台菲種豬聯合育種研討會
Empowering Technology for Pig Breeding – Philippines & Taiwan

豬精子染色體缺損篩除
Boar Sperm Chromosome Breakage Screening

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Outline

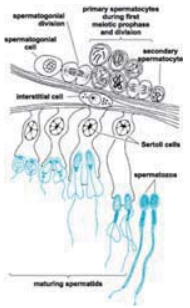
- 前言(Introduction)
- 目的 (AIM)
- 染色體缺損的原因 (Causes of sperm DNA damage)
- 染色體缺損的評估方法學 (Methods for assessment of sperm DNA fragmentation)
- SCSA法在家畜精子品質的應用 (SCSA for livestock sperm)
- 結論 (Conclusion)

前言
Introduction

- High standards of semen quality in boars are of economic revelence due to its association with fertility and offspring result.
- The control of quality in semen used for artificial insemination (AI) must be as precise as possible.
- The status of the DNA structure in the sperm cell, of revelence in human sterility, are not considered important in the boar, in spite of its economic revelence.
- Little is known about sperm DNA fragmentation in boars and even the normal threshold values for sperm DNA fragmentation and its influence on fertility have not yet been well established.

目的
AIM

- To establish sperm DNA fragmentation values which could be considered normal in a random population of breeding boars used for AI purpose.



http://www.fertilitycheck.ie/?q=Sperm-DNA-Fragmentation

染色體缺損的原因
Causes of Sperm DNA Fragmentation

A major causative factor for sperm DNA damage is oxidative stress. Other factors include abnormalities in the regulation of apoptosis, or defects in topoisomerase activity. Increased sperm DNA fragmentation is associated with:

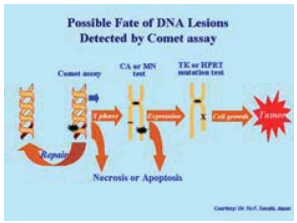
- Infection
- leucocytospermia
- sperm cytoplasmic droplets
- febrile illness
- elevated testicular temperature
- diet
- drug use
- exposure to environmental and occupational pollutants
- advanced age
- varicocoele
- Hormone factors
- Infrequent ejaculation

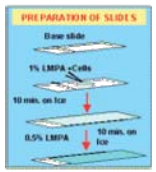
染色體缺損的評估方法學
Methods for assessment of sperm DNA fragmentation

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

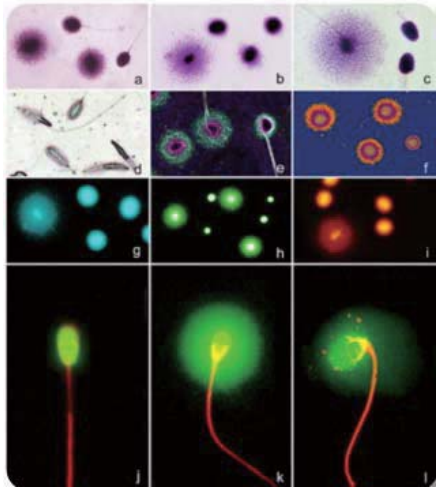
＊ the best experiments to determine what semen quality traits are most important for pregnancy outcome

COMET ASSAY





SCD Technology



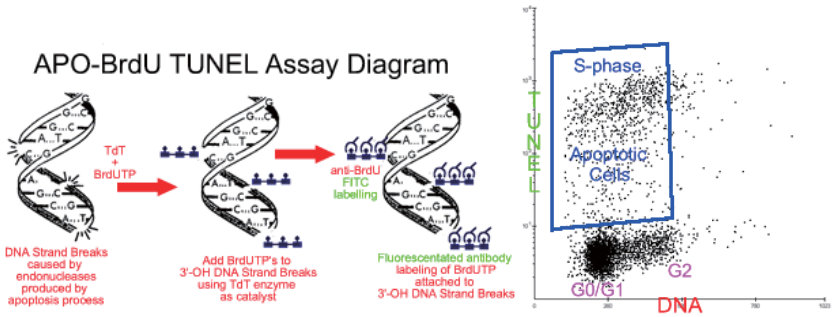
The products developed by Halotech are based on the SCD (Sperm Chromatin Dispersion) technology. The technique produces a differential chromatin decondensation pattern between cells with DNA fragmentation and those with intact DNA.

The differential decondensation protocol is simple and produces results that are analyzed by light or fluorescence microscopy.

The results are very easy to interpret: cells with intact DNA appear with a characteristic chromatin dispersion halo, whereas those with damaged DNA present no halo.

The results obtained using the SCD technique have been validated using the other techniques on the market such as the TUNEL Assay or the SCSA.

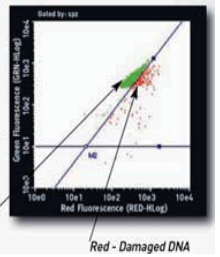
TUNEL Assay



DNA染色質結構完整性檢測
SCSA

5. Sperm Chromatin Structure Assay (SCSA):
Evaluates the degree of compaction of the DNA after an acid attack with Acridine orange
DNA nucleus condensation level assay

Evenson DP, Larson KL, and Jost LK, Sperm chromatin structure assay : its clinical use for detecting sperm DNA fragmentation in male infertility and comparison with other techniques (J. Androl, 2002;vol 23, n°1)
Morrel JM, Johannisson A, Dalin AM, Hamma L, Sandebert T, and Rodriguez-Martinez H. Sperm morphology and chromatin integrity in Swedish warmblood stallions and their relationship to pregnancy rates (acta veterinaria scandinavica, 2008;50:2, doi 10.1186/175-0147-50-2,)
Boe-Hansen GB, Christensen P, Vibjerg D, Nielsen MB, Hedeboe AM. Sperm chromatin structure integrity in liquid stored boar semen and its relationships with field fertility (Theriogenology, 2006;69(6):728-736)

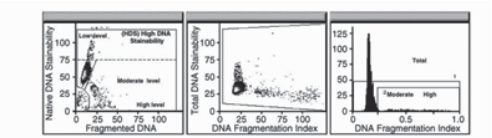


分析目的: 評估DNA被吖叮橙酸Acridine orange (AO)攻擊後其DNA凝集緻密之之狀態(degree of compaction of the DNA)。

分析原理: Acridine orange (AO) 與單股DNA結合產生紅螢光，而雙股DNA結合產生綠螢光。綠色-表示大多為完整的雙股DNA，或DNA較為緊實及緻密。紅色-表示大多為受損的單股DNA，或DNA較不緊實及緻密。

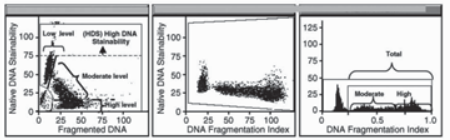
結果報告: 樣品內的精子其DNA染色質結構為不完整者所佔的百分比

Clinical aspects of sperm DNA fragmentation detection and male infertility



Patient	Date	Measurement	mean DFI	SD DFI	DFI (%)	HDS (%)
7272-87	####	1	213.5	111.6	6.8	5.0
		2	221.2	118.1	8.3	5.4
		mean	217.4	114.8	7.5	5.2
		sd	5.4	4.6	1.1	0.2

the outcome resulted in a pregnancy



Patient	Date	Measurement	mean DFI	SD DFI	Total DFI(%)	HDS(%)
7272-113	####	1	563.7	307.0	64.9	6.4
		2	561.4	304.8	64.9	7.2
		mean	562.6	305.9	64.9	6.8
		sd	1.2	1.1	0.0	0.4

the outcome did not result in a pregnancy

Donald P. Evenson et. Al., Theriogenology 65 (2006) 979–991

Thank You