

The Effects of the Spaceflight Environment on the Vaginal Mucin Layer of the Mouse

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ABSTRACT

It has been well documented that spaceflight has adverse effects on many tissues and systems throughout the body. Although this phenomenon is well documented, relatively little research has been done in the area of the female reproductive system. If spaceflight has harmful effects on the female reproductive system, the migration of the human species into space would be greatly compromised. The purpose of this study was to determine the effects of spaceflight on the thickness of the apical mucin layer in the vaginae of mice, as changes in this layer could have detrimental effects on sperm survival and, therefore, a profound impact on the animal's ability to reproduce. This study examined the thickness of the vaginal mucin lining from female mice that were exposed to 13 days of spaceflight and their concomitant controls. The tissues were stained using a technique commonly used to localize and analyze mucin varieties. The tissue was qualitatively analyzed for the type of mucin produced (i.e., acidic, neutral, acidic/neutral

mixture). Further, the tissue was quantitatively analyzed for the amount of mucins produced by measuring the thickness of the mucin layer. The results of this study indicate that spaceflight causes a thickening of the mucin lining of the vaginal canal. The results further indicate being housed in an Animal Enclosure Module also caused a thickening of the vaginal mucin layer — presumably due to internal cage environmental factors — but this effect was not as pronounced as that seen in the spaceflight mice.

INTRODUCTION

Many studies have documented the effects of spaceflight and simulated microgravity on various tissues and systems of the body. Some of these systems include, but are not limited to: skeletal muscle arterioles and regional blood flow (Arbeille et al., 1996; Delp, 1999), the skeletal system (Droppert, 1990; Ferguson et al., 2002; Milstead et al., 2004), the immune system (Armstrong et al., 1993; Chapes et al., 1993; Chapes et al., 1999; Sonnenfeld et al., 2003), the anterior pituitary (Pattison et al., 1991), and the seminiferous tubules of the male reproductive system (Kamiya et al., 2003; Motabagani, 2007; Forsman, 2012). Overall body fluid shifts have also been reported (Tipton et al., 1987). Unfortunately, investigations into the effects of spaceflight on the reproductive system—especially the female reproductive system—have been limited. If the reproductive system is adversely affected by spaceflight, human colonization of space would be problematic for our species.

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Due to the lack of research in this area, the 2011 Decadal Study on Biological and Physical Sciences in Space (National Research Council Decadal Survey, 2011) developed two overarching questions regarding the reproductive systems. The first overarching question of the Decadal Study regarding the reproductive systems was to determine whether successive life cycles could be completed in a microgravity environment. The second question was to determine if reproduction would be affected in spaceflight.

Reproduction — especially in regard to the female — is a multifaceted process. To fully investigate the questions brought forth in the Decadal Study, one must consider several linked factors. First, the production and ovulation of a viable secondary oocyte must be determined because reproduction cannot occur without a viable secondary oocyte. Second, the structures of the female reproductive system that will be responsible for the care and transport of the oocyte (as well as potential zygote/embryo), and subsequent implantation and development to term, must be investigated. This requires studies of the uterine (fallopian) tubes and the uterus. If the reproductive process proceeds to term, will the process of labor be affected by microgravity?

Additionally, it is imperative the female reproductive structures are studied in regard to their ability to interact with the male and, subsequently, successfully care for and transport spermatozoa to the secondary oocyte. This requires not only the study of the previously mentioned female structures, but also studies involving the vagina, as the vagina is responsible for receiving the sperm. If vagina/sperm interaction is modified, it could affect the ability of the sperm to travel farther into the female reproductive system. If sperm are not able to reach the secondary oocyte, there will be no fertilization and thus, no reproduction. Only a few of these factors have previously been addressed.

Mucins are glycoproteins that contain large numbers of O-linked oligosaccharides. Mucins are believed to provide several functions, such as lubrication and protection from pathogens. If one considers the fact that increased microbial virulence has been documented in spaceflight conditions (Klaus and Howard, 2006; Rosenzweig et al., 2010), the fact that mucins play a role in

protection from pathogens becomes increasingly important.

Mucins are found on the apical surface of many epithelia — including the uterine tissue (Gipson et al., 1995) — and are essential to reproduction in the female. Mucin genes are widely expressed in the tissue of the reproductive tract and each reproductive organ expresses a particular set of these genes (Lagow et al., 1999). It has been shown the expression of these genes can be influenced by steroid hormones and various disease states (Hebbbar et al., 2005). This entices the question: “Can gene expression be altered by the spaceflight environment?” The mucins that line the female reproductive tract provide an environment conducive to sperm maturation, gamete interaction, and early embryonic development (Gandolfi et al., 1989). Eight varieties of mucins have been related to the female reproductive tract in humans. These have been designated as MUC1-MUC7, with two subsets of mucins. All epithelia of the female reproductive tract express MUC1, which is a transmembrane mucin produced by most epithelia (Warren and Spicer, 1961). Mucins that contain sialic acid are commonly referred to as sialomucin complexes (SMC). MUC4/SMC is expressed at the apical surface of most epithelia of the female reproductive tract, including both uterine luminal and glandular epithelium. These SMCs have been shown to block cell and molecular recognition processes, which renders the apical surface of cells with this type of mucin non-adhesive (Carraway et al., 1992). This type of mucin has been found to be hormonally regulated in uterine luminal epithelium, but not in uterine glandular epithelium, vaginal epithelium, cervical epithelium, or the epithelium of the uterine tube (Idris and Carraway, 1999). McNeer et al. (1998) and Carraway and Idris (2001) reported SMC expression is tightly regulated in the uterus and its expression appears to block blastocyst implantation.

Studies of the apical mucin layer that lines the various regions of the female reproductive tract in mice indicate microgravity has varying effects on the mucin layer thickness, depending on the region involved. This is not unexpected since the mucin lining in each region of the female reproductive tract has a different function. Examination of the uterine tissue from STS-118

indicated spaceflight caused a thickening of the apical mucin layer (Forsman and Nier, 2013). This is a potentially significant finding since thickening of this layer would provide a greater barrier to blastocyst implantation (Carraway and Idris, 2001), resulting in a decreased chance of pregnancy.

Examination of the apical mucin layer of the uterine tubes from the same mice indicated the effect of spaceflight varied depending on the region of the uterine tube. Svalina and Forsman (2013) found in all regions of the uterine tube the baseline animals always had the thickest mucin layer. The mucin layer of the ampulla region from spaceflight animals was significantly thinner than that of the baseline animals. No significant change was found in the mucin layer of the infundibular region of the tube, regardless of treatment type. This is a potentially significant finding since the uterine tube functions for the transport of gametes and is an active secretory organ whose secretions provide a suitable environment for continued maturation of male gametes, interaction between gametes, and early embryonic development (Gandolfi et al., 1989). Modification of the mucin layer in the uterine tubes could have detrimental consequences on the survival of the oocyte, sperm, or embryo. If one or any combination of these three is impaired, the chances of a successful pregnancy are greatly reduced.

Mucins of the vaginal canal provide lubrication and protection from microbial invasion and infection (Idris and Carraway, 1999). The general acidity of vaginal mucin is the female reproductive system's first line of defense against possible microbial invasion. Normal vaginal pH is between 3.5-4.0 (Masters and Johnson, 1966). This pH range is generally inhospitable to many harmful bacteria and, therefore, is necessary to provide the female reproductive tract with some defense against possible invading harmful microorganisms. These vaginal and cervical mucins also play a role in sperm capacitation/motility. The optimal pH for sperm motility is between 7.0 and 8.5 (Tampion and Gobbons, 1963; Moghissi et al., 1964), and sperm motility has been shown to be reduced at pH values lower than 6.0 (Markler et al., 1981; Peek and Matthews, 1986). It has been shown that vaginal pH rises to approximately 7.0 within seconds after the introduction of ejaculate (Fox et

al., 1973). If vaginal mucins were to become more acidic than their normal state, it may result in vaginal pH remaining too low (below pH 7.0) and render an environment inhospitable to sperm, resulting in reduced fertilization (Brannigan and Lipshultz, 2008). With this in mind, this research project focused on changes in the female reproductive system by examining the mucin layer of the vaginal tissue of spaceflight mice.

MATERIALS AND METHODS

Twelve (12) C56BL/6 female mice (Charles River, Wilmington, MA) were flown on NASA space shuttle mission STS-118 in August 2007. The mice were approximately eight weeks old at the time of launch. The duration of the spaceflight subjected these animals to approximately 13 days of microgravity. The flight (FL) mice were housed in the animal enclosure module (AEM) of the Commercial Biomedical Testing Module-2 (CBTM-2), which was part of the payload in the shuttle's mid-deck flight locker. A set of 12 analogous mice — considered ground control (GC) mice — were housed in similar ground-based AEM/CBTM-2 enclosures. These ground-based enclosures and GC mice were run at a 48 hour delay in relation to the FL mice. This allowed for reproducing the same environmental factors as those experienced onboard the space shuttle (i.e., temperature, humidity, and light/dark cycles), except for the microgravity environment. A third set of 12 mice, considered baseline (BL) mice, were kept in standard rodent cages in standard conditions: 12/12 light-dark cycles at room temperature. Both BL and GC samples were maintained in the Space Life Sciences Lab, Kennedy Space Center, FL. Within a few hours of mission completion, the FL mice were transferred from the shuttle mid-deck flight locker to the Space Life Sciences Lab where, within minutes, the vaginal tissue was harvested from each mouse and the preservation steps initiated. Preservation involved immersion of tissues in 4% paraformaldehyde in 0.1 M PO_4 buffer (pH 7.4) for 12 hours at 4°C. This was followed with three washes in 0.1 M PO_4 with 2% sucrose and 50 mM NH_4Cl (pH 7.4) for 1 hour at 4°C and two washes in 0.1 M PO_4 with 50 mM NH_4Cl (pH 7.4) for 1 hour at 4°C. The tissues were then dehydrated using increasing concentrations of

ETOH and embedded in paraffin using standard embedding techniques. The tissue was stored until use in this study.

Each sample was sectioned at four microns using a Microm HM325 microtome, mounted on glass slides, and stained using an Alcian Blue Periodic Schiff procedure. The tissue was then dehydrated, cover-slipped, examined, and photographed using a Zeiss Axioskop 40 microscope equipped with a Cannon Powershot A640 camera. For each animal sample, three slides were prepared. Measurements were made using the Carl Zeiss AxioVision software, version 4.7.0. Using a randomization grid, a set of five random measurements of the thickness of the mucin layer was made from each of the three slides, giving a total of 15 measurements per sample. The average mucin thickness (in micrometers) was then calculated for each sample. A one-way ANOVA was made using MiniTab statistical software. The stained tissue was also qualitatively analyzed for the type of mucin present (i.e., acidic, neutral, a mixture of acidic and neutral) based on the color of the stained mucin. Using this staining technique, neutral pH mucins stain magenta, acidic mucins stain pale

blue, and mixtures of acidic and neutral mucins stain purple. The results are based on seven flight samples, 12 ground control samples, and 10 baseline samples.

RESULTS/DISCUSSION

Because this is a study of the female reproductive system, one must consider the stage of the estrous cycle between animals when making comparisons. Due to the tissue sharing nature of these animals, it was not possible to obtain blood hormone data or vaginal smears to indicate the stage of estrous of each individual animal. However, due to the numbers of female mice per cage and the absence of any male mouse or male mouse excreta, it is reasonable to assume that the Lee-Boot effect (Whitten, 1959) had synchronized all of these animals into an extended period of diestrus. Thus, hormonal changes due to estrus were eliminated and not considered as variables. The thickness and general pH of the apical mucin layer of the vaginal canals from BL mice (Figure 1), GC mice (Figure 2), and FL mice (Figure 3) were quantitatively and qualitatively compared.

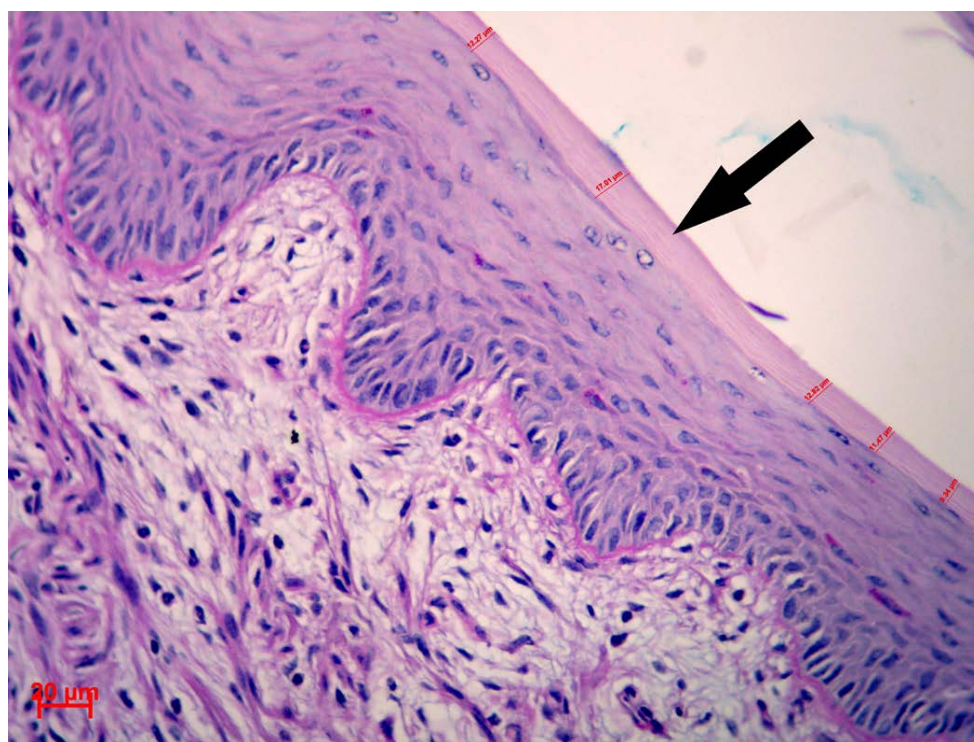


Figure 1. Vaginal epithelium from Baseline tissue (400X). Mean mucin layer thickness for Baseline tissue = 6.5633 μm . Note the pale blue staining of the mucin layer indicative of an acidic mucin (arrow).

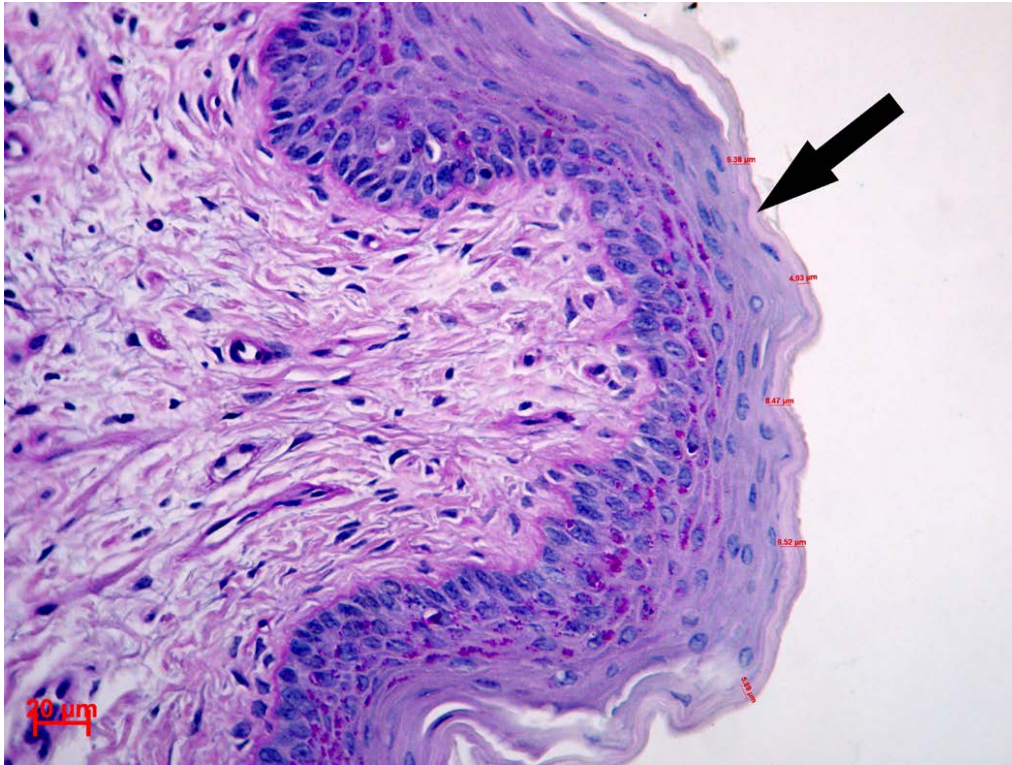


Figure 2. Ground Control Tissue (400X). Mean mucin layer thickness for Ground Control tissue = 10.0310 μm . Note the pale blue staining of the mucin layer indicative of an acidic mucin (arrow).



Figure 3. Flight tissue (400X). Mean mucin layer thickness for Flight tissue = 17.3520 μm . Note the pale blue staining of the mucin layer indicative of an acidic mucin (arrow).

Our studies indicate the mucin layer of the GC tissue was thicker than that of the BL, but this thickening was not statistically significant ($P=0.522$). However, the results showed the thickness of the mucin layer of the BL compared to the FL mice was significantly different ($P=0.040$). Interestingly, the mucin thickness of the FL mice was not significantly thicker than that of the GC mice ($P=0.211$) (Figure 4). The mucin layer of the FL mice was visually much thicker compared to the other two groups. For seven of

the BL tissues and six of the GC tissues, the mucin layer was too thin to be measured. It is important to note this was never the case for any of the FL tissues. The mucin layer for all samples was qualitatively examined to determine the general pH of the mucin. Neutral pH mucins stain magenta, acidic mucins stain pale blue, and mixtures of acidic and neutral mucins stain purple. All tissue samples in this study were found to be acidic in composition, regardless of treatment group.

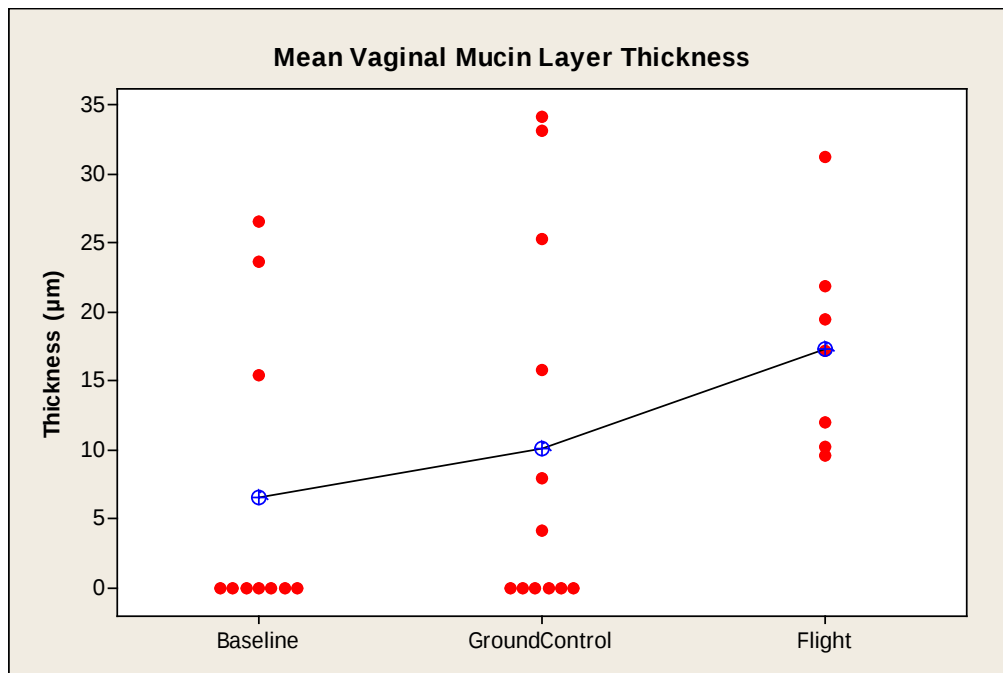


Figure 4. Graph comparing the mean apical mucin thickness between the BL, GC, and FL animals

These studies show there was a trend toward a thicker mucin layer of the vagina between the BL and GC mice. This is very interesting because it implies the AEM itself had some effect on the mucin layer — regardless of the gravitational situation — but that spaceflight also added to the thickening of the mucin layer. A possible explanation for this observation is mice are generally social animals that normally burrow into their bedding and sleep huddled with other mice. The AEM does not contain bedding; therefore, the mice (both GC and FL) are unable to bury themselves in bedding, possibly affecting the

animals' ability to maintain a normal body temperature. This could also explain the difference between the GC and FL mice, because the GC mice could still huddle and sleep together for warmth, whereas the FL mice — due to the nature of the microgravity environment — would have difficulty huddling while awake and would not be able to huddle while sleeping. This could make it even more difficult for the mice to adequately maintain normal body temperature, and consequently put these animals in a distressed state. There are also other environmental factors of the AEM that do not exist with standard rodent

cages, such as noise from the AEM ventilation system and a constant air flow, both of which could also present a stressful situation for the animals. Previous studies by Castagliuolo et al. (1996) indicated 30 minutes of immobilization stress can cause a significant increase in colonic mucin release in rats. This is a good indicator there is a need for further research to investigate the possible effects of stress on mucin release in the various regions of the female reproductive system.

The factor of spaceflight responsible for causing a thickening of the vaginal mucin layer is yet to be determined. It is possible the thickening is due to the microgravity of spaceflight, but it could also be caused by the high dosage of cosmic radiation experienced by the spaceflight mice. Experiments are currently being conducted to test this hypothesis. As previously stated, it has been well documented spaceflight has adverse effects on the immune system. One of the functions of vaginal mucin is to act as a barrier to possible invading microbes. Vaginal mucin is normally acidic in nature, which retards bacterial reproduction. Perhaps the thickening of the vaginal mucin in spaceflight is a response to ensure the animal will not contract an infection through the vaginal canal. Such an infection would be hard to fight for an animal with a depleted immune system. This thickening of the acidic vaginal mucin layer could also interfere with the ability of the female mouse to become pregnant, since acidic environments are highly spermicidal.

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