CLEAVAGE OF MOUSE OOCYTES AFTER THE INJECTION OF IMMOBILIZED, KILLED SPERMATOZOA

K. Goto[1], A. Kinoshita, A. Kuroda,
Y. Nakanishi and K. Ogawa

Laboratory of Animal Reproduction, Department of Animal Science
Faculty of Agriculture, Kagoshima University, Kagoshima 890, Japan

Summary

Immobilized (killed) mouse spermatozoa or sperm head were microinjected into mouse oocytes matured *in vivo* and cultured for 72h *in vitro*. When non-capacitated spermatozoon was injected, oocytes that developed to ≧ 2-cell and ≧ 4-cell was 27.8 (15/54) and 3.7% (2/54), respectively. When non-capacitated sperm head was injected, development to ≧ 2-cell and ≧ 4-cell was 21.3 (16/75) and 8.0% (6/75), respectively. When capacitated spermatozoon was injected, development to ≧ 2-cell and ≧ 4-cell was 21.4 (15/70) and 4.3% (3/70), respectively. When capacitated sperm head was injected, development to ≧ 2-cell and ≧ 4-cell was 29.9 (35/117) and 10.3% (12/117), respectively. In contrast, none developed beyond 4-cell in the sham-operated group. The results of this study demonstrated that mouse oocytes matured *in vivo* can undergo normal appearing cleavage to 4-cell stage by dead-sperm injection. Sperm treatment prior to injection did not affect the ability of mouse oocytes to cleave *in vitro*.

(Key Words: Mouse, Oocyte, Sperm, Injection)

Introduction

Mammalian eggs are normally fertilized in the ampulla of the oviduct. Sperm microinjection is the process of mechanically inserting whole spermatozoa or isolated sperm nuclei (head) into the ooplasm of an egg. Microinjection techniques have been used to explore features of fertilization and sperm decondensation that could not be studied using standard *in vitro* fertilization techniques (Uehara and Yanagimachi, 1976; Perreault et al., 1988; Keefer, 1989; Keefer et al., 1990). Mouse, rabbit and cow ova fertilized by injection can undergo cleavage development (Markert, 1983; Hosoi et al., 1988; Keefer, 1989; Keefer et al., 1990; Goto et al., 1990) and live offspring following embryo transfers have been obtained in the rabbit (Hosoi et al., 1988) and in the cow (Goto et al., 1990).

Recently Keefer (1989) reported that sperm treatment prior to injection could affect the ability of oocytes to activate and direct sperm decondensation and male pronuclear formation in rabbit. The present study was conducted to examine the effect of sperm treatment prior to injection on the cleavage development of mouse ova.

Materials and Methods

Oocyte collection

JCL-ICR strain of mice were maintained on a 14 h light; 10 h dark schedule with food and water *ad libitum*. Eight to 24 weeks old female mice were superovulated by injection of 7.5 IU PMSG (Teikoku-zoki, Tokyo, Japan) followed by 7.5 IU HCG (Teikoku-zoki, Tokyo, Japan) 48 h later. The female mice were killed 15 h after the HCG injection and their oviducts were removed and put into the dish containing BSA-free TYH-280 medium (Kasai et al., 1978) supplemented with 0.1% hyaludonidase (Sigma, St. Louis, MO, USA). The wall of the ampullar portion of the oviduct was dissected with a needle and the ova surrounded by cumulus cells were isolated. After removing cumulus, only the ova extruded 1st polar body were washed by TYH-280 medium and transferred into the 10 μl (TYH-280 medium) of injection microdrop covered with mineral oil (Squibb, Princeton, NJ, USA).

[1]Address reprint requests to Dr. K. Goto, Dept. of Animal Science, Faculty of Agriculture, Kagoshima University, Kagoshima 890, Japan

Received December 26, 1990
Accepted May 20, 1991

Preparation of spermatozoa

Sperm was obtained from the cauda epididymis of matured JCL-ICR male mice and suspended into 0.2 ml of the TYH medium (Toyoda et al., 1971) under mineral oil. After incubation for 1 h at 38.5℃ under 5% CO_2 in air, capacitated spermatozoa were killed by repeated (twice) freezing (−20℃) and thawing and stored in the freezer (−20℃) until use for injection. A part of the capacitated spermatozoa was sonicated (Bransonic, Model B-2200, Yamato, Japan) for 20 seconds to remove their tails and then treated as above before storage at −20℃. Non-capacitated spermatozoa (without incubation) were also received the same treatment as capacitated spermatozoa before storage at −20℃.

Injection procedure

Four types (non-capacitated, non-capacitated sonicated, capacitated, capaciated-sonicated) of spermatozoa or sperm head (nuclei) were used for injection. After thawing at room temperature, a small amount (1-2 μl) of sperm suspension was added to the drop of injection medium. Spermatozoa were non-motile (dead) due to frozen storage without cryoprotectant. The injection pipette was made such that the inner diameter of the tip was 3-4 μm. By this pipette individual sperm is attached by suction to the end of the injection pipette and then simply pushed through the zona pellucida and into the ooplasm of the egg itself that was held steady by a suction-controlled holding pipette. Only ova successfully injected (about 50% of ova treated) were cultured in the 200 μl drop (5-15 ova/drop) of TYH-280 medium containing 100 μM EDTA (Wako, Osaka, Japan) for 72 h at 38.5℃ under 5% CO_2 in air. This medium supported the development of *in vitro* fertilized mouse ova to blastocyst stage (Kasai et al., 1978; Authors, Unpublished). Sham-injected oocytes were prepared to assess the incidence of spontaneous (parthenogenetic) cleavage. All of the media used were sterilized through a Millipore filter (0.22 μm average pore size, Millipore Corp., MA, USA) and the mineral oil was sterilized by autoclave (121℃, 40 min).

Results

Results of this study are shown in table 1. When non-capacitated spermatozoon was injected, development to ≧ 2-cell and ≧ 4-cell was 27.8 (15/54) and 3.7% (2/54), respectively. When non-capacitated sperm head was injected, development to ≧ 2-cell and ≧ 4-cell was 21.3 (16/75) and 8.0% (6/75), respectively. When capacitated spermatozoon was injected, development to ≧ 2-cell and ≧ 4-cell was 21.4 (15/70) and 4.3 % (3/70), respectively. When capacitated sperm head was injected, development to ≧ 2-cell and ≧ 4-cell was 29.9 (35/117) and 10.3% (12/117), respectively. In contrast, none developed beyond 4-cell in the sham-operated (medium only) group. Figure 1 shows the 4-cell embryos obtained at 48 ~ 72 h after capacitated sperm-head injection.

TABLE 1. FATE OF INJECTED MOUSE OOCYTES

Substance injected	No.* of oocytes successfully injected	No. (%) of oocytes developed to 2 cell ≦	No. (%) of oocytes developed to 4 cell ≦
Non-capacitated sperm	54	15/54 (27.8)	2/54 (3.7)
Non-capacitated sperm head	75	16/75 (21.3)	6/75 (8.0)
Capacitated sperm	70	15/70 (21.4)	3/70 (4.3)
Capacitated sperm head	117	35/117 (29.9)	12/117 (10.3)
Medium only	69	9/69 (13.0)	0/69 (0.0)

*Sum of 3 to 5 trials per group.

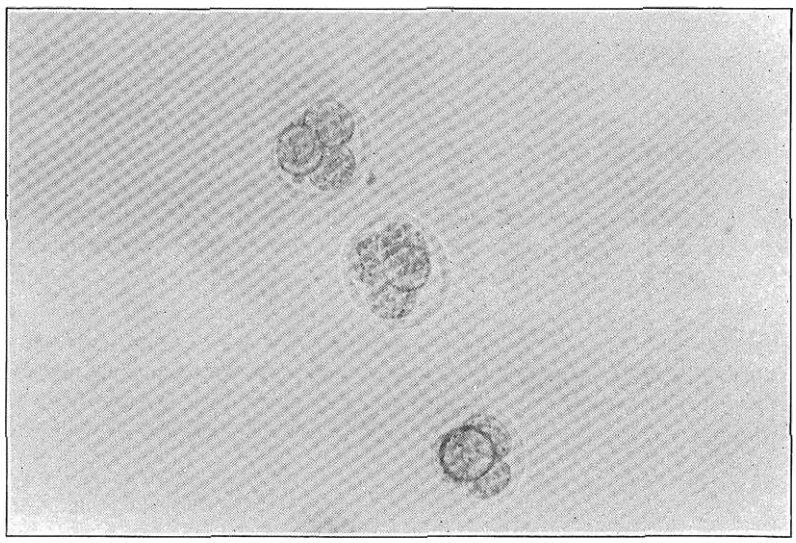

Figure 1. Four-cell stage mouse embryos obtained at 48 ~ 72 h after capacitated sperm-head injection.

Discussion

Results of this study demonstrated that mouse oocytes matured *in vivo* can undergo normal appearing cleavage to 4-cell stage by sperm injection. When capacitated sperm head was injected, two out of 117 oocytes developed to 8-cell stage during 72 h of culture (data are not shown in table 1). However none developed beyond 8-cell stage. In contrast, over 80% of *in vitro* fertilized mouse ova developed beyond 8-cell in the same medium (Authors, unpublished). Markert (1983) reported that when several hundred mouse oocytes were injected by sperm, great majority of these oocytes died without inducing any significant development of injected sperm. Nevertheless, several injected oocytes survived and induced normal sperm reactions, including the formation of a pronucleus, and these oocytes cleavage and some developed to blastocyst stage *in vitro*. However he did not show any figures in his paper. Although some of the injected oocytes may have developed parthenogenetically without participation of the spermatozoa, the higher incidence of sperm injected oocytes cleavaged to 2~4-cell stage than that of sham-injected group suggests that at least some of the embryos developed from oocytes containing an injected sperm (or sperm head) and were diploid. Recently it was shown that cow oocytes fertilized by sperm injection can undergo cleavage development (Keefer et al., 1990; Goto et al., 1990) and live offspring following embryo transfer have been obtained in the cow (Goto et al., 1990).

Keefer (1989) reported that sperm treatment prior to injection can affect the ability of oocytes to activate and direct sperm decondensation and male pronuclear formation in rabbit. In contrast, the conditions of the sperm injected were not related to ability of sperm injected oocytes to cleave in mouse in this study.

In sum, it may be possible to obtain mouse offspring by using dead-sperm injection technique in the future because some of the injected oocytes cleaved to clear 4-cell (each blastomere has a nucleus). Since we (Goto et al., 1990) have already obtained calves by dead-sperm injection technique, further refinement of the injection procedure may promise the success in mouse. Mouse oocytes are easier to be damaged by injection itself compared to cow oocytes (Authors, unpublished). It is the main reason why we have not yet succeeded to obtain blastocysts from sperm injected mouse oocytes as we did in cows.

Acknowledgements

This study was supported by Grant-in-Aid

(63560269) from the Ministry of Education, Science and Culture of Japan and by the First Purina Outstanding Research Award from AAAP to K. Goto

Literature Cited

Goto, K., A. Kinoshita, Y. Takuma and K. Ogawa 1990 *Fertilization of bovine oocytes by the injection of immobilised, killed spermatozoa.* Vet. Rec 127:517-522.

Hosoi, Y., M. Miyake, K. Uisumi and A. Iritani. 1988. Development of rabbit oocytes after microinjection of spermatozoa. Proc. 11th Int. Cong. Anim. Reprod. Artif. Insemination. Abstract 331.

Kasai, K., Y. Minato and Y. Toyoda. 1978. Fertilization and development *in vitro* of mouse eggs from inbred strains and F_1 hybrids. Jap. J. Anim. Reprod. 24:19-27.

Keefer, C. L. 1989. *Fertilization by sperm injection in the rabbit.* Gamete Res. 22:59-69.

Keefer, C. L., A. I. Younis and B. G. Brackett. 1990. *Cleavage development of bovine oocytes fertilized by sperm injection.* Mol. Reprod. Dev. 25:281-285.

Markert, C. L. 1983. Fertilization of mammalian eggs by sperm injection. J. Exp. Zool. 228:195-201.

Perreault, S. D., R. R. Barbee, K. H. Elstein, R. M. Zucker and C. L. Keefer. 1988. Interspecies differences in the stability of mammalian sperm nuclei *assessed in vivo by sperm injection and in vitro* by flow cytometry. Biol. Reprod. 39:157-167.

Toyoda, Y., M. Yokoyama and T. Hoshi. 1971. *In vitro* fertilization of eggs by fresh epididymal sperm. Jap. J. Anim. Reprod. 16:147-151.

Uehara, T. and R. Yanagimachi. 1976. *Microsurgical injection of spermatozoa into hamster eggs with subsequent trasformation of sperm nuclei into male pronuclei.* Biol. Reprod. 15:467-470.