Effects of gonadotrophins, hypophysectomy and prostaglandin F-2 α on corpora lutea and ovarian follicles, and evidence for the presence of PGF-2 α receptors in the ovarian follicles and interstitium of non-pregnant guinea-pigs

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Summary. Simultaneous administration of hCG (but not FSH or prolactin) with PGF-2 α prevented the luteolytic effect of PGF-2 α after Day 9 of the oestrous cycle both morphologically and in terms of progesterone secretion in hysterectomized guinea-pigs. Hypophysectomy on Day 5 or PGF-2 α administration on Days 6–9 were not luteolytic, but their combined effect resulted in an advanced state of luteolysis as early as Day 9. When PGF-2 α was injected simultaneously with FSH or prolactin (but not hCG), the proportion of atretic antral follicles was significantly increased, although the total number of antral follicles remained unchanged. When PGF-2 α receptors were measured in the membrane fractions of luteal tissue and ovarian stromal, interstitial and follicular tissue obtained from cyclic animals, the concentrations were 22.75 and 36.26, and 62.60 and 96.26 fmol/mg membrane protein on Days 5 and 12, respectively. It is concluded that (1) LH protected corpora lutea against the luteolytic action of PGF-2 α up to Day 9 of the cycle, (2) PGF-2 α receptors were present in follicles, and (3) these receptors and PGF-2 α were involved in the development of the normal complement of Graafian follicles.

Introduction

Prostaglandin (PG) F-2 α is luteolytic in guinea-pigs only after Day 9 of the oestrous cycle (Tso & Tam, 1977). This may be due to protection of the corpora lutea (CL) by the relatively high circulating levels of gonadotrophins (Blatchley, Donovan & ter Haar, 1976) before Day 9, or the development of luteal PGF-2 α receptors only after Day 9 of the cycle. LH and hCG have been reported to antagonize the luteolytic action of PGF-2 α in the rat (Behrman, MacDonald & Greep, 1971) and ewe (Bolt, 1979). However, PGF-2 α receptors are present in ovarian follicles; ovulation can be inhibited by suppression of follicular PGF-2 α synthesis and levels with intrafollicular administration of indomethacin and PGF antiserum (Armstrong, Greenwich, Moon & Zamecnik, 1974). If follicular PGF-2 α receptors remain the same after luteinization, PGF-2 α receptors would still be present in the newly formed CL. Moreover, because PGs are believed to be responsible for follicular rupture during ovulation (see review by Pharriss & Shaw, 1974), it would be of interest to see whether PGF-2 α has some other effects on the developing follicles. Accordingly, the aims of this study were to investigate whether (1) gonadotrophins protect the CL from the luteolytic action of PGF-2 α before Day 9, (2) PGF-2 α receptors are present in

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early and late CL as well as in the ovarian follicles, and (3) PGF-2 α has any effect on the developing follicles in guinea-pigs.

Materials and Methods

Animals, surgery and collection of specimens

The guinea-pigs used were nulliparous animals of the English short-haired strain, aged 2–4 months and weighing 450–800 g. The management of animals (Tam & Burgess, 1977) and the calculation of oestrous cycle length (16 ± 2 days) have been described elsewhere (Tso & Tam, 1977). Hysterectomy and sham hysterectomy (sectioning of the utero-vaginal junction) were performed under diethyl ether anaesthesia on Day 5 of the cycle. Hypophysectomy was performed on Day 5 under pentobarbitone sodium (Abbott Laboratories; Montreal, Quebec, Canada) anaesthesia (36 mg/kg body weight) and according to the procedure of McPhail & Parkes (1933). Immediately after hypophysectomy, the guinea-pigs were given an i.p. injection of 5 mg cortisone acetate suspended in 0.5 ml saline (0.154 M-NaCl). In sham hypophysectomy, the basisphenoid immediately ventral to the pituitary fossa was only drilled half way with a No. 8 dental burr. The sham-operated guinea-pigs were also given the cortisone acetate injection. Animals showing signs of brain damage by their unbalanced movement were excluded from the experiments. At the termination of the experiments, the guinea-pigs were placed under diethyl ether anaesthesia and systemic blood was collected by cardiac puncture into a heparinized syringe. Plasma was obtained by centrifugation and kept at -30°C until used for progesterone assay. The ovaries were removed and fixed in Bouin's fluid for routine histology. The hypothalamic region of the brain and the skulls of the first two hypophysectomized guinea-pigs were fixed, decalcified, serially sectioned and stained with haematoxylin and eosin. On examination, it was found that hypophysectomy was complete. In subsequent animals, only the histology of the ovaries was used as criterion for the completeness of the operation (see 'Results'). The ovaries of intact animals killed on Days 5 and 12 were removed and their CL were dissected out under a stereomicroscope. The remainder of the ovarian tissue (consisting of stromal and interstitial tissues and follicles) was referred to as residual tissue. Amounts of 114 and 172 mg luteal tissue and 1.1 and 1.4 g residual tissues were collected on Days 5 and 12 respectively. The tissues were stored at -80°C (Ultralow Freezer: Revco Inc., West Columbia, South Carolina, U.S.A.) until used for PGF-2 α receptor determination.

Treatment

Treatment with PGF-2 α consisted of two daily i.p. injections (09:00 and 17:00 h) each of 0.168 mg THAM salt of PGF-2 α (equivalent to 0.125 mg PGF-2 α ; Upjohn Co., Kalamazoo, Michigan, U.S.A.) in 0.5 ml saline (0.154 M-NaCl). Gonadotrophins were administered as one injection i.p. of 50 i.u. hCG (Sigma Chemical Co., St Louis, Missouri, U.S.A.) per day, two injections i.p. of 5 i.u. ovine prolactin (Sigma; contaminated with traces of STH) per day, or two injections i.p. of 2.5 Armour units porcine FSH (Sigma; contaminated with 0.03 Armour units of LH per Armour unit of FSH) per day, all in 0.1 ml saline. Suitable control groups, receiving the corresponding volumes of the saline carrier, were also included in the experiments.

Measurement of corpora lutea and follicles

The ovarian serial sections, stained with haematoxylin and eosin, were examined with a Bausch and Lomb microprojector. The outline of the maximal circumference of each CL was traced on paper and the two diameters set at right angles to each other were measured. The third diameter was obtained as the product of the thickness of the section (5 μm) and the number of

sections in which the CL appeared. After suitable adjustment was made to compensate for magnification, the volume of the CL was calculated as $0.524d^3 \text{ mm}^3$, where d^3 was the product of the 3 diameters measured. Follicular size and atresia (almost completely due to degeneration of the membrana granulosa) was observed with the light microscope.

Progesterone assay

The method for the competitive protein-binding assay of progesterone using progesterone-binding globulin obtained from the serum of 40–60-day-pregnant guinea-pigs was a modification of the method described previously (Tam, 1977). The working protein solution was a 1:1500 dilution of the serum with 0.1 M-sodium phosphate buffer containing, in each millilitre, 66 000 d.p.m. (0.11 ng) [1,2,6,7- ^3H]progesterone (sp. act. 89 Ci/mmol; Amersham Corp., Oakville, Ontario, Canada), which was purified by chromatography before use. Progesterone from 2 ml plasma was extracted three times with 5 ml fractionally distilled light petroleum (b.p. 38–55°C, A.C.S. Grade; Fisher Scientific Co., Don Mills, Ontario, Canada) and divided into 2 aliquots for duplicate assays. The recovery of the extraction was $81.92 \pm 1.14\%$ ($n = 12$). Blank values were always encountered, but these were subtracted from the amount of progesterone found in the plasma samples. Precision tests were carried out using male rabbit plasma treated with dextran-coated charcoal and made up to progesterone concentrations of 0, 1.0, 5.0, 25.0 and 100.0 ng/ml. The assayed progesterone concentrations were found to be respectively 0.3 ± 0.3 ($n = 6$), 1.1 ± 0.2 ($n = 4$), 4.5 ± 0.4 ($n = 13$), 26.3 ± 2.3 ($n = 7$) and 100.0 ± 5.6 ($n = 6$) ng/ml. At 5.0 ng/ml, the intra-assay coefficient of variation was 33% and the inter-assay coefficient of variation was 36%. At 100.0 ng/ml, these were reduced to 14%. Using systemic plasma pooled from 9 guinea-pigs killed on Day 9 of the oestrous cycle, the assayed progesterone was 2.7 ± 0.4 ng/ml, very similar to published values of 2.3–3.0 ng/ml (Feder, Resko & Goy, 1968; Challis, Heap & Illingworth, 1971; Blatchley *et al.*, 1976). Scintillation counting was done in 10 ml of an aqueous scintillation cocktail, Scintiverse (Fisher Scientific Co.).

Determination of prostaglandin F-2 α receptors

The luteal and residual tissues were homogenized in 4 volumes of 10 mM-Tris-HCl buffer (pH 7.0) with Potter-Elvehjem homogenizers. Membrane fractions were obtained from the homogenates using the method of Powell, Hammarström & Samuelsson (1975). Electron microscope examination showed that the plasma membranes were fragmentary or vesicular. After known aliquots were removed for protein determination (Lowry, Rosebrough, Farr & Randall, 1951), the membrane fractions were diluted with the Tris-HCl buffer to give protein concentrations of 1.350 mg/ml with the luteal fraction and 1.875 mg/ml with the residual tissue. For the assay of PGF-2 α receptors the general procedure of Powell *et al.* (1975) was followed, but the optimal amounts of unlabelled PGF-2 α and [^3H]PGF-2 α in each incubation were determined in a series of preliminary experiments. Three series of incubations were performed for each type of tissue to estimate (1) assay blanks, (2) non-specific and (3) specific binding. Each assay blank incubation contained 200 μl 0.5% gelatin in the Tris-HCl buffer, 0.2 ng [5,6,8,9,11,12,14,15- ^3H (N)]prostaglandin F-2 α (sp. act. 120 Ci/mmol; New England Nuclear, Boston, Massachusetts, U.S.A.; used without purification), and 5 μl ethanol. The non-specific binding incubations contained 200 μl of the diluted membrane fraction, 0.5 ng [^3H]PGF-2 α and 0.5, 1.0, 2.0 or 5.0 μg unlabelled PGF-2 α dissolved in 5 μl ethanol (saturation occurred at 10 μg). Each specific binding incubation contained 200 μl of the diluted membrane fraction, 0.2, 0.5, 1.0, 5.0 or 10.0 ng [^3H]PGF-2 α and 5 μl ethanol. These mixtures were incubated with agitation for 2 h at 24°C. After incubation, the bound and free [^3H]PGF-2 α in a few of the initial experiments were separated by the Millipore filtration (pore size 0.45 μm ; Millipore Corp.,

Bedford, Massachusetts, U.S.A.) method of Rao (1974). This method was slow and cumbersome due to the blocking of the Millipore filter by plasma membrane, and was later replaced by a centrifugation method (Rao, 1973), which gave the same results as Millipore filtration. Both bound and free [^3H]PGF-2 α fractions were counted in 10 ml Scintiverse.

Scintillation spectrometry

Radioactivity was counted spectrometrically (Beckman Model LS255). The duration of counting each sample was set so that the 95% confidence level error was <3%. Counting efficiency of ^3H was 32% with the aqueous Scintiverse cocktail. After adding known amounts of ^3H radioactivity and up to 450 μg protein (from the membrane fraction of guinea-pig liver) to 10 ml Scintiverse, no quenching was observed.

Statistical and data treatment

The results of Exps 1 and 3 were assessed by one-way analysis of variance and significant differences were determined by the *a posteriori* test of Student-Newman-Keuls (Sokal & Rohlf, 1969). Significant differences between means in Exp. 2 were calculated by Student's two-tailed *t* test. The methods of Scatchard plot and the calculation of dissociation constants and PGF-2 α receptor concentrations were based on those of Munck (1976). Regression analysis and test of significance in regression of the Scatchard plot were also based on procedures described by Sokal & Rohlf (1969). The results are expressed as means $\pm$ s.e.m. and $P < 0.05$ is taken as significant.

Results

Experiment 1: effects of hysterectomy, gonadotrophins and PGF-2 α on CL and progesterone levels at Day 14

As shown in Table 1, the relatively high progesterone levels in Groups B, D and F were associated with large and healthy CL. The ovaries of Group D animals were luteinized as reported by Reed & Hounslow (1971), and the ovaries also contained numerous small luteinized follicles with entrapped, degenerating ova. In Group B, the ovaries also contained many small luteinized follicles even though they were not as numerous as in Group D. No luteinized follicles were found in Group F animals. Luteal volumes in Groups E and G ($P <$

Table 1. Effects of hysterectomy (Day 5) and gonadotrophins (Days 8–13) and PGF-2 α (Days 9–13) treatment on corpora lutea of ovulation and systemic progesterone concentrations in cyclic guinea-pigs killed on Day 14 of the oestrous cycle

Group	Treatment	Luteal volume (mm ³)	Plasma progesterone conc. (ng/ml)
A	Saline and PGF-2 α	0.747 $\pm$ 0.124 (4, 8)	1.0 $\pm$ 0.6 (4)
B	hCG and saline	4.396 $\pm$ 0.237 (4, 14)	5.0 $\pm$ 1.0 (4)
C	hCG and PGF-2 α	1.636 $\pm$ 0.280 (4, 13)	5.5 $\pm$ 1.5 (4)
D	FSH and saline	4.248 $\pm$ 0.141 (4, 15)	10.3 $\pm$ 0.2 (4)
E	FSH and PGF-2 α	1.389 $\pm$ 0.169 (4, 13)	3.0 $\pm$ 0.3 (3)
F	Prolactin and saline	2.356 $\pm$ 0.157 (4, 15)	6.7 $\pm$ 0.6 (4)
G	Prolactin and PGF-2 α	0.972 $\pm$ 0.100 (4, 11)	3.0 $\pm$ 1.5 (3)

Values are mean $\pm$ s.e.m. for the no. of animals indicated and the no. of CL when two figures are given.

0.01 in both groups) and plasma progesterone concentrations ($P < 0.01$ and $P < 0.05$, respectively) were significantly reduced when compared with those of Groups D and F. However, this effect of PGF-2 α was not evident in Group C and progesterone concentrations in Groups B and C were virtually the same. There was no significant difference in luteal volume among Groups A, E and G, but luteal volume in Group C was significantly larger than that in Group A ($P < 0.05$). The luteal cells in Groups A, E and G, but not in Group C, showed crenated nuclei and cytoplasmic vacuolations.

Experiment 2: effects of hysterectomy, gonadotrophins and PGF-2 α on follicles at Day 14

The results are given in Table 2. The division of antral follicles into small, intermediate and large categories corresponded to follicles that had undergone 4–8 days, 8–12 days and more than 12 days of development respectively (Myers, Young & Dempsey, 1936). Virtually no intermediate and large antral follicles were found in Groups A and B and there were no large antral follicles in Groups C and D. Prolactin, hysterectomy or PGF-2 α treatment (Groups E, F and I) did not result in the obliteration of follicles of any particular size. In Group B there were significantly more ($P < 0.001$) atretic small antral follicles (91%) than in Group A (23%). The increase in atretic small antral follicles, though modest, was significant ($P < 0.02$), when PGF-2 α was administered to the prolactin-treated animals (Group F). In the absence of gonadotrophins and when compared with Group H, exogenous PGF-2 α (Group I) failed to increase significantly the proportions of atretic follicles in any of the three classes of follicles. However, the exogenous PGF-2 α significantly increased ($P < 0.05$) the total percentage of all atretic follicles from 34 to 43%. There was no increase in atresia in Group D, and the number of atretic small antral follicles was decreased ($P < 0.02$) when compared with Group C. PGF-2 α did not significantly increase the proportion of atretic large antral follicles in groups with large antral follicles. The total number of antral follicles in any one group was not influenced by gonadotrophins or PGF-2 α .

Table 2. Effects of hysterectomy (Hyst., Day 5) and gonadotrophins (Days 8–13) and PGF-2 α (Days 9–13) treatment of antral follicles in non-pregnant guinea-pigs killed on Day 14 of the oestrous cycle

Group	Treatment	No. of animals	Antral follicles*					
			Small		Intermediate*		Large	
			Total no. per animal	Atretic (%)	Total no. per animal	Atretic (%)	Total no. per animal	Atretic (%)
A	Hyst., FSH and saline	4	83.3 $\pm$ 14.8	23.2 $\pm$ 1.9	0.3 $\pm$ 0.3	0	0	0
B	Hyst., FSH and PGF-2 α	4	116.0 $\pm$ 14.8	91.3 $\pm$ 6.1	0	0	0	0
C	Hyst., hCG and saline	4	146.5 $\pm$ 29.9	44.9 $\pm$ 6.6	9.5 $\pm$ 4.6	49.2 $\pm$ 24.9	0	0
D	Hyst., hCG and PGF-2 α	3	101.3 $\pm$ 14.4	14.3 $\pm$ 2.9	2.7 $\pm$ 0.9	25.0 $\pm$ 25.0	0	0
E	Hyst., prolactin and saline	4	149.5 $\pm$ 65.6	33.2 $\pm$ 0.9	28.8 $\pm$ 10.6	64.6 $\pm$ 3.6	2.5 $\pm$ 0.6	25.0 $\pm$ 14.4
F	Hyst., prolactin and PGF-2 α	4	58.3 $\pm$ 6.6	38.9 $\pm$ 1.4	14.5 $\pm$ 4.1	68.4 $\pm$ 5.8	5.3 $\pm$ 0.9	35.2 $\pm$ 14.1
G	None (control)	5	42.8 $\pm$ 8.8	31.6 $\pm$ 5.0	18.0 $\pm$ 5.6	52.2 $\pm$ 7.4	4.2 $\pm$ 1.2	10.2 $\pm$ 4.3
H	Hyst. and saline	5	101.0 $\pm$ 18.5	28.9 $\pm$ 3.5	29.4 $\pm$ 8.9	50.6 $\pm$ 2.6	5.0 $\pm$ 2.2	57.0 $\pm$ 8.0
I	Hyst. and PGF-2 α	5	116.2 $\pm$ 28.6	36.8 $\pm$ 3.6	27.4 $\pm$ 3.4	61.6 $\pm$ 6.9	2.8 $\pm$ 0.7	65.2 $\pm$ 9.9

Values are mean $\pm$ s.e.m.

* Diameter of small antral follicles = 0.23–0.59 mm, intermediate antral follicles = 0.60–0.79 mm, large antral follicles = 0.80–1.15 mm.

Experiment 3: effects of hypophysectomy and PGF-2 α on CL and progesterone levels at Day 9

The results are presented in Table 3. In Groups A, B and C, large numbers of atretic and healthy antral follicles of all sizes were found. While no intermediate or large antral follicles were observed, 3–4 healthy small antral follicles (0.3 mm diameter) were found in the ovaries of 2 animals in Group D and 3 animals in Group E, indicating that hypophysectomy might not be complete in these guinea-pigs. All antral follicles in the other hypophysectomized animals were atretic. The CL in Groups A, B, C and D were large and healthy. However, those in Group E were shrunk and the mean luteal volume was significantly lower than those of the other groups ($P < 0.01$). The nuclei of the luteal cells in Group E were mostly crenated, although cytoplasmic vacuolation was not extensive. Progesterone values in Group E were significantly lower than those in Groups A, B ($P < 0.01$ for both), C and D ($P < 0.05$ for both). No distinction could be made between the progesterone concentrations in the completely and incompletely hypophysectomized animals in Groups C and D.

Table 3. Effects of hypophysectomy (hypox., Day 5) and PGF-2 α treatment (Days 6–9) on corpora lutea of ovulation and systemic progesterone concentrations in non-pregnant guinea-pigs killed on Day 9 of the oestrous cycle

Group	Treatment	Luteal volume (mm ³)	Plasma progesterone conc. (ng/ml)
A	None (control)	1.985 $\pm$ 0.099 (4, 14)	2.7 $\pm$ 0.4 (9)
B	Sham hypox. and saline	1.697 $\pm$ 0.109 (4, 15)	4.4 $\pm$ 0.9 (4)
C	Sham hypox. and PGF-2 α	1.654 $\pm$ 0.133 (4, 15)	3.1 $\pm$ 0.5 (4)
D	Hypox. and saline	1.637 $\pm$ 0.120 (5, 19)	3.1 $\pm$ 1.0 (4)
E	Hypox. and PGF-2 α	0.934 $\pm$ 0.052 (6, 22)	0.5 $\pm$ 0.3 (5)

Values are mean $\pm$ s.e.m. for the no. of animals indicated in parentheses and the no. of CL when two figures are given.

Experiment 4: determination of PGF-2 α receptors in luteal and residual tissue on Days 5 and 12

The Scatchard plot analyses of the results are shown in Text-fig. 1. The dissociation constants of the receptors on luteal tissues were calculated to be 2.86×10^{-8} M on Day 5 and 3.91×10^{-8} M on Day 12, while their concentrations were 22.75 and 36.22 fmol/mg membrane protein, respectively. For the residual tissues, the dissociation constants were 1.19×10^{-8} and 3.99×10^{-8} M, and the concentrations were 62.60 and 96.26 fmol/mg membrane protein, on Days 5 and 12 respectively.

Discussion

The detection of PGF-2 α receptors in the luteal tissue of Day 5 cyclic guinea-pigs clearly showed that these receptors were present early in the life of the CL. Neither PGF-2 α injections on Days 6–9 nor hypophysectomy on Day 5 alone (supporting the observations of Heap, Perry & Rowlands, 1967) were luteolytic. The luteolysis induced by PGF-2 α between Days 6 and 9 in animals hypophysectomized on Day 5 indicated that it was gonadotrophins that protected the CL in the PGF-2 α -treated, sham-hypophysectomized animals. According to the criterion of Perry & Rowlands (1962) that all antral follicles should be atretic 3 days after complete hypophysectomy, hypophysectomy was probably incomplete in some animals in this study. However, this should not distract from the validity of the interpretation of the observed effects, i.e. either the complete or partial removal of endogenous gonadotrophins during the first half of

Text-fig. 1. Scatchard plots of the specific binding of [^3H]prostaglandin F-2 α to the membrane fractions of (a) luteal tissue and (b) ovarian stromal and interstitial tissues and follicles obtained from non-pregnant guinea-pigs killed on Days 5 and 12 of the oestrous cycle.

the oestrous cycle would result in luteolysis by treatment with exogenous PGF-2 α . Of the three gonadotrophins used in Exp. 1, only hCG was antagonistic to the luteolytic effects of PGF-2 α in guinea-pigs, just as either hCG or LH was found to be antagonistic to PGF-2 α in rats (Behrman *et al.*, 1971; Thomas, Dorfinger & Behrman, 1978), ewes (Bolt, 1979), rhesus monkeys (Wallach, Bronson, Hamada, Wright & Stevens, 1975) and man (McNatty, Henderson & Sawers, 1975). The low plasma level of LH in guinea-pigs between Days 9 and 12 of the cycle (Blatchley *et al.*, 1976) therefore has a definite physiological significance in that it initiates the end of the oestrous cycle by allowing the rising levels of PGF-2 α (Blatchley, Donovan, Horton & Poyser, 1972) to induce luteolysis and perhaps also other changes in the ovary.

The dissociation constants of the PGF-2 α receptors in the luteal and residual tissues of the guinea-pig are not only very similar to one another, they are also of the same order of magnitude as those reported for luteal PGF-2 α receptors of cattle (Kimball & Lauderdale, 1975; Powell *et al.*, 1975; Rao, 1975), pigs (Powell *et al.*, 1974) and man (Rao, Griffin & Carman, 1977). Although the amounts of PGF-2 α in the luteal and residual tissues of the guinea-pigs were not compared, the amount of PGF-2 α receptors in the residual tissue was higher than that in the luteal tissue, a recognized target of PGF-2 α . As Koering & Kirton (1973) did not observe PGF-2 α to have any effect on ovarian interstitial cells, it is unlikely that PGF-2 α receptors are present in these cells. The most likely origins of the ovarian residual tissue PGF-2 α receptors in guinea-pigs are the epithelial cells of blood capillaries (Pharriss & Shaw, 1974) and the follicular cells. Even though the stromal tissue may be more well vascularized than luteal tissue, the substantially higher concentration of PGF-2 α receptors in the residual tissue probably suggests that some of these receptors came from the follicles. The follicular origin of some of the residual tissue PGF-2 α receptors is also supported by the correlation between PGF-2 α administration and increased follicular atresia in Exp. 2.

The changes in the proportions of healthy and atretic antral follicles observed in this study were evidently the combined effect of PGF-2 α and gonadotrophins. Although the atretic effect of hCG on medium-sized follicles in guinea-pigs has been reported (Peddie, 1980), this effect was not obvious in this investigation. Moreover, the administration of PGF-2 α in addition to hCG did have the opposite effect of diminishing the proportion of atretic small antral follicles from 45 to 14%. The reported protective effect of PMSG against atresia in rats (Braw & Tsafiri, 1980) did not prevent the drastically atretic effect of PGF-2 α on FSH-treated guinea-pigs. The mechanism and significance of these results cannot at present be explained. That there is a functional relationship between PGF-2 α and ovarian follicular development is already evident from the ability of PGF-2 α to induce the LH surge (Bono, Gaiani & Seren, 1980) and ovulation (Blatchley & Donovan, 1972; Lau, Saksena & Chang, 1974; Wallach *et al.*, 1975). It is possible that PGF-2 α may be part of a mechanism required for the development of the normal complement of Graafian follicles by encouraging the atresia of surplus and defective follicles, and that the interaction between PGF-2 α and follicles may be mediated by the receptors detected in the follicles in this study.

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