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A COMPARISON IN REPRODUCTIVE PERFORMANCE OF AMUR CARP DURING NORMAL AND LATE BREEDING SEASON IN TARAI REGION OF UTTARAKHAND

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Abstract: The present study was conducted for comparion of cryofreezed sperm and corresponding embryonic development, viability and fertilizing capacity in fish Amur carp (*Cyprinus carpio haematopterus*) during normal and late breeding season under Tarai conditions of Uttarakhand. The present observation reveals that there is difference in embryonic development in both the seasons for different proportion of extender and cryoprotectant solution. The combination of TRIS+DMSO+Glucose showed normal embryonic development where as comparatively better result was observed in RPMI+DMSO and late embryonic development was in PBS+DMSO treatment group in normal breeding season as well as in late breeding season. The result also reveals that sperm of Amur carp diluted with RPMI+DMSO have better post thaw motility, fertilization and hatching rate in both normal and late breeding season. The comparison between embryonic development showed significant difference being better in Amur carp by reference of motility, fertility and hatching percentage in comparison of normal and late breeding season. These observations indicate that Amur strain of common carp is suitable for replacing the Common carp in favorable conditions by virtue of its better reproductive potential.
Keywords: Amur carp, Normal and Late breeding season.

Introduction

The Amur wild carp is an ancient form, that originated from the Asian carp centre (Amur-China type of wild carp, *Cyprinus carpio haematopterus*) and spread to the water bodies of Western Asia. Artificial insemination can be applied to increase the production to meet this demand due to exploitation of natural stocks. Cryopreservation is a valuable technique to assist in the genetic improvement of cultured stocks as well as providing a continuous supply of good quality sperm for artificial insemination. Successful storage of fish sperm in liquid nitrogen has been reported for more than 200 species, but the protocol varies with species. Extender composition, cryoprotectant concentration, and freezing method are known to affect cryopreservation success. Cryopreservation of Amur carp sperm using saline extenders, TRIS, RPMI 1640, Phosphate buffer Saline, glucose and dimethyl sulfoxide (DMSO) produced variable results on post-thaw sperm motility and/or fertilization and hatching

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success. However, evaluation of the various cryopreservation methods is hindered by the different extenders, cryoprotectants and freezing methods that have been used. Seasonality in reproduction is one of the most striking characteristics of fish, which is synchronized with seasonal changes in climate, day length and food availability. Increased rainfall, low water temperature and adequate nutrition are believed to trigger final gonad maturation. It is rather a cumulative effect of environmental interactions that influence breeding. The main purpose of this study was to investigate the effects of various extenders containing different cryoprotectants on post-thaw viability, motility fertility of frozen spermatozoa and embryonic development from common carp and amur carp in normal and late breeding season.

Materials and methods

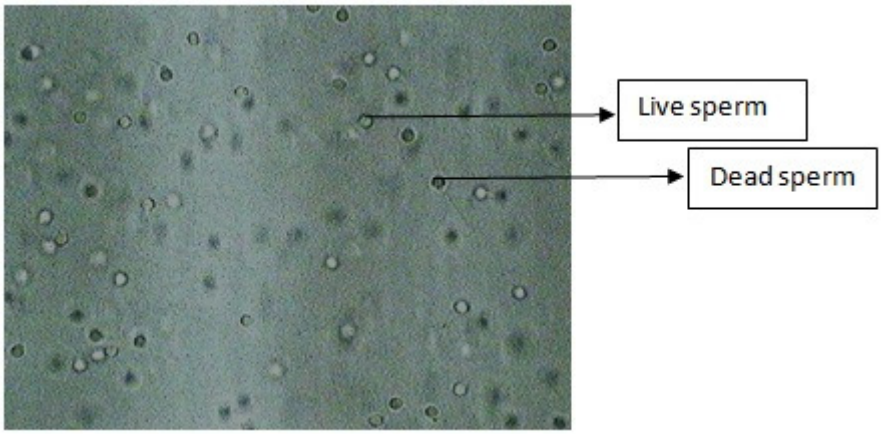
For the present study, sexually mature males of amur carp (2-2.5) kg of body weight were chosen randomly from Instructional Fish Farm of College of Fisheries. Males were examined to determine the sexual maturity by the presence of milt in the genital papilla after a light pressure of the abdomen. Only sperm samples showing more than 40% motility were used for cryopreservation. Diluents for the milt consisted of an extender plus a cryoprotectant in the v/v ratio 9 parts extender: 1 part cryoprotectant. The milt: extender was maintained at a ratio of 1:4. Carp brood stocks will also be maintained in indoor tanks supplied with a continuous flow of tap water. TRIS, RPMI and PBS were used as diluents and DMSO and glucose were taken as cryoprotectant. First we performed the dose approximation on common and amur carp for standardization of diluents concentration in second week of august (mid breeding season). Twelve combinations of extender and cryoprotectant were prepared. These combinations were Milt + (TRIS + DMSO), Milt + (TRIS + Glucose), Milt + (TRIS + DMSO + Glucose), Milt + (TRIS), Milt + (RPMI 1640+ DMSO), Milt + (RPMI 1640+ Glucose), Milt + (RPMI 1640+ DMSO + Glucose), Milt + (RPMI 1640), Milt + (PBS + DMSO), Milt + (PBS + Glucose), Milt + (PBS + DMSO + Glucose) and Milt + (PBS). Out of twelve combinations only three group were selected that were TRIS+ DMSO+Glucose, PBS + DMSO and RPMI + DMSO. So, only these groups were used for further study.

Freshly collected milt mixed with different diluents were discharged into polyvinyl straws. The straws were immediately sealed from open end, wiped and immediately transferred into biological freezer. The biological freezer holding the straws after allowing varying equilibration period were frozen by exposing them to liquid nitrogen vapour at about the surface of liquid nitrogen for 2-5 minutes, after which the straws were immersed and frozen at -196^0C. Fertility of preserved milt was tested using evoluted eggs stripped from

hypothesized females. Eggs were collected from selected spawners by dry stripping. Frozen milt was thawed by swirling straws in tap water (30^0C) and sperm tested for its fertilizing ability were applied immediately to fresh ova in a 250 ml beaker. Milt and ova were mixed by stirring with a feather. Tap water was added immediately after the milt was added. The ova were poured in large-sized beakers and kept under the running tap. Sample of ova were withdrawn at intervals and examined under a low power microscope.

Statistical analysis

To determine the effects of cryopreservation on sperm motility, fertilization and hatching capacity, a randomized block design (RBD) test was used to compare Amur carp frozen thawed sperm in different seasons. In all statistical analyses were performed on $P < 0.05$ considered significant.



Photoplate 1: Examination of live and dead sperm

Table 1: Post thaw sperm motility, fertilization and hatching percentage in response to different combinations of extenders and cryoprotectants of amur carp in normal and late breeding seasons.

		Normal breeding season			Late breeding season		
		Amur carp			Amur carp		
S.No.	Extenders with cryoprotectants	Motility percentage (%)	Fertilization Rate (%)	Hatching rate	Motility percentage	Fertilization rate	Hatching rate
1	TRIS + DMSO + Glucose	79	67	49	75	65	44
2	RPMI1640+ DMSO	84	73	55	81	72	50
3	PBS + DMSO	71	61	45	68	59	39

Results and discussion

Out of the three combinations of diluents, RPMI1640+ DMSO gave the best result in terms of post thawing motility closely followed by TRIS+ Glucose+ DMSO and PBS+DMSO. Maximum motility score was recorded with RPMI1640+DMSO. The capacity of the cryopreserved sperms to fertilise eggs was tested on Amur carp. The motility percentage was highest in RPMI1640+DMSO (84% NBS, 81% LBS) followed by TRIS + DMSO + Glucose i.e. (79% NBS, 75% LBS), PBS+DMSO (71% NBS, 68% LBS). Fertility evaluation conducted by sperm frozen with RPMI1640+DMSO on 50 ml of ova gave the highest fertilization rate of 73% (NBS),72%(LBS) and 55% (NBS), 50% (LBS) hatching rate followed by TRIS + DMSO + Glucose which gave 67% (NBS), 65% (LBS) fertilization rate with 49%(NBS),44% (LBS) of hatching rate and PBS+DMSO gave 61% (NBS) 59% (LBS) fertilization rate and 45% (NBS),39%(LBS) hatching rate. In case of late breeding season, motility, fertilizing and hatching percentage were same as in normal season only percentage was different.

Embryonic development in cryopreserved sperm-fertilized eggs of amur carp was observed from 0 hours to 70 hours and results shows that development of blastodisc started in 7 hours and development of embryo began in 11 hours. Differentiation of head region is completed in the developing embryo within 14 hours. Body somites are seen in 22 hours. Differentiation of brain parts are visible in 36 hours, followed by differentiation of eye ball, branchial and vascular bed within 46 hours, while yolk sac absorption within 60 hours. Actively functional digestive system was observed within 70 hours. By comparison of effect of three successful combinations of extenders and cryoprotectants on the basis of pattern of yolk sac absorption, it was found that combination of PBS+DMSO shown normal embryonic development whereas slightly better result was shown in TRIS+DMSO+Glucose and delayed embryonic development in RPMI+DMSO. Also, the result reveals that in case of combination of PBS+DMSO as well as TRIS+DMSO+Glucose, yolk absorption was normal while in RPMI+DMSO combination yolk absorption was slow and late. So, the combination of TRIS+DMSO+Glucose and PBS+DMSO could be used for successful embryonic development.

Long-term storage involves the use of diluting media and protective agents. Billard (1978) reported that the media in which fertilization naturally occurs didn't enhance the spermatozoan survival. Horton and Ott (1976) arrived at a formulation with three items viz. Sodium chloride for tonicity, Sodium bicarbonate for buffer and vegetable lecithin to protect

membrane. Other substance tried were Sodium citrate, Potassium chloride, Magnesium chloride, Eggyolk, Urea, Mannitol, Fructose and Dextrose. Among the protective agents the use of Ethyleneglycol (Polge *et al*. 1949) and Dimethyl sulfoxide (Lovelock and Bishop, 1959) have been reported. The best result have however, been achieved with DMSO. The present study was mainly aimed towards comparison of extenders and cryoprotectants for the survival of carp sperms under cryogenic conditions. Amongst all the combinations, TRIS+DMSO+Glucose, RPMI 1640+DMSO and PBS+DMSO gave satisfactory results but these were not consistent and cases of coagulation of thawed milt were quite common. As stated earlier, the incidence of coagulation of thawed milt was quite high with extender TRIS. Several reasons have been attributed by various workers for these clump formation after thawing the stored milt viz. improper concentration of the milt prior to their immersion in liquid nitrogen (Chao *et al.* 1975). Trials undertaken to study the relationship of DMSO concentration and clamp formation defy explanation (Withler, 1982). Along with the cryogenic preservation, motility 90% was recorded till one hour after storage thereafter it gradually declined. Stoss and Refstei (1983) have stored undiluted Rainbow trout milt for at least 23 days prior to loss of fertility. The motility score of 30-90% of diluted samples after storage period of 20 hours in the present study is quite significant as it shows that carp milt can be stored without adding any extender. Though there are several ways to detect live sperm viz. motility (Hodgins and Ridgway, 1964) differential staining (Fribourgh, 1966) the fertility evalution test is considered more accurate. In the present study only few fertility tests could be undertaken due to non-availability of sufficient number of oozing milters. The few trials conducted indicated that swirling sperm samples always give high fertilization rate while the fertilization done with negligible or even 20% motile sperms never fertilise the eggs. According to Truscott *et al.* (1968) the loss of activity in the sperm may be either due to loss of locomotion in which case the sperms are unable to find or penetrate the micropyle or to a general deterioration, as the capacity of the fertilization is lost.

The present study concludes that observations are indicative of successful cryopreservation of amur carp sperm by using either one of TRIS, RPMI1640 and PBS as extender with the addition of DMSO and Glucose together as cryoprotectants. The use of RPMI1640 diluent with DMSO is rated as the best combination for freezing amur carp sperm. The formation of sperm agglutination in amur carp sperm is likely affected by extender composition, type of cryoprotectant and cooling conditions. Thus, the use of TRIS, RPMI1640 and PBS with DMSO and Glucose for freezing amur carp sperm in the liquid nitrogen vapour may have

paramount importance being a viable protocol for captive breeding program in aquaculture. In case of embryonic development, TRIS+DMSO+Glucose and PBS+DMSO gave satisfactory result.

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