Semen Production: Review of techniques from collection to packaging

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Magapor SL

Which is one of the main targets of the semen production?

Maximize the sperm viability → **Maintain the reproductive potential of seminal doses**

Analysis

Collection

Dilution

Multiple factors affecting

Transport

Storage/Conservation

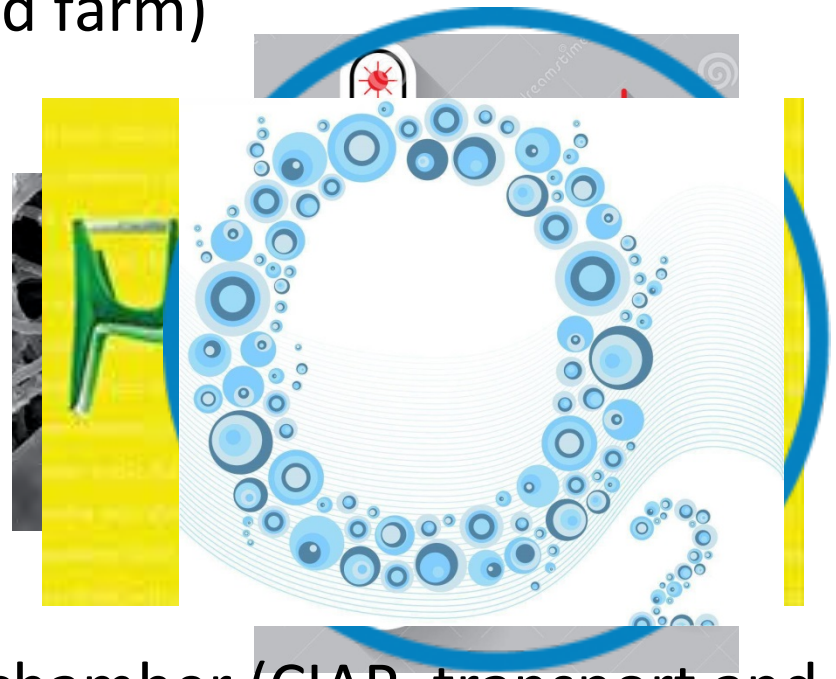
Enemies of seminal doses

Temperature (CIAP, transport and farm)

Workforce (CIAP)

Bacteria (CIAP)

Toxic materials (CIAP)



Storage: semen rotation and air chamber (CIAP, transport and farm)

Aspects to review

Quick and accurate osmolality check (workforce)

2 step dilution (temperature)

Effect of air chamber (storage)

Semen dose rotation (storage)

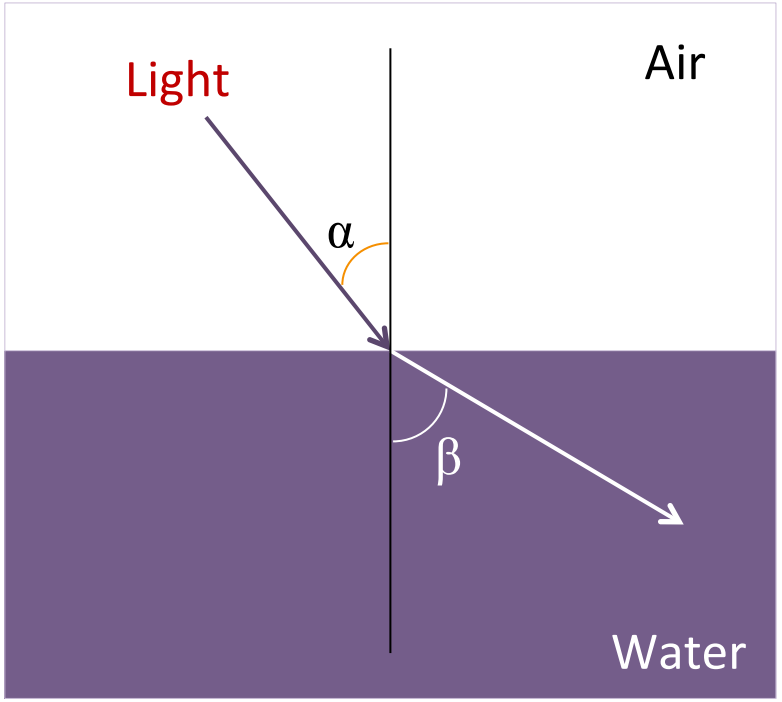
Quick and accurate osmolality check

Quick and accurate osmolality check

Measure based on the refraction of the light

Light changes its direction when it changes of medium

Depending on the media, the angle is different



Quick and accurate osmolality check

Correlates with osmolality

Fast and low cost

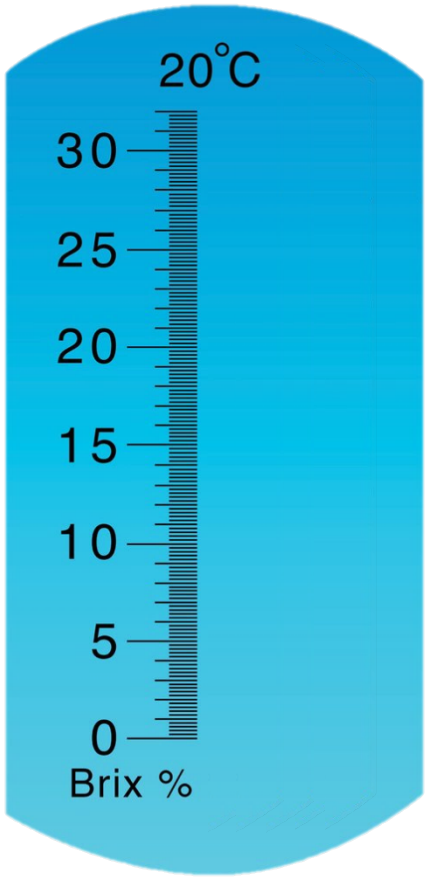
1.- Place sample to test (extender)

2.- Look through to measure

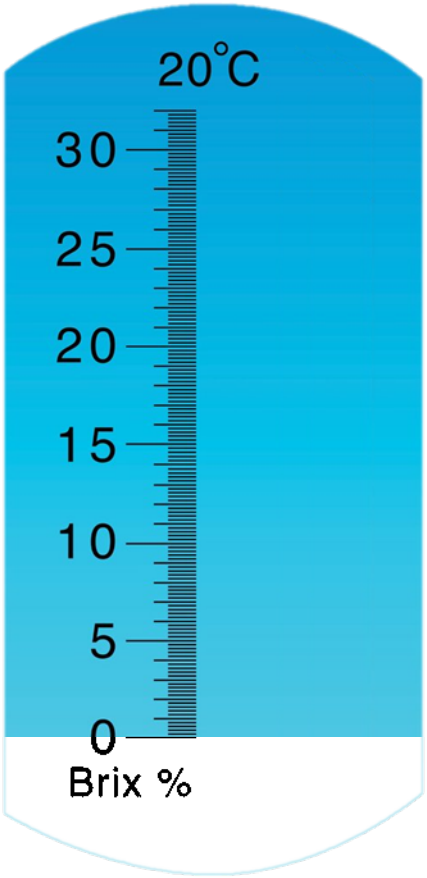
Refractometer

Quick and accurate osmolality check

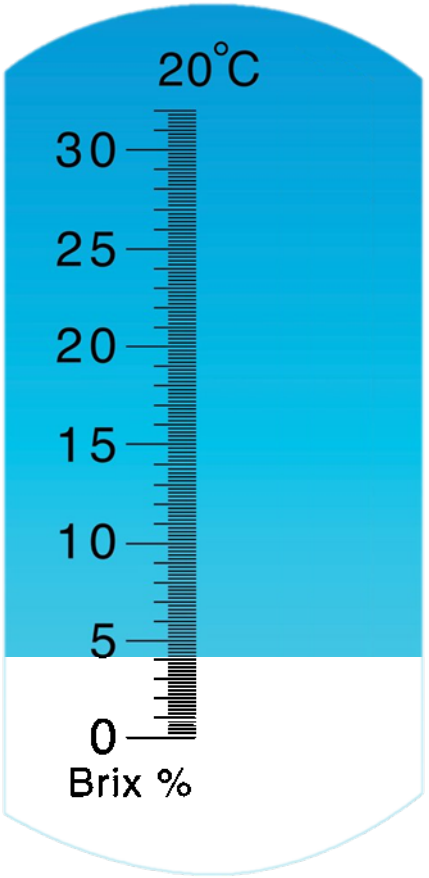
Empty



Water (set zero)

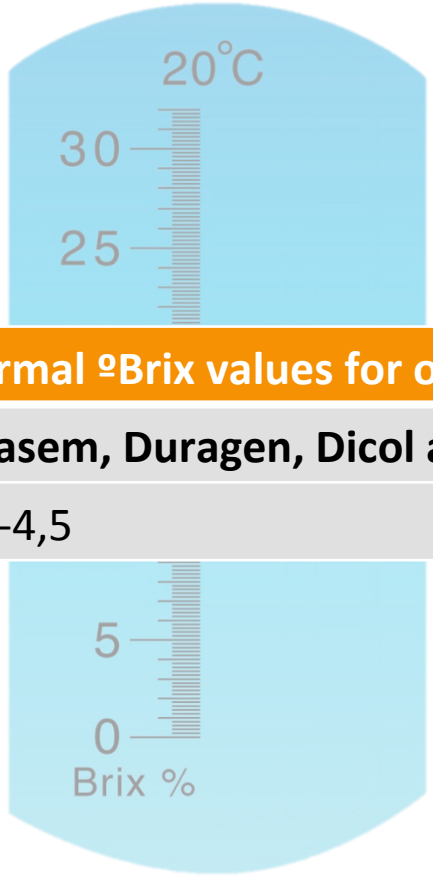


Extender



Quick and accurate osmolality check

Empty



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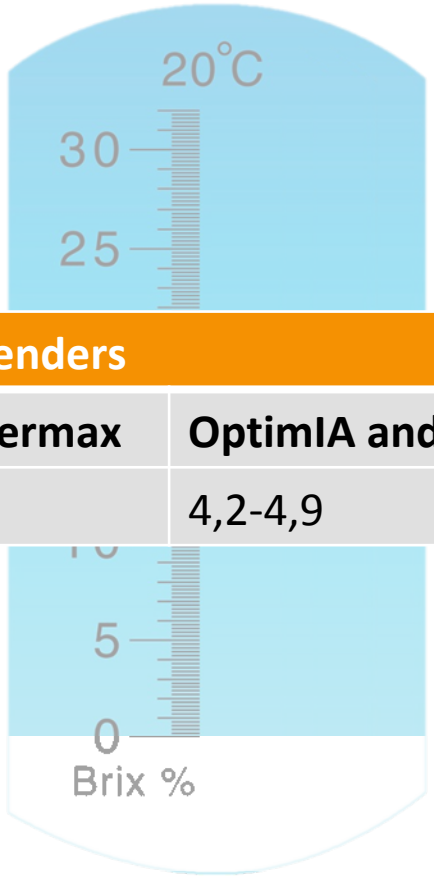
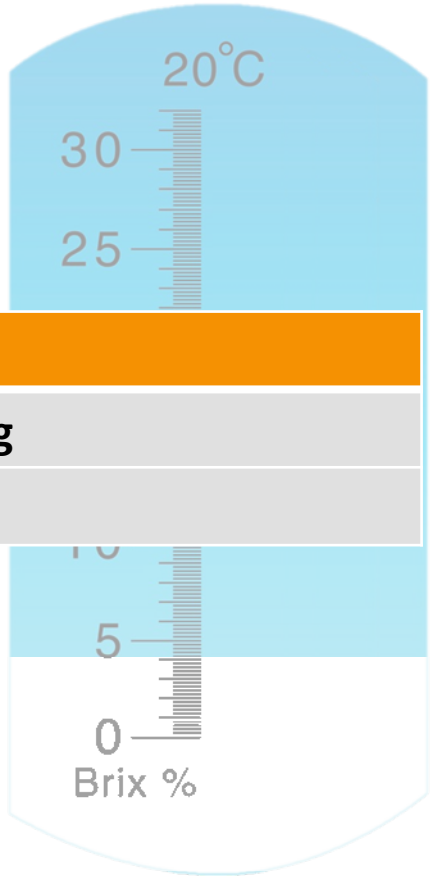


Extender



Normal ºBrix values for our extenders	
Vitasem, Duragen, Dicol and Spermax	**OptimIA and Biopig**
3,8-4,5	4,2-4,9

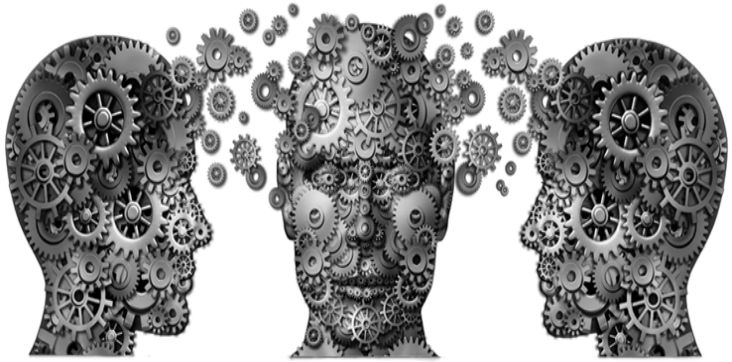
2 step dilution

2 step dilution

Introduction

The main paradigm in boar sperm dilution…

Difference of T^e between ejaculate and extender must not be more than ± 1-2°C



Sperm membrane damage

Introduction

Most common practices to avoid rapid chilling

One step dilution (33-37°C)

Isothermic two step dilution **(ITSD)**

1:1 Dilution 33-37°C

Final dilution 33-37°C

Hypothermic 2 step dilution (HTSD) Introduction

Raw ejaculate (35-37°C)

↓

1st: 1:1 dilution with extender (33-35°C)

↓ Keep at lab T^e

2nd: Final dilution with extender at lab T^e (21-25°C)

Advantages

Introduction

- Avoid **warming** large volume of **extender**
- **Doses stabilized** at 15-17°C before **delivery** →

  Avoid posible problems
- Posible **reduction** of the **contamination**????

2 step dilution

What we know up to now?

A. López et. al, 2012

M. Schulze et. al, 2013

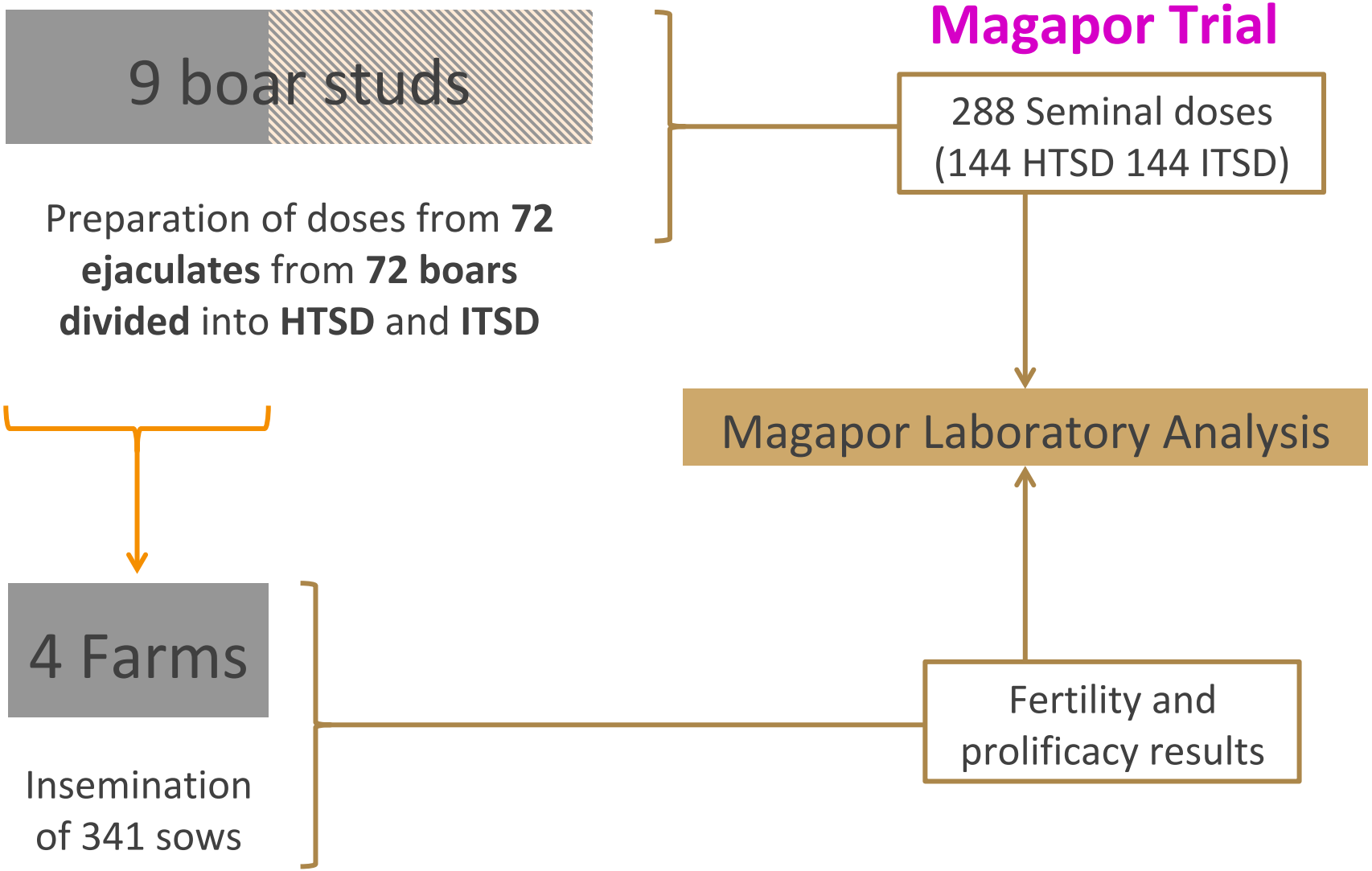
F. Bortolozzo et. al, 2015

M. Schulze et. al, 2018

Conclusions

- No differences in terms of sperm quality
- Differences between HTSD vs ITSD → Extender factor (BTS VS long term)
- Marked advantage with the one-step dilution

2 step dilution



2 step dilution

AI stud (9):

Raw ejaculate

Motility (>80%)
Morphology (>75%)

Semen dose:

Extender: Duragen®
Volume: 45 ml
Concentration: 1,5b
Tª every 15' (90 min)

Sow farms:

Fertility at 28 days
Farrowing rate
Prolificacy

Magapor laboratory analysis (days 1-3-7-9)

Sperm concentration and morphology

(1 day)

Motility

CASA System TM (>70%)-PM (>40%)

Flow citometry:

Viability (>80%)
Early apoptosis (<14%)
Reacted acrosome (<20%)
Mitochondrial activity (>70%)

Other test:

SHOST (>50%) and ORT (>70%)

2 step dilution

Use of bags instead of tubes


REVIEW Open Access

CrossMark

Boar management and semen handling factors affect the quality of boar extended semen

Alfonso Lopez Rodriguez*, Ann Van Soom, Ioannis Arsenakis and Dominiek Maes

It has been shown that the **type** of **container** will **influence cooling rate** and it seems that **bags need less time** to **reach 17 °C compared to tubes**

Willenburg K, Schindler J, Formo R, Gary B, Ozeboom K. Comparison of extended boar semen cooling rates for semen packaged in bags and tubes. In: Proceedings of the 42nd annual meeting of the American Association of Swine Veterinarians; 2011. p. 397.

Conclusions

No influence of the **dilution technique** in laboratory or farm results

Proper perform of all the **steps** of the **dilution** procedure is needed to assure the semen doses quality

Not all extenders are able to undergo this process

Membrane protectors and **antioxidants** of **high performance extenders** (Duragen®) could have an important **role**

Effect of air chamber

Effect of air chamber

Air Contact Influences the pH of Extended Porcine Semen

P Vyt[1], D Maes[2], SU Sys[3], T Rijsselaere[2] and A Van Soom[2]

[1]*Medic Lab, Aalst, Belgium;* [2]*Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University, Merelbeke;* [3]*Department of Pharmacology, Toxicology, Biochemistry and Organ Physiology, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium*

In this study, the **pH-rise during storage of extended porcine semen** was examined. This pH-rise was found to be **caused by CO2-loss from the buffering system in the extender** and was **more pronounced** with **increasing air volume** in the recipient. An **influence** on **sperm** motility parameters was observed between semen samples stored in the **presence** of **different amounts** of ambient **air** in the recipient. **Velocity parameters and percentage motile spermatozoa were significantly lower for semen stored in recipients with higher air volume and elevated pH**. Adjusting extender preparation by avoiding air contact in commercial AI-centres may minimize the pH-rise and its influence on sperm motility.

Effect of air chamber

Overproduction

ROS

- **< Antioxidant defence**
- **< Motility**
- **< Viability**
- **< Alt. DNA**

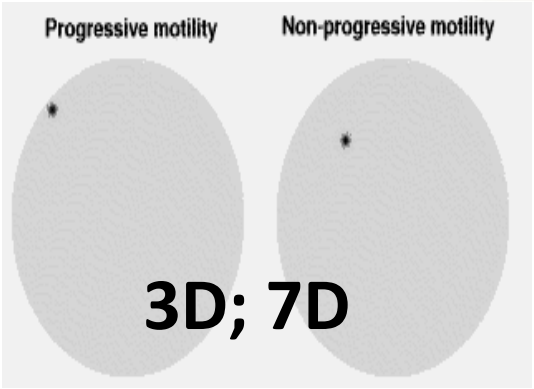
Experimental design:

Magapor Trial

N=26

AIR CHAMBER (1/3)

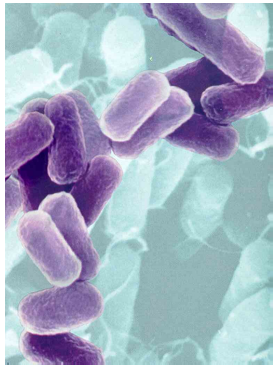
16ºC



3D; 7D

pH; 7D

MDA; 7D



7D

Effect of air chamber

Results:

D7

Magapor Trial

	With Chamber	Without chamber	p-value
TM	18,92 ± 31,57	46,30 ± 32,82	0,0029
PM	10,88 ± 20,35	23,65 ± 23,77	0,0302
pH	7,38 ± 0,58	7,14 ± 0,29	0,0384
MDA	9,38 ± 3,12	7,74 ± 2,30	0,0379

No differences in bacteria growth

Semen dose rotation

Semen dose rotation

Do we have to rotate the seminal doses?

Belief: Creation of toxic microenvironment (waste products of metabolism, ...) → Decrease pH

What do we know?

Rodríguez-Gil y Rigau (1995): they qualify it as beneficial

Martin Schulze (2015): Manual rotation twice per day or automatic system → **Loss** of **motility** and **changes** in the **kinetic movement**

Fernando Bortolozzo, ITM 2016:

- The homogenization was **not associated with** the **improvement** of the sperm parameters and the oxidative state of the seminal doses
- This process may **not** be **necessary** to **guarantee** sperm **quality during storage**

Semen dose rotation

Magapor Trial

	TM		PM		SHOST		ORT		VIABILITY		MIT. ACTIVITY		REACTED ACROSOME		EARLY APOPTOSIS	
	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD
Day 1																
Rotation	96,57	1,63	68,62	4,62	64,25	9,89	83,08	5,87	94,48	2,76	93,64	1,77	11,52	4,94	6,99	2,02
No rotation	96,45	1,77	66,94	5,29	70,00	10,24	83,50	6,46	95,29	2,61	94,20	2,55	11,12	7,03	6,79	2,99
Day 4																
Rotation	95,14	2,40	71,85	3,12	61,33	12,21	69,25	14,81	95,23	2,94	94,33	2,15	12,69	5,39	6,03	2,31
No rotation	95,21	2,80	69,81	5,36	66,50	16,47	71,08	20,96	95,39	1,87	94,68	2,07	12,34	4,20	6,15	2,84
Day 7																
Rotation	92,09	3,47	68,67	4,88	65,58	16,72	70,42	16,67	91,96	3,15	92,47	2,76	16,91	5,19	7,23	2,26
No rotation	94,57	2,50	68,72	3,44	66,75	16,66	73,17	21,07	92,35	1,18	93,10	1,79	19,74	5,69	6,97	2,18

*No statistical differences between methods

Conclusions

- Keep in mind the **sperm sensitiviy** when the ejaculates/ doses are **handled**
- **Multiple** steps/critical points when we could in **fall into** in **sperm damage**
- Some **effects** or **causes** are **unavoidable** → Try to minimize with **market tools** or **new techniques**

Thanks!

Raquel Ausejo Marcos