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Felinized murine intestinal organoids for gaining insight into sexual reproduction of *Toxoplasma gondii.*

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The apicomplexan parasite *Toxoplasma gondii* is the causative agent of Toxoplasmosis, a zoonotic disease affecting one-third of the human population which can cause severe fetal damage by vertical transmission in pregnancy. Toxoplasmosis has great impact in reproductive outcomes of productive species and thus economic losses worldwide. The life cycle of *T. gondii* encompasses sexual and asexual phases. The asexual cycle can occur in any warm-blooded animal while the sexual stage is restricted to felids. In the feline enterocytes, bradyzoites turn into merozoites, initiating sexual replication that will end in the formation of a zygote. Zygotes are key to the generation of diversity, as they allow the occurrence of genetic admixing and the generation of oocyst that will be disseminated in the environment with the feline feces. Until recently, limitations in the availability of appropriate experimental models had hindered the study of sexual stages despite its clear biological relevance. In the last year, the molecular basis involved in the species specificity of the sexual parasitic forms was identified: an excess of linoleic acid given by the lack of delta-6-desaturase activity in the felid´s intestine. Thus, mimicking these conditions in a murine intestine allowed *T. gondii* to sexually develop in a mouse model, providing the opportunity to answer biological questions relevant to *T. gondii* reproduction without the need of using feline animal models.

This work aimed to set up culturing systems based on "felinized" murine intestinal organoids in order to trigger *T. gondii's* differentiation into sexual stages *in vitro*. For this purpose, murine intestinal organoids generated from isolated intestinal stem cells and maintained in a 3D system inside a matrix, or trypsinized and seeded as a monolayer, were incubated in the presence of 20 µM delta-6-desaturase inhibitor and 200 µM linoleic acid. The cytotoxicity of felinizing compounds in 2D and 3D cultures was assessed showing no cytotoxicity for 5 days of culture. Optimization of the infection assays was performed by incubating intestinal organoids with bradyzoites, at two multiplicities of infection (1:1 and 1:10). The presence of the parasite was evaluated after 5 days of culture by immunofluorescence. Kinetic studies of the sexual differentiation of *T. gondii* were carried out and the evaluation of parasite switching efficiency to sexual stages is ongoing, both by immunofluorescence and qRT-PCR.

We put forward in *vitro* felinized intestinal organoids as a valuable tool for answering biological questions relevant to persistence and dissemination of *T. gondii.*