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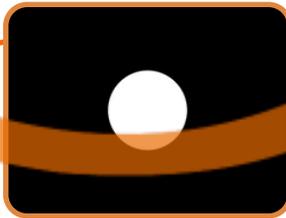
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Potential impact of biomedical techniques on the progression of boar fertility predicting methods

Prof. Ioannis Tsakmakidis, DVM, PhD

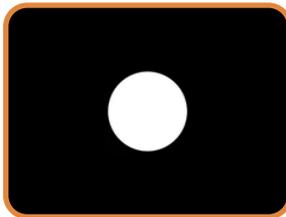
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Boar fertility plays key role in pig production efficiency



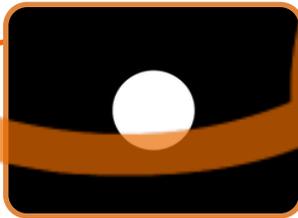
Accurate prediction of fertility remains a challenge in modern pig breeding programs



Emerging biomedical technologies offer promising avenues for improving prediction accuracy

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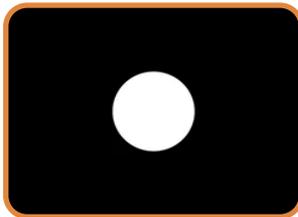
Boar is a critical component of pig industry



Each boar fertilizes more than a thousand sows per year



Each boar produces thousands of piglets per year



Economic study: the cost of purchasing a boar corresponds to about 0.5 % of the total value it brings back into the farm

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Literature reports / recommendations



Boars with highly motile and morphologically normal sperm are usually highly fertile



Boars that do not meet these criteria should be re-evaluated at 30 - 60 days intervals and, if not improved, replaced



Especially, boars in AI studs should be examined at least once a month after any disease or injury

Understanding the importance of the boar...



* 25% of the used boars provide farrowing rate < 80%

* One more piglet / farrowing increases the annually income about 120 million \$

* 1.700.000 \$ U.S. Department of Agriculture (USDA), prognosis of boar fertility



Understanding the importance of the boar...



* Data: 1193 boars - 116749 AIs

* Removal of 10% of less fertile boars results in 2.23 more piglets per litter

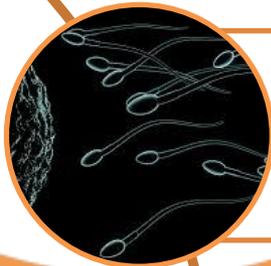
* More than 10000 euros/year for a farm of 1000 sows

* Uncovering the sub-fertile boars maximizing the pig farms' productivity



Source: Roca et al. (2015). *Reproduction in domestic animals*, 50, 48-55.

Traditional methods of boar fertility prediction



Basic semen analysis (volume, motility, morphology)



Clinical examination and testicular measurements



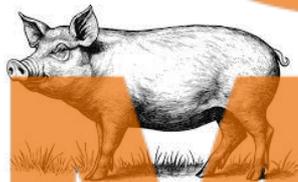
Data of reproductive performance and progeny testing

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Limitations of traditional methods



High variability among individuals



Inability to detect subfertile boars early



Lack of standardized, predictive biomarkers

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Basic semen analysis

All
Time
Classic



Subjective

Objective



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CASA analysis: VCL, BCF, VAP, VSL,
ALH positively correlated with
farrowing rate and litter size

Specialized traditional methods of boar fertility prediction



Evaluation and combination of multiple semen quality characteristics, e.g. sperm morphology and DNA integrity is positively related with farrowing rate[®]

Specialized traditional methods of boar fertility prediction

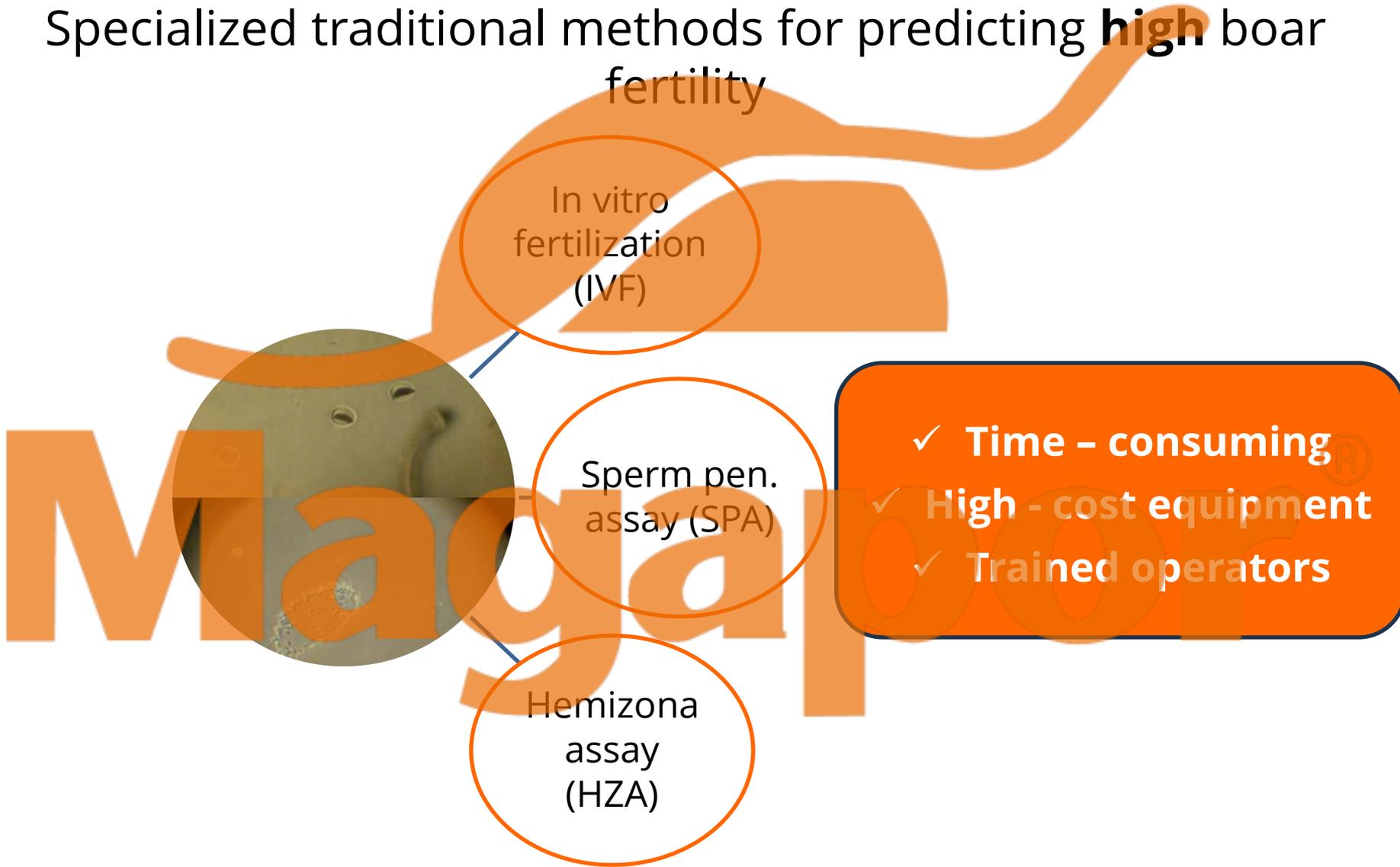


Sperm Morphology + DNA Integrity Combo:

this pairing predicts farrowing rates 12-18% more accurately than morphology alone [®]

* e.g. for a 500-sow farm: that's 85 more litters/year

Specialized traditional methods for predicting **high** boar fertility



In vitro fertilization (IVF)

Sperm pen. assay (SPA)

Hemizone assay (HZA)

- ✓ Time - consuming
- ✓ High - cost equipment
- ✓ Trained operators

New, but current in use methods

Molecular techniques, genetic background and potential biomarkers

Genomics, transcriptomics, proteomics, and metabolomics: a combination of complex and specialized techniques to recognize molecules that play a key role in major reproductive events



Molecular techniques, genetic background and potential biomarkers



Porcine seminal protein-I and II mRNA expression in boar spermatozoa is significantly correlated with fertility
Sae-han Kang, Won-Ki Pang, Do-Yeal Ryu, Won-Hee Song, Md Saidur Rahman, Yoo-Jin Park, Myung-Geol Pang*

© 2015 by The American Society for Biochemistry and Molecular Biology, Inc.
This paper is available on line at <http://www.mcponline.org>

Discovery of Predictive Biomarkers for Litter Size in Boar Spermatozoa*

Woo-Sung Kwon‡, Md Saidur Rahman‡, June-Sub Lee‡, Sung-Jae Yoon‡, Yoo-Jin Park‡, and Myung-Geol Pang‡§



Article Effect of Boar Sperm Proteins and Quality Changes on Field Fertility

Ilias Michos¹, Maria Tsantariotou¹, Constantin M. Boscos¹, Georgios Tsousis¹, Athina Basioura¹, Eleni D. Tzika¹, Panagiotis D. Tassis¹, Aristotelis G. Lymberopoulos² and Ioannis A. Tsakmakidis^{1,*}

OPN70
GPX5



PSP-1
PSP-2



AWN, TPI,
RAB2A, AQ3



Molecular techniques, genetic background and potential biomarkers

SCIENTIFIC REPORTS

OPEN Proteomic approaches for profiling negative fertility markers in inferior boar spermatozoa

Received: 18 June 2015
 Accepted: 05 August 2015
 Published: 08 September 2015
 Woo-Sung Kwon, Shin-Ae Oh, Ye-Ji Kim, Md Saidur Rahman, Yoo-Jin Park & Myung-Geol Pang

Spot No.	NCBI No.	Protein description	MASCOT score ^a	Expression level	
				Small litter size	Large litter size
2	1127023	pancreatic glycoprotein 2 (GP2)	158	▲	▼
3	47522754	Trifunctional enzyme subunit alpha, mitochondrial (HADHA)	202	▼	▲
4	359811347	60 kDa heat shock protein, mitochondrial (HSPD1)	243	▲	▼
5	73985642	Cytochrome b-c1 complex subunit 1, isoform2, mitochondrial (UQCRC1)	148	▲	▼
6	28336	mutant beta-actin (beta'-actin) (ACTB)	107	▲	▼
7	57086887	actin-related protein T2 (ACTRT2)	205	▲	▼
8	84579847	actin related protein T3 (ACTRT3)	197	▲	▼
9	3891849	Chain B, crystal structure of bovine mitochondria	105	▲	▼
10	300763221	pyruvate dehydrogenase: SUBUNIT = beta precursor (PDHB)	155	▲	▼
12	4929676	Homo sapiens CGI-104 protein mRNA, complete cds	236	▲	▼
13	109102081	cytosolic 5' nucleotidase 1B isoform 1	87	▲	▼
14	75052483	Acrosin-binding protein precursor (Proacrosin-binding protein sp32) (ACRBP)	335	▲	▼
16	190201	Porin	48	▲	▼
17	4505773	Prohibitin (PHB)	207	▲	▼
18	114053087	glutathione S-transferase Mu3 (GSTM3)	336	▲	▼
20	27807305	ATP synthase subunit d, mitochondrial (ATP5H)	145	▲	▼
21	311253799	Ras-related protein Rab-2A (RAB2A)	59	▲	▼
22	76779289	glutathione peroxidase 4 (GPx4)	147	▲	▼
23	13195731	glutathione peroxidase 4 (GPx4)	241	▲	▼
25	28417	Arginine vasopressin receptor 2 (AVPR2)	203	▲	▼

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Molecular techniques, genetic background and potential biomarkers

Using genomic markers instead of usually analyzed sperm parameters may be more accurate for assessing male fertility

Potential for **genomic selection** in AI studs

Early-life prediction of reproductive potential

Development of **diagnostic fertility kits**

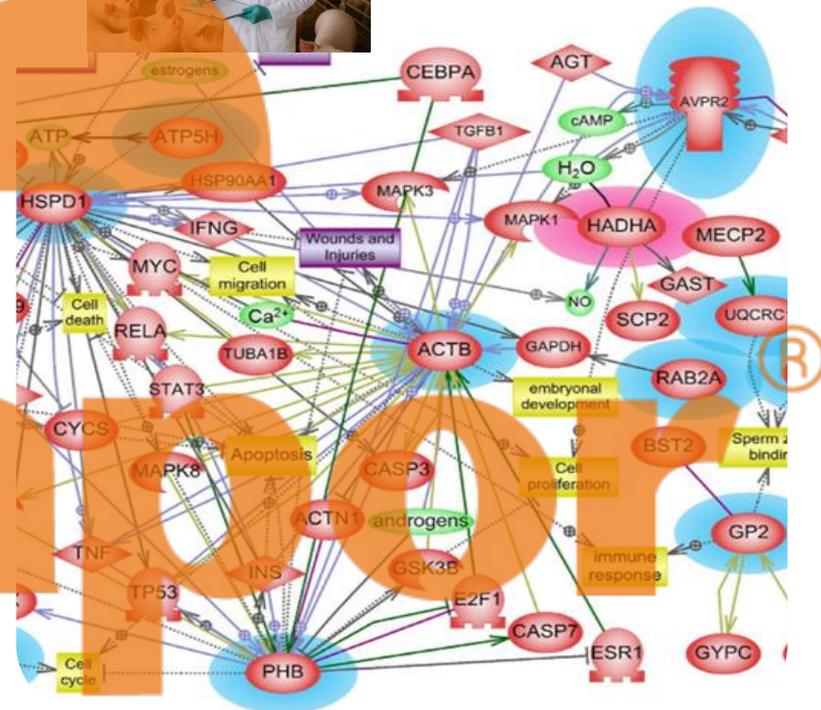
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Molecular techniques, genetic background and potential

Point of View



Signaling pathways associated with fertility-related proteins in boar spermatozoa



No stress and pain on animals

Cost and time friendly

Can be clinically applied

Statistically evaluated

Validated on field - fertility outcome



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Development of predictive models for boar fertility / semen quality



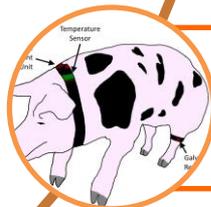
Statistical models - database of many influencing factors (animals, environmental, seasonal etc.)



AI - Machine Learning models



Biophysical methods: microfluidics and microchip technologies



On animal biomedical measurements in combination with semen quality variables

TREND

Magapor®

Development of predictive models for boar fertility / semen quality

Research project: Investigation of boar reproductive capacity using biomedical techniques

www.digipig.gr



This project has received funding from

ΕΛΙΔΕΚ
Εθνικό Ινστιτούτο Ερευνας Αγροτικών Οικονομιών

ΓΓΕΤ
ΓΕΝΙΚΗ ΓΡΑΜΜΑΤΕΙΑ
ΕΡΕΥΝΑΣ ΚΑΙ ΤΕΧΝΟΛΟΓΙΑΣ



DIGIPIG
Application of Biomedical techniques on boar semen

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An under-development method



Biomedical techniques:
applications of biological
and physiological principles
in clinical practice

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Knowledge and queries



Boar, critical important unit. The prediction of boar fertility saves resources



STRESS

Stress affects semen quality reducing the boar value



Periodic semen evaluation is required, waiting for the report of laboratory results

Questions to answer about the under-development method



Real time measurements?



Under boars' living conditions?



Specific stress factors of each farm / AI station?



Strong indications of semen quality before its' lab evaluation?

M **Magapor**®

The aim of the study



Is feasible to get reliable biomedical measurements of boar behavior?

Is feasible to develop a prognostic boar fertility model based on biomedical measurements?

Magjapoor®

Implementing the purpose of the study



Biomedical measurements of the boar behavior during semen collection within their living space



Objective boar semen evaluation



Correlation of biomedical and seminal data to establish a predictive model of boar semen production capacity

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Selected biomedical parameters / measurements

1

**Boar orientation and movements
/ magnetic fields**

Emotional status of the boar

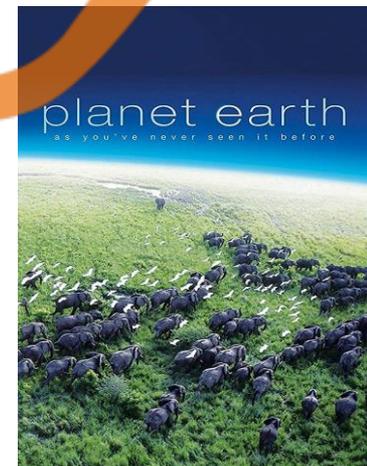
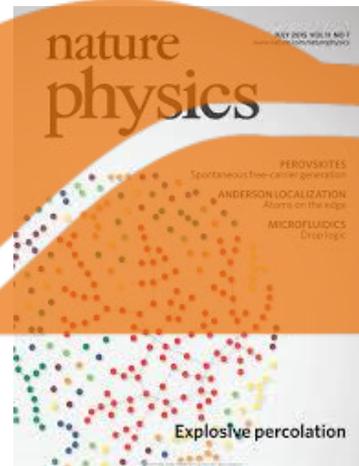
**Temperature (body, scrotal,
dummy's / environmental)**

Salivation of the boar

Scrotal contractions / relaxations

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How was the idea born?



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Animals have:

- Hyper-sensations and emotions
- Different perception of magnetic fields
- Orientation and navigation is based on the different perception of magnetic fields

Animals' migration based on magnetic fields

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Post, H., B. J. Ens, H. de Vries, M. A. H. Donners, M. R. Wernand, and J. M. Marquenie. 2008. Green light
for nocturnally migrating birds. *Ecology and Society* 13(2): 47. [online] URL: <http://www.ecologyandsociety.org/vol13/iss2/art47/>

E&S

Research Green Light for Nocturnally Migrating Birds

Hanneke Post¹, Bruno J. Ens², Han de Vries³, Maurice A. H. Donners⁴, Marcel R. Wernand⁵, and
Joop M. Marquenie⁶

ABSTRACT. The nighttime sky is increasingly illuminated by artificial light sources. Although this ecological light pollution is damaging ecosystems throughout the world, the topic has received relatively little attention. Many nocturnally migrating birds die or lose a large amount of their energy reserves during migration as a result of encountering artificial light sources. This happens, for instance, in the North Sea, where large numbers of nocturnally migrating birds are attracted to the many offshore platforms. Our aim is to develop bird-friendly artificial lighting that meets human demands for safety but does not attract and disorient birds. Our current working hypothesis is that artificial light interferes with the magnetic compass of the birds, one of several orientation mechanisms and especially important during overcast nights. Laboratory experiments have shown the magnetic compass to be wavelength-dependent: migratory birds require light from the blue-green part of the spectrum for magnetic compass orientation, whereas red light (visible long-wavelength) disrupts magnetic orientation. We designed a field study to test if and how changing light color influenced migrating birds under field conditions. We found that nocturnally migrating birds were disoriented and attracted by red and white light (containing visible long-wavelength radiation), whereas they were clearly less disoriented by blue and green light (containing less or no visible long-wavelength radiation). This was especially the case on overcast nights. Our results clearly open perspective for the development of bird-friendly artificial lighting by manipulating wavelength characteristics. Preliminary results with an experimentally developed bird-friendly light source on an offshore platform are promising. What needs to be investigated is the impact of bird-friendly light on other organisms than birds.



What about farm animals?

Cows: tendency to orient towards north or south when they are resting and grazing



Research included
8000 cows



Sense of magnetic
fields

Boar behavior, movements and orientation

Commercial
collars

On
boar

Self-made
collars

- Fitting collars with sensors
- Receive and send signals
- Processing, algorithms

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Boar behavior, movements and orientation



Sensor

Inertial measurement unit (IMU)

Triple axis gyroscope, accelerometer
and magnetometer

Barometric pressure and humidity

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A race to semen collection pen...

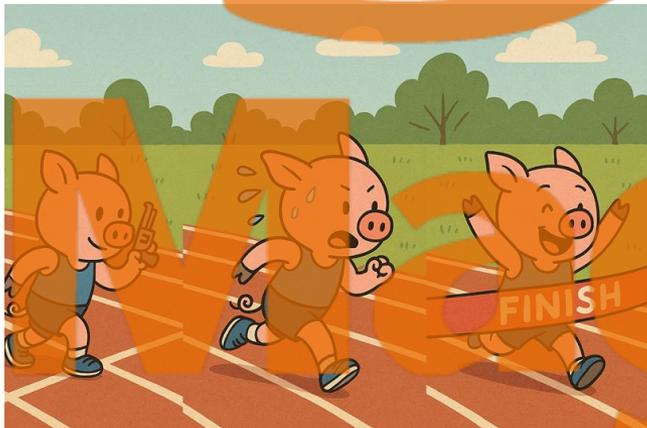
3...2...1...
GO!

M



Boar behavior, movements and orientation

3...2...1...
GO!



Boar orientation
approaching the dummy



Boar movements'
repeatability or not



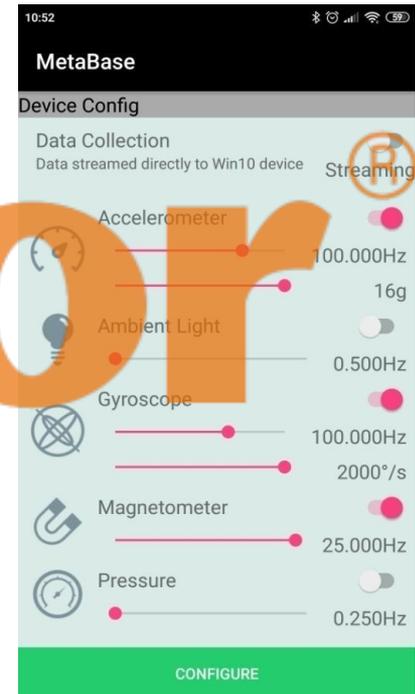
Number of circular
movements around the
dummy and mounting
attempts



DigiPIG – Methodology

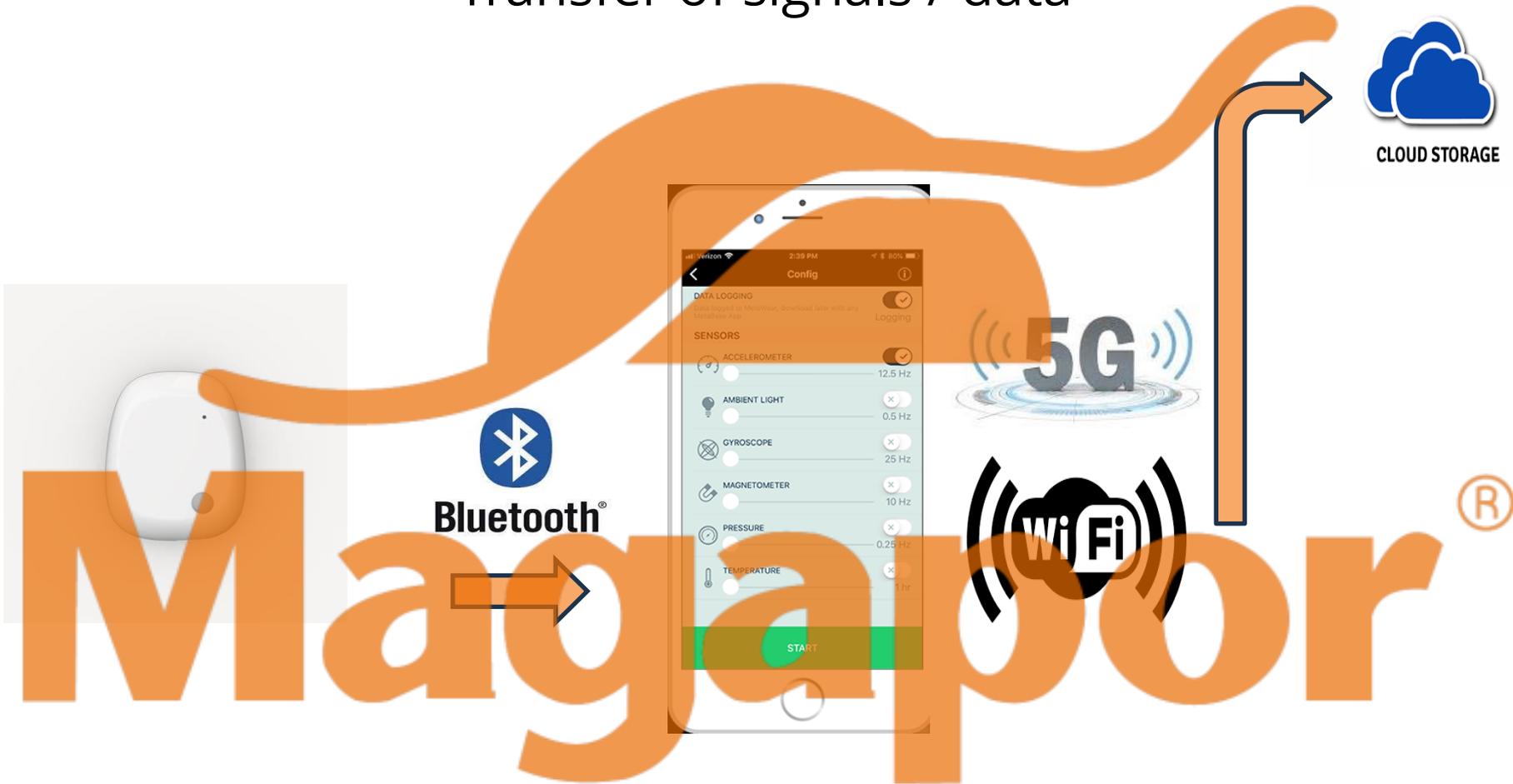


MetaMotionRL



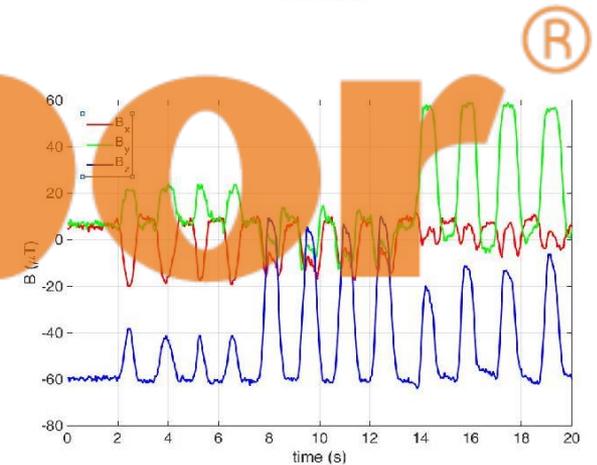
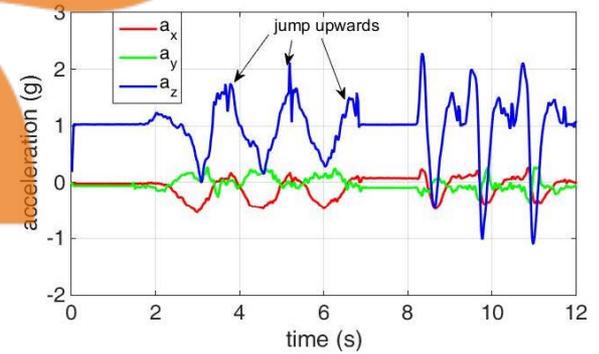
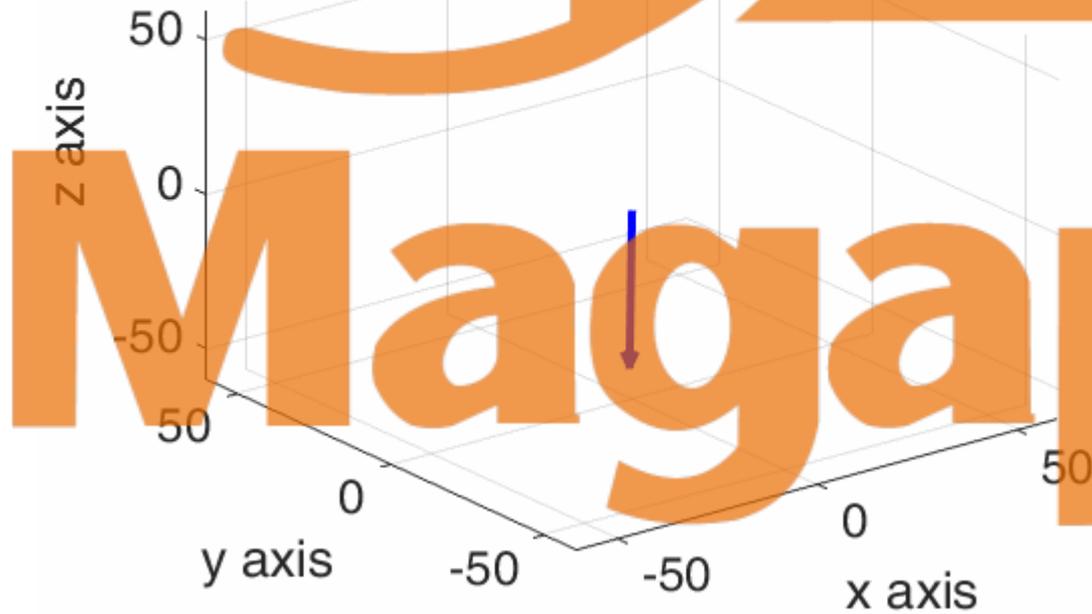
Magapor

Transfer of signals / data





$t = 0.0 \text{ s}$



Selected biomedical parameters / measurements

2

**Boar orientation and movements
/ magnetic fields**

Emotional status of the boar

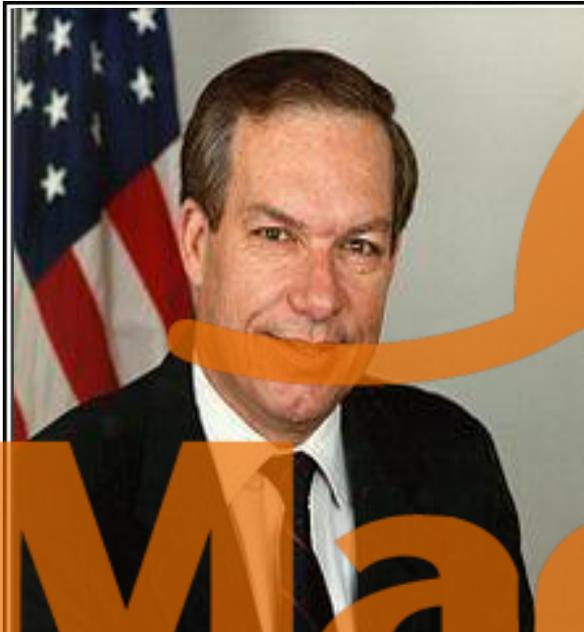
**Temperature (body, scrotal,
dummy's / environmental)**

Salivation of the boar

Scrotal contractions / relaxations

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Animals have emotions!!!



Never believe that animals suffer less than humans. Pain is the same for them that it is for us. Even worse, because they cannot help themselves.

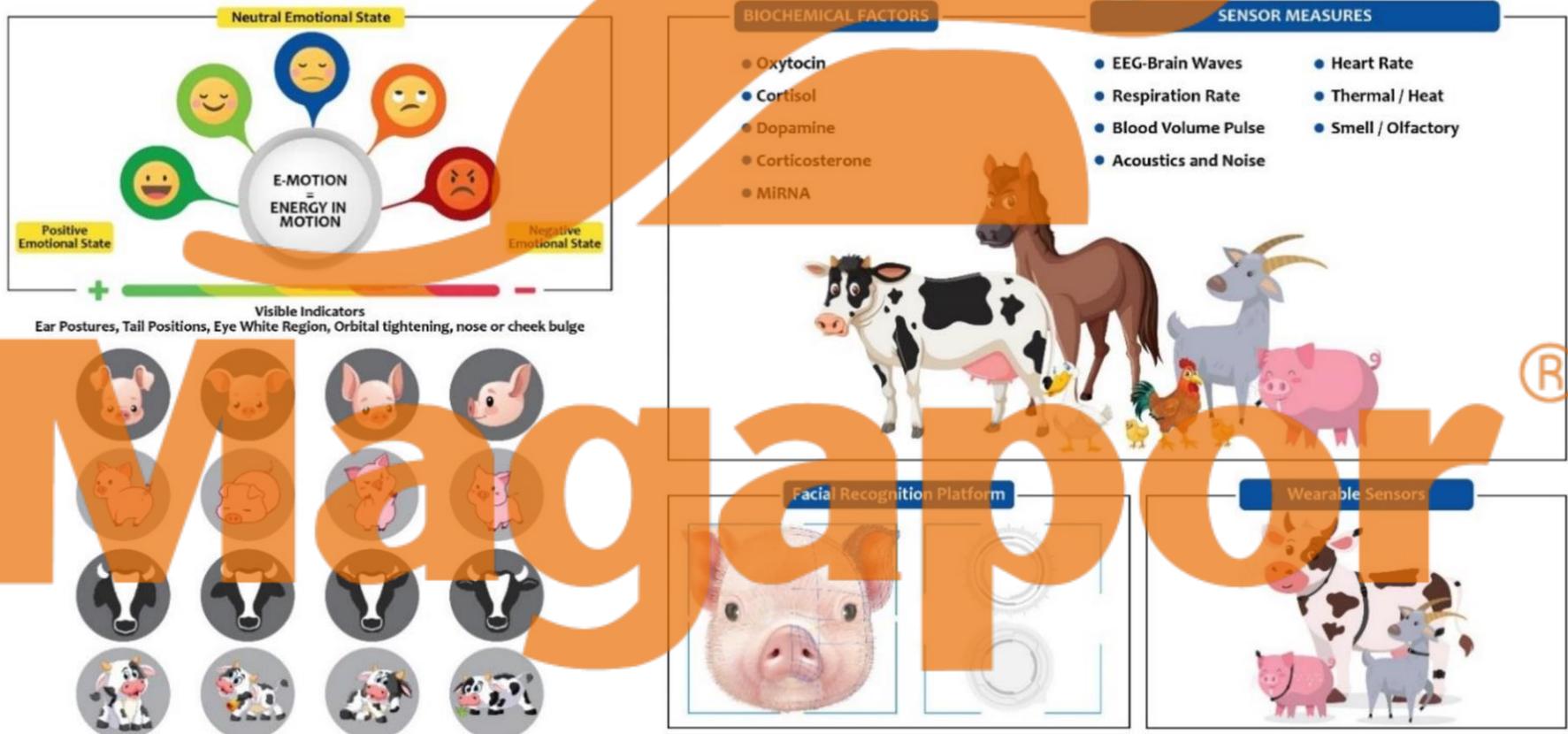
— Louis J. Camuti —

Louis J. Camuti: a New York City veterinarian and author

Magapor®

Animals have emotions!!!

MEASURING EMOTIONS IN FARM ANIMALS – SENSOR APPROACHES



Are the breeding boars happy???

The 5 freedoms - Farm Animal Welfare Council (FAWC)



Freedom from thirst, hunger and malnutrition



Freedom from thermal and physical discomfort



Freedom from pain, injury and disease



Freedom from fear and distress



Freedom to express normal behavior

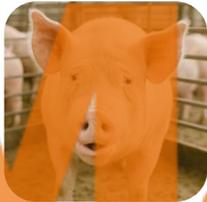


Breeding boars' emotional status...

They:



Recognize collection pens and handlers



Change vocalizations when they separated from mates



Stress Memory: Cortisol persists for > 72 hours after bad handling



How emotional status can be measured?



Electrodermal activity (EDA)
or Galvanic Skin Response
(GSR)



Variations in the electrical
properties of the skin in
response to sweat
secretion.

Magapor®

Electrodermal activity (EDA) or Galvanic Skin Response (GSR), the body property that causes continuous changes in the electrical characteristics of the skin

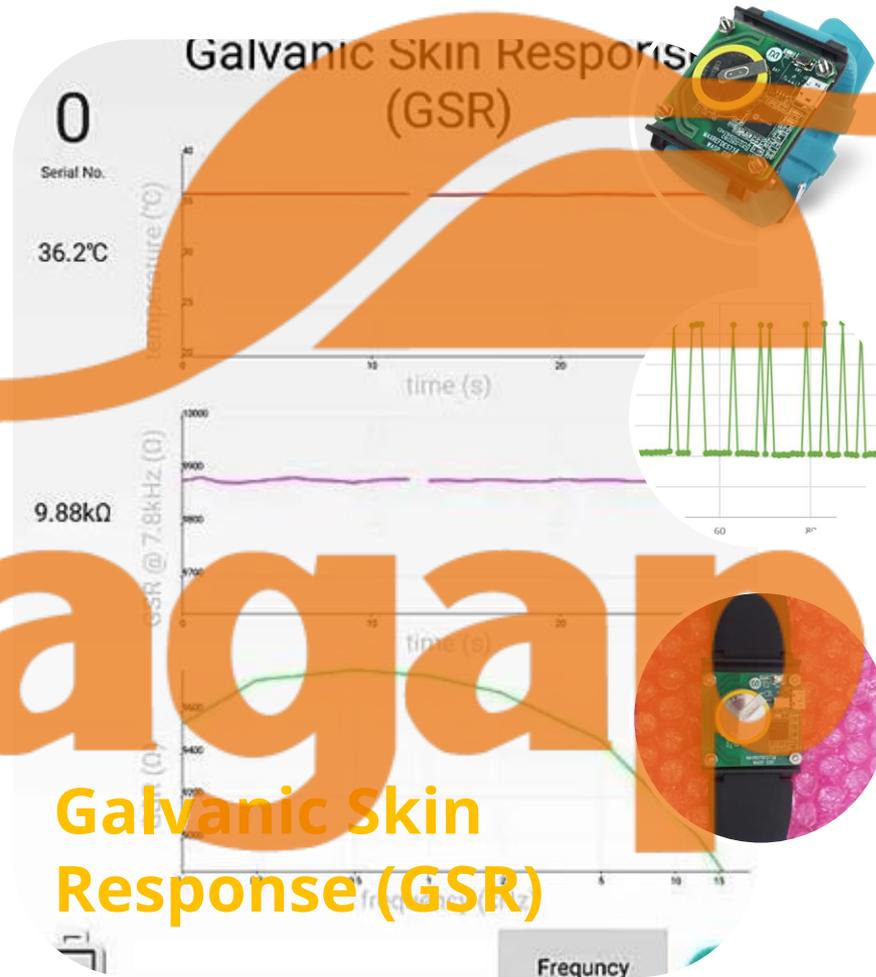
MAXREFDES73#



Magapor®



Electrodermal activity (EDA) or Galvanic Skin Response (GSR)



Magapor®

Galvanic Skin Response (GSR)

Selected biomedical parameters / measurements

**Boar orientation and movements
/ magnetic fields**

Emotional status of the boar

**Temperature (body, scrotal,
dummy's / environmental)**

Salivation of the boar

Scrotal contractions / relaxations

3 **Magapor**®

Temperature measurement by infrared thermo-camera



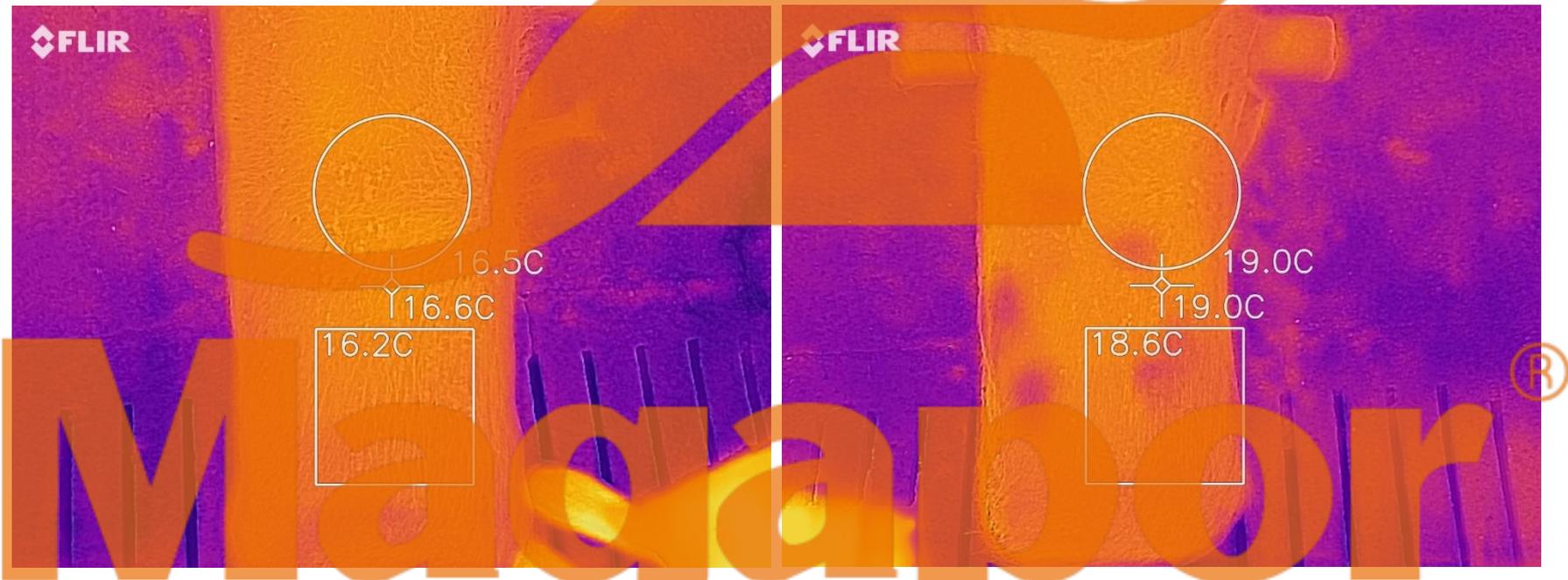
Sperm quality is affected by scrotal temperature



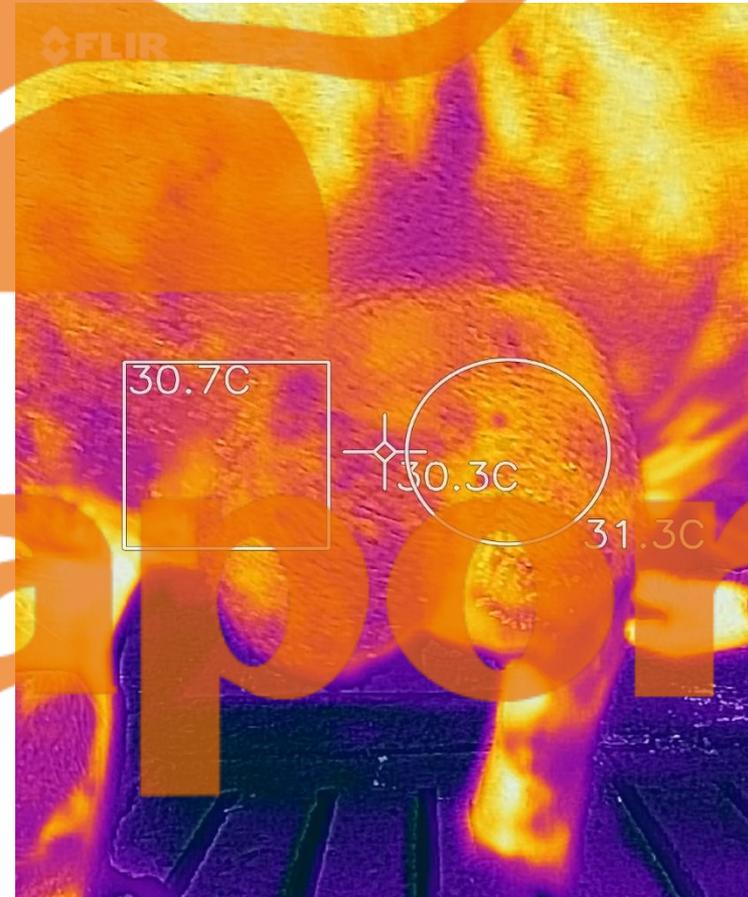
Dummy's temperature "maybe" affects the boar's mounting mood

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Dummy's temperature measurement



Scrotal temperature measurement



Selected biomedical parameters / measurements

**Boar orientation and movements
/ magnetic fields**

Emotional status of the boar

**Temperature (body, scrotal,
dummy's / environmental)**

Salivation of the boar

Scrotal contractions / relaxations

4

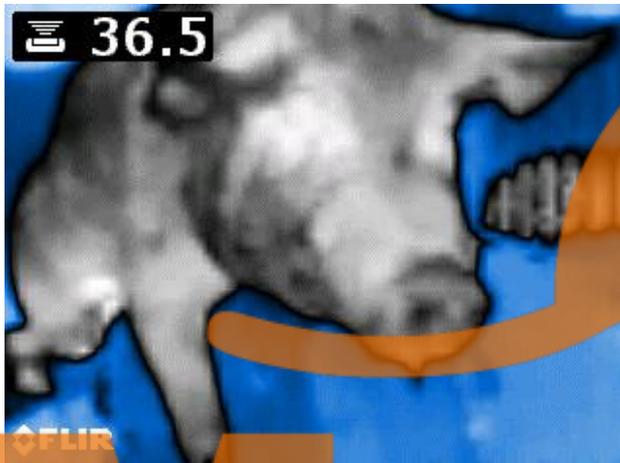


Estimation of salivation using an infrared (moisture) imaging camera



The boar during ejaculation shows more salivation

Estimation of salivation



Transfer of images / data

Selected biomedical parameters / measurements

**Boar orientation and movements
/ magnetic fields**

Emotional status of the boar

**Temperature (body, scrotal,
dummy's / environmental)**

Salivation of the boar

Scrotal contractions / relaxations

5

Magapor®

Recording of scrotal contractions using a video-camera



Contraction

Number
and intense
of scrotal
contractions



Relaxation

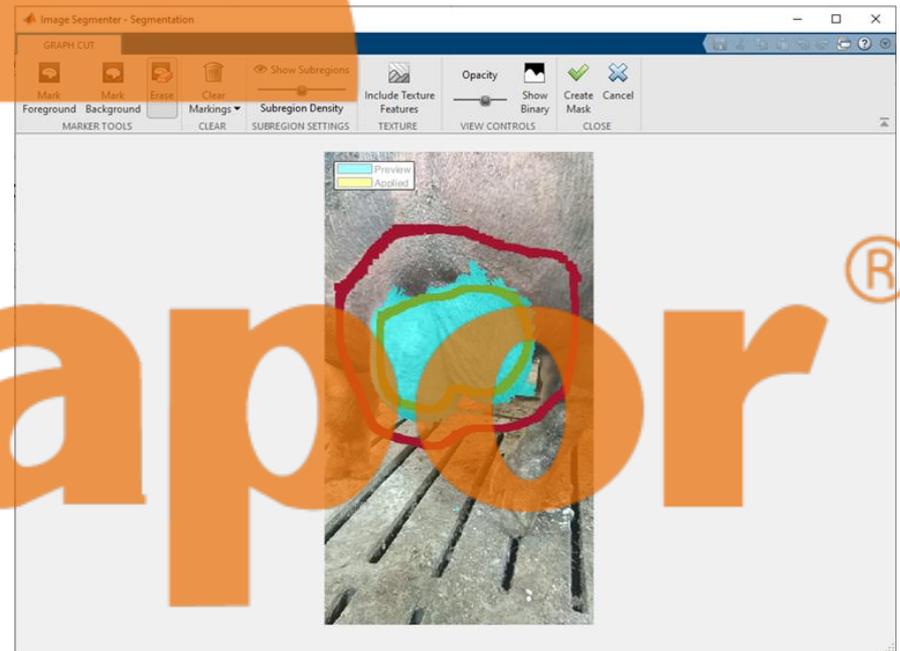
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DigiPIG – Scrotal contractions (Matlab)

Movie
Player

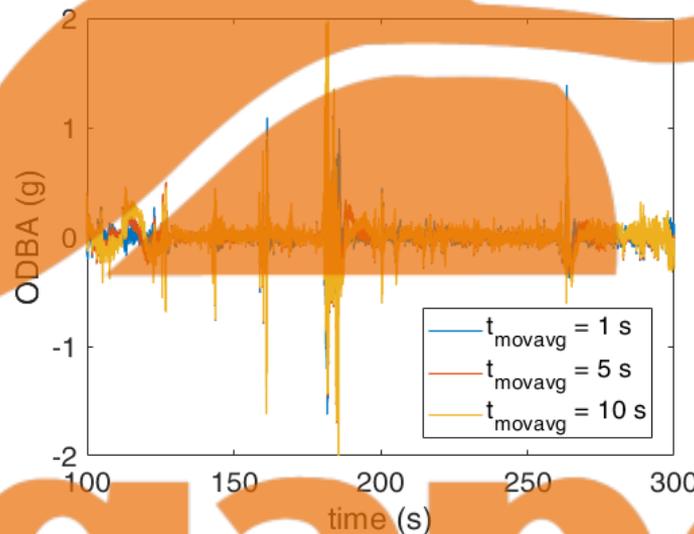
Image
Tool

Image
Segmenter



Magapor[®]

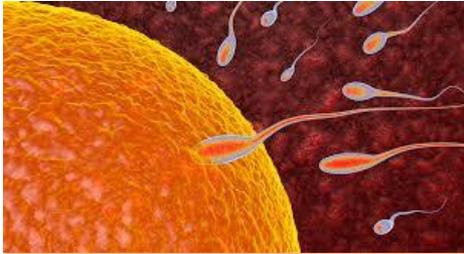
Processing of biomedical measurements



region (ms)	time (03:00)	elapsed (s)	x-axis (deg/s)	y-axis (deg/s)	z-axis (deg/s)
2	1507223296513	2018-05-07T13:01:3	0	-0.549	-0.427
3	1507223296523	2018-05-07T13:01:3	0.01	0.305	-0.976
4	1507223296533	2018-05-07T13:01:3	0.02	0.244	-0.732
5	1507223296543	2018-05-07T13:01:3	0.03	0.122	-0.549
6	1507223296553	2018-05-07T13:01:3	0.04	0.061	-0.549
7	1507223296563	2018-05-07T13:01:3	0.05	0.061	-0.488
8	1507223296573	2018-05-07T13:01:3	0.06	0.122	-0.549
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10	1507223296593	2018-05-07T13:01:3	0.08	0.061	-0.549
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12	1507223296613	2018-05-07T13:01:3	0.1	0.122	-0.61
13	1507223296623	2018-05-07T13:01:3	0.11	0.122	-0.732
14	1507223296633	2018-05-07T13:01:3	0.12	0.061	-0.488
15	1507223296643	2018-05-07T13:01:3	0.13	0.122	-0.427
16	1507223296653	2018-05-07T13:01:3	0.14	0.244	-0.488
17	1507223296663	2018-05-07T13:01:3	0.15	0.122	-0.61
18	1507223296673	2018-05-07T13:01:3	0.16	0	-0.549
19	1507223296683	2018-05-07T13:01:3	0.17	0.122	-0.732
20	1507223296693	2018-05-07T13:01:3	0.18	0.244	-0.549
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23	1507223296723	2018-05-07T13:01:3	0.21	0.183	-0.488
24	1507223296733	2018-05-07T13:01:3	0.22	0.366	-0.549
25	1507223296743	2018-05-07T13:01:3	0.23	0.244	-0.671
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27	1507223296763	2018-05-07T13:01:3	0.25	0.061	-0.488
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29	1507223296783	2018-05-07T13:01:3	0.27	0.122	-0.671
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Magapor

Objective semen evaluation



Semen variables

CASA analysis

Viability

Morphology

Sperm Membrane Biochemical Activity (HOST)

DNA integrity

Magapor®



Progressive motility

Rapid moving spermatozoa

VSL

Temperature of the body, dummy and scrotum

VCL

ALH



Article

The Use of Animal's Body, Scrotal Temperature and Motion Monitoring in Evaluating Boar Semen Production Capacity

Vasiliki Stravogianni ¹, Theodoros Samaras ², Constantin M. Boscos ¹, John Markakis ², Evdokia Krystallidou ³, Athina Basioura ⁴ and Ioannis A. Tsakmakidis ^{1,4}

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Morphology

Volume of the ejaculate

BCF

Temperature of the dummy and scrotum

Total time of ejaculation

Cytoplasmic droplets

Boar movements - Total dynamic acceleration

VAP

Intense of scrotal contractions

Magapor[®]

Five persons teamwork



a

a

a



Mobile app under development



Feasible methodology?



Feasible
measurements

Real time
signals'
collection

Related
with
semen
variables



Take home message (1)



Biomedical techniques complement our understanding of boar fertility



Biomedical techniques can be real time used in the boars' housing environment, reflecting the effects of any influential factor

Magapor®

Take home message (2)



Research about the development of new boar fertility prediction models is rising, providing encouraging results



Next steps include deeper collaboration between veterinarians, geneticists, data scientists, and animal behaviorists



By integrating biomedical, molecular, physiological, and computational methods, we move toward a new era of fertility prediction



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Inside DIGIPIG

Let's meet the people working in DigiPIG



Ioannis Tsakmakidis
Associate Professor



Constantine Boscos
Professor



Theodoros Samaras
Professor



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Eydokia Krystallidou



Vasiliki Stravogianni



Athina Basioura
Post-doctorate researcher



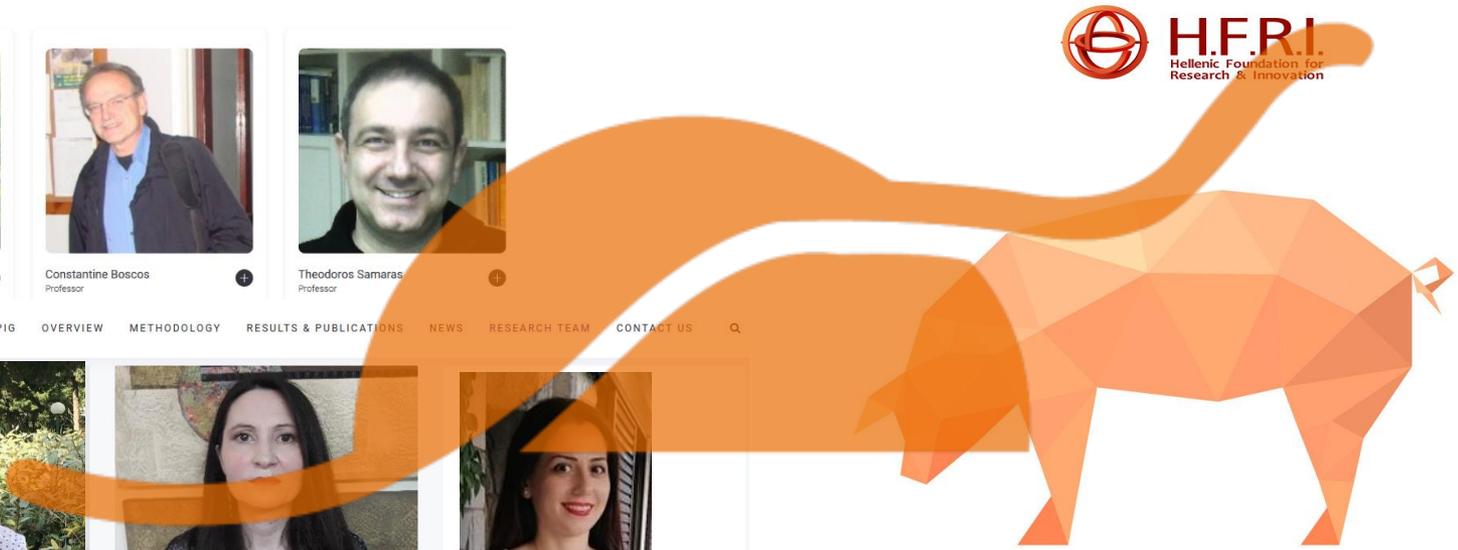
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Ioannis - Anestis Markakis



Vasileios Stravogiannis
Technician



DIGIPIG

Application of Biomedical techniques on boar semen



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Project Overview

Main Goals
Objectives and Challenges

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THANKS

GRACIAS

Ioannis Tsakmakidis

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