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Implantation of *in vitro* differentiated bone marrow derived mesenchymal stem cells into fibroblasts theca and granulosa cells on liver scaffold in mice model

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Background: Early menopause occurs before the age of 40, is an important medical condition necessitating life-long hormonal replacement therapy. It affects one out of every 1000 women aged 15 to 29 and about 1 out of every 100 women aged 30 to 39years. The causes include premature ovarian failure, chemotherapy and radiotherapy to the pelvis, surgical removal of the ovaries for either benign or malignant conditions, genetic factors and autoimmune diseases. This medical condition may include several risk factors as osteoporosis. Given the problems of poor compliance and dose adjustment, implantation of ovarian organoid is a great option for these patients.

Aim: To establish a protocol for ovarian tissue engineering using decellularized liver matrix seeded with bone marrow-derived MSCs in-vitro differentiated towards fibroblasts, granulosa and theca cells through coculture mechanism.

Materials and Methods: Ovarian Differentiation: Bone Marrow-derived mesenchymal stem cells were induced to differentiate into ovarian lineage by coculture mechanism. Cells were analyzed morphologically for the appearance of ovarian follicles. Genes were analyzed by PCR while proteins were evaluated by immunoblotting and immunofluorescence.

Organoid Preparation: Cultured ovarian cells were loaded on a decellularized porcine liver organoid and cultured in 6-well plates for 2weeks in the complete culture medium. Cellularization of the organoids was monitored morphologically.

Animal Model: 15 adult female mice were subjected to bilateral oophorectomy. Serial detection of ovarian hormones confirmed the reduction in ovarian hormones level.

Organoid Implantation: Ovarian organoids were implanted intraperitoneal in 5 mice and intramuscular in 5 mice, while the rest were left untreated. Animals were hosted for 4weeks with weekly evaluation of ovarian hormone levels in the blood. After 4weeks, animals were sacrificed and histological and immunochemical examination was done to the implanted organoids.

Results and Conclusions: All mice experienced a menopausal state after bilateral oophorectomy. After implantation, implanted organoids showed viability and function both in the peritoneum and intramuscular. However, intramuscular organoids showed better vascularity. All animals with implanted organoids showed correction of the menopausal state as compared to the sustained menopausal state in the control group. Ovarian organoids can present a replacement therapy option.

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