Assessment of Carbohydrate and Amino Acid Metabolism in Oocytes/Embryos

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Metabolism/Nutrition Profiling

- **Ovarian follicles**
- **Oocytes (immature and mature)**
- **Embryos**

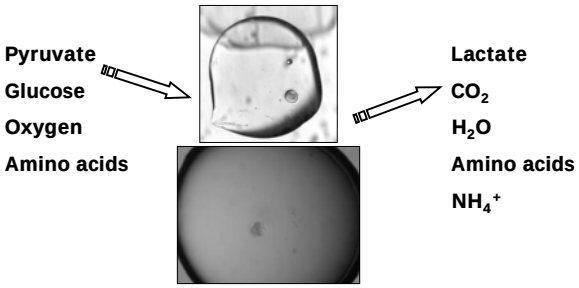
Metabolic Bio-markers of Quality

- **Carbohydrates, amino acids, oxygen.**
- **More complete understanding of metabolism profiles of follicles, oocytes and embryos in healthy and disease states, *in vitro* and *in vivo*.**
- **Non-invasive metabolic bio-markers are potentially useful to assist selection of embryos produced by ART.**
- **Metabolism profiling: identification of potential bio-markers.**
  - **Follicle: *in vitro* growth**
  - **Oocyte: *in vitro* maturation**
  - **Embryo: *in vitro* culture**

Metabolic Bio-markers of quality

- Several studies.
- Useful research tool.
- Associations of metabolic and biochemical markers with:
  - Meiotic maturation
  - Fertilisation potential
  - Embryo development: slow, arrested
  - Live birth rate
  - Chromosome abnormalities

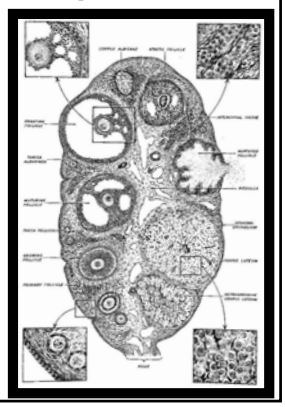
Non-invasive metabolism analysis



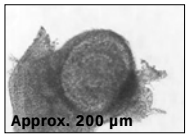
- Prospect of long-term follicle culture for production of oocytes:
  - Cancer patients – unsuitable to re-graft potentially malignant tissue
  - Source of oocytes for egg donation therapy
  - Source of oocytes for SCNT, stem cell production
- Metabolism:
  - Basic biology – involvement in selection processes?
  - Optimisation of culture protocols
  - Non-invasive bio-markers of quality?

Ovarian follicle development

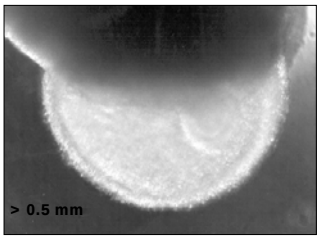
- Complete follicle growth requires several months (approx. 4-5 weeks: mouse; approx. 6 months: human).
- Changing nutritional and metabolic needs of growing follicle and oocyte.
- Follicle metabolism is influenced by signals from the oocyte.
- Oocyte maturation is regulated by cumulus metabolism.



Long-term follicle culture



IVG Day 0.

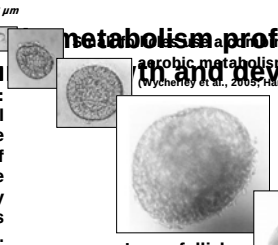


IVG Day 18.

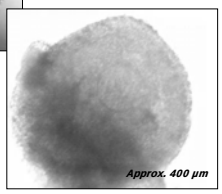
Possible to culture follicles for 30 or more days

Follicle metabolism profiles change during growth and development

Diffusion of nutrients across small distances: primordial follicles utilise a variety of carbohydrate energy substrates (Harris, 2002).



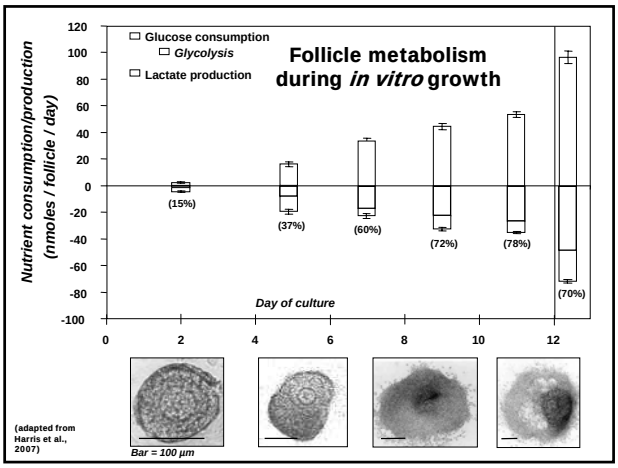
Large follicles become almost totally reliant on glycolytic glucose consumption (Boland et al., 1994; Harris et al., 2007).



Primordial – Graafian follicle

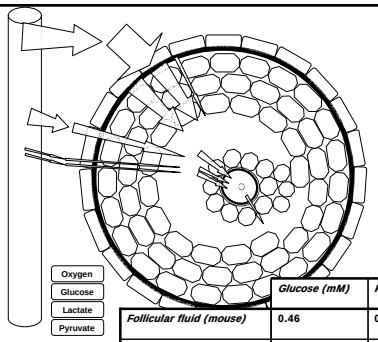
Mouse: >30,000 –fold increase in volume.

Human: >91,000,000 –fold increase in volume.



What pushes the metabolic switch?

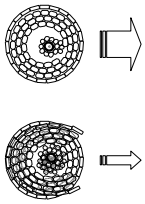
- **Small follicles use a combination of different pathways to metabolise glucose (glycolysis and aerobic metabolism). Sufficient oxygen reaches the oocyte.**
- **As follicles get bigger, diffusion of nutrients and oxygen to the centre becomes limited** (Gosden & Byatt-Smith, 1986).
- **To compensate, the follicle develops an antrum, increasing the effective surface area for diffusion over the follicle surface.**
- **The oocyte secretes factors (including BMP15 and FGFs) which promote follicular glycolysis** (Sugiura et al., 2005).
- **Oxygen is able to diffuse to the follicle core.**



	Glucose (mM)	*Pyruvate (mM)*	*L-Lactate (mM)*
Follicular fluid (mouse)	0.46	0.38	17.3
Plasma (mouse)	11.7	0.16	4.8
Follicular fluid (human)	3	0.26	6.06
Plasma (human)	5	0.1	0.6

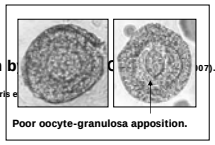
Adapted from: Harris & Picton, 2007

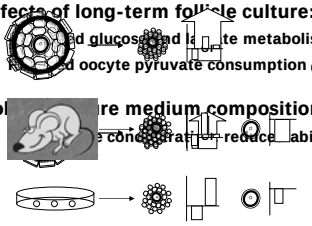
- ***In vitro*** **growth of follicles: useful time period over which to assess developmental characteristics:**
  - **Follicle lactate production during growth *in vitro* is higher by follicles not destined to ovulate** (Harris et al., 2007).



Culture environment influences follicle, oocyte and cumulus metabolism

- **Quality of follicles cultured long-term influences metabolism of the mature mouse oocyte-cumulus complex** (Harris et al., 2007).
  - **Reduced follicle theca coverage: increased glycolytic index in mature OCCs (cell stress).**
  - **Reduced follicle oocyte-granulosa apposition: reduced lactate metabolism by mature OCCs.**
- **Effects of long-term follicle culture:**
  - **Increased glucose and lactate metabolism by mouse OCCs** (Harris et al., 2007).
  - **Reduced oocyte pyruvate consumption** (Harris et al., 2007).
- **Follicle culture medium composition:**
  - **Low glucose concentration: reduced ability for follicle steroidogenesis**





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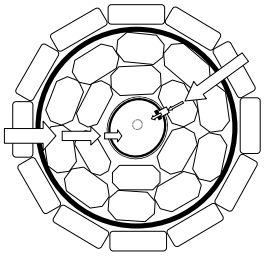
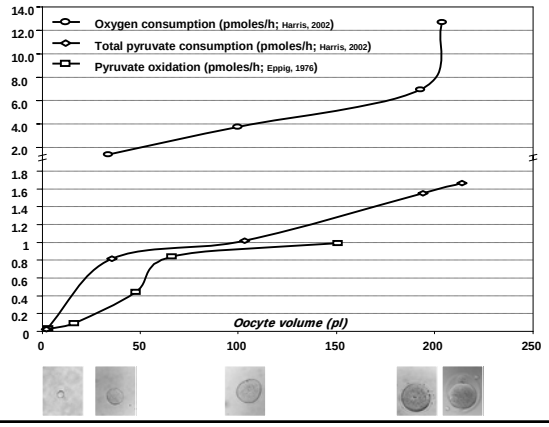
- **Follicle culture medium composition:**
  - **Low glucose concentration: reduced ability for follicle steroidogenesis** (Boland et al., 1994).

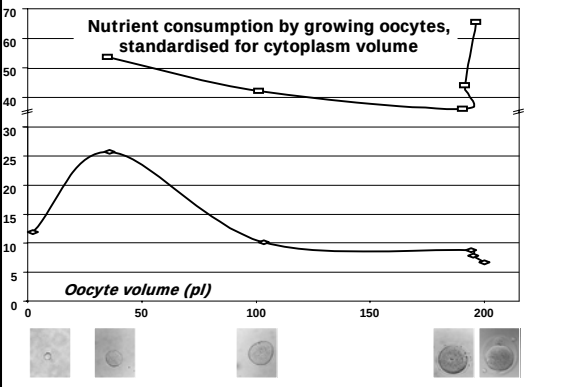
Oocyte Metabolism and Nutrition

- **During oogenesis, the oocyte obtains metabolites by direct uptake of metabolites from interstitial fluid / follicular fluid.**
- **Metabolites and molecules can also pass directly to the oocyte via gap junctions with granulosa / cumulus cells.**

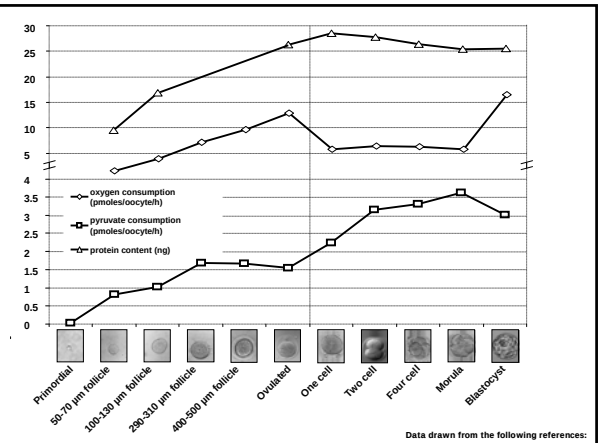


Nutrient consumption by growing mouse oocytes



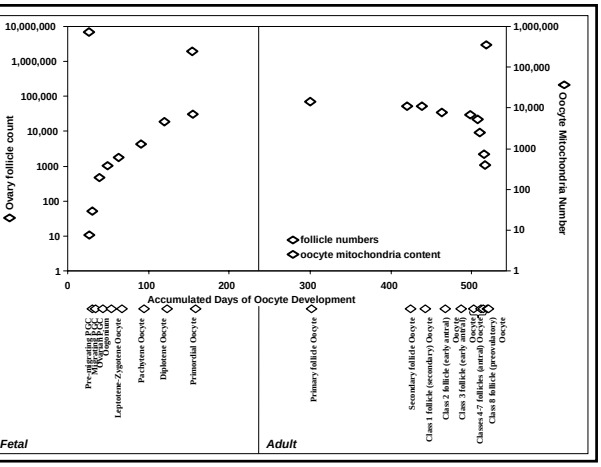


Adapted from Harris, 2002



Mitochondria: role in oocyte quality

- **Oocyte and preimplantation embryo metabolism: oxidative.**
- **Requirement for adequate cohort of good quality mitochondria.**
- **Bottleneck:**
  - **Mitochondria Restriction: expansion of a very small cohort of mitochondria.**
  - **mtDNA Segregation: expansion of a subset of segregated mitochondria.**

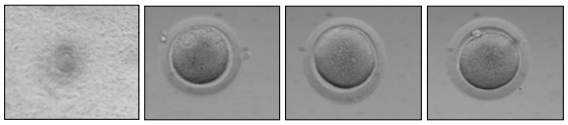


Mitochondria quality

- Accumulation of mtDNA mutations (Hsieh et al., 2002).
- Mitochondria activity (Thouas et al., 2004).
- Spindle assembly (Wilding et al., 2003; Eichenlaub-Ritter et al., 2003) – aneuploidy risk.
- Mitochondria morphology (Heng-Kien et al., 2005).
- MtDNA gene expression (Hsieh et al., 2004).
- Mitochondria density (May-Panloup et al., 2005; Santos et al., 2005).
- Repeated ovarian stimulation associated with mitochondria mutations in mouse oocytes (Chao et al., 2005).
  - Ageing (Wilding et al., 2001).
  - Ovarian failure (May-Panloup et al., 2005).
  - Diabetes, insulin resistance (Sheratt et al., 1999; Kyu-Lee et al., 2005; Maassen et al., 2005).
  - Cryopreservation (Jones et al., 2004).
- Identification of markers of mitochondria quality would be beneficial!

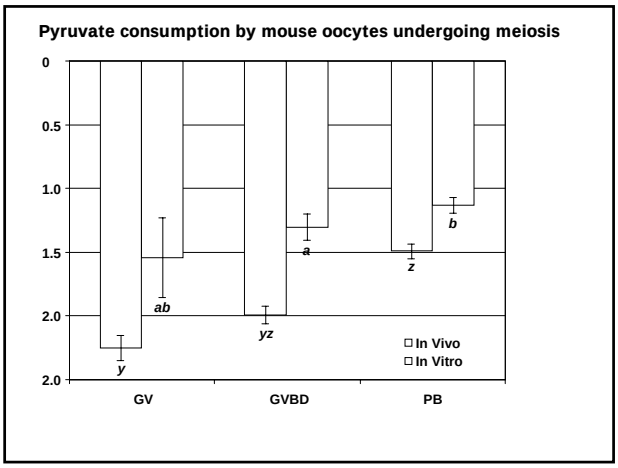
- Metabolic profiling: identification of possible bio-markers of quality:
  - Follicle: *in vitro* growth
  - Oocyte: *in vitro* maturation
  - Embryo: *in vitro* culture

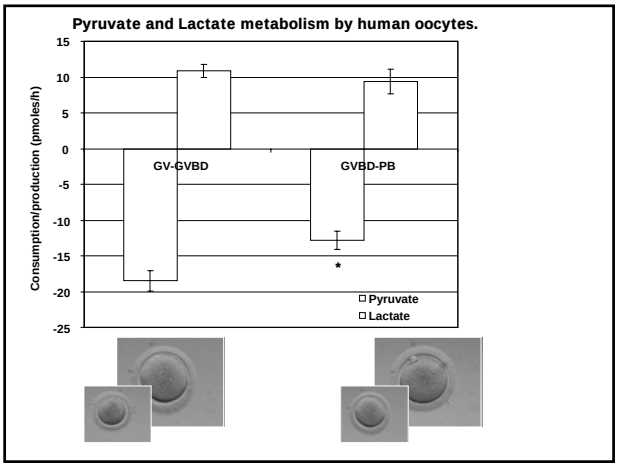
Oocyte meiotic maturation



Metabolism during meiotic maturation

- FSH stimulates PI3-K pathway. Translocation of GLUT-4 (Roberts et al., 2004).
- Ovulation induction stimulates glucose metabolism by the follicle (Boland et al., 1994; Harris et al., 2007) and cumulus cells (Downs & Utecht, 1999).
- More glucose diverted to the pentose phosphate pathway (Downs & Utecht, 1999).
- Generation of purine precursors.
- Glucose hyaluronic acid synthesis – cumulus expansion (Sutton-McDowell et al., 2004).
- Mouse oocyte pyruvate consumption rate changes during meiotic maturation (Downs et al., 2002).

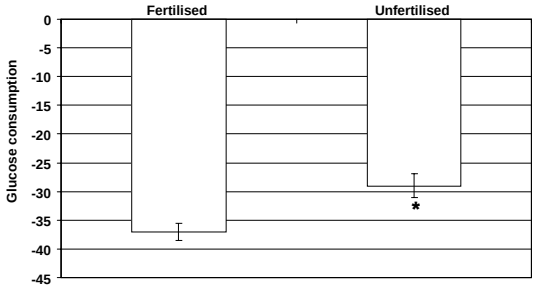




Metabolic markers during IVM

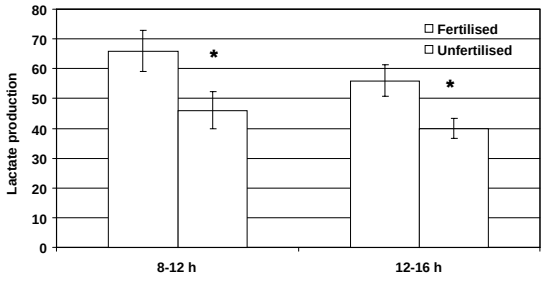
- **IVM – time window for metabolism analysis.**
- **Human oocytes competent to mature to MII have greater glutamine consumption** (Vyjayanthi et al., 2007)
- **Human oocytes with intermediate pyruvate consumption more likely to implant** (Turner et al., 1994)
- **Higher rates of mouse OCC glucose and lactate metabolism during maturation associated with oocyte fertilisation** (Preis et al., 2005)

Glucose consumption by mouse OCCs during hours 8-12 of 16 h IVM



Preis et al., 2005

Lactate production by mouse OCCs during hours 8-12 and 12-16 of 16 h IVM

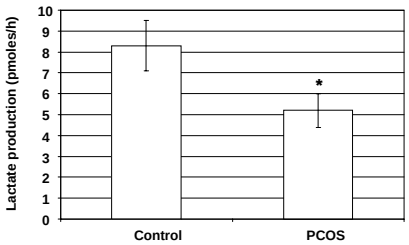


Preis et al., 2005

Metabolic disturbances during IVM

- **Reduction in mouse cumulus PPP glucose metabolism in diabetes – reduced oocyte maturation rate** (Colton et al., 2003)
- **Altered oocyte carbohydrate metabolism in polycystic ovary syndrome (PCOS)** (Harris et al., in prep.)

Lactate production by control and PCOS oocytes undergoing PB formation.



Oocytes progressing to MII.

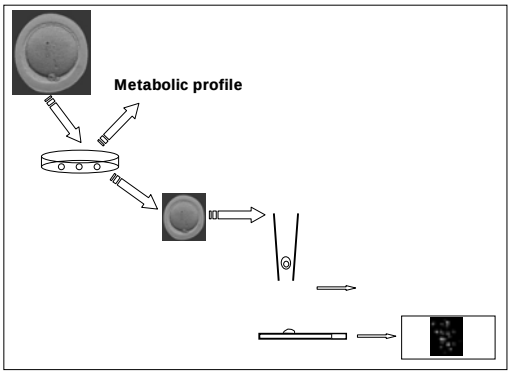
Harris et al., in prep.

- **Altered metabolism observed in oocytes from patients with different aetiologies (e.g. PCOS).**
- **Focus on certain patient groups?**
- **Is metabolism disturbed in other cases?**
- **Chromosome abnormality?**

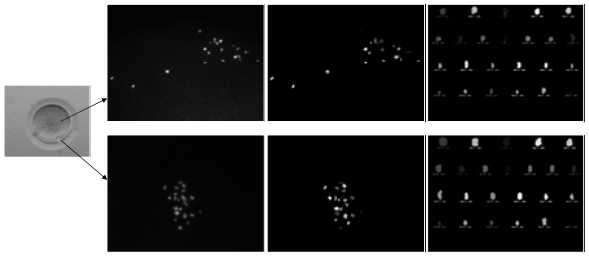
Oocyte chromosome abnormalities

- **High proportion of mature human oocytes harbour chromosome abnormalities.**
- **Arise from errors in meiosis I:**
  - **Nondisjunction**
  - **Premature Separation of Sister Chromatids (PSSC).**
- **Aneuploidy in embryo.**
- **Chromosome segregation and polar body formation are energy-dependent.**
- **Age-related increase in chromosome abnormalities.**
- **Associated with age-related decrease in mitochondria quality? (Schon et al., 2000).**
- **Non-invasive markers to predict chromosome abnormality?**

Investigation of oocyte chromosome abnormalities

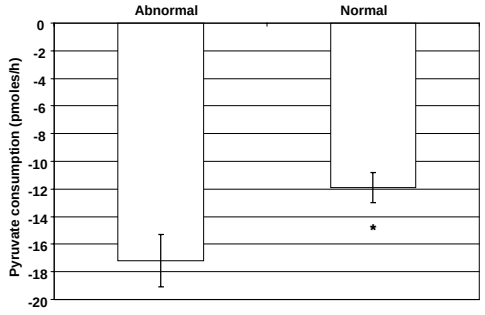


Oocyte karyotype assessed by WCP-mFISH



Chromosome abnormalities

Pyruvate consumption by oocyte with abnormal and normal karyotypes

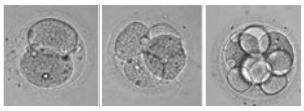


Markers?

- **FF oxygen content** (Van Blerkom et al., 1998)
- **Follicular vascularity** (Van Blerkom et al., 1998)
  - **Embryo chromosome abnormalities.**

- **Metabolic profiling: identification of possible bio-markers of quality:**
  - **Follicle: *in vitro* growth**
  - **Oocyte: *in vitro* maturation**
  - **Embryo: *in vitro* culture**

Embryo culture



Culture environment influences oocyte, cumulus and embryo metabolism and developmental capacity

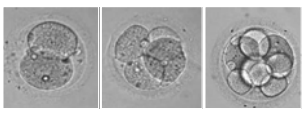
- **Embryo culture medium nutrient composition affects:**
  - **Embryo metabolism: cell stress responses are alleviated by using more physiological culture medium** (Gardner and Leese, 1990; Gardner and Sakkas, 1993).
  - **Blastocyst formation** (Rose-Hellekant et al., 1998).
  - **Cell number** (Van Soom et al., 1996).
  - **Implantation rate** (Barak et al., 1998).

Metabolic markers of embryo development

- **Pyruvate metabolism associated with:**
  - **Human embryo implantation rate** (Conaghan et al., 1993).
  - **Human embryo development, polyspermy, parthenogenesis** (Hardy et al., 1989).
- **Mouse blastocyst glycolytic profile and glucose consumption associated with pregnancy outcome** (Lane & Gardner, 1996).
- **Mitochondria activity correlated with glutamine turnover** (Harris et al., in prep.).
- **Too much variation to be used reliably.**

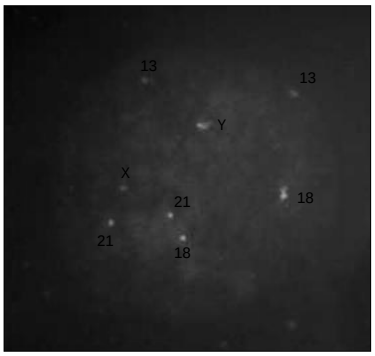
Biomarkers: Embryo Metabolism

- **Net turnover of certain amino acids (Alanine, Arginine, Glutamine, Methionine, Asparagine & Leucine) by cleavage stage embryos (day 2-3) predicts blastocyst development *in vitro*** (Houghton et al., 2002).
- **Turnover of 3 key amino acids (Asparagine, Glycine & Leucine) on day 1-2 was significantly correlated with pregnancy and live birth (data from 52 patients)** (Brison et al., 2004).



Embryo Chromosome Abnormalities

- **Up to 50% of embryos produced *in vitro* have chromosomal abnormalities, including: aneuploidy and mosaicism.**
- **13, 18, 21, X and Y are responsible for 65% of embryo chromosome abnormalities and >95% of chromosome aberrations in live births.**
- **Chromosome abnormalities cause approx. 50% of embryo wastage before implantation.**
- **Embryo chromosome errors can be introduced during:**
  - ***Oogenesis*** **(in cases of gonadal mosaicism)**
  - ***Meiosis*** **(segregation errors, non-disjuncion, premature chromatid separation)**
  - ***Embryo cleavage divisions*** **(failed mitotic checkpoints)**
  - ***Maternal/Paternal balanced translocations*** **causing germ cell unbalanced translocations**

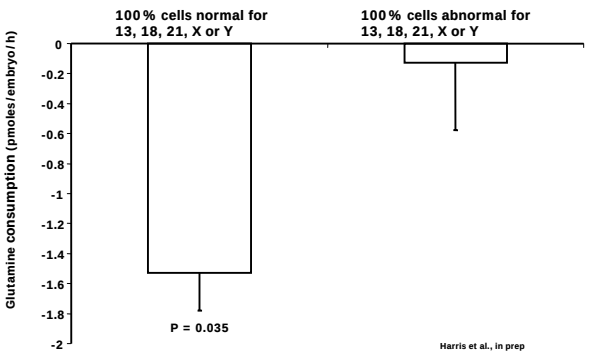


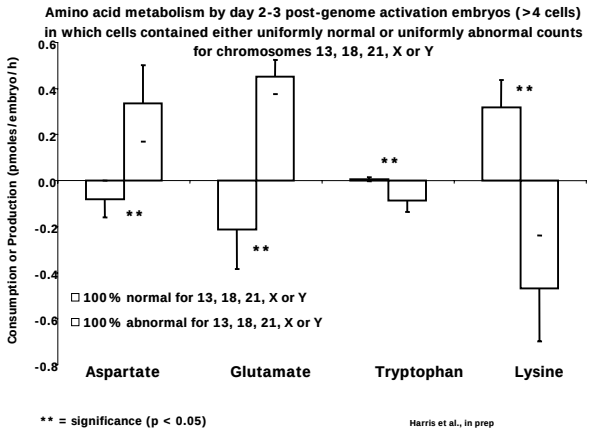
5 probe FISH on an embryo blastomere, assessing for the presence of chromosomes 13, 18, 21 , X and Y.

Red = 13
Aqua = 18
Green = 21
Blue = X
Gold = Y

This blastomere nucleus was from a male embryo and had normal counts for the chromosomes analysed.

Glutamine consumption by day 2-3 pre-genome activation (<4 cells), non-arrested embryos at the start of 24 hours culture





- **Plasma Lysine higher in children with Trisomy 21** (Lejuene *et al.*, 1992; HJ Heggarty *et al.*, 1996)
- **Deficiencies in Glutathione, Serine and Tryptophan** (Lejuene *et al.*, 1992)
- **Increased Methionine requirement** (K McLeod, 1996)
- **Trisomy (chr16) in mice certain up-regulates genes involved in metabolism of Glutamate, Aspartate, Alanine, Arginine, Tyrosine, Phenylalanine & Tryptophan metabolism** (Vaisman *et al.* 1981)

Summary

- **Metabolic and mitochondria activity may aid follicle/oocyte selection mechanisms during development *in vivo*.**
- **Metabolic profiling *in vitro* may prove to be beneficial in the selection of oocytes and embryos in ART.**
  - **Patient groups? E.g. Diabetes, PCOS, advanced maternal age?**
- **Metabolic profiling is a useful for optimisation of culture conditions *in vitro*.**
- **Prospective, controlled studies: further advances?**

Acknowledgements

- **Helen Picton, University of Leeds, UK**
- **Henry Leese, University of York, UK**
- **Kay Elder, Bourn Hall, Cambridge, UK**

- **Adam Balen, Leeds General Infirmary, UK**
- **Jan Hogg, Leeds General Infirmary, UK**
- **Judith Hawkhead, University of York, UK**
- **Ann Barker, University of York, UK**

- **Funding from: BBSRC and MRC, UK; White Rose, UK.**