

Biotechnology applied to fish reproduction: tools for conservation

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Abstract This review discusses the new biotechnological tools that are arising and promising for conservation and enhancement of fish production, mainly regarding the endangered and the most economically important species. Two main techniques, in particular, are available to avoid extinction of endangered fish species and to improve the production of commercial species. Germ cell transplantation technology includes a number of approaches that have been studied, such as the transplantation of embryo-to-embryo blastomere, embryo-to-embryo differentiated PGC, larvae to larvae and embryo differentiated PGC, transplantation of spermatogonia from adult to larvae or between adults,

and oogonia transplantation. However, the success of germ cell transplantation relies on the prior sterilization of fish, which can be performed at different stages of fish species development by means of several protocols that have been tested in order to achieve the best approach to produce a sterile fish. Among them, fish hybridization and triploidization, germline gene knockdown, hyperthermia, and chemical treatment deserve attention based on important results achieved thus far. This review currently used technologies and knowledge about surrogate technology and fish sterilization, discussing the stronger and the weaker points of each approach.

All the authors contributed equally to this work

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Introduction

Having more than 30,000 identified species, the Fish group is the most diverse group among vertebrates, representing approximately 50% of all known species (Nelson 2016). A major factor contributing to the success of Fish is the great capacity of its members to reproduce in a wide range of habitats with abiotic factors, such as predation pressure and water parameters, that vary considerably. As an example, fish species can reproduce and develop in water temperatures ranging from 40 to 0 °C, including the deepest waters in the ocean (Stein and Drazen 2014) and the highest altitudes, such as the Alpine Bunny Lake in California (Reimers 1964). This adaptability for reproducing in different habitats is due to the ampleness of reproductive strategies adopted by fish species, being the most diverse among vertebrate species (Vazzoler 1996).

However, in spite of the great number of species, natural fish populations are suffering a decrease, as suggested by stagnation of fishing activity since the 1980s. That is at odds with the world fish consumption per capita, which has increased from an average of 9.9 kg in the 1960s to 19.2 kg in 2012 (FAO 2016).

This reduction in natural fish populations might be related, mainly, to the overexploitation of their stocks and the degradation of their habitats by human activity. According to the last update (December 2016) of IUCN Red List of threatened species (IUCN 2016), 16,023 fish species are already extinct, extinct in the wild, critically endangered, endangered, vulnerable, or near threatened, which in total is more than 50% of all known species.

On the other hand, aquaculture production has steadily increased from 40 million tons in 1940s to almost 160 million tons in 2012 (FAO 2016). This reflects the response to the growing demand of fish consumption and accompanies the continuous growth of the world population, which is estimated at 9 billion people in 2050 (Cohen 2003). Therefore, the world demand for fish consumption may increase more and more, and concomitantly, aquaculture production must also increase.

One of the tools to boost aquaculture production and conservation of endangered species is the enforcement

and improvement of biotechnology approaches used in fish reproduction, such as germ cell transplantation and fish sterilization.

The former technique, also known as surrogate technology, comprises the production of a germline chimera via transference of germ cells of a target species to an appropriate host, aiming for the production of heterologous gametes (Okutsu et al. 2007; Saito et al. 2008; Lacerda et al. 2010; Nóbrega et al. 2010; Majhi et al. 2014; Hamasaki et al. 2017; Shang et al. 2018). Lin and colleagues firstly tried this approach in 1992 by transferring embryonic cells from one zebrafish embryo to another (Lin et al. 1992). Since then, this technique has been adjusted and improvements have been made, such as testis transplantation performed between specimens of rainbow trout (*Oncorhynchus mykiss*) (Nagler et al. 2001). In this technique, Nagler et al. (2001) removed the left testis of a donor specimen and immediately transplanted it into a host, whose testis was previously removed. The transplanted testis had increased in mass and was sexually mature, containing sperm. Moreover, the numbers of eyed embryos obtained from transplanted (84%) and intact testes (85%) was very similar after fertilization tests (Nagler et al. 2001). Despite the success, this technique has not been applied in other species, probably due to the great difficulty in management of the individuals and the impossibility in large-scale systems. Nevertheless, it suggested the possibility of storing frozen gonadal fragments for post-thawing isolation and transfer of germ cells into host animals (Lee et al. 2016), which represents an important strategy for conservation of endangered fish populations as we will discuss later.

Nowadays, transplantation can be performed by different methodologies, which include (1) blastomere transplantation from embryo to embryo (Lin et al. 1992), (2) differentiated primordial germ cell (PGC) transferred from embryo to embryo (Saito et al. 2008), (3) differentiated PGC transplanted from larvae to embryo and larvae (Takeuchi et al. 2003), (4) spermatogonial transplantation in larvae (Okutsu et al. 2006), (5) spermatogonial transplantation from adult to adult (Lacerda et al. 2006), and (6) oogonia transplantation (Yoshizaki et al. 2010).

The success of the germ cell transplantation approach, however, depends on the availability of a sterile host because donor cells require a niche within the germinal epithelium to colonize and develop without the competition from host germ cells. So far, there are

three main approaches for sterilization in fish: (1) treatments on eggs (hybridization) (Scheerer and Thorgaard 1983), triploidization (Cal et al. 2006), or germline gene knockdown (Ciruna et al. 2002); (2) treatments on embryos (hyperthermia, irradiation) (Tashiro 1972) or hormonal treatment (Hu et al. 2007); (3) treatment on adults (hyperthermia) (Ito et al. 2008), chemical treatment (Siqueira-Silva et al. 2015), or surgery (McBride et al. 1963).

Germ cell transplantation is not only useful for preparing host fish for transfer of germ cells. Because sterile fish are incapable of breeding with wild species, it may also contribute effectively to bio-containment in aquaculture programs in farms with large-scale commercial production. This is noted in case specimens accidentally escape from tanks, or the out flow of specific strains from inventors occurs. Moreover, infertile fish present an increase in somatic growth, since the energy normally directed to gonadal formation and development, as well as reproduction, is used to somatic increase (Nascimento et al. 2017).

Recent reviews on the field of germ cell transplantation have been provided in literature. The authors usually focus on a specific topic, such as Robles et al. (2016), which emphasized the biology of PGCs and spermatogonia and Golpour et al. (2016) that discussed the challenges of fish sterilization. More recently Majhi and Kumar (2017) also briefly summarized the main techniques used for germ cell transplantation approach. However, since new experiments on the field of germ cell transplantation have been performed all the time in quite different species and innovative methods are being proposed, such as the subcutaneous auto-grafting of immature testes in the rainbow trout, aiming earlier differentiation and production of functional gametes by surrogate broodstock approach (Hayashi et al. 2018) and sterilization of sterlet sturgeon by application of UV irradiation (Saito et al. 2018), this review will discuss the main advantages and disadvantages of such techniques in a new light, while presenting an update on the current status and the future perspectives of the application of these techniques in fish production and conservation.

Germ cell transplantation in fish

Germ cell transplantation in fish involves the production of heterologous gametes by production of germline

chimera (Okutsu et al. 2007). Such a technique, also known as surrogate technology, has emerged by investigations of Lin et al. (1992), who tested whether embryonic cells transplanted from one zebrafish embryo to another could contribute to the germline of the recipient. Four to six weeks after introducing cells from genetically pigmented (donor) embryos to albino recipients at the midblastula stage, pigmentation was observed on the body of 23 of 70 chimeras (Lin et al. 1992).

That successful result opened new perspectives on the study of fish reproduction and new protocols for germ cell transplantation in fish started to be developed and evaluated. This has been demonstrated in several cases, such as the transplantation of PGC cells precursors/differentiated PGC between embryos (Saito et al. 2008) or larvae (Takeuchi et al. 2003), the spermatogonial transplantation from juveniles/adults to embryo (Shang et al. 2018), larvae (Okutsu et al. 2006), or between adults (Lacerda et al. 2006), and also oögonia transplantation: Each one of these procedures presents its own specificity (Fig. 1; Table 1).

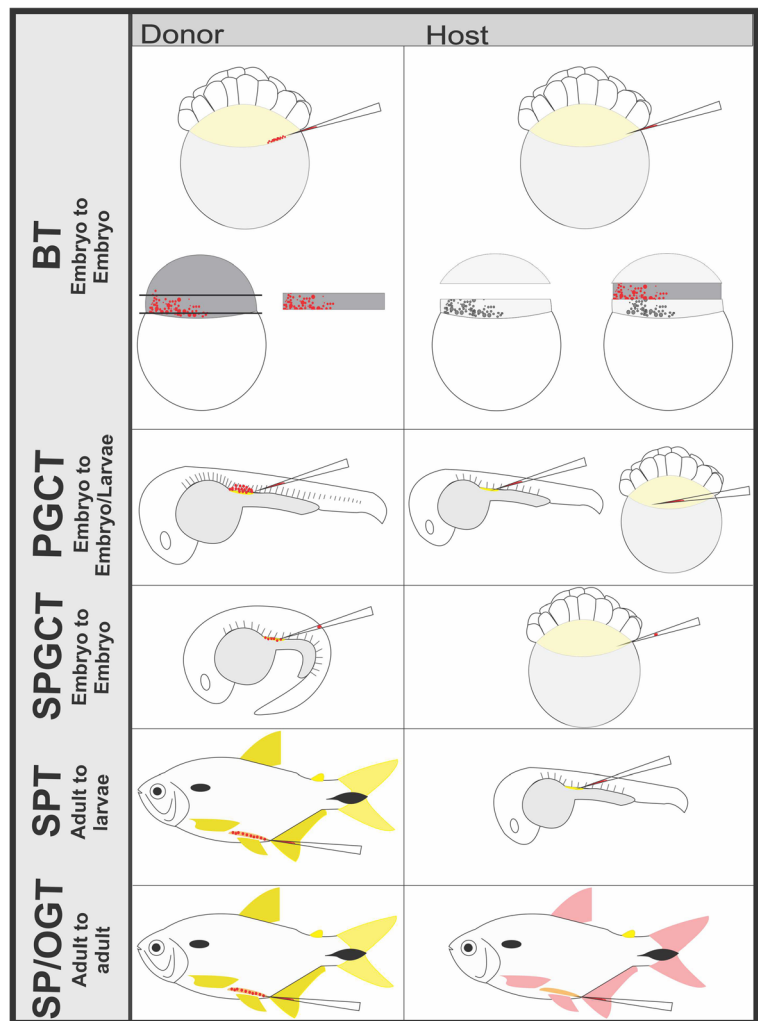
Blastomere transplantation between embryos

In this technique, a number of blastomere cells is aspirated using a Pasteur pipette (Lin et al. 1992) or, more usually, a glass microneedle connected to a microinjector (Saito et al. 2010) from the lower part of the blastoderm of a donor embryo at the midblastula stage. At this stage, blastomere cells still maintain pluripotency and the precursors of primordial germ cells (PGCs) were identified in goldfish *Carassius auratus* (Kazama-Wakabayashi et al. 1999), zebrafish *Danio rerio* (Yoon et al. 1997), medaka *Orizyas latipes* (Shinomiya et al. 2000), the loach *Misgurnus anguillicaudatus* (Okutsu et al. 2006), and rainbow trout *Oncorhynchus mykiss* (Takeuchi et al. 2001).

Donor blastomeres are transplanted into the host embryo blastoderm, and PGC precursors differentiate into PGC cells that are able to migrate into the genital primordium during the embryonic stage, differentiating into functional gametes in the host gonads (Yamaha et al. 2007).

One of the greatest advantages of this technique is the feasibility of the procedure, as it does not require the labeling step of donor germ cells by a fluorescent dye or mRNA before transplantation. Moreover, a large number of cells can be easily aspirated into a micropipette at

Fig. 1 Draft of the main approaches of germ cell transplantation. Red spots represent donor germ cells (*PGC* precursors, *PGCs*, spermatogonia and oogonia)



the blastula stage of the embryo, as there is no intercellular connection at this stage of development (Nakagawa et al. 2002).

However, many disadvantages can be pointed to this technique. Since *PGC* cell precursors comprise a scanty percentage of the pool of aspirated cells and their migration to genital region is low (Ciruna et al. 2002; Saito et al. 2010; Yamaha et al. 2001), germline chimeras are not always achieved. This was demonstrated by Saito et al. (2010) in interspecies blastomere transplantation between four cyprinid species [zebrafish/zebrafish, pearl danio (*Danio albolineatus*)/zebrafish, goldfish/zebrafish, and loach/zebrafish], in which the percentage of chimeras presenting donor *PGCs* at gonadal region were only 7, 4, 7, and 1%, respectively. Thus, few progeny specimens (around 0.24 to 14.6%) carrying

donor genetic features commonly arise from chimera parents (Nakagawa et al. 2002; Takeuchi et al. 2001). Additionally, somatic cells present in the transplantation suspension form aggregations in the host embryos that negatively influencing both *PGC* migration to the gonadal ridge and development of the embryos (Saito et al. 2010).

Since it is quite difficult to achieve a higher rate of germline transmission and, consequently, offspring holding donor-derived characteristics using blastomere transplantation, a new approach was proposed for transplantation between the triploid carp *Carassius auratus langsdorffii* and the diploid goldfish host, in which the donor blastoderm is removed from donor species and sandwiched between the blastoderm of host embryo at the mid- to late-blastula stage (Yamaha et al. 2001). In

Table 1 Approaches used in germ cell transplantation in fish. Main advantages and disadvantages

Approach	Advantages	Disadvantages
Blastomeres transplantation between embryos	<ul style="list-style-type: none"> • Does not require great skills to be performed • Many PGC precursors can be easily sucked into a micropipette at once 	<ul style="list-style-type: none"> • Requires a great supply of donor embryos • Domain of reproduction of both donor and host species • Synchrony of donor and host species reproduction • Months to years until target gamete and, consequently, production of the target offspring • Low rate of PGC migration to host genital region • Low percentage of donor-derived offspring
PGC transplantation from larvae to larvae and embryo	<ul style="list-style-type: none"> • Higher rate of PGC migration to host genital region • More efficient to reach to offspring production 	<ul style="list-style-type: none"> • Requires a great supply of donor embryos • Domain of the reproduction of both donor and host species • Synchrony of donor and host species reproduction time • Months to years to start target gamete and, consequently, offspring production • Sophisticated equipment is needed • Skills needed
Single PGC transplantation between embryos	<ul style="list-style-type: none"> • High efficiency to produce offspring • Do not require a great supply of donor embryo • One cell is enough to resume gametogenesis 	<ul style="list-style-type: none"> • Domain of the reproduction of both donor and host species • Synchrony of donor and host species reproduction time • Months to years to start target gamete and, consequently, offspring production • Sophisticated equipment is needed • Skills needed
Spermatogonial transplantation to embryo and larvae	<ul style="list-style-type: none"> • Many spermatogonia are present at male testes • Do not need to synchronize donor and host reproduction time • Donor cells can be harvested from a wild specimen, reducing necessity of reproduction in laboratory 	<ul style="list-style-type: none"> • Months to years to start target gamete and consequently offspring production • Sophisticated equipment is needed • Skills needed
Spermatogonial transplantation between adults	<ul style="list-style-type: none"> • Do not need sophisticated equipment • Do not need great skills • Donor cells can be harvested from a wild specimen, rejecting the necessity of reproduction in laboratory • Target gametes and offspring can be produced in few weeks or months • Donor germ cells can be injected any time with no possibility of rejection • Do not need to synchronize donor and host reproduction time 	<ul style="list-style-type: none"> • Sterile host is necessary • A considerable number of donor cells is needed • Low efficiency • Loss of maternal genetic material, such as mitochondrial DNA and germplasm
Oogonia transplantation between adults	<ul style="list-style-type: none"> • Do not need sophisticated equipment • Do not need great skills • Donor cells can be harvested from a wild specimen, reducing necessity of reproduction in laboratory • Target gametes and offspring can be produced in few weeks or months • Donor germ cells can be injected any time with no possibility of rejection • Do not need to synchronize donor and host reproduction time • Transference of maternal genetic material to offspring 	<ul style="list-style-type: none"> • Sterile host is necessary • A considerable number of donor cells is needed • Low efficiency

this study, PGC precursors comprised into transplanted blastoderm differentiated into mature oocytes in four females of chimeric fish. Yamaha et al. (2003) also used this approach showing sperm production derived from

chimeric fish between a sterile male hybrid host and fertile goldfish donor.

Since those approaches use embryos as donor and host, their success depends on the domain of the

reproduction techniques of the involved species that must synchronously happen. Furthermore, production of target gametes and the consequent donor-derived offspring takes months or years to arise from chimeras because they must develop and reach sexual maturation to start production of donor-derived-gametes.

Those transplantation approaches are considered the first step for mediated propagation of valued commercial or endangered fish species. However, neither technique is suitable for conservation purposes because a great supply of donor embryos is necessary to produce a large number of chimeras (Saito et al. 2010).

PGC transplantation from larvae to larvae and embryo

In order to overcome those problems, Takeuchi and colleagues (Takeuchi et al. 2003; Takeuchi et al. 2004) proposed the isolation of PGCs labeled by *green fluorescent protein* (GFP) from genital ridges of hatched larvae before their transplantation into the lower part of the blastula blastoderm, the shield of the gastrula embryonic stage, or into the peritoneal cavity of larvae into rainbow trout hosts. The suitability of those three developmental host stages were compared and identified hatched larvae as the best moment to perform transplantation because survival rate of embryos was higher (94%) in comparison to when blastula and gastrula embryos were used as hosts (26 and 62%, respectively) (Takeuchi et al. 2003).

In addition, all GFP-labeled cells in the embryos host were found in ectopic positions, whereas in hatched larvae recipients, genital colonization was observed in 21.6% (16 of 74) of the transplanted individuals. Even though the main objective for the described methodology was the improvement of the genetic donor transfer by exclusive transplantation of PGCs, a few dozen somatic cells were also injected together in the cell suspension. Thus, the non-colonization of host embryos in the genital ridge and the low survival rate might probably be due to the formation of aggregates of donor somatic cell, impairing PGC migration and embryo development (Saito et al. 2010), as already discussed in the previous section on transplantation methodology using embryos as host (Saito et al. 2010).

In host-transplanted-larvae, after migration and colonization, the donor-derived GFP-labeled cells were able to proliferate and differentiate into fully developed

gametes (spermatozoa and oocytes) within the host gonads. Additionally, recipient gametes were fertilized with gametes of the wild-type animal, producing offspring that presented the donor-derived phenotype (Okutsu et al. 2006).

To determine the production of offspring from spermatozoa derived from donor testicular cells, semen from GFP-positive fish was used to fertilize non-transgenic eggs from wild-type females. The milts produced by recipients were extracted by using abdominal pressure and their volumes were measured. In addition, the milt samples were diluted 1000 times with PBS, and the spermatozoa were counted with a hemocytometer. The number of donor-derived spermatozoa was obtained by using the following formula: whole milt volume (ml)/spermatozoa density (number per ml)/germline-transmission rate of donor-derived haplotype (%)/100. To determine whether transplanted testicular germ cells could differentiate into functional gametes, eggs obtained from 2-year-old female recipients were fertilized with spermatozoa from wild-type males (Okutsu et al. 2006).

Despite the great advances provided by this approach to germ cell transplantation technique in fish and mainly for the understanding of PGC biology and physiology (Nikolic et al. 2015; Robles et al. 2016), some problems still prevailed with regard to fish conservation purposes. The technique's performance involves steps that can make it unsuitable to be conducted in many fish species, such as the preparation of a strain containing germline cells labeled by GFP as performed by Linhartová and colleagues in the tench *Tinca tinca* (Linhartova et al. 2014) and Saito and Psenicka in sturgeons (Saito and Psenicka 2015). For these purposes, *in vitro* reproduction of parental fish is necessary, which could be extremely difficult for some species. This is particularly true for most of the endangered and/or threatened ones, whose reproduction is commonly not performed outside of natural conditions. Without this step, PGC isolation from donor genital ridges is almost impossible, since PGC must be identified. Furthermore, the size of some species such as zebrafish can make the excision of the genital ridge difficult.

Moreover, there is an optimal age in which PGC cells must be excised from donor genital ridge because their capacity of migration toward the host' gonadal ridge decreases as the time pass (Takeuchi et al. 2003; Yamaha et al. 2007). The host age also interferes with PGC migration, and the transplantation of cell suspension on host peritoneal cavity must be conducted before

the complete formation of the immunological system to avoid, therefore, their elimination (Takeuchi et al. 2003). Thus, interspecies PGC transplantation between larvae depends on the synchrony of recipient and donor reproduction time. Additionally, the choice of a small fish species as the host can also make the development of the transplantation approach difficult, since their peritoneal cavities can be too small to allow for insertion of the transplant needle (Takeuchi et al. 2003). Lastly, the contribution rate of the donor-derived phenotypic to offspring using this approach was estimated at no more than 2.08 to 4% (Takeuchi et al. 2003, 2004).

PGC transplantation between embryos

To increase the efficiency of germline chimera's formation and to eliminate host development problems caused by donor somatic cells co-transplanted with PGCs, as described previously, Saito et al. (2008) proposed the isolation and transplantation of one single differentiated PGC between embryos of two closely related cyprinid species, the pearl danio *D. albolineatus* and the zebrafish *D. rerio* (Meyer et al. 1993). Furthermore, they extended their study and transplanted single PGCs of goldfish *Carassius auratus* (Cyprinidae family, however from a different genus of zebrafish) and loach *Misgurnus anguillicaudatus* (Cobitidae family) into zebrafish host embryos, in which PGCs development was previously blocked by injection of *dnd* antisense morpholino. They showed that a single donor PGC ensures complete spermatogenesis replacement in host embryos, independent of the barriers imposed by the phylogenetic distance between the used species. No phenotypic females were observed among chimeric species because, as already stated by Slanchev et al. (2005), PGC-ablated fish invariably develop as males. Thus, approximately half of the zebrafish transplanted with pearl danio PGC formed normal sized testes, whereas eight germline chimeras were observed from goldfish and five from loach donors (Saito et al. 2008).

Three females were achieved from males of pearl danio/zebrafish chimeras treated with estradiol (E2) for 1 month, and fertile offspring owning donor DNA were obtained from natural spawning of this chimeras. The same treatment did not work in the other chimera species. However, their sperm proved to be functional and donor derived, fertilizing the oocytes of the

corresponding wild species to produce normal offspring that had the typical donor phenotype (Saito et al. 2008).

Single PGC transplantation between embryos proved to be a suitable methodology to propagate endangered species. Since the number of PGCs at the embryos genital ridge can reach more than 20 cells and seems to be conserved among teleost, as shown in medaka (20–27 PGCs) (Herpin et al. 2007) and sturgeons of *Acipenser* genus (23.5 cells on average) (Saito et al. 2014), this approach enables the use of just one donor individual cell to generate many chimeras and hundreds of offspring.

On the other hand, this technique is very sophisticated, requiring expensive equipment and material, in addition to the need for many steps and handling skills in order to achieve those results. Thus, it becomes impractical to be performed in fish farms and most research laboratories.

Spermatogonial transplantation in larvae and embryo

Okutsu et al. (2006) were pioneers in the research on the feasibility of the spermatogonial cell population in transmitting genetic information to the next generation through differentiation and fertilization. This approach, which was first studied in mice by Brinster's group (Brinster and Zimmermann 1994; Brinster and Avarbock 1994), is based on the self-renewal ability of spermatogonia and the continuous production of spermatozoa throughout the lifetime of male animals (Rooij and Russell 2000; Schulz et al. 2010). Thus, a testicular cell suspension containing spermatogonia was prepared from one 9-month-old transgenic rainbow trout carrying a GFP gene and transplanted into the peritoneal cavity of newly hatched allogeneic rainbow trout larvae (Okutsu et al. 2006). Donor spermatogonia were able to migrate toward the genital ridge, colonize, and resume gametogenesis in 39 and 37% of transplanted male and female, respectively, concurrently with endogenous host germ cells (Okutsu et al. 2006). Later, progeny tests showed that donor-derived germ cells were able to mature and produce functional spermatozoa and oocyte. The average germline transmission rate to offspring was 5.46% when spermatozoa of chimeric fish were used to fertilize wild rainbow trout oocytes and 2.14% when chimeric oocytes were fertilized by wild-type rainbow trout spermatozoa. Additionally, donor-derived offspring were

also obtained in the next reproduction season (Okutsu et al. 2006).

Those results showed that spermatogonial cells, alike PGC's, have the capacity to migrate toward and colonize host genital ridge. Moreover, in addition to being able to resume gametogenesis even in a microenvironment of competition with endogenous host germ cells, they showed a great developmental plasticity for differentiating into both spermatozoa and oocytes, depending on the somatic environment of the host. Finally, since donor-derived offspring were observed in successive seasons of reproduction, stem cell spermatogonia were believed to be capable of self-renewal in host gonads.

After those findings, spermatogonial transplantation in larvae was performed many other times involving species, such as the Nibe Croaker *Nibea mitsukurii* (Takeuchi et al. 2009; Yoshikawa et al. 2016), the yellowtail *Seriola quinqueradiata* (Morita et al. 2012), the Nile tilapia *Oreochromis niloticus* (Farlora et al. 2014), and the zebrafish *D. rerio* (Li et al. 2017), among others. However, since germline transmission rate to offspring was considered low because of niche competition between donor and endogenous germ cells, subsequent studies commonly used sterile hosts, such as Okutsu et al. (2007) that produced trout fry from triploid sterile salmon. Moreover, thinking about fish conservation, spermatogonial transplantations were successfully conducted between two different species, such as the flatfish *Senegalese sole* (donor) and *Scophthalmus maximus* (host) (Pacchiarini et al. 2014), between the sturgeons Siberian sturgeon *Acipenser baerii* (donor) and the starlet sturgeon *Acipenser ruthenus* (host) (Pšenička et al. 2015), between the salmonids the Rainbow trout *Onchorhynchus mykiss* (donor) and the salmon (host) (Yoshizaki et al. 2016), between the tiger puffer *Takifugu rubripes* (donor) and the grass puffer *Takifugu niphobles* (host) (Hamasaki et al. 2017), and between the Chinese *Acipenser sinensis* (donor) and *Acipenser dabryanus* Dabry's (host) sturgeons (Ye et al. 2017).

Spermatogonial transplantation presents some great advantages in relation to the previous methodologies. First, it is not necessary to synchronize reproduction time between donor and host species, since spermatogonia can be easily harvested from juvenile to adult specimens' testes, and therefore, individuals at different age can be used as the donor. Furthermore, since each stem spermatogonia can self-renewing before it becomes committed to spermatozoa formation (Leal

et al. 2009; Schulz et al. 2010), and testes have plenty of spermatogonial stem cells. That way, even one unique individual can be enough to save a target species from extinction. However, spermatogonial transplantation also needs to be performed before the complete formation of the immunological system of host species to avoid donor cell rejection.

Spermatogonial transplantation between adults

Concomitant to the emergence of spermatogonial transplantation in larvae (Okutsu et al. 2006), Lacerda et al. (2006) proposed the spermatogonial transplantation between sexually mature fish. The practical difference from this approach and that of the Okutsu's group is the use of adult individuals not only as donor but also as host. Thus, spermatogonial cell suspension was transplanted directly inside the host testes via urogenital papilla opening. Before transplantation, host spermatogenesis was suppressed using the alkylant agent busulphan associated with warm water (Leal et al. 2009) in order to ensure a niche for the donor spermatogonia to colonize and proliferate inside the germinal epithelium (Lacerda et al. 2010).

Maybe the greatest advantage of this transplantation method was the simplification of all the techniques previously discussed here. One of the reasons is the apparent presence of a functional blood-testis barrier formed by Sertoli cells, which shut off the testes compartment from the vascular system and makes its lumen an immune privileged site (Batlouni et al. 2009). Thus, isolated germ cells could be injected at any moment without rejection problems, which is different from transplantation into peritoneal cavity of larvae.

Another good point is that the testis compartment is easily reached by the simple method of cannulation, which is usually performed by fish farmers, aiming for sex determination in some species or even to evaluate gonadal maturation status in others. This way, germ cell transplantation in fish became also tangible to be performed in fish farms and not only in large research centers like the previous approaches that needed expensive and sophisticated equipment and great development skills. As performed in this laboratory group, this can be achieved using a simple syringe to transplant spermatogonia between the Characiform species the piracanjuba

Brycon orbignyanus and the yellowtail tetra *Astyanax altiparanae* (Fig. 2) (unpublished data).

Additionally, spermatogonial cells used for transplantation can be obtained from wild adult specimens, both eliminating the requirement to rear the donor in captivity and permitting the gametes of the target species to be generated in the same reproductive season in which the spermatogonial cell is transferred to the host. Ultimately, thanks to spermatogonium sex plasticity, both oocytes and sperm can be continuously produced using this procedure (Nóbrega et al. 2010; Majhi et al. 2014).

Since spermatogonial stem cell transplantation into sexually mature hosts has already been showed to be feasible between two different species such the Jundia catfish *Rhamdia quelen* (donor) and the Nile tilapia *O. niloticus* (host) (Silva et al. 2016), it has the potential to be the most efficient strategy for conservation of threatened species and recuperation of those already extinct in the shortest possible time. In conjunction with cryobanking, the cryopreservation of testes in these species can provide an effective backup of target species, since as already shown by Lee et al. (2013) and Yoshizaki and Lee (2018), spermatozoa and oocytes in salmonid fish were recovered after transplantation of frozen stem cell spermatogonia.

However, some problems still need to be addressed, such as the low production of donor-derived gametes that is mainly attributed to the transient status of suppression of host spermatogenesis, as shown in the yellowtail tetra (Siqueira-Silva et al. 2015). Thus, despite the capacity of donor spermatogonia to colonize the host epithelium when spermatogenesis is suppressed, proliferation of endogenous spermatogonial stem cells will take place in some moment, and germ cells arising/originating from mitotic and meiotic divisions will fill the gonad and produce the majority of obtained spermatozoa and oocytes. In addition, instead of production of both donor-derived oocytes and spermatozoa (Lee et al. 2013), genetic information such the germplasm is maternally inherited (Knaut et al. 2000) and, therefore, lost by spermatogonial transplantation.

Oogonia transplantation

Yoshizaki's group (Yoshizaki et al. 2010) were pioneer in the transplantation of ovarian germ cells in rainbow trout. In that study, ovarian cell suspensions prepared

from 6- to 9-month-old *pvasa-Gfp* transgenic trout were allogeneic transplanted into the peritoneal cavity of sterile triploid rainbow trout. Two years later, donor-derived sperm and oocytes obtained from germline chimeric fish were used in progeny tests. Approximately 50% of the offspring presented donor-derived phenotype.

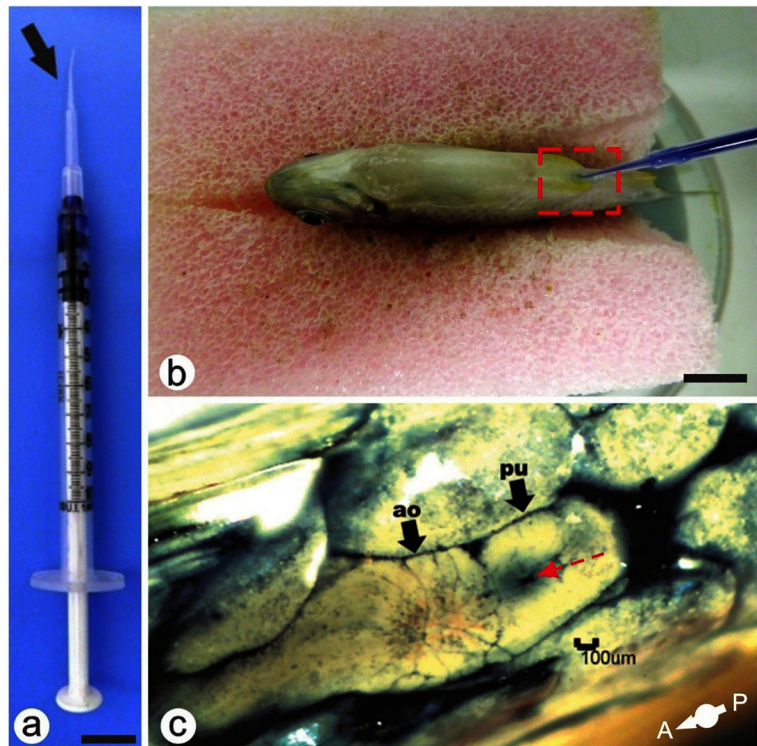
This approach aimed to evaluate the plasticity of ovarian germ cells, since spermatogonial plasticity and gonadal somatic cells role on sex differentiation in fish was already known (Okutsu et al. 2006). However, due to the successful production of donor-derived offspring, this novel technique expanded the versatility of fish germ cell transplantation (Yoshizaki et al. 2010). Its main contribution is the transference of mitochondrial DNA and germplasm constituents, since maternally inherited genetic information is lost in spermatogonial cell transplantation.

Following that experiment, Wong et al. (2011) investigated the presence of stem cells in ovarian cells of zebrafish. Transplantation of the ovarian germ cell suspension was performed into the peritoneal cavity of the sterile hybrid larvae between pearl danio and zebrafish cross. Nine chimeric male fish were produced. However, no chimeric females were observed, which could be the result of the development of a rudimentary ovary in the female hybrid, making the colonization and/or proliferation of transplanted ovarian cells difficult (Wong et al. 2011). On the contrary, Ye et al. (2017) demonstrated that ovarian germ cells harvested from the critically endangered Chinese sturgeons *A. sinensis* were able to colonize the genital ridge in 40% of the transplanted larvae of the closer related Dabry's sturgeon *A. dabryanus*.

Majhi and colleagues (2014) also proved that transplanted oogonia by a non-surgical procedure between the young pejerrey *Odontesthes bonariensis* (donor) and sexually mature Patagonian pejerrey *Odontesthes hatcheri* hosts via the urogenital papilla could produce viable donor-derived oocytes and spermatozoa. Moreover, 39.7 and 52.2% pure *O. bonariensis* offspring was obtained when surrogate spermatozoa and oocyte were used in crosses with wild *O. bonariensis*, respectively.

Even though this technique presents essentially the same advantages and disadvantages of spermatogonial transplantation, transplant of ovarian germ cells suspension might be preferably conducted before donor species reach sexual maturity and start production of

Fig. 2 **a** Hypodermic syringe adapted for germ cells transplantation, **b** SSC transplantation via *Astyanax altiparanae* urogenital papilla. **c** magnification of the region selected in image **b**, showing urogenital opening (red arrow). Urogenital papilla = pu; anus = ao; A = cranial direction; P = caudal direction. **a, b** Scale bars = 1 cm



vitellogenic oocytes, as the lipid content of mature oocytes may impair the isolation of oogonial cells.

Fish sterilization

The successful application of any of the above described surrogate technologies depends on the establishment of a sterile host species to ensure that transplanted germ cells, exclusively, have restrictive maturation. Non-sterile hosts may simultaneously produce endogenous and exogenous gametes (Yamaha et al. 2007). Since the exogenous transplanted cells are generally in reduced number in the host gonads, they may be suppressed by number and by the physiological conditions of maturing endogenous cells.

Thus, many protocols have been developed to achieve the “ideal host” (Golpour et al. 2016), whose gonads present only somatic cell niches. This enables the colonization, proliferation, and maturation of the transplanted donor germ cells, which may increase the colonization success and ensure the production of heterologous gametes. However, since each of those germ cell transplantation techniques presents its own particularity, the host sterilization also requires a specific approach.

In this section, we will address the main protocols used to sterilize fish in order to use them as a tool for conservation of endangered species.

Treatment on eggs

Fish sterilization by treatment on eggs can be performed by two main strategies, one involving chromosome manipulation (hybridization and triploidization) and the other interfering with PGC specification by knocking down germline-specific genes.

Sterilization by hybridization and triploidization

Hybrid and triploid fish are well known to be sterile in several species (Duhan 2004). Yamaha et al. (2003), for example, produced a hybrid from the cross between a female goldfish and male common carp. That hybrid was successfully used as a host, giving rise to a chimeric fish that produced sperm exclusively derived from the donor goldfish and showed that the hybrid was fully sterile. Another successful demonstration of the use of a sterile hybrid fish as host in germ cell transplantation was showed by

Wong et al. (2011) when they transplanted zebrafish ovarian germ cells into host hybrids larvae from the cross between male pearl danio and female zebrafish. F1 embryos produced by chimeric hybrids were confirmed to hold only donor-derived genetic traits. Yoshikawa et al. (2018) demonstrated that sterilization of Scianidae hybrids was caused by mitotic arrest of PGCs. Those cells were specified and migrated toward hybrid embryo gonads; however, they were not able to proliferate. Consequently, transplanted donor germ cells could colonize and differentiate in functional donor-derived gametes.

Similarly, some fully sterile fish were achieved when a protocol of triploidization was used, such as in the large yellow crocker *Pseudosciaena crocea*, whose gonadal development was significantly impaired in both sexes after hydrostatic pressure shock (Xu et al. 2008). Other methods to achieve sterile triploid specimen in fish involves temperature shocks by cold (Piferrer et al. 2003) or heated water (Adamov et al. 2016), and chemical treatment (Johnstone et al. 1989). Despite the differences between methods, all of those techniques aimed to block polar body extrusion in fertilized eggs of fish (Tiwarly et al. 2004).

As already stated here, Okutsu et al. (2007) transplanted spermatogonial stem cells from rainbow trout into sterile triploid masu salmon *Oncorhynchus masou*, which was achieved by heat water shock at 27 °C and allowed for obtaining only gametes from donor species after chimera sexual maturation.

The possibility of treating hundreds of eggs at the same time is one of the great advantages of fish sterilization by hybridization. However, its efficiency is very low because even while being unable to produce sperm and oocytes, endogenous germ cells can develop until pre-meiotic stage, as demonstrated by the massive presence of spermatogonia into the sterile triploid masu salmon gonads (Okutsu et al. 2007). This constraint may be a major limitation for the colonization of donor-transplanted cells, as demonstrated in a study in which only five out of the 50 species ovulated (Okutsu et al. 2007).

In addition, it was already reported that either some hybrids, such as the Cyprinidae *Rutilus alburnoides* (Alves et al. 1999) and triploid male individuals of the yellowtail tetra, could produce gametes (Nascimento et al. 2017). Moreover, “the ideal host” in tetra species was only achieved through the triploidization of the hybrid offspring resulting from the crossing between

Astyanax altiparanae and *Astyanax fasciatus* species. Interestingly, only male achieved sexual maturity.

Since accidental escapes are very common in fish farms, production of a fertile hybrid or triploid species can specially represent an environment problem of genetic introgression for natural populations of fish.

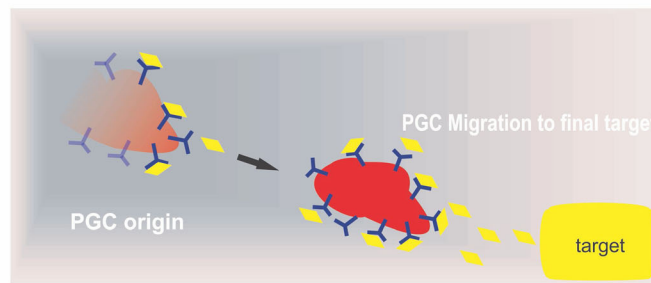
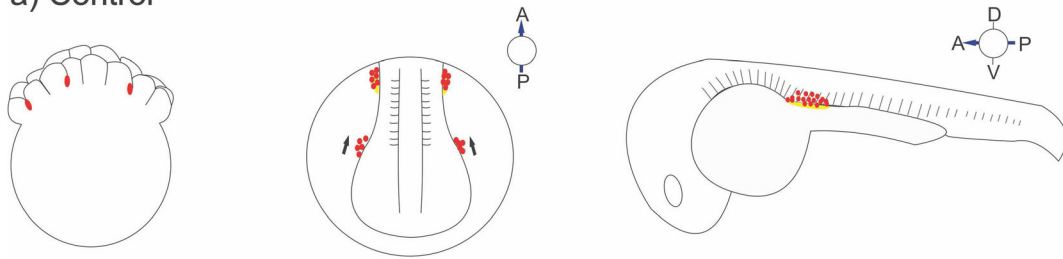
Sterilization by knockdown of specific genes

This approach is based on the biology of PGC cells, which originates from a region different from genital ridge to where they migrate to differentiate in both male and female gametes. Thus, PGCs migration toward the genital ridge can be blocked by suppression of the genes in charge. One of these genes, encoding a RNA-binding protein crucial for migration and survival of PGCs—the Dead end (*dnd*) gene—is effectively knocked down by injection of the chemically modified Morpholino oligonucleotide antisense (MO) into the eggs, as tested in the sterlet sturgeon *Acispenser ruthenus* (Linhartová et al. 2015). Many other fish were also proven to be fully sterile when *dnd* gene is suppressed by MO, such as zebrafish (Ciruna et al. 2002; Li et al. 2017) and rainbow trout *Oncorhynchus mykiss* (Yoshizaki et al. 2016). Although effective, as shown by Saito and colleagues that used zebrafish morphants as host (Saito et al. 2008), this technique cannot be applied at large-scale because the embryos must be individually injected, as well as resulting in higher percentage of males. Therefore, despite its efficiency, this method still needs to be improved to be used in conservation programs of endangered species.

Treatment on embryos

Novel approaches for fish sterilization has been tested by this laboratory group. One using the treatment of embryos by their incubation in chemicals that are known as antagonistic of the chemoattractive SDF-1a/CXCR system, responsible by PGCs direction toward genital ridge. Strong evidences suggest that PGCs migration toward genital ridge in fish embryo is controlled by a chemoattractive system, in which SDF1a (stromal derived factor) molecules and its receptors, the transmembrane proteins CXCR4 and CXCR7, play an essential role. The migration path of PGCs is strictly correlated with the dynamics of the patterns whose mRNA is expressed by the chemokine SDF-1a (Boldajipour et al.

a) Control



b) Chemical treatment

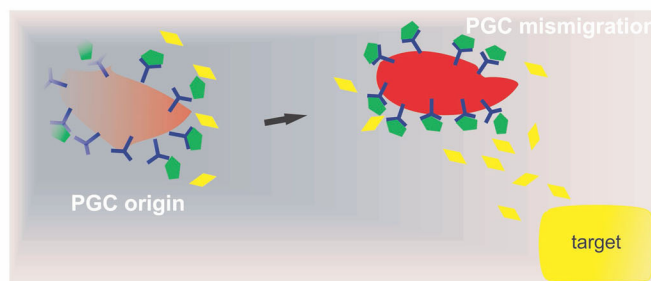
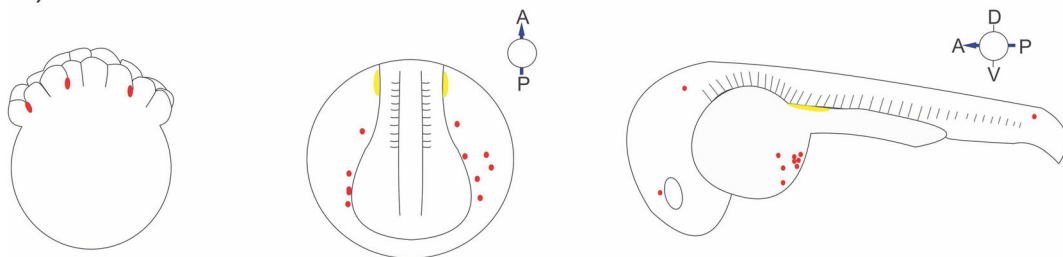


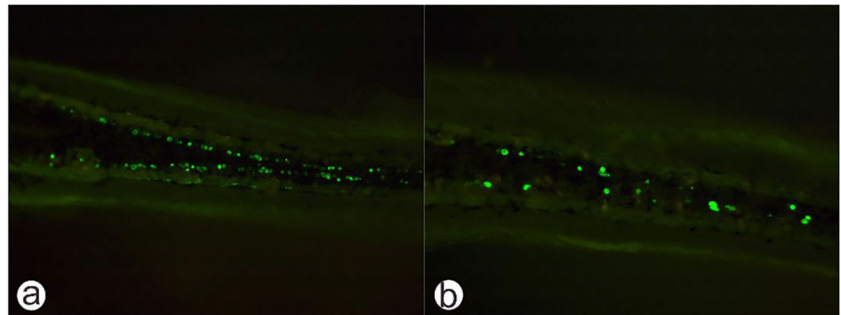
Fig. 3 Draft highlighting SDF-1a/CXCR4 system that controls PGC migration. **a** PGCs correct migration toward genital ridge. **b** “mis” migration caused by embryo submersion in chemical treatment antagonist to SDF-1a/CXCR4 system

2008). Doitsidou et al. (2002) have showed that by knocking down the activity of the receptor CXCR4 and its binder SDF-1a, one can trigger severe migration defects of zebrafish PGCs, highlighting that their

targeting depends on activation on their receptor system instead of the independence of PGCs movement (Fig. 3).

Thus, in a pilot experiment using sterlet sturgeon *A. ruthenus*, the migration of many PGC-like-cells into

Fig. 4 Genital ridge of sterlet sturgeon *A. ruthenus* embryo. **a** Control. **b** Embryo treated with chemicals antagonist to SDF-1 α /CXCR4 system



genital ridge was impaired. Nonetheless, some of these cells followed the corrected path and were observed in the gonadal anlage (Fig. 4). As one unique PGC is able to resume the total gametogenesis of a species (Saito et al. 2008), this technique needs to be improved to impair any PGC of reaching the genital ridge.

Another method involves the UV irradiation of embryos at 1 to 4 cells stage (Saito et al. 2018). As already known PGCs are specified by the inheritance of maternally supplied germplasm, which in embryos of sturgeons is localized at the peripheral layer of the vegetal pole (Saito et al. 2014). Thus, application of UV irradiation to the vegetal pole could disrupt the germplasm and, consequently, affect PGC formation, producing that way sterile specimens.

Most of the embryos irradiated at 1-cell stage were totally devoid of PGCs and their hatching rate was similar to that of control animals. However, when the embryos at 2- to 4-cells stage were irradiating, malformations were observed and they did not hatch. Therefore, 1-cell stage embryos seem to be the optimum stage for sterilizing starlet sturgeon by this methodology.

Although the successful sterilization of many embryos, the UV irradiation did not ensure total sterilization of all animals, since some of them presented few PGCs in the genital anlage at neurula stage embryos. This effect is probably due to physiological and morphological differences among embryos and/or because of the deficiency in uniformly apply UV irradiation to all embryos. Thus, new experiments must be conducted in order to overcome this problem and sterilize all embryos at once. These approaches have several advantages, such as the possibility of mass sterilization and small space to be executed, since fish embryo are used and does not present risks to the environment. However, UV irradiation cannot be used to sterilize all fish species, since in some of them, such as the zebrafish (Yoon et al. 1997; Olsen et al. 1997), PGCs are specified in the cleavage

planes of blastomere cells and UV irradiation in this region will certainly harm the animals.

Treatments on adults

Achieving fully sterilization in sexually mature fish is a huge problem yet to be solved because, by then, the germ cell lineage is completely established in their gonads, and total elimination of stem germ cells seems to be very improbable with the existent protocols (Siqueira-Silva et al. 2015). However, efforts must be focused in this sense, since getting sterile mature fish could be one, if not the best, strategy to be used in conservation programs. This is because transplanted donor germ cells can resume gametogenesis and produce donor-derived gametes few weeks after transplantation into sexually mature hosts (Lacerda et al. 2013).

Nonetheless, the protocols used until now—the hyperthermia (Ito et al. 2008; Pandit et al. 2015), which seems to cause apoptosis of germ cells by heat stress (Strüssmann et al. 1998; Ito et al. 2008) or the hyperthermia, associated with chemical treatment by alkylating drugs like busulphan (Bishop and Wassom 1986; Lacerda et al. 2010; Majhi et al. 2014), only assured a transient sterilization. After a prolonged stress caused by warm water or by chemical germ cell ablation, the stem cell spermatogonia mitotically proliferate many times to regenerate the damaged gonad and start a new meiotic cycle (Siqueira-Silva et al. 2015). Moreover, since germ cell apoptosis occurs preferentially during meiosis (Pacchiarini et al. 2014) and busulphan agent alkylates nitrogenized bases in division (Majhi et al. 2009), stem germ cells that are in a stage of low metabolism may be little affected (Schulz et al. 2010).

The studies in which sexually mature male were used as a host stated a low rate of donor genetic transmission

to offspring (Majhi et al. 2009; Lacerda et al. 2010; Majhi et al. 2014).

Perspectives

Biotechnology studies on germ cell transplantation in fish have been increasing at a steady rhythm since it started by Lin and colleagues in 1992 (Takeuchi et al. 2003). Proof of this is the new method proposed by (Yoshizaki and Lee 2018) to produce sperm by grafting immature fragment of testes into the subcutaneous space along the back of sexually mature fish. Auto-grafted testicular tissue underwent spermatogenesis and produced normal sperm and convenient spermatozoa were able to fertilize oocytes in vitro. However, testicular fragments were rejected at between 6 and 9 weeks after grafting in allogenic specimens. According to the authors, this method provides some advantages in relation to previous germ cell transplantation approaches, such the monitoring of spermatogenesis begins and sperm production just by external appearance of the host fish. Moreover, using of grafted testis will ensure isolation of 100% of donor sperm, which is an advantage in comparison to some methods whose endogenous gametes is still predominantly produced. Furthermore, this method does not require sterile hostess.

However, in spite of its advances, many things still need to be improved to make this technology practical to be used as a reliable tool in fish conservation programs. The scientific aspect that most of the approaches present remains one of its greatest hindrances, making their application in common fish farms difficult. Therefore, new strategies ought to be developed to make that important tool more handily and low cost, as well as more accessible to anyone who desired to use it to preserve fish species from extinction. Moreover, fish sterilization in a large and safe scales must be chased, since this step is the necklace to a prosper advance in the production of 100% donor-derived offspring.

Considering the increasing human population growth around the world, the enhancement of those techniques can be more than a tool used to preserve genetic resources in fish. It is useful to improve production of food from commercially important species by reduction of their generation cycle, as well as reduce the space used for keeping the specimens and investment on their somatic growth instead of reproduction, while also helping to reduce hunger in the world.

References

- Adamov NSM, Nascimento NF, Maciel ECS, Pereira-Santos M, Senhorini JA, Calado LL et al (2016) Triploid induction in the yellowtail tetra, *Astyanax altiparanae*, using temperature shock: tools for conservation and aquaculture. *J World Aquac Soc* 48:741–750. <https://doi.org/10.1111/jwas.12390>
- Alves MJ, Coelho MM, Prospero MI, Collares-Pereira MJ (1999) Production of fertile unreduced sperm by hybrid males of the *Rutilus alburnoides* complex (Teleostei, Cyprinidae): an alternative route to genome tetraploidization in unisexuals. *Genetics* 151:277–283
- Batlouni FSR, Nóbrega RH, França LR (2009) Cell junctions in fish seminiferous epithelium. *Fish Physiol Biochem* 35:207–217
- Bishop JB, Wassom JS (1986) Toxicological review of busulfan (Myleran). *Mutat Res Genet Toxicol* 168:15–45
- Boldajipour B, Mahabaleshwar H, Kardash E, Reichman-Fried M, Blaser H, Minina S, Wilson D, Xu Q, Raz E (2008) Control of chemokine-guided cell migration by ligand sequestration. *Cell* 132:463–473
- Brinster RL, Avarbock MR (1994) Germline transmission of donor haplotype following spermatogonial transplantation. *Proc Natl Acad Sci U S A* 91:11303–11307
- Brinster RL, Zimmermann JW (1994) Spermatogenesis following male germ-cell transplantation. *Proc Natl Acad Sci U S A* 91:11298–11302
- Cal RM, Vidal S, Gómez C, Álvarez-Blázquez B, Martínez P, Piferrer F (2006) Growth and gonadal development in diploid and triploid turbot (*Scophthalmus maximus*). *Aquaculture* 251:99–108
- Ciruna B, Weidinger G, Knaut H, Thisse B, Thisse C, Raz E et al (2002) Production of maternal-zygotic mutant zebrafish by germ-line replacement. *Proc Natl Acad Sci U S A* 99:14919–14924
- Cohen JE (2003) Population: the next half century. *Science* 302:1172–1175
- Doitsidou M, Reichman-Fried M, Stebler J, Köprunner M, Dörries J, Meyer D, Esguerra CV, Leung T, Raz E (2002) Guidance of primordial germ cell migration by the chemokine SDF-1. *Cell* 111:647–659
- Duhan RA (2004) Aquaculture and fisheries biotechnology: genetic approaches. CABI, Alabama
- FAO (2016) World Review of Fisheries and Aquaculture: the State of World Fisheries and Aquaculture (SOFIA). Rome. <https://doi.org/92-5-105177-1>
- Farlora R, Hattori-Ihara S, Takeuchi Y, Hayashi M, Octavera A, Alimuddin, Yoshizaki G (2014) Intraperitoneal germ cell transplantation in the Nile tilapia *Oreochromis niloticus*. *Mar Biotechnol* 16:309–320
- Golpour AM, Siddique AM, Siqueira-Silva DH, Pšenička M (2016) Induced sterility in fish and its potential and challenges for aquaculture and germ cell transplantation technology: a review. *Biologia* 71(8). <https://doi.org/10.1515/biolog-2016-0118>
- Hamasaki M, Takeuchi Y, Yazawa R, Yoshikawa S, Kadomura K, Yamada T, Miyaki K, Kikuchi K, Yoshizaki G (2017) Production of Tiger puffer Takifugu Rubripes offspring from triploid grass puffer Takifugu Niphobles parents. *Mar*

- Biotechnol 19(6):579–591. <https://doi.org/10.1007/s10126-017-9777-1>
- Hayashi M, Sakuma D, Yoshizaki G (2018) Production of functional sperm by subcutaneous auto-grafting of immature testes in rainbow trout. *Mol Reprod Dev* 85:155–162. <https://doi.org/10.1002/mrd.22949>
- Herpin A, Rohr S, Riedel D, Kluever N, Raz E, Scharlt M (2007) Specification of primordial germ cells in medaka (*Oryzias latipes*). *BMC Dev Biol*:1–10
- Hu W, Li S, Tang B, Wang Y, Lin H, Liu X, Zou J, Zhu Z (2007) Antisense for gonadotropin-releasing hormone reduces gonadotropin synthesis and gonadal development in transgenic common carp (*Cyprinus carpio*). *Aquaculture* 271:498–506
- Ito LS, Takahashi C, Yamashita M, Strüssmann CA (2008) Warm water induces apoptosis, gonadal degeneration, and germ cell loss in subadult pejerrey *Odontesthes bonariensis* (Pisces, Atheriniformes). *Physiol Biochem Zool* 81(6):762–774
- IUCN (2016) The IUCN red list of Threatened Species. <http://www.iucnredlist.org>. Accessed 31 Jan 2017
- Johnstone R, Knott RM, Macdonald AG, Walsingham MV (1989) Triploid induction in recently fertilized *Atlantic salmon* ova using anaesthetics. *Aquaculture* 78:229–236
- Kazama-Wakabayashi M, Yamaha E, Yamazaki F (1999) The elimination and duplication of lower part of blastoderm effects on the number of primordial germ cells in goldfish. *Fish Sci* 65:577–582
- Knaut H, Pelegri F, Bohmann K, Schwarz H, Nüsslein-Volhard C (2000) Zebrafish vasa RNA but not its protein is a component of the germ plasm and segregates asymmetrically before germline specification. *J Cell Biol* 149:875–888
- Lacerda SMSN, Batlouni SR, Silva S, Homem C, França L (2006) Germ cells transplantation in fish: the Nile-tilapia model. *Anim Reprod* 3:146–159
- Lacerda SMSN, Batlouni SR, Costa GMJ, Segatelli TM, Quirino BR, Queiroz BM et al (2010) A new and fast technique to generate offspring after germ cells transplantation in adult fish: the Nile tilapia (*Oreochromis niloticus*) model. *PLoS One* 5:1–9
- Lacerda SMSN, Costa GMJ, Campos-Junior PHA, 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
- Leal MC, Cardoso ER, Nobrega RH, Batlouni SR, Bogerd J, Franca LR et al (2009) Histological and stereological evaluation of zebrafish (*Danio rerio*) spermatogenesis with an emphasis on spermatogonial generations. *Biol Reprod* 81:177–187
- Lee S, Iwasaki Y, Shikina S, Yoshizaki G (2013) Generation of functional eggs and sperm from cryopreserved whole testes. *Proc Natl Acad Sci U S A* 110:1640–1645
- Lee S, Iwasaki Y, Yoshizaki G (2016) Long-term (5 years) cryopreserved spermatogonia have high capacity to generate functional gametes via interspecies transplantation in salmonids. *Cryobiology* 73:5–9. <https://doi.org/10.1016/j.cryobiol.2016.08.001>
- Li Q, Fujii W, Naito K, Yoshizaki G (2017) Application of *Dead End*-knockout zebrafish as recipients of germ cell transplantation. *Mol Reprod Dev.* (4):1–38. doi:<https://doi.org/10.1002/mrd.22870>
- Lin S, Long W, Chen J, Hopkins N (1992) Production of germ-line chimeras in zebrafish by cell transplants from genetically pigmented to albino embryos. *Proc Natl Acad Sci U S A* 89:4519–4523
- Linhartova Z, Saito T, Psenicka M (2014) Embryogenesis, visualization and migration of primordial germ cells in Tench (*Tinca tinca*). *J Appl Ichthyol* 30(6):29–39. <https://doi.org/10.1111/jai.12429>
- Linhartová Z, Saito T, Kašpar V, Rodina M, Prášková E, Hagihara S, Pšenička M (2015) Sterilization of sterlet *Acipenser ruthenus* by using knockdown agent, antisense morpholino oligonucleotide, against dead end gene. *Theriogenology* 84:1246–1255
- Majhi SK, Kumar S (2017) Germ cell transplantation: a potential tool for propagation of endangered fishes. *Annals Aqua Res* 4:4–7 <https://www.jsimedcentral.com/Aquaculture/aquaculture-4-1040>
- Majhi SK, Hattori RS, Yokota M, Watanabe S, Strüssmann CA (2009) Germ cell transplantation using sexually competent fish: an approach for rapid propagation of endangered and valuable germlines. *PLoS One* 4:1–8
- Majhi SK, Hattori RS, Rahman SM, Strussmann CA (2014) Surrogate production of eggs and sperm by Intrapapillary transplantation of germ cells in Cytoablated adult fish. *PLoS One* 9(4):e95294. <https://doi.org/10.1371/journal.pone.0095294>
- McBride J, Fagerlund U, Smith M, Tomlinson N (1963) Resumption of feeding by and survival of adult sockeye salmon (*Oncorhynchus nerka*) following advanced gonad development. *J Fish Board Canada* 20:95–100
- Meyer A, Biermann CH, Orti G (1993) The phylogenetic position of the zebrafish (*Danio rerio*), a model system in developmental biology: an invitation to the comparative method. *Proc Biol Sci* 252:231–236
- Morita T, Kumakura N, Morishima K, Mitsuboshi T, Ishida M, Hara T et al (2012) Production of donor-derived offspring by allogeneic transplantation of spermatogonia in the yellowtail (*Seriola quinqueradiata*). *Biol Reprod* 86:176
- Nagler JJ, Cloud JG, Wheeler PA, Thorgaard GH (2001) Testis transplantation in male rainbow trout (*Oncorhynchus mykiss*). *Biol Reprod* 64:644–646
- Nakagawa M, Kobayashi T, Ueno K (2002) Production of germline chimera in loach (*Misgurnus anguillicaudatus*) and proposal of new method for preservation of endangered fish species. *J Exp Zool* 293:624–631
- Nascimento NF, Pereira-Santos M, Piva LH, Manzini B, Fujimoto T, Senhorini JA et al (2017) Growth, fatty acid composition, and reproductive parameters of diploid and triploid yellowtail tetra *Astyanax altiparanae*. *Aquaculture* 471:163–171
- Nelson JS (2016) Fishes of the world [Internet]. *Bulletin of Marine Science*. Vol. 3rd edn, 601 p. Available from: http://www.worldcat.org/title/fishes-of-the-world/oclc/28965588&referer=brief_results
- Nikolic A, Volarevic V, Armstrong L, Lako M, Stojkovic M (2015) Primordial germ cells : current knowledge and perspectives. *Stem Cells Int* (2016)
- Nóbrega RH, Greebe CD, Van de Kant H, Bogerd J, de França LR, Schulz RW (2010) Spermatogonial stem cell niche and spermatogonial stem cell transplantation in zebrafish. *PLoS One* 5(9):1–16

- Okutsu T, Suzuki K, Takeuchi Y, Takeuchi T, Yoshizaki G (2006) Testicular germ cells can colonize sexually undifferentiated embryonic gonad and produce functional eggs in fish. *Proc Natl Acad Sci U S A* 103(8):2725–2729
- Okutsu T, Shikina S, Kanno M, Takeuchi Y, Yoshizaki G (2007) Production of trout offspring from triploid Salmon parents. *Science* 317(5844):1517
- Olsen LC, Aasland R, Fjose AA (1997) *vasa*-like gene in zebrafish identifies putative primordial germ cells. *Mech Dev* 66:95–105
- Pacchiarini T, Sarasquete C, Cabrita E (2014) Development of interspecies testicular germ-cell transplantation in flatfish. *Reprod Fertil Dev* 26(5):690
- Pandit NP, Bhandari RK, Kobayashi Y, Nakamura M (2015) High temperature-induced sterility in the female Nile Tilapia, *Oreochromis Niloticus*. *Gen Comp Endocrinol* 213(3):110–117. <https://doi.org/10.1016/j.ygcen.2015.01.028>
- Piferrer F, Cal RM, Gómez C, Bouza C, Martínez P (2003) Induction of triploidy in the turbot (*Scophthalmus maximus*): II. Effects of cold shock timing and induction of triploidy in a large volume of eggs. *Aquaculture* 220(1–4):821–831
- Pšenička M, Saito T, Linhartová Z, Gazo I (2015) Isolation and transplantation of sturgeon early-stage germ cells. *Theriogenology* 83(6):1085–1092. <https://doi.org/10.1016/j.theriogenology.2014.12.010>
- Reimers N (1964) Conditions of existence, growth, and longevity of brook trout in a small, high-altitude lake of the eastern sierra Nevada. *Calif Fish Game* 44(4):319–333
- Robles V, Riesco MF, Psenicka M, Saito T, Valcarce DG, Cabrita E, Herráez P (2016) Biology of teleost primordial germ cells (PGCs) and Spermatogonia: biotechnological applications. *Aquaculture* 472(2017):4–20. <https://doi.org/10.1016/j.aquaculture.2016.03.004>
- Rooij DG, Russell LD (2000) All you wanted to know about spermatogonia but were afraid to ask. *J Androl* 6512:776–798
- Saito T, Psenicka M (2015) Novel technique for visualizing primordial germ cells in sturgeons (*Acipenser Ruthenus*, *A. Gueldenstaedtii*, *A. Baerii*, and *Huso huso*). *Biol Reprod* 93(4):1–7. <https://doi.org/10.1095/biolreprod.115.128314>
- Saito T, Goto-Kazeto R, Arai K, Yamaha E (2008) Xenogenesis in teleost fish through generation of germ-line chimeras by single primordial germ cell transplantation. *Biol Reprod* 78(1):159–166
- Saito T, Goto-Kazeto R, Fujimoto T, Kawakami Y, Arai K, Yamaha E (2010) Inter-species transplantation and migration of primordial germ cells in cyprinid fish. *Int J Dev Biol* 54(10):1479–1484
- Saito T, Psenicka M, Goto R, Adachi S, Inoue K, Arai K et al (2014) The origin and migration of primordial germ cells in sturgeons. *PLoS One* 9(2):e86861
- Saito T, Guralp H, Iegorova V, Rodina M, Psenicka M (2018) Elimination of primordial germ cells in sturgeon embryos by UV-irradiation. *Biol Reprod*. <https://doi.org/10.1093/biolre/iy076/4964749>
- Scheerer PD, Thorgaard GH (1983) Increased survival in salmonid hybrids by induced Triploidy. *Can J Fish Aquat Sci* 40(11):2040–2044
- Schulz RW, de França LR, Lareyre J, LeGac F, Chiarini-Garcia H, Nobrega RH et al (2010) Spermatogenesis in fish. *Gen Comp Endocrinol* 165(3):390–411
- Shang M, Su B, Perera DA, Alsaqufi A, Lipke EA, Cek S, Dunn DA, Qin Z, Peatman E, Dunham RA (2018) Testicular germ line cell identification, isolation, and transplantation in two north American catfish species. *Fish Physiol Biochem* 44(2):717–733. <https://doi.org/10.1007/s10695-018-0467-3>
- Shinomiya A, Tanaka M, Kobayashi T, Nagahama Y, Hamaguchi S (2000) The *vasa*-like gene, *olvas*, identifies the migration path of primordial germ cells during embryonic body formation stage in the medaka, *Oryzias latipes*. *Develop Growth Differ* 42(4):317–326
- Silva MA, Costa GMJ, Lacerda SMSN, Brandão-Dias PFP, Kalapothakis E, Silva Júnior AF, Alvarenga ER, França LR (2016) Successful xenogenic germ cell transplantation from *Jundia* catfish (*Rhamdia Quelen*) into adult Nile Tilapia (*Oreochromis Niloticus*) testes. *Gen Comp Endocrinol* 230(2016):48–56. <https://doi.org/10.1016/j.ygcen.2016.03.012>
- Siqueira-Silva DH, Dos Santos Silva AP, Ninhaus-Silveira A, Verissimo-Silveira R (2015) The effects of temperature and busulfan (Myleran) on the yellowtail tetra *Astyanax altiparanae* (Pisces, Characiformes) spermatogenesis. *Theriogenology* 84(6):1033–1042
- Slanchev K, Stebler J, de la Cueva-Méndez G, Raz E (2005) Development without germ cells: the role of the germ line in zebrafish sex differentiation. *Proc Natl Acad Sci U S A* 102(11):4074–4079
- Stein DL, Drazen JC (2014) *Paraliparis hawaiiensis*, a new species of snailfish (Scorpaeniformes: Liparidae) and the first described from the Hawaiian archipelago. *J Fish Biol* 84(5):1519–1526
- Strüssmann CA, Saito T, Takashima F (1998) Heat-induced germ cell deficiency in the teleosts {*Odontesthes bonariensis*} and {*Patagonina hatcheri*}. *Comp Biochem Physiol A Mol Integr Physiol* 119(2):637–644
- Takeuchi Y, Yoshizaki G, Takeuchi T (2003) Generation of live fry from intraperitoneally transplanted primordial germ cells in rainbow trout. *Biol Reprod* 69(4):1142–1149
- Takeuchi Y, Yoshizaki G, Takeuchi T (2001) Production of germ-line chimeras in rainbow trout by blastomere transplantation. *Mol Reprod Dev* 59(4):380–389
- Takeuchi Y, Yoshizaki G, Takeuchi T (2004) Biotechnology: surrogate broodstock produces salmonids. *Nature* 430(7000):629–630
- Takeuchi Y, Higuchi K, Yatabe T, Miwa M, Yoshizaki G (2009) Development of Spermatogonial cell transplantation in Nibe croaker, *Nibea mitsukurii* (Perciformes, Sciaenidae). *Biol Reprod* 81(6):1055–1063
- Tashiro F (1972) Effects of irradiation 60Co γ ray on the maturation of rainbow trout. *Ippon Suisan Gakkai-Shi* 38(8):793–797
- Tiwary BK, Kirubakaran R, Ray AK (2004) The biology of triploid fish. *Rev Fish Biol Fish* 14(4):391–402
- Vazzoler AEA de M (1996) *Biologia da reprodução de peixes teleosteos: teoria e prática*. Maringá, EDUEM 169 p
- Wong T-T, Saito T, Crodian J, Collodi P (2011) Zebrafish germline chimeras produced by transplantation of ovarian germ cells into sterile host larvae. *Biol Reprod* 84(6):1190–1197
- Xu J, You F, Wu X, Zhang P, Lin Y, Jiang H, Zheng C (2008) Induction of triploidy in large yellow croaker *Pseudosciaena crocea* (Richardson, 1846): effects of pressure shocks and growth performance in the first rearing year. *Aquac Res* 39(13):1369–1376

- Yamaha E, Kazama-Wakabayashi M, Otani S, Fujimoto T, Arai K (2001) Germ-line chimera by lower-part blastoderm transplantation between diploid goldfish and triploid crucian carp. *Genetica* 111(1–3):227–236
- Yamaha E, Murakami M, Hada K, Otani S, Fujimoto T, Tanaka M, Sakao S, Kimura S, Sato S, Arai K (2003) Recovery of fertility in male hybrids of a cross between goldfish and common carp by transplantation of PGC (primordial germ cell)-containing graft. *Genetica* [Internet] 119(2):121–131
- Yamaha E, Saito T, Goto-Kazeto R, Arai K (2007) Developmental biotechnology for aquaculture, with special reference to surrogate production in teleost fishes. *J Sea Res* 58(1): 8–22
- Ye H, Li CJ, Yue HM, Du H, Yang XG, Yoshino T, Hayashida T, Takeuchi Y, Wei QW (2017) Establishment of intraperitoneal germ cell transplantation for critically endangered Chinese sturgeon *Acipenser Sinensis*. *Theriogenology* 94:37–47. <https://doi.org/10.1016/j.theriogenology.2017.02.009>
- Yoon C, Kawakami K, Hopkins N (1997) Zebrafish *vasa* homologue RNA is localized to the cleavage planes of 2- and 4-cell-stage embryos and is expressed in the primordial germ cells. *Development* 124(16):3157–3165
- Yoshikawa H, Takeuchi Y, Ino Y, Wang J, Iwata G, Kabeya N, Yazawa R, Yoshizaki G (2016) Efficient production of donor-derived gametes from triploid recipients following intraperitoneal germ cell transplantation into a marine teleost, Nibe croaker (*Nibea Mitsukurii*). *Aquaculture* 47:35–48. <https://doi.org/10.1016/j.aquaculture.2016.05.011>
- Yoshikawa H, Xu D, Ino Y, Yoshino T, Hayashida T, Wang J, Yazawa R, Yoshizaki G, Takeuchi Y. (2018) Hybrid sterility in fish caused by mitotic arrest of primordial germ cells. *Genetics*, April, genetics.300777.2018. <https://doi.org/10.1534/genetics.118.300777>
- Yoshizaki G, Lee S (2018) Production of live fish derived from frozen germ cells via germ cell transplantation. *Stem Cell Res* 29:103–110. <https://doi.org/10.1016/j.scr.2018.03.015>
- Yoshizaki G, Ichikawa M, Hayashi M, Iwasaki Y, Miwa M, Shikina S, Okutsu T (2010) Sexual plasticity of ovarian germ cells in rainbow trout. *Development* 137(8):1227–1230
- Yoshizaki G, Takashiba K, Shimamori S, Fujinuma K, Shikina S, Okutsu T, Kume S, Hayashi M (2016) Production of germ cell-deficient salmonids by dead end gene knockdown, and their use as recipients for germ cell transplantation. *Mol Reprod Dev* 83(4):298–311