BIOLOGY OF REPRODUCTION 27, 431–439 (1982)

Metabolism of Arachidonic Acid In Vitro by Bovine Blastocysts and Endometrium[1]

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ABSTRACT

Metabolism of arachidonic acid and prostaglandin $F_{2\alpha}$ by bovine blastocysts and endometrial slices recovered on Days 16 and 19 postmating was studied in vitro. In Experiment 1, arachidonic acid (10 μCi tritiated and 200 μg radioinert) was added to blastocysts and endometrial slices prior to incubation for 24 h. [^{3}H] arachidonic acid ([^{3}H] AA) and metabolites in extracts of culture medium and tissue homogenates were separated on columns of Sephadex LH-20. Elution profiles of [^{3}H] AA and metabolites in extracts of culture medium revealed that 13,14-dihydro-15-keto-$PGF_{2\alpha}$ (PGFM), PGE_2, $PGF_{2\alpha}$, and at least four unidentified compounds were produced by Day 16 and Day 19 blastocysts. Endometrial slices from both days of pregnancy produced ^{3}H-prostaglandins. Experiment 2 was conducted to quantify PGE_2, $PGF_{2\alpha}$ and PGFM in aliquots of culture medium from Day 16 and Day 19 blastocyst and endometrial incubates. These tissues were incubated with 200 μg of radioinert arachidonic acid. Day 16 blastocysts produced less (μg/blastocyst; $P<0.01$) of each prostaglandin than Day 19 blastocysts (PGE_2, 0.7 ± 0.4 vs. 4.2 ± 1.0; $PGF_{2\alpha}$, 2.1 ± 0.7 vs. 22.8 ± 4.1; PGFM, 0.03 ± 0.01 vs. 0.5 ± 0.2). Endometrial slices produced PGE_2, $PGF_{2\alpha}$ and PGFM, but quantities were not affected by day postmating or uterine horn-corpus luteum relationships. The third experiment was conducted to determine directly if Day 19 blastocysts and endometrial slices metabolized [^{3}H] $PGF_{2\alpha}$ to [^{3}H] PGFM. Blastocysts and endometrial slices produced [^{3}H] PGFM. Endometrial slices metabolized 34.3 ± 1.5% of the [^{3}H] $PGF_{2\alpha}$ to [^{3}H] PGFM, while blastocysts metabolized 7.5 ± 1.6% of the [^{3}H] $PGF_{2\alpha}$ to [^{3}H] PGFM. Results of this study indicate that bovine blastocysts and endometrial slices can metabolize [^{3}H] AA in vitro. It is postulated that prostaglandins of blastocyst and endometrial origin have a role in maintenance of early pregnancy in cattle.

INTRODUCTION

Unilateral hysterectomy of cattle prolonged the life span of the corpus luteum (CL) on the ipsilateral ovary (Ginther et al., 1967). This and other studies indicate that the uterus produces a luteolytic substance, probably prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; see Horton and Poyser, 1976, for review), that acts through a local venoarterial pathway (Mapletoft et al., 1976a). During early pregnancy in cattle, a conceptus in the uterus prevents luteolysis (Del Campo et al., 1977; Northey and French, 1980) and this antiluteolytic effect is exerted through a local utero-ovarian mechanism (Del Campo et al., 1977). Apparently, between Days 15 and 17 of pregnancy, the bovine conceptus produces or stimulates production of a substance that prevents luteolysis (Northey and French, 1980). The nature of this compound or group of compounds and involvement of the endometrium in prevention of luteolysis has not been determined.

Sheep conceptuses prevent luteolysis in a manner similar to that in cattle, i.e., through a local utero-ovarian mechanism (Mapletoft et al., 1976b). It has been proposed that prostaglandins of the E series may be involved in the antiluteolytic effects of the ovine conceptus (Pratt et al., 1977; Ellinwood et al., 1979; Huie et al., 1981; Reynolds et al., 1981). Similar studies have not been reported for the bovine. How-

Accepted April 7, 1982.
Received October 29, 1981.
[1] Florida Agricultural Experiment Station Journal Series No. 3426. This work was supported in part by U.S. Department of Agriculture, Cooperative State Research Service Agreement No. 616-15-162.
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ever, Shemesh et al. (1979) found that bovine blastocysts released PGE and PGF in vitro. This suggests the possibility that prostaglandins may be involved in maintenance of early pregnancy in cattle.

The studies reported here were undertaken to determine whether bovine blastocysts and endometrium had an active arachidonic acid cascade.

MATERIALS AND METHODS

General

A total of 42 Angus, Brown Swiss, or Angus X Brown Swiss heifers were used in three experiments. Heifers were observed in the morning and evening for standing estrus, and were mated to a Brown Swiss bull on the day of onset of estrus (Day 0). On the day of estrus, heifers were scheduled to be slaughtered on either Day 16 or 19 postmating.

At the time of slaughter, the reproductive tract from each heifer was recovered within 15 min of exsanguination, sealed inside a plastic bag and covered with ice. After returning to the laboratory, the ovary bearing the CL was noted and the contents of each reproductive tract were flushed out with sterile, isotonic saline using a procedure similar to ones described by Bazer et al. (1978). When a heifer was pregnant, a blastocyst in the uterine flushings was observed easily.

The blastocyst from each heifer was transferred from the uterine flushings to a sterile petri dish containing 15 ml modified minimum essential medium (MEM). The MEM was prepared by adding nonessential amino acids (1 ml of 100 X concentrate; GIBCO Labs., Grand Island, NY), antibiotic-antimycotic (10,000 U penicillin, 10,000 μg streptomycin and 25 μg amphotericin B; GIBCO Labs.), insulin from bovine pancreas (20 IU; Sigma Chemical Co., St. Louis, MO) and α-D(+) glucose (500 mg; Sigma Chemical Co.) to 100 ml of minimum essential medium with D-valine (GIBCO Labs.). Endometrium was dissected from the midpoint of both uterine horns from each pregnant heifer. Each sample of endometrium (467 ± 38 mg; mean ± SEM) was placed into a separate, sterile petri dish, sliced with scalpel blades into pieces approximately 3 mm X 3 mm, and 15 ml MEM was added to each sample. All incubates were placed into a culture chamber (Bellco Glass Inc., Vineland, NJ) on a rocking platform (Bellco Glass, Inc.) for 24 h at 37°C in an atmosphere of 50% N_2:45% O_2:5% CO_2. This incubation procedure is essentially the same as the one described by Basha et al. (1979) for porcine endometrial explants. Lewis et al. (1979), using this same procedure, found that Day 19 bovine blastocysts incorporated radioactive amino acids into macromolecules throughout a 24-h incubation period. At the end of the incubation period, tissue and MEM were separated by centrifugation at 4°C. Supernatants were transferred to individual glass storage vials. Blastocysts were weighed individually and placed into separate glass vials. Since endometrial samples were weighed prior to incubation, the samples were transferred directly to glass vials. All samples were frozen immediately and stored at −20°C.

Experiment 1

Blastocysts recovered from 5 heifers on Day 16 and 8 heifers on Day 19 postmating were incubated in this experiment. Ten microcuries of [5,6,8,9,11,12,14,15 (N)-^{3}H] AA (61 Ci/mmol; New England Nuclear, Boston, MA) in 100 μl ethanol and 200 μg of radioinert AA (Sigma Chemical Co.) in 20 μl ethanol were added to the MEM in each petri dish just prior to commencement of incubation. Three samples of endometrium from each uterine horn from 4 heifers 19 Days pregnant were treated with estradiol-17β (E_2-17β; Steraloids Inc., Wilton, NH) to determine if this treatment altered metabolism of arachidonic acid. Prior to incubation, MEM containing each of the endometrial samples received either 0, 200 or 1,000 pg E_2-17β in 20 μl ethanol.

To evaluate metabolism of [^{3}H] AA, tissues were homogenized (Pexton et al., 1975) and [^{3}H] AA and its metabolites were extracted (Pexton et al., 1975; Lewis et al., 1978) from the homogenates and from aliquots of MEM. The [^{3}H] AA and metabolites in the extracts were separated on columns containing 1.0 g of Sephadex LH-20 (Sigma Chemical Co.) using 50 ml of a mixture of 98% methylene chloride and 2% methanol (Christensen and Leyssac, 1976; Lewis et al., 1978). Columns were calibrated by determining the mobility of [5,6,8,9,11,12,14 (N)-^{3}H] 13,14-dihydro-15-keto-$PGF_{2\alpha}$ ([^{3}H] PGFM; 60 to 100 Ci/mmol; Amersham Corporation, Arlington Heights, IL), [5,6,8,11,12,14,15 (N)-^{3}H] PGE_2 (100 to 200 Ci/mmol; New England Nuclear) and [9(N)-^{3}H]-$PGF_{2\alpha}$ (5 to 15 Ci/mmol; New England Nuclear) in relation to the mobility of [^{3}H] AA. Each column was used for one extract and then discarded. The eluate from each column was collected in 100 X 0.5 ml fractions, evaporated to dryness at 45°C and disintegrations per minute (dpm) were determined in each chromatographic fraction.

Percentage of the total dpm for each fraction from each column was calculated by dividing dpm for each fraction by total dpm recovered from the respective column. Profiles of metabolism of [^{3}H] AA were evaluated by plotting percentage of total dpm against fraction number. Percentage of [^{3}H] AA metabolized was calculated for each sample using the equation: (total dpm in medium + total dpm in tissue) − ([^{3}H] AA dpm in medium + [^{3}H] AA dpm in tissue)/(total dpm in medium + total dpm in tissue).

Based upon Sephadex LH-20 profiles of metabolism of [^{3}H] AA by blastocysts, 8 regions were discerned. Percentage of total dpm for each fraction within each region was totaled so that 8 percentages were derived from each column. These percentages were then used for statistical analyses. The data from endometrial incubates were reduced in this same manner. General Linear Models procedure of the Statistical Analysis System (SAS; Barr et al., 1979) was used to compare percentages among regions within day postmating. Statistical comparisons were not made between endometrium and blastocysts.

Experiment 2

Three presumptive metabolites of [^{3}H] AA were identified in Experiment 1. Separate radioimmunoassays were used to quantify the release of these metabolites (13,14-dihydro-15-keto-$PGF_{2\alpha}$, Eley et

al., 1980; PGE_2, Lewis et al., 1978; $PGF_{2\alpha}$, Moeljono et al., 1977) by blastocysts and endometrial slices recovered from 5 Day 16 and 4 Day 19 pregnant heifers. The tissues from these heifers were incubated in MEM that contained 200 μg of radioinert arachidonic acid (no [^{3}H]AA was added). In addition, the three prostaglandins were quantified in aliquots of uterine flushings obtained from Day 16 (n = 10) and Day 19 (n = 12) pregnant heifers and from the Day 16 (n = 8) and Day 19 (n = 7) mated, but not pregnant, heifers used in Experiments 1 and 2. Radioimmunoassays for PGFM and $PGF_{2\alpha}$ were modified to include extraction, chromatography, and estimation of procedural losses by procedures described by Lewis et al. (1978).

General Linear Models procedure of SAS (Barr et al., 1979) was used to compare quantities of each prostaglandin between days of pregnancy. Also, wet weights of blastocysts and quantities of each prostaglandin adjusted for blastocyst wet weight were compared between days of pregnancy. Quantities of prostaglandins produced by blastocysts were not compared statistically to those produced by endometrial slices. Since prostaglandins were quantified by separate radioimmunoassays with separate sources of technical error, concentrations among prostaglandins were not compared.

Experiment 3

Prostaglandin $F_{2\alpha}$, 5 μCi of [9(N)-^{3}H] in 50 μl ethanol (no radioinert $PGF_{2\alpha}$ was added), was added just prior to commencement of incubation to MEM in each petri dish containing either endometrial slices or a blastocyst recovered from each of 5 heifers on Day 19 postmating. Metabolism of [^{3}H]$PGF_{2\alpha}$ was evaluated by extracting [^{3}H]$PGF_{2\alpha}$ and its metabolites from MEM, and then separating and identifying them using the procedures described in Experiment 1. Percentages of total dpm for [^{3}H]$PGF_{2\alpha}$ and [^{3}H]PGFM were determined as described in Experiment 1, and the sum of these two percentages was subtracted from 100 to give the total percentage of other metabolites of $PGF_{2\alpha}$. General Linear Models procedure of SAS (Barr et al., 1979) was used to compare percentages of [^{3}H]$PGF_{2\alpha}$, [^{3}H]PGFM and other [^{3}H]metabolites for each tissue type.

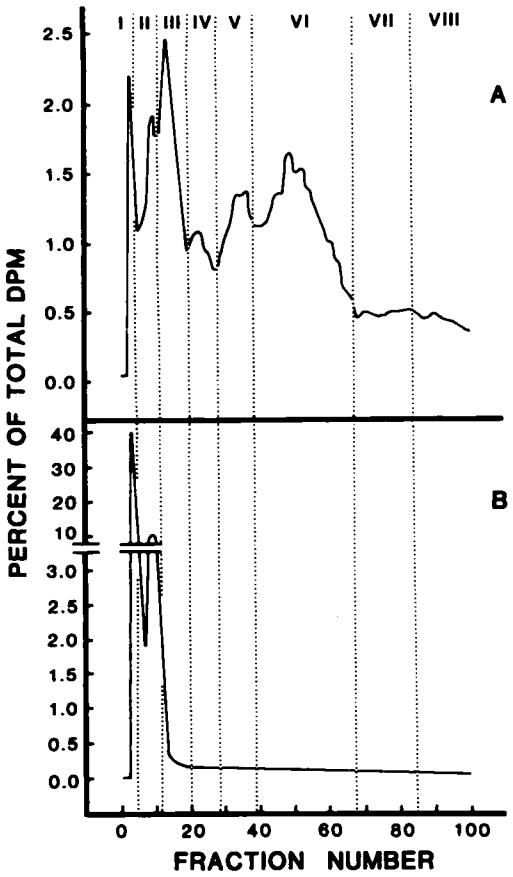

FIG. 1. Sephadex LH-20 elution patterns of metabolism of [^{3}H]AA by Day 19 bovine blastocysts incubated for 24 h. Panel *A* shows [^{3}H]AA and its metabolites in extracts of culture medium. Panel *B* illustrates [^{3}H]AA and metabolites in extracts of homogenized blastocysts. Data points used to construct the profiles are the means of percents of total dpm from eight blastocysts. Regions II, IV, V and VI migrated with authentic arachidonic acid, 13,14-dihydro-15-keto-$PGF_{2\alpha}$, PGE_2 and $PGF_{2\alpha}$, respectively.

RESULTS

Experiment 1

Blastocysts recovered on Day 19 postmating were heavier ($P<0.05$) than those recovered on Day 16 (122 ± 17 mg vs. 14 ± 0.2 mg, respectively). Patterns of [^{3}H]AA metabolism by blastocysts recovered 19 days after mating are shown in Fig. 1. Panel *A* shows [^{3}H]AA and metabolites in extracts of culture medium; Panel *B* shows these compounds in extracts of homogenized blastocysts. The elution profile in Panel A was divided into 8 regions. Based upon calibration of Sephadex LH-20 columns, regions II, IV, V and VI migrated with AA, PGFM, PGE_2 and $PGF_{2\alpha}$, respectively. It is possible that these regions also contained other compounds that were not resolved on the Sephadex LH-20 columns. The metabolites of [^{3}H]AA found in regions I, III, VII and VIII have not been identified. The patterns of [^{3}H]-AA metabolism by Day 16 blastocysts were similar to the Day 19 patterns.

The percentage of [^{3}H]AA metabolized by Day 19 blastocysts was greater ($P<0.05$) than that metabolized by Day 16 blastocysts (78 ± 3 vs. 51 ± 4), but these differences were due to differences in tissue weights. The greatest

TABLE 1. Distribution of percent of total dpm in extracts of homogenates of blastocysts recovered on Days 16 and 19 postmating and incubated with [^{3}H]arachidonic acid for 24 h and in extracts of medium from the incubates.[a]

	Percentages of total dpm			
	Culture medium		Blastocyst homogenates	
Region	Day 16	Day 19	Day 16	Day 19
I	5.7 ± 0.4^e	5.1 ± 0.2^f	81.8 ± 0.3^c	62.6 ± 1.5^c
II (AA)	54.1 ± 3.8^c	9.1 ± 0.1^e	12.6 ± 0.4^d	30.7 ± 1.3^d
III	10.4 ± 1.2^e	15.3 ± 0.3^d	0.9 ± 0.003^e	2.9 ± 0.2^e
IV (PGFM)	5.0 ± 0.3^e	7.8 ± 0.3^{ef}	0.6 ± 0.02^e	0.9 ± 0.05^e
V (PGE_2)	3.9 ± 0.3^e	13.0 ± 0.3^d	0.6 ± 0.03^e	0.6 ± 0.02^e
VI ($PGF_{2\alpha}$)	15.2 ± 2.3^d	34.0 ± 0.5^c	1.6 ± 0.1^e	0.9 ± 0.04^e
VII	2.7 ± 0.2^e	8.1 ± 0.4^{ef}	0.9 ± 0.04^e	0.5 ± 0.02^e
VIII	2.9 ± 0.4^e	7.4 ± 0.4^{ef}	0.9 ± 0.04^e	0.8 ± 0.05^e

[a]Percentages are means ± SEM of percentage of total dpm within each region discerned by Sephadex LH-20 chromatography. Different superscript letters within a column indicate differences ($P<0.05$) among regions within day postmating.

($P<0.05$) percentage of total dpm in extracts of MEM from incubations containing Day 16 blastocysts was in region II ([^{3}H]AA; Table 1). More ($P<0.05$) [^{3}H]$PGF_{2\alpha}$ (region VI) was produced by Day 16 blastocysts than any other metabolite of arachidonic acid (Table 1). The percentages of the other metabolites of [^{3}H]-AA did not differ among regions (Table 1). The Day 19 blastocysts metabolized the [^{3}H]AA primarily ($P<0.05$) to [^{3}H]$PGF_{2\alpha}$ (region VI), while smaller ($P<0.05$) amounts of [^{3}H]AA were metabolized to region III and to [^{3}H]-PGE_2 (region V; Table 1). Each of the other regions contained less ($P<0.05$) of the percentages of the total dpm than either region II, III, V or VI (Table 1). Region I was found in greater ($P<0.05$) quantities than [^{3}H]AA in extracts of homogenized Day 16 and Day 19 blastocysts (Table 1). Only a small percentage of the total dpm was in the other six regions (Table 1).

Relationship between the CL and the uterine horn from which endometrial samples were recovered did not affect metabolism of [^{3}H]-AA. Similarly, E_2-17β did not affect metabolism of [^{3}H]AA by endometrium recovered

TABLE 2. Distribution of percent of total dpm in extracts of homogenates of endometrial slices recovered on Days 16 and 19 postmating and incubated with [^{3}H]arachidonic acid for 24 h and in extracts of medium from the incubates.[a]

	Percentages of total dpm			
	Culture medium		Endometrial homogenates	
Region	Day 16	Day 19	Day 16	Day 19
I	2.0 ± 0.4^f	4.0 ± 0.9^e	49.5 ± 4.9^c	18.5 ± 1.5^d
II (AA)	62.6 ± 4.8^c	60.6 ± 3.3^c	43.1 ± 5.0^c	71.3 ± 1.5^c
III	10.8 ± 1.2^d	9.3 ± 0.7^d	2.8 ± 0.5^d	4.4 ± 0.2^e
IV (PGFM)	4.8 ± 0.7^{ef}	4.8 ± 0.4^e	0.8 ± 0.1^d	0.8 ± 0.06^f
V (PGE_2)	4.3 ± 0.7^{ef}	4.5 ± 0.4^e	0.7 ± 0.1^d	0.5 ± 0.04^f
VI ($PGF_{2\alpha}$)	8.7 ± 1.7^{de}	10.1 ± 1.0^d	1.8 ± 0.2^d	1.9 ± 0.2^f
VII	3.3 ± 1.0^{ef}	3.5 ± 0.4^e	0.8 ± 0.2^d	1.2 ± 0.2^f
VIII	2.6 ± 1.0^{ef}	3.1 ± 0.4^e	0.4 ± 0.2^d	1.0 ± 0.1^f

[a]Percentages are means ± SEM of percentage of total dpm within each region discerned by Sephadex LH-20 chromatography. Different superscript letters within a column indicate differences ($P<0.05$) among regions within day postmating.

TABLE 3. Quantities of prostaglandins released into culture medium during a 24-h incubation period by bovine blastocysts recovered 16 and 19 days postmating.

Prostaglandin	Quantity (μg)/ blastocyst[a]		Quantity (ng)/mg blastocyst	
	Day 16	Day 19	Day 16	Day 19
PGE_2	0.7 ± 0.4	4.2 ± 1.0	66.4 ± 37.3	17.8 ± 4.7
$PGF_{2\alpha}$	2.1 ± 0.7	22.8 ± 4.1	284.5 ± 76.4[b]	110.7 ± 32.3
13,14-dihydro-15-keto-$PGF_{2\alpha}$	0.03 ± 0.01	0.5 ± 0.2	3.2 ± 0.9	2.3 ± 0.8

[a]Quantity (means ± SEM)/blastocyst differed ($P<0.01$) between days postmating for each prostaglandin.

[b]Quantities of $PGF_{2\alpha}$ per mg blastocyst tended ($P<0.08$) to differ with day postmating.

on Day 19 postmating regardless of the relationship between the uterine horn and the corpus luteum. Therefore, endometrial data were pooled within day postmating.

Day 16 endometrial slices metabolized slightly, but significantly ($P<0.05$) more of the [^{3}H] AA added to incubation medium than did Day 19 endometrial slices (44 ± 5% vs. 32 ± 2%). The majority of radioactivity in extracts of MEM from endometrial incubates from both days postmating was found in region II ([^{3}H]-AA; Table 2). The amounts in regions III and VI ($PGF_{2\alpha}$) were approximately the same, while the remainder of dpm was distributed fairly uniformly among the other regions (Table 2).

The majority of dpm in extracts of homogenized endometrial slices was found in regions I and II regardless of the day postmating endometrium was recovered (Table 2). On Day 16, [^{3}H] AA (region II) and region I were found in approximately equal proportions, but on Day 19 far more ($P<0.05$) of the dpm were in region II than in region I (Table 2).

Experiment 2

Blastocysts recovered 19 days after mating produced more ($P<0.01$) PGE_2, $PGF_{2\alpha}$ and PGFM during the 24-h incubation period than did Day 16 blastocysts (Table 3). Wet weight of Day 16 blastocysts (12.2 ± 1.9 mg) was less ($P<0.001$) than that of Day 19 blastocysts (238.6 ± 35.2 mg). When PGE_2 and PGFM were expressed on a per milligram of blastocyst basis, the quantities of each prostaglandin no longer differed with day postmating. However, Day 16 blastocysts tended ($P<0.08$) to produce more $PGF_{2\alpha}$ per milligram of wet tissue than did Day 19 blastocysts.

Prostaglandin E_2, $PGF_{2\alpha}$ and PGFM were produced by endometrial slices. The quantities produced during the 24-h incubation period were not affected by day postmating, relationship of the CL to the uterine horns or the interaction of these two main effects (Table 4).

Of the 40 ml saline used to flush each reproductive tract, an average of 34.8 ± 1.3 ml were recovered. Content of $PGF_{2\alpha}$ tended ($P<0.15$) to be greater in flushings from preg-

TABLE 4. Quantities of prostaglandins released into culture medium during a 24-h incubation period by bovine endometrium recovered on Days 16 and 19 postmating from uterine horns adjacent and opposite the corpus luteum.

Prostaglandin	Relationship of uterine horn to corpus luteum			
	Adjacent		Opposite	
	Day 16	Day 19	Day 16	Day 19
	ng/mg tissue			
PGE_2	0.6 ± 0.1[a]	0.7 ± 0.3	0.7 ± 0.1	1.1 ± 0.7
$PGF_{2\alpha}$	2.1 ± 0.4	2.0 ± 0.4	1.4 ± 0.5	2.2 ± 0.4
13,14-dihydro-15-keto-$PGF_{2\alpha}$	3.0 ± 0.9	5.2 ± 1.7	2.5 ± 0.7	4.5 ± 0.8

[a]Mean ± SEM.

TABLE 5. Quantities of prostaglandins in uterine flushings collected from heifers 16 and 19 days after mating.

Prostaglandin	Pregnant		Mated, not pregnant	
	Day 16	Day 19	Day 16	Day 19
	ng/uterine flushing			
PGE_2	205.2 ± 54.4[a]	225.3 ± 59.4[b]	36.0 ± 4.7	68.7 ± 15.6
$PGF_{2\alpha}$	148.7 ± 41.3	425.2 ± 125.8[c]	61.3 ± 20.0	104.7 ± 46.4
13,14-dihydro-15-keto-$PGF_{2\alpha}$	65.6 ± 8.6	146.0 ± 23.3[d]	37.0 ± 3.1	109.8 ± 35.3

[a]Mean ± SEM.

[b]Content of PGE_2 in uterine flushings differed ($P<0.05$) with pregnancy status.

[c]Content of $PGF_{2\alpha}$ in uterine flushings tended ($P<0.15$) to differ with pregnancy status.

[d]Content of 13,14-dihydro-15-keto-$PGF_{2\alpha}$ in uterine flushings differed ($P<0.05$) with day postmating.

nant than in flushings from mated, but not pregnant heifers (Table 5). Content of PGE_2 in uterine flushings was increased ($P<0.05$) by pregnancy (Table 5). Quantity of PGFM in flushings collected 19 days after mating was greater ($P<0.05$) than in flushings recovered on Day 16 postmating (Table 5).

Experiment 3

Blastocysts and endometrium recovered on Day 19 postmating metabolized [^{3}H]$PGF_{2\alpha}$ to [^{3}H]PGFM and to other, unidentified compounds. Corpus luteum-uterine horn relationship did not affect the distribution of percent of total dpm in extracts of endometrial incubation medium. Therefore, the endometrial data were combined. Endometrial slices metabolized 34.3 ± 1.5% of the [^{3}H]$PGF_{2\alpha}$ to [^{3}H]PGFM, while blastocysts metabolized only 7.5 ± 1.6% of the [^{3}H]$PGF_{2\alpha}$ to [^{3}H]PGFM (Table 6). The percentages of unidentified ^{3}H-material in MEM extracts from blastocyst and endometrial incubates were approximately the same (Table 6).

DISCUSSION

Bovine blastocysts recovered 16 and 19 days after mating synthesized prostaglandins in vitro. This is consistent with data reported by Shemesh et al. (1979) indicating measurable amounts of immunoreactive PGF and PGE in homogenized blastocyst incubates. Since the blastocyst incubates of Shemesh et al. (1979) were assayed for PGF and PGE only, it is not known whether they contained other metabolites of arachidonic acid. In the present study, however, at least seven metabolites of [^{3}H]AA were discerned chromatographically. Three of these metabolites had mobilities corresponding to PGE_2, $PGF_{2\alpha}$ and PGFM.

In this study, as well as in the one by Shemesh et al. (1979), blastocyst production of $PGF_{2\alpha}$ and PGE_2 increased with day of pregnancy. Blastocysts in both studies produced more $PGF_{2\alpha}$ than PGE_2. The quantity of PGE_2 and $PGF_{2\alpha}$ produced, as measured by radioimmunoassay, by Day 16 blastocysts in the present study was almost twice that reported by Shemesh et al. (1979), but these differences may be due to different incubation procedures.

Based upon radioimmunoassay and Sephadex LH-20 chromatography, Day 16 and Day

TABLE 6. Distribution of percent (means ± SEM) of total dpm in extracts of culture medium in which Day 19 blastocysts or endometrial slices were incubated for 24 h with [^{3}H]$PGF_{2\alpha}$.[ab]

Prostaglandin	Percentage of total dpm	
	Blastocyst	Endometrium
$PGF_{2\alpha}$	70.3 ± 4.3[c]	37.6 ± 1.4[c]
13,14-dihydro-15-keto-$PGF_{2\alpha}$	7.5 ± 1.6[d]	34.3 ± 1.5[c]
Unidentified	22.2 ± 3.2[e]	28.1 ± 1.2[d]

[a]Percentages did not differ between endometrial slices recovered from uterine horns ipsilateral and contralateral to the corpus luteum, so the data were combined.

[b]Percentages of [^{3}H]$PGF_{2\alpha}$ and its metabolites were compared within tissues. Different superscript letters within a column indicate differences ($P<0.001$) among prostaglandins within tissue type.

19 blastocysts produced 13,14-dihydro-15-keto-$PGF_{2\alpha}$, which is consistent with the report on porcine blastocysts by Maule Walker et al. (1977). Maule Walker et al. (1977) found also that porcine blastocysts produced 13,14-dihydro-15-keto-PGE_2. Production of PGFM suggests that bovine blastocysts contain the 15-hydroxyprostaglandin dehydrogenase and Δ^{13}-prostaglandin reductase enzymes (Anggard, 1971).

It is possible that extracts of blastocyst incubation medium may have contained the 15-keto and/or 13,14-dihydro metabolites of PGE_2 and $PGF_{2\alpha}$ and may have contained 13,14-dihydro-15-keto-PGE_2, but it is difficult to separate a parent prostaglandin from metabolites of another parent prostaglandin (Crutchley and Piper, 1976). In addition, 13,14-dihydro-15-keto-PGE_2 is unstable (Granstrom et al., 1980; Fitzpatrick et al., 1980). Isakson et al. (1976) reported that [^{14}C] arachidonic acid was incorporated into phospholipids by intact kidney and heart tissue and subsequently metabolized to [^{14}C] prostaglandins. Bridges and Coniglio (1970) showed that rat testes have enzymes allowing elongation of arachidonic acid to less polar fatty acids. Since, in addition to its role in prostaglandin biosynthesis, arachidonic acid may be incorporated into several lipid fractions (Bridges and Coniglio, 1970), there are numerous possibilities for identities of the unidentified regions in extracts of blastocyst and endometrial incubation medium. Furthermore, regions which contain identified compounds also may contain compounds not resolved by Sephadex LH-20 chromatography.

The finding that extracts of homogenized Day 16 and Day 19 blastocysts contained substantial amounts of [^{3}H] AA and region I only indicates that the metabolites of AA, except for region I, were not stored appreciably in the tissue. Most of the radioactivity in extracts of blastocyst homogenates was in region I. Perhaps bovine blastocysts utilize an appreciable proportion of available AA for synthesis of more complex less polar lipids.

The physiological functions of AA and its metabolites in growth and development of bovine blastocysts are unknown. Dey et al. (1980) reported that rabbit blastocysts produced prostaglandins F and E-A-B (the antisera in their radioimmunoassay cross-reacted significantly with the three classes of prostaglandins), while Biggers et al. (1978) suggested that prostaglandins produced by rabbit conceptuses are involved, through their effects on water transport (Barth and Barth, 1972), in blastocyst expansion and implantation.

According to Psychoyos (1973), either an implanting blastocyst or an artificial deciduogenic stimulus can increase endometrial vascular permeability. Recent studies have shown that increased endometrial vascular permeability observed in several mammalian species prior to implantation is mediated by prostaglandins (Hoffman et al., 1977; Kennedy, 1977, 1980; Evans and Kennedy, 1978; Hoos and Hoffman, 1980), and it is possible that this also may occur in cows prior to blastocyst attachment. Perhaps prostaglandins produced by blastocysts decreased endometrial vascular resistance which would have increased blood flow to the gravid uterine horn in the cows studied by Ford et al. (1979). This occurred between Days 14 and 19 of pregnancy which is the period in cows when a blastocyst in the uterus is necessary for luteal maintenance (Northey and French, 1980).

Ovine endometrium has been shown to contain (Wilson et al., 1972; Lewis et al., 1977, 1978) and produce in vitro PGE_2 and $PGF_{2\alpha}$ (Ellinwood et al., 1979). Similarly, bovine endometrial slices used in this study produced PGE_2, $PGF_{2\alpha}$ and PGFM. Prostaglandin production was not affected by day postmating, relationship of the endometrium to the CL, or E_2-17β added to MEM. That E_2-17β did not have detectable effects on metabolism of [^{3}H] AA is at variance with data from studies of other species (Castracane and Jordan, 1975; Ford et al., 1975; Small et al., 1978). The lack of detectable effects of E_2-17β may be because tissues in vitro cannot undergo growth and developmental changes that may be necessary for increased prostaglandin secretion. Another explanation is that the endogenous pool of endometrial AA was so large that the specific activity of the ^{3}H-pool of AA was not sufficient to provide adequate labeling of metabolites.

It appeared from the three experiments that endometrial slices were capable of producing more PGFM than were blastocysts. This apparent difference between blastocyst and endometrial activity of the prostaglandin metabolic enzymes may be a mechanism for controlling the amount of prostaglandin of blastocyst origin that leaves the uterus. That is, the endometrium may metabolize $PGF_{2\alpha}$ it produces as well as $PGF_{2\alpha}$ produced by blastocysts.

Bartol et al. (1981) reported that the quan-

tity of immunoreactive PGF recovered from the uterine lumen of cows on Days 16 and 19 of pregnancy was greater than that recovered on the same days of the estrous cycle, and the amount recovered on Day 19 of pregnancy was greater than the amount recovered on Day 16 of pregnancy. The greater content of PGE_2 in uterine flushings from pregnant than from nonpregnant heifers in the present study, the data from the study of Bartol et al. (1981), and the fact that blastocysts produced PGE_2 and $PGF_{2\alpha}$ suggests that blastocysts, in addition to the endometrium, make a substantial contribution to the uterine milieu. Also, blastocysts probably contribute to the composition of the uterine venous effluent. Perhaps PGE_2 in the bovine, as may be the case in the ovine (Pratt et al., 1977; Ellinwood et al., 1979; Huie et al., 1981; Reynolds et al., 1981), has some role in preventing luteolysis during early pregnancy.

This study shows that bovine blastocysts and endometrial slices are capable of metabolizing AA in vitro to several compounds. Prostaglandin E_2, $PGF_{2\alpha}$ and PGFM produced by blastocysts and endometrial slices, as well as the unidentified metabolites produced by blastocysts, may be involved in growth and development of blastocysts, endometrial proliferation and control of changes in uterine vascularity and blood flow during early pregnancy. Metabolites of AA originating in blastocysts may be involved in preventing luteolysis during early pregnancy. This could be a direct effect on the CL, an indirect effect via stimulation of production of an antiluteolysin/luteotropin by the endometrium and/or blastocyst or an alteration of endometrial transport of luteolysin. Additional studies of blastocyst and endometrial metabolism and how each tissue might effect the action of the other are essential for an understanding of the control of luteal function during early pregnancy in cattle.

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