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Successful xenogeneic germ cell transplantation from Jundia catfish (*Rhamdia quelen*) into adult Nile tilapia (*Oreochromis niloticus*) testes

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ABSTRACT

Fish germ cell transplantation presents several important potential applications for aquaculture, including the preservation of germplasm from endangered fish species with high genetic and commercial values. Using this technique in studies developed in our laboratory with adult male Nile tilapias (*Oreochromis niloticus*), all the necessary procedures were successfully established, allowing the production of functional sperm and healthy progeny approximately 2 months after allogeneic transplantation. In the present study, we evaluated the viability of the adult Nile tilapia testis to generate sperm after xenogeneic transplant of germ cells from sexually mature Jundia catfish (*Rhamdia quelen*) that belong to a different taxonomic order. Therefore, in order to investigate at different time-periods post-transplantation, the presence and development of donor PKH26 labeled catfish germ cells were followed in the tilapia seminiferous tubules. From 7 to 20 days post-transplantation, only PKH26 labeled spermatogonia were observed, whereas spermatocytes at different stages of development were found at 70 days. Germ cell transplantation success and progression of spermatogenesis were indicated by the presence of labeled PKH26 spermatids and sperm on days 90 and 120 post-transplantation, respectively. Confirming the presence of the catfish genetic material in the tilapia testis, all recipient tilapias evaluated ($n = 8$) showed the genetic markers evaluated. Therefore, we demonstrated for the first time that the adult Nile tilapia testis offers the functional conditions for development of spermatogenesis with sperm production from a fish species belonging to a different order, which provides an important new venue for aquaculture advancement.

1. Introduction

For fish, it has been assumed that preservation of the genetic resources is limited to the rearing of live individuals (Zhang et al., 2007). However, although the cryopreservation of fish milt is feasible, several other alternative reproductive technologies have been developed to efficiently produce functional gametes and offspring from endangered and commercially important species, which are normally difficult to breed in captivity (Majhi et al., 2014). Among these approaches, the induction of ovulation, *in vitro* gametogenesis and germ cell transplantation are the most popular (Kawasaki et al., 2015; Lacerda et al., 2013a; Mylonas et al., 2010). The latter is an important technique that involves the isolation of spermatogonial stem cells (SSCs) from a donor animal and the transplantation of these cells into a recipient testis (Brinster and Avarbock, 1994), where they will be able to develop and form mature fertile sperm carrying the donor's genetic characteristics (Brinster, 2002; Shinohara et al., 2006).

In several fish species, intraspecific allogeneic germ cell transplantation has been performed by microinjecting primordial germ cells and/or spermatogonial cells into blastula-stage fish embryos, as well as in the coelomic cavity of newly hatched fish larvae (Ciruna et al., 2002; Kise et al., 2012; Kobayashi et al., 2007; Okutsu et al., 2006; Takeuchi et al., 2009, 2003; Wong et al., 2011). Although these methodologies allow the production of fertile donor sperm in the recipient gonads, a long time-interval is required to generate functional gametes, mainly because recipient animals need firstly to reach puberty (reviewed by Lacerda et al., 2013a; Yoshizaki et al., 2012). Studies developed in our laboratory

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by Lacerda et al. (2013b, 2010, 2006) standardized all the necessary methodologies to perform successful SSCs transplantation using, as recipient, the adult Nile tilapia (*Oreochromis niloticus*). Donor SSCs were injected through the common spermatic duct located in the urogenital papilla and this procedure provided a faster (approximately 2 months) generation of donor-derived spermatozoa and the production of normal offspring carrying the donor genetics characteristics. Additionally, in association with the Utrecht University (The Netherlands), we performed intraspecific germ cells transplantation in zebrafish (Nóbrega et al., 2010). Although the transplantation efficiency was very low, the recipient male zebrafish were able to develop donor-derived gametogenesis (Nóbrega et al., 2010).

The feasibility of interspecific (xenogeneic) spermatogonial transplantation was also determined in several fish species using embryos, newly hatched larvae, and adult fish as recipient (reviewed by Lacerda et al., 2013a). In this regard, the key studies related to xenogeneic germ cell transplantation, using embryos and newly hatched larvae, were performed respectively in zebrafish (Ciruna et al., 2002; Wong et al., 2011) and rainbow trout (Kise et al., 2012; Kobayashi et al., 2007; Okutsu et al., 2006; Takeuchi et al., 2003). From the obtained results, the authors concluded that the spermatogonial transplantation technique is valid for interspecies applications, resulting in donor-derived sperm and offspring (reviewed by Lacerda et al., 2012), even for some fish species (donor and recipients) that are phylogenetically distant. In the first study where xenogeneic germ cell transplantation was successfully developed in adult fish, using *Odontesthes hatcheri* as recipients and *Odontesthes bonariensis* as donors, a surgical procedure was used (Majhi et al., 2009). More recently, using the same species, Majhi et al. (2014) described a successful production of eggs and sperm by intrapapillary transplantation of germ cells in cytoablated adult fish.

Based on the results obtained for xenogeneic germ cell transplantation in adult fish, a very promising scenario has emerged, as this approach can be used to assist the preservation of the genetic resources of endangered species or valuable genetic strains. Therefore, in the present study, we evaluated the feasibility of xenogeneic germ cell transplantation using two species belonging to different taxonomic orders, using the adult Nile tilapia (*O. niloticus* from the Perciformes order) as a recipient and sexually mature Jundia catfish (*Rhamdia quelen* from the Siluriformes order) as a germ cell donor.

2. Materials and methods

2.1. Animals

Eighteen sexually mature male Nile tilapia (*O. niloticus*; mean body weight = 208 g) and ten Jundia catfish (*R. quelen*; mean body weight = 289 g) were used, respectively, as recipient and donor for germ cells transplantation. These fish were obtained from the Aquaculture Laboratory (School of Veterinary Sciences or Veterinary School, Federal University of Minas Gerais, Belo Horizonte, Brazil) and from commercial farmers located nearby Belo Horizonte city (state of Minas Gerais, Southeastern Brazil). The experimental design used in the present study is shown in Fig. 1. All experimental procedures were performed in accordance with the guidelines approved by the local ethics committee on animal experimentation – CEUA, UFMG (protocol # 89/2012).

2.2. Recipient preparation

The adequate preparation of the recipient fish, in which endogenous spermatogenesis is naturally absent or experimentally deprived, is crucial for the success and efficiency of germ cells transplantation. In this condition, niches for germ cells colonization and development are available. In the present study, the endogenous spermatogenesis was depleted according to the procedures already described by Lacerda et al. (2006). Eighteen tilapias were kept at temperatures of 35 °C (Fig. 1A) for at least 2 weeks before receiving intraperitoneally two injections of busulfan (Sigma, St. Louis, MO, USA) (18 mg/kg body weight and 15 mg/kg body weight), with a two-week interval between the injections (Fig. 1B and C). The germ cell transplantation was performed 1 week after the second injection (Fig. 1D).

2.3. Donor germ cell isolation

Donor spermatogonial cells were harvest from the testes of eight adult Jundia catfish (mean testis weight = 8 g; Fig. 2B) through enzymatic digestion according to standardized protocols (Lacerda et al., 2010). Briefly, testes were dissociated with 2% collagenase (Sigma, St. Louis, MO) in Dulbecco Modified Eagle medium/Ham F-12 medium (DMEM/F12 – Gibco, Grand Island, NY) for 4 h at 28 °C. The dispersed testicular tissue was then incubated with 0.25% trypsin/1 mM EDTA (Sigma, St. Louis, MO) and 0.03% DNase I (Sigma, St. Louis, MO) under constant shaking at 28 °C for 30 min. After this step, an equal volume of DMEM containing 10% of fetal bovine serum (FBS, Gibco) was added to the cell suspension in order to inactivate the trypsin. The testicular cell suspension was sequentially filtered using 70 and 40 μm mesh and then centrifuged at 400*g* for 10 min and re-suspended in DMEM/F12 (Fig. 2C). An enriched type A spermatogonia cell suspension, characterized according to the germ cell morphology in Jundia (Fig. 2A) was obtained after centrifugation, at 800*g* for 30 min, using a 40% percoll gradient, followed by another centrifugation using 35% percoll. The pellet was discarded and the supernatant (Fig. 2D) was subjected to differential plating (Fig. 2F–H), in order to deplete testicular somatic cells that adhere to the culture bottle (Fig. 2G) (Lacerda et al., 2010). A total of 22×10^7 cells was obtained, and 7.3×10^7 cells per culture bottle (75 cm^2, TPP, Switzerland) were cultured in FSM (fish spermatogonial medium – DMEM/F12 supplemented) for 18 h, at 28 °C in an atmosphere of 5% CO_2. These methodologies allowed an efficient enrichment of germ cell for transplantation (Fig. 2H). Additionally, in order to morphologically characterize Jundia germ cells along spermatogenesis, testes samples from donors were fixed in 4% glutaraldehyde and embedded in plastic for routine histological analysis (Alvarenga and França, 2009; Leal et al., 2009).

2.4. Donor germ cell labeling and transplantation

Before germ cell transplantation into recipient Nile tilapia testes, donor germ cells were incubated with the fluorescent membrane dye PKH26-GL (Sigma, St. Louis, MO), an adequate molecular tracer for the investigation of transplanted donor germ cell fate and location into the recipient seminiferous tubules (Lacerda et al., 2013b, 2010, 2006; Majhi et al., 2014) (Fig. 3A and B). The cell labeling was performed following the manufacture's guidelines with the final optimal concentration of 9 mM PKH26. The collected cells were then suspended in DMEM/F12 at a concentration of 10^7 - cells/mL and approximately 1 mL of cell suspension was injected in each recipient. The cells were transplanted using a non-surgical access, via the common spermatic duct that opens in the urogenital papilla through the urogenital pore (Fig. 3C). For this purpose, the recipient Nile tilapias were anesthetized with Quinaldin solution (1:5000, added in the aquarium water; Merck & Co.) and the donor germ cells were transplanted using a glass micropipette (outside diameter 70 μm) under a stereomicroscope (Olympus SZX-ILLB2-100). As Nile tilapia usually reproduce at a temperature of 26–

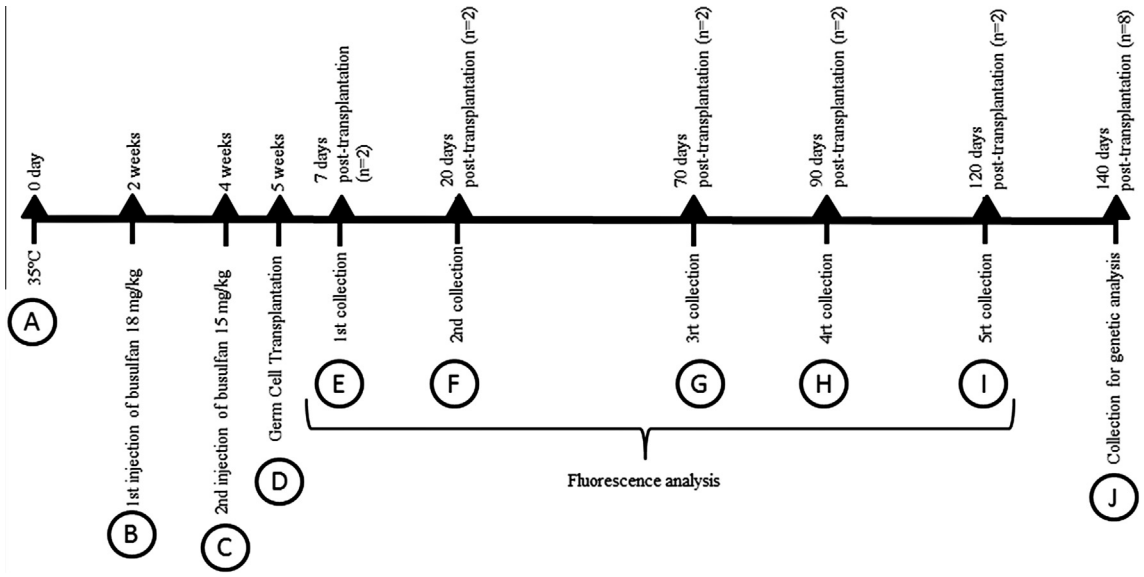

Fig. 1. Experimental design timeline. To deplete the endogenous spermatogenesis of recipient tilapia, the fish (n = 18) were kept at temperatures of 35 °C for at least 2 weeks (A) before receiving intraperitoneally two busulfan injections (B; 18 mg/kg body weight and C; 15 mg/kg body weight), with a two-week interval between injections. Three weeks after the first busulfan injection, recipient tilapia received PKH26-labeled donor germ cells through the common spermatic duct (D). To evaluate the development (proliferation and differentiation) of donor germ cells in the recipient gonads, the testes were collected at specific time periods (E-I; from 7 to 120 days). In order to demonstrate the presence of Jundia catfish DNA in the recipient testis 140 days post germ cell transplantation, genetic analysis was also performed (J).

28 °C, the water temperature was gradually decreased 1–2 °C per day after the transplantation, until 28 °C was reached.

2.5. Microscopic evaluation of donor-derived spermatogenesis in the recipient Nile tilapia testis

Testes were collected from ten recipient tilapia for analysis of PKH26 positive donor spermatogonia behavior in the Nile tilapia testis (Fig. 1E–I). In order to evaluate the progression of spermatogenesis and the eventual spermatozoa formation after germ cell transplantation, the recipient fish testes were collected at 7, 20, 70, 90 and 120 days post germ cell transplantation. The testes were immediately embedded in Jung Tissue Freezing Medium (Leica Instruments, Nussloch, Germany), frozen in liquid nitrogen and stored at −80 °C freezer. These testis samples were serially cryosectioned (7 μm of thickness), stained with DAPI (targeting DNA in the cell nucleus) and then analyzed under the fluorescence microscope (Olympus IX-70).

2.6. Genetic analysis

A PCR approach was used to detect donor germ cell DNA in eight recipient Nile tilapia testes (Table 1 and Fig. 1J). The primers consisted of three (outer, middle and inner) pairs amplifying the Rhq7 microsatellite sequence for *R. quelen* (GenBank access number KC117544.1); primer specificity and sensitivity was tested both *in silico* and *in vitro*. *In silico* tests consisted of a primer-BLAST specificity tool (Ye et al., 2012) against GenBank *O. niloticus* database and no evidence of a cross amplification was observed. *In vitro* tests comprised serial dilutions of small quantities of total *R. quelen* genomic DNA (from 0.000089 to 2.23 ng μL^{-1}) into large amounts of total *O. niloticus* genomic DNA (from 2.53 to 7.04 ng μL^{-1}), and no evidence of an unspecific amplification was detected. Total DNA was extracted from the controls (Nile tilapia and Jundia), and from the recipient Nile tilapia testes following a standard proteinase k, phenol/chloroform extraction (Sambrook and Russell, 2001), and PCR reactions were run in a Veriti 96-Well thermal cycler (Applied Biosystems). The best reaction cycle was an initial outer primer pair reaction consisting of a 95 °C denaturation step (5 min), followed by 5 cycles of 92 °C denaturation (20 s), 64 °C annealing (30 s) and 72 °C of extension (30 s). One μL of first amplification product was then used as template DNA for a second reaction with middle primers, which differed from the first one by adding 20 additional cycles (total 25 cycles) of denaturation, annealing and extension steps. Immediately after that, a $1{:}10^{-3}$ dilution of the second PCR product was applied as a template for an inner primer reaction, using again 25 cycles.

3. Results

3.1. Jundia germ cell morphology and Jundia PKH26 labeled germ cells in the recipient testis

As depicted in Fig. 2A, type A spermatogonial cells show a large ovoid nucleus with a prominent nucleolus and these cells are noticeably larger than early and late type B spermatogonia.

At 1 week post-transplantation (Fig. 4A–C), labeled donor germ cells were observed in the recipient tilapia seminiferous tubules epithelium. Based on established morphological criteria (Fig. 2A), these cells were considered spermatogonia. Twenty days post-transplantation, a higher number of labeled germ cells, similar to spermatogonial cells, were also identified in the seminiferous tubules epithelium (Fig. 4D–N).

Demonstrating the progression and differentiation of donor-derived germ cells through spermatogenesis, 70 days after transplantation, PKH26 labeled germ cells cysts at different stages of development were observed in the recipient seminiferous tubules (Fig. 5A–G). The number and size of the germ cells present in these cysts indicated that they were spermatocytes (meiotic cells). At 90 (Fig. 5H–J) and 120 days (Fig. 6) post-transplantation, labeled germ cells presented morphological characteristics of spermatids and sperm, respectively. These cells were arranged in cystic structures (90 days) inside the seminiferous tubules (Fig. 5J) and at 120 days in the tubular lumen (Fig. 6). All recipient tilapia testes presented PKH26 positive donor germ cells. Particularly in the later time-periods, cysts containing germ cells in different developmental

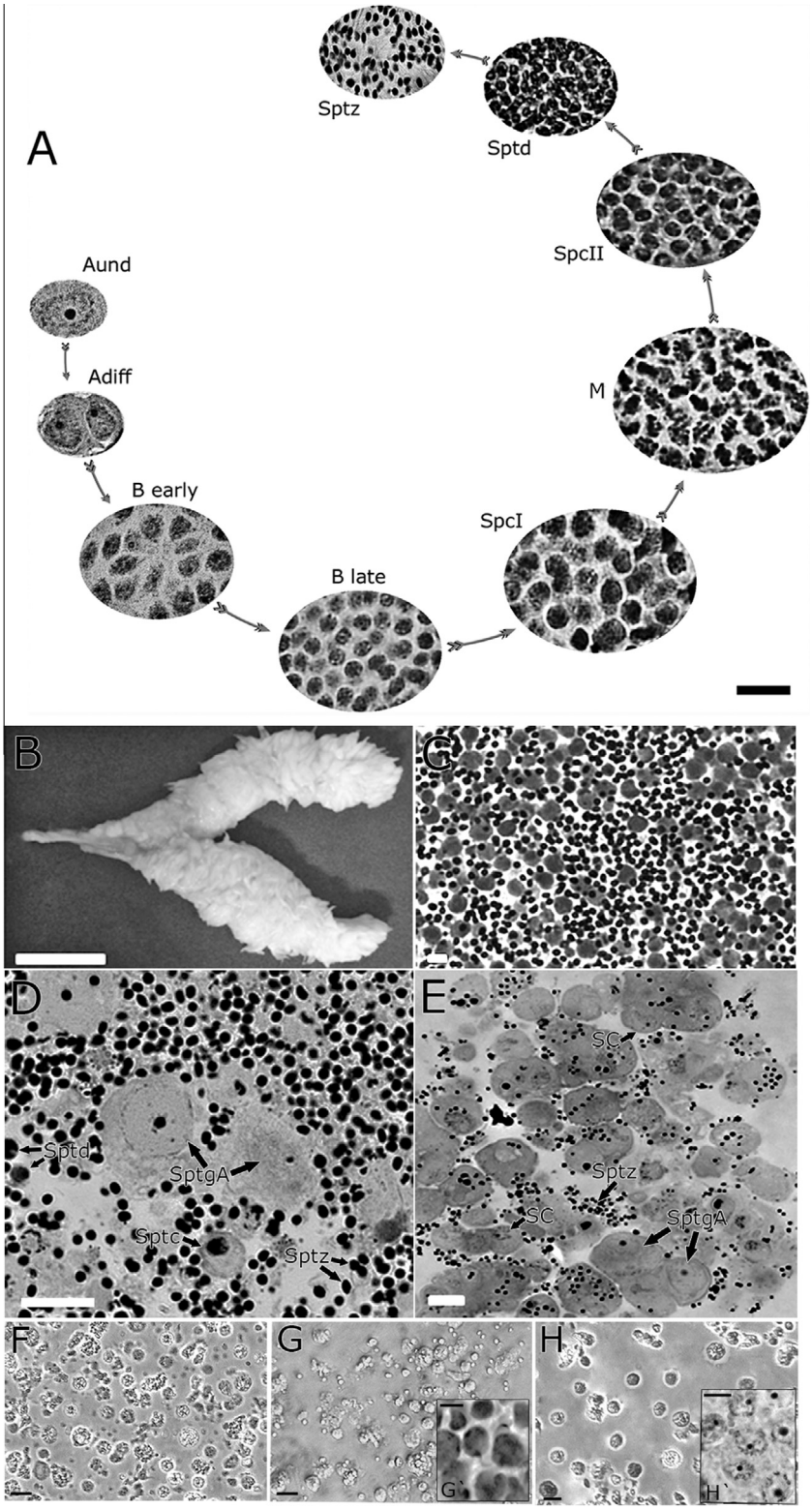

Fig. 2. Germ cell morphology and preparation of enriched spermatogonia cell suspension from Jundia catfish for transplantation. (A) Morphological illustration of germ cell cysts along spermatogenesis in adult Jundia: type A undifferentiated spermatogonia (Aund), type A differentiated spermatogonia (Adiff), type B early spermatogonia (Bearly), type B late spermatogonia (Blate), primary spermatocyte (SpcI), meiotic divisions (M); secondary spermatocyte (SpcII), spermatid (Sptd) and spermatozoa (Sptz) located in the tubular lumen. The nuclei of type A spermatogonia are much larger than the nuclei of all other germ cell types. For transplantation, donor germ cells were harvest from the testes of adult males (B) through enzymatic digestion. The cell suspension (C) was filtered and submitted to percoll gradient centrifugation. After 40% percoll, the pellet contained only erythrocytes and sperm (not shown), whereas the supernatant (D) contained type A spermatogonia (SpgA), spermatocytes (Spc), spermatids (Sptd) and sperm (Sptz). This cell suspension (D) was then subjected to the 35% percoll and the supernatant (E) showed a high concentration of type A spermatogonia (SpgA); however, sperm (Sptz) and somatic cells (SC) were also observed. A further enrichment of type A spermatogonia was obtained through differential plating (F) for 12 h: testicular somatic cells adhered firmly to the culture dish and hence were drastically reduced (G), whereas spermatogonia remained in suspension (H). Inset photos in G and H show the morphology of adherent (G′; somatic cells) and non-adherent (H′; type A spermatogonia) cells, after differential plating. Scale bars: A, C, D, and E = 10 μm; F, G, and H = 15 μm; B = 2 cm; G′ and H′ (inserts) = 10 μm.

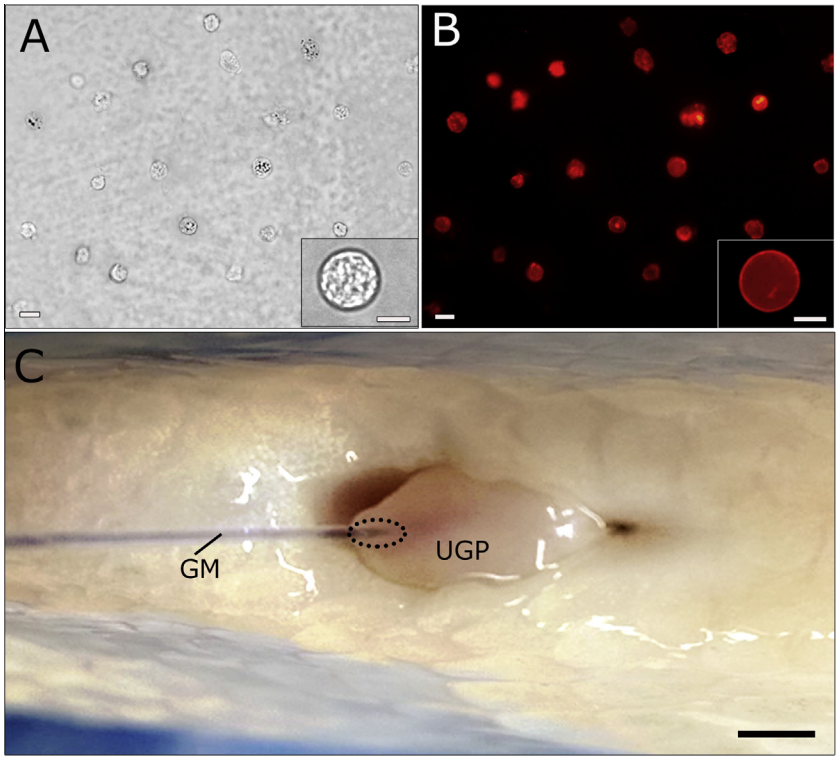

Fig. 3. Jundia catfish spermatogonial cell labeling and transplantation. (A and B) Isolated donor spermatogonia were labeled with 9 μM red fluorescent dye (PKH26) and all cells in the brightfield (A) are strongly stained as shown in the fluorescent image (B). The inset photos in A and B at higher magnification show the typical PKH26 membrane-associated labeling. (C) Transplantation of PKH26-labeled Jundia spermatogonial cells into the adult Nile tilapia testis. Before transplantation, the cells were mixed with trypan blue and then injected using a glass micropipette (GM) into the common spermatic duct, located in the urogenital papilla (UGP). The dotted circle delimits the urogenital pore opening. Scale bars: A and B = 15 μm; inserts = 7 μM; and C = 3 mm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Primer pairs used in the genetic analysis to detect donor Jundia catfish germ cells DNA in the Nile tilapia recipient testis.

Primer		Sequence
Outer	Forward	5′-GAC GCC TTT CTG GGG TCT AC-3′
	Reverse	5′-CTC TGC AGC CTC AGT CCT TC-3′
Middle	Forward	5′-GCC TTT CTG GGG TCT ACC TG-3′
	Reverse	5′-AGC CTC AGT CCT TCC AGA GA-3′
Inner	Forward	5′-GGA AGG TCT CTG TCC AAC AGG-3′
	Reverse	5′-TAA ACA CCC CTC GTA GCC CA-3′

stages (spermatogonia, spermatocytes and spermatids) were noted. Some germ cells at later stages may have been present among the transplanted cells, but it seems unlikely that these more differentiated germ cells contributed to the long-term spermatogenesis in the recipient testis, for which usually stem cell properties would be required.

3.2. Genetic analysis of the Nile tilapia recipient testes

All recipient fish ($n = 8$; Fig. 1J) showed the presence of Jundia catfish genetic material in their testis (Fig. 7E–L). The evaluated testis samples displayed different intensities of the amplicon bands, and although no qPCR was performed in the present study, these results suggest different transplantation/colonization efficiencies. In all cases, however, the data strongly suggest a successful donor germ cell colonization and spermatogonial progression in the Nile tilapia testis. Importantly, the primers, which were designed for Jundia specific sequences (Fig. 7B), did not recognize any tilapia sequences (Fig. 7C).

4. Discussion

Previous studies developed in our laboratory using Nile tilapias demonstrated that this species represents an excellent recipient for fish SSCs transplantation (Lacerda et al., 2013b, 2010, 2006). This finding motivated us to investigate if the Nile tilapia testis environment could support the development of germ cells from phylogenetically distant fish species. In the present investigation we evaluated the success of xenogeneic germ cell transplantation using *R. quelen* (a species belonging to the Siluriformes order) as a donor and the Nile tilapia (species belonging to the Perciformes order) as a recipient. The formation of *R. quelen* sperm in the tilapia seminiferous tubules strongly suggests that the Nile tilapia has high plasticity as a recipient, providing an excellent possibility for use in aquaculture to preserve the germplasm of endangered fish species and the production of gametes in species with high commercial and genetic values.

Although Majhi et al. (2014, 2009) have already carried out a xenogeneic transplantation in adult fish, the species used (donor and recipient) belonged to the same genus. In their study, spermatogonia and oogonia from *O. bonariensis* were transplanted, respectively, in male and female *O. hatcheri*. After germ cell transplantation, the establishment of spermatogenesis and oogenesis was observed in the recipient species with the donor sperm and oocytes production. The gametes were functionally tested, leading to healthy offspring generation. In our study, although we have demonstrated the presence of *R. quelen* sperm in the Nile tilapia testis, their viability was not evaluated due to the lack of information about the induction of ovulation and/or artificial insemination in the *R. quelen*. Therefore, in order to demonstrate the fertilization capacity and generation of healthy embryos derived from these

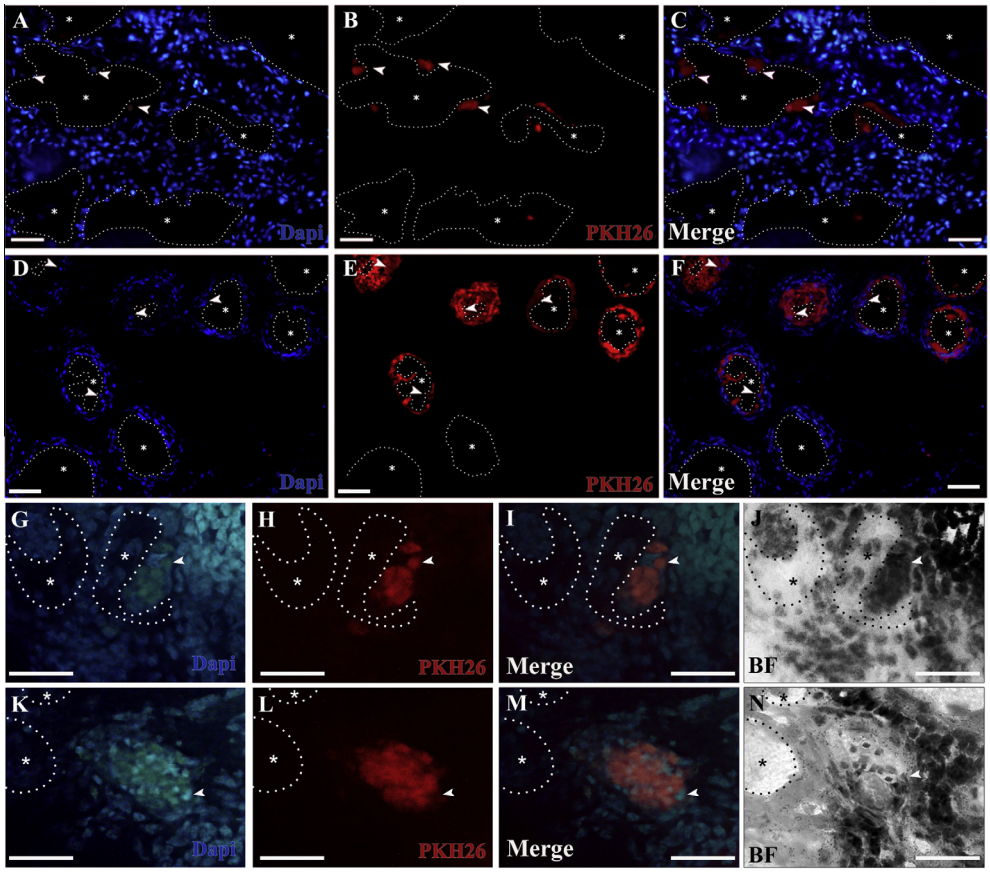

Fig. 4. Microscopic evaluation of recipient tilapia testes following 1 week and 20 days after Jundia catfish spermatogonial cells transplantation. At one-week (A–C) post-transplantation, labeled spermatogonial cells (white arrowheads) were observed in the tilapia seminiferous tubules epithelium. Twenty days (D–N) after transplantation, labeled spermatogonial cells were also identified in the seminiferous tubules epithelium (white arrowheads). PKH26 labeled spermatogonial cells are observed in red (B, C, E, F, H, I, L, and M). Cell nuclei labeled in blue with DAPI are also visualized (A, C, D, F, G, I, K, and M). The brightfield images (J and N) correspond to the fluorescent images shown in I and M (merge images). The dashed lines delimit the tubular lumen (*). Scale bar = 50 μm in all images. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

gametes produced after germ cell transplantation, further studies are being conducted.

As reviewed by Lacerda et al. (2012), the successful production of xenogeneic sperm was also described from germ cell transplantation using zebrafish embryos (Saito et al., 2010, 2008) and salmon larvae (Takeuchi et al., 2004) as recipients. Based on the literature for higher vertebrates (Honaramooz and Yang, 2011), it is possible that xenotransplantation in mammals is less efficient than that performed in fishes, which could be based on represent a higher plasticity provided by the testicular somatic cells. In fact, oogonia can differentiate into spermatogonia in the fish testis, resulting in viable gamete production (Wong et al., 2011; Yoshizaki et al., 2011, 2010) and the opposite is also true (Okutsu et al., 2007; Nóbrega et al., 2010). From these results, it can be concluded that the somatic cells in the fish testis are crucial for gametogenesis development when different cell lineages (male or female) or germ cells from distinct fish species are transplanted.

The duration of spermatogenesis is considered species-specific and controlled by the germ cell genotype (França et al., 1998). Although the time required for sperm formation, from preleptotene primary spermatocytes, takes about 11 days in the Nile tilapia (Vilela et al., 2003), in this species sperm production after allogeneic germ cell transplantation is observed approximately 9 weeks post-transplantation (Lacerda et al., 2010). Studies developed in mammals have shown that the commitment of SSCs to differentiation occurs at approximately 2 weeks after germ cell transplantation due to the time-period required for colonization of the seminiferous epithelium by these cells (Ishii et al., 2014; Nagano et al., 1999; Ohta et al., 2000; Parreira et al., 1998). In the present investigation, the required time to form *R. quelen* sperm in the Nile tilapia testis was approximately 17 weeks, which is a much longer time than that described for intraspecific germ cell transplantation in tilapias. A long time-period (24–34 weeks) for sperm production was also observed when germ cells from *O. bonariensis* were transplanted to the *O. hatcheri* seminiferous tubules (Majhi et al., 2014). Although this time could be considered as rather long, the production of spermatozoa using adult fish as recipients is much faster in comparison to germ cell transplantation using embryos or larvae as recipients (Kise et al., 2012; Okutsu et al., 2007; Saito et al., 2011, 2010, 2008; Takeuchi et al., 2004; Yazawa et al., 2010; Yoshizaki et al., 2010). In order to evaluate whether the duration of spermatogenesis in Jundia *R. quelen* is longer than in Nile tilapias, or if their spermatogonial SSCs need a longer time-period to colonize and differentiate in the tilapia testis, further studies related to these aspects are being developed in our laboratory.

The species *R. quelen* is an important source of protein for human consumption (Sampaio and Sato, 2006), and therefore has been used as germ cell donor in the present study. The phylogenetic distance between this species and the Nile tilapia is approximately 110 million years (Wittbrodt et al., 2002) and a similar distance separates man from mouse (Nagano et al., 2002). However, while in the present investigation we could observe *R. quelen* sperm formation in the Nile tilapia testis, human spermatogonia

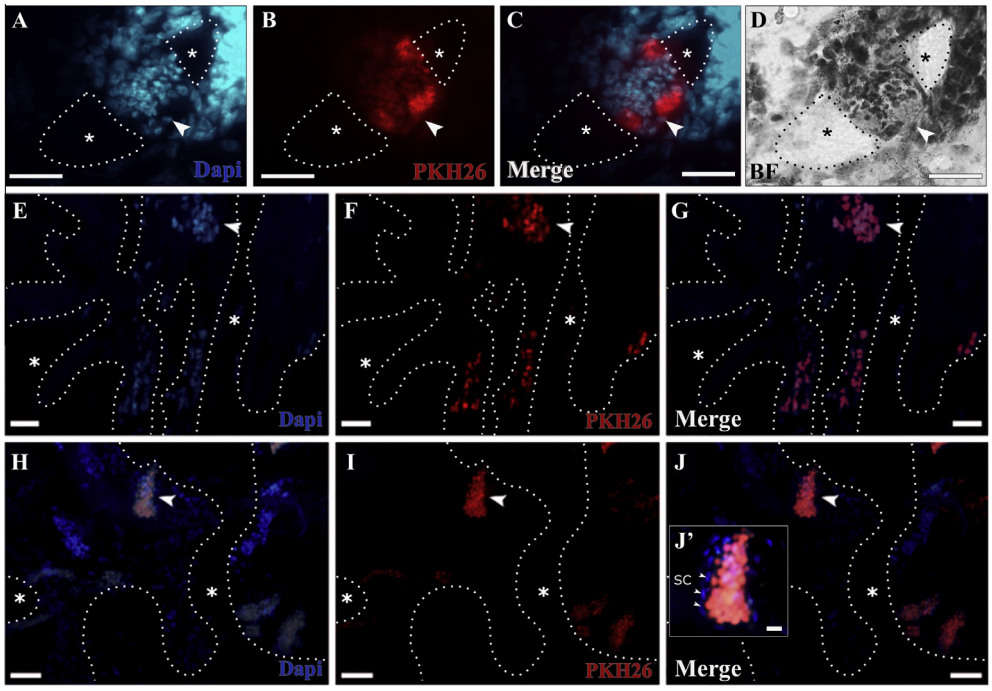

Fig. 5. Microscopic evaluation of recipient tilapia testis following 70 and 90 days after Jundia catfish spermatogonial cells transplantation. At 70 days (A–G) post-transplantation, labeled spermatocytes (white arrowheads) were observed in the tilapia seminiferous tubules epithelium. Ninety days (H–J) after transplantation, evident labeled spermatids were also present in the seminiferous epithelium (white arrowheads). PKH26 labeled spermatocytes (B, C and F, G) and spermatids (I, J) are observed in red. Cell nuclei labeled in blue with DAPI are also visualized (A, C, E, G, H, and J). The brightfield image (D) corresponds to the fluorescent image shown in C (merge image). The dashed lines delimit the tubular lumen (*). The insert (J′) shows PKH26 negative Sertoli cells (SC – white arrowhead) enveloping PKH26 positive spermatids. Scale bar = 50 μm in all images, except for the insert (5 μm). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

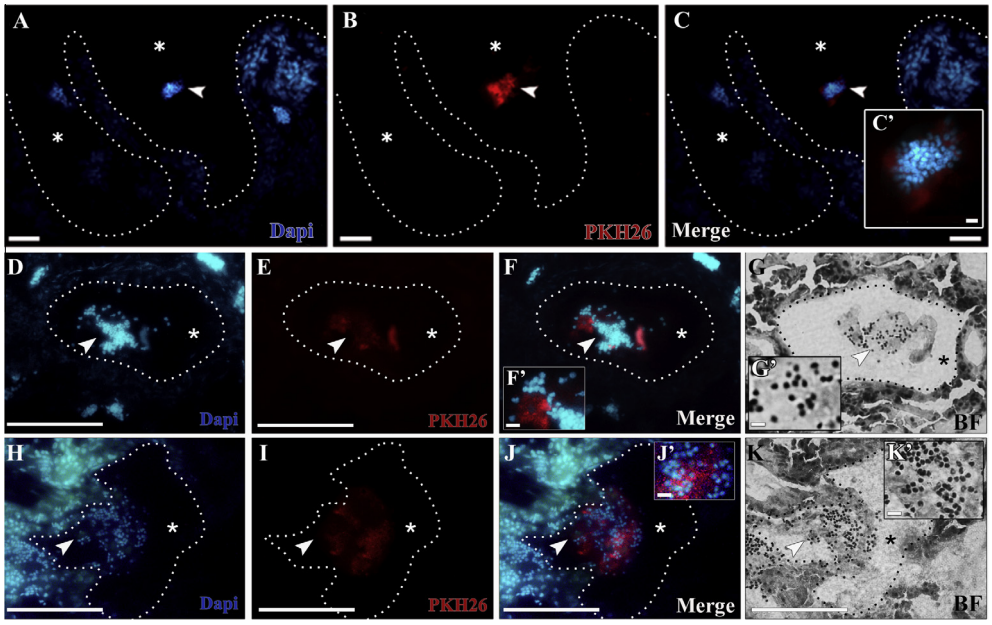

Fig. 6. Microscopic evaluation of recipient tilapia testis following 120 days after Jundia catfish spermatogonial cells transplantation. In all images, labeled spermatozoa (white arrowheads) are observed in the tilapia seminiferous tubules lumen. PKH26 labeled spermatozoa (B, C, E, F, I, and J) are observed in red. Cell nuclei labeled in blue with DAPI are also visualized (A, C, D, F, H, and J). The brightfield images (G and K) correspond to the fluorescent images shown in F and J (merge images). The dashed lines delimit the tubular lumen (*). The inset photos (C′, F′, G′, J′, and K′) show at higher magnification labeled Jundia spermatozoa. Scale bar = 50 μm in all images, except for the inserts (5 μm). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

were only able to colonize the mouse testis without any further progression of spermatogenesis (Nagano et al., 2002). These xenogeneic germ cell transplantation studies suggest that, different from mammals (Dobrinski and Hill, 2007; González and Dobrinski, 2015), fish apparently have no tight phylogenetic barrier that would limit the success of this very important biotechnology.

In the present study, we applied the technique of spermatogonial transplantation using Jundia as the donor and Nile tilapia as the recipient species. We demonstrated that the somatic microen-

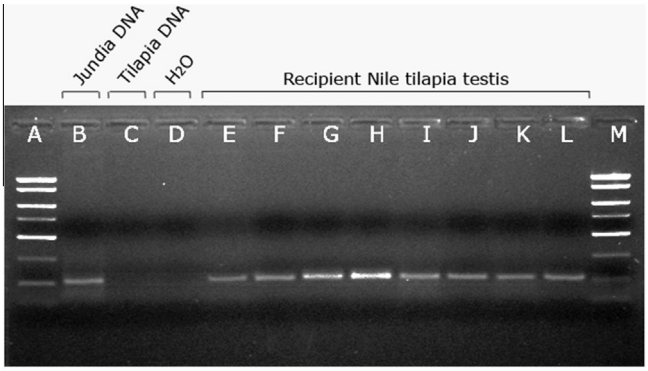

Fig. 7. Electrophoresis in 2% agarose gel for amplicons of the region corresponding to Rhq7 microsatellite sequence for *Rhamdia quelen* (Jundia) by nested PCR. Ladder molecular weight of 100 pb (lanes A and M); positive control with Jundia DNA (lane B); negative control with tilapia DNA (lane C); negative control with H_2O (lane D); and DNA from recipient Nile tilapia testis (lanes E–L). All recipient testes (lanes E–L) show the presence of Jundia genetic material (bands), but the amplicon bands display different intensities, suggesting different degrees of germ cell transplantation colonization/efficiency.

vironment of the Nile tilapia gonad can support colonization, survival, proliferation and the differentiation of transplanted progenitor spermatogonial cells. The novelty of our report lies in the phylogenetic distance between donor and recipient, which are species from different taxonomic orders. These findings indicate a remarkable plasticity in the capacity of the somatic cells in the teleost testis to support xenogenic germ cells. A functional test of catfish sperm matured in tilapia testis must await the availability of catfish eggs and the development of an *in vitro* fertilization protocol.

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