Poster Presentation

Retinol improves *in vitro* differentiation of neonatal murine spermatogonial stem cells into haploid germ cells

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Abstract

One in 650 children is affected by cancer and about 20% of survivors suffer infertility due to gonadotoxic cancer treatments. Before starting treatments, testis tissue biopsies can be collected and cryopreserved for future use to uphold biological fatherhood. Currently, the only safe approach to produce haploid germ cells in the cryopreserved testis biopsies is through in vitro spermatogenesis, because auto-transplantation/grafting of biopsies runs the risk of reintroducing cancer cells that may have remained in these pre-treatment biopsies. The aim of the present study was to evaluate the effect of retinol on in vitro spermatogenesis using immature mouse spermatogonial stem cells (SSC).

Testis cells were obtained from 6-8 day-old ICR mouse pups, enriched for SSC, and cultured (2.0×10e3/well, in 24-well plates) for >30 days in a differentiation medium (DMEM+2%KSR+8%FBS+10ng/mL GDNF+10ng/mL bFGF), with or without retinol (0.0286 ng/mL=low-RE; 286 ng/mL=high-RE; no RE=control; n=4 replications). Eight wells in each 24-well plate represented a given media composition per time-point.

Immunostaining of CD9 confirmed that all resultant colonies were SSC. At days 10 and 20 of culture, RE-supplemented groups had high levels of *Stra8* (a meiotic stage-specific gene, $P<0.001$). Starting at day 10, *Sycp3* (post-mitotic stage-specific) increased in the low-RE group ($P<0.05$), while it increased at day 30 in high-RE ($P<0.001$). Expression of the haploid male germ cell-specific gene *Acrosin* was up-regulated at day 30 in both RE-supplemented groups but low-RE had the highest levels ($P<0.001$). Round and elongating spermatids were observed in both RE-supplemented groups, but spermatozoa-like cells were only seen in low-RE. Flow cytometry showed that low-RE had the highest population (8.1%) of haploid male germ cells compared with high-RE (4.9%) and control (1.9%).

We concluded that supplementation of 0.0286 ng/mL of retinol is sufficient to improve the in vitro production of haploid germ cells.