PREDICTIVE POWER OF
OXYGEN CONSUMPTION
IN ASSESSMENT OF
OOCYTE /EMBRYO QUALITY

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*** No commercial activities are related to this presentation**

ESHRE Campus Symposium
Salzburg, Austria, 2 April 2011

OBJECTIVE

↑success rates of IVF treatments

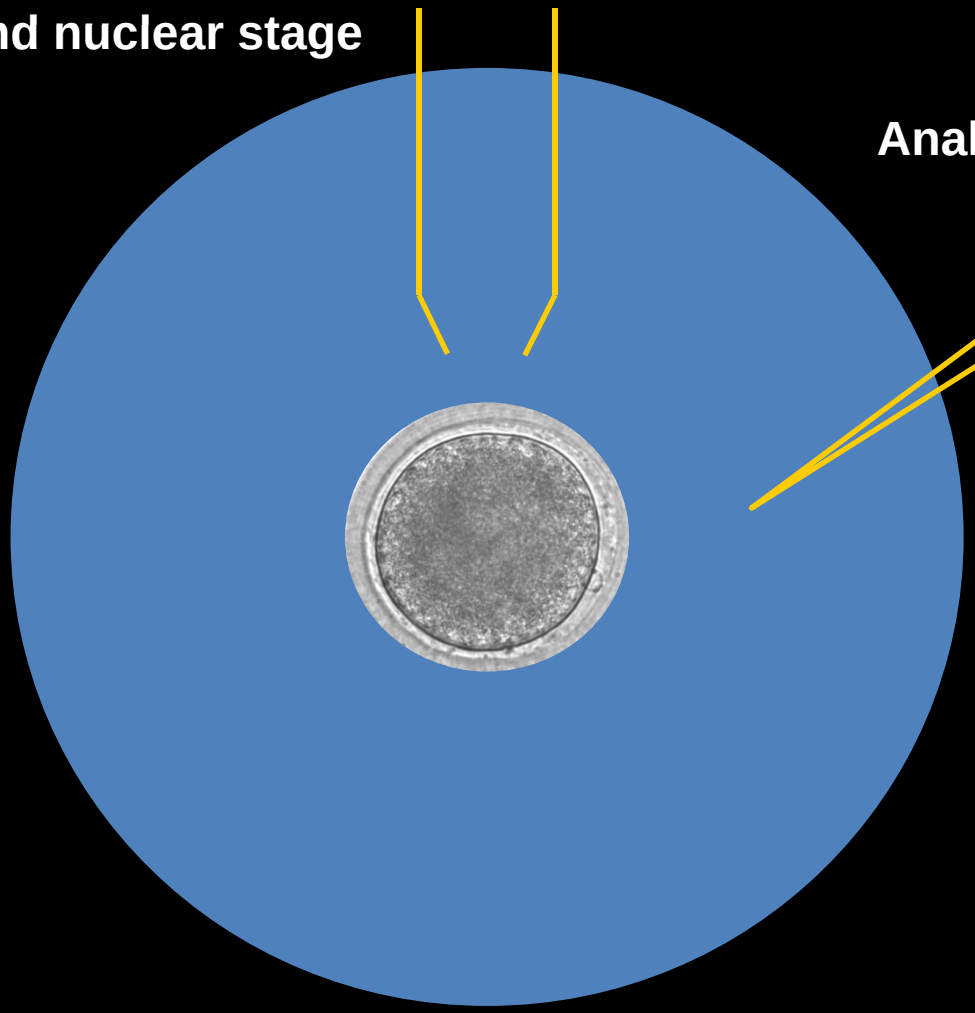
Only 50% of all transferred embryos get implanted

↓ number transferred embryos by treatment cycle

Multiple gestations → obstetrical & neonatal complications

OOCYTE SELECTION

Morphology and nuclear stage



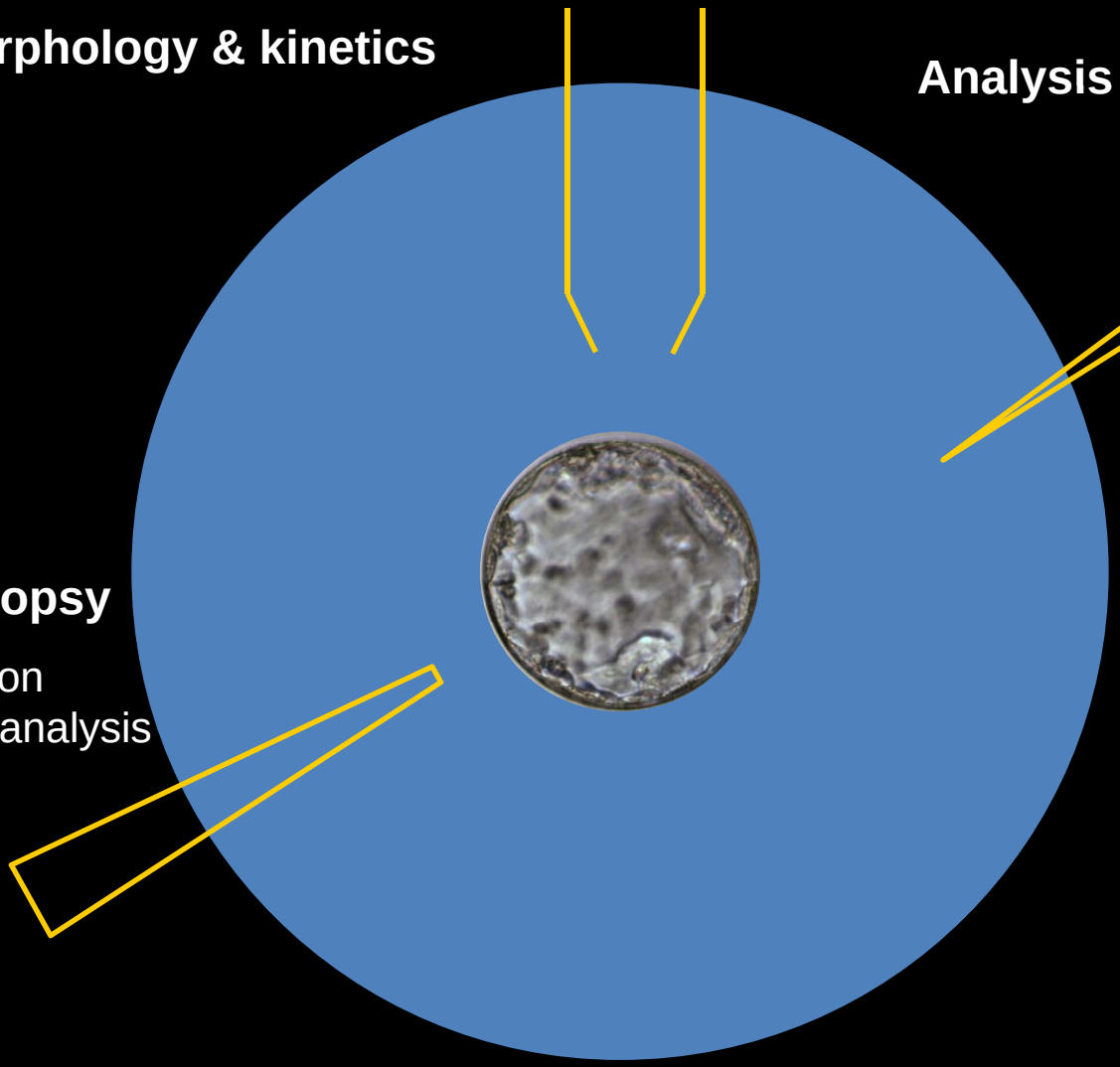
Analysis culture medium

- Pyruvate
- Glucose
- O2
- Lactate
- Albumin
- CO2
- Amino acids
- NH3
- Enzymes
- Early pregnancy factor
- PAF
- Oxidative stress

EMBRYO SELECTION

Morphology & kinetics

Analysis culture medium



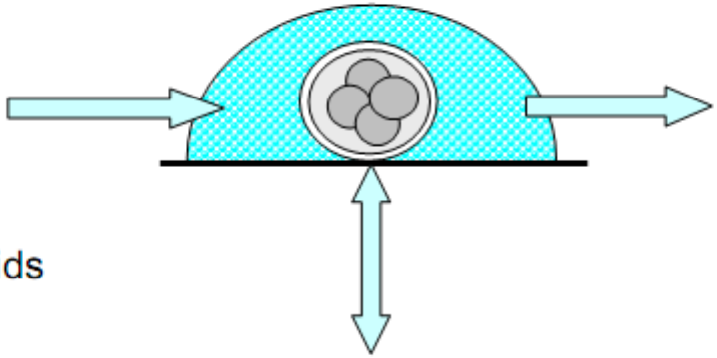
- Pyruvate
- Glucose
- O2
- Lactate
- CO2
- Amino acids
- NH3
- Enzymes
- Early pregnancy factor
- PAF
- Oxidative stress

Blastomere biopsy

- Gene expression
- Chromosomal analysis

Nutrition and metabolism of early embryos: Non-invasive assessment

Depletion	***Appearance***
Pyruvate	H_20
Lactate	CO_2
Glucose	Lactate
Lipid	Amino acids
Amino acids	NH_4^+
Oxygen	Enzymes
	Cytokines
	Proteins



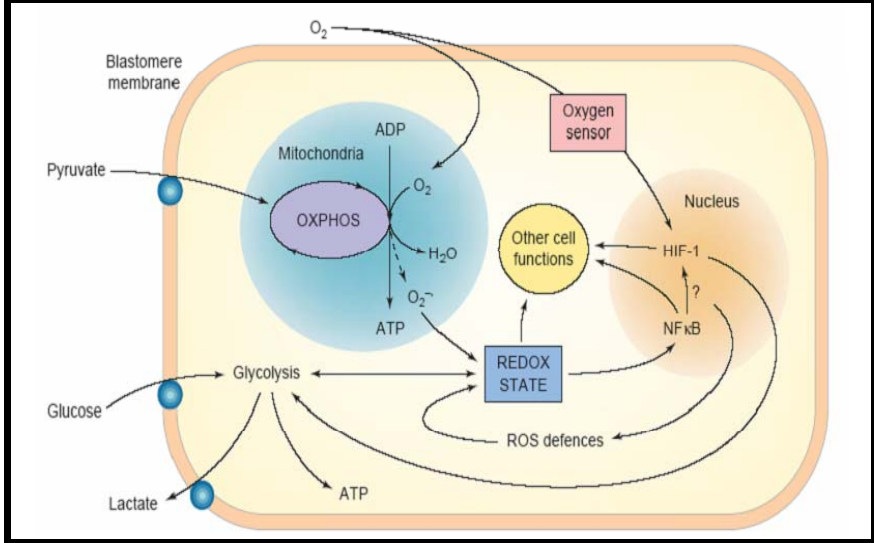
Gardner DK. and Leese HJ. (1999). Assessment of embryo metabolism and viability. In: Handbook of In vitro Fertilization Trounson A, Gardner DK (eds). Pp 348-366. CRC Press.

WHY OXYGEN???

Oxygen provides the best indication of overall metabolic activity

BECAUSE

is directly related to ATP production via oxidative phosphorylation.

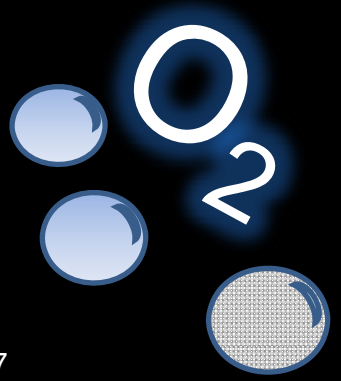


Harvey et al. (2002)

AND ....

An adequate cohort of good quality mitichondria is mandatory for a GOOD quality oocyte and embryo

MEASURING OXYGEN CONSUMPTION ...



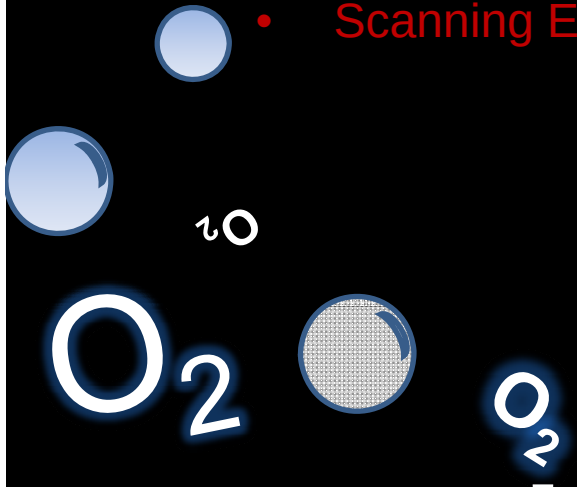
a) Cartesian diver techniques Fridhandler *et al*.1957: Mills & Brinster 1967

b) Spectrophotometric Methods Magnusson *et al.* 1977; Nilsson *et al.* 1982

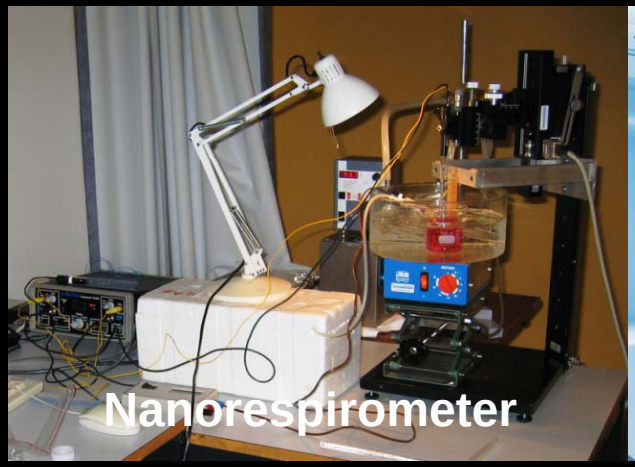
c) Ultramicrofluorescence techniques Houghton *et al.* 1996; Thompson *et al.* 1996

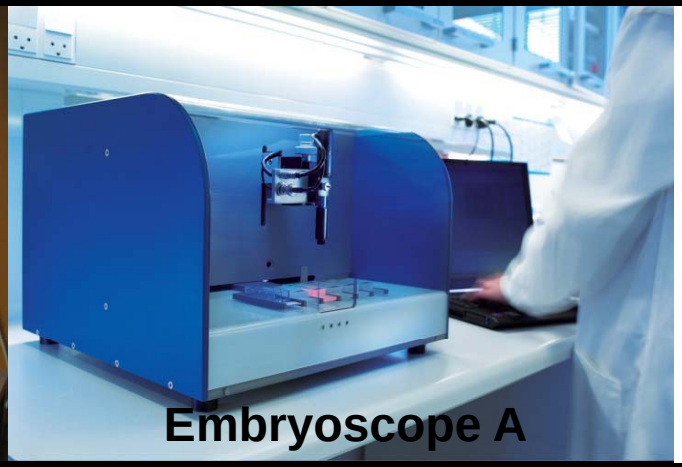
d) Eletrochemical techniques

- Overström method Overström 1987
- Scanning Electrode Technique Smith *et al.*1995,1999; Trimarchi *et al.* 2000
- Scanning Electrochemical microscopy Shiku *et al.* 2001; Koike *et al.* 2010



The EmbryoScope


Nanorespirometer


Embryoscope A

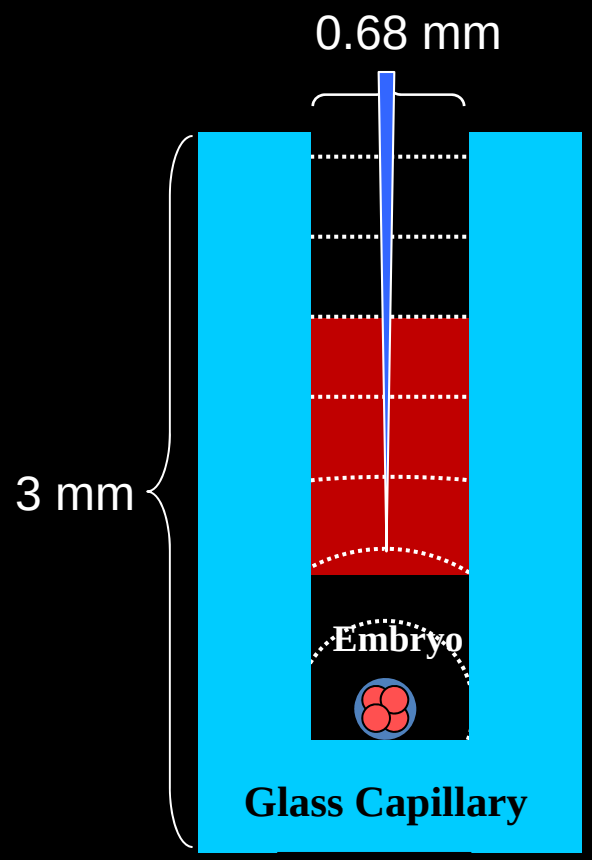

Embryoscope C

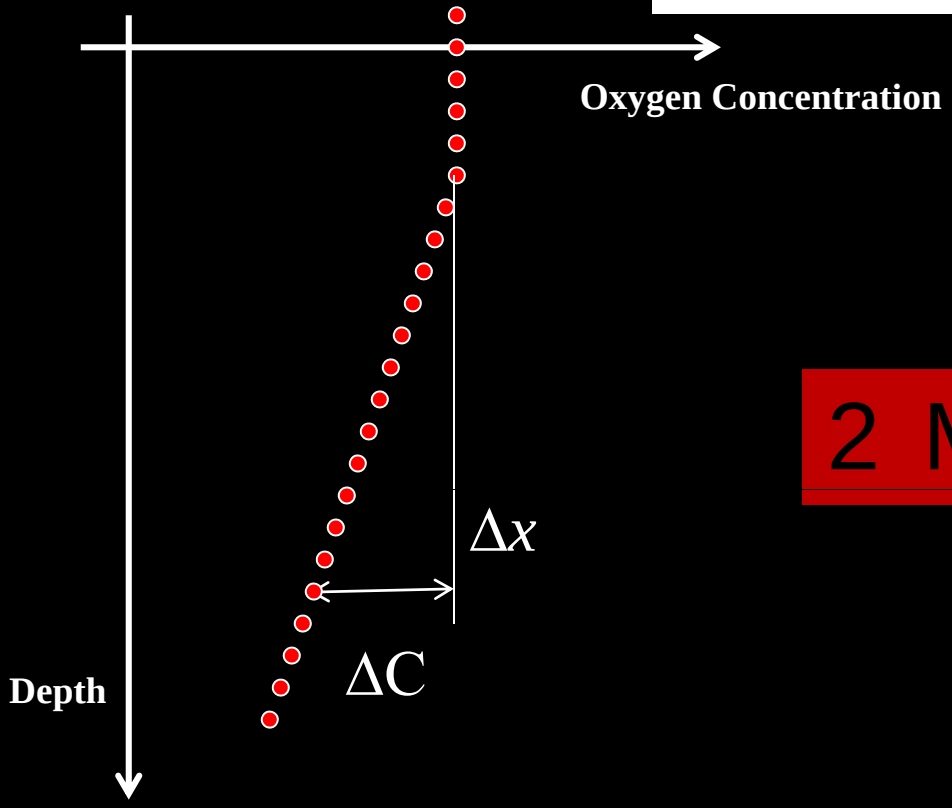
Unisense Fertilitech

- Non invasive
- Rapid
- Simple and practical
- Adapted to measure individual embryos
- Accurate and consistent (reproducible)
- Highly sensitive
- Does not interfere with viability

THE PRINCIPLE

$$\text{Respiration} = - D \frac{\Delta C}{\Delta x} A$$





2 Min

Lopes *et al.* (2005) Reproduction 130 669-679.

Oocytes (human)

GV, MI (Day 0 and 1) and MII oocytes (Day 1, aged oocytes)

Rates ranged between = 0.6 - 0.7 nl/h

Differences in oxygen consumption

- **Oocytes**
- **Patients**
- **Oocytes developing in vitro vs. arrested in vitro**

Scott et al. (2008) RMOnline

Scott et al. (2008) Proc. ESHRE Campus

Oocytes (human)

Age and hormonal levels

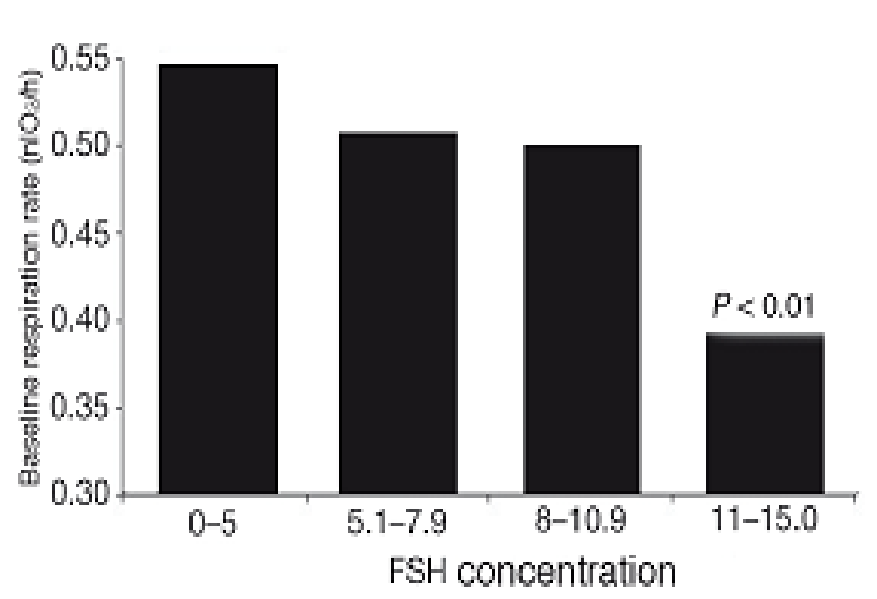


Table 4. Baseline respiration rates of day 0 (D0) oocytes according to the age of the patient.

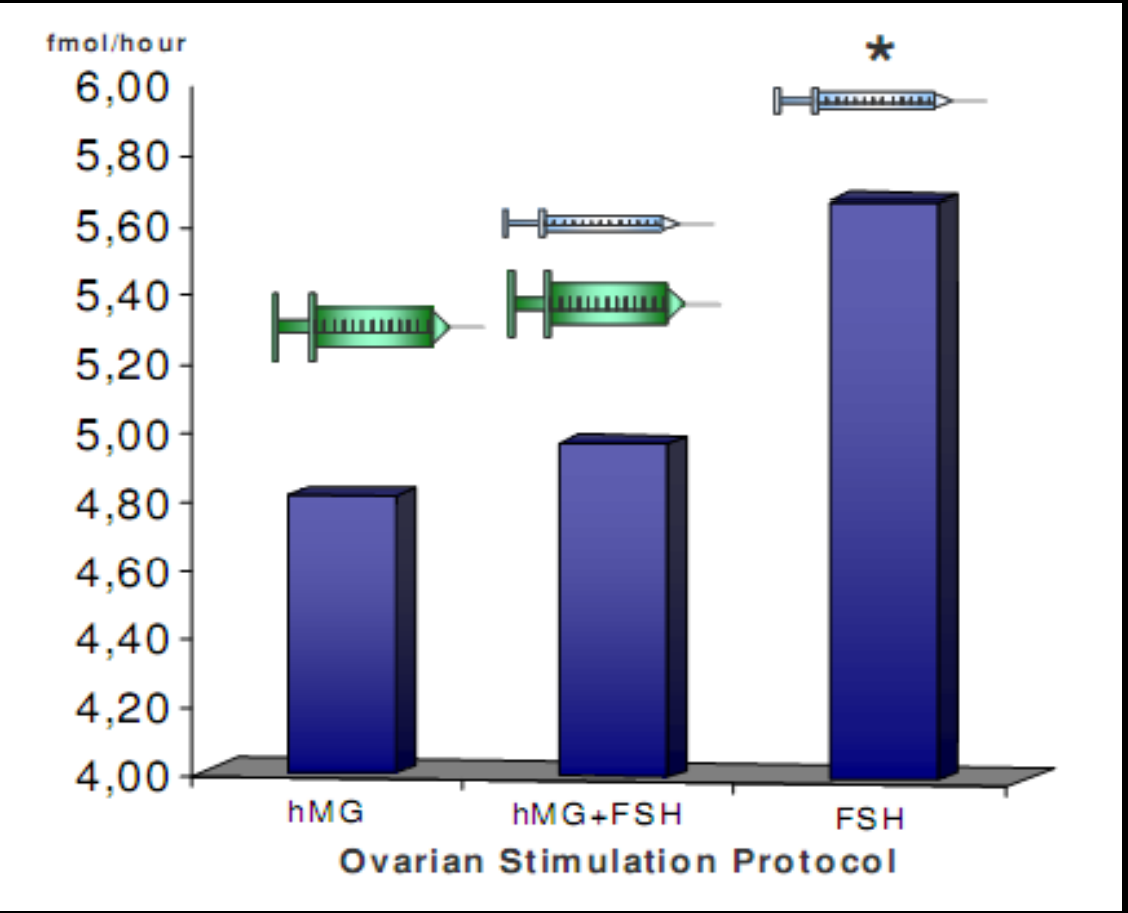
Patient age (years)	*D0-GV* n	*Baseline respiration rate (nl O_2/h)*[a]	*D0-MI* n	*Baseline respiration rate (nl O_2/h)*[a]
<35	42	0.575 (0.014)	32	0.569 (0.016)
35–37	25	0.608 (0.008)	9	0.628 (0.014)
38–40	16	0.532 (0.012)	8	0.499[b] (0.021)
>40	16	0.461[b] (0.098)	12	0.466[b] (0.013)

- **FSH (lower oxygen consumption in patients with high FSH levels)**
- **Age (higher oxygen consumption in oocytes of younger women)**

Scott et al. (2008) RMOnline

Oocytes (human)

Stimulation protocol

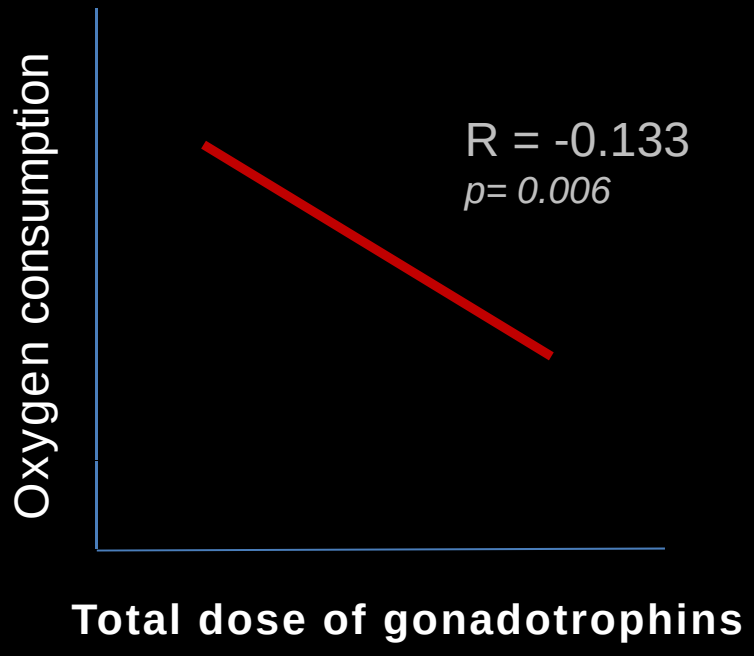


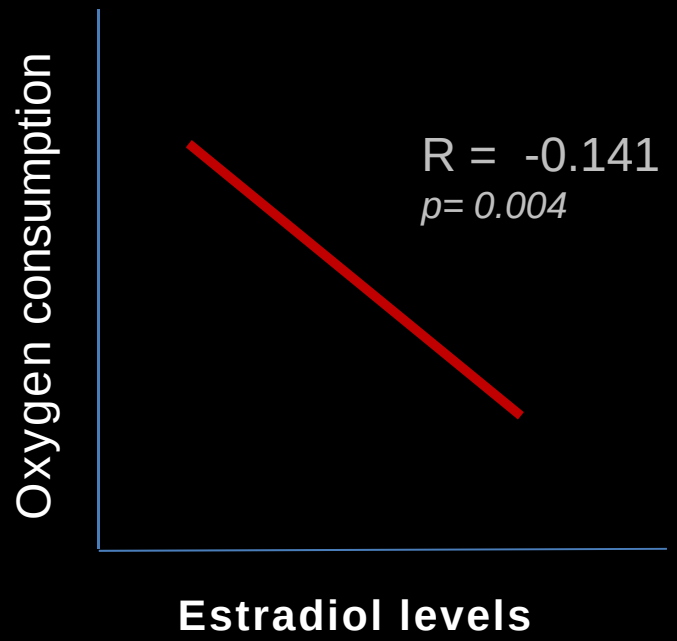
Higher oxygen consumption of oocytes in patients stimulated with FSH

Tejera et al. 2010
ESHRE Rome

Oocytes (human)

Stimulation protocol & hormonal levels

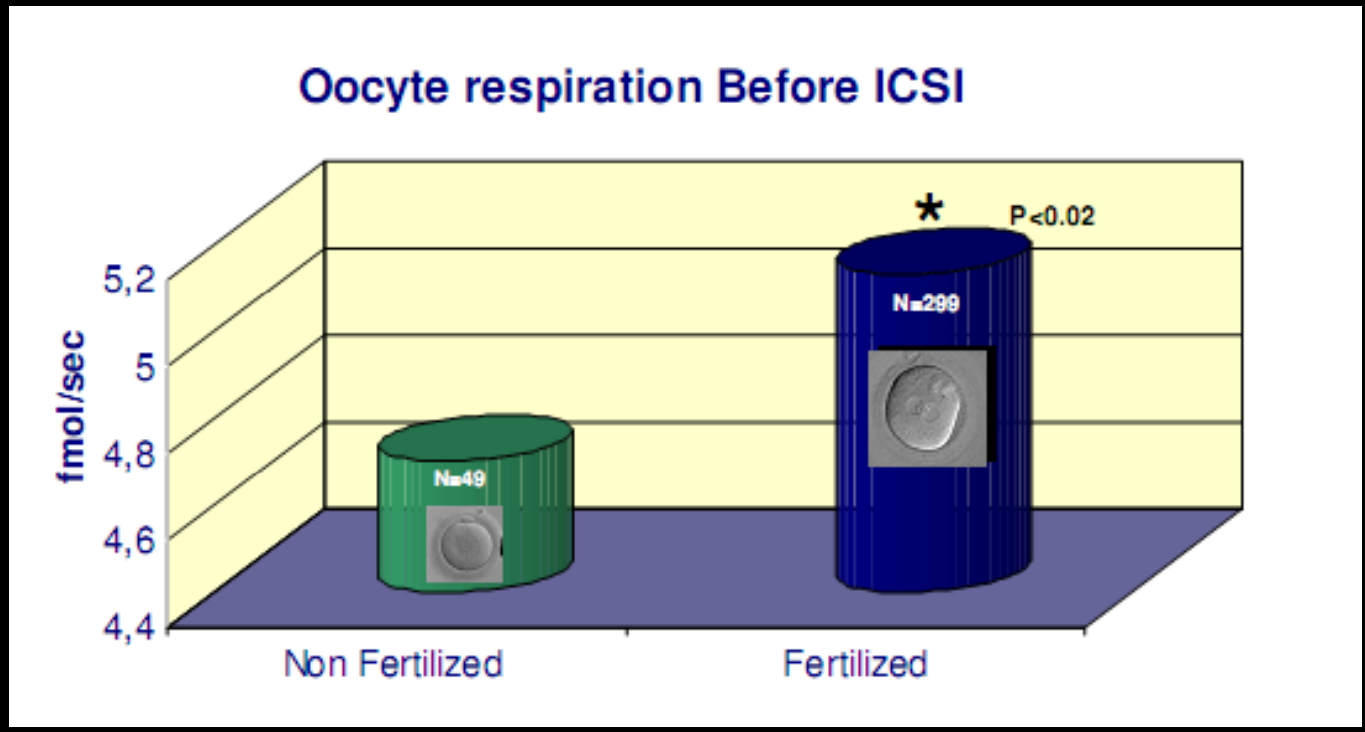




Tejera et al. 2011
(in preparation)

Oocytes (human)

Fertilization

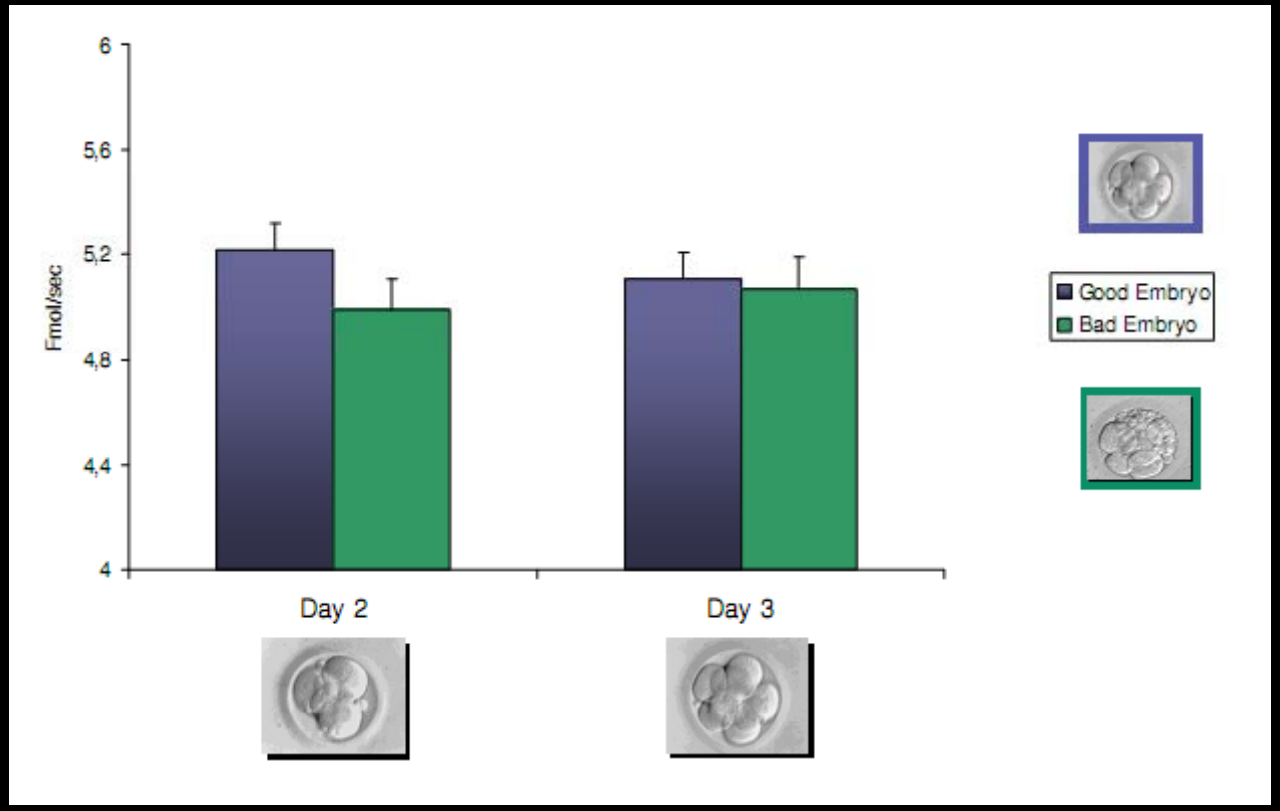


Oxygen consumption higher in oocytes with correct fertilization

Tejera et al. 2010
ESHRE Rome

Oocytes (human)

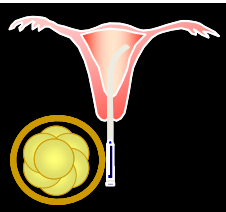
Embryo quality

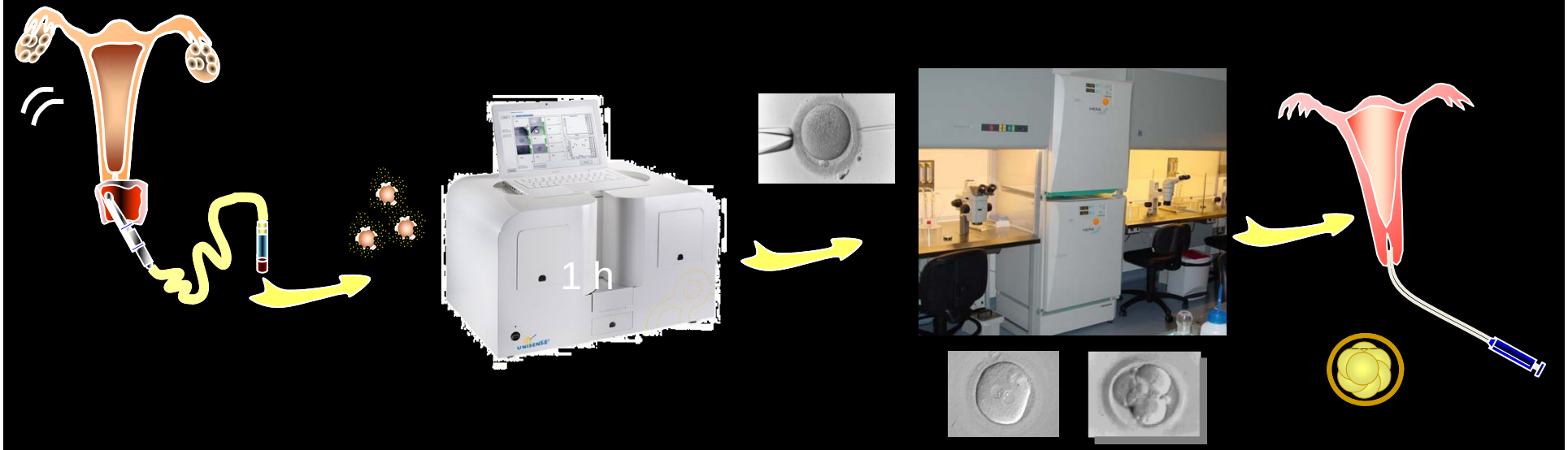


Higher oxygen consumption in oocytes producing good quality embryos (not significant)

Tejera et al. 2010
ESHRE Rome

Oocytes (human)

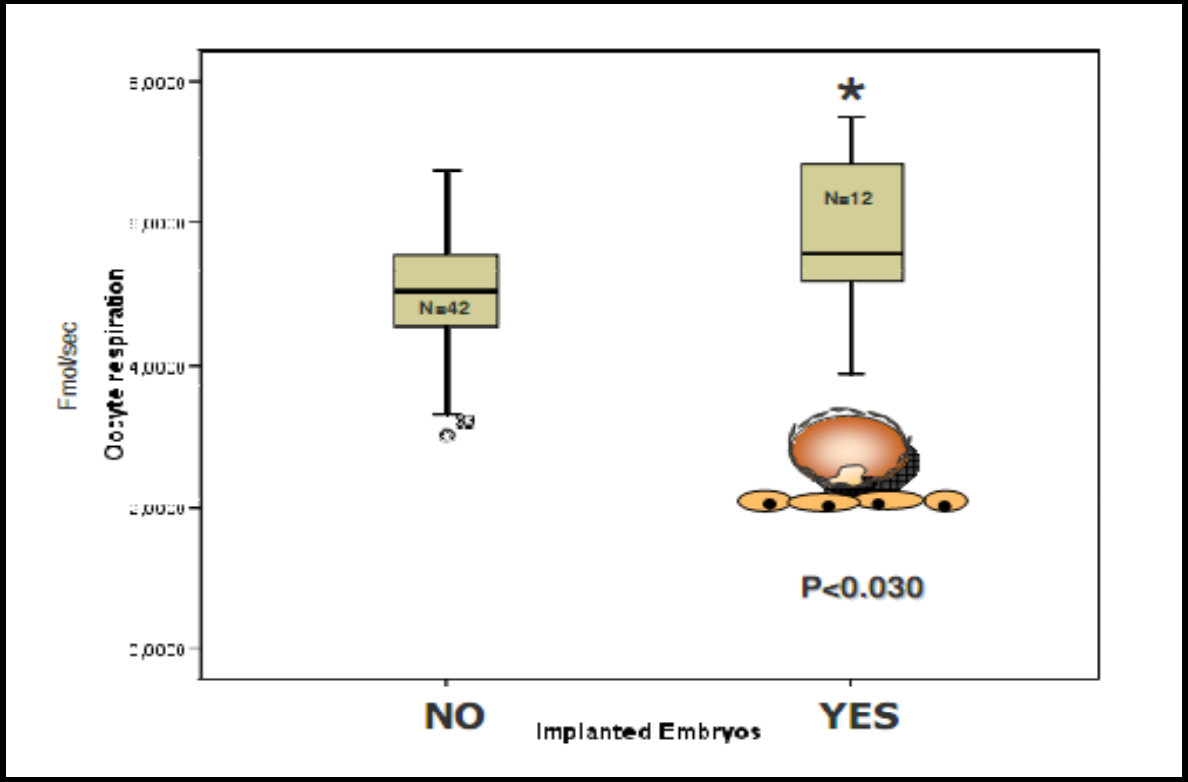
56 couples	348 oocytes	248 embryos	Day-3
			



Tejera et al. 2010
ESHRE Rome

Oocytes (human)

Implantation



Higher oxygen consumption in oocytes producing embryos that sucessfully implanted

Tejera et al. 2010
ESHRE Rome

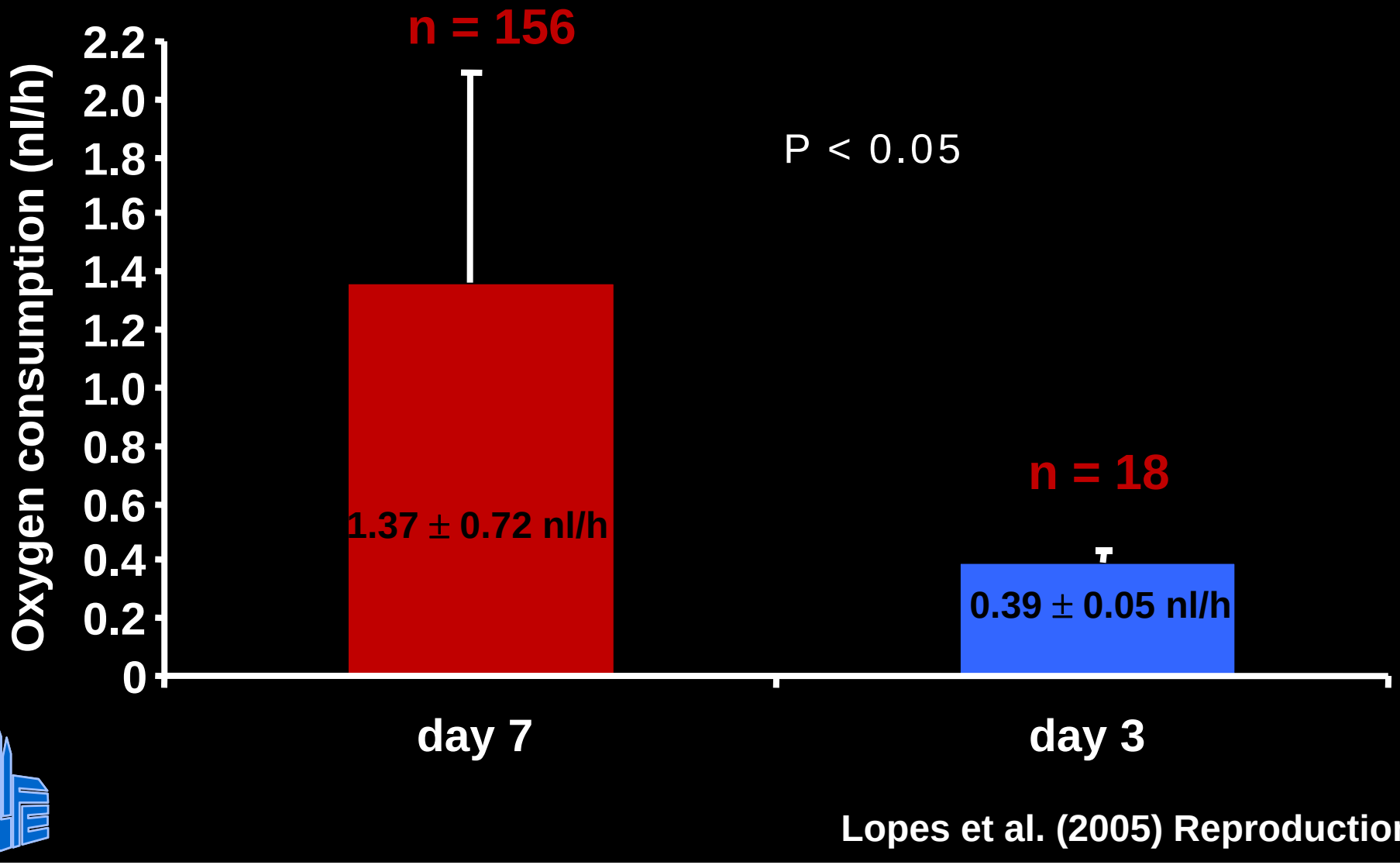
Embryos (bovine)

- In vitro produced embryos
  (day 3: 8- cell stage)
  (day 7: blastocyst stage)

- In vivo produced embryos

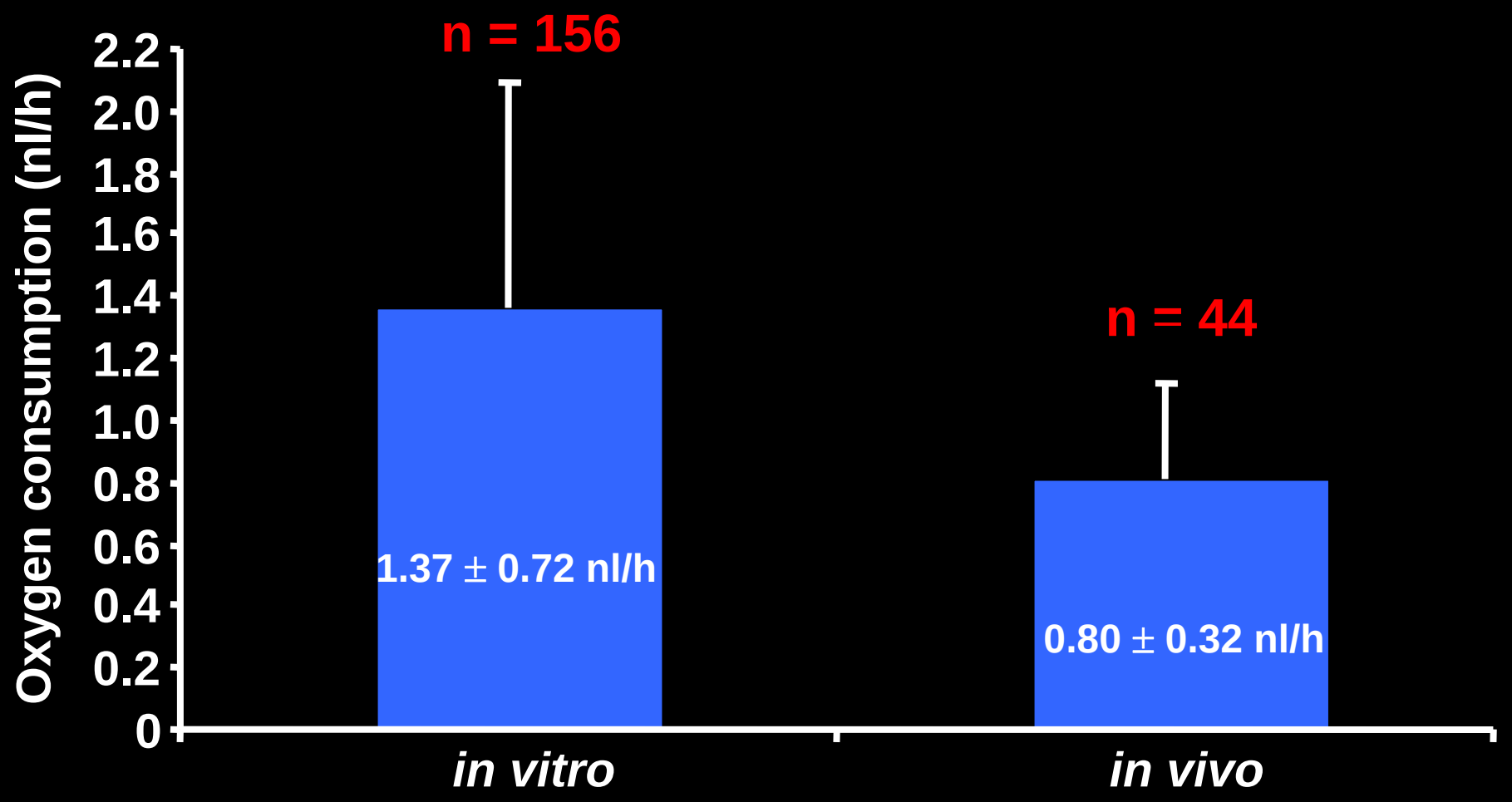
Lopes et al. (2005) Reproduction
Lopes et al. (2007) Human Reproduction

Embryos (bovine)



Lopes et al. (2005) Reproduction

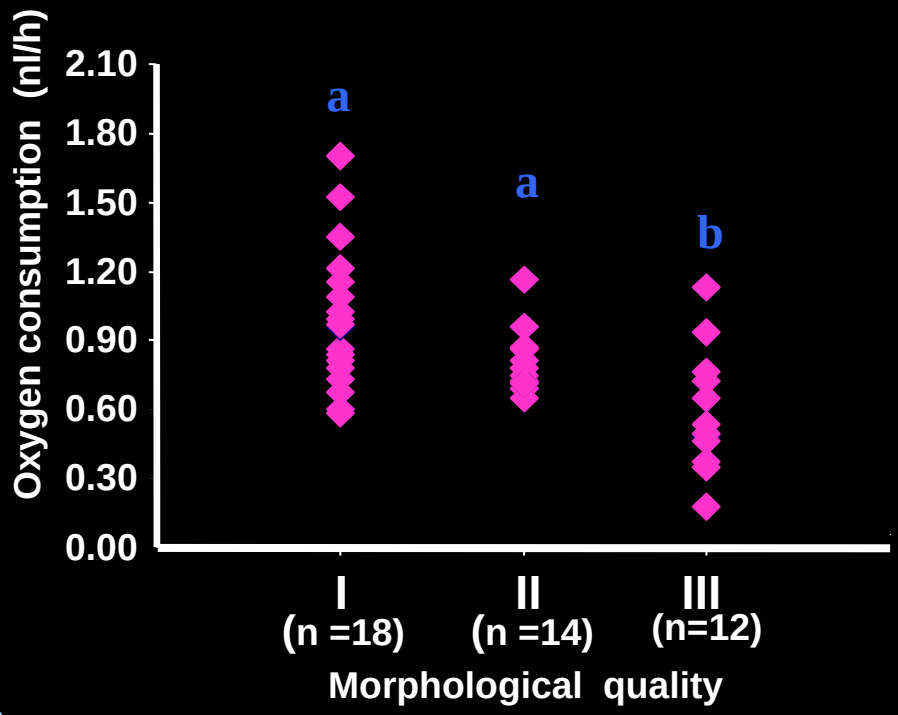
Embryos (bovine)



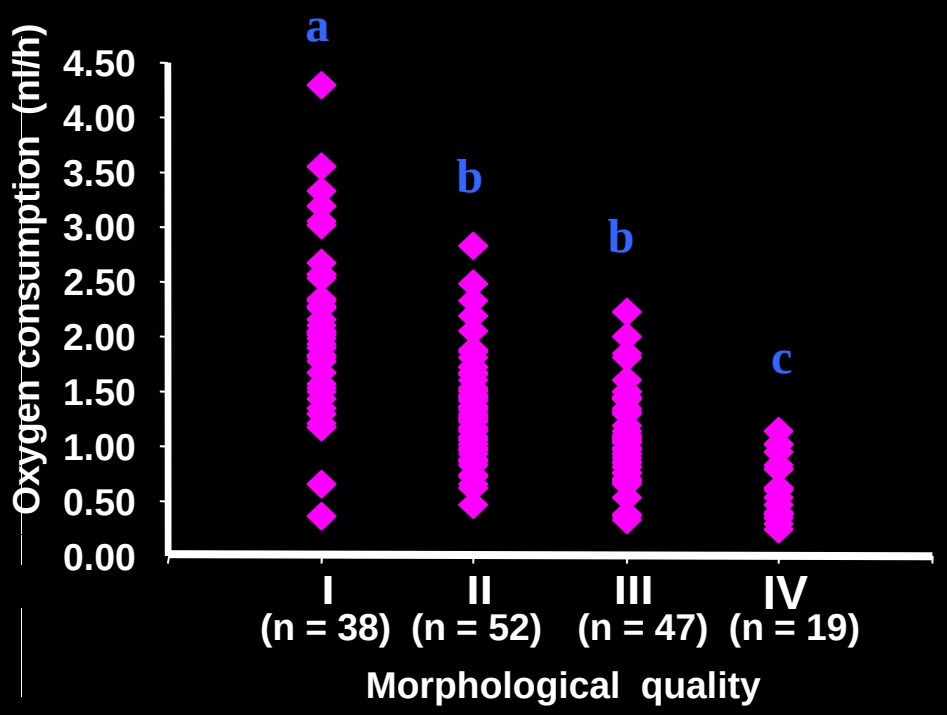
Lopes et al. (2007) Human Reproduction

Embryos (bovine)

IN VIVO EMBRYOS

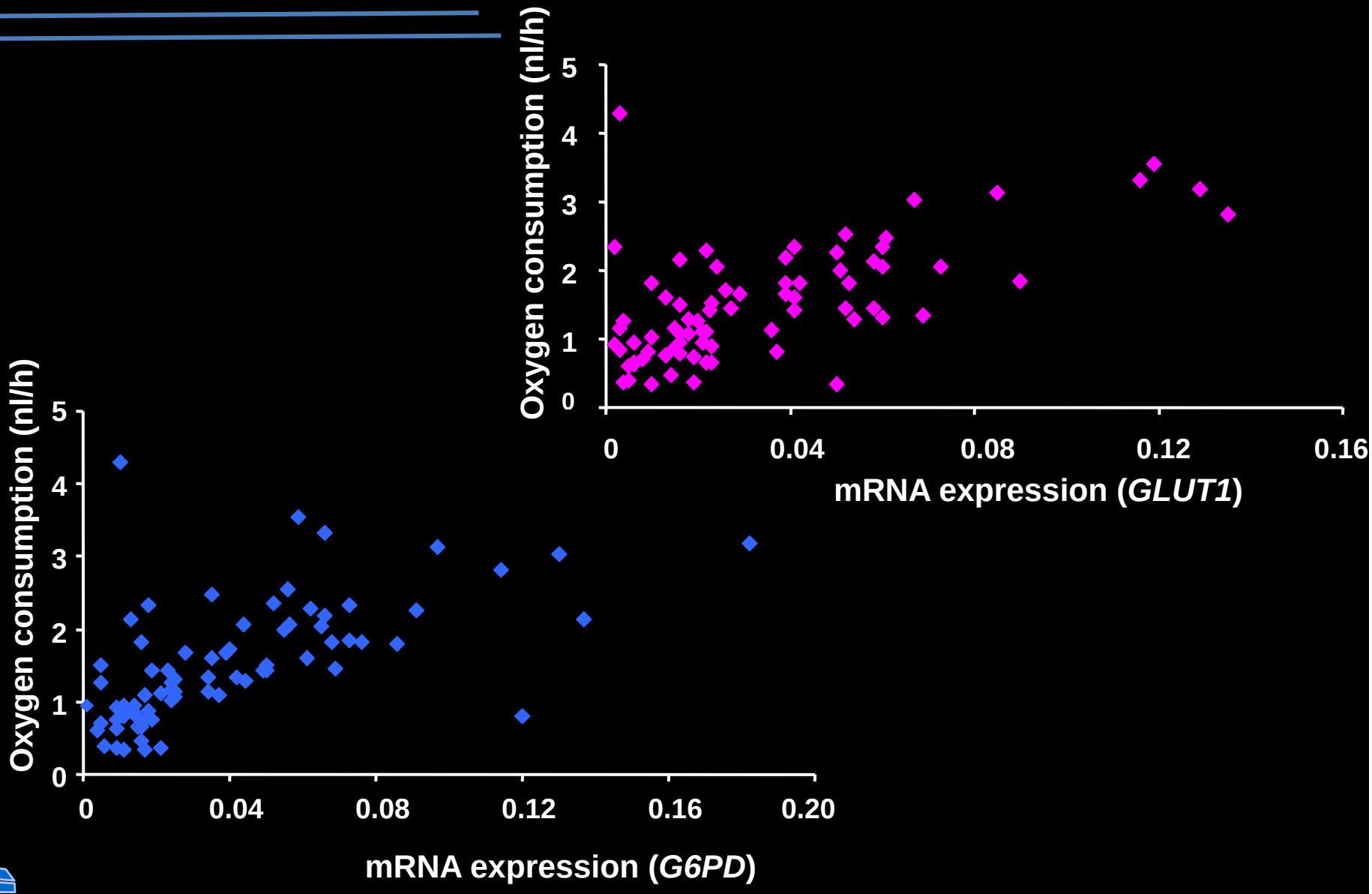


IN VITRO EMBRYOS



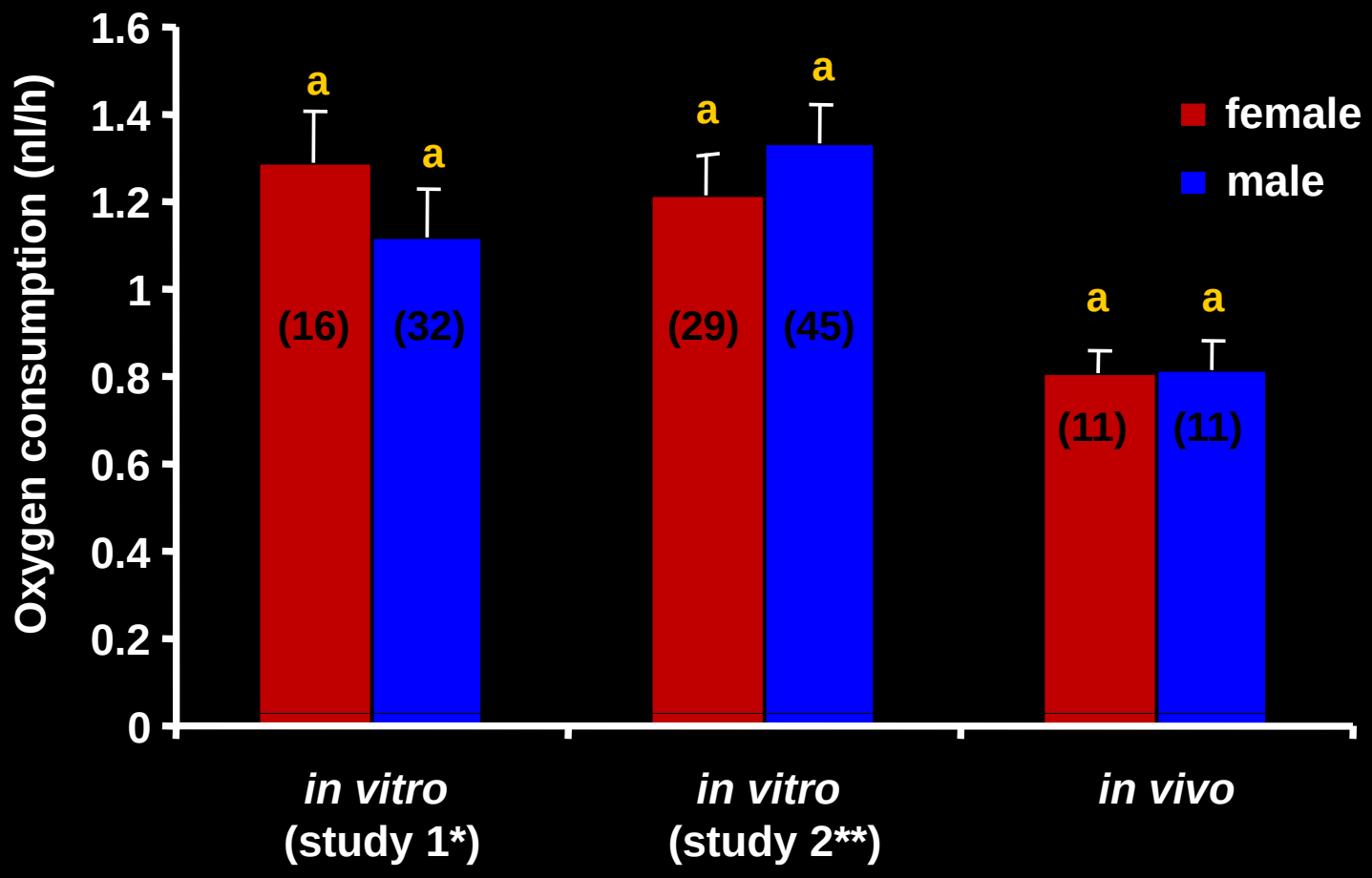
Lopes et al. (2007) Human Reproduction

Embryos (bovine)



Lopes et al. (2007) Theriogenology

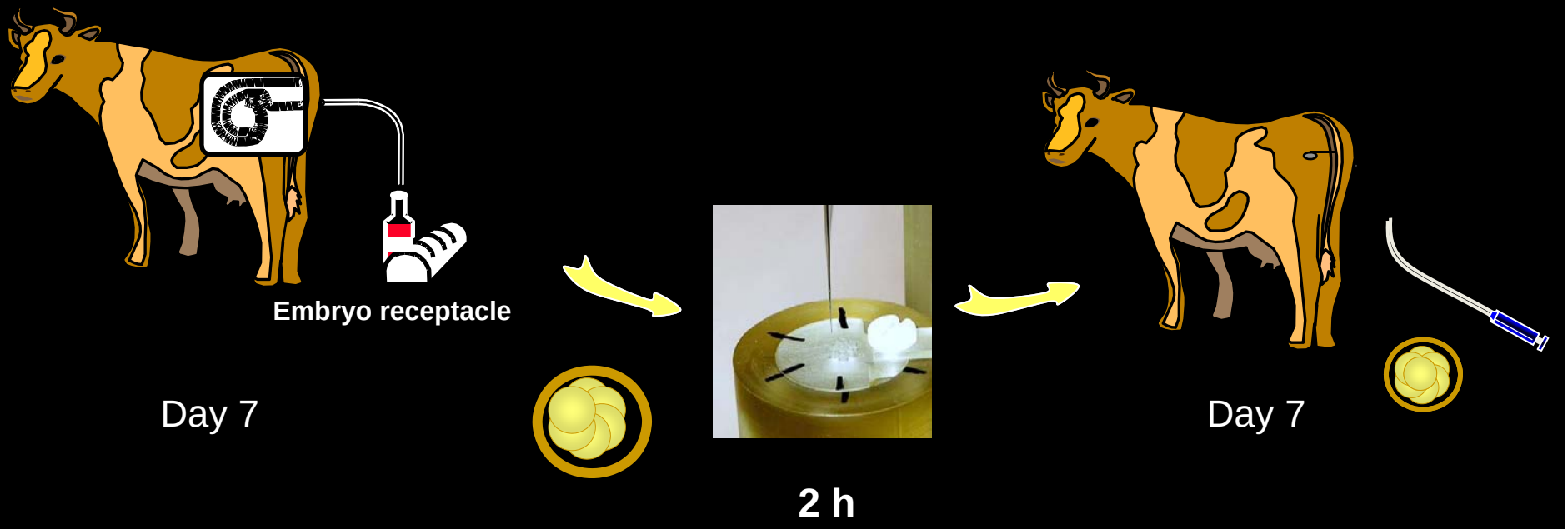
Embryos (bovine)



* Lopes et al. (2005) Reproduction

** Lopes et al. (2007) Human Reproduction

Embryos (bovine)



Lopes et al. 2007
Human Reproduction

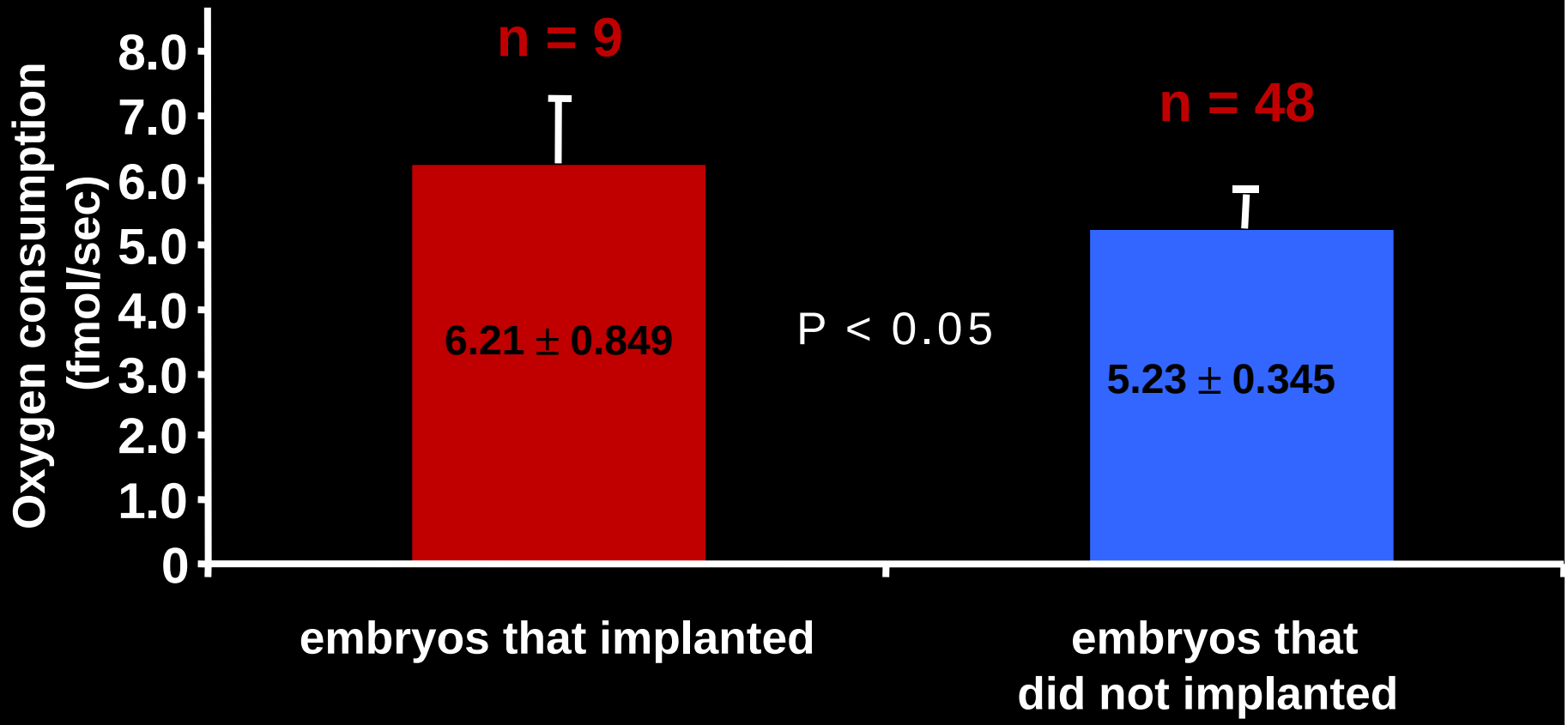
Embryos (bovine)

Pregnancy status

OXYGEN CONSUMPTION CATEGORY	PREGNANT	NON-PREGNANT
High (> 1.0 nl/h)	25 % (n = 1)	75 % (n = 3)
Medium (0.78 - 1.10 nl/h)	100 % (n = 13)	0 % (n = 0)
Low (< 0.78 nl/h)	48 % (n = 11)	52 % (n = 12)

Lopes et al. (2007) Human Reproduction

Embryos (human)



Tejera et al. (2010) Fertility & Sterility

Embryos (human)

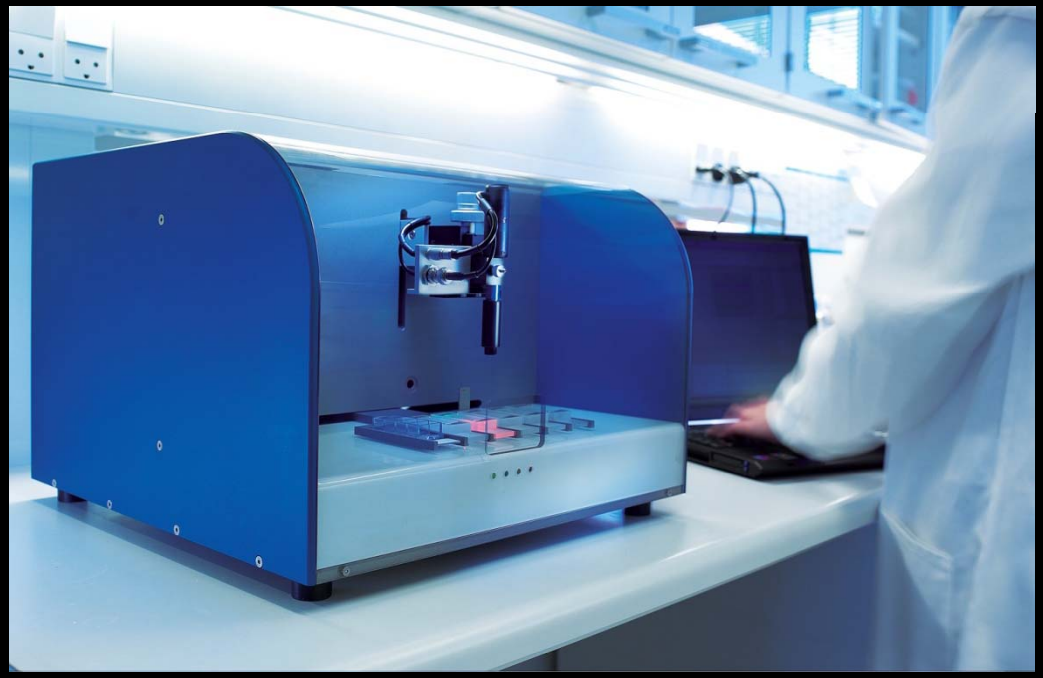
Scanning Electrochemical Microscopy (SECM)

MEASUREMENT OF OXYGEN CONSUMPTION RATE OF EMBRYOS TO SELECT THE BEST EMBRYO FOR E-SET. M. Koike, Y. Kumasako, K. Goto, H. Ito, T. Utsunomiya, H. Abe. St-Luke Clinic, Oita, Japan; Department of Obstetrics and Gynecology Faculty of Medicine Fukuoka University, Fukuoka, Japan; Graduate Program of Human Sensing and Functional Sensor Engineering, Graduate School of Science and Engineering, Yamagata University, Yonezawa, Yamagata, Japan.

RESULTS: Among the cycles (N=72) that embryos showed morphologically exactly same grade by Veeck's method, the pregnancy rate in cycles with an embryo elected based on morphological evaluation plus measuring the oxygen consumption rate had a significantly higher pregnancy rate of 50.0%(18/36). compare with an embryo elected by conventional morphological method was 25.0%(9/36). Further more, the abortion rate of the oxygen measured group(5.6% 1/18) was less than the conventional group(22.2% 2/9). Finally, on-going pregnancy rate was significantly higher in the oxygen measured group(47.2% 17/36) than the conventional morphological method group(19.4% 7/36).

Koike et al. (2010) Fertility and Sterility

Repetitive measurements of oxygen consumption



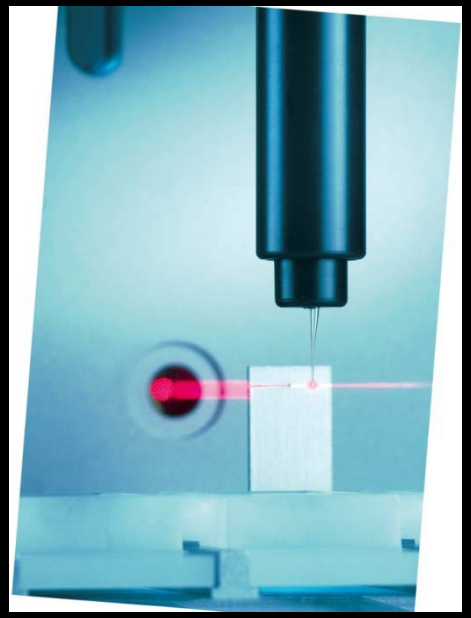
Embryoscope™ Unisense

Repetitive measurements with acquisition of digital images from each embryo

Embryos (bovine)

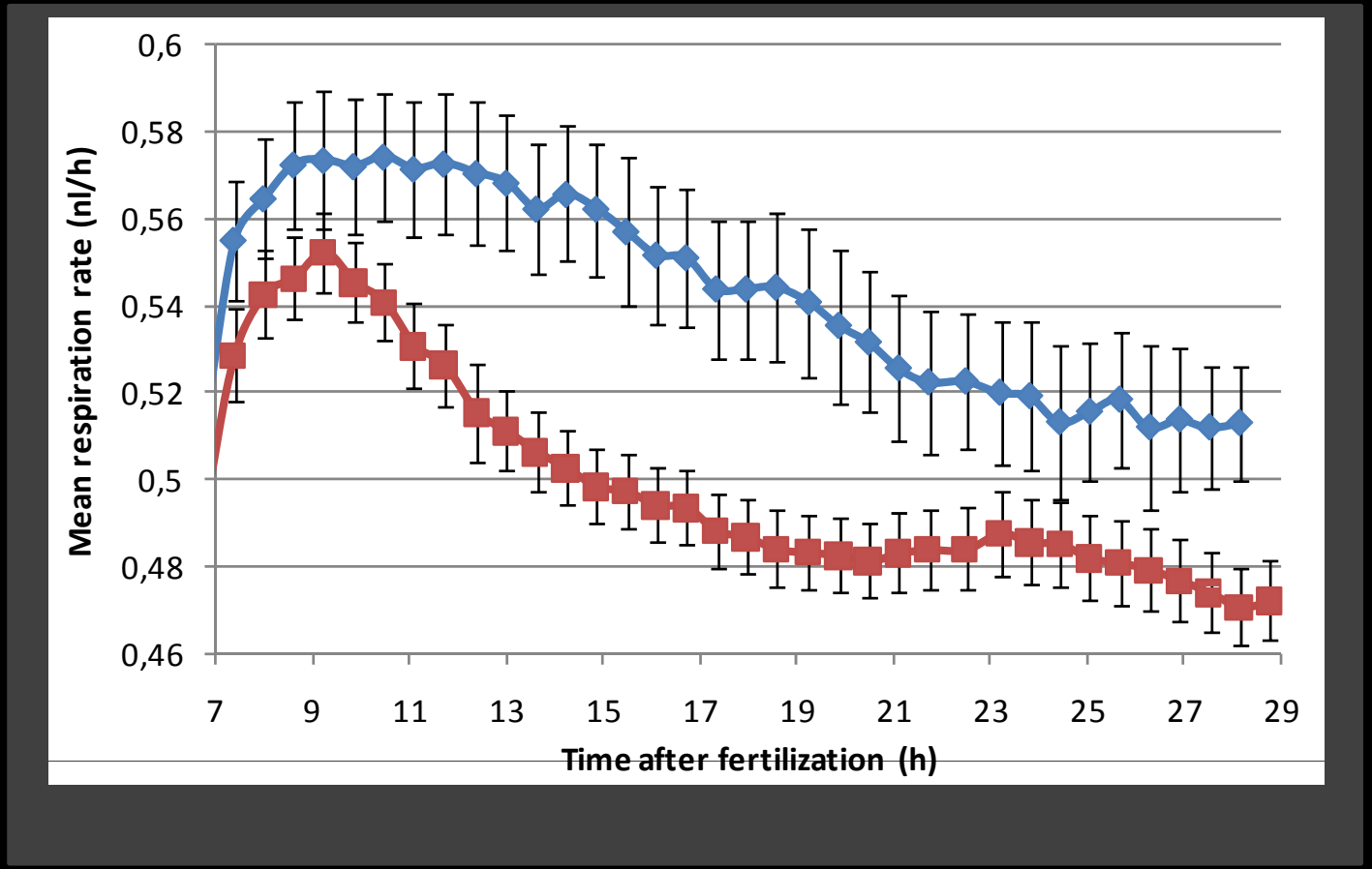
Denuded zygotes
(previous IVF for 6 h)

Loaded in the Embryoslide



Repetitive O2 measurements
+ Digital pictures

Embryos (bovine)

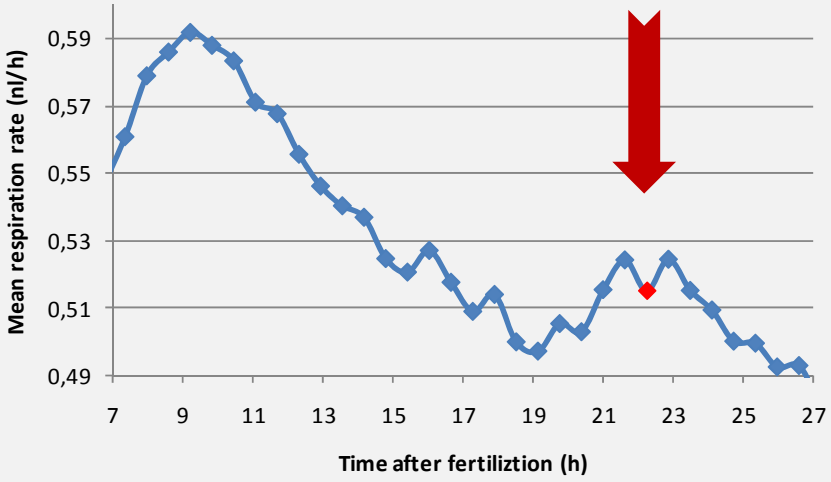


non-fertilized *in vitro*-matured oocytes

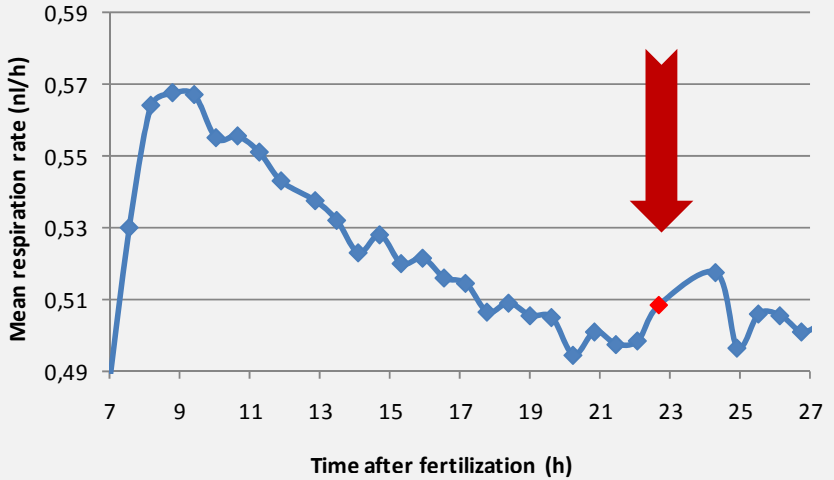
in vitro-produced zygotes

Lopes *et al.* (2010) Human Reproduction

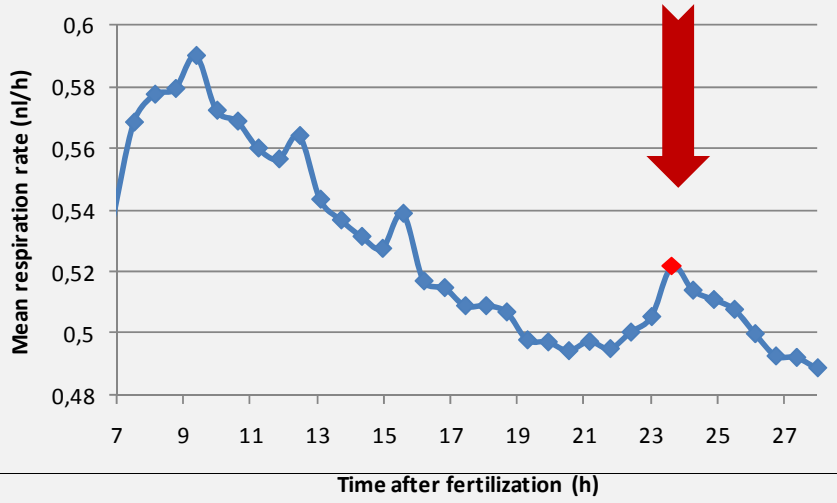
Embryos (bovine)



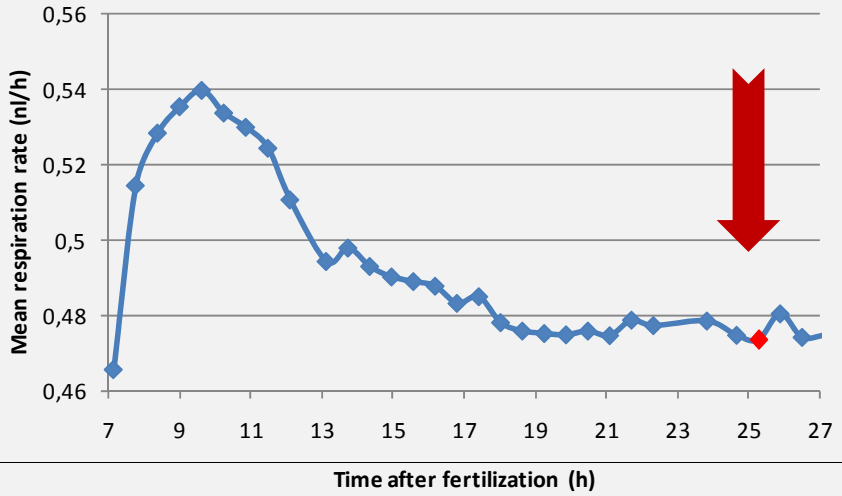
a)



b)



c)



d)

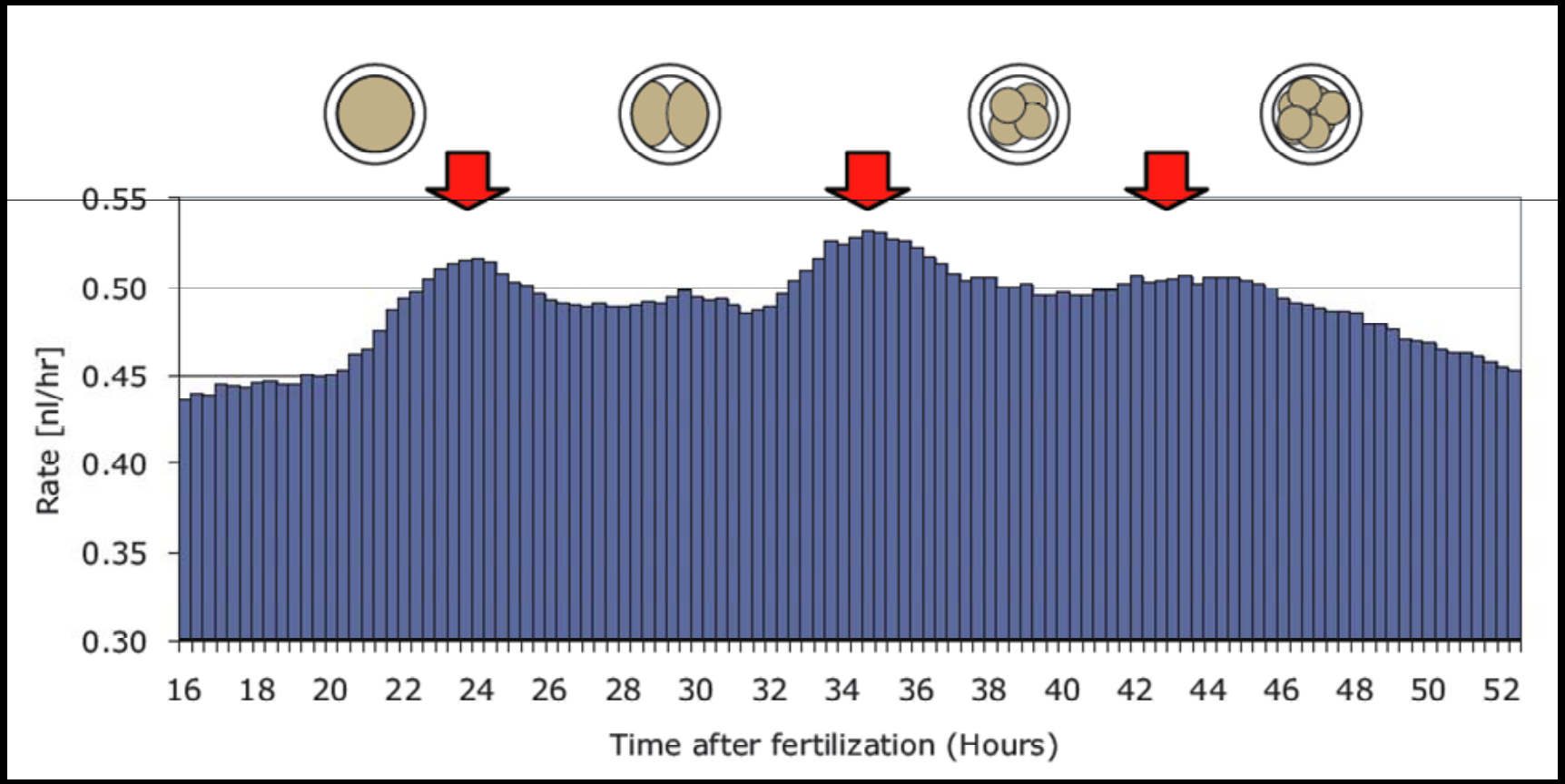
Lopes *et al.* (2010) Human Reproduction

Embryos (bovine)

Time in relation to cleavage (min)	*Mean oxygen cons. (nl/h)*	*SEM*
-72	0.4988[a]	0.0099
-36	0.4911[b,c]	0.0107
0	0.4920[b,d]	0.0100
36	0.4925[b]	0.010
72	0.4873[c,d]	0.0099

Lopes *et al. (*2010) Human Reproduction

Embryos (bovine)



= cell division observed in recorded images

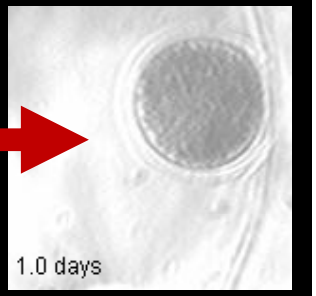
Callesen et al. 2006

Depart. Genetics & Biotechnology, Aahrus University, Denmark

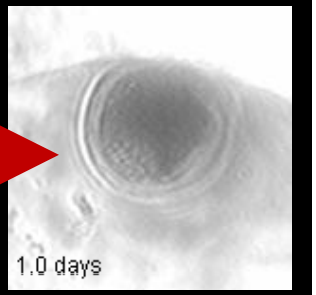
Embryos (bovine)

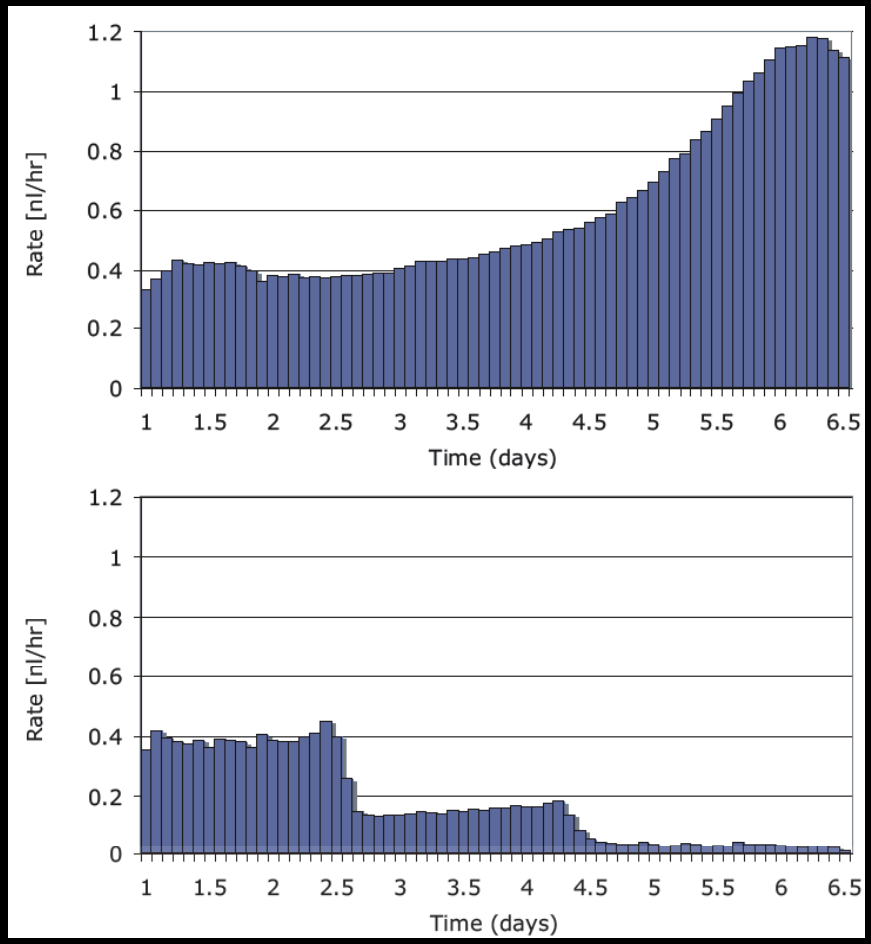
O2 consumption during DEVELOPMENT

Zygote developing to the expanded blastocyst stage



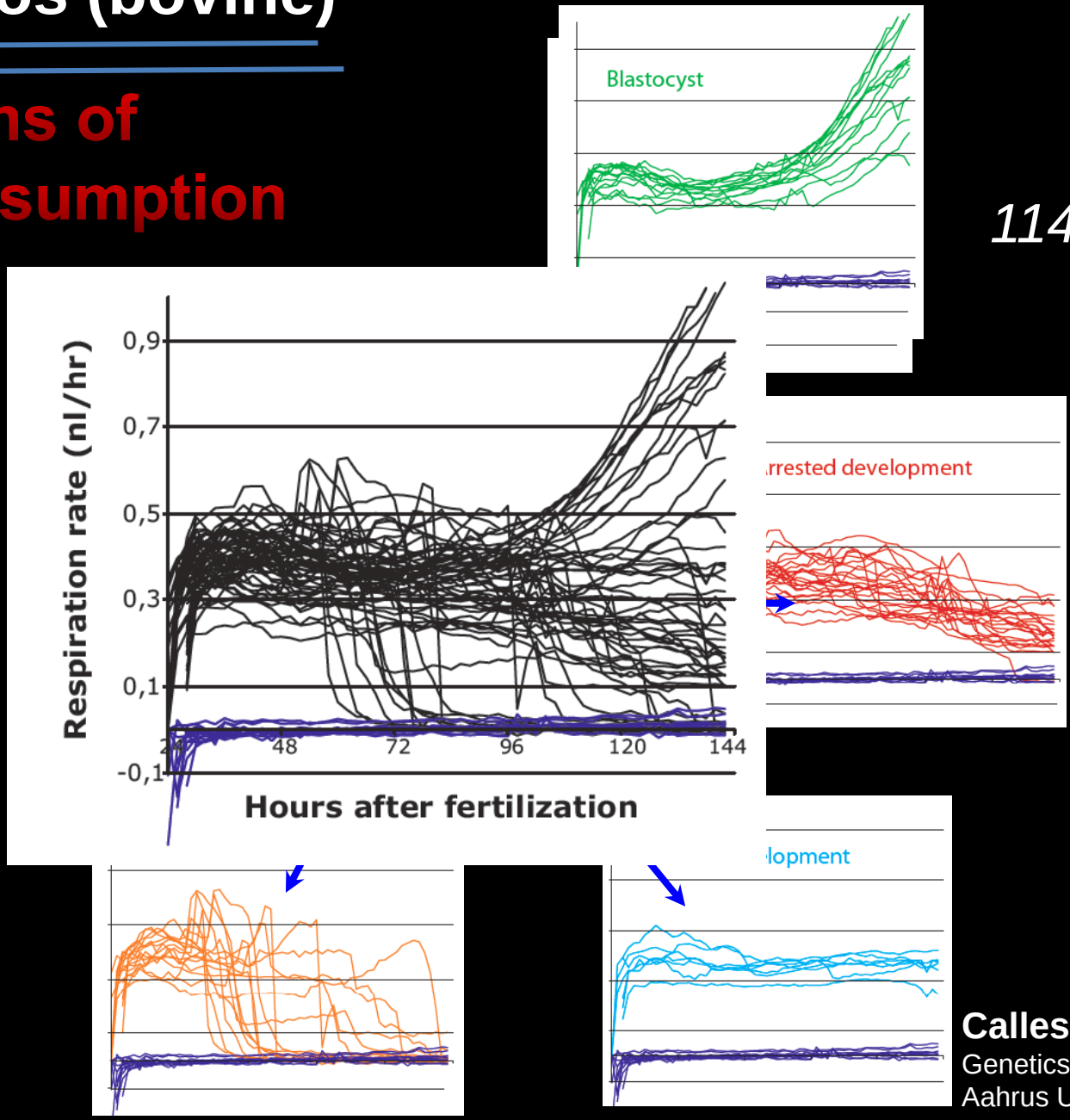
Degenerated embryo





Embryos (bovine)

Patterns of O2 consumption



114 embryos

Callesen et al. 2006
Genetics & Biotechnology
Aahrus University, Denmark

Embryos (murine)

Table 1 Mean individual oxygen consumption rates ± standard error of the estimate

Developmental stage	Mean oxygen consumption (nl O_2 h^{-1} $embryo^{-1}$±SE) All embryos	Mean oxygen consumption (nl O_2 h^{-1} $embryo^{-1}$±SE) embryos reaching exp blast	Mean oxygen consumption (nl O_2 h^{-1} $embryo^{-1}$±SE) embryos arrested before exp blast
2 cell	0.162±0.0043 (*n*=93)	0.162±0.0073 (*n*=20)	0.162±0.0052 (*n*=73)
4 cell	0.166±0.0038 (*n*=128)	0.172±0.0053 (*n*=55)	0.161±0.0053 (*n*=73)
7–8 cell	0.197±0.0089 (*n*=33)	0.218±0.0115 (*n*=15)	0.179±0.0120 (*n*=18)
Morula	0.234±0.0056 (*n*=142)	0.261±0.0070 (*n*=71)	0.207±0.0074 (*n*=71)
Expanded blastocyst	0.464±0.0185 (*n*=69)	–	–

N number of embryos with a recorded oxygen consumption.

Cleavage stage (2-8cell) individual oxygen consumption ranges between 0.16 - 0.20 nl/h

Oxygen consumption rises slightly at the morula stage and significantly at the expanded blastocys stage

Ottosen et al. 2007 J Assist Reprod Genet

Embryos (murine)

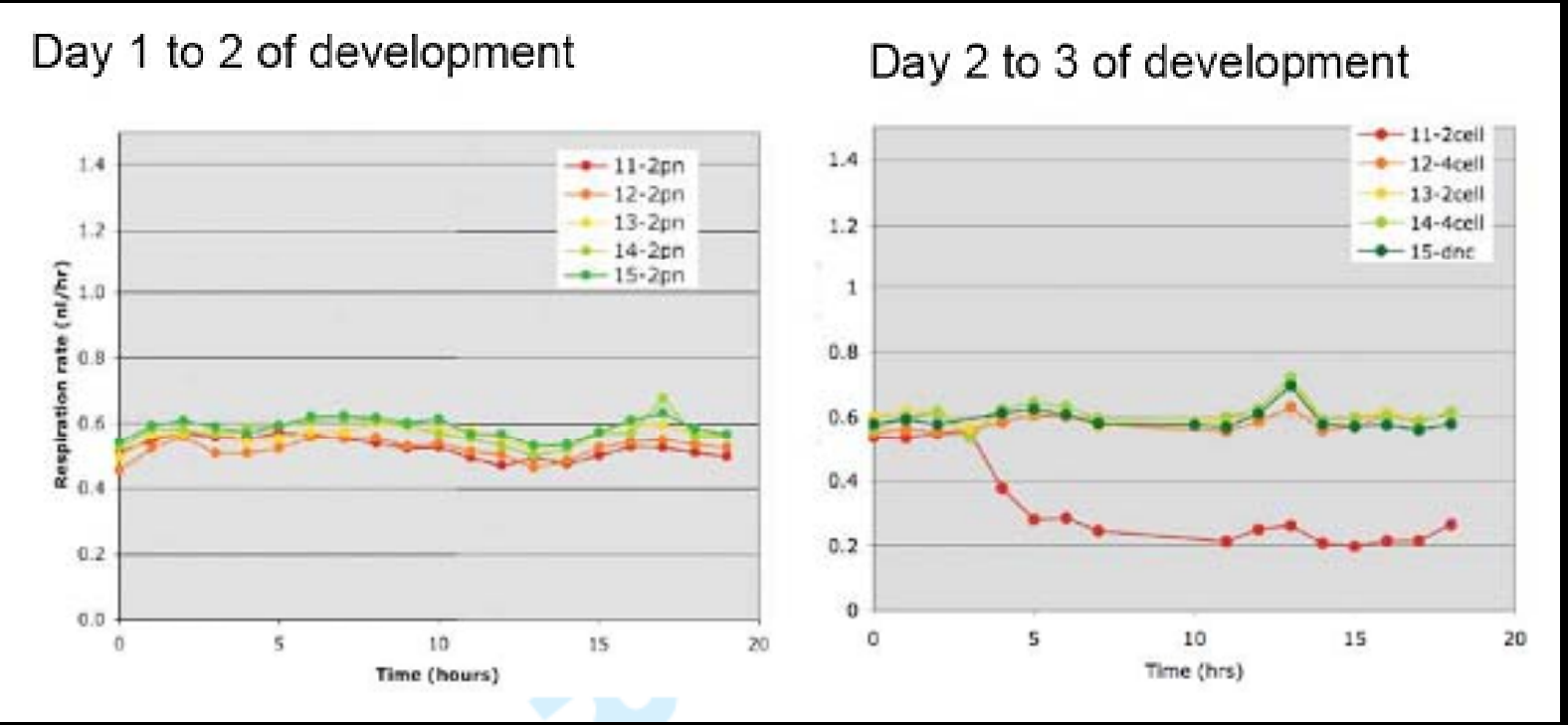
Table 2 Grouping of embryos according to their oxygen consumption, and Odds Ratios (with 95% confidence intervals) for development to expanded blastocysts among various consumption groups

Developmental stage	Low consumption group nl O_2 h^{-1} $embryo^{-1}$	High consumption group nl O_2 h^{-1} $embryo^{-1}$, (n)	OR (95% CI) High consumers for development to expanded blastocyst
2 cell	<0.145 (n = 39)	>0.145 (n = 54)	1.93 (0.67–5.57)
4 cell	<0.145 (n = 43)	>0.145 (n = 85)	2.25 (1.04–4.90)
7–8 cell	<0.190 (n = 14)	>0.190 (n = 19)	4.07 (0.85–19.4)
Morula	<0.230 (n = 72)	>0.230 (n = 70)	3.18 (1.60–6.32)

ONLY at the Morula stage was higher oxygen consumption associated with subsequent development to the blastocysts stage.

Ottosen et al. 2007 J Assist Reprod Genet

Embryos (human)

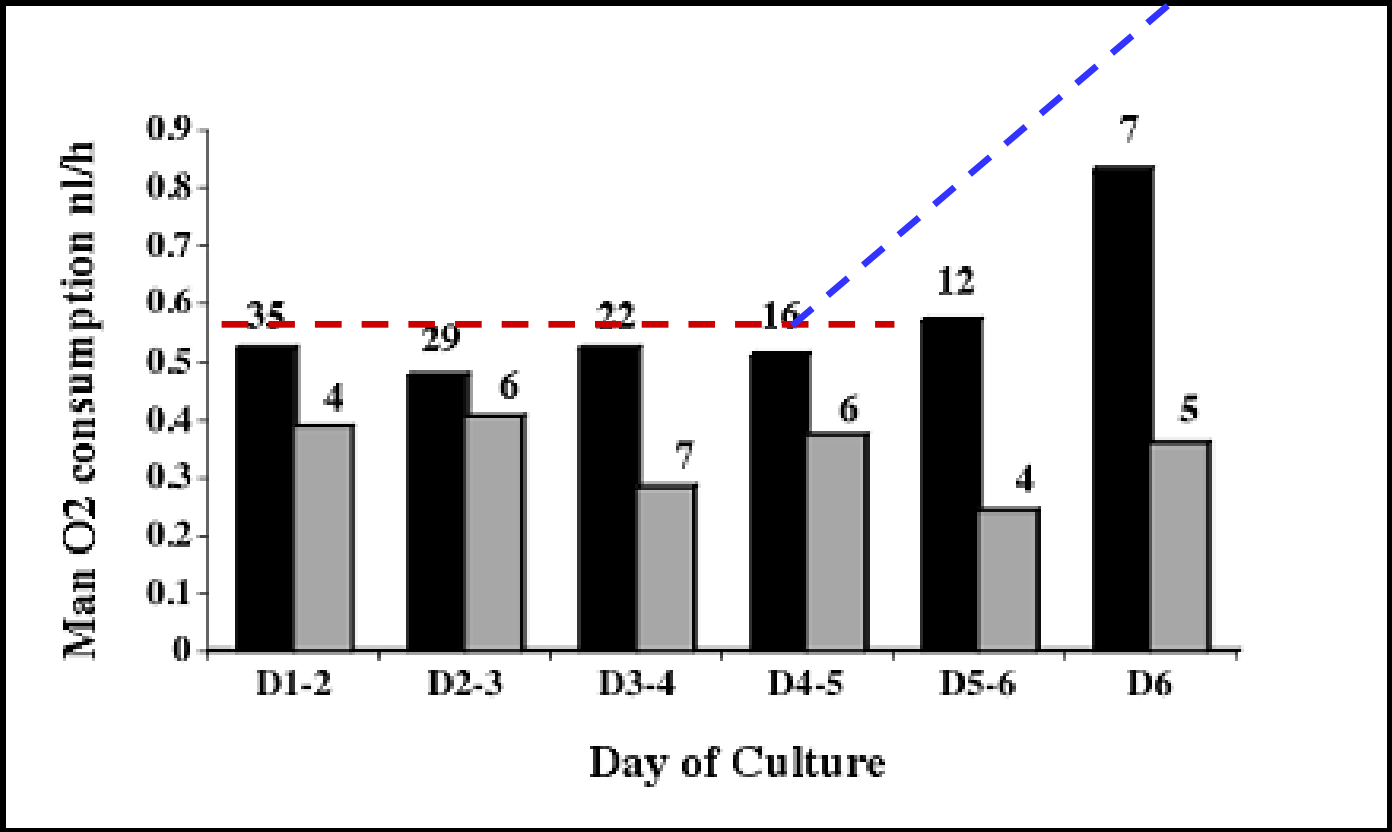


Different O2 rates and profiles for embryos developing vs. arresting in vitro

- Non used thawed embryos (2PN to blastocysts)
- 1 & 3PN embryos
- Day 2 and 3 discarded embryos
- Day 4 abnormal embryos from PGD

Scott et al. (2008) Proc. ESHRE Campus

Embryos (human)



Oxygen consumption is steady until day 4, ranging bt 0.5- 0.6 nl/h

Oxygen consumption rises is consistent with blastocyst formation (range 1.0 -1.3 nl/h)

Scott et al. (2008) Proc. ESHRE Campus

THUS.....

- **Oxygen consumption measured once/continuously is a valuable parameter for assessing oocyte/embryo metabolism**
- **Combining oxygen consumption with other viability criteria may improve in the selection of superior embryos before transfer.**
- **Patterns of oxygen consumption around the time of cleavage may also help in selecting better quality embryos**
- **Analysis of metabolism (and thus oxygen) can be crucial for implementation of the SET policies (mostly embryos similar morphology).**

ACKNOWLEDGMENTS

Henrik Callesen

TRANS EMBRYO GENETICS
Denmark

Søren Madsen

Torben Greve

Marcus Messeguer
AlbertoTejera

Jeremy Thompson
Michelle Lane

Niels Ramsing

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