# BOVINE EMBRYOS CAN USE KETONE BODIES AS ENERGY SUBSTRATES AT DIFFERENT DEVELOPMENTAL STAGES IN VITRO. 

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In vitro produced (IVP) bovine embryos can develop in presence of the ketone bodies (KB) acetoacetate and $\beta$-D-hydroxybutyrate. The lipid store in embryonic cells suggest that KB presumably play a role during embryo development. The effects of KB, added on different periods of the early bovine embryo development, were examined.

Bovine cumulus-oocyte complexes (COCs) from slaughtered Asturiana de los Valles cow ovaries were incubated for 24 h in TCM-199, FSH, LH, $\mathrm{E}_{2}$ and FCS in $5 \% \mathrm{CO}_{2}$ in air and high humidity throughout. Matured COCs were fertilized with swim-up separated frozen-thawed sperm for $18 \pm 1 \mathrm{~h}$. Presumptive zygotes were vortexed for 3 min and cultured in mSOF suplemented either with lactate/pyruvate $3.3 \mathrm{mM} / 0.3 \mathrm{mM}$, as usual, or with a single KB 3.6 mM at 3 periods: 0 to $48 \mathrm{~h}, 48$ to 120 h and 120 to 216 h . Results of embryo development are shown in table 1. Data were analyzed by ANOVA and Tukey's test, and expressed as means士SEM.

TABLE 1: Development up to the hatched blastocyst stage of bovine IVP embryos cultured in mSOF with acetoacetate or $\beta$-D-hydroxybutyrate replacing lactate and pyruvate during 3 different periods of culture.

| Culture period/ <br> Treatment | Embryos | \% Blastocysts <br> Day 8 (168 h) | \%Expanded <br> blastocysts | \%Hatched <br> Blastocysts |
| :--- | :--- | :--- | :--- | :--- |
| $0-48 \mathrm{~h} /$ |  |  |  |  |
| Acetoacetate | 106 | $36.84 \pm 9.01$ | $34.94 \pm 7.94$ | $24.54 \pm 9.79$ |
| Hydroxybutyrate | 111 | $27.13 \pm 2.86$ | $19.26 \pm 3.11$ | $13.82 \pm 2.91$ |
| Lactate/Pyruvate | 108 | $37.63 \pm 0.79$ | $34.51 \pm 2.45$ | $27.28 \pm 4.48$ |
| $48-120 \mathrm{~h} /$ | $8-16$ cell |  |  |  |
| Acetoacetate | 89 | $60.02 \pm 11.73$ | $48.46 \pm 7.66$ | $19.74 \pm 1.23$ |
| Hydroxybutyrate | 90 | $52.84 \pm 4.67$ | $32.96 \pm 6.79$ | $15.82 \pm 6.22$ |
| Lactate/Pyruvate | 90 | $46.47 \pm 7.12$ | $34.22 \pm 5.51$ | $18.38 \pm 5.31$ |
| 120-216 h/ | $\mathrm{M}+\mathrm{EB}$ |  |  |  |
| Acetoacetate | 58 | $74.75 \pm 6.46$ | $56.46 \pm 16.37$ | $21.89 \pm 9.76$ |
| Hydroxybutyrate | 56 | $51.10 \pm 12.71$ | $49.34 \pm 13.77$ | $15.16 \pm 10.06^{\mathrm{a}}$ |
| Lactate/Pyruvate | 56 | $64.31 \pm 8.57$ | $53.64 \pm 5.78$ | $27.40 \pm 6.88^{\mathrm{b}}$ |

Period 0-48 h: 3 replicates; period 48-120 h: 4 replicates; period $120-216$ h: 3 replicates. M+EB= morulae + early blastocysts.Values with different superscripts within each column and culture period differ $(P<0.05)$

Results demonstrate that acetoacetate and hydroxybutyrate can be used as energy substrates at different periods along the early bovine embryo development in vitro. This means that embryos are enabled to make a metabolic use of a eventual lipid breakdown.

