ROLE OF ENDOGENOUS DOPAMINE IN REGULATION OF ANTERIOR PITUITARY HORMONE SECRETION DURING EARLY POSTPARTUM AND VARIOUS STAGES OF THE ESTROUS CYCLE IN HOLSTEIN COWS

BY

AMIN AHMADZADEH

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APPROVED:

M. A. Barnes, Chairman

R. M. Akers

F. C. Gwazdauskas

M. L. McGilliard

D. M. Denbow

W. E. Vinson, Department Head

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Department of Dairy Science

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ABSTRACT

The role of endogenous dopamine, utilizing a dopamine antagonist (fluphenazine; FLU), in modulation of gonadotropin, growth hormone (GH) and prolactin (PRL) secretion during the early postpartum period and various stages of the estrous cycle was investigated in Holstein cows. Experiment 1 was conducted in anovulatory early postpartum cows. Fluphenazine caused a decrease ($P < .05$) in mean serum LH concentration and LH pulse frequency. Likewise, FLU caused a ($P < .05$) decrease in mean GH concentration. These results suggest that endogenous dopamine, at least in part, is responsible for regulation of LH and GH secretion in anovulatory Holstein cows. Experiment 2 was conducted in cyclic lactating Holstein cows during the mid-luteal phase of the estrous cycle. Mean serum LH and FSH concentrations, pulse frequencies, and peak amplitudes did not change in response to FLU. FLU did not affect mean serum GH concentration. These results suggest that a dopamine-mediated mechanism for modulation of gonadotropin and GH secretion is absent or perhaps overridden by high progesterone concentration during the luteal phase of the estrous cycle in lactating dairy cows. Experiment 3 was conducted during the early follicular phase of the estrous cycle in Holstein cows. During the follicular phase, FLU caused a decrease ($P < .05$) in mean serum LH concentration and LH pulse frequency. However, FLU had no effect on mean serum FSH concentration or pulse frequency. Further, FLU increased ($P < .05$) GH concentrations during the follicular phase. Experiment 4 was conducted during the early

metestrus phase of the estrous cycle. During the metestrus phase, FLU tended to decrease ($P < .1$) mean LH concentration and suppressed ($P < .05$) LH pulse frequency but had no effect on FSH secretion. Fluphenazine caused a transient increase ($P < .05$) in mean serum GH concentration. The results of the third and fourth experiments suggest that, during the early follicular and metestrus phases of the estrous cycle, when progesterone concentration is low, modulation of LH and GH secretion, at least in part, is modulated by endogenous dopamine. However, a dopamine mediated mechanism for FSH secretion is absent during both phases of the estrous cycle in lactating Holstein cows. In all experiments FLU increased ($P < .01$) PRL secretion indicating that endogenous dopamine suppresses PRL secretion in cattle regardless of ovarian status. It is concluded that: 1) endogenous dopamine plays a stimulatory role in LH secretion during the anovulatory postpartum period and during the estrous cycle only when serum progesterone is low. 2) FLU decreased GH secretion in anovulatory postpartum Holstein cows but it increased GH secretion during the follicular and metestrus phases of the estrous cycle. However FLU had no effect on GH secretion during the luteal phase of the estrous cycle. Thus it appears that, modulation of GH secretion is dependent upon reproductive status and ovarian hormones secretion.

DEDICATION

I dedicate this dissertation to Judith Elizabeth and Kevin C. Cullen, Doylestown, Pennsylvania my second family, for their love and support.

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INTRODUCTION

Following parturition in cattle, there is a transitional period during which animals are anestrous. During this period of uterine involution, reproductive organs recover from the effects of pregnancy. During pregnancy the hypothalamic-pituitary axis is suppressed, in part, by carry-over effects of high concentrations of gonadal steroids (Nett, 1987). The inadequate discharge of gonadotropin releasing hormone (GnRH) from the hypothalamus results in inadequate LH secretion to initiate cyclicity during the early postpartum period (Edgerton and Hafes 1973;Nett, 1987). Thus, prolongation of the postpartum anestrous period may be attributable to suppression of the mechanism which stimulates GnRH release.

Ovarian steroids act as modulators of secretion of gonadotropins during the estrous cycle in the bovine (Stumpft et al., 1988). Ireland and Roche (1982) reported a negative correlation between level of exogenous progesterone administered and secretion of LH in cattle, with low levels of progesterone associated with an increase in frequency of LH pulses. These authors suggested that progesterone is part of a negative feedback complex on LH secretion in cattle. Walters et al. (1983) suggested that LH and FSH pulses stimulate pulses of estradiol and progesterone from the ovary, which in turn feed back upon the pituitary and hypothalamus to regulate the frequency and amplitude of LH and FSH pulses. Therefore, it appears that establishment of normal estrous cycles and ensuing fertility depends upon stimulatory input from pituitary gonadotropins and feedback effects of steroids from the ovaries. Because of the significance of this interaction in control of the estrous cycle, scientists have studied different aspects of the hypothalamic-pituitary-gonadal axis. The exact mechanism by which gonadotropin secretion is regulated by ovarian steroids is not clear. However, because of the episodic discharge characteristic of LH secretion, the involvement of neural elements is probable (Goodman and Karsch, 1980). Extensive studies have established that monoamines, such as dopamine, influence secretion of gonadotropins in laboratory animals and ewes (see Barraclough and Wise, 1982; Dailey et al., 1987 for reveiw). Furthermore, it is known

that steroids influence synthesis and turnover rates of monoamines in hypothalamic tissues (Coppola, 1969; Beattie et al., 1972). Additionally, it appears that the median eminence, mediobasal hypothalamus, the preoptic area, and the periventriciular nuclei in the cow contain dopaminergic neurons (Kizer et al., 1976; Leshin et al., 1995). Some of these regions contain GnRH (Kizer et al., 1976; Leshin et al., 1991). The possibility therefore exists that dopamine interacts with GnRH neurons and plays a role in regulating gonadotropin secretion during the early postpartum period, and may play a part in neuroendocrine regulation of steroid feedback during the various stages of the estrous cycle in dairy cows. Our basic understanding of mechanisms that control the secretion of gonadotropins and ovarian steroids during the estrous cycle may have profound implications relative to the management of ovarian follicles for the synchronization of estrous cycles and subsequent fertility. This information would aid in the development of new and better techniques for artificial control of reproduction in cattle to improve reproductive efficiency. Thus, experiments designed to clarify the possible role of dopamine in regulation of gonadotropin secretion, the most important signals which control the reproductive cycle, can be beneficial to achieve these goals.

Increasing milk yield and thus improving the productivity and profitability of dairy farms requires a better understanding of regulation of hormones which regulate mammary gland function. Coordinated action of several pituitary hormones such as growth hormone (GH) and prolactin (PRL) govern growth and development of the mammary gland and ultimately milk secretion (lactation). Circulating concentrations of GH are closely related to body maintenance and enhanced milk yield (Akers, 1994). Administration of GH increased milk yield in cattle (Peel and Bauman, 1987). Such increases are associated with increased mammary blood flow, increased partitioning of dietary nutrient energy toward the mammary gland, and increased voluntary feed intake and feed efficiency (Peel et al. 1983; Akers 1994).

It is known that GH secretion is regulated by a central nervous system mechanism involving GH-releasing hormone (GRH) and GH-inhibiting factor (GIF) released from the hypothalamus (Buonomo and Baile, 1990;Malven, 1993a). The release of these hypothalamic hormones is in turn influenced by a network of monoaminergic neurons.

However, findings concerning the role of specific monoamines such as dopamine, norepinephrine, and serotonin in the control of GH secretion are equivocal. The role of dopamine in regulation of GH secretion is not clear and studies in ruminants are limited. Some believe dopamine to be the major stimulatory monoamine responsible for release of GH while others believe that dopamine is inhibitory to GH release (see Buonomo and Baile, 1990; Spencer et al., 1991 for review). Therefore, improving our basic understanding of neuroendocrine control of GH, which is critical for successful lactation, may provide some profound implications concerning mammary growth and improvement of milk yield in the dairy cow. Experimental treatments designed to clarify the neural pathway(s) which control secretion of GH can be beneficial. Elucidation of the role of dopamine in modulation of GH may provide evidence and enhance our knowledge of neuroendocrine regulation of GH secretion in dairy cattle.

The overall objective of this study was to investigate the role of endogenous dopamine in control of gonadotropin, GH, and PRL secretion during the anovulatory postpartum period and during various stages of the estrous cycle in lactating Holstein cows.

REVIEW OF LITERATURE

Introductory Remarks

The importance of dopamine as a monoamine neurotransmitter in the mammalian brain has been recognized only in the last four decades. Until the 1950's, dopamine was exclusively considered to be an intermediate in the biosynthesis of the two catecholamines, norepinephrine and epinephrine. A high level of dopamine was first found in peripheral organs of ruminant species (Cooper et al., 1996). In the late 1950's scientists found that dopamine was also present in the bovine and rat brain (Cooper et al., 1996). The difference in regional distribution of dopamine and norepinephrine, both in bovine peripheral tissues and within the central nervous system (Cooper et al., 1996), led investigators to suggest biological roles for dopamine apart from its role as an immediate precursor for norepinephrine biosynthesis. The relationship between basal ganglia and motor control, extrapyramidal symptoms in Parkinson's disease, and profound depletion of dopamine in basal ganglia of Parkisonians patients supported the hypothesis that in fact dopamine was a separate neurotransmitter (Cooper et al., 1996). With the advent of fluorescent histochemical and retrograde tracing techniques, dopamine-containing neurons were identified in the tuberoinfundibular system of the hypothalamus. This system includes the arcuate and periventricular nuclei that are known to play an important role in the synthesis of several releasing factors, i.e. GnRH, GRF, and somatostatin (Thiery and Martin, 1991; Leshin et al., 1994). Dopamine containing cell bodies in the arcuate and periventricular nuclei of the hypothalamus send projections into the median eminence, the area in which the aforementioned releasing factors are stored and released. Major advancement in understanding the biochemistry, physiology, and pharmacology of dopamine has come through bioassay procedures and the development of sensitive radioimmunoassay and high performance liquid chromatography.

Dopamine and its synthesis

Dopamine belongs to a class of organic compounds known as catecholamines. The term catecholamine refers to all organic compounds that contain a catechol nucleus and an amine group. The catechol group consists of a benzene ring with two adjacent hydroxyl groups (Figure 1).

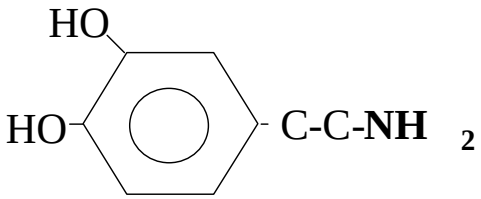

Figure 1. Dopamine

Dopamine is synthesized originates from the amino acid tyrosine. Tyrosine must be transported across the blood brain barrier into dopamine neurons. Once tyrosine enters the neuron, it is converted to L-dihydroxyphenylalanine (L-DOPA) by action of tyrosine hydroxylase. This is the rate-limiting step in dopamine synthesis. L-DOPA is subsequently converted to dopamine by the aromatic amino acid decarboxylase enzyme, DOPA decarboxylase (Figure 2). Turnover rate of the latter enzyme is very rapid and as a result amount of L-DOPA in the brain is relatively low. In the processes of dopamine biosynthesis, tyrosine hydroxylase is the rate-limiting enzyme and plays the key role for the formation of dopamine. As a result, it is not surprising that this enzyme is susceptible to physiological regulation and pharmacological manipulation. Activity of this enzyme is tightly regulated in several ways. For example, dopamine itself functions as the end-product inhibitor of tyrosine hydroxylase (Cooper et. Al., 1996). Also, presynaptic dopamine receptors modulate the rate of tyrosine hydroxylation within dopamine neurons and thus, dopamine turnover rate. It is obvious that pharmacological manipulation of these receptors influence the physiological action

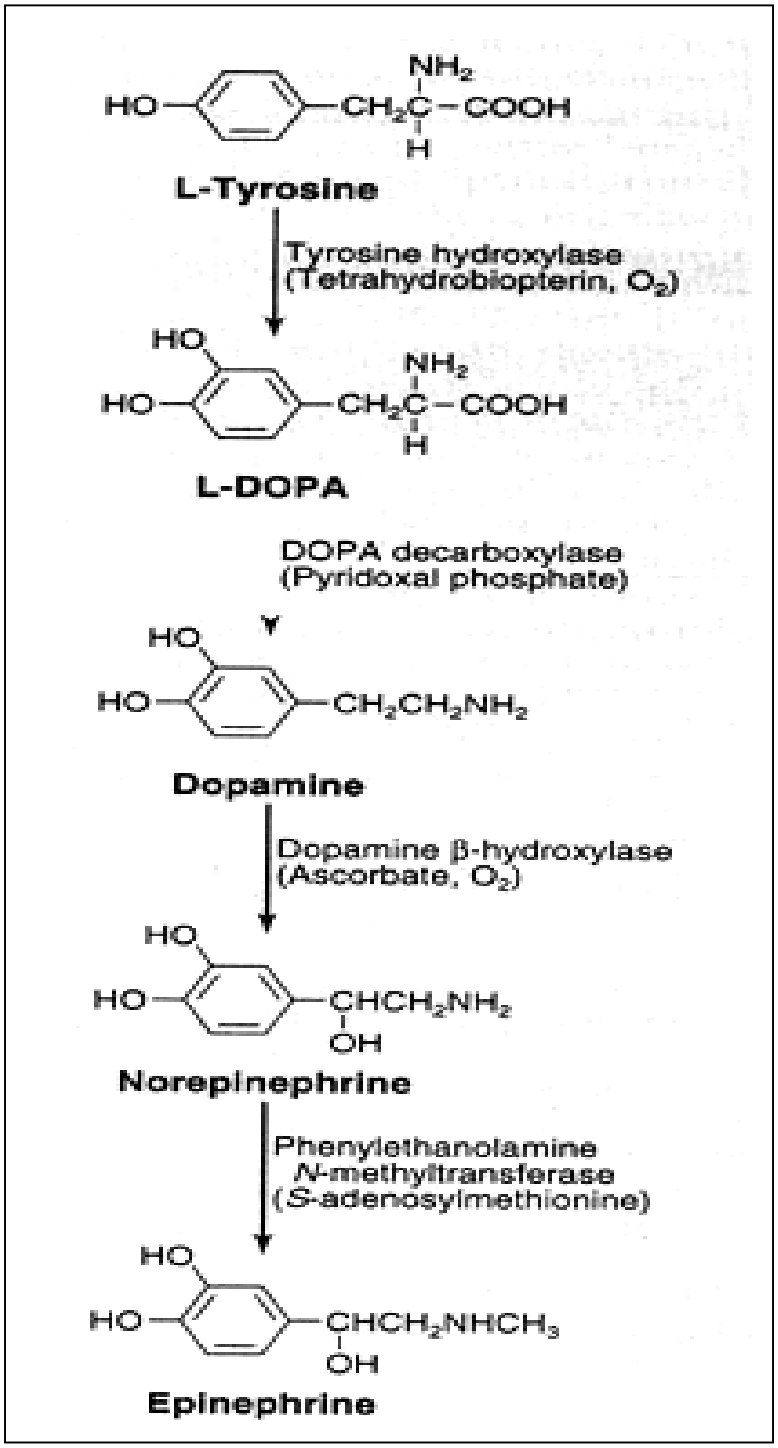

Figure 2. Biochemical pathway for Dopamine synthesis

of endogenous dopamine. This issue will be discussed in more detail later in this chapter.

Dopamine Release, Transport, Uptake and Metabolism

In dopamine neurons, conversion of tyrosine to L-DOPA and L-DOPA to dopamine occurs in the cytosol. Dopamine then is taken up into storage vesicles by vascular amine transporters. This transport mechanism is essential for maintaining the supply of dopamine at the presynaptic terminals because it decreases cytoplasmic concentration of dopamine (free dopamine) and prevents its metabolism by monoamine oxidase (MAO). Dopamine is released from presynaptic terminals by a calcium-dependent mechanism. When an action potential takes place at the terminal, Ca^{+2} channels open, allowing Ca^{+2} entry into the terminal. The increase in intracellular Ca^{+2} concentration causes fusion of dopamine containing vesicles to the plasma membrane. Vesicles then release dopamine into the synaptic cleft (Weiner and Molinoff, 1994)

Despite marked fluctuation in the activity of dopamine containing neurons, the level of dopamine within nerve terminals remains relatively constant. This phenomenon is accomplished by an efficient regulatory mechanism, i.e. feedback inhibition and reuptake mechanisms. These mechanisms involve transport proteins, presynaptic receptors, and tyrosine hydroxylase activity. Dopamine uptake plays an important physiological role in inactivation and recycling of dopamine in the synaptic cleft and in the presynaptic terminals, respectively. Advancement in understanding of uptake mechanisms came about through development of selective uptake blockers such as nomifensine, amphetamine, and GBR (Cooper et al., 1996).

Dopamine Metabolism

Monoamine oxidase (MAO) and catechol-O-methyl transferase (COMT) are primarily responsible for inactivation of dopamine. Intraneuronal MOA converts dopamine to dihydoxyphenyl acetic acid (DOPAC). At the extracellular site, COMT and

MAO convert dopamine to homovanilic acid (HVA; Cooper et al., 1996; Weiner and Molinoff, 1994). Monoamine oxidase and COMT play an important role in regulatory mechanisms of dopamine turnover. Therefore, chemical compounds that affect activity of these two enzymes they also influence dopamine turnover rate. Dopamine antagonists are able to influence dopamine turnover. Dopamine antagonists, mainly via autoreceptors, affect MAO and its metabolite concentrations within the brain and therefore influence dopamine turnover rate. For example, some dopamine antagonists increase MAO concentration leading to an increase in DOPA concentration within the striatum and subsequently changing impulse flow in dopaminergic neurons (Henson et al., 1987;Cooper et al., 1996). The fact that dopamine antagonists can influence dopamine turnover rate is important because in pharmacological studies these compounds may function in a biphasic manner. For example, low doses of dopamine antagonists may affect postsynaptic receptors and thereby impair dopamine action, whereas higher doses may affect autoreceptors and increase dopamine turnover rate.

Dopamine Receptors

The concept of specific cell receptors for a substance arises from the ability of a tissue to bind and physiologically respond to minute quantities of a substance in some particular way (Kebabian and Calne, 1979). Physiological, pharmacological, and biochemical studies revealed the existence of multiple classes of receptors for dopamine and that different subtype dopamine receptors serve different physiological roles in different target tissues. Understanding the exact physiological role of each subtype of dopamine receptor is important because it provides scientists with more specific and improved ways of manipulating dopaminergic activity in various target tissues.

Like other catecholamines, dopamine mediates its effects through interaction with plasma membrane receptors. Dopamine receptors are members of the G protein-linked receptor family with seven-hydrophobic domain, an extracellular N terminus, and an intracellular C terminus. Consensus sequences for phosphorylations are found in the third intracellular loop and the C-terminal tail (Weiner and Molinoff, 1994). Dopamine

receptors are generally divided into presynaptic and postsynaptic categories.

Postsynaptic receptors are on non-dopamine cell types and presynaptic receptors, which are also called autoreceptors, are located on dopamine cells. Neuronal autoreceptors are so named because they regulate the activity of their own transmitter and therefore control dopamine turnover rate within dopamine neurones. Dopamine receptors can be further classified on the basis of physiological, pharmacological, and biochemical studies. It is well established that dopamine can act via two receptors, D1 and D2 subtypes (Kebabian and Calne, 1979; Weiner and Molinoff, 1994). Separation of dopamine receptors is mainly based on presence or absence of positive coupling of receptors to the enzyme adenylate cyclase (AC). D1 receptors stimulate AC activity, whereas D2 receptors either inhibit AC activity or function through mechanisms independent from AC (Kebabian and Calne, 1979; Cooper et al., 1996). These mechanisms may include direct effects on potassium and calcium channels, as well as modulation of inositol phosphate production. The signal transduction mechanisms associated with these two receptor types are summarized in Table 1. Furthermore, while D1 receptors have mM affinity for dopamine antagonists, D2 receptors have pM affinity for antagonists (Cooper et al., 1996). The development of selective dopamine agonists and antagonists has been helpful in determining whether physiological effects of endogenous dopamine are mediated by D1 or D2 receptors.

Table 1. Signal transduction mechanisms associated with dopamine receptors [a].

D2 receptors	D1 receptors
Inhibition of AC[b]	Stimulation of AC
Inhibition of Ca^{2+} entry through voltage gated channels	Stimulation of PI turnover
Modulation of PI[c] metabolism	
Enhancement of K^+ conductance	

[a] Adapted from Cooper et al. (1996).
[b] Adenylate cyclase
[c] PI = Phosphoinositide.

Recent developments in molecular biology, identified multiple D1-like and D2-like receptors. D1-like subtypes include D1 and D5 receptors and D2-like subtypes include D2, D3, and D4 receptors. The major differences among these subtype receptors are number of amino acids, regional distribution of mRNA encoding amino acids within the CNS, and their affinity for different dopamine agonists and antagonists (see Cooper et al., 1996 for review).

Dopamine Receptor Distribution

Currently there are few pharmacological or immunological tools available for measuring distribution of dopamine receptors. Most information became available using *in situ* hybridization experiments in primates and rats. It is evident that postsynaptic receptors, D1 and D2 mRNAs are present in high levels in the caudate putamen and olfactory tubercle and lower levels in the septum and entire hypothalamus. D2 receptor mRNA has also been found outside the CNS, in the anterior pituitary gland, adrenal gland and retina (Stafford et al., 1993; Dahmar and Senolgles, 1996; Jensen and Daw, 1986). D1 receptor mRNA has been found in very low levels in the substanitia nigra, where dopamine perikarya are mostly located (Cooper et al., 1996). This finding led investigators to believe that the D1 receptor may not play a role as an autoreceptor and thus may not be involved in autoregulation of dopamine synthesis and turnover.

Dopamine receptor agonists and antagonists can affect dopamine cell activity, dopamine turnover, and dopamine catabolism. Nonetheless, the effects of dopamine agonists or antagonists depend on affinity of these compounds for D1 or D2 receptors. For example, compounds with high affinity for D2 receptors interact with autoreceptors and dramatically affect dopamine turnover rate, whereas compounds with affinity for D1 primarily induce a physiological action on postsynaptic receptors with little or no effect on dopamine turnover. This raise a point in experimental procedure in which dopamine agonists and (or) antagonists are utilized to investigate the role of endogenous dopamine in regulation of hypothalamic neurohormones. Depending upon the chemical properties

of dopamine agonist or antagonist that is used, investigators must pay close attention to interpretation of their data. This issue of receptor specificity will be further discussed in the rationale for utilizing a dopamine antagonist to study the role of dopamine in regulation of pituitary hormone secretion.

Anatomical Distribution of Dopamine Neurons

Based on the anatomy of dopaminergic neurons and length of their efferent fibers, dopamine neurons can be divided into three major systems (Cooper et al., 1996): Ultra-short Systems, Intermediate Systems, and Long-length Systems. The emphasis of the following discussion will be on the Intermediate-length System because of its anatomical relationship to hypophysiotrophic neurohormones such as GnRH.

Intermediate-length dopaminergic systems include: tuberohypophysial dopamine neurons, A13 and A14 dopaminergic neurons, and A15 dopaminergic neurons.

a) *tuberohypophysial dopamine neurons* (tuberoinfundibular system). The neurons in this system originate from arcuate and periventricular nuclei and their terminals project into the pars intermedia of the pituitary and stalk/median eminence (ME). In sheep the tuberoinfundibular dopamine neurons also project into the pre-optic area (POA) of the hypothalamus (Thiery and Martin, 1991). In the rat, in addition to the POA these neurons also project into the pars nervosa of the pituitary (Barraclough and Wise, 1982). In cattle, dopamine concentration was high throughout the ME region (Kizer et al, 1976) as evidenced from high activity of tyrosine hydroxylase, which is a fairly specific marker for dopamine neurons in this region. Results from studies using immunocytochemical staining techniques for presence of catecholaminergic-synthesizing enzymes suggest that the periventricular, arcuate, and retrochiasmatic nuclei as well as the pituitary-stalk-median eminence of cattle contain dopaminergic neurons (Leshin et al., 1995)

b) *A 13 and A14 dopaminergic nuclei:* These nuclei are components of incertohypothalmic neurons. This system links the dorsal and posterior hypothalamus

with the dorsal anterior hypothalamus (Cooper et al., 1996). At least in the rat, the A14 cell group is distributed in the periventricular, suprachiasmatic, and mediobasal preoptic nuclei (Barraclough and Wise, 1982)

c) The *A15 dopaminergic neurons* consist of the third subdivision of the intermediate length system. These neurons are situated in the mediobasal hypothalamus. There is evidence that in sheep, some axons of the A15 system terminate in the POA of the hypothalamus and in the septum (Thiery and Martin, 1991;Cooper et al., 1996). In the bovine the reterochiasmatic division of supraoptic nuclei correspond to the ventral A15 nuclei, which have been shown to contain specific dopamine-producing cells (Leshin et al., 1995).

Existence of dopaminergic neurons in the area of periventricular, arcuate, and retrochiasmatic nuclei and in the pituitary-stalk median eminence of cattle is of a particular interest because hypothalamic releasing-hormones which control gonadotropin and GH secretion are also found in the same regions (Kizer et al. 1976; Leshin et al., 1991; Leshin et al., 1995). The next section describes the relationship between hypothalamic releasing factors, gonadotropin and GH secretion, and dopaminergic neurons.

Hypothalamic Releasing Factors and Their Relationship to Dopamine

Distribution of Dopamine and Gonadotropin Releasing-hormone in the Brain

It is known that the hypothalamus is the center which controls secretion of anterior pituitary hormones. In the early 1970's development of specific antibodies against GnRH and immunocytochemical techniques helped scientists to localize GnRH perikarya in the hypothalamus. In the various species studied, proportion of GnRH cell bodies found in different regions of the brain differed, but there was a relatively consistent pattern of distribution. It appears that most GnRH cell bodies are located in the anterior hypothalamus in the area between the POA, the anterior POA area and the mediobasal hypothalamus (Thiery and Martine, 1991). For example, in the rat most GnRH cells are

found in the POA (Kawano and Daikoku, 1981), whereas in the rhesus monkey GnRH cells are mostly present in the mediobasal hypothalamus (Silverman et al., 1982). In domestic ungulates, the mediobasal hypothalamus contains relatively few LHRH perikarya and most are located in the POA (Malven, 1993c). In the ewe, 95% of GnRH cell bodies are located in a region covering the anterior hypothalamus, the POA, the diagonal band of broca, and septum (Thiery and Martin, 1991). Half of these neurons terminate in the (ME). It is interesting to note that in the ewe, contacts between GnRH cells and catecholaminergic terminals have been shown in both the POA and ME (Kuljis and Avis, 1989). Kizer et al. (1976) reported a high concentration of LHRH in the middle lateral and anterior part of the bovine ME. In the same study, dopamine was present in highest concentration in the same subdivisions of the bovine ME that were rich in LHRH. Similarly, Leshin et al. (1991) found high concentrations of LHRH neurons in the ME as well as in the POA region of the anterior hypothalamus in the bovine. It should be noted that the anterior hypothalamus is adjacent to the retrochiasmatic, and as mentioned previously, this area was stained with anti-tyrosine hydroxylase, indicating the presence of dopamine (Leshin et al., 1995). Although there is no evidence in the cow, an interaction between hypothalamic GnRH and dopamine has been shown in the ewe. The dopamine system acts in the retrochiasmatic and (or) ME to suppress GnRH pulse frequency in the ovary-intact anestrous ewe because placement of dopamine antagonist in this region significantly increased LH pulse frequency and mean LH concentrations. (Havern et al., 1991). It has been proposed that long-day inhibition of LH secretion by estradiol in the ewe results from stimulation of tyrosine hydroxylase activity in the A15 nucleus dopaminergic neurons (Gayrard et al., 1994). The above studies provide evidence that ovarian steroids, the dopaminergic system and GnRH may interact with each other to regulate LH secretion.

The above discussion, at least from an anatomical point of view, brings up a logical question: Is there an interaction between dopamine and GnRH in cattle? In light of findings in sheep, it would be surprising if GnRH and subsequently LH activity, are not affected by changes in dopaminergic activity in the cow.

Exogenous Dopamine and Dopamine Antagonist Influence on LH Secretion

The anterior pituitary gland secretes pulses of LH in response to GnRH released by the hypothalamus into hypophysial portal blood. Tonic secretion of pituitary LH is the result of interplay between stimulatory input from the brain and inhibitory steroid feedback from the gonads, and involvement of neural components is clearly evident in view of the episodic discharge characteristics of tonic LH secretion (Goodman and Karsch, 1980). The pulsatile nature of secretion is important because frequency of pulses is directly related to activity of GnRH neurons.

Extensive studies, primarily in laboratory rodents, have established that biogenic amines, particularly norepinephrine, dopamine, and serotonin influence gonadotropin secretion (Barraclough and Wise, 1982). However, the role of dopamine in control of gonadotropin secretion remains controversial. Some believe that the effect of dopamine on LH secretion is stimulatory and some believe the opposite is true. Although these differences may relate to differences in experimental methodology, the picture regarding the role of dopamine is very complex and many other factors may play a part. Among these factors, some might directly influence the activity of GnRH neurons, whereas others might act indirectly via other interneurons; some might be stimulatory while others may be inhibitory (Thiery and Martin, 1991). Furthermore, steroids influence the dopaminergic system and may excite or inhibit the effects of dopamine. In addition, factors such as species, age relative to puberty (at least in laboratory species), ovarian status, seasonality, and pheromonal effects may modify dopamine effects on gonadotropin secretion (Thiery and Martin, 1991).

Initial studies suggested a stimulatory role of dopamine in LH secretion. Schnieder and McCann (1969) used an *in vitro* system in which large pieces of hypothalamus were coincubated with anterior pituitary glands. Addition of pharmacological amounts of dopamine increased LH release from 161% to 236% of the controls. In progesterone-treated ovariectomized rats, a 10-fold increase in plasma LH occurred after intraventricular dopamine injection, and dopamine induced 8- to 10-fold

increases in LH secretion in proestrus rats (Schnieder and McCann, 1970). These authors proposed that dopamine is the catecholamine responsible for triggering release of LHRH. This idea was supported by Kamberi et al. (1970) who showed that infusion of dopamine into the third ventricle stimulated LH secretion in female rats. Rotszteju et al., (1977) demonstrated that dopamine stimulates release of GnRH by mediobasal hypothalamic tissue of male rats *in vitro*. It should be noted that one could not definitely conclude that these stimulatory effects involve a dopaminergic system because dopamine can be converted to norepinephrine in tissue by the enzyme dopamine hydroxylase (Figure 2). Therefore, observed increases in LH secretion could be attributable to the effect of norepinephrine and not dopamine. Unfortunately, the picture regarding dopamine effects on LH secretion is not as clear as has been shown in above studies. For example, administration of various doses of dopamine (intraventricularly) to progesterone- and estradiol-primed ovariectomized rats did not affect LH secretion, whereas norepinephrine significantly increased LH secretion (Krieg and Sawyer, 1974). Sawyer et al (1974) reported that intraventricular norepinephrine stimulated LH release in the rabbit, whereas dopamine not only failed to increase plasma LH, but also blocked the stimulatory effect of norepinephrine. In another study (Lamb et al., 1993), dopamine antagonist did not affect LH secretion in rabbits.

Most of the research investigating effects of dopamine on LH in ruminants has been conducted with sheep because of the relative ease of experiments and lowere expenses. However, that choice of animal model added other dimensions including seasonality and photoperiods, which make the picture even more complicated. Because of limited information concerning the role of dopamine on LH secretion in other domestic animals, most of the following review will concentrate on studies conducted on ewes. One point to make is that in various studies, exogenous dopamine, dopamine agonist, and dopamine antagonist influenced, or had no effect on LH secretion. These differing results led investigators to develop various hypotheses regarding the role of endogenous dopamine in control of LH secretion. As emphasized by Deaver and Dailey (1982) interpretation of results by these approaches is influenced by pharmacological agent used, route of administration, and physiological status of the animals.

Role of dopamine in LH secretion in the anestrous- ovariectomized-ewe

The role that dopamine plays in control of LH secretion in ovariectomized animals is not clear and drawing conclusive remarks concerning the interaction between steroid status of animals and dopamine action on LH using this animal model is difficult.

In ovariectomized ewes, a high dose of pimozide (a dopamine antagonist) did not affect LH pulse frequency or mean LH values, but when animals were implanted with estradiol, the same dose of pimozide increased both LH pulse frequency and mean concentration (Meyer and Goodman, 1985). These results may indicate that in the absence of ovarian steroids dopaminergic neurons may not be fully active and thus do not act upon GnRH neurons. However, other findings may dispute this hypothesis. For instance, pimozide had no effect on LH in intact or estradiol-treated ovariectomized ewes during seasonal anestrous (Kao et al., 1992). Early work by Jackson (1977) demonstrated that pimozide suppressed tonic secretion of LH in ovariectomized ewes. A .5 mg/kg BW dose of fluphenazine (another dopamine antagonist) did not affect LH pulse frequency, but it suppressed LH peak amplitude and mean LH concentrations in ovariectomized ewes (Goodman, 1985). Kao et al. (1992) also reported similar results utilizing pimozide. Hill and co-workers (1980) reported that intramuscular injection of bromocryptin, a dopamine agonist, decreased LH secretion in ovariectomized ewes. Moreover, in ovariectomized ewes, response of LH to intravenous infusion of dopamine was dose-dependent with the lower dose increasing, and the higher dose decreasing, plasma LH (Deaver and Dailey, 1982).

It is possible that the dose and types of pharmacological compound used, and (or) the experimental protocol may account for some of the variability observed in the above studies. Because of this variation, it is very difficult to predict effects of dopamine or its antagonists under different physiological status, but it does appear that circulating steroids affect the activity of the dopaminergic system.

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Role of dopamine in LH secretion in the intact anestrous ewe

It has been hypothesized that steroid-dependent suppression of LH in seasonally

anestrous ewes may be mediated via dopaminergic neurons. Meyer and Goodman (1985) used intact seasonally anestrous ewes and showed that a high dose of pimozide significantly increased LH pulse frequency and mean LH concentration indicating involvement of endogenous dopamine in suppression of LH secretion. This finding was further supported (Meyer and Goodman, 1986) using another potent dopamine receptor antagonist, fluphenazine. In that study, .5 mg/kg BW of fluphenazine significantly increased LH pulse frequency. Przekop et al. (1975) reported that intraventricular infusion of dopamine did not influence release of LH in anestrous ewes. Similarly Deaver and Dailey (1982) used intact anestrous ewes and found out that various doses of dopamine did not affect LH concentration. It has been suggested (Dailey et al., 1987) that dopaminergic systems were operating at maximum capacity in intact ewes and that further stimulation of the post-synaptic pathway was simply ineffective. Lack of LH response to dopamine in the studies by and Deaver and Dailey (1982) does not contradict findings of Meyer and Goodman (1985, 1986) because exogenous dopamine utilized in the studies by Deaver and Dailey (1982) and Przekop et al (1975) could have been converted to norepinephrine in the brain and thus, there may not have provided enough dopamine stimulation at synaptic clefts. Further, it should be recalled that dopamine cannot readily cross the blood brain barrier and thus the sites of action are limited to the ME and pituitary, whereas pimozide can penetrate the blood brain barrier and affect the hypothalamus. Perhaps, using pure dopamine to test the role of dopaminergic system on gonadotropin secretion is not the best choice. In general, dopamine appears to play an inhibitory role on tonic LH release during seasonal anestrous in ewes. In other words suppression of LH secretion by strong negative feedback of estradiol during seasonal anestrous may be mediated via inhibitory dopaminergic neurons (Meyer and Goodman, 1985).

In addition to seasonal anestrous, ewes can become anestrous after prolonged exposure to induced-short day length (photorefractory; Kao et al., 1992). It has been suggested that the role of the dopaminergic system in control of gonadotropin secretion may be different in seasonally anestrous from that in short-day anestrous ewes. The effect of dopamine antagonist, pimozide, on LH secretion in artificial short-day,

photorefractory ewes is opposite to those found in seasonally anestrous ewes (Meyer and Goodman 1985). Pimozide decreased LH pulse frequency and LH mean concentrations in intact photorefractory ewes (Kao et al., 1992). It seems that where dopamine may play an inhibitory role on LH secretion in long-day seasonally anestrous ewes, it may play a stimulatory role in short-day photorefractory anestrous ewes. The contradictory actions of dopamine antagonist in two different physiological states raised the possibility that at least two different catecholaminergic systems may be involved in regulating episodic secretion of LH. Activity of these two systems depends upon steroid feedback and sensitivity of the GnRH pulse generator to this feedback (Dailey et al, 1987; Kao et al., 1992).

The role of dopamine in other farm animals during anestrous periods has not been thoroughly investigated. Daily administration of dopamine D2 antagonist, sulpride, advanced mean time to first ovulation in seasonally anestrous mares, and increased mean FSH concentration and pulse frequency. However, sulpride had no effect on LH concentration or LH pulse frequency (Besognet et al., 1996). In postpartum anestrous beef cows, a bolus injection of metocloramide, another D2 dopamine antagonist, did not alter mean serum LH concentration, pulse frequency, or peak amplitude (Thompson et al., 1992).

Role of dopamine in LH secretion during the estrous cycle

There is evidence that both estradiol and progesterone act on the hypothalamus to modify secretion of GnRH (Goodman, 1987; Clark et al., 1989). It is known that progesterone has a suppressive effect on LH secretion in mammals (Hansel and Convey, 1983). This negative feedback effect of progesterone is prominent during the luteal phase of the estrous cycle when blood progesterone concentration is high. The exact mechanism by which LH secretion is reduced by progesterone is not clear. It appears that LH response to steroids is rapid, but it is unlikely that steroids alter GnRH secretion by directly affecting GnRH neurons, because steroid receptors have not been found in GnRH cells (Malven, 1993c; Thiery and Martin, 1991). Therefore, at least one other set of

neurons must exist in the brain to mediate the effects of these ovarian hormones. From an anatomical and endocrine viewpoint many studies have revealed the significance of interaction between dopamine and steroids on secretion of LH. However, the role of dopamine, and gonadotropin response, may vary dramatically with different levels of circulating steroids (i.e. estradiol and progesterone ratio) during the estrous cycle.

Dopamine and estradiol negative feedback on LH In an *in vitro* system, where hypothalamic tissue was coincubated with pituitary tissue, releasing action of dopamine on LH was blocked by estradiol (Schneider and McCann, 1970). Administration (i.c.v.) of the dopamine agonist, apomorphine, increased plasma LH in estradiol- and progesterone-primed ovariectomized rats (Vijayan and McCann, 1978) indicating that perhaps negative feedback of estradiol is partially mediated via dopamine neurons. In support of this theory estradiol implants in the regions containing dopaminergic cells inhibited LH pulse frequency in rats, and pimozide, a dopamine receptor antagonist, blocked this effect of estradiol (Tadakoro et al., 1986). Schneider and McCann (1970) proposed that the dopaminergic system might be involved in negative feedback mechanism of estradiol on LH secretion in that estradiol exerted negative feedback on tonic secretion by blocking stimulatory effect of dopamine.

During the follicular phase of the estrous cycle, estradiol is the predominant steroid that controls tonic secretion of LH (Goodman and Karsch, 1982). However, the role of dopamine in regulation of gonadotropin during this phase is not clear. Deaver and Dailey (1983) demonstrated that in cyclic ewes intravenous infusion of dopamine reduced LH concentration during the early induced-luteal regression. However, the same dose of dopamine had no effect on LH during the late follicular phase of the estrous cycle. These authors concluded that during periods of estrogen domination, dopamine has no detectable effect on secretion of LH in ewes.

Dopamine and positive feedback of estradiol on LH Schneider and McCann (1970) hypothesized that the dopaminergic system might be also involved in triggering the preovulatory LH surge. In sheep there is evidence that positive feedback of estradiol is mediated via dopaminergic neurons. For instance, it was reported (Robertson and Rakha, 1965) that ovulation could be blocked by chlorpromazine, a dopamine inhibitor.

Jackson (1977) has suggested that dopaminergic mechanisms are involved in regulating estrogen-induced release of LH because estrogen-induced surge of LH, as well as tonic secretion of LH in ovariectomized ewes, was blocked by injection of a high dose of pimozide, a dopamine receptor antagonist (Jackson, 1977). These findings provide evidence for a facilitatory role of dopamine in control of GnRH and LH release prior to preovulatory surge of LH when estradiol exerts a positive feedback on gonadotropin secretion.

Dopamine and negative feedback of progesterone on LH McNneily (1980) suggested that dopamine might be important as an inhibitor of LH secretion in ewes during the breeding season. This author suggested that progesterone might increase activity of hypothalamic dopaminergic pathways and inhibit release of LHRH. During the luteal phase of the estrous cycle, dopamine antagonist (domperidone) administration (i.m.) increased LH pulse frequency and peak amplitude compared to control animals. Similarly, Goodman (1985) utilized (i.v.) different potent dopamine antagonists (fluphenazine and trifluoperazine) and observed an increase in LH pulse frequency compared to the control group. In contrast, low and high doses of pimozide did not affect LH pulse frequency and peak amplitude during the mid-luteal phase of the estrous cycle in ewes (Meyer and Goodman, 1985). Goodman repeated a similar experiment using haloperidol (D2 dopamine antagonist) and observed no change in LH pulse frequency during the luteal phase in ewes. The ability of different dopaminergic antagonists, i.e. fluphenazine and trifluoperazine, to increase secretion of LH during the luteal phase raised the possibility that the dopaminergic neural system actively inhibits release of LH during this phase of the cycle in ewes (Dailey et al., 1987). It is not clear why pimozide and haloperidol were not effective, however the observed difference may be related to differing affinity of these antagonists for subclasses of dopamine receptors. Pimozide and haloperidol have a higher affinity for D2 dopamine receptors than D1 subtype receptors, whereas fluphenazine and trifluoperazine bind to both D1 and D2 receptors (Goodman, 1985), and in fact fluphenazine has a higher affinity for D1 receptors (Jenesen and Daw, 1986). Therefore it is possible that endogenous dopamine exerts its effect on LH secretion via D1 receptors during the luteal phase in sheep. Overall, based

on the results of treatment of luteal-phase ewes with dopamine receptor antagonists, it appears that progesterone may exert its inhibitory effect on LH through a dopaminergic system (Dailey et al., 1987).

Based on the effects of dopamine, dopamine agonists, and dopamine antagonist on LH secretion in seasonally anestrous ewes, ovariectomized ewes, photorefractory anestrous ewes, and cyclic ewes, a conceptual model has been developed (Dailey et al., 1987; Kao et al. 1992). The apparent contradictory action of dopamine antagonists in ewes with different ovarian status (i.e. steroid milieu) raised the possibility that at least two dopaminergic systems (subsystems A and B) are involved in episodic secretion of LH (Figure 3). Subsystem A is steroid independent and is stimulatory to LH (e.g. in anestrous ovariectomized ewes and photorefractory anestrous ewes). Subsystem B is inhibitory to LH secretion and regulated by ovarian steroids (e.g. in seasonally anestrous ewes and during the luteal phase of the estrous cycle)

Although it has been proposed that dopamine inhibits LH secretion during the luteal phase of the estrous cycle in sheep (Dailey et al., 1987), studies in rats and women have shown different results. In progesterone-treated rats, intraventricular dopamine injection increased plasma LH, suggesting a stimulatory role for dopamine (Schneider and McCann, 1970). In healthy women, dopamine antagonist metoclopramide, did not affect serum LH concentration during the mid-luteal phase of the menstrual cycle (Rossmanith et al., 1989). In contrast, serum LH concentration increased after a bolus dose of a dopamine antagonist during the mid-luteal phase of the cycle (Ropert et al., 1984; Seki and Nagata, 1990).

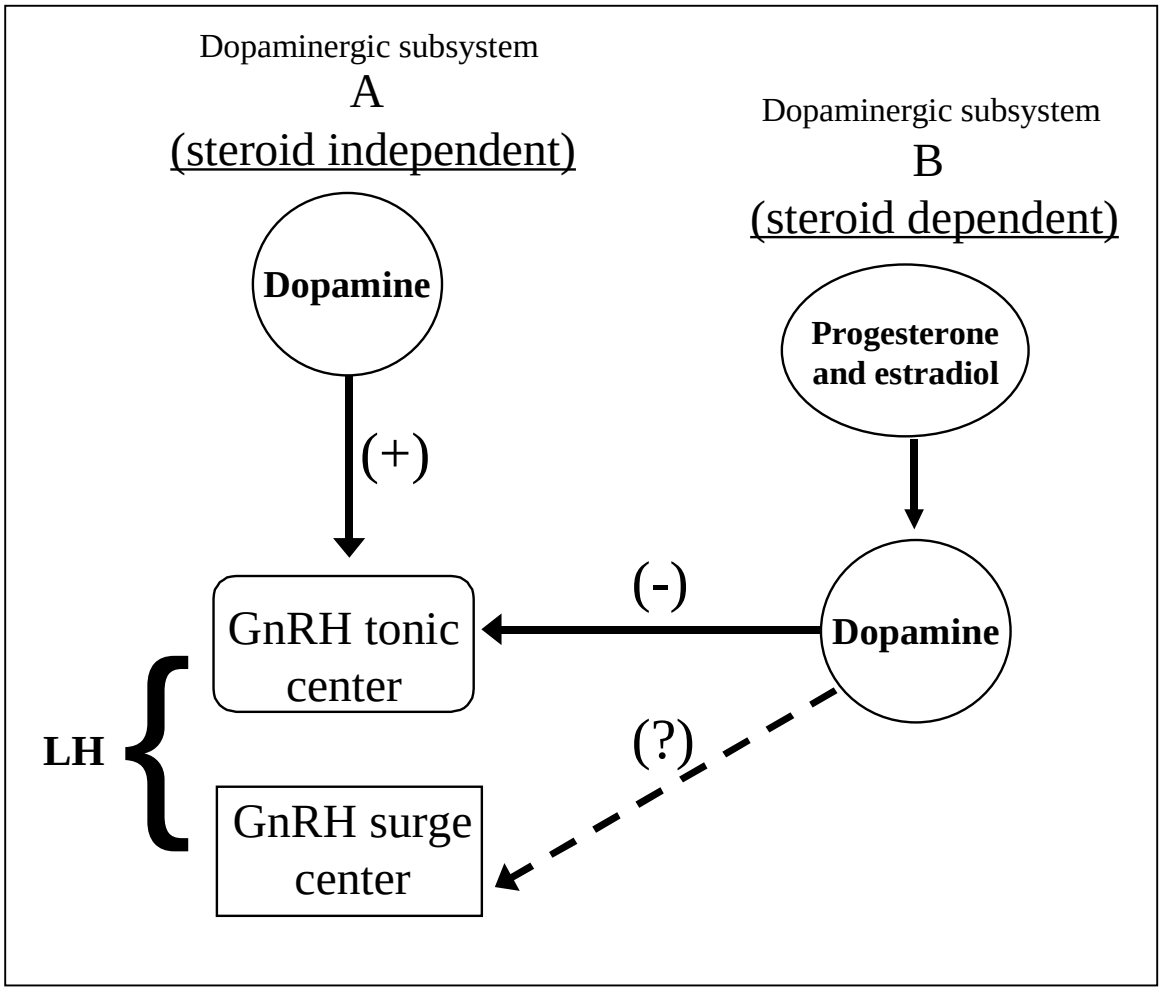

Figure 3. Conceptual model of possible interaction between two dopaminergic systems and ovarian steroids on regulation of episodic secretion of luteinizing hormone in the ewe.

There is limited information concerning the role of dopamine in regulation of gonadotropin secretion during the estrous cycle in cattle. Ergot alkaloids (ergotamine and ergonovine), which have dopamine like activity, decreased LH concentration during the late luteal phase of the estrous cycle in Holstein cows (Browning et al., 1998). It was concluded that dopamine may play an inhibitory role in LH secretion during this phase of the estrous cycle in cattle. However, this effect of ergotamine or ergonovine on LH secretion (Browning et al., 1998) may not be due to activation of the dopaminergic system by these compounds because both of these alkaloids exhibit partial agonistic and antagonistic action on α-adrenergic, and serotonergic receptors (Rall and Schleifer, 1980). Therefore, decrease in LH secretion observed by Browning et al. (1998) could be due to activation of other chatecholaminergic systems and not dopaminergic systems. Although feedback effects of progesterone may be mediated via dopaminergic neurons in the ewe, it is not clear whether the same mechanism exists in cattle. More experiments, utilizing specific dopamine agonists and antagonists, need to be conducted to elucidate the role of dopamine in regulation of LH secretion during this phase of the estrous cycle in cattle.

Dopamine and Follicle Stimulating Hormone Secretion

Follicle stimulating hormone is required for follicular growth, maturation, and subsequent development of an anovulatory follicle. This hormone is a key gonadotropin for steriodogenesis and folliculogenesis, and therefore responsible for triggering physiological events necessary for reproduction in cattle (Ireland, 1987). As discussed in previous sections, there is evidence that dopamine influences LH secretion by influencing GnRH release from the hypothalamus. If GnRH is responsible for release of pituitary FSH, it is also possible that dopamine, by affecting GnRH release, plays a role in control of FSH secretion. Our basic understanding of mechanisms that control secretion of FSH during the estrous cycle may have profound implication relative to management of follicular growth and improved synchronization of the estrous cycle and fertility.

There is limited information concerning actions of biogenic amines on secretion

of FSH. The role of dopamine in control of FSH secretion remains unclear and interpretation of results of previous studies is even more difficult compared to those which pertain to dopamine and LH interaction. The following information reviews some findings of previous studies regarding interactions between FSH and dopamine.

Extracts of Angus Castus, which has dopamine-like activity via D2 receptors, did not affect FSH release from rat pituitary cells, *in vitro* (Jarry et al., 1994). Haloperiodol, an anti-dopaminergic drug, increased FSH secretion in intact 12 day-old female rats (Lacau-megido et al., 1993). In the same study, bromocriptine (ergot alkaloid), blocked haloperidol-induced FSH increase. In males, haloperidol did not affect FSH secretion. However, if males were orchidectomized at birth or at 9 d of age, haloperidol was effective in increasing of FSH during the infantile period. It was concluded that antidopaminergic-induced gonadotropin release is modulated by serum testosterone (Lacau-megido et al., 1993).

Metoclopramide, a D2 receptor antagonist, did not alter serum FSH during the menstrual cycle (Seki and Nagata, 1990). Similarly, metoclopramide did not affect FSH secretion in postmenopausal women (Parra et al., 1997). Similar results were obtained when these subjects were treated with transdermal estradiol. These authors concluded that the dopaminergic system may not be involved in control of gonadotropin release in women.

In sexually inactive rams, bromocriptine (ergot alkaloid) administration in the mediobasal hypothalamus induced a decrease in concentrations of FSH (Tortonse and Lincoln, 1995). It was concluded that the inhibitory effect of bromocriptine is likely mediated through activation of hypothalamic dopamine receptors linked to GnRH neurons regulating FSH secretion. Further, it has been shown that a superovulatory dose of FSH increased plasma dopamine 24 to 48 h after administration in the ewe (Pastorova and Varady, 1996a). Administration of FSH also increased dopamine levels in the POA and ME (Pastorocva and Varady, 1996b). These authors suggested that FSH administration affects catecholamine level and activity of MOA in the POA and ME by means of a feedback mechanism. In contrast concentrations of FSH were not affected when dopamine was infused (i.v.) in ovariectomized, pituitary stalk-transected ewes

(Donnelly and Dailey, 1991).

Besognet et al. (1996) proposed that dopamine inhibits FSH secretion in seasonally anestrous mares because long-term dopamine antagonist (sulpride) administration increased FSH pulse frequency on the first day of treatment and increased mean FSH concentration on day 11 of treatment. In contrast, s.c. administration of sulpride did not affect basal FSH concentration or FSH response to GnRH in geldings (Thompson and DePew, 1997).

Overall, it appears that depending upon species, sex, route of drug administration, animal models, and pharmacological agent used, endogenous dopamine may or may not influence FSH secretion. Because of the extremely important role of FSH in the process of reproduction in cattle, a better understanding of neuroendocrine regulation of FSH secretion may lead to more effective ways to enhance reproductive efficiency in cattle.

Does dopamine play a role in regulation of gonadotropin secretion in cattle?

It is clear that dopamine acts as a neuromodulator of LH secretion in the ewe. As discussed, dopamine mediates effect of steroids on LH secretion and their stimulatory or inhibitory effect on LH secretion appears to be related to stage of the estrous cycle and (or) particular circulating steroid level that is predominant at time of the experiment. Furthermore, dopamine plays an inhibitory role in LH secretion in seasonal anestrous ewes, whereas it plays a stimulatory role in LH secretion in photorefractory anestrous ewes. Whether dopamine acts as a neuromodulator of LH secretion during the estrous cycle and postpartum anestrous period in cattle is not clear. Luteinizing hormone releasing hormone (LHRH) perikarya was concentrated in middle external and lateral anterior subdivisions of the bovine ME as well as the POA (Kizer et al., 1976;Leshine, 1991). Also, dopamine was present in highest concentration in the same subdivisions of the bovine ME and hypothalamus (Kizer et al., 1976;Leshine, 1995). These authors suggested that at the level of hypothalamus and ME, central neuroendocrine regulation of LHRH release may be regulated by dopamine. Currently there is no information to elucidate involvement of dopamine in mediating feedback action of steroids on LH secretion in dairy cattle. Furthermore, although some studies have established that

biogenic amines influence secretion of FSH, there is no information concerning action of dopamine on secretion of FSH. If neural elements mediate the suppressive effect of ovarian steroids on gonadotropins, then the possibility exists that endogenous dopamine may play a role in modulating the effect of progesterone and estradiol on LH and FSH secretion during the estrous cycle in dairy cattle. Figure 4 depicts a conceptual model of possible interactions between the dopaminergic system, ovarian steroids, and GnRH neurons in regulating LH secretion.

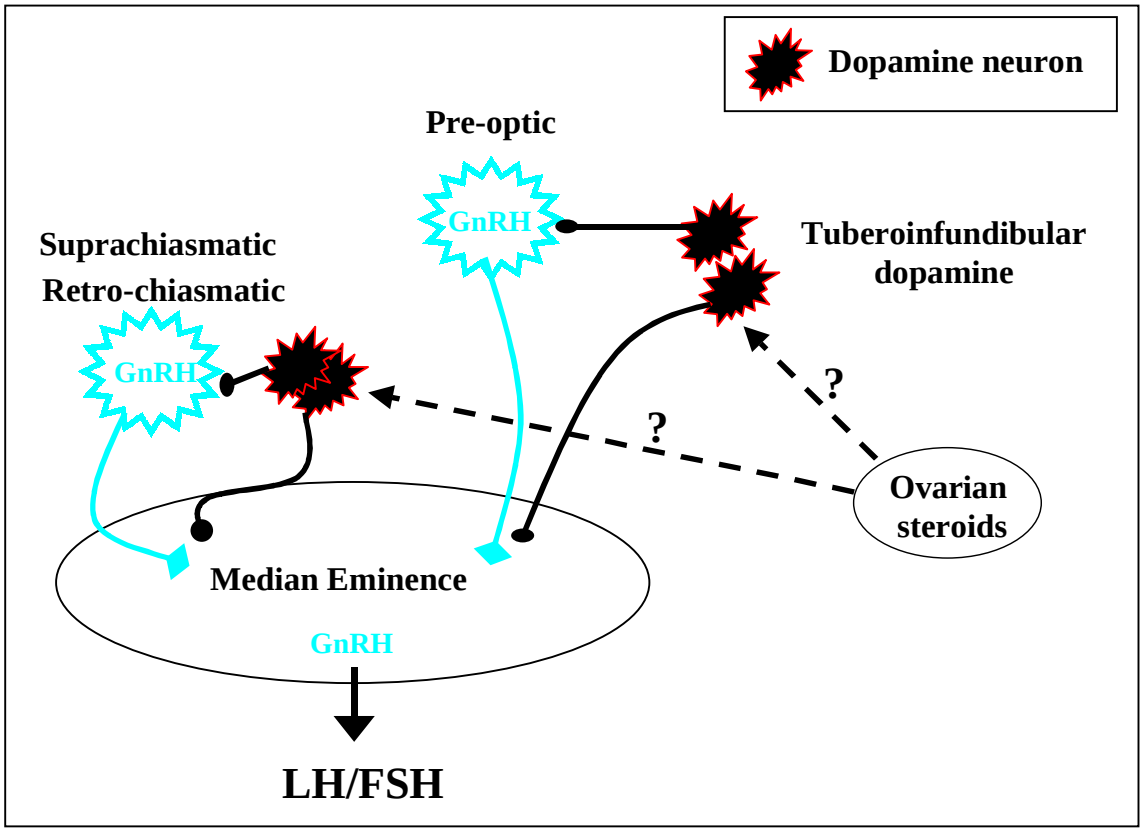

Figure 4. Conceptual model of possible interaction between dopaminergic systems, ovarian steroids, and GnRH neurons on regulation of gonadotropin secretion. Ovarian steroids may stimulate or inhibit dopamine neurons and dopamine neurons in turn synapse on GnRH neurons in the hypothalamus and modify GnRH and gonadotropin secretion.

Role of Dopamine in Growth Hormone Secretion

Growth and development of the mammary gland and ultimately milk secretion (lactation) is governed by coordinated action of several pituitary hormones including GH. Circulating concentrations of GH are closely related with maintenance of lactation and enhanced milk yield (Akers, 1994). It is well documented that GH is galactopoetic in cattle because administration of GH has been shown to increase milk yield (see Peel and Bauman, 1987, for review). Administration of GH is associated with increased mammary blood flow, increased partitioning of dietary nutrient energy toward the mammary gland, and increased voluntary feed intake and feed efficiency (Peel et al. 1983; Akers 1994). Thus, developing strategies to increase secretion of this galactopoetic hormone may enhance lactation yield in cattle. However, successful manipulation of GH secretion from the pituitary will require a better understanding of mechanisms controlling function of the hypothalamic-pituitary axis.

Growth hormone release is controlled by two hypothalamic peptides: GRF and GIF. Many important advances in our understanding of GH secretion have occurred during the last twenty years. It is now evident that secretion of these hypothalamic peptides is regulated by a number of neurotransmitters and peptides present both within and outside the CNS (Buonomo and Baile, 1990). Although scientists have found that various neurotransmitters and neuropeptides are implicated in the control of GRF and somatostatin release, the role of many of these compounds (e.g. serotonin, gamma-amino butyric acid, and dopamine) is still a matter for discussion. It is not clear whether these compounds regulate GH secretion by directly affecting the pituitary or by influencing GRF, and (or) GIF release from the hypothalamus.

The role of dopamine in regulating GH secretion is not well understood and there is disagreement in the literature. Some researchers believe that dopamine is the stimulatory monoamine responsible for release of GH, while other researchers believe that dopamine inhibits GH secretion (see Spencer et al., 1991; Buonomo and Baile, 1990

for review). In humans, there is evidence that supports the existence of a stimulatory role of dopamine in release of GH. Apomorphine, a dopamine agonist, caused a significant increase in GH secretion in normal human subjects (Lal et al., 1973; 1996). Furthermore, L-DOPA, the immediate precursor of dopamine, increased GH secretion and this increased secretion of GH was completely abolished after administration of the dopamine receptor antagonist, pimozide (Liuzzi et al., 1976). These results indicated not only that dopamine might regulate GH secretion, but also that effects more likely were mediated through dopamine receptors. Similar results have been reported in infant rats where L-DOPA increased GH secretion and pretreatment of animals with dopamine antagonist blocked L-DOPA-induced increase of GH secretion (Liuzzi et al., 1976). Also, in adult rats, dopamine agonists, apomorphine and pibedil, caused an increase in GH secretion (Muller et al., 1976), whereas dopamine antagonists inhibited GH secretion (Eden et al., 1979). In contrast, in rats (Chihara et al., 1979) intraventricular injection of dopamine stimulated release of GIF, which may imply that dopamine suppressed GH secretion. Furthermore, in the adult rat, dopamine inhibited GH secretion (Kateo et al., 1973 in Liuzzi et al., 1976). These different results may indicate that the role of dopamine in modulation of GH secretion apparently changes by age and (or) puberty.

Information regarding the role of dopamine in central control of GH secretion in domestic animals is limited, ambiguous, and perhaps species dependent. Hart (1973) reported that a long-acting dopamine agonist (ergocryptine) increased circulating GH in the goat. In contrast, Thompson et al. (1992) showed that administration of a dopamine antagonist, sulpride, did not affect GH secretion in mares. Further, in ovariectomized and pregnant ewes, systemic administration of pure dopamine did not affect basal GH concentration and administration of haloperidol, a dopamine receptor antagonist, was ineffective in changing GH secretion (Elsasser and Bolt, 1987). Similarly, Smith et al. (1974) reported that ergocryptine did not affect GH secretion in lactating Holstein cows indicating that dopamine may not play a role in regulation of GH secretion. Parallel to this finding, Fernandez et al. (1998) reported that fluphenazine, a dopamine receptor antagonist, did not affect GH secretion during 12 and 14 wk of age in prepubertal bulls, indicating that endogenous dopamine may not be the neuromodulator of GH secretion in

prepubertal bulls.

Although *in vivo* studies in cattle appear to indicate that dopamine may not be important in regulating GH secretion, *in vitro* studies clearly indicate that dopamine is an important neuromodulator of GH secretion in cattle. For instance, West et al., (1997) in an *in vitro* study using bovine hypothalamic slices, hypothesized that dopamine plays an inhibitory role on GH secretion. In that experiment, activation of D1 dopaminergic receptors by a potent dopamine agonist (SKF 38393) caused release of GIF and decreased release of GRF from bovine hypothalamic tissue. Furthermore, a D1 receptor antagonist blocked SKF 38393-induced release of somatostatin. Therefore, it is possible that dopamine acts via D1 receptors to suppress GH secretion by stimulating GIF and suppressing GRF. Recently, McMahon et al. (1998) showed that fasting-induced release of GH was blocked by D1 dopamine receptor agonist in meal-fed steers. In addition, GH response to exogenous GRF was lower in the dopamine agonist-treated group compared to the control group. These authors concluded that stimulation of D1 receptors by D1 dopamine agonist increases activity of somatostatin, and this increased activity is associated with suppressed basal- and GRF-induced release of GH in serum of meal-fed steers.

Immunocytochemical staining techniques further support the theory that dopamine plays a role in regulation of GH secretion in cattle. In steers, heifers and cows, dopamine neurons were found throughout periventricular regions, ventrolateral aspects of the arcuate nucleus, and the ependymal layer of the ME (Leshin et al., 1995). In addition, in the bovine, GRH and GIF are synthesized in cell bodies that are located primarily in the arcuate and periventricular nucleus and are released from end terminals in the pituitary stalk/ME (Leshin et al, 1994). Because dopamine neurons, GRF, and GIF perikarya are in such close proximity within the hypothalamus, it is possible that dopamine influences release of GRF and (or) GIF from the bovine hypothalamus and affects GH secretion. Clarification of the role of dopamine in modulation of GH in cattle can enhance our knowledge of neuroendocrine regulation of this galactopoetic hormone and provide some profound implications concerning mammary growth and improvement of milk yield in dairy cows. On the basis of findings from *in vitro* studies (Leshin et al.,

1994), Figure 5 provides a theoretical model which shows possible interactions between the dopaminergic system, GRH, and somatostatin, in regulating GH secretion in cattle.

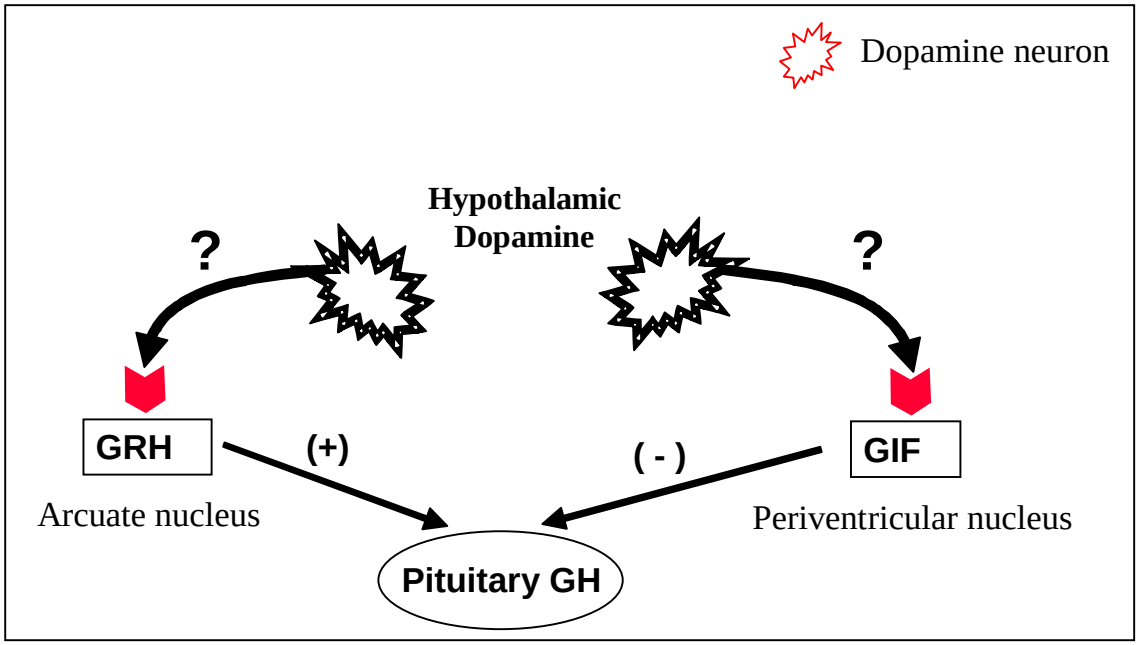

Figure 5. Theoretical model which shows the possible interaction between dopaminergic system, growth hormone releasing hormone (GRH), and somatostatin (GIF) in regulating GH secretion in cattle. There are hypothalamic dopamine neurons which may interact with GRH and GIF neurons in the arcuate and periventricular nuclei, respectively and modify GH secretion.

Rationales for Utilizing Dopamine Antagonist in Studying Dopamine Regulation of Hypothalamic-Pituitary Axis Hormones

The classical approach for studying the role of an endogenous neurotransmitter in control of pituitary hormone secretion consists of administration of pure neurotransmitter or its agonist, or neurotransmitter receptor antagonist, and (or) the combination of both agonist and antagonist. In studying the role of endogenous dopamine in modulation of pituitary hormone secretion, one may use pure dopamine or its agonist (e. g. apomorphine) to determine whether exogenous dopamine affects hormone secretion. Dopamine antagonist (e.g. pimozide, fluphenazine) alone, and (or) its combination with dopamine agonist can be utilized to determine whether endogenous dopamine is involved in regulating hormone secretion and whether any effects are specifically mediated through dopamine receptors. However, the use of pure dopamine poses problems in studying dopamine regulation of hypothalamic-pituitary axis hormones for the following reasons:

1) The liable nature of dopamine limits its use for intravenous infusion because its presence in peripheral circulation is extremely short-lived. For instance, it has been reported that plasma dopamine concentration in sheep declined to 2% of initial concentration within 9 min (Elsasser and Bolt, 1987).

2) The structure of dopamine is such that it cannot penetrate the blood brain barrier (Dailey et al., 1987). Thus, the only sites of dopamine action are limited to the ME and the pituitary gland. If the investigator's hypothesis is that endogenous dopamine regulates anterior pituitary hormones by affecting releasing hormone cell bodies (e.g. GnRH, somatostatin perikarya, etc.) then, exogenous dopamine cannot be used to test the hypothesis because exogenous dopamine cannot reach hypothalamic sites.

3) Exogenous dopamine in brain tissue can be converted into norepinephrine, by β-hydroxylase enzyme, and activate noradrenergic system. Therefore, one cannot be certain whether observed physiological changes after pure dopamine infusion is

attributable to α-adrenergic activity or dopaminergic activity.

The classical dopamine agonist, apomorphine, is easily available and affordable for *in vivo* experiments. This compound is a potent dopamine agonist and can penetrate the blood brain barrier. However, apomorphine has a high affinity for D2 receptors and thus can affect presynaptic receptors (autoreceptors). As previously mentioned, these receptors are the major autoregulators of dopamine turnover rate. Apomorphine can decrease dopamine turnover rate rather drastically and quickly (Cooper et al., 1996). Even if apomorphine administration influences pituitary hormone secretion, this will not provide information as to whether the effect was due to mimicking the action of dopamine at postsynaptic receptors or decreasing endogenous dopamine turnover rate by acting upon presynaptic receptors.

These reasons against using a dopamine agonist justify use of a dopamine receptor antagonist to examine effects of endogenous dopamine in regulation of anterior pituitary hormones in Holstein cows during the early postpartum period and various stages of the estrous cycle.

Characteristics of Fluphenazine Hydrochloride: the dopamine antagonist of choice for conducting experiments

Fluphenazine (FLU) hydrochloride ($C_{22}H_{26}F_3OS.2HCL$; MW=510.4) is a white, odorless crystalline powder that is soluble at dilution of 1:10 in water. Fluphenazine is a high potency typical neuroleptic drug. The common name of this drug in the U.S. is Prolixin. Other common names include Anatensol, Dapotum, Flunazine, Funazine, Moditen,l, Pacinol, Permitil, and Sediten (Long, 1998).

Fluphenazine differs from some other phenothiazine derivatives in several respects: It has less effect on CNS depressants and anesthetics than do some phenothiazines and appears to be less sedating. However, FLU is among the group of phenothiazines which exhibit a greater propensity for producing extrapyramidal reactions such as muscle tremors, rigidity, spasm, and irregular, involuntary movement. A study to determine absorption and fate of FLU in humans indicated that after a single dose of FLU

was administered i.m., plasma half life was 14.9-15.3 h. Also it has been reported that following i.m. administration, onset of action takes place in approximately 1 h and has a duration of 6 to 8 h from a single dose (Long, 1998). Fluphenazine is widely distributed into body tissues and fluids, and it does cross the blood-brain barrier. Fluphenazine metabolism in the liver is extensive, with metabolites contributing about 50% of antipsychotic activity (Long, 1998).

It has been shown that FLU has a high affinity for D1 subtype receptors; (Johnson et al., 1995). However, other studies have shown that FLU also binds to D2 receptors (Kokay and Mercer, 1996). Coirini et al. (1997) proposed that in the rat basal ganglia, FLU binds with D2 receptors uniformly, whereas it alters D1-recpetor binding in a region-dependent manner. Jensen and Daw, (1986) and Sawaguchi et al. (1990) have shown that FLU has equal affinity for both subtype receptors. Overall, it appears that FLU has affinity for both subtype receptors and its binding ability may change depending upon dose and different brain regions (Coirini et al., 1997;Oneill et al., 1991).

Experimental Objectives and Rationale

Objective 1

Luteinizing hormone plays an important role in regulating reproductive activity of dairy cattle and dopamine may be involved in LH modulation. Circulating concentrations of LH are low during the early postpartum period in dairy cows (Edgerton and Hafs, 1973), apparently due to a decreased frequency of LH pulses. The role of endogenous opioids in suppression of LH during this period is evident (Ahmadzadeh et al., 1998a). It is also apparent that in certain situations opioids may exert their effect on LH secretion via dopaminergic neurons (see Barraclough and Wise, 1982;Thiery and Martin, 1991 for review). However, the effect of dopamine in regulation of LH secretion has been implicated mostly in ewes and there is no information available concerning effects of endogenous dopamine on pituitary LH secretion in early postpartum, anovulatory dairy cattle. Thus, the first objective of this study was:

To investigate the role of endogenous dopamine in modulation of pituitary LH secretion by characterizing serum LH concentration and pulse frequency in response to administration of a dopamine antagonist (FLU) in primiparous and multiparous anovulatory dairy cows during the early postpartum period.

Objective 2

Circulating concentrations of GH are closely related with maintenance and enhancement of milk yield in cattle (Akers, 1994). Thus, developing strategies to alter secretion of this galactopoetic hormone may enhance lactation in cows. However, successful manipulation of GH secretion from the pituitary gland requires a better

understanding of mechanisms controlling function of the hypothalamic-pituitary axis. Although there is evidence that dopamine plays a role in modulation of GH secretion in some species (Buonomo and Baile, 1990), there is very limited information concerning action of dopamine on secretion of GH in lactating dairy cattle. Thus, the second objective was:

To investigate the role of endogenous dopamine in modulation of pituitary GH secretion by characterizing serum GH in response to administration of a dopamine antagonist (FLU) in primiparous and multiparous anovulatory dairy cows during the early postpartum period.

Objective 3

Compelling evidence indicates that dopamine is involved in LH modulation during the estrous cycle in the ewe (Dailey et al., 1987). These observations suggest that in certain situations (varies with ovarian status and steroid milieu) steroid modulation of the LH pulse generator may be mediated via dopaminergic systems. However, involvement of endogenous dopamine in mediating feedback actions of progesterone and estradiol on gonadotropin secretion in dairy cattle has not been investigated. The third objective was:

To determine the effect of the dopamine antagonist, FLU, on LH and FSH secretion during the luteal, follicular, and metestrus phases of the estrous cycle in Holstein cows.

Objective 4

It is known that sex steroids (estrogen and progesterone) can affect GH secretion (see Gluckman et al., 1987 for review). It also appears that various neurotransmitters

including dopamine affect GH secretion (Buonomo and Baile, 1990). There is evidence that steroids and some of their metabolites (catecholestrogen) may influence action of dopamine either by affecting dopamine synthesis and turnover, or by affecting dopamine receptors. For example, Fuxe et al., (1980) showed that estradiol 17β directly activated dopaminergic neurons in the lateral tuberoinfundibular system. Therefore, it is possible that gonadal steroids affect GH secretion and these effects, in part, are modified by endogenous dopamine. Studies investigating interactions between steroids, dopamine and GH secretion are nonexistent. Therefore, the fourth objective was:

To examine the role of endogenous dopamine in regulation of GH secretion by characterizing serum GH response to the dopamine antagonist, FLU, during the luteal, follicular, and metestrus phases of the estrous cycle in lactating Holstein cows and determine whether the effects differ from those in anovulatory cows during early postpartum period.

To achieve these objectives, Four experiments were conducted at the Virginia Tech Dairy Facilities, Blacksburg Virginia. The study was initiated in spring, 1995 and completed in fall, 1997.

CHAPTER 1

INVOLVEMENT OF DOPAMINE IN MODULATION OF LUTEINIZING HORMONE, GROWTH HORMONE, AND PROLACTIN SECRETION IN ANOVULATORY HOLSTEIN COWS DURING THE EARLY POSTPARTUM PERIOD

ABSTRACT

Two experiments investigated the effect of fluphenazine (FLU), a dopamine antagonist, on pituitary LH, growth hormone (GH), and prolactin (PRL) secretion in anovulatory primi- and multiparous Holstein cows. In Experiment 1, 12 primiparous cows were randomly assigned to receive either saline (n=6) or .3 mg FLU/kg BW (n=6) in wk 2 postpartum. Blood samples were collected at 15 min intervals for 4 h before and 4 h after saline or FLU. Immediately thereafter, all cows received 25 ug of GnRH and 90 ug of growth hormone releasing hormone (GRH) and blood collection continued for an additional 1.5 h. Mean serum progesterone concentration was .13 ± .08 ng/ml. There was no difference in mean serum LH and GH concentrations between groups prior to treatments. Fluphenazine caused a transient decrease ($P < .05$) in mean serum LH concentration and decreased ($P < .01$) LH pulse frequency and pulse amplitude. Mean serum LH remained unchanged in saline-treated cows. Fluphenazine caused a decrease ($P < .05$) in mean serum GH concentration (from 5.8 ± .3 to 3.9 ± .3 ng/ml). Mean serum GH concentration remained unchanged in saline-treated cows. In Experiment 2, 6 anovulatory multiparous cows (wk 2 postpartum) were used and all cows received FLU (.3 mg/kg BW). Experimental procedures were the same as used with primiparous cows, however the GRH dose used was increased to 110 ug. Mean progesterone concentration was .12 ± .04 ng/ml. Fluphenazine decreased ($P < .05$) mean serum LH concentration and LH pulse frequency, but not LH pulse amplitude. Mean serum GH concentration decreased ($P < .05$) in response to FLU (from 5.7 ± .3 to 3.2 ± .3 ng/ml) and mean PRL concentration increased ($P < .01$; from 8.2 ± 8.9 to 75.6 ± 8.9 ng/ml) in all cows. Exogenous GnRH increased ($P < 0.01$) LH concentration in all cows in both experiments

and there was no effect of treatment on LH response to GnRH. Exogenous GRH increased ($P < .1$) serum GH concentration in both experiments. Mean serum PRL concentration increased ($P < .01$) after FLU administration in both experiments. These results suggest that modulation of LH and GH secretion, at least in part, is mediated via endogenous dopamine in anovulatory cows during the early postpartum period regardless of parity. Data also confirm that endogenous dopamine plays an inhibitory role in PRL secretion in lactating cows.

Key Words: Dopamine antagonist, Luteinizing hormone, Growth hormone, Anovulatory dairy cattle

INTRODUCTION

Circulating concentration of LH is low during the early postpartum period in dairy cattle (Edgerton and Haffs, 1973). The amount of LH secreted from the anterior pituitary is insufficient to initiate cyclicity during the early postpartum presumably due to infrequent discharge of GnRH into the hypothalamic-hypophysial portal circulation (Walters et al., 1982). The anterior pituitary gland secretes pulses of LH in response to episodic release of GnRH from the hypothalamus, and involvement of neural components is evident from the episodic discharge characteristics of GnRH and LH secretion (Goodman and Karsch, 1980). Thus, suppression of the mechanism which stimulates GnRH release may be responsible for prolongation of the postpartum period.

Extensive studies, primarily in laboratory rodents, have shown that dopamine stimulates secretion of LH (see Barraclough and Wise, 1982 for review). However, the role of dopamine in control of LH secretion remains controversial. For instance, Kamberi et al. (1970) showed that infusion of dopamine into the third ventricle stimulated LH secretion in female rats. Sawyer et al. (1974) reported that intraventricular norepinephrine stimulated LH release in the rabbit, whereas dopamine not only failed to increase plasma LH but also blocked the stimulatory effect of norepinephrine. Dopamine injection into the third ventricle had no effect in cyclic rats, but caused an increase in LH secretion in progesterone-treated ovariectomized rats (Schinder and McCann, 1970). These authors concluded that depending upon the physiological status of the animals, dopamine effects on LH secretion were different.

In farm animals most studies regarding the role of dopamine in LH secretion have been conducted in ewes (see Dailey et al., 1987 for review). Early work by Jackson (1977) demonstrated that pimozide (dopamine antagonist) suppressed tonic LH secretion in ovariectomized ewes. A single dose of FLU (.5 mg/kg BW) did not affect LH pulse frequency but suppressed peak amplitude and mean LH concentrations in ovariectomized ewes (Meyer and Goodman, 1986) indicating that, in the absence of ovarian steroids, dopamine is stimulatory to LH secretion. Additionally, Kao and coworkers (1992) showed that pimozide suppressed LH pulse frequency and amplitude in both ovary-intact and ovariectomized photorefractory ewes. In contrast, dopamine antagonists exerted an opposite effect on LH secretion and increased pulsatile secretion of LH in intact anestrous ewes (Meyer and Goodman, 1985), and dopamine agonist suppressed LH pulse frequency in both anestrous and breeding season ewes which were ovariectomized (Meyer and Goodman, 1986). Metoclopramide, a D2 dopamine receptor antagonist, had no effect on LH secretion in anestrous primiparous beef cows (Thompson et al., 1992). In seasonally anestrous mares, daily administration of the dopamine D2 antagonist, sulpride, shortened mean time to first ovulation but had no effect on LH concentration or LH pulse frequency (Besognet et al., 1996). It appears that depending on the species and (or) physiological status of the animal, dopaminergic input under one set of circumstances may be stimulatory and under another set of circumstances may be inhibitory (Thiery and Martin, 1991).

The interaction between hypothalamic GnRH secretion and dopamine has been shown in the ewe. The dopamine system acts in the retrochiasmatic and (or) ME to suppress GnRH pulse frequency in the ovary-intact anestrous ewe because placement of dopamine antagonist in these regions increased LH pulse frequency and mean LH concentrations (Havern et al., 1991). In cattle, the ME and POA adjacent to the retrochiasmatic region contain LHRH neurons (Kizer et al., 1976; Leshin et al., 1988; 1991). Retrochisamatic divisions of the supraoptic nucleus, suprachiasmatic and pituitary stalk-median eminence contained dopaminergic neurons in cattle (Leshin et al., 1995). Although there is no evidence in the cow, it is likely that dopamine neurons within the

brain act to modify secretion of GnRH and LH.

Growth hormone release is controlled by two hypothalamic peptides: growth hormone releasing hormone (GRH) and somatostatin or growth hormone inhibiting factor (GIF). The secretion of GRH and GIF is regulated by a variety of neurotransmitters and peptides present within and outside the central nervous systems (Buonomo and Baile, 1990). There is ample evidence that neurotransmitters including dopamine, noradrenaline and serotonin are involved in regulation of GH secretion. The role of dopamine on GH secretion is not clear and there is disagreement in the literature. Some researchers believe dopamine to be the major stimulatory monoamine for release of GH while others believe the exact opposite is the case (see Buonomo and Baile, 1990; Spencer et al., 1991 for review). In humans, there is evidence that dopamine stimulates GH secretion because apomorphine, a dopamine agonist, increased GH secretion (Lal et al. 1972 and 1996). Furthermore, L-DOPA, the immediate precursor of dopamine, increased GH secretion, and this increased secretion of GH was completely abolished after administration of the dopamine receptor antagonist, pimozide (Liuzzi et al., 1976). In adult rats dopamine agonists, apomorphine and pibedil, caused an increase in GH secretion (Mueller et al., 1976), whereas dopamine antagonists inhibited GH secretion (Eden et al., 1977). However, it has been shown in rats (Chihara et al., 1979) that intraventricular injection of dopamine stimulates release of GIF, which may imply that dopamine suppresses GH secretion in rats. In farm animals, investigations to determine the effect of dopamine on GH are ambiguous and perhaps species dependent. Administration of L-DOPA alone had no effect on plasma GH release in sheep (Davis and Borger, 1973). Smith et al. (1974) showed that an ergot alkaloid, which has dopamine-like activity, did not affect GH secretion in lactating Holstein cows, whereas the same compound increased circulating GH in goats (Hart, 1973). Further, it has been shown (Elsasser and Bolt, 1987) that in ovariectomized and pregnant ewes, systemic administration of pure dopamine did not affect basal GH concentration and administration of a dopamine receptor antagonist was ineffective in changing GH secretion. Moreover, Thompson et al. (1992) reported that administration of a dopamine antagonist did not affect GH secretion in mares.

Growth hormone-releasing hormone and GIF are synthesized in the arcuate and periventricular nuclei, respectively, and released from axon terminals in the pituitary stalk/median eminence (Leshin et al., 1994). In the bovine, immunocytochemical techniques revealed that dopaminergic neurons innervate the periventricular, arcuate nuclei, and the pituitary stalk/median eminence (Leshine et al., 1995). West et al. (1997) in an *in vitro* study, using heifer hypothalamic slices, showed that activation of the D1 dopaminergic receptor by a potent dopamine agonist (SKF 38393) caused release of GIF and decreased release of GRF. These *in vitro* studies suggest that dopamine may be inhibitory to GH secretion in cattle.

There is very limited information concerning action of dopamine in regulation of gonadotropin and GH secretion in lactating dairy cows. Thus, the objective of the present study was to investigate the role of dopamine in regulation of gonadotropin and GH secretion by characterizing serum LH and GH response to the dopamine receptor antagonist, FLU, in anovulatory Holstein cows during the early postpartum period. Because it is well established that dopamine inhibits PRL secretion in cattle, serum PRL concentrations in response to FLU were measured as positive controls to ensure that dopamine receptors were effectively blocked.

MATERIALS AND METHODS

Experiment 1

Twelve early postpartum primiparous Holstein cows between 24 and 31 mo of age (mean BW = 486 ± 68 kg) from the Virginia Tech Dairy Center, Blacksburg, VA were used. The experiment was conducted from February through April. All cows were fed a total mixed ration which was balanced for all nutrient requirements for milk yield of 40 kg/d according to NRC recommendation starting at parturition (DM: 53%, CP: 17.4%, ADF: 20%, NEL: 1.65 Mcal/kg of DM). Calves were removed from their dams within 24 h after parturition. On the day before the experiment all cows were weighed and the ovaries were examined by ultrasound scanner. Subsequently, cows were fitted with jugular catheters (Johnson et al., 1993) and randomly assigned to receive either .9%

saline (n=6) or .3 mg FLU/kg BW (Sigma Chemical Corp., St. Louis, MO; n=6). On d 13 or 14 postpartum, beginning at 0500 h blood samples were collected at 15 min intervals for 4 h before and 4 h after FLU or saline. Fluphenazine was dissolved in 10 ml of 25^{o}C physiological saline. Four h post-treatment (1300 h), all cows received (i.v.) 25 ug GnRH (Cystorelin®, Sanofi, Overland Park, KS) and 90 ug bovine GRH (Sigma Chemical Crop., St. Louis, MO; dissolved in physiological saline) and blood collection continued for another 1.5 h. This low dose of GnRH and GRH was administered to test pituitary competency to release of LH and GH and also to observe if FLU treatment effects LH and GH response to GnRH and GRH, respectively. Blood samples were placed in ice immediately after withdrawal and then stored at 4^{o}C for 24 h to allow clotting. All blood samples were centrifuged for 30 min at 2,200 × g at 4^{o}C. Serum was harvested and stored at -20^{o}C until assayed for LH, PRL, and progesterone content. During blood sampling all cows were tied in individual stalls with access to a total mixed ration and water *ad libitum.*

Experiment 2.

Six lactating multiparous Holstein cows (mean BW = 590 ± 44 kg) were used in this experiment. On d 13 or 14 postpartum, all cows received .3 mg FLU/kg BW. Blood samples were collected for 4 h as baseline sampling periods and thus each animal served as its own control. Blood samples were collected for 4 h after FLU administration. Immediately thereafter, cows received GnRH (25 ug) and 110 ug GRH and blood collection continued for an additional 1.5 h. Experimental procedures were the same as those used with primiparous cows

Hormone Assay

Concentration of LH was determined by a double-antibody RIA as described by Bolt and Caldwell (1993). This assay was performed in non-equilibrium condition. Purified LH (USDA-bLH-B-6) was used as the reference standard and for radioiodination. Sheep anti-rabbit gammaglobulin was used as the precipitating second

antibody. The primary antiserum (USDA-309-684p) bound approximately 40% of radiolabeled LH in the absence of unlabeled hormone. Sensitivity of the assay was .08 ng/ml reference standard LH and defined as the concentration corresponding to 2 SD less than mean zero dose tubes. All samples were assayed in duplicate, and intra- and inter-assay CV calculated from serum pools averaged 8.3% and 13.2%, respectively.

Concentration of GH was determined by a double-antibody RIA by method of Barnes et al. (1985). Purified bovine GH [bGH Cynamide 6952 (-42A)] and (USDA-bGH-I-1) were used as the reference standard and for radioiodination, respectively. Sheep anti-rabbit gammaglobulin was used as the precipitating second antibody. Primary antibody was raised in rabbits using bovine GH (NIH-GH-B18). The primary antiserum bound 40% of radiolabeled GH in the absence of unlabeled hormone. The sensitivity of the assay was less than .3 ng/ml reference standard GH and defined as the concentration corresponding to 2 standard deviations less than mean zero dose tubes. All samples were assayed in duplicate, and intra- and inter-assay CV calculated from serum pools averaged 6.9% and 8.2%, respectively.

Concentration of PRL was determined by a double-antibody RIA by the method of Barnes et al. (1985). Sensitivity of the assay was less than 1.25 ng/ml reference standard. All samples were assayed in duplicate and intra- and inter-assay CV were 6% and 14.4%, respectively.

Concentration of progesterone was quantified using a solid-phase RIA (Diagnostic Products Corp.; Los Angeles, CA) which has been previously validated in our laboratory (Holt et al., 1989). The standard curve ranged from 0.1 to 20 ng/ml. All samples were assayed in duplicate (1 assay) and the intra-assay CV was 4.1%.

Statistical Analysis

Experiment 1. Serum LH, GH and PRL data were analyzed by least-squares analysis of variance by the General Linear Model (GLM) procedure using Statistical Analysis Systems (SAS® Institute, Cary, NC). To determine the effect of FLU on LH and GH, blood samples were categorized to one of three periods. Period 1 represented blood

samples collected for 4 h before saline or FLU administration and was considered the pre-treatment period. Period 2 represented blood samples collected for 2 h immediately after FLU or saline administration; and samples thereafter (2 h) represented period 3. To determine the effect of FLU on PRL, blood samples were also assigned to one of three periods. However, period 1 represented blood samples collected for only 2 h before saline or FLU administration and was considered the pre-treatment period. Period 2 represented blood samples collected for 2 h immediately after FLU or saline administration, and blood sample thereafter (2 h) represented Period 3. The experiment was designed to determine the effects of FLU on LH and PRL concentrations in period 1 compared to periods 2 and 3. The statistical model included treatment, cow within treatment, period, and all the possible interactions. Period by cow within treatment was used as error term to test the effects of period and period by treatment. If period or period by treatment effects were significant ($P < .05$), non-orthogonal contrasts were used to compare least squares means for periods 1 vs 2, 1 vs 3, and 2 vs 3 within each treatment group using the improved Bonferroni F-test (1977).

To determine the effect of GnRH on serum LH concentration, the sampling interval was divided into two periods. The pre-GnRH period represented serum samples collected 1.5 hr prior to GnRH administration, and the post-GnRH period represented samples collected for 1.5 hr after GnRH treatment. Differences between the two periods were tested using the same statistical model as above.

To determine the effect of GRH on serum GH concentration, the sampling interval was divided into 2 periods. The pre-GRH period represented the serum samples collected 1.5 hr prior to GRH administration and the post-GRH period represented samples collected for 1.5 hr after GRH treatment. Differences between the two periods were tested using the same statistical model as above.

Experiment 2. Hormone data were analyzed as in Experiment 1 except that the statistical model for testing the dependent variables included period, cow and cow by period. Cow by period interaction was used as the error term to test the period effect.

Pulses of LH were determined as described by Goodman and Karsch (1980) and

modified by Richards et al. (1991). Briefly, any value of LH greater than two SD above the mean for a cow followed by at least 2 values of lesser concentration was considered a pulse. In addition, a peak had to occur within two samples of previous nadir and the amplitude of the peak had to be greater than the sensitivity of the LH assays. The amplitude of the hormone pulse was the difference between the greatest concentration during the pulse and the nadir within 30 min before the pulse. Pulse frequency and peak amplitude of LH were analyzed by least-squares analysis of variance by the GLM procedure using Statistical Analysis Systems (SAS Institute, Cary, NC). The statistical model for Experiment 1 included treatment, cow within treatment, period, and period by treatment interaction. Using the improved Bonferroni F-test (1977), the least square means for LH pulse frequency and peak amplitude between pre- and post-treatment periods were compared. For Experiment 2, LSM of LH pulse frequency and peak amplitude between pre- and post-treatment period were compared by student *t*-test.

RESULTS

Experiment 1

Results of ultrasonography conducted on the day before the experiment verified the absence of a corpus luteum in all cows. Mean diameter of the largest follicle was 11 ± 5 mm. Mean serum progesterone concentration on the day of experiment was .13 ± .08 ng/ml. None of the cows exhibited any estrous behavior prior to, or on the day of experiment. This information indicated a lack of ovarian activity and that the experiment was conducted in anestrous cows.

Serum PRL concentrations were similar for both treatment groups during the pre-treatment period (Table 1). Fluphenazine elicited an increase ($P < .01$) in mean serum PRL concentration compared to the pre-treatment period (Table 1). Serum PRL concentration increased by more than 8 fold from 14.4 ± 3.1 before FLU to 118.1 ± 3.1 ng/ml during the first 2 h after FLU and remained elevated (89.2 ± 5.1 ng/ml) throughout the experiment (Figure 1). Serum PRL concentration remained unchanged in saline-treated cows (Table 1). These results indicated that the dose of FLU was sufficient to block dopamine receptors and cause a physiological change in cows.

Table 1. Mean prolactin (PRL) concentrations[a] in primiparous Holstein cows treated with saline or fluphenazine (FLU) on d 13 or 14 postpartum.

	Mean serum PRL concentration (ng/ml)		
Treatment	Period [b] 1	Period 2	Period 3
Saline (n=7)	18.4 ± 3.1	22.4 ± 3.1	13.4 ± 5.3
FLU (.3 mg/kg BW;n=8)	14.4 ± 3.1 [c]	118.1 ± 3.1 [d]	89.2 ± 5.1 [d]

[a] Least squares means ± SEM.

[b] Period 1= Mean hormone concentration of 8 blood samples collected before fluphenazine or saline administration from 0715 to 0900 h; period 2= Mean hormone concentration of 8 blood samples collected after fluphenazine or saline administration from 0915 to 1100 h; period 3= Mean hormone concentration of 4 blood samples collected from 1130 to 1300 h (every 30 min sample).

[c,d] Means with different superscripts differ within the same treatment row ($P < .01$).

Mean serum LH concentrations during the sampling period for both treatment groups are shown in Table 2. Serum LH concentrations were similar for saline- and FLU-treated groups during the pretreatment period. Fluphenazine caused a decrease ($P < .05$) in serum LH concentration (Table 2). Mean serum LH concentration decreased from .26

Table 2. Mean LH concentrations[a] in primiparous Holstein cows treated with saline or fluphenazine (FLU) on d 13 or 14 postpartum.

	Mean serum LH concentration (ng/ml)		
Treatment	Period [b] 1	Period 2	Period 3
Saline (n=6)	.30 ± .02	.30 ± .03	.24 ± .03
FLU (.3 mg/kg BW;n=6)	.26 ± .02 [c]	.19 ± .03 [c]	.14 ± .03 [d]

[a] Least squares means ± SEM.

[b] Period 1= Mean hormone concentration of 17 blood samples collected before fluphenazine or saline administration from 0500 to 0900 h; period 2= Mean hormone concentration of 8 blood samples collected after fluphenazine or saline administration from 0915 to 1100 h; period 3= Mean hormone concentration of 8 blood samples collected from 1115 to 1300 h.

[c,d] Means with different superscripts differ within the same treatment row ($P < .05$).

± .02 in period 1 to .19 ± .03 ng/ml during period 2 and then further decreased ($P < .05$) to .14 ± .03 ng/ml during period 3. Saline administration did not affect LH concentration (Table 2; Figure 2).

Mean LH pulse frequency and peak amplitude were also decreased ($P < .05$) after FLU administration (Table 3). LH pulse frequency was completely abolished in 4 cows after FLU. However, LH pulse frequency and peak amplitude were unaffected by FLU in 2 other cows. Mean serum LH concentration, pulse frequency, and peak amplitude were not altered by saline treatment (Table 3).

Table 3. Mean LH pulse frequency and peak amplitude[a] in primiparous Holstein cows treated with saline or fluphenazine (FLU) on d 13 or 14 postpartum.

	Mean LH pulse frequency (pulses /4 h)		Mean LH peak amplitude (ng/ml)	
Treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
Saline (n=6)	1.3 ± .2	1.0 ± .2	.41 ± .09	.30 ± .09
FLU (.3 mg/kg BW; n=6)	1.3 ± .2	.3 ± .2 *	.35 ± .09	.06 ± .09*

[a] Least squares means ± SEM.

* Different from pre-treatment mean ($P < .05$).

Exogenous GnRH increased ($P < .01$) serum LH concentration in both treatment groups (Table 4). Although the magnitude of LH response was slightly higher for FLU-treated cows, there was no significant effect of FLU on LH response to GnRH (Figure 3).

Serum GH concentrations were similar for both FLU- and saline-treated groups during the pre-treatment period. Fluphenazine caused a decrease ($P < .05$) in serum GH concentration within the last 2 h after FLU administration compared to the pre- treatment period (Table 5). Serum GH concentration decreased ($P < .1$) from 5.8 ± .3 ng/ml during period 1 to 4.3 ± .4 ng/ml in period 2, and then further decreased ($P < .05$) to 3.9 ± .4 ng/ml in period 3 (Figure 4). Serum GH concentration was unaffected by saline treatment (Table 5).

Table 4. Mean serum LH concentrations[a] in primiparous Holstein cows treated with saline or fluphenazine (FLU) before and after GnRH (25 ug) administration on d 13 or 14 postpartum.

	Mean serum LH concentration (ng/ml)	
Treatment	Pre-GnRH [b]	Post-GnRH
Saline (n=6)	.24 ± .34 [c]	1.74 ± .34 [d]
FLU (.3 mg/kg BW; n=6)	.14 ± .34 [c]	1.94 ± .34 [d]

[a] Least squares means ± standard error.

[b] Pre-GnRH=Mean hormone concentration of 6 blood samples collected 1.5 h prior to GnRH administration; Post-GnRH=Mean hormone concentration of 6 blood samples collected 1.5 h after GnRH administration.

[c,d] Means with different superscripts differ within the same treatment row ($P < .01$).

Table 5. Mean GH concentrations[a] in primiparous Holstein cows treated with saline or fluphenazine (FLU) on d 13 or 14 postpartum.

	Mean serum GH concentration (ng/ml)		
Treatment	Period [b] 1	Period 2	Period 3
Saline (n=6)	6.4 ± .3	7.2 ± .4	7.1 ± .4
FLU (.3 mg/kg BW;n=6)	5.8 ± .3 [c]	4.3 ± .4 [c]	3.9 ± .4 [d]

[a] Least squares means ± SEM.

[b] Period 1= Mean hormone concentration of 17 blood samples collected before fluphenazine or saline administration from 0500 to 0900 h; period 2= Mean hormone concentration of 8 blood samples collected after fluphenazine or saline administration from 0915 to 1100 h; period 3= Mean hormone concentration of 8 blood samples collected from 1115 to 1300 h.

[c,d] Means within the same treatment row with different superscripts differ ($P < .05$).

Administration of GRH increased ($P < .1$) serum GH in all cows (Table 6; Figure 4). There was a significant ($P < .05$) time by treatment interaction in that, GH response to exogenous GRH was delayed for 30 min in FLU–treated group, whereas all saline-treated

Table 6. Mean serum GH concentrations[a] in primiparous Holstein cows treated with saline or fluphenazine (FLU) before and after GRH (90 ug) administration on d 13 or 14 postpartum.

	Mean serum GH concentration (ng/ml)	
Treatment	Pre-GRH [b]	Post-GRH
Saline (n=6)	6.8 ± 3.2 [c]	15.2 ± 3.2 [d]
FLU (.3 mg/kg BW; n=6)	3.9 ± 3.2 [c]	12.6 ± 3.2 [d]

[a] Least squares means ± standard error.

[b] Pre-GRH= Mean hormone concentration of 6 blood samples collected 1.5 h prior to GRH administration; Post-GRH= Mean hormone concentration of 6 blood samples collected 1.5 h after GRH administration.

[c,d] Means within the same treatment row with different superscripts differ ($P < .1$).

cows responded immediately to GRH with an increase in GH secretion (Figure 5).

Experiment 2.

Similar to Experiment 1, ultrasonography confirmed the absence of a corpus luteum in all 6 cows on the day before the experiment. No estrous behavior was observed prior to or on the day of experiment. The mean diameter of the largest follicle was 17 ± 2.8 mm and mean progesterone concentration was .12 ± .04 ng/ml.

Fluphenazine elicited an increase ($P < .01$) in serum prolactin concentration (Table 7). Serum PRL increased from 8.2 ± 7.2 before FLU to 78.7 ± 7.2 ng/ml during

Table 7. Mean prolactin (PRL) concentrations[a] in multiparous Holstein cows treated with fluphenazine (FLU) on d 13 or 14 postpartum.

	Mean serum PRL concentration (ng/ml)		
Treatment	Period [b] 1	Period 2	Period 3
FLU (.3 mg/kg BW;n=6)	8.2 ± .7.2 [c]	78.7 ± 7.2 [d]	69.9 ± 10.2 [d]

[a] Least squares means ± SEM.

[b] Period 1= Mean hormone concentration of 8 blood samples collected before fluphenazine or saline administration from 0715 to 0900 h; period 2= Mean hormone concentration of 8 blood samples collected after fluphenazine or saline administration from 0915 to 1100 h; period 3= Mean hormone concentration of 4 blood samples collected from 1130 to 1300 h (every 30 min sample).

[c,d] Means with different superscripts differ ($P < .01$).

the first 2 h after FLU. Serum PRL remained elevated (69.9 ± 10.2 ng/ml) throughout the sampling period (Figure 6).

Fluphenazine elicited a decrease ($P < .01$) in serum LH in multiparous cows (Table 8). Serum LH concentration decreased from .29 ± .01 ng/ml during pre-treatment period (period 1) to .22 ± .01 ng/ml during both periods 2 and 3 (Figure 7).

Table 8. Mean LH concentrations[a] in multiparous Holstein cows treated with fluphenazine (FLU) during d 13 or 14 postpartum.

	Mean serum LH concentration (ng/ml)		
Treatment	Period [b] 1	Period 2	Period 3
FLU (.3 mg/kg BW;n=6)	.29 ± .01 [c]	.22 ± .01 [d]	.22 ± .01 [d]

[a] Least squares means ± SEM.

[b] Period 1= Mean hormone concentration of 17 blood samples collected before fluphenazine or saline administration from 0500 to 0900 h; period 2= Mean hormone concentration of 8 blood samples collected after fluphenazine or saline administration from 0915 to 1100 h; period 3= Mean hormone concentration of 8 blood samples collected
from 1115 to 1300 h.

[c,d] Means with different superscripts differ ($P < .05$).

Fluphenazine suppressed ($P < .05$) LH pulse frequency but not peak amplitude in

Table 9. Mean LH pulse frequency and peak amplitude[a] in multiparous Holstein cows treated with fluphenazine (FLU) on d 13 or 14 postpartum.

	Mean LH pulse frequency (pulses /4 h)		Mean LH peak amplitude (ng/ml)	
Treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
FLU (.3 mg/kg BW; n=6)	1.33 ± .2	.50 ± .2 *	.19 ± .06	.11 ± .06

[a] Least squares means ± SEM.

* Different form Pre-treatment mean ($P < .05$).

multiparous cows (Table 9). After FLU administration, LH pulse frequency was completely abolished in 3 cows, decreased from 2 to 1 pulses/4 h in 2 cows, and did not change in the remaining cow. Exogenous GnRH increased ($P < .01$) serum LH concentration by approximately 9 fold (Table 10; Figure 8).

Table 10. Mean serum LH concentrations[a] in multiparous Holstein cows treated with fluphenazine (FLU) before and after GnRH (25 ug) administration on d 13 or 14 postpartum.

	Mean serum LH concentration (ng/ml)	
Treatment	Pre-GnRH [b]	Post-GnRH
FLU (.3 mg/kg BW; n=6)	.22 ± .26 [c]	1.91 ± .26 [d]

[a] Least squares means ± standard error.

[b] Pre-GnRH=Mean hormone concentration of 6 blood samples collected 1.5 h prior to GnRH administration; Post-GnRH=Mean hormone concentration of 6 blood samples collected 1.5 h after GnRH administration.

[c,d] Means with different superscripts differ ($P < .01$).

Mean serum GH concentration decreased ($P < .01$) after FLU administration (Table 11). Serum GH decreased from 5.5 ± .4 ng/ml during period 1 to 2.5 ± .6 and 2.0

Table 11. Mean GH concentrations[a] in multiparous Holstein cows treated with fluphenazine (FLU) on d 13 or 14 postpartum.

	Mean serum GH concentration (ng/ml)		
Treatment	Period [b] 1	Period 2	Period 3
FLU (.3 mg/kg BW;n=6)	5.5 ± .4 [c]	2.5 ± .6 [d]	2.0 ± .6 [d]

[a] Least squares means ± SEM.

[b] Period 1=Mean hormone concentration of 17 blood samples collected before fluphenazine or saline administration from 0500 to 0900 h; period 2=Mean hormone concentration of 8 blood samples collected after fluphenazine or saline administration from 0915 to 1100 h; period 3=Mean hormone concentration of 8 blood samples collected from 1115 to 1300 h.

[c,d] Means with different superscripts differ ($P < .01$).

± .6 ng/ml in period 2 and 3, respectively (Table 11; Figure 9). Exogenous GRH caused an increase (P =.07) in serum GH concentration (Table 12). Similar to Experiment 1, GH response to exogenous GRH was delayed for 30 min (Figure 9).

Table 12. Mean serum GH concentrations[a] in multiparous Holstein cows treated with saline or fluphenazine (FLU) before and after GRH (110 ug) administration on d 13 or 14 postpartum.

	Mean serum GH concentration (ng/ml)	
Treatment	Pre-GRH [b]	Post-GRH
FLU (.3 mg/kg BW; n=6)	2.1 ± 2.8 [c]	11.1 ± 2.8.[d]

[a] Least squares means ± standard error.

[b] Pre-GRH= Mean hormone concentration of 6 blood samples collected 1.5 h prior to GRH administration; Post-GRH= Mean hormone concentration of 6 blood samples collected 1.5 h after GRH administration.

[c.d] Means with different superscripts differ (*P* = .07).

DISCUSSION

As expected, FLU caused a significant increase in serum PRL concentrations in primi- and multiparous cows, verifying that the dose of FLU was sufficient to antagonize dopamine action (Figures 1and 6). Results of these experiments indicate that FLU (.3 mg/kg BW) decreased LH pulse frequency and LH concentrations in anovulatory postpartum dairy cows regardless of parity (Figures 2 and 7). Because FLU negates the action of dopamine by blocking dopamine receptors, the decrease in LH pulse frequency and mean concentrations after FLU administration appears to indicate that endogenous dopamine plays a role in modulation of LH secretion. Further, it appears that endogenous dopamine is stimulatory to LH secretion in anovulatory dairy cows during the early postpartum period.

Results of the present study are in agreement with findings of Jackson (1977) and Meyer and Goodman (1985) who have shown that dopamine antagonist suppressed tonic

secretion of LH and mean LH concentrations in ovariectomized ewes. Also, Kao and coworkers (1992) observed similar results and showed that pimozide suppressed LH pulse frequency and amplitude in ovary-intact photorefractory anestrous ewes.

In contrast to the present study, it has been shown (Thompson et al., 1992) that a dopamine receptor antagonist (metoclopramide) did not effect LH secretion in postpartum anestrous primiparous beef cows. Although in that study the dose of dopamine antagonist was sufficient to increase serum prolactin, it is not clear why it did not affect LH secretion. The lack of LH response may be due to the type of antagonist that was used. Metoclopramide is a specific D2 dopamine receptor antagonist (Thompson et al., 1992), whereas fluphenazine binds with both D1 and D2 receptors and has a higher affinity for the D1 subtype dopamine receptor (Sawaguchi et al., 1990; Johnson, 1995). Furthermore, cows in that study (Thompson et al., 1992) were 45-67 d postpartum compared to 13 to 14 d postpartum cows utilized in the present study.

The exact physiological mechanism which caused a decrease in LH pulse frequency and mean LH concentration in response to FLU, and site of stimulatory action of dopamine on LH secretion, cannot be derived from this study. Based on the fact that dopamine antagonist suppressed LH secretion in photorefractory anestrous ewes, Kao et al., (1992) proposed that there are sets of steroid insensitive dopaminergic neurons that synapse with GnRH, and these dopamine neurons may stimulate the GnRH pulse generator. Therefore, disruption of this system with dopamine antagonist would result in reduction of GnRH and LH pulse frequency as observed in anovulatory cows in the present experiment.

It can be argued that FLU inhibits LH secretion by antagonizing dopamine action at the level of the pituitary. It has been shown (Dailey et al., 1978) that dopamine directly inhibited LH secretion in the rabbit. However, Rotsztein et al. (1977) reported that dopamine stimulates the release of GnRH from the midiobasal hypothalamic tissue (*in vitro*) in male rats. Moreover, it is apparent that LH pulse frequency is directly related to the activity of GnRH cells and that LH pulses in the peripheral circulation corresponded, on a one to one basis, with GnRH pulses secreted by the hypothalamus (Thiery and Martin, 1991). Knowing that FLU can penetrate the blood brain barrier (Meyer and

Goodman, 1986; Long, 1998), suppression of LH pulse frequency after FLU administration is probably indicative of inhibition of GnRH release by this dopamine antagonist. In addition, LH response to exogenous GnRH was not affected by FLU compared to saline–treated cows (Figures 3 and 8). This appears to indicate that endogenous dopamine may exert its action at a site other than the pituitary, possibly the hypothalamus and the ME.

As previously mentioned LH pulse frequency did not change in 3 cows after FLU administration. Mean LH concentration decreased in 2 of 3 cows but remained unchanged in one cow. The lack of LH pulse response in these 3 cows is not attributable to insufficient dose of FLU because serum PRL increased immediately after FLU administration in both cows indicating that the dose of FLU was sufficient to cause a physiological response.

If endogenous dopamine plays a stimulatory role in LH secretion in anovulatory cows and this system is present during the early postpartum period, then what factor(s) may contribute to suppression of LH secretion in early postpartum dairy cows resulting in delayed cyclicity? It is apparent that in certain situations opioids may interact with dopaminergic neurons to modify LH secretion. For instance, Rotsztejn et al. (1978) proposed that both β-endorphin and enkephalin inhibit secretion of LH and they reduce the stimulatory effect of dopamine. Chandolia et al. (1997) proposed that opioid inhibition of gonadotropin secretion might be mediated through the dopaminergic system in bull calves. Recently, it has been shown (Ahmadzadeh et al., 1998a) that endogenous opioid plays an inhibitory role in LH secretion in anovulatory lactating cows during the early postpartum period. Therefore, it may be speculated that in anovulatory lactating cows, endogenous opioid decreases LH secretion by reducing the stimulatory effect of endogenous dopamine during the early postpartum.

Results of this experiment indicate that FLU (.3 mg/kg BW) decreased serum GH concentrations in anovulatory primi- and multiparous dairy cows during the early postpartum period. Growth hormone response to a bolus injection of FLU was gradual (Figures 4 and 9). Because FLU blocked the action of dopamine and suppressed GH secretion, the findings of this study indicate a stimulatory role for endogenous dopamine

in GH secretion in anovulatory early postpartum dairy cows. Results of this study are in agreement with findings in rats in which chlorpromazine and haloperidol (dopamine antagonists) inhibited episodic GH secretion (Eden et al., 1979). In addition, the findings of the present study are similar to those for human subjects, where chlorpromazine antagonized stimulatory effects of apomorphine on GH secretion (Lal et al., 1973; 1996). Conversely, lack of GH response to dopamine antagonists, sulpride and haloperidol, was reported in mares (Thompson et al., 1992) and ewes (Elssasar and Bolt, 1987), respectively. Furthermore, administration of L-DOPA alone (Davis and Broger, 1973) and pure dopamine (Elssasar and Bolt, 1987) did not alter GH secretion in sheep.

The reason for the variable results in sheep and horses versus cattle is not clear. In addition to species differences, there are several other points which may explain the lack of GH response to dopamine and its antagonists in the above studies compared to the GH decrease in response to FLU seen in the present experiment. First, L-DOPA and dopamine in brain tissue can be converted to norepinephrine by decarboxylase and methyltransferase enzymes. Therefore, the lack of GH response to L-DOPA (Davis and Broger, 1972) and dopamine (Elssasar and Bolt, 1987) could be attributable to the fact that these precursors had been converted to norepinephrine, and thus dopaminergic receptors were unaffected. Second, sulperide and haloperidol are considered as D2 receptor antagonists (Sawaguchi er al., 1990) whereas FLU blocks both D1 and D2 receptors and has a higher affinity for D1 receptors (Coirini et al., 1997). This difference in receptor selectivity may account for the variation in the response observed in the present study compared to others.

It has been shown that in grazing heifers (Bolt et al. 1983) and in wethers (Henson et al., 1987) the dopamine antagonist (spiperone) increased pituitary monoamine oxidase (MAO) and caused a decrease in plasma dopamine compared to control animals. Since MAO catalyzes the deamination of catecholamines in a variety of tissues (Martin, 1985) it has been hypothesized (Henson et al., 1987) that spiperone may have resulted in lowered levels of plasma dopamine by elevation of tissue MAO. Therefore, it is possible that the decrease in serum GH concentration in the present study is attributable to the fact that FLU caused a decrease in endogenous dopamine reducing its stimulatory effect on

GH secretion.

D1 dopamine receptor agonist (SKF 38893) increased GIF and decreased release of GRH from hypothalamic slices from yearling heifers *in vitro* (West et al., 1997). Furthermore, D1 antagonist blocked SKF-38393-induced release of GIF and suppression of GRH. These results support the hypothesis that dopamine modulates both somatostatin and GRH release. If a dopamine agonist exerts stimulatory action on GIF and inhibitory action on GRH, then it can be speculated that dopamine suppresses GH secretion. However, contrary to this hypothesis (West et al., 1997), results of the present experiments indicate that dopamine is stimulatory to GH secretion. There are major differences including animal model, experimental protocol and pharmacological agents used, which may explain the conflicting results between the present study and West et al. (1997).

The exact physiological mechanism that caused a decrease in GH secretion in response to FLU, and the site of inhibitory action of FLU on GH secretion cannot be derived from this study. Fluphenazine can penetrate the blood brain barrier and it may be speculated that the observed decrease in GH secretion after dopamine antagonist administration is attributable to blocking the action of endogenous dopamine within the hypothalamus. The fact that FLU inhibition of GH secretion was overridden by exogenous GRH and that GH response to GRH in FLU-treated cows was similar to that of the control group, support this hypothesis (Figures 4 and 9). Nonetheless, results of this study do not rule out the direct action of FLU on the ME and (or) the anterior pituitary.

Presynaptic dopamine receptors (autoreceptors) are present on dopamine neurons and are classified as D2 receptors. Blocking autoreceptors by D2 receptor antagonist increases the synthesis and release of endogenous dopamine (Cooper et al., 1996). Therefore, it can be argued that observed decrease in GH secretion after FLU is not attributable to blocking dopamine action via postsynaptic receptors, rather it is due to modulation of presynaptic receptors and thus an increase in dopamine turnover rate. However, there are several factors which suggest that FLU did not act presynaptically. First, FLU is not a specific potent D2 receptor and it may have a higher affinity for D1

receptors (Johnson et al., 1995). Second, if FLU blocked D2 presynaptic receptors and increased dopamine turnover, then serum PRL should not have remained elevated during the experiment because dopamine is a suppressor of PRL secretion. Third, even potent D2 blockers such as haloperidol did not change dopamine turnover until 15-17 h after administration in humans (Cooper et al., 1996). Therefore, it unlikely that FLU-induced decrease in GH secretion is attributable to dopamine presynaptic action and increased dopamine turnover.

In summary, FLU caused a significant decrease in mean LH concentration and LH pulse frequency in anovulatory primi- and multiparous cows during the early postpartum period. This finding supports the theory that dopamine plays a part in the control of LH secretion during the early postpartum and that endogenous dopamine is stimulatory to LH secretion. Furthermore, endogenous dopamine exerts its effect, directly or indirectly, on LH secretion via dopamine receptors. These data do not provide information in regard to the site or mechanism of action of endogenous dopamine. However, because a temporal correlation between pulses of GnRH and pulses of LH exists and FLU suppressed LH pulse frequency, it may be hypothesized that the site of stimulatory action of endogenous dopamine on LH secretion is the hypothalamus. Furthermore, the data from this study indicate that endogenous dopamine plays a role in regulation of GH secretion in dairy cows during the early postpartum period. In addition, because a dopamine receptor antagonist decreased GH secretion, it appears that endogenous dopamine is stimulatory to GH secretion in anovulatory primi- and multiparous cows during the early postpartum period. The increase in serum PRL concentration after FLU administration provides evidence that the dose of FLU was sufficient to antagonize dopamine suppression of pituitary PRL secretion and supports the hypothesis that dopamine is inhibitory to PRL in dairy cattle.

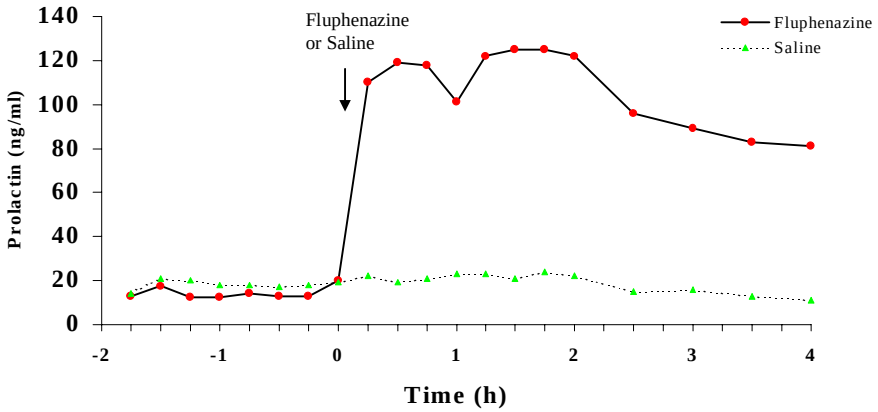

Figure 1. Mean Serum prolactin concentrations before and after fluphenazine (.3 mg/kg BW;n=6) or saline (n=6) administration on d 13 or 14 postpartum in primiparous Holstein cows.

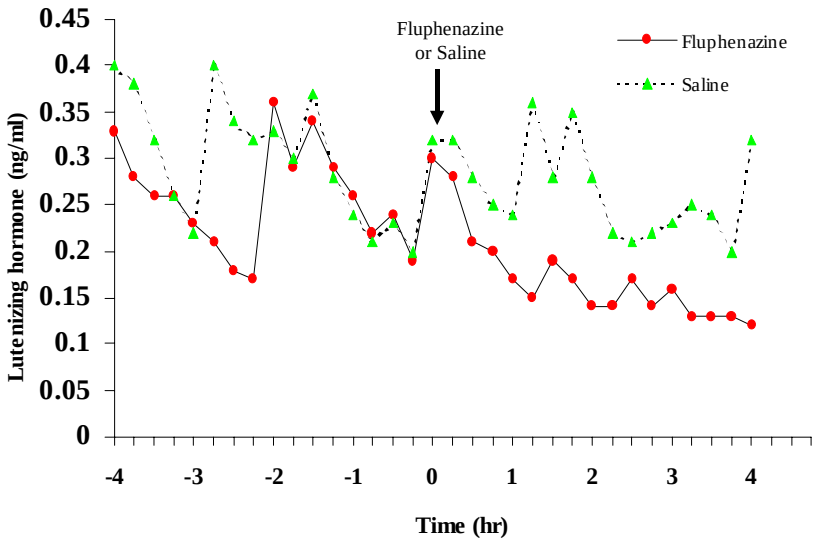

Figure 2. Mean Serum luteinizing hormone concentrations before and after fluphenazine (.3 mg/kg BW;n=6) or saline (n=6) administration on d 13 or 14 postpartum in primiparous Holstein cows.

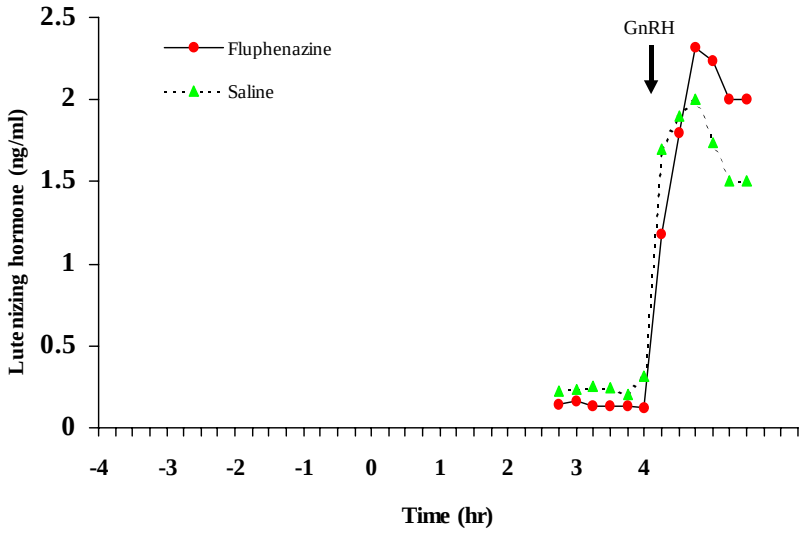

Figure 3. Mean serum luteinizing hormone concentrations before and after GnRH (25 ug) administration in fluphenazine- (n=6) and saline-treated (n=6) primiparous Holstein cows on d 13 or 14 postpartum.

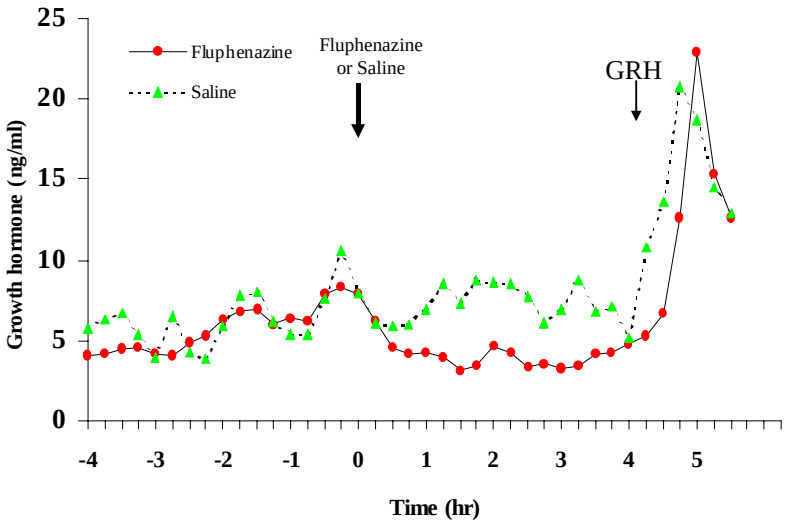

Figure 4. Mean Serum growth hormone concentrations before and after fluphenazine (.3 mg/kg BW;n=6) or saline (n=6) administration and after GRH (90 ug) during d 13 or 14 postpartum in primiparous Holstein cows.

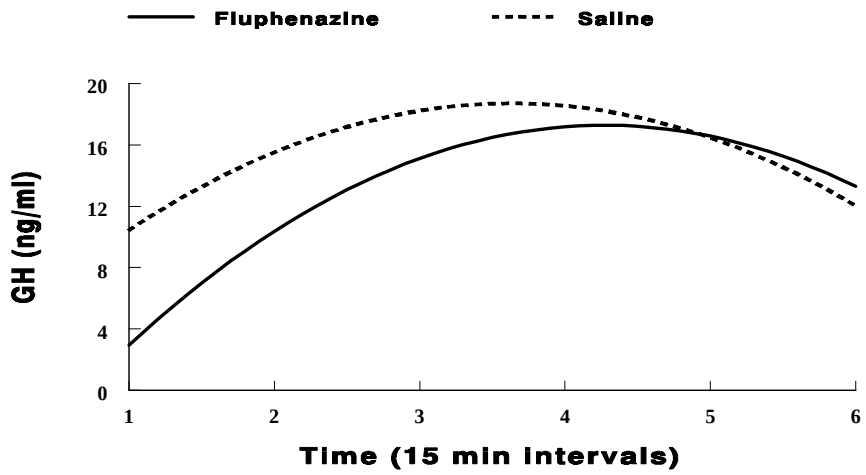

Figure 5. Changes in growth hormone response to growth hormone releasing hormone (GRH; 90 ug) after treatment with FLU (.3 mg/kg BW; n=6) or saline (n=6) on d 13 or 14 postpartum in Holstein cows. Time 1 refers to 15 min post-GRH administration.

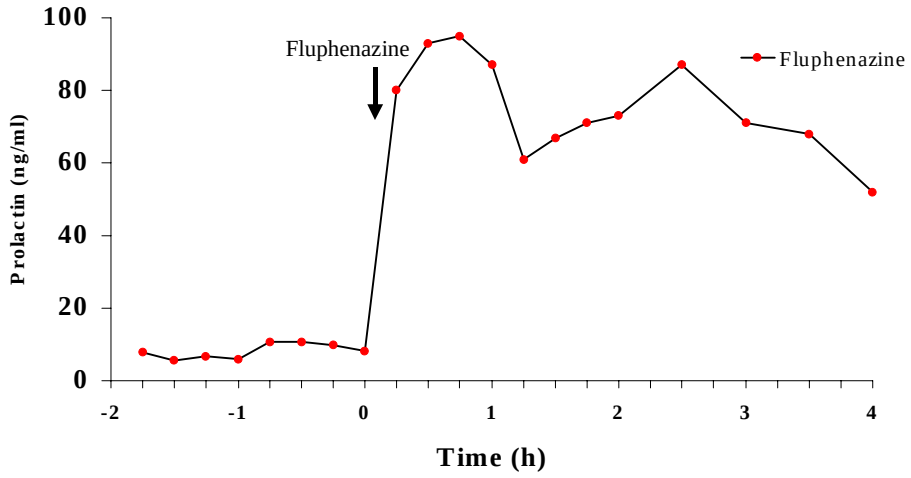

Figure 6. Mean Serum prolactin concentrations before and after fluphenazine (.3 mg/kg BW;n=6) administration on d 13 or 14 postpartum in multiparous Holstein cows.

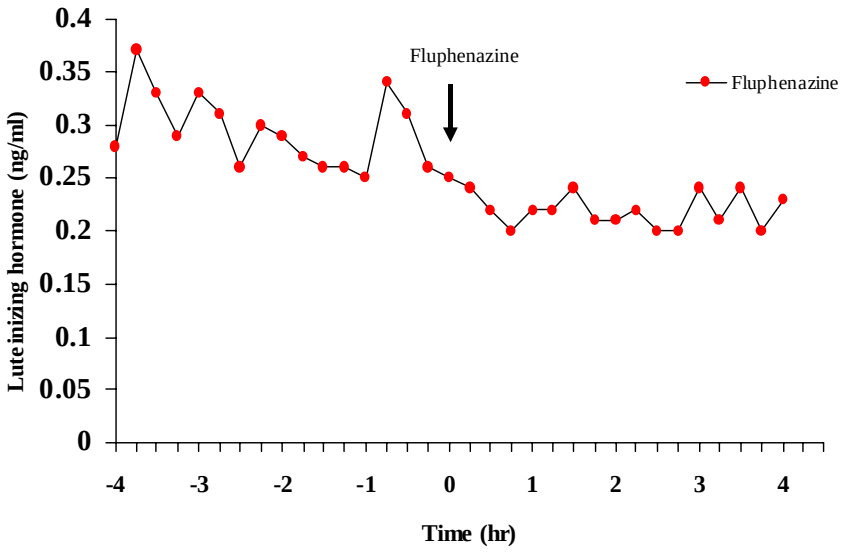

Figure 7. Mean Serum luteinizing hormone concentrations before and after fluphenazine (.3 mg/kg BW;n=6) administration on d 13 or 14 postpartum in multiparous Holstein cows.

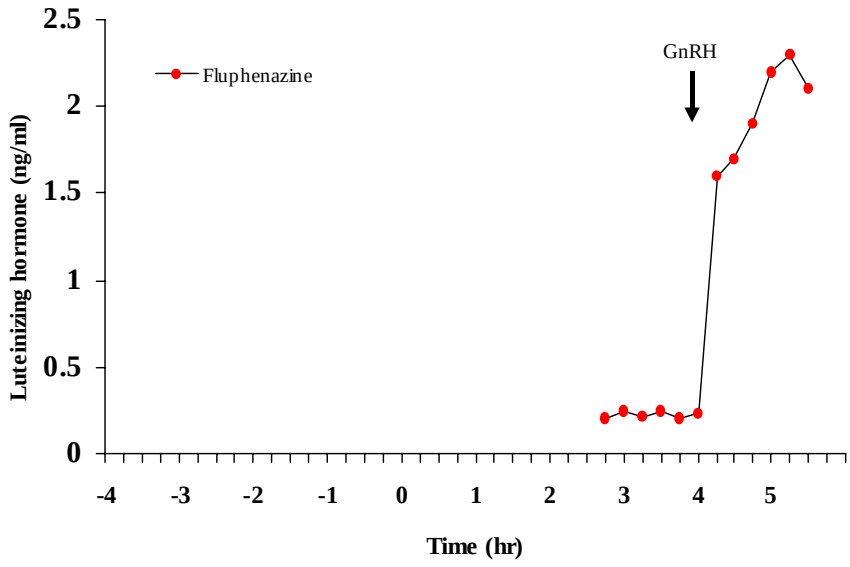

Figure 8. Mean serum luteinizing hormone concentrations before and after GnRH (25 ug) administration in fluphenazine-treated multiparous Holstein cows (n=6) on d 13 or 14 postpartum.

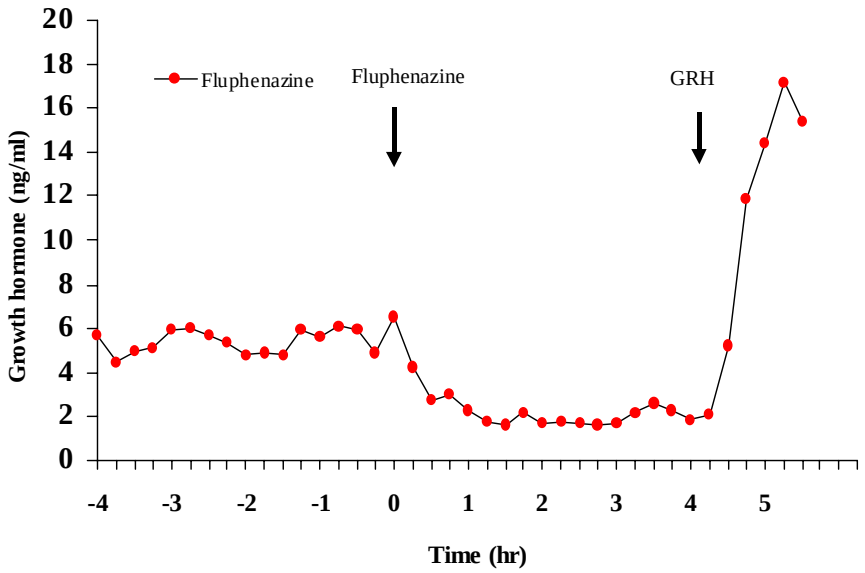

Figure 9. Mean Serum growth hormone concentrations before and after fluphenazine (.3 mg/kg BW;n=6) administration and after GRH (110 ug) during d 13 or 14 postpartum in multiparous Holstein cows.

CHAPTER 2

EFFECT OF DOPAMINE ANTAGONIST (FLUPHENAZINE) ON SERUM LUTEINIZING HORMONE, FOLLICLE STIMULATING HORMONE, AND GROWTH HORMOINE CONCENTRATIONS DURING THE LUTEAL PHASE OF THE ESTROUS CYCLE IN HOLSTEIN COWS

ABSTRACT

Eighteen lactating Holstein cows (76 ± 15 d postpartum) were used to determine the effect of different doses of fluphenazine (FLU), a dopamine receptor antagonist, on pituitary LH, FSH, growth hormone (GH), and prolactin (PRL) secretion during the mid-luteal phase of the estrous cycle. After detection of a corpus luteum, estrous cycles were synchronized by one dose of prostaglandin ($PGF_{2\alpha}$) administration (25 mg). On d 10 or 11 post-estrus, cows received (i.v.) either saline (C; n=6), .1 mg FLU/kg BW (LD; n=6), or .5 mg FLU/kg BW (HD; n=6). Blood samples were collected at 15-min intervals for 4 h before and 4 h after FLU or saline. Immediately thereafter all cows received 25 ug GnRH (i.v.) and blood collection continued for 1.5 h. Mean serum progesterone was 3.1 ± .3 ng/ml. Mean serum LH, FSH, GH and PRL concentrations did not differ among the groups prior to treatments. Both doses of FLU increased ($P < .01$) serum PRL concentration compared to saline-treated cows. Neither dose of FLU affected mean LH concentration compared to the saline-treated cows. Further, LH pulse frequency and peak amplitude did not change in response to treatments. No treatment altered mean FSH concentration, pulse frequency, or peak amplitude. Neither dose of FLU affected mean serum GH concentration. Likewise, serum GH concentration remained unchanged in saline-treated cows. Exogenous GnRH increased ($P < .05$) LH and FSH concentrations in all cows and FLU treatment did not alter gonadotropin response to exogenous GnRH. These results suggest that a dopamine-mediated mechanism for modulation of

gonadotropin secretion and GH is absent during the luteal phase of the estrous cycle in lactating dairy cows or perhaps overridden by the presence of elevated progesterone. However, endogenous dopamine does play an inhibitory role in PRL secretion in the luteal phase lactating dairy cow.
Key words: Dopamine antagonist, Luteinizing hormone, Follicle stimulating hormone, Growth hormone, Luteal phase, Dairy cattle

INTRODUCTION

Dopamine and Gonadotropin secretion The anterior pituitary gland secretes pulses of LH in response to GnRH released into the hypophysial portal blood by the hypothalamus. The pulsatile nature of LH secretion is very important because the frequency of the pulses is directly related to the activity of GnRH neurons. Gonadal steroids (estradiol and progesterone) act on the hypothalamus and affect frequency of GnRH and LH pulses (Goodman and Karsch, 1980). For instance, Ireland and Roche (1982) reported a negative correlation between level of progesterone administered and LH secretion in the bovine, where low levels of progesterone were associated with an increase in frequency of LH pulses. These authors suggested that progesterone is part of a negative feedback complex regulating LH secretion in cattle. Walters et al. (1983) suggested that pulses of LH and FSH stimulate pulses of estradiol and progesterone from the ovary which in turn feedback upon the pituitary and hypothalamus to regulate the frequency and amplitude of LH and FSH pulses.

The negative feedback effect of progesterone on LH secretion is prominent during the luteal phase of the estrous cycle when blood progesterone concentration is high. The exact mechanism by which progesterone reduces LH secretion is not clear; however, it is likely that steroids do not alter GnRH secretion by directly affecting GnRH neurons because steroid receptors have not been found in GnRH cells (Thiery and Martin, 1991; Malven, 1993c). Therefore, at least one other set of neurons are believed to mediate the effects of these ovarian hormones. There is evidence that dopaminergic, noradrenergic, serotonergic, and (or) opioidergic neurons may be involved (Brooks et al., 1989).

McNeily (1980) has suggested that progesterone might increase the activity of hypothalamic dopaminergic pathways, which inhibit the release of LHRH. It has been shown that intravenous infusion of dopamine decreased LH secretion during the luteal phase of the estrous cycle in ewes (Deaver and Daily, 1983). However, the effects of dopamine antagonists on LH secretion in ewes during the luteal phase of the estrous cycle were variable. Fluphenazine (Goodman, 1985) and domperidone (Deaver et al., 1987) markedly increased LH pulse frequency whereas pimozide, another dopamine antagonist, did not (Goodman and Meyer, 1985). Nonetheless, because of the ability of FLU and domperidone (dopaminergic antagonists) to increase secretion of LH during the luteal phase, it was suggested that the dopamine regulates LH release in this phase of the estrous cycle and progesterone might exert its inhibitory effect on LH through a dopaminergic system in ewes (Dailey et al., 1987). Serum LH concentrations increased after a bolus dose of metoclopramide, a dopamine antagonist, during the mid-luteal phase of the menstrual cycle in women (Ropert et al., 1984; Seki and Nagata, 1990). In contrast, in progesterone-treated rats, intraventricular dopamine injection increased plasma LH, suggesting a stimulatory role for dopamine (Schneider and McCann, 1970). Ergot alkaloids (ergotamine and ergonovine) which have dopamine-like activity, decreased LH concentration during the late luteal phase of the estrous cycle in Holstein cows (Browning et al., 1998).

Dopamine and GH secretion Increasing milk yield and thus improving the productivity and profitability of dairy farms requires a better understanding of regulation of hormones which control mammary gland function. Growth hormone governs growth and development of the mammary gland and circulating concentrations of GH are closely related with body maintenance and enhanced milk yield (Akers, 1994). It is known that sex steroids can affect GH secretion. For example, Trenkle (1970) suggested that estrogen stimulates GH secretion because feeding diethylstilbesteol increased plasma GH in yearling steers. Davis and Borger (1973) showed that progesterone and estradiol benzoate treatment of ovariectomized ewes enhanced GH secretion. Implantation of estrogenic anabolic compounds, such as zeranol and diethylstilbestrol, increased GH secretion in growing beef steers (Gopinath and Kitts, 1984). The mechanism that

promotes increased secretion of GH is unclear. It has been suggested that steroids play a role in GH secretion by regulating somatostatin (GIF) in female rats. For instance, ovariectomy caused a decrease in hypothalamic GIF content (Gabriel et al., 1989). Estupina and co-workers (1996) hypothesized that GIF release from the hypothalamus is influenced by gonadal steroids, and estrogen alone may not modulate GIF secretion. It was suggested (Estupina et al., 1996) that perhaps the progesterone to estrogen ratio is more important in this regulatory mechanism. Nonetheless, steroids may not alter GIF neuron activity directly because GIF neurons of the periventricular area, at least in rats, lack estrogen receptors (Herbison et al., 1993). Therefore, there may be other interneurons which mediate the effect of sex steroids on GIF secretion and subsequently GH secretion.

There is evidence that steroids affect catecholamine turnover rate in hypothalamic tissues (Beattie et al., 1972). Fuxe et al. (1980) showed that steroids and some of their metabolites (catecholestrogen) may influence dopamine release and synthesis in the area of the anterior hypothalamus and the arcuate nucleus in the rat. Furthermore, they showed that estradiol 17β markedly reduced dopamine turnover in the rat brain and directly activated dopaminergic neurons in the lateral tuberoinfundibular system. The arcuate nucleus and tuberoinfundibular nuclei are the areas which contain GRH and GIF neurons which regulate GH secretion (Leshine et al., 1995).

There is ample evidence to suggest that dopamine is involved in regulation of GH secretion in man and laboratory animals (see Liuzzi et al., 1976 for review). McMahon et al. (1998) showed that fasting-induced release of GH was blocked by D1 dopamine receptor agonist in meal-fed steers. These authors concluded that stimulation of D1 receptors by D1 dopamine agonist increases GIF neurons and this increased GIF activity is associated with suppression of GRH. Thus, GH secretion would be reduced.

As discussed, neurotransmitters may mediate the effect of progesterone on LH secretion. Whether dopamine acts as a neuromodulator of LH secretion during the luteal phase of the estrous cycle in cattle is not clear. Furthermore, there is limited information concerning action of biogenic amines such as dopamine on secretion of FSH in cattle. Thus, the first objective of this study was to investigate the role of endogenous dopamine

on gonadotropin secretion by characterizing serum LH and FSH response to the administration of a dopamine antagonist, FLU, during the luteal phase of the estrous cycle in lactating Holstein cows.

Steroids increased pituitary GH secretion in beef steers (Gopinath and Kitts, 1984) and hypothalamic dopamine turnover rate (Fuxe et al., 1980). Dopamine in turn was postulated to regulate the release of GH. Therefore, it is possible that gonadal steroid regulation of GH secretion is modified, in part, by endogenous dopamine. Information concerning the interaction between steroids, dopamine and GH secretion in cattle is lacking. It has been shown (Chapter 2) that in anovulatory postpartum dairy cows, a dopamine receptor antagonist decreased GH secretion indicating that endogenous dopamine may play a stimulatory role in GH secretion during the early postpartum period. It was of interest to investigate whether dopamine modulation of GH secretion changes with the resumption of ovarian cyclicity and the accompanying changes in steroid milieu. The second objective of the present study was to investigate the role of endogenous dopamine on GH secretion by characterizing serum GH response to a dopamine antagonist, FLU, during the luteal phase of the estrous cycle. Because it is well established that dopamine inhibits PRL secretion in cattle, serum PRL concentrations in response to FLU were measured as a positive control to ensure that FLU effectively blocked dopamine receptors and impaired dopamine action.

MATERIALS AND METHODS

Eighteen lactating Holstein cows, 76 ± 15 d postpartum, were used. Mean BW was 605 kg (± 65). Ovarian structures of cows were monitored by a real-time B-mode ultrasound scanner, equipped with a 5 MHz rectal transducer probe (Aloka Co., 6-22-1, Mure, Mitaka-shi, Tokyo , Japan) and upon detection of a corpus luteum, 25 mg of $PGF_{2\alpha}$ (Lutalyse®, The Pharmacia and Upjohn Co., Kalamazoo, MI) was administered (i.m.) to induce luteolysis. Estrous activity was monitored using an electronic pressure-sensitive device Heat Watch (HW ®, DDx Inc. Denver, CO). One day prior to the experiment (d 9 or 10 post-estrus) all cows were weighed and ovaries were examined by ultrasonography.

Shortly thereafter, using aseptic procedure, an indwelling jugular cannula (polyethylene tubing, #427420, Becton Dickinson, Sparks, MD) was inserted. A sample port equipped with a Luer stub adapter (#7564, Becton Dickinson, Franklin Lakes, NJ) was fixed to the end of the cannula for blood withdrawal. Clotting of blood in cannulation tubing was prevented by infusion of a 3.5% citrate solution after cannulation and immediately after each blood sample was drawn. External tubing with the sample port was securely taped to the side of the cow's neck for easy blood withdrawal.

On d 10 or 11 post estrus (mid-luteal phase of the estrous cycle) cows were randomly assigned to receive intravenously saline (n=6), 0.1 mg FLU/kg BW (LD; n=6), or 0.5 mg FLU/kg BW (HD; n=6). Starting at 0500 h, blood samples were collected at 15-min intervals for 4 h before and 4 h after saline or FLU (dissolved in 10 ml of 25^{o} C physiological saline). Immediately thereafter, all cows received (iv) 25 ug GnRH (Cystorelin®, Sanofi, Overland Park, KS) and blood collection continued for an additional 1.5 h. This low dose of GnRH was administered to test the competency of the pituitary to release of LH and FSH and to determine whether FLU treatment affected LH and (or) FSH response to GnRH. In each sampling interval, the initial 2 ml of each blood sample was discarded, and a 10 ml sample was then transferred into 13 ml collection tubes, which were immediately placed in ice and then stored at 4^{o} C for 24 h for clotting. All blood samples were centrifuged for 30 min at 2,200 $\times$g at 4^{o} C. Serum was harvested and stored at -20^{o}C until assayed for LH, FSH, PRL, and progesterone content. During blood sampling all cows were tied in individual stalls with access to a total mixed ration and water *ad libitum.*

Hormone Assay

Concentration of LH was determined by a double-antibody RIA as described by Bolt and Caldwell (1992). Purified LH (USDA-bLH-B-6) was used as reference standard and for radioiodination. Sheep anti-rabbit gammaglobulin was used as the precipitating second antibody. Rabbit anti-bovine primary antiserum (USDA-309-684p) bound approximately 35% of radiolabeled LH in the absence of unlabeled hormone. Sensitivity of the assay was 0.08 ng/ml reference standard LH and defined as the concentration

corresponding to 2 standard deviations less than mean zero dose tubes. All samples were assayed in duplicate, and intra- and inter-assay CV calculated from serum pools averaged 5.3% and 15.8%, respectively.

Serum FSH was quantified by a heterologous double antibody RIA using rabbit anti-ovine FSH antibody (NIDKK-anti-oFSH-1) in a non-equilibrium condition. Purified FSH (USDA-bFSH-I-2) was used as reference standard and for radioiodination. Sheep anti-rabbit gammaglobulin was used as the precipitating second antibody. Primary antiserum bound 17% of radiolabeled FSH in the absence of unlabeled hormone. Sensitivity of the assay was less than .06 ng/ml reference standard FSH and defined as the concentration corresponding to 2 standard deviations less than mean zero dose tubes. All samples were assayed in duplicate, and intra- and inter-assay CV calculated from serum pools averaged 8.1% and 4.2%, respectively.

Concentration of GH was determined by a double-antibody RIA by method of Barnes et al. (1985). Purified recombinant bovine GH [bGH Cynamide 6952 (-42A)] and (USDA-bGH-I-1) were used as the reference standard and for radioiodination, respectively. Sheep anti-rabbit gammaglobulin was used as the precipitating second antibody. Primary antibody was raised in rabbits using bovine GH (NIH-GH-B18). Primary antiserum bound 40% of radiolabeled GH in the absence of unlabeled hormone. Sensitivity of the assay was less than 2 ng/ml reference standard GH and defined as the concentration corresponding to 2 standard deviations less than mean zero dose. All samples were assayed in duplicate, and intra- and inter-assay CV calculated from serum pools averaged 3.7% and 8%, respectively. Sensitivity of the PRL assay was less than 1.25 ng/ml reference standard PRL and defined as the concentration corresponding to 2 standard deviations less than mean zero dose. Serum PRL was quantified in one assay and intra-assay CV was 7.6%.

Concentration of PRL was determined in one assay by a double-antibody RIA (Barnes et al., 1985). Sensitivity of the assay was less than 1.25 ng/ml reference standard. All samples were assayed in duplicate and intra-assay CV was 7.6%. Concentration of progesterone was quantified using solid-phase RIA (Diagnostic Products Corp.; Los Angeles, CA) which has been previously validated in our laboratory

(Holt et al., 1989). The standard curve ranged from 0.1 to 20 ng/ml. All samples were assayed in duplicate (1 assay) and the intra-assay CV was 5.4%.

Statistical Analysis

Serum LH, FSH and GH data were analyzed by least-squares analysis of variance by the General Linear Model (GLM) procedure using Statistical Analysis Systems (SAS® Institute, Cary, NC). To determine the effect of FLU on LH, FSH, GH, and PRL secretion, blood samples were categorized to one of three periods. Period 1 represented blood samples collected for 4 h before saline or FLU administration and was considered the pre-treatment period. Period 2 represented blood samples collected for 2 h immediately after FLU or saline administration; and samples thereafter (2 h) represented period 3. The experiment was designed to determine the effects of FLU administration by comparing LH and FSH, GH, and PRL concentration between period 1 and periods 2 and 3. The statistical model included dose, cow within dose, period, and all the possible interactions. Period by cow within dose was used as the error term to test the effects of period and period by dose. If the effects of period or period by dose were significant ($P <$ .05), non-orthogonal contrasts were used to compare least squares means for periods 1 vs 2 and 1 vs 3, and 2 vs 3 within each treatment using the improved Bonferroni F-test (1977). To determine the effect of GnRH on serum LH concentration, the sampling interval was divided into two periods. The pre-GnRH period represented serum samples collected 1.5 h prior to GnRH administration and the post-GnRH period represented samples collected for 1.5 h after GnRH treatment. Differences between the two periods were tested using the same statistical model as above.

Pulses of LH and FSH were determined as described by Goodman and Karsch (1980) and modified by Richards et al. (1991). Any value of LH and FSH greater than two SD above the mean for a cow, followed by at least 2 values of lesser concentration was considered a pulse. In addition, a peak had to occur within two samples of previous nadir and the amplitude of the peak had to be greater than the sensitivity of the LH and FSH assays. The amplitude of LH and FSH pulses was the difference between the greatest concentration during the pulse and the nadir within 30 min before the pulse.

Pulse frequency and peak amplitude of LH and FSH were analyzed by least-squares analysis of variance by the GLM procedure using Statistical Analysis Systems (SAS® Institute, Cary, NC). The statistical model included dose, cow within dose, period, and dose by period interaction. Using the improved Bonferroni F-test (1977), the least square means of LH and FSH pulse frequencies and peak amplitudes between pre- and post-treatment periods were compared.

RESULTS

None of the cows exhibited estrous behavior during 9 to 10 days prior to, or on the day of experiment. Results of ultrasonography on the day before the experiment indicated the presence of a corpus luteum in all cows. As was expected, serum progesterone concentrations of all cows were greater than 1 ng/ml and mean serum progesterone was 3.1± .3 ng/ml. This information confirmed that the experiment was conducted during the luteal phase of the estrous cycle when LH and FSH secretion was likely under negative feedback control of progesterone.

All FLU-treated cows showed some behavioral changes in response to treatment. Approximately 20 min after FLU treatments, cows became immobile and showed muscle tremors and some abnormal chewing behavior. These side effects lasted for approximately 2 h. Reduction in feed and water intake was also observed during the first 2 h after FLU treatment.

Effect of FLU on PRL secretion Mean serum PRL concentrations did not differ among groups prior to treatments. Both doses of FLU increased ($P < .01$) serum PRL concentrations from 6.2 ± 4.1 before FLU to 51.3 ± 4.2 ng/ml after FLU in LD group and from 4.9 ±4.1 before FLU to 50.2 ± 4.1 ng/ml after FLU in HD group (Table 1). Serum PRL concentrations remained elevated ($P < .01$) in response to FLU and did not return to pretreatment level during the sampling period (Figure 1). Serum PRL concentrations were unaffected in saline-treated cows (3.7 ± 4.1 and 4.7 ± 4.2 ng/ml, before and after

Table 1. Mean serum prolactin (PRL) concentrations[a] in Holstein cows treated with saline or fluphenazine (FLU) during the luteal phase of the estrous cycle

Treatment	Mean serum PRL concentration (ng/ml)		
	Period [b] 1	Period 2	Period 3
Saline (n=6)	3.7 ± 4.1	4.7 ± 4.2	2.2 ± 5.8
FLU (.1 mg/kg BW;n=6)	6.2 ± 4.1 [c]	51.3 ± 4.2 [d]	44.7 ± 5.9 [d]
FLU (.5 mg/kg BW;n=6)	4.9 ± 4.1 [c]	50.2 ± 4.2 [d]	45.2 ± 5.8 [d]

[a] Least squares means ± SEM.

[b] Period 1=Mean hormone concentration of 17 blood samples collected before fluphenazine or saline administration from 0500 to 0900 h; period 2=Mean hormone concentration of 8 blood samples collected after fluphenazine or saline administration from 0915 to 1100 h; period 3=Mean hormone concentration of 4 blood samples collected from 1115 to 1300 h (every 30 min sample).

[c,d] . Means within the same treatment row with different superscripts differ ($P < .01$).

saline, respectively; Table 1).

Effect of FLU on LH secretion Mean serum LH concentrations during the 4 h pre-treatment period and 4 h post-treatment period in both saline- and FLU-treated groups are shown in Table 2. Mean pre-treatment serum LH concentrations (period 1)

Table 2. Mean serum LH concentrations[a] in Holstein cows treated with saline or fluphenazine (FLU) during the luteal phase of the estrous cycle.

Treatment	Mean serum LH concentration (ng/ml)		
	Period [b] 1	Period 2	Period 3
Saline (n=6)	.18 ± .02	.17 ± .03	.24 ± .03
FLU (.1 mg/kg BW;n=6)	.25 ± .02	.22 ± .03	.19 ± .03
FLU (.5 mg/kg BW;n=8)	.18 ± .02	.16 ± .03	.24 ± .03

[a] Least squares means ± SEM.

[b] Period 1= Mean hormone concentration of 17 blood samples collected before fluphenazine or saline administration from 0500 to 0900 h; period 2 = Mean hormone concentration of 8 blood samples collected after fluphenazine or saline administration from 0915 to 1100 h; period 3= Mean hormone concentration of 8 blood samples collected from 1115 to 1300 h.

Table 3. Mean LH pulse frequency and peak amplitude[a] in Holstein cows treated with saline or fluphenazine (FLU) during the luteal phase of the estrous cycle.

	Mean LH pulse frequency (pulses /4 h)		Mean LH peak amplitude[b] (ng/ml)	
Treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
Saline (n=6)	1.00 ± .25	.83 ± .25	.23 ± .05	.19 ± .05
FLU (.1 mg/kg BW; n=6)	1.00 ± .25	.83 ± .25	.14 ± .05	.13 ± .05
FLU (.5 mg/kg BW; n=6)	.83 ± .25	.83 ± .25	.09 ± .05	.10 ± .05

[a] Least squares means ± SEM. [b] difference between the preceding nadir and maximum height of pulses.

were .18 ± .02, .25 ± .02, and .18 ± .02 ng/ml for the saline, LD, and HD groups, respectively, and were similar between treatment groups. Administration of FLU at either .1 mg/kg or .5 mg/kg BW did not affect mean serum LH concentrations (Table 2; Figure 2). Serum LH concentrations were unaffected by saline treatment. Mean LH pulse frequency and peak amplitude was similar among groups before saline or FLU (Table 3).

Table 4. Mean serum LH concentrations[a] in Holstein cows treated with saline or fluphenazine (FLU) before and after GnRH (25 ug) administration during the luteal phase of the estrous cycle.

	Mean serum LH concentration (ng/ml)	
Treatment	Pre-GnRH [b]	Post-GnRH
Saline (n=6)	.19 ± .17 [c]	1.24 ± .17 [d]
FLU (.1 mg/kg BW; n=6)	.20 ± .17 [c]	1.88 ± .17 [d]
FLU (.5 mg/kg BW; n=6)	.24 ± .17 [c]	1.46 ± .17 [d]

[a] Least squares means ± SEM.

[b] Pre-GnRH = Mean hormone concentration of 6 blood samples collected 1.5 h prior to GnRH administration; Post-GnRH = Mean hormone concentration of 6 blood samples collected 1.5 h after GnRH administration.

[c,d] Means within the same treatment row with different superscripts differ ($P < .01$).

administration. Neither dose of FLU affected LH pulse frequency or peak amplitude (Table 3). Pulse frequency and peak amplitude of LH did not change in response to saline administration (Table 3). Injection of GnRH (25 ug) increased ($P < .01$) mean LH concentrations in both saline- and FLU-treated cows (Table 4). There was no treatment effect on LH response to exogenous GnRH (Figure 2).

Effect of FLU on FSH secretion Table 5 depicts mean FSH concentrations for 4 h before and 4 h after saline or FLU. Mean serum FSH concentrations before treatments were similar among groups. Neither dose of FLU affected mean FSH concentrations and FSH concentrations remained unchanged in saline-treated cows (Table 5; Figure 3).
Mean FSH pulse frequency and peak amplitude was similar among the groups before saline or FLU administration (Table 6). Neither dose of FLU altered mean FSH pulse frequencies and mean peak amplitudes (Table 6). Follicle stimulating hormone pulse frequency and peak amplitude were also unaffected in saline-treated cows.

Exogenous GnRH caused an increase ($P < .01$) in mean FSH concentrations and

Table 5. Mean serum FSH concentrations[a] in Holstein cows treated with saline or fluphenazine (FLU) during the luteal phase of the estrous cycle.

	Mean serum FSH concentration (ng/ml)		
Treatment	Period [b] 1	Period 2	Period 3
Saline (n=6)	.23 ± .01	.26 ± .01	.25 ± .01
FLU (.1 mg/kg BW;n=6)	.25 ± .01	.25 ± .01	.26 ± .01
FLU (.5 mg/kg BW;n=6)	.22 ± .01	.21 ± .01	.24 ± .01

[a] Least squares means ± SEM.

[b] Period 1= Mean hormone concentration of 17 blood samples collected before fluphenazine or saline administration from 0500 to 0900 h; period 2= Mean hormone concentration of 8 blood samples collected after fluphenazine or saline administration from 0915 to 1100 h; period 3= Mean hormone concentration of 8 blood samples collected from 1115 to 1300 h.

Table 6. Mean FSH pulse frequency and peak amplitude[a] in Holstein cows treated with saline or fluphenazine (FLU) during the luteal phase of the estrous cycle.

Treatment	Mean FSH pulse frequency (pulses /4 h)		Mean FSH peak amplitude[b] (ng/ml)	
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
Saline (n=6)	.83 ± .27	1 ± .27	.07 ± .05	.10 ± .05
FLU (.1 mg/kg BW; n=6)	.67 ± .27	.50 ± .27	.13 ± .05	.05 ± .05
FLU (.5 mg/kg BW; n=6)	.83 ± .27	.50 ± .27	.15 ± .05	.03 ± .05

[a] Least squares means ± SEM. [b] difference between the preceding nadir and maximum height of pulses.

magnitude of FSH response to GnRH was similar in all treatment groups (Table 7). Mean FSH concentration for all treatment groups was approximately .26 ng/ml before GnRH and increased to .48 ng/ml after GnRH (Figure 3). Fluphenazine had no effect on FSH response to exogenous GnRH.

Table 7. Mean serum FSH concentrations[a] in Holstein cows treated with saline or fluphenazine (FLU) before and after GnRH (25 ug) administration during the luteal phase of the estrous cycle.

Treatment	Mean serum FSH concentration (ng/ml)	
	Pre-GnRH[b]	Post-GnRH
Saline (n=6)	.26 ± .07 [c]	.48 ± .07 [d]
FLU (.1 mg/kg BW; n=6)	.26 ± .07 [c]	.49 ± .07 [d]
FLU (.5 mg/kg BW; n=6)	.26 ± .07 [c]	.48 ± .07 [d]

[a] Least squares means ± standard error.

[b] Pre-GnRH = Mean hormone concentration of 6 blood samples collected 1.5 h prior to GnRH administration; Post-GnRH = Mean hormone concentration of 6 blood samples collected 1.5 h after GnRH administration.

[c,d] Means within the same treatment row with different superscripts differ ($P < .01$).

Effect of FLU on GH secretion Table 8 depicts mean GH concentrations 4 h before and 4 h after saline or FLU administration. Mean serum GH concentrations before treatments were similar among groups. Neither dose of FLU affected mean GH concentrations (Table 8). Likewise, serum GH concentrations were unaffected by saline-treatment (Figure 4).

Table 8. Mean serum growth hormone (GH) concentrations[a] in Holstein cows treated with saline or fluphenazine (FLU) during the luteal phase of the estrous cycle

	Mean serum GH concentration (ng/ml)		
Treatment	Period [b] 1	Period 2	Period 3
Saline (n=6)	4.5 ± .3	4.6 ± .5	4.9 ± .5
FLU (.1 mg/kg BW;n=6)	4.6 ± .3	5.5 ± .5	5.3 ± .5
FLU (.5 mg/kg BW;n=8)	4.8 ± .3	4.6 ± .5	4.5 ± .5

[a] Least squares means ± SEM.

[b] Period 1= Mean hormone concentration of 17 blood samples collected before fluphenazine or saline administration from 0500 to 0900 h;period 2= Mean hormone concentration of 8 blood samples collected after fluphenazine or saline administration from 0915 to 1100 h;period 3= Mean hormone concentration of 8 blood samples collected from 1115 to 1300 h.

DISCUSSION

The presence of a corpus luteum coupled with high serum progesterone concentrations (3.0 ± .3 ng/ml) confirmed that the experiment was conducted during the luteal phase of the estrous cycle and when the hypothalamic-pituitary axis was likely under the influence of negative feedback of progesterone. Mean LH pulse frequency during the pre-treatment period in this study was similar to those reported by Rahe et al. (1980) and Mahmoud et al. (1989) during the luteal phase of the estrous cycle in cattle.

The data from this study support the hypothesis that dopamine inhibits PRL secretion in cattle because the dopamine receptor blocker, FLU, elicited an abrupt increase in PRL secretion (Figure 1). The site of stimulatory action of FLU cannot be derived from this study; however, two possible mechanisms could account for stimulation

of PRL secretion. The more probable mechanism is direct interaction of FLU with pituitary dopamine receptors. It has been postulated that control of PRL secretion occurs through inhibitory mechanisms involving binding of dopamine to specific pituitary receptors (Calabro and McLeod, 1978; Elssaser and Bolt, 1987). It has been shown that the bovine anterior pituitary gland contains dopamine receptors (Chazot and Strange, 1992; Stafford et al., 1993). Thus, FLU, a specific dopamine receptor antagonist, may interact with these pituitary receptors and block the binding of endogenous dopamine to its respective receptors.

A second possible mechanism is a FLU-mediated decrease in blood concentration of endogenous dopamine and thus reduction of dopamine inhibitory effect on pituitary PRL secretion. It has been shown that spiperone, a dopamine receptor antagonist, caused a decrease in plasma dopamine probably by increasing the pituitary monoamine oxidase (MAO) concentration (Henson et al., 1987). Monoamine oxidase catalyzes the oxidative deamination of dopamine in a variety of tissues (Martin, 1985). It is possible that FLU treatment resulted in lowered level of dopamine by elevating tissue MAO. If this hypothesis is valid, then FLU-induced increase in serum PRL in the present study may be related to a decrease in blood concentration of dopamine. Dopamine modulation of gonadotropin secretion during the luteal phase of the estrous cycle was not evident because neither dose of dopamine antagonist, FLU, altered LH and FSH secretion (Figures 2 and 3). Lack of effect of FLU on serum LH secretion reported in the present study agrees with previous findings (Ahmadzadeh et al., 1998b) where pimozide, another dopamine antagonist, did not affect mean LH concentration or LH pulse frequency during the luteal phase of the estrous cycle in lactating dairy cows. Furthermore, the lack of LH pulse frequency response to pimozide (Meyer and Goodman, 1985) and haloperidol (Goodman, 1985) has been reported in luteal phase ewes. Similarly, a dopamine antagonist (metoclopramide) did not affect serum LH concentration during the luteal phase of the menstrual cycle of women (Rossmanith et al., 1989).

In contrast to the findings of this experiment, Browning et al. (1998) have shown that ergot alkaloids (ergotamine and ergonovine), which have dopamine-like activity, decreased mean LH concentration during the late luteal phase of the estrous cycle in

Holstein cows. Based on these findings, Browning et al. (1998) hypothesized that dopamine may play an inhibitory role in LH secretion during the luteal phase of the estrous cycle in cattle. However, it should be noted that this effect of ergotamine or ergonovine on LH secretion (Browning et al., 1998) may not be attributable to activation of a dopaminergic system by these compounds because both of these ergot alkaloids exhibit partial agonistic and antagonistic action on α-adrenergic, and serotonergic receptors (Rall and Schleifer, 1980). Therefore, alkaloid-induced decrease in LH in that study (Browning et al., 1998) could have been due to activation of other catecholamine or indolamine systems and not the dopamine system.

Results of the present experiment are also contrary to previous reports in ewes where dopamine antagonists, FLU (Goodman, 1985) and domperidone (Deaver et al., 1987) markedly increased LH pulse frequency during the luteal phase of the estrous cycle. Because these antagonists increased LH pulse frequency, it has been suggested that progesterone may exert inhibitory effects on LH secretion through dopaminergic system (Goodman, 1985; Dailey et al., 1987) in ewes. It appears that cyclic cows are different from ewes in that progesterone suppression of LH secretion may not be mediated via a dopaminergic mechanism or perhaps the negative influence of progesterone is sufficient to override the possible stimulatory effect of FLU on gonadotropin secretion.

McNeilly (1980) has suggested that in the ewe progesterone might increase the activity of hypothalamic dopamine. If this hypothesis holds true in cattle, then during the luteal phase of the estrous cycle, dopamine activity could be strong enough that the doses of FLU (.1 and .5 mg/kg BW) were insufficient to establish competitive inhibition and overcome the effect of endogenous dopamine. However, as indicated previously, the ability of FLU (either dose) to alter PRL secretion (Figure 1) indicates that a sufficient dose of FLU was administered to block at least some dopaminergic receptors and subsequently negate endogenous dopamine action. Moreover, plasma half life of FLU, at least in man, is approximately 15 h (Long, 1998) and serum PRL remained elevated throughout this experiment indicating that sufficient FLU concentration must have been present to maintain an effect during the sampling period.

The variability in LH response to different receptor antagonists (Goodman, 1985) may reflect the selectivity of subclasses of dopamine receptors in modulation of LH secretion during the luteal phase. Fluphenazine is not a potent D2 receptor antagonist and has a higher affinity for D1 receptors (Sawaguchi et al. 1990). Therefore, it may be hypothesized that in cows, dopamine modulation of gonadotropin secretion during the luteal phase of the estrous cycle may be mediated via D2 receptors which FLU is not capable of antagonizing. However, the fact that pimozide, which is relatively specific for D2 dopamine receptors (Goodman, 1985), did not affect LH secretion in luteal phase cows (Ahmadzadeh et al., 1998b) seems to negate that possibility.

Administration of FLU during the luteal phase of the estrous cycle at either .1 or .5 mg/kg BW did not affect serum FSH concentration, FSH pulse frequency, or FSH peak amplitude (Table 5 and Figure 2). These results indicate that the dopaminergic system does not appear to be involved in control of FSH release during this phase of the estrous cycle in cattle. The present data are in agreement with those reported in women, in which metoclopramide, a dopamine antagonist, did not alter serum FSH during the menstrual cycle (Seki and Nagata, 1990). Moreover, concentrations of FSH were not affected when dopamine was infused (i.v.) in ovariectomized, pituitary stalk-transected ewes (Donnelly and Dailey, 1991).

Unfortunately, there is no information regarding the role of dopamine and (or) its antagonist on FSH secretion in cattle. Moreover, in other studies in which the role of dopamine on gonadotropin secretion was investigated in ewes (Deaver and Dailey 1983; Goodman, 1985; Deaver et al., 1987), the effects of dopamine or dopamine antagonist on FSH secretion have not been studied. There is a possible explanation for the lack of FSH response to FLU. If one assumes that FLU exerts an affect on LH secretion possibly by altering hypothalamic GnRH secretion (Chapter 1), and accept the concept that GnRH is the releasing factor for both LH and FSH secretion (Arimura et al., 1976), then the lack of FSH response to FLU administration in this experiment is not surprising, given FLU did not affect LH secretion as described in the present experiment.

The present data show that bolus injection of FLU (.3 mg/kg BW) is ineffective in inducing a change in serum GH secretion during the luteal phase of the estrous cycle in

lactating Holstein cows (Figure 4). This finding suggests that endogenous dopamine may not be important in modulating GH secretion during the luteal phase when serum progesterone concentration is high in cattle. The present study further supports the previous observation by Ahmadzadeh et al. (1998b) concerning lack of GH response to a dopamine antagonist (pimozide) in luteal-phase lactating Holstein cows. The result of the present study is also similar to findings in pregnant ewes in which a dopamine receptor antagonist did not affect GH secretion (Elssaser and Bolt, 1987), and in mares in which administration of a dopamine antagonist (sulpride) was ineffective in changing GH secretion (Thompson et al., 1992). Further, Fernandez et al. (1998) did not observe any change in GH secretion after FLU administration in prepubertal bulls. Findings of the present study are contrary to those in humans and adult rats in which pimozide increased GH secretion (see Liuzzi et al., 1976 for review). Moreover, McMahon et al., (1998) showed that fasting-induced release of GH was blocked by D1 dopamine receptor agonist in steers, suggesting an inhibitory role for dopamine.

The lack of GH response to FLU could be attributable to dose insufficiency of FLU. In other words, .1 or .5 mg FLU/kg BW may be inadequate to block the possible suppressing effect of endogenous dopamine on GH secretion during the luteal phase of the estrous cycle. However, the ability of either dose of FLU to increase PRL secretion (Figure 1) indicates that sufficient dose of FLU was administered to block dopamine receptors and therefore block dopaminergic activity.

It has been hypothesized (McMahon et al., 1998; West et al., 1997) that dopamine inhibits GH secretion in steers and this inhibitory action of dopamine is specifically mediated through D1 dopamine receptors. If this hypothesis is valid, one would expect that dopamine antagonist (FLU) impair endogenous dopamine action and cause an increase in GH secretion. There is no clear explanation concerning the lack of GH response to FLU in the present study. It can be argued that the lack of GH response was attributable to lack of specificity of FLU for D1 receptors since FLU is not a potent D1 antagonist and has affinity for both D1 and D2 receptors (Sawaguchi et al., 1990). In other words, if dopamine regulates GH secretion specifically via D1 receptors (West et al., 1997; McMahon et al., 1998), then it is conceivable that the lack of GH response to

FLU in the present study is due to an inability of FLU to effectively block D1 receptors. However, the fact that the same dopamine antagonist decreased GH secretion during the early postpartum period in cattle (Chapter 2) negates the above hypothesis. In that study, bolus injection of FLU (.3 mg/kg BW) suppressed GH secretion in anovulatory early postpartum cows suggesting that endogenous dopamine is stimulatory to GH secretion during this period. There is no clear explanation for this different response to FLU in anovulatory postpartum cows versus the luteal-phase cows in the present study. It appears likely that dopamine modulation of GH secretion changes with time during the postpartum period and is abolished after resumption of ovarian cyclicity.

In conclusion, results of this study support our previous finding in cattle (Ahmadzadeh et al., 1998b), and indicates that while dopamine antagonist is capable of altering the dopaminergic system and increasing PRL secretion during the luteal phase of the estrous cycle, it does not affect gonadotropin secretion. These findings appear to indicate that dopamine modulation of gonadotropin secretion during the luteal phase of the estrous cycle is absent or overridden by high circulating progesterone. It appears likely that dopamine is not modulating gonadotropin secretion during this phase of the estrous cycle and progesterone suppression of LH secretion is mediated via different neural elements. Furthremore, These findings appear to indicate that dopamine modulation of GH secretion during the luteal phase of the estrous cycle is also absent or overridden by high circulating progesterone, suggesting that dopamine is may not be a neuromodulator of GH secretion during in this phase of the estrous cycle.

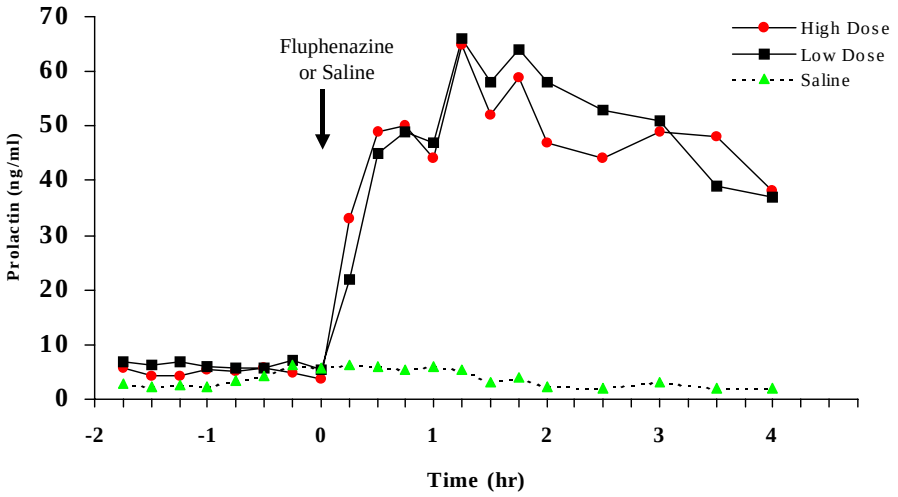

Figure1. Mean serum prolactin concentrations before and after low dose (.1 mg/kg BW;n=6)of fluphenazine, high dose (.5 mg/kg BW;n=6) of fluphenazine, or saline (n=6) administration during the luteal phase of the estrous cycle in Holstein cows.

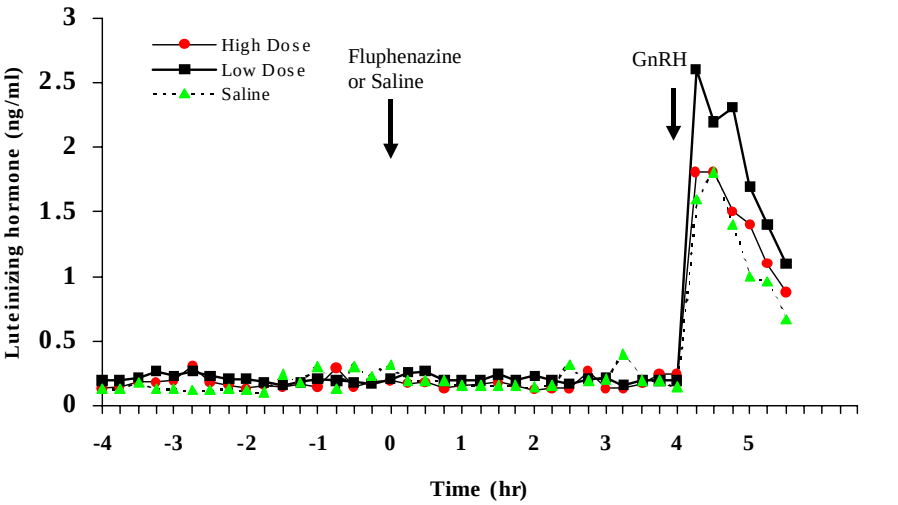

Figure 2. Mean serum luteinizing hormone concentrations before and after low dose (.1 mg/kg BW; n=6) of fluphenazine, high dose (.5 mg/kg BW; n=6) of fluphenazine, or saline (n=6) administration and after GnRH (25 ug) during the luteal phase of the estrous cycle in Holstein cows.

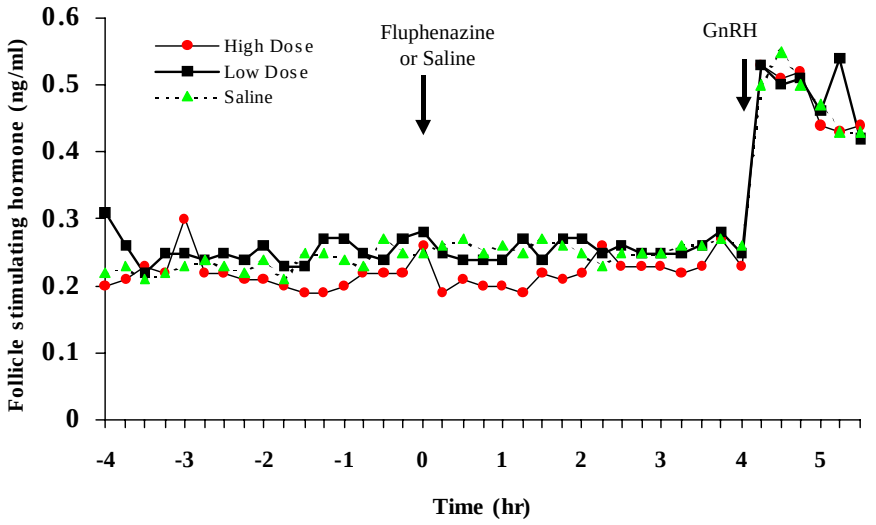

Figure 3. Mean serum follicle stimulating hormone concentrations before and after low dose (.1 mg/kg BW; n=6) of fluphenazine, high dose (.5 mg/kg BW; n=6) of fluphenazine, or saline (n=6) administration and after GnRH (25 ug) during the luteal phase of the estrous cycle in Holstein cows.

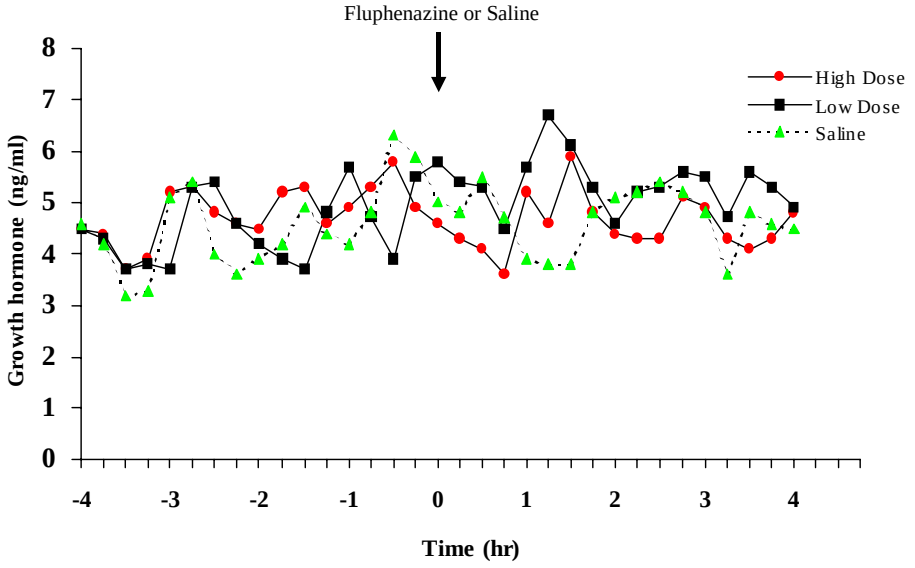

Figure 4. Mean serum growth hormone concentrations before and after low dose (.1 mg/kg BW;n=6) of fluphenazine, high dose (.5 mg/kg BW;n=6) of fluphenazine, or saline (n=6) administration during the luteal phase of the estrous cycle in Holstein cows.

CHAPTER 3

EFFECT OF DOPAMINE ANTAGONIST (FLUPHENAZINE) ON LUTEINIZING HORMONE AND FOLLICLE STIMULATING HORMONE SECRETION DURING THE FOLLICULAR AND METESTRUS PHASES OF THE ESTROUS CYCLE IN HOLSTEIN COWS

ABSTRACT

Fluphenazine (FLU), a dopamine receptor antagonist, was used to investigate the role of endogenous dopamine on serum LH and FSH concentrations during the early follicular and early metestrus phases of the estrous cycle. In Experiment 1, 15 lactating Holstein cows received 25 mg prostaglandin ($PGF_{2\alpha}$), and on d 9 or 10 post-estrus, after detection of a corpus luteum, all cows received a second treatment of $PGF_{2\alpha}$. Thirty-three h later (early follicular phase), cows were randomly assigned to receive either saline (n=7) or .3 mg FLU/kg BW i.v. (n=8). Blood samples were collected at 15-min intervals for 4 h before and 4 hr after saline or FLU. Immediately thereafter, all cows received 25 ug GnRH and blood collection continued for 1.5 h. Mean serum progesterone was .5 ± .3 ng/ml. Fluphenazine caused a decrease ($P < .05$) in mean serum LH concentration and LH pulse frequency. Fluphenazine did not affect mean serum FSH concentration or pulse frequency. Mean serum LH and FSH remained unaffected in saline-treated cows.

In Experiment 2, 15 lactating Holstein cows were used. Cows received 25 mg $PGF_{2\alpha}$, and after detection of estrus, occurrence of ovulation was confirmed using ultrasound. Fifteen to 20 h post-ovulation (metestrus phase) cows were randomly assigned to receive (i.v.) either saline (n=7) or .3 mg FLU/kg BW. Blood collection and drug administration methods were the same as used in Experiment 1, except that blood samples were collected at 12 min intervals. Mean serum progesterone was .16 ± .13 ng/ml. Fluphenazine decreased ($P < .01$) mean LH concentration and suppressed ($P < .05$) LH pulse frequency. Mean serum FSH concentration and pulse frequency were not

altered by FLU. In both experiments exogenous GnRH increased ($P < .01$) mean serum LH and FSH concentrations in all cows and there was no effect of FLU on gonadotropin response to GnRH. Fluphenazine increased ($P < .01$) PRL concentration in all cows, whereas saline had no effect. These results suggest that during the early follicular and metestrus phases of the estrous cycle, when progesterone concentration is low, LH secretion is modulated by endogenous dopamine. However, a dopamine mediated mechanism for FSH secretion is absent in both phases of the estrous cycle in lactating Holstein cows. The fact that dopamine antagonist increased PRL secretion indicates that endogenous dopamine does inhibit PRL secretion during both the follicular and metestrus phases of the estrous cycle.
Key words: Dopamine antagonist, Lutenizing hormone, Follicle stimulating hormone, Follicular phase, Metestrus phase, Dairy cow

INTRODUCTION

It is known that the inhibitory effect of the gonads on GnRH and gonadotropin secretion is the result of negative feedback action of both estradiol and progesterone (Goodman and Karsch, 1980). Ovarian steroids act as modulators of gonadotropin secretion during the bovine estrous cycle (Stumpf et al., 1988). It is also known that this effect of steroids on GnRH and LH secretion is not direct, and is mediated through another set of interneurons (Thiery and Martin, 1991). There is evidence that steroids affect catecholamine turnover rates in hypothalamic tissue (Beattie et al., 1972). Fuxe et al., (1980) showed that steroids and some of their metabolites (catecholestrogen) may influence dopamine release and synthesis in the area of the anterior hypothalamus in the rat. Furthermore, Fuxe et al. (1980) showed that estradiol 17β markedly reduced dopamine turnover in rat brains and directly activated dopaminergic neurons in the lateral tuberoinfundibular system.

Dopamine has been postulated to regulate release of LH (see Barraclough and Wise, 1982 for review). In an *in vitro* system, where hypothalamic tissue was coincubated with pituitary tissue, the releasing action of dopamine on LH was blocked by

estradiol (Schneider and McCann, 1970). They proposed that the dopaminergic system might be involved in negative feedback mechanism of estradiol on LH secretion, whereby estradiol exerts negative feedback on tonic LH secretion by blocking the stimulatory effect of dopamine. Administration (i.c.v.) of the dopamine agonist, apomorphine, increased plasma LH in estradiol- and progesterone-primed ovariectomized rats (Vijayan and McCann, 1978) indicating that perhaps negative feedback of estradiol is partially mediated via dopamine neurons. In support of this theory estradiol implants in the regions which contain dopaminergic cells inhibited LH pulse frequency in rats, and pimozide, a dopamine receptor antagonist, blocked this effect of estradiol (Tadakoro et al., 1986).

During the follicular phase of the estrous cycle, estradiol is the predominant steroid that controls tonic secretion of LH (Goodman and Karsch, 1982). However, the role of dopamine in regulation of gonadotropin during this phase is not clear. Deaver and Dailey (1983) demonstrated that in cyclic ewes intravenous infusion of dopamine reduced LH concentration during early induced-luteal regression. However, the same dose of dopamine had no effect on LH during the late follicular phase of the estrous cycle. These authors concluded that during periods of estrogen domination, dopamine has no detectable effect on secretion of LH in ewes.

The dopamine antagonists pimozide (Jackson, 1977) suppressed tonic secretion of LH in ovariectomized ewes. In contrast to the inhibitory effect of these dopamine antagonists in ovariectomized ewes, pimozide and FLU increased pulsatile secretion of LH in intact anestrous ewes (Meyer and Goodman, 1985; Meyer and Goodman, 1986). Therefore, it appears that, depending on presence or absence of ovaries, i.e. steroid milieu, the effect of dopamine antagonists on LH may vary. It has been shown (Chapter 1) that dopamine may play a stimulatory role in modulation of LH secretion in anovulatory early postpartum dairy cows because administration of a dopamine antagonist (FLU) suppressed LH secretion . In contrast dopamine antagonists had no effect on gonadotropin secretion during the luteal phase of the estrous cycle, when progesterone is the predominant circulating steroid in cattle (Ahmadzadeh et al., 1998b; Chapter 2). Estradiol 17β affects dopamine turnover rate in the brain in rats (Fuxe et al.,

1980) and the dopaminergic system is involved in the negative feedback mechanism of estradiol on LH secretion (Schneider and McCann, 1970). It not clear whether dopamine influence LH secretion when circulating progesterone is low in cyclic cattle and whether the effects differ from those in anovulatory postpartum and luteal-phase cows. Thus, the objective of this study was to investigate the role of endogenous dopamine on gonadotropin secretion by characterizing serum LH and FSH response to administration of a dopamine antagonist, FLU, during the follicular and metestrus phases of the estrous cycle in lactating Holstein cows. Since it is well established that dopamine inhibits PRL secretion, serum PRL concentrations in response to FLU were measured as positive controls to ensure that dopamine receptors were effectively blocked.

MATERIALS AND METHODS

Experiment 1.

Fifteen cyclic Holstein cows, 72 ± 29 d postpartum (mean BW= 585 ± 64), were used. Ovarian activity of cows was monitored weekly by real-time ultrasound scanner, equipped with a 5 MHz rectal transducer probe (Aloka Co., 6-22-1, Mure, Mitaka-shi, Tokyo , Japan) and upon detection of a corpus luteum, 25 mg of $PGF_{2\alpha}$ (Lutalyse®, The Upjohn Co., Kalamazoo, MI) was administered (i.m.) to induce luteolysis. Estrous activity was monitored using an electronic pressure-sensing device (HW®; Heat Watch, DDx, Denver, CO). On d 9 or 10 post-estrus (estrus = d 0), after detection of a corpus luteum by ultrasound, cows received a second dose of $PGF_{2\alpha}$ (25 mg/cow) at 2000 h. Twenty-four h later, cows were weighed and ovarian activity was monitored by ultrasound. All cows were fitted with jugular catheters as previously described (Johnson et al., 1993). Thirty-three h post-$PGF_{2\alpha}$ (early follicular phase), all cows were randomly assigned to receive either .9 % saline (n=7) or .3 mg FLU/kg BW (n=8) i.v. dissolved in 10 ml physiological saline. Beginning at 0500 h blood samples were collected at 15-min intervals for 4 h before and 4 h after FLU or saline. Immediately thereafter, all cows received (i.v.) 25 μg GnRH (Cystorelin®, Sanofi, Overland Park, KS) and blood

collection continued for an additional 1.5 h. This low dose of GnRH was administered to test pituitary competency to release of LH.

Experiment 2.

Fifteen lactating Holstein cows, 65 ± 24 d postpartum (mean BW = 576 ± 68), were used. Ovarian activity of cows was monitored by ultrasonography and upon detection of a corpus luteum, 25 mg of $PGF_{2\alpha}$ (Lutalyse®, The Upjohn Co., Kalamazoo, MI) was administered (i.m.) to induce luteolysis. Estrous activity was monitored using the HW® system. Following first mount recorded by HW®, ultrasonographic exams of ovaries were performed at 12 and 20 h and then every 4 h until ovulation or until 32 h post-estrus. Cows that did not ovulate by 32 h post-estrus were not used. Ovulation was defined as sudden disappearance of any follicle larger than 10 mm in diameter (Kaneko et al., 1991). Ovulation time was defined as the number of hours from the first recorded mount to the midpoint of the examinations between which the ovulatory follicle had disappeared (Walker et al., 1996). After detection of ovulation, cows were weighed, fitted with jugular catheters (Johnson et al., 1993), and randomly assigned to receive either .9 % saline (n=7) or .3 mg FLU/kg BW (n=8) i.v. dissolved in 10 ml physiological saline. Fifteen to 20 h post ovulation (early metestrus) blood samples were collected at 12-min intervals for 4 h before and 4 h after FLU or saline. Immediately thereafter, all cows received (i.v.) 25 μg GnRH (Cystorelin®, Sanofi, Overland Park, KS) and blood collection continued for an additional 1.5 h.

In both experiments, in each sampling interval, the initial 2 ml of each blood sample was discarded, and a 10 ml sample was then transferred into collection tubes which and were immediately placed in ice and then stored at 4°C for 24 h for clotting. All blood samples were centrifuged for 30 min at 2,200 ×g at 4°C. Serum was harvested and stored at -20°C until assayed for LH, FSH, PRL, and progesterone. During blood sampling all cows were tied in individual stalls with access to a total mixed ration and water *ad libitum*.

Hormone Assay

Concentration of LH was determined by a double-antibody RIA as described by Bolt and Caldwell (1992). This assay was performed in non-equilibrium condition. Purified LH (USDA-bLH-B-6) was used as reference standard and for radioiodination. Sheep anti-rabbit gammaglobulin was used as precipitating second antibody. The primary antiserum (USDA-309-684p) bound 40% of radiolabeled LH in the absence of unlabeled hormone. Sensitivity of the assay was less than 0.1 ng/ml reference standard LH and defined as the concentration corresponding to 2 standard deviations less than mean zero dose. All samples were assayed in duplicate, and intra- and inter-assay CV calculated from serum pools were 3.1% and 11.2%, respectively.

Serum FSH was quantified by a heterologous double antibody RIA using with rabbit anti-ovine FSH antiserum (NIDKK-anti-oFSH-1).). The assay performed in a non-equilibrium condition. Purified FSH (USDA-bFSH-I-2) was used as reference standard and for radioiodination. Sheep anti-rabbit gammaglobulin was used as the precipitating second antibody. The primary antiserum bound 15% of radiolabeled FSH in the absence of unlabeled hormone. Sensitivity of the assay was less than .06 ng/ml reference standard FSH and defined as the concentration corresponding to 2 standard deviations less than mean zero dose. All samples were assayed in duplicate, and intra- and inter-assay CV calculated from serum pools were 4.6% and 9.3%, respectively.

Concentration of PRL was determined by a double antibody RIA (Barnes et al., 1985). All samples were assayed in duplicate, and intra- and inter-assay CV calculated from serum pools were 4.6% and 9%, respectively.

Concentration of progesterone was quantified using solid-phase RIA (Diagnostic Products Corp.; Los Angeles, CA) which has been previously validated in our laboratory (Holt et al., 1989). The standard curve ranged from 0.1 to 20 ng/ml. All samples were assayed in duplicate (1 assay) and intra-assay CV was 4.1%.

Statistical Analysis

Serum LH and FSH data in both experiments were analyzed by least-squares analysis of variance by the General Linear Model (GLM) procedure using Statistical

Analysis Systems (SAS® Institute, Cary, NC). To determine the effect of FLU on LH and FSH, blood samples were categorized to one of three periods. Period 1 represented blood samples collected for 4 h before saline or FLU administration and was considered the pre-treatment period. Period 2 represented blood samples collected for 2 h immediately after FLU or saline administration; and samples thereafter (2 h) represented period 3. The experiment was designed to determine the effects of FLU administration by comparing LH and FSH concentration between period 1 and periods 2 and 3. The statistical model included treatment, cow within treatment, period, and all the possible interactions. Period by cow within treatment was used as the error term to test the effects of period and period by treatment. If the effects of period or period by treatment were significant ($P < .05$), non-orthogonal contrasts were used to compare least squares means for periods 1 vs 2, 1 vs 3 and 2 vs 3 within each treatment using the improved Bonferroni F-test (1977). To analyze the effect of GnRH on serum LH concentration, the sampling interval was divided into 2 periods. The pre-GnRH period represented the serum samples collected 1 hr prior to GnRH administration and the post-GnRH period represented samples collected for 1 hr after GnRH treatment. Differences between the two periods were tested using the same statistical model as above.

Pulses of LH and FSH were determined as described by Goodman and Karsch (1980) and modified by Richards et al. (1991). Briefly, any value of LH and FSH greater than two SD above the mean for a cow followed by at least two values of lesser concentration was considered a pulse. In addition, a peak had to occur within two samples of previous nadir and the amplitude of the peak had to be greater than the sensitivity of the LH and FSH assays. Amplitude of LH and FSH pulses defined as the difference between the greatest concentration during the pulse and the nadir within 30 min before the pulse. Pulse frequency and peak amplitude of LH and FSH were analyzed by least-squares analysis of variance by the GLM procedure using Statistical Analysis Systems (SAS® Institute, Cary, NC). The statistical model included treatment, cow within treatment, period, and treatment by period interaction. Using the improved Bonferroni F-test (1977), the least square means of LH and FSH pulse frequencies and

peak amplitudes between pre- and post-treatment periods were compared.

RESULTS

Experiment 1.

None of the cows exhibited estrous behavior during 36 h prior to, or on the day of experiment. Results of ultrasonography conducted approximately 10 h before the initiation of the experiment, verified the absence of organized luteal tissue (compared to pre-$PGF_{2\alpha}$). On the day of experiment, 33 h after $PGF_{2\alpha}$, all cows had serum progesterone concentrations less than 1 ng/ml and mean concentration was .51± .28. Mean number of follicles equal or greater than 5 mm in diameter in both ovaries was 4.3 ± 1.2 and mean diameter of the largest follicle was 14.8 ± 3.2 mm.

Mean serum PRL was similar between saline- and FLU-treated groups during period 1. As expected, serum PRL concentration increased ($P < .01$) in response to FLU and remained elevated ($P < .01$) and did not return to pretreatment level during the sampling period (Figure 1). Mean serum PRL concentrations remained unchanged during the sampling period in saline-treated cows (Figure 1).

During the follicular phase of the estrous cycle, FLU caused a small but significant decrease ($P < .05$) in serum LH concentration (Table 1; Figure 2). Serum LH concentration decreased from .27 ± .01 ng/ml during the pre-treatment period (Period 1) to .20 ± .01 during both periods 2 and 3 after FLU administration. Serum LH concentrations were unaffected in saline-treated cows (Table 1).

Mean LH pulse frequency was similar between groups before treatment. Mean LH pulse frequency decreased ($P < .05$) in response to FLU, but LH peak amplitude did not change (Table 2). Pulse frequency of LH was completely abolished in 6 of 8 FLU-treated cows, decreased from 2 to 1 pulses/4 h in one cow and did not change in the remaining cow. Mean LH pulse frequency, and peak amplitude were not altered by saline treatment (Table 2).

Exogenous GnRH elicited an increase ($P < .01$) in serum LH concentration in

Table 1. Mean LH concentrations[a] in Holstein cows treated with saline or fluphenazine (FLU) during the follicular phase of the estrous cycle.

	Mean serum LH concentration (ng/ml)		
Treatment	Period [b] 1	Period 2	Period 3
Saline (n=7)	.23 ± .01	.23 ± .02	.26 ± .02
FLU (.3 mg/kg BW;n=8)	.27 ± .01 [c]	.20 ± .01 [d]	.21 ± .01 [d]

[a] Least squares means ± SEM.

[b] Period 1= Mean hormone concentration of 17 blood samples collected before fluphenazine or saline administration from 0500 to 0900 h; period 2= Mean hormone concentration of 8 blood samples collected after fluphenazine or saline administration from 0915 to 1100 h; period 3= Mean hormone concentration of 8 blood samples collected from 1115 to 1300 h.

[c,d] Means within the same treatment row with different superscripts differ ($P < .05$).

Table 2. Mean LH pulse frequency and peak amplitude[a] in Holstein cows treated wth saline or fluphenazine (FLU) during the follicular phase of the estrous cycle.

	Mean LH pulse frequency (pulses /4 h)		Mean LH peak amplitude[b] (ng/ml)	
Treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
Saline (n=7)	1.9 ± .3	2.1 ± .3	.17 ± .04	.15 ± .04
FLU (.3 mg/kg BW; n=8)	1.9 ± .3	.5 ± .3 *	.23 ± .04	.16 ± .04

[a] Least squares means ± SEM. [b] difference between the preceding nadir and maximum height of pulses.

* Different from Pre-treatment mean ($P < .05$).

both FLU- and saline-treated cows (Table 3). The magnitude of LH response was similar for both treatment groups. Fluphenazine did not alter LH response to exogenous GnRH (Figure 3).

During the follicular phase, serum FSH concentrations were similar for both FLU- and saline-treated groups during the pre-treatment period (Table 4). Fluphenazine did not alter FSH concentration during the follicular phase of the estrous cycle (Table 4; Figure 4). Mean FSH concentration was .143 ± .002 before FLU and .136 ± .002 ng/ml after

Table 3. Mean serum LH concentrations[a] in Holstein cows treated with saline or fluphenazine (FLU) before and after GnRH (25 ug) administration during the follicular phase of the estrous cycle.

	Mean serum LH concentration (ng/ml)	
Treatment	Pre-GnRH [b]	Post-GnRH
Saline (n=7)	.26 ± .35 [c]	2.29 ± .35 [d]
FLU (.3 mg/kg BW; n=8)	.22 ± .33 [c]	2.56 ± .33 [d]

[a] Least squares means ± standard error.

[b] Pre-GnRH= Mean hormone concentration of 6 blood samples collected 1.5 h prior to GnRH administration; Post-GnRH= Mean hormone concentration of 6 blood samples collected 1.5 h after GnRH administration.

[c,d] Means within the same treatment row with different superscript differ ($P < .01$).

FLU treatment. Serum FSH concentration, pulse frequency, and peak amplitude was not affected by saline administration (Tables 4 and 5).

Table 4. Mean Serum FSH concentrations[a] in Holstein cows treated with saline or fluphenazine (FLU) during the follicular phase of the estrous cycle.

	Mean serum FSH concentration (ng/ml)		
Treatment	Period [b] 1	Period 2	Period 3
Saline (n=7)	.150 ± .002	.152 ± .004	.157 ± .004
FLU (.3 mg/kg BW;n=8)	.143 ± .002	.136 ± .003	.136 ± .003

[a] Least squares means ± SEM.

[b] Period 1= Mean hormone concentration of 17 blood samples collected before fluphenazine or saline administration from 0500 to 0900 h; period 2= Mean hormone concentration of 8 blood samples collected after fluphenazine or saline administration from 0915 to 1100 h; period 3= Mean hormone concentration of 8 blood samples collected from 1115 to 1300 h.

Table 5. Mean FSH pulse frequency and peak amplitude[a] in Holstein cows treated with saline or fluphenazine (FLU) during the follicular phase of the estrous cycle.

	Mean FSH pulse frequency (pulses /4 h)		Mean FSH peak amplitude[b] (ng/ml)	
Treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
Saline (n=7)	1 ± .18	.71 ± .18	.07 ± .02	.05 ± .02
FLU (.3 mg/kg BW; n=8)	.62 ± .17	.50 ± .17	.05 ± .02	.04 ± .02

[a] Least squares means ± SEM. [b] difference between the preceding nadir and maximum height of pulses.

Exogenous GnRH increased ($P < .01$) serum FSH concentration in both saline- and FLU-treated cows (Table 6). Follicle stimulating hormone concentration increased by approximately 2-fold after GnRH and FLU had no effect on FSH response to GnRH (Figure 4).

Table 6. Mean FSH concentrations[a] in Holstein cows treated with saline or fluphenazine (FLU) before and after GnRH (25 ug) administration during the follicular phase of the estrous cycle.

	Mean serum FSH concentration (ng/ml)	
Treatment	Pre-GnRH [b]	Post-GnRH
Saline (n=7)	.157 ± .017 [c]	.278 ± .017 [d]
FLU (.3 mg/kg BW; n=8)	.136 ± .016 [c]	.315 ± .016 [d]

[a] Least squares means ± standard error.

[b] Pre-GnRH= Mean hormone concentration of 6 blood samples collected 1.5 h prior to GnRH administration; Post-GnRH= Mean hormone concentration of 6 blood samples collected 1.5 h after GnRH administration.

[c,d] Means within the same treatment row with different superscript differ ($P < .01$).

Experiment 2

On the day of experiment (15-20 h post-ovulation, early metestrus), all cows had serum progesterone concentrations of less than 1 ng/ml and mean serum progesterone

concentration was .16 ± .13 ng/ml. Mean diameter of the ovulatory follicle was 18 ± 2.6 mm. The average time from onset of estrus to ovulation was 26.1 (± 5.1) h.

Mean serum PRL did not differ between saline- and FLU-treated cows during period 1 (Figure 5). During metestrus phase, FLU increased ($P < .01$) serum PRL by more than 7-fold during period 2 when compared to the pre-treatment period (Figure 5). Serum PRL concentration remained elevated ($P < .01$) throughout the experiment and did not returned to pre-treatment level. Serum PRL increased in response to FLU within 15 min after administration and all cows responded to treatment with an abrupt increase in PRL. Mean serum PRL concentrations remained unchanged during the sampling period in saline-treated cows (Figure 5)

In Experiment 2, mean serum LH concentrations did not differ between saline- and FLU-treated groups before treatment (Period 1; Table 7). During the early metestrus phase mean serum LH concentration tended to decrease ($P = .07$) after FLU administration (Table 7; Figure 6). Serum LH concentration decreased from .20 ± .01 before FLU, to .17 ± .01 ng/ml after FLU. Serum LH concentration in cows that received saline were not different between periods (Table 7).

Table 7. Mean LH concentrations[a] in Holstein cows treated with saline or fluphenazine (FLU) during the metestrus phase of the estrous cycle.

	Mean serum LH concentration (ng/ml)		
Treatment	Period [b] 1	Period 2	Period 3
Saline (n=7)	.19 ± .01	.19 ± .01	.20 ± .01
FLU (.3 mg/kg BW;n=8)	.20 ± .01 [c]	.17 ± .01 [d]	.17 ± .01 [d]

[a] Least squares means ± SEM.

[b] Period 1= Mean hormone concentration of 21 blood samples collected before fluphenazine or saline administration from 0500 to 0900 h; period 2= Mean hormone concentration of 10 blood samples collected after fluphenazine or saline administration from 0912 to 1100 h; period 3= Mean hormone concentration of 10 blood samples collected from 1112 to 1300 h.

[c,d] Means within the same treatment row with different superscripts differ ($P = .10$).

Table 8. Mean LH pulse frequency and peak amplitude[a] in Holstein cows treated with saline or fluphenazine (FLU) during the metestrus phase of the estrous cycle.

	Mean LH pulse frequency (pulses /4 h)		Mean LH peak amplitude[b] (ng/ml)	
Treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
Saline (n=7)	1.6 ± .3	1.7 ± .3	.12 ± .07	.18 ± .07
FLU (.3 mg/kg BW; n=8)	1.9 ± .3	.7 ± .3 *	.22 ± .06	.07 ± .06

[a] Least squares means ± SEM. [b] difference between the preceding nadir and maximum height of pulses.
* Different from Pre-treatment mean ($P < .05$).

Fluphenazine decreased ($P < .05$) LH pulse frequency but did not affect LH peak amplitude (Table 8). Pulse frequency of LH was completely abolished in 3 of 8 FLU treated cows, decreased in 3 cows, and did not change in the 2 remaining cows. Further, LH pulse frequency and peak amplitude were not different between pre- and post-saline injection (Table 8). Gonadotropin releasing hormone elicited an increase ($P < .01$) in LH concentration in both saline- and FLU-treated cows (Table 9; Figure 7). Serum LH response to GnRH was similar between the two treatment groups, indicating that FLU did not affect LH response to exogenous GnRH.

Table 9. Mean serum LH concentrations[a] in Holstein cows treated with saline or fluphenazine (FLU) before and after GnRH (25 ug) administration during the metestrus phase of the estrous cycle.

	Mean serum LH concentration (ng/ml)	
Treatment	Pre-GnRH [b]	Post-GnRH
Saline (n=7)	.20 ± .08 [c]	1.06 ± .08 [d]
FLU (.3 mg/kg BW; n=8)	.17 ± .08 [c]	.97 ± .08 [d]

[a] Least squares means ± standard error.
[b] Pre-GnRH= Mean hormone concentration of 7 blood samples collected prior to GnRH administration; Post-GnRH= Mean hormone concentration of 7 blood samples collected after GnRH administration.
[c,d] Means within the same treatment row with different superscripts differ ($P < .01$).

Table 10. Mean FSH concentrations[a] in Holstein cows treated with saline or fluphenazine (FLU) during the metestrus phase of the estrous cycle.

	Mean serum FSH concentration (ng/ml)		
Treatment	Period [b] 1	Period 2	Period 3
Saline (n=7)	.25 ± .01	.26 ± 0.1	.27 ± .01
FLU (.5 mg/kg BW;n=8)	.35 ± .01	.34 ± .01	.35 ± .01

[a] Least squares means ± SEM.

[b] Period 1=Mean hormone concentration of 21 blood samples collected before fluphenazine or saline administration from 0500 to 0900 h; period 2=Mean hormone concentration of 10 blood samples collected after fluphenazine or saline administration from 0912 to 1100 h; period 3=Mean hormone concentration of 10 blood samples collected from 1112 to 1300 h.

Similar to Experiment 1, mean serum FSH concentrations did not change after FLU administration during periods 2 and 3 compared to period 1 (Table 10; Figure 8). In addition, FLU did not affect FSH pulse frequency or peak amplitude (Table 11). Mean serum FSH concentration was lower during the pre-treatment period (period 1) in the saline group compared to the corresponding period in the FLU group. Mean serum FSH concentration, FSH pulse frequency and peak amplitude remained unchanged after saline injection (Table 10 and 11).

Table 11. Mean FSH pulse frequency and peak amplitude[a] in Holstein cows treated with saline or fluphenazine (FLU) during the metestrus phase of the estrous cycle.

	Mean FSH pulse frequency (pulses /4 h)		Mean FSH peak amplitude[b] (ng/ml)	
Treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
Saline (n=7)	.7 ± .2	.7 ± .2	.04 ± .02	.06 ± .02
FLU (.3 mg/kg BW; n=8)	.7 ± .2	.6 ± .2	.05 ± .02	.06 ± .02

[a] Least squares means ± SEM.

[b] difference between the preceding nadir and maximum height of pulses.

Table 12. Mean serum FSH concentrations[a] in Holstein cows treated with saline or fluphenazine (FLU) before and after GnRH (25 ug) administration during the metestrus phase of the estrous cycle.

	Mean serum FSH concentration (ng/ml)	
Treatment	Pre-GnRH [b]	Post-GnRH
Saline (n=7)	.27 ± .02 [c]	.42 ± .02 [d]
FLU (.3 mg/kg BW; n=8)	.35 ± .02 [c]	.54 ± .02 [d]

[a] Least squares means ± standard error.

[b] Pre-GnRH= Mean hormone concentration of 7 blood samples collected prior to GnRH administration; Post-GnRH= Mean hormone concentration of 7 blood samples collected after GnRH administration.

[c,d] Means within the same treatment row with different superscript differ ($P < .01$).

Exogenous GnRH increased ($P < .01$) FSH concentration by more than 1.5-fold in both treatment groups and there was no effect of FLU on FSH response to GnRH (Table 12; Figure 8)

DISCUSSION

In Experiment 1 (follicular phase), $PGF_{2\alpha}$ induced luteolysis and on the day of experiment (33 h post-$PGF_{2\alpha}$) mean serum progesterone concentration decreased to less than 1 ng/ml in all cows. Mean serum progesterone concentration was .51 ± .28 ng/ml compared to 3.6 ± .4 ng/ml prior to $PGF_{2\alpha}$ administration. Additionally, several follicles (greater than 5 mm in diameter) were present on the ovaries and no organized luteal tissue was detected. These observations, combined with decrease in serum progesterone concentration and lack of estrous activity before and on the day of the experiment, verified that the experiment was conducted during the follicular phase of the estrous cycle.

As expected, FLU administration abruptly increased PRL secretion (Figure 1) indicating that FLU antagonized the action of endogenous dopamine and at least some

dopamine receptors were blocked. Results of this experiment indicate that FLU (.3 mg/kg BW) decreased LH pulse frequency and LH concentrations (Figure 2) during the follicular phase of the estrous cycle. These results provide the first information concerning the negative effect of dopamine antagonist on release of pituitary LH and appear to indicate that endogenous dopamine plays a stimulatory role in LH secretion during the follicular phase of the estrous cycle in dairy cattle.

Results of the present experiment differ from those reported in studies utilizing sheep. Dopamine administration suppressed LH secretion during early luteal regression but had no effect on LH secretion during the late follicular phase of the estrous cycle in ewes (Deaver and Dailey, 1983). Furthermore, dopamine antagonist did not affect LH secretion during the follicular phase of the estrous cycle in ewes (Deaver et al., 1987). These authors hypothesized that during periods of estrogen domination (follicular phase), dopamine has no detectable effect on LH secretion in ewes. If this model holds true for cattle, then one would expect to observe that dopamine antagonist (FLU) administration exerted no effect on LH secretion during the follicular phase of the estrous cycle. However, in the present study FLU suppressed LH secretion. Therefore, unlike ewes, it appears that during the period of high circulating estradiol and low progesterone concentration (follicular phase), endogenous dopamine plays a role in regulation of LH secretion in cattle.

The exact mechanism for the inhibitory action of FLU on LH secretion in this experiment is not clear. As previously stated, dopamine may be stimulatory to LH secretion during the follicular phase of the estrous cycle and disruption of this system by a dopamine antagonist results in a decrease in LH secretion. It has been reported that in rats (Fuxe et al., 1980) and ewes (Gayrard et al., 1994) estradiol increased dopamine turnover in the hypothalamus. Therefore, it is speculated that perhaps high circulating estradiol during the follicular phase of the estrous cycle in cows, stimulates dopamine neurons and increases dopamine activity which in turn stimulates GnRH and LH secretion. This proposed interaction between estradiol and dopamine may explain, in part, the increase in LH pulse frequency during the follicular phase compared to the luteal phase of the estrous cycle as observed by Rahe et al. (1980) and Mahmaoud et al. (1989).

As previously mentioned, 7 of 8 FLU-treated cows experienced a decrease in LH pulse frequency. The lack of LH pulse response in one cow is not attributable to insufficient dose of FLU because serum PRL increased immediately after FLU administration in all cows indicating that the dose FLU was sufficient to cause a physiological change.

In Experiment 2, ultrasonography indicated the absence of a corpus luteum in all cows. As expected, mean serum progesterone concentration was less than 1 ng/ml in all cows (.16 ± .13 ng/ml). After completion of the experiment, no large follicles (> 10 mm in diameter) were observed in ovaries. These observations were similar to those by Kaneko et al. (1991) on d 3 post-estrus in cows. Kaneko et al. (1991) reported that plasma estradiol did not reach high levels until d 4 to 5 post-estrus. Further, the increase in plasma estradiol did not occur until non-ovulatory dominant follicles from the first follicular wave showed a marked increase in size from d 3 of the cycle onward. Experiment 2 was conducted between d 2 and 3 of the estrous cycle, likely before dominant follicles gain the ability to secrete substantial amounts of estradiol (Ireland and Roche, 1983; Ireland et al., 1984) during the early metestrus phase of the estrous cycle.

Similar to the follicular phase (Experiment 1), FLU administration during metestrus abruptly increased PRL secretion (Figure 5) indicating that FLU antagonized the action of endogenous dopamine and at least some dopamine receptors were blocked. Fluphenazine administration decreased LH pulse frequency (Table 8) and tended to decrease mean LH concentration (Table 7) during the early metestrus phase of the estrous cycle. To our knowledge, there is no information concerning the role of dopamine in control of LH secretion during this phase of the estrous cycle in cattle. Results of Experiment 2 provide evidence that a dopamine antagonist decreased LH secretion during the metestrus phase and appears to indicate that in this phase of the estrous cycle, when circulating concentrations of both progesterone and estradiol are low (Kaneko et al., 1991), endogenous dopamine plays a stimulatory role on LH secretion. As previously mentioned, LH pulse frequency did not change in 2 of 8 FLU-treated cows during the metestrous phase of the estrous cycle. The lack of LH pulse response in these cows is not attributable to insufficient dose of FLU because serum PRL increased immediately after

FLU administration in this cow indicating that the dose of FLU was sufficient to cause a physiological change

It was speculated (Experiment 1) that high circulating estradiol during the follicular phase of the estrous cycle in cows acts upon dopamine neurons, and increases dopamine activity which in turn stimulates LH secretion. However, based on findings in Experiment 2, it appears that endogenous dopamine is also stimulatory to LH secretion during the time when circulating estradiol is low (i.e. metestrus phase of the estrous cycle). The possibility exists that there are dopaminergic neurons in cattle that operate independently from estradiol or are estradiol insensitive, that stimulate pulses of GnRH and increase LH secretion. Therefore, in the absence of an active corpus luteum (low progesterone), disruption of this system by dopamine antagonist would result in reduction of GnRH and LH pulse frequency, as observed during both the follicular and early metestrus phases of the estrous cycle. The fact that FLU elicited a similar response in LH pulse frequency in anovulatory early postpartum cows (Chapter 1) supports the theory that endogenous dopamine stimulates LH secretion regardless of circulating estradiol level.

The site of inhibitory action of FLU on LH cannot be derived from these experiments. It is apparent that LH pulse frequency is directly related to activity of GnRH cells and that LH pulses in peripheral circulation corresponded, on a one to one basis, with GnRH pulses secreted by the hypothalamus (Thiery and Martin, 1991). Since FLU can penetrate the blood brain barrier (Meyer and Goodman, 1986; Long, 1998), suppression of LH pulse frequency after FLU administration during both the follicular and metestrus phases of the estrous cycle is probably indicative of inhibition of GnRH release by this dopamine antagonist. The fact that FLU administration did not alter LH response to exogenous GnRH compared to saline-treated cows in both experiments (Figures 3 and 7), combined with the suppressing effect of FLU on LH pulse frequency gives further support to the theory that FLU had acted at the hypothalamic/ME level. Therefore, it is likely that endogenous dopamine exerts its action at a site other than the pituitary, possibly the hypothalamus and ME.

In both experiments reported here, it was observed that FLU administration decreased LH secretion preferentially without affecting FSH secretion (Figures 4 and 8). Unfortunately, there is no information regarding the role of dopamine and (or) its antagonist on FSH secretion in cattle. Moreover, in other studies in which the role of dopamine on gonadotropin secretion was investigated in ewes (Deaver and Dailey 1983; Goodman, 1985; Deaver et al., 1987), the effects of dopamine or dopamine antagonist on FSH secretion have not been studied. Fluphenazine modified LH pulse frequency but did not alter LH response to exogenous GnRH during both follicular and metestrus phases of the estrous cycle. These findings led to the hypothesis that FLU had exerted its effects at the level of the hypothalamus and perhaps suppressed GnRH secretion. If this hypothesis is valid, then the different effects exerted by FLU on LH and FSH secretion appears difficult to reconcile, since hypothalamic GnRH is thought to be the sole controller of secretion of both LH and FSH. There are possibilities that may explain the difference between LH response and lack of FSH response to FLU in the present experiments. First, as was shown in rats (Kawakami and Higuchi, 1979), it is possible that there is a separate hypothalamic releasing factor for FSH (i.e. FSH-RH) that is different from the releasing factor for LH secretion (i.e. LHRH). Thus, while dopamine modulates LHRH secretion, it may not intervene with the control of FSH-RH. Second, it may be postulated that the neural control for FSH secretion is entirely different from that of LH secretion and therefore, while endogenous dopamine modulates LH secretion it does not play a role in modulation of FSH secretion. Perhaps other neurotransmitters such as opioids modulate FSH secretion (Koves et al., 1981; Muraki et al., 1979).

Nonetheless, from these studies several conclusions can be drawn:

1) FLU increased serum PRL concentrations during both the follicular and metestrus phases of the estrous cycle in lactating cows indicating that the dose of FLU was sufficient to impair endogenous dopamine action and that at least some dopamine receptors were blocked. Furthermore, dopamine inhibits PRL secretion during both phases of the estrous cycle.

2) Endogenous dopamine plays a role, at least in part, in modulating LH secretion during both the follicular and metestrus phases of the estrous cycle in lactating Holstein cows.

Moreover endogenous dopamine may play a stimulatory role on LH secretion. This conclusion is based on the observation that FLU administration suppressed LH pulse frequency during both phases of the estrous cycle.

3) In the absence of an active corpus luteum when progesterone is not the predominant steroid that exerts negative feedback effects on LH secretion, the suppressive effect of FLU on LH pulse frequency is consistent across both the follicular and metestrus phases of the estrous cycle in lactating cows.

4) Endogenous dopamine may not be the neuromodulator of FSH secretion during the follicular and metestrus phases of the estrous cycle in lactating Holstein cows, since FLU did not have any effect on FSH concentration, FSH pulse frequency, or FSH peak amplitude.

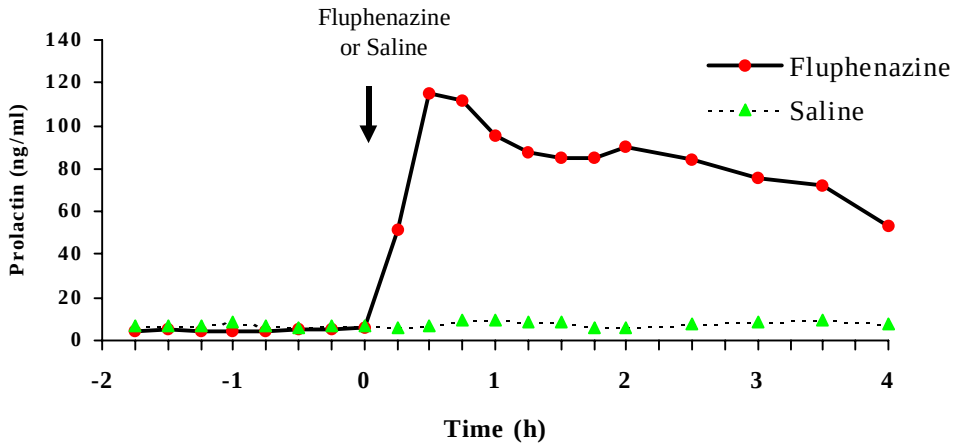

Figure1. Mean serum prolactin concentrations before and after fluphenazine (.3 mg/kg BW;n=8) or saline (n=7) administration during the follicular phase of the estrous cycle in Holstein cows.

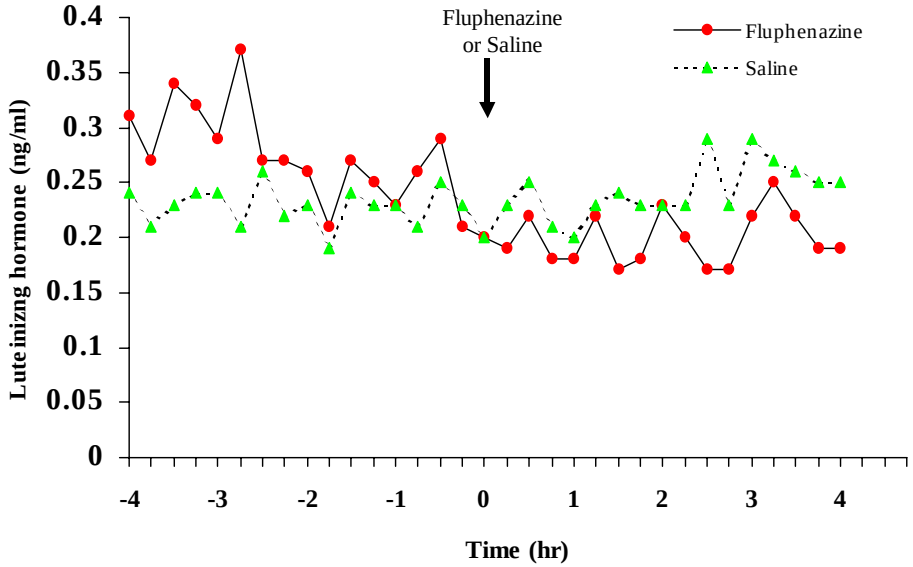

Figure 2. Mean serum luteinizing hormone concentrations before and after fluphenazine (.3 mg/kg BW; n=8) or saline (n=7) administration during the follicular phase of the estrous cycle in Holstein cows.

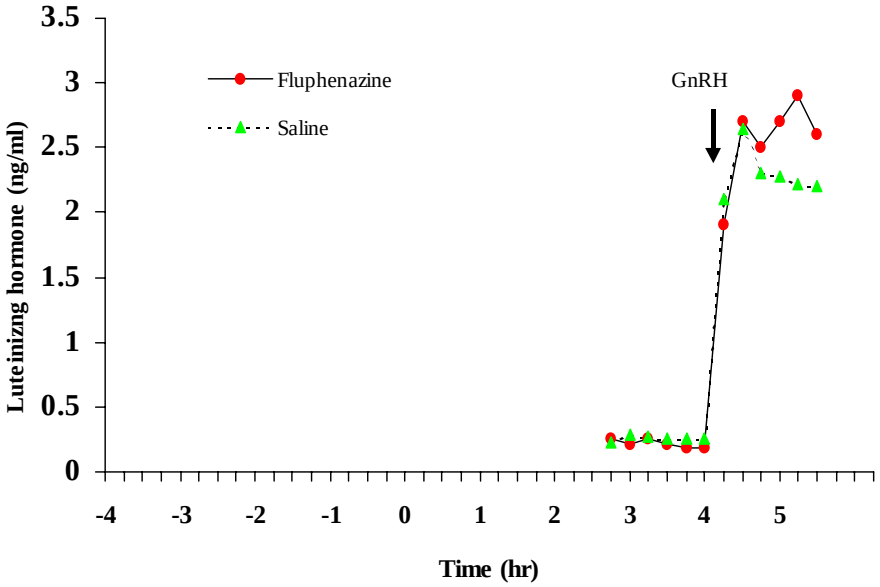

Figure 3. Mean serum luteinizing hormone concentrations before and after GnRH (25 ug) administration in fluphenazine- and saline-treated Holstein cows during the follicular phase of the estrous cycle.

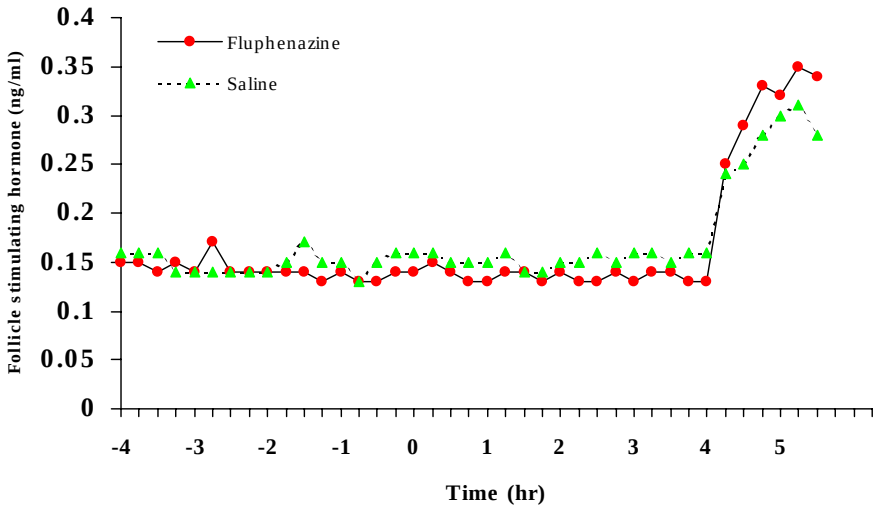

Figure 4. Mean serum follicle stimulating hormone concentrations before and after fluphenazine (.3 mg/kg BW; n=8) or saline (n=7) administration and after GnRH (25 ug) during the follicular phase of the estrous cycle in Holstein cows.

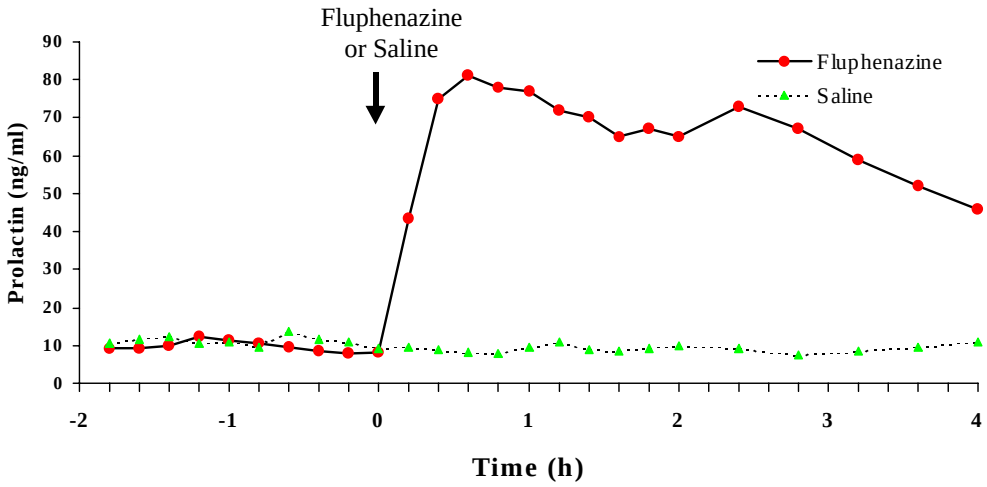

Figure 5. Mean Serum prolactin concentrations before and after fluphenazine (.3 mg/kg BW;n=8) or saline (n=7) administration during the metestrus phase of the estrous cycle in Holstein cows.

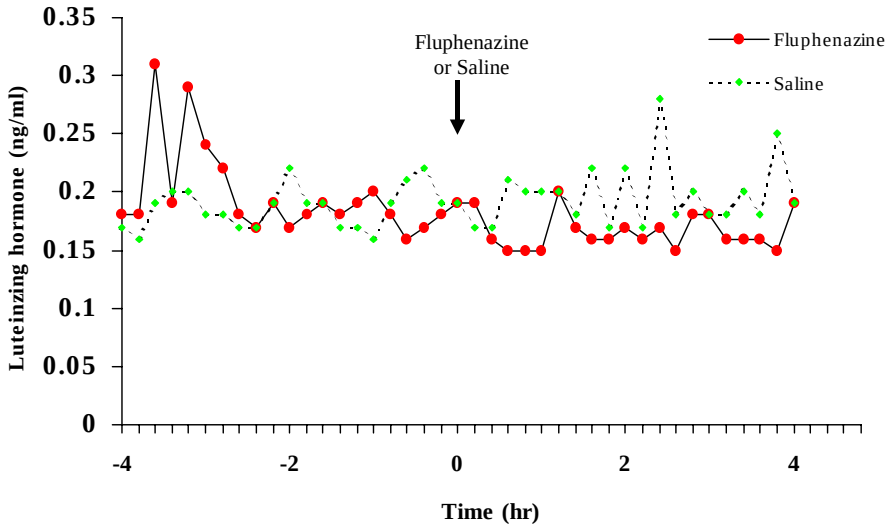

Figure 6. Mean serum luteinizing hormone concentrations before and after fluphenazine (.3 mg/kg BW; n=8) or saline (n=7) administration during the metestrus phase of the estrous cycle in Holstein cows.

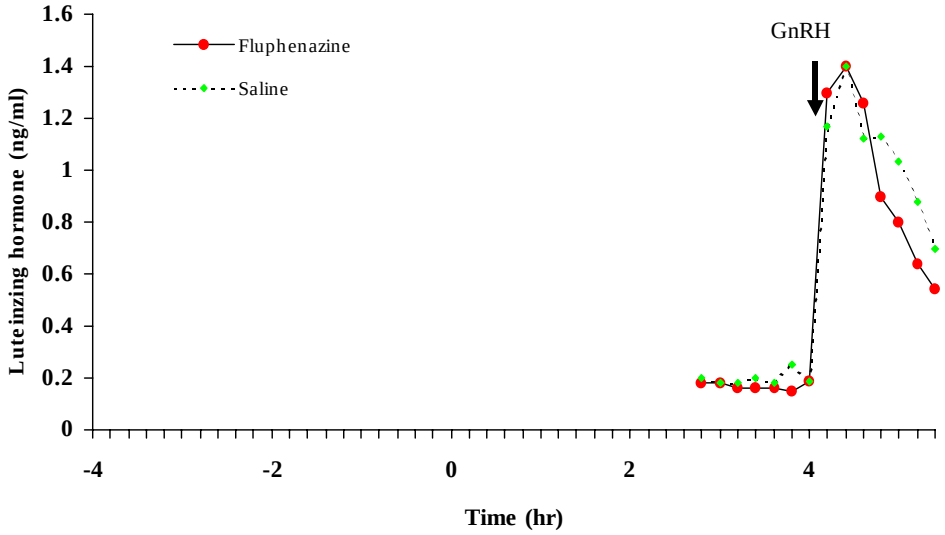

Figure 7. Mean serum luteinizing hormone concentrations before and after GnRH (25 ug) administration in fluphenazine- and saline-treated Holstein cows during the metestrus phase of the estrous cycle.

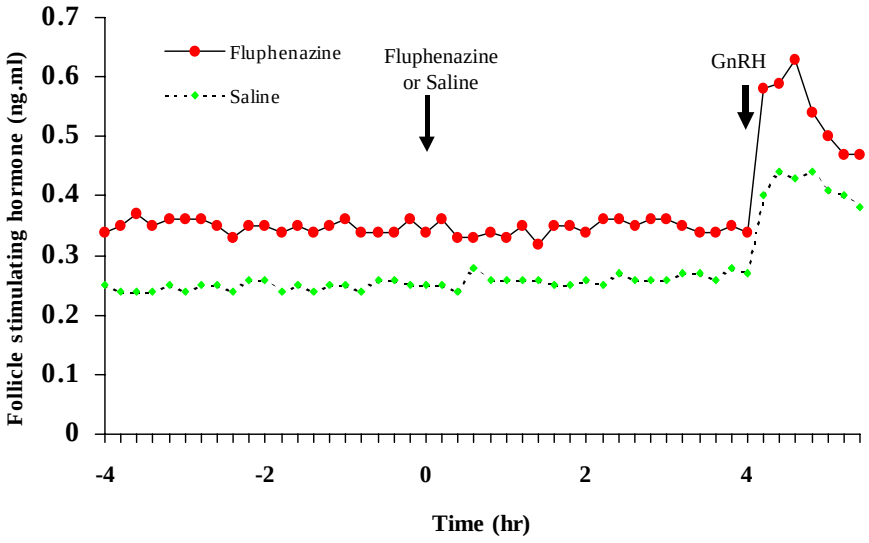

Figure 8. Mean serum follicle stimulating hormone concentrations before and after fluphenazine (.3 mg/kg BW; n=8) or saline (n=7) administration and after GnRH (25 ug) during the metestrus phase of the estrous cycle in Holstein cows.

SUMMARY ON EFFECT OF FLUPHENAZINE ON LH SECRETION

Chapters 1 to 3, contain findings which provide the first information pertaining to effects of a dopamine receptor antagonist, fluphenazine (FLU), on pituitary LH and FSH secretion in dairy cows. These chapters also provide preliminary information concerning the site of action of this drug to whether it occurs at the hypothalamus or the pituitary. These studies provide evidence that endogenous dopamine plays a role in modulating LH secretion during the anovulatory postpartum period, the follicular and metestrus phases of the estrous cycle. Moreover, endogenous dopamine appears to be stimulatory to LH secretion. This conclusion is based on the observation that FLU administration suppressed LH pulse frequency in anovulatory postpartum cows and during the follicular and metestrus phases of the estrous cycle (Figure 1). There was an evidence to indicate that endogenous dopamine regulates LH secretion during the luteal phase of the estrous cycle in lactating Holstein cows because blocking the action of endogenous dopamine had no effect on LH secretion. These results indicate that in the absence of an active corpus luteum, when progesterone is not the predominant circulating steroid that exerts negative feedback effects on LH secretion, the suppressive effect of FLU on LH pulse frequency is consistent (Figure 1). There are two hypotheses which may explain the lack of LH response to FLU during the luteal phase of the estrous cycle:

1) Dopamine is not the neuromodulator of gonadotropin secretion during the luteal phase of the estrous cycle and progesterone suppression of LH secretion is mediated via different neural elements.

2) In the absence of an active corpus luteum, dopamine may be stimulatory to LH secretion. Although there is no evidence, it is speculated that high circulating progesterone concentration during the luteal phase inhibits dopamine activity that controls LH secretion. This progesterone-induced inhibition of dopamine in turn suppresses GnRH and LH secretion. In fact, this proposed interaction between progesterone and dopamine may explain, in part, the decrease in LH pulse frequency during the luteal phase compared to the follicular phase of the estrous cycle as previously observed by Rahe et al. (1980) and Mahmaoud et al. (1989), and in the present

experiments. If progesterone inhibits dopamine activity then it would not be surprising that dopamine antagonist has no effect on LH secretion, since dopaminergic activity is already suppressed by high circulating progesterone (Figure 2). The administration of dopamine agonist alone and (or) combined with dopamine antagonist and subsequent measurement of LH response would provide more definite information as to whether dopamine is a neurmodulator of LH secretion during the luteal phase of the estrous cycle.

The site of inhibitory action of FLU on LH cannot be derived from these experiments. It is apparent that LH pulse frequency is directly related to activity of GnRH cells and that LH pulses in peripheral circulation corresponded, on a one to one basis, with GnRH pulses secreted by the hypothalamus (Thiery and Martin, 1991). Since FLU can penetrate the blood brain barrier (Meyer and Goodman, 1986; Long, 1998), suppression of LH pulse frequency after FLU administration during both the follicular and metestrus phases of the estrous cycle is probably indicative of inhibition of GnRH release by this dopamine antagonist. The fact that FLU administration did not alter LH response to exogenous GnRH compared to saline-treated cows in both experiments combined with the suppressing effect of FLU on LH pulse frequency, gives further support to the theory that FLU had acted at the hypothalamic/ME level. Therefore, it is likely that endogenous dopamine exerts its action at a site other than the pituitary, possibly the hypothalamus and ME.

The inadequate discharge of gonadotropin releasing hormone (GnRH) from the hypothalamus results in inadequate LH secretion to initiate cyclicity during the early postpartum period (Edgerton and Hafes 1973; Nett, 1987). Thus, prolongation of the postpartum period may be attributable to suppression of the mechanism which stimulates GnRH release. Moreover, in cyclic cows, ovulatory follicles must possess an enhanced capacity compared with subordinate follicles to synthesize and release steroids to trigger physiological events (i.e estrus and ovulation) necessary for reproduction (Ireland, 1987; Kaneko et al., 1991). Luteinizing hormone plays an important role in processes of selection and initial growth of a dominant follicle during folliculogenesis (Ginther et al., 1996). Luteinizing hormone is also responsible for attained diameter, maintenance, turnover, and maturation of the dominant follicle. In this process the life span of the

dominant follicle depends on adequate LH pulse frequency (Fortune et al., 1991; Savio et al., 1993). If the above hypotheses are true, then it is possible that endogenous dopamine plays an indirect role in folliculogenesis and maturation of the dominant follicle, and subsequent ovulation in cattle by regulating LH secretion. Nevertheless, the possible use of dopamine or its agonists to enhance LH secretion during early postpartum and estrous cycle requires more basic knowledge about endocrine and neuroendocrine mechanisms which regulate LH secretion.

The findings of the present studies raise questions which provide opportunity for future investigation; What subtype dopamine receptor is involved in modulation of LH secretion? What is the exact site of action of dopamine and its antagonist?

Fluphenazine is known to have affinity for both D1 and D2 dopamine receptors (Sawaguchi et al., 1990). However, the present studies do not provide specific evidence that show which subtype dopamine receptor is responsible for mediating the suppressive effect of FLU on LH secretion. The administration of dopamine agonist and (or) antagonist with specific affinity for each subtype receptor and subsequent measurement of LH response would provide more definite information as to which dopamine receptors are involved in modulating LH secretion.

Nevertheless, the current studies do provide evidence that endogenous dopamine plays a part in LH secretion in anovulatory postpartum cows, as well as during the follicular and metestrus phases of the estrous cycle.

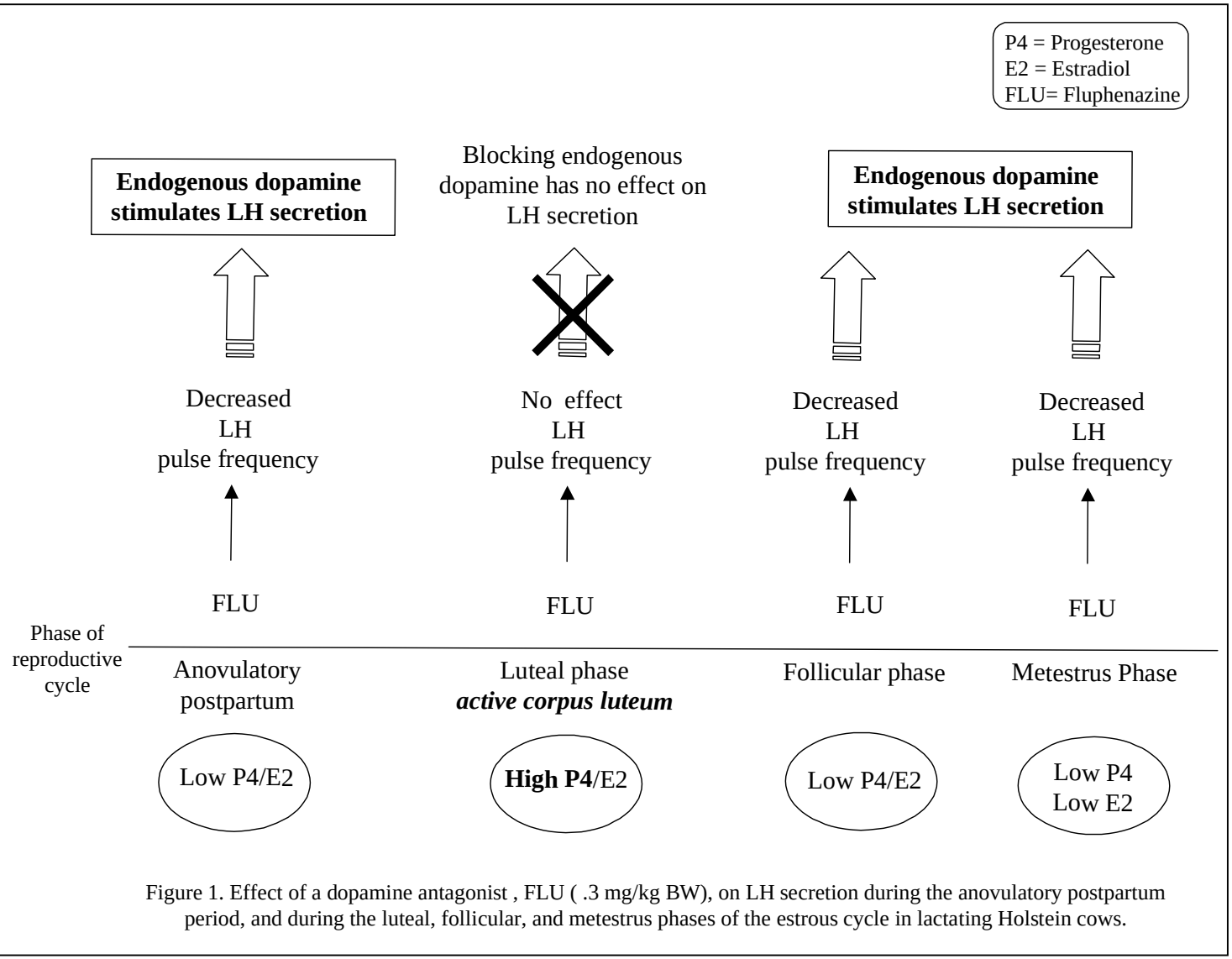

Figure 1. Effect of a dopamine antagonist , FLU (.3 mg/kg BW), on LH secretion during the anovulatory postpartum period, and during the luteal, follicular, and metestrus phases of the estrous cycle in lactating Holstein cows.

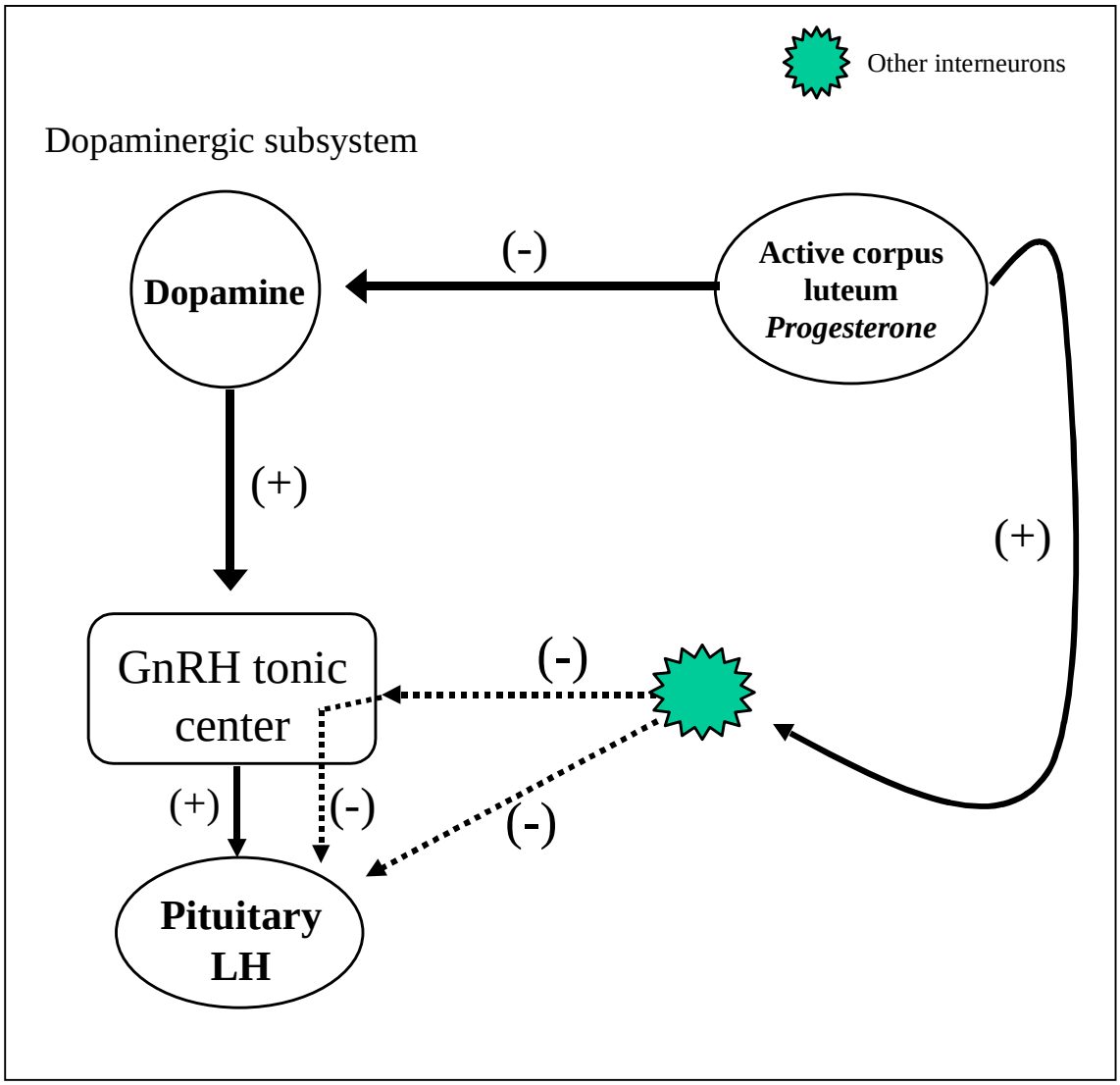

Figure 2. Conceptual model of possible interaction between the dopaminergic system, ovarian progesterone, and GnRH neurons affecting LH secretion.

CHAPTER 4

ROLE OF ENDOGENOUS DOPAMINE IN MODULATION OF GROWTH HORMONE AND PROLACTIN SECRETION DURING THE FOLLICULAR AND METESTRUS PHASES OF THE ESTROUS CYCLE IN HOLSTEIN COWS

ABSTRACT

Fluphenazine (FLU), a dopamine receptor antagonist, was used to investigate the role of endogenous dopamine on serum growth hormone (GH) and prolactin (PRL) concentrations during the early follicular and early metestrus phases of the estrous cycle. In Experiment 1, 15 lactating Holstein cows were used and upon detection of a corpus luteum 25 mg prostaglandin ($PGF_{2\alpha}$) was administered and estrous activity was monitored. On d 9 or 10 post-estrus, after detection of a corpus luteum, cows received a second dose of $PGF_{2\alpha}$ (25 mg). Thirty-three h post-$PGF_{2\alpha}$ (early follicular phase), cows were randomly assigned to receive either saline (n=7) or .3 mg FLU/kg BW i.v. (n=8). Blood samples were collected at 15-min intervals for 4 h before and 5.5 h after saline or FLU. Mean serum progesterone was .51 ± .28 ng/ml. Fluphenazine caused a transient increase ($P < .05$) in mean serum GH concentration. Mean serum GH did not change in saline-treated cows. Fluphenazine increased ($P < .01$) PRL concentration, while saline had no effect.

In Experiment 2, 15 lactating Holstein cows were used. Upon detection of a corpus luteum, cows received 25 mg $PGF_{2\alpha}$. After detection of estrus, occurrence of ovulation was confirmed using ultrasound. Fifteen to 20 h post-ovulation (metestrus phase) cows were randomly assigned to receive either saline (n=7) or .3 mg FLU/kg BW i.v. Blood collection and drug administration protocol were the same as used in Experiment 1, except that blood samples were collected at 12-min intervals. Mean serum progesterone was .16 ±.13 ng/ml. Fluphenazine increased GH and PRL concentrations

($P < .05$ and $P < .01$, respectively). Mean serum GH and PRL concentrations were not altered by saline. These results suggest that during the early follicular and metestrus phases of the estrous cycle, when serum progesterone concentration is low, GH and PRL secretion are modulated by endogenous dopamine and the effect of dopamine on both hormones is inhibitory.
Key words: Dopamine antagonist, Growth hormone, Prolactin, Estrous cycle, Dairy cattle

INTRODUCTION

Circulating concentrations of GH are closely related with maintenance and enhanced milk yield in cattle (Akers, 1994). Thus, developing strategies to alter secretion of this galactopoetic hormone may enhance lactation in cows. However, successful manipulation of GH secretion from the pituitary gland requires a better understanding of mechanisms controlling function of the hypothalamic-pituitary axis. It is known that sex steroids can affect GH secretion. For example, Davis and Borger (1973) showed that progesterone and estradiol benzoate treatment of ovariectomized ewes enhanced GH secretion. Implantation of estrogenic anabolic compounds, such as zeranol and diethylstilbestrol, increased GH secretion in growing beef steers (Gopinath and Kitts, 1984). The mechanism that promotes increased secretion of GH is unclear. It has been suggested that ovarian steroids play a role in GH secretion by regulating somatostatin (GIF) and (or) GRH in female rats. For instance, ovariectomy caused a decrease in hypothalamic GIF content (Gabriel et al., 1989). Estupina and co-workers (1996) showed that GIF release increased during diestrus compared to estrus in female rats. Further, it has been shown that pituitary responsiveness to GRH changed during the estrous cycle in rats and it was maximal during diestrus and minimal during proestrus (Aguilar and Pinilla, 1991; Ruiz et al., 1997).

There is evidence that steroids affect dopamine turnover rate in hypothalamic tissues (Beattie et al., 1972). Fuxe et al. (1980) showed that estradiol 17β directly activated dopaminergic neurons in the lateral tuberoinfundibular system in rats. The arcuate nucleus and tuberoinfundibular nuclei are the areas that contain GRH and GIF

neurons and regulate GH secretion (Leshine et al., 1995). There is also evidence that dopamine affects GH secretion (see Buonomo and Baile, 1990 for review). It has been suggested that dopamine may decrease GH secretion by increasing GIF and decreasing GRH hormone in cattle (West et al., 1997; McMahon et al., 1998). It has been hypothesized that endogenous dopamine may be stimulatory to GH secretion in anovulatory postpartum cows (Chapter 1). However, a dopamine mediated mechanism for modulation of GH secretion was absent or suppressed by high circulating progesterone during the luteal phase in cattle (Ahmadzadeh et al., 1998b; Chpater 2). Estrogenic compounds increased pituitary GH secretion in beef steers (Gopinath and Kitts, 1984) and regulation of GH secretion may vary depending upon circulating progesterone and estrogen levels during the reproductive cycle (Estupina et al., 1996). Furthermore, estradiol increased hypothalamic dopamine turnover rate in rats (Fuxe et al., 1980). It not clear whether dopamine influences GH secretion when circulating progesterone is low in cattle and whether the effects differ from those in anovulatory postpartum and luteal-phase cows.

The objective of the present study was to investigate the role of endogenous dopamine by characterizing serum GH response to a dopamine antagonist, FLU, during the follicular and metestrus phases of the estrous cycle. Because it is well established that dopamine inhibits PRL secretion in cattle, serum PRL concentrations in response to FLU were measured as a positive control to ensure that dopamine receptors were effectively blocked.

MATERIALS AND METHODS

Experiment 1

Fifteen cyclic Holstein cows, 72 ± 29 d postpartum (mean BW = 585 ± 64), were used. Ovarian activity of cows was monitored by real-time ultrasound scanner, equipped with a 5 MHz rectal transducer probe (Aloka Co., 6-22-1, Mure, Mitaka-shi, Tokyo , Japan) and upon detection of a corpus luteum (CL), 25 mg of $PGF_{2\alpha}$ (Lutalyse®, The Upjohn Co., Kalamazoo, MI) was administered (i.m.) to induce luteolysis. Estrous

activity was monitored using an electronic pressure-sensing device (HW ®, Heat Watch, DDx, Denver, CO). On d 9 or 10 post-estrus (estrus = d 0), after detection of a CL by ultrasound, cows received a second dose of $PGF_{2\alpha}$ (25 mg/cow) at 2000 h. Twenty-four h later, cows were weighed and ovarian activity was monitored by ultrasound. All cows were fitted with jugular catheters as previously described (Johnson et al., 1993). Thirty-three h post-$PGF_{2\alpha}$ (early follicular phase), all cows were randomly assigned to receive either .9 % saline (n=7) or .3 mg FLU/kg BW (n=8) i.v. dissolved in 10 ml physiological saline. Beginning at 0500 h blood samples were collected at 15-min intervals for 4 h before and 5.5 h after FLU or saline.

Experiment 2.

Fifteen lactating Holstein cows, 65 ± 24 d postpartum (mean BW = 576 ± 68) were used. Ovarian activity of cows was monitored by ultrasound and upon detection of a CL, 25 mg of $PGF_{2\alpha}$ (Lutalyse®, The Upjohn Co., Kalamazoo, MI) was administered (i.m.) to induce luteolysis. Estrous activity was monitored using the HW® system. Following first mount recorded by HW®, ultrasonographic exams of ovaries were performed at 12 and 20 h and then every 4 h until ovulation occurred or until 32 h post-estrus. Cows that did not ovulate by 32 h post-estrus were not used. Ovulation was defined as sudden disappearance of any follicle larger than 10 mm in diameter (Kaneko et al., 1991). Ovulation time was defined as the number of hours from first recorded mount to midpoint of the examinations between which the ovulatory follicle had disappeared (Walker et al., 1996). After detection of ovulation cows were weighed and fitted with jugular catheters (Johnson et al., 1993) and randomly assigned to receive either .9 % saline (n=7) or .3 mg FLU/kg BW (n=8) i.v. dissolved in 10 ml physiological saline. Fifteen to 20 h post ovulation (early metestrus) blood samples were collected at 12-min intervals for 4 h before and 5.5 h after FLU or saline.

In both experiments, in each sampling interval, the initial 2 ml of each sample was discarded, and a 10 ml sample was then transferred into collection tubes, which were immediately placed in ice and then stored at 4°C for 24 h for clotting. All blood samples

were centrifuged for 30 min at 2,200 ×g at 4°C. Serum was harvested and stored at -20°C until assayed for GH, PRL, and progesterone. During blood sampling all cows were tied in individual stalls with access to a total mixed ration and water *ad libitum*.

Hormone assays

Concentration of GH and PRL was determined by a double-antibody RIA by method of Barnes et al. (1985). Purified bovine GH [bGH Cynamide 6952 (-42A)] and was used as reference standard and for radioiodination. Sheep anti-rabbit gammaglobulin was used as precipitating second antibody. Primary antibody was developed in rabbits using pure bovine GH (NIH-GH-B18). This assay was performed in a non-equilibrium condition. Briefly, standard reference and serum samples (300 ul) in assay buffer (200 ul) were incubated with primary antiserum (100 ul) for 24 h at room temperature. Next, ^{125}I radio-labeled GH (100 ul; 35,000 cpm) was added and samples were incubated for an additional 24 h at room temperature. Second antibody (100 ul) was added to all sample tubes, which were then incubated for 72 h at 4°C. Samples were then centrifuged (2,200 × g) for 30 min at 4°C. To separate bound GH from free, supernatant was poured off, sample tubes drained and pellets were counted for 1 min in a gamma counter. The primary antiserum bound 40% of radiolabeled GH in the absence of unlabeled hormone. Sensitivity of the assay was less than .5 ng/ml reference standard GH and defined as the concentration corresponding to 2 standard deviations less than mean zero dose. All samples were assayed in duplicate, and intra- and inter-assay CV calculated from serum pools were 6.1% and 7.3%, respectively.

The sensitivity of the PRL assay was less than 1.25 ng/ml reference standard PRL and defined as the concentration corresponding to 2 standard deviations less than mean zero dose. All samples were assayed in duplicate, and intra- and inter-assay CV calculated from serum pools were 7.6% and 9%, respectively.

Concentration of progesterone was quantified using solid-phase RIA (Diagnostic Products Corp.; Los Angeles, CA) which has been previously validated in our laboratory (Holt et al., 1989). The standard curve ranged from 0.1 to 20 ng/ml. All samples were assayed in duplicate (1 assay) and the intra-assay CV was 4.1%.

Statistical Analysis

Serum GH and PRL data in both experiments were analyzed by least-squares analysis of variance by the General Linear Model (GLM) procedure using Statistical Analysis Systems (SAS® Institute, Cary, NC). To determine the effect of FLU on GH, blood samples were categorized to one of three. Period 1 represented blood samples collected for 4 h before saline or FLU administration and was considered the pre-treatment period. Period 2 represented blood samples collected for 2 h immediately after FLU or saline administration; and samples thereafter (2 h) represented Period 3. To determine the effect of FLU on PRL, blood samples were also categorized to one of three periods and hormone concentrations were averaged for each period. However, period 1 represented blood samples collected for only 2 h before saline or FLU administration and was considered the pre-treatment period. The experiment was designed to determine the effects of FLU on GH and PRL concentrations in periods 2 and 3 compared to period 1. The statistical model included treatment, cow within treatment, period, and all the possible interactions. Period by cow within treatment was used as error term to test the effects of period and period by treatment. If period or period by treatment effects were significant ($P < .05$), non-orthogonal contrasts were used to compare least squares means for periods 1 vs 2, 1 vs 3 within each treatment using the improved Bonferroni F-test (1977).

RESULTS

None of the cows exhibited estrous behavior during 36 h prior to, or on the day of experiment. Results of ultrasonography conducted approximately 10 h before initiation of the experiment, verified the absence of organized luteal tissue (compared to pre-$PGF_{2\alpha}$). As expected, on the day of experiment (33 h after $PGF_{2\alpha}$,) serum progesterone concentrations were less than 1 ng/ml for all cows and mean concentration was .51 ± 28 ng/ml. Mean number of follicles equal to or greater than 5 mm in diameter in both

Table 1. Mean prolactin (PRL) concentrations[a] in Holstein cows treated with saline or fluphenazine (FLU) during the follicular phase of the estrous cycle.

	Mean serum PRL concentration (ng/ml)		
Treatment	Period [b] 1	Period 2	Period 3
Saline (n=7)	6.9 ± 8.4	7.6 ± 8.4	8.4 ± .11.9
FLU (.3 mg/kg BW; n=8)	5.0 ± 7.9[c]	90.5 ± 7.9[d]	71.8 ± 11.1[d]

[a] Least squares means ± SEM.
[b] Period 1=Mean hormone concentration of 8 blood samples collected before fluphenazine or saline administration from 0715 to 0900 h; period 2=Mean hormone concentration of 8 blood samples collected after fluphenazine or saline administration from 0915 to 1100 h; period 3=Mean hormone concentration of 4 blood samples collected from 1130 to 1300 h (every 30 min sample).
[c,d] Means within the same treatment row with different superscripts differ ($P < .01$).

ovaries was 4.3 ± 1.2 and the mean diameter of the largest follicle was 14.8 ± 3.2 mm.

Mean serum PRL was similar between saline- and FLU-treated groups during period 1. Serum PRL concentration increased ($P < .01$) in response to FLU (Table 1). Serum PRL increased immediately after FLU administration, peaked approximately 30 min later, and declined gradually, but did not return to pre-treatment level during the experiment (Figure 1). In contrast, saline administration did not affect serum PRL and mean serum PRL concentrations remained unchanged during the 3 periods (Table 1).

Table 2 Mean GH concentrations[a] in Holstein cows treated with saline or fluphenazine (FLU) during the follicular phase of the estrous cycle.

	Mean serum GH concentration (ng/ml)		
Treatment	Period [b] 1	Period 2	Period 3
Saline (n=7)	4.6 ± .8	6.3 ± 1.0	7.1 ± 1.0
FLU (.3 mg/kg BW; n=8)	4.7 ± .6 [c]	7.3 ± 1.0 [cd]	9.9 ± 1.0 [d]

[a] Least squares means ± SEM.
[b] Period 1=Mean hormone concentration of 17 blood samples collected before fluphenazine or saline administration from 0500 to 0900 h; period 2=Mean hormone concentration of 8 blood samples collected after fluphenazine or saline administration from 0915 to 1100 h; period 3=Mean hormone concentration of 8 blood samples collected from 1115 to 1300 h.
[c,d] means within the same treatment row with different superscripts differ ($P < .05$).

During the follicular phase of the estrous cycle, FLU elicited an increase ($P < .05$) in serum GH concentration during the last 2 h after administration (period 3; Table 2). The increase in serum GH was not abrupt and occurred gradually (Figure 2). Serum GH increased from 4.7 ± .8 before FLU to 7.3 ± 1.0 ng/ml during the first 2 h after FLU. Serum GH continued to increase to 9.9 ± 1.0 during the last 2 h after FLU, which was higher ($P < .05$) than pre-treatment levels (Table 2). Serum GH concentration increased slightly, but not significantly, during period 2 and 3 after saline administration (Table 2).

Experiment 2

One the day of experiment (15-20 h post-ovulation), all cows had serum progesterone concentrations of less than 1 ng/ml and mean serum progesterone concentration was .16 ± .13 ng/ml. Mean diameter of the ovulatory follicle was 18 (± 2.6) mm. The average time from onset of estrus to ovulation was 26.1 h (± 5.1).

Mean serum PRL did not differ between saline- and FLU-treated cows during period 1 (Table 3). Fluphenazine increased ($P < .01$) serum PRL more than 7-fold during period 2 when compared to the pre-treatment period (Table 3). Serum PRL concentration remained elevated ($P < .01$) throughout the experiment and did not return to pre-

Table 3. Mean prolactin (PRL) concentrations[a] in Holstein cows treated with saline or fluphenazine (FLU) during the metestrus phase of the estrous cycle.

	Mean serum PRL concentration (ng/ml)		
Treatment	Period [b] 1	Period 2	Period 3
Saline (n=7)	11.1 ± 4.4	9.2 ± 4.4	9.2 ± 6.3
FLU (.3 mg/kg BW; n=8)	9.7 ± 4.2 [c]	69.4 ± 4.2 [d]	59.9 ± 5.9 [d]

[a] Least squares means ± SEM.

[b] Period 1=Mean hormone concentration of 10 blood samples collected before fluphenazine or saline administration from 0712 to 0900 h; period 2=Mean hormone concentration of 10 blood samples collected after fluphenazine or saline administration from 0912 to 1100 h; period 3=Mean hormone concentration of 5 blood samples collected from 1124 to 1300 h (every 24 min sample).

[c,d] Means within the same treatment row with different superscripts differ ($P < .01$).

treatment level. Serum PRL increased in response to FLU within 15 min and all cows responded to treatment with an abrupt increase in PRL (Figure 3). Saline administration did not affect serum PRL during period 2 and 3 compared to period 1 (Table 3).

Mean Serum GH concentrations were similar during the pre-treatment period (period 1) in saline and FLU treatment groups. Fluphenazine treatment increased ($P < .05$) serum GH levels during the last 2.5 h after administration (Table 4; Figure 4). Serum GH did not increase during the first 2 h post-FLU (period 2), when compared to the pre-treatment period. However, serum GH increased ($P < .05$) by approximately 2-fold during period 3 (Table 4). No difference in serum GH concentrations was observed after saline treatment during period 2 and 3 (Table 4).

Table 4. Mean GH concentrations[a] in Holstein cows treated with saline or fluphenazine (FLU) during the metestrus phase of the estrous cycle.

	Mean serum GH concentration (ng/ml)		
Treatment	Period [b] 1	Period 2	Period 3
Saline (n=7)	4.2 ± .9	5.6 ± 1.2	5.7 ± 1.1
FLU (.3 mg/ kg BW; n=8)	5.2 ± .9 [c]	6.0 ± 1.1 [c]	11.2 ± .1.1[d]

[a] Least squares means ± SEM.

[b] Period 1=Mean hormone concentration of 21 blood samples collected before fluphenazine or saline administration from 0500 to 0900 h; period 2=Mean hormone concentration of 10 blood samples collected after fluphenazine or saline administration from 0912 to 1100 h; period 3=Mean hormone concentration of 10 blood samples collected from 1112 to 1300 h.

[c,d] Means within the same treatment row with different superscripts differ ($P < .05$).

DISCUSSION

In experiment 1 (follicular phase), $PGF_{2\alpha}$ induced luteolysis and on the day of experiment (33 h post-$PGF_{2\alpha}$) mean serum progesterone concentration was .51 ± .28 ng/ml compared to 3.6 ± .4 ng/ml prior to $PGF_{2\alpha}$ administration. Additionally, several

follicles (greater than 5 mm in diameter) were present on the ovaries and no organized luteal tissue was detected. These observations, combined with decreases in serum progesterone concentration and lack of estrous activity before and on the day of the experiment, verified that the experiment was conducted during the follicular phase of the estrous cycle.

Mean serum PRL concentration during the pre-treatment period was comparable to basal PRL levels previously observed in lactating cows (Barnes et al., 1985). As expected, FLU administration abruptly increased PRL secretion (Figure 1). The data from this study support the hypothesis that dopamine inhibits PRL secretion in cattle and indicates that FLU antagonized endogenous dopamine and at least some dopamine receptors were blocked. Mean serum GH concentration during the pre-treatment period was similar to levels that was observed previously in 90-day postpartum cows (Kazmer et al., 1986). Results of this experiment indicate that FLU (.3 mg/kg BW) increased GH secretion during the follicular phase of the estrous cycle (Figure 2). These results provide the first information concerning the stimulatory effect of dopamine antagonist on release of pituitary GH in lactating cows and appear to indicate that endogenous dopamine plays an inhibitory role in GH secretion during the follicular phase of the estrous cycle in dairy cattle.

In experiment 2, ultrasonography indicated the absence of a corpus luteum and mean serum progesterone concentration was less than 1 ng/ml in all cows (.16 ± .13 ng/ml). After completion of the experiment, no large follicles (> 10 mm in diameter) were observed in any cow. These observations were similar to those by Kaneko et al. (1991) on d 3 post-estrus in cows. Kaneko et al. (1991) reported that plasma estradiol did not reach high levels (comparable to the follicular phase) until day 4 to 5 post-estrus. Further, the increase in plasma estradiol did not occur until non-ovulatory dominant follicles from the first follicular wave showed a marked increase in size from d 3 of the cycle onward. Experiment 2 was conducted between d 2 and 3 of the estrous cycle, likely before dominant follicles gain the ability to secrete substantial amounts of estradiol (Ireland and Roche, 1983; Ireland et al., 1984) during the early metestrus phase of the estrous cycle.

Systemic administration of FLU during metestrus abruptly increased PRL secretion (Figure 3) indicating that FLU antagonized the action of endogenous dopamine and at least some dopamine receptors were blocked. Fluphenazine administration caused a transient increased in mean GH concentration during the early metestrus phase of the estrous cycle (Figure 4). To our knowledge, there is no information concerning the role of dopamine in control of GH secretion during this phase of the estrous cycle in cattle. Results of Experiment 2 suggest that endogenous dopamine plays an inhibitory role in GH secretion during metestrus in dairy cattle and disruption of dopamine action by a dopamine antagonist (FLU) increased GH secretion. This observation appears to indicate that during metestrus when circulating concentrations of both progesterone and estradiol are low (Kaneko et al., 1991), endogenous dopamine plays a role in modulation of GH secretion.

The findings of the present experiments are contrary to those in humans (Liuzzi et al., 1976) and rats (Eden et al., 1977) which indicated the existence of a stimulatory role of dopamine in release of GH, since dopamine antagonists inhibited GH secretion. Moreover, Smith et al. (1974) reported that ergocryptine, which has dopamine-like activity, did not affect GH secretion in lactating Holstein cows indicating that dopamine may not play a role in regulation of GH secretion. The reasons for lack of GH response to ergocryptine in that study (Smith et al., 1974) is not clear, but ergocryptine has affinity for α-adrenergic receptors and may activate the noradrenergic system which has been shown to be stimulatory to GH secretion in rats (Martin et al., 1978). Thus, the lack of GH response to ergocryptine could be attributable to simultaneous action of this drug on both the inhibitory dopaminergic, and the stimulatory noradrenergic system (McMahon, 1998; Martin et al., 1978).

The site of stimulatory action of FLU on GH secretion cannot be determined from these experiments. It may be speculated that in the present study FLU increased GH secretion by acting at the hypothalamic level, because it has been shown (Chapter 1) that FLU administration did not alter GH response to exogenous GRH in anovulatory Holstein cows. Further, it has been shown that ergocryptine (Smith et al., 1974) and

monoamines (Vale in Martin et al., 1978) had no effect on growth hormone secretion from pituitary cell cultures *in vitro*.

Findings of the present experiments provide no evidence with respect to mode of action exerted by FLU on GH secretion. Assuming that FLU acts at the level of the hypothalamus, it is not clear whether FLU increased GH secretion by increasing GRH release or decreasing GIF release from the hypothalamus. It is possible that FLU caused an increase in GH secretion by decreasing GIF. There is a line of evidence which indicates that dopamine inhibits GH secretion by increasing hypothalamic GIF secretion. For instance, activation of D1 dopaminergic receptors by a potent dopamine agonist (SKF 38393) caused release of GIF and decreased release of GRF from bovine hypothalamic tissue *in vitro* (West et al., 1997). Furthermore, D1 receptor antagonist blocked SKF 38393-induced release of GIF. Recently, McMahon et al. (1998) showed that stimulation of D1 receptors selectively increases activity of GIF neurons in the periventricular nucleus, and this increased activity was associated with suppressed basal GHRH-induced release of GH in serum of meal-fed steers. Since FLU has ability to block D1 dopamine receptors (Sawaguchi et al., 1990), it possible that in the present experiments blockade of dopamine action by FLU inhibited hypothalamic GIF secretion. FLU-induced inhibition of GIF release in turn would lead to an increase in GH secretion as observed during the follicular and metestrus phases of the estrous cycle.

Fluphenazine did not affect GH secretion during the luteal phase of the estrous cycle in Holstein cows (Ahmadzadeh et al., 1998b; Chapter 2). The reason for lack of GH response to FLU during the luteal phase of the estrous cycle compared to the follicular and metestrus phases is not clear. It has been proposed that dopaminergic activity in the central nervous system is affected by stage of the estrous cycle, ovariectomy, and estradiol replacement in rats (Diaz-Veliz et al., 1994). Therefore, it is possible that during the luteal phase of the estrous cycle, when progesterone is the predominant steroid hormone, the dopaminergic system that regulates GH secretion is inactive and hence does not exert any affect on GH secretion. Nonetheless, it appears that in the absence of an active corpus luteum, regardless of circulating estradiol levels (i.e the follicular vs. metestrus phase of the estrous cycle), endogenous dopamine is

inhibitory to GH secretion. The possibility exists that there are dopaminergic neurons in cattle that operate independently from estradiol or are estradiol insensitive and inhibit GH secretion. Disruption of this system by dopamine antagonist would result in an increase in GH secretion, as was observed during both the follicular and early metestrus phases of the estrous cycle.

If FLU inhibits dopamine action and increases GH secretion when circulating progesterone is low, then the opposite effect exerted by same dose of FLU on GH secretion in anovulatory postpartum dairy cows (Chapter 1) appears difficult to explain. On the basis of previous observations in early postpartum cows (Chapter 1) and present observations in cyclic cows, a model is proposed to reconcile explain the different effects of FLU on GH secretion in anovulatory and cyclic cows: It is possible that there are two sets of dopaminergic neurons, subsystems A and B, which control GH secretion. Stimulatory subsystem A, which is steroid independent and is active during the anovulatory early postpartum period. Disruption of this system with FLU would result in suppression of GH secretion as observed in anovulatory postpartum dairy cows (Chapter 1). Inhibitory subsystem B, which becomes active after resumption of ovarian activity and recurrence of estrous cycle. In the absence of high circulating progesterone, blockade of dopamine action with FLU would reduce the inhibitory action of this subsystem and hence increase GH secretion, as observed during the follicular and metestrus phases of the estrous cycle.

Nonetheless, from these studies several conclusions can be drawn:

1) FLU increased serum PRL concentrations during both the follicular and metestrus phases of the estrous cycle in lactating cows indicating that dose of FLU was sufficient to impair endogenous dopamine action and that at least some dopamine receptors were blocked. Furthermore, dopamine inhibits PRL secretion during both phases of the estrous cycle.

2) Endogenous dopamine plays a role, at least in part, in modulating GH secretion during both the follicular and metestrus phases of the estrous cycle in lactating Holstein cows. Moreover endogenous dopamine may be inhibitory to GH secretion. This conclusion is

based on the observation that FLU administration increased GH secretion during both phases of the estrous cycle.

3) In the absence of an active corpus luteum when progesterone is not the predominant steroid the stimulatory effect of FLU on GH secretion is consistent across the follicular and metestrus phases of the estrous cycle in lactating cows.

4) The specific role of endogenous dopamine is different for anovulatory postpartum and cyclic dairy cows. Where endogenous dopamine appears to be stimulatory to GH secretion in anovulatory early postpartum cows, it seems to play an inhibitory role in GH secretion after resumption of ovarian cyclicity, during the follicular and metestrus phases of the estrous cycle.

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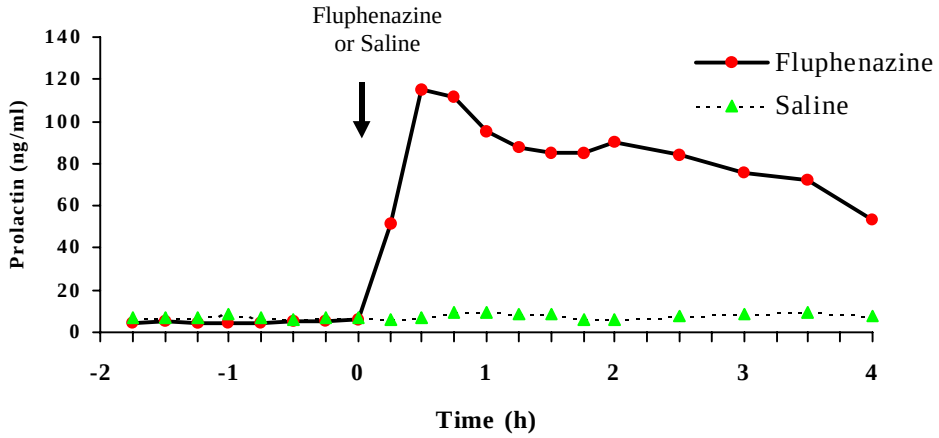

Figure 1. Mean serum prolactin concentrations before and after fluphenazine (.3 mg/kg BW;n=8) or saline (n=7) administration during the follicular phase of the estrous cycle in Holstein cows.

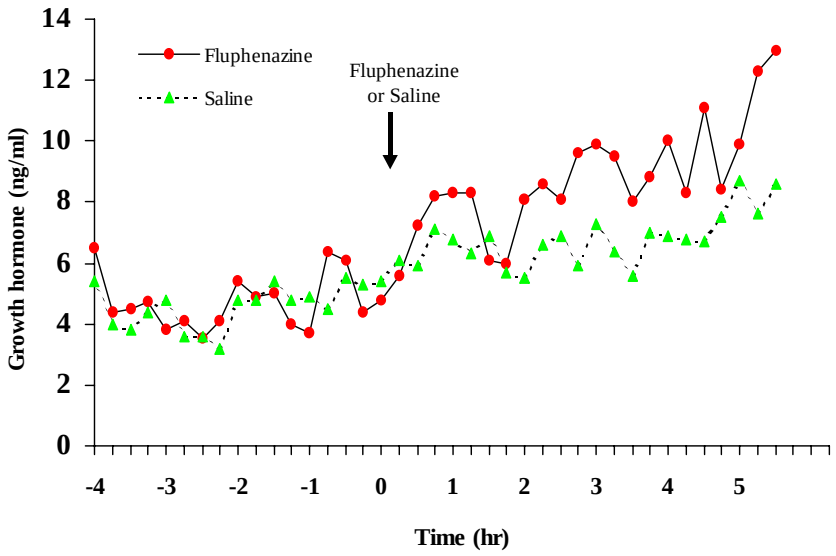

Figure 2. Mean serum growth hormone concentrations before and after fluphenazine (.3 mg/kg BW; n=8) or saline (n=7) administration during the follicular phase of the estrous cycle in Holstein cows.

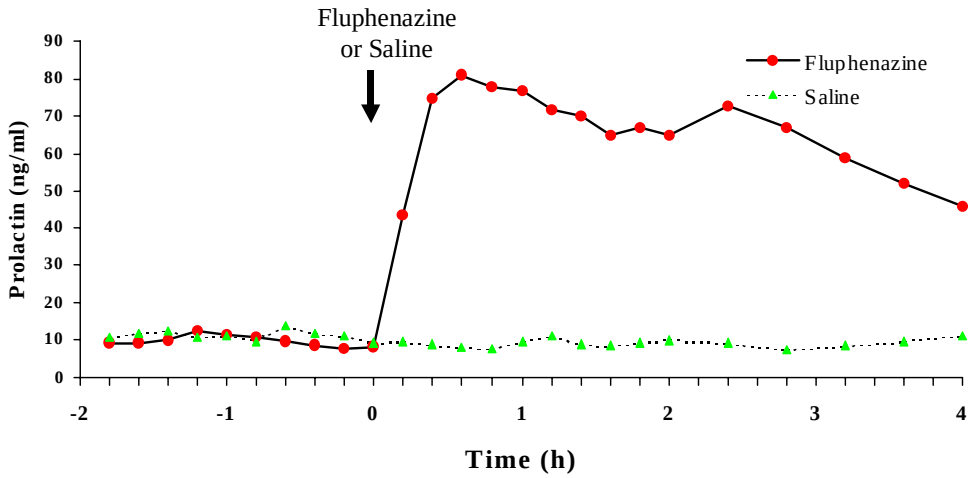

Figure 3. Mean Serum prolactin concentrations before and after fluphenazine (.3 mg/kg BW;n=8) or saline (n=7) administration during the metestrus phase of the estrous cycle in Holstein cows.

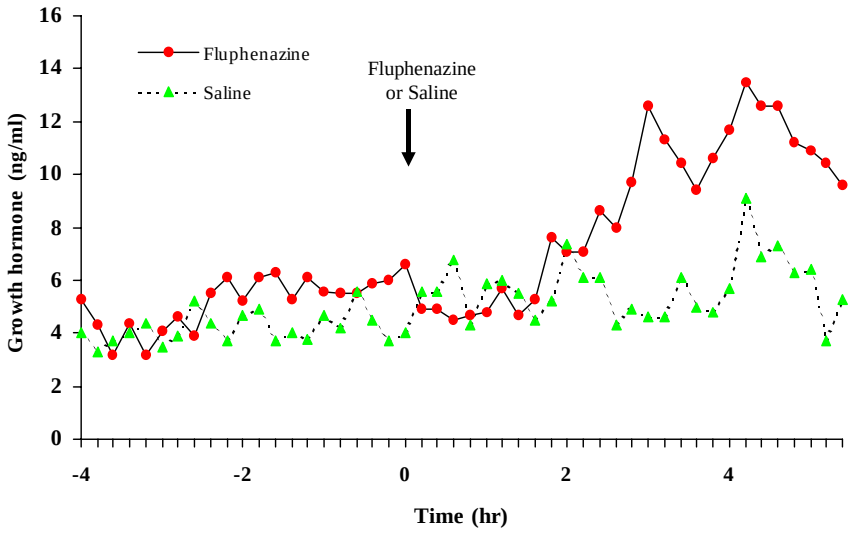

Figure 4. Mean serum growth hormone concentrations before and after fluphenazine (.3 mg/kg BW; n=8) or saline (n=7) administration during the metestrus phase of the estrous cycle in Holstein cows.

SUMMARY ON EFFECT OF FLUPHENAZINE ON GROWTH HORMONE SECRETION

Pituitary GH is responsible for growth and development of the mammary gland and circulating concentrations of GH are closely related with body maintenance and enhanced milk yield (Akers, 1994). Thus, developing strategies to alter secretion of this galactopoetic hormone may enhance lactation in cows. However, successful manipulation of GH secretion from the pituitary gland requires a better understanding of mechanisms controlling function of the hypothalamic-pituitary axis. Growth hormone release is controlled by two hypothalamic peptides: growth hormone releasing hormone (GRH) and somatostatin or growth hormone inhibiting factor (GIF). The secretion of GRH and GIF is regulated by a variety of neurotransmitters and peptides present within and outside the central nervous systems (Buonomo and Baile, 1990). There is ample evidence that dopamine is involved in regulation of GH secretion. Chapters 1 to 4 contain findings which provide the first information pertaining to effects of a dopamine receptor antagonist, fluphenazine (FLU), on pituitary GH secretion in lactating dairy cows and preliminary information concerning the site of action of this dopamine antagonist in the brain.

Data from chapter 1 indicate that dopamine regulates GH secretion in anovulatory postpartum dairy cows regardless of parity. Further, because FLU decreased GH secretion, it appears that endogenous dopamine is stimulatory to GH secretion in anovulatory primi- and multiparous cows during the early postpartum period (Figure 1).

The effect of FLU in cyclic cows differed from that of anovulatory cows and varied depending upon ovarian steroid secretion. Results of the study during the luteal phase (chapter 2) support the previous finding (Ahmadzadeh et al., 1998b) and indicated that dopamine modulation of GH secretion during the luteal phase of the estrous cycle is absent or overridden by high circulating progesterone, suggesting that dopamine may not be a neuromodulator of GH secretion during this phase of the estrous cycle. (Figure 1). This conclusion is based on the observation that FLU administration did not affect GH

secretion during the luteal phase of the estrous cycle. However, FLU stimulated GH secretion during the follicular and metestrus phases of the estrous cycle (chapter 6) indicating that endogenous dopamine may be inhibitory to GH secretion (Figure 1). Moreover, in the absence of an active corpus luteum, when progesterone is not the predominant steroid, the stimulatory effect of FLU on GH secretion is consistent across the follicular and metestrus phases of the estrous cycle in lactating cows.

The site of action of FLU on GH cannot be derived from these experiments. It may be speculated that FLU increased GH secretion by acting at the hypothalamic level, because FLU administration did not alter GH response to exogenous GRH in anovulatory Holstein cows (chapter 4). Further, it has been shown that ergocryptine (a dopamine agonist; Smith et al., 1974) and other monoamines (W. Vale in Martin et al., 1978) had no effect on growth hormone secretion from pituitary cell cultures *in vitro*.

Findings of the present experiments provide no evidence with respect to mode of action exerted by FLU on GH secretion. Assuming that FLU acts at the level of the hypothalamus, it is not clear whether FLU affected GH secretion by altering GRH release or GIF release from the hypothalamus. It may be speculated that during the follicular and metestrus phases of the estrous cycle FLU caused an increase in GH secretion by decreasing GIF. It has been shown (*in vitro*; West et al., 1996) that D1 dopamine agonist increased hypothalamic GIF secretion and D1 dopamine antagonist blocked this effect. Since FLU has the ability to block D1 dopamine receptors (Sawaguchi et al., 1990), it is possible that blockade of dopamine action by FLU inhibited hypothalamic GIF secretion. FLU-induced inhibition of GIF release in turn would lead to an increase in GH secretion as observed during the follicular and metestrus phases of the estrous cycle. However, if FLU stimulates GH secretion by inhibiting GIF release, it is difficult to reconcile the inhibitory action of FLU on GH secretion in anovulatory postpartum cows. Direct measurement of changes in GRH and GIF concentrations in hypothalamic-hypophysial portal blood after FLU administration would provide more direct evidence concerning the mode of action of this dopamine antagonist. Further, pituitary-stalk section would have been very useful in determining the site of action of dopamine on GH secretion in dairy cows. However, these approaches require special and expensive equipment and

extensive surgical skills (Leshin et al., 1990). Use of a different dopamine antagonist, such as domperidone, which cannot penetrate the blood brain barrier, along with immunization of animals against GRH and (or) GIF, and subsequent measurement of GH response to dopamine antagonist might provide more direct evidence concerning site and mode of action of endogenous dopamine in modulation of GH secretion.

In conclusion, FLU decreased GH secretion in anovulatory postpartum Holstein cows, but it increased GH secretion during the follicular and metestrus phases of the estrous cycle. However FLU had no effect on GH secretion during the luteal phase of the estrous cycle. The specific role of endogenous dopamine is apparently different for anovulatory postpartum and cyclic dairy cows. Where endogenous dopamine appears to be stimulatory to GH secretion in anovulatory early postpartum cows, it seems to play an inhibitory role in GH secretion after resumption of ovarian cyclicity, during the follicular and metestrus phases of the estrous cycle. Thus it appears that modulation of GH secretion is only partially dependent on dopaminergic regulation and may also be affected by reproductive status and ovarian hormone secretion.

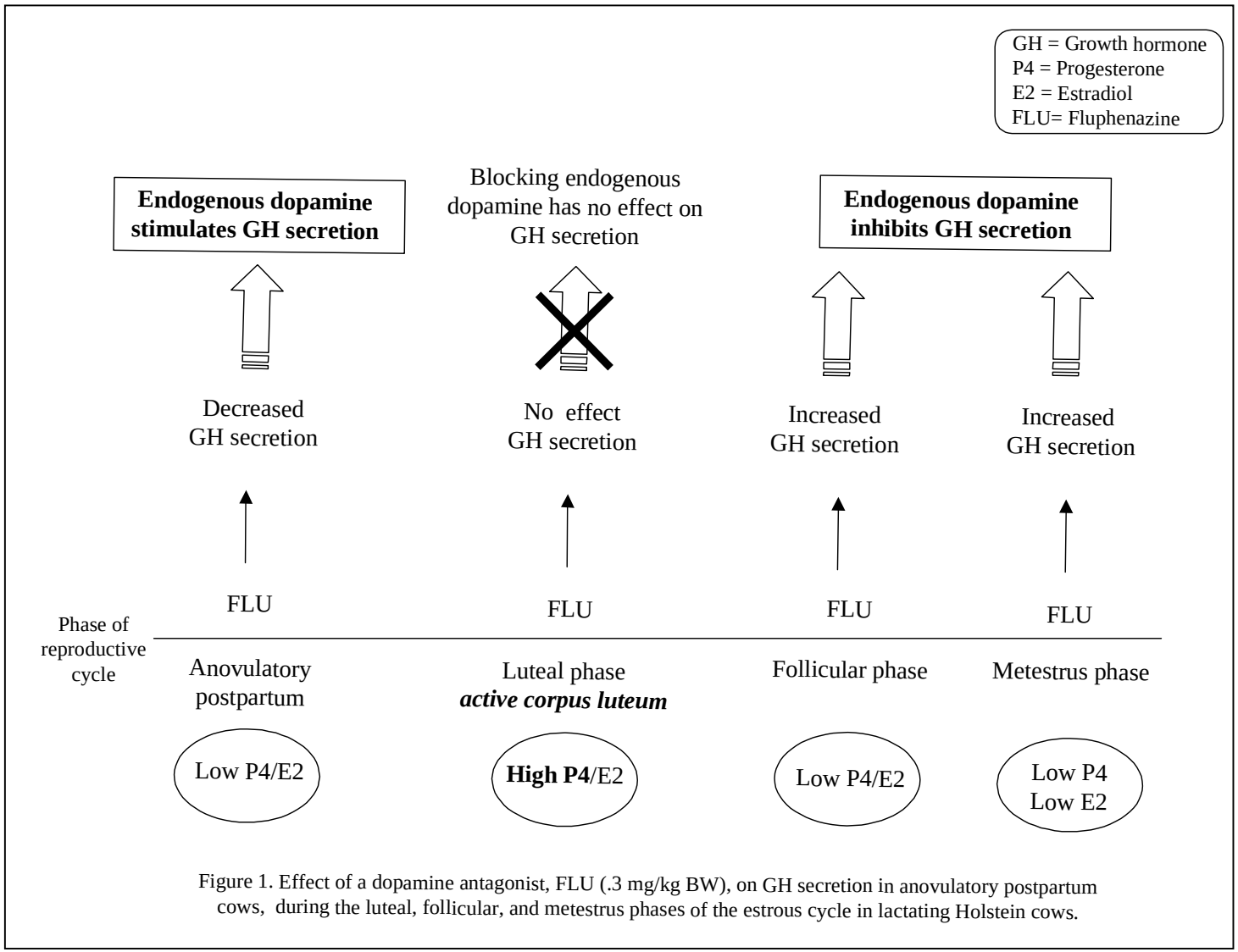

Figure 1. Effect of a dopamine antagonist, FLU (.3 mg/kg BW), on GH secretion in anovulatory postpartum cows, during the luteal, follicular, and metestrus phases of the estrous cycle in lactating Holstein cows.

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APPENDICES

APPENDIX A

PROCEDURE FOR RADIOIODINATING LUTENIZING HORMONE AND FOLLICLE STIMULATING HORMONE

Anion exchange columns preparation

To prepare AG 1X8 anion exchange columns, a small wad of glass wool was placed in the bottom of a 3 ml plastic syringe and approximately 1.5 ml (by volume) of anion exchange resin was added. The column was then rinsed twice with 1 ml of .05 M PO4 and one time with 1 ml of .5 M PO4. The column was then eluted with 3 ml of 1% bovine serum albumin (BSA) in 0.05 M PBS, which is also used as assay buffer. A rubber stopper was attached to the syringe and 1 ml .05 M PO4 was added to prevent the column from drying until iodination

Radioiodination of LH using Cholaramine T

Lyophilized pure bovine LH (USDA-bLH-B-6) was used for iodination. An aliquot of LH was weighed from lyophilized form and dissolved in deionized distilled H_2O (400 ng/ml). Fifty ul (20 ng) of dissolved LH was then dispensed into 1-ml microfuge vials, re-lyophilized, and stored at -20^{O}C. For iodination, 35 ul 0.5 M $P0_4$, pH 7.5 was added to the l-ml microfuge vial containing 20 ug of lyophilized LH. After the hormone was dissolved, approximately 500 uCi ^{125}I was added to the vial. A short time interval (no longer than 3 min) between the addition of hormone and ^{125}I is desirable. Immediately therafter, 3 to 5 ug cholaramine T (Fisher, MW=281.68, reagent grade), dissolved in 0.05 M PO_4 (0.5 mg/ml), was added to the vial containing the hormone and ^{125}I (use no more than 1 ug of choloramine T per ug of hormone). The solution was gently vortexed for 90 seconds and the reaction was stopped by addition of 5 ug Na-metabisulfite in 0.05 M PO_4 (0.5 mg/ml) to the vial. Immediately thereafter, .5 ml of 0.05 M PO_4 was added to the vial. The anion exchange AG 1X8 column was placed over a collection tube containing 2 ml of assay buffer. Using a 1-ml syringe equipped

with a 20g disposable needle, the iodinated hormone was transferred to the anion exchange column and then the column was rinsed with 2 ml of 0.05 M PO_4 and 1 ml of assay buffer. The iodinated fraction (2.5 ml; one fraction) was collected into a tube containing 2 ml of assay buffer. Iodinated hormone was brought up to 10 ml with 1% BSA-PBS. The desirable activity for the assay is 20,000 ± 2,000 cpm/100 ul.

ASSAY PROTOCOL FOR LH

A homologous, double antibody radioimmunoassay was developed to quantify LH in bovine serum. Specific rabbit anti-bovine LH antiserum was provided by USDA (USDA-309-684p) and used as the primary antibody. The second antibody was sheep anti-rabbit gammaglobulin and used at a dilution of 1:15 or 1:20 in 0.5 M phosphate buffer saline (PBS)-EDTA (pH=7.5). To prepare a standard curve, pure bovine LH (USDA-bLH-B-6) was dissolved in 1 % BSA-PBS (25 ng/ml). The standard curve consisted of six points (.04, .08, .16, .32, .64, 1.28 ng/tube) in quadruplicate. Lyophilized primary antiserum (USDA-309-684p) was initially reconstituted in .05 M PBS-EDTA (at a dilution of 1:400) and was further diluted with PBS-EDTA containing rabbit control serum (1:300). Primary antiserum (at a final dilution of 1:100,000) bound 35-40% of ^{125}I-LH in the absence of unlabeled LH. The assay was conducted in non-equilibrium condition. On day one, the standard curve was prepared and 200 ul serum samples were diluted with 300-ul-assay buffer in duplicate. Two- hundred ul of primary antiserum was added to the standard reference and serum samples, all tubes were vortexed, and were then incubated for 24 hr at 25^{O}C. On day two, 100 ul of ^{125}I-bLH (20,000 cpm ± 2000) was added to all tubes. All tubes were gently mixed and incubated for an additional 48 hr at 25^{O}C. On day four, 200 ul of second antibody was added to all tubes, and after mixing they were incubated for 48 hr at 4^{O}C. On day 6, samples were diluted with 1 ml of 0.05 M PBS and then centrifuged (2,300 $\times$g) for 30 min at 4^{O}C. To separate bound from free LH, supernatant (unbound LH) was poured off and tubes were drained for at least 3 h. The pellets (bound LH) in sample tubes were then counted in the gamma counter for 1 min.

Radioiodination for FSH using Cholaramine T

Lyophilized pure bovine FSH (USDA-bFSH-I-2) was used for iodination. For iodination, 20 ul 0.5 M $P0_4$, pH 7.5 was added to the l-ml glass vial containing 10 ug of lyophilized FSH. After the hormone was dissolved, approximately 500 uCi ^{125}I was added to the vial. A short time interval (no longer than 3 min) between the addition of hormone and ^{125}I is desirable. Immediately therafter, 3 ug cholaramine T (Fisher, MW=281.68, reagent grade), dissolved in 0.05 M PO_4 (0.5 mg/ml), was added to the vial containing the hormone and ^{125}I (use no more than 1 ug of choloramine T per ug of hormone). The solution was gently vortexed for 90 seconds and the reaction was stopped by addition of 5 ug Na-metabisulfite in 0.05 M PO_4 (0.5 mg/ml) to the vial. Immediately thereafter, 0.5 ml of 0.05 M PO_4 was added to the vial. The anion exchange AG 1X8 column was placed over a collection tube containing 2 ml of 1% BSA-PBS. Using a 1-ml syringe, equipped with a 20g disposable needle, the vial content was transferred to the anion exchange column and then the column was additionally rinsed with 2 ml of 0.05 M PO_4 and 1 ml of 1% BSA-PBS. The iodinated fraction (2.5 ml; one fraction) was collected into a tube containing 2 ml of 1% BSA-PBS. Iodinated hormone was brought up to 10 ml with 1% BSA-PBS. The desirable activity for the assay is 18,000 ± 2,000 cpm/100 ul.

ASSAY PROTOCOL FOR FSH

A heterologous, double antibody radioimmunoassay was developed to quantify FSH in bovine serum. Specific rabbit anti-ovine FSH antiserum was provided by Dr. A. F. Parlow (NIDKK-anti-oFSH-1) and used as the primary antibody. The second antibody was sheep anti-rabbit gammaglobulin and used at a dilution of 1:15 or 1:20 in 0.05 M phosphate buffer saline (PBS)-EDTA (pH=7.5). To prepare a standard curve, pure bovine FSH (USDA-bFSH-I-2) was dissolved in 1 % BSA-PBS (25 ng/ml). The standard curve consisted of seven points (.01, .02, .04, .08, .16, .32 and, .64 ng/tube) in quadruplicate. Lyophilized primary antiserum (NIDKK-anti-oFSH-1) was initially

reconstituted in 1 ml deionized distilled water (at a dilution of 1:8) and was further diluted with PBS-EDTA contained rabbit control serum (1:200). Primary antiserum (at a final dilution of 1:80,000) bound 15-20% of ^{125}I-FSH in the absence of unlabeled FSH. The assay was conducted in non-equilibrium condition. On day one, the standard curve was prepared and 100 ul serum samples were diluted with 400 ul assay buffer (.1 % Gel in 0.05 M PBS) in duplicate. Two- hundred ul of primary antiserum was added to the standard reference and serum samples, all tubes were vortexed, and were then incubated for 48 hr at 25^{O}C. On day three, 100 ul of ^{125}I-bLH (18,000 cpm ± 2000) was added to all tubes. All tubes were gently mixed and incubated for an additional 24 hr at 25^{O}C. On day four, 200 ul of second antibody was added to all tubes and after mixing they were incubated for 48 hr at 4^{O}C. On day 6, samples were diluted with 1 ml of 0.05 M PBS and, centrifuged (2,300 $\times$g) for 30 min at 4^{O}C. To separate bound from free FSH, supernatant (unbound FSH) was poured off and tubes were drained for at least 3 h. The pellets (bound FSH) in sample tubes were then counted in the gamma counter for 1 min.

APPENDIX B

Appendix B. Table 1. Analysis of variance for the effect of fluphenazine treatment on serum **luteinizing hormone** concentration in **primiparous** Holstein cows on **day 13 or 14 postpartum** (n=12).

Source of Variation	df	Mean Square	F Value	PR > F
Treatment*	1	0.632	5.06	0.0482
Cow (Treatment)	10	0.124	6.97	0.0001
Period**	2	0.276	7.67	0.0033
Treatment×Period**	2	0.044	1.22	0.3155
Period×Cow (Treatment)	20	0.036	2.00	0.0069
Error	360	0.018		

* Using type III MS for Cow (Treatment) as the error term.
** Using type III MS for Period×Cow (Treatment) as the error term.

Appendix B. Table 2. Analysis of variance for the effect of fluphenazine on **luteinizing hormone pulse frequency** in **primiparous** Holstein cows on **day 13 or 14 postpartum** (n=12).

Source of Variation	df	Mean Square	F Value	PR > F
Treatment*	1	0.666	2.00	0.1100
Cow (Treatment)	10	0.333	1.26	0.3655
Period**	1	2.666	10.00	0.0101
Treatment×Period	1	0.666	2.54	0.1449
Error	10	0.266		

* Using type III MS for Cow (Treatment) as the error term.

Appendix B. Table 3. Analysis of variance for the effect of fluphenazine on **luteinizing hormone peak amplitude** in **primiparous** Holstein cows on **day 13 or 14 postpartum** (n=12).

Source of Variation	df	Mean Square	F Value	PR > F
Treatment*	1	0.129	4.38	0.0629
Cow (Treatment)	10	0.029	0.58	07996
Period**	1	0.260	5.11	0.0474
Treatment×Period	1	0.045	0.88	0.3694
Error	10	0.051		

* Using type III MS for Cow (Treatment) as the error term.

Appendix B. Table 4. Analysis of variance for the effect of gonadotropin releasing hormone treatment on serum **luteinizing hormone** concentration in **primiparous** Holstein cows on **day 13 or 14 postpartum** (n=12).

Source of Variation	df	Mean Square	F Value	PR > F
Treatment*	1	0.061	0.02	0.9007
Cow (Treatment)	10	3.750	20.86	0.0001
Period**	1	97.948	23.16	0.0007
Treatment×Period**	1	0.825	0.20	0.6680
Period×Cow (Treatment)	10	4.229	23.62	0.0001
Error	120	0.179		

* Using type III MS for Cow (Treatment) as the error term.
** Using type III MS for Period×Cow (Treatment) as the error term.

Appendix B. Table 5. Analysis of variance for the effect of fluphenazine treatment on serum **luteinizing hormone** concentration in **multiparous** Holstein cows on **day 13 or 14 postpartum** (n=6).

Source of Variation	df	Mean Square	F Value	PR > F
Period*	2	0.093	12.49	0.0019
Cow	5	0.177	26.34	0.0001
Cow×Period	10	0.009	2.11	0.259
Error	180	0.004		

* Using type III MS for Cow × Period as the error term.

Appendix B. Table 6. Analysis of variance for the effect of fluphenazine on **luteinizing hormone pulse frequency** in **multiparous** Holstein cows on **day 13 or 14 postpartum** (n=6).

Source of Variation	df	Mean Square	F Value	PR > F
Period	1	2.083	7.35	0.0422
Cow	5	0.283	1.00	0.5000
Error	5	0.283		

Appendix B. Table 7. Analysis of variance for the effect of fluphenazine **on luteinizing hormone peak amplitude** in **multiparous** Holstein cows on **day 13 or 14 postpartum** (n=6).

Source of Variation	df	Mean Square	F Value	PR > F
Period	1	0.196	1.00	0.3629
Cow	5	0.116	0.59	0.7098
Error	5	0.196		

Appendix B. Table 8. Analysis of variance for the effect of **gonadotropin releasing hormone** treatment on serum **luteinizing hormone** concentration in **multiparous** Holstein cows **on day 13 or 14 postpartum** (n=6).

Source of Variation	df	Mean Square	F Value	PR > F
Period*	1	51.657	20.25	.0064
Cow	5	3.492	18.09	.0001
Cow×Period	5	2.551	13.20	0.0001
Error	60	0.193		

* Using type III MS for Cow × Period as the error term.

Appendix B. Table 9. Analysis of variance for the effect of fluphenazine treatment on serum **prolactin** concentration in **primiparous** Holstein cows on **day 13 or 14 postpartum** (n=15).

Source of Variation	df	Mean Square	F Value	PR > F
Treatment*	1	140718.31	112.99	0.0001
Cow (Treatment)	10	1245.45	9.37	0.0001
Period**	2	70440.29	151.29	0.0001
Treatment×Period**	2	62785.00	134.85	0.0001
Period×Cow (Treatment)	20	465.60	3.50	0.0001
Error	191	132.87		

* Using type III MS for Cow (Treatment) as the error term.
** Using type III MS for Period×Cow (Treatment) as the error term.

Appendix B. Table 10. Analysis of variance for the effect of fluphenazine treatment on serum **growth hormone** concentration in **primiparous** Holstein cows on **day 13 or 14 postpartum** (n=12).

Source of Variation	df	Mean Square	F Value	PR > F
Treatment*	1	431.295	0.96	0.3502
Cow (Treatment)	10	449.042	64.27	0.0001
Period**	2	11.342	1.14	0.3404
Treatment×Period**	2	79.566	7.98	0.0028
Period×Cow (Treatment)	20	9.968	1.43	0.1062
Error	360	6.987		

* Using type III MS for Cow (Treatment) as the error term.
** Using type III MS for Period×Cow (Treatment) as the error term.

Appendix B. Table 11. Analysis of variance for the effect of growth hormone releasing hormone treatment on serum **growth hormone** concentration in **primiparous** Holstein cows on **day 13 or 14 postpartum** (n=12).

Source of Variation	df	Mean Square	F Value	PR > F
Treatment*	1	269.753	0.49	0.4979
Cow (Treatment)	10	545.326	10.53	0.0001
Period**	1	2632.744	6.96	0.0248
Treatment×Period**	1	0.363	0.00	0.9758
Period×Cow (Treatment)	10	376.739	7.27	0.0001
Error	120	51.786		

* Using type III MS for Cow (Treatment) as the error term.
** Using type III MS for Period×Cow (Treatment) as the error term.

Appendix B. Table 12. Analysis of variance for the effect of fluphenazine treatment on serum **prolactin** concentration in **multiparous** Holstein cows **on day 13 or 14 postpartum** (n=6).

Source of Variation	df	Mean Square	F Value	PR > F
Period*	2	66401.75	26.54	0.0001
Cow	5	9087.58	26.50	0.0001
Cow×Period	10	2501.90	7.30	0.0001
Error	119			

* Using type III MS for Cow × Period as the error term.

Appendix B. Table 13. Analysis of variance for the effect of fluphenazine treatment on serum **growth hormone** concentration in **multiparous** Holstein cows on **day 13 or 14 postpartum** (n=6).

Source of Variation	df	Mean Square	F Value	PR > F
Period*	2	259.411	14.28	0.0012
Cow	5	34.860	13.65	0.0001
Cow×Period	10	18.164	7.11	0.0001
Error	180	2.554		

* Using type III MS for Cow × Period as the error term.

Appendix B. Table 14. Analysis of variance for the effect of growth hormone releasing hormone on serum **growth hormone** concentration in **multiparous** Holstein cows **on day 13 or 14 postpartum** (n=6).

Source of Variation	df	Mean Square	F Value	PR > F
Period*	1	1485.034	5.06	0.0743
Cow	5	345.265	6.13	0.0001
Cow×Period	5	293.358	5.21	0.0005
Error	60	56.290		

* Using type III MS for Cow × Period as the error term.

APPENDIX C

Appendix C. Table 1. Analysis of variance for the effect of fluphenazine serum **luteinizing hormone** concentration in Holstein cows in the **luteal phase** of the estrous cycle (n=18).

Source of Variation	df	Mean Square	F Value	PR > F
Dose*	2	98.983	0.91	0.4255
Cow (Dose)	15	109.351	2.88	0.0002
Period**	2	20.225	0.39	0.6792
Dose×Period**	4	53.999	1.05	0.4001
Period×Cow (Dose)	30	51.622	1.36	0.0976
Error	540	37.914		

* Using type III MS for Cow (Dose) as the error term.
** Using type III MS for Period×Cow (Dose) as the error term.

Appendix C. Table 2. Analysis of variance for the effect of fluphenazine **luteinizing hormone pulse frequency** in Holstein cows in **the luteal phase** of the estrous cycle (n=18).

Source of Variation	df	Mean Square	F Value	PR > F
Dose*	2	0.028	0.08	0.9274
Cow (Dose)	15	0.367	0.94	0.5446
Period	1	0.111	0.29	0.6008
Dose×Period	2	0.028	0.07	0.9314
Error	15	0.389		

* Using type III MS for Cow (Dose) as the error term.

Appendix C. Table 3. Analysis of variance for the effect of fluphenazine **luteinizing hormone peak amplitude** in Holstein cows in **the luteal phase** of the estrous cycle (n=18).

Source of Variation	df	Mean Square	F Value	PR > F
Dose*	2	0.045	3.31	0.0643
Cow (Dose)	15	0.014	1.07	0.4522
Period	1	0.001	0.15	0.7009
Dose×Period	2	0.002	0.20	0.8171
Error	15	0.013		

* Using type III MS for Cow (Dose) as the error term.

Appendix C. Table 4. Analysis of variance for the effect of **gonadotropin releasing hormone** treatment on serum **luteinizing hormone** concentration in Holstein cows in **the luteal phase** of the estrous cycle (n=18).

Source of Variation	df	Mean Square	F Value	PR > F
Dose*	2	0.166	1.18	0.3345
Cow (Dose)	15	0.141	6.71	0.0001
Period**	1	9.385	86.10	0.0001
Dose×Period**	2	0.206	1.88	0.1867
Period×Cow (Dose)	15	0.109	5.19	0.0001
Error	180	0.021		

* Using type III MS for Cow (Dose) as the error term.
** Using type III MS for Period×Cow (Dose) as the error term.

Appendix C. Table 5. Analysis of variance for the effect of fluphenazine treatment on serum **follicle stimulating** hormone concentration in Holstein cows in **the luteal phase** of the estrous cycle (n=18).

Source of Variation	df	Mean Square	F Value	PR > F
Dose*	2	0.519	0.28	0.7599
Cow (Dose)	15	1.867	97.59	0.0001
Period**	2	0.086	1.95	0.1603
Dose×Period**	4	0.074	1.68	0.1802
Period×Cow (Dose)	30	0.044	2.32	0.0001
Error	540	0.019		

* Using type III MS for Cow(Tmt×Parity) as the error term.
** Using type III MS for Per×Cow(Tmt×Parity) as the error term.

Appendix C. Table 6. Analysis of variance for the effect of fluphenazine **follicle stimulating hormone pulse frequency** in Holstein cows in **the luteal phase** of the estrous cycle (n=18).

Source of Variation	df	Mean Square	F Value	PR > F
Dose*	2	0.361	0.72	0.5018
Cow (Dose)	15	0.500	1.15	0.3926
Period	1	0.111	0.26	0.6200
Dose×Period	2	0.194	0.45	0.6467
Error	15	0.433		

* Using type III MS for Cow (Dose) as the error term.

Appendix C. Table 7. Analysis of variance for the effect of fluphenazine **follicle stimulating hormone peak amplitude** in Holstein cows in **the luteal phase** of the estrous cycle (n=18).

Source of Variation	df	Mean Square	F Value	PR > F
Dose*	2	0.000	0.00	0.9976
Cow (Dose)	15	0.014	0.86	0.6116
Period	1	0.031	1.91	0.1874
Dose×Period	2	0.018	1.07	0.3663
Error	15	0.016		

* Using type III MS for Cow (Dose) as the error term.

Appendix C. Table 8. Analysis of variance for the effect of **gonadotropin releasing hormone** treatment on serum **follicle stimulating** hormone concentration in Holstein cows in **the luteal phase** of the estrous cycle (n=18).

Source of Variation	df	Mean Square	F Value	PR > F
Dose*	2	0.054	0.04	0.9645
Cow (Dose)	15	1.486	30.25	0.0001
Period**	1	29.578	107.94	0.0001
Dose×Period**	2	0.024	0.09	0.9150
Period×Cow (Dose)	15	0.274	5.59	0.0001
Error	180	0.049		

* Using type III MS for Cow (Dose) as the error term
** Using type III MS for Per×Cow (Dose) as the error term

Appendix C. Table 9. Analysis of variance for the effect of fluphenazine on serum **prolactin** in Holstein cows in the **luteal phase** of the estrous cycle (n=18).

Source of Variation	df	Mean Square	F Value	PR > F
Dose*	2	32277.27	16.17	0.0002
Cow (Dose)	15	1996.33	28.90	0.0001
Period**	2	36086.86	44.93	0.0001
Dose×Period**	4	8818.15	10.98	0.0001
Period×Cow (Dose)	30	803.09	11.63	0.0001
Error	354	69.07		

* Using type III MS for Cow (Dose) as the error term.
** Using type III MS for Period×Cow (Dose) as the error term.

Appendix C. Table 9. Analysis of variance for the effect of fluphenazine treatment on serum **growth hormone** concentration in Holstein cows in the **luteal phase** of the estrous cycle (n=18).

Source of Variation	df	Mean Square	F Value	PR > F
Dose*	2	116.762	0.12	0.8895
Cow (Dose)	15	989.708	46.53	0.0001
Period**	2	46.842	0.45	0.6411
Dose×Period**	4	74.366	0.72	0.5874
Period×Cow (Dose)	30	103.817	4.88	0.0001
Error	538	21.271		

* Using type III MS for Cow (Dose) as the error term.
** Using type III MS for Period×Cow (Dose) as the error term.

APPENDIX D

Appendix D. Table 1. Analysis of variance for the effect of fluphenazine treatment on serum **luteinizing hormone** concentration in Holstein cows during **the follicular phase** of the estrous cycle (n=15).

Source of Variation	df	Mean Square	F Value	PR > F
Treatment*	1	0.017	1.00	0.3264
Cow (Treatment)	13	0.133	21.14	0.0001
Period**	2	0.051	3.09	0.0624
Treatment×Period**	2	0.122	7.33	0.0030
Period×Cow (Treatment)	26	0.017	2.26	0.0001
Error	450	0.006		

* Using type III MS for Cow (Treatment) as the error term.
** Using type III MS for Period × Cow (Treatment) as the error term.

Appendix D. Table 2. Analysis of variance for the effect of fluphenazine on **luteinizing hormone pulse** frequency in Holstein cows in **the follicular phase** the estrous cycle (n=15).

Source of Variation	df	Mean Square	F Value	PR > F
Treatment*	1	4.929	5.37	0.0375
Cow (Treatment)	13	0.918	1.38	0.2850
Period**	1	2.215	3.33	0.0912
Treatment×Period	1	5.149	7.74	0.0156
Error	13	0.665		

* Using type III MS for Cow (Treatment) as the error term.

Appendix D. Table 3. Analysis of variance for the effect of fluphenazine on **luteinizing hormone peak amplitude** in Holstein cows in **the follicular phase** the estrous cycle (n=15).

Source of Variation	df	Mean Square	F Value	PR > F
Treatment*	1	0.009	0.28	0.6027
Cow (Treatment)	13	0.033	2.34	0.0711
Period**	1	0.013	0.93	0.3476
Treatment×Period	1	0.005	0.35	0.5682
Error	13	0.014		

* Using type III MS for Cow (Treatment) as the error term.

Appendix D. Table 4. Analysis of variance for the effect of **gonadotropin releasing hormone** treatment on serum **luteinizing hormone** concentration in Holstein cows in **the follicular phase** of the estrous cycle (n=15).

Source of Variation	df	Mean Square	F Value	PR > F
Treatment*	1	0.637	0.11	0.7495
Cow (Treatment)	13	5.984	18.24	0.0001
Period**	1	215.003	41.94	0.0001
Treatment×Period**	1	1.114	0.22	0.6489
Period×Cow (Treatment)	13	5.126	15.62	0.0001
Error	150	0.328		

* Using type III MS for Cow (Treatment) as the error term.
** Using type III MS for Period × Cow (Treatment) as the error term.

Appendix D. Table 5. Analysis of variance for the effect of fluphenazine treatment on serum **follicle stimulating hormone** concentration in Holstein cows inuring **the follicular phase** of the estrous cycle (n=15).

Source of Variation	df	Mean Square	F Value	PR > F
Treatment*	1	0.234	0.11	0.7599
Cow (Treatment)	13	2.207	551.84	0.0001
Period**	2	0.002	0.29	0.7491
Treatment×Period**	2	0.021	2.96	0.0693
Period×Cow (Treatment)	260	0.007	1.75	0.0248
Error	450	0.004		

* Using type III MS for Cow (Treatment) as the error term.
** Using type III MS for Period × Cow (Treatment) as the error term.

Appendix D. Table 6. Analysis of variance for the effect of fluphenazine on **follicle stimulating hormone pulse** frequency in Holstein cows in **the follicular phase** the estrous cycle (n=15).

Source of Variation	df	Mean Square	F Value	PR > F
Treatment*	1	0.648	1.03	0.3278
Cow (Treatment)	13	0.627	2.59	0.0494
Period**	1	0.315	1.30	0.2750
Treatment×Period	1	0.048	.20	0.6630
Error	13	0.242		

* Using type III MS for Cow (Treatment) as the error term.

Appendix D. Table 7. Analysis of variance for the effect of fluphenazine on **follicle stimulating hormone peak amplitude** in Holstein cows in **the follicular phase** the estrous cycle (n=15).

Source of Variation	df	Mean Square	F Value	PR > F
Treatment*	1	0.0022	0.93	0.3527
Cow (Treatment)	13	0.0024	1.34	0.2674
Period**	1	0.0024	1.34	0.2674
Treatment×Period	1	0.0008	0.46	0.5104
Error	13	0.0018		

* Using type III MS for Cow (Treatment) as the error term.

Appendix D. Table 8. Analysis of variance for the effect of **gonadotropin releasing hormone** treatment on serum **follicle stimulating hormone** concentration in Holstein cows during **the follicular phase** of the estrous cycle (n=15).

Source of Variation	df	Mean Square	F Value	PR > F
Treatment*	1	0.029	0.03	0.8756
Cow (Treatment)	13	1.158	57.12	0.0001
Period**	1	10.116	80.95	0.0001
Treatment×Period**	1	0.371	2.97	0.1085
Period×Cow (Treatment)	13	0.125	6.16	0.0001
Error	150	0.020		

* Using type III MS for Cow (Treatment) as the error term.
** Using type III MS for Period × Cow (Treatment) as the error term.

Appendix D. Table 9. Analysis of variance for the effect of fluphenazine treatment on serum **luteinizing hormone** concentration in Holstein cows in **the metestrus phase** of the estrous cycle (n=15).

Source of Variation	df	Mean Square	F Value	PR > F
Treatment*	1	0.0322	0.64	0.4386
Cow (Treatment)	13	0.505	10.94	0.0001
Period**	2	0.0088	1.10	0.3475
Treatment×Period**	2	0.0341	4.26	0.0251
Period×Cow (Treatment)	26	0.0080	1.74	0.0139
Error	570	0.0046		

* Using type III MS for Cow (Treatment) as the error term.
** Using type III MS for Period×Cow (Treatment) as the error term.

Appendix D. Table 10. Analysis of variance for the effect of fluphenazine on **luteinizing hormone pulse frequency** in Holstein cows in **the metestrus phase** the estrous cycle (n=15).

Source of Variation	df	Mean Square	F Value	PR > F
Treatment*	1	0.815	0.78	0.3944
Cow (Treatment)	13	1.050	1.74	0.1663
Period**	1	1.800	2.98	0.1082
Treatment×Period	1	3.000	4.96	0.0443
Error	13	0.605		

* Using type III MS for Cow (Treatment) as the error term.

Appendix D. Table 11. Analysis of variance for the effect of fluphenazine on **luteinizing hormone peak amplitude** in Holstein cows in **the metestrus phase** the estrous cycle (n=15).

Source of Variation	df	Mean Square	F Value	PR > F
Treatment*	1	0.0002	0.01	0.9343
Cow (Treatment)	13	0.0293	0.85	0.6116
Period**	1	0.0186	0.54	0.4775
Treatment×Period	1	0.0926	0.12	0.1253
Error	13	0.0344		

* Using type III MS for Cow (Treatment) as the error term.

Appendix D. Table 12. Analysis of variance for the effect of **gonadotropin releasing hormone** treatment on serum **luteinizing hormone** concentration in Holstein cows in **the metestrus phase** of the estrous cycle (n=15).

Source of Variation	df	Mean Square	F Value	PR > F
Treatment*	1	0.171	0.42	0.5272
Cow (Treatment)	13	0.406	5.11	0.0001
Period**	1	36.179	103.74	0.0001
Treatment×Period**	1	0.042	0.12	0.7336
Period×Cow (Treatment)	13	0.348	4.38	0.0001
Error	180	0.079		

* Using type III MS for Cow (Treatment) as the error term.
** Using type III MS for Period×Cow (Treatment) as the error term.

Appendix D. Table 13. Analysis of variance for the effect of fluphenazine treatment on serum **follicle stimulating** concentration in Holstein cows in **the metestrus phase** of the estrous cycle (n=15).

Source of Variation	df	Mean Square	F Value	PR > F
Treatment*	1	1.0856	2.15	0.1662
Cow (Treatment)	13	0.5046	648.58	0.0001
Period**	2	0.0045	0.54	0.5908
Treatment×Period**	2	0.0055	0.65	0.5281
Period×Cow (Treatment)	26	0.0085	10.62	0.0001
Error	570	0.0007		

* Using type III MS for Cow (Treatment) as the error term.
** Using type III MS for Period × Cow (Treatment) as the error term.

Appendix D. Table 14. Analysis of variance for the effect of fluphenazine on **follicle stimulating hormone pulse frequency** in Holstein cows during **the metestrus phase** the estrous cycle (n=15).

Source of Variation	df	Mean Square	F Value	PR > F
Treatment*	1	0.005	0.01	0.9059
Cow (Treatment)	13	0.369	0.88	0.5880
Period**	1	0.029	0.07	0.7959
Treatment×Period	1	0.029	0.07	0.7959
Error	13	0.418		

* Using type III MS for Cow (Treatment) as the error term.

Appendix D. Table 15. Analysis of variance for the effect of fluphenazine on **follicle stimulating hormone peak amplitude** in Holstein cows in **the metestrus phase** the estrous cycle (n=15).

Source of Variation	df	Mean Square	F Value	PR > F
Treatment*	1	0.0044	0.13	0.7261
Cow (Treatment)	13	0.0343	1.45	0.2572
Period**	1	0.0164	0.69	0.4207
Treatment×Period	1	0.0004	0.02	0.8984
Error	13	0.0237		

* Using type III MS for Cow (Treatment) as the error term.

Appendix D. Table 16. Analysis of variance for the effect of **gonadotropin releasing hormone** treatment on serum **follicle stimulating hormone** concentration in Holstein cows in **the metestrus phase** of the estrous cycle (n=15).

Source of Variation	df	Mean Square	F Value	PR > F
Treatment*	1	0.5631	1.85	0.1973
Cow (Treatment)	13	0.3049	121.96	0.0001
Period**	1	1.5326	94.16	0.0001
Treatment×Period**	1	0.0275	1.69	0.2159
Period×Cow (Treatment)	26	0.0163	6.52	0.0001
Error	209	0.0025		

* Using type III MS for Cow (Treatment) as the error term.
** Using type III MS for Period×Cow (Treatment) as the error term.

APPENDIX E

Appendix E. Table 1. Analysis of variance for the effect of fluphenazine treatment on serum **prolactin** concentration in Holstein cows during **the follicular phase** of the estrous cycle (n=15).

Source of Variation	df	Mean Square	F Value	PR > F
Treatment*	1	155682.26	39.07	0.0001
Cow (Treatment)	13	11913.64	44.12	0.0001
Period**	1	60310.43	15.14	0.0001
Treatment×Period**	1	56928.07	14.29	0.0001
Period×Cow (Treatment)	13	3984.57	14.76	0.0001
Error	180	270.01		

* Using type III MS for Cow (Treatment) as the error term.
** Using type III MS for Period×Cow (Treatment) as the error term.

Appendix E. Table 2. Analysis of variance for the effect of fluphenazine treatment on serum **growth hormone** concentration in Holstein cows during **the follicular phase** of the estrous cycle (n=15).

Source of Variation	df	Mean Square	F Value	PR > F
Treatment*	1	228.243	0.51	0.4863
Cow (Treatment)	13	444.473	47.14	0.0001
Period**	2	768.089	9.49	0.0008
Treatment×Period**	2	84.701	1.05	0.3655
Period×Cow (Treatment)	26	80.936	8.58	0.0001
Error	532	9.42		

* Using type III MS for Cow (Treatment) as the error term.
** Using type III MS for Period×Cow (Treatment) as the error term.

Appendix E. Table 3. Analysis of variance for the effect of fluphenazine treatment on serum **prolactin** concentration in Holstein cows during **the metestrus phase** of the estrous cycle (n=15).

Source of Variation	df	Mean Square	F Value	PR > F
Treatment*	1	111847.91	80.34	0.0001
Cow (Treatment)	13	10037.82	91.57	0.0001
Period**	1	34530.30	24.80	0.0001
Treatment×Period**	1	38864.81	27.92	0.7336
Period×Cow (Treatment)	26	1392.14	12.70	0.0001
Error	180	109.62		

* Using type III MS for Cow (Treatment) as the error term.
** Using type III MS for Period×Cow (Treatment) as the error term.

Appendix E. Table 4. Analysis of variance for the effect of fluphenazine treatment on serum **growth hormone** concentration in Holstein cows in **the metestrus phase** of the estrous cycle (n=15).

Source of Variation	df	Mean Square	F Value	PR > F
Treatment*	1	861.074		
Cow (Treatment)	13	526.058	48.16	0.0001
Period**	2	927.049	7.19	0.0033
Treatment×Period**	2	406.831	3.16	0.0593
Period×Cow (Treatment)	26	128.936	11.80	0.0001
Error	675	10.924		

* Using type III MS for Cow (Treatment) as the error term.
** Using type III MS for Period×Cow (Treatment) as the error term.

VITA

Amin Ahmadzadeh, the son of Hashem and Mansoureh Ahmadzadeh, was born on May 22, 1964, in Mashhad, Iran. He graduated from high School in Mashhad, in 1982 and after working on the family owned dairy farm for 5 years, he came to the United States. In fall of 1988, the author enrolled at Delaware Valley College, Doylestown, Pennsylvania and received a Bachelor of Science degree in Dairy Sceince in 1992. In fall of 1992, he began his masters program at Virginia Tech, and received a Master of Science degree in Dairy Science (Reproductive Physiology) in November 1994. He then began his doctorate degree at Virginia Tech in Animal physiology and received his Ph.D. in October 1998.

The author is a member of the American Dairy Science Association and American Society of Animal Science. He is also member of Gamma Sigma Delta Agriculture Honor Society and Sigma Xi and Phi Kappa Phi Scientific Honor Societies.

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