# Ovarian Follicular and Luteal Development in the Spaceflight Mouse

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## ABSTRACT

The effects of space travel are relatively unexplored in regard to the female reproductive system. An important step in determining possible adverse effects on the human female reproductive system is the analysis of test animal data. This study analyzed the ovarian tissue of mice flown aboard space shuttle Endeavour on NASA mission STS-118. The experiment consisted of three groups of animals: two sets of control animals and a single set of flight animals. Each set consisted of twelve individual mice. The flight animals were housed in the Animal Enclosure Module (AEM) of the Commercial Biomedical Testing Module-2 (CBTM-2) over the 13 day flight. One set of control animals (baseline) were housed in standard cages at room temperature. The other set of control animals (ground control) were housed in ground based AEMs which were environmentally controlled to match the conditions aboard the shuttle Endeavour with a delay of 48 hours and subject to normal gravity. The ovarian tissue samples were fixed in 4% paraformaldehyde, paraffin embedded, sectioned, mounted, and stained using standard Hematoxylin and Eosin staining procedures, and cover-slipped. The gross morphology of the tissue was then qualitatively analyzed. The flight animals were compared to the baseline and ground control sets. The presence of developing follicles of all stages as well as the presence of corpora lutea in all three treatment groups indicates no significant gross morphological changes occur within ovarian tissue when exposed to spaceflight for 13 days or less.

## INTRODUCTION

The effects of spaceflight and simulated microgravity on various tissues and systems of the body have been well documented. Changes have been documented in the skeletal system (Droppert, 1990; Ferguson et al., 2002; Milstead et al., 2004), skeletal muscle arterioles and regional blood flow (Arbeille et al., 1996; Delp, 1999), immune system (Armstrong et al., 1993; Chapes et al., 1993; Chapes et al., 1999), and seminiferous tubules (Kamiya, et al. 2003; Motabagani, 2007; Forsman, 2012) to name a few. Overall body fluid shifts (Tipton et al., 1987) as well as changes in the anterior pituitary (Pattison et al., 1991) have also been documented. One major area that has not been extensively examined is the female reproductive system.

The report from a meeting of the Space Studies Board and National Research Council in 1987 stated that it was of particular importance to

determine whether or not the space environment would interfere with human and/or animal reproduction (Moody and Golden, 2000). This was reiterated in the 2011 Decadal Survey on Biological and Physical Sciences in Space; however, this report did not give reproductive studies high priority (National Research Council Decadal Survey, 2011).

A fully encompassing study of the effects of spaceflight on mammalian reproduction must consider all aspects of reproduction, including hormonal interactions, from the development and ovulation of a secondary oocyte to delivery and weaning of the newborn and all aspects in between. To date no study has been conducted that addresses all of these issues.

There have been spaceflight studies related to the reproductive system that focus on the development of a new organism across several levels of taxonomy. The vertebrate species studied include Medaka fish (Ijiri, 1998), amphibians (Smith and Neff, 1986; Huang and Johnson, 1995; Souza et al., 1995), birds (Skrobanek et al., 2008; Huss et al., 2010), and rats (Wong and DeSantis, 1997). The number of spaceflight studies on mammalian female reproduction has been very limited and most of these have been conducted on animals that were already pregnant before the onset of the flight. Renengar et al. (1995) reported no significant effects of spaceflight on the placental structure in rats. Studies conducted by Burden et al. (1995) evaluated ovarian follicles, corpora lutea, luteinizing hormone, and follicle stimulating hormone from pregnant rats subjected to spaceflight. These studies showed no effect of spaceflight on any of the parameters studied. Burden et al. (1998) found that myometrial smooth muscle decreased by 37% between the 20th day of gestation and postpartum in spaceflight rats compared to synchronous controls. Studies by Burden et al. (1999) reported more labor contractions in rats delivering immediately after spaceflight compared to control animals. This is presumed to be due to decreased uterine levels of connexin 43 reported in the study. The incidence of increased labor in post flight rats was also supported by the observations of Ronca and Alberts (2000). Bhat et al. (2001) reported reduced progesterone production by luteal cells from the pregnant rat. These findings

were obtained from cell cultures grown in simulated microgravity conditions, so it is yet to be determined if these same effects are seen in an intact system.

Very few studies have had the opportunity to examine the female reproductive structures in non-pregnant mammals. Gupta et al. (2010) and Tash et al. (2011) examined the reproductive tissues from mice flown on space shuttle mission STS-131 and found that most follicles from spaceflight ovaries were atretic and there were fewer numbers of corpora lutea compared to the baseline and ground control animals. A trend toward smaller uteri in flight animals was also reported. Nier and Forsman (2011) reported that spaceflight caused a thickening of the apical mucin layer in the uterus of mice flown on space shuttle mission STS-118.

The ovaries, being located in the abdominal cavity, may be affected by some of the previously documented changes.  Cephalad fluid shifts would undoubtedly change the microenvironment surrounding the ovaries, and via changes in regional vascular/interstitial pressures, perhaps the internal ovarian environment as well. Since the estrous cycle is under control of hormones (follicle stimulating hormone and luteinizing hormone) from the anterior pituitary, it is possible that changes in that gland may also cause changes in the ovaries. With the understanding that one would expect to see changes in the ovaries of mice subjected to spaceflight, this study focused on examining the gross morphology of the ovarian tissue from mice flown on shuttle mission STS-118 and tissues from corresponding control animals.

## MATERIALS AND METHODS

The animals used in this study were a subset of animals utilized by the Amgen Corporation (Thousand Oaks, CA). All mice used in these experiments were C56BL/6 female mice (Charles River, Wilmington, MA). The mice were initially divided into two groups of animals designated as drug treated mice (DM) and vehicle mice (VM). These groups were then subdivided into 3 treatment groups: flight (FL), ground control (GC), and baseline (BL). The drug treated group was proprietary and all tissues from this group were retained by Amgen. For all three treatment groups the VM were randomly mixed with the

DM. All of the FL and GC mice were housed in the animal enclosure module (AEM) of the Commercial Biomedical Testing Module-2 (CBTM-2). The FL AEMs were flown on shuttle mission STS-118 in the shuttle mid-deck locker. This exposed the FL mice to approximately 13 days of spaceflight. The GC AEMs (housed at the Space Life Sciences Lab at Kennedy Space Center) were populated with the same number of mice as the FL AEMs and were conducted at a 48 hour delay from the FL animals to allow for reproducing the environmental conditions experienced on board the shuttle. Each AEM contained 8 mice configured 4 to a side. There were 3 FL AEMs for a total of 24 FL mice; 12 proprietary DM and 12 VM which were available for this study. Accordingly, there were 3 GC AEMs for a total of 24 GC mice; 12 proprietary DM and 12 VM which were available for this study. The BL mice were housed in standard rodent cages at the same population density. These mice were also housed at the Space Life Sciences Lab at Kennedy Space Center. The 12 BL VM were available for this study. All mice were approximately 9 weeks old at the onset of the mission. Upon mission completion, the ovarian tissues were harvested from each animal,

fixed in 4% paraformaldehyde, dehydrated, and paraffin embedded using standard embedding techniques. The embedded tissue was stored until use in this study. Each sample was sectioned at approximately 6 microns, mounted, and stained using a standard H & E staining protocol. The tissue was then examined using light microscopy. The FL, BL, and GC tissue was qualitatively analyzed for possible alterations in the morphology of developing follicles and corpora lutea. Follicular stages were based on guidelines set forth by Oakberg (1979).

## RESULTS

Figures 1 and 2 depict light microscopic evaluation of the ovarian tissue from FL animals indicating the presence of stage 3a, stage 4b, and stage 7 follicles as well as corpora lutea. Similarly, evaluation of the ovarian tissue from GC animals depicted in Figures 3 and 4 indicates the presence of stage 4a and 4b follicles as well as stage 5a and stage 7 follicles and corpora lutea.

Figures 5 and 6 show that examination of baseline tissues indicates the presence of stage 3a follicles, 5b follicles, and stage 7 follicles as well as corpora lutea.
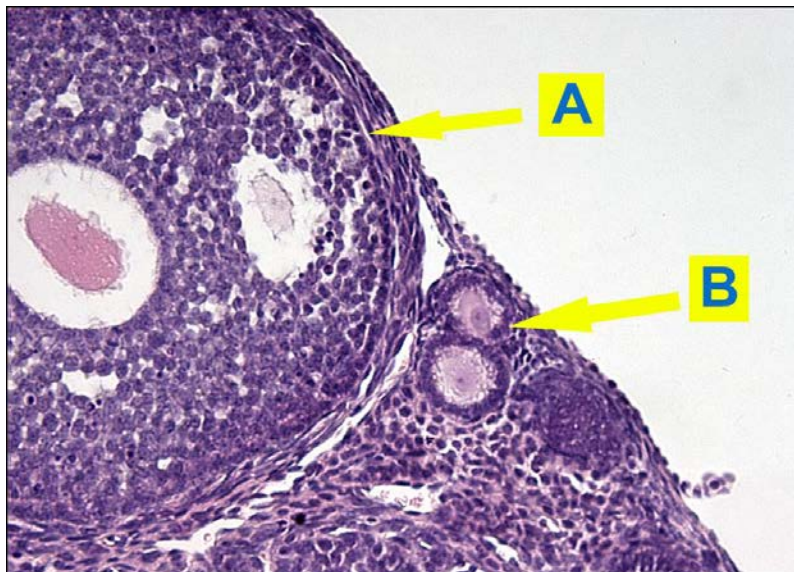


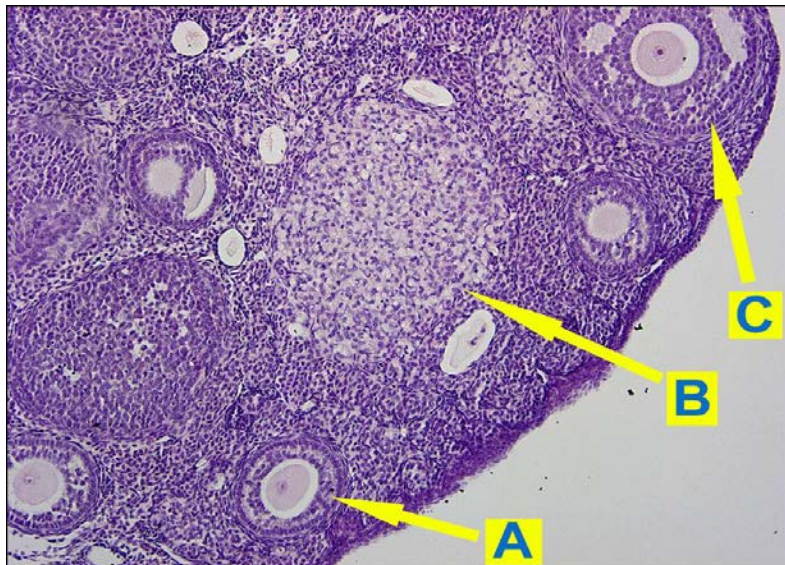**Figure 1. Ovarian tissue from FL animal #10, A – stage 7 follicle, B – stage 3a follicle (400X).**

**Figure 2. Ovarian tissue from FL animal #21. A- stage 4b follicle, B – corpus luteum, C – stage 7 follicle (200X).**
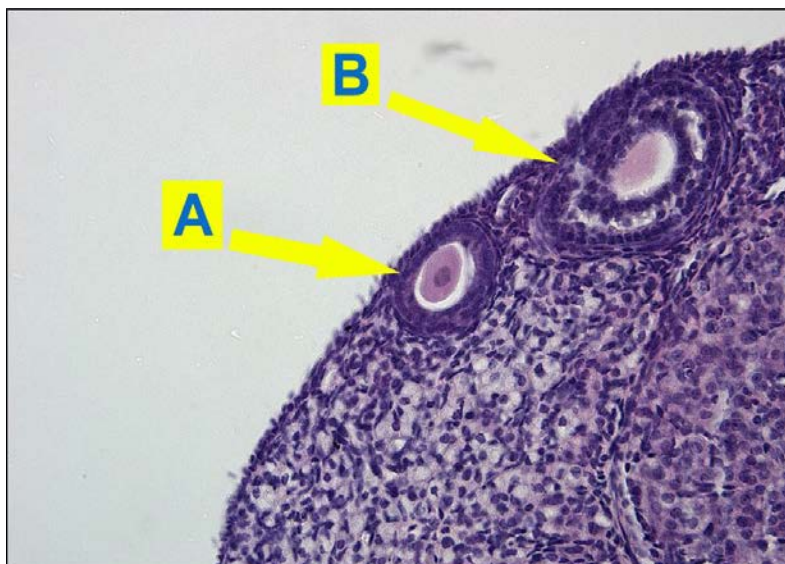


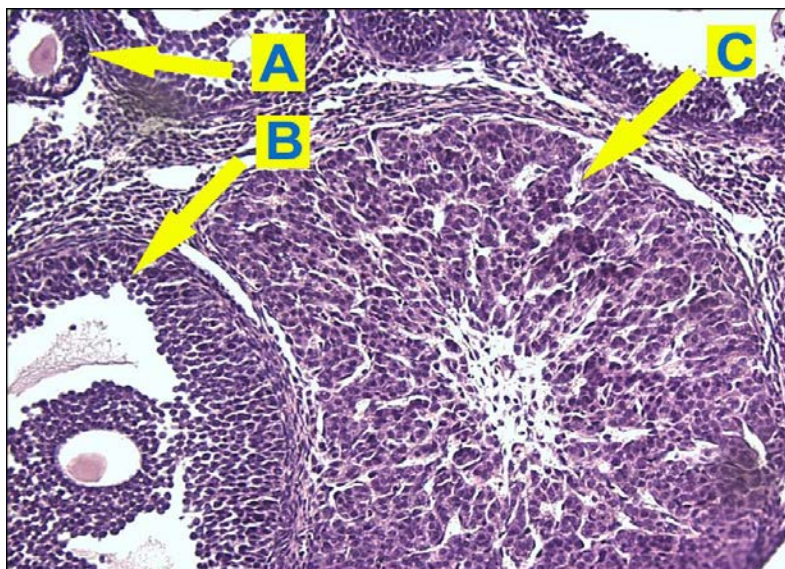**Figure 3. Ovarian tissue from GC animal #34. A –stage 4a follicle, B – stage 4b follicle (400X).**



**Figure 4. Ovarian tissue from GC animal #53. A – stage 5a follicle, B – stage 7 follicle, C – corpus luteum (200X).**
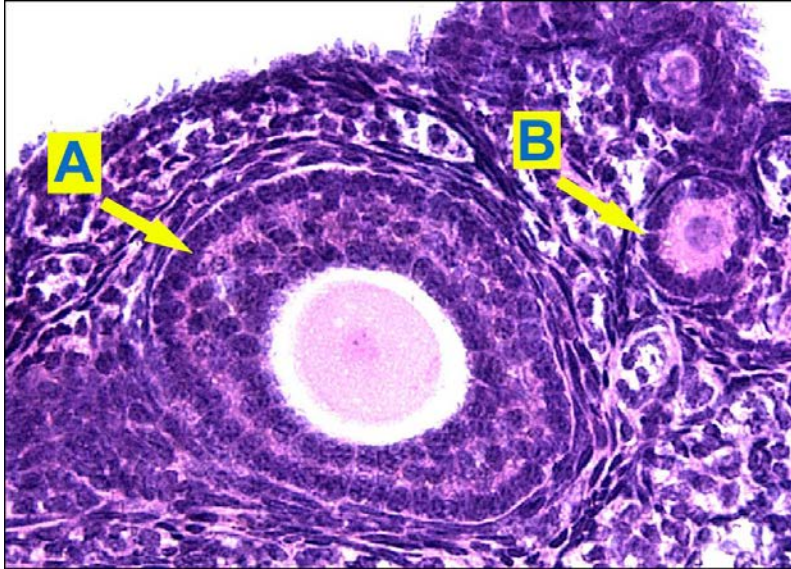
**Figure 5. Ovarian tissue from BL animal #68. A – stage 5b follicle, B – stage 3a follicle (400X).**
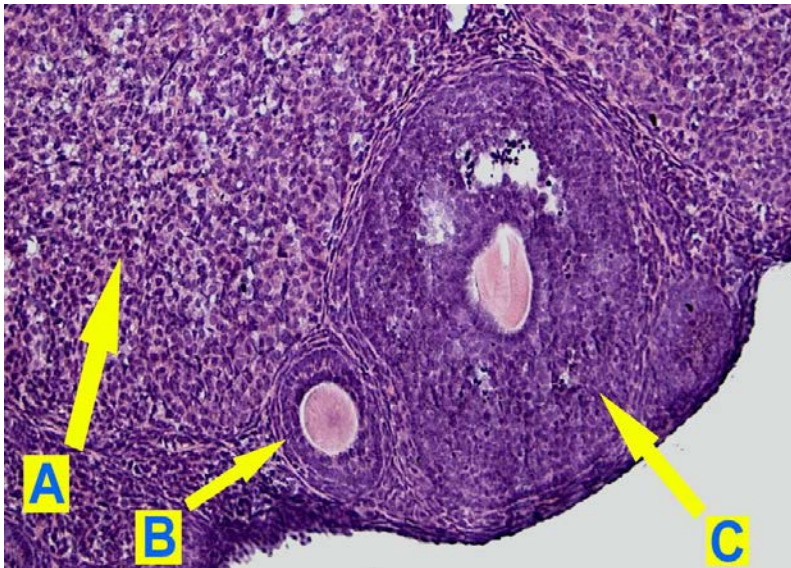


**Figure 6. Ovarian tissue from BL animal #70. A – corpus luteum, B – stage 5b follicle, C – stage 7 follicle (200X).**

## DISCUSSION

This study indicates that no gross morphological changes occur with regard to follicular development and the formation of corpora lutea in the ovarian tissue of mice exposed to the spaceflight environment for a period of 13 days or less. FL tissue was morphologically indistinguishable from both GC and BL tissue. GC and BL samples were indistinguishable from each other as well. The qualitative observations from this study indicate that ovarian follicles of each stage of development as well as corpora lutea were present in all ovaries from FL, GC, and BL mice. These findings are not surprising given the duration of this shuttle mission—12 days, 17 hours, 56 minutes. The findings of this study are in direct contrast with the findings of Gupta et al. (2010) and Tash et al. (2011). This clearly indicates that further experiments in this area are necessary. There are several factors that could account for the discrepancies. Future experimental designs should take into account the overall intricacies of the female reproductive system and its inter-relationship with the other systems of the animal. Factors that must be considered are outlined in the next few paragraphs.

Although the mouse is generally described as having a 4-6 day estrous cycle (Allen, 1922;

Parkes, 1928), follicular development spans several cycles. Using [3H]thymidine labeling, Pedersen (1970) estimated that it takes approximately 19 days for a stage 3 (primary) follicle to reach maturity and ovulate. This number may be greatly underestimated. Oakberg (1979), using labeling of the zona pellucida to follow follicular growth, estimated that it would take slightly more than 6 weeks for such a follicle to mature. Applying the numbers from either of these studies would indicate that many of the follicles and corpora lutea seen in our samples were already well along in their development before the launch of the shuttle. This raises the question as to whether or not spaceflight may have slowed the development of the follicles. Based on the number of stage 3a (primary) follicles seen in FL, GC, and BL tissues, this would not seem likely; however, primary follicles are seen at all stages of the estrous cycle (Bassett, 1943).

A further complication in this study is related to the population density of the AEMs. In the 1950s it was well documented that estrous cycles in mice were frequently interrupted by prolonged diestrous intervals. These intervals were initially believed to be spontaneous pseudopregnancies (van der Lee and Boot, 1955; van der Lee and Boot, 1956). Further investigation into this phenomenon indicated that it was caused by overcrowding of the animals (4 or more per cage) in the absence of a male or male excreta. Whitten (1959) showed that grouping of female mice produced a highly significant reduction in the incidence of estrous. The effect was consistently observed when the group was maintained for 3 months. This effect was reversed within a few days of introduction of a male. This indicated that the effect, which has been termed the Lee-Boot Effect, is not pseudopregnancy, since the duration of pseudopregnancy is not altered by the presence of a male. Since the mice used in our study were group housed in AEMs or standard rodent cages, and without the presence of a male, it is almost certain that the Lee-Boot effect either extended the length of the estrous cycle or arrested it in the diestrous stage. The fact that the ovaries did contain follicles from all stages, as well as corpora lutea, indicates that, from an ovarian aspect, reproduction in mice subjected to

spaceflight should not be significantly altered given interaction with a male mouse.

These findings also do not provide any data as to the ability of the observed corpora lutea to produce adequate levels of estrogens and progesterone and deliver these hormones into the circulation. The corpus luteum, being a temporary endocrine organ, requires an extensive blood supply. The vasculature of the ovaries of rats (Bassett, 1943) and hamsters (Forsman and McCormack, 1992) has been well documented and blood flow to the ovary is higher in the luteal phase than in the follicular phase (Varga and Greenwald, 1979). If the regional blood flow in the abdomen has been altered during spaceflight, it is conceivable that an increase in luteal blood vessels would be necessary to maintain its function. Anecdotal evidence obtained in our lab from antiorthostatically suspended female hamsters indicates that the capillary density of the corpora lutea may be increased.

While no morphological differences were determined in this study, the results do not preclude the possibility of underlying, long-term effects of spaceflight. Because the tissue obtained in this study was part of a tissue sharing program involving researchers from several areas of interest, it was not possible for us to obtain data involving blood hormone levels or vaginal smears, which would have made it possible to determine the specific stage/s of the estrous cycles. Simple histological studies such as this one are necessary, but can only give limited data. Hormone levels and vaginal smears to determine the exact stage of the estrous cycle would have significantly furthered the goals of this study.

As has been outlined in this paper, to be completely accurate, studies involving the female reproductive system must cover a wide range of issues. Future studies of this nature should consider the stages of the estrous cycle for each mouse both pre and post flight. This should be confirmed with hormonal data as well as by vaginal smears. Mice should be housed in as small groups as possible. The scent of male mouse excreta could be introduced to circumvent the Lee-Boot effect. Analysis of the ovarian tissue should go beyond gross morphology and should look at indicators such as capillary density and kit ligand expression, to name a few.

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