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Ovarian Steroidogenesis in the Proestrous Hamster

SRINIVAS K. SAIDAPUR[1] and GILBERT S. GREENWALD[2]

Department of Physiology,
University of Kansas Medical Center,
Kansas City, Kansas 66103

ABSTRACT

In the proestrous hamster, in response to the LH surge, there is a dramatic and sustained increase in serum progesterone (P) and transitory increases in testosterone (T), estrone (E_1) and estradiol-17β (E_2). Ovarian steroidogenesis in the proestrous hamster was analyzed in detail by studying: 1) concentrations of these steroids in the whole ovary, antral follicles (AF) and the nonantral follicular portion of the ovary (NAO) at 1200 h (prior to the LH surge), 1500 h (during the LH surge) and 1800 h (after the LH surge); 2) *in vitro* steroidogenic production by the AF and NAO, as well as the whole ovary (removed before 1200 h, 1500 h and 1800 h and 3) *in vitro* effects of LH, FSH and P on steroidogenesis of ovaries removed before the LH surge and incubated for 2 h.

P concentration was the same in AF and NAO at 1200 h but increased slightly in the AF with the onset of the LH surge. Both the concentration and production rate of P increased from a minimum at 1200 h (1 ng/mg/h) to a maximum by 1800 h (30 ng/mg/h) in the ovary, AF and NAO. On the other hand, the concentration of T, E_1 and E_2 was selectively greater in the AF at all times. Maximum T production occurred in the ovaries removed during the LH surge (150 pg/mg/h) followed by a significant decline by 1800 h. However, AF produced negligible amounts of T at all times. In the NAO, the production of T was essentially the same (about 20 pg/mg/h) in all 3 incubations. The production of E_1 and E_2 was much greater in the AF than in the NAO, especially between 1200-1500 h. By 1800 h, the levels and synthetic capacity for estrogens in the ovary, AF and NAO declined to baseline levels, while the production of P was still very high. These studies indicate that in the proestrous hamster, steroidogenesis in the NAO which largely represents the interstitial compartment is limited to the production of P and androgens and only a negligible fraction passes down the pathway to form estrogens. On the other hand, steroidogenesis is more complete in the AF where it can proceed efficiently as far as estrogens.

LH (5–250 ng/ml) added *in vitro* increased the production rate of all steroids whereas 1 ng LH increased only the synthesis of E_2. FSH (100–200 ng/ml) also stimulated E_2 production but not E_1. Higher concentration of FSH (250 ng/ml) resulted in overall increases in P, T, E_1 and E_2 possibly due to contamination with LH. Exogenous P (10, 100 ng/ml) had no effect on T or E_1 synthesis, but E_2 production was enhanced.

INTRODUCTION

The past 15 years have witnessed a great deal of research related to steroidogenesis by the mammalian ovary. Several excellent reviews have appeared on this subject (Short, 1964; Savard et al., 1965; Armstrong, 1968; Yoshinaga, 1973; Baird, 1977; Armstrong and Dorrington, 1977; Channing and Tsafriri, 1977; Dorrington, 1977). There are numerous reports on steroid levels in peripheral and ovarian venous blood and in the ovary *per se* for several laboratory species and for the human; the references can be found in the above cited reviews. Information concerning the ability of different ovarian cell types to synthesize steroid hormones is often approached by *in vitro* incubation and/or studies of isolated components in culture. While it is generally agreed that antral follicles are the main source of estrogens, the roles played by theca interna and granulosa cells are still controversial and often confusing. The relative contribution of antral follicles and other compartments of the ovary, for instance, interstitial gland cells, to different steroid hormones during the estrous cycle of rodents as well as other species is not clear.

In the hamster, the ability of corpora lutea (CL) and nonluteal ovary *in vitro* to synthesize P, T, E_1 and E_2 during the estrous cycle has

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[1] Permanent address: Department of Zoology, Karnatak University, Dharwar-580 003, India.

[2] Address reprint requests to: Dr. G. S. Greenwald, Department of Physiology, University of Kansas Medical Center, Kansas City, Kansas 66103.

been studied previously (Leavitt et al., 1973; Terranova et al., 1978). Similarly, isolated cell types from the hamster antral follicle have been shown to produce P, T, E_1 and E_2 (Makris and Ryan, 1975).

The pattern of changes in peripheral blood and ovarian levels of P, T, E_1 and E_2 during the estrous cycle of hamsters is now well established (references in Saidapur and Greenwald, 1978a). In the hamster, marked changes in gonadotropin levels (Bast and Greenwald, 1974) and steroid hormones (Saidapur and Greenwald, 1978a) occur on Day 4 (proestrus) of the cycle. The composite pattern of steroid and gonadotropins (Fig. 1) on proestrus in the hamster poses several interesting problems: 1) what compartments of the ovary are involved in steroidogenesis; 2) what factors are involved in "turning off" T and E_2 at 1500–1600 h and 3) what are the *in vitro* effects on ovarian steroidogenesis of LH, FSH and progesterone. The present studies were therefore designed to shed light on antral follicular vs interstitial steroidogenesis and the role of gonadotropins in *in vitro* ovarian steroidogenesis in the proestrous hamster.

MATERIALS AND METHODS

Adult female golden hamsters (*Mesocricetus auratus*) weighing 80–120 g and maintained on a 14 h light: 10 h dark schedule were used after 3–4 consecutive 4 day cycles. Day 1 is defined as the day of ovulation and Day 4 corresponds to proestrus. Day 4 animals were used in all experiments. Hamsters were killed at specified times by decapitation and the trunk blood was saved for steroid hormone assays. The ovaries were excised immediately and placed in ice-cold saline until freed of periovarian fat under a dissecting microscope.

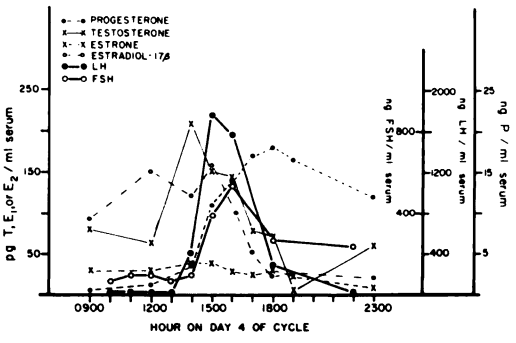

FIG. 1. Shows the interrelationship between the steroid hormones (P, T, E_1 and E_2) and the gonadotropins (LH and FSH) on Day 4 (proestrus) of the hamster cycle. The data on steroid levels is adapted from Saidapur and Greenwald (1978a), whereas the data on LH (ovine-ovine assay) and FSH is from S. K. Saidapur and G. S. Greenwald, unpublished.

Relative Concentration of Steroid Hormones in the Ovary, Antral Follicle and the Nonantral Ovary at Different Times on Day 4 of the Cycle

One ovary was weighed to the nearest 0.01 mg and stored at -20°C in vials containing 0.4 ml 95% alcohol. From the other ovary, all antral follicles (AF) were carefully dissected out using fine forceps and needles. The nonantral follicular portion of the ovary (NAO) and AF were weighed and likewise stored in alcohol. Animals were killed at 1145 h (before the onset of LH surge), 1445 h (60–90 min after the onset of LH surge) and at 1745 h (after the LH surge). The tissues were then processed for RIAs of steroid as described earlier (Saidapur and Greenwald, 1978a; Terranova and Greenwald, 1978).

Steroid Hormone Production by the Whole Ovary at 1200 h, 1500 h and 1800 h on Day 4 of the Cycle

Ovaries of animals killed at 1145 h, 1445 h and 1745 h were used. One ovary from each animal was weighed and stored in alcohol to determine the initial concentration of steroids. The other ovary was weighed and incubated in 1 ml Krebs Ringer bicarbonate buffer (KRB) at 37°C in an atmosphere of oxygen-carbon dioxide (95% O_2:5% CO_2) in a shaker bath for 2 h. Details of the incubation technique are described in an earlier paper from this laboratory (Terranova et al., 1978). At the end of incubation, the media were snap frozen and stored at -20°C. The tissues were washed gently using cold saline and stored in alcohol. The tissues and media were then processed for RIAs of steroids.

Steroid Hormone Production by AF and NAO at 1200 h, 1500 h and 1800 h on Day 4 of the Cycle

AF and NAO obtained as described in experiment 1 were incubated and processed for RIAs of steroids. At 3 different times, 2 h incubations were carried out as in experiment 2.

Effect of LH, FSH and P on Ovarian Steroidogenesis in vitro

Experiments with added LH (NIH-S-18), FSH (NIH-S-10) or P in the incubation medium were carried out using whole ovaries obtained from animals killed at 1145 h on Day 4 of the cycle (before the onset of the LH surge). The hormones were dissolved in KRB and added to vials containing one ovary and incubated. The concentrations of hormones used are shown in Tables 1–3. After a 2 h incubation, the tissues and media were processed for the determination of P, T, E_1 and E_2. The final volume of incubation media in these three experiments was also 1 ml as in the other incubations described above. Ovaries incubated without any hormone served as controls.

Calculations

The production rate of a given steroid hormone was calculated using the following formula:

$$\text{Production rate/hour} = \frac{(A + B) - C}{2}$$

where A = steroid content/mg tissue after incubation, B = steroid content of the medium/mg tissue incubated and C = mean steroid content/mg tissue before incubation.

Statistical Analysis

Statistical significance was determined by comparing A + B vs C (see above) using Student's t test. Data pertaining to changes in the steroid concentration and production at different times in the whole ovary, AF and NAO was analyzed by one way analysis of variance, whereas, the steroid production in the AF and NAO at different times was compared by two way analysis of variance. Specific differences were determined by Duncan's Multiple Range test (Steel and Torrie, 1960) following the analysis of variance. Differences were adjudged significant if $P<0.05$.

RESULTS

Progesterone

At any given time the concentration of P was about the same in unincubated ovary, AF and NAO (Fig. 2). However, two way analysis of variance indicated that at 1500 h and 1800 h the concentration of P was higher in AF ($P<0.05$) than in NAO. In the ovaries removed prior to the LH surge (1200 h) P was at the base line level of about 1 ng/mg of tissue. By 1500 h, P levels dramatically increased ($P<0.05$) to about 25 ng/mg, reaching a peak ($P<0.05$) by 1800 h to about 30–40 ng/mg of tissue.

Production of P was minimal (1 ng/mg/h) in the ovaries removed prior to the onset of endogenous LH surge (1200 h). On the other hand, ovaries removed during (1500 h) and after (1800 h) the LH surge produced 20 times ($P<0.05$) more P at 1500 h (20 ng/mg/h) and about 30 times ($P<0.05$) more at 1800 h (30 ng/mg/h) (Fig. 2). Production of P in both AF and NAO was the same at any given time (Fig. 2). The production of P was minimal at 1200 h followed by a significant rise ($P<0.05$) at 1500 h, reaching a peak ($P<0.05$) by 1800 h. The pattern of P production in isolated components of the ovary, the AF and the NAO was similar to that found when the whole ovary was incubated (Fig. 2).

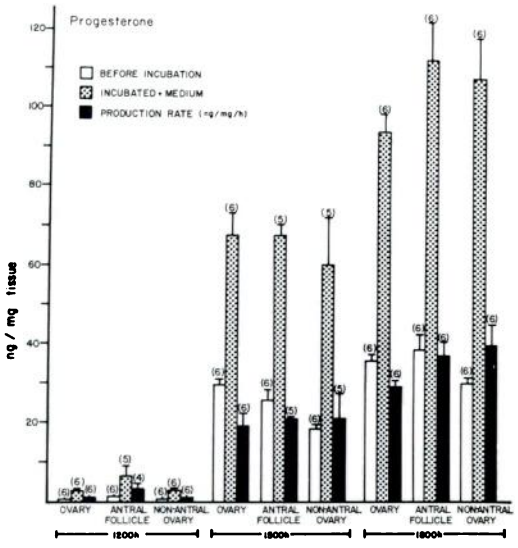

FIG. 2. Shows the initial concentration of P (before incubation), P produced in the tissue and the medium at the end of the incubation and the production rate of P in the ovary, AF and NAO at the specified times.

Testosterone

Unlike P, the initial concentrations of T in AF and NAO were distinctly different. Testosterone was 3–4 times greater in the AF at 1200 h and an even higher level was reached by 1500 h. The concentration of T increased at 1500 h to its peak in both AF ($P<0.05$) and NAO ($P<0.05$) and thus in the ovary ($P<0.05$) as a whole (Fig. 3). This was, however, followed by a sharp decline.

Testosterone production (Fig. 3) seemed to follow the pattern of P, in that it increased from a minimal rate at 1200 h (in fact, 2 ovaries did not produce any T) to a peak (150

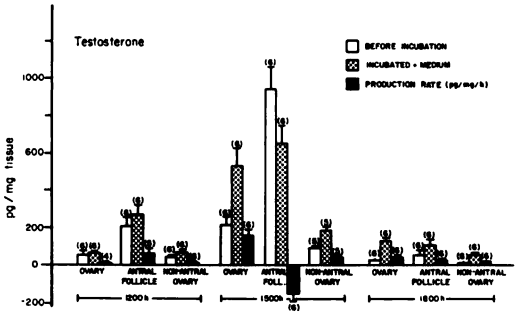

FIG. 3. Shows the initial concentration of T (before incubation), T produced in the tissue and the medium at the end of the incubation and the production rate of T in the ovary, AF and NAO at the specified times.

pg/mg/h) at 1500 h ($P<0.05$). However, at 1800 h, unlike P production which was maximal, T production dropped significantly ($P<0.05$) compared to 1500 h, but was similar to the production rate of 1200 h incubations.

Production rate of T in NAO was the same whether the ovaries were removed before, during or after the LH surge (Fig. 3). Similarly, T production in AF at 1200 h and 1800 h incubations was the same. In contrast, at 1500 h there was no production of T in AF and the production rate was significantly different ($P<0.05$) compared to the other times.

Estrone

The concentrations of E_1 were highest at 1200 h in both compartments of the ovary. These high levels were maintained until 1500 h in AF and in the ovary as a whole but not in the NAO (Fig. 4). Estrone levels at 1500 h were much lower in the NAO ($P<0.05$) compared to the AF. However, E_1 concentration declined markedly by 1800 h in the AF ($P<0.05$), the NAO and the entire ovary ($P<0.05$).

Production of E_1 was the same in ovaries at 1200 and 1500 h (Fig. 4). However, by 1800 h production rate of E_1 declined markedly ($P<0.05$). The production rate of E_1 was always significantly higher by AF than NAO (Fig. 4). There was a progressive decline in E_1 production in the NAO from 1200 h to 1500 h ($P<0.05$) and baseline levels were reached by 1800 h (5 pg/mg/h). On the other hand, E_1 production in the AF was higher at 1500 h ($P<0.05$) than at 1200 h and 1800 h.

Estradiol-17β

Like T and E_1, initial concentrations of E_2 were higher in AF. The E_2 levels were high in the ovary and AF, at 1200 h and 1500 h (Fig. 5) but declined markedly thereafter to baseline values by 1800 h ($P<0.05$). However, the AF still contained higher E_2 concentrations compared to the NAO. The decline in E_2 levels began as early as 1500 h ($P<0.05$) in NAO. In general, the pattern of changes in E_2 concentrations in the AF and the NAO was similar to that of E_1, while the actual concentration of E_2 was much higher than E_1.

While E_2 production was the same in 1200 h and 1800 h incubations, its production rate was 5–6 times greater in the ovaries incubated at 1500 h (Fig. 5). The E_2 production in NAO (Fig. 5) was the same at 1200 h and 1500 h but declined significantly thereafter. In AF, maximum E_2 production occurred at 1500 h followed by a marked decline at 1800 h. At any given time, E_2 production by the AF was significantly greater than in the NAO.

Effects of LH, FSH and P on Ovarian Steroidogenesis in vitro

Addition of LH (5–250 ng/ml) to the incubation medium had a stimulatory effect on P, T, E_1 and E_2 production *in vitro*, whereas

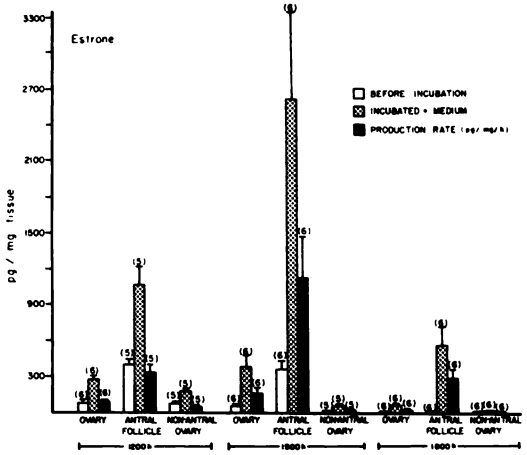

FIG. 4. Shows the initial concentration of E_1 (before incubation), E_1 produced in the tissue and the medium at the end of the incubation and the production rate of E_1 in the ovary, AF and NAO at the specified times.

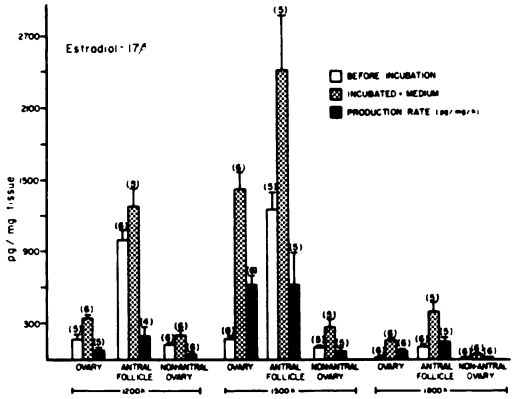

FIG. 5. Shows the initial concentration of E_2 (before incubation), E_2 produced in the tissue and the medium at the end of the incubation and the production rate of E_2 in the ovary, AF and NAO at the specified times.

LH at a concentration of 1 ng/ml enhanced only E_2 production significantly ($P<0.05$; Table 1). Five ng LH was as effective as 25 ng LH in stimulating overall steroidogenesis. However, 250 ng LH had a distinctly greater effect on the production rate of all of the steroids studied.

While P and E_1 production was not altered by 100 or 200 ng FSH, the production of T declined (Table 2). However, there was approximately a 3-fold increase ($P<0.05$) in E_2 production. Increasing the amount of FSH to 250 ng/ml caused a significant overall increase in the production rates of all steroids.

Addition of 10–100 ng P to the incubation media had no effect on T and E_1 production but enhanced E_2 production significantly ($P<0.05$) (Table 3).

DISCUSSION

Several previous studies have indicated that corpora lutea on Day 4 of the hamster cycle are functionally defunct (Leavitt et al., 1973; Chatterjee and Greenwald, 1976; Saidapur and Greenwald, 1978b; Terranova et al., 1978). Also, preantral follicles of the hamster ovary react negatively to the histochemical test for 3β-hydroxysteroid dehydrogenase (Saidapur and Greenwald, 1978b). Thus, on Day 4 of the hamster cycle, the steroidogenic capability in the ovary is mainly restricted to the interstitial gland cells (NAO) and the antral follicles (AF). In light of the above, the relative contribution by AF and NAO to various steroid hormones in the proestrous hamster is now considered.

There are several possible problems in the *in vitro* technique used in this study. 1) In large mammals, high concentrations of steroids are present in follicular fluid and presumably this pool is not capable of *de novo* synthesis. It is not known whether there are similarly elevated concentrations of steroids in rodent follicular fluid. If so, secretion of steroids into the medium and increases or decreases in steroid content of whole ovaries and AF, but not NAO, would be influenced by the background provided by follicular fluid levels. 2) Because different amounts of tissue were incubated there might be different rates of diffusion between tissue and medium. However, the fact that whole ovaries synthesized reasonable amounts of steroids compared to the smaller AF and NAO compartments militates against this argument. 3) It is obvious that we are measuring steroid content at one point in time versus initial content and that steroids may be synthesized

TABLE 1. Effect of LH (NIH-S-18) on *in vitro* ovarian steroidogenesis in the proestrous[a] cyclic hamster. Results expressed as mean ± SEM.

Steroid	Controls (without LH)	Production rate/h 1 ng/ml	5 ng/ml	25 ng/ml	250 ng/ml
Progesterone ng/mg	1.0 ± 0.1 (6)	1.8 ± 1.2 (5) ns	5.9 ± 0.8 (6) s	4.1 ± 0.6 (6) s	25.5 ± 1.5 (6) s
Testosterone pg/mg	7.9 ± 3.1[b] (4)	−5.4 ± 6.5 (4) ns 139.4 ± 46.5 (2) s	40.0 ± 6.5 (6) s	74.7 ± 13.5 (6) s	361.2 ± 39.5 (6) s
Estrone pg/mg	95.6 ± 18.0 (6)	179.1 ± 60.3 (6) ns	206.1 ± 18.6 (6) s	241.6 ± 46.3 (6) s	445.5 ± 55.7 (6) s
Estradiol-17β pg/mg	87.1 ± 12.4 (6)	253.5 ± 71.0 (5) s	656.9 ± 91.4 (6) s	666.5 ± 78.3 (6) s	1453.7 ± 118.4 (6) s

[a]The ovaries were removed from animals killed at 1145 h.

[b]Two ovaries did not produce T.

P values were calculated using Student's t test.

Figures in parentheses indicate number of animals.

s = significant ($P<0.05$) as compared to the controls.

ns = not significant.

and rapidly metabolized to compounds not detected by the RIAs.

Progesterone

As with other steroids, AF contain higher P levels (at 1500 h and 1800 h) than the NAO, although both exhibit the same capacity to produce P *in vitro*. It is apparent from Fig. 2 that P production is minimal in ovaries before the endogenous LH surge. With the onset of the LH surge, there is an abrupt increase in P levels in both AF and NAO (therefore in the ovary as a whole) and in the serum which is also reflected by the significant increase in the *in vitro* ability of these ovaries to produce P. The preovulatory rise and maintenance of P production over an extended period of time is clearly related to the LH surge. Previously, Norman and Greenwald (1971) and Bosley and Leavitt (1972) showed that blocking the LH release by phenobarbitol on Day 4 of the hamster cycle prevents the ovarian synthesis of P. The present studies indicate that even *in vitro* ability of the ovary or of isolated components (AF, NAO) to produce P is dependent on the exposure of ovaries to the endogenous LH surge prior to their removal as is the case of other species studied (references in Channing and Tsafriri, 1977). Similarly, the ovaries (removed before the LH surge), under the influence of exogenous LH *in vitro*, synthesize large amounts of P which mimic the P producing ability of the ovaries endogenously exposed to elevated levels of LH. It is also of interest that the *in vitro* effects of LH are manifested with as little as 1–5 ng of the hormone. On the other hand, addition of 100–200 ng FSH to the incubation medium was unable to stimulate P production. The fact that large amounts of FSH (250 ng/ml) did stimulate P production can perhaps be attributed to the LH contamination present in the FSH preparation.

The present studies also provide important clues with regard to the relative contribution of AF vs NAO to the preovulatory P levels. The AF represent less than 20% of the total ovarian weight. Therefore, although both AF and NAO possess the same ability to produce P (as was also shown by Leavitt et al., 1971), it is apparent that more than 80% of total P produced by the ovary on the afternoon of proestrus represents the contribution by the interstitial gland

TABLE 2. Effect of FSH (NIH-S-10) on *in vitro* ovarian steroidogenesis in the proestrous[a] cyclic hamster. Results expressed as mean ± SEM.

Steroid	Production rate/h			
	Controls (without FSH)	with added FSH		
		100 ng/ml	200 ng/ml	250 ng/ml
Progesterone ng/mg	1.0 ± 0.1 (6)	0.7 ± 0.2 (6) ns	0.7 ± 0.1 (6) ns	8.7 ± 0.9 (6) s
Testosterone pg/mg	7.9 ± 3.1[b] (4)	−13.8 ± 4.0[c] (4) s	−12.7 ± 3.6 (5) s	86.9 ± 20.6 (6) s
Estrone pg/mg	95.6 ± 18.0 (6)	75.3 ± 12.7 (6) ns	89.3 ± 18.4 (6) ns	204.1 ± 44.6 (6) s
Estradiol-17β pg/mg	87.1 ± 12.4 (6)	226.9 ± 39.1 (6) s	207.8 ± 51.8 (6) s	729.7 ± 124.9 (6) s

[a]The ovaries were removed from animals killed at 1145 h.

[b]Two ovaries did not produce T.

[c]One ovary produced T.

Figures in parentheses indicate number of animals.

P values were calculated using Student's t test.

s = significant ($P<0.05$) as compared to the controls.

ns = not significant.

cells (NAO). Earlier Norman and Greenwald (1971) reached a similar conclusion based on the fact that selective elimination of antral follicles by X-irradiation has little effect on the concentration of P in the proestrous hamster.

Testosterone

Changes in the pattern of T levels in the ovary and its production *in vitro* are well correlated. Under the influence of the endogenous LH surge, there is an initial transitory increase in T production. Likewise, the ovaries removed prior to the LH surge produce significant amounts of T *in vitro*, if LH (5–250 ng/ml) is added to the medium. Previous studies have shown that LH stimulates T production by theca isolated from rat follicles (Fortune and Armstrong, 1977). On the other hand, FSH (100–200 ng/ml) significantly reduced the production rate of T, which was, however, accompanied by a concomitant increase in E_2 production. It is now well established that FSH has an aromatizing ability (references in Channing and Tsafriri, 1977) and is considered to play an important role in converting T into estrogens (Dorrington, 1977; Dorrington et al., 1975; Fortune and Armstrong, 1978). The present findings are in agreement with this concept. However, stimulation of T production observed when greater amounts of FSH (250 ng/ml) were added is possibly owing to its contamination with LH. In the rat, P has no effect on androgen production by thecal cells (Fortune and Armstrong, 1977). Interestingly, P also had no effect on ovarian T production in the hamster.

Although the AF contain large quantities of T, the production rate of the hormone was insignificant ($P>0.05$), at least at the times used in this study. It is possible, however, that T produced in AF is rapidly converted to estrogens. This seems to be especially true at 1500 h. On the other hand, the conversion rate of T into estrogens in the NAO might be so low (since NAO produced very little estrogen) that one is able to detect the production of the hormone in the NAO, especially at 1500 h and 1800 h incubations. While it appears that both AF and NAO can synthesize T, the relative contribution by each of these components to the total ovarian and serum levels of the hormone is not clear.

Estrone

Unlike P and T, the production of E_1 in the ovaries is of the same magnitude whether the ovaries are removed before (1200 h) or during the LH surge (1500 h). However, ovarian E_1 production at 1800 h is at baseline levels. Thus, the ability of the ovaries *in vitro* to produce

TABLE 3. Effect of progesterone on *in vitro* ovarian steroidogenesis in the proestrous[a] cyclic hamster. Results expressed as mean ± SEM.

Steroid	Production rate/h		
	Controls (without P)	10 ng P/ml	100 ng P/ml
Testosterone pg/mg	7.9 ± 3.1[b] (4)	8.7 ± 0.9[b] (4) ns	16.0 ± 2.6 (6) ns
Estrone pg/mg	95.6 ± 18.0 (6)	76.4 ± 7.8 (6) ns	115.0 ± 11.4 (6) ns
Estradiol-17β pg/mg	87.1 ± 12.4 (6)	154.0 ± 12.9 (6) s	259.2 ± 37.5 (6) s

[a]The ovaries were removed from animals killed at 1145 h.

[b]Two ovaries did not produce T.

Figures in parentheses indicate number of animals.

P values were calculated using Student's t test.

s = significant ($P<0.05$) as compared to the controls.

ns = not significant.

E_1 is also correlated with *in vivo* changes in ovarian and serum levels of the hormone. A striking difference exists with regard to E_1 content in the NAO and AF at all times which is clearly reflected in their *in vitro* ability to produce the steroid. The content and *in vitro* ability to produce E_1 in the NAO is not only minimal but declines with time. On the other hand, AF contain high E_1 levels and also exhibit significantly greater ability to produce the steroid *in vitro*. Unlike the NAO, the AF respond clearly to the LH surge by producing large amounts of estrone (1500 h incubation). This, however, is transitory since the production rate drops significantly in the AF harvested from ovaries at 1800 h. From the above considerations, it is reasonable to conclude that AF are the main source of E_1 synthesis.

LH (5-250 ng/ml) also stimulates E_1 synthesis significantly *in vitro* as it does with regard to the other steroids. On the other hand, 100–200 ng FSH/ml was unable to increase E_1 production, even though it stimulated E_2 production significantly. However, higher concentration of FSH (250 ng/ml) stimulated E_1 production significantly which may be due to the combined effect of FSH and/or the LH contamination present in the FSH preparations. Addition of 10–100 ng P to the incubation medium also had no effect on the rate of E_1 production.

Estradiol-17β

The pattern in the ovarian content of E_2 and its *in vitro* ability to produce the steroid is similar to that of E_1. The ovaries, before the LH surge, produce significant amounts of E_2 and the ovaries removed during the LH surge exhibit greater ability to produce E_2 *in vitro*. As is the case with T and E_1, the LH surge has a transitory effect in stimulating E_2 production, since there is a significant decline in the production rate of E_2 in the ovaries removed at 1800 h.

The content of E_2 in the NAO is low at all times and shows a declining trend with time. In addition, even though the ovary as a whole, as well as the AF, respond to the LH surge and exhibit greater ability to produce E_2 *in vitro* in the ovaries removed during the LH surge, the NAO produces insignificant quantities of E_2. Thus, the present observations are in agreement with the view that AF are the major source of estrogens in the mammalian ovary (Moon et al., 1975; Young Lai, 1976; Channing and Tsafriri, 1977; Dorrington, 1977; Makris and Ryan, 1975, 1977).

The stimulation of E_2 production by LH may be regarded as a result of overall increase in steroidogenesis, whereas FSH and P had a specific stimulation of E_2 without causing any rise in T or E_1. The fact that FSH stimulated mainly E_2 rather than E_1 production (except when a large dose is used) suggests its specific effect on the conversion of T into E_2 rather than androstenedione to estrone in the hamster ovary. Similarly, the exogenous P enhanced E_2 production rather than T or E_1 which may be attributed to the fact that only low quantities of P were added to the medium.

The most intriguing question concerns the factors controlling the "turning off" of testosterone and estrogen synthesis on the afternoon of proestrus following the gonadotropin and P surge. Previous *in vitro* and *in vivo* studies on the rat (Hori et al., 1969; Lieberman et al., 1975; Hillensjo et al., 1976; Katz and Armstrong, 1976) and sheep (Moor, 1974) suggest that LH inhibits C-17–20 side chain cleavage and/or C-19 androgen aromatase leading to the decline in the estrogen production. The *in vitro* LH inhibitory effects in the above studies were shown by using microgram quantities of the hormone and long term incubations. In our present study we used low quantities of LH (1–250 ng/ml) and also only short term incubations were performed, wherein no inhibition of estrogen production was observed. However, it is possible that higher concentrations of LH and long term incubations might yield results similar to those reported for the rat and sheep. In the rabbit, interestingly, higher LH concentrations (10 μg/ml) had no effect on follicular estrogen production *in vitro*, whereas 1–10 ng LH/ml (low concentrations) caused a significant increase in estrogen synthesis (Young Lai, 1974).

Further, unpublished observations from this laboratory indicate that injection of P on the morning of Day 3 (G. S. Greenwald, unpublished) or Day 4 (S. K. Saidapur and G. S. Greenwald, unpublished) reduces serum E_2 levels significantly within 1 h. The LH surge on the afternoon of proestrus is also accompanied by the P surge. Therefore, whether the LH effects on the reduction of E_2 synthesis are brought about directly or mediated through P remains unknown, especially in view of our above unpublished observations. In the present short term incubations, however, P showed no inhibitory effect on E_2 production *in vitro*.

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