We are IntechOpen, the world's leading publisher of Open Access books
Built by scientists, for scientists

6,900
Open access books available

185,000
International authors and editors

200M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS

BOOK CITATION INDEX

INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Sperm Motility Regulatory Proteins: A Tool to Enhance Sperm Quality

Sandhya R. Dungdung, Arpita Bhoumik, Sudipta Saha, Prasanta Ghosh, Kaushik Das, Sandipan Mukherjee, Debjani Nath, Jitamanyu Chakrabarty, Chanakyanath Kundu, Bijay Shankar Jaiswal, Mahitosh Mandal, Arunima Maiti, Saswati Banerjee, Madhumita Roychowdhury, Debleena Ray, Debdas Bhattacharyya and Gopal C. Majumder

Additional information is available at the end of the chapter

Abstract

Sperm forward motility is an essential parameter in mammalian fertilization. Studies from our laboratory have identified and characterized a few unique sperm motility regulatory proteins/glycoproteins from the male reproductive fluids and mammalian blood serum. The purified sperm motility-initiating protein (MIP) from caprine epididymal plasma as well as the forward motility-stimulating factor (FMSF) and motility-stimulating protein (MSP) from buffalo and goat serum, respectively, have high efficacy to initiate or increase motility in nonmotile or less motile sperm. Antibody of sperm motility inhibitory factor (MIF-II) has the high potential to enhance sperm vertical velocity and forward motility by increasing intracellular cyclic adenosine monophosphate (cAMP) level. The appearance and disappearance of D-galactose–specific lectin and its receptor along the epididymis has been reported to be involved in motility regulation in spermatozoa. A novel synthetic cryopreservation method and role of lipid to protect membrane damage during cryopreservation have been demonstrated. Motility-promoting proteins may be extremely useful for improving cattle breeding and breeding of endangered species, thereby helping in enhanced production of animal products as well as in the conservation of animals. Isolated proteins and developed cryopreservation technology may also be beneficial in human infertility clinics to increase the chance of fertilization.

Keywords: Spermatozoa, Epididymis, Motility regulatory proteins, Cryopreservation, Reproduction

1. Introduction

Livestock is a very important subsector of Indian agricultural production system. The overall contribution of the livestock is almost 4.11%. India ranks first in milk production in the world (132.4 million tons), which is mostly contributed by cattle and buffalo. The bull concentrates in itself a high economic value and thus need to be maintained on proper nutrition and management to obtain optimum performance in terms of semen production [1]. The demand for the best males has increased considerably due to a shortage in the number of proven bulls having better semen characteristics for sustaining a successful breeding program [2].

Reproductive techniques facilitate the breeding of farm animals, which ultimately improve milk production and growth in dairy industry. Artificial insemination (AI) is a first-generation reproductive biotechnology that profoundly contributed to genetic improvement, particularly in dairy cattle. Such impact would not have been possible without successfully freezing bull semen. Quality control of frozen sperm is of utmost importance for the sperm to be used in AI [3]. Sperm cryopreservation allows prolonged preservation of semen and a wider use of a male gamete [4]. This technique is used for breeding of domestic animals, maintaining the genetic diversity, and establishing gene banks [5]. The success of fertilization with the use of frozen–thawed spermatozoa varies considerably between species and among individuals of the same species [6]. Semen cryopreservation relies on the use of cryoprotectants (such as glycerol and egg yolk), substances to maintain the osmolarity, sources of energy (such as glucose or fructose), and enzymes and antibiotics, which are essential for maintaining the viability of the spermatozoa during cooling, freezing, and thawing [7]. Still, the major disadvantage in the cryopreservation process is its harmfulness to spermatozoa; even using the best preservation techniques, only half of the sperm population survives after freezing and thawing procedures [8].

Mammalian sperm motility is a key factor to determine semen quality and fertilizing capability [9]. Motility of spermatozoa depends on various proteins in blood and male reproductive fluids [10, 11]. For the last few decades, studies in our laboratory focused on the identification and characterization of sperm motility regulatory protein (SMRP) molecules in caprine (*Capra indicus*) sperm [12]. Efforts have been made to raise antibodies against these purified proteins for further investigation of their immunological and functional role expecting to augment motility/fertility of male gametes. Our findings also showed the role of lipid to protect the spermatozoon membrane against damage during cryopreservation. A novel cryopreservation system has also been developed, which may help to improve the existing technology for preservation of spermatozoa across higher mammalian species such as bull and buffalo, as well as in endangered species.

A positive correlation between sperm concentration at semen collection and motility has been reported. In general, fertility rate of frozen thawed semen used for AI is poorer than that obtained with fresh semen, which is partially compensated by using insemination doses with greater numbers of live spermatozoa [13]. Several SMRPs have been isolated, which showed the ability to enhance the motility in cryopreserved semen samples. Based on the hypothesis that these motility enhancer proteins may not be species specific in mammals, it is suggested that these proteins could perform their biological functions in spermatozoa of various species. Therefore, they appear as an alternative to improve sperm fertility after cryopreservation, by incubating sperm with motility enhancing purified proteins.

Sperm motility is a major criterion in semen quality; it is also an important determinant for the success rate of fertilization. Sperm motility is related to the availability of Adenosine triphosphate (ATP): the frequency and amplitude of the tail movement of sperm is closely related to the dephosphorylation of ATP [14].

The epididymal lumen is a complex microenvironment in which synthetically inactive spermatozoa move from the proximal to the distal end and interact with proteins that are synthesized and secreted in a highly regionalized manner in the epididymis get gradually mature and acquire the capacity of progressive motility and fertility [15, 16]. In addition to the integration of epididymal secretory proteins, posttranslational modifications of existing sperm proteins are important for sperm maturation and acquisition of fertilizing potential [17].

2. Sperm Motility Regulatory Proteins (SMRPs)

The epididymis is essential for sperm development and maturation. Sperm taken from the caput show little motility and are infertile, whereas sperm from the cauda are motile and can achieve fertilization. How sperm motility is initiated in the epididymal duct is uncertain, but a sperm motility protein has been demonstrated in both the corpus and the cauda of the epididymis.

Besides having an influence through controlling the extracellular milieu of spermatozoa, proteins secreted by epididymal cells may be incorporated at the sperm surface to exert their regulation. Epididymal secretions suggested to functions as cholesterol carrying proteins that have been identified in the ram [18] and other farm animals [19].

Specific secretory proteins produced in the epididymis seem to remain associated with spermatozoa during the transit through the organ and may play a key role in the mammalian sperm maturation process by conferring to the male gamete the ability to recognize the oocyte [17–20].

2.1. Molecules with sperm motility regulatory properties

Several molecules showing motility regulatory effects on spermatozoa have been identified. Some of them were located in epididymis, but there are several reports also on the occurrence of various types of sperm motility regulatory protein factors in male reproductive fluids and blood serum as well as in follicular fluid [21, 22]. The sperm surface undergoes a sequence of alterations during the epididymal maturation and uterine capacitation process, allowing the male gametes to acquire the potential to fertilize the female egg [23, 24]. Available information on SMRPs has been reviewed below.

Motility of washed rabbit spermatozoa derived from fresh ejaculates was also greatly stabilized by blood serum albumin; rabbit serum albumin was more effective than bovine or human serum albumin [19], suggesting the existence of a species-specific effect.

Of the large number of proteins present in follicular fluid (FF), few have been discovered and most are yet to be nominated. A 52-kDa glycoprotein purified from porcine FF stimulates the

motility of boar spermatozoa [25]. We have partially purified a sperm motility enhancer from human FF, which significantly enhances the forward motility and vertical velocity of human and caprine spermatozoa.

Moreover, its importance in the acrosomal reaction and the interaction with specific receptors in oocyte surface at fertilization is also acknowledged [26]. Phosphorylation of proteins catalyzed by protein kinases is recognized as a major regulator of cell functions [27]. It has been demonstrated that it is a cAMP-independent protein kinase (ecto-CIK) and its substrate (MPS) on the external surface of goat epididymal spermatozoa that causes phosphorylation of endogenous membrane-bound phosphoproteins that are externally oriented [28–30]. The presence of lectins (e.g. galactose-specific and N-acetyl-D-galactosamine–specific lectins) on the surface of mature spermatozoa has been reported in few species [31, 32]. We have demonstrated for the first time the epididymal maturational profile of a sperm external surface lectin-like molecule.

2.1.1. Motility initiating protein and motility inhibitory factor

Hoskins and associates [18, 32], in a novel *in vitro* model, triggered forward motility in the immature (immotile) sperm derived from bovine caput epididymis by incubation in presence of epididymal or seminal plasma and theophylline. Subsequently, from caprine epididymal plasma, pursuing to identify the extracellular proteins and their role in biochemical regulation of sperm motility, Jaiswal et al. [33] and Das et al. [34] have purified and characterized a motility initiating protein (MIP) and a motility inhibitory factor (MIF), respectively.

Purification and characterization of the MIP from caprine epididymal plasma was achieved in our laboratory. MIP is a heat-stable, acidic, dimeric protein with a weight of close to 125 kDa, and presenting two subunits: 70 and 54 kDa, with an isoelectric point of 4.75 and maximal activity at pH 8 [33]. It contains mannose, galactose, and N-acetylglucosamine approximately in the ratios of 6:1:6. MIP loses activity by actions of alpha-mannosidase and beta-N-acetyl-glucosaminidase, thereby showing the sugar side chains responsible for the motility initiating potential. Epididymal plasma is the richest source of MIP. Intrasperm cAMP level was increased by MIP [33].

Immature caput spermatozoa do not show forward motility. Addition of MIP induced forward motility to a significant population of spermatozoa (Figure 1). The number of forward motile cells increased markedly with the increase in MIP concentration. The factor showed maximal activity at concentration as low as 30 μg/ml when it induces forward motility in nearly 22% of the immature spermatozoa.

We have also isolated and purified a different 57-kDa protein from caprine cauda epididymal plasma, which acts as a quiescence factor to immobilize cauda spermatozoa – the sperm motility quiescence factor (QF). This protein also decreases reactive oxygen species (ROS) concentration and thus helps to reduce oxidative stress in cauda spermatozoa, which is prone to damage by ROS due to the presence of high level of polyunsaturated fatty acids (PUFA) in sperm plasma membrane (unpublished data).
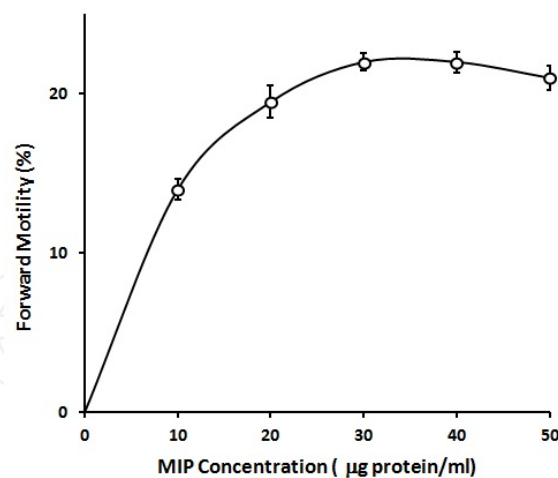
Figure 1. Dose course of MIP for initiation of forward motility in caput-sperm under the standard assay conditions. Reproduced with permission from Jaiswal et. al. (2010).

A rabbit polyclonal antibody raised against purified sperm motility inhibiting factor (MIF-II), at dilution 1:5000, allowed to increase sperm motility by 75% compared to the control within 30 min of incubation (Figure 2a). SPERMA, a sperm motility analyzer, showed a 40% increase in vertical velocity of MIF-II antibody-treated spermatozoa as compared to the control serum (Figure 2b). MIF-II antibody also enhanced the motility of immature caput spermatozoa under *in vitro* initiation media.
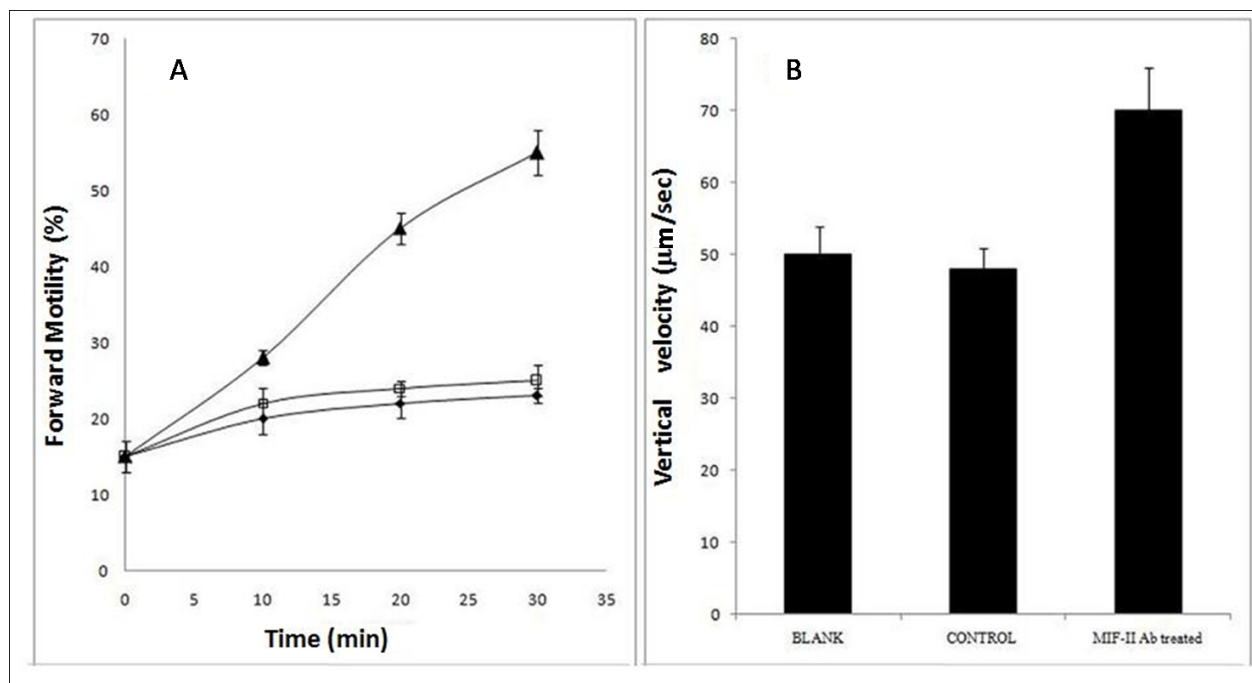

Figure 2. Effect of MIF-II antibody (1:5000) on goat cauda sperm forward motility. a) Microscopic analysis: Blank (untreated) (◆), Control with pre immune sera (□), MIF-II Ab (▲). b) Analysis by SPERMA. Reproduced with permission from Das et. al. (2010).

2.1.2. Forward motility-stimulating factor and motility-stimulating protein

A forward motility-stimulating factor (FMSF) purified to apparent homogeneity from buffalo blood serum, showed high protein specificity and affinity for stimulating forward motility of goat cauda epididymal spermatozoa [35]. A molecule exhibiting similar role was found in goat blood serum and some of its physical, biochemical, physiological, and immunological properties were characterized. This protein was named as sperm forward motility-stimulating protein (MSP) because it stimulated forward motility even in weakly motile spermatozoa [36].

Both FMSF and MSP are 66-kDa monomeric, heat-stable proteins. FMSF is acidic in nature with isoelectric point 3.7. Aspartate, glutamate, and leucine are the amino acids with higher representation in FMSF. FMSF is inhibited when treated with α-mannosidase, which acts on the sugar part of the protein.

In regard to its motility-promoting potential, FMSF is not species specific. Sperm surface has specific receptors of FMSF [37]. FMSF was also immunodetected in uterine fluids of cattle species. A maturation-dependent expression of FMSF receptor and consequential stimulation of forward motility were observed [38]. FMSF binds to the surface of the mature sperm cells to promote forward motility. FMSF initiates a novel signaling cascade to stimulate transmembrane adenylyl cyclase (tmAC) activity that augments intracellular cAMP, which through downstream cross talk of phosphokinases leads to enhanced forward motility in mature spermatozoa [36]. In *in vitro* fertilization, maximum activity of FMSF was observed at 0.5 μM level when nearly 60–70% of spermatozoa showed forward motility [37].

On the other hand, motility-promoting efficacy of MSP is markedly higher than theophylline or bicarbonate or their combination at 0.9 μM and also showed longer motility maintenance. MSP exhibits maximal activation in 1 min or less, whereas theophylline or bicarbonate takes about 3–5 min for maximal activation (Figure 3a). Addition of theophylline and bicarbonate does not activate further MSP-induced motility. Figure 3b shows the maintenance of motility for longer period. Theophylline and bicarbonate have been found to help in the maintenance of motility in a much stabilized manner when added along with MSP. These reagents helped in the storage of sperm cells in motile condition for at least 3 h at room temperature [36].

Studies with CASA or SPERMA evidenced a similar trend of increase in horizontal and vertical velocities, respectively. MSP action was found to be independent of cAMP, in which it differs from FMSF. MSP occurrence was higher in testis, although blood was its richest source.

MSP was localized throughout the cell surface of spermatozoa. *In vitro* fertilization studies were performed with antibody of MSP using mouse oocyte and spermatozoa. The control experiment did not show any inhibition of fertilization, but the MSP antibody treatment inhibited fertilization by 100% at 1:25 dilution [36].

2.1.3. Follicular Fluid Motility Enhancing Protein (FFMP)

FF has a pivotal effect on motility and chemotaxis of spermatozoa for a successful fertilization. FF has been shown to stimulate sperm motility [39, 40], capacitation [41] and acrosome reaction [42], as well as the sperm–oocyte fusion [43]. There are a large number of proteins present in this fluid that are associated with the regulation of sperm function; many of them are yet to be
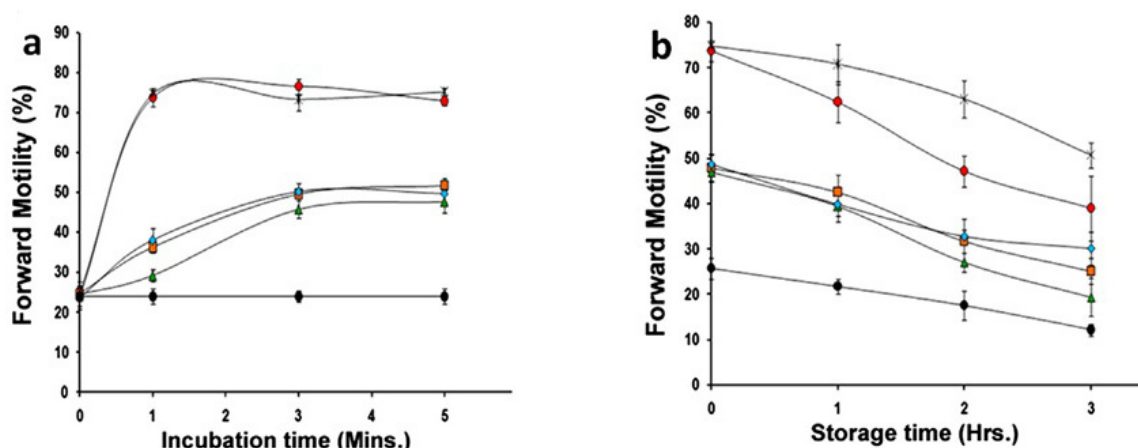
Figure 3. Effect of goat MSP, 0.9 M (-●-) (Red), theophylline 5 mM (-■-) (Orange), bicarbonate 20 mM (-▲-) (Green), theophylline + bicarbonate (-◆-) (Blue), and MSP + theophylline + bicarbonate (-X-) (Black) on sperm motility with respect to control (-●-) (Black). At different times up to 5 minutes MSP-induced motility was found to be more significant with respect to other initiators (panel a). At 30 seconds to the 1 minute time point the statistical significance level is P<.001. At storage up to 3 hours, even at the end of 2 hours, there are significant differences in motility in MSP versus theophylline (P<.05), MSP versus bicarbonate (P<.01), and MSP versus theophylline + bicarbonate (P<.05; panel b). Reproduced with permission from Saha et. al. (2013).

nominated or characterized. We have partially purified a sperm motility enhancer molecule from human FF. This protein stimulates sperm motility by more than 50% in a poor motile sample and also increases vertical velocity of human and caprine spermatozoa (unpublished data).

2.1.4. Lectin and lectin receptor

Maturing goat spermatozoa recovered at the distal corpus epididymis showed head-to-head autoagglutination when incubated *in vitro* in a modified Ringer's solution [44]. This is due to a lectin-like molecule located on the sperm surface that specifically interacts with its receptor of neighboring homologous cells. The D-galactose lectin and its receptor were partially purified from the plasma membrane of goat cauda and caput spermatozoa, respectively. The terminal stage of sperm maturation, that is, the induction of flagellar motility, is associated with a sharp disappearance or inactivation of the lectin receptor along with the appearance of the lectin [44], suggesting that the lectin-like molecule acquired by the mature sperm might induce sperm motility, whereas activation of its receptor suppresses the motility-mediating potential of the lectin.

2.2. Applied potential for the different SMRP

Sperm motility regulation is performed by the different SMRP by their respective mode of actions. The motility-promoting effect of all these proteins, including MIF antibody, could support its use to enhance motility in sperm samples with poor motility traits, which could be useful in infertility management. These are physiological activators of sperm motility and thus may be used in biomedical application in human infertility clinics, animal breeding centres, and animal conservation centres.

3. New synthetic model for sperm cryopreservation

Sperm cells are stored for long term by the cryopreservation method. Cattle breeding by AI technique requires ready supply of good quality sperm cells, which are only possible by cryopreservation. Sperm cells are susceptible to less viability and sublethal dysfunction by freezing and thawing events during cryopreservation. Cell deaths during cryopreservation are caused mainly due to membrane damage [45].

Cryopreservation of semen has long been foreseen as a tool to improve breeding of farm animals of economical importance, and it is recognized as a major asset for conservation of endangered species and for solving particular problems of human male infertility. During cryopreservation, a substantial portion of sperm cells undergo damage from thermal, mechanical, chemical, and osmotic stresses [46, 47].

We recently developed a simple sperm cryopreservation method for goat cauda epididymal sperm using a modified Ringer's solution [48, 49]. This cryopreservation model has been developed using a careful manipulation of cooling and freezing rates, with the help of a computer-controlled biofreezer. It is generally accepted that sperm cells were highly sensitive to cooling rates particularly during cooling from room temperature to 5°C and during freezing (5 to −20°C). In this model, the optimized protocol consisted of a drop of 0.25°C/min from ambient temperature to 5°C, a cooling rate of 5°C/min from 5 to −20°C, thereafter a decreased rate of 20°C/min to −100°C, and finally the plunging into liquid nitrogen (Table 1). The cryoprotective ability of several biomolecules, such as amino acids, carbohydrates, and polymers, was assessed. The best cryoprotection was offered by glycerol at the 0.87 M level. Glycerol, dimethyl sulfoxide (DMSO), and amino acids in combination allowed 52% of motility recovery in cryopreserved sperm samples (Figure 4). DMSO and amino acids when used in combination with glycerol increased the cryoprotectant ability of glycerol [50].

PROTOCOL	MOTILITY RECOVERY (%)	
	FORWARD	TOTAL
RT $\xrightarrow{5°C/m}$ 5°C $\xrightarrow{5°C/m}$ -20°C $\xrightarrow{20°C/m}$ -100°C	0	0
RT $\xrightarrow{1°C/m}$ 5°C $\xrightarrow{5°C/m}$ -20°C $\xrightarrow{20°C/m}$ -100°C	0	0
RT $\xrightarrow{0.5°C/m}$ 5°C $\xrightarrow{5°C/m}$ -20°C $\xrightarrow{20°C/m}$ -100°C	0	0
RT $\xrightarrow{0.5°C/m}$ 5°C $\xrightarrow{5°C/m}$ -20°C $\xrightarrow{20°C/m}$ -100°C	15 ± 1.2	23 ± 2
RT $\xrightarrow{0.2°C/m}$ 5°C $\xrightarrow{5°C/m}$ -20°C $\xrightarrow{20°C/m}$ -100°C	18 ± 1.5	35 ± 2.5
RT $\xrightarrow{0.2°C/m}$ 5°C $\xrightarrow{5°C/m}$ -20°C $\xrightarrow{20°C/m}$ -50°C	16 ± 2	34 ± 2
RT $\xrightarrow{0.25°C/m}$ 5°C $\xrightarrow{5°C/m}$ -20°C $\xrightarrow{20°C/m}$ -100°C	32 ± 3	35 ± 2

(Reproduced with permission from kundu et. al., 2000a)

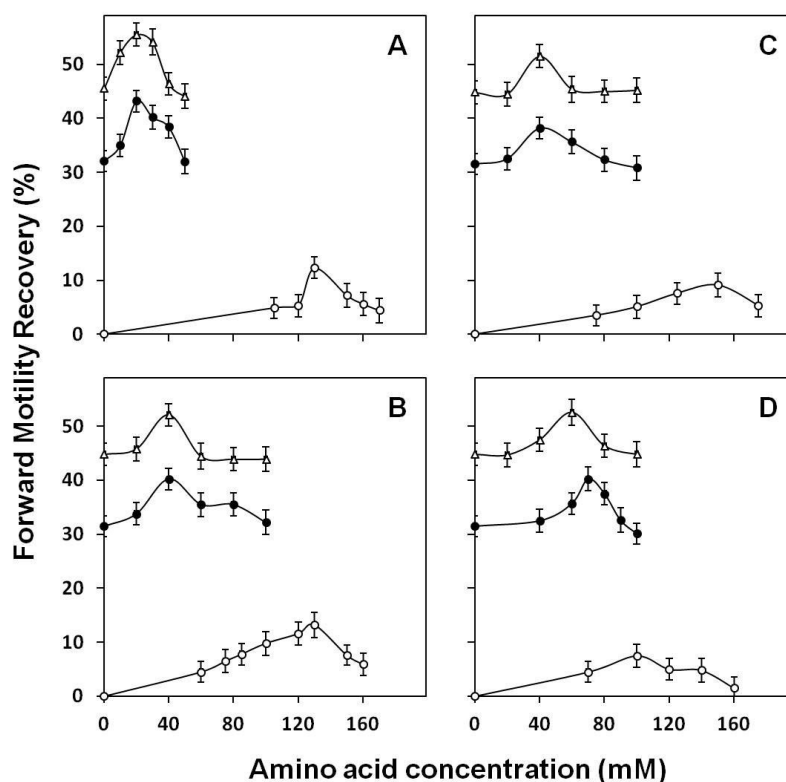**Table 1.** Effect of different cooling rate using 0.87 M glycerol as cryoprotectant

Figure 4. Effect of amino acids on forward motility recovery of spermatozoa after freezing and thawing of goat sper-
matozoa. (A, B, C and D). Results for L proline, L alanine, glycine and L glutamine, respectively. (○) Effect of amino
acids in absence of other cryoprotectants. (●) FM recovery (%) in the presence of glycerol. (Δ) FM recovery (%) in the
presence of glycerol + Me₂SO. The values shown are mean ± S.D. of five experiments. Reproduced with permission
from Kundu et. al. (2001).

To improve the motility of sperm cells at recovery (after thawing), the combined actions of
dextran, glycerol, and DMSO were tested. Addition of different concentrations of 10 kDa
dextran (2.0–6.27 mM) to a fixed concentration of glycerol (0.87 M) caused a concentration-
dependent increase of the recovery of motility (approximately 22–25%) of sperm cells. The
highest recovery of motility (forward motility percent 50 ± 3% and total motility percent 53 ±
3%, respectively) was found at 6.27 mM dextran. Addition of another dextran (40 kDa; at doses
between 0.5 and 2.0 mM) to the same concentration of glycerol (0.87 M) also caused a dose-
dependent increase of motility recovery [51]. We suggested that glycerol and other penetrating
compounds form H-bond with the polar phosphate head group of both sides (exoplasmic and
protoplasmic) of the cell membrane forming a protective cushion that protects membrane from
ice crystal-mediated damage [52]. The observed additive nature of cryoprotectant efficacies of
glycerol and dextran, a polymer of carbohydrate, suggests that these two molecules may exert
their cryoprotective effects through different mechanisms. We suggested that dextran cryo-
protects the cells by inhibiting ice crystal lattice formation [51].

Because of the high cryoprotectant efficacy, these cryoprotectants may have potential for the
cryopreservation of semen of various species, which may finally have a great potential in
animal reproduction.

4. Role of lipid to protect membrane damage during cryopreservation

Membrane damage is one of the main reasons for reduced motility and fertility of sperm cells during cryopreservation. Lipid is an important constituent of cell membrane. Dysfunction or malfunction of lipid component may cause lethal damage to different types of cells including spermatozoa. The lipid composition of sperm membrane differs from species to species. Lipid content in the plasma membrane of goat cauda epididymal spermatozoa changes significantly during maturation [53]. Cryopreservation is not a natural phenomenon and sperm cells are not "programmed" for it. So, when sperm cells are subjected to cryopreservation (including both freezing and thawing), they suffer from stress. As a result, a huge population of sperm undergoes irreversible damage during the process. Membrane impairment has been identified as one of the manifestations of such damage.

To study the changes in sperm plasma membrane lipid composition associated to the cryo-preservation process, a model system was developed using a synthetic medium, which was devoid of any lipid component [48]. It showed that the total lipid and its components of goat cauda epididymal sperm plasma membrane changed significantly after cryopreservation [54]. The composition in phospholipid (PL) and neutral lipid (NL) was severely altered. The unsaturated fatty acids decreased, whereas the saturated ones augmented; the cholesterol: phospholipid ratio was also increased causing profound enhancement of hydrophobicity of the sperm plasma membrane. Reports from our laboratory also showed that sperm cell membrane selectively sheds off the hydrophilic lipid molecules to remain viable against cryodamage (Table 2) [58]. Therefore, increasing the membrane hydrophobicity of the spermatozoon may enhance its resistance to cryodamage during freezing.

The role of fatty acids in cryopreservation of sperm cells of different species has been discussed elsewhere [55]. Egg yolk is used for a long time as a cryoprotectant [56] although in recent studies it was demonstrated the existence of detrimental effects of egg yolk extender over sperm cells [57, 58]. To circumvent these problems, phospholipids isolated from egg yolk [58] or from other commercial sources [59] were tried instead of the whole egg yolk. Soybean lecithin (phosphatidyl choline) has emerged as a popular choice for replacing egg yolk as cryoprotectant for goat [61, 52].

In a recent study, we have found that a lecithin from marine fish was also effective for cryopreservation of the goat spermatozoa (unpublished data). The loss of plasma membrane lipids was prevented to a great extent by adding this lecithin, prior to cryopreservation, to the sperm suspension in the synthetic media described above. While loss of total, neutral and phospholipids after cryopreservation was 29.5, 32.3, and 33.3%, respectively, addition of marine-origin lecithin reduced it to 7.9 and 19.6% for total lipid and phospholipid, whereas change in neutral lipid was insignificant. According to our observations, the cholesterol did not cryoprotect goat spermatozoa neither did the cholesterol: phospholipid ratio altered appreciably. However, it is possible that integration of exogenous PC into the cell membrane had enhanced its actual amount, suggesting that the integration of exogenous PC might trigger a remodeling of membrane structure to maintain homeostatic condition.

	Before Cryopreservation	After Cryopreservation	Sig. (2- tailed)
Total Lipid[a] (TL) (mg lipid /100 mg protein)	147.92 ± 1.83	115.31 ± 0.7	0.001
% w/w of total lipid			
Neutral Lipid (NL)	50.35 ± 0.57	53.82 ± 0.10	0.008
Glycolipid (GL)	13.28 ± 0.08	15.05 ± 0.16	0.005
Phospholipid (PL)	36.37 ± 0.42	31.13 ± 0.16	0.001
% w/w of total neutral lipid			
Hydrocarbons (HC)	27.94 ± 0.07	41.93 ± 0.45	<0.001
Sterols (ST)	44.55 ± 0.06	41.52 ± 0.16	<0.001
Steryl Esters (SE)	13.61 ± 0.05	6.06 ± 0.21	0.001
Wax Esters (WE)	2.89 ± 0.09	1.92 ± 0.06	<0.001
1-O-alkyl 2,3-diacyl glycerol (ADAG)	6.98 ± 0.08	4.54 ± 0.11	0.005
Triacyl glycerol (TG)	4.03 ± 0.06	4.03 ± 0.04	0.383
% w/w of total phospholipid			
Phosphatidyl choline (PC)	38.91 ± 0.10	36.85 ± 0.20	0.018
Phosphatidyl ethanolamine (PE)	26.95 ± 0.09	24.71 ± 0.03	0.021
Phosphatidyl inositol (PI)	15.81 ± 0.13	16.88 ± 0.05	0.014
Sphingomyelin (Sph)	18.33 ± 0.09	21.56 ± 0.05	0.004

(Reproduced with permission from Chakrabarty et. al., 2007)

Table 2. Lipid composition of sperm plasma membrane

5. Conclusion

Animal products have an important role in the global economy and any improvement in animal breeding will lead to production of more and better-quality animal products such as milk, butter, meat, wool, leather, etc. Motility promoting proteins such as MSP, FMSF, MIP, and FFMP have a direct role in motility enhancement of weak sperm cells. MIF antibody also enhances motility by neutralizing the activity of MIF, which is a potent motility inhibitor. Membrane proteins such as ecto-CIK and its substrate MPS regulate the sperm motility as well as increase the acrosomal reaction, thereby helping in fertilization. All these proteins have the potential for improving the breeding of animals in animal husbandry or poultry for better yield or for conservation purpose of almost extinct species in the world.

Motility regulatory proteins may be useful in human infertility clinics to solve some of the problems of human infertility. The purified motility regulatory proteins from our laboratory are expected to have potentially important applications because it will improve the quality of mammalian semen by activating sperm motility essential for fertility of the male gametes.

Cryopreservation has been widely used in the modern cattle industry for animal production and AI has been the most widely applied tool in facilitating the extensive utilization of frozen semen. The sperm cells of different species have different inherent resistance toward cryopre-

servation like high sensitivity to cooling rates particularly before and during freezing. The synthetic model developed by our laboratory has a great promise for better understanding the mechanism of cryoprotection. The development of an optimum cryoprotectant formulation of the combined action of glycerol, DMSO, Ficoll, and amino acids provided the high motility recovery.

Acknowledgements

Author details

Sandhya R. Dungdung[1*], Arpita Bhoumik[1], Sudipta Saha[1], Prasanta Ghosh[1], Kaushik Das[1], Sandipan Mukherjee[1], Debjani Nath[2], Jitamanyu Chakrabarty[3], Chanakyanath Kundu[4], Bijay Shankar Jaiswal[5], Mahitosh Mandal[6], Arunima Maiti[1], Saswati Banerjee[1], Madhumita Roychowdhury[7], Debleena Ray[7], Debdas Bhattacharyya[8] and Gopal C. Majumder[8]

*Address all correspondence to: srdungdung@iicb.res.in; sandhya2308@hotmail.com

1 Cell biology and Physiology Division, CSIR-Indian Institute of Chemical Biology, Kolkata, India

2 Department of Zoology, University of Kalyani, Kalyani, West Bengal, India

3 National Institute of Technology, Durgapur, West Bengal, India

4 School of Biotechnology, KIIT University, Odissa, India

5 Department of Molecular Biology, Genentech Inc., CA, USA

6 Indian Institute of Technology, Kharagpur, India

7 A. H. IVF & Infertility Research Centre, Kolkata, India

8 Centre for Rural and Cryogenic Technologies, Jadavpur University, Kolkata, India

References

[1] Naha BC, Chakravarty AK, Mir MA, Jamuna V, Singh AP, Maher D. Identifying factors affecting age at first semen freezing and age at first semen use in Sahiwal bulls. Veterinary World. 2015; 87: 928–931.

[2] Rodríguez-Martínez H. Semen evaluation techniques and their relationship with fertility. Anim Reprod. 2013; 10: 148–159.

[3] Kumaresan A. Advancements in quality assessment of frozen semen and correlations between lab tests and field fertility data. In: Proceedings of International workshop on Bovine Semen production and processing – Compendium on 16-17 Oct, 2014, at National Dairy Development Board, Anand, Gujarat, India.

[4] Haugan T, Grohn YT, Kommisrud E, Ropstad E, Reksen O. Effects of sperm concentration at semen collection and storage period of frozen semen on dairy cow conception. Anim Reprod Sci. 2007; 97: 1–11.

[5] Shivaji S, Kholkute SD, Verma SK, Gaur A, Umapathy G, Singh A, Sontakke S, et al. Conservation of wild animals by assisted reproduction and molecular marker technology. Indian J Exp Biol. 2003; 41(7): 710–723.

[6] Thurston LM, Siggins K, Mileham AJ, Watson PF, Holt WV. Identification of amplified restriction fragment length polymorphism markers linked to genes controlling boar sperm viability following cryopreservation. Biol Reprod. 2002; 66: 545–554.

[7] Vishwanath R, Shannon P. Storage of bovine semen in liquid and frozen state, Anim Reprod Sci. 2000; 62(1–3): 23–53.

[8] Mathevon M, Buhr MM, Dekkers JCM. Environmental, management and genetic factors affecting semen production in Holstein bulls. J Dairy Sci. 1998; 81: 3321–3330.

[9] Petrunkina AM, Waberski D, Günzel-Apel AR, Töpfer-Petersen E. Determinants of sperm quality and fertility in domestic species. Reproduction. 2007; 134: 3–17.

[10] de Souza FF, Barreto CS, Lopes MD. Characteristics of seminal plasma proteins and their correlation with canine semen analysis. Theriogenology. 2007; 68: 100–106.

[11] Tso WW, Lee WM. Seminal plasma and progressive motility of boar spermatozoa. Int J Androl. 2008; 3: 243–250.

[12] Majumder GC, Das K, Saha S, Nath D, Maiti A, Das S, Dey N, et al. Purification and characterization of novel sperm motility-related proteins. In: Benitez M, Aguiree V, editors. Protein Purification. Nova Science Publishers: USA. 2012. p. 1–90.

[13] Haugan T, Grohn YT, Kommisrud E, Ropstad E, Reksen O. Effects of sperm concentration at semen collection and storage period of frozen semen on dairy cow conception. Anim Reprod Sci. 2007; 97: 1–11.

[14] Gibbons BH, Gibbons IR. Flagellar movement and adenosine triphosphatase activity in sea urchin sperm extracted with Triton X-100. From the Pacific Biomedical Research Center, University of Hawaii, Honolulu, Hawaii 96822.

[15] Orgebin-Crist MC, Fournier-Delpech S. Sperm-egg interaction. Evidence for maturational changes during epididymal transit. J Androl. 1982; 3: 429–433.

[16] Cuasnicú PS, González Echeverría F, Piazza A, Piñeiro L, Blaquier JA. Epididymal proteins mimic the androgenic effect on zona pellucida recognition by immature hamster spermatozoa. J Reprod Fertil. 1984b; 71(2): 427–431.

[17] González-Echeverría F, Cuasnicú PS, Piazza A, Pineiro L, Blaquier JA. Addition of an androgen-free epididymal protein extract increases the ability of immature hamster spermatozoa to fertilize in vivo and in vitro. J Reprod Fertil. 1984; 71: 433–437.

[18] Hoskins DD, Brandt H, Acott TS. Initiation of sperm motility in the mammalian epididymis. Fed Proc. 1978; 37: 2534–2542.

[19] Muller B, Kirchner C. Influence of seminal plasma proteins on motility of rabbit spermatozoa. J Reprod Fertil. 1978; 54(1): 167–172.

[20] Rochwerger L, Cuasnicu P, Conesa D. Participation of a rat sperm epididymal glycoprotein in the acquisition of sperm-egg fusion ability during maturation. In: Alexander NJ, Griffin D, Spieler JM, Waites GMH, editors. Gamete interaction. Prospects for immunocontraception. John Wiley Liss & Sons, Inc: New York. 1990. p. 630–631.

[21] Mandal M, Banerjee S, Majumder GC. Stimulation of forward motility of goat cauda epididymal spermatozoa by a serum glycoprotein factor. Biol Reprod 1989; 41:983-9.

[22] Majumder GC, Mandal M, Banerjee S. A novel sperm motility-promoting protein from blood serum and its applied potential. In: Ghosal SK, Ray D, editors. Recent Advances in Animal Science Research. Orion Press International: Chandernagore, West Bengal. 2001. p. 61–67.

[23] Hammerstedt RH, Parks JE. Changes in sperm surfaces associated with epididymal transit. J Reprod Fertil Suppl. 1987; 34: 133–149.

[24] Majumder GC, Dey CS, Haldar S, Barua M. Biochemical parameters of initiation and regulation of sperm motility. Arch Androl. 1990; 24(3): 287–303.

[25] Lee SL, Kuo YM, Kao CC, Hong CY, Wei YH. Purification of a sperm motility stimulator from porcine follicular fluid. Comp Biochem Physiol B. 1992; 101(4): 591–594.

[26] Walsh DA, Cooper RH. The physiological regulation and function of cAMP dependent protein kinase. In: Litwack G, editor. Biochemical Actions of Hormones. Academic Press: New York. 1979; vol. 6: p. 2–75.

[27] Halder S, Majumder GC. Phosphorylation of external cell surface proteins by an endogenous ecto-protein kinase of goat epididymal intact spermatozoa. Biochem Biophys Acta. 1986; 887: 291–303.

[28] Halder S, Dey CS, Majumder GC. An ecto-cyclic AMP-independent protein kinase in goat spermatozoa and its change of activity during forward motility. Biochem Int. 1986; 13(5): 809–817.

[29] Mitra S, Nath D, Majumder GC. Purification and characterization of a protein kinase from goat sperm plasma membrane. Biochem Cell Biol. 1994; 72(5–6): 218–226.

[30] Goluboff ET, Mertz JR, Tres LL, Kierszenbaum AL. Galactosyl receptor in human testis and sperm is antigenically related to the minor C-type (Ca2+-dependent) lectin variant of human and rat liver. Mol Reprod Dev. 1995; 40: 460–466.

[31] Yuuji S, Harumi S, Takashi S. Purification of a sperm lectin extracted from spermatozoa of the sea urchin *Hemicentrotus pulcherrimus*. Dev Growth Differ. 1992; 34: 285–291.

[32] Acott TS, Hoskins DD. Bovine sperm forward motility protein: partial purification and characterization. J Biol chem. 1978; 253: 6744–6750.

[33] Jaiswal BS, Das K, Saha S, Dungdung SR, Majumder GC. Purification and characterization of a motility initiating protein from caprine epididymal plasma. J Cell Physiol. 2010; 222: 254–263.

[34] Das S, Saha S, Majumder GC, Dungdung SR. Purification and characterization of a sperm motility inhibiting factor from caprine epididymal plasma. PLoS One. 2010; 5: 1–12.

[35] Mandal M, Saha S, Ghosh AK, Majumder GC. Identification and characterization of a sperm motility promoting glycoprotein from buffalo blood serum. J Cell Physiol. 2006; 209: 353–362.

[36] Saha S, Das S, Bhoumik A, Ghosh P, Majumder GC, Dungdung SR. Identification of a novel sperm motility-stimulating protein from caprine serum: its characterization and functional significance. Fertil Steril. 2013; 100: 269–279.

[37] Dey S, Roy D, Majumder GC, Bhattacharyya D. Receptor expression is essential for forward motility in the course of sperm cell maturation. Int J Biochem Cell Biol. 2014a; 92: 43–52.

[38] Dey S, Roy D, Majumder GC, Bhattacharyya D. Extracellular regulation of sperm transmembrane adenylyl cyclase by a forward motility stimulating protein. PLOS One 2014b; 9(10): e110669.

[39] Chao HT, Ng HT, Kao SH, Wei YH, Hong CY. Human follicular fluid stimulates the motility of washed human sperm. Andrologia. 1991; 26: 61–65.

[40] Jeon BG, Moon JS, Kim KC, Lee HJ, Choe SY, Rho GJ. Follicular fluid enhances sperm attraction and its motility in human. J Assist Reprod Genet. 2001; 18: 407–412.

[41] Ravnik SE, Zarutskie PW, Muller CH. Lipid transfer activity in human follicular fluid lipid transfer protein that stimulates human sperm capacitation. J Androl. 1990; 11: 216–226.

[42] Suarez SS, Wolf DP, Meizel S. Induction of the acrosome reaction in human spermatozoa by a fraction of human follicular fluid. Gamete Res. 1986; 14: 107–121.

[43] Lambert H, Steinleitner A, Eiserman J, Serpa N, Cantor B. Enhanced gamete interaction in the sperm penetration assay after co-incubation with pentoxifylline and human follicular fluid. Fertil Steril. 1992; 58: 1205–1208.

[44] Banerjee S, Dungdung SR, Das K, Majumder GC. Synchronous modulation of cell-surface lectin and its receptor in a homologous cell population: a novel mechanism of cellular regulation. Exp Cell Res. 2006; 312: 2299–2308.

[45] Radojcic L, Vukotic-Maletic V, Balint B. Current knowledge on cryopreservation of spermatozoa, ovum cells and zygotes. Med Pregl. 1998; 51: 29–36.

[46] Watson PF. Recent developments and concepts in the cryopreser-vation of spermatozoa: the assessment of their post-thawing function. Reprod Fertil Dev. 1995; 7: 871–891.

[47] Kundu CN, Chakrabarty J, Dutta P, Bhattacharyya D, Ghosh A, Majumder GC. Development of a simple sperm cryopreservation model using a chemically defined medium and goat cauda epididymal spermatozoa. Cryobiology. 2000a; 40: 117–125.

[48] Kundu CN, Chakrabarty J, Dutta P, Bhattacharyya D, Ghosh A, Majumder GC. A novel epididymal sperm model for analyzing the cryoprotecting potential of various reagents. In: Proceedings of the 18th International Cryogenic Engineering Conference. Mumbai, India. 2000b; p. 695–699.

[49] Kundu CN, Das K, Majumder GC. Effect of amino acids on goat cauda-epididymal sperm cryopreservation using a chemically defined model system. Cryobiology. 2001; 1: 21–27.

[50] Kundu CN, Chakrabarty J, Dutta P, Bhattacharyya D, Ghosh A, Majumder GC. Effect of dextrans on cryopreservation of goat cauda epididymal spermatozoa using a chemically defined medium. Reproduction. 2002; 123: 907–913.

[51] Rana APS, Misra S, Majcunder GC, Ghosh A. Phospholipid asymmetry of goat sperm plasma membrane during epididymal maturation. Biochem Biophys Acta. 1993; 1210: 1–7.

[52] Kmenta I, Strohmayer C, Muller-Schlosser F, Schafer-Somi S. Effects of a lecithin and catalase containing semen extender and a second dilution with different enhancing

buffers on the quality of cold-stored canine spermatozoa. Theriogenology. 2011; 75: 1095–1103.

[53] Chakrabarty J, Banerjee D, Pal D, De J, Ghosh A, Majumder GC. Shedding off specific lipid constituents from sperm cell membrane during cryopreservation. Cryobiology. 2007; 54: 27–35.

[54] Mandal R, Badyakar D, Chakrabarty J. Role of membrane lipid fatty acids in sperm cryopreservation. Adv Androl. 2014; Article ID 190542, 9 pages.

[55] Phillips PH, Lardy HA. A yolk-buffer pabulum for the preservation of bull semen. J Dairy Sci. 1940; 23: 399–404.

[56] Benchmark D, Amirat L, Pascal O, Anton M, Schmitt E, Desherces S, Delhomme G, et al. The advantages of LDL (low density lipoproteins) in the cryopreservation of canine semen. Theriogenology. 2008; 70: 1478–1488.

[57] Forouzanfar MM, Sharafi SM, Hosseini S, Ostadhosseini M, Hajian L, Hosseini P, Abedi N, et al. In vitro comparison of egg yolk-based and soybean lecithin-based extenders for cryopreservation of ram semen. Theriogenology. 2010; 73: 480–487.

[58] Pillet E, Labbe C, Batellier F, Duchamp G, Beaumal V, Anton M, Desherces S, et al. Liposomes as an alternative to egg yolk in stallion freezing extender. Theriogenology. 2012; 77(2): 268–279.

[59] Röpke T, Oldenhof H, Leiding C, Sieme H, Bollwein H, Wolkers WF. Liposomes for cryopreservation of bovine sperm. Theriogenology. 2011; 76(8): 1465–1472.

[60] Vidal AH, Batista AM, da Silva ECB, Gomes WA, Pelinca MA, Silva SV, Guerra MMP. Soybean lecithin-based extender as an alternative for goat sperm cryopreservation. Small Ruminant Res. 2013; 109: 47–51.

[61] Salmani H, Towhidi A, Zhandi M, Bahreini M, Sharafi M. In vitro assessment of soybean lecithin and egg yolk based diluents for cryopreservation of goat semen. Cryobiology. 2014; 68(2): 276–280.