EFFECT OF INSULIN ON THE DEVELOPMENT OF BOVINE EMBRYOS FERTILIZED *IN VITRO*

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In order to provide information on the effect of insulin on the development of bovine embryos fertilized *in vitro*, the postulation that supplementation with insulin could enhance embryonic development has been investigated.

Supplementation with insulin (5 μg/ml) significantly ($p<0.05$) supported embryonic development into the morula stage compared with the control (27.3 vs. 13.8%, respectively), when *in vitro* fertilized embryos were cultured in modified synthetic oviduct fluid (mSOF) supplemented with 10% fetal calf serum (FCS).

In the next experiment, *in vitro* fertilized embryos were cultured in a chemically defined medium, mSOF supplemented with polyvinylalcohol (PVA) instead of FCS, to examine the effect of insulin and amino acids (AAs) on embryonic development into the morula stage. The developmental rate into the morula stage was increased by supplementing with insulin (31.4%) or AAs (23.0%). However, the stimulating effect insulin had on embryonic development was not synergistic with that of AAs.

When *in vitro* fertilized embryos were cultured in mSOF + AAs, the percentage of embryos that developed to the morula stage increased in proportion with insulin concentration. Insulin did not improve the developmental rate into the blastocyst stage. However, a significant increase in cell number of blastocysts was observed with the addition of 1mM glucose and 1 or 10 μg/ml insulin from the morula stage (5 days after insemination).

In conclusion, the present study shows that insulin and AAs support the development of *in vitro* fertilized bovine embryos to the morula stage. The stimulating effect of insulin is not synergistic with that of AAs. A beneficial synergistic effect of insulin and glucose supplementation on embryonic development from the morula stage was observed. Present experiments also demonstrate that in vitro fertilized bovine embryos can develop to the blastocyst stage in a chemically defined medium.