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Characterization of a Polarized Porcine Uterine Epithelial Model System[1]

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ABSTRACT

A porcine uterine epithelial cell (pUE) culture system that retains structural and functional properties of the surface epithelium in vivo was developed. Uterine luminal epithelial cells were isolated after pancreatin-dispase enzymatic release of epithelium from hysterectomized gilts. Cells were seeded on Millicell filters precoated with Matrigel in 24-well plates and subsequently allowed to proliferate to confluence. Purity of the isolation was confirmed by the presence of > 99% cytokeratin-positive cells. Epithelial cells became polarized in vitro and compared favorably in morphology to uterine epithelial cells in situ once a transepithelial resistance of > 600 Ωcm^2 was established. Microscopic analysis confirmed the presence of a simple columnar epithelium with prominent microvilli on the apical cell surface and a well-developed junctional complex containing tight junctions, belt and spot desmosomes, and interdigitating lateral cell processes. Indirect immunofluorescence of the tight junction-associated protein, ZO-1, indicated the formation of tight junctional complexes in the subapical region of the polarized cells. Functional polarity of epithelial cultures was also verified by 1) electrical resistance measurements, 2) basal preference for the secretion of prostaglandins $F_{2\alpha}$ and E_2, 3) apical preference for the release of ^{35}S-methionine-incorporated secretory proteins, and 4) apically and basally distinct secretory protein profiles. Steroid treatment (estrogen, progesterone, or estrogen plus progesterone) of the polarized pUE cells affected the release of radiolabeled methionine-incorporated secretory proteins. In addition, the protein profiles as compared to samples treated with fetal bovine serum or charcoal/dextran-stripped fetal bovine serum were altered. Steroid treatments did not alter the electrical resistance or the basal preference for prostaglandin secretion. This culture system may be useful for in vitro analysis of maternal recognition of pregnancy paradigms as well as the study of the direct actions of hormones, prostaglandin secretion, and epithelial-stromal interactions.

INTRODUCTION

The uterine endometrium in the pig responds to ovarian cues with orderly cycles of tissue proliferation, differentiation, glandular secretion, and prostaglandin production [1, 2]. The cyclic changes are programmed to be developmentally synchronous with blastocyst development so that blastocysts arrive in the uterus when histotroph production will support embryonic development and production of conceptus-derived estrogen [1, 2]. This embryonic signal is involved in preventing luteal regression by altering the production and release of luteolytic pulses of endometrial prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) [1, 2]. In addition, structural and functional alterations in the endometrial epithelium convert the pre-receptive epithelial cells to the "receptive" state, thereby allowing blastocyst attachment [3–8]. Even though luteal function has been maintained, a large number of embryos are lost at the stage of pregnancy when embryonic and uterine epithelial cells are closely apposed and cellular attachment is achieved between trophoblastic and endometrial epithelium [9, 10].

The goal of reducing early embryonic loss has led to the advancement of approaches for closely examining and experimentally manipulating cellular interactions occurring within tissue-level compartments of the uterus. The development of uterine endometrial epithelial cell model systems that exhibit structural and functional properties observed in situ, which can be regulated in vitro, would facilitate investigations of cellular responses to hormones as well as signaling mechanisms active during the periimplantation period [7, 11].

Insight into the specialized secretory functions and transport characteristics of uterine epithelial cells (including the vectorial secretion of proteins, proteoglycans, and prostaglandin) has resulted from study of rodent and ovine model systems in which structural and functional polarity are reestablished by growing cells on an extracellular matrix-coated filter [12–16]. Acquisition of polarity in this culture system provides access to apical and basal plasma membrane domains of the epithelial cells and to their respective secretions [17]. In the present study, methods developed for culturing polarized rodent cells were adapted for use with homogeneous populations of isolated porcine uterine epithelial cells (pUE). The development of polarity was evaluated by correlating electrical resistance measurements with epithelial cell morphology and development of continuous tight junctions. Analysis of prostaglandin production, incorporation of ^{35}S-methionine, and the secretion of proteins into apical and basal compartments of the culture system confirmed the polarity of these cells.

MATERIALS AND METHODS

Materials

Dulbecco's Modified Eagle's Medium with F-12 salts (DME/F12), estradiol-17β valerate (E_2), progesterone (P_4), fetal bovine serum (FBS), PBS, PBS without Ca^{2+} and Mg^{2+} (Ca/Mg-free PBS), trypsin, mouse monoclonal anti-cytokeratin antibody (clone K8.13, a broad-spectrum antibody recognizing both acidic and basic cytokeratins), goat biotinylated anti-mouse IgG, streptavidin-conjugated fluorescein, and general laboratory chemicals were purchased from Sigma Chemical Company (St. Louis, MO). Other reagents and sources included charcoal/dextran-stripped FBS (sFBS) from HyClone Laboratories, Inc. (Logan, UT), monoclonal anti-ZO-1 antibody from Chemicon (Temecula, CA), Matrigel from Collaborative Research Inc. (Bedford, MA), and Millicell-CM inserts (12 mm; CM; 0.4 μm pore size, microporous, transparent Biopore membrane) and Millicell-HA inserts (12 mm; HA; 0.45 μm pore size, mi-

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croporous, mixed esters of cellulose) from Millipore Corp. (Bedford, MA). The ^{35}S-methionine was obtained from Amersham Life Sciences (Arlington Heights, IL), and dispase was purchased from Boehringer Mannheim (Indianapolis, IN). All tissue culture supplies were purchased from Fisher Scientific (Pittsburgh, PA). Ampholytes and electrophoresis supplies were purchased from Bio-Rad Laboratories (Richmond, CA). Electron microscopy supplies were obtained from Electron Microscopy Sciences, Inc. (Fort Washington, PA).

Isolation of pUE Cells

Sexually mature gilts were observed for estrus (Day 0) in the presence of a boar. Day 15 pregnant gilts were hysterectomized, and the uterus was trimmed of the broad ligament and flushed with PBS to remove embryonic tissue. A 25- to 30-cm section was isolated, and the free ends were ligated. The uteri were transported to the laboratory in a plastic bag containing 500 ml PBS with 4% penicillin/streptomycin on ice.

The pUE cells were isolated according to procedures developed by Glasser and coworkers [12] with minor modifications. Briefly, the uterine lumen section was repeatedly flushed with approximately 40 ml of Ca/Mg-free PBS by inserting an 18-gauge needle into the lumen before trimming a ligated end of the uterine section, which was subsequently closed with surgical forceps. The pUE cells were released by luminal infusion of 4.8 mg/ml dispase with single-strength pancreatin in Ca/Mg-free PBS with an 18-gauge needle followed by incubation for 1 h at 37°C. During incubations, the uterine section was kept in a plastic bag containing approximately 300 ml PBS with 4% penicillin/streptomycin. The uterine section was gently massaged and flushed repeatedly with 40 ml Ca/Mg-free PBS, and the individual flushes were collected. Each flush was examined with an inverted microscope (Zeiss IM-35; Carl Zeiss, Inc., Thornwood, NY) for the presence of small rafts of pUE cells. Flushes that contained pure pUE cell rafts were pooled, washed, and subsequently pelleted by gentle centrifugation. Cells were then resuspended in DME/F12 medium and seeded into 75-cm^2 flasks and/or Millicell inserts and incubated at 37°C under an atmosphere of 5% CO_2:95% air. The medium was replaced the day after seeding and every other day thereafter.

Preparation and Evaluation of Polarized pUE Cells

Millicell inserts were thinly coated by placing 100 μl of a solution containing 100 μg/ml Matrigel diluted with medium onto the filter for approximately 5 min. Excess Matrigel was subsequently aspirated, leaving a moist residue that was allowed to air-dry under ultraviolet light in a laminar flow hood. Matrix-coated filters were rehydrated 1 h before use by being washed in culture medium containing 5% sFBS and were placed in 24-well plates. Approximately 500 μl of the epithelial cell suspension in medium was added to the inner chamber of the Millicell filter, and 500 μl medium was added to the outer chamber. The pUE cells were routinely cultured in phenol red-free DME/F12 medium (pH 7.4) with 5% (v:v) sFBS. Uterine luminal epithelial cells were grown to confluence on filter inserts, and electrical resistance measurements of the cell layer and filter inserts were obtained with a Millicell ERS system (Millipore Corp.) immediately after medium replacement on alternating days.

In one series of experiments, the electrical resistance measurements were correlated to morphological properties of the epithelial cell cultures, which were analyzed by light and electron microscopy. In a second series of experiments, the directional secretion of radiolabeled proteins and the secretion of prostaglandin E_2 and $F_{2\alpha}$ into apical and basal compartments of the Millicell inserts were evaluated in cultures treated for 10 days in phenol red-free DME/F12 (pH 7.4) containing 5% sFBS (v:v) without steroids, supplemented with either 10^{-8} M E_2, 10^{-6} M P_4, E_2 plus P_4 (E_2P_4), or medium with 5% complete FBS.

Purity of polarized pUE cells grown on filter inserts was evaluated using indirect immunofluorescence analysis of epithelial cytokeratins [12] and the tight junction-associated protein, ZO-1 [18]. Filter cultures were fixed in −20°C methanol for 10 min, rinsed in PBS, and blocked with 1% BSA/3% goat serum for 1 h at 37°C before incubation with the primary antibodies for 1 h at 37°C. After several washes in PBS containing 0.3% Tween-20, cultures were incubated with a biotinylated secondary antibody, washed repeatedly, and subsequently incubated with fluorescein-streptavidin. Portions of the filter measuring from 0.5 to 1.0 cm in diameter were placed on a glass slide and overlaid with a coverglass and mounting media prepared with 1% p-phenylenediamine in glycerol and PBS to retard quenching of fluorescence. Slides were viewed with a Zeiss Photomicroscope III and/or a Zeiss Axiovert equipped with a CCD camera and image-capturing software (CellScan; Scanalytics Inc., Bedford, MA), and were photographed on T-Max 3200 film (Kodak, Rochester, NY).

Additional microscopic analyses were performed on cross-sections of isolated uteri and polarized pUE cells on filter inserts. Preparations were fixed in 3% glutaraldehyde/2% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.3), post-fixed in 1% osmium tetroxide, dehydrated in a graded ethanol series, and embedded in Epon-Araldite. For bright-field light microscopy, 0.25-μm sections were stained with 1% toluidine blue dye and examined with a Zeiss Photomicroscope III. Ultrathin sections were stained with uranyl acetate and lead citrate, and examined with a Zeiss 10C transmission electron microscope. For scanning electron microscopy, the polarized pUE cells were fixed and dehydrated as described above, critical point-dried and sputter-coated with gold, and subsequently examined with a JEOL JSM 25S II scanning electron microscope (JEOL Inc., Peabody, MA).

^{35}S-Methionine Incorporation

The preferential secretion of ^{35}S-methionine-incorporated proteins from the apical and basal surfaces from nine polarized pUE filter cultures (i.e., three cultures per isolation, from three different animals) were analyzed. The polarized pUE filter cultures were treated for 10 days as described above. The cultures were washed with methionine-free DME/F12 medium, and 500 μl of ^{35}S-methionine (50 μCi/ml) in methionine-free DME/F12 medium was placed into both the apical and basal chambers for 24 h. Medium was collected from the apical and basal compartments of the filter system and dialyzed by ultrafiltration with Millipore Ultrafree-MC filters (3000 M_r cut-off). Non-dialyzable radioactivity was determined by scintillation spectroscopy. Labeled proteins secreted into the apical or basal chamber were determined for each filter culture. All samples were stored at −20°C until analyzed.

PAGE

The radiolabeled proteins obtained from the polarized pUE filter cultures were separated by two-dimensional SDS-PAGE (2D-PAGE) with 12.5% polyacrylamide gels as described previously [19]. Equal amounts (200 000 dpm) of radiolabeled proteins were subjected to isoelectric focusing (IEF) in the first dimension. Tube gels were equilibrated with IEF gel reducing buffer and run in the second dimension on 12.5% polyacrylamide gels. Gels were stained with 0.125% Coomassie Blue dye, destained, incubated in sodium salicylate, and dried. Fluorographs were obtained using X-OMAT AR (Kodak) film at −80°C.

RIA of Prostaglandins

Medium collected from the apical and basal compartments of the polarized pUE cells from three animals (i.e., three isolations) were analyzed in triplicate for PGE_2 and $PGF_{2\alpha}$ using a direct RIA procedure with antibodies against PGE_2 and $PGF_{2\alpha}$ characterized by Dubois and Bazer [20]. Standard curves were prepared in 0.05 M Tris-HCl with known amounts of PGE_2 or $PGF_{2\alpha}$. The linear portion of the curve was obtained with Inplot4 computer software (GraphPad Software, San Diego CA). Control samples included medium containing sFBS and FBS. For each sample, a 100-μl aliquot was added to each test tube along with 100 μl of Tris buffer. Subsequently, 100 μl of rabbit anti-PGE_2 or rabbit anti-$PGF_{2\alpha}$ (antiserum dilution of 1:5000 for both PGE_2 and $PGF_{2\alpha}$) and 100 μl of ^{3}H-PGE_2 or ^{3}H-$PGF_{2\alpha}$, respectively, were added to each tube and incubated overnight at 4°C. After charcoal-dextran stripping by centrifugation to remove unbound prostaglandins, the solution was transferred to scintillation vials and radioactivity was determined.

Statistical Analysis

All data are reported as means ± SEM. Data on the secretion of ^{35}S-methionine-labeled proteins and prostaglandin assays were analyzed to detect effects of treatment (sFBS, E_2, P_4, E_2P_4, or FBS), domain (apical vs. basal), treatment by domain interaction, and animal, using analysis of variance (ANOVA) and least squares means procedures. Significance was determined at $p < 0.05$. Statistical analyses was performed on SAS software or by InStat software (GraphPad Software).

RESULTS

Indices of Structural Polarity

Comparisons of growth properties of pUE cells on the CM and HA inserts indicated that, although CM inserts had better optical properties, HA inserts supported more rapid polarization of epithelial cells. The CM and HA inserts were employed in analyses of the structural properties of the pUE cells during the development of polarity. Transepithelial resistance measurements across cell-coated filter inserts were also monitored and correlated with the complexity of junctional complexes at the lateral border of cells. Cultures were typically confluent on CM filters by 8 days, although maximum resistance was achieved by 10 days and remained constant for up to 28 days. Cells grown on HA filters reached confluence by 6 days with maximal resistance established by 8 days. HA inserts were used in biochemical studies.

The morphology of isolated pUE cells during the course of growth on matrix-coated filter inserts was analyzed in conjunction with resistance measurements (Fig. 1). Cells maintained in culture for 2 days and achieving a resistance of about 150 Ωcm^2 exhibited a simple squamous morphology (Fig. 1a). In contrast, cultures reaching resistance values of over 600 Ωcm^2 exhibited a compact columnar morphology closely resembling the polarized phenotype of pUE cells in vivo (Fig. 1b).

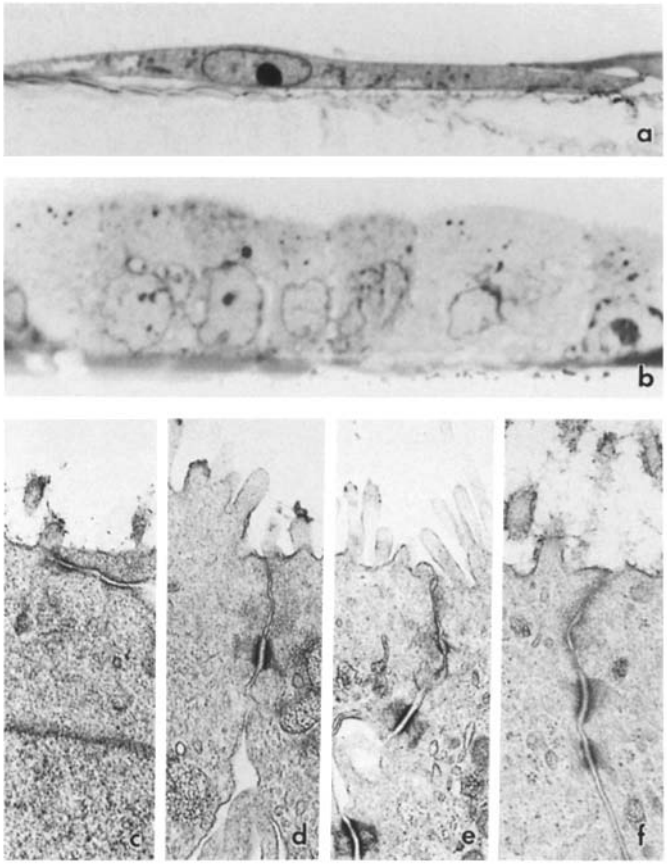

FIG. 1. Micrographs of pUE cells cultured on matrix-coated filter inserts. **a**) Light micrograph of pUE cells in preconfluent cultures (≈ 150 Ωcm²) exhibiting a squamous appearance with overlapping lateral cell borders and few points of contact between neighboring cells. ×1200. **b**) Light micrograph of polarized cultures (> 600 Ωcm²) exhibiting columnar-shaped cells with basally located nuclei, tight lateral borders, and increased cell height. ×1400. **c-e**) As electrical resistance increased, electron microscopy revealed increased complexity of the junctional contacts. ×22 500. **c**) Junctional complexes of low resistance pUE cells (≈ 150 Ωcm²) were very shallow and near the apical surface. **d**) At ≈ 300 Ωcm², the junctional complexes were deeper, and the lateral borders became tighter. **e**) Upon polarization (> 600 Ωcm²), all cells within the filter culture exhibited deep junctional complexes and closely resembled the junctional complexes observed in vivo (**f**).

Ultrastructural analysis of cultures from three different pUE cell isolations was performed as cells achieved resistance measurements of 150, 300, 450, and 600 Ωcm^2, and these were compared to pUE cells in vivo (Fig. 1, c-e). Prominent changes in apical and basolateral domains of isolated pUE cells accompanied increased transepithelial resistance. Apical plasma membrane acquired longer and more numerous microvilli and increased organization of junctional complexes and interdigitation of basolateral membranes. Tight junctions and belt desmosomes were detected in cells with resistance of 300 Ωcm^2 and greater; these structures became more complex and were located deeper in the apicolateral region of the cell with increasing

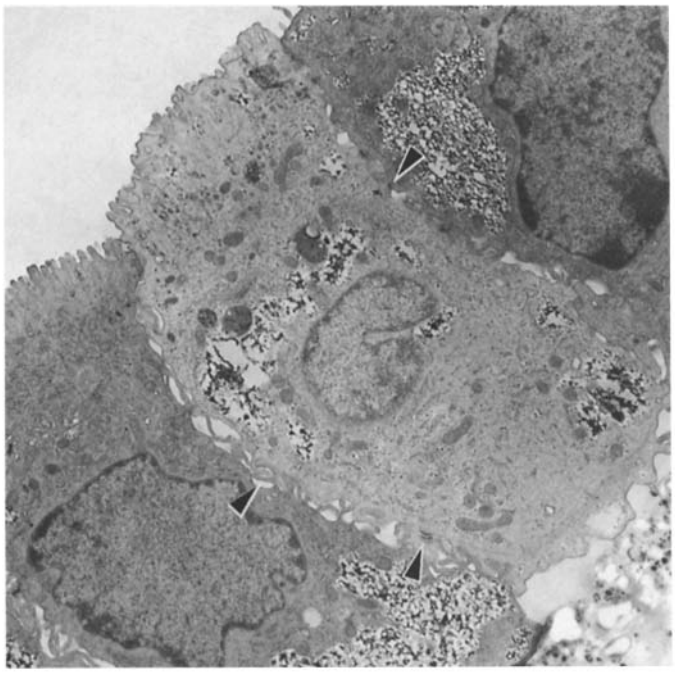

FIG. 2. Ultrastructure of polarized pUE cells cultured on matrix-coated filter inserts exhibited many characteristics seen in uterine epithelial cells in situ including abundant apical microvilli, tight lateral borders with complex interdigitation, and spot desmosomes (arrowheads). ×6500.

resistance (Fig. 1e). No further morphological changes in pUE cells were observed once 600 Ωcm^2 resistance values were reached on both HA and CM filters (Fig. 2). Scanning electron microscopy corroborated the ultrastructural and light microscopy analyses (data not shown). Therefore, 600 Ωcm^2 was used as the criterion for structural polarity in subsequent experiments designed to evaluate functional polarity of porcine endometrial epithelial cells.

Additional structural analyses employed to evaluate the purity of epithelial cells and the development and maintenance of polarization included indirect immunofluorescence analysis of epithelial cell-specific markers. Cytokeratin expression in pUE cells on filters was uniform (Fig. 3a), and all cells observed were cytokeratin-positive. Further, the tight junction-associated protein ZO-1 surrounded all cells and was restricted to a thin optical plane corresponding to the subapical membrane of cells (Fig. 3b). Light and electron microscopic observations indicated the absence of fibroblasts underlying the polarized pUE cells. Cytokeratin and ZO-1 expression therefore confirmed the efficacy of the epithelial cell isolation protocol and the polarized features of cells grown on matrix-coated inserts.

Indices of Functional Polarity

Secretion of ^{35}S-methionine-labeled proteins. Functional polarity of the pUE cells was determined by analysis of proteins secreted into either the apical or basal compartments which developed after establishment of junctional complexes in confluent cultures grown on matrix-coated filter inserts. Radiolabeled proteins were preferentially secreted into the apical chamber for all groups ($p < 0.01$; Fig. 4) except the P_4-treated cultures.

The radiolabeled protein profiles of apically and basally secreted proteins were determined by 2D-PAGE. The apically secreted proteins were distinctly different from their basal counterparts in all samples studied. The complexity

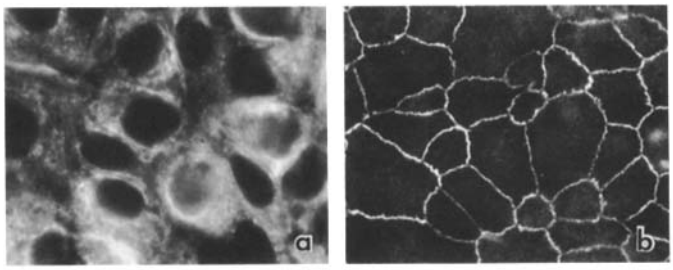

FIG. 3. Immunofluorescence staining of epithelial markers in polarized pUE cells. **a**) Cytokeratins were labeled with a broad-spectrum monoclonal antibody. ×650. **b**) Tight junctions were labeled with a monoclonal antibody directed against a 225-kDa peptide localized on the cytoplasmic surface of the tight junction. ×400. Uniform labeling of both markers reveals a homogeneous, polarized pUE cell culture system grown on matrix-coated filter inserts.

of higher molecular mass proteins present in the apical chambers (Fig. 5, upper panels) and the quadruplet of low molecular mass proteins (< 29 kDa) secreted predominantly into the basal chamber (Fig. 5, lower panels) indicates functional polarity of the pUE cells. The apically secreted proteins from samples containing no steroids (sFBS-treated) displayed a profile (Fig. 5a) similar to the steroid-treated samples (E_2; Fig. 5c: P_4; Fig. 5e: E_2P_4 not shown), except for the group of basic 20- and 25-kDa proteins present in the steroid-treated groups, which was not present in the sFBS-treated group. There was a difference in the basally secreted proteins between the sFBS-treated group (Fig. 5b) and the steroid-treated group (E_2; Fig. 5d: P_4; Fig. 5f: E_2P_4 not shown). Compared to sFBS, steroids increased the intensity and complexity of labeled proteins especially those of low molecular mass (< 29 kDa). However, all steroid-treated cultures (E_2, P_4, E_2P_4) exhibited similar protein profiles for the apical and the basal compartments. When the polarized pUE cells were cultured in complete serum (FBS), the apical (Fig. 5g) and basal (Fig. 5h) protein profiles demonstrated patterns more complex than for the

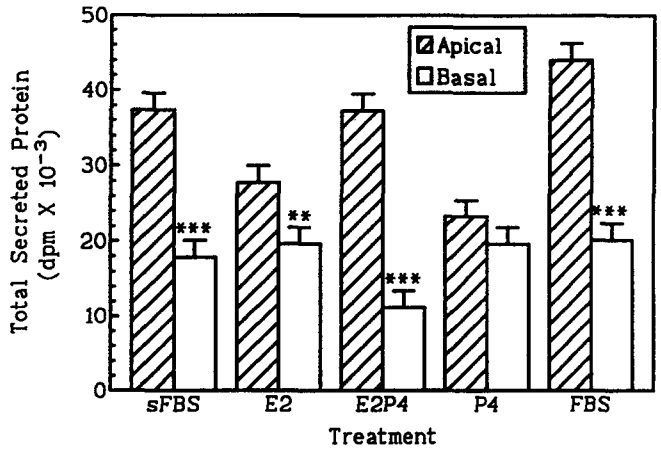

FIG. 4. Preferential secretion of proteins with incorporated ^{35}S-methionine by polarized pUE cells cultured on matrix-coated filter inserts. Polarized cultures treated with sFBS, FBS, E_2, and E_2P_4 demonstrated apical secretion of radiolabeled proteins when compared to the basal secretions ($n = 9$; ** $p < 0.01$; *** $p < 0.001$). However, the P_4-treated pUE cells did not exhibit a vectorial preference for the secretion of radiolabeled proteins. In addition, pUE cells treated with E_2P_4 secreted more radiolabeled proteins apically when compared to the E_2- or P_4-treated samples ($p < 0.05$).

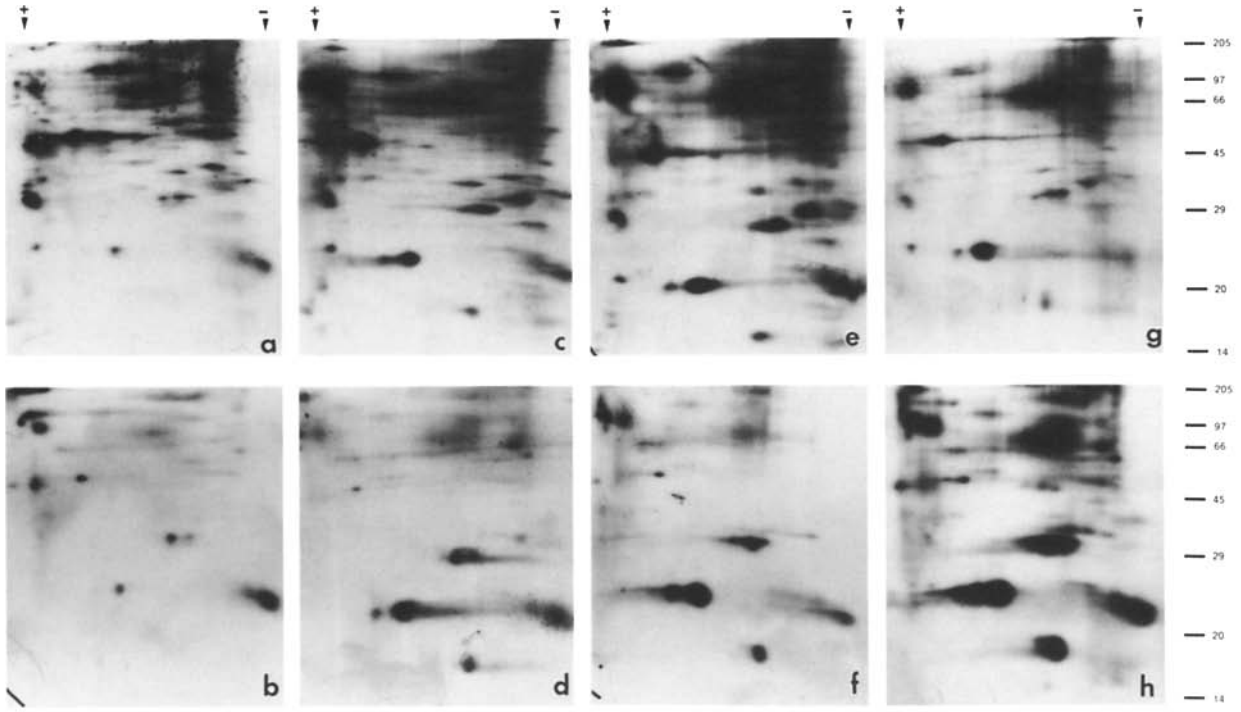

FIG. 5. Results of 2D-PAGE gels of ^{35}S-methionine-labeled secreted proteins comparing apical (upper panels: **a,c,e,g**) and basal (lower panels: **b,d,f,h**) secretions of polarized pUE cells treated with sFBS (**a,b**), E_2 (**c,d**), P_4 (**e,f**), and FBS (**g,h**). Note the increase in complexity of the higher molecular mass proteins from the apical chambers (top panels) compared to the basal chambers (bottom panels) for all treatment groups, which indicates functional polarity. The protein profiles obtained from the apical chambers of sFBS-treated cell cultures (**a**) did not contain the low molecular mass proteins (< 29 kDa) present in the steroid-treated samples (**c** and **e**). The protein profiles from the basal chambers of the steroid-treated cultures (**d** and **f**) were increased in intensity and complexity compared to the sFBS-treated culture, especially the quadruplet of low molecular mass proteins < 29 kDa (**b**). The protein profiles of the E_2P_4-treated samples were similar to the other steroid-treated pUE cultures. The FBS-treated cell cultures (**g** and **h**) displayed a protein profile more similar to that of the steroid-treated cultures than the sFBS-treated cell cultures.

sFBS-treated groups and similar to profiles for the steroid-treated samples.

Prostaglandin production. Ten polarized pUE filter cultures (four cultures from one isolation and three cultures each from two other isolations) were run in triplicate to quantify prostaglandin secretion into the apical and basal chambers. In all treatment groups studied, approximately two-thirds of the PGE_2 and $PGF_{2\alpha}$ were secreted into the basal compartment (Table 1). Steroid treatment did not alter the direction of prostaglandin secretion by the polarized pUE cell cultures.

The ratio of total prostaglandin secreted by each polarized pUE cell filter culture did not differ between or within treatment groups (Table 1). However, the ratio of prostaglandins within the basal compartment of E_2- and E_2P_4-treated cultures was higher ($p < 0.05$) than for the apical compartment. The other treatment groups (sFBS, P_4, and FBS) demonstrated a similar trend, although not significant at $p = 0.05$. Apical and basal ratios of prostaglandins between groups did not differ. The subtle changes observed in the percentage of PGE_2 secreted apically and basally by the E_2 and E_2P_4 groups was not significant. However, differences in PGE_2:$PGF_{2\alpha}$ ratio ($p < 0.05$) in the apical and basal compartments were detected, indicating that slight variations in the vectoring of prostaglandins has a significant effect on the PGE_2:$PGF_{2\alpha}$ ratio.

DISCUSSION

Using techniques developed for use with primary cultures of uterine epithelial cells from rats [12], a homogeneous pUE culture system that exhibits both structural and functional indices of polarization was developed. Cells grown on artificial basement membrane-coated porous filters developed morphological properties characteristic of porcine uterine epithelium observed in situ. Associated with the development of structural polarity was the elaboration of junctional complexes at the subapical border of cells, which develop continuous staining of the tight junctional protein, ZO-1 [18]. By relating the morphological indices of polarization with electrical resistance measurements, an endpoint of 600 Ωcm^2 provided a noninvasive and simple functional criterion for determination of polarity in pUE cultures grown on Millicell inserts, which was achieved after about 6 days of culture. Although the time in culture required to achieve polarity was comparable to that determined for cultured epithelial cells from the rat [12], the electrical resistance values in pUE cultures were higher and may reflect more elaborate junctional complexes.

Standards used to confirm the functional polarity of uterine epithelial cells [12] have been adapted from established epithelial cell lines [21, 22]. These include 1) transepithelial electrical resistance measurements, 2) uptake of ^{35}S-methionine, 3) incorporation of ^{35}S-methionine into secreted proteins, 4) the secretory protein profiles, and 5) secretion of prostaglandins [23]. The apical secretion of proteins is a common feature of polarized cells in vitro [12, 14, 23, 24] and can be used as a criterion for polarization of pUE cells. The majority of radiolabeled proteins were secreted into the apical chamber of the polarized pUE cells for all treatment groups except the P_4-treated cells. The differences in apically and basally secreted proteins are reflected in the pro-

TABLE 1. Prostaglandin secretion (ng/chamber/24 h) into the apical and basal chambers of polarized pUE cell filter cultures, the percent of total PGE_2 or $PGF_{2\alpha}$ secreted into each chamber, and the ratio of PGE_2 to $PGF_{2\alpha}$ (n = 10), treated with sFBS, E_2, E_2P_4, P_4, or FBS.[a]

Treatment	Chamber	PGE_2 Mean (±SEM)	% PGE	$PGF_{2\alpha}$ Mean (±SEM)	% PGF	PGE:PGF
sFBS	Apical	654.83 (±68.24)	31.46** (±3.19)	922.71 (±124.8)	35.56** (±2.14)	0.8542 (±0.1445)
	Basal	1511.77 (±170.53)	68.54 (±3.19)	1642.76 (±173.41)	64.44 (±2.14)	0.9792 (±0.1044)
E_2	Apical	654.01 (±76.27)	25.62** (±2.03)	1005.38 (±109.01)	32.71** (±2.4)	0.7407* (±0.1366)
	Basal	1942.95 (±196.15)	74.38 (±2.03)	2100.21 (±201.03)	67.29 (±2.4)	0.9571 (±0.1052)
E_2P_4	Apical	750.71 (±74.69)	23.69** (±1.57)	1112.78 (±109.13)	33.48** (±1.61)	0.7322** (±0.0967)
	Basal	2431.41 (±181.33)	76.31 (±1.57)	2199.40 (±169.74)	66.52 (±1.61)	1.1500 (±0.1185)
P_4	Apical	902.02 (±102.15)	30.56** (±2.15)	1050.90 (±95.22)	33.71** (±2.31)	0.9316 (±0.1440)
	Basal	2049.6 (±201.32)	69.44 (±2.15)	2072.31 (±146.23)	66.29 (±2.31)	1.0205 (±0.1176)
FBS	Apical	875.14 (±117.41)	30.28** (±2.37)	1231.38 (±134.68)	33.34** (±1.67)	0.7788 (±0.1319)
	Basal	1990.94 (±178.24)	69.72 (±2.37)	2388.92 (±183.29)	66.66 (±1.67)	0.8808 (±0.1077)

[a] Means are different from basal complement (* $p < 0.05$; ** $p < 0.001$).

tein profiles of the polarized pUE cell filter cultures. The autoradiographs of the 2D-PAGE gels indicated a more complex array of apically secreted radiolabeled proteins compared to the basally secreted proteins. The secretion of unique proteins by polarized pUE cells into the apical or basal compartments may serve as markers for functional polarity.

Previous studies have shown that HA filters do not adsorb proteins [12]; therefore, the differences observed are a result of the directional secretion of the proteins. The polarized pUE cell filter cultures treated with sFBS demonstrated fewer and less intensely labeled secretory products when compared to the steroid-treated cells, suggesting that these epithelial cells are responsive to steroids. The E_2-, P_4-, and E_2P_4-treated cells demonstrated similar apically secreted protein profiles, and the protein profiles from the basal compartment were also similar in steroid-treated cultures. Although the P_4-treated samples did not demonstrate a predominantly apical secretion of proteins, the protein profile did reflect differences in the secretion of proteins into the apical and basal chambers. When complete FBS was added to the medium, the polarized pUE cells showed a more complex protein profile, which was more similar to that in the steroid-treated samples than in the sFBS sample. These results suggest that polarized pUE cells respond to signals present within the medium, including steroids and possibly additional factors present in the complete FBS. The apical or basal preference of secretion for individual proteins along with distinct protein profiles for each chamber is consistent with the development of a polarized culture observed with other cell types [21, 22] including rat uterine epithelial cells [12].

The directional release of prostaglandins has also been used as a marker for polarization in uterine epithelial cells of rats and mice in vitro [23]. However, it is recognized that all endometrial cell types secrete prostaglandins [25]. In this study, luminal epithelial cells were evaluated for directional prostaglandin production as an index of polarity. Over two-thirds of PGE_2 and $PGF_{2\alpha}$ were secreted basally. Steroids did not alter the direction of prostaglandin secretion, although differences in the ratio of PGE_2 to $PGF_{2\alpha}$ were evident in the apical and basal chambers of E_2- and E_2P_4-treated samples. The utilization of a polarized cell system of luminal uterine epithelial cells and the recombination of the epithelial cells with uterine stroma cells may be useful in understanding epithelial-stromal interactions related to prostaglandin synthesis and secretion in the pig.

Experiments with isolated pUE cells are limited (e.g., [25–27]), and analysis of responses in these and the present study were restricted to a single dose of P_4. However, this polarized pUE cell model system will be useful for studying the response of the cells to a range of steroid and other hormone treatments, and the interaction between stromal and pUE cells. Studies are under way to examine luminal epithelial-trophectoderm interactions, including the control of uterine receptivity and epithelial responses to embryonic cues.

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