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Efficient Cryopreservation of Hanwoo (Korean Cattle) Blastocysts Derived from Nuclear Transfer with Somatic Cell Using Vitrification

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Cryopreservation by vitrification of Hanwoo blastocysts derived from nuclear transfer with Hanwoo adult ear cell was compared with that of in vitro fertilized blastocysts. For vitrification, day 7 or day 8 blastocysts were serially exposed in glycerol (G) or/and ethylene glycol (EG) mixtures (10% G for 5 min, 10% G plus 20% EG for 5 min, and 25% G plus 25% EG for 30 sec) which was diluted in 10% FBS added D-PBS. Thawing of straw was carried out in air for 10 sec and then in water bath of 25℃ for 20 sec. The contents of the straw (0.2 ml) was expelled into a culture dish contained with 0.5M sucrose and 10% FBS (S-DPBS) by cutting the cotton plug and then the recovered embryos were put into fresh 0.25M and 0.125M S-DPBS for 30 sec, respectively. The embryos were transferred into D-PBS with 10% FBS for 5 min. and were cultured in a 10ul droplet of co-culture environment (cumulus cell monolayer + 10% added CR1 medium) for 24 h. In the result, survival rates were 88.9% and 85.4% for nuclear transfer embryos and in vitro fertilized embryos, respectively. After transfer of vitrified-thawed blastocysts produced from nuclear transfer, 4 of 5 total recipients did not return to the subsequent estrus cycle at 30 days. It is concluded that the Hanwoo blastocysts derived from nuclear transfer can be successfully cryopreserved using simple and efficient vitrification method.

Key Words: ***IVF, Nuclear transfer, Vitrification, Cryopreservation***