

2012

## Development of an estradiol-dopamine anagonist protocol for inducing ovulation in seasonally anovulatory mares

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DEVELOPMENT OF AN ESTRADIOL-DOPAMINE ANTAGONIST  
PROTOCOL FOR INDUCING OVULATION IN SEASONALLY  
ANOVULATORY MARES

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  
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B.S., Louisiana State University, 2004  
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August 2012

## **ACKNOWLEDGMENTS**

I owe a huge debt of gratitude to so many people. First, I would like to thank my major professor, Dr. Donald L. Thompson, Jr. for all of his guidance, advice, direction, and patience during this adventure. I would never have gotten to where I am without him. I would also like to express my gratitude to the other members of my committee, Dr. Kenneth R. Bondioli, Dr. Glen T. Gentry, Dr. Dale L. Paccamonti, and Dr. Cathy C. Williams. I could not have asked for a better group of professors to help guide me through this process. I'd also like to thank Dr. Gary M. Hay for the opportunity he gave me to teach for the department; the experience was invaluable. Additionally, I owe thanks to Dr. Laura R. Gentry for all of her help in the lab and making sure I had needed supplies. And to Ms. Sally Turner – what would I have done without you? Thank you for everything!

There are also a lot of people who helped make my projects run smoothly out at the farm. Thank you so much to Mr. Randy Wright and Mr. Joe Paul at the LSU Horse Unit not only for always being accessible and making what I needed a reality, but for the priceless friendships we've formed over the years. I would also like to thank Dr. Glen Gentry, Mr. Jared Pitchford, Mr. James Sterling and Ms. Sonjya Thomas at the Reproductive Biology Center for allowing me to use their horses and their facilities and for being great friends in the process.

A very special thank you is due to Dr. Patrick J. Burns of Burns BioRelease Technologies in Lexington, KY. Dr. Burns provided the treatments for all experiments included herein, as well as invaluable input over the years. He passed away very suddenly in January of 2012. His passing is truly a loss to the field of equine reproductive research and he will be terribly missed. I am so grateful for his role in making my research a success.

I have been so fortunate to be surrounded by amazing fellow students. There have been many who have been a huge help over the years and I'd like to thank them, both former and current colleagues: Nicole Arana Valencia, Dr. Josh Cartmill, Thomas Caltabilota, David Carwell, Sarah Clavier, Lisa DiGiovani, Lisa Earl, Caitlin Hebert, Rebecca Hill, Dr. Nan Huff, Jeanne Lestelle, Dr. April Levy, Dr. Jairo Sarmiento, Dr. Kristian Schumacher, Brittany Scott, Jaclyn Steinmiller, Tommy Stevens, Dr. Bill Storer, and Dr. Cara Wright. You all are my extended family and have been irreplaceable in so many ways. Whether it was countless hours at the farm, suffering through classes together or struggling through life's curve balls – you all were always there and I'm so grateful! I'm also indebted to all of the undergraduate students who have helped me. I wish I could list you all by name, but you know who you are!

This list would never be complete without thanking my family. I could not have completed this journey without them. My deepest gratitude goes to my parents, Jackie and Joyce Boliew and Garry and Mary Jefferson for always being there with encouragement and support. I also want to thank my sister and her husband, Mark and Lori Seagroves and my niece and her husband, Adam and Stephanie McCravey. All of you were always there cheering me on when I stumbled and I appreciate it so much. To my sister-in-law and her family - Cullen and Pam Saucier and Stephanie, Rivers and Lainey – thank you also for being great cheerleaders for me and always supporting me.

Then there is the man without whom I would never have taken the first steps of this journey – my husband. David – you have been my rock. You have encouraged me when I failed, you have made me laugh when I wanted to cry and you have given me the push I needed when I was in a rut. You gave me Riley Grace, the most amazing daughter any mommy has ever had. You have taken impeccable care of her and me when my attention was focused elsewhere with

school. Having the two of you to come home to everyday has kept me going and made it all worth it. This degree should have your name on it, too. Thank you so much. I love you and my Bug.

To my Father in Heaven – thank you for guarding my steps and my decisions with your wisdom. Thank you for holding my little family in the palm of your hand through this adventure. And thank you most of all for placing so many wonderful people along my path who have walked the journey with me. I am truly blessed.

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## **ABSTRACT**

Five experiments were conducted to assess potential improvements in a protocol for inducing ovulation in seasonally anovulatory mares based upon estradiol pretreatment followed by dopamine antagonist injection. The first experiment compared various doses of estradiol cypionate (ECP) and domperidone (in biodegradable microparticles), as well as additions and deletions to the protocol. It was concluded that as little as 75 mg ECP and as little as 1.5 g of domperidone could be used with success, but that both components were required. In the second experiment, timing of the injection (1, 6, or 11 days apart) and ECP dose were assessed. It was concluded that administration of domperidone 1 day after ECP injection provided the best results, and that 50 and 100 mg ECP provided similar results. The third experiment, using geldings as a model for prolactin secretion, compared the prolactin responses to an alternate dopamine antagonist, sulpiride, to those with domperidone, both in biodegradable particles. The magnitude of the prolactin responses were similar for both antagonists, however the sulpiride effect was quicker to occur (1 day) and was shorter-lived (<10 days) than that of domperidone (about 18 days). In the fourth experiment, two doses of ECP (25 and 50 mg) were compared followed immediately (same day) by injection of domperidone or sulpiride in biodegradable microparticles. Both the luteinizing hormone (LH) and prolactin responses to treatment were poor or absent in all but a few mares, and only two mares had early ovulation (one control mare and one mare receiving 25 mg ECP and domperidone). The reason for the poor responses was unknown, but the experiment confirmed the need for responses in LH and prolactin for positive ovarian responses. The last experiment evaluated domperidone versus a new non-particle formulation of sulpiride given at two doses (0.75 versus 1.5 g) on days 1, 6, and 11 relative to ECP (100 mg). Also factored across those treatments was the administration of 50 mg thyroxin

in microparticles administered 6 days before ECP injection. High success rates (prolactin and ovulation) were obtained with the higher sulpiride dose. Thyroxin treatment had no effect.

## INTRODUCTION

The induction of early cyclicity and ovulation in the seasonally anovulatory mare is a hot topic in the realm of equine reproductive research. Given that the artificial January 1 birth date used by most major breed registries is in opposition the mare's natural breeding season, there is a need for therapies to assist in facilitating breeding mares earlier in the year. There are several effective treatments currently employed by horse breeders, such as the use of artificial photoperiod, either alone or in concert with other hormonal treatments, such as progestins, gonadotropin releasing hormone or its agonists, among others. Most of these alternatives are either drawn out over a very long period of time, or involve frequent administration of either oral or injectable treatments. They can also be expensive and labor intensive. A need exists for an alternative that is more streamlined and cost effective.

Researchers have had success using dopamine antagonists to induce early ovulation in anovulatory mares (Besonet et al., 1996, 1997; Brendemuehl and Cross, 2000; Daels et al., 2000; Mari et al., 2009). Prolactin elevation seems to be the key to success. Prolactin, a protein hormone secreted by the adenohypophysis, is higher during the breeding season in mares (Johnson, 1986a; Thompson et al., 1987b; Worthy et al., 1987) than in winter. In fact, prolactin concentrations increase in plasma along with the resurgence of LH prior to the onset of the breeding season and that concomitant rise has been hastened by increasing photoperiod in winter (Johnson, 1987a). Additionally, ovulation has been induced in mares by treatment with prolactin only (Nequin et al., 1993; Thompson et al., 1997).

More recently success was reported using an estradiol pretreatment in conjunction with the dopamine antagonist sulpiride (Kelley et al., 2006). That study combined every other day injections of estradiol benzoate in oil with daily injections of sulpiride in anovulatory mares and achieved consistent elevated prolactin and early ovulation in treated mares. While these results

were very promising, treatment still encompassed the use of frequent and numerous injections. A portion of the protocol was streamlined when Thompson et al. (2008) evaluated single dose formulations of estradiol compared to the every other day estradiol benzoate injections employed previously (Kelley et al., 2006). It was found that a single injection of ECP gave similar and consistent prolactin and luteinizing hormone responses in geldings (Thompson et al., 2008).

The following experiments were designed to continue the process of streamlining the protocol with the ultimate goal in mind of a reliable, non-labor-intensive and cost effective treatment regimen for inducing early cyclicity and ovulation in seasonally anovulatory mares. Experiments were performed to further evaluate various doses of both the estradiol component and the dopamine antagonist component. Dosing intervals between treatments were also evaluated as well as comparing the use of either sulpiride or domperidone as the recommended dopamine antagonist. Furthermore, determining the repeatability of successfully inducing ovulation with this treatment was also of interest.

## **CHAPTER 1**

### **REVIEW OF LITERATURE**

#### **Hypothalamic-Pituitary Axis**

Research has led reproductive scientists to the common understanding that the reproductive axis is regulated by the hypothalamic production of gonadotropin releasing hormone (GnRH). As opposed to the rat, GnRH is synthesized diffusely throughout the hypothalamus in the horse (Strauss et al., 1979). Following production, GnRH, a decapeptide hormone (Matsuo et al., 1971), is packaged and stored in secretory granules within the median eminence that are released into capillaries of the primary plexus upon neuronal stimulation; from there, GnRH travels to and acts upon receptors located on the gonadotropes in the pars distalis of the adenohypophysis (Alexander and Irvine, 1993; Kainer, 1993).

The ability of the gonadotropes to respond to GnRH depends on the density of GnRH receptors present (Wise et al., 1984). The density of receptors is dependent on stage of the ovulatory cycle with peak levels occurring just prior to ovulation and number of receptors is also influenced by estradiol, inhibin, and activin, and by GnRH itself (Rispoli and Nett, 2005). Some level of GnRH is required to maintain a full cohort of GnRH receptors, and increases in GnRH secretion stimulate expression of the GnRH receptor gene, thereby promoting the up-regulation of its own receptors (Clayton, 1989; Rispoli and Nett, 2005).

Gonadotropin releasing hormone stimulates the release of the two gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), by the gonadotropes of the adenohypophysis. Gonadotropin releasing hormone is released in a pulsatile fashion (Knobil, 1980). The frequency of the hormone pulses regulates the secretion of LH and FSH, with more frequent pulses (every 45 min) favoring secretion of LH and less frequent pulses (every 6 h) favoring FSH (Alexander and Irvine, 1987, Ginther, 1992). This is partially confirmed by the fact that during estrus, circulating LH concentrations are high corresponding with pulses of

GnRH occurring twice per hour, while FSH concentrations remain low. Conversely, during diestrus, circulating concentrations of FSH are high, corresponding to a much slower GnRH pulse rate of only two per day, and LH concentrations remain low (Alexander and Irvine, 1993).

The gonadotropins, LH and FSH, are glycoprotein hormones consisting of identical alpha subunit peptide chains and differing, function-specific beta subunit chains (Pierce and Parsons, 1981). The binding of the gonadotropins with specific ovarian receptors exert their actions by activating intracellular second messenger, cAMP (Sanborn et al., 1980). Receptors for FSH are located primarily on the granulosa cells of the ovarian follicles, whereas LH receptors are only found on the thecal cells of pre-antral follicles (Alexander and Irvine, 1993). After antrum development, LH receptors can also be found on granulosa cells as well (Alexander and Irvine, 1993). Follicle stimulating hormone serves the primary function of stimulating follicular growth (Guyton and Hall, 2000); however, LH controls the final stages of follicular development and ovulation (Alexander and Irvine, 1993). The production of estradiol and inhibin accompanies follicular growth. Additionally, following ovulation, a corpus luteum (CL) forms and secretes progesterone. All of these hormones provide negative feedback on the hypothalamus and the adenohypophysis (Alexander and Irvine, 1993). This ensures a closely regulated feedback loop to assure a balance of all hormones and their actions.

### **Estrous Cycle of the Mare**

The estrous cycle of mares is defined as the period of time from the beginning of one estrus to the start of the next (Daels and Hughes, 1993). The average estrous cycle in the mare is 21.7 days, with a range of 19.1 to 23.7 days (Ginther, 1992). The estrus phase of the cycle averages 6.5 days with a range of 4.5 to 8.9 days (Ginther, 1992). The entire cycle consists of the follicular phase, commonly called estrus, and the luteal phase, known as diestrus (Daels and Hughes, 1993). Circulating FSH causes the early follicular growth during diestrus that

eventually leads to the emergence of a dominant follicle and the onset of estrus. As the follicles grow, they begin to release the hormone inhibin, which has a negative feedback on FSH (Bergfelt and Ginther, 1985). Estrogenic hormones, primarily estradiol, are released by growing follicles, serving two purposes. First, they are responsible for the expression of sexual behavior and receptivity to the stallion (Daels and Hughes, 1993). Secondly, estradiol from the thecal cells of the growing follicles serves as a positive feedback mechanism on LH production and secretion in the pituitary gland. The resulting rise in LH causes ovulation, i.e., the expulsion of the oocyte from the dominant follicle. This process typically occurs 24 to 48 hours before the end of estrus (Daels and Hughes, 1993).

After ovulation, the follicle that ovulated will develop into a CL, which secretes progesterone, the hormone that dominates diestrus and is responsible for maintenance of any ensuing pregnancy. Ginther (1992) reported that the duration of diestrus is 12.1 to 16.3 days following ovulation. Progesterone suppresses sexual receptivity of the mare to the stallion (Daels and Hughes, 1993). The CL will persist for about 14 days, and then begin to regress if the mare is not pregnant, which allows the mare to return to the follicular phase (Daels and Hughes, 1993).

During the estrous cycle, there is a reciprocal pattern to the gonadotropins, with FSH concentrations low when LH concentrations are high. It is presumed that the negative feedback of progesterone secreted by the CL suppresses LH concentrations during diestrus and that the lack of negative feedback from the ovaries due to estradiol and inhibin allows FSH to reach higher concentrations (Freedman et al., 1979). Secretion of FSH begins to increase soon after ovulation, and may fluctuate in surges at 10- to 11-day intervals (Evans and Irvine, 1975), or may stay high throughout diestrus. The first surge of FSH in early diestrus is thought to be responsible for initial development of follicles up to 20 mm in diameter (Evans and Irvine,



1975). As the follicular phase nears, FSH secretion begins to wane due to the secretion of estradiol and inhibin from the growing follicles, and LH secretion undergoes a marked increase, which continues through approximately 1 day post-ovulation (Miller et al., 1980).

### **Seasonality**

Many species, including horses, have adapted their mating seasons to favor survival of offspring. Based on a gestation length of roughly 11 months, horses breed during spring and summer months, thereby ensuring birth of the foal during temperate weather and periods of high availability of forage the following year (Ginther, 1992). Mares are seasonally polyestrous breeders, meaning most non-pregnant mares will exhibit successive estrous cycles during the breeding season, with many becoming completely reproductively inactive during the winter months (Daels and Hughes, 1993). The phenomenon of seasonality is influenced by factors such as nutrition and climate, but it is primarily controlled by photoperiod (Daels and Hughes, 1993). The period of little to no reproductive activity is best described as the anovulatory period due to the fact that mares may, in fact, exhibit erratic signs of estrus during the winter, but generally do not ovulate.

The anovulatory period is divided into 3 phases by Ginther (1992): receding, inactive, and resurging. The first phase is also known as fall transition and coincides with the autumnal equinox (September 21). Mares enter this phase following the last ovulation of the breeding season due to inadequate LH secretion and lack of final growth of an ovulatory follicle (Snyder et al., 1979). This phase is characterized by the failure to ovulate large follicles due to the absence of an LH surge (Ginther, 1992). Pituitary reserves of LH have been shown to decline steadily from approximately the middle of the breeding season to approximately the middle of the anovulatory season (Silvia et al., 1986). Following the receding phase, mares enter the inactive phase, during which few follicles develop beyond 10 to 15 mm in diameter (Ginther,

1992). During this phase, ovaries of mares regress to a small size (2.4 x 1.6 x 1.6 cm; 17.5 g/ovary) and are found to be firm and kidney shaped (Van Neikerk et al., 1973). Mares then enter the resurging phase, which is referred to as vernal transition. This is the time when mares begin to recover from the anovulatory state. The ovaries begin to increase in size again (Van Neikerk et al., 1973) and the number of follicles developing beyond 20 mm increases (Ginther, 1992).

Sharp and Davis (1993) define the anovulatory period as a time of sexual incompetence and indifference during the winter months of the year. These authors state that the sharp decrease in GnRH secretion by the hypothalamus is the reason for this inactivity, based on the pioneering research of others (Strauss et al., 1979; Hart et al., 1984). Sharp and Grubbaugh (1987) used push-pull perfusion technique in pony mares to assess hypothalamic GnRH during the months of November and December and found no detectable levels of the hormone. Additionally, Thompson et al. (1986, 1987a) reported a distinct suppression of gonadotropins during the winter months, both in peripheral plasma as well as the pituitary gland itself. Despite the common absence of gonadotropins and follicular activity in most studies, it has also been reported that some mares will display weak, erratic signs of estrus during the anovulatory period (Ginther, 1992; Gentry et al., 2002a), and some will tend to cycle year-round.

During the resurging phase, the time between the initial rise in FSH and the first ovulation can be long and variable >60 days; Davis et al., 1987). Secretion of FSH will fluctuate, but increases in LH remain absent until just before ovulation (Hines et al., 1991). Additionally, as the first ovulation is approached, FSH secretion and pulse amplitude will actually decrease (Freedman et al., 1979; Silvia et al., 1986; Hines et al., 1991) due to the production of inhibitory factors (estradiol and inhibin) from large follicles (Miller et al., 1981). Additionally, the higher levels of estradiol associated with these follicles can lead to the mare

exhibiting long and erratic periods of estrus and ambiguous receptivity to a stallion (Hines et al., 1991). Luteinizing hormone levels begin to increase at this time due to the rise in GnRH secretion coupled with the development of steroidogenically (i.e., estrogen) competent follicles, which elevate peripheral estrogen concentrations (Sharp and Davis, 1993).

### **Folliculogenesis and Follicular Waves**

The primary functional unit of the ovary is the follicle. The process of growth and differentiation of ovarian follicles is called folliculogenesis. This process consists of 3 phases: selection, dominance, and then either ovulation or atresia (Pierson, 1993). Follicles serve both endocrine and exocrine roles in the reproductive system as follicles produce and secrete estrogens and protein hormones as well as expelling the oocyte at ovulation. There is a myriad of processes that control ovarian function: endocrine (systemic hormone release); paracrine (local intracellular diffusion); and autocrine (release of substances that bind to the cell's own receptors; Pierson, 1993).

Like other mammalian females, the mare is born with a finite number of primordial follicles in her ovaries (Greenwald and Terranova, 1988). Primordial follicles are oocytes surrounded by one layer of flattened granulosa cells. Ginther (1992) classifies follicles further into three categories based on size: small (2 to 10 mm), medium (11 to 24 mm) and large ( $\geq 25$  mm). The number of small follicles decreases during the preovulatory stage and increases during the postovulatory stage (Pierson, 1993). The number of medium follicles remains relatively constant throughout the cycle and the number of large follicles is dependent on follicular waves (Pierson, 1993).

According to Ginther (1992), there is a group of small follicles that is constantly growing and regressing regardless of reproductive status, producing a "cohort" from which follicles are selected for further growth. It is from this stage that pre-antral follicles develop. This stage of

the cycle is marked by growth of the oocyte, formation of the zona pellucida, and the division of granulosa cells into cuboidal epithelium (Pierson, 1993). The follicle is said to be a tertiary follicle upon formation of the antrum, which occurs at a diameter of around 300  $\mu\text{m}$  in the mare. Growth will continue in the form of thickening of the follicular wall and an increase in volume in the antrum (Pierson, 1993).

The vast majority, 99%, of follicles that develop to the antral stage are destined for atresia rather than ovulation. When a follicle becomes atretic, it undergoes a gradual decrease in size and activity, eventually disappearing from the ovary. The other 1% of follicles that develop will ovulate (Pierson, 1993).

Gonadotropins are highly involved in the regulation of follicular growth, despite the fact that they are not needed for early development to the antral stage (Pierson, 1993). However, they are necessary for continued growth past this stage. In pre-antral follicles, LH and FSH receptors are acquired in the thecal and granulosa cell membranes, respectively (Pierson, 1993). Thecal cells produce androgens under the influence of LH, which then pass through the basal lamina and are aromatized to estrogens by the granulosa cells, a process controlled by FSH. The increase in estrogen levels is stimulatory to LH secretion, which further increases production of estrogen. There is a corresponding increase in LH receptors. This dramatic increase in estrogen production marks the transition from the antral stage to the preovulatory stage. Without the capability to respond to the gonadotropins, a follicle will fail to reach the final stages of development (Pierson, 1993).

As in other species, growth and selection of large follicles seems to occur in “waves” in the mare (Ginther, 1992; Pierson, 1993). Bimodal and unimodal FSH surges are the causes for follicular waves (Pierson, 1993). Additionally, Ginther and Bergfelt (1993) state that increases in FSH concentrations are responsible for follicular waves. A major follicular wave occurs when

several follicles grow in synchrony (equal rate of growth and size) with eventual dissociation (Ginther, 1992). Dissociation involves the selection of a dominant follicle for continued growth. This process is also known as divergence. At this point, subordinate follicles will begin to undergo atresia (Ginther, 1992) as the dominant follicle grows larger. At least one follicular wave per cycle will be experienced by all mares, with some mares having two waves in a single estrous cycle. When two waves occur, it is the first wave that is considered secondary, taking place during late estrus to early diestrus. Dominant follicles that develop during the secondary follicular wave generally become static and regress; however, occasional diestrus ovulations can occur. The primary wave is the second follicular wave, occurring during mid-diestrus. This wave will produce the dominant follicle that will become the eventual ovulatory follicle (Ginther, 1992).

Once a follicle reaches approximately 13 mm in diameter, FSH concentrations decrease (Ginther et al., 2001). The follicles themselves cause this decrease. Just prior to deviation of the dominant follicle, estradiol, free IGF-1, activin-A, and inhibin-A increase differentially. Deviation occurs due to higher responsiveness by the dominant follicle to the gonadotropins. This process is marked by the two largest follicles reaching an average size of 19.0 to 22.5 mm. Only the most developed follicles, now responsive to LH, will continue to grow because FSH levels have already declined. Atresia of the remaining subordinate follicles is caused by the continued production of estradiol and inhibin by the dominant follicle (Ginther, 1992; Pierson, 1993).

### **Ovulation and the Luteal Phase**

Mares are unique among livestock species in the anatomy of the ovary and ovulation process. Where most species have an interior medulla and an exterior cortex entirely covered by surface germinal epithelium, the equine ovary is the opposite. The cortex is located more in the

central portion of the ovary and is surrounded by medullary tissue. Also, surface germinal epithelial cells are located only on the underside of the ovary, allowing ovulation to occur only at the ovulation fossa (Ginther, 1992; Pierson, 1993). Additionally, mares have LH receptors in the granulosa cells of the dominant follicle, allowing for response to increasing LH levels during estrus (Ginther, 1992). Concentrations of LH undergo a prolonged surge that begins a few days prior to the start of estrus and does not reach maximum levels in the mare until approximately 1 day post-ovulation. Return to low levels takes several days; the entire period of high LH concentrations is much longer in mares than in other mammalian species (Alexander and Irvine, 2011). It has been suggested that some threshold of LH concentrations occurring during the estrous rise of LH in the mare is sufficient to cause ovulation, rather than the peak levels per se, as in other species (Pierson, 1993).

The luteal phase begins with ovulation. Progesterone, secreted by the CL, is the dominant hormone of this phase. The CL is fully formed by 3 days post-ovulation, though growth continues until day 9, when progesterone secretion is at its maximum (Niswender and Nett, 1993). In the mare, the CL requires LH for continued function. This is evidenced by the fact that treatment with human chorionic gonadotropin (hCG), which is LH-like in horses, or equine pituitary extract will extend the life of the CL (Ginther, 1992). The population of LH receptors in the CL increases as progesterone concentrations increase and vice versa (Roser and Evans, 1983). Around day 14, the non-pregnant uterus will secrete prostaglandin- $F_{2\alpha}$ , which lyses the CL, resulting in a drop in progesterone levels, allowing the estrus cycle to begin again.

### **Reversing the Seasonal Anovulatory Period in Mares**

Lights and progesterone treatment. Due to the seasonal nature of the mare's natural breeding season, breed association-imposed January 1 birthdates for all horses born within a calendar year has created a dilemma for many horse breeders. Foals born early in the calendar

year often have a size and maturity advantage at 2 and 3 years of age. Thus, hastening the onset of cyclicity and breeding mares earlier in the year can be advantageous both in maximizing performance and prices obtained in the sale ring (Squires, 2008). There are several accepted methods in place for inducing early ovulation; however, many of them prove to be labor intensive and expensive.

A common practice within the horse industry is to expose mares to artificial photoperiod to induce cyclicity and ovulation earlier in the year. Generally, mares must receive this increased photoperiod beginning approximately December 1 of the preceding year to achieve cyclicity in the desired timeframe (Squires, 2008). Approximately 16 hours of light per day with 8 hours total darkness, with the artificial light added in the evening, seems to be most effective (Sharp, 2011). Even though this management practice is commonly used and is effective, it is problematic given that mares will still experience a transition period (Squires, 2008), a time of erratic estrus behavior with no accompanying ovulation (Hines et al., 1991; Ginther, 1992). Therefore, exogenous progesterone treatment is often coupled with artificial photoperiod. Typically, 12 to 15 days of exposure to an exogenous progestin (such as altrenogest, sold as Regumate<sup>®</sup>) is administered once a mare has been under lights for 30 to 60 days (Squires et al., 1979). This treatment is effective only in mares with at least medium-sized follicles on the ovaries (Squires, 2008). Progestin treatment is usually oral altrenogest, progesterone in oil injected daily, or more recently, long acting formulations developed to release progesterone or altrenogest for 7 to 10 days following a single injection (Burns et al., 2008; Squires, 2008). Numerous studies have shown that regardless of type of progesterone, only mares in mid to late transition will respond favorably to this treatment (Squires, 2008).

GnRH, GnRH agonists and gonadotropins. Another approach receiving considerable research attention in the 1970's was the use of GnRH and GnRH agonist treatments. When

GnRH was first administered to estrous and anestrus mares, it was observed that LH concentrations doubled (Ginther and Wentworth, 1974). Later, it was documented that treatment with GnRH increased both FSH and LH in the mare (Evans and Irvine, 1976). These same researchers were the first to use repeated injections of GnRH to induce follicular development and ovulation in anestrus mares (Evans and Irvine, 1977; Squires, 2011). Other studies have been performed using very frequent, very low volume injections of GnRH to stimulate follicular development alone or with an accompanying ovulation and corpus luteum development in mares (Johnson, 1987b; Turner and Irvine, 1991). One study showed that twice daily injections of the GnRH agonist, buserelin, hastened the first ovulation of the year (Harrison et al., 1990). Mares were injected at 12-hour intervals for 28 days or ovulation, whichever came first (Harrison et al., 1990).

Additionally, implants containing GnRH or GnRH analogs have been used successfully to induce cyclicity and ovulation in the anestrus mare (Allen et al., 1987; Harrison et al., 1990; Turner and Irvine, 1991). Another approach utilized osmotic mini-pumps containing GnRH or its analogs to bring mares into season. Researchers have used both continuous infusion of GnRH (Ainsworth and Hyland, 1991) and pulsatile infusion (Johnson, 1986b) successfully. The two protocols have been compared, and an advantage of pulsatile treatment was reported (Becker and Johnson, 1992). Subcutaneous implants of the GnRH analogue goserelin acetate increased ovulation over controls, but there was a high degree of variation (Fitzgerald et al., 1993). The effect of continuous low dose GnRH infusion has also been investigated as a means of confirming the lack of refractoriness in the mare that is a concern in other species (Ginther, 1992). Williams et al. (2007) inserted osmotic mini-pumps on day 12 post-ovulation that released 5 µg of GnRH/hour for 14 days. Progesterone levels were increased, as well as LH levels to some degree, but the interovulatory interval was not affected, indicating no



desensitization of the hypothalamic-pituitary axis. Williams et al. (2007) also looked at this regimen's efficacy on mares that were persistently anovulatory, that is, mares that had failed to develop a follicle of at least 35 mm by April 1 of the breeding season. All mares treated ovulated compared to controls. Interestingly, it has been noted that beginning treatment with an osmotic pump in late September/early October in an attempt to preclude an anovulatory season from occurring was unsuccessful (Collins et al., 2007).

Treatment with gonadotropins themselves has also been used with limited success. Purified equine FSH has been used successfully to stimulate earlier ovulation when compared to controls (Niswender et al., 2004). Mares were treated with 12.5 mg purified eFSH twice daily for up to 15 days. Eight of ten mares ovulated within the predetermined response window. It is important to note that these mares had been under lights since the first week of December and treatment did not commence until the mare was determined to be in transition, that is, the presence of a follicle  $\geq 25$  mm was detected. Again, this regimen has a disadvantage of handling the mares frequently as well as a great expense for the injected FSH (Squires, 2008).

## **Prolactin**

Prolactin is a polypeptide hormone, consisting of 199 amino acids (Nett, 1993), that is secreted from the adenohypophysis (Freeman et al., 2000). Its discovery stemmed from two observations. The first was in 1928 when researchers found that milk secretion in rabbits was stimulated by an extract of anterior pituitary (Stricker and Grueter, 1928). A few years later, it was discovered that administration of bovine pituitary extract resulted in increased crop sac size and crop milk production in pigeons, therefore it was given the name prolactin (Riddle et al., 1933). It has been reported that prolactin has over 300 different biological functions in various vertebrate species (Bole-Feysot et al., 1998).

Prolactin is produced primarily in the lactotropes, but it has also been found in the same secretory granules along with growth hormone in mammosomatotropes (Rahmanian et al., 1997). Depending upon stage of the estrous cycle, lactotropes make up 5 to 16% of pituitary cells and mammosomatotropes make up 10 to 16.5% (Rahmanian et al., 1997). Rather than being controlled by a stimulatory factor, as are most pituitary hormones, prolactin is instead held under constant inhibitory control by dopamine, secreted from the hypothalamus (Morresey, 2011). Pituitary lactotropes removed from the inhibition of dopamine will secrete large amounts of prolactin due to spontaneous depolarization (Hadley, 2000). It has been reported that treatment with the dopamine agonist bromocriptine decreases prolactin in stallions when administered prior to sexual stimulation (Thomson et al., 1996). Bromocriptine treatment also delayed puberty in female rats (Advis et al., 1981). In most mammals, including horses, thyrotropin releasing hormone (TRH) secreted by the hypothalamus is also stimulatory to prolactin secretion (Johnson, 1986a; Colborn et al., 1991; Gentry et al., 2002b) via TRH receptors on the lactotropes (Gerschengorn et al., 1979). However, the role of TRH in the day-to-day, hour-to-hour control of prolactin secretion in horses has yet to be determined.

Typically, secretion of prolactin is fairly constant, with occasional surges (Thompson et al., 1994). In most species, including the horse, day length affects prolactin secretion, with levels being lower in the winter than in summer, the breeding season for the horse (Johnson, 1986a; Thompson et al., 1987b; Worthy et al., 1987). Prolactin secretion in the horse has been shown to be increased by exercise (Thompson et al., 1994) as well as TRH (Thompson et al., 1986). Resting levels are higher in stallions and mares than in geldings (Thompson et al., 1994). Factors such as photoperiod and ambient temperature also influence prolactin secretion in the mare (Johnson, 1987a).

As previously stated, prolactin has a host of functions in various species. In the mare, as in other species, prolactin stimulates both mammary development and milk secretion (Worthy et al., 1986). There is a concomitant increase in plasma prolactin concentrations that occurs with the resurgence of LH prior to the onset of the breeding season that has been hastened by increasing photoperiod in winter (Johnson, 1987a). Also, treatment during a short photoperiod with melatonin decreased prolactin secretion (Fitzgerald and McManus, 2000). Prolactin secretion can be elevated by the administration of dopamine antagonists, such as sulpiride and domperidone. Follicle growth on the equine ovary during spring transition has been associated with this elevation in secretion, whether from endogenous or exogenous means (Nequin et al., 1993). These facts have led scientists to investigate the role of prolactin in control of seasonality in the mare (Ginther, 1992), including the experiments herein.

### **Dopamine Antagonists**

Recently, extensive study has gone into the use of dopamine antagonists as an alternative to the use of artificial photoperiod, progestogens, GnRH and its analogues, and gonadotropins to induce early cyclicity and ovulation in anovulatory mares.

Dopamine's role in reproductive seasonality. Studies performed in the ewe, another seasonal breeder, indicated that there may be a regulatory function of dopamine D2 receptors on seasonality as evidenced by the presence of synapses between dopaminergic and GnRH neurons in the median eminence of the pituitary and that suppression of these receptors increased LH during the anestrus period (Thiery and Malpoux, 2003; Ciechanowska, et al., 2008; Tibary, 2011). Dopamine concentration is higher in cerebrospinal fluid in mares during the anovulatory season than during the breeding season (Melrose et al., 1990). Additionally, it is inversely correlated to plasma prolactin levels (Johnson, 1986a).

Dopamine's exact mechanism of action on follicular dynamics is unclear, but it is presumed to be via its regulation of prolactin (Cross et al., 1995; Tibary, 2011). As stated before, it is known that prolactin levels are lower in fall and winter than in spring and summer in mares, when follicular activity resumes (Johnson, 1986a; Thompson et al., 1987b; Worthy et al., 1987). Also noted previously, there is a positive correlation between prolactin and follicular diameter (Nequin et al., 1993) during spring transition in mares. Researchers have been successful in inducing ovulation using treatment with either ovine or recombinant porcine prolactin (Nequin et al., 1993; Thompson et al., 1997). While LH is stimulated, FSH is not, indicating the response is not due to increased pituitary gonadotropin secretion (Tibary, 2011).

More recent work has confirmed the presence of dopamine receptors on both theca and granulosa cells in the equine ovary (King et al., 2005). Dopamine D1 and D2 receptors have also been identified in equine corpus luteum tissue (King et al., 2005) and it is assumed that primary and secondary pre-antral follicles have the ability to produce D2 receptors based on observed elevated mRNA levels in the equine ovarian cortex (King et al., 2008).

It has been suggested that dopamine may act via the D2 receptor to inhibit follicular growth (Tibary, 2011). This theory is supported by the fact that the dopamine antagonists sulpiride and domperidone have a positive effect on follicular growth in anovulatory mares, as well as the fact that treatment with these doesn't increase FSH secretion in these mares (Nequin et al., 1993; Thompson et al., 1997; Brendemuehl and Cross, 2000; Donadeu and Thompson, 2002; King et al., 2008; Tibary, 2011).

Use of dopamine antagonists. Though there are several available dopamine antagonists, the most commonly used in the horse are sulpiride (Besognet et al., 1996, 1997; Donadeu and Thompson, 2002; Duchamp and Daels, 2002) and domperidone (Brendemuehl and Cross, 2000). Two others used far less in the horse are fluphenazine (Nequin et al., 1993) and perphenazine

(Bennett-Wimbush and Loch, 1998; Bennett-Wimbush et al., 1998). All of these compounds have been documented to induce follicular growth or ovulation in seasonally anovulatory mares (Tibary, 2011). There is, however, a great deal of variation among the studies (Tibary, 2011).

The dopamine antagonist domperidone has a high affinity for both D2 and D3 receptors and a half-life of about 7 hours, being predominantly metabolized in the liver and intestine (Tibary, 2011). It is commonly administered orally but can also be administered by injection. It is frequently used to treat agalactia and fescue toxicity, and is becoming increasingly popular for use in inducing cyclicity and follicular growth in anovulatory and transitional mares (Tibary, 2011). While much success has been gained by using this drug in this way, it is important to note that results are often inconsistent and, in some experiments, it has been used with little to no success (McCue et al., 1999; McCue et al., 2007).

Sulpiride, another commonly used dopamine antagonist, is selective for D2 receptors and has also been used with success in the mare to induce cyclicity, although again with somewhat inconsistent results. Besognet et al. (1996, 1997) reported success using both a high dose, 1.0 mg/kg body weight once daily (in 1996), and a lower dose, 200 mg/mare (in 1997). Daels et al. (2000) used a 0.5 mg/kg dosage to decrease the interval to first ovulation. However, other studies have failed to show any influence on date of first ovulation despite increased prolactin levels (Nagy et al., 1999; Donadeu and Thompson, 2002). It is worth noting that Mari et al. (2009) compared sulpiride with domperidone under the same conditions, and sulpiride treated mares ovulated earlier than controls, while domperidone failed to have any effect.

## **Effects of Estradiol on Prolactin**

Many hypotheses have been put forth about why increasing prolactin secretion via the use of dopamine antagonists is not always successful in causing mares to begin cycling earlier in the year. One that has much merit is the suggestion that there is a lack of estrogen in these mares. Treatment with estradiol was previously shown to increase plasma prolactin concentrations in ovariectomized pony mares by approximately 35% (Thompson et al., 1991), but the greatest effect was on pituitary content (increased approximately 300%). Kelley et al. (2006) was the first to apply this information by using 10 days of estradiol benzoate pretreatment prior to daily sulpiride administration in seasonally anovulatory mares. In that experiment, estradiol pretreatment greatly increased the prolactin response to the injected sulpiride relative to sulpiride alone, and the ovarian response was rapid, with 8 of 9 mares ovulating an average of 30 days from the onset of treatments on January 11th.

In horses, in addition to the report of Thompson et al. (1991), Aurich et al. (1995) reported that estradiol benzoate stimulated serum concentrations of both prolactin and LH in mares, when administered in conjunction with naloxone, an opioid antagonist. The stimulatory effects of estradiol on prolactin production and secretion in other species has been well documented (del Pozo and Brownell, 1979).

## **Rationale for Present Experiments**

The present series of experiments was conducted to further study the use of estradiol pretreatment combined with dopamine antagonist administration for the induction of ovulation in seasonally anovulatory mares. Experiments included empirical testing of estradiol dose, type of dopamine antagonist, and effects of other hormonal treatments in conjunction with estradiol and the dopamine antagonist. The ultimate goal was to develop the simplest, easiest, and most cost

effective procedure that produced a high degree of success (ovulation) in mares of all stages of anovulation in winter in preparation for breeding on approximately February 15th.

## **CHAPTER II**

### **STIMULATION OF OVULATION IN SEASONALLY ANOVULATORY AND VERNAL TRANSITIONAL MARES WITH ESTRADIOL AND DOMPERIDONE: DOSE AND COMBINATION STUDIES<sup>1</sup>**

#### **Introduction**

One aim of reproductive research in the mare continues to be a development of simple method of inducing cyclicity in seasonally anovulatory mares. Administration of the dopamine antagonists, domperidone (Brendemuehl and Cross, 2000) and sulpiride (Besognet et al., 1997; Mari et al., 2009) has been shown to hasten ovulation in some studies, but not in others (McCue et al., 1999; Donadeu and Thompson, 2002). Kelley et al. (2006) demonstrated that pretreatment with estradiol benzoate greatly increased the prolactin response, as well as LH response, in anovulatory mares, which resulted in a greater ovulatory response compared with sulpiride treatment alone. Subsequently, Thompson et al. (2008) determined that a single injection of ECP (100 mg) yielded the most similar and consistent prolactin response to the previously reported protocol of every-other-day injections of estradiol benzoate (Kelley et al., 2006). The current experiments were designed to test the effectiveness of several protocols for the single injection of ECP combined with a single injection of domperidone for inducing ovulation in seasonally anovulatory mares (Experiments 1 and 2) and mares entering transition (Experiment 3). Specifically, the goal of the study was to evaluate the efficacy of various combinations and dosages of ECP, domperidone, and progesterone for inducing ovulation in seasonally anovulatory and transitional mares.

#### **Materials and Methods**

Three experiments were conducted. Experiments 1 and 2 were conducted in the winter (January and February) of 2007. The first experiment was conducted using 43 light horse mares

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housed at the Equine Reproductive Center of Equine Services Hospital in Simpsonville, KY. The second experiment was conducted using 24 mares housed at Louisiana Agricultural Experiment Station farms located in Baton Rouge, LA. In both experiments, mares were selected for use based on lack of luteal tissue and ovarian follicular sizes less than 20 mm based on ultrasound examination (Aloka 550V with 5-MHz linear-array transducer; Aloka Science and Humanity, Wallingford, CT) for 3 weeks before the start of the project. Groups were selected with body condition equally distributed, and treatment was then randomly assigned to group. Mares were housed on pasture; sufficient locally produced grass hay was supplied to maintain average to good body condition (5 or greater; Henneke et al., 1983).

Experiment 1 included the following treatment groups: control (no treatment, n = 9), domperidone in biodegradable particles (DPB) (Kelley et al., 2006) only (n = 9), 100 mg ECP (BET Pharm, BioRelease Estradiol Cypionate LA, Lexington, KY, USA) + DPB (n = 6), 150 mg ECP + DPB (n = 7), 150 mg ECP + DPB + progesterone (n = 7), and 150 mg ECP + progesterone (n = 5). Progesterone (1.5 g; BET Pharm P4 LA 300; 5mL) was administered at the same time as the DPB. Experiment 2 had two treatment groups: control (n = 13) and 150 mg ECP + DPB (n = 11). All injections of DPB were 3 g and were administered intramuscularly. Ten days after ECP injection, based on the results of Kelley et al. (2006). Experiment 1 began on January 19, 2007, while Experiment 2 began on January 13, 2007. In both experiments, mares were monitored through ultrasound every 3 days to assess follicular activity. Jugular blood samples were also drawn every 3 days for the measurement of plasma progesterone concentrations. On detection of a follicle 35 mm or greater, a mare was scanned and blood was drawn daily until she ovulated or the follicle regressed to < 25 mm. In both experiments, blood samples were taken for at least 5 days post-ovulation. Mares were considered to have responded if they ovulated within 35 days of the onset of treatment.

Blood samples from five control mares and five mares receiving ECP and domperidone (selected randomly from each group) in Louisiana were obtained in Experiment 2 every other day from 2 days before to 50 days after the first injection. Prolactin was measured in these samples to characterize the response to treatment.

Experiment 3 was conducted in late winter and early spring of 2008. Eighteen mares with ovarian sizes less than 20 mm and no evidence of a corpus luteum, housed in Baton Rouge, LA, were randomly assigned to treatments arranged in a 2 x 3 factorial design: ECP at 0, 75, or 150 mg injected intramuscularly on March 11, 2008, followed by DPB at 1.5 or 3.0 g injected 10 days later. Time to first ovulation was determined from progesterone concentrations determined in plasma samples drawn on days -2, -1, 0, 1, 2, 3, 4, 5, 6, 9, 10, 11, 12, 13, 14, 16, 20, 24, 28, 32, 36, 40, and 44 relative to ECP or control (vegetable oil) injections (as day of first high progesterone minus 1 day; progesterone values in non-ovulating mares were consistently less than 0.3 ng/mL, and a sample more than 0.5 ng/mL, followed by increasing progesterone values was considered indicative of ovulation). Because of the later starting date of the experiment, mares were considered to have responded if they ovulated within 21 days of treatment. Prolactin was measured in selected samples from all mares to characterize the response to treatment.

Blood samples from Experiments 2 and 3 were assayed for prolactin with radioimmunoassay as described previously (Colborn et al., 1991). Concentrations of progesterone in plasma were assayed by radioimmunoassay with commercially available reagents (Diagnostic Laboratory Systems, Webster, TX, USA). Intra- and interassay coefficients of variation and assay sensitivities were 7%, 12%, and 0.2 ng/mL for prolactin and 5%, 8%, and 0.05 ng/mL for progesterone, respectively.

For analysis of variance (ANOVA), successful ovulations within the selected time period were coded 1, and failure to respond was coded 0. Data for Experiments 1 and 2 were combined

and analyzed by one-way ANOVA through the general linear model procedure of SAS (SAS Institute, Cary, NC, USA). Contrast statements were utilized to make logical comparisons among groups. Data for Experiment 3 were analyzed by ANOVA for a 2 x 3 factorial arrangement of treatments; one contrast statement was included to compare all ECP-receiving groups to those receiving no ECP. Prolactin concentrations for Experiments 2 and 3 were analyzed by ANOVA that took into account the repetitive sampling. Because there was no effect in the ANOVA for domperidone dose in Experiment 3, data were pooled across domperidone doses for analysis; data were also pooled for the two groups receiving ECP for comparison with those receiving no ECP.

## **Results**

The number of mares ovulating within 35 days in Experiments 1 and 2 are shown in Table 2.1. Results of the contrast statements comparing groups and their associated mean success ratios are presented in Table 2.2. Over both experiments, treatment with ECP plus domperidone increased ( $P = .0002$ ) success rate relative to all other treatments. Treatment with domperidone (no ECP) did not alter success rate ( $P = .24$ ), nor did addition of progesterone to the ECP and domperidone regimen ( $P = .7$ ). Relative to controls only (no treatment), the ECP plus domperidone treatments increased success rate ( $P = .005$ ), whereas dose of ECP (100 vs. 150 mg) did not affect success rate ( $P = .88$ ). Success rates of control mares did not differ between Kentucky and Louisiana ( $P = .81$ ); however, there was a tendency for greater success rates for ECP plus domperidone treatments of mares in Kentucky as compared with that in Louisiana ( $P = .07$ ).

Mean prolactin concentrations in the control mares and those receiving ECP and domperidone are presented in Figure 2.1. There was a stimulatory effect of treatment starting on day 18 ( $P < .05$ ), and prolactin concentrations remained higher in treated mares than in control

**Table 2.1.** Treatment groups, total number of mares, and number of mares ovulating within 35 days in Experiments 1 and 2.

Treatment <sup>a</sup>	n	35-Day Success, n	Mean Success Value <sup>b</sup>
1. KY Controls	9	2	0.22
2. KY domperidone only	9	0	0
3. KY 100 mg ECP + 3 g domperidone	6	3	0.50
4. KY 150 mg ECP + 3 g domperidone	7	5	0.71
5. KY 150 mg ECP + 3 g domperidone + progesterone	7	3	0.43
6. KY 150 mg ECP + progesterone	5	0	0
7. LA controls	13	1	0.08
8. LA 150 mg ECP + 3 g domperidone	11	3	0.27

<sup>a</sup>Experiment 1 was conducted in Kentucky (KY) and Experiment 2 in Louisiana (LA).

<sup>b</sup>Mean success x 100 would be the percentage of mares in a treatment group that ovulated within the predetermined time frame.

**Table 2.2.** Associated mean success values and *P*-values for contrasts made for treatment groups in Experiments 1 and 2.

Contrast <sup>a</sup>	Mean 1 <sup>b</sup>	Mean 2 <sup>b</sup>	<i>P</i> - Value
All ECP + domperidone groups versus rest (3, 4, 5, 8 vs. 1, 2, 6, 7)	0.45	0.08	0.0002
KY domperidone only versus KY controls (2 vs. 1)	0.00	0.22	0.24
ECP + domperidone versus ECP + domperidone + progesterone (3, 4, 8 vs. 5)	0.46	0.43	0.70
Controls versus all ECP + domperidone (1, 7 vs. 3, 4, 5, 8)	0.14	0.45	0.005
KY controls versus KY ECP + progesterone (1 vs. 6)	0.22	0.00	0.32
100 mg ECP + domperidone versus 150 mg ECP + domperidone (3 vs. 4, 5, 8)	0.50	0.44	0.88
Controls, KY versus LA (1 vs. 7)	0.22	0.08	0.81
ECP + domperidone, KY versus LA (3, 4, 5 vs. 8)	0.55	0.27	0.07

<sup>a</sup>Numbers refer to treatments in Table 1; mean 1 is for the first group(s) listed and mean 2 is for the second group(s) listed.

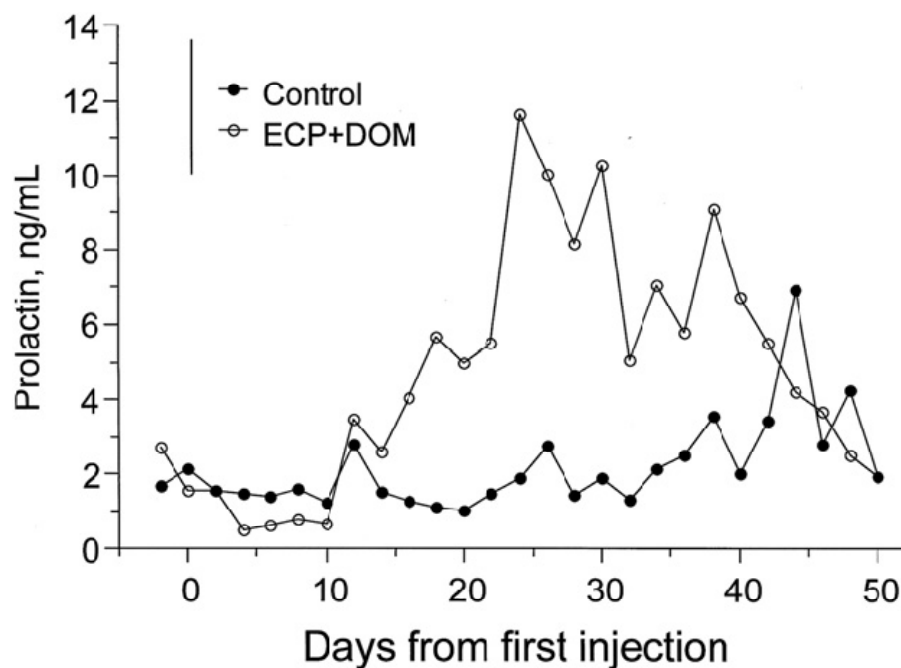
<sup>b</sup>Mean success x 100 would be percentage of mares in a treatment group that ovulated within the predetermined time frame.

mares until day 42. In Experiment 3, there was no effect of ECP dose ( $P = .15$ ), domperidone dose ( $P = .63$ ), or interaction ( $P = .22$ ) for success rate at 21 days (Table 2.3; interaction not shown); however, there was an effect ( $P = .055$ ) of ECP averaged over the two doses relative to no ECP (0 dose). There was an effect of ECP pretreatment ( $P = .04$ ) on prolactin concentrations in Experiment 3 (Fig. 2.2), as well as a tendency for an interaction between treatment and time ( $P = .07$ ); prolactin concentrations differed between groups on days 20 and 24.

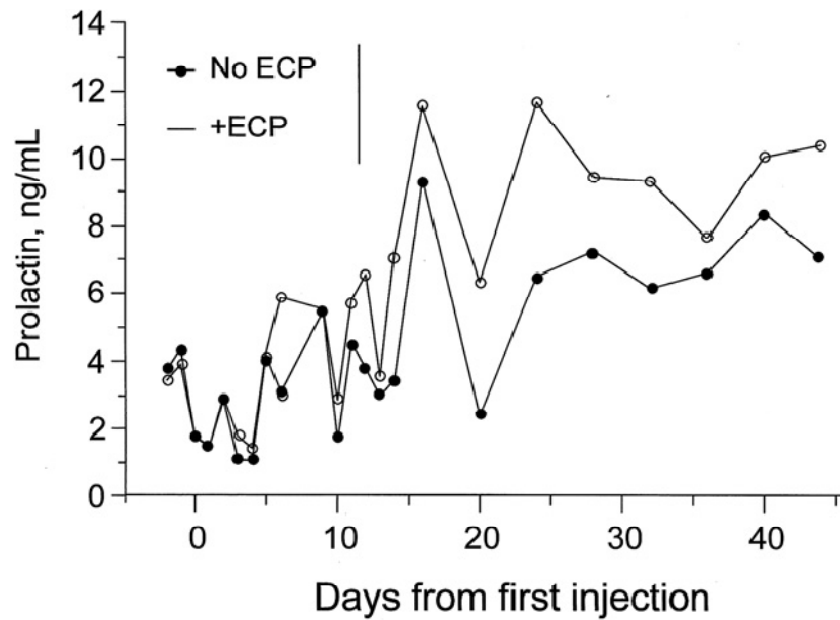
## **Discussion**

Studies on the stimulation of ovarian activity with dopamine D2 receptor antagonists in seasonally anovulatory mares have produced varied results. Besognet et al. (1997) and Brendemuehl and Cross (2000) reported good success rates with sulpiride and domperidone, respectively, whereas Donadeu and Thompson (2002) and McCue et al. (1999) had little to no success with the same compounds. Daels et al. (2000) reported that sulpiride treatment of mares maintained indoors in locations with cold climates was more effective than in mares maintained outdoors at the same location. Kelley et al. (2006) reported that pretreatment of seasonally anovulatory mares with estradiol benzoate for 10 days before the daily administration of 1 mg/kg sulpiride resulted in an earlier ovulation than administration of sulpiride alone. Donadeu and Thompson (2002) noted that administration of 1 mg/kg sulpiride alone to seasonally anovulatory mares did not stimulate follicular development or induce ovulation compared with placebo-treated mares.

Treatment of seasonally anovulatory mares with a single injection of ECP 10 days before a single injection of domperidone in microparticles provides a much more practical method of inducing ovulation in winter relative to the every-other-day injections of estradiol benzoate and daily injections of sulpiride used by Kelley et al. (2006). Although the dual-injection approach



**Figure 2.1.** Mean plasma prolactin concentrations in control mares and mares treated with a single injection of 150 mg ECP on day 0, followed by an injection of 3 g of domperidone in biodegradable particles on day 10 (ECP + DOM) in Experiment 2 (performed in Louisiana). There was a stimulatory effect of treatment starting on day 18 ( $P < .05$ ), and prolactin concentrations remained higher in treated mares than in control mares until day 42. Pooled SEM was 1.86 ng/mL. The vertical line indicates the least-significant difference ( $P < .05$ ) for comparison of control and treatment means within days.



**Figure 2.2.** Mean plasma prolactin concentrations in mares treated with no ECP or a single injection of ECP (75 or 150 mg; +ECP) on day 0 in Experiment 3 (performed in Louisiana); all mares received an injection of 1.5 or 3 g of domperidone in biodegradable particles on day 10. There was an effect of ECP pretreatment ( $P = .04$ ), as well as a tendency for an interaction between treatment and time ( $P = .07$ ). Pooled SEM was 1.3 ng/mL. The vertical line indicates the least-significant difference ( $P < .1$ ) for comparison of group means within days.

**Table 2.3.** Treatment and contrast means and  $P$ -values for Experiment 3.

Treatment	Dose, mg	Mean Success <sup>a</sup>	<i>P</i> -Value
ECP	0	0.17	.15
	75	0.67	
	150	0.67	
Domperidone	1500	0.44	.63
	3000	0.56	
Contrast			
All ECP	75 or 150	0.67	.055
No ECP	0	0.17	

<sup>a</sup>Mean success x 100 would be the percentage of mares in a treatment group that ovulated within the predetermined time frame.

used in the present experiments was superior to the results for mares not receiving the dual-injection protocol, the success rates were not equivalent to that reported by Kelley et al. (2006; eight of nine mares responding). It is assumed that the prolactin response is partially, if not wholly, responsible for the stimulation of ovulation at this time because a similar induction of ovulation resulted from injection of recombinant porcine prolactin in seasonally anovulatory pony mares (Thompson et al., 1997). Concentrations of LH are also stimulated by the ECP injection in the current procedures (Kelley et al., 2006); however, this was not the case for pony mares treated with recombinant prolactin (Thompson et al., 1997). The prolactin responses of mares in Experiments 2 and 3 were variable within treatments. Some treated mares in Experiment 2 experienced maximum prolactin concentrations of 5 ng/mL, whereas others reached 20 ng/mL (controls rarely reached 5 ng/mL, and generally were 2 ng/mL or less). Thus, the magnitude of the prolactin response per se does not seem to be a good predictor of whether a mare will ovulate within 35 days. The same seemed to be true for Experiment 3, in that there was no apparent relationship between magnitude of the prolactin response and success. This may indicate that sensitivity of a given mare to the increase in prolactin and LH concentrations is an important factor in whether she responds to some minimal, or threshold, increase in prolactin and LH.

Given that there was no difference in success rates for the doses of ECP and domperidone tested, it is possible that the minimal effective doses of these components have yet to be reached. Kelley et al. (2006) treated cyclic mares in the breeding season (starting on the first day of estrus) with the same dose of estradiol benzoate that was used in their winter experiment and found no perturbation in time to ovulation, subsequent luteal function, or cycle length compared with controls. They concluded that the dose of estradiol benzoate, which raised plasma estradiol concentrations to approximately 20 pg/mL in 21 days in a gelding model (Thompson et al.,



2008), was low enough to not perturb follicular activity, as opposed to higher doses (Woodley et al., 1979). The efficacy of lower doses of ECP needs to be tested in the future, as well as the timing of the domperidone relative to the ECP injection.

In retrospect, the average temperatures for the two regions during Experiments 1 and 2 were compared. The mean maximum temperature in Louisville, KY, during Experiment 1 (January 19 through February 23) was 35°F, and mean minimum was 21°F. An overall success rate of 55% under such unfavorable conditions is not ideal, but perhaps is understandable. For comparison, the mean maximum and minimum temperatures of Baton Rouge, LA, during the same period were 66°F and 34°F, respectively, yet the success rate tended to be lower than in Kentucky. Moreover, the average hours:minutes of daylight for the two locations during the experiments were 10:17 for Lexington and 10:48 for Baton Rouge. Other factors, such as nutritional plane and body condition score of the mares were similar for the two groups.

Domperidone alone had no beneficial effect on ovulation relative to controls. This is in contrast to the report of Brendemuehl and Cross (2000), who fed mares 1.1 mg/kg body weight domperidone daily, starting January 14. Mares fed domperidone ovulated an average of 25 days later (range, 15 to 55), whereas the average date of first ovulation for controls was 78 days beyond that of the treated mares. In contrast to the results of Brendemuehl and Cross (2000), Mari et al. (2009) reported no stimulatory effect of domperidone feeding on mares in deep anestrus (February 3 to 28); they did report success with daily sulpiride injections. The lack of response to domperidone alone in Experiment 1 may be because of the amount getting into the bloodstream, and hence any possible prolactin response; however, Mari et al. (2009) also reported a lack of response to orally administered domperidone, which has been reported to be effective by others. Brendemuehl and Cross (2000) reported very high prolactin concentrations (20 to 30 ng/mL averages from 7 to 63 days of domperidone feeding); this was relative to

feeding approximately 550 mg daily until ovulation (a total of 13.75 g for a 500-kg mare). From the response seen in Figure 2.1, a single injection of 3 g of DPB, coupled with pretreatment of ECP, resulted in elevated prolactin concentrations from 16 to 40 days (roughly 75 mg of domperidone per day, on average, for 40 days). It is likely that the domperidone injection alone in Experiment 1 raised plasma prolactin concentrations only slightly, similar to the responses reported by Kelley et al. (2006) for mares in summer and Donadeu and Thompson (2002) for mares administered daily sulpiride; LH concentrations would not be expected to be stimulated by domperidone alone in the first few days as they are by ECP (Kelley et al., 2006, Thompson et al., 2008).

In conclusion, pretreatment of mares with ECP followed by administration of DPB, as described in these experiments, can be used to stimulate a greater proportion seasonally anovulatory mares and transition period mares to ovulate than would otherwise. It is likely that as little as 75 mg ECP and as little as 1.5 g of domperidone can be used. Further research is needed to determine whether the 10-day delay between injections is optimal, and whether the doses tested to date can be reduced even further.

### **CHAPTER III**

## **COMPARISON OF ESTRADIOL CYPIONATE DOSES AND DOSING INTERVAL OF ESTRADIOL CYPIONATE AND DOMPERIDONE IN SEASONALLY ANOVULATORY MARES**

### **Introduction**

In previous studies (Kelley et al., 2006; Chapter II), estradiol treatment prior to dopamine antagonist administration was used successfully to enhance prolactin responses in mares to dopamine antagonist treatment. In the initial experiment (Kelley et al., 2006), seasonally anovulatory mares were injected with estradiol benzoate in oil every other day for 20 days, with daily injections of the dopamine antagonist sulpiride in vegetable shortening beginning on day 11 of estradiol treatment. Daily sulpiride injections were given until ovulation, or for 45 days. Compared to mares receiving only sulpiride, estradiol pretreated mares had much greater prolactin responses and ovulated an average of 30 days earlier.

In an attempt to simplify the treatment regimen (Chapter II), seasonally anovulatory mares were treated with a single 150 mg injection of ECP in place of daily injections (Thompson et al., 2008) 10 days before a single injection of the dopamine antagonist, domperidone (3 g), in biodegradable polymer. Although the prolactin response in ECP-treated mares was greater than in controls (no ECP or domperidone), it was not as great as that observed previously by Kelley et al. (2006). Moreover, the percentage of mares ovulating within 30 days of treatment was greater than controls, but not as successful as the response observed by Kelley et al. (2006).

Both of those previous studies used a similar delay between estradiol treatment and treatment with a dopamine antagonist. The current experiment was designed to answer two questions. First, how does the delay between ECP and domperidone injections affect the prolactin response, and secondly, would a lower dose of ECP (50 vs. 100 mg) produce an equivalent stimulation of prolactin and LH in these mares?

## Materials and Methods

Animals and treatments. Prior to the beginning of the project in January, mares in the resident herd at LSU Agricultural Center Horse Unit were examined via rectal ultrasonography (Aloka 550V with 5-MHz linear-array transducer; Aloka Science and Humanity, Wallingford, CT) to determine follicular activity and estrous cycle status. Only mares that were determined to be anovulatory based upon ovarian status were selected for use in the current project. Mares were blocked by age and body condition into 3 similar groups of 9 plus a control group of 5 mares. On day 0 (February 9, 2009), within each group (except controls), half (4 or 5) of the mares were treated with 50 mg ECP and half were treated with 100 mg of ECP. The three treatment groups were then subsequently treated with 1.5 g of domperidone in microparticles on day 1, 6 or 11 relative to ECP treatment. Control mares received injections of vehicle on treatment days.

All mares used were light horse type, ranging from 5 to 20 years of age with body conditions ranging from 5 to 8 (Henneke et al., 1983). They were maintained on native grass pastures and supplemented with hay as needed. They also had free access to water and trace minerals.

Ultrasound schedule and blood sample collection and analysis. Beginning on day -2, all mares were examined with ultrasound twice weekly until detection of a follicle > 30 mm, at which point the mare was placed on daily ultrasound either until ovulation or regression of the follicle to < 25 mm. Blood samples were collected in the morning on days -1, 0, 1, 2, 3, 4, 6, 7, 8, 9, 11, 12, 13, 14, 16, 18, 20, 22, 24, 26, 28, 30 and 32 relative to ECP treatment. Also, any mare not ovulating within that 32-day time frame had additional samples collected twice weekly until ovulation. Blood samples were collected via jugular venipuncture into sodium heparin tubes (Vacutainer, Becton and Dickinson, Franklin Lakes, NJ). Samples collected were analyzed for

progesterone levels using commercially available kits (Diagnostic Laboratory Systems, Webster, TX, USA), for prolactin and LH as previously described by Colborn et al. (1991) and Thompson et al. (1983), respectively. Intra- and interassay coefficients of variation and assay sensitivities were 5%, 8%, and 0.05 ng/mL for progesterone, 7%, 12% and 0.2 ng/mL for prolactin, and 6%, 9% and 0.2 ng/mL for LH.

Statistical analyses. Data were analyzed using the Proc GLM procedure of SAS (SAS Institute, Cary, NC, USA). Hormonal data from daily samples were analyzed for effects of treatment, time and treatment by time interactions with repeated measures ANOVA. Due to the unbalanced nature of treatments, separate analyses were run. The first compared all treatments as 7 individual treatments. Because there was no effect of ECP dose in the first analysis, the second series of ANOVA compared the three domperidone treatment groups individually against the controls. Also, data for LH were truncated at day 11 due to the confounding of responses. That is, mares that ovulated had suppressed LH concentrations due to the progesterone rising in the blood, whereas non-responders did not, and typically had increasingly higher LH in successive days.

For analysis of successful ovulations within the selected time period (28 days), mares ovulating were coded 1 and those failing to ovulate were coded 0; factorial ANOVA with ECP dose and day of domperidone, as well as the interaction, was used. Contrast statements were utilized in SAS to make logical comparisons among groups.

## **Results**

After final progesterone determinations, several mares (2 controls and 6 in the treated groups) were found to be not anovulatory prior to the onset of the experiment. Subsequently, those mares were excluded from the data analyses. The final numbers of mares within each group and their associated success rates (ovulation in the first 28 days) are shown in Table 3.1.

**Table 3.1.** Treatment groups, total number of mares, and number of mares ovulating within 28 days.

Treatment	n	28-day success, n	Mean success value <sup>a</sup>
1. 50 mg ECP + domperidone on d1	4	4	1.00
2. 50 mg ECP + domperidone on d6	4	3	0.75
3. 50 mg ECP + domperidone on d11	4	1	0.25
4. 100 mg ECP + domperidone on d1	3	3	1.00
5. 100 mg ECP + domperidone on d6	2	0	0.00
6. 100 mg ECP + domperidone on d11	4	3	0.75
7. Controls; no ECP or domperidone	3	0	0.00

<sup>a</sup>Mean success x 100 would be the percentage of mares in a treatment group that ovulated within the predetermined 28-day time frame.

**Table 3.2.** Associated mean success values and *P*-values for contrasts made between treatment groups.

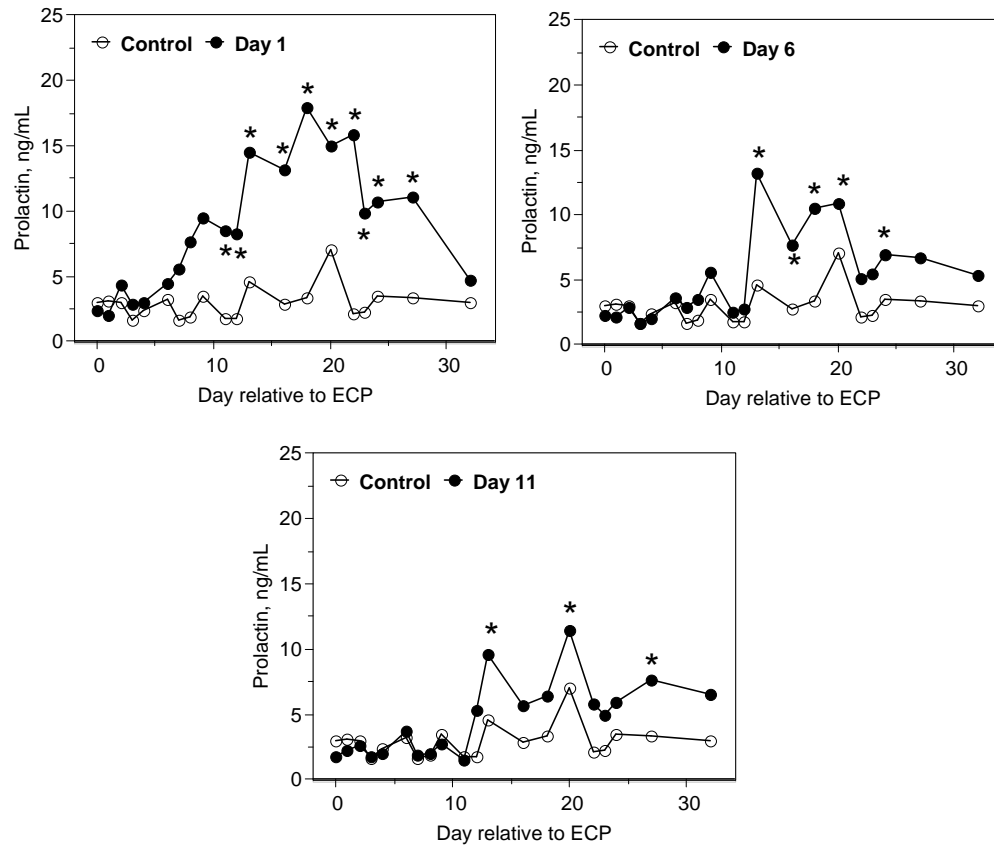
Contrast <sup>a</sup>	Mean 1 <sup>b</sup>	Mean 2 <sup>b</sup>	<i>P</i> -Value
All treated versus controls (1, 2, 3, 4, 5, 6, vs. 7)	0.67	0.00	0.0131
50 mg ECP vs 100 mg ECP (1, 2, 3 vs. 4, 5, 6)	0.67	0.67	0.6183
50 mg ECP vs controls (1, 2, 3 vs. 7)	0.67	0.00	0.0293
100 mg ECP vs controls (4, 5, 6 vs. 7)	0.67	0.00	0.0113
Domperidone d1 vs domperidone d6 (1, 4 vs. 2, 5)	1.00	0.50	0.0085
Domperidone d1 vs domperidone d11 (1, 4 vs. 3, 6)	1.00	0.50	0.0172

<sup>a</sup>Numbers refer to treatments in Table 3.1; mean 1 is for the first group(s) listed and mean 2 is for the second group(s) listed.

<sup>b</sup>Mean success x 100 would be percentage of mares in a treatment group that ovulated within the predetermined time frame.

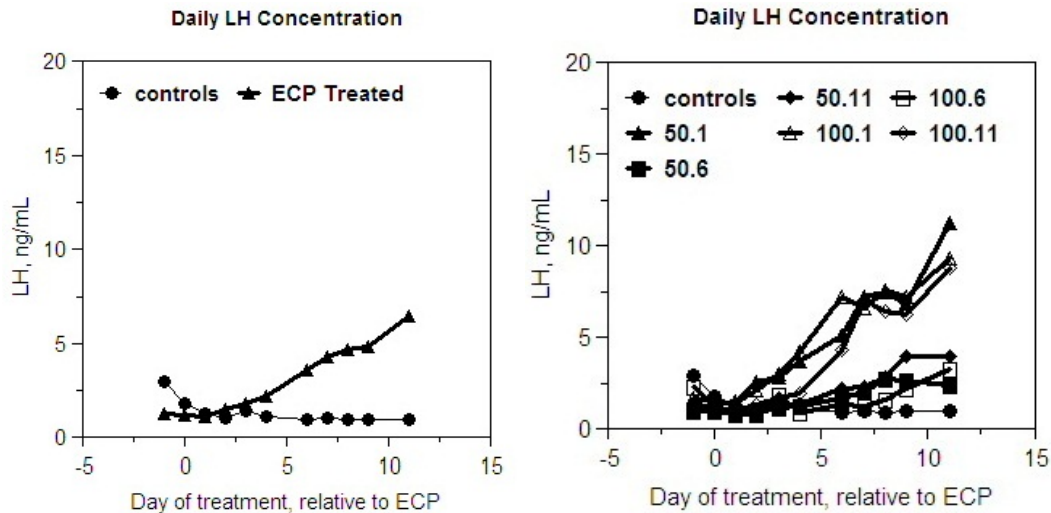
The contrasts among groups and their associated means and *P*-values are presented in Table 3.2.

There was an effect of treatment on date of first ovulation ( $P = 0.0092$ ), with treated (any combination of ECP + domperidone) mares ovulating earlier than controls. There was no difference in success due to ECP dosage ( $P = 0.62$ ). Domperidone treatment on day 1 was more effective than treatment on day 6 ( $P = 0.0085$ ) or day 11 ( $P = 0.017$ ). Daily prolactin means by



**Figure 3.1.** Mean daily prolactin concentrations for the three days of domperidone treatment analyzed separately versus control mares. Asterisks indicate differences ( $P < 0.1$ ) between treated and control mares for the specified day relative to ECP injection. Overall, the prolactin response tended to be better for day 1 than for days 6 or 11.

day of domperidone treatment are presented in Figure 3.1. Individual groups are presented versus the controls for clarity. There was no effect of ECP dosage on daily prolactin concentrations ( $P = 0.75$ ), nor any interaction with day of domperidone treatment, thus the individual ANOVA were run without this factor in the model. From those individual ANOVA for each day, differences ( $P < 0.1$ ) were detected between the treated mares and controls for differing days of



**Figure 3.2.** Mean LH concentrations in mares treated with ECP relative to controls (left panel); means for the seven individual groups (right panel). There was an interaction ( $P = 0.04$ ) between treatment and day relative to ECP injection. Individual means on the right illustrate the variation in mean responses among the groups receiving ECP. The greatest responses were in the groups treated on day 1 and one of the groups treated on day 11.

treatment, as indicated by the asterisks in the figure. In general, the prolactin responses to domperidone were more consistent and higher in magnitude in the mares treated on day 1 compared to the responses for the other two days.

Daily concentrations of LH analyzed through day 11 relative to ECP treatment are presented in Figure 3.2. In the first analysis, which compared all seven treatment groups, there was an overall effect of treatment ( $P = 0.067$ ) on LH response. In the second analysis, in which all ECP treated groups were compared to controls, there was an interaction ( $P = 0.04$ ) between treatment and day relative to ECP injection. Mean LH concentrations increased over time in ECP treated mares relative to controls and were higher from days 7 through 11.



## Discussion

Using dopamine antagonists by themselves as a means of inducing early cyclicity in mares has produced mixed results in previous studies. Besognet et al. (1996; 1997) observed a hastening of first ovulation in mares treated daily with sulpiride alone (1 mg/kg body weight and 200 mg per mare, respectively). Mari et al. (2009) successfully induced ovulation in mares with sulpiride, but not domperidone. Brendemuehl and Cross (2000) had success with daily oral domperidone. In contrast, Donadeu and Thompson (2002) and McCue et al. (1999) saw no hastening of first ovulation in mares treated daily with sulpiride alone.

Early ovulation has been induced in mares by treatment with prolactin only (Nequin et al., 1993; Thompson et al., 1997). While several studies have seen treatment with the dopamine antagonist sulpiride increase prolactin levels in anovulatory mares (Besognet et al., 1997; Donadeu and Thompson, 2002), early ovulation was not necessarily achieved. Also, there have been reports of the prolactin response to sulpiride treatment decreasing over time in geldings (Thompson and DePew, 1997) and in mares (Donadeu and Thompson, 2002), indicating that the induction of secretion was likely not accompanied by an increase in production.

Estradiol is known to be antidopaminergic (Raymond et al., 1978; Fitzgerald and Dinan, 2008). Additionally, it increases production of pre-prolactin mRNA in rats (Ryan et al., 1979; Maurer 1982) and has been reported to increase both circulating plasma concentrations of prolactin, as well as pituitary content in ovariectomized pony mares (Thompson et al., 1991). Therefore, it is likely that the high levels of prolactin seen by Kelley et al. (2006) and in this experiment, when compared to prolactin levels reported in previous dopamine antagonist only studies (Thompson and DePew, 1997; Donadeu and Thompson, 2002), were due to the employment of estradiol pretreatment prior to dopamine antagonist treatment. Thus,

pretreatment with estradiol appears to stimulate prolactin synthesis and ensures ample available hormone to be released upon dopamine antagonist treatment over an extended period of time.

In an effort to further simplify the protocol (i.e., fewer total injections), subsequent research by Thompson et al. (2008) indicated that replacing the frequent injections of EB in oil (Kelley et al., 2006) with a single shot of ECP in oil gave a similar and consistent response to the previously used regimen, which was implemented successfully in Chapter II. Both Kelley et al. (2006) and the experiments in Chapter II involved similar time intervals of 10 to 11 days between estradiol treatment and onset of dopamine antagonist treatment. Different timing of the two injections needed to be examined given the possibility another time interval might contribute to a superior response, hence the current experiment was devised. In general, treatment with domperidone 1 day after ECP tended to give the best prolactin and LH responses, as well as ovulation times. Thus, this data contributes to the goal of establishing a more stream-lined, less labor intensive protocol for inducing cyclicity in mares. It was interesting to note that the lower dose of ECP (50 mg) was not different than the higher dose (100 mg) with regard to the prolactin and LH responses.

While treatment did have an effect on LH concentration, it was not as dramatic as the effect seen by Kelley et al. (2006). Burns and Douglas (1981) found that treatment with estradiol stimulated LH secretion for up to two weeks after CL regression. Those circumstances are similar in anovulatory mares, as they have no active luteal tissue present, and therefore no influence of progesterone on LH concentrations. Garcia and Ginther (1978) also reported that treatment with estradiol increased LH concentrations in ovariectomized mares in both summer and in winter. One would expect the estradiol effect in this study and that of Kelley and co-workers (2006) to be similar given that the comparison made by Thompson et al. (2008)

indicated a comparable LH response in the two forms of estradiol (i.e., unconjugated estradiol versus ECP).

Mari et al. (2009) did not observe a main effect of treatment (sulpiride and domperidone) on LH concentration in anovulatory mares (no estradiol was given), but they did observe an effect of time and treatment by time interaction on LH concentrations. In that study, beyond day 18, LH was higher in sulpiride treated mares than domperidone treated mares, though neither group received any sort of estradiol treatment. That observation may have been due to the greater number of responding mares (since more had ovarian response to sulpiride than to domperidone) and hence more follicular estradiol circulating in the blood. Alternatively, these findings might mean that the more robust prolactin response observed by Kelley et al. (2006) could be due to the effect of sulpiride further stimulating prolactin production versus domperidone. Further studies will be needed to confirm effects of treatment on LH concentrations.

## **CHAPTER IV**

### **DOMPERIDONE VERSUS SULPIRIDE EFFECTS ON PROLACTIN SECRETION IN ESTRADIOL-PRIMED GELDINGS**

#### **Introduction**

Kelley et al. (2006) first reported the stimulatory effect of estradiol pretreatment on prolactin secretion in response to daily sulpiride administration in seasonally anovulatory mares. In Chapter II, the use of a single injection of ECP followed by a single injection of domperidone in biodegradable microparticles was tested, based on the selection of a single injection of ECP performed by Thompson et al. (2008). Results in the experiments in Chapter II were positive, but mixed. Treated mares ovulated earlier than their non-treated counterparts and had a higher prolactin response, but the results lacked the consistency that was achieved by Kelley et al. (2006). Sulpiride treated mares had significantly higher prolactin levels over controls one day after treatment (Kelley et al., 2006), whereas domperidone treated mares did not have significantly higher prolactin levels versus controls until 8 days after treatment (Chapter II).

Thus, the question arose whether the disparity in achieving early ovulation was due to the fact that sulpiride produced a better prolactin response and therefore more consistent results. Thus, the present experiment was designed to directly compare the prolactin responses to domperidone versus sulpiride in estradiol-primed geldings. It has been confirmed that geldings serve as a good model, in lieu of seasonally anovulatory mares for assessing the prolactin and LH responses to treatment (Thompson et al., 2008).

#### **Materials and Methods**

Animals and treatments. Eighteen geldings were used from the resident herd at the LSU Agricultural Center Horse Unit in Baton Rouge. They ranged from 9 to 24 years in age and had body condition scores ranging from 6 to 8 (Henneke et al., 1983). Horses were maintained on

local native grass pastures and supplemented with hay when required for maintenance of body condition. They had free access to water and mineralized salt blocks. The experiment was conducted in February and March, 2009.

Using data from previous experiments, geldings were grouped based on their prolactin responses in previous experiments, and then allotted to groups such that each group had approximately the same number of high versus low responders. The 3 groups were then randomly assigned to 3 treatments: 1) ECP only, receiving 100 mg ECP on day 0 and vehicle only on day 7; 2) ECP and domperidone, receiving 100 mg ECP on day 0 and 1.5 g domperidone on day 7; and 3) ECP and sulpiride, receiving 100 mg ECP on day 0 and 1.5 g of sulpiride on day 7. The ECP injections were intramuscular injections in 2 mL of oil-based vehicle, and the domperidone and sulpiride were intramuscular injections in microparticles mixed with the provided vehicle. All injections were given in the neck region in the splenius muscle.

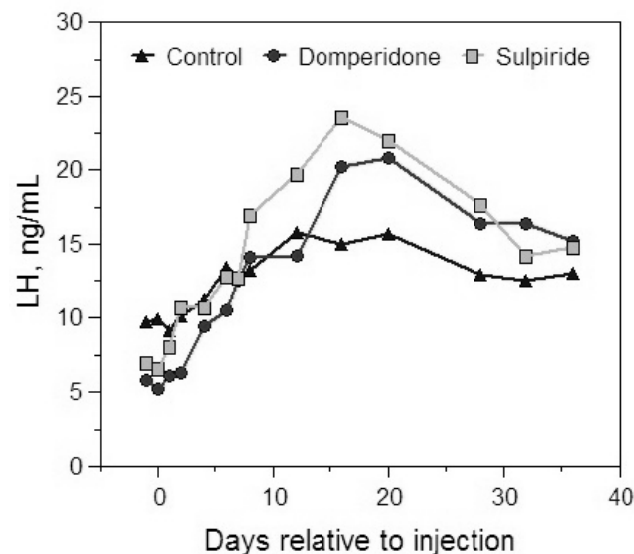
Blood sample collection and analysis. Jugular blood samples were collected on days -2 and -1, and immediately before injection of ECP on day 0 (February 28, 2009). Subsequent blood samples were collected on days 1, 2, 4, 6, 7, 8, 12, 16, 20, 24, 28, 32, 36, and 40 (April 9) relative to treatment. Samples were collected into 7 mL sodium heparin tubes (Vacutainer, Becton and Dickinson, Franklin Lakes, NJ) and centrifuged within 30 minutes of collection; plasma was harvested and stored frozen until assayed for prolactin (Colborn et al., 1991) and LH (Thompson et al., 1983). Intra- and interassay coefficients of variation and assay sensitivity were 7%, 12% and 0.2 ng/mL for prolactin, 6%, 9% and 0.2 ng/ml for LH.

Statistical analyses. Data were analyzed using the Proc GLM procedure of SAS (SAS Institute, Cary, NC, USA). Data from daily samples were analyzed for effects of treatment, time and treatment by time interactions with a repeated measures design ANOVA. Differences

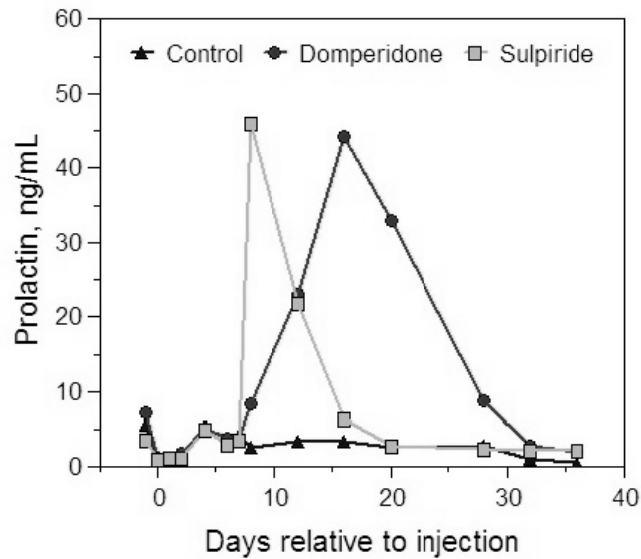
between means for the 3 treatment groups for any given day were compared by the lsd-test using the pdiff procedure of SAS.

## Results

There was an effect of day ( $P < 0.001$ ) on LH concentrations, but no effect of treatment or interaction with treatment ( $P > 0.1$ ; Figure 4.1). In contrast, treatment did affect prolactin concentrations (Figure 4.2). There was an effect of treatment ( $P = 0.022$ ) as well as an interaction of treatment and sampling day ( $P < 0.001$ ). Control geldings had relatively constant prolactin concentrations over the 32-day sampling period. Geldings treated with sulpiride in biodegradable microparticles had maximum prolactin response on the first day after injection, and prolactin concentrations had returned to baseline within about 10 days of injection. In contrast, geldings



**Figure 4.1.** Mean daily LH concentrations in estradiol-primed (100 mg ECP on day 0) geldings receiving an injection of vehicle (control) or domperidone or sulpiride in biodegradable microparticles on day 7. There was an effect of day relative to ECP injection ( $P < 0.001$ ) but no effect ( $P > 0.1$ ) of treatment or interaction.



**Figure 4.2.** Mean daily prolactin concentrations in estradiol-primed (100 mg ECP on day 0) geldings receiving an injection of vehicle (control) or domperidone or sulpiride in biodegradable microparticles on day 7. There was an effect of treatment ( $P = 0.022$ ) as well as an interaction ( $P < 0.001$ ) between treatment and day relative to ECP injection.

treated with domperidone in biodegradable microparticles had maximum prolactin response 9 days after injection, and prolactin concentrations returned to baseline in about 20 days after injection. The maximum responses of the two treated groups did not differ.

## Discussion

The results of this experiment are consistent with the results of Kelley et al. (2006) in that sulpiride provides a good prolactin response in estrogen-primed horses, and differs from domperidone only in duration of response. The mares used by Kelley et al. (2006) were treated daily with sulpiride, and the prolactin response lasted until the estrogen treatment had ceased. The sulpiride in the current experiment was in biodegradable microparticles, and was expected to provide several days of sulpiride release. In actuality, the response was only about half as long as the response to domperidone in microparticles, thus for equivalent prolactin elevation, the

sulpiride injections would need to be given twice as often as the domperidone injections.

However, the immediate prolactin response to the sulpiride formulation may provide a benefit in regimens for inducing ovarian activity and ovulation in seasonally anovulatory mares. It may be that a combination of sulpiride and domperidone in microparticles would provide an extended period of elevated prolactin concentrations, and this needs to be tested in future experiments.

Given that all 3 groups of geldings received the same dose of ECP, the similarity in LH responses was not unexpected. However, the lack of difference does indicate that there is little or no effect of sulpiride versus domperidone on the LH response to ECP, as was suggested in Chapter III. The elevation in LH concentrations serves as a good indicator of the biological activity (duration of activity) and the timing of hydrolysis to free estradiol of the injected ECP, which were apparently similar in the 3 treatment groups.

Although not directly comparable, the prolactin responses to domperidone in these geldings seemed much greater in magnitude than the prolactin responses observed in previous experiments in seasonally anovulatory mares (Chapter II, Chapter III). Mean prolactin concentrations in those experiments reached as high as approximately 15 ng/mL, and all were less than 20 ng/mL. The mean response in these geldings was approximately twice that of mares. The present experiment was conducted in late February through March, which may account for the greater responses; however, it needs to be noted that geldings used as a model may be better responders to the estradiol-domperidone combination than seasonally anovulatory mares. Alternatively, given the variability of prolactin responses in seasonally anovulatory mares to the estradiol-domperidone combination, it may be that geldings respond more consistently (fewer or no non-responders) than mares. This possibility needs to be tested directly in future experiments in winter including both mares and geldings as test subjects.



## **CHAPTER V**

### **DOMPERIDONE VERSUS SULPIRIDE INJECTION SIMULTANEOUS WITH ECP IN SEASONALLY ANOVULATORY MARES**

#### **Introduction**

Current reports in the literature indicate that the use of dopamine antagonists is a viable treatment for the successful induction of early cyclicity and ovulation in seasonally anovulatory mares (Besognet et al., 1996, 1997; Brendemuehl and Cross, 2000; Daels et al., 2000; Mari et al., 2009). The mechanism of action is presumably the increased prolactin concentration in mares that ovulate in response to treatment. Kelley et al. (2006) first reported a much higher success rate when combining the dopamine antagonist sulpiride with an estradiol pretreatment over dopamine antagonist treatment alone. Success in inducing early ovulation was also achieved in the experiments in Chapter II by combining a single ECP injection (Thompson et al., 2008) with a single injection of the dopamine antagonist domperidone in microparticles, albeit not to the degree seen by Kelley et al. (2006).

A subsequent experiment included in Chapter III sought to test the dosing interval between estradiol pretreatment and administration of the dopamine antagonist domperidone. Results of that experiment indicate that one day between treatments was superior to either 6 or 11 days, both in terms of prolactin response and day of first ovulation.

The experiment described in chapter IV compared a single injection of sulpiride and domperidone after ECP treatment in geldings to compare prolactin responses. As previously mentioned, Kelley et al. (2006) had higher success rates in terms of early ovulation with the use of sulpiride compared to domperidone as used in Chapter II, giving rise to the question if sulpiride was superior in terms of prolactin response as a precursor to early ovulation. Results presented in Chapter IV indicate that the amplitude of prolactin response to both sulpiride and

domperidone after ECP treatment was similar, but response to sulpiride was more immediate than that of domperidone, and more short-lived.

Based upon those results, the current experiment was designed to compare sulpiride and domperidone following ECP treatment in anovulatory mares to determine if one was better than the other in eliciting early ovulation. A second component involved comparing smaller doses of ECP than those used in previous experiments (Chapter II; Chapter III). The initial selection of 100 mg of ECP was based on the comparison performed by Thompson et al. (2008). The experiment in Chapter II used 100 versus 150 mg, with no difference detected between doses. In the experiment in Chapter III, 50 versus 100 mg of ECP were compared, again with no difference in ECP dose detected. From these results, it was apparent that the minimal effective dose of ECP had not yet been reached. Therefore, the current experiment was designed to compare 25 versus 50 mg of ECP as a pretreatment. Furthermore, given that 1 day between components of the protocol was superior over other intervals previously evaluated, simultaneous injection of ECP and the dopamine antagonist was evaluated in the current experiment. If successful, the treatment regimen could be streamlined into a single injection, meeting the ultimate goal of a non-labor intensive approach to inducing early cyclicity and ovulation in mares.

## **Materials and Methods**

Animals and treatments. Prior to the start of the project, all mares in the resident herds at the LSU Agricultural Center Horse Unit and the LSU Reproductive Biology Center had blood samples collected once per week for 3 weeks for progesterone analysis to confirm lack of any active luteal tissue. Mares were also examined with ultrasound scan of the ovaries (Aloka 550V with 5-MHz linear-array transducer; Aloka Science and Humanity, Wallingford, CT) prior to the beginning of the project to assess follicular activity. Only mares with no high progesterone ( $< 1$

ng/mL) and no follicles > 20 mm were selected for the project. Eligible mares were allotted to 3 equal groups (n = 10 each) based on ovarian activity and body condition score (Henneke et al., 1983). The groups were then randomly allotted to treatment. All mares used were light horse type, ranging from 5 to 20 years of age with body conditions ranging from 5 to 8. They were maintained on native grass pastures and supplemented with hay as needed. They also had free access to water and mineralized salt blocks.

The project began (day 0) on January 15, 2010. Control mares received an injection of vehicle only. A second group of mares received ECP + domperidone (1.5 g in microparticles) on day 0, with half of the group receiving 25 mg (n = 5) or 50 mg (n = 5) of ECP. A third group received ECP + sulpiride (1.5 g in microparticles) on day 0, with half of the group receiving 25 mg (n = 5) or 50 mg (n = 5) of ECP.

Blood sample collection, ultrasound, and analysis. Jugular blood samples were collected into 7 mL sodium heparin tubes (Vacutainer, Becton and Dickinson, Franklin Lakes, NJ) on days -3, -2, -1, 0, 1, 2, 3, 4, 6, 8, 10, 12, 14, 17, 19, 21, 23, 25, 27, 29, 31, 33, 38 for analysis of progesterone, prolactin, and LH. Samples were centrifuged within 30 minutes of collection; plasma was harvested and stored frozen until assayed. Progesterone analysis was performed using commercially available kits (Diagnostic Laboratory Systems, Webster, TX, USA) after day 38 to determine if any mares had responded to treatment. Prolactin and LH were analyzed via radioimmunoassay as previously described for horses (Colborn et al., 1991; Thompson et al., 1983). Intra- and interassay coefficients of variation and assay sensitivities were 5%, 8%, and 0.05 ng/mL for progesterone, 7%, 12% and 0.2 ng/mL for prolactin and 6%, 9% and 0.2 ng/mL for LH. Mares were also scanned via rectal ultrasonography on January 13 to determine follicular activity and a second time on February 22 (day 38) of the project.

Data were analyzed using the Proc GLM procedure of SAS (SAS Institute, Cary, NC, USA). Data from daily samples were analyzed for effects of treatment, time, and treatment by time interactions with a repeated measures design ANOVA. Differences between means for the treatment groups for any given day were compared by the lsd-test using the pdiff procedure of SAS.

## **Results**

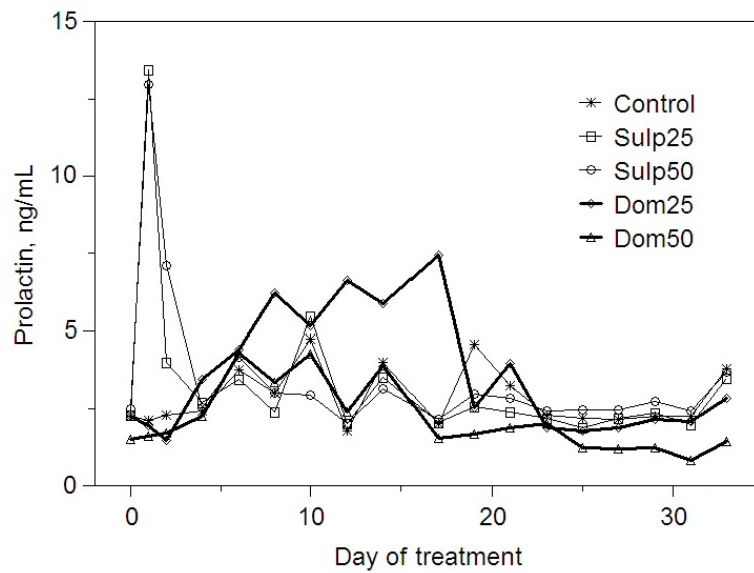
There was no effect of treatment on day of first ovulation ( $P = 0.66$ ). Only two mares ovulated during the timeframe considered for a mare to be responsive to treatment (first 30 days): one control mare and one mare in the 25 mg ECP group receiving domperidone.

There was no main effect of treatment on daily prolactin secretion ( $P = 0.29$ ). However, there was an effect of time ( $P < 0.0001$ ) and a treatment by time interaction ( $P < 0.0001$ ). Some perturbations in prolactin secretion occurred; however, elevated levels were not maintained. Daily prolactin secretion by treatment is presented graphically in Figure 5.1.

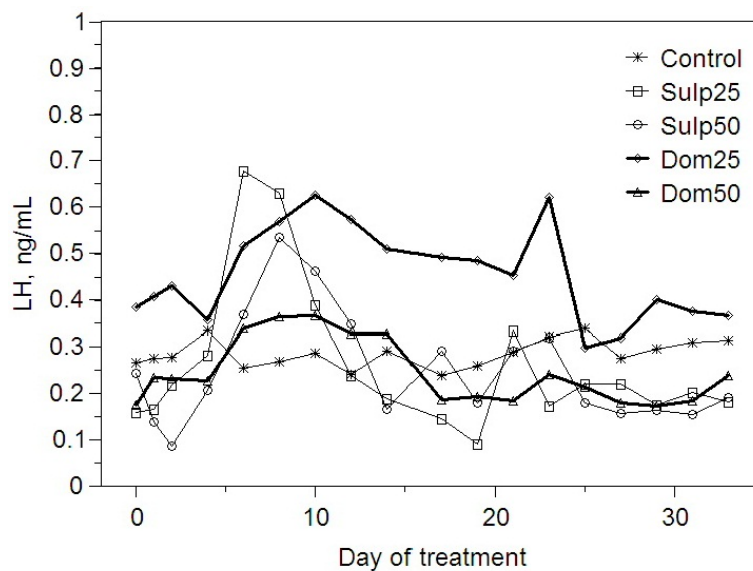
Upon LH analysis, data for two mares were removed from the analysis. These two mares had unusually high LH levels (10 to 20 ng/mL throughout the project) compared to the rest of the mares, who all had less than 1 ng/mL for the duration of the project. It was suspected that these mares had residual endometrial cup tissue from an earlier pregnancy, and it was equine chorionic gonadotropin interfering in the LH assay. In the remaining mares, there was no effect of treatment on daily LH secretion ( $P = 0.34$ ). Daily LH concentrations are presented graphically in Figure 5.2.

## **Discussion**

As previously discussed, results using dopamine antagonists for the induction of early cyclicity and ovulation in mares has produced mixed results (Besognet et al. 1996, 1997;



**Figure 5.1.** Daily PRL secretion in anovulatory mares receiving 25 or 50 mg of ECP simultaneous with either sulpiride or domperidone. There was no effect of treatment on prolactin secretion ( $P = 0.2927$ ). Time and treatment by time interaction were significant, both at ( $P < 0.0001$ )



**Figure 5.2.** Daily luteinizing hormone secretion in anovulatory mares receiving 25 or 50 mg of ECP simultaneous with either sulpiride or domperidone. There was no effect of treatment on prolactin secretion ( $P = 0.3436$ ).

McCue et al., 1999; Brendemuehl and Cross, 2000; Donadeu and Thompson, 2002; Mari et al., 2009). The current experiment was, however, the first attempt using estradiol pretreatment combined with dopamine antagonists that failed to produce early ovulations and poor prolactin response to treatment (Kelley et al., 2006; Chapter II; Chapter III).

Given the results of the study in Chapter III, in which 1 day between treatments proved superior over longer lapses, it was tested whether both components of treatment could be given simultaneously with the same results and, at first glance, the answer appears to be a resounding “no.” However, the failure of LH to be stimulated, even at the 50 mg dose of ECP, indicates that conditions for this experiment in some way differed from the previous experiments. Whether the ECP formulation was not as biologically active as it was thought to be (possibly due to degradation or error in preparation), or whether the environmental conditions produced less responsive mares, is not known. In every experiment conducted thus far, there have been at least one or more non-responders (little to no LH response to ECP) among mares that respond to treatment. Whatever factor(s) contribute to those mares being non-responsive could possibly have affected the majority of the mares in this experiment. Unfortunately, the factor(s) that have been responsible for non-responders in the past have yet to be identified.

In general, prolactin responded to the administration of the dopamine antagonists, but the magnitude of the response was mediocre. As expected, sulpiride-treated mares had an immediate, sharp spike in prolactin, but the elevation was not maintained and returned to pre-treatment levels in approximately 4 days. Domperidone treated mares had a slightly delayed and less dramatic rise in prolactin, but again the circulating concentrations of prolactin were both low and not maintained for very long. When the estradiol pretreatment plus dopamine antagonist protocol has been used successfully, prolactin levels in those mares were elevated for over 20

days (Kelley et al., 2006; Chapter II; Chapter III). The lackluster prolactin response may also reflect a lack of ECP effectiveness.

The lack of LH and prolactin stimulation in these mares, and the subsequent failure of all mares except two to ovulate, is consistent with the working hypothesis that an increase in both hormones is needed for the positive ovarian response. Retrospective analysis of the results of previous experiments has revealed that, in general, a robust response in both hormones seems to lead to the best ovulation rates. However, some responders have been observed with mediocre responses in one hormone or the other, and some mares with robust responses in both hormones have turned out to be non-responders. Knowing what factor(s) account for this variation would greatly improve the chances of developing a highly successful treatment.

## **CHAPTER VI**

### **THYROXIN EFFECTS ON RESPONSE OF SEASONALLY ANOVULATORY MARES TO STIMULATION WITH ECP AND DOMPERIDONE OR SULPIRIDE IN WINTER**

#### **Introduction**

Kelley et al. (2006) were the first to combine estradiol pretreatment with the dopamine antagonist sulpiride to induce early cyclicity and ovulation in seasonally anovulatory mares. Following that success, the experiment in Chapter II also effectively used a single injection of ECP with the dopamine antagonist domperidone to achieve early ovulation in mares in the winter. However, the success of that study was not equal to the previous research by Kelly et al. (2006). Experiments contained herein have sought to fine tune the protocol in order to make it a reliable option available to producers that is both cost effective and less labor intensive than other currently available options. Based on results herein, it became evident that 1 day between injections was most effective (Chapter III) and that 100 mg of ECP was the most useful dose for pretreatment (Chapter V).

It has been hypothesized that sulpiride might be superior for inducing early ovulation. Currently published studies certainly suggest this to be true, though they do not include the element of estradiol pretreatment (Besognet et al., 1996, 1997; Daels et al., 2000; Mari et al., 2009). These combined with the more consistent success rate of Kelley et al. (2006) compared to Chapter II, both with estradiol pretreatment, support the idea that sulpiride may indeed be the more effective dopamine antagonist for use in this protocol. It was attempted to test this theory in the experiment included in Chapter V, however presumably, the simultaneous administration of both components of the protocol led to nearly no induction of early ovulation and it therefore needed to be reevaluated.

Therefore, this experiment was designed utilizing the 100 mg dose of ECP administered 1 day prior to dopamine antagonist treatment. The results from Chapters IV and V indicated that



the prolactin response was more short-lived with sulpiride, so it was decided to use three injections of sulpiride spaced 5 days apart to sustain the elevated prolactin levels longer. Moreover, preliminary trials with a new sulpiride formulation based on a non-particulate carrier indicated that the new formulation produced a better prolactin response, thus it was incorporated into the present experiment.

After conducting experiments over several seasons utilizing this protocol in the same general population of mares, a perplexing question arose as to why some mares respond consistently, while others fail to respond year after year. Even more puzzling are some mares that have responded one or more times, yet have also failed to respond one or more times. Mares used are similar in breed type, body condition, and age and are housed under the same conditions. Therefore, a preliminary step toward investigating the patterns of response was included in this experimental design by adding treatment with thyroxin to determine the effects of increased metabolism on induction of early ovulation. Thyroxin is known to increase basal metabolic rate (Guyton and Hall, 2000). Also, it has been reported that thyroxin concentrations are higher in mares that cycle year round compared to anestrus mares (Fitzgerald and Davison, 1998). The possible effect of thyroxin on the response of mares to treatment was thus tested.

## **Materials and Methods**

Animals and treatments. Thirty-four light horse mares of average to high body condition (4.5 to 7.5; Henneke et al., 1983), ranging in age from 5 to 20 years and housed at the LSU Agricultural Center Horse Unit and the Reproductive Biology Center in St. Gabriel were used. They were maintained on local native grass pastures and supplemented with hay as needed. They also had free access to water and mineralized salt blocks.

Mares were selected from the resident herds based on 1) little to no significant progesterone in plasma samples collected from December 22 to January 6 prior to the start of the

project and 2) little to no significant follicular activity on the ovaries upon ultrasound exam on January 11 to 13. Mares were classified as either completely inactive (no progesterone or ovarian activity) or mostly regressed (some follicular activity, but no progesterone), and then grouped for allotment to treatments.

Mares were evenly divided based on these criteria, and then treatment was randomly assigned to group. The groups that resulted were: 1) Controls (n = 9); 2) 100 mg ECP + 1.5 g domperidone (n = 8); 3) 100 mg ECP + .75 g sulpiride (n = 8); 4) 100 mg ECP + 1.5 g sulpiride (n = 9). On January 21, 2011 (day - 6), half of the mares in each group received a single i.m. injection of 50 mg of thyroxin in biodegradable microparticles (BioRelease Thyroxine Microparticles LA; BETpharm.com). Six days later, all treated mares received an i.m. injection of 100 mg of estradiol cypionate (ECP; day 0, January 27); control mares received a similar injection of vehicle. The following day (day 1), mares in the domperidone and sulpiride-treated groups received either a single i.m. injection of 1.5 g of domperidone in biodegradable microparticles, or sulpiride, either 0.75 g or 1.5 g, respectively, in the new non-particle formulation; control mares received a similar injection of vehicle. Sulpiride-treated mares received repeat i.m. injections of 0.75 g or 1.5 g of sulpiride (same dose as the first injection) on days 6 and 11; all other mares received similar injections of vehicle on those days.

Blood sample collection and analysis. Samples of jugular blood were collected for hormonal analysis from all mares on days -6, 0, 1, 2, 4, 6, 7, 9, 11, 12, 14, 16, 18, 20, 22 into 10-mL sodium heparin vacutainers (Vacutainer, Becton and Dickinson, Franklin Lakes, NJ), which were centrifuged within 30 minutes of collection; plasma was harvested and stored frozen until assayed. Subsequent samples for progesterone analysis to confirm ovulation were taken every 3 days for mares not ovulating in the first 3 weeks.

The ovaries of each mare were examined by ultrasound scanning for follicular activity beginning on day 7, and then again every 5 days until detection of a follicle of 30 mm or larger. Mares were scanned daily thereafter until ovulation or regression of the follicle to < 25 mm. Blood samples for progesterone analysis were taken at each scan if not scheduled otherwise for that day. Progesterone was analyzed using commercially available kits (Diagnostic Laboratory Systems, Webster, TX, USA). Blood samples from day -6 through 22 were analyzed for prolactin (Colborn et al., 1991) and LH (Thompson et al., 1983) as previously described. Intra- and interassay coefficients of variation and assay sensitivity were 5%, 8%, and 0.05 ng/mL for progesterone, 7%, 12% and 0.2 ng/mL for prolactin, and 6%, 9% and 0.2 ng/mL for LH.

Data were analyzed using the Proc GLM procedure of SAS (SAS Institute, Cary, NC, USA). Data from daily samples were analyzed for effects of treatment, time and treatment by time interactions with repeated measures ANOVA. For analysis of successful ovulations within the selected time period (32 days), mares ovulating were coded 1 and those failing to ovulate were coded 0; factorial ANOVA with treatment (sulpiride or domperidone) and thyroxin treatment, as well as the interaction, was used. Contrast statements were utilized in SAS to make logical comparisons among groups.

## **Results**

One mare was injured in a manner not related to treatment and had to be removed from the project. There was an effect of dopamine antagonist treatment on day of ovulation ( $P = 0.056$ ) with treated mares ovulating earlier than control mares. Thyroxin treatment had no effect ( $P = 0.48$ ) on ovulation. Mean ovulation dates by treatment group and the number of mares ovulating within each treatment group is presented in Table 6.1. The treatment success

**Table 6.1.** Treatment groups, total number of mares, and number of mares ovulating within 32 days.

Treatment	n	Mean day of first ovulation	32-Day Success n	Mean Success Value <sup>a</sup>
1. Control (no ECP or dopamine antagonist)	9	74	1	0.11
Thyroxine treated	4		1	0.25
No thyroxine	5		0	0.00
2. 100 mg ECP + domperidone on day 1	8	69	2	0.25
Thyroxine treated	4		1	0.25
No thyroxine	4		1	0.25
3. 100 mg ECP + .75g sulpiride on days 1, 6, 11	7	72	1	0.14
Thyroxine treated	4		1	0.25
No thyroxine	3		0	0.00
4. 100 mg ECP + 1.5g sulpiride on days 1, 6, 11	9	35	7	0.77
Thyroxine treated	5		4	0.80
No thyroxine	4		3	0.75

<sup>a</sup>Mean success x 100 equals percentage of mares in a treatment group that ovulated within the predetermined time frame.

window was set at 32 days, meaning that mares deemed to have responded to the treatment ovulated by February 28, falling in line with the desire to breed mares so that they foal in January. Dopamine antagonist treatment had an effect on success ( $P = 0.005$ ) and again thyroxin treatment did not have any effect on success rates ( $P = 0.32$ ). Results of the contrast statements comparing groups and their associated mean success ratios are presented in Table 6.2.

Daily prolactin concentrations are presented graphically in Figure 6.1. There was an effect of dopamine antagonist treatment on daily prolactin secretion ( $P < 0.0001$ ). Thyroxin treatment did not have any effect on prolactin levels ( $P = 0.56$ ). The dopamine antagonist by thyroxin interaction indicated a trend toward statistical significance ( $P = 0.108$ ).

**Table 6.2.** Associated mean success values and *P*-values for contrasts made between treatment groups.

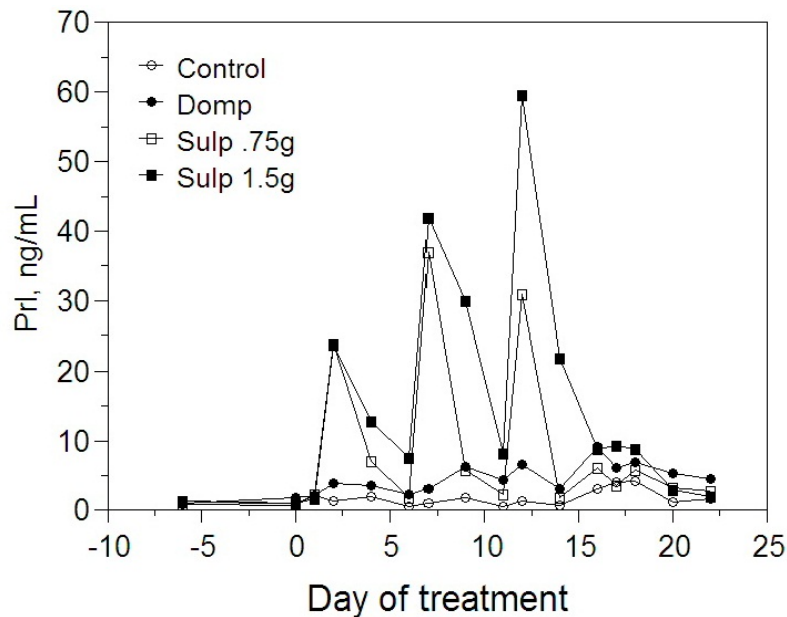
Contrast <sup>a</sup>	Mean 1 <sup>b</sup>	Mean 2 <sup>b</sup>	<i>P</i> - Value
Control vs. all treated (1 vs. 2, 3, 4)	0.11	0.42	0.1031
Domperidone vs. control (2 vs. 1)	0.25	0.11	0.5061
Control vs. .75g sulpiride (1 vs. 3)	0.11	0.14	0.9748
Control vs. 1.5g sulpiride (1 vs. 4)	0.11	0.77	0.0018
.75g sulpiride vs. 1.5g Sulpiride (3 vs. 4)	0.13	0.77	0.0025
Domperidone vs. 1.5g Sulpiride (2 vs. 4)	0.25	0.77	0.0123

<sup>a</sup>Numbers refer to treatments in Table 6.1; mean 1 is for the first group(s) listed and mean 2 is for the second group(s) listed.

<sup>b</sup>Mean success x 100 would be percentage of mares in a treatment group that ovulated within the predetermined time frame.

Because of very different absolute levels of response, separate graphs for prolactin concentrations were prepared for clarity (Figure 6.2). Prolactin levels for domperidone treated animals were higher than controls ( $P = 0.01$ ). Prolactin responses for mares receiving the two doses of sulpiride did not differ, but there was a tendency towards higher levels in the higher dose group ( $P = 0.108$ ).

Daily LH concentrations are presented graphically in figure 6.3. There was no effect of treatment on daily LH concentrations ( $P = 0.153$ ), nor did thyroxin treatment have an effect on LH response ( $P = 0.3196$ ). The interaction of dopamine antagonist treatment with thyroxin treatment was also not significant ( $P = 0.85$ ). Although there seems to be, at first glance, differences between groups in LH concentrations, there was a high degree of variation among horses within groups. That is, approximately half of the mares in the sulpiride groups had large LH responses to ECP, whereas the other half basically did not respond. Most of the mares in the domperidone group did not respond. Mares that were low versus high LH responders were evenly distributed among mares that received thyroxin and those that did not.

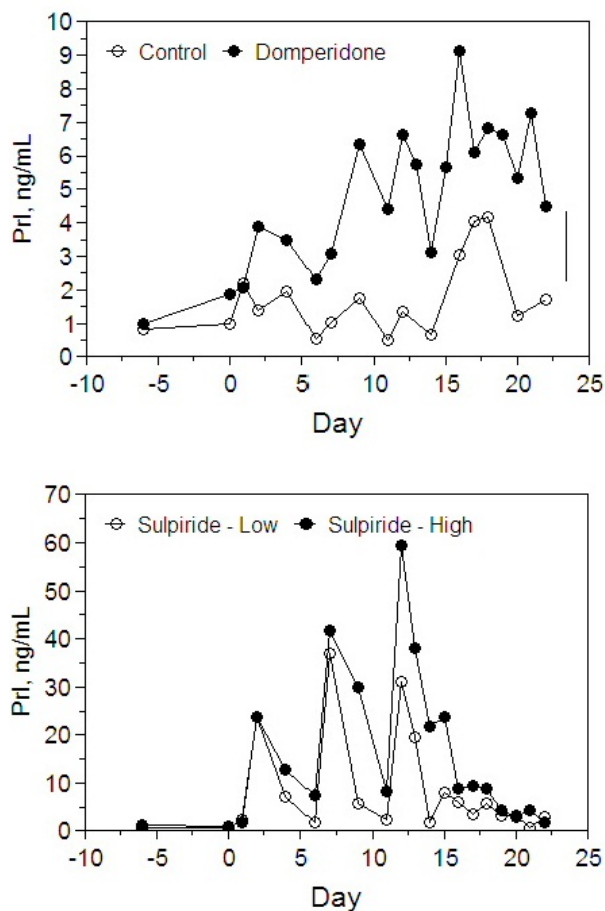


**Figure 6.1.** Daily prolactin secretion in anovulatory mares receiving vehicle, 100 mg ECP plus 1.5 g of domperidone, 0.75 g sulpiride or 1.5 g sulpiride. There was a main effect of treatment ( $P < 0.0001$ ). ECP treatment was on day 0; domperidone treatment was on day 1; sulpiride treatments were on days 1, 6, and 11.

## Discussion

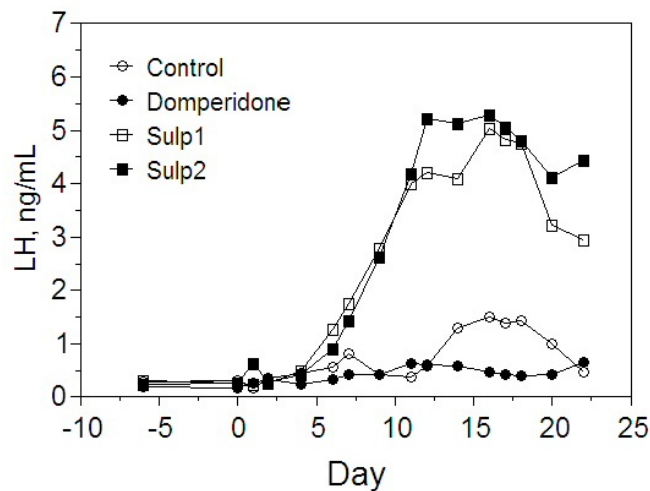
Similar to results seen by Kelley et al. (2006) and in Chapters II and III, mares that received ECP in combination with dopamine antagonist treatment ovulated earlier than control mares. In this experiment, mares that received domperidone or the lower dose of sulpiride (0.75 g) did not ovulate earlier than controls. However, the high success rate of 7 of 9 mares receiving the higher dose of sulpiride (1.5 g) was impressive. Repeat testing using the higher dose of sulpiride in larger of numbers of mares is certainly warranted.

Also consistent with previous results (Kelley et al., 2006; Chapters II and III), treatment with ECP plus dopamine antagonists increased daily prolactin concentrations. Once again, it



**Figure 6.2.** Daily prolactin secretion comparisons between domperidone and controls (top panel) and between low and high sulpiride doses (bottom panel). Anovulatory mares received vehicle, 100 mg ECP plus 1.5 g of domperidone, 0.75 g sulpiride or 1.5 g sulpiride. Domperidone treated mares differed from controls ( $P = 0.0103$ ). The vertical line indicated the least-significant difference ( $P < .1$ ) for comparison of group means within days. There was a trend ( $P = 0.108$ ) for higher levels in mares receiving the higher dose of sulpiride.

was noted that the effect of sulpiride on prolactin is immediate, yet persists for a shorter duration than the prolactin response of domperidone as seen in geldings in Chapter IV. By utilizing repeated injections of sulpiride in this experiment, the duration of high prolactin in sulpiride-treated mares was extended and 3 injections is still far more practical and less labor intensive than some currently employed hormonal therapies for induction of ovulation. Similar to results in Chapter V, the maximum prolactin response of domperidone treated mares was lower than



**Figure 6.3.** Daily LH secretion in anovulatory mares receiving vehicle, 100 mg ECP plus 1.5 g of domperidone, 0.75 g sulpiride or 1.5 g sulpiride. There was a main effect of treatment ( $P < 0.0001$ ). ECP treatment was on d 0; domperidone treatment was on day 1; sulpiride treatments were on days 1, 6, and 11.

those receiving sulpiride, yet remained elevated over controls for a longer duration. As noted in Chapter IV, these response patterns support the idea that a combination of sulpiride and domperidone may indeed produce a more consistent prolactin response, achieving higher levels of prolactin elevated for a longer duration. This type of treatment should be examined in future studies.

It was noted in Chapter IV that the maximum prolactin responses of geldings were higher than those of mares in Chapters II and III. In this study, the sulpiride treated mares had maximum responses in the same range as geldings in Chapter IV. However, mares receiving domperidone had maximum prolactin levels that were  $< 10$  ng/mL, whereas the geldings receiving domperidone had prolactin levels in the 30 to 40 ng/mL range. Though geldings and



mares are not directly comparable, it would be interesting to study both mares and geldings in the same experiment to further examine differences in prolactin responses.

The lack of effect on LH in some mares is puzzling. While there was an LH response in Chapter III, it was not very dramatic. In chapter IV, there was basically no effect of treatment on LH concentrations in geldings, but all groups received treatment with ECP. The similar LH response in those animals indicated little to no influence of sulpiride or domperidone on LH. All of this is in contrast to the dramatic difference in LH responses observed by Kelley et al. (2006), which was in line with previous observations that estradiol treatment stimulates LH response (Garcia and Ginther, 1978; Burns and Douglas, 1981). It is of particular interest how some mares had LH responses within all groups, while others had very little circulating LH in plasma. Further investigation into this variation among mares is definitely warranted.

The results of the present experiment are consistent with previous experiments in that robust prolactin responses tend to be associated with positive ovarian effects. The sulpiride formulations used herein provide a relatively cheap (compared to microparticles) alternative for stimulating prolactin concentrations. The success rate for the mares in the high sulpiride group was equivalent to the success reported by Kelley et al. (2006). Application of this protocol to a larger group of mares, with subsequent breeding to a stallion, is needed to confirm its potential usefulness in the horse industry.

## **SUMMARY AND CONCLUSIONS**

Experiments were conducted to evaluate modifications to a protocol for inducing early cyclicity and ovulation in seasonally anovulatory mares utilizing estradiol pretreatment followed by dopamine antagonist administration. With one exception, mares treated with the protocol have ovulated earlier than controls in their respective experiments. These mares have also begun to cycle and ovulate early enough in the year that they could be bred, become pregnant, and foal the following year more in line with the demands placed upon horse breeders by the horse industry. In the one experiment in which treatment failed to induce early ovulation, mares were treated with both components of the protocol simultaneously. It was determined that this method of treatment was incompatible to achieve advanced ovulation over controls and that one day between components of the protocols is the most effective dosing schedule.

In all experiments, prolactin responses have been higher in ECP plus dopamine antagonist treated animals. Some variation in prolactin response between sulpiride and domperidone was observed. Response to sulpiride treatment tends to be immediate and more short-lived than that of domperidone, which occurs a little slower, yet stays higher longer. Use of repeated injections (for a total of 3 injections, 5 days apart) of sulpiride in the last experiment resulted in very high prolactin that persisted for over 2 weeks. While that was an improvement, a better, more extended prolactin response might be possible by the use of a combined sulpiride-domperidone treatment.

Effects of treatment on LH response were mixed. In some experiments, there was an effect of treatment on LH, but that was not so in others. One of the greatest problems is the extreme amount of variability between mares within treatments in terms of LH response. Further investigation into this disparity of response is certainly warranted.

Though some results were mixed, these experiments indicate that the use of estradiol pretreatment combined with dopamine antagonists is an effective, less-labor-intensive method of inducing early cyclicity and ovulation in seasonally anovulatory mares. With subsequent research and fine-tuning of the protocol, it should be a very useful tool to horse producers.

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Pamela Boliew Mitcham, daughter of Jackie and Joyce Boliew of Minden, Louisiana, and Garry and Mary Jefferson of Benton, Louisiana, was born in 1978 in Bossier City, Louisiana. Pamela is the youngest of three and has two older sisters, Lori Boliew Seagroves and Kimberly Boliew Watkoske. Pamela grew up in north Louisiana and attended Haynesville High School her freshman year and Airline High School her sophomore year and onward, graduating near the top of her class in 1996. Pamela was offered the LSU Honor Scholarship and chose to pursue her higher education at Louisiana State University, where she earned a Bachelor of Science degree in animal, dairy and poultry sciences in May of 2004. In 2005, Pamela married William David Mitcham. She earned her Master of Science degree in animal, dairy and poultry sciences in May of 2007. That fall, Pamela began the pursuit of the doctoral degree at Texas A&M University, but returned to LSU in June of 2008 to complete her doctoral studies. Later that year, Pamela and William became the proud parents of Riley Grace. Following completion of her doctorate, Pamela plans to pursue a career teaching at the university level.