LOCALIZATION OF LUTEINIZING HORMONE-RELEASING HORMONE (LHRH) IN THE HYPOTHALAMUS OF FETAL BOVINE

THESIS

Presented to the Graduate Council of
Southwest Texas State University
In Partial Fulfillment of
The Requirements

For the Degree

Master of Education

By

Ivy DeSimone, B.S.

San Marcos, Texas
May, 2001

To Penny- the best friend a girl could ever have

4/12/1977-12/5/2000

ACKNOWLEDGEMENTS

Let me begin by expressing my appreciation to Dr. Hardin Rahe as my committee chair and advisor in throughout every aspect of graduate school. I also thank the members of my graduate committee Dr. Carl Igo and Dr. Michael Abel for continued direction and support on the writing of my thesis. My gratitude also goes out to the other professors of the Agriculture department, especially Dr. Aditi Angirasa and Dr. Lon Shell, who unselfishly offered infinitely helpful advice. Dr. Joe Koke and Dr. Dana Garcia will never be forgotten for their expert advice and use of their labs and equipment during experimentation.

Next, my sincere appreciation goes out to the H & B Packing Plant in Waco and the lab of Dr. Les Dees at Texas A&M for the collection of materials that made this project possible. Houston Livestock Show and Rodeo has my deepest gratitude for the assistantship that made it possible to commit my full devotion to this thesis project.

My biggest thanks to the undying support of my family and friends both near and far. Especially mom, my biggest advocate to get this degree for her sound urging, faith in my accomplishments and positive attitude. Kara Warner was my partner from the beginning and the only person who understood the ups and downs felt during this project. Thank you to Kara and Grace who gave support, knowledge and tons of help during the year. I owe you both a debt I could never repay.

To Matt Desjardin for inspiration to keep going and motivation to finish during the most demanding of times, you will forever have my heart. His strong spirit taught me the confidence to finish what I start and do it to the best of my ability. All of these people were integral links in what resulted as my thesis and deserve as much credit as the author.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

LOCALIZATION OF LUTEINIZING

HORMONE-RELEASING HORMONE (LHRH) IN THE

HYPOTHALAMUS OF FETAL BOVINE

By

IVY BETH DESIMONE, B.S.
Southwest Texas State University
May 2001

SUPERVISING PROFESSOR: C. HARDIN RAHE

Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) are mammalian gonadotropins released from the anterior pituitary by Gonadotropin Releasing Hormone (GnRH) in the hypothalamus. Various analogs of GnRH have been identified. Localization of these hormones is the first step in discovering the individual function of each analog. Immunohistochemistry and confocal microscopy were used to localize Luteinizing Hormone Releasing Hormone (LHRH), also known as mammalian GnRH, in the fetal bovine hypothalamus. This analog is believed to differentiate the pituitary primordia during embryonic development and initiate the release of LH throughout the life of the animal. Localization of LHRH bound to neuronal membranes began from embryonic day 100 of gestation through term. Spatially neurons were located throughout the hypothalamus. Specifically, LHRH neurons were identified in most of the tissue observed from the most anterior sections including the OVLT and preoptic, rostral hypothalamus, extending as far caudally as the medial mammillary nucleus and axons were observed in the median eminence and ventral to the third ventricle.

CHAPTER I.

INTRODUCTION

Follicle stimulating hormone (FSH) stimulates the development of follicles in female mammals whereas luteinizing hormone (LH) is responsible for ovulation. Testing various analogues of gonadotropin releasing hormone (GnRH), scientists developed a hypothesis that a single releasing hormone originating in the hypothalamus stimulates the secretion of both LH and FSH from the anterior pituitary gland. Luteinizing hormone releasing hormone (LHRH) is a decapeptide secreted from the hypothalamus that regulates the synthesis and release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) (Matsuo et al. 1971). McCann, et al. (1983) suggested the possibility of differential signaling hormones for LH and FSH at the hypothalamic level. Differential release was hypothesized because electrochemical stimulation of various areas of the hypothalamus elicited release of different hormones. The medial preoptic area only released LH, further caudal stimulation released equivalent amounts of LH and FSH, whereas dorsal anterior hypothalamic area only released FSH (MCCann, et al. 1983).

Luteinizing hormone releasing hormone (LHRH) is one several forms of GnRH (Sealfon et al. 1997). Several reports have indicated that LHRH is synthesized in many adult mammalian species and in the fetal hypothalamus of rats, mice, guinea pigs and humans. The precise role in the establishment of reproductive competency has yet to be determined (Aubert et al., 1985). Jennes (1985) and Aubert et al. (1990) reported that LHRH is first

identifiable by immunochemistry in fetal rats on day 15 and LH cells can be distinguished on embryonic day 16 and 17, respectively. Therefore, it was concluded that onset of fetal LH secretion is initiated by LHRH. It is highly debated if LHRH causes LH cell development, but the times during gestation of release is agreed upon by multiple sources.

LHRH has been identified in the hypothalamus of the adult bovine (Dees and McArthur, 1981 and Estes 1977), as well as in other livestock (Dees et al., 1981a and Dees et al., 1981b) using immunohistochemically and radioimmunoassay (RIA). In the adult bovine, LHRH was identified by RIA in the medial basal hypothalamus (Estes 1977) and by immunohistochemistry in the rostral to caudal (as far as the medial mammillary nucleus) hypothalamus (Dees and McArthur1981). However, LHRH has not been reported in the bovine fetal hypothalamus. Since most fetal work thus far has been done in rodents, this research will determine if LHRH is found in equivalent spatial and temporal patterns in the fetal bovine.

Although LHRH was identified decades ago, scientists have recently identified another GnRH that is capable of stimulating the release of FSH (identified as GnRH III Lamprey GnRH-III or Follicle stimulating hormone releasing factor (FSHRF)). Researchers have recently attempted to determine if FSHRF independently controls the release of FSH (Yu et al. 1997, Marubayashi et al. 1999). Unlike other mammals, gonadotropes in the bovine occur in separate LH- and FSH-producing cells. Thus, we believe that the bovine represents a model to study the independent regulation of the gonadotropins (Bastings et al. 1991). Therefore, LHRH must be identified in the hypothalamus of the fetal bovine hypothalamus to demonstrate its role in the reproductive process. Next, it will be necessary to determine if FSHRF is synthesized in the bovine hypothalamus. Finally, we will attempt to

determine both temporal and spatial patterns of LHRH and FSHRF synthesis in the fetal bovine hypothalamus.

Once identified during fetal development, we will be able to correlate the onset of LH and FSH production to the onset of LHRH and FSHRF production. Although the role of LHRH has been extensively studied in several laboratory animal species, limited information is available in cattle. Because studies have shown hormones are regulated differently in many species, it is prudent that we understand how LHRH is regulated in livestock. Once understood, biochemical compounds that mimic or inhibit the effects of LHRH can be synthesized as needed for production agriculture or medical science. The application of these hormones in livestock production would be to superovulate animals in a more natural and inexpensive way. In theory, it is not unreasonable to suspect that a more efficient and perhaps novel means of controlling multiple ovulations in livestock may emerge.

Statement of the Problem

The problem to be addressed is if LHRH can be localized by immunohistochemistry in the fetal bovine hypothalamus.

Goals and Objectives

In addition to the problem, this study also set out to find at what age in fetal bovine development could mammalian GnRH (LHRH) be localized in the hypothalamus by immunohistochemistry. The objectives of the study proposed here are to (i) localize LHRH in the fetal bovine hypothalamus and (ii) determine both temporal and spatial patterns of development of LHRH neurons.

CHAPTER II.

REVIEW OF LITERATURE

LHRH'S ROLE IN THE ENDOCRINE SYSTEM

Structure Related Function

Gonadotropin releasing hormone has been identified in multiple forms. Porcine LH and FSH releasing hormone was the original decapeptide sequenced. This became known as mammalian GnRH and is also referred to as LHRH (Matsuo et al. 1971). GnRH and LHRH are used interchangeably throughout the literature, as indicated by the title of a paper by Aubert et al. (1985) "Ontogeny of hypothalamic luteinizing hormone-releasing hormone (GnRH) and pituitary GnRH receptors in fetal and neonatal rats." The use of LHRH here is for clarification purposes since there are so many forms of GnRH. The second form of GnRH was discovered in chickens so it may be termed c-GnRH II (Miyamoto et al. 1982). It can also be written GnRH II to eliminate confusion since it is not only found in chickens, but also fish, amphibians, reptiles, birds and mammals (Sealfon et al. 1997). Other forms of GnRH are Chicken I (King and Millar 1982b), Seabream (Powell et al. 1994), Catfish (Ngamvongchon et al. 1992), Salmon (Sherwood et al. 1983), Dogfish (Sherwood et al. 1985), Lamprey I (Sherwood et al. 1986), Lamprey III (Sower et al. 1993), Tunicate I, and Tunicate II (Powell et al. 1996). Different types of GnRH are named for the animal the hormone was discovered in and the resemblance of the amino acid sequence to the original mammalian form (Sealfon et al. 1997).

	1	2	3	4	5	6	7	8	9	10	
Mammal	pGlu	His	Trp	Ser	Tyr	Gly	Leu	Arg	Pro	Gly	NH_2
Chicken I	pGlu	His	Trp	Ser	Tyr	Gly	Leu	Gln	Pro	Gly	NH_2
Seabream	pGlu	His	Trp	Ser	Tyr	Gly	Leu	Ser	Pro	Gly	NH_2
Salmon	pGlu	His	Trp	Ser	Tyr	Gly	Trp	Leu	Pro	Gly	NH_2
Catfish	pGlu	His	Trp	Ser	His	Gly	Leu	Asn	Pro	Gly	NH_2
Chicken II	pGlu	His	Trp	Ser	His	Gly	Trp	Tyr	Pro	Gly	NH_2
Dogfish	pGlu	His	Trp	Ser	His	Gly	Trp	Leu	Pro	Gly	NH_2
Lamprey I	pGlu	His	Tyr	Ser	Leu	Glu	Trp	Lys	Pro	Gly	NH_2
Lamprey III	pGlu	His	Trp	Ser	His	Asp	Trp	Lys	Pro	Gly	NH_2
Tunicate I	pGlu	His	Trp	Ser	Asp	Tyr	Phe	Lys	Pro	Gly	NH_2
Tunicate II	pGlu	His	Trp	Ser	Leu	Cys	His	Ala	Pro	Gly	NH_2

Table 2.1 Amino Acid structure of GnRH analogs. Major differences lie in the 5-8 position. Amino acids different from mammalian GnRH are highlighted in red.

Most vertebrate species have two or more analogs of which act differently on the endocrine system (Powell et al. 1994). The reason for their difference in behavior is due to the different amino acid structure. All forms of GnRH start with an NH_2-terminal domain of pGlu-His-Trp-Ser and end with a COOH-terminal domain of Pro-Gly-NH_2. While the NH_2-terminal domain and COOH-terminal domain tolerate very few substitutions, the central part of the chain has numerous substitutions and retains activity. The differences lie in the 5-8 position. Examples include mammalian (m) GnRH has Tyr-Gly-Leu-Arg in the 5-8 position (Matsuo et al. 1971), chicken (c) GnRH II has His-Gly-Trp-Try (King and Millar 1982a) and

Lamprey (l) GnRH III has His-Asp-Trp-Lys (Sower et al. 1993). Mammalian GnRH is the only form found to date with Arg in position 8.The highly active binding affinity in mammals to this LHRH form is attributed to Arg^8 because other GnRH's with a different amino acid in this position have shown poor binding affinity. The only exception is when a neutral amino acid is substituted for Arg, His is in position 5 and Trp is in position 7. Although His in the five position retains high receptor potency, LH release is reduced. The only two forms of GnRH with this sequence are lamprey and chicken which are currently being researched to prove the affinity for these forms by the mammalian pituitary receptors (Millar et al. 1989). Non-mammalian receptors have different requirements in the central domain from mammalian species (Sealfon et al.1997).

Hypothalamic Releasing Factor

Generally it is believed that the hypothalamus plays an important role in controlling gonadotropin release from the anterior pituitary. Sakar (1983) reports a hormone is a hypothalamic releasing factor when it is extractable from hypothalamic or stalk-median eminence tissue, resides in the pituitary portal blood in greater amounts than in systemic blood, and varying concentrations in portal vessel blood are related to varying secretion rates of one of the AP hormones. Schally (1978) began his research to isolate and identify LHRH by extracting material from beef and pig hypothalami that stimulated in vitro and in vivo release of LH, satisfying Sakar's first criteria. Sakar reports evidence to satisfy criteria two that LHRH is present in greater amounts in the pituitary portal blood than in systemic blood. The third criteria was fulfilled by Ching (1982) who revealed that LH surges in the systemic

blood of Sprague-Dawley rats during proestrus resulted from LHRH induced stimulation of the pituitary.

From his review, Sakar (1983) deduced events that lead to preovulatory gonadotropin release. Before the beginning of estrus, ovary estradiol secretion increases. Either directly or through stimulation of the facilitatory noradrenergic and incertohypothalamic dopaminergic systems, estradiol activates the LHRH neuronal system (Sakar 1983). Yu, Walczewska et al. (1997) studied the chain reaction that dopamine begins acting on Nitric Oxide which in turn activates leptin. Leptin is released by adipose tissue and travels through the blood to the hypothalamus then the pituitary. It has been shown to stimulate or inhibit the release of LHRH and LH. This is a type of regulator that only allows the animal to cycle if there is not too much or too little fat to inhibit proper fetal growth (Yu, Kimura et al. 1997). Once pulsatile LHRH is allowed to be secreted, the pituitary is primed for gonadotropin release. The result of the peak LHRH output is a massive release of gonadotropins and estrus is begun.

LH causes a rupture of the follicle to release the egg and in its place develops a corpus luteum that produces progesterone. When progesterone rises to a threshold level, the TIDA system is activated and LHRH release is inhibited. Sakar concludes the TIDA system inhibits LHRH by the activation of non-adenylate cyclase dependent dopaminergic receptors. The cycle is begun again when FSH promotes a new Graffian follicle to mature and the ovary produces estradiol in amounts to alert the LHRH neural signal to start again (Sakar 1983).

Role in Embryogenesis

In addition to causing the release of gonadotropins, Aubert et al. (1985) and Jennes (1990) studied the role of LHRH has in embryogenesis. Both studies conclude that LHRH initiates the release of LH at embryonic day 16 or17 but only Aubert believes it also differentiates the pituitary primordia. Jennes (1990) reported that GnRH (LHRH) must be present before first gonadotropin expression, LHRH must be able to reach the developing pituitary, and LHRH must bind to specific membrane receptors on the developing pituitary cells in order to prove LHRH is involved in differentiation of fetal gonadotropins. Florsheim and Rudko (1968) identified the hypothalamo-pituitary portal system for transport of blood and hormones from the hypothalamus to the pituitary. Aubert (1985) identified receptors on the fetal pituitary at embryonic day 15 satisfying the third criteria. The first criteria that LHRH is present before first gonadotropin expression is a conflicting argument leading to a difference of opinion on the exact role of LHRH in embryogenesis.

Aubert et al. (1985) report that at 10-12 days of gestation LHRH causes differentiation of the pituitary primordia. Schwanzel-Fukuda and Pfaff (1989) studied fetal tissue before day 12 and concluded that LHRH begins to be produced on day E12. Aubert et al. (1985) designed a study to prove that differentiation depended on LHRH by removing tissue that produced LHRH before embryonic day 13. No differentiation would occur when tissues were removed unless LHRH was added to the incubation medium. Jennes (1990) study concluded gonadotropes are differentiated much earlier since GnRH receptors are present on pituitary cells by day E13 and his research showed that LHRH is not first identifiable by immunohistochemistry until day E15. LHRH can not differentiate primordial pituitary cells because specific actions of LHRH are taking place in the pituitary receptors that would mean that the cells have already differentiated. Jennes suggests the possibility that

LHRH from the placenta can cross to the developing fetus and initiate the differentiation of gonadotropins since RIA identified LHRH is in the amnion fluid from day E12 to E 18. Aubert et al. (1985) found no evidence of LHRH in rat placenta.

Horacek et al. (1989) provide another explanation for the differentiation role of LHRH. In order to clear up the question as to whether LHRH differentiated gonadotrophs they studied the effects of LHRH in hamster differentiation. They studied the effect of LHRH on LH and FSH cells because they believed the fault with previous studies was that they mostly studied LH cell development. Their results were that LHRH not only played a significant role in the appearance of gonadotrophs containing FSH but also maintenance of FSH gonadotrophs. Curiously, there was no evidence of LHRH causing the differentiation of LH gonadotrophs. Their conclusion was that the vascular system connecting the hypothalamus to the developing pituitary is also in a stage of development while the pituitary is differentiating, satisfying Jennes's criteria that LHRH must be able to reach the developed pituitary is not being met. LHRH is only reaching the pituitary in small amounts during the stage of LH cell development so there is not a dependence on it for development, but the induction of FSH requires greater exposure to LHRH. This explains why FSH cells are developed later when the pituitary portal vessel is developed and can carry larger amounts of LHRH to the pituitary. Based on the theory that some cells contain FSH and LH releasing capabilities, Horacek et al. (1989) postulate that LHRH is responsible for differentiating these cells and causing FSH to be produced. It will be interesting to see if the new development of a FSHRF will clarify this physiology during fetal development.

Medical Uses

In ruminant livestock species that are monotocous, there is an interest in superovulating to preserve excellent genetics in a species. Like humans, monotocous livestock rarely ovulate more then 1 to 3 oocytes per cycle. Campbell et al. (1999) studied the effects of varying amounts of FSH and LH to learn what selects a follicle to rupture. FSH causes the development of 3 to 4 follicles each cycle and LH is the mechanism that selects the follicle to rupture in a mono-ovulatory species. Once FSH levels are increased for multiple follicles to mature, LH has no effect on the ovulation rate and superovulation is achieved (Campbell et al. 1999). Seminara et al. (2000) demonstrate that using LHRH increases both hormones together.

Seminara et al. (2000) had success causing normal ovulation in a patient karyotyped 46XX (Kallmann syndrome) defined by absence of spontaneous puberty by age 18, absence of pulsatile gonadotropin secretion, and no evidence of a mass lesion on brain imaging of the hypothalamic-pituitary region. This patient required doses much higher then other patients had tolerated before they exhibited superovulation or multiple gestation. At normal levels given, there was no response. A slightly higher dose supported follicle development but the luteal phase was irregular. Finally at a third dose of 250 ng/kg, the gonadotropin and sex steriod dynamics of the patient recapitulated those of normal ovulatory women. This patient developed a single dominant follicle in each cycle and conceived three times. This demonstrates the critical sensitivity of the corpus luteum to LHRH-induced gonadotropin secretion.

(Seminara et al 2000) acknowledge the risk of hyperstimulation and multiple gestation that Campbell et al. (1999) reported. They believe pulsatile GnRH therapy is a safe and effective therapeutic option for some patients with HH and GnRH receptor mutations of

$Gln^{106}Arg$ substitution in the first extracellular loop, decreasing hormone binding and $Arg^{262}Gln$ mutation impairing signal transduction (Seminara et a. 2000). As far as livestock species with normal cycles or humans with other forms of HH these hormones would most likely cause superovulation or multiple gestation as shown by Campbell et al. (1999). Other medical uses that have been around longer include differentiating pituitary and hypothalamic causes of hypogonadism, treatment for cryptorchidism, therapeutic induction of ovuluation in amenorrheic women and a possible male contraceptive by an inhibitory analog of LHRH (Schally, 1978).

LHRH (GnRH) LOCALIZATION

Historical Overview

Two studies in LHRH localization were done at about the same time in the mouse (Gross, 1976) and guinea pig (Silverman, 1976). Although, Gross reports distribution of GnRH and Silverman LHRH. Both conducted their studies because there was ambiguity in the findings of previous research conducted on mice, guinea pigs and rat. Using the same immunohistochemical technique labeling the sections with Sternberger et al. (1970) PAP complex results were complimentary. While both found the majority of staining concentrated in the medial portion of the post-infundibular median eminence (ME), Silverman points out the guinea pig's reproductive functions are more likely to model the monkey rather then rodents. Gross distinguishes differences between rats and mice. Therefore, each species must be studied to accurately localize the hormone in a particular species.

The next year results were published on primate species (Silverman et al. 1977). Silverman was the first to report immunohistochemical results in rhesus monkey's and did so

because wider distributions of LHRH was being found in guinea pigs, rabbits and other monkey species when compared to results of rats and mice. Once again using immunohistochemical techniques, he found reactive cell bodies concentrated in the anterior portion of the hypothalamus and in the medial pre-optic area. Another area of concentration was the infundibular nucleus and some neurons were present in smaller amounts in many other parts of the hypothalamus. These results of cell bodies from the preoptic area to the mammillary body complex are in compliance with the guinea pig's distribution. He goes on in his discussion to point out differences within different species of primates, concluding that vast differences occur among and between species of animals (Silverman et al. 1977).

Localization Differences Per Livestock Species

LHRH localization differences occur even among livestock species. Comparisons were made to other species in explaining the results of GnRH localization in the equine brain and infundibulum (INF), (Dees et al. 1981a). In some cases there are similarities. Cases such as the pony and sheep (Dees et al. 1981b) both contain GnRH primarily in the external layer of the rostral INF. The mouse (Gross 1976), sheep (Dees 1981b), rat (Gross 1980), and guinea pig (Silverman 1976) all showed an aggregation of the hormone bilaterally, adjacent to the tuberoinfundibular sulcus (TIS). The differences are in whether the hormone acculumates dorsal or ventral to the TIS in the intermediate INF. In the pony, the greatest amount of immunoreactive deposits lies both ventral and medial to the TIS. In the sheep (Dees 1981b) the hormone was concentrated almost entirely at the ventral portion of the TIS. The mouse (Gross 1976) localized most of the dorsal rather then ventral to the TIS.

Adult Bovine Localization

Since reports in sheep (Dees et al. 1981b), horses (Dees et al. 1981a), guinea pigs (Silverman, 1976), mice (Gross, 1976), rats (Gross 1980) and the rhesus monkey (Silverman et al. 1977) indicate species differences in precise LHRH localization in the INF and around the TIS of a variety of animals, the most important localization information should come from the cow, albeit the adult. Estes (1977) used radioimmunoassay (RIA) to localize gonadotropin releasing hormone (GnRH) within the bovine because there was little localization research beyond laboratory rodents and because of the availability and size of the bovine hypothalamus. Dees and McArthur (1981) were the first to study distribution of GnRH within the brain and infundibulum (INF) of adult mixed-breed beef cows by immunohistochemistry at the light micropscopic level. Both studies were conducted on mature cows and the RIA study included steers and a bull. The RIA results were reported by the concentration amount and distance from the pituitary stalk. They suggested that GnRH in the bovine hypothalamus is confined to the pituitary stalk and the rostral hypothalamic area anterior to the median eminence (Estes 1977). Immunohistochemistry found additional staining in the middle region of the hypothalamus and as far caudally as the medial mammillary nucleus (Dees and McArthur 1981). The immunohistochemical results more closely relate to results of other species like the guinea pig (Silverman 1976) and monkey (Silverman 1977). Kozlowski and Nilaver (1983) report that immunocytochemistry (ICC) is a more sensitive technique then RIA and may account for the differences.

Bovine Immunohistochemistry Methods

With that in mind, the methods and results reported by Dees and McArthur (1981) will be followed more closely while working with fetal cows. Methodology includes collecting brain tissue that has been pre-fixed with 10% phosphate-buffered formalin and Bouin's solution. Next tissue was divided into rostral, middle and caudal thirds, post fixed, cut into 10μm sections, mounted on glass slides and stained using the peroxidase-antiperoxidase technique described by Sternberger et al. (1970). Primary antibody was rabbit LHRH antiserum used at 1:700 to 1:1000 dilution rates for 24-48 hours at 4 degrees Celcius. Next, incubation with sheep anti-rabbit globulin serum used at a 1:200 dilution for 30 minutes at room temperature, rabbit PAP complex used at a 1:200 dilution for 30 minutes at room temperature and 0.05% 3.3' diaminobenzidine with 0.003% hydrogen peroxide for 5 to 10 minutes at room temperature. All steps were done on an automatic shaker and slides were washed between steps with Tris Buffer, pH 7.6. Control was replacing the primary antiserum, sheep anti-rabbit globulin serum, or the PAP complex with normal rabbit serum or Tris buffer.

Fetal Localization

Early in embryonic development neurons may not be found where they were localized in the adult brain. Since localization has not been done in the fetal bovine, the work in fetal mice and rats is the only indicator of the location of LHRH neurons. Schwanzel-Fukuda and Pfaff (1988) conducted LHRH localization research in fetal mice to find the migratory route for LHRH-expressing neurons. They were searching for the cause of Kallmann's syndrome (hypogonadotropic hypogonadism anosmia) and thought it was related to the lack of proper migration of the LHRH neurons from the origin to their destination.

They concluded failure of axonal and LHRH neuronal migration from the olfactory placode would explain anosmia and syndromes that involve gonadotropic deficiencies.

LHRH neurons began to be seen on embryonic day 11 and 12 (E 11 and E 12) in the placode which includes the epithelia of the olfactory placode, the olfactory pit and the analog of the vomeronasal organ. Stained cells were oval or fusiform in shape. No LHRH immunoreactive cells were observed in the forebrain bridging between the nasal cavity and forebrain at this early stage. It is not until day E 16 the neurons were identified where they would be seen through day E 20 and post-natally. This included the arch (central roots of the nervus terminalis), anterior olfactory nucleus, preoptic area, caudal preoptic area, and anterior hypothalamus (Schwanzel-Fukuda and Pfaff 1988).

Findings of Schwanzel-Fukuda and Pfaff (1988) were duplicated by Yoshida et al. (1995) who took the experiment one step further in rats and searched for the guide for migrating LHRH neurons. Yoshida et al. investigated the role of the developing vomeronasal nerve as the guide for migrating LHRH neurons because they found the vomeronasal nerve started and ended where the LHRH neurons were found to be migrating. Fluorescent, lipophilic dye was used to label the vomeronasal nerve (VNN) fibers and trace their migration. The vomeronasal nerve can be divided immunohistochemically into four spatially distinct subpopulations of fibers that selectively express different staining.

This study began with embryonic day 14 and found all four subpopulations start as several large fascicles that converge as they enter the region of the cribriform plate. The dorsal branch terminates in another area of the brain not concerned with the LHRH neurons. The caudal branch divides into CC6 terminating in the olfactory bulb and TAG-1(transient axonal surface glycoprotein), PSA-N-CAM (polysialated form of neural cell adhesion

molecule) which extends to the basal forebrain. Double labeling of LHRH neurons and caudally directed VNN fibers showed that LHRH neurons were consistently in contact with the caudal branches of the VNN. This migratory route across the nasal septum to the olfactory bulbs arching into the septal-preoptic area of the brain was in accordance with Schwanzel-Fukuda and Pfaff's 1988 report.

SYNTHESIS OF PREVIOUS RESEARCH

Review of the literature indicated LHRH is one of many forms of GnRH (Sealfon et al. 1997) and functions as a hypothalamic releasing factor (Sakar 1983). The cycle is begun with a threshold amount of estradiol from the follicle on the ovary activating LHRH to lead to rupture of the follicle (Sakar 1983). Nitric oxide and leptin act as regulators on LHRH and LH allowing the cycle to continue if the body is in the right condition (Yu, Walczewski et al. 1997 and Yu, Kimura et al. 1997). Once LH is released, the job of LHRH is over until the cycle starts again.

The more debatable role of LHRH is its affect on embryogenesis. LHRH undeniably intiates release of LH at embryonic day 16 in the rodent (Aubert et al. 1985 and Jennes 1990). Aubert et al. (1985) believe LHRH differentiates the pituitary primordia, Jennes (1990) believes pituitary cells are differentiated prior to LHRH presentation and Horacek et al. (1989) reported LHRH only differentiates cells that release FSH and not cells that release LH. As studies are conducted in the multiple forms of GnRH, this issue is sure to be resolved, as well as the issue of using LHRH as a viable means for inducing multiple ovulations in monotocous species (Campbell et al. 1999) and causing normal cycles in hypogonadotropic humans (Seminara et al. 2000).

LHRH has been localized in several adult animals (Gross 1976 and 1980, Silverman 1976 and 1977, Dees et al. 1981a and 1981b) including the bovine (Dees and McArthur 1981 and Estes 1977). Despite differences in precise LHRH localization, LHRH has been localized anterior to the median eminence with nerve fibers extending through the medial basal hypothalamus (Estes 1977 and Sétáló et al 1976) and as far caudally as the mammillary body (Dees and McArthur 1981). The present study will concentrate on searching the hypothalamus for time of gestation when LHRH can be localized. Migration of LHRH from the nasal septum and vomeronasal organ reported in mice (Schwanzel-Fukada and Pfaff, 1988), rats (Yoshida et al. 1995), guinea pigs (Schwanzel-Fukuda et al.1981) and humans (Kjaer and Fischer-Hansen 1996) will require a more extensive study. Fetal LHRH migration research will provide framework of when and where in the fetal bovine the current study will focus the search for LHRH.

Rodent LHRH production begins at the start of the third trimester (E11 mouse, E12 rat and E25 guinea pig) of a short gestation when compared to the 9 month gestation shared by humans and cows (Schwanzel-Fukuda and Pfaff 1988, Yoshida et al. 1995, and Schwanzel-Fukuda et al. 1981). Human embryology studies located LHRH beginning at 12 weeks or during the first trimester (Kjaer and Fischer-Hansen 1996). While it takes only a few days for LHRH in the rodent to travel to the hypothalamus, it takes 5 weeks in the human. This leads to the hypothesis that LHRH will be in the hypothalamus during the 2nd (25-45 cm fetal length) and 3rd (50-80 cm fetal length) trimester of the cow gestation and possibly in the last stages of the first trimester (6-20 cm fetal length) as well (Sakumato et al. 2000). The same Kelsh antibody used by Dees and McArthur (1981) and Gross (1976 and

1980) will be used along with a similar immunohistochemical protocol (Sternberger et al. 1970).

CHAPTER III.

MATERIALS AND METHODS

Tissue Preparation

Fetal calves were collected from H & B Packing Plant in Waco, Texas, soon after the cow was eviscerated. Information was recorded on the calf length based on crown to rump length. The blood was drained from the calf intracardially; then its head removed. In order to remove the brain, the dermal layers were transected to expose the cranium. An autopsy saw was used to cut across the frontal bone, laterally along the squamosal sutures and posterally at the lambdoidal suture. During the removal of the brain the olfactory and optic nerves were severed with special care taken to keep the optic chiasma intact. The brain stem was transected in the medulla oblongata region and the brain pulled free from the cranium cavity. The brain was placed intact, dorsal side down, in a dish with sterile phosphate buffered saline (PBS). Cerebral tissue was cut away anterior to the organum vasculosum of the lamina terminalis (OVLT), posterior to the mammillary bodies and the portion of the cerebrum lateral to the diencephalon. See figure 3.1.

The tissue retained for sectioning was further divided into rostral, intermediate, and caudal portions, including the preoptic region and OVLT, optic chiasma, and post diencephalon regions, respectively. See figure 3.1. The optic chiasma served as a marker the mid-diencephalon region. The most caudal section contained the mammillary bodies.

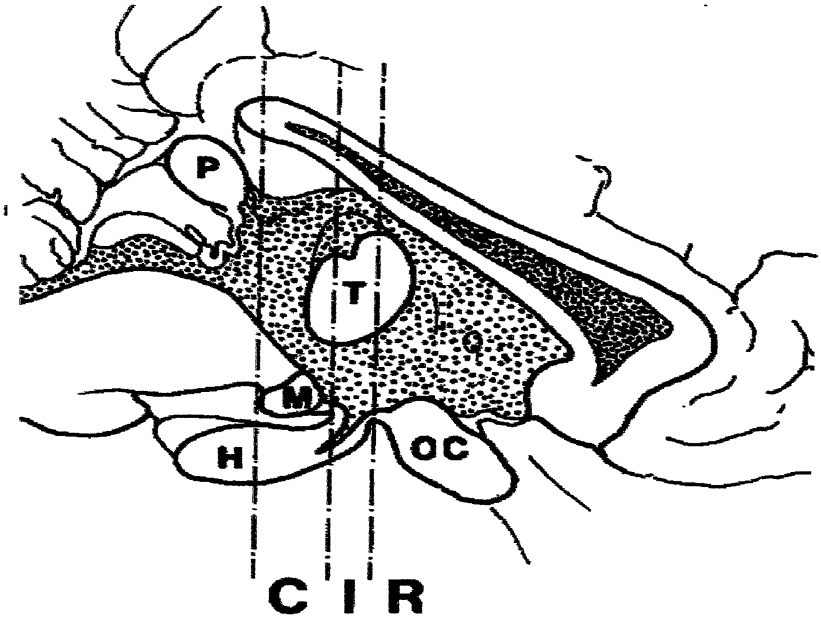

Figure 3.1 Cross-sectional drawing of the brain showing the division of the hypothalamus into rostral (R), intermediate (I), and caudal (C). *P* pineal; *T* thalamus; *M* mammillary body; *OC* optic chiasm. Reprinted from Dees et al. (1981b).

Tissue sections were rapidly fast frozen for one minute in a beaker of hexane emersed in dry ice slurry with 95% ethanol. Tissues removed from the hexane were placed in plastic collection bags and labeled for fetus and section number. Tissue was stored at –80° C. Twenty-four to 48 hours prior to cryosectioning the brain was transferred to a -25° C freezer to bring the tissue temperature to that equal that of the Micron cryotome.

Age identification

Before decapitation of the fetus, a crown to rump measurement was taken in inches with a metal measuring tape. Crown to rump length approximated the fetal age, sorted by trimester, with some variation due to breed size, nutrition and other factors (Sakumato 2000). The first trimester included calves from 16-20cm, the second trimester ranged from 25-45cm and the final trimester fetuses were larger than 50cm. (See table 3.1).

Trimester	Days of Gestation	Crown to Rump Length (in)	Crown to Rump Length (cm)
1st	≤ 90	≤ 5-7	≤ 16-20
2nd	110-120	9-11	25-28
	150-170	12-17	30-45
3rd	180	20-24	50-60
	210	24-32	60-80
	240-270	28-38	71-96

Table 3.1 Age identification from crown to rump measurement. Sakumato et al. (2000).

Cryosectioning and Immunohistochemistry

Tissue was embedded in Tissue Tek (OCT) and frozen onto a cryotome chuck at -25° C for 30 minutes to 60 minutes. Tissue was sectioned at 35-50μm. Every twentieth section was collected on collagen coated coverslips and adjacent sections were kept for negative controls. Tissue was allowed to dry overnight at 4° C. then rehydrated the next day with three washes in PBST (buffer with tween added for lysing the cell membranes). A protein blocker of 10% nonfat milk was added for 15 minutes. After rinsing off the protein blocker with three PBST washes, primary antibody was added for 24 hours at 4° C or two hours at room temperature. Kelch anti-LHRH was used at a 1:500 dilution rate and controls were treated with normal rabbit serum at the same dilution or PBST for the same amount of time. After another rinse with PBST, the secondary of goat anti-rabbit IgG tagged conjugated to Texas red tetramethylrodamine isothyocyanate (TRITC) was added for two hours at room temperature. TRITC is a light sensitive fluor and tissues were treated in light safe conditions. A second control using PBST instead of the secondary was administered. The last rinse was

in PBS, no Tween, and the tissue is set in distilled water until mounting on a slide with a drop of glycerol KF. All reagents were used in 150µl per coverslip increments, except the first rinse after dehydration sometimes required double on larger tissue sections to adequately hydrate the whole section.

Slides were examined with an Olympus IX-70 inverted confocal microscope. Images were collected using BioRad MRC 1024 Scan Head and software.

CHAPTER IV.

RESULTS/ANALYSIS OF DATA

As identified in Figure 3.2, sections were labeled rostral, intermediate and caudal. Rostral refers to areas including the preoptic area, supraoptic, anterior hypothalamus and paraventricular nucleus. Intermediate sections included the median eminence and areas around the third ventricle such as ventromedial nucleus and dorsomedial nucleus. The caudal sections included mammillary bodies and posterior hypothalamus.

Statistical analysis is not necessary for this particular study. Results were collected based on two criteria. First, whether or not the tissue stained for LHRH. Once the neurons have been identified, the location in the hypothalamus was identified. Conventional statistical analysis was inappropriate because the error variance was not equally distributed across treatments.

RESULTS by GESTATIONAL AGE

Third trimester

Fetuses collected from the third trimester of gestation ranged from 200 days (24" CRL) to 260 days (35" CRL). All specimens showed similar distribution of LHRH neurons. Rostrally axons were identified in the septal-preoptic area, OVLT and rostral hypothalamus. Larger numbers of axons were contained throughout the sections of the ventromedial hypothalamus and dorsalmedial nucleus, with occasional staining of bipolar neurons.

Caudally axons were scattered throughout the medial mammillary nucleus and caudal region of the hypothalamus.

Second trimester

Distributions of LHRH neurons in the fetuses from the second trimester of gestation were mostly the same as third trimester fetuses. Ages of the second trimester fetuses ranged from 150 days (13" CRL) to 180 days (21" CRL). The smallest 13" CRL fetus did not have rostral staining before the paraventricular nucleus as strong as in the other second trimester fetuses. Similar to the third trimester fetuses, the older second trimester fetuses had rostral staining in the preoptic area, supraoptic area and anterior hypothalamus. Intermediate and caudal staining of all second trimester fetuses matched that of the third trimester with axons extending from the middle region of the hypothalamus, lateral hypothalamic area and back to the mammillary bodies. (See figure 4.1).

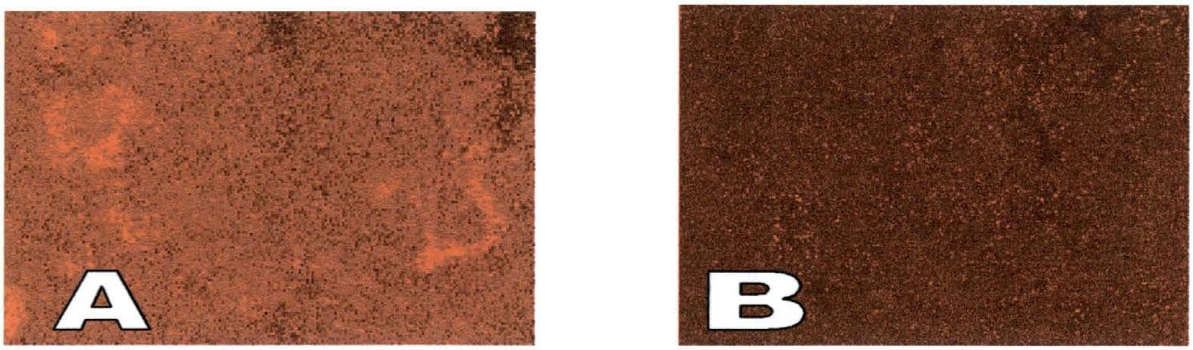

Figure 4.1. Brain tissue from a Second trimester fetus approximately 150 embryonic days.
A) Positive staining with Texas Red of two LHRH cells. Image located in the caudal region, ventral to the mammillary nucleus.
B) Adjacent Control section of tissue stained with Normal Rabbit Serum instead of primary antibody.

First Trimester

First trimester fetuses were under 100 days of gestation (<12" CRL). Landmarks in the brain became very difficult to distinguish at this young age. In the sections of the youngest fetus (8" CRL), there was no identifiable staining of LHRH neurons. Neurons were also difficult to identify in the 11" CRL fetus. There were a few axons identified in the rostral hypothalamic and caudal hypothalamic areas, anterior to the 3rd ventricle and lateral to the caudal parts of the 3rd ventricle. The oldest in this group showed the range of staining similar to the second and third trimester fetuses but not as abundantly distributed. Axons were identified from the preoptic area, OVLT and anterior hypothalamus through the ventromedial nucleus and dorsomedial nucleus to the posterior hypothalamus and medial mammillary nucleus. (See figure 4.2).

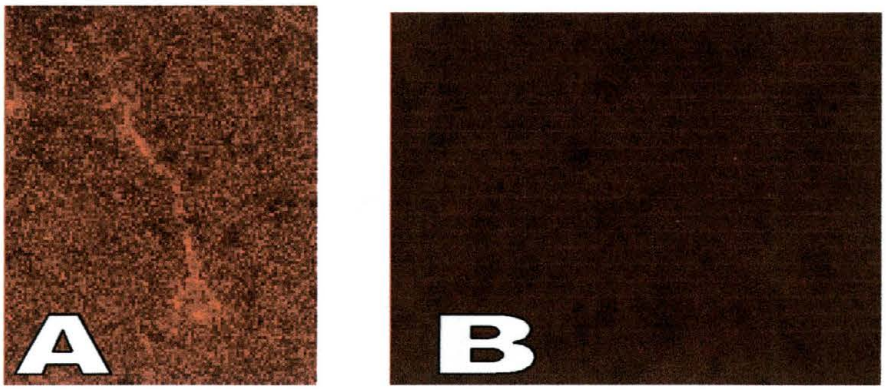

Figure 4.2. Tissue from a First Trimester Fetus approximately 120 embryonic days.
A) LHRH stained axon located in the posterior hypothalamus.
B) Adjacent section of Control tissue treated with PBS instead of primary antibody.

CHAPTER V.

DISCUSSION/RECOMMENDATIONS

Localization of LHRH in the fetal bovine brain was similar to that described by Dees and McArthur (1981) in the adult bovine brain. LHRH neurons were identified in most of the tissue observed from the most anterior sections including the OVLT and preoptic, rostral hypothalamus and extending as far caudally as the medial mammillary nucleus. This range of LHRH-staining neurons has also been reported in the equine (Dees et al. 1981a), ovine (Dees et al. 1981b), and rhesus monkey (Silverman et al. 1977). While localization in the anterior hypothalamus of the mouse (Gross 1976) and rat (Sétáló et al. 1976) was similar to the results of this study, axons have not been found as far caudally as the mammillary bodies. Axons in the median eminence and ventral to the third ventricle have been observed in all species mentioned above studied by immunohistochemistry including the fetal bovine.

Studies to determine when LHRH begins to be produced were completed in mice (Schwanzel-Fukuda and Pfaff 1988), rats (Aubert et al. 1985, Yoshida et al. 1995 and Jennes 1990), guinea pigs (Schwanzel-Fukuda et al. 1981) and humans (Kjaer and Hansen 1996). During the short gestation of the rodent, it was reported that LHRH begins to be localized on day E11 in the epithelia of the olfactory placode and takes one week to migrate across the nasal septum to the septal-preoptic area and anterior hypothalamus. This is completed in the final trimester of gestation. The fetal bovine has a much longer gestation and we hypothesized migration to the hypothalamus would be completed much earlier in gestation. This was proven when LHRH was localized in the septal-preoptic area and hypothalamus by

100 days of gestation, or the end of the first trimester. Humans have the same gestation as the bovine and our results agree with results of human LHRH localization (Kjaer and Hansen 1996). LHRH activity before 100 days is appeared to be migrating along the nasal septum and via the olfactory bulb to the hypothalamus. In their study of the vomeronasal organs, Kjaer and Hansen (1996) showed migration took four weeks and then around 100 days they no longer observed any immunoreactive LHRH. Assuming that at this time it has reached the hypothalamus and that is when we began to find it there in the bovine brain.

Recommendation for further studies to be conducted should determine if LHRH neurons follow the same migration pattern as found in the mouse (Schwanzel-Fukuda and Pfaff 1988), guinea pig (Schwanzel-Fukuda et al. 1981), rat (Yoshida et al. 1995) and human (Kjaer and Hansen 1996). Because the objectives of this study were to determine when LHRH neurons first appeared in the hypothalamus, younger fetuses were not studied. Before immunohistochemical study on bovine fetuses under 90 days can be conducted, a method of making the tissue less fragile will have to be found. Retrieving our specimens from the slaughterhouse prevented the tissue from being perfused with aqueous fixatives. Without fixatives, the tissues were very fragile, especially fetuses less than 100 days of age. Identification of brain landmarks after cryosectioning would be difficult had the area sectioned not been known before staining.

Moreover, the precise age of the fetus was unknown. Ideally, having cows bred at a specific date would have yielded fetuses of known gestational age when the tissues were harvested. Certainly other laboratory procedures could have been used to further determine the presence of LHRH neurons in the bovine fetal brain including RIA (Estes 1977, Jennes

1990), gel filtration and high performance liquid chromatography studies (King and Millar 1982, Sherwood et al. 1983, Powell et al. 1994).

In summary, results of this study indicate that the fetal bovine hypothalamus aquires LHRH neurons beginning around embryonic day 100. Comparing the location of the neurons found in this study to those reported in adult bovines by Dees and McArthur (1981), it is concluded these are the neurons that will continue through bovine adulthood. Implications from studying LHRH should include better knowledge of bovine physiology for the synthesis of superovulatory medications. In the realm of human medicine, understanding the GnRH family will lead to helping anestrous women conceive or the development of synthetic analogs that prohibit estrous as a method of birth control.

REFERENCES

Aubert, M.L., M. Begeot, B.P. Winiger, G. Morel, P.C. Sizonenko, and P.M. Dubois. 1985. Ontogeny of hypothalamic luteinizing hormone-releasing hormone (GnRH) and pituitary GnRH receptors in fetal and neonatal rats. *Endocrinology* 116: 1565-76.

Bastings, E., A. Beckers, M. Reznik and J. Beckers. 1991. Immunocytochemical evidence for production of luteinizing hormone and follicle-stimulating hormone in separate cells in the bovine. *Biology of Reproduction* 45: 788-796.

Campbell, B.K., H. Dobson, D.T. Baird, and R.J. Scaramuzzi. 1999. Examination of the relative role of FSH and LH in the mechanism of ovulatory follicle selection in sheep. *Journal of Reproduction and Fertility* 117: 355-367.

Ching, Melvin. 1982. Correlative surges of LHRH, LH and FSH in pituitary stalk plasma and systemic plasma of rat during proestrus. *Neuroendocrinology* 34: 279-285.

Dees, W.L. and N.H. McArthur. 1981. Immunohistochemical localization of gonadotropin releasing hormone (GnRH) in the bovine hypothalamus and infundibulum. *The Anatomical Record* 200: 281-285.

Dees, W.L., A.M. Sorensen Jr., W.M. Kemp and N.H. McArthur. 1981a. GnRH localization in the equine brain and infundibulum: An immunohistochemical study. *Brain Research* 208: 123-134.

Dees, W.L., A.M. Sorensen Jr., W.M. Kemp, and N.H. McArthur. 1981b. Immunohistochemical localization of gonadotropin-releasing hormone (GnRH) in the brain and infundibulum of the sheep. *Cell Tissue Research* 215: 181-191.

Estes, K.S., V. Padmanabhan, and E.M. Canvey. 1977. Localization of gonadotropin releasing hormone (GnRH) with bovine hypothalamus. *Biology Reproduction* 17: 706-711.

Florsheim, W.H. and P. Rudko. 1968. The development of portal system function in the rat. Neuroendocrinology 3: 89-98.

Greenstein, J.S. and R.C. Foley. 1955. Preliminary observations on the development of the normal bovine embryo from the late blastocyst stage to the differentiation of organ primordia. *Journal of Animal Science* 14: 1183.

Gross, D.S. 1976. Distribution of Gonadotropin-releasing hormone in the mouse brain as revealed by immunohistochemistry. *Endocrinology* 98:1408- 1417.

*Gross, D.S. 1980. Effect of castration and steroid replacement on immunoreactive gonadotropin releasing hormone in hypothalamus and preoptic area. *Endocrinology* 106: 1442-1450.

Horacek, M.J., G.T. Campbell, and C.A. Blake. 1989. Luteinizing Hormone (LH)-Releasing Hormone: Effects on induction of LH, Follicle-Stimulating Hormone, and Prolactin cell differentiation. *The Endocrine Society* 124:1800-1806.

Jennes, Lothar. 1990. Prenatal development of gonadotropin-releasing hormone receptors in the rat anterior pituitary. *Endocrinology* 126 (2): 942-947.

King, J.A. and R.P. Millar. 1982a. Structure of chicken hypothalamic luteinizing hormone-releasing hormone. I. Structural determination on partially purified material. *Journal of Biological Chemistry* 257(18): 10722-10728.

King, J.A. and R.P. Millar. 1982b. Structure of chicken hypothalamic luteinizing hormone-releasing hormone. II. Isolation and characterization. *Journal of Biological Chemistry* 257 (18): 10729-10732.

Kjaer, I. and B. Fischer Hansen. 1996. The human vomeronasal organ: prenatal developmental stages and distribution of luteinizing hormone-releasing hormone. *European Journal of Oral Sciences* 104: 34-40.

Kozlowski, G.P. and W.L. Dees. 1984. Immunocytochemistry for LHRH Neurons in the arcuate nucleus area of the rat: Fact or Artifact? *The Journal of Histochemistry and Cytochemistry* 32 (1): 83-91.

Kozlowski, G.P. and G. Nilaver. 1983. Immunoelectron microscopy of Neuropeptides: Theoretical and Technical Considerations. In *Current Methods in Cellular Neurobiology Vol. 1: Anatomical Techniques*, ed. J.L. and J. F. McKelvy, 133-146. New York: John Wiley and Sons.

Marubayashi, U., W.H. Yu, and S.M. McCann. 1999. Median eminence lesions reveal separate hypothalamic control of pulsatile follicle-stimulating hormone and luteinizing hormone release. *Proceedings of the Society for Experimental Biology and Medicine* 220 (3): 139-146.

Matsuo, H., Y. Baba, R.M.G. Nair, A. Arimura and A.V. Schally. 1971. Structure of the porcine LH- and FSH-releasing hormone. I. Proposed Amino Acid Sequence. *Biochemical and Biophysical Research Communiations* 43 (6): 1334-1339.

McCann, S.M., H. Mizunuma, W.K. Samson and M.D. Lumpkin. 1983. Differential hypothalamic control of FSH secretion: a review. *Psychoneuroendocrinology* 8 (3): 299-208.

Millar, R.P., C.A. Flanagan, R.C. deL. Milton and J.A. King. 1989. Chimeric analogues of vertebrate gonadotropin-releasing hormones comprising substitutions of the variant amino acids in positions 5,7, and 8. *Journal of Biological Chemistry* 264 (35): 21007-21013.

Miyamoto, K., Y. Hasegawa, T. Minegishi, M. Nomura, Y. Takahashi and M. Igarashi. 1982. Isolation and characterization of chicken hypothalamic luteinizing hormone-releasing hormone. *Biochemical and Biophysical Research Communications* 107 (3): 820-827.

Ngamvongchon, S., D.A. Lovejoy, W.H. Fischer, A.G. Craig, C.S. Nahornisk, R.E. Peter, J.E. Rivier, N.M. Sherwood. 1992. Primary structure of two forms of gonadotropin-releasing hormone, one distinct and one conserved, from catfish brain. *Molecular Cellular Neuroscience* 3: 17-22.

Powell, J.F.F, Y. Zohar, A. Elizur, M. Parks, W.H. Fischer, A.G. Craig, J.E. Rivier, D.A. Lovejoy, and N.M. Sherwood. 1994. Three forms of gonadotropin-releasing hormone characterized from brains of one species. *Proceedings of the National Academy of Sciences* 91: 12081-12085.

Powell, J.F.F., S.M. Reska-Skinner, M.O. Prakash, W.H. Fischer, M. Park, J.E. Rivier, A.G. Craig, G.O. Mackie and N.M. Sherwood. 1996. Two new forms of gonadotropin-releasing hormone in a protochordate and the evolutionary implications. *Proceedings of the National Academy of Sciences* 93: 10461-10464.

Sakar, D.K. 1983. Does LHRH meet the criteria for a hypothalamic releasing factor? *Psychoneuroendocrinology* 8 (3): 259-275.

Schally, A.V. 1978. Aspects of hypothalamic regulation of the pituitary gland. *Science* 202: 18-28.

Schwanzel-Fukuda, M., J.A. Robinson, and A.J. Silverman. 1981. The fetal development of the luteinizing hormone-releasing hormone (LHRH) neuronal systems of the guinea pig brain.

Schwanzel-Fukuda, M. and D.W. Pfaff. 1989. Origin of luteinizing hormone-releasing hormone neurons. *Nature* 338 (Mar): 161-164.

Sealfon, S.C., H. Weinstein and R.P. Millar. 1997. Molecular mechanisms of ligand interaction with the gonadotroping-releasing hormone receptor. *Endocrine Review* 18 (2): 180-205.

Seminara, S.B., M. Beranova, L.M.B. Oliveira, K.A. Martin, W.F. Crowley, Jr. and J.E. Hall. 2000. Successful use of pulsatile gonadotropin-releasing hormone (GnRH) for ovulation induction and pregnancy in a patient with GnRH receptor mutations. *The Journal of Clinical Endocrinology and Metabolism* 85 (2): 556-562.

Sétáló, G., S. Vigh, A.V. Schally, A. Arimura and B. Flerkó. 1976. Immunohistological study of the origin of LH-RH-containing nerve fibers of the rat hypothalamus. *Brain Research* 103: 597-602.

Sherwood, N., L. Eiden, M. Brownstein, J. Spiess, J. Rivier and W. Vale. 1983. Characterization of a teleost gonadotropin-releasing hormone. *Proceedings of the National Academy of Sciences* 80: 2794-2798.

Sherwood, N.M. and S.A. Sower. 1985. *Neuropeptides* 6: 205-214.

Sherwood, N.M., S.A. Sower, D.R. Marshak, B.A. Fraser, and M.J. Brownstein. 1986. Primary structure of gonadotropin-releasing hormone from lamprey brain. *Journal of Biological Chemistry* 261 (11): 4812-4819.

Silverman, A.J. 1976. Distribution of Luteinizing Hormone-Releasing Hormone (LHRH) in the Guinea Pig brain. *Endocrinology* 99: 30-41.

Silverman, A.J., J.L. Antunes, M. Ferin and E.A. Zimmerman. 1977. The distribution of Luteinizing Hormone-Releasing Hormone (LHRH) in the hypothalamus of the Rhesus Monkey. Light microscopic studies using immunoperoxidase technique. *Endocrinology* 101: 134-142.

Sower, S.A., Y.C. Chiang, S. Lovas, and J.M. Conlon. 1993. Primary structure and biological activity of a third gonadotropin-releasing hormone from lamprey brain. *Endocrinology* 132 (3): 1125-1131.

Sternberger, L.A., P.H. Hardy Jr., J.J. Cuculis and H.G. Meyer. 1970. The unlabeled antibody enzyme method of immunochemistry: Preparation and properties of soluble antigen-antibody complex (horseradish peroxidase-antihorseradish peroxidase) and its use in identification of spirochetes. *The Journal of Histochemistry and Cytochemistry* 18: 315-333.

Yoshida, K., S.A. Tobet, J.E. Crandall, T.P. Jimenez, and G.A. Schwarting. 1995. The migration of luteinizing hormone-releasing hormone neurons in the developing rat is associated with a transient, caudal projection of the vomeronasal nerve. *The Journal of Neuroscience* 15 (Dec): 7769-7777.

Yu, W.H., S. Karanth, A. Walczewska, S.A. Sower and S.M. McCann. A hypothalamic follicle-stimulating hormone-releasing decapeptide in the rat. *Proceedings of the National Academy of Science* 94 (17): 9499-9503.

Yu, W.H., M. Kimura, A. Walczewska, S. Karanth and S.M. McCann. 1997. Role of leptin in hypothalamic-pituitary function. *Proceedings of the National Academy of Sciences* 94 (Feb): 1023-1028.

Yu, W.H., A. Walczewska, S. Karanth and S.M. McCann. 1997. Nitric oxide mediates leptin-induced luteinizing hormone-releasing hormone (LHRH) and LHRH and leptin-induced LH release from the pituitary gland. *Endocrinology* 128 (11): 5055-5058.

APPENDIX

AP	Anterior Pituitary
Arg	Arginine
E	embryonic (for noting days of gestation)
FSH	Follicle stimulating hormone
FSHRF	Follicle stimulating hormone releasing factor
Gln	Glutamine
Glu	Glutamic acid
Gly	Glycine
GnRH	Gondatropin releasing hormone
HH	Hypothalamic hypogonadism
His	Histidine
INF	Infundibulum
Leu	Leucine
LH	Luteinizing hormone
LHRH	Luteinizing hormone releasing hormone
ME	Median eminence
OVLT	Organum vasculosum of the Lamina terminalis
PAP	Peroxidase-anti-horseradish peroxidase complex
Pro	Proline
PSA-N-CAM	Polysialated form of neural cell adhesion molecule
RIA	Radioimmunoassay
Ser	Serine
TAG-1	Transient axonal surface gycoprotein
TIDA	Tuberoindundibular dopaminergic
TIS	Tuberoinfundibular Sulcus

Trp	Tryptophan
Tyr	Tyrosine
VNN	Vomeronasal Nerve

Vita

Ivy Beth DeSimone was born in Providence, Rhode Island on April 19, 1977, the daughter of Beth Silva DeSimone and David Anthony DeSimone. The family transferred to Houston, Texas in 1981 and then finally settled in San Antonio in 1983. After graduating Cum Laude from James Madison High School in 1995, Ivy entered Southwest Texas State University with the desire to become a physical therapist. After one semester she changed her major to Animal Science which is the undergraduate degree she graduated with in May 1999. She went directly to graduate school at Southwest Texas in the Agriculture department and worked as a graduate assistant conducting research and helping with undergraduate classes. While completing her Masters in Education, she also completed the requirements to earn her secondary teacher certification in agriculture and student taught at James Bowie High School. Additional teaching experience came from substituting for San Marcos Consolidated Independent School District. Throughout her time at SWT, Ivy was a volunteer at a horseback riding therapy center for handicap children and adults in San Marcos.

Permanent address: 114 Moonlight Meadow
Cibolo, Texas 78108

This thesis was typed by Ivy Beth DeSimone.