Semen analysis by different technics

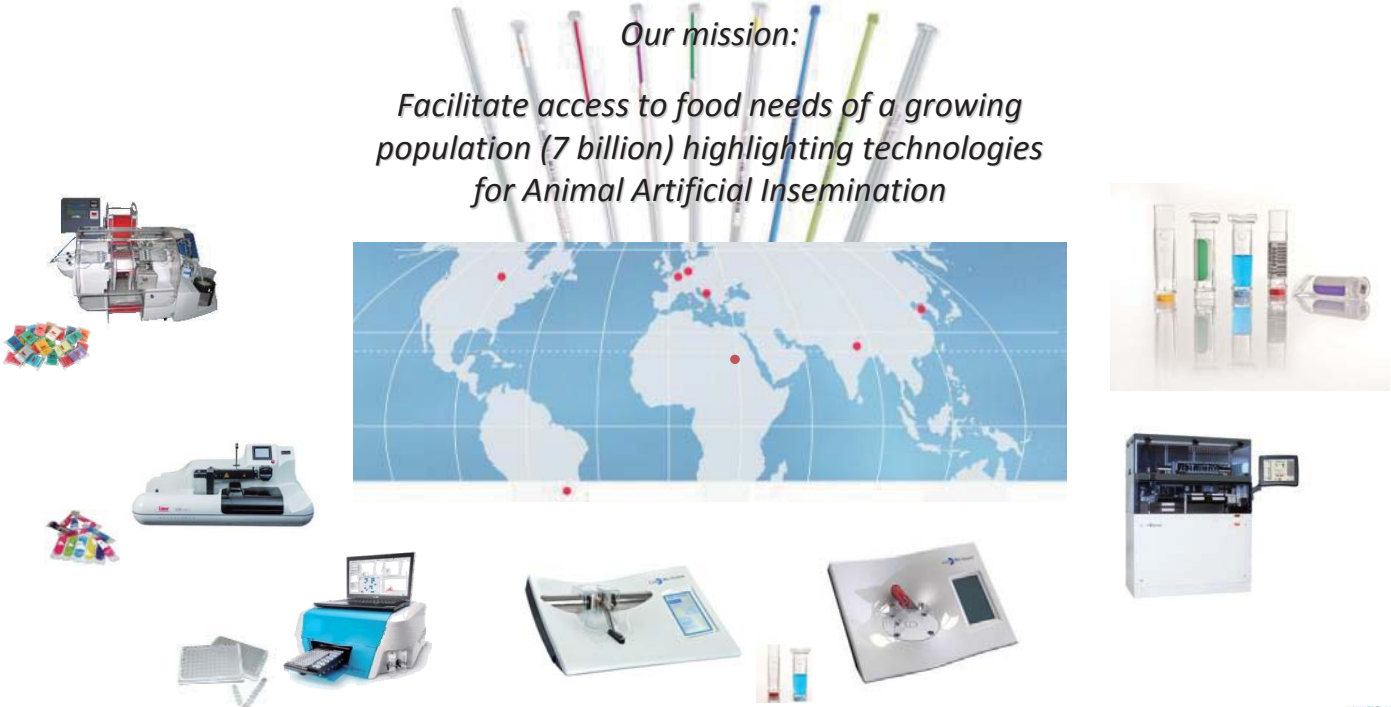
imv TECHNOLOGIES
www.imv-technologies.com

IMV Technologies

***I**nstruments de **M**edicine **V**eterinaire (veterinary medical instrument)*
World leadership in artificial insemination technologies and products, Biobanking solutions

Our mission:

Facilitate access to food needs of a growing population (7 billion) highlighting technologies for Animal Artificial Insemination



WORLDWIDE PRESENCE

275 employees.
IMV subsidiaries: Italy, Netherlands, USA, China, India.
Wide network of 120 agents distributors.

AREAS OF EXPERTISE

- ✓ 49 years of expertise in animal artificial insemination and embryo transfer technologies
- ✓ Plastic extrusion / Biochemistry / Media / Instrumentation
- ✓ Cryopreservation, biological sample freezing
- ✓ Reproductive physiology / Semen analysis / Sperm physiology
- ✓ R/D project development management

- ✓ I am Ludivine Chevrier. I have joined IMV since Januray 2011.
- ✓ Manager of R&D laboratory and project leader for semen analysis by flow cytometry.

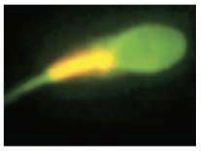
Introduction

Microscope/CASA observation

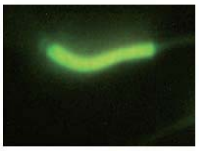
Only external parameters (motility, concentration)

Internal physiological parameters

Flow cytometry analysis



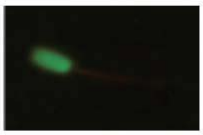
High potential



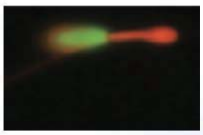
Low potential

Live

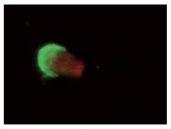
Dead



Normal organization



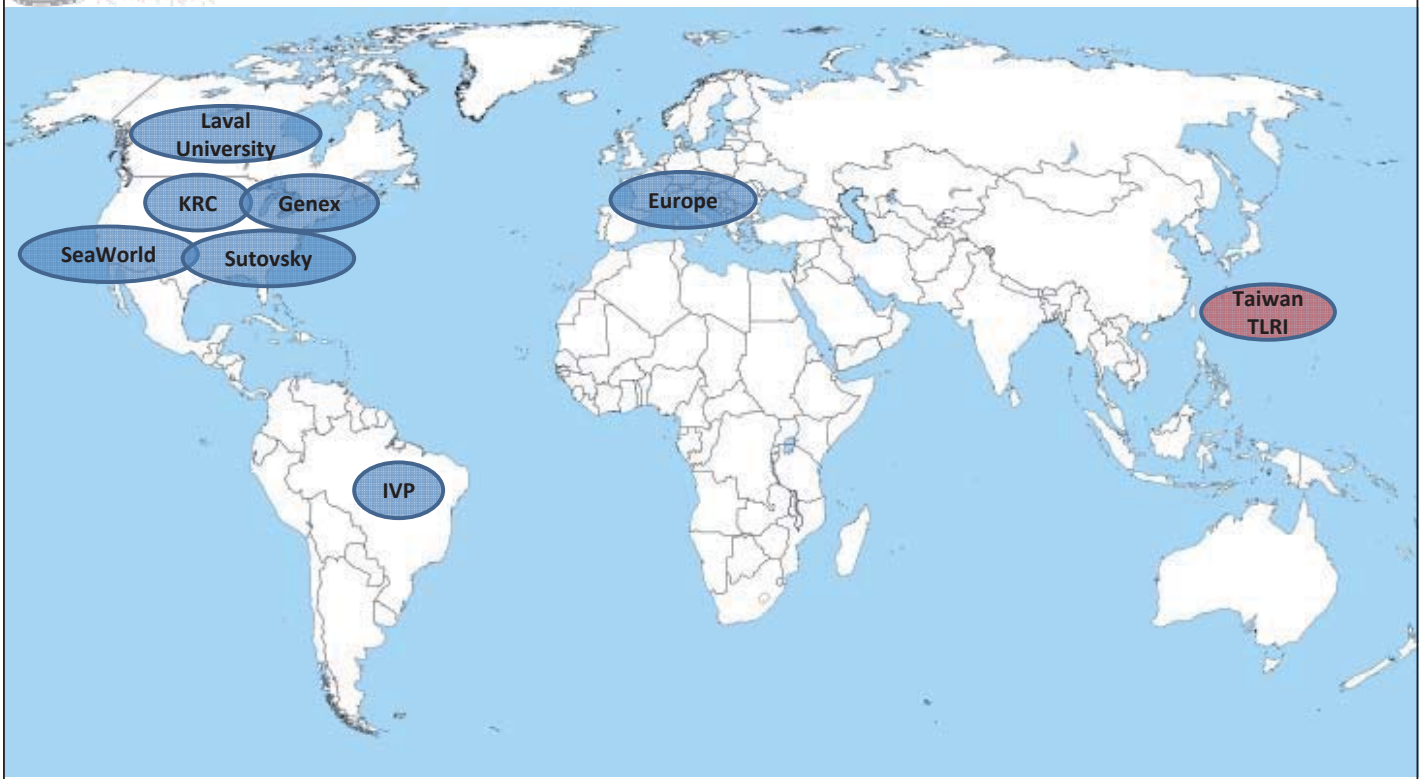
Membrane phospholipid disorder



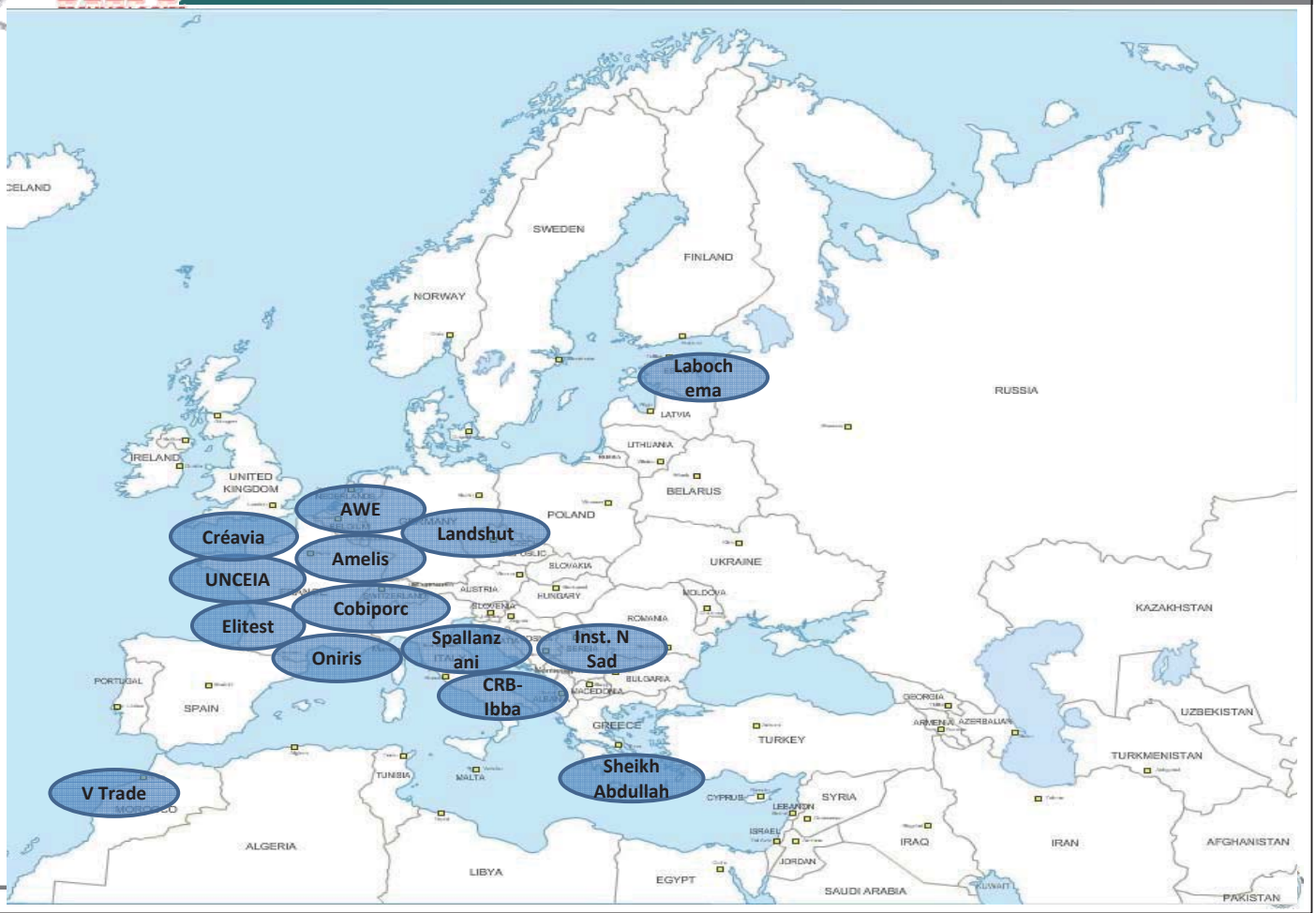
Disrupted acrosome

Helps a lot to take a decision on quality semen

Easycyte Sales Worlwide



Easycyte sales in Europe



Semen Analysis

1. Analysis of Motility, concentration and morphology
    a. Microscopes
    b. Photometers
    c. CASA systems
    d. Utrecht University / Topigs results

2. Analysis of different physiological parameters
    1. Flow cytometer: EasyCyte
    2. Spallanzani Institute results
    3. Rooster results
    4. UNCEIA and IMV results

1. **Analysis of Motility, concentration and morphology**
   - a. **Microscopes**
   - b. Photometers
   - c. CASA systems
   - d. Utrecht University / Topigs results

2. Analysis of different physiological parameters
   1. Flow cytometer: EasyCyte
   2. Spallanzani Institute results
   3. Rooster results
   4. UNCEIA and IMV results

Microscopes

Binocular microscope

UB 203 i
Ref. 023939

Basic; for small centers (swine, equine, bovine)

Nikon E 100 – Led
Ref. 016744

CIA all species
Nikon optical quality

Trinocular microscope

Nikon Eclipse Ci Series
Ref. 024374

Universities, Research centers
Nikon optical quality

→ Very good but limited to objective motility, morphology and concentration

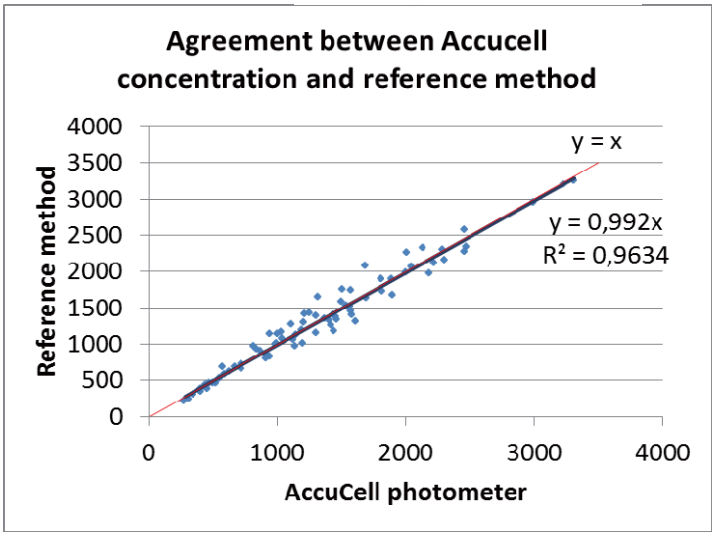
1. **Analysis of Motility, concentration and morphology**
   a. Microscopes
   b. Photometers
   c. CASA systems
   d. Utrecht University / Topigs results

2. Analysis of different physiological parameters
   1. Flow cytometer: EasyCyte
   2. Spallanzani Institute results
   3. Rooster results
   4. UNCEIA and IMV results

ACCUREAD

ACCUCELL

- Accuread and Accucell are
  - Reliable
    CV% between measure on Accuread and Accucell < NucleoCounter, Ultimate and Hemacytometer
  - Accurate
    Good correlation with all other method including reference method
  - Rapid
    Less than 15'' of sample preparation and analyze
  - Cost effective



Customer assay on Zebu semen

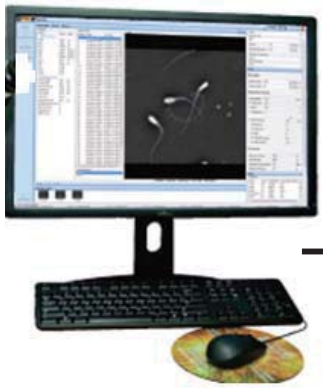
Semen Analysis

1. **Analysis of Motility, concentration and morphology**
   a. Microscopes
   b. Photometers
   c. CASA systems
   d. Utrecht University / Topigs results

2. Analysis of different physiological parameters
   1. Flow cytometer: EasyCyte
   2. Spallanzani Institute results
   3. Rooster results
   4. UNCEIA and IMV results

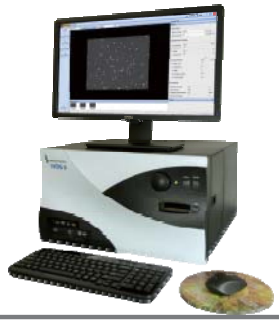
New generation of machine

CEROS II



Leja slide with sample → Analysis with a trinocular microscope → Infomation is sent to a software for analysis → Results

IVOS II

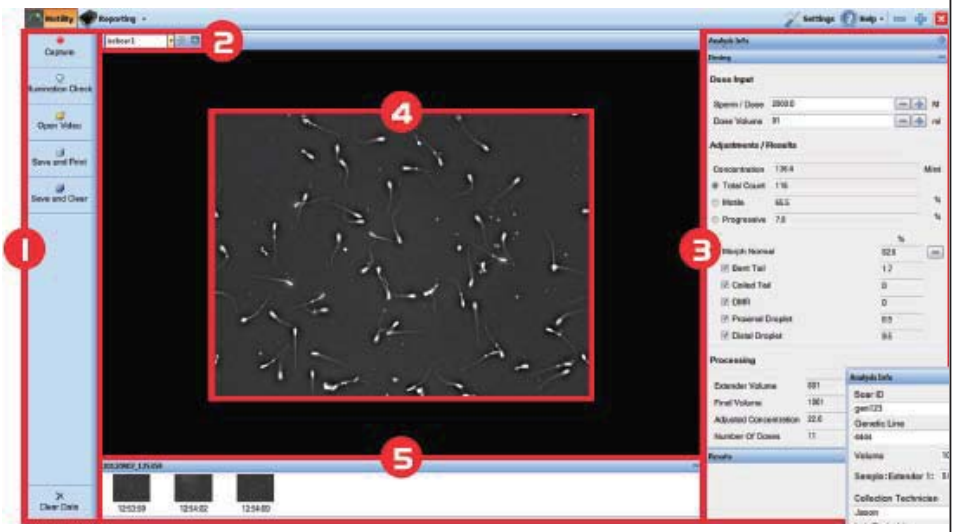


New generation of software

Intuitive software

Available in English, Russian, Chinese, Spanish (more coming soon)

1. Control for initiating analysis
2. Quick selection of analysis setup
3. Results
4. Image
5. Thumbnail gallery of captured video images

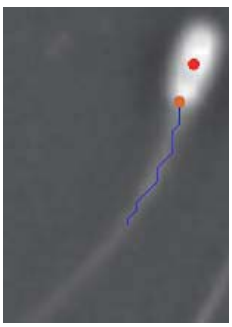


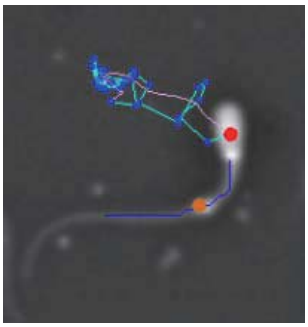
New analyzed parameters

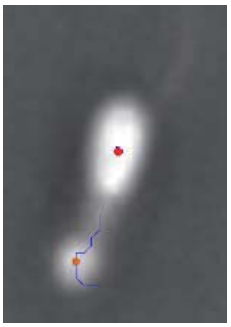
Automated morphologic abnormalities analysis

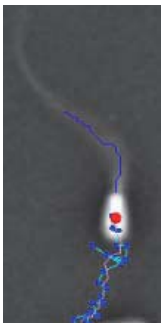
On life or dead semen

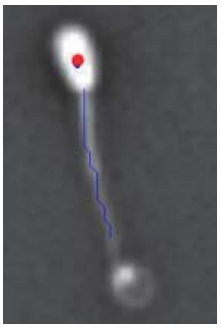
Species: swine, equine, bovine (clear media)


Proximal droplet


Distal droplet

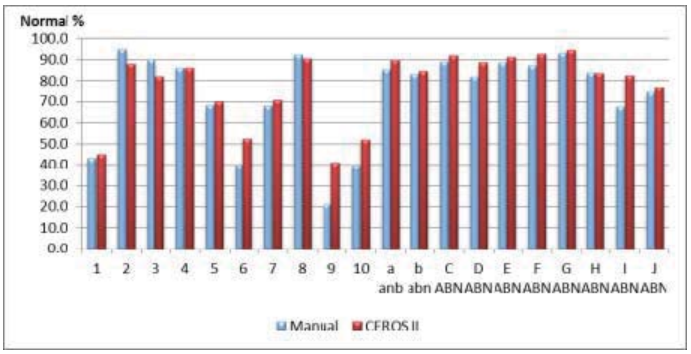

Distal Midpiece reflex

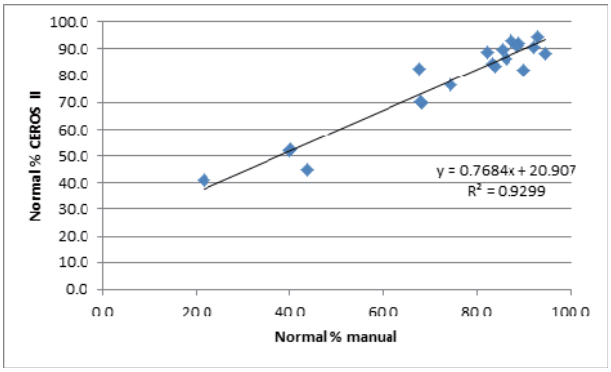

Bent tail


Coiled tail

New analyzed parameters

Proven results on automated morphological abnormalities detection





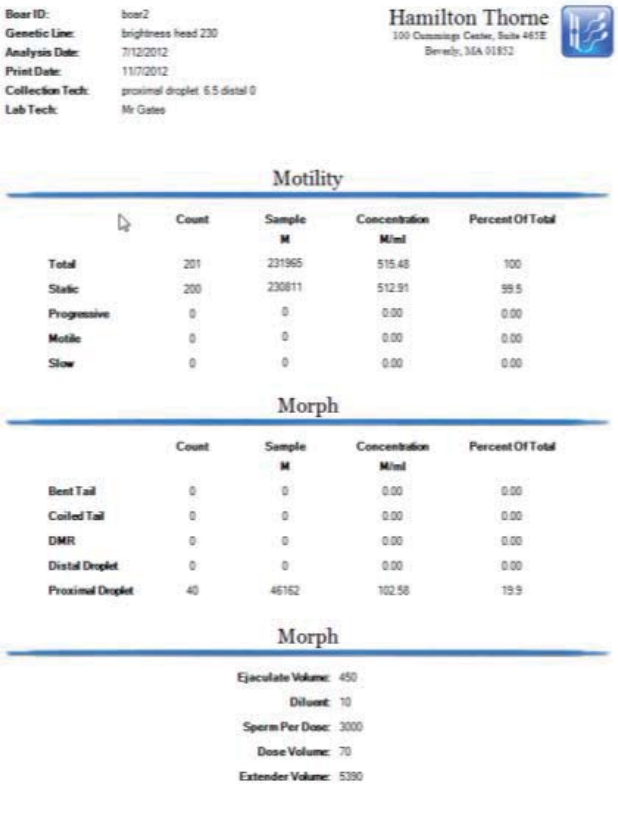
	P Normal %	Average difference %
CEROS 2 / Manual	0.319	9.8%

Good correlation between automated detection and manual couting.

User-friendly data analysis

Report viewer and designer

✓ Creation of entirely new form



Boar ID: boar2
Genetic Line: brightness head 230
Analysis Date: 7/12/2012
Print Date: 11/7/2012
Collection Tech: proximal droplet 6.5 distal 0
Lab Tech: Mr Gates

Hamilton Thorne
100 Cummings Center, Suite 465E
Beverly, MA 01852

Motility

	Count	Sample M	Concentration M/ml	Percent Of Total
Total	201	231965	515.48	100
Static	200	230811	512.91	99.5
Progressive	0	0	0.00	0.00
Motile	0	0	0.00	0.00
Slow	0	0	0.00	0.00

Morph

	Count	Sample M	Concentration M/ml	Percent Of Total
Bent Tail	0	0	0.00	0.00
Coiled Tail	0	0	0.00	0.00
DMR	0	0	0.00	0.00
Distal Droplet	0	0	0.00	0.00
Proximal Droplet	40	46162	102.58	19.9

Morph

Ejaculate Volume: 450
Diluent: 10
Sperm Per Dose: 3000
Dose Volume: 70
Extender Volume: 5390

1

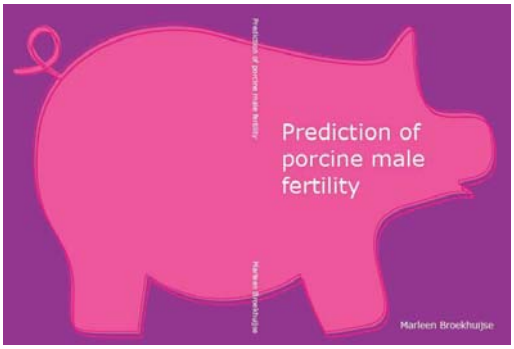
Semen Analysis

1. **Analysis of Motility, concentration and morphology**
   a. Microscopes
   b. Photometers
   c. CASA systems
   d. Utrecht University / Topigs results

2. Analysis of different physiological parameters
   1. Flow cytometer: EasyCyte
   2. Spallanzani Institute results
   3. Rooster results
   4. UNCEIA and IMV results

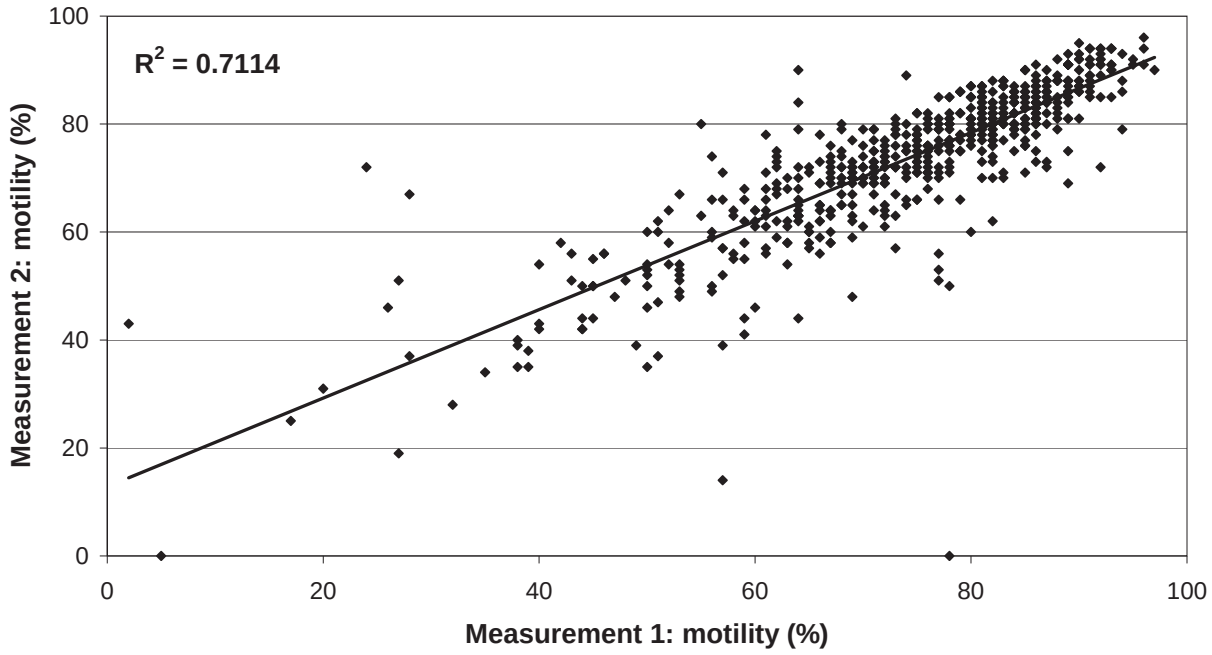
Utrecht University results

- Work done by Marleen Broekhuijse
- PhD: Prediction of porcine male fertility, 2012
  Utrecht University in cooperation with Pig AI Netherlands
- Current job: combining pigs and cattle
  - TOPIGS Research Center IPG
  - CRV



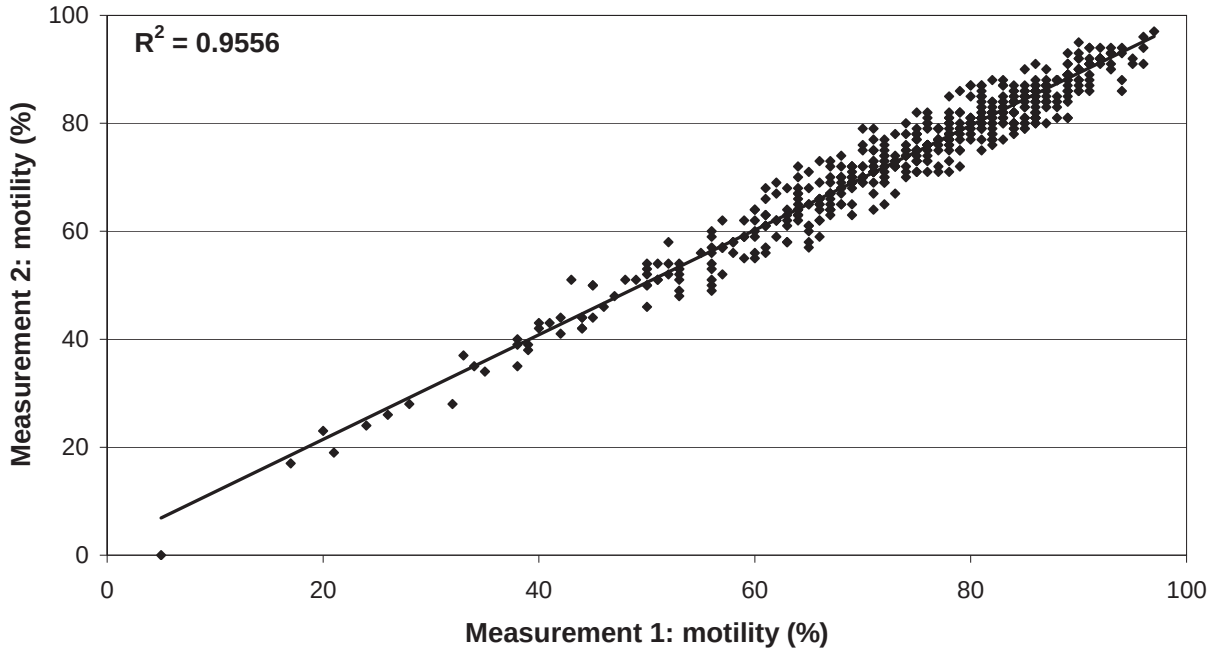
Repeatability of CASA system

Repeatability before training



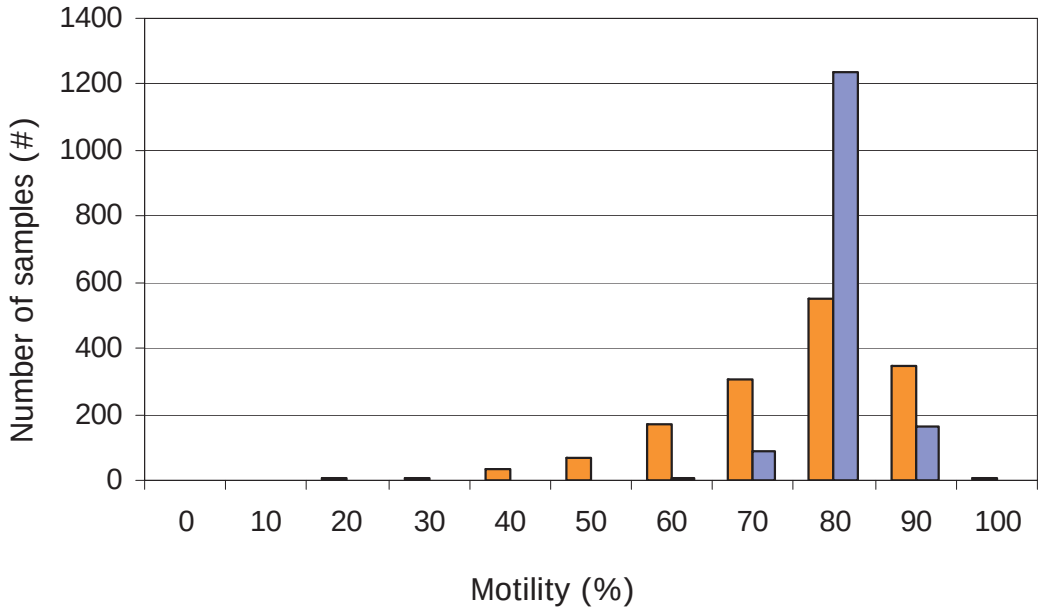
Repeatability of CASA system

Repeatability current situation



Microscope vs. CASA

1,500 ejaculates, microscope vs. CASA



Cost - benefit analysis

Potential benefits

↓ Cells / dose

↑ Doses produced per ejac.

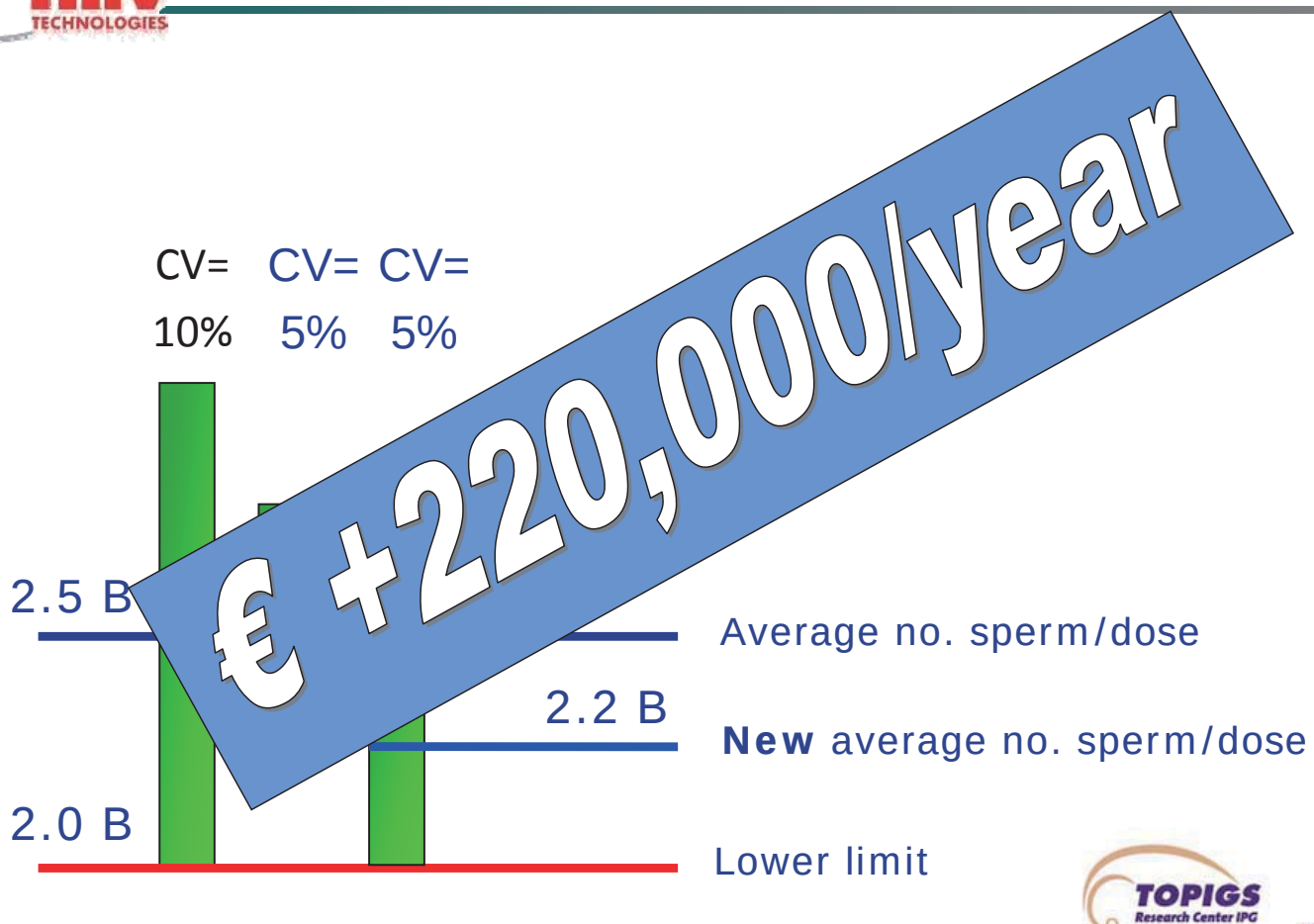
↓ Costs per year

↓ Collection costs per year

Some CRV data….

- Production doses/year: 3.5 million
- No. boars present: 1,560
- No. labs: 7
- No. systems required: 7 ph / 14 CASA
- CV photometer: 10%
- CV CASA (assumed) 5%
- No. sperm per dose: 2.5 billion
- No. doses per ejaculate: ± 32

Effect of CV



Conclusions

Why to use a photometer ?

- First step in semen analysis (concentration)
- Cost effective
- Rapid solution
- Reliable

Why to use a CASA system ?

- Standardization (if several lab technicians)
- More parameters analyzed AND automatically
- Data storage : traceability and help for decision
- Easier for training

Semen Analysis

1. Analysis of Motility, concentration and morphology
   a. Microscopes
   b. Photometers
   c. CASA systems
   d. Utrecht University / Topigs results

2. **Analysis of different physiological parameters**
   a. **Flow cytometer: EasyCyte**
   b. Spallanzani Institute results
   c. Rooster results
   d. UNCEIA and IMV results

Why to use a flow cytometer?

		
Light microscopy	**SpectroPhotometry**	**CASA**
Makes Invisible visible Basic, cheap	Estimates concentration (number of spermatozoa per ml) Quick, cheap	Objective interpretation of images/external aspect of spermatozoa Objective measures

- Very good but limited

- Optical methods = limited to motility, morphology and concentration → crucial physiological parameters for fertilisation are not encountered

Definition

CYTOMETRY

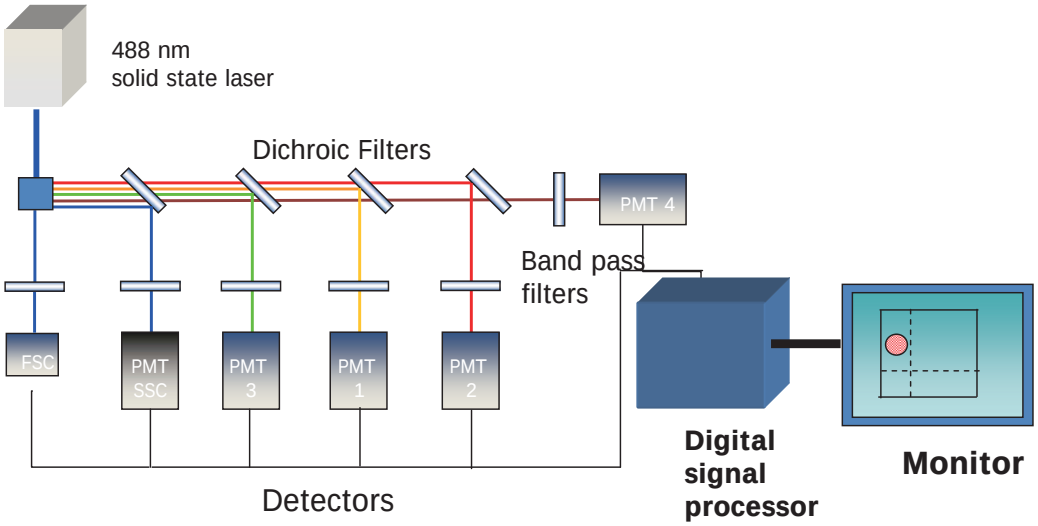
Cell Measurement

- Flow cytometry:

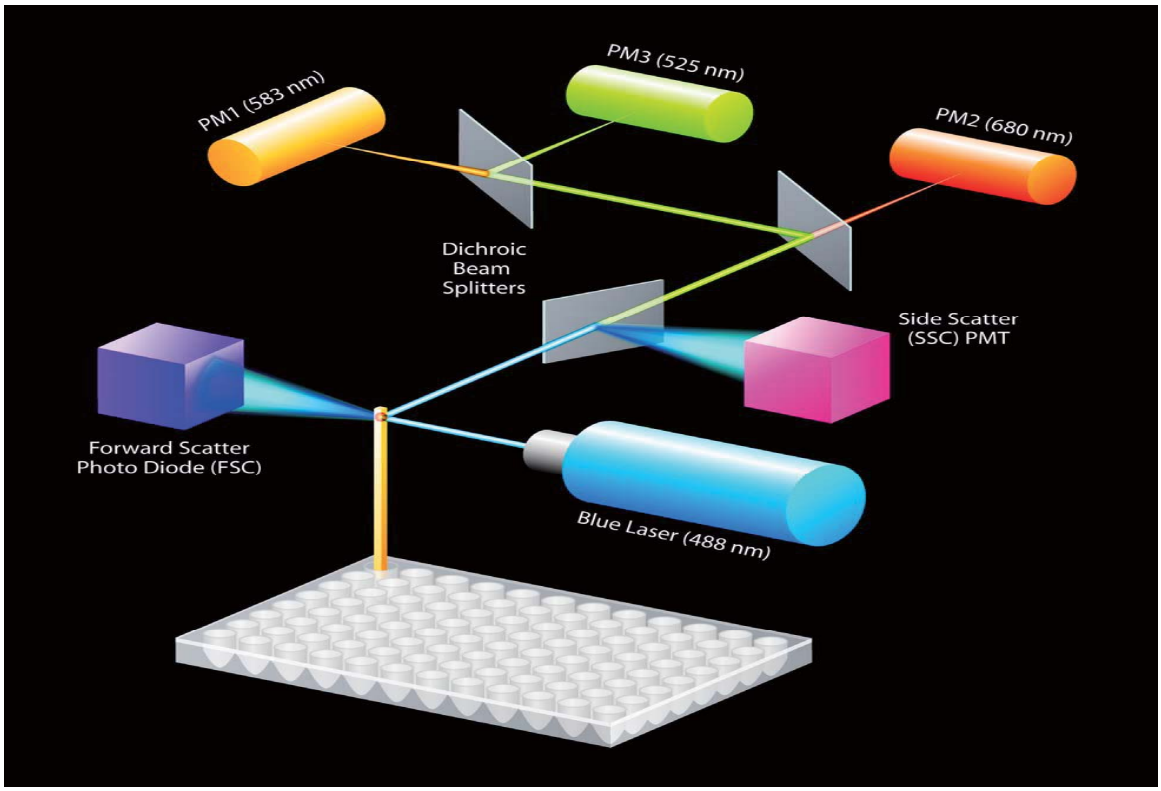
Flow cytometry is a powerful technique for the analysis of multiple parameters of individual cells within heterogeneous populations.

A typical flow cytometer layout

- A flow cytometer is made up of five main systems
- **Fluidic system**: presents samples in front of the laser and takes away the waste
- **The laser**: light source for scatter and fluorescence
- **Filter**: to route specified wavelengths of light to detectors
- **Detectors:** photodiodes and photomultiplier tubes (PMT) to receive the light
- **Electronics and peripheral computer system**: convert the signals from the detectors into digital data and perform the necessary analyses.



A typical flow cytometer layout



EasyCyte

IMV proposes a complete solution for semen analysis by flow cytometry :

- A range of flow cytometers
- Validated protocols for bull and boar semen
- Adapted softwares to analyze and store the data
- Technical support
- Ready to use kit
- Washing solution especially developed for semen

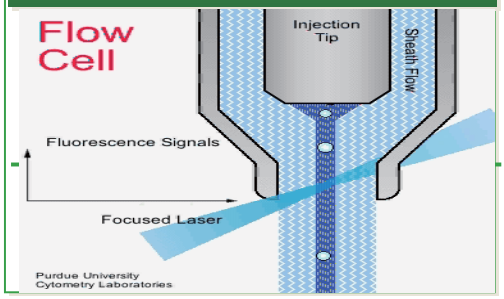
IMV flow cytometer Easy Cyte

- One machine (the flow cytometer)
- One computer (laptop with the machine)
- One software for data analysis and interpretation
- Installation, training, After sale service, technical support

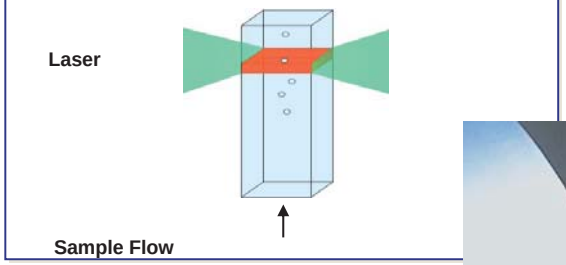
→ Easycyte 5HT: semen analyser, compact and user friendly

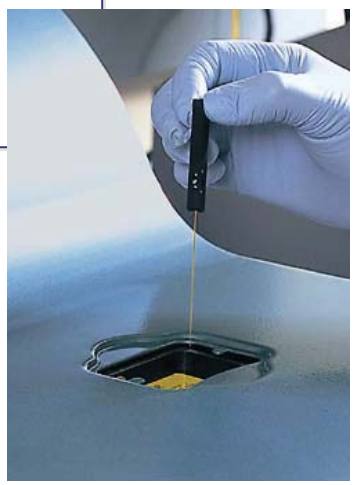
Fluidic system / capillary

Conventional system of "Sheath Fluid"



System patented by GUAVA





Advantages:

1. **Absolut count**
2. **Less sample**
3. **Less reagents**
4. **Less disposable liquid**
5. **Less training sessions**
6. **Less maintenance**

Why use a flow cytometer?

ASSAY	Microscope	CASA	Easy Cyte ®
Motility	++	+++	-
Concentration	+	+++	+++
Viability	+	+++	+++
Acrosome	+	+	+++
merocyanine	-	-	+++
oxydation	-	-	+++
mitopotential	-	-	+++
Other physiological tests	-	-	+++

→ new parameters for higher prediction of semen fertility

A range of flow cytometers



	easyCyte 8HT	easyCyte 6HT/2L	easyCyte 5HT
Cat #	0500-4008	0500-4007	0500-4005
Laser	Blue/Red	Blue/Red	Blue
Forward Scatter	X	X	X
Side Scatter	X	X	X
Green	X	X	X
Yellow	X	X	X
Red1	X	X	X
NIR1	X		
Red2	X	X	
NIR2	X		
96-well plate	X	X	X

Different machines to answer to different customers needs:

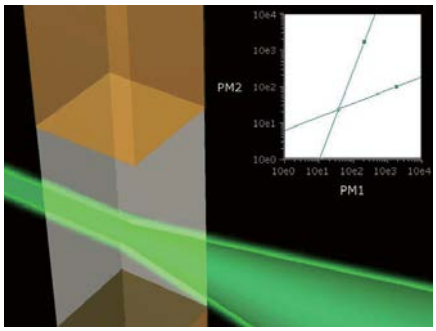
- Easycyte 5HT more for production centers, AI centers for quality control
- Easycyte 8HT et 6HT2L more for research laboratories

Easy Cyte: How does it work?

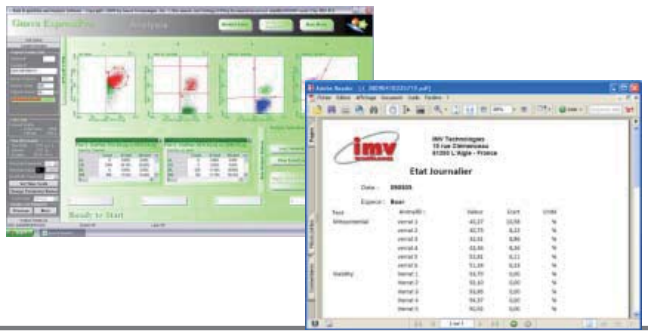
1/ PREPARE (validated protocols)

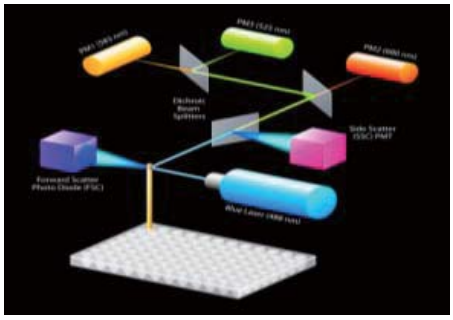
1. *Incubate the semen sample with the reagent*
2. *The reagents are going to attach the cell structure (membrane, nucleus) or react wih the cell enzymes*
3. *Introduce the 96 wells plate or tube in the flow cytometer*
4. *PMT (photomultiplicators) will measure the fluoresent light emitted by these reagents*

2/ ACQUISITION (pre-arranged settings)



3/ READ (cytosoft) & STORE RESULTS (easysoft)





Validation plan for an easycyte assay

Each protocol develop by IMV is validated according to this validation plan:

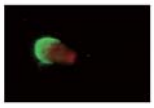
- Each assay should be validated on at least two species: boar and bull
- For each assay a positive control need to be done
- Define the optimum concentration of fluorochrome for a given number of spermatozoa.
- Compare the results from easycyte with epifluorescence microscope.
- Validate the test on a range : bad/good samples
- Test the repeatability between wells
- Assess the stability of the signal in the time (read the plate each 10minutes).
- Test the reproducibility of the assay

Material

Tests validated by IMV

Viability & Acrosome integrity assay

•% of dead sperm (PI red): membrane status
•% of reacted acrosome (PNA-FITC Green) : acrosome status

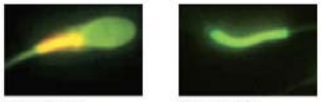


Viability

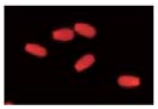
•Indicator of sperm membrane integrity
•Difference between live sperm (green)SYBR14/dead (red)PI

Mitochondrial activity assay

•Polarized Mitochondria (JC1 orange); depolarized Mitochondria (JC 1 green)

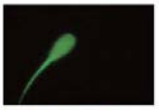


Sperm count total number of sperm cells



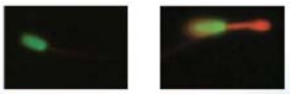
Oxidation level test

•Detection of free radicals in spermatozoa
•H2DCFDA (green)



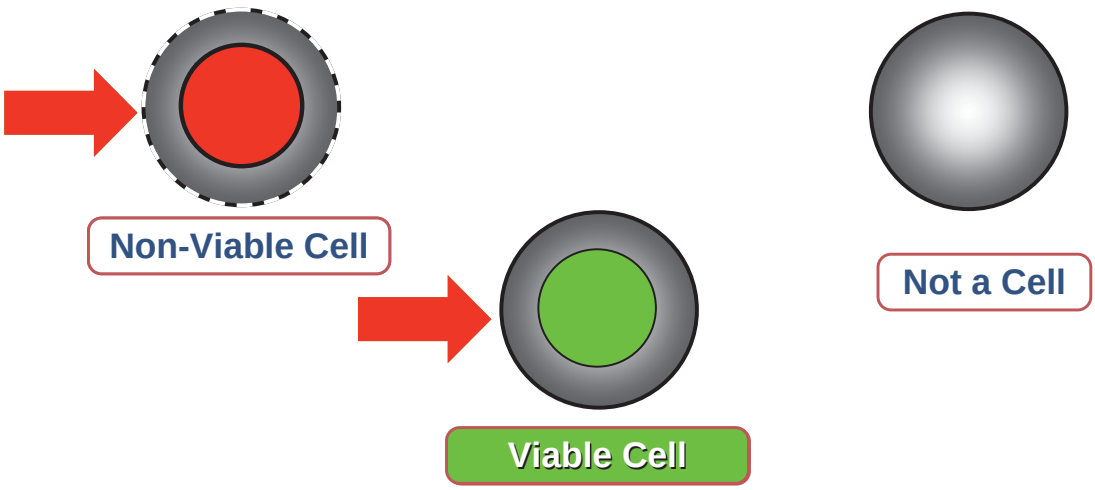
Membrane Fluidity test

. Phospholipid disorders (Merocyanine red)



IMV flow cytometer : easyCyte

➢ AIM: This test indicates the % of viable spermatozoa: cells with intact plasma membrane over the sperm head.
Intact plasma membrane is crucial for every cellular process, since spermatozoa lack the capacity of membrane damage repair.
➢ Protocol:
-X µL PI +Y µL SYbr14 + 0,5µL bull semen + 196 µL easybuffer
-Incubate 10 minutes at 37°C
-Acquire 5000 events

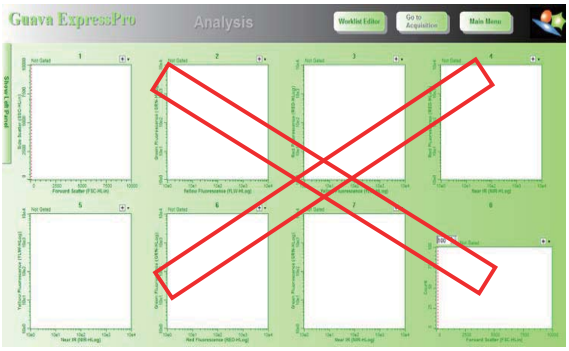


Adapted softwares to acquire and analyze

- Cytosoft: to acquire and analyze the samples.

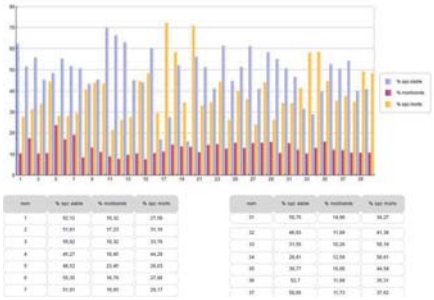
IMV's software: pre-arranged settings

conventional's software: no pre-arranged settings

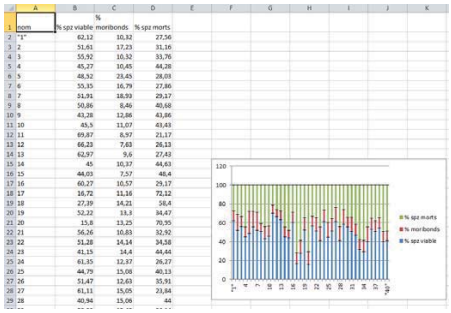


- Easysoft: to store the data and make reports

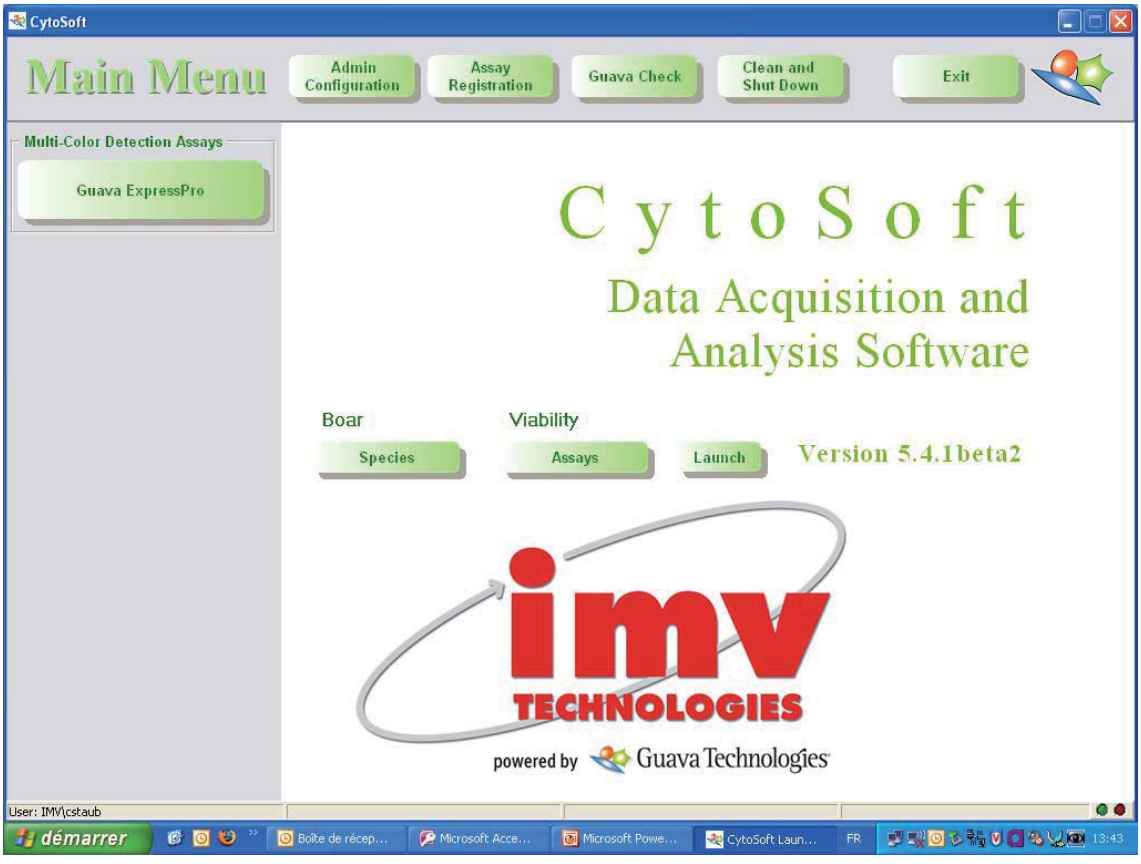
pdf



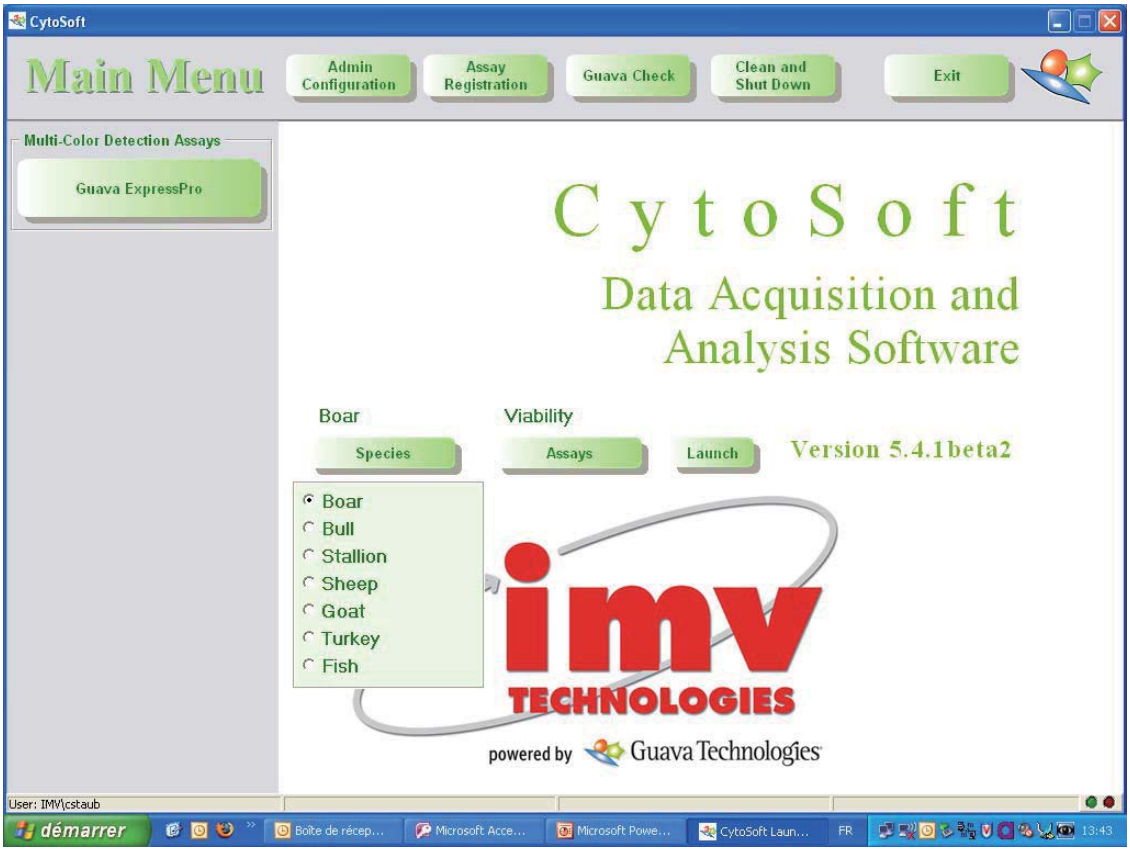
excel



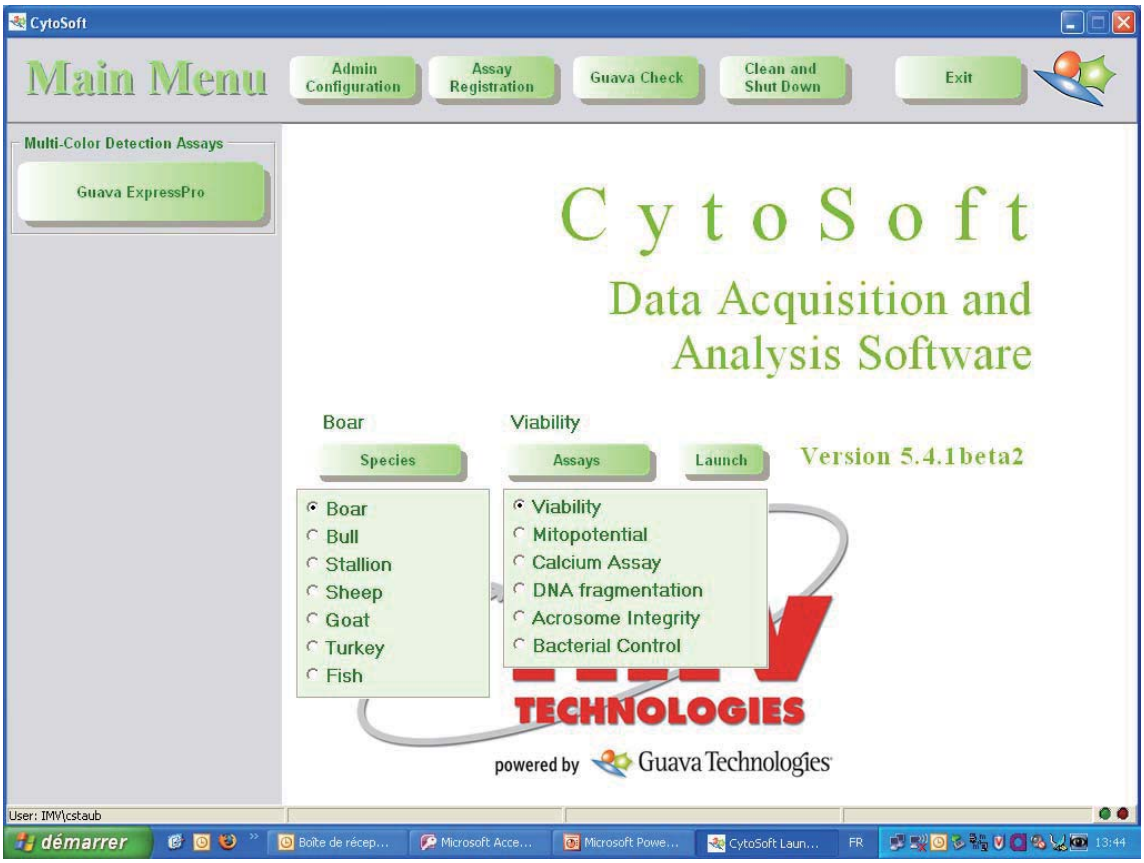
CytoSoft: pre-arranged settings prepared



Cytosoft: choice of the specie

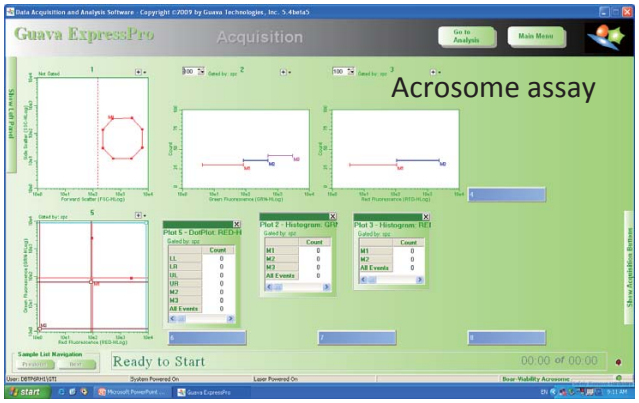


CytoSoft: choice of the assay

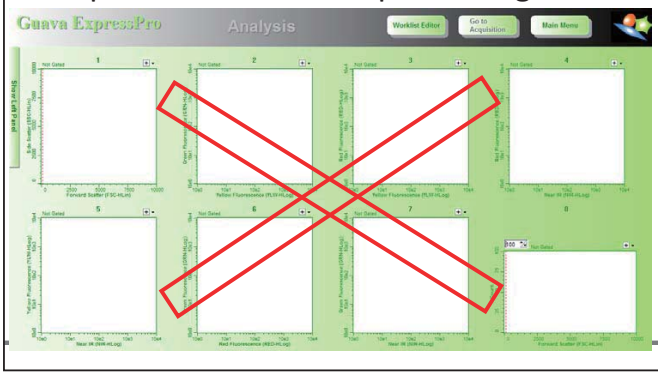


CytoSoft: pre-arranged settings prepared

IMV's software: 1 protocol = 1 specific setting



Millipore's software: no pre-arranged settings



Easysoft

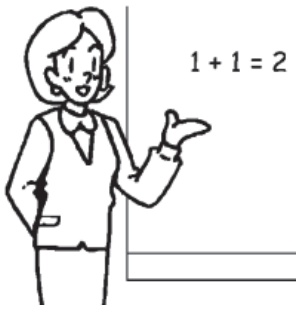
BEFORE THE EASYSOFT

Which value am I interested in?

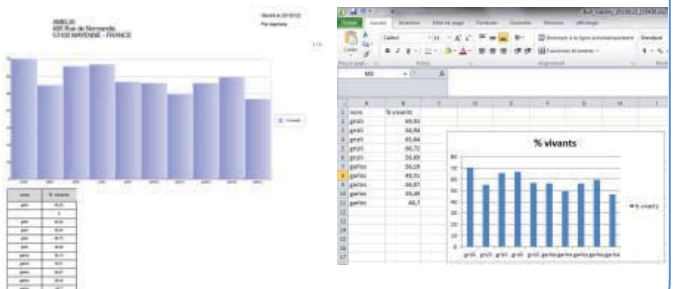
? ? ?

spz (Plot1)												
Count	%Total	%Gated	X-Geometric	Y-Geometric	X-Arithmetic	Y-Arithmetic	X-Median	Y-Median	X-%CV	Y-%CV	Cells/mL	Vertex1
5000	70.09	---	505.02	312.41	590.34	432.57	467.04	280.92	73.94	121.95	133499.94	226;5477
5000	72.41	---	501.20	335.43	587.12	476.66	464.86	296.93	75.70	120.51	125441.48	226;5477
5000	71.33	---	506.42	320.22	587.93	446.79	470.85	289.75	71.79	119.30	118635.05	226;5477
5000	71.10	---	527.91	340.09	629.29	500.37	483.21	298.18	78.99	126.16	108933.31	226;5477
3070	64.21	---	448.24	953.56	550.39	1453.72	439.44	1037.31	73.12	86.28	13645.59	226;5477

AFTER THE EASYSOFT



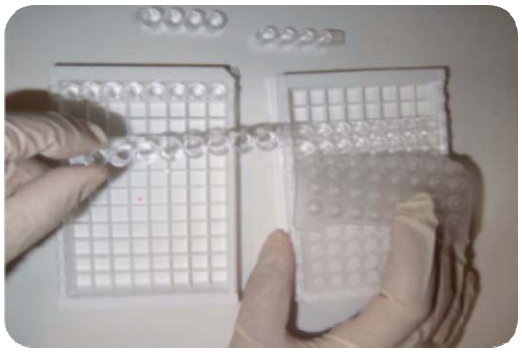
Really Easy: here is the report you want



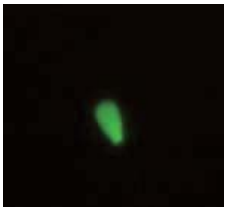
-Export in pdf or excel file
- bar chart already done

A real innovation : ready-to-use kits

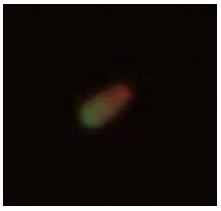
- Breakable wells
- Lyophilized fluorochromes
- Validated for bovine and porcine
- Safety : reduces handling of fluorochromes
- EasyKit contains :
  - 5 plates of 96 wells with lyophilized fluorochromes
  - 1 working base
  - 1 black lid
- Available in:
  - **EasyKit 1 : viability or concentration**
  - **EasyKit 2 : Mitochondrial activity**
  - **Easykit 3 and 4 : oxydation; coming soon**



Viable spz



Dying spz



Dead spz

A real innovation : ready-to-use kits

BEFORE THE EASYKIT

OR

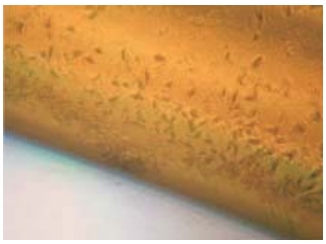
NOW WITH THE EASYKIT

EASYKIT makes your life easier and no risk of protocol error.

EasyClean : a washing solution

BEFORE THE EASYCLEAN

Millipore's product



dirty capillary

NOW WITH EASYCLEAN

IMV's product

clean capillary

- ✓ **Washing solution especially developed for semen application.**
- ✓ **The number of clogg decrease**
- ✓ **The shelf life of the capillary increase**

Semen Analysis

1. Analysis of Motility, concentration and morphology
   a. Microscopes
   b. Photometers
   c. CASA systems
   d. Utrecht University / Topigs results

2. **Analysis of different physiological parameters**
   a. Flow cytometer: EasyCyte
   b. Spallanzani Institute results
   c. Rooster results
   d. UNCEIA and IMV results

Spallanzani Instituts results

- ✓ Done by Dr Andrea Galli; Spallanzani Institute, Italy.
- ✓ Aim of the study: COMPARISON between NucleoCounter and EasyCyte for Concentration and Membrane Integrity measures using Bland Altman method

- ✓ 64 batches

  Extender →Transparent , Opaque

  4 straws per batch (thawed for 1' at 37°C and pooled)

Instruments - Description

NUCLEO COUNTER

The NucleoCounter SP-100 (ChemoMetec A/S, Allerød, Denmark) is an instrument with a fluorescence microscope and an integrated digital video camera. It uses "cassette" with PI and fixed volume.
Membrane Integrity is evaluated comparing the concentration of spermatozoa after disrupting and without disrupting plasma membranes.

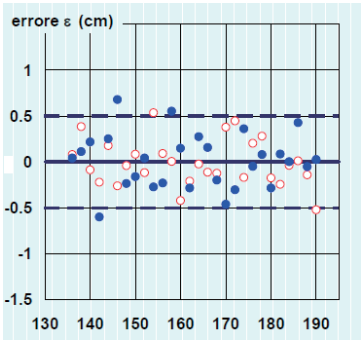
EASY CYTE

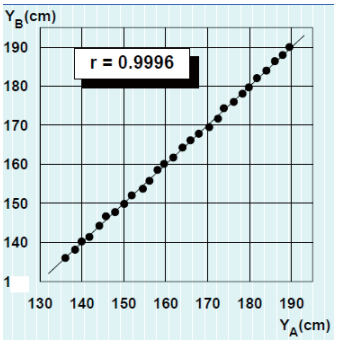
The EasyCyte™ Plus (IMV Technologies, L'Aigle, France) is a flow cytometer dedicated for semen analysis with microcapillary technology.

The fluid system is simpler than traditional instruments and utilizes smaller sample volumes.

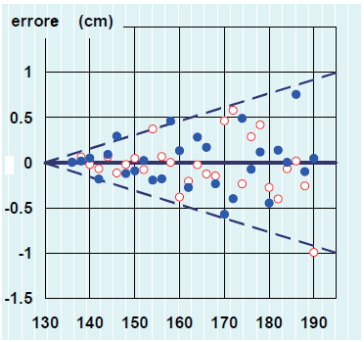
IMV has developed kits for several semen analysis.

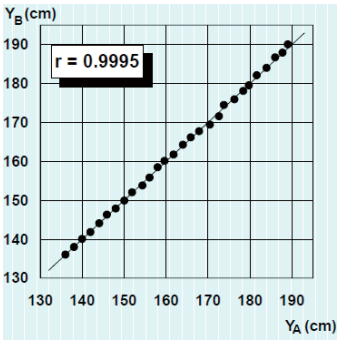
Agreement between instruments and Correlations





Good Agreement
High correlation





Poor Agreement
High correlation

CORRELATION COEFFICIENT
It does not measure the **degree of agreement** between two methods but only their **linear association**

Bland-Altman method

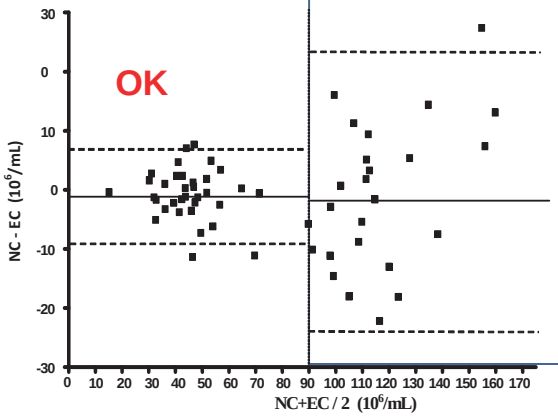
Alternative method, based on **graphical techniques** and **simple calculations**
Created in 1983 by Bland J M & Altman D G (*);

Used to assess **agreement** between two methods of clinical measurement.

I answer to the question:
By how much the new instrument is likely to differ from the old one?

(*) **Bland J M & Altman D G.** Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet 1:307-10, 1986.*

CONCENTRATION - Bland–Altman Plot



OK

Concentration ≥ **90** x 10^6 /mL

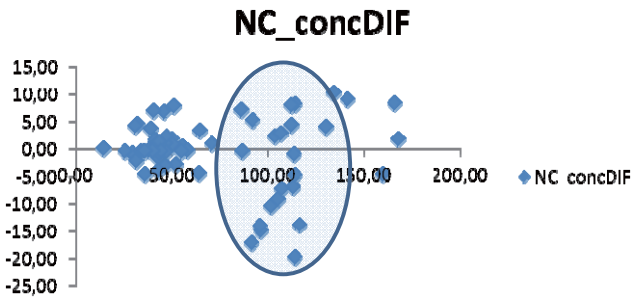
NOT GOOD AGREEMENT

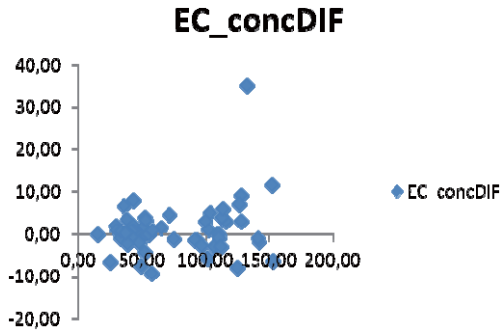
Concentration **< 90** x 10^6 /mL

GOOD AGREEMENT

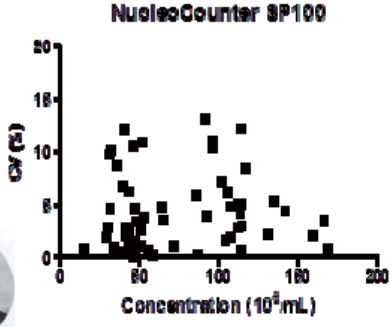
The agreement between the two methods of analysis varies with respect to values of concentration.

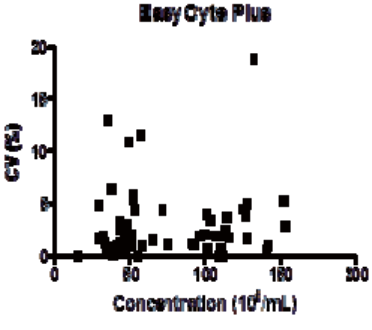
CONCENTRATION - Repeatability



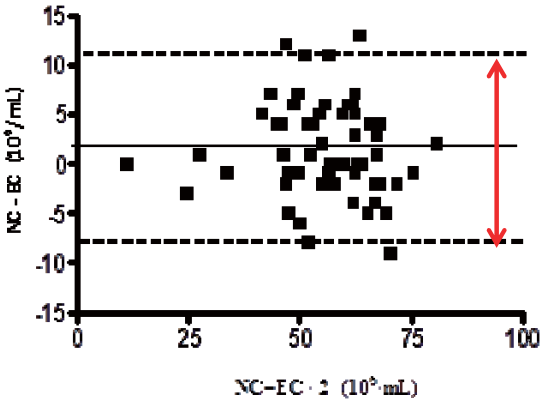


EasyCyte is more accurate than NucleoCounter
(mean CV → 2.90% *versus* 5.07%).





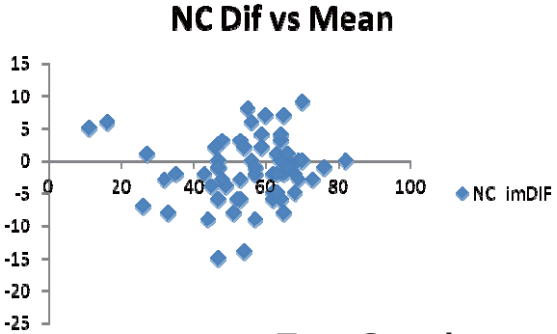
MEMBRANE INTEGRITY - Bland–Altman Plot

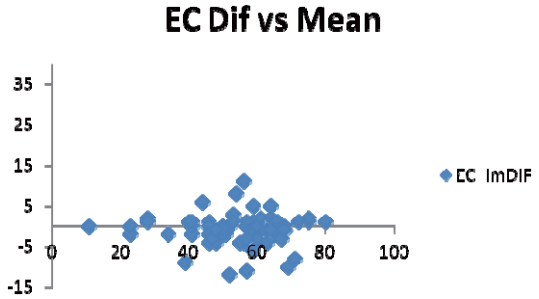


Overview of difference plots with mean differences (solid lines) and 95% limits of agreement (dashed lines; -7.9 to 10.8%).

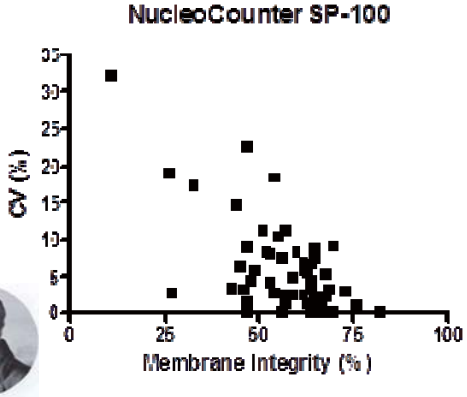
VERY GOOD AGREEMENT

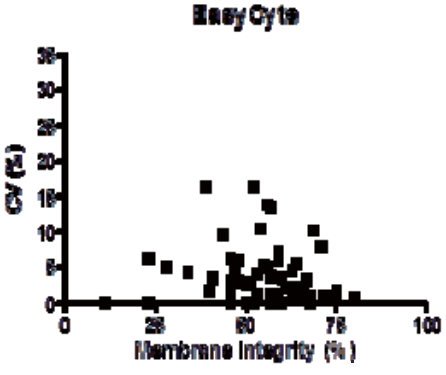
MEMBRANE INTEGRITY - Repeatability





EasyCyte is more accurate than NucleoCounter (mean CV → 3.78% *versus* 5.69%).





CONCLUSIONS

- ✓ **Eacy Cyte more accurate** (less variability between 1° and 2° measures).
- ✓ Good Agreement between Nucleo Counter and Easy Cyte for Concentration only for samples with < 90 x 10^6 spermatozoa / mL.
- ✓ Good Agreement between Nucleo Counter and Easy Cyte for Membrane Integrity.

Semen Analysis

1. **Analysis of Motility, concentration and morphology**
   a. Microscopes
   b. Photometers
   c. CASA systems
   d. Utrecht University / Topigs results
2. **Analysis of different physiological parameters**
   a. Flow cytometer: EasyCyte
   b. Spallanzani Institute results
   c. Rooster results
   d. UNCEIA and IMV results

Rooster semen quality : Material and Method

Semen :

•30 roosters = 10 roosters of 3 different strains (A, B and C)

***In vitro* parameters tested :**

MOTILITY : CASA Analysis D0 and D1

VIABILITY : Easycyte Analysis D0 and D1

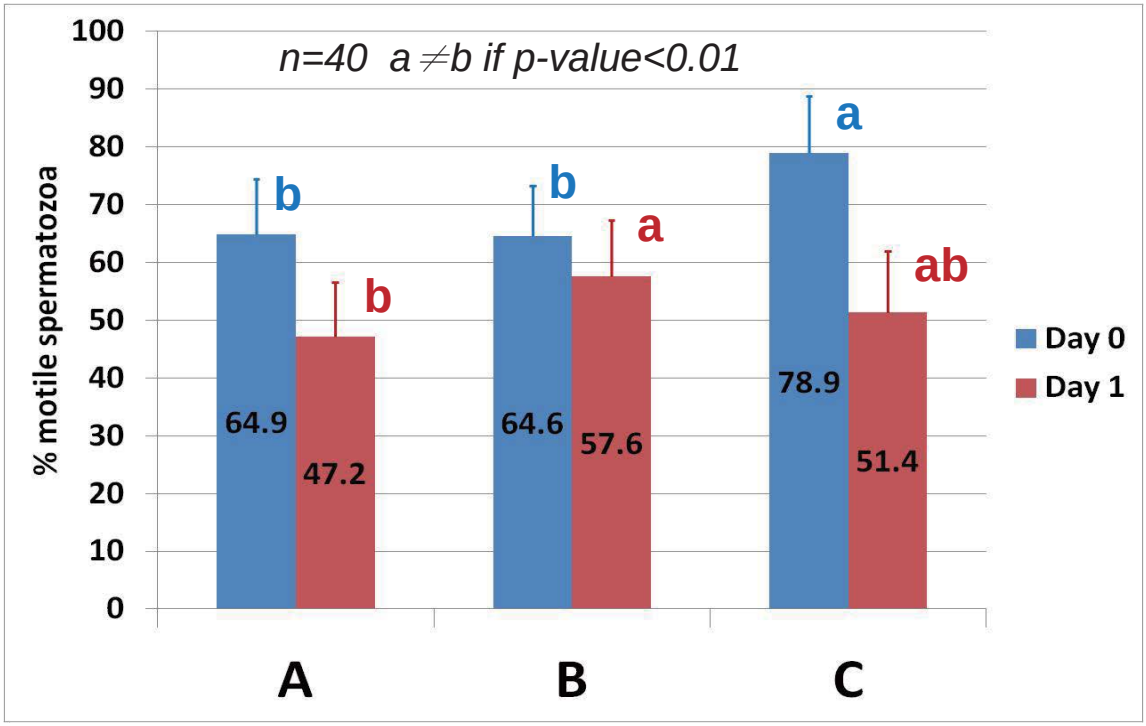
MITOPOTENTIAL : Easycyte Analysis D0 and D1

OXIDATION : Easycyte Analysis D0 and D1

MEROCYANINE : Easycyte Analysis after thermoresistance 2h 37°C D0

CALCIUM : Easycyte Analysis D0 + thermoresistance 2h 37°C D1

Rooster semen quality : MOTILITY

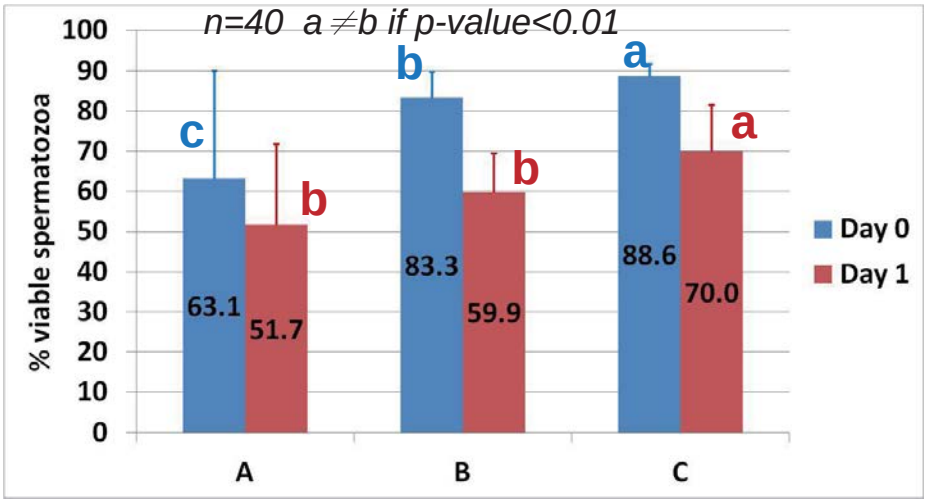


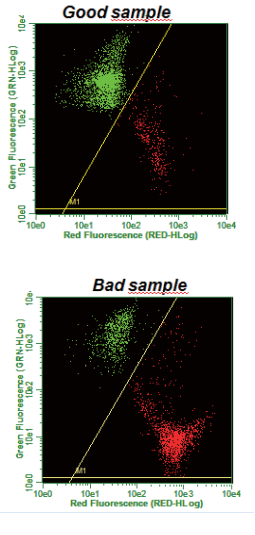
•strain#C shows the better motility at day 0

•strain#B keeps the most stable motility during 30 hours

•Bredd#A is significantly lower than C at day 0 and B at day 1

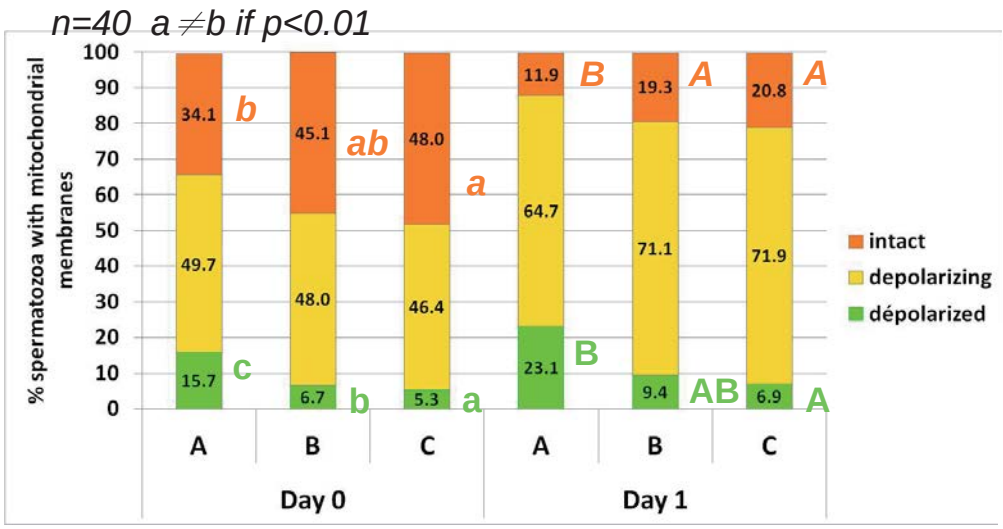
Rooster semen quality : VIABILITY

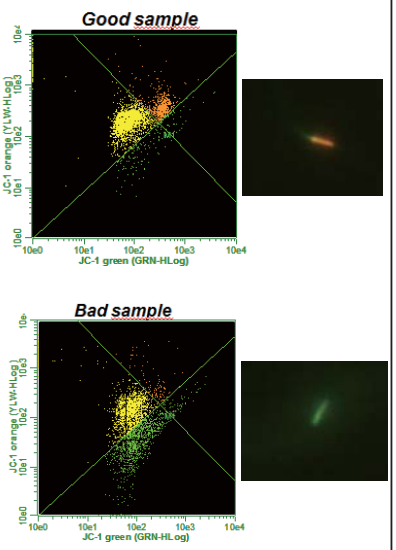




- strain#A shows a significantly lower membrane integrity than B and C at day 0 and than C at day 1
- strain#B is significantly lower than C at day 1
- strain#C has the significantly higher membrane integrity

Rooster semen quality : MITOPOTENTIAL

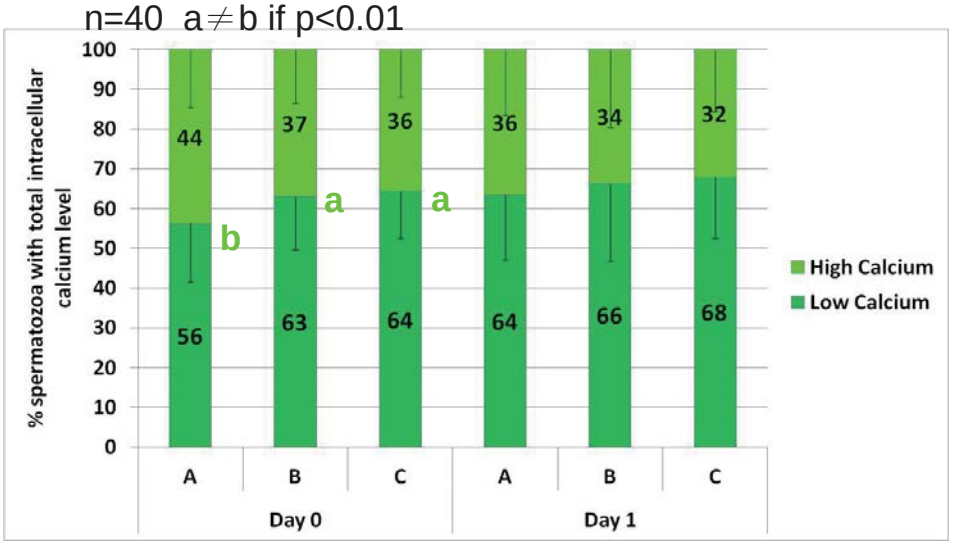


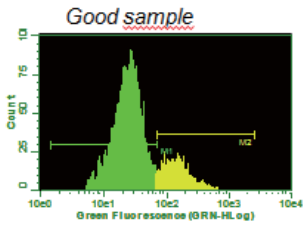


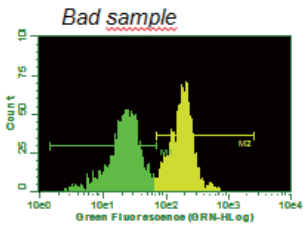
- strain#A shows a significantly higher depolarization of mitochondrial membranes at day 0 and day 1 than B and C
- strain#B is in the middle
- strain#C shows the higher integrity of mitochondrial membranes during 30 hours

Rooster semen quality : CALCIUM

n=40 a≠b if p<0.01







•strain#A contains significantly lower calcium at day 0 than B and C
•Individual effect (high standard deviations)

Rooster semen quality : CONCLUSIONS

Statistic notes for *in vitro* parameters tested : (NS=no significative difference)

Breed	motility		viability		mitopotential		merocyanine	oxidation		calcium	
Day	D0	D1	D0	D1	D0	D1	D0	basal	induced	D0	D1
A	b	b	c	b	b+c	b+b	NS	b	NS	b	a
B	b	a	b	b	ab+b	a+ab	NS	a	NS	a	a
C	a	ab	a	a	a+a	a+a	NS	a	NS	a	a

Considering a=2, b=1 and c=0, strains can be graded :

BREED	DAY	MOTILITY	VIABILITY	MITOPOTENTIAL	BASAL OXIDATION	CALCIUM	TOTAL
A	Day 0	1	0	1	1	1	0.8
B	Day 0	1	1	1.5	2	2	1.5
C	Day 0	2	2	2	2	2	2
A	Day 1	1	1	1	1	2	1.2
B	Day 1	2	1	2	2	2	1.8
C	Day 1	1.5	2	2	2	2	1.9

STRAIN	GRADE
A	1.4
B	2.4
C	2.95

It was confirmed by the producer that strain C is more fertile than the other strains

Semen Analysis

1. **Analysis of Motility, concentration and morphology**
   a. Microscopes
   b. Photometers
   c. CASA systems
   d. Utrecht University / Topigs results

2. **Analysis of different physiological parameters**
   a. Flow cytometer: EasyCyte
   b. Spallanzani Institute results
   c. Rooster resuts
   d. UNCEIA and IMV results

Correlation with fertility

« Use of in vitro assessed semen quality criteria to predict fertility of bull semen » by UNCEIA in collaboration with IMV

Experimental design

72 Holstein bulls
1 ejaculate / bull

- For each ejaculate, an adjusted Fertility Value (FV) estimated from non-return rates at 28 days (NRR28)
- NRR28 issued from 613±99 inseminations per bull (100 to 763), and adjusted on the same environment factors as the French breeding values model
- FVs ranged from -12,8 to +9,2 between bulls

Motility and morphological abnormalities

CASA parameters : VAP, VCL, ALH...

Viability, acrosom status, mitopotential, cellular oxidation, chromatin condensation
(Easycyte 5HT ; IMV Technologies)

Correlation between each parameter and FV

Multiple regression models (proc REG/stepwise SAS®) to estimate simultaneously parameters effects on FV

Correlation with fertility

Descriptive statistics and correlation coefficient (R) between individual parameter and FV

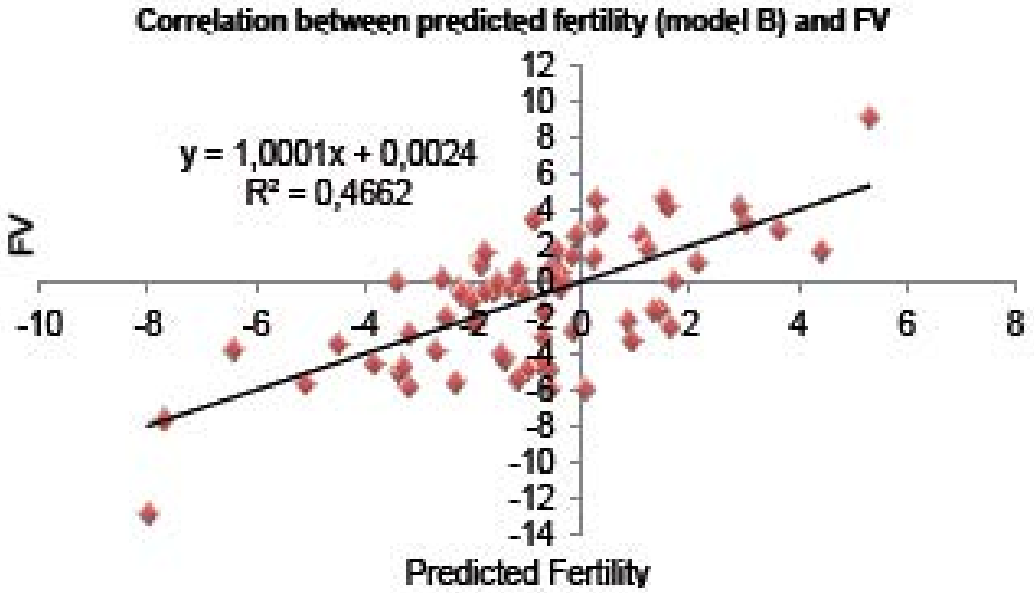
Parameter	Mean	St-D	Correlation coefficient with FV (p)
Acrosome damage in live sperm (%)	2,47	1,62	-0,032 (p=0,791)
Viability (%)	52,7	9,9	0,164 (p=0,169)
Mitopotential (mifu)	738	209	0,322 (p=0,006)
Oxidation (%)	66,1	8,28	0,294 (p=0,012)
Oxidation 2 (fluorescent unit)	73,1	29	0,28 (p=0,010)
Morphological abnormalities	21,5	5,8	-0,262 (p=0,026)
Chromatine condensation (mifu)	114,1	8,7	-0,331 (p=0,004)
CASA/ VAP (µm/s)	111,4	7,5	0,142 (p=0,233)

Correlation with fertility

Two multiple regression models with an accurate prediction of FV

Model A : 4 flow cytometer tests, R=0,64, p<0,0001

Model B : Model A + morphological abnormalities R=0,69, p<0,0001



Correlation with fertility

« Use of in vitro assessed semen quality criteria to predict fertility of bull semen » by UNCEIA in collaboration with IMV

Multiple regression models with an accurate prediction of FV : 4 flow cytometer tests

✓Develop the 4 ready-to-use kits

✓Create EasySoft fertility with UNCEIA algorithm

Conclusions

Why to use a flow cytometer ?

➢To improve the semen analysis
➢Standardization with high statistic power
➢For male management
➢For quality control (dose certification)
➢High value animal

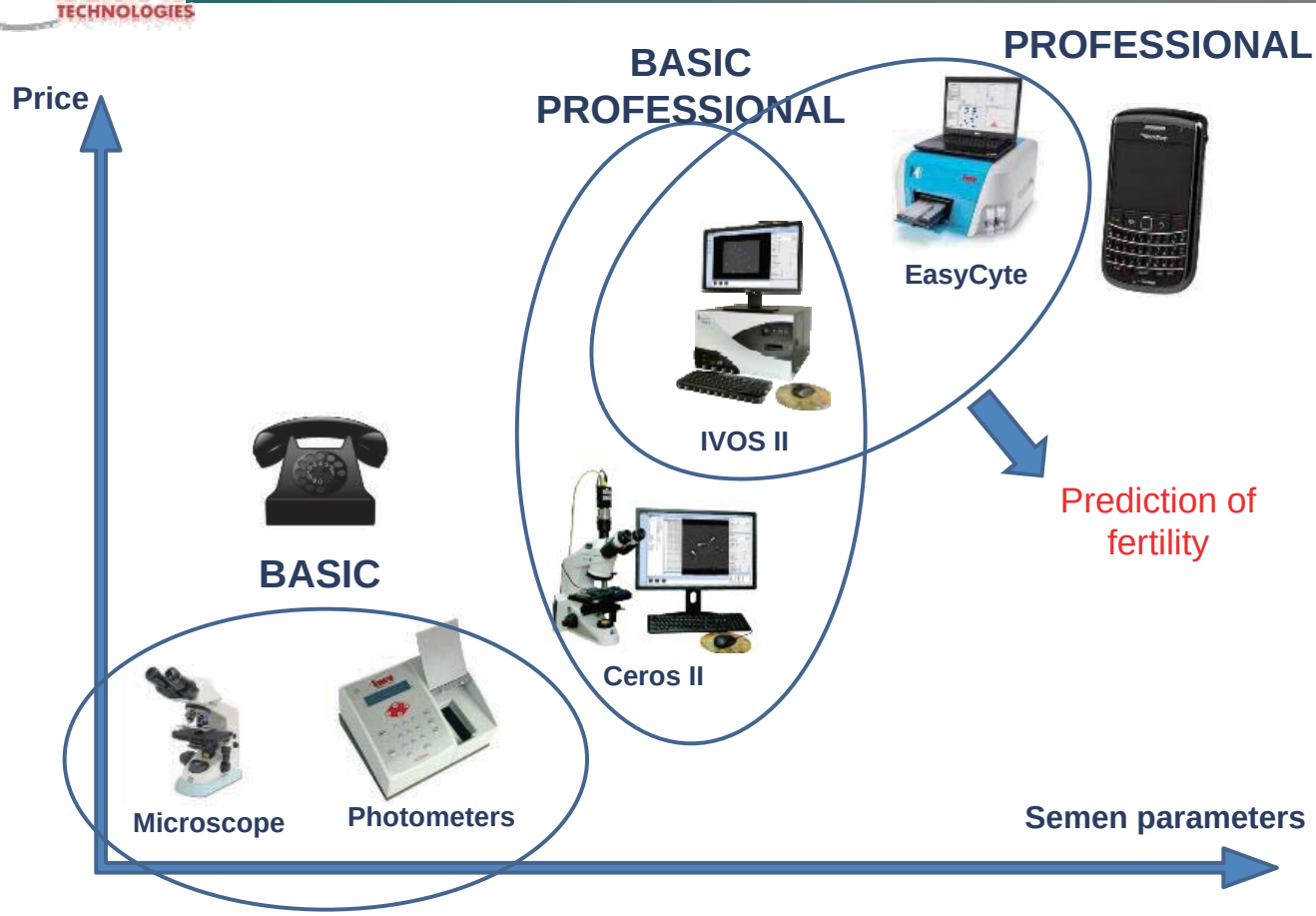
Main advantages of IMV flow cytometers

• Adapted for sperm analysis (PMT, laser, software…)
• IMV unique ready-to-use protocols
• Ready-to-use kits
• Intuitive software package
• Scientific technical support specialized in semen analysis

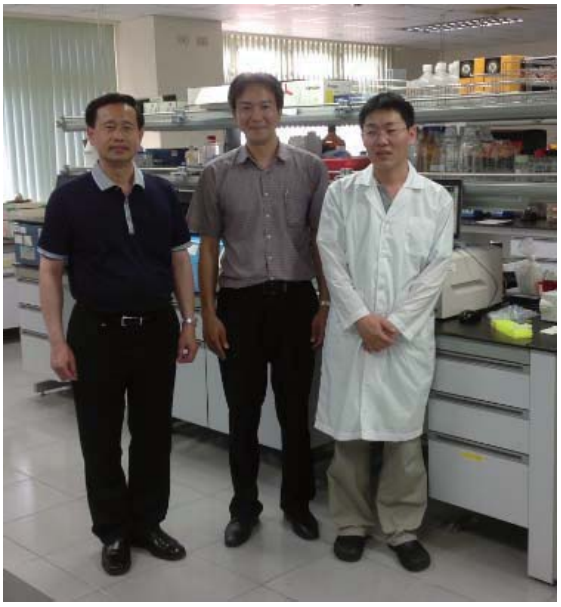
⇒Not only a flow cytometer but **a complete range adapted for semen analysis**

Some customers that are using IMV flow cytometer : **Genex (USA), KRC (USA), Seaworld (USA), UNCEIA (France), Créavia (Sersia/France), Cobiporc (France), Spallanzani Institute (Italy), Laval university (Canada), AWE (Belgium), Bayern Genetik (Landshut/Germany), TLRI (Taiwan) ...**

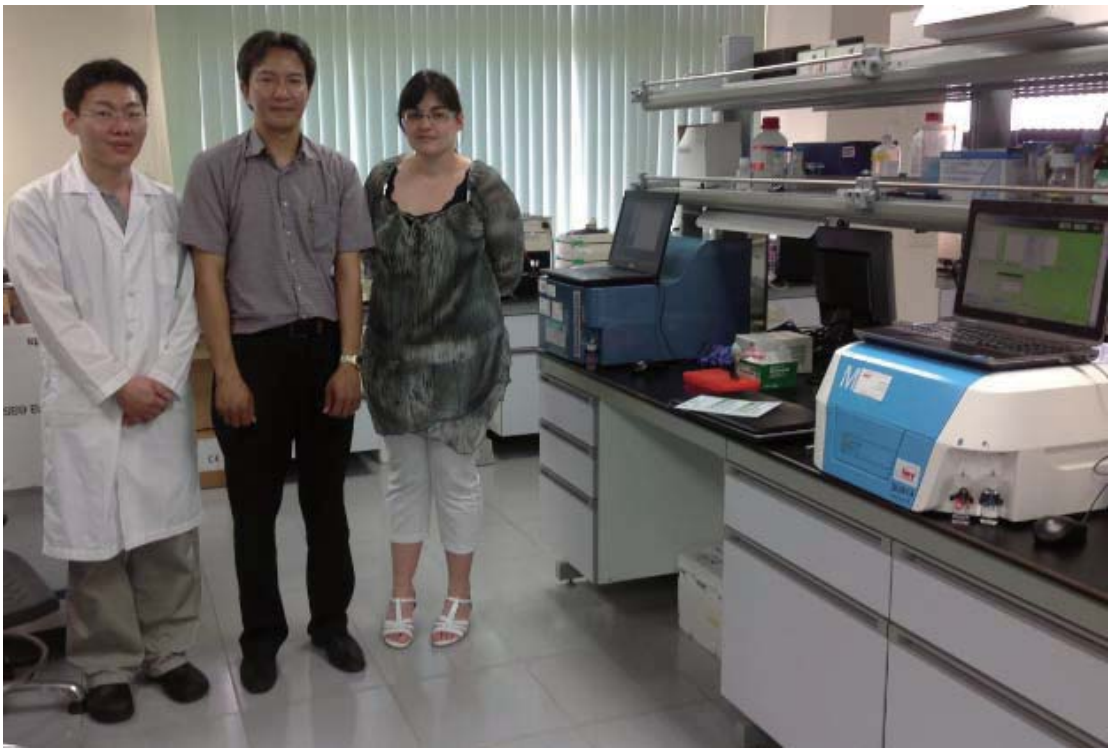
Conclusion

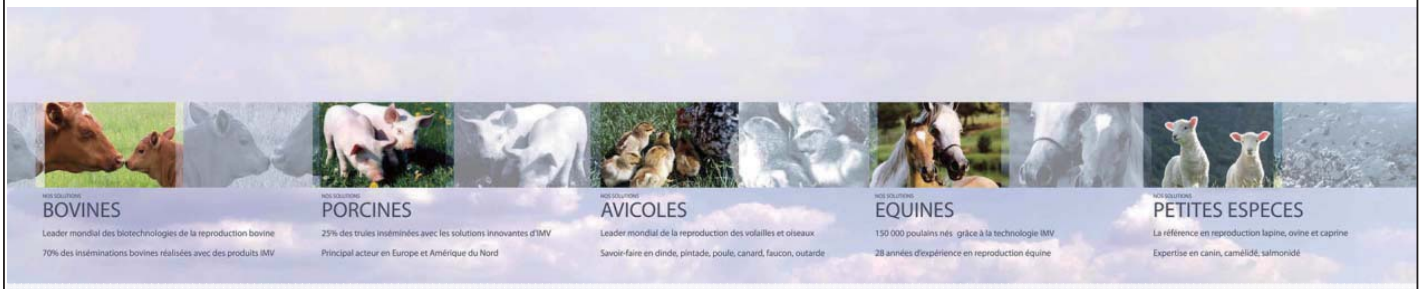


Thank you for welcoming me in your laboratory and making me discover Taiwan.



FLOW CYTOMETRY EXPERT TEAM





Thank you for
your attention!