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Studies on Nuclear Transplantation in Mouse Embryos
Ⅲ. Production of Cloned Mice from 2nd Generation Nuclear Transplant Embryos

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생쥐 수정란의 핵이식에 관한 연구
Ⅲ. 제2세대 핵이식에 의한 복제생쥐의 생산

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적 요

포유동물의 초기 발생단계에서 핵의 분화와 전능성을 규명하고 제2세대 핵이식 기법을 개발하고자 생쥐를 모델로하여 공핵란은 2-세포기에 있는 수정란의 핵을 사용하였으며, 수핵란은 zygote 및 2-세포기에 있는 수정란을 탈핵하여 제2세대 핵이식을 실시하여 electrofusion system으로 핵융합을 실시하고 cloned embryo를 작출하여 이를 24~48시간동안 체외에서 배양을 시킨 다음 위임신이 유기된 수란생쥐의 난관에 체내 이식을 실시하여 개체로의 발생 여부 등을 조사하였다.

핵이식후의 융합율은 zygote 및 2-세포기의 수정란을 수핵란으로 사용하였을 때 각각 84.7 및 84.0%으로서 차이가 없었으며, 제1세대의 86.8 및 85.4%로서 세대간에 차이가 없었다.

4-세포기 이상으로 발달한 제2세대 핵이식 수정란의 체외배양율은 수핵란을 zygote 및 2-세포기 수정란을 사용하였을때 각각 36.2 및 43.7%로서 제1세대 핵이식의 44.3 및 50.4% 보다는 다소 낮았다.

제2세대 핵이식 수정란을 위임신이 유기된 수란생쥐의 난관에 이식을 실시하여 얻은 산자생산율은 수핵란을 zygote 및 2-세포기 수정란을 사용하였을때 각각 23.0 및 25.0%로서 모두 25마리의 산자를 생산하였다.

INTRODUCTION

The technical development for the efficient production of large numbers of genetically identical offspring will be of great potential value for the multiplication, selection and evaluation of genotypes of superior domestic animals and for the reduction in the sample size of laboratory animals used no report has been found yet on the production of mice from the 2nd generation nuclear transplantation.

In this study, the developmental potential of single nucleus from nuclear transplanted two-cell mouse embryos, which were transplanted into the enucleated zygote and 2-cell embryos,

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was monitored by examining their development *in virto* and *in vivo* to term after embryo transfer to recipients.

MATERIALS AND METHODS

Preparation of eggs from immature ICR mice was similar to Choe *et al.*(1990). Pseudopregnant recipient females were obtained by mating superovulated females to vasectomized males. Nuclear recipient zygotes or 2-cell embryos were collected 20~22 or 44~45 hours after hCG injection. The ICR mouse embryos of two-cell stage or the 1st NT 2-cell ICR mouse embryos were used as nuclear donors.

The micromanipulation of embryos for nuclear transplantation was performed as described by Choe *et al.*(1990). Recipient embryos were prepared by removing nuclei from zygotes and both blastomeres of two-cell embryos. Single nucleus from two-cell embryos were fused into the enucleated zygote or one of blastomeres of two-cell recipient embryos using electrofusion by Kono and Tsunoda(1988).

Culture of nuclear transplant embryos *in vitro* for 24~48 hours and transfer of nuclear transplant 2-cell embryos into recipient mice were done as described previously (Park and Park, 1991 ; Choe *et al.*, 1990, 1992).

RESULTS AND DISCUSSION

1. Fusion of nucleus into recipient cytoplasm

The proportions of embryos that a nucleus from two-cell donor embryo was successfully injected and subsequently fused into the cytoplasm of recipient embryos are shown in Table 1.

The proportion of 2nd generation NT eggs that a nucleus was fused successfully into the enucleated recipient zygotes and 2-cell embryos were similarly high as 84.7 and 84.0%, respectively, and was not significantly decreased, compared with the results of the 1st generation, which was similar to the data from Kono and Tsunoda(1988).

2. *In vitro* development of nuclear transplant embryos

The fused embryos which received a nucleus from two-cell donor embryos were cultured *in vitro* for 24~48 hours. The developmental potential was shown in Table 2.

The *in vitro* development to 2 to 3-cell stage of the nuclear transplantation embryos was over 80% and not significantly different between the stages of recipient eggs or the 1st and 2nd generation. Stice *et al.* (1992) also reported no significant difference in *in vitro* development of bovine embryos between NT generation.

3. *In vivo* development of nuclear transplant embryos

Full-term development of 2nd generation nuclear transplant mouse embryos after transfer to pseudopregnant recipients was achieved suc-

Table 1. Successful electrofusion of nuclei with cytoplasts from mouse eggs at different development stages by the 1st and 2nd generation

Donor nucleus	Recipient eggs	Eggs fused/injected (%)	
		1st generation	2nd generation
2-cell	Zygote	105/121(86.8)	89/105(84.7)
2-cell	2-cell	94/110(85.4)	79/ 94(84.0)

Table 2. Preimplantation *in vitro* development of nuclear transplant mouse embryos by different different stages of recipient eggs and generation

Recipient eggs	Generation of nuclear transplant	Embryos developed to /fused (%)	
		2- to 3-cell	4-cell over
Zygote	1st	88 /103(85.4)	39 / 88(44.3)
	2nd	69 / 83(83.1)	25 / 69(36.2)
2-cell	1st	107 /125(85.6)	54 /107(50.4)
	2nd	80 / 95(84.2)	35 / 80(43.7)

Table 3. Production of live youngs after transfer of the first or second 2-cell donor nuclear transplanted embryos

Recipient eggs	Generation of nuclear transplant	Pregnant mice / mice transferred(%)	Young /embryos transferred(%)
Zygote	1st	11 /26(42.3)	21 /72(29.2)
	2nd	8 /22(36.4)	11 /48(23.0)
2-cell	1st	13 /28(46.2)	20 /62(32.2)
	2nd	10 /26(38.5)	14 /56(25.0)

cessfully. As shown in Table 3, the proportions of 2nd NT embryos developed to term were similarly 23.0 and 25.0% in recipient cytoplasm of zygote and 2-cell embryos respectively, which was found slightly lower than those of 1st generation. Although the success rate in production of recloned mice was considerably low, the present results suggest that the production of recloned mice could be possible and the production of recloned domestic animals from transplantation of blastomere could be more successful as shown by Bondioli *et al.* (1990).

SUMMARY

The single nucleus from nucleus transplanted two-cell mouse embryos were transplanted into enucleated zygote and two-cell embryos by micromanipulation and electrofusion(2nd NT). The developmental potential of these reconstituted embryos by fusion was examined *in vitro* and *in vivo*.

The fusion rate of 2nd NT of single nucleus to enucleated zygote and 2-cell embryos was 84.7 and 84.0%, respectively. However, the 2nd NT of single nucleus to enucleated zygote and two-cell embryos developed to full term *in vivo* after embryo transfer to recipient mice(in the rate of 23.0 and 25.0%, respectively.).

REFERENCES

Bondioli KR, Westhusin, ME and Looney CR. 1990. Production of identical bovine offspring by nuclear transfer. Theriogenol. 33:165-174.

Choe SY, Park CS, Lee HJ and Park HS. 1990. Studies on nuclear transplantation in mouse embryos. I. Functional differences between maternal and paternal genomes. Korean Vet. Res. 30:123-127.

Kono T and Tsunoda Y. 1988. Effects of induction current and other factors on large-scale electrofusion for pronuclear transplantation

of mouse eggs. Gamate Res. 19:349-357.

Stice SL, Keefer CL, Maki-Laurila M and Phillips PE. 1991. Producing multiple generation of bovine nuclear transplant embryos. Theriogenol 35:273.

박희성, 박충생. 1991. 저온 보존한 공핵란을 이용한 생쥐 수정란의 핵이식. 한국축산학회지 33:831-837.

최상용, 박희성, 이효종, 박충생. 1992. 생쥐 8-세포기 수정란의 핵이식에 의한 복제산자의 생산. 한국축산학회지 34:89-96.