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

EGFR mRNA in the blastocysts treated with TGF-α was higher than those of the control. In conditioned medium collected after 3 days of embryo culture, TGF-α induced the gelatinase activities of proMMP-9 in all groups and activated MMP-2 in 10 and 100 ng/ml TGF-α treated group. In conditioned medium collected after 5 days, TGF-α induced the gelatinase activities of proMMP-9 in all groups, activated MMP-9 in TGF-α treated group. TGF-α also induced the gelatinase activities of activated MMP-2 in 1 and 10 ng/ml TGF-α treated group and control.

Conclusions: These results suggest that the addition of TGF-α in vitro culture medium is proper to create a favorable environment for preimplantation embryo development and implantation.

P-7 일반배양액과 Vero Cell 공배양에서의 생쥐 배아발달에 관한 비교 연구

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목 적: 많은 배아의 17~30%만이 확산된 배반포시기까지 정상으로 난할하고 있으며 이것은 현 인간 배아 방법에 나타나는 다양한 환경조건이 최적의 상태가 아니라는 것을 의미한다. 그러므로 체외에서 높은 비율로 배반포까지 자랄 수 없는 세포장애 (cell blocks)를 극복하기 위해 여러 조력세포들 (somatic helper cells)과 배아를 공동배양 (coculture) 하므로써 배반포까지의 배아발달율을 높이고 있다. 이에 저자들은 인간 체외수정에 적용하는 공배양기법에 사용할 세포로 vero cell을 택하여 vero cell monolayer 상에서 생쥐배아를 공배양한 것과 일반배양액인 IVF-20 배양액에서만 생쥐배아를 배양하여 두 군의 배아양상에서 배아발달을 비교하여 그 차이를 밝혀보고자 본 실험을 실시하였다.

재료 및 방법: 1. 실험동물은 물과 먹이를 자유롭게 섭식시켜 사육한 ICR계 흰 생쥐를 사용하였다. 2. 공배양을 위해 vero cell line을 사용하여 배양하였다. 3. 배양액 - ① 배아채취용 배양액: Ham'sF-10 배양액 ② vero cell 배양액: vero cell 배양액을 위해 RPMI를 사용하였다. ③ 배아배양액: 일반배양액에서만 2세포기 배아를 배양할 때 사용한 배양액은 IVF-20 (Vitolife Co. IVF Science Scandinavia) 이었으며, 또한 vero cell과 2세포기 배아를 공배양할 때에도 IVF-20을 배아배양액으로 사용하였다.

결 과: 1. 배양초기인 48시간까지는 4세포기와 상실배까지의 발달이 IVF-20에서는 60.7%, 55.7% ($p<0.05$) 였으며 vero cell 공배양에서는 47.6%, 30.0%의 발달율을 보였다. 2. 배양중기인 72시간에는 배반포와 확산된 배반포까지의 발달이 IVF-20에는 51.6% ($p<0.01$), vero cell과 공배양에서는 25.9%의 발달을 보였다. 3. 배양후기인 배양 96시간과 120시간에는 확산된 배반포와 부화까지의 발달이 IVF-20에서는 37.7%와 36.9%, vero cell과 공배양에서는 32.6%와 37.4%의 비슷한 발달을 보였다.

결 론: 생쥐 2세포기 배아를 IVF-20 배양액에서만 배양하였을 때에도 vero cell과 공배양 했을 때와 같이 좋은 성적을 얻었으므로 인간배아를 배반포나 확산된 배반포까지 배양할 때에도 굳이 다른 세포들과 공배양할 필요없이 좋은 양질의 일반배양액을 선택하여 배양하면 좋은 결과를 얻을 수 있을 것으로 사료된다.