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# Increases in the Basal Secretion Rate of Follicle-Stimulating Hormone (FSH) Accompany Age-Associated Changes in Serum FSH Levels on Estrus[1] (42793)

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*Abstract.* This laboratory has recently reported that by 5–6 months of age, alterations in the secretion and production of follicle-stimulating hormone (FSH) occur in virgin female rats which precedes the age-related disruption of estrous cycles and attenuation of preovulatory gonadotropin surges. Specifically, circulating immunoreactive FSH levels are higher on estrus in rats 5 months and older compared to levels measured in 2- to 3-month-old rats. Therefore, the present study was conducted to explore a possible mechanism for this age-related increase in FSH levels. At 1400 hr on proestrus, estrus and diestrus-1, groups ($n$ = 6–12 rats/group) of 3- and 7-month-old, cyclic rats were decapitated, trunk blood was collected, and anterior pituitary glands were bisected and placed in incubation flasks containing 1 ml media (medium 199). Following a 30-min preincubation period, hemipituitary fragments were incubated for an additional 2 hr. Media and serum FSH levels were quantified by RIA. Levels of FSH were twofold higher in the serum of 7-month-old rats than 3-month-old rats on estrus. Similarly, the basal secretion rate (BSR) of FSH (expressed as ng FSH/ml/2 hr) was significantly ($P < 0.05$) higher from incubated hemipituitary fragments of 7-month-old estrous rats than from fragments obtained from younger estrous rats (7 month: 1637 ng/ml/2 hr vs 3 months: 1253 ng/ml/2 hr). Neither the serum FSH levels nor the BSR of FSH differed between age groups on proestrus or diestrus-1. These results show that age-associated increases in circulating FSH levels on estrus may be attributed to an enhanced basal secretion of FSH from the pituitary gland. 

The periovulatory profile of circulating follicle-stimulating hormone (FSH) levels during the rat estrous cycle is characterized by two periods of increased pituitary secretion of this gonadotropin (1). The first elevation in plasma FSH concentrations occurs during the afternoon of proestrus, and precedes ovulation by approximately 8–10 hr. This preovulatory increase in plasma FSH levels is primarily dependent on the hypothalamic discharge of luteinizing hormone–releasing hormone (LHRH) (2–5). A second increase in plasma FSH levels then commences late on proestrus and continues into estrus. This estrous rise in FSH seemingly occurs as a consequence of increases in the basal secretion rate (BSR) of FSH from the anterior pituitary gland (6). In contrast to FSH, no cyclic changes in the BSR of LH occur during the cycle (6). Since inhibin suppresses the BSR of FSH *in vitro* (7–9), and since a fall in serum inhibin concentrations precedes the second increase in circulating FSH levels (10, 11), it would appear that increases in the BSR of FSH on estrus are due to reduced ovarian inhibin secretion.

In an attempt to examine the effects of aging on periovulatory FSH secretion, this laboratory reported that plasma FSH concentrations on estrus were higher in 5-month-old than in 3-month-old 4-day cycling rats (12). It was subsequently observed that reductions in inhibin secretion accompanied the age-associated elevation in circulating FSH levels (13). Therefore, in view of this later finding, and the aforementioned effects of inhibin on the BSR of FSH, the following study was performed to determine whether age-related increases in circulating FSH levels could be due, in part, to changes in the BSR of FSH.

**Materials and Methods.** *Animals.* Female, Sprague–Dawley rats of the Crl:CD(SD)BR strain were purchased at approximately 2 months of age from Charles River Laborato-

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ries (Portage, MI). Animals used for the experiments were ordered in two staggered shipments so that the study could be conducted on both age groups (3- and 7-months-old) simultaneously.

Upon arrival, rats were housed in quarters environmentally controlled for temperature (22–24°C) and light–dark cycle (lights on 0500–1900 hr). Rat chow and tap water were continuously made available. Commencing at 6 months of age for rats studied at 7 months, and 2 weeks after arrival for animals studied at 3 months, daily vaginal lavages were obtained. Only those rats exhibiting more than 2-consecutive 4-day cycles were used for study. A total of 53 rats were used in this experiment.

*Hemipituitary incubations.* At 1400 hr on diestrus-1, proestrus, and estrus, rats were decapitated and trunk blood was collected. This time corresponded to the interval of time on estrus when age-related increases in circulating FSH levels are observed and is also prior to preovulatory rises in serum FSH levels on proestrus (12). Following decapitation and the collection of trunk blood, the pituitary gland was rapidly excised, and the posterior pituitary gland was dissected out and discarded. The remaining anterior pituitary gland was bisected and placed in a Hank's–Hepes-buffered solution. Each hemipituitary fragment then was blotted and weighed. Hemipituitary glands from each age group on a given day of the cycle were pooled, and randomly distributed into incubation flasks (two fragments/flask; 6–12 flasks/group) containing 1 ml medium 199 with unmodified Earle's salts and L-glutamine (Grand Island Biological Co., Grand Island, NY), and buffered to pH 7.2 with sodium bicarbonate. The flasks were placed in a Dubnoff metabolic shaking incubator maintained at 37°C under an atmosphere of 95% $O_2$/5% $CO_2$. After a 30-min preincubation period, the media was discarded, and the fragments were washed 2× with 1 ml fresh media which was discarded. One milliliter of media was then added to each flask, and the fragments were incubated for an additional 2 hr. Afterward, the media was collected on ice and spun at 1500*g* for 15 min in a refrigerated centrifuge. In order to avoid possible contamination with cellular debris, the upper nine-tenths of the centrifuged media were stored frozen (−20°C) for subsequent determination of FSH levels by radioimmunoassay.

*Radioimmunoassays.* Determinations of media and serum FSH concentrations were made using rat kit materials kindly provided by the NIDDK. For determination of FSH levels in media, the RP-2 standard was diluted with the incubation media instead of assay buffer. Concentrations of FSH were expressed in terms of the rat RP-1 standard which has a biological potency of 2.1 × NIH-FSH-S1. To avoid interassay variability, all samples were run in duplicate in one assay using standards diluted either in buffer or in media. The intraassay variability for serum samples determined on a pool of plasma from long-term ovariectomized rats at three different aliquots (50, 25, and 10 μl) was 4.9%. The intraassay variability for media samples based on duplicate determinations of a solution containing 2250 ng FSH/ml at three aliquots (25, 10, and 5 μl) was 9.2%.

Serum LH concentrations were determined by the ovine–ovine procedure of Niswender *et al.* (14) using the rat RP-2 as standard. Concentrations were expressed in terms of the rat RP-1 standard which has a biopotency of 0.03 × NIH-LH-S1. The intraassay variability was 5.1%.

*Statistical analysis.* All data were subjected to a two-way (age × day of cycle) analysis of variance. Differences in various parameters among time intervals within a given age group were evaluated by the Student–Neuman–Keuls multiple-comparison test. The Student *t* test was used to analyze possible differences between age groups.

**Results.** As shown in Fig. 1A, serum FSH levels were approximately twofold higher in 7-month-old estrous rats than FSH levels measured in younger rats on this day of the cycle. Similarly, hemipituitary fragments from 7-month-old estrous rats released significantly ($P < 0.05$) more FSH during a 2-hr incubation period than did fragments from 3-month-old estrous rats (Fig. 1B) (7 months: 1637 ng/ml/2 hr vs 3 months: 1253 ng/ml/2 hr). However, since the weight of the fragments was significantly ($P < 0.01$) higher in older than in younger rats on estrus

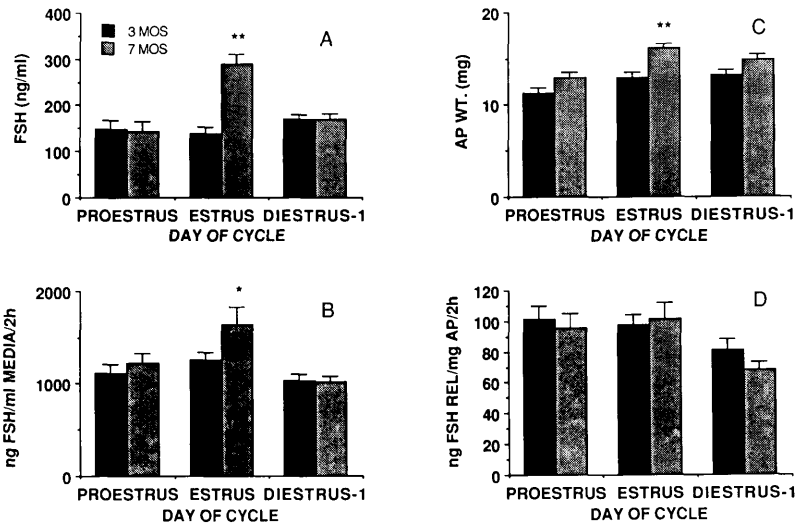


FIG. 1. Effects of aging on the BSR of FSH. Serum FSH levels (ng/ml) measured in 3- (solid column) and 7- (stippled column) month-old rats ($n$ = 6–12/group) are shown in A. The BSR of FSH measured *in vitro* from incubated hemipituitary fragments of 3- and 7-month-old rats is shown in B expressed as nanogram FSH per milliliter media per 2 hr and D expressed as nanogram FSH per milligram anterior pituitary (AP) weight per 2 hr. The AP weights (mg) presented in C reflect the total weights of the two hemipituitary fragments in each flask. Columns and vertical bars represent the means and SEM, respectively. *; $P < 0.05$ vs. 3-month-old group. **; $P < 0.01$ vs 3-month-old group.

(Fig. 1C), the BSR of FSH did not differ between young and old estrous rats when the amount of FSH secreted was expressed per milligram anterior pituitary weight (Fig. 1D).

No age-related changes in either the serum FSH levels or the BSR of FSH expressed as either nanogram per milliliter per 2 hr or nanogram per milligram per 2 hr were observed on other days of the cycle (Fig. 1). Furthermore, no significant differences in serum LH levels were observed between age groups on any day of the cycle studied (data not shown).

Both the serum FSH levels (Fig. 1A) and the BSR of FSH expressed per milliliter media (Fig. 1B) were significantly higher in 7-month-old rats on estrus than these parameters measured in 7-month-old rats on other days of the cycle ($P < 0.01$ for serum FSH levels vs proestrus and diestrus-1; $P < 0.05$ for BSR vs proestrus; $P < 0.01$ for BSR vs diestrus-1). In contrast, neither the serum FSH levels nor the BSR of FSH differed significantly among the days of the cycle in 3-month-old rats.

**Discussion.** Previous data from this laboratory has shown that circulating FSH levels are higher in rats age 5–6 months and older than levels measured in younger (2–3-months old) rats on estrus (12). Results now are presented to show that one possible mechanism for this age-related increase in serum FSH levels on estrus is an increase in the BSR (expressed per ml media) of FSH from the anterior pituitary gland. Furthermore, since the secondary FSH surge on estrus in young rats can be attributed to an increase in basal FSH secretion (6), the present data would seem to indicate that higher serum FSH levels in older rats on estrus reflect an age-associated prolongation of the secondary FSH surge. The consequence of this extended period of FSH secretion to subsequent aging of the reproductive axis remains to be determined.

As reported previously (12), age-associated increases in serum FSH concentrations are accompanied by an increased synthesis of acidic FSH isohormones in the anterior pituitary gland. Due to the increased incorporation of sialic acid residues onto the polypeptide subunits, these larger acidic FSH forms exhibit an increased half-life in the circulation (15). Assuming that these forms are re-

leased into the circulation, it was originally thought that the increase in circulating FSH levels with age may primarily reflect the secretion of FSH molecules that are more immunologically potent and less biologically active *in vitro,* but more biologically efficient *in vivo* (because of their longer half-life) as opposed to the release of more FSH molecules. The present observation that FSH immunoreactivity is higher in the media obtained from incubated hemipituitary fragments of older animals than from incubated fragments of younger animals on estrus would seem to support our original interpretation. However, this is not to say that increases in the actual number of FSH molecules do not contribute to age-related increases in circulating FSH levels. Along these same lines, one could speculate that the higher BSR of FSH found on estrus in younger cycling rats (6) may be a reflection of a qualitative change in the type of FSH secreted in addition to a quantitative one since it was observed that proportionately more acidic FSH isoforms are present in and secreted from hamster pituitary glands on estrus (and diestrus-1) than on other days of the cycle (16).

In our earlier study (12), it was shown that anterior pituitary glands from 7-month-old estrous rats were larger (approximately 2 mg) than these glands from 3-month-old rats on estrus. In the present study, hemipituitary fragments obtained from 7-month-old estrous rats weighed more than fragments obtained from younger estrous rats. Consequently, when expressed per milligram anterior pituitary weight, no age-associated changes in the BSR of FSH were observed. Although this finding could be interpreted to mean that increases in the BSR of FSH on estrus are due simply to a larger pituitary gland in older estrous rats, two observations point to a more intricate explanation. First, the weights of hemipituitary fragments from 7-month-old estrous and diestrous-1 rats were similar, yet the BSR of FSH was significantly higher from estrous than from diestrous-1 hemipituitary fragments. Second, age-related increases in circulating FSH concentrations on estrus first were observed in 5-month-old rats whose anterior pituitary weights did not differ significantly from weights measured in 3-month-old rats (12). Aside from these observations, we have reported that pituitary FSH concentration is lower in 7-month-old estrous rats than in 3-month-old estrous rats (12). To our knowledge, cytological studies on possible aging changes in the number and size of pituitary gonadotropes have not been performed in rats. However, it seems unlikely on the basis of the lower pituitary FSH stores (and unchanging LH stores) in 7-month-old rats that there would be an increase in the number and/or size of gonadotropes to explain the higher BSR of FSH at this older age.

In summary, our results demonstrate that higher circulating FSH levels in older estrous rats may be attributable to an increase in the BSR of FSH. In turn, the higher BSR of FSH in older rats may be related to the age-associated reduction in inhibin secretion on estrus. Since the basal rate of FSH release by the pituitary gland is independent of diencephalic influences, one may conclude that age-related changes in pituitary FSH secretion at this age involve perturbations in ovarian–pituitary interactions.

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