Rhamdia quelen's semen pre-cryopreservation processing and sperm viability time after thawing

Processo de pré-criopreservação de sêmen de *Rhamdia quelen* e tempo de viabilidade do esperma após o descongelamento

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ABSTRACT
The study's aim was to evaluate the sperm movement in the semen of Rhamdia quelen, cryopreserved at different times of exposure to nitrogen vapor, besides different equilibration times and with different concentrations of methanol, and in thawed semen kept at 25ºC for different periods of time. Three distinct experiments were carried out. In the first experiment, fresh semen aliquots were diluted in a solution containing 5% D-Fructose, 5% milk powder (Nestle®, Ninho Fortificado®) and 10% methanol, filled into 0.25 mL straws and immediately kept in nitrogen vapor by 0,5; 1.0; 4.0; 8.0; 12.0 and 18.0h. After these times, the straws were transferred to the liquid nitrogen (-196°C). After 72 h, the semen was thawed by immersing the straws in water at (25 ° C) for 10 seconds. The sperm activation was evaluated by diluting the thawed semen in distilled water in the proportion of 1:250 (semen:activating solution; v/v). Motility and spermatic velocity in thawed semen were measured using the CASA system. In the second experiment, the fresh semen was diluted in solutions with glucose and powdered milk (Nestle®, Ninho Fortificado®) and 5.0; 7.5; 10.0; 12.5% methanol. Immediately after, it was filled into 0.25mL straws and kept at 25ºC by equilibrium times corresponding to 0, 20, 40 and 60 minutes. Subsequently, part of the straws was used for spermatic evaluation before cryopreservation and others were submitted to cryopreservation in nitrogen vapor for 30 minutes and after transferred to liquid nitrogen (-196°C). The processes of thawing and sperm evaluation were performed as described above. In the third experiment, the cryopreserved semen was thawed and kept at 25°C for 0.0; 7,5; 15,0; 22.5 and 30.0 minutes. After each time, the spermatic movement was evaluated as described above. The exposure time of straws to nitrogen vapor and the kept time of the thawed semen at 25ºC did not affect ($p > 0.05$) the sperm movement. On the other hand, methanol concentrations and equilibrium time ($p < 0.05$) influenced the spermatic movement interactively both, before and after cryopreservation. The best results were achieved when the semen was cryopreserved immediately after dilution. The cryopreservation of Rhamdia quelen semen can be successfully performed when the semen is diluted in a solution containing 5% D-Fructose, 5% milk powder and 11.66% of methanol and immediately filled into

0.25 mL straws and kept in nitrogen vapor by 30 min before being transferred to liquid nitrogen. In addition, after thawing, the semen can be kept at 25ºC for up to 30 minutes without damage to spermatic movement.

Keywords: CASA, equilibrium time, motility, nitrogen vapor, spermatic velocity

RESUMO

O objetivo deste estudo foi avaliar o movimento espermático no sêmen do *Rhamdia quelen*, criopreservado em diferentes tempos de exposição ao vapor de nitrogênio, além de diferentes tempos de equilíbrio e com diferentes concentrações de metanol e, no sêmen descongelado mantido à 25ºC por diferentes períodos de tempo. Foram realizados três experimentos distintos, no primeiro experimento, alíquotas de sêmen fresco foram diluídas em solução contendo 5% de D-Frutose, 5% de leite em pó (Nestle®, Ninho Fortificado®) e 10% de metanol, envasadas em palhetes de 0,25mL e imediatamente mantidos em vapor de nitrogênio por 0,5; 1,0; 4,0; 8,0; 12,0 e 18,0h. Após estes períodos, os palhetes foram transferidos para o nitrogênio líquido (-196ºC). Depois de 72h, o sêmen foi descongelado por meio da imersão dos palhetes em água a (25°C) por 10 segundos, onde foi avaliado a ativação espermática por meio da diluição do sêmen em água destilada na proporção de 1:250 (sêmen:solução ativadora; v/v). A motilidade e a velocidade espermática no sêmen descongelado foram mensuradas por meio do sistema CASA. No segundo experimento, o sêmen fresco foi diluído em soluções com glicose e leite em pó (Nestle®, Ninho Fortificado®) e 5,0; 7,5; 10,0 e 12,5% de metanol, envasado em palhetes de 0,25mL, sendo mantidos à 25ºC pelos tempos equilíbrio correspondentes a 0, 20, 40 e 60 minutos. Posteriormente, parte dos palhetes foi utilizado para avaliação espermática antes da criopreservação e os demais foram submetidos à criopreservação em botijas de vapor de nitrogênio por 30 mim e transferidos para o nitrogênio líquido (-196°C). O processo de descongelamento, avaliação da atividade, motilidade e velocidade espermática foi realizado conforme descrito anteriormente. No terceiro experimento, o sêmen criopreservado foi descongelado e mantido à 25°C pelos períodos de tempo correspondentes à 0,0; 7,5; 15,0; 22,5 e 30,0 minutos. O movimento espermático foi avaliado pelo sistema CASA, após o descongelamento. O tempo de exposição dos palhetes ao vapor de nitrogênio e o tempo de manutenção do sêmen descongelado à 25ºC não afetaram (P>0,05) o movimento dos espermatozoides. Por outro lado, as concentrações de metanol e o tempo de equilíbrio influenciaram (P<0,05) de forma interativa o movimento espermático, tanto antes quanto após a criopreservação. Os melhores resultados foram alcançados quando o sêmen foi criopreservado imediatamente após a diluição. A criopreservação do sêmen *Rhamdia quelen* pode ser realizada com sucesso quando o sêmen for diluído em solução contendo 5% de D-Frutose, 5% de leite em pó e 11,66% de metanol e, imediatamente envasado e mantido em botijão de vapor de nitrogênio por 30 min antes de ser transferido para o nitrogênio líquido. Além disso, após o descongelamento o sêmen pode ser mantido à 25ºC por até 30 minutos sem prejuízos ao movimento espermático.

Palavras-chave: CASA, motilidade, tempo de equilíbrio, vapor de nitrogênio, velocidade espermática

1 INTRODUCTION

Rhamdia quelen is a neotropical specie of huge importance in South America (Koakoski et al., 2012). Considering its ecological and productive importance in aquaculture, it is necessary to develop genetic breeding programs and / or germplasm banks for biodiversity conservation (Adames et al., 2015).

Although cryopreservation is a technique applied in several fish species, from temperate (Cabrita et al., 2010) and neotropical (Maria and Carneiro, 2012) climates, the methods are still in the early stages of development. For many species, experimental protocols require methodological enhancement or still must be developed (Adames et al., 2015). It worsens when it comes to protocols for the use of cryopreserved semen on a large scale, since the high female fertility leads to a great demand for viable spermatozoa and, consequently, the need for large semen volumes (Maria and Carneiro, 2012).

The search for protocols that guarantee success with the use of cryopreserved semen should consider the appropriate use of freezing and thawing rates (Viveiros and Godinho, 2009) and also the protocols that allow the maximum action of cryoprotectants (Viveiros and Godinho, 2009). Among the various techniques, the use of protocols that use equilibrium time variations after dilution of semen can guarantee adequate cryoprotectant action within the cell and balance between the intra and extracellular media (He et al., 2004). In analogy to bulls, the benefits of equilibrium time may be related to the influence on the adaptation of cell membranes to the conditions of low temperatures and, consequently, to determine the rates or the absorption speed of the cryoprotectants by spermatozoa (Muiño et al., 2007). These effects are also related to the type or chemical nature of the cryoprotectants (Shaliutina-Kolesová et al., 2015) and their concentrations in the diluents (Kumar and Betsy, 2015).

The protocols used for cryopreservation of semen from neotropical fish usually employ semen dilution immediately after collection (Taitson et al., 2008), followed by pre-freezing in a nitrogen vapor canister (Viveiros et al., 2012a) for the period of up to 18 hours (Caroldsfeld et al., 2003) and finally storage in liquid nitrogen (Galo et al., 2011). Also, the use of semen in artificial fertilization routines is usually used immediately after thawing (Maria et al., 2015). Ingeneral, cryopreservation and thawing procedures limit the use of semen in commercial or large-scale routines. These limitations are usually related to the need for larger structures for cryopreservation, and more time to ensure adequate thawing of the reeds. This time leads to a loss of gamete's viability.

Therefore, the objective of this study was to evaluate the sperm movement in the cryopreserved *Rhamdia quelen* semen at different equilibration times and with different concentrations of methanol and in the thawed semen kept at 25ºC for different periods of time.

2 MATERIALS AND METHODS

2.1 FACILITIES, EXPERIMENTAL ANIMALS AND COLLECTION OF SEMEN

Thirty-eight Rhamdia quelen (0.196±0.078 kg) males in reproductive breeding were used as semen donors for an exercise in three cryopreservation experiments. The breeders were kept in an excavated tank, covered with concrete, soil bottom and size of 200 m2, with water supply for replenishment of water evaporated and infiltrated. The animals were fed with commercial extruded feed containing 32% of crude protein.

All fish used in this research had the characteristics of evidence of gonadal maturation, which released small plots of semen when submitted into a soft abdominal massage (Tessaro et al., 2012).

After the identification and selection of the breeders, fishes were transferred to the artificial breeding laboratory, stored in masonry tanks installed in a water recirculation system, containing mechanical, biological and electric heating filters (24.0±1.0 °C). All fishes were submitted to hormonal manipulation for sperm synchronization and increased of semen release by intramuscular application of 3.0mg pituitary extract of carp/kg (Bombardelli et al., 2006).

After 240 accumulated thermal units (10h, water at 24 ºC), the sperm of each breeder was collected by means of abdominal massage in the cephalocaudal direction (Tessaro et al., 2012). From each breeder, 3mL of semen were collected. Semen samples were stored under cooling (12 °C) for the necessary period to evaluate semen and sperm characteristics (Sanches et al., 2011).

2.2 SEMINAL AND SPERMATIC ANALYZES

Immediately after semen collection, samples of fresh semen from each breeder were submitted to the evaluation of seminal pH (7.50±0.23) by the colorimetric method with the use of litmus paper (Merck®).

Sperm motility (91.12±8.75%) and average path velocity (VAP) (73.41±11.87 μm/s) were evaluated by the Computer Assisted Sperm Analysis (CASA) in free software, adapted for the species (Adames et al., 2015). Analyzes were performed in 10s after the

beginning of sperm activation. The spermatic motion videos were obtained by optical microscope (Nikon Eclipse®, E200) in 10x objective, coupled to a video camera (Basler, A602fc). The images were captured at a rate of 100 frame/second (100fps) using the AMCap software. The obtained videos were processed and analyzed by the CASA plugin inserted in the free software ImageJ (Wilson-Leedy and Ingermann, 2007; Purchase and Earle, 2012).

Samples of semen were diluted in buffered saline formol (1: 1000; v/v) and submitted to the measurement of sperm concentration by the cell counting method in Neubauer's hematimetric chamber (Wirtz and Steinmann, 2006). From these samples, aliquots were obtained for an evaluation of the percentage of morphologically normal sperm cells (94.12±7.25%). For this, 300 cells stained in Bengal Rose were evaluated under light microscopy on 40X objects and classified as normal and abnormal spermatozoa according to Mewes et al. (2016).

2.3 CRYOPRESERVATION AND SEMEN FREEZING

After semen and sperm analysis of fresh semen, samples of this material were diluted 1: 3 (semen: diluent), in a solution containing 10% of methanol, 5.0% of D-Fructose and 5.0% of powder milk (Nestle®, Ninho Fortificado®). The diluted semen was packed in 0.25 mL vanes and immediately sent to pre-freezing in a nitrogen vapor canister (MVESC42C dry shipper) (Carolsfeldt et al., 2003), which allows temperatures of up to -170 °C to be reached at a rate Of -35.6 °C/min (Maria et al., 2006). Afterwards the vanes were transferred and stored in liquid nitrogen (-196 ºC). After 72h, the semen was thawed by immersing the vanes in water at (25°C) for 10 seconds (Adames et al., 2015).

2.4 EXPERIMENT I: EXPOSURE TIME OF THE DILUTED SEMEN TO THE NITROGEN VAPOR

In this experiment 16 breeding herds were used, which yielded four composite samples or semen pools, where each pool consisted of four fishes' semen. Semen plots from each "pool" were diluted and packed as previously described and submitted to exposure to nitrogen vapor for different periods of time.

The experimental design was completely randomized blocks, with six treatments and four replications. The treatments were constituted by exposure of the vanes to the nitrogen vapor for periods of 0.5; 1.0; 4.0; 8.0; 12.0 and 18.0h. The blocks were

constituted by the semen pools. After exposure to steam, the vanes were transferred to the liquid nitrogen. After thawing, the sperm activation was performed by diluting the semen in distilled water at a ratio of 1: 250 (semen: activating solution; v/v). From the thawed semen the variables of sperm motility rate and average velocity of displacement were evaluated, as previously described.

2.5 EXPERIMENT II: METHANOL CONCENTRATION AND EQUILIBRATION TIME OF SEMEN AFTER DILUTION

In a similar way to the previous experiment, 16 other breeders were used, which gave rise to four semen pools. In this experiment, a completely randomized experimental design was used in a 4x4 factorial scheme. The treatments were constituted by the use of diluents based on glucose and milk powder (Nestle®, Ninho Fortificado®), containing 5.0; 7.5; 10.0 and 12.5% methanol and, by exposing the diluted semen at 25 °C, for the equilibration times corresponding to 0, 20, 40 and 60 minutes. After these time periods, part of the reeds was used to evaluate the spermatic movement before cryopreservation, and the others were submitted to cryopreservation in a nitrogen vapor cylinder for 30 minutes. Then, the vanes were transferred to the liquid nitrogen. Semen cryopreservation and thawing, activation, and sperm analysis were performed as described above.

2.6 EXPERIMENT III: SPERM MOVEMENT IN THAWED SEMEN MAINTAINED AT 25ºC FOR DIFFERENT PERIODS OF TIME

In this experiment, semen samples from six other breeders were individually submitted to dilution in solution containing 5% D-Fructose, 5% milk powder and 10% methanol. After this process, all samples were immediately packed in 0.25 ml vanes. After that, the blades were submitted to pre-freezing in nitrogen vapor for 30 minutes, cryopreserved in liquid nitrogen and thawed as previously described. A completely randomized experimental design was used, consisting of five treatments and six replicates. The maintenance of the frozen semen at 25 ºC for the periods of time corresponding to 0,0 was considered as treatment; 7.5; 15.0; 22.5 and 30.0 minutes. The semen plots from each breeder were considered as an experimental unit. After each period of time, the thawed semen was submitted to sperm activation and sperm analysis as previously described.

2.7 STATISTICAL ANALYZES

The results of sperm motility rate and average velocity of displacement from unifactorial experiments were submitted to analysis of variance (ANOVA) and submitted to the multiple comparison test of Duncan's average if necessary, both at a level of 5% probability. The results from the bi-factorial experiments were evaluated by the response surface model, at a 5% probability level. In case of evidence interaction between the experimental factors, the data were plotted in 3D and 2D charts, where the lines of maximum performance were added. When the individual effect of the experimental factors was evidenced, the results were plotted in 2D charts, where the lines of maximum performance were added. The results expressed as a percentage were submitted to transformation by the sine arc of the square root.

The assumptions of homoscedasticity of the data were checked by the Levine test and the normality of the residues by the Shapiro-Wilk test (Kéry and Hatfield, 2003). All analyzes were performed using the software Statistica 7.0 ©.

3 RESULTS

3.1 EXPERIMENT I: EXPOSURE TIME OF THE DILUTED SEMEN TO THE NITROGEN VAPOR

The time the vanes were exposed to nitrogen vapor during pre-freezing did not change ($p > 0.05$) the motility rates or the sperm velocity in thawed semen (Table 1).

Table 1 - Motility and average spermatic velocity of displacement in cryopreserved semen of Jundiá (*Rhamdia quelen*) submitted to different times of exposure to nitrogen vapor.

			Exposure Time (hours)				
Variable	*p*						
	0,5	1,0	4,0	8,0	12	18	
Motility (%)	44,86±9,77	52,39±7,71	48,40±5,10	52,44±7,69	55,15±5,54	53,22±4,45	0,23
VAP (µm/s)	45,54±2,61	49,66±3,39	48,90±2,52	50,08±3,64	48,74±1,07	49,72±3,57	0,15

Values expressed as average ± standard deviation. VAP= Average sperm displacement velocity

3.2 EXPERIMENT II: METHANOL CONCENTRATION AND EQUILIBRATION TIME OF SEMEN AFTER DILUTION

Sperm motility in the diluted and non-cryopreserved semen had an interactive effect ($p<0.05$) between the methanol levels and the equilibrium time at which the vanes were maintained at 25 °C (Figure 1). The model suggests that higher motility rates were achieved when the semen was immediately diluted in solution containing 5% D-Fructose,

5% milk powder and 8.15% methanol (Figure 1). The combination of the experimental factors can guarantee the maximum sperm motility before cryopreservation, when using solutions containing up to 5% of methanol and keeping the vanes in equilibrium for up to 9.3 minutes at 25°C (Figure 1).

On the other hand, the average speed of sperm displacement was influenced in an inverse proportional way only with the equilibrium time at 25 ºC ($p<0,05$), where the highest speeds were verified immediately after dilution (Figure 2).

Figure 1- Sperm motility (%) in Jundiá semen (*Rhamdia quelen*) diluted in solutions containing different concentrations of methanol and maintained at different equilibration times at 25 ºC prior to cryopreservation. A) Graphic representation by means of response surface in 3D chart. B) Graphical representation by contours in 2D graph.

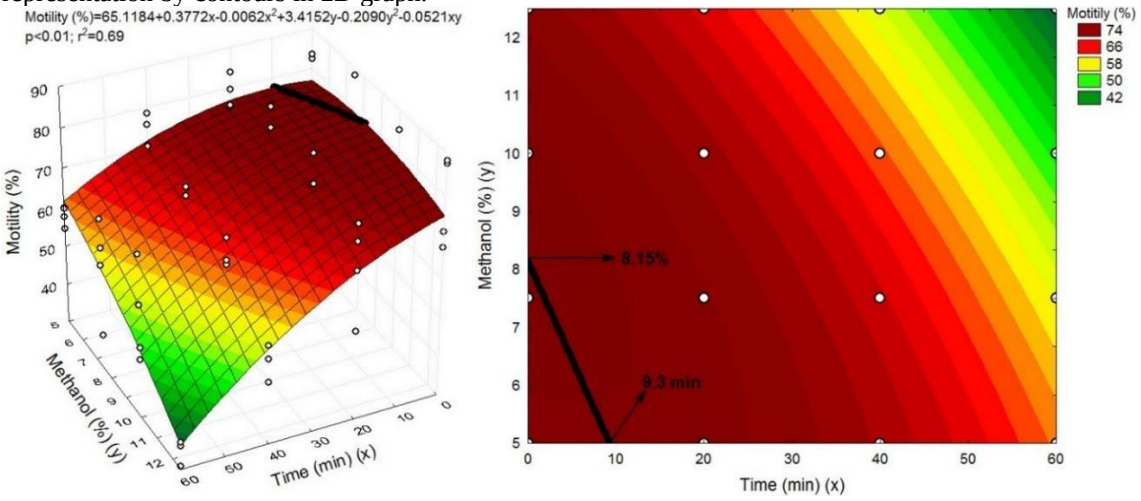
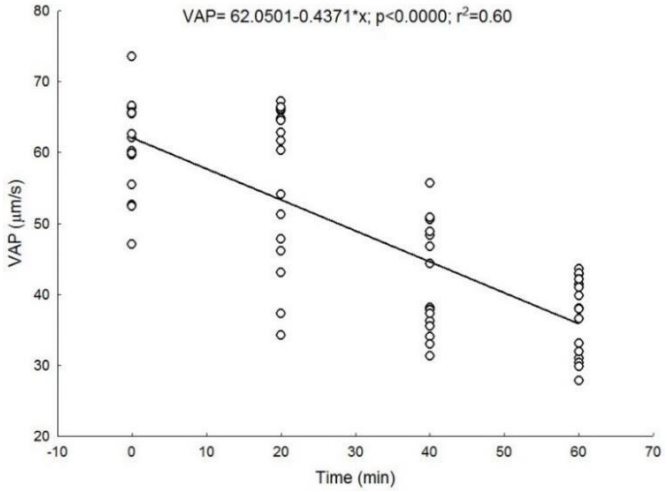
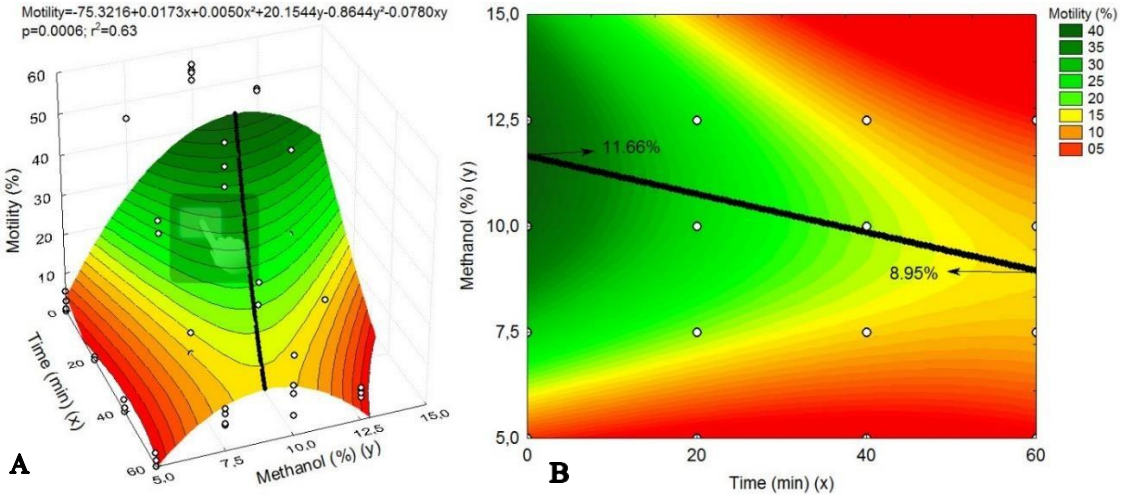


Figure 2 - Average path velocity (µm/s) (VAP) in Jundiá semen (*Rhamdia quelen*) diluted in solutions containing different concentrations of methanol and maintained at different equilibration times at 25 ºC before cryopreservation. This effect is inversely proportional only to the times when the vanes remained in equilibrium at 25 ºC.



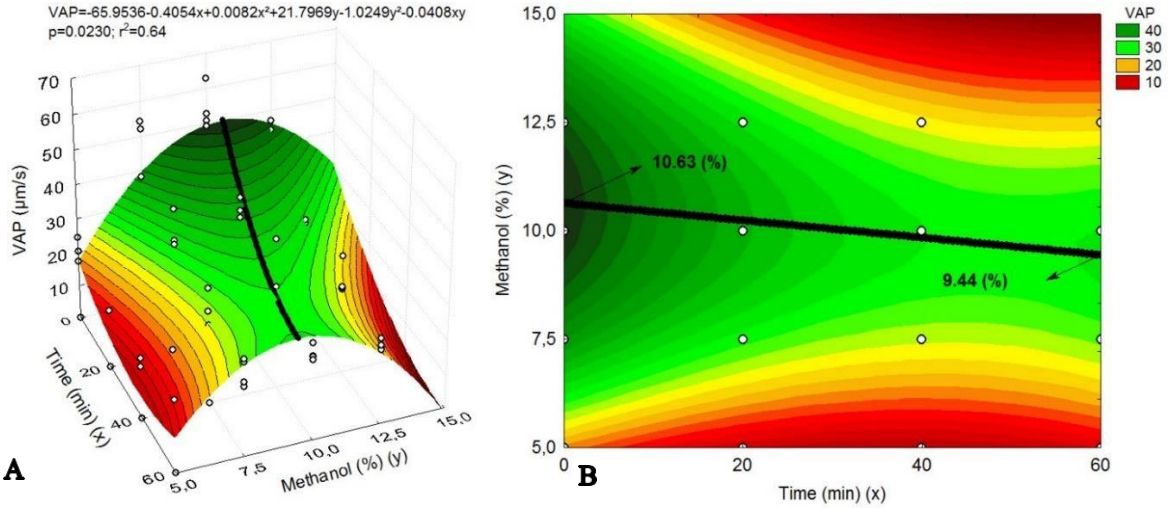
After defrosting, methanol concentrations and equilibration times interactively influenced ($p<0.05$) sperm motility. The model suggests that the highest values of sperm motility can be achieved with cryopreservation being performed immediately after the dilution of semen in solutions containing 5% D-fructose, 5% milk powder and 11.66% methanol. In addition, if necessary, the combination of the experimental factors can guarantee maximum sperm motility in defrosted semen at levels of up to 8.95% methanol and keeping the vanes in equilibrium for up to 60 seconds at 25 °C (Figure 3).

Figure 3 - Sperm motility (%) in thawed semen of Jundiá (Rhamdia quelen) diluted in solutions containing different concentrations of methanol and maintained at different equilibration times at 25 ºC. A) Graphic representation by means of response surface in 3D chart; B) Graphical representation by contours in 2D graph.



Sperm velocity in defrosted semen also had the same interactive effect of methanol concentrations and equilibrium times ($p<0.05$). The model suggests that the highest values of sperm velocity can be achieved with semen cryopreservation being performed immediately after dilution in solutions containing 10.63% of methanol (Figure 4). Similar to motility rates, the combination of methanol and equilibration times can guarantee maximum sperm velocity for up to 60 seconds at 25 °C when using a solution containing up to 9.44% methanol (Figure 4).

Figure 4 - Average sperm displacement velocity (µm/s) (VAP) in thawed semen of Jundiá (*Rhamdia quelen*) diluted in solutions containing different concentrations of methanol and maintained at different equilibration times at 25 ºC. A) Graphic representation by means of response surface in 3D chart; B) Graphical representation by contours in 2D graph.



3.3 EXPERIMENT III: SPERM MOVEMENT IN THAWED SEMEN MAINTAINED AT 25ºC FOR DIFFERENT PERIODS OF TIME

The sperm movement in the thawed semen was not influenced (p>0.05) by the time it was kept at 25 ºC (Table 2).

Table 2 - Motility and average spermatic velocity of displacement in thawed semen of Jundiá (*Rhamdia quelen*) maintained at 25 ºC for different periods of time.

Variable	Time (min)					*p*
	0,00	7,50	15,00	22,50	30,00	
Motility (%)	52,76±12,30	48,96±11,59	48,88±11,05	53,91±11,78	45,65±11,70	0,74
VAP (µm/s)	47,23±8,19	45,75±9,56	42,56±6,97	44,53±6,14	45,18±7,61	0,13

Values expressed as average ± standard deviation. VAP= Average sperm displacement velocity

4 DISCUSSION

The protocols of semen cryopreservation adopted for neotropical fish usually employ the freezing of the vanes, immediately after the dilution and packaging of the semen, keeping the samples exposed to the nitrogen vapor for periods of up to 18 or 24 hours (Carolsfeldt et al., 2003). Despite the lack of information that supports the establishment of effective protocols for semen cryopreservation of these species, long periods of exposure to nitrogen vapor limit their processing on a large scale.

Even though nitrogen vapor cylinders' exhibit temperature reduction rates of approximately -35.6 ° C/min (MARIA et al., 2006), cryopreservation of large volumes of semen can interfere with these rates of heat exchange and determine success of cryopreservation (Cabrita et al., 2001).

Although the results of this research suggest the use of at least 30 minutes of exposure of the vanes to the nitrogen vapor, possibly this period of time can be reduced, since in less than five minutes the internal temperature of the cylinders can reach -170 ºC (MARIA et al., 2006).

The use of efficient diluents in protecting the cells against the adversities of cryopreservation have also received attention. In general, low molecular weight organic substances with easy permeability to cell membranes have been employed as intracellular cryoprotectants (Viveiros et al., 2015). The main function of these substances is to promote the reduction of the microcrystals ice formation inside the cells and to guarantee the stability of cell membranes and proteins of biological importance in aqueous solutions (Zhiqing et al., 2014).

However, membrane permeability rates (Tsai et al., 2009) and the concentrations (Viveiros and Godinho, 2009) of cryoprotectants interfere interactively in the protection of cells and consequently in the success of cryopreservation. Especially in high concentrations, internal cryoprotectants can promote protein denaturation and compromise the functioning of different enzymes (Nascimento et al., 2012).

Depending on the purpose, the maintenance of the biological material in contact with the diluents for certain periods of time can potentiate the absorption and the functioning of the cryoprotectants, however, long periods of equilibrium time can cause irreversible damages to the cells (He and Woods, 2003). This research's results suggest toxic or deleterious effects on the spermatozoa, which impairs sperm movement when imposed on any time of contact of these cells with the diluents, suggesting that the cryopreservation should be performed immediately after the dilution of the semen. In addition, the results showed that when there is a need to keep the sperm in contact with the diluents at 25 °C for any period of time, methanol concentrations must be reduced to minimize sperm cell damage.

Considering the lower complexity of spermatozoa in freshwater fish compared to mammals (Muiño et al., 2007), the results of this research may be related to the high methanol permeability rates (Murgas et al., 2007) and Low osmolarity in relation to other cryoprotectants (Ribeiro and Godinho, 2003). In this case, the quick absorption of methanol by spermatozoa may promote protection of cells against cryoinjuries, but consequently damage the cellular structure when exposed in contact for long periods of time before cryopreservation (Nascimento et al., 2012). This hypothesis can be corroborated by the results of He and Woods (2003) who tested the effects of equilibrium

time and the use of Dimethyl sulfoxide, methanol and dimethylacetamide on the cryopreservation of striped bass (Morone saxatilis) semen and verified that the shortest equilibrium times with methanol promote the best results of sperm movement in thawed semen.

Although methanol is the most membrane-permeable cryoprotective agent, at high concentrations, this substance causes germ cell damage in several species of fish, except for tilapia spermatozoa (Viveiros et al., 2010). Usually all cell damages from exposure to methanol are associated with the occurrence of osmotic stress and/or biochemical and physiological changes that interfere in cellular functions (Viveiros et al., 2012b). Even though these deleterious effects can be minimized by maintaining the equilibrium time at low temperatures, under these conditions, cell dehydration is facilitated and the formation of ice crystals that causes cryoinjuries is reduced (Zhiqing et al., 2014).

Interestingly, the maintenance of the semen thawed at 25 ºC for periods of time up to 30 minutes did not influence the sperm movement. Although simple, this result has relevance and direct implication in the routines of artificial reproduction with the use of cryopreserved semen, because the high females' fecundity implies the thawing of a large number of vanes and consequently, in the expenditure of a considerable period of time for use of the semen plots after thawing. Therefore, the possibility of maintaining semen thawed for up to 30 minutes at 25 ºC, without loss of sperm movement, significantly facilitates the management of semen during artificial fertilization procedures.

5 CONCLUSION

Cryopreservation of the jundiá semen (Rhamdia quelen) can be successfully performed when the semen is diluted in a solution containing 5% D-Fructose, 5% milk powder and 11.66% methanol and immediately packaged and kept in Nitrogen vapor boiler for 30 min before being transferred to the liquid nitrogen. In addition, after thawing, the semen can be kept at 25 °C for up to 30 minutes without damaging the sperm movement and be potentially useful in artificial fertilization procedures.

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