Fiscal Year:	FY 2015	**Task Last Updated:**	FY 01/02/2015
PI Name:	Luderer, Ulrike M.D., Ph.D.		
Project Title:	Charged Particle Effects on the Ovary		
Division Name:	Human Research		
Program/Discipline:			
Program/Discipline--Element/Subdiscipline:			
Joint Agency Name:		**TechPort:**	No
Human Research Program Elements:	(1) **SR**:Space Radiation		
Human Research Program Risks:	None		
Space Biology Element:	None		
Space Biology Cross-Element Discipline:	None		
Space Biology Special Category:	None		
PI Email:	uluderer@uci.edu	**Fax:**	FY
PI Organization Type:	UNIVERSITY	**Phone:**	949-824-8081
Organization Name:	University of California - Irvine		
PI Address 1:	Center for Occupational and Environmental Health		
PI Address 2:	856 Health Sciences Rd, Suite 3200, Zotcode 1830		
PI Web Page:			
City:	Irvine	**State:**	CA
Zip Code:	92697	**Congressional District:**	45
Comments:			
Project Type:	Ground	**Solicitation / Funding Source:**	2013 Space Radiobiology NNJ13ZSA001N
Start Date:	03/01/2014	**End Date:**	08/31/2016
No. of Post Docs:	1	**No. of PhD Degrees:**	
No. of PhD Candidates:		**No. of Master' Degrees:**	
No. of Master's Candidates:		**No. of Bachelor's Degrees:**	
No. of Bachelor's Candidates:		**Monitoring Center:**	NASA JSC
Contact Monitor:	Simonsen, Lisa	**Contact Phone:**	
Contact Email:	lisa.c.simonsen@nasa.gov		
Flight Program:			
Flight Assignment:	NOTE: End date is now 8/31/2016 per NSSC information and PI (Ed., 12/1/15) NOTE: End date is now 2/28/2016 per NSSC information and PI (Ed., 2/25/15)		
Key Personnel Changes/Previous PI:	Dec. 2014 report: Dr. T. Shioda was not included as a key personnel on the funded one year pilot project and was deleted as CoI.		
COI Name (Institution):	Limoli, Charles Ph.D. (University of California, Irvine)		
Grant/Contract No.:	NNX14AC50G		
Performance Goal No.:			
Performance Goal Text:			

Task Description:	Fifteen percent of astronauts are women, but the risks of space radiation to women's reproductive health and risks of gynecological cancers remain poorly understood. Radiation treatment for cancer is known to cause temporary infertility and premature menopause. Premature menopause increases women's risks for cardiovascular disease, osteoporosis, and Alzheimer's disease. In addition, animal studies and studies of atomic bomb survivors have shown that radiation exposure increases the risk for ovarian cancer. Ovarian cancer has a high mortality rate and is the leading cause of gynecological cancer deaths in women. To best protect the health of women astronauts, it is important to understand whether space radiation has similar effects on the ovary as the types of radiation exposure that are common on Earth. We propose to test the effects of low dose charged particle radiation (low LET oxygen and high LET iron ions), typical of exposures in space, on ovarian follicles (the functional unit of the ovary) and on ovarian carcinogenesis in adult female mice. We will use histomorphometric methods to quantify the effects of charged particles on numbers of ovarian follicles and ovarian tumor multiplicity and size. We will use in situ methods to assess oxidative damage, DNA damage, apoptosis, and proliferation. Our previous studies have shown that oxidative stress plays a role in and the antioxidant glutathione is protective against radiation- and chemical-induced damage to ovarian cells and ovarian carcinogenesis. We will therefore also examine whether antioxidant supplementation is protective against the adverse ovarian effects of charged particle radiation. Our analyses will provide critical insights into whether ovarian damage and tumors caused by exposure to charged particles are biologically similar to those in other mouse models of ovarian cancers and identify potential targets for preventive or therapeutic intervention. These studies will help to fill important gaps in our understanding of the effects of space radiation on ovarian function and ovarian cancer and will lead to better ways to prevent ovarian cancer and protect reproductive health in women astronauts.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	Charged particles are currently being used in cancer therapy. The findings of our research on the ovarian effects of charged particles will increase our understanding of the possible adverse ovarian effects of charged particle radiation used for the treatment of cancer in women. Comparisons of our data on the ovarian effects of charged particles with prior data on ovarian effects of x-rays and gamma radiation will improve our understanding of the sensitivity of the ovary to these different types of radiation.
	CHARGED PARTICLE EFFECTS ON THE OVARY Introduction Currently about 15% percent of astronauts are women, but the risks to the ovary of exposure to galactic cosmic rays and solar particle events during space missions remain largely unknown. The ovary is highly sensitive to gamma radiation. Gamma irradiation for cancer treatment causes premature ovarian failure. Premature ovarian failure, also called premature menopause, has many adverse consequences, including early loss of fertility and increased risk of osteoporosis, cardiovascular disease, and Alzheimer's disease. Gamma irradiation has long been known to cause ovarian cancer. Sixty percent of women diagnosed with ovarian cancer will die of the disease; it is the leading cause of death from gynecological cancers. Because ovarian cancer tends to be asymptomatic until it has reached an advanced stage, treatment is often ineffective. For all of these reasons, it is important to understand the risks to the ovary of space radiation. Our own and others work showed that gamma irradiation chronically elevates cellular reactive oxygen species (ROS) production and oxidative stress and that ROS initiate apoptotic death of ovarian follicles. We therefore hypothesize that high charge and energy (HZE) particles typical of space radiation cause ovarian oxidative stress, resulting in premature ovarian failure and that this contributes to the pathogenesis of ovarian cancer. We are testing this overarching hypothesis in two specific aims. Aim 1: Exposure to low dose HZE particles induces ovarian oxidative stress, which initiates apoptotic destruction of ovarian follicles, causing premature ovarian failure. Adult female C57BL/6J mice will be exposed to low dose (0, 5, 30, and 50 cGy) oxygen (LET = 16.5 keV/μm) or iron (LET = 179 keV/μm) at energy of 600 MeV/u and analyzed 6 hours, 1 week, and 8 weeks after exposure. Two groups will be irradiated at the highest dose for each of the two charged particles, one fed normal control rodent chow and the other fed the same chow supplemented with the antioxidant alpha lipoic acid for the duration of the experiment. One ovary from each mouse will be used for detailed histomorphometric analyses of ovarian follicle numbers. The other ovary will be used for in situ analyses of apoptosis, proliferation, oxidative damage, and DNA double strand breaks. Aim 2: Exposure to low dose HZE particles causes epithelial ovarian tumors. Adult female mice of two strains, one sensitive to radiation-induced tumors (B6C3F1) and one thought to be less sensitive to radiation-induced tumors (C57BL/6J) will be irradiated with charged oxygen or iron particles at 50 cGy, 600MeV/u energy or sham-irradiated and aged to 18 months for evaluation of ovaries for tumors. Materials and Methods Three month old female mice (C57BL/6J) were divided into five groups: 1) sham-irradiated and fed with AIN-93M rodent chow (0 cGy group); 2) irradiated with 5 cGy charged iron particles at an energy of 600 MeV and fed AIN-93M rodent chow; 3) 30 cGy charged iron particles and fed AIN-93M rodent chow; 4) 50 cGy charged iron particles and fed AIN-93M rodent chow; 5) irradiated with 50 cGy charged iron particles and fed AIN-93M rodent chow supplemented with 150 mg/kg diet alpha lipoic acid. Additional groups of mice were irradiated with charged oxygen particles at the same doses with or without dietary lipoic acid supplementation. Feeding with supplemented chow began one week before irradiation and continued until euthanasia. Ovaries were collected 6h, 1wk, and 8 wks after irradiation. Estrous cycling was monitored by vaginal cytology for two weeks prior to euthanasia at the 8 wk time point. Ovarian follicles were counted in serial ovarian sections stained with hematoxylin and eosin. Apoptosis and DNA double strand breaks were assessed by immunostaining for activated caspase 3 and phosphorylated H2AX (gamma H2AX), respectively. Analyses are ongoing. Interim results from the 0 cGy control group and the two 50 cGy charged iron particles-exposed groups are presented here. To test whether charged iron particle or charged oxygen particle irradiation causes ovarian tumors, 3 month old female mice of two strains, one sensitive to radiation induced tumors (B6C3F1) and one thought to be less sensitive to radiation-induced tumors (C57BL/6J) were irradiated with 50 cGy charged iron particles at an energy of 600 MeV (LET = 179 keV/μm) or sham-irradiated. These mice are being aged to 18 months for evaluation of ovaries for tumors. Results

Task Progress:	Aim 1: Exposure to low dose HZE particles induces ovarian oxidative stress, which initiates apoptotic destruction of ovarian follicles, causing premature ovarian failure. Charged iron particles increase ovarian DNA double strand breaks and apoptosis To define the mechanism of ovarian damage by charged iron particles, we analyzed the localization of DNA double strand breaks using phosphorylated H2AX (gamma H2AX) immunostaining in ovarian sections 6h after irradiation. Positive gamma H2AX immunostaining was significantly higher in granulosa cells and/or oocytes of ovarian follicles at all stages of development in 50 cGy-treated mice compared to control 0 cGy-treated mice. Mice that received the lipoic acid supplemented diet along with 50 cGy charged iron particles had significantly decreased percentages of gamma H2AX immunostained granulosa cells and/or oocytes compared to 50 cGy charged iron particles-treated mice fed the normal diet, indicating that lipoic acid supplementation is protective. We assessed the percentage of follicles undergoing death by apoptosis at 6h after irradiation using activated (cleaved) caspase-3 immunostaining in the granulosa cells. The percentages of activated caspase 3-positive primordial and secondary follicles were significantly elevated in the 50 cGy charged iron particles group compared to the 0 cGy group. Mice that received the lipoic acid supplemented diet along with 50 cGy charged iron particles were completely protected against primordial follicle apoptosis and partially protected against secondary follicle apoptosis induction by 50 cGy charged iron particles. Effects of charged iron particles on ovarian follicle numbers At 1 week after irradiation, the numbers of healthy small (primordial and primary) and growing (secondary and antral) follicles per ovary were significantly reduced in the ovaries of mice treated with 50 cGy charged iron particles compared to 0 cGy controls. The lipoic acid supplemented diet partially prevented the decline in small ovarian follicle numbers at 1 week. Effects of charged iron particles on estrous cycling At the 8wk time point, estrous cycling was monitored by vaginal cytology. Estrous cycling in rodents is analogous to menstrual cycling in women. 12.5% and 25%, respectively, of mice treated with 50 cGy charged iron particles or 50 cGy charged iron particles plus lipoic acid had irregular estrous cycles. The remaining mice had regular estrous cycles, but mice treated with 50 cGy charged iron particles had non-significantly longer estrous cycles than other treated groups (5.2±0.3 days compared to 4.3±0.2 days in controls). Effects of charged iron particles on reproductive hormone concentrations If the hypothalamic-pituitary-ovarian axis were functioning normally, one would expect the depletion of ovarian follicles to result in decreased negative feedback to the hypothalamus and pituitary, decreasing the circulating levels of follicle stimulating hormone (FSH) and luteinizing hormone (LH) secreted from the anterior pituitary gland at 8 weeks post-irradiation. Serum FSH concentrations were significantly higher in the mice treated with 50 cGy charged iron particles compared to 0 cGy-treated mice, whereas concentrations of LH were non-significantly elevated. Aim 2: Exposure to low dose HZE particles causes epithelial ovarian tumors. No effects of charged iron or oxygen particles on weight gain C57BL/6J and B6C3F1 mice irradiated with charged iron or oxygen particles and respective controls have been followed for over 30 weeks. Mice of both strains have been gaining weight as expected. There have been no treatment-related differences in weight gain among groups. Ovaries will be collected when the mice are 18 months old and examined for tumors. Conclusion Our results reveal that acute exposure to charged iron particles induces DNA double strand breaks and apoptosis in ovarian follicles, resulting in depletion of ovarian follicles. With depletion of the ovarian follicles, ovarian hormone production is decreased, resulting in increased serum concentrations of the pituitary hormones FSH and LH. The data show that exposure to charged particles typical of space radiation is highly damaging to the ovary and causes premature ovarian failure. Our results further demonstrate that dietary supplementation with the antioxidant alpha lipoic acid is partially protective against the ovarian toxicity of charged iron particles, suggesting that such dietary supplementation may be beneficial to prevent premature ovarian failure in female astronauts exposed to space radiation.
Bibliography Type:	Description: (Last Updated: 06/20/2025)
Abstracts for Journals and Proceedings	Mishra B, Ortiz L, Luderer U. "Charged Iron Particle Exposure Increases Apoptosis and Depletes Ovarian Follicles in Mice." Presented at the 2015 NASA Human Research Program Investigators' Workshop, Galveston, TX, January 13-15, 2015. 2015 NASA Human Research Program Investigators' Workshop, Galveston, TX, January 13-15, 2015. Abstract #0104. , Jan-2015