eJBio

Electronic Journal of Biology, 2005, Vol. 1(1): 6-8

Cytoplasm Transfer in Mature Rabbit Oocytes

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Abstract

In order to evaluate the effects of ooplasm on oocyte fertilization and early embryonic development and study the mitochondrial DNA heterogeneity of early embryos, microinjection was performed to transfer a small amount (5% to 7%) of donor ooplasm into recipient oocytes and fertilize the eggs with rabbit sperm through intracytoplasmic sperm injection (ICSI). In Group 1 (homogeneous ooplasmic transfer), both the donor and recipient rabbit oocytes were at metaphase II. In Group 2 (heterogeneous ooplasmic transfer), the donor was mouse oocyte of metaphase II and the recipient was rabbit oocyte of metaphase II. In the control group, only ICSI was done on rabbit oocyte without ooplasmic transfer. Result showed that homogenous ooplasmic transfer had no significant influence on rabbit oocyte fertilization and early embryonic development, but heterogeneous ooplasmic transfer did cause notable reduction in blastocyst development rate. Compared with the control group, the embryonic quality declined after ooplasmic transfer operation in the present experiment.

Key words: Ooplasmic transfer; Intracytoplasmic sperm injection; Embryonic development

1. Introduction

In the process of mammalian oogenesis, the developing oocytes accumulate a great deal of maternal information in cytoplasm, such as proteins, mtDNA, mRNA, factors and their precursors. The quantity of the maternal information is vital for fertilization and embryonic development. It has been reported that to reduce the quantity of ooplasm will influence oocyte fertilization and embryonic development [1]. In human, using ooplasmic transfer to add a part of high-quality cytoplasm from a young woman into the cytoplasm of the aged oocyte of an old woman helped the oocyte to have the normal ability of fertilization and embryonic development, and the zygote even succeeded in developing into a healthy baby [2, 3].

In the research reported here, a small amount of ooplasm of homogeneous or heterogeneous animals was added into rabbit MII oocytes, which were then manipulated by ICSI, and the effect of ooplasmnic transfer on fertilization and early embryonic development was studied. The conditions of mtDNA heterogeneity and nucleus-cytoplasm interaction after ooplasmic transfer were studied by mtDNA detection and fingerprinting diagnosis.

2. Materials and methods

2.1 Animal and Oocytes Collection

Pregnant mare serum gonadotropin (PMSG, model 20021212; Huafu, China) and human chorionic gonadotropin (hCG, model 20021218; Huafu, China) were used on adult female rabbits (5 to 12 months old and 2.5 to 3.0kg) and mice to gain ovulation synchronization. Twelve to fourteen hours later, the mice were killed, their oviducts were cut off, and the expansion part of the oviduct was torn and Cumulus Oocyte Complexes (COCs) were collected under a stereoscopic microscope. COCs of the rabbit and the mouse were treated separately in M_2 culture medium containing 0.2% hyaluronidase (Model H3506; Sigma, USA) to get rid of cumulus cells. Having been washed three times with M_2 culture medium, the nude MII oocytes with the first polar body were placed in M_2 culture droplets that contained 10% FBS. Then they were incubated for one hour or longer in a CO_2 incubator (Model 3131; Forma, USA) at 38.5 ℃ and in a 5% CO_2 atmosphere.

2.2 Preparation of sperm

Fresh semen of adult rabbits (8 to18 months old and 3.5 to 4.5kg) was collected with a manufactural vagina and then was treated twice by centrifugal washing, using Krebs-Ringer bicarbonate medium. Sperm suspension with high activity was prepared by the swim-up method. Then 1μL of the suspension was added into 5μL M_2 culture droplet containing 10% PVP at the bottom border of the droplet to avoid its suspending in the upper layer of the droplet fluid.

2.3 Ooplasmic Transfer and Intracytoplasmic Sperm Injection

One donor oocyte and one recipient oocyte were placed into two separate 10L microdroplets containing M_2 with 5μg/mL cytochalasin B (CCB,

model C6762; Sigma, USA) and treated for 10min before micro–manipulation.

In Group 1 (homogenous ooplasmic transfer): Both the donor and the recipient were rabbit MII oocytes. Using injection needle that was filled with PVP fluid to trig sperm through drawing it into the injection needle from the sperm tail. The oocyte was stabbed at the direction of 3 o'clock (polar body located at the direction of 12 o'clock) and ooplasm was imbibed at the location opposite the polar body [4]. The cytoplasm in the needle was 2 to 3 times as long as the oocyte diameter and the quantity is equal to 5% to 10% volume of the cytoplasm. The donor ooplasm and single sperm were injected into recipient oocyte at the direction of 3 o'clock.

In Group 2 (heterogeneous ooplasmic transfer): The method of ooplasmic transfer and microinjection was the same as what was described above. The recipient was also rabbit MII oocyte, but the donor was mouse MII oocyte.

The control group: A single sperm was injected in rabbit MII oocyte only.

2.4 Culture in-vitro

The treated oocytes were washed 2 to 3 times in M_2 culture medium that contained 10% FBS, then they were cultured in the R1/R2 sequential culture medium [5]. The embryonic development was recorded.

2.5 Statistical Analysis

The data were analyzed using Chi-square test, and differences with a probability value of $P<0.05$ were considered significant.

3. Results

A total of 66 rabbit oocytes, 31 in Group 1 and 35 in Group 2, were treated by homogeneous or heterogeneous ooplasmic transfer, and the survival rate was above 70%. The survived oocytes were cultured for 24 hours in sequential culture in vitro. Fertilization rates in Group 1 were 69.6% and 66.7% in Group 2, with no significant difference as compared with the control group. Development rates of 2-cell and 8-cell embryos and morulae in Group 1 and Group 2 weren't significantly different from those of the control group ($P>0.05$). Development rates of blastocyst in the three groups were 13.0%, 0 and 16.7%, respectively. There was no significant difference between Group 1 and control group ($P>0.05$), but the difference between Group 2 and control group was significant ($P<0.05$) (Table 1).

Table 1. Effects of ooplasmic transfer on fertilization and development of rabbit oocytes.

Group	Num. of oocytes	Survival	Development stage (%)				
			Fertilization	2-cell	8-cell	Morula	Blastocyst
Group 1	31	23 (74.2) [a]	16 (69.6) [a]	9 (39.1) [a]	7 (30.4) [a]	4 (17.4) [a]	3 (13.0) [a b]
Group 2	35	27 (77.1) [a]	18 (66.7) [a]	10 (37.0) [a]	7 (25.9) [a]	2 (7.4) [a]	0 (0) [a]
Control	36	24 (66.7) [a]	16 (66.7) [a]	10 (41.7) [a]	6 (25.0) [a]	5 (20.8) [a]	4 (16.7) [b]

a: $P>0.05$; b: $P<0.05$.

Whether the embryos were operated by homogenous or heterogeneous ooplasmic transfer, the blastomeres were not divided equally and the fragments increased, and thus the embryonic quality declined markedly when compared with the control group.

4. Discussion

The quantity of cytoplasm is of vital importance to embryonic development and embryonic quality in animal early embryos. In humans, ooplasm transfer [3, 6] and mitochondrion transfer [7] can notably enhance the ability of fertilization of old women's oocytes and reduce the incidence rate of embryonic fragment. They can also improve the embryonic quality coincident with the ability of implantation. Nagai et al. [8] had done cytoplasmic transfer (CT) and intra-cytoplasmic sperm injection in the mouse system, Sibling oocytes were used to transfer 2, 4, or 6pL of ooplasm to recipient eggs respectively, every time along with a sperm head using piezo-actuated injection. The survival and fertilized eggs were those that had been injected 2pL or 4pl ooplasm, but the survival rate of 6pL group was rather low. Development to the blastocyst stage was also inversely related to CT volume, with some decline beginning at the 4pL CT group.

In our research, we did ooplasm transfer and micro-fertilization by micro-injection. Direct injection as was used in this research was simpler and had less interference to the procedure of ICSI. About 5% to 7% of the ooplasm was transferred, calculated according to the inside diameter of the injection needle and the length of ooplasm in the needle. In the present research, few fragments of ICSI embryos occurred in the control group and the embryos had normal shape, while embryonic fragments were obvious in both the homogeneous and the heterogeneous ooplasm transferred

embryos, the blastomeres were not divided equally and the development was slow after ooplasmic transfer and ICSI. The results above were not consistent with the research results of Cohen et al. [3] and Dale et al. [6]. Our results showed that the addition of a small amount of ooplasm into normal oocytes did not markedly improve their ability of fertilization and embryonic development; the operation caused a decline in embryonic development, instead.

The mechanism might be that increasing the quantity of ooplasm helped to recover the ability of fertilization and embryonic development in oocytes of deteriorated quality but it had no promoting effect on oocytes with normal ability of fertilization. It is notable that there was no statistical difference in the rates of fertilization and embryonic development between heterogeneous and homogeneous ooplasmic transfer groups after ICSI. We cannot affirm that heterogeneous ooplasmic transfer could promote or support the fertilization of the heterogeneous oocytes and the development of early embryo, but we can at least speculate that heterogeneous ooplasm transferred and micro-inseminated embryo can develop. But none of the heterogeneous ooplasm transferred embryos developed to blastocysts (0, 0/27). So, the embryonic development rate just before the stage of blastocyst is notably affected by transfer of mouse ooplasm into rabbit oocyte. The result indicates that mitochondrial incompatibilities and specific epigenetic have been the obstacles to development and this coincides with the results of Malter et al. [9]. Also, Ooplasmic transfer could cause donor mitochondria to exist in recipient oocyte and the consequences are the possible transmission of two mitochondrial DNA populations to the offspring [3, 6, 10]. This pattern of inheritance is in contrast to the strictly maternal manner in which mtDNA is transmitted following natural fertilization and ICSI. Therefore, it is necessary to investigate the transmission of mitochondrial DNA following cytoplasmic transfer, before it is used in assisted reproduction therapy in human.

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