

Identification of viable embryos by measurement of amino acid turnover.

Roger Sturmey
Hull York Medical School, UK

Conflict of interest



THE HULL YORK
MEDICAL SCHOOL

- None!

Outline

- Background
- The appeal of metabolic analysis
- Amino acids – putative markers
- How to...
- Review of the findings
- Future directions

Background

- Multiple births remain a concern
 - Serious risks to mother and children
- Single Embryo Transfer
 - Impact on success rates?
 - Subjective
- Strong desire to select the ‘best embryos’
 - What is ‘best’?

Criteria for embryo test

- Non-invasive
 - Sensitive
 - Individual embryo
 - Simple
 - Robust
 - Reliable
 - Cost-effective
 - Scientifically rigorous
 - Diagnostic information additional to morphology
- Use what is available
– the culture media

The 'appeal' of metabolism

H.Y.M.S

THE HULL YORK
MEDICAL SCHOOL



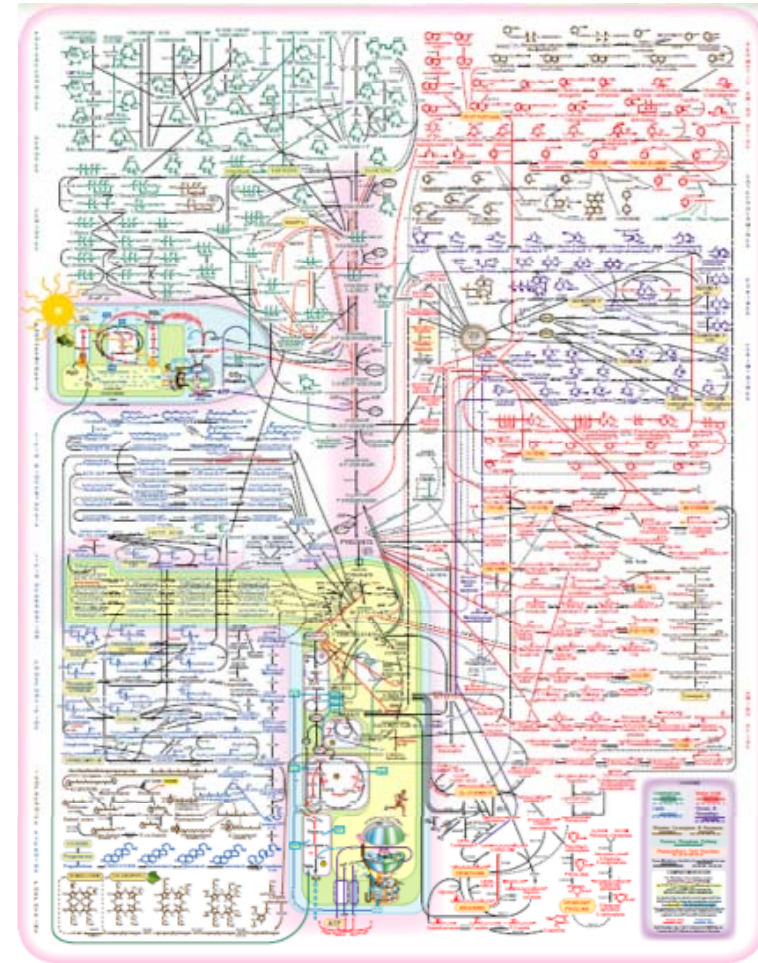
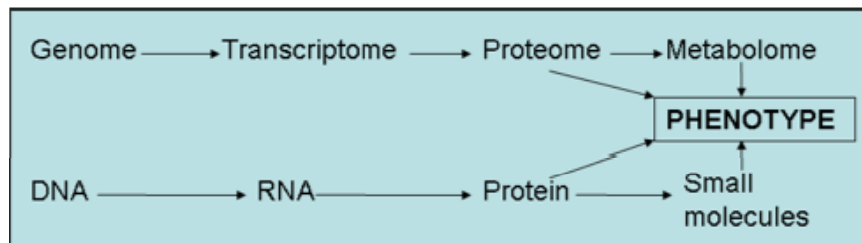
02 April 2011

Practical aspects of non invasive selection - ESHRE
Campus Symposium, Salzburg, Austria

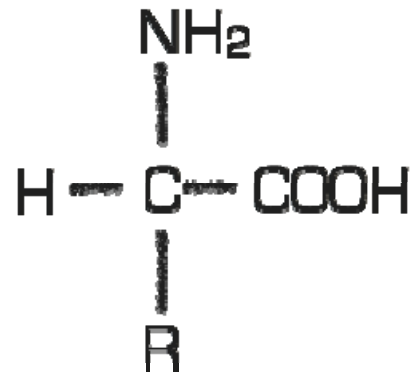
www.visitlondoncity.com

The 'appeal' of metabolism

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



Amino Acids



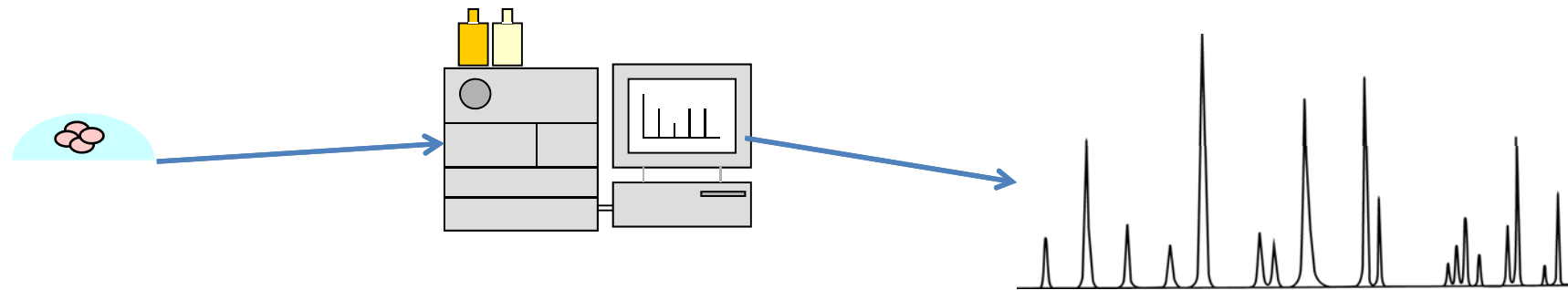
- 20 protein amino acids; all *L*-isomers
- Variety of roles
 - Protein synthesis
 - Osmolytes
 - Nucleotide synthesis
 - Provision of 1-C units
 - Precursors of signaling molecules
- More than one individual metabolic pathway
- Components of embryo culture medium
- Some depleted while others accumulate during culture

How?

- Typically by HPLC
 - High
 - Performance
 - Liquid
 - Chromatography
- Samples carried in the MOBILE PHASE
- Interact with the SOLID PHASE
- Eluted from the SOLID PHASE and detected



Workflow



- Embryos cultured for defined time period
 - Non-metabolisable standard
- Spent medium diluted and analysed by HPLC
 - 7min per sample

How?

H·Y·M·S

THE HULL YORK
MEDICAL SCHOOL

Mobile phase - Buffer

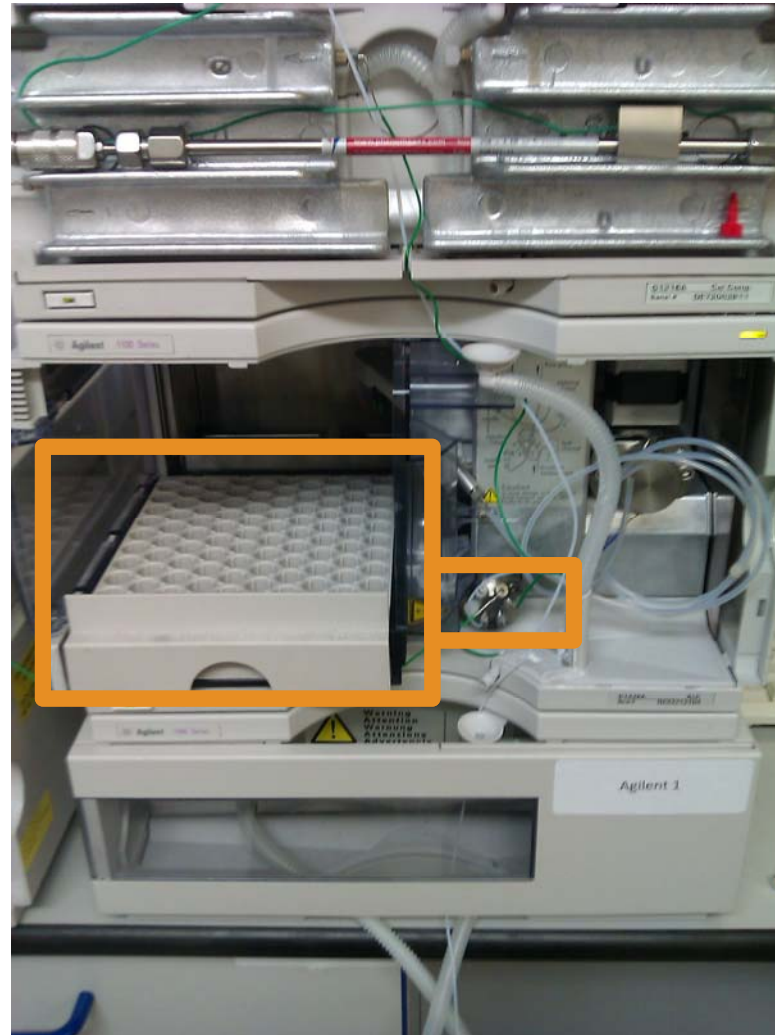
Pumps

Autosampler and
mixing seat



How?

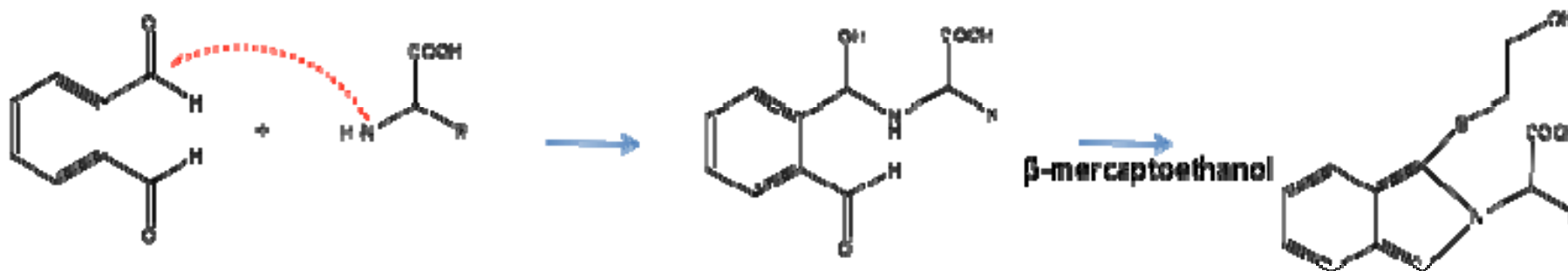
Samples



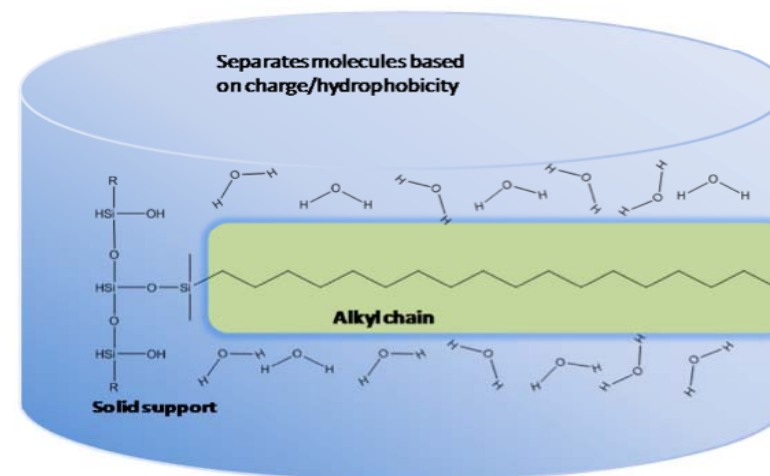
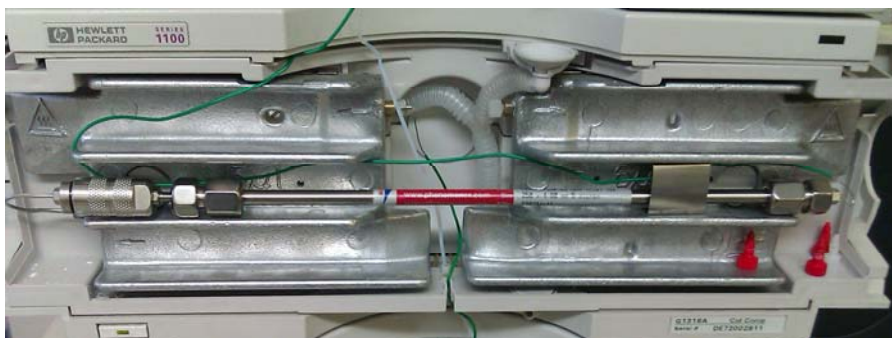
Sample mixed and
derivatised

How?

Amino acids are *derivatised* with O-Phthaldialdehyde



Interact with column – REVERSE PHASE

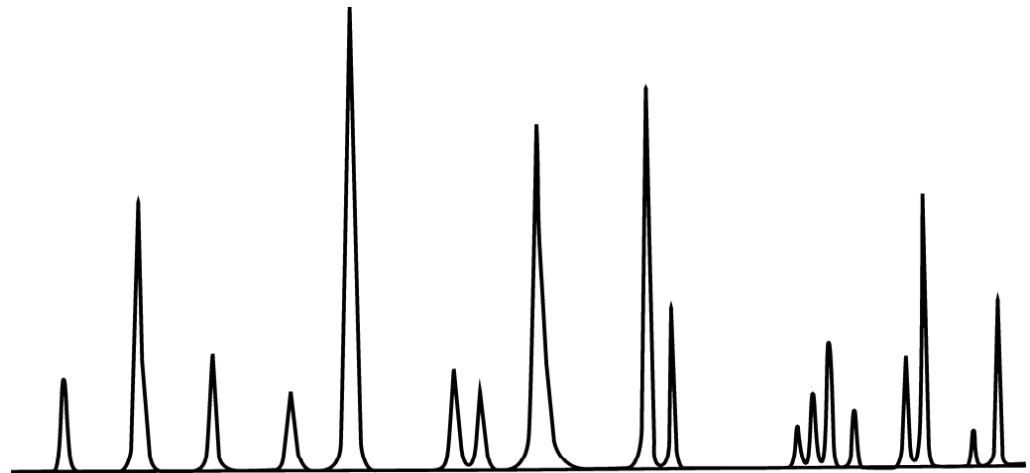


How?

Amino acids eluted from column
according to chemical interaction.

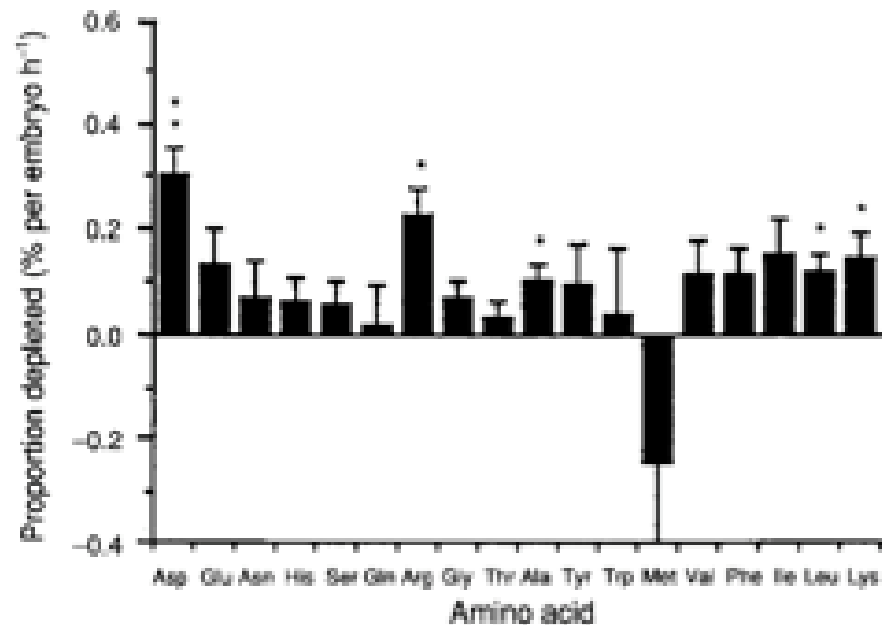
Area under the curve calculated and
related to areas of known, calibrated
standards.

All values related to internal standard
Non-metabolisable
Inert



Highly accurate and sensitive whilst
accounting for dilution/transfer errors
and non-specific changes (i.e.
evaporation)

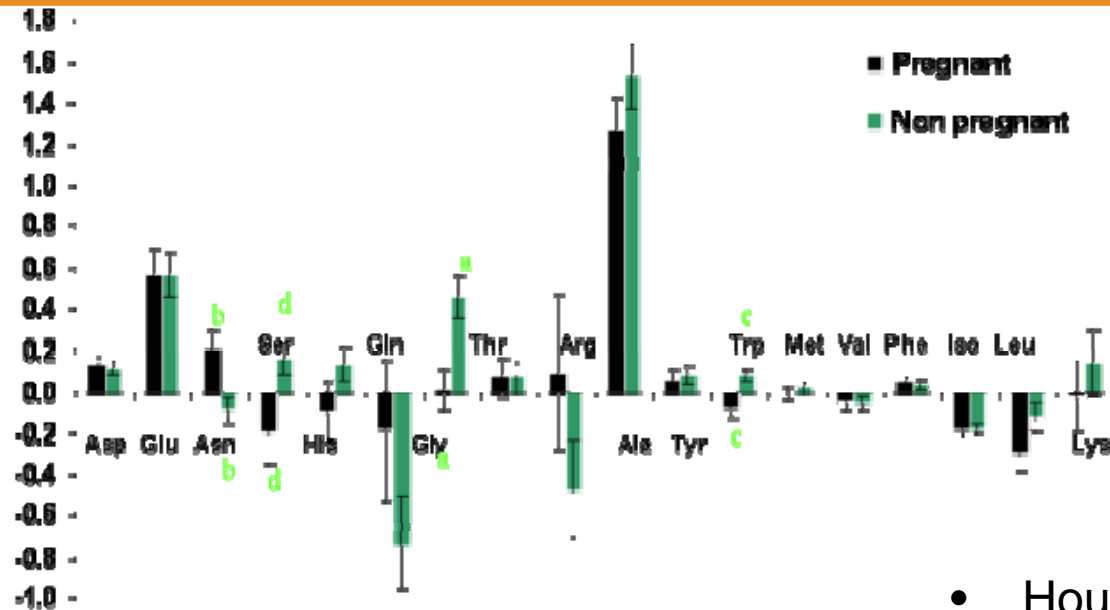
First reports



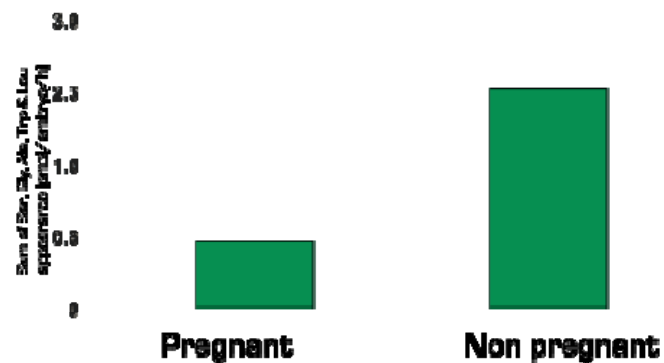
Lamb and Leese, 1994

- Day 4 mouse blastocysts in M16 medium + amino acids
- General appearance of amino acids in medium
- Methionine appears; all other amino acids depleted
- Note; no internal standard in this work

First reports

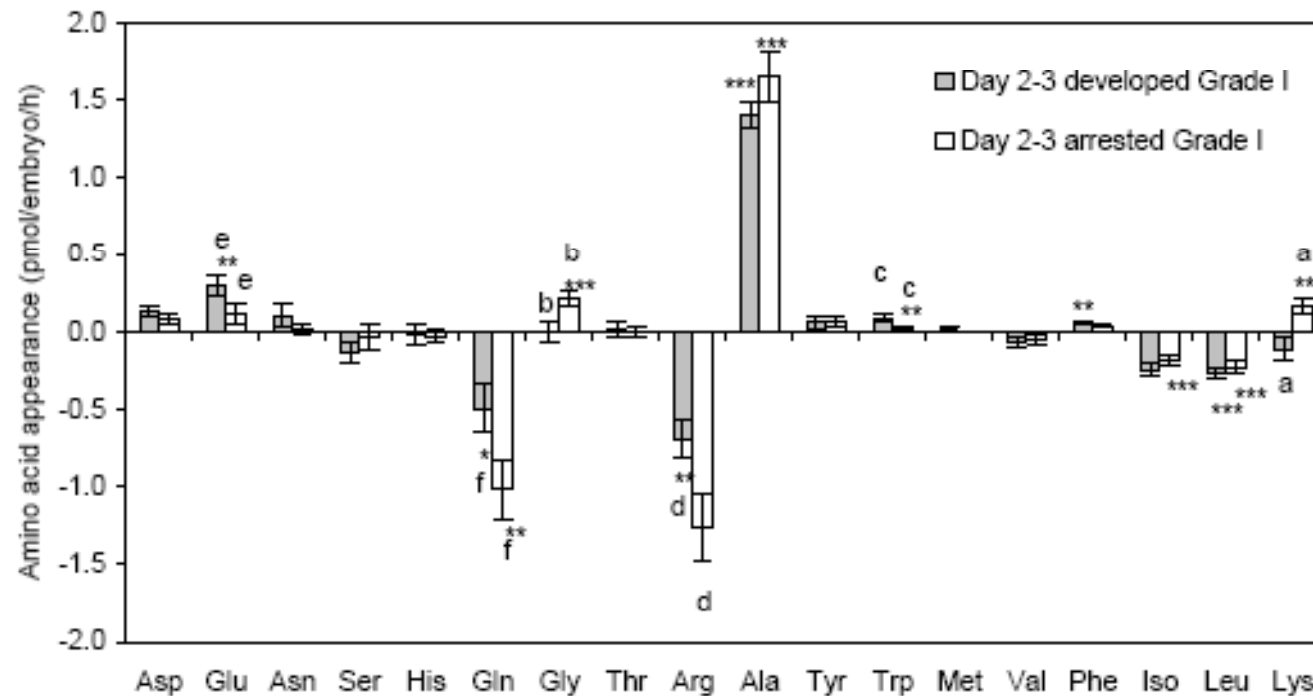


Brison et al, Hum Reprod, 19 pp2319-24 2004



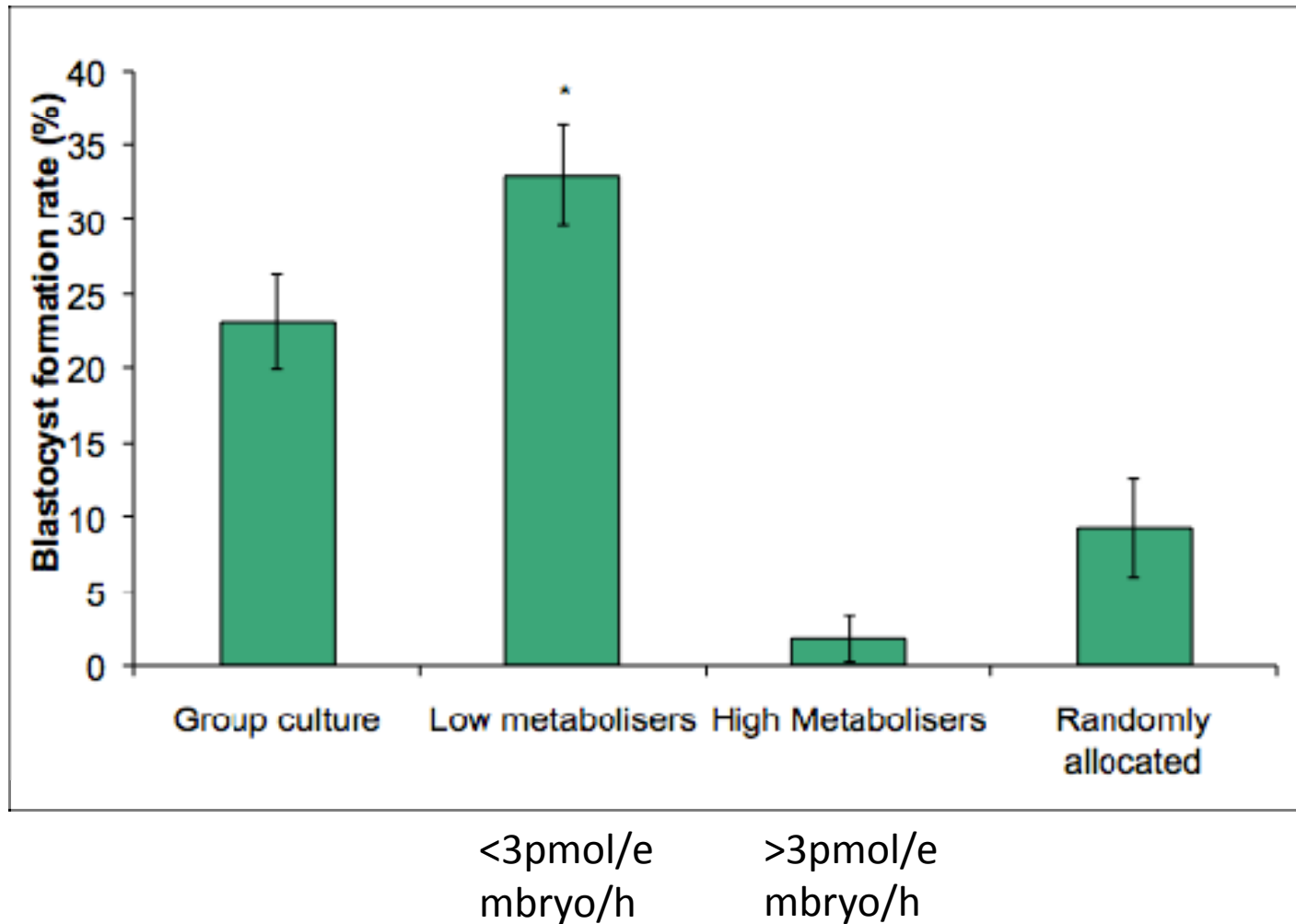
- Houghton et al (2002): AAP can predict blastocysts from d2-3 embryos
- Brison et al (2004): AAP correlated with clinical pregnancy
- Stokes et al (2007): AAP can predict outcome of cryopreserved embryos

First reports



- Can predict WITHIN grade 1 embryos those most likely to give blastocyst
 - More informative than morphology alone

Prospective analysis



- Bovine model
- Blastocyst rates of embryos assigned to groups
- Embryos with quieter metabolism more viable
 - Quiet hypothesis (Leese)

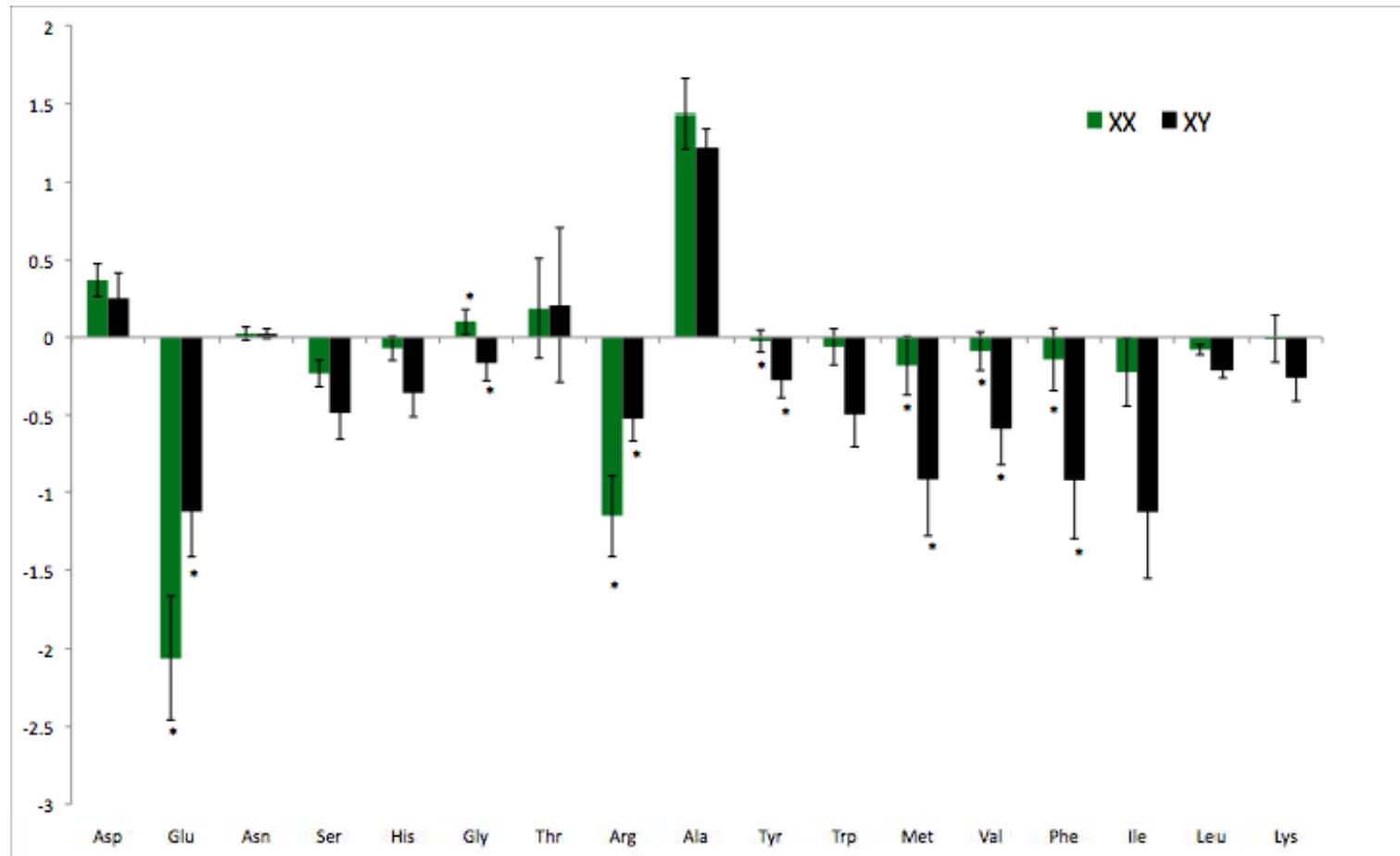
Added information



THE HULL YORK
MEDICAL SCHOOL

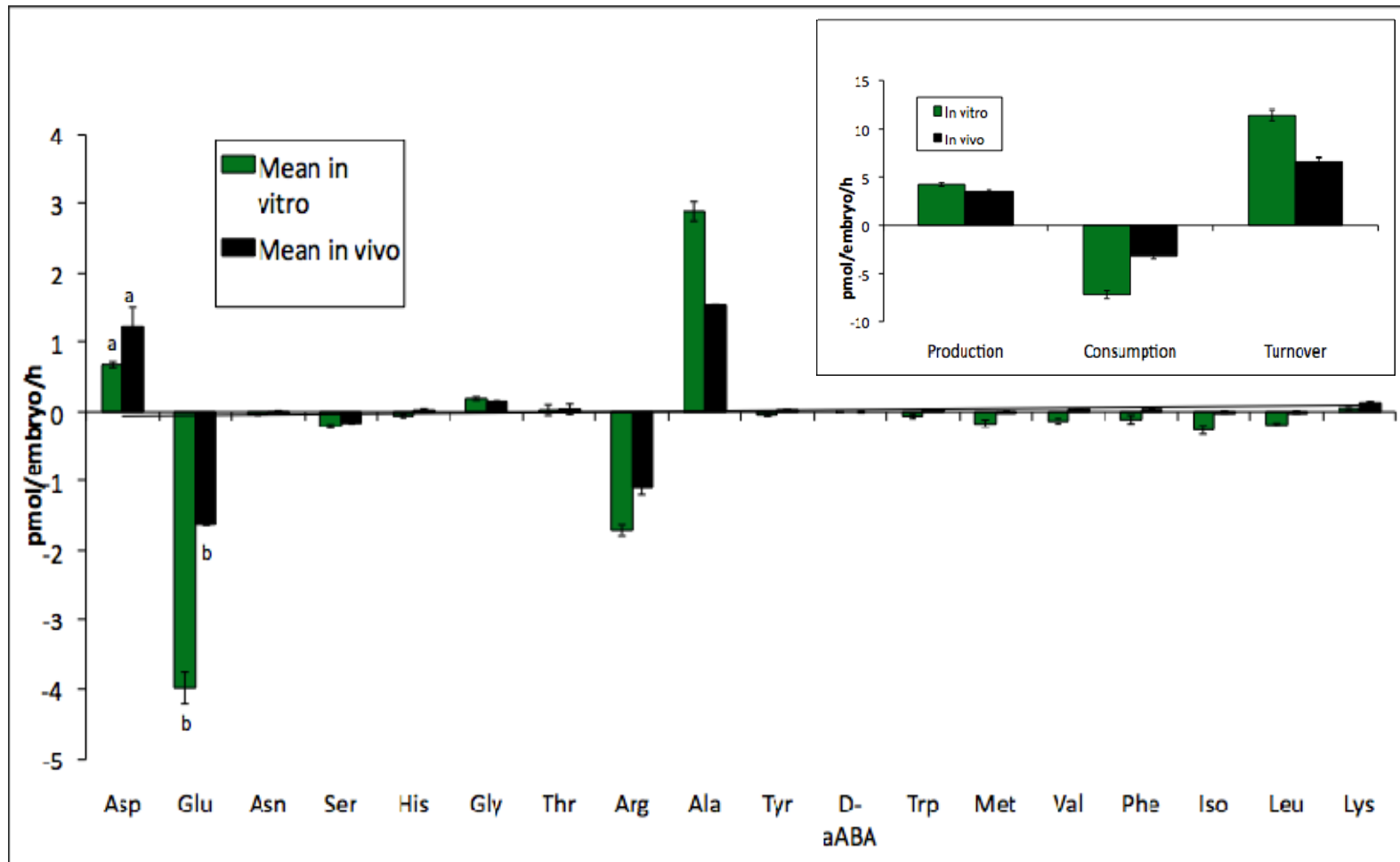
- Amino acids involved in various pathways
 - Physiology?
- Information other than viability?

Sex



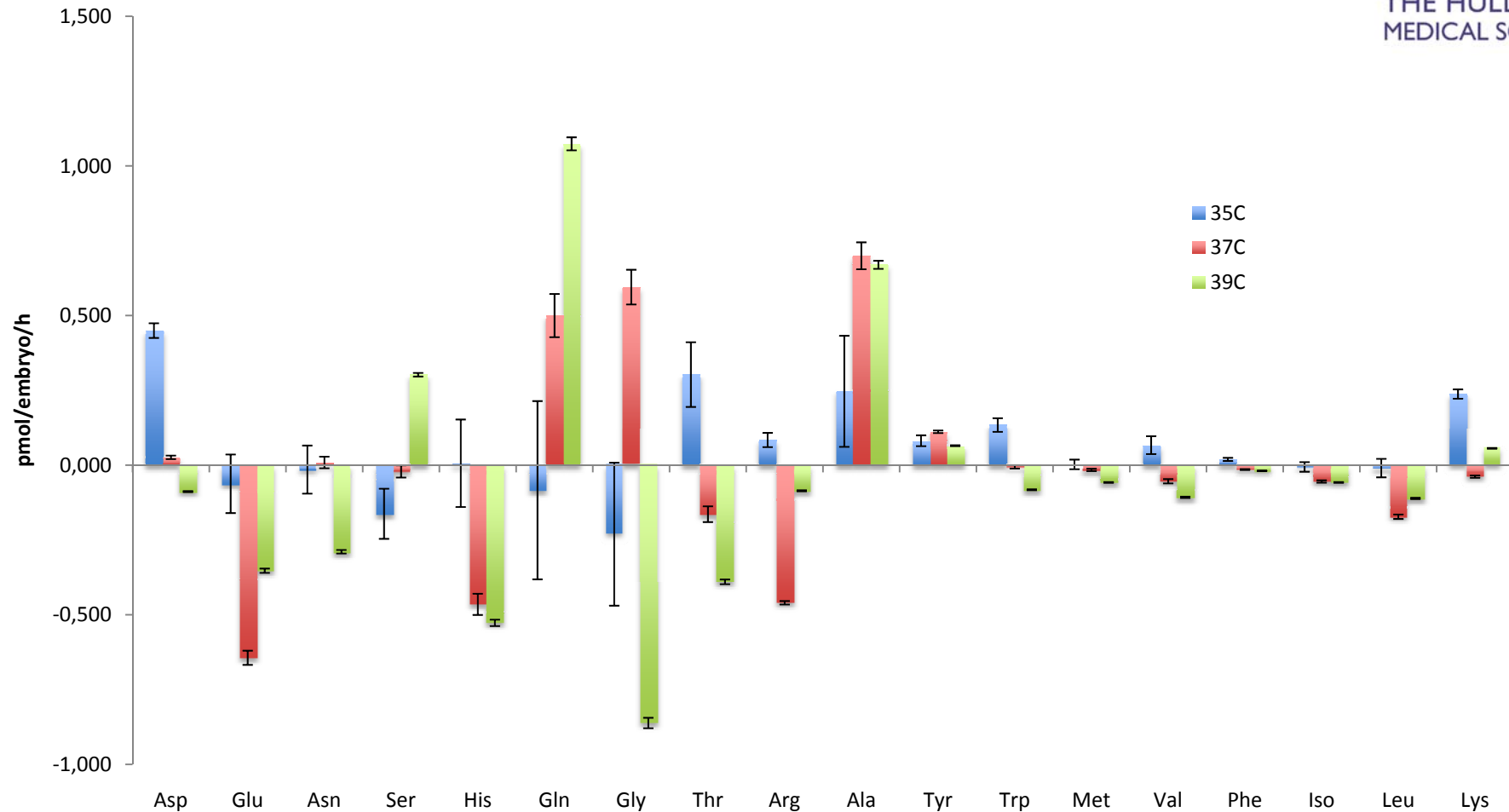
Bovine embryos sexed by PCR and retrospectively related to AAP

Origin



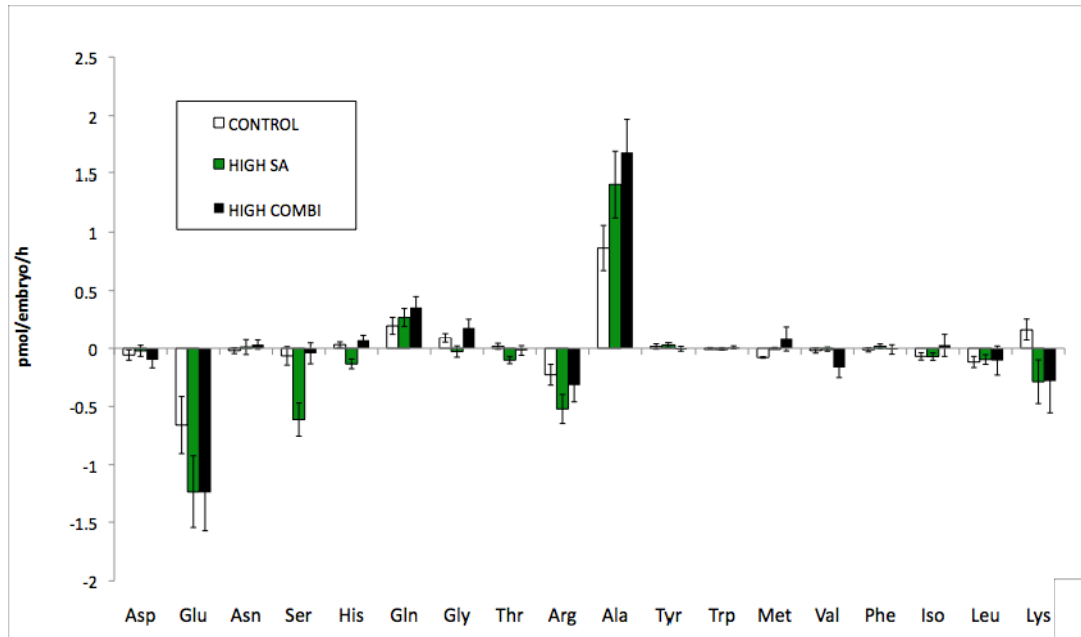
- AAP differs between in vivo- and in vitro-derived embryos

Culture conditions



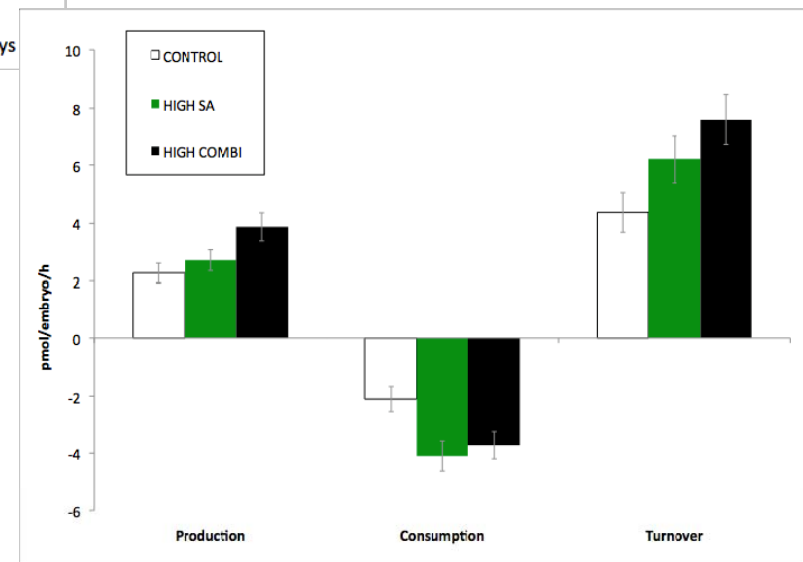
- Temperature alters amino acid metabolism

Maternal condition

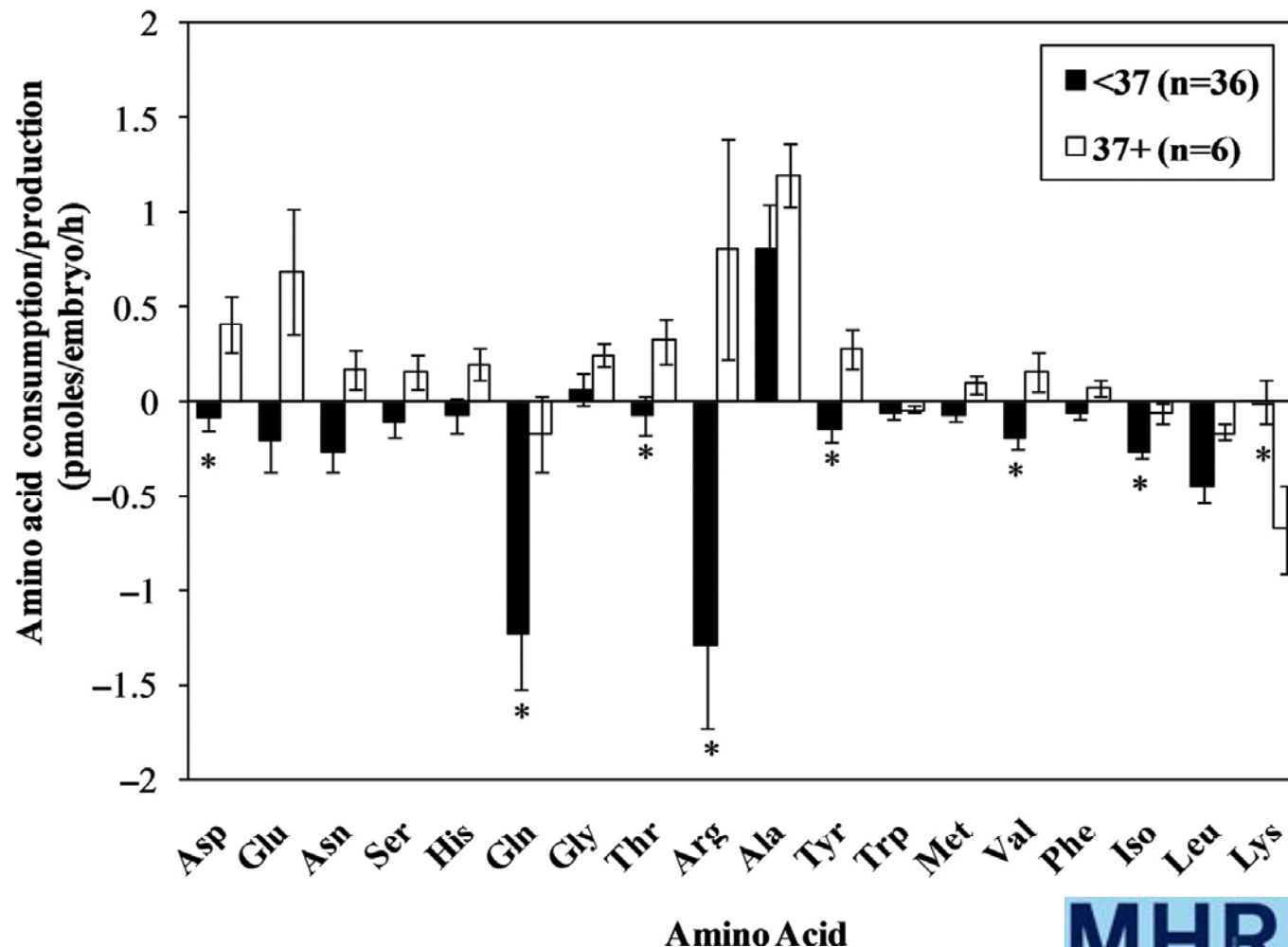


- Expose OCCs to elevated NEFA; similar to that in obese women

- Reduced viability reflected in Amino acid profiles



Maternal Age



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

Damage

Figure 4a

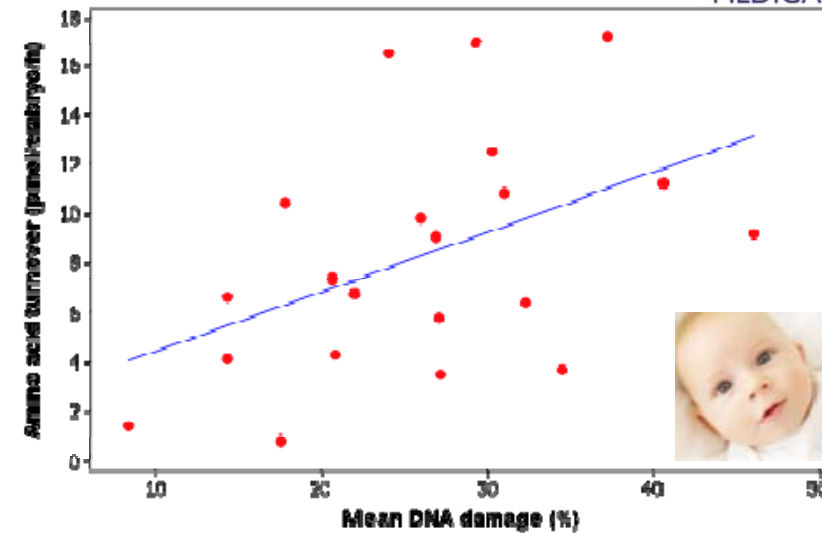
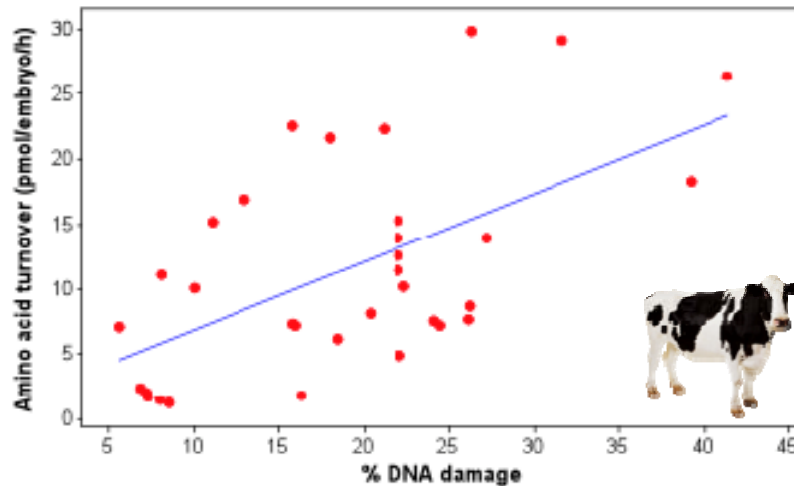
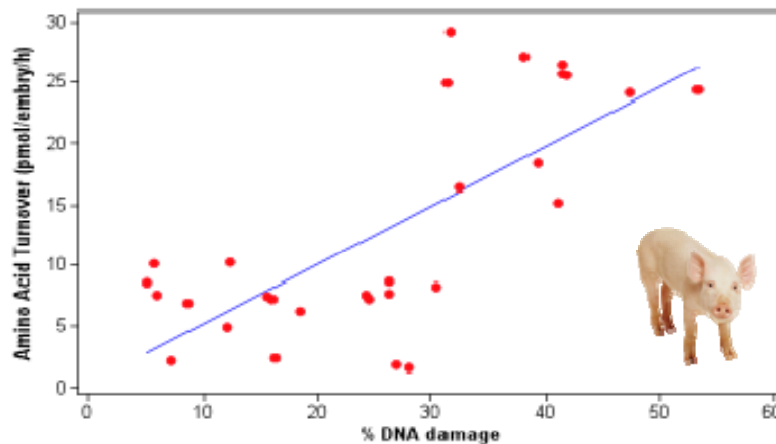
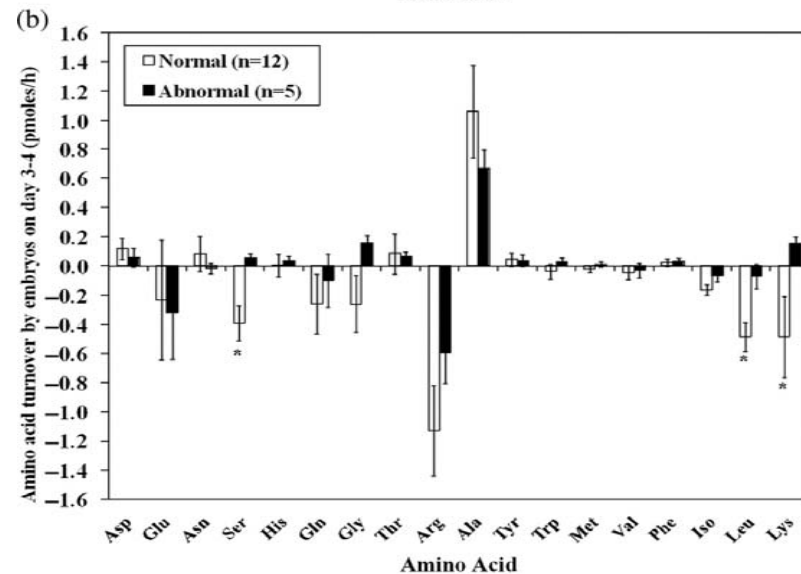
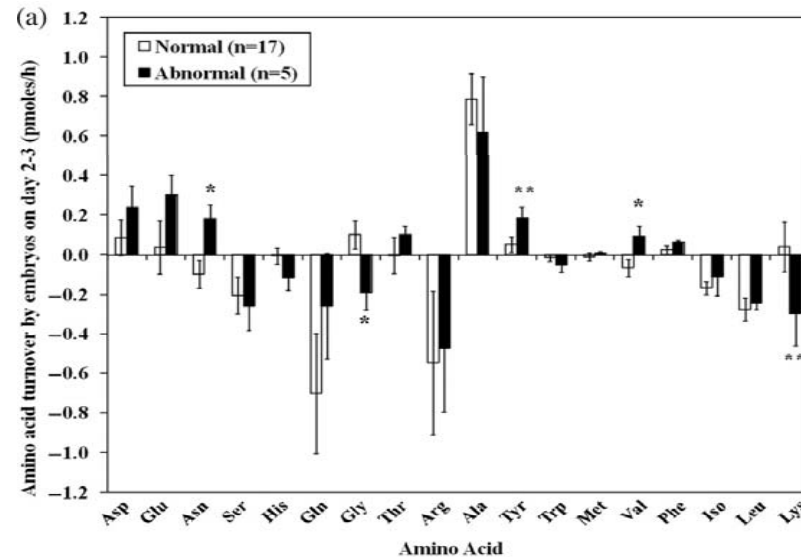


Figure 4b



- DNA damage levels in blastocysts relates to Amino acid metabolism

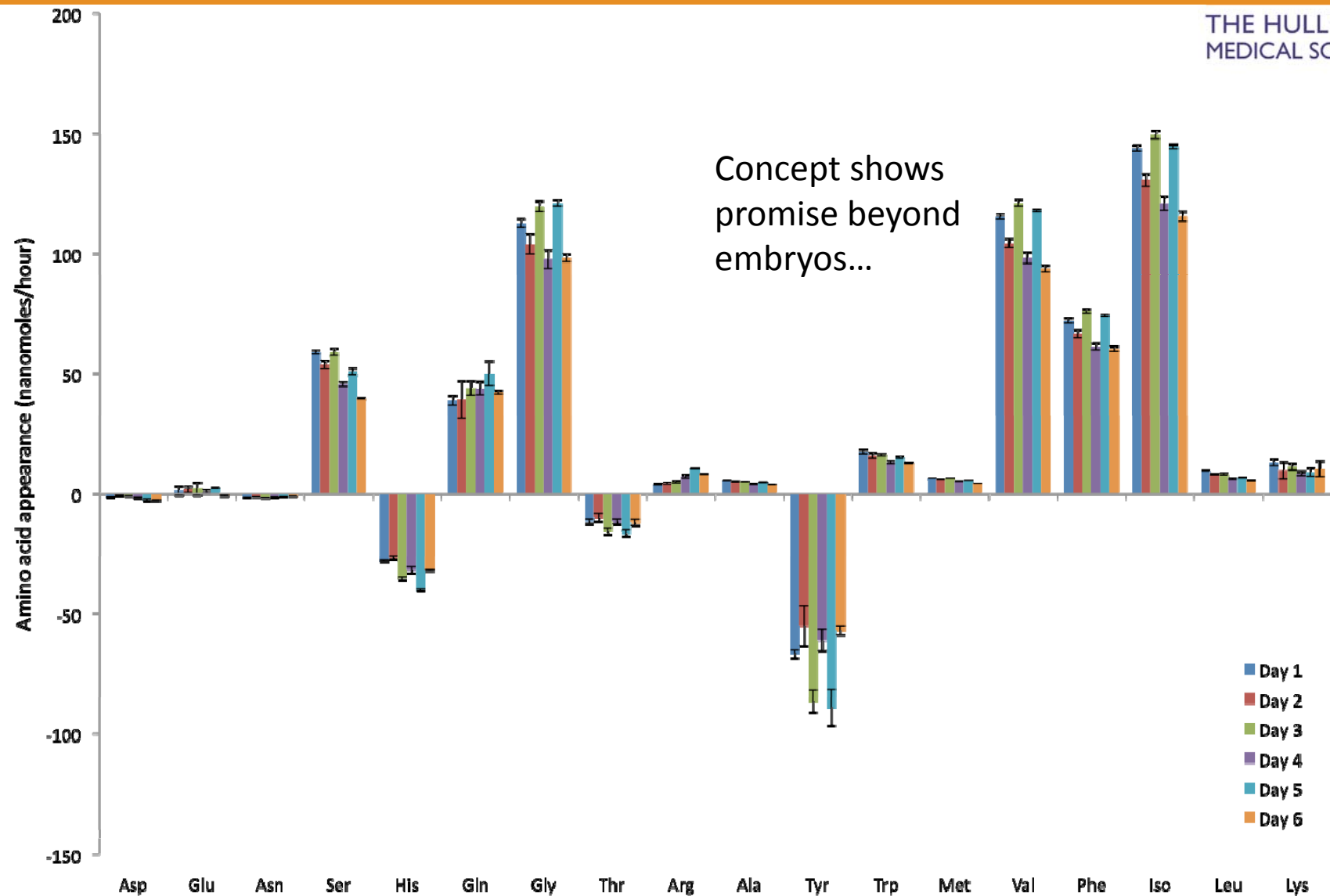
Aneuploidy



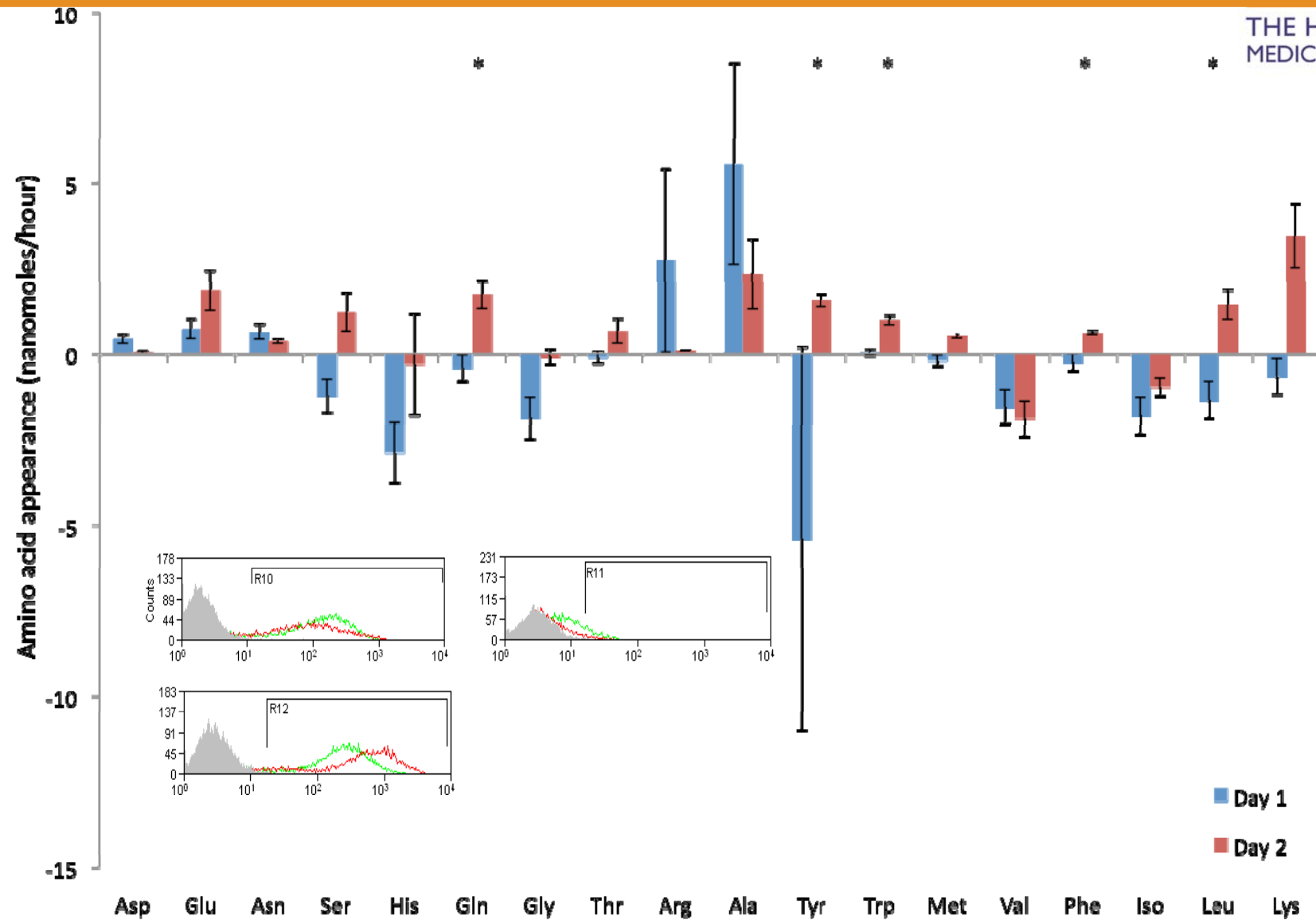
- Amino acid turnover by individual embryos in relation to abnormality of chromosomes 13, 18, 21, X and Y on Days 2–3 (a) and Days 3–4 (b).

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

Stem Cells



Stem Cells



- STILL no large scale prospective trial with human embryos
 - Origio in process
- Adaptation away from solvents
- Increased throughput of samples
- Must be shown to work in clinical setting...
- More samples, more observations will likely lead to additional markers
 - Beyond viability...

Acknowledgements



THE HULL YORK
MEDICAL SCHOOL

Henry Leese
Judith Hawkhead
Peter Humpherson
Rebecca Coates
Patty Sachamitr
Mark Coles
Christoph Baumann
Danielle Smith

Pablo Bermejo-Alvarez (Missouri)

Dimitrios Rizos (Madrid)
Alfonso Gutierrez-Adan (Madrid)

Patrick Lonergan (Dublin)

Helen Picton (Leeds)



Daniel Brison (Manchester)



Jo Leroy (Antwerp)
Veerle Van Hoeck (Antwerp)

