A noninvasive approach for human oocyte and embryo selection: new insights into the relationship between developmental competence and bioenergetics

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MAKING MITOCHONDRIA GREAT AGAIN
Current Focus in Clinical IVF is on Energy and the Role of Mitochondria in its Production

It is widely assumed that insufficient energy production creates a cytoplasmic bioenergetic deficiency making it unable to support essential developmental processes during preovulatory oocyte maturation, fertilization and preimplantation embryogenesis.

Proposed causes: abnormal mtDNA copy number (too low or too high) or subnormal mitochondrial mass, maternal age-related structural defects (limited evidence for); ATP analysis suggests no significant age-related decline in energetics at MII (Van Blerkom 2011, Mitochondrion).
This has largely been the rationale for the ‘bioenergetic rejuvenation’ enterprise by:

mitochondrial refreshment, reinvigoration, replacement, dietary supplementation, spindle or pronuclear transfer to donor oocyte—i.e., general use for non-OXPHOS diseases and recently touted in popular press for 60yr old+ women who can have baby with own genetics by replacing or supplementing their ‘worn out’ mitochondria--

responses in media---positive: a new world for patients; negative: living in an alternate universe
...but wait, there’s more....’natural’ herbal remedies and homeopathic cures for low energy to boost fertility

Reverend John’s PhD, D.D.: Mitochondrial Elixir, Tonic and Infertility Cure*

*MMGA Caps, sample bottles, and 55 gallon drums soon or be available
Focus of this presentation on the following:

Can mitochondrial organization and cytoplasmic bioenergetic state be assessed noninvasively in living oocytes and embryos for purposes of selection and diagnosis of maturation, fertilization or preimplantation failure?

Thermographic evidence supporting:

(i) disproportionate mitochondrial segregation during cleavage as a common and significant factor affecting developmental competence in clinical IVF.

(ii) compartmentalization of differential energy production as being central to understanding developmental competence and how it may support, or not, claims of restoring fertility.
Some Basics
If energy is central to reproductive success, in what form(s) and how used?

Energy is stored in chemical bonds—ATP/GTP—orthophosphate bond (ATP to ADP + Pi) is highest state—how used?

Used for work:

**Mechanical** - moving things in cells—chromosomes

**Chemical** - coupled with ‘thermodynamically unfavorable’ reactions—membrane pumps, transporters, receptors, enzymes such as Pi driven kinases—often have ATPase domains and function fails when energy deficiency occurs

Pi, often overlooked, is source of kinase phosphorylation required for a variety of critical activities including signal transduction pathway functions
It’s More Than Energy

Mitochondrial Functions That May Influence Human Oocyte and Embryo Developmental Competence

- Regulation of calcium homeostasis, REDOX potential
- Superoxide (ROS) generation and ROS-dependent signaling
- Thermoregulation, apoptosis, lipid modification
- Oxygen Sensor of Cell—HIF pathway, HUMMAR
- Fatty acid metabolism (*beta oxidation*)
- Signaling pathways (*nfκβ*)
- miRNAs of mitochondrial origin
- Novel cryptic polypeptides ‘hidden’ within mitochondrial proteins—with functions different from parental molecule
TYPICAL SOMATIC CELL MITOCHONDRION –’ORTHODOX’ FORM*

*indicative of high energetic output and high $\Delta \Psi_m$
What are these cellular ‘powerhouses’ actually like in the oocyte and early embryo that are the targets of such intense interest and hope to restore fertility?

Since they remain largely unchanged for decades, what wears out?
Mammalian Oocyte and Nascent Embryo Mitochondria Occur in the ‘Condensed’ Form*

*indicative of low energetic state and low $\Delta \Psi_m$—but there are lots of them compared to somatic cell

Makabe and Van Blerkom, 2006
All mitochondria maternally inherited, no numerical increase until days after implantation.

Possible reduction in numbers during latter preimplantation stages.

Current dogma = largely equivalent segregation/inheritance with each cell division during preimplantation stages.

mtDNA copy number and mitochondrial mass not equivalent.

**EM Morphometry MII Human Oocytes says: mitochondrial mass: 16-24K**
Can Relative Metabolic Levels Be Assessed Noninvasively in Living Oocytes and Embryos for Purposes of Selection in IVF?
Mitochondrial bioenergetics not uniform in blastocyst

5' adenosine monophosphate-activated protein kinase (AMPK)

GTP-dependent kinase activity

[ADP + protein serine/threonine phosphate, dependent on the presence of GTP]
Indirect means using enzymatic activities visualized in situ

Informative, but not practical (need confocal) or likely to be clinically acceptable
‘Novel’ Methods of Visualizing Oocyte and Early Embryo Energetics

• live-cell imaging: near infra-red—specialized laser line -- need confocal microscope

• conventional epifluorescence microscopy: excitation around 365nm and visualization of NADH and NADPH autofluorescence around 450nm---reveals different intensities and mitochondrial distributions/organizations

• benefit: can quantify relative fluorescent intensity as proxy for bioenergetic state using confocal microscopy and then apply to standard epifluorescence microscopy---we use scale of 1-5 at 0.5 intervals
live-cell imaging reveals different intensities and mitochondrial distributions within cohorts of MII oocytes
At MII, 90K-110K mtDNA copies
The results demonstrated that meiotic maturation occurs in both mouse and human oocytes over a wide range of ATP contents, and that the ATP content of normal appearing oocytes falls within the same range reported by Van Blerkom et al., 1995, for normal appearing MII oocytes in the same GIFT cohort that were not inseminated for IVF.
Average cytoplasmic ATP and RFI

RFI 1-3: 0.9-1.7 pM

RFI 4-5: 1.9-2.6pM

[ATP] and mtDNA content do not seem to be directly related

[ATP] more likely to be related to RFI

Need to correlate with mitochondrial mass
Utility of mitochondrial autofluorescence for selective purposes or assessment of competence requires ongoing validation after ICSI, with unfertilized MII oocytes after conventional IVF, and with embryos that prematurely arrest during cleavage for evidence of disproportionate mitochondrial segregation.
Recent studies indicate that temperatures as high as 50°C occur in orthodox mitochondria and may locally elevate ambient cytoplasmic temperature as heat is dispelled

(CHRETEN ET AL, 2018: Mitochondria are physiologically maintained at close to 50 °C. PLOS BIO 16: e2003992)

Can intracellular thermography be applied to human oocyte and embryo mitochondria and detect differential energy production spatially compartmentalized/localized within cells?

Confirmation of Differential Bioenergetics and Disproportionate Mitochondrial Segregation During Cleavage by Intracellular Thermography
A diffusive fluorescent polymeric thermometer for intracellular temperature mapping in living cells related to mitochondrial metabolism*

Polymeric molecule containing thermosensitive, hydrophilic and fluorescent domains, injected into oocyte or embryo, with thermal differences distinguished by fluorescent intensity using confocal (lookup table) or epifluorescent imaging (monochromatic intensity). Fluorescent lifetime imaging microscopy (FLIM) can also be performed but unnecessary as only need static (one-time) information, at present.

Diffusive Thermoprobe, Funakoshi, Tokyo (collaboration with Yumi Hoshino, Hiroshima University)
Differences in emission intensity indicate corresponding differences in mitochondrial metabolism-ATP generation.

Ambient temp in subplasmalemmal cytoplasm containing circumferential domain of high polarized mitochondria may be up to 1.5°C higher than rest of cytoplasm.
Thermography of Separated Blastomeres Related to Disproportionate Mitochondrial Segregation—Preliminary Findings
Abnormal GM1 phenotypes associated with failure to dock sperm and low metabolic activity in corresponding subplasmalemmal mitochondria (Van Blerkom and Caltrider, 2013, Van Blerkom and Zimmermann 2016,: RMBO)

docking permissive GM1 phenotype associated with high metabolism subplasmalemmal mitochondria
Bioenergetic Compartmentalization

Spatial location and $\Delta \Psi m$ are critical for developmental competence and likely determined by whether mitochondria focally generate sufficient ATP/GTP to support plasma membrane and subplasmalemmal activities required for fertilization and normal early preimplantation embryogenesis.

Whether elevated temperature in the subplasmalemmal cytoplasm from may enhance these developmental activities remains to be determined.
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