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The Effect of Stress on Serum Prolactin in the Female Rat[1, 2] (39415)

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The effect of stress on endocrine control mechanisms is receiving increased attention. Several investigators have demonstrated that various stressors can increase serum prolactin levels in both male and female rats other than on the afternoon of proestrus (1–6). The elevated serum prolactin levels on the afternoon of proestrus were not affected by at least one commonly used stress, ether vapor (2, 7). Stress has been associated with impaired reproductive performance in many species. These effects of stress on reproduction have included blockage of sexual cycles, decreased implantation rates, and increased embryonic mortality (8–11). Although stress is usually associated with increased serum prolactin concentrations, recent data from our laboratory showed decreased serum prolactin and inhibition of ovulation in female rats given restraint stress on the afternoon of proestrus (12). In addition a recent study has indicated that ether stress can inhibit prolactin release in rats on the afternoon of proestrus (13). The present study was undertaken to determine the effect of stressors of varying intensities on prolactin secretion in rats in a variety of physiological states, including cycling, castration, pregnancy, and lactation.

Material and methods. Young adult (4–8 months) female Long-Evans (Blue-Spruce Farms, Almont, N.Y.) were used in these studies. The rats were housed in a temperature-controlled (72 ± 2°F) and artificially lighted (12-hr light cycle) room and maintained on Wayne Lab-Blox and water supplied *ad libitum*. Daily vaginal smears were taken. Only rats exhibiting at least two normal estrous cycles before experimentation were included in the study.

All blood samples were collected by orbital sinus puncture. The volume of each blood sample was about 1 ml. The adequacy of orbital sinus bleedings in the determination of "control or nonstressed" serum prolactin concentrations was studied by comparing serum prolactin in blood samples taken by orbital sinus and by rapid decapitation techniques. At 4 PM on the days of estrus, proestrus, and diestrus Day 2, individual rats were rapidly transferred from their cages directly into ether chambers and an orbital sinus blood sample was taken between 40 and 60 sec after initial animal disturbance (designated as a 1-min blood sample). The rats were returned to their cages. At 4 PM on the third estrous cycle after orbital sinus sampling, the same rats at the same stages of their estrous cycles were individually removed from their cages, transported to an adjacent room and a blood sample was taken by decapitation within 15 sec of animal disturbance.

Separate groups of rats at proestrous, estrous, and diestrous 2 stages of their ovarian cycles were subjected to one of three types of stressors: ether vapor, ether vapor plus serial bleeding, and restraint. Rats exposed to ether vapor stress were removed from their cages and placed in ether chambers. Following anesthesia, they were removed from the chambers and allowed to regain consciousness. The same rats were then reintroduced into the ether chambers at 8, 15, and 25 min after removing them from their cages. A single blood sample was taken by orbital sinus puncture after the 25-min anesthesia. Control prolactin levels for this experiment were measured in orbital sinus blood taken within 1 min of animal disturbance from separate groups of rats. The second stress, ether vapor plus serial bleeding, utilized the same anesthesia scheme as described for ether vapor stress, except serial

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blood samples were taken by orbital sinus puncture at each anesthesia interval (1, 8, 15, and 25 min). Prolactin concentrations in the 1-min blood sample was designated as the controls for this experiment. The third stressor employed was restraint. Groups of rats were removed from their cages, placed in ether chambers and an orbital sinus blood sample taken within 1 min of initial disturbance. The rats were then restrained in the supine position for 2 hr. At the end of the 2-hr restraint, rats were released, placed back into ether chambers, and a second blood sample taken within 1 min from the end of the restraint period. Serum prolactin levels in the prerestraint blood samples were considered as controls for this experiment.

The effect of chronic stress on serum prolactin was determined by subjecting a group of rats on the first day of diestrus to 20 consecutive days of 2-hr restraint stress. Serum prolactin levels were measured in serial blood samples taken within 1 min of animal disturbance in their cages on Days 1, 9, and 19. These prestress prolactin levels were designated as control hormone levels. The effect of the chronic restraint on serum prolactin was measured in additional blood samples taken within 1 min of the end of the restraint period on Days 1, 5, 10, 15, and 20 of the treatment regime.

Another experiment measured the effect of 2-hr restraint stress on serum prolactin on the first day of pregnancy. Separate groups of rats received 2-hr restraint stress treatments from 12–2 PM or 3–5 PM on the day sperm were detected in the vaginal smear (Day 1 of pregnancy). Serum prolactin was measured in serial blood samples taken at 12, 2, 3:30, and 5 PM from the 12–2 PM stress groups, and at 3, 5, 6:30, and 8 PM from the 3–5 PM stress group. Control prolactin levels were measured in serial blood samples taken by the rapid sampling technique at the same time intervals from similar groups of rats that did not receive the restraint stress treatment.

The final experiment included in this study considered the effect of serial bleeding and restraint stressors on prolactin secretion in lactating rats. Rats included in this study were in the fourth to tenth day of lactation and were observed to be over their pups in the interval before experimentation. For the serial bleeding study, rats were removed from their cages, placed in ether chambers, and bled at 1, 8, 15, and 25 min following first disturbance. For the restraint portion of the experiment, rats were restrained for 2 hr after collection of the 1-min blood sample with a second blood sample taken within 1 min of the end of the restraint period.

Serum prolactin was measured in duplicate by a standard double-antibody radioimmunoassay procedure (14). NIAMD-Rat Prolactin, RP-1 was used as the reference standard. Differences between groups were analyzed by Student's t test and a probability of 0.05 was considered as significant.

Results. Comparison of control or nonstressed serum prolactin levels measured in the same rats by two experimental techniques, rapid orbital sinus puncture and decapitation is shown in Fig. 1. No differences were recorded in serum prolactin concentrations measured by these techniques at any stage of the estrous cycle.

Figure 2 illustrates the effect of exposure to a mild stress, ether vapor, in rats at proestrous, estrous, and diestrous 2 stages of their ovarian cycle. Serial exposure to ether vapor over the 25-min experimental interval (the rats were anesthetized at 1, 8, 15, and 25 min after removing them from their cages) did not significantly affect serum prolactin concentrations at any stages of the estrous cycle tested.

We next considered the effect of ether vapor plus serial blood sampling stress on serum prolactin levels (Fig. 3). Serum prolactin concentrations again were not in-

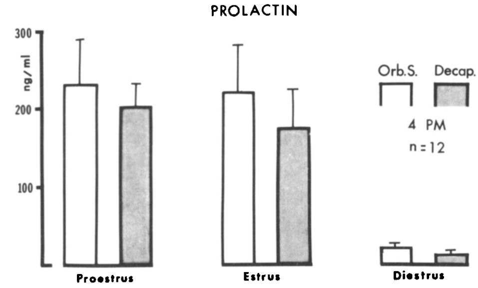

FIG. 1. Comparison of oribital sinus puncture and decapitation procedures of collecting blood for measurement of nonstressed levels of prolactin. Mean prolactin values and SEM are shown from blood collected at 4 PM at proestrous, estrous, and diestrous stages of the ovarian cycle.

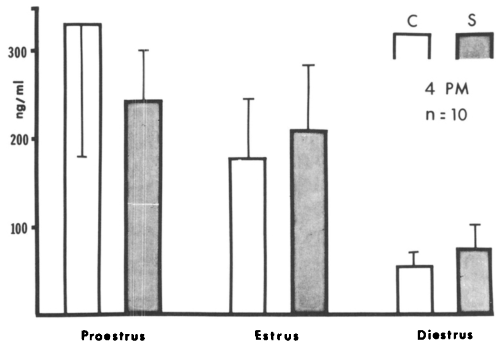

FIG. 2. Effect of serial ether vapor on rat serum prolactin at 4 PM on proestrous, estrus, and diestrus. Blood samples were taken by orbital sinus puncture under light ether anesthesia. Rats were exposed to ether at 1, 8, 15, and 25 min after cage disturbance. The experimental sample (S) was taken after the 25-min anesthesia. Control samples (C) were taken from similar rats not exposed to ether. Serum prolactin levels (ng/ml) are plotted as group means with indicated SEM.

creased by this treatment in any of the groups tested. The high levels of prolactin encountered on the afternoon of proestrus and estrus were reduced by this treatment. On the other hand, the low level of prolactin on the afternoon of diestrus Day 2 was not affected by this treatment, and although there was a reduction in the mean prolactin levels in the group stressed on the morning of estrus, these differences were not significant.

The effect of a single 2-hr restraint stress treatment on serum prolactin is shown in Fig. 4. The high level of prolactin in the proestrous group was again reduced by the stress treatment. On the other hand, serum prolactin levels were not affected by restraint stress in the estrous group, and the low control group levels of prolactin in both the castrate and diestrous group was sharply increased following the 2-hr restraint stress treatment.

Figure 5 indicates the effect of 20 days of chronic restraint stress on serum prolactin. Prolactin levels in blood samples before restraint was initiated on Days 1, 9, and 19 (designated as control samples), increased sharply during the experiment, rising from 27 to 242 ng/ml. Prolactin was increased in the blood sample taken at the end of the first day of the restraint treatment. On the other hand, prolactin levels were less than 50 ng/ml at the end of the restraint period on Days 5, 10, 15, and 20. These end of stress prolactin levels are markedly lower than the pretreatment prolactin concentration measured on Days 9 and 19. Rats in this group were on the first day of diestrus when the treatment was started. This stress regime was sufficient to block regular ovarian cyclicity in all the experimental animals.

The effect of restraint stress on serum prolactin on the afternoon of the first day of pregnancy is depicted in Fig. 6. The 2-hr stress was administered to separate groups of rats from 12–2 PM or 3–5 PM. Serial blood samples were taken from each stress group and similar control rats before and after the stress treatment and at $1^1/_2$ and 3 hr after the end of the stress regime. In the group receiving the stress treatment from 12–2 PM, prolactin levels were lower than control group concentrations only at the 3:30 PM sampling interval, $1^1/_2$ hr after the end of the treatment. Prolactin levels in the 3–5 PM stressed group were less than control group concentrations at the end of the stress period, but were similar to control groups hormone levels at both $1^1/_2$ and 3 hr after the end of the treatment.

The response of lactating rats to serial bleeding and restraint stress is depicted in Fig. 7. The pretreatment prolactin levels for both experiments were elevated. Both stress treatment regimes resulted in a prompt reduction in serum hormone levels. In the serial bleeding stress group, prolactin levels were only 1/3 as high by the 8-min sampling interval and continued to decrease until the end of the treatment. Prolactin levels in the group receiving the restraint treatment were reduced from 342 to only 68 ng/ml.

Discussion. These experiments indicate that the effect of stress on serum prolactin concentrations in the female rat varies markedly with the physiological state of the animal and the intensity of the stressor employed. Instead of uniformly stimulating prolactin secretion, in some instances stress regimes were found to have no effect on prolactin levels, and in other experimental situations stress was associated with a sharp reduction in serum prolactin. If the stress used was of relatively low intensity, such as periodic exposure to ether vapor alone at all stages of the estrous cycle we tested or ether

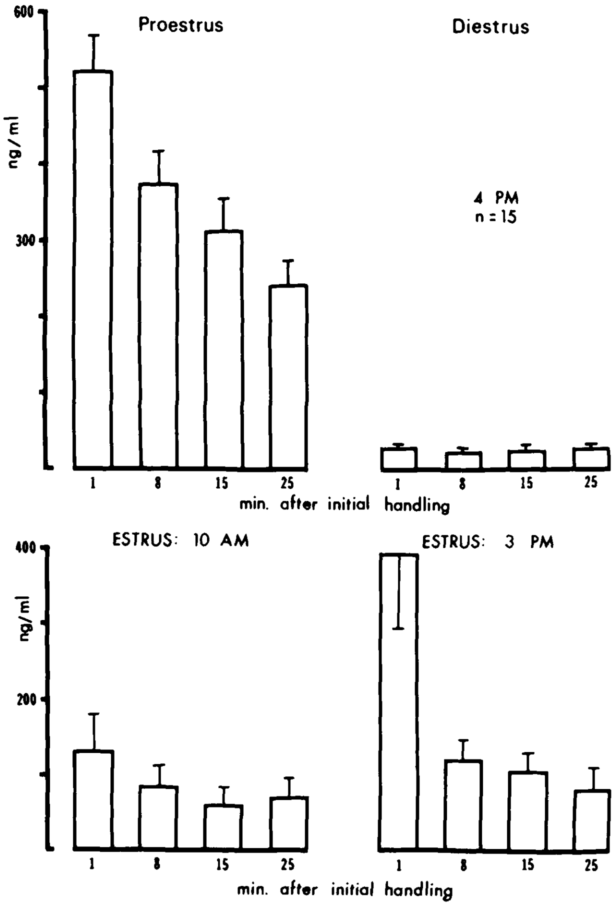

FIG. 3. Effect of ether vapor plus serial bleeding stress on rat serum prolactin at 4 PM on proestrus and diestrus and at 10 AM and 3 PM on estrus. Rats were ether anesthetized and serially bled by orbital sinus puncture at 1, 8, 15, and 25 min after cage disturbance. Serum prolactin levels (ng/ml) are plotted as group means with indicated SEM.

vapor plus serial bleeding stress in diestrous rats, serum prolactin concentrations were not affected. The effect of high-intensity stress was determined by the physiological state of the rat. Rats with high pretreatment serum prolactin concentrations, on the afternoon of proestrus, the afternoon of estrus, Day 1 of pregnancy, and during lactation, uniformly showed reduced prolactin levels after serial bleeding and all these groups except those on the afternoon of estrus had reduced serum prolactin following restraint stress treatment. These observations are in agreement with recent studies on the effects of ether stress on serum prolactin levels at proestrus (13) and the effects of serial bleeding on serum prolactin in lactating rats (15). On the other hand, rats with low pretreatment levels of prolactin, such as the diestrous and castrate groups, had increased serum prolactin levels following restraint stress. Thus, the initial level of prolactin in the serum appears to be important in determining the amount of prolactin released by a stress (13). Our Long-Evans rats show a diurnal peak of prolactin secretion in the afternoon of estrus similar to what we find on the afternoon of Day 1 of pregnancy (Fig. 6) and what has been previously reported on the first day of pseudopregnancy (16). The only group of rats that had lower serum prolactin levels on the afternoon of estrus than similar proestrus groups was that subjected to 2 hr of restraint stress (Fig. 4). It is of interest that this group which had initial serum prolactin levels intermediate between those of proestrous groups or other afternoon of estrous groups and diestrous prolactin levels did not have either increased or decreased serum prolactin after the restraint stress treatment.

These data do not indicate whether the changes in serum prolactin related to stress are caused by modification of the rate of pituitary secretion, a change in metabolism or clearance of circulating prolactin, or a combination of both effects. A recent study considering the effects of ether stress during the proestrus surge indicated that the fall in serum prolactin during acute stress was similar to the decline after hypophysectomy (13). Another report suggests that the changes in serum LH following acute stress in the male rat are not due to alterations in metabolism or clearance of LH from the blood or to alterations in pituitary responsiveness to hypothalamic gonadotropin-releasing hormone (17). Current understanding of hypothalamic regulation of prolactin secretion implies that several hypothalamic factors may be involved. It has been suggested that prolactin secretion is regulated by both hypothalamic inhibitory factors (PIF) and stimulatory factors (PRF) (18, 19). Prolactin secretion has been reported to be influenced by alterations in hypothalamic content of serotonin (20), dopamine (21, 22), norepinephrine (23), and acetylcholine (24) affecting PIF and PRF secretion, or possibly to the hypothalamic secretion of dopamine and norepinephrine acting directly on pituitary prolactin secretion (25). Recent reports have shown that reserpine can block ether stress reduction of prolactin on the afternoon of proestrus (13) and that acute stressors can reduce arcuate nucleus content of both dopamine and norepinephrine (26).

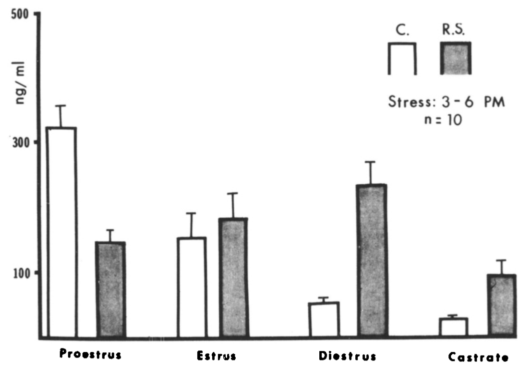

FIG. 4. Effect of 2-hr restraint stress on rat serum prolactin at proestrus, estrus, and diestrus, and in castrates. Control samples (C) were taken before restraint stress and the stressed samples (RS) at the end of the 2-hr stress regime. Blood samples were taken by orbital sinus puncture under light ether anesthesia. Serum prolactin levels (ng/ml) are plotted as group means with indicated SEM.

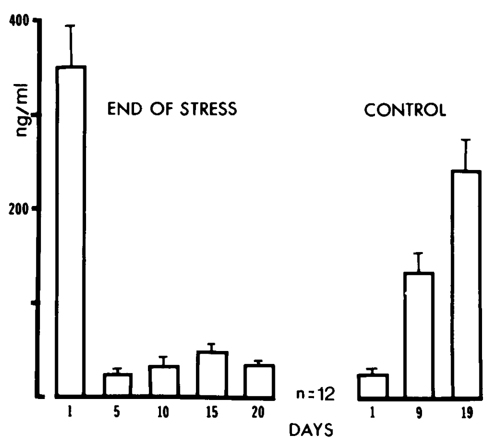

FIG. 5. Effect of chronic restraint stress (2 hr/day for 20 days) on serum prolactin. Prestress control samples were taken on Days 1, 9, and 19 of treatment. End of stress samples were taken on Days 1, 5, 10, 15, and 20 of the stress regime. Blood samples were taken by orbital sinus puncture under light ether anesthesia. Serum prolactin levels (ng/ml) are plotted as group means with indicated SEM.

It is clear from these experiments that serum concentrations of radioimmunoassayable prolactin can show prompt alterations with the initiation of stress treatments. If the alterations in serum prolactin are due to changes in hypothalamic PIF and PRF secretion, stress apparently affects their release differently in diverse physiological situations. When serum prolactin levels are high as during lactation or on the afternoon of proestrus, the effect of stress may involve either a decrease in PRF or an increase in PIF. On the other hand, the increased prolactin release seen after restraint stress in diestrous or castrate rats may be due to either an increase in PRF or a decrease in PIF. The effects of the different stressors on hypothalamic biogenic amines remain to be determined. In addition the sensitivity of the mechanisms controlling serum prolactin levels during stress was different in different physiological states. Pretreatment serum

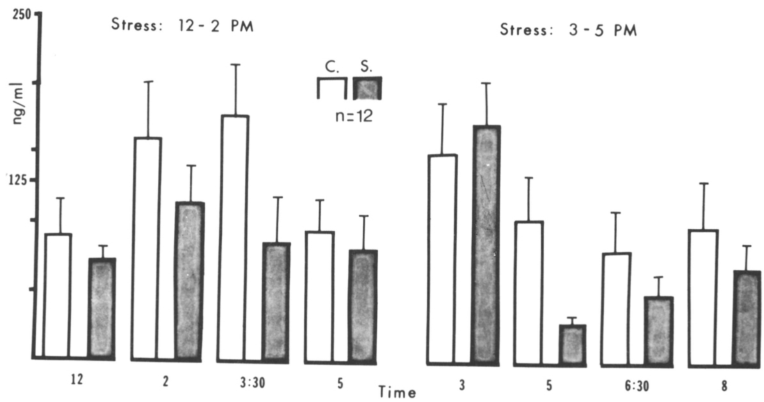

FIG. 6. Effect of restraint stress on serum prolactin on Day 1 of pregnancy. Rats received 2 hr of restraint stress from 12–2 or from 3–5 PM. Serial blood samples were taken from each rat before the stress, at the end of the stress, and $1^1/_2$ and 3 hr after the end of the stress. Blood samples were taken by orbital sinus puncture under light ether anesthesia. Serum prolactin levels (ng/ml) are plotted as group means with indicated SEM.

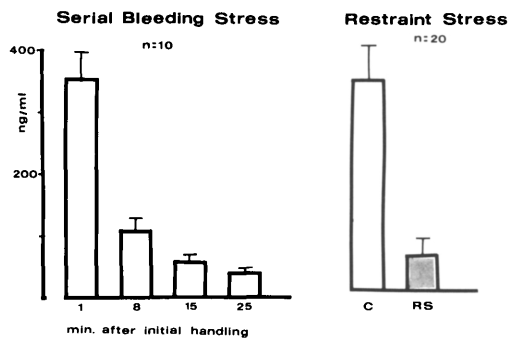

FIG. 7. Effect of serial bleeding and restraint stress on serum prolactin in rats on Days 4–10 of lactation. Blood samples were taken by orbital sinus puncture under light ether anesthesia. Blood samples were taken from the restraint stress group before stress and at the end of the 2-hr treatment. Blood samples were taken from the serial bleeding stress group at 1, 8, 15, and 25 min after removing them from their litters. Serum prolactin levels (ng/ml) are plotted as group means witl indicated SEM.

prolactin was elevated in the proestrous, estrous, pregnant, and lactating rats. The reduction of serum prolactin by stress treatments was greater in estrous rats subjected to serial bleeding and in the stressed lactating rats than after stress in the proestrous or pregnant groups and the estrous group subjected to the high intensity restraint stress. Although the latter had higher pretreatment prolactin levels than the diestrous group, their serum prolactin levels were lower than the proestrous group and they did not show any change in serum prolactin concentrations following the stress.

The effect of stress on serum prolactin on the first day of pregnancy also depended on the initial hormone levels. Serum prolactin levels of the control groups show a diurnal peak of serum prolactin at about 3 PM. The group of rats that received the stress treatment before the peak of serum prolactin (12–2 PM) did not show this diurnal peak and the group which received the stress during the diurnal peak (3–5 PM) had a prompt reduction of serum prolactin which was sustained throughout the sampling period.

The increase in pretreatment prolactin levels in the group of rats subjected to 20 days of restraint stress suggests that these rats were experiencing chronically increased prolactin levels through much of their treatment regime. On the other hand, the effect of the restraint stress on prolactin levels during the stress was markedly different during different periods of this treatment. On the first day of treatment, the elevated prolactin levels were typical of the data reported from the previous diestrous group exposed to this stressor. However, this high end of restraint stress prolactin level was not sustained during the remainder of the treatment period. The effect of restraint stress on this group of rats at Days 5, 10, 15, and 20 of treatment was to reduce an initially high prolactin level to the low end of treatment concentration, similar to the effect of this stressor on high pretreatment prolactin in other physiological states.

Although our data on the effect of simple exposure to ether vapor stress without serial bleeding are not in agreement with a recent report of decreased serum prolactin in proestrous rats following administration of this stress (13), our results from more intense stressors, serial bleeding with ether anesthesia and restraint stress, in groups of proestrous rats confirm this report. Although the sensitivity to stress may vary in different strains of rats and different investigators may alter the severity of a particular stress regime, these data reaffirm the importance of understanding the effects of stress on hormone control mechanisms, emphasize the importance of avoiding irregular or undue stress in animals used for studies of prolactin release, and point to the importance of maintaining uniform blood sampling times in experiments where hormone levels may be affected by stress.

Summary. Changes in serum prolactin in response to ether vapor, ether vapor plus serial bleeding, acute restraint and chronic restraint stresses were measured in cycling, castrate, pregnant, and lactating female Long-Evans rats. Serum prolactin was measured by radioimmunoassays in serial blood samples taken before and after administration of each stressor. Pretreatment prolactin levels were elevated in rats in the afternoon of proestrus and estrus, on Day 1 of pregnancy, and during lactation. Low initial prolactin concentrations were observed during diestrus, on the morning of estrus, and in castrate females. Low intensity stress, such as ether vapor alone, did not affect serum prolactin concentrations in any group tested. The effect of higher intensity stress was related to the pretreatment concentration of serum prolactin. If the initial prolactin levels were high, such as on the afternoon of proestrus and estrus, on Day 1 of pregnancy, and during lactation, high intensity stress was associated with a reduction in serum prolactin in all cases except for the group of rats on the afternoon of estrus which received the restraint stress treatment. On the other hand, if the pretreatment prolactin levels were low, such as in diestrous and castrate rats, high intensity stress was found to increase serum prolactin concentrations. Rats subjected to chronic restraint stess had increased pretreatment serum prolactin during the treatment regime. Although their initial prolactin concentrations were increased, these rats had lowered levels of prolactin at the end of their restraint periods. These data reaffirm the importance of understanding the effects of stress on hormone control mechanisms, and emphasize the importance of avoiding irregular or undue stress in animals used for studies of prolactin secretion.

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