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STEROIDAL AND GROWTH FACTOR REGULATION OF [^{3}H]THYMIDINE INCORPORATION BY CULTURED ENDOSALPINGEAL CELLS OF THE BOVINE OVIDUCT

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SUMMARY

Cultured cells from the bovine endosalpinx were used to evaluate effects of estradiol-17β, progesterone, epidermal growth factor, and insulinlike growth factors I and II on [^{3}H]thymidine incorporation. Cells were treated with hormones and growth factors when approximately 50% confluent. After 24 h, DNA synthesis was quantified by pulsing cells with [^{3}H]thymidine for 12 h and determining uptake into DNA. Cells prepared by mechanical dispersal incorporated more [^{3}H]thymidine than cells dispersed with collagenase. However, hormonal responses were the same for both types of cells. As compared to plastic, cells on a Matrigel substratum exhibited lower incorporation of [^{3}H]thymidine and were unresponsive to hormones. Estradiol-17β increased [^{3}H]thymidine incorporation slightly at 10^{-10} mol/liter and higher. Epidermal growth factor, insulinlike growth factor-I, and insulinlike growth factor-II also stimulated [^{3}H]thymidine incorporation. Effects of insulinlike growth factor-I were greater for cells treated with estradiol-17β. In the absence of estradiol, progesterone inhibited [^{3}H]thymidine incorporation at 1, 10, and 100 ng/ml. When estradiol-17β was present, progesterone stimulated [^{3}H]thymidine incorporation at 1 ng/ml and reduced incorporation at 100 ng/ml. In conclusion, [^{3}H]thymidine incorporation by cultured oviductal endosalpingeal cells can be regulated by ovarian steroids and growth factors. These molecules may represent signals through which the ovary, embryo, and oviduct regulate oviductal growth.

Key words: oviduct; bovine; progesterone; estrogen; growth factors; proliferation.

INTRODUCTION

Recent experiments have emphasized the importance of the mammalian oviduct for fertilization and early embryonic development. Oviductal endosalpingeal cells can prolong the survival and fertilizing ability of spermatozoa (25) and promote the growth of the early embryo (9,30). Additionally, proteins produced by the oviduct become associated with oocytes (34), spermatozoa (20), and developing embryos (2). Oviductal cells are under the control of ovarian steroids, with both estradiol-17β and progesterone regulating synthesis and secretion of specific oviductal proteins (5,16,23) and morphological characteristics of endosalpingeal epithelium (1,23,29). The role of these steroids in proliferation of the oviductal endosalpinx is not well understood, however. In a recent study, estradiol-17β was reported to increase [^{3}H]thymidine incorporation by bovine endosalpingeal cells while progesterone inhibited incorporation and was antagonistic to the stimulatory effects of estradiol-17β (17). Similar results were found when steroids were administered *in vivo* to rhesus monkeys (29). In other studies, however, these steroids were reported to be without effect on growth of endosalpingeal cells from human (24,30).

The oviduct is also exposed to various locally produced growth factors such as epidermal growth factor (EGF)(7,18), transforming growth factor-α (18,32) and insulinlike growth factors I and II (IGF-I and II) (28,32). While EGF has been reported to increase proliferation of human oviductal epithelial cells (30), little other work has been conducted to evaluate the effects of growth factors on the function of the oviduct.

The present investigations had two major objectives. The major goal was to evaluate regulatory effects of progesterone, EGF, IGF-I, and IGF-II on proliferation of oviductal endosalpingeal cells and to determine whether estradiol-17β stimulated proliferation or altered proliferative responses of oviductal cells to these other regulatory molecules. The second goal was to determine effects of method of cell dispersal (mechanical versus collagenase) and substrate (plastic versus a basement membrane extract) on oviductal cell growth. Bovine endosalpingeal cells were chosen as a model because of the ease of obtaining and culturing tissue, as well as the fact that the functional properties (9,25) of the bovine oviduct have been well described. The growth regulatory properties of the hormones and growth factors tested were measured indirectly by their effects on oviductal cell incorporation of [^{3}H]thymidine into newly synthesized DNA.

MATERIALS AND METHODS

Materials. Estradiol-17β, progesterone, and mouse EGF (purified from submaxillary glands) were from Sigma Chemical Co. (St. Louis, MO). Human recombinant insulinlike growth factor-I and II produced in *Saccaromyces cerevisiae* were obtained from Upstate Biotechnology (Lake Placid, NY). Matrigel™ was produced by Collaborative Biomedical (Bedford, MA). [^{3}H-

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methyl]thymidine ([^{3}H]TdR; spec. act. = 5.0 Ci/mmol) was from Amersham Corporation (Arlington Heights, IL). Monoclonal antibodies to cytokeratin (no. 18) and vimentin, and FITC-conjugated sheep anti-mouse IgG were obtained from Boehringer Mannheim Biochemicals (Mannheim, Germany). Collagenase (type IV), antibiotic-antimycotic (ABAM) (10 000 U/ml penicillin, 10 mg/ml streptomycin and 25 μg/ml amphotericin B), and other reagents for tissue culture were from Sigma.

Preparation of oviductal endosalpingeal cells. Sources of oviducts in this study were reproductive tracts collected from a local abattoir. Generally, cows were of unknown reproductive status, in which case only reproductive tracts with a functional corpus luteum on one ovary were used. Other tracts were collected from cows on Days 2, 13, 14, or 15 of the estrous cycle. Oviducts were transported at room temperature in Dulbecco's phosphate-buffered saline (PBS) containing 0.5% (wt/vol) bovine serum albumin (BSA). Oviducts were separated from connective tissue and the fimbria removed. Each oviduct was then stripped of endosalpingeal cells by use of a small forceps. The oviduct was stripped by moving the forceps from the uterotubal junction towards the infundibulum. The endosalpingeal cells were extruded through the opening in the infundibulum and then washed three times in Tissue Medium 199 supplemented with 1% (vol/vol) ABAM.

After collection, cells were dispersed either mechanically (by repeated pipetting through a Pasteur pipette) or enzymatically. Because cells separated mechanically were often in clumps, cells were resuspended in a modification of Eagle's minimal essential medium called Pig MEM (19) containing 1% (vol/vol) ABAM and 10% (vol/vol) fetal calf serum (FCS) to a density of 0.05 optical density units. Optical density was determined at a wavelength of 490 nm using a microtiter plate reader. For enzymatic separation, cells were washed once in Hank's balanced salt solution (HBSS) without Ca^{++} and Mg^{++} supplemented with 1% (vol/vol) ABAM and then resuspended in the same medium containing 0.1% (wt/vol) collagenase (5 ml per oviduct equivalent). Cells were incubated for 90 min at 37° C in a gas atmosphere of 5% CO_2; frequent gentle pipetting was performed to promote dissociation. After 90 min, cells were washed once in Eagle's minimal essential medium (MEM) and then resuspended in Pig MEM containing 1% (vol/vol) ABAM and 10% (vol/vol) FCS to a final concentration of 1×10^6 cells/ml (as determined by hemacytometer).

Wells of tissue culture plates were generally untreated although in one experiment, wells were coated with Matrigel (25 μl diluted 1:4 (vol/vol) in serum-free MEM). To initiate culture, 150 μl cell suspension were added to wells. Medium was changed after 2 d of culture. Cells were used in proliferation experiments when monolayers were approximately 50% confluent (Days 3–4 of culture for mechanically separated cells and Day 5 of culture for enzymatically separated cells).

Immunofluorescence. To evaluate the cell types present in culture, oviductal cells were analyzed by immunofluorescence using antibodies specific for epithelium (anticytokeratin) and fibroblasts (antivimentin). Mechanically dispersed endosalpingeal cells were grown on chamber slides (NUNC, Naperville, IL) in Pig MEM containing 1% (vol/vol) ABAM and 10% (vol/vol) FCS until reaching confluence. Cells were then washed in PBS, pH 7.4 (PBS), fixed with acetone at −20 °C, rinsed with PBS, incubated with FCS for 30 min at room temperature (to block nonspecific binding sites), and then incubated with mouse anticytokeratin or antivimentin (10 μg/ml in PBS) for 60 min at 37° C (anticytokeratin) or room temperature (antivimentin). Cells were then washed with PBS, incubated with sheep antimouse IgG coupled to FITC [12 μg/ml in PBS containing 0.5% (wt/vol) BSA], washed extensively in PBS, and viewed for fluorescence using a Zeiss epifluorescence microscope.

Studies on [^{3} H]TdR incorporation. When cells were about 50% confluent, they were washed three times with HBSS containing 1% (vol/vol) ABAM; then 200 μl RPMI 1640 (phenol red and serum-free) supplemented with 1% (vol/vol) ABAM and various hormonal treatments were added in quadruplicate. Cells were cultured an additional 24 h and then 0.1 μCi [^{3}H]TdR was added in a volume of 10 μl culture medium. After an additional 12 h, cells were harvested with a semiautomatic cell harvester onto glass-fiber filters. Filters were counted for radioactivity using scintillation spectrometry.

Four experiments were performed. Experiment 1 was designed to test whether estradiol-17β and EGF were mitogenic for cells cultured on plastic or Matrigel substrata. Cells were obtained separately from five cows and prepared by collagenase digestion (n = 4) or mechanical separation (n = 1). For each cow, cells were then plated on plastic or on Matrigel-coated plates. When cells on plastic became 50% confluent, cells in both types of wells (plastic and Matrigel) were treated with various concentrations of estradiol-17β (0, 10^{-10}, 10^{-9}, or 10^{-8} mol/liter) or EGF (0, 0.5, 5.0, and 50.0 ng/ml). After 24 h, cells were pulsed for 12 h with 0.1 μCi [^{3}H]TdR. The objective of experiment 2 was to evaluate the concentration-dependent stimulation of proliferation of endosalpingeal cells prepared by mechanical separation versus collagenase digestion. Cells were dispersed mechanically (n = 3 cows) or by collagenase (n = 2 cows) and treated when 50% confluent with 0, 10^{-12}, 10^{-11}, or 10^{-10} mol/liter estradiol-17β. For experiment 3, cells were prepared by mechanical separation (n = 3 cows) or by collagenase (n = 3). When 50% confluent, cells were treated in quadruplicate with 0 or 10^{-10} mol/liter estra-

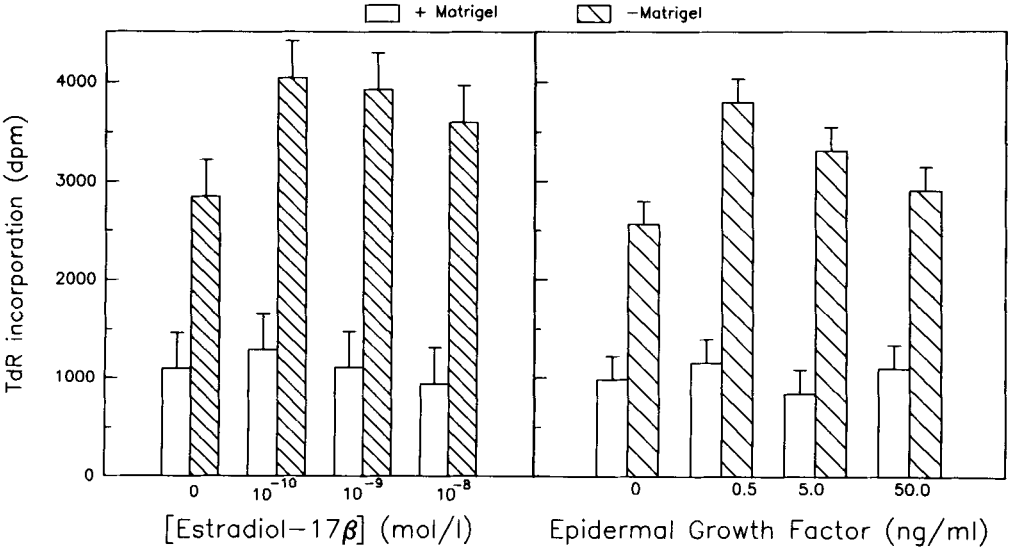

FIG. 1. Incorporation of [^{3}H]thymidine (TdR) by endosalpingeal cells of the bovine oviduct in response to estradiol-17β and epidermal growth factor (EGF) as affected by substratum. Cells were cultured on plastic coated with Matrigel (+Matrigel) or uncoated plastic (−Matrigel). Results represent least-squares means ± SEM (n = 5 cows). For the estradiol-17β experiment, [^{3}H]TdR incorporation was affected by Matrigel ($P = 0.10$) and the orthogonal contrast testing the linear effect of log estradiol concentration ($P = 0.08$). For the EGF experiment, [^{3}H]TdR incorporation was affected by Matrigel ($P < 0.05$), EGF (overall effect, $P = 0.10$; log-quadratic effect, $P = 0.05$) and Matrigel × EGF (Matrigel × log-quadratic, $P < 0.05$).

diol-17β and various concentrations of EGF, IGF-I, or IGF-II (0, 0.1, 0.5, 2.5, 5.0, and 50.0 ng/ml). Thus, treatments were arranged in a factorial array to determine whether estradiol-17β altered responses to growth factors. For experiment 4 (conducted to determine interactions between estradiol-17β and progesterone), the design was similar except that concentrations of progesterone tested were 0, 1, 10, and 100 ng/ml and cells were obtained by mechanical separation (n = 3) or by collagenase digestion (n = 1).

Statistical analysis. Data from replicate determinations were averaged before analysis. All data were analyzed by the method of least-squares analysis of variance (26). The mathematical model for experiment 1 included main effects of cow, substratum (plastic versus Matrigel), and estradiol-17β or EGF concentration. The model for experiment 2 included main effects of type of separation (mechanical versus collagenase), cow nested within separation, and estradiol-17β concentration. The model for experiment 3 included main effects of type of separation, cow nested within separation, estradiol-17β (+ or −), and growth factor concentration (i.e, concentration of EGF, IGF-I, or IGF-II). The model for experiment 4 included main effects of cow, estradiol-17β, and progesterone concentration. For each analysis, all interactions were included in the experimental model and oviduct cell donor (i.e., cow) was considered as a random effect; other main effects were considered fixed. Tests of significance were made using proper error mean squares as determined from calculation of expected mean squares. Variation associated with effects of growth factor concentration and interactions of concentration with other main effects was partitioned into individual degree-of-freedom comparisons using orthogonal contrasts. For estradiol-17β (experiments 1 and 2), EGF (experiment 1), and progesterone (experiment 4), orthogonal contrasts were constructed to test the linear, quadratic, and cubic effects of the log-transformed hormone or growth factor concentration. For experiment 3 (EGF, IGF-I, and IGF-II), concentrations were not equally spaced and, therefore, contrasts tested were as follows: contrast 1, 0 vs. other concentrations; contrast 2, 0.1 ng/ml vs. higher concentrations; contrast 3, 0.5 ng/ml vs. higher concentrations; contrast 4, 2.5 ng/ml vs. higher concentrations; contrast 5, 5.0 vs. 50 ng/ml.

Results

Characterization of cells by immunofluorescence. Approximately 50% of oviductal cells dispersed mechanically and grown to confluence on plastic reacted positively with antibody to cytokeratin, an epithelial cell marker (results not shown). Similarly, about 50% of the cells reacted with antibody to vimentin, an intermediate filament protein present in fibroblasts. Thus, the mechanically dispersed cell cultures grown on plastic contained a mixture of epithelial and stromal cells.

Incorporation of [³H]thymidine. In experiment 1, effects of estradiol-17β and EGF were tested in cells grown on plastic versus cells grown on Matrigel-coated plastic. Cells that attached to Matrigel showed very little evidence of growth and never became 50% confluent. Therefore, while cells on plastic were evaluated for hormonal responsiveness when cultures were about 50% confluent, cells on Matrigel were tested when cell density was less. As shown in Fig. 1, both estradiol-17β and EGF increased incorporation of [³H]TdR in cells grown on plastic ($P = 0.08$ for log-linear effect of estradiol-17β concentration; $P = 0.10$ for the overall effect of EGF; and $P = 0.05$ for the log-quadratic effect of EGF). For both estradiol-17β and EGF, all concentrations caused increased [³H]TdR incorporation. For cells on Matrigel, in contrast, neither estradiol-17β or EGF was mitogenic (matrigel × log-quadratic effect of EGF, $P < 0.05$).

Incorporation of [³H]TdR for experiment 2 (Fig. 2) was higher for cells prepared by mechanical separation than for cells prepared by collagenase digestion ($P < 0.07$). Estradiol-17β increased [³H]TdR uptake slightly (overall effect, $P < 0.05$; log-quadratic effect $P = 0.07$; log-cubic effect, $P < 0.05$) but stimulation was apparent only at the highest concentration tested (10^{-10} mol/liter). The stimulatory effect of 10^{-10} mol/liter estradiol-17β occurred for cells prepared by mechanical stimulation and cells prepared by enzymatic digestion.

Experiment 3 was designed to test interactions between 10^{-10} mol/liter estradiol-17β and various growth factors in regulating cell proliferation. Cells prepared by collagenase treatment incorporated less [³H]TdR than cells prepared by mechanical separation ($P < 0.03$ for the EGF experiment, $P < 0.01$ for the IGF-I experiment, and $P = 0.16$ for the IGF-II experiment). However, responses to growth factors and hormones were similar for cells prepared by both methods (i.e., interactions with type of separation were not significant). Therefore, least-squares means are presented as pooled across this classification. For all three growth factors tested, effects of estradiol-17β interacted with concentration of growth factor (Figs. 3 and 4). The main effect of estradiol-17β was significant only for the IGF-II experiment. Each growth factor enhanced [³H]TdR incorporation although to varying degrees. EGF was mitogenic at concentrations of 2.5 ng/ml and higher (Fig. 3; $P < 0.03$ for 0.5 ng/ml vs. higher concentrations). Estradiol-17β altered the effects of EGF ($P = 0.08$ for estradiol × 0.1 ng/ml versus higher concentrations) because the lowest concentration of EGF (0.1 ng/ml) inhibited [³H]TdR incorporation by untreated cells while having no effect in estradiol-treated cells (Fig. 3). For IGF-I (Fig. 4), concentrations of 2.5 ng/ml and higher increased [³H]TdR incorporation (overall effect of concentration, $P = 0.09$; 0.1 ng/ml versus higher concentrations, $P = 0.06$) and stimulatory effects of IGF-I were greater for cells with estradiol-17β ($P < 0.05$ for estradiol × 0.5 ng/ml versus higher concentrations). For IGF-II, all concentrations stimulated [³H]TdR incorporation (Fig. 4; $P < 0.05$ for untreated versus IGF). While not significant ($P = 0.16$ for estradiol × untreated versus IGF), responses to IGF-II tended to be of greater magnitude for cells treated with estradiol-17β.

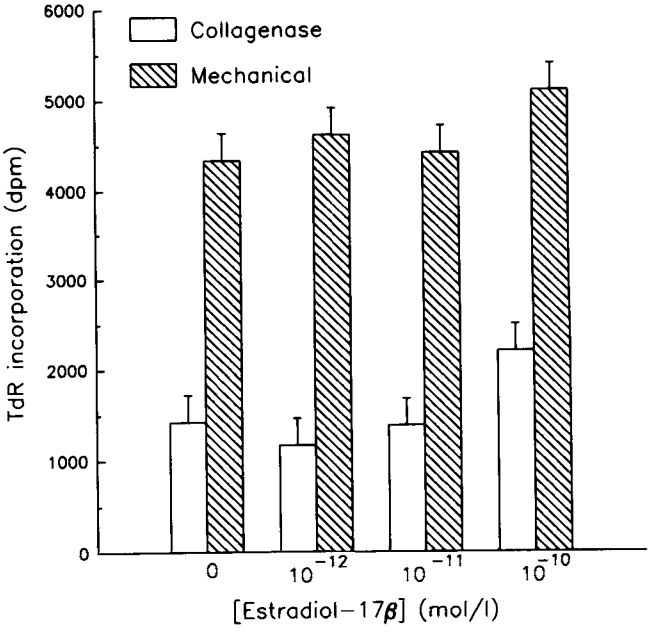

FIG. 2. Effect of method of cell preparation and estradiol-17β on [³H]thymidine (TdR) incorporation by endosalpingeal cells of the bovine oviduct. Cells were prepared either by digestion with collagenase (n = 2 cows) or by mechanical dispersion (n = 3 cows). Results represent least-squares means ± SEM. Incorporation of [³H]TdR was affected by method of preparation ($P = 0.07$) and concentration of estradiol-17β (overall effect, $P < 0.05$; log-quadratic, $P = 0.07$; log-cubic, $P < 0.05$).

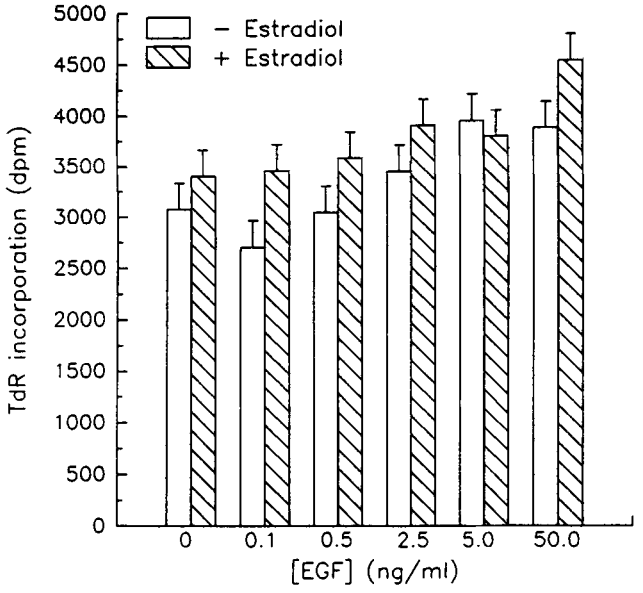

FIG. 3. Incorporation of [^{3}H]thymidine (TdR) by endosalpingeal cells of the bovine oviduct as affected by interactions between estradiol-17β and epidermal growth factor (EGF). Results represent least-squares means ± SEM (n=6 cows). Incorporation was affected by EGF (0.5 ng/ml versus higher concentrations, $P < 0.03$) and estradiol × EGF (estradiol × 0.1 ng/ml versus higher concentrations, $P = 0.08$)

The effect of increasing concentrations of progesterone on incorporation of [^{3}H]TdR in the presence and absence of 10^{-10} mol/liter estradiol-17β is presented in Fig. 5. The effect of progesterone depended on the presence of estradiol-17β (estradiol × log-quadratic effect of progesterone, $P = 0.09$). In the absence of estradiol-17β, progesterone inhibited [^{3}H]thymidine incorporation at 1, 10, and 100 ng/ml. When estradiol-17β was present, progesterone stimulated [^{3}H]thymidine incorporation at 1 ng/ml and reduced incorporation at 100 ng/ml. Estradiol-17β only increased incorporation of [^{3}H]TdR if cells were also treated with progesterone.

DISCUSSION

These results indicate that [^{3}H]TdR incorporation by cultured endosalpingeal cells of the bovine oviduct can be affected by steroids and growth factors. As reported earlier in the bovine (17) and rhesus monkey (29), estradiol-17β stimulated proliferation of oviductal endosalpingeal cells. These findings are in contrast to results with human (24,30) oviductal endosalpingeal cells. The lowest concentration of estradiol-17β that was effective in causing increased [^{3}H]TdR incorporation in the present study (10^{-10} mol/liter; i.e., 27.2 pg/ml) was higher than found previously (1 pg/ml) by Kamwanja and Hansen (17). However, that experiment was performed in medium supplemented with FCS and phenol red and these constituents could have altered the effective concentration of estradiol-17β. Estimates of the K_d of the uterine estrogen receptor vary from 10^{-11} to 10^{-10} mol/liter (15,21,31). Concentrations in peripheral blood at estrus vary from 10–30 pg/ml in the cow (8,27). Thus, an elevation of circulating estradiol-17β concentrations at estrus may be sufficient to induce increased growth of oviductal endosalpinx. Nonetheless, the magnitude of estradiol-induced [^{3}H]TdR incorporation in the present study and in the earlier study by Kamwanja and Hansen (17) was low. Therefore, other factors are likely to contribute to regulation of endosalpingeal proliferation.

Effects of estradiol-17β on growth of endometrial epithelial cells from mice was enhanced if cells were in contact with stroma (15). In the endosalpinx, estrogen receptors are present in both stromal cells and secretory epithelial cells (3) and perhaps similar epithelial-stromal cell interactions regulating growth occur in the oviduct. Cell cultures in the present study contained both epithelial and stromal cells and such interactions were possible.

There was some evidence from experiment 4 that estradiol-17β was more stimulatory in the presence of progesterone. The significance of this finding is uncertain, however, because estradiol-17β was capable of stimulating [^{3}H]TdR incorporation without other hor-

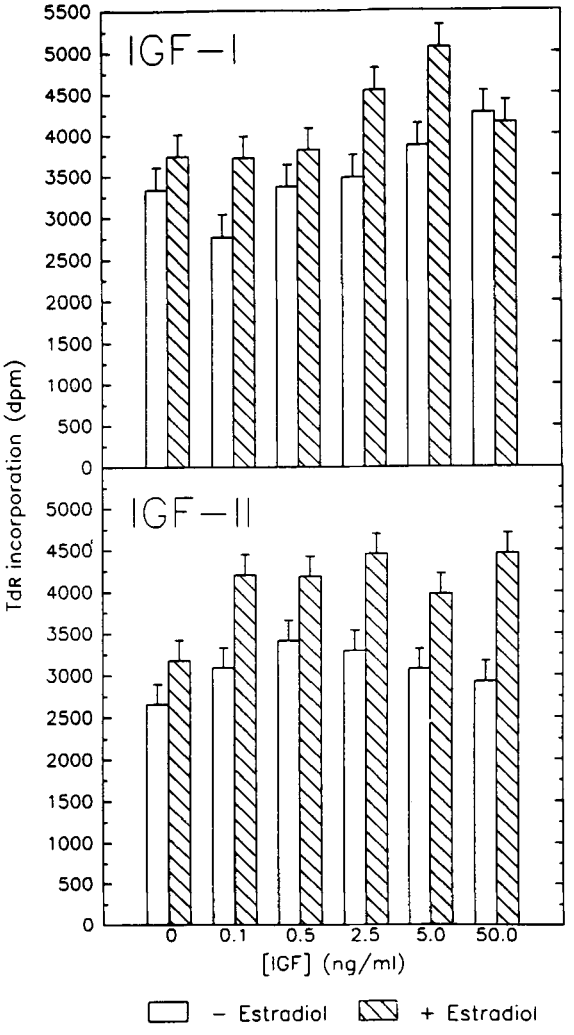

FIG. 4. Incorporation of [^{3}H]thymidine (TdR) by endosalpingeal cells of the bovine oviduct as affected by interactions between estradiol-17β and insulinlike growth factor-I (IGF-I) and insulinlike growth factor-II (IGF-II). Results represent least-squares means ± SEM (n=6 cows). For the IGF-I experiment, incorporation was affected by concentration of IGF-I (overall effect, $P = 0.09$; 0.1 ng/ml versus higher concentrations, $P = 0.06$) and estradiol × IGF (estradiol × 0.5 ng/ml versus higher concentrations, $P < 0.05$). For IGF-II, incorporation was affected by estradiol-17β ($P < 0.05$) and IGF (untreated versus IGF, $P < 0.05$).

mones in experiments 1–3. Moreover, progesterone was antagonistic to the stimulatory effects of estradiol-17β in earlier studies with cultured bovine oviductal endosalpingeal cells (17) and when administered *in vivo* to rhesus monkeys (29). As found earlier in the cow (17), progesterone was inhibitory to [^{3}H]TdR incorporation by endosalpingeal cells in the absence of estradiol-17β. This result contrasts with the reported refractoriness of human (24,30) oviductal endosalpingeal cells to progesterone. The response to progesterone was altered when cells were also treated with estradiol-17β. While all concentrations of progesterone inhibited [^{3}H]TdR incorporation by cells without estradiol-17β, only the highest concentration of progesterone tested, 100 ng/ml, reduced [^{3}H]TdR incorporation in estradiol-treated cells. Moreover, 1 ng/ml progesterone stimulated incorporation of [^{3}H]TdR by estradiol-treated cells. These results suggest that high concentrations of progesterone may be required to cause inhibition of proliferation if cells are also exposed to estradiol-17β and that low concentrations of progesterone may stimulate proliferation of cells exposed to estradiol 17β. While a concentration of progesterone as high as 100 ng/ml could be present in the oviduct ipsilateral to the corpus luteum (33), this concentration is much lower than circulating concentrations during the estrous cycle of cattle.

Both EGF (4) and IGF-I (22) have been implicated as mediators of the mitogenic effects of estradiol-17β in the endometrium. Growth factors from the early embryo could also regulate oviductal growth. In the present study, EGF, IGF-I, and IGF-II stimulated [^{3}H]TdR incorporation by endosalpingeal cells and may, therefore, represent possible mediators of oviductal growth. While EGF is not produced by the embryo (32), both EGF and its receptor have been identified in the human oviductal epithelium (18). Previously, EGF was reported to increase proliferation of cultured human oviductal epithelial cells (30). It is possible that EGF acts through stimulation of the estrogen receptor (13,14). This is the first report, however, indicating that insulinlike growth factors are mitogenic towards endosalpingeal cells. While not significant for IGF-II, estradiol-17β tended to increase responsiveness of oviductal endosalpingeal cells to both IGFs. Consistent with this finding are observations that estradiol-17β stimulates IGF-I receptors and proliferation in the rat uterus (10,22). Low levels of mRNA for IGF-I and IGF-II have been detected by dot-blot analysis of porcine oviducts (28). Additionally, mRNA for IGF-I and IGF-II have been identified in preimplantation bovine embryos as early as the one-cell stage (32). The oviduct also contains various IGF binding proteins (11,28). Thus, regulation of growth of the oviduct could be achieved by growth factors of oviductal or embryonic origin, as well as through changes in growth factor receptors or binding proteins.

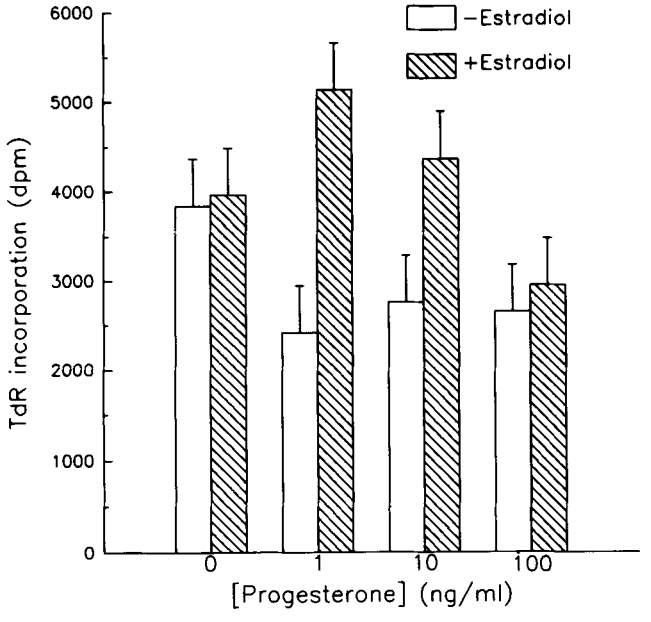

FIG. 5. Incorporation of [^{3}H]thymidine (TdR) by endosalpingeal cells of the bovine oviduct as affected by interactions between estradiol-17β and progesterone. Results represent the least-squares means ± SEM (n=4 cows). Incorporation was affected by progesterone (main effect, $P < 0.05$; log-linear effect, $P < 0.01$) and the estradiol × log-quadratic effect of progesterone $P = 0.09$).

Cells from one animal in experiment 3 were collected at Day 2 of the estrous cycle but otherwise cells were collected from animals that were under progesterone domination. The cells from the cow at Day 2 were included in results because responses of these cells to treatments were similar to cells from other animals. It is possible, however, that responses of the cultured cells to hormonal treatment depend on hormonal milieu of the animals used as oviduct donors. Similarly, cell cultures were established in media containing phenol red; it is not known to what degree exposure to this estrogenic medium constituent altered responses of cells to treatments added later in culture, when phenol red was absent.

One goal of the experiments was to identify culture conditions that are optimal for studying oviductal growth. Matrigel proved unworkable as a substratum for establishing growing cells; cells plated on Matrigel were apparently viable but grew very slowly. Also, cells on Matrigel were unresponsive to estradiol-17β and EGF. Slow growth was also observed when cells were grown on Matrigel-coated Millicell inserts (Millipore Corp., Bedford, MA, unpublished observations). Similarly, Joshi (16) found that cells on Matrigel grew slowly although they became differentiated with respect to polarity and junctional complexes. Thus, the cytoarchitecture of cells grown on Matrigel more closely mimics the situation *in vivo* than for cells on plastic. Growth of polarized oviductal epithelial cells may more closely represent what occurs in the oviduct than growth of cells on plastic. However, conditions for establishing polarity (i.e., type of matrix) must be altered from those used in the present experiments to allow cells to grow and respond to hormonal or growth factor treatment.

Collagenase treatment to disperse cells before culture, which has been used previously (30), caused a consistent reduction in the subsequent growth of plated cells. It is not clear whether differences in [^{3}H]TdR incorporation between cells prepared by mechanical versus enzymatic procedures represent differences in the relative amounts of stromal and epithelial cells or in ability of individual cell types to proliferate. In any case, hormonal responses of cells were similar for cells separated by collagenase and mechanical dispersion, suggesting that both procedures would be acceptable for establishing cell cultures to measure proliferation.

The method of preparation of cell cultures used in the present experiments is usually considered to produce a population of cells termed "epithelial" (6,12,17,25). Previously, this assumption has not been confirmed by immunohistochemical staining for stromal and epithelial cell markers. The fact that preparations of oviductal cells prepared by mechanical disruption contain both cell types illustrates

the importance of performing such immunohistochemical analyses before assuming that cell populations are homogeneous. Moreover, additional cell purification steps are warranted if it is desired to extrapolate results to specific oviductal cell populations.

Electron microscopy confirmed that cells grown on plastic were almost totally lacking in cilia by the time they became confluent (results not shown). Given that differentiation of ciliated cells is controlled by estrogen (1), it is possible that cilia could have been maintained in culture by continued presence of estradiol-17β. However, this treatment caused only temporary maintenance of ciliary beating in cultured bovine oviductal epithelial cells (12).

In conclusion, these results indicate that [^{3}H]TdR incorporation by cultured cells of the bovine oviduct can be affected by ovarian steroids and polypeptide growth factors. These molecules may, therefore, represent endocrine or paracrine regulatory signals for regulation of oviductal growth by the ovary, embryo and oviduct.

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