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Effect of Simulated Microgravity on Sexual Development of Male Japanese Quail

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Abstract

Hypodynamia can be used to simulate weightlessness in laboratory conditions. The objective of our study was to investigate the effects of chronic hypodynamia on the growth and development of the testes and cloacal gland, and plasma testosterone concentration in Japanese quail.

The testis weight in males reared under hypodynamia was significantly lower compared to age-matched control between 21 and 63 days of age ($P < 0.05$). The cloacal gland area of experimental birds calculated from its width and length was also smaller in comparison with control quail from 35 to 56 days of age ($P < 0.05$). The foam production was significantly lower in hypodynamia males at age 35, 42, 49 and 63 days ($P < 0.05$). The plasma testosterone concentration was significantly reduced in hypodynamia birds between 35 and 70 days of age ($P < 0.05$), with the exception of day 56.

These results provide further evidence that although hypodynamia negatively affects the examined variables, the male Japanese quail is able to develop normally under conditions of simulated weightlessness.

Hypodynamia, testes, cloacal gland, testosterone, age

Long-term manned missions into space require a detailed understanding of developmental biology of the species that serve not only as food or companion animals but as well as models for study of the influence of space environment on human organism. Japanese quail may be used as one of the models (Boďa 1993).

Slovak and Russian scientists have documented within several experiments that weightlessness (microgravity) does not have a negative impact on embryogenesis of Japanese quail (Boďa et al. 1992; Guryeva et al. 1993). On board the Mir space station newly hatched Japanese quail chicks had all the external characteristics of normal development (Sabo et al. 2001). Nevertheless, it remains unknown how microgravity may influence their further development during different ontogenetic phases. A partial answer to some of these questions may be provided by ground-based experiments using animal models under conditions of simulated microgravity (hypodynamia).

The first studies to examine the effect of hypodynamia were carried out on the adult Japanese quail (Juráni et al. 1983), followed by similar experiments aimed at post-hatching development of Japanese quail chicks (Škrobánek et al. 2001).

The objective of this study was to investigate the influence of simulated microgravity on development of the cloacal gland and testes of male Japanese quail reared under hypodynamia from day 3 post-hatch to 70 days of age.

Materials and Methods

One hundred and seventy 3-day-old male Japanese quail (Laying Line 01 Ivanka pri Dunaji) were individually weighted (mean body weight 10.15 ± 0.67 g) and assigned at random into experimental group (n = 85; exposed

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to hypodynamia) and control (n = 85). Hypodynamia is a method of weightlessness simulation where birds are placed in special individual slings suspended by a flexible metal device such that their legs cannot touch the floor (Škrobánek et al. 2004). Control quail chicks were placed in two rearing boxes (0.6 × 0.6 × 0.3 m). The birds of both groups were kept until 70 days in a windowless poultry room with controlled ventilation and heating by infrared lamps. The temperature in the rearing room was maintained at 35-36 °C for the first few days after hatching and then decreased gradually through the four weeks to about 22 °C, remaining on this level until the end of the experiment. A commercial starter mash HYD-13 and water were available *ad libitum*. The diet containing 260 g/kg protein and 11.5 MJ metabolisable energy/kg was fed from hatch to termination of experiment. Lighting in the rearing room was continuous. The care and use of animals were in accordance with laws and regulations of the Slovak Republic and were approved by the Ethics Committee of the Institute of Animal Biochemistry and Genetics, SASci, Ivanka pri Dunaji.

At 21, 28, 35, 42, 49, 56, 63 and 70 days of age, ten birds of each group were randomly selected and individual body weight was recorded. Subsequently, the length and width of the cloacal gland was measured by a calliper. The cloacal gland foam production was determined by subjective scaling of the amount of foam ejected upon manual expression (squeezing) of foam gland, using a scale of 1 (no foam expressed) to 5 (maximum amount of foam expressed). Then, the birds were euthanized by cervical dislocation and blood for testosterone analysis was collected into heparinized plastic tubes. The blood samples were immediately placed on ice, centrifuged at 1,500 *g* for 30 min at 4 °C to separate the plasma and stored at -20 °C until analyzed. Testosterone was determined by direct radioimmunoassay (RIA) partially modified by Zeman et al. (1986). Immediately after blood collection, the abdominal cavity was opened and the testes were removed and measured (length, width and weight). The samples of the testes for light microscopy were collected routinely at the age of 49 days. They were fixed in 4% neutral formaldehyde, embedded in paraffin, and the sections were stained with haematoxylin and eosin at room temperature. The stained cells were analyzed using a light microscope (JENAMED) with a camera attached.

Data from both groups were analyzed by two-way analysis of variance (ANOVA) with age and treatments as factors. In each analysis, significant differences among means were detected using Tukey multiple comparison test. In all cases, the level of significance was set at $P < 0.05$. All values are presented as mean ± SEM.

Results

The mean body weight of males in both groups increased with age (Fig. 1). However, the body weight of Japanese quail reared under hypodynamia was by 13 to 37% lower than that of age-matched control between 21 and 70 days of age ($P < 0.05$).

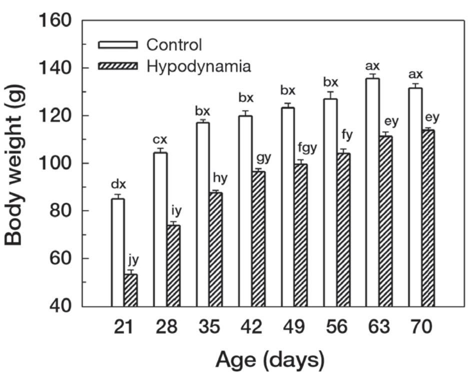
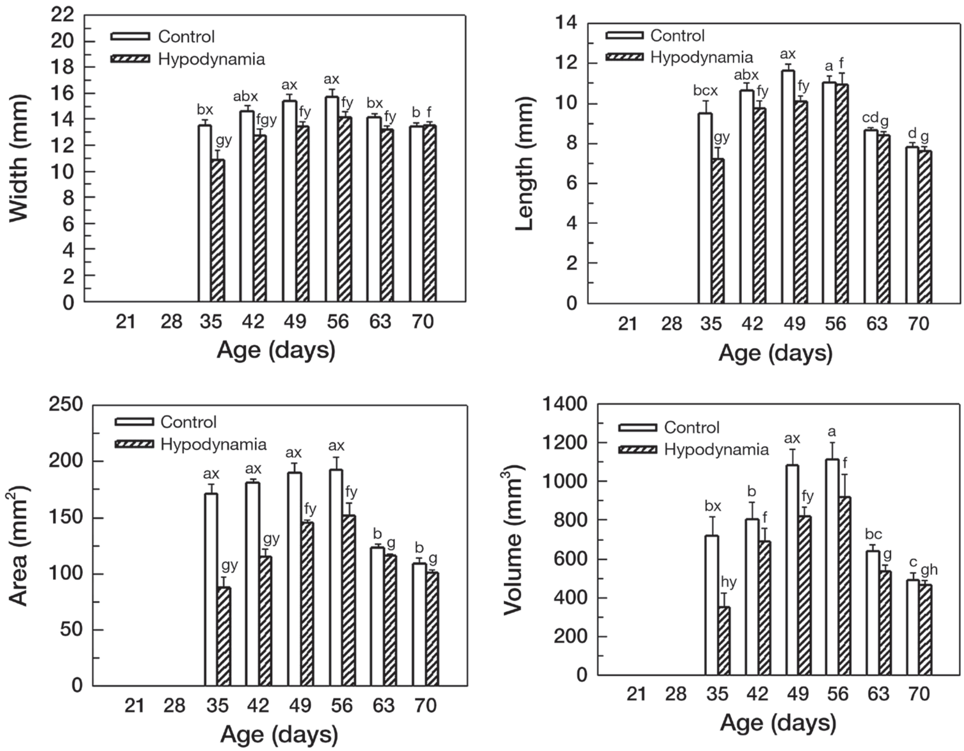


Fig. 1. The mean body weight of male Japanese quail developing in the simulated microgravity (hypodynamia). Values are means ± SEM, n = 10.
([a, b, c, d]) Means of control with different superscripts are significantly different ($P < 0.05$) ([f, g, h, i, j]) Means of hypodynamia with different superscripts are significantly different ($P < 0.05$)
([x, y]) Means of control and hypodynamia with different superscripts are significantly different ($P < 0.05$)

The size of the cloacal gland (width and length) in both groups increased from 35 to 56 days of age and was significantly reduced at 63 and 70 days of age (Fig. 2). The width and length of the cloacal gland of control males was significantly greater than those of hypodynamia ones between day 35 and 63 of age and between day 35 and 49 of age, respectively. Cloacal gland area of control calculated from these measurements was also significantly larger than that of hypodynamia birds from 35 to 56 days of age. However, significant differences in the cloacal gland volume between control and test birds were observed only on day 35 and 49 of age.

Foam production increased from 35 to 63 days of age in both groups. During this time, the foam production was significantly lower in hypodynamia males compared to the control, with the exception of day 56.

The rapid growth of the testes in Japanese quail is apparent from Fig. 3. Left and right testis enlarged quickly between 28 and 42 days of age. Later, from 49 to 70 days the testicular weight increased only slightly. However, the mean testicular weight in males reared under hypodynamia was significantly lower than



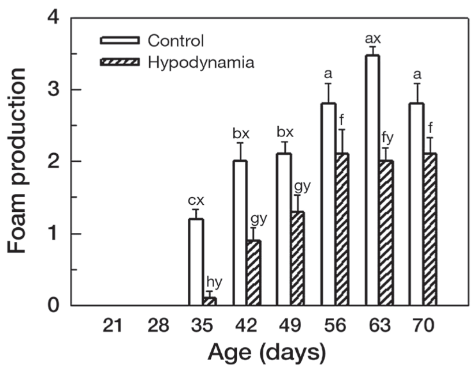


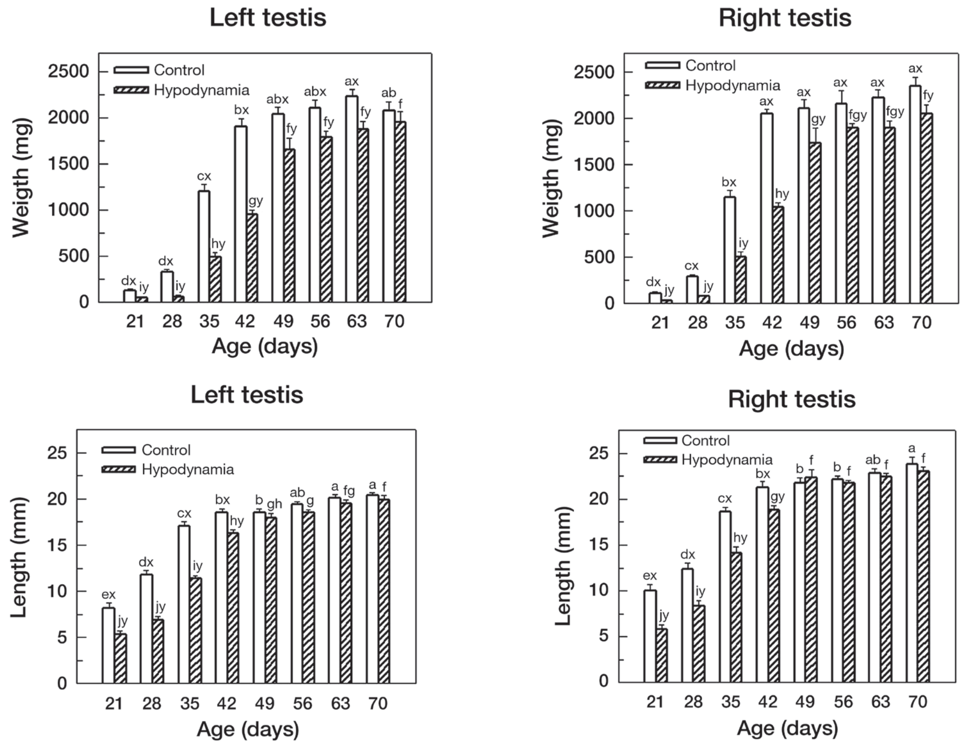
Fig. 2. The mean cloacal gland indicators and foam production of male Japanese quail developing in the simulated microgravity (hypodynamia). Values are means ± SEM, n = 10.
([a, b, c, d]) Means of control with different superscripts are significantly different ($P < 0.05$)
([f, g, h]) Means of hypodynamia with different superscripts are significantly different ($P < 0.05$)
([x, y]) Means of control and hypodynamia with different superscripts are significantly different ($P < 0.05$)

that of the age-matched control during the whole experiment, with the exception of the left testis at day 70 ($P < 0.05$).

The mean testicular length in both groups increased gradually until 42 days of age. Subsequent prolongation of testes from 49 day of age to the end of experiment was inconsiderable. The differences between hypodynamia and control group were significant from start to day 42 of age ($P < 0.05$).

The mean testicular width in both groups changed similarly as the testicular length. The testes of control birds, however, were wider than those of hypodynamia group starting from day 21 until day 63 ($P < 0.05$).

Histological analyses of testes revealed structural differences between both groups. In



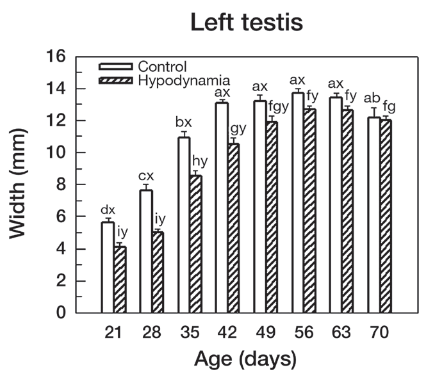


Fig. 3. The mean testes weight and testes indicators of male Japanese quail developing in the simulated microgravity (hypodynamia). Values are means ± SEM, n = 10.
([a, b, c, d, e]) Means of control with different superscripts are significantly different ($P < 0.05$)
([f, g, h, i, j]) Means of hypodynamia with different superscripts are significantly different ($P < 0.05$)
([x, y]) Means of control and hypodynamia with different superscripts are significantly different ($P < 0.05$)

the epithelium of seminiferous tubules of control birds, all the developmental stages of spermatogenic cells were found (Plate II, Fig. 4). Sustentacular (Sertoli) cells were high, slender, and in their apical part the maturing spermatides were immersed in the cytoplasm. Interstitial (Leydig) cells were polygonal and they were present in the interstitium, in groups close to blood vessels.

In the seminiferous epithelium of hypodynamia quail testis, the spermatogenic cells were observed in the stages of mitosis, meiosis, and spermiogenesis, testifying a normal course of spermatogenesis (Plate II, Fig. 5). However, this epithelium was lower in comparison with the control, with empty spaces among the cells. The desquamated immature cells of epithelium occurred in the lumen of the seminiferous tubules. The sustentacular cells had a

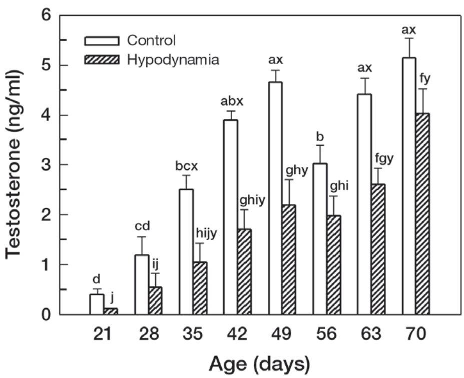


Fig. 6. Concentration of plasma testosterone in male Japanese quail developing in hypodynamia. Values are means ± SEM, n = 10.
([a, b, c, d]) Means of control with different superscripts are significantly different ($P < 0.05$)
([f, g, h, i, j]) Means of hypodynamia with different superscripts are significantly different ($P < 0.05$)
([x, y]) Means of control and hypodynamia with different superscripts are significantly different ($P < 0.05$)

normal structure. The number of maturating spermatides was pronouncedly lower than in the control. Interstitial cells were in groups of 2–4. Sporadically, necrotizing interstitial cells were observed with a shriveled nucleus and dark cytoplasm.

The mean concentration of testosterone in the blood plasma increased progressively in both groups between 21 and 49 days of age (Fig. 6). At day 56, the plasma testosterone was dramatically reduced in control ($P < 0.05$), whereas in males under hypodynamia only slight decrease was observed. Between days 63 and 70, concentration of testosterone increased again in both groups. The peak testosterone concentrations were observed at the end of experiment (day 70). There were significant differences between the level of plasma testosterone in the hypodynamia birds and control from 35 and 70 days of age, with the exception of day 56.

Discussion

The continual state of hypodynamia of male Japanese quail during development caused a decrease of body and tested weight, cloacal gland size and foam production, as well as plasma testosterone concentration, and affected the morphological structure of testes. Significant differences of the tested variables between hypodynamia and control group were mostly recorded between 28 and 49 days of age. Towards the end of experiment they persisted only in body weight, right testis weight and testosterone concentration.

The observed developmental deviations were not unexpected, considering the results from the study of effects of hypodynamia on sexual development of female Japanese quail (Škrobánek et al. 2008). We assume that the reason for these differences might be stress in the first days after hatching that caused a decrease of food conversion and induced subsequent delays in the early growth and development of organ systems and their functions. Moreover, the above mentioned differences could be caused by an absence of free movement that is essential for the development of the animal organism.

Nevertheless, it is difficult to compare our results with those of other authors, because to our knowledge, this is the first report studying the sexual development of male birds in conditions of simulated microgravity. Other studies investigated the effects of simulated microgravity on reproductive activity only in adult male Japanese quail (Juráni et al. 1984). However, more information is available from the studies of the influences of simulated and real microgravity on sexual functions of mammals. For example, the effect of long-term (6-wk) hind limb suspension on testicular function in adult male rats was examined by Tash et al. (2002). Results of their experiments showed that testicular weights and spermatogenesis were significantly reduced by hind limb suspension such that no spermatogenic cells beyond round spermatids were present and epididymides were devoid of mature sperm. By contrast, sustentacular and interstitial cell appearance, testosterone, luteinizing hormone and seminal vesicle weight were unchanged by hind limb suspension. In the rats exposed to a real microgravity for 14 days, circulating testosterone was significantly reduced compared with control exposed to simulated microgravity (Amann et al. 1992). Similarly, Sapp et al. (1990) observed significant reduction in testosterone contents, testes weight (> 50%), and number of spermatogonia in the flight animals versus the free-roaming controls. In mice, the effect of simulated microgravity on testosterone level was examined by Kamiya et al. (2003). The results of their experiments showed that testicular weight and testosterone concentration were significantly lower in the 7-days tail-suspended group compared to controls. In humans, a rapid decline in plasma testosterone concentration was consistently observed in astronauts during Space Shuttle flights (Strollo et al. 1998).

In conclusion, this report is the first study examining the effects of hypodynamia (simulated microgravity) on sexual development of male Japanese quail. The present results indicate that hypodynamia has a negative impact on the cloacal gland, testes and plasma testosterone concentration. Together with our previous experiments, this data may by useful for understanding Japanese quail ontogeny during exposure to altered gravitation in conditions of a real space flight.

Vplyv simulovanej mikrogravitácie na sexuálny vývin kohútikov prepelice japonskej

Cieľom nášho výskumu bolo skúmať vplyv nepretržitej hypodynamie, ako metódy simulácie beztiažového stavu v laboratórnych podmienkach, na rast a vývin semenníkov, kloakálnej žľazy a koncentráciu plazmatického testosterónu u prepelice japonskej.

Hmotnosť semenníkov kohútikov, ktorí boli odchovávaní v podmienkach hypodynamie, bola významne nižšia ako hmotnosť semenníkov kontroly medzi 21 a 63 dňami veku ($P < 0{,}05$). Plocha kloakálnej žľazy pokusných jedincov vypočítaná z jej šírky a dĺžky bola v porovnaní s kontrolou menšia od 35 do 56 dní veku ($P < 0{,}05$). Tiež produkcia peny kloakálnej žľazy bola významne nižšia u kohútikov chovaných v hypodynamii vo veku 35, 42, 49 a 63 dní ($P < 0{,}05$). Koncentrácia plazmatického testosterónu bola významne znížená u jedincov v hypodynamii v porovnaní s kontrolou medzi 35 a 70 dňami veku, s výnimkou 56 dní.

Dosiahnuté výsledky poskytli ďalší dôkaz o tom, že hoci hypodynamia negatívne ovplyvňuje skúmané reprodukčné ukazovatele, samci prepelice japonskej sú schopní normálne sa vyvíjať aj v špecifických podmienkach simulovaného beztiažového stavu.

Acknowledgement

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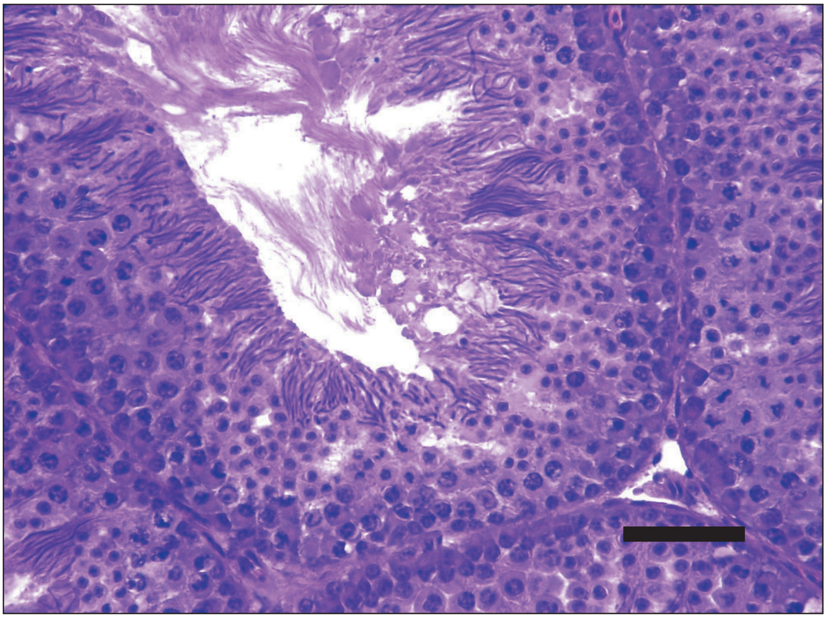

Fig. 4. Seminiferous tubules of Japanese quail developing under standard conditions (control). Scale bar represents 50 μm.

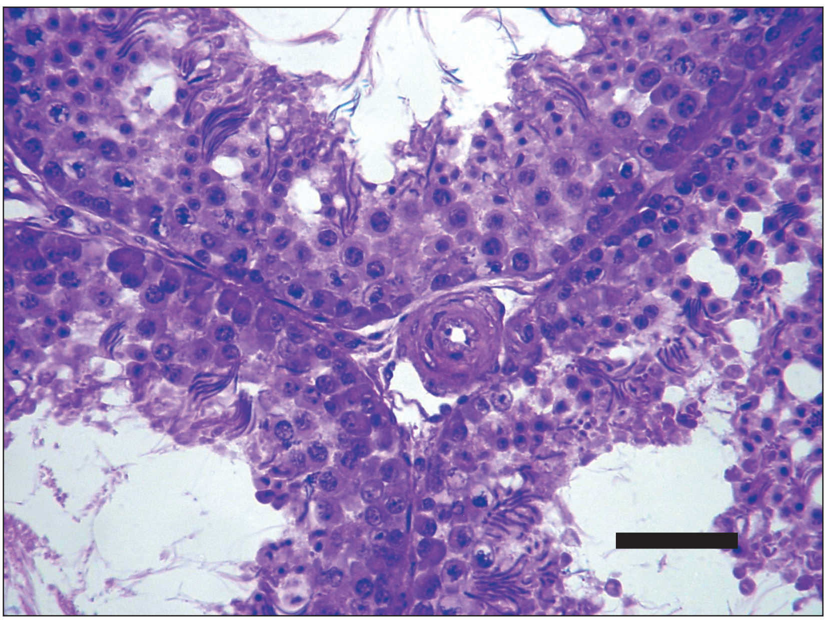

Fig. 5. Seminiferous tubules of Japanese quail developing in the simulated microgravity (hypodynamia). Scale bar represents 50 μm.