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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)