Spermiation induction of *Brycon amazonicus* with GnRHa and Carp Pituitary Extract

(Registration number: 17189506)

Rosilane Gomes de Souza de Oliveira* and Marle Angélica Villacorta Correa[1]

* [1]Federal University of Amazonas (UFAM) – Manaus, AM, Brazil. E-mail: rosilanegso@gmail.com

INTRODUCTION

Brycon amazonicus, know as matrinxã (Fig.1), belongs to the Characidae family and can be found in the Amazon River and in some Brazilian tributaries [1]. It is a rheophilic fish, with an annual reproductive cycle and total spawning, that does not spontaneously reproduce in captivity, requiring exogenous hormones. The pituitary extracts of mature fish are largely used on spermiation and spawning induction of rheophilic fish. However, among other disadvantages, it can offer sanitary risks to breeders, which propel researches with substitute synthetic hormones. The aim of this study was to analyze the sperm quality of *B. amazonicus* induced with two gonadotropin releasing hormone analogs and carp pituitary extract.

Fig. 1 *Brycon amazonicus*, matrinxã (Oliveira, 2014)

MATERIAL AND METHODS

The experiment was carried out at the Centre of Aquaculture Technology, Training and Production of Balbina – CTTPA – Presidente Figueiredo, Amazonas, Brazil (1 ° 55'10.01" S; 59 ° 27'54.67" W) (Fig. 2). The fish were kept in earthen ponds (2000m²) and fed twice daily to their apparent satiation with commercial fish feed that contained 32% crude protein.

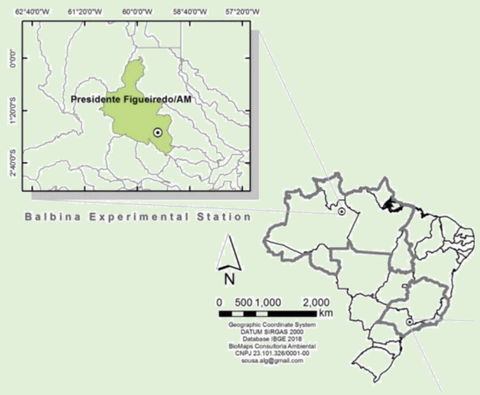


Fig. 2: CTTPA – Balbina

Twenty eight four-year-old males were used (1.27±0.04 kg bw) and randomized in three treatments groups (Table 1).

Table 1. Hormonal treatments

Treatment	Hormonal preparation	Content	Dosage (bw)	Number of fish
Ovaprim (sGnRHa)	D-Arg^{6}-Pro^{9}-NEt-sGnRH+domperidone	1 mL = 20 µg sGnRHa + 10 mg domperidone	0.5 mL.kg^{-1}	10
Ovopel (mGnRHa)	D-Ala^{6}-Pro^{9}-NEt-mGnRH+metoclopramide	1 pellet = 18–20 µg mGnRHa + 8–10 mg metoclopramide	2/3 pellet.kg^{-1}	8
CPE	Carp pituitary extract	-	2.0 mg.kg^{-1}	10

Each specimen was taken from the tank, placed in a container containing Eugenol solution to reduce stress during this procedure and collected the gametes. The milt was collected five hours after hormonal injection in mL-graduated tubes.

Seminal volume was collated in mL at the time of collection. Relative semen volume was calculated by the ratio between semen volume and fish body mass.

Sperm motility were evaluated in two steps as described by Billard and Cosson [2]. The first step consisted of diluting the semen in a 1% NaCl solution (1:40 - semen: diluter) and, in the second step, the previously diluted sample was activated with distilled water (1:20 - diluted sample:water), on a microscope slide coated with 0.05% bovine serum albumin, to prevent sperm from adhering to the slide, in an optical microscope, 40X objective.

Semen samples from each specimen were fixed with buffered saline formaldehyde at a ratio of 10:990 (semen:fixative), and spermatozoa concentration was determined in a Neubauer hematimetric chamber. The product between the sperm concentration and the seminal volume obtained the total sperm production.

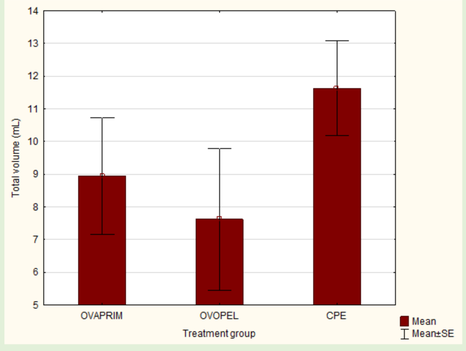
The cells were classified as normal or abnormal (%) in wet preparations with fixed semen stained with 3% Rose Bengal, placed on a slide and covered with a cover slip. Images were obtained using an optical microscope (Axiophot2, Zeiss) with an immersion objective.

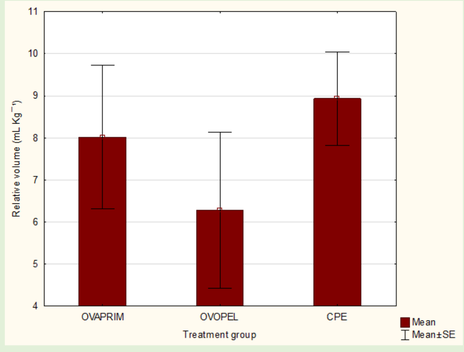
Statistical Analysis

All the data obtained within the study were first checked for normal distribution using the Kolmogorov-Smirnov test. The parametric data were subjected to one-way ANOVA followed by Holm-Sidak's multiple comparisons test. For non-parametric data, the Kruskal-Wallis test were used. All the data are presented as mean ± SEM. A threshold of $P < 0.05$ was set to infer statistical significance. Statistical analysis was performed using Statistica v.12 (StatSoft, EUA).

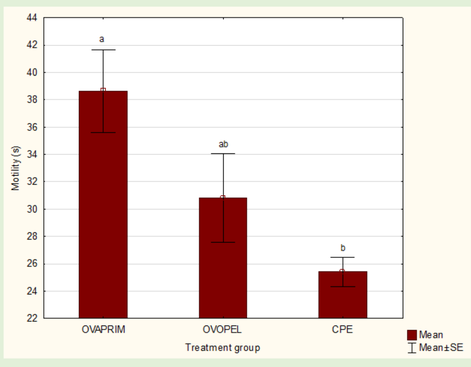
RESULTS

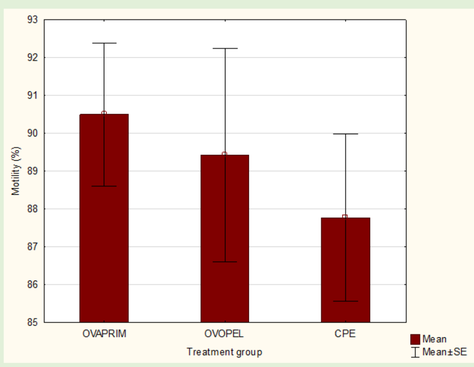
All treated males released semen. The total volume was 7.6±2.17 a 11.62±1.45 mL (P = 0.293; Fig. 4), and the relative volume was 6.3±1.8 to 8.9±1.1 mL.kg-1 (P = 0.507; Fig. 5).



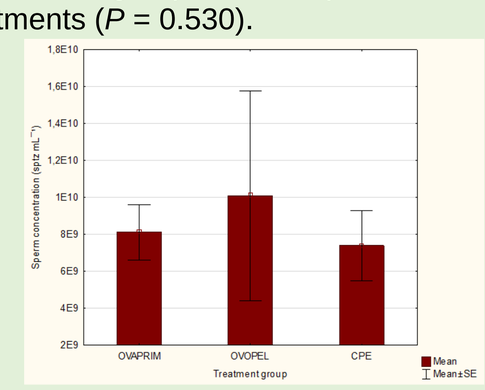


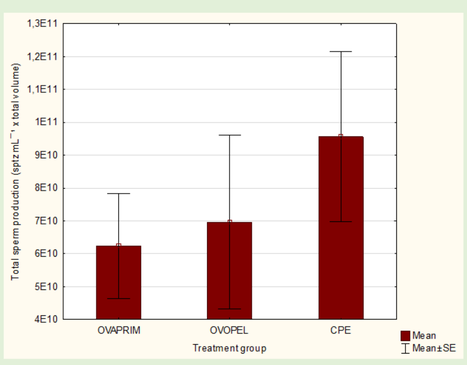
There was a significant increase in motility in sGnRHa-group (38.64±3.0 s) compared to CPE-group (22.8±2.7 s, P = 0.003; Fig. 6). Medium post-activation motility rate was 89.2±1.2 % (P = 0.737; Fig. 7).





Sperm concentration varied from 7.4±1.5 to 10.1±0.6 x 109 spt mL-1(P = 0.581; Fig. 8). Total sperm production was 9.6±2.6 to 6.2±1.6 x 1010 spt.mL.male-1 (P = 0.536; Fig. 9).Normal spermatozoa was higher than 58.3±2.4 % in all treatments (P = 0.530).





DISCUSSION

The control of the reproductive function through hormone protocols and the maximum utilization of the available gametes, with efficiency and economy, are some of the strategies to impactate aquaculture.

Seminal volume, motility and sperm concentration values were similar to those found by other authors for the species, after injection with CPE or GnRH analogues [3-6]. In this study, motility(s) was higher in the Ovaprim-treated group compared to the CPE-group. In general, hormonal therapies usually do not affect sperm motion performance [7]. However, it impossible to compare the results obtained by different laboratories, because each study uses different methodologies to assess sperm motility.

The Brazilian College of Animal Reproduction [8] established that abnormal sperm percentages above 30% for cattle, equine and swine, 20% for sheep and goats, and 10% for poultry, compromise artificial insemination. Although it has not been advocated by CBRA, it is estimated that the percentages of acceptable sperm defects are higher for fish because their sperm concentration is higher than the terrestrial species [9, 10]. Other species also had relatively high rates of deformities, varying from 32 to 65% [9, 11].

To sum up, the GnRHa induced spermiation of similar quality and quantity of the CPE, and can be used in broodstock farms with one single injection for the reproduction of *B. amazonicus*.

References

[1] Lima FCT (2003) Subfamily Bryconinae (Characins, Tetras). In Check List of the Freshwater Fishes of South and Central America (eds RE Reis, SO Kullander and CJ Ferraris Jr, pp. 174–81. Porto Alegre: EDPURCS.
[2] Billard R, Cosson MP (1992) Some problems related to the assessment of sperm motility in freshwater fish. *Journal of Experimental Zoology* 261, 122–131.
[3] Bashiyo-Silva C, da Silva Costa R, de Castro Ribeiro D, Senhorini JA, Veríssimo-Silveira R, Ninhaus-Silveira A (2015) Hormonal induction of *Brycon cephalus* (Characiformes, Characidae) to spermiation using D-ala6, pro9net-mGnRH+ metoclopramide. *Zygote* 24, 319–325.
[4] Ninhaus-Silveira A, Foresti F, Veríssimo-Silveira R, Senhorini JA (2006) Seminal analysis, cryogenic preservation, and fertility in matrinxã fish, *Brycon cephalus* (Günther, 1869). *Braz Arch Biol Techn* 49, 651–659.
[5] Cruz-Casallas PE, Medina-Robles VM, Velasco-Santamaría YM (2006) Evaluación de diferentes crioprotectores para la crioconservación de espermatozoides de yamú (*Brycon amazonicus*). *Rev Colomb Cienc Pec* 19, 152–159.
[6] Pardo-Carrasco S, Suarez-Mahecha H, Muñoz-Lara D, Arias-Castellanos J, Hernando GIL (2002) Inducción de la ovulación y del desove del yamú, *Brycon siebenthale*, con implantes de mGnRH-a. *Bol Inst Pesca* 28, 19–24
[7] Gallego V, Asturiano JF (2019). Fish sperm motility assessment as a tool for aquaculture research: a historical approach. *Rev Aquac* 11.
[8] Colégio Brasileiro De Reprodução Animal – CBRA (2013) Manual para exames andrológicos e avaliação de sêmen animal 3rd edn, Belo Horizonte, MG, 104 pp
[9] Streit DP Jr, Sirol R.N, Ribeiro RP, Digmayer M, Galo JM, Moraes GV and Povh JA (2012) Parâmetros seminais de reprodutores de *Pseudoplatystoma reticulatum*, em cativeiro, pré e pós-indução hormonal. *Rev Bras Reprod An* 36, 188–93.