World Journal of Fish and Marine Sciences 3 (3): 194-200, 2011
ISSN 2078-4589

Seminal Plasma Composition and Their Physiological Relationship with Spermatozoa Motility in Silver Carp *Hypophthalmichthys molitrix*

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Abstract: This study investigated the mineral and organic composition of the seminal plasma, physical spermatological parameters and their relationships especially with sperm motility in silver carp *Hypophthalmichthys molitrix*. The seminal plasma contained 100.44 ± 2.37 mM L^{-1} Sodium (Na^+), 46.01 ± 1.52 mM L^{-1} Potassium (K^+), 118.99 ± 2.72 mM L^{-1} Chloride (Cl^-), 173.09 ± 9.28 mg dL^{-1} Total protein, 58.85 ± 1.37 mg dL^{-1} Albumin, 3.41 ± 0.12 mM L^{-1} Cholesterol and 3.01 ± 0.09 mM L^{-1} Calcium (Ca^{2+}). The physical spermatological parameters were as follows: sperm motility 94.63 ± 0.40 %, concentration 2.82 ± 0.08 x10^9 mL^{-1}, pH 7.25 ± 0.04 and fertilizability 66.03 ± 1.74 %. Significant positive correlations were found between sperm motility and ionic and organic components of seminal plasma. Total protein and cholesterol were found positively correlated with spermatozoa motility ($r=0.344$, $P<0.05$ and $r=0.363$, $P<0.05$ respectively).While fertilizability and sperm concentration were positively correlated with spermatozoa motility ($r=0.324$, $P<0.05$ and $r = 0.594$; $P<0.05$, respectively), seminal pH was found to be negatively correlated with motility ($r=0.446$, $P<0.05$). Among mineral and organic components of seminal plasma, a highly significant positive relationship was found between Na^+ and Cl^- ($r=0.729$, $P<0.05$), whereas a significant negative relationship was detected between concentration and pH ($r=-0.649$, $P<0.05$). K^+ ion correlated negatively with cholesterol and fertilizability ($r=-0.071$, $P<0.05$ and $r=-0.019$, $P<0.05$).The results of this study has implications with developing procedures for either artificial fertilization or cryopreservation of sperm.

Key words: Bangladesh · Sperm Motility · Silver Carp · Seminal Plasma · Mineral and Organic Components · Spermatological Parameters

INTRODUCTION

Silver carp contributes nearly 12% of the total country's fish yield with the global production of 4.1 mega ton (MT) per anum [1, 2]. Silver carp spawn between late spring and summer (April-September) when the temperature of the water is relatively high. But it does not reproduce equally between early and late seasons of spawning cycles. The rate of fertilization has been observed only 10-15% in August-September (late season) compared to April-May-June (early season) period (over 80%) (Abdul Halim, Hatchery Manager, Jagorany Chakra Fish Hatchery, Jessore, Bangladesh; personal comm. 2006). Poor fry/fingerling production performance between spawning season's results in higher production cost, lower quantity of seed and lesser access by the end users. Lower rate of fertilization in the late season could be related to the seminal plasma parameters of the semen especially sperm motility.

Seminal plasma has a unique composition, containing substances supporting sperm cells and some substances reflecting the function of the reproductive system and spermatozoa [3]. The main role of seminal plasma is to create an optimal environment for the storage of spermatozoa. In addition, seminal plasma has beneficial functions for spermatozoa during external fertilization by creating a favorable microenvironment for sperm movement [4]. It plays a crucial physioendrocrinological role in supporting spermatozoa after the release of sperm from the testis into the sperm duct and subsequently after discharge of sperm into the aquatic environment [5-6]. The composition of seminal plasma and other biological fluids can be used as reference in preparing media for use as diluents or for gamete storage.

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The correlation between seminal plasma components and the spermatozoa motility has been investigated only in few species including freshwater cyprinids: *Cyprinus carpio* [7], *Alburnus alburnus* [8] and *Acipenser fulvescens* [9]. In this regard, compared with the physiology of reproduction of other fish, less attention has been paid to that of the male silver carp.

Therefore, the current study was designed to determine the spermatological parameters; major mineral and organic components of the seminal plasma and the relationships between the spermatological and the mineral and organic contents of the seminal plasma with spermatozoa motility in silver carp.

MATERIALS AND METHODS

Experimental Fish: Silver carp broods (57.9±2.2 cm; 2.2±0.2 kg; mean±SEM) of BRAC Fish and Prawn Hatchery, Rajendrapur, Gazipur, Bangladesh were used as the experimental fishes. The experiment was carried out during the commercial operation of this hatchery between April and September.

Conditioning and Hormonal Induction of Brood Fishes: Selected brood fishes were conditioned by holding them in 1600 L flow-through-tank system for 6 hours prior to inducing by hormone and given mechanical aeration. Hormonal solution was prepared and applied to the brood fishes following standard methods [10]. Human chorionic gonadotropin (HCG; Fuda Hormone Factory, Xiamen, China) and extract of the commercially available pituitary gland (PG; Ducamar Company, Cantabria, Spain) were used as stimulating agents.

Collection of Semen: The males were anaesthetized in 100 ppm of MS 222 (Agent Labs, Redmond, WA, USA) before stripping. The semen samples were collected by gentle abdominal massage from the anterior portion of the testis towards the genital papilla with plastic syringes to prevent contamination with urine, mucus, blood and faeces. The semen was stored in sterilized plastic vials separately for each fish and held on dry ice (4°C) until measurement of sperm density within 1 day after stripping.

Detection of Sperm Motility (%): Sperm motility was determined immediately after collection of fresh semen sample. Freshly collected semen sample was diluted with 0.3% NaCl (activating solution) at 10:1 ratio (10 ml activation solution and 1 ml milt sample). Diluted samples (4-5 μL) was placed on a slide covered by a cover slip (22 × 22 mm) and sperm motility was determined using a microscope (biomicroscope, XSZ21-5DN, China) connected with a laptop (DELL, Germany) at 1600x magnification and expressed as percentage.

Determination of Sperm Concentration ($\times 10^9$ mL^{-1}): Sperm concentration was determined using improved Neubauer counting chamber (area 1 mm^2 and depth 0.1 mm) (Germany). Semen sample was diluted with 0.3% NaCl solution at 1000:1 ratio. The diluted sample was placed on the counting cells of a Neubauer chamber with a cover slip and was left for approximately 10 minutes to allow the sperm cells to be settled on the counting chamber. The sperm cells were counted by a compound light microscope (LABOMED CXRII, USA) at 160x magnification connected with a CCD camera (Unican, HV-2616, Japan) and picture was displayed on a monitor (Samsung 17 inch CRT monitor, Japan) through a TV card (PERPECT Smart Power, China). Sperm concentration was calculated following standard method [6].

Seminal Plasma Assay: Semen samples were centrifuged (REMI RM12C, Laboratory Centrifuge, Germany) for 10 minutes at room temperature at 8000 *g* to separate the seminal plasma and preserved at -20°C until analysis. While seminal pH was measured using standard pH electrodes after 30 minutes of sampling, mineral (Na^+, K^+, Cl^-, Ca^{2+}) and organic components (total protein, albumin and cholesterol) were measured following the method [11].

Data Analysis: All percent data were transformed into square root before statistical analysis, while sperm concentration data into natural log. Correlations between spermatological parameters and seminal plasma composition were estimated using Pearson's correlation test. Data have been presented as mean ± SEM and analyzed by using the statistical software SPSS version 10.0 with the level of significance at $P<0.05$.

RESULTS

Correlations Between Seminal Plasma Composition and Spermatological Variables

Spermatological Parameters: Semen of silver carp was found to be viscous in consistency and creamy white in color in all samples. Spermatological parameters of the sampled sperm were found to be rather variable (Table 1).

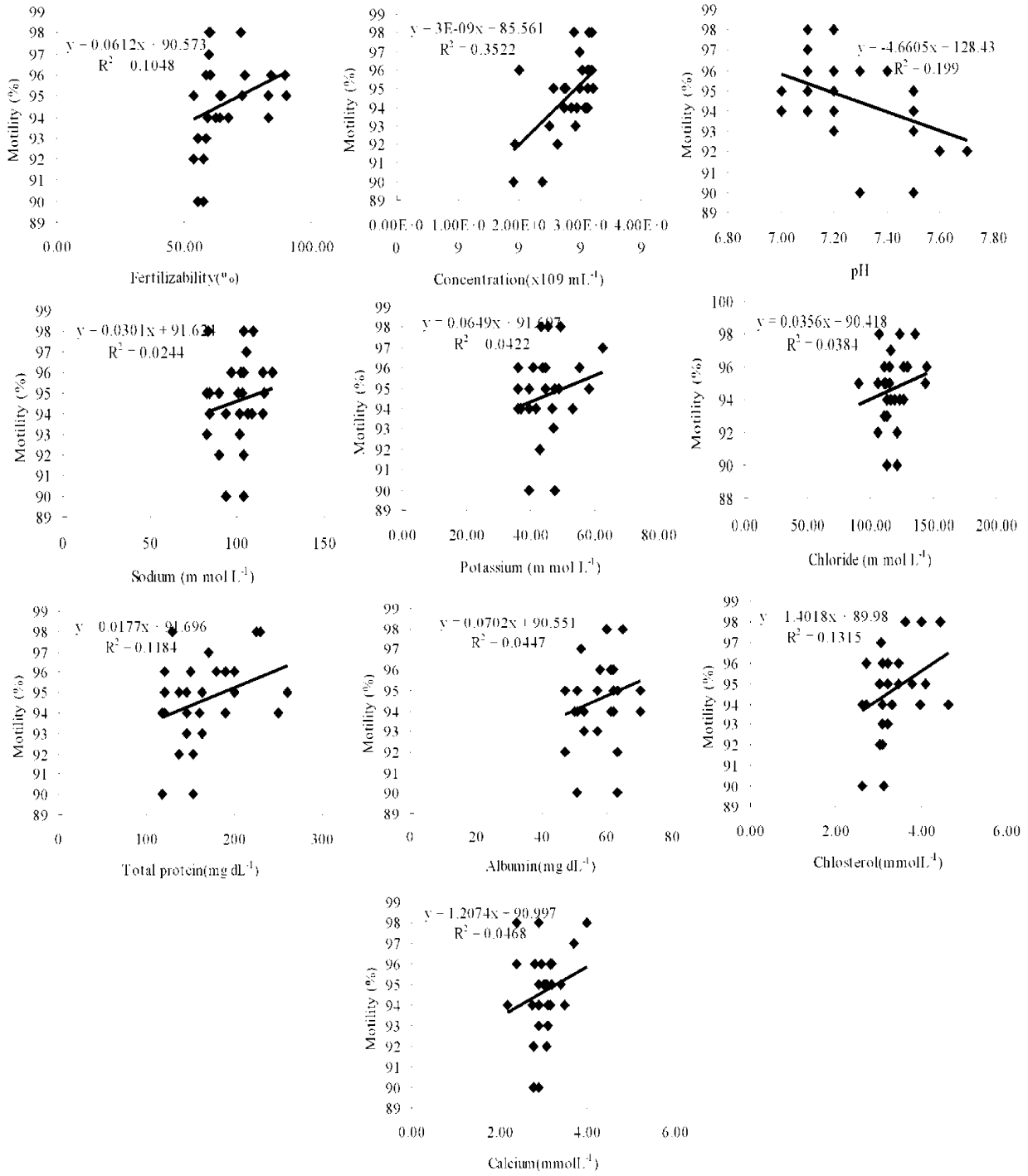

Fig. 1(a-j): Relationships between sperm motility and (a) Fertilizability (%), (b) Concentration (x10^9 mL^{-1}), (c) pH, (d) Sodium (mM L^{-1}), (e) Potassium (mM L^{-1}), (f) Chloride (mM L^{-1}), (g) Total protein (mg dL^{-1}), (h) Albumin (mg dL^{-1}), (i) Cholesterol (mM L^{-1}) and (j) Calcium (mM L^{-1}).

Motility of the spermatozoa ranged between 90% and 98% with an average value of 94.63±0.40 %. While spermatozoa motility was positively correlated with fertilizability and sperm concentration (Figure 1a & 1b), motility and semen pH was found to be negatively correlated (Figure 1c).

Table 1: Physical spermatological parameters of silver carp, *Hypophthalmichthys molitrix*

Spermatological parameters	Minimum	Maximum	Mean	SEM
Motility (%)	90.00	98.00	94.63	0.40
Concentration ($x10^9$ mL^{-1})	1.91	3.22	2.82	0.08
pH	7.00	7.70	7.25	0.04
Fertilizability (%)	53.98	90.35	66.03	1.74

Table 2: Seminal plasma ions and metabolite composition of silver carp *Hypophthalmichthys molitrix*

Variables	Minimum	Maximum	Mean	SEM
Sodium (mM L^{-1})	82.20	119.90	100.44	2.37
Potassium (mM L^{-1})	36.20	62.50	46.01	1.52
Chloride (mM L^{-1})	91.20	144.70	118.99	2.72
Total protein (mg dL^{-1})	118.00	260.00	173.09	9.28
Albumin (mg dL^{-1})	47.00	70.00	58.85	1.37
Cholesterol (mM L^{-1})	2.62	4.64	3.41	0.12
Calcium (mM L^{-1})	2.20	4.00	3.01	0.09

Table 3: Linear correlations (r) between physical spermatological variables and seminal plasma composition of silver carp *Hypophthalmichthys molitrix*

	Sodium	Potassium	Chloride	Total protein	Albumin	Cholesterol	Calcium	Concentration	Motility	Fertilizability	pH
Sodium	1										
Potassium	0.2780	1									
Chloride	0.7299	0.4614	1								
Total protein	0.3023	0.1412	0.3680	1							
Albumin	0.4617	0.0858	0.3934	0.6329	1						
Cholesterol	0.0185	-0.0715	-0.0549	0.7271	0.5414	1					
Calcium	0.2274	0.4412	0.2443	0.0873	-0.1953	-0.1062	1				
Concentration	0.0105	0.1739	0.0906	0.5209	0.1059	0.4485	0.1044	1			
Motility	0.1563	0.2053	0.1958	0.3441	0.2114	0.3626	0.2163	0.5935	1		
Fertilizability	0.2696	-0.0197	0.1198	0.6650	0.4514	0.5335	-0.0227	0.6176	0.3237	1	
pH	-0.2465	-0.2051	-0.1366	-0.4867	-0.4039	-0.5528	-0.1165	-0.6488	-0.4461	-0.6020	1

Significant at $P<0.05$

Seminal Plasma Composition: Ionic compositions of seminal plasma and metabolites of silver carp have also been determined which was found to be variable and high (Table 2). Spermatozoa motility was found to be positively correlated with minerals (Na^+, K^+, Cl^- Ca^{2+} ions) and total protein, albumin and cholesterol (Figure1d-j; Table 3). K^+ ion correlated negatively with cholesterol and fertilizability. A high significant positive relationship was also found between Na^+ and Cl^- whereas a significant negative relationship was detected between concentration and pH. Fertilizability correlated positively with total protein and concentration. Albumin showed a significant positive relationship with total protein. Cholesterol correlated positively with total protein, albumin and fertilizability.

DISCUSSION

Changes in sperm motility in silver carp over spawning seasons could have resulted from repeated use of the same male breeder for spawning, disturbances in spermatogenesis, time of spawning, collection technique and time of collection or dilution ratio. Discontinuous spermatogenesis may lead to deficiency in the formation of seminal plasma which may affect the sperm motility. However, individual variation in sperm motility has also been demonstrated depending on their maturity [12].

Sperm concentration of silver carp has a positive correlation with sperm motility. As the sperm concentration increased, sperm motility also increased. The observed difference in the concentration of sperm across spawning seasons could have resulted from discontinuous spermatogenesis. Sperm concentration might be related to gonadal development and maturation, which are also regulated by changing in climate, day length and food supply. Changes in sperms concentration could also be related to the differences in hormonal stimulation methods, environmental conditions, biological characters of the brood fish such as age and others [13].

The observed negative correlation of seminal pH with sperm motility in silver carp could be the result of complex chemistry among the seminal plasma components. Lower seminal pH indicates the best alkaline condition for fish sperm viable. Similar findings have been confirmed in common carp [14] and grass carp [15].

The positive correlation between fertilizabitily and sperm motility as found in silver carp is expected. The higher the sperm motility, the higher the possibility to be fertilized the egg. The difference in fertilizability in the spawning season has resulted due to sperm concentration. Repeated use of the same breeders could be responsible for lower quality of sperm. However, higher fertilization than the current experimental fishes has also been observed in catla *Catla catla* about 91-92% [16].

Knowledge of the physical and chemical constituents of spermatozoa and seminal plasma is a prerequisite for the successful evaluation of the reproductive ability of different fishes. Mineral and biochemical constituents of seminal plasma lead to a better understanding of mechanisms of fertilization. In contrast to that of higher vertebrates, fish seminal plasma is characterized by a low total protein concentration, substantial mineral compounds (Na^+, K^+, Cl^-, Ca^{2+}) and low concentration of other organic components (total protein, albumin and cholesterol).

The observed Na^+ content in seminal plasma of silver carp is higher than that of common carp (75 mML^{-1}) [17]; grass carp (98 mML^{-1}) [15] and rainbow trout (80 mML^{-1}) [18] but lower than perch (124 mML^{-1}) [19] and Asian catfish (164 $mM\,L^{-1}$) [20]. However, in this study, seminal K^+ level of silver carp is lower than in common carp (70 $mM\,L^{-1}$) [17] but higher than that of Atlantic salmon *Salmo salar* (28 $mM\,L^{-1}$) [21] and Asian catfish (18 $mM\,L^{-1}$) [20]. This difference in the seminal Na^+ and K^+ concentration denotes species-specific characteristics [22]. The electrolytes ensure the viability of the sperm. K^+ ion has a specific role in maintaining spermatozoa in quiescent state [23]. Low levels of Na^+ and K^+ ions are associated with low percentages of motile spermatozoa and such semen is considered to be of low quality. The observed low levels of Na^+ and K^+ may be related to the deficiency in the seminal plasma formation. K^+ has an inhibitory effect on the initiation of sperm motility in salmonids [24] which is associated with a reduction in sperm-fertilizing ability. But this situation is not clear in cyprinidae. In this current study, percentage of motile sperm cells in silver carps' semen has increased when electrolytes levels in the seminal plasma has increased. The findings of this research indicated that K^+ increased the motility of silver carps' spermatozoa. Similar result was also confirmed in carp *Cyprinus carpio* [25] and grass carp [15].

Seminal Cl^- ion concentration of silver carp is higher than that of ocean pout (9.04 - 21.11 $mM\,L^{-1}$) [6] but lower than in Caspian brown trout *Salmo trutta caspius* (133.04 ± 5.96 $mM\,L^{-1}$) [11]. However, Cl^- ion concentration has been reported to be in the range of 135 $mM\,L^{-1}$ in salmonids and 96-110$mM\,L^{-1}$ in cyprinids [26], which is close to our findings. The observed seminal Ca^{2+} level of silver carp seminal plasma was similar to brown trout *Salmo trutta fario* (3.50±0.67 $mM\,L^{-1}$) [27] However, seminal plasma of the studied species had higher Ca^{2+} ion concentration compared to that of Caspian brown trout (1.68±0.20 $mM\,L^{-1}$) [11] and barbel (0.3-0.4 $mM\,L^{-1}$) [28]. This variable concentration of seminal Cl^- and Ca^{2+} ion could be related to the secretion of seminal plasma from the spermatic duct epithelium [29]. The difference could also be related to several parameters including spawning time of the fish species and urine contaminated semen during stripping [30].

In this study, the observed high concentration of total protein and albumin in seminal plasma in silver carp indicates a high demand for protein. High level of protein might have a protective role in sperm motility. However, because of negative correlation of total protein with the sperm motility, the specific role of protein in fish semen remains unknown. Seminal plasma protein prolongs the viability of spermatozoa in rainbow trout as measured by sperm motility [31]. In this study, seminal cholesterol level of silver fish does not change much across the spawning seasons. Though it is identified in the seminal plasma of freshwater fish [32], its role is undefined. However, cholesterol could have a protective effect on environmental changes (especially on temperature) that might occur upon releasing fish semen into water. Different studies have showed that seminal plasma components not only show an inter-specific variation but also vary among different groups of fish of the same species [33].

CONCLUSIONS

The analysis of physical, mineral and bio-chemical components of semen revealed some species-specific characteristics, particularly K^+ and protein levels. The results of this study have shown that the quality of sperm changes in silver carp as the spawning seasons progressed. The current study on the quality of silver

carp seminal plasma and its relationship with sperm motility helps in determining the optimum quality of sperm to be used for artificial fertilization purposes. It also provides a better knowledge of the changes of ionic and bio-chemical components of seminal plasma, which could be very useful to improve cryopreservation techniques used for long term conservation of fish sperm for artificial fertilization.

ACKNOWLEDGEMENTS

The authors wish to thank Manager, BRAC Fish and Prawn Hatchery, Rajendrapur, Gazipur, Bangladesh for providing their facilities in this study.

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