Microgravity exposure induces antioxidant barrier deregulation and mitochondrial structure alterations in TCam-2 cells

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One of the hallmarks of microgravity-induced alterations in several cell models is an alteration of oxidative balance. Notably, also male germ cells, sensible to oxidative stress, have been shown susceptible to changes of gravitational force. To gain more insights into the mechanisms of male germ cell response to altered gravity, a 3D cell culture model was established from TCam2 cells, a seminoma cell line, and the only available in-*vitro* model to study mitotically active human male germ cells. TCam2 spheroids were cultured for 24 hours under unitary gravity (UG) or simulated microgravity conditions (SM), which were obtained using the Random Positioning Machine (RPM). Apoptosis and necrosis analyses performed on UG and SM exposed samples, revealed no significant differences of all the cell death markers**.**

Notably, Mitosox assay revealed a significant oxidation of mitochondria, after microgravity exposure, at least at this culture time. In SM treated samples, gene expression levels (evaluated by Real-time PCR) of the main enzymes of the antioxidant barrier, GPX1 and NCF1, are reduced indicating an influence of SM on mitochondria function. Notably, the expression of HMOX, involved in the heme catabolism of mitochondria cytochromes, is increased. SOD, XDH, CYBA, NCF-2, TXN and TXNRD genes were not affected. The ultrastructural analysis by Transmission Electron Microscopy revealed that SM significantly altered TCam2 spheroid mitochondria, which appeared swollen and, in some cases, disrupted. Indeed, mitophagy, or mitochondrial autophagy, appears to be more represented in samples exposed to simulated microgravity. This result seems to be in line with the increase, mediated by the simulated microgravity, of the enzyme HMOX1.

All together, these preliminary data demonstrate TCam2 spheroids sensitivity to acute SM exposure, strongly indicating a microgravity-dependent modulation of mitochondria morphology and activity and encourage to perform further investigations on chronical exposure to SM of TCam2 spheroids.