

## 9<sup>th</sup> Yazd International Congress and Student Award on Reproductive Medicine with 4<sup>th</sup> Congress of Reproductive Genetics

### Key Lectures

#### K-36

#### Potential regenerative effects of amniotic-fluid derived exosomes on the rat model of azoospermia

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Any defect during the spermatogenesis process may cause temporary or permanent male infertility. Cell-free therapies and by-products such as exosomes have been used as alternative modalities for the treatment of tissue injuries. There is no data on the use of extracellular vesicles to restore male fertility. This study aimed to explore the therapeutic effects of amniotic fluid-derived extracellular vesicles including exosomes (AF-Exos) on the recovery of sperm production capacity in a rat model of azoospermia. Exosomes were isolated from amniotic fluid samples via ultracentrifugation and characterized by scanning and transmission electron microscopy (SEM and TEM), dynamic light scattering (DLS), and western

blotting techniques. The induction of non-obstructive azoospermia (NOA) in rats was performed by intratesticular administration of 5 mg/kg/testes Busulfan. Azoospermia was confirmed with histological and spermiogram analysis. AF-Exos samples (10 and 40 µg exosomal protein) were injected into the testes of NOA rats. Two months after intervention, the spermatogenesis rejuvenation was evaluated via histopathology (H & E staining), spermiogram, and hormonal analysis. The expression level of a regeneration marker (OCT-3/4) was also studied via immunohistochemistry staining and the number of spermatogonial progenitors was as well evaluated. AF-derived Exos showed sphere-shaped morphology with  $50 \pm 7.521$  nm mean diameter and -7.16 mV zeta potential, and are positive in specific surface markers (CD63, CD9, and CD81). Histopathological and spermiogram data revealed that the spermatogenesis index and sperm parameters were significantly improved after AF-Exos injection compared to azoospermic groups. Also, after AF-Exos injection the OCT-3/4+ cells were increased in NOA rats exhibited spermatogenesis restoration. Both doses of exosome (10 and 40 µg) restored the testicular function in NOA rats. Except in a high dose of AF-Exos (40 µg) for testosterone and FSH, no statistically significant differences were found regarding hormonal levels post-exosome injection. Our study demonstrated that AF-Exos have the potential capacity to facilitate regeneration in the spermatogenesis process and improve sperm quality through paracrine effects via releasing potential restoratives factors into the site of injury. This study provides a novel therapeutic insight on the NOA treatment.