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Lipid analysis of immature pig oocytes

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Summary. The detailed analysis of the lipid composition of immature pig oocytes represents the first such study carried out on mammalian eggs. In order to undertake a large scale lipid analysis using conventional extraction and chromatographic techniques a procedure for mass harvesting relatively large numbers of pig oocytes (200–300 oocytes/ovary) was developed. The study revealed that triacylglycerol was the major lipid component (100·71 nmol/mg protein) followed by cholesterol (32·71 nmol/mg protein). Phosphatidylcholine constituted the major phospholipid component (27·83 nmol/mg protein). Pig oocytes contained relatively low proportions of phosphatidylethanolamine (16·41% total phospholipid) and relatively high proportions of lysophosphatidylcholine (4·68% total phospholipid). The free fatty acid pattern was strikingly similar to the fatty acid composition of phosphatidylcholine. This observation, in conjunction with the observed high levels of lysophosphatidylcholine and the low ratio of phosphatidylethanolamine to phosphatidylcholine, suggests a fast rate of phospholipid turnover in the immature pig oocyte. Analysis of fatty acids esterified to the individual phospholipids and neutral lipids has shown that in all the classes examined, particularly in the neutral lipid fractions, there are high levels of the saturated fatty acid palmitic acid (16:0) and the monounsaturated fatty acid oleic acid (18:1). Triacylglycerol, free fatty acids and most of the phospholipids, particularly phosphatidylethanolamine, are considerably enriched in *n*-6 polyunsaturated fatty acids, specifically linoleic (18:2), arachidonic (20:4) and adrenic (22:4) acids. This may indicate an ability of oocytes to synthesize prostaglandins and leukotrienes.

The results show that the lipid environment of the immature pig oocyte may be adapted to the highly specialized requirements of the cell, promoting growth and development with a potential role in the regulation of maturation.

Introduction

During the past decade, much attention has focussed on the protein composition of mammalian oocytes before, and during, the resumption of meiosis (reviewed by Schultz, Montgomery & Belanoff, 1983). Nevertheless, to date, there have been no biochemical analyses of the lipid composition of mammalian oocytes although an early study with single-cell mouse embryos revealed that 12·5% of the cytoplasmic dry mass was lipid (Loewenstein & Cohen, 1964).

Lipids not only provide a source of nutrient to the cell, but also play a vital role in modifying the physical properties and functions of biological membranes, and have potent effects on cell–cell interactions, cell proliferation and transport (reviewed by Stubbs & Smith, 1984). In addition, the activities of membrane proteins depend on their immediate lipid environment, and the ability of hormone receptor complexes to bind to effector molecules may be modulated by the headgroup and fatty acid composition of membrane lipids (Hirata, Strittmatter & Axelrod, 1979; Galo, Uñates & Farias, 1981). The importance of lipids in maintenance of normal cell function is therefore unequivocal.

The study of lipids in mammalian oocytes has been hampered by the relative insufficiency of cells required for standard lipid biochemical methods. However, we have modified a procedure designed for the collection of large amounts of pig zona pellucida (Dunbar, Wardrip & Hedrick, 1978), and have been able to harvest sufficient numbers of highly purified pig oocytes for such an analysis.

Materials and Methods

Oocyte collection

Pig reproductive tracts were obtained from Arizona Pork Products Inc. Ovaries containing follicles of >5 mm diameter were excluded, but no discrimination was made between ovaries with small or medium-sized follicles. The ovaries were macerated with doubled-edged razor blades in collecting buffer (0·87% (w/v) NaCl + 5 mM-EDTA + 2·5 mM-Hepes pH 7·4). Large tissue debris was removed by passing the suspension through a 210 μm stainless-steel filter. Cumulus-enclosed oocytes were collected on a 75 μm filter and blood cells and other small debris were washed through. Cumulus-enclosed oocytes were centrifuged for 5 min at 300 ***g*** and the supernatant was discarded. Cumulus cells were removed by addition of sodium citrate (1% w/v) in 10 mM-EDTA (pH 7·4) at 4°C, and vortexed for 5 min. The suspension was passed through a 75 μm filter. The denuded oocytes and remaining debris were collected from the top of the filter in collecting buffer and centrifuged at 300 ***g*** for 5 min. The pellet was resuspended in 4 ml chemically defined culture medium (2A-BMOC; McGaughey, 1977) and layered over 2 ml 30% (w/v) sucrose in this medium. After centrifugation at 1400 ***g*** for 4 min, the purified denuded oocytes were collected from the interphase and washed twice with Medium 2A-BMOC. This procedure resulted in a preparation of oocytes which was free of contaminating cells as shown by light microscopy at × 100 magnification. Less than 1% of the preparation contained broken oocytes. The cells were finally resuspended in 1–2 ml Medium 2A-BMOC. Samples (10 μl) were removed for cell counting and for protein determination according to the method of Lowry, Rosebrough, Farr & Randall (1951). Yields were usually between 200 and 300 oocytes per ovary, with an average protein content of 86 μg/10^3 oocytes.

Total lipid extraction

Purified denuded oocytes in Medium 2A-BMOC were incubated with 100 μg protease/ml (Sigma Chemical Co., St Louis, MO, U.S.A.; obtained from *Streptomyces griseus*, Type VI, repurified with no detectable RNase or DNase activity) for 15 min at 37°C to remove the zona pellucida. The oocytes were then extracted with chloroform and methanol according to the method of Bligh & Dyer (1959). To ascertain whether prior incubation with protease could lead to lipid artefacts in the oocyte lipid extracts, the enzyme was thoroughly checked for phospholipase activity on red blood cell ghosts or phosphatidylcholine liposomes under similar conditions. Gas–liquid chromatography analysis of total free fatty acids and thin-layer chromatography of phospholipids confirmed the absence of any detectable phospholipase contamination of the protease. Total lipid extracts from 10–20 × 10^3 oocytes were evaporated to dryness under oxygen free nitrogen and treated with 10% sulphuric acid in methanol in sealed vials for 4 h at 80°C (Marai & Kuksis, 1973). The fatty acid methyl esters were extracted with petroleum ether, evaporated to dryness and resuspended in hexane. The methyl esters were separated by gas–liquid chromatography using a glass column packed with 10% EGSS-X on gas chrome Q (The Foxboro Co., New Haven, CT, U.S.A.). Unknown fatty acids were identified using thin-layer chromatography silica gel G plates impregnated with 10% silver nitrate and a developing solvent of hexane/diethyl ether/glacial acetic acid (94:4:2 by vol.) as described by Dudley & Anderson (1975), followed by gas–liquid chromatography of eluted bands.

Separation and analysis of individual lipid classes

Total lipid extracts from 65–100 × 10^3 oocytes were pooled from collections on several days and were dissolved in 0·3 ml chloroform. The extracts were loaded onto silicic acid columns containing 1 g BIO SIL-HA (Sigma) equilibrated with chloroform. Neutral lipids were eluted with 20 ml chloroform and phospholipids were eluted with 12 ml methanol followed by 8 ml 1% water in methanol (Homa, Conroy & Smith, 1983).

Neutral lipids were further fractionated by silicic acid column chromatography with increasing proportions of diethyl ether in petroleum ether (Hirsch & Ahrens, 1958). Triacylglycerol was eluted with 15 ml 1% diethyl ether in petroleum ether, cholesterol and free fatty acids with 40 ml 8% diethyl ether in petroleum ether, diacylglycerol with 10 ml 25% diethyl ether in petroleum ether and monoacylglycerol with 12 ml 100% diethyl ether. Each fraction was assayed for purity using thin-layer chromatography on silica gel G plates developed in the solvent petroleum ether/diethyl ether/glacial acetic acid (90:10:1 by vol.). Each individual neutral lipid fraction was evaporated to dryness under oxygen-free nitrogen and saponified with 5 ml 33% KOH dissolved in ethanol (6:94, v/v) containing 40 μg hydroquinone/ml (Belin, Pettet, Smith, Thompson & Zilkha, 1971). After refluxing at 80°C for 1 h (with the exception of cholesteryl esters, which were heated at 90°C for 1·5 h) the saponified extract was washed twice with hexane. These washes were retained for cholesterol assay. Acidification of the aqueous phase and subsequent washing with hexane extracted the fatty acids. Fatty acids were methylated with boron trifluoride in methanol and were separated by gas–liquid chromatography as described above.

Phospholipids were separated into their individual classes by thin-layer chromatography. The phospholipid fraction was evaporated to dryness under oxygen-free nitrogen and spotted onto Whatman LHP-K (10 cm × 10 cm) thin-layer plates. The developing solvent was methyl acetate/chloroform/*n*-propanol/methanol/0·25% aqueous KCl (25:25:25:10:9, by vol.) (Vitiello & Zanetta, 1978). Individual phospholipid bands were visualized with 0·05% dichlorofluorescein, scraped off and eluted according to the method described by Arvidson (1968) using chloroform/methanol/glacial acetic acid/water (50:39:1:10, by vol.). Dichlorofluorescein was removed with 4 M-NH_4OH, followed by one wash with 50% methanol (Arvidson, 1968). Each individual phospholipid was hydrolysed by alkaline saponification (Belin *et al.*, 1971) followed by boron trifluoride methanolysis, or by sulphuric acid methanolysis (Marai & Kuksis, 1973) as described above. The former procedure is adequate for hydrolysing ester bonds, but the harsher treatment of the latter procedure is required to hydrolyse fatty acids in amide linkage in sphingomyelin, and to remove the aldehydes from plasmalogens. The fatty acid methyl esters and dimethyl acetals were separated by gas–liquid chromatography. As monounsaturated fatty aldehydes elute with saturated fatty acids on gas–liquid chromatography, proportions of these compounds were calculated by comparing gas–liquid chromatograms of phospholipid samples which were divided in half and which had undergone both sulphuric acid methanolysis and alkaline saponification.

Quantification of individual lipid classes

Phospholipids were quantified by phosphorus analysis using a modified method of Allen (1940). After extraction of the fatty acid methyl esters following sulphuric acid methanolysis, the aqueous phases, containing the phosphorus groups, were heated to 170°C for 1 h to eliminate water and any remaining organic solvents. The samples were digested with 0·77 ml perchloric acid for 45 min at 190°C followed by addition of 4 ml 0·83% ammonium molybdate after cooling. Then 0·5 ml $SnCl_2$ solution (0·25 ml 40% $SnCl_2$ in concentrated HCl diluted in 50 ml water) was added. Absorbance was measured spectrophotometrically at 620 nm and compared with standards of KH_2PO_4, which had undergone similar treatment. The standards contained 30 μl H_2SO_4 to compensate for an estimated volume of H_2SO_4 which remained after sulphuric acid methanolysis of the samples.

Cholesterol was assayed using the Liebermann–Burchard reaction (Huang, Chen, Wefler & Raftery, 1961). Cholesterol standards and samples were dissolved in glacial acetic acid. Acetic anhydride sulphuric acid mixture (2·5 ml) was added and after 20 min, absorbance was measured spectrophotometrically at 550 nm. Amounts of glycerol containing neutral lipids were estimated by enzymic analysis of glycerol using a test kit from Sigma Chemical Co., and a triolein standard.

Results

Individual lipid class content

Analysis of the phospholipid content of purified pig oocytes showed that there are measurable quantities of phospholipid, in the nanomolar range, per mg protein (Table 1). The most predominant phospholipid was phosphatidylcholine, which constitutes 27·83 nmol/mg protein, representing more than 46% of the total phospholipid content. Phosphatidylethanolamine represented 16·41% of the total phospholipid, whereas lysophosphatidylcholine constituted 4·68%. Phosphatidylinositol constituted more than 6% of the total phospholipid. Table 2 shows that triacylglycerol constituted the major portion of neutral lipid in pig oocytes, 100·71 nmol/mg protein. There was also a significant amount of cholesterol in these cells (32·71 nmol/mg protein). Pig oocytes contained about 3 times more neutral lipid than phospholipid. The calculated lipid ratios indicated a relatively low proportion of phosphatidylethanolamine:phosphatidylcholine (0·36 ± 0·05) but a high proportion of phosphatidylcholine:sphingomyelin (4·13 ± 0·59), while the ratio of phospholipid:cholesterol was 1·63 ± 0·04 ($n = 3$ in all cases).

Fatty acid profiles of total lipid and individual lipid classes

The fatty acid profiles are shown in Tables 3–5. Palmitic acid comprised 50·7% of the total lipid fatty acid content (Table 3). The monounsaturated fatty acid, oleic acid, constituted the largest portion of unsaturated fatty acids. Polyunsaturated fatty acids constituted only 16% of the total.

Table 1. Phospholipid content of denuded pig oocytes

Phospholipid	nmol/mg protein	% total phospholipid
Phosphatidylethanolamine	9·85 ± 1·11	16·41 ± 1·61
Phosphatidic acid	2·81 ± 0·40	4·63 ± 0·49
Phosphatidylinositol	3·72 ± 0·64	6·23 ± 1·03
Phosphatidylserine	5·83 ± 1·49	9·48 ± 2·05
Phosphatidylcholine	27·83 ± 0·51	46·77 ± 2·67
Sphingomyelin	7·12 ± 0·88	11·80 ± 1·18
Lysophosphatidylcholine	2·79 ± 0·07	4·68 ± 0·22

Values are means ± s.e.m. of 3 experiments.

Table 2. Neutral lipid content of denuded pig oocytes

Neutral lipid	Amount (nmol/mg protein)
Monoacylglycerol	5·08 (2·23, 7·92)
Diacylglycerol	16·69 (13·40, 19·98)
Triacylglycerol	100·71 (92·83, 108·58)
*Cholesterol	32·71 ± 3·1
*Cholesterol ester	12·60 ± 1·6

Values are the average of 2 experiments or the mean ± s.e.m. of 4* experiments.
Numbers in parentheses are the observed values from each of two experiments.

Table 3. Fatty acid composition of total lipid extract of pig oocytes

Fatty acid	% total fatty acids
14:0	0·98 ± 0·05
16:0	50·70 ± 0·78
16:1	3·90 ± 0·38
17:0	1·20 ± 0·42
18:0	8·47 ± 0·56
18:1	17·94 ± 0·51
18:2	4·44 ± 0·27
18:3 + 20:0	0·35 ± 0·05
20:1	0·29 ± 0·03
20:3 (n-6)	0·97 ± 0·09
20:3 (n-3)	0·88 ± 0·09
20:4 > 22:1	6·85 ± 0·48
22:4	3·03 ± 0·32

Values are means ± s.e.m. for 9 replicates and are expressed as a percentage of the total fatty acid composition. Separation of fatty acid methyl esters on silver nitrate thin-layer chromatography revealed that 22:1 constituted only 0·2% of the total fatty acids.

Table 4. Fatty acid composition of neutral lipids of pig oocytes

Fatty acid	Free fatty acids	Cholesteryl ester	Triacylglycerol	Diacylglycerol	Monoacylglycerol
14:0	0·77 ± 0·13	0·44 ± 0·02	0·49 ± 0·06	1·17 ± 0·11	2·49 ± 0·57
16:0	34·13 ± 2·12	53·06 ± 0·33	36·83 ± 1·78	44·58 ± 1·44	41·35 ± 0·76
16:1	4·24 ± 0·21	3·72 ± 0·12	5·01 ± 0·56	5·12 ± 0·35	8·43 ± 2·65
17:0	0·85 ± 0·09	1·14 ± 0·05	0·78 ± 0·04	0·93 ± 0·19	0·87 ± 0·03
18:0	10·50 ± 2·93	9·23 ± 0·41	6·47 ± 2·35	8·48 ± 0·97	12·87 ± 3·00
18:1	22·42 ± 1·18	16·32 ± 0·41	14·27 ± 1·91	18·89 ± 0·39	18·49 ± 0·84
18:2	6·24 ± 0·49	3·44 ± 0·19	7·16 ± 0·44	7·56 ± 1·88	5·49 ± 0·73
18:3 + 20:0	0·31 ± 0·08	0·29 ± 0·03	0·78 ± 0·09	0·30 ± 0·03	0·68 ± 0·14
20:1	0·50 ± 0·07	0·49 ± 0·03	0·34 ± 0·10	0·94 ± 0·25	1·11 ± 0·10
20:2	N.D.	0·06 ± 0·01	0·15 ± 0·07	N.D.	N.D.
20:3 (n-6)	1·28 ± 0·28	0·83 ± 0·10	1·13 ± 0·15	0·72 ± 0·04	N.D.
20:3 (n-3) + 22:0	1·33 ± 0·12	0·62 ± 0·04	1·67 ± 0·15	0·60 ± 0·07	N.D.
20:4	12·61 ± 1·88	4·56 ± 0·45	12·30 ± 1·19	4·70 ± 0·22	4·24 ± 1·07
20:5 (n-6) + 22:2	0·49 ± 0·05	N.D.	N.D.	0·52 ± 0·07	2·10 ± 0·64
20:5 (n-3)	N.D.	N.D.	0·49 ± 0·05	N.D.	N.D.
22:3	0·58 ± 0·09	0·70 ± 0·02	0·54 ± 0·12	0·68 ± 0·04	N.D.
22:4	3·77 ± 0·35	3·14 ± 0·10	6·48 ± 0·23	3·34 ± 0·20	1·88 ± 0·42
22:5 (n-6)	N.D.	0·94 ± 0·06	1·37 ± 0·21	1·48 ± 0·27	N.D.
22:5 (n-3)	N.D.	0·34 ± 0·10	1·92 ± 0·38	N.D.	N.D.
22:6	N.D.	0·26 ± 0·06	1·35 ± 0·34	N.D.	N.D.
26:0	N.D.	0·42 ± 0·03	0·50 ± 0·04	N.D.	N.D.

Values are the means ± s.e.m. of 4 analyses and are expressed as a % of the total fatty acid composition in each individual neutral lipid class.
N.D. = not detectable.

Table 4 shows the fatty acid composition of the individual neutral lipid classes. It is evident that palmitic acid was the major fatty acid in all the neutral lipid fractions of pig oocytes, followed by oleic acid. Oocyte monoacylglycerol appeared to be relatively rich in palmitoleic acid. Triacylglycerol and free fatty acids contained proportionately higher concentrations of unsaturated fatty acids, there being 45% and 46% saturated fatty acids, respectively. Both of these neutral lipid

Table 5. Fatty acid composition of phospholipids of pig oocytes

Fatty acid	PC (n = 5)	LPC (n = 4)	SM (n = 5)	PS (n = 3)	PA (n = 4)	PI (n = 3)	PE (n = 2)
14:0	0·46 ± 0·11	2·83 ± 1·23	0·96 ± 0·24	1·55 ± 0·49	1·23 ± 0·54	0·94 ± 0·35	1·51 (0·53, 2·49)
16:0	38·01 ± 0·71	32·97 ± 3·92	35·54 ± 2·50	25·66 ± 0·79	23·42 ± 4·07	32·97 ± 2·65	18·73 (17·66, 19·80)
16:1	2·02 ± 0·14	7·62 ± 2·22	2·54 ± 0·82	2·92 ± 0·54	3·84 ± 0·55	2·31 ± 1·31	6·31 (4·47, 8·14)
17:0	0·70 ± 0·05	1·11 ± 0·21	1·01 ± 0·12	0·65 ± 0·11	0·79 ± 0·09	1·28 ± 0·12	0·48 (0·17, 0·78)
18:0	9·43 ± 0·16	14·94 ± 1·50	13·41 ± 0·72	24·29 ± 2·57	17·52 ± 3·00	25·56 ± 2·72	13·23 (13·57, 12·89)
18:1	23·12 ± 0·37	26·98 ± 3·23	9·82 ± 1·83	24·35 ± 3·33	29·52 ± 2·71	14·00 ± 2·56	21·24 (21·73, 20·75)
18:2	6·00 ± 0·16	3·70 ± 0·50	1·43 ± 0·21	4·05 ± 0·17	14·11 ± 3·20	2·04 ± 0·34	3·37 (3·14, 3·60)
18:3 + 20:0	0·27 ± 0·02	N.D.	7·02 ± 0·61	N.D.	N.D.	1·75 ± 0·49	0·22 (0·14, 0·29)
18:4	N.D.	N.D.	1·29 ± 0·11	N.D.	N.D.	N.D.	N.D.
20:1	0·29 ± 0·03	N.D.	N.D.	N.D.	N.D.	0·85 ± 0·39	0·54 (0·39, 0·69)
20:3 (n-6)	0·37 ± 0·07	N.D.	N.D.	N.D.	N.D.	N.D.	0·93 (0·82, 1·04)
20:3 (n-3) + 22:0	0·64 ± 0·15	N.D.	*6·04 ± 0·58	N.D.	N.D.	N.D.	0·53 (0·49, 0·56)
20:4	14·28 ± 0·48	9·85 ± 2·47	N.D.	11·20 ± 3·77	9·57 ± 3·13	18·30 ± 0·72	24·74 (27·88, 21·60)
22:1	N.D.	N.D.	2·11 ± 0·36	N.D.	N.D.	N.D.	N.D.
20:5 (n-6) + 22:2	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0·93 (0·83, 1·03)
22:3	0·75 ± 0·35	N.D.	N.D.	1·80 ± 0·06	N.D.	N.D.	0·76 (0·82, 0·70)
22:4	2·64 ± 0·25	N.D.	N.D.	3·53 ± 1·22	N.D.	N.D.	5·75 (6·65, 4·84)
22:5 (n-6)	0·51 ± 0·05	N.D.	N.D.	N.D.	N.D.	N.D.	0·76 (0·72, 0·81)
22:5 (n-3)	0·52 ± 0·07	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
23:0	N.D.	N.D.	1·56 ± 0·16	N.D.	N.D.	N.D.	N.D.
24:0	N.D.	N.D.	10·08 ± 1·28	N.D.	N.D.	N.D.	N.D.
24:1	N.D.	N.D.	7·19 ± 0·62	N.D.	N.D.	N.D.	N.D.

Values are the means ± s.e.m. Figures are expressed as a % of the total fatty acid composition in each individual phospholipid class. Numbers in parentheses are the observed values from each of two experiments. PC = phosphatidylcholine, LPC = lysophosphatidylcholine, SM = sphingomyelin, PS = phosphatidylserine, PA = phosphatidic acid, PI = phosphatidylinositol, PE = phosphatidylethanolamine. * This value is representative of 22:0 in sphingomyelin as levels of 20:3 (n-3) in this lipid are negligible.
N.D. = not detectable.

fractions were enriched in polyunsaturated fatty acids, particularly in linoleic acid, arachidonic acid and adrenic acid. In contrast, the fatty acid profile of cholesteryl esters appeared to be relatively low in polyunsaturated fatty acids, with 64% of the total fatty acid consisting of saturated fatty acids. All the neutral lipid classes contained measurable levels of adrenic acid.

The fatty acid profiles of the individual phospholipids (Table 5) revealed that although the proportion of saturated to unsaturated fatty acids was similar in phosphatidylcholine, phosphati-

dylserine and phosphatidic acid, 66% of phosphatidylethanolamine fatty acids were unsaturated. However, in all these phospholipids, more than 20% of their total fatty acid composition was polyunsaturated fatty acid; 21% in phosphatidylserine, 24% in phosphatidic acid, 25% in phosphatidylcholine and 36% in phosphatidylethanolamine. With the exception of sphingomyelin, there was an enrichment of arachidonic acid in all the phospholipid classes. Adrenic acid also occurred in phosphatidylethanolamine and to a lesser extent in phosphatidylserine and phosphatidylcholine. Considerable levels of linoleic acid were found in phosphatidic acid but lower levels were also found in other phospholipids. Pig oocyte sphingomyelin was characteristically rich in long-chain saturated and monounsaturated fatty acids. Although oocyte phosphatidylinositol was rich in arachidonic and stearic acids, it also contained an unusually high proportion of palmitic acid.

The aldehyde composition of phosphatidylethanolamine plasmalogen revealed that the monounsaturated 18 carbon aldehyde was the most predominant (39·73%), with the saturated aldehydes being more or less equally distributed (21·7% for 16:0 aldehyde and 27·5% for 18:0 aldehyde) while the 16:1 aldehyde represented the lowest proportion (11·01%) ($n = 2$ in all cases). Aldehydes were negligible in phosphatidylcholine.

Discussion

The results of this study provide the first detailed lipid analysis of purified immature mammalian oocytes. While the lipid profile is in many respects similar to that of other cell types examined, there are some striking differences which may have a significant regulatory function on the resumption of meiosis.

Analysis of the total lipid content of pig oocytes revealed that triacylglycerol is the major constituent. This observation is consistent with results obtained in a previous histochemical study of cat and dog oocytes (Guraya, 1965). The molar ratio of phospholipid to cholesterol in immature pig oocytes is 1·63. This ratio influences the physical properties of the bilayer as increasing proportions of cholesterol lead to increased ordering of membrane acyl chains and an elevation in basal adenylate cyclase activity (Sinensky, Minneman & Molinoff, 1979). It would therefore be of interest to determine whether the phospholipid to cholesterol ratio changes during the maturation process. Most of the phospholipids in the immature oocyte occur in proportions similar to those found in other mammalian cells (White, 1973). However, there is a proportionately low level of phosphatidylethanolamine in pig oocytes (16·41% total phospholipid; Table 1); in most tissues, this phospholipid constitutes between 20 and 30% (White, 1973). Lower levels of phosphatidylethanolamine may be accounted for by an increased methylation of this phospholipid to phosphatidylcholine (Hirata *et al.*, 1979; Mozzi & Porcellati, 1979). This is supported by the relatively high amount of phosphatidylcholine in oocytes, resulting in a proportionately low ratio of phosphatidylethanolamine to phosphatidylcholine. A regulatory role for phospholipid methylation has been implicated in the elevation of intracellular adenosine 3′,5′-cyclic monophosphate (cAMP) as stimulation of β-adrenergic receptors results in methylation of phosphatidylethanolamine to phosphatidylcholine, preceding the coupling of receptors with adenylate cyclase (Hirata *et al.*, 1979). This is of particular interest regarding the immature mammalian oocyte, since high levels of cAMP are associated with maintenance of meiotic arrest (Racowsky, 1985). Although these results initially suggest that the phospholipid environment of the immature oocyte is conducive to maintaining high levels of cAMP, there is a high level of lysophosphatidylcholine in these cells, which has been demonstrated to have a potent inhibitory effect on both basal (Zwiller, Ciesielski-Treska & Mandel, 1976) and hormone-stimulated (Houslay & Palmer, 1979) adenylate cyclase activity. Lysophosphatidylcholine constitutes 4·68% of the total oocyte phospholipid, whereas in most tissues, it does not exceed more than 1% (White, 1973). An elevation in the amount of lysophosphatidylcholine may reflect an

increase in the activity of phospholipase A_2. This suggestion is supported by the observation that the free fatty acid composition of pig oocytes is strikingly similar to the fatty acid composition of phosphatidylcholine (see Tables 4 and 5), indicating a high turnover rate of this particular phospholipid. Further evidence for phospholipase A_2 activity has been demonstrated in a previous study in which rapid deacylation and turnover of phosphatidylcholine were observed in the preimplantation mouse embryo (Pratt, 1980). Alternatively, it could be argued that high lysophosphatidylcholine levels may occur due to a reduction of lysophospholipid acylation. Pig oocytes may therefore exhibit a decreased activity of acyl CoA ligase and/or acyl CoA lysophospholipid acyltransferase, which respectively catalyse esterification of fatty acids to CoA with subsequent transesterification of the acyl group to lysophospholipid (Dise, Goodman & Rasmussen, 1980).

Analysis of the fatty acid profiles of the individual lipid classes has revealed that free fatty acids in pig oocytes are probably derived from the ovarian follicular fluid, as the free fatty acid composition resembles that of pig follicular fluid rather than that of pig serum (Yao, Ryan & Dyck, 1980): the proportion of free saturated fatty acid is 46% in the oocyte compared with 41% in follicular fluid and 37% in serum, and free polyunsaturated fatty acids constitute 25% in the oocyte, 23% in follicular fluid and only 15% in serum. The unusually high level of free arachidonic acid in pig oocytes (12·6% total free fatty acid compared with 8·3% and 1·8% in follicular fluid and serum, respectively) may be accounted for in this manner, although it may result from an elevation in phospholipase A_2 activity as discussed above.

The fatty acid composition of pig oocyte neutral lipid is somewhat dissimilar to that of pig follicular fluid and serum (Yao *et al.*, 1980). The major fatty acid in all the oocyte neutral lipid classes is palmitic acid, followed by oleic acid, whereas unsaturated fatty acids constitute the major portion of follicular fluid and serum neutral lipid. The high levels of palmitic and oleic acids found in oocyte neutral lipids may serve as a storage pool of metabolic precursors, as both these fatty acids are required as substrates for fatty acid elongation and desaturation (Jeffcoat, 1979).

The most striking observation from the individual lipid fatty acid profiles, is that of an enrichment of linoleic (18:2 n-6), arachidonic (20:4 n-6), and adrenic (22:4 n-6) acids particularly in the free fatty acid, triacylglycerol and phosphatidylethanolamine fractions. This observation is consistent with studies on whole pig ovaries (Holman & Hofstetter, 1965) which demonstrated considerable quantities of linoleic, arachidonic and adrenic acids in phospholipid and neutral lipid classes.

Enrichment of arachidonic acid in esterified sites may be indicative of the capacity of oocytes to synthesize prostaglandins, thromboxanes and leukotrienes, as arachidonic acid, which is derived from linoleic acid, is the primary substrate for cyclooxygenase and lipoxygenase (Samuelsson, 1983). Although it has not yet been established whether mammalian oocytes can actively synthesize these products, it appears that prostaglandins are involved in follicular function (Armstrong, 1981).

The presence of adrenic acid in pig oocytes is of interest since, unlike linoleic and arachidonic acids, this fatty acid is not prevalent in other cells. Adrenic acid may be used as a substrate for cyclooxygenase and lipoxygenase (Tobias, Vane & Paulsrud, 1975; Cagan, Zusman & Pisano, 1979; Sprecher, Van Rollins, Sun, Wyche & Needleman, 1982; Van Rollins, Horrocks & Sprecher, 1985), and hence the possibility exists that it plays a central role in the production of dihomo-derivatives of prostaglandins and hydroxy fatty acids by the oocyte. Such a role in pig oocytes must be established, however, since conversion of adrenic acid to dihomo-prostaglandins and hydroxy fatty acids has only been demonstrated in sheep seminal vesicle microsomes (Tobias *et al.*, 1975), rabbit renal medulla (Cagan *et al.*, 1979; Sprecher *et al.*, 1982) and human platelets (Van Rollins *et al.*, 1985), although this fatty acid is present in several additional tissues. Since dihomo-prostaglandin E-2 stimulates adenylate cyclase activity (Sprecher *et al.*, 1982) and as cAMP classically has been associated with the maintenance of meiotic arrest in mammalian oocytes (reviewed by Racowsky, 1985), it is possible that adrenic acid plays a role in the regulation of this process. Alternatively, adenylate cyclase may be modified by the degree of unsaturation in the oolemma, since

incorporation of unsaturated fatty acids has been associated with stimulation of the activity of this enzyme in other cells (Engelhard, Glaser & Storm, 1978; Colard, Kervabon & Roy, 1980; Poon, Richards & Clark, 1981). With the exception of sphingomyelin, immature pig oocyte phospholipids are enriched in unsaturated fatty acids, particularly phosphatidylethanolamine (Table 4). Experiments are now in progress to determine the direct effects of fatty acids on spontaneous maturation of pig oocytes.

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