

ENDOCRINE AND REPRODUCTIVE RESPONSES TO IMPLANTS OF
DESLORELIN ACETATE IN HORSES

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Interdepartmental Program
in Animal and Dairy Sciences

by

Carrie Ann Johnson

B.S., Northeast Louisiana University, 1996

M.S., Northeast Louisiana University, 1998

August, 2002

ACKNOWLEDGEMENTS

I would like to thank the members of my graduate committee (Dr. David Foltz, Dr. Dennis French, Dr. Robert Godke, Dr. William Todd, and Dr. Cathleen Williams) for their participation and assistance in my graduate program. A special thank you goes to my committee chair, Dr. Donald Thompson, Jr. for his guidance and advice throughout my program. An additional thank you goes out to the staff and faculty of the Animal Science Department for their assistance during my program. To my fellow graduate students, you have been an enormous help at the farm and in the lab and your valued friendships will always be special. Thank you to Mr. Randy Wright and the horse unit staff for their assistance during experiments. Special appreciation is extended to my family and friends for their love and support over the last 10 years. To my mom and dad, your guidance, love, and support are appreciated beyond words. Finally, to my husband, Natie, and our precious daughter, Lena, thank you for being there.

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ABSTRACT

Four experiments were performed to study the effects of the gonadotropin releasing hormone (GnRH) analog, deslorelin acetate (Ovuplant™), on endocrine and reproductive characteristics in mares. The first experiment tested whether anecdotal field reports of Ovuplant causing extended interovulatory intervals would be detectable under controlled, experimental conditions. The use of Ovuplant to hasten ovulation in 13 mares, compared to 12 controls, increased ($P < 0.05$) the interovulatory interval by 6.2 d and suppressed ($P < 0.05$) plasma concentrations of both luteinizing hormone (LH) and follicle stimulating hormone (FSH) for approximately 11 d. Two mares receiving Ovuplant did not return to estrus within 30 d. In the second experiment, 10 control mares and 10 mares induced to ovulate with Ovuplant were administered GnRH (50 µg) on d 1, 4, 7, and 10 after ovulation. Again, treated mares had a longer (4.4 d, $P < 0.05$) interovulatory interval and suppressed LH and FSH concentrations in daily plasma samples. The gonadotropin response to GnRH was lower ($P < 0.05$) in the deslorelin mares on d 1, 4, and 7, indicating a lack of pituitary responsiveness. In the third experiment, 9 stallions and 12 steroid-treated geldings were used to determine if males were potential models for studying the deslorelin-induced gonadotropin suppression. In both cases, treatment with Ovuplant caused an initial rise in both gonadotropins followed by suppression for about 14 d. In the last experiment, 21 mares were used to determine if multiple doses of deslorelin would cause complete ovarian shutdown. Mares received either sham injections, three Ovuplant implants on the first day, or one implant per day for 3 d ($n = 7/\text{group}$). Treatment with multiple deslorelin implants increased ($P < 0.05$) the interovulatory interval by 14.8 d and suppressed LH and FSH concentrations for

approximately 25 d, however no mares exhibited complete ovarian shutdown. In conclusion, deslorelin acetate implants in horses in the form of Ovuplant induce short-term increases in LH and FSH secretion followed by long-term suppression of these concentrations and an insensitivity of the pituitary to GnRH. In a small percentage of mares, long-term ovarian shutdown is a possibility.

CHAPTER I

INTRODUCTION

The horse industry is concerned with reducing the number of breedings per cycle, shortening estrus, and getting mares in foal earlier. One means of accomplishing these goals is shortening the time to ovulation. The estrous cycle of the mare is extremely long in comparison to other domesticated livestock, with a mean of 5 to 7 d in length compared with less than 1 d for cows, ewes, and does, and 2 d for sows. This long estrus means numerous breedings per cycle to ensure the mare is mated close to the time of ovulation. Additional breedings per cycle result in more labor, overuse of the stallion, and more costs to the mare owner. If the time of ovulation could be more accurately predicted, mare owners could reduce their breeding costs and stallion owners could reduce the physical demands on their stallions.

Ovuplant™ (Fort Dodge Animal Health, Overland Park, KS), a commercially available implant, has been proven to hasten ovulation in estrous mares with a follicle greater than 30 mm in diameter. The active ingredient in Ovuplant is deslorelin acetate. Deslorelin is an analog of gonadotropin releasing hormone (GnRH) and shortens the time to ovulation by causing increased luteinizing hormone (LH) and follicle stimulating hormone (FSH) concentrations, resulting in a hastening of maturation of the ova and a fertile ovulation. The vast majority of mares treated with Ovuplant when they achieve a follicle greater than 30 mm will ovulate within 48 h; therefore, a mare can be treated with a deslorelin implant on d 1 (the day a follicle >30 mm is detected) and bred on d 2. The mare will most likely ovulate on d 2 or 3 and be going out of estrus by d 3 or 4. This

method of ultrasonography and deslorelin implantation has allowed breeders to reduce the number of breedings per cycle to one.

After the first year of commercial availability of Ovuplant in the United States, several reports began circulating that mares not getting pregnant to the Ovuplant-induced ovulation had a delayed return to estrus and subsequent ovulation. Moreover, it appeared that in some instances Ovuplant-treated mares went anestrous and were unbreedable for the season.

Based on these anecdotal reports, the series of experiments described herein were initiated 1) to determine, under controlled conditions, if there was in fact a suppression of cyclicity following an ovulation induced by deslorelin implantation, 2) to study the underlying endocrine changes involved in the suppression, and 3) to study possible mechanism(s) by which this anovulation occurred.

CHAPTER II

REVIEW OF LITERATURE

Hypothalamic-Pituitary Axis

The hypothalamus has been described as the 'master gland' (Ginther, 1992) because it serves as the collecting center for information and then converts neural signals to hormonal signals (Guyton and Hall, 1996). The hypothalamus produces GnRH, the pivotal regulatory hormone of reproduction. Hypothalamic GnRH is a decapeptide (10 amino acids) and has a short half-life (5 to 10 min; Conn et al., 1986, 1987). The sequence of the amino acids that make up GnRH is (1) glutamine, (2) histidine, (3) tryptophan, (4) serine, (5) tyrosine, (6) glycine, (7) leucine, (8) arginine, (9) proline, and (10) glycine. Synthesis of GnRH in some species occurs in neurons whose cell bodies are located in the arcuate nuclei of the hypothalamus (Guyton and Hall, 1996); however in the horse, synthesis is evenly distributed throughout the hypothalamus (Strauss et al., 1979). GnRH controls reproduction by acting on the adenohypophysis to cause a cascade of hormonal events. The major function of GnRH is to stimulate the secretion of the gonadotropins, LH and FSH. Another function of GnRH is to stimulate the production of its own receptor (Clayton, 1989). In response to appropriate stimuli, GnRH is released in a pulsatile manner, enters the hypothalamic-pituitary portal system, and travels to the adenohypophysis where it bathes the gonadotropes (Ginther, 1992). The pulsatile release of GnRH leads to pulsatile release of the gonadotropins (Dyer, 1989). It has been suggested that differential release of LH and FSH may be due to the pulse frequency of GnRH secretion (Savoy-Moore and Swartz, 1987).

Concentrations of GnRH in peripheral blood, such as in the jugular vein, are too low to be detected, making determination of release from the hypothalamus a difficult task. However, a technique involving the cannulation of the intercavernous sinus, into which pituitary venous blood flows, has allowed the direct study of GnRH secretion (Alexander and Irvine, 1987). Investigations into the seasonal changes in the hypothalamic-hypophyseal axis revealed minimal concentrations of GnRH during anestrus. GnRH concentrations gradually increased during transition to reach maximal levels during the breeding season (Hart et al., 1984; Sharp and Grubaugh, 1987). Concentrations of LH were low or undetectable during anestrus and progressively increased through transition to estrus, while FSH concentrations did not vary (Hart et al., 1984; Silvia et al., 1986). Similarly, mares immunized against GnRH had lower circulating concentrations of LH and FSH, an absence of estrus behavior, and a reduction in follicular activity (Garza et al., 1986; Safir et al., 1987). Concentrations of LH are low during diestrus, begin to increase before estrus, increase progressively during estrus, peak the day after ovulation, and decrease to baseline values over the next 4 to 6 d (Ginther, 1992). In contrast, concentrations of FSH are low during estrus, increase after ovulation and peak during mid-diestrus, and decrease with the advancing diameter of the next dominant, pre-ovulatory follicle (Ginther, 1992). During the periovulatory period, secretion of GnRH, LH, and FSH occurs continuously with synchronous pulses superimposed on the tonic background (Alexander and Irvine, 1993).

Treatment with exogenous GnRH causes a rapid increase in secretion of both gonadotropins. A single injection of GnRH to seasonally anovulatory mares, ovulatory mares, and stallions caused a transient increase in concentrations of LH (Ginther and

Wentworth, 1974). A single injection of GnRH caused FSH concentrations to increase to levels comparable with peak levels seen during the diestrous period (Evans and Irvine, 1979). Constant infusion of GnRH resulted in continuing LH release (Garcia and Ginther, 1978).

Treatment with GnRH or its analogs has been the subject of numerous studies into controlling the reproductive cycle of the mare. Synthetic GnRH has been reported to be successful at inducing ovulation in mares with inactive or sub-normally active ovaries (Heinze and Klug, 1975). Administration of synthetic GnRH during estrus stimulated the release of LH and reduced the duration of estrus (Irvine et al., 1975). These initial reports sparked interest in the potential of using GnRH for induction of the first ovulation of the breeding season and in the timing of ovulation in the estrous mare, and numerous experiments were subsequently carried out testing these possibilities. Regimes of native GnRH alone (0.4 mg/8 h for 14 d; Bailey and Douglas, 1977) or GnRH with progesterone (Evans and Irvine, 1976, 1977) caused ovulation near the end of the anovulatory season. Numerous other regimens of repeated or continuous GnRH/GnRH agonists have been shown to be successful for causing follicular development and ovulation during the anestrous season (summarized in Table 5.5, p. 167 in Ginther, 1992).

Down-Regulation

Research with female monkeys was used to show that GnRH secretion is “obligatorily intermittent with episodes of secretion separated by periods of nonsecretion” (Knobil, 1980). That is, normal gonadotropin secretion is dependent upon pulsatile, or intermittent, secretion of GnRH, as opposed to a continuous secretion. Continuous secretion exposes GnRH receptors to continuous stimulation, and an eventual

decrease in receptor numbers, which results in refractoriness (Knobil, 1980) that leads to a suppression of endogenous gonadotropins. The lack of gonadotropin inhibits reproductive function including follicular development, ovulation, and luteal function (Fraser, 1981). The decrease in receptor numbers on the gonadotropes has been termed “down-regulation,” which has been so effective in some species that it has been used as a method of contraception (Irvine, 1983). The possible causes of the reduction in plasma LH and FSH concentrations include reduced endogenous GnRH secretion from the hypothalamus (Crowder et al., 1986), desensitization of the pituitary to GnRH stimulation, and(or) depletion of LH and FSH in the pituitary. The prolonged gonadotropin suppression seen in pituitary down-regulation following constant high GnRH stimulation (Heber and Swerdloff, 1981; Nett et al., 1981; Sandow, 1983) is generally caused by reduced sensitivity of the gonadotropes to further GnRH stimulation (desensitization; Belchetz et al., 1978; Zilberstein et al., 1983).

Down-regulation in other species has been shown to involve a reduction in the number of GnRH receptors plus a perturbation of post-receptor mechanisms (Smith et al., 1983; Conn et al., 1987). In research with ewes, Crowder and coworkers (1986) induced down-regulation with continuous GnRH infusion and reported no dramatic alteration in pituitary LH and FSH concentrations.

In general, mares have been thought to be relatively refractory to down-regulation by GnRH analogs (Fitzgerald et al., 1993; Irvine and Alexander, 1993). In horses, constant treatment (28 d) with large doses (1.3 mg/kg/day) of a GnRH agonist suppressed LH concentrations similar to the suppression reported in other species (Fitzgerald et al., 1990). Treatment of horses with high doses (10 mg/d) of a GnRH analog resulted in a

reversible suppression of ovarian activity causing a lengthening of the estrous cycle (Palmer and Quellier, 1988). However, additional trials with prolonged continuous administration of GnRH suggested that horses are not as sensitive to prolonged treatment as other species. Constant infusion of GnRH (24 h) resulted in a continuous LH release in horses (Garcia and Ginther, 1978). Osmotic minipumps that constantly delivered GnRH for 28 d maintained LH secretion (Hyland et al., 1987) and induced prolonged gonadotropin secretion without refractoriness (Allen et al., 1987) even at relatively large doses (Turner and Irvine, 1992). Administration of up to 50 mg/d of a potent GnRH agonist had no suppressive effect on LH secretion, sexual behavior, or semen characteristics of stallions; in mares, luteal phase concentrations of LH were maintained, but follicular development was suppressed, although only after 30 d of treatment (Montovan et al., 1990). Compared to ruminants and humans, the horse does in fact seem less sensitive to the suppressive effects of continual high levels of GnRH or agonists.

GnRH Analogs

Once the structure of hypothalamic GnRH was known, considerable effort was put into finding modifications of the hormone that would produce more potent “analogs,” or agonists, for use in clinical medicine. Most GnRH analogs are produced by the substitution and(or) removal of one or more amino acids. Substitution of the glycine at position 6 with D-alanine, tryptophan, or serine confers more structural and metabolic stability, thereby prolonging the half-life and action of the analog (Monahan et al., 1973; Sandow et al., 1978). Removal of the terminal glycine (position 10) and amidation of the proline at position 9 also results in an increase in agonist activity (Sandow et al., 1978).

Substitutions near the N terminal, particularly of histidine in position 2, produce antagonists, which bind to the GnRH receptor but do not turn on the intracellular machinery to increase gonadotropin production and secretion (Sandow et al., 1978).

Several researchers have shown the utility of human chorionic gonadotropin (hCG) for hastening ovulation in the estrous mare (Loy and Hughes, 1966; Sullivan et al., 1973; Voss et al., 1975). Unfortunately, hCG is a human hormone, therefore foreign to the horse, and acts as an antigen allowing for the development of anti-hCG antibodies (Roser et al., 1979). Repetitive use of hCG has been shown to result in a diminishing response (Sullivan et al., 1973). Due to this problem with hCG, GnRH analogs have been tested widely as an alternative for inducing ovulation in the mare. The benefits of a GnRH analog include 1) its smaller molecular weight makes it less antigenic, 2) it may be used for alternate cycles with hCG, 3) it is a synthetic product, eliminating the possibility of viral contamination, and 4) its supply is not dependent upon pregnant women.

Several different GnRH agonists have been studied in the horse. One of these agonists, buserelin, has proven successful for inducing ovulation in estrous mares with a follicle >35 mm in diameter; however, twice daily injections were required (Squires et al., 1981).

Deslorelin

Deslorelin is a GnRH analog produced by substituting tryptophan for glycine at position 6, removing the glycine at position 10, and amidating the proline at position 9 (6-D-tryptophan-9-(N-ethyl-L-prolinamide)-10-Desglycinamide LHRH; Peptide Technology Limited, Dee Why, NSW 2099, Australia). Deslorelin is commercially available as Ovuplant, a short-term implant that releases 2.2 mg of deslorelin over 2 to 3

d. Ovuplant is a white cylindrical tablet (2.3 x 3.7 mm). Ovuplant has been shown to be effective in increasing LH concentrations and hastening ovulation in cyclic mares (McKinnon et al., 1993; Meinert et al., 1993; Jöchle and Trigg, 1994; Lubbecke et al., 1994; Squires et al., 1994; Mumford et al., 1995) and in inducing ovulation in transitional mares (McKinnon et al., 1997). Three studies have directly compared Ovuplant to hCG, and those studies reported a treatment-to-ovulation interval of 1.98 and 1.88 d (McKinnon et al., 1993), 46.9 and 43 h (Meinert et al., 1993), and 2.2 and 2.2 d (Vanderwall et al., 2001), respectively. Donadeu (1997) administered deslorelin implants to mares and reported an initial short-term increase in the concentrations of circulating gonadotropins. This increase in the gonadotropins coincided with the release of deslorelin from the implant. Deslorelin concentrations peaked (~400 pg/mL) at 3 to 6 h after implantation and declined to near baseline within 24 h. Mumford et al. (1995) treated mares with 3 or 5 times the recommended dose of deslorelin implants over three consecutive cycles; they reported an increase in the interovulatory interval for mares administered the “5x” dose and noted diminished follicular activity in the “3x” and “5x” dose groups. Some mares in the “5x” group never reached the criteria for the third implantation (a follicle >30 mm).

Rationale for Present Experiments

Even after the extensive testing of deslorelin in mares for use in hastening ovulation, after the first year of commercial availability of Ovuplant in the United States, word-of-mouth reports began circulating of problems in mares not becoming pregnant at the induced ovulation. When the present series of experiments was initiated, no published reports in the literature existed. Thus, the first step was to determine, under

controlled experimental conditions, whether the alleged problems were reproducible. Given the results of the first experiment (Chapter III), subsequent experiments were designed to further study the endocrine basis of the prolonged interovulatory interval and occasional anestrus produced by Ovuplant administration.

CHAPTER III

PROLONGED INTEROVULATORY INTERVAL AND HORMONAL CHANGES IN MARES FOLLOWING THE USE OF A DESLORELIN IMPLANT TO HASTEN OVULATION*

Introduction

The early induction of ovulation in the mare is a valuable management tool, and the various methods towards this end, including injections of HCG, prostaglandin- $F_{2\alpha}$ ($PGF_{2\alpha}$), and GnRH or one of its analogs, have been used with varying degrees of success (reviewed by Jöchle and Trigg in 1994). Ovuplant, a short-term implant containing the GnRH analog deslorelin acetate, recently became commercially available in the United States for hastening ovulation in mares. However, in its first year of use, there have been anecdotal reports from the field that mares not becoming pregnant after Ovuplant-induced ovulation often have delayed return to estrus and prolonged interovulatory interval, particularly after $PGF_{2\alpha}$ -induced luteal regression commonly used in embryo transfer programs. Similar effects were noted by Mumford et al. (1995) in mares administered 3 or 5 times the recommended dose of deslorelin implants over three consecutive cycles not altered by $PGF_{2\alpha}$ administration. Thus, the present experiment was designed 1) to determine if the field observations were repeatable under controlled experimental conditions and 2) to gather endocrine data that might yield information on the underlying cause(s) of this delayed return to estrus.

Materials and Methods

Twenty-five, non-lactating light horse mares were used. The mares were between 3 and 20 yr of age, were of good body condition (6 to 8; Henneke et al., 1983), and were

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known to have cycled normally the previous year. They were kept on native grass pasture and supplemented with grass hay as needed to maintain body condition. The experiment was performed in the months of April through July; the transition period in Louisiana normally starts around late February or early March.

Beginning in April, mares were checked each morning for behavioral estrus with a vigorous stallion. Once a mare came into estrus, her ovaries were examined daily via transrectal ultrasonography, and the diameter of all detectable follicles recorded. Based on these follicle counts, mares were generally classified as having either high or low follicle numbers, and their allotment to treatment took this into consideration such that high and low follicle numbers were evenly distributed between groups. The first day a mare achieved a follicle greater than 30 mm, she received either a single implant of Ovuplant (treated mares) in the neck region or was administered a sham injection (same size needle). Assignment to the treatment and control groups was random for the first mare and then alternated except when follicular number dictated; no more than three mares of either category were assigned consecutively to the treatment or control groups. Additionally, treatments were coded such that the assignments of individual mares were unknown to the personnel involved with data collection.

The ovaries of each mare were examined daily during the first estrus until ovulation was detected, after which each mare was examined on an every other day schedule. On d 7 after ovulation, each mare was administered PGF_{2α} (LutalyseTM, Upjohn, Kalamazoo, MI) to induce regression of the corpus luteum. Every other day examinations were continued until a mare returned to estrus or developed a follicle 30

mm or greater, at which time the mare was switched to daily examinations until ovulation was detected on this second estrus.

Samples of jugular blood were drawn each morning beginning on the first day of the first estrus and ending 3 d after ovulation on the second estrus. All blood samples were collected prior to estrus detection (except for the very first day) and prior to ultrasound examination. Heparinized plasma was harvested by centrifugation and was stored frozen. Plasma samples were analyzed for LH (Thompson et al., 1983a), FSH (Thompson et al., 1983c), progesterone (Diagnostic Systems Laboratory, Webster, TX), and estradiol (Diagnostic Systems Laboratory) by radioimmunoassay.

Two of the mares receiving Ovuplant had interovulatory intervals greater than 30 d after treatment. For statistical analyses, the data for these mares were excluded from the treated group. One control mare had incomplete luteal regression after PGF_{2α} administration on d 7, thus her data were not included with those of the control group. Data for single point variables (such as length of the interovulatory interval) were analyzed by one-way analysis of variance (Steel and Torrie, 1980) with PC-SAS (SAS Institute, Cary, NC). Data for variables from repetitive sampling (e.g., hormonal concentrations over time) were analyzed by ANOVA in a split-plot design (Gill and Hafs, 1971). Differences between groups in the treatment x time interactions from these latter analyses were assessed by the LSD-test (Steel and Torrie, 1980).

Results

For the 22 mares in the analysis, mean diameter of the largest follicle on the day of treatment or sham injection at the first estrus did not differ ($P = 0.713$) between groups

(Table 3.1). After treatment, the mean diameter of the pre-ovulatory follicle at the exam prior to ovulation was smaller ($P = 0.037$) for mares receiving Ovuplant than for control mares. Although there was no difference between groups for the mean interval from treatment or sham injection to ovulation on the treatment estrus ($P = 0.438$), the general trend was in the direction for shorter intervals in the treated group. When analyzed as percentage of mares ovulating within a specified interval after treatment, there were more mares ovulating within 48 ($P = 0.095$) and 72 ($P = 0.059$) h after Ovuplant administration than after sham injection. The interval between the first ovulation on the treatment estrus and that on the subsequent estrus (interovulatory interval) was greater ($P = 0.0001$) for mares administered Ovuplant compared to controls (Table 3.1).

Hormonal data were expressed relative to the day of ovulation (d 0) on the first estrus. Plasma concentrations of LH (Figure 3.1a) were greater ($P < 0.05$) in control mares than in mares receiving Ovuplant on the day of ovulation and for the next 4 consecutive days. They were again greater ($P < 0.05$) on d 9, 11, 19, and 20 after the first ovulation. Plasma concentrations of FSH (Figure 3.1b) were higher ($P < 0.05$) in mares receiving Ovuplant relative to controls on the day preceding the first ovulation. Within 4 d after ovulation, plasma FSH concentrations began to rise in control mares but actually dropped slightly in mares receiving Ovuplant, resulting in lower ($P < 0.05$) average concentrations relative to controls from d 4 through 11 after the first ovulation. Plasma progesterone concentrations increased in both groups of mares after the first ovulation (Figure 3.2a), but were lower ($P < 0.05$) in mares receiving Ovuplant than in control mares on d 5 and 6. Average concentrations dropped precipitously 1 d after $\text{PGF}_{2\alpha}$ administration on d 7, and average concentrations were again lower ($P < 0.05$) in

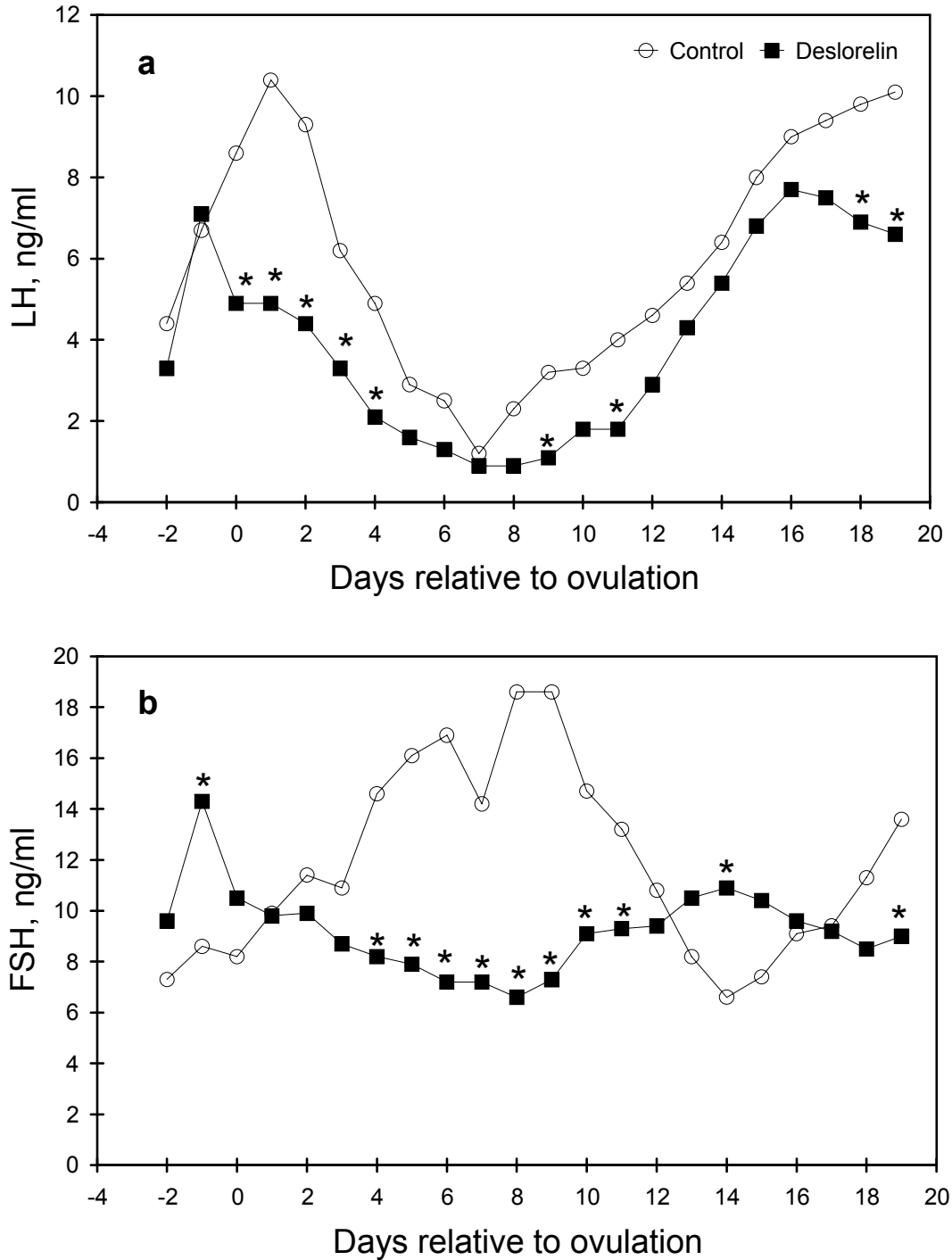


Figure 3.1 Plasma concentrations of LH (a) and FSH (b) in mares administered sham injections (control) or treated with a deslorelin implants (deslorelin) on the first estrus. Data are expressed relative to the day of ovulation on the first estrus (d 0) for both groups. Prostaglandin injection was administered to all mares on d 7. Asterisks indicate differences ($P < 0.05$) between groups on specific days. The pooled SEM from the analyses of variance were 0.7 and 1.2 ng/ml for LH and FSH, respectively.

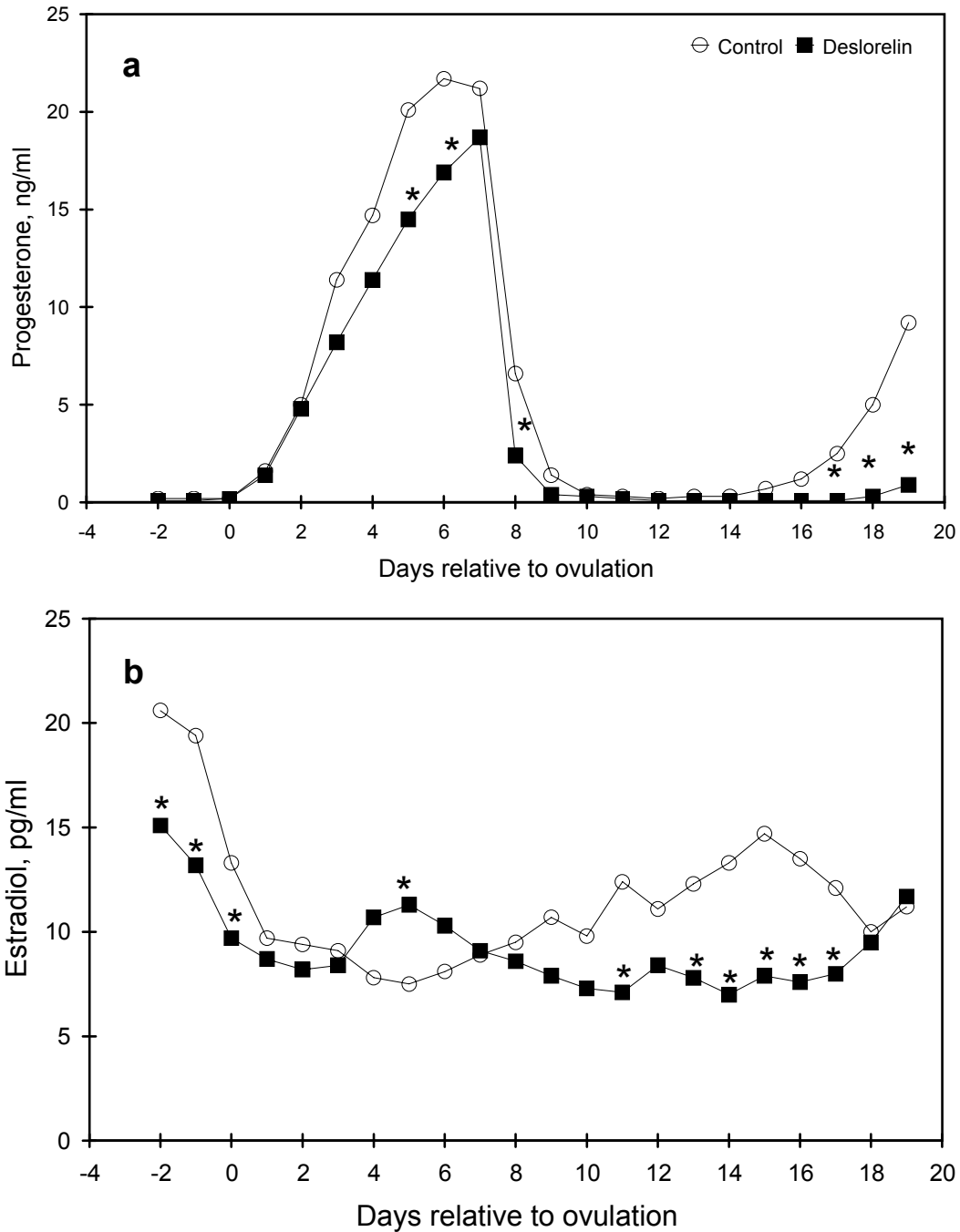


Figure 3.2 Plasma concentrations of progesterone (a) and estradiol (b) in mares administered sham injections (control) or treated with a deslorelin implants (deslorelin) on the first estrus. Data are expressed relative to the day of ovulation on the first estrus (d 0) for both groups. Prostaglandin injection was administered to all mares on d 7. Asterisks indicate differences ($P < 0.05$) between groups on specific days. The pooled SEM from the analyses of variance were 1.4 ng/ml and 1.2 pg/ml for progesterone and estradiol, respectively.

Table 3.1. Mean follicular diameter and ovulation data for mares administered sham injections (control) or treated with deslorelin on the first estrus.

Item	Control	Deslorelin	SEM	P-value
Number of mares	11	11		
Follicle diameter at treatment, mm	36.2	35.5	1.21	0.713
Follicle diameter before ovulation, mm	42.4	37.9	1.33	0.037
Treatment to ovulation, d	3.4	2.8	0.49	0.438
Number ovulating within 48 h (%)	3/11 (27.3%)	7/11 (63.6%)		0.095
Number ovulating within 72 h (%)	6/11 (54.5%)	10/11 (90.9%)		0.059
Interovulatory interval, d	15.8	22.0	0.60	0.0001

mares receiving Ovuplant than in control mares on d 8, 17, 18, and 19. Plasma estradiol concentrations (Figure 3.2b) were higher ($P < 0.05$) in control mares than in mares receiving Ovuplant on the day of the first ovulation and for the preceding 2 d and again on d 11 through 17.

Follicular data were compared relative to the following reference points: the day of treatment, the day of the first ovulation, the day of PGF_{2α} administration, and the day of ovulation on the estrus following PGF_{2α} administration (second estrus and ovulation). Diameter of the largest follicle (Figure 3.3a) was greater in control mares than in mares receiving Ovuplant 1 d before the first ovulation was detected (as indicated in Table 3.1), but did not differ between groups otherwise. Numbers of other large (25 mm and larger; Figure 3.3b) follicles were greater ($P < 0.05$) in control mares relative to mares receiving Ovuplant 1 d before ovulation on the second estrus. Relative to mares receiving Ovuplant, total number of medium (11 to 24 mm) follicles (Figure 3.4a) were lower ($P < 0.05$) in control mares on the day of the first ovulation, but were then higher ($P < 0.05$) on the day of the second ovulation and d 4 and 3 preceding it. Total number of small (10

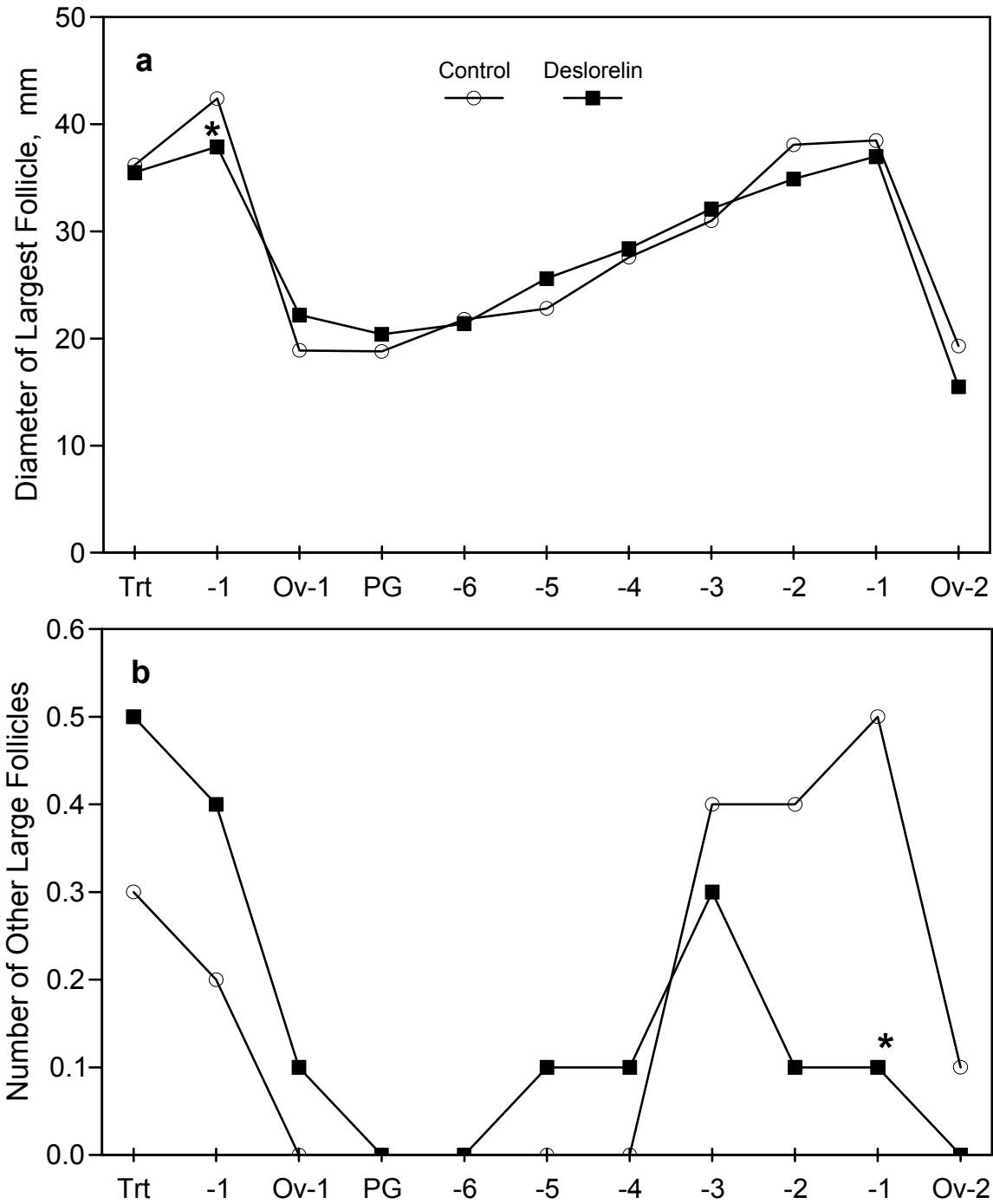


Figure 3.3 Diameter of the largest follicle (a) and total number of other large (25 mm and larger) follicles (b) on the ovaries of mares administered sham injections (control) or treated with a deslorelin implant (deslorelin) on the first estrus. Data are expressed relative to the day of treatment (trt), the day of ovulation on the treatment estrus (ov-1), the day of Prostaglandin administration (PG), and the day of ovulation on the second estrus (ov-2). Asterisks indicate differences ($P < 0.05$) between groups on specific days.

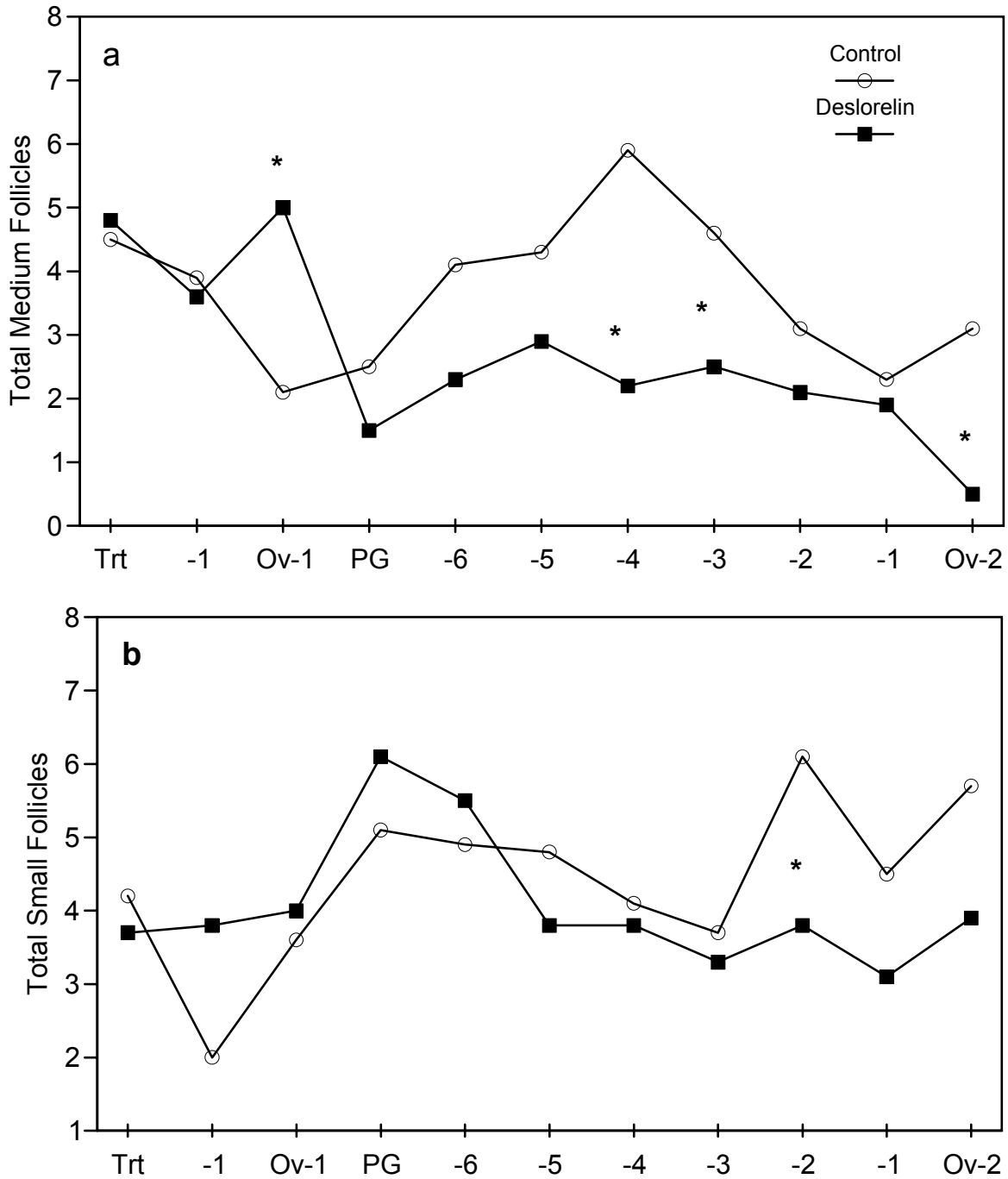


Figure 3.4 Total number of medium (11-24 mm) (a) and small (10 mm and smaller) (b) follicles on the ovaries of mares administered sham injections (control) or treated with a deslorelin implant (deslorelin) on the first estrus. Data are expressed relative to the day of treatment (trt), the day of ovulation on the treatment estrus (ov-1), the day of Prostaglandin administration (PG), and the day of ovulation on the second estrus (ov-2). Asterisks indicate differences ($P < 0.05$) between groups on specific days.

mm and smaller) follicles (Figure 3.4b) differed ($P < 0.05$) between groups only on d 2 preceding the second ovulation, when they were highest in control mares.

Of the two mares receiving Ovuplant that did not return to estrus within 30 d, one mare (#386, Figure 3.5) ovulated 48 h after treatment, had a normal appearing progesterone rise thereafter, and responded to $\text{PGF}_{2\alpha}$ with a rapid decline in progesterone concentrations. Her ovaries then became inactive, gaining no greater than a 10-mm follicle through 56 d after treatment, and estradiol and progesterone concentrations confirmed this inactivity. Concentrations of LH and FSH gradually increased through 30 to 40 and plateaued at elevated levels, indicative of a total lack of negative feedback from the ovaries (i.e., similar to an ovariectomized mare). This mare remained inactive through her last exam, which was several months after treatment.

The other treated mare that did not return to estrus within 30 d (#376, Figure 3.6) had a similar pattern in follicular activity and LH and FSH concentrations for 30 d, but eventually developed a 35-mm follicle and ovulated on d 31 after treatment. Moreover, she never had a rise in progesterone concentrations after her apparent first ovulation, which was 72 h after treatment.

Discussion

Deslorelin acetate, the active ingredient of the Ovuplant implant, has proven to be an effective alternative to hCG for inducing ovulation in mares with pre-ovulatory follicles of 30 mm or larger (McKinnon et al., 1993; Meinert et al., 1993; Jöchle and Trigg, 1994; Squires et al., 1994; Ganheim et al., 1995; Mumford et al., 1995; Meyers et al., 1997), and was tested extensively in field trials in North and South America, Europe and Australia (Jöchle and Trigg, 1994). The first anecdotal reports of mares not returning

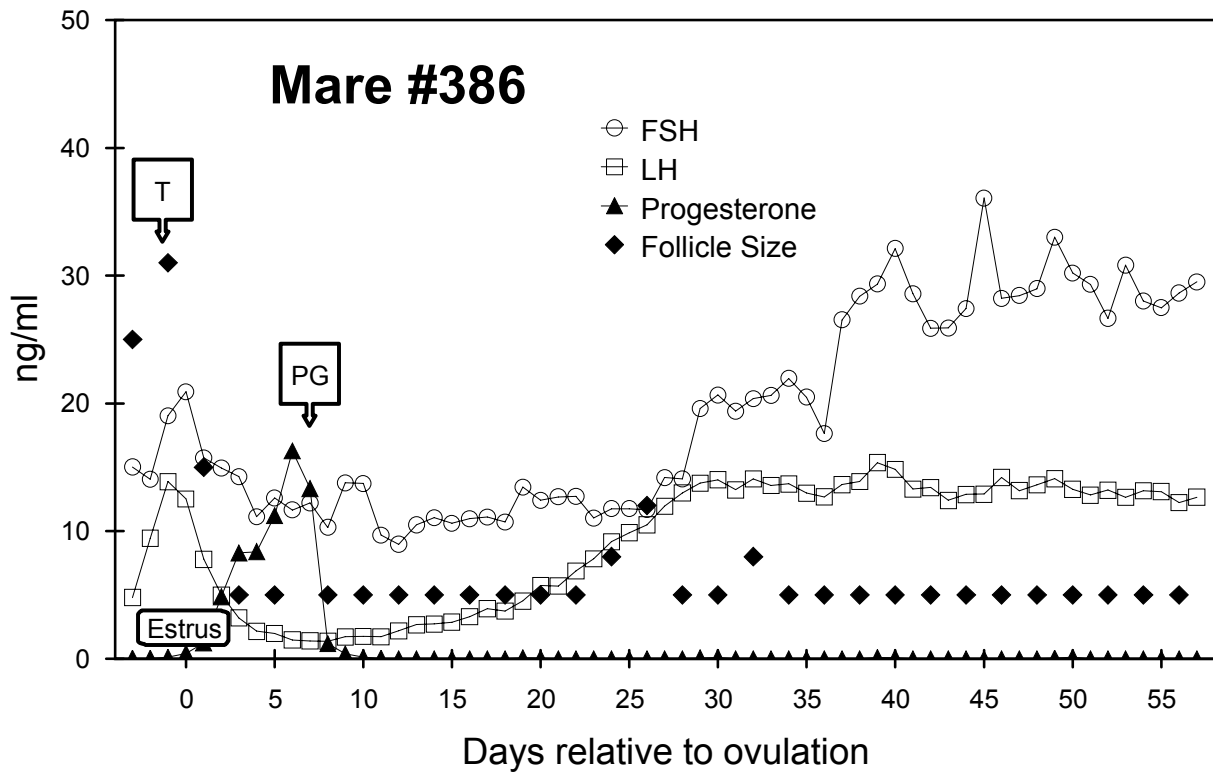


Figure 3.5 Plasma concentrations of LH, FSH, and progesterone in mare #386 after treatment with a deslorelin implant 2 days before ovulation (d 0). Estrus is designated, treatment is designated by T, and prostaglandin injection is designated by PG. Diameter of the largest follicle (mm) on either ovary is indicated by the solid diamonds. After an apparent normal ovulation, progesterone concentrations increased as expected, and subsequently decreased after injection of PG. Thereafter, little follicular activity was noted, and concentrations of LH and FSH increased to gonadectomized-like levels, indicating a lack of feedback from the ovaries. This condition persisted throughout the breeding season and was still present at the last examination in October.

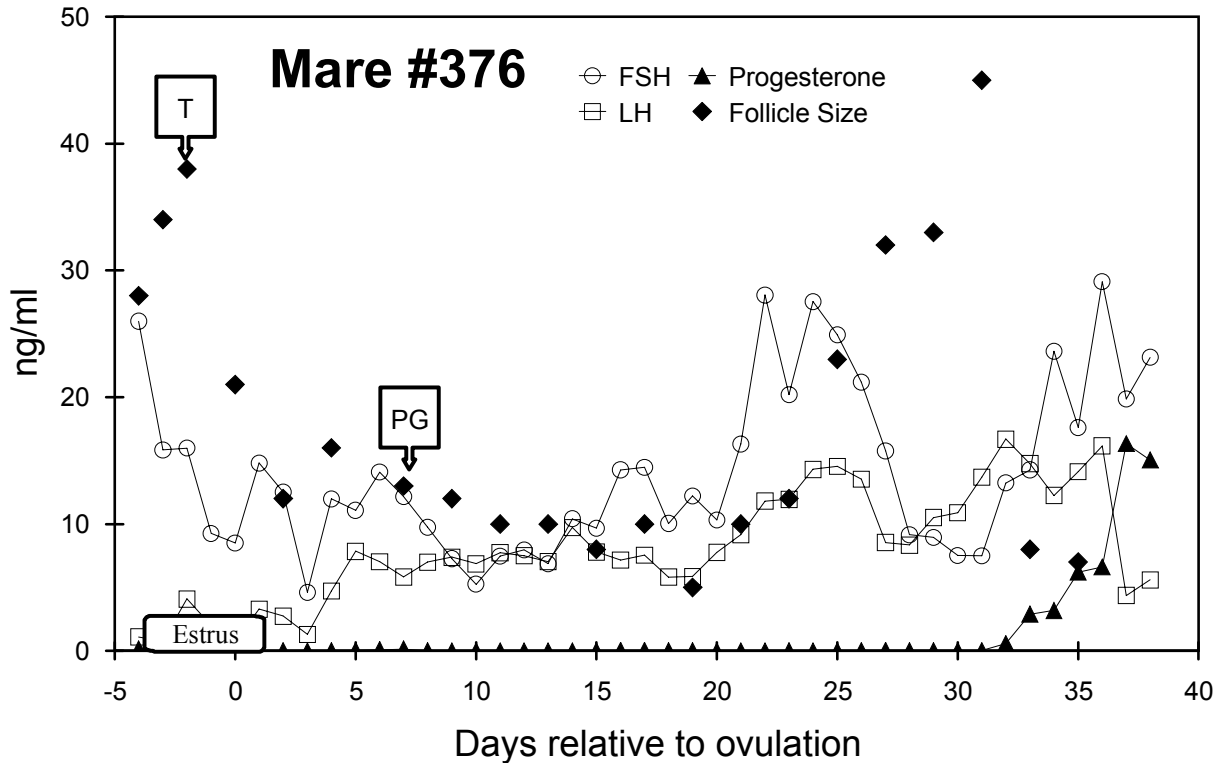


Figure 3.6 Plasma concentrations of LH, FSH, and progesterone in mare #376 after treatment with a deslorelin implant 2 d before ovulation (d 0). Estrus is designated, treatment is indicated by T and prostaglandin injection is indicated by PG. Diameter of the largest follicle (mm) on either ovary is indicated by the solid diamonds. After what appeared to be an ovulation (based on the morphological changes in a 38 mm follicle present on the left ovary), progesterone concentrations never increased and follicular activity decreased for approximately 23 days. Concentrations of LH and FSH increased to gonadectomized-like levels, and in this case the ovary responded with a pre-ovulatory follicle, a decrease in FSH concentrations, and ovulation on d 31. Progesterone concentrations increased thereafter in an apparent normal manner and LH concentrations began to decrease accordingly.

to estrus at the expected time began to circulate in the spring of 1999 when Ovuplant became commercially available in the United States. Allegedly, the degree to which mares were delayed in their return to estrus varied from slight to extensive, and those not returning for extensive periods were often associated with PGF_{2α}-induced short-cycling commonly used in embryo collection programs. Due to the potentially tenuous nature of such observations in the field, the present experiment was designed to gather information under experimentally controlled conditions on the occurrence and possible underlying cause(s) of this phenomenon.

Under the conditions of this experiment, the use of Ovuplant resulted in an interovulatory interval 6.2 d longer than that in control mares, not considering the two mares that had intervals of greater than 30 d. Those two mares were not included in the data analyses because their responses were so drastically different from the rest. Whether their responses were truly due to Ovuplant is difficult to confirm, and future studies with larger numbers of mares are needed to answer the question appropriately. However, the previous field observations did indicate the occurrence of long-term anestrus in a percentage of mares, and it is possible that these mares are indicative of that population. Moreover, Mumford et al. (1995) described several mares administered 3 or 5 implants of deslorelin that had extended interovulatory intervals, lack of follicular growth, and(or) total ovarian inactivity. Thus, it is possible that some mares are exceptionally sensitive to the GnRH analog and shut down in response to a single implant.

The alterations in plasma concentrations of LH and FSH in mares receiving Ovuplant likely led to the subsequent alterations in follicular growth. There was a short-lived increase in both hormones after insertion of the implant, but thereafter they

decreased gradually, indicative of suppression of secretion. Mumford et al. (1995) presented very similar LH and FSH patterns in response to 1, 3 or 5 implants of deslorelin, although they indicated that only the highest doses were suppressive. Given that the progesterone patterns after ovulation were similar for the two groups in this experiment up through d 6, the lower LH concentrations in the mares receiving Ovuplant on d 0 through 4 likely indicate some type of suppression other than progesterone. Moreover, FSH concentrations, which increased after ovulation as expected in control mares due to the release from feedback by follicular estradiol and inhibin, appeared to be suppressed for at least 8 d after ovulation in mares receiving Ovuplant. In general, mares are thought to be relatively refractory to down-regulation by GnRH analogs (Fitzgerald et al., 1993); however, these data are reminiscent of such an effect.

The major result of this suppression of LH and FSH after ovulation seemed to be the reduction in medium-sized follicles after $\text{PGF}_{2\alpha}$ -induced luteal regression. Because the $\text{PGF}_{2\alpha}$ injection was given to mares in both groups on d 7 after ovulation, the increased interovulatory interval in mares receiving Ovuplant was due to a delay in growth and emergence of a dominant pre-ovulatory follicle after this induced luteal regression. Once that follicle emerged, its diameter up to and at ovulation did not differ between groups, as indicated by the data in Figure 3.3a. Similarly, Mumford et al. (1995) made a subjective observation that mares administered 3 or 5 deslorelin implants had diminished follicular activity for 6 to 10 d after ovulation in non- $\text{PGF}_{2\alpha}$ shortened estrous cycles, and speculated that this might be due to suppressed follicular development or delayed recruitment of dominant follicles.

Given the results of the present experiment and those of Mumford et al. (1995) and Fitzgerald et al. (1993), it is concluded that the administration of Ovuplant at the recommended dosage does indeed alter the interovulatory interval of mares short-cycled with PGF_{2α}, and likely is responsible for a small percentage of mares becoming anestrus and anovulatory for extended periods of time. These latter mares are of particular concern, because they might be unbreedable for the entire breeding season. The mechanism by which long-term suppression of follicular activity in the face of elevated LH and FSH concentrations (indicative of a problem at the ovarian level), seen in the one mare in this experiment, needs to be studied further, because understanding that phenomenon may provide insight into the cause of other forms of ovarian lesions resulting in infertility.

CHAPTER IV

PITUITARY RESPONSIVENESS TO GNRH IN MARES FOLLOWING DESLORELIN ACETATE IMPLANTATION TO HASTEN OVULATION*

Introduction

Previous investigation (Johnson et al., 2000) into field reports of prolonged interovulatory intervals in mares following use of a commercially available implant of deslorelin acetate (Ovuplant) to hasten ovulation revealed that treated mares not only had a greater interovulatory interval but also had greatly reduced plasma LH and FSH concentrations for approximately 10 d after ovulation. Although the mares in that experiment (Johnson et al., 2000) were short-cycled with PGF_{2α}, similar effects were noted for estrous cycles not altered by PGF_{2α} administration by Mumford et al. (1995) in mares administered 3 or 5 times the recommended dose of deslorelin implants and by Farquhar et al. (2001) and Vanderwall et al. (2001) for mares administered a single implant.

The prolonged suppression of gonadotropin secretion after ovulation in deslorelin-treated mares was characteristic of pituitary down-regulation induced by constant high GnRH input (Heber and Swerdloff, 1981; Nett et al., 1981; Sandow, 1983), generally characterized by a reduced sensitivity of pituitary gonadotropes to further GnRH stimulation (desensitization; Belchetz et al., 1978; Zilberstein et al., 1983). The results of Farquhar et al. (2001), who reported a reduced gonadotropin response to GnRH at 10 d after ovulation in deslorelin-treated mares, are consistent with the concept of down-regulation. Alternatively, the observed reduction in gonadotropin secretion may

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have been due to reduced endogenous GnRH input to the pituitary in treated mares (Crowder et al., 1986). The objective of the present experiment was to characterize the pituitary responsiveness to exogenous GnRH in the first 10 d after ovulation following deslorelin acetate implantation at the normal dosage for hastening ovulation in mares.

Materials and Methods

Twelve nonlactating, light horse mares between 4 and 16 yr of age were used beginning in April. All mares were in good body condition (6 to 8, Henneke et al., 1983) and were maintained on a combination of rye grass (winter) and native grass pastures. Mares were determined to be through the vernal transition period and cycling normally before use. All mares were checked daily with a vigorous stallion throughout the experiment for signs of behavioral estrus. In addition, the ovaries of each mare were examined via transrectal ultrasonography three times weekly (Monday, Wednesday, and Friday) until detection of a follicle at least 25 mm in diameter or until the mare showed signs of estrus, at which time daily examinations were initiated as well as daily blood sampling via jugular venipuncture. Once a follicle > 30 mm was detected for a given mare, she was administered either a single Ovuplant implant (n = 6) s. c. in the neck region or a sham injection (same needle size, no implant; n = 6) given in the same manner. Assignment to treatment group and administration of treatment were unknown to the personnel involved with on-farm data collection. After treatment, mares were followed with daily ultrasound exams until ovulation.

On d 1, 4, 7, and 10 following this first ovulation, each mare was challenged i.v. with 50 µg GnRH (Sigma), 7-mL blood samples were collected via an indwelling jugular catheter at -10, 0, 10, 20, 30, 45, 60, 90, and 120 min relative to GnRH injection. The

dose of GnRH (50 μ g) was determined in preliminary trials to be the smallest amount that consistently caused a measurable response in both LH and FSH concentrations.

Ultrasound examinations were also performed on the day of each GnRH challenge, after all blood samples had been collected, to determine follicular activity. Daily ultrasound exams were resumed once a mare subsequently returned to estrus and were continued through the second ovulation. Daily blood sampling was continued for 4 d after the second ovulation to confirm ovulation via progesterone concentrations.

All blood samples in the experiment were placed on ice until centrifugation at 1,200 x g for 15 min at 5°C. Plasma was harvested and stored at -15°C until assayed via radioimmunoassay for LH (Thompson et al., 1983a), FSH (Thompson et al., 1983c), and progesterone (Diagnostic Systems Laboratory). Intra- and interassay coefficients of variation and assay sensitivities were 6%, 9%, and 0.2 ng/mL for LH; 7%, 11%, and 1.4 ng/mL for FSH; and 5%, 8%, and 0.05 ng/mL for progesterone.

Data were analyzed via the GLM procedure of SAS (SAS Inst. Inc.). Data from repetitive sampling over time (daily samples) were analyzed in a split-plot ANOVA (Gill and Hafs, 1971) for a completely randomized design (Steel and Torrie, 1980). The main effect of treatment was tested with the mare within treatments term; the time factor and its interaction with treatment were tested with the residual error term. For data from the repeated GnRH challenges, a second split was included (day of challenge), and the data were analyzed as a split-split-plot design (Steel and Torrie, 1980) with all appropriate interactions. Differences between treatment groups in the treatment x day x minute interaction were assessed by the LSD-test (Steel and Torrie, 1980). Net areas under the GnRH-response curves for LH and FSH were calculated by summing the time x

concentration increments after subtracting the pre-GnRH average for each mare in each challenge; areas were analyzed separately for each day by one-way ANOVA (Steel and Torrie, 1980) due to heterogeneity of variances among the four challenge days.

Results

The diameter of the largest ovarian follicle on the day of treatment ($P = 0.89$) and the day before ovulation ($P = 0.36$) did not differ between groups (Table 4.1). Mares receiving a deslorelin implant ovulated an average of 2.0 d earlier ($P = 0.0003$) and had an interovulatory interval 4.4 d longer ($P = 0.0361$) than control mares (Table 4.1). After the first ovulation, the diameter of the largest follicle present on the GnRH challenge day was larger in deslorelin-treated mares on d 1 ($P = 0.0067$), but smaller ($P = 0.021$) on d 10 (Table 4.1) relative to controls.

Plasma LH concentrations from daily blood samples in control mares followed the expected rise and fall around ovulation and into diestrus (Figure 4.1a). In mares receiving deslorelin, LH concentrations were lower ($P < 0.05$) relative to those in control mares from d 0 to 5, d 7, and again on d 20 to 24 (Figure 4.1a). Daily plasma concentrations of FSH in control mares were low during estrus and followed the expected rise after ovulation (Figure 4.1b). Plasma FSH concentrations in deslorelin-treated mares were lower ($P < 0.05$) relative to controls on d 3 to 13 (Figure 4.1b). Plasma progesterone concentrations were not altered ($P = 0.99$) by deslorelin treatment (Figure 4.1c).

There were interactions ($P < 0.001$) among treatments, days, and minutes of blood sampling in the ANOVA for both LH and FSH concentrations in the four GnRH challenges. Relative to control mares, the LH (Figure 4.2) and FSH (Figure 4.3)

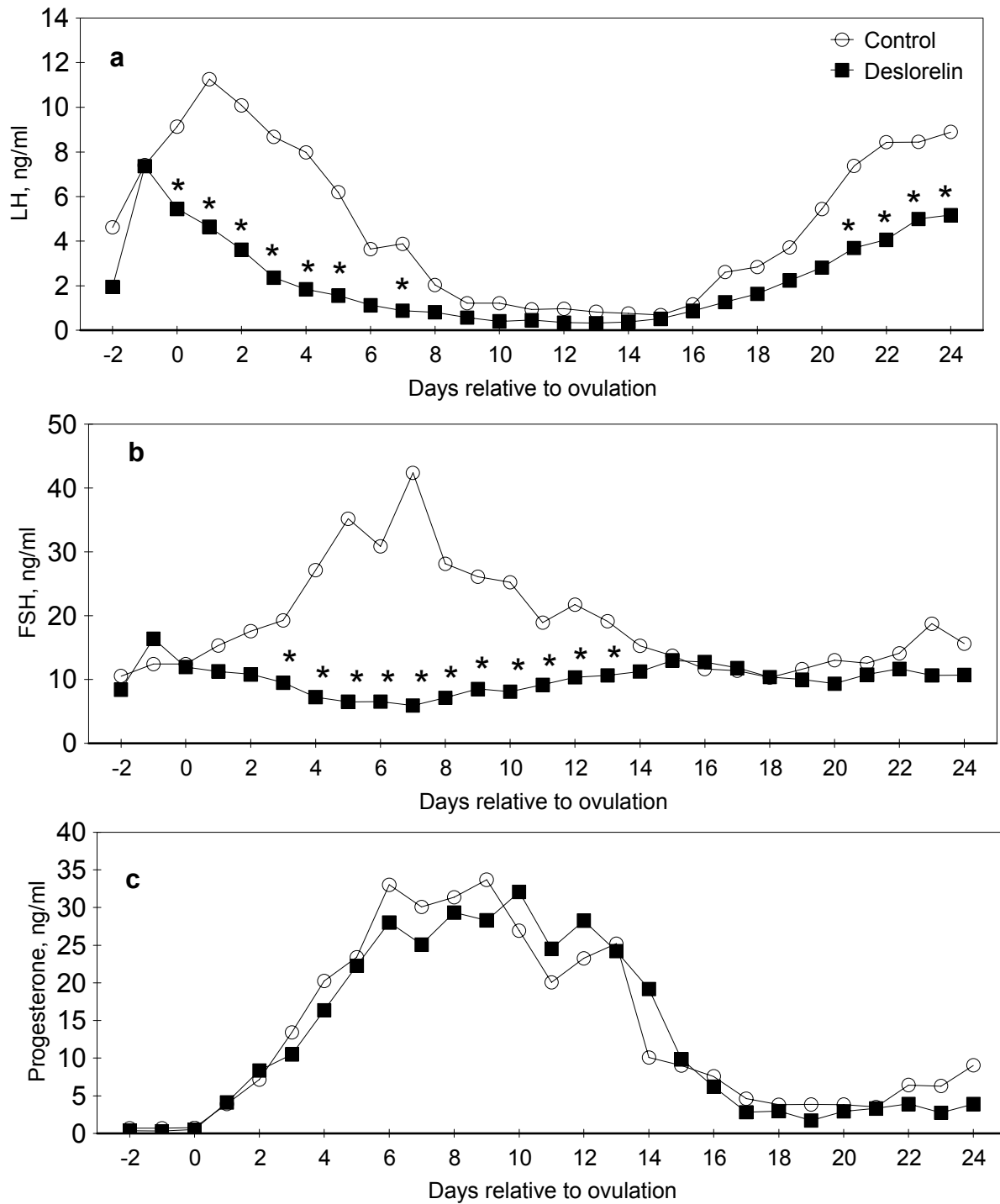


Figure 4.1 Plasma concentrations of LH (a), FSH (b), and progesterone (c) in mares administered a deslorelin implant or sham injection (control) during the first estrus. Data are expressed relative to the d of the first ovulation (d 0). Asterisks indicate differences ($P < 0.05$) between groups on specific days. The pooled SEM from the analyses of variance were 0.94, 2., and 3.6 ng/ml for LH, FSH and progesterone concentrations, respectively.

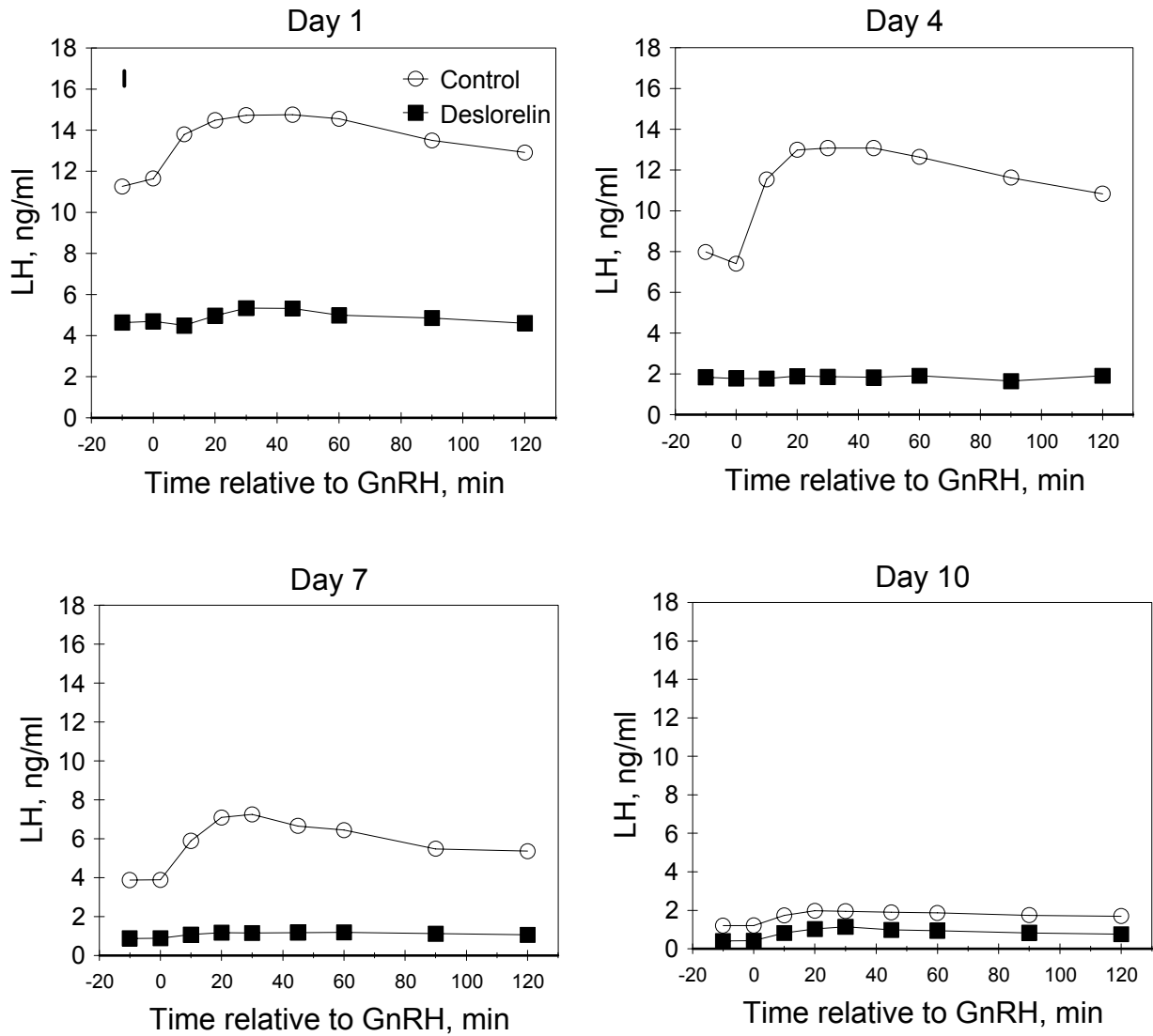


Figure 4.2 Plasma LH concentrations for GnRH challenges on d 1, 4, 7, and 10 following ovulation for mares administered a deslorelin implant or sham injection (control). There was a treatment x day x minute interaction ($P < 0.001$) in the analyses of variance. The vertical line indicates the $P < 0.05$ value. The pooled SEM was 0.32 ng/ml.

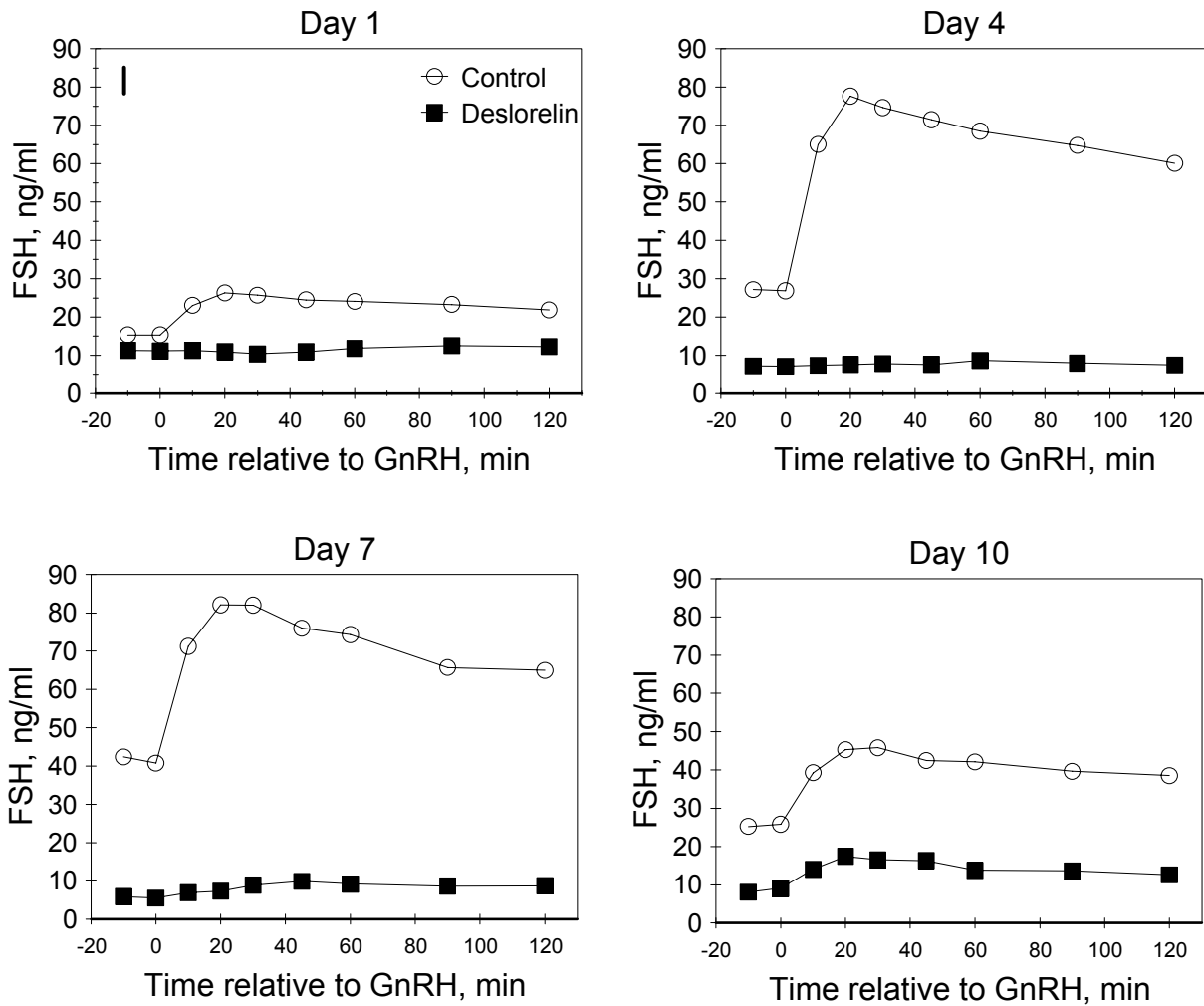


Figure 4.3 Plasma FSH concentrations for GnRH challenges on d 1, 4, 7, and 10 following ovulation for mares administered a deslorelin implant or sham injection (control). There was a treatment x day x minute interaction ($P < 0.001$) in the analyses of variance. The vertical line indicates the $P < 0.05$ value. The pooled SEM was 2.8 ng/ml.

Table 4.1. Intervals from treatment to ovulation, between first and second ovulation, and diameter of the largest follicle

Item	Control	Deslorelin	SEM	P-value
Treatment to ovulation, d	4.0	2.0	0.26	0.0003
Interovulatory interval, d	21.0	25.4	1.2	0.036
Diameter of the largest follicle, mm:				
Day of treatment	34.0	33.8	0.8	0.892
Day 1 before ovulation	41.5	40.0	1.1	0.36
Day of ovulation	17.7	20.7	2.2	0.356
Day 1 after ovulation	15.3	22.3	1.5	0.007
Day 4 after ovulation	19.7	15.3	3.1	0.339
Day 7 after ovulation	15.0	16.2	1.5	0.605
Day 10 after ovulation	19.2	13.0	1.6	0.021

responses to exogenous GnRH were suppressed in the deslorelin-treated mares on d 1, 4, and 7. In agreement, net areas under the curve for the LH ($P < 0.008$) and FSH ($P < 0.009$) responses to GnRH were lower in deslorelin-treated mares on d 1 and 4, and on d 7 for LH (Figure 4.4).

Discussion

Deslorelin acetate, as the commercially available implant, Ovuplant, has been proven many times to be effective at hastening ovulation in mares with pre-ovulatory follicles larger than 30 mm (McKinnon et al., 1993; Meinert et al., 1993; Jöchle and Trigg, 1994). Shortly after its commercial release in the United States, there were field reports that some treated mares displayed either prolonged interovulatory intervals or complete anestrus. The report of Johnson et al. (2000) confirmed, under controlled conditions, the deslorelin effect on interovulatory interval for mares short-cycled on d 7 with $\text{PGF}_{2\alpha}$ and extended the observations to include the reduction in LH and FSH concentrations following treatment. Similar results were reported by Farquhar et al. (2001) for mares not short-cycled with $\text{PGF}_{2\alpha}$, except that those authors found no

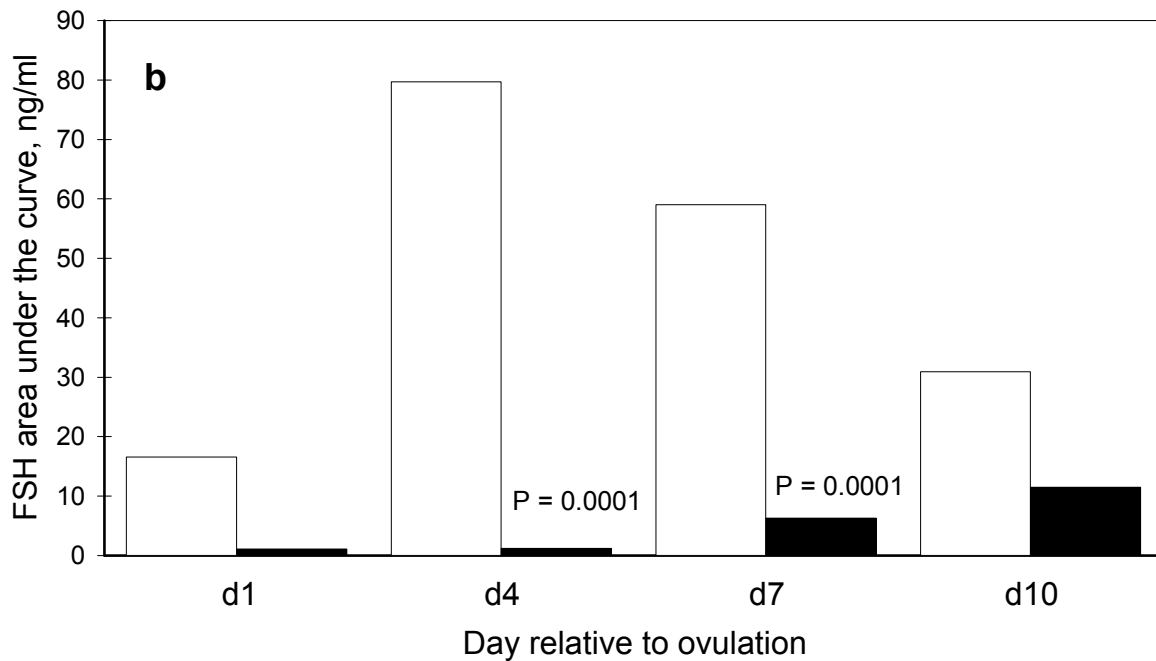
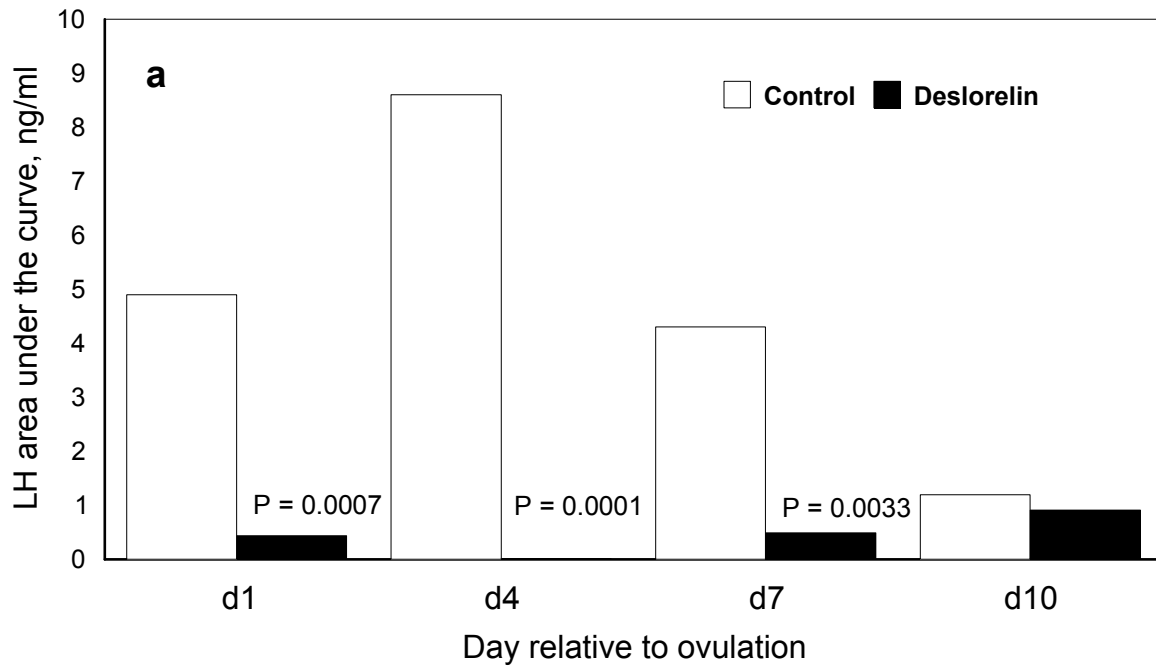


Figure 4.4 Area under the curve for LH (a) and FSH (b) responses to GnRH challenges performed on d 1, 4, 7, and 10 following ovulation for mares administered a deslorelin implant or sham injection (control). Each day was analyzed separately due to heterogeneity of variances; P-values for the differences between groups are shown. The pooled SEM were 0.69, 1.5, 0.81, and 0.31 ng/ml-1/h-1 for LH and 3.3, 12.4, 7.3, and 9.5 ng/ml-1/h-1 for FSH for d 1, 4, 7, and 10, respectively.

significant suppression of daily LH concentrations. Other researchers have reported similar extensions in the interovulatory interval of mares treated with deslorelin as compared with those receiving no ovulation induction drug and to those induced to ovulate with hCG (Morehead et al., 2000; Vanderwall et al., 2001).

The effects of deslorelin implantation on time to ovulation, interovulatory interval, and plasma concentrations of LH and FSH observed in the present experiment were virtually identical to previous results (Johnson et al., 2000). Because the mares in that experiment had been short-cycled with PGF_{2α} injection on d 7 after ovulation, it could not be determined whether the shortened diestrous period affected the results. It is now apparent that the normal, recommended dose of deslorelin has profound effects on gonadotropin secretion in mares not administered PGF_{2α} as well.

For both gonadotropins, there was an initial stimulation of plasma concentrations in response to deslorelin implantation. Although only daily blood samples were collected in the present experiment, the short-term release of LH and FSH after deslorelin implantation has been characterized (Donadeu, 1997; Johnson et al., 2001), with peaks occurring at 6 to 8 h after administration. This initial rise in both gonadotropins corresponds with the release of deslorelin: peak concentrations of deslorelin (about 400 pg/mL) occur at 3 to 6 h after implantation and decline to near baseline within 24 h (Donadeu, 1997). As in the present and previous (Johnson et al., 2000) experiments, concentrations of both gonadotropins decreased continuously for several days after the initial peak.

The diameter of the largest follicle on the ovaries did not differ between treatment groups on the day of treatment or 1 d before ovulation; however, the interval from

treatment to ovulation was shortened by 2 d. These data are consistent with previous studies with deslorelin implants (Jöchle and Trigg, 1994; Mumford et al., 1995). The diameter of the largest follicle was greater in the deslorelin-treated mares on d 1 following ovulation. This may have been due to incomplete suppression from the dominant follicle ovulating earlier or to the initial temporary rise in LH and FSH following implant administration. By d 10, the diameter of the largest follicle on the ovaries of the deslorelin-treated mares was smaller than on the ovaries of the control mares, which was likely due to the long-term gonadotropin suppression.

The possible causes of the reduction in plasma LH and FSH concentrations include 1) a reduction in endogenous GnRH secretion from the hypothalamus, 2) a desensitization of the pituitary that usually accompanies down-regulation, and 3) a depletion of LH and FSH in the pituitary gland itself. Previous research with continuous infusion of GnRH and the subsequent phenomenon of down-regulation in ewes indicated that pituitary LH and FSH contents were not dramatically altered (Crowder et al., 1986). The desensitization that occurred in those experiments was the major factor altering gonadotropin secretion, although a reduction in endogenous GnRH input was also concluded (Crowder et al., 1986). To differentiate between these two possibilities, the present experiment was performed.

Both the pre-GnRH concentrations of LH and the LH response to GnRH in control mares were characteristic of the stage of the estrous cycle (Alexander and Irvine, 1986; Ginther, 1992). Immediately after ovulation (d 1), plasma LH concentrations were at their peak, and the response to GnRH was minimal due to the high rate of secretion already occurring. With the drop in LH secretion as progesterone concentrations

increased, the LH response to GnRH increased, and maximal response occurred on d 4. Later, as the inhibitory effects of progesterone become greater, the LH response to GnRH diminished, and was very low by d 10. The elevated LH concentrations seen in control mares on d 20 to 24 coincided with their impending second ovulation, which was delayed in the deslorelin-treated mares.

Similar to LH, the pre-GnRH concentrations of FSH and the FSH response to GnRH in control mares were characteristic of the stage of the estrous cycle. Plasma FSH concentrations were low on d 1, due to residual effects of estradiol and inhibin from the dominant follicle (Ginther, 1992). On d 4 and 7, FSH had been freed of those inhibitory effects, and the response to GnRH was characteristic of diestrus. By d 10, the response had begun to diminish, likely due to the onset of the next follicular phase.

For mares receiving a deslorelin implant, there was virtually no LH or FSH response to the GnRH injections on d 1, 4, and 7 after ovulation. These results indicate that the pituitary glands of these mares were indeed relatively insensitive to the injected GnRH, which would support the concept of down-regulation as described in other species. The small dose of GnRH used for the challenges was chosen to approximate the endogenous (physiologic) amount of GnRH normally reaching the pituitary gland, as opposed to a saturating dose. In addition, it was felt that each challenge should be kept to a minimum to avoid carry-over effects from injection to injection (such that the challenges might become similar to replacement therapy for a quiescent hypothalamus). Although Alexander and Irvine (1986) calculated even smaller doses as physiologic, similar methods used in the present experiment determined 50 µg/mare to be the smallest dose that would give consistent responses for both LH and FSH.

Although down-regulation is a common complication in using GnRH and its analogs in other species, the horse was thought to be less susceptible to down-regulation (Irvine and Alexander, 1993). That is, horses continually infused with GnRH for 24 h had a steady increase in LH concentrations (Garcia and Ginther, 1975). Osmotic minipumps designed to deliver GnRH to horses constantly for 28 d maintained LH secretion (Hyland et al., 1987). Allen et al. (1987) administered long-term (28 d) GnRH agonist implants to horses and reported a prolonged gonadotropin secretion without refractoriness; Turner and Irvine (1992) reported similar results using higher doses. Daily administration of high levels of a GnRH agonist did not suppress LH levels in stallions, and luteal levels of LH were maintained in mares; however, follicular activity was diminished after 30 d of treatment (Montovan et al., 1990).

By d 10 after ovulation, the LH and FSH responses to GnRH were similar for the two groups of mares, indicating that the deslorelin effects were waning. Because the onset of the next estrus in the control mares thereafter began confounding the resting levels of LH and FSH and their responses to GnRH, subsequent GnRH challenges would not have been informative. One model that might be useful for determining the actual length of the deslorelin effect and the characteristics of recovery would be similar experiments in stallions or steroid-treated geldings (Johnson et al., 2001), which exhibit the same long-term inhibition of LH and FSH secretion.

Other than the hastening of the first ovulation (Jöchle and Trigg, 1994; Mumford et al., 1995) and few days delay in ovulation on the estrus subsequent to deslorelin administration, no other effects on the estrous cycle were observed. Given the major perturbation in plasma LH and FSH concentrations, one might expect a greater effect on

follicular populations and onset of the next estrus. The lack of effect on plasma progesterone concentrations after the first ovulation indicates that the formation and subsequent function of the corpus luteum are not dependent upon the normally high LH concentrations from the day of ovulation onward. Similar results have been reported for mares treated with testosterone propionate during mid-estrus (Thompson et al., 1983b) and the subsequent diestrus, in which LH concentrations during estrus were reduced by about 50%, whereas, the timing of ovulation and the function of the subsequent corpus luteum were unaffected.

In conclusion, the reductions in plasma LH and FSH concentrations following Ovuplant administration to mares are accompanied by an insensitivity to exogenous GnRH challenge for up to 7 d. Such desensitization is characteristic of the down-regulation phenomenon, which in other species has been shown to involve a reduction in GnRH receptor numbers and a perturbation of post-receptor mechanisms (Smith et al., 1983; Conn et al., 1987). Whether endogenous GnRH secretion is altered in these mares, as has been suggested for ewes (Crowder et al., 1986), and whether pituitary LH and FSH content is reduced, needs to be determined in future experiments.

CHAPTER V

EFFECTS OF DESLORELIN ACETATE IMPLANTATION ON LH AND FSH CONCENTRATIONS IN STALLIONS AND STEROID-TREATED GELDINGS

Introduction

The use of commercially available deslorelin acetate implants (Ovuplant) to hasten ovulation in mares is associated with an extended interovulatory interval and suppression of LH and FSH secretion for approximately 10 d after ovulation (Johnson et al., 2000) and an insensitivity of the pituitary to exogenous GnRH challenge (Johnson et al., 2002). Given that many of the LH and FSH responses to steroidal feedback in gonadectomized horses are similar for both sexes (Thompson et al., 1979, 1991; Reville-Moroz et al., 1984), male horses may provide a useful model for studying the suppressive effects of deslorelin implantation observed in mares. Thus, the two experiments described herein were designed to determine the effects of deslorelin implantation on LH and FSH secretion in stallions and in geldings administered progesterone and estradiol to mimic the normal estrous cycle changes in mares.

Materials and Methods

Experiment 1. Nine light horse stallions between 4 and 20 yr of age were used during June and July. They were housed in individual outdoor lots and were fed a commercially available, nutritionally balanced, pelleted ration plus free-choice grass hay to maintain body condition scores between 5 and 7 (Henneke et al., 1983).

The day of treatment was d 0. On that day, stallions were allotted to two groups such that age and breed type were evenly distributed in the groups. One group was then randomly selected to receive a single deslorelin implant (n = 5) and the other group a

sham injection (n = 4). All treatments were administered s.c. in the side of the neck. Samples of jugular blood were collected daily each morning from 5 d before through 13 d after injection. On d 0, additional blood samples were collected at 0, 4, 8, and 12 h relative to treatment. All blood samples were collected via jugular venipuncture into heparinized tubes and the plasma was harvested via centrifugation at 1,200 x g and stored at -15°C.

Experiment 2. Twelve light horse geldings between 6 and 16 yr of age were used during May and June. They were maintained as a group on native grass pasture and had body condition scores of 6 to 8 (Henneke et al., 1983).

All geldings were treated with estradiol and progesterone to mimic the normal changes in these steroid hormones during the estrous cycle (Ginther, 1992). Starting on d -20, all geldings received daily i.m. injections of progesterone (500 µg/kg BW; Sigma) in corn oil to mimic the diestrous period; these injections were given in the morning for a total of 17 d (through d -4). No injections were given on d -3. On d -2, -1, and 0, all geldings received twice daily i. m. injections (morning and evening) of estradiol (17.5 µg/kg BW; Sigma) to mimic the estrous period. On the morning of d 0, the geldings were randomly allotted to groups and administered either a single deslorelin implant (n = 6) or a sham injection (n = 6) s.c. in the neck. Daily (morning) injections of progesterone were again given on d 2 (125 µg/kg), 3 (250 µg/kg), and 4 through 15 (500 µg/kg). Samples of jugular blood were collected immediately before any injections every day from d -20 through d 15; additional blood samples were collected at 0, 4, 8, and 12 h relative to deslorelin or sham injections on d 0.

Sample and Statistical Analyses. All plasma samples in both experiments were analyzed for LH and FSH via radioimmunoassay previously validated for horse samples (Thompson et al., 1983a,c). Plasma samples from stallions in Exp. 1 were analyzed for testosterone using commercially available radioimmunoassay kit reagents (Diagnostic Systems Laboratory). Intra- and interassay coefficients of variation and assay sensitivities were 6%, 9%, and 0.2 ng/mL for LH; 7%, 11%, and 1.4 ng/mL for FSH; and 5%, 8%, and 0.02 ng/mL for testosterone.

Data were analyzed in each experiment via the GLM procedure of SAS (SAS Inst. Inc.). Data from repetitive sampling over time (daily samples) were analyzed in a split-plot ANOVA (Gill and Hafs, 1971) for a completely randomized design (Steel and Torrie, 1980). The main effect of treatment was tested with the horse within treatment term; the main effect of time and its interaction with treatment were tested with the residual error term. Differences between groups for each time period in the treatment x time interactions were assessed by the LSD-test (Steel and Torrie, 1980).

In Exp. 1, there was a large variation in gonadotropin concentrations among stallions before the onset of treatment, and in addition, their responses to treatment were multiplicative, not additive (varied proportionally with their pretreatment levels). To account for this, the first five data points were averaged for each stallion, and then all his subsequent data points (d 0 through 13) were expressed as a percentage of that mean (Steel and Torrie, 1980). Subsequent ANOVA as described above were performed on these data to determine treatment differences. In Exp. 2, gonadotropin concentrations for the two treatment groups differed ($P < 0.05$) during the first progesterone treatment phase (d -20 through -4), when all geldings were treated the same. To adjust for the

pretreatment differences between groups, the first 6 d of data were averaged for each gelding and subtracted from each of his subsequent data points; the resulting residuals were analyzed as described above to determine treatment differences (Steel and Torrie, 1980).

Results

Experiment 1. Plasma concentrations of both LH and FSH (Figure 5.1a,b), expressed as percent of pretreatment, were elevated ($P < 0.05$) in the treated stallions at 4, 8, and 12 h after deslorelin implant administration; in addition, plasma LH concentrations remained elevated ($P < 0.05$) at 24 h. After that, LH concentrations decreased ($P < 0.05$) to below control concentrations by d 3 and remained low through d 13. Plasma FSH concentrations in the treated stallions fell below ($P < 0.05$) control stallions on d 6 through 13. Plasma testosterone concentrations (Figure 5.1c) in the treated stallions followed a similar pattern to the gonadotropins in response to deslorelin implant administration, being elevated ($P < 0.05$) above controls at 4, 8, 12, 24, and 48 h. In contrast to the gonadotropins, testosterone concentrations only fell below ($P < 0.05$) control concentrations on d 11 and 13.

Experiment 2. On d -2, when all geldings began receiving estradiol injections, plasma LH concentrations (Figure 5.2a) began to increase. Above that increase, plasma LH concentrations in treated geldings increased ($P < 0.05$) rapidly above control concentrations at 4, 8, and 12 h after administration of the deslorelin-containing implant. Thereafter, plasma LH concentrations decreased and were below ($P < 0.05$) control concentrations on d 2 through 7, d 9, and d 11 through 15. During the mimicked diestrus

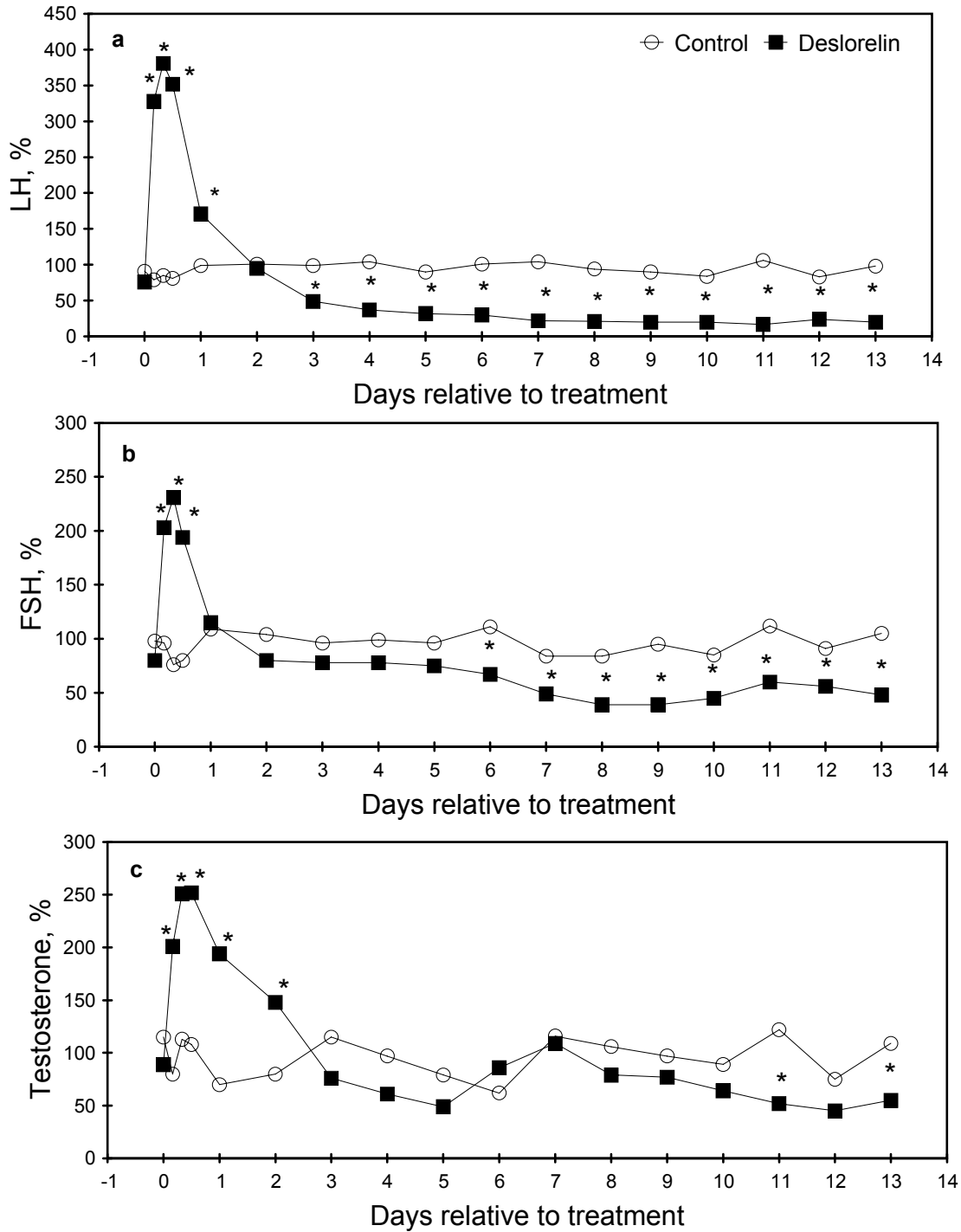


Figure 5.1 Plasma concentrations of LH (a), FSH (b), and testosterone (c) in deslorelin-treated and control stallions relative to the d of treatment (d 0). Data are expressed as a percentage of pretreatment values. Asterisks indicate differences ($P < 0.05$) between groups on specific days. The pooled SEM from the analyses of variance were 15.4, 9.9, and 20.6 percent for LH, FSH, and testosterone, respectively.

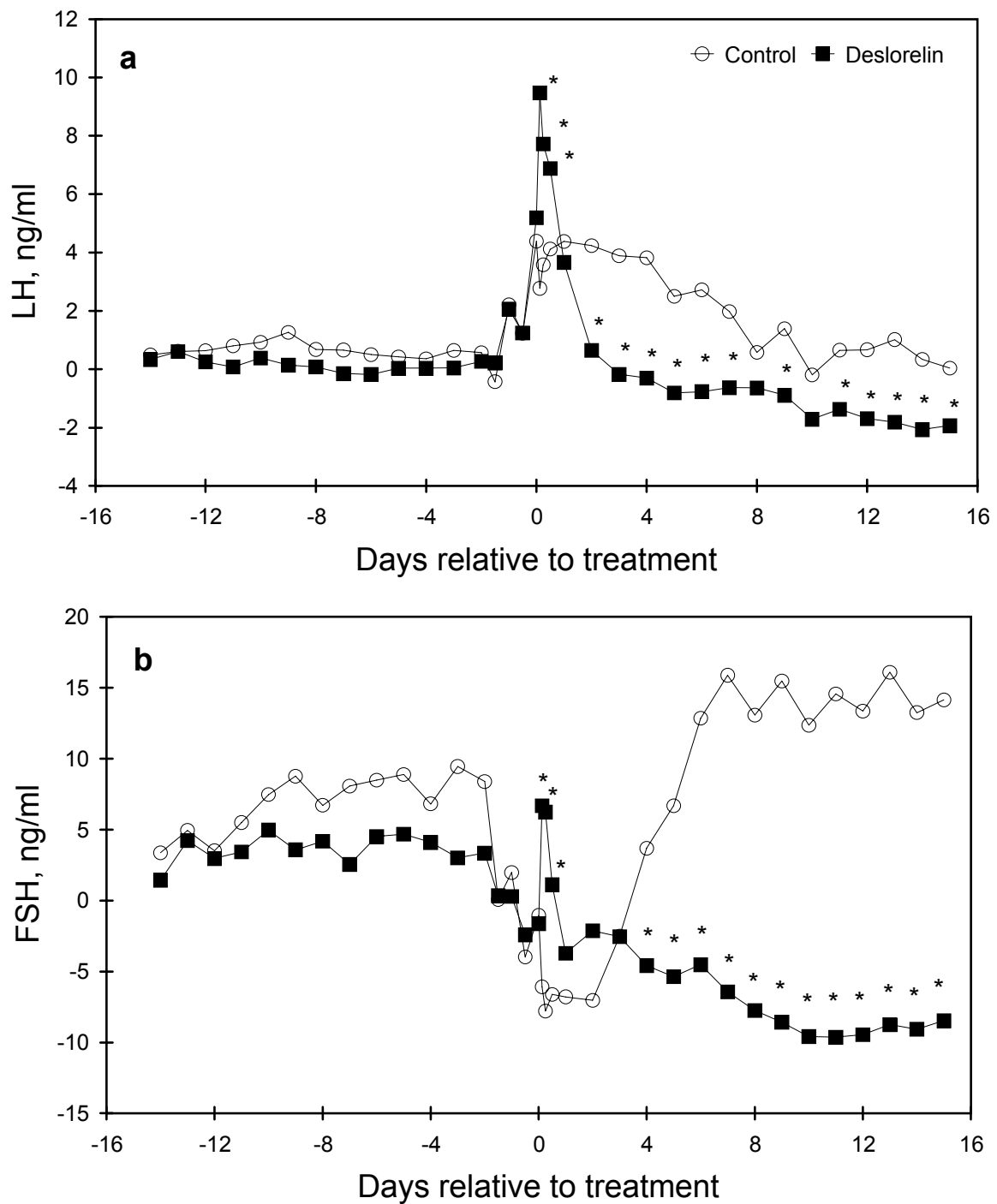


Figure 5.2 Plasma concentrations of LH (a) and FSH (b) in deslorelin-treated and control geldings relative to the d of treatment (d 0). Data are expressed as residuals due to pretreatment differences. Asterisks indicate differences ($P < 0.05$) between groups on specific days. The pooled SEM from the analyses of variance were 0.63 and 2.28 ng/ml for LH and FSH, respectively.

(d 2 through 15), when all geldings again received progesterone, plasma LH concentrations decreased in control geldings back to pre-estradiol concentrations.

Within 1 d after the geldings began receiving estradiol, plasma FSH concentrations (Figure 5.2b) began to decrease in both groups. Plasma FSH concentrations of treated geldings increased ($P < 0.05$) briefly on d 0 (4, 8, and 12 h) in response to the deslorelin implant, but began to decrease again by d 2 and were lower ($P < 0.05$) than control concentrations on d 4 through 12. Plasma FSH concentrations in control geldings rebounded after the switch from estradiol injections to progesterone injections.

Discussion

Administration of a deslorelin implant resulted in an initial short-term increase in the gonadotropin concentrations in the treated stallions just as seen in mares following administration of a deslorelin implant (Donadeu, 1997). This initial stimulation of LH and FSH secretion is coincident with the release of deslorelin from the implant (Donadeu, 1997). Deslorelin release from the implant reaches peak concentrations (approximately 400 pg/mL) at 3 to 6 h after implantation and declines to near baseline within 24 h. Concentrations of LH and FSH follow a similar pattern to deslorelin, reaching peak concentrations 6 to 8 h after implantation and reaching baseline by 24 to 48 h. After the initial increase, LH concentrations declined below controls for 11 d and were still suppressed when sampling was stopped. FSH concentrations did not decline as rapidly as LH; however, by d 6 the FSH concentrations of the treated stallions were below controls. Concentrations of FSH in the treated stallions were still below controls on d 13 when blood sampling was stopped. Testosterone concentrations followed the same initial

response as the gonadotropins following implant administration; however, there was no suppression of testosterone concentrations until the end of the sample period. It is unclear whether testosterone concentrations remained suppressed due to sampling having stopped after 13 d. If testosterone concentrations continued to be low, potential effects on libido and sperm production could occur, which may deserve further study.

The steroid treatment resulted in gonadotropin patterns similar to those seen during the estrous cycle in mares. The pretreatment with progesterone allowed both gonadotropins to stabilize. Estradiol treatment resulted in increasing LH concentrations in both groups prior to treatment with the deslorelin implant as well as decreasing FSH concentrations in both groups. Once progesterone treatment resumed LH concentrations declined in the controls while FSH concentrations increased. This model of steroid treatment appears successful in mimicking the gonadotropin changes seen in mares during an estrous cycle. The GnRH analogue implant was inserted on the morning of d 0, LH and FSH concentrations both increased in response to the deslorelin implant. After the initial increase, concentrations of both gonadotropins began to rapidly decline. The suppression of the gonadotropins is very similar to that reported in mares following treatment with the commercially available deslorelin implant, Ovuplant (Johnson et al., 2000, 2002). The suppression of the gonadotropins in the mares was from d 0 to 11 relative to ovulation, whereas the geldings' gonadotropins were still suppressed 15 d after the implant was administered.

In conclusion, after an initial short-term stimulation of LH and FSH secretion, deslorelin implantation caused a long-term suppression of both gonadotropins in stallions as well as in steroid-treated geldings. The suppression was very similar to that observed

in deslorelin-treated mares (Johnson et al., 2000, 2002). Because blood sampling was stopped after d 13 for the stallions and d 15 for the steroid-treated geldings, it is not clear exactly how long the suppression of LH and FSH may have persisted. Due to their responses, either of these male models may prove useful for studying this suppression of pituitary function in horses administered a slow-releasing deslorelin implant.

CHAPTER VI

EFFECTS OF MULTIPLE GNRH ANALOG (DESLORELIN ACETATE) IMPLANTS ON CYCLIC MARES

Introduction

Treatment with a commercially available GnRH analog (deslorelin acetate) implant (Ovuplant) results in a long-term (11 d) suppression of gonadotropin concentrations in mares (Johnson et al., 2000, 2002). Those mares treated with the deslorelin implant to hasten ovulation had a prolonged interovulatory interval (>4 d; Johnson et al., 2000, 2002), most likely due to the gonadotropin suppression. The gonadotropin suppression following treatment with a deslorelin implant was also observed in stallions and steroid-treated geldings (Chapter V). Although this resulting gonadotropin suppression is an important concern, the bigger problem is the small percentage of mares that exhibit complete ovarian shutdown following treatment (Johnson et al., 2000). This ovarian shutdown can last for weeks or months, resulting in mares being not breedable for the entire breeding season. During this ovarian shutdown, gonadotropin levels rebound and reach castrate-like levels (Johnson et al., 2000) even though the ovary remains quiescent.

Because only a small percentage of mares exhibit ovarian shutdown in response to Ovuplant, it has been hypothesized that such mares are more sensitive to the effects of deslorelin than the average mare. If this is true, then multiple implants may induce ovarian shutdown in a greater percentage of mares. A consistent means of producing ovarian shutdown is needed to study the underlying mechanism(s) responsible for its

occurrence. Thus, the present experiment was designed to determine whether multiple deslorelin implants would result in a higher portion of ovarian shutdown in mares.

Materials and Methods

Twenty-one light horse mares with body condition scores of 5 to 7 (Henneke et al., 1983) were used beginning in April. Mares were maintained on native grass pastures. All mares were determined to be though the transition period by progesterone concentrations >1 ng/mL for at least 12 d. Mares were teased daily with a vigorous stallion for signs of behavioral estrus. Ultrasound examinations were performed three times weekly for determination of follicular activity. Ultrasound exams were performed daily once a mare developed a follicle >25 mm in diameter or once she exhibited behavioral signs of estrus.

On the day a mare developed a follicle >30 mm, she was randomly assigned to one of three treatments: 1) 3 deslorelin implants administered s. c. in 1 d, 2) 1 deslorelin implant consecutively for 3 d, or 3) a single sham injection (control). Treatment assignments and administration were performed by personnel other than those performing the data collection and were unknown to those involved in data collection. Follicular activity was assessed on a daily basis from the first treatment through two ovulations or for 30 d. If a mare had not ovulated for the second time by d 30 relative to the first ovulation, ultrasound exams were performed twice weekly until she ovulated or until 90 d after the first ovulation.

Blood samples were collected via jugular venipuncture into heparinized tubes on a daily basis beginning when a follicle >25 mm was detected or when the mare showed behavioral signs of estrus; this sampling was continued until 4 d after the second

ovulation or 30 d after the first ovulation. If the mare had not ovulated by 30 d following the first ovulation, blood samples were collected twice weekly until the second ovulation or for 90 d from the first ovulation.

Additional samples of blood were collected at 0, 2, 4, 8, 12, 24, 26, 28, 32, 36, 48, 50, 52, 56, and 60 h relative to the first treatment. Plasma was harvested from all samples via centrifugation at 1,200 x g and stored at -15° C until assayed. All daily samples were analyzed for LH and FSH in radioimmunoassays previously validated for horses tissues (Thompson et al., 1983a,c). Progesterone was measured in daily samples using commercially available reagents (Diagnostic Systems Laboratory).

Data were analyzed via the GLM procedure of SAS (SAS Institute Inc.). Data from repetitive sampling over time (daily and hourly samples) were analyzed in a split-plot ANOVA (Gill and Hafs, 1971) for a completely randomized design (Steel and Torrie, 1980). Differences among treatment groups in the treatment x time interaction(s) were assessed by the LSD-test (Steel and Torrie, 1980).

Results

The interovulatory interval was extended ($P = 0.0004$) in both deslorelin-treated groups relative to control mares (36.8 vs 22.0 d, SEM = 2.1; Table 6.1), but the two treated groups did not differ from each other. The diameter of the largest follicle (Table 6.1) did not differ on the day of treatment, the day prior to ovulation (d -1), the day of ovulation (d 0), or on d 1 to 7 after the first ovulation. The diameter of the largest follicle in both groups of mares receiving deslorelin (Figure 6.1) was smaller ($P < 0.05$) than in control mares on d 8, 9, 11, and d 13 through 23 after the first ovulation. Mares receiving

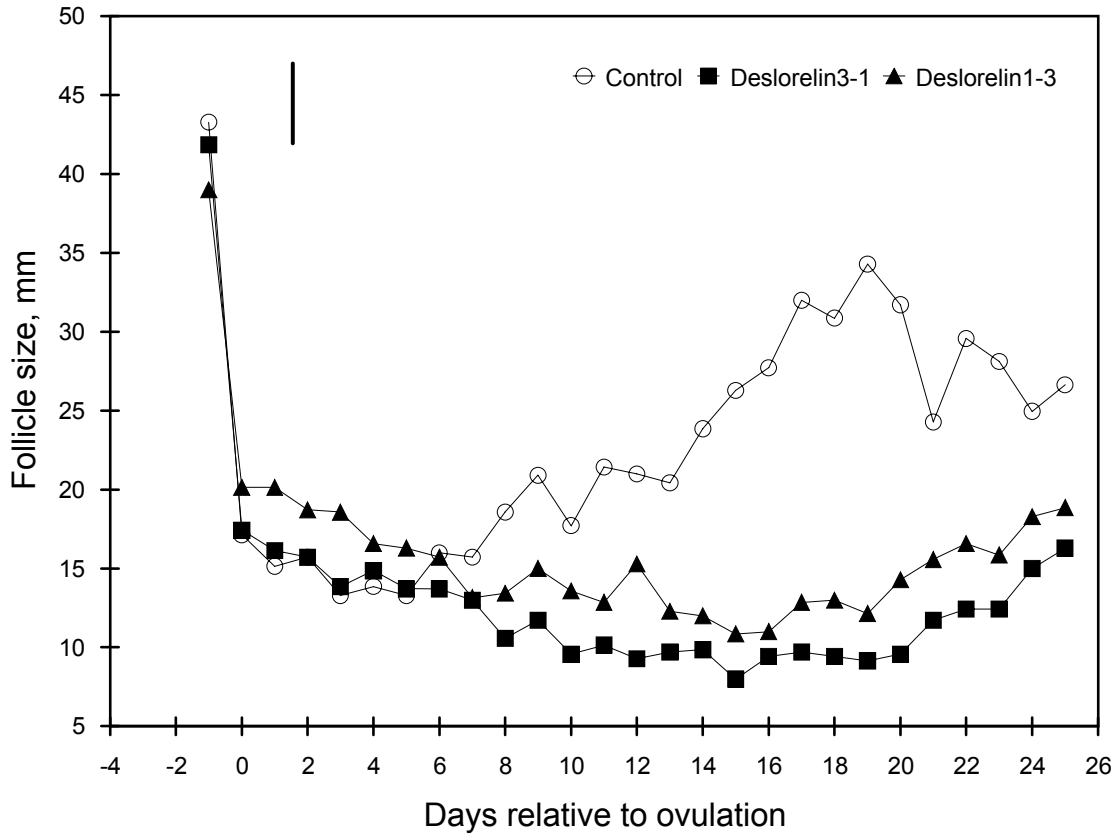


Figure 6.1 Diameter of the largest follicle for mares administered sham injections (control), treated with 3 deslorelin implants in 1 d (deslorelin3-1), or treated with 1 deslorelin implant per day for 3 consecutive days (deslorelin1-3) during the first estrus. Data are expressed relative to the day of ovulation on the first estrus (d 0) for all groups. The vertical line indicates the $P < 0.05$ value.

Table 6.1. Intervals from treatment to ovulation, between first and second ovulation, and diameter of the largest follicle.

Item	Control	Deslorelin3-1 ^a	Deslorelin1-3 ^b	SEM	P-value
Treatment to ovulation, d	3.9	3.3	2.1	0.79	0.32
Interovulatory interval, d	22.5	36.8	36.9	2.06	0.0004
First estrus length, d	6.33	7.14	4.71	1.08	0.297
Second estrus length, d	5.75	12.6	14.1	2.73	0.205
Return to estrus, d	16.0	27.0	23.0	2.57	0.089
Largest follicle on day of treatment, mm	32.4	32.7	34.1	0.60	0.128

^a Three implants administered in 1 d.

^b One implant administered daily for 3 d.

3 implants at treatment also had a reduced ($P < 0.05$) largest follicle than control mares on d 10 and 12. Diameter of the largest follicle was similar ($P > 0.05$) for the two treated groups throughout the experiment. There was no difference ($P > 0.05$) among groups in the length of estrus for the first or second ovulation (Table 6.1).

Relative to treatment, LH concentrations (Figure 6.2a) in mares receiving 3 implants at treatment were higher ($P < 0.05$) at 8 and 12 h following implantation than in control mares or in mares receiving one implant daily for 3 d. Concentrations of LH for both deslorelin treated groups were suppressed ($P < 0.05$) below control values on d 4, 5, and 6 relative to the day of treatment. Daily FSH concentrations for mares receiving 3 implants at treatment (Figure 6.2b) were elevated ($P < 0.05$) above controls at 4, 8, 12, 24, 26, 28, 32, and 36 h after implantation. Mares receiving three implants at treatment had higher ($P < 0.05$) FSH concentrations at 8 h following treatment than mares receiving one implant daily for 3 d. Concentrations of FSH for mares receiving 1 implant daily for 3 d were higher ($P < 0.05$) at 2, 4, 8, 12, 26, 32, and 36 h following treatment than those of control mares. Concentrations of FSH in mares receiving 3 implants at treatment were

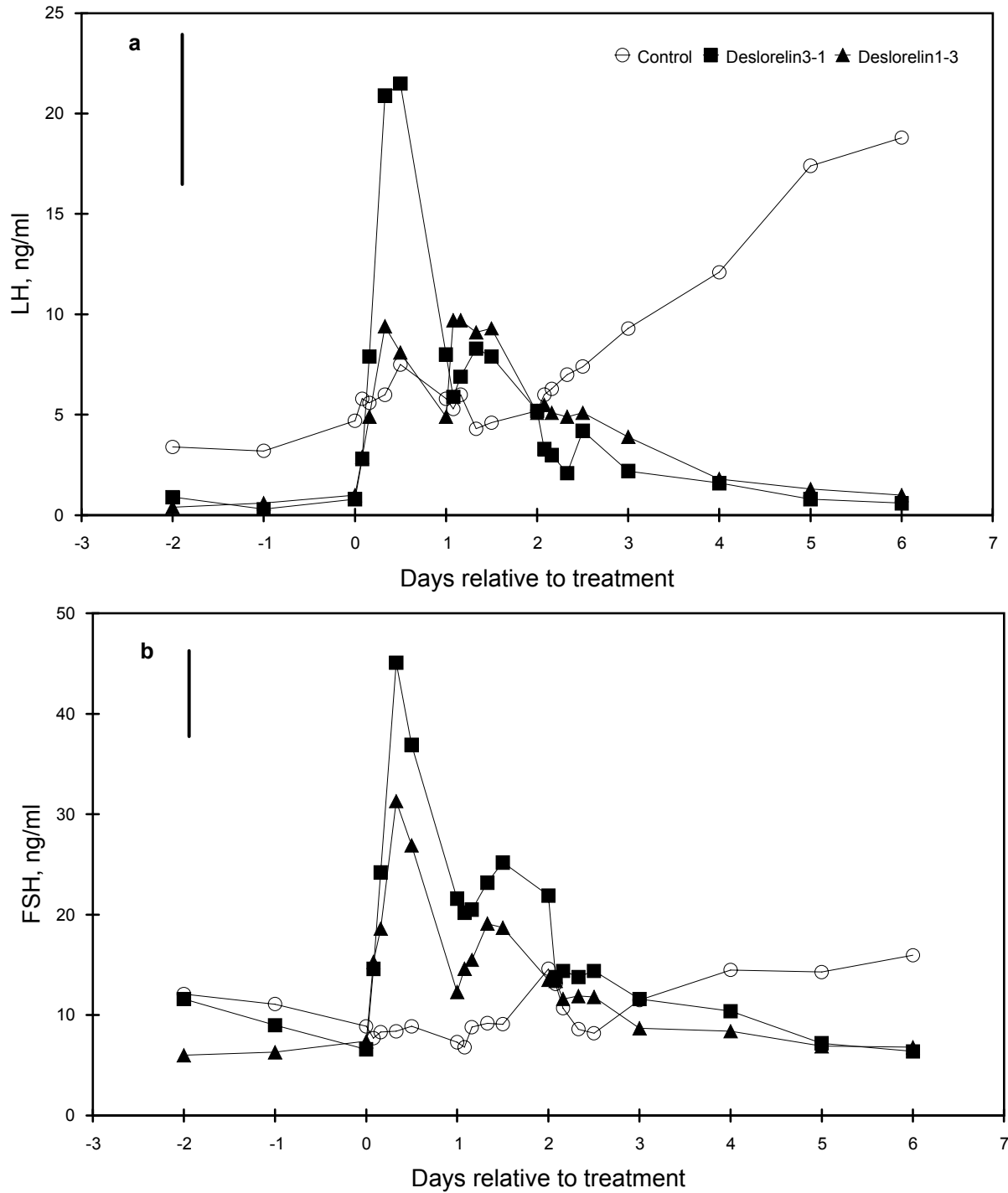


Figure 6.2 Plasma concentrations of LH (a) and FSH (b) in mares administered sham injections (control), treated with 3 deslorelin implants in 1 d (deslorelin3-1), or treated with 1 deslorelin implant per day for 3 consecutive days (deslorelin1-3) during the first estrus. Data are expressed relative to the day of treatment on the first estrus (d 0) for all groups. The vertical line indicates the $P < 0.05$ value. The pooled SEM from the analyses of variance were 2.42 and 2.76 ng/ml for LH and FSH, respectively.

suppressed ($P < 0.05$) below controls on d 6, while FSH concentrations in mares receiving one implant daily for 3 d were suppressed ($P < 0.05$) below controls on d 5 and 6.

Daily LH concentrations (Figure 6.3a) were lower ($P < 0.05$) in both deslorelin-treated groups than in controls on d -2, d 0 to 6, and d 18 to 25. Concentrations of LH in mares receiving one implant daily for 3 d were also lower ($P < 0.05$) than in controls on d -1. Daily plasma LH concentrations in the two deslorelin treated groups did not differ ($P > 0.05$) from one another throughout the study. Concentrations of FSH (Figure 6.3b) in the two treated groups were suppressed ($P < 0.05$) below control values on d 3, 5, 6, 7, 9 through 14, 17, 18, 19, and 20. Plasma FSH concentrations in mares receiving three implants at treatment were also lower ($P < 0.05$) than in controls on d 4, 8, 15, and 16. Daily plasma FSH concentrations in the two deslorelin-treated groups did not differ ($P > 0.05$) from each other throughout the study.

Concentrations of progesterone (Figure 6.4) were higher ($P < 0.05$) in the mares receiving 1 implant per day for 3 consecutive days than in controls on d 11, 12, and 13, and were higher ($P < 0.05$) than in the mares receiving 3 implants on the day of treatment on d 13. With the exception of d 11 to 13, progesterone concentrations were similar between all groups throughout the study. No mares exhibited complete ovarian shutdown as had been reported in the previous research.

Discussion

As in previous experiments (Johnson et al., 2000, 2002; Morehead et al., 2000 and Vanderwall et al., 2001), administration of the commercially available GnRH agonist, Ovuplant, to estrous mares resulted in an extension of the interovulatory interval

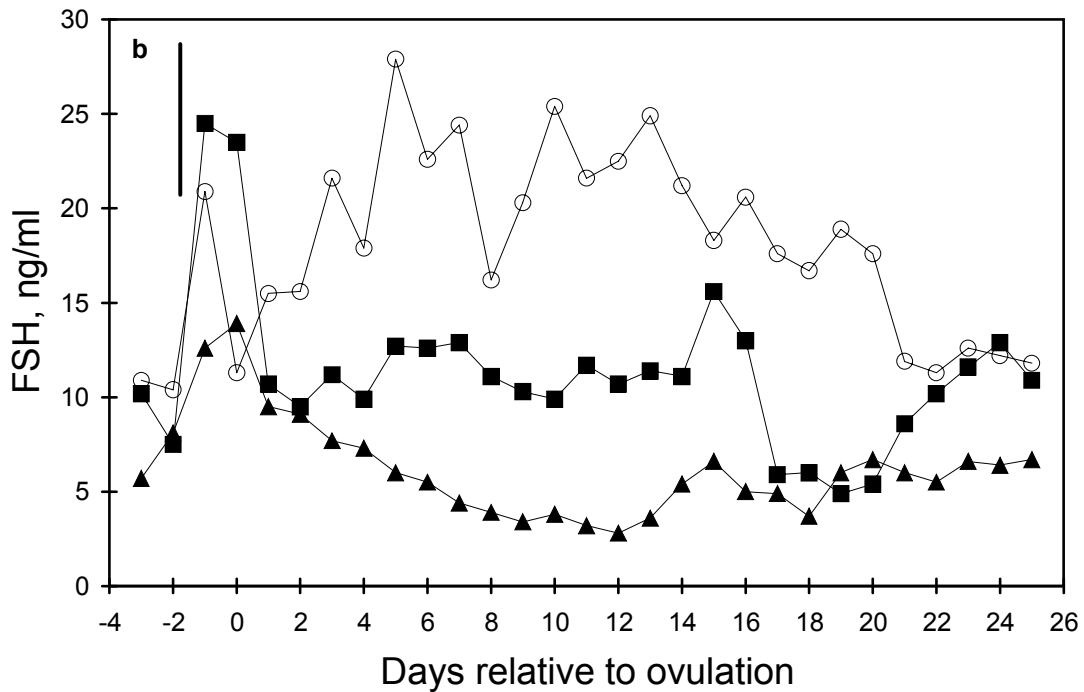
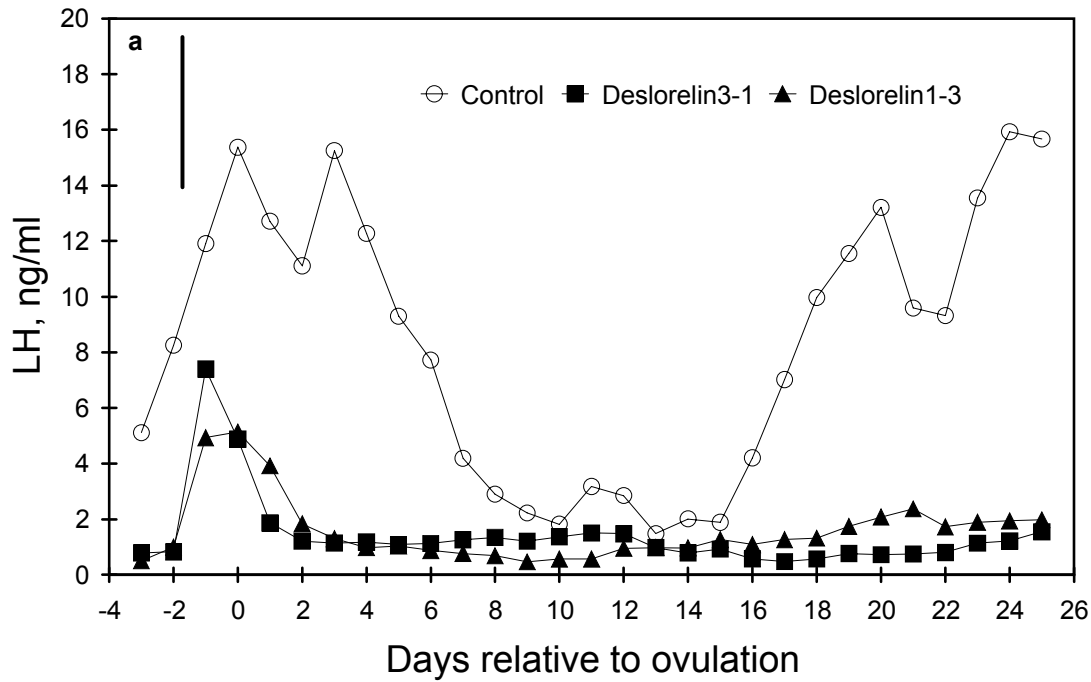


Figure 6.3 Plasma concentrations of LH (a) and FSH (b) in mares administered sham injections (control), treated with 3 deslorelin implants in 1 d (deslorelin3-1), or treated with 1 deslorelin implant per day for 3 consecutive days (deslorelin1-3) during the first estrus. Data are expressed relative to the day of ovulation on the first estrus (d 0) for all groups. The vertical line indicates the $P < 0.05$ value. The pooled SEM from the analyses of variance were 1.70 and 2.37 ng/ml for LH and FSH, respectively.

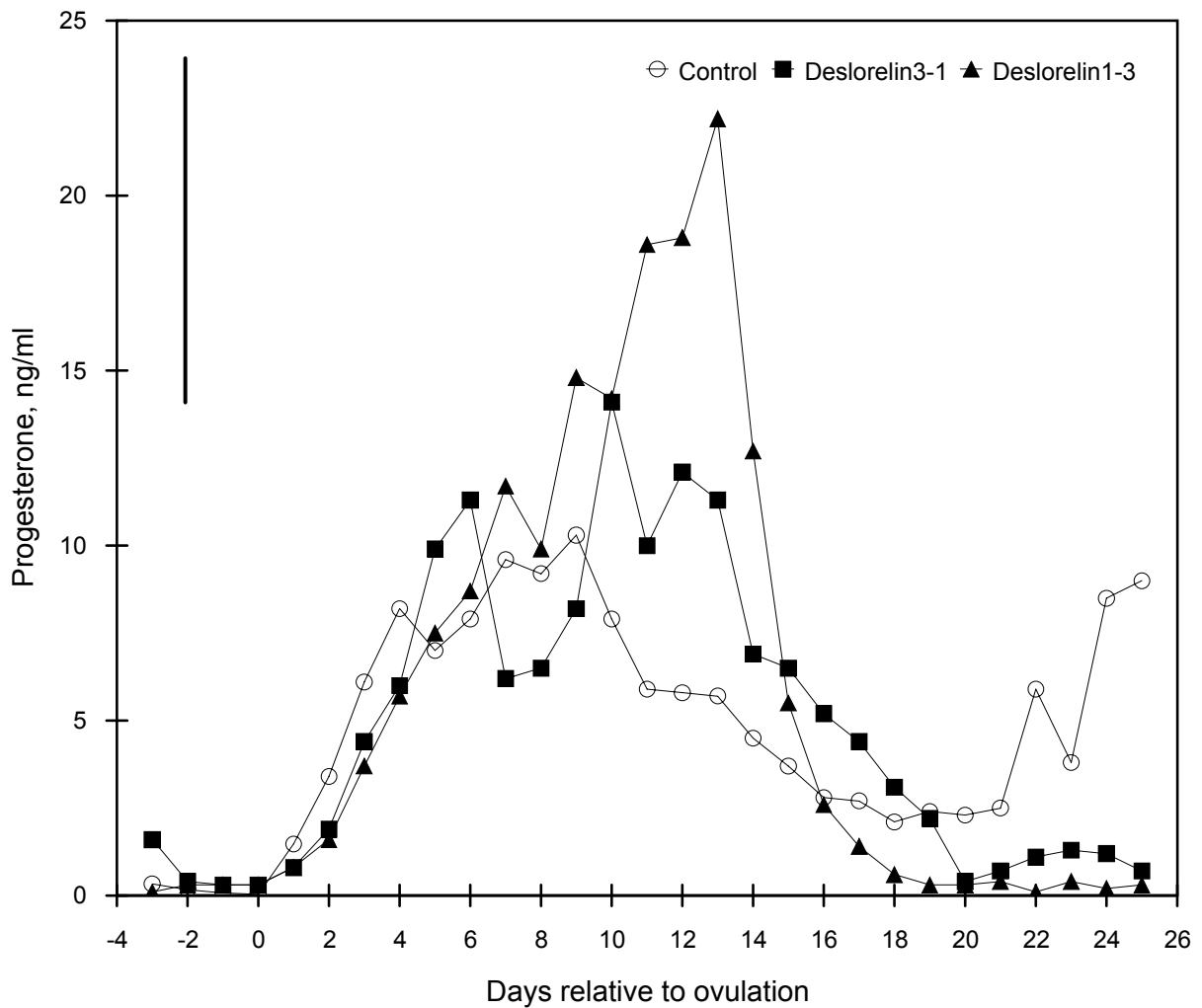


Figure 6.4 Plasma concentrations of progesterone in mares administered sham injections (control), treated with 3 deslorelin implants in 1 d (deslorelin3-1), or treated with 1 deslorelin implant per day for 3 consecutive days (deslorelin1-3) during the first estrus. Data are expressed relative to the day of ovulation on the first estrus (d 0) for all groups. The vertical line indicates the $P < 0.05$ value. The pooled SEM from the analysis of variance was 1.99 ng/ml for progesterone.

and a suppression of follicular activity and gonadotropin concentrations. In previous experiments (Johnson et al., 2000, 2002), a single deslorelin implant caused a consistent suppression in daily concentrations of both LH and FSH for 10 to 14 d. The suppression of the gonadotropins in the deslorelin-treated mares is likely the cause of the prolonged interovulatory interval seen in those mares (Johnson et al., 2000, 2002). The interovulatory interval for mares receiving a total of three deslorelin implants (36.8 d) was considerably longer than observed in mares receiving only one implant (25.4 d; Chapter IV). This agrees with the longer suppression of the gonadotropins (~20 to 25 d) seen in mares receiving 3 implants.

The reduced diameter of the largest follicle in the deslorelin-treated mares on d 8 though 23 is further evidence of the suppression of the gonadotropins, although the diameter of the largest follicle did not differ on d 24 or 25. This latter fact is due to the control mares already having ovulated the dominant follicle of the second estrus. The diameter of the largest follicle for the treated groups were similar throughout the study indicating that there is no difference in treating mares with three deslorelin implants at once or one deslorelin implant per day for three consecutive days.

Although treatment with a total of three deslorelin implants resulted in a longer suppression of follicular activity and gonadotropin secretion, it did not induce a long-term ovarian shutdown as seen in one mare previously (Johnson et al., 2000). That mare, which experienced total ovarian shutdown for the entire breeding season, was followed through the next breeding season. After an apparently normal ovulation, treatment with a single Ovuplant again resulted in complete ovarian shutdown for the second season. Thus, the question arises as to why certain mares exhibit complete ovarian shutdown in

response to a single deslorelin implant whereas the average, or typical, mare does not necessarily shut down after three implants. It is possible that the percentage of mares that are exceptionally sensitive to deslorelin is extremely small and by chance none were in the treatment groups in this experiment. This is reassuring for the breeder, because the chance of having a mare shut down in response to a deslorelin implant is minimal. However, some means of detecting the sensitive mares prior to treatment with deslorelin would be beneficial to avoid treating those mares.

CHAPTER VII

SUMMARY AND CONCLUSIONS

Mares treated with Ovuplant to hasten ovulation experience a prolonged interovulatory interval following the induced ovulation. This agrees with the field reports that began to circulate soon after the horse industry started using Ovuplant. Mares treated with the deslorelin containing implants had an extended interovulatory interval whether short-cycled with PGF_{2α} or allowed to cycle normally. The extended interovulatory interval reported in deslorelin-treated mares was accompanied by a decrease in follicular activity. Concentrations of LH and FSH were suppressed during the estrous cycle following deslorelin treatment. This suppression of gonadotropins is likely the reason for the diminished follicular activity as well as the lengthening of the interovulatory interval. The deslorelin-induced suppression of the gonadotropins is consistent in stallions and steroid-treated geldings.

Following treatment with deslorelin, mares experience a lack of pituitary responsiveness to exogenous GnRH stimulation. The insensitivity to exogenous GnRH and the suppression of the gonadotropins are indicative of the down-regulation seen in other species following treatment with GnRH or its analogs. Although the horse has been thought to be relatively resistant to down-regulation, it would appear that this analog, delivered in a slow-release implant, is capable of causing a short-lived (~10 to 14 d) down-regulation in the horse.

Treatment with multiple deslorelin implants on the same day or over three consecutive days results in a longer extension of the interovulatory interval. In spite of this, no mares exhibited the complete ovarian shutdown seen in one mare from the first

experiment. Therefore, the reason that some mares experience ovarian shutdown, characterized by anestrus-like ovaries under castrate-level gonadotropins, remains unknown.

REFERENCES

- Alexander, S. L., and C. H. G. Irvine. 1986. Effect of graded doses of gonadotrophin-releasing hormone on serum LH concentrations in mares in various reproductive states: Comparison with endogenously generated LH pulses. *J. Endocrinol.* 110:19-26.
- Alexander, S. L., and C. H. G. Irvine. 1987. Secretion rates and short-term patterns of gonadotrophin-releasing hormone, FSH and LH throughout the periovulatory period in the mare. *J. Endocrinol.* 114:351-362.
- Alexander, S. L., and C. H. G. Irvine. 1993. FSH and LH. Pages 45-56 in *Equine Reproduction*. A. O. McKinnon and J. L. Voss, eds. Lea & Fibiger, Philadelphia, PA.
- Allen, W. R., M. W. Sanderson, R. E. S. Greenwood, D. R. Ellis, J. S. Crowhurst, D. J. Simpson, and P. D. Rosedale. 1987. Induction of ovulation in anoestrous mares with a slow-release implant of a GnRH analogue (ICI 118 630). *J. Reprod. Fertil.* 35 (Suppl.):469-478.
- Bailey, V. E., and R. H. Douglas. 1977. Induction of ovulation in seasonally-anovulatory mares with GnRH. Page 336 in *Proc. Am. Soc. Anim. Sci.*, Madison, WI. (Abstr.)
- Belchetz, P. E., T. M. Plant, Y. Nakai, E. J. Keogh, and E. Knobil. 1978. Hypophysial responses to continuous and intermittent delivery of hypothalamic gonadotropin-releasing hormone. *Science* 202:631-633.
- Clayton, R. N. 1989. Gonadotrophin-releasing hormone: Its actions and receptors. *J. Endocrinol.* 120:11-19.
- Conn, P. M., D. Staley, C. Harris, W. V. Andrews, W. C. Gorospe, C. A. McArdle, W. R. Huckle, and J. Hansen. 1986. Mechanism of action of gonadotropin releasing hormone. *Annu. Rev. Physiol.* 48:495-513.
- Conn, P. M., W. R. Huckle, W. V. Andrews, and C. A. McArdle. 1987. The molecular mechanism of action of gonadotropin releasing hormone (GnRH) in the pituitary. *Recent Prog. Horm. Res.* 43:29-68.
- Crowder, M. E., R. D. Herring, and T. M. Nett. 1986. Rapid recovery of gonadotroph function after down-regulation of receptors for GnRH in ewes. *J. Reprod. Fertil.* 78:577-585.
- Donadeu, F. F. X. 1997. Evaluation of deslorelin during the breeding season and of sulpiride during seasonal anestrus for induction of ovulation in mares. M.S. Thesis, Louisiana State Univ., Baton Rouge.

- Dyer, R. G., and J. E. Robinson. 1989. The LHRH pulse generator. *J. Endocrinol.* 123:1-2.
- Evans, M. J., and C. H. G. Irvine. 1976. Measurement of equine FSH and LH: Response of anestrus mares to gonadotropin-releasing hormone. *Biol. Reprod.* 15:477-484.
- Evans, M. J., and C. H. G. Irvine. 1977. Induction of follicular development, maturation and ovulation by gonadotropin releasing hormone administration to acyclic mares. *Biol. Reprod.* 16:452-456.
- Evans, M. J., and C. H. G. Irvine. 1979. Induction of follicular development and ovulation in seasonally acyclic mares using gonadotrophin-releasing hormones and progesterone. *J. Reprod. Fertil. Suppl.* 27:113-121.
- Farquhar, V. J., P. M. McCue, T. M. Nett, and E. L. Squires. 2001. Effect of deslorelin acetate on gonadotropin secretion and ovarian follicle development in cycling mares. *J. Am. Vet. Med. Assoc.* 218:749-752.
- Fitzgerald, B. P., K. D. Peterson, and P. J. Silvia. 1993. Effect of constant administration of a gonadotropin-releasing hormone agonist on reproductive activity in mares: Preliminary evidence on suppression of ovulation during the breeding season. *Am. J. Vet. Res.* 54:1746-1751.
- Fitzgerald, B. P., A. F. Micka, and S. L. Meyer. 1990. Suppression of circulating concentrations of luteinizing hormone (LH) in ovariectomised (OVX) mares by constant administration of an agonist analogue (wy-40972) of gonadotrophin-releasing hormone (GnRH). Pages 106-107 in *Proc. Internatl. Symp. Equine Reprod.*, Deauville, France. (Abstr.)
- Fraser, H. M. 1981. Luteinizing hormone-releasing hormone and fertility control. *Oxford Rev. Reprod. Biol.* 3:1-48.
- Garcia, M. C., and O. J. Ginther. 1975. Plasma luteinizing hormone concentration in mares treated with gonadotropin-releasing hormone and estradiol. *Am. J. Vet. Res.* 36:1581-1584.
- Garcia, M. C., and O. J. Ginther. 1978. Regulation of plasma LH by estradiol and progesterone in ovariectomized mares. *Biol. Reprod.* 19:447-453.
- Garza, F., Jr., D. L. Thompson, Jr., D. D. French, J. J. Wiest, R. L. St. George, K. B. Ashley, L. S. Jones, P. S. Mitchell, and D. R. McNeill. 1986. Active immunization of intact mares against gonadotropin releasing hormone: Differential effects on secretion of luteinizing hormone and follicle-stimulating hormone. *Biol. Reprod.* 35:347-352.

- Gánheim, A., A. Gánheim, and W. Jöchle. 1995. Acceleration and timing of fertile ovulation in cyclic mares with a deslorelin implant. *Acta. Vet. Scand.* 36:393-400.
- Gill, J. L., and H. D. Hafs. 1971. Analysis of repeated measurements of animals. *J. Anim. Sci.* 33:331-336.
- Ginther, O. J. 1992. *Reproductive Biology of the Mare: Basic and Applied Aspects.* Equiservices, Cross Plains, WI.
- Ginther, O. J., and B. C. Wentworth. 1974. Effect of a synthetic gonadotropin-releasing hormone on plasma concentrations of luteinizing hormone in ponies. *Am. J. Vet. Res.* 35:79-81.
- Guyton, A. C., and J. E. Hall. 1996. *Textbook of Medical Physiology.* 9th ed. W. B. Saunders Co., Philadelphia, PA.
- Hart, P. J., E. L. Squires, K. J. Imel, and T. M. Nett. 1984. Seasonal variation in hypothalamic content of gonadotropin-releasing hormone (GnRH), pituitary receptors for GnRH, and pituitary content of lutenizing hormone and follicle-stimulating hormone in the mare. *Biol. Reprod.* 30:1055-1062.
- Heber, D., and R. S. Swerdloff. 1981. Down-regulation of pituitary gonadotropin secretion in postmenopausal females by continuous gonadotropin-releasing hormone administration. *J. Clin. Endocrinol. & Metab.* 52:171-172.
- Heinze, H., and E. Klug. 1975. The use of Gn-RH for controlling the oestrous cycle of the mare (preliminary report). *J. Reprod. Fertil. Suppl.* 23:275-277.
- Henneke, D. R., G. D. Potter, J. L. Kreider, and B. F. Yeates. 1983. Relationship between condition score, physical measurements and body fat percentage in mares. *Equine Vet. J.* 15:371-376.
- Hyland, J. H., P. J. Wright, I. J. Clarke, R. S. Carson, D. A. Langsford, and L. B. Jeffcoat. 1987. Infusion of gonadotrophin-releasing hormone (GnRH) induces ovulation and fertile oestrus in mares during seasonal anoestrus. *J. Reprod. Fertil. Suppl.* 35:211-220.
- Irvine, C. H. G., and S. L. Alexander. 1993. GnRH. Pages 37-44 in *Equine Reproduction.* A. O. McKinnon and J. L. Voss, eds. Lea & Fibiger, Philadelphia, PA.
- Irvine, C. H. G. 1983. Gonadotropin-releasing hormone. *J. Equine Vet. Sci.* 3:168-170.
- Irvine, D. S., B. R. Downey, W. G. Parker, and J. J. Sullivan. 1975. Duration of oestrus and time of ovulation in mares treated with synthetic Gn-RH (AY-24,031). *J. Reprod. Fertil. Suppl.* 23:279-283.

- Jöchle, W., and T. E. Trigg. 1994. Control of ovulation in the mare with Ovuplant, a short-term release implant (STI) containing the GnRH analogue deslorelin acetate: Studies from 1990 to 1994. (A review). *J. Equine Vet. Sci.* 14:632-644.
- Johnson, C. A., D. L. Thompson, Jr., and J. A. Cartmill. 2001. Effects of Ovuplant™ on LH and FSH secretion in stallions and steroid-treated geldings. Pages 464-465 in *Proc. 17th Equine Nutr. Physiol. Symp.* Lexington, KY.
- Johnson, C. A., D. L. Thompson, Jr., K. M. Kulinski, and A. M. Guitreau. 2000. Prolonged interovulatory interval and hormonal changes in mares following the use of Ovuplant™ to hasten ovulation. *J. Equine Vet. Sci.* 20:331-336.
- Johnson, C. A., D. L. Thompson, Jr., and J. A. Cartmill. 2002. Pituitary responsiveness to GnRH in mares following deslorelin acetate implantation to hasten ovulation. *J. Anim. Sci.* 80:(In press).
- Knobil, E. 1980. The neuroendocrine control of the menstrual cycle. *Recent Prog. Horm. Res.* 36:53-88.
- Lubbecke, M., E. Klug, H. O. Hoppen, and W. Jöchle. 1994. Attempts to synchronize estrus and ovulation in mares using progesterone (CIDR-B) and GnRH-analog deslorelin. *Reprod. Domest. Anim.* 29:305-314.
- Loy, R. G., and J. P. Hughes. 1966. The effects of human chorionic gonadotrophin on ovulation, length of estrus and fertility in the mare. *Cornell Vet.* 56:41-50.
- McKinnon, A. O., A. M. Nobellius, S. Tarrida del Marmol Figueroa, J. Skidmore, J. R. Vasey, and T. E. Trigg. 1993. Predictable ovulation in mares treated with an implant of the GnRH analogue deslorelin. *Equine Vet. J.* 25:321-323.
- McKinnon, A. O., J. R. Vasey, T. B. Lescun, and T. E. Trigg. 1997. Repeated use of a GnRH analogue deslorelin (Ovuplant) for hastening ovulation in the transitional mare. *Equine Vet. J.* 29:153-155.
- Meinert, C., J. F. Silva, I. Kroetz, E. Klug, T. E. Trigg, H. O. Hoppen, and W. Jöchle. 1993. Advancing the time of ovulation in the mare with a short-term implant releasing the GnRH analogue deslorelin. *Equine Vet. J.* 25:65-68.
- Monahan, M. W., M. S. Amoss, H. A. Anderson, and W. Vale. 1973. Synthetic analogs of the hypothalamic luteinizing hormone-releasing factor with increased agonist of antagonist properties. *Biochemistry* 12:4616-4620.
- Montovan, S. M., P. P. Daels, J. Revier, J. P. Hughes, G. H. Stabenfeldt, and B. L. Lasley. 1990. The effect of a potent GnRH agonist on gonadal and sexual activity in the horse. *Theriogenology* 33:1305-1321.

- Morehead, J. A., and T. L. Blanchard. 2000. Clinical experience with deslorelin (Ovuplant™) in a Kentucky Thoroughbred broodmare practice (1999). *J. Equine Vet. Sci.* 20:358-402.
- Mumford, E. L., E. L. Squires, W. Jöchle, L. A. Harrison, T. M. Nett, and T. E. Trigg. 1995. Use of deslorelin short-term implants to induce ovulation in cycling mares during three consecutive estrous cycles. *Anim. Reprod. Sci.* 39:129-140.
- Meyers, P. J., T. Bowman, G. Blodgett, H. S. Conboy, T. Gimenez, M. P. Reid, B. C. Taylor, J. Thayer, W. Jöchle, and T. E. Trigg. 1997. Use of the GnRH analogue, deslorelin acetate, in a slow-release implant to accerlerate ovulation in oestrous mares. *Vet. Rec.* 140:249-252.
- Nett, T. M., M. E. Crowder, G. E. Moss, and T. M. Duello. 1981. GnRH-receptor interaction. V. Down-regulation of pituitary receptors for GnRH in ovariectomized ewes by infusion of homologous hormone. *Biol. Reprod.* 24:1145-1155.
- Palmer, E., and P. Quellier. 1988. Uses of LHRH and analogues in the mare. Pages 339-346 in *Proc. 5th Internatl. Cong. Anim. Reprod. Artif. Insem.*, Dublin, Ireland.
- Reville-Moroz, S. I., D. L. Thompson, Jr., L. F. Archbald, and L. M. Olsen. 1984. Effects of in vivo administration of testosterone propionate on in vitro production of follicle-stimulating hormone and lutenizing hormone by pituitaries of pony mares. *Biol. Reprod.* 30:673-678.
- Roser, J. F., B. L. Kiefer, J. W. Evans, D. P. Nealy, and C. A. Pacheco. 1979. The development of antibodies to human chorionic gonadotrophin following its repeated injection in the cyclic mare. *J. Reprod. Fertil. Suppl.* 27:173-179.
- Sandow, J. 1983. The regulation of LHRH action at the pituitary and gonadal receptor level: A review. *Psychoneuroendocrinology* 8:277-297.
- Sandow, J. 1978. Structure-activity relationships in the LH-RH molecule. Pages 49-70 in *Control of Ovulation*. D. B. Crighton, N. B. Haynes, N. B. Foxcroft, G. R. Lamming, and G. E. Lamming eds. Butterworth, London.
- Safir, J. M., R. G. Loy, and B. P. Fitzgerald. 1987. Inhibition of ovulation in the mare by active immunization against LHRH. *J. Reprod. Fertil. Suppl.* 32:261-267.
- Savoy-Moore, F. T., and K. H. Swartz. 1987. Several GnRH stimulation frequencies differentially release FSH and LH from isolated, perfused rat anterior pituitary cells. *Adv. Exp. Med. Biol.* 219:641-645.

- Sharp, D. C., and W. R. Grubaugh. 1987. Use of push-pull perfusion techniques in studies of gonadotrophin-releasing hormone secretion in mares. *J. Reprod. Fertil. Suppl.* 35:289-296.
- Silvia, P. J., E. L. Squires, and T. M. Nett. 1986. Changes in the hypothalamic-hypophyseal axis of mares associated with seasonal reproductive recrudescence. *Biol. Reprod.* 35:897-905.
- Smith, M. A., M. H. Perrin, and W. W. Vale. 1983. Desensitization of cultured pituitary cells to gonadotropin-releasing hormone: evidence for post-receptor mechanism. *Mol. Cell. Endocrinol.* 30:85-96.
- Squires, E. L., R. A. Wallace, J. L. Voss, B. W. Pickett, and R. K. Shideler. 1981. The effectiveness of PGF 2α , HCG, and GnRH for appointment breeding of mares. *J. Equine Vet. Sci.* 1:5-9.
- Squires, E.L., D. M. Moran, M. E. Farlin, D. J. Jasko, T. J. Keefe, S. A. Meyers, E. Figueiredo, P. M. McCue, and W. Jöchle. 1994. Effect of dose of GnRH analog on ovulation in mares. *Theriogenology* 41:757-769.
- Steel, R. G. D. and J. H. Torrie. 1980. *Principles and Procedures of Statistics: A Biometrical Approach*. 2nd ed. McGraw-Hill Book Co., New York.
- Strauss, S. S., C. L. Chen, S. P. Kalra, and D. C. Sharp. 1979. Localization of gonadotrophin-releasing hormone (GnRH) in the hypothalamus of ovariectomized pony mares by season. *J. Reprod. Fertil Suppl.* 27:123-129.
- Sullivan, J. J., W. G. Parker, and L. L. Larson. 1973. Duration of estrus and ovulation time in nonlactating mares given HCG during three successive estrous periods. *J. Am. Vet. Med. Assoc.* 163:895-898.
- Thompson, D. L., Jr, B. W. Pickett, E. L. Squires, and T. M. Nett. 1979. Effect of testosterone and estradiol-17 β alone and in combination on LH and FSH concentrations in pituitary and blood serum of geldings and in serum after GnRH. *Biol Reprod* 21:1231-1237.
- Thompson, D. L., Jr., R. A. Godke, and E. L. Squires. 1983a. Testosterone effects on mares during synchronization with altrenogest: FSH, LH, estrous detection and pregnancy rate. *J. Anim. Sci.* 56:678-686.
- Thompson, D. L., Jr., S. I. Reville, D. J. Derrick, and M. P. Walker. 1983b. Effects of testosterone, dihydrotestosterone and estradiol on gonadotropin release after gonadotropin releasing hormone administration in cyclic mares. *Biol. Reprod.* 29:970-976.

- Thompson, D. L., Jr., S. I. Reville, M. P. Walker, D. J. Derrick, and H. Papkoff. 1983c. Testosterone administration to mares during estrus: Duration of estrus and diestrous and concentrations of LH and FSH in plasma. *J. Anim. Sci.* 56:911-918.
- Thompson, D. L., Jr., F. Garza, R. L. St. George, M. H. Rabb, B. E. Barry, and D. D. French. 1991. Relationships among LH, FSH and prolactin secretion, storage and response to secretagogue and hypothalamic GnRH content in ovariectomized pony mares administered testosterone, dihydrotestosterone, estradiol, progesterone, dexamethasone or follicular fluid. *Domest. Anim. Endocrinol.* 8:189-199.
- Turner, J. E., and C. H. G. Irvine. 1992. The effect of a potent GnRH agonist on gonadal and sexual activity in the horse. *Theriogenology* 33:213-225.
- Vanderwall, D. K., T. D. Juergens, and G. L. Woods. 2001. Reproductive performance of commercial broodmares after induction of ovulation with hCG or Ovuplant™ (deslorelin). *J. Equine Vet. Sci.* 21:539-542.
- Voss, J. L., J. J. Sullivan, B. W. Pickett, W. G. Parker, L. D. Burwash, and L. L. Larson. 1975. The effect of HCG on duration of oestrus, ovulation time and fertility in mares. *J. Reprod. Fertil. Suppl.* 23:297-301.
- Zilberstein, M., H. Zakut, and Z. Naor. 1983. Coincidence of down-regulation and desensitization in pituitary gonadotrophs stimulated by gonadotropin releasing hormone. *Life Sci.* 32:663-669.

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Agricultural Center
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Department of Animal Science
209 J. B. Francioni Hall
Louisiana State University
Baton Rouge, LA 70803-4210
Phone: 225-388-3445
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EMAIL: dthompson@agctr.lsu.edu

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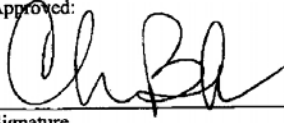
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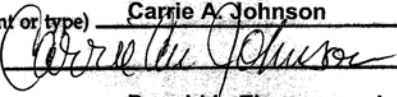
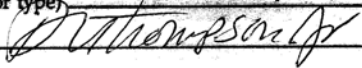
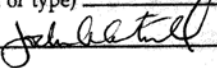
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VITA

Carrie Ann Johnson was born on April 19, 1974, to Donnie Strebeck and Stella Dannheim in Crossett, Arkansas. Carrie attended Crossett High School and received her academic diploma in May, 1992. In the fall of 1992, Carrie attended Northeast Louisiana University in Monroe, Louisiana, on a rodeo scholarship and obtained her bachelor of science degree in agribusiness in May, 1996. Carrie then began working on a master of science degree in biology under the direction of Dr. Ann Findley and Dr. William Hoefler. She obtained her master of science degree in August, 1998. In August, 1998, Carrie began her doctor of philosophy program in equine physiology and reproduction at Louisiana State University in Baton Rouge, Louisiana, under the direction of Dr. Donald L. Thompson, Jr. Carrie completed her requirements for the doctor of philosophy degree in the summer of 2002.