Hindlimb unloading (HU) is a rodent model system used to simulate weightlessness experienced in space. However, some effects of this approach on rodent physiology are under-studied, specifically the effects on ovarian estrogen production which drives the estrous cycle. To resolve this deficiency, we conducted a ground-based validation study using the HU model, while monitoring estrous cycles in 16-weeks-old female C57BL6 mice. Animals were exposed to HU for 12 days following a 3 day HU cage acclimation period, and estrous cycling was analyzed in HU animals \((n=22)\), normally loaded HU Cage Pair-Fed controls \((\text{CPF}; n=22)\), and Vivarium controls fed \textit{ad libitum} \((\text{VIV}; n=10)\). Pair feeding was used to control for potential nutritional deficits on ovarian function. Vagaline cells were sampled daily in all mice via saline lavage. Cells were dried and stained with crystal violet, and the smears evaluated using established vaginal cytology techniques by two individuals blinded to the animal treatment group. Estrous cyclicity was disrupted in nearly all HU and CPF mice, while those maintained in VIV had an average normal cycle length of \(4.8 \pm 0.5\) days, with all stages in the cycle visibly observed. CPF and HU animals arrested in the diestrous phase, which precedes the pre-ovulatory estrogen surge. Additionally, infection-like symptoms characterized by vaginal discharge and swelling arose in several HU animals, which we suspect was due to an inability of these mice to properly groom themselves, and/or due to the change in the gravity vector relative to the vaginal opening, which prevented drainage of the lavage solution. Pair-feeding resulted in similar weight gains of HU and CPF \((1.5\% \text{ vs } 3.0\%\), respectively\). The current results indicate that pair-feeding controlled weight gain and that the HU cage alone influenced estrous cyclicity. Thus, longer acclimation needs to be tested to determine if and when normal estrous cycling resumes in non-loaded mice in HU cages prior to HU testing. Future studies might also examine whether modifications to the vaginal lavage procedure might prevent the onset of the infection-like symptoms, and allow estrous cyclicity to be measured in this model system. Research supported by NNX15AB48G to JST.