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FISH SPERM COMPOSITION AND BIOCHEMISTRY

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O. Linhart, V. Šlechta and T. Slavík (1991) Fish sperm composition and biochemistry. *Bull. Inst. Zool., Academia, Monograph* **16**: 285-311. The spermatozoa of both chondrostean and teleostean fishes characterized by external fertilization have a simple structure. The main characteristics of chondrostean (studied in Acipenseridae) and those teleostean spermatozoa morphology are an elongated head with the acrosome-like structure and a spherical or slightly elongated (2-3 μm) sperm head with the absence of the acrosomal process, respectively. The reduced middle piece with some traces of cytoplasm and slightly modified mitochondria is typical for both subclasses, similarly as the conspicuous endpiece. Tail length varies from 40 to 60 μm and the plasmatic membrane often forms one or two fin-like ridges along the tail. The seminal plasma contains not only several different cations (Na^+, K^+, Mg^+, Ca^+) but also the organic compounds (glycids, proteins, lipids, etc.). Osmotic pressure, concentration of K^+ and sucrose, and pH of seminal plasma lower than 7 are the main factors inhibiting sperm motility of salmonids, and the osmotic pressure seems to be the major supressive factor in cyprinids. The depolarization of cell membrane is an activating factor initiating motility. Spermatozoa have cellular energetic reserves such as phospholipids, glucolipids, glycogen and enzymes necessary for the metabolism. The system of microtubules in the flagellum represents the motile aparatus of spermatozoon. Each of the peripheral double-tubules carries two arms which consist of an ATPase called dynein which is regulated by Ca^+ ions in such a way which results in an asymmetric flagellar movement. Energy necessary for spermatozoa movement originates as ATP from glycolytic and oxidative reactions. In fishes with external fertilization, limiting factors as primitive structure of spermatozoa, endogenous storage capacity of substrates and limited metabolic pathways can bound the optimal motility only to a very good environment.

The spermatozoa of those teleostean fishes characterized by internal fertilization have more developed structures. The spermatozoa have both head (3–4 μm) and midpiece (6–7 μm). The head is elongated and contains highly condensed chromatin. Spermatozoa of fishes with internal fertilization can metabolize both endogenous (glycogen) and exogenous (glucose) energy sources. The strategy of reproduction of both ovoviviparous fish and mammals is similar in a very general mode; therefore it has been possible to complete the missing or insufficient data on ovoviviparous fish spermatozoa with the data on those of mammals.

Key words: Fish spermatozoa, Reproductive biology, Sperm composition, Spermatozoa structure, Sperm metabolism, Seminal plasma, Sperm biochemistry.

To arrange the comprehensive review on morphology, external and internal structures, chemical composition and metabolism of fish spermatozoa is undoubtedly a very complex problem with respect to the number of more than 20,000 fish species estimated to occur (Cohen, 1970). The aim of this review is to summarize available data about structure and biochemistry of fish spermatozoa and sperm liquid to trace the future possible development of research activities in this field. We preferred to focus this review particularly on the differences between chondrosteans and teleosteans and between groups with different modes of fertilization. The review is based mainly on the data gathered for families Acipenseridae, Salmonidae, Cyprinidae (oviparous fishes) and Poeciliidae (ovoviviparous fishes). In the latter group, several unknown data were estimated on the basis of probable analogy with farm mammals.

DISCUSSION

Morphology and Structure of Spermatozoa

Oviparous species

Physiologically formed fish spermatozoon has typically flagellate shape and consists of head, middle- and neck-pieces.

Head of spermatozoon

The basic task of spermatozoan head is to keep and transfer genetic material localized in nucleoplasma. The prerequisite for good penetration of spermatozoon throughout egg vestments, especially micropyle opening, is the optimal shape and size of spermatozoan head (Ginsburg, 1968). Different shapes of spermatozoan

heads occur in chondrostean and teleostean fishes with external fertilization. Regular, ball-shaped spermatozoan head is that of northern pike (*Esox lucius*) (Mattei, 1969; Rötheli *et al.*, 1950); oval, egg- or heart-shaped one is in stone loach (*Noemacheilus barbatulus*), arctic charr (*Salvelinus alpinus*) (Retzius, 1905), channal catfish (*Ictalurus punctatus*) (Jasper *et al.*, 1976), grass carp (*Ctenopharyngodon idella*), common carp (*Cyprinus carpio*), bighead (*Aristichthys nobilis*), silver carp (*Hypophtalmichthys molitrix*) (Emeljanova and Makeeva, 1985; Stein, 1981; Billard, 1969b); ovoid-shaped in rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*) (Billard, 1969b, 1978, 1983); banana-shaped in Atlantic eel (*Anguilla anguilla*) (Billard and Ginsburg, 1973). In some species (*e.g.* in perch, *Perca fluviatilis*), the spermatozoan head is laterally flattened (Retzius, 1905). The greatest diversity in shapes of spermatozoan head has been found in sculpins (Cottidae) (Hann, 1930). Generally, the spermatozoan head in fishes is relatively small (Table 1) in relation to the total size of spermatozoa as compared with elasmobranchs, holocephalians or even mammals. The acrosomal head structures have not been found in chondrostean and teleostean fish species with external fertilization but acrosome-like structures is present in several acipenserids and Atlantic eel (Ginsburg, 1968; Tuzet and Fontain, 1937). Ginsburg and Billard (1973), Billard (1978), Todd (1976) confirmed the total absence of acrosomal process in sturgeon and Atlantic eel. Pasteels (1965a, 1965b) suggested that the absence of acrosomal process may be associated with the presence of a micropyle in the eggs of the teleost fish species. As reported by Furier (1962), the head of trout spermatozoon has no structure which can be identified as an acrosome. However, the surface of the chromatin, condensed against the inner layer of the nuclear membrane and formed during spermiogenesis shows a little hollow in the frontal part (Billard, 1983), which is found in other species in association with acrosome formation (Mattei and Mattei, 1978). The rudimental acrosome mentioned above is either rudimental or, maybe better said, primitive because this structure in phylogenetically much higher mammals is more developed and fully functional. In common carp and channel catfish, the chromatin is slightly granular (Stein, 1981; Jasper *et al.*, 1976). In rainbow trout, brown trout

Table 1
Morphology of fish spermatozoa (μm)

Species	Length of head	Breadth of head	Length of spermatozoa	Author
Acipenser guldenstaedti colchicus	8.9	1.9	58	Detlaf and Ginsburg (1954)
Acipenser stellatus	6.3	1.8	47	Detlaf and Ginsburg (1954)
Huso huso	7.4	1.1	55	Detlaf and Ginsburg (1954)
Clupea harengus pallasi	2.0	1.5	43	Yanagimachi (1957)
Oncorhynchus tschawytscha	2.6	2.0	19.6	Riddle (1917)
Oncorhynchus mykiss	2.5	1.5-2.0		Billard (1969)
Salmo trutta m. *lacustris*	2.0-2.4	1.5-2.0	31-34	Ginsburg (1968)
Coregonus lavaretus asperi	3.5	3.0	43.5	Rôtheli *et al.* (1950)
Cyprinus carpio	1.85		43	Emeljanova and Makeeva (1985)
	3.3	2.5		Billard (1969)
Carassius auratus	3.2	3.2	60	Fribourgh *et al.* (1970)
Ctenopharingodon idella	1.6		38	Emeljanova and Makeeva (1985)
Aristichthys nobilis	1.6		37	Emeljanova and Makeeva (1985)
Hypophthalmichthys molitrix	1.6		37	Emeljanova and Makeeva (1985)
Esox lucius	2.0	2.0	37-42	Rôtheli *et al.* (1950)
	2.0	1.8		Billard (1969)
Esox niger	1.84	1.86	31.3	McLean *et al.* (1982)
Rhodeus ocellatus	1.6		34	Emeljanova and Makeeva (1985)
Hemiculter eigenmanni	1.8		35	Emeljanova and Makeeva (1985)
Ictalurus punctatus	2.3	2.4	99	Jaspers *et al.* (1976)
Pseudorasbora parva	1.85		39	Emeljanova and Makeeva (1985)
Misgurnus anguillicaudatus	3.0	2.8	21.5	Kobayashi (1963)
Perca flavescens	1.7	1.6	22.6	McLean *et al.* (1982)
Anguilla anguilla	8-11		32-47	Billard and Ginsburg (1973)
Anguilla australis	6	2	32-36	Todd (1976)
Anguilla difenbachi	8	3	26-52	Todd (1976)
Anguilla japonica	6.3	1	36.8	Colak and Yamamoto (1974)
Oligocottus snyderi	4.0	0.8	38.6	Fink and Haydin (1960)
Oligocottus rubellio	5.5	1.1	53	Fink and Haydin (1960)
Lepomis macrochirus	2.15	2.0	39.4	McLean *et al.* (1982)
Poecilia reticulata	4.2	1.3	59	Ginsburg (1968)
	4.0	1.0		Billard (1969a)
Cymatogaster aggregata	4.0		50	Gardiner (1978b)

and northern pike chromatin occur as large granulars (Billard, 1969b; Drozdov *et al.*, 1981). The head consists of nucleus containing nucleoplasma and covering membranes, *i.e.*, nucleolemma and cytoplasmic membrane. The nucleus contains chromatin in such amount of DNA corresponding with haploid chromosome set.

Middle piece

The middle piece is firmly linked with head; it contains centriolar and mitochondrial segments. In chondrostean and teleostean fishes, only the mitochondrial segment is recognizable while centriolar segment is hidden in so-called intranuclear channel (Ginsburg, 1968). In a centriolar segment, a scheme of the relationships between the two centrioles has been proposed previously (Billard, 1969b). In rainbow trout the distal centriole attached to the transverse axis system includes a free portion nesting on the proximal centriole, which varies in form between a circle and an ellipse on some images (Billard, 1983). Through the mitochondrial segment, fibrilar system of flagellum passes excentrically or even nearly laterally (Ginsburg, 1968). The middle piece appears to be asymmetric, too, but it is never oriented in the same plane of asymmetry as the flagellum (identified by the pair of central fibers), as has been observed in many species (see André, 1982). In rainhow trout, the two centrioles, which are about the same size (length 30 nm, diameter 22 nm), are arranged rectangularly to the base of the head in a roughly cubical depression equivalent to an implantation groove (Billard, 1983). In coho salmon (*Oncorhynchus kisutch*), the mitochondrial segment forms a part of spermatozoan head and it is joined laterally with the flagellum at the head base but without any mutual connection (Lowman, 1953). In rainbow trout and brown trout, the mitochondrial segment is directly wedged to the base of head (Billard, 1969b, 1978, 1983). In channel catfish, it is usually referred to as cytoplasmic or mitochondrial colar which is low (mean length 1.6 μm), but broad (mean width 3.1 μm) (Jasper *et al.*, 1976). The single mitochondrion was found to be localized in the spermatozoan head in perch (Retzius, 1905) and Atlantic eel resembling the acrosome (Billard and Ginsburg, 1973). On the other hand, high number (more than 20) of mitochondria were found in the mid-piece of *Idus melanotus* (Ginsburg, 1968). In cyprinids, generally,

Table 2
The mitochondria of cyprinid species
(Emeljanovova and Makeeva, 1985)

Species	Number of mitochondria	Mean (nm)	Surface average (μm^2)
Cyprinus carpio	7-9	170× 160	0.17
Hemiculter eigenmanni	7-9	190× 180	0.22
Hypothalmichthys molitrix	4-5	250× 320	0.20
Aristichthys nobili	4-5	230× 200	0.17
Opsariichthys uncirostris	2-4	300× 290	0.21
Rhodeus ocellatus	1	1,300×1,000	1.02
Pseudorasbora parva	2-3	430× 390	0.33

the number of mitochondria varies from 2 to 10 (Table 2) and depends on the particular species (Baccetti *et al.*, 1984).

Tail (flagellum)

The tail of spermatozoon can be divided into proximal, central and terminal parts. In fishes, especially the central and terminal parts can be recognized. The tail has distinct narrow terminal part in clupeids, salmonids, perch and burbot (*Lota lota*), while tail without remarkable terminal part is present in northern pike, crucian carp (*Carassius carassius*) and sturgeon (Acipenseridae) (Ginsburg, 1968; Billard, 1969b; Mattei, 1969). The tail itself is composed of two central and nine peripheral fibriles, so-called "9+2 complex" (Mattei *et al.*, 1972; Billard, 1969b), covered with membrane forming two opposite, lateral cytoplasmatic extensions, which are usually symmetrical in rainbow trout (Billard, 1983). Central fibriles are of simple composition while peripheral ones consist of two filaments each. This characteristic composition, *i.e.*, 9+2, axonema with the central fibriles showing identical orientation presents in channel catfish, coho salmon, common carp or loach (*Misgurnus fossilis*) (Ginsburg, 1968; Lowman, 1953; Billard, 1969b; Jasper *et al.*, 1976). Each flagellum arises from an individual centriolar complex and is separated from the cytoplasmic canal. The diameter of central flagellum in coho salmon is 1.6 μm at the proximal part; with 1 μm at the end and terminal part of flagellum 0.3-0.6 μm (Lowman, 1953). The spermatozoon of Atlantic eel (*Anguilla anguilla*) is interestingly structured having an elongated head with mitochondria

localized at the anterior part in opposite position to the insertion of the 9+0 (*i. e.*, without central fibriles) axoneme (Billard and Ginsburg, 1973; Todd, 1976; Gibbons *et al.*, 1985) (Fig. 1). The flagellum from *Elopiformes* has no central microtubules, too (Mattei and Mattei, 1975). In rainbow trout the total length of flagellum is about 35 μm with 30 μm-long membrane (Billard, 1983) and in channel catfish has flagellum total length of about 94 μm (Jasper *et al.*, 1976). The flagellum of the channel catfish spermatozoon lacks a peripheral sheet or fringe as is found among others in the following fish species: coho salmon, common carp, northern pike, rainbow and brown trout (Jasper *et al.*, 1976; Lowman, 1953; Billard, 1969b). The fibriles of tail are very well recognizable after the treatment with distilled water. Using this method, Lowman (1953) found in coho salmon that peripheral fibriles have different lengths and are always shorter than the axial ones so that the tail (about 1 μ long) is formed by the central fibriles only. Cytoplasmatic cover of tail has typically flat membraneous structure unlike to coho salmon, where the membrane in central part of tail is spiralized having 12 to 15 coils (Lowman, 1953). The tail of spermatozoon in some species is covered by so-called free mantle membrane without any folding. Such kind of cytoplasmatic cover of tail was found in northern pike where it forms fringe of 1 μ width and 0.07 μ thick (Rôtheli *et al.*, 1950; Ginsburg, 1968). This structure very rapidly disintegrates after releasing sperm into water environment. In northern pike, this disintegration occurs within 4 minutes (Ginsburg, 1968).

Ovoviviparous species

The structure of the spermatozoon has previously been described for only four species in the family Poeciliidae, Jenysiidae, Pantodontidae and Embiotocidae (Stoss, 1984). The spermatozoa of those teleostean fishes characterized by internal fertilization have more developed structures. For example, the spermatozoa of guppy (*Poecilia reticulata*) possess head (3-4 μm) and the midpiece (6-7 μm). Head of spermatozoa is elongated and contains highly condensed chromatin. The middle piece contains large mitochondrial structures and intercentriolar material. Fibrillar complexes of the flagellum separate each other and have different lengths and the flagellum in spermatozoa of this fish ends indistinctly (Billard, 1970). The anterior part of the

flagellum of *Cytogaster aggregata* originates at the basal body (distal centriole) and is contained within an extracellular, flagellar tunnel-sheath-within the mitochondrial midpiece (Gardiner, 1978a).

Chemical and Biochemical Characteristics of Seminal Plasma (Chemical and Biochemical Analyses)

Oviparous species

Ionic composition of seminal plasma

Limited extent of chemical investigations on teleost sperm contrasts with the much more intensive studies on mammalian ones (Kucherova, 1972). A few data for salmonids, cyprinids and other fish species are summarized in Table 3, which enables comparison of both presence and concentrations of separate ions and differences among fish species. We can see clear differences in ionic Na^+/K^+ and Mg^{++}/Ca^{++} ratios between seminal plasma and spermatozoa in salmon (Hwang and Idler, 1969) and grass carp (Gosh, 1985). Ionic concentrations in seminal plasma of rainbow trout changes in dependence on the time of spawning (Table 4, Munkittrick and Moccia, 1987) and in some species can reach such low osmotic level that spermatozoa could probably be active

Table 3
Seminal plasma and sperm ion levels of fish sperm ($mmol \times l^{-1}$)

Species	Na^+	K^+	Mg^{++}	Ca^{++}	Cl^-	Author
A. Seminal plasma						
Oncorhynchus mykiss	104	25.3	1.1	1.4	135	Holtz *et al.* (1979)
	107	25.8	0.8	2.6		Holtz *et al.* (1977)
	133	20			130	Schlenk and Hahmann (1938)
Oncorhynchus keta	141	66	3.6	1.0	134	Morisawa *et al.* (1979)
Salmo salar	103	22	0.9	1.3		Hwang and Idler (1969)
Salmo clarki	107	38.6	1.5	0.3	156	Cruea (1969)
Cyprinus carpio	94	67.8	0.02	12.5		Clemens and Grant (1965)
Vimba vimba	107	38.7	1.2	0.3		Kusherova (1972)
Ctenopharyngodon idella	811	35.1	1.6	1.0		Gosh (1985)
Stizostedion vitreum	167	24.8	2.0	0.4	132	Gregory (1970)
B. Sperm						
Salmo salar	36.5	76.2	0.8	0.03		Hwang and Idler (1969)
Gadus morhua	77.4	60.6	0.2	0.4		Hwang and Idler (1969)
Ctenopharyngodon idella	35.7	2.1	1.3	2.6		Gosh (1985)

Table 4
The ionic levels in seminal plasma of rainbow trout
($mmol \times l^{-1}$; Munkittrick and Moccia, 1987)

Time after start of spermiation	Na^+	K^+	Cl^-	Osmolality ($mmol \times kg^{-1}$)
1-3 months	91.9-54	18.8-12.4	86.3-53.7	210.3-138.1
2-4 months	63.1-42.8	14.2-9.4	65 -39.6	159.1- 79.4
3-5 months	44.3-37.4	9.8-7.4	48.1-35	120.9-106.4

without activation by water. Level of pH in rainbow trout vary from 7.3 to 8.3 (Nomura, 1964; Schleng and Kahman, 1938; Bratanov and Dikov, 1961; Scheuring, 1928). Osmolarity of seminal plasma was found 232 mosmol in salmon (*Salmo salar*) (Hwang and Idler, 1969), 332 mosmol in chum salmon (*Oncorhynchus keta*) (Morisawa *et al.*, 1965), 297-280 mosmol (Saad *et al.*, 1988) and 258 mosmol (Redondo *et al.*, 1991) in common carp and in other species (tilapia, channel catfish, grass carp). Fish spermatozoa are immotile in the testis, and, in many species, in the seminal plasma. The environmental factors, such as ions, pH or osmolality, may depolarize the cell membrane and stimulate motility of spermatozoa.

Scheuring (1925) first reported that Na^+, Ca^{2+} and Mg^{2+} reduced the inhibitory action of K^+ with the bivalent cations being more effective than Na^+. Schleng and Kahman (1938) observed motility in environment with combined Na^+ and K^+. Some authors reported that the inhibition of motility by milimolar concentrations of K^+ was overcome by an increase of external Ca^{2+} (Bayens *et al.*, 1981; Cosson *et al.*, 1986; Tamino and Morisawa, 1988), and preliminary results indicated that the concentration of intracellular Ca^+ increased upon initiation of motility (Cosson, 1986; Cosson *et al.*, 1989). The spermatozoan motility was not initiated in activating media containing NaCl, KCl and manitol which have osmotic pressure of about 300 mosmol per kg^{-1} or more (Morisawa and Suzuki, 1980; Plouidy and Billard, 1982). Sperm movement was initiated in media solution containing 50 mM NaCl, 80-100 mM KCl and sperm movement was optimal in period of about 5-8 min (Grant *et al.*, 1980; Morisawa and Suzuki, 1980; Morisawa *et al.*, 1983). Redondo *et al.* (1991) showed that spermatozoa capability of movement in common carp was preserved

after dilution of the sperm with 200 mM KCl medium. The K^+ also regenerates the sperm capability to move with a minimal requirement of 50 mM KCl in media of high osmotic pressure (380 mosmol) (Redondo *et al.*, 1991).

Metabolites

In seminal plasma, the metabolites of glycolysis and Krebs' cycle are present. The levels of seminal plasma metabolites were very different in common carp and grass carp in dependence on duration of sperm storage (Gosh, 1985); concentrations of these metabolites were higher in seminal plasma of grass carp. During the storage of grass carp sperm for 24 h the levels of lactate, pyruvate and α-ketoglutarate increased, the concentrations of malate and isocitrate decreased, and in common carp, on the contrary, the concentration of lactate in seminal plasma was lowered, too (Table 5). A comparative study was carried out by Belova (1982) on proteins and lipids concentrations in some cyprinids treated or untreated by the injection of pituitary gland extracts. Levels of individual types of lipid compounds differ between species; response to "hypophysation" is different in individual species. Comparison of all these data is not easy because of different methods used by individual authors. From their results, the overall tendencies cannot be deduced and further investigations are necessary (Table 6, Billard and Cosson, 1990).

Table 5
The metabolite composition in seminal plasma of grass carp and common carp after short-term storage of sperm at temperature 4-5°C (average, nmol×l^{-1}; Gosh, 1985)

Species	Storage of sperm (h)	Pyruvate	Lactate	Malate	Isocitrate	α-keto-glutarate
Common carp	0	1.7	243	15	35.8	2.8
	24	2.8	187	10.6	32	2.9
	36	—	240	7.1	30	3.2
	48	—	307	16.8	—	3.8
Grass carp	0	6.1	390	28.1	46.0	4.1
	12	6.9	430.5	16.3	41	5.0
	24	—	500.4	—	—	—

Table 6
Organic composition of seminal plasma (Billard and Cosson, 1990)

Components	Content ($mg \times l^{-1}$)	Species	Author
Glucose	9–100	Common carp	Kruger *et al.* (1984)
	20–220	Rainbow trout	Piironnen and Hyvarinen (1983)
	8–218	Whitefish (*Coregonus lavaretus*)	Piironnen and Hyvarinen (1983)
	62–214	Tilapia (*O. mossambicus*)	Kruger *et al.* (1984)
Fructose	58–63	Common carp	Kruger *et al.* (1984)
	0–9	Rainbow trout	Piironnen and Hyvarinen (1983)
	78	Rainbow trout	Holtz *et al.* (1979)
	8–218	Whitefish	Piironnen and Hyvarinen (1983)
	47–156	Tilapia (*O. mossambicus*)	Kruger *et al.* (1984)
	0–12	Perch (*Perca fluviatilis*)	Piironnen and Hyvarinen (1983)
	20–79	Burbot (*Lota lota*)	Piironnen and Hyvarinen (1983)
	25–90	Brook trout (*Salvelinus fontinalis*)	Gregory (1968)
	21–80	Cutthroat trout (*Salmo clarki*)	Gregory (1968)
Lactate	3.9–50	Common carp	Kruger *et al.* (1984)
	243 ($nmol \times ml^{-1}$)	Common carp	Gosh (1985)
	390 ($nmol \times ml^{-1}$)	Grass carp	Gosh (1985)
	20	Rainbow trout	Holtz *et al.* (1979)
	0–56	Tilapia (*O. mossambicus*)	Kruger *et al.* (1984)
Cholesterol	0–40	Common carp	Kruger *et al.* (1984)
	0–4	Tilapia (*O. mossambicus*)	Kruger *et al.* (1984)
Lipids	98–1,316	Common carp	Kruger *et al.* (1984)
	34–374	Rainbow trout	Piironnen and Hyvarinen (1983)
	0–3	Tilapia (*O. mossambicus*)	Kruger *et al.* (1984)
Phospolipids	5.6	Common carp	Ploudy and Billard (1983)
Glycerol	35–391	Whitefish	Piironnen and Hyvarinen (1983)
Proteins	1,200	Common carp	Ploudy and Billard (1983)
	0.4–40	Common carp	Kruger *et al.* (1984)
	800–1,900	Rainbow trout	Sanchez-Rodriguez *et al.* (1978)
	700–2,800	Rainbow trout	Maisse *et al.* (1988)
	125	Rainbow trout	Cruea (1969)
	375	Cutthroat trout (*Salmo clarki*)	Cruea (1969)
Amino acids	36.7 $nM \times l^{-1}$	Common carp	Menezo *et al.* (1983)
	98–136	Common carp	Kruger *et al.* (1984)
	589 $nM \times l^{-1}$	Rainbow trout	Billard and Menezo (1984)
	84	Rainbow trout	Boafonte Zaracozano (1977–78)

Enzymes

For determination of overall metabolic activity, the concentrations of NAD co-enzymes and/or the values of NAD^+/NADH and $NADP^+$/NADPH are very informative. For common carp and grass carp, these levels are shown in Table 7.

The concentrations of NAD and NADP decreased and concentrations of NADH and NADPH increased during 24 h storage of common carp and grass carp sperms. The level of NADP decreased much more (Table 7). Decrease of NAD(P)/NAD(P)H is correlated with disorders of steady-state rate of glycolysis (Francis and Miller, 1972) and with lowered spermatozoan motility which lowered exploitation of energetic pools (Gosh, 1985).

From the seminal plasma, phosphatase, LDH and MDH, acetyl- and butyryl-esterases, alanyl- and leucyl-aminopeptidases and glucosaminidase were isolated (Breton *et al.*, 1974). Alkaline phosphatase presents in carp and tilapia (*O. mossambicus*) seminal plasma in spring with seasonal variation in carp (from 5 $mg \cdot l^{-1}$ in winter and late spring to 70 $mg \cdot l^{-1}$ in early spring) and in tilapia (1 to 3.6 $mg \cdot l^{-1}$) (Kruger *et al.*, 1984).

Ovoviviparous species

To our knowledge, for teleostean fish with internal fertilization there is no information concerning the composition of seminal plasma and secretions in the female genital tract. From some similarities with mammals we can presume several probable functions of seminal plasma, *e. g.*, assuring suitable environment for spermatozoa in female genital tract, at least as concerns the buffering function of seminal plasma.

Table 7
The level of NAD(P)/NAD(P)H in seminal plasma of grass carp and common carp (average, $nmol \times^{-1}$) after short-term storage of sperm at temperature 4-5°C (Gosh, 1985)

Species	Storage of sperm (h)	NAD^+	NADH	NAD^+/NADH	$NADP^+$	NADPH	$NADP^+$/NADPH
Common carp	0	639	20	31	27	40	0.68
	24	545	38	14.3	11	53	0.21
Grass carp	0	400	40	10	28.3	49	0.57
	12	350	51	6.9	13.4	56.0	0.24

Biochemistry of Spermatozoa

Oviparous Species

Ionic composition of spermatozoa and its influence on their motility

Ionic compositions of spermatozoa of some fish species are shown in Table 3. Fish spermatozoa are immotile in the testis, and, in many species, also in the seminal plasma. These phenomena have been especially studied in salmonids. Mann (1964) has reviewed the foregoing literature on this subject. The immobility of spermatozoa is influenced by various factors such as concentration of K^+ (Schlenk and Kahmann, 1937; Billard and Jalabert, 1974), and Ca^{++}, and, in rainbow trout, pH value of 9 (Billard and Cosson, 1990). Whereas K^+ ions retard the motility of spermatozoa, adding of Ca^+ ions stimulate it. The characteristics of sperm movement and short duration of the motility phase show some differences in various conditions; though in media with high external K^+ concentrations and low pH increasing of external Ca^{2+} or bivalent ions concentrations were shown to overcome K^+ and H^+ inhibition of sperm motility. Both conditions have been shown to depolarize the plasmatic membrane potential (Gatti *et al.*, 1990). The effect of Ca^{2+} on the axonemal movement mechanism seems not to be in the initiation of flagellar beating but probably in the classical regulation of movement of flagellum (Cosson *et al.*, 1991), while some unknown factor controls the asymmetry of this movement.

Lipid and glycid composition

The lipid composition of some cyprinid species has been mentioned by Belove (1982). In other fish groups, various types of lipids were also identified: neutral lipids and phospholipids in the rainbow trout (Terner and Korsh, 1963), various glycolipids and sulfated glycolipids in chum salmon and rainbow trout (Levins *et al.*, 1976).

Glycogen was detected histochemically in the toadfish (*Opsanus tau*) spermatozoa where it forms "β-particles" (Anderson and Personne, 1970), but it was not found in the spermatozoa of rainbow trout (Billard and Breton, 1970). Glycogen as an energy stock is probably important for spermatozoa movement because motility of marine fish spermatozoa (bearing glycogen particles) persists for a longer time after activation (Ginsburg, 1968).

Zhukinski and Gosh (1974) found in roach 97.7 mg% and in bream

(*Abramis brama*) 52.1 mg% of fructose. They confirmed the relationship between the level of fructose, rate of fructolyse, and spermatozoa fertility in roach (*R. rutilus heckeli*) and used this rate—like in mammals—as specific metabolic characteristics of spermatozoa.

Respiratory level

Zhukinski and Gosh (1974) found low level of aerobic processes in fish spermatozoa. Respiratory rates in spermatozoa of roach and bream are illustrated in Fig. 1 (Gosh, 1985). These rates in fish are during the first hour after stripping higher than in mammals at 20°C. In roach, the rate was 164 μl $O_2 \cdot ml^{-1}$ sperm, in bream 96 μl $O_2 \cdot ml^{-1}$ sperm, whereas consumption in bull was 30 μl $O_2 \cdot ml^{-1}$ sperm and in stallion 12 μl $O_2 \cdot ml^{-1}$ sperm (Gosh, 1985). Rainbow trout, Atlantic salmon and Atlantic cod (*Gadus morhua*) sperm consumed 20–40 μl $O_2 \cdot ml^{-1}$ sperm (Terner, 1962; Terner and Korsh, 1963; Mounib, 1967).

After activation of spermatozoa with water, respiratory rate increased 2.5 times in roach, *i.e.*, to 400 μl $O_2 \cdot ml^{-1}$ sperm (Zhukinski and Gosh, 1974). In carp, this rate in activated spermatozoa was 180 μl $O_2 \cdot ml^{-1}$ sperm and in grass carp 289 μl $O_2 \cdot ml^{-1}$ sperm (Gosh, 1985). In sturgeons *Acipenser güldenstadti* and *A. stellatus*, oxygen consumption was increased 2 to 3 times after activation (Burnashova, 1960). After the recalculation of respiratory rates

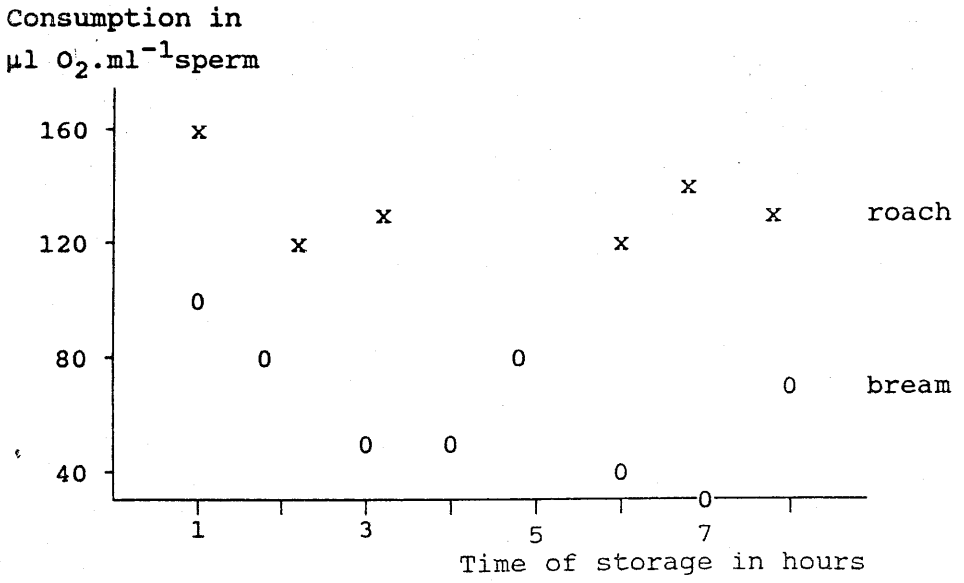

Fig. 1. Respiration dynamics in non-activated spermatozoa (Gosh, 1985).

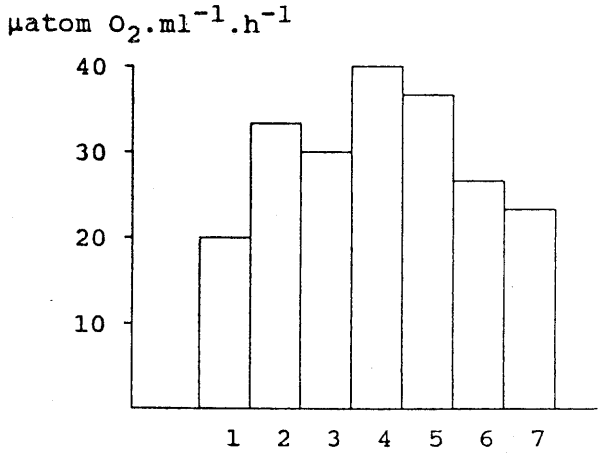

Fig. 2. Oxygen consumption after addition of pyruvate (2), lactate (3), malate (4), succinate (5), α-ketoglutarate (6), and citrate (7) as compared with control (1).

of different animal species to the same spermatozoa concentration we can see that in non-activated fish spermatozoa this rate is much lower than in ejaculated spermatozoa of farm animals (which are *de facto* activated). The respective values of respiratory rates are in roach 2.0; in grass carp 1.1; in bream 1.2; in common carp 0.9; on the other hand, in bull 8.4, and in stallion 4.3 μl O_2• 10^8 spermatozoa (Shergina, 1967; Zhukinski and Gosh, 1974).

Storage of spermatozoa at 4-5°C lowered the endogenous respiratory rate. This rate increases after addition of pyruvate, lactate, malate, succinate and α-ketoglutarate (Fig. 2).

Citric acid (Krebs') cycle and related metabolisms

Function of this cycle can be demonstrated by presence of some of its metabolites in fish spermatozoa. During the short-term storage of carp spermatozoa at 4-5°C, considerable

Table 8
The metabolite composition in common carp spermatozoa after short-term storage of sperm at temperature 4-5°C (average, nmol×10^9 spermatozoa; Gosh, 1985)

Storage of sperm (h)	Pyruvate	α-ketoglutarate	Malate	Isocitrate	Oxaloacetate
0	0.9	3.7	2.2	1.5	1.3
24	0.3	2.2	1.0	1.1	0.5

decrease of concentrations of some intermediate metabolites was observed (see Table 8). On the contrary, after addition of some metabolites, the metabolic turnover can be increased (see Fig. 2). Oxygen consumption after addition of these metabolites may, however, depend not only on the amount of metabolites, ATP/ADP, NAD/FAD, activity of citric acid cycle enzymes, but also on the capability of metabolites to pass through the mitochondrial membrane (Gosh, 1983, 1985).

These results indicate the disturbances of citric acid cycle during spermatozoa storage as a consequence of exhausting both initial and intermediate metabolites.

In anaerobic conditions, lactic acid is the end product of glycolysis. Standard concentrations in bream and roach are 22.3 mg% and 25.5 mg%, respectively (Zhukinski and Gosh, 1974) (Table 9). In anaerobic conditions lactic acid accumulates, pH value decreases rapidly, the membrane integrity is damaged owing to decreasing the amount of structural lipids and proteins. Water from seminal plasma passes into the spermatozoa causing their destroying (Belova, 1982).

Washed rainbow trout spermatozoa incubated under aerobic conditions in the presence of ^{14}C-acetate or -pyruvate, ^{14}C incorporated into various lipid fractions, especially diglycerids and triglycerids (Terner and Korsh, 1963); ^{14}C-labelled glyoxylate, pyruvate, and, to a lesser extent, acetate, were incorporated into lipids in the sperm of Atlantic salmon (Mounib and Eisan, 1968); spermatozoa of cod in the presence of $^{14}CO_2$ incorporated labelled carbon into organic acids, lipids, proteins, and nucleic acids (Mounib and Eisan, 1968). From these data follows that in aerobic conditions the metabolism

Table 9
The level of lactylacetate in spermatozoa of grass carp and common carp (nmol×10^9 spermatozoa) after short-term storage (4-5°C) of sperm (Gosh, 1985)

Species	Storage of sperm (h)	Lactylacetate	Species	Storage of sperm (h)	Lactylacetate
Common carp	0	10.5	Grass carp	0	15
	24	9.0		12	23
	36	20		24	30
	48	39		36	44

of spermatozoa persists intact for an appreciable time. These conclusions are supported by the results of Stoss (1979) and Billard (1981) obtained in rainbow trout and of Saad *et al.* (1988) in common carp when the survival and fertility of spermatozoa in aerobic conditions were significantly better.

Enzymes

Enzymes involved in the metabolism (ATPases, phosphatases, lipases, esterases and oxidases) were found in isolated carp and rainbow trout spermatozoa (Tibbs, 1959; Breton *et al.*, 1974). Detailed localization of lactate dehydrogenase (LDH) was made by Baccetti *et al.* (1975). Two malic enzymes, NAD- and NADP-dependent malic dehydrogenases, were identified in salmon and cod sperms (Mounib, 1974).

Rate of energetic metabolism is controlled by levels of NAD coenzymes which operate as cyclic hydrogen transmitters in all cells. In Table 10, concentrations of NAD(P) and NAD(P)H in spermatozoa of two fish species are shown (Gosh, 1985). In both species, level of NAD exceeded NADH whereas the level of NADP is lower than that of NADPH. During the storage (until 24 h) of sperm of both species, the amount of NAD(P) changes very little as compared with reduced forms where the amount of NADH in sperm of common carp increased 4.5 times and that of NADPH 3 times. After longer time of storage (more than 24 h), level of NAD, however, decreased 2.5 times. Under anaerobic conditions, therefore, rate of oxidative reactions is lowered and concentration of lactic acid increased. Glycolysis resulted in increasing of NADH concentration,

Table 10
The level of NAD(P)/NAD(P)H in spermatozoa after short-term storage of sperm (temperature of storage 4-5°C, nmol$\times 10^9$ spermatozoa) (Gosh, 1985)

Species	Storage of sperm (h)	NAD^+	NADH	NAD^+/NADH	$NADP^+$	NADPH	$NADP^+$/NADPH
Common carp	0	28.6	1.4	20.4	1.4	2	0.71
	24	29.8	6.2	4.8	1.1	6	0.18
Grass carp	0	12	3.1	4.0	0.8	2.6	0.3
	12	8.1	6.1	1.3	0.6	4.1	0.15

alteration of endogenous metabolite composition, and retardation or total suppression of metabolism.

The fish spermatozoa have the complex of lipid re-synthesis which is functioning during the sperm storage (Belova, 1982).

Amount of ATP and its role in spermatozoa motility

The system of microtubules in the flagellum represents the motion apparatus of a spermatozoa. Each of the peripheral double-tubules carries two arms which consist of an ATPase called dynein. The ATPase activity of perch spermatozoa was shown to be activated both by $MgCl_2$ and $CaCl_2$, and can be easily extracted from the flagella (Tibbs, 1954).

At the initiation of flagellar movement of the trout spermatozoa, transient increase of intracellular cyclic AMP was reported (Morisawa *et al.*, 1983), from a level of about 10 pmol·mg^{-1} protein to about 80 pmol·mg^{-1} protein within 1 sec after dilution; cAMP level in 20 to 30 sec after dilution falls back to 30-40 pmol. This transient peak was associated with the activation of adenylate cyclase at initiation of motility (from 0.4 pmol cAMP·sec^{-1}·mg^{-1} protein to 1.1 pmol cAMP·sec^{-1}·mg^{-1} protein) and with the activation of a phosphodiesterase with a 4 sec delay (Morisawa *et al.*, 1983). In trout spermatozoa, a tyrosine proteinkinase was suggested to be a key enzyme in controlling flagellar movement by phosphorylation of 15-kD protein under cAMP regulation (Billard and Cosson, 1990). More recent works demonstrated the occurrence of a rise in internal cAMP levels at the initiation of movement (Benau and Terner, 1980; Morisawa *et al.*, 1983; Morisawa and Ishida, 1987) and the cAMP-dependent phosphorylation of of a 15 kD axonemal protein has been proposed as the trigger initiating trout sperm motility (Morisawa and Hayashi, 1985; Morisawa and Morisawa, 1990), but Ca^{2+} may also play role if similarities with mammals exist. It is not quite clear if adenylate cyclase activation results in cAMP degradation because activators of adenylate cyclase and inhibitors of phosphodiesterase in fish spermatozoa are not known. In mammalian spermatozoa, some proteinkinases are activated by cAMP, whereas the others are controlled by Ca^{2+}.

The energy (in the form of ATP) necessary for spermatozoan movement originates from glycolytic and oxidative reactions. There are only

a few publications dealing with determining of ATP amount in spermatozoa (Felix *et al.*, 1956; Tibbs, 1962; Burnashova, 1960; Mohri, 1964; Christien *et al.*, 1987). In general, high correlation between ATP level and spermatozoan motility was found. Burnashova (1960) carried out examinations of activated spermatozoa of sturgeons, *Acipenser güldenstadti* and *A. stellatus,* and found before activation the ATP level 36 $mg \cdot ml^{-1}$ sperm with respiratory rate 17 $\mu l\ O_2 \cdot ml^{-1}$ sperm. During 1-2 min after activation with water the amount of ATP increased to 104 $mg \cdot ml^{-1}$, and respiratory rate increased to 37 $\mu l\ O_2 \cdot ml^{-1}$ sperm. After 5-10 min, the motility decreases considerably along with reversion of ATP level to the original value. Burnashova (1960, 1982) mentioned that after inhibition of glycolytic or oxidative processes in fish spermatozoa the level of ATP decreased rapidly and spermatozoa movement is slower.

Cytochromoxidase (cyt-A_3)

Cytochromoxidase is a member of respiratory chain together with some oxidoreductases and other enzymes and with about 20 different cytochromes. This enzyme catalyzes the last step of oxidative reactions in the cell. Zhukinski and Gosh (1974) found this enzyme in roach and bream spermatozoa. They stated, however, that the highest fertilization rate in roach was reached with spermatozoa possessing the lowest cyt-A_3 activity and that the lowest activity of this enzyme was found in the roach males with the highest sperm fertilization capability. On the contrary, the highest activity of cyt-A_3 was found in spermatozoa of both young and old adult roach males. These authors supposed that the correlation between the low cyt-A_3 activity and the amount of energetic pool in the most fertile sperm. Our opinion is that this result (if not incidental) is an expression of predominance of anaerobic metabolism over the aerobic one in activated spermatozoa. Very high motility of spermatozoa after activation—much higher than in mammals—leads us to this conclusion, too.

Ovoviviparous species

Surfperch (Embiotocidae) spermatozoa diluted in saline solution are motile for a few hours (Gardiner, 1978); guppy spermatozoa diluted in Ringer solution save their motility for 60 min (Billard, 1978). The same spermatozoa diluted in water moves only 1 min (Billard, 1969, 1978). The

long duration of motility found in spermatozoa of viviparous species results probably from glycolytic metabolism with consumption of substrates like carbohydrates most probably endogenous (glycogen) or exogenous (glucose or fructose from seminal plasma or secretions of the female genital tract) (Billard and Cosson, 1990). Viviparous fish spermatozoa may also metaobolize extracellular glucose *in vitro*. The metabolic rate measured by the production of $^{14}CO_2$ is low, but detectable in guppy and surfperch (*Cytogaster aggregata*) (1 and 3.5 mmol glucose utilized per 10^8 cells per hour, respectively) (Gardiner, 1978a).

CONCLUSION

The spermatozoa of those chondrostean and teleostean fishes characterized by external fertilization have a simple structure. The main characteristics of morphology of chondrostean (studied in Acipenseridae) and teleostean spermatozoa are the elongated head with acrosome-like structure, and spherical or slightly elongated (2-3 μm) head without acrosome, respectively. Part of histone and non-histone proteins are associated with highly condensed chromatin, some proteins, as protamines, play an important role in constitution of cytoskeleton. The reduced middle piece with limited amount of cytoplasm and slightly modified or unmodified mitochondria are typical for both fish subclasses, similarly as prominent end piece. The tail contains centrally placed axoneme which represents highly ordered complex of microtubules surrounded by dense fibers extending from the head near to posterior end of axoneme. Tail length varies from 40 to 60 μm and the plasmatic membrane often forms one or two fin-like ridges along the tail.

The spermatozoa of those teleostean fishes characterized by internal fertilization have a more developed structures. The spermatozoa have elongated both head (3-4 μm) containing highly condensed chromatin, and a midpiece (6-7 μm) which contains large mitochondrial structures and intercentriolar material.

The seminal plasma contains several different cations (Na^+, K^+, Mg^+, Ca^+) and organic compounds (glycids, proteins, lipids, etc.). The comparison of seminal plasma compositions of different species is difficult because they are very variable in dependence on various external and internal factors. Fish

spermatozoa are immotile in the testis and in the seminal plasma in many species. Osmotic pressure, concentration of K^+ and sucrose, and pH lower than 7 in seminal plasma are the main factors inhibiting spermatozoa motility of salmonids; the osmotic pressure seems to be the major supressive factor in cyprinids. The depolarization of cell membrane is the activating factor which initiates the motility. Spermatozoa have energetic cellular reserves such as phospholiphids, glycolipids and glycogen. Energy for both basic cell metabolism and motility are derived from the endogenous nutrients breakdown in the absence or presence of oxygen. In the case of external fertilization, fish spermatozoa are shed into an aqueous environment without metabolic substrates. Some enzymes as MDH as well as metabolites (pyruvate, acetate) present in spermatozoa are the main functional factors of the Krebs' cycle. Products of lipid and phospholipid metabolisms can be incorporated into this eycle, too. The possibilities of energy utilization from external sources of seminal plasma are more extensively discussed. However, no better results in motility after addition of ATP and cAMP to the sperm were assessed. In spermatozoa of species with external fertilization, the limiting factors, as primitive structure of the spermatozoa, endogenous storage capacity of substrates and limited metabolic cycles, can optimally be used only in a very good environment.

The well-developed mitochondrial sheath in the midpiece of spermatozoa in some species indicates the need for extensive metabolic activity. The spermatozoa of species with internal fertilization can metabolize both endogenous (glycogen) and exogenous (*e.g.* glucose or fructose) sources. The strategy of reproduction of both ovoviviparous fish and mammals is similar in a very general mode; therefore, it was possible to complete the missing or insufficient data with those of mammals.

Future objectives and applications

As shown above, this relatively unexploited ways of research in reproductive biology of fishes should further be focused as follows:

1. To collect and arrange all the necessary data concerning species-specific morphological characteristics of normal spermatozoa of those species with artificial propagation or of economical importance, maybe in the form of an atlas; the picture list

of known abnormalities of spermatozoa would be included there.

2. On the basis of collected data, the simple, practical procedures to determine defect spermatozoa should be worked out and standardized.

3. Analyses of the chemical and biochemical composition of seminal plasma of species with artificial propagation and/or of economical importance can be utilized for working up the particular media for storage, immobilization and/or cryopreservation of spermatozoa.

4. The studies focus on the energy sources and their utilization by spermatozoa after activation in different activation solutions may bring the new view of the energetics of active fish spermatozoa. These studies could enable us to determine the most suitable conditions for the highest fertilization rates.

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