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Seminal characteristics of piabanha before and after induction with different hormones

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ABSTRACT. The migratory species *piabanha* does not reproduce in lentic environments since it requires environmental stimuli for the maturation and extrusion of gametes, and therefore hormonal induction is mandatory. Current study compares the seminal characteristics of *Brycon insignis* without any hormonal induction (Control - Ctrl) and with two types of hormonal inductors, or rather, carp pituitary extract (T1 - 2.5 mg kg^{-1} body weight) and GnRH analogues, the latter applied in two different concentrations (T2 - 0.7 mg kg^{-1} body weight and T3 - 1.4 mg kg^{-1} body weight). Post-induction analyses showed that the hormones increased the motility rate - Ctrl (95%), T1 (100%), T2 (100%) and T3 (98%), although sperm concentration - Ctrl (11.52 x 10^9); T1 (4.37 x 10^9); T2 (4.34 x 10^9); T3 (4.01 x 10^9) decreased. Assessments for sperm vigor, motility time and spermatic morphology did not vary with hormonal induction. Hormonal inducer does not alter negatively the seminal characteristics of the piabanha, and the choice for the proper hormone depends on the preference of the dispenser.

Keywords: *Brycon insignis*, carp pituitary extract, GnRH analogues, sperm.

Características seminais de piabanha antes e após a indução com diferentes hormônios

RESUMO. A espécie migradora piabanha não possui a capacidade de reproduzir em ambientes lênticos devido à necessidade de estímulos ambientais para a maturação e extrusão dos gametas, por isso a necessidade da indução hormonal. No presente estudo, as características seminais do *Brycon insignis* foram comparadas sem indução hormonal (Ctrl) e utilizando dois tipos de indutores hormonais - Extrato de Hipófise de Carpa (T1 - 2,5 mg kg^{-1} de peso vivo) e Análogos de GnRH, sendo este último aplicado em duas concentrações distintas (T2 - 0,7 mg kg^{-1} de peso vivo e T3 - 1,4 mg kg^{-1} de peso vivo). As análises realizadas após a indução mostraram que os hormônios utilizados produziram um aumento da taxa de motilidade - Ctrl (95%), T1 (100%), T2 (100%) e T3 (98%), porém houve uma diminuição na concentração espermática - Ctrl (11,52 x 10^9), T1 (4,37 x 10^9), T2 (4,34 x 10^9) e T3 (4,01 x 10^9). Os restantes das avaliações, vigor espermático, tempo de motilidade e morfologia espermática não apresentaram variações com a indução hormonal. Portanto, a utilização do indutor hormonal não altera negativamente as características seminais de piabanha, e a escolha do mesmo se deve à preferência do manipulador.

Palavras-chave: *Brycon insignis*, extrato de hipófise de carpa, análogos de GnRH, sêmen.

Introduction

The species *Brycon insignis*, popularly known as piabanha in Brazil (FOWLER, 1951), is one of the most fished species in the country, mainly in the Paraíba do Sul region (MACHADO; ABREU, 1952). Although highly appreciated for its meat and high market value, the *B. insignis* is scantily exploited for commercial purposes and is rather found in fish farms for restocking and conservation purposes (SHIMODA et al., 2007). Precisely for such purposes, studies on the seminal characteristics of this Brazilian native species are of great importance, especially for the acquisition and improvement of its several reproductive traits, in captivity (VIVEIROS; GODINHO, 2009).

Like all rheophilic fish species, the piabanha needs hormone treatment to trigger and increase the release of gametes in captivity. Although hypophysation is one of the most costly methods of hormonal induction, it is the most used method in Brazil (WOYNAROVICH; HORVÁTH, 1983). Synthetic hormone compounds, such as mammalian GnRH analogues, have been successfully tested to induce fish reproduction (DAS, 2000; 2004; ULIKOWSKI, 2004; KRÓL et al., 2009; TARGOŃSKA; KUCHARCZYK, 2011), and have

proved to be easy-to-use and low-cost products when compared to pituitary hormones.

Current assay compares the seminal quality of *B. insignis* before and after induction with hypophysation and GnRH analogues.

Material and methods

Place and animals

The seminal collection and evaluation of the animals were performed at a commercial fish farm in Nova Mutum, Mato Grosso State, Brazil (13°49'44"S and 56°04'56"W). During the reproductive period twelve *B. insignis* males were selected and underwent mild abdominal massage in the craniocaudal direction to release the semen.

Hormonal induction

Before the hormonal treatment, the animals were fasted for 24 hours. Breeding fish were weighed separately and subdivided into three groups according to hormones and doses: T1 – Carp Pituitary Extract (CPE - 2.5 mg kg^{-1} body weight); T2 - GnRH analogues (0.7 mg kg^{-1} body weight) and T3 - GnRH analogues (1.4 mg kg^{-1} body weight). The hormone was administered near the base of the dorsal fin, with an interval of 7 hours between the application and the seminal collection.

The qualitative and quantitative seminal characteristics of each animal were evaluated before and after the administration of hormones, and the control group (Ctrl) was composed of the semen samples taken before hormonal induction.

Collection and evaluation of semen quality

Prior to the collection of the milt, the urogenital papilla was cleaned with paper towels to prevent contamination by feces or urine, and the premature activation of sperm cells. The semen was then collected by lightly massaging the coelomic wall in a craniocaudal direction and the samples were placed in sterile test tubes and kept at room temperature (23°C).

After collecting the semen, the samples, with and without hormonal induction, were analyzed by a single examiner, respecting the following protocols:

- Motility rate and sperm vigor: a 2 μL aliquot of the collected semen was diluted in 100 μL of distilled water. Subsequently, 20 µL of this dilution were placed between a slide and coverslip, and rates from 0 to 100% were assigned to the motility rate, depending on the percentage of motile sperm; and point rates from 1 to 5 were assigned to the vigor of the spermatic movement. The variables were analyzed with the 40X objective of an optical microscope.

- Motility time*:* when diluting the semen with distilled water in the previous analysis, a stopwatch was started and only stopped when the sperm's flagellar beats ceased. Motility rates were calculated in seconds.

- Concentration and sperm morphology: a 2 μL aliquot of semen was diluted in 2000 µL of saline-buffered formalin. Sperm concentration was obtained from this dilution by a Neubauer chamber under a 100X objective, counting the number of sperms present on the slide. In the case of sperm morphology, a sample of the initial dilution (100 μL) was placed on a histological slide and stained with Rose Bengal, following Streit Junior et al. (2004). Sperm morphology was evaluated according to Miliorini et al. (2011), sorting the sperm into the categories 'normal' and 'abnormal'.

Experimental design

The design was completely randomized, with control and three treatments (Ctrl - without induction; T1: CPE - 2.5 mg kg^{-1} body weight; T2: GnRH analogues 0.7 mg kg^{-1} body weight; T3: GnRH analogues 1.4 mg kg^{-1} body weight). A normal analysis for all dependent variables was performed with Shapiro-Wilk test. The sperm motility and vigor were converted into a rannor function and the variable "sperm concentration" was converted into a logarithm. Collected data were submitted to an F test for analysis of variance; when differences between the means were detected, Tukey´s test was performed at a significance level of 5%. Analyses were performed with the General Linear Model (GLM) by Statistical Analysis System, version 9.4 (SAS, 2013).

Results and discussion

Motility rate was higher ($p<0.05$) in treatments T1 and T2 when compared to control group, but sperm concentration was significantly reduced ($p<0.05$) by hormonal induction in all treatments. The variables sperm vigor and motility time showed no difference between treatments (Table 1).

Hormone inducement in reproduction increases plasma, sperm production and motility rates (CLEARWATER; CRIM, 1998). Improvement in sperm quality in current study may probably be justified by the composition of hormones chosen. In fact, carp pituitary extract is composed of 11-ketotestosterone, testosterone, 17α,20β-dihydroxy-4-pregnem-3-one or 17.20β,21-trihydroxy-4-pregnen-3-one (SCHULZ; MIURA, 2002), whereas GnRH

Table 1. Qualitative and quantitative parameters of the semen of *B. insignis* before and after induction, with different hormones and dosages.

Variable	Ctrl	T1	T2	T3	P*
MR (%)[(1)]	95.83 ± 6.10b	100.00 ± 0.00a	100.00 ± 0.00a	98.75 ± 2.50ab	0.0283
SC (x10^9)[(2)]	11.42 ± 6.69a	4.37 ± 1.69b	4.34 ± 2.41b	4.01 ± 0.53b	0.0012
SV (1-5)[(3)]	1.92 ± 0.90	3.00 ± 0.82	2.75 ± 0.96	3.00 ± 0.92	0.4179
MT (seg.)[(4)]	58.33 ± 8.07	63.25 ± 4.99	64.50 ± 10.63	65.50 ± 12.12	0.4197

Statistical differences for dependent variables are represented by letters on the same line. Ctrl - Control (without hormonal induction); T1 – Carp Pituitary Extract (CPE - 2.5 mg kg^{-1} body weight); T2 - GnRH analogues (0.7 mg kg^{-1} body weight) and T3 - GnRH analogues (1.4 mg kg^{-1} body weight). [(1)] - MR (Motility Rate); [(2)] - SC (Sperm Concentration); [(3)] - SV (Sperm Vigor); [(4)] - MT (Motility Time).

analogues comprise D-Ala6, Pro9Net-mGnRH, metoclopramide and dopamine (DAS, 2004).

Previous studies on hormonal administration show lower motility rates than those found in current analysis. Andrade-Talmelli et al. (2001) employed hCG and obtained a 90% rate in their evaluation of *Brycon insignis* semen, while Pardo-Carrasco et al. (2006) reported motility rates between 49 and 86% in the semen of *Brycon amazonicus* subjected to different hormonal inducers, respectively, CPE and GnRH-a. In a study on *Brycon nattereri* (OLIVEIRA et al., 2007) motility was evaluated at around 100% after hormonal induction with CPE, corroborating result in current assay.

Despite increased motility rate after induction, the spermatic vigor did not change. Response pattern in *B. insignis* was similar to that observed by Streit Junior et al. (2008b) in the species *Salminus maxillosus*, where they failed to report any difference before and after induction with CPE. Hormonal induction with CPE altered the sperm vigor in another rheophilic species, *Leporinus elongatus*, reducing it from 2.93 to 2.37 (STREIT JUNIOR et al., 2008a).

The duration of sperm motility generally coincides with the time the micropyle oocyte remains open (COSSON, 2004). In current study, sperm motility lasted about 65 seconds in hormone-induced animals. This time was considered sufficient for fertilization of oocytes in artificial reproduction since the micropyle closed minutes after hydration of the structure by water (NAKATANI et al., 2001). In another *Brycon* species, the reported average motility time was lower than that in current study, with approximately 34 seconds in *B. amazonicus* induced with GnRH-a (PARDO-CARRASCO et al., 2006), and with approximately 39 seconds in *B. orthotaenia* induced with CPE (MELO; GODINHO, 2006). Motility times are also quite variable in other South American neotropical fish species, and rates between 128 and 164 seconds have been reported for *Prochilodus lineatus* induced with GnRH-a (PAULA et al., 2012) and 486 seconds for *Piaractus mesopotamicus* induced with CPE (MARIA et al., 2004).

In the case of sperm concentration, a great variation exists among species of teleosts (VIVEIROS; GODINHO, 2009) and even within the same species. Semen of *B. insignis* induced with CPE (CPE 0.5 mg kg^{-1} body weight) was more concentrated than that in current study, or rather, 24.38 x 10^9 (SHIMODA et al., 2007).

When compared to that reported prior to hormonal induction in the control group (11,4 x 10^9), reduction in sperm concentration after induction in all treatments (average of 4.29 x 10^9) may be due to testicular hydration caused by hormonal induction (SCHULZ; MIURA, 2002; VIVEIROS et al., 2002;. MARIA et al., 2011). According to Maria and Carneiro (2012), testicular hydration increased seminal volume in assisted reproduction and a better handling of gametes. The importance of sperm concentration in determining the correct sperm:oocyte ratio is worth mentioning since it enhances a better fertilization rate (BOMBARDELLI et al., 2006; SANCHEZ et al., 2009; LEITE et al., 2013).

The morphological analysis of semen showed no significant difference between control and treatments ($p>0.05$) (Tables 2 and 3).

Cell morphology in current assay was not altered by hormonal inductors. Result was similar to assay on *Cyprinus carpio* and *Prochilodus lineatus* (MORAES et al., 2004) and *P. mesopotamicus* (STREIT JUNIOR et al., 2006). However, the CPE induction reduced the occurrence with abnormal sperm of *C. macropomum* from 25 to 15% after hormone administration (MARIA et al., 2011). According to Streit Junior et al. (2008b), sperm morphology is an important parameter for assessment and is related to the low rate of fertilization and motility.

Although varying among species, morphology may also be changed within the same species, according to treatment. The use of different hormones in *Leporinus macrocephalus* showed different rates for the number of normal sperm, namely, 51% with CPE and 31.2% with rabbit pituitary extract (MORAES et al., 2004).

Although a classification standard exists for sperm pathologies in mammalian semen (CBRA, 2013), there is still no standard of acceptable conditions in fish (MILIORINI et al., 2011) and the great variation in the neotropical species of South America makes it difficult to conduct comparative studies.

Table 2. Assessment of sperm morphology before and after hormone induction, with different dosages and hormones in *B. insignis*.

Variable	Ctrl	T1	T2	T3	P⋆
NS (%)[(1)]	39.99 ± 5.00	41.14 ± 4.80	28.35 ± 14.63	31.21 ± 13.61	0.2854
PC (%)[(2)]	2.64 ± 2.29	1.50 ± 0.93	2.12 ± 1.34	2.29 ± 1.44	0.2685
SC (%)[(3)]	57.36 ± 5.55	57.37 ± 4.28	69.53 ± 13.57	66.50 ± 13.23	0.3611

Ctrl - Control (without hormonal induction); T1 – Carp Pituitary Extract (CPE - 2.5 mg kg^{-1} body weight); T2 - GnRH analogues (0.7 mg kg^{-1} body weight) and T3 - GnRH analogues (1.4 mg kg^{-1} body weight). [(1)] - NS (Normal Sperm); [(2)] - PC (Primary Change); [(3)] - SC (Secondary Change).

Table 3. Percentage distribution of damages on semen before and after induction, with different hormones and dosages.

Variables[(1)]	Ctrl	T1	T2	T3	P⋆
Macro	0.25 ± 0.47	0.00 ± 0.00	0.25 ± 044	0.30 ± 0.52	0.0835
Micro	0.34 ± 0.66	0.51 ± 0.44	0.81 ± 0.84	0.99 ± 0.86	0.1601
PCD	0.24 ± 0.68	0.21 ± 0.37	0.25 ± 0.44	0.00 ± 0.00	0.1385
DCD	1.82 ± 2.40	0.78 ± 0.83	0.79 ± 0.84	1.00 ± 1.16	0.9922
IH	5.01 ± 2.37	2.55 ± 0.90	1.71 ± 1.57	2.07 ± 2.95	0.4873
DH	0.81 ± 0.97	1.20 ± 1.42	1.82 ± 0.61	2.80 ± 1.97	0.1239
MD	0.55 ± 0.66	0.44 ± 0.38	0.28 ± 0.49	0.98 ± 1.10	0.1601
FT	33.36 ± 6.29	35.31 ± 5.22	45.30 ± 10.46	37.94 ± 10.63	0.2116
BT	6.69 ± 4.19	8.68 ± 4.66	8.43 ± 5.94	6.63 ± 4.60	0.6919
CT	2.24 ± 1.50	3.16 ± 2.08	6.03 ± 6.11	5.22 ± 3.96	0.6689
DT	8.70 ± 6.51	6.01 ± 1.75	5.96 ± 2.16	10.85 ± 4.18	0.3716

Ctrl - Control (without hormonal induction); T1 – Carp Pituitary Extract (CPE - 2.5 mg kg^{-1} body weight); T2 - GnRH analogues (0.7 mg kg^{-1} body weight) and T3 - GnRH analogues (1.4 mg kg^{-1} body weight). [(1)] - Variables: Macro (Macrocephaly); Micro (microcephaly); PCD (Proximal Cytoplasmic Droplet) and DCD (Distal Cytoplasmic Droplet); IH (Isolated Head); DH (Degenerated Head); MD (Midpiece Degenerated); FT (Folded Tail); BT (Broken Tail); CT (Coiled Tail); DT (Degenerate Tail).

Conclusion

Hormone induction in *Brycon insignis* with carp pituitary extract and GnRH analogues enhanced a greater seminal motility rate when compared to that of specimens with no additional hormones. Contrastingly, induction caused an increase in seminal plasma, the effects of which could be observed in the low concentration of sperm cells in the semen of hormone-induced animals.

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