ment and survival of nuclear transplant (NT) embryos and also sexing and freezing of embryos is required for efficient production of NT embryos. Recently, it has been reported that *in vitro* developmental block could be partially overcome by co-culture with somatic cells and addition of growth factors (Yang et al., 1993; 1995), high accuracy for sexing achieved from PCR using male-specific DNA fragment from embryos (Utsumi et al., 1992), and male embryos developed more rapidly than female embryos (Carvalho et al., 1995). This study was designed to compare efficacy of EGF with BOEC co-culture system for supporting development of cloned embryos to M+B stage and to investigate suitable condition for sexing and freezing of embryos.

Materials and Methods

Recipient oocytes were collected from slaughterhouse ovaries and were co-cultured with granulosa cells for 22hr for *in vitro* maturation (IVM) in TCM199 containing FCS and hormones in a 5% CO_2 incubator of 39℃. Donor nuclei were obtained from IVM-IVF embryos which were co-cultured in TCM199 on BOEC monolayer. Micromanipulation (enucleation and nuclei insertion) was done at 24hr after start of IVM and electrofusion was carried out at 44hr of age in BTX fusion chamber using a single DC pulse of 0.8-1.0kV/cm for 70 μ sec. NT embryos randomly assigned to 4 culture treatments in TCM199 : 1) BOEC, 2) BOEC+10ng/ml EGF, 3) 10ng/ml EGF and 4) 50ng/ml EGF. Sex of isolated blastomeres was determined with bovine Y-specific DNA primer and morula stage embryos were frozen in 1.5M glycerol using a programmable freezer.

Results and Discussion

Cellular DNA amplified by PCR showed a 141 bp band in male cells but not in female cells (not shown data). Sex was successfully determined using one or two blastomeres as reported by Utsumi et al.(1992). However, no difference in development between sexes was attained. Addition of EGF had no any beneficial effects on morula-blastocyst development as well as viability after freezing varied slightly among co-culture systems (Voelkel et al., 1992) cleavage rate. This result was not coincident with Paria and Dey (1990) and Yang et al.(1995), but similar to Keefer(1992). Especially, Illera et al.(1992) showed that EGF had no effect on denuded eggs. Freezability of cloned embryos was very low as compared to result for IVM-IVF embryos(Yang et al., 1995).

Conclusions

These results indicate that high accuracy for sexing can be obtained by PCR using Y-specific primer and that EGF may be not proper for early bovine embryo development. Further investigation is necessary concerning cryopreservation of cloned embryos to determine its practicality.

P-33

Studies on Production and Efficient Utilization of Livestock Embryos by *In Vitro* Fertilization and Micromanipulation. Ⅵ. *In Vitro* Culture and Cryopreservation of Sexed and Nuclear Transplanted Bovine Embryos

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Aims of present work were to study effect of EGF and electric pulse on development of NT embryos and to compare freezability and sex of NT embryos during *in vitro* development. EGF had no positive effect during development and activation of enucleated oocytes by electric pulse resulted in stimulated development rate to morula-blastocyst. Freezability of 8-cell and morula stage NT embryos was very low, especially in 8-to 16-cell stage than in morula.

Sex ratio of embryos was 3.5:1(male 78%; female 22%) and NT male embryos showed preferential survival during *in vitro* culture. In conclusion, Activation treatment before electro-fusion appeared to provide higher percentage of M+B development rate and results of preferential survival in males and very low freezability in early NT embryos is of very considerable interest and must be studied further for increasing NT embryo production.