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Progesterone and Prostanoid Production by Bovine Binucleate Trophoblastic Cells[1]

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ABSTRACT

A procedure for preparing highly enriched suspensions of bovine binucleate trophoblastic cells was developed and data showing that these cells produce progesterone, prostacyclin (PGI_2), and prostaglandin E_2 (PGE_2) were obtained. Approximately 200 × 10^6 enzymatically dissociated cells from bovine cotyledons were applied to the surface of a density gradient of 2% to 4% Ficoll-400 using the Wescor CELSEP sedimentation chamber. After 90–120 min of sedimentation at unit gravity, fractions containing binucleate trophoblastic cells were obtained and washed in HEPES-buffered Medium 199. Preparations of 90% to 100% binucleate trophoblastic cells were obtained routinely; viability was 50% to 80%. After incubation at 37°C, concentrations (ng/10^5 cells) of progesterone were greater in those fractions containing binucleate cells than in those containing primarily smaller, mononucleate cells. Total progesterone secreted (mean ± SEM) after 4 h by 1 × 10^5, 2 × 10^5, 4 × 10^5, 8 × 10^5, and 1.6 × 10^6 binucleate cells was 0.27 ± 0.03, 1.01 ± 0.09, 4.02 ± 0.37, 10.31 ± 0.92, and 20.96 ± 2.23 ng, respectively (r=0.997). Addition of 10% fetal bovine serum (FBS) or normal anestrous cow serum increased (P<0.05) production of progesterone by binucleate trophoblastic cells. Luteinizing hormone, follicle-stimulating hormone, prolactin, thyrotropin, and 8-bromo-adenosine 3',5'-cyclic monophosphate had no effect. Binucleate trophoblastic cells also produced PGI_2 in relation to number of cells incubated (r=0.996). Time courses for production of PGI_2, PGE_2, and progesterone were similar. Aspirin inhibited production of PGI_2 and PGE_2 by about 50% at a dose of 100 μM; FBS stimulated production of both prostanoids.

INTRODUCTION

The trophoblastic component of the ruminant chorion consists of numerous cuboidal mononucleate cells and relatively few rounded binucleate cells (Wimsatt, 1951; Boshier and Holloway, 1977). The morphologic (Boshier and Holloway, 1977), histochemical (Boshier, 1969; Reddy and Watkins, 1978; Wooding, 1980), and migratory (Wooding, 1980; Wooding and Wathes, 1980) characteristics of binucleate trophoblastic cells in the ruminant placenta suggest a close relationship to the endocrinologically active syncytiotrophoblast of the deciduate placenta of humans (Wimsatt, 1951; Simpson and MacDonald, 1981).

Many previous studies of placental endocrine function in vitro have relied on the use of heterogeneous placental explants (Hoffman et al., 1979; Siler-Khodr and Khodr, 1981; Ling, 1983; Matt and MacDonald, 1984) or cell suspensions (Winkel et al., 1980; Branchaud et al., 1983; Jogee et al., 1983; Shemesh et al., 1984a,b). Although such studies have contributed to our knowledge of placental endocrinology, these heterogeneous preparations do not allow examination of endocrine functions of the individual cell types within the placenta. This article describes our techniques for preparing homogeneous populations of binucleate trophoblastic cells from bovine cotyledons and demonstrates that these cells produce and secrete progesterone and prostanoids in vitro.

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MATERIALS AND METHODS

Materials

Trypsin solution (0.25% in Hank's balanced salt solution without Ca^{2+} and Mg^{2+}), Medium 199 with Earle's salts and L-glutamine, Dulbecco's phosphate-buffered saline (D-PBS), penicillin (10,000 units/ml)-streptomycin (10,000 μg/ml) solution, and HEPES (*N* - 2 - hydroxyethylpiperazine - N^1 - 2 - ethanesulfonic acid) were purchased from GIBCO (Grand Island, NY). Ficoll-400 and deoxyribonuclease (400–600 Kunitz units/mg) were purchased from Sigma Chemical Co. (St. Louis, MO). The Wescor CELSEP apparatus and Masterflex peristaltic pump were purchased from Harvey Instruments (Buffalo, NY). Sterile cluster dishes were purchased from Costar (Cambridge, MA), and polystyrene culture tubes from Bio-Rad Laboratories (Rockville Centre, NY). Reagents for radioimmunoassays of progesterone and prostanoids were obtained from Micromedic Systems (Horsham, PA) and New England Nuclear (Boston, MA), respectively. Surgical instruments and glassware were sterilized by autoclaving; the CELSEP sedimentation chamber was sterilized with ethylene oxide gas.

Dissociation and Purification of Binucleate Trophoblastic Cells

Gravid bovine uteri were obtained from a local abattoir (Taylor Packing Co., Wyalusing, PA) and transported to the laboratory packed in ice. Uteri were cut open longitudinally and cotyledons and caruncles were separated manually. Cotyledons were rinsed in D-PBS containing 20,000 units of penicillin and 20,000 μg of streptomycin/1000 ml. Cotyledons were cut into pieces of approximately 1 cm^2 with scalpel blades and stored in a beaker maintained in an ice bath. Generally, 200 g of wet tissue were transferred into two 1000-ml Erlenmeyer flasks and trypsin solution (1 ml/g of tissue) and deoxyribonuclease (10 mg/100 ml of trypsin solution) were added to the tissue. Digestion was done in an oscillating water bath at 37°C for 15 min. The digest was filtered through two layers of cheesecloth and the liquid discarded. The solid tissue was digested with fresh trypsin solution and deoxyribonuclease a second time for 45 min. After filtering, dissociated cells were centrifuged at 100 × *g* for 15 min. If additional cells were needed, the remaining tissue was digested a third time for 45 min and cells from the second and third digestions were pooled. Cells were centrifuged and washed three times in Medium 199 containing 25 mM HEPES. After the final wash, the cells were suspended in HEPES-buffered Medium 199 and were used immediately or were stored overnight at 4°C. Cell viability at this stage of purification ranged from 60% to 90%.

The CELSEP apparatus was used to separate binucleate cells from other cell types on the basis of density sedimentation at unit gravity (Peterson and Evans, 1967). The apparatus includes the motor base unit, the sedimentation chamber consisting of upper and lower thermoplastic plates clamped to a short cylindrical section, a gradient maker, and the peristaltic pump. The CELSEP chamber in the "up position" was loaded with a continuous gradient of 2% to 4% Ficoll solution dissolved in HEPES-buffered Medium 199 using the gradient maker and peristaltic pump. The gradient was underlayered with a dense cushion of 7% Ficoll until the gradient began to exit the upper chamber port (Fig. 1-1). The dissociated, heterogeneous cell suspension in 1% Ficoll solution (approximately 200 × 10^6 cells in 25 ml) was filtered through artist's silk and then loaded onto the gradient by reversing the pump. An overlay of Medium 199 (20 ml) without Ficoll was loaded in the same manner (Fig. 1-2). The chamber was oriented to the horizontal, or "down," position (Fig. 1-3) and the cells were allowed to sediment at 20–22°C for 90–120 min (Fig. 1-4). Subpopulations of cells, separated on the basis of density, were unloaded from the lower port after reorientation of the chamber to the "up position" (Fig. 1-5).

Twenty 50-ml fractions were collected from the chamber and centrifuged at 150 × *g* for 15 min at 4°C. Supernatants were aspirated, leaving about 1 ml of medium in each tube. The cells were resuspended in the remaining medium and examined microscopically for size, appearance, and viability (trypan blue exclusion). Fractions containing binucleate cells were pooled and the cells were washed three times in Medium 199 at 4°C. Washed cells were suspended in 2–5 ml of Medium 199 to allow statistically reliable counting with a hemacytometer and were diluted to appropriate concentrations. Purity of the concentrated suspension of binucleate trophoblastic cells was confirmed by observing two nuclei per cell after heating approximately 500 μl of the suspension to kill the cells (Fig. 2). All data presented are on the basis of live binucleate trophoblastic cells.

Progesterone Production and Secretion by Binucleate Trophoblastic Cells

To determine initially whether binucleate or mononucleate trophoblastic cells produced progesterone, we obtained 14 fractions of cells from a single placenta of 235 days of gestation (estimated by crown-rump length; Evans and Sack, 1973). The cells, regardless of type, were counted and the concentration adjusted with Medium 199 to 5 × 10^5/ml. A 1-ml sample of each fraction was incubated in 12 × 75-mm polystyrene culture tubes at 37°C for 18 h in a humid atmosphere of 95% air/5% CO_2. The tubes were centrifuged and supernatants were stored at −20°C until assayed.

We next determined if production of progesterone by binucleate trophoblastic cells was related to the number of cells incubated (i.e., dose-response analysis). A suspension of binucleate trophoblastic cells was prepared from a placenta of 120 days of gestation. Cells were dispensed in 2 ml of HEPES-buffered Medium 199 into wells (16 mm diameter) of sterile cluster dishes in concentrations of 1 × 10^5, 2 × 10^5, 4 × 10^5, 8 × 10^5, and 1.6 × 10^6 cells per well (3 wells/concentration). Cells were incubated as above for 2 h. One milliliter of medium was aspirated carefully from each well and frozen. An equal volume of fresh medium was replaced and the cells were returned to the incubator. After 4 h, a second 1-ml sample of medium was aspirated and frozen. The remaining 1 ml of medium containing cells was also frozen to lyse the cells. Progesterone was measured in the first two samples of medium to determine the amount that was secreted by the cells and in the third

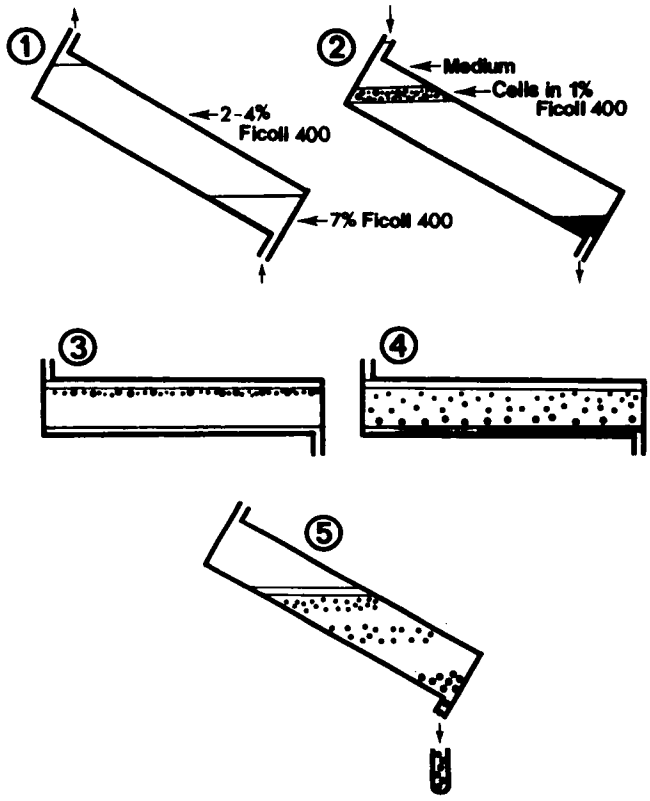

FIG. 1. Diagram of the CELSEP sedimentation chamber for preparation of populations of binucleate trophoblastic cells from the bovine placenta.

sample, after thawing and centrifugation, to reflect intracellular content of progesterone plus that which was secreted. The total quantity of progesterone secreted in 4 h was calculated as follows. Because the incubation volume in each well was 2 ml, the concentration (ng/ml) of progesterone in the 2-h sample of medium was doubled to represent the quantity secreted by the cells in 2 h. The concentration in the 4-h sample was doubled and the concentration at 2 h was subtracted to give the quantity secreted de novo between 2 h and 4 h. The sum of the quantity secreted in 2 h and that secreted between 2 h and 4 h was considered as the total quantity of progesterone secreted into the medium in 4 h (i.e., ng/4 h).

To determine more precisely the time course of progesterone production by binucleate trophoblastic cells, 5×10^5 binucleate cells (gestational age = 170 days) were incubated in triplicate for 1.5, 3, 6, 12, 18, or 24 h in 1 ml of medium in sterile cluster dishes. After each time period, the contents of appropriate dishes were frozen and stored at −20°C until assayed.

Progesterone was quantified in all samples by solid-phase radioimmunoassay similar to that described by Reimers et al. (1983). Validity of the assay for Medium 199 containing HEPES, antibiotics, and disrupted trophoblastic cells and/or their products was demonstrated in several ways. Serial dilutions of media from lysed (by freezing and thawing) trophoblastic cells (5×10^5/ml) containing 3.85, 6.03, or 8.88 ng/ml (means) of progesterone inhibited binding of ^{125}I-labeled progesterone to the antibody in a manner parallel with progesterone solutions in Medium 199 used as standards. When known amounts of progesterone were added to samples of medium from lysed binucleate cells (120, 170, and 250 days of gestation) and assayed, regression analysis of the amounts recovered by this assay gave the equation $y = 1.03x - 0.01$ and a correlation coefficient (r) of 1.0. Intraassay coefficients of variation (CV) for estimates of progesterone in samples of medium from cells containing 1.65, 6.50, and 11.27 ng/ml (means) were 5.2%, 9.2%, and 6.4%, respectively (n=10 duplicate determinations/sample). Interassay CVs for quality-control samples containing 0.42, 1.70, and 7.95 ng/ml (means) of progesterone estimated in 15 separate assays were 25.5%, 12.6%, and 5.6%, respectively. Sensitivity of 10 progesterone assays determined as described by McCann et al. (1983) was 0.16 ng/ml. Relative activities of cross-reacting steroids for this assay have been published (Reimers et al., 1983).

Prostanoid Production and Secretion by Binucleate Trophoblastic Cells

In the first study of prostanoid production, binucleate trophoblastic cells were purified from cotyledons of 150 days of gestation and 1×10^5, 2×10^5,

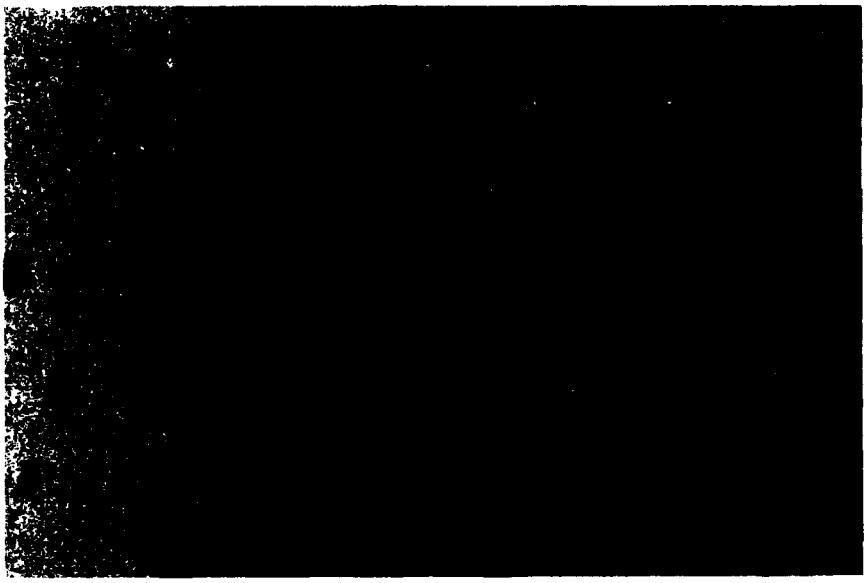

FIG. 2. Binucleate trophoblastic cells stained with trypan blue after killing by heating.

4×10^5, 8×10^5, or 1×10^6 of these cells were incubated in duplicate in 1 ml of Medium 199 for 24 h to establish a dose-response relationship. After incubation, media containing cells were frozen until assayed for 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$), the stable metabolite of prostacyclin (PGI_2). To determine the time course for production of PGI_2, 6-keto-$PGF_{1\alpha}$ was quantified in the same samples collected for time course analysis of progesterone production described above. Prostaglandin E_2 was also measured in these samples.

The radioimmunoassay kit for 6-keto-$PGF_{1\alpha}$ consisted of lyophilized rabbit anti-6-keto-$PGF_{1\alpha}$ serum, ^{125}I-labeled 6-keto-$PGF_{1\alpha}$ solution, assay buffer (0.9% NaCl, 0.01 M ethylenediaminetetraacetic acid, 0.3% bovine gamma globulin, 0.005% Triton X-100, and 0.05% sodium azide in 0.05 M phosphate buffer; pH 6.8), lyophilized 6-keto-$PGF_{1\alpha}$ standard, and precipitating reagent (16% polyethylene glycol 6000 and 0.05% sodium azide in 0.05 M phosphate buffer; pH 6.8). As prescribed in the package insert for the kit, 100 μl of each standard solution or sample in duplicate, 100 μl of ^{125}I-labeled 6-keto-$PGF_{1\alpha}$ solution, and 100 μl of antiserum solution were incubated at 4°C for 22–24 h in 12 × 75-mm polypropylene tubes. Precipitating reagent was then added (100 μl) and incubated at 4°C for 30 min. Tubes were centrifuged at 1000 × g for 30 min, the supernatants were decanted, and radioactivity in precipitates was quantified. Serial dilutions of media from lysed trophoblastic cells containing 2.52, 2.60, or 7.13 ng/ml (means) of 6-keto-$PGF_{1\alpha}$ produced inhibition curves that were parallel with the standards prepared in Medium 199. When known amounts of 6-keto-$PGF_{1\alpha}$ were added to samples of medium from lysed cells from placentas of 120, 170, and 250 days of gestation, regression analysis gave the equation $y = 1.27x + 0.27$ and r=0.999. Intraassay CVs for 10 duplicate estimates of 6-keto-$PGF_{1\alpha}$ concentrations in samples containing 0.11 and 1.10 ng/ml (means) were 5.1% and 10.0%, respectively. Sensitivity determined from three assays was 0.03 ng/ml. According to the package insert for the kit, cross-reactivity of the antiserum with other prostanoids was minimal (2.6% for $PGF_{1\alpha}$, 1.9% for PGE_1, 1.4% for thromboxane, and 1.1% for PGE_2; all other compounds tested were <1%).

The kit for assay of PGE_2 consisted of lyophilized rabbit anti-PGE_2 serum, ^{125}I-labeled PGE_2 solution, assay buffer as above, PGE_2 standard solution, and precipitating reagent as above. Volumes of reagents and assay conditions for PGE_2 were the same as those for 6-keto-$PGF_{1\alpha}$. Serial dilutions of medium from trophoblastic cells containing 1.91, 2.72, and 5.32 ng/ml (means) of PGE_2 generated inhibition curves parallel with the standards. Quantitative recovery of added PGE_2 from samples described above yielded the equation $y = 0.95x + 0.09$; r=0.996. Intraassay CVs for samples containing 0.26 and 1.58 ng/ml of PGE_2 were 11.0% and 8.43%, respectively. Sensitivity of the PGE_2 assay was 0.02 ng/ml. Cross-reactivity of the antiserum was 3.7% with PGE_1 and <1% with all other compounds tested by the kit manufacturer.

Extraplacental Factors Affecting Progesterone and Prostanoid Production

Finally, we wanted to determine if extraplacental factors affected production of progesterone and prostanoids. In the first experiment, binucleate trophoblastic cells (1×10^6/ml; 212 days of gestation) were incubated in 1 ml of Medium 199 containing 500, 50, 5, or 0.5 ng of bovine luteinizing hormone (NIAMMD-bLH-4), bovine follicle-stimulating hormone (FSH; LER-1640-3), bovine prolactin (Prl; NIH-P-B6), or bovine thyrotropin (NIAMMD-bTSH-9), or 10%, 1%, or 0.1% fetal bovine serum (FBS) or normal anestrous

cow serum (NCS). All treatments were done in triplicate. Progesterone was measured in samples of medium after incubation at 37°C for 4 h.

In the second experiment, binucleate trophoblastic cells (5×10^5/ml) from a placenta of 190 days of gestation were incubated in triplicate in 1 ml of Medium 199 containing 50 or 500 ng of bLH, 1 or 10 mM 8-bromo-adenosine 3',5'-cyclic monophosphate (8-Br-cAMP), 0.5 or 5% FBS, or 10 or 100 mM aspirin. Prostanoids as well as progesterone were measured in samples of media after 18 h of incubation.

RESULTS

Concentrations of progesterone in 14 fractions obtained by sedimentation of dissociated placental cells are shown in Fig. 3. Fractions A through C consisted mostly of aggregates of mononucleate and binucleate cells. Although not specifically quantified, fractions D through G consisted almost entirely of purified single binucleate cells, fractions H through J contained a mixture of binucleate and mononucleate cells and fractions K through N contained mononucleate placental cells, erythrocytes, and other blood cells. Progesterone production in Fig. 3 is expressed as nanograms per 1×10^5 cells, regardless of cell type in each fraction. Fractions ascertained microscopically to contain the greatest proportion of binucleate trophoblastic cells contained the highest concentrations of progesterone.

Populations of 90% to 100% binucleate cells were used routinely to generate the results described below. Estimates of viability ranged from 50% to 80%. Numbers of binucleate trophoblastic cells per incubation were correlated with progesterone concentrations in the medium after 2 h (r=0.994) and 4 h (r=0.997; Fig. 4), with progesterone concentrations in the lysed cells after 4 h (r=0.986), and with total progesterone secreted into the 3 ml of medium during the 4-h period (r=0.997; Fig. 5). Binucleate trophoblastic cells produced progesterone rapidly for 12 h in our incubation system (Fig. 6); thereafter, production stabilized. Addition of 10% FBS, 10% NCS (Fig. 7), or 5% FBS (Fig. 8) increased ($P<0.05$) concentrations of progesterone in media from incubations of binucleate trophoblastic cells, whereas bLH, bFSH, bPrl, bTSH, 8-Br-cAMP, and aspirin had no effect.

That binucleate trophoblastic cells actively synthesized prostanoids is reflected in the relationships between concentrations of 6-keto-$PGF_{1\alpha}$ and numbers of cells incubated (r=0.996, Fig. 9) and the time courses for production of 6-keto-$PGF_{1\alpha}$ and PGE_2 (Fig. 6), which closely paralleled that for production of progesterone. Aspirin inhibited production of 6-keto-$PGF_{1\alpha}$ and PGE_2 by about 50% at a dose of 100 μM (Fig. 8; $P<0.05$). Whereas 0.5% FBS stimulated production of both prostanoids ($P<0.05$), addition of 5% FBS only stimulated production of PGE_2. Neither bLH nor 8-Br-cAMP affected prostanoid production.

DISCUSSION

The relative importance of the placenta and corpus luteum as sources of progesterone during pregnancy in the cow remains equivocal. When the ovary bearing the corpus luteum was removed between 200 and 268 days of gestation, 13 of 21 cows delivered live calves, albeit with shortened gestations, dystocia, retained placentas, and metritus (Estergreen et al., 1967). Dispersed luteal cells from late gestation produced little progesterone in vitro, and this was associated with degeneration of the cells, particularly of the large cells (Alila, 1984). Although Wagner et al. (1974) and Hoffmann et al. (1979) minimized the importance of the bovine placenta as a source of peripheral progesterone, production of the steroid by the placenta and its localized effect on the uterus may be significant for maintenance of pregnancy. Enzymatically dispersed, heterogeneous

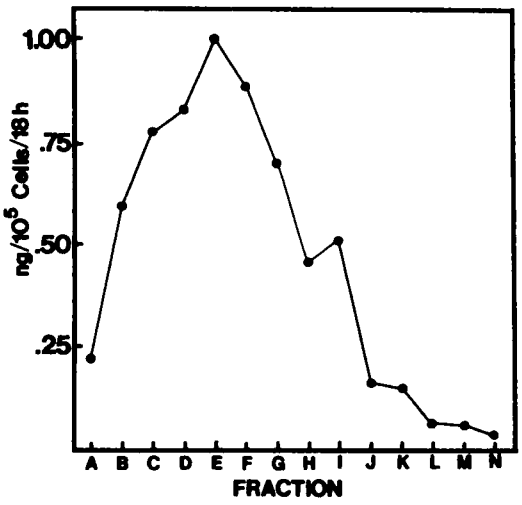

FIG. 3. Progesterone concentrations in media from fractions obtained after sedimentation of dissociated placental cells (235 days of gestation) in the CELSEP chamber.

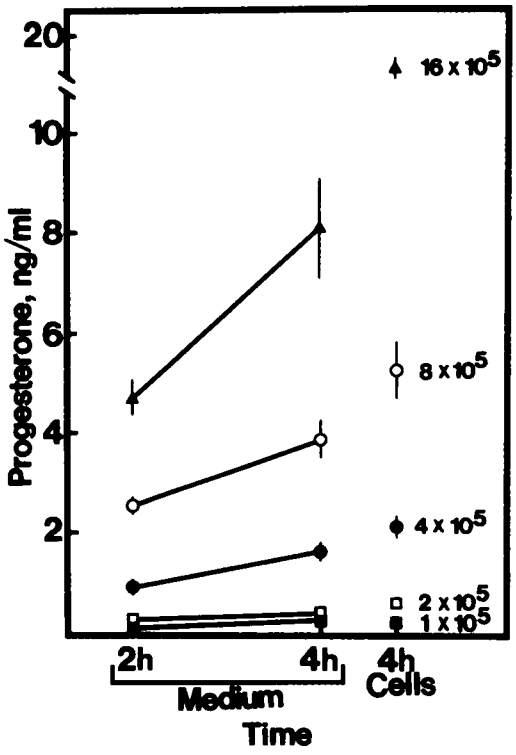

FIG. 4. Concentrations of progesterone in samples of medium after 2 and 4 h of incubation of 1 X 10^5, 2 X 10^5, 4 X 10^5, 8 X 10^5, and 1.6 X 10^6 binucleate trophoblastic cells (120 days of gestation) and in samples of medium in which cells had been lysed by freezing and thawing. Each point represents the mean ± SEM of 3 incubations.

populations of bovine placental cells were shown previously to produce progesterone in culture (Shemesh et al., 1983, 1984a). Our initial indication that the binucleate trophoblastic cells produced this steroid came from quantifying progesterone in fractions obtained by sedimentation of dissociated placental cells. Highly enriched populations of binucleate cells from placentas as early as 120 days and as late as 235 days of gestation showed a remarkable ability to produce progesterone. However, production and secretion by these fetal cells must be reconciled with the very low concentrations of progesterone in fetal blood (Reimers, unpublished data). Possibly, these cells are inhibited from producing progesterone in cotyledons and only produce it after they migrate into the maternal caruncle, where they contribute to maternal blood concentrations of progesterone. Shemesh et al. (1983) reported the extraction and partial purification of a placenta-derived inhibitor of luteal and placental progesterone production. However, this inhibitor was found in caruncles as well as in cotyledons. In our experience, it is very difficult to collect caruncles without significant contamination by cotyledonary villi. Therefore, the reported presence of the inhibitor in maternal tissue may be due to contamination. Isolation and incubation of binucleate trophoblastic cells as described by us may be artifactual because the inhibitor had been removed or hydrolyzed by trypsin.

Neither addition of human chorionic gonadotropin (hCG; Demers et al., 1973) or neutralization of endogenous hCG with an antiserum (Simpson and MacDonald, 1981) affected secretion of progesterone by cultures of human trophoblastic cells. Thus, our data support the general concept that production of

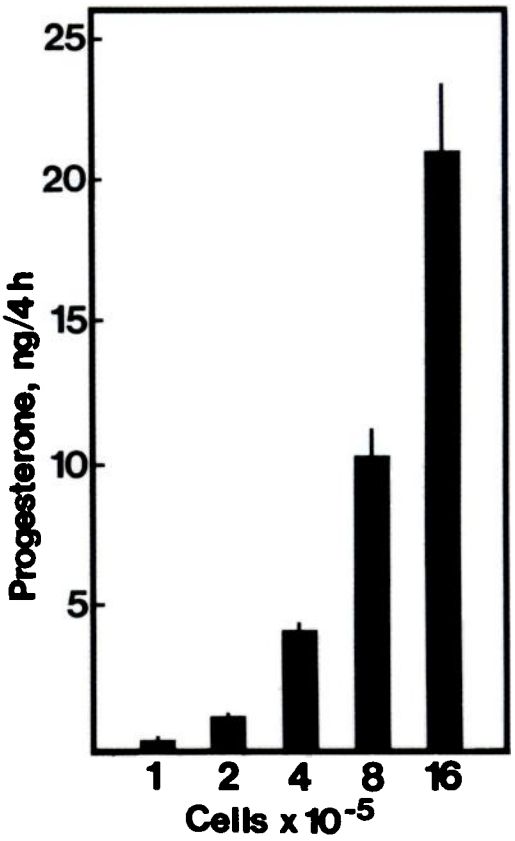

FIG. 5. Total progesterone secreted by binucleate trophoblastic cells (120 days of gestation) during 4 h of incubation. Each bar represents the mean ± SEM of 3 incubations.

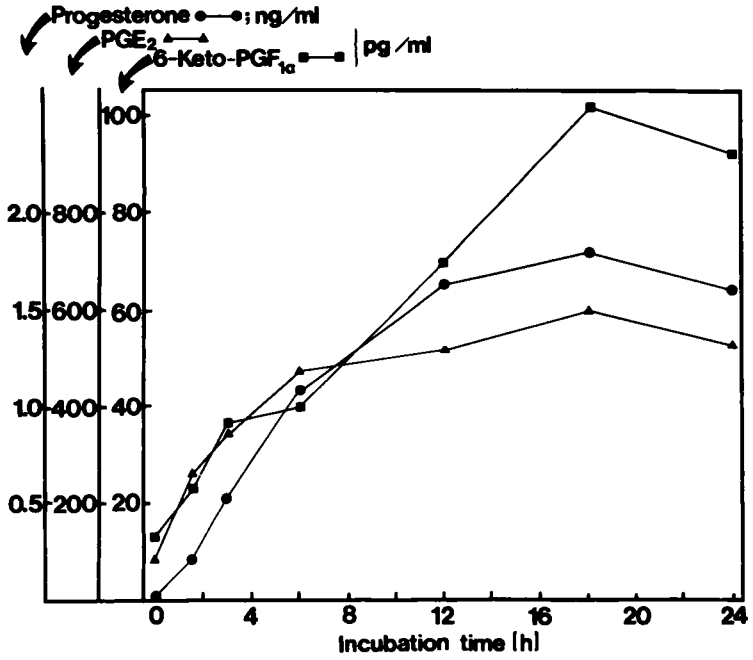

FIG. 6. Time course for production of progesterone, PGI_2 (reflected in concentrations of 6-keto-$PGF_{1\alpha}$), and PGE_2 by binucleate trophoblastic cells (170 days of gestation). Each point represents the mean ± SEM of 3 incubations each consisting of 5 × 10^5 cells in 1 ml of medium.

progesterone by the placenta is autonomous (Simpson and MacDonald, 1981). Only addition of serum to incubations affected progesterone production and secretion. Proteins in serum may simply bind progesterone and facilitate its removal from the cells' microenvironment (Westphal, 1970; Condon and Pate, 1981; Bruot and Collins, 1983) or the serum may furnish a tropic hormone or other secretagogue that stimulates progesterone production. Apparently the stimulatory substance was not LH, FSH, Prl, TSH, or cAMP because addition

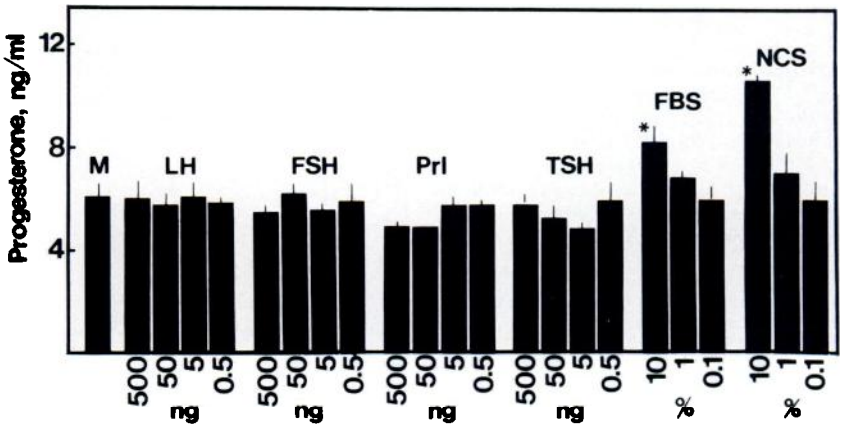

FIG. 7. Progesterone concentrations in medium from binucleate trophoblastic cells (212 days of gestation) after treatment with bLH, bFSH, bPrl, bTSH, FBS, and NCS; M = Medium 199 only. Cells were incubated at 37°C for 4 h. Each bar represents the mean ± SEM of 3 incubations each consisting of 1 × 10^6 cells in 1 ml of medium. *$P<0.05$.

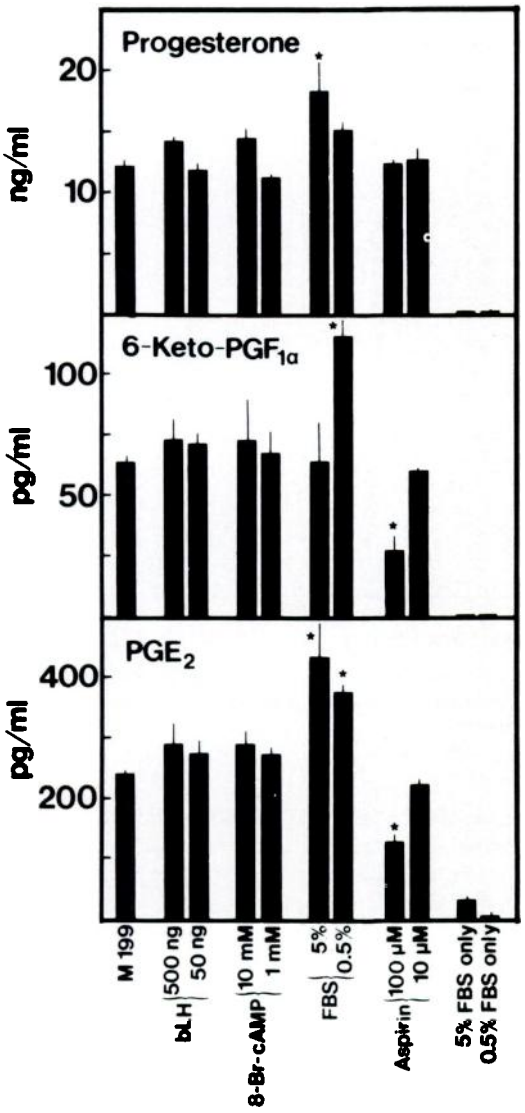

FIG. 8. Progesterone, 6-keto-$PGF_{1\alpha}$, and PGE_2 concentrations in medium from binucleate trophoblastic cells (190 days of gestation) after treatment with bLH, 8-Br-cAMP, FBS, and aspirin. Cells were incubated at 37°C for 18 h. The last pair of bars represents concentrations of progesterone and prostanoids in FBS alone. Each bar represents the mean ± SEM of 3 incubations each consisting of 5×10^5 cells in 1 ml of medium. *$P<0.05$.

of these substances had no effect on progesterone concentrations. Progesterone production by heterogeneous bovine placental cells also was not affected by LH or 8-Br-cAMP (Shemesh et al., 1984a).

Prostanoids are very important for maintenance and regression of the corpus luteum (McCracken et al., 1981; Silvia et al., 1984b), ovulation (Armstrong and Grinwich, 1972; Hamada et al., 1977), embryonal implantation (El-Banna et al., 1976; Johnson and Dey, 1980; Kennedy and Lukash, 1982; Hoos and Hoffman, 1983), maternal recognition of pregnancy (Ellinwood et al., 1979; Lacroix and Kann, 1982; McCracken et al., 1984; Ottobre et al., 1984; Sharp et al., 1984; Silvia et al., 1984a; Thatcher et al., 1984), regulation of placental blood flow (Rankin and Phernetton, 1976; Clark et al., 1982; Ylikorkala et al., 1983), and parturition (Edqvist et al., 1981). Prostaglandin E_2 and/or PGI_2 have been measured in or shown to be secreted by whole gravid uteri (Chan, 1983), endometrium (Wilson et al., 1972; Ellinwood et al., 1979; Evans et al., 1981; Findlay et al., 1981; Marcus, 1981; Lacroix and Kann, 1982; Lewis et al., 1982; Thatcher et al., 1984), preimplantation embryos (Marcus, 1981; Lewis et al., 1982; Shemesh et al., 1984b), placental tissue (Mitchell and Flint, 1977; Chan, 1983; Jogee et al., 1983), and embryonal-fetal membranes (Evans et al., 1981; Marcus, 1981). Our data

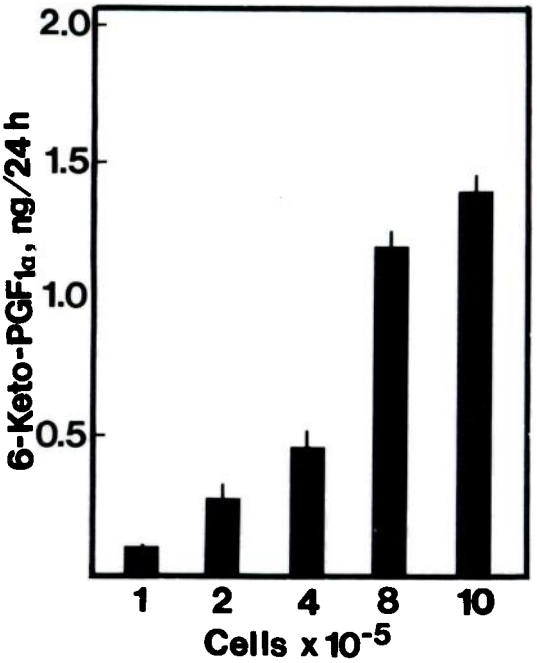

FIG. 9. Concentrations of 6-keto-$PGF_{1\alpha}$ in medium after 24 h of incubation of 1×10^5, 2×10^5, 4×10^5, 8×10^5, and 1×10^6 binucleate trophoblastic cells (150 days of gestation). Each bar represents the mean ± SEM of 3 incubations.

indicate that binucleate trophoblastic cells produce these prostanoids, as well as progesterone.

Whereas 8-Br-cAMP reduced production of PGE_2 by cultured trophoblastic cells from preimplantation bovine embryos (Shemesh et al., 1984b), 8-Br-cAMP did not affect production of PGE_2 or PGI_2 in our incubations of binucleate trophoblastic cells. As with human placental cells (Jogee et al., 1983), aspirin was a potent inhibitor of prostanoid production by bovine binucleate cells. It is not clear why 5% FBS had no effect on production of PGI_2 and 0.5% did.

In summary, we have prepared highly enriched populations of binucleate trophoblastic cells using a relatively inexpensive, yet efficient, technique and have shown that they produce progesterone, PGI_2, and PGE_2 in vitro. However, the possibility that mononucleate trophoblastic cells also produce progesterone and prostanoids has not been ruled out. Whether binucleate cells also produce peptide and protein hormones is being investigated.

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