결 과: P/Q-type Ca^{2+}-channel에 대한 염색 실험에서 초기 2-세포기, 체외배양 2-세포기, Ni^{2+} 처리한 2-세포기 배 대부분이 부분적인 분포를 나타내는 localized staining이었고 4-세포기 배에서는 세포막 전체로 퍼져 나타나는 homogeneous staining이 많았으나 체외에서 배양한 4-세포기 배나 Ni^{2+} 처리로 2-cell block을 극복한 배에서는 homogeneous staining 보다 localized staining이 많이 나타남으로서 체내 발생된 세포와는 다른 양상을 보임을 알 수 있었다.

N-type Ca^{2+}-channel에 대한 염색 실험에서는 초기 2-세포기 배에서부터 2-cell block에 걸린 세포와 Ni^{2+}로 극복된 2-세포기 배, 체내 발생 4-세포기 배와 체외 발생 4-세포기 배, Ni^{2+} 처리로 2-cell block이 극복된 4-세포기 배로 갈수록 homogeneous staining이 약 90%로 나타나 발생과정이 진행될수록 세포 전체에 N-type Ca^{2+}-channel이 퍼져 존재한다고 사료된다. 항체 Anti-α_{1C}를 이용한 L-type Ca^{2+}-channel은 초기 2-세포기 배를 비롯한 발생정지 현상이 나타난 배, 자연적으로, Ni^{2+} 처리로 인해 극복된 배 등 모두에서 대부분이 localized staining으로 나타났으나 2-세포기 때는 2개의 localized staining이 나타난 것이 가장 많았고 4-세포기 배가 되어서는 4개의 localized staining을 나타낸 것이 가장 많아 2-세포기 배에서 4-세포기 배로 발달하면서 각 할구에 1개씩의 localized staining이 나타나도록 발달한다는 것을 알 수 있었다. L-type Ca^{2+}-channel이지만 항체 Anti-α_{1D}의 경우 homogeneous staining과 homogeneous와 localized staining이 함께 나타나는 mixture staining이 많이 나타나 항체 Anti-α_{1C}와는 다른 분포를 나타내었다.

결 론: 본 실험에서 자연적으로, Ni^{2+}을 처리하여 2-cell block 극복 전후의 배에 대한 Ca^{2+}-channel 분포양상은 2-세포기에서 4-세포기로 발생된 배라도 2-cell block이 완전하게 극복된 것이 아니라 포배기에 이르지 못하고 발생정지 현상을 나타냄으로서 체내에서 발생한 배들과는 분포양상에 차이가 있음이 확인되었다. 또한 Ni^{2+}을 처리하여 2-cell block이 극복된 경우에서도 Ni^{2+}의 영향으로 Ca^{2+}-channel 형성이 체내 발생한 배와 다르게 나타나고 이것은 Ni^{2+}이 세포내 대사에 영향을 미친 것으로 추정할 수 있었다.

P-5 Systems for Production of Calves from Hanwoo IVM/IVF/IVC Blastocyst: IV. Direct Transfer of Vitrified and One-Step Diluted Hanwoo Blastocysts

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Objective: This study was to examine whether the vitrified, one-step diluted and direct transferred Hanwoo IVM/IVF/IVC blastocysts can be successfully survived in vivo and they were succeeded into the live birth.

Materials and Methods: For vitrification, blastocysts were serially exposed in glycerol (G) or/and ethylene glycol (EG) mixtures [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] which is 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. One-step dilution within the straw was done in water bath of 25℃ for 1 min.

Results: Vitrified and one-step diluted embryos were directly transferred into 36 (natural or hormone induced synchronized) recipient cows in 6 areas of Kyungsang Buk-Do. Pregnancies were confirmed at first when recipient cows did not return to the subsequent estrus cycle, and later by manual palpation per rectum on day 45, 90 and then living calves were derived into parturition. Overall pregnancy was 33.3% (12/36). However, higher pregnancy was obtained when the recipients exhibited estrus one day earlier than the age of transferred embryos (53.3 vs 25.0~27.3%), irrespective of synchronization methods. Also, parous recipients became pregnant higher than nulliparous heifers. And, there were not different in pregnancy rates by the aspect of corpus luteum (CL) quality of recipients (good, 29.4; fair, 37.5; poor, 33.3%). One hundred eight of frozen-thawed Hanwoo blastocysts were directly transferred into 36 recipent cows. In 12 of pregnant cows, 3 cows were aborted and 9 cows were calved [single, 66.7% (6/9); twin, 33.3% (3/9)]. Total embryo implantation rate was 11.1% (12/108). However, 9 Hanwoo calves were lived.

Conclusion: These results demonstrated that direct transfer technique of vitrified and one-step diluted bovine blastocysts can be applied easily and effectively with high pregnancy rate on field trial without the equipment and embryological skills.

P-6 Influence of Transforming Growth Factor-α on Expression of Matrix Metalloproteinase-2, 9 and Epidermal Growth Factor Receptor Gene in the Mouse Blastocysts

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Objectives: To investigate the influence of transforming growth factor-α (TGF-α) on preimplantation development, the expression of MMP-2, 9 and epidermal growth factor receptor (EGFR) mRNA in mouse blastocysts and the effect on the production and activation of MMP-2, 9 during trophoblast outgrowth.

Materials and Methods: Two-cell mouse embryos were cultured for 96 hr in the absence or presence of 1, 10 and 100 ng/ml TGF-α. Reverse transcription-polymerase chain reaction (RT-PCR) was used to examine the expression of MMP-2, 9 and EGFR mRNA in vitro cultured blastocysts. To investigate the effect on the production and activation of MMP-2, 9 during trophoblast outgrowth, the conditioned medium collected after 3 days of embryo culture (120 hr after hCG) and 5 days of embryo culture were assayed for MMP activity by gelatin zymography.

Results: The relative expression level of MMP-2, 9 mRNA in the blastocysts treated with TGF-α was higher than those of the control in a concentration-dependent manner. The relative expression level of