



Sperm and Spermatozoa Characteristics in the Siberian Sturgeon

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Abstract

Sperm and spermatozoa in Siberian sturgeon are very interesting and specific from several points of view. Siberian sturgeon usually produces high volume of semen with relatively low sperm and protein concentration, which is partially explained by the atypical testicular morphology where spermatozoa are mixed with urine during passage through the kidneys to the Wolffian ducts. Sodium and chloride ions contribute most to the osmolality of the seminal fluid. Potassium ions are critical for immobilization of spermatozoa, while its antagonist is calcium ion, which triggers spermatozoa motility. The motility period is relatively long (2–3 min) with flagellum beat frequency about 50 Hz. The main characteristics of sturgeon spermatozoa are an elongated head with an acrosome containing acrosomal proteins. The flagellum is equipped with a fin for more efficient movement. During penetration into the egg micropyle, the acrosome undergoes acrosomal reactions, which include formation of fertilization filament and opening of posterolateral projections. The fertilization filament activates the egg and causes the formation of a perivitelline space, while the posterolateral projections serve as an anchor against release from the micropyle. The acrosomal reaction has been recognized to be important for fertilization and development.

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Keywords

Semen composition • Spermatozoon ultrastructure • Acrosomal reaction • Siberian sturgeon

Introduction

Siberian sturgeon semen is characterized by high volume and low numbers of spermatozoa and ions as well as low protein concentration (Tables 15.1 and 15.2). Significant variation in these values is observed and most likely relates to age, time after hormonal stimulation, number of sperm collections, and maintenance. The lowest volume (8 mL) was reported from a 34-month-old male, whereas older males of 7+ years produced ejaculates >200 mL (Glogowski et al. 2002). Sperm concentration can be as low as 0.2×10^9 spermatozoa, but values ten times higher have been recorded. Such variation can be partially explained by dilution of semen with urine in the Wolffian ducts (Gallis et al. 1991). Some variation of seminal plasma osmolality and ionic composition are observed as well (Table 15.2).

The mode of spermatozoa-ova gamete interaction is unique in sturgeons. The spermatozoa possess an acrosome, which is generally used to penetrate through the egg envelopes after entering one of the eggs several micropyles so as to provide increased access for spermatozoa.

15.1 Ions of Seminal Plasma

Basic parameters of Siberian sturgeon seminal plasma are shown in Table 15.2. Sodium and chloride ions are the most significant components that contribute to the seminal osmolality, which is generally lower than in teleostean species. Potassium

Table 15.1 Semen volume and sperm concentration of Siberian sturgeon milt

Feature	Mean \pm sd	Reference
Semen volume (mL)	25.9 \pm 14.3	Li et al. (2011)
	73 \pm 58	Sieczyński et al. (2012)
	8 ($n = 1$)	Glogowski et al. (2002)
	267.5 \pm 38.9	Glogowski et al. (2002)
	85–300	Piros et al. (2002)
	356 \pm 135	Tsvetkova et al. (1996)
Sperm concentration ($\times 10^9$)	0.20 \pm 0.17	Li et al. (2011)
	0.22 ($n = 1$)	Glogowski et al. (2002)
	0.58 \pm 0.37	Sieczyński et al. (2012)
	0.61 \pm 0.37	Pšenička et al. (2008a)
	0.64 \pm 0.42	Sarosiek et al. (2004)
	1.21 \pm 0.45	Judycka et al. (2015a)
	1.21 \pm 0.41	Glogowski et al. (2002)
	1.68 \pm 0.33 (1) ^a	Piros et al. (2002)
2.42 \pm 0.78 (2) ^a	Piros et al. (2002)	

^aFirst and second semen collections as described in Piros et al. 2002. All values are mean \pm SD with the exception of sperm concentration data of Piros et al. 2002 which are expressed as mean \pm SEM

Table 15.2 Basic parameters of Siberian sturgeon seminal plasma

Feature	Mean \pm sd	Reference
pH	7.97 \pm 0.42	Li et al. (2011)
	8.16 \pm 0.18	Pšenička et al. (2008a)
	8.70 \pm 0.13	Judycka et al. (2015a)
Osmolality	38 \pm 3	Gallis et al. (1991)
	46.2 \pm 11.6	Li et al. (2011)
	77.2 \pm 52.8	Pšenička et al. (2008a)
	93.6 \pm 7.3 (1) ^a	Piros et al. (2002)
	95.7 \pm 5.4 (2) ^a	Piros et al. (2002)
Na ⁺ (mM)	14.6 \pm 6.1	Li et al. (2011)
	28 \pm 0.7	Gallis et al. (1991)
	31.4 \pm 10.2	Pšenička et al. (2008a)
K ⁺ (mM)	2.5 \pm 0.3	Gallis et al. (1991)
	3.5 \pm 1.1	Pšenička et al. (2008a)
	4.5 \pm 1.1	Li et al. (2011)
	0.24 \pm 0.06	Pšenička et al. (2008a)
Ca ²⁺ (mM)	0.27 \pm 0.09	Li et al. (2011)
Mg ²⁺ (mM)	0.48 \pm 0.22	Li et al. (2011)
Cl ⁻ (mM)	6.2 \pm 1.2	Li et al. (2011)
	14.0 \pm 4.3	Pšenička et al. (2008a)
Protein conc. (mg mL ⁻¹)	0.38 \pm 0.16	Judycka et al. (2015a)
	0.39 \pm 0.19	Sarosiek et al. (2004)
	0.58 \pm 0.11 (1) ^a	Piros et al. (2002)
	0.57 \pm 0.06 (2) ^a	Piros et al. (2002)

^aFirst and second semen collections as described in Piros et al. 2002. All values are mean \pm SD with the exception of osmolality data of Piros et al. 2002 which are expressed as mean \pm SEM

ions are critical for immobilization of spermatozoa in Wolffian duct (Gallis et al. 1991; Toth et al. 1997). However, inhibition of sperm motility is not always complete because some movement is often observed in non-diluted semen (Glogowski et al. 2002). Inhibition of motility by K⁺ ions can be modulated by Ca²⁺ ions which are also present in seminal plasma (Alavi et al. 2012a). Values of pH seem to be less variable and are alkaline (8.0 and higher) which is a characteristic for sperm maturation in fishes (Morisawa and Morisawa 1988). Lower values of pH have been attributed to contamination with urine (Li et al. 2011).

15.2 Proteins of Seminal Plasma

15.2.1 Electrophoretic Pattern

Protein concentration in seminal plasma of Siberian sturgeon is low. Generally, in sturgeon it is not higher than 1 mg mL⁻¹ (Table 15.2). Two-dimensional electrophoresis of seminal plasma has not been performed yet, and at present only a one-dimensional SDS-PAGE protein profile is available (Li et al. 2011). The latter distinguished five mutual protein bands with molecular weight ranging from 29 to

71.3 kDa; a band of 71.3 kDa is speculated to be β -N-acetylglucosaminidase which was isolated and characterized by Sarosiek et al. (2008).

15.2.2 Enzymes and Inhibitors

Data on biochemical composition of Siberian sturgeon seminal plasma were described by Piros et al. (2002). Activities of lactic dehydrogenase (LDH), arylsulfatase, acid phosphatase, and β -N-acetylglucosaminidase were found; the presence of the latter had been postulated by Li et al. (2011; see above). These activities seem to be low, because values several times higher were found in spermatozoa (Piros et al. 2002). This seems to reflect a low concentration of protein in seminal plasma, and it is possible that the presence of these enzymes in seminal plasma is related to their release from damaged spermatozoa. It has been postulated that similar to mammals, Siberian sturgeon arylsulfatase may modify the spermatozoan surface charge by removal of sulfate groups of galactosyl conjugates (Piros et al. 2002). Superoxide dismutase, glutathione reductase, and glutathione peroxidase were detected both in seminal plasma and spermatozoa of Siberian sturgeon (Shaliutina et al. 2013). This indicates the existence of protection against oxidative stress.

Despite the presence of proteolytic enzymes in spermatozoa, anti-proteinase activity (APA) in seminal plasma is also low, but seems to be exclusively present in seminal plasma, because APA does not change after freezing-thawing of semen (Piros et al. 2002). Activity of APA is clearly affected by the season (Słowińska et al. 2015), being highest in December ($87.3 \pm 17.0 \text{ U mL}^{-1}$) compared to February ($12.6 \pm 1.4 \text{ U mL}^{-1}$) and April ($12.4 \pm 2.7 \text{ U mL}^{-1}$). Before stimulation, fish were transferred from ponds (water temperatures of 1.2 and 5.3 °C, for December and April, respectively) to tanks. The water temperature in the tanks was gradually increased (1 °C day⁻¹) to 16 °C and then maintained at that temperature for 7 days. This suggests that protease inhibitors are especially important during spermatogenesis and sperm maturation. Target protease for APA are unknown at present, but it is likely that some of several proteases recently identified in seminal plasma, including serine proteases and metalloproteases, can be controlled by APA (Słowińska et al. 2015).

15.3 Proteins of Spermatozoa

15.3.1 Electrophoretic Pattern

Contrary to information on seminal plasma, two-dimensional electrophoretograms of spermatozoa extracts are available (Li et al. 2010, 2011). The latter identified 95 protein spots (Li et al. 2011) to over 100 (Li et al. 2010); these can be divided into those common for sturgeons, presumably highly conserved proteins, and protein spots which seems to be species-specific. At present, a lack of data on cDNA and gene sequence hampers identification of protein spots, but several isoforms of enolase B and lactate dehydrogenase have been identified, which seem to be highly conserved in sturgeon sperm.

15.3.2 Enzymes

The main proteinase in the acrosome of sturgeons is acrosin which was first discovered in white sturgeon (Ciereszko et al. 1994, 1996), and recently, it was reported from sterlet by immunohistochemistry (Pšenička et al. 2009, see Sect. 15.5.2.) and Siberian sturgeon using Western blotting (Słowińska et al. 2015). The latter have demonstrated the presence of four bands of acrosin in sperm extracts which likely represent a proacrosin-acrosin system. Proacrosin is present in an inactive zymogen form which is converted to mature enzyme through a series of proteolytic cleavages. The role of acrosin is likely involved in the acrosomal reaction. The role of the acrosome reaction in sturgeon fertilization is paradoxical because of the presence of micropyles in the eggs.

The presence of enzymes other than acrosin-acrosomal in Siberian sturgeon acrosome is unclear. It is possible that arylsulfatase and β -N-acetylglucosaminidase are acrosomal enzymes (Piros et al. 2002); however, direct evidence is not yet available. There are some indirect indications for the presence of both enzymes in the acrosome. Both enzymes are localized in mammalian acrosomes (Nikołajczyk and O’Rand 1992; Brandon et al. 1997). Moreover, arylsulfatase was not found in anacrosomal spermatozoa of teleost fishes. On the other hand, β -N-acetylglucosaminidase is present in teleostean sperm (Sarosiek et al. 2012), which complicates the understanding of its localization and role. Recent data suggest that arylsulfatase, β -N-acetylglucosaminidase, and acid phosphatase are related to sperm fertilizing ability in Siberian sturgeon (Sarosiek et al. 2014; Sarosiek et al. 2015).

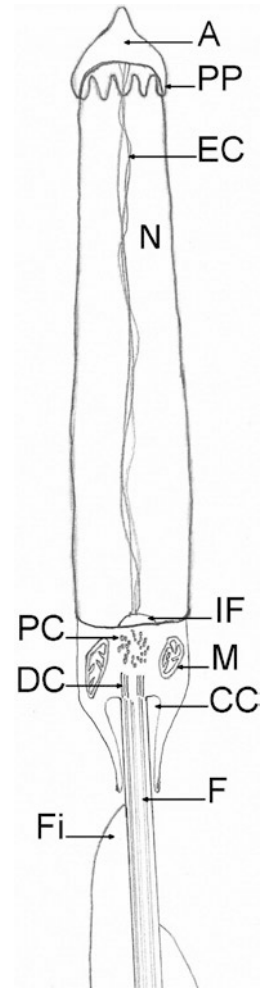
Other enzymes identified in spermatozoa (enolase, LDH) are related to metabolism (glycolysis). Their role is likely related to provide energy (ATP) for sperm movement. This can be demanding task for sturgeon sperm, because duration of motility is much longer, compared to teleostean fish spermatozoa (see below).

15.4 Spermatozoa Morphology

Generally, the spermatozoon function in the transport of the male haploid chromosome sets into the oocyte. For this purpose, it is composed of four different compartments—the acrosome, the head, the midpiece, and the flagellum (Callard and Callard 1999; Knobil and Neill 1999). The acrosome has lytic activities so as to enable the entrance of the spermatozoon into the oocyte through egg envelope; the head contains the nucleus and therefore the DNA material. The mitochondria are located in the midpiece, which delivers the energy for flagellar beating. The flagellum itself is the motor of the spermatozoon. The centriolar complex consists of the proximal and the distal centriole whereby the latter is the basal body of the flagellum. This centriolar complex anchors the flagellum at the sperm cell and is normally located in close proximity to the nucleus (Figs. 15.1 and 15.4a).

Fish gametes are diverse in morphology and ultrastructure, including the number and location of different organelles (Baccetti et al. 1984; Baccetti 1986; Jones and Butler 1988). Species-specific morphological and physiological features have been shown in spermatozoa and eggs of several fish species, which reflect

Fig. 15.1 Shows basic structure of surgeon spermatozoa. *A*, acrosome; *PPs*, posterolateral projections; *EC*, endonuclear canals; *IF*, implantation fossa; *N*, nucleus; *PC*, proximal centriole; *DC*, distal centriole; *M*, mitochondria; *CC*, cytoplasmatic canal; *F*, flagellum; *Fi*, fin



differences in functional capabilities and adaptations for spawning strategy. Morphology and fine structure are considered to be the major sources of information in comparative spermatology (Baccetti 1986; Jamieson 1991; Jamieson 1999; Lahnsteiner and Patzner 1997).

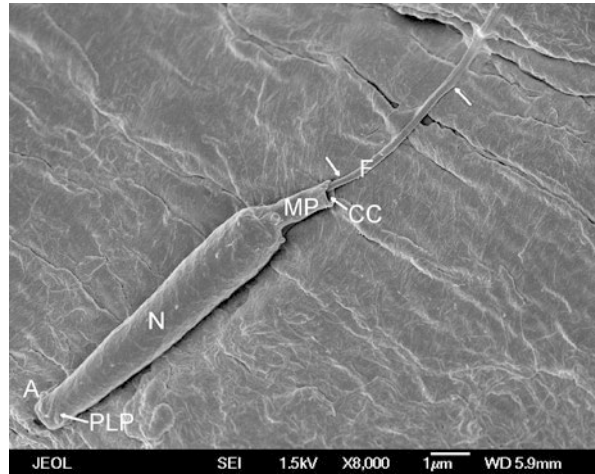
Spermatozoa of sturgeons have characteristic differences compared to those of teleost fishes in terms of morphology (Jamieson 1991), by the presence of acrosomal structure elongate nucleus and a flagellum with fins (Cherr and Clark 1984; Dettlaff et al. 1993; Pšenička et al. 2007; Wei et al. 2007). Moreover, there are also significant differences in parameters of spermatozoa among sturgeon species. Morphological parameters of Siberian sturgeon spermatozoa are compared with other sturgeon species (Table 15.3). It follows that Siberian sturgeon spermatozoa belong to middle-size sturgeon spermatozoa. Although Pšenička et al. (2010a)

Table 15.3 Comparison of morphological parameters of spermatozoa among sturgeon species

Sturgeon species	AL	AW	HL	ANW	PNW	ML	MW	FL	TL	n	References
Beluga	1.12 (0.14)	0.87 (0.10)	7.14 (0.47)	0.69 (0.06)	0.98 (0.08)	2.10 (0.42)	0.61 (0.09)	42.21 (3.82)	51.27 (4.71)	4	Linhartova et al. (2013)
Russian sturgeon	1.18 (0.01)	1.05 (0.01)	6.84 (0.04)	1.10 (0.01)	1.48 (0.01)	1.64 (0.03)	0.92 (0.01)	49.42 (0.37)	57.08 (0.79)	5	Hatef et al. (2012)
Persian sturgeon	1.15 (0.15)	1.16 (0.15)	7.05 (0.51)	1.24 (0.15)	1.54 (0.17)	1.81 (0.46)	0.90 (0.12)	50.31 (5.87)	59.18 (6.23)	5	Hatef et al. (2011)
Sterlet	0.79 (0.07)	0.75 (0.07)	3.30 (0.31)	0.67 (0.07)	0.85 (0.08)	0.97 (0.23)	0.64 (0.12)	42.47 (1.89)	47.61 (1.89)	3	Pšenička et al. (2009)
Chinese sturgeon	0.54 (0.15)	0.68 (0.06)		0.59 (0.05)	1.84 (0.45)	2.17 (0.36)	1.57 (0.27)	33.26 (2.74)	38.7		Wei et al. (2007)
Siberian sturgeon	0.95 (0.17)	0.93 (0.12)	4.98 (0.83)	0.87 (0.13)	1.14 (0.18)	1.09 (0.43)	0.80 (0.25)	44.75 (4.93)	51.76	8	Pšenička et al. (2007)
Pallid sturgeon	1.07 (0.10)	0.82 (0.06)	3.78 (0.33)	0.68 (0.04)	0.89 (0.06)	1.23 (0.16)	0.67 (0.08)	37.16	43.23	16	DiLauro et al. (2001)
Lake sturgeon	0.73 (0.14)	0.81 (0.07)	5.69 (0.43)	0.68 (0.07)	1.04 (0.08)	2.68 (0.43)	0.70 (0.08)	47.53	56.63	14	DiLauro et al. (2000)
Shortnose sturgeon	0.78 (0.08)	0.91 (0.06)	6.99 (0.83)	0.75 (0.11)	1.21 (0.12)	1.91 (0.35)	0.81 (0.09)	36.7	46.41	15	DiLauro et al. (1999)
Atlantic sturgeon	0.83 (0.11)	1.00 (0.07)	3.15 (0.36)	0.92 (0.06)	0.55 (0.08)	1.37 (0.16)	0.51 (0.07)	37.08	42.74	12	DiLauro et al. (1998)
White sturgeon	1.31	1.34	9.21	1.25	1.44	2.13	1.08	30-40	41.82- 51.82	1	Cherr and Clark (1985)
Stellate sturgeon	0.97	1.22	6.66	0.98	1.49	3.43	1.38	40-70	51.05- 81.05	1	Ginsburg (1977)

Measurements are shown as means \pm SD (in parentheses) for n sperm. Data are measured in μm . AL, acrosome length; ANW, anterior nucleus width; AW, acrosome width; FL, flagellum length; HL, head length; ML, midpiece length; MW, midpiece width; PNW, posterior nucleus width; TL, total length (head with acrosome, midpiece, and flagella)

Fig. 15.2 Micrograph from scanning electron microscopy shows acrosome (A), cytoplasmic channel (CC), flagellum (F), midpiece (MP), and nucleus (N). The arrows indicate the development of fins along the flagellar length. Scale bar is 1 μm . (Pšenička et al. 2007)



showed highly significant differences, with deviation up to 30%, in size determination of all measured values for one specimen (sterlet spermatozoa) using different electron microscopic methods, they suggested that for correct comparative studies, the samples should be prepared in the same way and at the same time, which unfortunately cannot be done (a posteriori). A scheme of common structure of Siberian sturgeon spermatozoa is illustrated (Fig. 15.1).

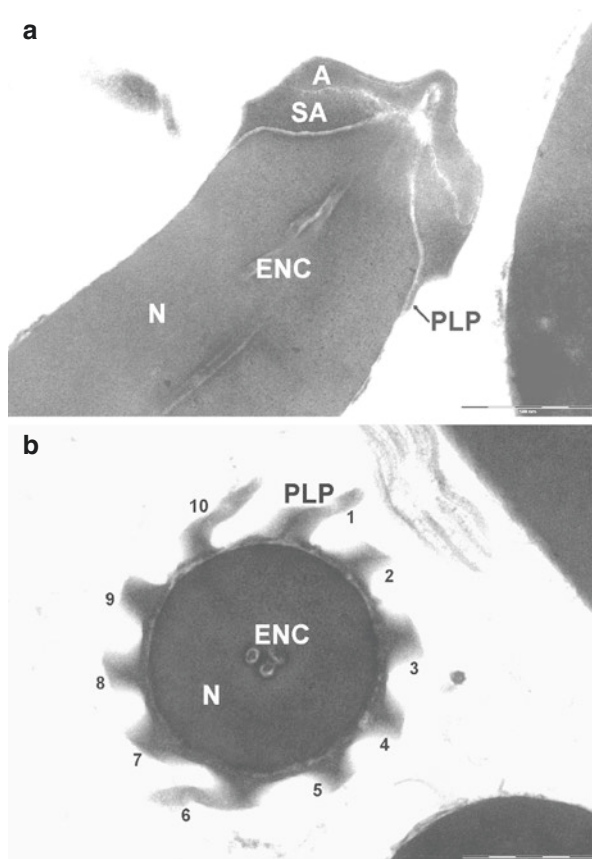
15.4.1 The Sturgeon Sperm Head

The shape of Siberian sturgeon sperm head has an elongated trapezoidal shape, tapering from the anterior end to the posterior end, similar to other sturgeon species spermatozoa (Fig. 15.2). The head is composed of the acrosome, endonuclear canals (ECs), an implantation fossa, and a nucleus with electron dense and slightly granular material surrounded by a nuclear membrane.

The acrosomal components include the acrosome, a subacrosome, and postero-lateral projections (PPs); the latter are located at the posterior part of acrosome. Numbers and sizes of PPs vary between sturgeon species; the acrosome of Siberian sturgeon possesses a high number (10) and size (940 nm) of PPs (Fig. 15.3a, b) (Pšenička et al. 2007). For comparison, Chinese sturgeon *A. sinensis* has 10 (370 nm) (Wei et al. 2007), and there are 9–10 (295 nm) in sterlet *A. ruthenus* (Pšenička et al. 2009), 8 (760 nm) in pallid sturgeon *Scaphirhynchus albus* (DiLauro et al. 2001), and 7–9 (490 nm) in beluga *Huso huso* (Linhartova et al. 2013).

Ciereszko et al. (1996) found that the trypsin-like activity in sturgeon spermatozoa shares many properties with mammalian acrosin. Acrosin appears to be a widely distributed and conserved protein (Baccetti et al. 1989), which developed a billion years ago during the early period of eukaryote evolution (Klemm et al.

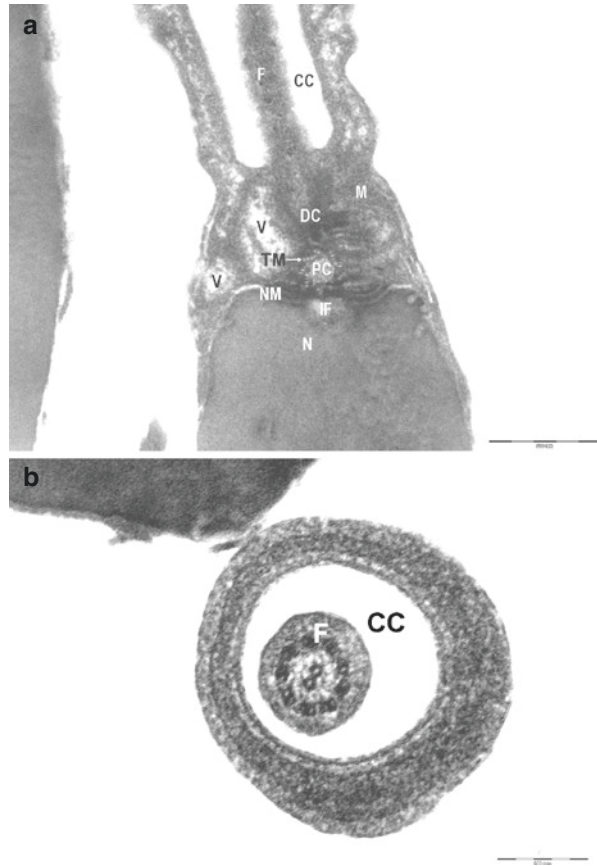
Fig. 15.3 Longitudinal sagittal section (a) and cross section (b) captured by transmission electron microscopy shows the acrosome (A) and the subacrosome (SA), with the ten posterolateral projections (PLPs) and the endonuclear canals (ENC) traversing the nucleus (N). Scale bar, 500 nm. (Pšenička et al. 2007)



1991). For this reason, the presence of acrosin in sturgeon sperm was expected (see Sect. 15.4.2). Immunolabeling shows that acrosin is present in sturgeon (sterlet) spermatozoa and is localized in the acrosome and the implantation fossa. In addition, scanning electron microscopy on cryofracture shows evidence of the opening of ECs to the acrosome and implantation fossa as well. That means that the acrosome is connected to the implantation fossa by the ECs. Therefore it is suggested that the ECs and implantation fossa are components of the acrosome (Pšenička et al. 2009).

Except for the Atlantic sturgeon (DiLauro et al. 1998), which has two ECs, all other sturgeon species have three ECs in their spermatozoa (Ginsburg 1977; Cherr and Clark 1984; DiLauro et al. 2000, 2001; Pšenička et al. 2007; Wei et al. 2007; Linhartova et al. 2013). Pšenička et al. (2008a) also found that some spermatozoa of Siberian sturgeon had two to four ECs. There also are differences in the canal diameters between sturgeon species, with values of 35, 97, 49, 57, 40, and 44 nm in Atlantic, shortnose, lake, pallid, sterlet, and Siberian sturgeons, respectively.

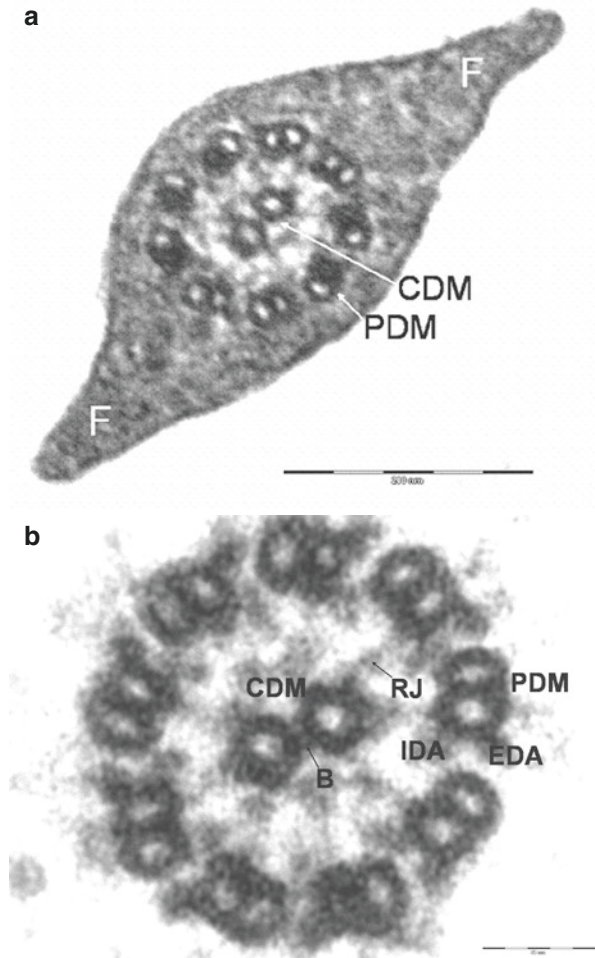
Fig. 15.4 Longitudinal sagittal section (a) and cross section (b) of the midpiece captured by transmission electron microscopy shows the distal (DC) and proximal (PC) centrioles in the implantation fossa (IF) with triplets of microtubules (TM). Numerous mitochondria (M) and vesicles (V) were irregularly dispersed in the cytoplasm. The nucleus (N) is surrounded by the nuclear membrane (NM). The flagellum (F) is separated by the cytoplasmic channel (CC). Scale bars are 500 nm (a) and 200 nm (b). (Pšenička et al. 2007)



15.4.2 The Sturgeon Sperm Midpiece

The midpiece has a cylindrical shape and an elongated caudal base. Siberian sturgeon spermatozoa contain from three to six mitochondria in a peripheral section of the midpiece. The mitochondria provide energy in the form of ATP for sperm movement. The proximal centriole (185.28 nm × 147.42 nm) is posteriorly the implantation fossa in nucleus. Both centrioles are composed of nine peripheral triplets of microtubules in a cylindrical shape. The axoneme is formed as an extension of the distal centriole. Around the flagellum, there is a plasma membrane with cytoplasmic channel, which is formed by an invagination of the membrane. There is an extracellular space between the cytoplasmic sheath and the flagellum called cytoplasmic channel (Fig. 15.4a, b) (Pšenička et al. 2007).

Fig. 15.5 Cross section of the flagellum (a) and axoneme (b) captured by transmission electron microscopy shows the peripheral doublets of microtubules (PDM) and central doublets of microtubules (CDM) with radial joints (RJ) and a bridge (B). The propelling machinery is the internal (IDA) and external (EDA) dynein arms. The fins (F) make the flagellum more effective. Scale bars are 200 nm (a) and 50 nm (b). (Pšenička et al. 2007)



15.4.3 The Sturgeon Flagellum

In sturgeon, the fibrillar part of the flagellum, called axoneme, consists of nine peripheral doublets and a central pair of single microtubules. The pair of central microtubules are linked by bridges and they are encased in a central sheath. Nine radial spokes, or joints, connect the two central and peripheral doublets. From the end of the cytoplasmic sheath of the midpiece to the terminal region of the flagellum, two independent lateral extensions of the flagellar plasma membrane gradually taper to form the fins (Figs. 15.2 and 15.5a, b). These fin structures are

oriented along the horizontal plane, parallel to the central microtubules (Billard 1970), and probably help to increase the efficiency of wave propagation (Cosson et al. 2000). In silver salmon *Oncorhynchus kisutch*, the spermatozoon tail has the membrane in central part formed into a spiral with 12–15 coils (Lowman 1953), but Pšenička et al. (2007) described this fin as (straight) in Siberian sturgeon spermatozoa, indicating no spiral (rotation), but exactly parallel to the plane of the two central microtubules of the flagellum. This means that the central microtubules also are orientated in one plane. The fins are not formed the same on either side of the flagellum. The first and second fins start 0.7 and 5.3 μm post-midpiece, respectively, and continue along the flagellum. The first and second fins end 3.4 and 5.1 μm from the end of the flagellum, respectively. They extend distally up to 705.87 ± 220.04 nm in the middle part of flagellum. The sperm flagellar membrane in fishes with this unusual fin-shaped structure also significantly increases the membrane surface area, thus, favoring increased water exchange. This has considerable implications for water exchange/osmotic regulation at activation (Cosson et al. 2000; Pšenička et al. 2007).

The length of the flagellum and content of mitochondria are positively correlated with the swimming velocity in tench spermatozoa (Pšenička et al. 2010b). Pšenička et al. (2008a) compared sperm characteristics in Siberian and sterlet sturgeons. There also was a correlation between the flagellum length and velocity, and therefore motility, especially at the end of motility.

15.5 Sperm Motility Characteristics

Sperm motility of sturgeons differs markedly from that of teleosts. Duration of sturgeon sperm movement is longer, for example, about 2–3 min for Siberian sturgeon, and also the number of active spermatozoa regularly declines to 5–10% at 2–3 min post-activation (Billard et al. 1999). During this period, the flagellum beat frequency is stable at about 50 Hz for 30 s and then drops to about 30 Hz after 60 s. Beat frequency is affected by cryopreservation; flagellar waves are asymmetrical in post-thawed spermatozoa (Billard et al. 2000). During the motile period, a constant decline in ATP concentration takes place, but it seems that the efficiency of swimming performance of Siberian sturgeon spermatozoa is also improved by the fins. Swimming performance of Siberian sturgeon spermatozoa is facilitated by the presence of the paddle-like fins that extend along most of flagella length (Gillies et al. 2013).

Initiation of sperm motility consists of a cascade of events, starting from a decrease in osmolality and potassium ion concentrations, which result in an efflux of these ions from spermatozoa (Alavi et al. 2012b). Next, membrane hyperpolarization occurs, followed by Ca^{2+} ion influx and membrane depolarization. A rise in Ca^{2+} and calmodulin induces initiation of sperm movement through the activation of phosphodiesterase.

Due to low osmolality of sturgeon semen (see Table 15.2), activating solutions are characterized by low osmolality as well; for example, Tsvetkova et al. (1996) used 50 mM Tris-HCl, pH 8.0, and Billard et al. (1999) 30 mM Tris-HCl, pH 8.0,

Table 15.4 Sperm motility parameters of Siberian sturgeon semen

Feature	Mean \pm sd	Reference
Motility (%)		
<i>Before activation</i>	0.47 \pm 1.1	Glogowski et al. (2002)
	2.4 \pm 2.5	Sieczynski et al. (2012)
<i>After activation</i>	41.3 \pm 14.1	Sieczynski et al. (2012)
	61.7 \pm 17.6	Glogowski et al. (2002)
	70	Urbanyi et al. 2004
	76	Sarosiek et al. (2004)
	80	Judycka et al. (2015b)
	88 \pm 4.4	Tsvetkova et al. (1996)
	90	Billard et al. (1999)
	95–100	Shaliutina et al. 2013
	95.2	Pšenička et al. (2011)
	100	Pšenička et al. (2008a)
	100	Judycka et al. (2015a)
Duration of motility	2 min	Shaliutina et al. (2013)
	2 min.	Pšenička et al. (2008a)
Velocity ($\mu\text{m s}^{-1}$)	123.7 \pm 42.6	Li et al. (2011)
	165 \pm 14	Shaliutina et al. (2013)
	181.0 \pm 3	Pšenička et al. (2011)
VCL ($\mu\text{m s}^{-1}$)	121.9 \pm 17.3	Sieczynski et al. (2012)
	311.5 \pm 12.9	Judycka et al. (2015a)
VAP ($\mu\text{m s}^{-1}$)	97.6 \pm 14.0	Sieczynski et al. (2012)
	292.6 \pm 16.1	Judycka et al. (2015a)
VSL ($\mu\text{m s}^{-1}$)	82.5 \pm 14.3	Sieczynski et al. (2012)
	237.5 \pm 17.9	Judycka et al. (2015a)
LIN (%)	58.56 \pm 11.0	Sieczynski et al. (2012)
	72.9 \pm 4.8	Judycka et al. (2015a)
ALH (μm)	10.4 \pm 2.7	Sieczynski et al. (2012)
	2.3 \pm 0.2	Judycka et al. (2015a)

for motility analysis of Siberian sturgeon spermatozoa. Values of pH for optimal movement are 8.0 and higher; these values resemble pH of seminal plasma. Sperm motility parameters are shown in Table 15.4. Although spermatozoa remain quiescent under inhibitory action of K^+ ions, some motility in undiluted semen has been observed, possibly due to mixture with urine. Recently, Judycka et al. (2015a) established that spontaneous motility of undiluted semen may be related to season. These authors observed a 40% spontaneous sperm motility (estimated subjectively by one experimenter, as in Williot et al. 2000) in samples collected in December. In this context, CASA analysis (computer-assisted sperm analysis) showed that 1/3 of the sperm population obtained in December was motile upon activation in seminal plasma compared to only single spermatozoon in semen from April.

Parametric data for sperm motility obtained with CASA are now available for Siberian sturgeon. This system is capable of measuring several parameters related to speed and trajectory of movement. The most popular parameters for

characterizing spermatozoa are straight-line velocity (VSL), curvilinear velocity (VCL), average path velocity (VAP), linearity ($\text{LIN} = 100 \times \text{VSL}/\text{VCL}$), amplitude of lateral head displacement (ALH) and percentage of motile sperm. Values of sperm motility parameters clearly indicate, that the percentage of motility can reach 100%, which translates into elevated values of sperm velocity (up to $300 \mu\text{m s}^{-1}$) and high linearity of movement (LIN values 70% and higher). These values are lower for poor-quality semen as can be demonstrated by comparison of the data of Judycka et al. (2015a) with data of Sieczynski et al. (2012); they recorded 100% sperm motility after activation versus 41% of sperm motility, respectively.

15.6 The Acrosome Reaction

In general, the typical organelle of many vertebrate spermatozoon, the acrosome, is considered to be responsible for enabling the spermatozoon to traverse the investment coats surrounding an egg. Most spermatozoa undergo an acrosomal reaction in response to an egg component; this acrosomal reaction exposes the contents of the acrosome, which includes enzymes and binding proteins (Dan 1967; Shapiro and Eddy 1980; Lopo 1983). Tunicate, lampreys, hagfish (*Eptatretus burgeri* and *E. stouti*), and sturgeons are reported to possess sperm that form fertilization filaments during the acrosomal reaction (Cherr and Clark 1984; Dettlaff et al. 1993; Morisawa 1995, 1999a, 1999b; Pšenička et al. 2009). Lamprey eggs are covered by an impenetrable envelope with no micropyles (Kille 1960). Morisawa and Cherr (2002) described the acrosomal reaction in hagfish, where the spermatozoa must penetrate a U-shape layer that fills the bottom of the micropyle (Morisawa 1999b). However, sturgeon eggs possess an impenetrable envelope, which is perforated by numerous micropyles (3–16 in Siberian sturgeon, Debus et al. 2002). The latter provide spermatozoon direct access to the oolemma. The multiplicity of micropyles could be advantageous to facilitate egg location by spermatozoon in fast-flowing water; however, at the same time, it also increases the risk of polyspermy. Therefore, the presence of acrosomes and long acrosomal processes in sturgeon spermatozoa is inconclusive, and the contradictions to others do not agree with models of acrosomal evolution (Baccetti and Afzelius 1976; Baccetti 1979). The micropyles of sturgeon eggs are filled with spermatozoa within about 15 s postfertilization. The spermatozoon, which reached the bottom vicinity of the micropyle, merges with a cytoplasmic projection of the egg that is located in the micropyle. The membrane of the acrosome decomposes, starting from the apical part. During the acrosomal reaction, material in the three ECs of the sperm head is ejected forward through the acrosome like a fine harpoon-like acrosomal filament (Fig. 15.6a–c; Pšenička et al. 2010c). With reference to multiplicity of micropyles, it has been speculated that the filament serves as a quick signal transducer, conveying information to the egg about the presence of spermatozoon in close proximity, and induces a cytoplasmic projection from the egg for fusion with the spermatozoon and blocking polyspermy through the formation of a perivitelline space in all other micropyles (Pšenička et al. 2010c).

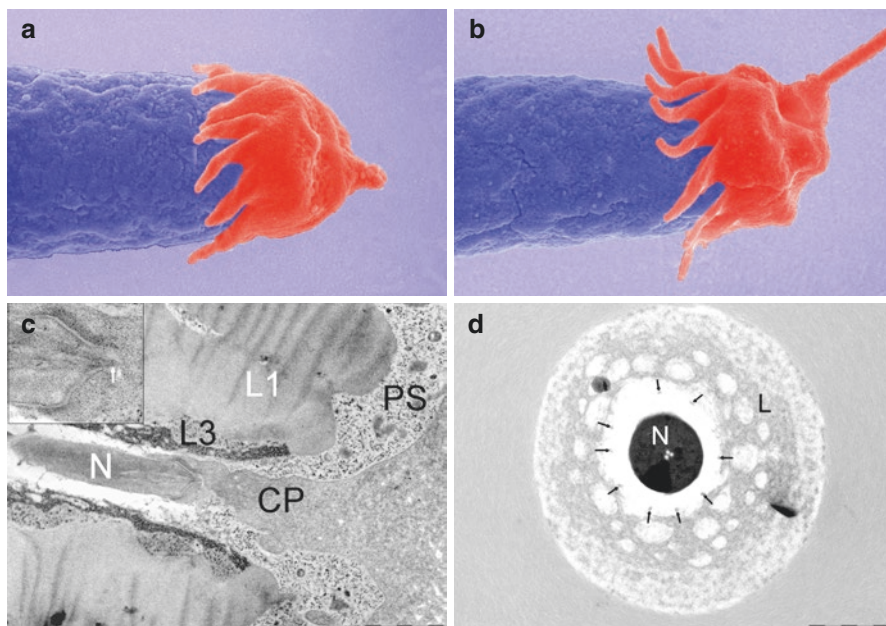


Fig. 15.6 Micrographs show acrosome before (a) and after (b) acrosomal process (scale bar, 100 nm); spermatozoon into the egg 60 s after fertilization showing the fusion of spermatozoon acrosome and nucleus (N) with egg cytoplasmic projection (CP) and the formation of perivitelline space (PS) under egg layers (L1 and L3). Inset: formation of fertilization filament (*arrow*) (c, scale bar, 2 μm); cross section at the level of ten extended posterolateral projections (*arrows*) of spermatozoon in egg micropyle with nucleus (N) and egg layer (L) (d, scale bar, 1 μm) (Pšenička et al. 2010c)

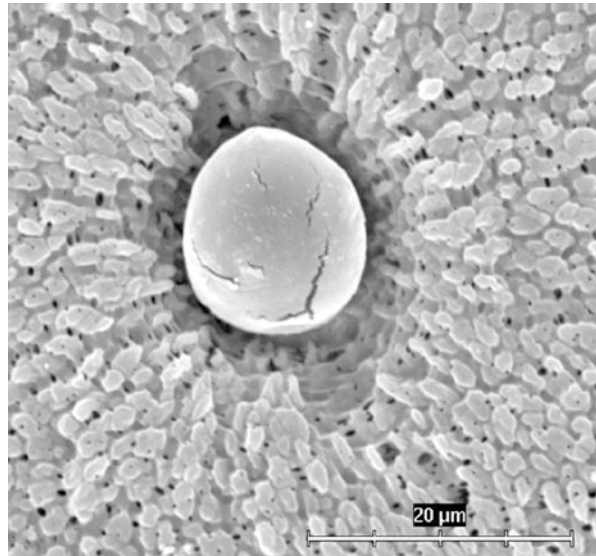
15.6.1 Function of Posterolateral Projections

The PPs of the acrosomes of sturgeon spermatozoa are radially distributed lobes. The diameter of the micropylar base in Siberian sturgeon eggs (1.95 μm) is only slightly larger than the diameter of the spermatozoon's head (1.13 μm). As a result, no more than one sperm can enter the micropyle. When a spermatozoon of Siberian sturgeon penetrates through the micropyle into the egg, the ten PPs open and function as an anchor; this is a unique feature of sturgeon spermatozoa. To confirm the opening of the PPs, the distance of PPs from the nucleus was measured in cross sections in the PP region of *in vitro* activated spermatozoa ($0.19 \pm 0.12 \mu\text{m}$) and nonactivated spermatozoa ($0.14 \pm 0.04 \mu\text{m}$). The difference was highly significant ($p < 0.01$) as well as the distance of PPs of spermatozoa in micropyles ($0.20 \pm 0.16 \mu\text{m}$) (Fig. 15.6a, b, d; Pšenička et al. 2010c).

15.6.2 Prevention of Polyspermy

Immediately after fusion, when the first spermatozoon penetrates the oocyte plasma membrane, the entire egg envelope separates from the oocyte plasma membrane by

Fig. 15.7 Scanning electron micrograph shows fertilization cone in the micropyle 180 s after fertilization. Scale bar, 20 μm



the circumferential spreading of a perivitelline space which is filled with the material from cortical granules; this agglutinates other spermatozoa. The perivitelline space expands around the oocyte from the site of sperm attachment to the vegetal part of the oocyte (Fig. 15.6c) (Cherr and Clark 1985; Dettlaff et al. 1993; Pšenička et al. 2010c).

Mature eggs of several fish species respond to sperm entry by the formation of fertilization cone at the sperm entry site (Kudo 1980; Kobayashi and Yamamoto 1981; Iwamatsu et al. 1991; Linhart and Kudo 1997). In Siberian sturgeon, approximately 60 s postfertilization, the fertilization cone extends to about 20–30 μm in width, with the ball-like enlarged apex of the cone which reaches the micropylar vestibule. Spermatozoa are never able to fuse with the cone membrane (Fig. 15.7, Pšenička et al. 2010c).

15.6.3 Monitoring and Importance of the Acrosomal Reaction

Pšenička et al. (2008b) tested a soybean trypsin inhibitor conjugated with Alexa Fluor® 488 for screening of acrosomal reaction in sturgeon spermatozoa. This method seems to be the most objective for acrosomal integrity evaluation (see chapter Siberian sturgeon sperm cryoconservation). There were differences in the integrity of acrosome in sperm within males. Moreover, while using this staining, suitable acrosome activation medium containing 2.5 mM Ca^{2+} and 15 mM tris with pH 10 was selected for sturgeon spermatozoa. Sperm was treated with the medium and used for fertilization. The hatching percentage rapidly decreased and in addition was negatively correlated within males with percentage of disrupted acrosomes; nevertheless, the ability of movement was sustained. These results strongly suggest a usefulness of acrosome for fertilization and development in sturgeon (Pšenička et al. 2008b).

Conclusions

The characteristics of Siberian sturgeon semen are considerably affected by dilution with urine in the Wolffian ducts. The concentrations of spermatozoa, proteins, ions, and, therefore, osmolality are lower than in teleostean species, and semen volume is higher. Moreover, the structure of spermatozoa as well as fertilization process is unique among fishes. Sturgeon spermatozoa possess an acrosome which contains typical acrosomal proteins, and while sperm penetration is facilitated by a number of egg micropyles which disagrees with evolutionary biology in fish gametes. It is suggested that the acrosome with the posterolateral projections has a more likely function of an anchor within micropyle instead of a mechanism for penetration.

Acknowledgments The study was financially supported by COST Office (Food and Agriculture COST Action FA1205: AQUAGAMETE); by the Ministry of Education, Youth and Sports of the Czech Republic, projects “CENAKVA” (No. CZ.1.05/2.1.00/01.0024) and “CENAKVA II” (No. LO1205 under the NPU I program); and by the Czech Science Foundation (No. P502/13/26952S), the National Science Centre granted for research project (No. 2011/01/D/NZ9/03738) and funds appropriated to Institute of Animal Reproduction and Food Research. Authors also express thanks to prof. MSc. William L. Shelton, PhD, for English corrections.

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