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Physical and Biochemical Characteristics of Semen and Ultrastructure of Spermatozoa in Six Carp Species

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Abstract

The aim of the study was to investigate the physical difference in biochemical characteristics of spermatological and seminal plasma and also ultrastructure of spermatozoa in six carp species, viz. catla, *Catla catla*, rohu, *Labeo rohita*, kalbasu, *Labeo calbasu*, mrigal, *Cirrhinus mrigala*, silver carp, *Hypophthalmichthys molitrix* and grass carp, *Ctenopharyngodon idella.* Semen yield from different carps ranged from 6.6 to 8.9-ml/kg body weight at the peak-spawning season. Sperm density (spermatocrit) showed a range of 69-81% among the six carps. Similarly, the sperm count varied between 2.6 x 10^{10}/ml to 3.5 x 10^{10}/ml in six species of carps. The motility of carp spermatozoa ranged from 80 to 110 sec in different species. Maximum motility of 110 sec was observed in mrigal. The mean pH and osmolality ranges were 7.3-8.1 and 269-289 mOsm/kg. The ion concentrations were: Na^+ 106±1.2, 85.2±1.0, 81.8±3.6, 94±2.27, 64±1.2, 110 ± 0.81 mEq/L, K^+ 25±1.2, 32.1±1.1, 48.6±2.4, 51±3.67, 25±0.8, 36 ±1.2 mEq/L, Cl^- 246 ±2.0, 175±4.3, 174±5.2, 245±7.7, 226 ±0.8, 253±0.4 mEq/L for catla, rohu, mrigal, kalbasu, silver carp and grass carp, respectively. The other parameters of seminal plasma were: total protein; 0.2±0.006, 0.1±0.006, 0.4±0.06, 0.4±0.04, 0.6±0.08, 0.8±0.14 g/dl; cholesterol 14.1±0.8, 17.4±1.7, 22.8±1.3, 15.4±0.61, 14.0±0.7, 12.7±0.7 mg/dl and glucose 1.2±0.11, 1.4±0.07, 1.8±0.16, 1.2±0.12, 2.0±0.34, 2.0±0.35 mg/dl for catla, rohu, mrigal, kalbasu, silver carp and grass carp, respectively. Ultra structure by electron microscopy revealed that spermatozoon of carp is composed of head without any acrosomal complex with circular to elliptical nucleus and tail or flagellum with a midpiece consisting of mitochondrial ring. The size of the spermatozoa varied significantly among different carp species ($P<0.05$). All six carp flagellum has a typical 9+2 axoneme arrangement. Indian major carps, silver carp and grass carp are seasonal breeders and to make artificial propagation successful by utilizing the available brood fish in an efficient way, the present information on the normal physical and chemical characteristics of semen of these carps presented in this paper will eventually help in selecting good milters and devising improved protocols for cryopreservation and artificial propagation methods.

Keywords: CASA, carp, ion composition, motility, osmolality, semen, ultrastructure.

Introduction

The study of fish spermatozoa has revealed a great variety in both morphology and ultrastructure, which has been useful in establishing phylogenetic relationships among species (Jamieson, 1991). To date, much of the fish gamete research has been centred on the male germ cells, the spermatozoa and many excellent studies concerning the biology, physiology and preservation of the spermatozoa have been performed (Scott and Baynes, 1980; Stoss, 1983). Fish sperm might be varied from aflagellate to biflagellate and shows an enormous range of shapes and sizes and structures; the number and location of organelles might also be varied (Baccetti *et al*., 1984, Baccetti, 1986; Jones and Butler, 1988). Similarly, seminal plasma is an important constituent of semen that has a vital role in sperm metabolism, function, survival and sperm motility. The cations such as Na^+, K^+ and Cl^- in the seminal plasma establish osmotic balance, while essential trace elements are components of many important enzymes. Thus, biochemical evaluation of seminal plasma is an important criterion for assessment of milt quality (Billard *et al*., 1995). The structure of sperm and the biochemical composition of seminal plasma might be varied more or less widely within families. For instance, the spermatozoa of seven cyprinid species revealed that each was characterized by a specific organization of sperm organelles within a general pattern common to the whole family (Baccetti *et al*., 1984). However, there are several variations in the size, sperm count, duration of motility and biochemical composition among different species of carps. Moreover, knowledge of sperm structure may also be useful for evaluation of possible damage consequent to either cryopreservation (Laveroni Calvi *et al*., 1994; Lahnsteiner *et al*., 1996) or exposure to contaminants (Van Look and Kime, 2003). Knowledge of the species-specific spermatozoon ultrastructure is not only important in systematic and phylogeny but has also applications related to artificial fertilization and sperm preservation (Billard, 1978; Suquet *et al*., 1998).

Indian carps such as catla, *Catla catla*, rohu, *Labeo rohita*, kalbasu, *Labeo calbasu,* mrigal, *Cirrhinus mrigala* and other carps such as silver carp, *Hypophthalmichthys molitrix* and grass carp,

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Ctenopharyngodon idella are economically important food fish widely cultured and propagated in the Indian sub-continent. Though these fish are widely propagated in hatcheries at many places, their semen characteristics either biochemical or ultra structural are not studied systematically which has a major role in the success of artificial fertilization and cryopreservation. Successful cryopreservation techniques have been established for several fish species but there is a wide variation in the fertilization ability of post-thawed spermatozoa. This in turn has been presumed to be due to sperm quality (Linhart *et al*., 2000). Therefore, the use of high quality gametes from captive fish brood stock is of great importance for ensuring production of valuable offspring and increased production in aquaculture (Bromage, 1995). In order to have controlled and successful production in aquaculture systems, it is necessary to have adequate knowledge of the physical and chemical characteristics of the semen and spermatozoon structure of cultivated fish.

Therefore, the aim of the present study was to investigate the physical and biochemical characteristics of semen and ultra structure of spermatozoa in six carps at peak spawning season (June and July).

Materials and Methods

Brood Stock Management

The brood fish of Indian major carps and exotic carps in the three years age group (mean weight 1.9±0.8 kg) were reared in earthen ponds of each 0.1 ha (50 x 20 m, 1.5-2 m depth) with a stocking rate of 1000-1500 kg/ha at the farm facility of Central Institute of Freshwater Aquaculture, Bhubaneswar, India. Pond preparation procedure remained similar to that of Nandi *et al*. (2007). Essentially the procedure involved the periodic fertilization of pond using raw cow dung manure at 2500 kg/ha and single super phosphate at 100 kg/ha in ten equal instalments followed by liming at 200 kg/ha after seven days fertilization. Total water replenishment was 20-30% during the pre-spawning period (February-April) (Nandi *et al.*, 2007). Physico-chemical analysis of pond water was conducted fortnightly following APHA (1992). A standard feed containing groundnut oil cake, rice meal, fishmeal, roasted soybean meal, vegetable oil, fish oil and vitamin mineral premix was provided to the brood fish.

Collection of Milt

For semen collection, two year matured male brood fish of *Catla catla* (2.2± 0.4 kg), *Labeo rohita* (1.8±0.5 kg), *Labeo calbasu* (1.0±0.2 kg), *Cirrhinus mrigala* (1.6±0.4 kg), *Hypophthalmichthys molitrix* (2.3±0.6 kg) and *Ctenopharyngodon idella* (2.8±0.8 kg) were administered intraperitoneal hormone Ovaprim (Salmon GnRH + domperidone, Ovaprim Syndel Laboratories, Canada) at a rate of 0.2 ml/kg body weight. Semen samples were collected in ice cooled sterilized test tubes after 4 h of hormone administration. During semen collection, attention was paid to prevent contamination by faecal matter, urine, blood or scales; to provide enough oxygenation to the sperm by keeping enough head space in the tubes and maintain the temperature of the collected semen at 4°C until further analysis.

Motility Assessment

The collected milt of carps was evaluated for sperm yield kg^{-1} body weight, motility, pH, spermatocrit percentage and sperm count. Spermatozoa motility assessment was carried out by diluting milt with sterile water (1: 100) at room temperature (31°C) on glass slide, observed immediately under an inverted microscope (200 X) (Zeiss, Germany) with a CCD camera attachment. Estimation of spermatozoa motility was started immediately (approximately 10 s) after dilution and the movement was observed till 2 min. The motility was recorded in a computer by using computer aided motility software (Biovis motility software, Expert Vision Pvt. Ltd, India) and computer assisted sperm analyzer (CASA). The percentage of rapid, vigorous and forward (RVF) motility was observed and calculated in relation to the total number of observed (immotile and poorly motile) spermatozoa in each field of vision from the time activator and was added until the motility ceased. The following parameters were recorded: number of sperm exhibiting motility (%), progressive motility (%), average path velocity (VAP) (µm/s), curvilinear velocity (VCL) (µm/s), straight line velocity (VSL) (µm/s), amplitude of lateral head displacement (ALH) (µm), beat cross frequency (BCF) (Hz) and linearity (LIN) (%).

Estimation of Sperm Count, Spermatocrit and Osmolality

Sperm density was determined by measuring spermatocrit value and also through microscopic sperm counting. Microhaematocrit capillary tubes (75 mm length and 1.2 mm diameter) were filled (approximately 75%) with semen and one end of each tube was sealed for tube centrifugation in a microhaematocrit centrifuge at 10,000 x g (Hermle, USA) for 5 minutes. Measurements were taken in triplicate for each sample and the average of the three measurements was used for the results immediately after semen collection to avoid abnormal reading due to cellular swelling induced by CO_2 release (Wedemeyer and Yasutake, 1977). The sperm count was done by diluting it 1000 times with an Extender C (Gupta *et al*., 1995) and adding 20 µl of mixture to the haemocytometer slide and observed under an inverted microscope and expressed as number of

spermatozoa /ml. The osmolality of seminal plasma was measured simultaneously in an osmometer (Model 3250, Advanced Instruments Inc, Massachusetts-02062, USA) and expressed as mOsmol /kg.

Biochemical Analysis of Seminal Plasma

Milt from each sample was centrifuged (10,000 x g, 10 min) and the seminal plasma was removed and kept at -20°C for further analysis in the laboratory of Biochemistry at the S.C.B. Medical College and Hospital, Cuttack, India. All electrolytes, metabolites and enzymes were determined using an automated system with adequate standards (Flexor-XL ISE, Netherlands). The following parameters were measured and expressed in the following units: albumin (g/dl), glucose (mg/dl) (Srikanth *et al*., 2004), urea, uric acid (Fei *et al*., 2006), cholesterol, triglycerides (Sullivan *et al*., 1985), bilirubin, urea, creatinine (mg/dl) alanine aminotransferase (ALP or GPT), aspartate aminotransferase (AST or GOT), alkaline phosphatase (U/l), chloride, potassium, sodium (mEq/l) (Ng *et al*., 1985), albumin and total protein (g/dl) (Kinsgsley, 1939). Alanine aminotransferase and aspartate aminotransferase were measured by following modified IFCC method (without pyridoxal phosphate) (Henderson and Donald, 2001) and kinetic colorimetry using p-nitrophenylphosphate (modified IFCC) method, respectively.

Sperm Ultrastructure

The semen from six species of carps was collected as described earlier for study of ultrastructual morphology by scanning electron microscope (SEM) and transmission electron microscope (TEM). A fixation technique for transmission microscopy was done as used by Lahnsteiner and Patzner (1991). For TEM, semen samples were fixed in modified Karnovsky's fluid (0.2 M phosphate or cacodylate Buffer 500 ml and Paraformaldehyde 40 g in 960 ml double distilled water and 40 ml of 25% glutaraldehyde) (David *et al*., 1973) buffered with 0.1 M sodium phosphate buffers at pH 7.4. Fixation was for 10-18 h at 4°C temperature, after which the tissues were washed in fresh buffer and post fixed for two hours in 1% osmium tetraoxide in the same buffer at 4°C. After several washes in 0.1 M Sodium phosphate buffer, the specimens were dehydrated in graded acetone solutions and embedded in CY 21 araldite. Ultrathin sections of 60-80 nm thickness were cut using an ultracut E (Reichert Jung) ultra-microtome and the sections were stained in 2% alcoholic uranyl acetate (10 min) and lead citrate (10 min) before examining the grids in a transmission electron microscope (Philips, CM-10) operated at 60-80 KV.

The SEM fixation was done as described by Marquez and Ogasawara (1975). Milt smears were fixed in modified Karnovsky's fluid buffered with 0.1 M sodium phosphate buffer at pH 7.4. Fixation was for 3 h at 4°C temperature, after which the sample was washed with fresh buffer and washed three times in double distilled water for 15 min each. The sample was air dried and coated with 20nm gold palladium (SEM Leo 435 UP) in a sputter coater and observed under a SEM (Morgangi-268 D) at 80 KV.

Statistical Analysis

The data for semen parameters were analyzed for each characteristic using triplicate samples taken from 6 different species (n=30 for each species). Statistical evaluation was performed by Duncan's multiple range test (DMRT). A *P* value of $P<0.05$ was considered as statistically significant.

Results

Semen Characteristics and Seminal Plasma Composition

The semen of Indian major carps, silver carp and grass carp was white in colour with little fishy odour. The semen yield, spermatocrit value, sperm count, seminal plasma osmolality and motility status of semen of six carps are shown in Table 1.

The semen yield from different carps ranged from 6.6 to 8.9 ml/Kg-body weight at the peak-spawning season (June and July). Maximum semen yield was recorded from mrigal (8.9 ml/kg body weight). The sperm density (spermatocrit) showed a range from 69 to 81% among the six carps. Similarly, the sperm count varied between 2.6 x 10^{10}/ml to 3.5 x 10^{10}/ml in six species of carps. The minimum sperm count was recorded in silver carp and maximum in kalbasu. The mean pH and osmolality value ranges were 7.3-8.1 and 269-289 (mOsm/kg) in all the six carps. The spermatozoa remained immobile in the semen and were activated after dilution with freshwater. The motility of carp spermatozoa ranged from 80 to 110 sec in different species. Maximum motility of 110 sec was observed in mrigal whereas minimum values observed in silver carp (80 sec) and in catla (85 sec). The computer aided semen analysis showed different kinds of motility of carp spermatozoa as shown in Figure 1. Among the different types of motility of spermatozoa, the type that exhibited more were: VCL (58) and linearity (64) in catla, progressive (60) and linearity (74%) in rohu, VAP (53) and linearity (65%) in mrigal, VCL (64) and linearity (65%) in kalbasu, progressive (93%) and linearity (71%) in silver carp and progressive (71) and linearity (73%) in grass carp. The detailed biochemical parameters of seminal plasma of carps are shown in the Table 2.

Spermatozoa Ultrastructure

Ultrastructure by electron microscopy revealed

Table 1. Semen characteristics of six carp species

Parameters	Catla	Rohu	Mrigal	Kalbasu	Silver carp	Grass carp
Mean semen yield (ml/kg body weight	7.3±0.3[a]	8.3±0.9[a]	8.9±0.7[a]	6.6±2.1[b]	6.9±0.4[b]	7.1±2[a]
Spermatocrit (%)	72±3.2[b]	67±3.5a	81±2.8[a]	80±2.6[a]	69±1.5[b]	72±1.8[b]
Sperm count (x 10^{10}/ml)	2.8±0.19[b]	2.71±0.7[b]	3.2±0.2[a]	3.5±0.3[a]	2.6±0.2[b]	3±0.2[b]
Motility (%)	90±1.5[a]	90±2.3[a]	92±1.4[a]	88±3[a]	93±1.7[a]	89±3.2[a]
Maximum duration of motility (seconds)	80±4.5[d]	90±5.5[c]	110±5.0[a]	100±5.5[b]	75±3.5[d]	85±2.5[d]
Semen pH	7.8±0.07[b]	7.3±0.06[c]	7.9±0.05[b]	8.1±0.09[a]	7.8±0.03[b]	7.9±0.06[b]
Seminal plasma osmolality (mOsm/kg)	278±7.0[c]	269±5.5[d]	284±7.5[a]	289±8.0[a]	276±4.3[c]	269±1.8[d]

Data are expressed as mean±SEM. (n=9).
Values having different letters differ significantly in a row (P<0.05).

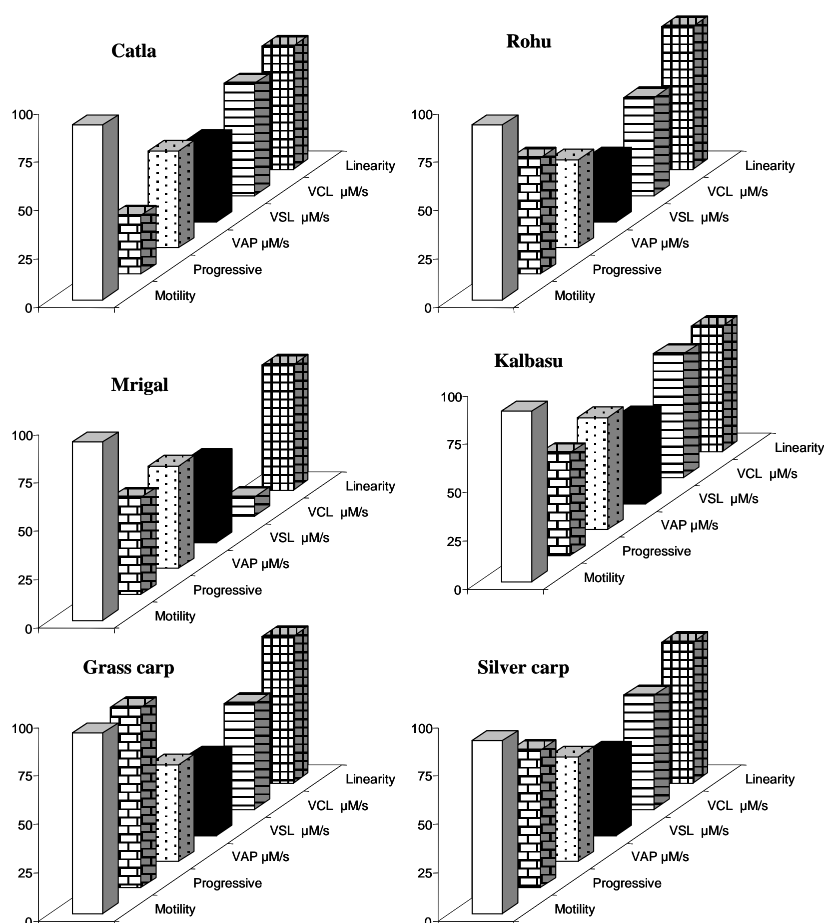

Figure 1. Motility patterns of spermatozoa in six carp species. (motility, progressive motility, VAP : average path velocity, VSL: straight line velocity, VCL: curvilinear velocity and linearity LIN). Data are expressed as mean±SEM.

that spermatozoon of carp is composed of head without any acrosomal complex with circular to elliptical nucleus and tail or flagellum with a midpiece consisting of mitochondrial ring. The size of the spermatozoa differs significantly among different carp species (P<0.05). The morphometric analyses of spermatozoa of six caps are shown in Table 3. The mean length of carp spermatozoa was in the range of 15±2.6 µm to 24±2.4 µm. The catla spermatozoa length was the lowest among the group (15 µm) and maximum 24 µm was observed in silver carp and rohu spermatozoa. The spermatozoa morphology as revealed by SEM is shown in Figure 2. The spermatozoa of these carps are uniflagellated and have circular to elliptical nucleus (Figure 3). A nucleus with electron dense granular chromatin occupied the head nuclear region. The centriolar apparatus was asymmetrically attached to the nucleus. The mid-piece was cylindrical, rich in cytoplasmic material, containing spherical mitochondria arranged around a post nuclear canal (Figure 4). The mid piece is joined with the posterior portion of the head,

Table 2. Biochemical characteristics of seminal plasma of carps

Parameters	Catla (Mean±SEM)	Rohu (Mean±SEM)	Mrigal (Mean±SEM)	Kalbasu (Mean±SEM)	Silver carp (Mean±SEM)	Grass carp (Mean±SEM)
Albumin (g/dl)	0.1±0.006^b	0.1±0.02^b	0.2±0.02^a	0.11±0.02^b	0.13±0.08^b	0.19±0.01^c
Protein (g/dl)	0.2±0.006^d	0.1±0.006^d	0.4±0.06^d	0.4±0.04^c	0.6±0.08^b	0.8±0.14^a
Glucose (mg/dl)	1.2±0.11^b	1.4±0.07^b	1.8±0.16^c	1.2±0.12^b	2.0±0.34^a	2.0±0.35^a
Urea (mg/dl)	1.0±0.12^c	2.7±0.25^b	3.4±0.37^b	3.0±0.39^b	3.0±0.06^b	5.0±1.03^a
Creatinine (mg/dl)	0.6±0.04^d	0.42±0.03^d	1.34±0.23^b	2.2±0.29^a	1.1±0.02^c	1.3±0.12^c
Total bilirubin (mg/dl)	0.2±1.08^b	0.09±0.01^b	0.2±0.04^b	0.2±0.02^b	0.1±0.81^b	0.1±0.5^a
Cholesterol (mg/dl)	14.1±0.8^c	17.4±1.7^b	22.8±1.3^a	15.4±0.61^b	14.0±0.7^c	12.7±0.7^c
HDL-Cholesterol (mg/dl)	6.0±1.08^c	8.2±1.3^c	7.0±1.2^a	4.0±0.40^c	5.0±1.2^c	4.0±0.3^c
Triglycerides (mg/dl)	3.0±0.4^d	11.7±1.1^c	19.4±0.1^c	15.0±1.22^b	18±0.2^b	39±2.44^a
Acid uric (mg/dl)	0.1±0.006^b	0.1±0.007^b	0.4±2.27^b	1.0±0.10^a	1.0±1.6^a	1.0±0.3^a
Sodium (mEq/L)	106±1.2^a	85.2±1.0^c	81.8±3.6^a	94±2.27^b	64±1.2^d	110±0.81^a
Potassium (mEq/L)	25±1.2^b	32.1±1.1^b	48.6±2.4^a	51±3.67^a	25±0.8^b	36±1.2^b
Chloride (mEq/L)	246±2.0^b	175±4.3^d	174±5.2^d	245±7.7^b	226±0.8^c	253±0.4^a
GOT (U/I)	12±1.6^c	23±1.5^a	21±1.18^a	15.9±7.6^b	21±1.6^a	11±1.22^c
GPT (U/I)	9±2.2^a	4.2±2^d	2.4±0.2^c	8.0±0.4^a	4.0±0.7^d	7.0±0.81^c

Data are expressed as mean±SEM. Values having different letters differ significantly in a row ($P<0.05$)

Table 3. Morphometric analysis of spermatozoa of different carps

Parameters	Catla	Rohu	Mrigal	Kalbasu	Silver carp	Grass carp
Mean length of Spermatozoa (µm)	15.0±2.6^d	24.0±2^a	19.0±1.8^c	22.0±3^b	24.0±2.4^a	23.0±2.1^a
Mean head length (µm)	1.5±0.3^b	1.9±0.2^a	2.0±0.1^a	1.5±0.1^b	2.0±0.16^a	2.0±0.2^a
Mean head width (µm)	1.5±0.3^c	1.6±0.2^c	1.8±0.1^b	1.3±0.1^d	1.8±0.15^b	2.0±0.2^a
Mean mid-piece length (µm)	0.4±0.08^c	0.6±0.04^a	0.5±0.06^b	0.5±0.03^b	0.5±0.02^b	0.6±0.02^a
Mean mid-piece width (µm)	0.7±0.05^b	0.5±0.08^c	0.9±0.06^a	0.5±0.02^c	0.5±0.02^c	0.5±0.03^c
Mean flagellum length (µm)	13.1±0.9^d	21.5±1.2^a	16.5±1.4^c	20.0±2.4^b	21.5±3^a	20.4±2.9^b

Data are expressed as mean±SEM. Values having different letters differ significantly in a row ($P<0.05$)

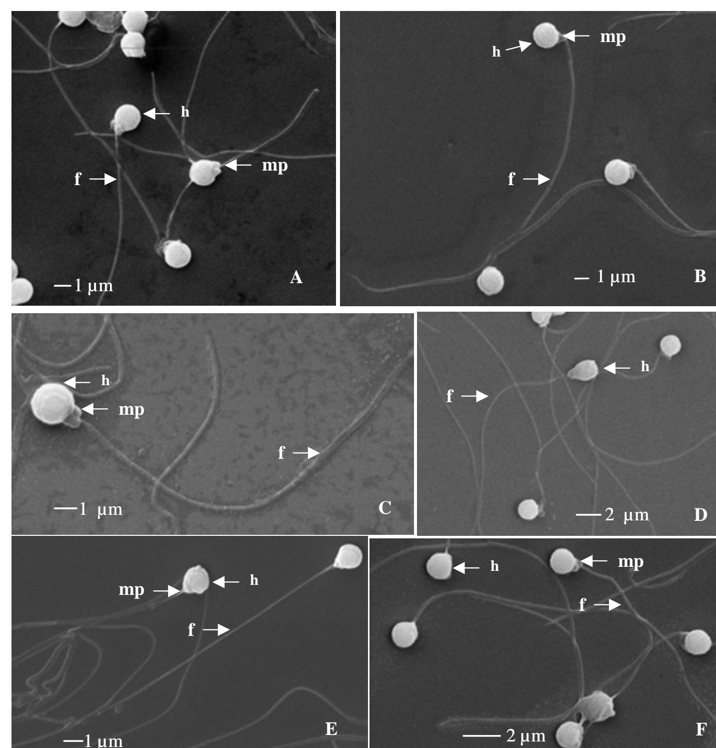

Figure 2. Scanning electron micrograph showing the morphological structures of six carp species. (A: catla, B: rohu, C: mrigal, D: kalbasu, E: grass carp, F: silver carp). (h: head, mp: mid piece, f: flagellum).

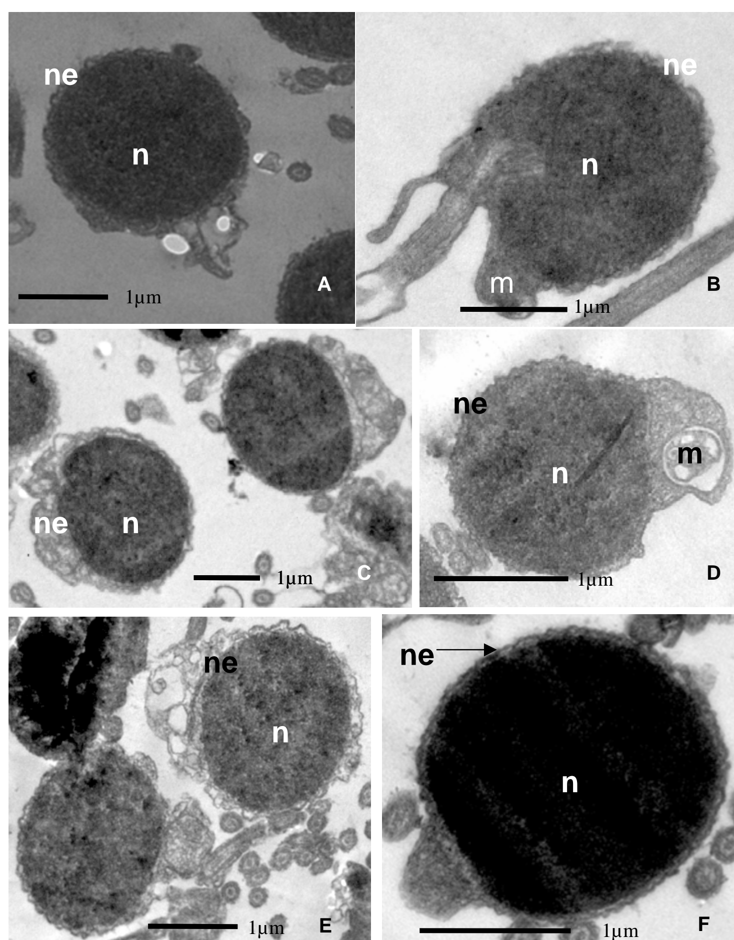

Figure 3. Transmission electron micrographs of head of the mature spermatozoon of carps. A: Catla, B: Rohu, C: Mrigal, D: Kalbasu, E: Silver carp, F: Grass carp. n: nucleus, ne: nuclear envelope, m: mitochondria.

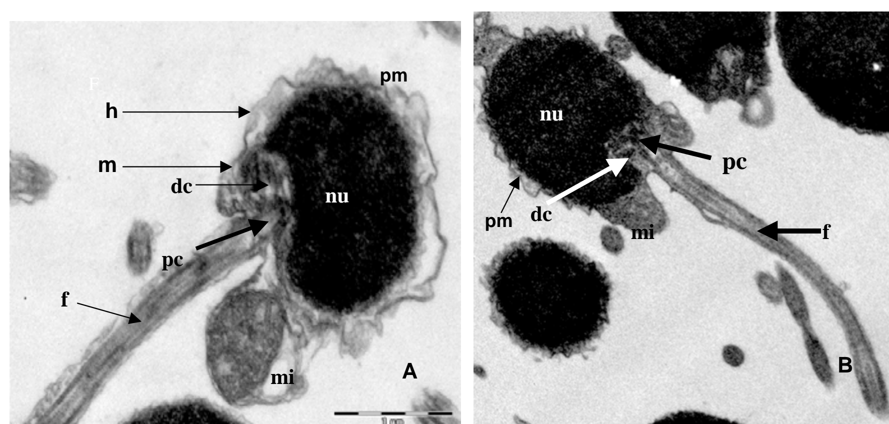

Figure 4. Transmission electron micrograph of a spermatozoon of (A) mrigal and (B) Rohu; h: head, m: midpiece, f: flagellum, pc: proximal centriole, dc: distal centriole, nu: nucleus, pm: plasma membrane, mi: mitochondria.

consists of a mitochondrial ring and centrioles (Figure 4). An axoneme with the typical pattern of two central microtubules surrounded by a ring of nine doublets originated from basal body of the distal centriole and pervaded the mid-piece (Figure 5). This pattern was found in all the six carps. The mean length of sperm head and flagellum for catla, rohu, mrigal, kalbasu, silver carp and grass carp is shown in Table 3. A long flagellum was noticed in case of grass carp and silver carp.

Discussion

It is well known that the amount of milt produced from a fish is of vital importance in fertilization process, as large amount of spermatozoa

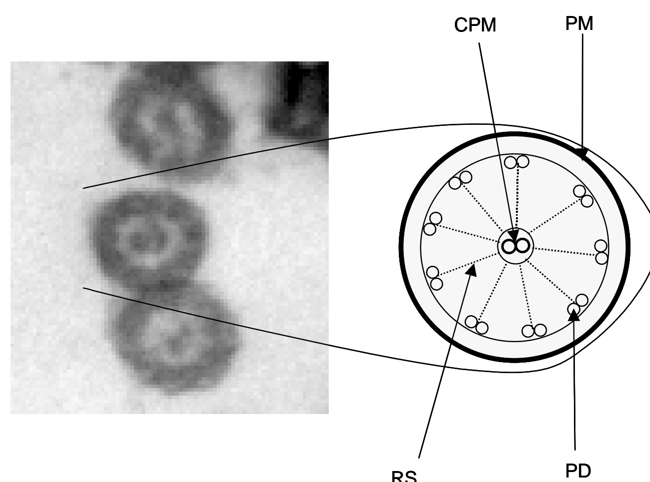

Figure 5. Ultrastructure of the flagellum of Indian major carp with a schematic representation of the inner structure showing the 9+2 arrangement; CPM: central microtubule, PM: plasma membrane, RS: radial spokes, PD: peripheral doublets.

gets wasted due to several problems associated with the external environment and short motility time of spermatozoa. Similarly, the semen characteristic also varies from species to species. These parameters are important for devising various breeding and cryopreservation protocols of fish spermatozoa (Routray *et al*., 2006; Routray *et al*., 2007). In the present study, we assessed the semen characteristics and ultrastructure of six important carp species for generating an information database for future research in the carp gamete research. The variation in the semen yield from different carps was evident due to the individual characteristics of species. However, in any case the semen yield from these carps was not less than 6 ml per kg body weight. The semen yield from jundiá, *Rhamdia quelen* has been reported to be in the range of 0.24 to 0.95 ml per kg body weight (Borges *et al*., 2005). Similarly, semen yield from *Labeo rohita* injected with pituitary gland extract has been reported to be 3.63-ml/kg body weight (Khan *et al*., 1992). In the present study, all the species were injected with synthetic GnRH + Dopamine antogonist hormone (Ovaprim) that has resulted in better semen output from carps.

A wide variation in the sperm density (spermatocrit) and sperm count was noticed among the different carp species. These variations are due to the spermatozoa size and species-specific nature of carps. Significant correlation between spermatozoa density and spermatocrit was reported in rainbow trout (Baynes and Scott, 1985) and for several other teleost species (Bouck and Jacobson, 1976; Piironen, 1985; Ciereszko and Dabrowski, 1993; Ratikin *et al*., 1999; Tvedt *et al*., 2001). The present study also showed a trend that the higher the sperm count the more was the spermatocrit value in carps and recommends using spermatocrit value as a method of determining the sperm density. In *Labeo rohita* spermatocrit value of more than 70% is generally recommended for utilization in cryopreservation and fertilization process (Routray *et al*., 2006).

The motility of spermatozoa upon activation was more than 90% in carps and the motility remains for 75 to 110 sec. After this time, most of the spermatozoa becomes immobile. The duration of spermatozoa motility of several cyprinids is till 120 seconds (Suzuki, 1959). Similarly, here in case of Indian major carps, silver carp and grass carp, it was observed till 110 sec. The different types of motility exhibited by carp spermatozoa have been enumerated by CASA. The most obvious parameters that are useful in assessing sperm quality are motility, progressive motility and duration of movement. In mammals, the straight line velocity (VSL) *i.e.,* the progressive velocity in a specific direction of sperm is the most reliable indicator of fertility (Moore and Akhondi, 1996). In fish, the trajectory was generally more curved than mammals and fish sperm can move three dimensionally in the aqueous medium, so the duration of progressive movement decreases rapidly. This is an important factor for the sperm ability to enter the egg. Here, the progressive motility was more than 60% in spermatozoa of Indian major carps as well as in silver and grass carps. Carp spermatozoa in this study exhibited mostly four motility patterns; namely, progressive, VSl, VCL and linear and some spermatozoa also showed haphazard movement. The motility pattern and duration of motility demonstrated pronounced time dependent motility after activation. This study provides a first hand report about the motility patterns of spermatozoa of six species of carps. Motility patterns of carp spermatozoa following short-term storage of semen demonstrated a time dependent decrease in VCL, VAP, VSL and ALH following activation with water (Ravinder *et al*., 1997). The CASA system is a useful tool to achieve reliable results to analyze sperm quality; because it takes several images of sperm samples and using an

automatic process, analyzes the motility parameters that are essential to determine the quality of the studied sperm.

Moreover, studies focused on the biochemical composition of seminal plasma of carps species during the spawning season are scarce or limited. Billard *et al*. (1995) have reviewed the biochemical composition of seminal plasma of some fish. The sodium and potassium levels in the seminal plasma of six carps were high as shown in Table 2 and most probably responsible for the suppression of sperm motility due to their osmotic effect. The Na^+ and K^+ levels are known to suppress the sperm motility in jundiá, *Rhamdia quelen* (Borges *et al*., 2005; Billard, 1975; Benau and Terner, 1980; Morisawa, 1985). When the seminal plasma ionic composition was compared with the data reported by the review of Linhart *et al*. (1991) on four salmonid species (*Oncorhynchus mykiss*, *O. keta*, *Salmo salar*, *Salmo clarki*) and four cyprinid species (*Cyprinus carpio*, *Vimba vimba*, *Ctenopharyngodon idella*, *Stizostedium vitreum*), it was found that the Na^+ and K^+ levels were comparable to our studies. The seminal plasma osmolality plays an important role in spermatozoa activation. The osmolality of seminal plasma was used as a controlling point to develop extenders for semen of many fish species and reversibly suppresses the spermatozoa activation (Ohta and Izawa, 1996). Osmolality of any extenders used for artificial propagation of fish seminal plasma is generally adjusted by the use of Na^+ and K^+ levels. Sperm quiescence in undiluted semen occurs roughly in the range of 270-300 mOsm/kg. The seminal plasma osmolality in the European eel has been reported to be in the range of 325-330 mOsm/kg and extenders with this range of values helped in reversibly suppressing the motility of spermatozoa (Asturiano *et al*., 2004). The glucose content of seminal plasma is also an important biochemical parameter; because it provides membrane protection to spermatozoa and serves as an external cryoprotectant as well (Maisse, 1996). Sugar extenders are also used successfully in African catfish, *Clarias gariepinus* (Steyn and Van Vuren, 1987; Urbányi *et al*., 1999). The present study has estimated the glucose level in seminal plasma of six species of carps that may be used for future reference while preparing extenders for carps.

Ultrastructural studies of spermatozoa of Indian major carps, silver carp and grass carp were not reported earlier. The morphological structure exhibited by sperms of these carps described here show similarity to those described in most of the teleostean fishes in which the absence of acrosome is a common character (Mattei, 1970). A variety of acrosomal structures are found in fish spermatozoa (Stanley, 1971; Mattei, 1970); however, carp spermatozoa lacked an acrosome. The acrosome reaction occurs inside or on the surface of the egg envelope to allow sperm penetration. In case of teleostean fish such as carps, spermatozoa reach the egg plasma membrane through a narrow micropyle because the sperm lacks an acrosome (Morisawa, 1995). The sperm has a slightly elliptical nucleus always eccentrically placed on the tail; two variously oriented centrioles and post nuclear cytoplasmic region of varying size which contains the mitochondrion and surrounds the periaxonemal post nuclear canal (Jamieson, 1991).

To date all the fish spermatozoa examined show some structural homogeneity which supports the idea that the ultrastructural features of spermatozoon can be useful for taxonomic and phylogenetic studies (Baccetti *et al*., 1984; Mattei and Mattei, 1984; Gwo *et al.,* 1996). Spermatozoa of species that exhibit external fertilization, including the Indian major carps usually have a few mitochondria in the short mid piece surrounding the centrioles and the 9+2 axoneme without accessory structures in the flagellum. The structure of carp flagellum is highly conserved in the teleostean group and is composed of a number of cytoskeletal elements whose proper assembly is critical for sperm motility. The axoneme is a cytoskeletal structure composed of a ring of 9 microtubule doublets surrounding a central pair. The flagellum of spermatozoa of Indian major carps as well as that of silver and grass carp was comparatively longer (13-24 µm in length) when compared to their body size. This could be probably countered the vagaries of nature. In aquatic species with external fertilization the spermatozoa are released into a hostile environment where they typically become activated, then survive for a short period of 1-2 min in case of freshwater fish (Holt and van Look, 2004). The duration of carp spermatozoa motility in the present study also observed a duration from 1-3 min after activation with water.

Indian major carps, silver carp and grass carp are seasonal breeders and to make artificial propagation successful by utilizing the available brood fish in an efficient way, the present information of the normal physical and chemical characteristics of semen of these carps presented here will eventually help in selecting good milters and devising improved protocols for cryopreservation and artificial propagation methods.

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