Embryo metabolism prior to implantation: what does it really mean?

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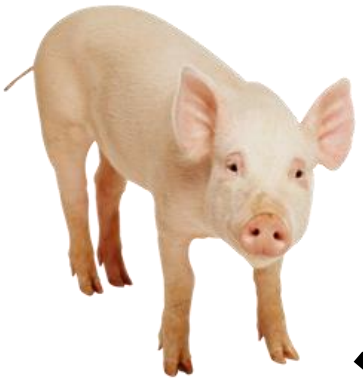
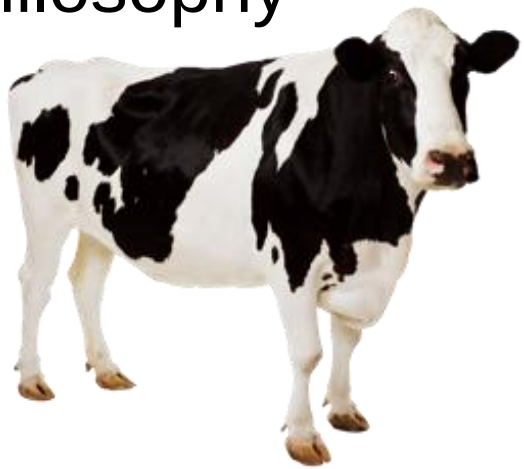
Les recherches sur l'embryon humain *in vitro* : aspects scientifiques et éthiques INSERM, Paris, October 7. 2015

Disclaimer

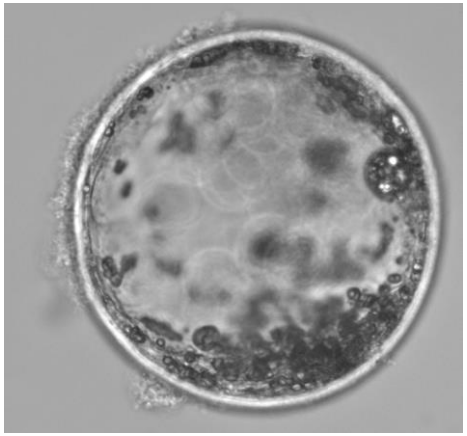
- I have no commercial interests or conflicts of interest to declare.
- All studies on human embryos have been performed with full ethical approval, and informed patient consent, in accordance with UK HFEA license R/0067/9/a

Lab philosophy





- Similar genome activation (4c)
- Similar size embryo
- Similar physiology (mono-gastric omnivore)
- Readily available
- Free of ethical considerations



Maximise data generation from spare human embryos

- Similar embryo metabolism
- Similar size embryo
- Mono-ovulator
- Readily available
- Free of ethical considerations

Overview

- What is metabolism?
- Why measure it?
- The importance of metabolism
- Closing thoughts

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What is metabolism?

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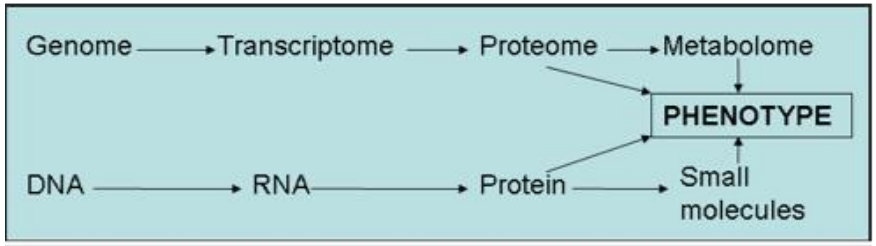
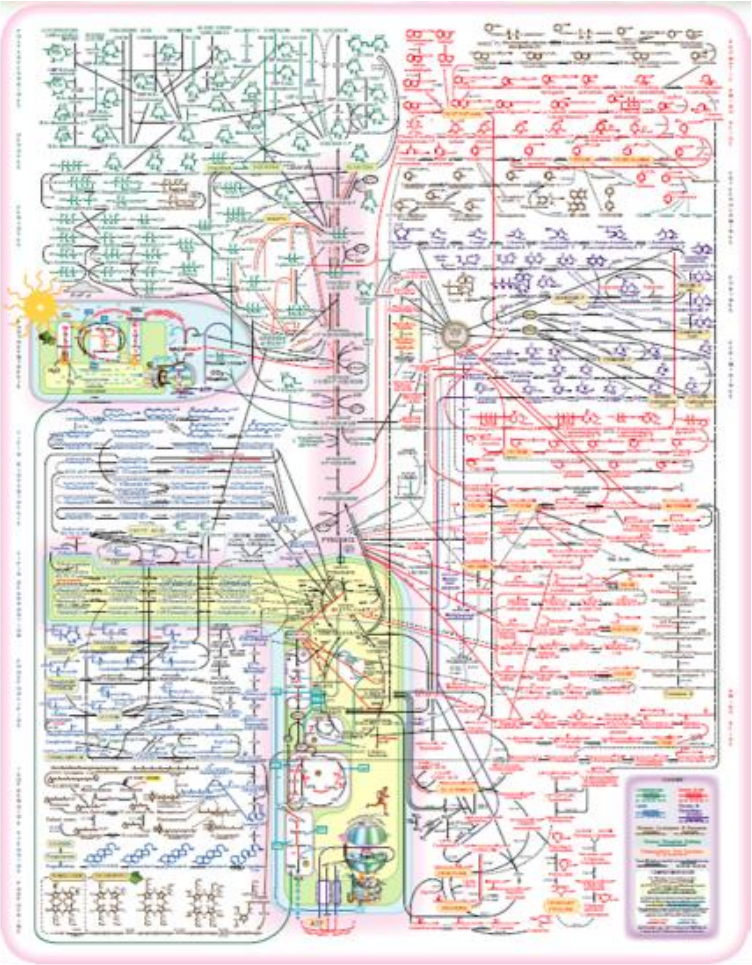
The ‘appeal’ of metabolism

- Metabolism = complex
- Diverse targets
  - Chemistry
  - Structure
- Abundance
- *Essential* for cellular function
- <u>Snapshot of physiology</u>





Overview

- What is metabolism?
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Why?

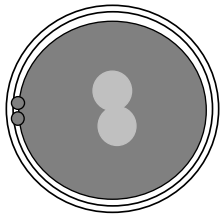
- Interest
  - “desire to understand the beginnings of the self”
    - Lewis Wolpert

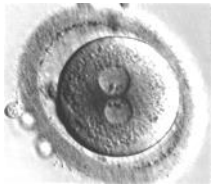
- The metabolic life course of the embryo

Developmental milestones

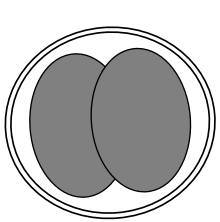
5-7 days, depending on species

Mitochondrial number fixed

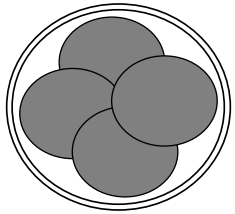




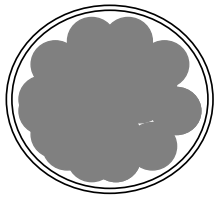
- Genetic material combined

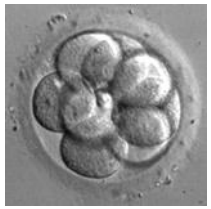


- Increase cell number

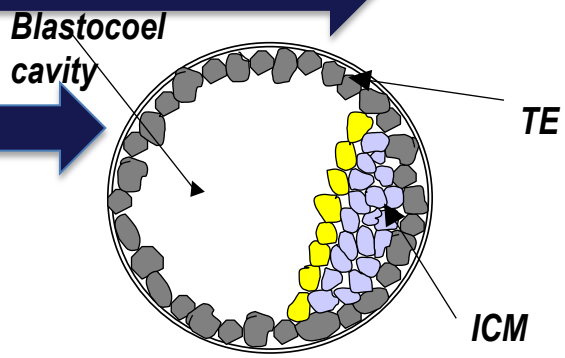


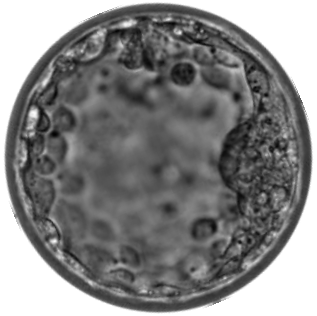
- Genome activated





- Cell-cell junctions begin to form





- Differentiation
- Protein synthesis
- Na^+/K^+ ATPase
- True growth
- Prep for implantation

Smith and Sturmey, 2013, Biochem Soc Trans

Cell number

Uptake

Pyruvate

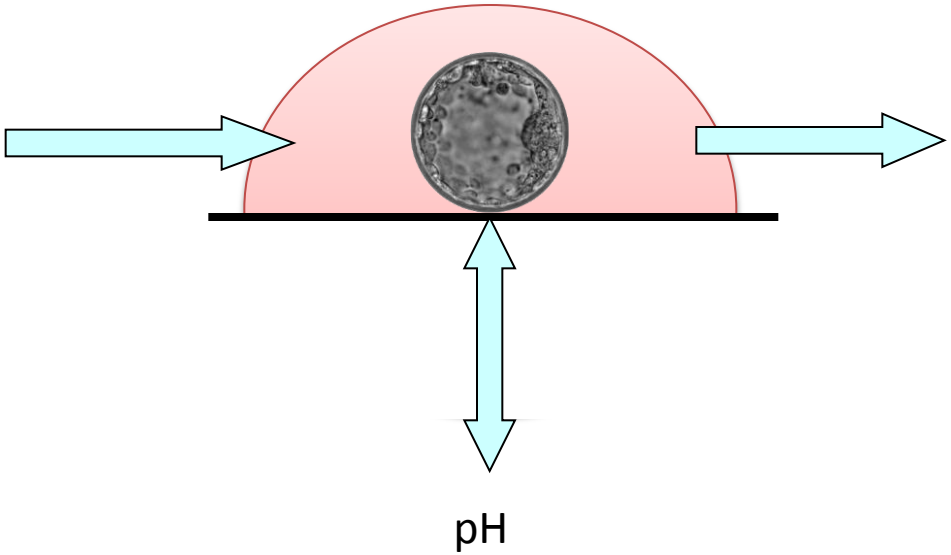
Lactate

Glucose

Lipid

Amino acids

Oxygen



Production

H_20

CO_2

Lactate

Amino acids

NH_4^+

Enzymes

Hormones

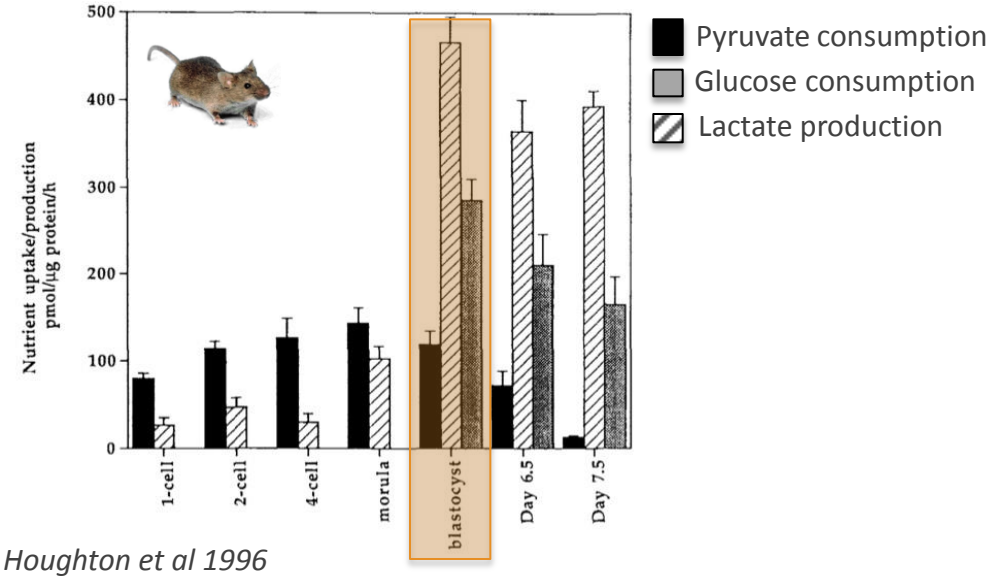
Cytokines

Proteins

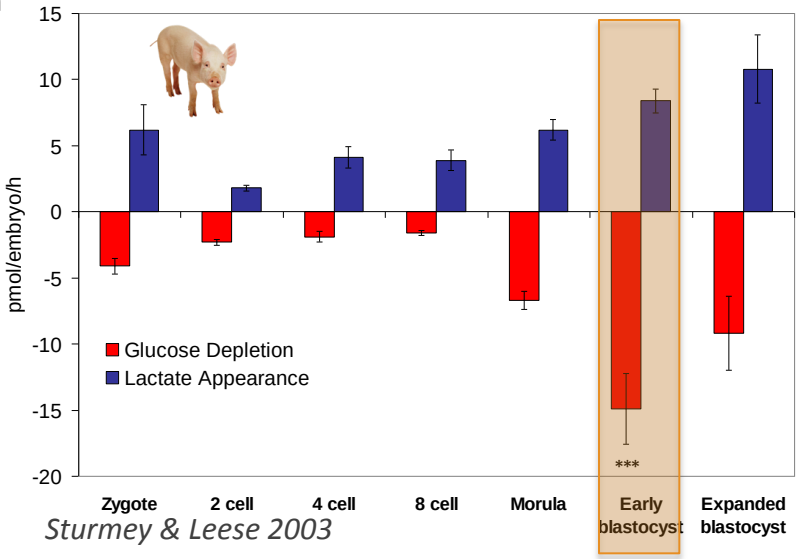
Most studies do not measure:

- pathways
- flux
- interaction
- signalling events
- regulation

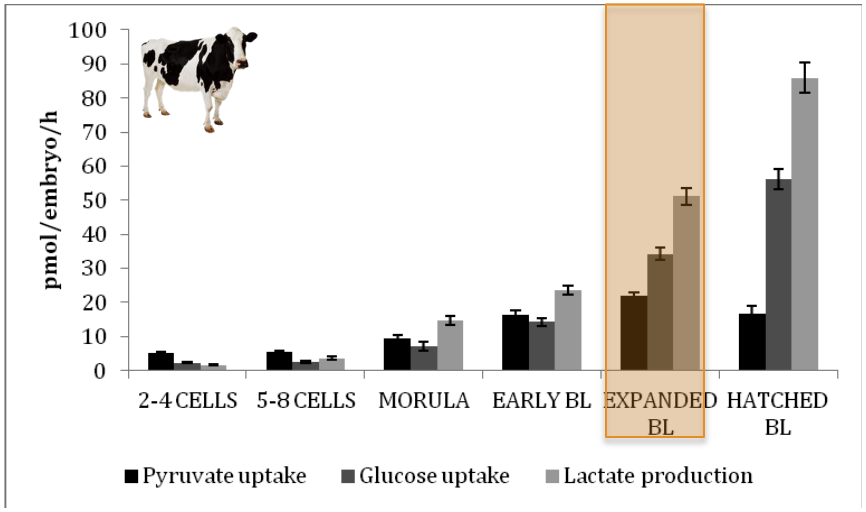
Glucose, Lactate, Pyruvate



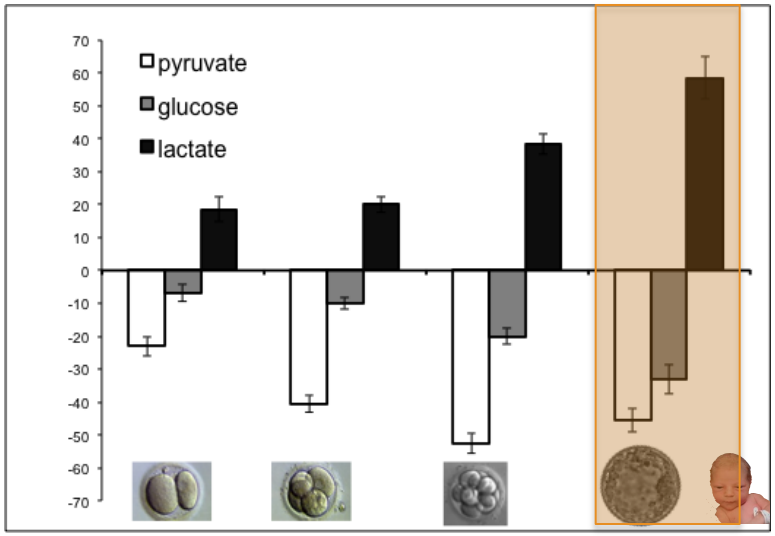
Houghton et al 1996



Sturmey & Leese 2003



Guerif et al 2013



Leary et al unpublished

Powering development

"Pyruvate appears to be the central energy substrate in those species in which energy source requirements of the embryo have been examined. During the first day or two of the embryo's life, *glycolysis* has a very low capability, but after blastocyst formation there is a sharp increase in glycolytic ability.

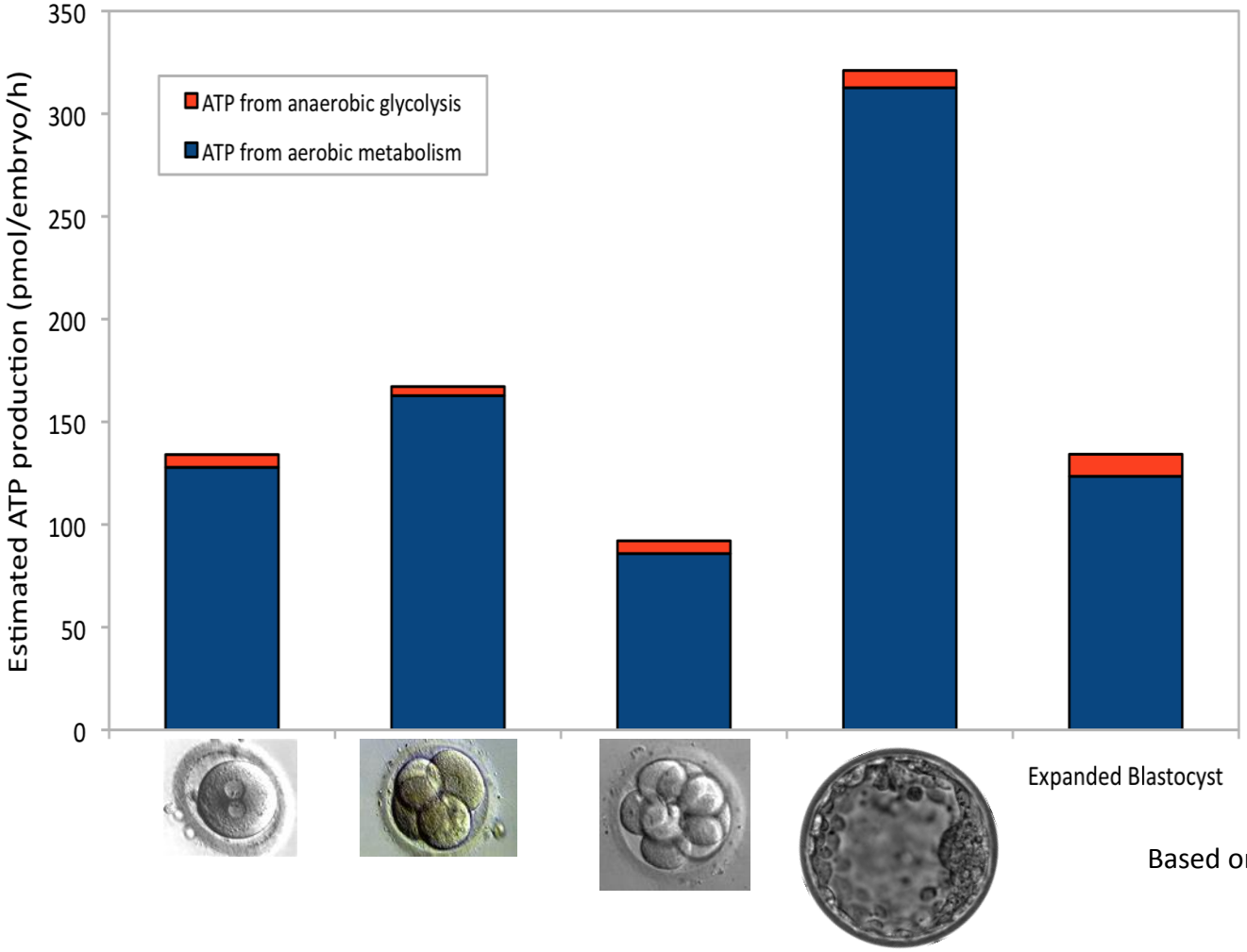
The Krebs cycle is the main source of energy throughout the preimplantation period. Large increases in oxygen consumption and uptake and incorporation of carbon occur at about the time of blastocyst formation.

The embryo goes from a relatively inactive metabolic tissue at ovulation to a rapidly metabolizing tissue at implantation."

Ralph Brinster **(1973)** *Nutrition and metabolism of the ovum, zygote and blastocyst. Handbook of Physiology (R.O. Greep ed)*

Embryos have lipid stores as well… another story…

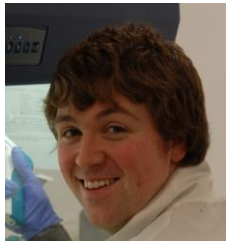
ATP production throughout development



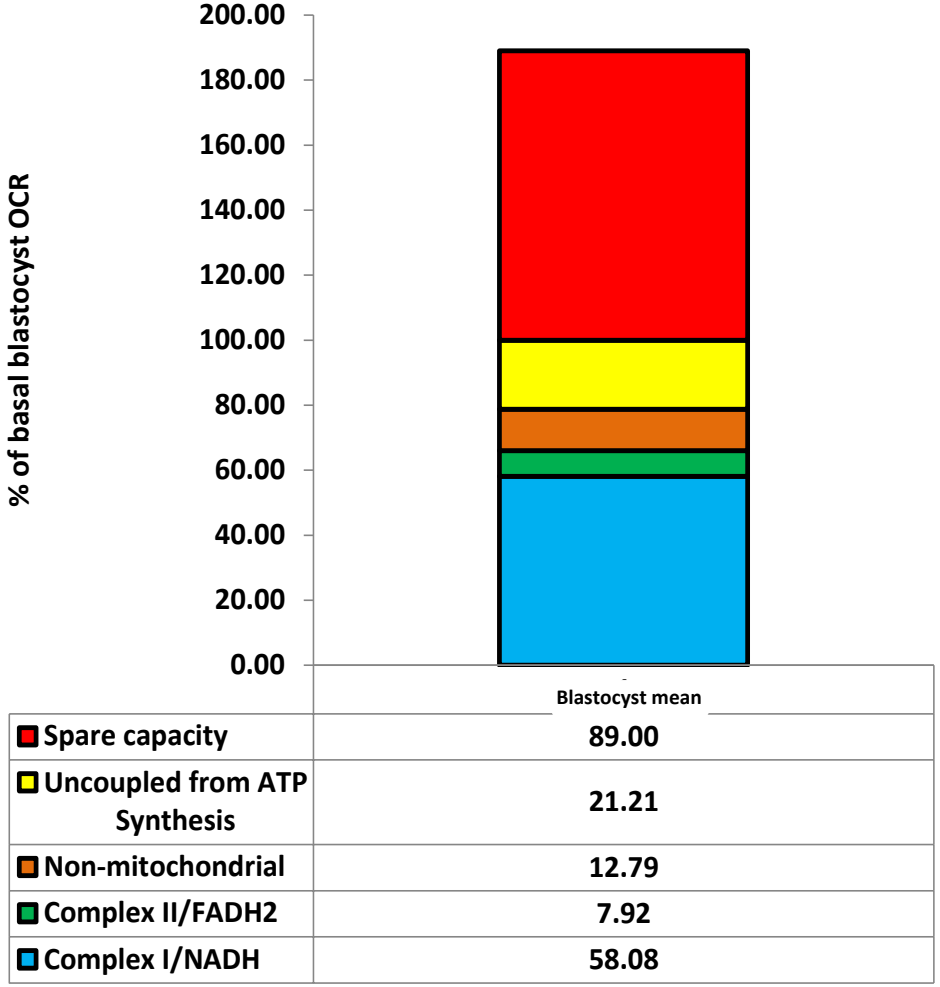
- *Increase in ATP requirements throughout development*
- *Increase glucose consumption*
- *Principle route for generating ATP remains oxidative*

Based on Sturmey and Leese 2003

NOT ALL OXYGEN CONSUMED BY EMBRYOS IS USED 'PRODUCTIVELY'!



Not all oxygen makes energy

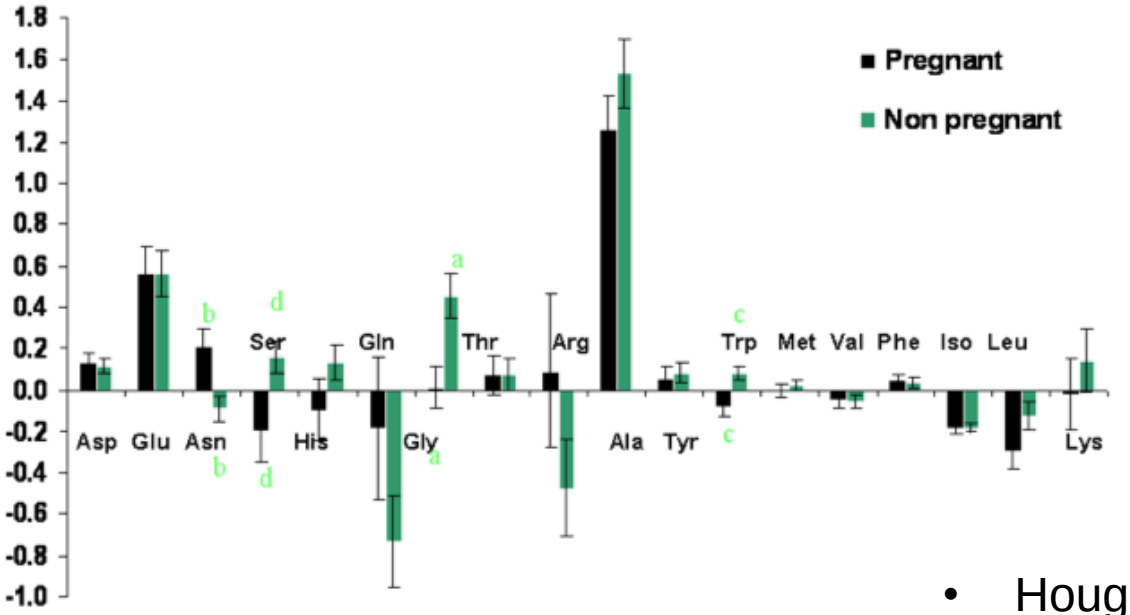


- A new systematic profile of blastocyst OCR, using nanorespirometry
- New information on the fate of energy substrates from the TCA cycle
- Enables review of previously published data
  - ATP production has been overestimated
  - e.g. 120pmol/embryo/hr (Sturmey et al. 2003)
  - If 66% OCR coupled to ATP production, 63.36 pmol/embryo/hr
- These data highlight the plasticity of embryo metabolic regulation

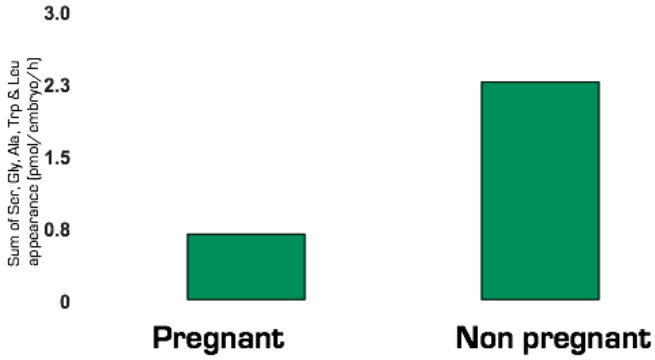
Self correcting nature of science!

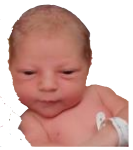
McKeegan & Sturmey *in prep*

Biomarkers of viability?



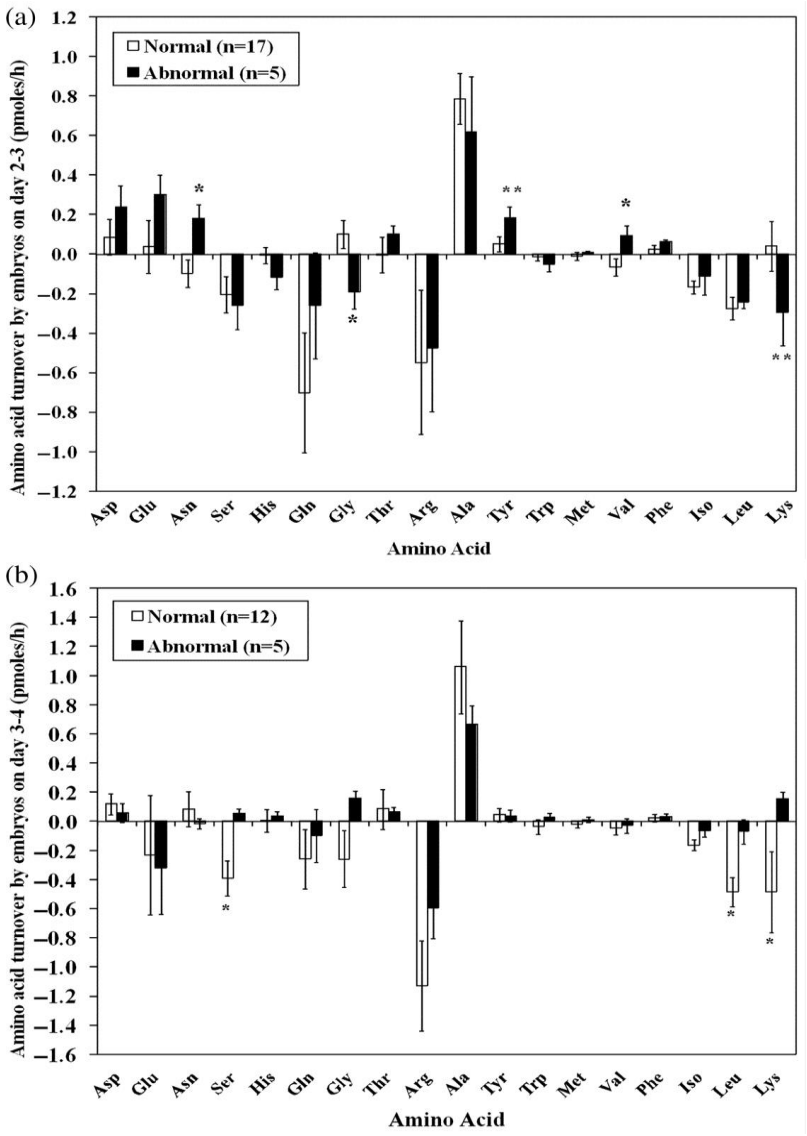
Brison et al, Hum Reprod, 19 pp2319-24 2004





- Houghton et al (2002): AAP can predict blastocysts from d2-3 human embryos
- Brison et al (2004): AAP correlated with clinical pregnancy
- Stokes et al (2007): AAP can predict outcome of cryopreserved embryos

Aneuploidy



- Amino acid turnover by individual embryos in relation to abnormality of chromosomes 13, 18, 21, X and Y on Days 2–3 (a) and Days 3–4 (b).

Picton H M et al. Mol. Hum. Reprod. 2010;16:557-569

Human Reproduction, Vol.29, No.3 pp. 455–461, 2014
Advanced Access publication on January 8, 2014 doi:10.1093/humrep/det456

human reproduction

META-ANALYSIS *Embryology*

No evidence that embryo selection by near-infrared spectroscopy in addition to morphology is able to improve live birth rates: results from an individual patient data meta-analysis

C.G. Vergouw[1,*], M.W. Heymans[2], T. Hardarson[3], I.A. Sfontouris[4], K.A. Economou[5], A. Ahlström[3], L. Rogberg[3], T.G. Lainas[4], D. Sakkas[6], D.C. Kieslinger[1], E.H. Kostelijk[1], P.G.A. Hompes[1], R. Schats[1], and C.B. Lambalk[1]

R.G. STURM

Overview

- What is metabolism?
- Why measure it?
- The importance of metabolism
- Closing thoughts

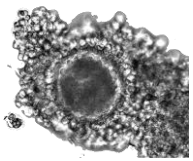
Question:
Does metabolism programme development?

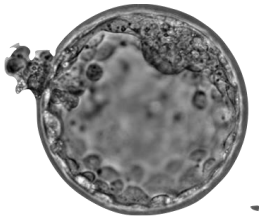
Answer pending, but evidence provided by Dr Veerle Van Hoeck, Prof Jo Leroy, Dr Paul McKeegan and Christine Leary



Impact of excessive fat exposure

- Bovine model where OOCYTES were exposed to elevated, but physiological NEFA





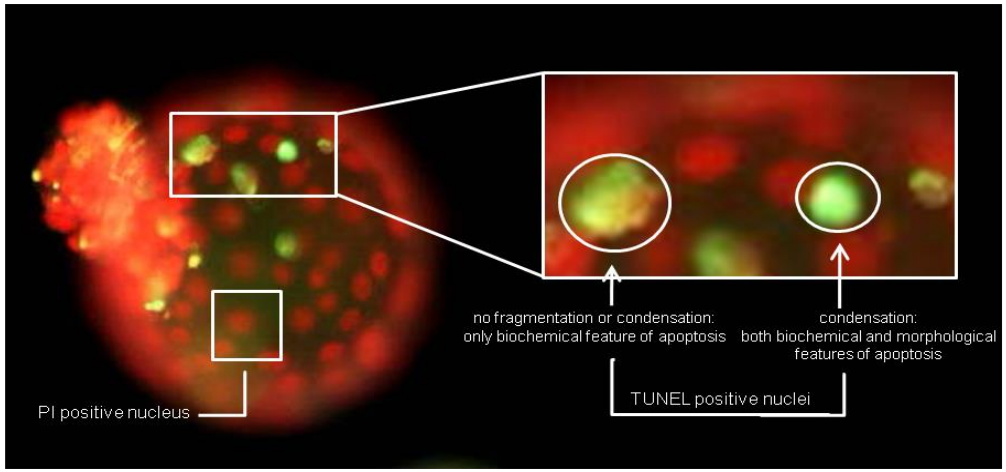
Cell count/allocation
Apoptosis
Amino acid profile
Energy metabolism
Gene expression

Elevated Non
Esterified
Fatty Acids

Cell count and apoptotic cell ratio

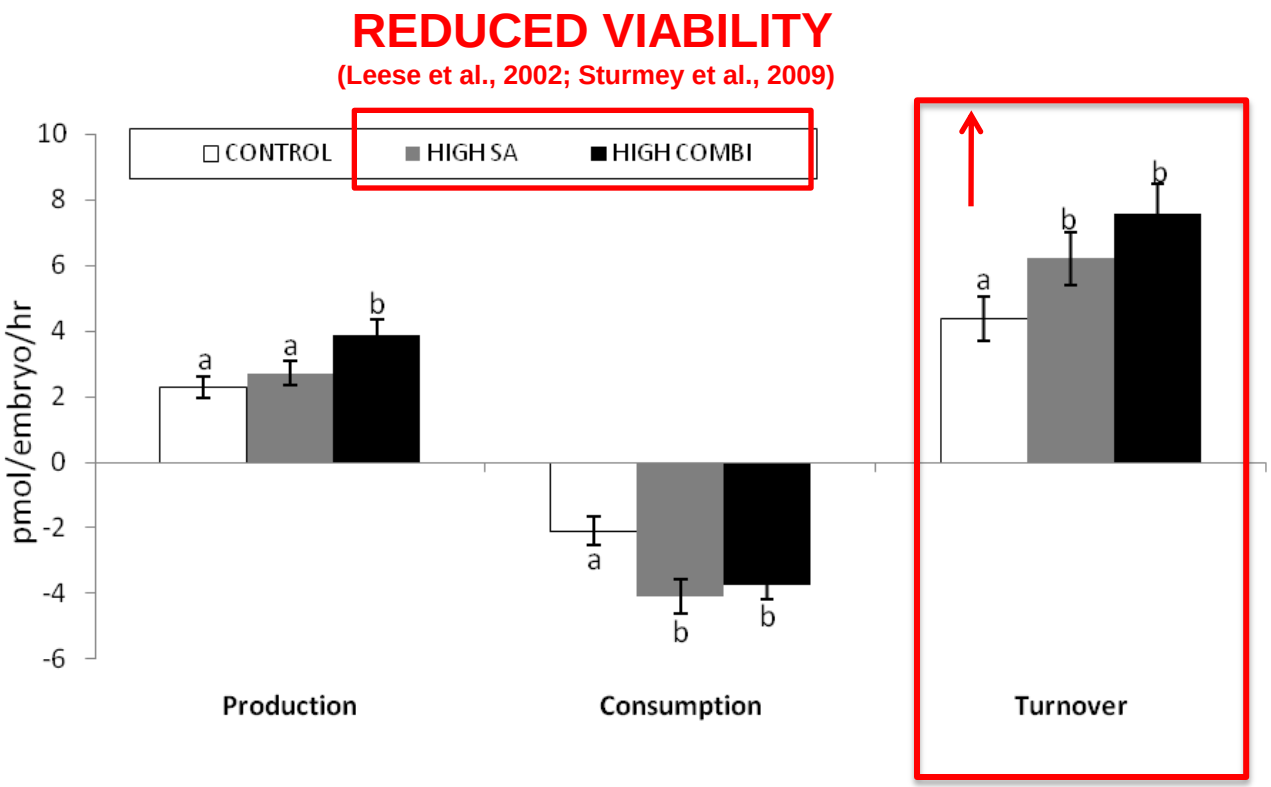
	CONTROL	HIGH SA	HIGH COMBI
		REDUCED QUALITY	
CELL NUMBER	125.8 ± 29.4[a]	105.4 ± 24.7[b]	104.7 ± 26.1[b]
APOPTOTIC CELL RATIO	0.085 ± 0.053[a]	0.18 ± 0.078[b]	0.14 ± 0.12[a]

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



Van Hoeck et al 2011

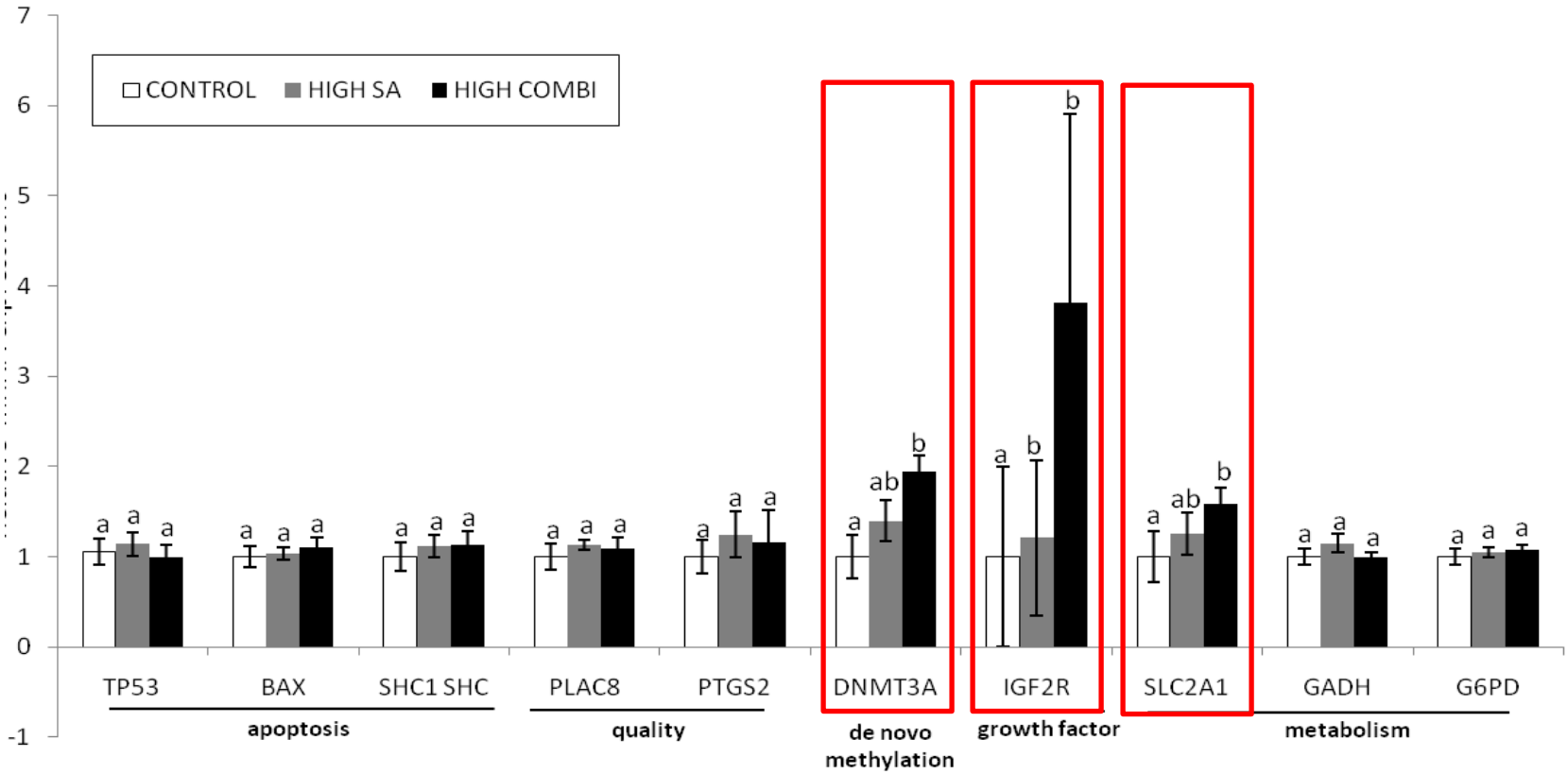
Blastocyst metabolic ‘quality’ reduced



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

Van Hoeck et al 2011

Metabolic strategy



REPRODUCTION
RESEARCH

Oocyte developmental failure in response to elevated nonesterified fatty acid concentrations: mechanistic insights

V Van Hoeck, J L M R Leroy, M Arias Alvarez[1], D Rizos[2], A Gutierrez-Adan[2], K Schnorbusch, P E J Bols, H J Leese[3] and R G Sturmey[3]

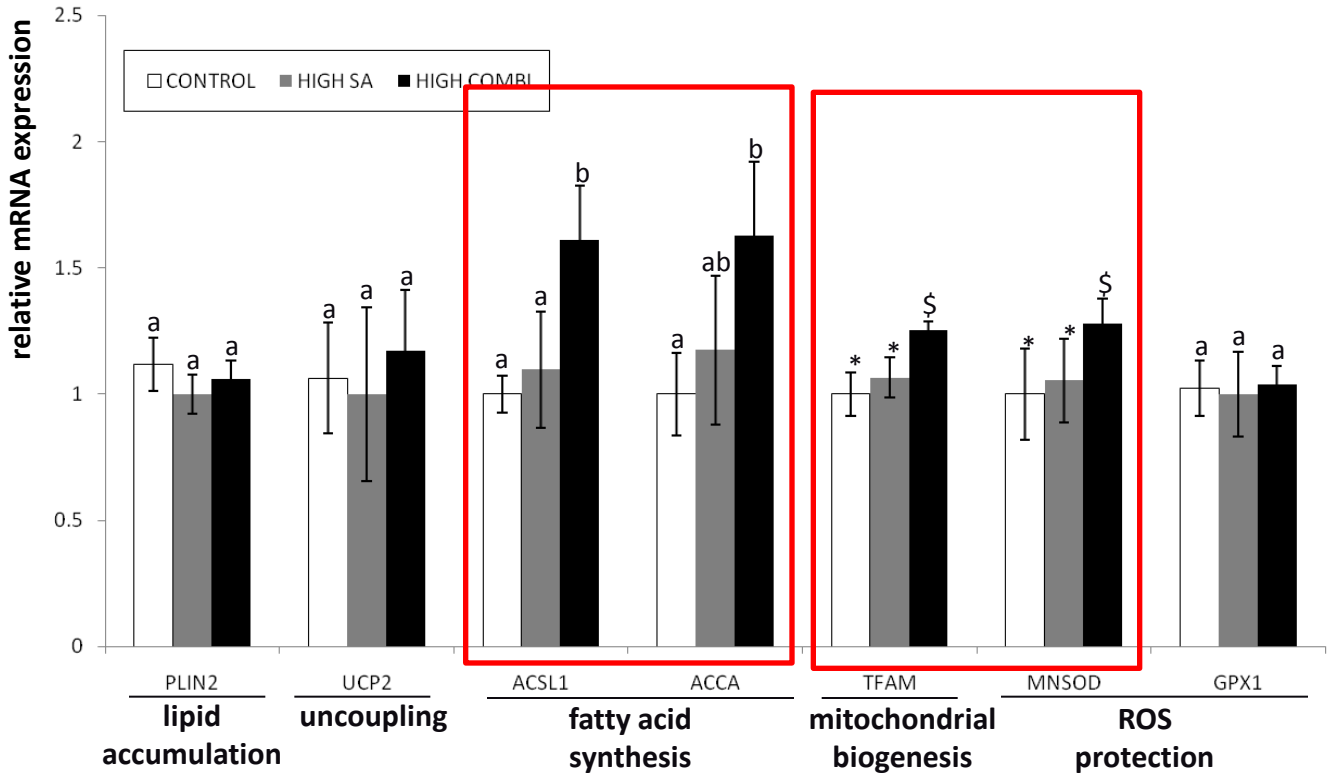
OPEN ACCESS Freely available online
PLoS one

Elevated Non-Esterified Fatty Acid Concentrations during Bovine Oocyte Maturation Compromise Early Embryo Physiology

Veerle Van Hoeck[1]*, Roger G. Sturmey[2], Pablo Bermejo-Alvarez[3], Dimitrios Rizos[3], Alfonso Gutierrez-Adan[3], Henry J. Leese[2], Peter E. J. Bols[1], Jo L. M. R. Leroy[1]

1 Laboratory for Veterinary Physiology and Biochemistry, Department of Veterinary Sciences, Faculty of Biomedical, Pharmaceutical and Veterinary Sciences, University of Antwerp, Wilrijk, Belgium, **2** Hull-York Medical School, University of Hull, Hull, United Kingdom, **3** Departamento de Reproducción Animal y Conservación de Recursos Zoogenéticos, INIA, Madrid, Spain

Increased expression of FA Synthesis genes



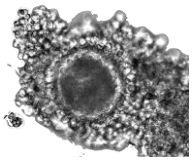
superscripts a, b: $P < 0.05$. superscripts *,$: $P < 0.1$

Van Hoeck et al 2013

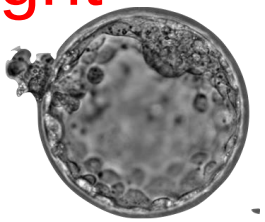
…and in the human?

Human embryos at risk?

- Human embryos from oocytes collected from overweight/obese women compared to those from women of healthy body weight



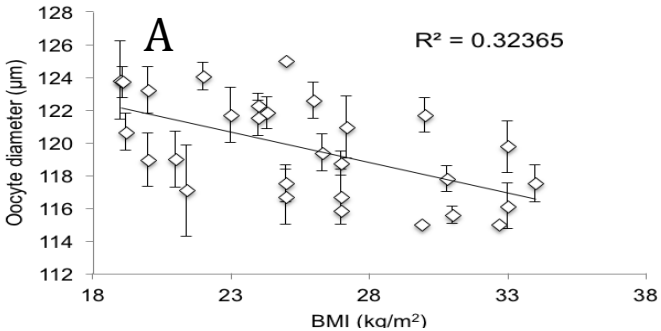
Matched fertilisation and culture environment

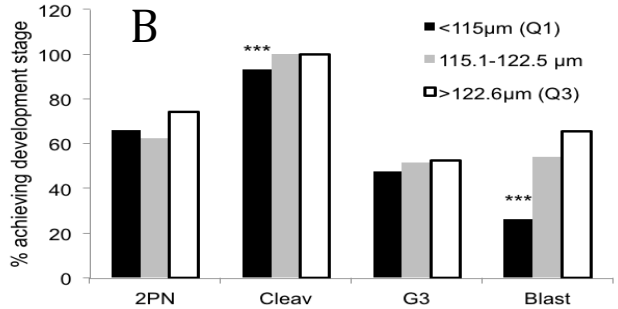


Cell count/allocation
Apoptosis
Amino acid profile
Energy metabolism
Birth weights

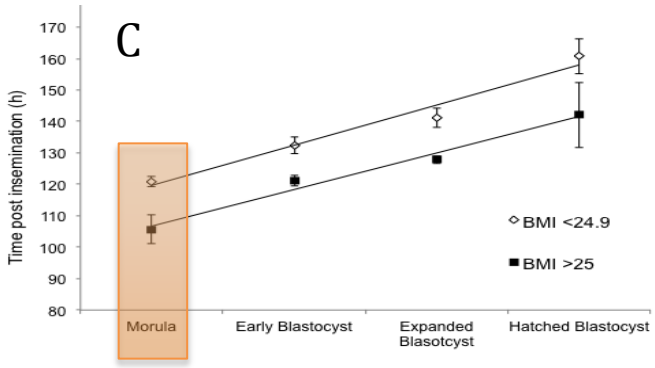
Follicle differences due to maternal physiology

Human oocyte size inversely correlated to BMI





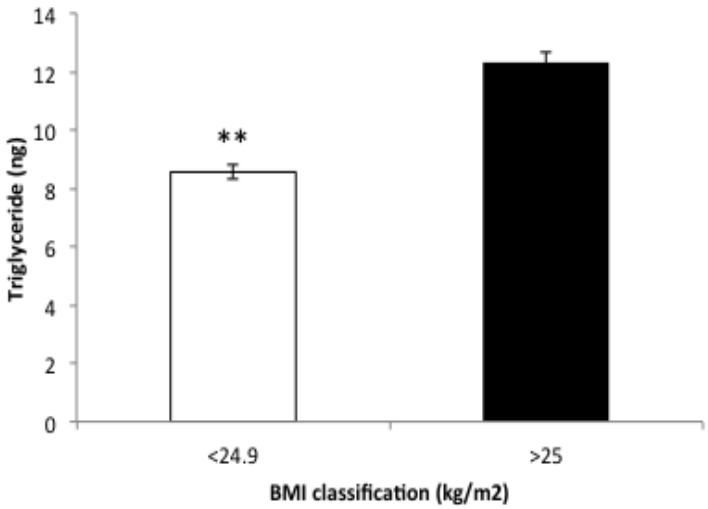
Correlations (*r*)	Normal fertilisation (2PN)	Zygote cleavage (Cleav)	Day3 embryo quality (G3)	Day5 Blastocyst development (Blast)	BMI (kg/m^2)
Oocyte diameter (µm)	0.13	0.23***	0.05	0.28***	-0.46***

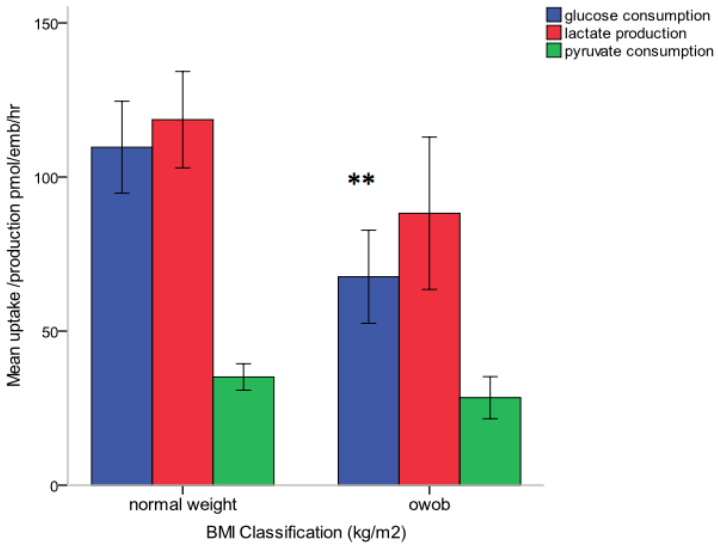


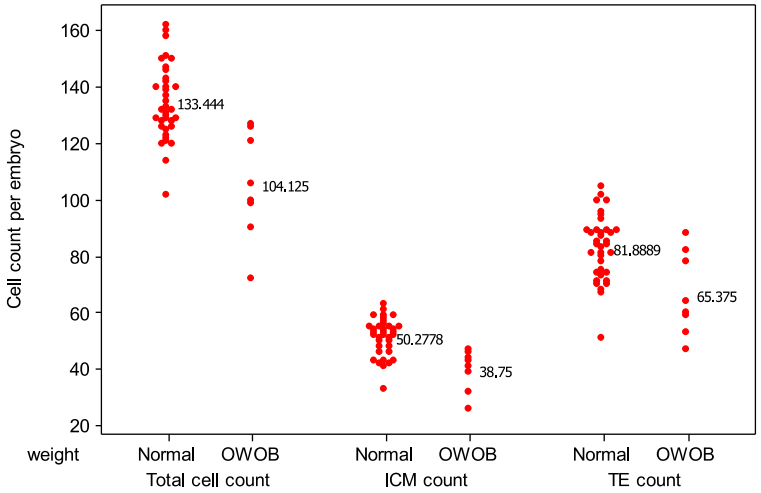
- 169 MII oocytes, from 22 patients.
- Mean of 9.24 ± 3.63 (SD) oocytes per patient.
- Mean oocyte diameter was 134.25 ± 3.49µm (SD).
- Mean diameter of OWOB oocytes was 133.1 ± 3.63µm (SD) compared to those of a healthy BMI 135.78 ± 2.82µm (SD).

- Morphokinetic differences – embryos from overweight women show precocious development to morula

Embryos from overweight women have altered energy metabolism



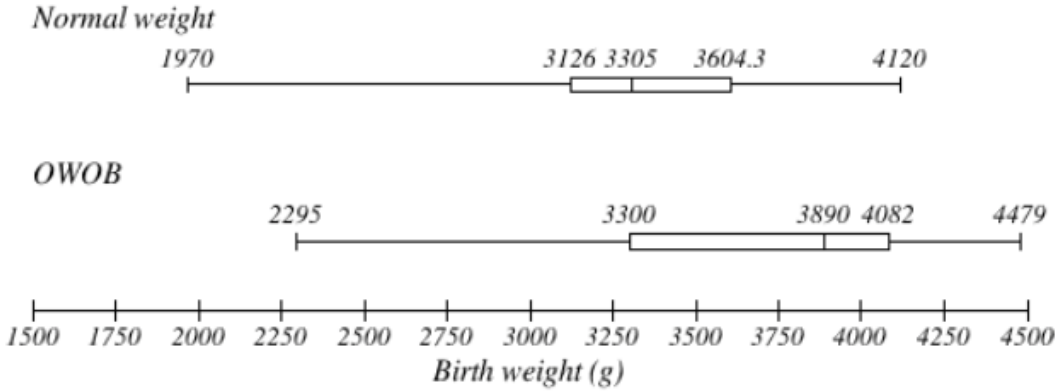




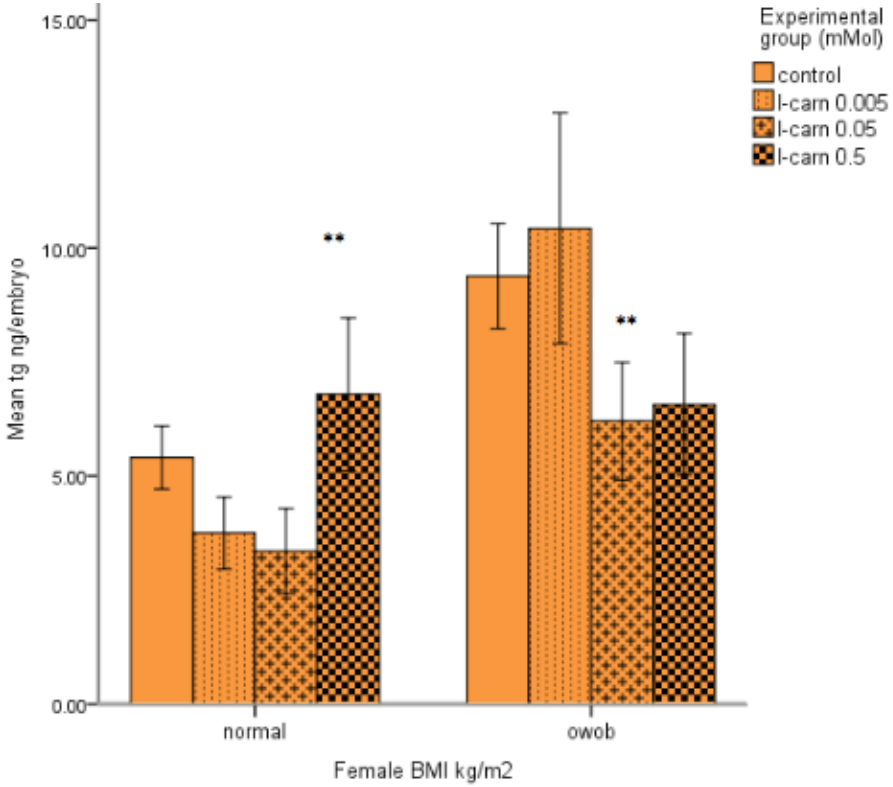
- Increased intracellular TG
- Diminished capacity for glucose consumption
- Replicated in 3 independent studies with over 900 human embryos
- Independent of male

Leary et al Hum Rep 2015

Impact?



+L-carnitine

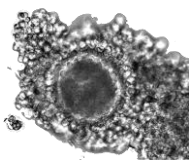


- Heavier babies at birth
- L-Carnitine in medium reduces TG content

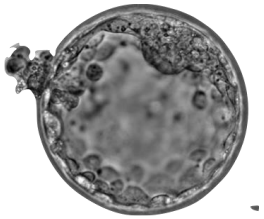
Leary et al Unpub

Conclusion

HOW?



No metabolic insult here



Lets consider what does happen here

Changes:
Development
Cell number
Phenotype
Gene expression patterns

Metabolic insult here

Rewriting the genome

LETTER

doi:10.1038/nature13544

The DNA methylation landscape of human early embryos

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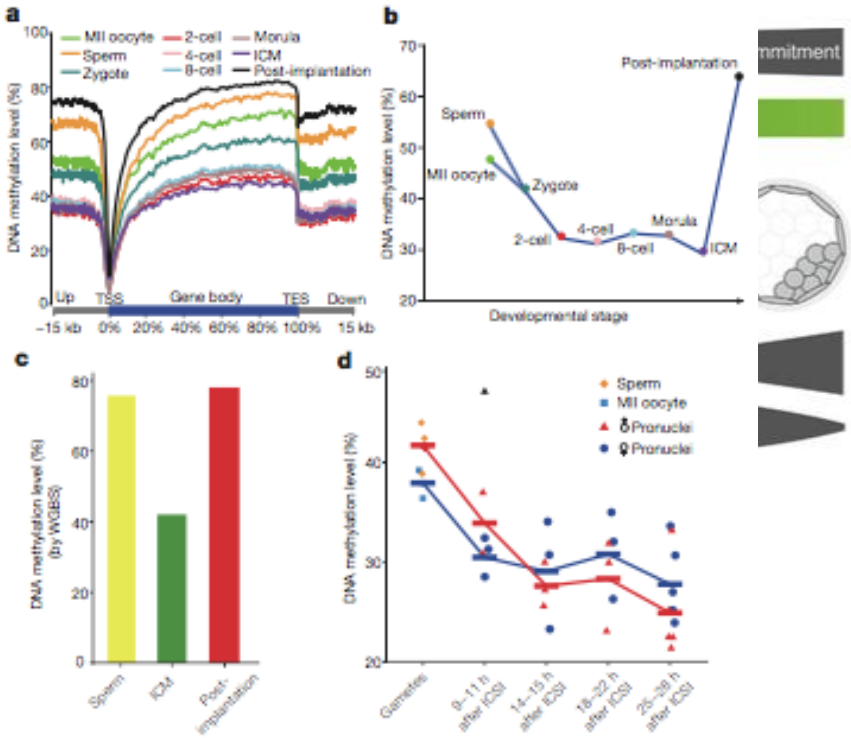

Cell

Leading Edge
Review

Influence of Metabolism on Epigenetics and Disease

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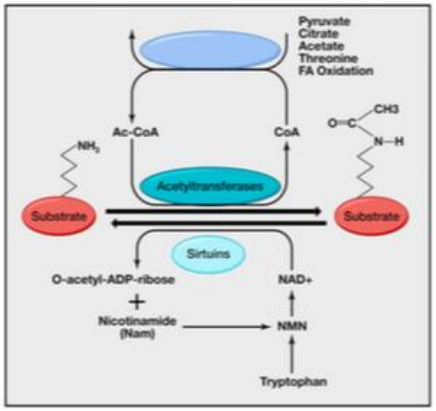
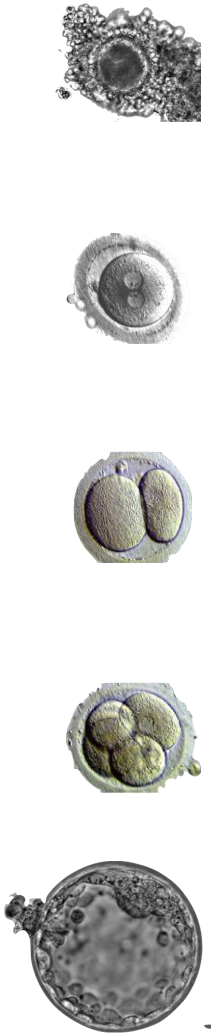


Figure 1. Metabolism and Acetylation/Deacetylation
Histone acetylases use acetyl-CoA (Ac-CoA) as an acetyl donor, whose synthesis requires coenzyme A (CoA). Ac-CoA can be regenerated in chemical reactions involving pyruvate, citrate, acetate, and various amino acids such as threonine and by fatty acid beta oxidation. Deacetylation by Sirtuin family histone deacetylases requires NAD+, leading to the generation of O-acetyl-ADP ribose and nicotinamide (NAM). NAD+ is produced from NMN (nicotinamide mononucleotide), which can be salvaged from NAM or produced de novo from tryptophan. For simplicity, enzymes catalyzing the various reactions are not shown.

- Two highly specialised, differentiated cells form undifferentiated totipotent cells
- All* genome marks are removed and replaced

Genetic/epigenetic consequences

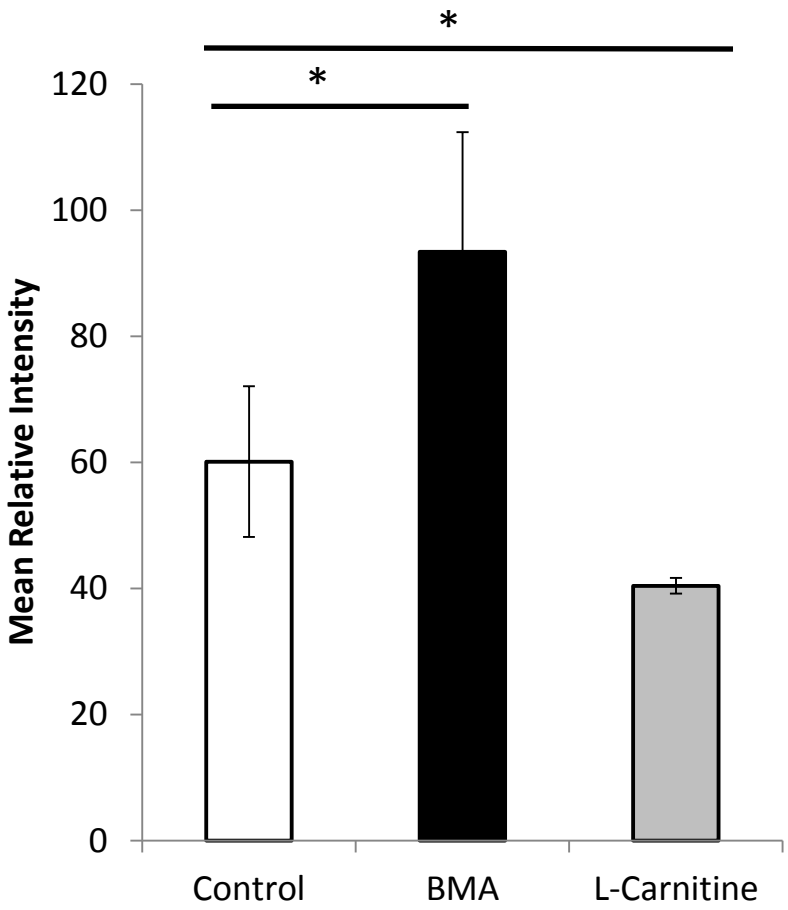


Compared transcriptome and epigenome using Embryogene platform

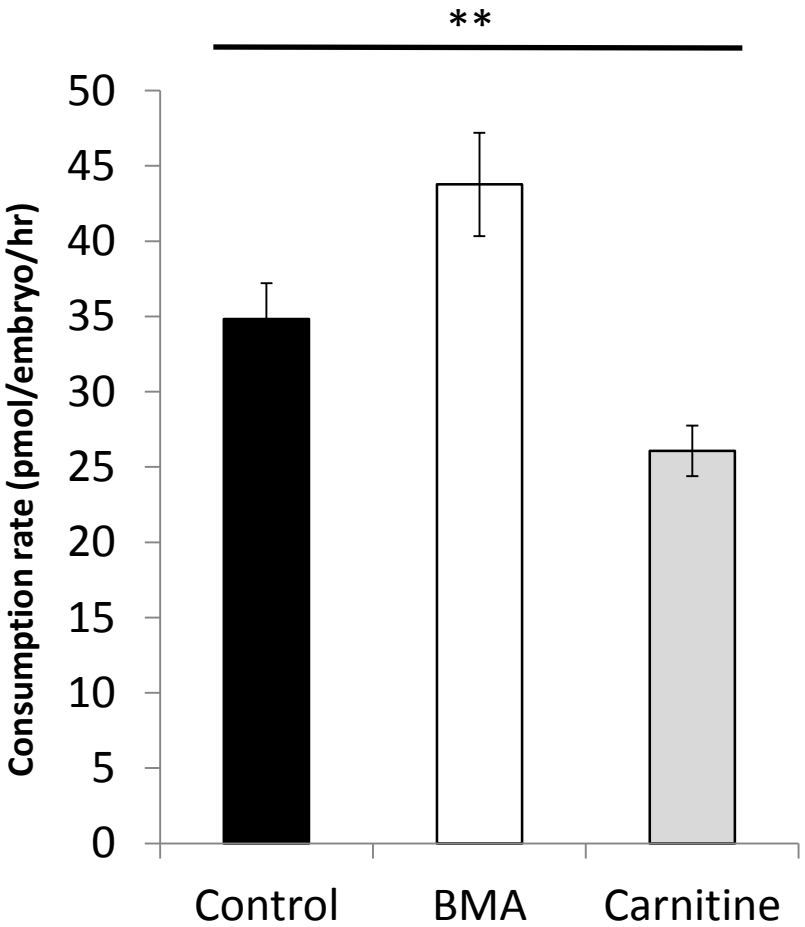
Activated T
metabo
(L-carniti

Measured pyruvate, glucose and oxygen consumption

Metabolic phenotype



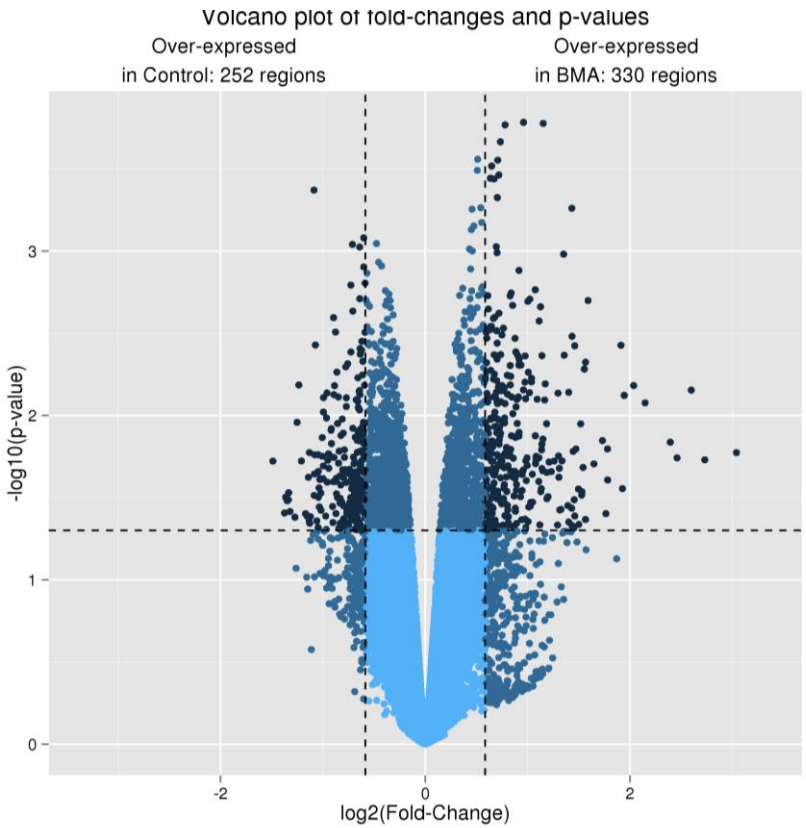
Lipid content



Oxygen consumption

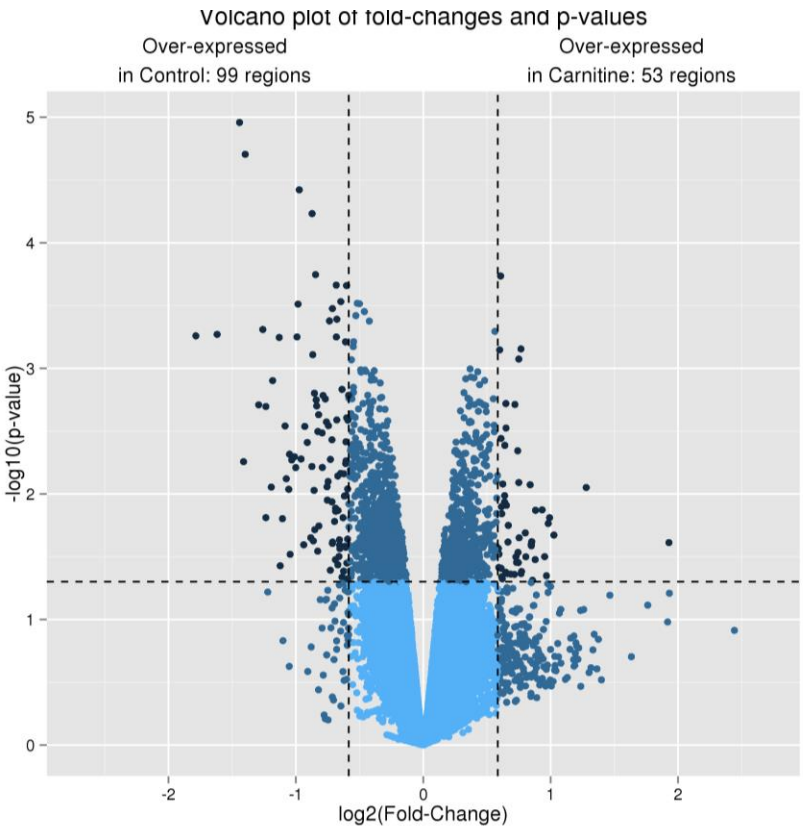
Transcriptomic differences

BMA (KO FAO)



440 genes differentially expressed from controls

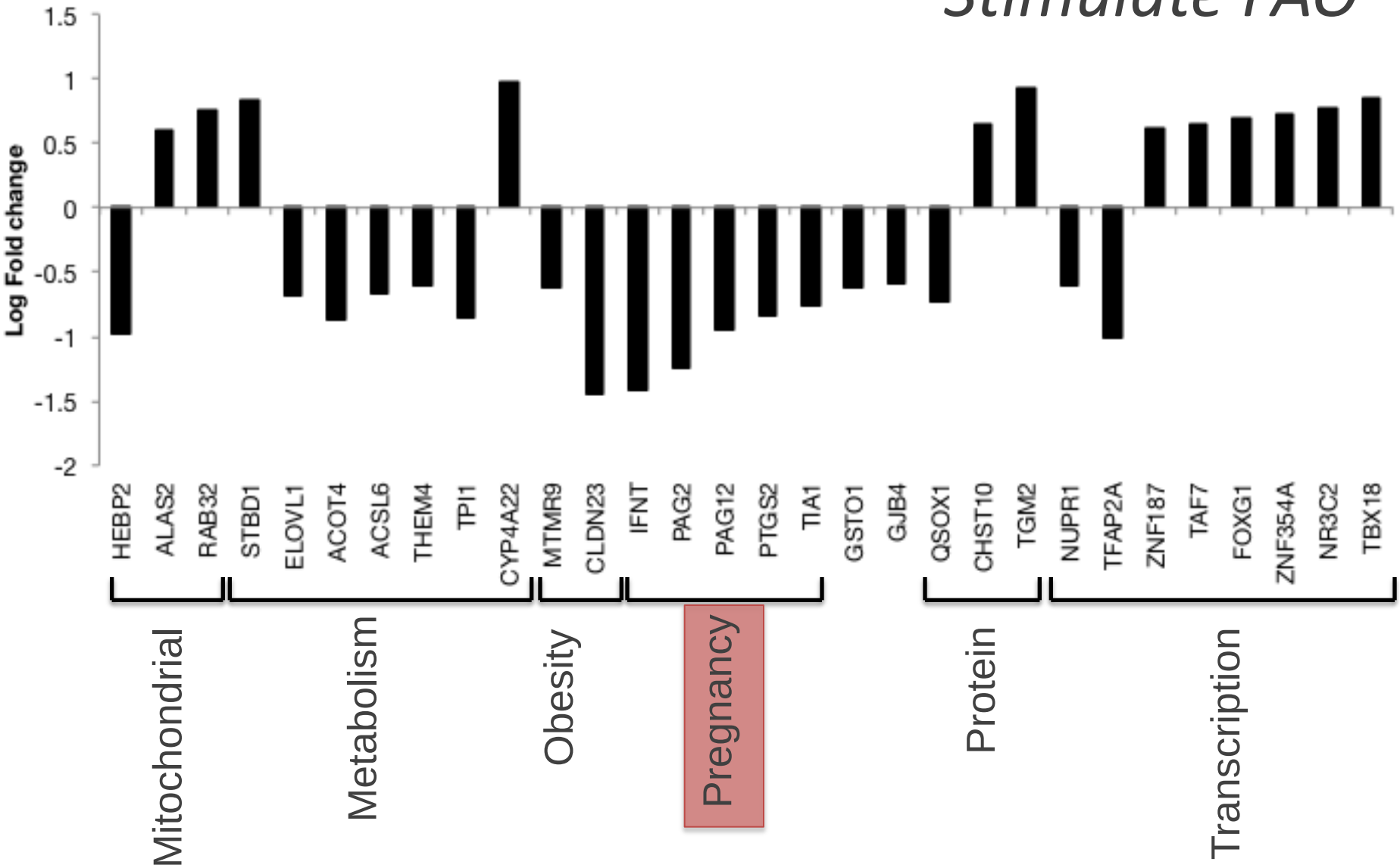
L-Carnitine (stimulate FAO)



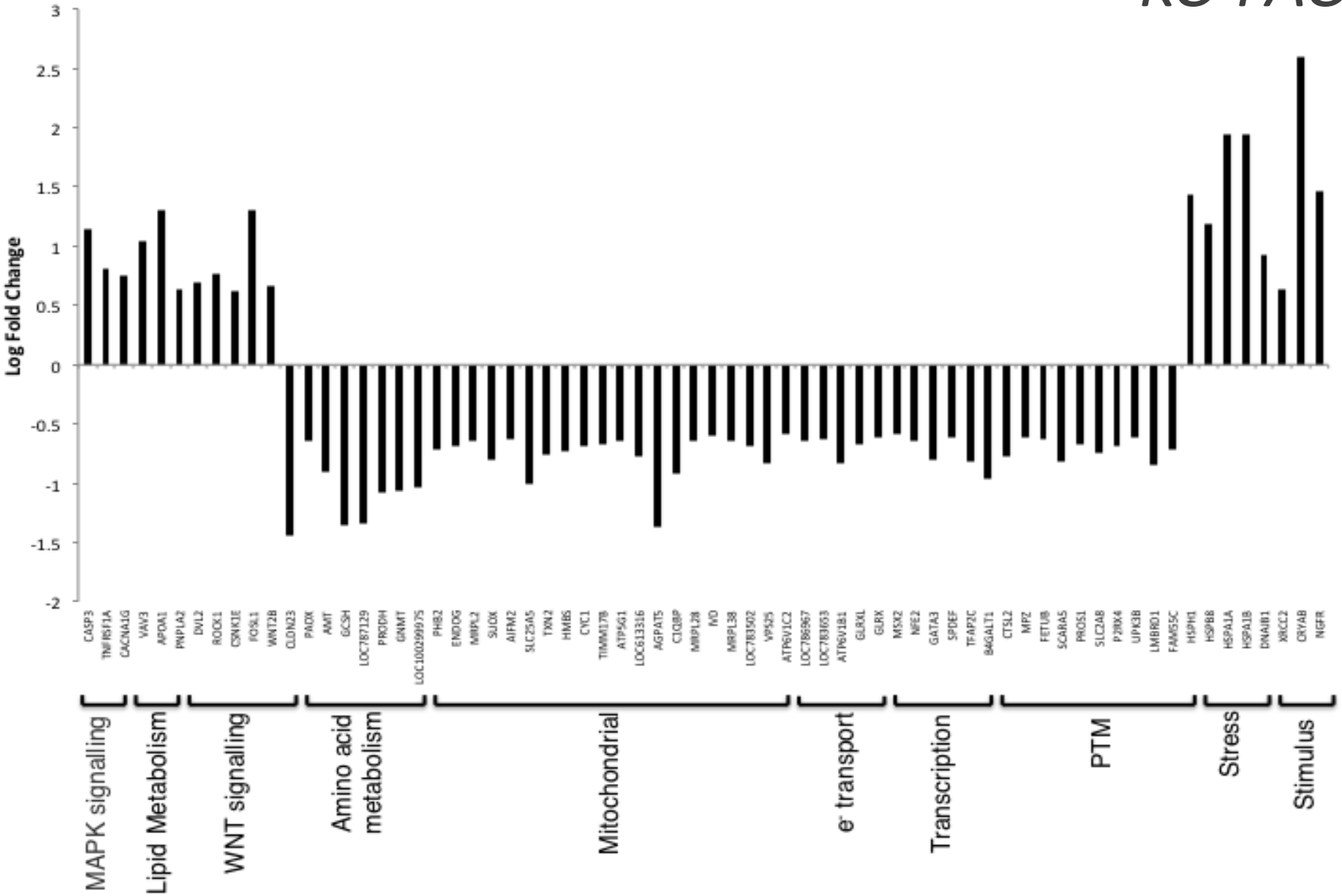
152 genes differentially expressed from controls

Differential expression in L-carnitine

Stimulate FAO

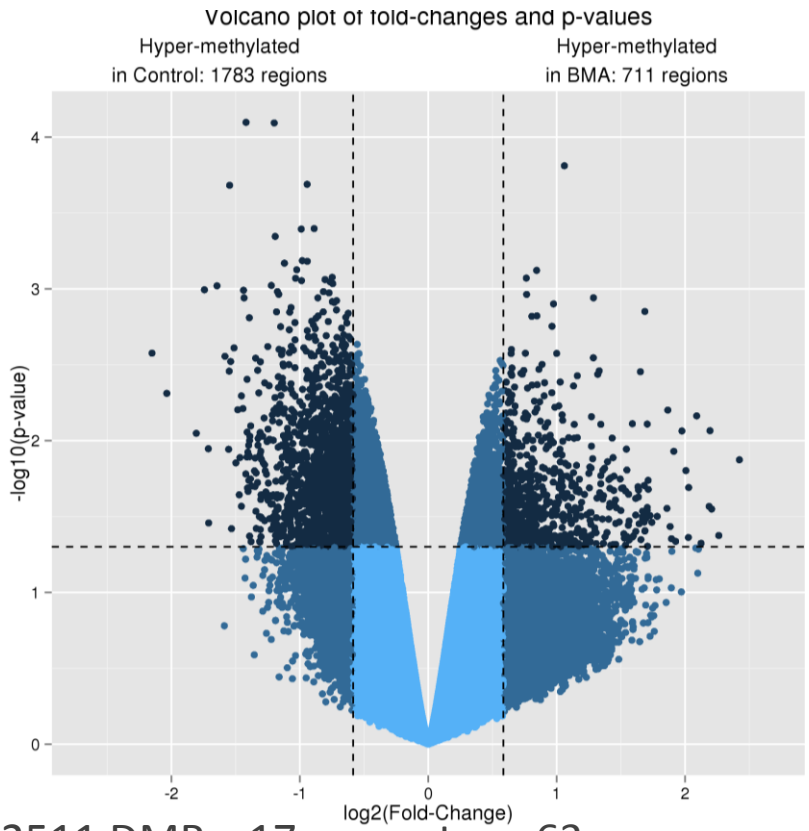


Differential expression in β-Mercaptoacetate *KO FAO*



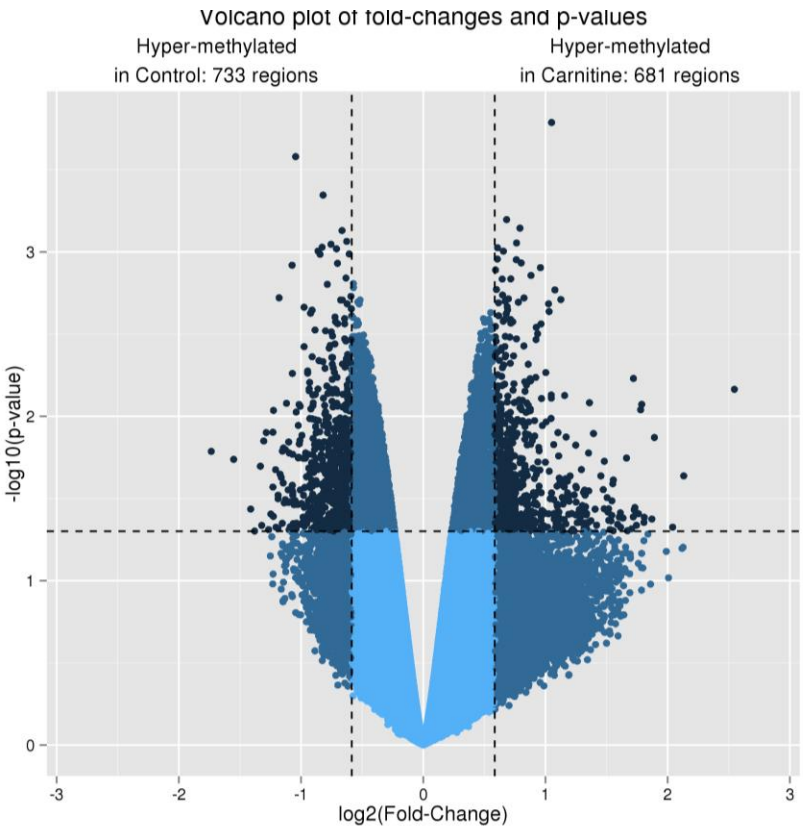
Differentially methylated regions…???

BMA (KO FAO)



2511 DMRs; 17 promoters, 63 intragenic CGIs

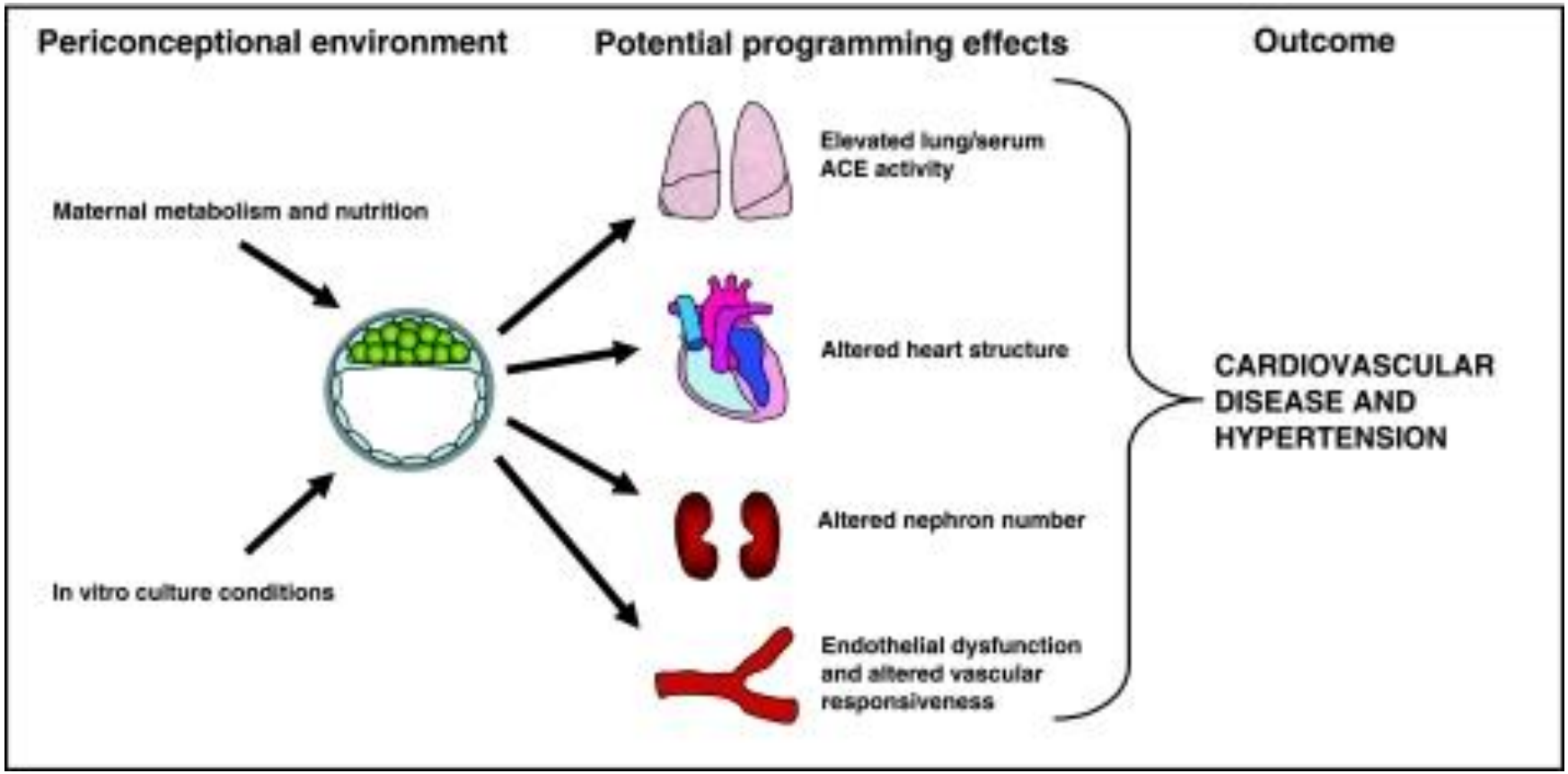
L-Carnitine (stimulate FAO)



1414 DMRs; 18 imprinted genes, 5 promoters, 67 distal promoters, 24 intragenic CGIs

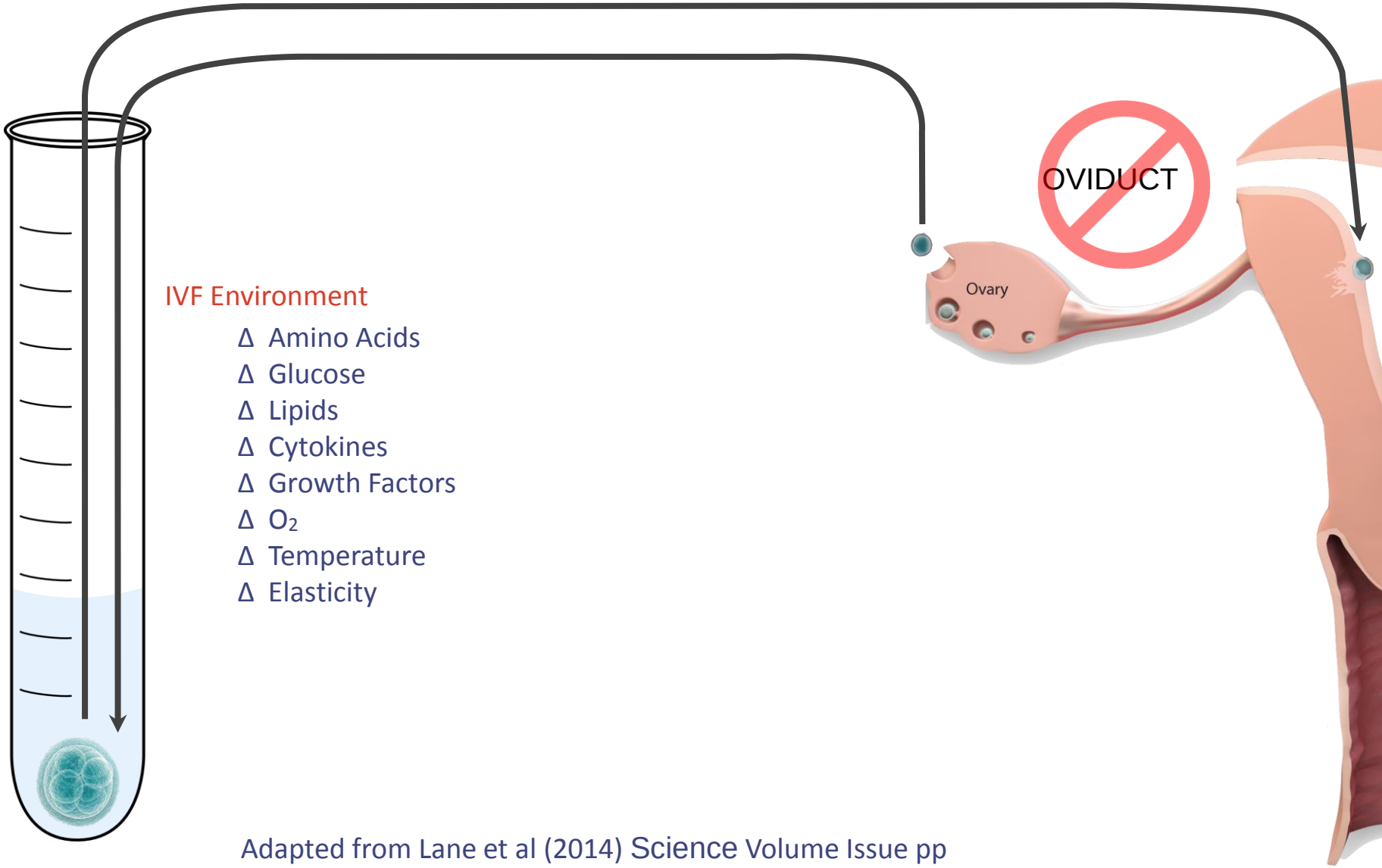
Blastocyst environment and its influence on offspring cardiovascular health: the heart of the matter

Watkins and Fleming: *Journal of Anatomy* 215: 52-59 (2009)



What are the easiest ways to change embryo metabolism?

Assisted Reproduction



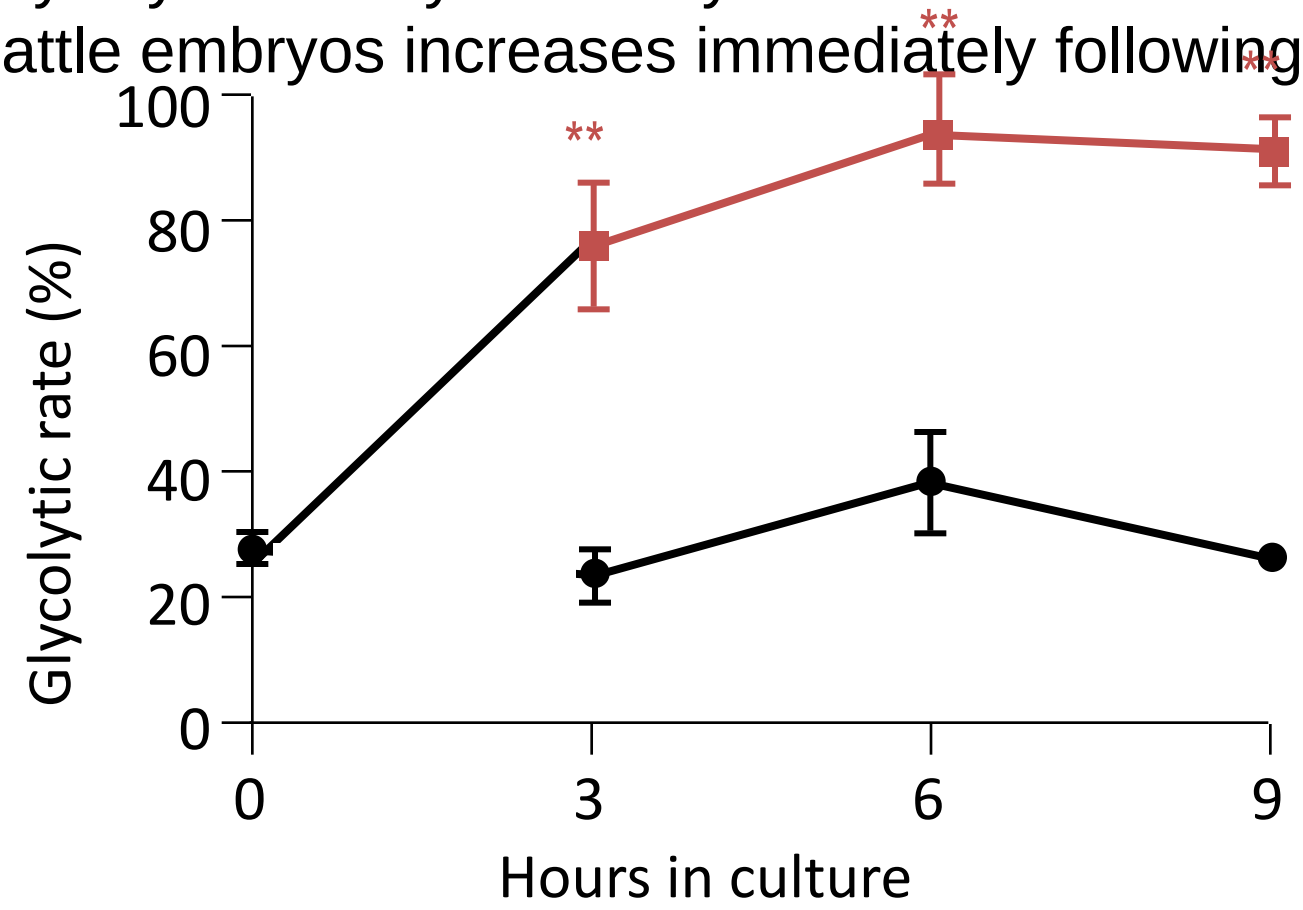
IVF Environment

- Δ Amino Acids
- Δ Glucose
- Δ Lipids
- Δ Cytokines
- Δ Growth Factors
- Δ O_2
- Δ Temperature
- Δ Elasticity

Adapted from Lane et al (2014) Science Volume Issue pp

Dynamic metabolism

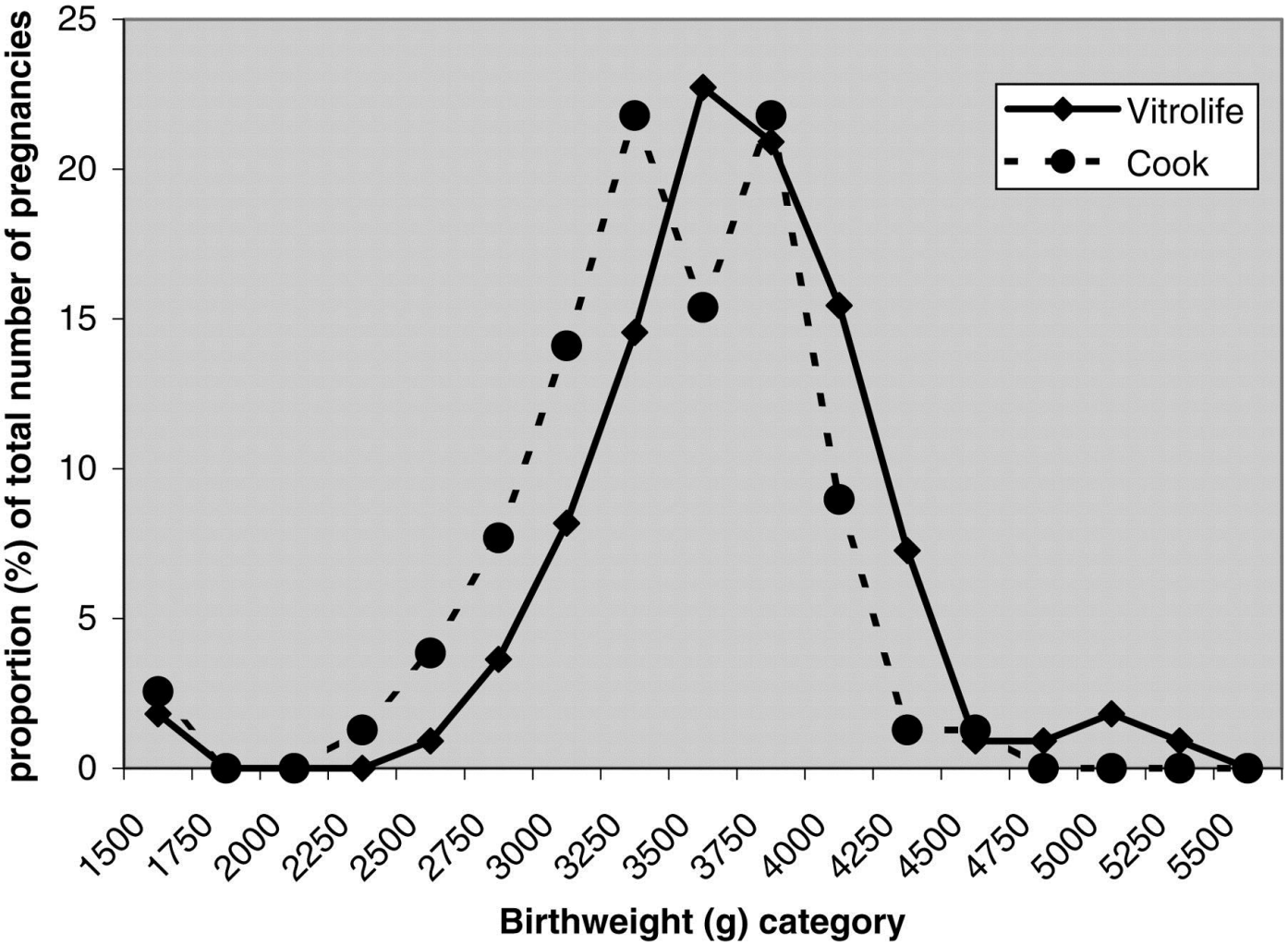
- Glycolytic activity of freshly-flushed mouse embryos and cattle embryos increases immediately following culture



Gardner & Leese 1990

Change the medium…

Birthweight distributions of live born singletons resulting from embryo culture in either Vitrolife or Cook sequential media



Dumoulin, J. C. et al. Hum. Reprod. 2010 25:605-612; doi:10.1093/humrep/dep456

Human Reproduction

Differences in gene expression profiles between human preimplantation embryos cultured in two different IVF culture media†

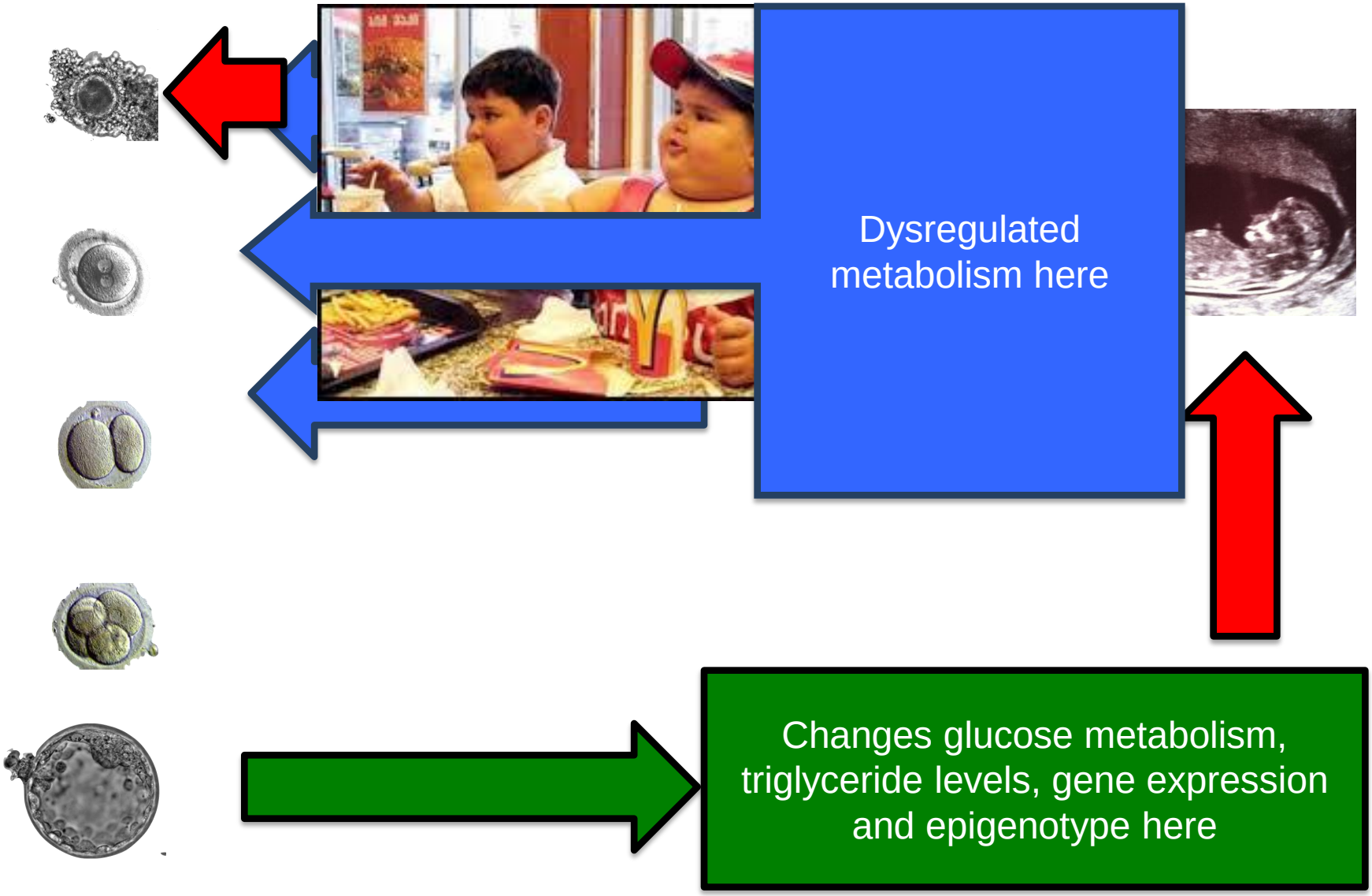
Hum Rep 2015

Sander H.M. Kleijkers[1], Lars M.T. Eijssen[2], Edith Coonen[1], Josien G. Derhaag[1], Eleni Mantikou[3,4], Martijs J. Jonker[4], Sebastiaan Mastenbroek[3], Sjoerd Repping[3], Johannes L.H. Evers[1], John C.M. Dumoulin[1], and Aafke P.A. van Montfoort[1,*]

Table IV Pathways with a significant overrepresentation of DEGs clustered per biological process.

Pathway	Positive (*r* genes)	Measured (*n* genes)	Total genes	%	Z-score
Cell-cycle regulation					
RB in Cancer	20	95	104	21.05%	5.66
Gastric cancer network 2	5	25	33	20.00%	2.67
G1 to S cell-cycle control	9	62	69	14.52%	2.48
DNA Replication	6	38	50	15.79%	2.25
Apoptosis					
Apoptosis	11	73	85	15.07%	2.88
TP53 Network	4	17	23	23.53%	2.78
DNA Damage Response	9	61	75	14.75%	2.53
DNA Damage Response (only ATM dependent)	11	85	101	12.94%	2.33
miRNA Regulation of DNA Damage Response	9	66	106	13.64%	2.27
Protein processing					
Parkin-Ubiquitin Proteasomal System pathway	9	58	75	15.52%	2.70
Proteasome Degradation	8	54	67	14.81%	2.40
Metabolism					
Glycerophospholipid Biosynthetic Pathway	6	23	94	26.09%	3.73
Folate Metabolism	8	50	139	16.00%	2.65
Vitamin B12 Metabolism	6	36	118	16.67%	2.40
SREBF and miR33 in cholesterol and lipid homeostasis	3	15	19	20.00%	2.06
Nuclear Receptors in Lipid Metabolism and Toxicity	4	23	45	17.39%	2.06
Oxidative phosphorylation	7	52	69	13.46%	1.96
Development					
Hedgehog Signaling Pathway	3	13	17	23.08%	2.37

Legacy…


Dysregulated metabolism here
Changes glucose metabolism, triglyceride levels, gene expression and epigenotype here

Summary

- Metabolism is a broad term that refers to the processes to provide the fuel and blocks to support '*everything'*
- We have a picture of embryo metabolism
- That picture is evolving
- Embryo metabolism can be changed, but with consequences…
- Little changes, 'insignificant choices', can have big impact…
  - Epigenetics?
  - Signaling?
  - Life long health?

Thanks!

Fabrice Guerif

Veerle Van Hoeck

Henry Leese
Daniel Brison
Judith Hawkhead
Maryam Ghavideldarestani

Jo Leroy
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