

<b>Focus</b>	Production of functional gametes derived from cryopreserved germ-line stem cells using a surrogate broodstock system in medaka
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<b>Period</b>	FY2012 - 2013
<b>Overview</b>	<p>Although numerous inbred lines and endangered wild populations of medaka currently exist, the only method currently available for preserving medaka genetic resources is to rear live individuals, as protocols for cryopreserving fish eggs or embryos have not yet been successfully established. However, rearing live individuals is always associated with the risk of mutation due to mobile elements, and the loss of parental fish through infectious disease or accidents related to rearing conditions and equipment. To overcome these obstacles, as in a previous experiment using trout, we will attempt to cryopreserve germ-line stem cells, which we will then transplant into recipient medaka. The proposed study will therefore involve the following steps: 1) develop a method to vitrify whole medaka testes, 2) optimize techniques for transplanting germ-line stem cells into the body cavity of newly hatched larvae, which have poorly developed immune systems. After transplantation, the donor-derived germline stem cells are expected to migrate towards the gonads of the recipients where they will undergo gametogenesis. Finally, we intend to establish a method for mass producing sterile triploid medaka which can then be used as recipients to obtain surrogates that produce only donor-derived sperm and eggs. These techniques will facilitate the establishment of a stable and reliable system for preserving valuable medaka strains semi-permanently.</p>
<b>Progress</b>	<p>(written in Japanese)</p> <p>メダカ精巣を緩慢凍結、およびガラス化することで、メダカ遺伝子資源を長期間保存する技術の構築に成功した。また、これらの精巣組織から細胞懸濁液を調整し、これを宿主メダカの孵化仔魚の腹腔内へと移植することで、ドナー精巣から得られた生殖幹細胞が宿主の卵巣、精巣へと移動し、そこで機能的な卵、精子にまで分化することを確認した。さらに、得られた宿主の雌雄を交配することで、その次世代にドナーに由来する個体を作出することが可能となった。以上のように液体窒素内で長期保存したメダカ精巣組織から機能的な卵、精子を、ひいては授精を介して正常な個体を作り出す技術を構築することに成功した。(プロトコル集 出版予定)</p>