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Studies on nuclear transplantation in mouse embryos. I. Functional differences between maternal and paternal genomes

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생쥐 수정란의 핵이식에 관한 연구 Ⅰ. 모성 및 부성 genome의 기능차이에 관한 연구

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초록 : 모성 및 부성 genome의 기능을 알아보기 위하여 미세조작기법과 Sendai virus를 이용한 핵융합 기술을 이용하여 2개의 자성전핵만으로 구성된 2배체의 gynogenetic 수정란을 그리고 2개의 웅성전핵만으로 구성된 2배체의 androgenetic 수정란을 인위적으로 작출하였다. 이들의 작출효율은 biparental 수정란에서는 56%, gynogenetic 수정란에서는 50% 그리고 androgenetic 수정란에서는 56% 이었다. 이들을 체외에서 배양한 결과 gynogenetic 및 androgenetic 수정란은 2-세포기 이후에는 biparental 및 intact 수정란에 비하여 그 발달능이 매우 저조하였으나 이들 중 25% 이상이 포배까지 발달할 수 있음을 확인하였다. Gynogenetic 및 androgenetic 수정란을 동기화된 수란생쥐의 난관내에 이식하였던 바, androgenetic 수정란은 전혀 착상 되지 않았으나, gynogenetic 수정란에서는 착상이 확인되었다. 핵이식기법으로 인위조작된 2배체의 biparental 수정란으로부터 28마리의 생쥐 신생자를 생산하였다.

Key words: nuclear transplantation, genome, mouse embryo.

Introduction

The applicability of nuclear transplantation to cloning of embryos was first demonstrated by Gurdon (1962), who suceeded in producing young frogs from blastula or gastrula cell nuclei transplanted to enucleated eggs. However, little has been done in applying this technique towards developing a method of cloning mammalian embryos. Illmensee and Hoppe (1981) were the first to report the successful nuclear transplantation in mouse embryo.

In behalf of the enormous potential usefulness of this technology for the efficient production of large numbers of gentically identical domestic animals, several researchers have attempted to produce the cloned animals by this technology, and some of them succeeded in rabbit (Stice et al., 1988), sheep (Willadsen, 1986; Smith and Wilmut, 1989) and cattle (Prather et al., 1987; Robl et al., 1987). The other application of this technology is to the production of parthenogenetic, gynogenetic and androgenetic embryos. Gynogenetic and androgenetic embryos have been reported to develop *in vivo* to midgestation (MaGrath and Solter, 1984). It has been generally

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believed that both the parental sexes contribute equivalent nuclear information to the zygous nucleus since in many animal species viable parthenotes exist. Equivalency of the maternal and paternal contributions to the zygote genome in mammalian species, however, is uncertain. In this experiment of nuclear transplantation, We have also found in the mouse that the biparental, gynogenetic and androgenetic eggs are equally able to develope *in vitro* up to blastocyst stage, but the biparental and gynogenetic eggs develop *in vivo* up to term and to day 10 of pregnancy, respectively, and the androgenetic eggs fail to be implanted after tranfer.

Materials and Methods

Preparation of eggs: Immature ICR and C_{57} BL/6J mice were superovulated by injection of 5 IU PMSG(Sigma, USA), followed 48 hours later by injection of 5 IU hCG(Sigma, USA). Pseudopregnant recipient females were obtained by mating superovulated ICR females to vasectomized ICR males. Nuclear donor and recipient eggs were obtained from ICR or C_{57} BL/6J females mated with the same strain males. Eggs were collected from mated females, which were confirmed by vaginal plug detection, by puncturing the ampullary region of excised oviducts with forceps 20 hours after hCG injection. The eggs with cumulus cells were treated with hyaluronidase (300 NF/ml, Sigma, USA).

Micromanipulation of eggs: Egg micromanipulation was performed on an inverted microscope (Olympus, Japan) using micromanipulators (Goodfellow, England). Eggs were incubated at 37°C for 15~60 min under 5% CO_2 in air in Hepes-BMOC-3 containing 5μg/ml cytochalasin B(Sigma, U.S.A.) and 0.1 μg/ml colcemid (Sigma, U.S.A.) prior to micromanipulation.

One drop of Hepes-BMOC-3 containing cytochalasin B and colcemid was prepared separately for donor and recipient eggs, and another one drop of buffer containing inactivated Sendai virus (1,000 HAU/ml) was placed between the above two drops, and all the drops were covered with light paraffin oil. Biparental eggs were produced by injecting both male and female pronuclei into perivitelline space of enucleated mouse eggs of the same strain. Diploid gynogenetic or androgenetic eggs were produced by injecting a female or male pronucleus enucleated from donor eggs into perivitelline space of haploid gynogenetic or androgenetic recipient eggs. The karyoplast enucleation, injection and fusion techniques and preparation of haploid gynogenetic or androgenetic eggs were performed as described by McGrath and Solter (1983) (Fig 1).

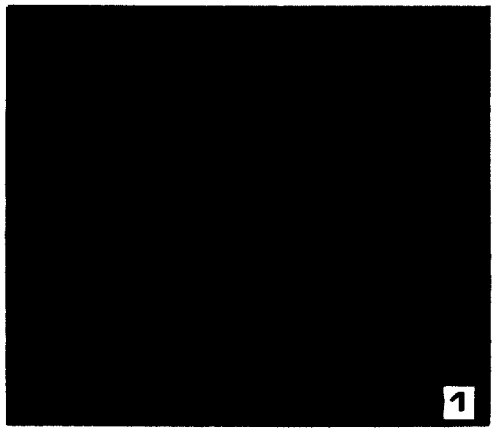
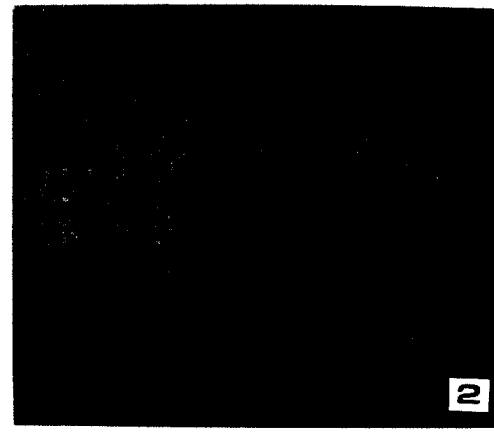
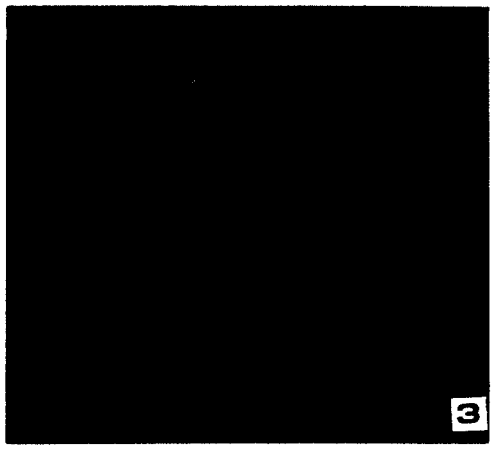
Culture and transfer of eggs: After micromanipulation for nuclear transplantation the eggs were washed several times and cultured for 4 days with $NaHCO_3$-BMOC-3 containing 100 μM EDTA in an atmosphere of 5% CO_2 and 95% air at 37°C. Eggs fused with the pronuclear karyoplast were either *in vitro* cultured or transfered to the oviduct of females on day 0.5~1.0 of pseudopregnancy. The recipients were allowed to go to term or autopsied on 10 days after transfer and the number of youngs, normal fetuses and/or resorptions were recorded in each uterine horn of the recipient.

Statistical analysis: The significance of the differences in mean success rate of embryo micromanipulation or development of the eggs *in vitro* and *in vivo* were tested statistically by chi-square test.

Results and Discussion

Of 406 micromanipulated embryos, 326(80.3%) were successfully enucleated, and of the 326 pronuclear karyoplasts obtained, 272(83.4%) were successfully injected into the perivitelline space of enucleated zygotes. After incubation at 37°C for an hour, 216(81.8%) of these karyoplasts fused into the cytoplasm of the egg (Table 1). The technique for enucleation seemed to be the most limiting factor in the whole procedure for nuclear transplantation in mice. No significant ($p<0.05$) differences in the overall successful nuclear transplantation were shown between these two strains of mouse, and also between the types of zygote produced: biparental eggs (56%), gynogenetic eggs (50%) and androgenetic eggs(56%).

After nuclear transplantation both the nuclear transferred and intact eggs were cultured for 95 hours in 5% CO_2 incubator and their *in vitro* development


Legends for figures

Fig 1. Injection of karyoplast to an enucleated mouse embryo at pronuclear stage (X 200).
Fig 2. A 2-cell-stage embryo produced by nuclear transplantation and ***in vitro*** culture (X 200).
Fig 3. A blastocyst-stage embryo produced by nuclear transplantation and ***in vitro*** culture (X 200).
Fig 4. C57BL mice (black coated) produced by nuclear transplantation and embryo transfer into uterine tubes of ICR foster mouse.

Table 1. Successful enucleation, karyoplast injection and fusion by biparental, gynogenetic and androgenetic zygotes[1)]

Type of zygotes	Strain of mouse	No. and (%) of eggs enucleated	No. and (%) of eggs injected	No. and (%) of eggs fused
Biparental	ICR	58/ 70(82.8)	49/ 58(84.5)	35/ 43(81.4)
	C57BL/6J	53/ 65(81.5)	44/ 53(83.0)	36/ 44(81.8)
Gynogenetic	ICR	98/123(79.6)	84/ 98(85.7)	68/ 84(81.0)
	C57BL/6J	13/ 18(72.2)	10/ 13(76.9)	8/ 10(80.0)
Androgenetic	ICR	78/ 98(79.5)	64/ 78(82.0)	52/ 64(81.2)
	C57BL/6J	26/ 32(81.2)	21/ 26(80.7)	17/ 19(89.5)
Total		326/406(81.2)	272/326(83.4)	216/264(81.8)

1) There are no significant ($p<0.05$) differences between the strain or zygote means.

Table 2. Preimplantation *in vitro* development of biparental, gynogenetic and androgenetic zygotes after nuclear transplantation[1)]

Type of zygotes	Strain of mouse	No. of eggs used	No. and (%) of eggs develop to		
			2-cell	Morula	Blastocyst
Intact	ICR	120	116(96.6)a	102(85.0)a	99(82.5)a
	C57BL/6J	115	112(97.3)a	100(86.9)a	97(84.3)a
Biparental	ICR	21	16(76.0)a	10(47.6)b	9(42.8)b
	C57BL/6J	20	17(85.0)a	8(40.0)b	7(35.0)b
Gynogenetic	ICR	44	39(88.6)a	8(18.2)b	8(18.2)b
	C57BL/6J	10	9(90.9)a	3(30.0)b	3(30.0)b
Androgenetic	ICR	36	31(86.1)a	10(27.7)b	10(27.7)b
	C57BL/6J	18	17(86.1)a	7(38.9)b	5(27.7)b

1) There are no significant ($p<0.05$) differences between zygote means with the same letters within the same strain.

Table 3. Implantation after transfer of biparental, gynogenetic or androgenetic zygotes into recipient mice[1)]

Type of zygote	Strain of mouse	No. of eggs transferred	No. of mice transferred	No. and (%) of mice pregnant at day 10	No. and (%) of eggs implanted at day 10
Intact	ICR	81	10	6(60.0)aA	32(39.5)aA
	C57BL/6J	70	6	2(33.3)aA	7(10.0)aB
Biparental	ICR	25	5	2(40.0)aA	3(12.0)abA
	C57BL/6J	22	3	1(33.3)aA	2 (9.1)aA
Gynogenetic	ICR	63	15	2(13.3)a	2 (3.2)b
Androgenetic	ICR	61	14	0 (0.0)a	0 (0.0)b

1) There are no significant ($p<0.05$) differences between the same small (zygote means within strain) or capital (strain means within zygote) letters.

Table 4. Production of live youngs after transfer of nuclear transplanted eggs into recipient mice[1)]

Type of zygote	Strain o mouse	No. of eggs transferred	No. of mice transferred	No. and (%) of mice pregnant	No. and (%) of youngs
Intact	ICR	65	9	4(44.4)a	24(36.9)a
	C57BL/6J	77	8	3(37.5)a	11(14.3)b
Biparental	ICR	58	13	5(38.4)a	22(37.9)a
	C57BL/6J	36	8	3(37.5)a	6(16.6)a

1) There are no significant ($p<0.05$) differences between zygote means within strain and between strain means with the same letters within zygote.

potential was assessed (Table 2). Of 120 intact ICR eggs, 99(82.5%) developed to blastocyst and of 115 intact C_{57} BL/6J eggs, 97(84.3%) developed to blastocyst. However, no more than half of the nuclear transferred eggs were developed. Especially in gynogenetic and androgenetic eggs 11(20%) of 54 and 15(29%) of 54 developed to blastocyst, respectively, while 16(39%) of 41 heterozygous biparental zygotes developed to blastocyst (Fig 3).

The general *in vitro* development of the nuclear transplanted eggs in the present experiment was too low, compared with the results of McGrath and Solter (1984). As shown in Tale 2 there was found no significant ($p<0.05$) difference in the *in vitro* culture of nuclear transplanted eggs upto blastocyst stage between the gynogenetic and androgenetic

zygotes. The exact reason for such a low culture result from the present trial cannot be fully understood. The technical improvement in the egg micromanipulation should be an important factor for the higher rate of *in vitro* development of the nuclear transferred eggs.

After fusion of the pronuclear karyoplast in the enucleated eggs, the fused eggs were transferred into one side of oviduct of synchronized pseudopregnant mice. Their implantation in the uterus was recorded on day 10 of pregnancy (Table 3). The conception rates of recipient mice after egg transfer into the oviduct, which was determined by autopsy at day 10, were 8/16, 3/8. 2/15 and 0/14 in intact, biparental, gynogenetic and androgenetic zygotes, respectively. The similar results were also obtained in the eggs implanted at day 10. The implantation rate from transfer of intact eggs in the present trial was similar to the result of McGrath and Solter (1984). However, the gynogenetic and androgenetic eggs showed a quite low implantation rate of 3.2% and null, respectively. After transfer of 94 reconstituted biparental eggs to 21 pseudopregnant ICR recipient mice, 9 mice were pregnant and among them 28 (30%) live youngs were delivered(Table 4 and Fig 4). This result was comparable to the results of McGrath and Solter (1984) and Barton et al (1984).

From the results of this experiment, it is assumed that the paternal and maternal genomes apparently play complementary roles for normal embryogenesis and fetal development in mouse and and both genomes are essential for development until term.

Summary

By nuclear transplantation technology twenty eight mice have been produced after transfer of heterozygous biparental eggs. Also heterozygous gynogenetic eggs with two female pronuclei and heterozygous androgenetic eggs with two male pronuclei have been obtained by injecting a male or female pronucleus with Sendai virus into the perivitelline space of enucleated haploid zygotes at pronuclear stage. The success rate of enucleation, karyoplast injection and fusion of both the pronuclei was 80.3, 83.4 and 81.8%, respectively. The overall pronuclei fusion rates by this technique were 56, 50 and 56% in biparental, gynogenetic and androgenetic eggs, respectively. The evidence was ascertained that the gynogenetic and androgenetic eggs were also able to develop *in vitro* up to blastocyst stage, even though their developmental potential was greatly diminished beyond 2-cell stage. The gynogenetic eggs were able to develop *in vivo* up to day 10 of pregnancy, while the androgenetic eggs failed to develop *in vivo* during the same period.

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