

# Phospholipids and Their Hydrolysing Enzymes in Bull Semen as a Factor Concerned With Sperm Motility

By JUNJI MASAKI

Animal Reproduction Division,  
National Institute of Animal Industry

Studies on the bull semen have been developed in recent years in relation to its extensive use in the field of artificial insemination (A.I.). The semen preparation available for the A.I. must contain a number of motile spermatozoa to bring about good results in fertility.

As a factor concerned with sperm motility, this article summarizes main results of our studies on the phospholipids and their hydrolysing enzymes in bull semen and their physiological roles.

Phospholipid is known as a major constituent of plasma membrane. In addition, it can act as a metabolic reserve to maintain sperm motility under the environment lacking extra-cellular substrate such as fructose in seminal plasma. Accordingly, damage of phospholipid portion in spermatozoa leads to loss of their motility.

## Distribution and classes of phospholipids in bull semen

Phospholipids in mammalian semen distribute mainly to the spermatozoa. Seminal plasma contains remainder either secreted from accessory genital organs or leaked from spermatozoa. Our results<sup>7)</sup> show that fresh bull semen of  $10 \times 10^8$ /ml in sperm concentration contains about 8 mg phospholipid-P/100 ml, of which 75% are detected in spermatozoa. In the disintegrated sperm sample of a Guernsey bull, distribution of phospholipids in the heads and midpiece-tails is approximately 3:7<sup>8)</sup>.

Phospholipid composition of bull spermatozoa does not much differ from that of seminal plasma. Our results<sup>10)</sup> of thin-layer chromatography show that it consists of 36% choline plasmalogen (phosphatidyl choline), 24% lecithin (phosphatidyl choline), 13% sphingomyelin and several minor phospholipids, including phosphatidyl ethanolamine and ethanolamine plasmalogen (phosphatidyl ethanolamine). Choline plasmalogen seems to be major phospholipid in spermatozoa of ruminants.

## Changes in content and composition of phospholipids of bull spermatozoa during maturation and ageing

It has been reported that phospholipid composition of ram spermatozoa changes during the passage through the male genital tract<sup>10)</sup>. Marked decrease occurs in the lecithin content, particularly. Our results<sup>10)</sup> show that there is definite species difference in the change of the phospholipid composition during sperm maturation (Fig. 1). In this respect, it is probable that bull and ram spermatozoa differ from boar spermatozoa.

After ejaculations, spermatozoa loses their motility with ageing in a relatively short time unless some proper treatment for preservation is provided them. As an example, cold-shocking or freeze-thawing of fresh bull semen results in a lethal effect on the spermatozoa. In such a case, marked loss of sperm

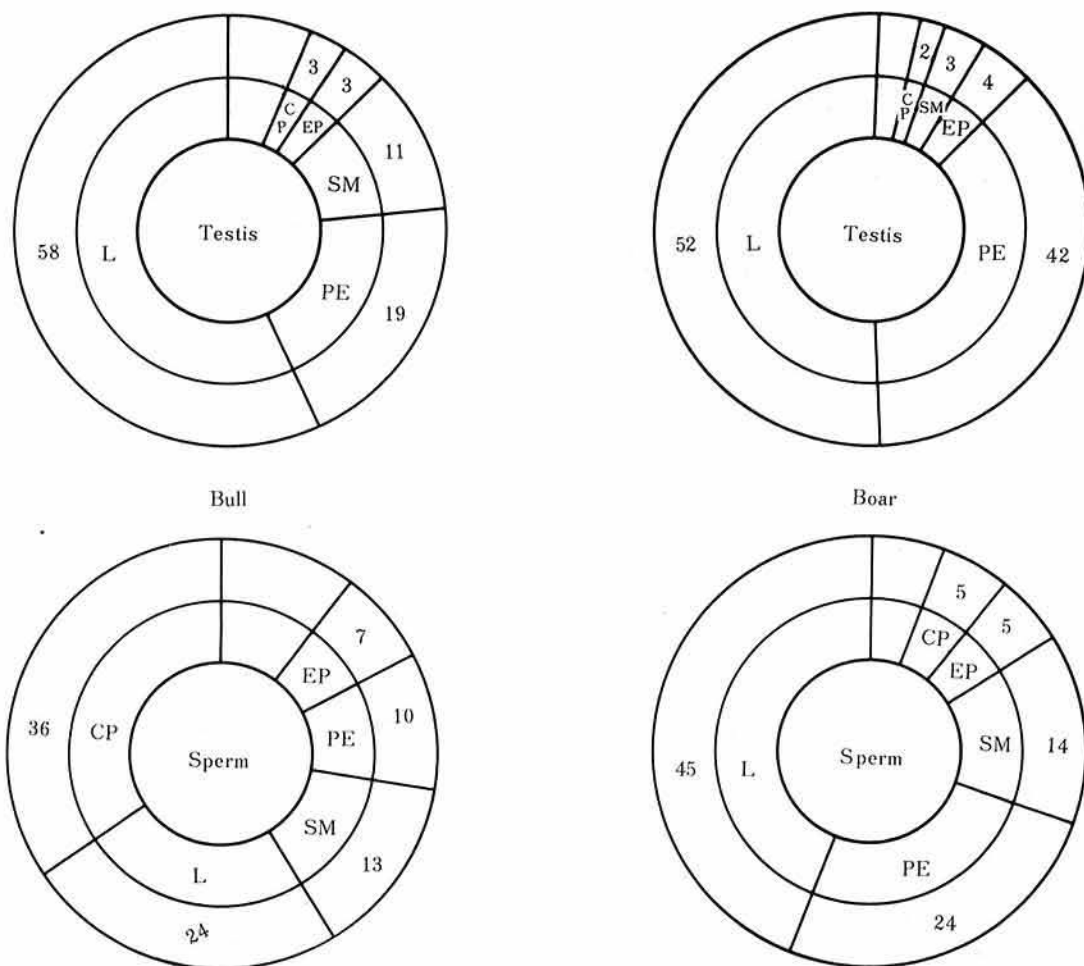


Fig. 1. Phospholipid compositions of testicular tissue and ejaculated spermatozoa  
L, Lecithin; PE, Phosphatidyl ethanolamine; SM, Sphingomyelin;  
EP, Ethanolamine plasmalogen; CP, Choline plasmalogen

phospholipids occurs, followed by corresponding increase in the lipid content of seminal plasma<sup>8),12)</sup>.

### Metabolic activity and phospholipid content of bull spermatozoa

Seasonal variation is often seen in the semen characteristics of bull particularly imported breeds although domestic cattle is not a seasonal breeder. In some parts of Japan, climatic condition of summer causes lower quality of bull semen. Sometimes, the detrimental effect

is observed in the other season, too.

In these periods, the semen presents a figure of falls in sperm motility and metabolic activity, decrease in sperm phospholipids and occasionally increase in glyceryl-phosphorylcholine (GPC) in seminal plasma.

There is a close relationship between seasonal variations in plasmalogen content and metabolic activity of bull spermatozoa (Fig. 2), although individual phospholipid available for energy reserve has not fully been clarified in bull spermatozoa as choline plasmalogen is in ram spermatozoa<sup>9)</sup>.

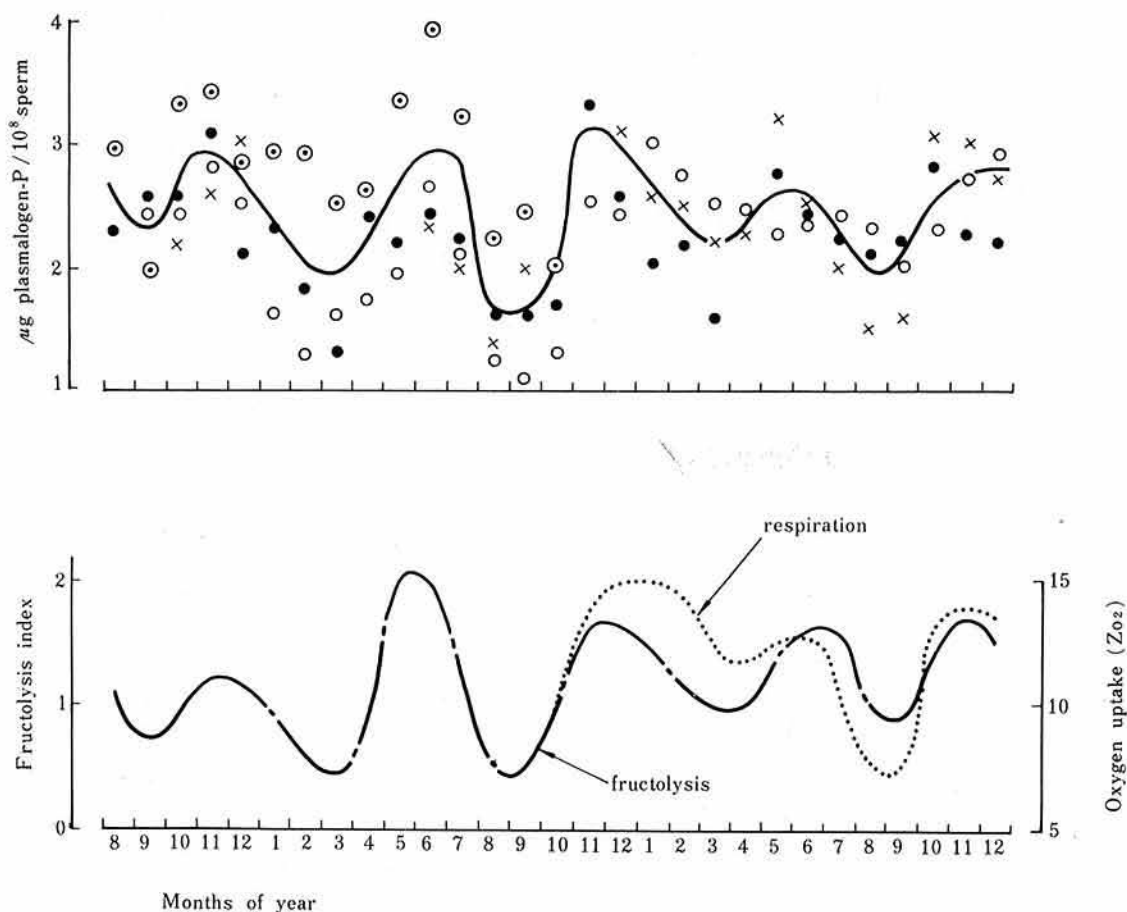


Fig. 2. Seasonal variations in phospholipid content and metabolic activity of bull spermatozoa

### Phospholipase activity of bull seminal plasma

Phospholipid hydrolysing enzymes have been detected widely in biological materials, i.e. phospholipase A in venom of snakes, phospholipase B in pancreas, phospholipase C in bacterial kingdom and phospholipase D in higher plants<sup>1)</sup>.

However, there is few information on the enzymes in spermatozoa although phospholipase A must play a role for hydrolysing the intra-cellular substrate to free fatty acid.

In contrast, we have observed that bull seminal plasma has high activity of phospholipase A and B as the following results show. Also, we have confirmed that the enzyme

activity in seminal plasma is originated mainly to vesicular secretion.

#### 1) Hemolytic activity

It is well known that bull seminal plasma causes hemolysis of erythrocytes when incubated together. Seminal plasma of other species of farm animals has less or no activity in the same experimental condition.

#### 2) Sperm-lipid removing activity

As already described, freeze-thawing of fresh semen results in lipid loss of spermatozoa. The decreasing rate in phospholipid content of spermatozoa of farm animals is higher in the order of cattle (usually more than 40%), goat, stallion, sheep and boar (less than 5%). From the following results

**Table 1. Lipid loss of epididymal spermatozoa by freeze-thawing in the presence of seminal plasma**

Epididymal spermatozoa	Sperm count ( $\times 10^6/\text{ml}$ )	Ratio in vol. of sperm suspension to seminal plasma or Ringer sol.	Lipid loss of spermatozoa (%)		
			Addition of bull seminal plasma	Addition of boar seminal plasma	Addition of Ringer sol.
Bull (b)	7.8	1 : 1	44	4	17
Stallion (b)	11.0	1 : 1	45	0	10
Boar (a)	39.6	1 : 2.5	51	0	8
Goat (b)	13.4	1 : 2.5	46	6	17
Goat (b)	19.5	1 : 2.5	61	11	19
Mean			49	4	14

a : undiluted; b : diluted with Ringer solution

we have come to the conclusion that such a species difference is due to constituents of seminal plasma rather than those of spermatozoa.

We have observed that epididymal spermatozoa of each species, bull, stallion, boar and goat, lose more than 40% of phospholipids without exception by adding bull seminal plasma before freezing, whereas by boar seminal plasma, less than 10% (Table 1).

### 3) Egg yolk clearing activity

Phospholipase A attacks lipoprotein of egg yolk and make clear the suspension. We have found that similar change occurs in the egg yolk suspension in the presence of bull seminal plasma. Fig. 3 shows an individual difference in this activity of seminal plasma in nine ejaculates from three bulls. It is also detected in the uterine fluid of cow and seminal plasma of goat occasionally but not in boar seminal plasma.

### 4) Vitellus lytic activity

Addition of 1-2 drops of bull seminal plasma causes collapse in vitelline membrane of mammalian eggs within a few minutes and transparent change in vitellus in the successive time. This reaction is readily observed under microscope, suggesting enzyme-substrate relation between the lytic factor in bull seminal plasma and lipid portion of egg.

### 5) Confirming enzyme activity

Chromatography on Sephadex column reve-

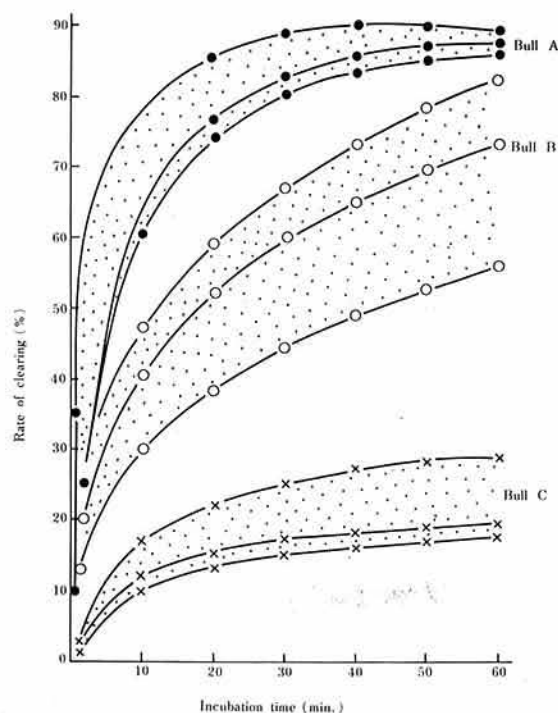


Fig. 3. Yolk-clearing activity of bull seminal plasma

Reaction mixture consists of 0.1 ml seminal plasma, 1.0 ml working suspension of egg yolk<sup>5)</sup> and 4.9 ml saline solution. After incubation at 37°C, turbidity is measured at 900 nm.

als that those lytic actions are probably induced by a protein factor which attacks phospholipid. We have obtained the following results by the enzyme assay.

#### (1) Decrease of lecithin

1 ml bull seminal plasma caused 46% de-

crease for 5.3  $\mu$  moles (calculated by P content) ovoid lecithin on their incubation at 37°C for 30 min. However, corresponding increase of lysolecithin, hydrolyzed product of lecithin by phospholipase A, was not observed.

(2) Decrease of lysolecithin

1 ml bull seminal plasma (a) and (b) caused 66% and 44% decrease, respectively for 1.9  $\mu$  moles lysolecithin on their incubation at 37°C for 30 min.

(3) Production of GPC

1.3  $\mu$  moles lecithin produced 1.2  $\mu$  moles GPC on the incubation with 2 ml bull seminal plasma at 37°C for 60 min.

These results will show participation of enzymes in bull seminal plasma to the following reactions; lecithin  $\xrightarrow{(i)}$  lysolecithin  $\xrightarrow{(ii)}$  GPC or lecithin  $\xrightarrow{(iii)}$  GPC. The enzymes for (i), (ii) and (iii) should be phospholipase A, lysophospholipase and phospholipase B, respectively.

### Possible role of phospholipases of bull seminal plasma

There is an indication that the phospholipases of bull seminal plasma may participate in the sperm life through the action for releasing lysosomal enzymes from the cell. The lysosomal enzymes have been detected in acrosome and cytoplasmic droplet of spermatozoa<sup>21</sup>.

Acid phosphatase, one of the enzymes, is released from epididymal spermatozoa of vari-

ous species on the incubation with either bull seminal plasma or bull vesicular secretion (Table 2). Same action is detected in the uterine fluid of cow at estrus.

If such an action means release of fertilization-participating enzymes from spermatozoa, it could be a phenomenon relating to 'capacitation'. In another word, bull spermatozoa may be needed to contact with either the seminal plasma or the uterine fluid before fertilization.

Besides this, it may be probable that the enzymes have roles such as decomposition of surplus and aged spermatozoa, encouragement of sperm migration by removing some lipid constituents in genital tract, providing spermatozoa with extra-cellular substrate produced by hydrolysis of phospholipid (i.e., spermatozoa can utilize GPC in the presence of GPC-diesterase and oxygen).

### Evaluation of semen quality based on the phospholipids and their hydrolysing enzymes in bull semen

In the field of A.I., phospholipids have indispensable role in preserving motility of spermatozoa as a component of semen diluent, mostly in a form of egg-yolk and lecithin preparation. These exogenous phospholipids protect spermatozoa from loss of endogenous lipids which are directly or indirectly concerned with

Table 2. Release of acid phosphatase from epididymal spermatozoa on their incubation with genital secretions

Mixture of 0.1~0.2 ml epididymal semen and 0.4 ml genital secretions was incubated at 37°C for 30 min.

Epididymal semen (A)	Genital secretion (B)	Increase of acid phosphatase activity in (B) after incubation
		King-Armstrong Unit
Boar	Bull vesicular secretion	137
	Boar vesicular secretion	15
Goat	Bull seminal plasma	203
	Goat seminal plasma	0
Bull	Bull seminal plasma	291
	Cow uterine secretion at estrus	177

**Table 3. Decrease in phospholipid content of bull spermatozoa during storage at 4°C**

Diluted (1 : 10) with	Storage time at 4°C (days)	Plasmalogen-P ( $\mu\text{g}/10^8$ sperm)	Score of sperm motility
—	0	3.09	4
Ringer solution	5	1.95	1
5% glucose solution	5	2.14	1
Egg-yolk diluent	5	2.36	3

their motility.

Accordingly, phospholipid content of spermatozoa could be an index of sperm motility or metabolic activity (Table 3). Its estimation will give us more precise data on the effect of handling and diluent for bull semen (Fig. 4), and seasonal effect.

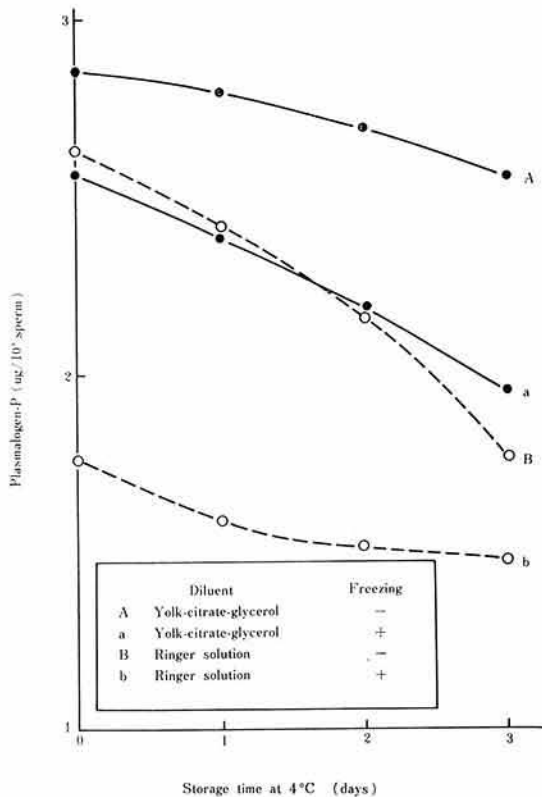
It is preferable that the phospholipase activity of bull seminal plasma is assayed by the yolk clearing activity (YCA) as a clinical method. It is likely that this activity has a

relation to some qualities of the ejaculated spermatozoa, such as maintenance of the motility during storage and even freezability.

Since these enzymes are mainly originated to seminal vesicles, the estimation of YCA in bull seminal plasma may be useful for diagnosis of the function of that organ in the same manner as well-known fructose and citric acid tests<sup>4)</sup>.

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**Fig. 4.** Effects of diluent and pre-treatment of freeze-thawing on the lipid loss of bull spermatozoa during storage at 4°C



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