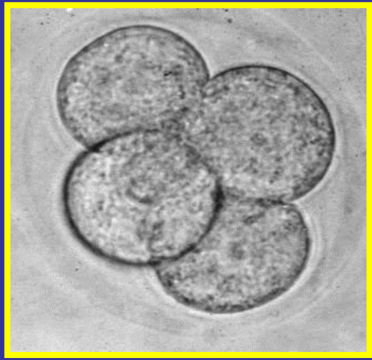
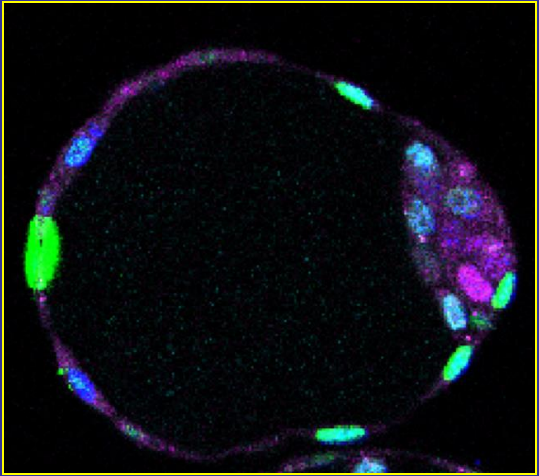
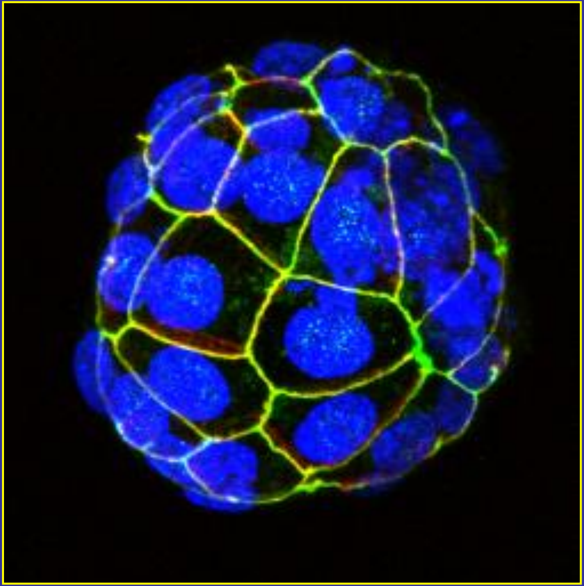
Southampton
UNIVERSITY OF
Centre for Biological Sciences

The effect of nutrition and environment on the pre-implantation embryo

Tom Fleming
University of Southampton, UK
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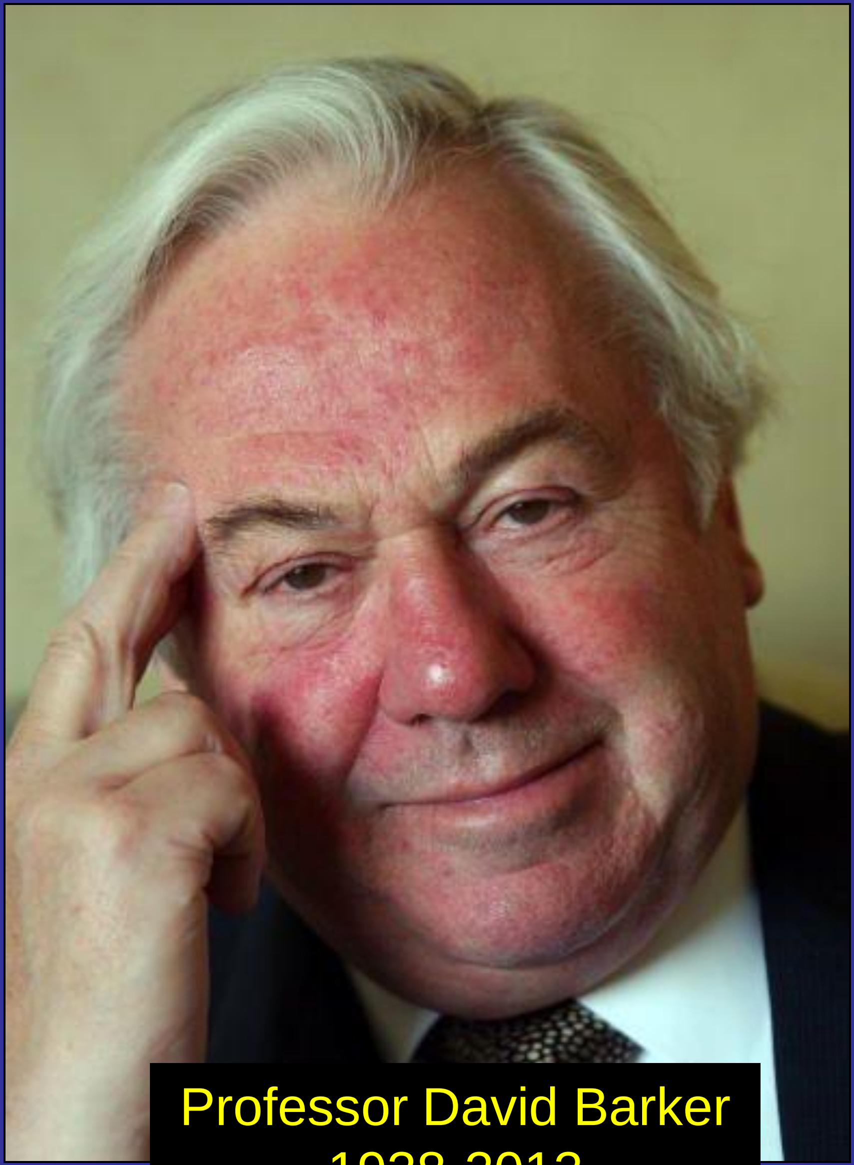
Dutch Society of Clinical Embryologists, January 2015



Professor David Barker
1938-2013
University of Southampton
Oregon Health & Science Uni

Developmental Origins of Health and Disease (DOHaD)

www.mrc.soton.ac.uk/dohad

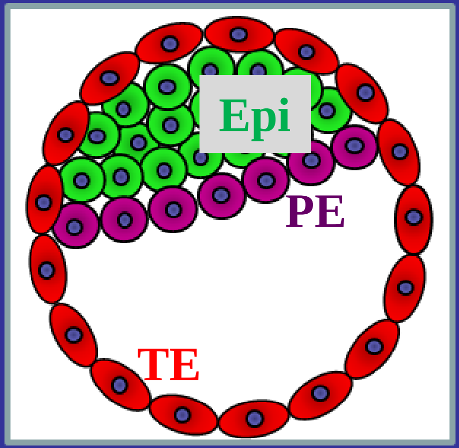
An early life origin of adult chronic diseases?

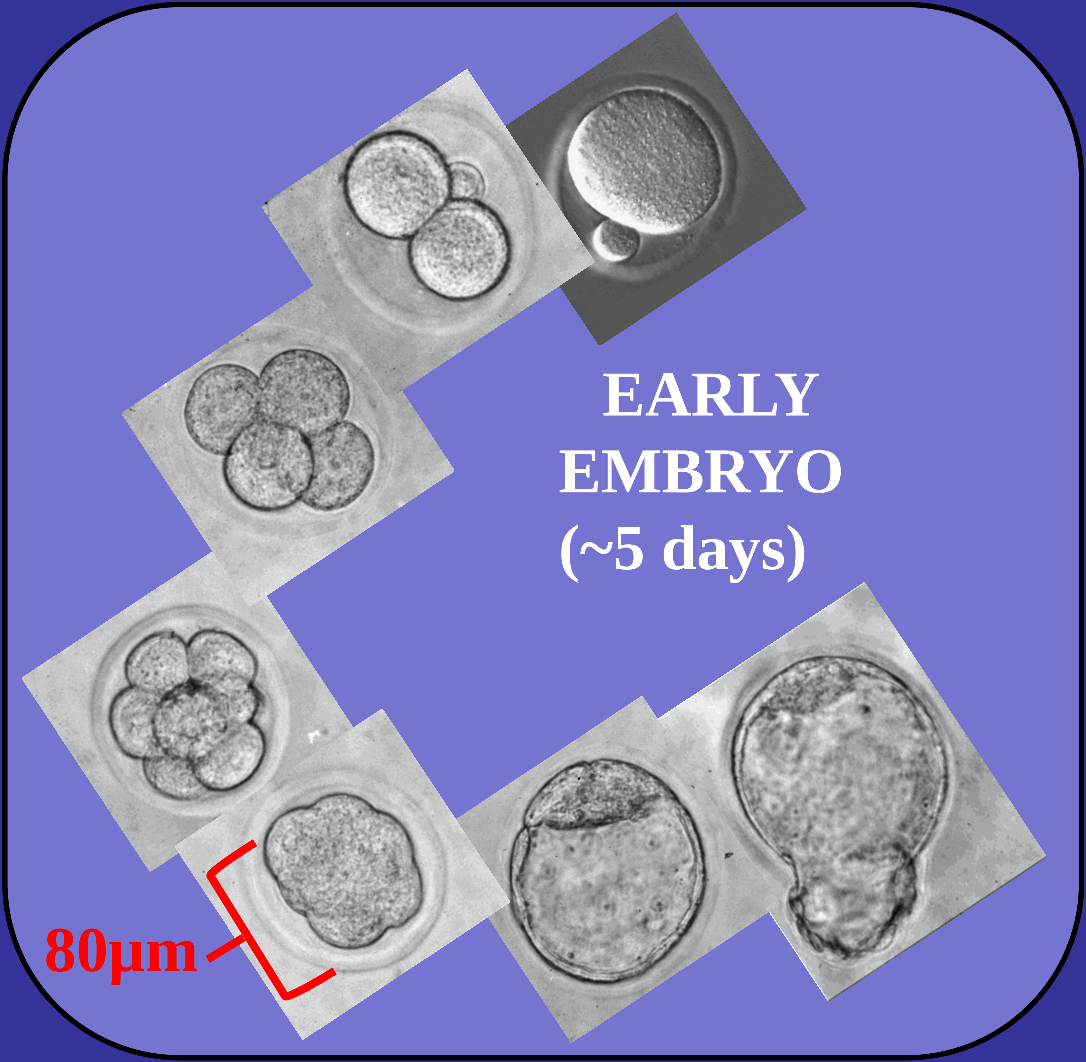
Epidemiological evidence:

Risk of adult chronic disease (eg, stroke, cardiovascular disease, coronary artery disease, diabetes, obesity, osteoporosis, metabolic syndrome) increases with poor maternal nutrition in utero and rapid ‘catch-up’ growth in early childhood

Peri-conceptional environment?

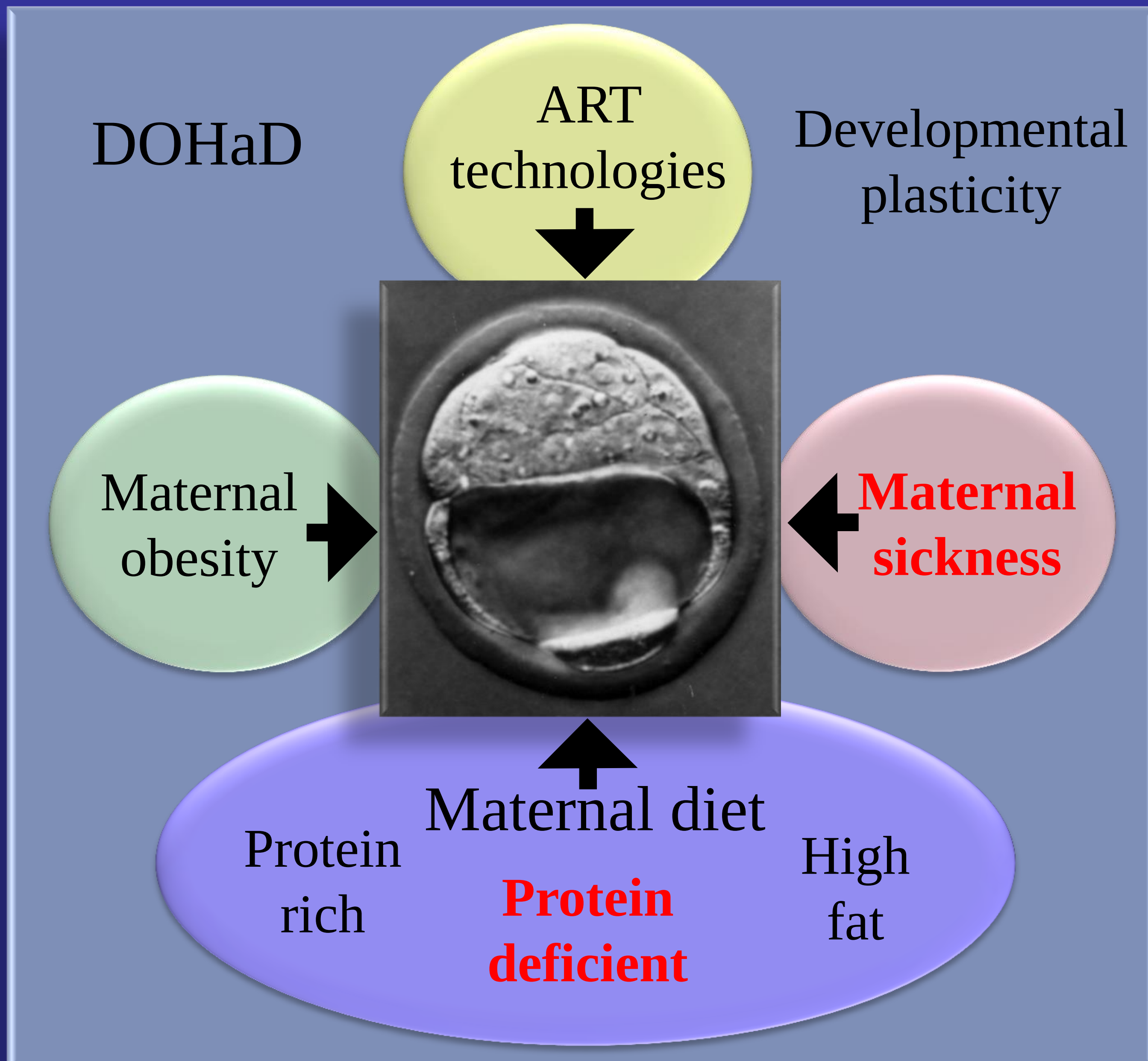
WHY EMBRYOS ?





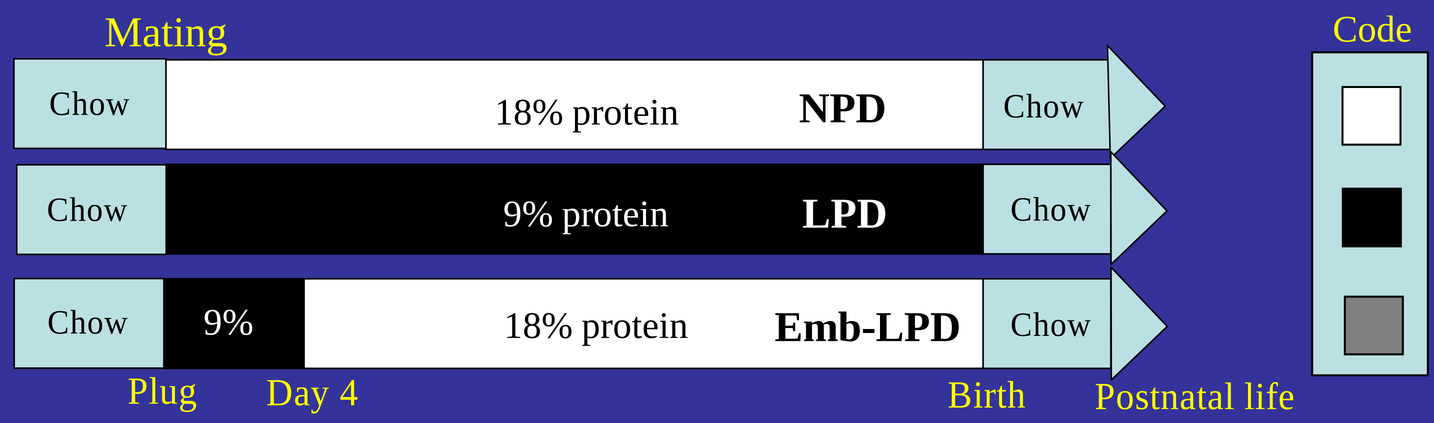
- Small ‘shifts’ at the beginning, large changes at the end
- Totipotency/pluripotency
- Epigenetics
- Do little embryos make big decisions?
- Plasticity

Periconceptional Environment – Mouse Models



Mouse maternal protein restriction model

Watkins et al. (2008) Biol Reprod 78:299



Diet Composition (g/kg)

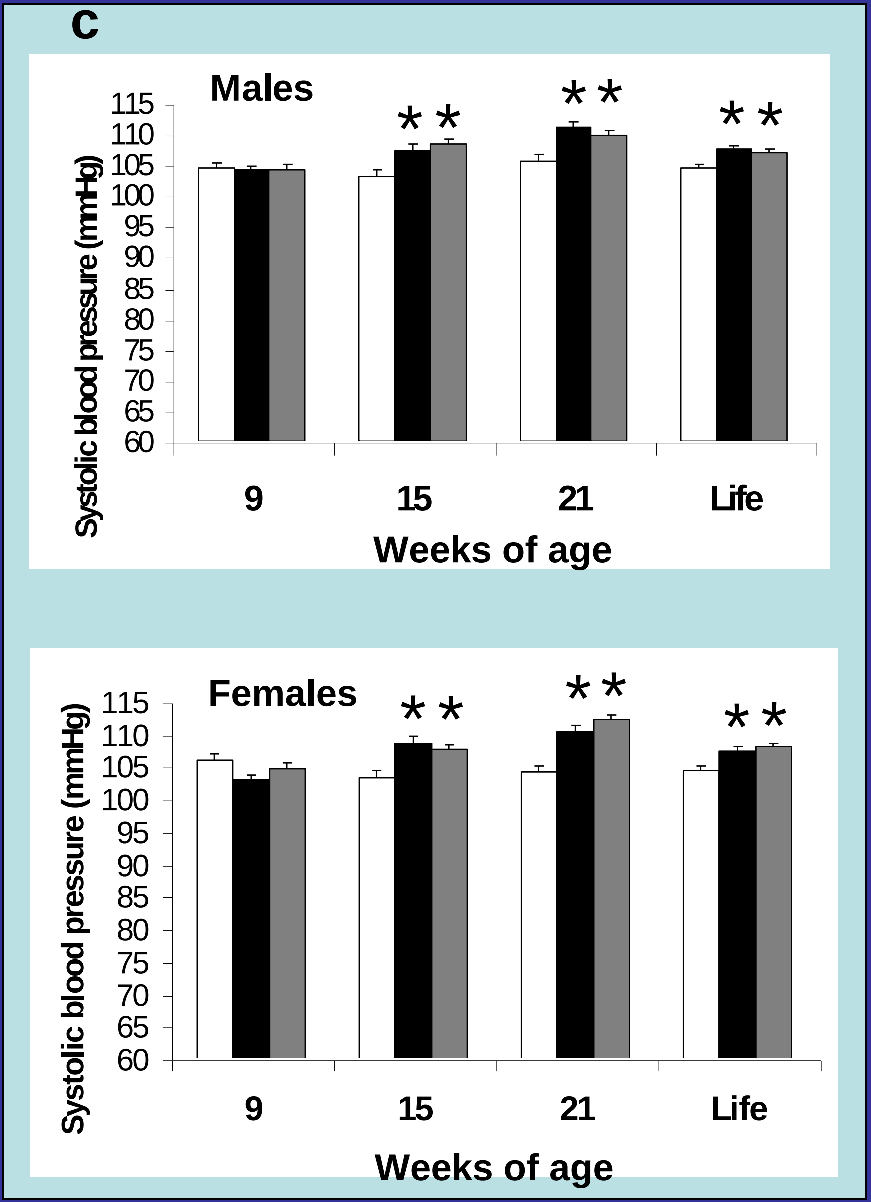
	18%	9%
Casein	180	90 *
Corn starch	425	485 *
Fibre	50	50
Sucrose	213	243 *
Choline chloride	2	2
DL-Methionine	5	5
AIN-76 mineral mix	20	20
AIN-76 vitamin mix	5	5
Corn oil (gm/kl)	100	100

- Iso-caloric
- Derived from Langley & Jackson, 1994

Low protein diet

- **Mild challenge**: 9% protein is sufficient for non-pregnant rodent – therefore not starvation but within normal range
- **Large study**: (19 mothers, 114 offspring per treatment) allows detailed associations to be identified
- No effect on gestation length, litter size or male:female ratio

Maternal Emb-LPD and Postnatal Cardiovascular Phenotype



Adult Emb-LPD offspring exhibit:

- Relative hypertension
- Smaller heart mass (females)
- Increased vasoconstriction factors (eg, lung ACE activity)
- Reduced arterial vasodilation

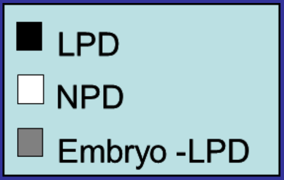
Watkins et al. (2008) BOR 78:299
Watkins et al (2010) Br J Nutr 103:1762-70
Watkins et al al (2011) PLOS One 6:e28745

Similar datasets:

Rat Emb-LPD [Kwong et al, 2000, Development 127: 4195-4202]

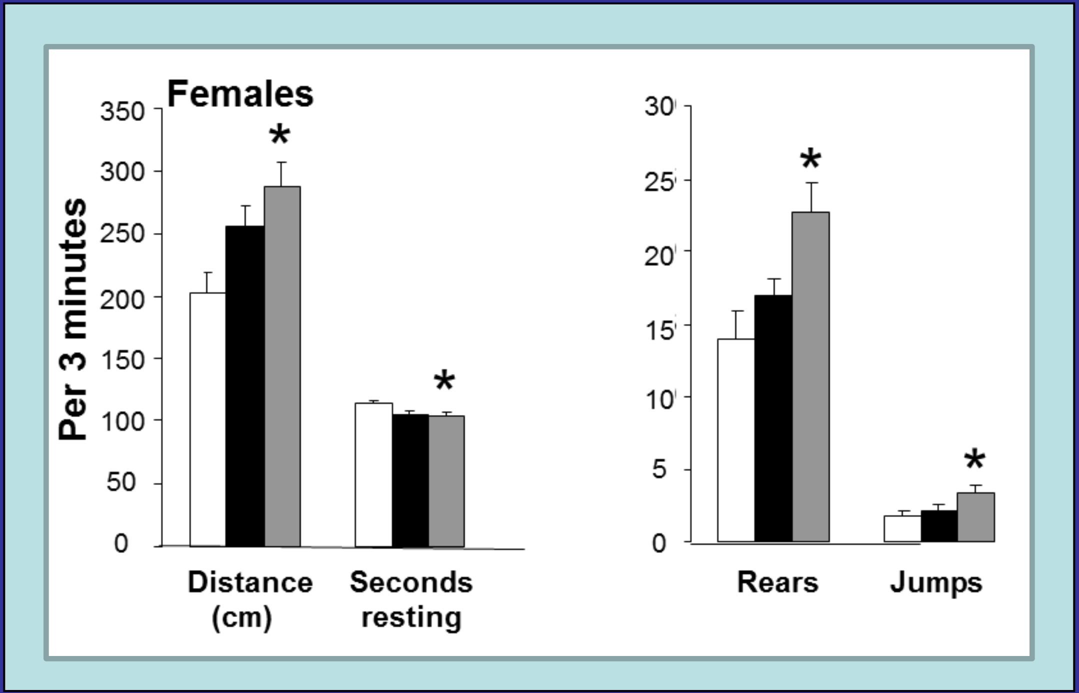
Mouse LPD during oocyte maturation, [Watkins et al, 2008, J Physiol 586:2231-2244]

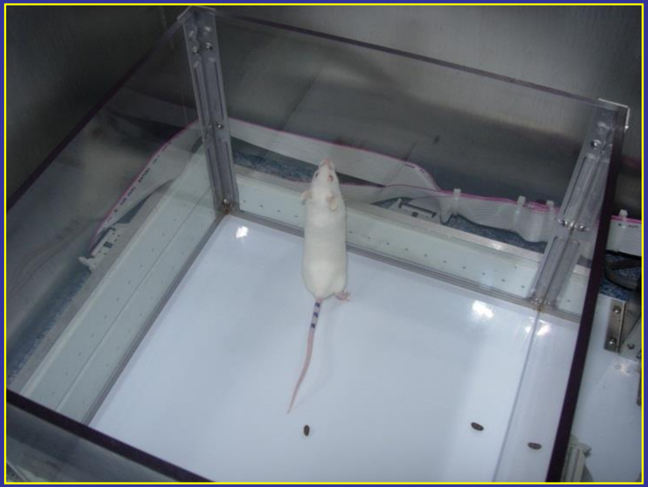
Mouse ART culture, [Watkins et al, 2007, PNAS 104:5449-54]



Maternal Emb-LPD and postnatal behaviour

- Assays measure anxiety-related locomotor and exploratory activities
- Mean of tests repeated five times over weeks 8, 11, 14, 17 and 20 after acclimatization
- **Emb-LPD offspring exhibit 'hyperactive' behaviour**



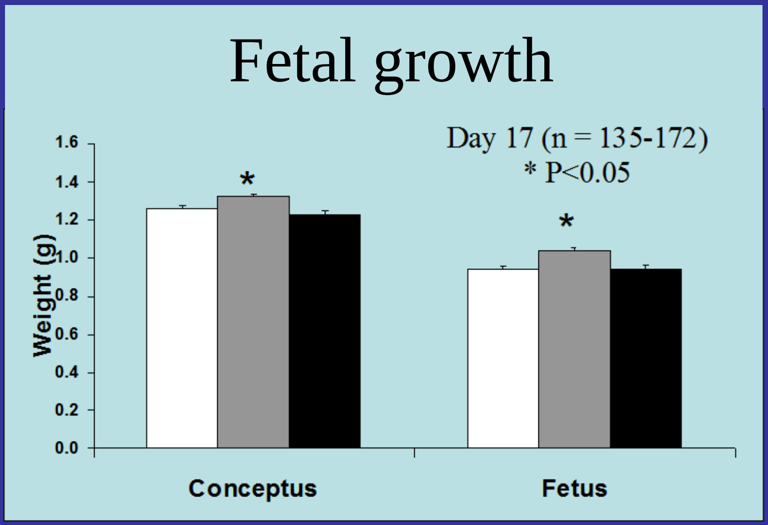


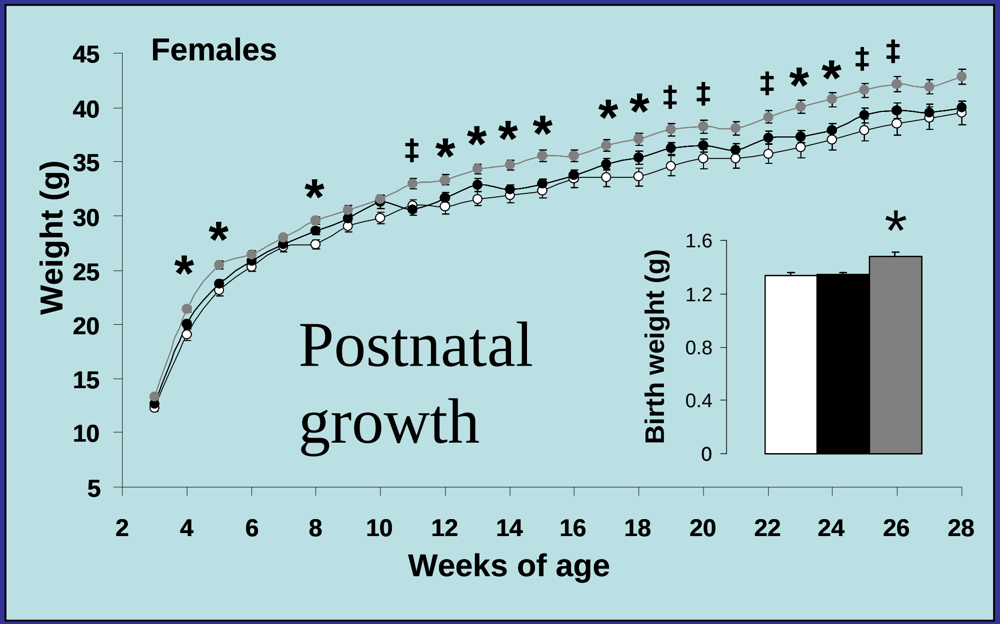
LPD
NPD
Embryo-LPD

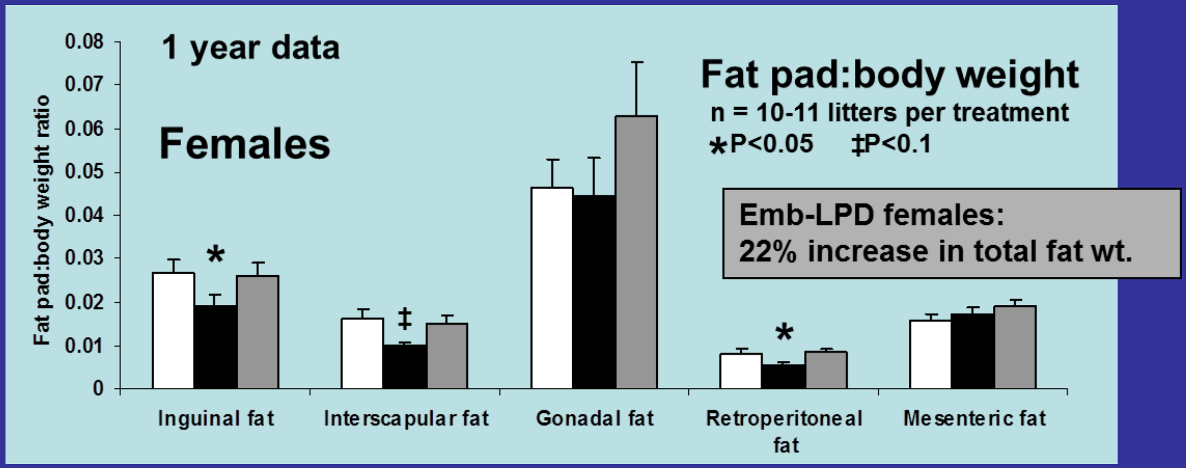
with Hugh Perry and colleagues

Watkins et al. (2008) BOR 78:299

Maternal Emb-LPD affects fetal and postnatal growth leading to adult adiposity







Watkins et al. (2008) BOR 78:299

Watkins et al. (2011) PLOS-One

Poor nutrition during periconceptional period and long-term outcomes

Mouse: Emb-LPD model:

- adult hypertension and dysfunctional CV system
- adult hyperactive behaviour
- increased adiposity in later life
- increased gestational and postnatal growth

Sheep: Periconceptional below maintenance feeding (50-70% control diet)

- adult hypertension, increased arterial vasoconstriction, reduced vasodilatation (Torrens et al. 2009)
- impaired glucose tolerance (Todd et al. 2009).
- suppressed behavioural reactions to stress, abnormal HPA axis (Hernandez et al. 2009, 2010)

Human: Is it a similar outcome?

The Dutch hunger winter 1944/45

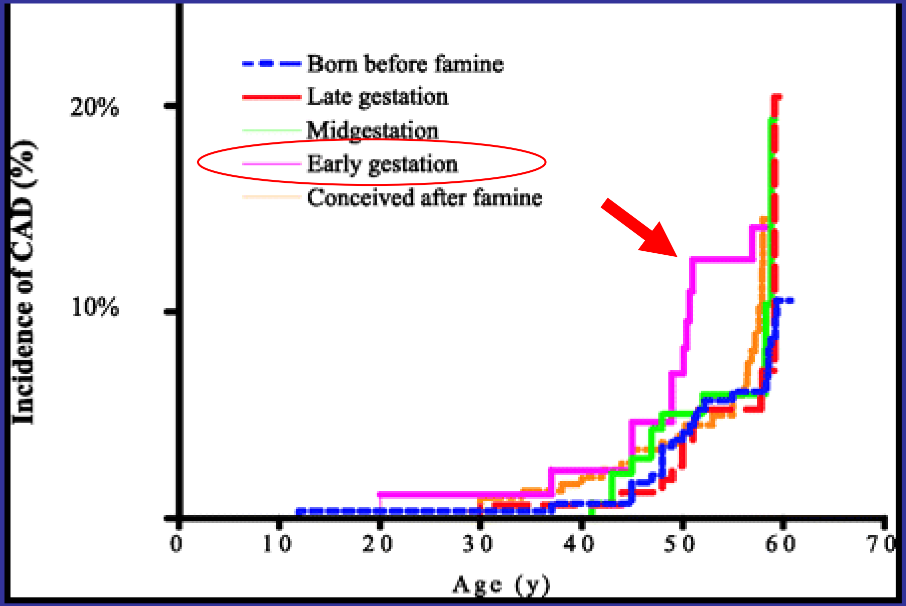
Dutch winter famine

A 5 month period between 1944-45 in Amsterdam.

Effects of the famine experienced during pregnancy have revealed an **elevated risk of cardiometabolic and neurological disease in later life** than for pregnancies occurring outside the famine period

Roseboom, Painter et al. (2011) *Maturitas* 70(2), 141-5.

The Dutch hunger winter 1944/45



Incidence of coronary artery disease in persons exposed to the Dutch Hunger Winter during different stages of gestation.

Persons ***conceived*** during the famine (versus later gestation) exhibit in adulthood:

- double the rate of coronary heart disease
- increased blood pressure response to psychological stress
- increased BMI and glucose intolerance
- more atherogenic plasma lipid profile,
- increased risk of schizophrenia and depression,
- more responsive to stress and performed worse in cognitive tasks,
- stronger evidence of accelerated ageing.

Data are independent of gender, smoking, social class, birth size factors.

Painter et al, 2006, *Am J Clin Nutr* 84:322-7.
Roseboom, Painter et al. (2011) *Maturitas* 70(2), 141-5.

Periconceptional protein restriction and adult health

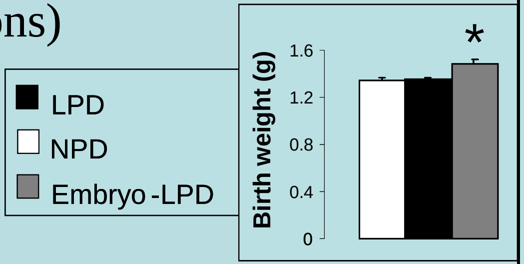
What are the mechanisms?

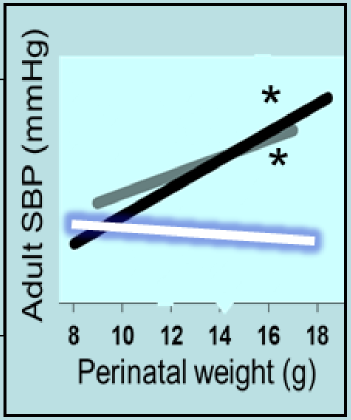
Emb-LPD causes: (1) hypertension and dysfunctional CV system
(2) hyperactive behaviour
(3) increased gestational and postnatal growth
(4) increased adiposity

Increased growth:

• Evident during fetal development – an early response, long before disease

• Weight around birth is **predictive** of adult weight, CV and behavioural disease (positive correlations)





What might be happening?

• Growth response is 'compensatory'

• Poor diet causes embryo to increase nutrient supply from mother throughout gestation – needed for LPD, not needed for Emb-LPD

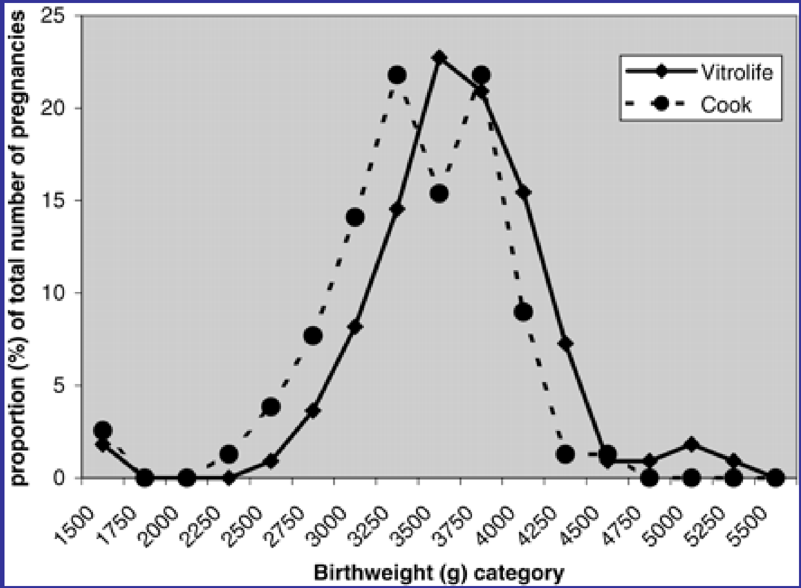
• This makes evolutionary sense - protects fetal growth and maintains offspring competitive fitness

• Later disease is a 'trade-off' – less evolutionary significance

Does periconceptional environment influence fetal growth and later health in the human?

Some evidence from ART

- **Periconceptional environment affects birth weight of IVF children** (adjusted for gestational age, gender) dependent upon commercial embryo culture medium used; Cook v Vitrolife – sustained up to 2 years of age (Dumoulin et al 2010; Nelissen et al 2012, 2013; Kleijkers et al 2014)

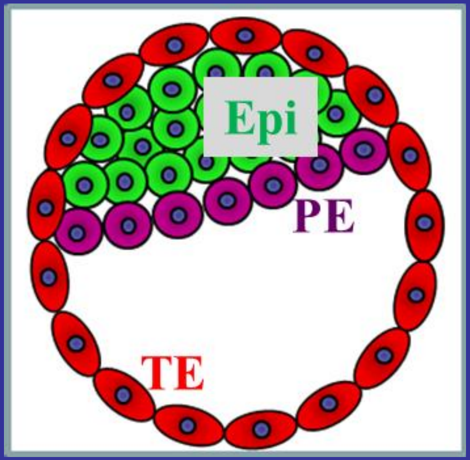


- **Systolic and diastolic blood pressure** levels higher in IVF children (8-18 years) than controls ($P < 0.001$) (Ceelen M et al 2008)
- **Growth velocity** higher in IVF children and is predictive of later elevated blood pressure (Ceelen et al, 2009)
- **Increased early growth** ⟶ **CV dysfunction**

Emb-LPD

- Maternal Low protein diet just for preimplantation period (0-4d)
- Maternal-embryonic interactions before implantation

Sequence of events



Emb-LPD changes the composition of maternal serum and then uterine fluid (insulin, amino acids)

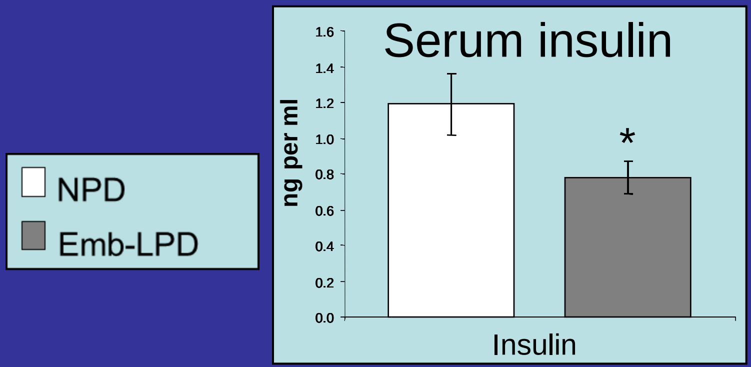
Early embryo in uterus has sensing mechanisms to 'assess' the quality of maternal nutrients (mTOR pathway)

Induction of nutritional programming by blastocyst stage (embryo transfer expts)

Early responses are *compensatory*, promote development and function of extra-embryonic lineages (TE, PE) at expense of embryonic (Epi)

Emb-LPD: changing maternal environment

Insulin and amino acids depletion by Emb-LPD



Depleted maternal insulin and branched chain AAs following Emb-LPD at E3.5

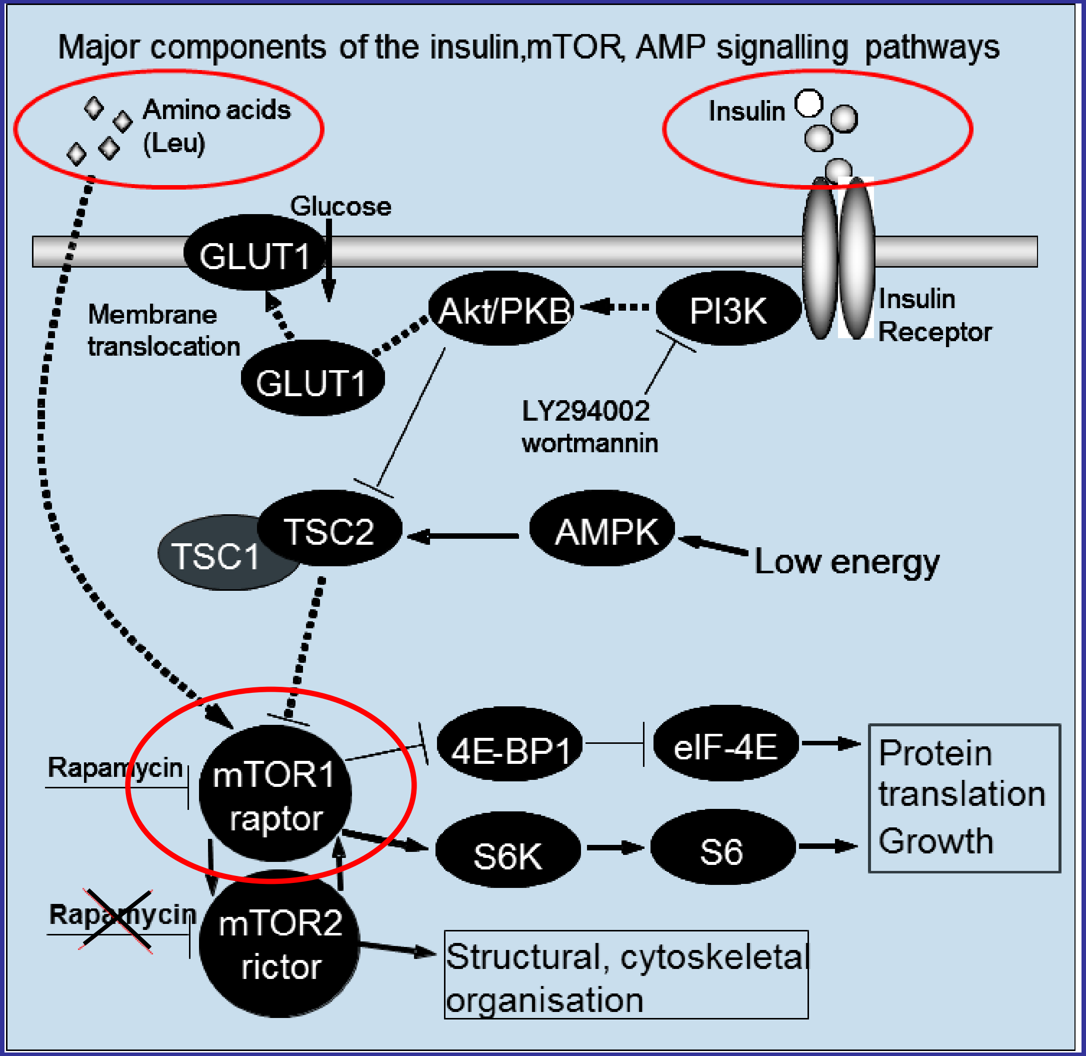
Amino acid mM

Uterine Fluid E3.5	**NPD**	**Emb-LPD**	**% Reduction**	
Branched chain	0.993±0.082	0.716±0.079	↓ 27.9%	*
Essential	2.623±0.180	2.201±0.173	↓16.1%	*
Non-essential	31.098±2.217	24.724±2.217	↓ 20.5%	*
Total	33.747±2.365	26.639±2.365	↓ 21.1%	
Leucine	0.324±0.030	0.243±0.029	↓ 25.0%	*
Isoleucine	0.212±0.018	0.163±0.017	↓ 23.2%	*
Valine	0.457±0.040	0.311±0.039	↓ 32.0%	*

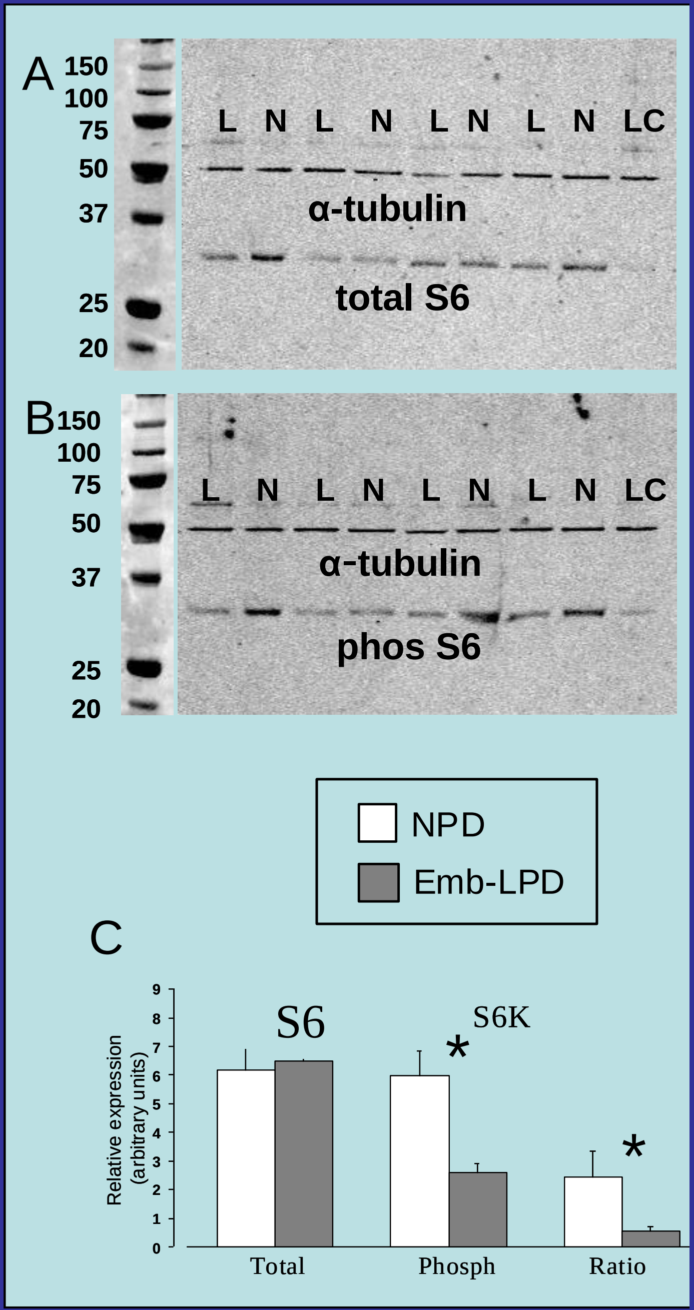
n = 9-15 mothers per treatment
* P<0.05

Eckert et al 2012 PLOS-One 7:e52791

Emb-LPD: sensing by embryos via mTOR pathway



Emb-LPD: sensing by embryos via mTOR pathway



Emb-LPD leads to reduced mTOR1 signalling via S6 in blastocysts

Deficient insulin and AA availability is detected by embryos

The moment an embryo decides to do something about its mother?!

Activates an altered developmental programme

Results in a series of compensatory growth mechanisms

Eckert et al 2012 PLOSOne 7:e52791

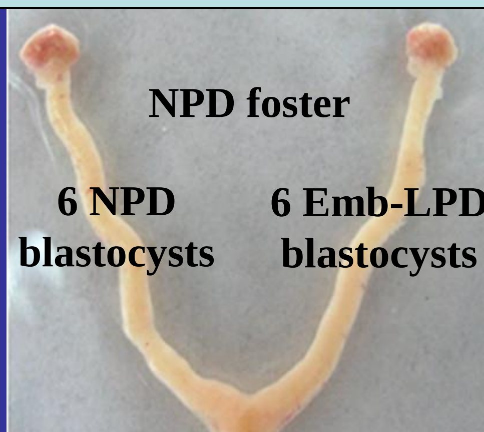
25 blastocysts per sample
8 replicates per treatment

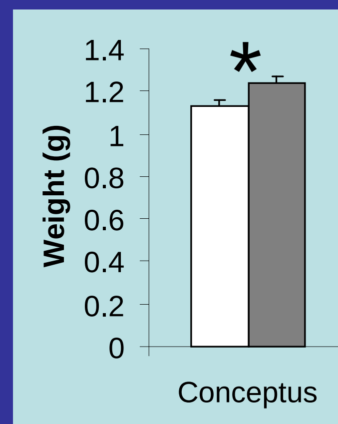
Emb-LPD: Induction of altered developmental programme

Emb-LPD
vs NPD
E0-3.5

Collect blastocysts
Immediate embryo transfer
NPD foster mothers

Blastocyst uterine transfer





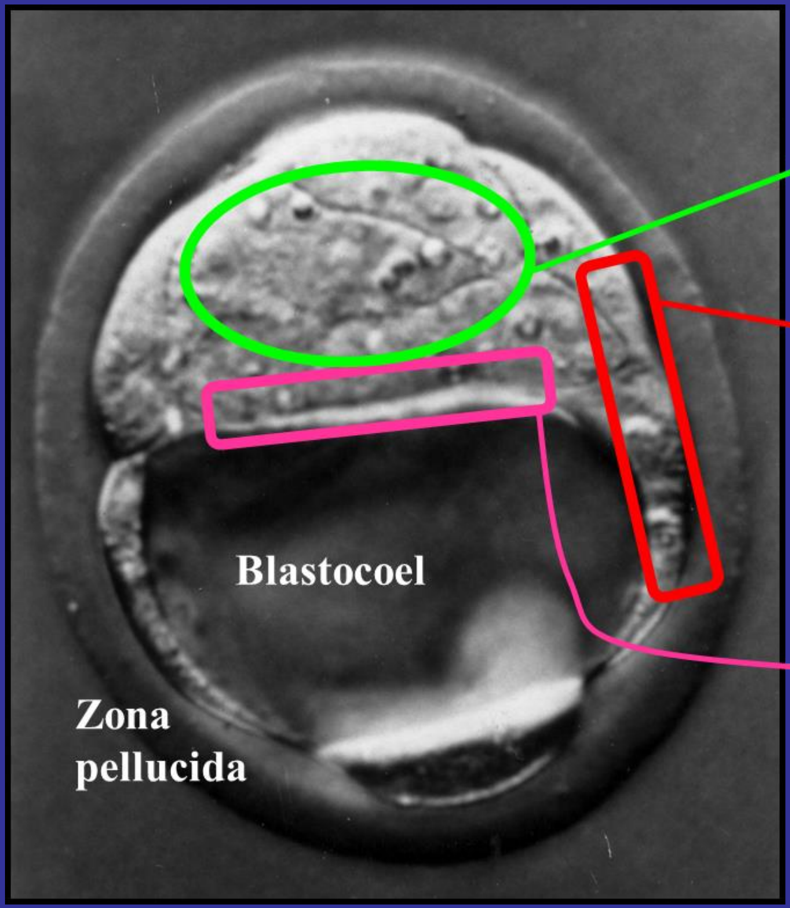
NPD
Emb-LPD

Increased perinatal growth in Emb-LPD conceptuses is induced by the blastocyst stage independent of later maternal environment

Watkins et al. (2008) BOR 78:299

How do embryos respond to their mother's nutritional status to protect fetal growth?

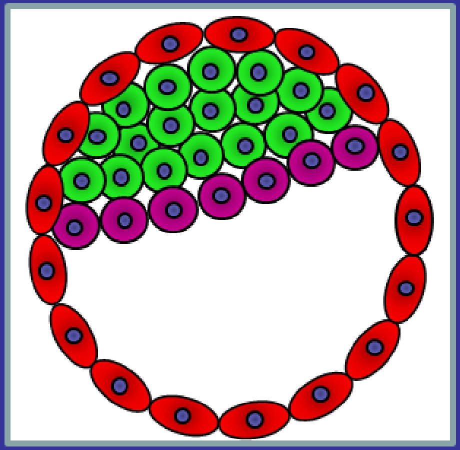
A role for extra-embryonic lineages



Inner cell mass
→ Fetus

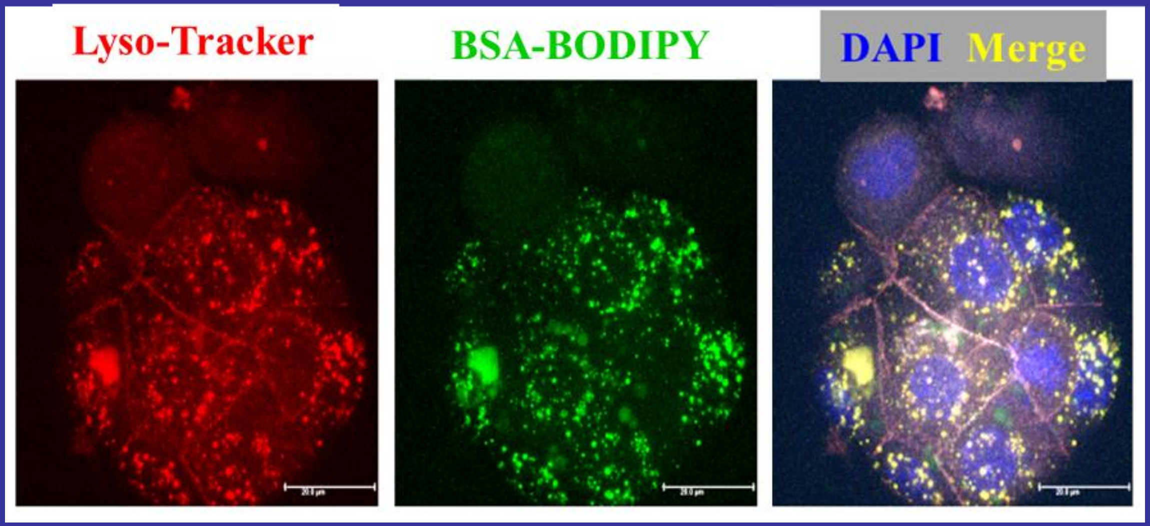
Trophectoderm
→ Chorio-allantoic placenta

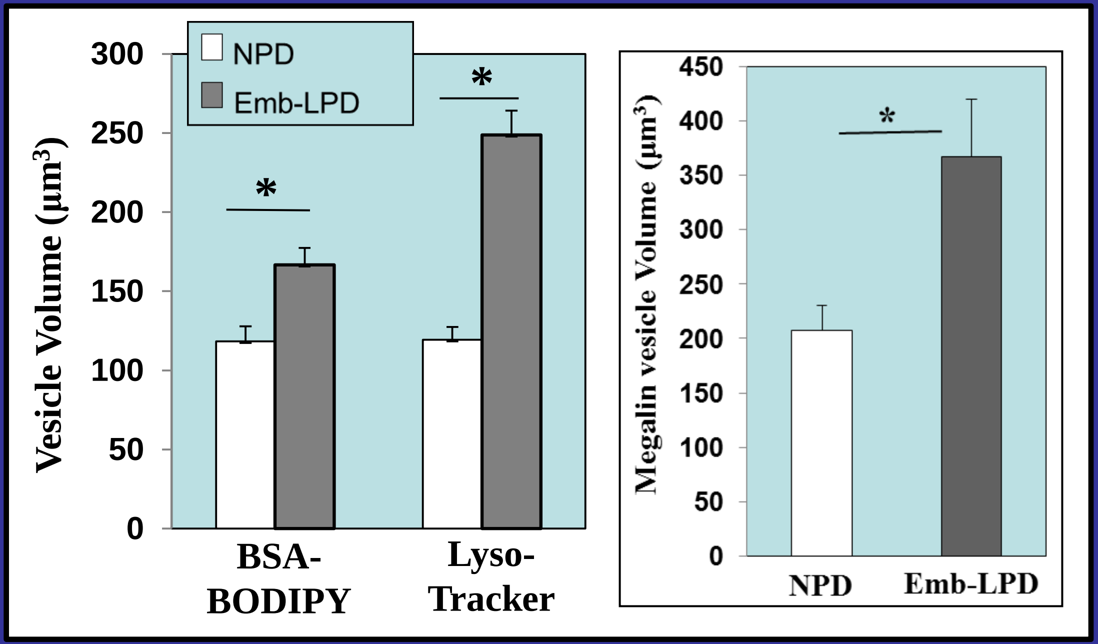
Primary endoderm
→ Yolk sac placenta



How do Emb-LPD embryos activate compensatory growth?

1. Stimulate endocytosis in **trophectoderm**





Confocal microscopy and *Volocity* image analysis; immunoblotting

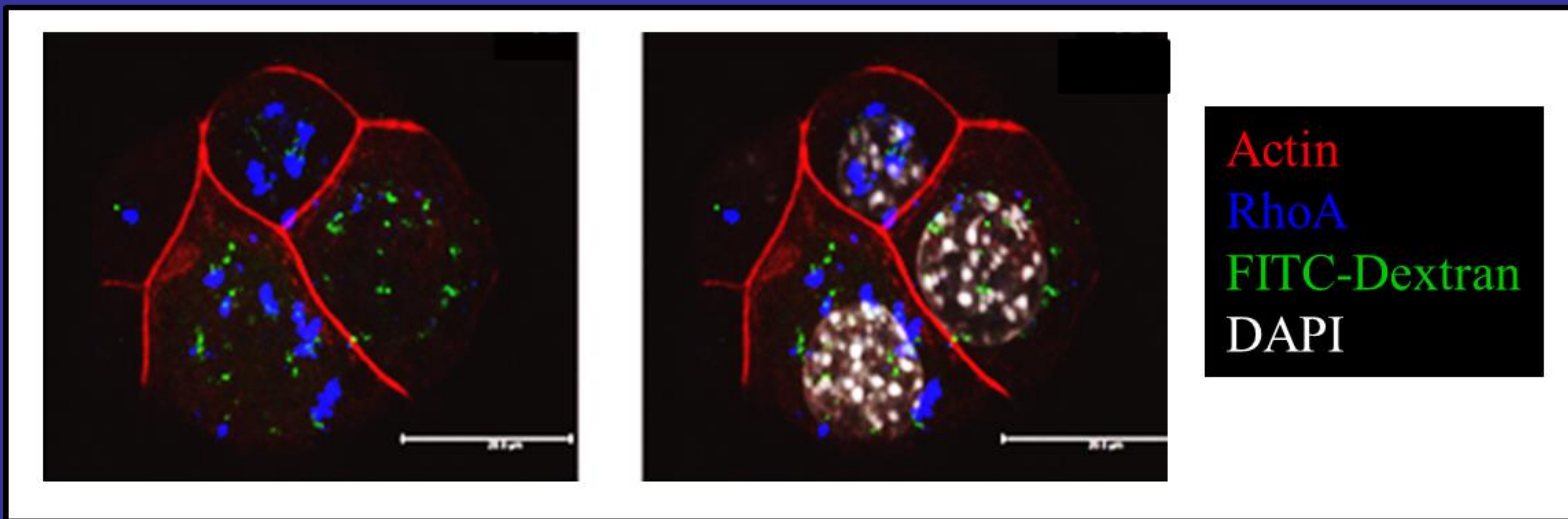
Emb-LPD:

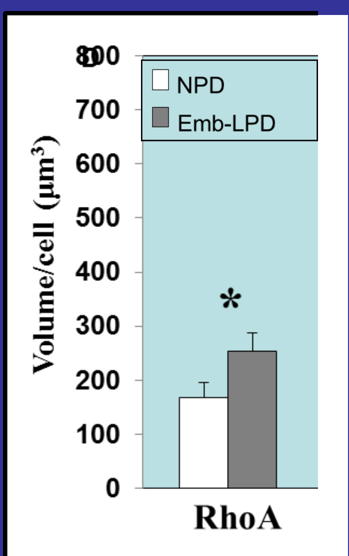
- Increased ligand (BSA-Bo-Dipy), lysosome (LysoTracker) and receptor (megalin) vesicles in TE
- Activated by Rho A GTPase and actin

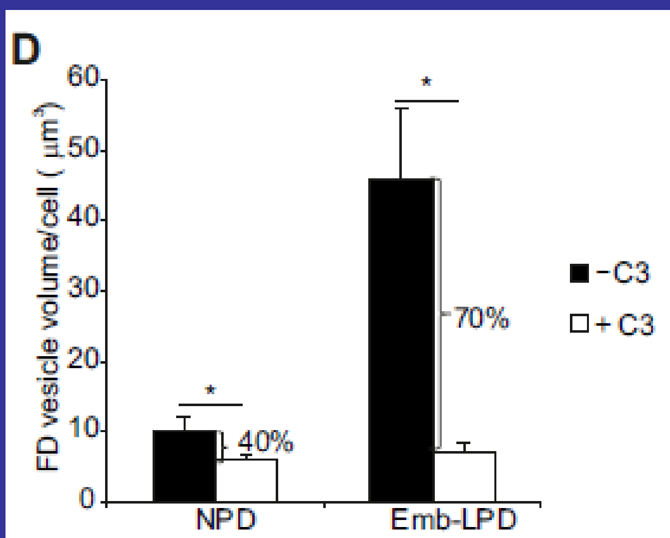
Sun et al 2014 *Development* 141: 1140-1150

How do Emb-LPD embryos activate compensatory growth?

1. Stimulate endocytosis in **trophectoderm**







Emb-LPD Blastocyst TE:

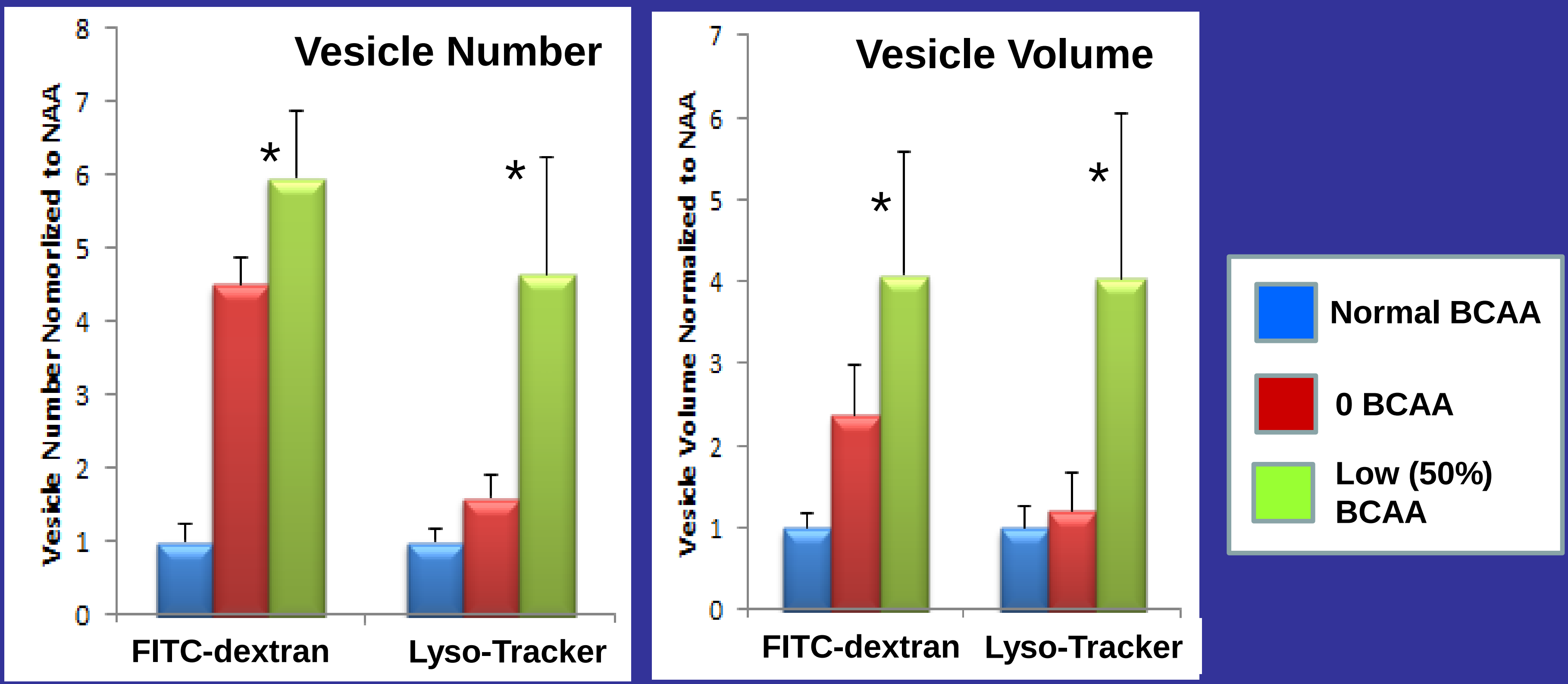
- Increased RhoA GTPase
- Rho A inhibitor C3 transferase reverses the stimulation of FD endocytosis
- RhoA modulates actin to stimulate endocytosis

Sun et al 2014 *Development* 141: 1140-1150

How do Emb-LPD embryos activate compensatory growth?

1. Stimulate endocytosis in **trophectoderm**

Activated by low levels of Branched Chain Amino Acids (BCAAs)

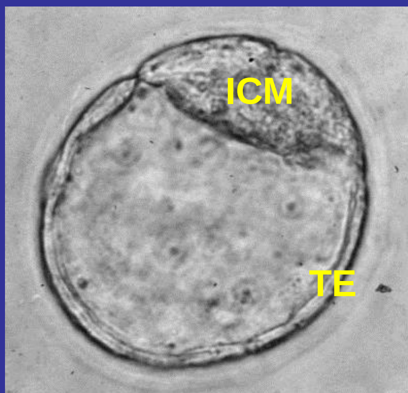


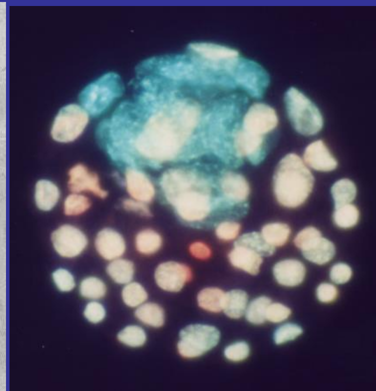
Embryo culture from 2-cell - blastocyst stage in KSOM + defined AA conc
Normal-BCAA = BCAA as in NPD Uterine Fluid (~1mM); Low-BCAA = 50%

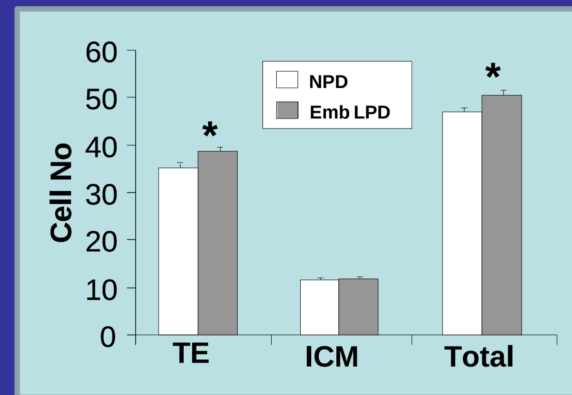
Sun et al 2014 *Development* 141: 1140-1150

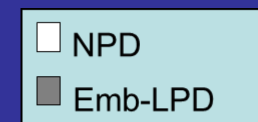
How do Emb-LPD embryos activate compensatory growth?

2. Stimulate **trophectoderm** proliferation and outgrowth motility







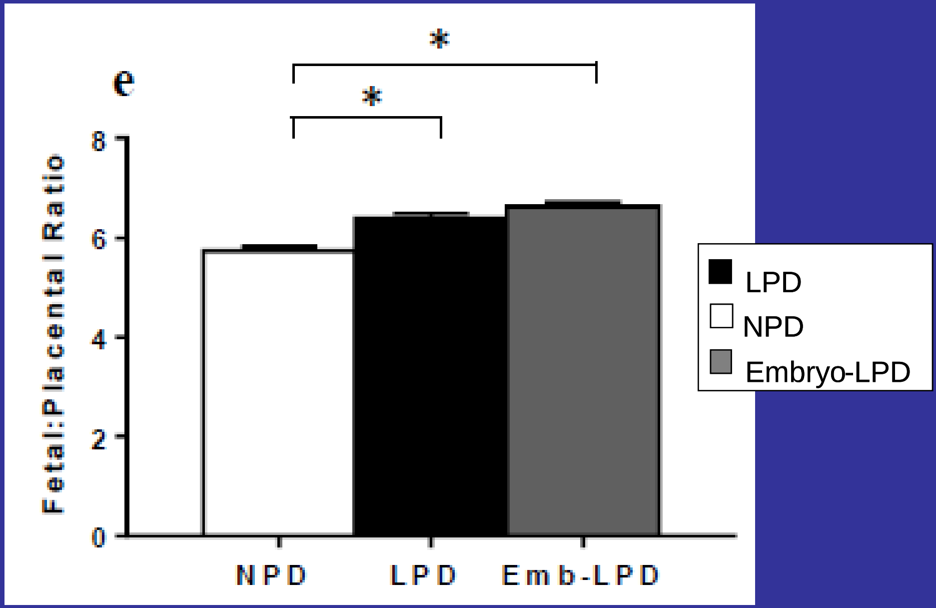


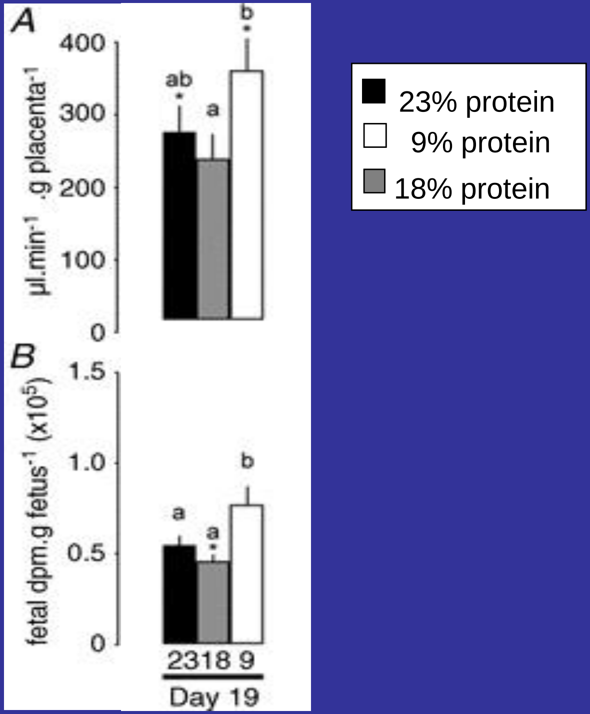
Emb-LPD increases proliferation and invasiveness of trophectoderm

Eckert et al 2012 7:e52791

How do Emb-LPD embryos activate compensatory growth?

2. Enhance efficiency and transport of placenta during later pregnancy





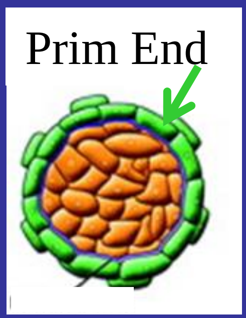
Placental efficiency:
Emb-LPD and LPD fetal/placental ratio increased in late gestation d17.5
Watkins et al, under review

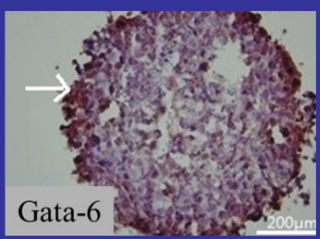
Placental transport:
LPD increased glucose transport across placenta (A) and into fetus (B); plus transporter expression
Coan et al (2011) J Physiol

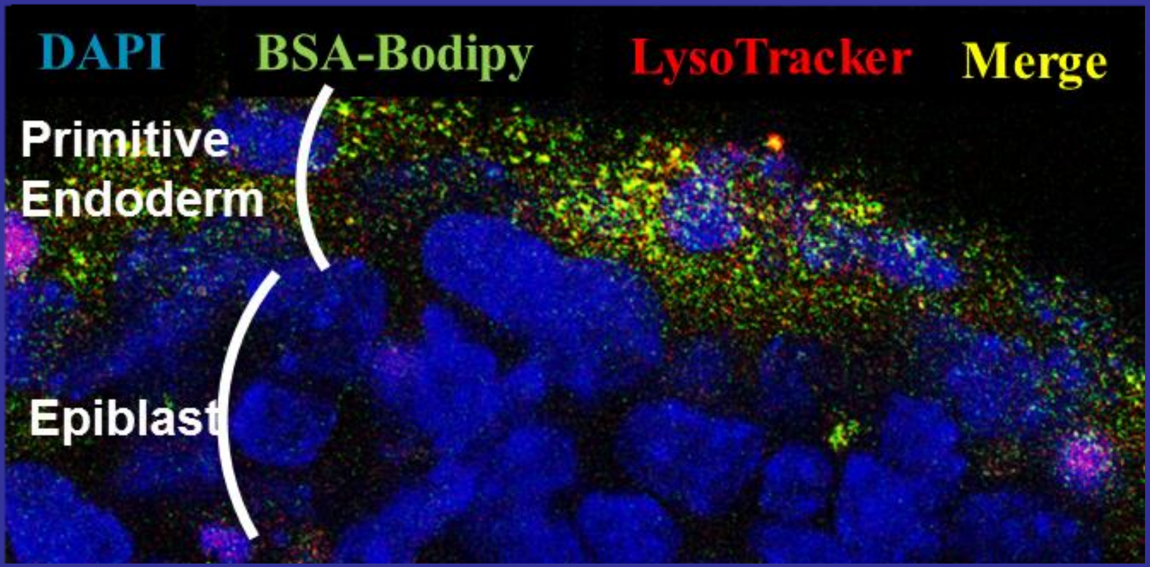
How do Emb-LPD embryos activate compensatory growth?

3. Stimulated endocytosis in **primitive endoderm** – visceral yolk sac lineage

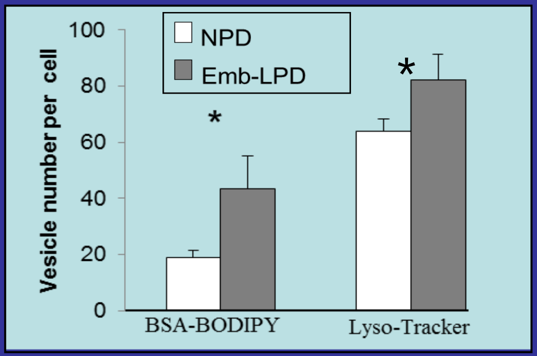
Model: Derive **embryonic stem cell lines** from Emb-LPD and NPD blastocysts
Use for embryoid body formation with outer primitive endoderm







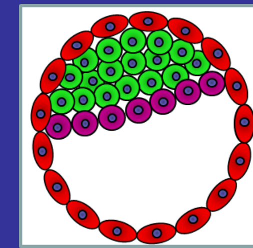
Sun et al 2014
Development
141: 1140-

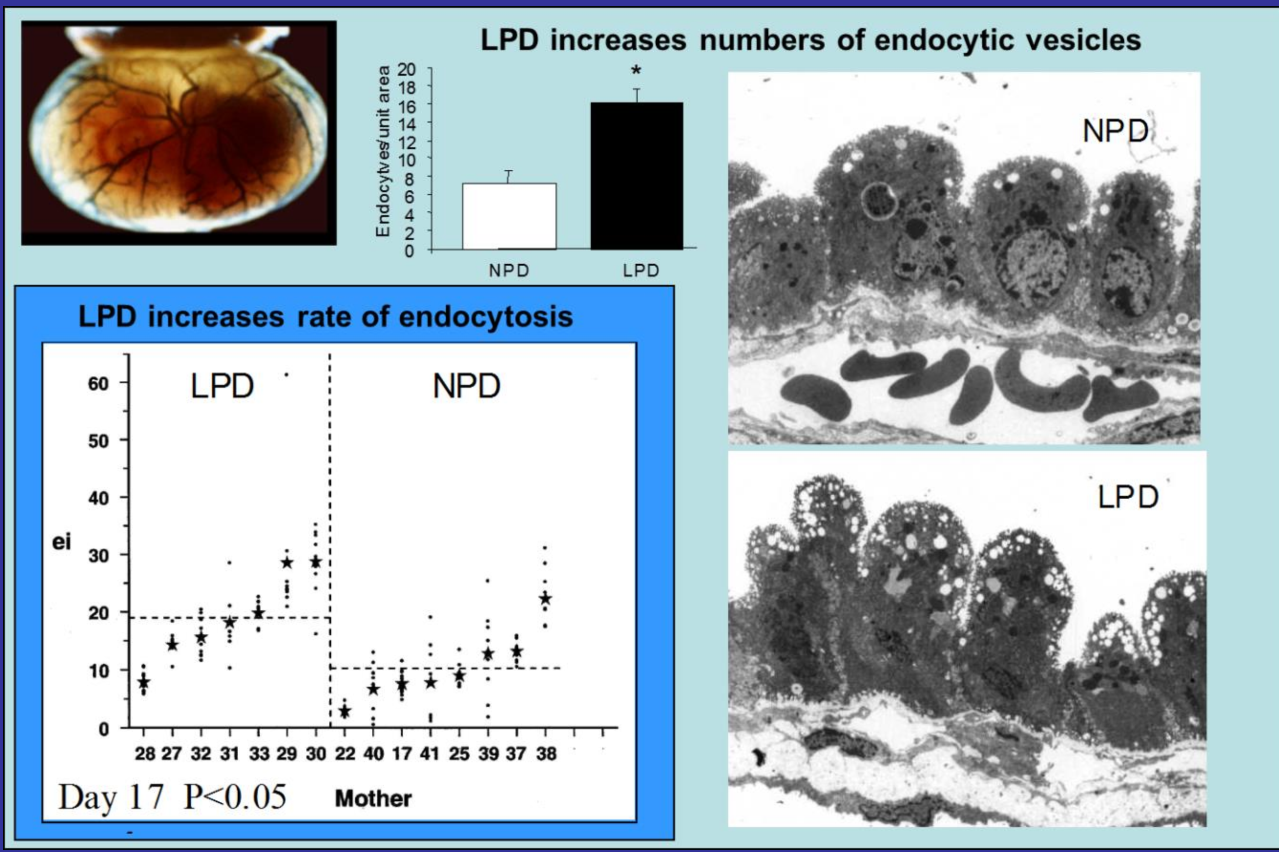


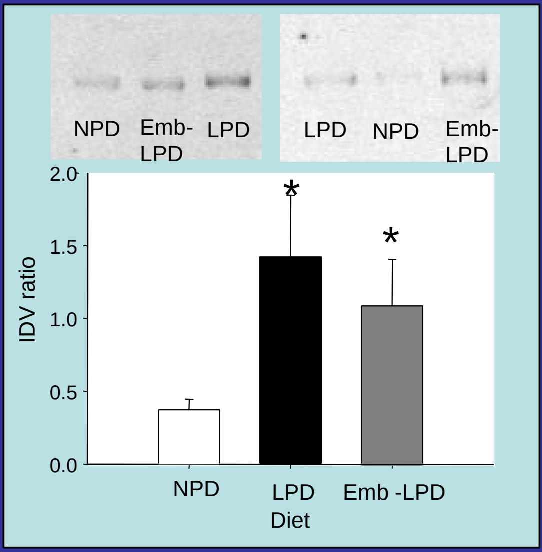
- **Primitive endoderm from Emb-LPD embryoid bodies show enhanced endocytosis – just like trophectoderm**
- **Stem cell lines show stability of induced programming - phenotype heritable over many passages in standard medium**

How do Emb-LPD embryos activate compensatory growth?

3. Stimulated endocytosis in **primitive endoderm** – visceral yolk sac lineage







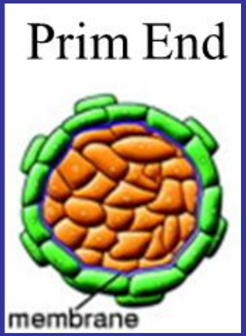
Increased endocytosis maintained throughout gestation in PE → visceral endoderm yolk sac lineage

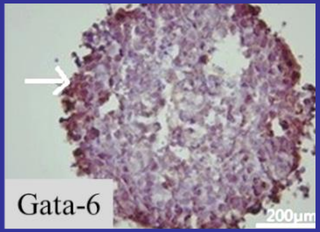
Liz Ursell in Watkins et al. (2008) BOR 78:299

Epigenetic regulation of Emb-LPD developmental programming

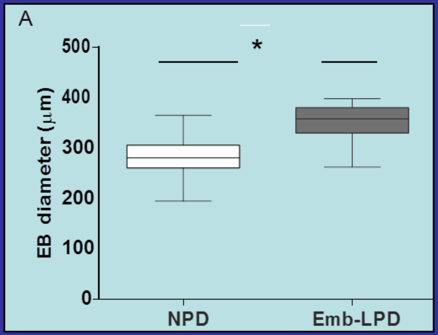
Emb-LPD stimulates growth of primitive endoderm by down-regulation of *Gata6* transcription factor expression.

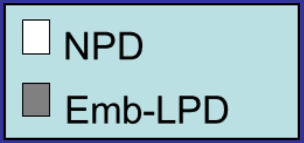
Gata6 downregulation - known mechanism of growth promotion in ovarian cancer models



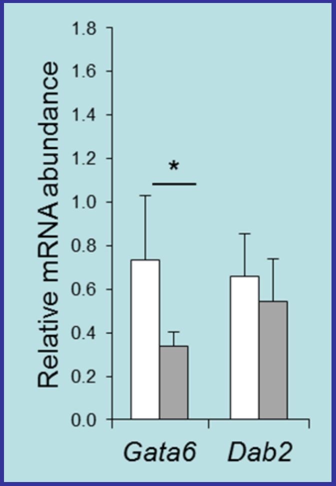


Embryoid Body Growth

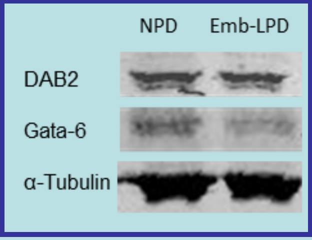


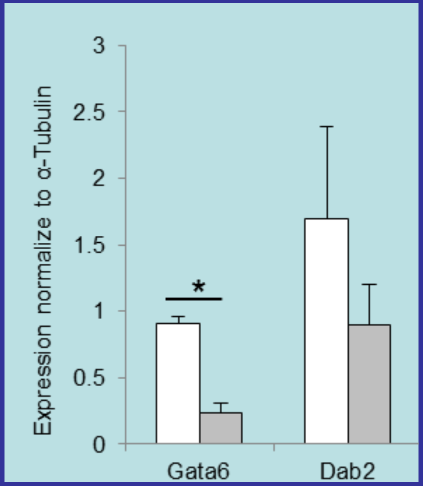


mRNA
Embryoid Bodies

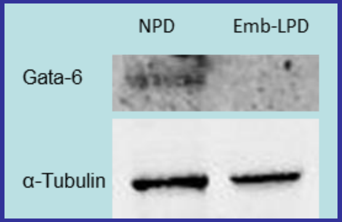


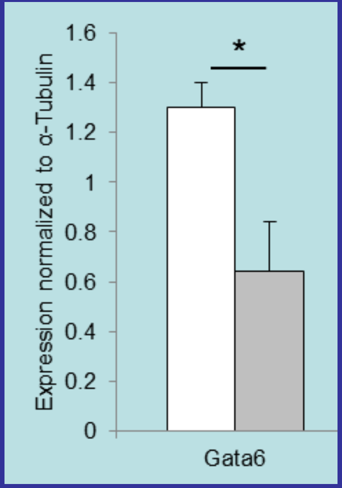
Protein
Embryoid Bodies





Protein
d17 Yolk Sacs





Sun et al 2014, under review

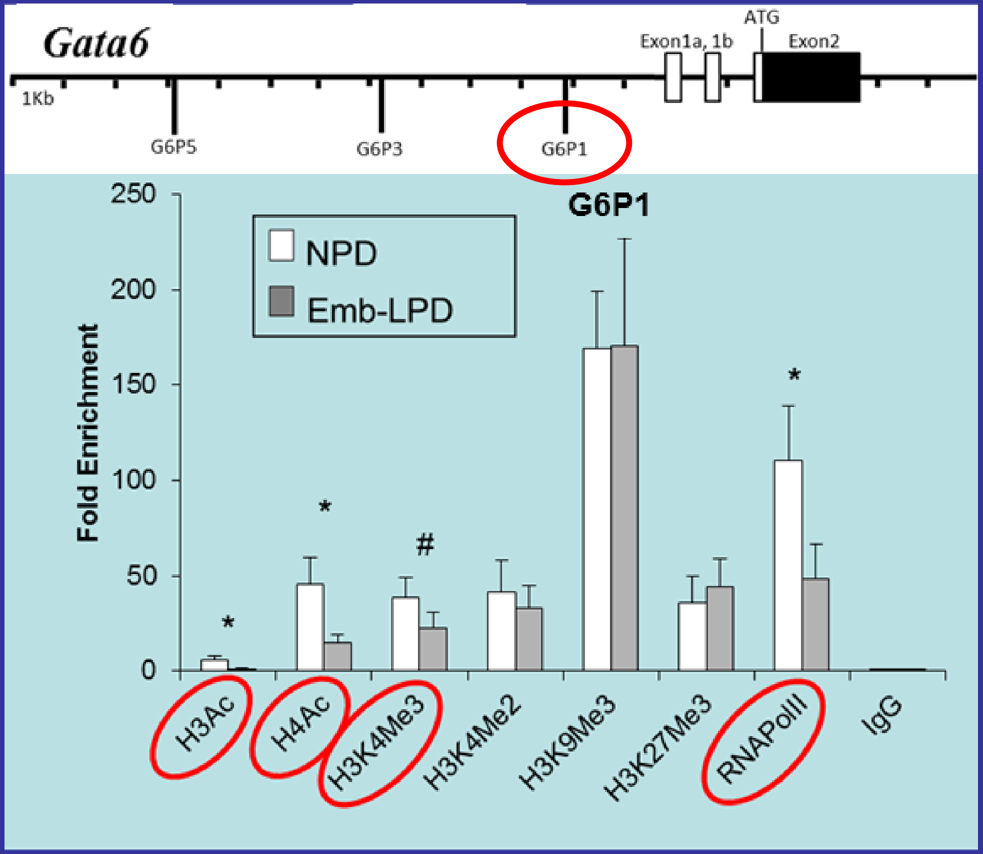
Epigenetic regulation of Emb-LPD developmental programming

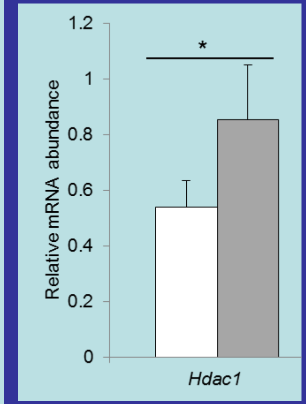
Embryoid bodies

ChIP analysis:
***Gata6* promoter G6P1**

Emb-LPD:
Reduced
H3+H4 acetylation,
H3/lysine K4 tri-methylation
and RNA Pol II binding

Emb-LPD:
Increased
HDAC1expression

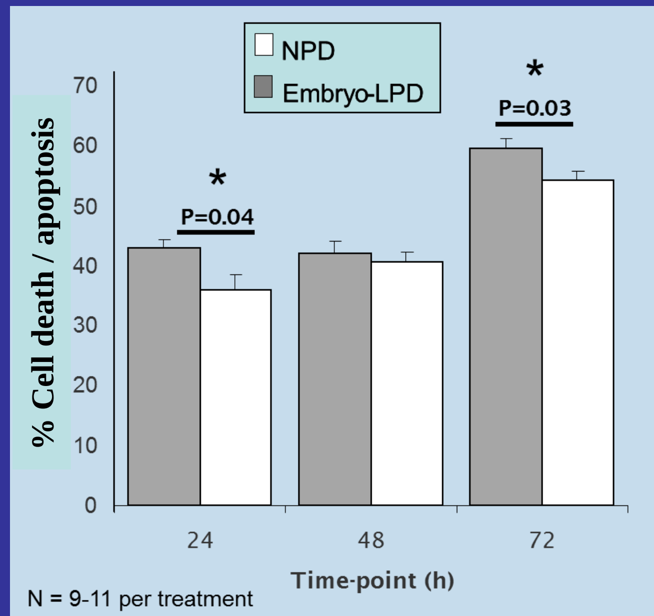




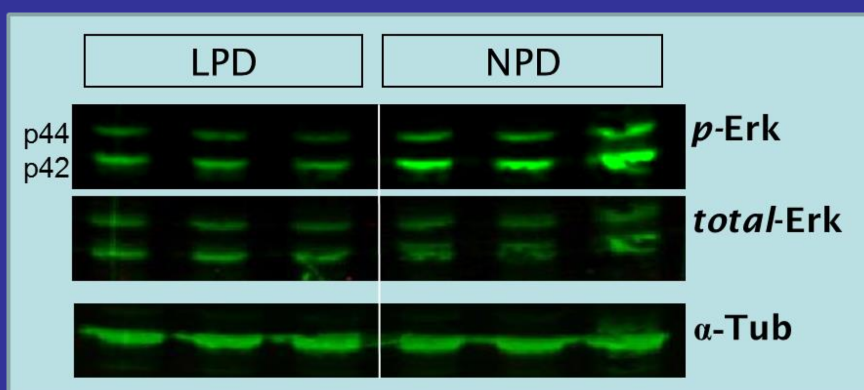
All epigenetic modifications are characteristic of <u>reduced</u> gene expression.
Modifications persist from blastocyst induction over many passages

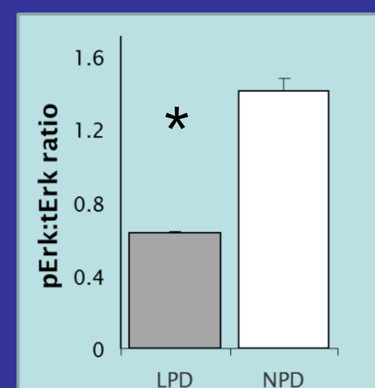
Sun et al 2014, under review

Early **embryonic** lineages: a different story
Emb-LPD reduces ES cell survival



Cell death / Apoptosis





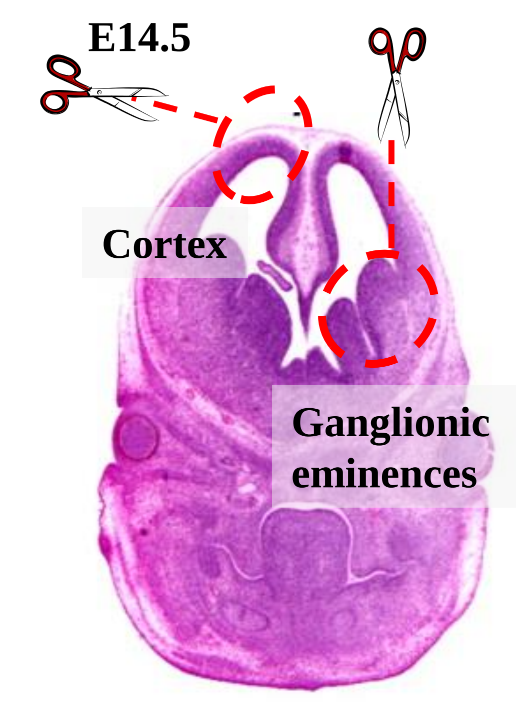
Erk1/2 signalling

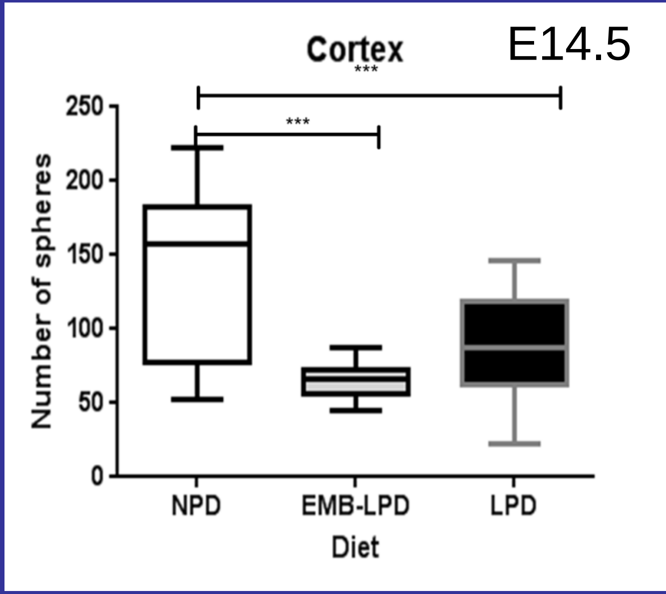
Emb-LPD increases level of apoptosis in ESCs, possibly mediated through reduced p:total ERK-1,2 survival signalling

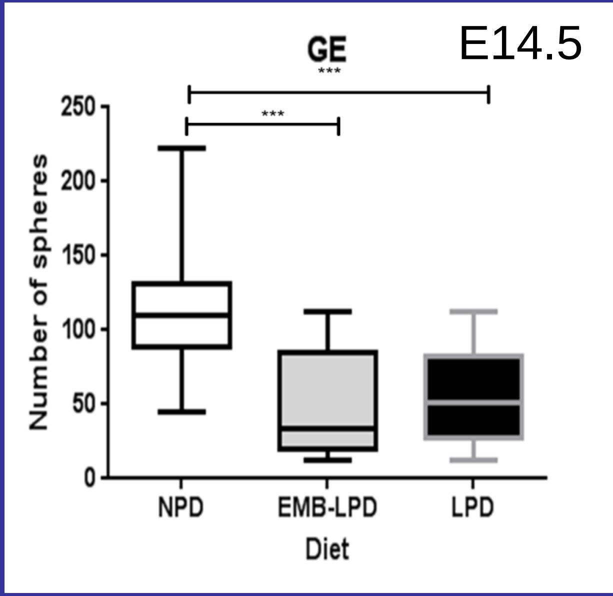
Andy Cox, Neil Smyth

LPD, neurogenesis and neural stem cell derivation

- Neural development relies on the tight regulation of **neural stem cells** (NSC) to generate the appropriate number of progeny to populate the nervous system
- Emb-LPD causes behavioural phenotype in adults







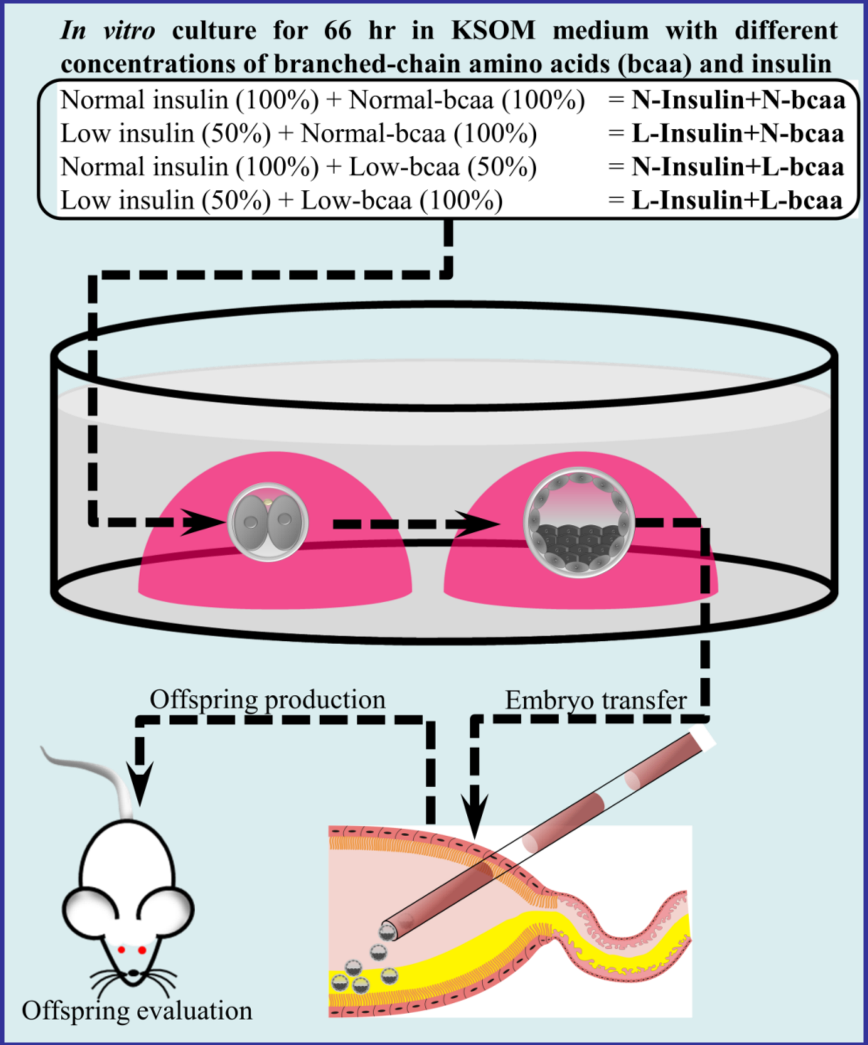
Reduced primary neurosphere formation (comprising NSCs) in cortex and ganglionic eminances in E14.5 Emb-LPD and LPD fetal brain - cultured over 7 days.

Sandrine Willaime-Morawek

Joanna Gould, Chris Airey

An in vitro model of Emb-LPD programming

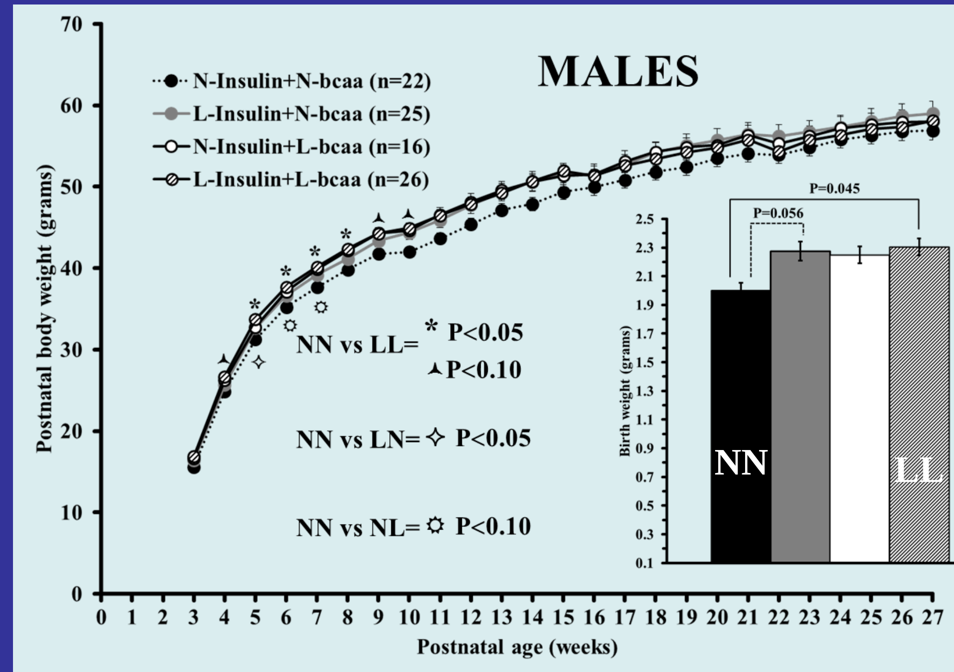
Hypothesis: Low insulin and BCAA induce blastocyst programming of adverse offspring health

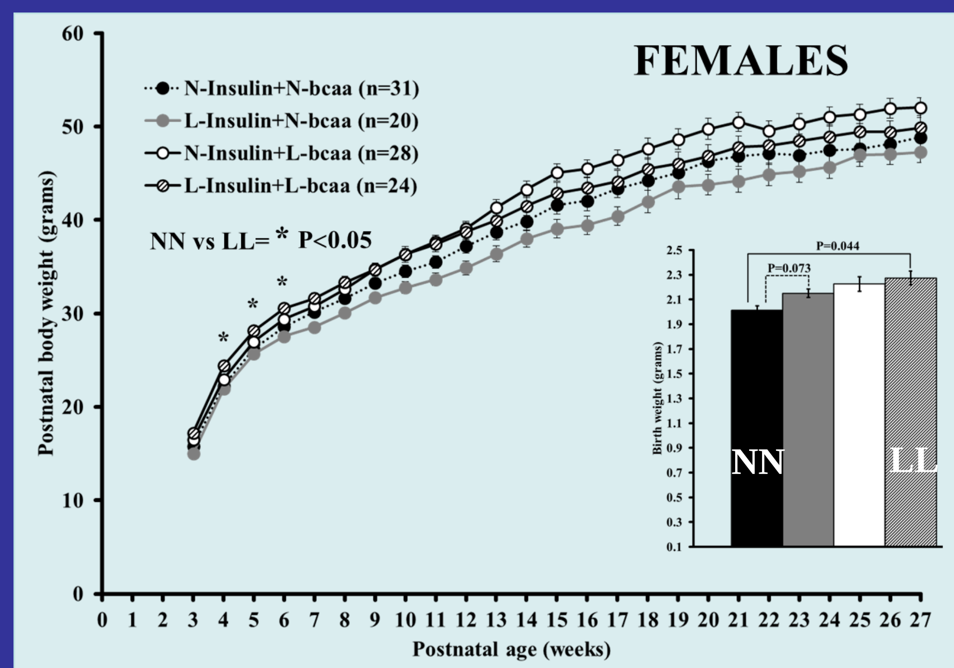


Miguel Velazquez

In vitro model of Emb-LPD programming

Low insulin and BCAA up to blastocyst stage induce increased birth weight and early postnatal growth

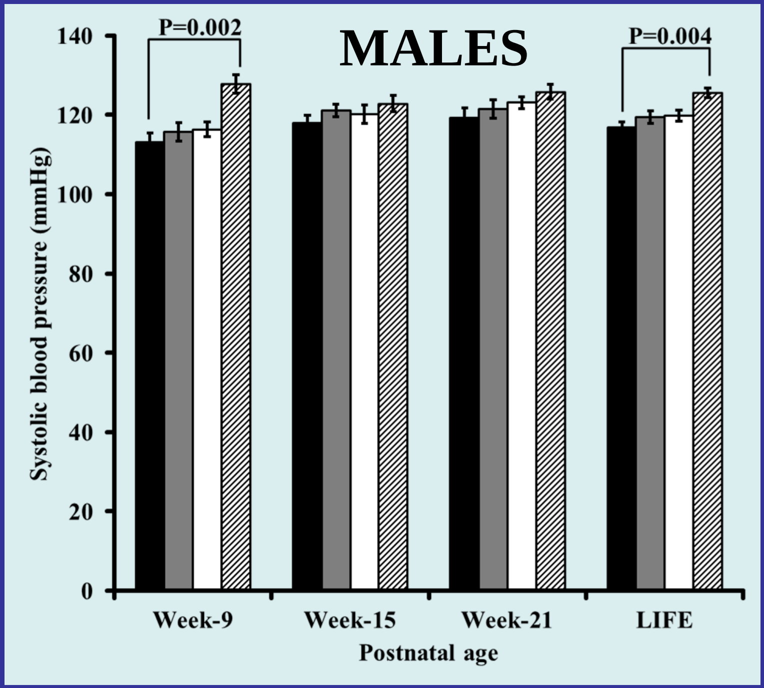


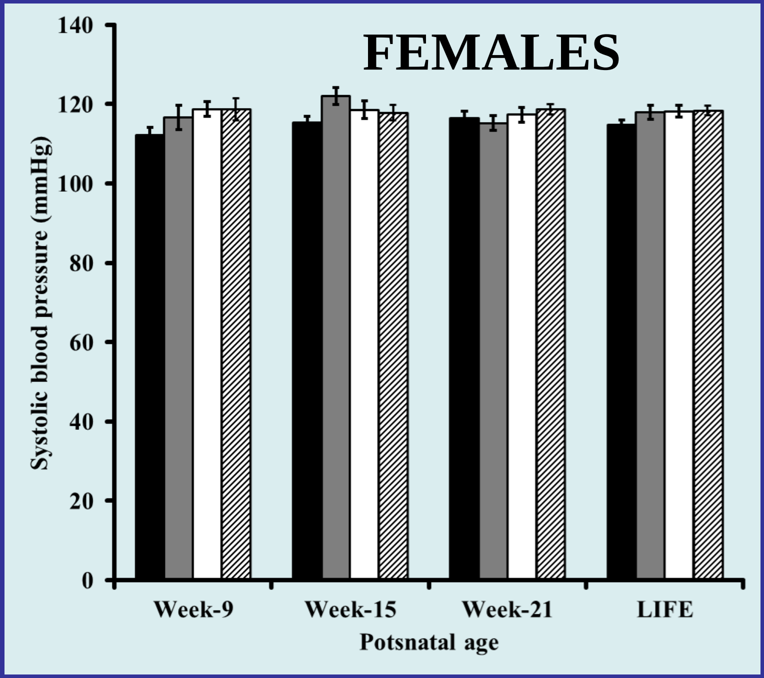


Miguel Velazquez

In vitro model of Emb-LPD programming

Low insulin and BCAA up to blastocyst stage induce increased systolic blood pressure in male offspring





Miguel Velazquez

Maternal Emb-LPD model: In Summary

- Emb-LPD leads to adult CV, metabolic, behavioural dysfunction
- Diet alters circulating nutrient levels and composition of uterine fluid
- Nutrient levels sensed by embryos (BCAA, insulin) via mTOR1 signalling
- Induction of altered developmental programming
- Activation of multiple compensatory mechanisms by blastocyst stage within extra-embryonic lineages to enhance nutrient capture during pregnancy
- Stimulate endocytosis in trophectoderm and primitive endoderm
- Stimulate trophectoderm cell proliferation and invasiveness
- Compromised survival and increased apoptosis within early embryonic lineages and stem cells
- Correlation between perinatal compensatory growth and later disease
- **Combination of epigenetic, cell biological and physiological changes underlie periconceptional developmental programming**

Periconceptional Environment

DOHaD

In vitro culture

Developmental plasticity

Maternal obesity

Maternal sickness

Maternal diet

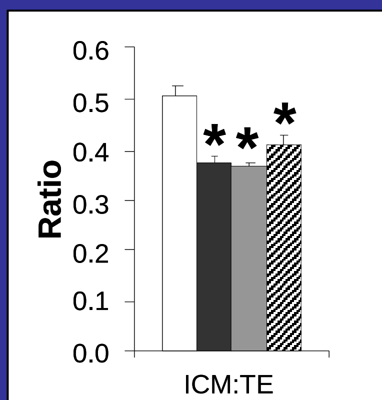
Protein rich

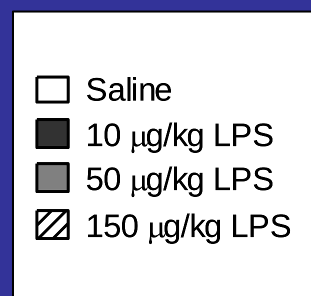
Protein deficient

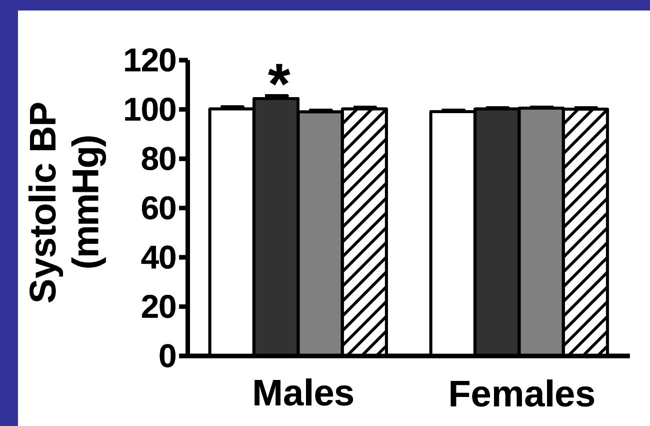
High fat

Maternal sickness at conception affects development and health into adulthood

- Induce mouse maternal sickness and inflammatory response
  - ➢ Bacterial LPS i.p. injection on Day 1 (zygote)
  - ➢ 10, 50 or 150 μg/kg *Salmonella enterica enteritidis* LPS or saline control







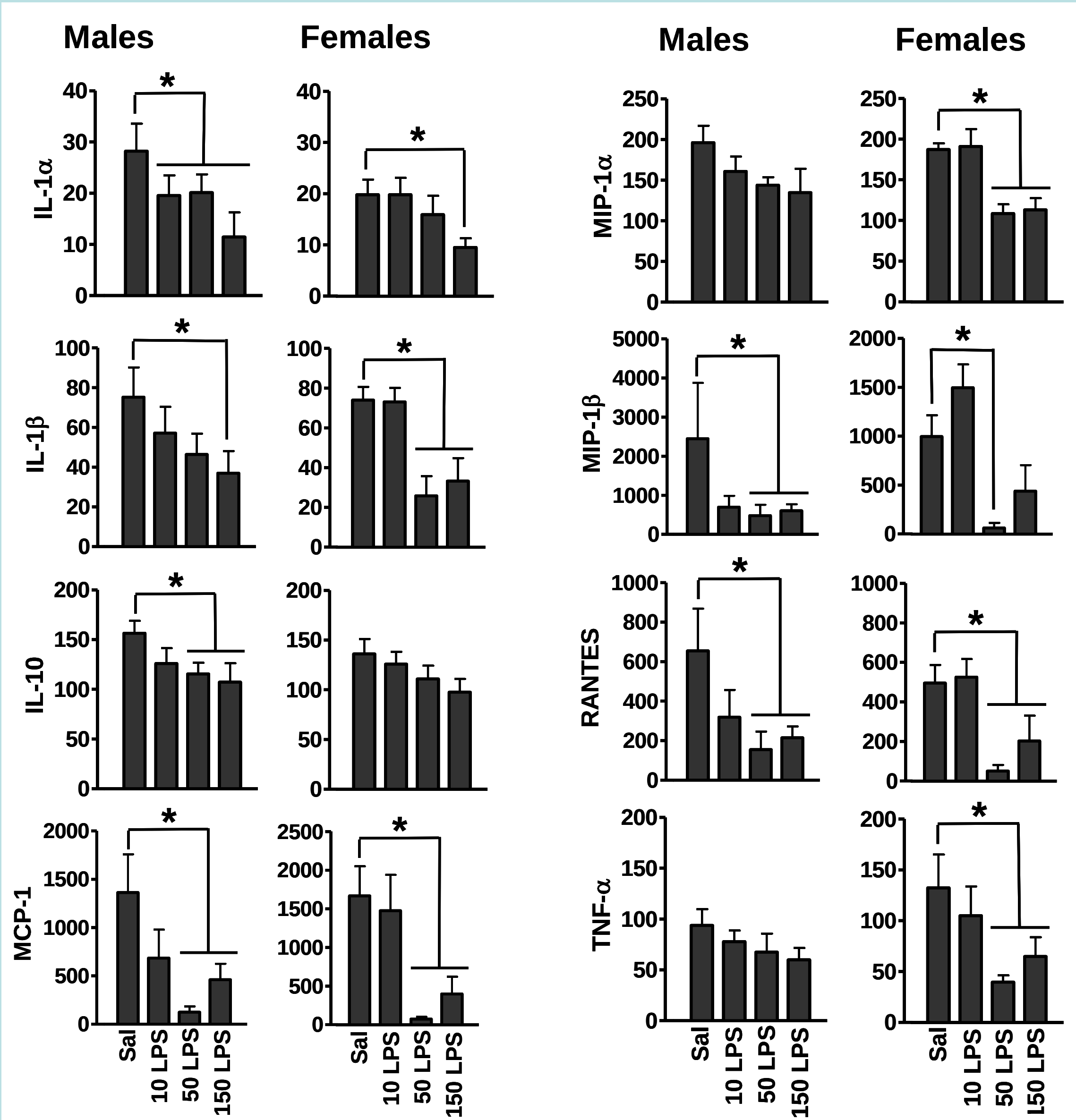
Blastocyst ICM:TE reduced
Fewer ICM cells

Blood pressure normal
Postnatal growth normal

Distinct phenotype from LPD model

Williams et al, 2011 BMC Biology 9:49

Systemic maternal LPS at zygote stage – offspring effects



Adult offspring:

- **Blunted cytokine responsiveness to LPS**
- Dose-dependent with respect to maternal LPS concentration
- Evidence of adult innate immunity being programmed from periconceptional maternal environment
- Is immune suppression an adaptive response to enhance postnatal tolerance within anticipated pathogen-rich environment?

Williams et al, 2011
BMC Biology 9:49

Thanks!

<u>Embryo Laboratory</u>

Andy Cox
Emma Lucas
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Liz Ursell
Miguel Velazquez
Adam Watkins
Charlotte Williams

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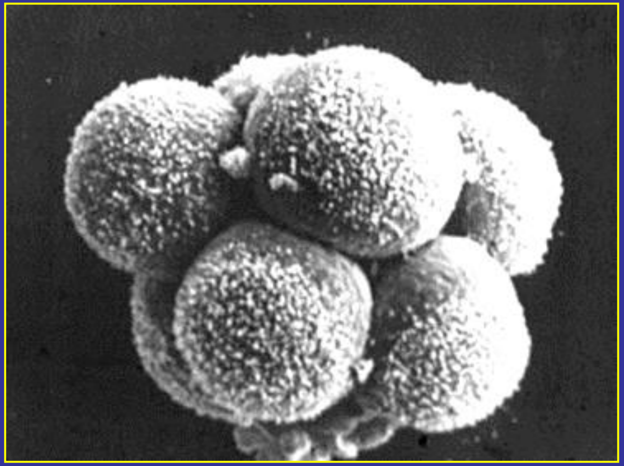
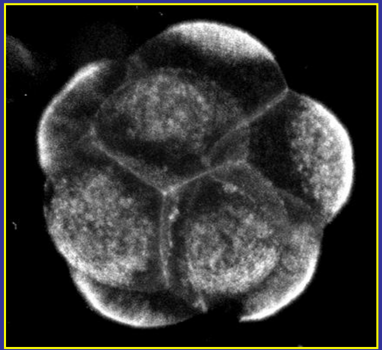
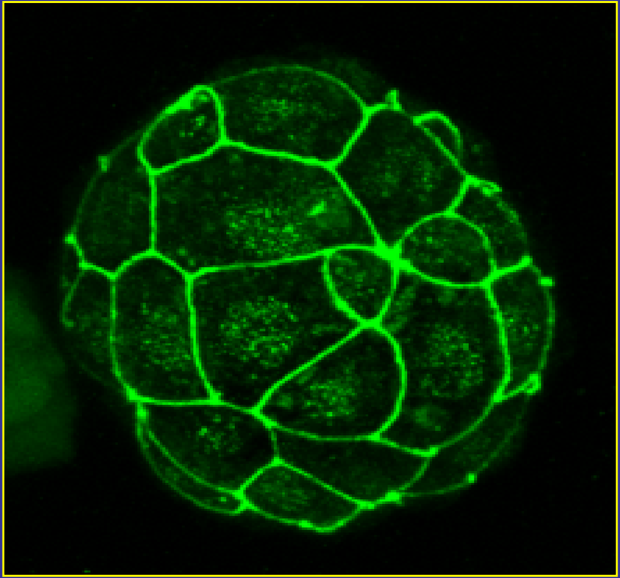
BBSRC bioscience for the future
cost
MRC Medical Research Council
SEVENTH FRAMEWORK PROGRAMME
NATIONAL INSTITUTES OF HEALTH
GK
EpiHealthNet







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