AgriCos
e-Newsletter
ISSN: 2582-7049

AgriCos e-Newsletter
Open Access Multidisciplinary Monthly Online Magazine
Volume: 06 Issue: 02 February 2025 Article No: 02

Revolutionizing Fisheries with Surrogate Broodstock Technology

Panneerselvam Dheeran
ICAR- Central Institute of Fisheries Education, Mumbai

SUMMARY

Surrogate broodstock technology is a reproductive biotechnological approach that uses stem cell transplantation to generate surrogate broodstock to preserve and multiply fish genetic resources. This technology has great potential for stem cell treatment and reproductive biotechnology. To produce donor gametes from the surrogate male, donor spermatogonial stem cells are transplanted into the surrogate's testis. The use of surrogacy in research is defined as the use of one test species in place of another test or target species. Surrogate broodstock technology involves two main steps: isolating and enriching the precursors of gametes, germline stem cells (GSCs), and transplanting GSCs into sterile recipients. The transplantation of a testis or ovary cell solution containing germline stem cells into larvae right after hatching can enable this approach. The recipient larvae do not require transplanting donor-derived germline stem cells into their ovary or testis. Surrogate broodstock technology has wide applications in fisheries resource management and aquaculture.

INTRODUCTION

One practical reproductive biotechnology for preserving and multiplying fish genetic resources is the generation of surrogate broodstock using stem cell transplantation. The method's massive potential for use in stem cell treatment and reproductive biotechnology has drawn much interest. To produce donor gametes from the surrogate male, donor spermatogonial stem cells are transplanted into the surrogate's testis. Eventually, this technology might help conserve species and make it easier to produce important fish commercially.

Surrogate Broodstock

The word "surrogate" comes from the Latin word "surrogatus," which means "a replacement or substitute." In general, surrogacy speaks of "the act of acting as a surrogate mother." Regarding research, surrogacy is defined as "the use of one test species in place of another test or target species." An egg is taken from the intended mother (donor) and fertilized with the sperm of the intended father (donor) in mammalian gestational surrogacy. The fertilized egg, or embryo, is then transferred to a surrogate (receiver) who carries the offsprings to next generation. Live offspring are produced when recipient primordial germ cells (PGCs) are transformed into functional gametes. One technique that can expeditiously prepare surrogate males and females for fish breeding is surrogacy. Adult fish receivers that can host implants and quickly produce surrogate sperm and egg cells from them can be created using thermo-chemical procedures. When immature germ cells from one species are transferred to another, the target species' sperm and eggs can be produced (Yoshizaki *et al.,* 2002). The grafting of testicular fragments into isogeneic fish resulted in donor-derived spermatogenesis in the initial attempts to perform germ cell transplantation (GCT) in fish, which were done on rainbow trout. Smaller host species are more efficient since they require less room and food to maintain. Furthermore, little fish can accelerate maturation (big fish often take longer). Previous studies showed the yellowtail kingfish (*Seriola lalandi*) as a potential host for tuna in the perciform order.

Methods to produce surrogate broodstock

Creating donor-derived gametes in a surrogate fish (recipient individual) involves grafting donor germ cells onto a recipient belonging to a different strain or species. This technique is known as surrogate broodstock technology. The transplantation of a testis or ovary cell solution containing germline stem cells, which will eventually develop into sperm or eggs, respectively, into larvae right after hatching, can enable this approach. Rejection can be prevented even when recipients receive transplants of allogeneic or xenogeneic donor cells since freshly hatched larvae cannot reject foreign substances due to their immature immune systems (Takeuchi *et al*. 2004; Okutsu *et al*. 2007). Furthermore, recipient larvae do not require transplanting donor-derived germline stem cells into their ovary or testis. Following their transplantation via a tiny glass pipette into the intraperitoneal cavity, they move independently to the immature testis and ovaries, where they are incorporated and start the

corresponding processes of spermatogenesis and oogenesis (Okutsu *et al*. 2006). Moreover, the germline stem cells used in transplants don't need to be purified. Only germline stem cells move to the recipient's genital ridges for incorporation when testis or ovary tissues are fragmented by proteinase to prepare the cell solution for transplantation; the remaining cells eventually die in the abdominal cavity (Okutsu *et al*. 2006). Therefore, germline cell transplantation is a straightforward microscopic procedure with a stereomicroscope and a coarse motion micromanipulator.

Steps Involved in Surrogate Broodstock Technology

Surrogate broodstock technology comprises two main steps: a) isolation and enrichment of the precursors of gametes, germline stem cells (GSCs), and b) transplantation of GSCs into sterile recipients.

1) Isolation and enrichment of donor germ cells

Primordial germ cells and gonial cells are the two basic types of GSCs that can be separated and injected into surrogate hosts. While the latter can be separated from sexually differentiated animals, the former can be isolated from embryos. Applying surrogate broodstock technology begins with isolating these GSCs; the techniques for isolating, cultivating, and transplanting these cell types are described below.

1.1) Primordial germ cells (PGCs)

PGCs are determined by maternal germplasm and set aside during the cleavage stage in the primary groupings of aquaculture species, which include teleost fish, Bivalvia, and the majority of Crustacea (Extavour and Akam, 2003; Yamaha *et al*., 2010). During development, the PGCs migrate into the gonadal anlagen, combining with gonadal somatic cells to produce gametes. PGCs are usually labelled (either permanently or temporarily) with reporter proteins and sorted according to the reporter signals to isolate and enrich PGCs from embryos. PGCs have been effectively extracted from transgenic rainbow trout (*Oncorhynchus mykiss*) expressing green fluorescent protein (GFP), which is driven by the vasa gene promoter (vasa is uniquely expressed in animal germ cells) (Takeuchi *et al*., 2004). Reporter labelling has made it possible to examine GSC transplantation in great detail, particularly in salmonid fish, and shows promise for the mass isolation of PGCs. Nonetheless, there are alternate techniques for PGC labelling available for certain species, and the creation of transgenic lines presents difficulties. The most popular method involves injecting chimeric messenger RNA (mRNA) into the cytoplasm of zygote cells, which combines a reporter protein (like GFP) with the 3' untranslated region (3'UTR) of germline-specific genes (like vasa or nanos3). The reporter gene is only produced in PGCs because of the germline-specific regulatory sequence (the construct is destroyed in somatic cells) (Yoshizaki *et al*., 2005; Saito *et al*., 2008). The main limitation to the use of PGCs for surrogate technology in several teleost fish is that each embryo has on average only 13-43 PGCs (based on studies including zebrafish (*Danio rerio*), pearl danio (*Danio albolineatus*), loach (*Misgurnus anguillicaudatus*), goldfish (*Carassius auratus*), medaka (*Oryzias latipes*) and ice goby (*Leucopsarion petersii*) (Saito *et al*., 2006). Although a single transplanted PGCs can generate germline chimera across species, genera and families (Saito *et al*., 2008), the low number of PGCs impedes large-scale surrogate production and genetic manipulation. In this case, PGC development and culture in vitro would be required to make further applications possible. It is still challenging to consistently culture PGCs in most teleost species; hence, additional research in this crucial field is needed. Making PGCs in vitro from an embryonic stem cell (ESC) line is also an option (Robles *et al*., 2017).

1.2) Gonial Cells

Spermatogonia and oogonia, or gonial cells, can also be transferred and settle in the gonads or gonadal ridges of sterile surrogate species. Even though their gonads are sexually distinct, they exhibit sexual bipotency, producing either sperm or eggs based on the recipient surrogate animal's phenotypic sex (Hamasaki *et al*., 2017). The primary benefit of gonial cells over PGCs is their number; sexually developed fish can yield hundreds or even thousands of gonial cells. This abundance offers another benefit for cryopreservation: complete ovaries or testes can be preserved, and recipient fish can create functional gametes from thawed gonial cells. Although PGCs can also be cryopreserved, they require a lengthy procedure, and the low number of cells is a limitation (Robles *et al*., 2017). Although labelling of gonial cells would be helpful for isolation and monitoring of the transplanted germ cells, gonial cells can be isolated without labelling procedure from testes or ovaries using their physiochemical and biochemical properties such as size, density and specific receptors (Xie *et al*., 2020). Gonial cells are a better option for donor germ cells than PGCs because 1) they can be separated in large enough numbers to allow the use

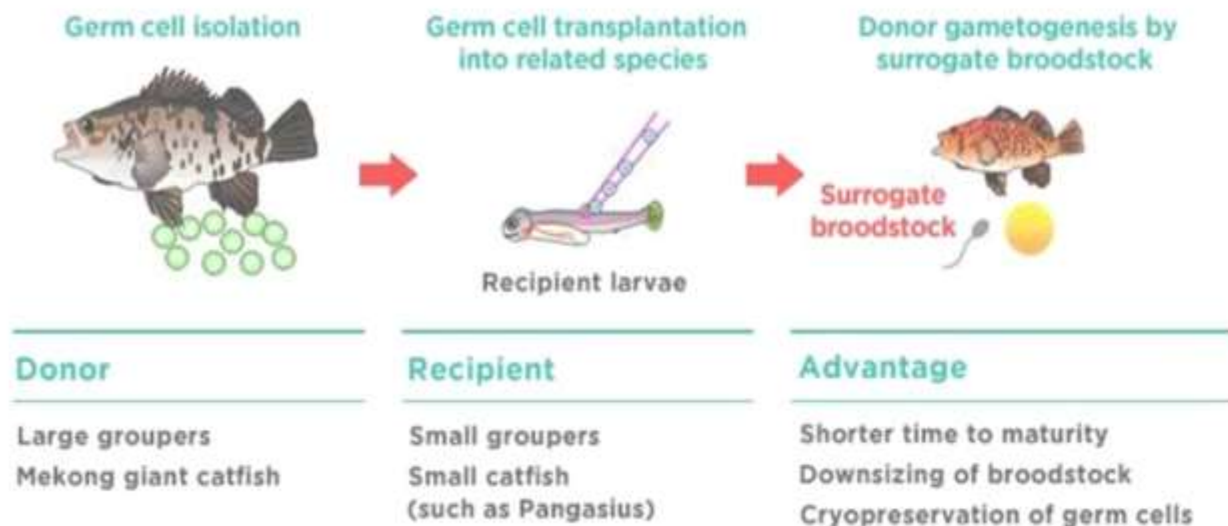
of surrogate broodstock even in the absence of a culturing stage, and 2) their in vitro culture is more manageable, and more optimized than that of PGCs.

2) Transplantation into different life stages of sterile surrogate recipients

Isolated germ cells can be transplanted into the sterilised recipient animals at the different life stages: 1) blastula, 2) hatchlings and 3) adults. During the blastula stage, germ cells can be transplanted by inserting a graft of donor blastoderm containing PGCs between the blastodermal cells of the recipient (Yamaha *et al*., 2003) or injecting donor PGCs into the marginal region of the blastodisc (Saito *et al*., 2008). However, this method requires using PGCs isolated at the early somite stage to achieve migration into the host gonadal ridges (Saito *et al*., 2008), making this approach impractical for large-scale surrogate production due to the limited number of donor PGCs at early stages, and the difficulty in culturing them. At the hatchling stage of the surrogate host, germ cell transplantation is usually carried out. As more numerous gonial cells can be employed for transplantation and are more culturable, transplantation at this stage favours the large-scale creation of gametes from surrogates.
Furthermore, this strategy is less likely to result in immunological rejection of the transplanted germ cells than in adult recipients since newly formed embryos have a comparatively undeveloped immune system (Okutsu *et al*., 2006). However, it can be difficult in animals whose peritoneal cavity is too small for injection, so that blastula stage transplantation may be preferable (Saito *et al*., 2008). In conclusion, the production of donor-derived germ cells can be accelerated by gonial cell transplantation at the adult stage of sterile recipient fish via urogenital papilla injection (Lacerda *et al*., 2013); however, the rate of germline transmission is reduced in comparison to transplantation at the blastula or hatchling stage. For species like zebrafish (Slanchev *et al*., 2005), medaka (Kurokawa *et al*., 2007), three-spined stickleback (*Gasterosteus aculeatus*) (Lewis *et al*., 2008), and Nile tilapia (*Oreochromis niloticus*) (Li *et al*., 2014), where PGC depletion induces masculinization, adult broodstock surrogates can be helpful. This is because it generates recipients of both sexes for producing sperm and oocytes.
Endogenous germ cells of recipient fish must be inhibited or ablated to improve surrogate production of donor-derived gametes, regardless of the surrogate's life stage, as they will outcompete the gametogenesis of donor-derived gametes. Sterilization methods are the knockdown of crucial genes for the formation of germ cells, such as dead-end 1 (*dnd1*), triploidy, and interspecies hybrids. Additionally, cytostatic medications and heat exposure can sterilise adult fish.

Procedure:

Significant loss of germ cells occurs from the therapy that involves continuously raising the animals in water that is 30 degrees Celsius and injecting them with busulfan (40 mg/kg body weight) every two weeks. The testes shrink, and there are noticeably fewer oogonia in the ovarian regions. Male donor fish are killed by overdosing on anaesthesia, and their testes are removed and then cleaned in saline buffered with phosphate. After chopping, the testicular tissue is incubated for two hours at 22°C in a dissociating solution containing 0.5% Trypsin (pH 8.2), 5% Fetal Bovine Serum, and 1 mM Ca2+ in PBS (pH 8.2). The trypan blue (0.4% w/v) exclusion experiment was used to examine the cell viability of the targeted germ cells, which had been identified in initial trials based on cell size measurements.



Application in Fisheries Resource Conservation and Management

Stem cell transplantation holds great potential for managing fisheries resources in open-water environments like lakes and reservoirs. Native fish populations may be declining due to invasive fish species in lakes and reservoirs that can potentially harm the ecology. A classic example is the introduction of African catfish (*Clarias gariepinus*) and Channel catfish (*Ictalurus punctatus*) into Asian water bodies, which have had such an impact that some of the region's valuable native fish species are in danger of going extinct. It is also true that these invasive fish species are very challenging to control in large water bodies; in this case, adopting the invasive fish species as surrogate parents could be a workable solution to stop the population of these fish species from growing farther than necessary. Many fish are endangered on the IUCN Red List due to overfishing in the oceans. Thus, it is urgently needed to develop technology that will support biodiversity conservation and improve, enhance, and restore the declining stocks. This technology ensures the production of transgenic animals. It is a powerful tool for preventing the biodiversity of indigenous ornamental fish. Surrogate broodstock technology is a shortcut to achieving conservation goals.

Application in Aquaculture

Although surrogate broodstock technology is still in its early stages as far as practical uses go, it has enormous potential as a research tool and as a means of accelerating genetic improvement in aquaculture. Specifically, surrogate broodstock can support the use of genetic technologies and resources in aquaculture by (i) enabling genome editing research applications to overcome current limitations, (ii) reducing the effective generation interval in aquaculture breeding programs, (iii) facilitating the dissemination of customized and potentially edited production animals of high genetic merit for farming; (iv) retaining genetic resources of both commercially important and endangered species along with germ cell cryopreservation technology; and (v) producing gametes of difficult-to-raise in captivity in easier-to-breed recipient species.

Constraints

Some constraints of the surrogate broodstock technology include the initial capital investment, the captive environment that induces behavioural abnormalities in the animals, and the problem of hatchery-raised stock’s survival in the wild.

CONCLUSION

In conclusion, surrogate broodstock technology can facilitate the reproduction of high-value aquaculture species that would otherwise be challenging to raise in captivity and contribute to conserving significant aquatic genetic resources. The combination of surrogate broodstock, genome selection, and genome editing can revolutionize aquaculture research and production in the following decades, even if more study is still needed.

REFERENCES

Extavour, C.G. and Akam, M., 2003. Mechanisms of germ cell specification across the metazoans: epigenesis and preformation.

Hamasaki, M., Takeuchi, Y., Yazawa, R., Yoshikawa, S., Kadomura, K., Yamada, T., Miyaki, K., Kikuchi, K. and Yoshizaki, G., 2017. Production of tiger puffer Takifugu rubripes offspring from triploid grass puffer *Takifugu niphobles* parents. *Marine Biotechnology*, *19*, pp.579-591.

Lacerda, S.M.S.N., Costa, G.M.J., Campos-Junior, P.H.A., Segatelli, T.M., Yazawa, R., Takeuchi, Y., Morita, T., Yoshizaki, G. and França, L.R., 2013. Germ cell transplantation as a potential biotechnological approach to fish reproduction. *Fish physiology and biochemistry*, *39*, pp.3-11.

Lewis, Z.R., McClellan, M.C., Postlethwait, J.H., Cresko, W.A. and Kaplan, R.H., 2008. Female-specific increase in primordial germ cells marks sex differentiation in threespine stickleback (*Gasterosteus aculeatus*). *Journal of morphology*, *269*(8), pp.909-921.

Li, M., Yang, H., Zhao, J., Fang, L., Shi, H., Li, M., Sun, Y., Zhang, X., Jiang, D., Zhou, L. and Wang, D., 2014. Efficient and heritable gene targeting in tilapia by CRISPR/Cas9. *Genetics*, *197*(2), pp.591-599.

Okutsu, T., Shikina, S., Kanno, M., Takeuchi, Y. and Yoshizaki, G., 2007. Production of trout offspring from triploid salmon parents. *Science*, *317*(5844), pp.1517-1517.

Okutsu, T., Yano, A., Nagasawa, K., Shikina, S., Kobayashi, T., Takeuchi, Y. and Yoshizaki, G., 2006. Manipulation of fish germ cell: visualization, cryopreservation and transplantation. *Journal of Reproduction and Development*, *52*(6), pp.685-693.

Robles, V., Riesco, M.F., Psenicka, M., Saito, T., Valcarce, D.G., Cabrita, E. and Herraez, P., 2017. Biology of teleost primordial germ cells (PGCs) and spermatogonia: Biotechnological applications. *Aquaculture*, *472*, pp.4-20.

Saito, T., Fujimoto, T., Maegawa, S., Inoue, K., Tanaka, M., Arai, K. and Yamaha, E., 2006. Visualization of primordial germ cells in vivo using GFP-nos1 3'UTR mRNA. *International Journal of Developmental Biology*, *50*(8), p.691.

Saito, T., Goto-Kazeto, R., Arai, K. and Yamaha, E., 2008. Xenogenesis in teleost fish through generation of germ-line chimeras by single primordial germ cell transplantation. *Biology of reproduction*, *78*(1), pp.159-166.

Slanchev, K., Stebler, J., de la Cueva-Méndez, G. and Raz, E., 2005. Development without germ cells: the role of the germ line in zebrafish sex differentiation. *Proceedings of the National Academy of Sciences*, *102*(11), pp.4074-4079.

Takeuchi, Y., Yoshizaki, G. and Takeuchi, T., 2004. Surrogate broodstock produces salmonids. *Nature*, *430*(7000), pp.629-630.

Xie, X., Nóbrega, R. and Pšenička, M., 2020. Spermatogonial stem cells in fish: characterization, isolation, enrichment, and recent advances of in vitro culture systems. *Biomolecules*, *10*(4), p.644.

Yamaha, E., Goto-Kazeto, R., Saito, T., Kawakami, Y., Fujimoto, T., Adachi, S. and Arai, K., 2010. Primordial germ cell in teleost fish with special references to its specification and migration. *Journal of Applied Ichthyology*, *26*(5), pp.816-822.

Yoshizaki, G., Tago, Y., Takeuchi, Y., Sawatari, E., Kobayashi, T. and Takeuchi, T., 2005. Green fluorescent protein labeling of primordial germ cells using a nontransgenic method and its application for germ cell transplantation in salmonidae. *Biology of Reproduction*, *73*(1), pp.88-93.

Yoshizaki, G.O.R.O., Takeuchi, Y.U.T.A.K.A., Sakatani, S.H.I.G.E.K.O. and Takeuchi, T.O.S.H.I.O., 2002. Germ cell-specific expression of green fluorescent protein in transgenic rainbow trout under control of the rainbow trout vasa-like gene promoter. *International Journal of Developmental Biology*, *44*(3), pp.323-326.