Preservation of structural integrity and cell viability in feline testicular tissue under prolonged organotypic culture conditions

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Male fertility preservation, especially in prepubertal individuals, remains a major concern in reproductive medicine. Organotypic culture of testicular tissue is a promising tool to maintain germ cells and potentially support in vitro spermatogenesis (IVS). These approaches have been successful in rodents (1,2), but adaptation to other species, particularly felids, remains challenging (3). Beyond providing physical support, successful culture also relies on optimized media and supplements to maintain tissue and cell viability. This study evaluated the effects of a rodent-IVS base medium (BM) (2) and its supplementation with retinol (RET: 1 μM) and taurine (TAU: 60 μM)—known for their roles in germ cell differentiation and antioxidant activity—alone or combined (RET+TAU), on the survival and integrity of domestic cat cultured testicular tissue.

Testicular tissue fragments (~1.8 mm^3) from juvenile domestic cats (testes weight <0.30 g; n=10) were fixed immediately (CTR) or cultured on agarose gel blocks in different media at 35°C with 5% CO_2 for 10, 16, 19 and 22 days, time points chosen to encompass early phases of spermatogonial and spermatocyte development**.** Samples were processed for histomorphometric analyses (area of tubular, necrotic and fibrotic interstitial tissue, analyzed relative to total tissue area) and immunohistochemical detection of apoptosis (caspase-3), proliferation (MCM-7), spermatogonial (PGP9.5) and spermatocytes (SYCP3) markers, expressed as the number of positive cells per intratubular area (cells/mm^2). Data (mean±SD) were analyzed using the Kruskal–Wallis test followed by the DSCF post-hoc test ($p<0.05$).

Histomorphometric evaluation indicated that tubular, necrotic and fibrotic areas did not significantly change among culture conditions or over time (ranging from 0.311±0.163 to 0.422±0.126, 0.195±0.135 to 0.303±0.194, and 0.312±0.108 to 0.391±0.113, respectively; $p>0.05$), indicating preserved seminiferous structure.

Consistent with these findings, immunohistochemical analysis revealed that apoptotic cells remained stable throughout the culture period and across media (ranging from 6.78±6.83 to 58.9±87.8 cells/mm^2, $p>0.05$), reflecting limited progression of tissue degeneration. Similarly, spermatogonial and proliferating cells were maintained at comparable levels during the entire culture period and in all experimental conditions (ranging from 763±520 to 1756±1065 cells/mm^2 for PGP9.5, $p>0.05$; from 416±319 to 897±473 cells/mm^2 for MCM-7, $p>0.05$), indicating preservation of the germ cell population and sustained proliferative activity. SYCP3-positive cells were already present in some seminiferous tubules in CTR samples and were occasionally observed at later time points ($p>0.05$), without evidence of further meiotic progression.

All tested media preserved tissue integrity and germ cell populations throughout 22 days of culture, highlighting the system's potential for long-term culture and representing a promising step toward the development of IVS in the feline model.

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