Human preimplantation development *in vitro* is not adversely affected by biopsy at the 8-cell stage

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**Question:** If you remove one or two cells from an 8-cell stage embryo, how is preimplantation development affected?

**Context of Study**

In 1990, the only way to screen for genetic defects in a baby was chorion villus sampling or amniocentesis. These procedures can be done between 8 and 20 weeks of gestation. If genetic defects are present, the only option of terminating the pregnancy is an abortion.

*Why Study Preimplantation Development?*

If preimplantation diagnosis could identify genetic disease after *in vitro* fertilization, doctors could select which “healthy” embryo to implant in the uterus.

*Why Study 1 & 2 cell removal?*

DNA amplification by PCR may not always be successful given the nature of the sequence, therefore two cells might be needed for a more accurate diagnosis.

**Development Terms & Facts**

- ICM = inner cell mass
- TE = trophectoderm
- IVF = in-vitro fertilization

*In Vitro* Fertilization → late Day 3 compaction between 8 & 16 cell stage → Day 5 or 6 blastolation → Implantation *in vivo* Day 7-10 (from Hardy, 1989b)

Pyruvate and glucose uptake in normal embryo increases between days 2.5 & 4.5. Pyruvate uptake continues to increase from day 4.5 to blastocyst stage. Lower pyruvate uptake is associated with arrest in cleavage stages. (from Hardy, 1989a)

**Experimental Design**

- Take in-vitro fertilized embryos, and remove either 1/8 or 2/8 cells.
- Follow development until Day 5 or 6 post-fertilization.
- Use glucose and pyruvate uptake as a measure of cell health and growth.
- Differentially label blastomere nuclei to distinguish and count ICM vs. TE cells.
- Measure the mitotic index and dead cell index of both ICM & TE cells.

**Questions on Experimental Design**

- Is there any bias in how they initially selected embryos for the study?
- Are the culture methods the most accurate *in vitro* system?
- How did they measure glucose and pyruvate uptake? Is this a good way to measure cell health?
- How does the differential labeling work?
- What is the procedure for counting live vs. dead cells?
What statistical measure was used to distinguish differences in data between manipulated embryos and control embryos?

**Ethical Issues**
- Study approved by the Interim Licensing Authority for Human In-Vitro Fertilization and Embryology and the ethics committees of the Royal Postgraduate Medical School and the University of York.
- Is this study ethical?
- Would it have been approved in the United States?

**Results**
- A large number of the 6/8 and 7/8 embryos developed to the Day 5 or Day 6 blastocyst stage without arrest.
- Pyruvate uptake declined, and glucose uptake increased from days 3.5 to 5.5 in manipulated and control embryos.
- There is no statistically significant difference in cell number between 7/8 embryos and control, however there is a statistically significant difference (P < 0.05) between 6/8 embryos and the control.
* The ratio of ICM:TE cells is the same as the controls, although overall cell numbers for ICM and TE are lower (but not significantly) in the 7/8 and 6/8 embryos. Important because fetus is derived from ICM after implantation.
* Most all deviations in cell number & in nutrient uptake are proportional to the reduced cellular mass of the manipulated embryos.

**Discussion Questions**
- Is the sample size big enough?
- Are the statistically significant differences in Table II a cause for concern, since it is suggested that two cells might really be needed for an accurate diagnosis?
- What further studies could be done? The authors suggest a clinical trial, should any further in vitro studies be conducted beforehand?
- Do the further applications of this study rely on the embryo compensating for reduced cell mass after implantation?
- Will in vitro findings translate to an in vivo situation?
- Should pre-implantation diagnosis be used for diseases that are not life-threatening or for “desirable” characteristics?

**Current Medical Practice**
Common practice now is to remove two cells from the 8-cell stage embryo. Genetic analysis takes on average two days. (From Gilbert, Developmental Biology, 7th Edition)