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

## Poster Presentations

### P-45

### In vitro mouse spermatogenesis on artificial testis engineered by 3D printing of extracellular matrix

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**Background:** Male infertility accounts for about 50% of all infertility cases, and 25 % of infertile men are azoospermic. Due to the very small number of spermatogonia stem cells (SSCs) in testicular tissue biopsy specimens, SSCs culture for infertile patients can be important.

**Objective:** The proliferation of SSCs on printed scaffold derived from the extracellular matrix (ECM) of testicular tissues evaluated.

**Materials and Methods:** Ram testicular tissue was decellularized using hypertonic solution -Triton X-100 for 30 min. The extracted ECM (5% ratio) was used as a bio-ink for the fabrication of artificial testes along with alginate and gelatin. Testicular cells were then isolated from the testes of 3-7 days old neonate mice after enzymatic digestion. The nature of SSCs was confirmed by flow cytometry and RT-PCR for specific markers Plzf, Id4, Gfrα1, and Prm1. Finally, cell viability evaluated using MTT test and testicular cell proliferation process on printed alginate-gelatin scaffolds (group I) and ECM-alginate-gelatin scaffolds (group II) using immunocytochemistry, flow cytometry, and real-time PCR techniques was assessed.

**Results:** The MTT test indicated that the cell viability on the composite scaffold was significantly higher than the hybrid scaffolds and control group ($p > 0.05$). The results of 2 wk of proliferation on the printed system showed that the expression of *Plzf, Id4, Gfrα1* gene using real-time PCR in group II was significantly higher than group I ($p > 0.05$). Flow cytometry analysis also showed that the number of Plzf-positive cells in group II was significantly higher than group I ($p > 0.05$). Immunocytochemistry results confirmed that Plzf, Id4, and Gfrα1 markers were expressed in both groups, but their expression in group II was significantly higher than group I ($p > 0.05$).

**Conclusion:** We concluded that the culture of testicular cells on scaffolds containing ECM increases the viability, colonization, and proliferation of SSCs and achieves a high number of cells for differentiation in vitro. Therefore, 3D printing using the ECM of the testis can be an ideal strategy for the regeneration of seminiferous tubules.

***Key words:*** *Spermatogonia stem cells, Extracellular matrix, 3D printing, Proliferation.*