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Effect of medium change and embryo density on  
*in vitro* development of mouse and bovine 1-cell embryos

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The objective of this study is to determine the effect of medium change and embryo density on *in vitro* development of mouse and bovine 1-cell embryos, and to evaluate the correlation between embryo development and ammonia concentration in the culture medium.

In experiment 1, different numbers of ICR mouse 1-cell embryos (1, 10 and 20 embryos in a 20  $\mu$ l drop) were cultured in the presence of amino acids for five days. The change of culture medium at 48 h after initiation of culture had no effect on embryo development to blastocysts in each embryo density. However, regardless of medium change, increasing embryo density significantly enhanced mouse embryo development.

In experiment 2, ICR mouse embryos were cultured for five days in the media supplemented with two distinct amino acid mixtures, basal medium Eagle and minimum essential medium. The change of culture medium at 48 h after initiation of culture had no effect on embryo development in each medium. Ammonia concentration in the culture medium was significantly reduced by medium change at 120 h of culture. However, there was no significant effect of amino acids treatment on embryo development and ammonia concentration in culture media.

In experiment 3, different numbers of *in vitro* matured and fertilized bovine 1-cell embryos (0, 3 and 30 embryos in a 30  $\mu$ l drop) were

cultured in the presence of amino acids for seven days. The change of culture medium at 90 h post insemination had no effect on the development of bovine embryos in each embryo density. However, regardless of medium change, bovine embryos cultured in groups of 30 had greater potential to develop to blastocysts with increased cell numbers compared to the other groups. Ammonia concentration in the culture medium in each group was increased linearly during the culture and significantly reduced by medium change. When embryos cultured in groups of 30, ammonia concentrations at 90 and 171 h post insemination were greater compared to the other groups.

In conclusion, ammonia concentration in culture media at the end of culture was significantly reduced by medium change. However, we found no advantage of changing medium during the culture as determined by developmental rate of embryos to blastocysts. These data indicated that the detrimental effect of ammonia in the culture medium is neglectable from this study in ICR mouse and bovine embryo development. On the other hand, high embryo density enhanced development of mouse and bovine 1-cell embryos to blastocysts. These results may indicate that embryos produce factor(s) which stimulate their own development.