Developing testicular organoids for assessing reproductive toxicity of antidepression drugs

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Introduction Evaluating the reproductive toxicity of antidepressants is particularly important for those depressed male patients of childbearing age and crucial for the rational use of drugs in clinic [1,2]. Traditional methods are unable to precisely discern how antidepressants affect sperm [3,4]. In this study, we developed an improved testicular organoid [5] which had all cells (germ cells, spermatocytes, supporting cells) related to sperm production and developed the blood testicular barrier to maintain the testicular microenvironment. Most importantly, this improved testicular organoid could prolong the life span. Using this improved testicular organoid, we evaluated in detail the effects of two most clinical used antidepression drugs, amitriptyline and mirtazapine on sperm development.

Aim and methods We introduced a new 3-layer gradient system (LGS) by changing the testicular organoid culture conditions such as the number of cells and the concentration of Matrigel. By doing so, the area of cytokine exchange around the organoid was greatly increased. This provided more nutrients for the growth and the culture of the organoid. These changes consequently facilitated a long-term culture of the organoid. We next used this improved testicular organoid to assess the reproductive toxicity of antidepression drugs, in particularly to mimic the long-term effect of the drugs on sperm. Two most used clinical drugs, amitriptyline and mirtazapine, were chosen to directly observe their effects on spermatogenic cells so that their reproductive toxicities can be evaluated.

Results

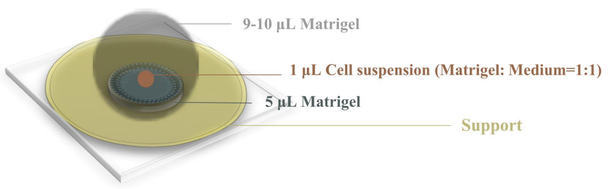

Figure 1. The testicular organoid model. The model has 3-layer gradient system with careful selection of cell culture conditions shown in the figure. A layer of 5 μL Matrigel is on the support. 1 μL volume of cell suspension (medium: Matrigel=1:1) is on the Matrigel. The outermost layer is wrapped by 9-10 μL Matrigel.

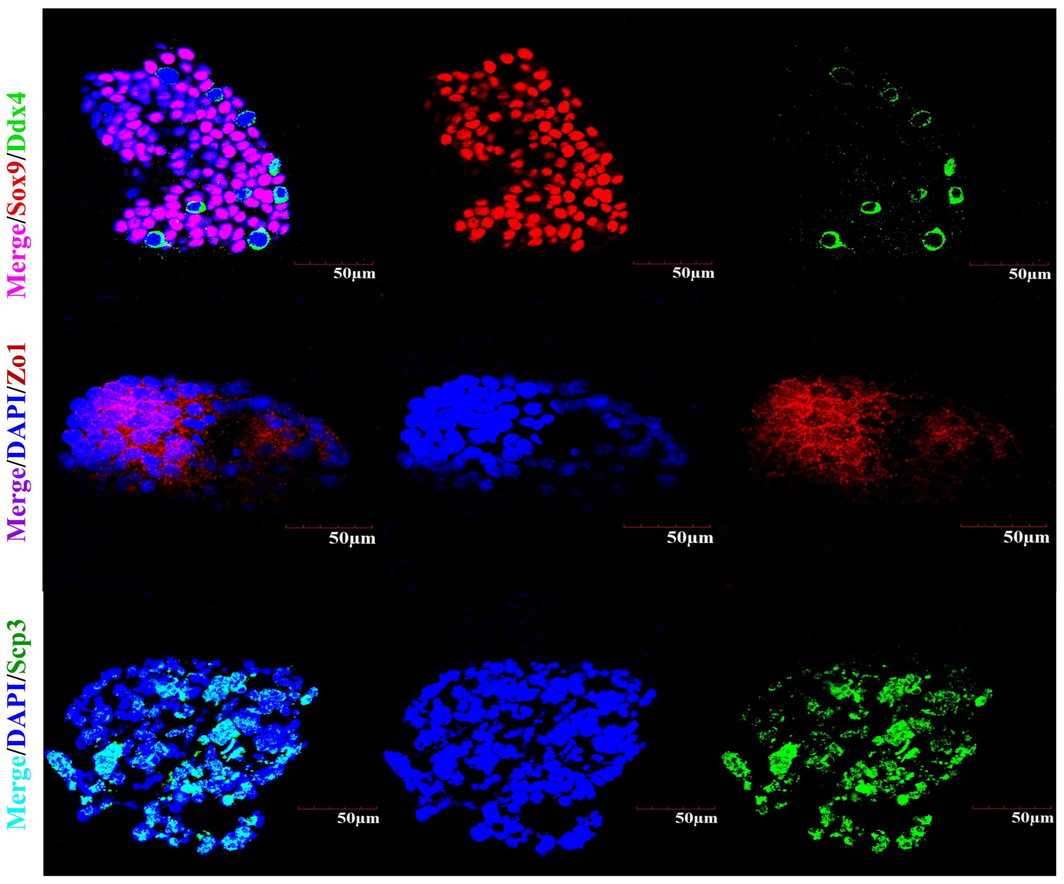

Figure 2. The characterization of organoids. Immunostaining images of Ddx4 (green), Sox9 (red), Zo1 (red) and Scp3 (green) genes for 7-day cultured organoid. Scale bars = 50 μm.

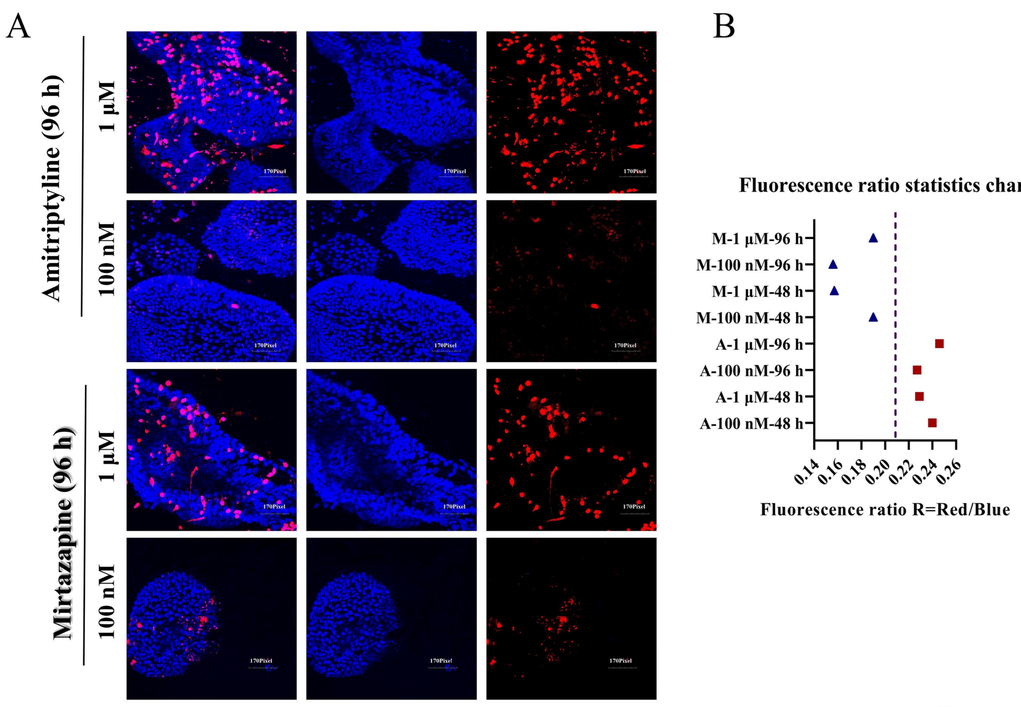

Figure 3. (A) Staining images of Hoechst (Blue)& PI (Red) after treating organoids with 1 μM and 100 nM amitriptyline and mirtazapine for 96 h. (B) The fluorescence ratio statistics chart, R=red/blue=PI/Hoechst. R value was less than 0.21 for the organoids treated with mirtazapine while greater than 0.21 for those organoids treated with amitriptyline.

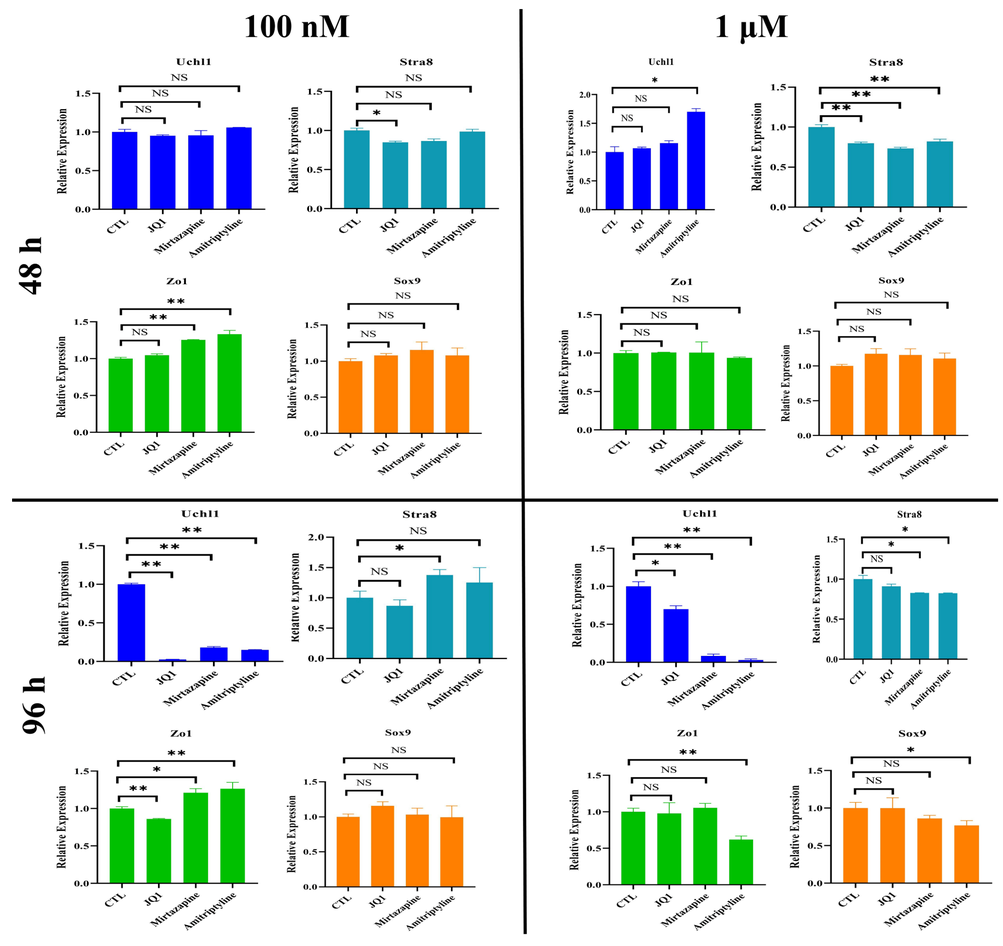

Figure 4. The mRNA expression of Zo1, Sox9, Stra8 and Uchl1 after treatment with 1 μM and 100 nM mirtazapine and amitriptyline for 48 h and 96 h. JQ1 was used a positive control. Gapdh is used as an internal reference gene for qPCR. The number of animals in each group n=3, *p<0.05, **p<0.01.

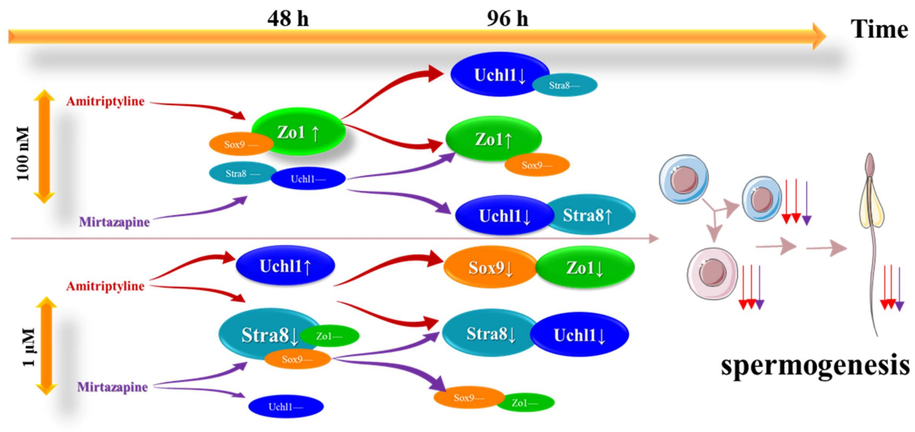

Figure 5. A schematic diagram of the blockade of spermatogenesis with amitriptyline and mirtazapine. "↓" means down regulation, "↑" means up regulation, "-" means no change, "↓ (purple)" means the degree to which mirtazapine affects spermatogenesis, "↓ (red)" means the degree to which amitriptyline affects spermatogenesis, "↓ (purple/red)" the more Indicates that mirtazapine/amitriptyline has a greater impact on spermatogenesis.

Conclusion The testicular organoids developed in this study can mimic the sperm development and then be successfully used to assess the reproductive toxicity. It can sensitively discern the changes between different drugs and their effects on the spermatogenesis process. This provides a powerful tool to observe more comprehensive dynamic process of spermatogenesis and to analyze key signal pathways affected by drugs on specific type of cells. This feasible solution for the evaluation of reproductive toxicity in the drug development certainly will attract further expedition to develop human testicular organoid and of course provide insight knowledge of reproductive toxicity of clinical drugs.

References

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