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Epithelial cell function during blastocyst implantation

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Abstract. In response to the ovarian secretion of progesterone and estrogen during early pregnancy, the mammalian uterus develops the capacity to perform complex cellular activities which occur before and after blastocyst implantation. Luminal epithelial cells participate in regulation of the metabolism of the blastocyst through the control of its humoral environment, provide an appropriate matrix for changes to occur at the interface between trophoblast and epithelium, and appear to transmit information from the blastocyst to the underlying stroma to initiate decidualization. With the completion of these functions during implantation in rodents, the epithelial cells self-destruct and are removed by phagocytic activity of the trophoblast. Control of both the endocytotic and secretory activity of luminal epithelial cells and their eventual self-destruction would require regulation of the Golgi-endoplasmic reticulum-lysosomes system within these cells. Progesterone secretion during early pseudo-pregnancy increases levels of cathepsin D, a lysosomal proteinase, in luminal epithelial cells by increasing the rate of enzyme synthesis. Progesterone pretreatment of ovariectomized rats followed by estradiol treatment results in the development of uterine sensitivity to deciduogenic stimuli. The number of proteins which are synthesized by luminal epithelial cells in response to estradiol to achieve this sensitivity has been determined. Epithelial cytosol proteins from rats treated with medroxyprogesterone acetate (3·5 mg sc) or medroxyproges-terone acetate plus estradiol (200 ng sc) were separated by two dimensional polyacrylamide gel electrophoresis. The synthesis of two proteins increased after 8 h of estradiol treatment and the synthesis of another three was increased by 12 h. The increased synthesis of these proteins could be related to changes in the capacity of the luminal epithelial cell for prostaglandin synthesis. The epithelial capacity for prostaglandin synthesis increases during pseudopreg-nancy to maximum levels at the time of maximum sensitivity to deciduogenic stimuli. Epithelial prostaglandin synthetic capacity may also depend upon the accumulation of prostaglandin precursors within these cells. Estradiol treatment of medroxyprogesterone acetate pretreated ovariectomized rats increased the arachidonic acid content and composition of epithelial phosphatidyl choline but the increases were not statistically significant. These changes in protein and lipid synthesis controlled by progesterone and estrogen would appear to contribute to the cellular activities of the luminal epithelium during early pregnancy.

Keywords. Cell function; blastocyst; implantation.

During early pregnancy, epithelial cells lining the uterine lumen perform several cellular functions vital to blastocyst implantation. By secretion and endocytosis, epithelial cells control the uterine environment of the blastocyst. Changes in the composition of the epithelial apical membrane enable the adhesion and attachment of the blastocyst. Arrival of the blastocyst initiates the transmission of a message, which increases capillary permeability and initiates the decidualization of underlying stromal cells. The accumulation of lysosomal hydrolase activity within the luminal epithelial

Abbreviations used: GERL, Golgi-endoplasmic reticulum-lysosomes; MPA, medroxyprogesterone acetate; IMP, intramembraneous particles; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; PGE_2, prostaglandin E_2; E, estradiol; sc, subcutaneous; PC, phosphatidyl choline; PE, phosphatidyl ethanolamine.

cells could provide enzymatic capacity for several of these functions and for the eventual self-destruction of the epithelial cells which occurs during blastocyst implantation in some species. This brief review will not consider all of these topics equally but will tend to focus on more recent data obtained in rodents.

From species to species there is considerable diversity in the length of time that the blastocyst remains free within the uterus and in the cellular response of the luminal epithelium to the blastocyst (Enders and Given, 1977). The amount and kinds of protein secreted by the luminal epithelium are presumably related to these kinds of diversity. Protein and other components of uterine fluid provide nutrition for the free blastocyst, and a means of control of blastocyst metabolism and development. Lumina of uterine glands are dilated by the progressive accumulation of carbohydrate material during the periimplantation period indicating increasing secretory activity (Given and Enders, 1980,1981). Numerous studies have examined the composition of uterine fluid and its hormonal control to determine the mechanisms by which blastocyst development and uterine sensitivity are synchronized. These studies have been recently reviewed (Surani and Fishel, 1980) and the topic will be considered in more detail by other contributors to this symposium. The suggestion that delayed implantation in rodents results from the secretion of inhibitors of blastocyst metabolism and that activation following estradiol results from a loss of activity of the inhibitors continues to be actively investigated (McLaren, 1973; Psychoyos, 1973; Weitlauf, 1976, 1978; O'Neill and Quinn, 1983).

In addition to secretion, epithelial cells can control the environment of the blastocyst by endocytotic uptake of material from the uterine lumen. The morphology of two endocytotic pathways which are active during the period of uterine sensitivity have been described (Parr, 1980, 1982a,b). Pinopod vacuoles and pinocytotic vesicles formed at the apical surface of luminal epithelial cells are channelled into secondary lysosomes. In the second pathway, pinocytotic vesicles formed at the basal and lateral membranes move across the cell towards the luminal surface, where they may fuse with the apical membrane to release their contents into the uterine lumen by exocytosis. Vesicles derived from the Golgi complex, also appear to move toward the apex of the cell and fuse with the surface membrane.

The endocytotic and secretory activity of uterine luminal epithelial cells requires intense intracellular activity by the Golgi-endoplasmic reticulum-lysosomes (GERL) system. Material taken into the cell by endocytosis is sequestered and digested within lysosomes (Parr, 1982a). Proteins for secretion synthesized on ribosomes on the rough endoplasmic reticulum are inserted through the membrane into the microsomal lumen. During this insertion, proteolytic cleavage and glycolytic processing of the protein determine whether the protein will be secreted or sequestered within the lysosomes of the cell. Histochemical studies have identified concentrations of lysosomal enzymes in endometrial epithelial cells which are controlled by progesterone and estrogen (Woessner, 1969; Roy *et al.,* 1983; Moulton and Koenig, 1983, Sengupta *et al.,* 1979). During early pregnancy, both multivesicular bodies and more typical lysosomes are found in luminal and glandular epithelial cells (Enders and Given, 1977). Our studies have demonstrated that cathepsin D, a lysosomal proteinase, is concentrated in luminal and glandular epithelial cells of the uterine endometrium (figure 1). During early pseudopregnancy, the levels of cathepsin D activity and the rate

Figure 1. Rat uterine cross section through non-implantation site on day 6 of pregnancy. Uterus was fixed in Bouin's solution, dehydrated, and embedded in paraffin. Deparafinized sections (6 μm) were pretreated with methanolic H_2O_2 and then with goat anti-rat serum to cathepsin D, donkey anti-goat IgG, and goat peroxidase anti-peroxidase. Sections were reacted with diaminobenzidine and H_2O_2 , washed, dehydrated, and mounted.

of synthesis of this enzyme increase to maximum levels at the time of maximum uterine sensitivity to deciduogenic stimuli (Moulton and Koenig, 1983). Considerable evidence implicates the lysosome as an important site of intracellular protein degradation (Segal and Doyle, 1978). Accumulation of cathepsin D within the epithelium could contribute to the degradation of extracellular protein brought to lysosomes by endocytosis and to the eventual programmed cell death of the luminal epithelium which is a significant feature of blastocyst implantation in rodents.

Adhesion and attachment of the blastocyst to luminal epithelial cells during early pregnancy could depend upon the insertion or deletion of specific proteins from the apical membrane of the epithelium. Although this potential mechanism of uterine sensitization has not been thoroughly examined, there is considerable morphological change in surface structures of luminal epithelium during the periimplantation period (Nilsson, 1980; Enders *et al.,* 1980; Takahashi *et al.,* 1980). Prior to implantation, the surface of the luminal epithelium is covered by regular digitiform microvilli. Later the microvilli are flattened and bulbous cytoplasmic projections appear for a limited period of time. With the loss of uterine sensitivity, these projections disappear and the epithelial cells bear ordered microvilli once again. These changes are largely under the control of the maternal ovarian hormones. Pronounced morphological differences between the microvilli of the implantation chamber and those of the rest of the uterus

have been observed, but these differences could not be related to differences in the chemical properties of the surface coat of the uterus (Enders *et al.,* 1980). The glycocalyx on the surface of rat uterine luminal epithelial cells contains both acidic and neutral carbohydrates under several different hormonal conditions. The acidic carbohydrates appear to be influenced by hormones, but no evidence could be found for a similar influence of hormones on neutral carbohydrates as revealed by concanavalin A binding (Murphy and Rogers, 1981). Further study may identify other sugars or specific acceptor proteins in the apical membrane of the epithelium. After separating labelled uterine epithelial proteins by 2 dimensional gel electrophoresis, we can detect the increased synthesis of several proteins and the altered proteolytic processing or glycosylation of several others during uterine sensitization to deciduogenic stimuli (figure 2). However, the location of these proteins within the luminal epithelial cells and their identity has not been determined.

More recently, freeze fracture electron microscopy has been used to examine more fine structure of the apical membrane and the number and density of intramembranous particles (IMP's), in particular (Murphy *et al.,* 1982a). IMP's are thought to represent globular proteins, or proteins complexed with small amounts of lipid and embedded deeply in the hydrophobic interior of the lipid bilayer. Aggregations of IMP's within the membranes may have some significance in promoting cell to cell contacts and several studies have implicated aggregations of IMP's in membrane transport. During early pregnancy, the density of IMP's in the apical membrane of luminal epithelial cells increase and by day 6, complex arrays of the IMP's were observed. If aggregates of IMP's participate directly in blastocyst implantation, then increase in the fluidity of the apical membrane to enhance the formation of these aggregates could be a significant prerequisite of implantation (Murphy *et al.,* 1982b). Increased membrane fluidity could also enable the aggregation of epithelial receptors for histamine, prostaglandins, or estradiol which have been proposed as mediators of communication between the blastocyst and the uterus. Receptors for prostaglandins (Kennedy *et al.,* 1983) have been measured in the endometrium, but their presence in epithelial cells has not been demonstrated. Progesterone pretreatment necessary for the development of uterine sensitivity to deciduogenic stimuli does not appear to increase estrogen binding in luminal epithelial cells (Martel and Psychoyos, 1982; Quarmby and Martin, 1982).

For many cells, the methylation of membrane phospholipid appears to be an initial common pathway for the transduction of receptor mediated signals through the membranes (Hirata and Axelrod, 1980). Various amines and peptides interacting with cell surfaces initiate a cascade of biochemical and physical changes in membranes including increased phospholipid methylation, increased mobility of receptors because of decreased membrane viscosity, the generation of cAMP, histamine release, mitogenesis, and chemotaxis. In several cell types examined, phospholipid methylation was associated with calcium influx, the release of arachidonic acid, and the formation of lysophosphatidylcholine. Contact of the blastocyst with the cell membrane of luminal epithelial cells initiates histamine release, the generation of cAMP, increased calcium influx, and increased prostaglandin synthesis.

In recent studies, we have examined changes in the activity of phosphatidyl methyltransferase activity in luminal epithelial cells following hormonal treatment to induce uterine sensitization to deciduogenic stimuli. Ovariectomized rats were treated

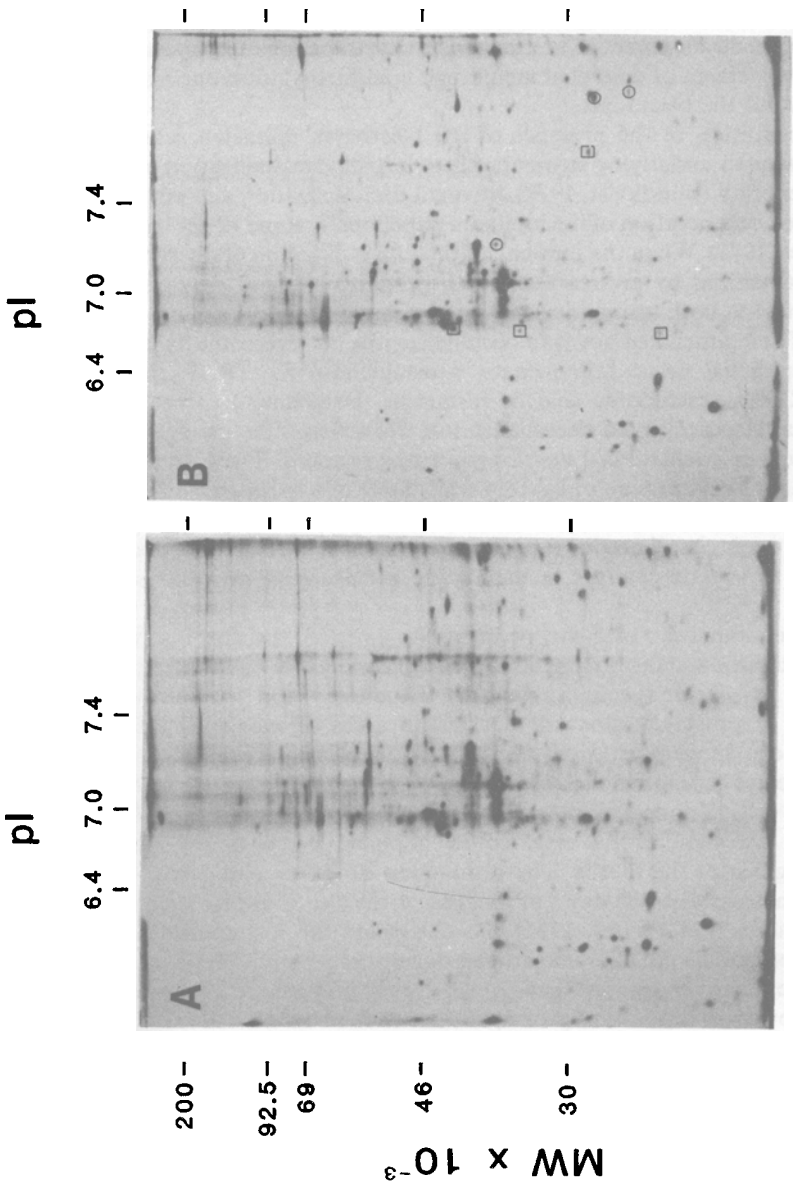

Figure 2. Effect of estradiol on pr rotein synthesis in the uterine luminal epithelium of rats pretreated with progestin. On successive days ovariectomized rats were treated with E, E, 0, 0, MPA, 0, and vehicle or estradiol for 12 h (E, estradiol, 500 ng, sc; 0, no treatment; MPA, medroxyprogesterone acetate, 3·5 mg, sc; e, estradiol, 200 ng, sc). Uterine horns were incubated in phosphate-buffered sa line containing [^{35}S]-methionine (400 μCi/ml) for 1 h. Epithelial cell extracts were prepared as described by Garrels (19 79), and then subjected to sodium dodecyl sulphate-polyacrylamide gel electrophoresis and isoelectric focusing electrophor resis.

with progestin (medroxyprogesterone acetate, 3·5 mg) for 48 h before treatment with estrogen (estradiol, 200 ng). Estradiol treatment had no effect on levels of either phosphatidyl methyltransferase I or II activity in uterine luminal epithelial cells. Although increases in the activity of these enzyme activities do not appear to be prerequisite for the development of uterine sensitivity, these preliminary results do not preclude the involvement of epithelial membrane lipid methylation during the earliest uterine response to the blastocyst.

With the recognition of the presence of the blastocyst, epithelial cells appear to transmit a message to underlying stromal cells to initiate decidualization and increase capillary permeability (Lundkvist, 1978). Stromal decidualization and edema preceed deterioration and degeneration of the luminal epithelium by some 12–24 h (Finn, 1977; Finn and Porter, 1975). When the luminal epithelium is experimentally removed from uterine horns sensitized by pretreatment with progesterone and estrogen, the uteri become insensitive to both traumatic and non-traumatic stimuli (Lejeune *et al.,* 1981). The inability of de-epitheliated horns to decidualize was not overcome by intraluminal injections of epithelial tissue homogenate, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) or prostaglandin E_2 (PGE_2), arachidonic acid or histamine. Detachment of the epithelium without removal also prevented decidualization. However, after the epithelium had regenerated, most of decidual cell development was restored. These studies indicate that the luminal epithelium is an obligatory transducer of a message from the luminal surface of the cell to the underlying stromal cells (Lejeune *et al.,* 1981). Luminal cells at the site of blastocyst implantation appear to synthesize and release deciduogenic substance(s) or to initiate changes in membrane permeability in response to these substances.

Increases in endometrial vascular permeability initiated by the blastocyst may involve both histamine and the prostaglandins, but considerable research implicates the prostaglandins as deciduogenic substances (Kennedy and Armstrong, 1981). Concentrations of prostaglandins are elevated in areas of increased vascular permeability after deciduogenic stimuli, and inhibition of prostaglandin synthesis by indomethacin delays or inhibits increases in vascular permeability and the decidualization of stromal cells (Kennedy and Armstrong, 1981). Concentrations of PGE, PGF, and 6-keto-F_{1a} are significantly elevated in implantation sites relative to non-implantation regions of the uterus. The production of PGE_2 and 6-keto-PGF_{1a} by uterine homogenates peaked on day 5 of pregnancy, the day of implantation (Fenwick *et al.,* 1977; Phillips and Poyser, 1981). To determine the histological site of this production, we prepared epithelial cell samples during early pseudopregnancy with and without indomethacin. When epithelial cells were prepared in the presence of indomethacin, lower tissue levels of prostaglandins were observed and these values did not change during pseudopregnancy (figure 3). When epithelial cells were prepared in the absence of indomethacin, levels of PG's were 4 to 5 times greater and the greatest increase occurred on day 5 of pseudopregnancy.

Changes in prostaglandin synthetic capacity could result from changes in the activity of enzymes necessary for synthesis or from changes in levels of lipid precursor for prostaglandin synthesis. During early pregnancy and pseudopregnancy, the content, composition, and distribution of lipid stores in luminal epithelial cells respond to progesterone and estrogen secretion (Enders and Given, 1977; Beall, 1972). Specific

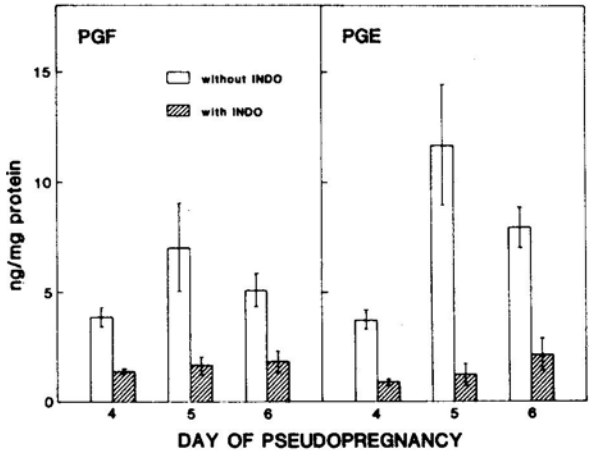

Figure 3. Levels of prostaglandins in uterine luminal epithelial cells during pseudopregnancy. Epithelial cells were removed, homogenized with or without indomethacin (20 μg/ml), and centrifuged (6000*g*, 20 min). Prostaglandin levels in the supernatants were determined by radioimmunoassay using $PGF_{2\alpha}$ and PGE_2 as standards. Each value is the mean ± the SEM of at least 7 samples with 3 rats/sample.

histochemical techniques demonstrated that triacylglycerol, the predominant neutral lipid in intracellular lipid droplets of uterine epithelial cells, decreased during early pregnancy (Boshier *et al.,* 1981). Further decreases in stores of neutral lipid were observed in implantation sites (Boshier, 1976). Neutral and unsaturated lipids decreased in response to estradiol as phospholipid granules appeared in the apical cell cytoplasm of luminal epithelial cells (Gould *et al.,* 1978). Biochemical assays of homogenates of uterine horns demonstrated that progesterone increased total uterine lipid content by increasing levels of phospholipid and neutral lipid (Manimekalai *et al.,* 1979). Estrogen treatment resulted in decreases in total uterine lipid content, but increased levels of phospholipids (Manimekalai *et al.,* 1979; Sakai *et al.,* 1980). The development of endometrial sensitivity to deciduogenic stimuli could depend upon these effects of progestin and estrogen on uterine lipid.

Rats ovariectomized for 3 weeks were pretreated with estradiol and progestin to develop uterine sensitivity to a deciduogenic stimulus. On each successive day, rats received the following treatments: E, E, 0, 0, MPA, 0, e or vehicle (E, estradiol, 500 ng, sc; 0, no treatment; MPA, medroxyprogesterone acetate, 3·5 mg, sc; e, estradiol, 200 ng, sc). After 18 h, control and e treated animals were sacrificed, phospholipids were extracted from the luminal epithelial cells, and separated by two dimensional tlc (Portoukalian *et al.,* 1978; Skipski *et al.,* 1964). The fatty acid composition of each phospholipid was determined by gas chromatography (MacGee and Allen, 1974). The precursor of prostaglandin synthesis, arachidonic acid, was detected only in phosphatidyl choline (PC) and phosphatidyl ethanolamine (PE). Estradiol increased the level of arachidonic acid in PC and the per cent of composition but neither result was statistically significant ($p < 0{\cdot}1$, nil). These data do not support the hypothesis that uterine sensitivity to deciduogenic stimuli or the capacity for prostaglandin synthesis depend upon changes in uterine lipid content or composition.

With the completion of various cellular functions prior and during blastocyst implantation, the epithelial cells self-destruct and are removed by phagocytic activity of the trophoblast (Schlafke and Enders, 1975). Although the trophoblast initiates cellular death of luminal epithelial cells, these cells appear programmed by progesterone and estrogen secretion to self-destruct given an appropriate stimulus (Finn, 1977; Finn and Publicover, 1981). Both the destruction of luminal epithelial cells during implantation in several rodent species and the digestion of endocytosed material depend upon the intracellular activity of lysosomes and their content of hydrolytic enzymes (El-Shershaby and Hinchliffe, 1975; Parr and Parr, 1978). In several examples of epithelial cell death, early lysosomal participation takes the form of first autophagy and then autolysis as acid hydrolases leak out of the autophagic vacuoles and damage the cytoplasm (Wyllie, 1981). It is not clear whether a lysosomal mechanism initiates cell death, but lysosomes are clearly involved in mammary gland involution (Helminen and Ericsson, 1970), prostate regression (Helminen and Ericsson, 1971), and blastocyst implantation (El-Shershaby and Hinchliffe, 1975; Abraham *et al.,* 1970; Elangovan and Moulton, 1980a; Moulton and Ingle, 1981).

In our early experiments, we measured the activities of several lysosomal enzymes in blastocyst implantation sites and in non-implantation uterine tissue. Of the five lysosomal enzymes examined, only cathepsin D activity decreased following implantation (Moulton, 1974; Moulton *et al.,* 1978). Immunohistochemical localization was used to determine whether the change in cathepsin D activity involved a specific change in one lysosomal enzyme in all uterine cells or a change in the enzyme content of a specific cell type. Cathepsin D was concentrated in luminal and glandular epithelial cells, and the enzyme content of luminal epithelial cells decreased as these cells deteriorated in response to either the blastocyst or an artificial stimulus (Elangovan and Moulton, 1980a; Moulton and Ingle, 1981). Lysosomal activity and cathepsin D activity have been shown to increase prior to cell death associated with anuran metamorphosis (Lockshin, 1981), involution of the mammary gland (Helminen and Ericsson, 1970), and prostate regression (Helminen and Ericsson, 1971). The concentration of cathepsin D in uterine luminal epithelial cells suggested that epithelial cell death during blastocyst implantation might depend in part upon the accumulation of this lysosomal protease.

Using a method for removing uterine luminal epithelial cells (Fagg *et al.,* 1979), we examined changes in the activity and rates of synthesis of cathepsin D in these cells during early pseudopregnancy (Moulton and Koenig, 1983). The activity of epithelial cathepsin D increased during pseudopregnancy and attained maximal levels as the uterus developed sensitivity to deciduogenic stimuli. The rate of epithelial cathepsin D synthesis increased dramatically between days 2 and 3 of pseudopregnancy and remained elevated. The activity and rates of synthesis of cathepsin D in stromal-myometrial tissues, however, remained unchanged during early pseudopregnancy. To determine the hormonal control of the rate of epithelial cathepsin D synthesis, ovariectomized rats were treated with MPA progesterone or estradiol (figure 4). The progestins increased the activity and rates of synthesis of cathepsin D in luminal epithelial cells. Estradiol had little effect on the relative rate of synthesis of cathepsin D and did not enhance the uterine response to progestins (Elangovan and Moulton, 1980b; Moulton, 1982). Estradiol treatment following progestin pretreatment, however, was necessary for the decrease in cathepsin D activity in response to a

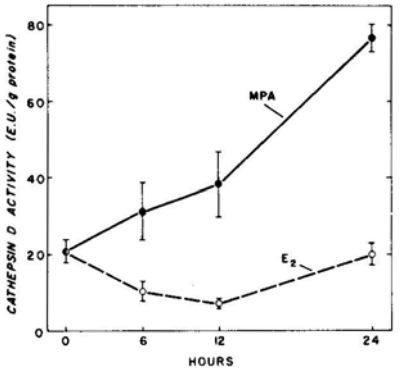

Figure 4. Effect of MPA or estradiol (E_2) on cathepsin D activity in uterine luminal epithelial cells. Ovariectomized rats were injected with MPA (2 mg, sc) or estradiol (5 μg, ip) and 15 animals were sacrificed at each interval thereafter. Each point represents the mean ± SEM of at least 6 assays. Each assay determined the cathepsin D activity of 5 pooled uterine horns.

deciduogenie stimulus (Moulton, 1982). The effect of estradiol could be inhibited by compounds which stabilize lysosomal structure. Collectively, these studies suggested that progesterone and estrogen might control autophagic activity and cell death in luminal epithelial cells during implantation by control of both lysosomal enzyme content and intracellular activity.

Despite the diversity of the morphology of implantation among species, cells lining the uterine lumen in all species must perform similar and essential functions during early pregnancy. While the timing and appearance of these cellular functions may vary, the expectation persists that biochemical processes held in common may still be found. Elucidation of these biochemical processes in luminal epithelial cells and their hormonal control continues to have considerable merit.

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