PRELIMINARY FINDINGS ON CARBOHYDRATE METABOLISM OF INTACT EQUINE CUMULUS-OOCYTE COMPLEXES DURING *IN VITRO* MATURATION

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Abstract.
Production of equine embryos *in vitro* is gaining popularity, and many differences exist in composition of *in vitro* maturation (IVM) media. Metabolism of the cumulus-oocyte complex (COC) is essentially unknown in the horse. Here, we describe preliminary data on carbohydrate metabolism of the equine COC during IVM. COC, collected by scraping of the granulosa layer of all visible follicles of abattoir-derived ovaries, were held overnight (12–18 h) at room temperature (~20°C) and then placed in Maturation Medium (M199 with Earle's salts, 10% FBS, with 25 μg mL^{-1} gentamicin and 5 mU mL^{-1} FSH). They were incubated singly in 10-μL droplets under mineral oil for 30 h at 38.3°C in 5% CO_2 in air. Control droplets without COC were incubated in the same dish. After incubation, COC were removed and spent media kept at –80°C for later analysis. Oocytes were denuded of cumulus cells by pipetting in the presence of hyaluronidase and evaluated by light microscopy at 500×. Those with a visible polar body were classified as metaphase II (MII); oocytes with an intact oolemma and no polar body were classified as immature intact (INT) and those with an irregular or unapparent oolemma, or shrunken cytoplasm, were classed as degenerating (DEG). To adjust for variation in cumulus cell number, the stripped cumulus cells were frozen at –20°C and later analysed for DNA content using Picogreene. The spent media was analysed for depletion of glucose and appearance of lactate on a BMG Fluostar spectrophotometer using an enzyme-linked ultrafluorometric method. Data were expressed as pmol/ng DNA/hr and analysed by *t*-test, x^2 and logistic regression. Thirty COC were cultured and analysed; 14 were classified as MII, 2 INT and 14 DEG. Seven COC (23%) depleted all the available glucose, indicating that the rate of glucose consumption in those 7 complexes was ≥1866 pmol/COC per hour. DNA content was positively correlated with glucose depletion ($P = 0.02$). In the COC that did not deplete available glucose, the ratio of glucose consumption:lactate production was 1.82, indicating that the major fate of exogenous glucose was production of lactate by glycolysis. In the 7 oocytes that depleted all the glucose, the ratio of glucose consumption:lactate production was 1.22. One explanation for this may be that when glucose was no longer available, it was conserved for other pathways. It was noteworthy that these COC had more cumulus cells ($P < 0.01$) and the maturation rate was 4/7 (57%). In the group of COC that did not deplete all of the glucose, there was no significant difference in glucose consumption (13.17 *v.* 12.25 pmol/ng DNA per hour; $P > 0.4$) or lactate production (21.48 *v.* 20.28 pmol/ng DNA per hour; $P > 0.4$) between COC in which the oocyte reached MII (10/23; 43%), and those which contained a degenerated oocyte at the end of culture, respectively. To the best of our knowledge, this is the first report documenting the metabolism of equine COC. These data underline the importance of further studies to determine optimal conditions for *in vitro* maturation of equine COC, especially in terms of glucose availability.