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ISOLATION OF FUNCTIONAL HUMAN LEYDIG CELLS: A DIFFERENTIAL-ADHESION APPROACH WITH MULTI-MODAL PHENOTYPIC AND STEROIDOGENIC VALIDATION

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INTRODUCTION

Leydig cells (LCs) are the principal source of testosterone in males, underpinning male reproductive health and androgen-dependent physiology. Precise isolation of viable human LCs is essential for mechanistic steroidogenesis research and cell-based therapeutic development. Existing protocols rely on density-gradient centrifugation, which is technically demanding and often compromises yield and viability. Here, we describe a simplified density-gradient-free approach using differential adhesion to enrich functional human LCs from testicular tissue.

METHODOLOGY

Human testicular fragments (~5 mm^3) were minced and enzymatically digested with collagenase IV (2 mg/mL) at 37°C for 20 min under gentle agitation (100 RPM). The suspension was filtered (45 μm) and plated onto poly-L-lysine-coated T25 flasks. After 24 hours, non-adherent cells were removed by PBS washing. Viability exceeded 90% by trypan blue exclusion. Cells were maintained in DMEM/F12 with 10% FBS, 1% Antibiotic-Antimycotic, and 10 ng/mL luteinizing hormone (LH) to preserve the mature LC phenotype. Characterization employed immunofluorescence and flow cytometry using antibodies against SF-1, StAR, LHCGR, PDGFRA, and TEM-1. Testosterone secretion was quantified by ELISA under basal conditions and LH-stimulated conditions (10 ng/mL).

RESULTS

The protocol yielded ~1.25 × 10^6 LCs per gram of tissue with >80% purity. Adherent HLCs displayed dense cytoplasmic lipid granules and intercellular networks consistent with active steroidogenesis. Flow cytometry confirmed 84.7% $StAR^+$ cells, indicative of a robust steroidogenic population. A distinct progenitor subpopulation ($PDGFR\alpha^+$/$TEM\text{-}1^+$) comprising ~28% of primary cultures, suggests retention of regenerative capacity. Basal testosterone secretion averaged 8.88 ng/mL per 24 hours, confirming preserved functional activity post-isolation.

CONCLUSION

This differential-adhesion protocol efficiently isolates functional human LCs without density-gradient media. Multi-modal validation integrating immunofluorescence, flow cytometry, and ELISA confirms both phenotypic identity and steroidogenic competence, providing a reproducible and accessible platform for LC research and translational applications in male hypogonadism and androgen replacement.