VIABILITY OF BOVINE BLASTOCYSTS OBTAINED AFTER 7, 8 OR 9 DAYS OF CULTURE FOLLOWING VITRIFICATION AND ONE-STEP REHYDRATION

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This study examined the morphological appearance, hatching rates and live:dead cell ratios following vitrification of in vitro produced (IVP) bovine blastocysts. Expanded blastocysts obtained after 7, 8 or 9 d of culture were vitrified following 2-step equilibration in 10% ethylene glycol (EG) plus 0.3M trehalose in Dulbecco's PBS (DPBS) supplemented with 10% calf serum and 0.6% BSA(m-PBS) for 5min at 22^0C and then a vitrification solution consisting of 40% EG plus 0.3M trehalose and 20% PVP in DPBS supplemented with 0.3% BSA for 1 min. Embryos were aspirated into 0.25 ml insemination straws and cooled rapidly by plunging into liquid nitrogen. Straws were thawed in 30^0C water for 20 sec and the contents were expelled into 2 ml of m-PBS. Embryos were washed 2 to 3 times in fresh m-PBS and their immediate post-thaw survival was assessed morphologically. Then, embryos were cultured in TCM199 and viability of frozen-thawed embryos recorded visually by re-expansion/hatching of embryos at 24, 48 and 72 h. The live:dead cell ratios of hatched blastocysts was determined by differential fluorochrome staining. Briefly, hatched blastocysts were rinsed in fresh culture medium, incubated in PBS containing propidium iodine (PI, 10 ug/ml) and bisbenzimide (10 ug/ml) for 30 min in an incubator at 38.5^0C and 5% CO_2 in air. Embryos were washed in PBS supplemented with 3% BSA and squashed on a microscope slide and examined with a fluorescence microscope. The total cell number and live:dead cell numbers of 10 embryos were determined for each day of culture (Day 7, 8 or 9).

Table 1. Embryo viability following vitrification and dilution

Days in Culture	No. of Embryos	No. Surviving	No. Hatching	No. of Cells	Live:Dead Ratio
7	36	31 (86%)[a]*	28 (78%)[a]	92±18	89:3
8	71	51 (72%)[a]	27 (38%)[b]	87±10	84:3
9	65	30 (46%)[b]	6 (9%)[c]	85±16	80.5
Control (unvitrified)	40	40 (100%)[a]**	35 (88%)[a]	88±31	85:3

[abc] Values within columns with different superscripts are significantly different ($P<0.01$)
[a]* with [a]** ($P<0.05$, Chi-Square test)

The average number of cells and live:dead cell ratio of hatched blastocysts was not significantly different. Hatching rate was significantly ($P<0.01$) influenced by the number of days in culture prior to vitrification. These results indicate that Day 7 blastocysts survive vitrification and direct dilution after thawing.