



Connected to...

Reproduction Biotechnologies



IMV Technologies 2015

World leadership in artificial insemination technologies and products, Biobanking solutions

Mission:

Facilitate access to food needs of a growing population developing innovative Biotechnologies for Animal Artificial Reproduction.





Areas of Expertise

- 52 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



Worldwide Presence - > 120 countries

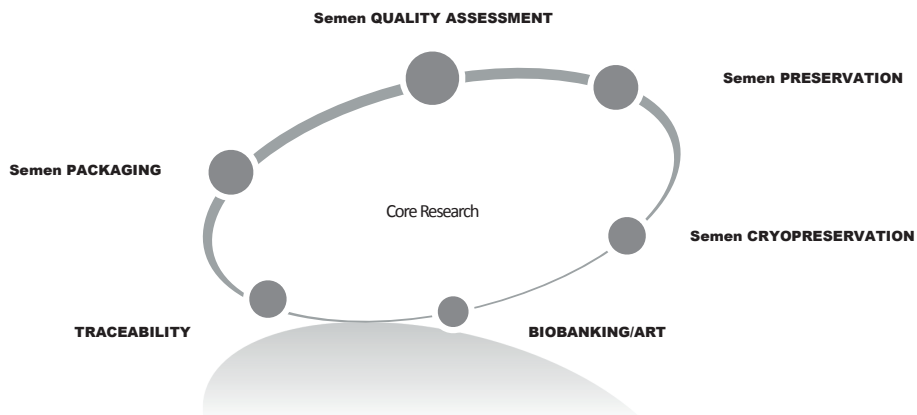


Key Figures

SUBSIDIARIES	USA - India - Italy - China - Netherlands
MANUFACTURING SITE	ISO 9001:2008 - ISO 13485:2003 90% made in France
PATENTS	233
WORLDWIDE PRESENCE	120 Countries
MARKET PRESENCE - VET	Bovine, Swine, Equine, Poultry and 13 others species
MARKET PRESENCE - HUMAN	Biobanking / ART
MARKET SHARE VET	20% to 60%
CAPEX	1.8 M€/year
R&D SPENDING	5 % of sales

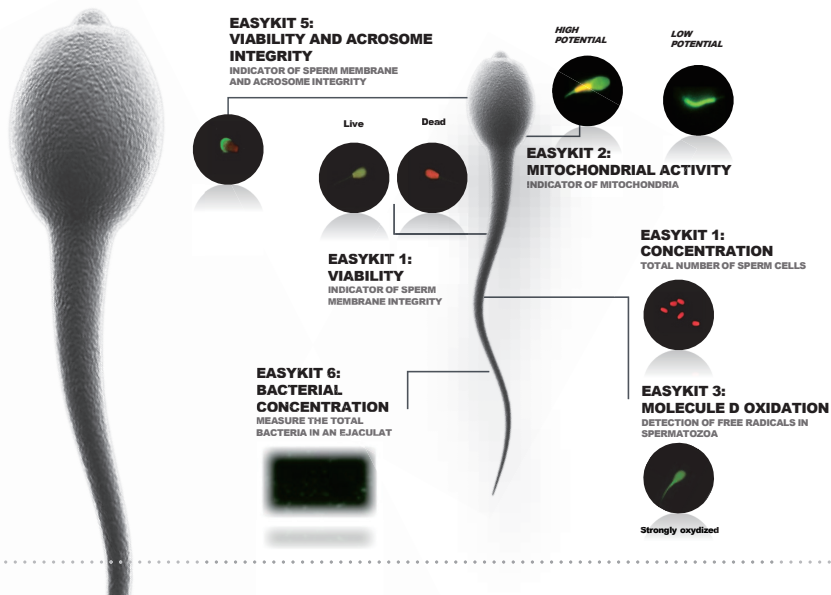


Strategic Research Areas



233 patents... 60 patent families

R&D Focus - Semen And Fertility Analysis



Bench-top flow cytometer for semen analysis
IMV unique ready-to-use protocols
Intuitive software package
Easy-to-use technology



SOFTWARE



EASYSKIN EASYCLEAN



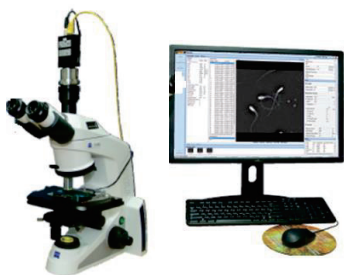
READY-TO-USE KITS

Connected To... Semen Analysis by different technics

1. Analysis of Motility, concentration and morphology
 - a. CASA systems: ceros II and ivos II
 - b. Utrecht University / Topigs results
2. Analysis of different physiological parameters
 - a. Flow cytometer: EasyCyte
 - b. ALLICE, CRV and IMV results
3. R&D products in development
 - a. Freezer
 - b. Free antibiotic extender: CoolpigXcell

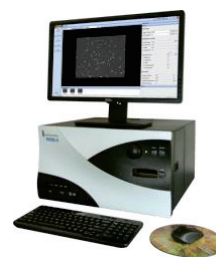


CEROS II



External microscope
Warming stage

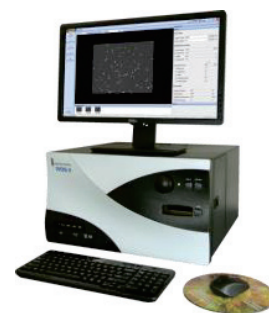
IVOS II



New integrated built in box
Automated warming stage
User friendly front panel

or

- IVOS II: microscope and software image analysis
 - Quantitative evaluation of sperm quality:
 - Concentration
 - Motility
 - Morphology
 - Objective automated method, replace subjective manual analysis
 - 800 spz analyzed / ejaculate within 20sec
 - 40 ejaculates analyzed/ h
- Useful during semen production
- Useful for semen quality control
- Currently 300 production centers use this equipment.

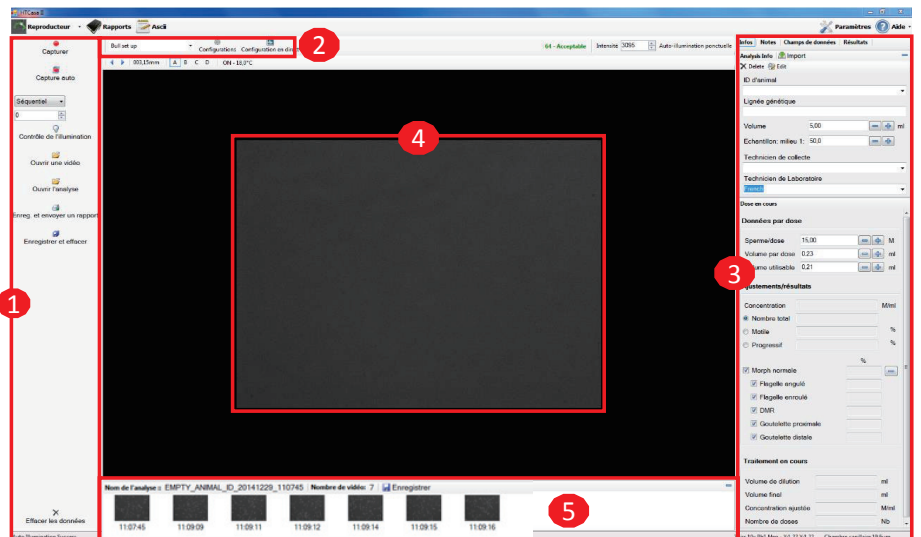




Intuitive software

Available in English, French, 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

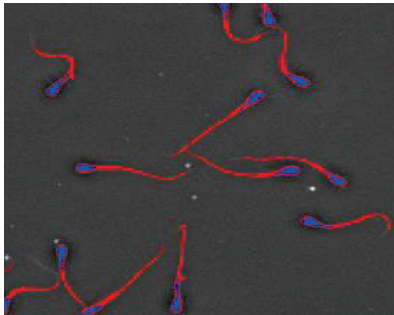


Adjustement

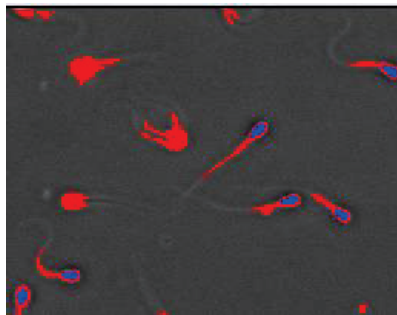
Interactive illumination check (consistency between all users)

Optimize identification of the sperm:

- ✓ Head : blue
- ✓ Tail : red



Good focus

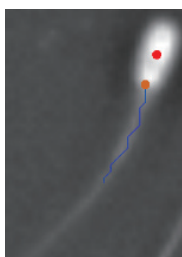


Out of focus

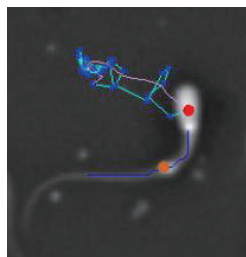
Automated morphologic abnormalities analysis

On life or dead semen

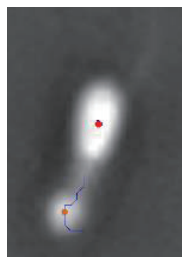
Species: swine, equine, bovine



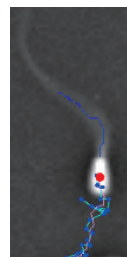
Proximal droplet



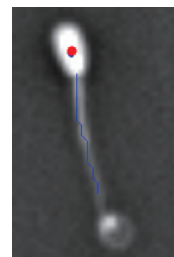
Distal droplet



Distal Midpiece reflex



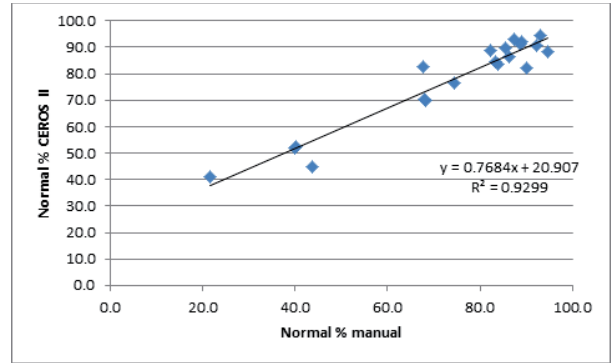
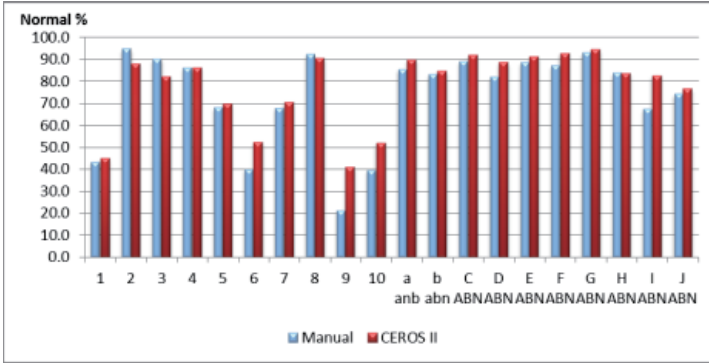
Bent tail



Coiled tail



Proven results on automated morphological abnormalities detection



Good correlation between automated detection and manual counting.



Can choose the number of doses calculation

Calculation depending on

- Total concentration
- Motile spermatozoa percent
- Progressive spermatozoa percent
- Morphological abnormalities percent

Ajustements/résultats

Concentration 995,88 M/ml

Nombre total 50

Motile 72

Progressif 66

Morph normale 92

Flagelle angulé 2

Flagelle enroulé 0

DMR 2

Goutelette proximale 4

Goutelette distale 0

Traitement en cours

Volume de dilution 59,13 ml

Volume final 64,13 ml

Concentration ajustée 77,64 M/ml

Nombre de doses 278 Nb

Ajustements/résultats

Concentration 995,88 M/ml

Nombre total 50

Motile 72

Progressif 66

Morph normale 97,2

Flagelle angulé 0

Flagelle enroulé 0

DMR 0

Goutelette proximale 2,8

Goutelette distale 0

Traitement en cours

Volume de dilution 43,80 ml

Volume final 48,80 ml

Concentration ajustée 102,04 M/ml

Nombre de doses 212 Nb

Report viewer and designer

- ✓ Creation of entirely new form

Animal ID: bull
 Genetic Line:
 Analysis Date and Time: 2011/2014 11:57:32
 Print Date: 29/12/2014
 Collection Tech: d
 Lab Tech: d
 IMV Technologies
 2310 EST
 61300 FAUGLE
Animal Breeder Report

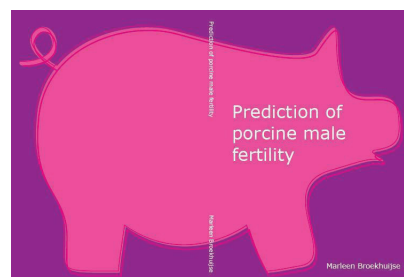
Motility				
	Count	Sample M	Concentration M/ml	Percent Of Total
Total	691	14	66.45	100
Static	481	10	46.25	66.6
Progressive	119	2	11.45	17.2
Mobile	210	4	20.20	30.4
Slow	23	0	2.21	3.3

Morph				
	Count	Sample M	Concentration M/ml	Percent Of Total
Bent Tail	229	7	31.64	47.6
Coiled Tail	46	1	4.42	6.7
DMR	0	0	0.00	0.00
Distal Droplet	0	0	0.00	0.00
Proximal Droplet	0	0	0.00	0.00
Normal Fraction:			45.7	%

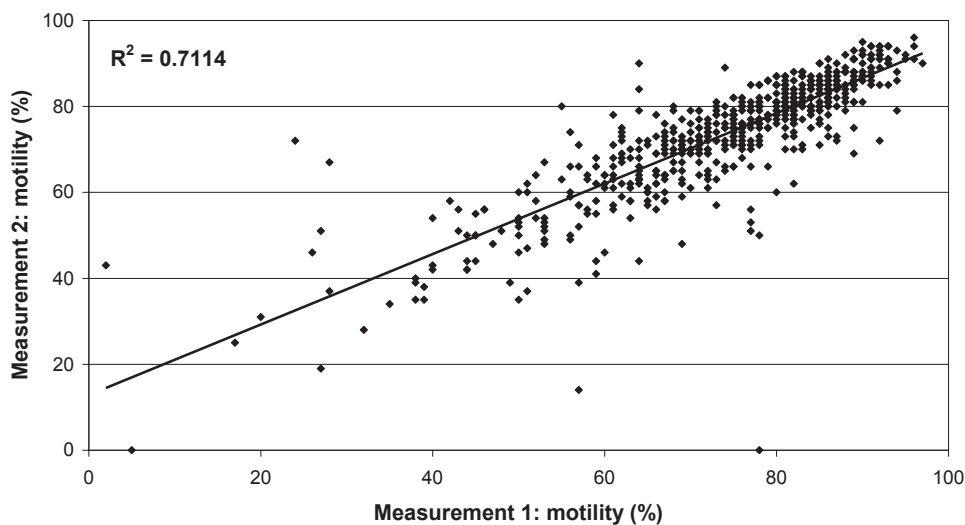
Dosing			
Ejaculate Volume (ml):	0.21	Selected Population:	Total
Sample Extender l:	4	Bent Tail, % of Selected:	47.6
Sperm Per Dose (M):	15	Coiled Tail, % of Selected:	6.7
Dose Volume (ml):	0.23	DMR, % of Selected:	0
Usable volume (ml):	0.21	Proximal, % of Selected:	0
Extender Volume (ml):	-0.01	Distal, % of Selected:	0
Number Of Doses:	0	Adjusted Concentration:	71.43

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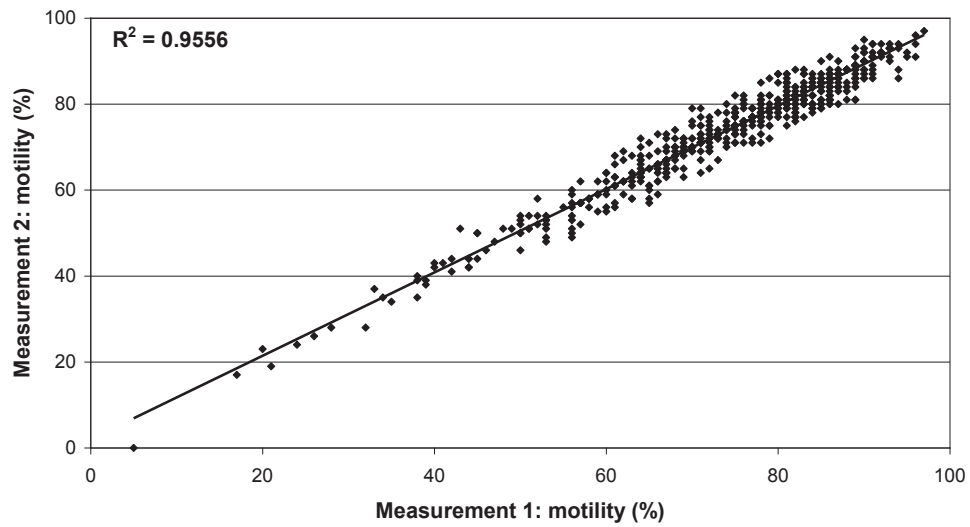
- Work done by Marleen Broekhuijse
- PhD: Prediction of porcine male fertility, 2012
Utrecht University in cooperation with Pig AI Netherlands
- Current job: combining pigs and cattle
 - TOPIGS Research Center IPG
 - CRV



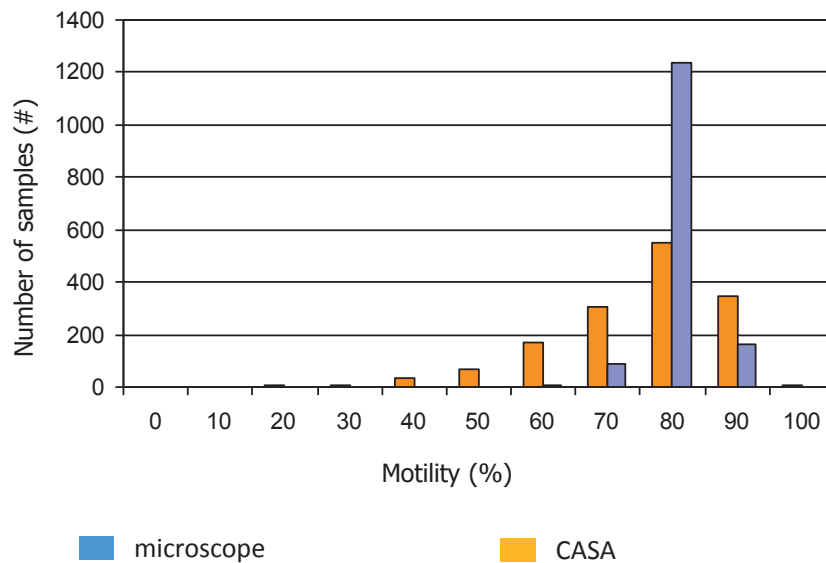
Repeatability before training



Repeatability current situation



1,500 ejaculates, microscope vs. CASA

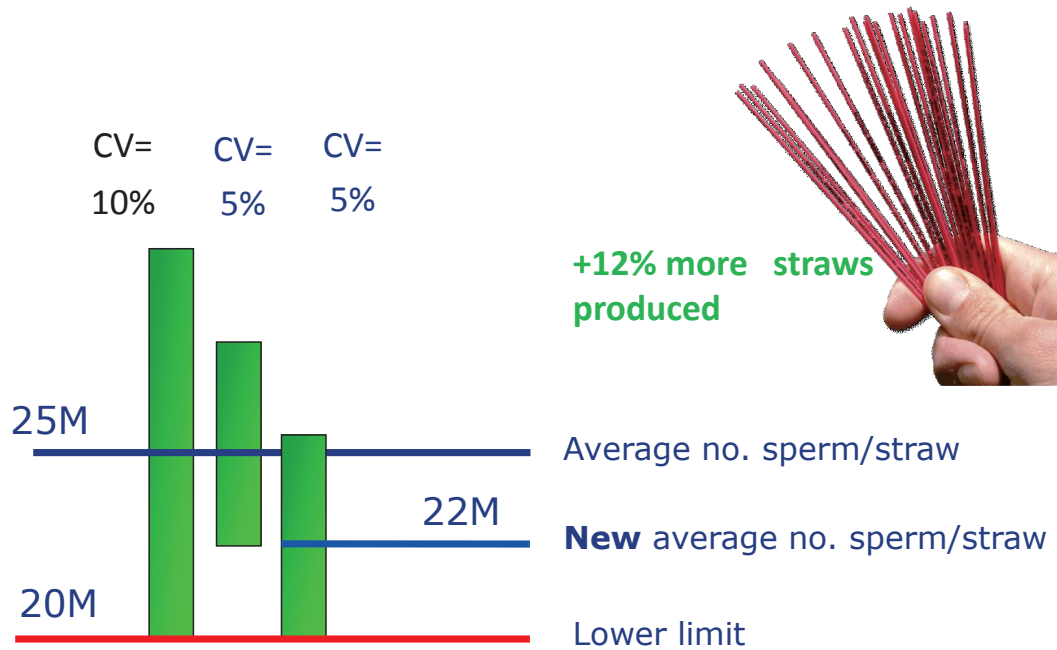


Potential benefits

- ↓ Cells / dose
- ↑ Doses produced per ejac.
- ↓ Costs per year
- ↓ Collection costs per year



- 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



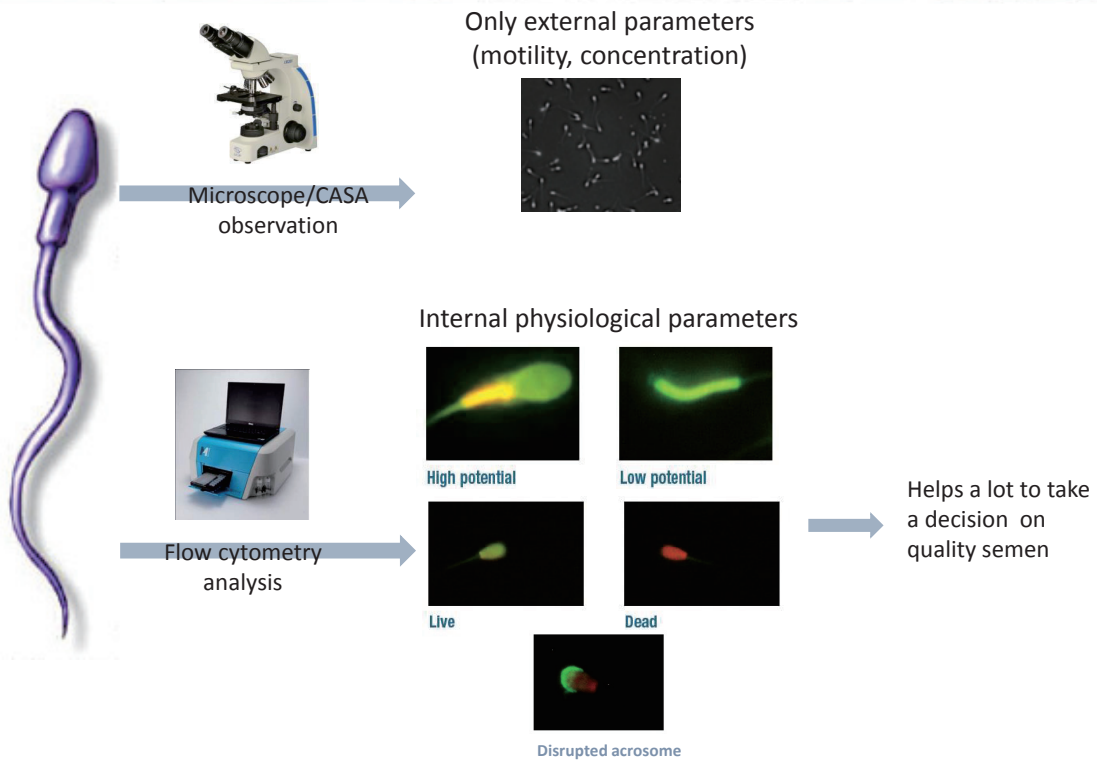
Why to use a CASA system ?

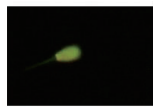
- Standardization (if several lab technicians)
- More parameters analyzed AND automatically
- Data storage : traceability and help for decision
- Easier for training

1. Analysis of Motility, concentration and morphology
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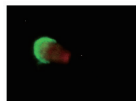
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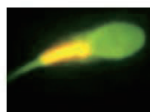




Live



Disrupted acrosome



High potential

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

→ new parameters for higher estimation of semen fertility

- Easycyte: flow cytometer

- Analysis of one spz per one spz in a flow
- 5000 spz counted per ejaculate in 30 sec
- 96 ejaculates analyzed in 1h30 (ex: viabilité).



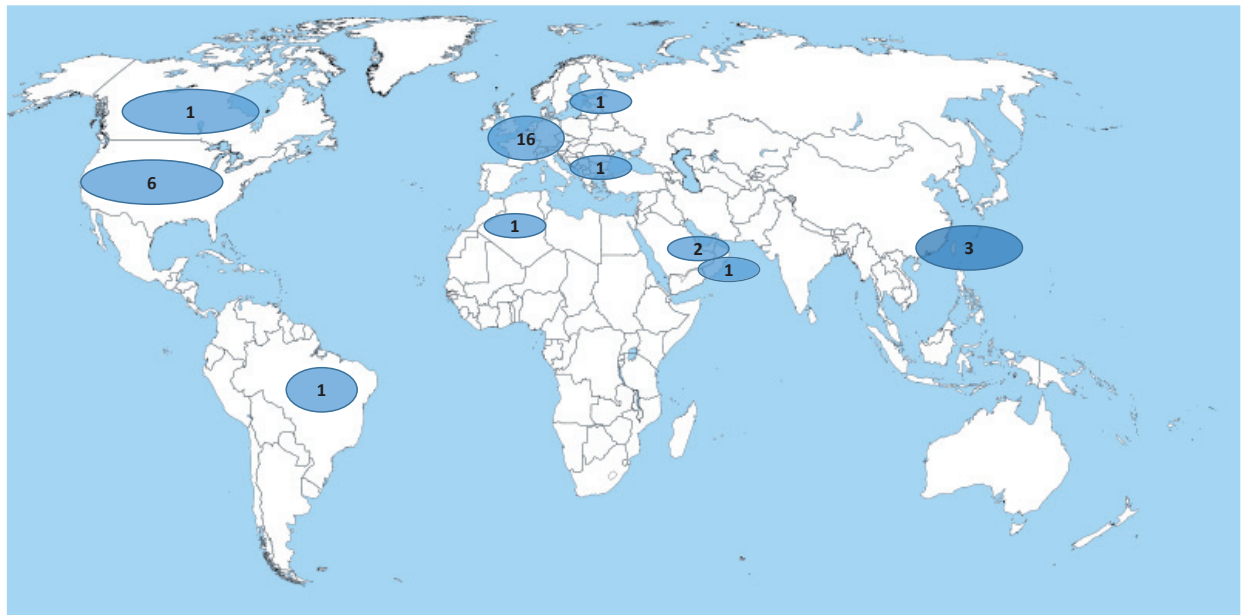
- Simple analysis with ready to use kits: 5 Easykits available

- Viability
- Mitochondrial activity
- Oxydation
- Acrosome integrity
- Bacterial concentration



- 35 production and R&D centers use this equipment





CYTOMETRY



Cell

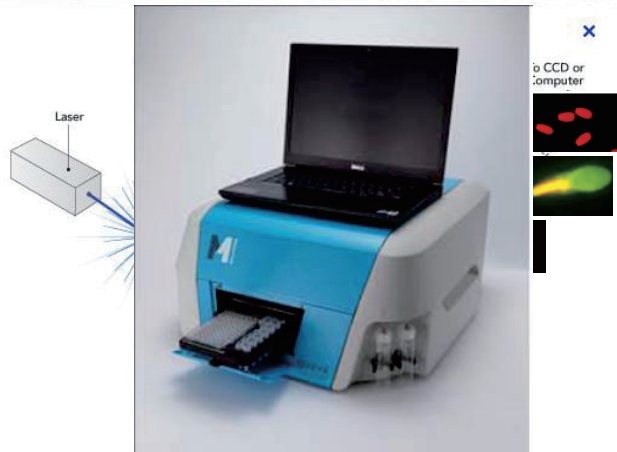


Measurement

➤ Flow cytometry:

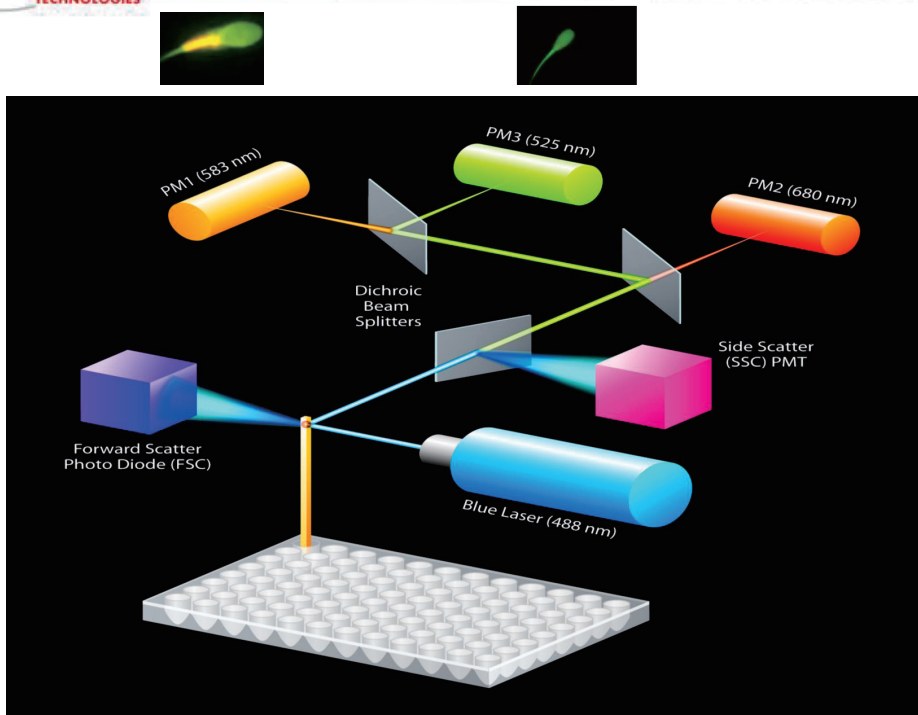
Flow cytometry is a powerful technique for the analysis of multiple parameters of individual cells within heterogeneous populations.

Connected To... 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.

Connected To... A typical flow cytometer layout

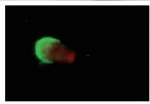
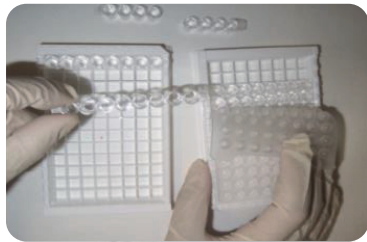


Plot 3 - DotPlot: RED-HLog vs GRN-HLog
Gated by: spz 2

	Count	%Total	%Gated	
LL	0	0,00%	0,00%	
LR	0	0,00%	0,00%	
UL	1807	22,32%	55,23%	live
UR	1465	18,09%	44,77%	dead

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 with the cell enzymes
3. Introduce the 96 wells plate or tube in the flow cytometer
4. PMT (photomultipliers) will measure the fluorescent light emitted by these reagents



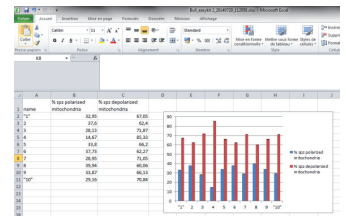
Easykits

2/ ACQUISITION (pre-arranged settings)



Cysoft

3/ STORE RESULTS



Easysoft

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

- A range of flow cytometers
- Ready to use kits for bull and boar semen
- Adapted softwares to analyze and store the data
- Technical support





EasyCyte II plus (IMV name)
96-w plate loader
plus 10 tubes



EasyCyte II mini (IMV name)
Just for tubes

Lasers:

Blue Laser (488 nm)

Detectors:

Forward Scatter – FSC

Side Scatter – SSC

Green – 525/30 nm

Yellow – 583/26 nm

Red1 – 655/50 nm



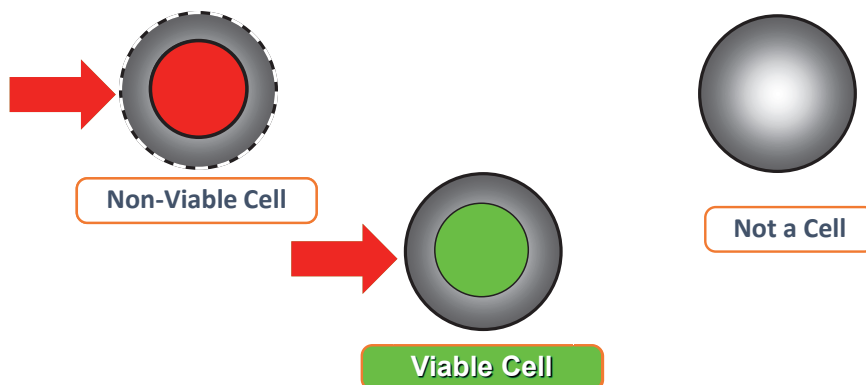
Robotics:

Automated 96 well plate and/or tubes

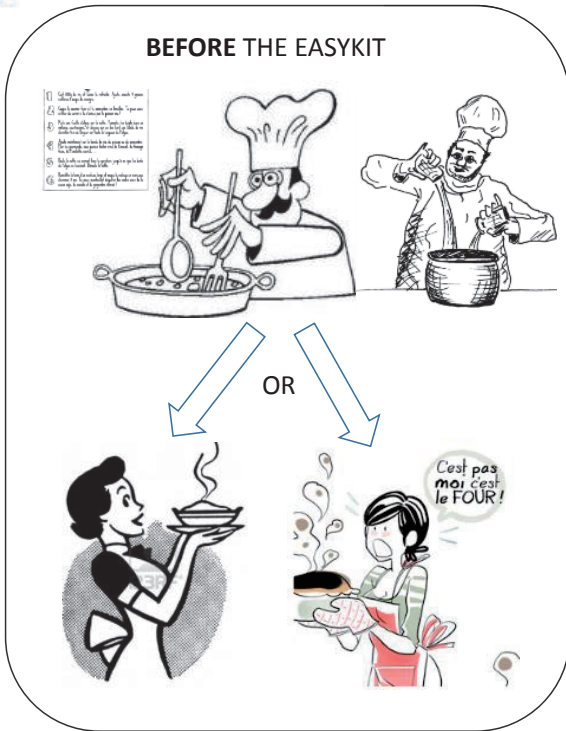
➤ This test indicates the % of viable spermatozoa

➤ Protocol:

- X μL red fluorochrome + Y μL green fluorochrome + 0,5μL bull semen + 196 μL Easybuffer
- Incubate 10 minutes at 37°C
- Acquire 5000 events



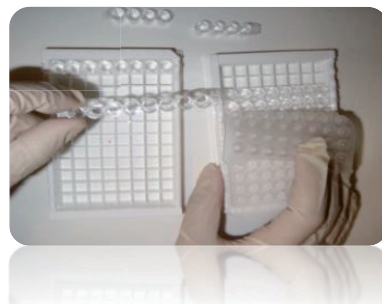
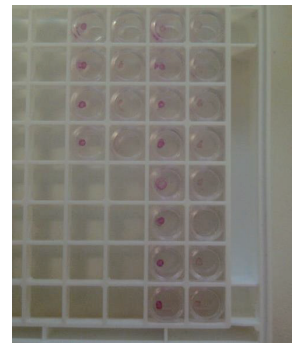
BEFORE THE EASYKIT



NOW WITH THE EASYKIT

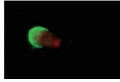


- Breakable wells
- Lyophilized fluorochromes
- Validated for bovine and porcine
- Safety : reduces handling of fluorochromes
- EasyKit contains :
 - 5 plates of 96 wells with lyophilized fluorochromes
 - 1 working base
 - 1 black lid



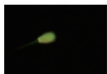
Easykit 5 Viability & Acrosome integrity

•Indicator of sperm membrane and acrosome integrity



Easykit 1: Viability

•Indicator of sperm membrane integrity



Live

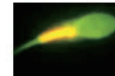


Dead

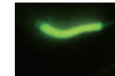


Easykit 2: Mitochondrial activity

• indicator of mitochondria



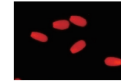
High potential



Low potential

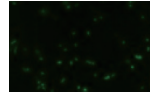
Easykit 1: concentration

total number of sperm cells



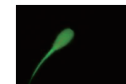
Easykit 6: Bacterial concentration

• Measure the total bacteria in an ejaculat



Easykit 3: Oxidation molecule D

•Detection of free radicals in spermatozoa



Strongly oxydized

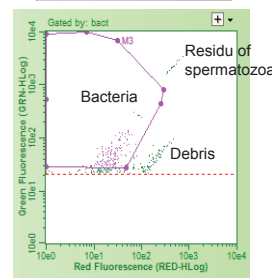
Bacteriosperma in semen can reduce sperm motility and viability and provoke a premature acrosome reaction

Agar Plate method



Three days to have:
Subjective concentration (Around x FUC/ml)
 Count only **viable** bacteria

Easycyte method



Plot 2 - DotPlot: RED-HL	
Gated by: Bacteries	
	Cells/mL
M1	(4.61e03)
All Events	7.76e03



20min to have:
Precise concentration (x bacteria/ml)
 Count **viable AND dead** bacteria

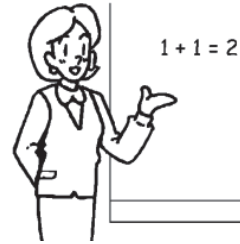
BEFORE THE EASYSOFT



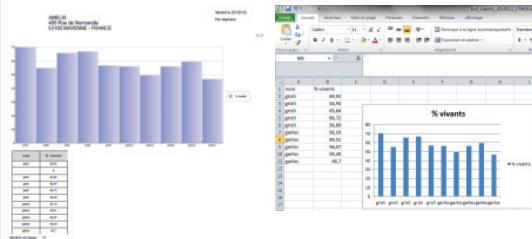
Which value am I interested in?

Count	%Total	%Gated	X-Geometric	Y-Geometric	X-Arithmetic	Y-Arithmetic	X-Median	Y-Median	X-%CV	Y-%CV	Cells/ml	Vertex1	Va
5000	70.89	---	505.02	312.41	590.34	432.57	467.04	285.92	73.94	121.95	133499.94	2265477	64
5000	72.41	---	592.20	335.43	587.12	476.66	464.86	296.93	75.70	120.31	125441.45	2265477	64
5000	71.33	---	506.42	320.22	587.93	446.79	470.85	288.75	71.79	119.30	118635.05	2265477	64
5000	71.10	---	527.91	340.09	629.29	500.37	483.21	298.18	78.99	126.16	108933.31	2265477	64
3070	64.21	---	446.26	303.56	500.99	343.72	439.46	197.31	71.12	86.20	13465.09	2265477	64

AFTER THE EASYSOFT



Really Easy: here is the report you want



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

Select your search conditions

Recorded searches: easykit 2

Protocol: Bull_easykit 2

Beginning date: 02/22/2012

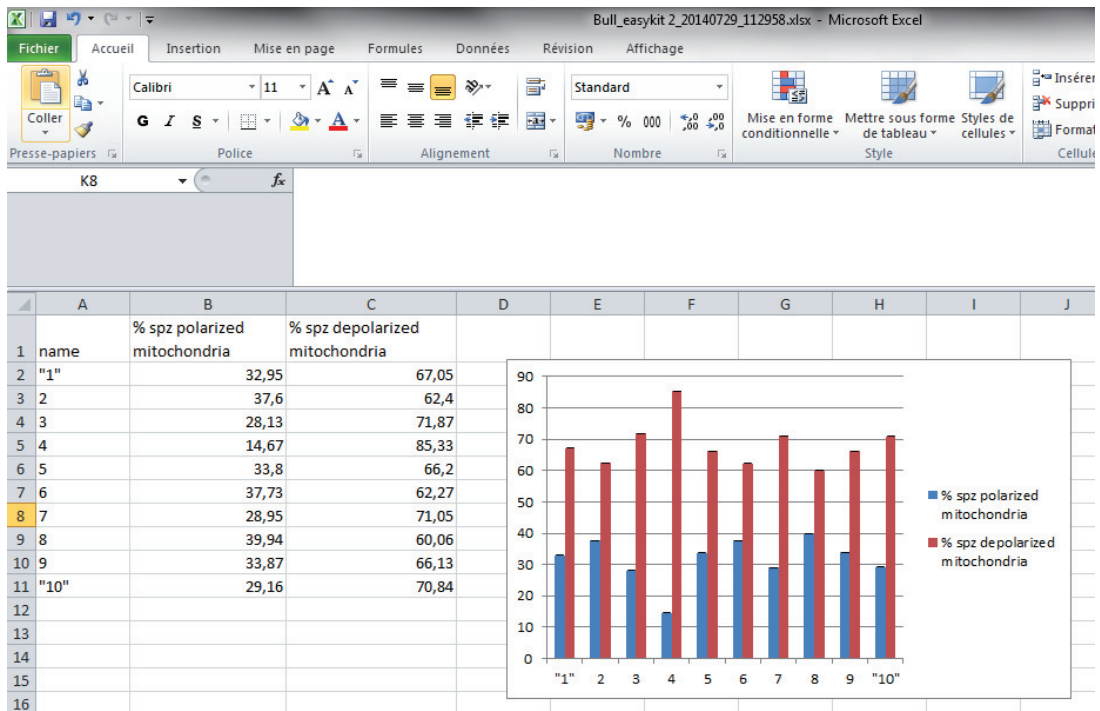
Ending date: 02/22/2012

On the Cytosoft files: Bull_easykit 2_131217_1035.PRO.CSV

Define the columns to display

Display	Column title	Sort	Display	Graph
Sample Number	name	Increasing	1 line per value	Bar graph on X axis
P3.M1 - UR (Plot3): %Gated	% spz mitochondries polarisées		1 line per value	Bar graph on Y axis
P3.M1 - LR (Plot3): %Gated	% spz mitochondries depolarisées		1 line per value	Bar graph on Y axis

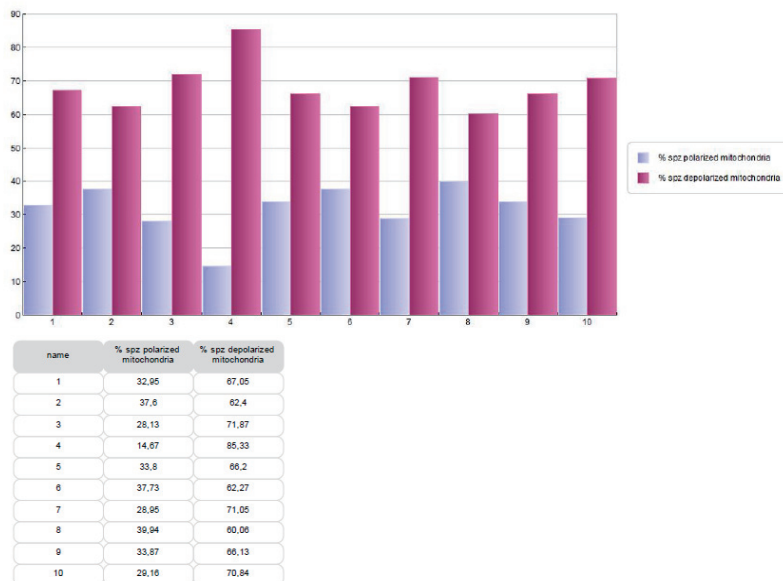
Definition of the parameters of the protocol of interest



IMV-TECHNOLOGIES
ZI N°1 Est
61300 SAINT OUEN SUR ITON - France

Created on 29/07/2014
By LUDIVINE

1



Number of lines : 10

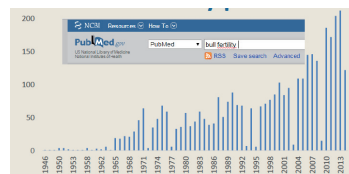
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Very few papers about the fertility prediction of bulls...Why ???

- Difficult to obtain semen from bulls with known fertility
- Studies based on a small data set...miss-confirmation of the prediction on more large population
- Weak relation between one physiological parameter and fertility. Multiparametric analysis is needed.



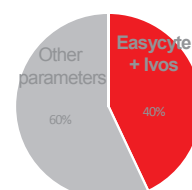
IMV, CRV and ALLICE assay: (submission in progress in Theriogenology)



155 ejaculats analyzed



fertility prediction



- Work done in collaboration with ALLICE (France) and CRV (Netherland)
- Presented in AAAA congress in Australia, 2014
- Submission in Theriogeneology in progress



In vitro assessment of semen quality criteria to predict fertility of bull semen

M.L.W.J. Broekhuijse⁽¹⁾, E. Sellem⁽²⁾, L. Chevrier⁽³⁾,
S. Camugli⁽³⁾, E. Schmitt⁽³⁾, L. Schibler⁽²⁾, E.P.C. Koenen⁽¹⁾

Introduction

Assessment of various sperm quality criteria to predict in vivo fertility of bulls remains a challenge for the breeding industry.

Technologies, such as CASA and flow cytometry, allow a more accurate and objective analysis of semen and may help improving semen quality control as well as fertility prediction.

The aim of this study was to evaluate the relation between semen quality characteristics and field fertility of bull ejaculates.

Experimental design

155 ejaculates analyzed from 19 Holstein bulls

Non return rate at 56 days (NRR56) issued from 601 ± 226 inseminations per ejaculate

For each ejaculate NRR56 is adjusted on the environment factors (adjNRR56)

adjNRR56 ranged from 44% to 71% between ejaculates (mean $65.6 \pm 3.1\%$)



Flow cytometry

Viability
Acrosome integrity
Mitopotential
Cellular oxidations
DNA fragmentation (Easycte 5HT; IMV Technologies)
(Easykits 1 to 5; IMV Technologies)

CASA

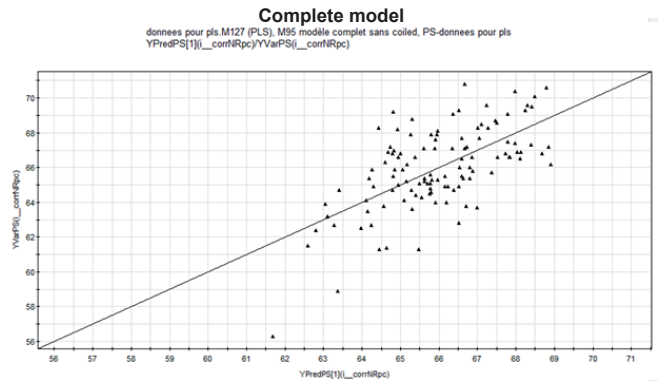
Motility track speed
Morphological abnormalities (IVOS II; Hamilton Thorne Inc., USA)

Basal and stressed conditions

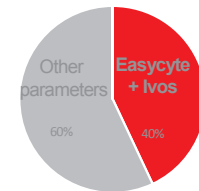
Correlation between each parameter and adjNRR56

Multiple regression models to estimate simultaneously parameters effects on adjNRR56 (PLS Simca software, SOLADIS)

model	protocol	condition	R ²
Ivos only	Morphology VSL	Basal Basal / induced	0,241
Easycyte only	Easykit mitopotential Easykit oxydation Easykit acrosome DNA compaction	Basal Induced Basal / induced Basal / induced	0,325
IVOS + Easycyte Simplify model 8 parameters: 1 kit 1 liquid protocol CASA	Easykit oxydation DNA compaction Morphology VSL	Induced Basal / induced Basal Basal	0,337
IVOS + Easycyte Complete model 12 parameters: 4 kits 1 liquid protocol Ivos	Easykit viability Easykit mitopotential Easykit oxydation Easykit acrosome DNA compaction Morphology VSL	Basal Basal / induced Induced Basal / induced Basal / induced Basal Basal	0,402



fertility estimation



Easysoft: analysis of the data and creation of reports



Easysoft fertilité: easysoft + contains the algorithm of fertility

FERTILITY PREDICTION

IMV-TECHNOLOGIES
ZI N°1 Est
61300 SAINT OUEN SUR ITON - France

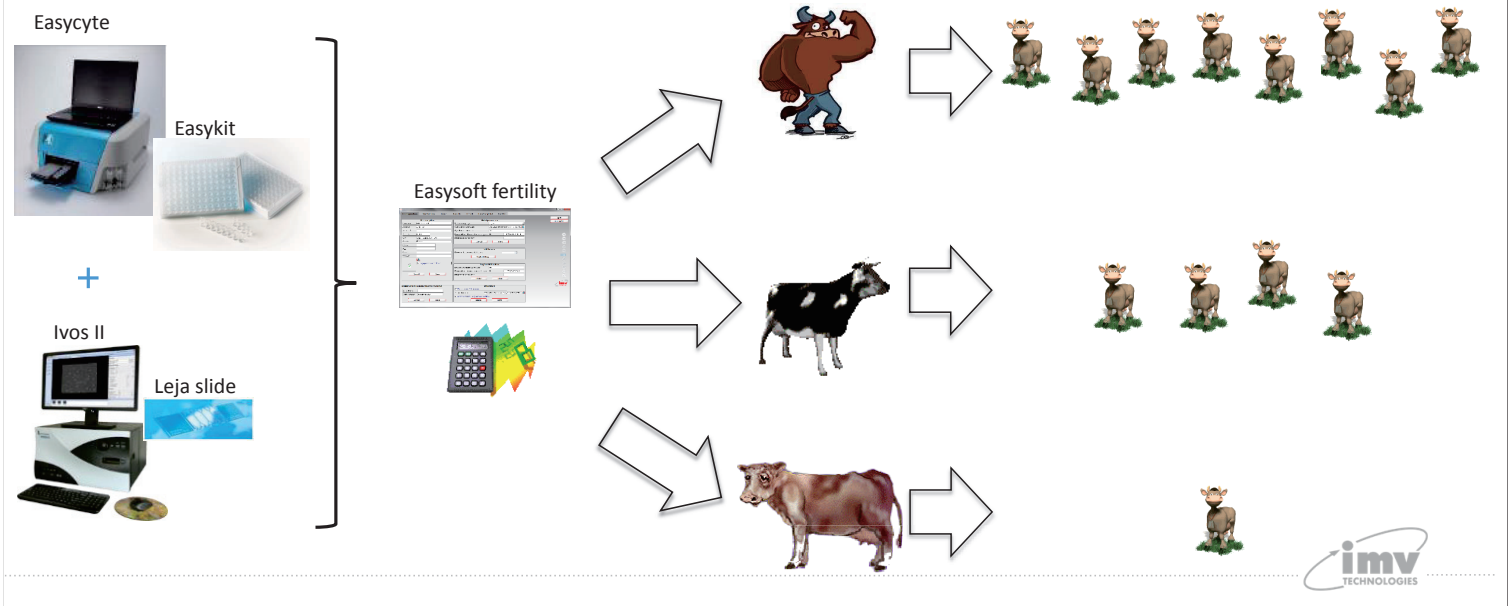
Généré le 22/01/2014
Par LUDIVINE

EasySoft fertility

Results.
FI=1 to 20: really good bulls
FI= -3,9 to 1: good bulls
FI= -30 to -3,9: bad bulls

Animal + N° lot	Valeur	Résultat
# 1	2,4	20
# 2	0,7	20
# 3	-1,4	20
# 4	-4,7	20
# 5	-5,5	20
# 6	-5,7	20
# 7	-3,7	20

Animal + N° lot	Valeur	Résultat
# 35	0,3	20
# 36	-7,3	20
# 37	-3,4	20
# 38	-4,7	20
# 39	-4,4	20
# 40	-1,7	20
# 41	-5,0	20



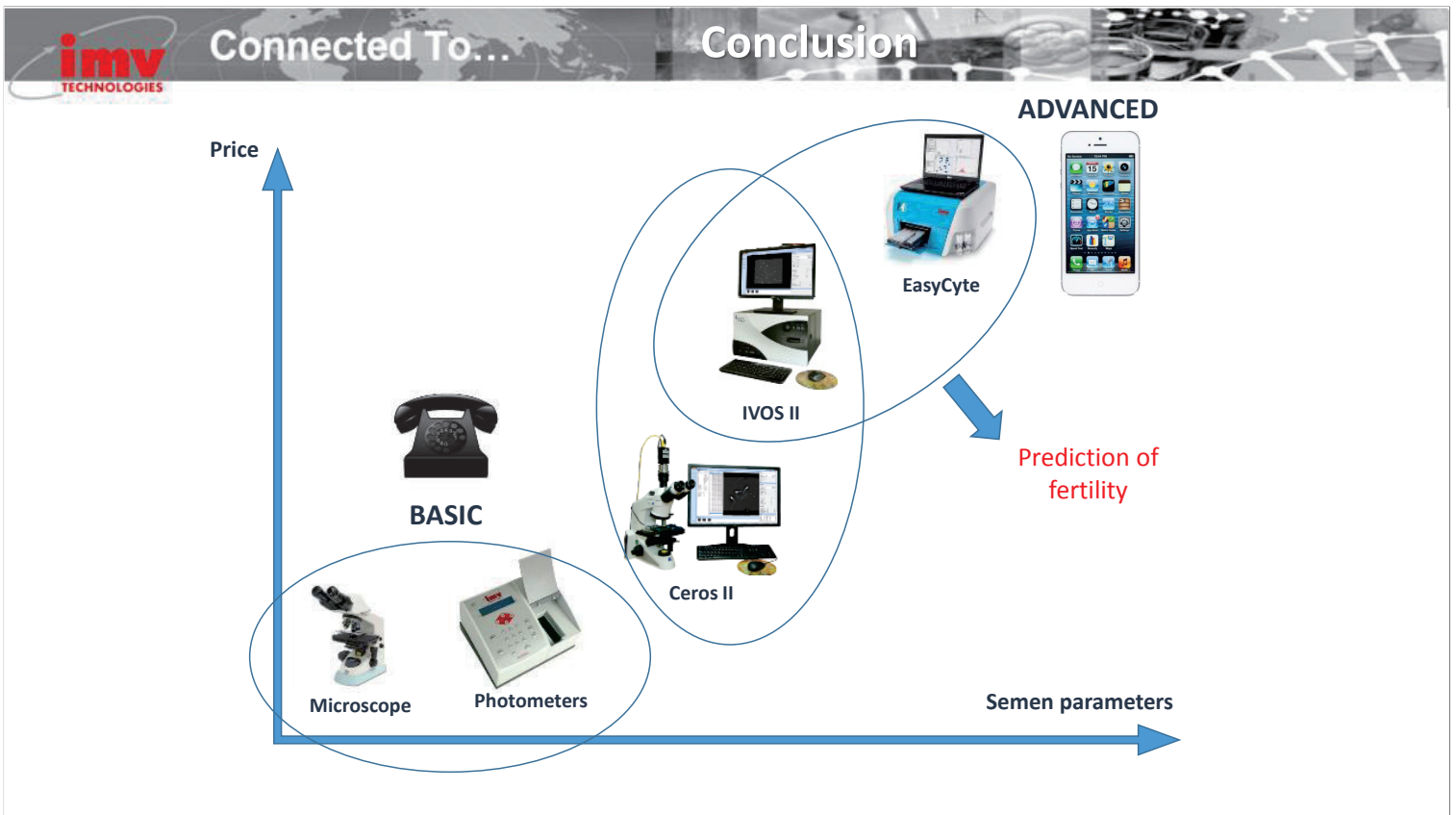
Why to use a flow cytometer ?

- 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

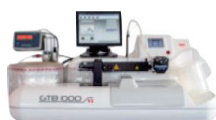
- Adapted for sperm analysis (PMT, laser, software...)
- IMV ready-to-use kits
- Intuitive software package
- Scientific technical support specialized in semen analysis

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



- imv TECHNOLOGIES** Connected To... Semen Analysis by different technics
1. Analysis of Motility, concentration and morphology
 - a. CASA systems: ceros II and ivos II
 - b. Utrecht University / Topigs results
 2. Analysis of different physiological parameters
 - a. Flow cytometer: EasyCyte
 - b. ALLICE, CRV and IMV results
 3. R&D products in development
 - a. Freezer
 - b. Free antibiotic extender: CoolpigXcell

Recent Innovative Products



GTB 1000 V3



NEXCELL
Antibiotic-free



GREENGLOVE
« home compost »



ALPHA SHEATH



ISEVO



COOLPIGXCELL
Antibiotic-free



DISPOSABLE NEEDLES



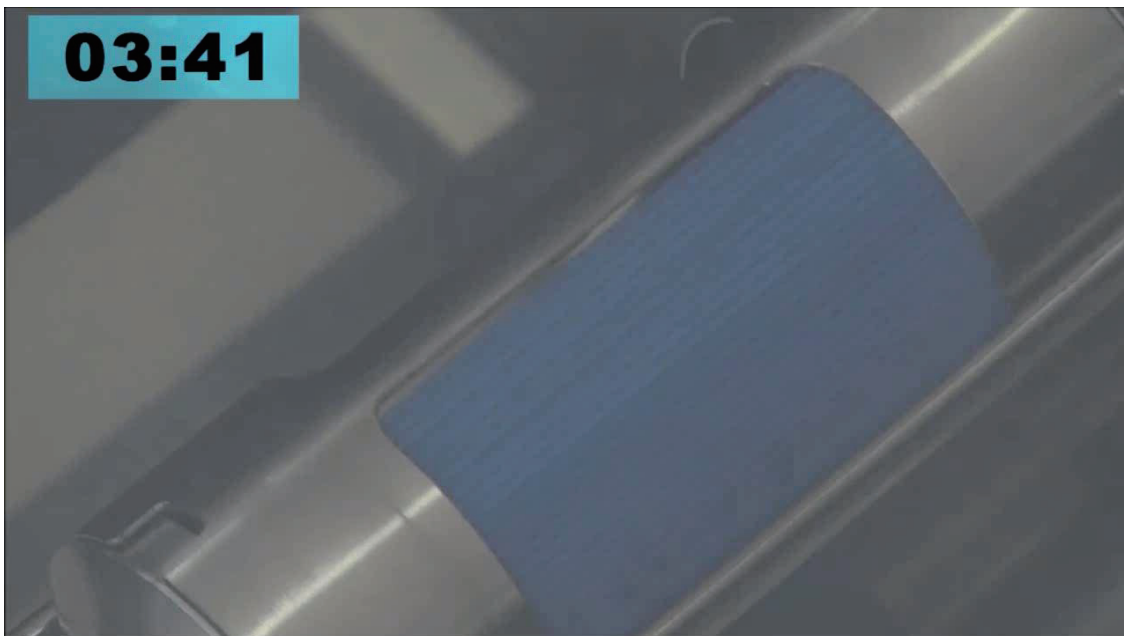
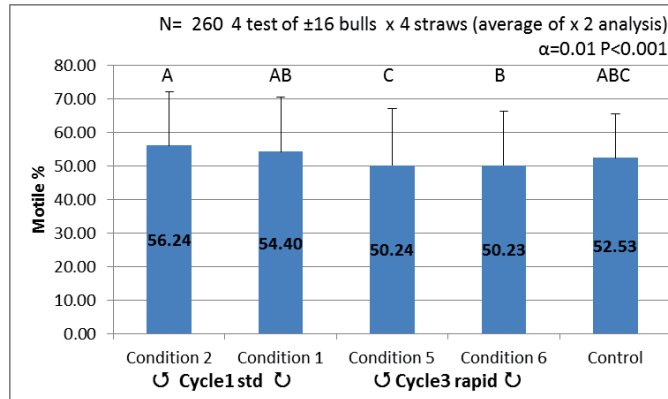
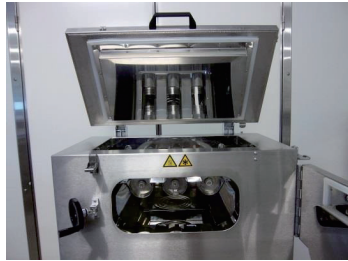
EASYKIT 6
Bacterial concentration



OPTO
ISEVO straw

Connected To... Semen Analysis by different technics

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3. R&D products in development
 - a. Freezer
 - b. Antibiotic Free semen preservation media: CoolpigXcell

- Boar ejaculate: not sterile.
- Mixture of different bacteria, the majority, enterobacteriaceae (Sone et al., 1989).

Table 1
Common bacterial flora isolated from the neat boar ejaculate

Tamuli et al. [7]	Danowski [3]	Dagnall [2]	Sone et al. [5]
<i>E. coli</i>	<i>Staphylococcus</i> spp.	<i>Citrobacter</i> spp.	<i>Pseudomonas</i> spp.
<i>Pseudomonas</i> spp.	<i>Pseudomonas</i> spp.	<i>Pseudomonas</i> spp.	<i>Micrococcus</i> spp.
<i>Bacillus</i> spp.	<i>E. coli</i>	<i>Corynebacterium</i> spp.	<i>Staphylococcus</i> spp.
<i>Staphylococcus</i> spp.	<i>Citrobacter</i> spp.	<i>Streptococcus</i> spp.	<i>Klebsiella</i> spp.
<i>Klebsiella</i> spp.	<i>Providencia</i> spp	<i>E. coli</i>	<i>E. coli</i>
<i>Proteus</i> spp.	<i>Neisseria</i> spp.	<i>Actinomyces</i> -like spp.	<i>Citrobacter</i> spp.
<i>Enterobacter</i> spp.	<i>Proteus</i> spp.	<i>Bacteroides</i> spp.	<i>Proteus</i> spp.
<i>Pasteurella</i> spp.		<i>Lactobacillus</i> spp.	<i>Actinomyces</i> spp.
<i>Citrobacter</i> spp.		<i>Acinetobacter</i> spp.	<i>Serratia</i> spp.
		<i>Bacillus</i> spp.	<i>Enterobacter</i> spp.
		<i>Actinobacillus</i> spp.	<i>Bacillus</i> spp.
		<i>Staphylococcus</i> spp.	<i>Streptococcus</i> spp.
		<i>Flavobacterium</i> spp.	
		<i>Klebsiella</i> spp.	
		<i>Micrococcus</i> spp.	
		<i>Proteus</i> spp.	

Althouse, 2004

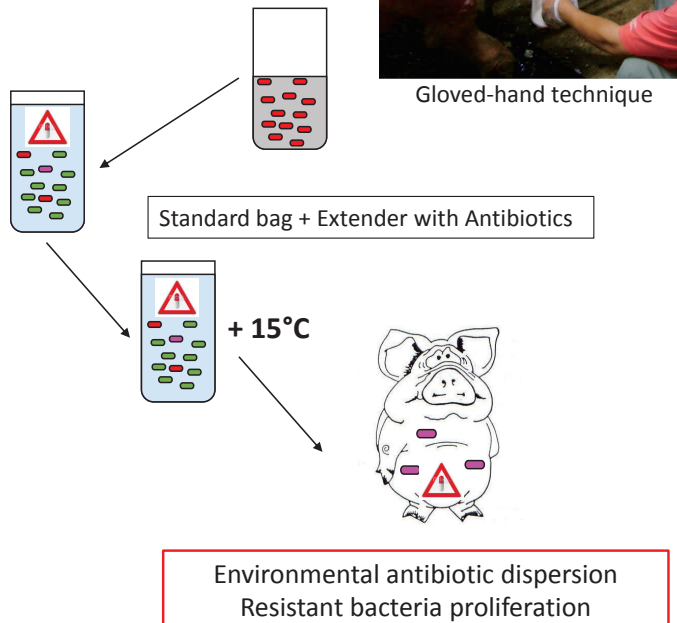
Purpose of antibiotics in extenders

- ✓ To prevent negative effects from bacterial contamination on semen quality
- ✓ To meet EU directive for exchange of genetics between EU countries

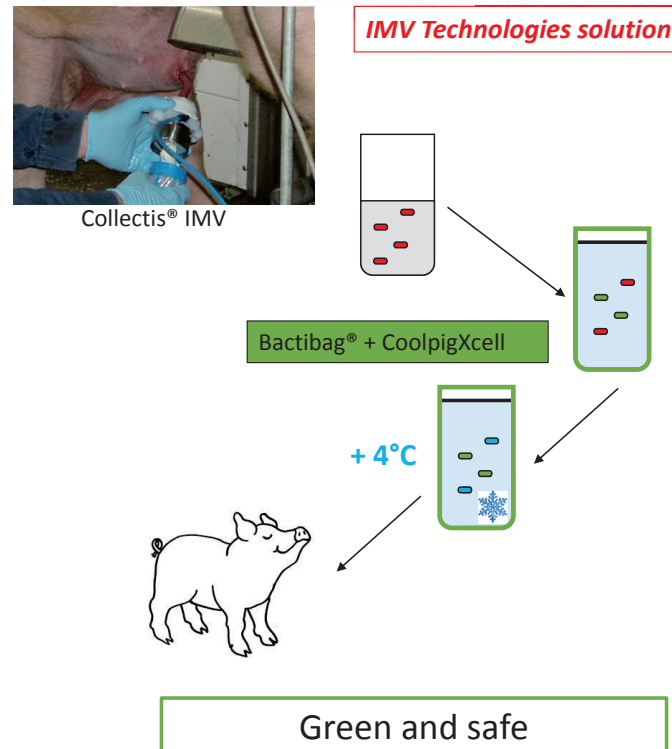
Antibiotic resistance

- ✓ **Resistance** to classic antibiotics such as **penicillin, aminoglycoside, lincomycin** and **spectinomycin** (Bolarin 2010) (Úbeda et al. 2013) (Althouse et al. 2000; 2008), (Schulze et al. 2012).
- ✓ 50% of bacteria also resistant to next generation antibiotics (**enrofloxacin** and **ceftiofur**) (Bolarin 2010; Mozo-Martin et al. 2012).
- ✓ More and more antibiotics will be banned from use in semen extenders in the (near) future.

Standard technique



IMV Technologies solution



Benefit for industry

- ✓ Reduction of antibiotics in total production chain
- ✓ Antibiotic free semen
- ✓ Production chain at 4°C
- ✓ Transport chain at 4°C; best economical option and better transport options of fresh semen export; reduces ecological footprint



BOVINES

Leader mondial des biotechnologies de la reproduction bovine
70% des inséminations bovines réalisées avec des produits IMV

PORCINES

25% des truies inséminées avec les solutions innovantes d'IMV
Principal acteur en Europe et Amérique du Nord

AVICOLES

Leader mondial de la reproduction des volailles et oieaux
Savoir-faire en dindes, pintade, poule, canard, faucon, outarde

EQUINES

150 000 poulains nés grâce à la technologie IMV
28 années d'expérience en reproduction équine

PETITES ESPECES

La référence en reproduction lapine, ovine et caprine
Expertise en canin, caméléon, salmonidé

Thank you for
your attention

