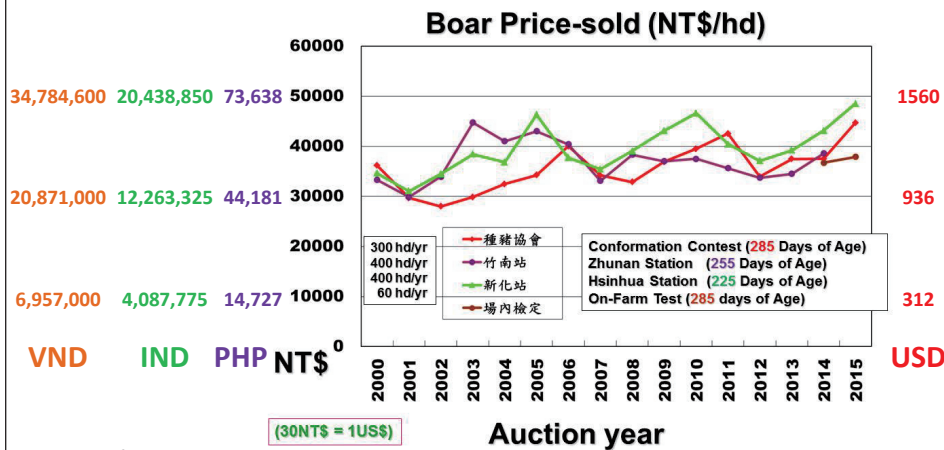


Mr. Kuo Ting-Yung

Division of Breeding and Genetics, TLRI

2017/10/25

Elite breeding swine of Taiwan 台灣公豬拍賣價格趨勢圖



By Simon kuo

2015/12/16 made by Ming-Che Wu

Outline

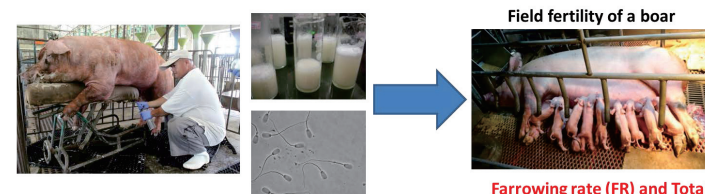
- ✓ ELITE BREEDING SWINE OF TAIWAN
- ✓ INTRODUCTION
- ✓ EXAM SPERM QUALITY ?
- ✓ METHODOLOGY OF SEMEN EVALUATION
- ✓ FLOW CYTOMETRY SPERM INTEGRITY ANALYSIS
- ✓ CAN WE RELATE SEMEN/SPERM QUALITY WITH FIELD FERTILITY?
- ✓ PREDICTION OF MALE FERTILITY
- ✓ PREDICTING MALE FERTILITY: WHAT IS THE ADDED VALUE FOR THE PIG INDUSTRY?
- ✓ FUTURE AND PROSPECTIVE

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



Male genetic contribution to normal offspring

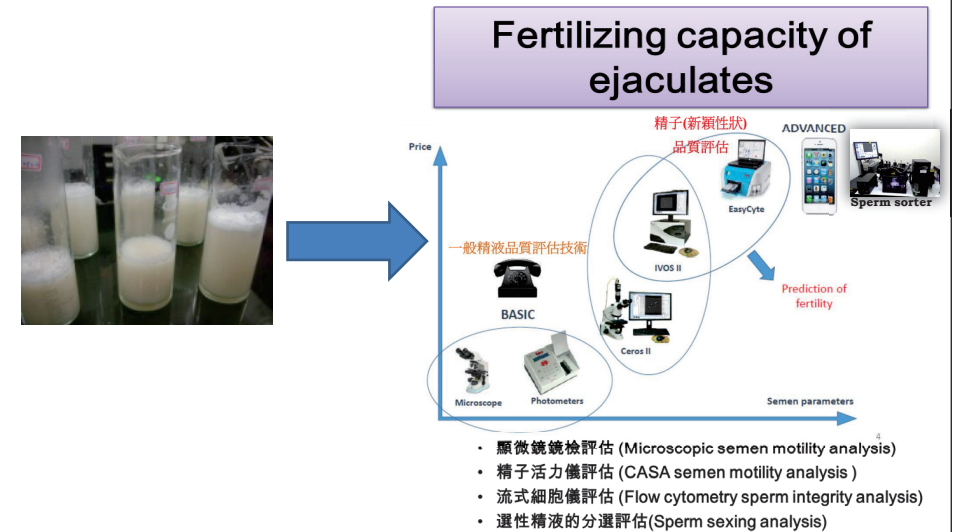
Farrowing rate (FR) and Total number of piglets born (TNB)

Sperm selection/Boar Fertility

- Boar fertility is a multifactorial process, relying on semen quality, female fertility, herd management, and accurate timing when using artificial.
- Boar fertility has received much less consideration.
- Subfertility and infertility boars can lead to significant financial loss.
- Semen quality may vary along the boar career, age, or environment and it must be monitored by regular examination of breeding records and assessment of semen quality.
- Ensuring an optimal quality of semen doses/straws or boar itself is thus a key concern for breeding farm and AI centers.
- To ensure optimal fertility of boar or after AI, major advances have been done for years in the selection of boar and several quality control procedures have been proposed to guarantee their semen fertility.
- Fewer tests relating to sperm factors and fertility have been developed for boar sperm than for other domestic species.
- To explore the relationship between field fertility data obtained for boar sperm intact and functional (new sperm parameter traits) assessments with flow cytometry parameters.

~24~

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/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)

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
- ◆ 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

Easykit 5: Viability & Acrosome integrity
- indicator of sperm membrane and acrosome integrity

Easykit 1: Viability
- indicator of sperm membrane integrity

Easykit 2: Mitochondrial activity
- indicator of mitochondria

Easykit 1: concentration
- total number of sperm cells

Easykit 3: Oxidation molecule D
- detection of free radicals in spermatozoa

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

流式細胞儀評估 (FLOW CYTOMETRY SPERM INTEGRITY ANALYSIS)



New Sperm parameters

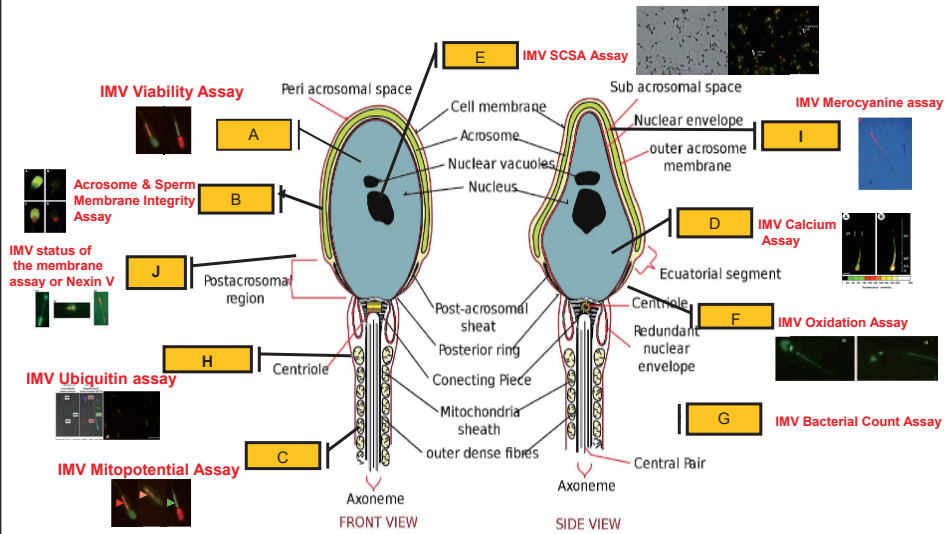
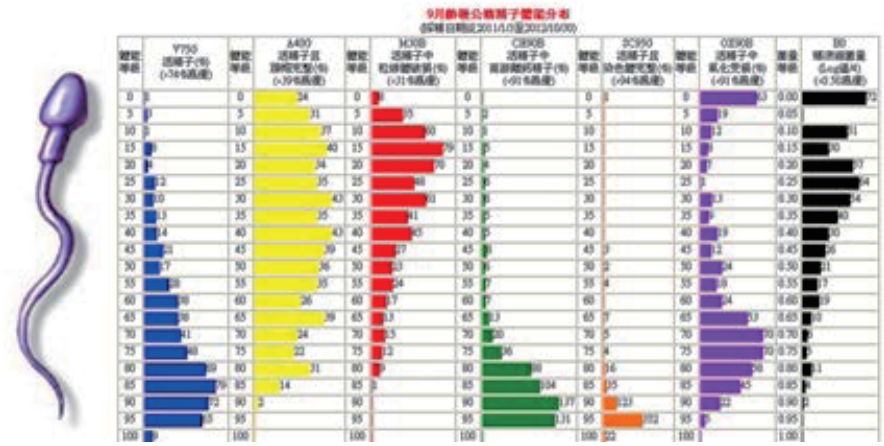


Fig. sperm structure and function related parameters Simon Kuo, 2012

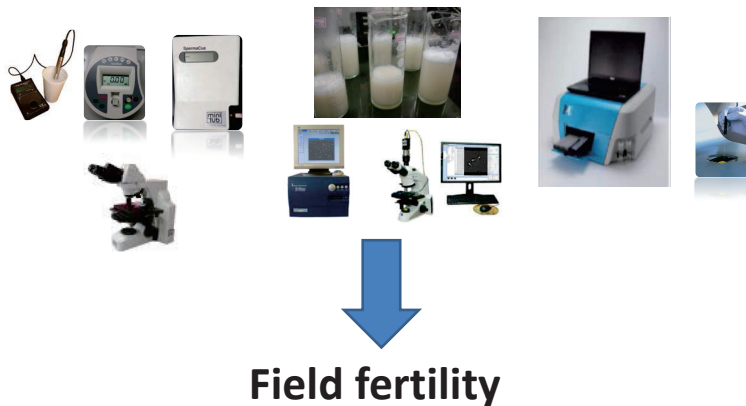
Variability of young boar semen quality parameters among 350 ejaculates, based flow cytometry analyses

2012年種公豬精子體能分布



~25~

Can we relate semen quality with field fertility?
(Which one is the best predictor of semen)



What is the right fertility predictor ?

Microscope

- ✓ Motility %
- ✓ Morphology

CASA

- ✓ Morphology
- ✓ Motility%
- ✓ Progressive%
- ✓ VSL, VCL, VAP
- ✓ ALH, BCF

Flow Cytometry

- ✓ Viability
- ✓ Mitochondrial status
- ✓ Oxidation level
- ✓ Acrosome integrity
- ✓ DNA compaction

WHAT SEMEN QUALITY TRAITS ARE MOST IMPORTANT FOR INSEMINATION/PREGNANCY OUTCOME?
(種公畜禽的何種精液品質參數對配種與分娩成績的好壞扮演重要的角色)

What semen quality traits are most important for insemination/pregnancy outcome?
Estimates fertility prediction value of the conventional, CASA and cytometry methods

ASSAY	Photometer	Microscope	CASA	Flow Cytometry
Concentration	+++	+	+++	+++
Morphology	-	+++	++	+
Motility	-	++	+++	-
Viability	-	+	+++	+++
Acrosome	-	+	+	+++
Mitopotential	-	-	-	+++
Chromosome	-	-	-	+++
Oxydation	-	-	-	+++
Merocyanine	-	-	-	+++
Other Physiological Assays	-	-	-	+++
Fertility predictor	5%	15%	25%	35%

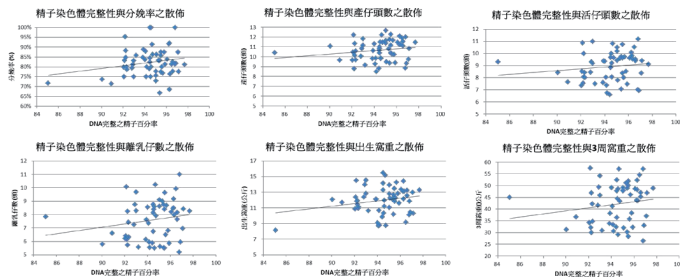
- Microscopic semen motility assessment only minimally relates to field fertility
- CASA is a tool to objectively discriminate between fertilizing capacity of ejaculates
- Flow cytometric semen quality assessment gives possibilities for an AI company and farm

Estimates of heritability between semen and sperm novel traits via bivariate animal model in boar

ASSAY	Photometer	Microscope	CASA	Flow Cytometry
Concentration	0.28/0.49	-	-	-
Morphology	-	0.38/0.34	-	-
Motility	-	-	0.37	-
Viability	-	-	-	0.45/0.33
Acrosome	-	-	-	0.21/0.42
Mitopotential	-	-	-	0.38/0.22
Chromosome	-	-	-	0.41/0.1
Oxydation	-	-	-	0.33/0.4
High Calcium	-	-	-	0.91
Merocyanine	-	-	-	-
Other Physiological Assays	-	-	-	BAC =0.18

Smital et al., 2005
TLRI 2015 on farm test n=180
Chung 2014 young boars n=480

New sperm parameters and the relationship to fertility of bore semen in Taiwan



圖、公豬精子染色體新穎性狀品質參數值與相關生育力性狀之散佈趨勢

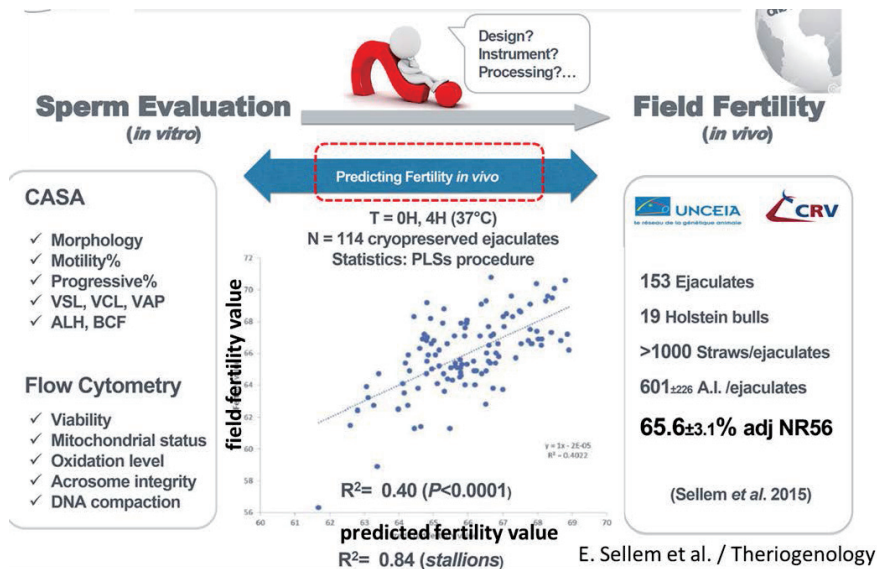
	平均受孕率	平均分娩率	平均產仔頭數
精子細胞膜完整率	0.518*** (SD=0.152)	0.531* (SD=0.075)	-0.282 (SD=0.328)
精子粒線體受損率	-0.715** (SD=0.003)	-0.592*** (SD=0.019)	0.025 (SD=0.934)
精子DNA完整率	0.637* (SD=0.019)	0.514 (SD=0.127)	0.699** (SD=0.0054)

WHAT IS THE RIGHT EQUATION FOR FERTILITY PREDICTION (最佳指標組合以預測種畜生育力是近年重要的研究)

What is the right equation ?

$$\text{Fertility} = \frac{(\text{Motility} + \text{Prog} + \text{VSL} + \text{LIN})}{4} \times \text{Viab} \times \text{Acro} \times \text{Mito} \times \text{SCSA}^2 \times \text{Ubi} \times \dots$$

Use of combinations of in vitro quality assessments to predict fertility of bovine semen



Horse fertility prediction (FDA) 馬的生育力預測(法國)

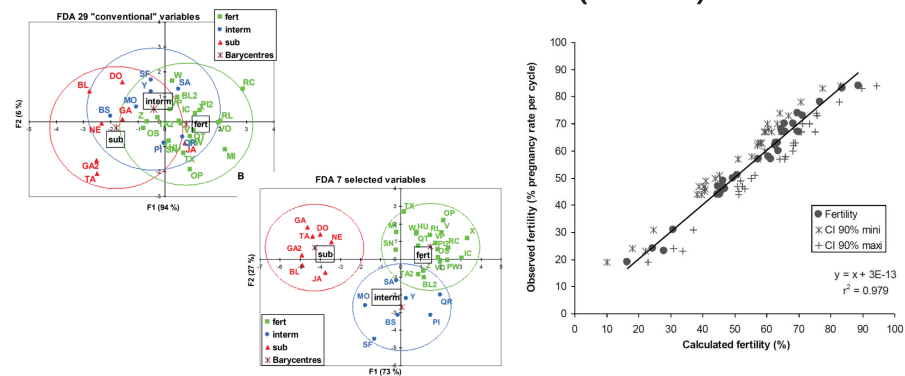


Fig. 2. Results of factorial discriminant analysis (FDA) using: (A) a combination of 29 "conventional" variables related to sperm motility, morphology, and viability assessed by eosin staining; (B) a combination of seven variables: percentage of polarized mitochondria at T24, percentage of alive acrosome-reacted spermatozoa without ionophore treatment at T0, percentage of dead acrosome intact spermatozoa after ionophore treatment without seminal plasma at T24, standard deviation alpha-t at T24, percentage of swollen spermatozoa after HOS test at T0, critical osmolality "tube" at T0 and critical osmolality "tube" at T24. T0: analysis within 2 hours after semen dilution, T24: analysis after 24-hours storage at 4 C. Stallions (n ?34) were divided into three fertility groups: fert: pregnancy rate per cycle (PRC) > 55%, intermediate: 44% < PRC < 55%, subfertile: PRC < 45%. Each stallion is represented by a dot and a coded name. Barycentres and confidence ellipses of the three fertility groups are indicated. Six stallions were misclassified using the combination of "conventional" variables: stallions PI, QR, and SA were classified as fertile, whereas they were intermediate (PRC ?45%, 49%, and 47%, respectively), stallion Z was classified as intermediate, whereas he was fertile (PRC ?62%), and stallion BS was classified as subfertile, whereas he was intermediate (PRC ?46%). HOS, hypoosmotic swelling; PI, propidium iodide.

Barrier Battut et al. / Theriogenology (2016)

Future and prospective The main challenges to correlate in vitro sperm evaluation with field fertility

- Design a meaningful field fertility trial/accuracy of field data recording/設計一個富含意義的場生育力評估試驗/準確的場內資料紀錄
- Ejaculate to ejaculate VARIABILITY for a given sire/每頭公畜個體精液品質的差異性
- Validation of semen assessment INSTRUMENTS and disposables/精液評估所用儀器及耗材的確認
- Standardization of semen assessment THRESHOLDS procedures/精液評估質好壞閾值作業的標準化訂定
- TRAINING of the sperm quality assessing operator/精液品質管人員的訓練
- Control for semen HANDLING in the field/Inseminator training/監控場內精液處理及人工授精的訓練
- Control of semen PROCESSING and storage/監控精液生產程序及其儲存穩定度

Pig Genetic Networking – Taiwan & Philippines

Thank you !

