Respiration measurements of Human Oocytes and Embryos: Potential for Selection?

Lynette Scott[1], Joseph Hill[1]
Neil Ramsing[2], Jens Gundersen[2],
Fertility Centers of New England
Unisense/ Fertilitech, Denmark

FERTILITY CENTERS of NEW ENGLAND

UNISENSE

Disclosure

- **The FCNE (Scott and Hill)have no commercial or financial interest in this technology, we are a site tester for its effectiveness**
- **Unisense/Fertilitech (Gundersen, Ramsing) have a financial and commercial interest in the technology. They provided the equipment, technical support, training and some data analysis**
- **The project is generously supported by an unrestricted educational grant from Organon Pharmaceuticals to Scott**

FERTILITY CENTERS of NEW ENGLAND

UNISENSE

Introduction

- **Oocyte health is hard to measure**
- **Oocytes requite ATP for development**
- **Oocyte mitochondrial activity and health are critical for development -** *VanBlerkom et al. 2008*

Embryos require ATP for development
BUT
"The Quiet Hypothesis"

- **So how metabolically active should human oocytes and embryos be?**

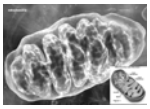
Mitochondrial DNA and Oocytes

- **138-315K copies per oocyte** *Steurwald et al, 2000*
- **11 - 903K/oocyte** *Reynier, 2001*
- **Range is 11-900K and the lower levels = abnormal oocytes**

- **GV 276K**
- **M1 227K**
- **M2 256K**

The mtDNA load is fixed at Ovulation It is not influenced by Nuclear Maturation

- **There are differences between oocytes within a cohort and between cohorts** *May-Panaloup et.al., 2007*



Mitochondria

- **Generate ATP, which is essential for maturation in the oocyte**
- **Regulates Ca+ release from ER, which is essential for the Ca+ oscillations which drive fertilization**
- **Involved in maintenance of the internal redox potential of the cell/oocyte**

- ***"It has been proposed that the viability of early mammalian embryos is associated with a metabolism that is "quiet" rather than "active" "***
  - *Leese HJ. 2002:BioEssays*
  - *Leese et al., 2007: Hum Reprod.*
- **In the bovine system blastocysts with very high or very low respiration rates are non-viable.**
  - *Lopes et al., 2007: Hum Reprod.*

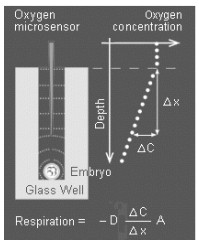
EmbryoScope- Non-invasive measurements of oxygen consumption

A sensor measures the concentration of an *analyte* by a current generated by an electrochemical reaction involving the analyte. The current in the circuit depends on the *concentration* of the *analyte*,

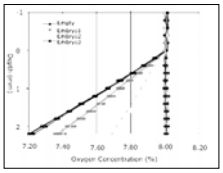
<u>oxygen</u> in this case.

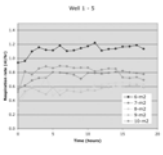
Which is expressed as use nl/hour

Respiration Measurements



The oocyte/embryo at the bottom of the well. uses O2, a diffusion gradient is established down the length of the well. Respiration is proportional to the slope of this gradient. Measurements are obtained over a set time.





Respiration Rate = -Diffusion Coefficient x Slope x Area

Alternative techniques

- **Proteomics, or what compounds is the oocyte/ embryo producing?**
- **Dip stick technology for a specific product**
- **Glucose consumption in later developmental stages**
- **AA utilization/ media depletion**

o ***Houghton, 2002; Leese 2003; Gott, 1990; Reiger, 1992; Lopes, 2005***

Oocytes

- Oocytes are very active, then arrest at M11
- Early embryos do not "grow" but metabolize and begin gene activation
- They switch from aerobic respiration using pyruvate, amino acids, to oxidative metabolism and aerobic glycolysis *(Martin, 2000; Sakkas & Gardner, 2005: Leese, 2000)*
- Lack of ATP in the oocyte results in deregulation of Ca+ homeostasis, which will = high cystolic Ca+
- This is the first step in apoptotic cell death, which may not be manifested until after fertilization and during embryo development

Hypothesis

- Respiration Measurements of oocytes
  - Or Oxygen Consumption
- May indicate the mitochondrial DNA load
- or MT which can activate which will = ability to grow and sustain development
- May be a means of selecting oocytes with the appropriate developmental competence

Experimental Runs with Human Oocytes

- Oocytes were non-clinical
- Either GV or MI on the day of ICSI (D0)
- Or GV, MI or MII not fertilized on D1
- All were read individually
- Measurements taken at 40 h post hCG for D0 and 58 h post hCG for D1 oocytes

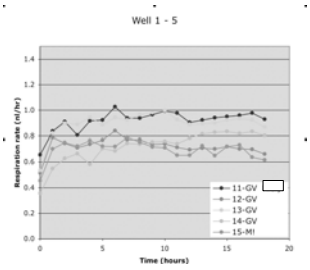
- The initial or Base Respiration Rate (first 1-3 hours of measuring) were used in analysis
- Oocytes were kept in culture for a further 18 h and scored for end state (mature, arrest, atretic)

Oocytes-Design

- **502 oocytes were used**
- **Data was analyzed and compared for initial stage of development**
- **Ability to mature in vitro (GV-M1)**
- **By patient age, FSH, infertility, stimulation, cohort fate**

-Scott et al., 2008, RBMOnline

Day 0 Oocytes 40-41 h Post hCG



11-GV to M2

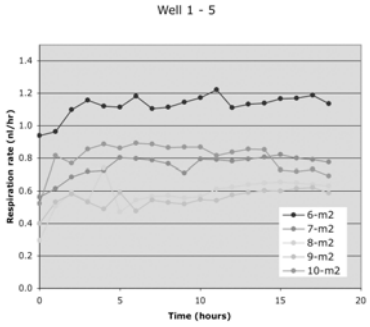
13, 14-GV to M1

12-Remained GV

15-M1 to M2

Mean rates varied between oocytes

M2 Non-Fertilized Oocytes Day 1, 58 h Post hCG



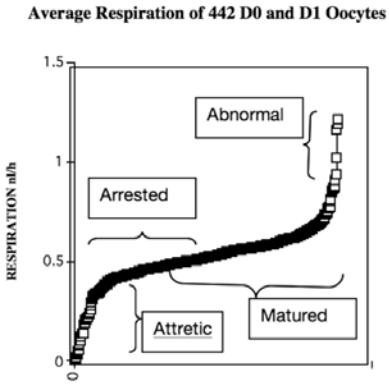
6, 7, 8 Patient 1

9, 10 Patient 2

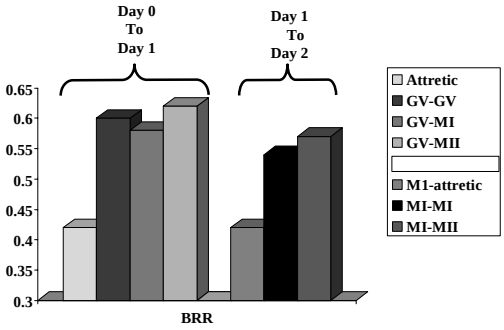
Average Rates
0.48 to 1.1 nL/H

- **Oocyte Differences**
- **Patient Differences**

BRR by stage of development and fate



BRR For All Oocytes By Fate (422)



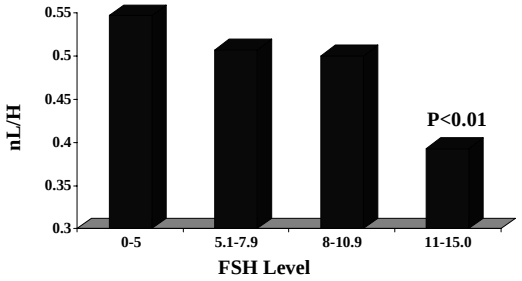
BRR on D 1 *vs.* D0 were lower but it was NS
RR are decreased with *in vitro* aging/maturation

FSH and Age vs. BRR

- **Increasing FSH levels are associated with decreased pregnancy outcome**
- **Increasing age results in declining fecundity**

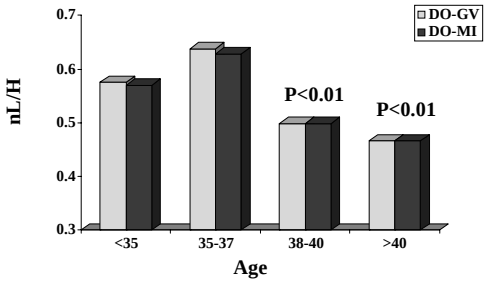
❖**Do either affect the BRR of oocytes?**

BRR for all oocytes (n=442) by FSH levels



BRR decreases as FSH increases
Consistent with clinical data where FSH affects outcome

BRR vs. Age



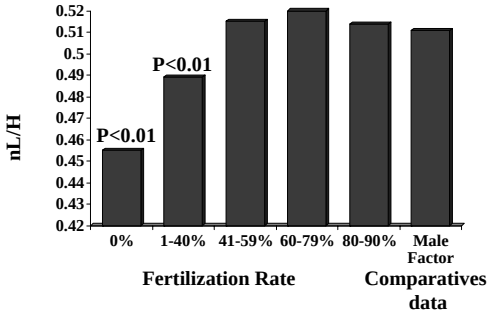
BRR decreases with age regardless of oocyte stage

M2 Oocytes that Fail to Fertilize

- **The mtDNA load is lower in oocytes that fail to fertilize when there is no male factor** *Reynier et al., 2001*
- **Will BRR also reflect this decrease?**

- **All failed fertilized M2 oocytes on D1 were analyzed from non-male factor, with fertilization rates divided by 0% to 90%. N= 134**
- **Comparative rates were M2 failed fertilized from male factor patients.**

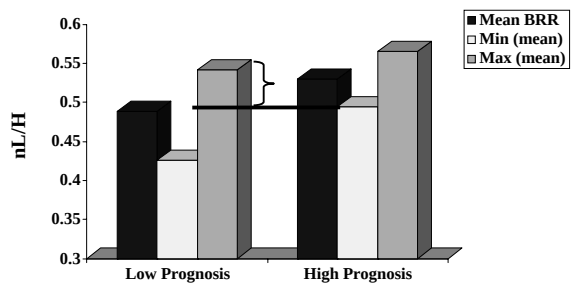
BRR and Fertilization



High vs. Low Prognosis Patients

- High prognosis were <38 years, had no severe ovarian disease (endometriosis, PCOS), BMI< 35, were on cycle 1 or 2, and who had at least 3 oocytes studied.
- Low prognosis patients included those with age >37, any age with ovarian disease, on cycle >2 with no pregnancy, but also had to have at least 3 oocytes for study

Mean, Minimum and Maximum BRR in High and Low Prognosis Patients



Conclusions

- **Oocyte Base Respiration Rates may correlate with oocyte health**
- **BRR rates correlate with ability to mature in vitro, and with FSH levels and Age**
- **ATP is required for fertilization and low BRR in non-male factor cases are consistent with failed and low fertilization rates**
- **Low prognosis patients have oocytes with low BRR, but a small cohort of these oocytes can be identified which fall in the high prognosis range**

Scott et al., 2008, RBMOnline

EMBRYOS

- ***"It has been proposed that the viability of early mammalian embryos is associated with a metabolism that is "quiet" rather than "active" "***
  - ***Leese HJ. 2002:BioEssays***
  - ***Leese et al., 2007: Hum Reprod.***
- **In the bovine system blastocysts with very high or very low respiration rates are non-viable.**
  - ***Lopes et al., 2007: Hum Reprod.***

What does this mean for human embryos and can this technology be used clinically to select embryos with increased potential based on their respiration?

Embryos- Source

- **Thawed embryos, 2PN to Blastocysts stage, donated to research***
- **1PN and 3PN abnormal fertilized oocytes***
- **Day 2 and Day 3 embryos not used in cryopreservation and donated for research***
- **Day 4 abnormal embryos from PGD/PGS and donated to research****

This work was under both informed consent* and IRB*

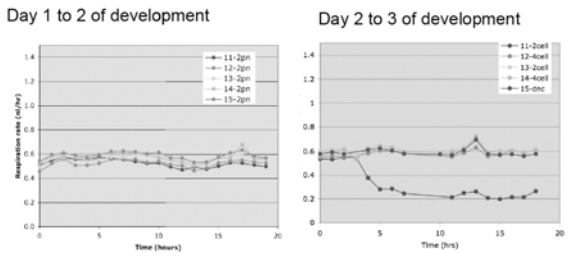
Design

- **RR monitored as the embryo developed, for as long as 120 hours of culture**
- **The mean rate at the beginning of the culture period and the end of the culture period were calculated**
- **All media kept constant (Sage) using 5% CO2/Air**
- **Data was analyzed accorrding to embryo fate for the preceding 24 hours:**
  - **Did it continue cleaving as expected?**
  - **Did it arrest or become atretic?**

TIMING

- **ER** **36 h post hCG**
- **Insemination** **40-41 h post hCG**
- **Day 1, 2PN (1PN/3PN)** **16-18 h post insem.**
- **Day 2, 2-4 cell** **42-44 h post insem.**
- **Day 3, 5-8 cell** **66-68 post insem.**
- **Day 4, morula** **90-92 h post insem.**
- **Day 5** **114-116 h post insem.**

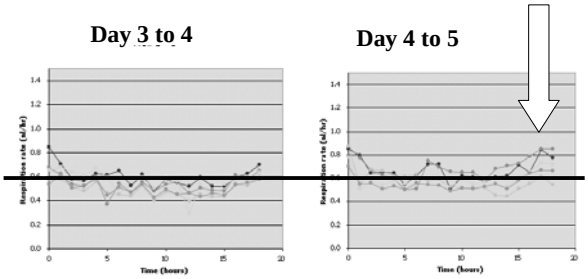
Rates from Thawed 2PN's during development



11- Z3 to 2 cell to 6 cell, then arrested•—•
12- Z2 to 4 cell to blastocyst •—•
13- Z3 to 2 cell to blastocyst •—•
14- Z1 to 4 cell to blastocyst•—•
15- Z1, thick zona, did not cleave, fragmented•—•

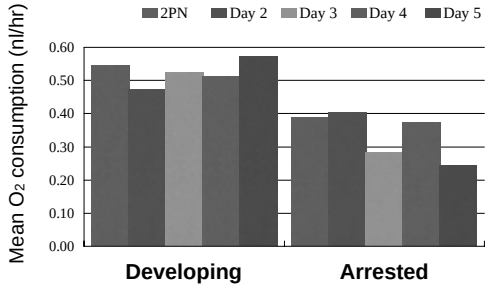
Increase in Respiration with Blastocyst Formation

Day 3 to 4 **Day 4 to 5**

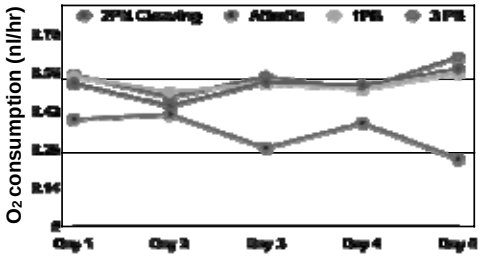


Mean respiration rate 0.5 nl/h **Mean respiration rate 0.7 nl/h**

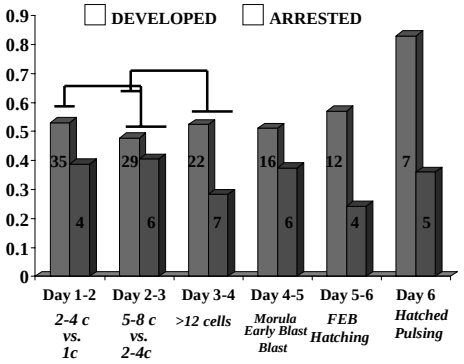
Mean Respiration Rates Based on Developmental Ability
All Embryos- Fresh and Thawed



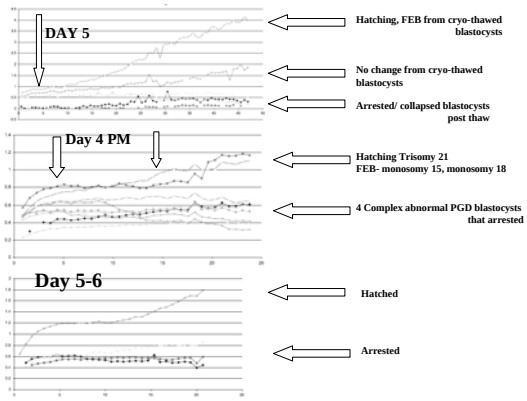
Mean Respiration Rates at the beginning of culture on each day



Mean Respiration Rates of 39 Thawed 2PN embryos during development over 120 hours



Blastocyst Respiration Rates



Cleaving Embryos- Conclusions

- **Respiration rates measured on the EmbryoScope indicate stable or "quiet respiration" for viable embryos with developmental potential.**
- **Abnormal embryos or embryos that are destined to stop growing have lowered rates, when measured at the beginning of a culture period**
- **Respiration, as measured with the EmbryoScope, only ramps up after the time human embryos would be used clinically**
- **RR could be used clinically when ideal levels at each developmental stage are validated in a clinical setting**

Conclusions

- **Respiration measurements on single human oocytes and embryos are feasible in the EmbryoScope**
- **The technology is non-invasive and *may* become compatible with ART laboratory procedures**
- **Differences in Respiration Rates between cohorts of oocytes, oocytes and embryos in a cohort and between sources of oocytes were found which could be the basis of defining limits for future selection criteria**
- **The Initial or base RR of oocytes and embryos can indicate their fate, if they will continue to grow or not, even when morphology does not indicate arrested development.**