PIG ENDOMETRIAL CELLS IN PRIMARY CULTURE:
MORPHOLOGY, SECRETION OF PROSTAGLANDINS
AND PROTEINS, AND EFFECTS OF PREGNANCY1,2

Z. Zhang, B. C. Paria3 and D. L. Davis4

Kansas State University5, Manhattan 66506

ABSTRACT

Luminal epithelial, glandular epithelial, and stromal cells were isolated from pig endometrium by enzymatic dispersion and sieve filtration. The three cell types, maintained in primary culture, showed distinctly different morphologies when viewed by light and scanning electron microscopy. Immunocytochemical staining indicated that luminal and glandular epithelial cells were positive for both cytokeratin and vimentin. However, stromal cells were positive only for vimentin. Acid phosphatase activity was detected in the culture medium of glandular cells and increased \((P < .05)\) when progesterone \((.1 \mu M)\) was included in the culture medium. The secretion of uteroferrin by glandular cells was also indicated by one-dimensional PAGE and Western blot analysis. Stromal cells produced more \((P < .01)\) prostaglandin E \((PGE)\) than prostaglandin \(F_2\alpha\) \((PGF_{2\alpha})\), whereas glandular cells secreted more \((P < .01)\) PGF\(_{2\alpha}\) than PGE. Pregnancy status affected prostaglandin secretion in that stromal cells secreted less \((P < .01)\) PGE and PGF\(_{2\alpha}\) and glandular cells secreted less \((P < .05)\) PGF\(_{2\alpha}\) when they were harvested from pregnant vs cyclic pigs. Furthermore, the PGE:PGF\(_{2\alpha}\) ratio in medium from stromal cells was greater \((P < .01)\) for cells collected from pregnant pigs. This culture system provides an in vitro model for studying the hormonal regulation of the endometrium and potentially may be useful for studying interactions between endometrial cells and embryos in the pig.

Key Words: Endometrium, Cell Culture, Acid Phosphatase, Prostaglandins, Pigs


Introduction

Methods for isolating and initiating primary cultures of endometrial cells have been reported for the rat (McCormack and Glasser, 1980), rabbit (Ricketts et al., 1983; Fortier et ai., 1987), cow (Fortier et al., 1988), guinea pig (Chaminadas et al., 1989), and human (Satyaswaroop et al., 1979; Schatz and Gupide, 1983; Centola et al., 1984; Bongso et al., 1988). Studies using cell culture have allowed the evaluation of distinct properties in different cell types with regard to prostaglandin secretion and adenylate cyclase activity (Fortier et al., 1987, 1988), RNA synthesis and estrogen receptors (McCormack and Glasser, 1980), and localization of uteroglobin synthesis (Ricketts et al., 1983). Modulating roles of progesterone and estrogen on ultrastructure, protein synthesis, and secretion in guinea pig endometrial glandular cells grown in primary culture have been documented (Alkhalaf et al., 1989; Chaminadas et al., 1989). Endometrial stromal cell culture also has been used as an in vitro model for studying human decidulization (Irwin et al., 1989).
Pig endometrium synthesizes prostaglandins and secretes a variety of proteins with uteroferrin as a predominant product (for review, see Bazer et al., 1984; Roberts and Bazer, 1988). The diffuse epitheliochorial placentation in this species renders the embryo dependent on endometrial histotroph throughout pregnancy (Amoroso, 1951). Studies conducted in vivo (Chen et al., 1973; Geisert et al., 1982a,b) and in tissue explant culture (Basha et al., 1979) have contributed to the understanding of endometrial secretory activities. The endometrium is a complex tissue consisting of a variety of cell types. Evaluation of the unique roles of different cell types and the potential interactions between cell types could be done in vitro using primary cell cultures. The objectives of this study were to obtain stromal cells and glandular epithelial and luminal epithelial cells from pig endometrium and to determine their morphology (light and scanning electron microscopy) and secretory characteristics. We also investigated the effects of pregnancy on prostaglandin (PG) secretion by endometrial cells in vitro.

Materials and Methods

Endometrial Cell Separation and Culture. Endometrial tissue was collected from multiparous sows (160 to 230 kg; n = 20) on d 13 of pregnancy or the estrous cycle (first day of estrus is d 0) by surgery as described by Rosenkrans et al. (1990). Cell populations were separated aseptically under a laminar flow hood using modifications of procedures previously described by Fortier et al. (1987) and Schatz and Gurpide (1983).

Tissue was placed in sterile incomplete tissue culture medium (IHBSS, Ca++ and Mg++ free, pH 7.4) containing penicillin (100 U/ml), streptomycin (100 μg/ml), and gentamicin (50 μg/ml). After removal of blood clots, the tissue was placed in 10 ml of IHBSS containing 0.3% trypsin (type XII-S)6 in a 50-ml conical flask and digested for 150 min at room temperature with occasional shaking to release luminal epithelial cells. Digested tissue was rinsed with 20 ml of IHBSS, cut into small strips (~1 mm in diameter), and resuspended in 10 ml of IHBSS with trypsin (.04%), collagenase (.06% type II)6, and DNase-I (.01% type II)6. The flask was incubated at 37°C for 50 min with vigorous shaking every 10 min. Then the tissue was rinsed with 50 ml of IHBSS and discarded.

The 10-ml cell suspension from the .3% trypsin digestion was combined with that of the 20-ml IHBSS rinse, and luminal epithelial cells were pelleted by centrifugation at 500 x g for 10 min. Cells were washed twice with IHBSS and resuspended in 2 ml of RPMI 16406 (without phenol red) supplemented with 10% heat-inactivated fetal bovine serum (FBS)6 and antibiotics as in IHBSS (basal medium). The cell suspension was then placed over 8 ml of Ficoll-Paque7 (5.7% Ficoll 400) and centrifugated at 2,000 x g for 10 min to separate erythrocytes. Epithelial cells were retained as a band above the Ficoll. They were pipetted and resuspended in basal medium.

Stromal cells and fragments of glands in the suspension from the second digestion (trypsin-collagenase-DNase) were separated by passing the suspension through a 38-μm stainless steel sieve8. Stromal cells in the filtrate were pelleted by centrifugation at 500 x g. Cells were washed and resuspended in basal medium. Glandular fragments retained on the sieve were washed free of stromal cells with IHBSS, collected by backwashing, pelleted by centrifugation at 200 x g for 10 min, resuspended in basal medium, and equally distributed among various culture dishes9 (21 cm2), each with 5 ml of basal medium.

Cell numbers in luminal epithelial and stromal cell suspensions were counted in three 20-μl aliquots and plated at a density of approximately 5 x 10⁴ cells/cm². Luminal epithelial cells, whether single or in clumps of up to 10 cells, were all included in hemacytometer counts. Glandular fragments were also counted in a hemacytometer and approximately 4 x 10⁴ clumps were plated in each dish. Cells were incubated at 38°C with a gas phase of 5% CO2 and 95% air in a humidified chamber9.

Glandular and stromal cells were cultured in basal medium for 48 h to allow completion of attachment and formation of a monolayer. Then medium was replaced with RPMI 1640 supplemented with 5% charcoal-stripped FBS. The medium was changed every 2 d. Luminal epithelial cells did not complete attachment.
until 3 d after seeding. In these cultures, the medium was replaced 3 d after seeding with RPMI 1640 containing 5% charcoal-stripped FBS; this was changed every 2 d thereafter for the duration of culture.

**Immunofluorescent Staining.** Indirect immunofluorescent staining of cytokeratin and vimentin was performed as described by Sun and Green (1978). Briefly, cells were grown on 18- × 18-mm glass coverslips for 2 d (glandular and stromal cells) and 3 d (luminal epithelial cells), fixed for 30 min, rinsed briefly with water, and then returned to PBS. Antiserum for cytokeratin and vimentin (prepared in guinea pig and goat, respectively) were diluted 1:30. Forty microliters of antiserum was placed on each coverslip and incubated for 1 h at 37°C. In control staining, nonimmune sera were applied. The serum was then removed by thorough rinsing in PBS. Fluorescein-isothiocyanate (FITC)-coupled antibodies were also performed. These cells showed no fluorescence.

**Scanning Electron Microscopy.** For scanning electron microscopy (SEM), glass coverslips on which cells had been grown were fixed in 3% glutaraldehyde (prepared in PBS, pH 7.4) for 45 min. Cells were washed twice in buffer and then dehydrated in a gradient of ethanol:water (30, 50, 70, 80, 95, and 100% ethanol). The dehydrated specimens were transferred to liquid CO₂ for critical-point drying. Gold-sputtered specimens were examined under an ETEC Autoscan electron microscope.

**Acid Phosphatase.** Acid phosphatase activity in culture medium was determined for cells exposed to 0 or 1 μM progesterone using p-nitrophenol phosphate as a substrate as described by Schlosnagle et al. (1976) and Bash et al. (1979). One unit of enzyme activity was defined as the amount of enzyme required to liberate, in 1 min, sufficient p-nitrophenol to yield an absorption of 1.0 at 410 nm. Donor pigs (n = 3) served as blocks and provided two to four dishes for each treatment (control vs progesterone-supplemented).

**Polyacrylamide Gel Electrophoresis and Western Blot Analysis.** For secretory protein analysis, glandular and stromal cells (three to four dishes per cell type from each of four sows) were grown in basal medium for 48 h. The medium was then changed to RPMI 1640 with 10 μg/ml of insulin. After an additional 48 h of culture, media were collected. Cell debris was precipitated by centrifugation at 10,000 × g for 30 min. Media were concentrated with Centricon™ (molecular cut-off of 10,000). Proteins were dissociated by boiling for 2 min in 2% (wt/vol) SDS solution containing 1 M dithiothreitol and 1.2% (wt/vol) Tris and subjected to SDS-PAGE according to the procedure of Laemmli (1970) using a 15% resolving gel and 3% stacking gel. After electrophoresis, the gel was stained with silver nitrate (Oakley et al., 1980). Molecular weight standards were rabbit muscle phosphorylase b (97.0 kDa), bovine serum albumin (66.0 kDa), hen egg white ovalbumin (42.7 kDa), bovine carbonic anhydrase (31.0 kDa), soybean trypsin inhibitor (21.5 kDa), and hen egg white lysozyme (14.4 kDa). For comparison the uteri of sows (d 13 of pregnancy) were flushed with PBS. Uterine flushings were centrifuged (10,000 × g for 30 min) and subjected to the same electrophoretic procedures.

The procedure of immunoblotting is essentially that of Sambrook et al. (1989). The proteins previously fractionated by SDS-PAGE were electrophoretically transferred onto nitrocellulose membrane, using 25 mM Tris, 192 mM glycine, 20% (vol/vol) methanol buffer. Following transfer, nonspecific binding was blocked by an incubation in PBS containing 1% (wt/vol) BSA, 0.2% sodium azide, and 0.02% Tween 20 (PBS-Tween) for 1 h at room temperature. The nitrocellulose was incubated

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10IgG ImmunoBiologicals, Lisle, IL.
11Kodak Co., Rochester, NY.
12Amicon Division of W. R. Grace & Co., Danvers, MA.
13Bio-Rad Laboratories, Richmond, CA.
for 1 h at room temperature in rabbit anti-uteroferrin (1:100 in PBS-Tween) or normal rabbit serum (1:100). Uteroferrin antiserum (Simmen et al., 1991) was kindly provided by F. W. Bazer (The University of Florida). The membranes were washed for 45 min in PBS-Tween with several changes, then incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG for 45 min at room temperature. Membranes were washed extensively in PBS-Tween, and reactions were visualized in color development buffer containing 30 mg of 4-chloro-1-naphthol in 10 ml of methanol and 10 µl of 30% H2O2 in 50 ml of PBS.

Prostaglandin Secretion. Glandular and stromal cells were recovered from pregnant (n = 7) and cyclic (n = 4) sows and cultured in basal medium for 2 d. Then media were changed to RPMI 1640 with 5% charcoal-stripped FBS, and cells were cultured for an additional 24 h. Culture media were collected for PG assays. Prostaglandin E and F2α in the culture medium were quantified by RIA as described by Rosenkrans et al. (1990) without extraction. Increasing volume (0 to 200 µl) of cell culture media produced binding curves that paralleled the standard curves. Adding 10, 25, 50, 100, and 250 pg of PGE or PGF2α to culture medium resulted in 92 and 95% immunorecoveries for PGE and PGF2α, respectively. Intra- and interassay CV were 9.5 and 13.2% for PGE and 6.2 and 7.0% for PGF2α.

Recovery of Cells, Protein and DNA Determinations. After collection of culture medium, 5 ml of IHBSS was added to culture dishes, and cells were removed by scraping dishes with a plastic policeman. The dish was rinsed with 3 ml of IHBSS, and cells were recovered by centrifugation at 1,500 x g for 15 min. Cellular protein contents were estimated using Folin-phenol reagent (Lowry et al., 1951). The DNA contents were determined using the procedure described by Labarca and Paigen (1980).

Statistical Analysis. Prostaglandins in culture media are expressed as picograms per microgram of cellular protein. The PGE: PGF2α (PG ratio) was calculated. Acid phosphatase activity was presented as milliunits.

Figure 1. Light micrographs of pig endometrial cells in vitro. (A) Fragments of glands after separation, (B) luminal epithelial cells 3 d after seeding, (C) glandular cell monolayer after 3 d in culture, (D) stromal cell monolayer after 3 d in culture. Bar = 20 µm.
(mU) per microgram of medium protein. Statistical analyses were conducted using the GLM procedure (SAS, 1982). Donor sows and cell type were included in statistical models. Pregnancy-status effects on PG secretion were tested using pig (pregnancy status) as the error variance; otherwise, the residual variance was used as the error variance for calculating $F$-statistics.

Results

Viability, Growth, and Morphology. Cell viability after separation was monitored by trypan blue dye exclusion. The test revealed a cellular viability of $88 \pm 1.2\%$ for luminal epithelial cells, $90 \pm 1.3\%$ for glandular cells, and $94 \pm 1.0\%$ for stromal cells. Plating efficiencies, defined as DNA content in attached cells as a percentage of DNA content at

Figure 2. Immunofluorescent staining for cytokeratin in luminal epithelial cells (A). Fluorescence was not observed when nonimmune serum was used (B).
time of seeding and tested at 48 h for glandular and stromal cells and at 72 h for luminal epithelial cells were 87, 83, and 78%, respectively. After trypsin dispersion, luminal epithelial cells were released as single cells or small clumps. These cells began to attach to culture dishes 24 h after seeding, grew slowly, and reached confluence after 7 to 8 d in culture. Limited numbers of luminal epithelial cells were obtained using these procedures. This characteristic combined with their slow growth limited the ability to test their PG secretory characteristics. Therefore, only the morphological characteristics and acid phosphatase secretory activity of luminal epithelial cells are described. Isolated glands were observed as tubular structures with a basement membrane (Figure 1 A) and attached between 12 and 48 h after seeding. Cells with beating cilia were frequently seen in gland cultures. Single stromal cells attached to culture dishes as early as 1 h after plating and completed attachment by 24 h. Glandular and stromal cells reached confluence by 72 to 96 h.

When cultured in 5% FBS-supplemented medium, endometrial cells retained their morphology for more than 4 wk. After culture in basal medium for 2 d to allow attachment and formation of a monolayer, endometrial cells could be kept in serum-free medium supplemented with insulin for 8 to 10 d and still maintain their morphology. After 8 to 10 d some cells became vacuolar, and gradually a small portion of cells detached from the dish. Cellular morphology and cytokeratin immunocytochemical staining revealed that the cultures were approximately 95% pure.

Luminal and glandular epithelial cells grew in colonies and were polygonal or spherical in shape (Figure 1 B, C). Stromal cells exhibited fibroblastic appearance after attachment (Figure 1 D). Immunocytochemical staining revealed that luminal and glandular epithelial cells were positive for both cytokeratin and vimentin (Figures 2A and 3A). Stromal cells stained positively for vimentin (Figure 3 B) but were negative for cytokeratin. Cells did not fluoresce if treated with nonimmune sera.

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Figure 3. Immunofluorescent staining for vimentin in endometrial cells. Glandular epithelial (A) and stromal (B) cells were stained with anti-vimentin antiserum. When glandular (C) and stromal (D) cells were treated with nonimmune serum, no fluorescence was observed.
instead of antisera (Figures 2 B, 3 C, and 3 D).

Figure 4 shows scanning electron micrographs of the three different cell types in vitro. Glandular cells had many apical protrusions (Figure 4 A) and were characterized by being closely associated and having numerous microvilli. Some cells had both cilia and microvilli. Luminal epithelial cells also were covered with microvilli but had few apical protrusions and no cilia (Figure 4 B). Stromal cells had a flattened, spindle-shaped appearance with no surface structures. Long cytoplasmic processes were observed in stromal cells (Figure 4 C).

**Secretion of Proteins.** Acid phosphatase activity was detected in media of glandular but not of luminal epithelial or stromal cultures. Progesterone supplement (1 μM) in glandular cultures increased \( P < .05 \) acid phosphatase activity (progesterone vs control = 1.59 ± .2 vs .91 ± .2 mU/μg of medium protein) in the culture medium. The SDS-PAGE of the medium of glandular cultures (Figure 5 C) revealed similarities to uterine flushings, although each contained some unique bands. A band (arrow) of approximately 35,000 Da, corresponding to the molecular weight of uteroferrin, was present in uterine flushings.

![Figure 4. Scanning electron micrographs of endometrial cells in vitro. (A) Glandular cells were covered with numerous microvilli. Cilia and apical protrusions were also present. Bar = 10 μm. (B) Luminal epithelial cell with numerous microvilli on cell surface. Bar = 1 μm. (C) Stromal cells with cytoplasmic processes. Bar = 10 μm.](image-url)
(Figure 5 B) and in the culture medium of glandular cells (Figure 5 C). However, a band corresponding to a trypsin/plasmin inhibitor (molecular radius $[M_r] - 14,000$) observed by Mullins et al. (1980) in uterine flushings was not found in the medium of glandular cultures. Major differences in secretory proteins were observed between glandular (Figure 5 C) and stromal cells (Figure 5 D), including the absence of a band corresponding to the molecular weight of uteroferrin in the culture medium of stromal cells. Two bands (arrow head) of $M_r < 30,000$ were present in glandular-cell but not stromal-cell culture media. The Western blot results of protein samples of uterine flushing and endometri al cell culture medium using the uteroferrin antiserum are shown in Figure 6. The 35-kDa protein observed in SDS-PAGE by silver staining (Figure 5 B, C) was detected on the immunoblot in samples from uterine flushings and glandular cell culture medium (Figure 6 A, C). No corresponding protein was found in the stromal cell culture medium (Figure 6 B). When normal rabbit serum instead of uteroferrin antiserum was used, no protein bands were detected.

Secretion of Prostaglandins. Secretion of PG was affected ($P < .05$) by cell type and pregnancy status. Glandular cells produced more ($P < .05$) PGF$_{2\alpha}$ than PGE. In contrast, stromal cells secreted more ($P < .05$) PGE than PGF$_{2\alpha}$ (Figure 7). Cells harvested on d 13 of pregnancy and the estrous cycle differed in their production of PGE and PGF$_{2\alpha}$. Glandular cells from pregnant pigs secreted less ($P < .05$) PGF$_{2\alpha}$ and stromal cells secreted less ($P < .01$) PGE and PGF$_{2\alpha}$/µg of cellular protein. The effect of pregnancy status was relatively greater for PGF$_{2\alpha}$ than for PGE, an increased ($P < .01$) PGE:PGF$_{2\alpha}$ ratio was secreted by the stromal cells from pregnant pigs (Figure 7). Indomethacin (10 µM) decreased ($P < .001$) PG production more than 95% (from 120 ± 36 to 6 ± 3 and 225 ± 117 to 3 ± 1 µg/µg of cellular protein for PGE and PGF$_{2\alpha}$, respectively, in glandular cells and from 65 ± 46 to 4 ± 2 and 59 ± 36 to 1 ± 1 for PGE and PGF$_{2\alpha}$, respectively, in stromal cells).
Discussion

Methods for separation and culture of endometrial cells have been reported for several species, but not for pigs. In this study, we report the separation and culture of three distinct cell populations from pig endometrium using modifications of methods previously described for rabbit (Fortier et al., 1987) and human (Schatz and Gurpide, 1983) endometrium. Based on cell morphology and cytokeratin immunocytochemical staining, the modified procedure yielded homogeneous cell populations with purity of approximately 95%. Morphological and functional characterization revealed distinct properties among the three cell populations.

Luminal and glandular epithelial cells were polygonal or spherical in appearance, as expected for epithelial cells. The cell surface was covered with numerous microvilli, and sometimes glandular cells also possessed cilia. Sinowatz and Friess (1983) observed that pig glandular epithelial cells sometimes have both cilia and microvilli. Many apical protrusions were observed in glandular cells. These protrusions are similar to those observed in vivo and have been considered to be involved in secretion (Stroband and Van der Lende, 1990). Stromal cells in vitro were typically fibroblastic in shape with no apparent structures on their surfaces. Immunocytochemical analysis revealed that stromal cells were positive for vimentin but negative for cytokeratin, an intermediate filament specific for epithelial cells. Luminal and glandular epithelial cells stained positively for both cytokeratin and vimentin in vitro. Although vimentin was originally thought to be a specific marker for mesenchymal cells (for review, see Franke et al., 1982), a growing body of evidence indicates that some epithelial cells also express this intermediate filament (Czemobilisky et al., 1985; Kasper and Karsten, 1988; Kasper and Stosiek, 1989). It is not known whether vimentin is expressed in porcine endometrial epithelial cells in vivo. However, this intermediate filament was demonstrated in normal human endometrial epithelial cells (Dabbs et al., 1986). Because vimentin has been observed in a variety of epithelial cells in vitro (Franke et al., 1979, 1981), it may not be appropriate to use it as a marker to distinguish epithelial and stromal cells.

Uteroferrin is a progesterone-induced glycoprotein that is synthesized and secreted by glandular cells of the porcine endometrium and accounts for the acid phosphatase activity measured in uterine flushings (Chen et al., 1973; Raub et al., 1985; Roberts and Bazer, 1989).

Figure 7. Prostaglandin (PG) E and F\textsubscript{2\alpha} secretion by primary cultures of glandular and stromal cells. Data are the amounts of PG released into the medium during 24 h of culture and are expressed as picograms per microgram of cellular protein and the PGE-PGF\textsubscript{2\alpha} ratio (PGR) (*P < .05; **P < .01). Asterisks designate PGE, PGF\textsubscript{2\alpha} or PGR differences between pregnant and cyclic sows.
in our endometrial cell cultures, acid phosphatase activity was only detected in the medium of glandular cells, not stromal or luminal epithelial cell cultures. The presence of acid phosphatase on days 7 and 8 of culture (Zhang et al., 1990) in serum-free medium suggests that uteroferrin is synthesized by glandular cells in vitro. In addition, the enzyme activity in the glandular cell culture medium was increased by progesterone treatment, suggesting that these cells are responsive to hormonal stimulation in vitro. Western blot analysis and SDS-PAGE also revealed the presence of uteroferrin in the culture medium of glandular cells.

Pig uterine secretory proteins contain plasmin/trypsin inhibitory activity (Mullins et al., 1980). The absence of a band corresponding to a plasmin/trypsin inhibitor (M₆ – 14,000) in the culture medium of glandular cells is consistent with its lack of trypsin-inhibitory activity (Zhang et al., unpublished observations). The plasmin/trypsin inhibitor has been localized to the luminal epithelium (Fazleabas et al., 1984) and, therefore, would not be expected among the secretions of uterine gland cells. Two bands of lower molecular weight (<30,000) were present in the glandular cell culture medium but not in stromal cell medium. The small numbers and slow growth of luminal epithelial cells did not permit electrophoretic analysis of secretory proteins.

Both glandular and stromal cells synthesized and released PGE and PGF₂α. However, preferential production of specific PG was observed. Glandular cells produced more PGF₂α than PGE. In contrast, stromal cells secreted more PGE than PGF₂α. Fortier et al. (1988) observed similar characteristic differences in PG secretion by bovine epithelial and stromal cells. Indomethacin suppressed PG in the culture medium greater than 95%, indicating de novo PG synthesis.

There is growing evidence indicating that extracellular matrix has an effect on epithelial morphology and function. Structural polarity is vital for the expression of differentiated function in epithelium (for review, see Rodriguez-Boulan and Nelson, 1989). Glasser et al. (1988) showed that Matrigel, a reconstituted basement membrane derived from Engelbreth-Holm Swarm tumor, is able to maintain the morphological and functional polarity of rat endometrial epithelial cells in vitro. The isolated glandular fragments observed in the present study were partially enclosed in basement membrane. The presence of basement membrane may allow glandular cells to maintain differentiated function.

Implications

Three distinct cell types can be isolated from pig endometrium by enzymatic dispersion and can be maintained in primary culture. Endometrial cells in vitro possess distinct properties that are consistent with their function in vivo. These methods should be useful for studying the hormonal regulation of endometrial function and the interactions between endometrial cells and embryos.

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