# GENETIC MODEL OF CONGENITAL MYASTHENIC SYNDROME DOES NOT AFFECT ESTROUS CYCLE OF FEMALE MICE

PASSOS, Laura G. A[1*].; PESSOA, Pedro W. M[1]; ZANETTI, Gustavo[1]; Carolina Batista[1,2]; MANTOVANI, Henrique[1]; SIMÕES, MARQUES, Gabriel[3]; MAGALHÃES-GOMES, Matheus P. S.[3]; WANNER, Samuel P.[1]; SOARES, Danusa[1], GUATIMOSIM, Cristina[3], GONÇALVES, Dawit A. P.[1,2].

[1]Exercise Physiology Laboratory, School of Physical Education, Physiotherapy and Occupational Therapy, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

[2]Sports Training Center, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

[3] Departament of Morphology, Institute of Biological Sciences, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil

* Corresponding author: lauragapassos@gmail.com

**Introduction:** Congenital Myasthenic Syndromes (CMSs) are part of a rare and diverse group of hereditary genetic disorders characterized by alterations in neuromuscular junction proteins and consequent progressive muscle weakness. Previous studies with transgenic male mice with reduced expression (knockdown, KD) of the Vesicular Acetylcholine (ACh) Transporter (VAChT), an antiporter that transfers ACh from the cytoplasm into synaptic vesicles, demonstrated deleterious effects on skeletal muscle and impairment in physical performance. However, it is unknown the effects of CMS model in female mice and on estrous cycle, which is important in female physiology and its disorder may exacerbate symptoms and alterations in CMS. Although this knowledge is extremely important for health in CMS women, data about

menstrual cycle in such patients are poorly available and about estrous cycle in female mice are unknown. **Objective:** To characterize the frequency of estrous cycle stages in murine model of CSM from 2 to 6 months of age. **Methods:** Transgenic female mice KD for VAChT (VKD) and wildtype (WT) animals from the C57BL/6J background (6-week-old) were used in the experiment. The murine reproductive cycle is divided in four stages (proestrus, estrus, metestrus and diestrus) that can be determined non-invasively by staining cells (i.e., nucleated epithelial cells, cornified squamous epithelial cells, and leukocytes) with crystal violet solution in vaginal smears. Daily assessment of the relative ratio of cells present in vaginal smears was used to identify murine estrous stages. This procedure was performed for 14 days in each month from 2 to 6 months of age. Statistical analysis was performed using the chi-square test. All experiments and protocols were approved by the Ethics Committee on Animal Use from UFMG. **Results**: We found in the analyses of average frequency (14 days in each month) of estrous cycle phases that WT mice in proestrus, estrus, metestrus and diestrus were 18.1% 67.7%, 4.4% and 9.9%, respectively, while VKD mice were 13.3%, 68.9%, 7% and 10.8% in the respective phases, indicating that estrous cycle was unaffected in VKD mice. There was no statistical difference ($P>0.05$) in the frequency of the estrous cycle stages between VKD and WT mice from 2 to 6 month-old. Estrus was the predominant phase of the estrous cycle in both VKD and WT groups in all analyzed time points. Body mass was similar between WT and VKD mice and did not significantly change during the period of the study. **Conclusion:** These results show that CMS did not cause any alteration in estrous cycle of young, adult VKD mice. However, further studies are needed to investigate female physiology in older animals.

Supported by FAPEMIG (APQ-01268-2), PRPq/UFMG (27764*27) and CNPq/PROANTAR (442645/2018-0)

**Keywords:** estrous cycle, vaginal smears, congenital myasthenic syndromes