Symbiosis

www.symbiosisonline.org
www.symbiosisonlinepublishing.com
ISSN Online: 2377-4274

Research Article | International Journal of Genetic Science | Open Access

Germ Cell Transplantation in Teleost Fishes: A Viable Approach for Germline Conservation

Sullip Kumar Majhi*

**ICAR-National Bureau of Fish Genetic Resources, Canal Ring Road, Dilkhusa P.O., Lucknow 226 002, Uttar Pradesh, India*

Received: March 28, 2018; Accepted: September 11, 2018; Published: September 20, 2018

Corresponding author: *Dr. Sullip Kumar Majhi, ICAR-National Bureau of Fish Genetic Resources, Canal Ring Road, Dilkhusa Post Office, Lucknow 226 002, Uttar Pradesh, India. Tel: + 91-522-2442441; Fax: + 91-522-2442403; Email: sullipkm@gmail.com*

Abstract

Germ cell Transplantation, a powerful assisted reproductive technology, is widely used for basic and applied research. This technique, initially developed for mammals, has been recently used for teleost fishes for conservation and propagation of elite germplasm. In this technique, germ cells derived from donor fish are transplanted into the gonads of sterile recipients through surgical and non-surgical interventions (through genital papilla). The recipients upon attending sexual maturity are crossed through artificial insemination and natural spawning to generate surrogate offspring. The vast fishery resources could be effectively managed by germ cell Transplantation technique, especially to rejuvenate the population of endangered fish species or 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.

Keywords: Fish; Conservation; Surrogacy; Germ cell Transplantation; Endangered;

Introduction

The great diversity of aquatic ecosystem, from marine to freshwater, harbors almost 30,700 fish species. However, rapidly changing climate and associated factors such as pollution, habitat loss and increasing anthropogenic pressure on the water bodies have played a crucial role in turning a number of fish species to become extinct or endangered (see http://www.iucnredlist.org).

There are two major approaches to conserve these threatened fish species such as ex situ and in situ approach. The in situ approach involve the preservation of habitat on a large scale, thereby protecting the species within the ecosystem [1,2]. Contrary, the ex situ approach involve conservation of these valuable germlines and propagate the individual species through breeding in captivity [3,4]. Although, there has been debate about the relative efficacies of these strategies, both obviously have merit. Ideally, habitat preservation should always be the highest priority, that help to protect entire ecosystems and many species simultaneously while concurrently retaining the inherent 'wildness' of nature, animals and aquatic plants [5]. However, the ability to conserve habitat long term is itself under constant threat from natural stochastic factors, local changes in land use and natural resource management choices. For sustainable management of genetic resources, it is my understanding that, both in situ and ex situ approaches should be complementary rather than competitive.

Germ Cell Transplantation

Germ Cell Transplantation (GCT) appears to be a viable and promising approach for ex situ conservation, a technique originally devised for use in mammals. The GCT was first demonstrated in mice and was performed with dispersed testicular Cell suspensions containing unknown numbers of spermatogonia derived from donor males and microinjected into sterilized male recipients, leading to establishment of donor-derived spermatogenesis [6]. Since then, the technique has been extensively used for the purpose of basic research [7], reproductive medicine [8] and treatment of infertility [9], but surprisingly remains, in part, unexplored in fish, even though GCT has potential applications in conservation and propagation of species facing eminent extinction (Figure 1-3).

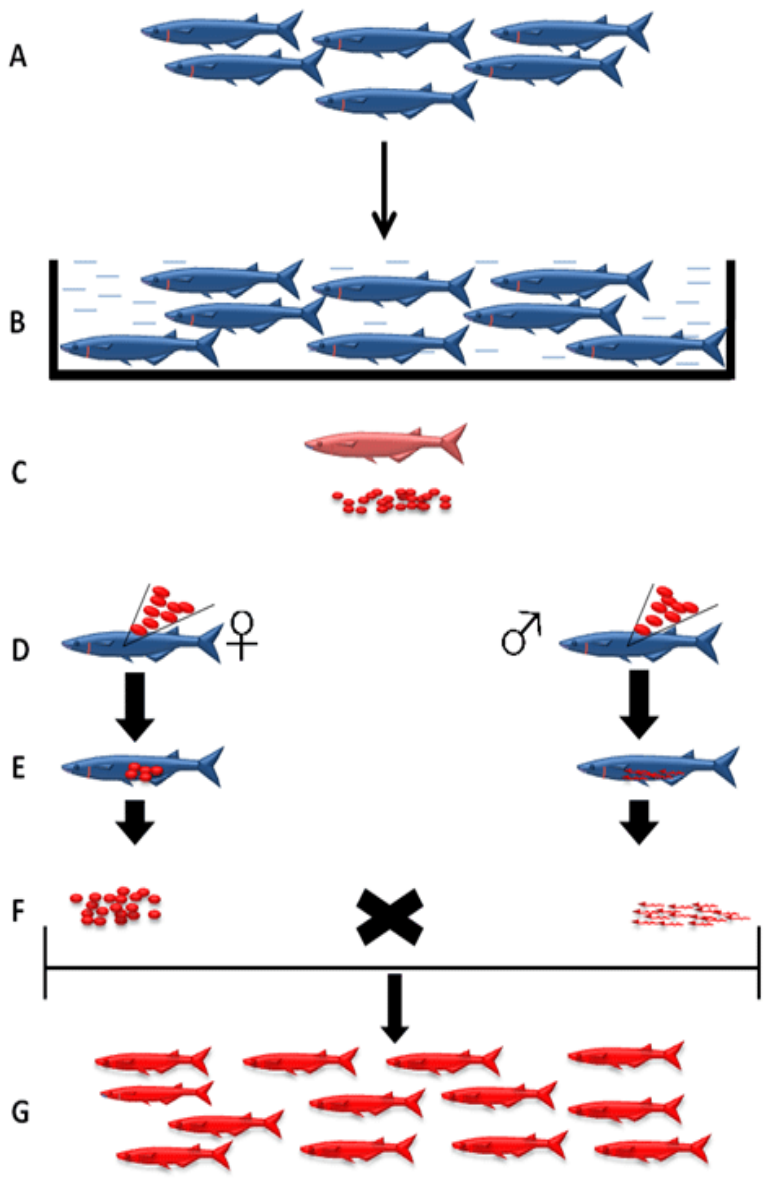

Figure 1: Potential applications of GCT in production of surrogate offspring. Using this technique, commercially important species and/or difficult to bred fish species can be quickly propagated by transplanting GCs from target species into closely related recipient, preferably the one that can be easily bred in captivity. **A)** The recipient fish. **B)** The endogenous germ cell of recipient fish is depleted by heat-chemical treatments. **C)** Germ cells are harvested from the donor fish. **D)** The cells are labeled with fluorescent dye and transplanted into recipient gonad through genital opening. **E)** Months after the procedure, transplanted cells differentiated into functional gametes; **F)** Surrogate parents are artificially inseminated or allowed natural spawning. **G)** Production of donor-origin progeny.

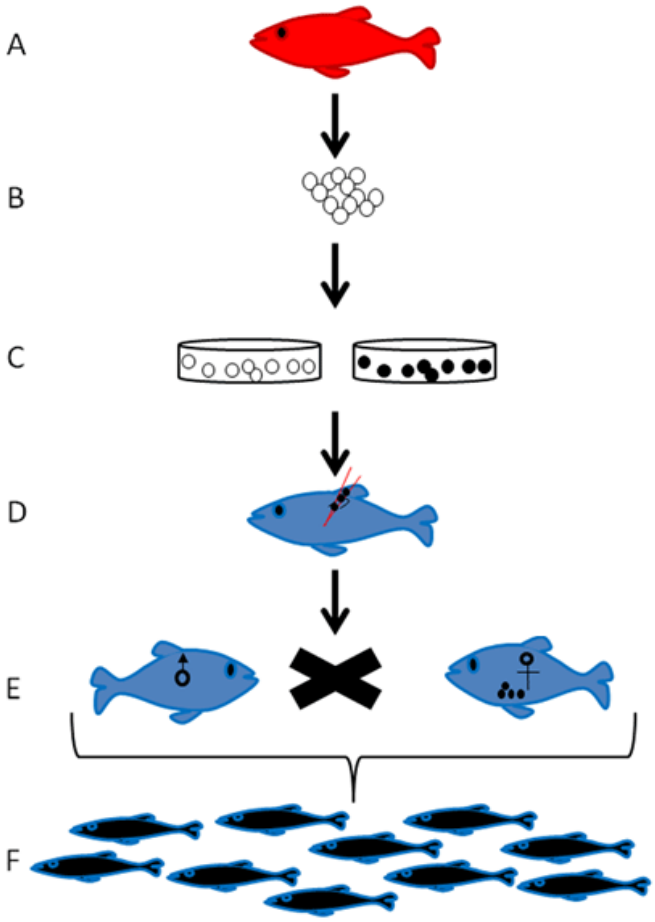

Figure 2: Schematic presentation of germ cell transplantation technique in transgenic fish production. **A)** Selection of a donor fish. **B)** Germ cells are harvested from the gonads of donor fish. **C)** The germ cells are transfected in vitro. **D)** The transfected cells are transplanted into allogeneic recipients those are prior depleted of endogenous germ cells. **E)** After attaining sexual maturity, the surrogate animal are crossed with its pure counterpart. **F)** Mass-scale production of transgenic progeny.

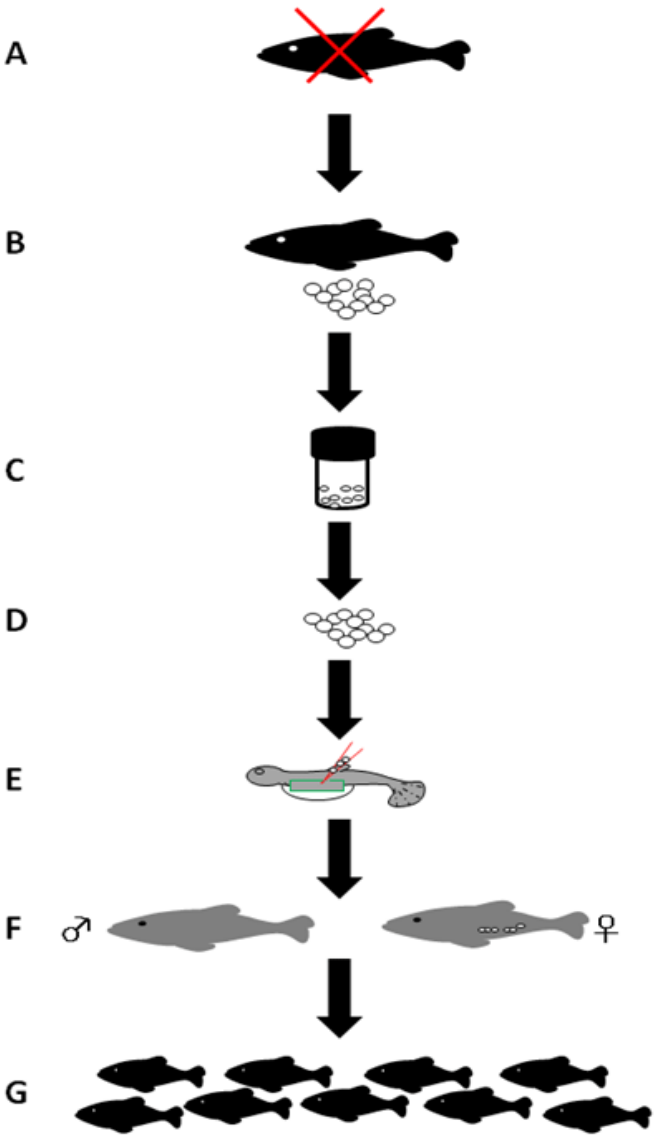

Figure 3: Schematic presentation of revitalizing the fishery of endangered species. The GCs from endangered species transplanted into xenogeneic sexually competent adult recipients can make it possible to regenerate them, even in case of last one survivor. **A)** Selection of an endangered fish. **B)** Germ cells are harvested from the endangered fish species. **C)** The gonadal cells are cyopreserved in liquid nitrogen for posterity use. **D, E)** The stored cells are recovered by thawing and labeled with fluorescent dye and transplanted into recipients. **F)** After attaining sexual maturity, the surrogate parents are induced breed or allow natural spawning. **G**) Generation of donor-derived progeny, thereby reviving the fishery of endangered species.

However, early beginning of this century has given us a much awaited breakthrough, a similar approach that was originally proposed in mammalian model, was developed in fish using Transplantation of Primordial Germ Cells (PGC) carrying GFP (Green Fluorescent Protein) into peritoneal cavity of rainbow trout hatchlings resulted in production of sperm with donor genetic characteristics [10]. Further, using the same methodology, xenogeneic Transplantation between rainbow (*Onchorhynchus mykiss*) and masu (*O.masu*) trout were also successfully performed [11]. Also, it was shown that PGCs can be cryopreserved for prolonged periods of time before Transplantation and still establish spermatogenesis in the recipient testis [12]. However, GCT using embryos and/or hatchlings requires very sophisticated instruments for the GCs isolation and quantum of labor for their Transplantation to the target site. Above all, the transplanted embryos and/hatchlings take considerably long time to reach adulthood and to produce the donor-derived functional gametes, adding considerably to the cost of producing surrogate gametes. Consequently, the hatchery units, which are the end user of the technique might reluctant to adopt the technique for the commercial production of valued fish seeds. In contrast, development of surrogate broodstock development involving Transplantation of spermatogonia Cells derived from target species into a closely related adult fish species, depleted of endogenous germ Cells using suitable ablative strategy and, for which captive breeding technique are well developed, has considerably shorten in production of donor-derived gametes and, make the technique of GCT more simple and viable to be practically feasible for the end users [13-15]. In our previous studies, we examined the feasibility of xenogeneic GCT in adult fish using two congeneric model species of atherinopsid fishes, the pejerrey (*Odontesthes bonariensis*) and the Patagonian pejerrey (*O. hatcheri*) as donor and recipient, respectively [13,15]. We had chosen these two species as experimental model due to the wealth of basic information available on their reproductive physiology, can be easily bred in captivity and availability of several genetic markers to distinguish them [16,17].

In those studies we had reported that the heat-chemical treatment successfully induces germ Cell depletion in Patagonian pejerrey [18], obtention of surrogate sperm following surgical implantation of donor pejerrey germ Cells into recipient Patagonian pejerrey testes [13] and, Transplantation of donor pejerrey germ Cells into recipient Patagonian pejerrey gonads through genital papilla produce surrogate eggs and sperm [15]. The results of those studies have collectively explore in-depth in understanding efficient preparation of sexually competent recipients, process of donor Cells colonization inside the recipients' gonads and functional properties of donor-derived gametogenesis in terms of germline transmission. To my knowledge, those were the first reports on GCT using adult fish as recipients that generated viable offspring by both artificial fertilization and natural spawning, which demonstrates the viability of the technique.

Conclusion

The investigations we reported earlier are believed to have broader implications in the vast fishery resources management. For example, the treatment protocol we developed for recipient preparation might also be handy for preparation of sterilized population with minor modification, which, beside useful for GCT can also be applied in aquaculture for sex control and/or control of nuisance fish species (yet a hypothesis). Further, the protocol we developed for GCT might also help in conservation of endangered and/or extinct fisheries resources and revive the fishery of such species in a considerably short period. Also, the technique can be useful in speedy propagation 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.

References

1. Margules CR and Pressey RL. Systematic conservation planning. Nature. 2000;405:243-253.
2. Hanks J. Conservation strategies for Africa's large mammals. Reprod Fertil Dev. 2001;13:459-468.
3. Holt WV and Pickard AR. Role of reproductive technologies and genetic resource banks in animal conservation. Rev Reprod. 1999;4(3):143-150.
4. Wildt DE and Wemmer C.. Biodiverse Conserv. 1999;8(7):965-976.
5. Pukazhenthi BS, Comizzoli P and Travis A, Wildt DE. Applications of emerging technologies to the study and conservation of threatened and endangered species. Reprod Fertil Dev. 2006;18(1-2):77–90.
6. Brinster RL and Averbock MR. Germline transmission of donor haplotype following spermatogonial transplantation. Proc Natl Acad Sci USA. 1994;91(24):11303-11307.
7. Brinster RL and Zimmermann JW. Spermatogenesis following male germ cell transplantation. Proc Natl Acad Sci USA. 1994;91(24):11298-11302.
8. Vassena R, Eguizabal C, Heindryckx B, Sermon K, Simon C, van Pelt AMM. Et al. Stem cells in reproductive medicine: ready for the patient?. Human Reproduction. 2015;30(9):2014–2021. DOI: 10.1093/humrep/dev181.
9. Brinster CJ, Ryu BY, Avarbock MR, Karagenc L, Brinster R and Orwig KE. Restoration of fertility by germ cell transplantation requires effective recipient preparation. Biol Reprod. 2003;69(2):412-420.
10. Takeuchi Y, Yoshizaki G and Takeuchi T. Generation of live fry from intraperitoneally transplanted primordial germ cells in rainbow trout. Biol Reprod. 2003;69(4):1142-1149.
11. Okutsu T, Shikina S, Kanno M, Takeuchi Y, Yoshizaki G. Production of trout offspring from triploid salmon parents. Science. 2007;317(5844):1517.
12. Okutsu T, Suzuki K, Takeuchi Y, Takeuchi T and Yoshizaki G. Testicular germ cells can colonize sexually undifferentiated embryonic gonad and produce functional eggs in fish. Proc Natl Acad Sci USA. 2006;103(8):2725–2729.

13. Majhi SK, Hattori RS, Yokota M, Watanabe S, Strüssmann CA. Germ cell transplantation using sexually competent fish: an approach for rapid propagation of endangered and valuable germlines. PLOS ONE. 2009;4(7):e6132.
14. Lacerda SMSN, Batlouni SR, Costa GMJ, Segatelli TM, Quirino BR, Queiroz BM. et at. A new and fast technique to generate offspring after germ cells transplantation in adult fish: The nile tilapia (*Oreochromis niloticus*) model. PLOS ONE. 2010;5(5):e10740.
15. Majhi SK, Hattori RS, Rahman SM, Strüssmann CA. Surrogate production of eggs and sperm by intrapapillary transplantation of germ cells in cytoablated adult fish. PLOS ONE. 2014;9(4):e95294.
16. Strüssmann CA, Akaba T, Ijima K, Yamaguchi K, Yoshizaki G, Takashima F. Spontaneous hybridization in the laboratory and genetic markers for the identification of hybrids between two atherinid species, *Odontesthes bonariensis* (Valenciennes 1835) and *Patagonina hatcheri* (Eigenmann 1909). Aquaculture Research. 1997;28(4):291-300.
17. Yoshizaki G, Yamaguchi K, Oota T, Strüssmann C A, Takashima F. Cloning and characterization of pejerrey mitochondrial DNA and its application for RFLP analysis. J Fish Biol. 1997;51(1):193-203.
18. Majhi SK, Hattori RS, Rahman SM, Suzuki T, Strüssmann CA. Experimentally-induced depletion of germ cells in sub-adult Patagonian pejerrey (*Odontesthes hatcheri*). Theriogenology. 2008;71(7):1162–1172.