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Reproduction in fishes is controlled by hormones and external factors. The quality of semen also has a role in the effective fertilization of the ova as the fertilization is in vitro and there are many possibilities for the failure. Like higher animals, in fishes too the spermatological parameters assume importance due to the reasons of need for high quality seeds and stock size fishes for the farm production. Seminal plasma together with the gametes has been studied well for about half century and notable findings have been documented. These results and observations in various fishes help planning for the breeding programs and also preservation of the spermatozoa. Reports from different authors have been compiled aspect wise for different species of fishes. A general opinion on the fish spermatozoa can be derived from results and observations were discussed in this line.

**Keywords:** Fish spermatozoa, Density, motility, Milt pH, Dilution, Motility duration

**INTRODUCTION**

World aquaculture production has been in the increasing trend year by year with an average annual rate of 6.3% during the past 5 years. Rapid growth in the areas of hybridization and development of new varieties has paved way for expanded market and assured returns in the farming. Cryopreservation of fish spermatozoa has been given due importance after its application in fish seed production and genetic improvement in the seeds. Research in the cryopreservation has been done for many cultivable species of fishes and reported from many parts of the world.

In the cryopreservation of fish gametes, sufficient importance has been attached to the spermatological parameters of the collected gametes as this only can help in the validation of the spermatozoa that are selected for cryopreservation. According to Bozkurt et al. (2009), sperm quality data are required for successful artificial insemination and handling the semen. Parameters such as density, viscosity, volume, color, pH and motility related factors are
considered important spermatological issues for fish semen many. It is worth pointing out that the spermatological parameters vary from species to species and individual to individual and it depends on so many factors (Alavi et al., 2008).

According to Santos (2011), sperm quality is the ability of the sperm sample to effect fertilization and the production of normal offspring. However, testing the fertilizing capacity is difficult and time-consuming, and therefore different parameters that allow a more rapid evaluation are necessary. Some authors have tried to relate fertilization with other parameters that are more or less easy to monitor (Rurangwa et al., 2004; Cabrita et al., 2008). In this context, among different factors, motility is the most commonly used parameter to evaluate sperm quality (Billard et al., 1995; Lahnsteiner and Patzner, 1998).

**OBSERVATION OF THE SPERMATOLOGICAL PARAMETERS**

Dilution is necessary for the observation of the spermatological parameters (density, motility pattern and percentage of motile spermatozoa) due to difficulties in determining the parameters in the raw semen. But dilution of semen itself has an effect on motility. The difference was significant between dilutions 1:10 and 1:1000 in terms of initial motility and duration of movement as observed by Suquet et al. (1992).

**Sperm Density**

Sperm density is an important factor in the determination of sperm quality (Suquet et al., 1992). Its role in the fertilization of spermatozoa is well documented and reported (Aas et al., 1991; Pool and Dillanea, 1998). Density of spermatozoa is observed to be highly varying depending on the species as evident from the reports on carp where it has been reported to be from 1 to 5×10⁹ cells/mL, in trout it was from 5 to 15×10⁹ cells/mL and in sturgeon it was from 0.1 to 4×10⁹ cells/mL (Cosson et al., 1991; Redondo et al., 1991; Tsvetkova et al., 1996). Babiak et al. (1997) estimated the sperm density of *C. carpio* as 20×10⁹ numbers per ml. Chutia et al. (1998) also mentioned that the sperm density of *C. carpio var. communis* was 6.6×10⁹ cells per mL of milt. Lahnsteiner et al. (2000) found that the sperm density of *C. carpio* was 0.5 to 1.0×10¹¹ cells per mL of milt. Akcay et al. (2004) reported sperm density of mirror carp to be 17.33±1.22×10⁹ mL/L. Sperm density of grass carp was reported by Bozkurt et al. (2008) as 15.43±0.72×10⁹ mL/L. Similar variations and ranges might be existing in other fish species also.

Number of spermatozoa can be counted accurately with a Haemocytometer (Kolmer and Boerner, 1941). Bouck and Jacobson (1976) modified and evaluated a rapid alternative procedure based on widely used microhematocrit technique. Billard et al. (1983) and Takashima et al. (1984) estimated the number of spermatozoa by densitometric method. The density of the spermatozoa is found to be influenced by external and internal factors in fishes.

Jaspers (1972) noted that age seemed to be the major factor influencing sperm concentration with 3 years old fish producing more spermatozoa per gram wet testicular tissue than 2 years old fishes of the same species. Sanchez-Rodriquez
et al. (1978) showed that the spermatocrit (packed cell volume/ total semen volume) remained constant throughout the spawning period. In rainbow trout, Billard et al. (1977) and Munkittrick and Moccia (1987) reported that sperm density declined as the season advanced. In salmonid species, sperm density and milt volume were highest at their peak spawning season and declined as the season progress (Billard, 1983). Whereas Sanchez-Rodriguez et al. (1978) and Piironen and Hyvarinen (1983) noted that spermatocrit values increased over the stripping season.

Based on the observations on various species, it can be said that spermatozoa concentrations in fishes can range from \(2 \times 10^6\) to \(5.3 \times 10^{10}\) cells per mL (Leung and Jamieson, 1991). A rare phenomenon of increased spermatozoa density to the decreasing volume of milt was observed and reported by Reenaselvi (1991), Nalliappan (1992) and Degraff et al. (2004). This needs to be further investigated.

Density of the spermatozoa in the semen can be a factor that will decide the dilution of the milt before cryopreservation. Sarder et al. (2009) contended that counting of spermatozoa and determination of the density is essential standardize the degree of dilution of milt and to determine the density of spermatozoa per straw for maintaining the desired egg: sperm ratio during fertilization.

**Motility of Spermatozoa**

As stated by Terner (1986) motility is a parameter to decide the quality of the spermatozoa. Sperm quality is usually assessed by the intensity of motility (Sanchez-Rodriguez and Billard, 1977). Motility can also be estimated by quasi elastic-light scattering (Craig et al., 1983) and Stroboscopy (Cosson et al., 1985) methods. A spermatic value of 70 and above is considered good as it implies that motility percentage of spermatozoa is above 70% (Thomas et al., 2003).

Fish spermatozoa are immotile in the seminal fluid (Ciereszko et al., 2000). In order to activate the spermatozoa, it has to be transferred into an activating solution which contains solutes that affect the osmotic pressure of the fluid surrounding the spermatozoa. In case of marine species, the osmotic pressure of the activating solution should be higher than the seminal fluid, whereas in freshwater species it should be lower.

The motility duration of fish spermatozoa ranges from 30 to 300 s (Stoss, 1983). Since many fish species have shorter motility period ranging from 30 to 120 seconds (Alavi and Cosson, 2005, 2006), it is necessary to avoid the preactivation of motility by urine contamination during stripping (Perchec et al., 1995a; Dreanno et al., 1998).

It was first shown by Scheuring (1924), Gaschott (1924) and later by Schlenk and Kahmann (1938) that the potassium in the seminal plasma prevents motility in fish sperm. Scheuring (1924) also found out that sodium, calcium and magnesium reduced the inhibiting action of potassium. This was confirmed by Holtz et al. (1977) with additional revelation that the antagonism is more effective by the presence of calcium and magnesium ions than sodium ions.
Sneed and Clemens (1956) stated that a higher concentration of potassium ions is required for the inhibition of *C. carpio* spermatozoa. It was also found and reported that the motility of sperm was extended to a longer period in saline and ovarian fluid of matured female than in water (Fredrich, 1984). Percentages of motile spermatozoa decrease significantly with increasing storage period in diluted and undiluted sperm during spawning season (Bozkurt *et al*., 2009).

Morisawa *et al.* (1983) demonstrated that sperm from common carp and crucian carp can be kept immotile in media like NaCl, KCl, mannitol and glucose media having 300 mosmol/kg which is isotonic to the seminal plasma of these species. Salinity of activating medium plays a role in inducing sperm motility; freshwater teleosts' sperm were motile only if salinity was lower than 15 ppt (Billard, 1978).

According to Redondo *et al.* (1991), motility can be initiated by decreasing the osmolality of the seminal plasma with freshwater or in a saline solution (45 mM NaCl, 5 mM KCl, tris 30 mM, pH 8) and it can last for 45 to 90 seconds. Although NaCl has properties similar to that of KCl, the recovery ability for motility is faster with KCl (Perchec *et al*., 1995b). Activation of carp spermatozoa is not dependent on broad range external pH (6.0-10.0) (Redondo *et al*., 1991).

It was also shown that external factors, such as pH or ions present, may polarize the cell membrane and stimulate motility of fish spermatozoa (Morisawa *et al*., 1999). The depolarization of the membrane potential is an important step in the initiation of sperm motility (Blaber and Hallet, 1988; Boitano and Omoto, 1991). In carp, osmolality-dependent permeability and structural changes are induced in the sperm membrane by hypo-osmolality, and reorganization of lipid structure has been proposed as a possible mechanism (Marian *et al*., 1993). Osmolalities isotonic to seminal plasma suppress sperm motility in marine and freshwater teleosts. Exposure of sperm to hypertonicity of seawater or hypotonicity of freshwater, induces the initiation of sperm motility at spawning (Takai and Morisawa, 1995).

In trout, the inhibition of sperm motility is mainly due to K+ ion concentration (Gatti *et al*., 1990 and Billard and Cosson, 1992). In turbot, anaerobiosis and high CO₂ content within the genital tract contribute to the inhibition of spermatozoa motility (Dreanno *et al*., 1995). However, most studies suggest that water is not a suitable activation medium (Billard and Cosson, 1992 and Cosson *et al*., 2000). In endorsing the above statement, it was shown that the sperm motility of Shovelnose sturgeon *Scaphirhynchus platorynchus* was significantly increased by activation in a buffered media instead of activation in distilled water (Cosson *et al*., 2000). The time of motility was prolonged and there were fewer damaged sperm cells in buffered media than in distilled water. At activation, sperm cells are exposed to a hostile environment, i.e., low or high osmolality compared with that of the seminal fluid. Simple physiological solutions and various complicated media are currently used in hatcheries. But it should be admitted that no thorough study has defined the best medium for each species.

Cosson *et al.* (1985) observed that in salmonids and cyprinids, temperature affected...
the sperm beat frequency. In trout, higher temperature increased the beat frequency and decreased the duration of forward movement (Billard and Cosson, 1992) while the lower temperature that trout experience during natural spawning of trout increases the duration of sperm movement (Van Look, 2001). In African catfish, low temperature (4°C) also prolonged motility and viability of spermatozoa compared to the culture temperature (25°C) (Mansour et al., 2002).

In fishes spawning in brackishwater and marine water, motility of spermatozoa is more long lasting than that of freshwater species (Hines and Yashouv, 1971; Ginzburg, 1972). Though there are several ways to detect live spermatozoa, viz., motility (Hodgins and Ridgway, 1964; Sneed and Clemens, 1956) and differential staining (Fribourgh, 1966), the fertility evaluation test is considered more exact and accurate.

It is noted and reported that the motility duration is influenced by the spawning preparedness of the brooders.

During the peak spawning season activated rainbow trout spermatozoa remained motile for 30-55 seconds. But during the end of the spawning season the duration of the motility declined to 15 s (Benau and Terner, 1980). In sea bass, *Dicentrarchus labrax* the duration of motility decreased from 5 min at the start of the season to 30 s at the end of the season (Billard et al., 1977). In rainbow trout, the proportion of spermatozoa that are activated may also gradually decrease as the spawning season progresses (Munkittrick and Moccia, 1987). The concentration and the ratio of ions such as K and Na (Morisawa and Suzuki, 1980) which are implicated in the initiation of sperm motility decreases as the season progresses (Munkittrick and Moccia, 1987).

To prolong the duration of motility, minimize osmotic shock during fertilization and disperse the sperm cells around the ova, inseminating solutions are advocated. The compositions of such solutions are similar to ovarian fluid (Ginsberg, 1963; Scott and Baynes, 1980) or have a salinity of 5% or 20% for fresh and sea water species respectively. Buffered 0.1-0.15 M sodium bicarbonate or chloride is commonly used for salmonids (Stoss, 1983). The addition of compounds such as isobutyl-1-methylxanthine (Benau and Terner, 1980) and theophylline (Scheerer and Thorgaard, 1989; Wheeler and Thorgaard, 1991), to buffered fertilizing medium may also help to reduce osmotic swelling and prolong the duration of sperm motility. Stoss and Holtz (1981) have increased the motility of pink salmon spermatozoa from 30 s to 10 min by activating with a 120 nm NaHCO₃ solution to which 1 BMX (3-isobutyl-1-methylxanthium) had been added.

**Premature Motility Activation and Temporary Immotility**

Accurate quantitative assessment of spermatozoa motility requires that all sperm are simultaneously activated. Since a high dilution (more than 1000-fold) is required for induction of synchronous motility in 100% spermatozoa, a two-step procedure is necessary, with an initial dilution of 1 to 100 in a medium that keeps the spermatozoa immotile and allows good mixing
of the viscous milt (Billard and Cosson, 1992). The second dilution (1 to 20) in the activating solution can be made directly under the microscope.

Glogowski et al. (2002) observed motility in undiluted milt of sturgeon. Many researchers have noticed “premature activation” of spermatozoa, the reason for which may be urine contamination, hormonal stimulation, stress, changes in seminal plasma composition and osmolality. Allyn et al. (2001) reported that 38% of semen samples collected from transported white bass (Morone chrysops) exhibited 10-25% motility prior to activation with water. Thus stress can induce premature activation of fish spermatozoa.

According to Scott and Baynes (1980), the motility and fertility do not reside on the same part of the sperm. Therefore, not all the motile spermatozoa can fertilize the eggs. This has been found true in carp and cod (Mounib et al., 1968; Kossman, 1973). It has been pointed out that fertilization could be obtained with sperm which did not show any sign of motility on dilution.

**Milt pH**

Hamner (1970) might be one of the oldest in reporting the pH related finding in semen of higher animals. He reported optimum survival of sperm at a pH of 7 and a progressive decline in motility and metabolism below that optimum pH in higher animals. Similarly the pH of fish milt was also found to affect the motility of spermatozoa and maturation process (Billard et al., 1995; Liley et al., 2002).

In cyprinids, it has been shown that extracellular and intracellular pH as well as the ionic composition of the seminal plasma influences the initiation and duration of sperm motility (Marian et al., 1997). Sahinoz et al. (2008) opined that the seminal plasma pH may affect final maturation of spermatozoa in fishes and added that duration of sperm motility in males could be influenced by the changes of semen pH.

Chao et al. (1987) measured the milt pH of Oreochromis species using Bromothymol blue pH test paper and found that the values varied from 6.2 to 8.2. Lahnsteiner et al. (1998) noted increased fertility in Oncorhynchus mykiss when milt pH was increased from 8.0 to 8.2. Faruk et al. (2007) found a milt pH range of 7.70 to 8.40 for O. mykiss. They used pH indicator strips to determine the pH of the milt of rainbow trout (O. mykiss) and himri (Carasobarbus luteus).

Bozkurt et al. (2009) evaluated pH of grass carp sperm using standard pH papers and mentioned that it was around 7. They also opined that determination of fluctuation in sperm pH could provide necessary information on fertilization capacity of spermatozoa. Verma et al. (2009) reported that the pH values of Catla catla, Labeo rohita, Labeo calbasu, Cirrhinus mrigala, Hypophthalmichthys molitrix and Ctenopharyngodon idella ranged from 7.3 to 8.1.

There has been an observation on the premature motility of the spermatozoa in salmonid species that has been attributed to the increase in external pH during the passage of spermatozoa from the testis to the spermatic duct (Morisawa
and Morisawa, 1986, 1988; Billard et al., 1995). This endorses the fact that the seminal fluid pH may also affect the final maturation of spermatozoa as stated by Lahnsteiner et al. (1998).

**CONCLUSION**

The importance of research on the spermatological issues of fishes has been well understood now. Presently the cryopreservation of fish gametes invariably preludes the study on the spermatological parameters to achieve success in the cryopreservation. Besides the need in the cryopreservation, knowledge on the speramntological parameters also help in the planning of breeding programs with quality males and thereby the quality of the seeds can be assured.

**REFERENCES**


40. Hodgin Ridgway H O (1964), "Recovery of
viable salmon spermatozoa after fast-freezing”, *Prog. Fish. Cult.*, Vol. 26, pp. 95.


54. Morisawa S and Morisawa M (1986), “Acquisition of potential for motility in rainbow...


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83. Tsvetkova L I, Cosson J, Linhart O and


