P-8 Efficient Cryopreservation of Hanwoo (Korean Cattle) Blastocysts Derived from Nuclear Transfer with Somatic Cell Using Vitrification

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Objective: This study was to compare the *in vitro* survival after vitrification and thawing of Hanwoo blastocysts derived from nuclear transfer with Hanwoo adult ear cell and *in vitro* fertilized blastocysts.

Materials and Methods: 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 0.5 M sucrose and 10% FBS (S-DPBS) by cutting the cotton plug and then the recovered embryos were put into fresh 0.25 M and 0.125 M S-DPBS for 30 sec, respectively. The embryos were transferred into D-PBS with 10% FBS for 5 min and were cultured in a 10 ul droplet of co-culture environment (cumulus cell monolayer + 10% added CR1 medium) for 24 h.

Results: 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.

Conclusion: It is concluded that the Hanwoo blastocysts derived from nuclear transfer can be successfully cryopreserved using simple and efficient vitrification method.

P-9 성숙이 정지된 생쥐난자의 Ca^{2+}-channel에 관한 연구

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목 적: 근육세포 및 신경세포 등에서 크게 (1) voltage-dependent Ca^{2+}-channel (① P/Q-type Ca^{2+}-channel, ② N-type Ca^{2+}-channel, ③ T-type Ca^{2+}-channel, ④ L-type Ca^{2+}-channel 및 ⑤ R-type Ca^{2+}-channel) (2) ligand-gated Ca^{2+}-channel (3) Ca^{2+}-leak channel (B_1, B_2 and B_3-type Ca^{2+}-leak channel) 등의 세 종류가 알려져 있으나 포유동물의 난자나 수정 후 embryo에서 어떤 type의 Ca^{2+}-channel이 존재하는지에 대해서는 많이 알려지지는 않았다.

본 실험에서는 정상적인 여포난자 (germinal vesicle, GV)와 여포난자를 3시간 배양하여 GVBD (germinal vesicle breakdown)가 일어난 난자의 Ca^{2+}-channel의 분포 양상과 17시간 배양하여 제 1극체를 형성하지 못하고 GV나 GVBD에서 정지한 난자를 voltage-dependent Ca^{2+}-channel 단백질에 대한 항체를