Prostaglandin production by the early pregnant guinea-pig uterus in relation to implantation and luteal maintenance, and the effect of oestradiol

N. L. Poyser

Department of Pharmacology, University of Edinburgh, 1 George Square, Edinburgh EH8 9JZ, U.K.

Summary. The outputs of prostaglandin (PG) F-2 α and PGE-2, but not of 6-oxo-PGF-1 α , from the guinea-pig uterus were significantly lower on Days 7 and 15 of pregnancy than on the corresponding days of the cycle. Uterine PGF-2 α output increased 28-fold between Days 7 and 15 of the cycle but only 4- to 5-fold between these same days of pregnancy. Uterine PGE-2 and 6-oxo-PGF-1 α outputs increased 2- to 3-fold between Days 7 and 15 of the cycle and of pregnancy. Endometrial PGF-2 α synthesizing capacity was 60–70% lower on Days 7 and 15 of pregnancy than on the corresponding days of the cycle, although it increased 2-fold and 2.5-fold between these days of pregnancy and of the cycle, respectively. Endometrial PGE-2 and 6-oxo-PGF-1 α synthesizing capacities showed no significant variation amongst Days 7 and 15 of the cycle and of pregnancy, except that endometrial PGE-2 synthesizing capacity was lower on Day 7 of the cycle.

Oestradiol treatment (10 μ g s.c. daily from Days 10 to 14 of pregnancy) did not affect plasma progesterone concentrations, uterine 6-oxo-PGF-1 α output, and endometrial PGF-2 α , PGE-2 and 6-oxo-PGF-1 α synthesizing capacities in 9/12 guinea-pigs when examined on Day 15. Uterine PGF-2 α and PGE-2 outputs increased 3- and 1.5-fold, respectively, in these guinea-pigs, but were still much lower than the outputs from the Day-15 non-pregnant uterus. The pregnancies appeared unaffected in these oestradiol-treated guinea-pigs. In the other 3 oestradiol-treated animals, uterine PGF-2 α output was 20- to 30-fold higher than in untreated, pregnant guinea-pigs on Day 15, and 2- to 3-fold higher than in Day-15 non-pregnant guinea-pigs. Uterine PGE-2 and 6-oxo-PGF-1 α outputs also tended to be higher in these treated guinea-pigs. In these 3 guinea-pigs, endometrial PGF-2 α , PGE-2 and 6-oxo-PGF-1 α synthesizing capacities were 4.0-, 3.4- and 2.5-fold higher, respectively, than in untreated, pregnant guinea-pigs on Day 15, and tended to be higher than in Day-15 non-pregnant guinea-pigs. Plasma progesterone concentrations were much lower in these 3 animals than in the other 9 treated with oestradiol, and also much lower than in untreated, pregnant guinea-pigs on Day 15. However, only 1 of these 3 guinea-pigs was in the process of aborting, and this had the lowest progesterone concentration (0.5 ng/ml).

In the pregnant guinea-pig a large increase in output of PGF-2 α from the uterus after Day 11 does not occur, uterine PGE-2 does not appear to be involved in luteal maintenance and general uterine PG production is not increased at the time of implantation.

Introduction

Prostaglandin (PG) F-2 α is the uterine luteolytic hormone in several mammalian species (see Horton & Poyser, 1976; Poyser, 1981) including the guinea-pig (Poyser, 1976). PGF-2 α

concentrations in the utero-ovarian venous plasma of guinea-pigs increase after Day 11 of the cycle (Blatchley, Donovan, Horton & Poyser, 1972; Earthy, Bishop & Flack, 1975) without any increase in the concentrations of PGE-2 (Antonini, Turner & Pauerstein, 1976). This increase in uterine PGF-2 α output is accompanied by an increase in uterine, particularly endometrial, PGF-2 α synthesizing capacity (Poyser, 1972, 1983a). Oestradiol acting on a progesterone-primed uterus stimulates relatively specific increases in uterine PGF-2 α output and endometrial PGF-2 α synthesizing capacity in the guinea-pig (Blatchley & Poyser, 1974; Naylor & Poyser, 1975; Wlodawer, Kindahl & Hamberg, 1976; Poyser, 1983a, b). Oxytocin is apparently not involved in this species (Poyser & Brydon, 1983). Oestradiol output from the ovary increases after Day 10 of the cycle (Joshi, Watson & Labhsetwar, 1973), just before the increase in uterine PGF-2 α output.

If pregnancy occurs, the luteolytic influence of the uterus has to be prevented since ovarian progesterone is necessary for the maintenance of pregnancy in the guinea-pig for the first 4–5 weeks (Heap & Deanesly, 1966; Csapo, Puri & Tarro, 1981). The increase in concentration of PGF-2 α in the uterine venous plasma after Day 11 does not occur if the guinea-pig becomes pregnant (Blatchley, Maule Walker & Poyser, 1975a, b; Antonini *et al.*, 1976). It is not known whether this is due to reduced production of PGF-2 α by the early pregnant guinea-pig uterus, or to a re-direction of PGF-2 α secretion into the uterine lumen as proposed for the pig (Bazer & Thatcher, 1977). PGE-2 delays luteal regression induced by PGF-2 α in sheep (Henderson, Scaramuzzi & Baird, 1977). Consequently, many studies have been performed on the sheep to investigate whether PGE-2 produced by the uterus is part of the stimulus for maintaining luteal function during early pregnancy (see Lacroix & Kann, 1982). Uterine PGE-2 may have a role in maintaining luteal function in other species also.

The present study has investigated PG production by the Day-15 pregnant uterus and the effect of oestradiol treatment, in order to obtain a better understanding as to how the conceptuses maintain luteal function over the 'critical period'. In addition, since implantation in the rat is dependent upon increased PG production by the uterus (see Kennedy & Armstrong, 1981), PG production by the guinea-pig uterus on Day 7 (the day of implantation) has also been studied. Superfusion of the guinea-pig uterus *in vitro* has been used previously for accurately monitoring changes in uterine PG output during the cycle and following in-vivo treatment with steroid hormones (Poyser & Brydon, 1983; Poyser, 1983a). Consequently, this technique has now been used to monitor PG output from the pregnant guinea-pig uterus.

Materials and Methods

Virgin guinea-pigs weighing 600–900 g were examined daily and a vaginal smear taken when the vaginal membrane was perforate. The first day of the cycle (oestrus) was taken as the day before the post-ovulatory influx of leucocytes when cornification was at a maximum. All animals used had exhibited normal oestrous cycles. Guinea-pigs were mated by placing them separately on Day 14 of the cycle with a male guinea-pig. The first day spermatozoa were present in the smear was designated Day 1 of pregnancy. The guinea-pigs were killed on Day 7 or 15 of pregnancy by stunning and incising the neck. A blood sample was collected into a heparinized (20 U/ml) syringe from the incised neck of each guinea-pig. The blood was centrifuged at 2500 *g* and the plasma was withdrawn and stored at –20°C before being assayed for progesterone. The uterus from each guinea-pig was removed and used in one of the following experiments.

Experiment 1. The uteri from 5 Day-15 pregnant guinea-pigs were divided into their two uterine horns, which were then 'opened' by cutting longitudinally. The conceptuses in one horn from each guinea-pig were removed by cutting around the point of attachment to the endometrium, and were discarded. Both uterine horns from each guinea-pig were weighed, and were then suspended in an organ bath and were attached to an isotonic lever, under a load of 2 g if the conceptuses had been removed or 4 g if the conceptuses were still present. Each uterine horn was superfused separately

(10 ml/min) with Krebs' solution (for composition see Mitchell, Poyser & Wilson, 1977) at 37°C and pre-gassed with 5% CO₂ and 95% O₂. After an initial settling period of 60 min, samples of superfusate were collected for 10-min periods consecutively over the next 90 min (i.e. 9 samples). The amounts of PGF-2 α , PGE-2 and 6-oxo-PGF-1 α in the samples were measured by radioimmunoassay (RIA).

Experiment 2. Experiment 1 was repeated using a further 5 guinea-pigs, except that the calcium ionophore, A23187 (1 μ g/ml) was added to the Krebs' solution during the collection of samples 4, 5 and 6.

Experiment 3. The uterine horns from 5 Day-15 pregnant guinea-pigs were opened longitudinally, and the conceptuses were removed but were not discarded. For one uterine horn from each guinea-pig, the endometrium was cut away from the myometrium by using a fine pair of scissors (Leaver & Poyser, 1981). The endometrium, myometrium, the other uterine horn, and two pairs of conceptuses were all separately weighed and homogenized in 15 ml Krebs' solution, and were incubated at 37°C for 90 min in an atmosphere of 5% CO₂ and 95% O₂. Arachidonic acid (2 μ g/ml) was added to one pair of homogenized conceptuses before the incubation. The amounts of PGF-2 α , PGE-2 and 6-oxo-PGF-1 α in the incubates were measured by RIA.

Experiment 4. Twelve guinea-pigs were injected subcutaneously (s.c.) with 10 μ g oestradiol benzoate in 0.5 ml arachis oil on each of Days 10–14 of pregnancy. On Day 15, the uterine horns were opened longitudinally, and the conceptuses were removed from both horns. Two pairs of conceptuses were weighed, homogenized and incubated as in Exp. 3. One uterine horn from each guinea-pig was weighed, suspended in an organ bath and superfused with Krebs' solution, as in Exp. 1, except that only 7 samples of superfusate were collected. The endometrium and myometrium in the other horn from each animal were separated, homogenized and incubated as in Exp. 3. The amounts of PGF-2 α , PGE-2 and 6-oxo-PGF-1 α in the superfusates and incubates were measured by RIA.

Experiment 5. The uterine horns from 5 guinea-pigs on Day 7 of pregnancy were separated and treated as in Exp. 4 (i.e. one uterine horn was superfused, and the other uterine horn was separated into endometrium and myometrium which were homogenized and incubated). The amounts of PGF-2 α , PGE-2 and 6-oxo-PGF-1 α present were measured by RIA.

Assay procedures

Solvent extraction. The pH of the superfusates and incubates was adjusted to 4.0 by adding HCl. Each sample was extracted by shaking twice with ethyl acetate. The ethyl acetate fractions were combined and taken to dryness at 45°C on a rotary evaporator. Each dried extract was redissolved in 5 ml (incubates) or 10 ml (superfusates) ethyl acetate and stored at –20°C before assay. The recoveries of PGF-2 α and PGE-2 are >90%, and the recovery of 6-oxo-PGF-1 α is >80% by this method (Poyser & Scott, 1980; Swan & Poyser, 1983). The results are not corrected for recoveries.

Radioimmunoassays. PGF-2 α , PGE-2 and 6-oxo-PGF-1 α were measured using antibodies raised in rabbits and tested in this laboratory (Dighe, Emslie, Henderson, Rutherford & Simon, 1975; Poyser, 1980; Poyser & Scott, 1980; Lytton & Poyser, 1982). The only significant cross-reactivities are PGF-1 α (100%) with the PGF-2 α antiserum, and PGE-1 (94%), PGB-2 (73%) and PGA-2 (24%) with the PGE-2 antiserum. Previous studies involving analysis by gas chromatography and mass spectrometry have shown that the guinea-pig uterus synthesizes very little PGF-1 α in comparison to PGF-2 α , and that there is no detectable synthesis of PGE-1, PGB-2 and PGA-2 (Poyser, 1983a). Consequently, the PGF-2 α and PGE-2 antisera are measuring predominantly the PG to which they have been raised. The intra-assay coefficients of variation, calculated from the variation between the duplicate results obtained for the samples, were 9.5%

(PGF-2 α), 10.6% (PGE-2) and 10.8% (6-oxo-PGF-1 α). The inter-assay coefficients of variation, calculated from the results obtained by incorporating a known amount of the appropriate PG into each assay, were 8.1% (PGF-2 α), 9.2% (PGE-2) and 12.3% (6-oxo-PGF-1 α). The limits of detection were 30 pg (PGF-2 α), 40 pg (PGE-2) and 40 pg (6-oxo-PGF-1 α) per tube.

Progesterone was measured as described by Poyser & Horton (1975), using an antibody raised against progesterone-11 α -hemisuccinate-bovine serum albumin in rabbits in this laboratory (Poyser, 1983a). The antiserum used had the following cross-reactivities; 11 α -hydroxyprogesterone, 37.5%; 11 β -hydroxyprogesterone, 29.2%; 17 α -hydroxyprogesterone, 2.0%; oestradiol-17 β , 0.55%; oestrone, 0.014%; oestriol, 0.067%; 20 α -hydroxy- Δ^4 -pregnen-3-one, 1.4%; 20 β -hydroxy- Δ^4 -pregnen-3-one, 1.9%; Δ^5 -pregnen-3 β -ol-20-one 1.4%; testosterone, 0.12%; Δ^4 -androstene-3,17-dione, 0.43%; cortisone, 1.5%; hydrocortisone, 0.28%; cholesterol, 0.23%. To test the performance of this antiserum in measuring plasma progesterone concentrations in guinea-pigs, blood samples were taken from 3 guinea-pigs by cardiac puncture using a heparinized syringe every Monday, Wednesday and Friday from early in one cycle to the end of the next cycle. Each blood sample was centrifuged, and the plasma was withdrawn and stored at -20°C , as described previously. The progesterone content of each sample was measured (Poyser & Horton, 1975). The values obtained were in the expected range (Challis, Heap & Illingworth, 1971; Poyser & Horton, 1975) and plasma progesterone concentrations started to decline 5 days before oestrus in each guinea-pig. The antiserum therefore measured progesterone concentrations in guinea-pig plasma satisfactorily. The detection limit was 30 pg per tube. The intra-assay coefficient, calculated from the variation between the duplicate results for each sample, was 12.6%. All samples were measured in two assays.

Statistical tests

Changes in the output of a particular PG with time during the superfusion experiments were analysed by Duncan's multiple range test. The paired *t* test was used to analyse the effect of adding exogenous arachidonic acid on PG production by the conceptuses. For all comparisons, Student's *t* test was employed, which was used in a modified form (Steel & Torrie, 1980) if the variances were unequal by the variance *F* ratio test. All results are expressed as the mean ($\pm$ s.e.m.).

Results

The outputs of PGF-2 α , PGE-2 and 6-oxo-PGF-1 α from the Day-15 pregnant guinea-pig uterine horns, with and without conceptuses, were low. PGF-2 α output tended to increase slightly with time, and the output after 90 min of collecting samples was significantly higher ($P < 0.05$) than during the first 30 min (Text-fig. 1). A23187 caused a significant increase ($P < 0.05$) in the outputs of PGF-2 α and 6-oxo-PGF-1 α , but not of PGE-2, from the Day-15 uterine horns irrespective of the presence or absence of conceptuses. The maximum outputs of PGF-2 α and PGE-2 were lower ($P < 0.05$), and the maximum output of 6-oxo-PGF-1 α was higher ($P < 0.05$) in pregnant than in non-pregnant guinea-pigs following stimulation of the uterus with A23187. Peripheral plasma progesterone concentrations (ng/ml) in these pregnant guinea-pigs on Day 15 were 10.1 ± 1.3 (Exp. 1) and 11.4 ± 2.0 (Exp. 2).

Since the basal output of each PG from the Day-15 pregnant guinea-pig uterus did not vary significantly with time during the first 70 min of sample collection, the average output of each PG from each uterine horn over the time period was calculated and the mean average outputs from the uterine horns in each group were determined. The outputs of each PG from the Day-7 pregnant guinea-pig uterus, and the Day-15 oestradiol-treated pregnant guinea-pig uterus (two groups A and B, see below) also did not vary significantly over the 70-min period; therefore the mean average outputs were determined. The mean outputs of PGs from the Day-7 and Day-15 non-pregnant

Text-fig. 1. Mean ($\pm$ s.e.m. for 5 observations) concentrations of prostaglandins (PGs) from both uterine horns (the conceptuses having been removed from one uterine horn) from Day-15 pregnant guinea-pigs treated (solid line) as indicated or not treated (broken line) with A23187 (1 μ g/ml). $\dagger$ Value significantly higher ($P < 0.05$) than the first 3 values in any one particular series.

guinea-pig uterus have been reported previously (Poyser & Brydon, 1983), and all the values for Day-7 and Day-15 non-pregnant and pregnant guinea-pigs are compared in Table 1.

Uterine PGF-2 α output increased 28-fold between Days 7 and 15 of the cycle, but only 4- to 5-fold between Days 7 and 15 of pregnancy. The output of PGF-2 α was 32% lower on Day 7 of pregnancy than on Day 7 of the cycle, but was 86-89% lower on Day 15 of pregnancy than on Day 15 of the cycle. Uterine outputs of PGE-2 and 6-oxo-PGF-1 α increased 2- to 3-fold between Days 7 and 15 of pregnancy and of the cycle, but uterine PGE-2 output was 60-65% lower during pregnancy than during the cycle. Peripheral plasma progesterone concentrations were not significantly different between Day 7 of the cycle and Day 7 of pregnancy. Plasma progesterone concentrations fell ($P < 0.05$) between Days 7 and 15 of the cycle, but increased ($P < 0.05$) between Days 7 and 15 of pregnancy.

Oestradiol treatment of guinea-pigs between Days 10 and 14 of pregnancy had two effects when examined on Day 15. In 3 (25%) of the animals (Group A), peripheral plasma progesterone concentrations fell ($P < 0.05$), and uterine PGF-2 α output was 20- to 30-fold higher than in Day-15 untreated, pregnant guinea-pigs and 3-6-fold higher than in Day-15 non-pregnant guinea-pigs. The uterine outputs of PGE-2 and 6-oxo-PGF-1 α also tended to be higher. In the remaining 9 oestradiol-treated pregnant animals (Group B), peripheral plasma progesterone concentrations were not significantly different from those in Day-15 untreated pregnant guinea-pigs. Uterine PGF-2 α output was 3-fold higher in Group B than in untreated pregnant animals, but was 65-75% lower in Group B animals than in Day-15 non-pregnant guinea-pigs.

Table 1. Mean ($\pm$ s.e.m.) average prostaglandin (PG) outputs from the uterus superfused *in vitro* during the first 70 min of sample collection from Day-7 and Day-15 non-pregnant guinea-pigs, Day-7 pregnant guinea-pigs, Day-15 pregnant guinea-pigs, with (+) and without (–) conceptuses (Con.), and Day-15 oestradiol-treated pregnant guinea-pigs without conceptuses (Groups A and B, see text)

	Non-pregnant†			Pregnant			Pregnant, oestradiol-treated	
	Day 7	Day 15	Day 7	Day 15 + Con.	Day 15 – Con.	Day 15, Group A	Day 15, Group B	
Output (ng/min/uterine horn)								
PGF-2 α	0.082 $\pm$ 0.011 ^a	2.2 $\pm$ 0.06 ^b	0.056 $\pm$ 0.001 ^c	0.30 $\pm$ 0.03 ^d	0.25 $\pm$ 0.01 ^d	7.9 $\pm$ 2.0 ^e	0.78 $\pm$ 0.15 ^f	
PGE-2	0.12 $\pm$ 0.02 ^a	0.21 $\pm$ 0.04 ^b	0.050 $\pm$ 0.006 ^c	0.074 $\pm$ 0.003 ^d	0.085 $\pm$ 0.009 ^d	0.48 $\pm$ 0.21 ^{a,b,c,d}	0.14 $\pm$ 0.01 ^a	
6-oxo-PGF-1 α	0.054 $\pm$ 0.011 ^a	0.15 $\pm$ 0.02 ^b	0.062 $\pm$ 0.005 ^a	0.15 $\pm$ 0.02 ^b	0.11 $\pm$ 0.01 ^b	0.40 $\pm$ 0.14 ^{a,b}	0.16 $\pm$ 0.02 ^b	
Output (ng/min/100 mg tissue)								
PGF-2 α	0.015 $\pm$ 0.001 ^a	0.42 $\pm$ 0.08 ^b	0.0094 $\pm$ 0.0012 ^c	—	0.038 $\pm$ 0.002 ^d	0.81 $\pm$ 0.19 ^e	0.11 $\pm$ 0.02 ^f	
PGE-2	0.024 $\pm$ 0.002 ^a	0.040 $\pm$ 0.004 ^b	0.0071 $\pm$ 0.0007 ^c	—	0.013 $\pm$ 0.001 ^d	0.046 $\pm$ 0.017 ^{a,b,c,d}	0.020 $\pm$ 0.002 ^a	
6-oxo-PGF-1 α	0.0094 $\pm$ 0.0017 ^a	0.029 $\pm$ 0.003 ^b	0.0089 $\pm$ 0.0004 ^a	—	0.016 $\pm$ 0.001 ^c	0.037 $\pm$ 0.011 ^{a,b,c}	0.022 $\pm$ 0.003 ^{b,c}	
Uterine wt (mg)	558 $\pm$ 46 ^{a,b}	515 $\pm$ 60 ^a	717 $\pm$ 55 ^{b,c}	1810 $\pm$ 210 [*]	656 $\pm$ 48 ^{a,b,c}	1020 $\pm$ 70 ^d	688 $\pm$ 37 ^c	
Peripheral plasma progesterone conc. (ng/ml)	3.4 $\pm$ 0.4 ^a	0.77 $\pm$ 0.06 ^b	4.1 $\pm$ 0.04 ^a	10.3 $\pm$ 0.8 ^c	—	1.4 $\pm$ 0.7 ^b	9.7 $\pm$ 1.0 ^e	
No. of animals	8	8	5	5	5	3	9	

Mean ($\pm$ s.e.m.) uterine weights and peripheral plasma progesterone concentrations are also shown.
In any one horizontal line, values with the same superscript letter are not significantly different ($P > 0.05$).
* Includes weight of conceptuses and not included in statistical analysis.
† Data taken from Poyser & Brydon (1983).

Table 2 compares the amounts of PGs synthesized by homogenates of uterine tissues after incubation from Day-7 and Day-15 non-pregnant and pregnant guinea-pigs, and Day-15 oestradiol-treated pregnant guinea-pigs (data for non-pregnant animals from Poyser, 1983a). Endometrial PGF-2 α synthesizing capacity increased 2.5-fold and 2-fold between Days 7 and 15 of the cycle and of pregnancy, respectively. However, endometrial PGF-2 α synthesizing capacity was 60–70% lower on Days 7 and 15 of pregnancy than on the corresponding days of the cycle. Endometrial PGE-2 and 6-oxo-PGF-1 α synthesizing capacities did not vary significantly amongst Days 7 and 15 of the cycle and of pregnancy, except that endometrial PGE-2 synthesizing capacity was significantly lower ($P < 0.05$) on Day 7 of the cycle.

Endometrial PGF-2 α , PGE-2 and 6-oxo-PGF-1 α synthesizing capacities were 4.0-fold, 3.4-fold and 2.5-fold higher, respectively, in Group A of the oestradiol-treated Day-15 pregnant guinea-pigs than in Day-15 untreated pregnant guinea-pigs. These synthesizing capacities were also higher

Table 2. Mean ($\pm$ s.e.m.) in-vitro prostaglandin (PG) production (ng/100 mg tissue) by homogenates of uterine tissues from Day-7 and Day-15 non-pregnant and pregnant guinea-pigs, and Day-15 oestradiol-treated pregnant guinea-pigs (Groups A and B, see text)

	Non-pregnant*		Pregnant		Pregnant, oestradiol-treated	
	Day 7	Day 15	Day 7	Day 15	Day 15, Group A	Day 15, Group B
Endometrium						
PGF-2 α	63.8 $\pm$ 9.2 ^a	162.3 $\pm$ 16.7 ^b	26.9 $\pm$ 3.8 ^c	54.5 $\pm$ 11.0 ^a	216.3 $\pm$ 28.3 ^b	42.5 $\pm$ 5.9 ^{a,c}
PGE-2	3.5 $\pm$ 0.2 ^a	7.7 $\pm$ 1.2 ^b	7.2 $\pm$ 0.6 ^b	7.2 $\pm$ 0.7 ^b	24.5 $\pm$ 1.7 ^c	6.9 $\pm$ 0.4 ^b
6-oxo-PGF-1 α	13.5 $\pm$ 1.0 ^a	19.0 $\pm$ 2.9 ^a	11.9 $\pm$ 1.5 ^a	18.1 $\pm$ 2.5 ^a	44.7 $\pm$ 9.6 ^b	16.8 $\pm$ 2.2 ^a
Myometrium						
PGF-2 α	21.4 $\pm$ 5.1 ^a	17.1 $\pm$ 2.4 ^a	14.8 $\pm$ 3.2 ^a	21.1 $\pm$ 5.2 ^a	33.9 $\pm$ 9.5 ^a	21.2 $\pm$ 2.8 ^a
PGE-2	5.4 $\pm$ 0.9 ^{a,b}	4.2 $\pm$ 0.6 ^a	7.1 $\pm$ 0.7 ^b	6.4 $\pm$ 0.4 ^b	5.9 $\pm$ 1.5 ^{b,c}	5.7 $\pm$ 0.8 ^{a,b}
6-oxo-PGF-1 α	40.1 $\pm$ 4.4 ^a	38.5 $\pm$ 4.4 ^{a,b}	32.5 $\pm$ 6.8 ^{a,b}	26.1 $\pm$ 3.2 ^b	30.6 $\pm$ 10.1 ^{a,b}	35.2 $\pm$ 4.4 ^b
Whole uterus						
PGF-2 α	86.8 $\pm$ 11.1 ^a	171.5 $\pm$ 13.7 ^b	—	44.7 $\pm$ 8.0 ^c	—	—
PGE-2	12.1 $\pm$ 2.8 ^a	14.7 $\pm$ 2.6 ^a	—	10.1 $\pm$ 1.1 ^a	—	—
6-oxo-PGF-1 α	29.4 $\pm$ 2.5 ^a	48.1 $\pm$ 6.7 ^b	—	19.7 $\pm$ 2.3 ^c	—	—
No. of animals	6	6	5	5	3	9

* Data from Poyser (1983a).

In any one horizontal line, values with the same superscript letter are not significantly different ($P > 0.05$).

Table 3. Mean ($\pm$ s.e.m.) in-vitro prostaglandin (PG) production (ng/100 mg tissue) by homogenates of conceptuses from Day-15 untreated, and oestradiol-treated pregnant guinea-pigs (Groups A and B, see text) in the presence (+AA) and absence (control) of exogenous arachidonic acid (2 μ g/ml)

	Pregnant, oestradiol-treated					
	Pregnant, non-treated		A		B	
	+ AA	Control	+ AA	Control	+ AA	Control
PGF-2 α	16.1 $\pm$ 3.6 ^{a,b,c}	9.1 $\pm$ 1.1 ^a	25.1 $\pm$ 4.8 ^{a,b,c}	19.5 $\pm$ 2.4 ^b	17.1 $\pm$ 1.7 ^b	12.1 $\pm$ 1.2 ^c
PGE-2	2.8 $\pm$ 0.2 ^{a,b}	2.0 $\pm$ 0.2 ^a	5.8 $\pm$ 0.8 ^c	4.3 $\pm$ 0.6 ^{a,d}	4.6 $\pm$ 0.5 ^c	3.1 $\pm$ 0.3 ^{b,d}
6-oxo-PGF-1 α	2.6 $\pm$ 0.2 ^{a,b}	2.1 $\pm$ 0.3 ^a	4.0 $\pm$ 0.8 ^{b,d}	3.7 $\pm$ 0.6 ^{b,d}	3.5 $\pm$ 0.2 ^{c,d}	2.9 $\pm$ 0.3 ^{a,b}
No. of animals	5		3		9	

In any one horizontal line, values with the same superscript are not significantly different ($P > 0.05$).

($P < 0.05$ for PGE-2 and 6-oxo-PGF-1 α) than in Day-15 non-pregnant guinea-pigs. In Group B of the oestradiol-treated Day-15 pregnant guinea-pigs, endometrial PGF-2 α synthesizing capacity was not significantly different from that in Day-15 untreated pregnant guinea-pigs, but was 74% lower than in Day-15 non-pregnant guinea-pigs. Endometrial PGE-2 and 6-oxo-PGF-1 α synthesizing capacities did not vary amongst these 3 groups of guinea-pigs.

Homogenates of the conceptuses synthesized predominantly PGF-2 α , followed by PGE-2 and 6-oxo-PGF-1 α (Table 3). Addition of exogenous arachidonic acid to the homogenates tended to increase the amounts of PGs synthesized, but these increases were significant ($P < 0.05$) only in oestradiol-treated, pregnant guinea-pigs of Group B. Basal PG output from the Day-15 pregnant guinea-pig uterus was unaffected by the presence or absence of the conceptuses (Table 2), indicating that the conceptuses contributed very little PG to the amounts secreted by the pregnant uterus. Oestradiol treatment generally increased the PG synthesizing capacities of the conceptuses (Table 3).

Discussion

The output of PGF-2 α from the guinea-pig uterus superfused *in vitro* is much lower on Day 15 of pregnancy than on Day 15 of the cycle. Consequently, the lack of increase in the concentration of PGF-2 α in the uterine venous plasma after Day 11 of pregnancy (Blatchley *et al.*, 1975a, b; Antonini *et al.*, 1976) is due to a lack of increase in endometrial PGF-2 α synthesis and release rather than due to a re-direction of PGF-2 α secretion from the venous drainage into the uterine lumen. The lower endometrial PGF-2 α synthesizing capacity on Day 15 of pregnancy than on Day 15 of cycle is consistent with this conclusion. Previous studies have shown that the lower production of PGF-2 α by homogenates of Day-15 guinea-pig uterus is not due to increased PG metabolism or lack of substrate (Maule Walker & Poyser, 1974, 1978). The large increase in the amount of PG synthetase (probably the cyclo-oxygenase component) in the endometrium which occurs after Day 11 of the cycle, probably in response to increased oestradiol output from the ovary (Poyser, 1983a), does not occur in the pregnant guinea-pig.

Uterine PGE-2 output was lower during pregnancy than during the cycle, and PGE-2 concentrations in the utero-ovarian venous plasma do not increase after Day 11 of pregnancy (Antonini *et al.*, 1976). It is unlikely, therefore, that uterine PGE-2 forms part of the stimulus for maintaining luteal function during early pregnancy in the guinea-pig. Uterine 6-oxo-PGF-1 α output did not vary between the non-pregnant and pregnant states indicating that PGI-2 is not involved in luteal maintenance. In addition, although homogenates of the conceptuses had the ability to synthesize PGs from endogenous arachidonic acid (with PGF-2 α being the major one formed), there was little secretion of any PG by the intact conceptus on Day 15 indicating that PGs from the conceptus are not involved in luteal maintenance.

On Day 7 of pregnancy, uterine PGF-2 α and PGE-2 outputs were lower than, and 6-oxo-PGF-1 α output was similar to, the outputs on Day 7 of the cycle. Uterine PGs appear, therefore, not to have a role in implantation in this species, although the possibility that there is an increase in PG concentration only at the implantation sites cannot be ruled out.

Oestradiol treatment of guinea-pigs from Day 10 to 14 of pregnancy had two distinct effects. In 25% of the guinea-pigs, plasma progesterone concentrations fell significantly, and there were large significant increases in uterine PGF-2 α output and endometrial PGF-2 α synthesizing capacity on Day 15. In the remaining 75% of the guinea-pigs treated with oestradiol, plasma progesterone concentrations remained high despite a small but significant increase in PGF-2 α output. However, endometrial PGF-2 α synthesizing capacity was not increased. Despite this apparent lack of any immediate effect the treatment of pregnant guinea-pigs with oestradiol on Days 10 and 11 does adversely affect the pregnancy from Day 30 onwards (Evans, Sin, Merrick & Kelleher, 1981). In non-pregnant guinea-pigs, the oestradiol treatment used universally stimulates increases in uterine

PGF-2 α output and endometrial PGF-2 α synthesizing capacity, and a decrease in ovarian progesterone output (Blatchley *et al.*, 1972; Blatchley & Poyser, 1974; Naylor & Poyser, 1975; Wlodawer *et al.*, 1976; Poyser, 1983a, b). To understand how the conceptuses can largely prevent the luteolytic action of oestradiol, one must first consider how uterine PGF-2 α output is normally stimulated in the non-pregnant guinea-pig.

The rate-limiting step in PG synthesis by tissues is the release of arachidonic acid from phospholipids by the calcium requiring enzyme, phospholipase A-2 (PLA-2; Vogt, 1978). Endometrial PLA-2 activity and PGF-2 α synthesizing capacity in guinea-pig endometrium increase 1.5-fold and 2.5-fold, respectively, between Days 7 and 15/16 of the cycle (Downing & Poyser, 1983; Poyser, 1983a). However, changes in these enzyme activities alone cannot account for the 22-fold increase in uterine PGF-2 α between Days 7 and 15 of the cycle. A23187 stimulates increased PGF-2 α output from the Day-7 and Day-15 non-pregnant guinea-pig uterus (Poyser & Brydon, 1983), an action which is abolishing by removing Ca²⁺ from the extracellular fluid (Poyser, 1984). It is therefore considered (Downing & Poyser, 1983) that activation of PLA-2 by raising intracellular free Ca²⁺ levels in the endometrium is of more importance in stimulating an increase in PGF-2 α production than changes in the absolute activity of the enzyme.

In platelets, increased thromboxane (TX) A-2 synthesis induced by thrombin is apparently due to stimulation of the phosphatidylinositol cycle (see Lapetina, 1982). However, activation of this cycle does not appear to be involved in increased PGF-2 α synthesis in the guinea-pig endometrium (A. C. W. S. Ning & N. L. Poyser, unpublished observation), so an increase in intracellular free Ca²⁺ levels for activation of PLA-2 in the endometrium must occur in some other way. Many actions of oestradiol are mediated by increased protein synthesis (Notebottom & Gorski, 1963). Luteolysis in guinea-pigs is prevented by the intrauterine administration of the protein synthesis inhibitor, actinomycin D, an effect which is not overcome by simultaneous oestradiol treatment (Poyser, 1979). It is proposed, therefore, that oestradiol stimulates the synthesis in the endometrium of a 'calcium-mobilizing' protein which, on a uterus primed with progesterone, causes the influx of extracellular Ca²⁺ and/or release of intracellular Ca²⁺. PLA-2 is then activated by the Ca²⁺ to release arachidonic acid, which is then metabolized predominantly into PGF-2 α . Support for this proposal is provided by the previous finding that uterine fluid in sheep contains high concentrations of PGF-2 α , calcium and protein (Harrison, Heap & Poyser, 1976). In early pregnancy, the conceptuses must prevent this proposed process in some way. Increased PGF-2 α release from the Day-15 pregnant guinea-pig uterus still occurred following an increase in intracellular free Ca²⁺ levels induced by A23187. Consequently, the conceptuses apparently need to prevent the proposed increase in free Ca²⁺ levels due to oestradiol and progesterone.

Previous studies have indicated that the conceptuses secrete an antiluteolytic factor which prevents increased uterine PGF-2 α production, in part by preventing increased ovarian oestradiol secretion (Blatchley *et al.*, 1975a, b). However, administration of oestradiol from Days 10 to 14 of pregnancy, in doses sufficient to cause luteolysis, had only a small stimulatory effect on uterine PGF-2 α output and no effect on plasma progesterone concentrations in the majority of guinea-pigs treated. It appears that the antiluteolytic factor greatly reduces the synthesis or action of the proposed calcium mobilizing protein. The former effect seems more probable since the increase in PG synthetase levels is also prevented in the pregnant uterus, an action mimicked by actinomycin D (Poyser, 1979). However, in 25% of the oestradiol-treated pregnant guinea-pigs, oestradiol did cause large increases in uterine PGF-2 α output and endometrial PG synthetase levels, and a decrease in plasma progesterone concentrations. Nevertheless, only one conceptus in one of these affected guinea-pigs was being resorbed, and this guinea-pig had the lowest progesterone concentration (0.5 ng/ml). In the other two affected guinea-pigs, plasma progesterone concentrations were still above 1 ng/ml which may have been sufficient to maintain the pregnancy at that stage. Consequently, the corpora lutea appear more resistant to the luteolytic action of PGF-2 α in the pregnant guinea-pig, suggesting that the conceptuses secrete also a luteoprotective factor. A human chorionic gonadotrophin (hCG)-like substance is present in guinea-pig placenta from at

least Day 10 of pregnancy, with maximum activity occurring on Day 18 (Humphreys, Hobson & Wide, 1982). Since hCG does antagonize the luteolytic action of PGF-2 α in non-pregnant guinea-pigs (Tam, Beveridge & Tso, 1982), placental hCG produced during early pregnancy in the guinea-pig may be responsible for this luteoprotective effect. In addition, the luteotrophic factor secreted from the guinea-pig placenta after Day 15 (Heap, Illingworth & Perry, 1973) may also be hCG. However, it is unlikely that the antiluteolytic factor responsible for suppressing uterine PGF-2 α production is hCG, since PGF-2 α output from the guinea-pig uterus increases after Day 26 of pregnancy (Granström & Kindahl, 1976) despite the continued production of hCG by the guinea-pig placenta at this time (Humphreys *et al.*, 1982).

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