SPACE SPERM – The Importance of Gravity in Sperm Navigation, Fertilisation and Early Embryo Formation.

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Space and space exploration attracts curiosity, excitement, and innovation nationally and internationally, with growing interest in Mars settlement, deep space exploration and Moon mining. However, effective long-term human settlement of celestial bodies and exploration of deep space poses many challenges, including but not limited to the requirement for mammalian reproduction in micro-gravity environments. It is crucial to understand the impact of low gravity on the earliest stages of space-born life, including the viability of conception and healthy fetal development in mammals, processes of reproductive success which are normally dependant on Earth's gravity. Utilising a gamete and embryo culture suitable dual-axis 3D clinostat to simulate microgravity we aimed to determine if exposure of mammalian sperm and embryos to simulated micro-gravity altered functional and developmental capacities. In this study non-capacitated mouse (N=10) and human (N=6) sperm were challenged to swim through an Ibidi 6-channel μ-slide for 1-6 h at either normal gravity (NG) or stimulated micro-gravity (0G), in conditions that mimic the female reproductive tract (media – G-IVF PLUS, 37^{o}C, 6% CO_2, 5% O_2). Sperm recovery and motility kinetics were assessed by computer-assisted semen analyser (CASA). Additionally, non-capacitated mouse sperm (N=7) underwent *in vitro* fertilisation (IVF) for either a 4 or 24 h fertilisation window at NG or 0G and zygotes subsequently cultured in GT-L at NG for 5-days. 2-cell cleavage rates were assessed at 24 h and on-time blastocyst development and cell numbers/allocation (Oct4 – inner cell mass and Nanog – epiblast fetal cells) at 96 h post-insemination. A subset of embryos underwent time-lapse culture to assess timing of PN fading, PN to 2-cell cleavage, and PN to blastocyst development. Additionally, porcine sperm (N=3) underwent *in vitro* fertilisation for a 6 h fertilisation window at NG or 0G, and the zygotes subsequently cultured in NCSU23-plg and NCSU-G at NG for 7-days at 38.5ºC, 6% CO_2, 5% O_2. Porcine 2-cell cleavage rates were assessed at 96 h and on-time blastocyst development at 144 h post-insemination. Exposure of both human and mouse sperm to 0G reduced its ability to navigate a channel slide, with a 40% reduction in sperm recovery after 1 hour (both $P<0.05$) and a reduction in both amplitude lateral head movement and curve linear velocities in the mouse (-33%, $P<0.05$), although total motility was unchanged in both species. Fertilisation rates in the mouse were reduced by 33% following 4 h fertilisation at 0G ($P=0.003$), although unchanged during a 24 h fertilisation window. On-time blastocyst rates were reduced by 22% (all $P<0.05$) following any fertilisation period at 0G. Interestingly, in those embryos that did make it to blastocyst, exposure to 0G increased epiblast fetal cells (+30%, $P<0.05$), although, blastocyst total cell numbers were unaffected. Similar effects were observed in the pig, where there was a 20% reduction in fertilisation rate ($P=0.067$) and a 23% reduction in on-time blastocyst rate ($P=0.046$). This

study indicates that microgravity can negatively influences sperm's ability to navigate and fertilise an egg. Even after return to normal gravity, embryos exposed to microgravity during fertilisation have reduced on-time blastocyst rates, and an increased fetal cell cellular allocation. The ability of mammals to reproduce in micro-gravity environments currently seems limited.