THREE-LAYER GRADIENT SYSTEM TO GENERATE TESTICULAR ORGANOIDS AND TO MODEL GERM TO SOMATIC CELL INTERACTIONS IN VITRO.

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Abstract Body

Abstract: Spermatogonial stem cell (SSC) proliferation and differentiation are complex processes governed by a broad network of factors and somatic cells. These signaling pathways and cell-to-cell interactions have been exhaustively studied, but a lot still remain unknown. A broad range of *in vitro* approaches such as organ culture or *de novo* formation of seminiferous-like structures from primary testicular cells have been applied to investigate the mechanisms that govern SSC fate decision into proliferation or differentiation. However, a more efficient and controlled model which recapitulate the germ-to-somatic cell associations is still need to study the SSC niche *in vitro*.
Lately, we have developed a novel method, the three-layer gradient system (3-LGS), that allows the reorganization of rat germ and Sertoli cells in spherical-tubular structures (STSs), demonstrating similarities with the seminiferous tubules organization. The characterization of these testicular organoids revealed that they are mainly formed by epithelized Sertoli cells. Additionally, germ cells could be maintained for 21 days in culture on the STSs. Furthermore, undifferentiated germ cells were observed to proliferate and form cellular chains in a similar way as observed *in vivo*.
The 3-LGS constitutes a new method to generate testicular organoids representing a unique model of germ-to-somatic cell association *in vitro* with possible application to search for the role of testicular somatic cells and novel factors involved in the SSC niche regulation and testicular development. Moreover, we propose a broader application of the 3-LGS in the development of organ-like structures in other stem-cell related areas.