To the Graduate Council:

I am submitting herewith a thesis written by Aleta Lynn Gordon entitled "The Effects of Exogenous Luteinizing Hormone Releasing Hormone (LHRH) on the Reproductive Function of Laying Hens and Cockerels." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

Hugo Eiler

H. Eiler, Major Professor

We have read this thesis
and recommend its acceptance:

R L Murphree

Michael H. Sims

Accepted for the Council:

L Evans Roth

Vice Chancellor
Graduate Studies and Research

4

THE EFFECTS OF EXOGENOUS LUTEINIZING HORMONE RELEASING HORMONE (LHRH) ON THE REPRODUCTIVE FUNCTION OF LAYING HENS AND COCKERELS

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Aleta Lynn Gordon

December 1982

ACKNOWLEDGMENTS

The author wishes to express her sincere gratitude and appreciation to the following persons who were instrumental in the successful preparation of this manuscript:

To Dr. Hugo Eiler, major professor, for his guidance, professional expertise, support, and friendship;

To Dr. R. L. Murphree. for his time and advice, his patience, and for reviewing this manuscript;

To Dr. M. H. Sims, for his assistance in the preparation and review of this manuscript and for his guidance and support throughout the graduate program;

To Janice Keller, Dr. Steve Kincaid, Dr. Craig Cullen, and Dr. Terry Schultz for their invaluable instruction in the preparation of histological sections and photomicrographs;

To Cindy Armstrong, Chris Stearns, Joan Hembree and other fellow Animal Science graduate students for their friendship, support and concern;

And especially to my parents, Jere and Wanda Gordon, and to Warren Brown for their guidance, understanding, encouragement, and constant support.

ABSTRACT

It has been demonstrated that a single LHRH injection will produce elevated LH levels in the hen and cockerel, and will induce premature ovulation in the laying hen (Furr et al., 1973a and Tanaka et al., 1974). However, reports on the effects of continuous infusion of LHRH over a relatively long period in both the laying hen and the cockerel have not been found in the literature. The purpose of this research was to determine, by tritiated estradiol metabolism studies and histological evaluation of various reproductive tissues, and by observing the laying performance, the effect on the laying hen of a continuous infusion of LHRH over a six day period. A companion study was conducted in cockerels, consisting of tritiated testosterone uptake studies and histological evaluation of various endocrine organs, to determine whether LHRH acts directly on the pituitary or on the gonads themselves in the bird. Twenty-four 64 week old laying hens were intrajugularly infused with either 857 or 429 μg synthetic LHRH using a subcutaneously implanted Alza (model 2ML1, 10 μl/hr infusion rate) osmotic pump. Egg production was recorded six days prior to and six days during the infusion period. Eighteen 12 week old cockerels were similarly infused with 214 μg synthetic LHRH. At the end of the six day period, one hour before death, hens and cockerels were intravenously injected with 10 μCi tritiated estradiol or 10 μCi tritiated testosterone, respectively. Immediately after death the hen oviduct, ovary, and pituitary were removed and weighed and either tritiated estradiol was extracted and counted or histological sections were prepared and

evaluated. The testes, adrenals, ductus deferens, and pituitary were removed and weighed in cockerels, tritiated testosterone uptake was determined and histological sections were made of these organs. LHRH treated hens and sham hens exhibited decreased egg production and significantly decreased ($P<0.05$) whole ovarian weight without a reduction in stroma ovarian weight. Magnum function was severely reduced as shown by significantly ($P<0.05$) reduced tissue cross sectional area, increased ciliated and secretory cell number, and absence of luminal secretions. Circular tunica muscluaris thickness and villi diameter were significantly reduced in the shell gland. The numbers of normal microscopic and atretic microscopic follicles per 100 μm^2 and the size of these microscopic follicles were not significantly affected ($P>0.05$) by LHRH treatment. Pituitary alcian blue periodic acid-Schiff positive basophil cell number (gonadotrophs) per 1000x field was not significantly different between control and LHRH hens. LHRH treated cockerels exhibited significantly ($P<0.05$) reduced number of basophil cells per 1000x field, and significantly increased chromaffin cell number percentage as compared to control cockerels. There were no significant differences ($P>0.05$) observed in body, comb, pituitary, testis, or adrenal weights in LHRH treated cockerels as compared to controls. These results indicate that in the laying hen LHRH infusion could not overcome the effect of stress induced follicular and oviductal atrophy, and that probably the results observed were not due to an LHRH deficiency. Results of the study in cockerels indicate that LHRH may act directly at the level of the pituitary in the bird.

TABLE OF CONTENTS

LIST OF TABLES

TABLE PAGE

LIST OF FIGURES

CHAPTER I

I. INTRODUCTION

A. Statement of the Problem

Previous research concerning the laying hen indicates that luteinizing hormone releasing hormone (LHRH) partly regulates ovulation, in combination with other endocrine processes. In mammals it is known that stimulation of the pituitary gland by hypothalamic releasing factors is conducive to pituitary release of gonadotropic hormones, which in turn activates both ovulation and the release of gonadal steroids. Specific tissue receptors for gonadal steroids have been postulated in both the oviduct and the hypothalamus in mammals as well as in birds. In fact it has been shown that a single intramuscular injection or a short intrapituitary infusion of LHRH will induce premature ovulation in the laying hen. The regulation of the male reproductive function is also under the influence of hypothalamic releasing factors. In the cockerel the release of gonadotropins has been attained by a single intravenous injection of LHRH. To our knowledge, the effect on the reproductive physiology of birds of continuous intravenous infusion of hypothalamic releasing factors over a relatively long period has not been reported. There is a possibility that continuous infusion of LHRH may result in increased laying rates in the domestic hen. In this experiment, laying performance, histological morphology, and metabolism of tritiated estradiol were evaluated to determine the effects of continuous infusion of LHRH in laying hens. Histological morphology and

metabolism of tritiated testosterone were evaluated in cockerels to determine if sex differences exist in the response to continuous infusion of LHRH, since reproductive functions in cockerels seem to be more stress independent as compared to laying hens.

B. Review of the Literature

Hormonal Control of Ovulation in the Laying Hen

Luteinizing hormone (LH), produced by the anterior pituitary, induces ovulation of the largest mature ovarian follicle (Opel and Nalbandov, 1961). Furthermore, it has been postulated that the pre-ovulatory release of LH is caused by release of luteinizing hormone releasing hormone (LHRH) from the hypothalamus (Reeves et al., 1973). Numerous investigators have shown that LH levels rise four to six hours prior to ovulation (Furr et al., 1973a; Rothchild and Fraps, 1949; Senior and Cunningham, 1974; Shahibi et al., 1975). However, the hormonal mechanisms involved in the release of LHRH and LH are still unclear. Using radioimmunoassay, plasma levels of various hormones have been measured throughout the ovulatory cycle, and results indicate that progesterone and estradiol are directly involved in ovulation. Peak levels of estradiol, progesterone, and LH all occur during the six hour interval prior to ovulation (Peterson and Common, 1972; Lague et al., 1975). Some investigators have found that both progesterone and estradiol levels increase before the pre-ovulatory LH release and have hypothesized that these steroids may activate the hypothalamic-pituitary axis such that LH is consequently released (Furr et al., 1973a; Lague et al., 1975). Before LHRH was characterized, Fraps (1965) postulated that the ovulatory cycle of the hen was controlled by LH and its interaction with

feedback hormones. He suggested that the feedback hormones were probably progestins, since progesterone has been shown to induce premature ovulation in the laying hen (Ralph and Fraps, 1960). He proposed an open period of eight hours, during which time the threshold of the neural component involved in LH release was low. When progesterone levels were high during the open period, LH was released and ovulation occurred. He also proposed a closed period of sixteen hours duration, during which time the threshold of the neural component was high and even though progesterone levels were high, LH was not released. Nalbandov (1976) agreed with the Fraps theory, suggesting that progesterone is involved in a feedback mechanism between the ovary and the hypothalmic-pituitary axis and that estradiol was not directly involved in this feedback system. Bonney and Cunningham (1977b) demonstrated that the sensitivity of the pituitary to LHRH was influenced by estradiol. Studies have shown that LHRH is present in the hypothalamus of the hen and that it will induce LH release both in vivo and in vitro (Jackson and Nalbandov, 1969; Opel and Lepore, 1972). No new theories have been proposed to explain the ovulatory mechanism to the laying hen in relation to LHRH, but this hypothalamic releasing factor is definitely involved.

Localization and Characterization of Avian LHRH

Although the hypothalamic nuclei associated with LHRH have not been positively identified, the preoptic region, the tuberal hypothalamus, and the posterior median eminence of the bird have been implicated. By electrically producing lesions in these areas and then noting any changes in the ovulation sequence, investigators have determined that

they are involved in the LH release mechanism, but as yet have not definitely proved that they produce LHRH (Opel, 1979). Experiments involving the intrapituitary infusion of median eminence extracts and tuberal hypothalamic extracts indicate that these areas contain LHRH or LHRH-like substances which will induce premature ovulation (Clark and Fraps, 1967; Opel and Lepore, 1972). To our knowledge, immunohistochemical studies such as were conducted in the rat (Palkovits et al., 1974) which indicate that LHRH was concentrated in the median eminence and arcuate nucleus, have not been conducted in birds.

Using ion-exchange chromatography, Jackson (1971) partially purified LHRH from chicken median eminence tissue, demonstrated that it would cause LH release in cultured rat pituitary cells, and demonstrated that the effects of LHRH were not due to arginine vasotocin. Based on Sephadex-G25 mobility and ion-exchange and thin layer chromatography patterns of cockerel, rat, rabbit, and synthetic LHRH, Jeffcoate et al. (1974) concluded that all four LHRH compounds were of similar structure, namely (pyro)-Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2. They also demonstrated that the cockerel hypothalamus contains the least LHRH, when compared to that of the rabbit and the rat. Results by King and Millar (1980), using radioimmunoassay, cation exchange chromatography, affinity chromatography and high pressure liquid chromatography indicate that chicken LHRH differs at the seventh amino acid, leucine, from the mammalian type. We have not found reports of the sequence of avian LHRH in the literature to support the results of either of these groups.

The Effects of Short Term Treatment with Mammalian LHRH in Birds

(1). The Effects of LHRH on Luteinizing Hormone Levels and Ovulation

Van Teinhoven and Schally (1972) demonstrated that mammalian LHRH will induce ovulation in chickens. Several routes of administration of LHRH have been used. Single intravenous injections of 20 μg of LHRH caused significant elevation of LH levels in laying hens (Bonney et al., 1974), broody bantams (Sharp and Lea, 1981), and intact and castrated cockerels (Tanabe et al., 1981). Intramuscular injection of 5 to 60 μg LHRH resulted in premature ovulation in laying hens. Intrajugular infusion of median eminence extracts 11 hours prior to ovulation induced premature ovulation in laying hens (Opel and Lepore, 1972). However, Bonney et al. (1974) were unable to induce ovulation in laying hens with 20 μg LHRH injected at different times throughout the cycle, and concluded that the duration of the LH peak produced by LHRH injection was too short to cause ovulation. They also found that LH levels increased significantly after LHRH injection at any time during the ovulatory cycle except when injected four to seven hours prior to ovulation. Tanaka and Kamiyoshi (1976) induced premature ovulation in 50 percent of 5 μg LHRH analog treated laying hens while only 30 percent of 10 μg synthetic LHRH treated birds ovulated prematurely.

(2). The Effects on Ovarian and Oviduct Function

The effects of LHRH on the oviduct and ovary of birds have not been extensively studied. Intramuscular injection of 20 μg LHRH produced no significant increase in the size of the 10 largest follicles in the non-laying hen (Reeves et al., 1973). In non-laying turkeys,

daily intramuscular LHRH injections for seven days or two-times daily intravenous LHRH injections for seven days failed to increase ovary and oviduct weights, while intramuscular injections of a combination of LH and FSH did so (Burke and Cogger, 1977).

(3). The Effects on Pituitary Function

In the male Japanese quail, single daily subcutaneous injections of 10 µg synthetic LHRH for 10 days caused an increase in the size and granule content of anterior pituitary gonadotrophs, as studied both by electron microscopy and conventional light microscopy using periodic acid-Schiff, alcian blue and orange G stains. Gonadotrophic cells were localized both in the cephalic and caudal lobes of the pituitary. No other cell type responded to LHRH treatment (Wada, 1975). Another study using the Japanese quail was conducted by Mikami et al. (1975) where 5 µg LHRH was injected intravenously, and the gonadotrophs were observed over a one hour period. At 10 minutes after injection degranulation had occurred; at 20 minutes hypertrophy and vacuolization were apparent. At 30 minutes the cells were large and spherical and contained only a few secretion granules. At 60 minutes post-injection the gonadotrophs were reduced in size, and lysosomes and vacuoles were predominant in the cytoplasm. Other pituitary cells were unaffected.

Factors Modulating the Putuitary Response to LHRH in the Bird

(1). Age

Bonney et al. (1974) studied the effects of LHRH on LH release in 11 to 21 week old pullets. They found in younger pullets that single LHRH injection tended to produce highly elevated LH levels, similar to

those produced in cockerels injected with LHRH. As the pullets became more mature, the LH increase produced in response to LHRH injection tended to be depressed, until at 21 weeks, just prior to the onset of lay, the LH response was not significantly different from that of mature laying hens. These results suggest that there may be a maturation of the hypothalamic-pituitary axis along with the initiation of a gonadal feedback system at maturity, to control the release of LH and ovulation. Williams and Sharp (1978) found no significant difference in the LH response in 10 month old as compared to 24 month old hens injected with 20 µg/kg LHRH four to nine hours after ovulation, which suggests that once the feedback system is operational the system responds much the same throughout the laying period.

(2). Sex

The LH response to LHRH in cockerels tends to be much greater than that of the mature laying hen (Furr et al., 1973b). The dose of LHRH required to stimulate the release of LH in the cockerel is much lower (1 to 4 µg) than that required in the laying hen (20 µg) (Bonney et al., 1974; Furr et al., 1973b). It is interesting that in the cockerel the basal LH levels remain stable over short periods, which is in contrast to that of most mammals. LHRH injection in the cockerel produces significantly elevated LH levels within five minutes, but within one hour the LH secretion returns to basal levels. These observations suggest that in the cockerel there exists some fundamental difference in the hypothalamic-pituitary axis or in a feedback mechanism whereby LH is released (Furr et al., 1973b).

(3). Feedback Mechanisms Involving Gonadal Steroids

It is well known that a feedback mechanism exists in most mammals between the gonads and the hypothalamic-pituitary axis to regulate the release of LH and FSH (Jones and Boyns, 1973). It may be that in the immature bird this feedback mechanism is not yet fully functional, since basal LH levels are higher and the response to LHRH is greater in the young hen and in the cockerel as compared to the mature hen (Furr et al., 1973b). The effects of estradiol on LH release and ovulation were investigated in vivo and in vitro by Bonney and Cunningham (1977b). They found that when dispersed chicken anterior pituitary cells were incubated with LHRH and 1 µg or 10 µg estradiol of 100 µg testosterone or progesterone were added, the expected increase in LH release was suppressed. When the cells were incubated with steroids and later LHRH was added, a potentiation of LH release was produced. An intermediate effect on LH release of a combination of estradiol and progesterone was observed. In vivo experiments using immature hens demonstrated that estradiol benzoate injected before LHRH significantly increased the LH response, but that similar amounts of testosterone and progesterone did not produce a similar effect. These results suggest that progesterone probably initiates LH release by its action on the hypothalamus, but that its effect is modified by estradiol's influence on the pituitary (Bonney and Cunningham, 1977b).

(4). Nutritional State

Tanabe et al. (1981) induced molting in laying hens by starvation for seven days, and observed plasma LH levels in response to LHRH. Basal LH secretion was significantly depressed during the starvation

period, follicular atresia and cessation of laying also occurred. The LH response to LHRH injection was significantly depressed at three days of starvation and also at seven days, as compared to the pre-starvation LHRH response. After seven days of re-feeding, the LH response to LHRH injection was similar to that observed before starvation. This study suggests that, as would be expected, the nutritional state of the bird has a marked effect on the hormonal balance and the response to LHRH. The effects of other environmental factors on the response to LHRH treatment have not been investigated in the bird.

The Effects of LHRH and LHRH Analogs in Mammals

(1). The Effects on Ovarian Function

The effects of LHRH and its analogs on ovarian function have been investigated in various species. Clayton et al. (1979) established that LHRH receptors are present both on granulosa and luteal cells. Several groups have reported an inhibitory effect of LHRH on ovarian steroidogenesis (Clayton and Catt, 1981; Hsueh et al., 1980; Jones and Hsueh, 1981; Massicotte et al., 1980). The inhibition of steroidogenesis may be mediated by changes in the kinetics of the dehydrogenase enzyme system (Hsueh et al., 1980; Jones and Hsueh, 1981) or by inhibition of the adenyl cyclase-cyclic AMP system (Harwood et al., 1980; Hillier et al., 1981; Massicotte et al., 1980). In relation to ovarian function, LHRH and LHRH analogs have been shown to terminate pregnancy at five to 10 days in the rat (Rivier et al., 1978), cause luteolysis during the mid-luteal phase of women (Sheenan et al., 1982), depress LH levels, and inhibit ovulation in rats (de la Cruz et al., 1976). Conversely, Arimura et al. (1976) demonstrated that

et al., 1976). Conversely, Arimura et al. (1976) demonstrated that endogenous LHRH is essential for implantation in the rat by injecting females with anti-LHRH gamma globulin at day one of pregnancy; at eight days post-treatment no implantation sites were found in treated rats as compared to an average of 14 in control rats.

(2). The Effects on Testicular Function

Much research has been conducted, primarily in the rat, to determine the effects of LHRH and LHRH analogs on testicular function. Labrie et al. (1980) demonstrated the presence of LHRH receptors on Leydig cells but not on Sertoli cells or seminiferous tubules. Several groups have shown that LHRH and its agonists will inhibit testicular steroidogenesis (Hsueh et al., 1981; Sandow et al., 1980. Seguin et al., 1981). Others have shown that LHRH causes a significant reduction in Leydig cell LH receptors (Auclair et al., 1977; Sandow et al., 1980), reduced testosterone production (Labrie et al., 1980; Rivier et al., 1981; Seguin et al., 1981), and decreased testis and accessory gland weights (Auclair et al., 1977; Rivier et al., 1981). However, it seems that these effects may be produced in a dose-response manner since low doses of synthetic LHRH (0.05-1.0 μg/min) continuously infused for four to six hours decrease accessory gland weights but not testis weight and no morphological changes were produced (Oshima et al., 1975). Similar results were obtained using 5 ng LHRH analog Buserelin, but at higher dosages (500 ng) testicular function was inhibited (Sandow et al., 1980). At present three theories have been proposed to explain the effects of LHRH on testicular function. (1) LHRH treatment makes the pituitary refractory to further LHRH stimulation; (2) LHRH produces a

large LH release which has a desensitizing effect on the testis, or (3) LHRH acts directly on the testis itself (Hsueh and Erickson, 1979). At this time there is no conclusive evidence to exclusively support any one of these theories.

(3). The Effects on Pituitary Function

Most researchers agree that the pituitary gland contains LHRH receptors (Chan et al., 1981). LHRH has been demonstrated to be associated with the secretory granules and the granules of the endoplasmic reticulum in the gonadotroph (Sternberger and Petrali, 1975). Continuous infusion or single subcutaneous injection of LHRH causes down regulation of pituitary LHRH receptors (Beltchetz et al., 1978; Ferland et al., 1981). The feedback mechanism of gonadal steroids to the pituitary may also affect the pituitary LHRH receptor number (Median and Koch, 1981). Even though down regulation may occur, in all studies LH levels have been elevated after LHRH treatment (Wilkinson and Moger, 1981). Continuous infusion of LHRH for 72 hours in post-menopausal women (Heber and Swerdloff, 1981) produced elevated LH and FSH levels which became greatest 12 hours after the start of infusion. However, even while more LHRH was being infused LH and FSH levels declined and returned to basal levels by the end of the infusion period. This suggests that the pituitary became desensitized to LHRH either by a persistent binding effect or by a down regulation effect. It seems that the response of the pituitary to LHRH is associated but not completely correlated with the LHRH receptor number; it may be that other mechanisms are involved in this response (Adams and Spies, 1981).

The Histology and Morphology of Selected Reproductive Organs in the Laying Hen and the Cockerel

(1). The Oviduct

The hen's oviduct consists of five regions, namely the infundibulum, the magnum, the isthmus, the shell gland, and the vagina. The function of the infundibulum is to retrieve the ovum from the abdominal cavity after ovulation; the magnum secretes albumen or egg white. The isthmus produces the soft shell membranes, the shell gland produces the calcified shell and the vagina allows passage of the egg to the outside of the body (Sturkie, 1976). Since this study was primarily concerned with the magnum and the shell gland, only these regions will be discussed.

The magnum consists of a thin connective tissue coat surrounding a thick muscle layer. Beneath the tunica muscularis are numerous tubular glands which are referred to as the submucosa. Covering the submucosa are columnar secretory and cilated epithelial cells (see Figure 1). The submucosal glands synthesize and package albumen into granules and the epithelial cells produce mucous (Hodges, 1974). In a normal laying hen the oviduct weighs approximately 40 grams and the height of the secretory epithelial cells is approximately 25 μm (Davidson et al., 1968). The secretory activity of the magnum varies with the stage of the laying cycle, but is generally continuous (Richardson, 1935). The tremendous increase in size and weight of the magnum from that of the chick to the adult is primarily due to hyperplasia and secondarily to hypertrophy (Brown and Badman, 1961; Yu and Marquardt, 1973). Estrogen and progesterone are required for normal development and continued

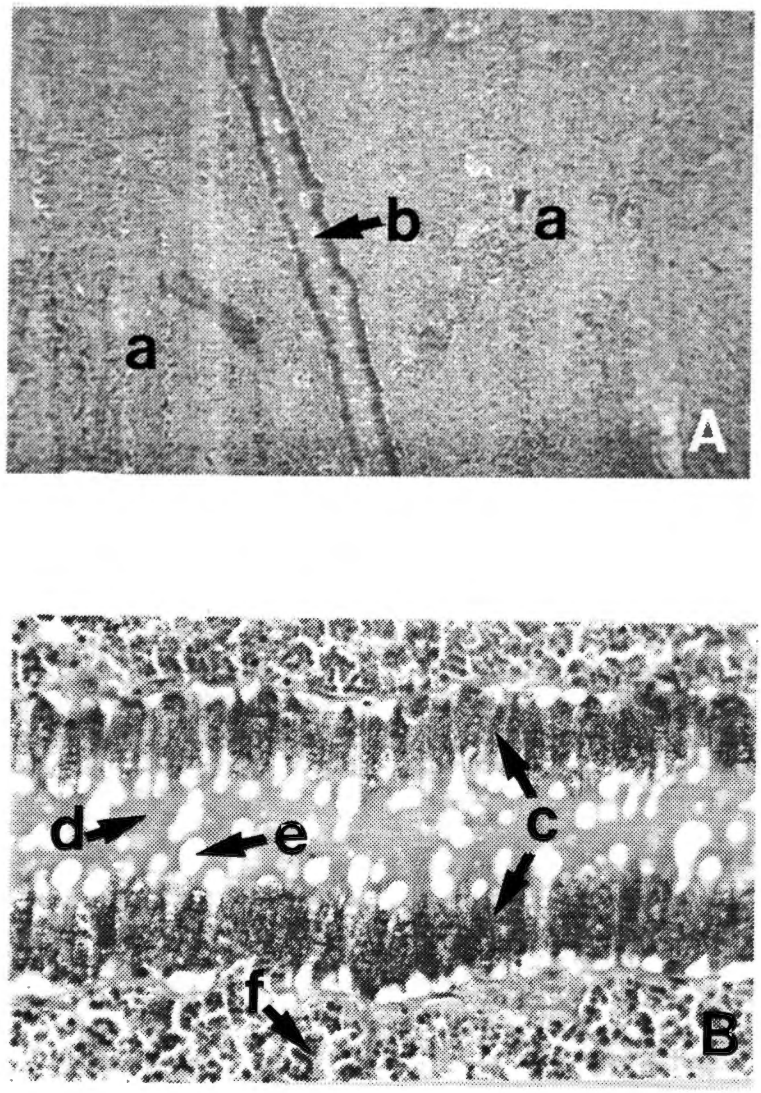

Figure 1. Photomicrographs of transverse sections of laying hen oviduct in the magnum region demonstrating the general structure. A. A low power photomicrograph of the magnum. Note the extensive submucosa containing numerous tubular glands (a), and a short section of anastomosing epithelial cell layers (b). Alcian blue, periodic acid-Schiff, and hematoxylin stained. 160x. B. A high power photomicrograph of two submucosal folds covered by columnar epithelium. Note the epithelial cells (c), the albumen (d), and mucous (e) secretions in the lumen, and the numerous submucosal glands containin periodic acid-Schiff positive granules (f). Alcian blue, periodic acid-Schiff, and hematoxylin stained. 400x.

function of the magnum (Brandt and Nalbandov, 1965; Kohler et al., 1969; Oka and Shimke, 1969). Regression quickly occurs when hormone levels are thrown into imbalance, all secretory activity ceases, and oviduct weight may decrease 75 percent.

The shell gland, or uterus, is a large pouch-like organ involved in the calcification of the egg (Navickis et al., 1979). Histologically the shell gland consists of an outer layer of connective tissue covering a circular and longitudinal muscle layer (see Figure 2). Beneath the muscularis are numerous villi-like projections covered with an epithelium consisting of apical (ciliated) and basal (non-ciliated) cells. Within these villi are numerous branched tubular glands (Hodges, 1974). The function of each of these cell types is not fully understood. It is thought that the apical cells are involved in the production of the shell matrix, a delicate layer of interwoven fibers upon which calcium carbonate is deposited. The basal cells probably are involved in the cuticle formation (Johnston et al., 1963). That each of these cell types is involved in the different phases of shell formation has been determined based on the time of their maximum activity. For example, the apical cells are most active during the early period of shell formation, with their villi becoming bulbous during this time (Breen, 1966). The function of the tubular glands, which are quite different from those of the magnum, is not known, however, their lumens do possess faint staining secretion granules at various times. The surface epithelium is approximately 30 μm in height (Richardson, 1935).

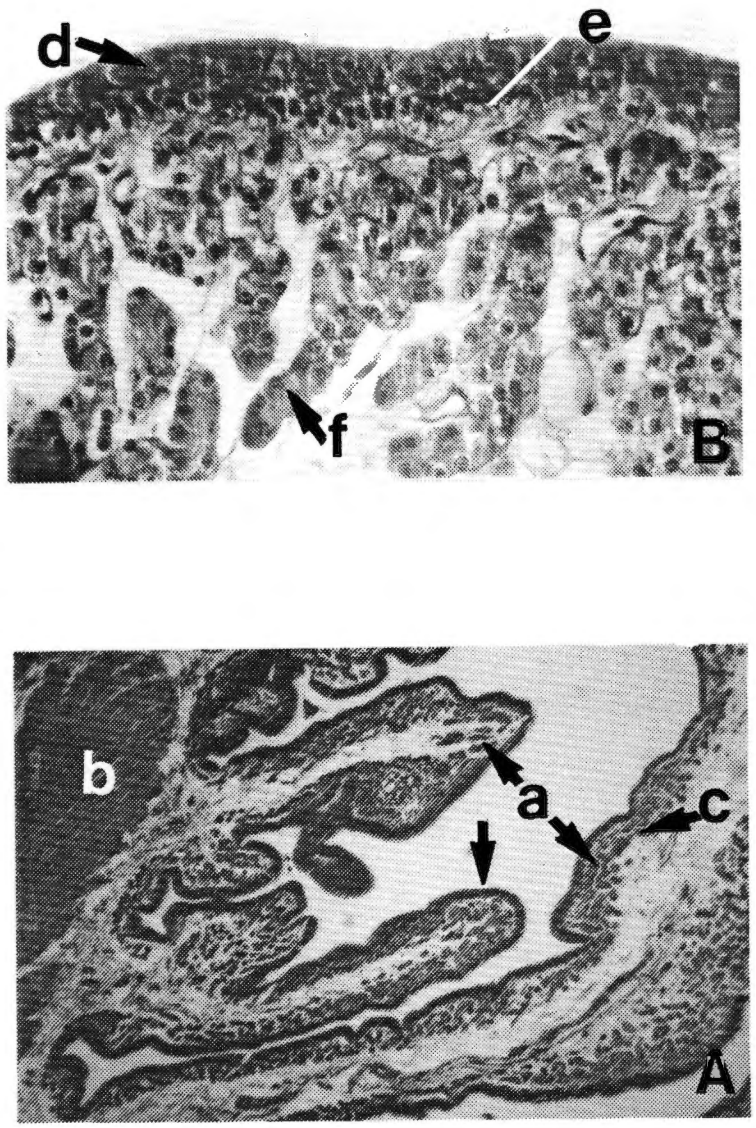

Figure 2. Photomicrographs of transverse sections of laying hen shell gland demonstrating the general structure. A. A low power photomicrograph of the shell gland. Note the numerous villi (a) which project from the tunica muscularis (b). Villi are covered by a columnar epithelium (arrow) and tubular glands (c) are present within each villous. Hematoxylin and eosin stained. 160x. B. A high power photomicrograph of a shell gland villous. Note the apical (d) and basal cells of the epithelium (e) and the numerous tubular glands (f) located beneath the epithelium. Hamatoxylin and eosin stained. 400x.

(2). The Ovary

Only the left ovary in the laying hen is functional, and it consists of a medulla containing thousands of immature follicles and a cortex containing several large follicles which are destined to ovulate or undergo atresia (Hodges, 1974). The follicle grows rapidly from the immature stage of a 40 mm follicle within five to eight days prior to ovulation (Wyburn et al., 1965). The medulla, also called the stroma, consists of connective tissue, immature follicles, and interstitial cells (see Figure 3). The pre-ovulatory follicle consists of a large yellow yolk surrounded by the follicular epithelium, the thin theca interna, the thick theca externa containing thecal glands, the blood vessels and nerves embedded in connective tissue, and the germinal epithelium. The zona radiata and the vitelline membrane are located immediately between the ovum and the follicular epithelium. The main areas of steroid secretion in the ovary are the medullary interstitial cells, the thecal glands of the theca externa, and the theca interna cells (Hodges, 1974). Large mature follicles undergo atresia by a bursting phenomenon where the follicular membranes rupture, the yolk is forced into the stroma, and phagocytosis, vacuolization, and proliferation of red blood cells occurs. This process is distinctly different from that occuring in mammals. Within four weeks the atretic follicle can barely be detected (Davis, 1942).

(3). The Anterior Pituitary

At present six cell types have been identified in the cephalic and caudal lobes of the chicken anterior pituitary, based on staining affinity and immunocytochemistry. These are the gonadotrophs (LH/FSH),

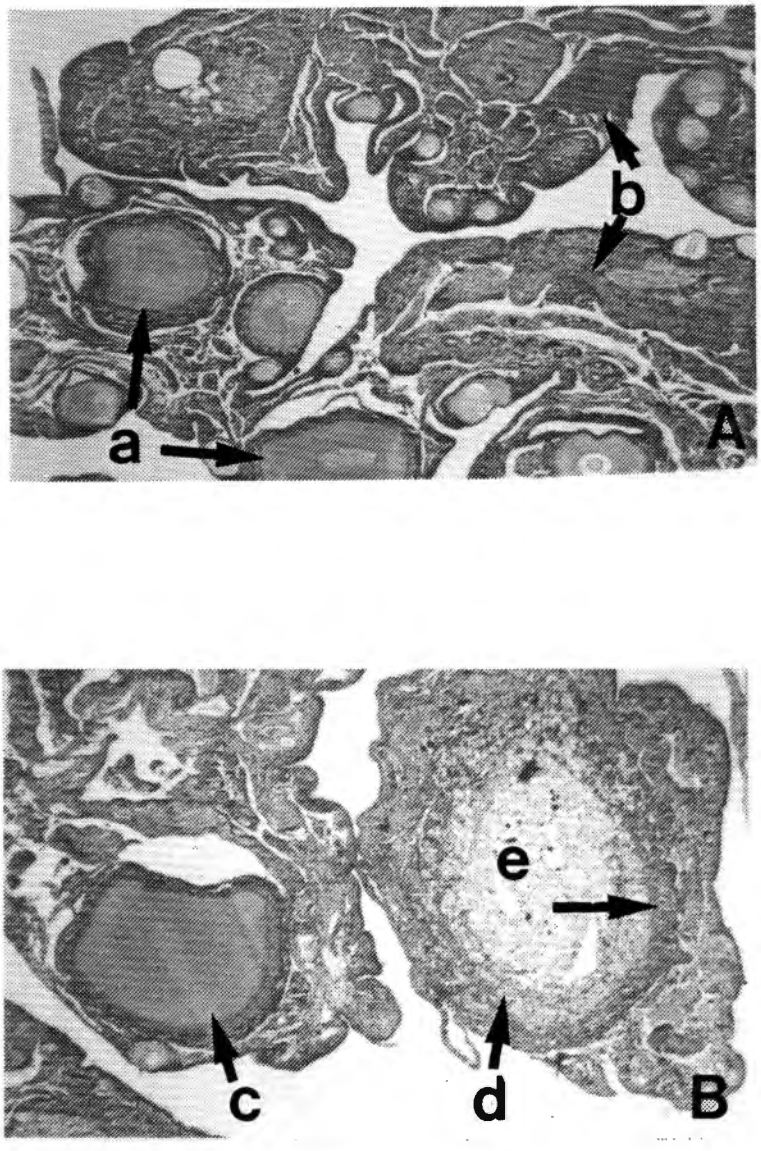

Figure 3. Photomicrographs of transverse sections of laying hen ovary demonstrating normal and atretic follicles. A. A low power photomicrograph of the ovary. Note the numerous immature follicles (a) of various sizes embedded in the connective tissue stroma (b). Hematoxylin and eosin stained. 64x. B. A low power photomicrograph of a normal follicle (c) and an atretic follicle (d). Note the breakdown of the follicular matrix (e) and the disruption of the follicular wall (arrow) in the atretic follicle. Hematoxylin and eosin stained. 64x.

the thyrotrophs (TSH), the corticotrophs (ACTH), the mammotrophs (PRL), the somatotrophs (GH), and the melanotrophs (MSH) (Sturkie, 1976). Since this study is primarily concerned with the gonadotrophs, only this cell type will be discussed.

The gonadotrophs are found both in the cephalic and caudal lobes (Reddy et al., 1977). They can be identified by electron microscopy based on granule size and by light microscopy based on positive reaction to periodic acid-Schiff (PAS) and alcian blue (AB) staining (see Figure 4). (Gilbert and Amin, 1969; Wada, 1975). These cells have been positively identified by immunocytochemistry, utilizing anti-LH sera, as the ones which produce LH and FSH (Midgley, 1963; Nakane, 1970). As previously mentioned, treatment in vivo with LHRH affected only the gonadotrophs (Mikami et al., 1975; Wada, 1975). Luck and Scanes (1980) demonstrated that calcium is required for normal gonadotroph function. Bonney and Cunningham (1977a) demonstrated that cyclic AMP mediates the effect of LHRH on the gonadotrophs.

(4). The Testis

The testis are paired spermatogenic and steroidogenic structures located near the cephalic lobe of the kidney, attached to the dorsal body wall (Sturkie, 1976). A thin tunica albuginea covers an anastomosing network of seminiferous tubules; the testes are not lobulated and are soft to the touch compared to mammalian testes (Lake, 1957) (see Figure 5A). Testis size normally is maximum at 12 to 14 weeks, but is influenced by several factors (Parker et al., 1942). Age, breed, nutrition, as well as social status and crowding can influence testis size (Bennet, 1961; Flickinger, 1966). The average weight of the testis

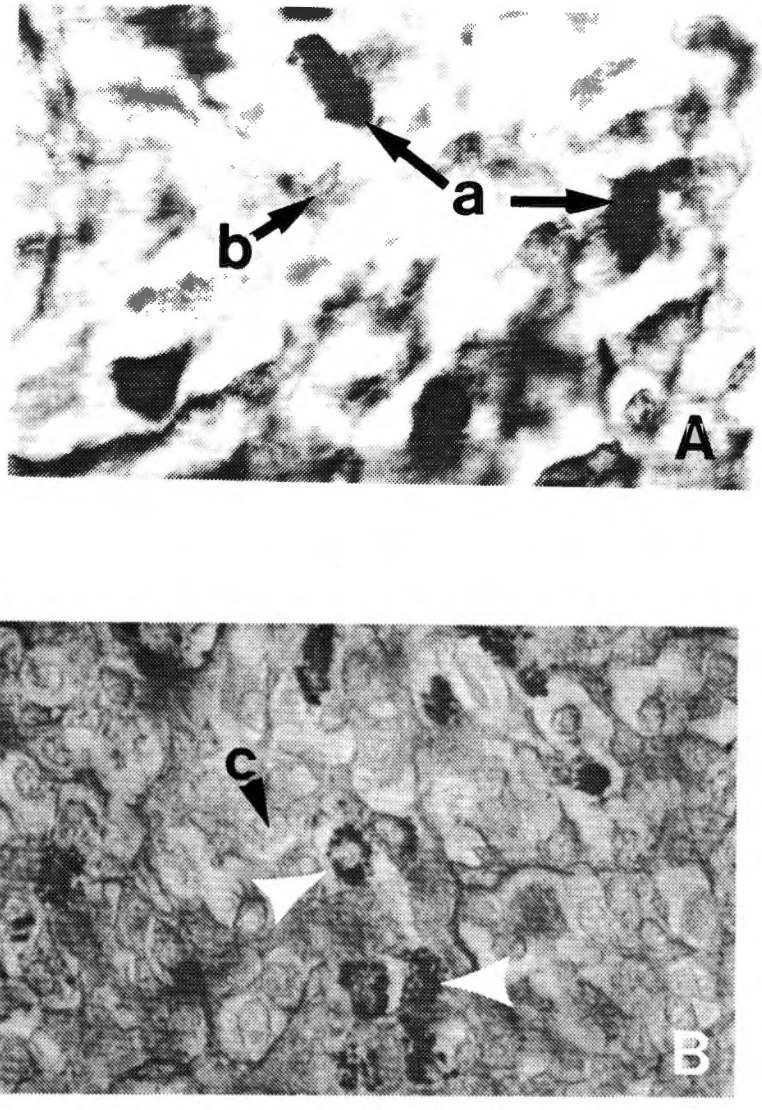

Figure 4. Photomicrographs of transverse sections of laying hen and cockerel anterior pituitary glands demonstrating the gonadotrophs (LH and/or FSH producing cells). A. A high power photomicrograph of the hen anterior pituitary gland. Note the gonadotrophs which stain intensely with alcian blue and periodic acid-Schiff stains (a) and the remaining cellular components which stain with light green (b). Alcian blue, periodic acid-Schiff, and light green stained. 1008x. B. A high power photomicrograph of the cockerel anterior pituitary gland. Note the darkly stained, alcian blue and periodic acid-Schiff positive granules within the gonadotrophs (arrow). The other cellular components stain with light green (c). Alcian blue, periodic acid-Schiff, and light green stained. 1008x.

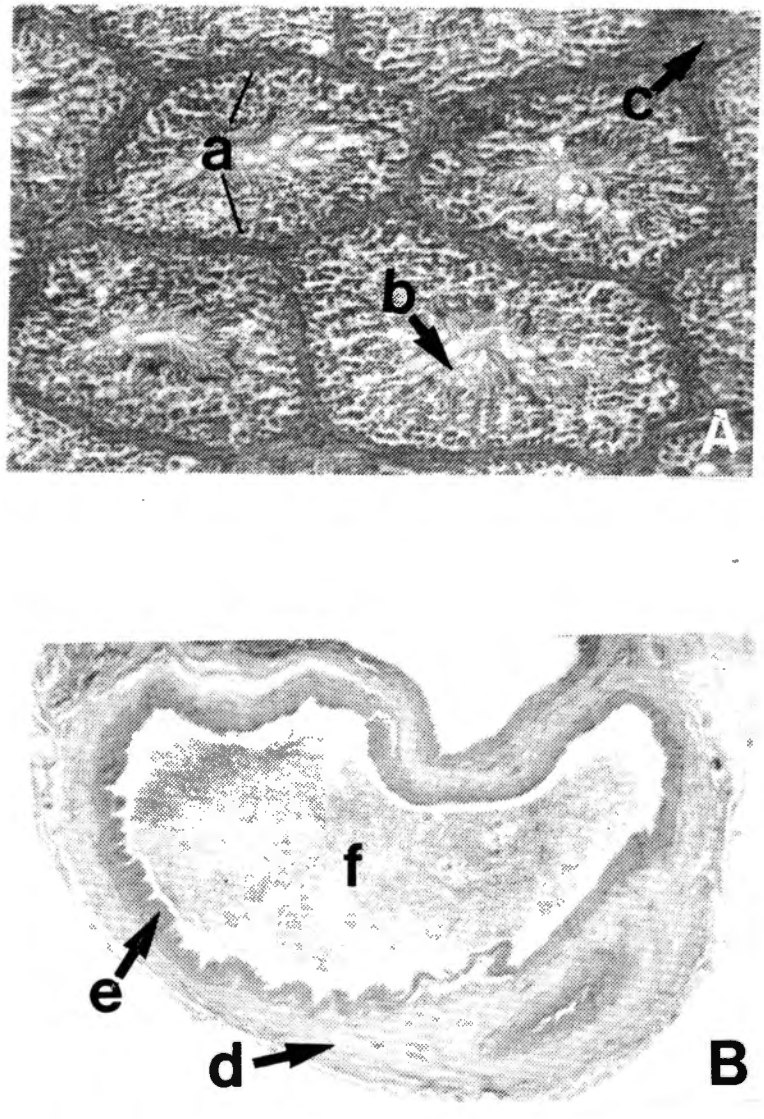

Figure 5. Photomicrographs of transverse sections of cockerel testis and ductus deferens demonstrating their general structures. A. A medium power photomicrograph of the cockerel testis. Note the arrangement of the seminiferous tubules (a) and the presence of spermatids in the lumen of the seminiferous tubules (b). The Leydig cells (c) are located between the seminiferous tubules in the connective tissue. Hematoxylin and eosin stained. 160x. B. A lower power photomicrograph of one half of the convoluted ductus deferens. Note the thick tunica muscularis (d) which is covered by a pseudostratified epithelial cell layer (e). The lumen contains numerous spermatozoa (f). Alcian blue, periodic acid-Schiff, and hematoxylin stained. 160x.

in a 12 week old bird is five grams (Leonard and Righter, 1936). Primary and secondary maturation divisions begin from six to ten weeks. Spermatids and some mature sperm are present at 12 weeks and the cockerel is usually able to fertilize at 24 weeks (Kumaran and Turner, 1949; Parket et al., 1942). The structure and function of the avian and mammalian testis are much the same, however, the seminiferous tubule diameter is slightly larger (250 μm) and there exist fewer Leydig cells in the avian testis (Parker et al., 1942).

(5). The Ductus Deferens

The ductus deferens is a highly convoluted tubular structure which transports spermatozoa from the ductus epididymis to the copulatory organ (Hodges, 1974). The ductus deferens consists of a fibrous connective tissue covering, a smooth muscle layer, and a pseudostratified epithelial cell layer bordering the lumen (Gray, 1936) (see Figure 5B). Individual epithelial cells have been found in the lumen with the sperm but their source is unknown (Tingiri, 1971). The diameter markedly increases toward the caudal end of the ductus deferens, due to increased fibrous connective tissue and muscle mass (Hodges, 1974). The spermatozoa travel through the ductus deferens bathed in a lipomucoprotein secretion. The ductus deferens is the major area of sperm storage in the bird (Lake, 1957).

(6). The Adrenal Gland

The adrenal glands are small paired endocrine organs closely associated with the kidney and the ovary, consisting of intermingled interrenal (cortical) and chromaffin (medullary) cells (see Figure 6). These cells are not arranged in discrete layers as occurs in mammals

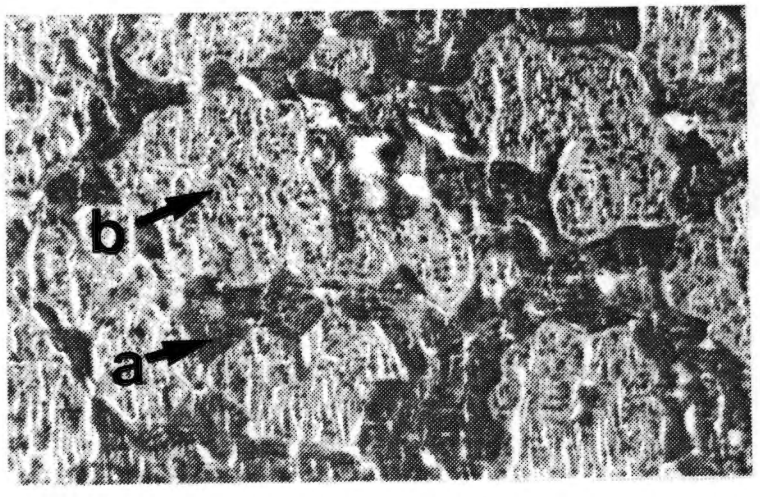

Figure 6. Photomicrograph of a transverse section of a cockerel adrenal gland demonstrating the general structure. Note the darkly basophilic chromaffin cells (a) which are intermingled with the palely stained interrenal cells (b). These cells are not arranged in discrete layers as occurs in the mammalian adrenal gland. Hematoxylin and eosin stained. 64x.

(Sturkie, 1976). The interrenal cells, arranged in cords, predominately secrete corticosterone and also aldosterone (Hodges, 1974; Sivaram, 1965). The chromaffin cells, which make up at least 70 percent or more of the total cell number, produce norepinephrine and epinephrine (Hodges, 1974; Sivaram, 1965). The cellular composition is influenced by sex, age, health, and genetics (Latimer and Landwer, 1924; Oakberg, 1951).

CHAPTER II

I. EXPERIMENTS AND RESULTS

A. Experiment I

Introduction

Mammalian LHRH injected intramuscularly, intravenously, or infused into the jugular vein, is capable of inducing ovulation in laying hens (Opel and Lepore, 1972; Tanaka et al., 1974). However, in some studies intravenous routes of administration have not proven successful in inducing ovulation, possibly because the rate of LH release was too short as compared with that occuring during the normal ovulatory process (Bonney et al., 1974). Research both *in vitro* with incubated pituitary cells and *in vivo* using anti-LHRH sera strongly suggests that LHRH is involved in the ovulation process in birds (Fraser and Sharp, 1978; Tanaka et al., 1974). Unless given immediately after the pre-ovulatory LH peak, the time of injection of LHRH has no effect on the amount of LH released (Bonney et al., 1974). It is of interest that daily intramuscular injections of LHRH for seven days or twice daily intravenous injections of LHRH for seven days failed to cause ovarian and oviductal growth in the non-laying turkey, while intramuscular injection of a combination of LH and FSH did (Burke and Cogger, 1977). Since persistent delivery of LHRH is expected to prolong the release of LH, which in turn causes ovulation, the objective of this study was to test the effect of continuous infusion of LHRH over a six

day period, using a mini-osmotic pump, on the rate of lay and on some anatomical components of the reproductive system of the laying hen.

Materials and Methods

White Leghorn hens, 64 weeks of age, were obtained from The University of Tennessee, Knoxville flock. All hens were individually housed in 8" x 16" battery cages in a 26' x 48' hen house. Hens were fed a 16.4% protein layer ration with water *ad libitum* and were held under a 16 hour light-8 hour dark lighting regimen for the duration of the experiment. Each individual hen's egg production was determined daily for 10 weeks prior to the start of the experiment. Egg production data was recorded specifically six days prior to the start of treatment and six days during the treatment period. At the outset of the experiment, all hens had been in production approximately 50 weeks. On the basis of prior egg production and present clutch sequence, six hens each were allotted to four treatment groups: LHRH 429 µg, LHRH 857 µg, control and sham.

Synthetic mammalian luteinizing hormone-follicle stimulating hormone releasing hormone, hereafter referred to as LHRH, was generously supplied by the National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health, Bethesda, MD. Mini-osmotic infusion pumps (model 2ML1, 2.0 ml capacity, Alza Corp., Palo Alto, CA) were charged with either 500 µg or 1000 µg LHRH in 2.0 ml double-distilled water and were subcutaneously implanted in the neck region of LHRH 429 and LHRH 857 group hens, respectively. Preliminary experiments were carried out to insure that the mini-pump would function correctly utilizing the reduced water content of hen subcutaneous

tissues. A total of 429 µg and 857 µg LHRH, as determined by difference, was infused via a polyethylene catheter attached to the mini-pump into the jugular vein of each hen over a six day period, at a rate of 10 µl per hour. Control hens were not implanted with mini-pumps and sham hens were implanted with mini-pumps but no hormone was delivered. At the end of the six day infusion period, one hour prior to killing, each hen was intravenously injected with 10 µCi [2, 4, 6, 7, 16, 17-^{3}H(N)]-17β estradi l (New England Nuclear, NEN-517, Boston, MS; 140 Ci/mMol). One minute prior to killing by cervical dislocation, 15 ml blood was collected via cardiac puncture and plasma was recovered. Immediately after death the pituitary was removed, weighed, and placed in 10% neutral buffered formalin (NBF). Magnum, shell gland, and ovary were removed, weighed, and samples were placed either in NBF or were frozen for histological analysis or for tritiated estradiol uptake studies, respectively.

Tissue and plasma content of tritiated estradiol was determined by ether extraction and by tissue solubilization methods. For ether extraction, tissues were homogenized in a Wheaton teflon-glass homogenizer (Wheaton Scientific Co., Millville, NJ), extracted twice with diethyl ether (84% extraction efficiency), and counted in a Searle Isocap liquid scintillation counter (56% counter efficiency). For tissue solubilization, tissue samples were minced and added to 750 µl Protosol tissue and gel solubilizer (New England Nuclear, Boston, MS) in glass scintillation vials, incubated at 50^{o}C for 12 hours, and counted. Dry weights for all tissues were determined and results are presented as counts per minute (CPM) per dry weight gram of tissue.

Ovary stroma, magnum, shell gland, and pituitary were processed

through graded alcohols (Fisher Histomatic Tissue Processor, Model 165 Fisher Scientific Co., Atlanta, GA), embedded in paraffin-plastic (Tissue-Prep, Fisher Scientific Co., Atlanta, GA), and sectioned at five microns. Magnum, shell gland and ovarian stroma were stained with hematoxylineosin and alcian blue (AB)-periodic acid-Schiff (PAS)-hematoxylin stains. Measurements were taken using a standard occular micrometer and a list of the criteria evaluated for each tissue appears in Table 1. Slides were prepared from four different regions of the ovary and from three different regions of the magnum. The anterior pituitary of each hen was semi-serial sectioned in five different areas throughout the gland, stained with alcian blue periodic acid-Schiff, utilizing a light green counterstain and the number of AB-PAS positive basophils per 1000x field (10 fields per slide, 100 fields per bird and 600 fields per group) was determined.

Design and Statistical Analysis

The design of the experiment was a dose-response relationship. Mean separation was accomplished using Student's t test, with significance reported at the 0.05 level.

Results

The egg production of laying hens continuously infused with two levels of LHRH for six days, control, and sham hens is presented in Table 2. The egg production of both LHRH treated hens was considerably less than that of controls, although egg production during the six day control period prior to infusion was not different between groups. Egg

Table 1. List of Tissues and Criteria Used in the Histological Evaluation of Laying Hens Continuously Infused with LHRH for a 6 Day Period.

Magnum:

- external diamater (mm)
- cross sectional area (mm^2)
- tissue cross sectional area (mm^2)[1]
- tunica muscularis thickness (μm)
- luminal secretions
  - albumen (PAS positive)[2]
- epithelial cell height
- epithelial cell number (per 50 μm)
  - ciliated cells
  - secretory cells
- secretory activity of submucosa
  - number of glands per 4375 μm^2
  - number of PAS positive granules per 450 μm^2

Shell gland:

- tunica muscularis thickness (μm)
  - circular
  - longitudinal
- villi
  - height (μm)
  - diameter (μm)
  - number per 1250 μm
  - epithelial cell height (μm)

Ovary:

- normal microscopic follicles
  - number per 100 μm^2
  - diameter (μm)
- atretic microscopic follicles
  - number per 100 μm^2
  - diameter (μm)

Anterior pituitary:

- AB-PAS positive basophils per 1000x field

---

[1]Tissue cross sectional area = total area-luminal area

[2]PAS positive = periodic acid-Schiff positive staining PAS material

Table 2. Egg Production of Laying Hens Continuously Infused with LHRH for a 6 Day Period.

Period	Control	Sham	LHRH 429 μg[1]	LHRH 857 μg[2]
6 days prior to treatment	24[x]	26	29	26
6 days during treatment	26	9 (6)[y]	5 (6)	11 (6)
percentage laid during treatment	108.3	34.6	17.2	42.3

[x]Numbers are total production of eggs per group during 6 days preceeding infusion and 6 days during infusion. Each group included 6 selected hens.

[y]Number of hens affected by treatment.

[1,2]LHRH 429 and LHRH 857 indicate 72 and 143 μg LHRH infused daily, respectively.

production declined to 17-42% of that of control hens during the treatment period. Egg production in sham hens decreased to 35% of controls.

The uptake of tritiated estradiol by the reproductive tract of laying hens continuously infused with two levels of LHRH for six days, control, and sham hens as determined by ether extraction is shown in Table 3. The first follicular membrane of LHRH 857 µg treated hens contained more tritiated estradiol than did controls; however, no significant differences in uptake of tritiated estradiol between treated and control groups were demonstrated in magnum, shell gland, ovarian stroma, or second follicular membrane. The shell gland consistently retained the most and the second follicular membrane the least tritiated estradiol of all tissues studied.

The uptake of tritiated estradiol by the reproductive tract of laying hens continuously infused with two levels of LHRH for six days, control, and sham hens as determined by the tissue solubilization technique is shown in Table 4. Uptake of tritiated estradiol in magnum, shell gland, and ovarian stroma was not significantly different as compared to controls.

The weight of the reproductive organs of laying hens continuously infused with 2 levels of LHRH for six days, control and sham hens is shown in Table 5. Whole ovary weights of both LHRH treated hens and sham hens were significantly decreased. Ovarian stroma weights tended to decrease but the differences were not significant ($P>0.05$). Mean total oviduct weights (including shell gland) were slightly higher but not significant, for LHRH treated hens as compared to controls. The pituitary weights of sham hens were significantly higher as compared to

Table 3. Uptake of ^{3}H-17β Estradiol by the Reproductive Tract of Laying Hens Continuously Infused with LHRH for a 6 Day Period as Determined by Ether Extraction Technique.

Tissue	CPM/g Dry Wt.			
	Control	Sham	LHRH 429 μg[1]	LHRH 857 μg[2]
Magnum	1351.1 ± 616.2[a]	1105.9 ± 330.3[a]	568.8 ± 111.2[a]	1588.1 ± 308.6[a]
Shell gland	1335.3 ± 318.5[a]	1325.0 ± 472.9[a]	1512.7 ± 191.8[a]	1758.9 ± 231.2[a]
Ovary				
Stroma	1403.0 ± 392.0[a]	645.9 ± 123.9[a]	793.3 ± 84.6[a]	778.4 ± 25.7[a]
1st follicular membrane	138.8 ± 23.5[a]	216.5 ± 53.9[a]	353.2 ± 104.3[a]	494.8 ± 115.9[b]
2nd follicular membrane	219.8 ± 17.4[a]	257.8 ± 115.8[a]	152.9 ± 25.2[a]	---------

Numbers are mean ± S.E.M. (n = 6).

[1,2]LHRH 429 and LHRH 857 indicate 72 and 143 μg LHRH infused daily, respectively.

[a,b]Similar superscripts indicate no significant ($P \geq 0.05$) differences, while different superscripts indicate significant differences as compared to controls.

Table 4. Uptake of ^{3}H-17β Estradiol by the Reproductive Tract of Laying Hens Continuously Infused with LHRH for a 6 Day Period as Determined by Tissue Solubilization Technique.

Tissue	CPM/g Dry Wt.			
	Control	Sham	LHRH 429 μg[1]	LHRH 857 μg[2]
Magnum	1167.5 ± 314.1[a]	1274.6 ± 164.5[a]	1304.3 ± 123.2[a]	1148.5 ± 247.3[a]
Shell gland	1591.9 ± 245.1[a]	1640.8 ± 280.4[a]	890.7 ± 213.6[a]	986.2 ± 370.2[a]
Ovary stroma	1747.8 ± 369.2[a]	1394.0 ± 373.3[a]	1484.5 ± 207.8[a]	1766.8 ± 111.6[a]

Numbers are mean ± S.E.M. (n = 6) of 2 determinations per hen.

[1,2]LHRH 429 and LHRH 857 indicate 72 and 143 μg LHRH infused daily, respectively.

[a,b]Similar superscripts indicate no significant (P>0.05) differences, while different superscripts indicate significant differences as compared to controls.

Table 5. Weight of Reproductive Organs of Laying Hens Continuously Infused with LHRH for a 6 Day Period.

Organ	Control	Sham	LHRH 429 µg[1]	LHRH 857 µg[2]
Oviduct (g) (including shell gland)	16.8 ± 1.7[a]	16.6 ± 5.0[a]	26.1 ± 3.9[a]	20.9 ± 2.3[a]
Ovary (g)				
whole	46.6 ± 8.2[a]	10.6 ± 1.2[b]	10.9 ± 0.7[b]	15.6 ± 5.4[b]
stroma	10.6 ± 5.4[a]	4.1 ± 0.6[a]	5.7 ± 1.2[a]	4.7 ± 1.1[a]
Pituitary (mg)	8.1 ± 0.4[a]	5.2 ± 0.8[b]	10.8 ± 1.2[a]	6.8 ± 1.0[a]

Numbers are mean ± S.E.M. (n = 6).

[1,2]LHRH 429 and LHRH 857 indicate 72 and 143 µg LHRH infused daily, respectively.

[a,b]Similar superscripts indicate no significant ($P>0.05$) differences, while different superscripts indicate significant differences as compared to controls.

control hens; there were no significant differences in pituitary weights between LHRH treated group hens and control hens.

The histological evaluation of the magnum of laying hens continuously infused with two levels of LHRH for six days, control, and sham hens is shown in Table 6. External diameter, cross sectional area and tissue cross sectional area were significantly decreased in all groups as compared to controls. Tunica muscularis thickness, ciliated, and secretory epithelial cell number per 50 microns were all significantly ($P<0.05$) greater in treated hens and sham hens versus controls. Tubular gland luminal secretions were completely absent in all sham and LHRH treated hens. The number of submucosal glands secretions (PAS positive) per 4375 square microns was significantly greater in all groups as compared to controls. Significantly fewer tubular glands per section were noted in LHRH 857 µg treated hens. No significant differences were noted in tubular gland epithelial cell height or number of PAS positive submucosal glands per 450 square microns.

The histological evaluation of the shell gland of laying hens continuously infused with two levels of LHRH for six days, control, and sham hens is shown in Table 7. Circular tunica muscularis thickness and villi diameter were significantly decreased in both LHRH treated groups as compared to controls. Longitudinal tunica muscularis thickness, villi height, number of villi per 1250 microns and villous epithelial height were not significantly different as compared to controls. Villi height tended to decrease in sham, LHRH 429 µg and LHRH 857 µg treated hens in a progressive fashion but the differences were not significant.

The histological evaluation of the ovary of laying hens

Table 6. Histological Evaluation of Magnum of Laying Hens Continuously Infused with LHRH for a 6 Day Period.

Item	Control	Sham	LHRH 429 µg[1]	LHRH 857 µg[2]
External diameter (mm)	17.2 ± 1.2[a]	10.9 ± 1.7[b]	10.6 ± 0.4[b]	9.4 ± 1.0[b]
Cross sectional area (mm^2)	452.2 ± 37.8[a]	235.8 ± 51.3[b]	170.7 ± 16.7[b]	162.3 ± 28.0[b]
Tissue cross sectional area (mm^2)[3]	411.0 ± 28.2[a]	214.5 ± 48.9[b]	150.6 ± 18.2[b]	111.3 ± 20.6[b]
Tunica muscularis thickness (µm)	46.7 ± 2.7[a]	77.7 ± 3.2[b]	103.9 ± 16.5[b]	78.3 ± 3.0[b]
Luminal secretions albumen (PAS positive)[4]	-	-	-	-
Epithelial cell height (µm)	24.0 ± 1.2[a]	25.9 ± 1.8[a]	22.9 ± 1.4[a]	23.3 ± 3.5[a]
Epithelial cell number (per 50 µm) ciliated cells	6.9 ± 0.5[a]	7.9 ± 0.6[a]	9.4 ± 0.4[b]	9.9 ± 0.6[b]
secretory cells	6.8 ± 0.2[a]	7.7 ± 0.5[a]	10.1 ± 0.8[b]	9.9 ± 0.8[b]
Secretory activity of submucosa, number of glands per 4375 $µm^2$	5.0 ± 0.3[a]	7.7 ± 1.0[b]	8.1 ± 0.6[b]	6.8 ± 0.2[b]
number of PAS positive granules per 450 $µm^2$	48.2 ± 4.4[a]	39.8 ± 3.7[a]	42.3 ± 1.7[a]	32.4 ± 2.2[a]

Numbers are mean ± S.E.M. (n = 6) of determinations made in 3 regions of the magnum.

[1,2]LHRH 429 and LHRH 857 indicate 72 and 143 µg LHRH infused daily, respectively.

[3]Tissue cross sectioned area = total area-luminal area.

[4]Periodic acid-Schiff staining procedure ('+' indicates presence, '-' indicates absence).

[a,b]Similar superscripts indicate no significant (P>0.05) differences, while different superscripts indicate significant differences as compared to controls.

Table 7. Histological Evaluation of Shell Gland of Laying Hens Continuously Infused with LHRH for a 6 Day Period.

Item	Control	Sham	LHRH 429 µg[1]	LHRH 857 µg[2]
Tunica muscularis				
Thickness (µm)				
circular	474.2 ± 25.9[a]	448.8 ± 58.4[a]	304.8 ± 33.5[b]	278.3 ± 34.0[b]
longitudinal	322.3 ± 50.3[a]	408.5 ± 42.9[a]	392.1 ± 61.8[a]	329.0 ± 43.9[a]
Villi				
Height (µm)	2134.4 ± 205.9[a]	2200.0 ± 184.1[a]	1974.0 ± 159.9[a]	1785.4 ± 150.1[a]
Diameter (µm)	401.7 ± 14.8[a]	342.5 ± 26.7[a]	306.7 ± 30.5[b]	342.3 ± 20.9[b]
Number per 1250 µm	4.5 ± 0.3[a]	5.1 ± 0.3[a]	4.7 ± 0.2[a]	5.0 ± 0.1[a]
Epithelial cell height (µm)	24.9 ± 1.2[a]	26.2 ± 1.6[a]	23.4 ± 1.6[a]	23.7 ± 2.0[a]

Numbers are mean ± S.E.M. (n = 6) of sections stained with hematoxylin and eosin.

[1,2]LHRH 429 and LHRH 857 indicate 72 and 143 µg LHRH infused daily, respectively.

[a,b]Similar superscripts indicate no significant (P>0.05) differences, while different superscripts indicate significant differences as compared to controls.

continuously infused with two levels of LHRH for six days, control, and sham hens is shown in Table 8. The number and diameter per 100 μm^2 of normal microscopic follicles were not significantly affected by either level of LHRH treatment, although mean diameter tended to increase progressively from that of controls to LHRH 429 µg treatment to LHRH 857 µg treatment. The number and diameter per 100 μm^2 of atretic microscopic follicles were not significantly different in both LHRH treated groups hens as compared to the control group. The number of atretic follicles per 100 μm^2 was only four percent of the number of normal follicles per 100 μm^2. The diameter of atretic follicles was approximately five times that of normal follicles.

The histological evaluation of the anterior pituitary gland of laying hens continuously infused with two levels of LHRH for six days, control, and sham hens, is shown in Table 9. Sham hens possessed significantly more AB-PAS positive basophils per 1000x field as compared to controls. There were no significant differences in basophil number between both LHRH treated groups and controls.

Discussion

The results from experiment 1 indicate that the observed differences between LHRH treated and control hens were primarily due to stress induced by experimental manipulation and that continuous infusion of LHRH was not able to overcome the effects of stress. Rothchild and Fraps (1945) demonstrated that surgical stress induced follicular atresia and inhibition of laying. The possibility that LHRH, as used in this experiment, was not recognized by the reproductive system of the hen is

Table 8. Histological Evaluation of Ovary of Laying Hens Continuously Infused with LHRH for a 6 Day Period.

Follicle Type	Control	Sham	LHRH 429 μg[1]	LHRH 857 μg[2]
Normal microscopic follicles				
Number per 100 μm^2	236.1 ± 43.3[a]	265.2 ± 85.7[a]	159.6 ± 25.3[a]	270.5 ± 64.3[a]
Diameter (μm)	283.5 ± 18.9[a]	262.2 ± 10.6[a]	292.4 ± 18.2[a]	310.0 ± 37.8[a]
Atretic microscopic follicles				
Number per 100 μm^2	8.6 ± 0.8[a]	11.3 ± 1.7[a]	9.2 ± 1.4[a]	10.0 ± 1.5[a]
Diameter (μm)	1217.3 ± 56.2[a]	1341.4 ± 79.4[a]	1403.2 ± 120.0[a]	1273.9 ± 63.2[a]

Numbers are mean ± S.E.M. (n = 6) from determinations made in 4 regions of the ovary using the alcian blue-PAS-hematoxylin staining procedure.

Atretic follicles were defined according to Davis, D. E., Anat.Rec.153-165, 1942.

[1,2]LHRH 429 and LHRH 857 indicate 72 and 143 μg LHRH infused daily, respectively.

[a,b]Similar superscripts indicate no significant (P>0.05) differences, while different superscripts indicate significant differences as compared to controls.

Table 9. Histological Evaluation of Anterior Pituitary Gland of Laying Hens Continuously Infused with LHRH for a 6 Day Period.

Cell Type	Control	Sham	LHRH 429 µg[1]	LHRH 857 µg[2]
AB-PAS positive basophil cells per 1000x field[3]	6.5 ± 0.2[a]	9.0 ± 0.3[b]	5.9 ± 0.2[a]	6.9 ± 0.4[a]

Numbers are mean ± S.E.M. (n = 6) of 100 determinations per hen, and 600 determinations per group.

[1,2]LHRH 429 and LHRH 857 indicate 72 and 143 µg LHRH infused daily, respectively.

[3]Alcian blue-periodic acid-Schiff staining procedure used with light green counterstain. Basophil cells are postulated to be the gonadotrophic cells in birds. (Wada, M., Cell. Tissue Res. 159: 167-178, 1975; Sharp, P. J., Gen. Comp. Endocr. 22: 265-266, 1974.)

[a,b]Similar superscripts indicate no significant (P>0.05) differences, while different superscripts indicate significant differences as compared to controls.

small, since van Teinhoven and Schally (1972) demonstrated that synthetic LHRH induced ovulation in laying hens. It is possible that the dosages of LHRH infused were either too high or too low to maintain normal gonadotropin secretion. It is also possible, although not probable, that LHRH inhibits reproductive function in the hen, through an indirect effect on the pituitary or a direct effect on the gonads themselves.

Egg production declined drastically in all LHRH treated and sham hens. It is possible that stress played the major role in depressing ovulation and that LHRH treatment had no effect. This may indeed be the case since the percentage egg production of sham and LHRH treated hens was not different.

No significant differences in tritiated estradiol uptake were observed between control and LHRH treated hens in magnum, shell gland, or ovary. These results suggest that the net uptake of estradiol was not affected by LHRH treatment. From this experiment it is not possible to conclude whether endogenous estradiol levels were influenced by LHRH treatment.

Mean whole ovary weight was significantly decreased in all sham and LHRH treated hens while mean stroma ovary weight was not affected, which suggests a stress effect on the ovary inducing follicular atrophy. Oviduct weight was not significantly different between control and LHRH treated groups. Pituitary weight was not significantly different between groups; however, changes in pituitary weight alone are not good indicators of morphology or function.

The magnum was dramatically altered both histologically and functionally in both LHRH treated and sham hens as determined by

significant changes in all criteria evaluated. The cause or causes of the reduced magnum function cannot be ascertained by this study. It is possible, since estrogen and progesterone are known to be the major hormones involved in magnum development and function (Brandt and Nalbandov, 1956), that reduced gonadotropin output caused a reduction of gonadal hormone secretion. It is also possible that stress induced follicular atrophy was the major cause of decreased gonadotropin secretion.

In the shell gland, villi diameter and circular tunica muscularis thickness were reduced, and all other criteria measured were not significantly different between LHRH treated and control birds. Possibly the reduced activity of the organ itself produced these changes, since, as already noted, magnum and ovarian function were drastically reduced. These results indicate that, of all reproductive organs studied, the magnum and ovary were significantly affected as compared to the shell gland and pituitary.

No significant differences were observed in the number per 100 μm^2 or the diameter of both normal and atretic follicles. The number of atretic follicles in control and treated ovaries is not unusual, since follicular atresia is a normal, continuous process and all ovaries possess a certain number of atretic follicles (Davis, 1942). These results, coupled with a decrease in mean total whole ovarian weight without a change in mean stroma ovarian weight, indicate that once again stress induced follicular atresia played the major role in the results observed in the ovary and that the ovary was not significantly affected by LHRH treatment.

The number of AB-PAS positive basophil cells per 1000x field (gonadotrophs) was not significantly different between control and treated hens. The results suggest that the pituitary may have become refractory to continued LHRH stimulation as was demonstrated in rats after short term LHRH treatment (Ferland et al., 1981). It is also possible that initially these cells responded to LHRH stimulation and eventually were depleted of stored LH and FSH but by six days had returned to a normal state.

B. Experiment II

Introduction

In the rat single or multiple injections of synthetic mammalian LHRH or LHRH analogs reduced testicular LH receptor number, testosterone production and testis and accessory gland weights (Auclair et al., 1977). However, plasma LH and FSH levels significantly increased after LHRH injection (Seguin et al., 1981). Twelve week treatment with LHRH analogs in the rat produced degenerative changes in the seminiferous tubules and inhibited spermatogenesis, but these effects were reversible (Labrie et al., 1980). In man synthetic LHRH injections caused an increase in testosterone levels in 78 percent of the subjects and no adverse effects were produced (Judd et al., 1974). Research in cockerels indicates that within five minutes of an intracarotid or intravenous injection of LHRH, plasma LH peaked and returned to basal levels within one hour (Furr et al., 1973b). It has been demonstrated that the basal LH levels in cockerels are higher than those of laying hens. Furthermore, the LH levels in cockerels tend to be stable for short periods of time, which

is in contrast to the pattern seen in most mammals. It is possible that there exists a modified mechanism for controlling LH release in the cockerel (Furr et al., 1973b). In the Japanese quail, the size and granule content of pituitary gonadotrophs increased when single subcutaneous LHRH injections were given for ten days (Wada, 1975). To our knowledge, at the present time the effects of continuous infusion of LHRH on the testis, pituitary and other endocrine organs of the cockerel have not been investigated. The objective of this study was to determine the effects of continuous infusion of LHRH over a six day period, using a mini-osmotic pump, by evaluating the histologic morphology and the metabolism of tritiated testosterone of various endocrine organs in LHRH treated and control cockerels.

Materials and Methods

Twelve week old White Leghorn cockerels were obtained from The University of Tennessee, Knoxville flock. All were group housed in a 10' x 10' pen in a brooder house and were fed an 18 percent growing ration and water ad libitum. Six cockerels each were randomly allotted to three treatment groups: LHRH 214 µg, control, and sham.

Mini-osmotic infusion pumps (model 2ML1, 2.0 ml capacity, Alza Corp., Palo Alto, CA) were charged with 250 µg synthetic mammalian LHRH (NIAMDD), diluted in 2.0 ml double-distilled water, and were subcutaneously implanted into the neck region of each LHRH treated cockerel. A total of 214 µg LHRH was infused over a six day period into the jugular vein of each cockerel; an average of 36 µg/day was delivered at a rate of 10 µl/hour. Control cockerels received no pump and sham cockerels

received a pump which delivered no hormone. Ten μCi [1, 2, 6, 7, -^{3}H(N)]-testosterone (New England Nuclear, Boston, MS, NEN-370, 98.9 Ci/mMol) was injected intravenously at one hour before death. Plasma was recovered one minute before death as in Experiment I. Immediately after death, the comb was removed and weighed; the testes, adrenals, ductus deferens, and pituitary were removed, weighed, and placed in 10 percent neutral buffered formalin or frozen. Samples of each tissue were taken for histological analysis and for tritiated testosterone uptake studies.

The amount of tritiated testosterone contained by the testis was determined by tissue solubilization methods using Protosol tissue and gel solubilizer (New England Nuclear, Boston, MS), as were conducted for laying hen tissues in Experiment I.

Testis halves, adrenals, and ductus deferens tissues were processed through graded alcohols, embedded and sectioned as in Experiment I, and stained with hematoxylin-eosin and alcian blue (AB) periodic acid-Schiff (PAS) hematoxylin stains. Slides were evaluated using a standard occular micrometer and criteria evaluated for each tissue are listed in Table 10. Sectioning, staining, and evaluation of the anterior pituitary of cockerels was carried out according to procedures used in Experiment I for the anterior pituitaries of laying hens.

Design and Statistical Analysis

The design of this experiment was similar to that of Experiment I, however, only a time-response relationship was investigated. Mean separation was accomplished by Student's t test and significance was reported at the 0.05 level.

Table 10. List of Tissues and Criteria Used in the Histological Evaluation of Cockerels Continuously Infused with LHRH (214 µg/6 days).

Testis:
Width at the center
Seminiferous tubules
diameter (µm)
number (per 100x field)
containing spermatids (percentage)
Ductus deferens:
Cross sectional diameter (µm)
Tunica muscularis thickness (µm)
Height of epithelium (µm)
Epithelial cell density (cell number in 100 µm)
Adrenal gland:
Interrenal cells (percentage)
Chromaffin cells (percentage)
Anterior pituitary:
AB-PAS positive basophils per 1000x field

The uptake of tritiated testosterone by the testes of cockerels continuously infused with 214 µg LHRH for six days, control, and sham cockerels as determined by the tissue solubilization method is presented in Table 11. The testicular uptake was not significantly different in treated versus control groups. The testes in LHRH treated cockerels retained less tritiated testosterone as compared to control cockerels but this difference was not significant ($P>0.05$).

The organ weights of cockerels continuously infused with 214 µg LHRH for six days, control, and sham cockerels is shown in Table 12. There were no significant differences between body, comb, pituitary, testis, or adrenal weights of LHRH treated cockerels versus controls. Mean weight of the testes of LHRH treated cockerels tended to be less than that of control cockerels but this difference was not significant ($P>0.05$). Pituitary weights of LHRH treated and control cockerels were almost identical.

The histological evaluation of the testes of cockerels continuously infused with 214 µg LHRH for six days, control, and sham cockerels is shown in Table 13. No significant differences were observed in testes width, seminiferous tubule diameter, number of seminiferous tubules per 100x field or percentage of seminiferous tubules containing spermatids. The number of seminiferous tubules per 100x field tended to be much less in control cockerels as compared to LHRH treated and sham cockerels, but this difference was not significant at the 0.05 level.

The histological evaluation of the ductus deferens of cockerels

Table 11. Uptake of ^{3}H-Testosterone by the Testes of Cockerels Continuously Infused with LHRH (214 µg/6 days) as Determined by Tissue Solubilization Technique.

Item	CPM/g Wet Wt.		
	Control	Sham	LHRH
Testis	1122.4 ± 194.7[a]	1259.9 ± 171.2[a]	689.1 ± 157.4[a]

Numbers are mean ± S.E.M. (n = 6) of 2 determinations per cockerel.

[a,b]Similar superscripts indicate no significant (P>0.05) differences, while different superscripts indicate significant differences as compared to controls.

Table 12. Organ Weights of Cockerels Continuously Infused with LHRH (214 µg/6 days).

Tissue	Control	Sham	LHRH
Body (g)	1733.2 ± 72.4[a]	1532.2 ± 58.3[a]	1668.0 ± 85.7[a]
Comb (g)	17.9 ± 2.8[a]	17.0 ± 2.0[a]	14.3 ± 3.6[a]
Pituitary (mg)	11.3 ± 1.3[a]	19.2 ± 5.7[a]	11.9 ± 1.3[a]
Testes (2) (g)	3.2 ± 0.6[a]	1.7 ± 0.5[a]	1.5 ± 0.5[a]
Adrenals (2) (mg)	191.7 ± 76.8[a]	145.0 ± 44.0[a]	179.6 ± 75.4[a]

Numbers are mean ± S.E.M. (n = 6).

[a,b]Similar superscripts indicate no significant (P>0.05) differences, while different superscripts indicate significant differences as compared to controls.

Table 13. Histological Evaluation of Testes of Cockerels Continuously Infused with LHRH (214 µg/6 days).

Item	Control	Sham	LHRH
Testes (fixed)			
Width at the center (mm)	11.5 ± 1.2[a]	9.9 ± 1.5[a]	10.2 ± 1.4[a]
Seminiferous Tubules			
Diameter (µm)	152.9 ± 28.3[a]	134.4 ± 57.9[a]	165.9 ± 24.2[a]
Number (per 100x field)	80.0 ± 36.6[a]	134.5 ± 37.1[a]	109.4 ± 46.5[a]
Containing spermatids (percentage)	73.0 ± 16.5[a]	58.8 ± 18.7[a]	43.4 ± 7.5[a]

Numbers are mean ± S.E.M. (n = 6).

[a,b]Similar superscripts indicate no significant (P>0.05) differences, while different superscripts indicate significant differences as compared to controls.

continuously infused with 214 µg of LHRH for six days, control, and sham cockerels is shown in Table 14. There was a significant ($P<0.05$) increase in cross sectional diameter in LHRH treated cockerels as compared to control cockerels. Cross sectional diameter in sham cockerels was significantly less than that of controls. Tunica muscularis thickness, ethithelial cell height, and epithelial cell density were not significantly different in LHRH treated cockerels versus controls, although there was a trend in the thickness of the tunica muscularis and in epithelial cell height to decrease progressively in control, sham, and LHRH treated birds.

The histological evaluation of the adrenal glands of cockerels continuously infused with 214 µg LHRH for six days, control, and sham cockerels is shown in Table 15. There was a significant ($P<0.05$) increase in the number percentage of chromaffin cells in LHRH treated cockerels as compared to control cockerels. The number percentage of interrenal cells was less in LHRH treated birds as compared to controls, but this decrease was not significant. In all groups the number percentage of chromaffin cells was greater than the number percentage of interrenal cells.

The histological evaluation of the anterior pituitary gland of cockerels continuously infused with 214 µg LHRH for six days, control, and sham cockerels is shown in Table 16. There was a significant ($P<0.05$) decrease in PAS positive basophil cell number per 1000x field in LHRH treated cockerels as compared to control cockerels. Sham cockerels also possessed significantly fewer AB-PAS positive basophils than did control cockerels.

Table 14. Histological Evaluation of Ductus Deferens[1] of Cockerels Continuously Infused with LHRH (214 μg/6 days).

Item	Control	Sham	Sham
Cross sectional diameter (μm)	904.2 ± 63.1[a]	500.0 ± 48.1[b]	1366.7 ± 187.8[b]
Tunica muscularis thickness (μm)	57.1 ± 6.5[a]	52.9 ± 6.3	38.5 ± 6.0
Height of epithelium (μm)	30.7 ± 6.2[a]	25.1 ± 2.6[a]	20.7 ± 3.1[a]
Epithelial cell density (cell number in 100 μm)	26.1 ± 2.3[a]	24.8 ± 1.6[a]	29.3 ± 3.9[a]

Numbers are mean ± S.E.M. (n = 6).

[a,b]Similar superscripts indicate no significant (P>0.05) differences, while different superscripts indicate significant differences as compared to controls.

[1]Tissue sections were taken from the middle region.

Table 15. Histological Evaluation of Adrenal Glands of Cockerels Continuously Infused with LHRH (214 µg/6 days).

Cell Type	Control	Sham	LHRH
Chromaffin Cells (percentage)	59.3 ± 2.2[a]	62.7 ± 1.2[a]	67.9 ± 1.6[b]
Interrenal Cells (percentage)	40.7 ± 2.2[a]	37.3 ± 1.2[a]	32.1 ± 1.6[a]

Numbers are mean ± S.E.M. (n = 6) of 25 determinations per cockerel and 150 determinations per group.

[a,b]Similar superscripts indicate no significant (P>0.05) differences, while different superscripts indicate significant differences as compared to controls.

Table 16. Histological Evaluation of Anterior Pituitary Gland of Cockerels Continuously Infused with LHRH (214 µg/6 days).

Item	Control	Sham	LHRH
AB-PAS positive basophil cells per 1000x field	11.9 ± 0.5[a]	9.5 ± 0.6[b]	7.4 ± 1.0[b]

Numbers are mean ± S.E.M. (n = 6) of 100 determinations per cockerel, and 600 determinations per group.

[a,b]Similar superscripts indicate no significant (P>0.05) differences, while different superscripts indicate significant differences as compared to controls.

[1]Periodic acid-Schiff staining procedure used with light green counterstain. Basophil cells are postulated to be the gonadotrophic cells in birds (Wada, M., Cell. Tissue. Res. 159: 167-178, 1975; Gilbert, A. B., and S. O. Amin, J. Reprod. Fert. 20: 363, 1969).

Disscussion

The results obtained in Experiment II demonstrate that although the number of pituitary gonadotrophs was significantly decreased, LHRH treatment seemed not to significantly affect the histologic morphology of reproductive organs in the cockerel. Stress may have also affected the cockerel since there was a significant increase in the number percentage of chromaffin cells but not an increase in the number percentage of interrenal cells in LHRH treated cockerels. Although it is possible that the dosage of LHRH infused was not high enough to produce a significant effect at the level of the gonads, the results may indicate that the main effect of LHRH is at the level of the pituitary.

The uptake of tritiated testosterone by the testis of LHRH treated and control cockerels was not significantly different, which suggests that the relative testosterone uptake was unaffected by LHRH treatment. There were no significant differences in body, testis, comb, adrenal, and pituitary weights between groups which suggests that within six days LHRH produced no marked changes in any of these organs. The histological evaluation of the testis, which involved determining the number of seminiferous tubules per 100x field, the percentage of seminiferous tubules containing spermatids, the diameter of seminiferous tubules, and the testis width, demonstrated that LHRH treatment did not significantly affect these tissues. However, since testis weight showed a non-significant decrease in LHRH treated cockerels as compared to controls, it is possible that if infusion had been continued past six days significant differences would have been observed.

Histological evaluation of the ductus deferens of LHRH treated,

sham, and control cockerels revealed that the cross sectional diameter significantly increased in LHRH treated cockerels. However, this difference could be attributed to sampling error since tunica muscularis thickness and epithelial cell height decreased non-significantly in LHRH treated birds.

The number percentage of chromaffin cells was significantly elevated in LHRH treated cockerels. In all groups the number percentage of interrenal cells was greater than the number percentage of chromaffin cells, which agrees with studies conducted by Siviram (1965).

As previously stated the number of AB-PAS positive basophils (gonadotrophs) was significantly decreased in LHRH treated cockerels. It is possible that continued LHRH stimulation of the gonadotrophs induced complete degranulation, but that by six days some cells had recovered so that they reacted positively within AB-PAS stains.

CHAPTER III

GENERAL DISCUSSION

The objective of this research was to determine the effects of continuous infusion of LHRH on the reproductive tract of the laying hen. Experiment II supplemented this work by using the less complex, less stress affected cockerel to act as a positive control in order to determine at what level of the reproductive system of the bird LHRH was directly acting upon. The reproductive physiology of the female is relatively complex, and stress tends to have a much more pronounced effect since there are many more sites where stress can interfere with the feedback system. The results from Experiment I suggest that LHRH could not overcome the stress induced anovulation, follicular atrophy, and oviductal atrophy. Since egg laying was greatly reduced in LHRH treated hens it is possible that LHRH deficiency was not involved in the marked reduction in laying rate. The results obtained in Experiment II indicate that the main effect of LHRH is at the level of the pituitary in the male, since the number of gonadotrophs per 1000x field was significantly decreased in LHRH treated cockerels.

The results of this study indicate that continuous infusion of LHRH in the laying hen for six days does not increase the ovulation rate. Refractoriness of the ovary and the pituitary induced by LHRH treatment combined with stress tended to produce the opposite effect where ovulation rate was reduced, follicular atresia and oviductal atrophy were produced.

LITERATURE CITED

LITERATURE CITED

Adams, T. E. and H. G. Spies. 1981. Binding characteristics of gonadotropin-releasing hormone receptors throughout the estrous cycle of the hamster. Endocr. 108: 2245.

Arimura, A., N. Nishi and A. V. Schally. 1976. Delayed implantation caused by administration of sheep immuno gamma globulin against LHRH in the rat. Proc. Soc. Exp. Biol. Med. 152: 71.

Auclair, C., P. A. Kelly, F. Labrie, D. H. Coy, and A. V. Schally. 1977. Inhibition of testicular luteinizing hormone receptor level by treatment with a potent luteinizing hormone-releasing hormone agonist or human chorionic gonadotropin. Biochem. Biophys. Res. Comm. 76: 855.

Belchetz, P. E., T. M. Plant, Y. Nakai, E. J. Keogh, and E. Knobil. 1978. Hypophyseal responses to continuous and intermittent delivery of hypothalamic gonadotropin-releasing hormone. Science 202: 631.

Bennett, C. H. 1961. Relation between size and age of the gonads in the fowl from hatching date to sexual maturity. Poultry Sci. 40: 99.

Bonney, R. C. and F. J. Cunningham. 1977a. A role for cyclic AMP as a mediator of the action of LHRH on chicken anterior pituitary cells. Mol. Cell. Endocr. 7: 233.

Bonney, R. C. and F. J. Cunningham. 1977b. The sensitizing effect of oestradiol on the response of the anterior pituitary gland of the domestic fowl to luteinizing hormone releasing hormone *in vitro* and *in vivo*. J. Endocr. 72: 16P.

Bonney, R. C., F. J. Cunningham, and B. J. A. Furr. 1974. Effect of synthetic luteinizing hormone releasing hormone on plasma luteinizing hormone in the female domestic fowl, *Gallus domesticus*. J. Endocr. 63: 539.

Brandt, J. W. A. and A. V. Nalbandov. 1956. Role of sex hormones in albumen secretion by the oviduct of chickens. Poultry Sci. 35: 692.

Breen, P. C. 1966. The fine structure of the secretory cells of the uterus (shell gland) of the chicken. Anat. Rec. 154: 321.

Brown, W. O. and H. G. Badman. 1961. The influence of estradiol on the composition and respiration of oviduct tissue in the immature chick. Endocr. 69: 275.

Burke, W. H. and E. A. Cogger. 1977. The effect of luteinizing hormone releasing hormone (L.H.R.H.) on serum L.H. and ovarian growth in turkeys. Poultry Sci. 56: 234.

Chan, V., R. N. Clayton, G. Know, and K. J. Catt. 1981. Ontogeny of pituitary GnRH receptors in the rat. Endocr. 108: 2086.

Clark, C. E. and R. M. Fraps. 1967. Induction of ovulation in the chicken with median eminence extracts. Poultry Sci. 46: 1245.

Clayton, R. N. and K. J. Catt. 1981. Regulation of pituitary gonadotropin-releasing hormone receptors by gonadal hormones. Endocr. 108: 887.

Clayton, R. N., J. P. Harwood, and K. J. Catt. 1979. Gonadotropin-releasing hormone analogue binds to luteal cells and inhibits progesterone production. Nature 282: 90.

Davidson, M. F., M. H. Draper, and E. M. Leonard. 1968. Structure and function of the oviduct of the laying hen. J. Physiol. 202: 2.

Davis, D. E. 1942. The bursting of avian follicles at the beginning of atresia. Anat. Rec. 82: 153.

de la Cruz, A., D. H. Coy, J. A. Vilchez-Martinez, A. Arimura, and A. V. Schally. 1976. Blockade of ovulation in rats by inhibitory analogs of luteinizing hormone-releasing hormone. Science 191: 195.

Ferland, L., B. Marchetti, C. Seguin, F. A. Lefebvre, J. J. Reeves and F. Labrie. 1981. Dissociated changes of pituitary luteinizing hormone releasing hormone (LHRH) receptors and responsiveness to the neurohormone induced by 17β-estradiol and LHRH in vivo in the rat. Endocr. 109: 87.

Flickinger, G. L. 1966. Response of the testes to social interaction among grouped chickens. Gen. Comp. Endocr. 6: 89.

Fraps, R. M. 1965. Twenty-four hour periodicity in the mechanism of pituitary gonadotropin release for follicular maturation and ovulation in the chicken. Endocr. 77:5.

Fraser, H. M. and P. J. Sharp. 1978. Prevention of positive feedback in the hen (Gallus domesticus) by antibodies to luteinizing hormone releasing hormone. J. Endocr. 76: 181.

Furr, B. J. A., R. C. Bonney, R. J. England, and R. J. Cunningham. 1973a. Luteinizing hormone and progesterone in peripheral blood during the ovulatory cycle of the hen, Gallus domesticus. J. Endocr. 57: 159.

Furr, B. J. A., G. I. Onuora, R. C. Bonney, and R. J. Cunningham. 1973b. The effect of synthetic hypothalamic releasing factors on plasma levels of luteinizing hormone in the cockerel. J. Endocr. 59: 495.

Gilbert, A. B. and S. O. Amin. 1969. Preliminary studies on the functional cytology of the anterior pituitary of the hen with the light and electron microscope. J. Reprod. Fert. 20: 363.

Gray, J. C. 1936. The anatomy of the male genital ducts in the fowl. J. Morph. 60: 393.

Harwood, J. P., R. N. Clayton, and K. J. Catt. 1980. Ovarian gonadotropin-releasing hormone receptors. I. Properties and inhibition of luteal cell function. Endocr. 107: 407.

Heber, D. and R. S. Swerdloff. 1981. Down regulation of pituitary gonadotropin secretion in postmenopausal females by continuous gonadotropin releasing hormone administration. J. Clin. Endocr. Metab. 52: 171.

Hiller, S. G., L. E. Reichert, Jr., and E. V. van Hall. 1981. Modulation of FSH-controlled steroidogenesis in the rat granulosa cells: direct in vitro effects of LHRH and ICI-118630. Mol. Cell. Endocr. 23: 193.

Hodges, R. D. 1974. The reproductive system. In "The Histology of the Fowl," Hodges, R. D., ed., Academic Press, New York, pp. 300-347.

Hsueh, A. J. W. and G. F. Erickson. 1979. Extra-pituitary inhibition of testicular function by luteinizing hormone releasing hormone. Nature 281: 66.

Hsueh, A. J. W., J. R. Schreiber, and G. F. Erickson. 1981. Inhibitory effect of gonadotropin releasing hormone upon cultured testicular cells. Mol. Cell. Endocr. 21: 43.

Hsueh, A. J. W., C. Wang, and G. F. Erickson. 1980. Direct inhibitory effect of gonadotropin-releasing hormone upon follicle stimulating hormone induction of luteinizing hormone receptor and aromatase activity in rat gramulosa cells. Endocr. 106: 1697.

Jackson, G. L. 1971. Avian luteinizing hormone-releasing factor. Endocr. 89: 1454.

Jackson, G. L. and A. V. Nalbandov. 1969. Luteinizing hormone releasing activity in the chicken hypothalamus. Endocr. 84: 1262.

Jeffcoate, S. L., P. J. Sharp, H. M. Fraser, D. T. Holland, and A. Gunn. 1974. Immunochemical and chromatographic similarity of rat, rabbit, chicken and synthetic luteinizing hormone releasing hormones. J. Endocr. 62: 85.

Johnston, H. S., R. N. C. Aitken, and G. M. Wyburn. 1963. The fine structure of the uterus of the domestic fowl. J. Anat. 97: 333.

Jones, G. E. and A. R. Boyns. 1973. Effect of sex steroids on the pituitary responsiveness to synthetic luteinizing hormone releasing hormone in the male dog. J. Endocr. 53: 15.

Jones, P. B. C. and A. J. W. Hsueh. 1981. Regulation of ovarian 20 alpha hydroxysteroid dehydrogenase by gonadotropin releasing hormone and its agonists in vitro and in vivo. J. Steroid Biochem. 14: 1169.

Judd, H. L., R. Rebar, G. Vandenberg, and S. S. C. Yen. 1974. Effect of luteinizing hormone-releasing factor on leydig cell function. J. Clin. Endocr. Metab. 39: 8.

King, R. D. and R. P. Millar. 1980. Comparative aspects of luteinizing hormone structure and function in vertebrate phylogeny. Endocr. 106: 707.

Kohler, P. O., P. M. Grimley, and B. W. O'Malley. 1969. Estrogen induced cytodifferentiation of the ovalbumin-secreting glands of the chick oviduct. J. Cell Biol. 40: 8.

Kumaran, J. D. S. and C. W. Turner. 1949. The normal development of the testes in the white plymouth rock. Poultry Sci. 28: 511.

Labrie, F., L. Cusan, C. Seguin, A. Belanger, G. Pelletier, J. Reeves, P. A. Kelly, A. Lemay, and J. P. Raynaud. 1980. Antifertility effects of LHRH agonists in the male rat and inhibition of testicular steroidogenesis in man. Int. J. Fert. 25: 157.

Lague, P. C., A. van Teinhoven, F. J. Cunningham. 1975. Concentrations of estrogens, progesterone and luteinizing hormone during the ovulatory cycle of the laying hen. Biol. Reprod. 12: 590.

Lake, P. E. 1957. The male reproductive tract of the fowl. J. Anatomy 91: 116.

Latimer, H. B. and M. F. Landwer. 1924. The relative volumes and the arrangement of the cortical and medullary cells of the suprarenal gland of the chicken. Anat. Rec. 29: 389.

Leonard, S. L. and J. W. Righter. 1936. Endocrine weights of the bantam fowl. J. Heredity 27: 363.

Luck, M. R. and C. G. Scanes. 1980. Ionic and endocrine factors influencing the secretion of luteinizing hormone by chicken anterior pututitary cells in vitro. Gen. Comp. Endocr. 41: 260.

Massicotte, J., R. Vielleux, M. Lavoie, and F. Labrie. 1980. An LHRH agonist inhibits FSH-induced cyclic AMP accumulation and steroidogenesis in porcine granulosa cells in culture. Biochem. Biophys. Res. Comm. 94: 1362.

Median, R., and Y. Koch. 1981. Variations in luteinizing hormone-releasing hormone receptor in pituitary cells from immature and mature cycling female rats. F.E.B.S. Letters: 132: 114.

Midgley, A. R., Jr. 1963. Immunofluorescent localization of human pituitary luteinizing hormone. Exp. Cell. Res. 32: 606.

Mikami, S., T. Jurosu, and D. S. Farner. 1975. Light and electron microscopic studies on the secretory cytology of the adenohypophysis of the Japanese quail Coturnix coturnix japonica. Cell. Tis. Res. 159: 147.

Nakane, P. K. 1970. Classifications of anterior pituitary cell types with immunoenzyme histochemistry. J. Histochem. Cytochem. 18: 9.

Nalbandov, A. V. 1976. The reproductive physiology of the hen. In "Reproductive Physiology of Mammals and Birds," Nalbandov, A. V., ed., W. H. Freeman and Company, San Francisco, pp. 143-155.

Navickis, R. J., B. S. Katzenellenbogen and A. V. Nalvandov. 1979. Effects of the sex steroid hormones and vitamin D_3 on calcium-binding proteins in the chick shell gland. Biol. Reprod. 21: 1153.

Oakberg, E. F. 1951. Genetic differences in quantitative histology of the adrenal, organ weights, and inter-organ correlations in white leghorn chickens. Growth 15: 57.

Oka, T. and R. T. Shimke. 1969. Interaction of estrogen and progesterone in chick oviduct development. J. Cell Biol. 41: 816.

Opel, H. 1979. The hypothalamus and reproduction in the female. Poultry Sci. 58: 1607.

Opel, H. and P. D. Lepore. 1972. In vitro studies of luteinizing hormone releasing factor in the chicken hypothalamus. Poultry Sci. 51: 1004.

Opel, H. and A. V. Nalbandov. 1961. Ovulability of ovarian follicles in the hypophysectomized hen. Endocr. 69: 1029.

Oshima, H., H. R. Nankin, D. Fan, T. Yanaihara, N. Niizato, K. Yoshida, and K. Ochiai. 1975. Delay in sexual maturation of rats caused by synthetic LH-releasing hormone: enhancement of steroid delta^4-5 alpha-hydrogenase in testes. Biol. Reprod. 12: 491.

Parker, J. E., F. F. McKenzie and H. L. Kempster. 1942. Development of the testes and combs of white leghorn and new hampshire cockerels. Poultry Sci. 21: 35.

Palkovits, M., A. Arimura, M. Brownstein, A. V. Schally, and J. M. Saavedra. 1974. Luteinizing hormone-releasing hormone (LH-RH) content of the hypothalamic nuclei in the rat. Endocr. 95: 554.

Peterson, A. J. and R. H. Common. 1972. Estrone and estradiol concentrations in peripheral plasma of laying hens as determined by radiommunoassay. Canad. J. Zool. 50: 395.

Ralph, C. L. and R. M. Fraps. 1960. Induction of ovulation in the hen by injection of progesterone into the brain. Endocr. 66: 269.

Reeves, J. J., P. C. Harrison, and J. M. Casey. 1973. Ovarian development and ovulation in hens treated with synthetic (porcine) luteinizing hormone releasing hormone/follicle stimulating hormone releasing hormone (LH-RH/FSH-RH). Poultry Sci. 52: 1883.

Reddy, P. R. K., H. P. van Drey, and B. P. Siegel. 1977. Ultrastructural observations of the adenohypophyseal gonadotrophs from growth selected lines of chickens. Poultry Sci. 56: 1237.

Richardson, K. C. 1935. The secretory phenomena in the oviduct of the fowl, including the process of shell formation examined by the microincineration technique. Phil. Trans. Roy. Soc. London (B) 225: 149.

Rivier, C., J. Rivier, and W. Vale. 1978. Chronic effects of (D-Trp^{6}-Pro^{9}-NEt) luteinizing hormone releasing factor on reproductive processes in the female rat. Endocr. 103: 2299.

Rivier, C., J. Rivier, and W. Vale. 1981. Effect of a potent GnRH agonist and testosterone propionate on mating behavior and fertility in the male rat. Endocr. 108: 1998.

Rothchild, I. and R. M. Fraps. 1945. The relation between ovulation frequency and the incidence of follicular atresia following surgical operations in the domestic hen. Endocr. 37: 415.

Rothchild, I. and R. M. Fraps. 1949. The interval between normal release of ovulating hormone and ovulation in the domestic hen. Endocr. 44: 134.

Sandow, I., W. Rechenberg, G. Jerzabek, K. Engelbart, H. Kuhl and H. Fraser. 1980. Hypothalamic-pituitary-testicular function in rats after supraphysiological doses of a highly active LRH analogue (Buserelin). Acta Endocr. 94: 489.

Seguin, C., L. Cusan, A. Belanger, P. A. Kelly, F. Labrie, and J. Raynaud. 1981. Additive inhibitory effects of treatment with LHRH agonists and antiandrogen on androgen-dependent tissues in the rat. Mol. Cell. Endocr. 21: 37.

Senior, B. E. and F. J. Cunningham. 1974. Oestradiol and luteinizing hormone during the ovulatory cycle of the hen. J. Endocr. 60: 201.

Shahabi, N. A., W. H. Norton, and A. V. Nalbandov. 1975. Steroid levels in follicles and in the plasma of hens during the ovulatory cycle. Endocr. 96: 962.

Sharp, P. J. and R. W. Lea. 1981. The response of the pituitary gland to luteinizing hormone releasing hormone in broody bantams. Gen. Comp. Endocr. 45: 131.

Sheenan, K. L., R. F. Casper, and S. S. C. Yen. 1982. Induction of luteolysis by luteinizing hormone releasing factor (LRF) agonist: sensitivity, reproducibility, and reversibility. Fert. Sterl. 37: 209.

Siviram, S. 1965. Structure and development of the adrenal gland of Gallus domesticus. Gen. Comp. Endocr. 43: 1021.

Sternberger, L. A. and J. P. Petrali. 1975. Quantitative immunocytochemistry of pituitary receptor for luteinizing hormone-releasing hormone. Cell Tis. Res. 162: 141.

Sturkie, P. D. 1976. In "Avian Physiology," Sturkie, P. D. ed., Academic Press, New York, pp. 290-344.

Tanabe, Y., T. Ogawa and T. Nakamura. 1981. The effect of short-term starvation on plasma LH, plasma estradiol and progesterone, and on pituitary response to LH-RH in the laying hen (Gallus domesticus). Gen. Comp. Endocr. 43: 392.

Tanaka, K. and M. Kamiyoshi. 1976. Induction of ovulation in hens by an analogue of LH-RH. J. Reprod. Fert. 46: 243.

Tanaka, K., M. Sakaida, and M. Kamiyoshi. 1974. Release of gonadotropins from the chicken pituitary by synthetic LH-RH/FSH-RH. Poultry Sci. 53: 72.

Tingari, M. D. 1971. On the structure of the epididymal region and the ductus deferens of the domestic fowl (Gallus domesticus). J. Anat. 109: 423.

van Teinhoven, A. and A. V. Schally. 1972. Mammalian LH-RH induces ovulation in the domestic fowl. Gen. Comp. Endocr. 19: 594.

Wada, M. 1975. Cell types in the adenohypophysis of the Japanese quail and effects of injection of luteinizing hormone-releasing hormone. Cell Tis. Res. 159: 167.

Wilkinson, M. and W. H. Moger. 1981. Development of a quotidian increase in pituitary responsiveness to GnRH in prepuberal female rats. J. Reprod. Fert. 62: 393.

Williams, J. B. and P. J. Sharp. 1978. Age-dependent changes in the hypothalamo-pituitary-ovarian axis of the laying hen. J. Reprod. Fert. 53: 141.

Wyburn, G. M., R. N. C. Aitken and H. S. Johnston. 1965. The ultrastructure of the zona radiata of the ovarian follicle of the domestic fowl. J. Anat. 99: 469.

Yu, J. Y. and R. R. Marquardt. 1973. Development, cellular growth and function of the avian oviduct. Biol. Reprod. 8: 283.

Yu, J. Y. and R. R. Marquardt. 1974. Hyperplasia and hypertrophy of the chicken (Gallus domesticus) oviduct during a reproductive cycle. Poultry Sci. 53: 1096.

VITA

Aleta Lynn Gordon was born in Memphis, Tennessee on October 18, 1959. She graduated from Kenton High School in 1977. In June 1977, she entered The University of Tennessee at Martin. While there she was elected to membership in Alpha Zeta national agricultural honor society, Block and Bridle national animal science organization, and Phi Kappa Phi national honor society. She received a Bachelor of Science degree in Agriculture, with a major in Animal Science, in June 1980. In September 1980 she entered the Graduate School of The University of Tennessee, Knoxville. She completed the requirements for the degree in August 1982 and was awarded a Master of Science degree with a major in Animal Science in December 1982. In September 1982 she entered The University of Tennessee, Knoxville, College of Veterinary Medicine and began working on a Doctor of Veterinary Medicine degree.