

Review Article

Germ Cell Transplantation: A Potential Tool for Propagation of Endangered Fishes

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Abstract

Germ cell transplantation, a powerful assisted reproductive technology, is widely used to study the functional characterization of stem cells. The technology is also used for conservation and propagation of elite germ lines. In teleost fish model, the technique has been successfully established to generate donor-derived gametes by transplanting primordial germ cells (PGC) or spermatogonial cells at various developmental stages such as embryonic, hatchling and adult stage. Various studies have confirmed, irrespective of developmental stages and methods of transplantation, the recipient fish have produced the surrogate gametes. The most potential application of this technique are 1) propagation of endangered and commercially important fish species those are difficult to breed in confinement, 2) control of invasive fish species, those pose threats to the native species and, 3) production of transgenic individual by transfecting the donor spermatogonial cells before transplantation. Very recently, the technique is also used to provide stem cell therapy to senile fishes those have lost their reproductive competence because of old age. The germ cell transplantation technique appears to be most valuable at the moment as other assisted reproductive technologies such as cryopreservation and in vitro gametogenesis fails to conserve or propagate the female gametes.

Keywords

- Germ cell transplantation
- Endangered
- Conservation
- Propagation
- Fish

INTRODUCTION

The diversified aquatic ecosystem, from freshwater to marine, is inhabited by over 30,700 fish species. However, in recent years, a number of fish species have reported to become extinct or endangered (<http://www.iucnredlist.org>) due to various known and unknown factors such as aquatic pollution, habitat loss, increasing anthropogenic pressure on the water bodies and most importantly sudden global climate changes.

To conserve these threatened fish species, two major approaches could be adopted. The first, *in situ* conservation, protecting the species within the ecosystem [1]. The second, *ex situ* conservation of these valuable germ lines and propagate the individuals of the species through captive breeding [2,3]. Obviously both the approaches have the merit to safeguard the fishes of declining population. However, ideally, the highest priority would always be habitat preservation as it will ensure to protect the entire ecosystems and many species simultaneously. But, the challenge would be sustainability to conserve the habitat for long term; given the fact that the habitats are under constant threat from natural stochastic factors and local changes in land use and natural resource management choices. Nevertheless, for managing the vast fishery resources, both *in situ* and *ex situ* approaches should be complementary rather than competitive [4].

Very recently, one of the most promising approach for *ex situ* conservation appears to be development of surrogate

broodstock for the commercially important and/or threatened fish species by germ cell transplantation [5-7]. The viability of the technique was first demonstrated in mice model where spermatogonia derived from donor males were microinjected into sterilized male recipients, leading to establishment of donor-derived spermatogenesis [8]. Since then, the technique has been extensively used for the purpose of basic research, reproductive medicine and treatment of infertility. In teleost fish model, the technique was successfully applied in rainbow trout during the early beginning of this century. In that case, primordial germ cells (PGC) carrying GFP (green fluorescent protein) was transplanted into peritoneal cavity of rainbow trout hatchlings and resulted in production of sperm with donor genetic characteristics [9]. Further, using the same methodology, xenogeneic transplantation between rainbow (*Onchorhynchus mykiss*) and masu (*O. masu*) trout were also successfully performed [10]. Also, it was shown that PGCs can be cryopreserved for prolonged periods of time before transplantation and still establish spermatogenesis in the recipient testis [11]. In fishes, germ cell transplantation could be performed in three distinct developmental stages; such as embryonic, hatchling and adult stage. Aim of this review is to discuss the various methods of germ cell transplantation used in various fish species, at different developmental stages, to generate surrogate gametes. The various assisted reproductive technologies discussed will pave the way to derive functional gametes from the endangered and/or commercially important fishes which are difficult to propagate in confinement.

Germ cell transplantation at various developmental stages

Embryos (blastula stage): Here, PGCs harvested from the donor hatchling are transplanted into recipient embryo by micromanipulator (blastula stage; Figure 1) that have had endogenous PGC development blocked by the injection of a dead end antisense morpholino oligonucleotide. Ever since this technique was proposed, the method was successfully applied into zebrafish [12] and pearl danio [13]. The major advantage of this method is that, no immunorejection of transplanted PGCs was observed because the transplantation was performed before the sex determination stage in recipient fish [13]. This technique is considered one of the viable approach as it allows to generate millions of gametes of fish species, with desired traits such as growth, disease resistance, fecundity etc. by using only one donor PGC [14].

Hatchling stage: In this case, the donor germ cells are

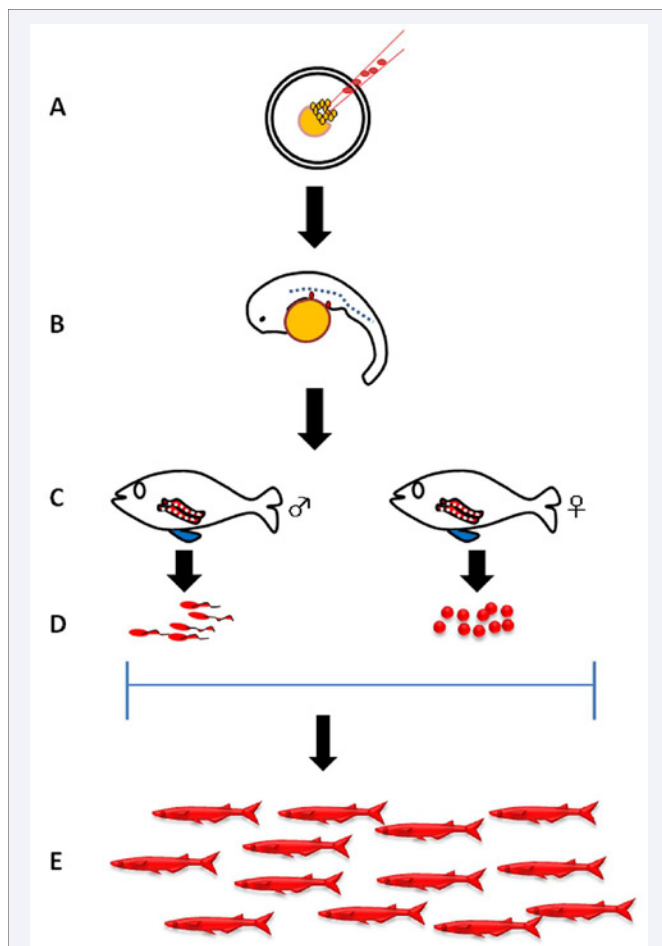


Figure 1 Graphical illustration of Primordial germ cell transplantation in fish embryo. A) Primordial germ cell are microinjected into the blastodisc of blastula-stage embryos. B) The donor PGCs divide prior to settling at the gonadal ridge (future gonads) of the recipient. C) The PGCs migrate and colonize the gonads. The cells proliferate and differentiate into the functional donor-derived gametes after the fish attain sexual maturity. D) The mature surrogate parents release functional donor-origin gametes. E) Generation of surrogate progeny by artificial insemination or natural spawning.

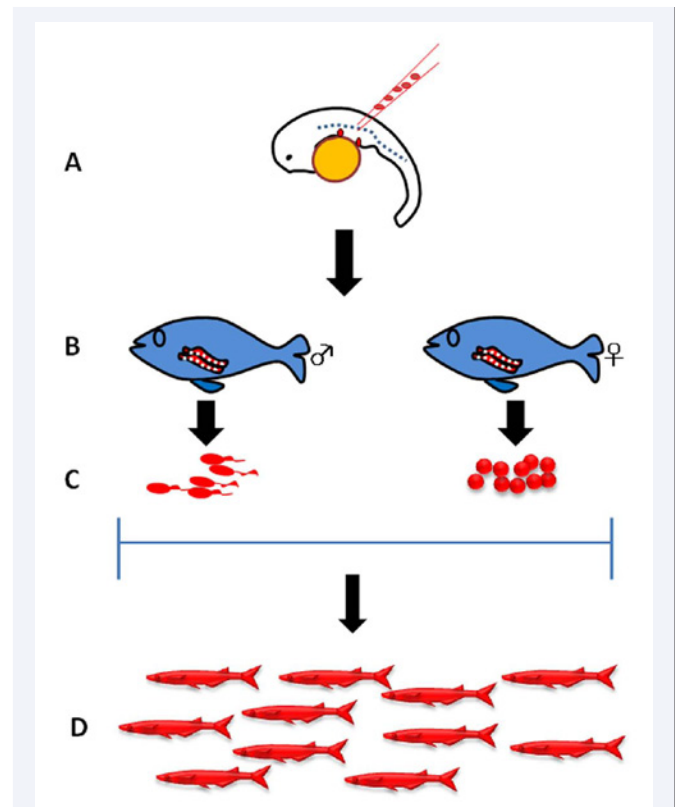
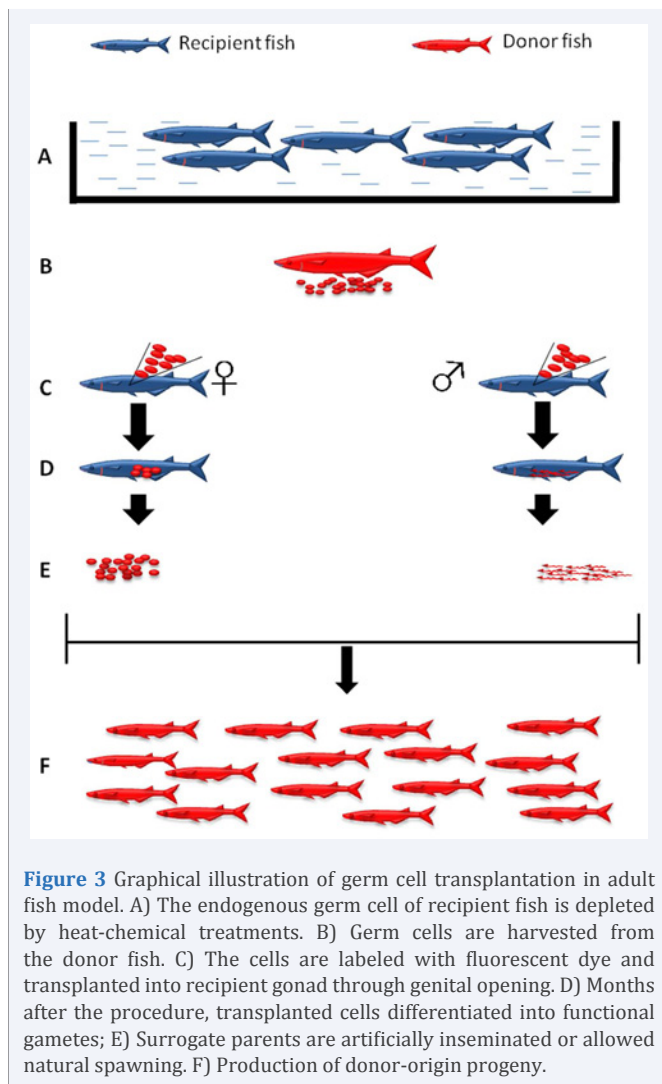


Figure 2 Graphical illustration of germ cell transplantation in newly hatched fish larvae. A) Donor germ cells are transplanted into the coelomic cavity of the hatchling during the time-period in which endogenous recipient larvae PGCs are still actively migrating. B) The transplanted cells migrate and colonize the genital ridges of the recipient. C) After attaining sexual maturation the recipient fish produce donor-derived gametes. D) Generation of surrogate progeny by artificial insemination or natural spawning.

transplanted into the peritoneal cavities of newly hatched larvae using a micro injector [15] (Figure 2). The newly hatched larvae are selected as recipients because they lack a functional immune system indicated by the absence of differentiation in both their thymus and T cells [16]. Lack of a functional immune system means absolutely no immunorejection of exogenous (donor-derived) germ cells. The PGC derived from transgenic rainbow trout were transplanted into the body cavity of masu salmon hatchlings [14]. These hatchlings upon reaching adulthood, produce the xenogenic donor derived offspring [5]. Later, the approach was also successfully applied to trout model where spermatogonial stem cell from vasa-Gfp rainbow trout were transplanted into sterile triploid masu salmon hatchling that resulted in establishment of gametogenesis in male and female recipients [10]. However, in this approach the only disadvantage is that the donor-derived gametes could be generated approximately 2-3 years after the transplantation, depending on the attainment of gonadal maturity of the animal.

Adult stage: This method of germ cell transplantation in teleost fish was proposed by Lacerda and her colleagues [7], using Nile tilapia fish (*Oreochromis niloticus*) model, in which spermatogonia were transplanted through the urogenital papilla of adult fish [17]. In this method, recipient adult fish was made partially sterile by treatments of cytotoxic drug, busulfan



(1,4-butanediol dimethanesulfonate), in association with elevated water temperature. An enriched type A spermatogonial cell suspension harvested from donor testes and labelled with the fluorescent lipophilic dye such as PKH-26 was injected into the adult testes of the recipient through the common spermatic duct (Figure 3). This method confirmed the possibility of using sexually mature adult fish as recipients in germ cell transplantation [7]. Making refinement to this approach, Majhi and his colleagues developed an intra-gonadal surgical method for xenogeneic germ cell transplantation in sexually competent adult fish. They used germ cells from juvenile pejerrey (*Odontesthes bonariensis*) as donor, while sexually competent adult Patagonian pejerrey (*Odontesthes hatchery*) as recipients for germ cell transplantation [6]. The endogenous spermatogenesis of recipient fish was depleted using busulfan and high temperature treatments [18]. Donor germ cells isolated by discontinued percoll gradient were labelled with CFDA-SE (green fluorescent dye) and introduced into the recipient gonad. Injection of the germ cell suspension was made possible in this procedure by a long midline cut made in the abdomen of the recipient fish followed by the exposition of the gonads from the coelomic cavity. The transplanted spermatogonial cells were able to colonize the gonads of recipient

Patagonian pejerrey and generate donor-derived sperm within 6 months post transplantation [6]. Later, the same group have simplified the transplantation approach by developing a non-surgical method and produced the functional surrogate eggs and sperm capable of fertilization in very short time [18]. The major advantage of using adult as the recipient for germ cell transplantation is that, it considerably reduces the time needed to obtain viable gametes and offspring of donor genotype.

CONCLUSION

It is believed that the germ cell transplantation technique beside application in functional characterization of stem cell has also potential applications in germline conservation and in the propagation of valued and/or endangered fish species [19,20]. Also, the technique can be useful in speedy propagation of commercially important species which are too large for hatchery rearing and, that do not spawn due to the stress of confinement, or whose maturation cycle is associated with complex migratory behavior which cannot be reproduced in captivity.

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