HUMAN UMBILICAL CORD PERIVASCULAR CELLS (HUCPVCS) MAINTAIN THEIR VIABILITY AND REGENERATIVE PROPERTIES FOLLOWING EXPOSURE TO ALKYLATING CHEMOTHERAPY, AND PRE-TREATMENT WITH THESE CELLS PREVENTS CHEMOTHERAPY-INDUCED GONADAL DAMAGE.

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Abstract Body

Introduction: When injected post-chemotherapy, mesenchymal stromal cells (MSCs) from various sources induce regenerative effects in rodent models of chemotherapy-induced gonadal injury. Here, we evaluated properties of first trimester (FTM) human umbilical cord perivascular cells (HUCPVCs), a novel source of MSCs (with increased regenerative capacity compared to older sources), that may render them a promising candidate for chemotherapeutic gonadal injury prevention. 1) their ability to resist the cytotoxic effects of alkylating chemotherapeutic agents *in vitro,* as compared to term HUCPVCs and bone marrow cells (BMSCs); and 2) their ability to prevent gonadal dysfunction if delivered prior to gonadotoxic therapy *in vivo*.

Methods: BMSCs, FTM HUCPVCs, term HUCPVCs were treated with cytotoxic doses of cyclophosphamide (CTX) *in vitro*. Viability, proliferative capacity, mesenchymal cell lineage differentiation capacity, immunogenicity, and paracrine gene expression were assessed. CTX was administered to Wistar rats 2 days following intra-ovarian injection of FTM HUCPVCs. Busulfan was administered to CD1 mice 3 days following an intra-testicular injection of FTM HUCPVCs, term HUCPVCs and BMSCs. HUCPVC survival, ovarian follicle numbers (female model) and the proportion of tubules with active spermatogenesis (male model) were assessed using histological methods. Fertility profiles were assessed in the males.

Results: Following CTX treatment *in vitro*, the viability and expression of MSC markers was unaltered in all MSC types. Both FTM and term HUCPVCs resumed a normal population doubling time by passage 2; retained their ability to differentiate towards chondrogenic, adipogenic and osteogenic lineages; maintained low immunogenicity; and maintained expression of MSC-associated pro-angiogenic, pro-survival and immunomodulatory genes. When compared to media only controls, females receiving FTM HUCPVCs prior to CTX treatment showed a significant recovery in the number of primordial follicles, and males receiving FTM, term HUCPVCs and BMSCs prior to busulfan treatment showed increased proportion of tubules with recovered spermatogenesis ($p<0.05$). FTM HUCPVC- and term HUCPVC-treated animals showed improved mating profiles when compared to busulfan-treated control counterparts ($P<0.05$).

Conclusion: FTM and term HUCPVCs maintain key regenerative properties following chemotherapy exposure and pre-treatment with these cells prevents gonadal damage *in vivo*. Therefore, HUCPVCs are promising candidates for fertility preservation.