Chapter III

Results

1. Viability of isolated rat luteal cells in culture.

1.1 Viability of isolated rat luteal cells obtained during estrous cycle.

Table 2 and Fig. 9 show the viability of luteal cells from cycling rats which incubated for 11 days.

The viability of luteal cells which obtained from estrus, diestrus-1 and diestrus-2 showed no significant difference between stages. The means of viable luteal cells were 90.68±4.24, 82.83±1.26, 80.78±5.28, 77.73±1.94, 71.64±4.14 and 69.33±3.30% on day 1, 3, 5, 7, 9 and 11 after incubation, respectively.

1.2 Viability of isolated rat luteal cells obtained during pregnanyc, PSP and lactating PSP.

Table 3 and Fig. 10 show the viability of luteal cells from pregnancy, PSP and lactating PSP stages during 11 days of incubation.

The viability of luteal cells which obtained from pregnancy stages L_2, L_6, L_{12} and L_{20} showed no difference in statistics in all groups. The means of viable luteal cells were 89.54±4.16, 83.35±8.14, 83.10±4.40, 78.13±4.30, 67.09±3.51 and 64.27±1.55% on day 1, 3, 5, 7, 9 and day 11 of incubation.

The viability of luteal cells from PSP stage L_2, L_6 and L_{12} showed the similar pattern during 11 days of incubation. The mean of viable luteal cells were 85.93±3.33, 82.22±1.03, 80.29±4.69, 76.33±2.42,

70.94±4.89 and 68.61±1.70% on day 1, 3, 5, 7, 9 and day 11, respectively.

The viability of luteal cells showed no difference in statistics whether they obtained from lactating stage L_2, L_{12} and L_{20}. The mean of viable luteal cells were 90.97±3.46, 83.80±1.17, 84.55±4.85, 81.22±3.73, 73.77±3.97 and 66.59±2.11% on day 1, 3, 5, 7, 9 and day 11 of incubation, respectively.

1.3 Effects of hCG, PRL, $PGF_{2\alpha}$ and their combinaitons on viability of isolated rat luteal cells obtained during various reproductive stages.

Viability of rat luteal cells from various reproductive stages in the control, treated with hCG (0.5 iu/ml)o-PRL (5 µg/ml) and $PGF_{2\alpha}$ (250 ng/ml) which were incubated for 11 days are shown in Table 4.

The viability of rat luteal cells on day 11 of incubation which obtained from estrus, diestrus-1 and diestrus-2 in the control group were 71.00±1.41, 74.25±7.43 and 67.25±3.18%, from PSP stage L_2, L_6 and L_{12} were 70.75±3.18, 68.25±1.77 and 74.50±3.54%, from pregnancy stage L_2, L_6, L_{12} and L_{20} were 74.00±1.41, 72.00±5.66, 72.00±2.83 and 67.50±2.10%, and from lactating stage L_2, L_{12} and L_{20} were 65.50±4.95, 70.75±3.89 and 71.00±3.54% respectively. In o-PRL, hCG + PRL and PRL + $PGF_{2\alpha}$ treated group, a high viability of luteal cells from various reproductive stages in cultures were observed and most were presented significantly different from the control. Otherwise, there were no significantly different in hCG, $PGF_{2\alpha}$ and hCG + $PGF_{2\alpha}$ treated group from the control.

2. P secretion of isolated rat luteal cells from various reproductive stages.

P secretion of isolated rat luteal cells which obtained from

Table 2. Percentages of cell viability and P secreting ability of luteal cells from dioestrous cycle during 11 day incubation (mean±S.E., n = 3).

Stages / Day of incubation	estrus		diestrus-1		diestrus-2		mean of % cell viability
	% cell viability	P content pmol/10^5cells	% cell viability	P content pmol/10^5cells	% cell viability	P content pmol/10^5cells	
1	89.25±1.75	21.0±0.6	92.0±5.75	34.4±2.4	90.78±0.5	15.42±2.0**	90.68±4.29
3	84.26±3.75	35.6±2.9*	81.87±3.25	45.3±0.5	82.14±4.75	20.2±2.2**	82.83±1.26
5	84.50±5.0	26.0±1.2	83.10±0.5	33.9±0.3	74.74±1.75	18.7±2.2*	80.78±5.28
7	79.81±3.75	22.6±6.2	77.41±5.75	23.8±5.8	75.97±4.0	14.5±4.4	77.73±1.94
9	72.65±2.5	19.8±5.0	75.19±4.0	18.6±7.0	67.09±3.5	9.4±1.0	71.64±4.14
11	69.68±0.5	12.2±1.4	73.16±4.75	11.0±3.0	63.15±4.0	6.2±2.1	69.33±3.30

** = $P < 0.01$, * = $P < 0.05$ significantly different from diestrus-1.

Table 3. Percentages of cell viability and P secreting ability of luteal cells from PSP, pregnancy and lactating PSP stages during 11 day incubaiton (mean±S.E., n = 3).

Stages / day of incubation	L_2		L_6		L_{12}		L_{20}		mean ($\bar{X}$±S.E.)
	% cell viability	P content pmol/10^5cells	% cell viability	P content pmol/10^5cells	% cell viability	P content pmol/10^5cells	% cell viability	P content pmol/10^5cells	% cell viability
PSP									
1	86.1±0.25	34.4±1.6*	86.06±2.00	49.0±5.0	85.55±3.50	13.3±2.7**	-	-	85.93±3.33
3	81.07±2.50	53.4±0.6	82.93±4.25	52.4±7.6	82.65±1.50	15.75±0.5**	-	-	82.22±1.03
5	78.88±4.75	38.3±7.3	79.81±5.0	35.2±4.0	81.17±7.00	12.9±2.3**	-	-	80.29±4.69
7	73.54±1.25	25.4±0.2	77.89±1.0	20.7±6.3	77.57±1.75	9.1±3.7*	-	-	76.33±2.42
9	70.15±1.75	14.5±0.9	71.39±0.25	13.9±2.1	71.29±0.75	7.9±2.5	-	-	70.94±4.89
11	67.23±2.25	7.4±2.7	68.75±1.0	7.7±0.3	69.84±1.75	4.6±1.0	-	-	68.61±1.70
pregnancy									
1	94.58±1.00	41.2±6.9	89.25±3.15	56.9±2.6	88.24±1.00	55.5±3.3	86.74±0.90	22.0±1.0*	89.85±4.16
3	84.12±5.50	45.9±1.3	84.26±3.75	66.4±2.8	91.08±2.00	59.4±0.6	74.86±3.45	11.0±3.8*	83.35±8.14
5	82.38±2.75	34.2±4.75	78.88±4.75	32.8±2.4	87.76±0.80	32.5±6.7	79.44±9.25	7.8±3.1*	83.19±4.40
7	73.42±2.25	17.9±2.3	76.00±4.00	17.6±6.4	81.83±1.25	14.61±1.0	79.15±4.05	2.7±1.3	78.13±4.30
9	70.68±2.50	12.0±3.4	75.50±4.50	10.2±3.2	63.33±2.30	15.0±3.0	68.27±4.55	2.9±1.7	67.09±3.51
11	66.45±7.75	9.0±1.2	70.55±1.95	7.5±2.7	63.09±4.50	11.6±0.8	63.26±2.70	2.7±0.9	64.22±1.55
lactating PSP									
1	90.51±1.25	26.4±0.8*	-	-	92.25±1.25	34.50±0.4	91.42±1.75	14.4±0.8**	90.97±3.46
3	84.87±5.0	30.6±2.6*	-	-	86.50±5.50	38.8±0.4	83.99±0.25	17.4±1.4**	83.80±1.17
5	83.64±8.25	19.8±0.2*	-	-	85.25±8.25	28.7±8.5	85.22±3.50	12.4±2.4**	84.55±4.85
7	79.22±5.75	11.0±4.0	-	-	80.75±5.75	13.6±2.8	78.91±5.21	9.6±3.2	81.22±3.73
9	71.13±1.50	2.8±0.0	-	-	72.50±1.50	14.0±1.6	71.85±4.50	9.5±2.7	73.77±3.97
11	64.27±2.50	2.9±2.50	-	-	65.50 2.50	10.0 0.0	69.37±6.0	8.1±4.5	66.59±2.11

* $P < 0.05$, ** = $P < 0.01$ significantly different, during PSP = significantly differnt from stage L_6, pregnancy = significantly different from stage L_2, L_6 amd L_{12}, lactating PSP = significantly different from stage L_{12}; infants in each lactating PSP stage = 9±1 pups

Table 4. Comparision of the viability of rat luteal cells in culture on day 11 of incubation among treatment group.(mean±S.E., n = 3).

stage	treatment	Cell viability (%)						
		control	hCG	PRL	hCG+PRL	$PGF_{2\alpha}$	hCG+$PGF_{2\alpha}$	PRL+$PGF_{2\alpha}$
estrous cycle	E	71.00±1.41	73.25±3.89	81.50±2.12*	78.00±2.12 NS	68.75±1.77	76.25±1.77	78.25±3.89 NS
	Di-1	74.25±7.43	82.50±7.78	85.50±12.73*	86.50±1.41*	75.25±6.72	72.75±8.13	83.00±10.61
	Di-2	67.25±3.18	68.00±7.07	80.50±2.12**	82.00±1.41**	67.50±1.41	75.00±3.54	79.50±2.12**
pregnancy	L_2	74.00±1.41	74.10±2.83	90.00±1.41**	86.50±3.54**	74.50±3.54	77.50±7.78	87.50±3.54**
	L_6	72.00±5.66	69.50±0.71	84.50±0.71**	82.00±2.83*	73.50±7.78	72.00±2.83	81.00±1.41*
	L_{12}	72.00±2.83	73.00±7.07	81.00±4.24**	83.00±4.24**	71.00±1.41	73.50±7.78	80.00±5.66**
	L_{20}	67.50±2.12	71.00±4.24	79.50±0.71**	78.50±0.71**	68.00±1.41	72.50±2.12	78.50±2.12**
PSP	L_2	70.25±3.18	70.75±7.43	80.25±0.35**	83.50±6.36**	69.00±1.41	73.75±4.60	8.100±2.12**
	L_6	68.25±1.77	67.76±1.06	77.25±3.89*	81.30±2.12**	68.75±1.77	71.25±1.77	76.50±4.24 NS
	L_{12}	74.50±3.54	73.25±3.89	88.25±0.35**	84.50±5.66*	69.25±3.89	73.50±4.24	78.25±1.06 NS
laction	L_2	67.50±4.95	73.00±7.78	81.00±1.41**	79.75±3.18**	67.50±6.36	72.75±6.72	75.00±7.07 NS
	L_{12}	70.75±3.89	75.00±2.83	82.50±7.07*	77.00±6.36 NS	66.00±15.57	72.85±6.86	78.25±11.67 NS
	L_{20}	71.00±3.54	72.50±3.54	82.00±7.07*	82.75±7.43*	66.50±5.66	71.50±0.71	76.00±4.95

* = P < 0.05, ** = P < 0.01 significantly different from the control

E = estrus, di-1 = diestrus-1, di-2 = diestrus-2 hCG = 0.5 iu/ml,

PRL = 5 µg/ml, $PGF_{2\alpha}$ 250 ng/ml

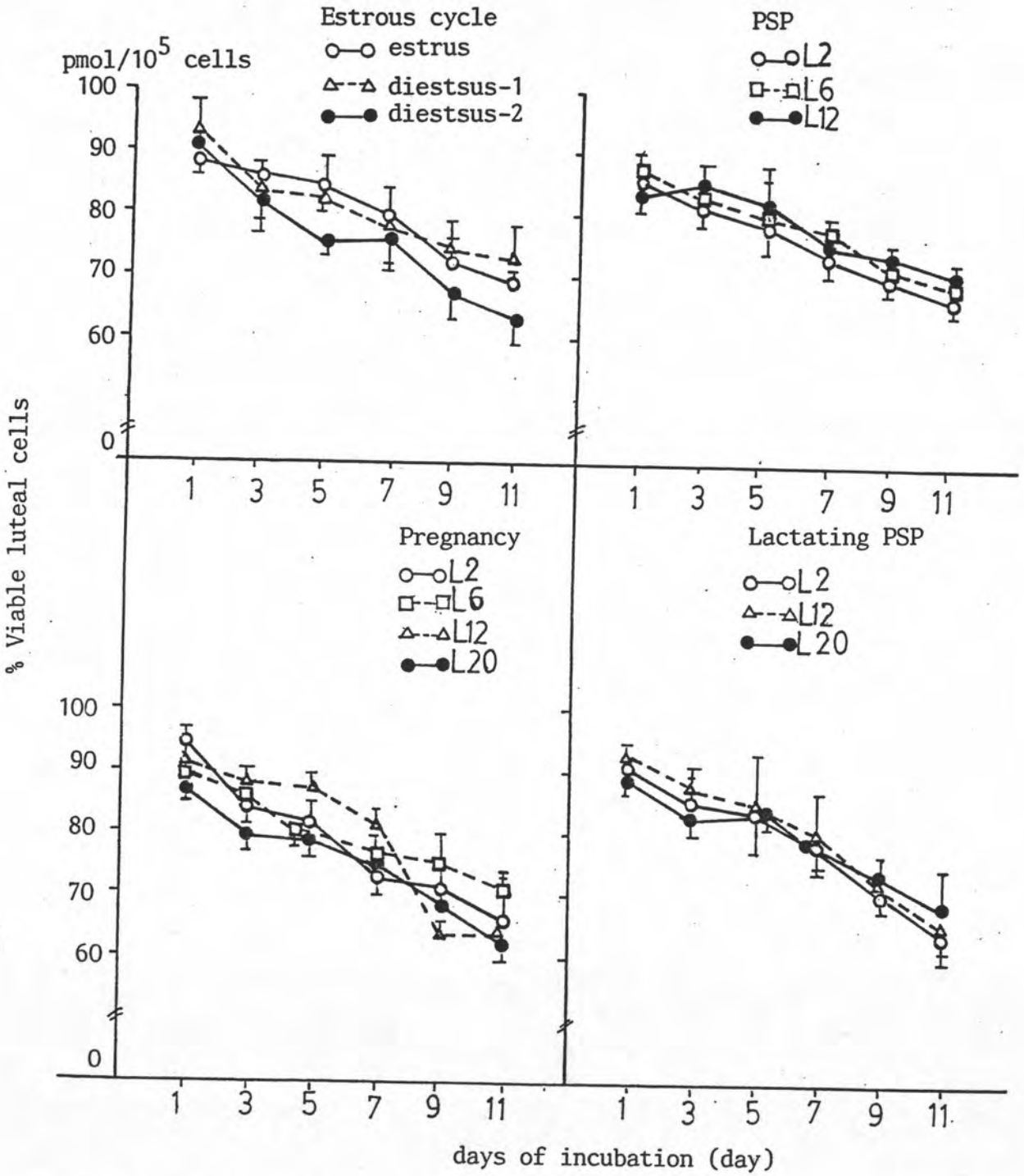

Figure 9 Viability of rat luteal cells from estrous cycle, pregnancy, PSP and lactating PSP during 11 day incubation (mean±S.E., n = 3).

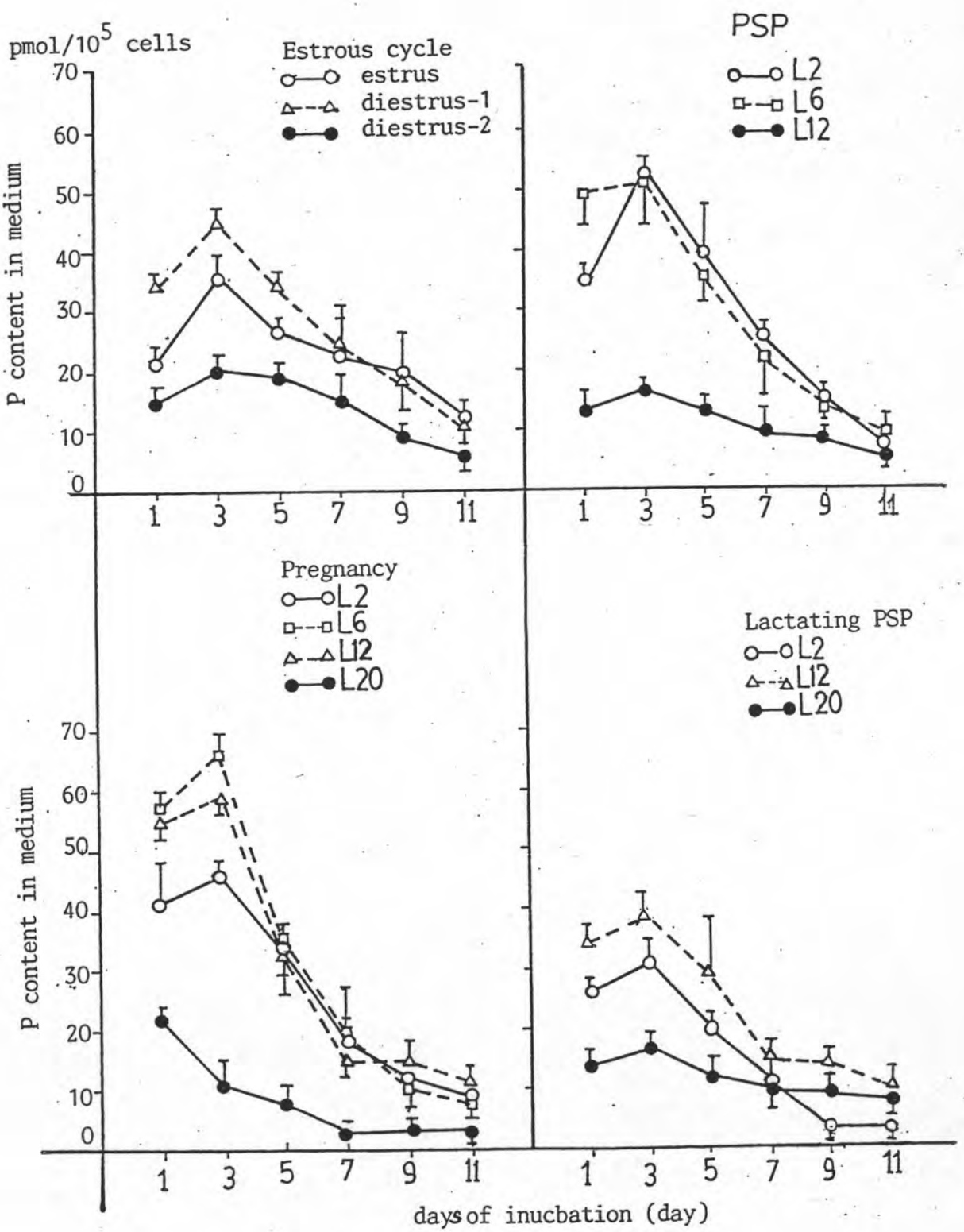

Figure 10 P secreting ability of rat luteal from estrous cycle, pregnan PSP and lactating PSP during 11 day incubation (mean±S.E., n = 3).

estrous cycle, pregnancy, PSP and lactating PSP during 11 day of incubation are shown in Fig. 10 and Tables 2 and 3.

On day 1 of incubation, P content in the culture medium of cyclic rat luteal cells from diestrus-1 was as high as 34.40±2.40 pmol/10^5 cells and was higher than P from estrus (21.0±0.6 pmol/10^5 cells, $P<0.05$) and diestrus-2 (15.4±2.0 pmol/10^5cells, $P < 0.01$). P content in the culture medium of L_6 and L_{12} pregnant rat luteal cells were 56.90±2.60 and 55.50±3.30 pmol/10^5 cells, these levels were higher than those of L_2 (41.20±6.90 pmol/10^5 cells, non significantly different), and L_{20} (22.00±1.00 pmol/10^5 cells, $P < 0.05$). P content of L_6 PSP rat luteal cells were to 49.00±5.00 pmol/10^5 cells,which was higher than P those of L_2 PSP (34.40±1.60 pmol/10^5 cells, $P < 0.01$) and L_{12} PSP (13.30±2.70 pmol/10^5 cells, $P < 0.01$). Similarly, P content of L_{12} lactating luteal cells (34.80±0.40 pmol/10^5 cells) was higher than those of L_2 (20.40±0.80 pmol/10^5 cells, $P < 0.05$) and L_{20} (14.40±0.80 pmol/10^5 cells, $P < 0.01$). P content in all culture media increased sharply on day 3 of incubation and gradually declined on the following day, but still remained detectable until day 11 of incubation.

3. Effects of hCG, PRL, $PGF_{2\alpha}$ and their combinations on P and E_2 secretion from isolated rat luteal cells.

3.1 Short term effects of hCG, PRL and $PGF_{2\alpha}$ and their combinaiton on P and E_2 secretions from isolated rat luteal cells obtained during estrous cycle.

P and E_2 production rate of isolated rat luteal cells from estrous cycle treated with hCG (0.5 iu/ml), o-PRL (5 µg/ml) and $PGF_{2\alpha}$ (250 ng/ml) hCG+PRL (0.5 iu + 5 µg/ml), hCG + $PGF_{2\alpha}$ (0.5 iu + 250 ng/ml) and PRL + $PGF_{2\alpha}$ (5 µg + 250 ng/ml) during 3 hours incubation are presented in Fig. 11.

Basal P production of luteal cell from estrus was 3.38±0.10 pmol/10^5 cells/3 hrs. Either PRL or hCG alone was capable to increase basal P secretion to 5.11±0.43 pmol/10^5 cells/3 hrs ($P < 0.05$) or 5.08±0.70 pmol/10^5 cells/3 hrs ($P < 0.05$), respectively. hCG + PRL further increased P production to the level as high as 5.61±0.07 pmol/10^5 cells/3 hrs, although this was not statistically difference from these produce by either PRL or hCG alone.

P production of luteal cells from diestrus-1 in the control was 6.35±0.55 pmol/10^5 cells/3 hrs. In the presence of hCG, it was capable to elevate basal P production up to 9.55±0.65 pmol/10^5 cells/3 hrs ($P < 0.01$) and up to 8.49±1.31 pmol/10^5 cells/3 hrs ($P < 0.01$) in PRL-treated cultures. No further increment was detected in hCG+PRL treated group.

In early diestrus-2 and late diestrus-2, basal P production of luteal cells were as low as 3.29±0.10 and 2.77±0.16 pmol/10^5 cells/3 hrs. Neither hCG nor PRL was capable to alter basal P production. Furthermore, $PGF_{2\alpha}$ did not affect basal P production of luteal cells from all stages.

None of these agents, except hCG was significantly stimulated basal increment of E_2 production from solated rat luteal cells. However, the capability to stimulate E_2 production was not observed during late diestrus-2.

3.2 Long term effects of hCG, PRL, $PGF_{2\alpha}$ and their combinations on P and E_2 secretions from isolated rat luteal cells obtained during estrous cycle.

Figure. 12 shows P and E_2 secreting ability of luteal cells from estrus, diestrus-1 and diestrus-2 treated with hCG (0.5 iu/ml) o-PRL (5 µg/ml), $PGF_{2\alpha}$ (250 ng/ml) hCG+PRL (0.5 iu + 5 µg/ml),

hCG+$PGF_{2\alpha}$ (0.5 iu + 250 ng/ml), and PRL + $PGF_{2\alpha}$ (5 µg + 250 ng/ml) during 11 days of incubation.

In control culture, P secreting ability of estrous luteal cells was initially near 21.00±0.60 pmol/10^5 cells on day 1 of incubation. This level increased markedly to 35.60±2.90 pmol/10^5 cells on day 3, then gradually declined on the following days to the level of 12.20±1.40 pmol/10^5 cells on day 11 of incubation. hCG, PRL or $PGF_{2\alpha}$ alone was capable to increase basal P secreting ability of luteal cells on dyas 3 and 5 of incubation significantly. These P levels increased to 60.80±4.80 ($P < 0.01$) and 49.50±11.10 pmol/10^5 cells ($P < 0.01$), to 52.60±1.78 ($P < 0.05$) and 46.2±6.6 pmol/10^5 cells ($P < 0.05$), or to 58.20±5.80 ($P < 0.05$) and 48.90±11.90 pmol/10^5 cells ($P < 0.01$) in the presence of hCG, PRL or $PGF_{2\alpha}$ respectively. Moreover, the presence of hCG + PRL further increased P secreting ability to 76.20±7.80 pmol/10^5 cells ($P < 0.01$) and 58.75 15.40 pmol/10^5 cells ($P < 0.05$). Basal E_2 secretion of estrous luteal cells was 0.54±0.02 pmol/10^5 cells on day 1 of incubation. This level increased to 0.62±0.04 pmol/10^5 cells on day 3, then declined gradually on the following and to 0.30±0.04 pmol/10^5 cells on day 11 of incubation. None of the agents added into the medium except hCG developed a significant ($P < 0.05$) increment of basal E_2 secretion on day 3-day 9 of incubation.

Basal P secretion of diestrous-1 luteal cells was 34.00± 2.40 pmol/10^5 cells on day 1 of incubation, the level increased sharply to 45.33±0.54 pmol/10^5 cells on day 3, then gradually declined on the following days to 11.00±3.00 pmol/10^5 cells on day 11 of incubation. hCG, PRL or $PGF_{2\alpha}$ alone was capable to elevate basal P secreting ability significantly. These P levels increased to 71.50±12.50 pmol/

10^5 cells ($P < 0.01$) and 52.60±1.80 pmol/10^5 cells ($P < 0.05$) in the presence of hCG on day 3 and day 5, being 68.70±11.30 pmol/10^5 cells ($P < 0.05$) on day 3 in PRL treated culture and being 60.70±17.10 pmol/10^5 cells ($P < 0.01$) on day 5 of incubation in $PGF_{2\alpha}$ treated groups. Furthermore, hCG + PRL further increased P secretion to the level as high as 75.60±8.40 pmol/10^5 cells on day 3, but such increment showed no statistically difference from hCG or PRL treated alone. Basal E_2 secretion of diestrous-1 luteal cells was 0.61±0.01 pmol/10^5 cells on day 1 increased slightly on day 3, declined on the next day and down to 0.40±0.05 pmol/10^5 cells on day 11 of incubation. In all cases, only the hCG treated culture developed a stimulatory effect on basal E_2 secretion significantly ($P < 0.01$) during day 3, and day 5 of incubation.

Basal P secretion of diestrous-2 luteal cells was 15.00± 2.00 pmol/10^5 cells, slightly elevated up to 20.20±2.20 pmol/10^5 cells on day 3, declined gradually on the following day and being 6.20±2.10 pmol/10^5 cells on day 11 of incubation. Basal E_2 secretion was 0.37± 0.05 pmol/10^5 cells on day 1 of incubation, decreased gradually on the next day and being 0.14±0.02 pmol/10^5 cells on day 11 of incubation. None of the agents added into the medium was capable to stimulate basal increment of P and E_2 secretion in all cases.

Figure 13 shows the total responsiveness of luteal cells from cyclic rat to hCG (0.5 iu/ml), o-PRL (5 µg/ml), $PGF_{2\alpha}$ (250 ng/ml) hCG + PRL (0.5 iu + 5 µg/ml), hCG + $PGF_{2\alpha}$ (0.5 iu + 250 ng/ml) and PRL + $PGF_{2\alpha}$ (5 µg + 250 ng/ml) on P and E_2 secretion during day 1 - day 7 of incubation.

In control group, P secretion in medium of diestrus-1 luteal cells was higher than estrous luteal cells ($P < 0.01$) and diestrous luteal cells ($P < 0.01$). Either estrous luteal cells or diestrus-1 luteal cells was capable to respond to hCG, o-PRL and $PGF_{2\alpha}$ by increasing P secretion. The presence of hCG + PRL enhanced P secretion of estrous luteal cells to the level as high as 188.10 20.90 pmol/10^5 cells/7 days ($P < 0.01$). Otherwise, diestrous-2 luteal cells were refractory to all agents absolutely. Futhermore, luteal cells of cyclic rats secreted E_2 autonomously until the morning of diestrus-2 and only hCG was capable to increase basal E_2 secretion of luteal cells until the morning diestrus-1.

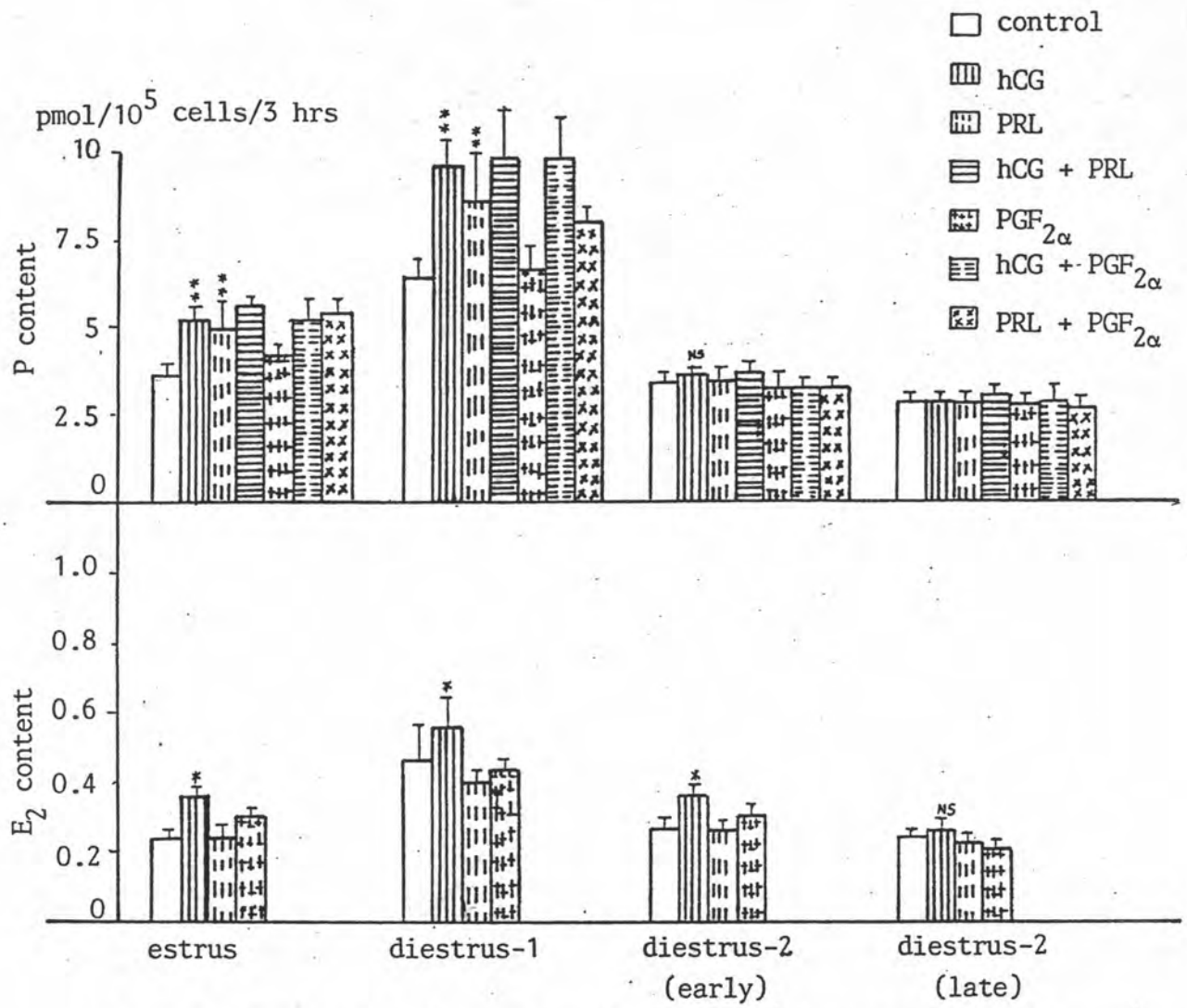

Figure 11 Short term effects of hCG, PRL and $PGF_{2\alpha}$ on in vitro P and E_2 production of rat luteal cells of estrous cycle during 3 hours incubation (mean ± S.E., n = 3).

(** = $P < 0.01$, * = $P < 0.05$ significantly different, NS=non significantly different from the control).

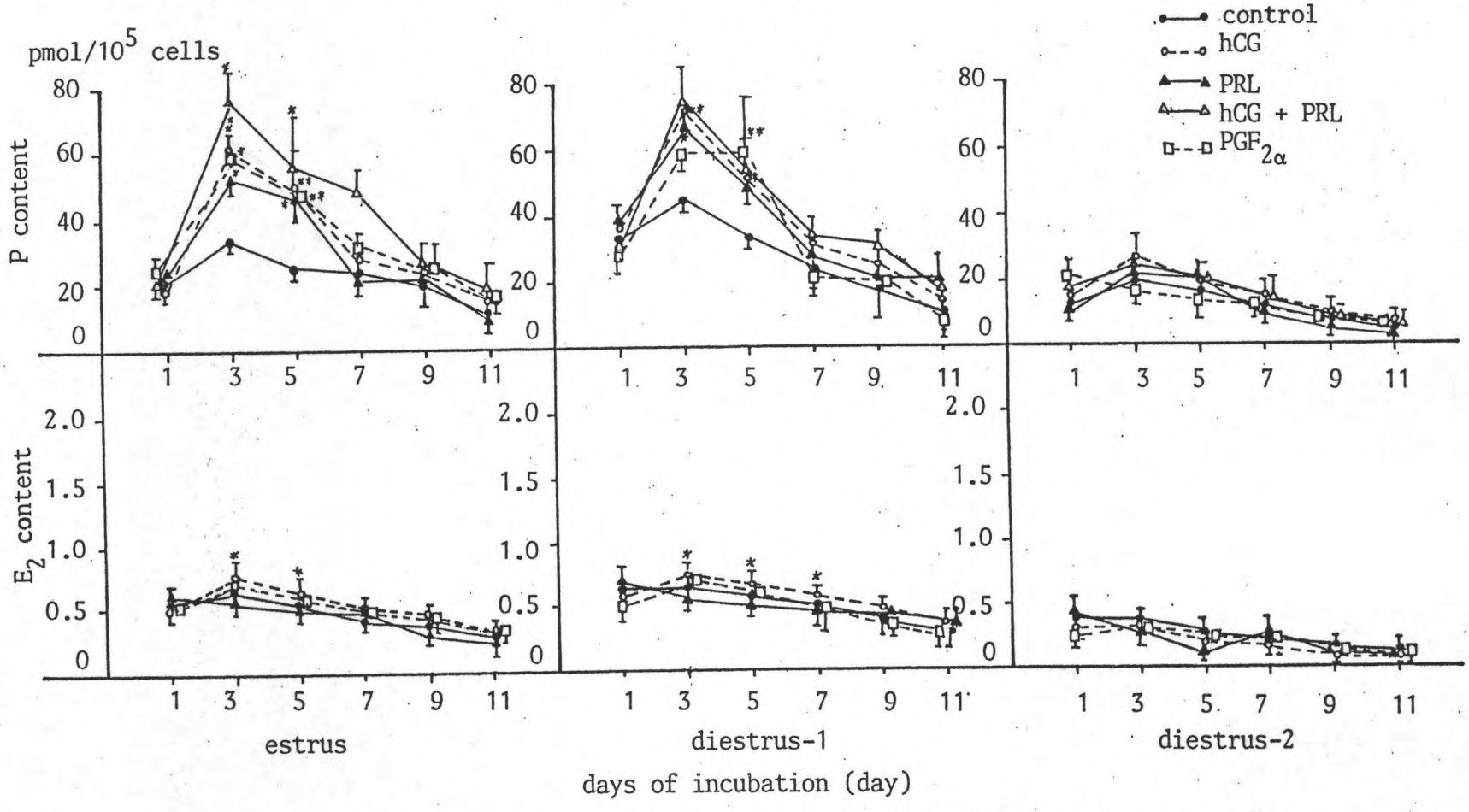

Figure 12 Long term effect of hCG, PRL and $PGF_{2\alpha}$ on in vitro stimulation of P and E_2 secretion of rat luteal cells from estrous cycle during 11 day incubation (mean ± S.E., n = 3).
(** = $P < 0.01$, * = $P < 0.05$ significantly different from the control.)

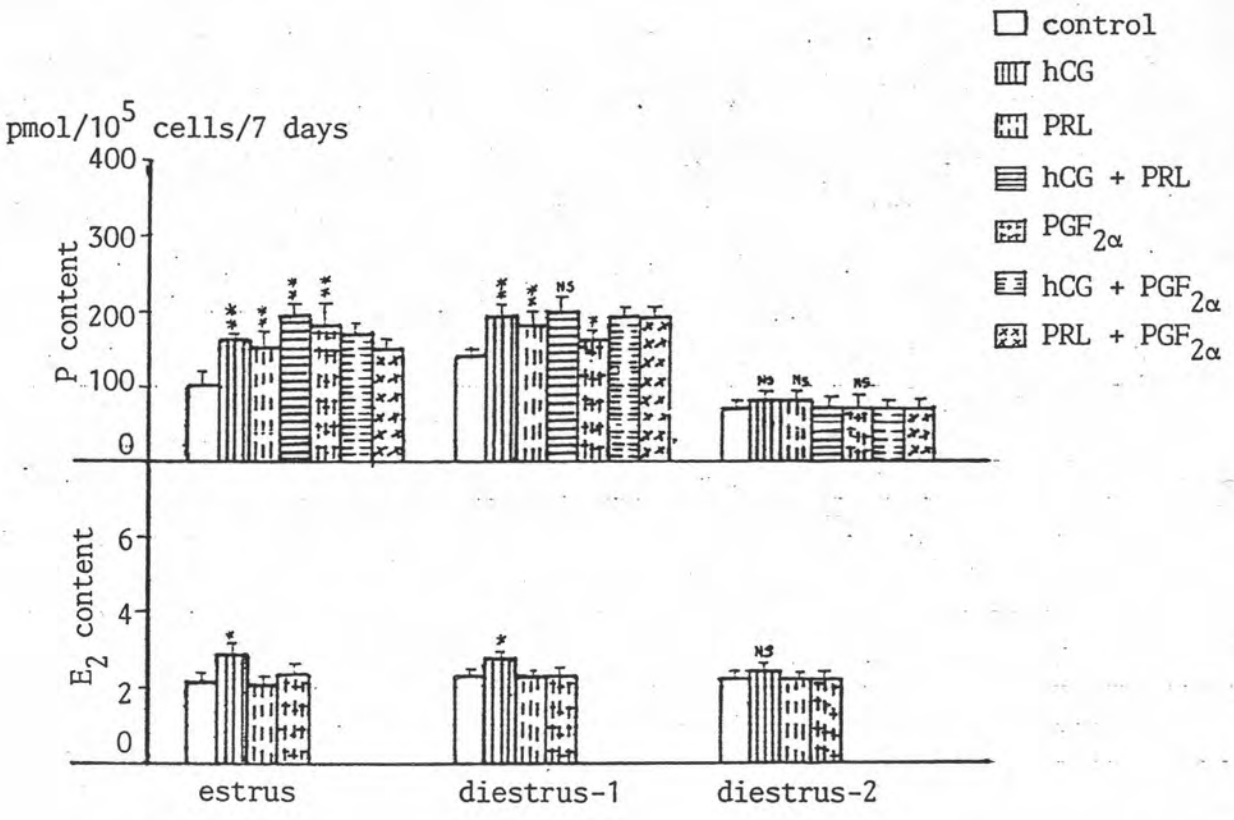

Figure 13 Responsiveness of luteal cells from cyclic rat to hCG, PRL and $PGF_{2\alpha}$ on P and E_2 secretion in 7 day incubation (mean±S.E., n = 3).
(** = $P < 0.01$, * = $P < 0.05$ significantly different from the control, NS = non-significantly different).

3.3 Short term effects of hCG, PRL and $PGF_{2\alpha}$ on P and E_2 secretion from isolated rat luteal cells obtained during pregnancy. PSP and lactating PSP.

Figure. 14 shows P and E_2 production rate of isolated rat luteal cells from pregnancy, PSP and lactating PSP rats which were treated with hCG (0.5 iu/ml), o-PRL (5 μg/ml), $PGF_{2\alpha}$(250 ng/ml),hCG + PRL (0.5 iu + 5 μg/ml),hCG + $PGF_{2\alpha}$ (0.5 iu + 250 ng/ml) and PRL + $PGF_{2\alpha}$ (5 μg + 250 ng/ml) during 3 hours incubation.

Basal P production of luteal cells from pregnant stage L_2 was 10.31±1.89 pmol/10^5 cells/3 hrs. Either hCG or PRL alone was capble to elevate basal P production to 23.92±4.69 pmol/10^5 cells/3 hrs ($P < 0.01$) or 19.63±4.98 pmol/10^5 cells/3 hrs ($P < 0.05$) respectively. The presence of hCG and PRL further increased P production to the level as high as 32.42±6.82 pmol/10^5 cells/3 hrs ($P < 0.05$). Basal P production of luteal cells from pregnant stage L_6 was 18.44± 3.50 pmol/10^5 cells/3 hrs. hCG or PRL alone raised the basal P production to 38.49±8.01 pmol/10^5 cells/3 hrs ($P < 0.01$) or 29.64±7.49 pmol/10^5 cells/3 hrs ($P < 0.05$) respectively. Furthermore, the presence of hCG + PRL further enhanced P production ability to the level of 44.87±7.74 pmol/10^5 cells ($P < 0.05$). In pregnant stage L_{12}, basal P production rate of luteal cells was 14.66±0.16 pmol/10^5 cells/3 hrs. hCG or PRL alone was significantly capable enhanced the basal P production to 29.07±0.24 pmol/10^5 cells/3 hrs ($P < 0.01$) or 22.97±1.74 pmol/10^5 cells/3 hrs ($P < 0.05$) respectively. However hCG + PRL showed to enhance P production to the level as high as 33.53±3.27 pmol/10^5 cells/3 hrs, but such increment showed no statistical difference. All cases of luteal cells from pregnant stage L_{20}, none of these agents

significantly stimulated increment of the basal P production (3.55±0.15 pmol/10^5 cells/3 hrs). Furthermore, the presence of $PGF_{2\alpha}$ did not showed alternation on basal P production rate of luteal cells from all stages of pregnancy.

E_2 production rate of pregnant rat luteal cells showed an increment which corresponed to the progressing stages. These E_2 production were 0.32±0.01, 0.60±0.02, 0.68±0.07 and 0.71±0.08 pmol/10^5 cells/3 hrs from pregnant rat stages L_2, L_6, L_{12} and L_{20} respectively. None of the agents added into the medium, except hCG significantly increased the basal E_2 production of luteal cells from stages L_2, L_6 and L_{12} ($P < 0.05$). Otherwhile such increment of basal E_2 production of luteal cells from stage L_{20} showed nonstatistically different.

Basal P production of luteal cells from PSP rat stage L_2 was 5.66±0.77 pmol/10^5 cells/3 hrs. hCG significantly elevated the basal P secretion to 12.51±0.45 pmol/10^5 cells/3 hrs ($P < 0.05$), while PRL resulted in slightly elevated P produciton to 8.36±1.00 pmol/10^5 cells/3 hrs ($P < 0.05$). Furthermore, a synergistic effect was found in hCG+PRL treated groups ($P < 0.05$). Basal P production of luteal cells from PSP stage L_6 was 12.29±0.01 pmol/10^5 cells/3 hrs. Either hCG or PRL significantly increased basal P secretion to 21.79± 1.51 ($P < 0.01$) or 20.49 5.81 ($P < 0.05$) pmol/10^5 cells/3 hrs, respectively. No further increment was detected in hCG+PRL treated group. In all cases of luteal cells from PSP stage L_{12} did not showed alternation of P secreting ability and low levels of P production were detected. Furthermore, the presence of $PGF_{2\alpha}$ in the medium showed a slight effect on basal P production but was not statistical different to other groups. None of these agents added into the medium, except hCG

significantly elevated basal E_2 production of luteal cells from PSP stage L_2 ($P < 0.05$) and L_6 ($P < 0.01$) but failed to alter basal E_2 production of luteal cells from PSP stage L_{12}.

Basal P production of luteal cells from lactating stage L_2 were 4.94±0.02 nmol/10^5 cells/3 hrs. Either hCG or PRL significantly enhanced basal P production to 7.46±0.21 ($P < 0.05$) or 6.29 0.04 pmol/10^5 cells/3 hrs ($P < 0.05$), respectively. However a further increase was not observed in hCG+PRL treated group. In lactating stage L_{12}, the basal P production was 6.41±0.25 pmol/10^5 cells/3 hrs. The presence of hCG or PRL alone elevated basal P production to 16.30±2.10 ($P < 0.01$) and 10.14±0.74 pmol/10^5 cells/3 hrs ($P < 0.01$), respectively. However, no additive effect was detected in the hCG+PRL treated group. Incubation of lactating luteal cells from stage L_{20}, basal P production was 4.25±0.45 pmol/10^5 cells/3 hrs. None of these agents applied into the culture, except hCG significantly increased basal P production to 7.35±0.45 pmol/10^5 cells/3 hrs ($P < 0.01$). Otherwise, the presence of $PGF_{2\alpha}$ was uncapable to altering basal P production of luteal cells of lactating rats from all stages but exhibited a significant inhibitory effect on hCG stimulated P production of luteal cell from stage L_{20}. Basal E_2 production of lactating luteal cells from stages L_2, L_{12} and L_{20} were 0.22±0.01, 0.41±0.04 and 0.51±0.02 pmol/10^5 cells/3 hrs, respectively. None of these agents added into the medium, except hCG significantly enhanced basal E_2 production up to 0.37±0.05 ($P < 0.01$), 0.57±0.03 ($P < 0.01$) and 0.64±0.03 ($P < 0.05$) pmol/10^5 cells/3 hrs of luteal cells from stages L_2, L_{12} and L_{20} respectively.

Similarly, the responsiveness of luteal cells from estrous cycle, pregnancy, PSP and lactating PSP were also shown by presenting in percent increment of P and E_2 secretion (Table 5).

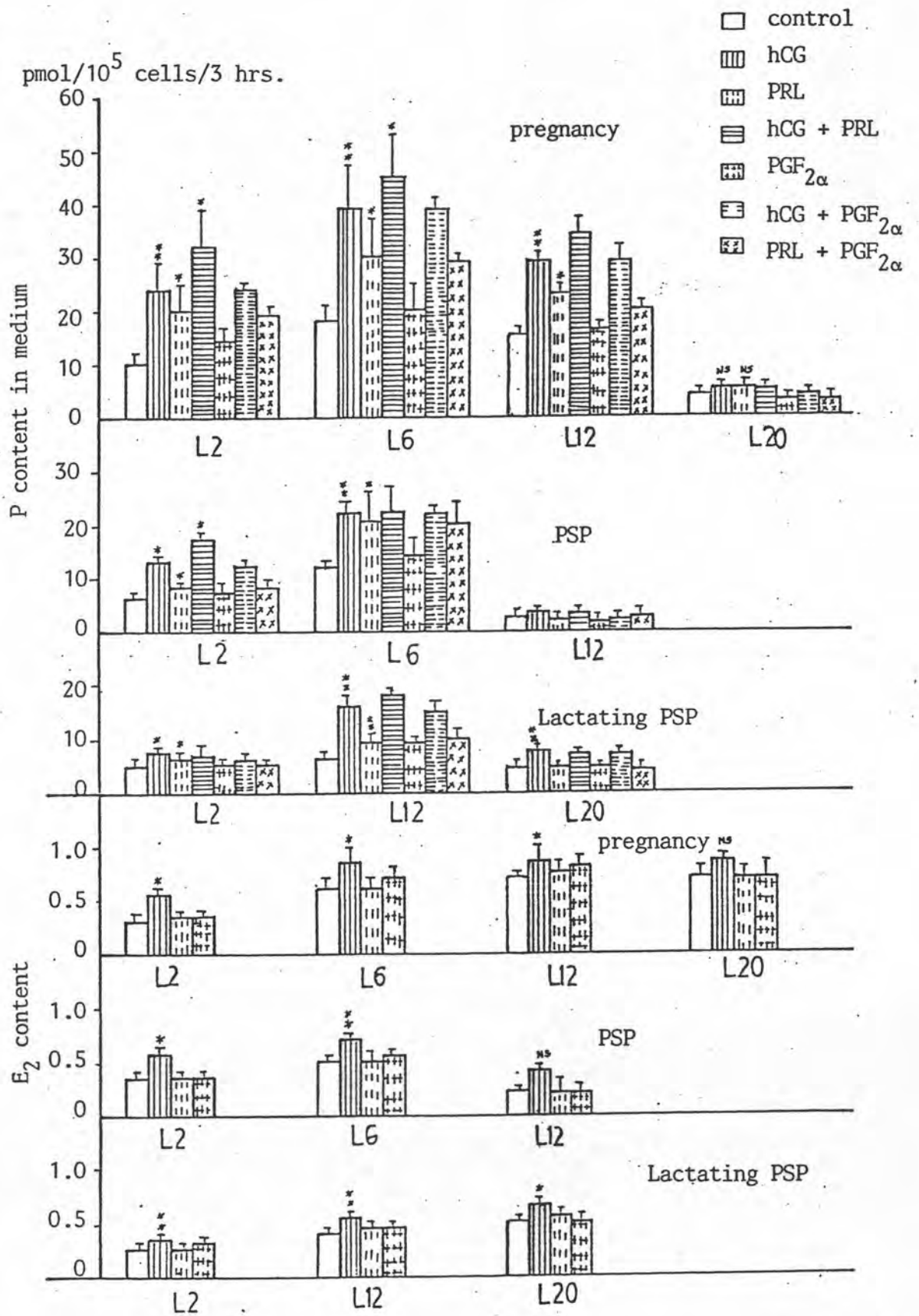

Figure 14 Short term effects of hCG, PRL and $PGF_{2\alpha}$ on in vitro P and E_2 production from rat luteal cells of pregnancy, PSP and lactating PSP during 3 hour incubation. (mean±S.E., n = 3) (**=P<0.01, *=P<0.05 significantly different, NS=non significantly different)

Table.5 Effects of hCG, o-PRL and $PGF_{2\alpha}$ on % increment of P and E_2 of rat luteal cells during 3 hour incubation (mean S.E., n = 3). * = $P < 0.05$, ** = $P < 0.01$, NS.= non-significantly different from the control.)

Treatments / Stages	pmol/10^5 cells control	% increment of P			pmol/10^5 cells control	% increment of E_2		
		hCG	o-PRL	$PGF_{2\alpha}$		hCG	o-PRL	$PGF_{2\alpha}$
Estrous cycle								
estrus (E)	3.4±0.1	50.9±11.6**	76.5±15.4*	21.9±8.6*	0.23±0.0	54.45±13.6**	3.09±1.1	10.43±1.8
diestrus-1(Di-1)	6.4±0.7	52.4±23.5**	36.5±32.4*	6.3±2.1NS	0.47±0.1	34.32±20.1*	-0.12±0.0	8.99±4.1
diestrus-2(Di-2) (8.30-9.00)	3.3±0.1	9.9±1.0NS	-7.4±2.4NS	-8.2±3.2NS	0.26±0.0	60.79±3.9**	3.92±1.8	13.40±0.6
diestrus-2(Di-2) (15.30-16.00)	2.8±0.2	2.6±1.2NS	-3.2±1.0NS	-0.6±0.3NS	0.23±0.0	50.82±1.1**	-2.0±1.1	-13.38±5.6
pregnancy								
L_2	10.3±1.8	131.3±4.4**	87.7±1.3**	40.9±6.9*	0.31±0.0	48.30±16.5*	12.46±5.8	12.77±0.8
L_6	18.4±3.5	107.9±5.6**	32.1±1.5*	8.2±1.1	0.60±0.1	42.86±0.9*	8.25±4.1	2.39±1.1
L_{12}	14.7±0.2	98.2±5.4**	56.7±10.8**	31.3±8.6*	0.48±0.1	44.31±7.7*	6.41±1.7	6.31±2.7
L_{20}	3.6±0.2	38.2±4.2*	30.4±22.5*	-10.9±1.2	0.41±0.1	31.68±15.1*	-3.20±1.9	-8.86±4.1
PSP								
L_2	5.7±0.7	123.6±19.6**	29.9±2.3*	50.7±9.7*	0.34±0.0	67.90±35.4**	-2.88±1.0	8.98±7.6
L_6	12.3±0.0	77.6±12.2**	66.7±47.1*	33.3±31.7*	0.48±0.0	29.13±22.2**	9.56±1.4	12.21±8.1
L_{12}	2.1±0.4	50.9±28.8*	20.1±10.5NS	0.7±0.0NS	0.21±0.0	79.73±4.9**	-9.61±5.1	9.57±5.1
Lactating PSP								
L_2	4.9±0.2	31.3±9.4*	7.3±3.4NS	2.9±0.9NS	0.22±0.0	67.47±23.0**	15.35±13.5	4.56±1.2
L_{12}	6.4±0.2	53.8±22.8*	43.4±17.0**	39.2±11.4*	0.41±0.0	36.19±5.0*	13.23±9.0	6.04±3.0
L_{20}	4.3±0.4	76.0±29.2**	4.9±0.9NS	2.2±0.9NS	0.31±0.0	45.46±21.2*	7.02±5.9	-5.23±1.9

3.4 Long term effects of hCG, PRL and $PGF_{2\alpha}$ on P and E_2 secretion from isolated rat luteal cells obtained during pregnancy.

Figure 15 shows P and E_2 secreting ability of luteal cells from pregnant rat stages L_2, L_6, L_{12} and L_{20} when treated with hCG (0.5 iu/ml), PRL (5 µg/ml) and $PGF_{2\alpha}$ (250 ng/ml) hCG+PRL (0.5 iu + 5 µg/ml), hCG+$PGF_{2\alpha}$ (0.5 iu + 250 ng/ml), PRL+$PGF_{2\alpha}$ (5 µg + 250 ng/ml) during 11 day incubation.

In control cultures, P secretion of luteal cells from pregnant rat stage L_2 was 41.20±6.90 pmol/10^5 cells on day 1, slightly increased to 45.90±1.30 pmol/10^5 cells on day 3 and declined gradually to 9.00±1.20 pmol/10^5 cells on day 11 of incubation. Either hCG, PRL or $PGF_{2\alpha}$ significantly increased basal P secretion to 111.80±4.20 pmol/10^5 cells ($P < 0.01$), 56.00±4.80 pmol/10^5 cells ($P < 0.05$) or 130.80±22.00 pmol/10^5 cells ($P < 0.01$), respectively, However, the presence of hCG+PRL resulted in enhancing P secretion to 130.80±22.00 ($P < 0.01$) and 70.20±1.00 pmol/10^5 cells ($P < 0.05$) on day 3 and day 5 of incubation. No additive effect was observed in hCG+$PGF_{2\alpha}$ or PRL+$PGF_{2\alpha}$ treated groups. Basal E_2 secretion was 0.59±0.07 pmol/10^5 cells on day 1, slightly increased to 0.69±0.05 pmol/10^5 cells on day 3 of incubation and gradually declined on the following day. None of these agents added into the medium, except hCG developed a significantly increment of basal E_2 secretion on day 3 - day 11 of incubation.

Basal P secretion of luteal cells from pregnant rat stage L_6 was as high as 56.90±2.30 pmol/10^5 cells on day 1, and further slightly increase to 66.40±2.80 pmol/10^5 cells on day 3, gradually declined on the following day and was 7.50±2.70 pmol/10^5 cells at day 11 of incubation. Either hCG, PRL or $PGF_{2\alpha}$ alone significantly elevated basal P secretion

up to 126.00±28.90 pmol/10^5 cells ($P < 0.01$), 89.70±5.10 ($P < 0.05$) or 86.10±14.80 pmol/10^5 cells ($P < 0.05$) on day 3 after incubation, respectively. Similarly, the presence of hCG+PRL further enhanced P secretion to 191.61±61.50 pmol/10^5 cells ($P < 0.01$). Otherwise, no additive effect was detected in hCG+$PGF_{2\alpha}$ or PRL+$PGF_{2\alpha}$ treated groups. Basal E_2 secretion was 0.83±0.04 pmol/10^5 cells on day 1, gradually declined on the following day and to 0.23±0.01 pmol/10^5 cells on day 11 of incubation. In treated cultures, the presence of hCG caused a significant increment of E_2 secretion on day 3 ($P < 0.01$) and day 5 ($P < 0.05$) after incubation in all cases.

In control cultures, initial P secretion of luteal cells from pregnant rat stage L_{12} were 55.50±3.30 pmol/10^5 cells on day 1, slightly increased to 59.40±0.60 pmol/10^5 cells on day 3 and led to a diminution on the following day of incubation.

Either hCG or PRL alone significantly elevated P secretion to 127.00±7.00 pmol/10^5 cells ($P < 0.01$) or 107.00±13.00 pmol/10^5 cells ($P < 0.01$) on day 3 of incubation respectively. Meanwhile, the presence of $PGF_{2\alpha}$ showed a non significantly different increment of basal P secretion to 75.30±10.80 pmol/10^5 cells. The presence of hCG+PRL further enhanced P production to the level as high as 138.70±10.70 pmol/10^5 cells but such increment did not statistically different. Basal E_2 secreiton was 1.68±0.23 pmol/10^5 cells on day 1, gradually declined on the following day and was 0.63±0.05 pmol/10^5 cells on day 11 of incubation. None of these agents added into the medium, except hCG significantly increased basal E_2 secretion on day 3 ($P < 0.01$) and day 5 ($P < 0.05$) of incubation.

Basal P secretion of luteal cells from pregnant rat stage L_{20} was 22.20±1.00 pmol/10^5 cells on day 1, gradually declined on the

following day and to 2.70±0.90 pmol/10^5 cells on day 11 of incubation. hCG or PRL alone significantly elevated P secretion to 36.00±2.40 pmol/10^5 cells ($P < 0.01$) or 23.50±8.50 pmol/10^5 cells ($P < 0.01$) on day 3 of incubation respectively. No further increment was observed in hCG+PRL treated group. Otherwise in the presence of hCG+$PGF_{2\alpha}$ showed a significant inhibitory effect on hCG stimulated P secretion on day 3 of inucation 21.80±1.60 pmol/10^5 cells, ($P < 0.01$). In control culture, E_2 secretion was 1.92±0.16 pmol/10^5 cells on day 1, sharply declined on the following day and down to 0.51±0.03 pmol/10^5 cells at the end of incubation. None of these agents added into the medium resulted in altering the basal E_2 secretion in all cases.

3.5 Long term effects of hCG, PRL and $PGF_{2\alpha}$ on P and E_2 secretion from isolated rat luteal cells obtained during PSP.

Figure. 16 shows P and E_2 secreting ability of CL cells from PSP rats stages L_2, L_6 and L_{12} which were treated with hCG (0.5 iu/ml), PRL (5 µg/ml) and $PGF_{2\alpha}$ (250 ng/ml), hCG+PRL (0.5 iu + 5 µg/ml), hCG+$PGF_{2\alpha}$ (0.5 iu + 250 ng/ml) and PRL+$PGF_{2\alpha}$(5 µg + 250 ng/ml) during 11 day incubaiton.

Basal P secreting ability of luteal cells from PSP stage L_2 was 34.40±1.60 pmol/10^5 cells on day 1 of incubation. These P levels elevated to 53.40±0.60 pmol/10^5 cells on day 3, then gradually decreased from 39.30±7.30 pmol/10^5 cells on day 5 to 7.40±2.70 pmol/10^5 cells on day 11 of incubaiton. hCG, PRL or $PGF_{2\alpha}$ alone significantly increased P secretion. These P levels increased to 98.20±3.80 ($P < 0.01$) and 61.80±16.60 pmol/10^5 cells ($P < 0.01$ in hCG-treated group on day 3 and 5 of incubation, and being 94.40 8.40 pmol/10^5 cells ($P < 0.01$) on day 3 of incubation when added PRL into the medium, and being 74.80±17.20 ($P < 0.05$) and 64.20±15.80 pmol/10^5 cells ($P < 0.01$)

in the presence of PGF_2 on day 3 and 5 of incubaiton, respectively. Furthermore, hCG+PRL showed an additive effect to enhance P secretion up to 83.80±17.40 pmol/10^5 cells ($P < 0.05$) on day 5 of incubation. In control culture, E_2 secretion was 0.08±0.04 pmol/10^5 cells on day 1. These levels increased to 0.88±0.12 pmol/10^5 cells on day 3, gradually declined on the following day and to 0.37±0.05 pmol/10^5 cells on day 11 of incubation. None of these agents added into the medium, except hCG significantly increased basal E_2 secretion on day 3 after incubation (1.09±0.19 pmol/10^5 cells, $P < 0.05$).

In control culture, initial P secreting ability of luteal cells from PSP stage L_6 was 49.00±5.00 pmol/10^5 cells on day 1 of incubation, slightly increased to 52.40±7.60 pmol/10^5 cells on day 3 and led to diminution on the following day. Either hCG, PRL or $PGF_{2\alpha}$ significantly elevated basal P secretion of luteal cells. These levels increased to 101.00±3.00 ($P < 0.01$) and 68.00±23.60 pmol/10^5 cells ($P < 0.05$) in hCG-treated group, up to 92.00±20.00 ($P < 0.01$) and 64.80±15.20 pmol/10^5 cells ($P < 0.05$) in PRL-treated group and up to 78.40±9.60 and 67.40±1.40 pmol/10^5 cells ($P < 0.05$) in $PGF_{2\alpha}$ treated group on day 3 and 5 of incubaiton, respectively. hCG+PRL further increased P secretion to the level as high as 120.90±4.90 pmol/10^5 cells on day 3, but such increment showed no statistical difference from either hCG or PRL alone. Furthermore, an additive effect was not found either hCG+$PGF_{2\alpha}$ or PRL+$PGF_{2\alpha}$ treated group. None of these agents added into the medium, except hCG significantly enhanced the basal E_2 secreting ability on day 3 and day 5 of incubation.

In the control, P secretion of luteal cells from PSP stage L_{12} was as low as 13.30±2.70 pmol/10^5 cells on day 1, slightly elevated to 15.70±0.50 pmol/10^5 cells on day 3 and gradually declined to 4.60±

1.00 pmol/10^5 cells at the end of incubation. None of these agents added into medium, except hCG significantly raised E_2 secreting ability on day 3 of incubation (18.00±0.40 pmol/10^5 cells, $P < 0.05$), Meanwhile the presence of $PGF_{2\alpha}$ showed a significant inhibitory effect on hCG-stimulated P secretion (15.90±1.70 pmol/10^5 cells, $P < 0.05$).

None of thse agents showed a significant alternation of basal E_2 secretion and this low level of E_2 secretion of luteal cells was detected in all cases.

3.6 Long term effects of hCG, PRL and $PGF_{2\alpha}$ on P and E_2 secretion from isolated rat luteal cells obtained during lactating PSP.

Figure 17 shows P and E_2 secreting ability of luteal cells from lactating PSP rats stages L_2, L_{12} and L_{20} which were treated with (0.5 iu/ml), PRL (5 µg/ml) and $PGF_{2\alpha}$ (250 ng/ml) hCG+PRL (0.5 iu + 5 µg/ml), hCG+$PGF_{2\alpha}$ (0.5 iu + 250 ng/ml) and PRL+$PGF_{2\alpha}$ (5 µg + 250 ng/ml) during 11 day incubation.

In control culture, P secretion of luteal cells from lactating PSP stage L_2 was 26.40±0.80 pmol/10^5 cells on day 1, slightly increased up to 30.60±2.60 pmol/10^5 cells on day 3, sharply declined on the following day and drop to 2.90 0.50 pmol/10^5 cells on day 11 of incubation. Either hCG or PRL alone significantly increased basal P secretion. These P secretions were 39.40±4.20 ($P < 0.05$), 27.20±0.20 ($P < 0.05$) and 25.70±5.70 ($P < 0.01$) pmol/10^5 cells in the presence of hCG, and were 37.10±3.30 ($P < 0.05$), 26.10±3.40 ($P < 0.05$) and 20.80±2.80 ($P < 0.01$) pmol/10^5 cells in PRL-treated cultures on day 3, 5 and 7 after incubation, respectively. However no additive effect was observed in hCG+PRL treated cultures. Similarly the presence of $PGF_{2\alpha}$ was capable to increase basal P secreting ability of the level

33.70±4.80 pmol/10^5 cells, but such increment did not statistically different.

Basal E_2 secretion was 0.44±0.01 pmol/10^5 cells on day 1, gradually increased on day 3 and day 5, slightly declined on the following day and to 0.32±0.02 pmol/10^5 cells on day 11 of incubation None of these agents added into the medium, except hCG showed a significant increase on the basal E_2 secretion on day 3 of incubation.

In control culture, the basal P secretion of luteal cells from lactating PSP stage L_{12} was 34.80±0.40 pmol/10^5 cells on day 1, slightly increased up to 38.50±0.40 pmol/10^5 cells on day 3, declined on the following day and to 10.00±0.01 pmol/10^5 cells on day 11 of incubation. Either hCG, PRL or $PGF_{2\alpha}$ significantly to increased basal P secretion of luteal cells on day 3, 5 and day 7 of incubation. The presence of hCG increased basal P secretion to 54.00±2.40 ($P < 0.01$), 47.20±0.80 ($P < 0.01$) and 22.70±1.70 ($P < 0.05$) pmol/10^5 cells respectively, otherwhile PRL raised basal P secretion up to 45.30±3.30 ($P < 0.05$) pmol/01^5 cells on day 5 of incubation The presence of $PGF_{2\alpha}$ caused an increment of basal P secretion to 48.10 4.90 ($P < 0.05$) pmol/10^5 cells on day 3 and being 42.00±0.40 ($P < 0.01$) pmol/10^5 cells on day 5 after incubation. No additive effects were observed in hCG+ $PGF_{2\alpha}$ or PRL+$PGF_{2\alpha}$ treated groups. Basal E_2 secretion was 0.57±0.03 pmol/10^5 cells on day 1, gradually increased to 0.61±0.05 pmol/10^5 cells on day 3, led to a diminution on the following day and to 0.28± 0.04 pmol/10^5 cells on day 11 of incubation. None of these agents added into the meddium, except hCG significantly elevated basal E_2 secretion throughout incubation peroid.

Basal P secretion of luteal cells from lactating PSP stage L_{20} was 14.40±0.80 pmol/10^5 cells on day 1 and gradually declined

on the following day until the end of incubation. None of these agents, except hCG was capable to increased basal P secretion to 24.80±0.61 pmol/10^5 cells ($P < 0.01$) on day 3 of incubation. Otherwhile the presence of $PGF_{2\alpha}$ showed a significant inhibitory effect on hCG-stimulate P secretion to 19.50±5.00 pmol/10^5 cells ($P < 0.05$).

In control culture, basal E_2 secretion was 0.77±0.05 pmol/10^5 cells on day 1 of incubation, sharply decreased on the following day and dropped to 0.36±0.05 pmol/10^5 cells on day 11 of incubation. The presence of hCG, PRL or $PGF_{2\alpha}$, only hCG significantly increased basal E_2 secretion.

Figure 18 shows the total responsiveness of rat luteal cells from pregnancy, PSP and lactating PSP to hCG (0.05 iu/ml), PRL (5 µg/ml) and $PGF_{2\alpha}$ (250 ng/ml) hCG+PRL (0.5 iu + 5 µg/ml), hCG+$PGF_{2\alpha}$ (0.5 iu + 250 ng/ml) and PRL+$PGF_{2\alpha}$ (5 µg + 250 ng/ml) on P and E_2 secretion during day 1 - day 7 of incubation.

In pregnancy, P secretion of rat luteal cells from stage L_6 (173±6.4 pmol/10^5 cells/7 days) and stage L_{12} (161.8±14.7 pmol/10^5 cells/7 days) were no statistical difference and were higher than P secretion of luteal cells from stage L_2 (139.1±15.7 pmol/10^5 cells/7 days $P < 0.01$) and L_{20} (43.6±7.1 pmol/10^5 cells/7 days $P < 0.01$). The presence of hCG increased basal P secretion of luteal cells from all stages of pregnancy. Similarly, PRL also increased basal P secretion of luteal cells from stage L_2, L_6 and L_{12}. While $PGF_{2\alpha}$ increased basal P secretion of luteal cells from stage L_2 and L_{12} only and inhibited on hCG-stimulated P secretion of luteal cells from stage L_{20}. The presence of hCG, PRL or $PGF_{2\alpha}$, only hCG significantly increased E_2 secretion of CL from all stages except stage L_{20}.

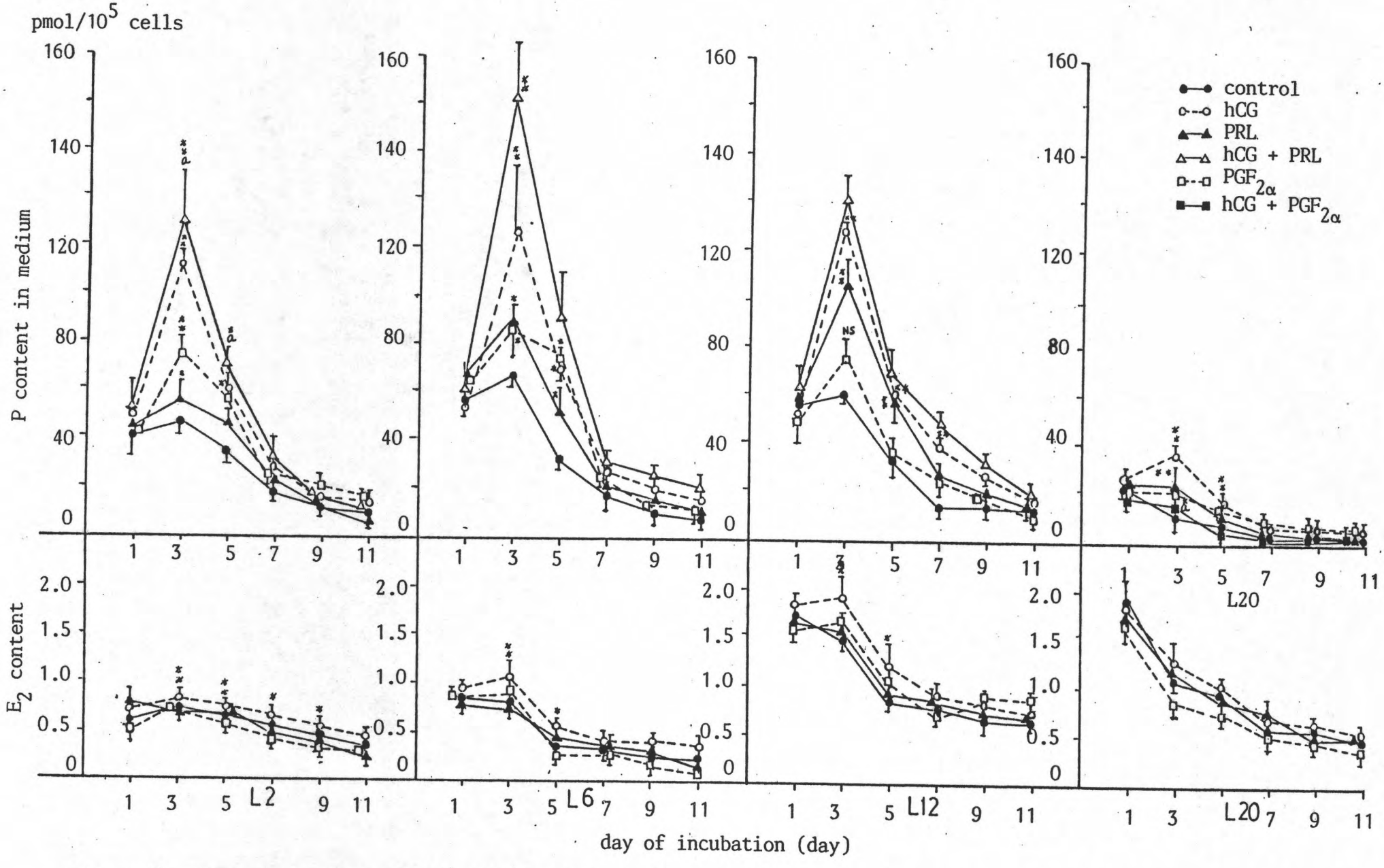

Figure 15 Long term effect of hCG, PRL and $PGF_{2\alpha}$ on in vitro stimulation of P and E_2 secretion of rat luteal cells from pregnancy during 11 day incubation (mean ± S.E., n = 3).
(** = P<0.01, * = P<0.05 significantly different from the control, a* = inhibition on hCG-stimulated P secretion)

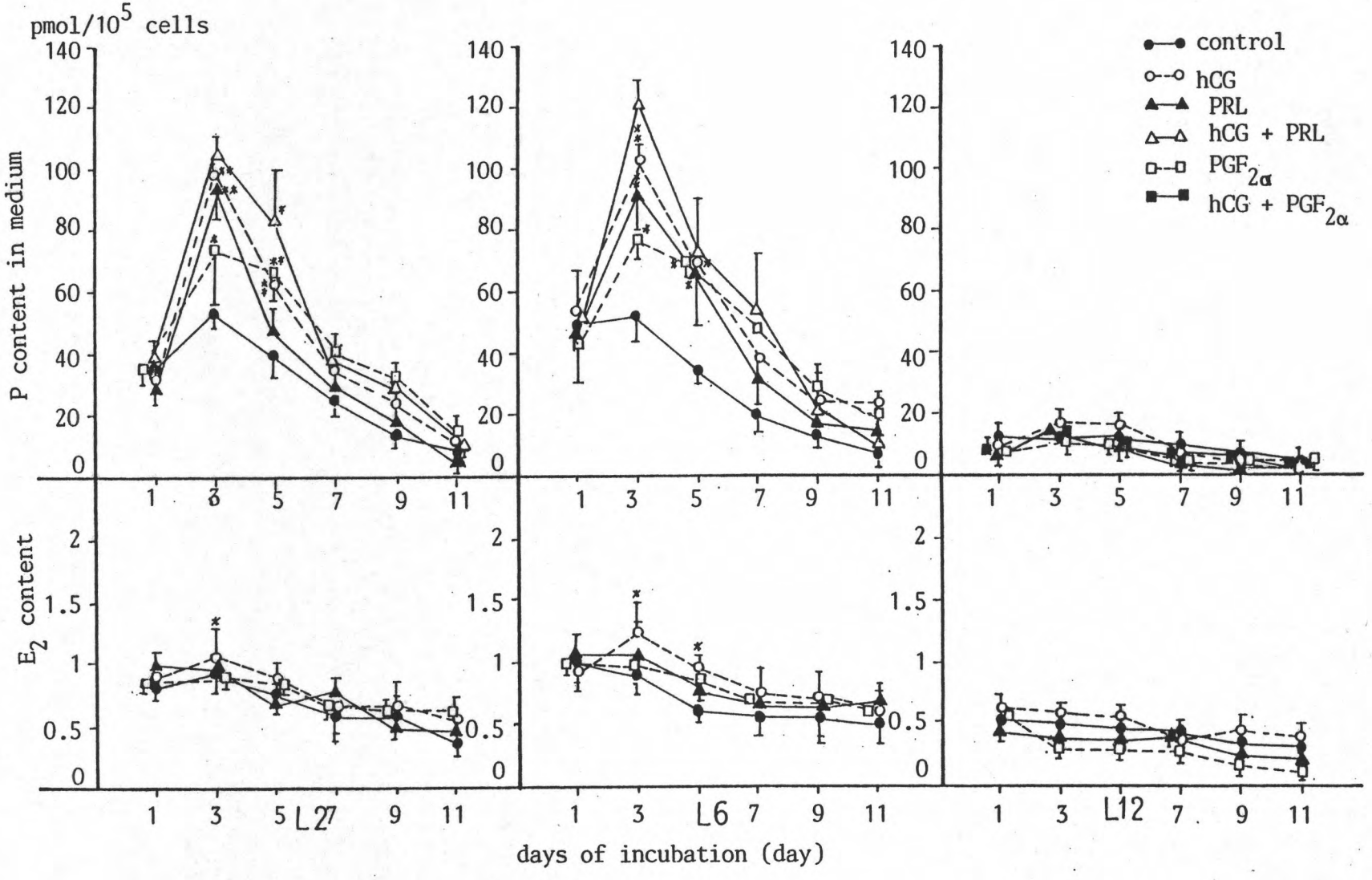

Figure 16 Long term effects of hCG, PRL and $PGF_{2\alpha}$ on in vitro stimulation of P and E_2 secretion of rat luteal cells from PSP during 11 day incubation (mean±S.E., n = 3).
(** = P<0.01, * = P<0.05 significantly from the control)

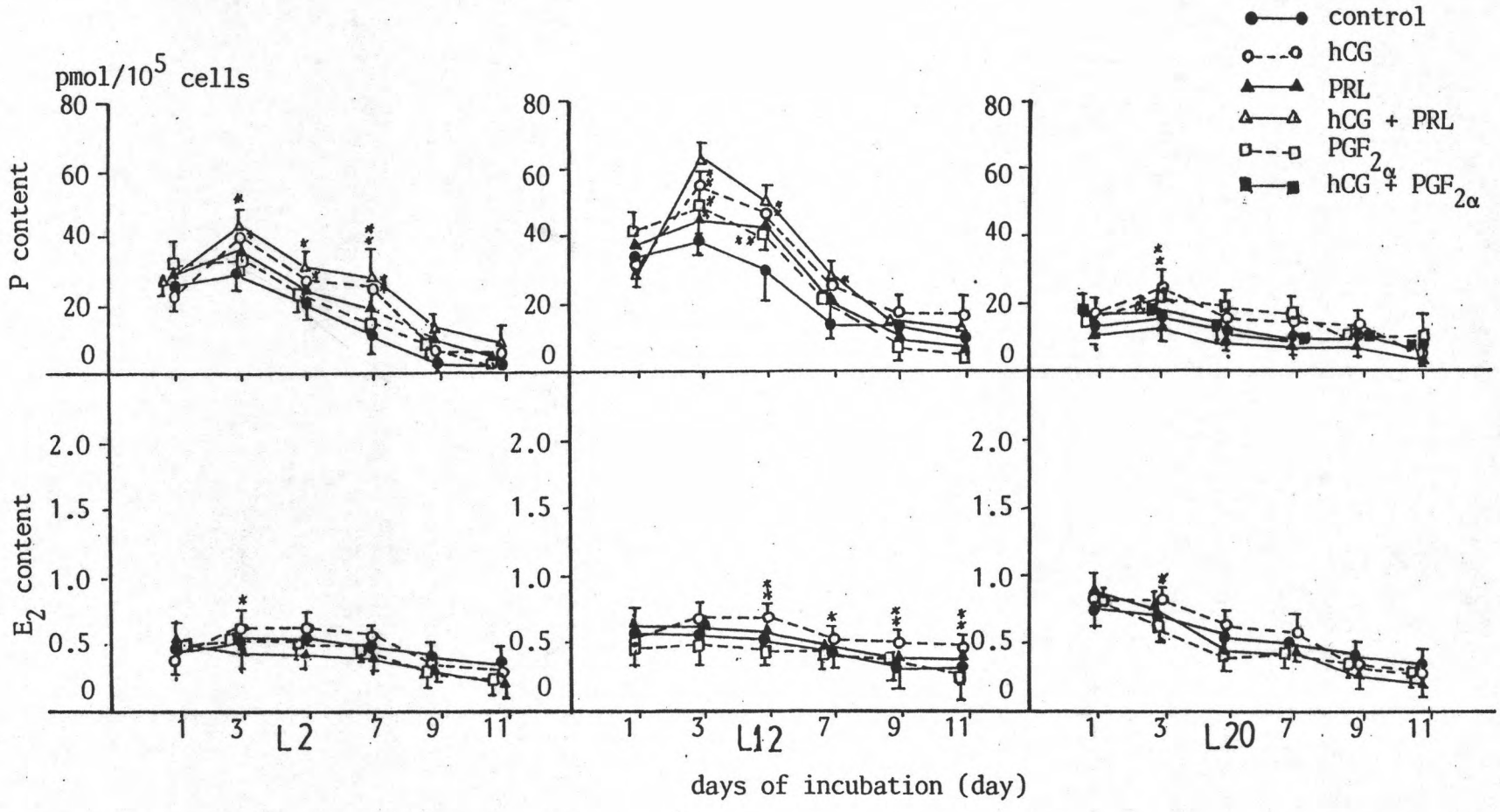

Figure 17 Long term effects of hCG, PRL and $PGF_{2\alpha}$ on in vitro stimulation of P and E_2 secretion of rat luteal cells from lactating PSP during 11 day incubation (mean±S.E., n = 3).

(** = $P < 0.01$, * = $P < 0.05$ significantly different from the control, a = compared with hCG-treated group).

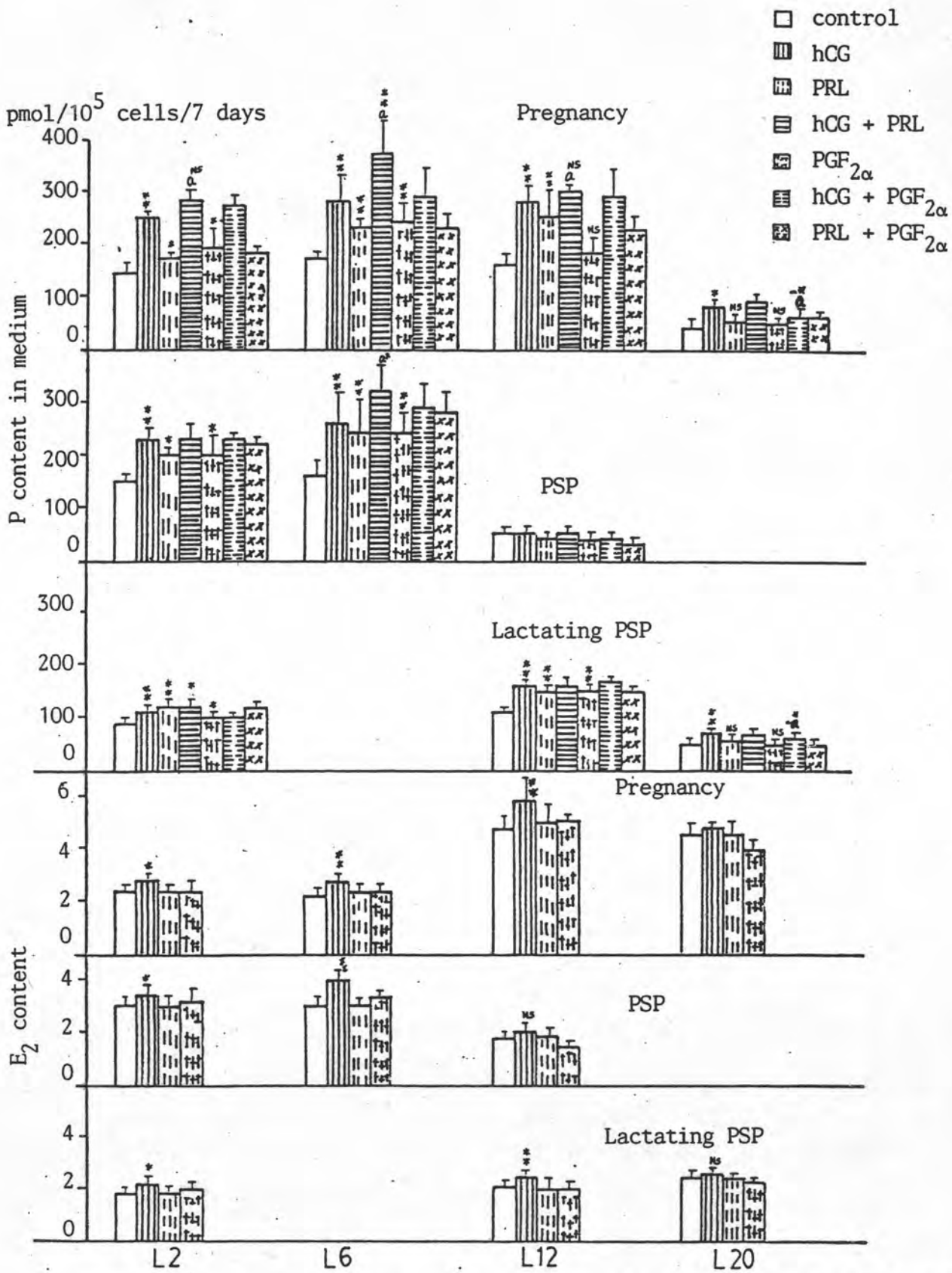

Figure 18 Responsiveness of rat luteal cells from pregnancy, PSP and lactating PSP to hCG, PRL and $PGF_{2\alpha}$ on P and E_2 secretion in 7 day incubation (**=P<0.01, *=P<0.05 significantly different from the control, NS = non significantly different, a = compared with hCG-treated group)

Table.**6** Effects of hCG, o-PRL and $PGF_{2\alpha}$ on % increment of P and E_2 of rat luteal cells during 7 day incubation (mean±S.E., n = 3)

Treatments / Stages	pmol/10^5 cells control	% increment of P			pmol/10^5 cells control	% increment of E_2		
		hCG	o-PRL	$PGF_{2\alpha}$		hCG	o-PRL	$PGF_{2\alpha}$
Estrous cycle								
estrus (E)	103.2±10.8	55.1±25.5**	42.2±1.1*	49.6±12.2*	2.11±0.0	18.47±3.2NS	3.54±1.9	9.72±0.7
diestrus-1(Di-1)	137.4±4.2	39.5±7.2*	33.5±6.9*	20.1±0.0NS	2.32±0.1	13.10±11.4NS	-5.62±2.1	3.37±1.5
diestrus-2(Di-2)	68.7±6.9	17.4±6.9NS	2.5±1.2NS	-5.6±2.2NS	1.25±0.1	3.41±1.1NS	1.00±0.1	-2.55±0.9
Pregnancy								
L_2	139.1±11.1	78.8±14.5**	19.9±4.5NS	35.5±14.8*	2.42±0.2	12.41±1.0NS	0.42±0.0	6.31±2.4
L_6	173.7±6.4	59.6±15.8*	37.3±5.6*	39.7±14.5*	2.27±0.1	24.91±10.8*	4.64±0.0	5.95±5.5
L_{12}	161.8±14.7	71.2±0.5**	53.2±13.4*	13.6±12.9	4.83±0.4	19.8±9.0*	3.85±1.1	3.11±1.8
L_{20}	43.6±7.1	93.1±18.5**	-3.67±5.2NS	7.7±2.5	4.67±0.4	2.21±0.4NS	-0.31±0.0	13.91±5.6
PSP								
L_2	152.5±4.9	48.7±8.0*	31.9±2.1*	38.0±13.3	3.11±0.3	11.9±1.3NS	2.47±1.0	3.26±0.3
L_6	157.3±22.9	164.3±12.2**	48.78±16.0*	53.7±2.3	3.04±0.0	30.97±12.0*	4.61±1.3	5.05±2.1
L_{12}	51.0±5.4	3.3±1.0	-17.4±5.8NS	-19.8±3.8	1.89±0.2	6.57±0.8NS	-0.50±0.0	-3.78±0.7
Lactating PSP								
L_2	91.8±0.4	13.3±0.4NS	24.1±6.9*	10.4±1.5NS	1.85±0.1	15.27±6.1NS	-8.61±4.2	3.46±1.8
L_{12}	115.9±8.1	35.5±9.7*	28.2±3.9*	29.5±3.0*	2.07±0.2	18.28±4.7NS	1.59±0.9	4.53±2.9
L_{20}	53.8±11.0	26.6±15.2*	3.2±1.0NS	-9.4±2.3NS	2.52±0.2	5.51±2.1NS	-2.51±1.0	1.21±0.4

* = P < 0.05, ** = P < 0.01, NS = non-significantly different from the control.

Table. 7 summarizing effect of hCG, PRL and $PGF_{2\alpha}$ on stimulation of rat CL steroid production during 7 day incubation

stages / treatments	L_2		L_6		L_{12}		L_{20}	
	E_2	P	E_2	P	E_2	P	E_2	P
PSP								
hCG	+	+	+	+	-	+ NS		
PRL	-	+	-	+	-	-		
$PGF_{2\alpha}$	-	+	-	+	-	-		
hCG + $PGF_{2\alpha}$		-		-	-	+ a NS		
pregnancy								
hCG	+	+	+	+	+	+	-	+
PRL	-	+	-	+	-	+	-	-
$PGF_{2\alpha}$	-	+	-	+	-	-	-	-
hCG + $PGF_{2\alpha}$		-		-		-		+ a
Lactation								
hCG	+	+			+	+	-	+
PRL	-	+			-	+	-	-
$PGF_{2\alpha}$	-	+			-	+	-	-
hCG + $PGF_{2\alpha}$		-				-		+ a
estrous cycle	estrus		diestrus-1		diestrus-2			
	E_2	P	E_2	P	E_2	P		
hGC	+	+	+	+	-	-		
PRL	-	+	-	+	-	-		
$PGF_{2\alpha}$	-	+	-	+	-	-		
hCG + $PGF_{2\alpha}$		-		-		-		

a = inhibition on hCG-stimulated P secretion of luteal cells

In PSP, basal P secretion of luteal cells from stage L_2 (152.5±6.9 pmol/10^5 cells/7 days) and L_6 (157.3±3.8 pmol/10^5 cells/7 days) were higher than stage L_{12} (51.0±3.8 pmol/10^5 cells/7 days, $P < 0.01$). hCG increased basal P and E_2 secretion of luteal cells from stage L_2 (227.2±19.6 pmol/10^5 cells/7 days, $P < 0.01$) and L_6 (261.2± 56.8 pmol/10^5 cells7 days, $P < 0.01$). Similarly, either PRL or $PGF_{2\alpha}$ also significantly increased P secretion of luteal cells from stage L_2 and L_6. Otherwhile, luteal cells from stage L_{12} of PSP were refractory to all agents.

In lactating PSP, P secretion of luteal cells from stage L_{12} (115.9±8.1 pmol/10^5 cells/7 days) was higher than P secretion of luteal cells from stage L_2 (91.8±0.6 pmol/10^5 cells/7 days, $P < 0.01$), and L_{20} (53.8±11.1 pmol/10^5 cells/7 days, $P < 0.01$). The presence of hCG significantly increased basal P and E_2 secretion of luteal cells from stage L_2, L_{12} and L_{20}. Similarly, PRL increased basal P of luteal cells from stage L_2 and L_{12}. While $PGF_{2\alpha}$ significantly increased basal P of luteal cells from stage L_2 and L_{12} and inhibited on hCG-stimulated P secretion of luteal cells from stage L_{20}.

Similarly, the responsiveness of luteal cells also presented in precent increment of P and E_2 secretion (Table 6.) and it was summarized in Table 7. by showing the effects of hCG, PRL and $PGF_{2\alpha}$ on stimulation of luteal cells steroid production during 7 day incubation.

4. Plasma P and E_2 levels in various reproductive stages of rats.

Plasma P and E_2 levels in various reproductive stage of rats are summarized in Table 8.

Plasma P levels in cyclic rats were 46.50±2.96, 62.25±7.22, 42.00±8.44 and 29.00±3.94 nmol/L during estrus, diestrus-1, early diestrus-2 and late diestrus-2 respectively, while plasma E_2 levels were quite low and highest at the late diestrus-2. During PSP, plasma P levels were 145.25±8.70, 272.75±22.61 and 70.00±7.18 nmol/L., similarly, plasma E_2 concentration were 0.03±0.17, 0.24±0.04 and 0.22±0.01 nmol/L during PSP stage L_2, L_6 and L_{12} respectively. In preganant rats, plasma P levels were 129.75±13.41, 185.75±8.11, 301.50±27.29 and 126.25±21.87 nmol/L, as well as, plasma E_2 concentration were 0.18±0.02, 0.27±0.02, 0.28±0.05 and 0.51±0.25 nmol/L during pregnancy stage L_2, L_6, L_{12} and L_{20}. Furthermore, plasma P levels of lactating rats were 82.00±10.00, 161.75±19.50 and 80.00±11.22 nmol/L, while plasma E_2 concentration were 0.21±0.05, 0.26±0.03 and 0.35±0.09 nmol/L during lactating stage L_2, L_{12} and L_{20} respectively.

Table.8 Plasma progesterone (P) and estradiol-17β (E_2) levels from various reporductive stages of rats

stages		P concentration (nmol/l)	E_2 concentration (nmol/l)
estrous cycle	estrus (8±1 hrs old CL)	46.50±2.96	0.28±0.06
	diestrus-1 (32±1 hrs old CL)	62.25±7.22	0.22±0.05
	diestrus-2 (56±1 hrs old CL)	42.00±8.44	0.32±0.07
	diestrus-2 (62±1 hrs old CL)	29.00±3.94	0.35±0.02
PSP	L_2	145.25±8.70	0.30±0.02
	L_6	275.75±22.61	0.24±0.04
	L_{12}	70.00±7.18	0.22±0.01
pregnancy	L_2	129.75±13.42	0.18±0.02
	L_6	185.75±8.11	0.27±0.03
	L_{12}	301.50±27.29	0.28±0.05
	L_{20}	126.25±21.87	0.51±0.13
lactating PSP	L_2	82.00±10.30	0.21±0.05
	L_{12}	161.75±19.51	0.26±0.03
	L_{20}	80.00±11.23	0.35±0.09

5. Effect of partial lutectomy on ovulation patterns and menstrual cycle in adult female monkeys.

Possible ovulation pattern in the cynomolgus monkeys were shown in Table 8.

Monkey #24, #75 and #101 exhibited regular menstrual cycle before and after lutectomy. The ovulation patterns of cynomolgus monkeys were dectected by the presence of CL in the ovary and these presentation of CL was alternate side of the ovary. The cycle length of monkey #24, #75 and #101 were 26.4±2.7, 35.1±5.7 and 33.9±41 days, respectively.

6. Plasma levels of P and E_2 during menstrual cycle in monkeys.

Table 9 summarizes plasma P and E_2 levels during menstrual cycle of monkey #101, #75 and #24.

Plasma P levels of three cynomologus monkeys were 4.61±0.90 -5.76±0.98 nmol/L during early luteal phase (15-18 days prior menses), 6.80±1.05 - 13.60±0.37nmol/L during mid luteal phase (8-9 days prior menses), 4.59±0.65 - 7.6±1.01 nmol/L during late luteal phase (2-5 days prior menses) and 0.89 ± 0.01 - 1.80 ± 0.02 nmol/L during luteolytic phase (0-2 days post menses). These P levels dropped sharply after lutectomy, and maintained at low levels were detected until day 5 after operation.

Plasma E_2 concentration were 0.46±0.09 to 0.80±0.12, 0.41± 0.05 to 0.88 ±0.11, 0.47±0.05 to 0.75±0.16, 0.33±0.03 to 0.38±0.03 nmol/L during early, mid, late luteal phase and luteolytic phase, respectively. These E_2 levels slightly dropped on day 1 after lutectomy and returned to basal level on day 5 after operation.

Table 9. Ovulation pattern of cynomolgus monkeys assessed by laparoscopy.

year	month	monkey # 24			monkey # 75			monkey # 101		
		Cycle length (day)	CL		Cycle length (day)	CL		Cycle length (day)	CL	
			R	L		R	L		R	L
1985	Jan-Feb							37		
	Feb-Mar							35		
	Mar-Apr							33		L
	Apr-May							35		R
	May-June							38		-
	June-July							38		-
	July-Aug							37		L
	Aug-Sep				32		-	32		-
	Sep-Oct				47		-	32		L
	Oct-Nov	32		-	45		-	49		-
	Nov-Dec	28		-	-			-		
	Dec-Jan	27		-	45		L	30		-
1986	Jan-Feb	25		-	30		-	30		R
	Feb-Mar	27		R	-			32		L
	Mar-Apr	25		-	32		-	30		-
	Apr-May	27		-	33		R	33		-
	May-June	21		L	33		-	33		-
	June-July	25		R	34		R	31		-
	July-Aus	29		-	36		-	32		-
	Aus-Sep	22		R	33		-	34		-
	Sep-Oct	26		-	31		-	33		-
	Oct-Nov	28		-	31		-	31		-
	Nov-Dec	28		-	31		-	31		-
		26.4±2.7			35.1±5.7			33.9±4.1		

CL = Corpus luteum, R = right side, L = left side

Table.10 Plasma levels of P and E_2 of cynomolgus monkey on the day of lutectomy, 1 and 5 days after lutectomy.

stages	day after lutectomy	# 101		# 75		# 24	
		P nmol/L	E_2 nmol/L	P nmol/L	E_2 nmol/L	P nmol/L	E_2 nmol/L
early lutel phase	0	4.61±0.90	0.80±0.02	5.76±0.98	0.46±0.09	-	-
	1	2.83±0.54	0.73±0.02	1.00±0.04	0.30±0.05	-	-
	5	2.17±0.37	0.93±0.02	0.86±0.02	0.37±0.08	-	-
mid luteal phase	0	13.60±0.37	0.88±0.01	12.80±1.01	0.58±0.02	6.80±1.05	0.41±0.05
	1	4.81±0.80	0.48±0.01	5.26±0.94	0.50±0.01	1.60±0.05	0.26±0.03
	5	4.59±0.63	0.77±0.01	2.93±0.05	0.56±0.02	1.20±0.03	0.58±0.09
late luteal phase	0	4.59±0.63	0.75±0.02	7.39±1.20	0.34±0.09	7.61±0.01	0.47±0.05
	1	1.85±0.04	0.46±0.02	3.22±0.98	0.37±0.08	3.00±0.04	0.36±0.04
	5	-	-	-	-	1.25±0.04	0.44±0.06
luteolytic phase	0	0.89±0.10	0.38±0.03	-	-	1.80±0.02	0.33±0.03
	1	0.26±0.04	0.43±0.05	-	-	-	-
	5	0.22±0.05	1.05±0.20	-	-	-	-

7. Viability of isolated monkey luteal cells in culture.

Table 11. shows the viability of monkey luteal cells from early, mid and late luteal phase on day 11 of incubation which were treated with hCG (0.5 iu/ml), o-PRL (5 µg/ml), $PGF_{2\alpha}$ (250 ng/ml), hCG+PRL (0.5 iu + 5 µg/ml), hCG+$PGF_{2\alpha}$ (0.5 iu + 250 ng/ml) and PRL+$PGF_{2\alpha}$ (5 µg + 250 ng/ml).

The viability of CL cells of cynomolgus monkey #101 from early, mid, late luteal phase and luteolytic phase in the control cultures were 60.00±5.66, 65.00±1.41, 59.00±1.42 and 51.00±1.41%. Monkey #75, the viability of luteal cells from early, mid and late luteal phase were 60.00±2.83, 66.00±1.41 and 61.00±4.24%, and monkey #24 the viability of luteal cells from mid, late luteal phase and luteolytic phase were 59.00±12.73, 64.00±5.66 and 52.50±3.54% respectively. In o-PRL, hCG+PRL and PRL+$PGF_{2\alpha}$ treated group showed a high viability of luteal cells from early, mid and late luteal phase of monkey #101, #75 and #24, while there were not statistically different in hCG, $PGF_{2\alpha}$ and hCG+$PGF_{2\alpha}$ treated group as compare to control. The viability of luteal cells from luteolytic phase showed no statistical difference in all cases.

8. Ability of isolated monkey luteal cells in P and E_2 secretion in culture.

Figure 19 shows P and E_2 secreting ability of luteal cells of monkey #101, #75 and #24 from early, mid, late ltueal phase and luteolytic phase during 24 hours of incubation.

P secreting ability of luteal cells form monkey #101, #75 and #24 during early luteal phase were 6.21±0.25 - 8.79±1.31 pmol/$5x10^4$ cells, during mid luteal phase were 5.61±2.10 - 82.0±5.81 pmol/$5x10^4$ cells, during late luteal phase were 30.00±5.35 - 36.4±5.10 pmol/$5x10^4$ cells and during luteolytic phase were 1.66±0.05 - 2.56±0.01 pmol/$5x10^4$ cells.

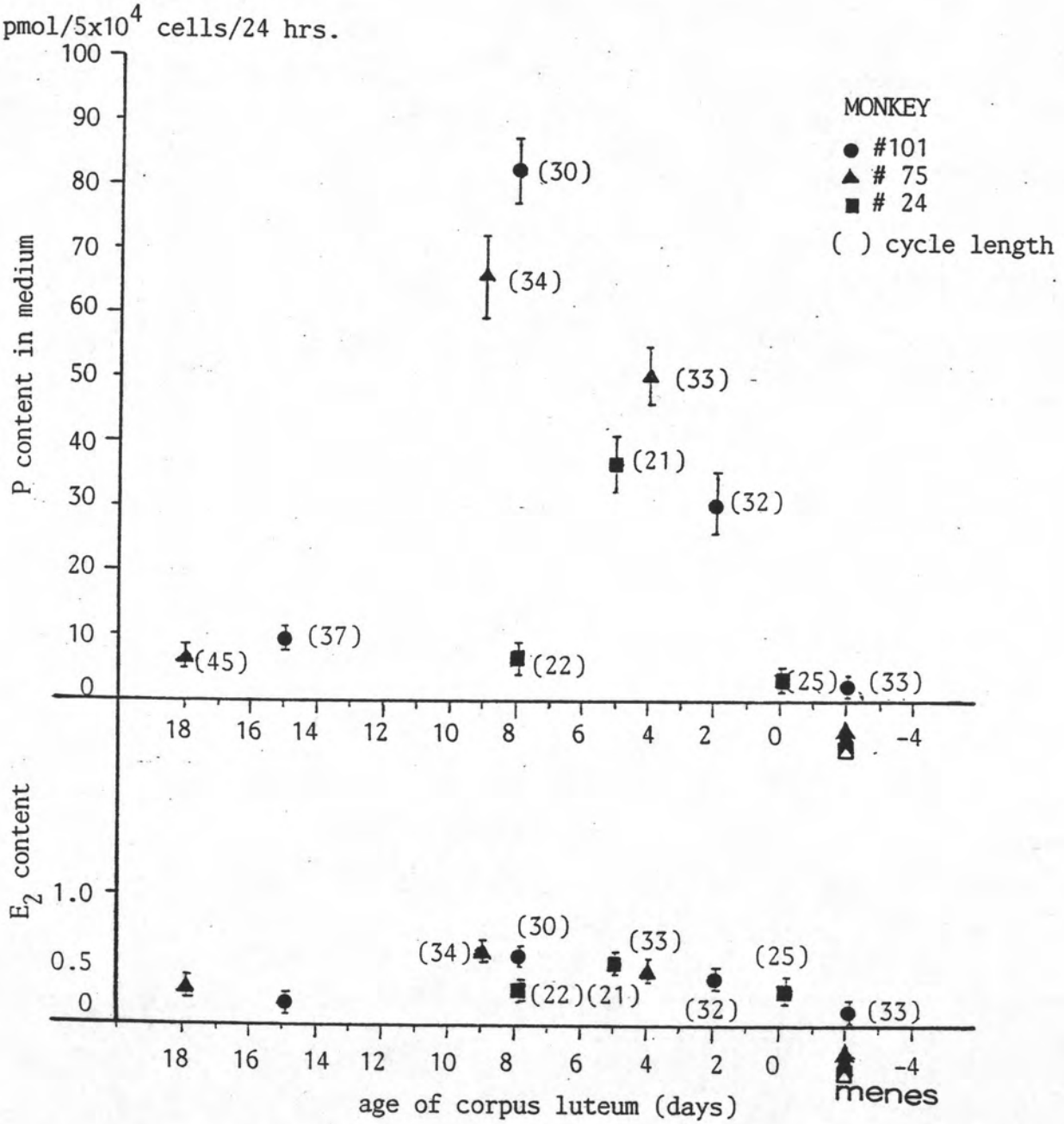

Figure 19 P and E_2 secreting abitity of luteal cells obtained from different age of corpus luteum during 24 hour incubation (mean±S.E., n = 3).

E_2 secreting ability of CL cell from monkey #101, #75 and #24 during early luteal phase were 0.16±0.02 - 0.27±0.02 pmol/5x10^4 cells, during mid luteal phase were 0.23±0.02 - 0.52±0.01 pmol/5x10^4 cells, during late luteal phase were 0.36±0.01 - 0.46±0.01 pmol/5x10^4 cells and during luteolytic phase were 0.13±0.00 - 0.27±0.02 pmol/5x10^4 cells.

9. Effects of hCG, PRL and $PGF_{2\alpha}$ on P and E_2 secretion from monkey isolated luteal cells obtained during early, mid, late and luteolytic phase.

Figure 20 shows effects of hCG (0.5 iu/ml), PRL (5 µg/ml) $PGF_{2\alpha}$ (250 ng/ml) and hCG+$PGF_{2\alpha}$ (0.5 iu + 250 ng/ml) on P and E_2 secretion from luteal cells of monkey # 101.

During early luteal phase (age of CL : 15 days prior menses of cycle 37 days). Basal P secretion was 8.79±1.31 pmol/5x10^4 cells on day 1, increased to 9.20±1.15 pmol/5x10^4 cells on day 3, gradually declined and down to 4.17±0.35 pmol/5x10^4 cells on day 11 of incubation. Either hCG or $PGF_{2\alpha}$ significantly increased P secretion, these P secretions increased to 24.95±0.43 ($P < 0.01$) and 21.85±1.32 pmol/5x10^4 cells ($P < 0.05$) in the presence of hCG and to 16.41 0.45 ($P < 0.01$) and 14.81±0.70 ($P < 0.01$) pmol/5x10^4 cells in the presence of $PGF_{2\alpha}$ on day 3 and day 5 of incubation respectively. No additive effect was observed in hCG+$PGF_{2\alpha}$ treated groups. Otherwise, PRL did not alter basal P secretion. None of these agents added into the medium on day 3 and day 5 of incubation were capable to increase basal E_2 production significantly, except hCG.

During mid luteal phase (age of CL : 8 day prior menses of cycle 30 days). The basal P secretion was 82.01±5.81 pmol/5x10^4 cells on day 1, decreased gradually on the following day and drop to 14.01±

3.34 pmol/5x10^4 cells on day 11 of incubaiton.

hCG was capable to increase basal P levels of luteal cells significantly throughout the incubation peroid. The presence of $PGF_{2\alpha}$ elevated basal P secretion to 96.3±1.40 ($P < 0.05$) and 90.22±11.40 ($P < 0.05$) pmol/5x10^4 cells on day 3 and day 5 respectively. Otherwise PRL showed no effect on basal P secretion in all cases. None of these agents, except hCG was capable to raise basal E_2 secretion significantly throughout incubation period.

During late luteal phase (age of CL : 2 days prior menses of cycle 32 days). The basal P secretion luteal cells were 30.00±5.35 on day 1, increased slightly on day 3 and gradually declined on the following day until the end of incubaiton. The presence of hCG significantly increased basal P secretion, these P secretions were 49.20±9.80 ($P < 0.01$), 63.57±9.50 ($P < 0.01$), 36.9±1.30 ($P < 0.01$) and 27.01±1.50 ($P < 0.05$) pmol/5x10^4 cells respectively. Otherwise, $PGF_{2\alpha}$ exhibited a significant inhibitory effect on hCG-stimulated P secretion and to 35.29±6.80 ($P < 0.05$) and 42.99±7.60 ($P < 0.01$) pmol/5x10^4 cells on day 3 and day 5 of incubation while PRL showed no effect. None of these agents added into the medium, except hCG significantly stimulated basal E_2 secretion on day 3 and day 5 after incubaiton.

During luteolytic phase (age of CL : 2 days post menstrual bleeding), basal P and E_2 secretion were quite low and refractory to any exogenous hormones.

Figure 21 show effects of hCG (0.5 iu/ml), PRL (5 µg/ml), $PGF_{2\alpha}$ (250 ng/ml) and hCG+$PGF_{2\alpha}$ (0.5 iu + 250 ng/ml) on P and E_2 secretion from luteal cells of monkey #75.

During early luteal phase (age of CL : 15 days prior menses of cycle 45 days), basal P secretion was 6.21±0.25 pmol/5x10^4 cells on day 1 of incubation, gradually increased on day 3 and day 5 of incubation and gradually declined on the following day and down to 6.40±0.47 pmol/5 x 10^4 cells on day 11 of incubaiton. These P levels were 48.00± 5.00 ($P < 0.01$) and 49.46±3.75 ($P < 0.01$) pmol/5 x 10^4 cells in hCG-treated group on day 3 and day 5 and were 34.59±5.25 ($P < 0.01$) pmol/ 5 x 10^4 cells in $PGF_{2\alpha}$-treated group on day 3 of incubation. It was of interest that PRL was not capable to alter these basal P secretions. None of these agents, except hCG significantly raised basal P secretion on day 3 and day 5 of incubation.

During mid luteal phase (age of CL : 9 days prior menses of cycle 34 days), basal P secretion was 64.78±7.78 pmol/5 x 10^4 cells on day 1, gradually decreased on the following day and down to 5.89±1.25 pmol/55 x 10^4 cells at the end of incubation. None of the agent, except hCG significantly increased basal P secretion on day 3, 5 and day 7 of incubation, these P secretions were 151.21±8.75 ($P < 0.01$), 139.43±7.64 ($P < 0.01$) and 63.57±3.66 ($P < 0.01$) pmol/5 x 10^4 cells respectively. The presence of $PGF_{2\alpha}$ increased P secretion to 77.07± 4.80 pmol/5 x 10^4 cells on day 3 of incubation but such increment showed no statistical difference, while RPL showed no effect. None of the agents, except hCG significantly raised basal E_2 secretion during day 3 - day 7 of incubation.

During late luteal phase (age of CL : 4 days prior menses of cycle 33 days), basal P secretion was 50.00±4.90 pmol/5 x 10^4 cells on day 1 and gradually declined on the following day until the end of incubaiton. hCG significantly elevated basal P secretion which were 86.79±3.50 ($P < 0.01$) and 68.89±8.00 ($P < 0.05$) pmol/5 x 10^4

cells on day 3 and day 5 of incubation. Otherwhile, the presence of $PGF_{2\alpha}$ showed a significant inhibitory effect on hCG-stimulated, P secretion which downed to 58.79±6.80 ($P < 0.05$) and 58.79±1.00 ($P < 0.05$) pmol/5 x 10^4 cells on day 3 and day 5 of incubation. Similarly, PRL did not effect on basal P secretion. None of these agents, except hCG significantly stimulated basal E_2 secretion on day 3 and day 5 of incubaiton.

Figure 22 shows effects of hCG (0.5 iu/ml), PRL (5 µg/ml), $PGF_{2\alpha}$ (250 ng/ml) and hCG+$PGF_{2\alpha}$ (0.5 iu + 250 ng/ml) on P and E_2 secretion from isolated luteal cells of monkey #24.

During mid luteal phase (age of CL : 8 days prior menses of cycle 22 days), basal P secretion was 5.60±2.10 pmol/5 x 10^4 cells on day 1, slightly increased on day 3 and gradually decreased until the end of incubation. hCG significantly increased basal P secretion these P secretions were 36.80±5.15 ($P < 0.01$), 40.08±3.90 ($P < 0.01$) and 24.20±4.50 ($P < 0.01$) pmol/5 x 10^4 cells on day 3, 5 and day 7 of incubation, respectively. Similarly, these P secretion was elevated up to 17.60±2.55 ($P < 0.05$) pmol/5 x 10^4 cells in $PGF_{2\alpha}$ treated group on day 3 of incubation. Whereas, PRL exhibited no effect on these basal P secretion. None of these agents added into the medium, except hCG significantly increased basal E_2 secretion on day 3 day 5 of incubation.

During late luteal pahse (age of CL : 5 days prior menses of cycle 21 days), basal P secretion was 36.40±5.10 pmol/5 x 10^4 cells on day 1 of incubaiton, gradually decreased until the end of incubation. hCG significantly increased basal P secretion throughout the incubation peroid. $PGF_{2\alpha}$ stimualted basal P secretion to 33.63±2.50

($P < 0.01$) pmol/5 x 10^4 cells on day 3 of incubation while PRL showed no effect. None of these agents added into the medium, except hCG significantly elevated basal E_2 secretion throughout incubation peroid.

During luteolytic pahse (age of CL : first day of menstrual bleeding), basal P and E_2 secretion were quite low and refractory to any exogenous hormones.

It can be summarized in Table 11, 13 and Fig. 23 showing the effects of hCG, PRL and $PGF_{2\alpha}$ on P and E_2 secreting ability of monkey isolated luteal cells from early, mid, late and luteolytic phase during 7 days of incubation.

Figure 23 shows that during early luteal phase P secretion of isolated from cynomolgus monkey #101, #75 and #24 were 32.0±4.8 to 47.43±5.52 pmol/5 x 10^4 cells/7 days, during mid luteal phase were 18.9±3.8 to 254.9±31.2 pmol/5 x 10^4 cells/7 days, during late luteal phase were 88.6±5.1 to 129.3±16.6 pmol/5 x 10^4 cells/7 days and during luteolytic phase were 5.10±1.1 to 7.1±0.2 pmol/5 x 10^4 cells/7 days. The presence of hCG significantly stimulated basal P secretion of isolated luteal cells obtained during early, mid and late luteal phase. Similarly, $PGF_{2\alpha}$ significantly stimulated basal P secretion of monkey luteal cells obtained during early and mid luteal phase, and inhibited hCG stimulated on P secretion of monkey luteal cells obtained during late luteal pahse. However, only hCG was capable to stimulate E_2 secretion of luteal cells obtained from all stages, except luteolytic phase. While PRL unaffected to P and E_2 secretion of monkey luteal cells from all stages of menstrual cycle. However, monkey isolated luteal cells obtained during luteolytic phase secreted low P and E_2 concentration in culture and refractory to all agents.

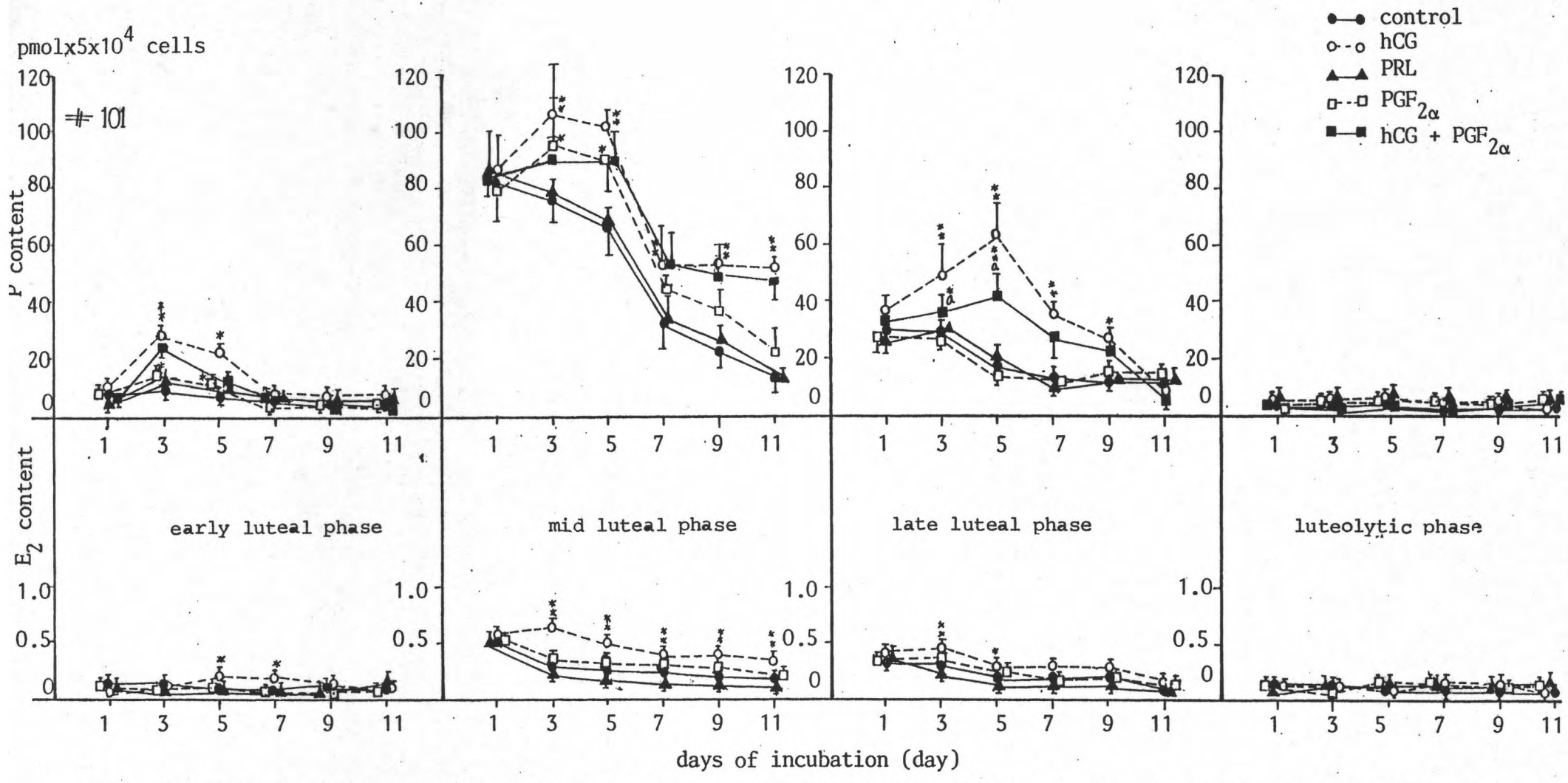

Figure 20 Long term effects of hCG, PRL and $PGF_{2\alpha}$ on in vitro stimulation of P and E_2 secretion of cynomolgus monkey luteal cells from early, mid, late luteal phase and luteolytic phase during 11 day incubation (mean±S.E., n = 3)
(** = P<0.01, * = P<0.05 significantly different from the control, a * = inhibition on hCG-stimulated P production)

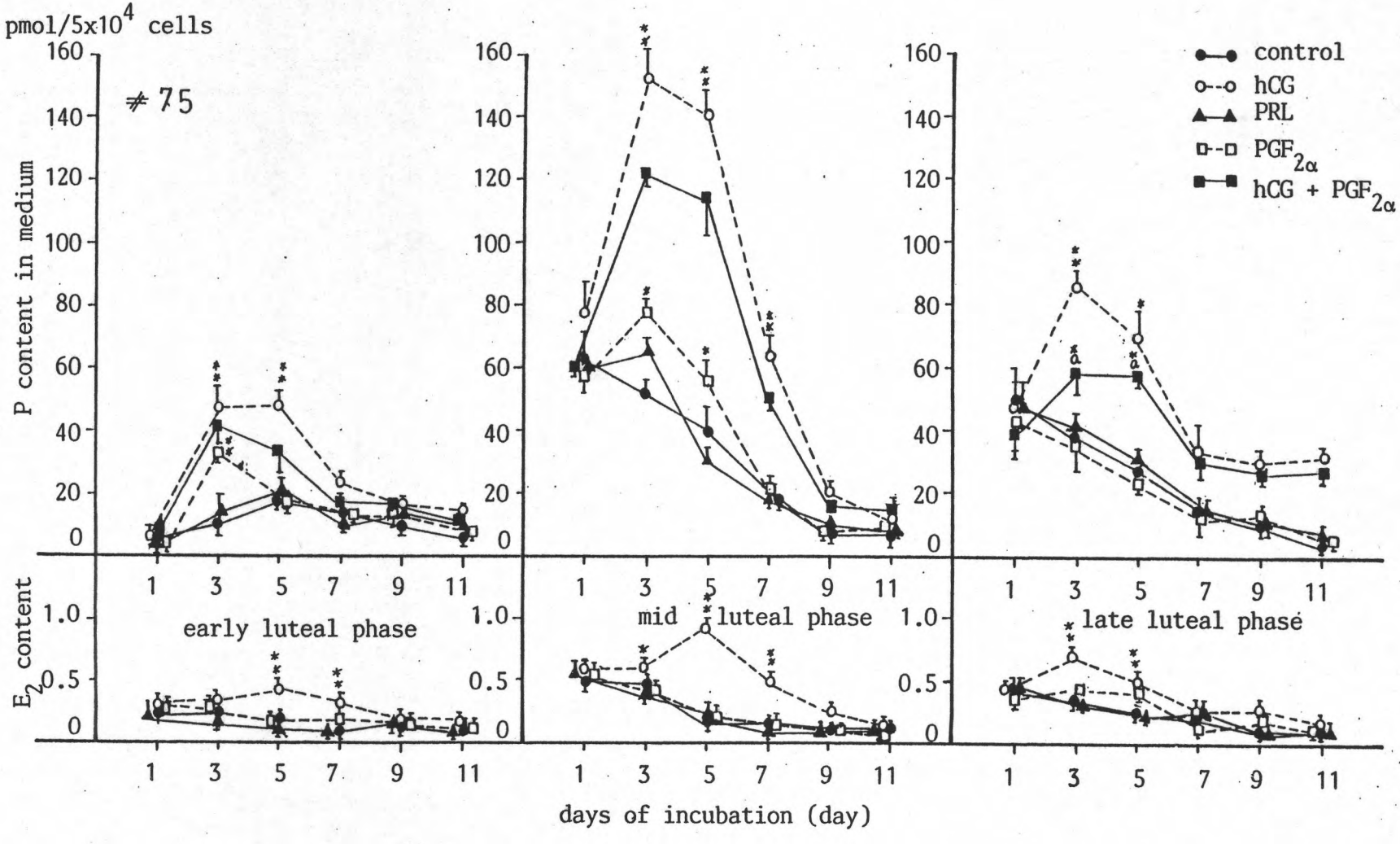

Figure 21 Long term effects of hCG, PRL and $PGF_{2\alpha}$ on in vitro stimulation of P and E_2 secretion of cynomolgus monkey luteal cells from early, mid and late luteal phase during 11 day incubation (mean±S.E., n=3) (**=P<0.01, *=P<0.05 significantly different from the control, a* = inhibition on hCG-stimulated P secretion).

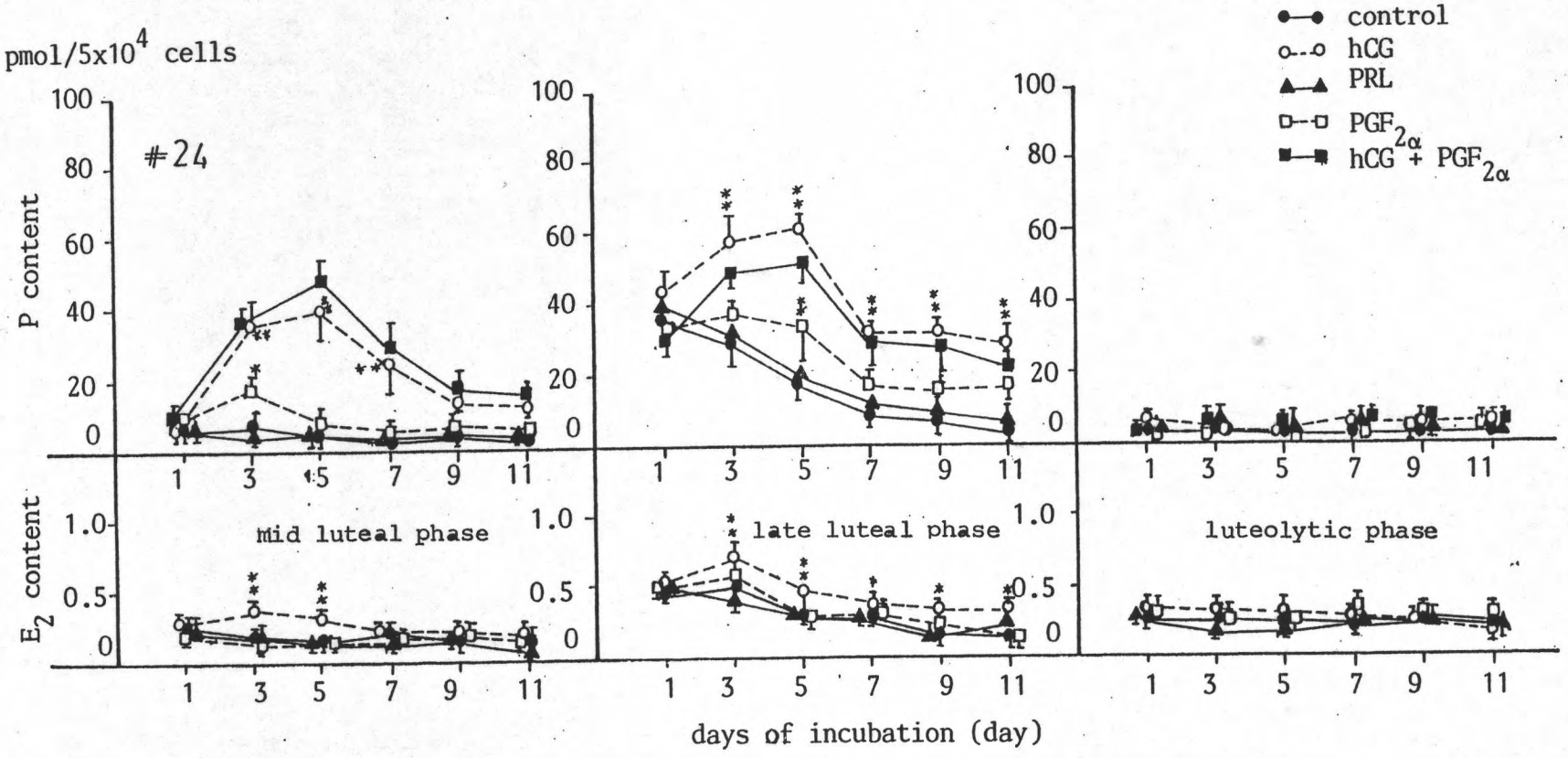

Figure 22 Long term effects of hCG, PRL and $PGF_{2\alpha}$ on in vitro stimulation of P and E_2 secretion of cynomolgus monkey luteal cells from mid, late luteal phase and luteolytic phase during 11 day (mean±S.E., n = 3)
(** = P<0.01, * = P<0.05 significantly different from the control)

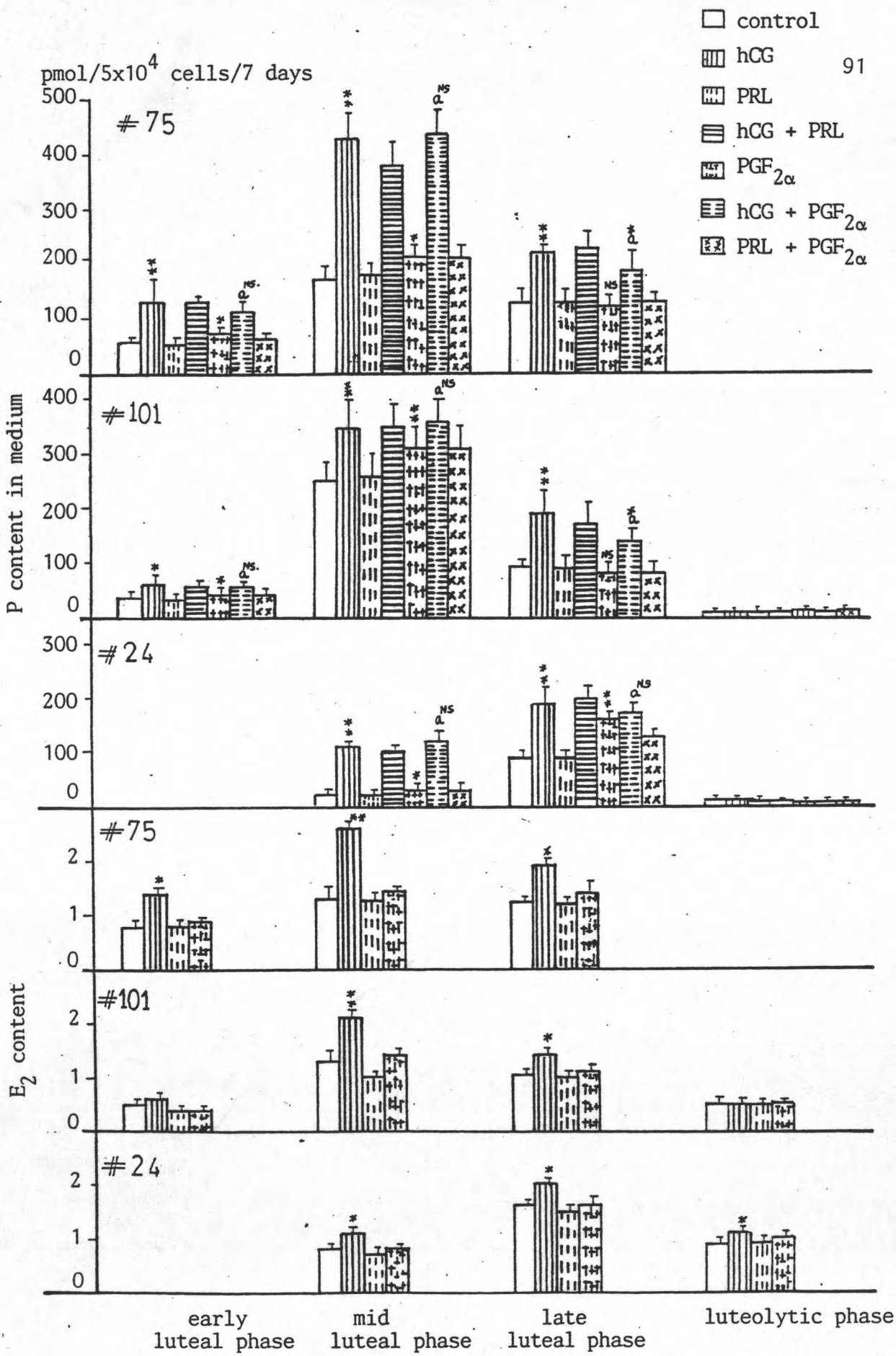

Figure 23 Responsiveness of cynomolgus monkey luteal cells to hCG, PRL and $PGF_{2\alpha}$ on P and E_2 secretion in 7 day incubation (mean±S.E.) (**=P<0.01, *=P<0.05 significanitly different from the control, NS = non significatly different , a = compared with hCG-treated group).

Table.11 Viability of monkey luteal cells in culture on day 11 of incubation; comparison among treatment groups and the control group

treatments / stages	cell viability (%)							Remarks :- day prior to the next bleeding/cycle length
	Control	hCG	PRL	hCG+PRL	$PGF_{2\alpha}$	hCG+PGF	$PRL+PGF_{2\alpha}$	
Monkey # 101								
early luteal phase	60.00±5.66	71.00±1.41	78.00±2.83**	80.00±5.66**	59.00±1.42	69.00±1.41	76.00±5.66	15/37
mid luteal phase	65.00±1.41	67.00±1.41	75.00±1.42**	76.00±5.66**	60.00±2.83	70.00±2.83	76.50±5.66**	8/30
late luteal phase	59.00±1.42	64.00±5.86	76.00±2.82**	76.00±5.66**	56.00±5.66	64.00±11.31	77.50±3.54**	2/32
luteolytic phase	51.00±1.41	55.00±4.24	55.50±7.78 NS.	60.00±2.83 NS	51.5±4.95	56.00±5.66	56.50±12.02 NS.	-2/33
Monkey # 75								
early luteal phase	60.00±2.83	63.00±4.24	81.50±4.95**	79.50±0.71**	58.00±5.66	67.00±12.73	81.00±4.24**	18/45
mid luteal phase	66.00±1.41	61.51±3.54	81.00±4.24**	75.00±1.41*	61.00±4.24	70.00±2.83	78.90±0.71**	9/34
late luteal phase	61.00±4.24	67.00±9.90	76.50±3.54**	79.00±1.41**	69.00±1.41	69.00±2.41	76.00±2.83**	4/33
Monkey # 24								
mid luteal phase	59.00±12.73	64.50±6.36	76.50±2.12**	74.50±0.71**	56.50±2.12	62.00±2.83	78.50±0.70**	8/22
late luteal phase	64.00±5.66	67.00±9.90 NS.	76.00±2.83* NS.	77.00±4.24* NS.	66.00±8.49	70.50±2.12	76.50±4.95* NS.	5/21
luteolytic phase	52.50±3.54	55.00±4.24	55.00±7.07	58.00±5.66	54.00±2.83	54.00±8.49	57.50±6.36	0/25

24 = animal with short cycle length in the colony.

(** = P<0.01, * = P<0.05 significatly different, N.S. = non significantly different from the control)

Table.12 Effects of hCG, o-PRL and $PGF_{2\alpha}$ on % increment of P and E_2 of cynomolgus monkey luteal cells during 7 day incubation (mean ± S.E., n = 3).

Treatments / Stages	pmol/5x10^4 cells control	% increment of P			pmol/5x10^4 cells control	% increment of E_2			days prior to menses/ cycle length
		hCG	o-PRL	$PGF_{2\alpha}$		hCG	o-PRL	$PGF_{2\alpha}$	
#101									
early lp	32.0±4.8	98.4±17.9 *	5.0±1.1 NS	33.1±6.1 *	0.48±0.0	22.92±5.1 NS	-7.08±5.1	0.00±0.0	15/27
mid lp	254.9±31.2	37.7±7.6 *	4.5±0.9 NS	22.4±8.8 *	1.30±0.2	60.77±13.2 *	-2.31±1.1	6.15±2.1	8/30
late lp	92.9±12.1	102.1±24.2 *	-5.4±1.9 NS	-10.6±4.3 NS	1.07±0.0	28.97±11.9 *	-9.35±2.4	7.48±2.4	2/32
luteolytic p	5.1±1.1	0.0±0.0 NS	3.4±0.5	17.6±3.2 NS	0.52±0.1	-1.92±0.0 NS	0.00±0.0	-3.85±0.9	-2/33
#75									
early lp	47.4±5.2	168.4±48.3 **	1.9±0.9 NS	50.5±10.1 *	0.84±0.1	65.48±1.9 *	-5.95±1.9	7.14±2.2	10/45
mid lp	174.8±21.9	147.5±52.1 **	0.3±0.0 NS	22.14±2.1 *	1.27±0.1	101.57±41.2 **	3.15±0.9	7.09±2.1	9/34
late lp	129.3±16.6	69.8±12.3 *	2.8±1.0 NS	-10.9±1.9 NS	1.25±0.3	52.80±12.9 *	4.00±2.1	10.40±3.3	4/33
#24									
mid lp	18.4±3.8	485.9±71.2 **	9.8±1.2 NS	89.7±5.6 *	0.78±0.0	44.87±11.1 *	6.41±2.9	2.56±0.9	8/22
late lp	88.6±5.1	116.0±21.4 **	1.6±0.0 NS	77.2±18.9 *	1.56±0.1	28.85±8.9 *	1.28±0.0	3.21±1.2	5/21
luteolytic p	7.1±0.2	22.5±0.9 NS	-7.0±2.0 NS	-5.6±0.9 NS	0.94±0.0	21.28±0.9 NS	2.13±0.9	8.51±0.9	0/25

lp = luteal phase, ** = $P < 0.01$, * $P < 0.05$ significantly different, NS = non-significantly from the control.

Table.13 summarizing effect of hCG, PRL and $PGF_{2\alpha}$ on stimulation of cynomolgus monkey CL steroid production during 7 day incubation.

stages / treatments	early luteal phase		mid luteal phase		late luteal phase		luteolytic phase	
	E_2	P	E_2	P	E_2	P	E_2	P
Monkey # 101								
hCG	+	+	+	+	+	+	-	-
PRL	-	-	-	-	-	-	-	-
$PGF_{2\alpha}$	-	+	-	+	-	-	-	-
hCG + $PGF_{2\alpha}$		-		-		+[a]		
# 75								
hCG	+	+	+	+	+	+		
PRL	-	-	-	-	-	-		
$PGF_{2\alpha}$	-	+	-	+	-	-		
hCG + $PGF_{2\alpha}$						+[a]		
# 24								
hGC			+	+	+	+	-	-
PRL			-	-	-	-	-	-
$PGF_{2\alpha}$			-	+	-	+	-	-
hCG + $PCF_{2\alpha}$							-	

a = inhibition on hCG-stimulated P secretion of CL cells