Exogenous progesterone reduces follicular prostaglandin E and 6-keto prostaglandin F-1 α in the cyclic hamster*

J. D. Brannian and P. F. Terranova

Department of Physiology, Ralph L. Smith Research Center, University of Kansas Medical Center, Kansas City, Kansas 66103, USA

Summary. In cyclic hamsters, exogenous progesterone (100 μ g) administered s.c. at 09:00 h on the day of dioestrus II reduced prostaglandin (PG) E and 6-keto PGF-1 α but not PGF concentrations in preovulatory follicles measured at 09:00 h of pro-oestrus. The injection of 10 μ g ovine LH (NIADDK-oLH-25) concurrently with 100 μ g progesterone on dioestrus II prevented the decline in follicular PGE and 6-keto PGF-1 α values. Administration of LH alone did not significantly alter follicular PG concentrations. Inhibition of follicular PGE accumulation by progesterone was due to a decline in granulosa PGE concentration and not thecal PGE. Progesterone administration also reduced follicular oestradiol concentrations. Administration of oestradiol-17-cyclopentanepropionate (ECP) (10 μ g) with progesterone did not prevent the decline in follicular PGE and 6-keto PGF-1 α but did increase follicular PGF concentrations. However, ECP given alone on dioestrus II reduced follicular PGE and increased PGF concentrations in preovulatory follicles on pro-oestrus. It is concluded that exogenous progesterone administered on dioestrus II inhibits granulosa PGE and 6-keto PGF-1 α accumulation in preovulatory follicles, probably by reducing serum LH concentrations, and that the granulosa cells, which are LH-dependent, are a major source of follicular PGE.

Keywords: prostaglandins; progesterone; ovary; follicles; hamster

Introduction

In a number of species, follicular prostaglandin (PG) E-2 and F-2 α concentrations increase in preovulatory follicles, are reportedly causal in ovulation (Ainsworth *et al.*, 1975, 1979; Armstrong & Zamecnik, 1975; Hamada *et al.*, 1977; Murdoch *et al.*, 1986) and are dependent on LH (LeMaire *et al.*, 1973; Bauminger & Lindner, 1975; Shemesh & Hansel, 1975; Clark *et al.*, 1978; Koos *et al.*, 1983; Evans *et al.*, 1983; Tsang *et al.*, 1988). Preovulatory follicles of rats (Koos & Clark, 1982) and pigs (Hunter & Poyser, 1985) also produce substantial amounts of PGI-2 but the importance of PGI-2 in ovulation is unknown. Thecal and granulosa cells produce PGs but the relative contributions of each cell type and their responsiveness to LH stimulation are species-dependent (Erickson *et al.*, 1977; Armstrong, 1981; Evans *et al.*, 1983; Ainsworth *et al.*, 1984) and largely unknown.

Administration of exogenous progesterone on the morning of dioestrus II in the cyclic hamster reduces circulating concentrations of LH and oestradiol and delays ovulation by 1 day (Greenwald, 1978). The decline in circulating and follicular oestradiol values induced by exogenous progesterone is due to inhibition of thecal androstenedione synthesis resulting from reduced LH stimulation (Garza & Terranova, 1984). Since follicular PG production is LH-dependent, it was of interest to ascertain whether exogenous progesterone inhibited PG synthesis in preovulatory follicles and, if

*Reprint requests to: Dr Paul F. Terranova.

so, to determine whether there was a differential effect on theca and granulosa cells. In addition, since oestradiol alters uterine PG metabolism (Ham *et al.*, 1975; Kogo *et al.*, 1977), the effects of reduced oestradiol and replacement therapy on follicular PGs after progesterone administration were also of interest.

The aims of this study were therefore (1) to determine the effect of a single injection of exogenous progesterone on follicular accumulation of PGE, PGF and 6-keto PGF-1 α (the stable metabolite of PGI-2); and (2) to compare follicular PG and steroid concentrations and (3) to determine whether PG synthesis in theca and/or granulosa cells was differentially altered.

Materials and Methods

Animals and treatments. Adult cyclic hamsters (Sasco, Inc., Omaha, NE, USA) were maintained in a 14 h light:10 h dark photoperiod at 21–23°C. Hamsters were used for experimentation only after exhibiting 3 consecutive 4-day cycles as monitored by a vaginal discharge on the morning of oestrus. Food and water were provided *ad libitum*.

At 09:00 h on the morning of dioestrus II, animals weighing 100–130 g were injected s.c. with one of the following treatments: (1) 100 μ g progesterone, (2) 100 μ g progesterone + 10 μ g ovine LH (NIADDK-oLH-25), (3) 100 μ g progesterone + 10 μ g oestradiol-17-cyclopentanepropionate (ECP: Upjohn, Kalamazoo, MI, USA), (4) 10 μ g ECP, (5) 10 μ g ovine LH, or (6) saline and peanut oil. Progesterone and ECP were dissolved in peanut oil; LH was dissolved in 25% polyvinylpyrrolidone in 0.9% (w/v) NaCl. At 09:00 h on the day of pro-oestrus (24 h after injection), hamsters were anaesthetized with ether and decapitated. Blood was collected and allowed to clot overnight at 4°C and serum was stored at –20°C until assayed for progesterone, oestradiol and LH by radioimmunoassay.

Ovaries were removed from the bursae in ice-cold 0.01 M-phosphate-buffered saline (PBS), pH 7.0. Preovulatory follicles were dissected out and trimmed of adhering tissues (interstitium and small follicles) and placed in 1 ml ice-cold methanol or were microdissected to separate the theca and the granulosa cells. Follicles were punctured with a fine needle and gently squeezed with jeweller's forceps to discharge the granulosa cells from the antrum. Granulosa cells ($\sim 5 \times 10^5$ /ovary) were washed twice in PBS followed by centrifugation at 50 g for 3 min, counted and resuspended in 1 ml ice-cold methanol. Theca shells were partly bisected, adhering granulosa cells were removed by mechanical agitation (Terranova *et al.*, 1982) and placed in 1 ml cold methanol. The ovarian tissues were homogenized, centrifuged at 1000 g for 10 min and the supernatant fluids were stored at –20°C until assayed for PGE, PGF, 6-keto PGF-1 α , progesterone, androstenedione and oestradiol.

Prostaglandin radioimmunoassays. PGE-2, PGF-2 α and 6-keto PGF-1 α antisera were purchased from Seragen, Inc. (Boston, MA, USA). Cross-reactivities of the antisera were as follows: anti-PGE-2: PGE-2 and PGE-1 = 100%, PGF-2 α = 1.3%, PGD-2 <1.0%, 6-keto PGF-1 α <1.0%; anti-PGF-2 α : PGF-2 α and PGF-1 α = 100%, PGE-1 = 1.1%, PGE-2 <1.0%, PGD-2 <1.0%, 6-keto PGF-1 α = 1.1%; anti-6-keto PGF-1 α : 6-keto PGF-1 α = 100%, PGF-1 α = 7.8%, 6-keto PGE-1 = 6.8%, PGF-2 α = 2.2%, PGE-2 <1.0%, PGD-2 <1.0%. [5,6,8,11,12,14,15-³H(N)]-PGE-2 (sp. act. 169.5 Ci/mmol), [5,6,8,9,11,12,14,15-³H(N)]-PGF-2 α (sp. act. 195.4 Ci/mmol) and [5,8,9,11,12,14,15-³H(N)]-6-keto PGF-1 α (sp. act. 160 mCi/mmol) were purchased from New England Nuclear, Boston, MA, USA. Since the PGE-2 and PGF-2 α antisera cross-reacted significantly with PGE-1 and PGF-1 α , the terminology of PGE and PGF values was used.

Duplicate 0.1 ml samples of supernatants from tissue extracts and standards in methanol were dried under low pressure at 40°C. Antisera and tritiated PGs diluted in PBS with 0.1% gelatin (PBSG) were added to dried residues and incubated at 4°C for 18 h. Free and bound label were separated with dextran-coated charcoal as previously described for steroid assays (Terranova & Greenwald, 1978). Inter- and intra-assay coefficients of variation were <7.5% for all 3 assays based on duplicate determinations of 4 assays. Lower limits of sensitivity were 2 pg/tube. Parallelism was evident in each assay between serial volumes (50, 100, 200 μ l) of samples and standards.

Steroid radioimmunoassays. Progesterone, androstenedione and oestradiol concentrations were measured in methanol extracts and standards: 25- μ l samples of extract or standard were dried under low pressure at 45°C and resuspended in 20 μ l PBSG, antisera and labelled steroids were added and incubated and the free and bound steroids were separated as described previously (Terranova & Greenwald, 1978).

Serum samples (10 μ l for the progesterone assay, 300 μ l for the oestradiol assay) were made up to a volume of 1 ml with distilled H₂O and subsequently extracted with 2 volumes anhydrous diethyl ether. Ether extracts were dried under low pressure at 45°C and incubated with antisera and radiolabelled steroid as described previously (Terranova & Greenwald, 1978).

Antisera and characteristics of the steroid assays have been previously described (Terranova, 1981). Inter- and intra-assay coefficients of variation were <8.5% for each assay.

LH radioimmunoassay. The LH RIA followed methods previously described (Bast & Greenwald, 1974). Anti-ovine LH (GDN No. 15 from Dr Gordon Niswender, Colorado State University, Fort Collins, CO, USA), ¹²⁵I-labelled ovine LH (NIADDK-oLH-I-3) and rat LH (NIADDK-rLH-RP-2) were used. Sensitivity was 0.2 ng/ml and inter- and intra-assay coefficients of variation were 6.3% and 9.0%, respectively, for duplicates of 3 assays.

Statistics. Data were analysed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. In cases of unequal variance within a set of data, ANOVA was replaced with the Kruskal-Wallis non-parametric test followed by Dunn's distribution-free multiple comparisons procedure. Statistical differences were considered significant at $P < 0.05$.

Results

Effects of progesterone and LH on follicular prostaglandins and steroids

Administration of 100 µg progesterone at 09:00 h on dioestrus II induced significant declines of PGE ($P < 0.001$) and 6-keto PGF-1α ($P < 0.05$) concentrations in preovulatory follicles by 09:00 h of pro-oestrus (Fig. 1). Follicular PGF concentrations were not significantly altered by exogenous progesterone. Injection of 10 µg ovine LH concurrently with progesterone prevented the decline in intrafollicular PGE and 6-keto PGF-1α concentrations which were brought to levels that were not significantly different from controls (Fig. 1). Administration of 10 µg LH alone had no significant effect on PGE, PGF or 6-keto PGF-1α concentrations.

Fig. 1. Follicular concentrations in hamsters of PGE, PGF and 6-keto PGF-1α at 09:00 h on pro-oestrus after s.c. injection of progesterone (100 µg), ovine LH (10 µg) or progesterone + LH at 09:00 h on dioestrus II. Values are mean $\pm$ s.e.m. for 6 animals (6–10 follicles/animal). Different letters denote groups that are significantly different from each other.

Progesterone administration significantly reduced intrafollicular concentrations of androstenedione and oestradiol without affecting follicular concentrations of progesterone (Table 1). LH given with progesterone returned androstenedione to values not significantly different from those of controls and significantly increased the oestradiol concentration compared to that in progesterone-treated hamsters but this value was still significantly lower than the control follicular oestradiol concentrations (Table 1). Progesterone given with LH had no effect on progesterone concentrations in the follicles. Follicular concentrations of progesterone and androstenedione were not altered by 10 µg LH but oestradiol values were slightly elevated.

When 100 µg progesterone were given there was a significant increase ($P < 0.001$) in serum progesterone and decline ($P < 0.05$) in serum concentrations of oestradiol by 09:00 h of pro-oestrus, but serum levels of LH were similar to those of controls (Table 2). Progesterone given in combination with LH further elevated the serum concentrations of progesterone compared with animals receiving progesterone alone and serum oestradiol values remained lower than in controls.

Table 1. Follicular concentrations in hamsters of androstenedione, progesterone and oestradiol at 09:00 h on the day of pro-oestrus after treatment at 09:00 h on dioestrus II

Treatment	Steroid conc. (pg/follicle)		
	Androstenedione	Progesterone	Oestradiol
Control (oil)	150 ± 10	81 ± 3	207 ± 8
100 µg progesterone	49 ± 8 ^a	77 ± 8	88 ± 20 ^a
100 µg progesterone + 10 µg LH	117 ± 24	75 ± 29	138 ± 12 ^b
10 µg LH	165 ± 20	56 ± 9	256 ± 18 ^c
100 µg progesterone + 10 µg ECP	10 ± 2 ^b	30 ± 4	12 ± 4 ^d
10 µg ECP	109 ± 22	60 ± 8	163 ± 10
ANOVA	<i>P</i> < 0.001	NS	<i>P</i> < 0.001

Values are mean ± s.e.m. for 6 animals/group and 8–10 follicles/animal. Superscript letters indicate values significantly different from the control group and group with a different letter.

but LH values were unchanged (Table 2). LH given alone on dioestrus II increased serum concentrations of progesterone but had no significant effect on serum oestradiol and LH values by the next day.

Table 2. Serum concentrations in hamsters of progesterone, oestradiol and LH at 09:00 h on the day of pro-oestrus after treatment at 09:00 h on dioestrus II

Treatment	Progesterone (pg/ml)	Oestradiol (pg/ml)	LH (ng/ml)
Control (oil)	< 150	46 ± 7	1.3 ± 0.3
100 µg progesterone	697 ± 59 ^a	18 ± 3 ^{a,b}	0.9 ± 0.2
100 µg progesterone + 10 µg LH	1607 ± 296 ^b	8 ± 2 ^a	1.5 ± 0.3
10 µg LH	242 ± 23 ^c	49 ± 4	1.1 ± 0.3
100 µg progesterone + 10 µg ECP	2955 ± 528 ^d	24 ± 6 ^b	1.8 ± 0.2
10 µg ECP	290 ± 35 ^c	35 ± 3	1.7 ± 0.2
ANOVA	<i>P</i> < 0.001	<i>P</i> < 0.001	NS

Values are mean ± s.e.m. for 6 animals/group. Superscript letters indicate values significantly different from the control group and group with a different letter.

Since a reduction in serum LH was not evident 24 h after progesterone injection, additional animals (3 controls, 3 treated with progesterone) were killed 6 h after treatment (15:00 h, dioestrus II). In these animals, serum LH concentrations were significantly (*P* < 0.001) reduced in the treated animals (0.38 ± 0.02 ng/ml) compared to controls (0.75 ± 0.05 ng/ml), as were oestradiol values (control = 112 ± 11 pg/ml, progesterone treated = 63 ± 7 pg/ml, *P* < 0.001).

Effects of progesterone and LH on granulosa cell and thecal prostaglandins

Administration of exogenous progesterone on dioestrus II significantly (*P* < 0.05) reduced granulosa PGE values and LH given with progesterone returned PGE concentrations to control

levels (Fig. 2a). Progesterone given with or without LH had no effect on thecal PGE, PGF or 6-keto PGF-1 α concentrations (Fig. 2b). Concentrations of 6-keto PGF-1 α were not significantly reduced by progesterone in the granulosa or thecal compartments.

Fig. 2. Concentrations in hamsters of PGE, PGF and 6-keto PGF-1 α in granulosa (a) and theca (b) at 09:00 h on the day of pro-oestrus after s.c. injection of progesterone (100 μ g) or progesterone + LH (10 μ g) at 09:00 h on dioestrus II. Values are mean $\pm$ s.e.m. for 6 animals (6–10 follicles/animal). *Significantly different from control.

Effects of progesterone and oestrogen on follicular prostaglandins and steroids

ECP given with progesterone did not prevent the decline in follicular PGE values (Fig. 3). In fact, ECP, like progesterone, given alone reduced follicular PGE concentration ($P < 0.001$) compared with controls while ECP with or without progesterone increased ($P < 0.005$) PGF concentrations (Fig. 3). Concentrations of 6-keto PGF-1 α were unaffected by ECP alone.

ECP given with progesterone on dioestrus II did not prevent the progesterone-induced declines in follicular androstenedione and oestradiol (Table 1). ECP plus progesterone lowered follicular concentrations of oestradiol and androstenedione more than that induced by progesterone alone; follicular progesterone also tended to be reduced with the combined treatment. Animals given ECP alone had follicular androstenedione, progesterone and oestradiol concentrations that were not significantly different from those of oil-treated controls (Table 1).

ECP administered with progesterone did not prevent the progesterone-induced decline in serum oestradiol concentrations (Table 2). Serum levels of oestradiol in animals given ECP alone were not different from those of controls. Animals given ECP with progesterone exhibited elevated serum progesterone concentrations compared to progesterone-treated hamsters. ECP administered with

Fig. 3. Follicular concentrations in hamsters of PGE, PGF and 6-keto PGF-1 α at 09:00 h on the day of pro-oestrus after s.c. injection of progesterone (100 μ g) oestradiol-17-cyclopentane-propionate (ECP) (10 μ g) or progesterone + ECP at 09:00 h on dioestrus II. Values are mean $\pm$ s.e.m. for 6 animals (6–10 follicles/animal). Different letters denote groups that are significantly different from each other.

or without progesterone on dioestrus II did not alter serum LH concentrations measured at 09:00 h on pro-oestrus.

Discussion

The decline in follicular PGE and 6-keto PGF-1 α was apparently due to a lack of LH stimulation on dioestrus II. Although serum LH concentrations measured on the morning of pro-oestrus were not different between controls and progesterone-treated hamsters (Table 2), LH was reduced 6 h after progesterone injection. Greenwald (1978) showed that this treatment in the cyclic hamster inhibited LH release only for 6–9 h on the day of dioestrus II and then serum LH concentrations increased to control levels late on dioestrus II. This implies that LH may regulate follicular prostaglandin production on dioestrus II and probably contributes to maintaining prostaglandin levels in preovulatory follicles on the day of pro-oestrus. Induction of granulosa prostaglandin synthase by LH/hCG has been demonstrated in PMSG/hCG-treated immature rats (Curry *et al.*, 1987; Hedin *et al.*, 1987). Immunocytochemical localization of prostaglandin synthase in the cyclic hamster indicates that it is present in granulosa cells only in preovulatory follicles beginning on dioestrus II (P. F. Terranova & J. D. Brannian, unpublished data) and so suppression of LH-induced granulosa prostaglandin synthase by exogenous progesterone may partly explain the decline in granulosa PGE values.

The decline in 6-keto PGF-1 α concentration in intact preovulatory follicles after administration of progesterone (Fig. 1) could not be demonstrated in isolated theca or granulosa cells (Fig. 2). The reason for this inconsistency is uncertain, but possibly the reduced intrafollicular concentrations of 6-keto PGF-1 α may have been due to reduced accumulation in follicular fluid which was not significantly reflected in intracellular levels of the theca and granulosa.

Follicular concentrations of PGF were not significantly reduced by progesterone; however, PGF concentrations appeared to be lowest when compared with PGE and 6-keto PGF-1 α on the morning of pro-oestrus (Fig. 1). Detection of a decrease in PGF may therefore be difficult when an

overall suppression of prostaglandin synthesis is induced by progesterone because PGF values are already very low.

Administration of exogenous LH alone in this study did not increase follicular prostaglandin concentrations, although it is well known that LH stimulates thecal and granulosa prostaglandin production *in vitro* (Clark *et al.*, 1978; Koos & Clark, 1982; Evans *et al.*, 1983; Tsang *et al.*, 1988). Perhaps endogenous circulating LH levels in the cyclic hamster are sufficient to stimulate maximally follicular prostaglandin production and additional exogenous LH may have little effect or LH may have had a stimulatory effect over a shorter time course which was not evident 24 h after treatment. Similarly, 10 µg exogenous LH treatment did not significantly increase follicular androstenedione concentrations (Table 1), but LH given with progesterone prevented the progesterone-induced inhibition of follicular PGE, 6-keto PGF-1 α , androstenedione and oestradiol (Fig. 1, Table 1). LH given alone did, however, increase follicular oestradiol (Table 1) and serum progesterone concentrations (Table 2) compared to controls. Surprisingly, LH given with progesterone administration did not bring follicular or serum oestradiol levels to control values (Tables 1 and 2), although follicular oestradiol values were significantly higher than in progesterone-treated hamsters. A larger dose of LH may therefore be required to overcome the inhibition of oestradiol secretion by progesterone. The high serum progesterone concentration resulting from LH replacement therapy (Table 2) may have further inhibited oestradiol synthesis and/or secretion since it has been shown that exogenous LH and progesterone can inhibit oestradiol synthesis in the hamster, LH apparently acting via elevated progesterone concentrations (Saidapur & Greenwald, 1979).

ECP given with progesterone failed to prevent the progesterone-induced decline in follicular PGE and 6-keto PGF-1 α values. In fact, ECP alone reduced PGE and increased PGF concentrations in preovulatory follicles (Fig. 3) as reported for the rat uterus in which exogenous oestrogen increased PGF-2 α and decreased PGE-2 production (Ham *et al.*, 1975). Greenwald (1975) demonstrated in the hamster that 50 µg ECP injected s.c. on the day of oestrus significantly reduced circulating LH concentrations which could account for the observed decrease in PGE (Table 1). However, it appears unlikely in the present study that 10 µg ECP appreciably inhibited LH secretion since follicular androstenedione and oestradiol concentrations were not significantly lower than control values (Table 1). These results suggest that oestradiol may preferentially stimulate PGF production at the expense of PGE (PGF increases while PGE decreases), possibly by an activation of PGE-9-ketoreductase which converts PGE-2 to PGF-2 α . In the rat, renal PGE-9-ketoreductase activity was enhanced with oestradiol treatment and progesterone antagonized this effect (Cagen & Baer, 1987). PGE-9-ketoreductase activity in the ovary has been reported (Watson *et al.*, 1979) but the hormonal regulation and functional role of this enzyme in follicular development and ovulation have not been determined.

This research was supported by NICHD grants 20839 and RCDA 00478 to P.F.T. and a grant (02528) from the NICHD to the Kansas Center for Human Development. We thank the National Hormone and Pituitary program for the gift of ovine LH.

References

- Ainsworth, L., Baker, R.D. & Armstrong, D.T. (1975) Preovulatory changes in follicular fluid prostaglandin F levels in swine. *Prostaglandins* **9**, 915–925.
- Ainsworth, L., Tsang, B.K., Downey, B.R., Marcus, G.J. & Armstrong, D.T. (1979) Effect of indomethacin on ovulation and luteal function in swine. *Biol. Reprod.* **21**, 401–411.
- Ainsworth, L., Tsang, B.K., Marcus, G.J. & Downey, B.R. (1984) Prostaglandin production by dispersed granulosa and theca interna cells from porcine preovulatory follicles. *Biol. Reprod.* **31**, 115–121.
- Armstrong, D.T. (1981) Prostaglandins and follicular functions. *J. Reprod. Fert.* **62**, 283–291.
- Armstrong, D.T. & Zamecnik, J. (1975) Preovulatory elevation of rat ovarian prostaglandins F and its blockade by indomethacin. *Molec. cell. Endocr.* **2**, 125–131.
- Bast, J.D. & Greenwald, G.S. (1974) Serum profiles of follicle-stimulating hormone, luteinizing hormone

- and prolactin during the estrous cycle of the hamster. *Endocrinology* **94**, 1295–1299.
- Bauminger, S. & Lindner, H.R.** (1975) Periovalutary changes in ovarian prostaglandin formation and their hormonal control in the rat. *Prostaglandins* **9**, 737–750.
- Cagen, L.M. & Baer, P.G.** (1987) Effects of gonadectomy and steroid treatment on renal prostaglandin 9-ketoreductase activity in the rat. *Life Sciences* **40**, 95–100.
- Clark, M.R., Marsh, J.M. & LeMaire, W.J.** (1978) Mechanism of LH regulation of prostaglandin synthesis in rat granulosa cells. *J. biol. Chem.* **253**, 7757–7761.
- Curry, T.E., Malik, A. & Clark, M.R.** (1987) Ovarian prostaglandin synthase: immunohistochemical localization in the rat. *Am. J. Obstet. Gynec.* **157**, 537–543.
- Erickson, G.F., Challis, J.R. & Ryan, E.J.** (1977) Production of PGF by rabbit granulosa cells and thecal tissue. *J. Reprod. Fert.* **49**, 133–134.
- Evans, G., Dobias, M., King, G.J. & Armstrong, D.T.** (1983) Production of prostaglandins by porcine preovulatory follicular tissues and their roles in intra-follicular function. *Biol. Reprod.* **28**, 322–328.
- Garza, F. & Terranova, P.F.** (1984) Inhibition of thecal androstenedione production by exogenous progesterone in the cyclic hamster. *J. Reprod. Fert.* **70**, 493–498.
- Greenwald, G.S.** (1975) Proestrous hormone surges dissociated from ovulation in the estrogen-treated hamster. *Endocrinology* **97**, 878–884.
- Greenwald, G.S.** (1978) Modification by exogenous progesterone of estrogen and gonadotropin secretion in the cyclic hamster. *Endocrinology* **103**, 2315–2322.
- Ham, E.A., Cirillo, V.J., Zanetti, M.E. & Kuehl, F.A.** (1975) Estrogen-directed synthesis of specific prostaglandins in uterus. *Proc. natn. Acad. Sci. USA* **72**, 1420–1424.
- Hamada, Y., Bronson, R.A., Wright, K.H. & Wallach, E.E.** (1977) Ovulation in the perfused rabbit ovary: the influence of prostaglandins and prostaglandin inhibitors. *Biol. Reprod.* **17**, 58–63.
- Hedin, L., Gaddy-Kurten, D., Kurten, R., DeWitt, D.L., Smith, W.L. & Richards, J.S.** (1987) Prostaglandin endoperoxide synthase in rat ovarian follicles: content, cellular distribution and evidence for hormonal induction preceding ovulation. *Endocrinology* **121**, 722–731.
- Hunter, R.H.F. & Poyser, N.L.** (1985) Ovarian follicular fluid concentrations of prostaglandins E-2, F-2 α and I-2 during the preovulatory period in pigs. *Reprod. Nutr. Develop.* **25**, 909–917.
- Kogo, H., Yamada, K. & Aizawa, Y.** (1977) Effect of estradiol on prostaglandin metabolism in rat uterus. *Prostaglandins* **13**, 785–794.
- Koos, R.D. & Clark, M.R.** (1982) Production of 6-keto-prostaglandin F-1 α by rat granulosa cells *in vitro*. *Endocrinology* **111**, 1513–1518.
- Koos, R.D., Clark, M.R., Janson, P.O., Ahren, K.E.B. & LeMaire, W.J.** (1983) Prostaglandin levels in preovulatory follicles from rabbit ovaries perfused *in vitro*. *Prostaglandins* **25**, 715–724.
- LeMaire, W.J., Yang, N.S.T., Behrman, H.R. & Marsh, J.M.** (1973) Preovulatory changes in the concentration of prostaglandins in rabbit Graafian follicles. *Prostaglandins* **3**, 367–375.
- Murdoch, W.J., Peterson, T.A., Van Kirk, E.A., Vincent, D.L. & Inskeep, E.K.** (1986) Interactive roles of progesterone, prostaglandins and collagenase in the ovulatory mechanism of the ewe. *Biol. Reprod.* **35**, 1187–1194.
- Saidapur, S.K. & Greenwald, G.S.** (1979) Regulation of 17 β -estradiol synthesis in the proestrous hamster: role of progesterone and luteinizing hormone. *Endocrinology* **97**, 878–884.
- Shemesh, M. & Hansel, W.** (1975) Stimulation of prostaglandin synthesis in bovine ovarian tissues by arachidonic acid and luteinizing hormone. *Biol. Reprod.* **13**, 448–452.
- Terranova, P.F.** (1981) Steroidogenesis in experimentally induced atretic follicles of the hamster: a shift from estradiol to progesterone synthesis. *Endocrinology* **108**, 1885–1890.
- Terranova, P.F. & Greenwald, G.S.** (1978) Steroid and gonadotropin levels during the luteal-follicular shift of the cyclic hamster. *Biol. Reprod.* **18**, 170–175.
- Terranova, P.F., Martin, N.C. & Chien, S.** (1982) Theca is the source of progesterone in experimentally induced atretic follicles of the hamster. *Biol. Reprod.* **26**, 721–727.
- Tsang, B.K., Aradi, J., Ming, L., Ainsworth, L., Srikandakumar, A. & Downey, B.R.** (1988) Gonadotropin regulation of prostaglandin production by ovarian follicular cells of the pig. *Biol. Reprod.* **38**, 627–635.
- Watson, J., Shepherd, T.S. & Dodson, K.S.** (1979) Prostaglandin E-2-9-ketoreductase in ovarian tissues. *J. Reprod. Fert.* **57**, 489–496.

Received 29 August 1989