P-23 *In Vitro* Development of Reconstructed Bovine eggs using Male Haploid Somatic Cell Derived from Sequential Nuclear Transfer: II. The Efficiency of Haploidization According to the Meiotic Stage as 1st Recipient Oocyte

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Objective: This study was to evaluate whether bovine eggs reconstructed using male haploid somatic cell derived from sequential nuclear transfer (NT) without sperm could be normally developed *in vitro*. Also, we compared the efficiency of haploidization according to the meiotic stage as 1st recipient oocytes.

Materials and Methods: Bovine GV oocytes were recovered from slaughtered bovine ovary and matured in TCM-199 supplemented with 10% FBS. For haploidization of oocytes, GV or 22 hrs cultured IVM recipient oocytes were stained using 5 μg/ml Hoechst and their GV or 1st polar body (PB) and MII plate were removed by enucleation micropipette under UV filter, respectively. Then, G0/G1 stage bovine male ear skin cells were introduced into two types of enucleated recipient oocytes. Reconstructed eggs were activated using ionomycin. Forty-eight hrs in GV oocytes and 18 hrs in IVM oocytes after activation, each nucleus of the 1st constituted eggs containing 2 sets of chromosomes from somatic cells was again direct injected into fresh MII oocytes, respectively. Sequentially reconstructed eggs were activated again using ionomycin and D-MAP, and then they were cultured in CR1aa medium supplemented with FAF-BSA. After 24 hrs, developed 2-cell embryos were transferred into co-culture drop prepared with cumulus cells and cultured in 10% FBS added CR1aa medium for 7 to 8 days.

Results: The rates of fusion between male somatic cells and GV and MII stage as 1st recipient oocytes after activation were 43.2 and 74.5%, respectively. After the sequential NT of 8 and 48 haploid pronuclei, their recovery rates were 87.5 and 79.1%, respectively. Also, 1 (14%) and 13 (34.2%) eggs with 2 sets of chromosomes were normally extruded 2nd PB, respectively. The rates of cleavage and blastocyst from reconstructed with sequential NT by using MII oocytes were 23 and 33.3%. With GV oocytes as 1st recipient oocytes, they were not developed beyond two-cell stage after sequential NT.

Conclusions: This result suggested that reconstructed bovine eggs derived from sequential NT using male haploid somatic cell can be developed into blastocysts *in vitro*, if MII oocytes were used as 1st recipient oocytes for haploidization. The advantage of this technique is for men who can not produce sperm in human IVF-ET program.