Revista Ciência Agronômica, v. 55, e20228660, 2024
Centro de Ciências Agrárias - Universidade Federal do Ceará, Fortaleza, CE
www.ccarevista.ufc.br

Scientific Article
ISSN 1806-6690

Evaluation of different hormone inducers on the reproduction of *Prochilodus brevis* males[1]

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ABSTRACT - The use of carp pituitary extract (CPE) in fish reproduction may have drawbacks, whereas analogues of mammalian (aGnRHm) or salmon (aGnRHs) gonadotropins containing dopamine inhibitor (DI) may offer greater advantages. The objective of this study was to evaluate the effect of different hormone inducers on the reproduction of *Prochilodus brevis* males. A total of 44 specimens of *P. brevis* were divided into six groups, namely, CPE 4.0 mg; saline solution 1.0 mL; aGnRHm+DI 0.3 mg and 0.5 mg; and aGnRHs+DI 0.25 mL and 0.35 mL kg^{-1} of live weight. The number of ejaculates, seminal volume, curvilinear velocity (VCL), straight-line velocity (VSL), average path velocity (VAP), sperm morphology and concentration, and membrane and DNA integrity were determined. Sample normality was examined using the Shapiro-Wilk test, and homogeneity using the Levene test. Analysis of variance (ANOVA) was conducted, followed by Tukey's test to compare means, with statistical significance set at $p < 0.05$. Notably, all fish induced with CPE 4.0 and aGnRHm+DI demonstrated ejaculation, yielding a greater seminal volume. Sperm concentration under aGnRHs+DI 0.35 exceeded that with aGnRHm+DI 0.3 and CPE 4.0. Regarding velocity, aGnRHm+DI 0.3 outperformed aGnRHs+DI 0.35 in VCL, while aGnRHm+DI 0.3 and 0.5 and aGnRHs+DI 0.25 led to higher VSL than aGnRHs 0.35. Additionally, VAP was elevated under aGnRHm+DI 0.3 and CPE, in contrast to aGnRHs+DI 0.35. Improved sperm morphology was observed with aGnRHm+DI 0.5 and aGnRHs+DI 0.25 compared to aGnRHm+DI 0.3. The remaining parameters did not differ significantly.

Key words: Brazilian bocachico. Carp pituitary. Gonadotropins.

DOI: 10.5935/1806-6690.20240004
Editor-in-Chief: Prof. Alek Sandro Dutra - alekdutra@ufc.br
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Received for publication 13/12/2022; approved on 14/08/2023
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INTRODUCTION

Prochilodus brevis, commonly known as the Brazilian bocachico or common curimatã, holds significant ecological importance within the fluvial ecosystems it inhabits due to its detritivore feeding behavior (NUNES *et al*., 2016). Moreover, it enjoys commercial value, particularly in the northeast region of the country (ALMEIDA-MONTEIRO *et al*., 2020). However, due to the unique characteristics of its eggs, which experience an increase in production during the reproductive period from December to May, the species faces a threat to survival, necessitating protection during the closed period (ALMEIDA-MONTEIRO *et al*., 2020).

As a rheophilic fish, the Brazilian bocachico migrates kilometers against the current, particularly during the rainy season, to initiate reproduction. This migration serves as a crucial trigger for the hormonal cascade, influenced by environmental stimuli (NUNES *et al*., 2016). Hormonal spikes are released in the hypothalamic-pituitary-gonadal axis through the bloodstream, leading to testicular hydration and heightened seminal plasma in males, and migration as well as the rupture of the germinal vesicle in females (MYLONAS; DUNCAN; ASTURIANO, 2017).

In confined or hormonally induced conditions within artificial environments, rheophilic fish are capable of releasing gametes (MYLONAS; DUNCAN; ASTURIANO, 2017). The most frequently employed hormone inducer for rheophilic fish, including the Brazilian bocachico, is carp pituitary extract (CPE) (BALDISSEROTO, 2018). This natural inducer contains gonadotropins from donor fish, stimulating gamete release in recipient fish (BALDISSEROTO, 2018). However, CPE is both costly and has been associated with disadvantages (SOUZA *et al*., 2018).

An alternative option lies in using gonadotropin analogues (aGnRH) that possess high potency, capable of stimulating pituitary hormones like luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (MARTINS *et al*., 2017; SOUZA *et al*., 2018; VIVEIROS *et al*., 2015). These analogous inducers, such as mammalian GnRH (aGnRHm) or salmon (aGnRHs), are cost-effective and readily available in the fish market. These inducers may also be associated with a dopamine inhibitor (DI), a GnRH antagonist (MARTINS *et al*., 2017; VIVEIROS *et al*, 2015).

Despite their widespread use, pituitaries from mature fish can lead to immune reactions and disease transmission to recipient fish (MYLONAS; DUNCAN; ASTURIANO, 2017). Therefore, the use of natural hormone inducers like CPE, derived from the pituitary of other fish, can pose risks to the reproduction of the Brazilian bocachico, potentially transmitting diseases and causing batch-to-batch variability in commercial products (MYLONAS; DUNCAN; ASTURIANO, 2017). Conversely, mammalian GnRH analogues associated with a dopamine inhibitor offer advantages by targeting early stages of the hormonal cascade, directly affecting the recipient fish's pituitary gland and stimulating other gonadotropic hormones at a lower cost (MARTINS *et al*., 2017; SOUZA *et al*., 2018; VIVEIROS *et al*., 2015). This study aimed to test different hormone inducers to enhance the release and quality of seminal fluid in *P. brevis*.

MATERIAL AND METHODS

Animals, facilities, and authorization

Conducted in March 2022, coinciding with the reproductive period of the species, the experiment took place at the Laboratory of Biotechnology of Fish Reproduction (LBRP) on the Itaperi Campus of the State University of Ceará (UECE), Fortaleza, Ceará, Brazil. Ethical approval was obtained from the UECE Ethics Committee (approval no. 03463435/2022). The animals were provided with commercial feed (32% protein), both macerated and in grain form, and were housed in a 7000-L fiberglass tank equipped with a continuous water recirculation system and aeration facilitated by an air compressor using porous stones.

Hormonal induction and semen collection

For hormone induction, a total of 44 males were utilized, possessing an average weight of 160 g and an average length of 20 cm. These specimens were divided into six distinct groups: Group 1 received a placebo induction via the injection of saline solution (SS) at a rate of 1.0 mL kg^{-1} live weight, serving as the negative control; Group 2 was administered carp pituitary extract at a dosage of 4.0 mg kg^{-1} live weight, serving as the positive control; Group 3 received aGnRHm + DI (OVOPEL®) at the dose of 0.3 pellet kg^{-1} live weight; Group 4 received aGnRHm + DI (OVOPEL®) at 0.5 pellet kg^{-1} live weight; Group 5 was subjected to aGnRHs + DI (OVAPRIM®) at 0.25 mL kg^{-1} live weight; finally, Group 6 underwent aGnRHs + DI (OVAPRIM®) at 0.35 mL kg^{-1} live weight. This experimental setup was carried out under a completely randomized design.

Due to the distinct characteristics of hormonal action, the induction procedures for the various groups occurred at different times. Specifically, at 17h00, the administration of saline solution at a rate of 1.0 mL kg^{-1} live weight, CPE at 4.0 mg kg^{-1} live weight, and aGnRHm + DI 0.3, and aGnRHm+DI 0.5 were conducted for the first set of groups. Semen collection was performed after a span of 14 h

following induction (PEREIRA *et al*., 2020). Subsequently, at the time of seminal collection for these groups, the hormone induction of the aGnRHs + DI groups took place at 07h00. This involved administering aGnRHs + DI 0.25 and aGnRHs + DI 0.35, with the collection of semen performed six hours post-induction (VIVEIROS *et al*., 2015). All applications were accomplished through intracoelomic injections (LOPES *et al*., 2014; VIVEIROS *et al*., 2015).

In the semen collection process, animals were individually sedated using a solution containing eugenol, alcohol, and water in a ratio of 1:10:10,000 (LOPES *et al*., 2014; NUNES *et al*., 2016). When exhibiting signs of imbalance, animals were transferred to the collection area where their ocular region was covered with a damp cloth and the urogenital papilla was carefully cleaned and dried to prevent contamination (LOPES *et al*., 2014). Soon after, an abdominal massage was performed in a craniocaudal direction to facilitate semen release (NUNES *et al*., 2016; PEREIRA *et al*., 2020). Seminal content was collected using a syringe or graduated microtube. Collected samples were promptly placed in a thermal box with ice at around 4 °C for preservation until further analysis (NUNES *et al*., 2016).

Seminal analysis

Semen volume (mL) was quantified by measuring the collected amount in each graduated microtube (NUNES *et al*., 2016). Ejaculation status was categorized based on visible semen presence; animals with even minimal semen were considered "ejaculated," while those lacking visible semen were deemed "non-ejaculated."

Kinetic analysis was conducted within one hour of collection using computerized semen analysis software, specifically (Sperm Class Analyser, SCA) by Microptics®, configured for fish. A microvolume (1 µL) of semen from each animal was placed on a Makler chamber, activated with NaCl (220 mM), and subjected to parameter analysis, including total motility (%), curvilinear velocity (VCL, µm s^{-1}), straight-line velocity (VSL, µm s^{-1}), and average path velocity (VAP, µm s^{-1}) (ALMEIDA-MONTEIRO *et al*., 2020).

For assessing Brazilian bocachico sperm morphology, semen was fixed using a 4% formalinized citrate solution (1:100; semen:fixative). A stained aliquot was prepared using Rose Bengal dye (4:20 ratio; dye:fixed semen) following Nunes *et al*. (2016). Using the smear technique, two slides were prepared with 100 spermatozoa each, and examined under an optical microscope (400x) (ALMEIDA-MONTEIRO *et al*., 2020). Classification followed the method outlined by Miliorini *et al*. (2011).

The integrity of sperm cell plasma membranes was evaluated using the smear technique and eosin-nigrosin staining. A mixture of semen, eosin, and nigrosin (1:2:2 ratio) was applied to slides, and 200 spermatozoa per slide were assessed for membrane integrity using a light microscope (400 x). Those with an intact membrane were considered to be colorless and those with a ruptured membrane were considered to have a pinkish/red tinge (ALMEIDA-MONTEIRO *et al*., 2020).

DNA integrity was determined using the Sperm Chromatin Dispersion (SCD) test, based on the fragmentation rate of sperm chromatin, according to Fernández *et al*. (2005), following adaptations by Almeida-Monteiro *et al*. (2020) and adaptations from this very study. Two hundred spermatozoa were analyzed per slide using a phase contrast microscope coupled to a camera (Nikon Eclipse 50i, Tokyo, Japan) in a CASA system. The presence of a halo around the sperm head was analyzed, and cells with an outer halo indicated intact DNA, while those without a halo indicated DNA fragmentation.

Sperm concentration was determined by fixing semen samples in saline formalin (1:1000 ratio; semen:formaldehyde). A Neubauer chamber was used for counting, with 10 µL of each sample (LOPES *et al*., 2014).

Statistical analyses

Statistical analyses were performed using the R statistical environment (R CORE TEAM, 2019). Data are presented as means ± standard deviation and were calculated using the dplyr package (WICKHAM *et al*., 2019).

Fisher's Exact Probability Test, available in the stats package (R CORE TEAM, 2019), was employed to assess the influence of treatments and concentrations on ejaculation frequency. One-tailed tests were utilized to compare probabilities ($P < 0.05$).

For variables requiring transformation, such as Frequency, DNA integrity, motility, and morphology, the square root of the arcsine was applied (Equation 1: y=sin^(-1)⁡[√x×(180⁄π)), to achieve normal distribution. Continuous variables (VCL, µm s^{-1}; VSL, µm s^{-1}, VAP, µm s^{-1}, and concentration) were kept in their original magnitudes.

Data normality was tested using the Shapiro-Wilk test, and homogeneity was assessed using the Levene test. Once the assumptions of normality were met, significant differences were evaluated between the aGnRHm+DI and aGnRHs+DI treatments and their respective concentrations, as well as CPE (positive control) and SS (negative control) using one-way ANOVA analysis. Tukey's test was employed for mean comparisons ($P < 0.05$).

RESULTS AND DISCUSSION

This study marks the pioneering comparison of hormonal induction of reproduction in *P. brevis*, utilizing mammalian and salmon GnRH in conjunction with antidopaminergic agents. Table 1 presents the reproductive characteristics of *P. brevis* semen, including ejaculation parameters, volume, and concentration, following various hormonal treatments in comparison to controls.

Mean percentage and standard error (±) values for volume, concentration, and ejaculation based on induced animals. Statistical significance level: $p < 0.05$. Different superscript letters indicate significant differences ($p < 0.05$). SS - saline solution; CPE - carp pituitary extract; aGnRHm + DI - mammalian GnRH analogue with dopamine inhibitor; aGnRHs + DI - salmon GnRH analogues with dopamine inhibitor. *n/nt – number of animals that ejaculated over the total number of animals induced

Semen collection from *P. brevis* was attempted using SS 1.0 (placebo), resulting in only two out of four animals ejaculating, with negligible semen volume, rendering measurement unfeasible (Table 1). Conversely, CPE 4.0 induced a seminal volume of 0.62 mL, with successful ejaculation in all eight animals. The aGnRHm + DI treatment group demonstrated superior seminal volume efficiency compared to the SS 1.0 group (Table 1). Furthermore, a higher number of animals ejaculated in the aGnRHm + DI group compared to the aGnRHs + DI group.

The number of animals that ejaculated in the aGnRHs + DI group was unexpected. This could be attributed to the potential potentiating effect of the antidopaminergic agent on the reproductive response of GnRH. Antidopaminergics inhibit dopamine, a dopaminergic neurotransmitter that restrains the synthesis and release of GnRH into the middle eminence and portal hypophyseal circulation (MYLONAS; DUNCAN; ASTURIANO, 2017). A study involving the induction of reproduction using aGnRH hormones (gonadorelin) in *Hypancistrus zebra* reported variations in semen release among animals, contrasting with the uniform gametic release seen with carp pituitary homogenate (CPH) as control (CALDAS *et al*., 2021). This is akin to the outcomes of the current study using GnRHs in conjunction with the antidopaminergic domperidone. In the *H. zebra* study, the disparity in ejaculation was attributed to immunological factors (CALDAS *et al*., 2021). In the present research, using metoclopramide as the antidopaminergic (aGnRHm + DI), all animals achieved semen release, often in larger volumes than those treated with domperidone (aGnRHs + DI), suggesting that the specific type of dopamine inhibitor could influence outcomes.

In certain fish species, two doses of inducing hormones are necessary for complete maturation and ejaculation. For instance, in *P. brevis*, two doses of CPE 4.0 were applied to prompt release (NUNES *et al*., 2016), differing from our study, where a single dose led to 100% semen release. This variation could stem from inconsistencies in CPE 4.0 composition and a lack of control over hormone proportion and presence due to collection from animals in varying reproductive states.

Seminal volume is known to be influenced by the type and dosage of hormones employed for reproductive induction. Despite lacking intrinsic fertilization effects, seminal volume corresponds to the quantity of cells contained (FERNANDES *et al*., 2020; SOLIS-MURGAS *et al*., 2011). The increase in seminal volume following hormone application is attributed to heightened hydration of the testicular ducts under stimulation (BERNARDES JÚNIOR; BOMBARDELLI; NUÑER, 2017; MYLONAS; DUNCAN; ASTURIANO, 2017). This increase results from luteinizing activity stimulated by inducers, promoting augmented fluid production in sertoli cells and seminal vesicles, thus enhancing maturation-inducing steroid synthesis (CALDAS *et al*., 2021; MYLONAS; DUNCAN; ASTURIANO, 2017). Consequently, increased seminal volume occurs through hydrostatic pressure within the testes, facilitating greater displacement through the ducts (CALDAS *et al*., 2021; RAINIS; BALLESTRAZZI, 2005).

Table 1 - Hormone induction effects on *Prochilodus brevis* sperm parameters

Treatment	Ejaculation (n/nt*)	Volume (mL)	Concentration (sperm/mL)
SS 1.0	2/4	0 ± 0 b	26.00 ± 9.43 ab
CPE 4.0	8/8	0.62 ± 0.03 ab	24.30 ± 2.17 b
aGnRHm + DI 0.3	8/8	0.88 ± 0.04 a	17.03 ± 9.37 b
aGnRHm + DI 0.5	8/8	0.86 ± 0.86 a	29.61 ± 2.46 ab
aGnRHs + DI 0.25	4/8	0.5 ± 0.12 ab	44.06 ± 9.84 ab
aGnRHs + DI 0.35	7/8	0.22 ± 0.01 ab	59.89 ± 5.93 ab

Sperm concentration knowledge is crucial for successful fertilization due to spermatozoa-to-oocyte ratio (SANCHES *et al*., 2015). In this study, CPE 4.0 provided lower sperm concentration (109 spermatozoa/mL) compared to aGnRHs + DI 0.35 ($p < 0.05$) (Table 1). Among the inducers, aGnRHs + DI 0.35 outperformed aGnRHm + DI 0.3 ($p < 0.05$) (Table 1). Previous studies on *P. brevis* showed sperm concentrations induced by CPE 4.0 at 27.89 ± 6.13 (NUNES *et al*., 2016) and 41.19 ± 8.35 spermatozoa/mL (ALMEIDA-MONTEIRO *et al*., 2020). Comparatively, *P. lacustres* displayed 4.1 ± 2.0 spermatozoa/mL (no hormonal induction) (FERNANDES *et al*., 2020), while *P. lineatus* showed 13.6 ± 9.9 spermatozoa/mL (aGnRHs + DI 0.25 mg kg^{-1}) (VIVEIROS *et al*., 2015).

Considering the foregoing, it becomes evident that there exists a variation in sperm concentration in *P. brevis* or between species of the same genus. This variability stems from genetic diversity between individuals, alongside factors such as the timing of semen collection, management practices, and unique environmental conditions specific to each region (FERNANDES *et al*., 2020). Moreover, the general attributes of semen are subject to a multitude of influences, including nutritional status, age, weight of the animals, health condition, exogenous hormonal interventions, frequency and methodology of semen collection, intervals of sexual rest, and prevailing climatic conditions (SOLIS-MURGAS *et al*., 2011), which can also be subject to disruptions due to confinement conditions (ALMEIDA, 2013). Furthermore, the application of aGnRH serves to elevate the production of seminal fluid, as well as stimulate processes such as spermatogenesis, spermiogenesis, and spermiation (MARTINS *et al*., 2017). This surge in seminal fluid production may potentially contribute to the elimination of intratesticular spermatozoa, providing a rationale for the higher sperm concentration identified in this study.

Sperm kinetics constitutes a crucial parameter for the characterization of fish semen, given its direct correlation with the fertilization rate (FIGUEROA *et al*., 2016). In this study, no significant difference was noted in VCL between the positive control (CPE 4.0) and the other treatment groups (Table 2). However, when comparing the aGnRH + DI treatments, VCL demonstrated a lower value with aGnRHs + DI 0.35 than with aGnRHm + DI 0.3 (Table 2). Regarding VAP, both CPE 4.0 and aGnRHm + DI 0.3 yielded higher results compared to aGnRHs + DI 0.35 ($p > 0.05$) (Table 2). Similarly, when comparing the aGnRH + DI treatments, VCL was lower under aGnRHs + DI 0.35 than under aGnRHm + DI 0.3, and CPE 4.0 and aGnRHm + DI 0.3 demonstrated superior VAP values to aGnRHs + DI 0.35 ($p > 0.05$) (Table 2).

Among sperm kinetic parameters, VCL emerges as a paramount factor in seminal evaluation, as its curvilinear movement is instrumental in facilitating the sperm's entry into the oocyte (NUNES *et al*., 2016). In fact, a study conducted on a species from the same genus, *P. lineatus*, revealed that VCL exhibited the most significant positive correlation with the fertilization process, in cryopreserved semen. This relationship is corroborated by VCL, as it represents the actual trajectory of fish sperm (VIVEIROS *et al*., 2010).

While VCL is the most important velocity examined in the analysis of seminal dynamics within fish, notably *P. brevis*, due to the anticipated curvilinear path that spermatozoa traverse to locate the oocyte's micropyle (NUNES *et al*., 2016), it is crucial to recognize that the parameters evaluated in sperm kinetics are interconnected (SANCHES *et al*., 2015). Both VAP and VSL, when combined with VCL, assume significance in the fertilization process (VIVEIROS *et al*., 2010).

Regarding total motility, there were no significant differences between treatments ($p < 0.05$) (Table 2). The total motility achieved with CPE

Table 2 - Velocity and motility parameters of *Prochilodus brevis* spermatozoa following the application of different hormone inducers

Treatment	VCL (μm s^{-1})	VAP (μm s^{-1})	VSL (μm s^{-1})	Total motility (%)
SS 1.0	73.20 ± 18.31 ab	41.35 ± 10.92 ab	22.50 ± 5.79 ab	87.65 ± 1.23
CPE 4.0	102.91 ± 2.27 ab	72.83 ± 1.43 a	46.31 ± 0.97 a	95.20 ± 0.33
aGnRHm + DI 0.3	111.20 ± 5.14 a	69.35 ± 2.55 a	42.36 ± 1.59 a	92.97 ± 1.74
aGnRHm + DI 0.5	100.73 ± 3.28 ab	62.73 ± 1.79 ab	38.33 ± 1.23 ab	96.27 ± 0.58
AGnRHs + DI 0.25	102.20 ± 2.80 ab	65.92 ± 3.97 ab	45.07 ± 2.96 a	95.25 ± 0.50
aGnRHs + DI 0.35	61.18 ± 5.16 b	36.54 ± 3.60 b	21.85 ± 2.27 b	68.92 ± 5.29

Mean ± standard error. Statistical significance level: $p < 0.05$. Different superscript letters indicate significant differences ($p < 0.05$). SS - saline solution; CPE - carp pituitary extract; aGnRHm+DI - mammalian GnRH analogue with dopamine inhibitor; aGnRHs + DI - salmon GnRH analogues with dopamine inhibitor; VCL - curvilinear velocity; VAP - average path velocity; VSL - straight-line velocity

Table 3 - Normal morphology, membrane integrity, and DNA integrity of *Prochilodus brevis* spermatozoa following the application of different hormone inducers

Treatment	Normal morphology	Membrane integrity	DNA integrity
SS 1.0	71.25 ± 0.17 ab	84.75 ± 3.71	66.75 ± 11.49
CPE 4.0	67.00 ± 0.58 ab	87.50 ± 0.46	50.62 ± 1.2
aGnRHm + DI 0.3	49.50 ± 0.87 b	77.00 ± 4.00	65.43 ± 2.92
aGnRHm + DI 0.5	74.75 ± 1.41 a	83.93 ± 0.72	64.87 ± 3.06
aGnRHs + DI 0.25	80.12 ± 2.21 a	88.37 ± 0.32	74.37 ± 6.57
aGnRHs + DI 0.35	62.00 ± 2.64 ab	87.50 ± 0.48	61.28 ± 3.95

Mean ± standard error. Statistical significance level: $p < 0.05$. Different superscript letters indicate significant differences ($p < 0.05$). SS - saline solution; CPE - carp pituitary extract; aGnRHm + DI - mammalian GnRH analogue with dopamine inhibitor; aGnRHs + DI - salmon GnRH analogues with dopamine inhibitor

induction in *P. brevis* aligned with previous studies, recording values of 95.54 ± 3.63% (LOPES *et al*., 2014) and 99.08 ± 0.36% (ALMEIDA-MONTEIRO *et al*., 2020). In *P. lineatus*, CPE at 4.0 resulted in a total motility of 65 ± 21%, comparable to aGnRHs at 0.25 mL (76 ± 11%) (VIVEIROS *et al*., 2015).

In terms of sperm morphology, the percentage of normal sperm differed only from the aGnRHm + DI 0.3 treatment, being higher (Table 3). Among the aGnRH treatments, aGnRHm 0.3 displayed lower values than aGnRHm 0.5 and aGnRHs 0.25, though not differing from other treatments ($p < 0.05$) (Table 3). Earlier investigations reported *P. brevis* sperm morphology as 85.67 ± 6.52% (ALMEIDA-MONTEIRO *et al*., 2020) and 74.18 ± 10.63% normal cells (NUNES *et al*., 2016) following CPE induction.

Morphological normality holds significance, as even though CPE-induced sperm exhibit viable motility, reduced normality could hinder successful oocyte penetration, critically affecting fertilization potential (NUNES *et al*., 2016). The lower normality percentage observed with aGnRHm + DI 0.3 in comparison to other aGnRH treatments might be linked to the maturity stage of cells transitioning from spermatids to spermatozoa, released without complete gamete maturation (KAVAMOTO *et al*., 1999).

In terms of membrane and DNA integrity, no significant differences were observed between treatments (Table 3). Consequently, hormone treatments did not impact the plasma membrane or DNA of *P. brevis* sperm cells. Notably, this study marked the first instance of DNA integrity analysis being employed in seminal analysis of fish post hormone induction, warranting further investigation of this parameter.

The use of CPE has been effectively integrated into confined fish reproduction (GODINHO; GODINHO, 1986; SAHOO; GIRI; CHANDRA, 2008) due to its composition containing LH and FSH, pivotal for ejaculation success (QUESNEL; BRETON, 1995). Nonetheless, adverse effects from pituitary gland hormone inducers have been observed (MARTINS *et al*., 2017), prompting consideration of aGnRH as a viable alternative for maintaining favorable seminal quality. The commendable seminal quality achieved at specific aGnRH concentrations in this *P. brevis* study supports their application in this rheophilic species. This could stem from the pituitary being prompted to release endogenous gonadotropins, differing from the mechanism of CPE (BALDISSEROTO, 2018; MARTINS *et al*., 2017; SOUZA *et al*., 2018).

Furthermore, treatment cost per kilogram of live weight stood at BRL 1.19 for CPE 4.0, BRL 0.36 for aGnRHm 0.3, BRL 0.60 for aGnRHm 0.5, BRL 1.43 for aGnRHs 0.25, and BRL 2.00 for aGnRHs 0.35. Consequently, the most cost-effective concentrations in terms of kinetics were those below CPE commercial prices. In confinement-based reproduction of rheophilic species, induction is indispensable, with hormonal application being the most commercially widespread technique. Therefore, cost consideration is pivotal when opting for hormone-based methods, ensuring optimal cost-benefit in fish farming endeavors.

CONCLUSIONS

For the reproduction of *P. brevis* through hormonal induction, the use of GnRH analogues associated with a dopamine inhibitor can replace existing protocols with CPE. The treatments involving aGnRHm at concentrations of 0.3 or 0.5 mg kg^{-1} of live weight are particularly recommended based on their favorable cost-effectiveness. Importantly, these treatments exhibit minimal detrimental impacts on *P. brevis* sperm cells when compared to the use of carp pituitary extract.

ACKNOWLEDGMENTS

The authors extend their gratitude for the support received from the Cearense Foundation for Scientific and Technological Development (FUNCAP) and the National Council for the Improvement of Higher Education Personnel (CAPES).

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