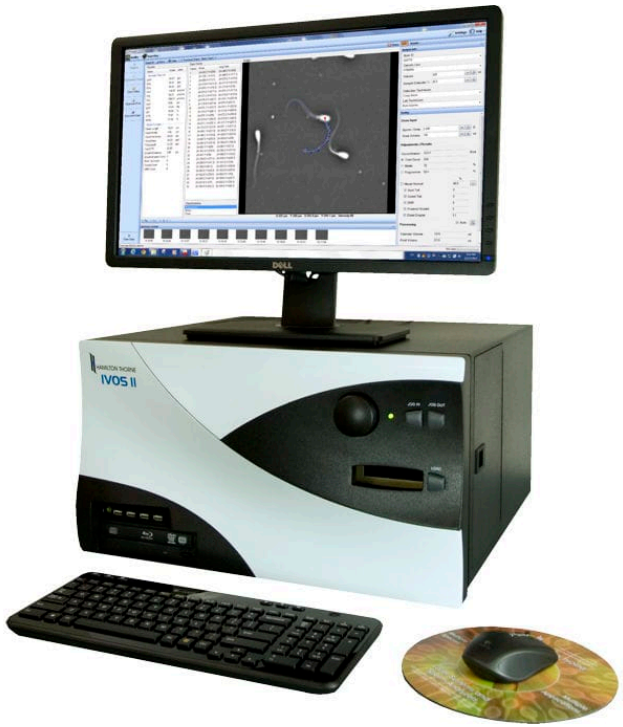
Webinar #01

Training department, v. 20200505

WHY ASSESSING SPERM QUALITY ?
WHY USING A CASA SYSTEM ?

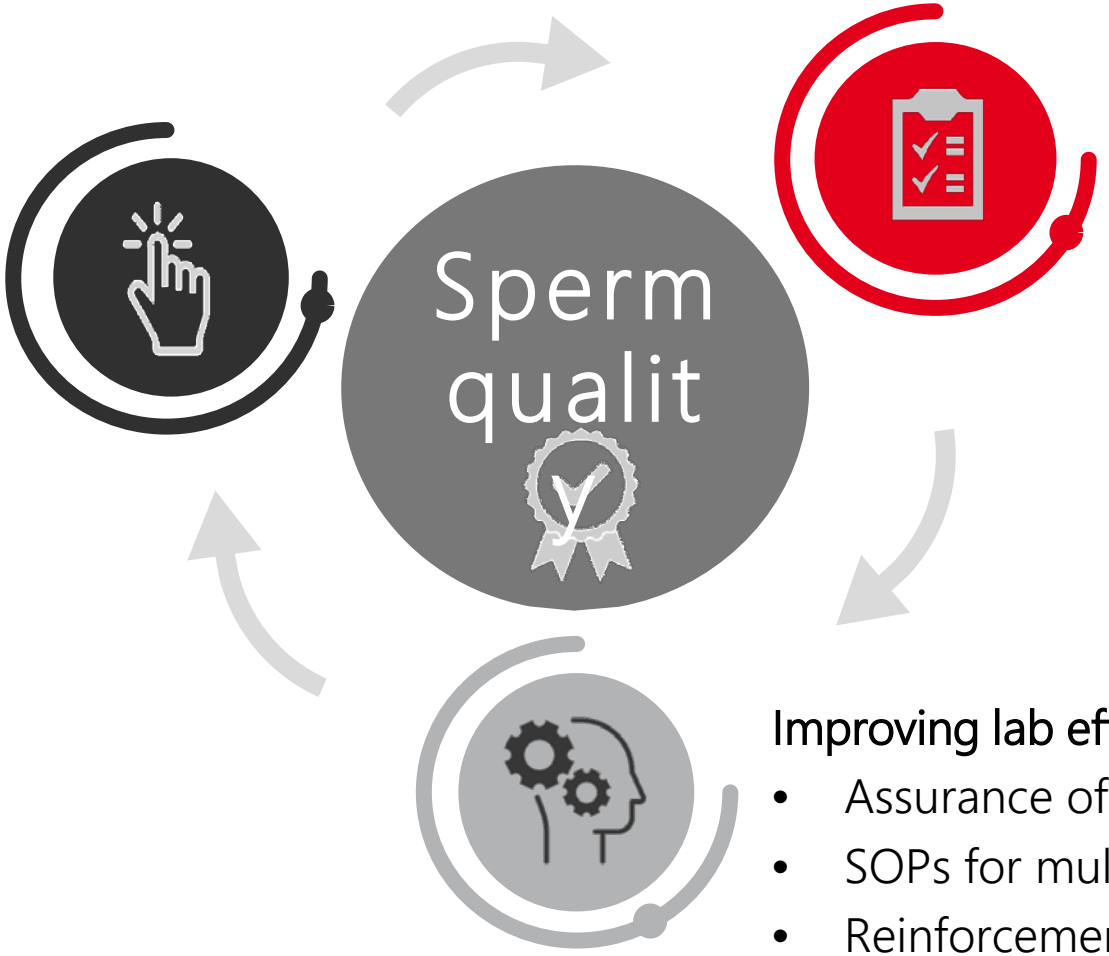


Why assessing sperm quality ?

Hossain et al., 2011; Amann et Waberski, 2014

Enhancing livestock management

- Decision tool to optimize sperm production and to better use high quality genetics
- Diagnosis of the health of the male reproductive organs
- Indication of environment conditions



Standardized tool for protocols testing

- To improve sperm storage conditions
- To optimize fertility rates
- To enhance breeding/rearing management methods

Improving lab efficiency

- Assurance of product quality
- SOPs for multi-sites management
- Reinforcement of the traceability
- To increase the number of doses produced thanks to a more accurate analysis of useful sperm

Why assessing sperm quality ?

Verstegen et al., 2002; Amann et Waberski, 2014; David et al., 2015

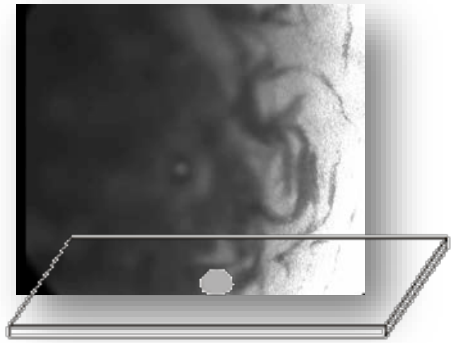
Sperm **motility** as an indicator of quality

Subjective motility

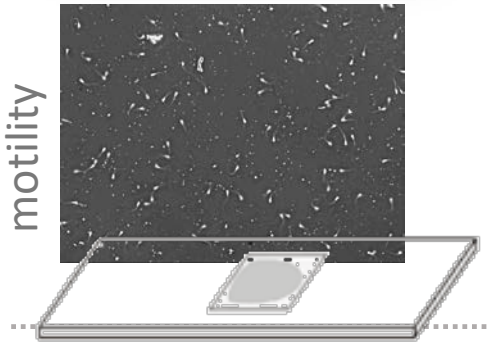
Use of a miscroscope and a human eye

- Time-consuming
- Operator-dependant
- Factors leading to mis-interpreting the results such as :
  - Sperm velocity
  - Sperm density
  - Sperm drift

Mass motility



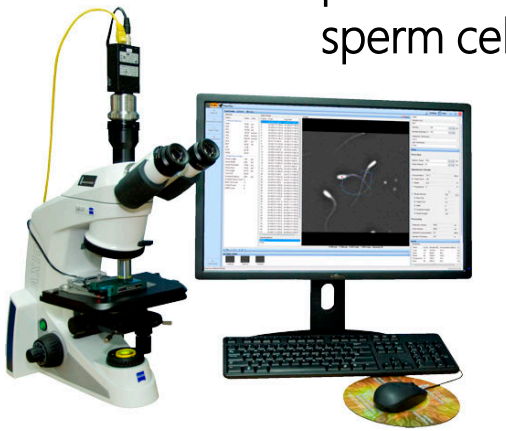
Individual motility



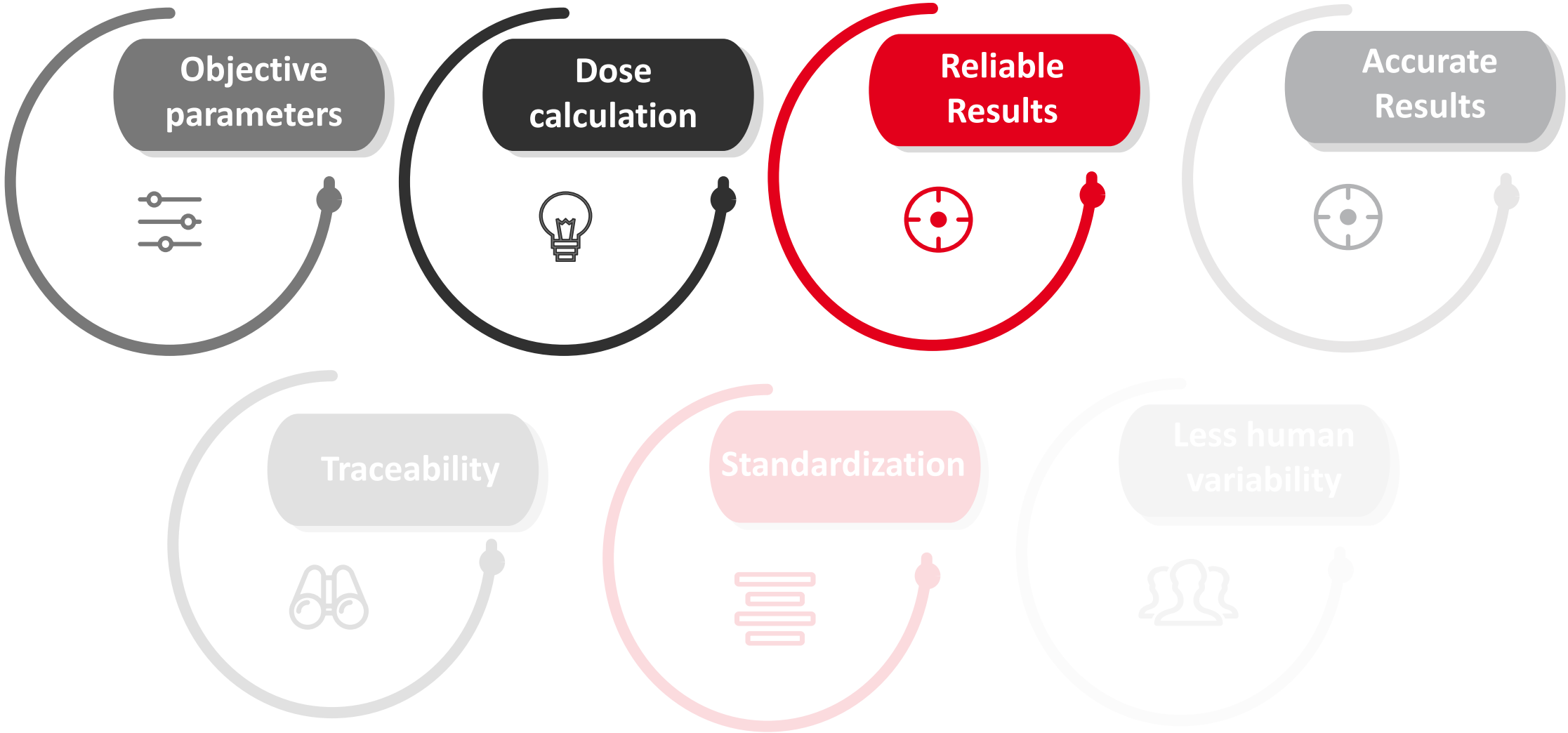
Objective motility

Use of a Computer-Assisted Sperm Analysis (CASA)

- Microscope linked to a camera
- A software able to quantify **multiple motility & kinetics parameters** of a **high number of sperm cells**, from a video



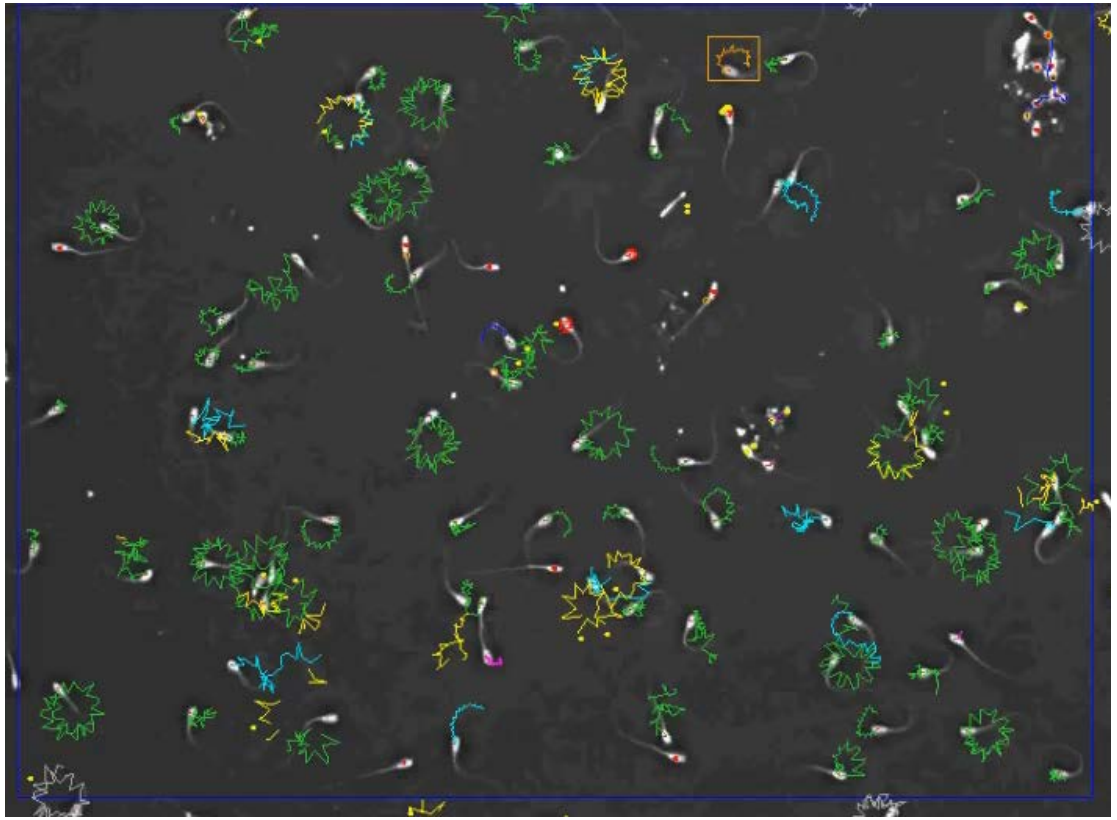
Why using a CASA system ?

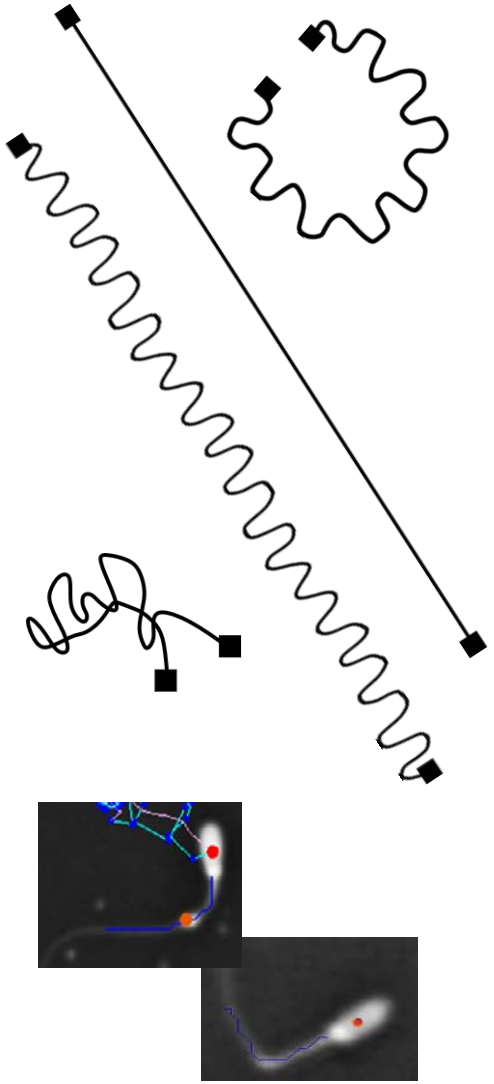


Why using a CASA system ?

Objective parameters

- Sperm tracking (motile/static)
- Kinematic (velocities, head movement)
- Concentration
- Morphologies





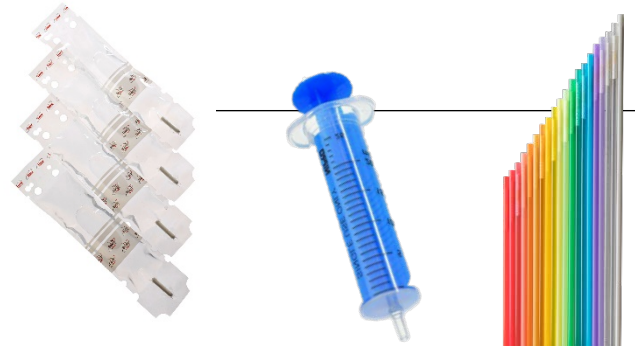
Why using a CASA system ?

Dose calculation

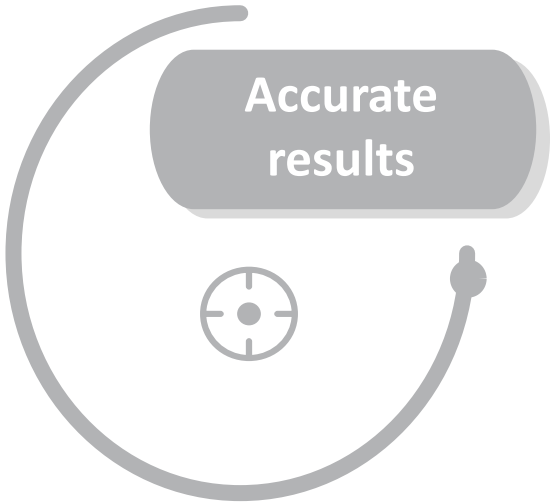
Input Your objectives	Output – Calculation by the computer
Initial concentration	Extender volume
Initial volume	Extender volume
Dose volume Sperm per dose Useful sperm per dose : % motile and/or normal morphology per dose	Dose number

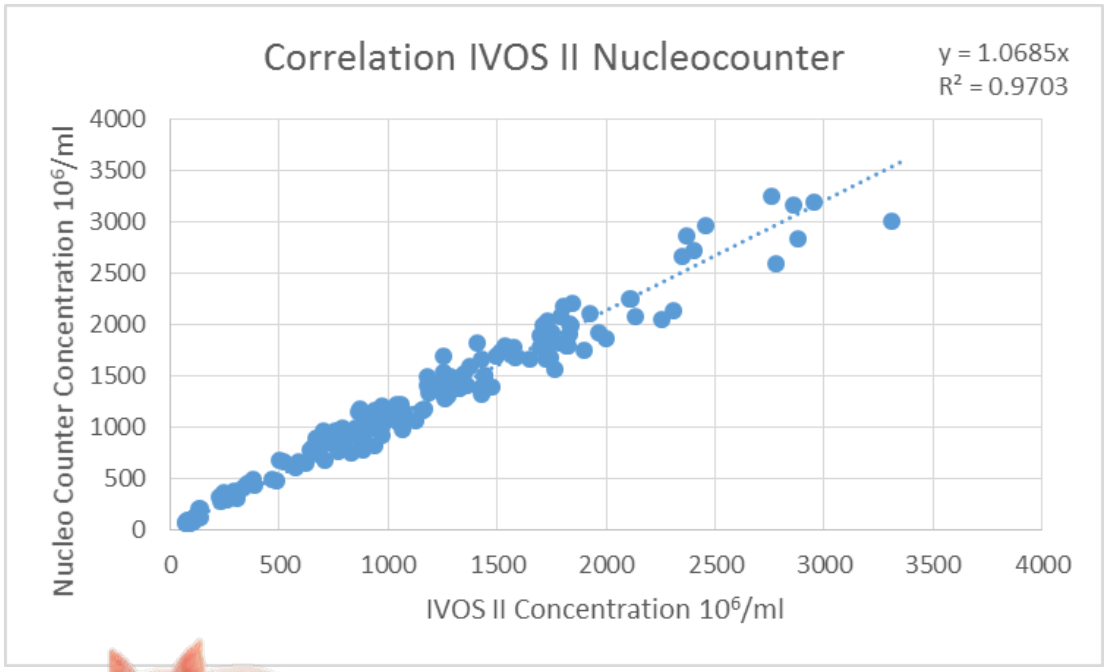
For an optimized stud production

Dose packaging



Why using a CASA system ?



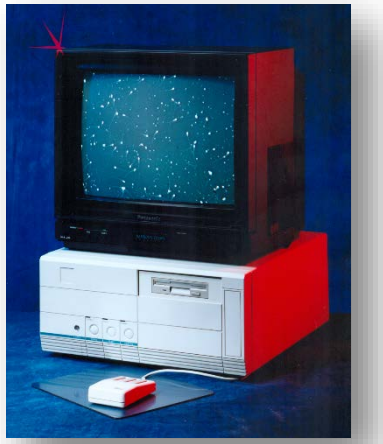


- Accuracy of parameters / results
- Very low CV = SD/Mean (< 10%)

	Pearson correlation coefficient	P	CV%
Concentration	0.96	P<0.0001	5.0
0Motility	0.98	P<0.0001	1.3
Morphology	0.98	P<0.0001	1.1

Why using our IVOS II / CEROS II?

Technical support and training provided



More than 30 years of expertise in sperm analysis

- Strong supplier collaboration
- **High expertise** in reproductive biotechnologies
- Large distribution in bull stud labs of a **standardized setup in Europe** (QualiVet, In Press)

High level training (2 days minimum)

- **Mandatory** for IVOS II & CEROS II installation

Technical & biological support

- **Service contract** for maintenance & re-training (anticipating team turnover)

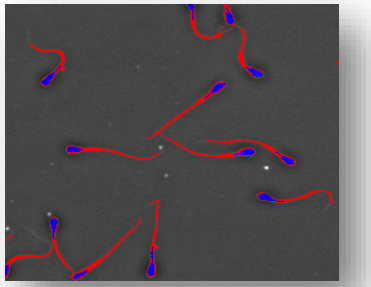
Why using our IVOS II / CEROS II?

DEMO

Unique features of our IVOS II and CEROS II

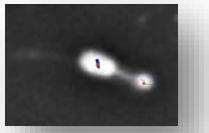
Before analysis

- 'Live overlay' → **heads/background differentiation**
- **Automated illumination** LED regulation (IVOS II)
- **Automated stage** (IVOS II)



During and after analysis

- Automated morphologies analysed, ie. **DMR**
- **Playback** of each field and of **single spermatozoa**
- Several digital image storage (mp4 + hmv)



Fast analysis !

Intuitive software !

Settings

- **Highly customizable** setup
- Use of **egg yolk-based medium** possible
- Possible playback reanalysis with other setup

Why using our IVOS II / CEROS II?

Mortimer & Maxwell, 2004

Options available

Fluorescence options

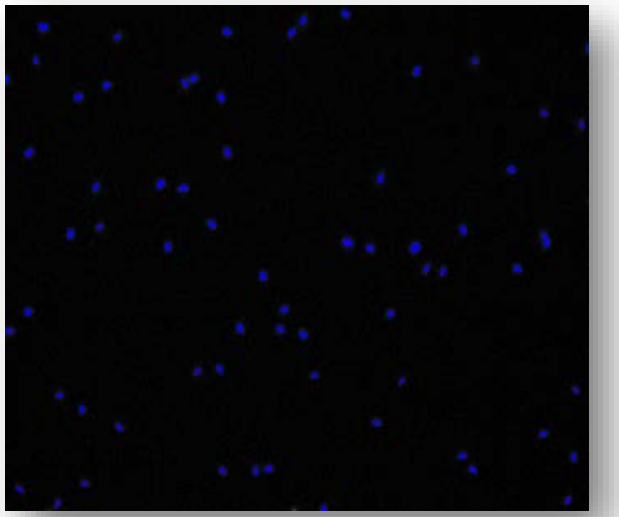
- Viadent® and Ident® = **DNA target** staining
- DNA Frag®

Equipment

- Scale – volume input
- Footswitch – quicker analysis
- eSmile – stud management software

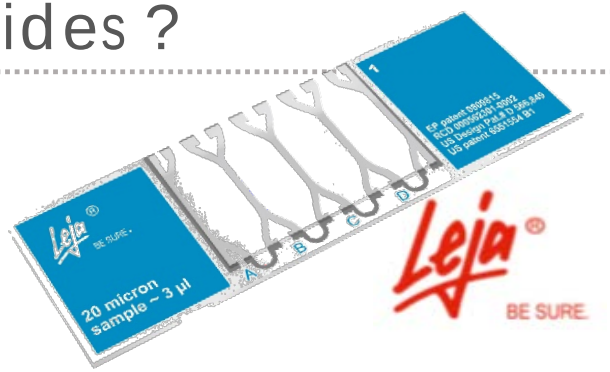
Sort option

- Isolation of sub-populations of cells with customized setup
- Algorithm to detect hyperactivated sperm (Fractal Dimension, ie. FDM)



Dead sperm cells dyed with Viadent® on IVOS II

Why using Leja slides ?



Concentration estimation

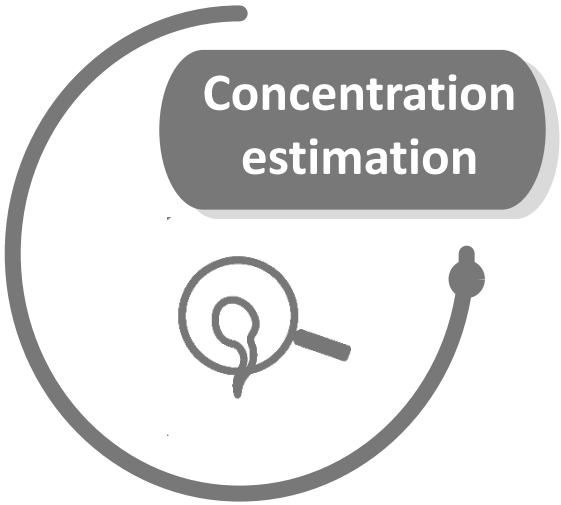
Glue quality

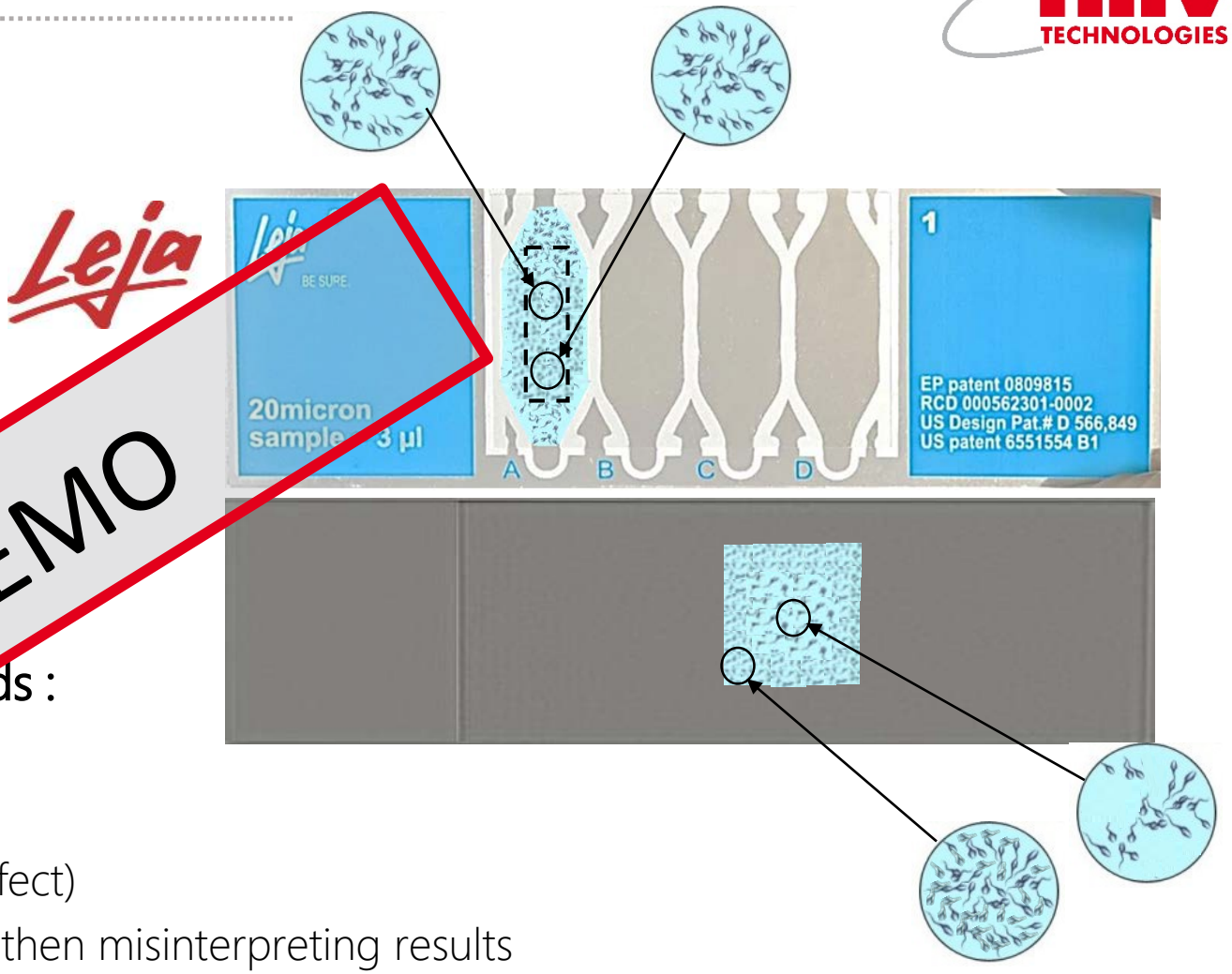
Glass quality

Economic gain

… Resulting in doses at accurate concentration !

Why using Leja slides ?



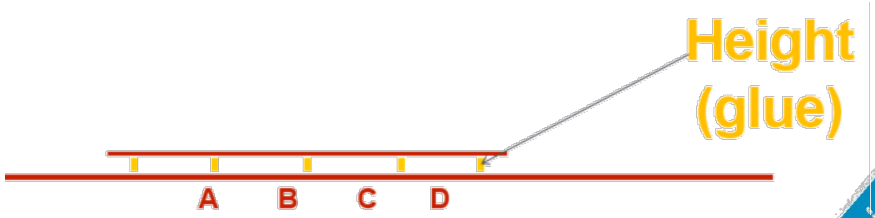


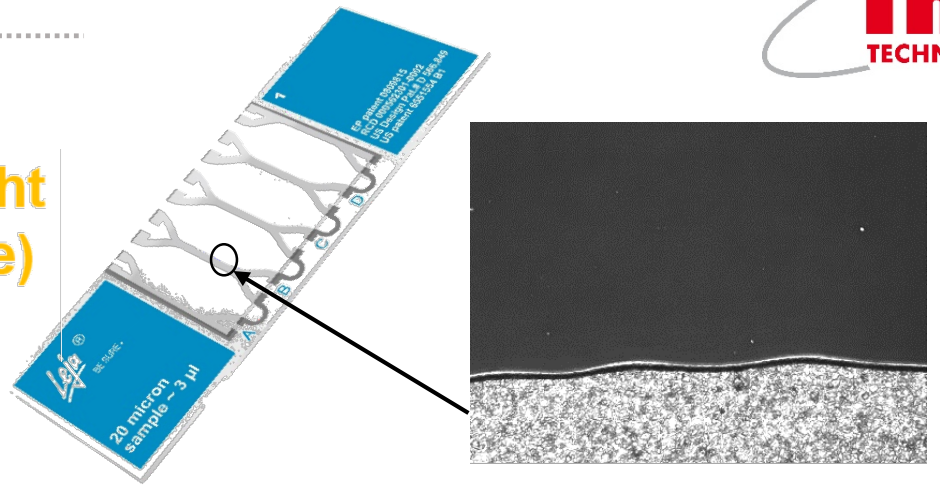
DEMO

- ✓ Compared to slides and coverslips methods :
  - Accurate concentration estimation
  - No air bubbles or agglomerates
  - Homogeneous repartition (no rheotaxis effect)
  - No risk of looking for the best frames and then misinterpreting results
  - Constant height…

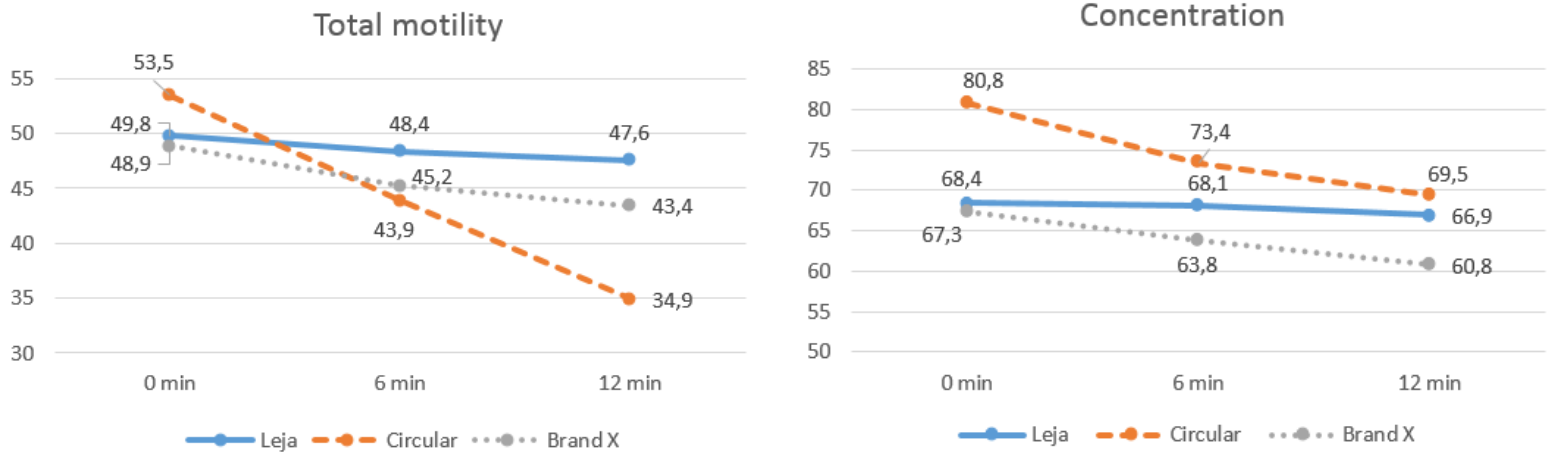
Why using Leja slides ?

Ibanescu et al., 2016





- High quality glue resulting in :
  - Fixed depth counting chamber
  - No coating marks
  - No air bubble development
  - No sperm toxicity
  - Accurate concentration estimation



Total sperm motility and concentration according to the chamber type and time after filling (n = 60).

Adapted from Ibanescu et al., 2016

Why using our range of sperm quality assessment ?

Conclusion

Accurate and reliable control quality of sperm

- Better use of **high quality genetics**
- **High standardization** of your lab procedures
- **Entire range** for sperm analyses for an **enhanced efficiency at the lab**
  (Associated equipment, **Leja® slides**, eSmile, etc.)
- **High level** of technical and **biological support**

Thank you for your attention

imv TECHNOLOGIES

Questions ?

References

Sample loading

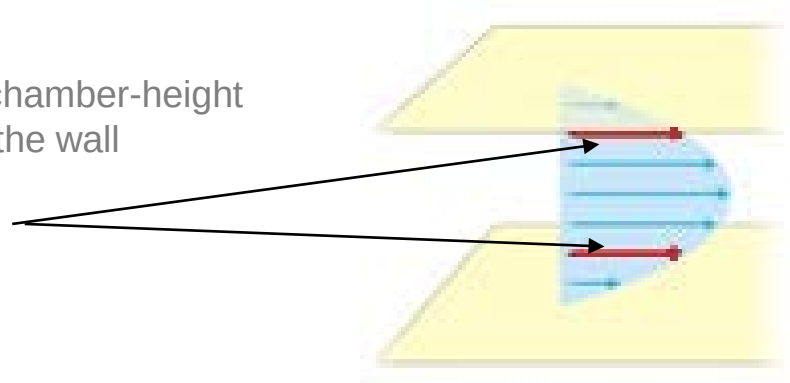
SERGE SILBERBERG EFFECT

The distance of these planes from the wall (β) is depending on a few parameters:

- Development of full Poiseuille flow
- Chamber height
- Surface properties of the counting chamber
- Surface tension
- Fluid viscosity
- Flow velocity
- Diameter of sperm-cell head

Sperm cells in the middle of the chamber-height **move faster** than the ones near the wall

Sperm cells move to two planes at equidistance from each chamber wall

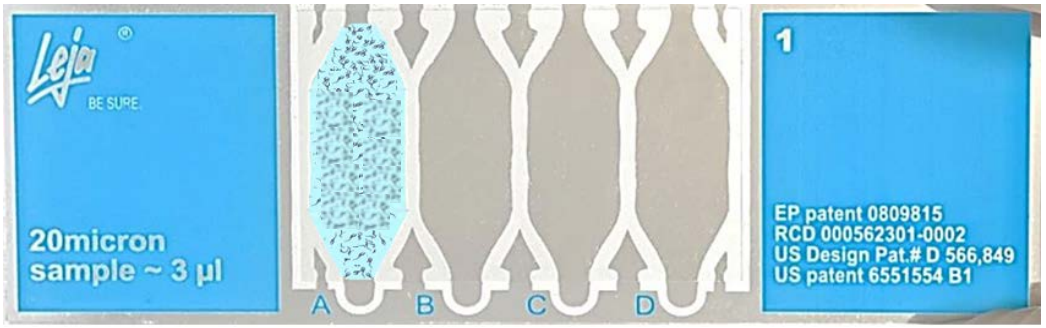


References (to download at www.leja.nl):
Douglas-Hamilton, D.H.; Smith, NG; Kuster, C.E.; Vermeiden, J.P.W.; Althouse, G.C.: Particle Distribution in Low-Volume Capillary-Loaded Chambers, *Journal of Andrology.* 2005;26(1):107-114
Douglas-Hamilton, D.H.; Smith, NG; Kuster, C.E.; Vermeiden, J.P.W.; Althouse, G.C.:Capillary-Loaded Particle Dluid Dynamics: Effect on Estimation of Sperm Concentration, *Journal of Andrology.* 2005;26(1):115-122

Sample loading

SERGE SILBERBERG EFFECT

Sperm cells in the 2 Segre Silberberg planes move faster than the average fluid velocity → Accumulation of sperm cells at the filling front.



When measuring the sperm concentration in the center of the Leja slide, an **underestimation of the concentration** takes place.

This is a **constant underestimation** that can be **corrected**!

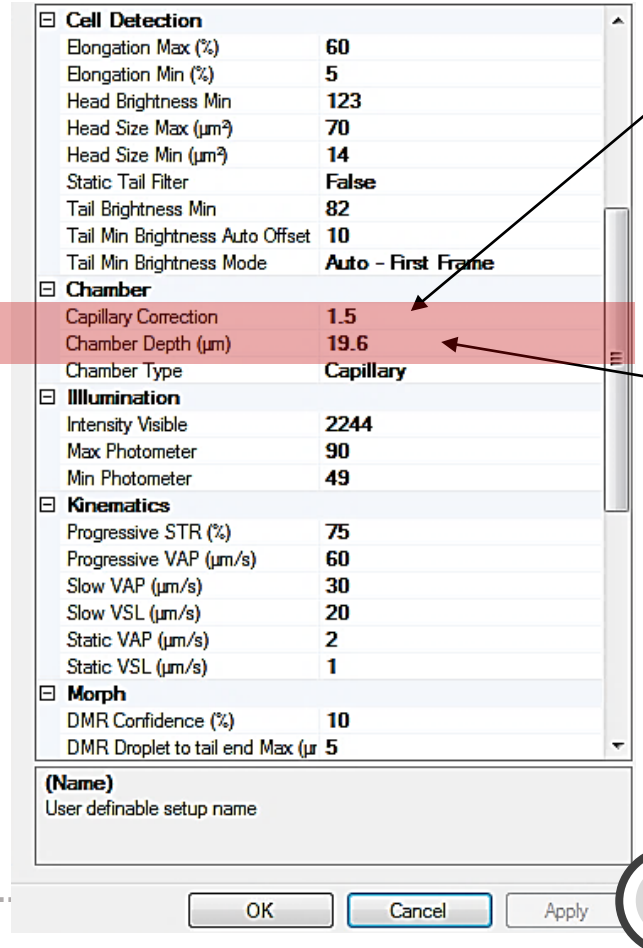
References (to download at www.leja.nl):

Douglas-Hamilton, D.H.; Smith, NG; Kuster, C.E.; Vermeiden, J.P.W.; Althouse, G.C.: Particle Distribution in Low-Volume Capillary-Loaded Chambers, *Journal of Andrology.* 2005;26(1):107-114

Douglas-Hamilton, D.H.; Smith, NG; Kuster, C.E.; Vermeiden, J.P.W.; Althouse, G.C.:Capillary-Loaded Particle Dluid Dynamics: Effect on Estimation of Sperm Concentration, *Journal of Andrology.* 2005;26(1):115-122

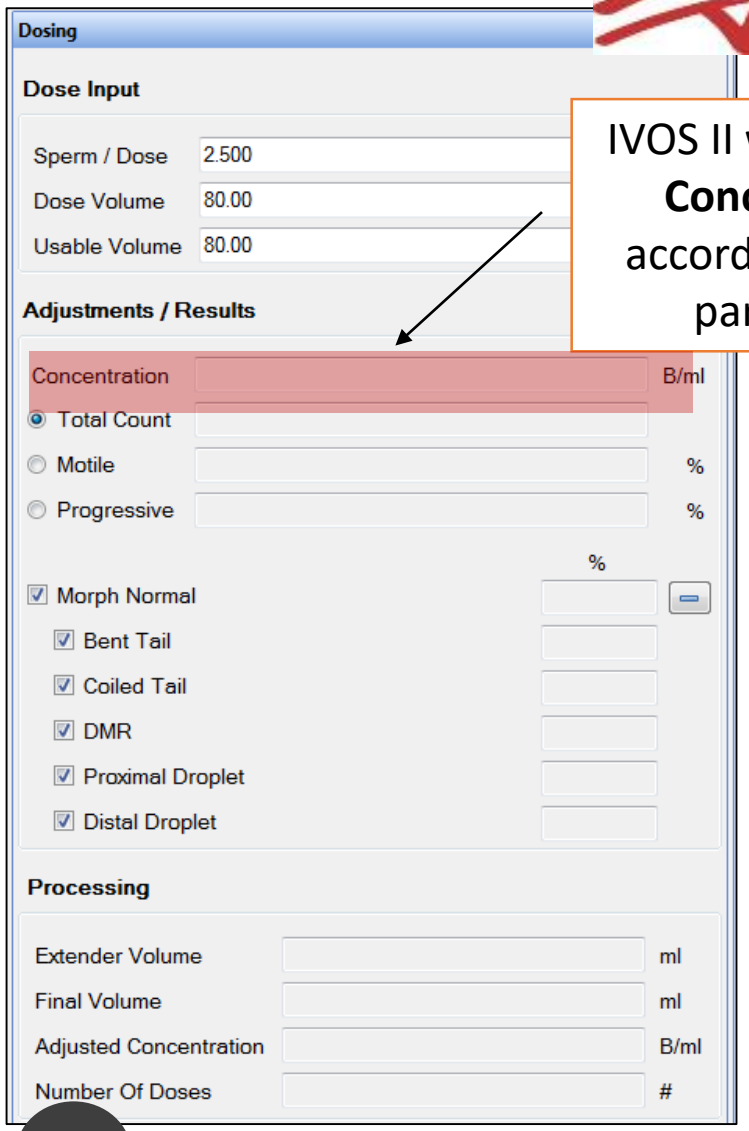
Sample loading

SERGE SILBERBERG EFFECT



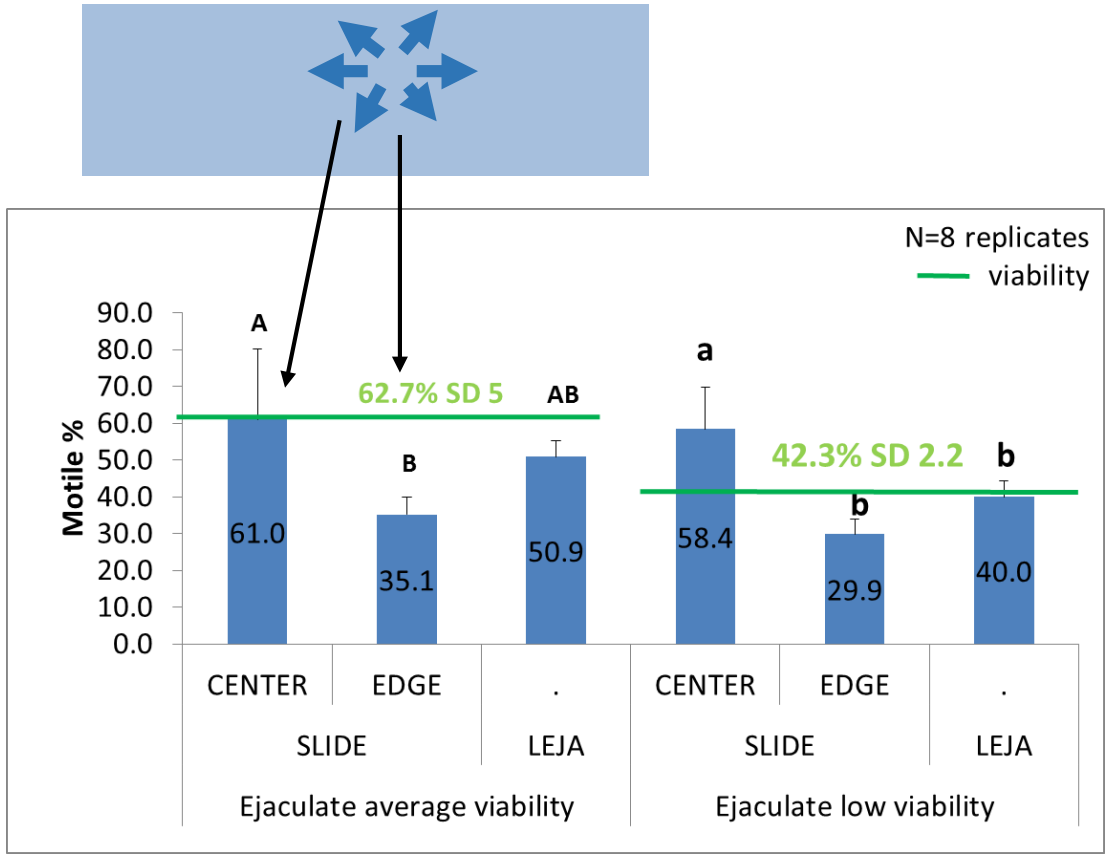
This Sildeberg effect is **taken into account in** the software

Chamber Depth has to be correct



IVOS II **will calculate Concentration** according to these parameters

Sample loading

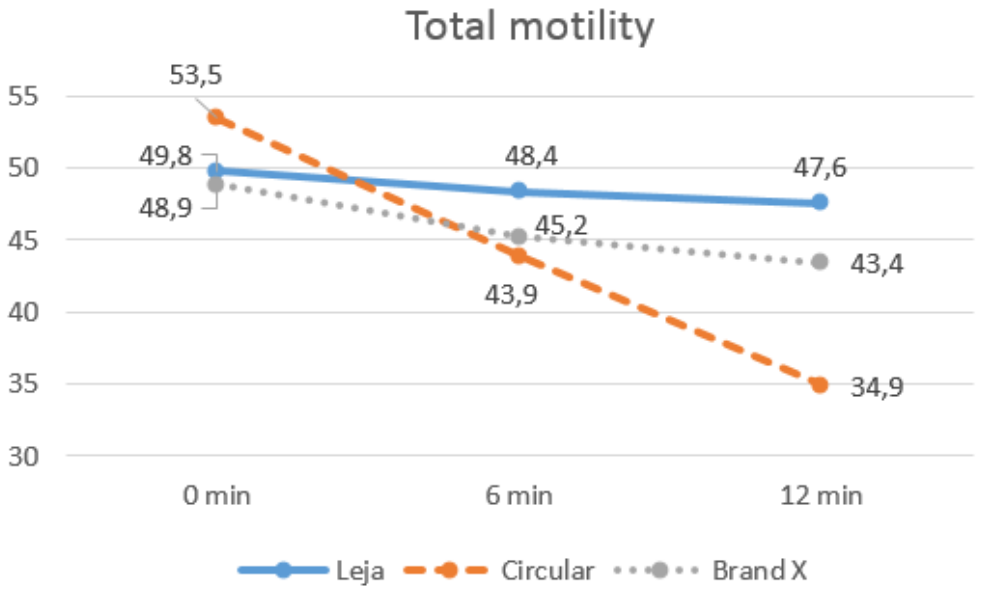


Slide + Coverslip leads to **misinterpretation**

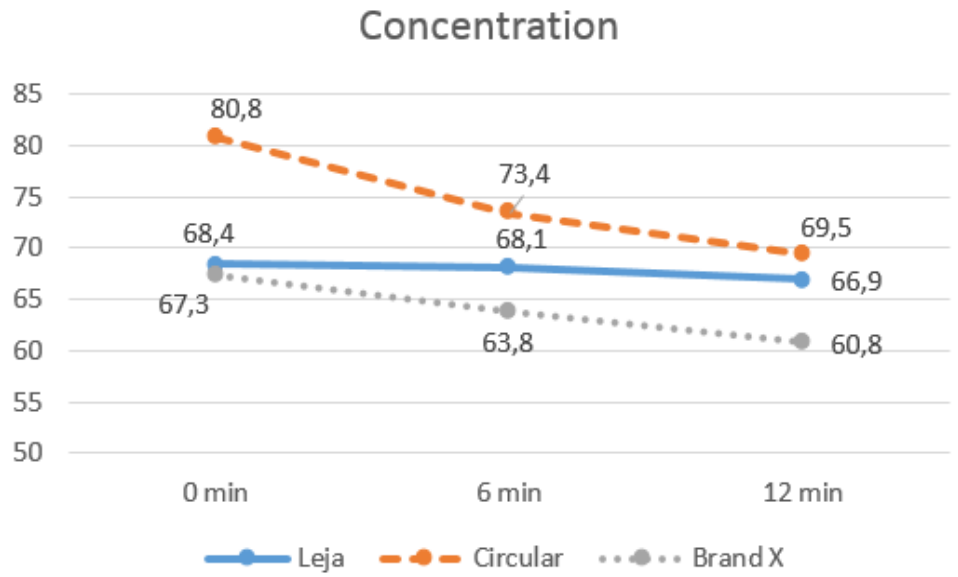
Leja® gives more precise and repeatable results

Sample loading

Ibanescu et al., 2016



Total sperm motility according to the chamber type and time after filling (n = 60).

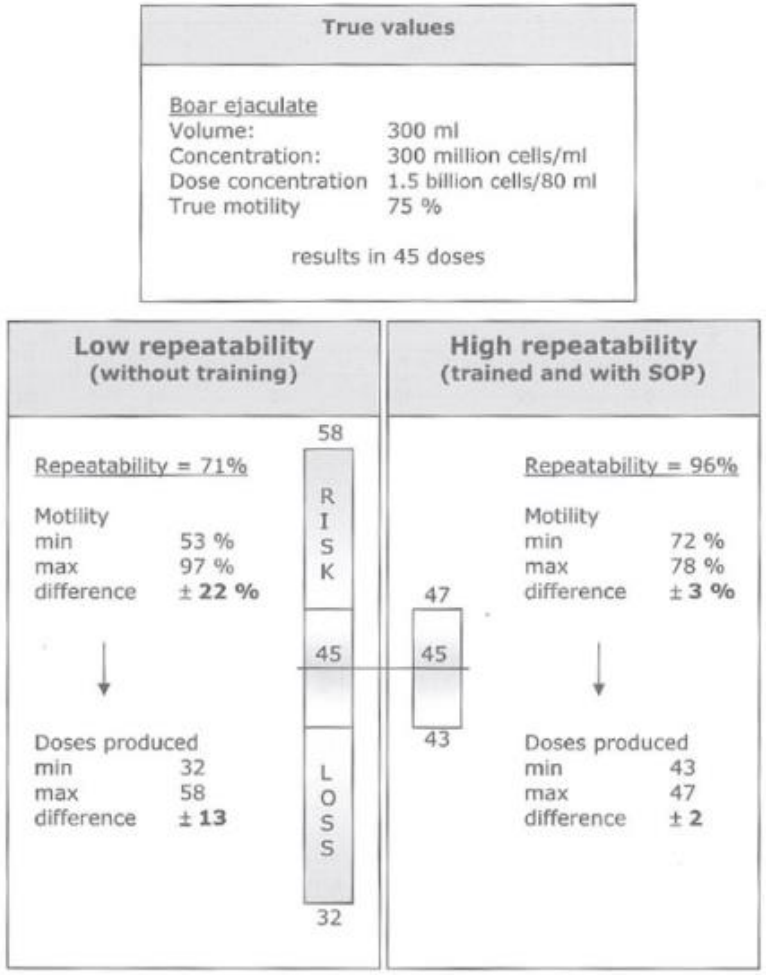


Sperm concentration according to chamber type and time after filling (n = 60).

"Reliable results (motility and conc.) for Leja after 6 and 12 min ($p < 0.05$)"

Circular and Brand X effect of **toxicity** over the time and sperm distribution

CASA TRAINING



Low repeatability consequence :

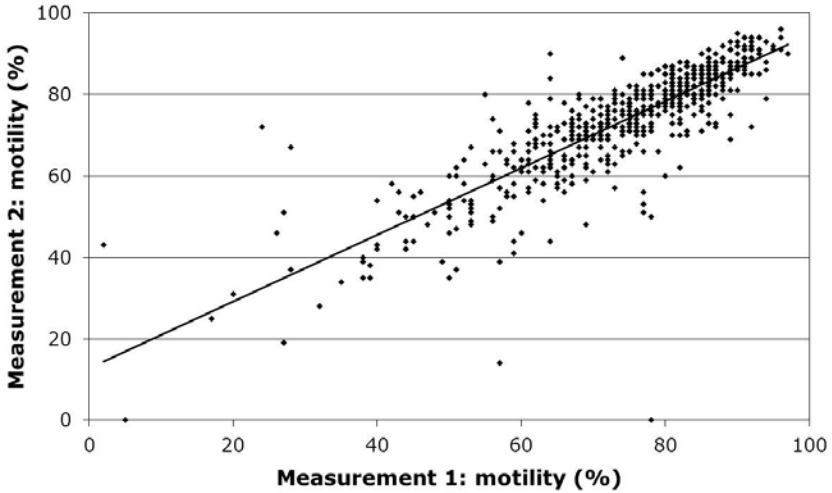
overestimated values for the number of doses
⇒ risk of insufficient fertility (insufficient concentration)

underestimated values for the number of doses
⇒ risk economic losses for the center (insufficient number of doses produced)

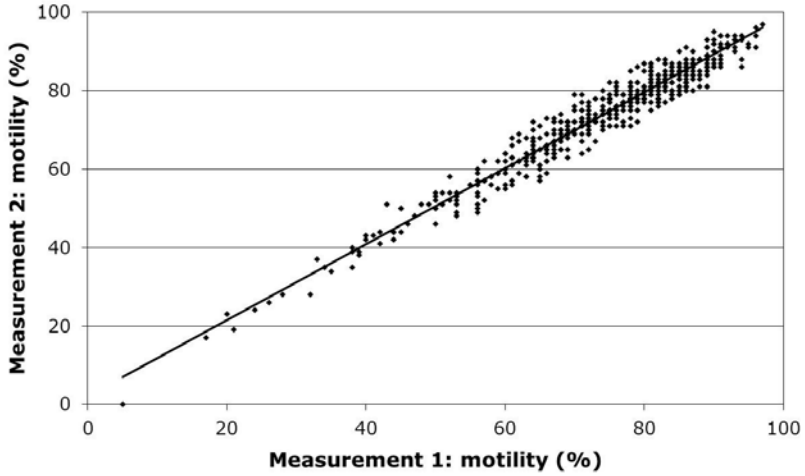
Broekhuijse et al., 2011

CASA TRAINING

Broekhuijse et al., 2011



Before training



After training