9th Yazd International Congress and Student Award on Reproductive Medicine with 4th Congress of Reproductive Genetics

Poster Presentations

P-40

Application of platelet-rich plasma increases in vitro proliferation of human spermatogonial stem cells in two-dimensional and three-dimensional culture systems

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Background: According to the American Cancer Society, the annual incidence rate of childhood tumors aged birth to 19 years old is 186.6 per 1 million adolescents. Regarding the improvement in life expectancy through the efficient medical procedures, side effects of treatment such as life quality and infertility are of grown importance. Spermatogonial stem cells (SSCs) are very sensitive to chemotherapy and radiotherapy, so male infertility is a great challenge for prepubertal cancer survivors. Cryoconservation of testicular cells before cancer treatment can preserve SSCs from treatment side effects. Different two-dimensional (2D) and three-dimensional (3D) culture systems of SSCs have been used in many species as a useful technique to in vitro spermatogenesis.

Objective: Since there is no available data on the proliferative effect of platelet-rich plasma (PRP) on SSCs, this research focused on the self-renewing of adult human SSCs in two-dimensional and three-dimensional culture systems of PRP.

Materials and Methods: Human testes samples taken from four brain-dead donors at 17, 21, 25, and 26 years old from November 2018 to September 2019. Approval from the family of each donor was acquired by the Organ Procurement Unit (OPU) of Imam Khomeini Hospital affiliated to Tehran University of Medical Science. Testicular cells cultivated in 2D pre-culture system, characterization of SSCs performed by RT-PCR and flow cytometry analysis. PRP prepared and dosimetry carried out to determine the optimized dose of PRP. After preparation of PRP scaffold, SSCs cultivated into three groups: Control, 2D culture by optimized dose of PRP and PRP scaffold. Finally, the diameter and number of colonies measured.

Results: After 2D pre-culture of testicular cells a significant increase in expression of OCT4, Vimentin, and VASA observed in comparison to after digestion ($p < 0.01$). Our results indicated that 16.2 % of all cells were positive for PLZF after enzymatic digestion, whereas after the 2D pre-culture significantly increased the purity of SSCs to 80.2%. After cultivation of SSCs in experimental groups, the number and diameter of colonies in the PRP-2D group increased significantly ($p < 0.01$) as compared to the control group. Interestingly, in the PRP- scaffold group only the mean number of colonies increased significantly ($p < 0.01$) related to the control group.

Conclusion: Our results suggested that PRP scaffold can reconstruct a suitable structure to the in vitro self-renewal and proliferation of human SSCs. The cytokines and growth factors obtained.

Key words: *Spermatogonial stem cell, Proliferation, Two dimensional culture system.*

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