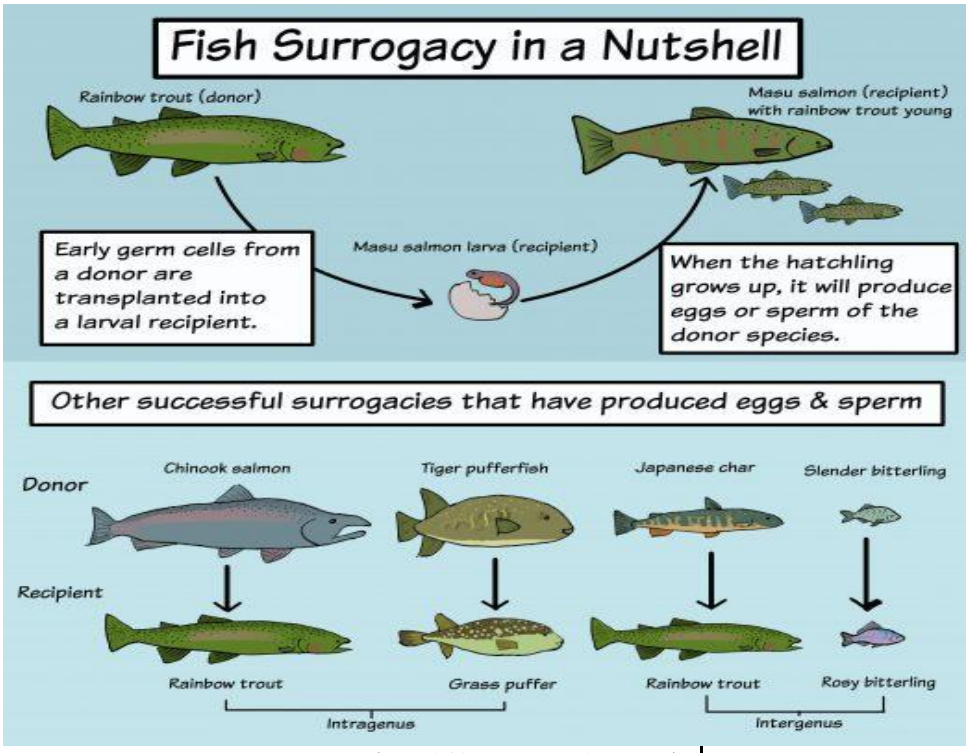
Surrogate Technology and its Applications in Aquaculture

***Gowhar Iqbal [1], Shahid Gul[1], Ubaid Qayoom[1], Showkat Ahmad Dar[2], Nail M. Bhat, Zahoor Mushtaq[1] and Amrita Mohanty[1]**

[1]ICAR - Central Institute of Fisheries Education, Mumbai, MH, India

[2]College of Fisheries Kishanganj Bihar Animal Science University, Patna, Bihar, India

*Corresponding Author: drgowhariqbal@gmail.com



Source (Yoshikawa et al., 2018)

The process of creating donor-derived gametes in a surrogate fish through the transplantation of donor germ cells into a recipient of a different strain or species is known as "surrogate broodstock technology." Utilising germline chimaeras, surrogate propagation is a methodical process for creating donor-derived gametes. In the 1990s, zebrafish (*Danio rerio*) and medaka (*Oryzias latipes*), two model fish species, were employed to research germ cell development in fish. For effective gamete generation in model fish species and endangered species, surrogate propagation has been intensively researched. This method can be made possible by implanting a cell suspension from the testis or ovary into larvae as soon as they hatch. These cell suspensions include germline stem cells, which will eventually develop into sperm or eggs. It is crucial to extend the use of surrogate broodstock technology to more fisheries species and boost the effectiveness of donor-derived gamete production when doing so. Additionally, recipient larvae do not require the transplantation of donor-derived germline stem cells into their ovary or testis. A thin glass pipette is used to implant them into the intraperitoneal cavity, where they move on their own to the immature testis and ovary, where they are incorporated and start the spermatogenetic and oogenesis processes, respectively. The germline stem cells used for transplantation do not need to be purified (Yoshizaki *et al*., 2019).

When testis or ovary tissues are dissociated by proteinase in preparing the cell suspension for transplantation, only germline stem cells migrate to the recipient's genital ridges for incorporation. In contrast, the remaining cells eventually die in the abdominal cavity. Therefore, germline cell transplantation comprises an extremely simple microscopic operation using a stereomicroscope and a coarse motion micromanipulator. In general, marine fish are kept as broodstock in sizable land-

based tanks, and fertilised eggs are collected using an egg collection net placed near the water exit of the tank. However, this approach is problematic for effective breeding techniques, such as selective breeding, which sometimes call for pairing certain parents with desired genetic features.

Some fish species' superior males and females can be artificially created using a maturity induction procedure that involves exogenous hormones. While collecting eggs or sperm, priceless parent fish can be lost because some marine species are extremely sensitive to handling stress. In vitro fertilised eggs typically have a lower survival rate than spontaneously produced eggs. Therefore, one must rely on spontaneous oviposition for fish species in which in vitro fertilization is difficult (Lacerda *et al*., 2013). It is difficult to get fertilised eggs from a few well-chosen individual fish in a tank of group-spawning marine fish (especially if only one male and one female are present). Extreme circumstances may prevent mating between males and females with superior qualities because they do not mature at the same time.

The lengthy generation times of many desirable aquaculture fish species are one of the main challenges to fish breeding. In order to minimise the generation time for breeding species for the aquaculture sector and fish research, mating experiments are essential in selective breeding programmes. It is feasible to significantly reduce the time required, as demonstrated by the example above, which describes the development of bluefin tuna from a little mackerel. We have successfully produced eggs and sperm of tiger puffer more quickly by transplanting germline stem cells of this species into grass puffer *Takifugu niphobles*. Generally, male tiger puffer requires 2 years to mature, whereas female tiger puffer fish require 3 years to mature. Reportedly, both sexes of the grass puffer can mature within a year when the water temperature is controlled and photoperiods. Similarly, it is now possible to produce eggs and sperm of Chinook salmon *Oncorhynchus tshawytscha* (which normally require 3–5 years to mature) in 1 and 2 years, respectively, when using rainbow trout as surrogate broodstock. Cryopreservation has not yet been fully adapted for fish because fish eggs are relatively large and rich in lipids and egg yolk (Yoshikawa et al., 2018).

Germline stem cells are tiny (about 10 m) and do not contain much lipid or egg yolk, making it simple to cryopreserve them in liquid nitrogen. It is well documented to freeze in liquid nitrogen the testis of immature individuals, which contains a significant number of immature spermatogonia, and it is potentially conceivable to keep germline stem cells within the testis in a frozen condition forever. In fact, our research team demonstrated that there was no decrease in the survival rate even after we thawed the rainbow trout testicles five years after they had been frozen in liquid nitrogen (Lee *et al*., 2013)

Additionally, it has been established that cryopreserved cells can create sperm and eggs even after they are thawed and transplanted into recipient fish. Gametes can also develop normally in the gonads of surrogate fish. As a result, cryopreservation is a potent technique for safeguarding priceless genetic resources because it doesn't call for any specialised or expensive equipment and is practical as long as liquid nitrogen and cryo containers are accessible. A combination of cryopreservation and transplanting of germline stem cells may be crucial for protecting the genetic resources of endangered species because there is currently no method for cryopreserving fish eggs.

Cryopreservation is a powerful method for preserving precious genetic resources as this technology requires no special and expensive

equipment and is feasible as long as liquid nitrogen and cryo containers are available. Because a cryopreservation technique for fish eggs is not yet available, a combination of cryopreservation and transplantation of germline stem cells could be extremely important for preserving the genetic resources of endangered species. Cryopreservation is a powerful method for preserving precious genetic resources as this technology requires no special and expensive equipment and is feasible as long as liquid nitrogen and cryo-containers are available. Because a cryopreservation technique for fish eggs is not yet available, a combination of cryopreservation and transplantation of germline stem cells could be extremely important for preserving the genetic resources of endangered species (Hayashi et al., 2014).

Application of surrogate broodstock technology in aquaculture

The following applications of this technology are expected in the field of aquaculture:

- This technology efficiently and reliably produces offspring carrying superior genetic traits by transplanting donor germ cells from a single selected fish with superior traits into many recipient fish.
- Time required to breed fish by using a recipient species with a short generation time to produce gametes of a species with a long generation time.
- The long-term storage of valuable species or strains as genetic resources by cryopreserving germ cells for transplantation.
- The mass production of genetically sterile fish by transplanting germ cells of a donor fish that is sterile due to a mutation in the somatic cells into normal recipients without this mutation.

Combining these techniques is expected to accelerate the breeding of aquaculture species greatly. It is important to adapt surrogate broodstock technology to a wider range of fishery species and further improve the efficiency of donor-derived gamete production when using surrogate broodstock (Yoshizaki et al., 2019).

References

Hayashi M, Sato M, Nagasaka Y, Sadaie S, Kobayashi S, Yoshizaki G (2014) Enrichment of spermatogonial stem cells using side population in teleost. Biol Reprod 91:23

Lacerda SM, Costa GM, Campos-Junior PH, Segatelli TM, Yazawa R, Takeuchi Y, Morita T, Yoshizaki G, França LR (2013) Germ cell transplantation as a potential biotechnological approach to fish reproduction. Fish Physiol Biochem 39:3–11

Lee S, Iwasaki Y, Shikina S, Yoshizaki G (2013) Generation of functional eggs and sperm from cryopreserved whole testes. Proc Natl Acad Sci USA 110:1640–1645

Yoshikawa, H., Ino, Y., Shigenaga, K., Katayama, T., Kuroyanagi, M. and Yoshiura, Y., 2018. Production of tiger puffer *Takifugu rubripes* from cryopreserved testicular germ cells using surrogate broodstock technology. *Aquaculture*, *493*, 302-313.

Yoshizaki, G. and Yazawa, R., 2019. Application of surrogate broodstock technology in aquaculture. *Fisheries science*, *85*, 429-437.

* * * * * * * * * * * * *