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MDPI

Supplementary Materials

Successful Cryopreservation of Spermatogonia Stem Cells of Neotropical Catfish (*Rhamdia quelen*) and Enriched Germ Cell Transplantation into Common Carp (*Cyprinus carpio*) Testes

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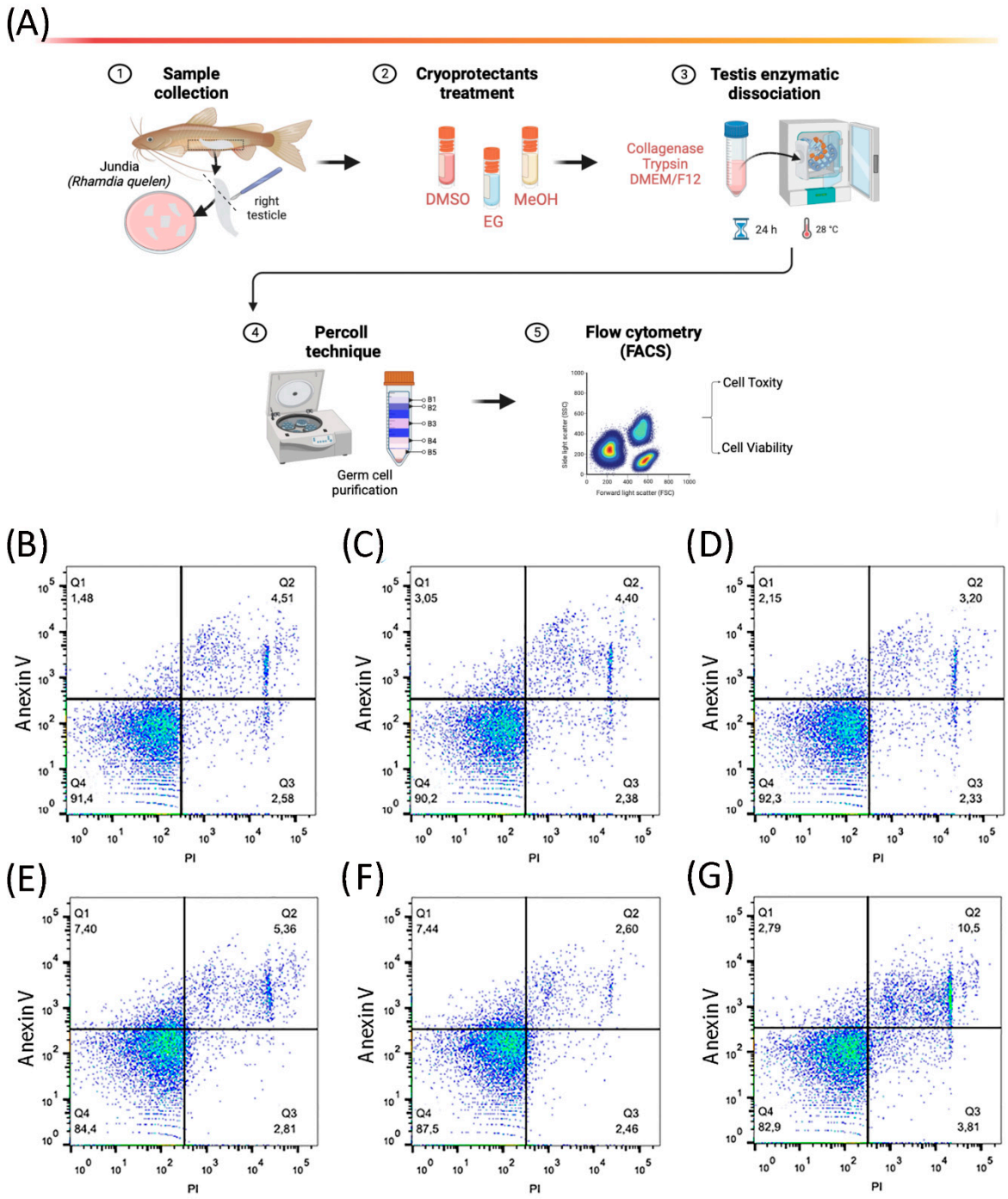

Figure S1. (**A**) Histograms of the cell viability analysis used to measure the toxicity of the cryoprotectants before freezing. (**B**) Cryoprotectant solution with DMSO for dissociated testes. (**C**) Cryoprotectant solution with MeOH for dissociated testes. (**D**) Cryoprotectant solution with EG for dissociated testes. (**E**) Cryoprotectant solution with DMSO for band 3. (**F**) Cryoprotectant solution with MeOH for band 3. (**G**) Cryoprotectant solution with EG for band 3. Dimethyl sulfoxide (DMSO), methanol (MeOH), ethylene glycol (EG), annexin V, and propidium iodide (PI). Quadrants: Q1: cells in initial apoptosis; Q2: cells in late apoptosis; Q3: cells in necrosis; Q4: living cells.

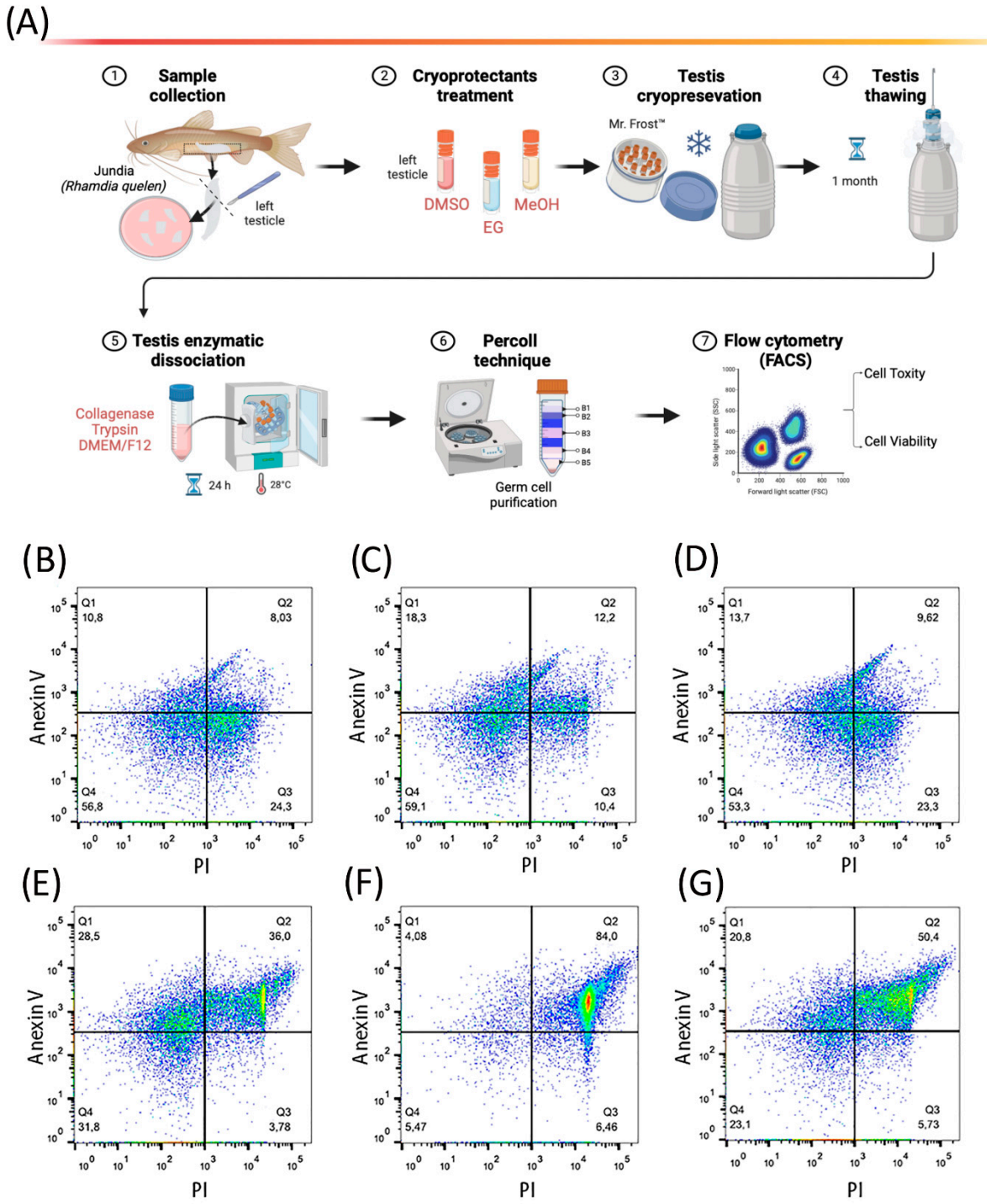

Figure S2. (**A**) Histograms of cell viability after thawing. (**B**) Cryoprotectant solution with DMSO for dissociated testes. (**C**) Cryoprotectant solution with MeOH for dissociated testes. (**D**) Cryoprotectant solution with EG for dissociated testes. (**E**) Cryoprotectant solution with DMSO for band 3. (**F**) Cryoprotectant solution with MeOH for band 3. (**G**) Cryoprotectant solution with EG for band 3. Dimethyl sulfoxide (DMSO), methanol (MeOH), ethylene glycol (EG), annexin V, and propidium iodide (PI). Quadrants: Q1: cells in initial apoptosis; Q2: cells in late apoptosis; Q3: cells in necrosis; Q4: living cells.

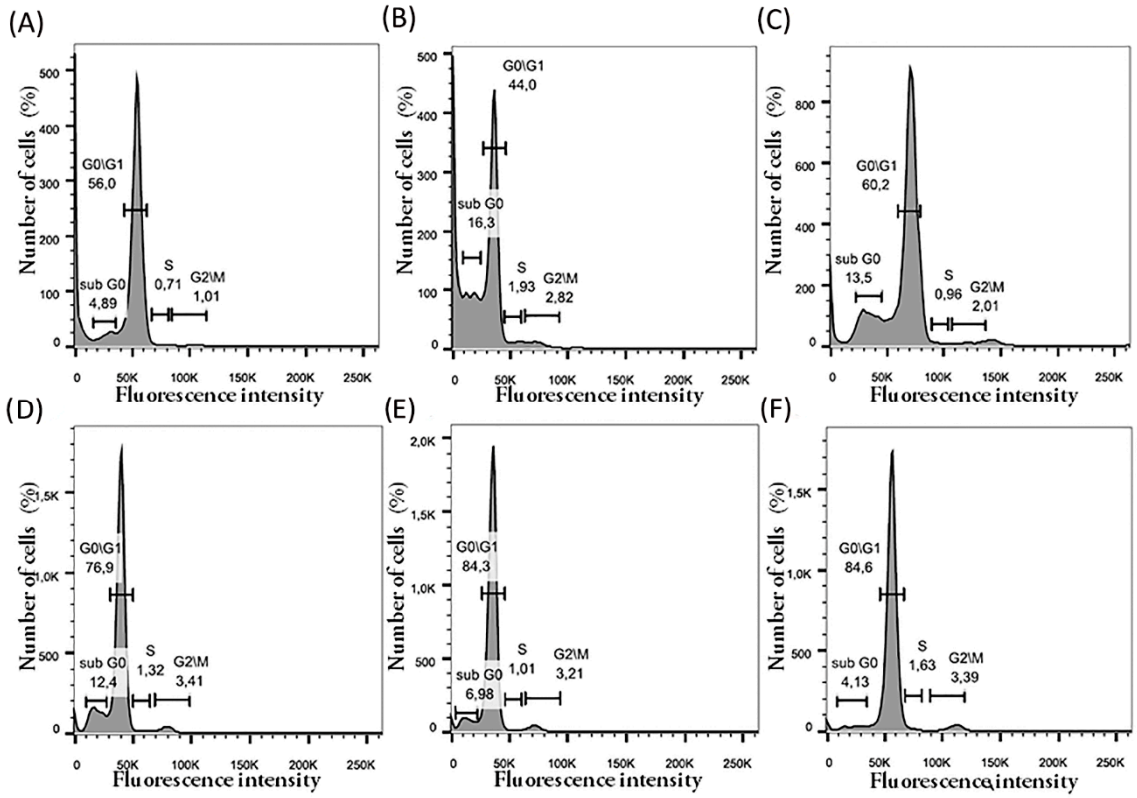

Figure S3. Flow cytometry analysis using propidium iodide to delineate cell cycle phases and distribution of cells from cell suspension poll of 3 animals. (**A**) Cell suspension (CS). (**B**) Band 1 (B1). (**C**) Band 2 (B2). (**D**) Band 3. (**E**) Band 4. (**F**) Band 5..

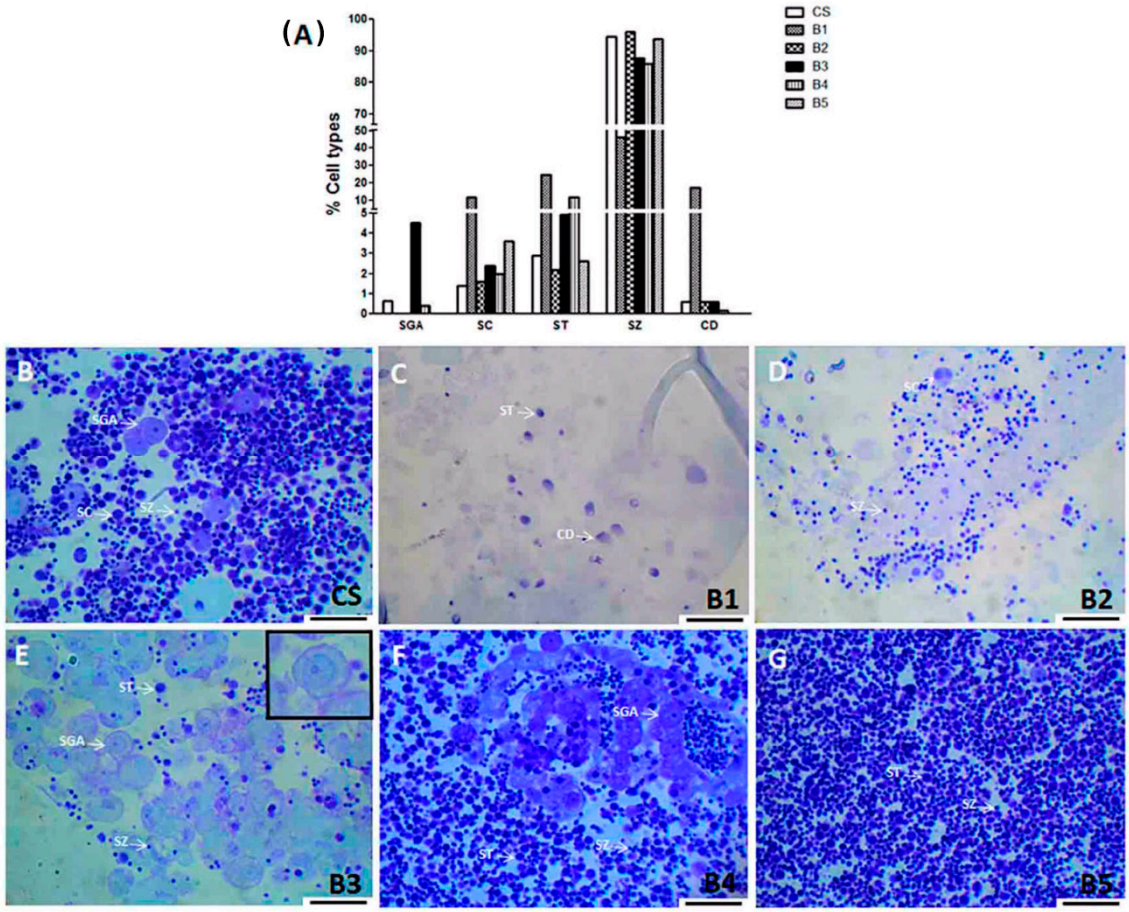

Figure S4. Characterization of germ cell types in percoll gradient ands and histological sections. (**A**) Percentage of germ cell types in the cell suspension (CS) and different Percoll bands (B1: Band 1; B2: Band 2; B3: Band 3; B4: Band 4; and B5: Band 5). Type A undifferentiated spermatogonia (SGA); spermatocytes (SC); spermatids (ST); spermatozoa (SZ) and cell debris (CD). (**B–G**) Histological section stained with toluidine blue. Type A undifferentiated spermatogonia (SGA); spermatocytes (SC); spermatids (ST); spermatozoa (SZ) and cell debris (CD). Inset in Figure E shows a higher magnification of type A undifferentiated spermatogonia in band 3. Scale bars: 25 μm.

Table S1. Groups for cellular toxicity and cell viability.

	Groups
1	Non-cryopreserved testicular cells from whole testes
2	Cryopreserved testicular cells from whole testes
3	Non-cryopreserved enriched testicular cells (Band 3)
4	Cryopreserved enriched testicular cells (Band 3)

Table S2. Spermatogenesis depletion process performed on recipient common carp specimens.

Week	**°C/Dose**
0	35°C (starting temperature)
2	1st dose of busulfan
4	2st dose of busulfan
6	Germ cell transplant

Table S3. Collection period and the number of carp specimens for histological analysis after transplantation of jundiá spermatogonial cells.

Collection Period	Number of Animals
24 h	3
1 week	2
2 weeks	3
3 weeks	2
4 weeks	2
5 weeks	2
6 weeks	2
12 weeks	2