Bull. Inst. Zool., Academia Sinica **20**(1): 75-82 (1981)

EFFECTS OF ESTROGEN ON SERUM GONADOTROPINS IN THYROIDECTOMIZED-OVARIECTOMIZED RATS[1]

W. CHIA-MO WAN, CHAO-ING LEE, FON-YI YIN
and CHING-FONG LIAO

Endocrinology Laboratory
Institute of Zoology, Academia Sinica
Taipei, Taiwan 115,
Republic of China

(Received December 5, 1980)

W. Chia-Mo Wan, Chao-Ing Lee, Fon-Yi Yin and Ching-Fong Liao (1981) Effects of estrogen on serum gonadotropins in thyroidectomized-ovariectomized rats. *Bull. Inst. Zool., Academia Sinica* **20**(1): 75-82. This study was designed to examine the responsiveness of pituitary of athyroid-ovariectomized rats (Tx-Ovx) upon the first contact with estrogen, and to observe the response of FSH release in a dose of the steroid which is sufficient to induce LH release in those rats. The gonadotropins were measured by radioimmunoassay. The ovaries of 4-day cycling rats were removed after 30 days of thyroidectomy. The first single dose of estradiol benzoate (EB, 10 μg/100 g) was injected sc 14 days following ovariectomy. Serum LH in Tx samples was found less than 50% of the controls, while in samples following Ovx reached 17 times of the controls. By 4, 28 and 50 hrs after the 1st EB, the LH decrease to 4, 3.6 and 8 times of the controls. Three days later, another EB of the same dose was injected, 21 and 63 times of LH increase were observed 4 and 24 hrs later. This additional increase of serum LH in Tx-Ovx is slightly higher than that of sham-thyroidectomized-Ovx rats ($0.1>p>0.05$). Parabolic curve-fitting for the changes of serum LH following the 1st EB revealed that the hourly-change ratio in Tx-Ovx group ($b=1.88$) is 3.4 times as that of the sham-operated controls ($b=0.54$). No obvious FSH change was observed, except in the increments following Ovx. It was reported previously by this laboratory that a decrease of hypothalamic LHRH content was found in athyroid rats, presumably resulting from enhanced release of the hormone. Thus, it is suggested that the pituitary in a higher LHRH 'environment' of athyroid rats response more vigorously than in a lesser LHRH 'environment' to the treatment of estrogen.

Gonadotropins of hypothyroid rats were reported to be deficient both in pituitary and in serum[5,7,10,19]. Administration of estrogen to ovariectomized (Ovx) rats has been reported to cause a surge in LH[4], and this surge was enhanced in the absence of thyroid hormones and it was suggested that the thyroid hormones may play a role in modulating the secretion[9,13]. In order to inspect the estrogen-induced surge of LH in thyroidectomized (Tx) and Ovx rats, consecutive injections of estrogen were given to the rats for priming[9]. However, sprayed rats injected with a single does of estrogen showed significant decrease in serum LH at least in 7 days and the lowest values were found in the first two days. [6] It is possible that the responsiveness of the pituitary of athyroid rats following

1. Paper No. 216 of the Journal Series of the Institute of Zoology, Academia Sinica.

Ovx may not the same as that of euthyroid-Ovx rats in the first contact with estrogens, continual injections of estrogen for several days may mask the true ability of pituitary gland in responding to estrogen in thyroid deficiency. In this report, estrogen induced LH surge in Tx-Ovx rats is reexamined by two single injections, and the changes of serum LH following the first estrogen injection are specially emphasized.

MATERIALS AND METHODS

Animal treatments. Sprague-Dawley female rats from National Laboratory Animal Resources -National Taiwan University-Medical College Hospital, Taipei, were transferred to our animal room (6:00 AM-6:00 PM light on, $23 \pm 1°$ C) for one month period of acclimation. The rats, at 175-220 g and 90-93 days-old with 4-day cycling, were subdivided at random for Tx and sham-thyroidectomy (S). Twenty-eight days after the operation, ovaries of all the rats were removed (Ovx). Blood samples were obtained by heart puncture before and after the operations and also in the following experiments. The rats were then subjected for two subcutaneous injections of estradiol benzoate (EB; Sigma Co.) homogenated in olive oil in a dosage of 10 μg/100 g body weight[4]. The first injection was at 14 days following Ovx and bleeding at 24 (10:30-11:20 AM), 28 and 50 hrs after the injection. Three days elapsed before the second injection was given[8], and blood samples were then obtained 4 (4:00-4:45 PM) and 28 hrs following the injection.

Thirty-five days after the last injection of EB, all the rats were subjected for 2 olive oil injections (0.1 ml/rat) subcutaneously with three days in between. Blood samples were obtained 24 and 28 hrs after the first and 4 and 28 hrs after the second injection. The oil administrations were at the same time of the day as EB treatments.

Radioactive iodine-131 uptake for all the rats were done 8-10 days following the oil administration with a modified process from other investigators[15]. A dose of 0.2 μC/0.1 ml saline of I^{131} was injected to the rats, intraperitoneally, and an identical sample was save for total injected count. Forty-eight hrs after the injection the rats were killed, thyroid glands and/or trachea, a piece of thigh muscle and serum were collected and weighed. The count rate of all the specimens were obtained over a scintillation counter (Beckman gamma 8000). The estimation of tissue percent uptake was calculated as follows.

$$U\% = \frac{\text{Tissue cpm/g} - \text{muscle cpm/g}}{\text{Total cpm} - \text{Background cpm}} \times 100$$

All the data of Tx rats with a tracheal U% higher than that of serum were eliminated, and data of S rats with thyroid U% lower than 20% were not included in this report.

Radioimmunoassay. Serum samples were stored at $-20°$C until hormone assay. Serum LH and FSH were measured by specific radioimmunoassay (RIA) using materials obtained from the NIAMDD Rat Pituitary Distribution Program (USA). LH and FSH concentrations in serum from individual sample were assayed in triplicates followed the instructions of NIAMDD. The results are expressed as NIAMDD-Rat LH-RP-1 or in terms of NIAMDD-Rats FSH-RP-1. The statistical quality control for assays was followed the description of Rodbard *et al.*[16]. Radioactive iodine-125 were purchased from the Radiochemical Center, Amersham, England.

Statistical analyses were followed the descriptions of Sokal and Rohlf[17].

RESULTS

Serum LH and FSH levels in Tx and Tx-Ovx rats. As shown in Tables 1 and 2 (groups SE and TxE), both serum LH and FSH levels in rats after the sham Tx operation show no difference from that of control measurements, while after Tx, LH level is significantly lower than that of control but not in FSH. Following Ovx, LH increases 8 times and FSH 7 in S group, 17 and 8 times in Tx group as compared with the control samples. There is significant difference in LH, but not in FSH, between S and Tx groups. It is obvious that the rate of increment of LH is much faster in Tx group

TABLE 1.
Effects of estradiol benzoate (EB) on serum LH (ng/ml) of thyroidectomized (Tx) and ovariectomized (Ovx) rats

Intact control value=67.0±3.9(15)*

Groups	Tx	Tx+Ovx	EB (10 μg/100 g) or oil (0.1 ml) injected				
			Hours after 1st injection			after 2nd injection	
			24	28	50	4	24
SO	—	743.2 ±121.6 (4)	913.8 ±167.3 (3)	775.8 ±137.2 (3)	—	984.4 ±286.3 (3)	601.8 ±284.9 (3)
SE	68.8 ±18.8 (8)	562.4 ±109.1 (6)	568.6 ±120.0 (7)	297.6 ±47.4 (6)	640.8 ±123.8 (5)	1181.2 ±130.3 (7)	3491.5 ±617.8 (7)
TxO	—	1364.9 ±240.4 (8)	1120.9 ±38.5 (4)	1260.6 ±124.1 (7)	—	1107.9 ±150.9 (6)	1582.8 ±183.9 (8)
TxE	30.0 ±5.8 (10)	1155.7 ±118.1 (10)	282.4 ±40.1 (9)	244.1 ±30.7 (9)	561.1 ±164.4 (9)	1406.1 ±331.4 (9)	4207.4 ±446.2 (9)

* Mean±SEM, numbers in parentheses indicate the sample size.
SO: Sham-operated thyroidectomy with olive oil injections.
SE: Sham-operated thyroidectomy with estradiol benzoate injections.
TxO: Thyroidectomized with oil injections.
TxE: Thyroidectomized with estradiol benzoate injections.
Analysis of variance: Significant level at 0.05.
Nonsignificant in all SO groups and TxO groups.
Significant in TxE and SE groups.
LSR: (groups numbered as 1~7, for Tx, Tx+Ovx, etc. in the main body of the table). Please refer to text for significant between SE and TxE groups.

SE	1	2	3	4	5	6	7
TxE	1	2	3	4	5	6	7

than in S group in a 14-days period following Ovx.

Effects of EB on serum LH and FSH of Tx-Ovx rats. LH Serum LH of Tx-Ovx rats is suppressed to a level below that of Tx-Ovx samples in 24 and 28 hrs by first EB injection (EB 1), but not to the intact control level. Fifty hrs after EB 1, LH increase to a level double that of 28 hrs. The EB 1 suppression in S-Ovx rats only shows in samples 28 hrs after the injection. Four hrs after the second EB injection (EB 2), serum LH level of Tx-Ovx rats reaches to twice as much as 50 hrs following EB 1 and 24 hrs to 7 times. In S group, the increment of LH level following EB 2 are 2 and 5 times respectively. The EB 2-LH levels of Tx rats are slightly higher than that of S rats ($0.1 > p > 0.05$; Table 1 and Fig. 1). **FSH** As shown in Table 2, obvious increase of serum FSH following Ovx can be observed, but the influence of EB on FSH level after Ovx is not clearly demonstrated in this report (Table 2, TxO vs TxE). A slight but significant higher FSH level ($p < 0.05$) was observed in Tx rats 24 hrs than 4 hrs after EB 2.

Oil Controls. Neither LH nor FSH in all of

TABLE 2.
Effects of estradiol benzoate (EB) on serum FSH (ng/ml) of thyroidectomized (Tx) and ovariectomized (Ovx) rats

Intact control value=256.6±78.7(6)*

Group	Tx	Tx+Ovx	EB (10 μg/100 g) or oil (0.1 ml) injected				
			Hours after 1st injection			after 2nd injection	
			24	28	50	4	24
SO	—	2179.2 ±544.7 (4)	2261.9 ±110.8 (3)	1744.6 ±303.5 (3)	—	1896.7	1546.7 ±224.8 (3)
SE	298.7 ±100.1 (3)	1753.9	1574.5 ±141.4 (7)	1791.7 ±137.5 (7)	1476.1 ±108.1 (6)	1503.0 ±131.1 (6)	2411.6 ±504.3 (7)
TxO	—	3482.4 ±329.9 (7)	3563.8 ±290.0 (6)	3629.6 ±376.8 (6)	—	2904.5 ±308.6 (5)	2674.7 ±199.3 (6)
TxE	207.2 ±31.4 (7)	2147.0 ±219.3 (7)	1978.1 ±137.3 (9)	2102.0 ±123.9 (9)	1870.5 ±155.1 (5)	1445.9 ±98.2 (9)	2603.7 ±416.1 (8)

See Table 1 for other explanations.
Nonsignificant in SO and Tx groups,
Significant in SE and Tx groups
LSR:

SE	1	2	3	4	5	6	7
TxE	1	2	3	4	5	6	7

the samples with oil administration shows any significant variation, but the hormonal level in all Tx samples are significant higher than that of S group (SO and TxO in Tables 1 and 2).

DISCUSSIONS

A significant decrease of serum LH level was observed in Tx rats but not in S rats (Table 1). This is in agreement with the findings of other investigators[5,7,9,10,13,18,19]. On the contrary, our earlier findings in Tx proestrus rats an increase of LH following Tx was observed[20]. It is proposed that the difference is due to the variations of estrogens in blood. In this report, the LH level in Tx (diestrus) rats is lower than in S (diestrus) rats, where the estrogen concentration may not be in a great difference. Following the 2nd injection of EB, which was intended to mimic the estrogen level in proestrus rats, there is a slight serum LH increase. This slight increment of serum LH may become even obvious if one scrutinizes the individual data (Fig. 1), and it was observed that individual variations in LH release following estrogen administration is rather large[4]. It is suggested that the estrogen changes in phenomenal proestrus (such as examined by vaginal smear) is coincident with the changes of the responsiveness of pituitary gland to the positive feedback stimulation of gonadal steriods, thus LH level is higher in Tx proestrus rats. Further investigation is needed in this aspect.

This investigation was designed to have the pituitary synthesis and/or release as much as TSH during the time after Tx (30 days) and to observe the serum LH changes after Ovx.

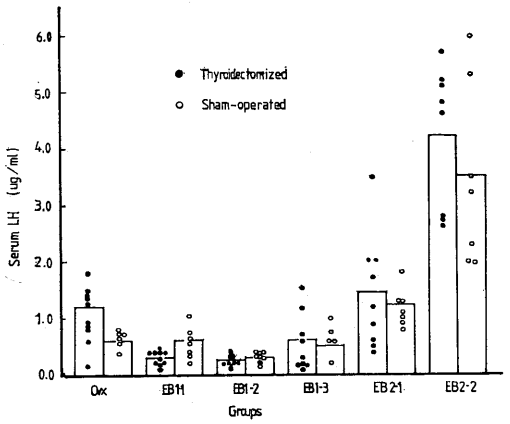

Fig. 1. Serum LH concentrations in rats after thyroidectomy and ovariectomy, and sham thyoidectomy operation and ovariectomy. Ovx: ovariectomy EB1-1 to EB1-3: 4, 28 and 50 hrs after first estradiol benzoate injection (10 μg/100 g). EB2-1 and EB2-2, 4 and 24 hrs after 2nd estradiol benzoate injections. Time between 1st and 2nd injection was 3 days.

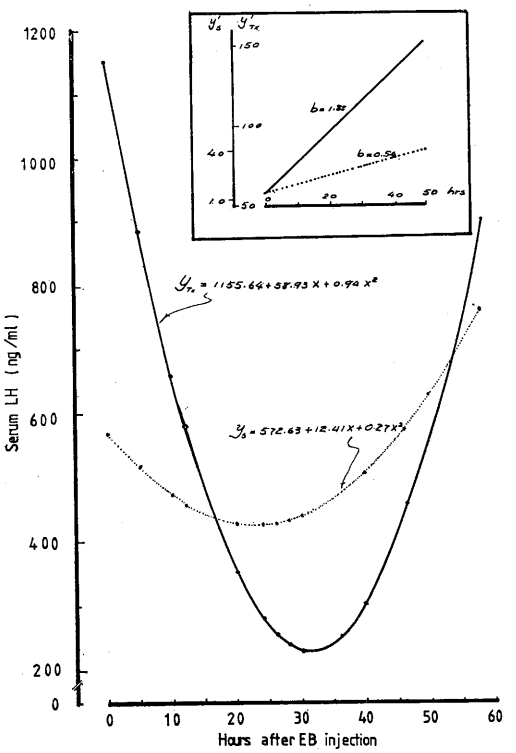

Fig. 2. Parabola curve fitting of serum LH with time pre- and post- first estradiol benzoate injection, and first derivatives of parabola equations (insert) indicating the hourly-change ratio (b).

Owing to the similarity of alpha subunits in TSH and LH, the TSH manufacture following Tx may utilized most of the sources of the subunits, this may be one of the reasons for lowering LH after Tx. If this is the case, the postovariectomy increase of LH should not have been seen in the rats after thyroidectomy. In the present report, a 38 (1155.7/30.0) times increase of LH is demonstrated in Tx rats after Ovx and 17 (1155.7/67.0) times as compared with controls (Table 1). This is in agreement with previous investigators[5,11,13].

The present results indicate that also estrogen injection is effective in inducing LH release from pituitary of Ovx rats either with or without thyroid hormones. Following first injection of EB (10 μg/100 g body weight) a significant decrease of serum LH was observed in 24 and 28 hrs later in Tx-Ovx rats, 28 hrs in S group; and at 50 hrs the LH were all increased. Twenty-four hrs after the second injection of EB of the same dosage, LH increased tremendously both in Tx-(17 times) and in S-(11 times) Ovx rats in compared with the lowest suppressed level (e. g. 63 and 52 times of control values, Table 1).

Single injection of estrogen was reported to keep serum LH low for at least 7 days in Ovx rats, but variated with individuals and time of the day[4,6]. It was reported that after estrogen priming the Ovx-Tx rats with thyroxine replacements needed more estrogen to induce a preovulatory surge, and it was suggested that the thyroxine modulating the control center in hypothalamus[9].

By parabolic curve-fitting for serum LH changes in Tx- and S-Ovx rats after 1st EB injection, it is interested to note that the changes in Tx-Ovx group is more rapid than in S-Ovx group. The first derivatives of parabolic equations show that the hourly-change ratio of

serum LH in Tx-Ovx rats is 1.88, and in S-Ovx, 0.54. This indicates that pituitary responses to estrogen stimulation are more vigorously in 'environment' without than with thyroid hormones (Fig. 2). It was found in our laboratory that LHRH content in female rats gradually decreased to 36% of the normal control 70 days after Tx in proestrus[20], and the lowering of LHRH implied an increase of the hormone release. This can be explained by the decrease of adenohypophyseal LH content in hypothyroid rat [7,10,18~20]. It is possible that in the present report the pituitary of Tx rats is in a 'environment' of higher LHRH concentration than in S rats, and the excess amount of LHRH may potentiate the responsiveness of pituitary to estrogen. The higher responsiveness are indicated by (1) the faster increase of serum LH upon the removal of ovaries in Tx rats (Table 1), (2) the higher hourly-change ratio following the 1st injection of EB (Fig. 1). This explanation is under the presumption that the effect of thyroid hormone is acted by way of hypothalamus through a biogenic amine process[9,21], however, the direct effect of thyroid hormones on pituitary LH release can not be excluded. Example of cross-effect between pituitary-thyroid axis and ovarian steroid had been reported where TSH content of pituitary decreased following estrogen administration[1]. The bioassayble LH of pituitary was not changed in Tx-Ovx rats[23], but the total immunoreactive LH was higher and the release of the LH in response to LHRH was greater in Ovx-Tx rats than Ovx-sham thyroidectomized rats[22]. Preincubated with estrogen the synthesis and release of LH of pituitary was reported to be increased following LHRH stimulation[2,3,8,12]. The potentiation of LHRH on estrogen effect *in vitro* remains to be investigated.

Serum FSH levels showed no variation after Tx, but increased significantly following Ovx. Except the samples of 28 hrs after 2nd EB injection in Tx group, no change of FSH was observed following EB treatments. The effect of estrogen on FSH surge still uncertain, a rise of plasma FSH was reported to occur in Ovx rats following the administration of estradiol but the mechanism has not been elucidated[14]. In the present report, it was designed to study that a dose of estrogen which is sufficient to induce LH surge upon injection would also change the FSH release in Tx-Ovx rats. The results indicate that the withdrawal of thyroid hormones only enhancing responsiveness of pituitary to estrogen in LH release.

Acknowledgement: This study is supported in part by a grant from National Science Council. The materials for LH and FSH radioimmunoassay is a gift from NIAMDD Pituitary Distribution Program, their generosity is highly appreciated. Thanks also extend to Dr. H. P. Wu, Biometry Laboratory, Institute of Botany, for his advices in statistics.

REFERENCES

1. AMESBURY, O. F., A. N. CONTOPOULOS and A. A. KONEFF (1965) Effects of oestrogen on pituitary function in the throidectomized rat. *Acta Endocrinol.* Kbh. **48**: 355-368.
2. APFELBAUM, M. E. and S. TALEISNIK (1976) Interaction between oestrogen and gonadotrophin-releasing hormone on the release and synthesis of luteinizing hormone and follicle-stimulating hormone from incubated pituitaries. *J. Endocrinol.* **68**: 127-136.
3. APFELBAUM, M. E. and S. TALEISNIK (1977) Influence of oestrogen administration *in vivo* on the release and synthesis of LH and FSH from incubated pituitaries. *Acta Endocrinol.* Kbh. **86**: 704-713.
4. BLAKE, C. A. (1977) Effects of estrogen and progesterone on luteinizing hormone release in ovariectomized rats. *Endocrinology* **101**: 1122-1129.
5. BRUNI, J. F., S. MARSHALL, J. A. DIBBET and J. MEITES (1975) Effects of hyper- and hypothyroidism on serum LH and FSH levels in intact and gonadectomized male and female rats. *Endocrlnology* **97**: 558-563.
6. CALIGARIES, L., J. J. ASTRADA and S. TALEISNIK (1971) Release of luteininzing hormone induced by estrogen injection into ovariectomized rats. *Endocrinology* **88**: 810-815.
7. CHOONG, M. Y. and W. C-M. WAN (1975) Adenohypophyseal gonadotrophin concentration in thyroidectomized female rats. *Bull. Inst. Zool., Academia Sinica* **14**: 49-53.

8. DE KONING, J., J. A. M. J. VAN DIETEN cnd G. P. VAN REES (1976) Inhibitory and augmentative effects of oestradiol on LHRH induced release of LH by anterior pituitary glands from intact female rats *in vitro*. *Mol. Cell. Endocrinol.* **5**: 321-330.
9. FREEMAN, M. E., F. T. LAROCHELLE, JR., and R. B. MOORE (1976) Effect of thyroid status on spontaneous and induced surges of luteinizing hormone. *Endocrinology* **99**: 713-719.
10. HWANG, J. C., P. H. LI and W. C-M. WAN (1974) Effect of induced hypothyroidism on pituitary luteinizing hormone (LH) concentration in female rats. *J. Formosan Med. Assoc.* **73**: 227-231.
11. KALLAND, G. A., A. VERA, M. PETERSON and R. S. SWERDLOFF (1978) Reproductive hormonal axis of the male rat in experimental hypothyroidism. *Endocrinology* **102**: 476-434.
12. LABRIE, F., J. DROUIN, L. FERLAND, L. LAGACE, M. BEAULIU, A. DE LEAN, P. A. KELLY, M. G. CARON and V. RAYMOND (1978) Mechanism of action of hypothalamic hormones in the anterior pituitary gland and specific modulation of their activity by sex steroids and thyroid hormones. *Recent. Prog. Horm. Res.* **34**: 25-93.
13. LAROCHELLE, F. T. JR. and M. E. FREEMAN (1974) Superimposition of thyroid hormone regulation of gonadotropin secretion. *Endocrinology* **95**: 379-387.
14. PIVA, F., M. MOTTA, and L. MARTINI (1979) Regulation of hypothalamic and pituitary function: Long, short, and ultrashort feedback loops. in "Endocrinology" vol 1. (L. J. DeGroot, G. F. Cahill, Jr., W. D. Odell, L. Martini, J. T. Potts, Jr., D. H. Nelson, E. Steinberger, and A. I. Winegrad). Grune and Stratton, N. Y., Chapter **3**: 21-33.
15. ROBERTSON, H. A. and I. R. FALCONER (1961) The estimation of thyroid activity: An evaluation of certain parameters. *J. Endocrin.* **21**: 411-420.
16. RODBARD, D., P. L. RAYFORD, and G. T. ROSS (1970) Statistical quality control of radioimmunoassay. in "Statistics in Endocrinology" (J. W. McArthur and T. Colton) The MIT Press, Mass, USA.
17. SOKAL, R. R. and F. J. ROHLF (1969) "Biometry" W. H. Freeman and Co.
18. WAN, W. C-M. and J. C. HWANG (1973) Effect of thyroidectomy on pituitary luteinizing hormone (LH) concentration in female rats. *Bull. Inst. Zool.*, Academia Sinica **12**: 39-44.
19. WAN, W. C-M. and H. T. CHEN (1974) Adenohypophyseal interstitial-cell stimulating hormone (ICSH) in rats after thyroidectomy. *J. Formosan Med. Assoc.* **73**: 387-392.
20. WAN, W. C-M., Y. F. CHEN and C. L. CHEN (1978) Changes of hypothalamic gonadotropin (GnRH), adenohypophyseal (AP) and serum LH and FSH following thyroidectomy (Tx) in female rats. (Abstract) in Fed. Proc. The 62nd annual meeting, Atlantic City, New Jersey, April 9-14, 1978.
21. WAN, W. C-M., W. K. WANG and N. C. TING (1979) Feedback loop on LRH release. (Abstract) in XIth International Congress of Biochemistry, 8-13, July Toronto, Canada, 1979.
22. WANG, P. S., T. C. LIU and G. L. JACKSON (1980) Effects of thyroidectomy and thyroxine therapy on biosynthesis and release of luteinizing hormone by rat anterior pituitary glands *in vitro*. *Biol Reprod.* **23**: 752-759.
23. WANG, P. S. G. and W. C-M. WAN, (1978) Biological activity of luteinizing hormone in adeno hypophyses of ovarietomized-thyroidectomized rats. *Bull. Inst. Zool.*, Academia Sinica **17**: 81-84.

動情素對去卵巢去甲狀腺大鼠生殖控制激素之影響

萬家茂　李昭英　殷鳳儀　廖欽峯

雌性成熟大白鼠於去除甲狀腺後 30 日，再將卵巢切去，凡 14 日後以動情素(苯化雌二醇) 10μg/100 克體重注入（此稱第一次注入)，第一次注入後 3 日再作第二次注入，血中 LH 及 FSH 以放射免疫測定法測定。本次實驗發現在無甲狀腺之雌鼠可因卵巢激素之缺如及動情素之注入而產生較有甲狀腺鼠爲大之 LH 變化，FSH 變化不明顯。以拋物線繪製時，一次微分的結果可知無甲狀腺鼠 LH 的血中濃度因切去卵巢及一次注射的每小時變化爲 $b=1.88$，而有甲狀腺者爲 $b=0.54$，相差 3.6 倍。此可能由於切除甲狀腺後增加 LHRH 分泌，而使腦下腺對動情素敏感的結果。