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Reproductive Endocrinology in Hatano High- and Low-Avoidance Rats During the Estrous Cycle

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The high- and low-avoidance animals (HAA and LAA rats) were originally selected from Sprague–Dawley rats for their shuttle-box task. Reproductive endocrinology during the estrous cycle was compared between HAA and LAA rats. All HAA rats showed a regular 4-d estrous cycle, whereas most LAA rats (70.8%) showed a regular 5-d estrous cycle. The peak level of preovulatory luteinizing hormone (LH) surge level was significantly lower in LAA rats than in HAA rats on the day of proestrus. In contrast, the peak level of prolactin surge on the day of proestrus was significantly higher in LAA rats than in HAA rats. Plasma concentrations of follicle-stimulating hormone (FSH) and estradiol-17β were significantly lower in LAA rats as compared with HAA rats at 12 h on the day of estrus and from 24 h on the day of diestrus to 18 h on the day of proestrus. On the other hand, plasma concentrations of progesterone were significantly higher in LAA rats compared with HAA rats on the day of diestrus. The number of antral follicles (300–600 μm in diameter) at 12 h on the day of proestrus was significantly fewer in LAA rats than in HAA rats. The size and number of corpus luteum at 12 h on the day of estrus were significantly greater in LAA rats than in HAA rats. These results clearly demonstrated that apparent differences are observed in reproductive endocrinology between two Hatano strains. These strain differences probably originated from neural regulation of pituitary hormones.

Key Words: Estrous cycle; ovary; Hatano rat strains; prolactin; progesterone.

Introduction

To estimate the risk of neurobehavioral teratogens, the high-avoidance animal (HAA) and low-avoidance animal (LAA) strains have been bred and selected from Sprague–Dawley rats in a shuttle-box task at the Hatano Research Institute *(1)*. HAA rats were selected on the basis of their high rate of avoidance response, and LAA rats for their low rate. These rats show differences in some respects in addition to divergence shuttle-box performance, such as maternal behavior *(2)*, adrenal weights, and plasma concentrations of adrenocorticotropic hormone (ACTH) under stressful conditions *(3)*. It has been well established that the hypothalamo-pituitary-adrenal axis (HPA) correlate with the hypothalamo-pituitary-gonadal axis (HPG). In vitro, corticotropin-releasing hormone (CRH) inhibits release of gonadotropin-releasing hormone (GnRH) at the level of neurosecretory terminals in median eminence *(4)*. There is immunocytochemical evidence for direct synaptic connections between CRH- and GnRH-containing neurones in the preoptic area of the rat *(5)*. Glucocorticoid also influenced the gonads directly *(6–9)*.

The present study, therefore, was designed to clarify the difference in reproductive endocrinology during the estrous cycle in two Hatano lines.

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Results

Length of the Estrous Cycle

All HAA rats showed a regular 4-d estrous cycle ($n = 41$). On the other hand, 6.3% and 70.8% of LAA rats exhibited regular 4- and 5-d estrous cycles, respectively; the rest of the LAA rats (22.9%) showed irregular estrous cycles ($n = 48$). The irregular estrous cycle showed on the prolonged day of diestrus or repeated 4-d cycle and 5-d cycle.

Organ Weights

There was no significant difference in body weight in the two strains (Fig. 1A). The weight of the pituitary glands of LAA rats were significantly heavier compared with that of HAA rats throughout the estrous cycle (Fig. 1B). Ovarian weights were significantly heavier in LAA rats than HAA rats throughout estrous cycle (Fig. 1C). In contrast, uterine weights (Fig. 1D) and adrenal gland weights (Fig. 1E) were significantly lower in LAA rats than HAA rats throughout the estrous cycle.

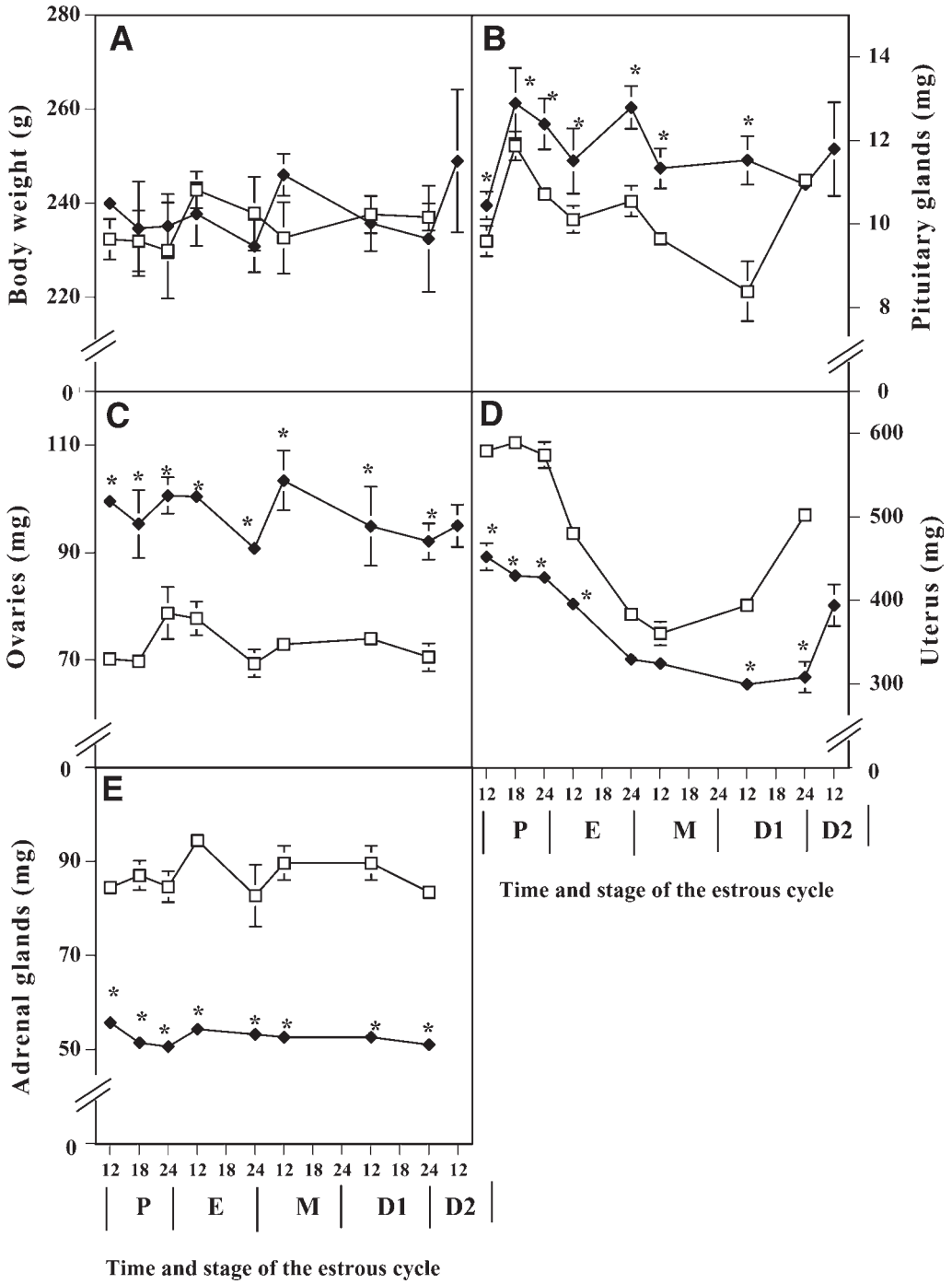

Fig. 1. Body weight (**A**) and weights of pituitaries (**B**), ovaries (**C**), uteri (**D**), and adrenal glands (**E**) in high-avoidance animals (HAA) (□) and low-avoidance (LAA) (◆) rats during the estrous cycle. P: proestrus, E: estrus, M: metestrus, D1: d 1 of diestrus, D2: d 2 of diestrus. Values are means ± SEM of five rats. *$p < 0.05$ compared with HAA rats (Tukey–Kramer test).

Hormone Levels

In both strains, the luteinizing hormone (LH) and prolactin surge occurred at 18 h on the day of proestrus in the 4- and 5-d estrous cycles (Fig. 2A,C). The peak level of LH surge was significantly lower in LAA rats than in HAA rats, whereas there was no significant difference in basal levels of LH between two strains (Fig. 2A).

Plasma concentrations of follicle-stimulating hormone (FSH) were significantly lower in LAA rats than in HAA rats at 12 h on the day of estrus (Fig. 2B). In HAA rats, the first and second surges of FSH were clearly separated at 18 h on the day of proestrus and at 12 h on the day of estrus, whereas in LAA rats, a single surge of FSH was observed at 18 h on the day of proestrus.

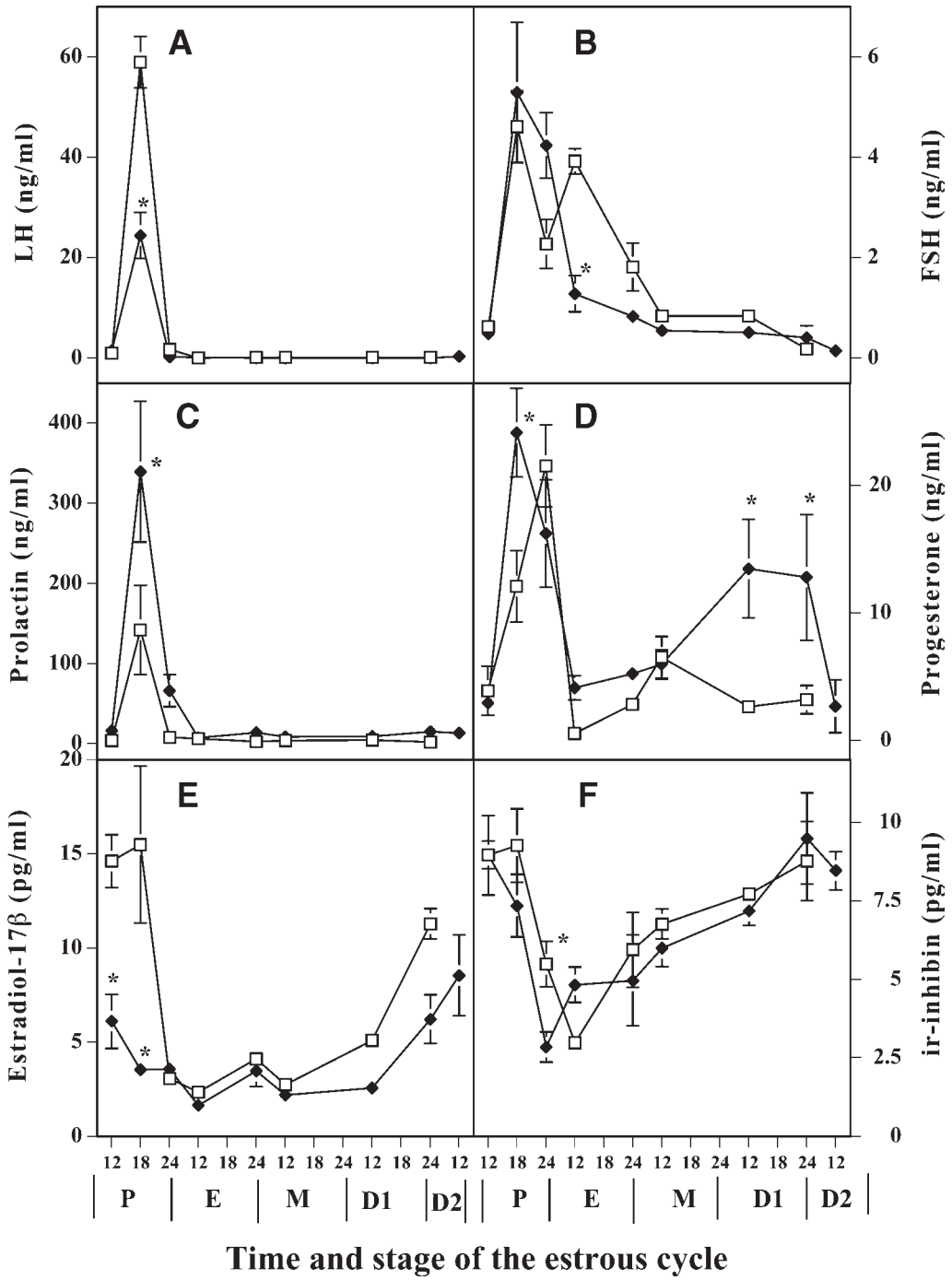

Fig. 2. Plasma concentrations of LH (**A**), FSH (**B**), prolactin (**C**), progesterone (**D**), estradiol-17β (**E**) and immunoreactive (ir-) inhibin (**F**) in HAA (□) and LAA(◆) rats during the estrous cycle. P: proestrus, E: estrus, M: metestrus, D1: d 1 of diestrus, D2: d 2 of diestrus. Values are means ± SEM of five rats. *$p < 0.05$ compared with HAA rats (Tukey–Kramer test).

The peak level of prolactin surge was significantly higher in LAA rats than in HAA rats on the day of proestrus, whereas there was no significant difference in basal levels of plasma prolactin between the two strains (Fig. 2C).

Plasma concentrations of progesterone were significantly higher in LAA rats than in HAA rats at 18 h on the day of proestrus and on the day of diestrus. The concentrations of progesterone tended to be higher in LAA rats than in HAA rats on the day of estrus, but the difference was not significant (Fig. 2D).

Plasma concentrations of estradiol-17β in HAA increased from 12 h on the day of diestrus to 18 h on the day of proestrus (Fig. 2E). An increase in plasma concentrations of estradiol-17β was also observed in LAA rats from 24 h on the

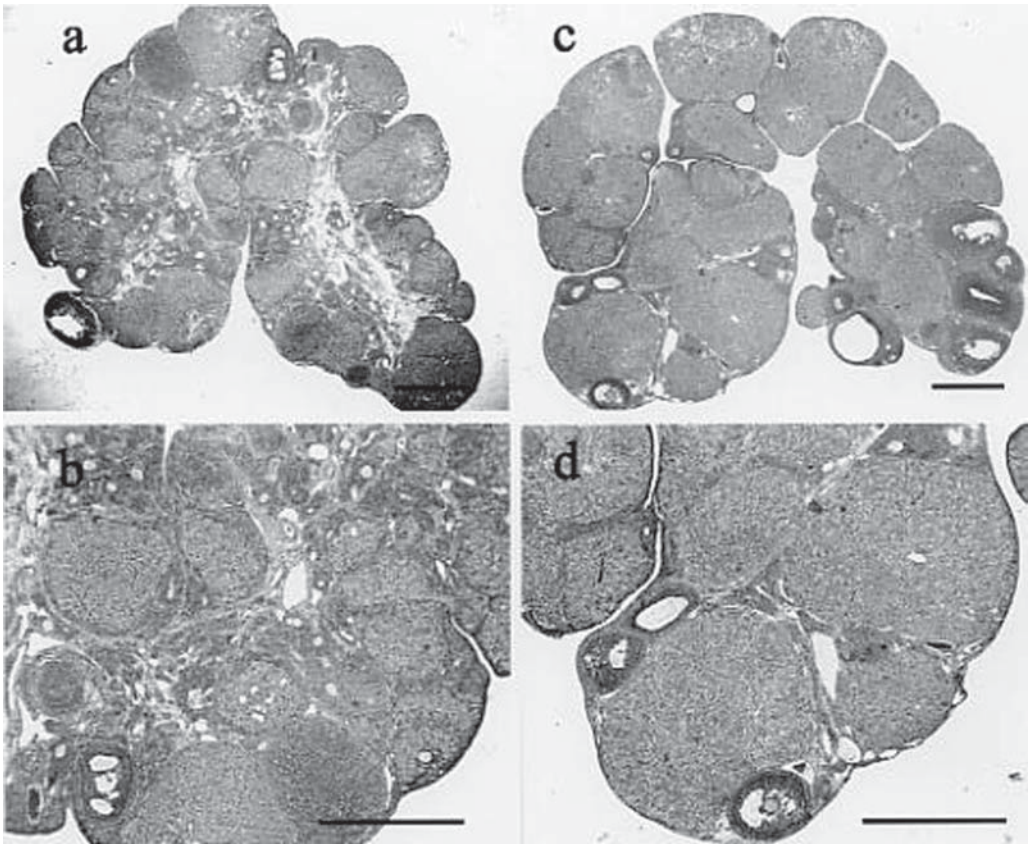

Fig. 3. Hematoxylin–eosin staining of ovary at 12 h on the day of estrous. (**a, b**) ovaries in HAA rats; (**c, d**) ovaries in LAA rats. The bar in each panel represents 500 μm.

day of first day of diestrus to 12 h on the day of proestrus, although the concentrations were significantly low in LAA rats compared with HAA rats on the day of proestrus (Fig. 2E).

No significant difference in circulating ir-inhibin levels was observed in two strains of rats throughout the estrous cycle except at 18 h on the day of proestrus (Fig. 2F).

Corpora Lutea and Follicles

The number of corpora lutea in HAA rats (23.3 ± 3.0, $n = 5$) was also significantly fewer than in LAA rats (30.7 ± 1.5, $n = 5$). In addition, the size of each corpus luteum of LAA rats was larger than in HAA rats (Fig. 3a–d). The number of ovulated oocytes was significantly fewer in LAA rats (14.3 ± 1.3, $n = 8$) than in HAA rats (16.0 ± 0.7, $n = 9$). The mean number of follicles 301–400 μm, 401–500 μm, and 501–600 μm in diameter was significantly lower in LAA rats than in HAA rats at 12 h on the day of proestrus (Fig. 4).

Discussion

The present study clearly demonstrated that HAA rats and LAA rats have differences in the follicular development, luteal function, and pattern of hormonal secretion during estrous cycle. The number of antral follicles in the ovary during estrous cycle was fewer, but the number of corpora lutea was larger in LAA rats than in HAA rats. These differences correspond to circulating concentrations of estradiol-17β and progesterone in both strains. The uterine weights were also parallel with circulating levels of estradiol-17β in these animals. In addition, circulating levels of progesterone correspond to luteal function. In LAA rats, ovaries have many functional corpora lutea compared to HAA rats. It has been well established that luteal progesterone secretion was more prolonged in 5-d cycling rats than in 4-d cycling rats *(10–12)*. Previous reports have shown that an injection of exogenous progesterone postponed the time of the preovulatory LH surge and ovulation by 24 h *(13)*. It has been also shown that injection of a sufficient amount of antiserum against progesterone at 23 h on the day of metestrus in 5-d cycle advanced ovulation and completion of the estrous cycle by 1 d in the most of animals *(12)*.

The concentrations of plasma prolactin from 15 h to 23 h on the day of proestrus remained consistently higher in 5-d cycling rats than in 4-d cycling rats *(14)*. Progesterone secretion also remained significantly higher during diestrus in 5-d cycling rats than in 4-d cycling rats *(12)*. In addition, the plasma prolactin and progesterone levels in early pseudopregnant rats continuously increased, and the pseudopregnancy was interrupted by injection of antiserum against pro-

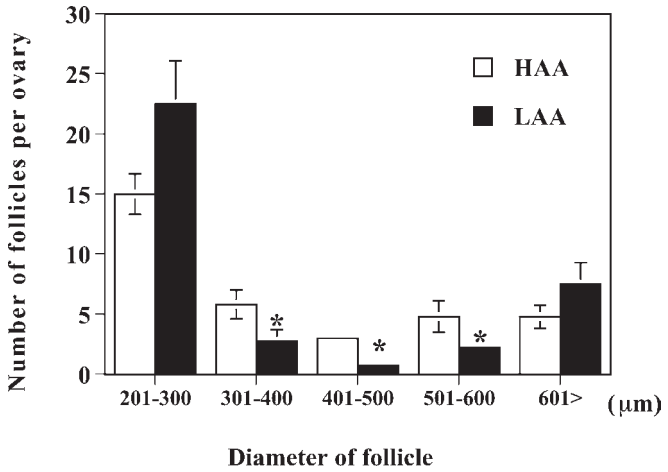

Fig. 4. The number of follicles per ovary at 12 h on the day of proestrus. Values are means ± SEM of five rats. *$p < 0.05$ compared with HAA rats (Student's t -test).

gesterone serum *(15)*. It has been shown that progesterone acts as an important factor that can change the sensitivity of corpus luteum to prolactin and suppresses prolactin-induced luteal cell apoptosis via reduction of the expression level of Fas mRNA in the corpus luteum *(16)*. Thus, these results suggest that the prolongation of luteal progesterone secretion in LAA rats in the present study probably relates to luteal activation by prolactin. A previous report has showed that prolactin is either an apoptosis inducer or suppressor, depending on the functional state of luteal cells in rats *(17)*. As shown in Fig. 2A,C, LAA rats showed a small LH surge and large prolactin surge on the day of proestrus. The elevated concentration of plasma progesterone is known to inhibit the secretion of LH *(13,18)*. Therefore, as long as the plasma concentrations of progesterone remain elevated, follicular growth will be retarded and a small LH surge will be occur because of the low levels of circulating estradiol-17β *(18)*. It was reported that the size of the FSH surge during the preovulatory phases is an important factor for subsequent follicular growth following ovulation *(19)*. In the present study, the FSH surge lasted about 1 d longer in HAA rats than in LAA rats on the day of estrus. The difference in the duration of the pre-ovulatory FSH surge probably involved the difference of follicular development in two strains of Hatano rats. A previous report showed that prolactin dose did not suppress LH and FSH secretion directly, but reduced the number of luteinizing hormone releasing hormone (LHRH) receptors in anterior pituitary *(20)*.

It has been reported that plasma concentrations of corticosterone were significantly higher in LAA rats than HAA rats of both sexes *(3)*. Previous reports have shown that CRH inhibits GnRH-mediated gonadotropin secretion *(21, 22)*, and also glucocorticoid acts to depress the sensitivity of LH release from pituitary glands *(23)*. Moreover, glucocorticoid inhibited the increase in aromatase activity by FSH *(6)* and FSH-mediated LH receptors induced in rat granulosa *(7)*. Glucocorticoid may influence the follicles directly and depress follicle development and ovulation because the granulosa cells have glucocorticoid receptor *(9)*. Thus, the line difference in HAA and LAA during the estrous cycle may mediate not only central regulation of hormonal secretion but also local regulation of hormone action in the ovary. These strains may be effective in studying the role of neural and hormonal mechanisms.

In summary, the present study clearly demonstrated the strain differences of the characteristics of circulating hormones and ovarian functions between two Hatano lines during the estrous cycle. These two Hatano rats may be useful animals in studying the role of neural and local regulatory mechanisms of hormones or chemicals.

Materials and Methods

Animals and Treatments

Adult female rats from each strain, HAA rats ($n = 41$) and LAA rats ($n = 48$) were used. Animals were kept under a 12-h light–dark cycle (lights on from 7:00 AM to 7:00 PM), at a temperature of 23–25ºC and relative humidity of 55 ± 5%. Food (CE-2; Clea Japan Inc., Tokyo, Japan) and water were available ad libitum. Daily vaginal smears were taken to determine the stage of the ovarian cycle for 2 wk from the age of 8 wk and only those rats that had shown more than two consecutive regular 4- or 5-d estrous cycles were used in the present study. Animals were killed by decapitation at various times during the estrous cycle. Trunk blood was collected in heparinized tubes and centrifuged immediately and plasma was separated and stored at –20ºC until assayed for LH, FSH, prolactin, immunoreactive (ir-) inhibin, estradiol-17β, and progesterone. After the decapitation, pituitary glands, adrenal glands, ovaries, and uteri were weighed. Oviducts were examined for oocytes on the day of estrus.

Radioimmunoassay

Concentrations of LH, FSH, and prolactin in plasma were measured using NIDDK kits for rat LH, FSH, and prolactin. Hormones for iodination were rat LH-I-7, rat FSH-I-7 and rat prolactin-I-5. The antisera used were anti-rat LH-S-10, anti-rat FSH-S-11, and anti-prolactin-S-9. Results were expressed in terms of NIDDK rat LH-RP-2, FSH-RP-2, and prolactin-RP-2. The intra-assay and inter-assay coefficients of variation were 7.2% and 11.2% for LH, 3.4% and 5.3% for FSH, 3.4% and 5.2% for prolactin, respectively. Concentrations of ir-inhibin *(24)*, estradiol-17β, and progesterone *(25)* in plasma were measured by double-antibody radioimmunoassays (RIAs) using ^{125}I-labeled radioligans as described previously. The intra-assay and inter-assay coefficients of variation were 7.0% and 11.4% for ir-inhibin, 4.1% and 6.6% for estradiol-17β, and 9.5% and 16.4% for progesterone, respectively.

Ovarian Histology

Ovaries were preserved in 4% paraformaldehyde solution for histological examination. The organs were embedded in paraffin wax and sectioned serially at 10 μm. The sections were stained with hematoxylin and eosin. Serial sections of the ovaries were scanned at a magnification of 40×, and follicles larger than 201 μm in diameter were measured with an ocular micrometer at 12 h on the day of proestrus. The size of the follicles was determined by measuring the diameters in the section that passed through the nucleus of an oocyte by averaging vertical and horizontal measurements of the membra granulosa to obtain a diameter reading. The follicles were classified into five arbitrary groups according to size *(26)*. The number and maximum size of corpora lutea were counted in the section at 12 h on the day of estrus.

Statistical Analysis

All results were expressed as the means ± SEM. Data on the number of corpora lutea and follicles were analyzed by the Student's *t*-test, but when more than two means were compared, an analysis of variance was carried out and the significance of the difference between means was determined by the Tukey–Kramer test. Differences were judged significant if p was less than 0.05.

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