Calving Production from Hanwoo (Korean Cattle) IVM/IVF/IVC Blastocysts: Direct Transfer of Vitrified and Quick One-Step Diluted Hanwoo Blastocysts

Sae Young Park, Deok Im Kim[1], Jin Cheol Tae,
Eun Young Kim, Won Don Lee[2], Sepill Park, Jin Ho Lim[2]
Maria Infertility Hospital Medical Institute/Maria Biotech,
[1]Hanwoo Improvement Center, NLCF, [2]Maria Infertility Hospital

This study was to examine whether vitrified Hanwoo (Korean cattle) IVM/IVF/IVC blastocysts can survive *in vitro/in vivo* by a quick one-step dilution method and these embryos result in live births. Blastocysts produced *in vitro* were vitrified by serial exposure to glycerol (G) and/or ethylene glycol (EG) mixtures of 10% (v/v) G for 5 min, 10% G plus 20% EG (v/v) for 5 min, and 25% G plus 25% EG (v/v) for 30 sec. The blastocysts were then loaded in straw, placed in cold nitrogen vapor for 3 min and plunged into LN2 (-196℃). One-step dilution within the straw was done in 25℃ and/or 36℃ water baths for different times (from 1 min to 3.5 min). *In vitro* survival of vitrified embryos was 76.9~93.5% after the one-step dilution. However, 48 h after thawing, the *in vitro* development rates in the beyond hatching or hatched state in 1 min dilution group (the quick one-step dilution method; 73.3 and 56.7%, respectively) were better than those of the other treatment groups (55.6~61.3% and 8.1~36.1%, respectively). Direct transfer of quick one-step diluted embryos into recipient cows resulted in an overall pregnancy incidence of 33.3% (12/36). The good pregnancy incidence was obtained when the recipients estrus cycle was one day earlier than the age of the transferred embryos (53.3 vs. 25.0~27.3%), irrespective of synchronization methods or condition of the corpus luteum. We obtained nine offspring from 12 pregnant cows. Therefore, quick one-step dilution and direct transfer of vitrified bovine IVM/IVF/IVC blastocysts could be applied as an efficient method for the field trials.

Key word: Hanwoo IVM/IVF/IVC blastocysts, Vitrification, One-step dilution, Direct transfer