Improvement of embryonic development and production of offspring by transferring meiosis-II chromosomes of senescent mouse oocytes into cytoplasts of young mouse oocytes

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Abstract

Purpose The effects of reciprocal transplantation of meiosis-II chromosomes between senescent and young mouse oocytes were evaluated based on pre- and post-implantation development ability of resultant embryos.

Methods Karyoplasts including meiosis-II chromosomes of oocytes from senescent Rockefeller mouse/Ms-Rb(6, 15) females (10 to 12 months, age-related infertile mice) were transferred into cytoplasts of oocytes from young F₁ females (3 to 5 months). Reconstructed oocytes were fertilized in vitro, and then the resultant embryos were cultured in vitro and transferred to recipient mice.

Results The reconstructed oocytes that consisted of aged-karyoplasts and young-cytoplasts showed significantly

improved embryonic development (from 23.2% to 30.0%) and development to term (from 6.3% to 27.1%, $P < 0.05$) as compared with the oocytes reconstructed from young-karyoplasts and aged-cytoplasts.

Conclusions The present study showed successful rejuvenation for age-related infertility using transplantation of meiosis-II chromosomes in animal experimental models.

Keywords In vitro fertilization · Meiosis-II chromosome · Mouse · Nuclear transfer · Senescence

Introduction

Decreasing reproductive potential in females has been associated with maternal age and oocyte quality [1]. Both poor quality cytoplasm resulting from abnormal oocyte maturation and aneuploidy caused by non-disjunction during meiosis-II are responsible for age-related infertility [2]. Transplantation of meiosis-II chromosomes (M-II-t) of poor-quality oocytes into developmentally competent oocytes has been suggested as a way to improve embryonic development [3]. Until now, although live births derived from embryos produced by M-II-t have been reported, the success rate of electrofusion is extremely low due to a suboptimal procedure [4]. A modified electrofusion method could successfully improve the fusion rate and decrease the incidence of chromosomal abnormalities in embryos derived from oocytes reconstructed by M-II-t [5], enabling its application for reproduction of invaluable animals and as assisted reproductive technology in humans.

When the nucleus of a developmentally incompetent oocyte is reconstructed with the maximum volume of good-

Capsule Transplantation of meiosis-II chromosomes of senescent mouse oocytes into cytoplasts of young mouse oocytes improves subsequent embryonic and fetal development until birth.

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quality cytoplasm, the oocyte appears to show better embryonic development than with cytoplasmic transfer [6]. Such nuclear transplantation by reciprocal exchange of ooplasm has recently been reported to rescue embryonic development for mouse oocytes aged in vitro [3] and to rejuvenate fertility in older women [7]. Previous reports using animal models could not practically use aged animals [3], thus they were incapable of assessing the developmental competence of nuclei in oocytes from aged animals and the actual efficacy of M-II-t. Therefore, we investigated embryonic development of oocytes reconstructed by M-II-t between senescent and young mice and clarified the competence of nuclei of oocytes from aged mice. Oocytes from aged Rockefeller (RF) mice were used as a model of age-dependent infertility. The present study is the first to derive normal offspring from aged-model mouse oocytes using M-II-t.

Materials and methods

Animals

Female, 3- to 5-month-old, (BALB/c×C57BL/6J) F1 hybrid mice and 10- to 12-month-old RF mouse/Ms-Rb [6, 15] females were used as oocyte donors, and 3- to 5-month-old RF males were used as sperm donors. The RF mice used in the present study have 38 chromosomes containing one pair of Robertsonian translocated chromosomes (38 tr 6/15) as marker chromosomes. In a pre-experiment in our laboratory, pregnancy rates by natural mating in RF females were 87.2% (34/39) in 2- to 4-month-old females, 73.1% (19/26) in 5- to 7-month-old females, 42.9% (3/7) in 8- to 9-month-old females, and 0% in >10-month-old females. The pregnancy rate was significantly lower in 8- to 9-month-old than in 2- to 4-month-old females ($P<0.05$, chi-square test). The mean litter size per female in 8- to 9-month-old females was 1.7 ± 1.5 , which is significantly lower than in 2- to 4-month-old (5.5 ± 2.1) and 5- to 7-month-old females (5.4 ± 2.5 ; $P<0.05$, one-way analysis of variance followed by Fisher's Protected Least Significant Difference test). Since the RF females that are >10 months old are infertile,

10- to 12-month-old RF females were used as oocyte-donor age-related infertile models.

Meiosis-II chromosome transplantation

Oocyte collection and preparation of karyoplasts and cytoplasts for M-II-t were conducted as described previously [5]. Aged-karyoplasts and aged-cytoplasts were prepared from oocytes of aged RF females by superovulation, and young karyoplasts and young cytoplasts were prepared from oocytes of superovulated young F1 hybrid females. A total of 4 groups of complexes were prepared: Aged-Karyoplast + Young-Cytoplast (Ak-Yc); Young-Karyoplast + Aged-Cytoplast (Yk-Ac); Young-Karyoplast + Young-Cytoplast (Yk-Yc); and Aged-Karyoplast + Aged-Cytoplast (Ak-Ac).

The complexes were electrically fused by a direct current pulse of 2.0 KV/cm for 60 μ s at room temperature using an ET-3 GOKU (Fujihira Industry, Tokyo, Japan). The fused oocytes were subjected to in vitro fertilization, and the fertilized embryos were cultured in vitro. Two-cell embryos or blastocysts were transferred into oviducts of day 2 pseudopregnant ICR females or uteri of day 4 pseudopregnant ICR females, respectively, according to the previous method [8]. All experiments using mice in the present study were carried out according to the Guideline for Care and Use of Laboratory Animals at Utsunomiya University.

Statistics

Statistical evaluation was conducted using the chi-square test to compare the success rates of electrofusion, in vitro fertilization, preimplantation development, and full-term development. Significance was assessed at $P<0.05$.

Results

Success rates for producing karyoplast-cytoplast complexes did not differ significantly among the 4 groups (95.5–100%, Table 1). The electrofusion success rate was significantly lower in Group Ak-Yc, 70.6% (89/126) than

Table 1 Rates of fertilization and embryonic development in reconstructed oocytes

Group	Numbers of oocytes				Numbers of zygotes developed to	
	Treated	Survived (%)	Fused (%)	Fertilized (%)	2-cell stage (%)	Blastocyst stage (%)
Ak-Yc	132	126 (95.5)	89 (70.6 ^a)	70 (78.7)	60 (85.7)	21 (30.0 ^a)
Ak-Ac	61	61 (100)	50 (82.0 ^b)	39 (78.0)	32 (82.1)	4 (10.3 ^b)
Yk-Ac	132	128 (97.0)	105 (82.0 ^b)	69 (65.7)	59 (85.5)	16 (23.2 ^{ab})
Yk-Yc	248	240 (96.8)	202 (84.2 ^b)	147 (72.8)	137 (93.2)	85 (57.8 ^c)

^{a-c} Values with different superscripts in the same column are significantly different ($P<0.05$).

Fig. 1 Mouse fertilized eggs derived from in vitro fertilization of oocytes reconstructed by meiosis-II chromosome transplantation in Group Ak-Yc. Six hours after insemination, the second polar body lay in the perivitelline space and pronuclei were found in the cytoplasm, showing normal fertilization

in Groups Ak-Ac (82.0%, 50/61), Yk-Ac (82.0%, 105/128), and Yk-Yc (84.2%, 202/240; $P<0.05$). The success rates of in vitro fertilization in reconstructed oocytes were 65.7–78.7%, with no significant difference among the 4 groups (Table 1). The fertilized eggs are shown in Fig. 1. One second polar body and two pronuclei were observed within 6 h after insemination.

There was no significant difference in the cleavage rates into the 2-cell stage among the 4 groups (82.1–93.2%). While the rate of development into the blastocyst stage was significantly higher in Group Ak-Yc (30.0%, 21/70) than in Group Ak-Ac (10.3%, 4/39; $P<0.05$), Group Yk-Yc had

Fig. 2 Blastocysts in Group Ak-Yc after in-vitro fertilization. Hatching and pre-hatched blastocyst on day 3 of in vitro culture were observed and all blastocysts hatched within day 5 of in vitro culture

the highest rate (57.8%, 85/147). Good morphology blastocysts were obtained in Group Ak-Yc, and their quality are equivalent to the blastocysts in Group Yk-Yc under microscopic observation (Fig. 2).

Since no mice became pregnant when 16 blastocysts of Group Ak-Yc and 13 blastocysts of Group Yk-Ac were transferred into uteri of day-4 pseudopregnant females, transfer of the 2-cell embryos into oviducts was conducted as the next experiment. In Group Ak-Yc, 48 embryos at the 2-cell stage were transferred into oviducts of day 2 pseudopregnant ICR females, and 13 fetuses (27.1%) developed to full term, which was significantly more than in Group Yk-Ac (4/63, 6.3%; $P<0.05$; Table 2).

Discussion and conclusion

Since RF mice have 38 chromosomes containing one pair of Robertsonian translocation chromosomes as a marker chromosome [8], they are useful for identifying the origins of polyploidy and aneuploidy in embryos produced in vitro in combination with gametes from normal strains having a normal chromosome complement of $2n=40$ [9, 10].

In the present study, 10- to 12-month-old RF females were used as oocyte-donor models that were age-related infertile, since the pilot study showed that 10- to 12-month-old RF females had severe infertility (0 pup /female), earlier than hybrid mice.

The rates of development to the blastocyst stage and to term were significantly higher in Group Ak-Yc than in Group Ak-Ac. The significantly increased rate in Group Ak-Yc means that development of embryos derived from senescent RF mice was rescued by the cytoplasm from a young mouse oocyte. As compared with the results of Bai et al. [3], who used oocytes aged in vitro, perhaps causing premature chromosome separation, the rate of full-term development in Group Ak-Yc (27.1%) was considerably higher. This fact indicates that nucleus competence (integrity of chromosome alignment) is normal in M-II-stage oocytes from aged RF females, though the cytoplasm is

Table 2 Development rates to term after embryo transfer of 2-cell embryos derived from meiosis-II chromosome transplantation

Group	Numbers of			
	Recipients	Pregnant mice (%)	2-cell embryos transferred	Fetuses (%)
Ak-Yc	3	2 (66.7)	48	13 (27.1 ^a)
Yk-Ac	5	1 (20.0)	63	4 (6.3 ^b)

^{a,b} Values with different superscripts in the same column are significantly different ($P<0.05$).

involved in the low development rate of aged RF mouse oocytes.

However, the equivalence of blastocyst development between Groups Ak-Yc and Yk-Ac permits us to speculate on the importance of the volume of healthy cytoplasm for full-term development. Reconstructed oocytes in Group Yk-Ac mainly consisted of cytoplasm from oocytes of aged females and a small amount of cytoplasm of young mice oocytes, resembling oocytes subjected to cytoplasmic transfer [6]. In oocytes subjected to cytoplasmic transfer, the volume of donor cytoplasm injected to recipient oocytes is extremely small as compared with M-II-t [11]. Many accumulated maternal mRNAs and proteins that affect embryonic and fetal development have been reported, and embryonic and fetal development is compromised when such factors are insufficient in the ooplasm [12, 13]. It seems that injection of a small volume of ooplasm in cytoplasmic transfer or Yk-Ac reconstruction can only improve embryonic development before implantation, but not fetal development, while exchanging cytoplasm by nuclear transfer enables rejuvenation of karyoplasts of aged females.

Embryos in Groups Ak-Yc and Yk-Ac are in the state of cytoplasmic heteroplasmy. Recent related reports claimed that their fetal growth is affected by mitochondrial heteroplasmy that may compromise epigenetic modifications [14]. However, in the present study, no malignant effects on fetal growth and postnatal development were found. To date, normal offspring as a result of cytoplasmic transfer [15] and mitochondrial heteroplasmy mice, mito-mice, have been reported [16]. Moreover, no defects in respiration have been observed in human somatic hybrids carrying heteroplasmic mtDNAs [17] or in mice carrying heteroplasmic mtDNAs derived from different individuals [18]. These studies support the notion that mitochondrial heteroplasmy does not compromise epigenetic modifications of embryos because of normal germ line transmission of cells with mitochondrial heteroplasmy [19]. Such evidence and the present result relieve the concerns about the clinical hazards of using oocytes with mitochondrial heteroplasmy. Furthermore, instead of nuclear transplantation by electrofusion, micro-manipulation methods have been developed [20] to prevent the transmission of mutated mtDNA possibly contained in a karyoplast. Based on these reports, M-II-t can be used as an assisted reproduction technique for invaluable animal reproduction and practical use.

In conclusion, the present study showed successful rejuvenation of age-related infertility in an animal experimental model using meiosis-II chromosome transplantation.

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