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Germ Cell Transplantation in Fisheries: A Promising Step Toward Conservation

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Abstract

Surrogate broodstock technology based on germ cell transplantation provides a powerful approach for conservation of aquatic genetic resources and enhancement of aquaculture production at scale. In fish, germline stem cells or primordial germ cells from donor gonads or embryos can be cryopreserved, transplanted into sterilized or germ cell-depleted recipients and then reconstituted as functional gametes of donor origin under controlled conditions. The strategy avoids technical limitations of oocyte cryopreservation, maternal inheritance of mitochondria, and long generation intervals for large-bodied or late-maturing species. Successful intra- and interspecific applications have been reported in commercially valuable taxa like pufferfish and sturgeons and also in Indian major carps where cryopreserved spermatogonia are revived in allogeneic hosts. The integration of surrogate broodstock systems with genome editing and environmentally benign sterilisation tools such as thermal manipulation further creates opportunities for secure cryopreservation, rapid propagation of elite germplasm and safe restoration of endangered populations over coming decades.

Keywords: Germ cell transplantation, Surrogate broodstock, Cryopreservation, Indian major carps, Conservation aquaculture

Introduction

Conservation of genetic resources in fishes has largely focused on sperm cryopreservation. However, fish oocytes, which contain substantial yolk reserves essential for embryogenesis, are difficult to cryopreserve due to their large size and high yolk content. Revival of species using sperm alone is intrinsically incomplete because mitochondrial DNA is transmitted maternally via the oocyte. In contrast, surrogate propagation can regenerate entire species from cryopreserved gonadal tissue or germ cells, restoring both nuclear and mitochondrial genomes, and offering a powerful strategy for safeguarding endangered and commercially important fishes. The manipulation of germline stem cells (GSCs) in fish is a relatively new reproductive biotechnology that takes advantage of the developmental potential of gonadal GSCs to generate donor-derived gametes. Donor GSCs isolated from the gonads and transplanted into recipient larvae home and establish in the genital ridge, then transdifferentiate according to the host gonadal environment: donor spermatogonia in female hosts are able to generate functional oocytes, while donor oogonia in male hosts can generate spermatozoa. Surrogate hosts thus produce gametes made up entirely of donor genetics (Goto and Saito, 2019). Cryopreservation can be combined with surrogacy as male (Franěk et al., 2019a) and female (Franěk et al., 2019b) GSCs can be frozen efficiently and re-established in sterilised recipients (Lee et al., 2013; Yoshizaki and Lee, 2018; Marinović et al., 2019). Recent studies have also shown that GSC-based methods can produce genome-edited donor gametes without inducing lethality or reduced fitness in edited adults (Zhang et al., 2020; Zhang et al., 2021), thus establishing surrogate broodstock technology as a flexible platform for conservation and genetic enhancement in aquaculture.

Methods of Germ Cell Transplantation

Several germ cell transplantation strategies have been developed and tested to generate germline chimeras in fish. In general, these approaches can be divided into those based on experimental embryology and those based on reproductive biology.

Approaches Based on Experimental Embryology

These techniques involve manipulation of the early stages of the embryo before full differentiation and migration of the germ cells.

- **Blastomere transplantation (BT):** At blastula stage blastomeres are aspirated from a donor embryo and injected into a host embryo at the same stage. At this stage the germplasm distribution is not completely outlined or separated yet. The transplanted population of cells usually comprises a mixture of primordial germ cells (PGCs) and somatic blastomeres. Historically, BT resulted in relatively low efficiencies of donor-derived offspring production (approximately 11–31%). More recent refinements using PGC-enriched donor embryos and sterile recipient hosts, however, have greatly improved the efficiency of producing donor-derived progeny.
- **Blastoderm transplantation (BdT):** BdT is a more refined form of blastomere transplantation. Researchers can take a piece of the lower part of the donor blastoderm enriched for PGCs and transplant it directly into a host blastula. Localization of the marginal region of the blastodisc containing PGCs, often identified using vasa

transcripts, this method has proven to be highly efficient and has been successful in achieving mono-sex and donor-only gamete production in conjunction with sterile hybrid recipients.

Approaches Based on Reproductive Biology

Reproductive biology–driven methods, on the other hand, depend on the transplantation of specific germ cell populations such as PGCs, spermatogonia (testicular germ cells), or oogonia (ovarian germ cells) into recipients at subsequent developmental stages (larval or adult) rather than on embryology-based techniques. These methods typically follow a sequence of steps:

- **Cell isolation and visualization:** Germ cells are isolated from donor gonads or embryos. Molecular and imaging tools, including visualization of vasa expression and fluorescent labeling (e.g., green fluorescent protein [GFP]), are employed to identify, track, and enrich pure germ cell populations while minimizing contamination with somatic tissue.
- **Microinjection into post-embryonic hosts:** The isolated germ cells are microinjected into the peritoneal cavity of hatched larvae or directly into the gonads of juvenile or adult fishes instead of blastula-stage embryos.
- **Migration and incorporation:** After transplantation, donor germ cells display a characteristic homing behavior, migrating toward the recipient's genital ridge or developing gonads, colonizing these tissues, and integrating into the recipient germline.
- **Surrogate gametogenesis:** The surrogate host provides the proper microenvironment to support the maturation of the donor germ cells. The host ends up producing functional gametes (eggs or sperm) of only the donor genotype.

The second class of methodologies is especially useful for aquaculture and conservation programs as it allows the use of cryopreserved germ cells from mature or endangered species and their reconstitution in another more manageable surrogate species.

Case Studies of Germ Stem Cell Technology

Global Applications

Commercially Important Species: Tiger puffer (*Takifugu rubripes*)

Seed production of the high-value tiger puffer (*Takifugu rubripes*) has been achieved using the grass puffer (*Takifugu niphobles*) as a surrogate host. Grass puffers are smaller, easier to handle, and more practical to rear under commercial or laboratory conditions. This surrogate system enables efficient, large-scale seed production of a premium aquaculture species using a more tractable host (Hamasaki et al., 2017).

Endangered Species: Large sturgeons

Surrogate parent technologies have been applied in the Czech Republic to regenerate large critically endangered sturgeon species. Germ cells isolated from these large sturgeons were transplanted into the much smaller sturgeon species sterlet (*Acipenser ruthenus*. Large sturgeons take a long time to reach sexual maturity and need large rearing facilities. Thus, using sterlet as a surrogate host circumvents the spatial limitation and accelerates conservation efforts to maintain the genetic diversity of these threatened taxa) (Pšenička *et al*., 2015).

Indian Scenario

Germ cell transplantation (GCT) has been tried on economically important indigenous fish species in India, with a few notable case studies and ongoing research programmes. Indian researchers and their international collaborators have concentrated on Indian major carps (IMCs) - Rohu (*Labeo rohita*), Catla (*Catla catla*) and Mrigal (*Cirrhinus mrigala*), which constitute the basis of aquaculture in South Asia. Due to their large size, long maturation periods and the fact that their eggs cannot be cryopreserved due to their size and yolk content, GCT is being promoted as a revolutionary tool for cryo-banking and surrogate seed production in carp aquaculture.

Rohu-to-Catla Allogeneic Transplantation

In an important study, spermatogonial germ cells were isolated from Rohu (*Labeo rohita*), an important commercial species, and cryopreserved with cryoprotectants such as dimethyl sulfoxide (DMSO). These germ cells were thawed and tested for viability before transplantation into larvae of allogeneic host species, Catla (*Catla catla*) (Patra et al., 2016). The transplanted cells migrated to recipient Catla gonads and successfully colonized and proliferated. The present work has demonstrated that germ cells from elite or selectively bred IMC strains can be cryopreserved in liquid nitrogen and recovered in surrogate carp hosts providing an effective insurance policy for valuable commercial breeding lines.

Recipient Preparation via Thermal Manipulation in Rohu

To generate gametes derived only from the donor, the endogenous germ cells of the surrogate host must be depleted or functionally inactivated. In many studies worldwide, sterility is induced by cytotoxic agents (e.g., Busulfan) or genetic modification. On the other hand, Indian scientists have explored the possibilities of germ cell depletion in Rohu through thermal regimes. They used fluorescent membrane dyes (PKH 26 and PKH 67) to study depletion dynamics and spatial distribution of endogenous germ cells by sequentially raising water temperature up to 36 °C (Majhi and Borah, 2024). This work lays a foundation for the development of environment friendly, chemically free "blank slate" surrogate hosts from the Indian carps through controlled thermal manipulations, thereby improving the biosafety and sustainability of the surrogate-based seed production systems for commercial realization.

Conclusion

Germ cell transplantation and surrogate broodstock technology overcome limitations of oocyte cryopreservation and long generation intervals, enabling cryobanking, efficient seed production, and conservation of endangered and high-value fishes across global and Indian case studies, while eco-friendly sterilization advances support safe, sustainable commercial aquaculture systems worldwide for future genetic resource management.

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