

Fatty acyl chains of phosphatidyl choline and ethanolamine as diacyl, alkyl and alkenyl forms during *in vitro* capacitation of goat spermatozoa

SUMAN DHANDA¹ and S K ATREJA²

National Dairy Research Institute, Karnal, Haryana 132 001 India

Received: 23 March 2010; Accepted: 28 November 2010

ABSTRACT

The fluidity of sperm plasma membrane increases during capacitation and acrosome reaction as these processes involve the remodeling of many proteins and phospholipid components. Therefore, we were interested in comparing the fatty acyl chains associated with phosphatidylcholine (PC) and phosphatidylethanolamine (PE) in capacitated and acrosome reacted spermatozoa of goat. PC and PE were separated from total phospholipids (PL) during *in vitro* capacitation and acrosome reaction. Radyl acetates were prepared from diradyl derivatives after phospholipase C treatment of PC and PE in the presence of pyridine and acetic anhydride. The corresponding fatty acyl chains were analysed by gas liquid chromatography (GLC) in diacyl, alkenyl and alkyl radyl acetate by separation and methyl ester preparation. C₁₂:0, C₁₄:0, C₁₆:0, C₁₈:0, C₂₀:0 and C₂₄:0 are saturated and C₁₆:1, C₁₈:1, and C₂₀:4 were the main unsaturated fatty acyl chains. Moreover, the major fatty acyl chains of spermatozoa are different from those of surrounding fluid during the course of *in vitro* capacitation and acrosome reaction. The results clearly indicated the rapid turnover of these 2 sperm phospholipids in terms of active deacylation and reacylation reactions. These changes would definitely alter the sperm fertilizing ability. This may also generate bioactive PLs, viz. autocoids, fusogens, cis-unsaturated fatty acids, secondary messengers and detergents to accomplish the processes preceeding and succeeding fertilization.

Key words: Acrosome reaction, Capacitation, Goat sperm, Phosphatidylcholine, Phosphatidylethanolamine, Phospholipids

Fertilization is achieved only after the sperm is capacitated and undergoes acrosome reaction which is the first step of fertilization. Signal transduction pathways are initiated by binding of agonists to the receptors accompanied by membrane phospholipid (PL) turnover altering membrane fluidity, stability and permeability to accomplish the required capacitation and acrosomal responsiveness both *in vitro* as well as *in vivo* (Roldon 1998). Some types of bioactive lipids are known to be derived from membrane PL remodeling (Waku and Inoue 1996). Role of PLs in sperm capacitation and acrosome reaction were studied (Vazquez *et al.* 1997) and efflux of PLs in the surrounding medium was reported during sperm capacitation and acrosome reaction (Singh and Atreja 1999) but the information on the role of individual membrane PLs is not available. Accordingly, 2 important membrane PLs, viz. phosphatidylcholine (PC) and phosphatidylethanolamine (PE) were separated from sperm PLs and analyzed for fatty acyl chains in the present study.

Present address: ¹Assistant Professor (e mail: dhanda.suman999@gmail.com), Department of Biochemistry, Kurukshetra University, Kurukshetra, Haryana 136 119 India.

²Principal Scientist (e mail: suresh_atreja@yahoo.co.in), Division of Animal Biochemistry.

The fatty acyl chains were studied in PC and PE occurring as diacyl, alkyl and alkenyl forms in the buck spermatozoa and surrounding fluid during *in vitro* capacitaion and acrosome reaction.

MATERIALS AND METHODS

Animals: Alpine × Sannan crossbred bucks (*Capra hircus*) weighing 50–60 kg, aged 2–3 years and kept in normal housing conditions in individual pens at National Dairy Research Institute (NDRI) were used for the study.

Preparation of sperm sample: Semen collected from crossbred bucks using artificial vagina was processed within 30 min at room temperature. Sperms showing good forward motility were washed thrice (800 g for 10 min) using modified Kreb's Ringer bicarbonate buffered medium at pH 7.4–7.5 (Wolf and Inoue 1976). The sperm count was made using haemocytometer and it was adjusted to 1–4 × 10⁸ cells/ml.

In vitro capacitation and acrosome reaction: *In vitro* capacitation and acrosome reaction was done as per Anand *et al.* (1989). Freshly washed spermatozoa were suspended in modified Kreb's Ringer bicarbonate buffered medium with 20 mg/ml BSA and incubated for 4 h at 37°C at 5% CO₂ in

air. Loss of acrosome was checked for acrosome reaction using Giemsa staining (Hancock 1952) 15 min after addition of 10 mM calcium to capacitated spermatozoa. Aliquots were saved as control, capacitated and acrosome reacted at specified intervals and centrifuged at 800g for 10 min to separate spermatozoa from the culture medium for analysis. Samples were frozen under N_2 and pooled together for having sufficient material for lipid extraction.

Extraction of lipids: Total lipids were extracted from the sperms and culture fluid by method of Folch *et al.* (1957) and phospholipid content was calculated on the basis of phosphorus content multiplied by 25 (Ahovocova and Odavic 1969). Aliquots were fractionated on silica gel G coated TLC plates and separation of PC and PE phosphoglycerides was done as described by Singh and Atreja (1999).

Preparation of samples for fatty acyl chain analysis in diacyl, alkenyl and alkyl phospholipids: Pure PC and PE were dissolved in 1 ml of ethyl ether and 1 ml of 0.1 M Tris, pH 7.4 and treated with phospholipase C (1–3 units) and stirred at room temperature for 16 h. Ether layer was collected and water layer was extracted thrice with ether. Ether was evaporated with N_2 and sample was redissolved in 0.5 ml ether. Samples were applied on TLC plates and developed using petroleum ether: diethyl ether: acetic acid (70: 30: 1, v/v/v). Diglyceride spots were scraped off separately for choline and ethanolamine and extracted with diethyl ether. Diacylglycerols were acetylated to form radyl acetates by treating them with 0.5 ml of acetic anhydride and 0.1 ml of pyridine. This was stirred intermittently for 16 h at room temperature and extracted thrice with ether. Sample was dried and applied on TLC plates which were developed with petroleum ether: ethyl ether: acetic acid (90: 10: 1, v/v/v) and then with toluene. Upper spot of 1-alkenyl-2-acyl-3-Sn-radyl acetate, followed by 1-alkyl-2-acyl-3-Sn-radyl acetate and lower 1, 2-diacyl-3-Sn-radyl acetate was scraped off and extracted using hexane.

Preparation of methyl esters and dimethylacetals for gas liquid chromatography (GLC) analysis: All radyl derivatives were converted into methyl esters and dimethylacetals by adding 0.5 N methanolic HCl so that final solvent to solute ratio was 20: 1 (Gray 1969) and were kept for GLC analysis.

GLC analysis of fatty acyl chains: Dimethylacetals (fatty aldehydes) and methyl esters were run on a GLC-AIMIL-NUCON-5700 series equipped with flame ionization detector (FID). The stationary column glass was maintained at 198°C while injector and detector temperatures were maintained at 230°C and 240°C, respectively. Nitrogen was used as carrier gas at a flow rate of 30 ml/min. Flow rate of hydrogen and air was 33 ml/min and 330 ml/min, respectively. The fatty acids were identified through retention times of reference standards. The fatty acids were quantified by triangulation method and expressed as percent fatty acids.

Statistical analysis: Statistical analysis was done using Prism 3.0 software. Unpaired student t-test was used to

compare the 2 groups and ANOVA was used to compare 3 groups. Comparisons between means were tested by Turkey.

RESULTS AND DISCUSSION

Sperm lipids undergo changes during both capacitation and interaction with ova (Stojanoff *et al.* 1988 and Seki *et al.* 1992) but fatty acyl composition of PC and PE fraction was determined for the first time in goat spermatozoa and culture fluid during capacitation and acrosome reaction. Fatty acids of PC and PE exhibit a wide range from C_8 to C_{24} (Table 1–4) though Dietz *et al.* (1963) reported the fatty acid chains only upto 18-C atoms in mammalian spermatozoa. Major fatty acyl chains in phospholipids of sperms are $C_{12:0}$, $C_{14:0}$, $C_{16:0}$ and $C_{16:1}$ while $C_{18:1}$, $C_{18:2}$ and $C_{20:4}$ are present in traces (Table 1a). Major fatty acyl chains of culture fluid are $C_{12:0}$, $C_{14:0}$ and $C_{16:0}$ (Table 1b) while $C_{8:0}$ and $C_{10:0}$ are reported only in PC of acrosome reacted spermatozoa (Table 1a). Our studies indicated that goat spermatozoa have higher proportion of saturated fatty acids which is in agreement with reports in boar (Grogan *et al.* 1966), bull (Hammerstedt *et al.* 1979) and goat spermatozoa (Rana *et al.* 1991). Higher values of $C_{16:0}$, $C_{18:0}$, $C_{20:0}$ and lower values of $C_{14:0}$, $C_{18:1}$ and $C_{22:0}$ are reported in porcine sperm during *in utero* and *in vitro* incubation (Evans *et al.* 1980). Our results are in agreement with Evans *et al.* (1980) except $C_{14:0}$ which is present in higher amounts in goat spermatozoa. Results reveal a significant turnover of membrane choline and ethanolamine PLs with respect to quantity and types of acyl chains. The decrease in fatty acyl content in sperm cells is accompanied by their release in surrounding culture fluid. $C_{12:0}$, $C_{14:0}$ and $C_{16:0}$ are major components in culture fluid in both PC and PE. $C_{12:0}$ in culture fluid is of significance as dilaurylphosphatidylcholine is found to be very effective in inducing sperm motility (Graham *et al.* 1991) and also capacitate stallion and bull spermatozoa sufficiently so that the acrosome reaction may take place (Landim *et al.* 2001). Although amounts of $C_{14:0}$ was found to increase in sperms during epididymal maturation of boar spermatozoa (Nikolopoulou *et al.* 1985) but during capacitation and acrosome reaction, its decrease is accompanied by its release in culture fluid. The presence of $C_{14:0}$ and $C_{16:0}$ in culture fluid is important because incubations of rabbit sperm with liposomes containing dimyristoyl- or dipalmitoyl-PC plus cholesterol interfered with the ability of sperms to fertilize ova (Davis 1976). So release of these moieties in culture fluid may facilitate process of capacitation and acrosome reaction which have also been proved in bull spermatozoa (Pursel and Graham 1967). $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$ were detected in PC only in spermatozoa but not in culture fluid of PC and PE. Fatty acyl chains at Sn-2 position of alkyl ether phospholipids in PC are dominated by $C_{12:0}$, $C_{14:0}$ and $C_{16:0}$ (Table 2a) and in PE by $C_{14:0}$ and $C_{16:0}$ (Table 2a). $C_{14:0}$ decreased in sperms PC and increased in PE but constitutes more than 70% of total

Table 1. Changes in fatty acyl chains of diacyl choline and ethanolamine phospholipids during *in vitro* capacitation and acrosome reaction in

(a) Spermatozoa

Fatty acid	Control		Capacitated		Acrosome reacted	
	Choline	Ethanolamine	Choline	Ethanolamine	Choline	Ethanolamine
C _{12:0}	12.5 ± 0.32 ^a	10.69 ± 0.02 ^a	10.32 ± 0.28 ^b	16.21 ± .05 ^b	6.77 ± 0.317 ^c	30.4 ± 0.02 ^c
C _{14:0}	30.4 ± 0.18 ^a	48.13 ± 0.12 ^a	22.42 ± 0.22 ^b	63.24 ± 0.57 ^b	18.53 ± 0.21 ^c	47.18 ± 0.02 ^a
C _{16:0}	20.79 ± 0.18 ^a	25.74 ± 0.14 ^a	31.06 ± 0.11 ^b	10.51 ± 0.27 ^b	34.83 ± 0.09 ^c	8.18 ± 0.01 ^c
C _{16:1}	8.00 ± 0.09 ^a	8.52 ± 0.06 ^a	7.40 ± 0.08 ^b	3.58 ± 0.02 ^b	6.80 ± 0.02 ^c	8.73 ± 0.02 ^c
C _{18:0}	6.69 ± 0.04 ^a	2.82 ± 0.02 ^a	11.57 ± 0.6 ^b	2.92 ± 0.002 ^b	2.7 ± 0.04 ^c	3.0 ± 0.02 ^c
C _{18:1}	0.9 ± 0.04 ^a	-	0.8 ± 0.01 ^{ab}	-	0.75 ± 0.03 ^b	-
C _{18:2}	2.0 ± 0.06 ^a	-	1.5 ± 0.02 ^b	-	1.3 ± 0.06 ^b	-
C _{18:3}	1.0 ± 0.02 ^a	-	0.5 ± 0.03 ^b	-	0.37 ± 0.2 ^c	-
C _{20:0}	3.5 ± 0.03 ^a	1.2 ± 0.01 ^a	3.1 ± 0.05 ^b	0.9 ± 0.02 ^b	2.8 ± 0.02 ^c	0.8 ± 0.01 ^c
C _{20:4}	1.3 ± 0.04 ^a	-	0.80 ± 0.01 ^b	-	0.75 ± 0.02 ^b	-
C _{22:0}	3.67 ± 0.18 ^a	1.4 ± 0.01 ^a	3.2 ± 0.02 ^b	1.1 ± 0.01 ^b	2.8 ± 0.02 ^b	0.9 ± 0.02 ^c
C _{24:0}	3.0 ± 0.04 ^a	1.6 ± 0.02 ^a	2.5 ± 0.02 ^b	1.2 ± 0.02 ^b	2.0 ± 0.04 ^c	0.8 ± 0.03 ^c

(b) Culture fluid

Fatty acid	Control		Capacitated		Acrosome reacted	
	Choline	Ethanolamine	Choline	Ethanolamine	Choline	Ethanolamine
C _{12:0}	-	-	30.31 ± 0.38 ^a	31.92 ± 0.17 ^a	14.20 ± 0.66 ^b	29.60 ± 0.62 ^b
C _{14:0}	-	-	29.45 ± 0.23 ^a	53.44 ± 0.02 ^a	59.58 ± 0.51 ^b	55.93 ± 0.02 ^b
C _{16:0}	-	-	27.62 ± 0.08 ^a	11.11 ± 0.03 ^a	13.02 ± 0.14 ^b	8.350 ± 0.02 ^b
C _{16:1}	-	-	6.92 ± 0.04 ^a	2.830 ± 0.02 ^a	7.30 ± 0.07 ^b	2.280 ± 0.06 ^b
C _{18:0}	-	-	-	-	-	3.04 ± 0.03
C _{20:0}	-	-	1.80 ± 0.04 ^a	-	2.10 ± 0.03 ^b	-
C _{20:4}	-	-	-	-	0.2 ± 0.02	-
C _{22:0}	-	-	2.60 ± 0.03 ^a	0.7033 ± 0.02 ^a	2.30 ± 0.07 ^b	0.8000 ± 0.03 ^b
C _{24:0}	-	-	1.00 ± 0.03	-	1.30 ± 0.21	-

Values with different superscripts are significantly different.

phospholipids of culture fluid. C_{18:1} was the only unsaturated fatty acid in both PC and PE at Sn-1 of alkenyl PLs (Table 3). C_{20:0} and C_{20:4} from Sn-2 of alkenyl PLs are released in the culture fluid of PC (Table 4). C_{22:0} constitutes about 10% of total fatty acids of PE and is released in culture fluid in both PC and PE. Oleic and linoleic acids in both PC and PE in capacitated and acrosome reacted spermatozoa as reported by us, were also reported in bovine and ovine semen (Neill and Masters 1973). C_{20:4} present in PC of sperm and culture fluid is potential prostaglandin precursor and plays important role in reproductive processes (Cross 1994). Polyunsaturated fatty acids reportedly increase membrane fluidity and spontaneous acrosome reaction (Nissen and Kreysel 1983). Oleic acid significantly improved the motility and viability, whereas oleic and arachidonic acid together improved acrosome reaction in boar spermatozoa (Hossain *et al.* 2007). Unsaturated fatty acids in culture fluids may be contributed by sperm mitochondria during capacitation and acrosome reaction because they are important constituents of mitochondrial lipids (Stoffel 1967). Further studies are

needed to explore the role of other fatty acids during these processes.

The changes in fatty acyl chains of PLs of spermatozoa and culture fluid indicate the metabolism of PLs during *in vitro* capacitation and acrosome reaction. The presence of C_{12:0}, C_{14:0} and C_{16:0} in the PLs of culture fluid (Table 1b) suggest that these fatty acids maintain motility and provide energy during capacitation (Hartree and Mann 1959). It is important as mammalian spermatozoa survive many hours between deposition and fertilization in female reproductive tract (Yanagimachi 1994). Presence of 18:1, 18:2 and 18:3 only in sperm PLs indicate that they may be metabolised by phospholipases to release unsaturated and lysophospholipids required for fusogenic activity for acrosome reaction and also during fusion of sperm with oocyte (Yanagimachi 1994). Lysophosphatidylcholine may be a substrate for platelet activating factor (PAF) (Baldi *et al.* 1993) which enhance acrosome reaction in most of the sperms (Huo and Yang 2000). Desnoyers and Manjunath (1993) suggested that PLs are the binding sites for bovine seminal plasma proteins

Table 2. Changes in fatty acyl chains at Sn-2 of choline and ethanolamine containing 1-O-alkyl phospholipids during *in vitro* capacitation and acrosome reaction in

(a) Spermatozoa

Fatty acid	Control		Capacitated		Acrosome reacted	
	Choline	Ethanolamine	Choline	Ethanolamine	Choline	Ethanolamine
C _{12:0}	7.0± 0.02 ^a	-	8.3± 0.02 ^b	-	10.85± 0.04 ^c	-
C _{14:0}	57.12± 0.05 ^a	62.42± 0.02 ^a	53.29 ± 0.03 ^b	70.43± 0.01 ^b	46.83 ± 0.10 ^c	71.74± 0.34 ^c
C _{16:0}	27.61± 0.02 ^a	32.58± 0.01 ^a	28.71± 0.03 ^b	22.37± 0.01 ^b	30.56 ± 0.02 ^c	20.32± 0.02 ^c
C _{16:1}	3.2± 0.02 ^a	1.7± 0.03 ^a	4.71± 0.02 ^b	2.2± 0.04 ^b	5.66± 0.02 ^c	2.44± 0.02 ^c
C _{18:0}	1.3± 0.04 ^a	3.3± 0.02 ^a	1.8± 0.01 ^b	5.12± 0.14 ^b	2.2± 0.03 ^c	5.2± 0.03 ^c
C _{18:1}	1.5± 0.02 ^a	-	1.2± 0.02 ^b	-	0.8± 0.02 ^c	-

(b) Culture fluid

Fatty acid	Control		Capacitated		Acrosome reacted	
	Choline	Ethanolamine	Choline	Ethanolamine	Choline	Ethanolamine
C _{14:0}	-	-	70.57 ± 0.11 ^a	79.29 ± 0.02 ^a	77.28 ± 0.03 ^b	81.39 ± 0.03 ^b
C _{16:0}	-	-	22.53 ± 0.02	16.50 ± 0.02 ^a	22.42 ± 0.02	14.12 ± 0.01 ^b
C _{18:0}	-	-	-	4.20 ± 0.03 ^a	-	4.50 ± 0.02 ^b

Values with different superscripts are significantly different.

Table 3. Changes in alkenyl fatty acyl chains at Sn-1 of choline and ethanolamine containing ether phospholipids during *in vitro* capacitation and acrosome reaction in

(a) Spermatozoa

Fatty acid	Control		Capacitated		Acrosome reacted	
	Choline	Ethanolamine	Choline	Ethanolamine	Choline	Ethanolamine
C _{12:0}	2.0± 0.02 ^a	6.2± 0.04 ^a	3.3± 0.02 ^b	7.3± 0.02 ^b	4.2± 0.02 ^c	7.5± 0.02 ^c
C _{14:0}	44.44± 0.02 ^a	42.17± 0.03 ^a	46.0± 0.02 ^b	47.10± 0.06 ^b	41.7± 0.33 ^c	51.08± 0.03 ^c
C _{16:0}	40.27± 0.02 ^a	33.33± 0.04 ^a	38.7± 0.03 ^b	30.69± 0.04 ^b	42.8± 0.02 ^c	29.21± 0.3 ^c
C _{18:0}	9.3± 0.02 ^a	11.99± 0.03 ^a	8.7 ± 0.03 ^b	9.8± 0.02 ^b	8.3± 0.02 ^c	7.6± 0.03 ^c
C _{18:1}	4.0± 0.04 ^a	6.3± 0.03 ^a	3.3± 0.00 ^b	5.2± 0.04 ^b	2.7± 0.03 ^c	4.6± 0.02 ^c

(b) Culture fluid

Fatty acid	Control		Capacitated		Acrosome reacted	
	Choline	Ethanolamine	Choline	Ethanolamine	Choline	Ethanolamine
C _{12:0}	-	-	-	9.30± 0.03 ^a	-	12.10 ± 0.05 ^b
C _{14:0}	-	-	64.29 ± 0.02 ^a	54.02 ± 0.02 ^a	65.70 ± 0.33 ^b	57.09 ± 0.04 ^b
C _{16:0}	-	-	30.51 ± 0.03 ^a	24.37 ± 0.04 ^a	29.70 ± 0.03 ^b	25.70 ± 0.02 ^b
C _{18:0}	-	-	5.20 ± 0.02 ^a	2.30 ± 0.02 ^a	4.30 ± 0.02 ^b	5.20 ± 0.03 ^a

Values with different superscripts are significantly different.

and their specific interaction might be important in modifying plasma membrane during capacitation and acrosome reaction.

The present study revealed the changes in the fatty acyl chains of PLs of the spermatozoa and culture fluid during *in vitro* capacitation and acrosome reaction in goat spermatozoa. This in turn reflects the PLs remodeling

during these processes in terms of active acylation and reacylation reactions to reflect the bioactivity associated with the PLs to attain the ultimate function of fertilization after achieving capacitation, acrosome reaction and gamete interaction. These are the preliminary findings which need to be strengthened by further systematic studies on other species.

Table 4. Changes in fatty acyl chains at Sn-2 of choline and ethanolamine containing alkenyl phospholipids during *in vitro* capacitation and acrosome reaction in

(a) Spermatozoa

Fatty acid	Control		Capacitated		Acrosome reacted	
	Choline	Ethanolamine	Choline	Ethanolamine	Choline	Ethanolamine
C _{12:0}	8.2± 0.02 ^a	4.9± 0.02 ^a	9.3± 0.02 ^b	4.4± 0.08 ^b	11.08± 0.01 ^c	4.7± 0.02 ^a
C _{14:0}	54.53± 0.03 ^a	27.25± 0.03 ^a	45.18± 0.04 ^b	26.33± 0.06 ^b	45.08± 0.01 ^b	38.89± 0.02 ^c
C _{16:0}	16.67± 0.05 ^a	22.75± 0.07 ^a	24.12± 0.03 ^b	27.5± 0.04 ^b	20.29± 0.04 ^c	25.15± 0.07 ^c
C _{16:1}	7.03± 0.06 ^a	1.4± 0.03 ^a	5.3± 0.03 ^b	3.17± 0.03 ^b	4.24± 0.03 ^c	2.67± 0.07 ^c
C _{18:0}	4.03± 0.05 ^a	7.0± 0.07 ^a	4.9± 1.00 ^a	6.3± 0.07 ^{ab}	3.3± 0.03 ^a	6.2± 0.29 ^b
C _{18:1}	1.3± 0.02 ^a	2.6± 0.04 ^a	2.33± 0.04 ^b	1.3± 0.03 ^b	2.1± 0.03 ^c	1.7± 0.04 ^c
C _{20:0}	3.02± 0.07 ^a	12.7 ± 0.04 ^a	4.3± 0.04 ^b	11.87 ± 0.04 ^b	4.1± 0.02 ^b	11.76± 0.07 ^b
C _{20:4}	2.7± 0.04 ^a	-	3.11± 0.07 ^b	-	3.3± 0.03 ^b	-
C _{22:0}	0.70± 0.04 ^a	12.1± 0.09 ^a	0.90± 0.04 ^b	10.3± 0.04 ^b	1.2± 0.02 ^c	11.7± 0.01 ^c
C _{24:0}	2.1± 0.06 ^a	9.3± 0.02 ^a	2.5± 0.04 ^b	8.7± 0.04 ^b	2.3± 0.02 ^c	8.5± 0.05 ^c

(b) Culture fluid

Fatty acid	Control		Capacitated		Acrosome reacted	
	Choline	Ethanolamine	Choline	Ethanolamine	Choline	Ethanolamine
C _{12:0}	-	-	-	15.03 ± 0.06 ^a	-	17.20 ± 0.03 ^b
C _{14:0}	-	-	47.44 ± 0.03 ^a	66.04 ± 0.05 ^a	64.19 ± 0.02 ^b	53.39 ± 0.03 ^b
C _{16:0}	-	-	26.11 ± 0.08 ^a	20.26 ± 0.03	18.41 ± 0.03 ^b	20.78 ± 0.28
C _{18:0}	-	-	1.80 ± 0.04 ^a	-	1.30 ± 0.03 ^b	-
C _{20:0}	-	-	7.30 ± 0.03 ^a	-	8.70 ± 0.01 ^b	-
C _{20:4}	-	-	5.30 ± 0.02 ^a	-	4.20 ± 0.03 ^b	-
C _{22:0}	-	-	6.93 ± 1.69 ^a	15.03 ± 0.07 ^a	7.90 ± 2.01 ^b	17.20 ± 0.02 ^b
C _{24:0}	-	-	8.40 ± 0.02 ^a	-	9.30 ± 0.02 ^b	-

Values with different superscripts are significantly different.

ACKNOWLEDGEMENT

We are thankful to National Dairy Research Institute for providing the necessary facilities to carry out this work.

REFERENCES

- Ahovocova J K and Odavic R. 1969. A simple method for quantitative analysis of phospholipids reported by thin layer chromatography. *Journal of Chromatography* **40**: 90–96.
- Anand S R, Atreja S K, Chauhan M S and Behl R. 1989. *In vitro* capacitation of goat spermatozoa. *Indian Journal of Experimental Biology* **27**(2): 921–24.
- Baldi E, Falsetti C, Krausz C, Gervasi G, Carloni V, Casano R, Forti G. 1993. Stimulation of platelet-activating factor synthesis by progesterone and A23187 in human spermatozoa. *Biochemical Journal* **292**: 209–16.
- Cross N L. 1994. Phosphatidylcholine enhances the acrosomal responsiveness of human spermatozoa. *Journal of Andrology* **15**: 484–88.
- Davis B K. 1976. Inhibitory effect of synthetic phospholipid vesicles containing cholesterol on the fertilizing ability of rabbit spermatozoa. *Proceedings on Society of Experimental Biology and Medicine* **152**: 257–61.
- Desnoyers L and Manjunath P. 1993. Interaction of a novel class of phospholipid binding-proteins of bovine seminal fluid with different affinity matrices *Archives in Biochemistry and Biophysics* **305**: 341–49.
- Dietz R W, Pickett P W, Komarek R J and Jensen R G. 1963. Fatty acid composition of bovine semen. *Journal of Dairy Science* **46**: 468–72.
- Evans R W, Weaver D E and Clegg E D. 1980. Diacyl, alkenyl and alkyl ether phospholipids in ejaculated, *in utero* and *in vitro* incubated porcine spermatozoa *Journal of Lipid Research* **21**: 223–28.
- Folch J, Lees M, Sloane-Stanley G H. 1957. A simple method of isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry* **226**: 497–509.
- Graham J K, Nolan J P and Hammerstedt R H. 1991. Effect of dilauroylphosphatidylcholine liposomes on motility, induction of the acrosome reaction, and subsequent egg penetration of ram epididymal sperm. *Biology Reproduction* **44**: 1092–99.
- Gray G M. 1969. The preparation and assay of long chain fatty aldehydes. *Methods in Enzymology* **14**: 678–84.
- Grogon D E, Mayer D T and Sikes J D. 1966. Quantitative differences in phospholipids of ejaculated spermatozoa and spermatozoa from three levels of the epididymis of the boar. *Journal of Reproduction and Fertility* **12**: 431–36.
- Hammerstedt R H, Keith A D, Hay S, Deluca N and Amann R P. 1979. Changes in ram sperm membranes during epididymal transit. *Archives in Biochemistry and Biophysics* **196**: 7–12.
- Hancock J L. 1952. The morphology of bull spermatozoa *Journal*

- of *Experimental Biology* **29**: 445–53.
- Hartree E F and Mann T. 1959. Plasmalogens in ram semen and its role in sperm metabolism. *Biochemical Journal* **71**: 427–34.
- Hossain M D S, Tareq K M A, Hammano Ko-ichi and Hirolada T. 2007. Effect of fatty acids in boar sperm motility, viability and acrosome reaction. *Reproduction Medicine Biology* **6** (4): 235–39.
- Huo L J and Yang Z M. 2000. Effects of platelet-activating factor on capacitation and acrosome reaction in mouse spermatozoa. *Molecular Reproduction and Development* **56**: 436–40.
- Landim-Alvaraenga F C, Alvaraenga M A, Seidel G E, Squires E L and Graham J K. 2001. Penetration of zona-free hamster, bovine and equine oocytes by stallion and bull spermatozoa pretreated with equine follicular fluid, dilaurylphosphatidylcholine or calcium ionophore A23187. *Theriogenology* **56** (5): 937–53.
- Neill A R and Masters C J. 1973. Metabolism of fatty acids by ovine spermatozoa. *Journal of Reproduction and Fertility* **34**: 279–87.
- Nikolopoulou M, Soucek D A and Vary J C. 1985. Changes in the lipid content of boar sperm plasma membranes during epididymal maturation. *Biochimica Biophysica Acta* **815**: 486–98.
- Nissen H P and Kreysel H W. 1983. Polyunsaturated fatty acids in relation to sperm motility. *Andrologia* **15**: 264–69.
- Ozku E and Yanagimachi R. 1982. Acceleration of the acrosome reaction in hamster spermatozoa by lysolecithin. *Journal of Experimental Biology* **32**: 224–59.
- Pursel V G and Graham E F. 1967. Phospholipid of bovine spermatozoa and seminal plasma. *Journal of Reproduction and Fertility* **14**: 203–11.
- 24 Rana A P S, Majumder G C, Misra S and Ghosh A. 1991. Lipid changes of goat sperm plasma membrane during epididymal maturation. *Biochimie* **27**: 75–78.
- Rolden E R. 1998. Role of sperm phospholipases during sperm acrosomal exocytosis. *Frontiers in Bioscience* **3**: D1109–19.
- Seki N, Toyama Y and Nagano T. 1992. Changes in the distribution of filipin-sterol complexes in the boar sperm plasma membrane during epididymal maturation and in the uterus. *Anatomical Record* **232**: 221–30.
- Singh S and Atreja S K. 1999. Distribution and changes of alkyl, alkenyl and diacyl phospholipids during *in vitro* capacitation and acrosome reaction of goat spermatozoa. *Indian Journal of Dairy Science* **52**(4): 237–42.
- Stoffel W. 1967. The chemistry of mammalian lipids. *Lipid and Lipases*. pp 1–39. (Ed) Schettler G, Springer-Verlag press, Heidelberg.
- Stojanoff A, Bourne H, Andrews A G and Hyne R V. 1988. Phospholipid composition of isolated guinea pig outer acrosome membrane and plasma membrane during capacitation *in vitro*. *Gamete Research* **21**: 297–311.
- Vazquez J M and Roldan R S. 1997. Phospholipid metabolism in boar spermatozoa and role of diacylglycerol species in the *de novo* formation of phosphatidylcholine. *Molecular Reproduction Development* **47**: 105–12.
- Waku K and Inoue K. 1996. Lipid messengers in the nervous system. *Journal of Lipid Mediators Cell Signal* **14** (1): 374.
- Wolf D P and Inoue M. 1976. Sperm concentration depending in the fertilization and zona sperm binding properties of mouse egg inseminated *in vitro*. *Journal of Experimental Zoology* **196**: 27–38.
- Yanagimachi R. 1994. Mammalian fertilization. *The Physiology of Reproduction*. pp. 189–317. (Eds) Knobil E and Neill J D. Raven Press, New York.