Effects of FSH and LH on incorporation of [3 H]thymidine into follicular DNA*

S. K. Roy and G. S. Greenwald

Department of Physiology, Ralph L. Smith Research Center, University of Kansas Medical Center, Kansas City, Kansas 66103, U.S.A.

Summary. To assess the roles of FSH and LH on follicular growth, after various experimental manipulations, hamster follicles were sorted into 10 stages and incubated for 4 h with [3 H]thymidine. Stages 1–4 correspond to follicles with 1–4 layers of granulosa cells, respectively; Stage 5 = 5 or 6 layers of granulosa cells plus theca; Stage 6 = 7–8 layers of granulosa cells plus theca; Stage 7 = early formation of the antrum; Stages 8–10 = small, intermediate and large antral follicles, respectively.

Phenobarbitone sodium injected at 13:00 h on pro-oestrus blocked the normal rise of blood FSH and LH concentrations at 15:00 h and prevented the increase of [3 H]thymidine incorporation into follicles of Stages 1–9. The optimal treatment to reverse the effects of phenobarbitone was 1 μ g FSH and 2 μ g LH injected i.p. at 13:00 h which restored DNA replication to follicles of Stages 2–10: FSH acted primarily on Stages 2–5 and LH on Stages 5–10. Injection of phenobarbitone at 13:00 h on pro-oestrus followed by 2.5 μ g FSH at 22:00 h restored DNA synthesis by the next morning to follicles at Stages 1–8. In hamsters hypophysectomized at 09:00 h on the day of oestrus (Day 1), injection on Day 4 of 2.5 μ g FSH restored DNA synthesis 6 h later to Stage 2–6 follicles. Unilateral ovariectomy on Day 3 resulted 6 h later in an acute rise in FSH and LH and change of follicles from Stage 4 to Stage 5 but, paradoxically, there was decreased synthesis of DNA in follicles of Stages 5–10. The incorporation of [3 H]thymidine was at normal low levels for Stage 5–10 follicles by 24 h after unilateral ovariectomy with significant increases in follicles of Stages 1–4. We conclude that small preantral follicles are already dependent on FSH for DNA synthesis and LH action is manifested when the follicles acquire a theca (Stage 5 onwards) and when LH receptors appear in granulosa cells.

Introduction

We have recently developed a method to dissociate intact follicles from the hamster ovary by microdissection to recover large follicles and by an enzymic treatment to isolate small follicles (Roy & Greenwald, 1985). The follicles were classified into 10 stages based on histological criteria: Stages 1–4 consist of 1–4 layers of granulosa cells; Stage 5 has 5–6 layers of granulosa cells with early formation of thecal cells at the 6 layered follicle; Stage 6 has 7–8 layers of granulosa cells; Stage 7 shows incipient development of an antral cavity; Stages 8–10 are small, intermediate and large antral follicles, respectively. Using this methodology, follicular incorporation of [3 H]thymidine was observed throughout the oestrous cycle: there was a significant increase in incorporation for follicles at Stages 1–4 on the afternoon of pro-oestrus whereas follicles of Stages 1–8 showed a peak in incorporation throughout the morning and afternoon of oestrus (Roy & Greenwald, 1986). These

*Reprint requests to Dr G. S. Greenwald.

changes during the periovulatory period suggested that fluctuations in blood gonadotrophin concentrations might not only account for the recruitment of the next set of follicles destined to ovulate but also stimulate the growth of small preantral follicles. The present study was designed to test this possibility by using hamsters in which ovulation was blocked by injecting phenobarbitone at the onset of the critical period on the day of pro-oestrus (Siegel, Bast & Greenwald, 1976), which were hypophysectomized and subsequently treated with FSH (Moore & Greenwald, 1974) and those which were unilaterally ovariectomized (Bast & Greenwald, 1977).

Materials and Methods

Adult, golden hamsters were used after at least 3 consecutive 4-day oestrous cycles were monitored (Day 1 = oestrus; Day 4 = pro-oestrus). The animals were maintained under 14 h light:10 h dark (lights on 05:00 h). Three experiments were performed.

Experiment 1. On Day 4 13:00 h, 42 hamsters received a s.c. injection of phenobarbitone (phenobarbitone sodium, 6.5 mg/100 g body weight: Elkins Inc., Cherry Hill, NJ) and the animals were treated (6 animals per group) as follows:

Group 1, no further treatment and killed at Day 4 15:00 h;

Group 2, an i.p. injection of 1.0 or 2.5 µg FSH (NIH-ovine-FSH-S16) at Day 4 13:00 h and killed at Day 4 15:00 h;

Group 3, an i.p. injection of 2 µg LH (NIH-ovine-LH-S24) on Day 4 13:00 h and killed at Day 4 15:00 h;

Group 4, i.p. injections of 1.0 µg FSH and 2 µg LH at separate sites on Day 4 13:00 h and killed at Day 4 15:00 h;

Group 5, killed at Day 1 09:00 h;

Group 6, a s.c. injection of 2.5 µg FSH on Day 4 22:00 h, and killed at Day 1 09:00 h.

Untreated animals killed on Day 4 15:00 h and Day 1 09:00 h served as controls for the respective groups (6 per group). To check on the effects of phenobarbitone injected at 13:00 h on serum FSH and LH values, two groups of 6 animals each were used, one was injected with phenobarbitone, the other served as controls. Unanaesthetized animals were bled on Day 4 at 12:00, 14:00 and 15:00 h by cardiac puncture (300 µl blood withdrawn at each time) and trunk blood was saved after decapitation at 16:00 h. Serum progesterone concentrations were measured in 15:00 h samples by RIA for both groups.

Experiment 2. On Day 1 09:00 h, 18 hamsters were hypophysectomized via the parapharyngeal approach (Zarrow, Yochim & McCarthy, 1964). Six animals were killed on Day 4 09:00 h and at Day 4 15:00 h; the remaining 6 animals received a s.c. injection of 2.5 µg FSH on Day 4 09:00 h and were killed at Day 4 15:00 h. The sella turcica was examined and animals in which there were any pituitary fragments were discarded. Two intact control groups (N = 6) were killed on Day 4 09:00 h and 15:00 h, respectively.

Experiment 3. On Day 3 09:00 h, under ether anaesthesia, alternate right and left ovaries were removed from 24 hamsters via a dorsal mid-line incision. Two ovaries from two different animals of the same group were used to isolate follicles and these ovaries served as time zero controls. The rationale for pooling two ovaries is that this was the minimal number needed to yield enough follicles for in-vitro incubation with [³H]thymidine (see below). Unilaterally ovariectomized and control (not sham-operated) hamsters were decapitated at Day 3 15:00 h or Day 4 09:00 h (12 for each group). To assess changes in gonadotrophins after unilateral ovariectomy, trunk blood was assayed for FSH and LH. Serum FSH was measured by a rat-rat assay system originally described by Bast & Greenwald (1974) for the hamster and LH was assayed by an ovine-ovine assay method described previously (Siegel *et al.*, 1976). The gonadotrophin concentrations were expressed in terms of NIAMDD rat

FSH-RP-1 ($2.1 \times \text{NIH-FSH-S1}$) and rat LH-RP-2 ($0.03 \times \text{NIH-LH-S1}$), respectively. The sensitivity of the rat-rat radioimmunoassay for FSH was 0.045 ng/tube and 0.0195 ng/tube for LH in the ovine-ovine assay. The intra-assay coefficient of variation was 9.8% for FSH and 10.8% for LH and the samples were run in a single assay for each hormone. The lower limit of sensitivity in the progesterone assay was 5 pg/tube . Serum from chronically hypophysectomized and ovariectomized hamsters contained $0.6 \text{ ng immunoreactive progesterone/ml}$. The intra-assay coefficient of variation for progesterone was 5.2%.

Follicular separation and in-vitro incubation with [^3H]thymidine. Follicles in different stages of development were isolated from the ovaries as described previously (Roy & Greenwald, 1985). Briefly, ovaries were cleared of extraneous fat and follicles from Stages 6–10 (preantral follicles with well developed theca to antral stages) were dissected by hand under a dissecting microscope. The rest of the ovaries (pair per animal) were minced and incubated in a mixture of collagenase, DNase and pronase in Krebs' Ringer with Hepes (KRHG), pH 7 for 20 min at 37°C . The suspension was centrifuged at $45 g$ and the pellet resuspended in Ca^{2+} - Mg^{2+} -free KRHG with 1% bovine serum albumin. Follicles at different stages of development were harvested with precalibrated pipettes (Roy & Greenwald, 1985).

Follicles of all stages from one animal (with the exception of those in Exp. 3) were incubated in 1 ml KRHG with 1% BSA in conical polystyrene tubes without capping at 37°C for 4 h in a Dubnoff metabolic shaker in the presence of $0.5 \mu\text{Ci}$ [^3H]thymidine (sp. act. 29 Ci/mmol ; Amersham, Arlington Heights, IL). The incubation was terminated by adding $50 \mu\text{g}$ unlabelled thymidine and placing the tubes in an ice-bath. The medium was removed after centrifugation at $2300 g$ at 4°C and follicles were washed twice with fresh medium and finally resuspended in DNA assay buffer, pH 7.0. Follicles were processed for counting of radioactivity and DNA was assayed as described by Roy & Greenwald (1986). The results were expressed as $\text{pg thymidine per } \mu\text{g DNA}$. All data were analysed statistically using analysis of variance with the help of a Biomedical Data Package (BMDP) program and Duncan's multiple range test; the level of significance was at 5%.

Results

Experiment 1: effects of injecting phenobarbitone at pro-oestrus, with or without gonadotrophins

In control serially bled hamsters, serum FSH increased at 15:00 h and peaked at 16:00 h while LH rose to significantly higher levels by 15:00 h and was maximal at 16:00 h (Table 1). However, injection of phenobarbitone on Day 4 at 13:00 h prevented the normal rise in serum FSH and LH.

Table 1. Serum concentrations of FSH, LH and progesterone in hamsters treated with or without phenobarbitone at Day 4 13:00 h

Hormone measured (ng/ml)	Group	12:00 h	14:00 h	15:00 h	16:00 h
FSH	Control		$495 \pm 158 (7)$	$*653 \pm 43 (5)$	$*1575 \pm 104 (6)$
	Phenobarbitone	$387 \pm 32 (11)$	$419 \pm 43 (5)$	$324 \pm 44 (6)$	$248 \pm 45 (5)$
LH	Control		$*13.7 \pm 0.4 (4)$	$*132.5 \pm 6 (4)$	$*171 \pm 2.7 (4)$
	Phenobarbitone	$7.3 \pm 0.8 (6)$	$6 \pm 0.6 (4)$	$8.6 \pm 0.7 (4)$	$9 \pm 0.5 (4)$
Progesterone	Control	—	—	$7.6 \pm 1.3 (6)$	—
	Phenobarbitone	—	—	$2.0 \pm 0.7 (6)$	—

Values are mean $\pm$ s.e.m. for the number of animals indicated in parentheses.

* $P < 0.05$ compared to 12:00 h value.

The suppression of the preovulatory LH surge by phenobarbitone was further substantiated by a concomitant deficiency in serum progesterone (Table 1). The phenobarbitone block of gonadotrophin release was associated with a significant fall in the incorporation of [3 H]thymidine into DNA of follicles at Stages 1–9 but with no change in Stage 10 (Fig. 1). Injection of 2.5 μ g FSH with phenobarbitone increased thymidine incorporation at 15:00 h for all classes of follicles except for Stages 1 and 9. Very few Stage 9 follicles were present and hence few were available for incubation. The FSH preparation used contains 4% LH contamination according to the NIADDK. When the

Fig. 1. Incorporation of [3 H]thymidine into DNA of follicles at different stages of development on Day 4 15:00 h in hamsters treated with or without phenobarbitone, FSH and or LH (at 13:00 h). Within-stage comparisons were done between control and phenobarbitone groups (a–a) and between phenobarbitone and hormone replacement groups (b–b), respectively. Only significantly different ($P < 0.05$) groups are designated by a common superscript. Follicular stages are as defined in 'Introduction'. Each bar represents a mean $\pm$ s.e.m. from 6 hamsters.

Table 2. Incorporation of [³H]thymidine into follicular DNA (pg [³H]thymidine/ μ g DNA/4 h) from hamsters treated with phenobarbitone* with or without FSH†

Treatment	Stages of follicular development									
	1	2	3	4	5	6	7	8	9	10
Day 1, 09:00 h (control)	74 $\pm$ 7 ^a	51 $\pm$ 9 ^{a,b}	30 $\pm$ 3 ^a	23 $\pm$ 3 ^a	34 $\pm$ 3 ^a	31 $\pm$ 5 ^a	22 $\pm$ 2 ^a	19 $\pm$ 3 ^a		
Phenobarbitone	47 $\pm$ 7 ^{a,b}	15 $\pm$ 2 ^a	14 $\pm$ 4 ^{a,b}	10 $\pm$ 1 ^{a,b}	8 $\pm$ 1 ^{a,b}	10 $\pm$ 2 ^{a,b}	15 $\pm$ 2 ^{a,b}	12 $\pm$ 1 ^b	4 $\pm$ 1 ^a	5 $\pm$ 1 ^a
Phenobarbitone + 2.5 μ g FSH	69 $\pm$ 11 ^b	17 $\pm$ 1 ^b	21 $\pm$ 2 ^b	30 $\pm$ 2 ^b	18 $\pm$ 3 ^b	24 $\pm$ 4 ^b	25 $\pm$ 1 ^b	29 $\pm$ 3 ^{a,b}	29 $\pm$ 3 ^a	13 $\pm$ 0.5 ^a

Values are mean $\pm$ s.e.m. for 6 animals in each group.

*Phenobarbitone was injected at 13:00 h on Day 4 (pro-oestrus); † FSH was injected s.c. at 22:00 h on pro-oestrus and the animals were killed on Day 1 (oestrus) at 09:00 h.

Within stages, values with common superscript letters are significantly different, $P < 0.05$.

Table 3. Incorporation of [³H]thymidine into follicular DNA (pg [³H]thymidine/ μ g DNA/4 h) from unilaterally ovariectomized* hamsters

Day of cycle	Stages of follicular development									
	1	2	3	4	5	6	7	8	9	10
Day 3 09:00 h (control)	57 $\pm$ 7	22 $\pm$ 5	19 $\pm$ 3	10 $\pm$ 1	10 $\pm$ 3	11 $\pm$ 2	10 $\pm$ 3	4 $\pm$ 1	2 $\pm$ 1	2 $\pm$ 0.5
Day 3 15:00 h (control)	44 $\pm$ 4	38 $\pm$ 6 ^a	25 $\pm$ 5 ^a	20 $\pm$ 3	17 $\pm$ 3 ^a	16 $\pm$ 2 ^a	10 $\pm$ 1 ^a	6 $\pm$ 1 ^a	4 $\pm$ 0.5 ^a	2 $\pm$ 0.2 ^a
Day 3 15:00 h (ULO)	57 $\pm$ 12	10 $\pm$ 1 ^a	17 $\pm$ 3 ^a	15 $\pm$ 2	6 $\pm$ 1 ^a	7 $\pm$ 1 ^a	6 $\pm$ 1 ^a	1 $\pm$ 3 ^a	1 $\pm$ 2 ^a	1 $\pm$ 2 ^a
Day 4 09:00 h (control)	24 $\pm$ 2 ^a	11 $\pm$ 1 ^b	10 $\pm$ 1 ^b	8 $\pm$ 2 ^a	9 $\pm$ 1	5 $\pm$ 1	4 $\pm$ 1	1 $\pm$ 0.4	0.5 $\pm$ 0.1	3 $\pm$ 0.7
Day 4 09:00 h (ULO)	86 $\pm$ 1 ^a	25 $\pm$ 4 ^b	25 $\pm$ 3 ^b	21 $\pm$ 3 ^a	4 $\pm$ 0.5	5 $\pm$ 1	3 $\pm$ 0.6	1 $\pm$ 0.1	1 $\pm$ 0.1	2 $\pm$ 0.3

Values are mean $\pm$ s.e.m. for 6 animals in each group (see text for details).

*Unilateral ovariectomy (ULO) was performed on Day 3 at 09:00 h.

For each stage at Day 3, 15:00 h and Day 4, 09:00 h values with common superscript letters are significantly different, $P < 0.05$.

Fig. 2. Incorporation of [^3H]thymidine into follicular DNA in hypophysectomized hamsters on Day 4 15:00 h with or without FSH treatment at Day 4 09:00 h. The hypophysectomized group was compared with control and FSH-treated groups, respectively. Only significantly different ($P < 0.05$) values are designated by asterisks. Each bar is mean $\pm$ s.e.m. from 6 hamsters.

dose of FSH was reduced to 1 μg and injected with phenobarbitone, FSH action was restricted to follicles up to Stage 5. However, 2 μg LH plus phenobarbitone resulted in significant increases in DNA synthesis in follicles of Stages 5–7 and 10 (Fig. 1). After LH treatment no increase in [^3H]thymidine was observed in follicles below Stage 5. The FSH contamination in the ovine LH preparation is less than 0.5% by weight. Therefore, 2 μg LH contains at most 10 ng FSH activity. Concurrent injection of 1 μg FSH and 2 μg LH to phenobarbitone-treated hamsters restored follicular DNA synthesis to all stages except for Stages 1 and 9. In the doses used, FSH and LH raised [^3H]thymidine incorporation by Stage 10 follicles to levels far in excess of normal values (Fig. 1). For Stage 1 follicles (1 layer of granulosa cells), none of the hormonal regimens was able to restore follicular DNA synthesis to control levels. Stage 3 follicles showed a marked but borderline increase in [^3H]thymidine incorporation in response to 1 μg FSH.

In the next part of this experiment, after injecting phenobarbitone at Day 4 13:00 h, 2.5 μg FSH was injected s.c. at Day 4 22:00 h. This resulted, the next morning, in significant increases in [^3H]thymidine incorporation by follicles of Stages 1–10 (except for Stage 2) especially for Stages 9 and 10 which had not ovulated because of the phenobarbitone treatment (Table 2). Stage 1 follicles showed DNA synthesis comparable to that of controls at Day 1 09:00 h.

Experiment 2: effects of hypophysectomy and FSH treatment

Hypophysectomy at Day 1 09:00 h arrested follicular development on the morning at Day 4 to

Stages 1–6 and there were no significant differences in DNA synthesis compared to intact control animals (Fig. 2). After the surges of FSH and LH on the afternoon of Day 4, Stage 1–4 follicles of intact hamsters showed dramatic increases in [^3H]thymidine incorporation in agreement with the previous results (compare Figs 1 & 2). For the untreated hypophysectomized hamsters, however, DNA synthesis did not differ between 09:00 h and 15:00 h on Day 4. The s.c. injection of 2.5 μg FSH at Day 4 09:00 h resulted 6 h later in significant increases in [^3H]thymidine for all stages of follicular development except for Stage 1. In contrast to intact controls, for the FSH injected hypophysectomized hamsters there were marked increases in DNA synthesis in follicles at Stages 5 and 6 follicles (Fig. 2).

Experiment 3: effects of unilateral ovariectomy

Unilateral ovariectomy at Day 3 09:00 h resulted 6 h later in significant increases in serum FSH and LH (controls: 173 ± 26 ng FSH/ml; 28 ± 0.8 ng LH/ml; unilaterally ovariectomized: 416 ± 42 ng FSH/ml; 58 ± 9 ng LH/ml). Although a significant mobilization and shift in follicular numbers between Stages 4 and 5 was evident by Day 3, 15:00 h (Chiras & Greenwald, 1978a; Roy & Greenwald, 1985), incorporation of [^3H]thymidine declined significantly for Stages 5–10 compared to controls (Table 3). By 24 h after operation, incorporation into follicular DNA increased significantly for Stages 1–4 compared to control and was comparable to the normal low levels for the larger stages in intact animals except for Stage 10 (Table 3).

Discussion

The first experiment (Fig. 1) confirmed our previous finding that the pro-oestrous surge of gonadotrophins initiates an increase in DNA synthesis in follicles, especially in the smaller preantral stages (Roy & Greenwald, 1986). The ability of phenobarbitone to block the gonadotrophin surges provided an excellent approach to analyse the roles of FSH and LH in the periovulatory period. In response to phenobarbitone DNA replication was reduced in follicles of all sizes except for the large antral follicles (Stage 10), the group destined to ovulate the next morning (Fig. 1). It is likely that cells of the large preovulatory follicles were already differentiating and had ceased active multiplication. Absence of surge levels of gonadotrophins therefore had no effect on cell proliferation of Stage 10 follicles.

The LH contamination in 2.5 μg ovine FSH is about 100 ng and this dose of FSH was able to restore DNA synthesis for Stages 2–8 and with an overshoot response by Stage 10 follicles. However, with 1 μg FSH only Stage 3–5 follicles overcame the blocking action of phenobarbitone; evidently LH contamination was no longer a factor. In contrast, 2 μg LH was effective in follicles of Stage 5 and greater. Stage 5 follicles probably responded to both FSH and LH because they represent a transitional group with 5 and 6 layers of granulosa cells and early differentiation of the theca interna. Stage 9 follicles are intermediate antral follicles, few in number and usually atretic and this was reflected in their inability to respond to any dose or combination of FSH and LH.

Topical autoradiography of the hamster ovary has shown that binding sites for FSH, hCG and prolactin are present in the granulosa cells of large preantral and antral follicles with FSH receptors absent from theca cells (Oxberry & Greenwald, 1982). Hence, the effects of LH on follicles from Stage 5 and onwards can be exerted on both granulosa and thecal cells.

The secondary rise in serum FSH, beginning late on Day 4 and continuing through the afternoon of Day 1, is responsible for the recruitment of the large preantral follicles that will ovulate 4 days later (Greenwald & Siegel, 1982). This concept is reinforced by the present study: injection of 2.5 μg FSH (with significant LH contamination) at 22:00 h on pro-oestrus overcame the phenobarbitone block and DNA synthesis at oestrus was restored to the normal range for Stage 1–8 follicles. Stage 9 and 10 follicles, which normally would have degenerated or ovulated, showed significant increases

in DNA synthesis, perhaps because of the prolonged period of FSH action for 11 h (Table 2). It is possible that some of the 'old' stage 9 follicles were replaced by younger follicles from the pool of stages 7 and 8.

Since ovarian follicles in intact animals are constantly exposed to low tonic levels of FSH and LH, our study was extended to the hypophysectomized hamster in which follicles are presumably deprived of circulating gonadotrophins (but see below). Follicles with fewer than 7–8 layers of granulosa cells lack LH receptors in granulosa cells (hamster: Oxberry & Greenwald, 1982; rat: Bortolussi, Marini & Reolon, 1979). Therefore, the effect of FSH (i.p.) alone was tested in the hypophysectomized hamster. This treatment led to increase of [^3H]thymidine incorporation in follicles of Stages 2–6 within 6 h although it did not stimulate DNA synthesis in Stage 1 follicles (Fig. 2). This corroborates previous autoradiographic findings that, in the hypophysectomized hamster, FSH but not LH stimulates follicular development and thymidine uptake, as well as labelling index and intensity, especially in follicles with 2–3 layers of granulosa cells (Chiras & Greenwald, 1978b); in the latter study Stage 1 follicles were not counted. Thus, in conjunction with the present results it is evident that periovulatory changes in FSH affect follicular recruitment and growth of all stages of development. The striking increase in [^3H]thymidine incorporation in Stage 5 and 6 follicles in hypophysectomized hamsters after FSH treatment agrees with our view that the normal endogenous surge of LH at pro-oestrus dampens the action of FSH in stimulating DNA synthesis of larger follicles. At first glance, it is surprising that 4 days after hypophysectomy there is still considerable incorporation of [^3H]thymidine by Stage 1–6 follicles (Fig. 2). However, although serum FSH is undetectable by Day 4 in untreated hypophysectomized hamsters, the binding affinity for FSH by small preantral follicles is enhanced and the number of occupied FSH receptors is high (Kim & Greenwald, 1986). Thus, 3 days after hypophysectomy, the follicles are still being influenced by FSH already present on the receptor sites.

The normal pro-oestrous synthesis of DNA was not restored to Stage 1 follicles by FSH treatment for 2–6 h in Exps 1 and 2 although Stages 2–4 responded quite nicely. However, after s.c. injection of FSH on the evening of Day 4, DNA synthesis was increased in stage 1 follicles 11 h later. This suggests that other factors may be necessary to account for the dramatic increase in [^3H]thymidine incorporation by the primary follicles between 09:00 h and 15:00 h on Day 4.

In all polytocous mammals that have been investigated, unilateral ovariectomy results in prompt follicular compensation from the remaining ovary. As found by Bast & Greenwald (1977), unilateral ovariectomy resulted within 6 h in significant increases in both serum FSH and LH. This was accompanied by a rapid shift of follicles from Stages 4 to 5 (Roy & Greenwald, 1985). However, on Day 3, 15:00 h there was actually a significant decrease in [^3H]thymidine incorporation into DNA for Stages 5–10 (Table 3). Using autoradiography as an endpoint, at 4–10 h after unilateral ovariectomy on Day 3 follicles with 2–5 layers of granulosa showed no increase in numbers or percentage labelled (Chiras & Greenwald, 1978a). It is possible that the concomitant rise after unilateral ovariectomy in serum LH counteracts the stimulating effect of FSH on cellular DNA synthesis. For example, PMSG, which has both FSH- and LH-like actions, causes follicular recruitment in the hypophysectomized hamster but diminishes uptake of [^3H]thymidine (Chiras & Greenwald, 1978b). LH also has been shown to reduce the in-vitro uptake of [^{14}C]thymidine by the mouse ovary (How, Chaplin & Ryle, 1970). We therefore suggest that, whereas FSH stimulates DNA synthesis (S phase), LH exerts a positive influence on cell mitosis (M phase) leading to the transformation of preantral to antral follicles. It is quite possible that the granulosa cells of rapidly growing follicles enter the S phase before 6 h after unilateral ovariectomy. Hence, examination at 1–3 h after unilateral ovariectomy might have increased the percentage of granulosa cells entering into the S phase. This is consistent with the observation that the doubling time for follicles can be as short as 6 h, after in-vitro exposure to pulsatile LH (Peluso, Luttmer & Gruenberg, 1984). After unilateral ovariectomy, the long term effect (24 h) on Stage 1–4 follicles is to stimulate significantly [^3H]thymidine incorporation into follicular DNA (Table 3). This is most probably due to sustained elevation of serum FSH concentration until 23:00 h on Day 3 accompanied by low values of LH

(Bast & Greenwald, 1977). In the intact pro-oestrous hamster, follicles at stages 5–8 (the developing follicles recruited to ovulate) do not show increased DNA synthesis until the morning of Day 1. Evidently, the high FSH and LH levels on the afternoon of pro-oestrus exert antagonistic actions and it is only when the FSH:LH ratio swings widely in favour of FSH that DNA synthetic activity is manifested.

S.K.R. was supported by a grant from the Rockefeller Foundation. The research was supported by NIH (HD00596). We thank the National Hormone Pituitary Program (NIADDK) for providing ovine FSH and LH and the RIA kit for FSH.

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Received 13 January 1986