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The effects of growth hormone or melatonin on the reproductive axis of stallions

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**THE EFFECTS OF GROWTH HORMONE OR MELATONIN ON THE
REPRODUCTIVE AXIS OF STALLIONS**

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirement for the degree of
Master of Science

in

The Interdepartmental Program in Animal
and Dairy Sciences

by

William Andrew Storer
B.S., McNeese State University, 1999
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ABSTRACT

Two experiments were conducted to determine the effects of growth hormone (GH) or melatonin on the reproductive axis of the stallion. In Experiment 1, nine stallions were treated with GH (20 μ g/kg BW) or saline for 21 d starting in January. During the last week of treatment, stallions were subjected to low and high dose injections of luteinizing hormone (LH), as well as low and high dose combined injections of gonadotropin releasing hormone (GnRH) and thyrotropin releasing hormone (TRH). Two months after the onset of GH treatment, semen was collected from all stallions every other day for 2 weeks. Treatment with recombinant equine GH increased ($P < 0.001$) daily IGF-I concentrations, but had no effect ($P > 0.1$) on concentrations of LH, follicle stimulating hormone (FSH), or testosterone. The testosterone responses to injections of LH were similar ($P > 0.1$) between treatments. Likewise, the LH, FSH, prolactin, and testosterone responses to the injections of GnRH/TRH were similar ($P > 0.1$) between groups. Stallions treated with GH exhibited greater volumes of gel-free semen ($P < 0.01$) and gel ($P < 0.05$) and had decreased time until ejaculation ($P < 0.05$). In Experiment 2, nine stallions were given corn syrup containing either melatonin (0.06 mg/kg BW) or nothing for 90 d starting in July. Between d 68 and 75 of treatment, stallions were given injections of LH and combined injections of GnRH and TRH, similar to Experiment I. Semen was collected from all stallions for three days during the last week of treatment. Treated stallions exhibited decreased daily concentrations of prolactin ($P < 0.01$) and FSH ($P < 0.05$), and tended to have lower ($P = 0.07$) LH concentrations for the first 30 d. Testosterone concentrations were similar between groups. In treated stallions, the low dose administration of GnRH/TRH was not as effective ($P < 0.01$) at increasing plasma concentrations of FSH and testosterone, and the response in plasma prolactin concentrations to a high dose administration of GnRH/TRH was decreased ($P < 0.01$). Melatonin treatment did not alter seminal characteristics or libido. In conclusion, GH may alter the long-term accessory gland contribution to seminal volume, but does not appear to interact with other constituents of the reproductive axis in the stallion. Long-term melatonin administration decreases plasma concentrations of gonadotropins and prolactin,

but the role of melatonin in perturbation of hypothalamic interaction with the pituitary deserves further study.

INTRODUCTION

One of the main goals in equine science is to gain the ability to manipulate reproduction in the horse. In efforts to better understand equine reproduction, the endocrinology and predisposing environmental stimuli behind the reproductive cycle of the horse have been carefully scrutinized. Growth hormone and melatonin are two hormones possibly related to variations in the reproductive status of the horse.

With the availability of recombinant equine GH, studies were initiated to investigate the possible role of GH in the reproductive physiology of the horse. Cochran et al. (1999a, b; 2000) reported that administration of GH increased the number of small follicles on the ovaries of the cyclic mare, and the combined treatment of seasonally anovulatory mares with a subthreshold daily GnRH analog treatment resulted in follicular growth to a size that could be induced to ovulate with human chorionic gonadotropin. One explanation for these observations was that GH treatment potentiates the ovaries of the mare such that they responded to much lower gonadotropin concentrations than they normally would, perhaps through increasing the number of gonadotropin receptors.

Evidence of a relationship between photoperiod and melatonin concentrations created interest in melatonin as a possible mediator of the reproductive seasonality in the horse. During the non-breeding season, equine melatonin concentrations are the highest (Guerin et al., 1995), the stallion exhibits partial testicular atrophy, decreased libido, and decreased accessory fluid production (Thompson et al., 1977), and the mare becomes anovulatory with decreased levels of steroids and gonadotropins (Ginther, 1993). Exogenous melatonin administration is reported to decrease plasma testosterone concentrations in the stallion (Argo et al., 1991) and alter the chronological events of the breeding season in the mare (Peltier et al., 1997). The following two experiments were undertaken to assess the effects of GH administration during the nonbreeding season or melatonin administration through the summer and autumnal transition on the reproductive axis of the stallion.

CHAPTER I

REVIEW OF LITERATURE

Hypothalamic-Hypophyseal-Gonadal Axis of the Stallion

Equine reproduction is primarily controlled by the neuroendocrine system, including the hypothalamus, pituitary, and gonads. This group of organs and their hormonal interactions are collectively termed the hypothalamic-hypophyseal-gonadal axis (Amann, 1993). The hypothalamus is responsible for secretion of GnRH, the primary mediator of gonadotropin secretion. Luteinizing hormone and FSH, the gonadotropins, are generated in the adenohypophysis (glandular portion of the pituitary gland). The hypophyseal-portal system is the anatomical section of the circulatory system that links the hypothalamus and the pituitary gland and transmits their products into the peripheral circulation. Gonadotropins interact with the testes in the male to control androgen production and secretion as well as spermatogenesis. Equilibrium of the male reproductive system is maintained through the negative feedback of steroids, predominately testosterone, on the hypothalamus and pituitary (Amann, 1993; Hadley, 2000).

Gonadotropin Releasing Hormone. Gonadotropin releasing hormone is a decapeptide produced in and secreted by neurons of the hypothalamus. Once termed luteinizing hormone releasing hormone for its effects on LH secretion, it was found also to modify FSH secretion and has since been referred to as GnRH. It has an approximate half-life in the peripheral blood stream of 30 min, however this figure can vary with the amount of albumin binding that occurs in circulation (Irvine and Alexander, 1993a).

Patterns of secretion for GnRH have been difficult to study due to its highly diluted circulating levels. Peripheral plasma GnRH levels exist in immeasurable subfemtomolar concentrations, and the only measurable concentrations of GnRH are located in the median eminence of the hypothalamus. Push-pull perfusion of the mediobasal hypothalamus, a method of extracting small amounts of hypothalamic venous effluent, has been one effective means for

the study of GnRH secretory patterns (Irvine and Alexander, 1993a). Sharp and Grubaugh (1987), as well as Irvine and Alexander (1991), developed facial vein catheterization methods for sampling hypothalamic venous effluent in the horse. Sharp and Grubaugh (1987) reported GnRH pulse frequency is irregularly episodic with the most infrequent secretion occurring during periods of reproductive quiescence. Moreover, Irvine and Alexander (1991) reported breeding season GnRH secretion is continuous with superimposed pulses occurring up to twice hourly.

Immunization against GnRH, as well as treatment with GnRH or analogs, has helped to expand the understanding of how GnRH affects gonadotropin concentrations. Immunization against GnRH has been proven to lower plasma gonadotropin concentrations (Schanbacher, 1984; Garza et al., 1986). Garcia and Ginther (1975) reported that constant infusion of mares with GnRH over a 24-h period induces a steady increase in LH concentrations. In addition, pulsatile administration of GnRH (Blue et al., 1991) and implantation of GnRH agonists (Boyle et al., 1991) have elevated plasma LH concentrations in stallions. Administration of GnRH to stallions has also elicited responses in FSH and variable responses in testosterone indirectly through LH (Seamans et al., 1991; Blue et al., 1991). Furthermore, the frequency of GnRH pulses seems to be important in regulating the proportions of gonadotropins released in the mare. Gonadotropin releasing hormone pulses every 45 min seem to favor LH secretion, yet pulses every 6 h causes increased secretion of FSH (Turner and Irvine, 1991).

Luteinizing Hormone and Follicle Stimulating Hormone. The gonadotropins, LH and FSH, are glycoproteins secreted by the gonadotrope cells of the adenohypophysis. The two hormones each have an approximate molecular weight of 34,000 (Braselton and McShan, 1970). Gonadotropins consist of a species specific α subunit and a biologically active β subunit (Pierce and Parsons, 1981). Secretion of gonadotropins is generally pulsatile in nature and is primarily controlled by GnRH stimulation. The role of the gonadotropins is to stimulate steroid and gamete production in both the male and female (Irvine and Alexander, 1993b).

The two gonadotropins play separate roles in the regulation of male reproduction. Follicle stimulating hormone affects Sertoli cell function and inhibin production. Also, FSH functions along with LH-induced testosterone secretion to initiate and maintain spermatogenesis. In contrast, LH controls production and secretion of steroids by the Leydig cells and subsequently the development of secondary sex characteristics. Moreover, FSH acts synergistically with LH to increase testosterone production (Hadley, 2000).

Regulation of gonadotropin secretion is accomplished through the stimulation of GnRH and the negative feedback of hormones secreted from the testes. Individual gonadotropin responses to hypothalamic GnRH pulses allow for differences in LH and FSH plasma concentrations (Hadley, 2000). Surges in plasma concentrations of LH, as opposed to FSH, seem more often to be concurrent with GnRH pulses in the stallion (Thompson et al., 1985). Dissimilar patterns of FSH and LH concentrations may result from three distinct types of gonadotrope cells characterized in the equine pituitary (Rahmanian et al., 1998). Also, while plasma concentrations of both gonadotropins are decreased by a tonic inhibitory steroid feedback at the pituitary and hypothalamus, FSH concentrations are altered at the pituitary by inhibin and activin as well (Irvine and Alexander, 1993b).

In the mare, plasma LH and FSH concentrations follow a seasonal pattern with maximal concentrations occurring in waves during the steroid induced cyclic reproductive season (Freedman et al., 1979; Garcia and Ginther, 1979). In the stallion, plasma LH and FSH concentrations rise with the advent of the breeding season, and begin to fall again as the winter months approach (Irvine and Alexander, 1982; Johnson and Thompson, 1983). Patterns of secretion of LH and FSH in the stallion are generally not pulsatile and are tightly coupled to one another (Thompson et al., 1985).

Testosterone. Testosterone is responsible for male sexual characteristics, accessory sex gland function, and synergistic maintenance of spermatogenesis with FSH (Amann, 1993). In the stallion, steroid hormones are primarily produced by the Leydig cells of the testicular interstitium. These steroids include testosterone, progesterone, estradiol, and their derivatives.

The precursor to steroids is cholesterol. In the male, cholesterol is converted to pregnenolone by the Leydig cells of the testes. Subsequently, testosterone is derived from pregnenolone by either the delta 4 or delta 5 steroid pathway. Luteinizing hormone stimulates steroidogenesis by the Leydig cells (Amann, 1993). The possible mechanism of LH stimulation of testosterone is the increased synthesis of pregnenolone from cholesterol (Hadley, 2000). Plasma testosterone concentrations in the stallion vary according to seasonal reproductive state. Thus, plasma testosterone concentrations are higher in the summer and lower in the winter (Johnson and Thompson, 1983). Variations in concentrations of testosterone in the stallion are reported to be episodic and are not consistently exhibited with pulses in LH concentrations (Thompson et al., 1985).

Seminal and Testicular Characteristics

Seminal evaluation can be an effective method of measuring reproductive competence in the stallion. Evaluation of trends in volume, concentration, and sperm motility of semen and associating these trends with changes in endocrine patterns have expanded the understanding of stallion reproductive physiology. The stallion is a seasonal breeder exhibiting changes in seminal characteristics over the course of the year. Reproductive capacity in stallions is greatest during the summer and diminished during the winter months (Pickett et al., 1976; Thompson et al., 1977; Clay et al., 1987). Leydig and Sertoli cell numbers and testosterone concentration of the equine testes are greatest during the breeding season (Johnson and Thompson, 1983, 1987). Consequently, mean volumes of gel-free semen and gel (Thompson et al., 1977) and number of spermatozoa per ejaculate (Pickett et al., 1976) are greatest in the summer in correlation with the breeding season of the mare.

Some seminal characteristics can be manipulated by an artificial photoperiod. An increasing artificial photoperiod imposed during the winter months resulted in decreased gel-free semen and increased spermatozoa per volume of gel-free semen, but no effect was seen on number spermatozoa per ejaculate or percentage of motile spermatozoa (Thompson et al., 1977).

Others have reported increases in sperm output and total scrotal width in response to an increased artificial photoperiod during the winter months (Clay et al., 1987)

Factors other than season affect seminal characteristics. Increased total scrotal width is correlated with an increase in daily sperm output (Thompson et al., 1979). Seminal volume, largely the product of accessory sex glands, and total scrotal width tend to increase with age. Conversely, concentration and sperm motility seem to remain similar with increased age (Amann et al., 1979). Sexual arousal has also been reported to increase gel-free seminal volume (Thomson et al., 1996).

Growth Hormone

Growth hormone (GH) is a protein consisting of 191 amino acids and is secreted by the somatotrope cells of the adenohypophysis (Hadley, 2000). Plasma GH concentrations analyzed in the horse are episodic in nature with no diurnal patterns (Thompson et al., 1992; Stewart et al., 1993). Concentrations of GH have been reported to increase upon refeeding after fasting (Nadal et al., 1997), after feed restriction (Sticker et al., 1995), and after acute exercise (Thompson et al., 1994). Administration of recombinant equine GH to the horse results in hyperglycemia, hyperinsulinemia, insulin insensitivity, and mobilization of fatty acids (Smith et al., 1999). Deficiency of GH production in young growing animals results in dwarfism. Conversely, hypersecretion of GH occurring early in life results in gigantism. Acromegaly occurs with hypersecretion of GH in mature animals (Hadley, 2000). Secretion of GH from the pituitary is inhibited by somatostatin and stimulated by GH releasing hormone (Hadely, 2000). Growth hormone response to GH releasing hormone administration has been reported in the horse (Thompson et al., 1994). These regulatory hormones are generated in the hypothalamus. Somatostatin is released in response to negative feed back by high levels of insulin-like growth factor I (IGF-I; Hadley, 2000). Furthermore, chronic administration of GH in the horse inhibits the endogenous GH response to secretagogue (Smith et al., 1999).

Many physiological effects of GH are carried out indirectly through somatomedin secretion by the liver (Hadley, 2000). Insulin-like growth factor-I is the prime somatomedin of

study. Growth hormone and IGF-I play a role in gonadal function of various species. Growth hormone deficient humans and those immunized against GH have experienced delayed puberty and lack of response to human chorionic gonadotropin. In contrast, GH deficient children have experienced accelerated pubertal maturation after GH treatment (Kulin et al., 1981). Similarly, immunization of heifers against GH releasing factor results in delayed puberty (Cohick et al., 1996). In dwarf mice, GH deficiency prevents full differentiation of Leydig and Sertoli cells (Hochereau-de Reviers et al., 1987). Growth hormone and IGF-I receptors have been identified in the Leydig and Sertoli cells of rat testes (Smith et al., 1987). In addition, elevated IGF-I levels have been linked to increased steroidogenesis in response to LH in mice (Chatelain et al., 1991). Mediation of testicular response to LH is attributed to GH in humans (Kulin et al., 1981) and experimental mice (Chatelain et al., 1991).

When recombinant equine GH became available, studies were conducted on the reproductive effects of GH administration to horses. Cochran et al. (1999b) reported that administration of recombinant equine GH increased the number of small follicles on the ovaries of the cyclic mare. Similarly, treatment of seasonally anovulatory mares with GH resulted in an increased number of small follicles not large enough to ovulate (Cochran et al., 1999a). However, the combined treatment of seasonally anovulatory mares with a GH and subthreshold daily GnRH analog treatment (Cochran et al., 2000) resulted in follicular growth to a size that could be induced to ovulate with human chorionic gonadotropin. Hence, GH treatment possibly potentiates the ovaries of seasonally anovulatory mares allowing an ovarian response to much lower gonadotropin concentrations than normal. One explanation for such a response is the up-regulation (increase in number) of gonadotropin receptors on the ovaries.

Photoperiod and Melatonin

Photoperiod. In a variety of species, reproduction is physiologically confined to a certain period of the year to ensure that the progeny will be born during optimum environmental conditions. Long day breeders including the hamster, brown bear, and horse experience diminished reproductive capacity during the decreased photoperiod of the winter months,

whereas, short day breeders, such as the sheep, are positively affected during the same period (Gerlach and Aurich, 2000). The predominating environmental factor regulating seasonal trends in reproduction is photoperiod in both long and short day breeders alike (Gerlach and Aurich, 2000).

The horse exhibits increased reproductive capacity during the summer in response to increased photoperiod. Sharp and Ginther (1975) exposed pony mares to either prevailing photoperiod from October to February or an artificial photoperiod equivalent to transition from March to July. Mares under increasing photoperiod exhibited increased number and size of follicles when compared to those under ambient conditions. This hastening of the onset of the ovulatory season in response to an artificially increased photoperiod has been consistently reported (Kooistra and Ginther, 1975; Freedman et al., 1979). The stallion also exhibits seasonal changes with increased total scrotal width and sexual behavior (Clay and Clay, 1992).

Response to photoperiod is also reflected by the patterns in hormonal secretion over the duration of the year. In the stallion, plasma LH concentrations rise at the advent of the breeding season, and begin to fall again as the winter months approach (Irvine and Alexander, 1982; Johnson and Thompson, 1983). Stallions under an artificial photoperiod of increasing daylight during the winter months experienced an accelerated seasonal increase in LH concentrations and increased plasma testosterone concentrations (Thompson et al., 1977). Likewise, mares exhibit increased plasma LH concentrations during the spring and summer (Freedman et al., 1979), and similar to the stallion, mares exposed to constant light for 28 d during the winter experienced increased daily plasma LH concentrations (Cleaver et al., 1991). Annual profiles of plasma FSH and testosterone concentrations in the stallion fluctuate similarly to those of LH but with smaller magnitude of change (Clay and Clay, 1992).

Photorefractoriness plays a role in the seasonal changes in the equine reproductive state. Clay and Clay (1992) give a good description of the photorefractoriness in the stallion. Stallions chronically exposed to an increased artificial photoperiod during the winter initially respond with a short-term testicular recrudescence, but then become refractory and return to the state of

untreated stallions. This implies that premature induction of sexual recrudescence results in a premature reproductive quiescence, or perhaps, photorefractoriness is the cause for normal regression at the advent of autumnal transition. Thus, the short days at the onset of winter may not be the stimulus for autumnal regression, but perhaps a period of lessened photostimulation in which stallions regain sensitivity to long days.

Melatonin. Melatonin, an indole amine, was first isolated from bovine pineal tissue and is secreted by the pineal gland of nearly all mammals (Reiter, 1981; Grubaugh et al., 1982; Gerlach and Aurich, 2000). Melatonin secretion by the equine pineal gland was determined to be circadian in nature with elevated levels occurring during the night (Kilmer, 1982; Guerin et al., 1995). In addition, the release of melatonin from the pineal gland is negatively influenced by photoperiod in rats (Quay, 1963; Wurtman et al., 1964), sheep (Rollag and Niswender, 1976; Karsch et al., 1986), and cattle (Sanchez-Barcelo et al., 1991). Melatonin secretion, therefore, provides a physiological unambiguous circannual rhythm with longest periods of secretion in the winter (long nights) and shortest periods of secretion in the summer (short nights).

Synthesis of melatonin in the mammalian pineal gland is the result of the precursor tryptophan being converted to serotonin and then to melatonin through a series of steps. Two enzymes involved in melatonin synthesis are hydroxyindole-O-methyl-transferase and the rate-limiting factor, N-acetyltransferase (Reiter, 1981). Hydroxyindole-O-methyl-transferase activity in the horse is highest in the fall and winter and lowest during the spring and summer. In addition, the percentage of ovulatory mares is inversely related to the amount of hydroxyindole-O-methyl-transferase activity (Wesson et al., 1979).

Reiter (1981) describes the route of innervation for photoregulation of the pineal gland. As light strikes the retina of the eye, a neural signal travels via the optic nerves to the suprachiasmatic nuclei. Optic nerves synapse with neurons of the suprachiasmatic nuclei leading to superior cervical ganglia. Finally, melatonin secretion by the mammalian pineal gland is inhibited by the post-ganglionic neurons diverging from the superior cervical ganglia. Interrupting this pathway through bilateral superior cervical ganglionectomy of pony mares

during winter anestrus results in delay of the second ovulatory season (Sharp et al., 1979). This is consistent with the “zeitgeber” phenomenon of free-running rhythm after the loss of an environmental time-keeping factor (Sharp and Cleaver, 1993). Similarly, pinealectomy of mares resulted in delay of the first ovulation for the second post-operative season and abolished the response to a stimulatory artificial photoperiod (Grubagh et al., 1982). Thus, the effect of melatonin on the reproductive axis may be to synchronize the endogenous physiological status of the horse to changes in photoperiod.

Short-day breeders, such as sheep, exhibit an increase in reproductive capacity in response to melatonin administration. Luteinizing hormone, FSH, and testicular function have been reported to increase in response to melatonin treatment in the ram (Lincoln and Maeda, 1992). Pinealectomized ewes treated with a short-day regimen of melatonin exhibit an initial increase in plasma LH concentrations (Karsch et al., 1986). Also, melatonin administration advances the onset of cyclic activity in lambs (Nowak et al., 1990).

Administration of melatonin to long-day breeders in a manner that mimics short days results in adverse effects on sexual competence. In the hamster, melatonin administration induces regression of the testes (Carter and Goldman, 1983) and suppresses plasma LH concentrations (Reiter, 1981). Similarly, the mare experiences a delay of first ovulation of the year after melatonin treatment (Guillaume and Palmer, 1991). Furthermore, exogenous melatonin administration decreased plasma testosterone concentrations in the stallion (Argo et al., 1991) and altered the chronologic events of the breeding season in the mare (Peltier et al., 1998). Plasma melatonin concentrations in ovariectomized pony mares under constant light initially decrease (Cleaver et al., 1991). Accordingly, melatonin administration to mares has been reported to decrease plasma prolactin concentrations (Aurich et al., 1997; Fitzgerald et al., 2000). Thus, a possible suppression of gonadotropin activity may be attributed to periods of increased melatonin secretion in the horse.

Receptors for melatonin have been characterized in the hypothalamus and pituitary of the rabbit, sheep (Stankov et al., 1991), cattle, donkey (Nonno et al., 1995), and horse (Stankov et

al., 1991; Nonno et al., 1995). With only slight differences between species, minute melatonin receptor concentrations exist in the mediobasal portion of the hypothalamus and increase in number distally to the pars tuberalis, pars distalis, and pars intermedia. The location of melatonin receptors in the hypothalamus is coincident with the equine median eminence, the area of highest GnRH concentrations (Strauss et al., 1979; Cleaver et al., 1991). In addition, the pars distalis is the location of equine gonadotrope cells (Rhamanian et al., 1998). The localization of melatonin receptors in the proximity of the hypothalamic-hypophyseal axis points to a possible interaction of melatonin in regulation of reproduction.

Prolactin

Prolactin is a 199 amino acid protein very similar in composition to GH, with an approximate molecular weight of 25,000, and is produced in the lactotropes of the adenohypophysis (Hadley, 2000). Equine prolactin secretion is reported as episodic in nature (Roser et al., 1987). In the horse, plasma prolactin concentrations increase in late pregnancy (Ginther, 1993) and are highest during the breeding season; they fall in winter and are correlated with both photoperiod and temperature (Johnson, 1986; Thompson and Johnson, 1987). However, increasing the photoperiod during the winter reproductive quiescence hastens the onset of high summer prolactin concentrations (Johnson, 1987). Plasma prolactin concentrations have also been reported to increase with age in horses (Thompson and Johnson, 1987).

Effects on a variety of physiological functions have been attributed to prolactin. In certain species, prolactin exhibits tropic effects on mammary glands, sebaceous glands, luteal tissue, and pelage (Hadley, 2000). Prolactin is likely responsible for light- and temperature-induced hair shedding in the horse (Kooistra and Ginther, 1975; Sharp and Ginther, 1975; Thompson et al., 1997) and in the sheep (Lincoln and Tortonesi, 1995). Also, plasma prolactin concentrations have been reported to increase with feeding (Sticker et al., 1995; Nadal et al., 1997), exercise, stress (Colborn et al., 1991a,b), and sexual stimulation (Colborn et al., 1991a,b; Thomson et al., 1996).

Regulation of prolactin secretion is achieved through inhibition by dopamine from the hypothalamus (Hadely, 2000). In the horse, administration of dopamine antagonists increase plasma prolactin concentrations (Colborn et al., 1991b; Nequin et al., 1993), and dopamine agonists exert inhibitory effects on prolactin concentrations (Thomson et al., 1996). Dopamine pathways have also been reported to be involved in prolactin synthesis in the ram (Lincoln and Tortonese, 1995). Though the existence of a true prolactin releasing hormone has not been elucidated, TRH has been reported to increase plasma prolactin concentrations (Johnson, 1986; 1987; Colborn et al., 1991b; Gentry et al., 2002). The response of prolactin to TRH administration is affected by season, with greater responses occurring in the summer (Johnson, 1987). In addition, TRH has been reported to bind directly to receptors on the lactotrope cells (Gerschegorn et al., 1979).

Prolactin may be a melatonin-mediated regulator of reproductive competence in seasonal species. Melatonin implants in the mediobasal hypothalamus of the ram caused a significant increase in prolactin (Lincoln and Maeda, 1992). Prolactin was also reported to increase in correlation with increased follicular activity and increased plasma LH concentrations in the mare (Nequin et al., 1993). Prolactin concentrations are significantly lower during the non-breeding season, the duration of highest plasma melatonin concentrations, than during the breeding season of the horse (Thompson et al., 1986; 1987). Nequin et al. (1993) reported that administration of a dopamine antagonist or exogenous prolactin during anestrus increased follicular activity on the ovaries of mares. Correspondingly, melatonin administration to mares decreases plasma prolactin concentrations during the reproductive season (Aurich et al., 1997; Fitzgerald et al., 2000), though the prolactin response to melatonin administration may be less sensitive during the winter (Fitzgerald et al., 2000).

Rationale for Present Experiments

The effects of GH on reproduction are apparent in various mammalian species (Kulin et al., 1981; Chatelain et al., 1991; Cohick et al., 1996). However, there is a lack of information on the effects of GH administration on reproduction in the horse. Recently, with the availability of

equine somatotropin, research endeavors have been undertaken to assess the role of GH in equine reproduction. Studies previously conducted in mares (Cochran et al., 1999a, b; 2000) indicated that GH administration increased the number of small ovarian follicles in cycling and anovulatory mares, and possibly increased ovarian sensitivity to gonadotropins. Given the nature of effects resulting from GH administration in the mare, a similar testicular response to GH is plausible.

Melatonin is another hormone implicated as a mediator of reproduction in the horse (Argo et al., 1991; Aurich et al., 1997; Peltier et al., 1997). Exogenous melatonin administration is reported to decrease plasma testosterone concentrations in the stallion (Argo et al., 1991) and alter the chronologic events of the breeding season in the mare (Peltier et al., 1997). Melatonin administration to mares has also been reported to decrease plasma prolactin concentrations (Aurich et al., 1997; Fitzgerald et al., 2000). As with GH administration, studies concerning the effects of melatonin administration on the reproductive function in the stallion are lacking.

Given the nature of GH and melatonin as mediators of reproductive function, the following two experiments were undertaken to assess the effects of GH administration during the non-breeding season or melatonin administration through the summer and autumnal transition on reproduction related hormonal and seminal characteristics of the stallion.

CHAPTER II

EFFECTS OF GROWTH HORMONE TREATMENT ON HORMONAL AND SEMINAL CHARACTERISTICS IN STALLIONS

Introduction

Growth hormone is reported to alter the reproductive physiology of humans (Kulin et al., 1981), mice (Chatelain et al., 1991), and cattle (Cohick et al., 1996), and with the availability of recombinant equine GH, understanding of the effects of GH administration on equine reproduction is currently developing. Cochran et al. (1999b) reported that administration of recombinant equine GH increased the number of small follicles on the ovaries of the cyclic mare. Similar treatment of seasonally anovulatory mares resulted in an increase in number of small follicles (Cochran et al., 1999a) but did not result in follicles large enough to ovulate. However, the combined treatment of seasonally anovulatory mares with a subthreshold daily GnRH analog treatment (Gentry et al., 1999) and GH resulted in follicular growth to a size that could be induced to ovulate with human chorionic gonadotropin (Cochran et al., 2000). One explanation for this latter observation was that the GH treatment potentiated the ovary of the seasonally anovulatory mares such that they responded to much lower gonadotropin concentrations than they normally would, perhaps via increasing the number of gonadotropin receptors on the ovary. The purpose of this experiment was to determine if similar daily GH administration to stallions would increase the testosterone response to a minimal dose of LH and to assess the subsequent effects of combined injections of GnRH and TRH.

Materials and Methods

Nine adult, light horse stallions were maintained in similar outdoor paddocks and fed daily hay and grain rations. Stallions were randomly assigned to GH-treated (n = 5) or control (n = 4) groups. Growth hormone-treated stallions were administered recombinant equine GH (BresaGen, Ltd., Adelaide, Australia) at 20 µg/kg BW as an i.m. injection daily for 21 d beginning in January and control stallions were administered saline in a similar manner. Blood

was collected via jugular venipuncture into heparinized tubes every 48 h at dawn prior to daily injections. On d 15 of GH treatment, frequent (every 30 min) blood samples were collected via indwelling jugular catheters from all stallions for a 6-h period following the daily treatments to assess the resting plasma concentrations of LH, FSH, and testosterone. Immediately after the 6-h sample was drawn, all stallions were administered a standard, “low” dose LH injection (0.2 μg LH/kg BW i.v.) to assess the testosterone responsiveness of the testes. The dose of LH used was determined in preliminary trials to produce an LH rise similar to naturally occurring, endogenous rises in stallions. On d 17 of treatment, a low dose GnRH/TRH combination (0.1 μg GnRH and 0.4 μg TRH/kg BW i.v.) was administered to all stallions to assess the pituitary responsiveness to these secretagogues. Subsequently, a high dose of LH (0.3 μg LH/kg BW i.v.) and a high dose GnRH/TRH combination (1.0 μg GnRH and 4.0 μg TRH/kg BW i.v.) were administered on d 19 and 21, respectively. Frequent blood sampling was conducted via indwelling jugular catheters around all secretagogue injections at -15, 0, 5, 10, 20, 30, 60, 90, 120, 150, 180, 210, and 240 min relative to treatment.

Blood collected during frequent and daily sampling was immediately centrifuged, and plasma was harvested and stored at -15°C until assay. All samples were analyzed by radioimmunoassay as previously described for LH (Thompson et al., 1983a), FSH (Thompson et al., 1983b), prolactin (Colborn et al., 1991), and testosterone (Diagnostic Systems Laboratories, Webster, TX). Daily blood samples were also analyzed for IGF-I (Sticker et al., 1995) and GH (Thompson et al., 1992) concentrations.

Two months after the onset of GH treatment, semen was collected from all stallions by means of an artificial vagina every 2 d for 2 weeks. Each collection was evaluated for length of time from arousal until ejaculation, number of mounts per ejaculate, gel-free seminal volume, volume of gel, concentration of spermatozoa per ejaculate, total spermatozoa per ejaculate, motility, and morphology (percentages of live/dead, normal, head abnormalities, tail abnormalities, mid-piece abnormalities, protoplasmic droplets).

Data were analyzed using the GLM procedures of SAS (SAS Inst. Inc., Cary, NC). Daily hormonal concentrations were analyzed by split-plot ANOVA (Gill and Hafs, 1971) for the effects of treatment, day, and treatment by day interactions. Hormonal concentration data from the period of frequent sampling and around the challenges were analyzed by ANOVA (Gill and Hafs, 1971) for the effects of treatment, minute, and treatment by minute interactions. Data at the seminal collections were also evaluated by split-plot ANOVA (Gill and Hafs, 1971) for the effects of treatment, day, and treatment by day interactions.

Results

Hormonal Data. Treatment with recombinant equine GH increased ($P < 0.001$) plasma IGF-I concentrations in samples collected every 48 h; plasma GH concentrations in the same samples were similar ($P > 0.1$) for the two groups through d 13, but diverged thereafter (Figure 2.1). Also, no treatment effect ($P > 0.1$) was observed for concentrations of testosterone in 48 h blood samples or during frequent blood collections on d 15 (Figure 2.2). Similarly, basal concentrations of LH and FSH during the period of frequent sampling on d 15 were not affected ($P > 0.1$) by treatment, however, treated stallions had greater ($P > 0.001$) GH concentrations for the duration of the sampling period (Figure 2.3). Furthermore, the testosterone concentrations in samples collected around both the low and high doses of LH and GnRH were virtually identical ($P > 0.1$) for the two groups of stallions (Figure 2.4), although testosterone concentrations for both groups increased over time ($P < 0.001$) in response to secretagogue administration. Likewise, the LH, FSH, and prolactin responses to combined GnRH and TRH administration at both the low and high doses, on d 17 (Figure 2.5) and d 21 (Figure 2.6) respectively, increased over time ($P < 0.001$) but were not different ($P > 0.1$) between groups.

Seminal Data. When semen was collected from these stallions 2 months later, GH-treated stallions had higher ($P < 0.001$) volume of gel-free semen, higher ($P < 0.05$) volume of gel, and decreased ($P < 0.05$) time until ejaculation; no other differences ($P > 0.05$) between groups were observed (Table 2.1).

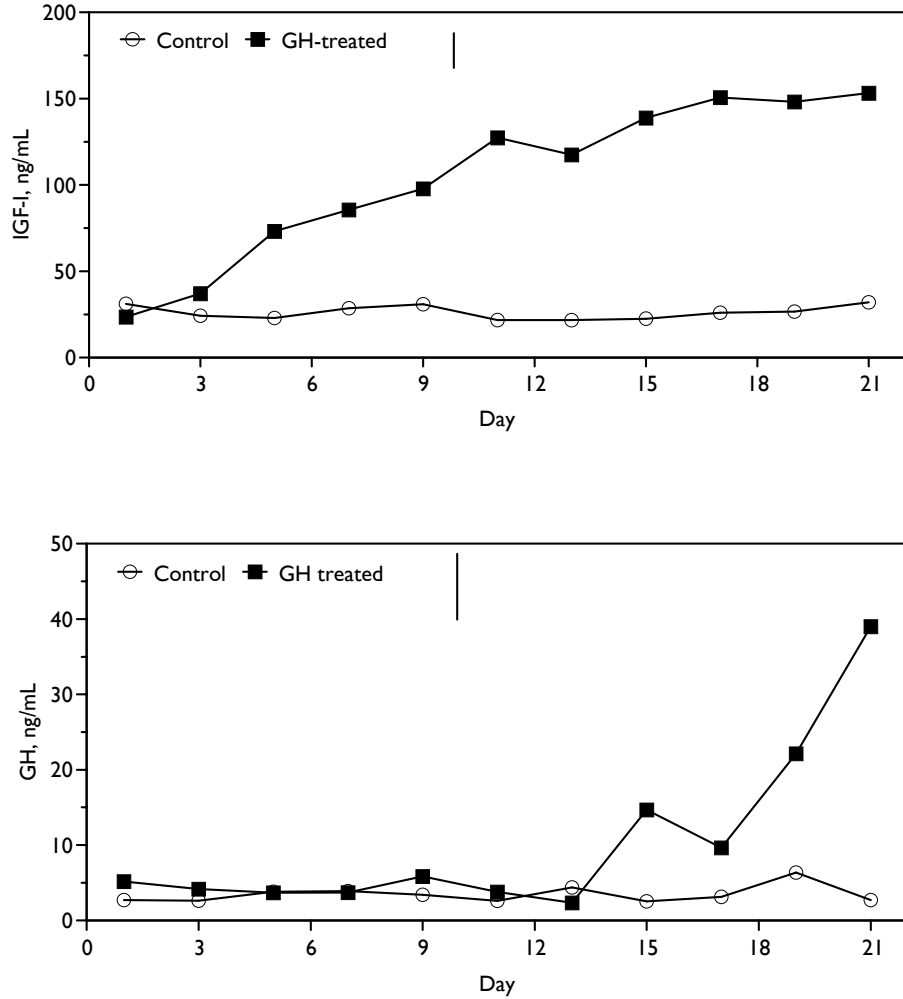


Figure 2.1. Mean plasma concentrations of GH and IGF-I for samples collected every 48 hr in response to daily GH (GH-treated) or saline (Control) administration. There was a main effect of treatment ($P < 0.001$) as well as a treatment by time interaction ($P < 0.001$) for plasma concentrations of IGF-I. The least significant difference (LSD) for comparison of means is indicated by the vertical bar.

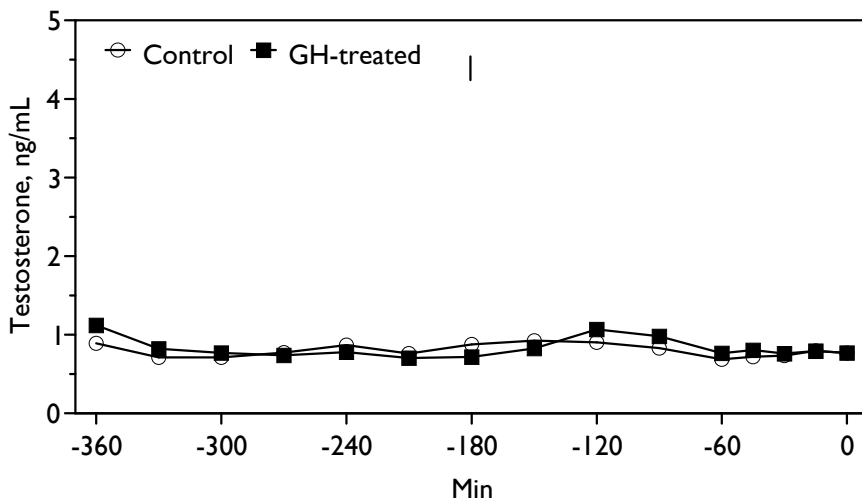
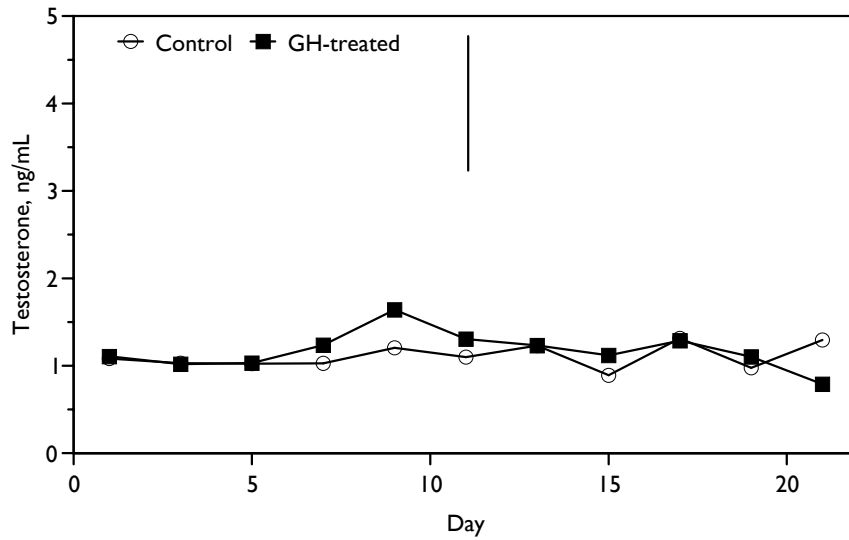


Figure 2.2. Mean plasma concentrations of testosterone for every 48 hr blood sampling and frequent blood collection on d 15 after treatment with daily GH (GH-treated) or saline (Control). No effect ($P > 0.1$) of treatment was observed. The least significant difference (LSD) for comparison of means is indicated by the vertical bar.

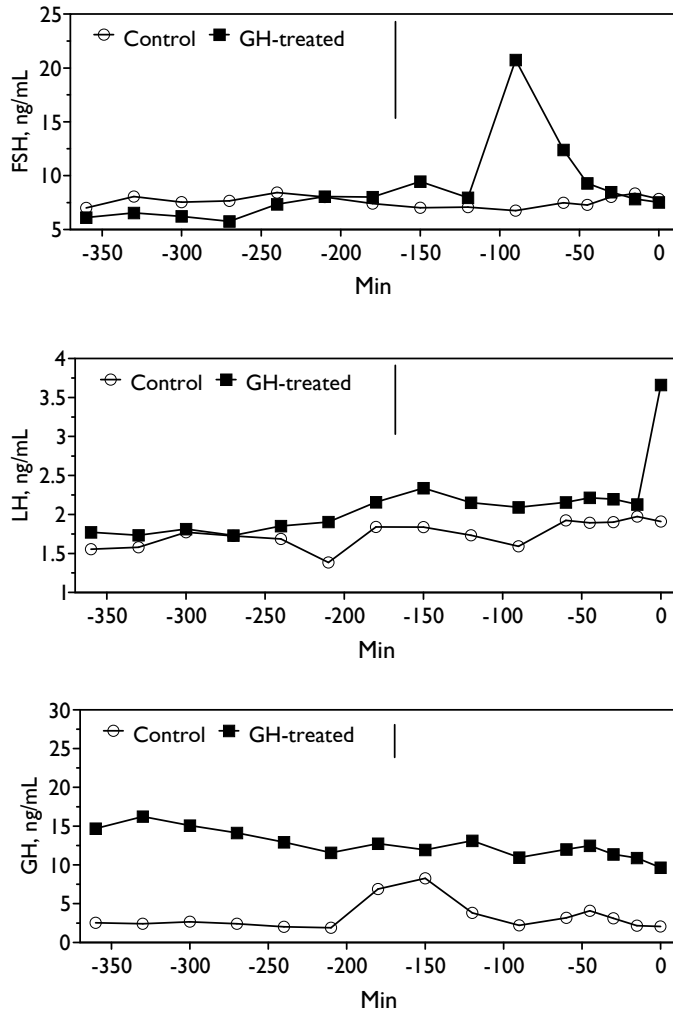


Figure 2.3. Mean plasma concentrations of FSH, LH, and GH during frequent blood sampling on d 15 after daily treatment with GH (GH-treated) or saline (Control). There was a main effect of treatment ($P < 0.001$) for plasma GH concentration. The least significant difference (LSD) for comparison of means is indicated by the vertical bar.

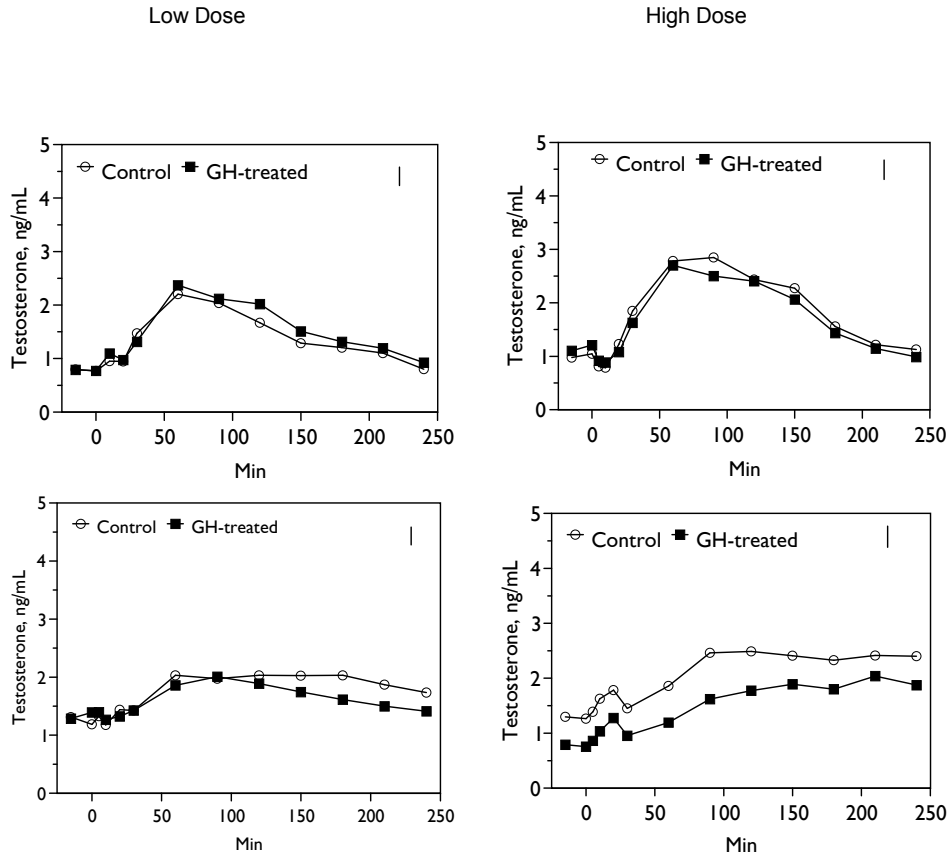


Figure 2.4. Mean plasma concentrations of testosterone in GH treated and control stallions relative to administration of low and high doses of LH (top) or GnRH (bottom) administration at time 0. No effect ($P > 0.1$) of treatment was observed. The least significant difference (LSD) for comparison of means is indicated by the vertical bar.

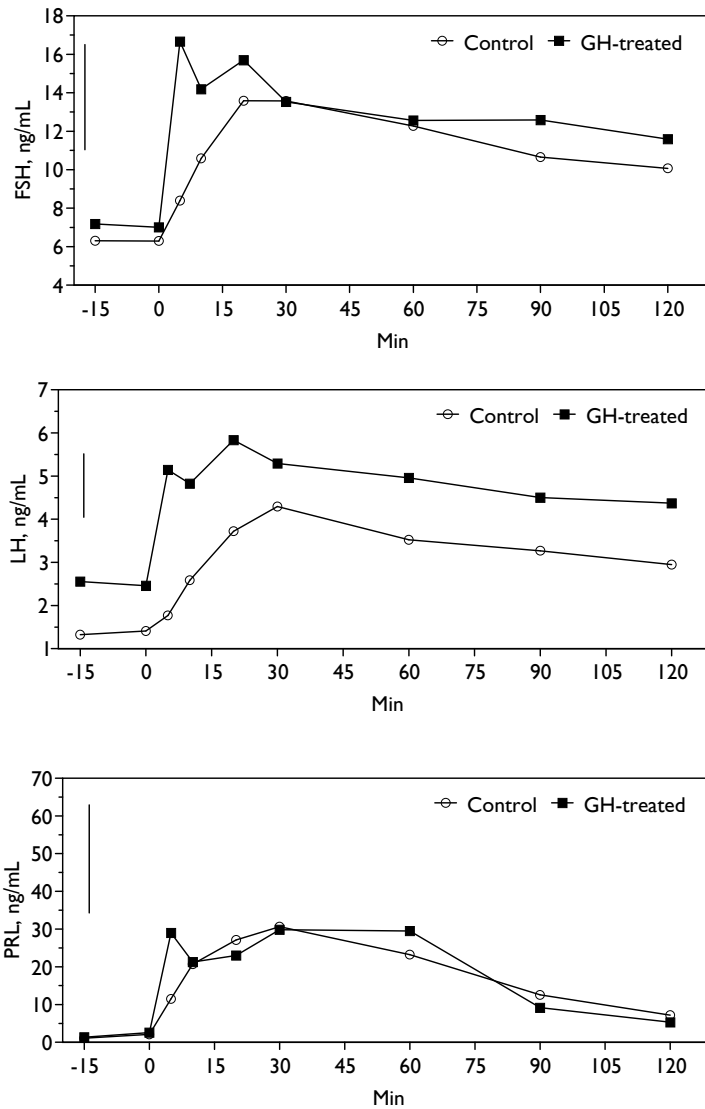


Figure 2.5. Mean plasma concentrations of FSH, LH, and prolactin in GH treated and control stallions after a low dose administration of GnRH/TRH at time 0. No effect of treatment ($P > 0.1$) was observed. The least significant difference (LSD) for comparison of means is indicated by the vertical bar.

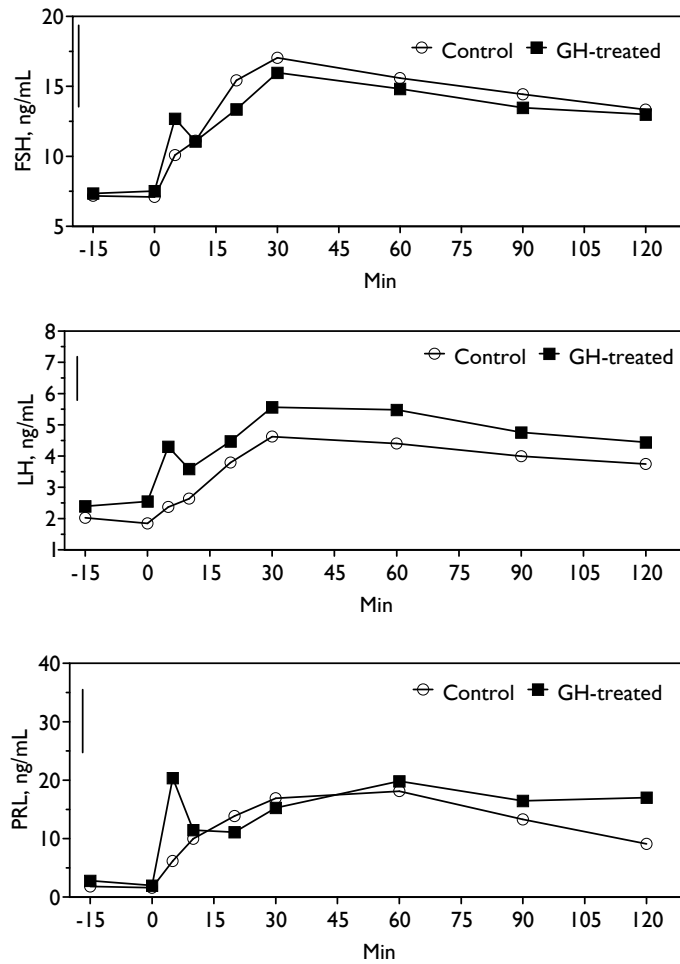


Figure 2.6. Mean plasma concentrations of FSH, LH, and prolactin in GH treated and control stallions after a high dose administration of GnRH/TRH at time 0. No effect of treatment ($P > 0.1$) was observed. The least significant difference (LSD) for comparison of means is indicated by the vertical bar.

Table 2.1 Semen collection data for control and GH-treated stallions expressed as mean \pm SEM.

	Control	GH
Number of Mounts	1.3 +/- 0.28	1.5 +/- 0.25
Gel-Free Volume, ml*	83.1 +/- 7.3	37.4 +/- 6.6
Gel Volume, ml*	20.1 +/- 6.4	2.7 +/- 5.7
Concentration, millions/ml	140 +/- 35.4	270 +/- 31.6
Total Sperm/Ejaculate, billions	11.2 +/- 2.0	8.4 +/- 1.8
Motility, %	61.7 +/- 8.2	60.5 +/- 7.36
Ejaculation Time, min*	2.5 +/- 1.1	5.1 +/- 0.9
Live spermatozoa, %	85.4 +/- 2.9	84.1 +/- 2.6
Normal spermatozoa, %	72 +/- 3.6	70.35 +/- 3.2
Head Abnormalities, %	13.96 +/- 2.8	13 +/- 2.5
Tail Abnormalities, %	8.4 +/- 2.0	10.9 +/- 1.8
Midpiece Abnormalities, %	4.72 +/- 1.8	4.8 +/- 1.6
Distal Droplet, %	0.92 +/- 0.8	0.95 +/- 0.7

Characteristics with superscript (*) differ ($P < 0.05$) between treatments.

Discussion

As in previous experiments (Cochran et al., 1999b; Smith et al., 1999), administration of equine GH increased daily plasma IGF-I concentrations in treated stallions, confirming the biological activity of the injected hormone. Also, frequent sampling on d 15, subsequent to daily GH administration, verified the maintenance of high levels of GH for a minimum of 6 h after injection. These elevated plasma GH concentrations returned to levels of controls by the following 48 h sample, demonstrated by the similar plasma concentrations in daily samples. However, in spite of high circulating levels of IGF-I and GH in treated stallions, plasma testosterone concentrations in samples collected every 48 h were similar to those of controls.

All stallions exhibited testosterone responses to the low and high doses of LH used in the challenges on d 15 and 19, however no alteration in response was observed due to previous GH treatment. If the number of LH receptors in the testes had been increased by GH treatment, a greater sensitivity of the testis to the small LH challenge (i.e., more testosterone secreted) would have been expected. The similar response in plasma testosterone concentrations indicates that there was likely no alteration in testis sensitivity, thus no alteration in LH receptor numbers. Certainly other explanations exist, and direct measurement of LH receptor content in the testes would better test the receptor-number hypothesis. These results in stallions differ from the results of others studying dwarf mice (Chatelain et al., 1991) and prepubertal boys (Kulin et al., 1981), in which treatment with GH or IGF-I resulted in an increase in the testosterone response to human chorionic gonadotropin. The low dose injection of LH in the current experiment was devised to avoid the possibility of “overwhelming” the testes with higher doses of LH or human chorionic gonadotropin commonly used in diagnostic testing in stallions.

In addition to the lack of perturbation of testosterone response to LH, there was no alteration in the LH or FSH response to GnRH in either challenge due to GH treatment or in the basal concentrations of LH, FSH, and testosterone during the period of frequent sampling. Thus, exogenous GH treatment does not appear to alter the pituitary sensitivity to GnRH during the stallion nonbreeding season.

Exogenous GH has been reported to enhance spermatogenesis in boars (Deaver and Bryan, 1999). Our assessment of total spermatozoa per ejaculate in these stallions indicated no difference between treatments. Administration of GH to castrated mice did not affect accessory sex gland weights (Keenan and Thomas, 1975). The increases in volume of gel-free semen and gel in the stallions in this experiment may indicate a long-term effect on accessory sex gland activity. These observations, as well as the possible effect on sexual behavior (decreased time to ejaculation), deserve further study.

Previous experiments reported the existence of mammosomatotropes, cells staining for both prolactin and growth hormone, in the equine pituitary (Rahmanian et al., 1997). Therefore, frequent sampling periods were conducted around TRH administration to analyze the effects of a possible concurrent inhibition of prolactin and GH in response to prior exogenous GH treatment (Smith et al., 1999). In both groups, administration of TRH resulted in an immediate increase in plasma prolactin concentrations at both high and low doses. This is consistent with previous reports on TRH administration (Gentry et al., 2002). However, the similarity in response exhibited between the two groups is contradictory to a concurrent inhibition of prolactin and GH at the mammosomatotropes. These data suggest that the mechanism controlling prolactin response to TRH is unassociated with the inhibition of endogenous GH concentrations following administration of exogenous GH administration.

In summary, no direct effect of exogenous GH administration on plasma concentrations of LH, FSH, and testosterone in the stallion were observed under the present experimental design. Furthermore, GH treatment did not affect the ability of TRH to stimulate plasma concentrations of prolactin. Therefore, the possible role of GH in maintenance of reproduction in the stallion may be confined to a long-term increase in the activity of the accessory sex glands.

CHAPTER III

EFFECTS OF MELATONIN TREATMENT ON HORMONAL AND SEMINAL CHARACTERISTICS IN STALLIONS

Introduction

One of the main goals in equine science is to manipulate the seasonal reproduction of the horse. Photoperiod was discovered to be a key player in the reproductive recrudescence in the horse. Increased daily light exposure has been shown to hasten the onset of the ovulatory season in pony mares (Sharp and Ginther, 1975) and increase mean LH and testosterone levels in stallions (Thompson et al., 1977). In elucidating the mechanism for photoperiod as a seasonal regulatory stimulus, the hormone melatonin became implicated as a link between neural and endocrine pathways. Melatonin secretion by the equine pineal gland is circadian in nature with elevated levels occurring during the night (Kilmer et al., 1982; Sharp, 1982). As recognition of the relationship between photoperiod and melatonin secretion progressed, the potential for melatonin mediation of the reproductive seasonality in the horse became evident. Subsequently, exogenous melatonin administration was shown to decrease plasma testosterone concentrations in the stallion (Argo et al., 1991) and alter the chronologic events of the breeding season in the mare (Peltier et al., 1997). Furthermore, melatonin receptor characterization within the equine pituitary indicates a possible direct interaction of melatonin at the hypothalamic-hypophyseal axis (Stankov et al., 1991). There is still much to learn about the involvement of melatonin as a mediator of seasonality in the horse. The present experiment was designed to assess the effects of an imposed regimen of melatonin that mimics the short days of winter on plasma concentrations of prolactin, the response of testosterone to exogenous LH, and the response of LH and FSH to exogenous GnRH in the stallion.

Materials and Methods

Nine adult, light horse stallions were used in this experiment. All stallions were maintained in similar outdoor paddocks and fed daily grain and hay rations. Stallions were

randomly assigned to melatonin treated (n = 5) or control (n = 4) groups. Beginning in July, all stallions were given 400 g sweet feed at 1400 daily for 90 d with treated stallions receiving melatonin (0.06 mg/kg BW) in 5 ml corn syrup top-dressing and controls receiving corn syrup top-dressing alone. Blood samples were taken twice weekly 30 min prior to treatment via jugular venipuncture into heparinized tubes with two consecutive daily samples taken prior to the initiation of treatment. On d 2 of treatment, frequent blood samples were collected from all stallions hourly for 24 h starting at 0800, and at 30 min intervals for the first 2 h following melatonin treatment to characterize the plasma hormone concentrations in response to oral melatonin administration. Low dose (0.2 µg/kg BW i.v.) and high dose (0.3 µg/kg BW i.v.) LH injections were administered to all stallions on d 68 and 73 respectively. Also, low dose (0.1 µg GnRH and 0.4 µg TRH/kg BW i.v.) and high dose (1.0 µg GnRH and 4.0 µg TRH/kg BW i.v.) GnRH/TRH combination injections were administered on d 70 and 75, respectively. Frequent blood samples were collected around secretagogue infusions via indwelling jugular catheter into heparinized tubes at -20, -10, 0, 10, 20, 30, 45, 60, 90, 120, 150, 180, 210, and 240 min relative to treatment..

On d 83, 84, and 85 of melatonin treatment, semen was collected from all stallions via artificial vagina and was evaluated for total volume, volume of gel, gel-free volume, concentration of spermatozoa per ml of gel-free volume, time until ejaculation, and number of mounts.

Blood collected during frequent and daily sampling was immediately centrifuged, and plasma was extracted and stored at -15°C until assay. All samples were analyzed by radioimmunoassay as previously described for LH (Thompson et al., 1983a), FSH (Thompson et al., 1983b), prolactin (Colborn et al., 1991), and testosterone (Diagnostic Systems Laboratories, Webster, TX).

Data were analyzed using the GLM procedures of SAS (SAS Inst. Inc., Cary, NC). Daily hormonal concentrations were analyzed by split-plot ANOVA (Gill and Hafs, 1971) for the effects of treatment, day, and treatment by day interactions. Hormonal concentration data

from the period of frequent sampling and around the challenges were analyzed by ANOVA (Gill and Hafs, 1971) for the effects of treatment, minute, and treatment by minute interactions. Data at the seminal collections were also evaluated by split-plot ANOVA (Gill and Hafs, 1971) for the effects of treatment, day, and treatment by day interactions.

Results

Hormonal Data. Treatment with melatonin decreased ($P < 0.001$) daily plasma concentrations of prolactin (Figure 3.1). Also, prolactin levels decreased ($P < 0.05$) over time in both groups as expected with the change in season. Melatonin treated stallions exhibited decreased ($P < 0.05$) prolactin response to the high dose combination of GnRH and TRH (Figure 3.2), yet there was no difference ($P > 0.05$) in the prolactin response between groups to the combination injection at the low dose. In both challenges, however, there was an increase ($P < 0.001$) in plasma prolactin concentrations over time.

Plasma concentrations of LH and FSH in daily samples decreased over time ($P < 0.001$) as expected with the change in season (Figure 3.3). Treatment with melatonin did not affect ($P > 0.05$) daily LH plasma concentrations over the 90-d period, however the plasma LH concentrations tended to be greater in control stallions ($P = 0.07$) during the first 30 d of treatment. After the initial 30-d suppression, the plasma concentrations of LH regained similarity between groups. Conversely, melatonin treatment was coincident with a significant decline ($P < 0.001$) in daily plasma concentrations of FSH. Administration of the high and low dose combination injections of GnRH and TRH increased ($P < 0.001$) plasma concentrations of LH and FSH (Figure 3.4). Melatonin treatment altered ($P < 0.001$) the response in plasma FSH concentrations to the low dose GnRH/TRH combination injection, yet the responses in plasma FSH concentrations at the high dose GnRH/TRH injection were similar ($P > 0.05$) for the two groups. No treatment by time interaction ($P > 0.05$) was observed for LH plasma concentrations at the high dose GnRH injection, yet plasma LH concentrations tended to be higher ($P < 0.1$) in control stallions during the low dose administration of GnRH.

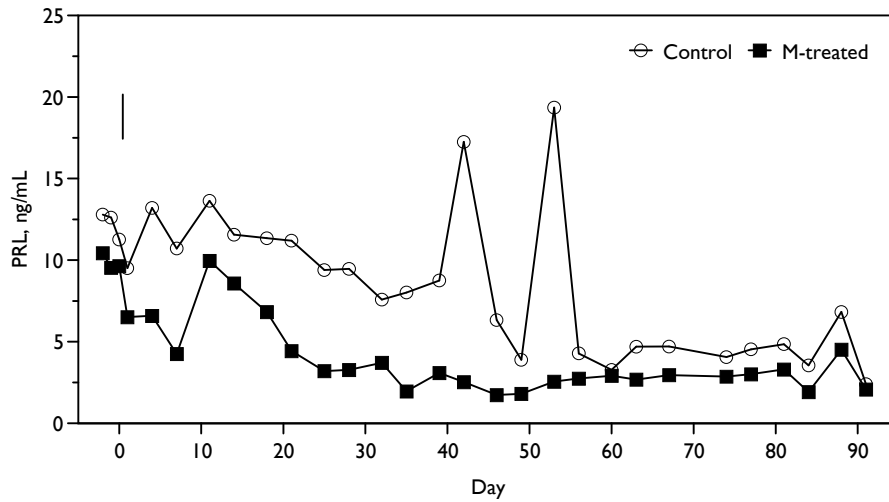


Figure 3.1. Mean plasma concentrations of prolactin in biweekly blood samples collected from melatonin (M) treated and control stallions. There was a treatment by time interaction ($P < 0.05$). The least significant difference (LSD) for comparison of means is indicated by the vertical bar.

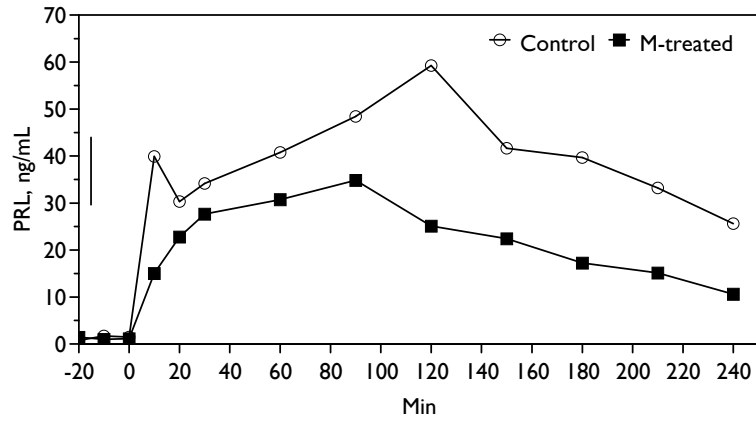
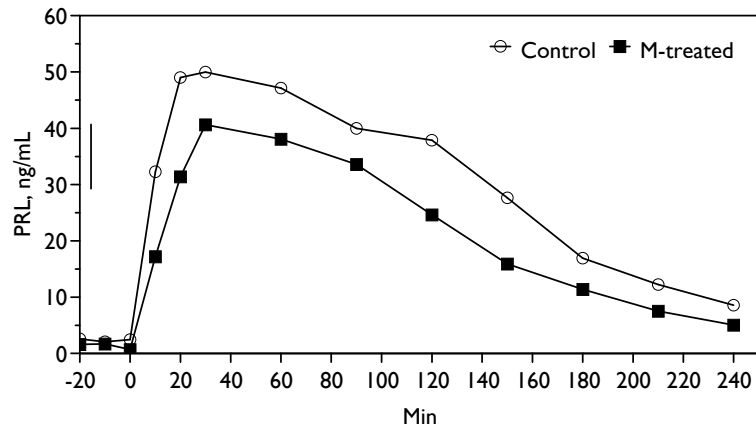


Figure 3.2. Mean plasma concentrations of prolactin in melatonin (M) treated and control stallions in response to low (top) and high (bottom) doses of a combined injection of GnRH and TRH at time 0. There was a treatment by time interaction ($P < 0.05$) at the high dose administration. The least significant difference (LSD) for comparison of means is indicated by the vertical bar.

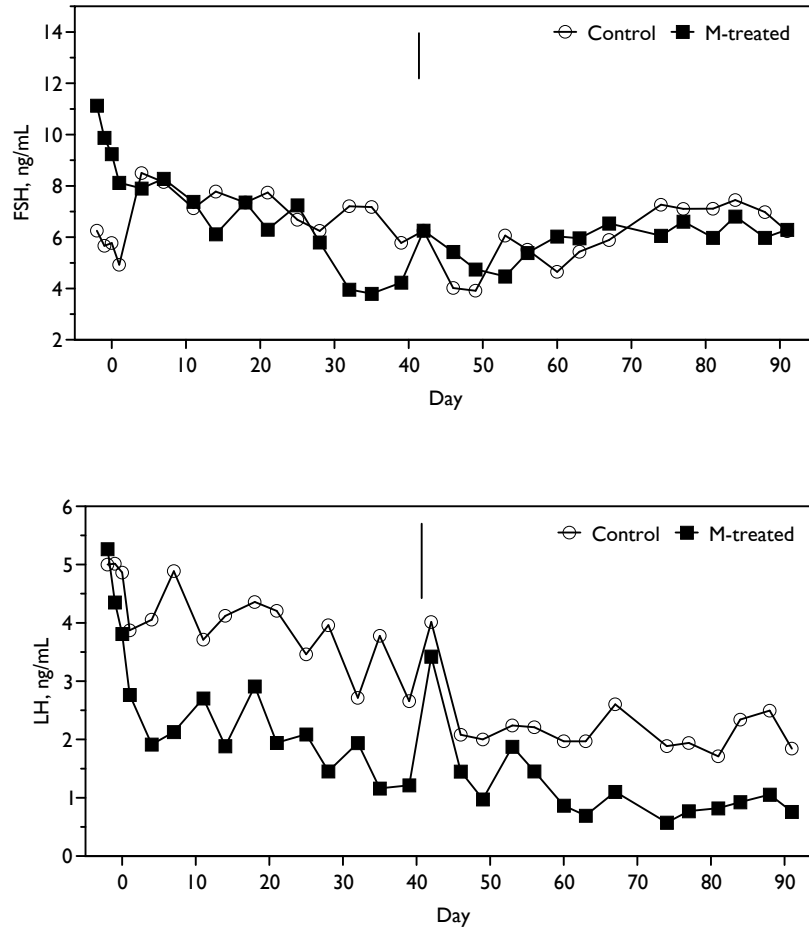


Figure 3.3. Mean plasma concentrations of FSH and LH in biweekly blood samples collected from melatonin (M) treated and control stallions. There was a treatment by time interaction ($P < 0.001$) for FSH and treatment by time interaction ($P < 0.1$) during the first 30 d for LH. The least significant difference (LSD) for comparison of means is indicated by the vertical bar.

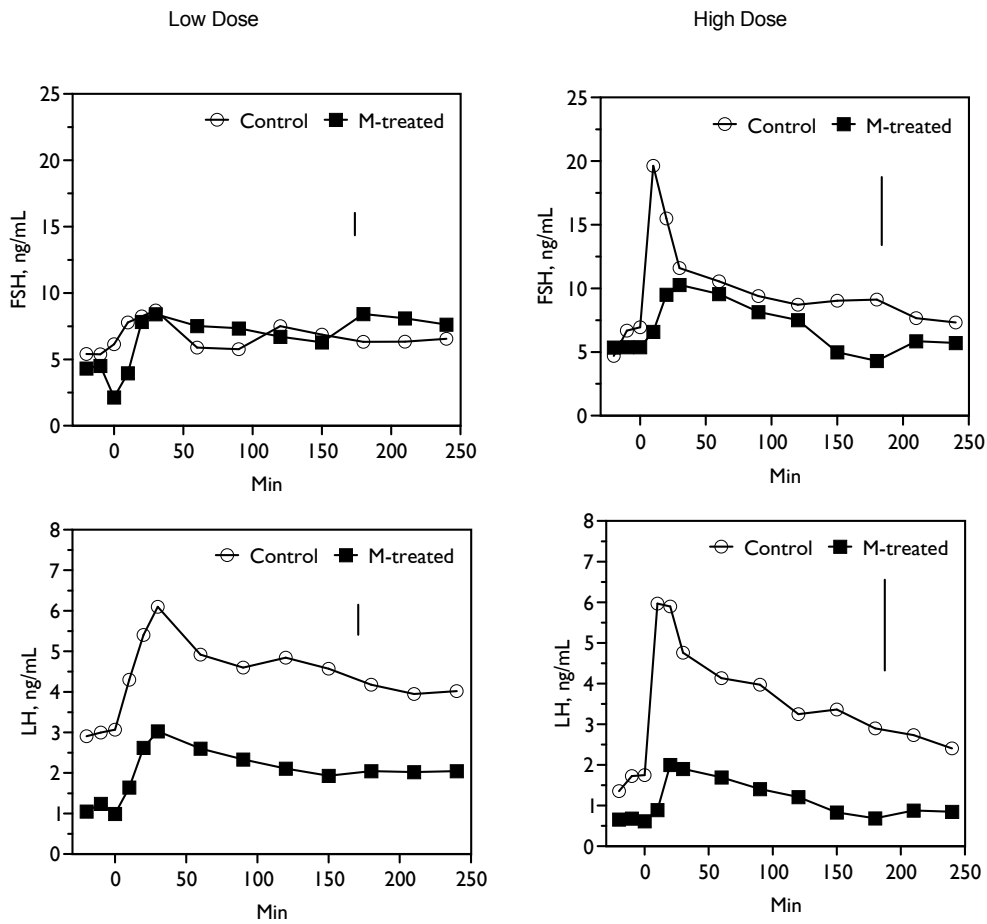


Figure 3.4. Mean plasma concentrations of FSH and LH in melatonin (M) treated and control stallions in response to low and high doses of GnRH at time 0. There was a treatment by time interaction ($P < 0.001$) at the low dose for FSH. The least significant difference (LSD) for comparison of means is indicated by the vertical bar.

Daily plasma testosterone concentrations decreased over time ($P < 0.001$) in the two groups (Figure 3.5), but the decline in plasma testosterone concentrations was similar ($P > 0.05$) for both groups. Administration of scrotogones at all four frequent sampling periods increased ($P < 0.001$) plasma testosterone concentrations in the two groups (Figure 3.6). The testosterone response to the low dose GnRH administration was decreased ($P < 0.001$) in melatonin treated stallions, but there was no difference ($P > 0.05$) in the response between groups to other low and high dose secretagogue administrations.

Collection Data. Semen collected on d 83, 84, and 85 did not differ ($P > 0.1$) in total volume, volume of gel, gel-free volume, and concentration of spermatozoa per ml of gel-free volume. Also, time until ejaculation and number of mounts were similar ($P > 0.1$) between groups (Table 3.1).

Discussion

Exogenous melatonin administration has been reported to decrease plasma testosterone concentrations in the stallion (Argo et al., 1991). This is contrary to our results in which similar treatment with melatonin failed to reduce plasma testosterone levels. Perhaps this lack of perturbation is due to administration of melatonin during a period of the year in which testosterone levels are naturally falling (Johnson and Thompson, 1983), such that a melatonin induced decrease in plasma testosterone concentrations was coincident with the naturally occurring seasonal decline in testosterone levels. Furthermore, plasma testosterone concentrations were similar for treated and control stallions after administration of both low and high dose LH injections. This suggests that the sensitivity of the Leydig cells to stimulation by LH was not altered by treatment with melatonin. However, the response in plasma testosterone concentrations for the melatonin treated stallions to the low dose GnRH injection was less than that of control stallions. This diminished response in testosterone concentrations observed in the treated stallions after the low dose GnRH injection was coincident with a reduced response in plasma FSH concentrations and a tendency for lower plasma LH concentrations. Hence,

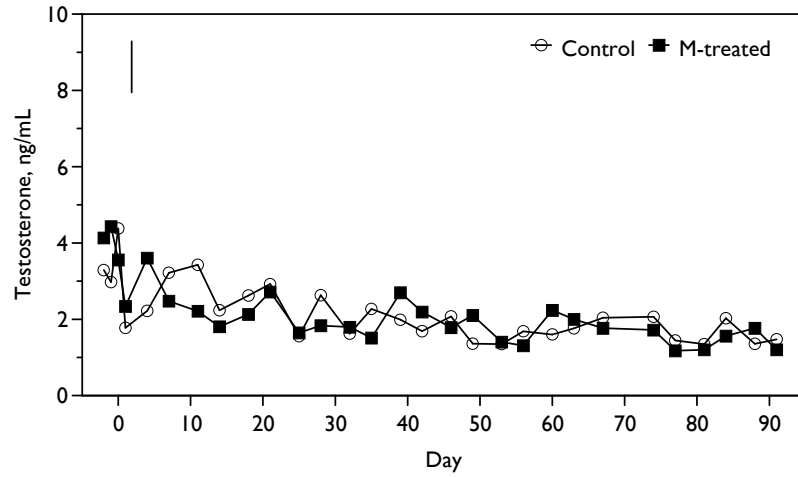


Figure 3.5. Mean plasma concentrations of testosterone in biweekly samples collected from melatonin (M) treated and control stallions. No effect of treatment ($P > 0.1$) was observed. The least significant difference (LSD) for comparison of means is indicated by the vertical bar.

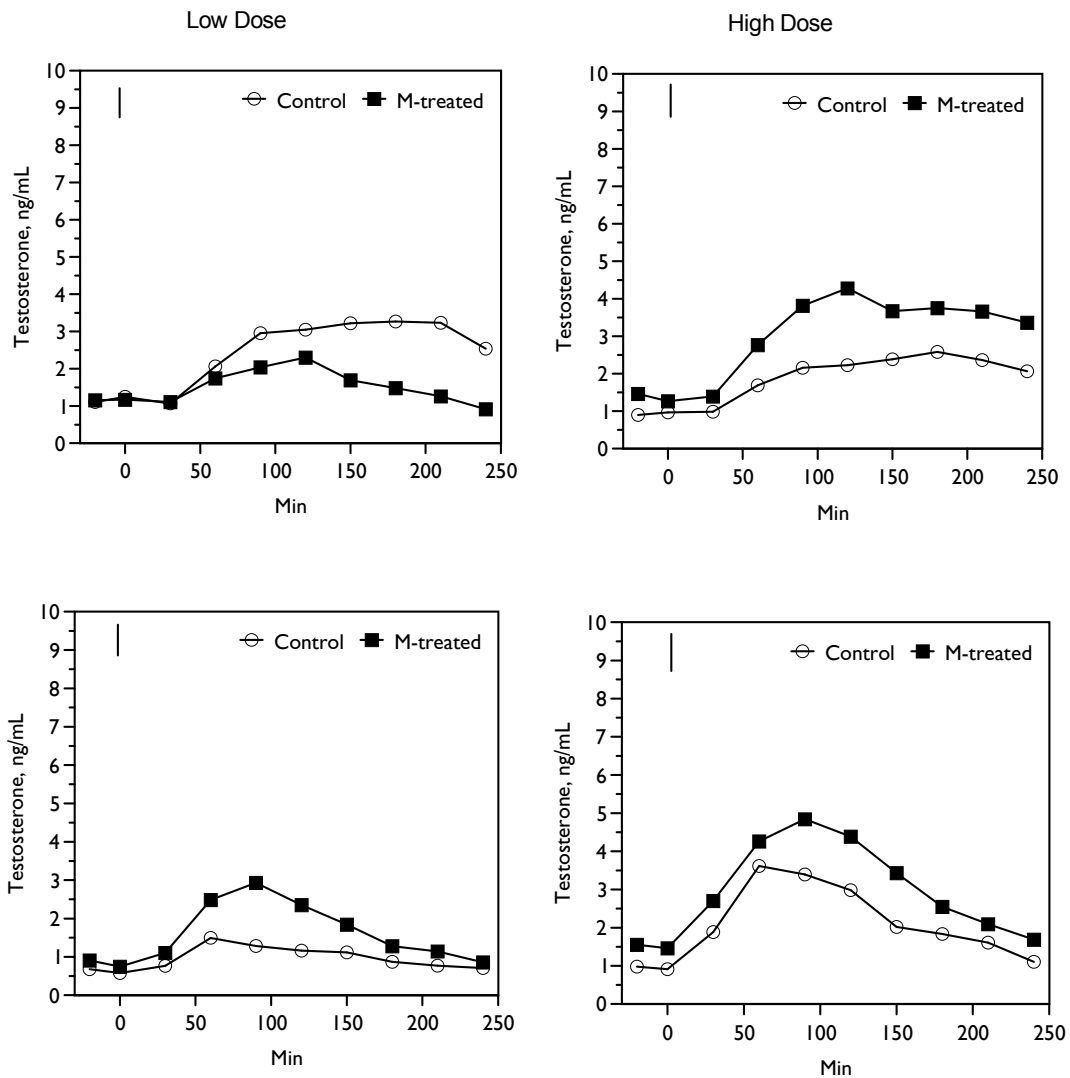


Figure 3.6. Mean plasma concentrations testosterone in melatonin (M) treated and control stallions in response to low and high doses of GnRH (top) or LH (bottom) administered at time 0. There was a treatment by time interaction ($P < 0.001$) at the low dose GnRH administration. The least significant difference (LSD) for comparison of means is indicated by the vertical bar.

Table 3.1 Semen collection data for control and melatonin treated stallions expressed as mean \pm SEM.

	Control	Melatonin
Number of Mounts	1.3 \pm 0.28	1.5 \pm 0.25
Gel-Free Volume, ml	51 \pm 7.1	54 \pm 6.4
Gel Volume, ml	10.7 \pm 5.0	11.1 \pm 4.4
Concentration, millions/ml	1.6 \pm 0.5	1.5 \pm 0.4
Total Sperm/Ejaculate, billions	7.4 \pm 0.88	7.4 \pm 0.78
Motility, %	64 \pm 2.7	61 \pm 2.4
Erection time, min	0.67 \pm 0.35	1.5 \pm 0.31

melatonin treatment possibly decreased the sensitivity of the pituitary to stimulation by GnRH, subsequently resulting in a lessening of the response in plasma testosterone concentrations. In contrast, administration of melatonin did not alter the testosterone response to the high dose GnRH administration. This is not in agreement with the melatonin interaction at the low dose GnRH administration, but may have resulted from overwhelming the hypothalamic pituitary axis with supraphysiological levels of GnRH.

The short days of winter, when melatonin concentrations are high for the longest duration each day, have been correlated to decreased plasma concentrations of LH and FSH in long-day breeders such as the hamster (Reiter, 1981) and the horse (Irvine and Alexander, 1982; Johnson and Thompson 1983; Clay and Clay, 1992). Similarly, plasma concentrations of LH and FSH in these stallions decreased with the onset of the autumnal transition. Moreover, melatonin treated stallions experienced an accelerated decline in plasma concentrations of FSH almost immediately after administration of melatonin began. Plasma LH concentrations in treated stallions also tended to fall below controls, however, by d 35 of melatonin administration, LH concentrations in both treated and control stallions had begun to converge. This precipitous decline in gonadotropin concentrations immediately after the initiation of melatonin administration is evidence of a possible role of melatonin in inhibition of GnRH pulse frequency or a reduction of GnRH receptors on the gonadotropes. The response in plasma concentrations of LH and FSH to a high dose of GnRH was not altered by melatonin treatment. This was not the case, however, for the low dose administration of GnRH. Melatonin-treated stallions exhibited a diminished response in plasma FSH concentrations and tended to have lower concentrations of LH in samples collected around the low dose GnRH administration. The absence of perturbation by melatonin treatment on the gonadotropins at the high dose GnRH administration may be due to an overwhelming of the hypothalamic pituitary axis by introducing supraphysiological levels of GnRH, such that the subtle differences observed at the low dose administration were not apparent. The responses exhibited in conjunction with the low dose administration of GnRH may further indicate an interaction of melatonin at the level of the

pituitary. Evidence that melatonin may interact at the pituitary has also been established by localization of melatonin receptors in the equine pituitary (Nonno et al., 1995). Certainly, other possibilities exist considering the similarity in gonadotropin concentrations at the high dose administration of GnRH.

In accordance with previous reports (Johnson, 1986; Thompson and Johnson, 1987), plasma concentrations of prolactin decreased in control stallions with the onset of the autumnal transition. Melatonin has been reported to decrease plasma concentrations of prolactin in the mare (Fitzgerald et al., 2000). Correspondingly, melatonin administration hastened the decline of plasma prolactin concentrations in treated stallions, though the two groups began to converge by d 60 of treatment. Plasma concentrations of prolactin increased in response to both low and high dose administrations of TRH, similar to previous reports (Gentry et al., 2002). In addition, melatonin-treated stallions exhibited a diminished response to the high dose TRH administration, however plasma concentrations of prolactin at the low dose administration of TRH were similar for the two groups. Evidently, melatonin administration was not effective at suppressing the prolactin response to the low dose administration of TRH, but the prolactin secretion required at the high dose TRH administration was physiologically unattainable by the treated stallions. This may indicate that the prolactin axis is impeded by the short days of winter not only by a long-term suppression of plasma concentrations of prolactin, but also by inhibiting the prolactin response to hypothalamic stimulation by TRH.

Prolactin has been implicated in regulation of seminal volume in the horse (Thomson et al., 1996), and FSH participates in the maintenance of spermatogenesis in the rat (Hadley, 2000). However, the decreased plasma concentrations of prolactin and FSH in the treated stallions did not alter the total volume, gel-free volume, or concentration of spermatozoa per ml of gel-free volume. Perhaps, a more demanding collection regimen may have helped reveal variation between treatment groups. Time until ejaculation and number of mounts were also similar between groups, indicating melatonin administration did not inhibit libido. Ejaculates were collected during the fall, the period when the natural decline in reproductive capacity occurs

(Pickett et al., 1976; Thompson et al., 1977; Clay et al., 1987). Therefore, the control stallions were beginning to exhibit a decline in reproductive status along with the treated stallions. This was also evident in the daily hormone profiles in treated stallions for LH, FSH, and prolactin concentrations, all of which became similar to control levels by d 60. Therefore, the ejaculates on d 83, 84, and 85 may have been collected too far into the autumnal transition to assess differences due to melatonin treatment.

In summary, melatonin has been previously reported to alter the reproductive status in various species (Reiter, 1981). In this experiment, long-term melatonin administration decreased plasma concentrations of prolactin and FSH. The effect of melatonin on plasma concentrations of LH was less evident, and plasma concentrations of testosterone were not altered. While the effects of melatonin on the basal levels of these pituitary hormones was evident, the role in which melatonin interacts within the hypothalamic pituitary axis to modify these hormones was not clearly established by our experimental design. It seems plausible, given the decreased responses observed in plasma concentrations of FSH and prolactin to administration of the combined injections of GnRH and TRH, that some inhibitory role of melatonin in the hypothalamic regulation of the pituitary exists.

CONCLUSIONS

Administration of recombinant equine GH was previously found to increase follicle numbers and ovulation rates in mares by possibly increasing the number of LH receptors present on the ovaries (Cochran et al., 2000, 1999a,b). In the first experiment, effects of similar treatment with recombinant equine GH on the testicular sensitivity of the stallion was studied, anticipating that the GH administration would increase the sensitivity of the Leydig cells by possibly up-regulating the number of receptors for LH. Therefore, treated stallions would exhibit a greater testosterone response to subsequent administration of LH. Daily administration of GH to the stallion did not alter the response in plasma testosterone concentrations after administration of LH or GnRH. Thus, GH treatment in the stallion apparently does not increase the sensitivity of the Leydig cells to stimulation by LH. However, when stallions were collected 60 d after the initiation of GH treatment, treated stallions had increased seminal volume and libido. Furthermore, GH treatment did not affect the ability of TRH to stimulate plasma concentrations of prolactin. Therefore, the role of GH in maintenance of reproduction in the stallion may be confined to a long-term increase in the activity of the accessory sex glands.

In the second experiment, long-term melatonin administration decreased plasma concentrations of prolactin and FSH. However, the effect of melatonin on plasma concentrations of LH was less evident, and plasma concentrations of testosterone were not altered. Melatonin perturbation of the basal levels of these pituitary hormones was evident. However, the role in which melatonin interacts within the hypothalamic pituitary axis to modify these hormones was not clearly established by our experimental design. It seems plausible, given the decreased responses observed in plasma concentrations of FSH and prolactin to administration of the combined injections of GnRH and TRH, that some inhibitory role of melatonin in hypothalamic regulation of the pituitary exists.

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VITA

William Andrew Storer, son of George and Courtney Storer, was born in Moss Bluff, Louisiana, on April 4, 1976. William is the oldest of four children. He resided in Moss Bluff until the age of eighteen and attended high school at Sam Houston High. After graduating in 1994, William was accepted to McNeese State Univeristy in Lake Charles, Louisiana, where he completed a Bachelor of Science degree in agriculture. In August of 2000, William began working towards a Master of Science degree in Animal Science at Louisiana State University and became married to Kristina Hope Barnett the following year. Upon graduation, he plans to pursue a doctorate degree.