EVALUATION OF MOTILITY OF THE SHORT-TERM CRYOPRESERVED SPERM OF THE AFRICAN GIANT CATFISH (*CLARIAS GARIEPINUS* BURCHELL, 1822)

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ABSTRACT

The motility of African giant catfish (Clarias gariepinus Burchell, 1822) sperm cryopreserved at –35^{0}C using different cryoprotectants were investigated. The study was carried out in the rainy season between June and September 2005. Ten percent (10%) of each of three permeating cryoprotectants: methanol, glycerol and dimethyl sulfoxide (DMSO); with 15% non permeating cryoprotectant (skim milk) and 75% phosphate-buffered saline (PBS) as extender were used to prepare three different types of cryoprotectant combinations. The milt which was mixed with cryoprotectants at the ratio 1:1 and stored in 2 ml cryotubes was short-term cryopreserved in a deep freezer at -35^{0}C. The sperm was then revived at various intervals to evaluate the effect of cryoprotectants and periods of cryopreservation on its viability and motility. The results showed that the type of cryoprotectant combination had significant effect on sperm motility ($p<0.05$). Cryoprotectant combinations containing glycerol-preserved sperm better than those with DMSO and methanol in that order. This result demonstrated that short-term cryopreservation technique could be used as method of storage and management of sperm of the African catfish especially in Nigeria where supply of male broodstock is limiting.

Keywords: Cryopreservation, sperm motility, *Clarias gariepinus*, African giant catfish

INTRODUCTION

Artificial induced spawning of the giant African catfish (*Clarias gariepinus*) is now a common practice in Nigeria among the fish farmers who produced fingerlings (Olaleye, 2005). The milt is stripped out of the male genital papillae while the female are induced to ovulate by using hormones either from fish pituitary extracts (Fagbenro *et al.*, 1992; Fafioye and Adeogun, 2005)) or imported fish hormone preparations such as Ovaprim (Syndel, Inc., Canada). The testes in the African catfish are located in the abdominal region which made it difficult for the milt to be stripped during induced spawning process. Usually, the male fish has to be sacrificed and the abdomen cut open to remove the testes which must be minced to release the milt which contains the sperm (Lamai, 1996). The practice has led to loss of many viable male catfish of desirable traits, even though only a few thousands of sperm cells are needed to fertilize one gram of oocytes (Viveiros *et al.*, 2000). The need to sacrifice the male fish necessitates devising means of preserving sperm cells from good quality male catfish. through cryopreservation (a molecular biological method of *in vitro* conservation).

Cryopreservation is the process of storing biological materials at very low temperatures for lengthy period of time using an extender and cryoprotectant agents which protect cells from damage during freezing (vitrification) and subsequent thawing cycles (CryoBioSystem, 2005). Cryopreservation can be used to overcome constraints that limit captive breeding by providing sperm on demand and simplifying the timing of induced spawning during hatchery operations. This method could also enhance efficient use of hatchery facilities, thus obviating the need to maintain live male broodstock. This method could also ensure safe reserve of genetic stock for genetic diversity and development of 'sperm bank' of highly valued individual fish for future stocking or in 'gene bank' development for conservation and preservation (Hiemstra *et al.*, 2006).

Cryopreservation also protects the spermatozoa from sudden environmental changes or disaster and disease translocation during transportation of broodstock over a long distance. (Billard *et al.*, 1995). This study is aimed at identifying the possible suitable cryoprotectant combinations for African catfish *C. gariepinus* sperm under short-term cryopreservation conditions and determine the effect of the period of cryopreservation on sperm motility

MATERIALS AND METHODS

Sperm Collection

The study was carried out between June and September 2005 using matured male catfish obtained from ZARTECH in Ibadan, Nigeria which ages were between 8 to 9 months and weighing 500-1200g were selected based on reddish or pinkish vascularised papilla (Richter *et al*, 1995). The fish were sacrificed, and dissected open and the testes were surgically removed and carefully lacerated to release the sperm using the new aseptic razor blade (Lamai, 1996).

Cryoprotectant preparation and administration

The cryoprotectant combinations were prepared by using phosphate-buffered saline (Kwantong and Amrit, 2000). Fifteen percent (15%) skim milk (DANO®, Holland) was used as non-permeating cryoprotectant base diluent for the three different cryoprotectant combinations (Chao *et al.*, 1987). Ten percent (10%) each of the 3 permeating cryoprotectants (methanol, glycerol and dimethyl sulphoxide (DMSO) were mixed with the base diluent to produce three cryoprotectant combinations labelled E-CPA A (containing methanol), E-CPA B (glycerol) and E-CPA-C (DMSO) (Table 1).

Table 1. Percentage composition of three different types of extender-cryoprotectant combinations (E-CPA) used.

Composition	E-CPA A	E-CPA B	E-CPA C
PBS	75	75	75
Skim milk	15	15	15
A- Methanol	10		
B- Glycerol		10	
C- DMSO	-	-	10

PBS- Phosphate buffered saline
DMSO-dimethyl sulphoxide
Means with the same superscript are not significantly different $p < 0.05$.
N- number of squares of sperm cells counted
DMSO-dimethyl sulphoxide

The milt obtained by careful laceration of the testis was observed under a compound microscope (Olympus, Japan) using high power objective 40X and the motility was assessed. Motile sperm cells were counted by using haemocytometer and the good and highly motile sperm were cryopreseved. The milt which was mixed with the different cryoprotectants combinations at ratio 1:1 (Stoss, 1983) were cryopreserved in 2 ml cryotubes. The tubes were arranged in a special plexi-glass container and placed in the refrigerator (NULEC) for 15 minutes before fast freezing (Viveiros *et al.*, 2000) in the IGNIS deep freezer at a stable freezing temperature of -35^{0}C.

The sperm cells were cryopreserved and motility was evaluated after 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 14 days and 28 days respectively by counting the number of motile sperm using a haemocytometer (SIGMA, 1994). Period 0 represents the first day when the milt was still fresh. The viability and motility of the sperm cells were evaluated by taking the percentage of motile sperm after period of cryopreservation (Hafez, 1985). The data collected were subjected to analysis of variance to determine the level of significance at $p < 0.05$

RESULTS

Cryoprotectant composition and sperm motility

The motility of fresh sperm was found to be significantly higher ($p<0.05$) than those of the cryopreserved sperms (Table 2). The result also showed that methanol-based cryoprotectant (E-CPA A) had a mean value that was significantly lower than glycerol-based (E-CPA B) and DMSO-based (E-CPA C) cryoprotectants ($p<0.05$). The percentage motile sperms after cryopreservation was found to be insignificantly different in glycerol- and DMSO-based cryoprotectants. The result also showed that the sperm cells that were motile were not significantly different ($p<0.05$) in methanol- and DMSO-based cryoprotectants after 28 days.

Table 2. Effect of type of cryoprotectant used on sperm viability after 28 days.

Cryopreservative	N	Mean Sperm Motility	Std. Deviation
E-CPA A (Methanol)	10	38.20^c	6.43
E-CPA B (Glycerol)	10	46.20^b	12.52
E-CPA C (DMSO)	10	45.20^{bc}	2.57
Fresh	10	67.70^a	8.25

Length of cryopreservation and sperm motility

The effect of the length of cryopreservation on *C. gariepinus* sperm motility was shown in Figure 1. There was a sharp decline in sperm motility after 24 h of cryopreservation (from 92 to 46%). Motility motility was 4 %. However, between day 7 up to day 28, motility decreased gradually at the rate of 0.1 %.

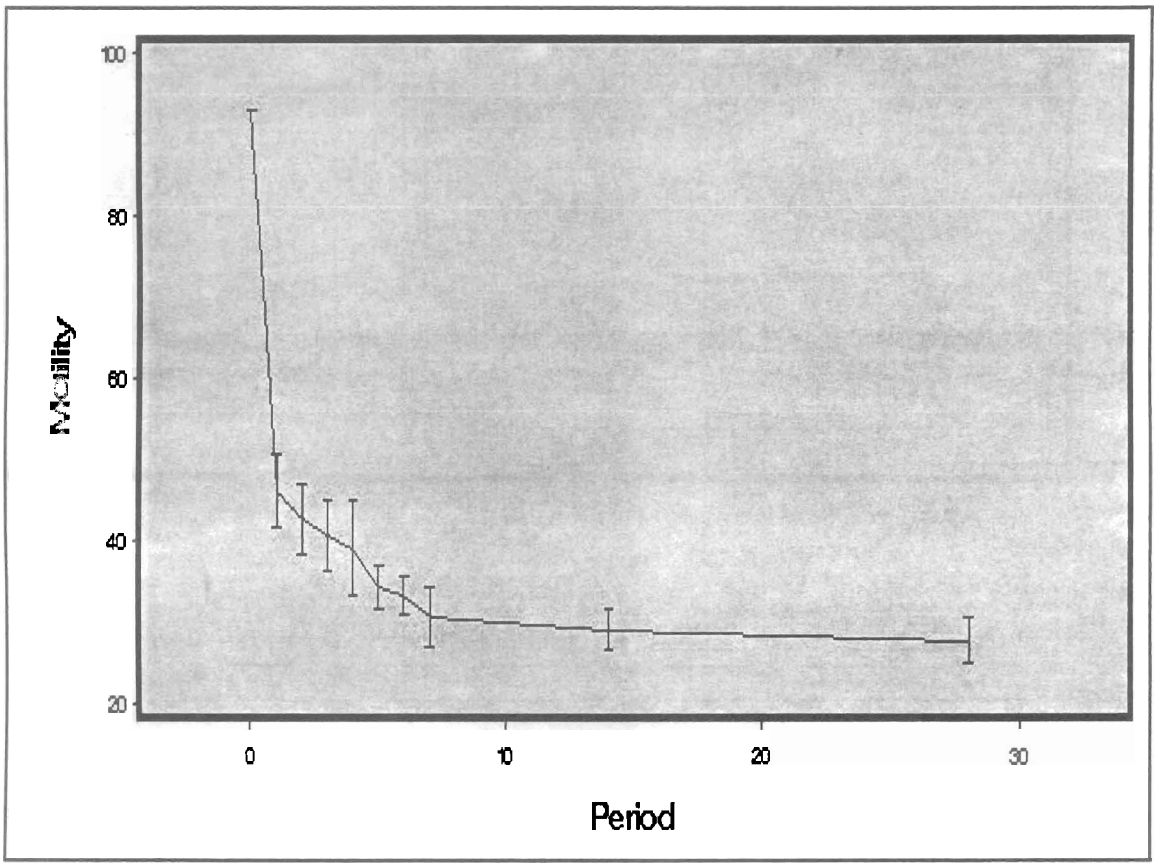

Figure 1. Effect of period (number of days) of cryopreservation on sperm otility (%). Vertical lines represent standard deviation

Cryoprotectant combinations, duration and sperm motility

During period 0 that represents the first day when the milt was still fresh, sperm cells in each of the cryoprotectant combinations had the same motility percentage which was the highest. The E-CPA B (glycerol-based cryoprotectant) had the highest sperm motility percentage (%) for days 1, 2, 3, 4 and 5. The E-CPA C (DMSO-based cryoprotectant) was found to preserve the sperm best from day 6 to day 28. The E-CPA A (methanol-based cryoprotectant) had the least motility percentage from day 1 to day 28 (Figure 2).

DISCUSSION

The type of cryoprotectant combinations used for this type of short-term cryopreservation in a deep freezer had a significant effect on sperm motility. The numbers of the motile sperm in the three cryopreservatives were significantly lower compared with the freshly-obtained sperm. The length of cryopreservation also had a noticeable effect on the motility of the sperm. The longer the sperm were kept in the frozen state the lower the motility. This may mean that the cryopreserved sperm in deep freezer must be reactivated in order for it to fertilize the fresh oocytes.

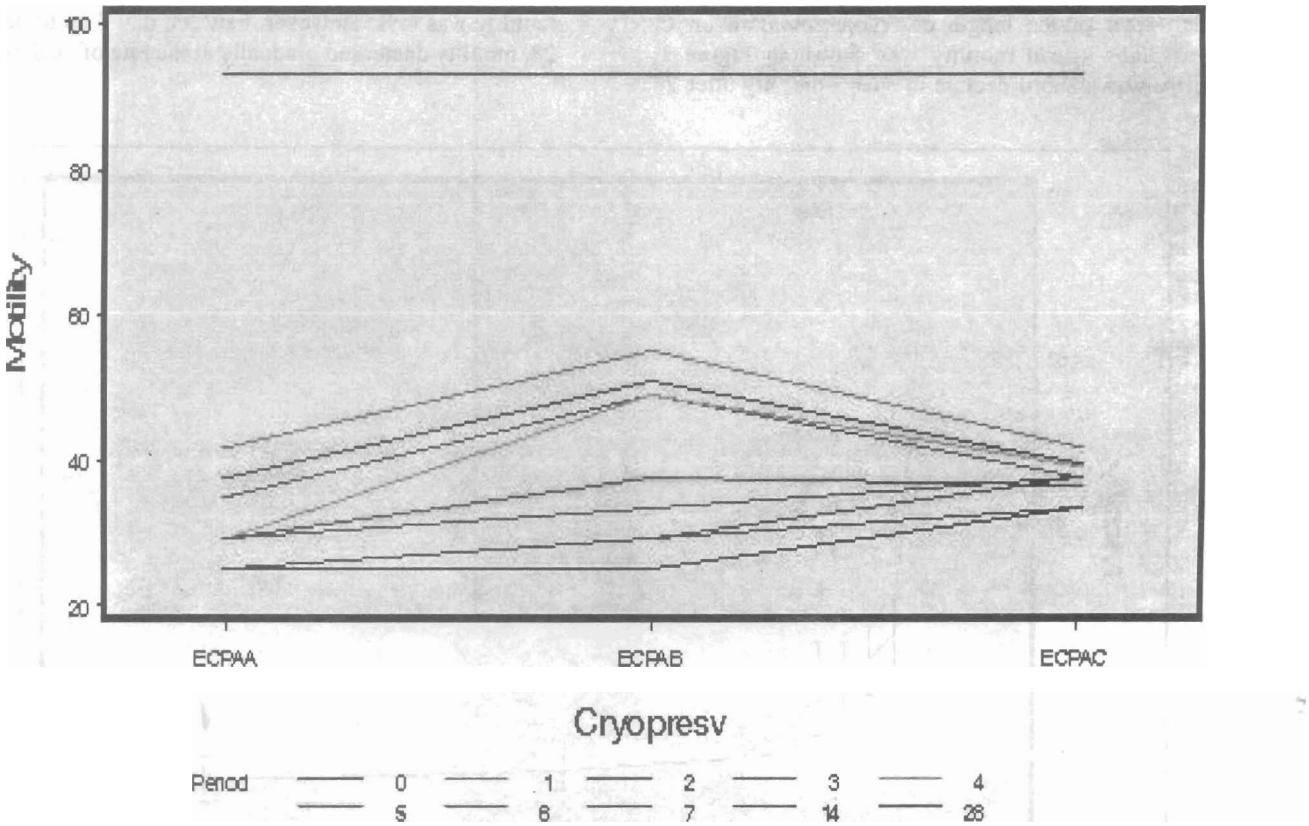

Figure 2: Comparison of sperm motility with different cryoprotectant combinations at different periods.

Our preliminary hatchability experiments, *i.e.*, using the three types of cryopreserved sperm, DMSO-preserved sperm even at day 8 were able to fertilize the eggs with appreciable hatchability rate of 50% (Omitogun *et al*, 2006). Attention must also be paid to the freezing and thawing processes, which obviously determine how motile and potent the sperm will be after cryopreservation. It could also be deduced from the results of this study that cryoprotectant combination B (glycerol) preserved sperm motility best for the first five days of cryopreservation while cryoprotectant combination C (DMSO) had the most beneficial effect from day 6 to day 28. This suggests that cryoprotectant combination B could be good for short-term cryopreservation while C could be used for a longer-term cryopreservation, however, this need to be confirmed. A study can also be made in the future to consider the possibility of combining glycerol and DMSO, the two active reagents in cryoprotectant combinations B and C for a long-term cryopreservation of the sperm of the African Catfish,

Clarias gariepinus.

In summary, these results demonstrate that short-term cryopreservation technique can be used as method of sperm storage and stock management of African catfish. The results from the study suggested that the cryoprotectant combinations with glycerol and DMSO have appreciable positive effect for both short and long term cryopreservation of the African giant catfish sperm. Further studies in freezing and thawing procedure need to be done to avoid vitrification of cryopreserved catfish sperm that can substantially reduce its fertilizing ability.

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REFERENCES

Billard, R., Cosson, J.., Crim, L.W. and Suquet, M. 1995. Sperm physiology and quality. **In**: Broodstock Management and Egg and Larval quality. (Eds: Bromage, N.R. and Roberts, R. J.) Blackwell Sc. Ltd., Osney Mead, Oxford, pp 25-52.

Chao, N. H., Chao, W.C, Liu, K.C, and Liao, I.C. 1987. The properties of tilapia sperm and its cryopreservation. *Journal of Fish Biology* 30; pp 117-118.

CryoBiosystem, 2006. Fundamentals of Cryobiology. http://www. cryobiosystem-imv.com /CBS/ Cryobiology/coms_cbs_asp.

Fafioye,O.O. and Adeogun,O.A. 2005. Homoplastic hypophysation of African catfish. *Nigerian J. Anim. Production* 32(1): 153-157.

Fagbenro, O.A., Salami, A.A. and Syndeham, D.H.J. 1992. Induced ovulation and spawning in the Catfish, *Clarias isheriensis* using pituitary extracts from non-piscine sources. *J. Applied Aquaculture* 1(4): 15-20.

Hafez, E.G. E. 1985. Preservation and cryopreservation of gamete and embryo techniques for improving reproductive efficiency, **In**: *Reproductive Physiology of Farm Animals*. Lea & Fabiger, USA. pp 571-600.

Hiemstra, S.J., Van der lende, T. and Woeldors, H. 2005. The potential of cryopreservation and reproductive technologies for animal genetic resources conservation strategies. **In**: Proceedings of the International Workshop on Role of Biotechnology for the Characterization and Conservation of Crop, Forestry, Animal and Fishery Genetic Resources. Villa Gualino Turin, Italy-5-7 March 2005, pp 25-35.

Kwantong, S. and Amrit, N.B. 2000. Effect of cryopreservation, extender and freezing rate on fertilization rate of frozen stripped catfish *Pangassius hypothalamus* sperm. http/www.Blackwell-synergy.com//link/dio/10-104obj;1365–2109. 2003.0089 /; labs/; jresswind.

Lamai, S.L. 1996. Successful hand stripping of hatchery breeding reared male African Catfish *Clarias gariepinus*. The 13th Annual Conference of the Fisheries Society of Nigeria, New Bussa, 1999, pp 159-162.

Olaleye, V.F. 2005. A review of reproduction and gamete management in the African Catfish *Clarias gariepinus* (Burchell). *Ife J. Science* : 63-70.

Omitogun, O.G., Olaleye, O.O. Ojiokpota, C. and Betiku, C.O. 2006. Potentials of short-term cryopreserved sperm of the African giant catfish (*Clarias gariepinus* Burchell, 1822) for aquaculture in Nigeria. **In:** Eds: Olakojo, S.A., Ogunbodede, B.A. and Akande, S.R. Proceedings of the 31st Annual Conference of the Genetics Society of Nigeria, NACGRAB, Moor Plantation, Ibadan. November 6-9, 2006. pp. 141-146.

Richter, C.J.J., Edding, E.H., Verreth, J.A.J. and Fleuren, W.L.G. 1995. African Catfish (*Clarias gariepinus*). **In**: Broodstock Management and Egg and Larval Quality. (Eds: Bromage, N.R. and Roberts, R. J.) Blackwell Sc. Ltd., Osney Mead, Oxford, pp. 242-276.

SIGMA, 1994. Cell counting and cell viability. Saint Louis, MO 63178 USA. pp 1634-1635.

Stoss, J. T. 1983. Short-term storage and cryopreservation of milt from Atlantic salmon and sea trout. *Aquaculture* 30:229-236.

Viveiros A.T, So, N., and Komen, J. 2000. Sperm cryopreservation of African catfish, *Clarias gariepinus*: cryoprotectants, freezing rates and sperm: egg dilution ratio. Theriogenology 54:(9).