GRAVITY AND ANIMAL EMBRYOS

Lynn M. Wiley
University of California
Davis, CA 95616

What is the likelihood that mammalian development might be affected by conditions encountered during space flight?

After more than 4,500 "rat hours" in space, there has been only one attempt (Cosmos 1129) at mating with an apparent absence of fertilization, implantation and subsequent development to partuition. However, segments of the cycle have occurred successfully, at a gross level (Final Reports of U.S. Monkey and Rat Experiments flown on the Soviet Satellite Cosmos 1514. R.C. Mains and E. W. Gomersall, eds. NASA Technical Memorandum 88223. p. 189, 1986). Specifically, later gestation and parturition in the rat proceeded during Cosmos 1167. However, on an earlier flight (Cosmos 1129) copulation and subsequent events did not proceed in the rat. It is not possible, unfortunately, to conclude whether the observed reproductive failure resulted from perturbations of biological process per se or for trivial reasons such as improper photoperiod.

Subsequent ground tests at NASA/Ames (1981; NASA-N81-32852) were performed to determine whether the following flight-related factors might have contributed towards the observed reproductive failure on Cosmos 1129:

a) effects of re-entry stresses on timed pregnant rats
b) effects of launch stresses on the male rat mating ability
c) effects of full flight simulation on viable pregnancies
d) Soviet paste diet
e) launch vibration, noise and acceleration
f) group housing in a confined volume
g) competition for limited food
h) restricted illumination and airflow
i) re-entry shock and acceleration
j) post-flight handling and isolation

None of these factors was able to cause all animals to fail to establish and maintain pregnancy to term. Furthermore, there were no significant differences in number of live fetuses and births or in adrenal weight ratios between the group exposed to launch and re-entry stresses, to the animal husbandry aspects of the flight conditions, and the control group. NASA's conclusion was that the failure of some flight- and control females to bear young was probably not solely due to the stresses of launch and re-entry.

These observations are consistent with the hypotheses that i) in a small mammal, the endocrinologic, cellular and molecular mechanisms comprising embryonic development after implantation, fetal development and parturition may occur in space, and ii) events preceding an established pregnancy might be impaired in space. To examine the second hypothesis, one can ask the following two experimental questions:

1. Is the hypothesized developmental impairment direct and/or indirect; i.e., is the impairment a result of direct effects of the space environment on

the gametes and the embryo and/or a result of indirect effects stemming from the maternal and/or paternal response(s) to the space environment?

2. Which developmental process(es) is/are imparied; i.e., are female factors, male factors, or a combination of male and female factors involved? Female factors might include oogenesis, ovulation, gamete transport, fertilization, embryo transport to the uterus, implantation and luteal function. Male factors might include spermatogenesis, sperm transport and ejaculation. Combined factors might include copulation together with any of the other factors named above.

Proposed experiment: examine in vitro and in vivo development in parallel in space.

As a starting point, we could conduct this experiment using two groups of female mice that have been mated on the ground 24 h prior to a 5-day flight. During the flight, one group of mice could be sacrificed when their embryos were at the 2-cell stage of development and these embryos cultured until ground control parallel cultures of embryos had attained the blastocyst stage. Mice from the remaining group could be sacrificed at various timepoints to compare the developmental progress of their embryos with that of the embryos developing in vitro in flight and on the ground and in vivo on the ground. The advantages of thie strategy are:

1. we eliminate behavioral factors related to copulation, increasing the likelihood of obtaining some useful information from the experiment.
2. we circumvent the need for in vitro fertilization and embryo transfer.
3. we take advantage of the fact that only preimplantation embryos of the mouse can be cultured throughout cleavage.
4. mouse embryonic development from the 2-cell stage to implantation normally takes 3.5 days; therefore a 5-day flight should be long enough for preimplantation development to implantation to take place, even if it is delayed somewhat by flight conditions.

Selecting experimental parameters for evaluating preimplantation development: 1) cleavage rate, 2) embryo cell number and 3) blastocyst formation accompanied by inner cell mass/trophectoderm differentiation.

Preimplantation development prepares the embryo for two events, embryogenesis and implantation. Each event is mediated by two different cell lineages, the inner cell mass and the trophectoderm, respectively. These two cell types are normally present by the blastocyst stage when the embryo finds itself within the uterus. To form both cell types requires that the embryo sustain a cleavage rate that will be fast enough to produce the minimum number of cells required for both cell types to develop adequately by the blastocyst stage. If embryo cell number is less than 16 when the two cell types begin to differentiate, then not enough cells may be available to form an inner cell mass and only trophectoderm will form or an inner cell mass of insufficient cell number might be formed. An inner cell mass that is missing or of insufficient cell number results in an implantation that is resorbed.

This is the rationale for selecting these 3 parameters for assessing the normalcy of preimplantation development in space flight. In addition, these parameters have been used traditionally for assessing preimplantation development and are easy to follow and quantitate and require only a dissection microscope for scoring.

Epigenetic influences on preimplantation development.

Many non-genetic influences from the environment can be reflected by these parameters. One is the concentrations of ions in the extraembryonic milieu--potassium and sodium specificially. Low levels of potassium will accelerate the onset of blastocyst formation and may cause it to begin before the embryo has 16 cells. Since space flight alters the ionic composition of serum--and perhaps also of oviductal fluid, abnormal ion concentrations might be a factor during preimplantation development during space flight.

Other epigenetic influences consist of cytoplasmic asymmetries. For example, the blastomere of the 2-cell embryo that inherits the remnant of the sperm tail will contribute more of its progeny cells to the inner cell mass than will its sister blastomere (Bennett J. 1982. J Cell Biol 163a).

Yet other influences are provided by extra-cytoplasmic, environmental asymmetries, the best-known one being asymmetric cell-cell contacts. At the 8-cell stage, asymmetric cell-cell contacts are established as a result of 'compaction' when the formerly spherical blastomeres flatten against one another. The blastomeres in the post-compaction embryo have basolateral surfaces that are apposed against adjacent blastomeres and apical surfaces that face the oviductal fluid. Consequently, the blastomeres exhibit an apical-basal axis of polarity. When such a polar blastomere divides so that one daughter cell inherits its apical half and the other daughter cell inherits its basal half (differential cell division), the apical daughter gives rise to trophectoderm while its basal sibling gives rise to inner cell mass. This is how the two cell lineages, inner cell mass and trophectoderm, are formed (review Johnson MH and Pratt HPM 1983 in Time, Space and Pattern in Embryonic Developent, Alan R. Liss, Inc. NY pp 287-312). Processes that impair the development of blastomere polarity--like the impairment of the cell shape changes and the increase in cell-cell adhesiveness that accompany compaction-will reduce the incidence of differential divisions and it is only differential divisions that will produce inner cell mass. (When the apical and basal cytoplasm of a parent polar blastomere are divided equally between the two daughters--conservative division--both daughters give rise to trophectoderm).

Aside from asymmetric cell-cell contacts, extracellular d.c. electric fields can also influence the developmental polarity of isolated blastomeres and their incidence of differential cell divisions. There is no information that indicates whether other physical environmental asymmetries such as an asymmetric gravity vector (such as 1 g)--or the lack thereof--can influence blastomere polarity and the likelihood of differential cell divisions.

Hardware considerations for accomodating preimplantation development in vitro in space.

In the conventional laboratory, embryos are obtained by flushing excised oviducts with a syringe of culture medium attached to a 30 g needle, with the aid of a dissection microscope (200X). Small numbers of embryos (about 20 2-cell embryos) are obtained per mouse so that several mice must be on hand to provide the 100 or so 2-cell embryos that would be necessary for one experiment envisioned by Dr. D. Wolgemuth and myself.

All embryo manipulations are performed with the aid of dissection microscopes (200X), including scoring for cleavage rate and incidence of blastocyst formation and embryo cell number. Manipulations include

transferring embryos from one medium to another during oviduct flushing and pooling prior to establishing cultures and fixation for morphology or for obtaining embryo cell numbers. Embryos are normally handled by mouth pipetting while they are submerged in cultured medium and cannot be allowed to contact an air-fluid interface.

Embryo temperature must be maintained between 35°C and 37°C for reproducibility and their culture medium is normally bicarbonate-based so that a carbon dioxide incubator is necessary. All manipulations, from excising oviducts from the female to establishing embryo culture, must be done using sterile technique.

It normally takes about a year before a person has acquired sufficient technical skill and judgement about embryo morphology to reliably flush oviducts and culture embryos with any consistency. Herein lies a major concern: handling these embryos on the ground--much less in space--can present a formidable challenge. I think that flushing the oviducts might prove the most frustrating aspect of this experiment. Using frozen embryos based on present embryo-freezing technology will not substitute for non-frozen embryos. ... Out of curiosity, with respect to frozen embryos, how does freezing/thawing react to microgravity?