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Determination of a Cryoprotective Solution for the Cryopreservation of Oreochromis Niloticus (Linnaeus, 1758) Bouake Strain Sperm Without Freezing

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Abstract | **Original Research Article**

Cryopreservation of sperm in freshwater Fish species requires different protocols. Therefore, studies are needed to establish reliable procedures for each species. Experiments have been realizedto identify the optimal cryoprotective solution that preserves high sperm motility for *Oreochromis niloticus* Bouake strain before cryopreservation. For this study, a batch of 29 male breeders with gonad weights ranging from 160.6 to 278.3 g were maintained in a thermostated aquaponic system whose water temperature varied from 25.41°C to 28.48°C. The gonad weights of these ales fluctuated between 0.293 and 2.263 g. A total of six different types of cryoprotectants, namely methanol (MeOH), dimethyl sulfoxide (DMSO), glycerol (Glyc), egg yolk (JO), coconut juice (ECO) and coconut milk (LCO), were tested at three concentration levels, namely 10%, 20% and 30% in two dilution media, including Ca free-HBSS 500 mOsmol/kg (saline solution) and Glucose 500 mOsmol/kg (sugar solution). The type and concentration of the cryoprotectant had a significant effect on sperm motility of *O. niloticus* **($p < 0.05$).** The best results were obtained with Ca-free HBSS (500 mOsmol/kg) and glucose (500 mOsmol/kg) extenders supplemented with 20% coconut juice (ECO). The highest motility values were obtained with 20% coconut juice, justifying its recommendation for sperm cryopreservation of *Oreochromis niloticus.*

Keywords: Sperm cryopreservation; Cryoprotectant; *Oreochromis niloticus*; Diluent; Motility; Coconut juice.

INTRODUCTION

Sperm cryopreservation allows fish to manage Fish genes resources for aquaculture within cryobanks. This for the lineage management in breeding schemes, or for managing domestic and wild diversity. Evaluation after freezing/thawing of sperm motility and fertilizing ability is most often considered sufficient to ensure the safety and effectiveness of genome transmission to offspring (Labbé, 2016). It is a process by which biological materials such as cells and tissues are preserved by cooling to very low temperatures in nitrogen liquid of around -196°. These cells The sperm cells remain viable throughout the process, after subsequent warming to temperature above 0°C (Agarwal, 2011).

Generally, cryopreservation reversibly halts cell metabolism while maintaining their integrity and functionality. The first successful sperm cryopreservation was carried out on breeding bulls over 60 years ago (Polge *et al*., 1949). Which led to numerous trials on other species, notably Fish species, with the first report of Fish sperm cryopreservation published by Blaxter in 1953 (Maisse *et al*., 1998). Following the breakthrough of this new technique, many publications demonstrated the feasibility of sperm cryopreservation in several freshwater Fish species, mainly Salmonids, sturgeons, and carps. However, this method of sperm preservation at low temperature (-196°C) must be carried out in the presence of cryoprotectants to limit or even prevent damage to living cells during storage.

Thus, the objective of this study is to identify the optimal cryoprotective solution that preserves highest sperm motility of *Oreochromis niloticus* Bouake strain, prior to cryopreservation. Specifically, it aims to evaluate the effect of cryoprotectants at different dilution

rates in saline or sugar extenders on the sperm motility of this Fish species.

MATERIALS AND METHODS

Study site

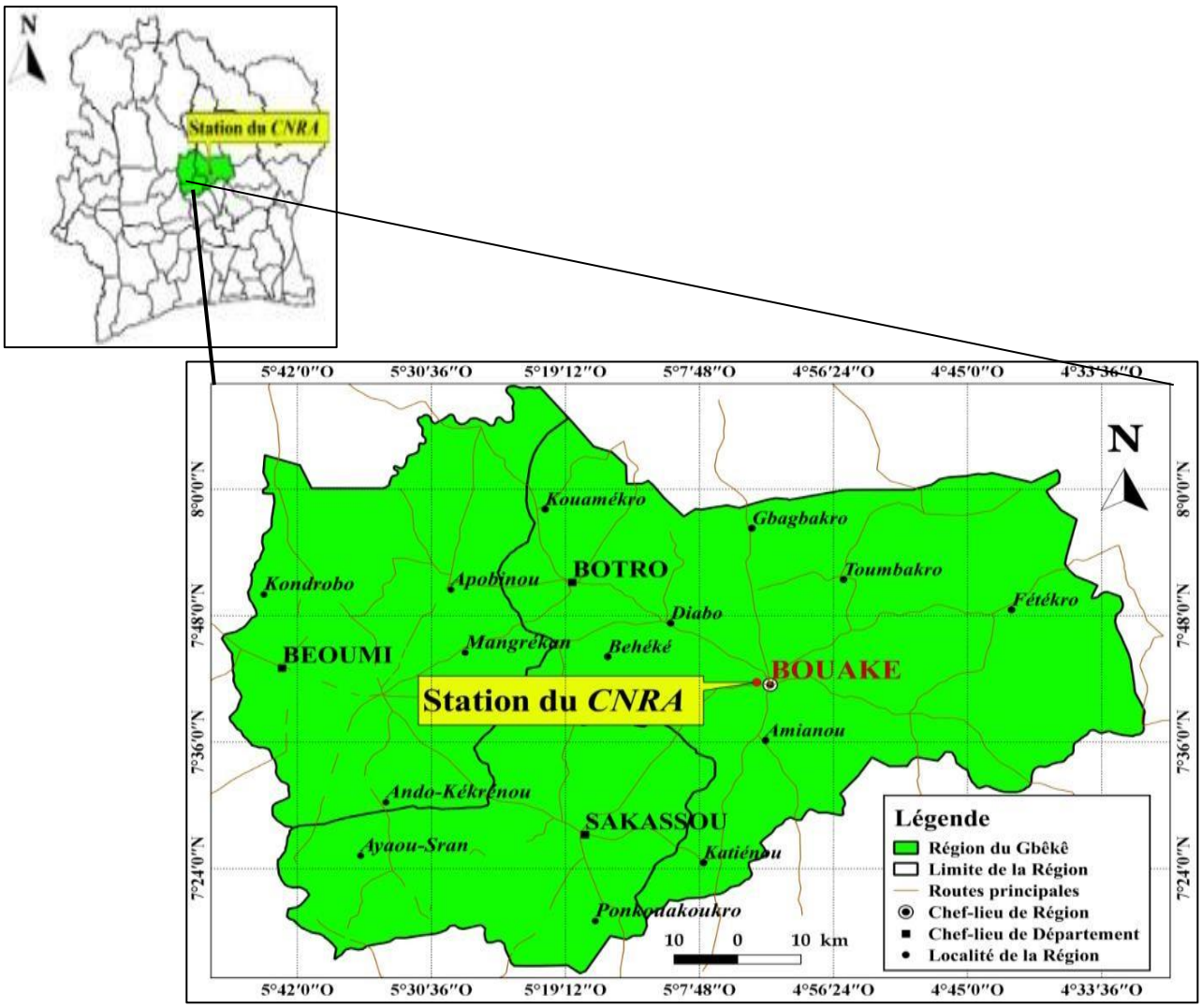

Figure: Location of the experimental study area

This work was carried out from January 2024 to May 2024 at the Laboratory of Ichthyology and Fish Genes Cryoconservation (LICGP) of the Research Station on Inland Fisheries and Aquaculture (SRPAC) of the Cote d'Ivoire National Center for Agronomic Research (CNRA). This research unit, initially named Kongodekro Fish farm of the Savanah institute (IDESSA), was created in 1956. It is located in the center of Cote d'Ivoire, in the Kongodekro classified forest (7°37' North latitude and 5°02' South longitude) at 6 km from Bouaké on the Bouake - Yamoussoukro road.

Handling of *Oreochromis niloticus* broodstock at the hatchery

For each experiment, a batch of 29 males specimens of *Oreochromis niloticus* weighing from 160.6 to 278.3 g stored in ponds, were selected and transfered to the station hatchery in a thermostated circular tank. This system was supplied with water from the CNRA-SRPAC forage. The temperature, dissolved oxygen and hydrogen potential of the water were measured daily using a multiparameter device.

Biological material and gamete collection

For this study, the biological material used consists, respectively, of a pool of male specimens of *Oreochromis niloticus*, acclimatized in tne thermostaed circular tank at 28 – 30 °C and spermatozoa of that Fish species. After a feeding period of at least two weeks with a 3A feed, the mature males were sampled from this broodstock, and transferred to the laboratory for anesthesia in a solution of 0.02 mL of clove essential oil + 4 mL of ethanol/L of water. Then, the anesthetized individuals are removed from the anesthesia tank and wrapped in paper towels to remove all traces of water and urine. These Fish specimens were then dissected and the testes collected and stored in a Petri dish at 4-5°C on ice in a polystyrene container. At this step, the testes are cleaned of any remaining tissue and blood capillaries with tissue paper and transferred to a new Petri dish, also

on ice, and weighed using a precision balance to 0.0001 g. Sperm is collected by grinding testicles in a sugar solution or a saline solution at the optimal dilution ratio obtained (1 :9).

Identification of a cryoprotective solution for the cryopreservation of *Oreochromis niloticus* sperm

This experiment was conducted according to the experimental protocol established by Vachon *et al*. (2013) for the cryopreservation of copper redhorse sperm in Quebec (Canada). For this trial, twenty-four (24) specimens of O. niloticus from the generator stock were used. For each test, the sperm from each male was stored in a graduated test tube, diluted and/or crushed in two sperm immobilization solutions, notably, a sugar solution (glucose, 500 mOsmol/kg) or a saline solution (Ca-free HBSS, 500 mOsmol/kg) at the optimal dilution ratio obtained (1: 20). Then, 18 subsamples of 50 µL of sperm solution were stored in their respective 1.5 mL cryotubes on ice. These sperm subsamples were mixed with equivalent volumes (50 µL) of cryoprotectants (methanol – MeOH; dimethyl sulfoxide – DMSO; glycerol – Glyc; egg yolk – YO; coconut juice – ECO; and coconut milk – LCO) to a final concentration of 10%, 20% and 30%. After mixing the sperm solution with the cryoprotectant solution, the sperm motility rate was determined by microscopic observation at 100x magnification. The maximum period (24 h) was tested and found to be sufficient to allow for all freezing procedures. The effectiveness of these cryoprotectant solutions was measured by monitoring sperm motility at incubation times of t_0 (initial time after sperm collection), t_2 (02 hours after sperm collection), t_4 (4 hours after sperm collection), t_6 (06 hours after sperm collection) then t_{24} (24 hours after sperm collection) after the test performed at t_0.

- ***Statistical analysis of data***

A one-way analysis of variance (ANOVA) was performed on the collected data to evaluate the effect of cryoprotectant concentrations on sperm motility. If the effect was significant, a Student-Newman-Keuls test was performed to identify the optimal diluent. The significance level was set at 5%. All analyses were performed using IBM SPSS software.

RESULTS

Physicochemical parameters in the handling water of *O. niloticus* broodstock

During the study period from January to May 2022, the water quality in the rearing circuit at the hatchery was verified by regularly measuring three physicochemical parameters (temperature, dissolved oxygen, and water potential). In the broodstock handling tank of *O. niloticus*. Concerning the physicochemical parameters measured in the broddstock specimens handling tank, water temperature fluctuated between 25.41°C and 28.48°C during the month of May. Oxygen levels varied from 1.49 mg/l (May) to 5.83 mg/l (March), and the pH fluctuated between 6.12 in January and 7.44 in April.

Table 1: Average evolution of the physicochemical parameters of the thermostated recirculating water in the broodstock handling tank during the study period (January 2024 to May 2024)

		Jan-24	Feb-24	March 24	Apr-24	May 24
Temperature	**Max**	26,66	27,61	27,52	28,15	28,48
	Min	25,49	25,50	26,75	27,20	25,41
	Average type ± Deviation	26,12 ± 0,42	26,82 ± 0,99	27,06 ± 0,33	27,68 ± 0,67	27,42 ± 1,20
Oxygen	**Max**	4,45	4,85	5,83	3,44	2,67
	Min	2,92	3,39	2,74	1,86	1,49
	Average type ± Deviation	3,69 ± 0,54	4,34 ± 0,65	4,35 ± 1,35	2,65 ± 1,12	1,93 ± 0,50
pH	**Max**	7,06	7,01	7,30	7,44	7,43
	Min	6,12	6,47	6,55	7,30	6,79
	Average type ± Deviation	6,46 ± 0,32	6,66 ± 0,24	7,07 ± 0,35	7,37 ± 0,10	7,08 ± 0,23

Identification of a cryoprotective solution for the cryopreservation of *O. niloticus* sperm

Tests carried out immediately (t_0) after sperm collection

The tests carried out at t_0 immediately after sperm collection at a ratio of 1 : 9 have shown a relatively satisfactory results. Initial comparisons showed, that the best motility rates were obtained in the storage container with Ca-free HBSS 500 mOsmol/kg, JO (10%), MeOH (10%), ECO (20%), LCO (20%), and DMSO (10%), with mean motility rates of 65.0 ± 0.0%, 55.0 ± 0.7.0%, 87.5 ± 0.35%, 80.0 ± 14.1%, and 67.5 ± 0.3.5%, with respective mean motility times of 10 ± 0.4 min, 17 ± 0.4 min, 13 ± 0.2 min, 11 ± 0.1 min, and 0.5 ± 0.1 min. However, statistical tests reveal a significant difference (ANOVA) between these different concentrations of the different cryoprotectants tested. ($p = 0.000000000067138$). Regarding the second medium (Glu500 mOsmol/kg), tests performed with the same cryoprotectants showed significant motility rates with Glu500 mOsmol/kg ECO (10%), DMSO (10%), ECO (20%), and ECO (30%), with mean motility rates ranging from 90.0 ± 7.0%, 90.0 ± 0.0%, 92.5 ± 3.5%, and 87.5 ± 3.5%, and mean motility durations of 7 ± 1 min, 5 ± 4 min, 15 ± 4 min, and 17 ± 13 min. However, statistical tests revealed a significant difference between these different concentrations of the various cryoprotectants tested.($p = 0.001569$).However, the best sperm motility rates after the addition of cryoprotectants were recorded with Ca-free HBSS 500 mOsmol/kg - ECO (20%) and

ECO (20%) for the second medium, Glu 500 mOsmol/kg, with respective mean motility rates of 87.5 ± 03.5% and 92.5 ± 03.5% (Tables 2 and 3).

Tests carried out two hours (t_2) after sperm collection

Two hours after sperm collection at a dilution ratio 1 : 9, the reactivation tests of *O. niloticus* sperm reveal, that the best motility rates were obtained with the Ca-free HBSS 500 mOsmol/kg storage container : 10% JO, 10% ECO, 10% LCO, 20% ECO, 20% LCO, 30% ECO, 30% LCO, and 10% DMSO, with respective varying mean motility rates of 55.0 ± 7.0%, 72.5 ± 3.5%, 65.5 ± 31.8%, 77.5 ± 3.5%, 70.0 ± 28.3%, 70.0 ± 14.1%, 57.5 ± 38.9%, and 62.5 ± 3.5%. The motility duration was, respectively, of 12 ± 1 min, 12 ± 01 min, 09 ± 02 min, 11 ± 04 min, 08 ± 03 min, 08 ± 04 min, 07 ± 03 min, and 05 ± 05 min. However, statistical tests (ANOVA) show a significant difference between these different concentrations of various cryoprotectants giving the best motility rates at t_2 ($p = 0.000005$). Regarding the second medium (Glu500 mOsmol/kg), the tests performed showed significant motility rates with Glu500 mOsmol/kg ECO (10%), DMSO (10%), ECO (20%), and ECO (30%), with respective mean motility rates of 87.5 ± 3.5%, 77.5 ± 10.6%, 92.5 ± 3.5%, and 92.5 ± 3.5%, at respective mean motility times of 8 ± 1 min, 6 ± 1 min, 9 ± 1 min, and 16 ± 15 min. However, statistical tests (ANOVA) showed a significant difference between these different concentrations of the different cryoprotectants giving the best motility rates at t_2 ($p = 0.000000015995$). From these results, the best sperm motility performance after the addition of cryoprotectants is recorded with Ca free HBSS 500 mOsmol/kg- ECO (20%), Glu 500 mOsmol/kg ECO (20%) and Glu 500 mOsmol/kg ECO (30%) with an average motility rate of 92.5 ± 03.5% and 92.5 ± 03.5% (Tables 2 and 3).

Tests carried out four hours (t_4) after sperm collection

The Table 2 presents the sperm motility rates of the species *Oreochromis niloticus* activated, four hours after sperm collection (time t_4) at a dilution ratio of 1:9. The test results revealed that the best motility rates were obtained only with the storage containers containing Ca-free HBSS 500 mOsmol/kg JO (10%), ECO (20%), LCO (20%), and ECO (30%), with respective mean motility rates of 47.5 ± 3.5%, 70.0 ± 0.0%, 60.0 ± 21.2%, and 65.0 ± 7.0% at respective mean motility times of 15 ± 6 min, 10 ± 1 min, 9 ± 2 min, and 7 ± 0 min. Statistical tests (ANOVA) showed a significant difference between these different concentrations of the different cryoprotectants tested at t_4($p = 0.00000001669$). The same tests performed in the Glu500mOsml/kg medium gave better motility rates with the storage container respective concentrations of Glu500mOsml/kg ECO (10%), DMSO (10%), ECO (20%), and ECO (30%) were 75.0 ± 7.0%, 45.0 ± 35.3%, 77.5 ± 10.6%, and 52.5 ± 45.9%, respectively, at mean motility times of 15 ± 8 min, 5 ± 1 min, 10 ± 8 min, and 11 ± 7 min. Statistical tests (ANOVA) showed a significant difference between these different concentrations of the various cryoprotectants tested at t_4 ($p = 0.000871$) (Table 3). From these experiments, the best sperm motility performance after the addition of cryoprotectants is recorded with ECO (20%) in both preservation media, with respective mean motility rates of 70.0 ± 0.0% and 77.5 ± 10.6%.

Tests carried out six hours (t_6) after sperm collection

Reactivated sperm, six hours after sperm collection (time t_6) at a dilution ratio of 1:9 are presented in Tables 2 and 3. The test results revealed that the best motility rates were obtained with the storage containers containing Ca-free HBSS 500 mOsmol/kg JO (10%), ECO (20%), and LCO (20%), with respective mean motility rates of 35.0 ± 7.0%, 50.0 ± 0.0%, and 47.5 ± 17.6% and mean motility times of 13 ± 5 min, 5 ± 3 min, and 7 ± 1 min, respectively. However, statistical tests (ANOVA) showed a significant difference between these different concentrations of the various cryoprotectants tested.t_6 ($p = 0.000004$). Tests conducted in the second storage medium, Glu500 mOsmol/kg, yielded results in the following storage containers : Glu500 mOsmol/kg ECO (10%), ECO (20%), and ECO (30%), with significantly higher motility rates of 70.0 ± 0.0%, 72.5 ± 0.35%, and 42.5 ± 53.0%, respectively, at mean motility times of 11 ± 0.4 min, 6 ± 0.1 min, and 6 ± 0.4 min, respectively. The performed statistical tests (ANOVA) showed a significant difference between the different concentrations of the various cryoprotectants tested at t_6 ($p = 0.000196$). However, the best sperm motility performance after the addition of cryoprotectants was recorded with ECO (20%) in both preservation media with respective mean motility rates of 50 ± 00% and 73 ± 04%.

Tests carried out twenty four hours (t_{24}) after sperm collection

The tables 2 and 3 present the sperm motility rates of the *Oreochromis niloticus* activated twenty-four hours after sperm collection (time t_{24}) at a dilution ratio of 1 : 9. The test results revealed that the best motility rates were obtained only with the storage containers containing Ca-free HBSS 500 mOsmol/kg JO (10%) and ECO (20%), with respective mean motility rates of 17.5 ± 3.5% and 20.0 ± 28.2% at respective mean motility times of 8 ± 4 min and 4 ± 5 min. Statistical tests (ANOVA) showed no significant difference between these different concentrations of the different cryoprotectants giving the best motility rates at t_{24} ($p = 0.131887$). The tests performed in the second preservation medium, Glu500mOsmol/kg, yielded significant results only in the Glu500mOsmol/kg JO (10%) preservation container, with a mean motility rate of 17.5 ± 24.7% at a mean motility duration of 0.1 ± 0.1 min. However, the best sperm motility performance after the addition of cryoprotectants was recorded with the Ca-free HBSS500mOsmol/kg-ECO (20%), with a mean motility rate of 20.0 ± 28.2%.

Table 2: O. niloticus sperm motility rate and duration after dilution in Ca free-HBSS 500 mOsmol/kg associated with cryoprotectants at different concentrations after first activation (t_0) and reactivation at t_2 to t_{24} at ratio 1: 9 with NaCl 74 mOsmol/kg

Milieu 1	Concentration cryo	Motility (%) t_0	Duration of motility (min) t_0	Motility (%) t_2	Duration of motility (min) t_2	Motility (%) t_4	Duration of motility (min) t_4	Motility (%) t_6	Duration of motility (min) t_6	Motility (%) t_{24}	Duration of motility (min) t_{24}
Ca free-HBSS 500mOsmol/kg	JO10%	65,0 ± 00,0^{ab}	10 ± 04^{ab}	55,0 ± 07,0^{a}	12 ± 01^{a}	47,5 ± 03,5^{abcd}	15 ± 06^{a}	35,0 ± 07,0^{a}	13 ± 05^{a}	17,5 ± 03,5^{a}	08 ± 04^{ab}
	JO20%	55,0 ± 07,0^{bc}	09 ± 01^{ab}	45,0 ± 07,0^{ab}	11 ± 05^{a}	30,0 ± 00,0^{cd}	09 ± 01^{b}	32,5 ± 10,6^{ab}	08 ± 03^{b}	15,0 ± 00,0^{a}	09 ± 02^{a}
	JO30%	42,5 ± 10,6^{c}	12 ± 06^{ab}	35,0 ± 07,0^{ab}	09 ± 04^{ab}	25,0 ± 07,0^{de}	08 ± 02^{bc}	22,5 ± 10,6^{ab}	08 ± 03^{b}	15,0 ± 07,0^{a}	06 ± 01^{abc}
	Gly10 %	00,0 ± 00,0^{e}	00 ± 00^{b}	00,0 ± 00,0^{b}	00 ± 00^{b}	00,0 ± 00,0^{e}	00 ± 00^{d}	00,0 ± 00,0^{b}	00 ± 00^{c}	00,0 ± 00,0^{a}	00 ± 00^{c}
	Gly20 %	00,0 ± 00,0^{e}	00 ± 00^{b}	00,0 ± 00,0^{b}	00 ± 00^{b}	00,0 ± 00,0^{e}	00 ± 00^{d}	00,0 ± 00,0^{b}	00 ± 00^{c}	00,0 ± 00,0^{a}	00 ± 00^{c}
	Gly30 %	00,0 ± 00,0^{e}	00 ± 00^{b}	00,0 ± 00,0^{b}	00 ± 00^{b}	00,0 ± 00,0^{e}	00 ± 00^{d}	00,0 ± 00,0^{b}	00 ± 00^{c}	00,0 ± 00,0^{a}	00 ± 00^{c}
	MeOH10%	55,0 ± 07,0^{bc}	17 ± 04^{a}	07,5 ± 00,0^{b}	05 ± 02^{b}	00,0 ± 00,0^{e}	00 ± 00^{d}	00,0 ± 00,0^{b}	00 ± 00^{c}	00,0 ± 00,0^{a}	00 ± 00^{c}
	MeOH20%	22,5 ± 03,5^{de}	07 ± 00^{ab}	00,0 ± 00,0^{b}	00 ± 00^{b}	00,0 ± 00,0^{e}	00 ± 00^{d}	00,0 ± 00,0^{b}	00 ± 00^{c}	00,0 ± 00,0^{a}	00 ± 00^{c}
	MeOH30%	00,0 ± 00,0^{e}	00 ± 00^{b}	00,0 ± 00,0^{b}	00 ± 00^{b}	00,0 ± 00,0^{e}	00 ± 00^{d}	00,0 ± 00,0^{b}	00 ± 00^{c}	00,0 ± 00,0^{a}	00 ± 00^{c}
	ECO10 %	80,0 ± 00,0^{ab}	13 ± 10^{ab}	72,5 ± 03,5^{a}	12 ± 01^{a}	47,5 ± 10,6^{abdc}	07 ± 01^{bc}	30,0 ± 00,0^{ab}	03 ± 00^{bc}	05,0 ± 07,0^{a}	01 ± 01^{c}
	ECO20 %	87,5 ± 03,5^{a}	13 ± 02^{ab}	77,5 ± 03,5^{a}	11 ± 04^{a}	70,0 ± 00,0^{a}	10 ± 01^{b}	50,0 ± 00,0^{a}	05 ± 03^{bc}	20,0 ± 28,2^{a}	04 ± 05^{bc}
	ECO30 %	75,0 ± 07,0^{ab}	10 ± 05^{ab}	70,0 ± 14,1^{a}	08 ± 04^{ab}	65,0 ± 07,0^{ab}	07 ± 00^{bc}	47,5 ± 24,7^{a}	04 ± 01^{bc}	10,0 ± 14,1^{a}	02 ± 02^{c}
	LCO10 %	77,5 ± 10,6^{ab}	12 ± 01^{ab}	65,5 ± 31,8^{a}	09 ± 02^{ab}	52,5 ± 17,6^{abc}	07 ± 01^{bc}	35,0 ± 07,7^{a}	06 ± 01^{bc}	00,0 ± 00,0^{a}	00 ± 00^{c}
	LCO20 %	80,0 ± 14,1^{a}b	11 ± 01^{ab}	70,0 ± 28,3^{a}	08 ± 03^{ab}	60,0 ± 21,2^{ab}	09 ± 02^{b}	47,5 ± 17,6^{a}	07 ± 01^{bc}	00,0 ± 00,0^{a}	00 ± 00^{c}
	LCO30 %	77,5± 17,6^{ab}	10 ± 03^{ab}	57,5 ± 38,9^{a}	07 ± 03^{ab}	40,0 ± 14,1^{bcd}	07 ± 01^{bc}	27,5 ± 10,6^{ab}	06 ± 01^{bc}	00,0 ± 00,0^{a}	00 ± 00^{c}
	DMSO 10%	67,5 ± 03,5^{ab}	05 ± 01^{ab}	62,5 ± 03,5^{a}	05 ± 00^{ab}	22,5 ± 3,5^{de}	03 ± 01^{cd}	00,0 ± 00,0^{b}	00 ± 00^{c}	00,0 ± 00,0^{a}	00 ± 00^{c}
	DMSO 20%	37,5 ± 03,5^{cd}	03 ± 00^{b}	00,0 ± 00,0^{b}	00 ± 00^{b}	00,0 ± 00,0^{e}	00 ± 00^{d}	00,0 ± 00,0^{b}	00 ± 00^{c}	00,0 ± 00,0^{a}	00 ± 00^{c}
	DMSO 30%	12,5 ± 03,5^{e}	03 ± 01^{b}	00,0 ± 00,0^{b}	00 ± 00^{b}	00,0 ± 00,0^{e}	00 ± 00^{d}	00,0 ± 00,0^{b}	00 ± 00^{c}	00,0 ± 00,0^{a}	00 ± 00^{c}
Probability		**6,71E-11**	**9,01E-04**	**0,000005**	**0,000015**	**0,00000001669**	**1,61E-07**	**0,000004**	**0,000006**	**0,131887**	**0,000281**
Meaning		**THS**	**THS**	**THS**	**THS**	**THS**	**THS**	**THS**	**THS**	**NS**	**THS**

The values of the same column bearing the same letters are not significantly different at the 5% threshold ($P < 0.05$) according to the Student-Newman-Keuls test

Table 3 : Detailed results of sperm motility (rate and duration) O. niloticus dilute in Glu500 mOsmol/kg associated with different cryoprotectants at different concentrations after activation at $t_0$and reactivation at $t_2$until t_{24}at a ratio of 1 : 9 with Sacc 100 mOsmol/kg

Milieu 2	Concentration cryo	Motility (%) t_0	Duration of motility (min) t_0	Motility (%) t_2	Duration of motility (min) t_2	Motility (%) t_4	Duration of motility (min) t_4	Motility (%) t_6	Duration of motility (min) t_6	Motility (%) t_{24}	Duration of motility (min) t_{24}
Glucose 500mOsmol/kg	JO10%	52,5 ± 24,7^{ab}	10 ± 01	07,5 ± 03,5^{d}	03 ± 01^{a}	05,0 ± 07,0^{b}	02 ± 02^{a}	00,0 ± 00,0^{b}	00 ± 00^{b}	17,5 ± 24,7^{a}	01 ± 01^{a}
	JO20%	40,0 ± 14,1^{ab}	06 ± 01^{ab}	07,5 ± 10,6^{d}	04 ± 06^{a}	06,0 ± 08,4^{b}	04 ± 05^{a}	02,5 ± 03,5^{b}	02 ± 03^{b}	15,0 ± 21,2^{a}	05 ± 07^{a}
	JO30%	32,5 ± 24,7^{ab}	06 ± 01^{ab}	02,5 ± 03,5^{d}	04 ± 06^{a}	00,0 ± 00,0^{b}	00 ± 00^{a}	00,0 ± 00,0^{b}	00 ± 00^{b}	07,5 ± 10,6^{a}	02 ± 01^{a}
	Gly10 %	00,0 ± 00,0^{b}	00 ± 00^{b}	00,0 ± 00,0^{d}	00 ± 00^{a}	00,0 ± 00,0^{b}	00 ± 00^{a}	00,0 ± 00,0^{b}	00 ± 00^{b}	00,0 ± 00,0^{a}	00 ± 00^{a}
	Gly20 %	00,0 ± 00,0^{b}	00 ± 00^{b}	00,0 ± 00,0^{d}	00 ± 00^{a}	00,0 ± 00,0^{b}	00 ± 00^{a}	00,0 ± 00,0^{b}	00 ± 00^{b}	00,0 ± 00,0^{a}	00 ± 00^{a}
	Gly30 %	00,0 ± 00,0^{b}	00 ± 00^{b}	00,0 ± 00,0^{d}	00 ± 00^{a}	00,0 ± 00,0^{b}	00 ± 00^{a}	00,0 ± 00,0^{b}	00 ± 00^{b}	00,0 ± 00,0^{a}	00 ± 00^{a}
	MeOH10%	80,0 ± 21,2^{ab}	07 ± 02^{ab}	57,5 ± 31,8^{bc}	07 ± 03^{a}	30,0 ± 42,4^{ab}	04 ± 06^{a}	17,5 ± 24,7^{b}	01 ± 01^{b}	00,0 ± 00,0^{a}	00 ± 00^{a}
	MeOH20%	67,5 ± 38,8^{ab}	09 ± 02^{ab}	20,0 ± 28,2^{d}	01 ± 01^{a}	00,0 ± 00,0^{b}	00 ± 00^{a}	00,0 ± 00,0^{b}	00 ± 00^{b}	00,0 ± 00,0^{a}	00 ± 00^{a}
	MeOH30%	45,00 ± 63,6^{ab}	07 ± 10^{ab}	00,0 ± 00,0^{d}	00 ± 00^{a}	00,0 ± 00,0^{b}	00 ± 00^{a}	00,0 ± 00,0^{b}	00 ± 00^{b}	00,0 ± 00,0^{a}	00 ± 00^{a}
	ECO10 %	90,0± 07,0^{a}	07 ± 01^{ab}	87,5 ± 03,5^{a}	08 ± 01^{a}	75,0 ± 07,0^{a}	15 ± 08^{a}	70,0 ± 00,0^{a}	11 ± 04^{a}	00,0 ± 00,0^{a}	00 ± 00^{a}
	ECO20 %	92,5± 03,5^{a}	15 ± 04^{ab}	92,5 ± 03,5^{a}	09 ± 01^{a}	77,5 ± 10,6^{a}	10 ± 08^{a}	72,5 ± 03,5^{a}	06 ± 01^{b}	01,5 ± 02,1^{a}	02 ± 03^{a}
	ECO30 %	87,5± 03,5^{a}	17 ± 13^{ab}	92,5 ± 03,5^{a}	16 ± 15^{a}	52,5 ± 45,9^{ab}	11 ± 07^{a}	42,5 ± 53,0^{ab}	06 ± 04^{b}	00,0 ± 00,0^{a}	00 ± 00^{a}
	LCO10 %	82,5± 03,5^{ab}	09 ± 01^{ab}	40,0 ± 00,0^{cd}	05 ± 01^{a}	02,5 ± 03,5^{b}	01 ± 01^{a}	00,0 ± 00,0^{b}	00 ± 00^{b}	00,0 ± 00,0^{a}	00 ± 00^{a}
	LCO20 %	77,5± 03,5^{ab}	06 ± 00^{a}	30,0 ± 00,0^{cd}	04 ± 00^{a}	00,0 ± 00,0^{b}	00 ± 00^{a}	00,0 ± 00,0^{b}	00 ± 00^{b}	00,0 ± 00,0^{a}	00 ± 00^{a}
	LCO30 %	77,5± 03,5^{ab}	07 ± 01^{ab}	12,5 ± 03,5^{d}	05 ± 01^{a}	00,0 ± 00,0^{b}	00 ± 00^{a}	00,0 ± 00,0^{b}	00 ± 00^{b}	00,0 ± 00,0^{a}	00 ± 00^{a}
	DMSO 10%	90,0± 00,0^{a}	05 ± 04^{ab}	77,5 ± 10,6^{ab}	06 ± 01^{a}	45,0 ± 35,3^{ab}	05 ± 01^{a}	00,0 ± 00,0^{b}	00 ± 00^{b}	00,0 ± 00,0^{a}	00 ± 00^{a}
	DMSO 20%	80,0± 00,0^{ab}	03 ± 03^{ab}	20,0 ± 00,0^{d}	04 ± 02^{a}	02,5 ± 03,5^{b}	02 ± 02^{a}	00,0 ± 00,0^{b}	00 ± 00^{b}	00,0 ± 00,0^{a}	00 ± 00^{a}
	DMSO 30%	57,5± 31,8^{ab}	02 ± 04^{ab}	00,0 ± 00,0^{d}	00 ± 00^{a}	00,0 ± 00,0^{b}	00 ± 00^{a}	00,0 ± 00,0^{b}	00 ± 00^{b}	00,0 ± 00,0^{a}	00 ± 00^{a}
Probabilité		0,001569	0,037373	1,60E-08	5,67E-02	0,000871	0,01451	0,000196	0,000145	0,595708	0,554703
Signification		HS	S	THS	NS	HS	S	THS	THS	NS	NS

NB: The values of the same column bearing the same letters are not significantly different at the 5% threshold ($P < 0.05$) according to the Student-Newman-Keuls test

DISCUSSION

The establishment of sperm cryobanks is a promising alternative in the context of producing biological material for restocking threatened and/or endangered species in african countries. However, sperm cryopreservation requires the use of a cryoprotectant and a diluent. The use of cryoprotectant issue from local products is also needed. Furthermore, these cryoprotectants must be applied in an optimal dilution ratio within the straws and adapted to the physiological characteristics of the fish in order to preserve sperm quality. (But *et al* (1998).

In our study, we aimed to determine the tolerance of six cryoprotectants at different concentrations on the spermatozoa of the Fish species *Oreochromis niloticus* Bouaké strain. During this study, the recorded physicochemical parameters were optimal during specimen handling prior to testing. The recorded temperature fluctuated between 25.41°C and 28.48°C. Huet (1970) indicates that the optimal temperature for reproduction ranges between 22 and 30°C. For the considered Fish species in our experiements, the thermal regime within the broodstock handling structures was

favorable to gonadal maturation. Indeed, temperature appears to be the most important environmental modulating factor of testicular function, including spermatogenesis.(Quintana *et al.*,2004; De Alvarenga & De França, 2009; Baroiller & D'Cotta, 2016; Dadras *et al*., 2017; Kouamé *et al.*, 2017; Yao *et al.*, 2017; Mugwanya *et al.,* 2022). As for dissolved oxygen, it varied between 1.49 and 5.83 mg/l. Malcolm *et al.* (2000) indicates that, with regard to dissolved oxygen concentration, this species tolerates both significant deficits and saturations. Thus, up to 3 mg/l of dissolved oxygen, *O. niloticus* does not exhibit any particular disorders, but when the dissolved oxygen concentration is below 3 mg/l, respiratory stress occurs, although mortality only occurs after 6 hours of exposure to levels of 3 mg/l. Nevertheless, this species can tolerate low dissolved oxygen concentrations for short periods. The optimum required is 5 mg/l. Our results corroborate those of these authors. Regarding water potential (pH), it fluctuated between 6.12 and 7.44. Our data fall within the range established by Malcolm *et al*. (2000), whose tolerance to pH variations is very high since the species is found in waters with pH values from 5 to 11. As well as the ideal pH being between 6.5 and 8.5.

The tests carried out demonstrate the lightness of six cryoprotectants before freezing at different concentrations (10%, 20% and 30%) in two different preservation media (Cafree HBSS 500 mOsmol/kg and Glucose 500 mOsmol/kg) of t_0(initial time after sperm collection) until t_{24}(24 hours of sperm balance supplemented with cryoprotectants). Of the six cryoprotectants used in this study, five gave satisfactory results, particularly 20% coconut juice (ECO 20%) in both preservation media (Cafree HBSS 500 mOsmol/kg and Glucose 500 mOsmol/kg). Our results corroborate those of Ndyomugyenyi & Malanda (2022) who worked on boars (pigs). Indeed, these authors indicate that the high concentration of sodium and potassium in green coconuts effectively promotes sperm motility and longevity in boars. These mineral ions, abundant in coconut water, create a favorable environment that prolongs sperm lifespan, extending their viability up to 96 hours, well beyond the usual 4 hours outside the boar's body. This extension allows for better management of artificial insemination, making it possible to divide the semen produced to inseminate several sows from a single collection. Thus, coconut water is a valuable natural medium for the preservation and effectiveness of sperm in this species. *O. niloticus* in artificial insemination. However, studies conducted in Quebec by Vachon *et al*. (2019) comparing sperm motility in copper knightfish with Hank's saline solution (HBSS) using other types of cryoprotectants showed that HBSS storage containers supplemented with cryoprotectant (DMSO) resulted in a significant motility rate of 95% at t_0Then, at the following activation times of 5 and 15 minutes, sperm reactivation results in a motility rate of 90%, and at activation times of 30 and 45 minutes, the same cryoprotectant applied by Jenkins *et al.*, (2011) in their work carried out for *Xyrauchen texanus* (Abbott, 1860), a 27% improvement in motility rate after freezing with DMSO at a concentration of 10% in combination with HBSS 500 mOsmol/kg. Also, with *Moxostoma robustum* (Cope, 1870), the cryopreservation of the milt is carried out using DMSO at a concentration of 10%. (Zelko, 2013).

Furthermore, this study showed that the cryoprotectant glycerol exhibited no motility regardless of the concentration and dilution medium used. This could be explained by the fact that the glycerol concentrations used in our study exhibit a high tolerance for spermatozoa of this Fish species. Our results are similar to those of Huiping *et al.* (2007), which were shown in a study on sperm cryopreservation in zebra fish *Danio rerio* (Hamilton, 1822), the effect of four cryoprotectants (methanol, DMSO, DMA, and glycerol) on sperm motility in this species during incubation before freezing. Indeed, their work shows that glycerol reduces zebrafish sperm motility within 15 min, indicating acute hypertension and a lack of suitability as a cryoprotectant.

We also observe, that sperm viability decreases as the concentration of the cryoprotectant increases and also over time. This hypothesis is supported by Pérez Cerezales *et al* (2010) who in their study on rainbow trout, despite the addition of cryoprotectant after cryopreservation, viability was reduced by more than 50%.

CONCLUSION

The study revealed the effect of six cryoprotectants at different concentrations on sperm motility in Nile tilapia (*Oreochromis niloticus*) Bouaké strain before storage in liquid nitrogen at -196 °C (freezing). However, storage containers containing Ca-free HBSS with an osmolality of 500 mOsmol/kg and Glucose with an osmolality of 500 mOsmol/kg, supplemented with 20% coconut juice (ECO), resulted in high motility rates with the milt of this species. The results obtained suggest a possibility for the cryopreservation (freezing) of sperm of *O. niloticus*. Furthermore, additional research is needed to examine the growth and survival of larvae from cryopreserved sperm with these preservation containers (Ca free-HBSS 500 mOsmol/kg and Glucose Osmolality 500 mOsmol/kg supplemented with 20% coconut juice).

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