

## Effects of space flight on ovarian–hypophyseal function in postpartum rats

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The effect of space flight in a National Aeronautics and Space Administration (NASA) shuttle was studied in pregnant rats. Rats were launched on day 9 of gestation and recovered on day 20 of gestation. On day 20 of gestation, rats were unilaterally hysterectomized and subsequently allowed to go to term and deliver vaginally. There was no effect of space flight on pituitary and ovary mass postpartum. In addition, space flight did not alter healthy and atretic ovarian antral follicle populations, fetal wastage *in utero*, plasma concentrations of progesterone and luteinizing hormone (LH) or pituitary content of follicle stimulating hormone (FSH). Space flight significantly increased plasma concentrations of FSH and decreased pituitary content of LH at the postpartum sampling time. Collectively, these data show that space flight, initiated during the postimplantation period of pregnancy, and concluded before parturition, is compatible with maintenance of pregnancy and has minimal effects on postpartum hypophyseal parameters; however, none of the ovarian parameters examined was altered by space flight.

### Introduction

Most mature ovarian follicles in rats release one ovum at ovulation (Brambell, 1956; Bocharov, 1966) and if the reproductive process is 100% efficient, the number of corpora lutea of pregnancy will equal the number of young delivered. In rats this usually does not occur because of conceptus loss during the pre- and postimplantation phases of pregnancy (Nalbandov, 1964). Nonetheless, the ratio of corpora lutea to young delivered (fecundity ratio) is a useful index of fecundity and changes in this index, relative to controls, provide a means of evaluating effects of different treatments during pregnancy on the number of live young delivered. During pregnancy, the rat ovary contains various sizes of antral follicles, and there is a continuous partial elimination of these follicles by atresia (Greenwald, 1966; Osman, 1986). Atresia occurs by apoptosis (Hsueh *et al.*, 1994) and this important process, which regulates the number of oocytes available for ovulation, is influenced by many extra- and intraovarian factors (Guraya, 1985). The rat has a full complement of preovulatory follicles soon after delivery (Rebar *et al.*, 1969; Ying *et al.*, 1973; Mori *et al.*, 1974; Osman, 1986) in preparation for the postpartum oestrus and ovulation that occur in this species (Blandau and Soderwall, 1941).

In the present study, we examined the effects of space flight on the postpartum fecundity ratio and numbers of healthy and atretic ovarian antral follicles. In addition, the concentration of plasma progesterone, LH, and FSH and the pituitary content of LH and FSH is reported. The animals had experienced space

flight during days 9–20 of gestation in a National Aeronautics and Space Administration (NASA) space shuttle.

### Materials and Methods

#### *Animals and treatments*

This study was conducted in accordance with all regulations specified in *Principles of Laboratory Animal Care* (National Institutes of Health publication No. 86-23, revised 1985). Sperm positive Sprague–Dawley rats (Taconic Farms, Germantown, NY) were shipped from the vendor via air freight to Kennedy Space Center (KSC), Florida, on day 2 of gestation (day 1 = morning on which spermatozoa are present in the vagina). Pregnancy was confirmed on day 7 of gestation by anaesthetizing animals with isoflurane (IsoFlo, Abbott Labs, North Chicago, IL) vapour using a nonbreathing rodent anaesthesia unit (Viking Products, Medford Lakes, NJ) and counting and recording the number of decidual swellings in each uterine horn. On day 8 of gestation, ten pregnant rats, each with at least five decidual swellings in each uterine horn, were placed into NASA flight cages (animal enclosure modules, AEMs, five rats per cage), loaded onto the mid-deck of the space shuttle, and launched into orbit on day 9 of gestation. On day 20 of gestation, the space shuttle landed at Edwards Air Force Base, CA. Approximately 3 h after landing, flight animals were recovered, anaesthetized with isoflurane, and subjected to unilateral hysterectomy to provide fetal material from one horn to investigators identified by NASA to study selected fetal parameters. The number of healthy fetuses in the

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removed horn was recorded. After unilateral hysterectomy, the animals were allowed to recover and deliver vaginally. There were three groups ( $n = 10$  each group) of control animals at KSC in the study. A synchronous control group, delayed 24 h with reference to the flight group, received the same surgeries and was housed five rats per AEM and exposed to all flight conditions (identical temperature, lighting and humidity) except microgravity. Vivarium control group 1 did not receive any surgery and was housed in a vivarium in clear polycarbonate cages. Vivarium control group 2 received only a unilateral hysterectomy on day 20 of gestation but otherwise was housed like the vivarium control group 1. Body masses of synchronous control and vivarium control 2 groups were comparable with those of the flight group on day 8 of gestation, the day the flight group was loaded into the shuttle (flight,  $216.6 \pm 2.8$  g; synchronous control,  $223.0 \pm 5.7$  g; vivarium control 2,  $222.8 \pm 3.3$  g). For determination of the duration of pregnancy, zero hour was designated as 12:00 midnight prior to detection of spermatozoa in the vagina.

#### Blood and tissue processing

Approximately 3 h after delivery, rats were anaesthetized with isoflurane and blood was withdrawn from the abdominal aorta into heparinized syringes. Blood was centrifuged at 2000 g and plasma stored at  $-70^\circ\text{C}$  until FSH, LH, and progesterone analysis.

The pituitary was removed, weighed to the nearest 0.1 mg, frozen on dry ice, and stored at  $-70^\circ\text{C}$  until FSH and LH analysis. Ovaries were removed, trimmed, weighed to the nearest 0.1 mg, and immersed in Bouin's fixative. After fixation for at least 48 h, ovaries were dehydrated, embedded in paraffin wax, serially sectioned at 10  $\mu\text{m}$ , and stained with haematoxylin and eosin. Follicles and corpora lutea were counted in both ovaries of each animal. The procedure for counting follicles has been reported by Burden *et al.* (1986). Briefly, each section was examined and antral follicles containing an oocyte with a nucleolus were measured. The maximum diameter and a diameter at right angles to it were measured to obtain a mean diameter for each such follicle. Follicles were designated as atretic if they contained either 10 or more granulosa cells with pyknotic nuclei or if the oocyte nucleus was pyknotic. Additional changes noted, but not always present, were resumption of meiosis, infiltration of the follicle with white blood cells, and fragmentation of the oocyte. All healthy and atretic antral follicles  $> 200 \mu\text{m}$  were measured and assigned to one of three size classifications: 201–400, 401–570, and  $> 570 \mu\text{m}$  diameter. Since absolute numbers of follicles varied, numbers of atretic and healthy follicles are expressed as percentages of the total follicular population counted.

#### LH and FSH radioimmunoassay

Pituitaries were homogenized in PBS, pH 7.4. Pituitary content and plasma concentration of LH and FSH were measured by radioimmunoassay (RIA) using procedures previously validated in our laboratory (Burden *et al.*, 1980). Reagents for the RIA were supplied by the Hormone Distribution Program of the National Institutes of Health and values

are expressed in terms of LH or FSH RP1 standards. Assays were performed by the double-antibody method using the instructions enclosed with the kits. All samples were run in the same RIA. The sensitivity of the LH and FSH RIA was  $12.5 \text{ ng ml}^{-1}$  and  $20 \text{ ng ml}^{-1}$ , respectively. Within assay variance was less than 10%.

#### Progesterone radioimmunoassay

Plasma concentrations of progesterone were measured using RIA procedures previously validated in this laboratory (Renegar *et al.*, 1992). Sensitivity, blanks, accuracy, and precision were determined in quadruplicate by measuring, respectively, standard quantities of hormone, blank test tubes, and pooled plasma by the procedure used for plasma samples.

#### Statistical analysis

Percentage data were subjected to arcsin transformation before analysis. Multisample numerical data between groups were analysed by one way ANOVA. If significance was found, group differences were ascertained by the Newman–Keuls test.

## Results

#### Body mass, duration of pregnancy, and organ mass

Space flight during days 9–20 of gestation did not affect body mass gain during this interval of pregnancy. At recovery on day 20 of gestation, the flight group had gained  $45.7 \pm 2.0\%$  body mass during this period, while the synchronous control group gained  $42.4 \pm 1.7\%$ . Spaceflight had no effect on the duration of pregnancy (Table 1). Animals delivered pups vaginally from the remaining horn in all groups during the interval from 13:00 h on day 22 of gestation to 15:00 h on day 23 of gestation (all deliveries reported in Eastern time). Two animals in the flight group and one in the synchronous control group had not delivered at 15:00 h on day 23 of gestation and pups from these three animals were delivered by caesarean section at 15:00 h on day 23 of gestation. Space flight did not alter the mass of the pituitaries and ovaries in postpartum rats (Table 1).

#### Ovarian follicles

Space flight during days 9–20 of gestation did not alter the number of postpartum healthy ovarian follicles in any of the size ranges studied (Table 2). In addition, there was no effect of space flight on the number of atretic follicles in the size ranges evaluated (Table 2).

#### Ovarian corpora lutea: fecundity ratios

The mean number of corpora lutea (both ovaries) in postpartum rats varied from 16.0 to 18.7 in different groups. These differences were not significant ( $P > 0.05$ ). The mean number of decidual swellings at day 7 of gestation in the flight and synchronous control groups was identical (Table 3). By

**Table 1.** Duration of pregnancy (h), and wet mass (mg  $\pm$  SEM) of pituitary and ovaries in flight, synchronous controls (delayed 24 h; same caging, temperature, humidity as flight), vivarium control 1 (no surgery, standard laboratory housing), and vivarium control 2 (unilateral hysterectomy day 20 of gestation, standard laboratory housing) postpartum rats

Group	Duration of pregnancy	Pituitary	Left ovary	Right ovary
Flight	528.8 $\pm$ 2.5 <sup>a</sup>	12.2 $\pm$ 0.5 <sup>a</sup>	48.5 $\pm$ 3.0 <sup>a</sup>	55.7 $\pm$ 2.3 <sup>a</sup>
Synchronous control	533.1 $\pm$ 3.1 <sup>a</sup>	12.9 $\pm$ 0.4 <sup>a</sup>	51.5 $\pm$ 2.9 <sup>a</sup>	50.2 $\pm$ 3.7 <sup>a</sup>
Vivarium control 1	532.6 $\pm$ 3.0 <sup>a</sup>	12.4 $\pm$ 0.3 <sup>a</sup>	48.6 $\pm$ 3.8 <sup>a</sup>	51.9 $\pm$ 2.5 <sup>a</sup>
Vivarium control 2	534.1 $\pm$ 1.5 <sup>a</sup>	13.3 $\pm$ 0.3 <sup>a</sup>	55.1 $\pm$ 4.1 <sup>a</sup>	53.5 $\pm$ 4.3 <sup>a</sup>

<sup>a</sup>Within each column, means bearing identical superscripts are not significantly different ( $P > 0.05$ ).

**Table 2.** Percentage of healthy and atretic ovarian follicles in flight, synchronous controls (delayed 24 h; same caging, temperature, humidity as flight), vivarium control 1 (no surgery, standard laboratory housing), and vivarium control 2 (unilateral hysterectomy day 20 of gestation, standard laboratory housing) postpartum rats

Group	Follicles 201–400 $\mu$ m diameter (%)		Follicles 401–570 $\mu$ m diameter (%)		Follicles over 570 $\mu$ m diameter (%)	
	Healthy	Atretic	Healthy	Atretic	Healthy	Atretic
Flight	35.9 $\pm$ 1.9 <sup>a</sup>	46.8 $\pm$ 1.6 <sup>a</sup>	8.1 $\pm$ 1.0 <sup>a</sup>	4.7 $\pm$ 0.9 <sup>a</sup>	4.1 $\pm$ 0.8 <sup>a</sup>	0.2 $\pm$ 0.1 <sup>a</sup>
Synchronous control	35.6 $\pm$ 2.2 <sup>a</sup>	49.0 $\pm$ 2.0 <sup>a</sup>	6.8 $\pm$ 1.0 <sup>a</sup>	3.7 $\pm$ 0.6 <sup>a</sup>	4.1 $\pm$ 0.6 <sup>a</sup>	0.5 $\pm$ 0.4 <sup>a</sup>
Vivarium control 1	37.4 $\pm$ 1.4 <sup>a</sup>	47.8 $\pm$ 1.8 <sup>a</sup>	7.6 $\pm$ 0.9 <sup>a</sup>	2.4 $\pm$ 0.4 <sup>a</sup>	4.3 $\pm$ 0.6 <sup>a</sup>	0.2 $\pm$ 0.1 <sup>a</sup>
Vivarium control 2	38.6 $\pm$ 1.6 <sup>a</sup>	46.1 $\pm$ 1.6 <sup>a</sup>	6.7 $\pm$ 0.6 <sup>a</sup>	4.0 $\pm$ 0.5 <sup>a</sup>	3.8 $\pm$ 0.8 <sup>a</sup>	0.4 $\pm$ 0.1 <sup>a</sup>

Values are mean  $\pm$  SEM.

<sup>a</sup>Within each column, means bearing identical superscripts are not significantly different ( $P > 0.05$ ).

**Table 3.** Number of corpora lutea, decidual swellings at day 7 of gestation, fecundity ratio at day 7 of gestation, live conceptuses at day 20 of gestation plus term and fecundity ratio at day 20 of gestation plus term in flight, synchronous controls (delayed 24 h; same caging, temperature, humidity as flight), vivarium control 1 (no surgery, standard laboratory housing), and vivarium control 2 (unilateral hysterectomy day 20 of gestation, standard laboratory housing) postpartum rats

Group	Number of corpora lutea*	Number of decidual swellings*	Fecundity ratio <sup>†</sup>	Number of live conceptuses*	Fecundity ratio <sup>‡</sup>
Flight	16.0 $\pm$ 1.0	13.1 $\pm$ 0.3	84.4 $\pm$ 4.9	11.8 $\pm$ 0.6	77.0 $\pm$ 5.7
Synchronous control	17.7 $\pm$ 1.3	13.1 $\pm$ 0.2	76.6 $\pm$ 4.1	11.7 $\pm$ 0.6	67.7 $\pm$ 3.3
Vivarium control 1	18.7 $\pm$ 1.6	—	—	11.2 $\pm$ 0.4	64.5 $\pm$ 5.0
Vivarium control 2	16.3 $\pm$ 1.3	—	—	11.3 $\pm$ 0.7	73.3 $\pm$ 7.0

\*Mean  $\pm$  SEM.

<sup>†</sup>Number of decidual swellings divided by number of corpora lutea.

<sup>‡</sup>Number of live conceptuses removed at unilateral hysterectomy plus number of live young delivered from contralateral horn divided by number of corpora lutea.

comparing numbers of corpora lutea postpartum with numbers of decidual swellings at day 7 of gestation, it can be seen (Table 3) that the resulting fecundity ratios between the two groups is not different. Likewise, by comparing numbers of corpora lutea postpartum to the number of live young at term (numbers of live young at term = number of fetuses in the horn removed at day 20 of gestation plus the number of live young delivered from the remaining horn), it can be seen (Table 3)

that the resulting fecundity ratios between the two groups are not different.

#### Plasma hormone concentrations

Space flight had no effect on the plasma concentration of progesterone or LH postpartum but increased ( $P < 0.04$ ) the

**Table 4.** Concentration of plasma progesterone, LH, FSH and pituitary content of LH and FSH in flight, synchronous controls (delayed 24 h; same caging, temperature, humidity as flight), vivarium control 1 (no surgery, standard laboratory housing), and vivarium control 2 (unilateral hysterectomy day 20 of gestation, standard laboratory housing) postpartum rats

Group	Progesterone (ng ml <sup>-1</sup> )	LH (ng ml <sup>-1</sup> )	FSH (ng ml <sup>-1</sup> )	Pituitary LH (µg mg <sup>-1</sup> )	Pituitary FSH (µg mg <sup>-1</sup> )
Flight	4.2 ± 0.8 <sup>a</sup>	126.7 ± 55.9 <sup>a</sup>	173.4 ± 32.5 <sup>a</sup>	9.3 ± 1.1 <sup>a</sup>	9.9 ± 0.8 <sup>a</sup>
Synchronous control	5.2 ± 0.6 <sup>a</sup>	129.8 ± 32.4 <sup>a</sup>	100.9 ± 19.2 <sup>ab</sup>	15.1 ± 1.7 <sup>b</sup>	9.0 ± 0.5 <sup>a</sup>
Vivarium control 1	3.9 ± 0.9 <sup>a</sup>	73.6 ± 15.8 <sup>a</sup>	83.9 ± 10.1 <sup>b</sup>	15.7 ± 1.4 <sup>b</sup>	9.9 ± 0.5 <sup>a</sup>
Vivarium control 2	4.8 ± 0.5 <sup>a</sup>	213.4 ± 55.1 <sup>a</sup>	100.3 ± 23.0 <sup>ab</sup>	11.9 ± 1.8 <sup>a</sup>	7.9 ± 0.6 <sup>a</sup>

Values are means ± SEM.

<sup>ab</sup>Within each column, values with different superscripts are significantly different ( $P < 0.05$ ).

plasma concentration of FSH (Table 4) relative to the vivarium control 1 group but not the synchronous control or vivarium control 2 groups.

#### Pituitary content of FSH and LH

Space flight had no effect on the pituitary content of FSH but decreased ( $P < 0.02$ ) the pituitary content of LH postpartum (Table 4).

### Discussion

The present study investigated the effects of space flight on the postpartum structure and function of the rat ovary and pituitary gland. Space flight during days 9–20 of gestation had no effect on the mass of these organs at postpartum on days 22–23. Rats have a period of oestrus and ovulation after delivery (Blandau and Soderwall, 1941), and the mechanisms that mediate postpartum ovulation are the same as those that control ovulation in normal cyclic rats (Ying *et al.*, 1973). For postpartum ovulation to occur, there must be a sufficient quantity and quality of preovulatory follicles present. We were interested in whether space flight affected the number of preovulatory follicles and the number of growing follicles that would supply oocytes for subsequent ovarian cycles. In addition, we wished to determine whether the rate of atresia was altered by space flight. Since the fate of about 77% of rat ovarian follicles is atresia (Arai, 1920; Mandl and Shelton, 1959; Byskov, 1978), any disruption of this fundamental ovarian regulatory process could result in an increased or decreased number of ovulatory follicles and a resultant altered fecundity. However, space flight during days 9–20 of gestation had no effect on the numbers of ovarian follicles in any size range studied, either healthy or atretic, in ovaries approximately 3 h postpartum. This indicates that space flight had no effect on the capacity of the ovary to produce the normal number of preovulatory follicles at the end of pregnancy.

During the normal oestrous cycle of rats, ovulatory follicles are converted into corpora lutea. In the absence of pregnancy or pseudopregnancy induction, corpora lutea secrete progesterone for only 2 days and then regress rapidly (Hashimoto *et al.*, 1968; Smith *et al.*, 1975). If the rat becomes pregnant or pseudopregnant, corpora lutea enlarge and secrete large

quantities of progesterone which maintain pregnancy (see review by Gunnet and Freeman 1983). The complex of events that occurs before delivery includes a precipitous drop in circulating progesterone (Wiest *et al.*, 1968; Louis *et al.*, 1978; Renegar *et al.*, 1992), a major controlling event in the delivery process in rats (Csapo and Wiest, 1969). In the present study, flight animals delivered at the same times as controls and as expected, circulating progesterone concentrations were low. Since corpora lutea develop from ovulatory follicles, and since most rat follicles contain only one oocyte (Brambell, 1956), the number of corpora lutea postpartum reflects the number of ova ovulated. Most embryo wastage occurs before implantation (Nalbandov, 1964). Because of the laparotomy at day 7 of gestation, the number of implanted embryos was known. By comparing the number of corpora lutea postpartum with the number of implanted embryos at day 7 of gestation, we determined that there was no significant difference in embryonic wastage between flight and synchronous controls at day 7 of gestation. The ratio of the number of corpora lutea:implantation sites is referred to as the fecundity ratio at day 7 of gestation. For this study, we made the assumption that the number of decidual swellings at day 9 of gestation was the same as recorded at day 7 of gestation. Thus, by comparing the number of corpora lutea postpartum with the number of live fetuses (at day 20 of gestation) and young born at day 22 or day 23 of gestation, we could ascertain the effects of space flight on fetal survival during days 9–20 of gestation. Space flight during this interval of pregnancy had no effect on this fecundity ratio at term. Postpartum gonadotrophin surges occur between 3.5 and 13 h after delivery (Rebar *et al.*, 1969; Mori *et al.*, 1974), although some rats show rises in circulating gonadotrophin concentrations as early as 2 h postpartum (Rebar *et al.*, 1969). Sequential blood samples would need to be taken for approximately 2–24 h postpartum to obtain meaningful data on circulating gonadotrophins at this time. The imposed logistics of the experimental design in the current study allowed only a single plasma sample postpartum for analysis. There was no effect of space flight on plasma concentrations of LH at the postpartum sampling, but statistically, space flight increased plasma concentrations of FSH. Because LH and FSH are released in a pulsatile manner, there is considerable variance and the biological relevance of this observation is equivocal.

The pituitary content of LH in rats is low postpartum relative to concentrations at day 20 of gestation (Greenwald, 1966). An inverse relationship between pituitary content and circulating peripheral plasma concentrations of LH has been demonstrated during the first 10 h after delivery in this species (Rebar *et al.*, 1969). In the present study, space flight rats had a lower pituitary content of this peptide postpartum than did the control groups. It has been hypothesized that low gravity affects hormone packaging within the cytoplasmic secretory granules within cells of the anterior pituitary (Hymer *et al.*, 1992). However, as mentioned above, space flight had no effect on circulating LH. Clearly, more studies need to be conducted to clarify any possible biological significance of this observation.

In conclusion, the results of the present study show that space flight, when initiated after implantation, when pregnancy is well established, and concluded before delivery, is compatible with fetal growth and development, pregnancy maintenance, and parturition in the rat. Although some statistical differences were noted in hypophyseal parameters, no clear pattern of change was evident and the structure and function of the postpartum ovary was normal in all respects.

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