Oocyte and follicle metabolic requirements in vitro

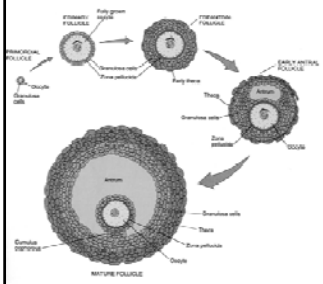
Roger Sturmey
Centre for Cardiovascular and Metabolic Research
Hull-York Medical School

7th Workshop on Mammalian Folliculogenesis and Oogenesis.
ESHRE Campus Symposium
April 20, 2012

Overview

- Follicle
- Glucose
- Evidence for the use of endogenous stores
  - Metabolism of endogenous Triglyceride
  - Mitochondrial activity
- Exposure of oocytes to suboptimal metabolic conditions
  - Impact on resulting embryos

Follicle



From Vander, Sherman & Luciano (1998)
Human Physiology McGraw Hill

- Steroidogenic
- Oocyte support
- Link between systemic supply and oocyte development
- Prolonged growth period
- In vitro development possible

Follicle

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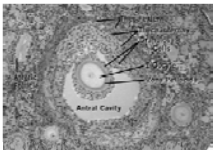

- Few reports on *follicle* metabolism
- Focus on culture methods
- Sequential culture medium with provision of hormones

- Requirements likely to be similar to most other cell types:
  - Energy source – *glucose/pyruvate*
  - Buffer
  - Amino Acids
  - Macromolecule*
  - Vitamins

Energy sources available to the oocyte

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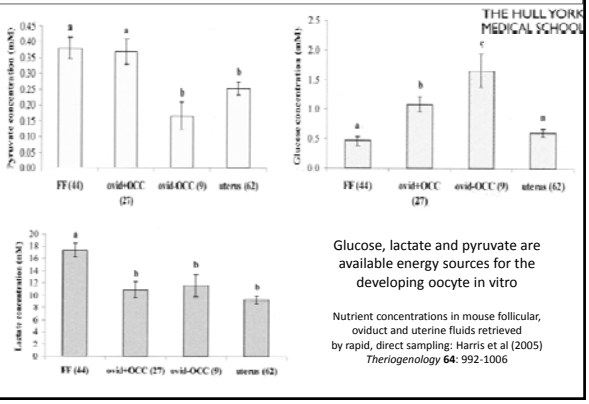

Glucose, lactate and pyruvate are available energy sources for the developing oocyte in vitro

Nutrient concentrations in mouse follicular, oviduct and uterine fluids retrieved by rapid, direct sampling: Harris et al (2005) *Theriogenology* **64**: 992-1006

Glucose - summary

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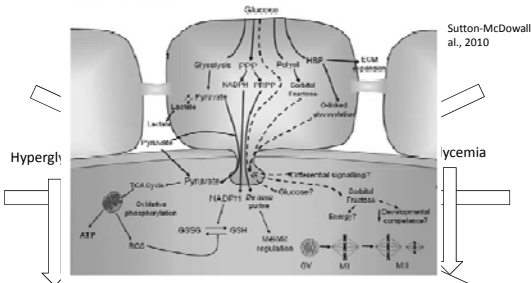
- Principally metabolised by cumulus
  - Probably to pyruvate ➔ oxidised by oocyte
- Glycolysis low and fairly constant in oocytes, but has been linked to developmental competence
- PPP important in resumption of meiosis and ongoing oocyte development
- HBP utilises glucose for synthesis of ECM
- Glucose of fundamental importance for oocyte maturation and ongoing development

Maternal metabolism and oocyte quality



Sutton-McDowall et al., 2010

Hypergl ycemia

Poor cytoplasmic and nuclear maturation, ROS formation, smaller oocytes, reduced embryo quality,

ACUTE
CHRONIC

Leroy et al., 2006; Jungheim and Moley, 2008

Delayed nuclear maturation, reduced development

Oocyte amino acid metabolism

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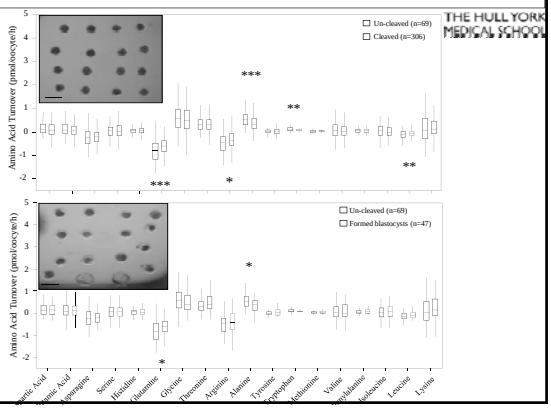

Endogenous energy stores – the other available energy source

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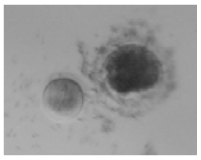
- Until recently, largely overlooked
- Oocyte largest cell in female body
  - Target for spermatozoa?
  - More efficient to make large cell and cleave, rather than small cell and grow?
  - Legacy of common ancestry?
  - Provision of endogenous energy store?
- Lipid stored in the ooplasm in the form of droplets
- Other endogenous energy sources, i.e. glycogen, present in very low quantities



Endogenous lipid in early embryos

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Species	Amount of Fat (ng)	Ref
Mouse	4	Lowenstein & Cohen, 1964
Cow	58	Ferguson & Leese, 1999
Pig	156	McEvoy *et al.* 2000
Sheep	89	Coull *et al.* 1997

In the absence of exogenous nutrients:

Mouse arrests within 15h

Bovine can complete maturation and generate blastocysts post IVF

Porcine can complete maturation and generate blastocysts post IVF

Indirect evidence that endogenous TG can support oocyte maturation

Endogenous lipid in oocytes

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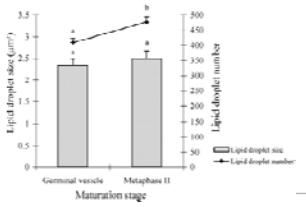

Lipid profile of oocytes is dynamic and can be modified by the environment!

- Free fatty acids taken from in vitro environment
- Principally distributed as TG or products of TG oxidation

Oocyte lipid fraction	Radioactivity (%)[a] 100 μM + [³H] palmitic acid	100 μM + [³H] oleic acid
Cholesterol esters	2 ± 0.7	2 ± 0.5
Triacylglycerol	50 ± 0.4	36 ± 0.4
Free fatty acid	5 ± 0.6	4 ± 0.8
Diacylglycerol	12 ± 1.6	14 ± 1.1
Phospholipids and oxidation products of fatty acids	31 ± 4.3	45 ± 1.0

[a] Distribution of radioactivity in different lipid fractions from oocytes after 2 h of maturation with 100 μM unlabeled C9, 10 (n) – [³H] palmitic acid or 100 μM unlabeled C9, 10 (n) – [³H] oleic acid; results are presented as mean ± standard deviation.

Aardema H et al. Biol Reprod 2011;85:62-69

Triglyceride metabolism

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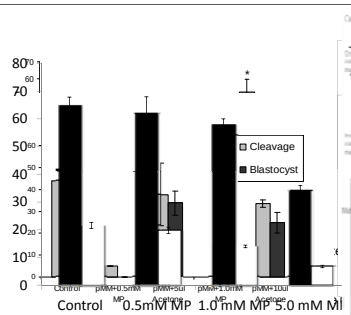

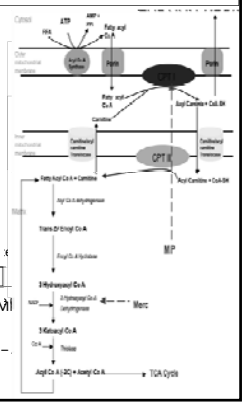

Control 0.5mM MP 1.0 mM MP 5.0 mM MP

This equates to 0.612nl O_2 ≡ 27 pmol O_2

O_2 consumption of oocytes = 25-30 pmol/embryo/h – *observed depletion of TG*

Sturmey & Leese 2003

Even mice…

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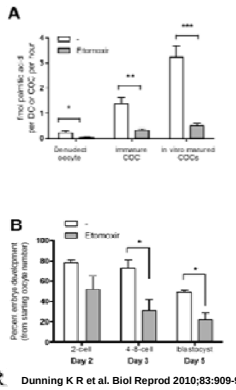

- Inhibition of CPT1B by Etomoxir during mouse IVM significantly reduces survivial rates (Dunning et al 2010)
- Can be rescued by addition of palmitic acid or carnitine (Downs et al, 2009, 2010)
- Promotion of β-oxidation during mouse IVM promotes development
- All three species show decreased development when b-oxidation inhibited
  - Prevention of ATP synthesis?
  - Other mechanism?

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Dunning K R et al. Biol Reprod 2010;83:909-918

Cytoplasmic organisation changes to support metabolism of triglyceride

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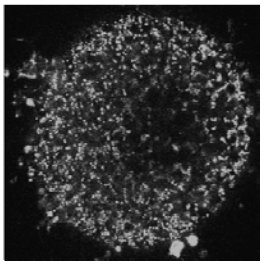
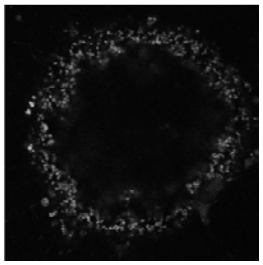


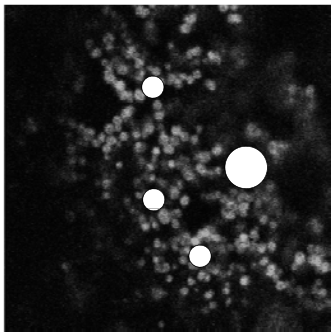
Immature porcine oocyte

Z-slice through the mid section

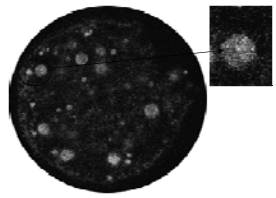


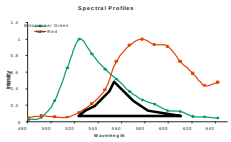
in vitro-matured oocyte

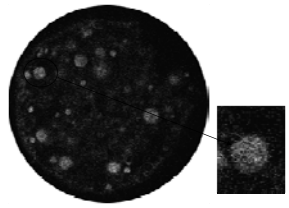
Z-slice through the mid section

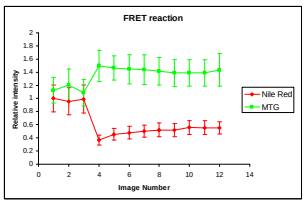


5x zoomed movie of mitochondrial movement in an *in vitro*- matured oocyte. Movement around unlabelled spheres is apparent, with very occasional disassociation









Sturmey et al Reproduction 132 pp829-37, 2006

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Metabolic consequences?

Too much of a good thing?

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- Increasing concern about high fat diets and impacts on reproductive outcome
- Bovine model where OOCYTES were exposed to elevated, but physiological NEFA
  - Control - 25µM SA, 50µM PA, 75µM OA
  - 75µM SA
  - High - 75µM SA, 150µM PA, 200µM OA
- Measured impact on subsequent embryos

Cell count and apoptotic cell ratio

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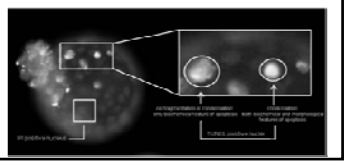
	CONTROL		REDUCED QUALITY HIGH SA	HIGH COMBI
CELL NUMBER	125.8 ± 29.4[a]	>	**105.4 ± 24.7[b]**	104.7 ± 26.1[b]
APOPTOTIC CELL RATIO	0.085 ± 0.053[a]	<	0.18 ± 0.078[b]	0.14 ± 0.12[a]

[a,b] Different superscripts per row indicate a significant difference between treatment groups ($P < 0.05$).



Van Hoeck et al 2011

Blastocyst metabolic 'quality'

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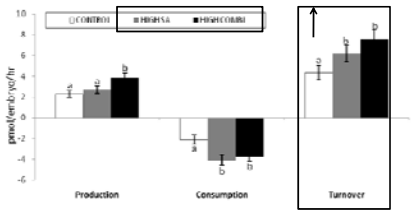
REDUCED VIABILITY
(Leese et al., 2002; Sturmey et al., 2009)



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Van Hoeck et al 2011

Blastocyst Oxygen, Pyruvate, Glucose & Lactate

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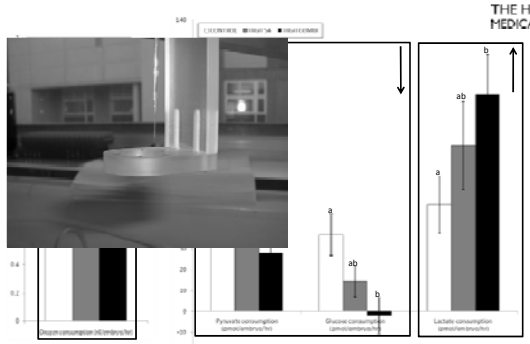

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Van Hoeck et al 2011

Blastocyst gene expression

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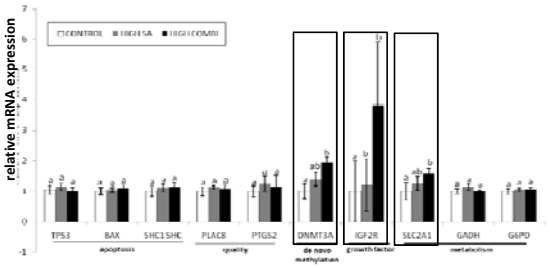

[a,b] Different superscripts indicate a significant difference between treatment groups ($P < 0.05$).

Van Hoeck et al 2011

Effect inhibition β-oxidation

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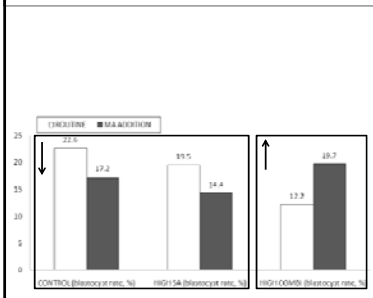

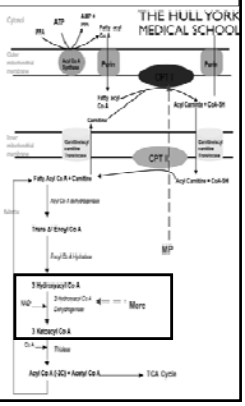

In the HIGH COMBI treatment blastocyst production increased after supplementation of <u>0.1mM β-mercapto-acetate</u> to the maturation medium.

Van Hoeck et al In prep

Mitochondria?

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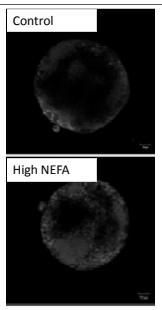

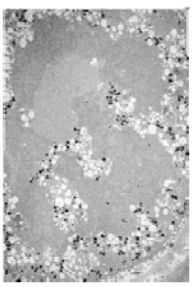

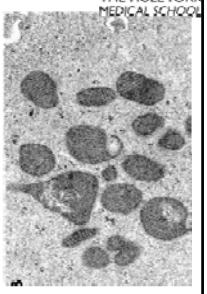

No difference in **oxygen consumption at zygote** level between zygotes originating from oocytes exposed to elevated NEFA concentrations and the CONTROL zygotes.

Gene expression - oocytes

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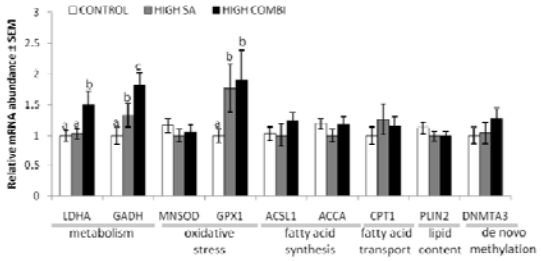

Gene expression – resulting blastocysts

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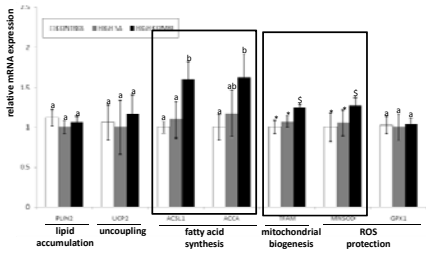

superscripts a, b: $P < 0.05$. superscripts *,$: $P < 0.1$

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Fatty acid metabolism, oocytes and embryos

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OOCYTES

- Inhibit fatty acid metabolism detrimental to ongoing development
  - Mouse, cow, pig
- No compensation
  - Low capacity for glucose metabolism in oocytes
- Increase fatty acid exposure (metabolism?) reduces development
  - Reduces glucose uptake
  - Reduces oxygen consumption
  - Implied altered methylation
- Regulation of metabolism

EMBRYOS

- Inhibit fatty acid metabolism has little obvious effect on development
  - Increased glucose oxidation
  - Short-term rise in oxygen consumption

Oocyte sensitive to fatty acid metabolism

Follicle lipid metabolism

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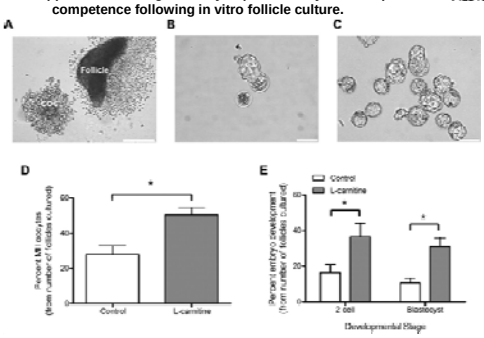
l-carnitine supplementation significantly improves oocyte developmental competence following in vitro follicle culture.



Dunning K R et al. Biol Reprod 2011;85:548-555

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Follicle-lipid metabolism

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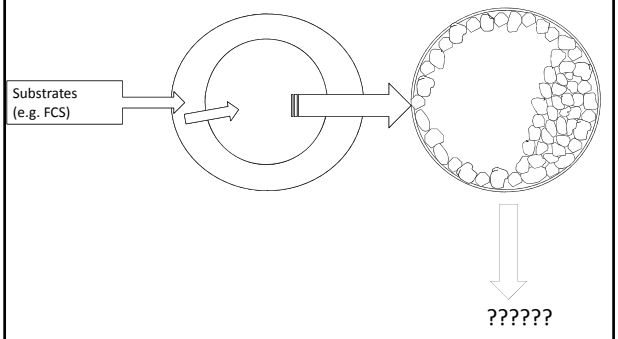

Closing remarks

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- Metabolism of exogenous substrates vital for successful oocyte development and ongoing embryo viability
- Endogenous stores play a vital role
- Disruption of oocyte metabolism has legacy effects in resulting embryos
  - Metabolic dysregulation
  - Mitochondrial dysfunction
  - Gene expression
- Metabolic interaction between follicle and oocyte requires further investigation
- Consequences for human oocyte development still unclear

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