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Immunotherapy using inhibin antiserum enhanced the efficacy of equine chorionic gonadotropin on superovulation in major inbred and outbred mice strains

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ABSTRACT

Improvement of the superovulation technique will help to enhance the efficiency of embryo and animal production. Blocking inhibin using inhibin antiserum (IAS) is known to promote follicular development by increasing the level of FSH. Previously, we reported that coadministration of IAS and eCG produced more than 100 oocytes from a single female C57BL/6 mouse at 4 weeks old. The oocytes derived from the IAS + eCG (IASe) treatment were able to fertilize and develop normally into offspring. In this study, we examined the effect of IASe treatment on the numbers of ovulated oocytes in major inbred (A/J, BALB/cByJ, C3HeJ, DBA/2J, and FVB/NJ) and outbred (CD1) mice strains at 4 weeks old. We confirmed the fertilization and developmental ability of the IASe-derived oocytes. IASe treatment ovulated 1.5 to 3.2 times higher numbers of oocytes than eCG treatment alone. The fertilization rate of IASe-derived oocytes was similar to that of eCG-derived oocytes. *In vitro* and *in vivo* developmental rates of the embryos derived from IASe were similar to the rates of embryos derived from eCG. We have shown that superovulation by IASe is very effective in obtaining high numbers of ovulated oocytes from small numbers of oocyte donor in a number of mice strains. The superovulation technique will contribute to the archiving of cryopreserved embryos of genetically engineered mice using small numbers of donors and has the potential to produce more live animals for rederivation of the archived mouse lines in mouse repositories.

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1. Introduction

Mouse repositories that support the production, preservation, and distribution of genetically engineered mice have been established to improve the accessibility of these mice to the scientific community [1]. The archived lines of genetically engineered mice are published by the International Mouse Strain Resource (IMSR; http://www.findmice.org/). Researchers can order and obtain mouse lines *via* the IMSR.

Reproductive technology is a powerful tool to efficiently manage genetically engineered mice in mouse repositories [2]. IVF using sperm and oocytes harvested from genetically engineered mice can readily produce a huge number of embryos. The embryos can be transferred to recipients to obtain the numbers of offspring required for a planned animal experiment or they can be preserved in liquid nitrogen for later use. The application of reproductive techniques for animal production allows the precise management of size and schedule of experiments using genetically engineered mice.

Summary sentence: Coadministration of inhibin antiserum and equine chorionic gonadotropin improves superovulation technique and is very effective in obtaining high number of ovulated oocytes in major inbred and outbred mice strains.

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The number of ovulated oocytes is a bottleneck that impacts the numbers of embryos and animals that can be produced by reproductive technology. It is important, therefore, to increase the number of ovulated oocytes using superovulation techniques, which artificially promote folliculogenesis and induce ovulation, by hormone treatment. Superovulation has been used successfully to obtain oocytes for the production of genetically engineered mice [3]. Traditionally, a regimen of eCG and hCG has been used to induce superovulation in mice [4]; however, the response to this technique is known to vary among inbred and hybrid mouse strains [5,6].

Administration of inhibin antiserum (IAS) has been shown to induce superovulation in several species [7]. Inhibin antiserum neutralized inhibin, which is a nonsteroidal hormone that acts on pituitary cells to suppress the production of FSH [8]. The blocking of inhibin by IAS promoted follicular development by facilitating the production of FSH [9,10]. Recently, we developed a novel superovulation technique by the coadministration of IAS and eCG [11], which yielded more than 100 oocytes from a single female C57BL/6 mouse at 4 weeks old. The ovulated oocytes were fertilized *in vitro*, and live pups developed after IVF and embryo transfer [11]. However, the efficacy of the IAS + eCG (IASe) treatment on female mice of major inbred and outbred strains has not been examined so far.

In this study, we investigated the effect of IASe treatment on the number of ovulated oocytes in major inbred and outbred mouse strains of A/J, BALB/c, C3H, CD1, DBA, and FVB mice. The ovulated oocytes were used for IVF, and the embryos were cultured and transferred to confirm their fertilization and developmental ability.

2. Materials and methods

2.1. Animals

Inbred mouse strains of A/J (SLC, Shizuoka, Japan), BALB/cByJ (CLEA Japan, Tokyo, Japan), C3HeJ (CLEA Japan), DBA/2J (CLEA Japan), and FVB/NJ (CLEA Japan) and an outbred mouse strain of CD1 (Charles River Japan, Yokohama, Japan) were purchased and used as donors of sperm (from male mice at 12 to 15 weeks old) and oocytes (from female mice at 4 weeks old). ICR mice (CLEA Japan) which were 8- to 16-week old were used as recipients of two-cell embryos. All the mice were housed in a specific-pathogen–free room under a 12-hour dark-light cycle (light from 07:00–19:00) at a 22 $\pm$ 1 °C with *ad libitum* food and water. The Animal Care and Use Committee of the Kumamoto University School of Medicine approved the protocols for the experiments using animals. The mice were properly handled and euthanized by the person who was admitted and directed by the Animal Care and Use Committee of Kumamoto University.

2.2. Medium

Inhibin antiserum was prepared as described previously and preserved at −20 °C before use [9,10]. Sperm were preincubated in a modified Krebs–Ringer bicarbonate solution containing 1.0-mg/mL polyvinyl alcohol (Sigma) and 0.75-mM methyl-β-cyclodextrin (Sigma) as sperm preincubation medium [12,13]. Calcium-enhanced human tubal fluid containing 0.25-mM reduced glutathione (Sigma) was used as fertilization medium [14–16]. Potassium simplex optimized medium was used to handle and culture the two-cell embryos to the blastocyst stage [17]. All media were prepared and stored at 4 °C no longer than 3 months before use. Sperm preincubation medium, fertilization medium, and embryo culture medium were preincubated in CO_2 incubator (5% CO_2 and 95% air) at 37 °C for 30 minutes before use.

2.3. Superovulation

Superovulation was performed as described in our previous study [11]. Briefly, immature female mice, which were 4-week old, were administered 7.5 IU eCG (ASKA Pharmaceutical Co. Ltd, Japan) or IASe (mixed solution of 0.1 mL IAS and 3.75 IU eCG) intraperitoneally. Forty-eight hours after the injection of eCG or IASe, the mice were administered 7.5 IU hCG (ASKA Pharmaceutical Co. Ltd, Japan) intraperitoneally. At 17 hours after hCG administration, the mice were euthanized by cervical dislocation, and the oviducts were collected quickly and transferred to a fertilization dish covered with paraffin oil. Under microscopic observation, cumulus–oocyte complexes were collected from the oviducts by dissecting needle and transferred to a 200-μL drop of fertilization medium. The numbers of ovulated oocytes and their ability to be fertilized were examined in each group.

2.4. In vitro fertilization

Male mice were euthanized by cervical dislocation, and the cauda epididymides were collected and transferred to a dish of sperm preincubation medium covered with paraffin oil. Aliquots of sperm were collected from the cauda epididymides using a dissecting needle and transferred to a 100-μL drop of sperm preincubation medium. Sperm were preincubated for 60 minutes to induce capacitation and then added to the fertilization drop containing the cumulus–oocyte complexes and cultured with oocytes at 37 °C in an atmosphere containing 5% CO_2 for 3 hours. The concentration of motile sperm was measured by computer-assisted sperm analysis (IVOS, Hamilton Thorn Inc.) before insemination and then adjusted to 400 to 800 motile sperm/μL in fertilization medium. At 3 hours after insemination, oocytes were washed in three drops of calcium-enhanced human tubal fluid (80 μL), and the number of ovulated oocytes was examined. At 24 hours after insemination, fertilization rates were calculated as the total number of two-cell embryos divided by the total number of inseminated oocytes multiplied by 100.

2.5. Embryo culture and transfer

To evaluate the development of the two-cell embryos produced by IVF, we performed embryo culture and transfer. After IVF, the two-cell embryos were divided into two groups; one that was cultured to the blastocyst stage in

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