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Hormone Secretion by Euthyroid and Hypothyroid Rat Ovaries during the Early Stages of hCG-Induced Ovarian Cyst Development (42469)

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Abstract. This study was undertaken to examine ovarian steroid production during the early stages of hCG-induced ovarian cyst formation in the hypothyroid rat. Rats were placed into two groups with one group made hypothyroid by adding thiouracil to their diet. After 10 days, each group was divided into two subgroups with one subgroup receiving daily injections of hCG for 2 days and the other subgroup receiving saline. On the morning of Day 13, ovaries were removed and incubated for 2 hr. No significant difference in progesterone secretion was observed. However, ovaries from hypothyroid, hCG-treated rats secreted significantly more testosterone and estradiol than ovaries from vehicle-treated, hypothyroid rats and euthyroid, hCG-treated rats. In a second experiment, ovaries from euthyroid and hypothyroid rats treated with hCG were incubated in medium supplemented with 100 n*M* androstenedione and 0 or 100 ng FSH/ml. FSH failed to affect progesterone, testosterone, and estradiol secretions by ovaries from euthyroid, hCG-treated rats. In contrast, FSH significantly enhanced testosterone and estradiol secretion by ovaries from hypothyroid, hCG-treated rats. These results support the hypothesis that increased levels of testosterone and estradiol secretion have a central role in the induction of polycystic ovaries by hCG in the hypothyroid rat.

The formation of cystic ovaries in hypothyroid rats given daily injections of human chorionic gonadotropin (hCG) was documented nearly 30 years ago (1). Several studies have assessed changes in serum hormone levels in rats during the development of cystic ovaries (2) and in rats with fully developed cystic ovaries (3, 4). However, little is known about the capacity of these ovaries to synthesize steroids. Callard and Leathem (5) reported that although fully developed cystic ovaries synthesize *in vitro* large quantities of 20α-dihydroprogesterone, they synthesized very little androgen or estrogen. However, others suggested that fully developed cystic ovaries synthesize *in vitro* more estrogen from androgen precursor than noncystic ovaries (6). Furthermore, there is also a paucity of information on the effect of gonadotropins on steroidogenesis by cystic ovaries from hypothyroid rats. Copmann and Adams (7) examined hCG and follicle-stimulating hormone (FSH) binding to ovarian homogenates during cyst development and found enhanced binding of FSH in the hypothyroid, hCG-treated rat compared to euthyroid, hCG-treated controls. No differences in hCG binding were observed. There have been no *in vitro* studies examining basal or FSH-stimulated steroidogenesis during the early development of cystic ovaries in the hypothyroid rat. This dearth of information is surprising in view of the fact that hormonal changes during this period may have a central role in ovarian cyst formation. Therefore, the present study was undertaken to examine basal and FSH-stimulated steroid production during the early stages of the induction of cystic ovaries in the hypothyroid, hCG-treated rat.

Materials and Methods. *Animals.* Female Long–Evans rats weighing 90–130 g were placed into two groups. One group of 15 rats was fed a semisynthetic diet of 20% casien and 27% fat (8). A second group of 15 rats was fed the diet with thiouracil at a level of 0.5%. The rats were fed *ad libitum* either the control or the thiouracil diet for 13 days. After 10 days, each group was divided into two subgroups. On the mornings of Days 11 and 12, rats in one of the subgroups from each dietary group were injected with 10 IU hCG (Sigma, St. Louis, MO). The other subgroup received saline. On the morning of Day 13, the rats were sacrificed by decapitation and the ovaries were removed for incubation. In a second experiment, two groups of 10 rats were fed the diets as previously described. After 10 days, the rats in both groups were given daily injections of 10 IU hCG. The rats were sacrificed and the

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ovaries were removed for incubation on the morning of Day 13. Day 13 was chosen because previous work from this laboratory has shown that serum progesterone, testosterone, and estradiol from hypothyroid, hCG-treated rats are elevated at this time (9). Gross inspection of these ovaries showed numerous follicles although no significant accumulation of cyst fluid could be detected. In addition, no new corpora lutea were observed. In contrast, ovaries from euthyroid, hCG-treated rats contained developing follicles and many new corpora lutea. Body and thyroid gland weights of five rats from each group were determined to confirm goiter formation in those rats fed the diet containing thiouracil (Table I).

Incubation procedure. The ovaries from each subgroup were pooled and each ovary was divided into four to eight sections weighing 6 to 7 mg. Some of these sections were used in experiments not described in this report. Three randomly selected sections of ovaries from the first experiment were placed in a 35-mm culture dish containing 2 ml of 25 m*M* Hepes buffered minimum essential medium (MEM; KC Biochemicals, Kansas City, MO) supplemented with 0.6% BSA, 100 IU penicillin/ml, and 100 μg streptomycin/ml. Sections of ovary from the second experiment were incubated in medium that was also supplemented with 100 n*M* androstenedione (Sigma) and 0 or 100 ng FSH/ml (NIAMDD oFSH-13). The ovaries from both experiments were incubated for 2 hr at 37°C in a humidified atmosphere of 95% air–5% CO_2. Preliminary experiments showed steroidogenesis to be linear at 2 hr. After incubation, the ovaries were removed, dried, and weighed. The medium was frozen and stored at −20°C until analyzed for progesterone, testosterone, and estradiol by radioimmunoassay.

TABLE I. EFFECT OF DIET ON BODY AND THYROID WEIGHTS

Diet/treatment	Body weights (g)		Thyroid weights (mg)
	Day 1	Day 13	Day 13
Control/hCG	116 ± 3	143 ± 4	18 ± 2
Thiouracil/hCG	114 ± 4	120 ± 2[a]	43 ± 3[a]

Note. Mean ± SE. $N = 5$ per group. [a] Significantly ($P < 0.05$) different than control.

Steroid analysis. Steroids secreted into the incubation medium were measured directly without extraction by radioimmunoassay. Progesterone was analyzed with a specific antiserum previously described (9, 10). This antiserum cross-reacted 4.6% with pregnenolone, 1.0% with androstenedione, 0.4% with 20α-dihydroprogesterone, and less than 0.1% with testosterone. When 71 and 214 pg progesterone were added to the incubation medium, the accuracies of the assay were 71 and 205 pg, respectively. The interassay and intraassay variabilities were 11 and 7%, respectively. Testosterone was analyzed using an antiserum generated in this lab (8) that cross-reacted less than 10% with dihydrotestosterone, less than 1% with androstenedione, and less than 0.1% with pregnenolone, progesterone, 20α-dihydroprogesterone, androsterone, estradiol, and estrone. When 36 and 72 pg of testosterone were added to the incubation medium, the accuracies of the assay were 38 and 76, respectively. The interassay and intraassay variabilities were 6 and 3% pg, respectively. Estradiol was analyzed using a specific antiserum previously described (11). This antiserum cross-reacted 3% with estrone, 0.4% with testosterone, and less than 0.1% with progesterone. When 35 and 70 pg of estradiol were added to the incubation medium, the accuracies of the assay were 38 and 76 pg, respectively. The interassay and intraassay variabilities were 8 and 4%, respectively.

Statistical analysis. Significant differences between mean hormone levels were determined using the two-way analysis of variance and the Newman–Keuls multiple comparison test (12). Comparison of the effects of diet on thyroid and body weights were done using the Student *t* test. A difference was considered significant if the *P* value was less than 0.05. All results are expressed as the means ± SE.

Results. Ovaries from hypothyroid and euthyroid rats given daily injections of hCG or saline were incubated for 2 hr to examine differences in the basal rate of steroid secretion (Fig. 1, left side). Although no signficant differences in progesterone secretion were observed, significant differences in testosterone and estradiol secretion were found. Ovaries from hypothyroid rats treated with hCG secreted 14 times more testosterone than those from vehicle-injected hypothyroid rats and five

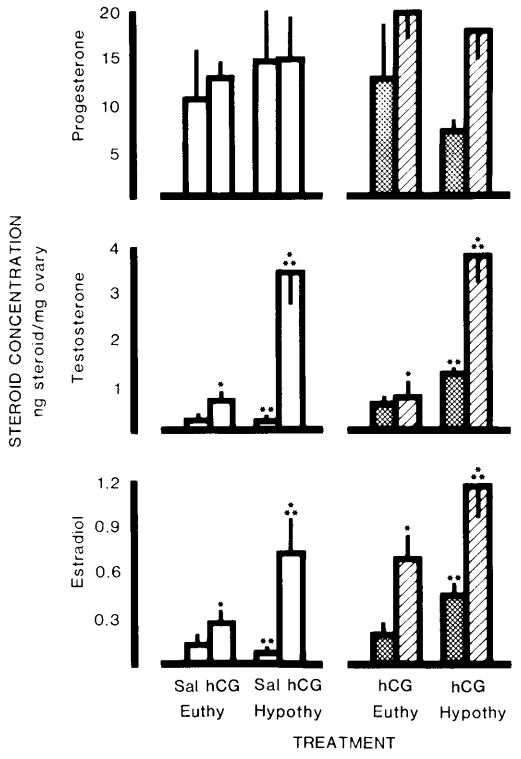

FIG. 1. Secretion of progesterone, testosterone, and estradiol by ovaries from euthyroid and hypothyroid rats incubated for 2 hr. The rats were given daily injections of 10 IU hCG or saline (left side) or 10 IU hCG (right side) for 2 days and sacrificed 24 hr after the last hCG injection. The ovaries were incubated in MEM (open bars), MEM supplemented with 100 n*M* androstenedione (shaded bars), or MEM supplemented with 100 n*M* androstenedione and 100 ng FSH/ml (cross-hatched bars). Each bar represents the mean ± SE of five replicates except the hypothyroid, hCG-treated group incubated in MEM which represents four observations. Similar symbols indicate significant ($P < .05$) differences among groups.

times more testosterone than ovaries from euthyroid, hCG-treated rats. Estradiol secretion by ovaries from hypothyroid, hCG-treated rats also was elevated significantly ($P < 0.05$) some 18-fold more than ovaries from vehicle-treated, hypothyroid rats and twice the amount from ovaries of euthyroid, hCG-treated rats.

Ovaries from euthyroid and hypothyroid rats treated with hCG were incubated for 2 hr in androstenedione supplemented medium to determine the effects of FSH on steroidogenesis (Fig. 1, right side). FSH had no effect on progesterone secretion. Moreover, FSH had no significant effect on testosterone or estradiol secretion by hCG-stimulated ovaries from euthyroid animals. The addition of exogenous androstenedione to the incubation medium suppressed testosterone production by hypothyroid, hCG-treated rat ovaries (Fig. 1, right side) compared to similarly treated ovaries incubated without androstenedione (Fig. 1, left side). However, ovaries from hypothyroid, hCG-treated rats incubated with FSH produced three and five times more testosterone than ovaries from hypothyroid, hCG-treated rats incubated without FSH and euthyroid, hCG-treated rats incubated with FSH, respectively. FSH also significantly enhanced the production of estradiol by ovaries from hypothyroid, hCG-treated rats. Ovaries from these rats produced more than twice as much as ovaries from similarly treated hypothyroid rats incubated without FSH and euthyroid, hCG-treated rats incubated with FSH.

Discussion. A previous study from this laboratory found elevated levels of serum progesterone during this stage of ovarian cyst development (9). However, no differences in progesterone production by ovaries from euthyroid and hypothyroid rats were observed. It is possible that differences in progesterone were not observed in this study due to the catabolism of progesterone to 20α-dihydroprogesterone which would be consistent with the report by Callard and Leathem (5) that fully developed cystic ovaries secrete more 20α-dihydroprogesterone than progesterone. The failure of FSH to increase progesterone synthesis significantly in the present study may have been the result of the rapid conversion of progesterone to other progestins, androgens, and/or estrogens. It is also possible that the elevated level of serum progesterone found in the previous study was not of ovarian origin. The adrenal is known to secrete significant amounts of progesterone (13). Furthermore, prolactin is believed to stimulate progesterone release (13). In the previous study (9), the hypothyroid, hCG-treated rats were the only group to have elevated levels of both prolactin and progesterone. Thus, it is possible that the increased progesterone secretion observed in the previous study was of adrenal origin.

Although hCG slightly increased basal testosterone production by euthyroid rat ovaries,

basal testosterone production by ovaries from hypothyroid, hCG treated rats was significantly elevated compared with that of the other groups. Previous results from this laboratory showed elevated levels of serum testosterone in the euthyroid and hypothyroid rats treated with hCG (9). This difference between *in vivo* and *in vitro* results suggests that the continued presence of hCG is required to sustain the elevated levels of testosterone synthesis by ovaries from euthyroid, hCG-treated rats. The addition of exogenous androstenedione to the incubation medium suppressed testosterone production by hypothyroid, hCG-treated rat ovaries compared with that of similarly treated ovaries incubated without androstenedione. This observation suggests the possibility of substrate inhibition of 17β-hydroxysteroid dehydrogenase (17β-HSD) in ovaries from hypothyroid, hCG-treated rats. The addition of FSH to the incubation medium overcame the inhibitory effect of androstenedione on granulosa cell 17β-HSD and increased testosterone production to levels similar to those secreted by ovaries incubated without the exogenous androgen. This is not surprising in light of a recent report that FSH stimulates 17β-HSD activity (14). Others have reported elevated serum androgen levels (15, 16) in women with polycystic ovaries and increased secretion of androgens by cultured human polycystic ovaries (17). Collectively, these results suggest that aberrant testosterone synthesis is an important concomitant of the development of cystic follicles. However, it remains to be determined whether its role is limited to serving as a substrate for enhanced estrogen synthesis or whether it has etiological function in the development of cystic ovaries.

Conflicting reports concerning the role of estrogens in the development of cystic ovaries have appeared over the past 20 years. Leathem and Adams (3) found that the estrogen antagonist ethamoxytriphetol (MER-25) prevented the formation of cystic ovaries in the hypothyroid, hCG-treated rat. Adams and Senseman (6) reported an increase in the conversion of androstenedione to estradiol and estrone by fully developed cystic ovaries. Moreover, previous results from this laboratory (9) found serum levels of estradiol to be significantly elevated during the early development of cystic ovaries. However, Callard and Leathem (5) found estradiol synthesis reduced in the fully developed cystic ovary compared with that of ovaries from euthyroid, hCG-treated rats. The results reported in the present paper show increased estradiol synthesis during the early development of the cystic ovary. Furthermore, FSH enhanced the already elevated production of estradiol. Others have reported that estrogens may be important in the development of ovarian cysts in aging rats (18), dehydroepiandrosterone-treated rats (19), and rats exposed to constant light (20). Furthermore, the administration of the long-acting estrogen, estradiol valerate, to young adult rats results in the development of polycystic ovaries (21, 22). Collectively, these results suggest that estrogens also play a central role in the induction of cystic ovarian follicles by hCG in the hypothyroid rat.

In conclusion, the results reported in this paper support the hypothesis that increased testosterone and estrogen production are associated with the early development of cystic ovaries in the hypothyroid rat. Furthermore, ovaries from the hypothyroid, hCG-treated rats are more responsive to FSH stimulation. This observation is consistent with a report by Copmann and Adams (7) that membrane preparations from hypothyroid rat ovaries obtained during the first 5 days of hCG treatment bind two to three times more FSH than ovarian membrane preparations from similarly treated euthyroid rats. These differences in steroidogenesis during this period may contribute to the subsequent development of cystic ovaries. Studies are currently underway investigating the mechanisms responsible for elevated estrogen and androgen production in this polyendocrine syndrome.

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