Topical: Mammalian Multi-Generational Studies in Space

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Mammalian Multigenerational Success in Space will Comprise a Repeating Cycle of Necessary Milestones

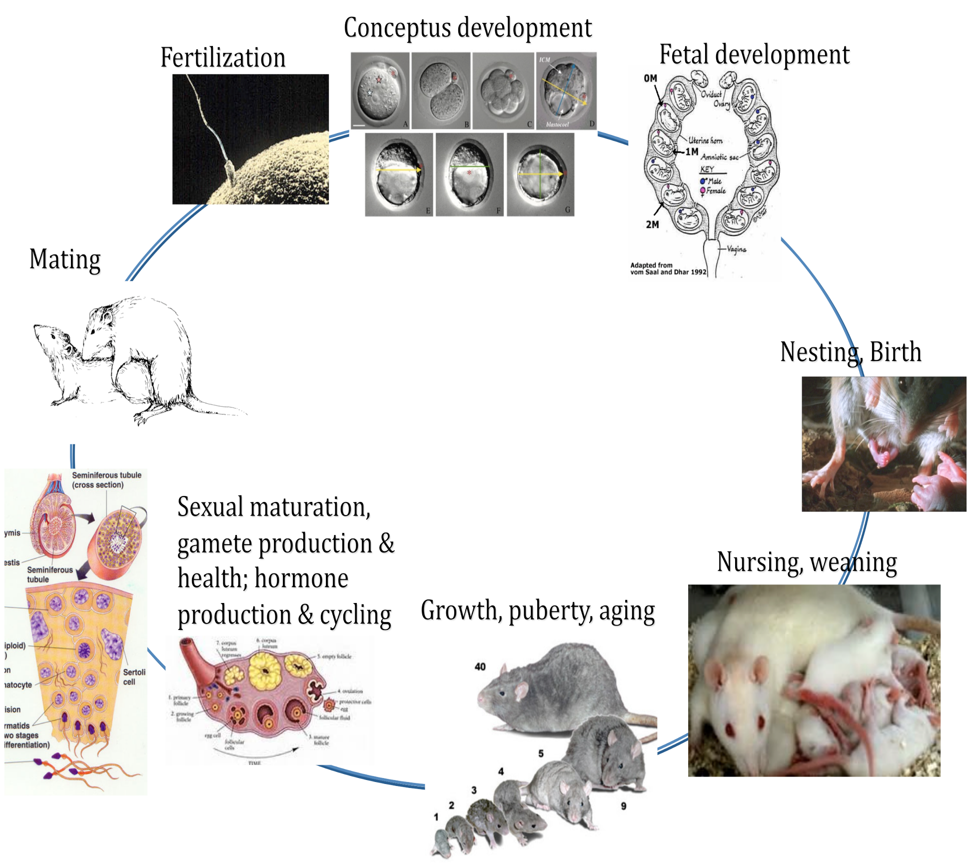

Illustration: J. Tash (In Ronca, A.E., et al., Mammalian Reproduction and Development on the International Space Station (ISS): Proceedings of the NASA Rodent Mark III Habitat Workshop. Gravitational and Space Research, 2013)

Reproductive and developmental biology have been at the forefront of most fundamental biomedical advancements over the last several decades, including stem cell technology, treatments for genetic disorders, influence of epigenetics and genetics, and are likely to play key roles in preservation of species as our world changes. Studying mammalian life cycles in space promises to uncover astounding new insights into how gravity shaped life on Earth and the adaptability of humans and animals to life beyond Earth. The reproductive system (i.e., germline) is the most sensitive tissue to irradiation and is hence a bellwether. This has been recognized by multiple National Research Council (NRC) (1987, 1991, 1998, 2011) panels convened to guide NASA Space Biology research, these panels and others have emphasized *the fundamental importance of research on development and reproduction of mammals in space*, specifying that animals should be studied both within and across generations, completing *two full life cycles in space* to produce truly space-developed progeny. Mammalian reproductive and developmental studies in various gravitational fields are important for multiple reasons:

1. Earth's gravitational field and protection from cosmic radiation, have marked influences on the evolution of species. Development of life on Earth includes at least two periods when its resident lifeforms had the opportunity to feel a gravitational/mechanical change—the transitioning of living creatures onto land, the return of some of them to the sea and now a third opportunity exists as we penetrate into space (Serova and Denisova, Physiologist, 1982). Earth's diversity gives evidence that in the first two cases, living organisms were capable of adapting successfully, however, we are only now learning how plants, animals, and humans may adapt to the cosmic environment. *Successful reproduction is a hallmark of adaptation to a novel environment.* As ISS research capabilities have expanded to support long duration studies of adult rodents (up to 180 days currently), there is an opportunity to set the foundation for multigenerational spaceflight studies by embarking on well-designed, highly controlled studies of the adaptability of reproducing and developing animals in space. Such experiments offer unprecedented opportunities to study reproduction and developmental biology of all major organ systems in mammals never exposed to the omnipresent force of Earth's gravity.
2. Ultimately, understanding the impact of the extraterrestrial environment on human reproduction is vital to the success of space travel and colonization, for astronauts returning to Earth after long-duration spaceflight, and potentially for all terrestrial humans. Not only may the information learned catalyze new insight and discovery into the origins and evolution of reproductive morbidity on Earth, but it may one day be critical for survival of the species. Reproductive biology studies of animals exposed for extended periods to the space environment are critically important to the health and safety of the next generation of male and female astronauts and to the offspring of these pioneers.
3. Development is a key to unlocking the door to understanding fundamental principles of how bodies and brains organize, maintain, and adapt. Genes are expressed during development and developmental processes regulate and guide genetic expression. Mammalian rodent reproduction and development can be viewed as a 'model' through which a complete life cycle (*mating, gestation, birth, infancy → adulthood, mating and subsequent development*) can be expressed in space and in altered gravitational environments. This capability will be rewarded by discoveries in a multitude of disciplines that are already important in the space biological sciences: musculoskeletal development and function, cardiovascular function, immunology, and neurovestibular function, to name a few. In each of these areas the questions are 'classic' and clear. There are roles of gravity to be discovered and analyzed in terms of direct effects as

well as activity-based regulation of development. Each of these systems is known to be actively 'adaptive', showing alterations in the adult system. Here, developmental analysis can be often used as a tool with which to magnify and clarify a process detected in the adult system, because the underlying processes are often accelerated in the young, rapidly forming system.

4. Numerous studies from ISS (including NASA and JAXA research), the Space Shuttle and Russian satellites (e.g., Cosmo, Mir & BION) suggest areas of highly productive and meaningful research relevant to health in space and on Earth. As an example, during prenatal development in rats, the cellular and pathway organization of the vestibular nuclei in the brainstem appear dramatically altered by nine days of microgravity exposure. Following the spaceflight exposure, early behavior of offspring on Earth showed commensurate effects (Ronca et al., Behavioral Neuroscience, 2008). *These observations point directly to the possibility of using gravity as a tool in understanding the organizing principles of the vestibular system.* Further work is needed to delineate early critical periods for vestibular system development and function in addition to later life outcomes. The benefits could be enormous, not only for astronauts, but for many humans who are recovering from motor system damage or who are adjusting to poorly understood changes in the vestibular system during aging. Likewise, studying mice with disrupted vestibular systems in altered gravitational environments will lead to a deeper understanding of the molecular processes of brain and body development.
5. Reproductive and developmental analyses can contribute significantly to predicting and understanding long-term effects on adult systems, effects that will not be possible to observe until sufficient long-term experiments are attempted. Thus, in the near term, research in these areas can set the stage for safe and reliable space exploration in the future, when humans will endure their first long, uninterrupted exposures to reduced and microgravity conditions, as well as radiation.

Knowledge Gaps

Fertility: Bisha and Luderer recently reviewed spaceflight effects on fertility (Nat Rev Endocrin, 2019); unfortunately, there is a paucity of studies examining the effects of cosmic radiation, microgravity, or other stressors of spaceflight on gonadal function and fertility. Not only is this of critical concern for female astronauts who often delay parity until post-flight and have increasing maternal age at first flight but is important to our understanding of fecundity in the cosmic environment in general (Steller JG, et al, Aerosp Med Hum Perform. 2020). Only one mammalian fertility study, the 1979 COSMOS 1129 spaceflight has carried paired male and female rats into space. Following an 18.5-day flight (sufficient time for 2-3 matings) and return to Earth, no pregnancies were observed. This lack of pregnancy could simply be due to an inability to copulate in space, or to more complex endocrine and embryonic developmental causes (Serova and Denisova, Physiologist 1982). Recent evidence shows that female mice on-orbit for 37 days and dissected in microgravity (thus, eliminating re-entry stressors) are experiencing estrous cycles, essential for fertility (Hong, et al, npj Microgravity, 2021). In males, a recent JAXA study, showed that after 35 days on orbit (two spaceflight groups: one experiencing microgravity and another artificial gravity at 1G via centrifugation while on ISS) and subsequent return to Earth, that sperm harvested from both males were successful in fertilizing and siring healthy offspring and, further, that offspring appeared fecund (Matsumura, et al, Sci Rep, 2019). A recent NASA collaboration with Russian scientists, characterized mRNA, protein, and epigenetic modifications in reproductive tissues of space-flown males, showing some epigenetic alterations (Ogneva, et al, Sci

Rep, 2019). For both sexes, longer duration studies are needed to monitor and understand effects over the full duration of oogenesis and spermatogenesis.

Pregnancy and Parturition: Three studies have examined the effect of spaceflight on mammalian pregnancy (reviewed by Ronca, Advances in Biology & Medicine, 2005). In 1982, COSMOS 1514 examined the effect of a 4.5-day flight exposure on gestational day (E) 13-18 of a 21-day rat pregnancy. Dams euthanized immediately upon landing and the fetuses were noted to be smaller with bone development affected. Four of five dams successfully delivered their litters post-flight. Additional findings included poor maternal weight gain and evidence of fetal growth restriction in comparison to controls, possibly related to the potato diet used to provision both water and food, but no change in litter dynamics. NIH.R1 (STS-66) and NIH.R2 (STS-70) missions followed in 1994-1995, which also evaluated dams and offspring exposed to spaceflight from mid-to-late gestation. Spaceflown pregnant rats had twice as many lordosis contractions during labor and decreased uterine myometrial connexin 43 (gap junction) protein expression in comparison to controls, while maternal weight gain, miscarriage/stillbirth rate, litter size, neonatal birthweight, placentophagia, and maternal care characteristics, were not significantly different. Offspring delivered from the COSMOS 1514, NIH.R1 and NIH.R2 missions were viable and normal, however, it is noted all three studies flew for a short period of the pregnancy and all after offspring organogenesis and returned to Earth for parturition. While these studies were hypothesis generating and foundational for pan-pregnancy mammalian studies in space, no further studies involving mammalian pregnancy in space have occurred 1995. Similarly, there are a paucity of studies investigating terrestrial analogs for microgravity such as hindlimb unloading or cosmic radiation during pregnancy.

Neonatal Development & Weaning The 1996 NIH.R3 (STS-72) and 1998 NeuroLab (STS-90) studies evaluated neonatal survival following 8–16-day spaceflight exposure of rat pups ranging from 5-30 days of age. Amongst the earliest pups flown a very high rate of neonatal demise was observed, however, pups launched after 15 days of age exhibited 100% survival. Further studies have not been performed since investigating the ability to neonates to adapt and develop in the microgravity environment.

Developmental Studies Outlined below are several key areas where reproduction and subsequent early embryonic development in space will be essential to establish the consequences of how space impacts these key systems.

Vestibular Development Gaps exist in knowledge of gravity's influence on (1) vestibular system development from peripheral end organs to central nervous system targets, including ground-based studies focused on genetically altered models and selective ablation of peripheral organs during development; (2) vestibular interactions with other neural systems (i.e., homeostatic and circadian systems, autonomic system, formation and maintenance of neural maps, affective system); (3) influence of efferent systems on the development and maintenance of peripheral vestibular apparatus; (4) development of posture, gait, and locomotion; (5) mechanisms for vestibular compensation; and (6) role of vestibular experience in complex navigational behavior. Space and ground-based studies are needed to address these gaps.

Motor Development and Sensory-Motor Integration A major void exists in understanding how developmental exposure to the space environment affects the formation, development, function, and maintenance of the motor system. It is important to ascertain whether these effects reflect reorganization of a motor system developing in ways that are appropriate to the environment within which it has developed and the extent to which post-development adaptation to a different environment can occur. Gravitational loading may be required for proper development and integration of vestibulomotor function. Identifying mechanisms underlying these changes will shed important new light on motor development and its controls.

<u>Neuroplasticity</u> Knowledge is lacking regarding how experience with gravity influences neural development and neuroplasticity, the changes, through new experiences, in neurons, neural networks, and their function. During development, neuroplasticity is shaped by sensory input during critical periods of development. Neurotrophins (i.e., nerve growth factors) play major roles in central nervous system development, plasticity, and experience, including learning by controlling neuronal survival, target innervation, and synaptogenesis. Studies in the space environment and ground-based platforms are needed to address this major gap in understanding how gravity shapes brain development.

<u>Epigenetic Developmental Programming</u> In the past two decades, major conceptual and technological advances in genetics, molecular conservation, and genome sequencing have considerably expanded the scope and depth of developmental biology in ways that are significantly enhancing the ability to uncover fundamental biological principles governing how bodies and brains organize, develop, maintain, and adapt under the constant force of gravity. For example, epigenetic ("above the genome") analysis refers to gene-environment interactions that play a role establishing an individual's phenotype beginning early in life. Epigenetic analysis provides the first mechanistic representation of how environmental factors modulate genetic expression without alterations in DNA and is increasing basic knowledge of how observable molecular changes (e.g., chromatin remodeling, DNA methylation, histone modifications of gene products and others) result in stable, heritable changes in gene expression and later-life phenotypes. An explosion of mainstream biomedical research on developmental epigenetic programming in rodents and humans has shown clear and profound effects of early environmental exposures on reproductive, metabolic, neural and behavioral outcomes that persist across generations. Virtually nothing is known about spaceflight effects on these critical processes within and across generations.

<u>Lifespan and Multigenerational Studies</u> Lifespan studies of mammals to enable identification of gravity-dependent processes in the organization and development of the central nervous system and other organ systems, including their integration, function and maintenance, and transmission across generations, have yet to be performed. The existing data is derived from brief (4.5-16 day) spaceflight developmental studies with post-flight analyses at 1g. High-priority studies include lifespan and multigeneration studies of rodents in the space environment, incorporating developmental programming, epigenetics, omics systems biology and behavioral approaches during key reproductive and developmental phases spanning conception, pregnancy, embryonic and fetal development, birth, postnatal maturation and aging. Throughout these studies, it will be important to determine effects of gravity in relation to those of other space-related factors (e.g., radiation) and their interactions.

Research Questions and Forward Plans

A reproductive-developmental research roadmap and high-priority spaceflight experiments relevant to Reproductive Biology, Neuroscience and Behavior, Musculoskeletal, General

Physiology, Immunology and Commercial Interests was generated in the NASA Rodent Mark III Habitat Workshop (Ronca, et al, 2013, Gravitational Research). It was noted that, for successful multigenerational studies of rodents in space to commence, significant knowledge gaps related to impacts of long-term space flight on reproductive health of males and females must be addressed. Some of the initial questions on female fertility aim to be answered by our team in the upcoming RR-20 mission (presently slated for 2023), though longer duration studies are needed in both males and females to characterize and understand germline effects. Further, multiple habitats with differing capabilities will be required to meet requirements for successful breeding, birthing and nursing, maturation and aging.

In summary, we believe that the study of reproduction and development of mammals in space can serve as springboard for better understanding how gravitational forces and radiation impacts life on earth. Studies in these two areas have been instrumental in much of our current understanding of genetics, epigenetics, stem cell and organismal biology. Moving forward, no agency is better suited than NASA for establishing Earth's impact on biological systems by studying mammalian development in the weightless of space. We have defined the key intellectual foundations and believe that the ISS and emerging space platforms can be equipped for these important scientific pursuits.