Synthesis and secretion of lipids by bovine oviduct mucosal explants

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The objectives of this study were to determine the types of lipid synthesized and secreted by the bovine oviduct, and to determine whether lipid synthesis and secretion varied with stage of the ovarian cycle and oviductal region. Oviduct explant cultures were prepared from cows killed during either the follicular or luteal stage of the oestrous cycle. Both stage of ovarian cycle and oviductal region affected lipid synthesis by oviductal explants in vitro. More lipid was synthesized by explants from follicular than from luteal-stage cows. Ampullar explants synthesized the greatest quantity of total lipid, followed by the preampulla and isthmus. Separation of extracted lipids from cultured tissue by high performance thin-layer chromatography (HPTLC) resolved phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, cardiolipin, free cholesterol, free fatty acid, triglyceride and esterified cholesterol, all of which were synthesized during culture. The ampulla synthesized significantly more phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol than did the other regions. Culture supernatants from ampullary explants contained the most newly synthesized cholesterol when compared with other regions. The histochemical location of neutral lipid droplets in the epithelium of cultured explants paralleled the localization of radioactivity in autoradiographs of explant extracts. The results suggest that the oviduct synthesizes a variety of lipids, and that some of these are released into culture supernatants.

Introduction

Lipid droplets have been detected in epithelial cells of the bovine oviduct and their numbers are reported to fluctuate with endocrine status (Witkowska, 1979) or remain constant (Wordinger et al., 1977; Henault et al., 1993). Although their origin and function in the oviduct are unknown, lipid droplets vary in composition, size and function in other cell types. They are produced in large quantity by mammary epithelia and consist mainly of triglyceride (Timmen and Patton, 1988; Valivullah et al., 1988). Lipid droplets consisting of mostly esterified cholesterol, surrounded by pools of free cholesterol, can accumulate in macrophages incubated with low density lipoproteins (McGookey and Anderson, 1983). Lipid droplets have also been reported in the intestinal mucosa, heart, liver and adipose tissue (Fawcett, 1967). These droplets perform various functions such as retinyl ester storage in liver stellate cells (Yamada et al., 1987; Moriwaki et al., 1988), triglyceride storage in adipose tissue (Fawcett, 1967), and pathological accumulation of esterified and free cholesterol in smooth muscle cells and macrophages during formation of atherosclerotic plaques (Minor et al., 1989).

The lipid composition of fluid recovered from the lumen of the bovine oviduct differs considerably from that of blood serum (Killian et al., 1989). Oviductal epithelia are known to direct fluxes of macromolecules from the vasculature to the lumen, indicating that some molecules found within the epithelial cells

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Received 31 July 1992.
rinsed in fresh transport medium, trimmed free of fat and connective tissue, and divided into preampullary, ampullary and isthmic regions. Oviducts were opened longitudinally, and 1 g of tissue was taken from each region and cut into 5 mm square explants. Explants were placed on scored areas of 35 mm dishes with the mucosal side up. Two dishes containing 0.5 g of tissue were prepared for each region. Explants were incubated at 39°C (5% CO₂, in air) with minimal culture medium (3.5 ml) to cover the bottom of the dish, but still allow air exchange to the explants. The protein-free culture medium (RDG) used in these experiments was composed of equal parts of RPMI-1640 (Sigma) and Dulbecco's Modified Eagle's Medium (Gibco Laboratories, Grand Island, NY) plus glucose (3 g l⁻¹), L-glutamine (0.292 g l⁻¹), insulin–transferrin, sodium selenite medium supplement (Sigma), epidermal growth factor (Sigma), sodium bicarbonate (2.85 g l⁻¹) and 1% antibiotic–antimycotic. The culture medium used at the initiation of the experiments contained [¹⁴C]acetate (5 μCi ml⁻¹) as substrate for lipid synthesis (Amersham Corporation, Arlington Heights, IL). After incubation for 6 h, one dish from each region was sampled. All medium from this dish was collected, centrifuged at 700 g for 15 min to remove cellular debris, and frozen. Tissue explants were washed in PBS and a portion of the tissue was frozen for histochemistry and tissue autoradiography. The remaining tissue was reweighed and frozen until later analysis. The supernatant was collected from the other dish for each region; the explants were washed; and 3.5 ml of medium without [¹⁴C]acetate was added. After 14 h, the medium and tissue from these dishes were sampled as before. Explants of bovine corpus luteum, a tissue known to synthesize lipids (Gwynne and Strauss, 1982), were cultured as described above and lipids were extracted and separated by HPTLC for comparison with oviductal tissue.

Quantitation of total lipid

The entire tissue aliquot from each time point and for each oviduct region was suspended in 2 ml of distilled water and completely homogenized by using a Kinematica tissue homogenizer (Brinkmann Instruments Co., Westbury, NY). Homogenates and 2.5 ml of supernatant medium from each time point were extracted by the Folch wash procedure (Folch et al., 1957). The lower phase was collected, dried completely and lipids were dissolved in 1 ml of 2:1 (v:v) chloroform:methanol. An aliquot (100 μl) of this extract was dried and then mixed with 10 ml of Ecosint (National Diagnostics, Manville, NJ) scintillation cocktail and radioactivity of the samples was measured in a Beckman liquid scintillation counter. Background radioactivity was determined by quantitation of the radioactivity (d.p.m.) present in 10 ml of scintillation cocktail (16.0 ± 0.4 d.p.m.). These values were assumed to be correct and any quenching accounted for, as when [¹⁴C]toluene was added to 10 of the samples and radioactivity measured again. 97.7 ± 3.6% of the added radioactivity was detected.

High performance thin-layer chromatography

Aliquots of extracts containing 2500 d.p.m. (tissue) and 750 d.p.m. (culture supernatants) were dried, rediluted in chloroform and kept in air tight vials under nitrogen at 0°C until analysis. Aliquots of extracts were dried completely and diluted in 8 μl chloroform. After rotating the tube to dissolve any lipids adhering to the sides, the remaining volume (3–4 μl) was applied to the preadsorbent region of a Whatman silica HPTLC plate (Alltech North/Applied Science Labs., State College, PA) using a disposable microcapillary tube. Before sample application, plates were activated by heating for 1 h at 110°C. The plates were then washed in mobile phase A (CHCl₃:MeOH:HOAc:H₂O (25:15:2:1)) and allowed to dry. After application of the samples, plates were developed by a modified two-step procedure for separation of neutral lipids and phospholipids (Skipski and Barclay, 1969). Plates were predeveloped twice using mobile phase A to move the applied samples to the edge of the preadsorbent region. After drying, the plates were developed in mobile phase B (petroleum ether/ethyl ether:HOAc (80:20:1)) to the top edge of the plate to separate the neutral lipids. After drying, the plates were dipped for 3 s in a solution of 10% CuSO₄ in 8% phosphoric acid, dried and charred at 140°C for 40 min (Bitman and Wood, 1982). This charring procedure resulted in adequate visualization of all components of diverse lipid mixtures and did not cause the plate to become highly hydroscopic after treatment. Because the coloration faded slightly over time, the plates were photographed immediately after charring with LPD-4 positive film (Kodak, Rochester, NY) and a Nikon 35 mm camera. After photographing, the plates were sprayed with Resolution TLC autoradiography enhancer (EMCorp, Chestnut Hill, MA) and allowed to dry for 15 min. Plates were then placed in optical contact with X-OMAT X-ray film (Kodak) and exposed for 7 days (tissue samples) or 14 days (supernant samples) at −70°C. The X-ray film was then developed in GBX developer (Kodak) at room temperature for 5 min with intermittent agitation, washed in water for 30 s, fixed in GBX fixer for 4 min and washed for 10 min. Autoradiographs were photographed on a DuPont Cronex Lighting Plus pad with LPD-4 positive film (Kodak).

Spots on TLC plates that corresponded to areas of radioactivity detected by autoradiography were scraped onto weighing paper and adsorbent was placed into scintillation vials. After addition of scintillation fluid, the radioactivity of individual radioactive lipids was measured.

Statistical analyses

Analysis of variance (SAS, 1985) was performed on radioactivity (d.p.m. values) of extracts and individual lipids using the model Y = stage cow(stage) time reg stage*time stage*reg time*reg stage*time*reg, with stage = ovarian cycle stage, time = culture incubation time and reg = oviductal region. Cow(stage) was specified as the error term to use when testing the main effect stage. Least square means were also obtained for information about higher level interactions and Bonferroni comparisons were used to determine significance among comparisons within the main effects.

Histochemistry

Frozen oviductal tissues from the various regions and experimental time points were prepared for histochemical detection of neutral lipids with Oil Red O (EM Sciences, Fort Washington, PA). After staining, dried slides were dipped in...
Table 1. Total lipid synthesis by bovine oviductal explants in vitro

<table>
<thead>
<tr>
<th>Source</th>
<th>Follicular*</th>
<th></th>
<th>Luteal*</th>
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<tbody>
<tr>
<td></td>
<td>6 h</td>
<td>20 h</td>
<td>6 h</td>
<td>20 h</td>
</tr>
<tr>
<td>Tissue (10^6 d.p.m. g⁻¹)</td>
<td>P 2.3 ± 0.6ᵃ</td>
<td>A 3.5 ± 0.4ᵇ</td>
<td>1.2 ± 0.8ᵇ</td>
<td>P 0.9 ± 0.02</td>
</tr>
<tr>
<td>Culture medium (10⁴ d.p.m. ml⁻¹ g⁻¹ of tissue)</td>
<td>P 6.2 ± 1.1ᵃ</td>
<td>A 11.7 ± 5.4ᵇ</td>
<td>I 9.3 ± 2.3ᵇ</td>
<td>P 0.1 ± 0.7ᵇ</td>
</tr>
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<td>n</td>
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<td>2</td>
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</tbody>
</table>

*Follicular and luteal refer to the ovarian cycle stage of the experimental animals.
Values with different superscripts within a row and section are significantly different (P < 0.05).
P: preampulla; A: ampulla; I: isthmus.

Fig. 1. Thin layer chromatograph of luteal phase oviduct explant tissue extracts. Lane 1: mixed standards; lanes 2 and 3 cow 1: explants of bovine corpus luteum at 6 and 20 h; lanes 4 and 5 cow 1 preampullary tissue at 6 and 20 h; lanes 6 and 7 cow 1 ampullary tissue at 6 and 20 h; and lanes 8 and 9 cow 1 isthmic tissue at 6 and 20 h. CE: cholesteryl ester; TG: triglyceride; FA: fatty acid; C: cholesterol; PC: phosphatidyicholine.

Results

Total lipid synthesis by oviductal explants

There was a significant effect of stage of ovarian cycle (P < 0.05) and oviductal region (P < 0.01) on the amount of radioactivity incorporated into tissue lipids (d.p.m. g⁻¹; Table 1). Explants from cows at the follicular stage contained more radioactivity (P < 0.05) than did luteal explants. Overall, and in follicular tissue explants incubated for 6 h, the ampullary region had more radioactive lipids than did the isthmus (P < 0.05) and the preampulla was intermediate to and not significantly different from the other regions. Oviductal explants from cows at the follicular stage that were incubated for 20 h demonstrated similar amounts of radioactivity in preampullary and ampullary tissues which were significantly higher than those of isthmic tissue (P < 0.05). Incorporation of radioactive acetate by luteal oviductal explants was similar among regions at both 6 and 20 h.

The radioactivity of culture supernatants from follicular explants (d.p.m. ml⁻¹, normalized by tissue weight) showed a significant effect of region at 20 h which paralleled that of the melted photographic emulsion (Ilford, K.5D), dried and packed in lightsafe boxes with desiccant. Emulsion was exposed for 4 days at 4°C and developed in 1:1 Dektol (Kodak) for 2 min, followed by 4 min in Rapid Fixer diluted for plates and films (Kodak). Coverslips were mounted on washed slides with glycerol–gelatin and sections were observed using a Leitz Ortholux microscope. Stained tissue sections were photographed with Ektachrome 200DL film (Kodak) using a Leitz Orthomat automatic microscope camera.
results from 6 h tissue. Overall, the media from ampullary cultures contained significantly more radioactivity than that of the isthmus \((P < 0.05)\). Overall and at 20 h (follicular explants) the d.p.m. recorded for the preampullary supernatants were intermediate to and not significantly different from the other regions (Table 1).

It is unlikely that the radioactivity measured in the extracts was the result of unincorporated \(^{14}C\)acetate, because extraction of culture medium plus \(^{14}C\)acetate (with no exposure to cells) resulted in an organic phase with radioactivity that was not greater than background values. The d.p.m. equivalent to those present in the culture medium were recovered in the aqueous phase.

Fig. 2. (a) Thin layer chromatograph of follicular phase oviduct explant tissue extracts. Lanes 1 and 8: mixed standards; lanes 2 and 3 cow 4 preampullary tissue at 6 and 20 h; lanes 4 and 5 cow 4 ampullary tissue at 6 and 20 h; lanes 6 and 7 cow 4 isthmic tissue at 6 and 20 h. (b) Autoradiograph of the TLC plate. CE: cholesteryl ester; TG: triglyceride; FA: fatty acid; C: cholesterol; PC: phosphatidylcholine.
Table 2. Individual lipids (expressed as % of total) synthesized by bovine oviductal explants in vitro

<table>
<thead>
<tr>
<th>Lipids</th>
<th>6 h</th>
<th>20 h</th>
<th>6 h</th>
<th>20 h</th>
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<tr>
<td></td>
<td>P</td>
<td>A</td>
<td>I</td>
<td>P</td>
</tr>
<tr>
<td>CE</td>
<td>4.0</td>
<td>2.0</td>
<td>4.0</td>
<td>3.0</td>
</tr>
<tr>
<td>±1.0</td>
<td>±1.0</td>
<td>±1.0</td>
<td>±1.0</td>
<td>±1.0</td>
</tr>
<tr>
<td>TG</td>
<td>5.0</td>
<td>4.0</td>
<td>6.5</td>
<td>8.5</td>
</tr>
<tr>
<td>±1.0</td>
<td>±1.9</td>
<td>±0.5</td>
<td>±1.5</td>
<td>±1.5</td>
</tr>
<tr>
<td>FA</td>
<td>9.5</td>
<td>6.5</td>
<td>12.0</td>
<td>6.5</td>
</tr>
<tr>
<td>±3.5</td>
<td>±1.5</td>
<td>±2.0</td>
<td>±0.5</td>
<td>±0.5</td>
</tr>
<tr>
<td>CH</td>
<td>16.0</td>
<td>9.0</td>
<td>15.5</td>
<td>19.0</td>
</tr>
<tr>
<td>±7.0</td>
<td>±3.0</td>
<td>±0.5</td>
<td>±1.5</td>
<td>±1.5</td>
</tr>
<tr>
<td>CL</td>
<td>15.5</td>
<td>15.5</td>
<td>15.5</td>
<td>14.5</td>
</tr>
<tr>
<td>±2.5</td>
<td>±4.5</td>
<td>±0.5</td>
<td>±1.5</td>
<td>±1.5</td>
</tr>
<tr>
<td>PE</td>
<td>8.0</td>
<td>14.0</td>
<td>9.5</td>
<td>8.5</td>
</tr>
<tr>
<td>±3.0</td>
<td>±0.0</td>
<td>±0.5</td>
<td>±3.5</td>
<td>±1.5</td>
</tr>
<tr>
<td>PS + PI</td>
<td>10.0</td>
<td>14.0</td>
<td>9.0</td>
<td>9.0</td>
</tr>
<tr>
<td>±4.0</td>
<td>±3.0</td>
<td>±2.0</td>
<td>±4.0</td>
<td>±3.0</td>
</tr>
<tr>
<td>PC</td>
<td>32.0</td>
<td>35.5</td>
<td>27.5</td>
<td>30.5</td>
</tr>
<tr>
<td>±6.0</td>
<td>±8.5</td>
<td>±4.5</td>
<td>±10.5</td>
<td>±4.5</td>
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<tr>
<td>n</td>
<td>2</td>
<td>2</td>
<td>2</td>
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</table>

Values are means ± SEM.

*Follicular and luteal refer to the ovarian cycle stage of the experimental animals.

+Values with different superscripts within a row and section are significantly different (P < 0.05).

CE: cholesterol ester; TG: triglyceride; FA: fatty acid; CH: cholesterol; CL: cardiolipin; PE: phosphatidylethanolamine; PS: phosphatidylserine; PI: phosphatidylinositol; PC: phosphatidylcholine.

phase of the extraction. In addition, spots were not seen at the origin (the location of free acetate) on autoradiographs of samples from these experiments.

Synthesis of individual lipids by the oviduct

Thin layer chromatography of lipids from oviductal tissue resolved five different phospholipids and at least four neutral lipids (Fig. 1). Phosphatidylinositol and phosphatidylserine had similar K_{r} values, were located above and adjacent to phosphatidylcholine and were not always separated. Phosphatidylinositol and phosphatidylserine were combined when the radioactivities of the individual lipids were measured. The thin layer chromatograms (Fig. 2a) and corresponding autoradiographs (Fig. 2b) of tissue extracts were nearly identical for all samples. Both neutral lipids and phospholipids were synthesized by all oviducral regions (Table 2). The results of this study showed no significant effects of time, stage, region or any higher level interaction on the synthesis of cholesteryl ester, triglyceride, cholesterol, cardiolipin or phosphatidylcholine (PC). There was an overall effect of time on the synthesis of free fatty acid (P < 0.05) and phosphatidylinositol plus phosphatidylserine (PI + PS) (P < 0.01) with more radioactive lipids present at 6 h than at 20 h. There was also an overall effect of oviducral region on the synthesis of phosphatidylethanolamine (PE) (P < 0.01) and PI + PS (P < 0.05). The amputla synthesized more PE and PI + PS (P < 0.05) than did the isthmus, and synthesis of these lipids by the preampulla was intermediate to and not different from the other regions. When explant regions within stage and time subgroups were considered, amputlar explants from only follicular phase cows produced significantly more PE than did the other two regions. This was also true for the amount of PI + PS at 6 h but not at 20 h. Luteal explants also showed regional differences in amounts of PI + PS. At 6 h, the amputlar oviduct had produced more PI + PS, differing significantly from the isthmus, but not from the preampulla. At 20 h, most PI + PS was measured in luteal preampullary explants which differed significantly from the ampulla, but not from the isthmic regions.

Lipids in culture supernatants did not resolve well on thin layer chromatographs. This may have resulted from the large amount of extract that had to be concentrated to load enough radioactivity to affect the X-ray film. Because the resolution of individual phospholipids was poor in culture supernatants, they were analysed as total phospholipid.

The thin layer chromatographs of culture supernatants from the different oviducral regions were similar to each other (data not shown) and contained mostly neutral lipids. Autoradiographs of these chromatograms displayed only one faint band appearing in the same location as cholesterol. Autoradiographs indicated differences in the amount of de novo synthesis of cholesterol resolved for the different culture times and oviducral regions. However, when the radioactivity of the individual supernatant lipids from these experiments was measured, there were no significant differences due to ovarian cycle stage, time, region or any higher level interaction.
Tissue autoradiography

The autoradiographs of sectioned oviductal tissue from these experiments, stained for neutral lipids, showed silver grain accumulation exclusively in the epithelium of all oviductal regions. No differences were apparent in the number of silver grains present in tissues from different cows or regions. Serial sectioning for histochemistry and autoradiography showed that the location of the silver grains was similar to the histochemical staining pattern of neutral lipid in the tissues. Comparison of alternating serial sections from the preampulla indicated that neutral lipid staining with Oil Red O (Fig. 3a) and autoradiograph silver grains (Fig. 3b) occupied similar tissue locations.

Discussion

This study demonstrates that the bovine oviduct is capable of significant de novo synthesis of a variety of lipids in vitro, and that this synthesis is affected by the stage of the ovarian cycle. These observations are in agreement with the report that

Fig. 3. (a) Oil Red O histochemical localization of neutral lipid droplets in cultured luteal phase explant from the oviductal preampulla. Note the concentration of Oil Red O staining in the epithelium (arrows). Magnification × 360. (b) Autoradiography and Oil Red O staining done simultaneously on serial section. Note similar location of lipid droplets and silver grains. Magnification × 360.
cellular lipids and dehydrogenase activity increased in oviduct epithelial monolayers in response to oestradiol treatment (Witkowska, 1979), and the increase in total lipid present in oviductal fluid recovered by cannula around oestrus (Killian et al., 1989).

Regional differences in the relative ability of oviducal explants to synthesize lipid were also apparent. Ampullar explants incorporated more $[^{14}C] $acetate into lipid than did those from the isthmus. Autoradiography of the explants suggests that most of the lipid synthesis occurred in the epithelium. However, the relative amount of epithelium present does not necessarily predict the amount of lipid synthesis observed. Although the preampulla had the greatest amount of epithelium compared with the other oviducal regions, it was intermediate to the ampulla and isthmus, when incorporation of $[^{14}C] $acetate into lipids was considered. This may be due to relative differences in the ciliated and secretory cells present in each region, and differences in their capacities to synthesize lipids. It is known that the preampullary epithelium is composed of a higher percentage of ciliated cells than the ampulla (Nilsson and Reinus, 1969). However, in the present study with frozen tissue sections, it was not possible to determine whether the concentration of silver grains in the autoradiograph, or lipid detected histochemically in the epithelium, was correlated with a particular cell type. Nevertheless, supernatants from the ampullary explants contained more radioactivity than did those from the isthmus, a result which paralleled the lipid synthesized by each region.

The bovine oviduct can synthesize a wide variety of lipids from $[^{14}C] $acetate within 6 h. The observation that more isotope was incorporated into fatty acids and PS + PI at 6 h than at 20 h could be explained if fatty acids were synthesized initially from acetate and then used as components of other lipids having fatty acid moieties. Cultured bovine mammary cells respond similarly when exposed to $[^{14}C] $acetate, and produce triglycerides as the main endproduct (Kinsella and McCarthy, 1968). Phosphatidylserine may also be converted to phosphatidylethanolamine by the action of phosphatidylserine decarboxylase (Vance, 1985).

The predominant lipid synthesized and detected in culture supernatants was cholesterol. On the basis of autoradiographic analyses of cultured tissues, culture supernatants and the localization of OI Red O staining neutral lipids, it is likely that the cholesterol was synthesized by the epithelium and mostly retained by the cells. The present study was unable to determine, however, whether the synthesized cholesterol found in the explant supernatants was in droplet form, or merely the result of nonspecific membrane loss. Accumulation of lipid droplets in oviduct monolayers and their passage as intact droplets into the culture medium has been reported (Witkowska, 1979). Similar observations could have been made with oviduct explant cultures in the study reported here if the culture period had been extended. Thin layer chromatographs of culture supernatants have resolved significant amounts of the same lipids that were detected in lipid droplets isolated from oviductal epithelial cells (Henault et al., 1992).

Because the oviduct is actively involved in the synthesis of lipid, and this activity differs with the stage of the ovarian cycle and oviducal region, it is possible that these events are important in reproduction. The membrane cholesterol:phospholipid ratio has been implicated as a factor in sperm capacitation (Davis et al., 1980) and the ability to undergo the acrosome reaction (Langlais and Roberts, 1985). A decrease in the plasma membrane cholesterol:phospholipid ratio is thought to favour capacitation and the acrosome reaction (Davis et al., 1979). Conversely, exposure of spermatozoa to cholesterol via phospholipid vesicles inhibits fertility of rabbit cauda epididymal spermatozoa. Desmosterol sulfate is a potent inhibitor of capacitation in vitro (Bleau et al., 1975) and cholesterol sulfate has been implicated as a membrane stabilizer and enzyme inhibitor during epididymal sperm maturation (Langlais et al., 1981). Cholesterol-3-sulfate was also found to inhibit fertility of capacitated rabbit spermatozoa in vivo (Fayrer-Hosken et al., 1987). Several studies have demonstrated that exogenous lipids can affect sperm membranes. Exogenous phospholipids become associated with rat, boar and bull spermatozoa (Evans and Setchell, 1978) and incubation with various lysosphospholipids accelerated capacitation in guinea-pig (Fleming and Yanagimachi, 1981) and bull (Wheeler and Seidel, 1989) spermatozoa.

The synthesis of fatty acids by the bovine oviduct is also likely to be important in early embryo development, since fatty acids are essential for embryo survival in other species (Kane, 1979; Quinn and Whittingham, 1982). Mouse embryos have also been shown to synthesize phospholipids (Pratt, 1980, 1982). Improvements of in vitro techniques have been made by mimicking the environment in vivo. The present study has defined more clearly the lipids that are present in the bovine oviducal environment. Future studies involving the addition in vitro of physiological concentrations of lipid components synthesized by the oviduct may lead to improved systems for fertilization and embryo development in vitro.

The authors thank R. H. Hammerstedt for his modifications of lipid separation by HPTLC (Skipski and Barclay, 1969), J. Alvarez for his help with HPTLC, and Y. Akin of the Penn State Statistical Consulting Service for assistance with statistical analyses. This research was supported in part by USDA Grants 85-CRCR-1-1841 and 89-372-4771.

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