Effects of Vitamin B12-fortified Culture Medium on Proliferation and Adipogenic Differentiation of 3T3-L1 Preadipocytes

6. Cultivation of eukaryotic cells

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Abstract text

Although cultivated meat (CM) is emerging as a potential alternative protein source, evidence suggests that it may lack certain micronutrients that are abundant in conventional meat products, especially vitamin B12. This study investigated the effect of vitamin B12-fortified proliferation medium on 3T3-L1 preadipocytes by evaluating cell viability (Alamar Blue assay), membrane integrity (lactate dehydrogenase; LDH), and cell morphology during proliferation (Days 1 and 2) and differentiation (Day 10). Furthermore, four key genes associated with adipogenic differentiation (CEBPa, CEBPb, FASN, PLIN) were assessed after complete differentiation on Day 10 for selected concentrations. During proliferation, preadipocytes were cultured in medium containing (i) vitamin B12 (0.1–100 µM) with 1% fetal bovine serum (FBS), (ii) 10% FBS (positive control) or (iii) 1% FBS (negative control). Subsequently, cells were switched to adipogenic differentiation medium (10% FBS, 0.5 mM IBMX, 1 µM dexamethasone, 10 µg/mL insulin) until Day 10. Our preliminary results showed that these concentrations (0.1–100 µM) did not significantly alter viability or membrane integrity ($p > 0.05$). Based on the absence of significant cytotoxicity across the tested range, three representative concentrations, 100, 50, and 3 µM with 1% FBS, were selected for further gene expression analysis. Our preliminary RT-qPCR analysis showed that increasing concentrations of vitamin B12 were associated with decreased expression of differentiation-related genes. Further investigation of vitamin B12 accumulation in the cells is needed.