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Serum Levels of Luteinizing Hormone, Prolactin, Estradiol and Progesterone in Laying and Nonlaying Mallards (Anas platyrhynchos)[1]

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ABSTRACT

Temporal changes of circulating serum hormones were measured to compare the reproductive endocrinology of laying and nonlaying mallards. In this study all sixteen control mallards left with their mates laid eggs, while only one of sixteen mallards stressed by daily movement into new pens, laid eggs. Serum levels of luteinizing hormone (LH), prolactin, estradiol, and progesterone were significantly lower ($P<0.05$) in stressed nonlaying mallards than in laying mallards over the 7-week period. Within 1 week of the rotation treatment, LH concentrations in stressed mallards averaged ($\bar{x}$ ± SEM) 2.72 ± 0.19 ng/ml and were significantly lower ($P<0.05$) than LH levels in the controls (3.62 ± 0.18 ng/ml).

After 7 weeks, injections of luteinizing hormone releasing hormone (LHRH) induced a greater change in circulating LH levels in stressed mallards (2.1 ± 0.3 ng/ml) than in breeding control mallards (0.9 ± 0.2 ng/ml). These data demonstrate that the lack of reproduction in stressed mallards was associated with LHRH-sensitive pituitary pools of LH, despite their low concentrations of serum LH. These data suggest that the block in reproduction is a failure of the hypothalamus to produce or release releasing hormones.

The serum hormone levels of the control mallards varied temporally with stages in the nesting cycle. LH levels increased with the onset of nesting activity, and showed marked fluctuations during the laying period. LH levels fell at the onset of incubation but increased after loss of clutch. Estradiol levels were highest prior to the laying of the first egg and their peak coincided with the initial nest building behavior of the females. Progesterone levels increased sharply with the laying of the 2nd-4th eggs, decreased sharply with the laying of the 6th egg, and then increased slightly at the end of the nesting cycle. Prolactin levels were initially low but gradually increased with laying and incubation activity, declined with loss of clutch, and increased again with renesting activity. Prolactin levels in the stressed mallards also increased ($P<0.01$) over the 7-week period, but significantly less ($P<0.05$) than in layers.

INTRODUCTION

Domestic mallards have been extensively used as models for the study of ecophysiological and neuroendocrine mechanisms of annual cycles (Jallageas et al., 1974; Haase et al., 1975a,b; Assenmacher et al., 1977; Donham, 1979). In contrast to the extensive research on domestic mallards, few investigations have been conducted on wild mallards. The few studies that do exist have identified several differences in the reproductive physiology of wild and game-farm mallards (Donham, 1979), and wild and domestic mallards (Haase et al., 1975a,b; Paulke and Haase, 1978). For example, differences were found in absolute hormone levels (Paulke and Haase, 1978) and in the temporal patterning of the hormone fluctuations (Donham, 1979). Since the reproductive endocrinology of wild mallards has not been thoroughly investigated, our first objective was to describe the changes in serum prolactin, luteinizing hormone (LH), estradiol, and progesterone occurring throughout the reproductive cycle in these birds.

Reproduction in wild mallards is periodic. This periodicity is attributed to the discontinu-

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ous nature of the environmental events that serve as cues for ovulation, incubation, and parental care of young. Due to the diversity of cues required for reproduction, it is not surprising that wild ducks usually fail to reproduce or delay their reproduction in response to adverse environmental conditions. The physiological basis for this reproductive failure is poorly understood. Therefore, our second objective was to elucidate the physiological mechanisms of inhibition of ovarian development in mallards that occurred in response to adverse environmental conditions. Our experimental approach was to inhibit laying in a group of mallards by manipulating their social environment. By comparing the levels of serum LH, prolactin, estradiol, and progesterone between groups of laying and nonlaying hens, we intended to elucidate which components in the hypothalamo-adenohypophysio-gonadal system caused reproductive failure in mallards.

Results of an earlier study of nonbreeding captive pintails (*Anas acuta*) demonstrated that reproductive failure was associated with large stores of pituitary gonadotropins but small gonads (Phillips and van Tienhoven, 1960). One could speculate that these birds were not releasing gonadotropins or that the gonads were insensitive to the levels of gonadotropins that were normally sufficient to stimulate the gonads of wild birds. If the former were true, it might be suggested that the pituitary glands of these birds were refractory to hypothalamic releasing stimuli or, alternatively, that the central nervous system (CNS) mechanisms that normally induce luteinizing hormone-releasing hormone (LHRH) release were inoperative. On the other hand, one might speculate that stress induced hyperprolactinemia, and that prolactin was exerting an antigonadal effect. While stressors have been shown to increase prolactin levels in some mammals (Neill, 1970; Ajika et al., 1972; Seggie and Brown, 1975), and prolactin has often been shown to exert antigonadal effects in birds (Bates et al., 1935, 1937; Tanaka et al., 1971; Camper and Burke, 1977b,c), there is only one study reporting stress-induced hyperprolactinemia in birds (Opel and Proudman, 1982).

The techniques available to earlier investigators precluded an examination of these possibilities. With the advent of radioimmunoassays (RIA) for avian LH and prolactin, and the general availability of RIA's for steroid hormones, some of these questions could be addressed. Thus, these studies were undertaken to compare levels of LH, prolactin, estradiol, and progesterone in mallards that successfully laid eggs to those that failed to lay. In addition, the responsiveness of the pituitary glands of mallards in each group were tested by treatment with LHRH.

MATERIALS AND METHODS

Mallards were reared from eggs collected from the wild in south central Manitoba. The eggs were hatched, and the mallards reared and maintained using standard procedures (Ward and Batt, 1973) at the Delta Waterfowl Research Station (50°11′ N, 19°19′ W). All were overwintered indoors and were exposed only to natural daylight.

The ducks were randomly paired in April, 1973. These same pairs were placed together, in individual nesting compartments within an outdoor pen, in April of 1974, 1975, and 1976. In this (1977) experiment, thirty-two mallard females, which had laid eggs in each of the 3 previous years, were randomly placed into either a control group or an experimental group. The experiment was started before the onset of egg laying. On April 12, 1977, control females were placed with their previous mates in breeding compartments that visually isolated them from other ducks. Females in the experimental group were also placed in isolated breeding compartments but were placed with another female as a pen partner instead of a drake. Each mallard in the experimental group was caught by hand, moved to a new compartment, and placed with a new female pen partner daily. One day a week, each experimental mallard was further stressed by being moved into a group of eight female mallards. This treatment was started on April 13 and continued until May 26. It was discontinued for 4 days (May 27-May 31), resumed on June 1, and continued until June 7.

The individual compartments were 3.5 m × 2.0 m, one-half of which was swimming water and one-half dry concrete floor. Compartments were separated by 0.4-m high walls that prevented physical and visual contact between pairs but allowed them to hear each other. Each pen contained a nest box and a feed dish. A standard diet of commercially prepared high-protein duck pellets (Ward and Batt, 1973) mixed with wheat was supplied freely. This diet was supplemented with oyster shells and grit.

Eggs

The wild mallards used in this study were experienced breeders that had laid eggs in 1974, 1975, and 1976. Once laying commenced in the control hens in 1977, each hen laid one egg per day, usually between 0500 and 1000 h. After eight to ten eggs had been laid, the females deposited down and started to spend more time on the nest incubating their eggs. All of the eggs of a hen's first clutch were numbered and left in the nest until the hen had not laid a new egg for 5 consecutive days, during which time she began incubation. The eggs were then collected and the nesting material was replaced. Mallard hens usually renested and started laying a second clutch of eggs within 7–10 days. Hens that laid a second clutch of eggs were

allowed to incubate them. Some hens were good nesters in that they deposited eggs in a normal sequence, tended their nests and eggs, and incubated until their eggs were taken. Other hens were poor nesters and deserted their nests at some stage during laying. Information on each hen's nesting behavior was recorded.

Schedule for Collection of Blood Samples and Radioimmunoassays

Blood samples (3–5 ml) were collected once a week for 7 weeks between 0800 and 1030 h. Levels of LH and prolactin were determined for all thirty-two ducks (sixteen experimentals and sixteen controls) using heterologous radioimmunoassays for turkey LH (Burke et al., 1979) and prolactin (Burke and Papkoff, 1980). Blood samples from half of the birds in each group (eight experimentals and eight controls) were assayed for progesterone. Estradiol values for four experimental and four control hens were also determined.

Dose-response relationships for turkey LH, mallard serum, and a purified mallard gonadotropin (Farmer and Papkoff, 1979) were examined to determine the validity of using the turkey LH RIA for measuring mallard LH. Furthermore, the levels of immunoreactive "LH" in mallard serum were compared before and after injection of LHRH. Further support for the validity of this assay for measuring mallard LH was generated in the course of the studies by the correlation of immunoreactive "LH" levels with reproductive events. The within-assay coefficient of variation for the LH assay, based on differences between duplicate potency estimates in the midrange of the assay (45–65% bound), was 7.72%. The between-assay coefficient of variation for LH based on repeated analysis of pooled serum from laying turkey hens was 17.86%.

The turkey prolactin RIA described by Burke and Papkoff (1980) was validated for use with duck serum by comparing the dose-response relationship of serum from incubating mallards to that of purified turkey prolactin. The within-assay coefficient of variation for the prolactin assay was 9.14%. The between-assay coefficient of variation for the prolactin assay, based on the average potency of duplicate tubes from a pool of broody turkey serum, was 16.08%.

Progesterone was measured with antiprogesterone sera S49 #6 (Abraham et al., 1971) following procedures described in detail by Camper and Burke (1977a). Estradiol was assayed using antiserum described by Wineland and Wentworth (1975). The extraction, chromatography and assay were conducted as described by Camper and Burke (1977a). The within-assay coefficients of variation for the progesterone and estradiol assays were 5.70% and 12.33%, respectively. The between-assay coefficient of variation for progesterone was 6.68% and for estradiol was 16.67%.

LHRH Injections

Each of the thirty-two female mallards (sixteen stressed, sixteen controls) was injected intravenously with 12 μg of LHRH (Beckman) dissolved in 0.25 ml of 0.15 M saline on 6/7/78 between 0800–1145 h. Preinjection blood samples were taken from the brachial vein, and the ducks were immediately injected with LHRH using the same vein. Ten minutes after the injection, another blood sample was taken from the other brachial vein. Blood samples were allowed to clot overnight at 4°C, and then centrifuged for 20 min at 2000 rpm. The serum was pipetted off the samples and then frozen until the samples were assayed for LH.

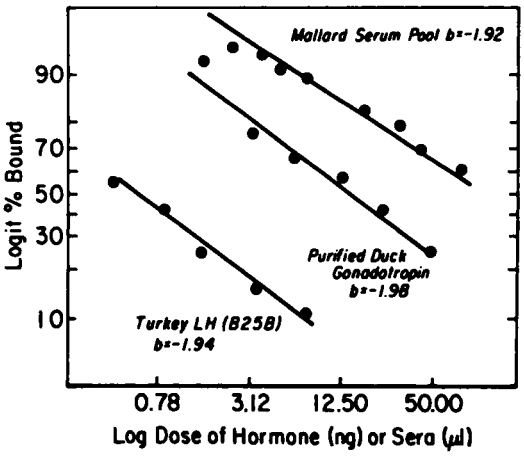

FIG. 1. Radioimmunoassay dose-response curves of turkey LH, a purified duck gonadotropin, and a mallard serum pool.

Statistics

A split-plot analysis of variance for an unbalanced data set (SAS, 1979) was used to compare the means of each of the two groups of mallards for each hormone over the 7-week sampling period. Duncan-Waller *t* tests (SAS, 1979) were used to compare the means of selected sampling dates for LH. The LH values from the LHRH injection experiment were analyzed using a split-plot analysis of variance (SAS, 1979).

RESULTS

Assay Validations

The purified mallard duck gonadotropin W247B and purified turkey LH (B25B) yielded dose-response curves that were not significantly different. The slope obtained with the two hormones was −1.98 and −1.94 (Fig. 1), respectively. Mallard serum, assayed in triplicate at 8 doses from 2.96 μl to 66.6 μl yielded a dose-response curve with a slope of −1.92 (Fig. 1). LHRH injection resulted in an increase in serum LH. The preinjection levels of all birds (± SEM) averaged 4.43 ± 0.42 ng/ml while 10 min later the levels had risen to 5.78 ± 0.35 ng/ml. All individuals responded to the injection with increases in LH.

The purified turkey prolactin standard and

the pooled mallard sera yielded dose-response curves with slopes of −2.34 and −2.44, respectively (Fig. 2). These slopes were not significantly different.

Egg Laying

All sixteen control birds laid eggs. The first egg was laid by these birds within 7–10 days after being placed outside. In contrast, only one of the sixteen stressed birds laid eggs and that individual did not begin laying until 6 weeks after being placed outside. Nine of the sixteen control birds laid a second clutch of eggs, beginning within 7–10 days after removal of the first clutch.

Luteinizing Hormone

Nonlaying stressed mallards had lower levels of LH than controls after only 1 week of the rotation treatment ($P<0.05$) (Fig. 3). These differences in LH levels between the two groups persisted for most of the study and were statistically significant ($P<0.05$) when analyzed over the 7-week duration of the experiment. LH levels in the stressed mallards had increased dramatically on June 1. Five days prior to this, the rotation stress treatment had been stopped for 4 days (May 27-May 30), and then had been resumed for 1 day prior to the June 1 blood sampling date. The LH levels in the experimental birds decreased in response to the continuation of the stress treatment as shown in Fig. 5 (preinjection LH levels).

The LH levels in the control group of laying mallard hens fluctuated over the 7-week period. The LH levels of all the laying hens were averaged together on each sampling date (irrespective of their stage in the nesting cycle) as shown in Fig. 3 and described in Table 1. This was done to compare the serum hormone levels of the control group with the experimental group on a weekly basis. When averaged over the population of sixteen control mallard hens, LH levels were highest on May 10 and May 17, dates corresponding to the seasonal midpoint in laying for the population. Half of the total

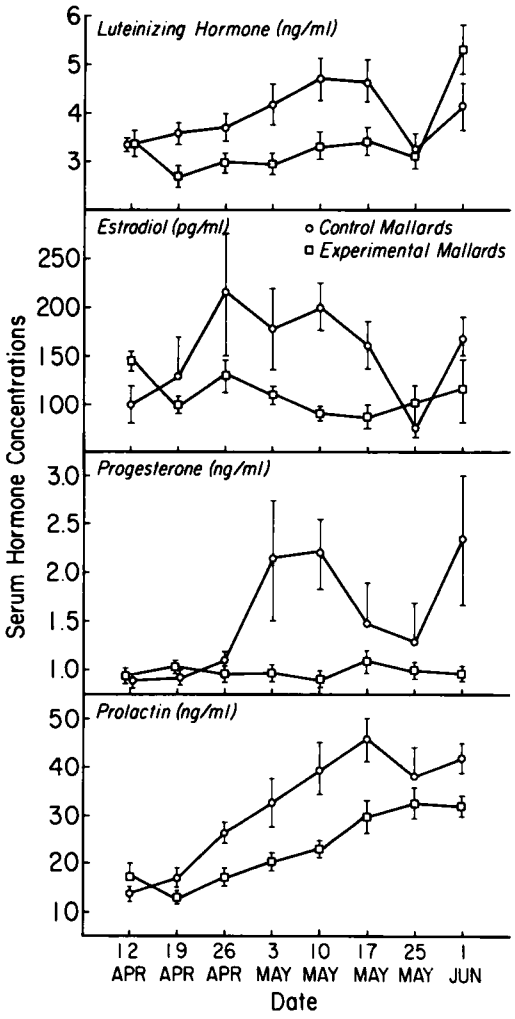

FIG. 3. Serum levels of LH, estradiol, progesterone, and prolactin ($\bar{x}$ ± SEM) at different dates through the spring for laying and nonlaying female mallards (*Anas platyrbynchos*). See Table 1 for key to stage in the cycle and sample sizes. The sample number for the dates varies with the hormone measured.

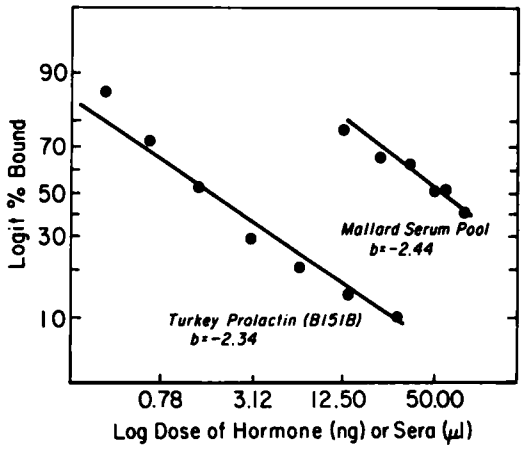

FIG. 2. Radioimmunoassay dose-response curves of turkey prolactin and a mallard serum pool.

number of eggs laid by controls were laid before May 12. The decrease in LH levels in the May 25 blood samples corresponded to the cessation of laying of the first clutch for this population. On this date, thirteen of sixteen mallards were terminating laying and were beginning to incubate their eggs. The remaining three females were laying their second clutch. The subsequent rise in LH (June 1) occurred at a time when eight of the thirteen females were renesting after the first clutch of eggs was removed. The temporal changes in LH levels of the control mallards (Fig. 3) were highly significant ($P<0.001$); serum levels of LH increased with nesting (May 3) and renesting activity (June 1), and decreased with the onset of incubation of the first clutch of eggs (May 17–25).

While the data shown in Fig. 3 are plotted in relation to calendar date, the same data for a subsample of eight of the control hens are plotted in relation to the stage of each bird's reproductive cycle (Fig. 4). All of the eight hens in this sample laid a first clutch of eggs, incubated them, and renested after their first clutch of eggs was removed. Descriptions of the stages of the nesting cycle, dates, and sample sizes, are found in Table 2. LH levels of control females fluctuated widely during the prelaying and first nesting stages of the reproductive cycle (Fig. 4). LH levels increased and decreased four times during the time that hens were laying their first clutch of eggs. LH levels then decreased when the females were terminating laying their first clutch and beginning to incubate and deposit down. LH levels increased again prior to the production of the second clutch of eggs.

Estradiol-17β

Estradiol levels of the stressed birds were significantly lower ($P<0.05$) than those of the control mallards. The first week of the rotation treatment resulted in a slight decrease in estradiol levels, suggesting that slight ovarian growth had occurred before the birds were moved outside, and ovarian regression had resulted from the stressful rotation procedure.

Estradiol levels in laying mallards fluctuated widely throughout the reproductive cycle. All mallards (four of four) in this sample had

TABLE 1. Bleeding dates and nesting activity.

Date[a]	Description of nesting activity in control mallard hens
12 April	All mallards were placed outdoors in individual nesting compartments.
19 April	Six out of sixteen hens formed nests in their nesting boxes within 2 days of this date. Two of these hens started to lay eggs.
26 April	Five new hens formed nests and four more hens started laying eggs, bringing the total number of layers to six.
3 May	Three additional hens formed nests. Ten of the sixteen hens were laying eggs.
10 May	Fourteen of the sixteen females were laying eggs by May 12th. Six of the sixteen females had laid eight-twelve eggs. Three of these six had deposited down in their nests.
17 May	All sixteen females had laid eggs by this date. Five of the sixteen hens were incubating their first clutch of eggs.
25 May	Thirteen of the sixteen females were incubating their eggs. The first clutch of eggs from each of these thirteen females was removed and the nesting material replaced from the 25th-30th of May. Two other females had laid six-eight eggs and one female was laying the second egg in her second clutch.
1 June	Eight of the thirteen females, which previously had their first clutch of eggs removed, had formed new nests. Five of these females had laid eggs in their second clutch by June 5th.

[a]Dates represent the date on which sixteen control and experimental mallards were blood sampled. Hormone data for these blood samples are shown in Fig. 3.

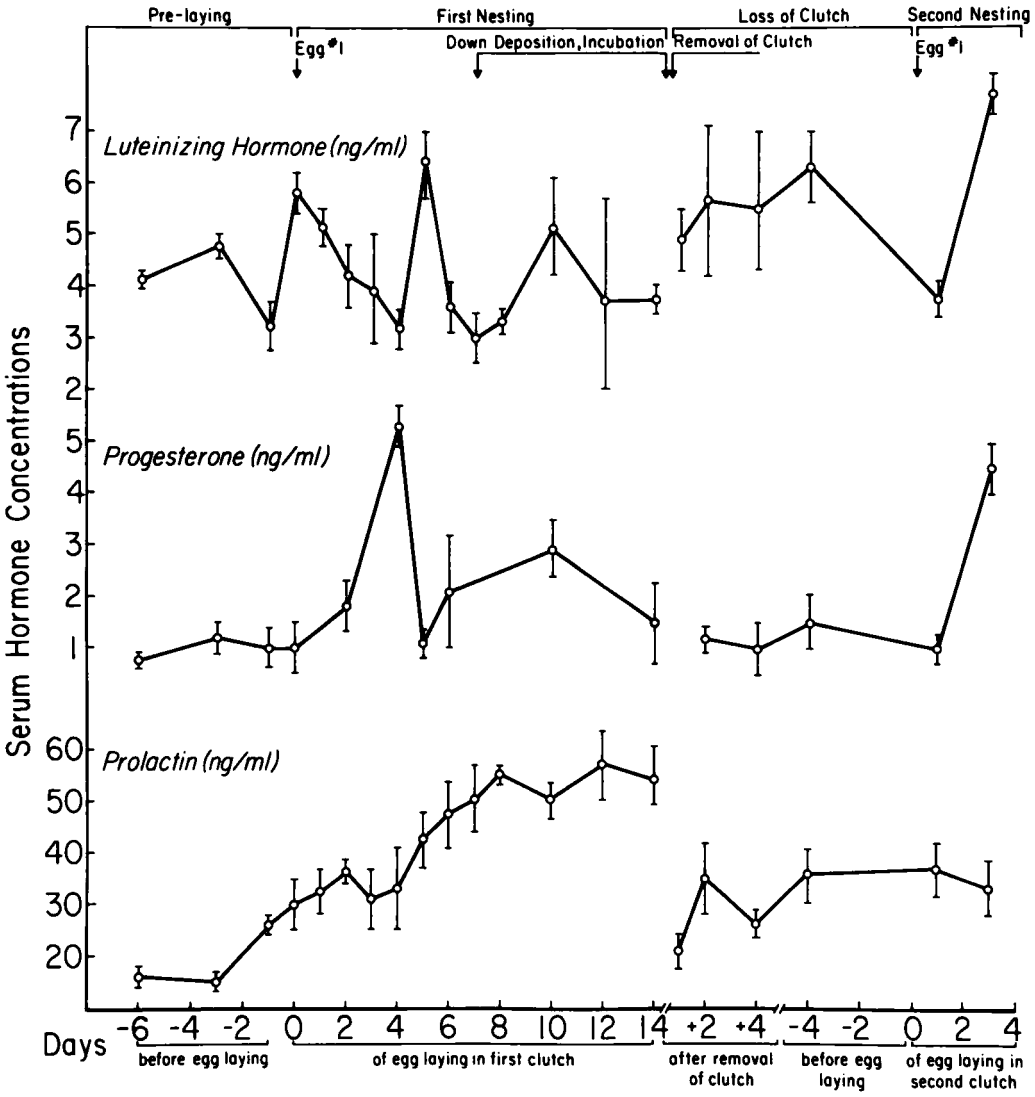

FIG. 4. Serum levels of LH, progesterone, and prolactin ($\bar{x}$ ± SEM) at different stages of the nesting cycle for eight control mallards. Data are plotted in relation to the onset of egg laying. See Table 2 for key to stage in the nesting cycle and sample sizes for the hormones measured.

formed nest bowls and half laid their first egg on April 25. The formation of the nest bowl usually preceded laying by 4–5 days.

Progesterone

The stressed nonlaying mallards had significantly lower ($P<0.01$) levels of progesterone than did the controls (Fig. 3). These depressed levels of progesterone would be expected if the nonlaying birds had small inactive ovaries. The progesterone data above suggested that fluctuations in the levels of serum progesterone in the controls paralleled the changes in the reproductive activity; at the April 26 sampling date, six of the hens in the sample had laid their first egg. By May 3, seven out of eight hens had laid between three and six eggs. The other female had formed a nest bowl but had not laid any eggs.

Progesterone levels fluctuated throughout the nesting cycle as shown in Fig. 4. They were low prior to laying, increased dramatically on the 3rd-4th day of egg production, and decreased

dramatically with the laying of the fifth egg. Progesterone levels increased with the laying of the sixth and tenth eggs but decreased slightly with the laying of the fourteenth egg. Progesterone levels decreased further after removal of the clutch but increased dramatically with the laying of the third egg in the second clutch.

Prolactin

Levels of serum prolactin in experimental mallards were significantly lower ($P<0.01$) than in control mallards (Fig. 3). Prolactin levels increased significantly ($P<0.001$) in both groups over the 7-week period (Fig. 3), but the rate of increase was less in the stressed than in the control birds. This resulted in a significant treatment × time interaction ($P<0.01$). Circulating levels of serum prolactin in the stressed mallards decreased slightly after 1 week of the pen-rotation treatment.

Prolactin levels of control mallards increased prior to egg laying and continued to increase during laying and early incubation (Figs. 3 and 4). Seven of eight control mallards were beginning to incubate their first clutch of eggs coincident with the peak prolactin levels (Fig. 3) of the population of control mallards. In the controls, a decrease in serum prolactin and the other hormones coincided with the removal of eggs from five of the six incubating control mallards (Fig. 3).

When the prolactin data are graphed in relation to the stage of each individual bird's reproductive events (Fig. 4), it is evident that circulating prolactin levels gradually increased

TABLE 2. Stages in the nesting cycle.

Stage	Dates[a]	Description of nesting activity	Sample size[b]
Pre-laying	April 12-May 5	6 days prior to egg laying	8(4)
		3 days prior to egg laying	2(2)
		1 day prior to egg laying	2(2)
First nesting	April 20-May 7	Laying of the first egg	3(2)
		Laying of the second egg	5(2)
		Laying of the third egg	5(5)
		Laying of the fourth egg	3(3)
		Laying of the fifth egg	3(2)
		Laying of the sixth egg	2(4)
		Laying of the seventh egg	6
		Laying of the eighth egg	2
		Laying of the ninth egg	2
Down deposition and incubation	May 1-May 14	Laying of the tenth egg	4(3)
		Laying of the twelfth egg	2
		Laying of the fourteenth egg	3(3)
Egg removal	May 14-May 30	Removal of eggs and nesting material	
		1 day after egg removal	3(2)
		2 days after egg removal	2
		4 days after egg removal	4(3)
Pre-laying of second clutch	May 11-June 2	4 days prior to egg laying	2(2)
Second nesting	May 12-June 9	Laying of the first egg	3(2)
		Laying of the third egg	2

[a]Dates represent the period in which birds in this stage of the cycle were blood sampled and do not necessarily represent the period for each stage in the population as a whole. Data for hormone levels are presented in Fig. 4.

[b]The sample size for LH and prolactin estimations were equal and are given. Numbers in parentheses are sample sizes for estimations of progesterone.

throughout the nesting cycle. Prolactin levels were increased 3-fold coincident with the termination of laying and incubation of the first clutch. Removal of the eggs of the first clutch was followed by an abrupt and significant decrease ($P<0.01$) in circulating prolactin levels. Circulating levels of prolactin increased later with renesting activity.

LHRH Elevation of LH Levels

LHRH injections resulted in a highly significant increase (Fig. 5) in LH in both stressed and control groups of mallards. Furthermore, the change in release of LH (both amount and percent of initial value) for the stressed mallards was greater than in control mallards ($P<0.001$) and resulted in a highly significant treatment × time interaction between the two groups.

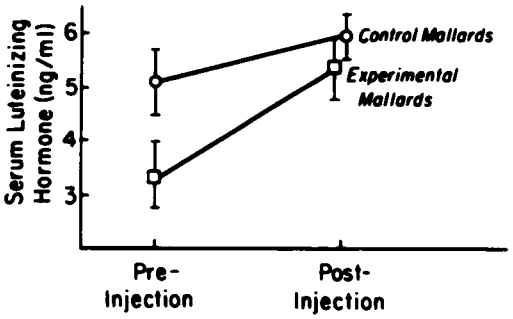

FIG. 5. Levels of serum LH for sixteen control and ten experimental female mallards before and after LHRH (12 μg/duck) injections.

DISCUSSION

The data obtained support the validity of using turkey LH and prolactin assays for measuring these hormones in mallard sera. The parallel dose-response curves obtained with turkey LH, mallard duck sera, and purified mallard (*Anas platyrhynchos*) gonadotropin indicate immunoreactive mallard duck LH can be quantitated using reagents derived from turkeys. The increase in serum LH levels following LHRH injection show the pituitary glands of mallards are responsive to appropriate stimuli and further support the contention that the LH RIA does indeed measure duck LH. Likewise, the seasonal change in LH in those birds that laid are in line with expectations and evidence from other avian species.

The parallelism between the incubating mallard serum and the turkey prolactin support the validity of the turkey prolactin assay for measuring mallard prolactin. In another study (Bluhm, 1981), the elution patterns of immunoreactive duck serum "prolactin" (from canvasback ducks, *Aythya valisineria*) and turkey prolactin from a Sephadex G75 column were essentially identical.

The results clearly demonstrate that most of the female mallards rotated into new pens on a daily basis did not lay eggs. Failure of the reproductive mechanism might be due to a failure of the hypothalamus to release releasing factors, failure of the pituitary to respond to hypothalamic stimulation, or failure of the ovary to respond to gonadotropin stimulation. One or more basic mechanisms might be invoked to explain reproductive failure at these possible loci. One might speculate that the stress of pen rotation would cause the release of prolactin and/or ACTH and these could directly or indirectly block reproductive activity.

Our results show that the reproductive failure produced by pen rotation was associated with lowered serum levels of LH, estradiol, progesterone, and prolactin. The fact that LH levels were low suggests that the primary site of reproductive failure was at or above the level of the pituitary gland. Clearly the ovaries were in an undeveloped state as shown by the low levels of estradiol and progesterone. We suggest that this is due to the lack of LH stimulation, but other possibilities exist. For example, the follicle-stimulating hormone levels may also be low or adrenal corticoids levels high. No information on these hormones are available. It does appear that a stress-induced hyperprolactinemia is not involved in the reproductive failure, since prolactin levels remained lower in the stressed birds than in the controls.

The observation that the pituitary glands of stressed birds released substantial quantities of LH in response to LHRH is strong evidence that the pituitary gland is competent to release LH if stimulated. This finding agrees with those of Phillips and van Tienhoven (1960), who showed a high content of gonadotropin in pituitary glands of nonlaying captive pintails (*Anas acuta*) with small gonads. These data suggest that reproductive failure in the stressed mallards is due to inadequate stimulation of the pituitary by hypothalamic releasing factors. Whether this failure lies with the LHRH secreting neurons or at some higher levels of control is not addressed in these studies.

In sharp contrast, the serum hormone levels of control mallards, which laid eggs, fluctuated with the stages of the reproductive cycle. The increase in LH in the control mallards that coincided with the initiation of egg laying parallels similar findings in chickens (Furr et al., 1973; Shodono et al., 1975; Wilson and Sharp, 1975), turkeys (Mashaly et al., 1976), Japanese quail (Doi et al., 1980), snow geese (Campbell et al., 1978), and domestic and wild mallards (Donham et al., 1976; Donham, 1979; Tanabe et al., 1980), herring gulls (Scanes et al., 1974) and white-crowned sparrows (Mattocks et al., 1976; Wingfield and Farner, 1978b).

The abrupt decline of serum LH in female mallards 2 days prior to the beginning of incubation that we observed (Days 6–8 of egg laying, Fig. 4) has been previously reported in wild and game-farm mallards by Donham et al. (1976) and by Goldsmith and Williams (1980). A fall in LH secretion at the onset of incubation has also been found in female (Cheng and Follett, 1976) and male (Silver et al., 1980) ring doves, bantam hens (Sharp et al., 1979), turkeys (Cogger et al., 1979; Burke and Dennison, 1980), white-crowned sparrows (Wingfield and Farner, 1978a,b) and snow geese (Campbell et al., 1978).

The removal of the eggs and nesting materials from the incubating control mallards resulted in renesting activity. LH levels increased 2-fold (Fig. 3) in the renesting control mallards within 7 days of the removal of nesting material. In the present study, two of the renesting mallards laid the first egg of their second clutch 7 days after the removal of the first clutch and nesting material. Similarly, Donham et al. (1976) found a significant increase in plasma LH levels of female mallards within 1 day in response to the loss of their eggs; mature follicles developed within 6 days.

Fluctuations in ovarian steroid levels that paralleled changes in reproductive state in the control mallards were in general agreement with data from other studies on a number of avian species (see Sharp, 1980, for review). Thus levels of both estradiol and progesterone rose preceding the onset of lay, remained elevated during the laying period, and declined with the onset of incubation. The estradiol levels appear to be elevated before progesterone levels increased. The rise in estradiol coincident with nest-building behavior, at a time when serum progesterone was still low, may indicate a causal relationship in this species as others (Cheng, 1974; Hutchison, 1975; Lehrman, 1965) have shown in other avian species.

The changing levels of prolactin observed in the control ducks is in agreement with the emergent pattern being reported for a variety of avian species. In particular, it appears that prolactin levels rise in the prelaying period (Sharp et al., 1979; Burke and Dennison, 1980; Lea et al., 1981), continue to rise or remain elevated in laying birds (Sharp et al., 1979; Etches et al., 1979; Burke and Dennison, 1980; Lea et al., 1981), and increase substantially coincident with the onset of incubation (Sharp et al., 1979; Burke and Dennison, 1980; Proudman and Opel, 1980; Lea et al., 1981). The fall in LH levels at the onset of incubation has likewise been observed in several groups of birds (Cheng and Follett, 1976; Donham et al., 1976; Campbell et al., 1978, Wingfield and Farner, 1978b; Cogger et al., 1979; Burke and Dennison, 1980; Goldsmith and Williams, 1980) but appears not to be an unequivocal pattern since Goldsmith and Williams have reported that LH levels decline at the onset of incubation in some mallards without a rise in prolactin. However, the results of Lea et al. (1981) showing an increase in LH levels of incubating bantam hens after injection of prolactin antisera do suggest that prolactin may directly or indirectly suppress LH in this species.

In summary, these data clearly show that behavioral or environmental stressors can effectively block reproduction in captive mallards and the hormonal characteristics of these birds (low LH levels, low estradiol and progesterone levels, and an increased pituitary responsiveness to LHRH) suggest the failure to be at a level of control above the pituitary gland. We have not identified the locus or mechanism responsible for the failure. It could be hypothalamic or at higher CNS levels. It clearly is not due to hyperprolactinemia.

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