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Recent progress in reproduction of whale oocytes

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Summary

Whale oocytes recovered from follicles can be matured *in vitro.* Whale sperm and mature oocytes can be used for *in vitro* fertilization (IVF), and IVF embryos have the ability to develop to morula stage. Whale sperm injected into bovine or mouse oocytes can activate the oocytes and form pronucleus. Interspecies somatic cell nuclear transfer embryos have been reconstructed with whale somatic cell nucleus and enucleated bovine or porcine oocytes, and interspecies cloned embryos can develop *in vitro.* This paper reviews recent progress in maturation, fertilization and development of whale oocytes.

Keywords: Embryo, Fertilization, Maturation, Oocyte, Whale

Introduction

In vitro embryo production through *in vitro* maturation (IVM), *in vitro* fertilization (IVF) and *in vitro* culture (IVC) has been possible in many kinds of mammalian species. Whale is an important mammalian animal. More information on reproductive events in follicular development, oocyte maturation, fertilization, and embryonic development should be obtained from male and female minke whale (Fukui *et al.*, 1997a). Research on IVM, IVF and IVC of whale embryos will contribute to the basic understanding of reproductive physiology of cetaceans (Bhuiyan *et al.*, 2008). Recently, whale oocytes have been used for cryopreservation, IVM, IVF and *in vitro* production of embryos.

Whale oocytes

Whale ovaries can be classified into three types according to the number and size of follicles. Type A has more than 200 follicles, and its diameter is <5 mm. The majority of the follicles are antral follicles. Types B and C have 50–200 and <50 follicles, respectively. The diameters of types B and C are up to 10 mm (Tetsuka *et al.*, 2004). Whale follicles can be classified into three types by their sizes. Small, medium and large follicles are <5, 5–10, and >10 mm in diameter, respectively. The diameters of oocyte ooplasm from the three follicle types are different, and there is no difference in the diameter of the whole oocyte and thickness of the zona pellucida among the three follicle types. The osmolarity of whale follicular fluid (wFF) from the three follicle types is 363.3–388.9 mOsmol. Follicular fluid from large follicles has a lower concentration of lactic acid than that from the small follicles, but the estradiol-17beta (E_2) concentration of wFF increases as the follicle size increases (Nagai *et al.*, 2007).

Whale oocytes are classified into four grades by the number of surrounding cumulus cells. Grades A and B have >5 and 1–3 layers of cumulus cells, grade C is naked oocyte or partially surrounded by cumulus cells, and grade D is surrounded by expanded cumulus cells (Fukui *et al.*, 1997a). The recovery rates of cumulus–oocyte complexes (COCs) per sei and Bryde's whales are 16–30.6 and 6.7–26.8, respectively (Bhuiyan *et al.*, 2008). The total proportions of grades A and B oocytes in the three follicle types are not different. The rates of germ vesicle (GV)-stage oocytes from the three follicle types are 55.9–72.1%. There are some degenerated oocytes in the small follicles. The rates of GV-stage oocytes are 25–81.1% for the four oocyte grades. Some grades C and D oocytes have resumed meiosis to metaphase II (MII) stage (Fukui *et al.*, 1997a).

In vitro maturation of whale oocytes

Whale COCs can be matured in the medium *in vitro,* and the oocytes extruding the first polar body are

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considered to be mature. A portable CO_2 incubator is a useful device for IVM of whale oocytes. The gas atmosphere of the portable incubator is 5% CO_2 and 8–10% O_2. The low oxygen tension reduces free oxygen radicals, and may promote cytoplasmic maturation and subsequent developmental competence of oocytes. The maturation rate of whale oocytes was 26.7% using the portable incubator (Iwayama *et al.*, 2005).

Whale oocytes were cultured in a dish containing IVM medium supplemented with fetal whale serum (FWS), follicle stimulating hormone (FSH) and E_2 for 96 h, and the maturation rate was 27.3% (Fukui *et al.*, 1997a). Maturation rate of whale oocytes matured in medium with FSH/E_2 for 30 h was 26.7% and the cumulus mass showed the maximum expansion (Iwayama *et al.*, 2005). Adding FWS to the maturation medium improved the proportion of whale oocytes at MII stage, and whale COCs cultured in medium with 20% FWS had a maturation rate of 31.8% (Asada *et al.*, 2001a). Whale COCs can also be matured using the Well of the Well (WOW) method in medium supplemented with wFF, FSH, E_2 and epidermal growth factor (EGF), and the maturation rate of oocytes cultured for 40 h was 30.4% (Fukui *et al.*, 2007). Supplementation of wFF in IVM medium may improve maturation rate of whale oocytes due to containing beneficial hormones and growth factors in it. The IVM medium of whale oocytes can be adjusted to 390 mOsmol by addition of D-sorbitol. The increased osmolarity of medium may provide with physiological condition for oocyte maturation (Bhuiyan *et al.*, 2008).

Cryopreservation of whale oocytes

Whale oocytes can be cryopreserved using ethylene glycol (EG).The morphologically viable proportion of post-thawed oocytes was 39.7%. Some damage is induced by the freezing and thawing procedures in the cryopreserved oocytes, such as rupture of the ooplasm membrane, vacuolation of microvilli, migration of cortical granules, and presence of vacuolated mitochondria. The presence of cumulus cells improves the proportion of oocytes at metaphase I, anaphase I, and telophase I stages. Thirty per cent of cryopreserved oocytes can resume meiosis *in vitro*, and four of 194 post-thawed oocytes can mature to the MII stage (Asada *et al.*, 2000). Whale oocytes can also be vitrified using EG + dimethylsulfoxide, and the maturation rate after warming was 30.2%. Whale oocytes can be treated with cytochalasin B before vitrification, and the maturation rate after warming was 30.4% (Fujihira *et al.*, 2006). Cryotop and open-pulled straw (OPS) have been used as the cryodevice for vitrification of whale COCs. The Cryotop is a better device than the OPS for vitrification of immature oocytes from adult minke whales (Iwayama *et al.*, 2004).

In vitro fertilization of whale oocytes

The total length of whale spermatozoa is 56.7 μm. The common forms of whale sperm heads are conical and elliptic (Mogoe *et al.*, 1998). Forty percent of spermatozoa frozen and thawed were motile, and 44% of spermatozoa frozen and thawed were vital. The motility and vitality of whale spermatozoa are correlated with the serum E_2 levels (Fukui *et al.*, 1996). Whale IVM oocytes can be fertilized in the fertilization medium with 20% FWS or 0.6% bovine serum albumin, sperm penetration and two-pronuclei formation occur in the whale oocytes (Asada *et al.*, 2001a). The rates of sperm penetration and pronuclear formation were higher in the whale oocytes matured for 120 h than in those matured for 96 h (Fukui *et al.*, 1997b). Whale sperm pretreated with dithiothreitol (DTT) can be injected into IVM oocytes, and intracytoplasmic sperm injection (ICSI) embryos have the ability to develop to 2–4-cell stage (Asada *et al.*, 2001b).

In vitro development of whale embryos

Whale IVM oocytes can be injected with sperm, and two from 21 sperm-injected oocytes can develop to two-cell stage (Asada *et al.*, 2001b). No cleavage occurs in whale oocytes without insemination. Whale IVM oocytes inseminated with post-thawed spermatozoa were cultured with cumulus cells, and the inseminated oocytes cleaved to 2–16-cell stages (Fukui *et al.*, 1997b). The cleavage, 4-cell and 8-cell rates of whale IVF embryos were not different between embryos from grades A and B oocytes. IVF embryos from whale grade B oocytes were cultured in FWS-supplemented medium, and 1.1% of the embryos developed to morula stage (Bhuiyan *et al.*, 2008). Whale IVF embryos from grade A oocytes had higher cleavage and morula rates than those from grade B oocytes; 4.2% of grade A oocytes can develop to morula stage, but no morula can be formed from grade B oocytes (Asada *et al.*, 2001a).

Interspecies fertilization of whale sperm

Interspecies microinsemination has been used to examine the ability of whale haploid spermatogenic

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cells to induce Ca^{2+} oscillations. Whale round spermatids (RS), early-stage elongating spermatids (e-ES), late-stage elongating spermatids (1-ES) and testicular spermatozoa (TS) were injected into mouse oocytes, and the repetitive increases of intracellular Ca^{2+} concentration occurred in oocytes injected with the e-ES, 1-ES and TS. RS can not induce Ca^{2+} oscillations. Whale spermatogenic cells acquire sperm-borne oocyte-activating factor (SOAF) activity, which is related to their Ca^{2+} oscillation-inducing ability (Amemiya *et al.*, 2007). SOAF activity in the whales is required during the early phase of spermiogenesis. Whale spermatid microinjected into mouse oocytes can induce the oocytes to be activated and resume meiosis. The RS can not activate mouse oocytes, but mouse oocytes can be activated by e-ES, l-ES and TS (Amemiya *et al.*, 2004).

Whale spermatozoa centrosome introduced into bovine oocytes can contribute to the microtubule-organizing centre. Bovine mature oocytes injected with DTT-treated whale spermatozoa and activated with ethanol + 6-dimethylaminopurine formed a whale sperm aster. Assembly of the microtubule network is promoted by oocyte activation. The ratio of aster diameter to oocyte diameter is 0.57 in injected and activated oocytes (Kobayashi *et al.*, 2006).

Whale spermatozoa injected into mouse oocytes can lead to oocyte activation, and the sperm nucleus can transform into a male pronucleus (Watanabe *et al.*, 2007). Whale frozen–thawed spermatozoa injected into bovine oocytes can participate in fertilization activities in bovine oocytes after the injection. Sperm head decondensation and male pronucleus formation occurred after ICSI. Male pronuclear formation rate was 39.1% in the injected oocytes. The development of male and female pronuclei is synchronous, and the mean diameters of male and female pronuclei are 30.4 μm and 29.3 μm, respectively (Wei & Fukui, 2000).

Whale interspecies somatic cell nuclear transfer

Whale interspecies somatic cell nuclear transfer (iSCNT) embryos can be reconstructed using bovine and porcine oocytes. Minke whale granulosa-cumulus cells (MWGC) and minke whale cumulus cells (MWCC) have been used as donor cells. Minke whale somatic cell nuclei transferred into enucleated bovine oocytes formed pseud-pronucleus (PPN), and the proportions of PPN were not different among interspecies and intraspecies SCNT oocytes. Minke whale iSCNT embryos had whale genomic DNA, and the embryos developed to 2–4–cell stages. There was no difference in the cleavage rates of minke whale iSCNT embryos from viable and dead cells (Ikumi *et al.*, 2004). Sei whale iSCNT embryos have been reconstructed using whale fetal fibroblasts as donor nuclei and bovine oocytes as recipient cells. Both intracytoplasmic cell injection (ICI) and subzonal cell insertion (SUZI) are equally effective with respect to PPN formation and cleavage of sei whale iSCNT embryos. Bovine oocytes have the ability to support development of sei whale nuclei up to the 6-cell stage (Bhuiyan *et al.*, 2010). Sei whale iSCNT embryos can also be reconstructed using porcine oocytes. Porcine oocytes supported development of sei whale iSCNT embryos to the 4-cell stage, indicating that porcine oocytes can induce the nuclear reprogramming of sei whale somatic cells (Lee *et al.*, 2009). The high rates of developmental blockage in whale iSCNT might be due to failure in timely activation of the whale embryonic genome. Further studies are needed on reprogramming mechanism of whale nuclei at the molecular level to gain a better understanding of development in iSCNT embryos (Bhuiyan *et al.*, 2010). Thus, development of iSCNT embryos may provide some information about whale embryo development.

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