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Hypothermic storage of isolated spermatogonia and oogonia from rainbow trout (*Oncorhynchus mykiss*)

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ABSTRACT

A growing number of fish species are endangered due to human activities. A short- or long-time preservation of gametes could conserve genetic resources of threatened fish species. The aim of this study was to evaluate a hypothermic condition for short-term preservation of spermatogonia and oogonia cells isolated from immature transgenic rainbow trout, *Oncorhynchus mykiss*, and to determine the maximum time point for further transplantation. Viability rate of germ cells was investigated after isolation and during storage at 4 °C up to 24 h. Dulbecco's modification of Eagle's medium supplemented with Hepes fetal bovine serum and L-glutamine was used as hypothermic storage media. The results showed that while viability decreased following 24 h storage, the remaining viable cells did not vary morphologically as well as GFP intensity retained similar to those observed in freshly isolated cells. The hypothermal storage study indicated that culture medium is suitable for preserving germ cells in the short periods of time. Simplicity, easily available culture media and low cost provide new insight into hypothermic conditions for preserving and transporting of germ cells for next applied and basic studies.

Fish stocks have become globally threatened because of overfishing and environmental pollution [5,10] and there is no more wild fish for the markets. Therefore, aquaculture is an important activity to fill this gap to provide high quantity and quality food in order to meet the growing protein demands and also protects the wild populations from over exploitation [6].

Several studies have attempted to develop novel techniques for conservation of wild fish from extinction. Short- and long-term preservation of germ cells brings the likelihood preserving genome of threatened species, increasing the introduction of genetically valuable animals for husbandry purposes and avoiding genetic losses through diseases and disasters [2,3,19]. While sperm cryopreservation has been available for many fish species, the methods for preserving fish embryos and mature oocytes have been unsuccessful due to their particular structure and high yolk content [7]. Whilst the maternally inherited materials including mitochondrial DNA cannot be preserved using only sperm cryopreservation [13]. Recent attentions have, therefore, been focusing on the low temperature banking of immature germ cells as possible alternatives with the aim to restore wild fish population or to use culture technology for further investigations [12,14,15].

Early stages of spermatogonia (SG) and oogonia (OG) cells, the basis for continuous sperm and egg production in male and female, respectively, retain their migration and differentiation capacities after transplantation in a recipient embryo [14,15,17,18,20,22,23]. These cells can also be used for surrogate reproduction through germline chimera [13–16]. In other words, manipulation of SG and OG seems to be more operative in transplantation and showed a practical potential to use in breeding programs of fish.

Although a great deal of research has been conducted on the long-term storage of early-stage germ cells, no study has reported the evaluation of quality and quantity of germ cells stored in a hypothermic condition. Hypothermic conditions are defined as those in which the temperature is lower than normal physiological temperature, but higher than the freezing point of the storage solution [1]. Hypothermic preservation of cell is ideal for field collection where access to a laboratory apparatuses such as

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Table 1
Characteristics of transgenic rainbow trout used for the present study.

Sex (n)	Body weight (g)	Body length (cm)	Gonad weight (g)	Gonado-somatic index (%)
Male (6)	17.5 ± 7.7	11.2 ± 1.2	0.014 ± 0.002	0.051 ± 0.004
Female (7)	23.4 ± 6.1	12.2 ± 1.0	0.059 ± 0.016	0.19 ± 0.04

microinjector, liquid nitrogen and deep freezer can be hours or days away. This technique is also very advantageous for hatchery management since it allows synchronizing the development of embryos collected from various spawning trials for surrogate reproduction via germline chimera. The aim of this study was therefore to investigate the hypothermic condition for a short-term preservation of SG and OG cells isolated from rainbow trout.

All of the experimental protocols and procedures were carried out in accordance with the Guidelines for the Care and Use of Laboratory Animal of Tokyo University of Marine Science and Technology. Rainbow trout used for this experiment were held and reared at the Oizumi Station of the Field Science Center of Tokyo University (Yamanashi, Japan). Immature gonads were obtained from *pvasa-GFP* transgenic rainbow trout [21] on October. The characteristics of fish sampled during the trial are shown in Table 1.

Testes and ovaries were excised from immature transgenic males (n = 10) and females (n = 5). The gonads were dissociated with 0.5% trypsin (Worthington Biochemical, Lakewood, NJ, USA) in phosphate-buffered saline (PBS) containing 1 mM Ca^{+2} for 2 h at 20 °C [11]. During enzymatic digestion, gentle pipetting was

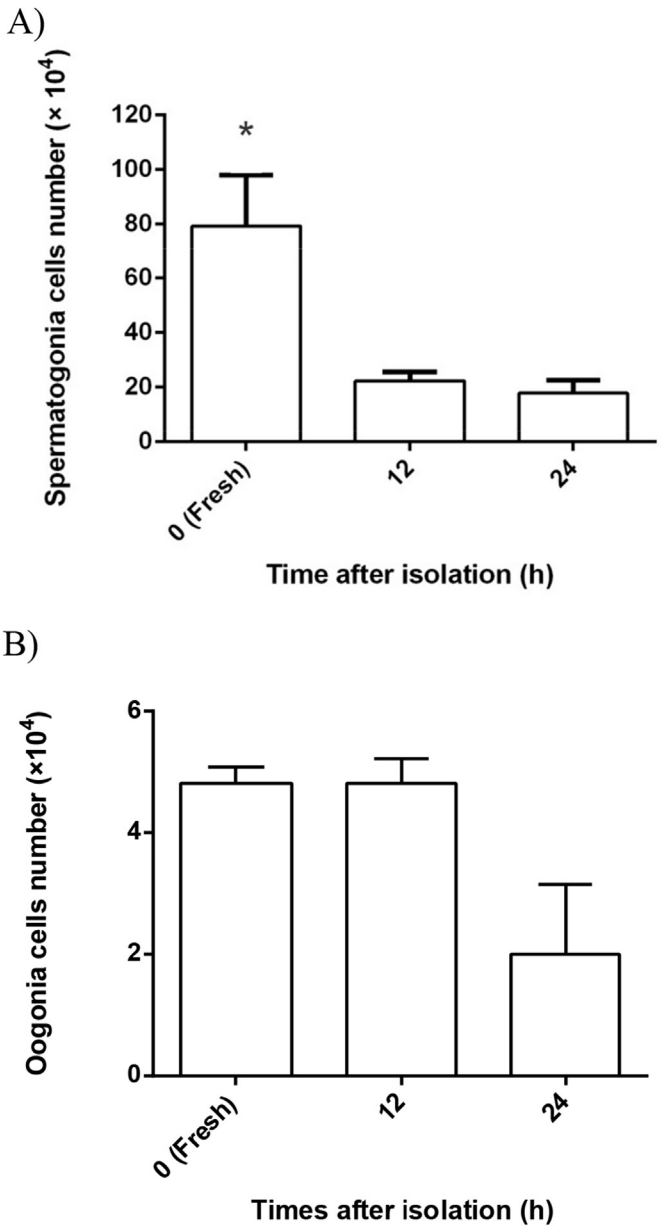

Fig. 1. Number of isolated spermatogonia (A) and oogonia (B) cells in transgenic male and female rainbow trout, respectively, during 24 h at 4 °C (mean ± SD; n = 5–10). Columns with asterisk show significant difference among the various times at $P < 0.05$.

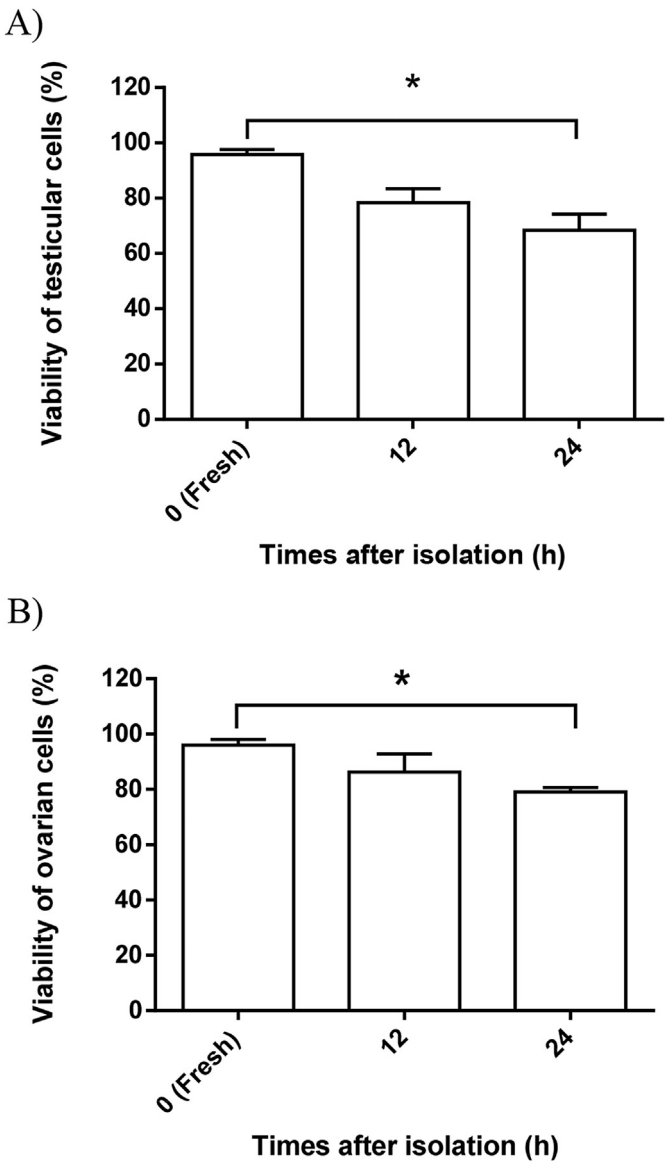

Fig. 2. Viability (%) of isolated testicular (A) and ovarian (B) cells in transgenic female and male rainbow trout during 24 h at 4 °C (mean ± SD; n = 5–10). Columns with asterisk show significant difference among the various times at $P < 0.05$.