

**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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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. 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°C for 20 sec. One-step dilution within the straw was done in water bath of 25°C for 1 min. Vitrified and one-step diluted embryos were directly transferred into 36 (natural or hormone induced synchronized) recipient cows in 6 areas of Kyung-sang 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 recipient 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. Therefore, these results demonstrate that direct transfer technique of vitrified and one-step diluted bovine blastocysts can be applied easily and effectively with the higher pregnancy rate on field trial without the equipment and embryological skills.

Key words: *Hanwoo IVM/IVF/IVC blastocyst, Vitrification, One-step dilution, In vitro/In vivo survival, Pregnancy*