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Oral Presentations

O-18
Culture in perfusion mini bioreactor can enhance in vitro spermatogenesis

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Background: In vitro spermatogenesis is one of the main aim of male infertility treatment, which proves critical in cancer patients who undergo treatment with gonadotoxic drugs and methods. Conventional methods of culture cannot support organs or tissues removed from body for a long time. A biomimetic system is achieved by a bioreactor capable of culturing tissues under in vivo-like conditions. Overall, the controlled parameters are fluid flow, pH, temperature, waste removal, nutrition flow. Application of a perfusion flow is for mimicking native testicular microenvironment.

Objective: In this study, we intend to evaluate the progression of spermatogenesis after in vitro transplantation (IVT) of spermatogonial stem cells (SSCs) isolated from mouse fresh testis tissue in mini perfusion bioreactor.

Materials and Methods: Adult mouse azoospermia model was used to remove testis tissue. SSCs isolation was carried out using two enzymatic digestion methods. The cell identification was confirmed via detection of promyelocytic leukaemia zinc finger (PLZF) protein. After being labeled with DiI, the cells were transplanted into azoospermic adult mice. After being fragmented, host testes were incubated for two and eight weeks in a bioreactor. Histological, molecular and immunohistochemical assessments were done after two and eight weeks. Data were statitically analyzed using analysis of variance (ANOVA) test and significancy was considered at ($p < 0.05$).

Results: Histological analysis suggested successful maintenance of spermatogenesis in host testis tissues grown in the bioreactor. Molecular analysis indicated that PLZF, Tekt1 and Tnp1 genes were expressed and that their expression in the experimental IVT group was significantly more than the control group (without transplantation) and 0-day cell suspension ($p < 0.05$). Immunohistochemical evaluation of host testis fragments in the experimental group showed that PLZF, synaptonemal complex protein (SCP3) and acrosin binding protein (ACRBP) proteins were expressed in spermatogonial cells, spermatocytes and spermatozoa, respectively.

Conclusion: Dynamic culturing methods appear to be capable of enhancing spermatogenesis in vitro. Such three- dimensional (3D) culturing system is able to give rise to haploid cells and can offer conditions similar to those of native like physiological microenvironment of testicular tissue. The current bioreactor, thus, can potentially provide an enhanced culturing system for testicular organ culture. Our findings reveal that following two weeks of SSCs transplantation in vitro, and 3D dynamic organ culture, these cells had migrated to basement membrane of seminiferous tubules and settled down through homing and initiated the spermatogenesis process. Perfusion bioreactor dynamic culturing system fosters spermatogenesis induction to generate haploid cells, in which long term (56 days) culturing host testicular tissue segments of the IVT group permitted spermatogenesis completion, giving rise to morphologically intact and mature spermatozoa.

Key words: *Spermatogonial stem cells, Mouse, Transplantation, Azoospermia, Perfusion bioreactor.*