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Immunohistochemical evaluation of spermatogenesis after three-dimensional culture of frozen-thawed neonatal mouse testicular tissue

Ahmad Alrahel

Avicenna Research Institute, ACECR, Iran

Abstract

Objective: In fact, one of the most important improvements in the Germ cell transplantation process is the creation of sperm with frozen cells. Applications of this technique is clinically important specially in young patients with cancer, which should be subjected to severe chemotherapy, which is often used in long-term form and sometimes the irreversible, the amount of sperm production in them is reduced. In immature boys who have not yet started spermatogenesis, it is possible to maintain their spermatogonial activity against anticancer and radiotherapy drugs by frozen spermatogenic cells in liquid nitrogen and re-injected them into the testes. In this study, three-dimensional cultures of testicular tissue were studied in two control and experimental groups to evaluate the spermatogenesis process.

Methods and materials: In this survey, there was two groups as follow: Control group: The culture of frozen-thawed neonatal mouse testicular tissue fragments in the first day after thawing, and Test group: the culture of frozen-thawed neonatal mouse testicular tissue fragments on the agarose cube, in-vitro α -MEM culture and KSR %10 for 8 weeks. Initially, the fragments of neonatal testicular tissues were dissected and transferred to laboratory. The testicular tissue fragments are placed in the special freezing culture, then by using the planner (KYRO 360-1.7 UK) programmable freeze device performs all freezing steps in a slow and stair manner automatically. Finally, the frozen tissue is ready to put in liquid nitrogen. At each stage of the study, the tissue was examined from the aspect of morphology using hematoxylin-Eosin staining. To evaluate the immunohistochemical feature, PLZF, SCP3, ACRBP antibodies were assessed to identify spermatogonia, spermatocyte and sperm-like cell respectively. In this study, three-dimensional culture of frozen-thawed testicular tissue components was studied to evaluate the progression of spermatogenesis process.

Results: The study of tissue sections prepared from the frozen-thawed testicular prepared from the tissue fragments at first day after thawing, and also eight weeks after the cultivation of the studied group revealed that the size and diameter of the seminiferous duct was increased compared to the control group and this increase in size showed the growth of testicular tissue. In two evaluated groups, fresh and frozen-thawed testicular, different categories of germ cells including spermatogonia stem cells and spermatocytes were observed. Although, based on the quantitative results obtained from the software image J, the expression of PLZF proteins in the control group (50.06 ± 8.35), and experimental group (12.85% ± 5.21) was not statistically significant (P≥0.05), the expression level of ACRBP and SCP3 in the control group were (0% ±0) (1.21 ± 1.97) and in the experimental group were (7.85%±2.47) (4.54% ±3.08) which were statistically significant (p≤0.05).

Conclusion: The slow programmed freeze of the testicular tissue and agarose testicular tissue culture appears to be a useful method to maintain long-term testicular tissue preservation, for children with cancer before initiating treatment with chemotherapy or radiotherapy.

Biography:

Ahmad Alrahel has completed his PhD at 2017 from Tarbiat Modares University and MD studies from Tehran of Medical sciences University at 2012. He is the director of Arab Patients Department in Ibn sina IVF clinic. He has published an ISI article and participate in more than 10 congress related reproductive medicine like ESHRE 2016 in Helsinki, Finland. He is a Referee in Iranian Biomedicine Journal.

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