Bovine Trophectoderm has the Metabolic Capacity to Convert Glucose into Glycogen

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Mouse and bovine blastocysts have surprising flexibility in their use of different energy substrates. While studies have shown that bovine blastocysts metabolize glucose primarily to lactate and pyruvate, not all metabolized glucose is accounted for. In this study we asked whether bovine blastocyst could convert glucose into glycogen as a means of energy storage for later use. Two approaches were taken. The first involved staining Day 7-8 blastocysts with the Periodic Acid-Shiff (PAS) method to determine if they stained positive for glycogen. The second used gas chromatography-mass spectrometry (GC-MS) to determine if stable isotope labeled [$^{13}C_6$]-glucose could be incorporated into glycogen.

In vitro produced bovine embryos on day 6 post fertilization were obtained from ART, Madison WI and cultured in SOFBE1 at 38.5°C in 6% $C0_2$, 6% O_2 and balance N_2 until they became expanded blastocysts on Day 7 or 8. Blastocysts were fixed with 4% formaldehyde and stained according to the PAS kit directions (Abcam).

Strong positive staining was observed in both trophectoderm and inner cell mass. The diastase treated controls, which should eliminate staining due to glycogen, retained strong staining suggesting that the stained substances were predominantly mucins (i.e., neutral and acid simple non-sulfated and acid complex sulfated types). To resolve the question as to if any of the glucose was being stored as glycogen, the metabolic processing of glucose in bovine trophectoderm cell line (CT-1) was assessed using stable isotope-based GC-MS analysis. Feeder-free CT-1 cells were grown in six-well Corning Cellbind™ plates and cultured in DMEM with 4.5 g per L glucose and 4 mM glutamine, 2 mM sodium pyruvate, 0.1mM non-essential amino acids, 50 U per ml penicillin/streptomycin and 10 % FBS at 37.5°C in 5% CO_2. Prior to treatment, cells were washed with SOFBE1 lacking pyruvate, lactate, and hexose. Cells were incubated in 2 ml of SOFBE1 supplemented with 0-, 1-, 5-, and 25-mM glucose containing 50% [$^{13}C_6$]-glucose. Cells were incubated for six hours, collected, washed twice with PBS, and stored at -20°C before being processed for GC-MS. Briefly, the glycogen pellet was extracted with ice-cold ethanol (95%), washed twice with ice-cold ethanol to remove any residual-free glucose, and dried at room temperature. The glucose units in glycogen were liberated by incubation (1 h at 55°C) with 0.5mg of amyloglucosidase (31.2 units/mg of solid) followed by di-O-isopropylidene acetate derivatization of glucose units and GC-MS analysis of the ^{13}C-isotopomers of glucose units from glycogen.

Increasing amounts of enrichment in M+3 and M+6 isotopomers of glucose units from glycogen were observed in a concentration dependent manner with 25 mM>5 mM>1mM glucose in the media (percent mole excess for M+3: 1.36±0.47; 1.03±0.29; 0.51±0.08 [R^2=0.36, F(3, 24) =4.555, p=0.0116] and for M+6: 2.89±0.97; 2.28±0.61; 0.57±0.11 [R^2= 0.41, F(3, 24)=5.503. p=0.0051], respectively). No labelled M+3 or M+6 isotopomers were detected in the control (no glucose supplementation). These results suggest that bovine trophectoderm cells have the metabolic capacity to incorporate and store available glucose into glycogen.

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