INCREASING PREGNANCY RATES IN DAIRY COWS THROUGH THE USE OF CHRONIC ADMINISTRATION OF A GnRH AGONIST: A NEW HORMONAL STRATEGY

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Authorization to Submit Thesis

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Abstract

The objectives of the present studies were to determine if chronic administration of a gonadotropin-releasing hormone (GnRH) agonist, Deslorelin, would increase circulating progesterone (P_4) concentrations and subsequently increase pregnancy rates in dairy cattle. Administration of Deslorelin for 12 days increased luteal volume and circulating P_4 concentrations in primiparous dairy cows, but increased only luteal volumes in multiparous cows. Treatment with Deslorelin increased day 45 pregnancy rates in cows compared to untreated controls. Chronic treatment with Deslorelin in dairy cattle: 1) increased luteal volume of the primary CL; 2) induced accessory CL; 3) increased circulating P_4 concentration; 4) did not lengthen the estrous cycle; and 5) increased pregnancy rates. This hormonal strategy may represent a suitable model to address local effects of P_4 and GnRH/luteinizing hormone on uterine environment and embryonic survival.

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Dedication

This is dedicated to my family; without all of their love and support I would not be who and where I am today

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Chapter 1: Review of Literature

Introduction

Reproductive efficiency is a contributing factor of economic profitability in most production livestock systems. Over the last four decades, milk production has increased in lactating dairy cattle; however, fertility has greatly declined (Faust et al., 1988; Nebel and McGilliard, 1993; Butler, 1998; Diskin and Morris, 2008). The value of a pregnancy is estimated to range between $300-$650 with an average of $450; factors influencing this value include future expected milk production, current days in milk, price of milk, age of the cow, stage of pregnancy, disease status, the value of cull cows, and cost of replacement heifers (Fricke et al., 2010). As milk prices are currently high and feed costs are low, milk production is expected to rise. In order to meet the demand in production, alternative reproductive management strategies are needed to increase pregnancy rates and the number of calves produced, thereby increasing the number of lactating animals. Although not all producers may need to implement reproductive management strategies, producers who struggle with reproductive efficiency in their dairy herds may benefit economically when using alternative reproductive management strategies.

Reasons for this decline in fertility are multifaceted. Dystocia, retained placenta, uterine infections, season of calving and subsequent rebreeding, lactation, and stress affect the fertility of dairy cows (Thatcher et al. 2006; Moore and Thatcher, 2006). Metabolic diseases, like ketosis and milk fever, and poor body condition may also factor into the decline. Nutrition, metabolic status, environment, age, parity, and lactation all play pivotal roles in the ability of the cow to reproduce efficiently (Moore and Thatcher,

2006; Sartori et al., 2002; Lucy, 2001, 2007). Four primary components of infertility in dairy cows are: 1) anovulatory or behavioral anestrous; 2) suboptimal progesterone (P_4) concentrations and irregular estrous cycles; 3) abnormal embryo development; and 4) uterine and placental incompetence (Lucy, 2007). Low circulating P_4 concentrations are of special concern as a potential cause of decreased pregnancy rates in lactating dairy cows. This literature review will provide further insight on reasons for low fertility and potential strategies to increase circulating P_4 during early embryonic development to overcome suboptimal P_4 concentrations and increase pregnancy rates in lactating dairy cattle.

Overview of the Estrous Cycle

The normal length of the bovine estrous cycle is 21 days. Recruitment of a cohort of follicles on the ovary consistently occurs around day 0 to 1 of the cycle, and again on day 10 with two follicular waves or days 9 and 16 with three follicular waves (Savio et al., 1988; Sirois and Fortune, 1988; Ginther et al., 1989). Recruitment of follicles is initiated by an increase of follicle stimulating hormone (FSH) from the anterior pituitary (Richards, 1980) 1 to 2 days preceding the emergence of a new wave (Adams et al., 1992). As this cohort of follicles begins to develop on the ovary, stimulated by FSH and luteinizing hormone (LH) from the anterior pituitary, follicles begin to produce estradiol-17β (E_2). Estradiol-17β, produced and secreted from the dominant follicle, feeds back in a negative manner to the anterior pituitary to suppress the secretion of FSH (Channing et al., 1982; Burger, 1988). Selection of a dominant follicle is preceded by the presence of luteinizing hormone receptors (LHR) expressed on the granulosa cells of the follicle (Xu et al., 1995). The shift in gonadotropin dependence, from FSH- to

LH-dependence, is a pivotal event in selection of the dominant follicle; by producing and secreting Inhibin, the dominant follicle disrupts FSH secretion from the anterior pituitary, suppressing growth of subordinate follicles (Xu et al., 1995; Pawson and McNeilly, 2005). Upon selection of the dominant follicle, follicles recruited within the cohort undergo atresia. In a three-wave cycle, the dominant follicle from the third wave becomes the pre-ovulatory follicle. Around day 17 to 18 of the estrous cycle, P_4 begins to fall as the corpus luteum (CL) undergoes luteolysis, induced by pulsatile release of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) from the uterine endometrium (McCracken et al., 1999). With the demise of the CL, P_4 negative inhibition on the hypothalamus is removed, allowing for stimulation of GnRH release by E_2 secreted from the dominant follicle. As the dominant follicle continues to develop, E_2 feeds back to the hypothalamus in a positive manner to increase the release of GnRH (Imakawa et al., 1986). Estradiol-17β increases until a threshold is met and initiates the preovulatory surge of GnRH. This leads to a surge of LH and subsequent ovulation of the dominant ovarian follicle roughly 24 to 34 hours later (Walker et al., 1996). Upon ovulation, granulosa and theca interna cells of the follicle differentiate into large and small luteal cells, respectively (O'Shea et al., 1987). Steroidogenic luteal cells, under the stimulation of pulsatile LH, begin to synthesize and secrete P_4 as the CL develops (Armstrong and Black, 1966). Fully functional around day 8 to 9, size of the CL and P_4 production plateaus and remains relatively constant until day 16 to 17. If the signal for maternal recognition of pregnancy (MROP) from the conceptus is not present, luteolysis will occur. Oxytocin (OT) released episodically from the posterior pituitary, binds to its receptor, up regulated by E_2 in the uterus, inducing $PGF_{2\alpha}$ release (McCracken et al., 1999). Binding

of $PGF_{2\alpha}$ to its receptor in the CL also induces the release of oxytocin from large luteal cells (Silvia et al., 1991). With the demise of the CL, P_4 negative inhibition on the hypothalamus is removed. Estradiol-17β induces the preovulatory surge of GnRH, and the estrous cycle begins again.

Estrous Synchronization Techniques

Understanding the series of time sensitive events that occur in follicular development has led to major advancements in reproductive management, including manipulation of the estrous cycle. One of the major synchronization protocols is Ovsynch, which can be initiated at any stage of the estrous cycle (Pursley et al., 1997). Ovsynch consists of administration of 100 μg of GnRH intramuscularly (i.m.), followed by an injection of 25 mg of $PGF_{2\alpha}$ i.m. after seven days. Animals receive 100 μg GnRH i.m. 30-36 hours following $PGF_{2\alpha}$ and are timed artificially inseminated (TAI) 16-20 hours later (Pursley et al., 1997). In a comparison of control cows receiving only injections of 25 mg of $PGF_{2\alpha}$ i.m. and receiving AI upon detection of estrus and cows receiving Ovsynch and TAI, Pursley et al (1997) reported no difference in pregnancy rates in lactating dairy cows. This protocol has allowed producers to synchronize ovulation without estrus detection and without detriment to pregnancy rates, as more cows synchronized at the same time, which reduces labor required for heat detection. Since the development of the Ovsynch protocol, numerous other protocols have been developed to synchronize ovulation. Peters and Pursley (2003) reported shortening the duration, from 36 to 0, 12, or 24 hours, between $PGF_{2\alpha}$ and final GnRH lowered pregnancy rates as smaller ovulatory follicles were induced to ovulate. Brusveen et al (2008) reported an increase in pregnancy rates when extending the time between

$PGF_{2\alpha}$ injection and final GnRH from 30 hours to 56 hours with TAI occurring at 72 hours after $PGF_{2\alpha}$. Due to the increase in pregnancy rates, this is the estrus synchronization protocol that is most widely used. Presynchronization consisting of two injections of 25 mg of $PGF_{2\alpha}$ i.m. fourteen days apart, prior to initiation of Ovsynch, increased pregnancy rates between presynchronized cows (46.9%) and untreated controls (34.4%; Moreira et al., 2001; Navanukraw et al., 2004). Synchronization rates were increased with the inclusion of a progesterone-releasing intravaginal device (PRID) from initial GnRH injection to $PGF_{2\alpha}$ in the Ovsynch protocol; however, pregnancy rates were no different in cows receiving a PRID compared to untreated control cows (Xu et al., 1996).

Fertilization rates in heifers, non-lactating, and moderate yielding dairy cows are between 90 to 100% (Maurer and Chenault, 1983; Wiebold, 1988; Sartori et al., 2002; Diskin and Morris, 2008); however, conception rates are approximately 45% following AI after observed spontaneous estrus (Lucy, 2001). Previously, incorporation of TAI into a majority of synchronization protocols may have contributed to even lower conception rates, as low as 35% (Pursley et al., 1997; Lucy, 2001). Currently, better manipulation of the estrous cycle, more specifically follicular waves, may result in increased conception rates. Double Ovsynch, consisting of the Ovsynch protocol without TAI followed by Ovsynch with TAI 7 days later, increased conception rates (49.7% vs. 41.7%) as more cows were at the same stage of the estrous cycle at the initiation of Ovsynch in which TAI was performed (Souza et al., 2008).

Ovulation of the dominant follicle occurs between 24 and 34 hours after onset of detectable estrus (Walker et al., 1996; Pursley et al., 1997). Cattle bred on detection of

estrus have greater conception rates (Kasimanickam et al., 2005). When breeding on estrus detection, cows are provided semen at the more appropriate time relative to when ovulation will occur. In a TAI situation, cattle are not always observed to be in estrus; this may be due to a small, less estrogenic dominant follicle in which the cow fails to show behavioral estrus (Brantmeier et al., 1987). Response to the initial GnRH injection of the Ovsynch protocol varies by day of the estrous cycle in which Ovsynch is began, with greatest rates of ovulation on days 5 to 9 of the estrous cycle due to presence of a dominant follicle (Vasconcelos et al., 1999). When follicles are induced to ovulate, smaller sized follicles may be induced to ovulate when compared to follicles that ovulate spontaneously (Vasconcelos et al., 1999). Smaller ovulatory follicles develop into smaller sized CL, which leads to decreased concentrations of circulating P_4 (Vasconcelos et al., 2001; Peters and Pursley, 2003). Although there is no set threshold of P_4 that will maintain a pregnancy, researchers consider a successful CL to secrete >1 ng/ml; not all cows have the same threshold and it may even differ in an individual animal between estrous cycles.

Development of the Embryo and Associated Membranes

Diskin and Morris (2008) report fertilization rates between 90 to 100% when semen from high fertility bulls is used in AI of heifers and moderate-producing dairy cows. Similar fertilization rates occur in high-producing lactating dairy cows (Wiebold, 1988; Ryan et al., 1993; Sartori et al., 2002); however, at higher ambient temperatures, fertilization rates are lower with 55% in lactating cows compared to 100% in heifers (Sartori et al., 2002). The zygote is formed shortly after fertilization and develops into a morula embryo. The embryo at the morula stage enters the uterine lumen between

days 5 or 6 with formation of the blastocyst on day 7 or 8 (Guillomot, 1995). The blastocyst consists of the inner cell mass, which develops into the fetus, the trophectoderm that develops into the extraembryonic membranes, and the fluid filled blastocoele. After hatching, around day 9, the blastocyst grows from a round cluster of cells into an elongated filamentous structure (Dorniak et al., 2013). By day 15 to 16 the filamentous conceptus (the embryo and associated extraembryonic membranes) will occupy the full length of the ipsilateral uterine horn to the CL and extend into the contralateral horn (Guillomot, 1995).

The trophectoderm, a single-cell layer that forms the extraembryonic membranes, elongates and plays a pivotal role in maternal-fetal communication that is critical for the establishment and continuance of pregnancy (Bazer et al., 1991). Interaction between the conceptus and the uterus prior to implantation is initiated by P_4 and is mediated through differential gene expression (Spencer et al., 2004). Expression of P_4-stimulated endometrial genes promotes survival, growth, and development of the blastocyst (Spencer et al., 2008). Translated proteins from these stimulated genes from the endometrium regulate conceptus growth and elongation (Forde et al., 2012). As the conceptus begins to grow and develop, the trophoblast layer synthesizes and secretes interferon tau (IFNT), prostaglandins (PG), and cortisol, which regulate expression of elongation- and implantation-related genes in the endometrial epithelia (Bazer et al., 2009; Dorniak et al., 2011; Spencer et al., 2013; Dorniak et al., 2013). Implantation of the conceptus in cattle is initiated by day 25 when trophoblast cells come in close contact with uterine epithelium sites, known as caruncles. Adhesion

is the final step, which gives rise to the cellular structure of an epithelio-chorial placenta (Guillomot, 1995).

Early Embryonic Loss

As stated previously, there is no one cause of decreased pregnancy rates in lactating dairy cattle. The Committee on Bovine Reproductive Nomenclature (1972) defines embryonic mortality to losses during the embryonic period, which extends from conception to differentiation, approximately 45 days of gestation. Early embryonic loss in cattle is approximately 40%, with 80% occurring between days 8 and 16 post-insemination (Dunne et al., 2000). Reasons for early embryonic mortality in dairy cattle are multifaceted. Oocyte quality is a major indicator of future embryonic development. Oocytes flushed from cows of low body condition score, an indicator of metabolic status, had lower rates of cleavage and blastocyst formation compared to cows with higher body condition scores (Snijders et al., 2000). Lactation may also affect embryonic quality. Embryos collected from heifers graded higher and had fewer degenerate embryos when compared to embryos recovered from lactating cows (Sartori et al., 2002). Sartori et al. (2002) reports higher body temperatures in lactating cows compared to heifers when exposed to similar environments. Lenz et al. (1983) reports oocytes exposed to high temperatures in vitro lowered oocyte maturity and capacity of the oocyte to become fertilized. Lactation also increases nutrient demand and metabolism to meet the increase in production.

Dry matter intake (DMI) by the cow must increase to meet energy and nutrient requirements as milk production increases. Metabolism of steroid hormones occurs in the liver, which is the major site of P_4 clearance from circulation (Parr et al., 1993). As

the plane of nutrition increases, liver blood flow (LBF) increases dramatically in sheep (Burrin et al., 1989). Dunne et al. (1999) reports in beef heifers, an increase in feed intake before AI decreased embryonic survival. Sangsritavong et al. (2002) reports an increase in LBF in lactating dairy cows increased metabolic clearance rate of P_4. Lactating dairy cows may have an increased risk of early embryonic loss due to a decrease in circulating P_4 concentrations resulting from increased P_4 clearance (Sangsritavong et al., 2002). Embryonic mortality is greater in cows when compared to heifers, a reason for lower fertility in cows (Sartori et al., 2002; Diskin et al., 2006; Berg et al., 2010). High milk producing cows have an increased risk of early embryonic mortality than moderate- and low-producing cows and non-lactating heifers (Sartori et al., 2002; Diskin et al., 2006; Moore and Thatcher, 2006; Diskin and Morris, 2008); as milk production increases, nutrient metabolism must increase to meet the nutrient demand, thereby lowering circulating P_4 concentrations due to increased P_4 metabolism.

Lesser circulating concentrations of P_4 in cows might contribute to subfertility or infertility (Ahmad et al., 1996; Butler et al., 1996; McNeill et al., 2006; Stronge et al., 2005). McNeill et al. (2006) shows a positive correlation between milk P_4 concentrations as early as day 4 post-ovulation and embryo survival in dairy cattle. Similar findings of early embryonic loss likely due to luteal insufficiency have been reported in beef cows and heifers (Hill et al., 1970; Maurer and Echternkamp, 1982). Sheep with greater P_4 concentrations before day 5 resulted in increased conceptus development, with 78% of conceptuses at the filamentous stage on day 13, when compared to ewes with lesser P_4 concentrations before day 5 (0%; Nephew et al.,

1991). The use of a P_4 receptor antagonist, RU486, in sheep results in lack of recovered blastocysts, indicating P_4 is critical during early embryonic development (Satterfield et al., 2006). Not only do lesser P_4 concentrations presumably contribute to fertility issues, but the rate at which P_4 concentrations increase during the post-conception period does as well. A delayed rise in P_4 concentrations after ovulation delays embryonic development in cattle (Ahmad et al., 1996; Green et al., 2005; Henricks et al., 1971; Mann et al., 2006; Mann and Lamming, 2001; McNeill et al., 2006).

Pre-implantation development of the conceptus plays a major role in survival of the pregnancy. Embryonic development is dependent on circulating P_4 concentrations, which regulate gene expression and uterine secretions (Spencer and Bazer, 2004). A delayed rise in P_4 after ovulation may delay the required down-regulation of the progesterone receptor (PGR) in the endometrium, altering embryonic development (Ahmad et al., 1996; Mann et al., 1998; Spencer et al., 2007). Lesser concentrations of circulating P_4 seem to contribute to infertility, but the rate at which P_4 increases after ovulation during the early luteal phase may have the greatest impact. A delayed rise in P_4 after ovulation during the early luteal phase delays embryonic development in cattle (Henricks et al. 1971; Ahmad et al. 1996; Mann and Lamming, 2001; Green et al. 2005; Mann et al. 2006; McNeill et al. 2006). Slow developing embryos may not be able to inhibit luteolysis and resumption of the estrous cycle; because an underdeveloped embryo might not produce adequate amounts of INFT to signal MROP and prevent luteolysis.

Maternal Recognition of Pregnancy and Role of IFNT and P_4

Maternal recognition of pregnancy is the physiological process by which the conceptus signals its presence to the maternal system (Bazer et al., 1991). The conceptus secretes IFNT for maintenance of CL function and establishment of pregnancy in ruminants (Bazer et al., 1991). Secretion of IFNT from the trophectoderm occurs between days 10 to 25 with maximal secretion around days 14 to 16 of pregnancy (Roberts et al., 1999). Interferon tau is the antiluteolytic signal that acts in a paracrine manner between the trophectoderm and the endometrium to prevent pulsatile release of $PGF_{2\alpha}$ from the endometrium, which initiates luteolysis and subsequently lowers circulating concentrations of P_4 in cattle (Thatcher et al., 1989; Spencer et al., 2007). Oxytocin, released from the posterior pituitary, binds to the oxytocin receptor (OTR) in the endometrium and induces the pulsatile release of $PGF_{2\alpha}$ from the uterine endometrium, which initiates luteolysis (McCracken et al., 1999). The antiluteolytic role of IFNT is to inhibit transcription of estrogen receptor alpha (ESR1), which subsequently inhibits the expression of OTR in the endometrium (Dorniak et al., 2013). Thus, the endometrium cannot produce luteolytic pulses of $PGF_{2\alpha}$ in response to oxytocin (Spencer et al., 2004); therefore, the CL and pregnancy are sustained.

Interferon tau regulates endometrial gene expression within the luminal epithelium (LE), glandular epithelium (GE), and stroma (Spencer et al., 2004). Genes stimulated by IFNT, known as IFN-stimulated genes (ISGs), are presumed to play a major role in endometrial differentiation and conceptus implantation (Spencer et al., 2008). Many ISGs, like ISG15 and WNT7A, are expressed in early pregnancy, day 10, become down-regulated around days 12 to 13, and are then stimulated by the presence

of IFNT after day 13 (Spencer et al., 2008). Some genes are induced by P_4 in the LE and GE and further stimulated by IFNT (Spencer and Bazer, 2004; Dorniak et al., 2013) all of which apparently aid in the preservation and continuance of pregnancy. All of the positive actions towards maintenance of pregnancy depend on development and ability of the conceptus to prevent luteolysis. Lesser growth of the conceptus reduces IFNT production, maintenance of the CL and P_4 production, and viability of the conceptus and continuance of pregnancy.

Methods of Increasing Circulating P_4

Enhancing P_4 concentrations either through administration of exogenous P_4 or induction of accessory CL to increase endogenous P_4 are strategies that have been examined. Garrett et al. (1988) administered daily injections of P_4 early in the cycle, days 1 through 5, and increased circulating P_4 concentrations and advanced conceptus development and subsequent IFNT production. Insertion of a controlled internal drug release (CIDR) to non-lactating dairy cows on day 5 (Mann et al., 2006) or a PRID on day 3 in beef heifers (Carter et al., 2008; Clemente et al., 2009) has similar results as daily injections of P_4 (Garrett et al., 1988). Others using CIDRs (Rhodes et al., 2001) or PRIDs (Hanlon et al., 2005a) after insemination of cows did not improve pregnancy rates. Insertion of a CIDR on day 2 of pregnancy is detrimental to embryo survival (Van Cleeff et al., 1996), likely due to alterations in oviductal transport of embryo and potential incidence of short-cycling. Ottobre et al. (1980) reported injections of P_4 eight hours after detection of estrus shortened cycle length (12.5 days) when compared to untreated controls (16.3 days) likely due to P_4 negative inhibition on the hypothalamus and release of GnRH. Development of embryos treated with P_4 in vitro and implanted

into recipient cows is not altered, indicating an effect of P_4 on the uterine environment and not on the embryo (Clemente et al., 2009). Morula and blastocysts recovered from cows receiving a CIDR beginning day 3 of pregnancy were not altered in terms of the morphology of the embryos recovered (Carter et al., 2008). It is hypothesized that early exposure to P_4 following ovulation alters endometrial transcriptome and uterine luminal protein content that provides an adequate environment for development of the embryo (Forde and Lonergan, 2012). Lesser concentrations of P_4 during this same period alter the endometrial transcriptome as well, resulting in an inadequate uterine environment to support conceptus development and elongation (Forde et al., 2011; Dorniak et al., 2013).

Human chorionic gonadotropin (hCG) induces accessory CL and increases P_4 concentrations, but has inconsistent effects on pregnancy rates in cattle (Kerbler et al., 1997; Santos et al., 2001; Rizos et al., 2012; Maillo et al., 2014). Administration of hCG on day 5 after AI increased pregnancy rates in dairy cows with those losing body condition being most responsive (Moore and Thatcher, 2006). Santos et al. (2001) reported that treatment with hCG (3,300 IU i.m.) on day 5 after AI induced an accessory CL, increased P_4 concentrations, and improved conception rates in lactating dairy cows by 7.1% on day 28. Human chorionic gonadotropin treatment on day 5 after TAI improves pregnancy rates in primiparous cows compared to controls, whereas older cows receiving hCG did not differ in pregnancy rates when compared to controls (Nascimento et al., 2013). Further, use of hCG 5 days after insemination does not improve pregnancy rates in anovulatory anestrous dairy cows (Hanlon et al., 2005b). Use of hCG on day 5 after AI or estrus requires a dominant follicle to be present on the

ovaries to respond to hCG, which has LH-like activity, for ovulation to occur and subsequent formation of an accessory CL.

Numerous studies using a GnRH agonist during different days of the estrous cycle or pregnancy have been conducted to determine the subsequent effects of the treatment on P_4 concentrations and embryonic loss in dairy cows. Use of a GnRH agonist, Deslorelin, in estrous synchronization programs to induce ovulation has shown to have little to no effect on circulating P_4 concentrations (Bartolome et al., 2004) or decrease pregnancy rates depending on the dose of Deslorelin (Santos et al., 2004). Pregnancy rates in cows treated with a 450μg implant of Deslorelin 48 hours past $PGF_{2\alpha}$ injection of the Ovsynch protocol to induce ovulation were similar to untreated controls (41.3% and 39%, respectively). Conversely, cows implanted with 750μg had lower pregnancy rates (27.5%; Santos et al., 2004). Use of GnRH agonist implants in Holstein cows to induce accessory CL, increases P_4 concentrations, but does not increase pregnancy rates (Rajamahendran et al., 1998). Insertion of a 2.1 mg Deslorelin implant on day 27 of pregnancy results in the formation of an accessory CL, increased P_4 concentrations, but no reduction in pregnancy loss between days 45 and 90 compared to controls (Bartolome et al., 2006). Administration of GnRH 5 days after AI induces accessory CL, increases P_4, but does not improve pregnancy rates (Howard et al., 2006). Similar to hCG, use of GnRH or an agonist of GnRH on day 5 requires a dominant follicle responsive to LH for ovulation to occur and form an accessory CL. Chronic administration of a GnRH agonist, beginning day 3 (Day 0 = estrus) of the estrous cycle at a dose of 1μg/kg of body $weight^{-1}$ per day^{-1}, increases basal LH secretion, area of the

CL, and circulating P_4 concentrations in beef heifers (Davis et al., 2003). Further investigation of this hormonal strategy is the focus of the thesis.

Conclusion

The success of a pregnancy is determined by many factors, however, Lucy (2007) has stated four primary components contributing to lower pregnancy rates in lactating dairy cows; 1) anovulatory or behavioral anestrous; 2) suboptimal P_4 concentrations and irregular estrous cycles; 3) abnormal embryo development; and 4) uterine and placental incompetence. Of these four components, circulating concentrations of P_4 are of special concern in lactating dairy cattle. Progesterone is required for preparation of the uterine environment for pregnancy by altering endometrial gene expression and stimulating synthesis and secretion of histotroph. A later or lesser rise in P_4 will lead to delayed growth of the conceptus as the uterine environment may not be conducive to pregnancy. An underdeveloped conceptus may secrete inadequate amounts of IFNT to inhibit luteolysis and maintain the pregnancy. The objectives of the current study were to implement the model developed by Davis et al. (2003) to overcome sub-luteal function during this period of critical embryonic development, increase luteal volume and subsequent circulating P_4 concentrations, and improve pregnancy rates in dairy cattle.

References

Adams GP, Matteri RL, Kastelic JP, Ko JC, Ginther OJ. Association between surges of follicle-stimulating hormone and the emergence of follicular waves in heifers. *J Reprod Fert.* (1992) 94:177-188.

Ahmad N, Beam SW, Butler WR, Deaver DR, Duby RT, Elder DR, Fortune JE, Griel LC Jr., Jones LS, Milvae RA, Pate JL, Revah I, Schreiber DT Jr., Townson DH, Tsang PCW, Inskeep EK. Relationship of fertility to patterns of ovarian follicular development and associated hormonal profiles in dairy cows and heifers. *J Anim Sci.* (1996) 74:1943-1952.

Armstrong DT, Black DL. Influence of luteinizing hormone on corpus luteum metabolism and progesterone biosynthesis throughout the bovine estrous cycle. *Endocrinology.* (1966) 78:937-944.

Bartolome JA, Santos JEP, Pancarci SM, Melendez P, Arteche ACM, Hernandez O, Archbald LF, Trigg T, Thatcher WW. Induction of ovulation in non-lactating dairy cows and heifers using different doses of a Deslorelin implant. *Theriogenology.* (2004) 61:407-419.

Bartolome JA, Kamimura S, Silvestre F, Arteche ACM, Trigg T, Thatcher WW. The use of a Deslorelin implant (GnRH agonist) during the late embryonic period to reduce pregnancy loss. *Theriogenology.* (2006) 65:1443-1453.

Bazer FW, Thatcher WW, Hansen PJ, Mirando MA, Ott TL, Plante C. Physiological mechanisms of pregnancy recognition of ruminants. *J Reprod Fert Suppl.* (1991) 43:39-47.

Bazer FW, Spencer TE, Johnson GA, Burghardt RC, Wu G. Comparative aspects of implantation. *Reproduction.* (2009) 138:195-209.

Brantmeier SA, Bellin ME, Boehm SK, Bushmeyer SM, Kubajak CL, Dentine MR, Grummer RR, Ax RL. Influence of stage of cycle, corpus luteum location, follicle size, and number of large follicles on estradiol-17 beta concentrations in bovine follicles. *J Dairy Sci.* (1987) 70:2138-2144.

Brusveen, DJ, Cunha AP, Silva CD, Cunha PM, Sterry RA, Silva EPB, Guenther JN, and Wiltbank MC. Altering the time of the second gonadotropin-releasing hormone injection and artificial insemination (AI) during Ovsynch affects pregnancies per AI in lactating dairy cows. *J. Dairy Sci.* (2008) 91:1044–1052.

Burger HG. Inhibin: definition and nomenclature, including related substances. *J Endocrinology.* (1988) 117:159-160.

Burrin DG, Ferrell CL, Eisemann JH, Britton RA, Nienaber JA. Effect of level of nutrition on splanchnic blood flow and oxygen consumption in sheep. *Br J Nutr.* (1989) 62:23-34.

Butler WR, Calaman JJ, Beam SW. Plasma and milk urea nitrogen in relation to pregnancy rate in lactating dairy cattle. *J Anim Sci.* (1996) 74:858-865.

Butler WR. Effect of protein nutrition on ovarian and uterine physiology in dairy cattle. *J Dairy Sci.* (1998) 81:2533-2539.

Carter F, Forde N, Duffy P, Wade M, Fair T, Crowe MA, Evans ACO, Kenny DA, Roche JF, Lonergan P. Effect of increasing progesterone concentration from day 3 of pregnancy on subsequent embryo survival and development in beef heifers. *Reprod Fertil Dev.* (2008) 20:368-375.

Channing CP, Anderson LD, Hoover DJ, Kolena J, Osteen KG, Pomerantz SH, Tanabe K. The role of nonsteroidal regulators in control of oocyte and follicular maturation. *Recent Prog Horm Res.* (1982) 38:331-400.

Clemente M, de La Fuente J, Fair T, Al Naib A, Butierrez-Adan A, Roche JF, Rizos D, Lonergan P. Progesterone and conceptus elongation in cattle: a direct effect on the embryo or an indirect effect via the endometrium? *Reproduction.* (2009) 138:507-517.

Davis TL, Mussard ML, Jimenez-Severiano H, Enright WJ, Kinder JE. Chronic treatment with an agonist of gonadotropin-releasing hormone enhances luteal function in cattle. *Biol Reprod.* (2003) 69:398-403.

Diskin MG, Murphy JJ, Sreenan JM. Embryo survival in dairy cows managed under pastoral conditions. *Anim Reprod Sci.* (2006) 96:297-311.

Diskin MG, Morris DG. Embryonic and early foetal losses in cattle and other ruminants. *Reprod Dom Anim.* (2008) 43:260-267.

Dorniak P, Bazer FW, Spencer TE. Prostaglandins regulate conceptus elongation and mediate effects of interferon tau on the ovine uterine endometrium. *Biol of Reprod.* (2011) 84:1119-1127.

Dorniak P, Bazer FW, Spencer TE. Physiology and endocrinology symposium: Biological role of interferon tau in endometrial function and conceptus elongation. *J Anim Sci.* (2013) 91:1627-1638.

Dunne LD, Diskin MG, Boland MP, O'Farrell KJ, Sreenan JM. The effect of pre- and post-insemination plane of nutrition on embryo survival in beef heifers.
Anim Sci. (1999) 69:411–417.

Dunne LH, Diskin MG, Sreenan JM. Embryo and foetal loss in beef heifers between Day 14 of gestation and full term. *Anim Reprod Sci.* (2000) 96:297-311.

Faust MA, McDaniel BT, Robison OW, Britt JH. Environmental and yield effects on reproduction in primiparous Holsteins. *J Dairy Sci.* (1988) 71:3092-3099.

Forde N, Beltman ME, Duffy GB, Duffy P, Mehta JP, Gaora PO, Roche JF, Lonergan P, Crowe MA. Changes in the endometrial transcriptome during the bovine estrous cycle: effect of low circulating progesterone and consequences for conceptus elongation. *Biol Reprod.* (2011) 84:266-278.

Forde N, Mehta JP, Minten M, Crowe MA, Roche JF, Spencer TE, Lonergan P. Effects of low progesterone on the endometrial transcriptome in cattle. *Biol Reprod.* (2012) 87:124, 1-11.

Forde N, Lonergan P. Transcriptomic analysis of the bovine endometrium: What is required to establish uterine receptivity to implantation in cattle? *J Reprod Dev.* (2012) 58:189-195.

Fricke P, Stewart S, Rapnicki P, Eicker S, Overton M. Pregnant vs. Open: Getting cows pregnant and the money it makes. (2010) http://www.extension.org/pages/11007/pregnant-vs-open:-getting-cows-pregnant-and-the-money-it-makes#.Uz8Dulel165

Garrett JE, Geisert RD, Zavy MT, Morgan GL**.** Evidence for maternal regulation of early conceptus growth and development in beef cattle. *J Reprod Fert.* (1988) 84:437–446.

Green MP, Hunter MG, Mann GE. Relationship between maternal hormone secretion and embryo development on day 5 of pregnancy in dairy cows. *Anim Reprod Sci.* (2005) 88:179-189.

Ginther OJ, Knopf L, Kastelic JP. Temporal associations among ovarian events in cattle during oestrous cycles with two and three follicular waves. *J Reprod Fert.* (1989) 87:223-230.

Guillomot M. Cellular interactions during implantation in domestic animals. *J Reprod Fertil Suppl.* (1995) 49:39-51.

Hanlon DW, Davidson PJ, Hittmann AR, Joe AK. Supplementing previously treated anestrous dairy cows with progesterone does not increase first-service conception rate. *Theriogenology*. (2005a) 63:239-245.

Hanlon DW, Jarratt GM, Davidson PJ, Millar AJ, Douglas VL. The effect of hCG administration five days after insemination on the first service conception rate of anestrous dairy cows. *Theriogenology.* (2005b) 63:1938-1945.

Henricks DM, Lamond DR, Hill JR, Dickey JF. Plasma progesterone concentrations before mating and in early pregnancy in the beef heifer. *J Anim Sci*. (1971) 33:450-454.

Hill JR, Lamond DR, Henricks DM, Dickey JF, Niswender GD. The effects of under nutrition on ovarian function and fertility in beef heifers. *Biol Reprod.* (1970) 2:78-84.

Howard JM, Manzo R, Dalton JC, Frago F, Ahmadzadeh A. Conception rates and serum progesterone concentration in dairy cattle administered gonadotropin-releasing hormone 5 days after artificial insemination. *Anim Reprod Sci.* (2006) 95:224-233.

Imakawa K, Day ML, Zalesky DD, Garcia-Winder M. Kittok RJ, Kinder JE. Regulation of pulsatile LH secretion by ovarian steroids in the heifer. *J Anim Sci.* (1986) 63:162-168.

Kasimanickam R, Cornell JM, Nebel RL. Fertility following fixed time AI or insemination at observed estrus in Ovsynch and Heatsynch in lactating dairy cows. *Theriogenology.* (2005) 63:2550-2559.

Kerbler TL, Buhr MM, Jordan LT, Leslie KE, Walton JS. Relationship between maternal plasma progesterone concentration and interferon-tau synthesis by the conceptus in cattle. *Theriogenology.* (1997) 47:703-714.

Lenz RW, Ball GD, Leibfried ML, Ax RL, First NL. In vitro maturation and fertilization of bovine oocytes are temperature-dependent processes. *Biol Reprod.* (1983) 29:173-179.

Lucy MC. Reproductive loss in high-producing dairy cattle: where will it end? *J Dairy Sci.* (2001) 84:1277-1293.

Lucy MC. Fertility in high-producing dairy cows: reasons for decline and corrective strategies for sustainable improvement. *Soc Reprod Fertil Suppl.* (2007) 64:237-254.

Maillo V, Duffy P, O'Hara L, de Frutos C, Kelly AK, Lonergan P, Rizos D. Effect of hCG administration during corpus luteum establishment on subsequent corpus luteum development and circulating progesterone concentrations in beef heifers. *Reprod Fertil Dev*. (2014) 26:367-374.

Mann GE, Lamming GE, Payne JH. The role of early luteal phase progesterone in the control of the timing of the luteolytic signal in the cow. *J Reprod Fertil.* (1998) 113:47-51.

Mann GE and Lamming GE. Relationship between maternal endocrine environment, early embryo development and inhibition of the luteolytic mechanics in cows. *Reproduction.* (2001) 121:175-180.

Mann GE, Fray MD, Lamming GE. Effects of time of progesterone supplementation on embryo development and interferon-tau production in the cow. *Vet J.* (2006) 171:500-503.

Maurer RR, Echternkamp SE. Hormonal asynchrony and embryonic development. *Theriogenology*. (1982) 17:11-22.

Maurer RR and Chenault JR. Fertilization failure and embryonic mortality in parous and nonparous beef cows. *J Anim Sci.* (1983) 56:1186-1189.

McCracken JA, Custer EE, Lams JC. Luteolysis: a neuroendocrine-mediated event. *Physiol Rev.* (1999) 79:263-323.

McNeill RE, Diskin MG, Sreenan JM, Morris DG. Associations between milk progesterone concentration on different days and with embryo survival during the early luteal phase in dairy cows. *Theriogenology.* (2006) 65:1435-1441.

Moore K and Thatcher WW. Major advances associated with reproduction in dairy cattle. *J Dairy Sci.* (2006) 89:1254-1266.

Moreira F, Orlandi C, Risco CA, Mattos R, Lopes F, Thatcher WW. Effects of presynchronizaiton and bovine somatotropin on pregnancy rates to a timed artificial insemination protocol in lactating dairy cows. *J Dairy Sci.* (2001) 84:1646-1659.

Nascimento AB, Bender RW, Souza AH, Ayres H, Araujo RR, Guenther JN, Sartori R, Wiltbank MC. Effect of treatment with human chorionic gonadotropin on day 5 after timed artificial insemination on fertility of lactating dairy cows. *J Dairy Sci.* (2013) 96:2873-2882.

Navanukraw C, Redmer DA, Reynolds LP, Kirsch JD, Grazul-Bilska AT, Fricke PM. A modified presynchronization protocol improves fertility to timed artificial insemination in lactating dairy cows. *J Dairy Sci.* (2004) 87:1551-1557.

Nebel RL and McGilliard ML. Interactions of high milk yield and reproductive performance in diary cows. *J Dairy Sci.* (1993) 76:3257-3268.

Nephew KP, McClure KE, Ott TL, Dubois DH, Bazer FW, Pope WF. Relationship between variation in conceptus development and difference in estrous cycle duration in ewes. *Biol Reprod*. (1991) 44:536-539.

Ottobre JS, Lewis GS, Thayne WV, Inskeep EK. Mechanism by which progesterone shortens the estrous cycle of the ewe. *Biol Reprod.* (1980) 23:1046-1053.

O'Shea JD, Rodgers RJ, Davis KE. Morphometric estimation of the numbers of granulosa cells in preovulatory follicles of the ewe. *Aus J of Biol Sci.* (1987) 40:451-457.

Pawson AJ and McNeilly AS. The pituitary effects of GnRH. *Anim Reprod Sci.* (2005) 88:75-94.

Parr RA, Davis IF, Miles MA, Squires TJ. Liver blood flow and metabolic clearance rate of progesterone in sheep. *Res Vet Sci.* (1993) 55:311-316.

Peters MW and Pursley JR. Timing of final GnRH of the Ovsynch protocol affects ovulatory follicle size, subsequent luteal function, and fertility in dairy cattle. *Theriogenology.* (2003) 60:1197-1204.

Rajamahendran R, Ambrose JD, Schmitt EJ-P, Thatcher J-J, Thatcher WW. Effects of buserlin injection and Deslorelin (GnRH-agonist) implant on plasma progesterone, LH, accessory CL formation, follicle and corpus luteum dynamics in Holstein cows. *Theriogenology*. (1998) 50:1141-1155.
Rhodes FM, McDougall S, Morgan SR, Verkerk GA. Supplementing treated anoestrous dairy cows with progesterone does not increase conception rates. *NZ Vet J.* (2001) 49:8-12.

Richards JS. Maturation of ovarian follicles: Actions and interactions of pituitary and ovarian hormones on follicular cell differentiation. *Physiol Rev.* (1980) 60:51-89.

Rizos D, Scully S, Kelly AK, Ealy AD, Moros R, Duffy P, Al Naib A, Forde N, Longergan P. Effects of human chorionic gonadotropin administration on day 5 after oestrus on corpus luteum characteristics, circulating progesterone and conceptus elongation in cattle. *Reprod Fertil Dev.* (2012) 24:472-481.

Roberts RM, Ealy AD, Alexenko AP, Han CS, Ezashi T. Trophoblast interferons. *Placenta.* (1999) 20:259-264.

Ryan DP, Prichard JF, Kopel E, Godke RA. Comparing early embryo mortality in dairy cows during hot and cool seasons of the year. *Theriogenology.* (1993) 39:719-737.

Sangsritavong S, Combs DK, Sartori R, Armentano LE, Wiltbank MC. High feed intake increases liver blood flow and metabolism of progesterone and estradiol-17β in dairy cattle. *J Dairy Sci.* (2002) 85:2831-2842.

Santos JE, Thatcher WW, Pool L, Overton MW. Effect of human chorionic gonadotropin on luteal function and reproductive performance of high-producing lactating Holstein dairy cows. *J Anim Sci.* (2001) 79:2881-2894.

Santos JEP, Bartolome JA, Cerri RLA, Juchem SO, Hernandez O, Trigg T, Thatcher WW. Effect of a Deslorelin implant in a timed artificial insemination protocol on follicle development, luteal function and reproductive performance of lactating dairy cows. *Theriogenology*. (2004) 61:421-435.

Sartori R, Sartor-Bergfelt R, Mertens SA, Guenther JN, Parrish JJ, Wiltbank MC. Fertilization and early embryonic development in heifers and lactating cows in summer and lactating and dry cows in winter. *J Dairy Sci.* (2002) 85:2803-2812.

Satterfield MC, Bazer FW, Spencer TE. Progesterone regulation of preimplantation conceptus growth and galectin 15 (LGALS15) in the ovine uterus. *Biol Reprod*. (2006) 75:289-296.

Savio JD, Keenan L, Boland MP, Roche JF. Pattern of growth of dominant follicles during the oestrous cycle of heifers. *J Reprod Fert.* (1988) 83:663-671.

Sirois J and Fortune JE. Ovarian follicular dynamics during the estrous cycle in heifers monitored by real-time ultrasonography. *Biol Reprod.* (1988) 39:308-317.

Silvia WJ, Lewis GS, McCracken JA, Thatcher WW, Wilson, Jr. L. Hormonal regulation of uterine secretion of prostaglandin $F_{2\alpha}$ during luteolysis in ruminants. *Biol Reprod.* (1991) 45:655-663.

Snijders SEM, Dillon P, O'Callaghan D, Boland MP. Effect of genetic merit, milk yield, body condition, and lactation number on in vitro oocyte development in dairy cows. *Theriogenology.* (2000) 53:961-969.

Souza AH, Ayres H, Ferreira RM, Wiltbank MC. A new presynchronization system (Double Ovsynch) increased fertility at first postpartum timed AI in lactating dairy cows. *Theriogenology.* (2008) 70:208-215.

Spencer TE, Burghardt RC, Jonson GA, Bazer FW. Conceptus signals for establishment and maintenance of pregnancy. *Anim Reprod Sci.* (2004) 82:537-550.

Spencer TE and Bazer FW. Conceptus signals for establishment and maintenance of pregnancy. *Reprod Biol and Endo.* (2004) 2:49-63.

Spencer TE, Johnson GA, Bazer FW, Burghardt RC. Fetal-maternal interactions during the establishment of pregnancy in ruminants. *Soc Reprod Fert Supp.* (2007) 64:379-396.

Spencer TE, Sandra O, Wolf E. Genes involved in conceptus-endometrial interactions in ruminants: insights from reductionism and thoughts on holistic approaches. *Reproduction.* (2008) 135:165-179.

Spencer TE, Forde N, Dorniak P, Hansen TR, Romero JJ, Lonergan P. Conceptus-derived prostaglandins regulate gene expression prior to pregnancy recognition in ruminants. *Reproduction*. (2013) 146:377-387.

Stronge AJ, Sreenan JM, Diskin MG, Mee JF, Kenny DA, Morris DG. Post insemination milk progesterone concentration and embryo survival in dairy cows. *Theriogenology*. (2005) 64:1212-1224.

Thatcher WW, Hansen PJ, Gross TJ, Helmer SD, Plante C, Bazer FW. Antiluteolytic effects of bovine trophoblast protein-1. *J Reprod Fert.* Suppl. (1989) 37:91-99.

Thatcher WW, Bilby TR, Bartolome JA, Silvestre F, Staples CR, Santos JEP. Strategies for improving fertility in the modern dairy cow. *Theriogenology.* (2006) 65:30-44.

Van Cleeff J, Macmillan KL, Drost M, Lucy MC, Thatcher WW. Effects of administering progesterone at selected intervals after insemination of synchronized heifers on pregnancy rates and resynchronization of returns to service. *Theriogenology.* (1996) 46:1117-1130.

Vasconcelos JLM, Silcox RW, Rosa GJM, Pursley JR, Wiltbank MC. Synchronization rate, size of the ovulatory follicle, and pregnancy rate after synchronization of ovulation beginning on different days of the estrous cycle in lactating dairy cows. *Theriogenology.* (1999) 52:1067-1078.

Vasconcelos JLM, Sartori R, Oliviera HN, Guenther JG, Wiltbank MC. Reduction in size of the ovulatory follicle reduces subsequent luteal size and pregnancy rate. *Theriogenology.* (2001) 58:307-314.

Walker WL, Nebel RL, McGilliard M. Time of ovulation relative to mounting activity in dairy cattle. *J Dairy Sci*. (1996) 79:1555-1561.

Wiebold JL. Embryonic mortality and the uterine environment in first-service lactating dairy cows. *J Reprod Fert.* (1988) 84:393-399.

Xu ZZ, Garverick HA, Smith GW, Smith MF, Hamilton SA, Youngquist RS. Expression of FSH and LH receptor mRNA in bovine follicles during the first follicular wave. *Biol Reprod.* (1995) 53:951-957.

Xu ZZ, Burton LJ, Macmillan KL. Reproductive performance of lactating dairy cows following oestrus synchronization with progesterone, oestradiol, and prostaglandin. *N Z Vet J.* (1996) 44:99-104.

Chapter 2: Effect of Chronic Administration of a GnRH Agonist on Luteal Function and Pregnancy Rates in Lactating Dairy Cattle

Abstract

Increased embryonic losses may be associated with inadequate progesterone (P_4) concentrations in high-producing lactating dairy cattle. The objectives of the present studies were to determine if chronic administration of a gonadotropin-releasing hormone (GnRH) agonist, Deslorelin, would increase circulating P_4 concentrations and subsequently increase pregnancy rates in dairy cattle. Administration of Deslorelin for 12 days increased ($P < 0.05$) luteal volume and circulating P_4 concentrations in lactating dairy cows. Treatment with Deslorelin increased Day 45 pregnancy rates in cows as compared to untreated controls. Chronic treatment with Deslorelin in dairy cattle: 1) increased luteal volume of the primary CL; 2) induced accessory CL; 3) increased circulating P_4 concentration; 4) did not lengthen the estrous cycle upon removal of treatment; and 5) increased pregnancy rates. This hormonal strategy may represent a suitable model to address local effects of P_4 and GnRH/luteinizing hormone on uterine environment and subsequent embryonic survival.

Introduction

In cattle, maternal recognition of pregnancy (MROP) must occur by day 16 to inhibit luteolysis so P_4 production and pregnancy is maintained. The elongating conceptus must secrete interferon tau (IFNT) for MROP (Spencer et al., 2007). It has been speculated that fertilization rates are greater than 90% in cows (Kidder et al., 1954; Bearden et al., 1956; Hill et al., 1970; Diskin et al., 2006; Diskin and Morris, 2008) and early embryonic loss is 40% with approximately 80% of embryonic loss occurring

primarily between days 8 and 16 post-insemination (Dunne et al., 2000). Early embryonic loss is considered loss of the embryo before day 16, while embryonic loss is during the embryonic period. Maurer and Chenault (1983) reported 67% of embryonic loss occurs by day 8, and 33% of the loss occurs between days 8 and 16 in both parous and non-parous beef cattle. It has been speculated that the oviductal and uterine environments on Days 3 to 7 post-conception are important for embryonic development and survival (Stronge et al., 2005; McNeill et al., 2006; Diskin and Morris, 2008). The Committee on Bovine Reproductive Nomenclature (1972) defined the embryonic period of gestation as conception to 42 days of gestation, or end of differentiation.

Lesser concentrations of circulating P_4 in lactating dairy cows might be one component of infertility (Ahmad et al., 1996; Butler et al., 1996; Stronge et al., 2005; McNeill et al., 2006; Starbuck et al., 2006). McNeill et al. (2006) showed a positive correlation between milk P_4 concentrations as early as day 4 post-ovulation and embryo survival in dairy cattle. Early embryonic loss likely due to luteal insufficiency occurs in beef cows and heifers (Henricks et al., 1971; Maurer and Echternkamp, 1982). The importance of P_4 during early embryonic development was confirmed when administration of RU486, a P_4 receptor antagonist, resulted in lack of recovery of blastocysts from ewes (Satterfield et al., 2006). Lesser concentrations of circulating P_4 seem to contribute to infertility, but the rate at which P_4 increases during the early luteal phase may have a greater impact. A slow increase in P_4 after ovulation during the early luteal phase delays embryonic development in cattle (Henricks et al., 1971; Ahmad et al., 1996; Mann and Lamming, 2001; Green et al., 2005; Mann et al., 2006;

McNeill et al., 2006). Garrett et al. (1988) showed that daily injections of P_4 on Days 1, 2, 3, and 4 of pregnancy increased circulating concentrations of P_4 on Days 2 thru 5, conceptus length on Day 14, and subsequent uterine concentrations of IFNT.

Chronic administration of a GnRH agonist increases basal and mean concentrations of luteinizing hormone (LH) in beef heifers (Davis et al., 2003). Elevated concentrations of LH provided greater luteotropic support and the CL in GnRH agonist-treated heifers was greater in size with subsequent greater concentrations of P_4 in circulation (Davis et al., 2003). This impact of a GnRH agonist on the CL is evident only when the agonist is administered when the CL is developing, rather than once the CL is fully developed (Davis et al., 2003). The present objective was to determine if chronic administration of a GnRH agonist will alter luteal function and initiate an increase in P_4 concentrations in lactating dairy cattle. The hypothesis was that chronic administration of a GnRH agonist would increase circulating progesterone concentrations earlier in the estrous cycle and improve pregnancy rates in dairy cattle.

Materials and Methods

Experiment 1: Alteration of Luteal Size and Function by Chronic Administration of a GnRH Agonist in Primiparous Lactating Dairy Cows

All animal experimentation was performed in compliance with regulations by the Office of Research Assurances, Institutional Animal Care and Use Committee, University of Idaho, Moscow, Idaho (Appendix A). Primiparous Holstein and Holstein x Jersey cows (476.7 ± 12.8 kg) from the University of Idaho Dairy Center were randomly assigned to this experiment. Cows were housed in a tie-stall barn and fed 35 kg of a total mixed ration diet twice daily after milking. Cows were milked at 0600 and 1800

daily with samples taken at each time, placed on ice to be transported to the laboratory and frozen at -20°C until time of analysis for P_4, E_2, and Deslorelin quantification. Daily blood samples were taken and plasma frozen at -20°C until analysis for P_4 and E_2. The average days in milk at the time of the experiment were 72.8 ± 2.8 and milk production throughout the experiment averaged 32.0 ± 1.2 kg. The experimental design is shown in Figure 2.1. Animals were synchronized using the Ovsynch protocol (Pursley et al. 1997). Cows were not inseminated, however, the day TAI would have occurred was considered day 0 of the experiment. Cows were randomly assigned as untreated controls (n = 6) or treated (n = 6) with a GnRH agonist (Deslorelin, [Pyr-His-Trp-Ser-Tyr-D-Trp-Leu-Arg-Pro-NHEt]) administered beginning on day 3 following observation of ovulation and continuing through day 15 via an osmotic pump placed under the skin near the seventh rib. Size of the ovulatory follicle and changes in luteal volume were determined by trans-rectal ultrasonography conducted from day -3 through the ovulation of the following estrous cycle (Figure 2.1).

Experiment 2: Alteration of Luteal Size and Function by Chronic Administration of a GnRH Agonist in Multiparous Lactating Dairy Cows

Lactating multiparous cows (n = 18; 6 second lactation, 8 third lactation, 3 fourth lactation, 1 fifth lactation) were used for the second experiment to determine if Deslorelin would alter the size and function of the CL. Cows were managed as described in experiment 1. The cows were of similar body weight (602.1 ± 12.7 kg) and were 129 ± 11 days in milk with milk production averaging 22.9 ± 0.9 kg throughout the experiment. Estrus was synchronized using the Ovsynch protocol (Pursley et al., 1997). Timed artificial insemination (day 0) 24 hours after final GnRH injection was done by a

single technician using semen from the same sire to reduce variability. Daily blood and milk samples were taken as described previously and frozen at -20°C until analysis. Size of the ovulatory follicle and changes in luteal volume were determined by trans-rectal ultrasonography from day -3 to day 0 and day 3 through the subsequent ovulation, except on day 14 (Figure 2.2). Cows were evenly distributed by parity and then randomly assigned by parity to untreated controls (n = 9) or Deslorelin-treated (n = 9) between days 3 and 15. On day 15 the conceptuses were recovered by flushing both uterine horns to measure conceptus development and interferon-tau quantification. Uterine flushings were immediately examined for conceptus tissue. All cows received an injection of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; 25 mg i.m., Lutalyse, Pharmacia & Upjohn Co., Kalamazoo, MI) following the uterine flush.

Experiment 3: Effects of Chronic Administration of a GnRH Agonist on Pregnancy Rates

Holstein and Holstein x Jersey cows at the University of Idaho Dairy Center were used to determine if Deslorelin treatment would increase pregnancy rates in dairy cows in a small pilot study. Lactating multiparous, primiparous, and non-lactating nulliparous animals (Table 2.1, n = 30) were used in this experiment. Animals were synchronized using a modified Ovsynch protocol (Pursley et al. 1997). The protocol was the initial administration of GnRH (100 μg) followed seven days later with two administrations of $PGF_{2\alpha}$ (25 mg each dose) 12 hours apart, followed by administration of GnRH (100 μg) 36 hours later. Animals were TAI at 16 hours following the 2nd GnRH injection and administered a second dose of GnRH (100 μg) was at TAI. A second TAI was performed again 12 to 16 hours later (GnRH-7d-$PGF_{2\alpha}$-12h-$PGF_{2\alpha}$-36h-GnRH-16h-TAI+GnRH-12-16h-TAI). This synchronization protocol is routinely used at the university dairy,

developed by the herd manager. The semen used was chosen by the herd manager as part of the routine management of the herd. The herd manager, who is normally responsible for AI, performed artificial insemination. Animals were randomly assigned as untreated controls (n = 10), 7 days of Deslorelin treatment (Days 3 – 10; n = 10), or 12 days of Deslorelin treatment (Days 3 – 15; n = 10). Ultrasonography was conducted on day 45 to determine pregnancy.

Administration of GnRH Agonist

Deslorelin was graciously provided by Dr. Paul Schober (Peptech Animal Health Pty Limited, Australia). Cows were treated with Deslorelin at a concentration of 1-µg/kg body weight^{-1} day^{-1} using Alzet osmotic pumps (DURECT Corp., Cupertino, CA). Lyophilized Deslorelin was re-suspended in sterile 0.15 M NaCl (J.T. Baker, VWR International) at 0.01 mg/µl for a stock solution. The Deslorelin stock solution was filter sterilized. The 14-day Alzet 2ML2 osmotic pump model was used with an average pumping rate of 4.96 µl/h (12 days of treatment). The 7-day Alzet 2ML1 osmotic pump model was used for 7 days of Deslorelin treatment in the pregnancy study (experiment 3) with an average pumping rate of 10.0 µl/h. Filled pumps were incubated at 37^{o}C in sterile 0.15 M NaCl overnight prior to implantation into the cows to initiate delivery of Deslorelin at the onset of insertion. Osmotic pumps were inserted subcutaneously near the seventh rib using a local anesthetic of lidocaine. Pumps were implanted on day 3 and removed on day 10 (7 days of treatment) or day 15 (12 days of treatment) of the experiment. The remaining volume of solution was aspirated from the pumps to ensure that the appropriate amount of Deslorelin had been delivered to the animal.

Ultrasonography

Transrectal ultrasonography was performed using an Aloka 500 V ultrasonographic monitor and 7.5-MHz linear probe (Corometrics, Wallingford, CT). Using a frozen image of the maximal size of ovarian follicles and CL, agreed upon by the person conducting the ultrasonography and another experienced with the procedure, the largest diameter (mm) was recorded for follicles. Size of the ovulatory follicle was the last recorded measurement for the structure before ovulation and followed for subsequent presence of luteal tissue in the same location on the ovary. Size of the CL was calculated using the maximal area measurements converted to volume using the formula $V = 4/3 \times \pi \times R^3$ with the radius being calculated by the formula $R= \sqrt{area}/\pi$. Presence of a cavity within the luteal tissue was subtracted from the total volume. Palpation of the reproductive tract causes release of $PGF_{2\alpha}$ and decreases embryo viability (Green et al., 2005; Looney et al., 2006). Others have reported release of $PGF_{2\alpha}$ and loss of pregnancy due to manipulation of the reproductive tract (Wann and Randel, 1990; Vaillancourt et al., 1979). Starbuck et al. (2004) reported no detriment to pregnancy with frequent manipulation of the reproductive tract. Daily palpation and ultrasonography of the reproductive tract was not performed in experiment 3 to prevent the potential release of $PGF_{2\alpha}$.

Blood Sampling

Jugular blood samples were collected daily for experiments 1 and 2 and coccygeal venipuncture was conducted for experiment 3. Blood samples were collected in Vacutainer tubes containing sodium heparin (BD Diagnostics, Franklin Lakes, NJ). Samples were immediately placed on ice to be transported to the laboratory. Samples

were centrifuged at 1,500 x g for 15 min, plasma collected, and stored at -20°C until assayed for hormone concentrations. Blood samples were collected daily beginning on day 0 and continued until ovulation occurred for the next estrous cycle (experiment 1). Sampling began on day -3 and continued until animals returned to estrus after discontinuation of treatment in experiment 2. Blood samples were collected from day -3 through day 21 and day 45 in experiment 3.

Radioimmunoassays

All assays were completed at the Endocrine Laboratory, Animal Reproductive and Biotechnology Laboratory, Colorado State University. Concentrations of P_4 in plasma for all experiments were determined by RIA (Niswender, 1973). Intra-assay coefficients of variation (CV) averaged 4.0%, and the inter-assay CV was 13.2%. Sensitivity averaged 50.7 pg/ml. Steroid hormones were extracted from milk collected in experiment 1 using 5 ml of petroleum ether (Sigma Aldrich, St. Louis, MO). Samples sat for 5 min to allow phase separation and then were frozen in a dry-ice methanol bath. The solvent was decanted into another glass tube and dried under nitrogen. Lipid precipitation of the sample was performed using 2 ml of HPLC grade methanol. Samples were vortexed for 2 min and incubated for 60 min at -80°C. Lipids were pelleted by centrifugation for 10 min at 1,500 x g at 0°C in pre-chilled carriers (-20°C). The supernatant was decanted carefully into a fresh glass tube so not to disrupt the lipid pellet. Removal of methanol was done by drying in a heating block (45°C) under nitrogen. The sample was reconstituted with 1.0 ml 0.01 M PBS (pH 7.0) containing 0.01% gelatin, vortexed for 2 min, and allowed to set overnight at 4°C before being assayed. Bulk tank milk samples were spiked with either [^{3}H]-E_2 or [^{3}H]-P_4 overnight at

4°C to determine recovery of steroid hormones following lipid extraction. Progesterone recovery was 65%, and E_2 recovery was 77%. Concentrations of P_4 in milk were then determined by RIA (Niswender, 1973). The intra-assay CV was 3.3 %. Concentrations of E_2 in milk were determined by RIA as previously described (Thompson et al., 1978). The intra-assay CV for E_2 was 3.2% and sensitivity was 1.4 pg/ml. Deslorelin concentrations were determined in milk samples for experiment 1. An initial extraction using methanol was done followed by incubation of samples for 24 hours at -20°C to precipitate and remove remaining lipid in samples. Samples were centrifuged for 15 min at -5°C at 1,500 x g in chilled carriers (-20°C). Supernatant was decanted into fresh glass tubes and the lipid extraction was repeated. Following the second centrifugation and pour off, methanol was removed by drying under nitrogen in a heated block (45°C). Deslorelin was measured using an anti-rabbit leuprolide primary antibody (CSU R7), anti-rabbit gamma globulin as the secondary antibody, and the radiolabeled hormone was ^{125}I-DAla^{6}-GnRH. The intra-assay CV averaged 2.6% and the inter-assay CV was 11.9%.

Statistical Analysis

Plasma P_4 and CL volume were examined by analysis of variance (ANOVA) using repeated measures of the mixed procedure of Statistical Analysis System (SAS; SAS Institute Inc., Cary, NC version 9.3). The statistical model included the treatment, the repeated factor day, and the treatment x day interaction. Cow within treatment was designated as a random effect and the covariance structure used was heterogeneous autoregressive, as it offered the best-fit statistic. Differences in least square means between the control group and treatment groups were determined using PDIFF

statement of SAS. Pregnancy status at day 45 was evaluated using Chi-square, procedure frequency, of SAS. Size of ovulatory follicle and length of estrous cycle, as determined from ovulation to ovulation, were analyzed by Student T-test. Two cows in the Deslorelin treated group were removed from the analysis for experiment 1, as the osmotic pump for one cow failed to deliver the GnRH agonist, and the other cow had a short-lived CL that was the first CL post-partum. In experiment 2, two control cows were removed from the analysis as double ovulation occurred; two Deslorelin-treated cows were removed from the analysis due to treatment for mastitis; one Deslorelin-treated cow was removed from the analysis as the pump failed to deliver the GnRH agonist.

Results

Experiment 1: Alteration of Luteal Size and Function by Chronic Administration of a GnRH Agonist in Primiparous Lactating Dairy Cows

The size of the ovulatory follicle did not differ between the untreated control (16.9 ± 1.3 mm) and Deslorelin-treated cows (17.5 ± 1.4 mm). Luteal volume in cows treated with Deslorelin was greater ($P < 0.5$) on days 5 through 18 compared to untreated controls (Figure 2.2*a*). Circulating concentrations of P_4 tended ($P = 0.06$) to be greater on days 8 through 10 for the Deslorelin-treated cows and were greater ($P < 0.05$) on days 11 thru 15 (Figure 2.2*b*). All Deslorelin-treated cows developed accessory CL (Table 2.2). The size of the ovulatory follicle for the subsequent estrous cycle upon removing treatment was 13.1 ± 1.0 mm for untreated controls and 12.8 ± 1.3 for Deslorelin-treated cows. The duration of the estrous cycle as determined from

ovulation to ovulation was 20.5 ± 0.9 days for untreated control cows and 23.0 ± 0.7 days (P = 0.08) for Deslorelin-treated cows.

Steroid hormones and Deslorelin concentrations were measured in daily milk samples to determine if treatment had any residual effect on hormone concentrations in milk. Progesterone concentrations were greater (P = 0.03) on day 8 in milk from Deslorelin-treated cows (6.6 ± 0.9 ng/ml) compared to untreated controls (4.0 ± 0.8 ng/ml). Estradiol-17β concentrations were greater (P < 0.05) on day 10 in untreated controls (1.0 ± 0.1 pg/ml) compared to Deslorelin-treated cows (0.5 ± 0.06 pg/ml). Deslorelin was undetectable in milk samples.

Experiment 2: Alteration of Luteal Size and Function by Chronic Administration of a GnRH Agonist in Multiparous Lactating Dairy Cows

Volume of the CL was greater (P < 0.05; Figure 2.3*a*) for the Deslorelin-treated cows compared to untreated controls days 7 and 9-13. Circulating P_4 concentrations were greater on days 8, 10, and 13 (P = 0.05; Figure 2.3*b*) and tended to be greater on days 9, 11, and 12 (P < 0.10; Figure 2.3*b*) for the Deslorelin-treated cows compared to untreated controls. All Deslorelin-treated animals developed accessory CL (Table 2.2). Conceptus tissue was collected from four (4/7, 57%) control and three (3/6, 50%) Deslorelin-treated cows; the conceptuses collected were multiple fragments of filamentous tissue and were unable to be measured.

Experiment 3: Effects of Chronic Administration of a GnRH Agonist on Pregnancy Rates

Circulating P_4 concentrations did not differ in cows treated with Deslorelin for 7 days (days 3 through 10; Figure 2.4*a*) compared to controls. Circulating P_4 concentrations were greater on days 10, 11, 12, 14, and 15 in cows treated with

Deslorelin for 12 days (days 3 through 15; $P < 0.05$; Figure 2.4*b*) compared to controls. Day 45 pregnancy rates were increased in animals treated with Deslorelin for 7 days (9/10, 90%) compared to control animals (4/10, 40%; $P = 0.02$; Table 2.3). Day 45 pregnancy rates were no different in animals treated with Deslorelin for 12 days (6/10, 60%) compared to untreated controls (4/10, 40%; $P = 0.37$; Table 2.3). Calving rates tended to be different in animals treated with Deslorelin (7/10, 70%) compared to controls (3/10, 30%; $P = 0.07$; Table 2.3). Calving rates did not differ between 12-day Deslorelin-treated animals (6/10, 60%) compared to controls (3/10, 30%; $P = 0.18$; Table 2.3). Pregnancy losses occurred after 45 days in one control animal and in two 7-day Deslorelin treated animals. Pregnancy losses were assumed to have occurred through abortion and resorption of the fetal tissues. One cow in the Deslorelin-treated group was not rebred and was removed from the herd at the herd manager's discretion. The control cow and the other Deslorelin-treated cow were rebred and became pregnant after the first breeding following the loss of their previous fetus. Parity differences for pregnancy rates are shown in Table 2.4.

Discussion

The experiments presented were designed to determine if chronic administration of a GnRH agonist, Deslorelin, beginning early in the estrous cycle would alter luteal volume, circulating P_4 concentrations, or pregnancy rates in lactating dairy cattle. The main findings from the present experiments were that chronic administration of Deslorelin: 1) increased luteal volume of the primary CL; 2) induced accessory CL in experiments 1 and 2; 3) increased circulating P_4 concentrations when

treated through day 15; and 4) increased pregnancy rates on Day 45 with short duration of treatment.

Administration of an agonist of GnRH beginning on day 3 or day 12 of the estrous cycle (day 0 = estrus) increased basal and mean concentrations of LH in beef heifers (Davis et al., 2003). Luteal area was increased only when GnRH agonist was administered chronically beginning on day 3. Luteal weights were nearly doubled when cows were treated chronically with a GnRH agonist beginning day 3 of the estrous cycle, although luteal cell size and ratio of steroidogenic cells were not altered (Davis, unpublished data).

The same method, chronic administration of a GnRH agonist, Deslorelin, was examined in the present experiments in both lactating and nonlactating dairy cattle relative to circulating P_4 concentrations and pregnancy rates. In experiment 1, chronic administration of Deslorelin increased luteal volumes, induced the formation of accessory CL, and increased circulating P_4 concentrations in primiparous lactating dairy cows as reported previously for beef heifers (Davis et al., 2003). Numerous scenarios to increase circulating P_4 concentrations have been performed to overcome luteal insufficiency, which is thought to be an important contributor to early embryonic loss (Lucy, 2007). Most of the efforts have focused on providing exogenous P_4 to supplement endogenous P_4 concentrations from the cow's CL. Daily progesterone injections beginning on day 1 of pregnancy advanced the development of the conceptus and increased IFNT concentrations (Garrett et al., 1988). Addition of Deslorelin into estrous synchronization programs to induce ovulation had little to no effect on circulating P_4 concentrations (Bartolome et al., 2004) or had no effect or even decreased pregnancy

rates depending on the dosage (Santos et al., 2004). Implantation of Deslorelin on day 27 of pregnancy resulted in the formation of an accessory CL and increased circulating P_4 concentrations, but did not reduce pregnancy loss between days 45 and 90 compared to controls (Bartolome et al., 2006). Deslorelin implants administered on day 5 of the estrous cycle increased circulating P_4 concentrations by induction of an accessory CL (Rajamahendran et al., 1998).

Other efforts to enhance circulating P_4 concentrations include inducing accessory CL early in the estrous cycle. Administration of GnRH on day 5 to cause ovulation of a dominant follicle during the first ovarian follicular wave and subsequent formation of an accessory CL has been tried numerous times with various responses (Sreenan and Diskin, 1983; Santos et al., 2001; Starbuck et al., 2006). Human chorionic gonadotropin (hCG) has been used to increase circulating P_4 concentrations and subsequent pregnancy rates. Administration of hCG on day 5 of the estrous cycle in cattle induced accessory CL and increased circulating concentrations of P_4 (Kerbler et al., 1997). Progesterone concentrations increased with the presence of the accessory CL and improved pregnancy rates on days 28, 42, and 90 (Santos et al., 2001). Rizos et al. (2012) administered hCG on day 5 after estrus; that treatment induced formation of accessory CL, hypertrophy of the primary CL, increased circulating P_4 concentrations from day 7 onward, increased size of the conceptus, and increased IFNT production by conceptus tissue in vitro. The use of hCG or GnRH agonist to induce an accessory CL depends upon the presence of a dominant follicle responsive to LH; therefore, the timing of administration is critical and may require ultrasonography to examine ovarian structures for the greatest efficacy of either hormone to induce accessory CL.

Elevated concentrations of circulating P_4 in the immediate post-conception period (days 3 thru 5) are associated with advanced embryonic development as determined by greater elongation of the conceptus, greater IFNT production, and pregnancy rates in cattle (Garrett et al., 1988; Mann and Lamming, 2001; Inskeep, 2004; Stronge et al., 2005; McNeill et al., 2006; Carter et al., 2008) and sheep (Ashworth et al., 1989; Satterfield et al., 2006). Beltman et al. (2009) reported that early P_4 supplementation increased circulating P_4 concentrations and embryo survival, but did not alter conceptus size in beef heifers. Overall, the crucial period to elevate circulating P_4 concentrations to avoid early onset of luteolysis and to advance conceptus development by insertion of a CIDR or PRID was day 3 in cattle (Carter et al., 2008; Forde et al., 2009; Forde et al., 2011). Earlier exposure to P_4 alters the endometrial transcriptome and components of uterine secretions (McNeill et al., 2006; Forde et al., 2009; Carter et al., 2010; Forde et al., 2011) likely providing a more conducive environment for advancement of embryo development and survival. In the present studies, circulating P_4 concentrations were increased, but did not begin to deviate until day 8, several days past the immediate post-conception period that seems critical to alter endometrial transcriptome and uterine environment.

Circulating P_4 concentrations were similar in beef heifers classified as infertile, subfertile, or high fertile providing evidence that P_4 in circulation may not have a direct role in embryonic loss (Minten et al., 2013). Beef heifers, however, do not have fertility issues the same as lactating dairy cows. Decreased P_4 concentrations are of special concern for lactating dairy cows, which would be expected to require greater dry matter intake. Increased dry matter intake by dairy cows results in greater liver blood

flow and P_4 and E_2 metabolism (Sangsritavong et al., 2002). Lactating cows have an increased risk of early embryonic death due to decreased circulating P_4 concentrations as a result of increased liver blood flow leading to greater metabolic clearance of P_4 (Diskin et al., 2006). No measurement has been identified to indicate the systemic P_4 threshold to sustain pregnancy. Luteal insufficiency did not appear to be an issue in the present studies because circulating P_4 concentrations were greater than 1 ng/ml (<1ng/ml typically concentration to indicate no functional CL present) in all the animals used.

Progesterone concentrations were not elevated during the critical post-conception period. A direct effect of an agonist of GnRH or LH on the uterus cannot be dismissed. Gonadotropin-releasing hormone receptor mRNA and protein has been identified in luminal and glandular endometrial epithelium and oviductal epithelial cells (Singh et al., 2008). The presence of GnRH has been described in pig, rat, and human endometrial tissue (Li et al., 1993a, b; Ikeda et al., 1996; Dong et al., 1998; Raga et al., 1998) and oviducts (Casan et al., 2000). However, the Type I GnRH receptor has not been examined in the bovine uterus. The Type II GNRH receptor mRNA has been identified in bovine endometrium (Thomas E. Spencer, personal communication), but these receptors are non-functional due to a frameshift giving rise to a stop codon (Millar, 2003). The presence of LH receptors and its mRNA have been described in bovine endometrium (Shemesh et al., 2001; Fields and Shemesh, 2004). Luteinizing hormone receptor numbers increased during the luteal phase and binding of LH to its receptor, increased endometrial cAMP and inositol phosphates, which further stimulated prostaglandin H-synthase 2 and $PGF_{2\alpha}$ synthesis (Shemesh et al., 2001). The

potential direct role of Deslorelin or LH on early embryonic development, oviductal tissue, or uterine endometrial tissue requires further exploration.

The implications of the present studies include: 1) chronic treatment with Deslorelin increased luteal volume of the primary CL; 2) induced accessory CL formation; 3) increased circulating P_4 concentrations with treatment for 12 days; 4) did not lengthen the estrous cycle upon removal of treatment; 5) did not alter steroid hormone concentrations in milk (nor was Deslorelin detected in milk); and 6) increased pregnancy rates. Although luteal insufficiency appears to be a highly probable reason for early embryonic loss, luteal insufficiency did not seem to be an issue among any of the animals across the three experiments. Finally, it is understood that too few animals were used in the present studies. Further, heifers should not have been included in experiment 3, because these animals do not have reproductive inefficiencies as do mature cows. However, these pitfalls do not take away from the apparent increase in pregnancy rates with chronic treatment with a GnRH agonist. This data is exciting and provides adequate evidence that more research should be conducted to determine the mechanisms by which Deslorelin treatment, increased LH, or increased P_4 potentially alters endometrial genomics, composition of histotroph, and development and elongation of the conceptus and subsequent IFNT production. Discretion in interpreting the pregnancy results due to low animal numbers is expected; however, the impacts of the hormonal strategy to control luteal function without altering length of the estrous cycle are promising.

References

Ahmad N, Beam SW, Butler WR, Deaver DR, Duby RT, Elder DR, Fortune JE, Griel LC Jr., Jones LS, Milvae RA, Pate JL, Revah I, Schreiber DT Jr., Townson DH, Tsang PCW, Inskeep EK. Relationship of fertility to patterns of ovarian follicular development and associated hormonal profiles in dairy cows and heifers. *J Anim Sci.* (1996) 74:1943-1952.

Ashworth CJ, Sales DI, Wilmut I. Evidence of an association between the survival of embryos and the preovulatory plasma progesterone concentration in the ewe. *J Reprod Fert.* (1989) 87:23-32.

Bartolome JA, Kamimura S, Silvestre F, Arteche ACM, Trigg T, Thatcher WW. The use of a Deslorelin implant (GnRH agonist) during the late embryonic period to reduce pregnancy loss. *Theriogenology*. (2006) 65:1443-1453.

Bartolome JA, Santos JEP, Pancarci SM, Melendez P, Arteche ACM, Hernandez O, Archbalk LF, Trigg T, Thatcher WW. Induction of ovulation in nonlactating dairy cows and heifers using different doses of a Deslorelin implant. *Theriogenology.* (2004) 61:407-419.

Bearden HJ, Hansel W, Bratton RW. Fertilization and embryonic mortality rates of bulls with histories of either low or high fertility in artificial breeding. *J Dairy Sci.* (1956) 39:312-318.

Beltman ME, Lonergan P, Diskin MG, Roche JF, Crowe MA. Effect of progesterone supplementation in the first week post conception on embryo survival in beef heifers. *Theriogenology*. (2009) 71:1173-1179.

Butler WR, Calaman JJ, Beam SW. Plasma and milk urea nitrogen in relation to pregnancy rate in lactating dairy cattle. *J Anim Sci*. (1996) 74:858-865.

Carter F, Forde N, Duffy P, Wade M, Fair T, Crowe MA, Evans ACO, Kenny DA, Roche JF, Lonergan P. Effect of increasing progesterone concentration from Day 3 of pregnancy on subsequent embryo survival and development in beef heifers. *Reprod Fertil Dev.* (2008) 20:368-375.

Carter F, Rings F, Mamo S, Holker M, Duzmany A, Besenfelder U, Havlicek V, Mehta JP, Tesfaye D, Schellander K, Lonergan P. Effect of elevated circulating progesterone concentration on bovine blastocyst development and global transcriptome following endoscopic transfer of in vitro produced embryos to the bovine oviduct. *Biol Reprod*. (2010) 83:707-719.

Casan EM, Raga F, Bonilia-musoles F, Polan ML. Human oviductal gonadotropin releasing hormone: possible implication in fertilization, early embryonic development, and implantation. *J Clin Endocrinol Metab.* (2000) 85:1377-1381.

Committee on Bovine Reproductive Nomenclature. Recommendations for standardizing bovine reproductive terms. *Cornell Vet*. (1972) 62:216-237.

Davis TL, Mussard ML, Jimenez-Severiano H, Enright WF, Kinder JE. Chronic treatment with an agonist of gonadotropin-releasing hormone enhances luteal function in cattle. *Biol Reprod.* (2003) 69:398-403.

Diskin MG, Murphy JJ, Sreenan JM. Embryo survival in dairy cows managed under pastoral conditions. *Anim Reprod Sci.* (2006) 96:297-311.

Diskin MG, Morris DG. Embryonic and early foetal losses in cattle and other ruminants. *Reprod Dom Anim.* (2008) 43: 260-267.

Dong DW, Marcelin K, Hsu MI, Chiang CM, Hoffman G, Roberts JL. Expression of gonadotropin-releasing hormone (GnRH) gene in human uterine endometrial tissue. *Mol. Hum. Reprod*. (1998) 4:893-898.

Dunne LH, Diskin MG, Sreenan JM. Embryo and foetal loss in beef heifers between Day 14 of gestation and full term. *Anim Reprod Sci.* (2000) 96:297-311.

Fields MJ, Shemesh M. Extragonadal luteinizing hormone receptors in the reproductive tract of domestic animals. *Biol Reprod*. (2004) 71:1412-1418.

Forde N, Carter F, Fair T, Crowe MA, Evans ACO, Spencer TE, Bazer FW, McBride R, Boland MP, O'Gaora P, Lonergan P, Roche JF. Progesterone-regulated changes in endometrial gene expression contribute to advanced conceptus development in cattle. *Biol Reprod*. (2009) 81:784-794.

Forde N, Beltman ME, Duffy GB, Duffy P, Mehta JP, O'Gaora P, Roche JF, Lonergan P, Crowe MA. Changes in the endometrial transcriptome during the bovine estrous cycle: effect of low circulating progesterone and consequences for conceptus elongation. *Biol Reprod*. (2011) 84:266-278.

Garrett JE, Geisert RD, Zavy MT, Morgan GL. Evidence for maternal regulation of early conceptus growth and development in beef cattle. *J Reprod Fert.* (1988) 84:437-446.

Green MP, Hunter MG, Mann GE. Relationship between maternal hormone secretion and embryo development on day 5 of pregnancy in dairy cows. *Anim Reprod Sci*. (2005) 88:179-189.

Henricks DM, Lamond DR, Hill JR, Dickey FJ. Plasma progesterone concentrations before mating and in early pregnancy in the beef heifer. *J Anim Sci.* (1971) 33:450-454.
Hill JR, Lamond DR, Henricks DM, Dickey JF, Niswender GD. The effects of undernutrition on ovarian function and fertility in beef heifers. *Biol Reprod*. (1970) 2:78-84.

Ikeda M, Taga M, Sakakibara H, Minaguchi H, Ginsburg E, Vonderhaar BK. Gene expression of gonadotropin-releasing hormone in early pregnant rat and steroid hormone exposed mouse uteri. *J Endocrinol Invest*. (1996) 19:708-713.

Inskeep EK. Preovulatory, postovulatory, and postmaternal recognition effects of concentrations of progesterone on embryonic survival in the cow. *J Anim Sci.* (2004) 82:E24-39.

Kerbler TL, Buhr MM, Jordan LT, Leslie KE, Walton JS. Relationship between maternal plasma progesterone concentration and interferon-tau synthesis by the conceptus in cattle. *Theriogenology.* (1997) 47:703-714.

Kidder HE, Black WG, Wiltbank JN, Ulberg LC, Casida LE. Fertilization rates and embryonic death rates in cows bred to bulls of different levels of fertility. *J Dairy Sci.* (1954) 37:691-697.

Li WI, Jiao S, Chin PP. Immunoreactive gonadotropin-releasing hormone in porcine reproductive tissues. *Peptides*. (1993a) 14:543-549.

Li WI. Jiao S, Chen CL, Bazer FW. Secretion of immunoreactive β-endorphin and GnRH by the pig uterus: effects of reproductive state and breed. *J Reprod Fertil*. (1993b) 99:253-258.

Looney CR, Nelson JS, Schneider HJ, Forrest DW. Improving fertility in beef cow recipients. *Theriogenology.* (2006) 65:201-209

Lucy MC. Reproductive loss in high producing dairy cattle: Where will it end? *J Dairy Sci.* (2001) 84:1277-1293.

Lucy MC. Fertility in high-producing dairy cows: Reasons for decline and corrective strategies for sustainable improvement. *Soc Reprod Fertil Suppl*. (2007) 64:237-254.

Mann GE, Lamming GE. Relationship between maternal endocrine environment, early embryo development and inhibition of the luteolytic mechanism in cows. *Reproduction*. (2001) 121:175-180.

Mann GE, Fray MD, Lamming GE. Effects of time of progesterone supplementation on embryo development and interferon-tau production in the cow. *The Veterinary Journal*. (2006) 171:500-503.

Maurer RR, Echternkamp SE. Hormonal asynchrony and embryonic development. *Theriogenology*. (1982) 17:11-22.
Maurer RR, Chenault JR. Fertilization failure and embryonic mortality in parous and nonparous beef cattle*. J Anim Sci.* (1983) 56:1186-1189.

McNeill RE, Diskin MG, Sreenan JM, Morris DG. Associations between milk progesterone concentration on different days and with embryo survival during the early luteal phase in dairy cows. *Theriogenology*. (2006) 65:1435-1441.

Millar RP. GnRH II and type II GnRH receptors. *Trends Endocrinol Metab*. (2003) 14:35-43.

Minten MA, Bilby TR, Bruno RGS, Allen CC, Madsen CA, Wang Z, Sawyer JE, Tibary A, Neibergs HL, Geary TW, Bauersach S, Spencer TE. Effects of fertility on gene expression and function of the bovine endometrium. *PLOS ONE*. (2013) 8:e69444.

Niswender GD. Influence of the site of conjugation on the specificity of antibodies to progesterone. *Steroids*. (1973) 22:413-424.

Pope WF, Maurer RR, Stormshak F. Distribution of progesterone in the uterus, broad ligament, and uterine arteries of beef cows. *Anat Rec*. (1982) 203:245-250.

Pursley JR, Wiltbank MC, Stevenson JS, Ottobre JS, Garverick HA, Anderson LL. Pregnancy rates per artificial insemination for cows and heifers inseminated at a synchronized ovulation or synchronized estrus. *J Dairy Sci.* (1997) 80:295-300.

Raga F, Casan EM, Wen Y, Huang H, Nezhat C, Polan ML. Quantitative gonadotropin-releasing hormone gene expression and immunohistochemical localization in human endometrium throughout the menstrual cycle. *Biol Reprod.* (1998) 59:661-619.

Rajamahendran R, Ambrose JE, Schmitt EJ-P, Thatcher M-J, Thatcher WW. Effects of buserelin injection and Deslorelin (GnRH-agonist) implants on plasma progesterone, LH, accessory CL formation, follicle and corpus luteum dynamics in Holstein cows. *Theriogenology*. (1998) 50:1141-1155.

Rizos D, Scully S, Kelly AK, Ealy AD, Moros R, Duffy P, Al Naib A, Forde N, Longergan P. Effects of human chorionic gonadotropin administration on day 5 after oestrus on corpus luteum characteristics, circulating progesterone and conceptus elongation in cattle. *Reprod Fertil Dev.* (2012) 24:472-481.

Sangsritavong S, Combs DK, Sartori R, Armentano LE, Wiltbank MC. High feed intake increases liver blood flow and metabolism of progesterone and estradiol-17β in dairy cattle. *J Dairy Sci.* (2002) 85:2831-2842.

Santos JEP, Thatcher WW, Pool L, Overton MW. Effect of human chorionic gonadotropin on luteal function and reproductive performance of high-producing lactating Holstein dairy cows. *J Anim Sci*. (2001) 79:2881-2894.

Santos JEP, Bartolome JA, Cerri RLA, Juchem SO, Hernandez O, Trigg T, Thatcher WW. Effect of a Deslorelin implant in a timed artificial insemination protocol on follicle development, luteal function and reproductive performance of lactating dairy cows. *Theriogenology*. (2004) 61:421-435.

Satterfield MC, Bazer FW, Spencer TW. Progesterone regulation of preimplantation conceptus growth and galectin 15 (LGALS15) in the ovine uterus. *Biol Reprod*. (2006) 75:289-296.

Shemesh M, Mizrachi D, Gurevch M, Shore LS, Reed J, Chang SM, Thatcher WW, Fields MJ. Expression of a function luteinizing hormone (LH) receptor and its messenger ribonucleic acid in bovine endometrium: LH augmentation of cAMP and inositol phosphate in vitro and human chorionic gonadotropin (hCG) augmentation of peripheral prostaglandin in vivo. *Reprod Biol.* (2001) 1:13-32.

Singh R, Graves ML, Roskelley CD, Giritharan G, Rajamahendran R. Gonadotropin-releasing hormone receptor gene and protein expression and immunohistochemical localization in bovine uterus and oviducts. *Domest Anim Endocrinol*. (2008) 34:319-326.

Spencer TE, Johnson GA, Bazer FW, Furghardt RC, Palmarini M. Pregnancy recognition and conceptus implantation in domestic ruminants: roles of progesterone, interferons and endogenous retroviruses. *Reprod Fertil Dev*. (2007) 19:65-78.

Sreenan JM, Diskin MG. Early embryonic mortality in the cow: its relationship with progesterone concentration. *Vet Rec.* (1983) 112:517-521.

Starbuck MJ, Dailey RA, Inskeep EK. Factors affecting retention of early pregnancy in dairy cattle. *Anim Reprod Sci*. (2004) 84:27-39.

Starbuck MJ, Poole DH, Inskeep EK. (2006). Ability of induced corpora lutea to maintain pregnancy from the third month of gestation to term in cattle. *Theriogenology*. (2006) 66:383-386.

Stronge AJ, Sreenan JM, Diskin MG, Mee JF, Kenny DA, Morris DG. Post insemination milk progesterone concentration and embryo survival in dairy cows. *Theriogenology*. (2005) 64:1212-1224.

Thompson DL, Pickett BW Jr., Nett TM. Effect of season and artificial photoperiod on levels of estradiol-17β and estrone in blood serum of stallions. *J Anim Sci*. (1978) 47:184-187.

Vaillancourt D, Bierschwal CJ, Ogwu D, Elmore RG, Martin CE, Sharp AJ, Youngquist RS. Correlation between pregnancy diagnosis by membrane slip and embryonic mortality. *J Am Vet Med Assoc.* (1979) 175:466-468.

Wann RA, Randel RD. Effect of uterine manipulation 35 days after parturition on plasma concentration of 13, 14-dihydro-15-keto prostaglandin $F_{2\alpha}$ in multiparous and primiparous Brahman cows. *J Anim Sci.* (1990) 68:1389-1394.

Tables

Table 2.1: Distribution of Dairy Cattle by Parity in the Untreated Controls and Cattle Treated with Deslorelin for 7 or 12 Days for Experiment 3.

Parity	Untreated Controls	7 Day Deslorelin Treatment	12 Day Deslorelin Treatment
Nulliparous	N = 4	N = 3	N = 3
Primiparous	N = 2	N = 3	N = 3
Multiparous	N = 4	N = 4	N = 4

Table 2.2: Development of Accessory CL in Deslorelin-Treated Cows in Experiment 1 and 2.

	Experiment 1	**Experiment 2**
Day of estrous cycle of first observation of first accessory CL**	Day 11 – 12	Day 7 – 8
Maximal size of first accessory CL	269.2 ± 64.8 mm^3	121.0 ± 27.8 mm^3
Incidence of first accessory CL	4/4 cows	7/7 cows
Day of estrous cycle of first observation of second accessory CL	Day 15	Day 13
Maximal size of secondary accessory CL	140.3 ± 30.9 mm^3	84.6 ± 0.0 mm^3
Incidence of secondary accessory CL	3/4 cows	2/7 cows
Side of accessory CL relative to primary CL	5/7 CL same 2/7 CL opposite	5/9 CL same 4/9 opposite

******Day of first observation is when the CL was definitively confirmed as luteal tissue following observation of an ovulated follicle and ability to acquire an accurate measurement done by ultrasonography.

Table 2.3. Pregnancy (Day 45) and Calving Rates for Untreated Control Animals and Animals Treated with Deslorelin for 7 days (Days 3 – 10) or 12 days (Days 3 – 15).

Treatment Groups	Day 45 Pregnancy Rates	Calving Rates
Untreated Controls	4/10 (40%)[a]	3/10 (30%)[c]
Deslorelin-treated 7 days	9/10 (90%)[b]	7/10 (70%)[d]
Deslorelin-treated 12 days	6/10 (60%)[a.b]	6/10 (60%)[c,d]

Differences in column are denoted by superscript.
[a,b] indicates P = 0.02.
[c,d] indicated P = 0.07

Table 2.4. Pregnancy Rates Based on Parity.

	Untreated Control		7 Day Deslorelin		12 Day Deslorelin	
	N per group	Pregnant	N per group	Pregnant	N per group	Pregnant
Nulliparous	4	2	3	3	3	3
Primiparous	2	1	3	3	3	1
Multiparous	4	1	4	3	4	2

Figures

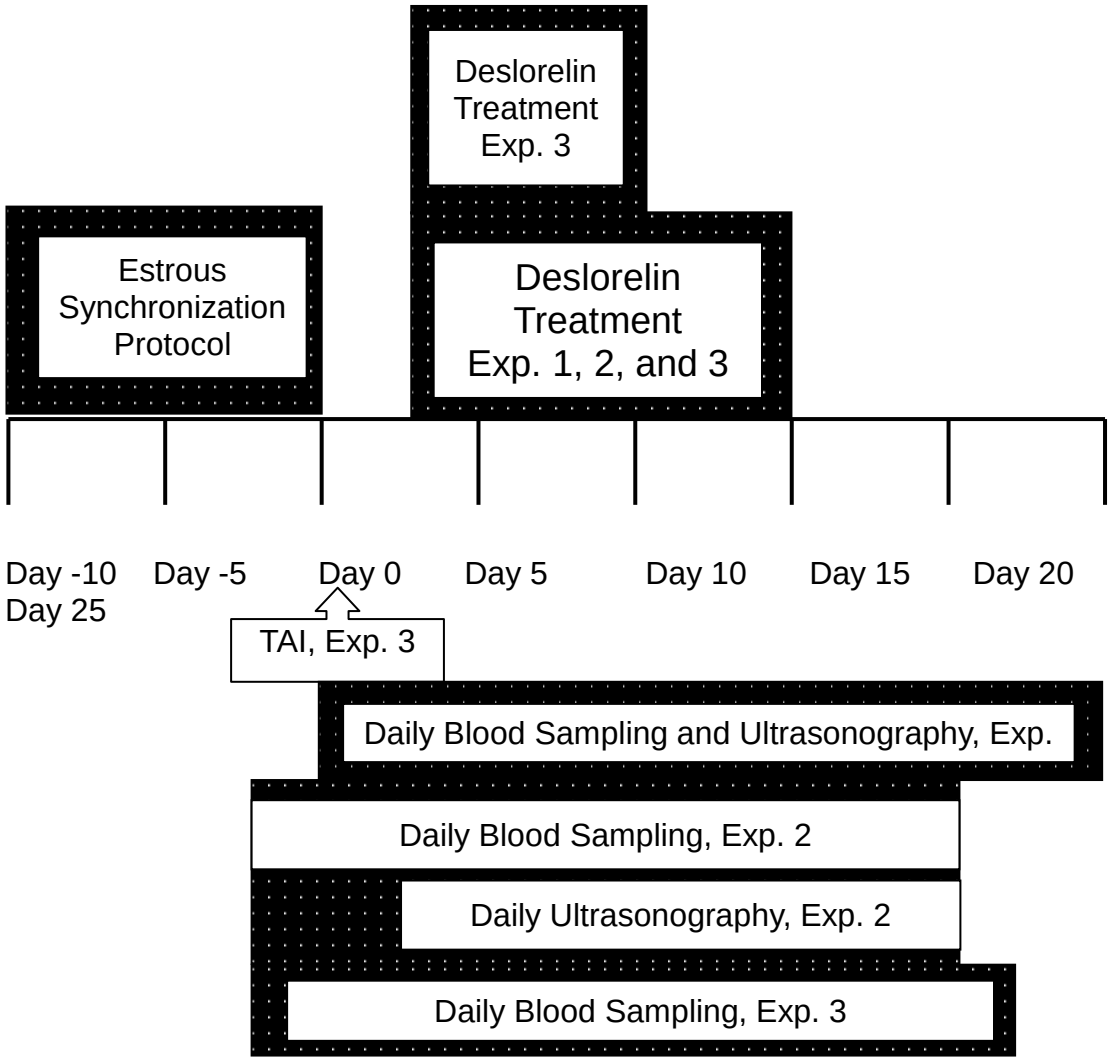


Figure 2.1: Experimental design for experiment 1 using primiparous cows, experiment 2 using multiparous cows, and experiment 3 using animals of various parity to determine effects of Deslorelin treatment on pregnancy rates.

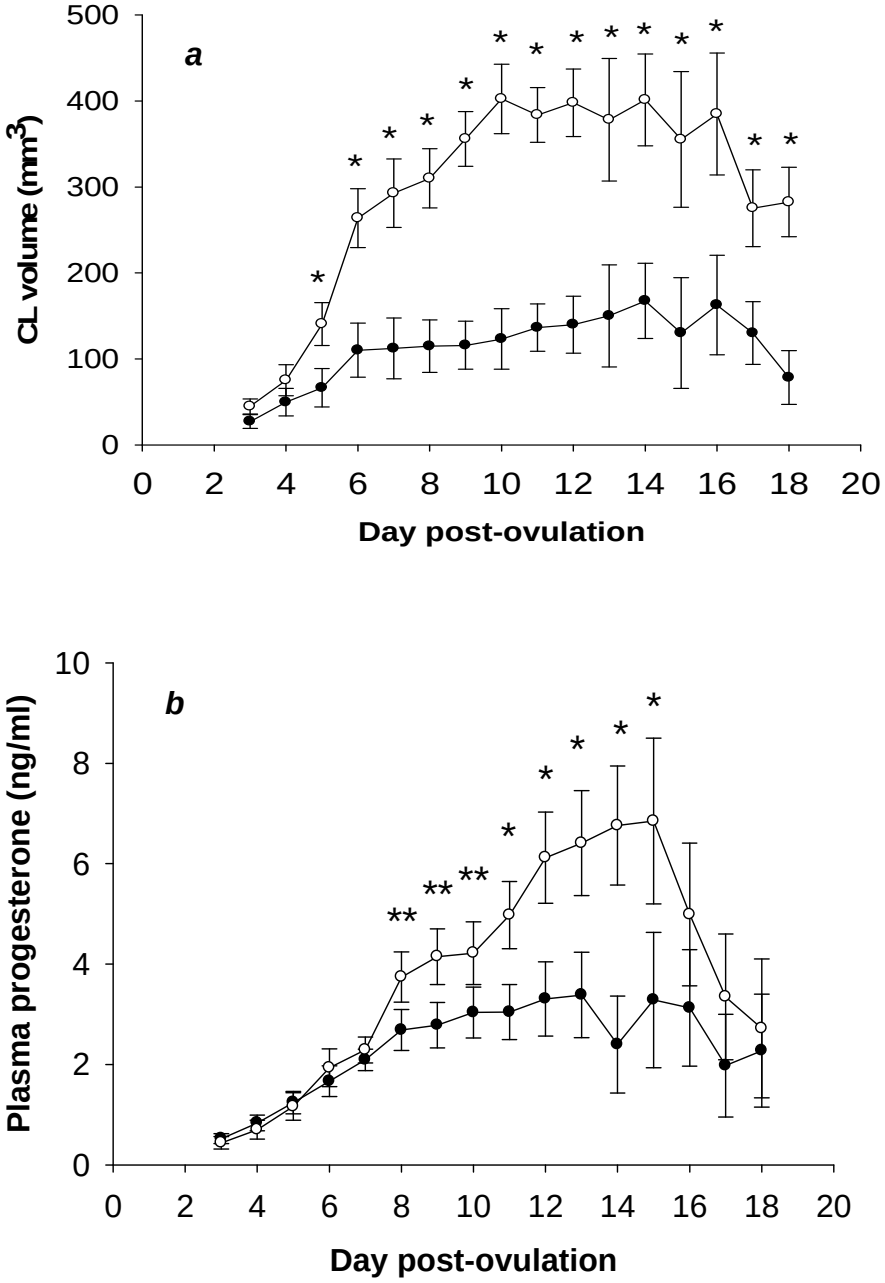


Figure 2.2: Effects of chronic administration of Deslorelin treatment on luteal volumes (*a*) and circulating concentrations of P_4 (*b*) in primiparous cows. Cows were left untreated (n = 6; closed circles) or treated with Deslorelin for 12 days beginning day 3 (n = 4; open circles). Data are represented as mean ± SEM. *P < 0.05; **P = 0.06

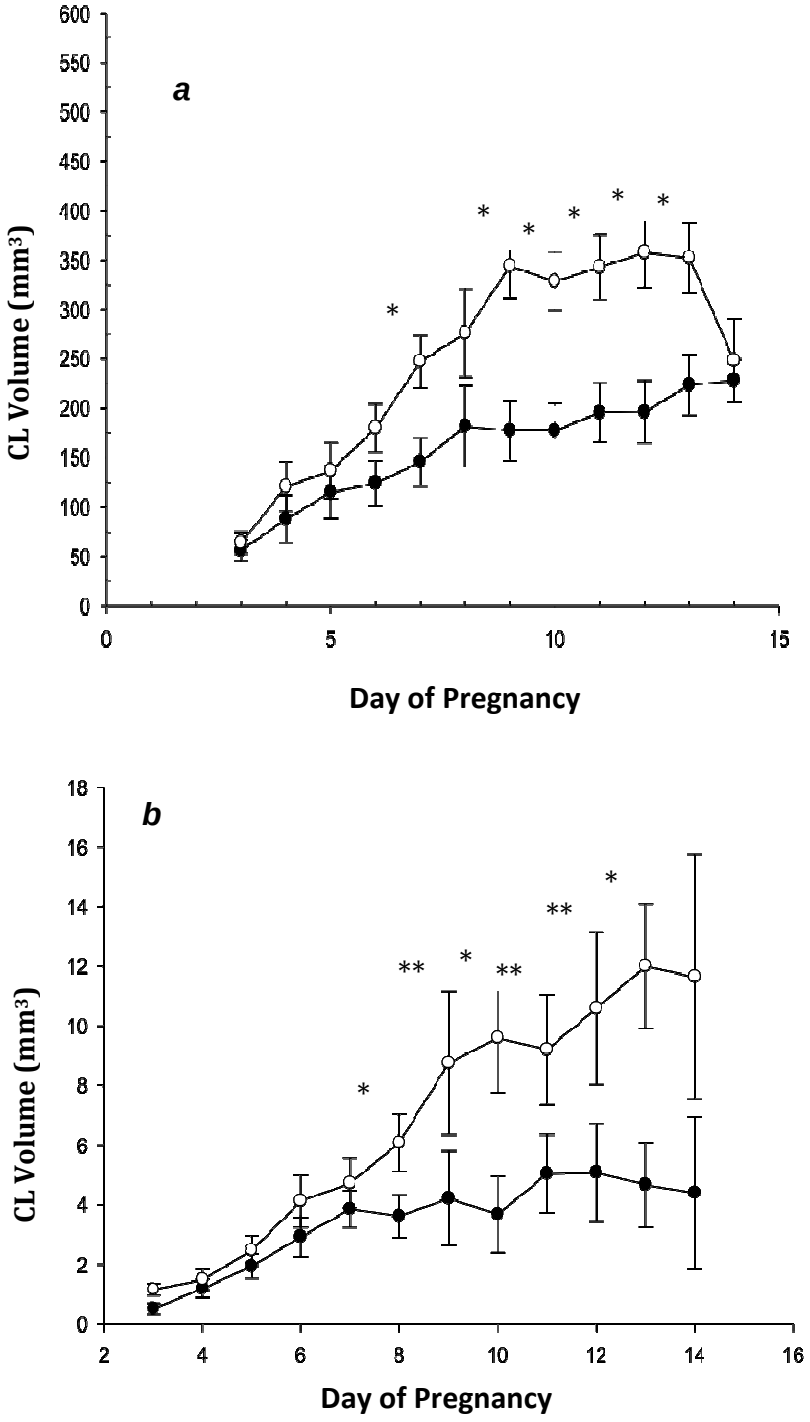


Figure 2.3: Effects of chronic administration of Deslorelin treatment on luteal volumes (*a*) and circulating concentrations of P4 (*b*) in multiparous cows. Cows were left untreated (n = 7; closed circles) or treated with Deslorelin for 12 days (n = 6; open circles). Data are represented as mean ± SEM. *P < 0.05, **P <0.10.

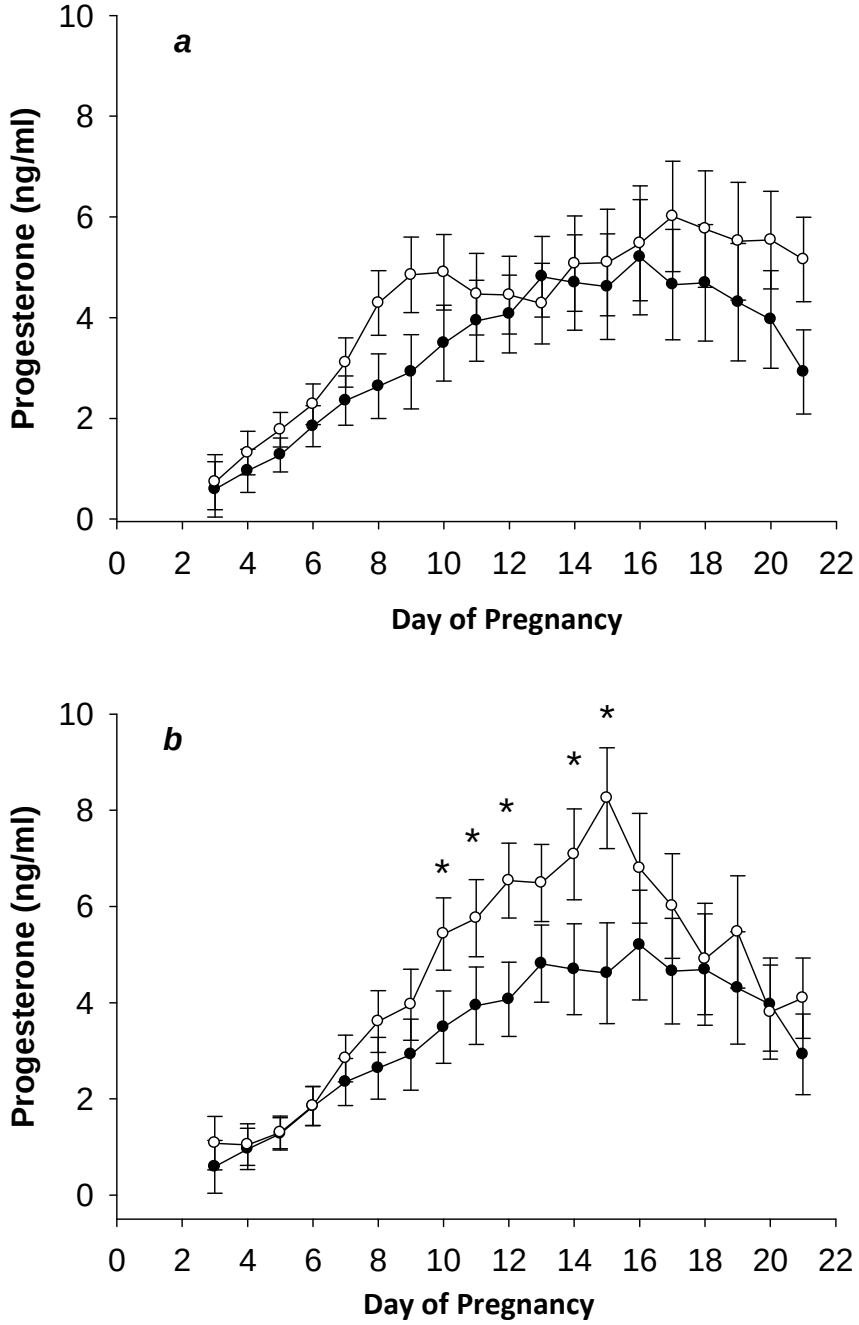


Figure 2.4: Effects of chronic administration of Deslorelin treatment (open circles) for 7 days on circulating P4 concentrations (*a*) or 12 days (*b*). The same controls (closed circles) are shown in figure *a* and *b*. Data are represented as mean ± SEM. *P < 0.05

Chapter 3: Limitations of Present Research and Future Research Direction

When performing research, there is no such thing as a perfect study, there are always limitations. It is understood that too few animals were used in these studies as funding and animals available were restricted. Although statistical significance was found in increasing P_4 concentrations in Deslorelin-treated animals, luteal volumes in Deslorelin-treated primiparous and multiparous cows, and pregnancy rates in 7-day Deslorelin-treated animals when compared to controls, an increase in animal numbers would provide for a stronger foundation on which to base results. In experiment 3, the pregnancy study, heifers were included to increase numbers in treatment groups. Heifers have higher pregnancy rates when compared to lactating dairy cows (Lucy. 2007) and should not have been included. Another downfall in both experiment 2 and 3 were the use of TAI. Cows bred at detection of estrus have increased conception rates when compared to cows bred at TAI (Pursley et al., 1997; Lucy, 2001). Animals should have been AI at estrus rather than TAI to ensure the animals were bred at the proper time relative to ovulation. Albeit, the pregnancy rates for the control animals in experiment 3 were similar to national pregnancy rates (Lucy, 2007) and the 5 year herd average at the University of Idaho Dairy Facility. This could also explain the lack of conceptuses collected in experiment 2. These pitfalls however, do not take away from the overall increase in pregnancy rates observed in the third experiment; these experiments provide a foundation for future research.

To strengthen statistical difference, I would perform another pregnancy study. Pregnancy rates in both primiparous and multiparous cows were increased with chronic GnRH agonist treatment; however, P_4 was not increased during the critical

post-conception period. A direct effect of a GnRH agonist or LH on the uterus cannot be dismissed. Gonadotropin-releasing hormone receptor mRNA and protein has been identified in luminal and glandular endometrial epithelium and oviductal epithelial cells (Singh et al. 2008). Type I GnRH receptor has not been described in the bovine uterus; the type II GnRH receptor mRNA has been identified in bovine endometrium (Thomas E. Spencer, personal communication), but these receptors are non-functional due to a frameshift, giving rise to a stop codon (Millar, 2003).

The presence of LH receptors and its mRNA have been described in bovine endometrium (Shemesh et al. 2001; Fields and Shemesh, 2004). Luteinizing hormone receptor numbers are increased during the luteal phase and binding of LH to its receptor, increased endometrial cAMP and inositol phosphates, which further stimulated prostaglandin H-synthase 2 and $PGF_{2\alpha}$ synthesis (Shemesh et al. 2001). The increase in mean and basal LH concentrations may increase PGs important in histotroph and growth and development of the conceptus. The potential direct role of Deslorelin or LH on early embryonic development, oviductal tissue, or uterine endometrial tissue requires further exploration.

Finally, another area that might benefit from the use of Deslorelin would be embryo transfer (ET). In ET, donor cows are superovulated to increase the number of follicles that ovulate and potentially fertilized. This results in multiple CL and a large increase in P_4 production (Forde et al., 2012). These embryos are collected on day 6 from a uterine environment exposed to higher than normal P_4 concentrations. Embryos are then frozen for later use or implanted into recipient cows, in the same stage of the estrous cycle as donor cows. These cows only have one CL; their uteri may not be at the

same developmental state as that from the donor cow. Use of Deslorelin in recipient cows to increase luteal volume and P_4 concentrations may alter the uterine environment to be more conducive to pregnancy and increase pregnancy rates.

References

Fields MJ, Shemesh M. Extragonadal luteinizing hormone receptors in the reproductive tract of domestic animals. *Biol. Reprod*. (2004) 71:1412-1418.

Forde N, Carter F, di Francesco S, Mehta JP, Garcia-Herreros M, Gad A, Tesfaye D, Hoelker M, Schellander K, Lonergan P. Endometrial response of beef heifers on day 7 following insemination to supraphysiological concentrations of progesterone associated with superovulation. *Physiol Genomics.* (2012) 44:1107-1115.

Lucy MC. Reproductive loss in high producing dairy cattle: Where will it end? *J Dairy Sci* (2001) 84:1277-1293.

Lucy MC. Fertility in high-producing dairy cows: Reasons for decline and corrective strategies for sustainable improvement. *Soc. Reprod. Fertil. Suppl*. (2007) 64:237-254.

Millar RP. GnRH II and type II GnRH receptors. *Trends Endocrinol. Metab*. (2003) 14:35-43.

Pursley JR, Wiltbank MC, Stevenson JS, Ottobre JS, Garverick HA, Anderson LL. Pregnancy rates per artificial insemination for cows and heifers inseminated at a synchronized ovulation or synchronized estrus. *J. Dairy Sci.* (1997) 80:295-300.

Shemesh M, Mizrachi D, Gurevch M, Shore LS, Reed J, Chang SM, Thatcher WW, Fields MJ. Expression of a function luteinizing hormone (LH) receptor and its messenger ribonucleic acid in bovine endometrium: LH augmentation of cAMP and inositol phosphate in vitro and human chorionic gonadotropin (hCG) augmentation of peripheral prostaglandin in vivo. *Reprod. Biol.* (2001) 1:13-32.

Singh R, Graves ML, Roskelley CD, Giritharan G, Rajamahendran R. Gonadotropin-releasing hormone receptor gene and protein expression and immunohistochemical localization in bovine uterus and oviducts. *Domest. Anim. Endocrinol*. (2008) 34:319-326.

Appendix A: Approval from Animal Care and Use Committee from the University of Idaho

Davis, Tracy

From: iacuc@uidaho.edu
Sent: Wednesday, November 16, 2011 9:44 AM
To: Davis, Tracy
Cc: Institutional Animal Care and Use Committee
Subject: Protocol 2012-4 - Increased Luteal Function on Subsequent Conceptus Growth and Pregnancy Rates in Lactating Dairy Cows

University of Idaho
Animal Care and Use Committee

Date: Wednesday, November 16, 2011
To: Tracy Davis
From: University of Idaho
Re: Protocol 2012-4
Increased Luteal Function on Subsequent Conceptus Growth and Pregnancy Rates in Lactating Dairy Cows

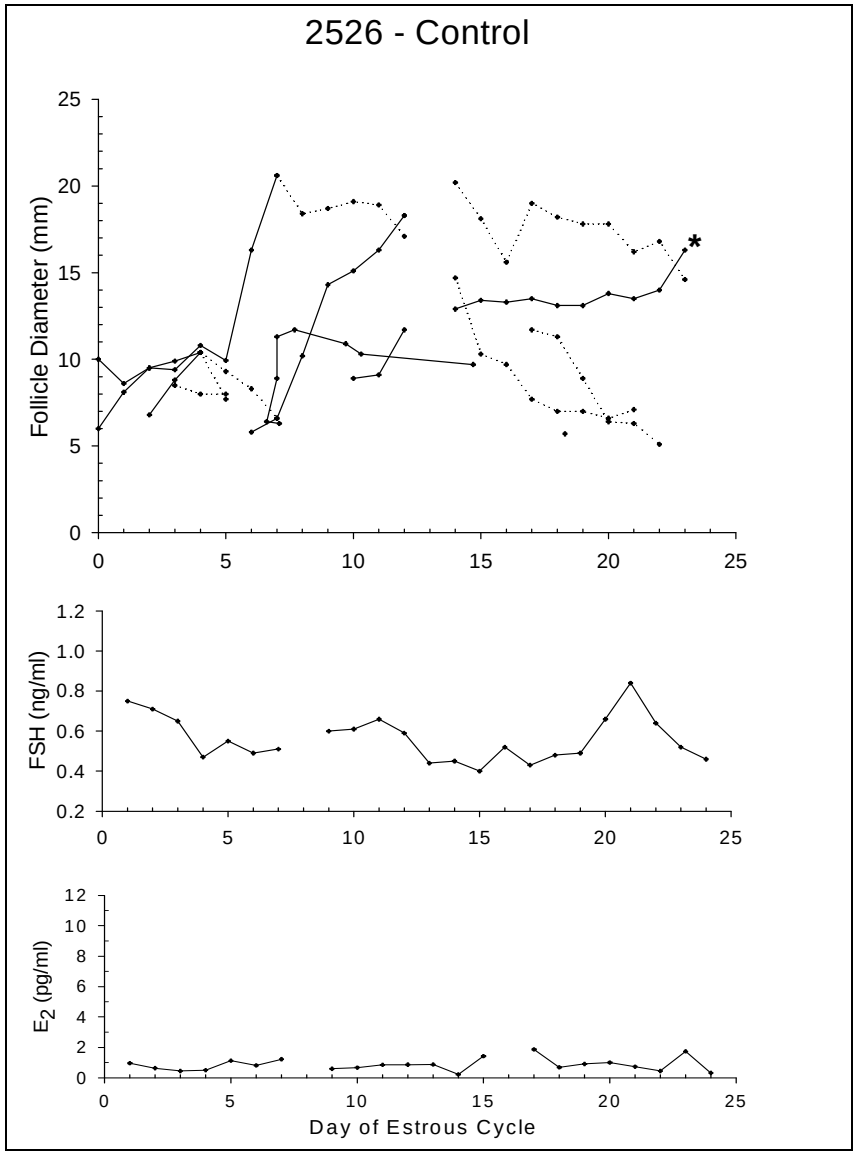
Your requested amendment to the animal care and use protocol shown above was reviewed and approved by the University of Idaho on Wednesday, November 16, 2011.

This protocol was originally submitted for review on: Monday, July 11, 2011
The original approval date for this protocol is: Tuesday, July 26, 2011
This approval will remain in affect until: Friday, November 16, 2012
The protocol may be continued by annual updates until: Saturday, July 26, 2014

Federal laws and guidelines require that institutional animal care and use committees review ongoing projects annually. For the first two years after initial approval of the protocol you will be asked to submit an annual update form describing any changes in procedures or personnel. The committee may, at its discretion, extend approval for the project in yearly increments until the third anniversary of the original approval of the project. At that time, the protocol must be replaced by an entirely new submission.

Brad Williams, DVM
Campus Veterinarian
University of Idaho
208-885-8958

Appendix B: Ovarian Follicular Waves and Hormone Profiles for Experiments 1 and 2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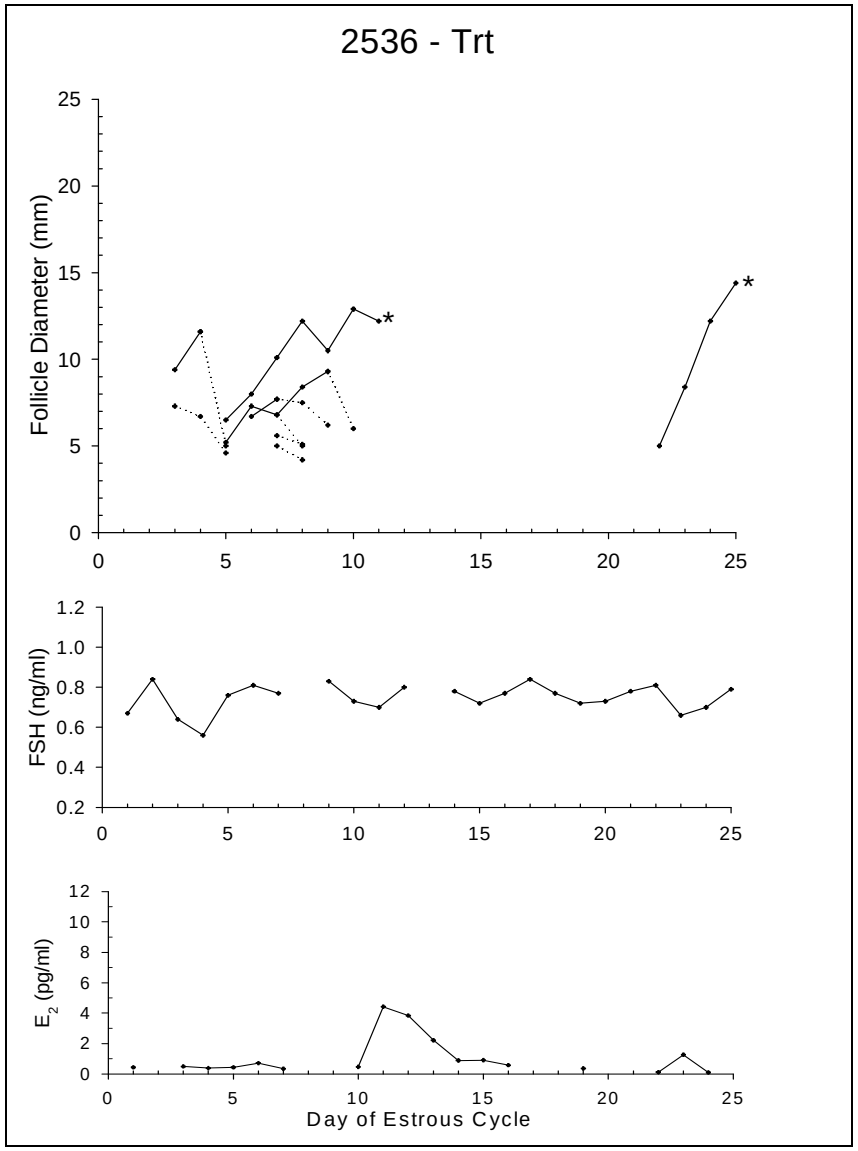
Follicle Diameter (mm)
FSH (ng/ml)
E2 (pg/ml)
Day of Estrous Cycle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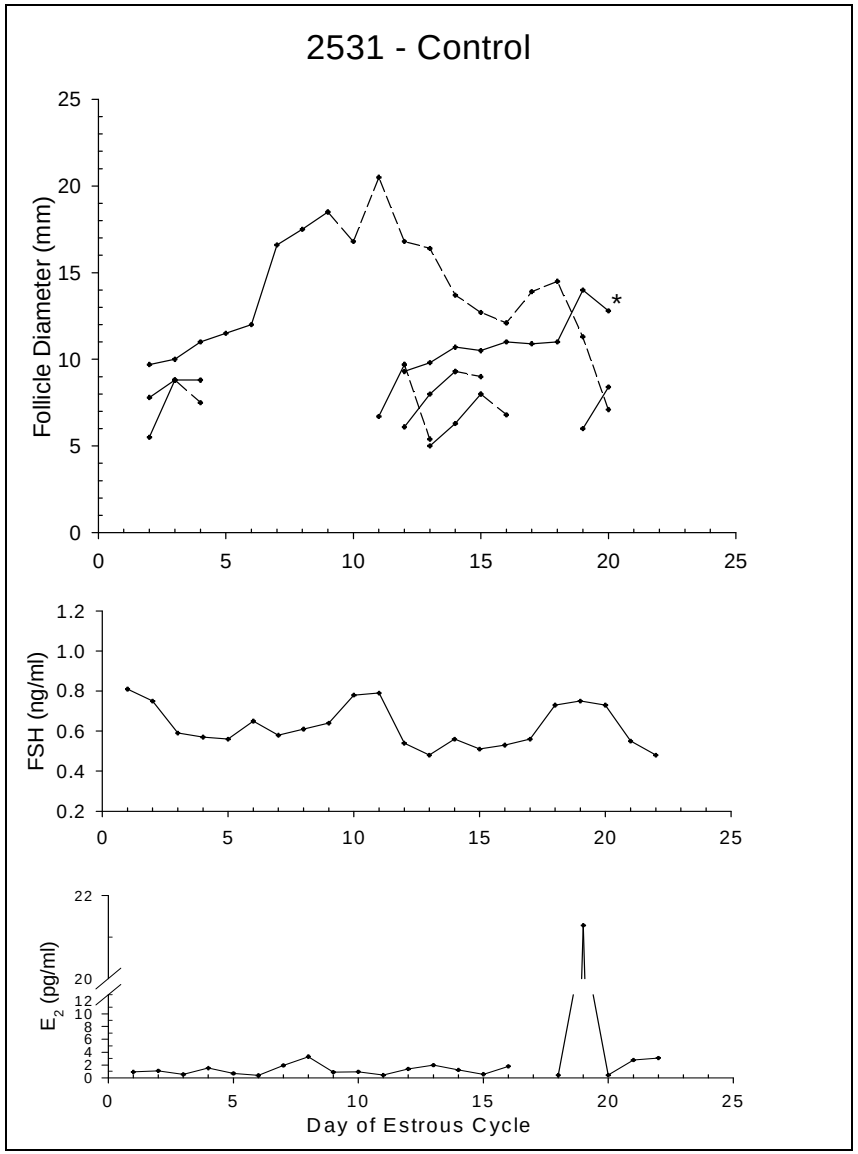
FSH (ng/ml)
E2 (pg/ml)
Day of Estrous Cycle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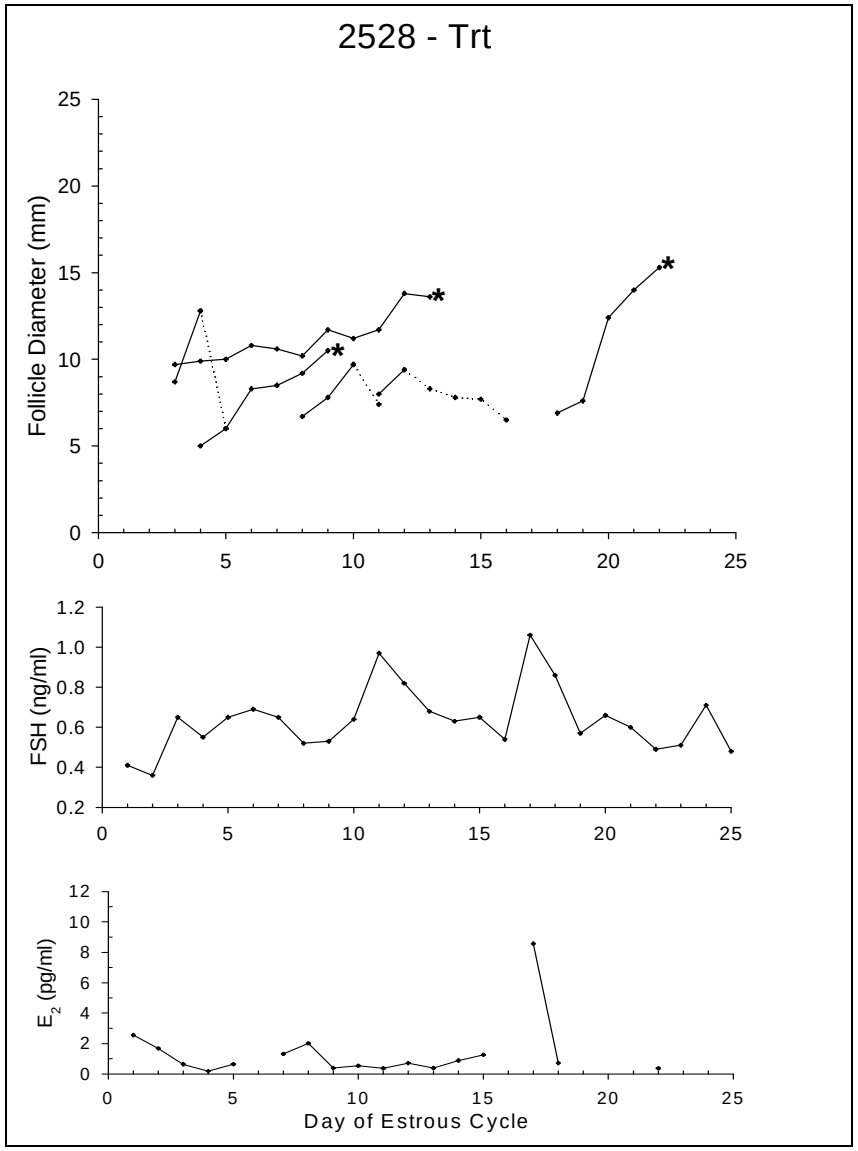
FSH (ng/ml)
E2 (pg/ml)
Day of Estrous Cycle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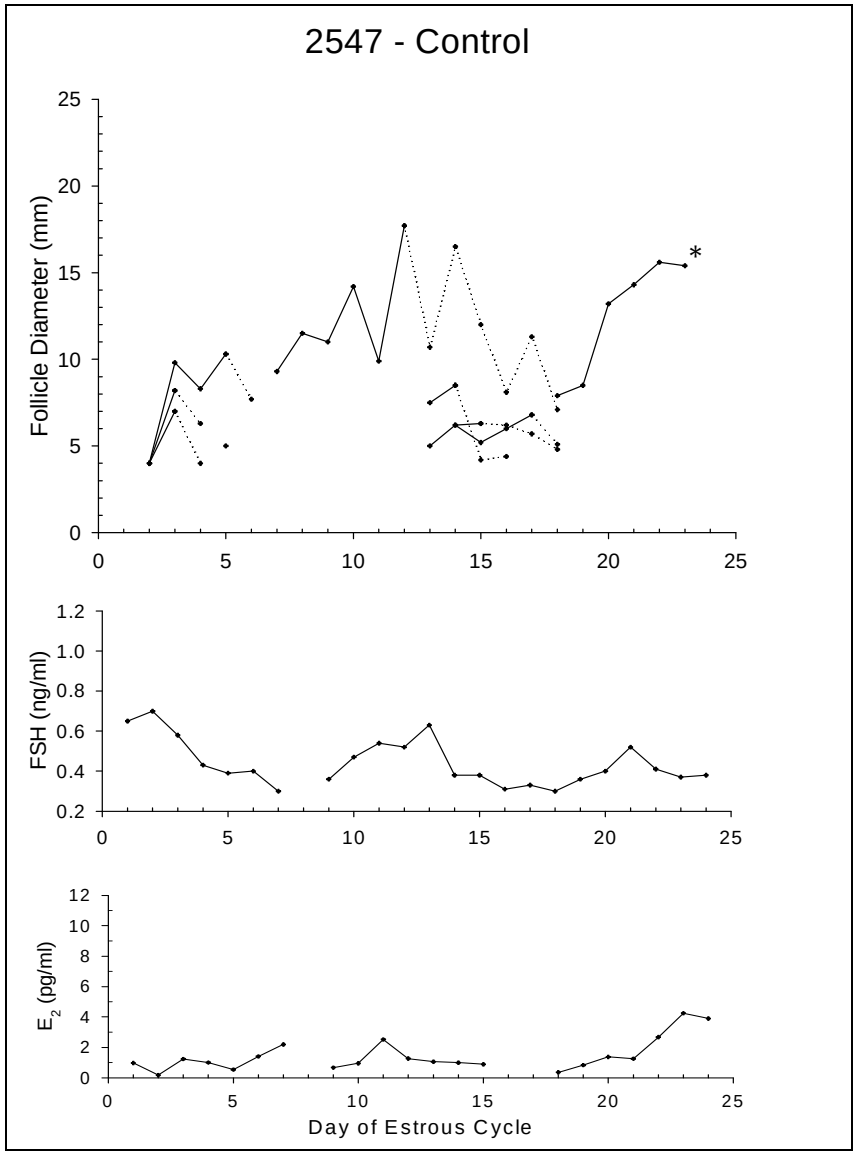
Follicle Diameter (mm)
FSH (ng/ml)
E2 (pg/ml)
Day of Estrous Cycle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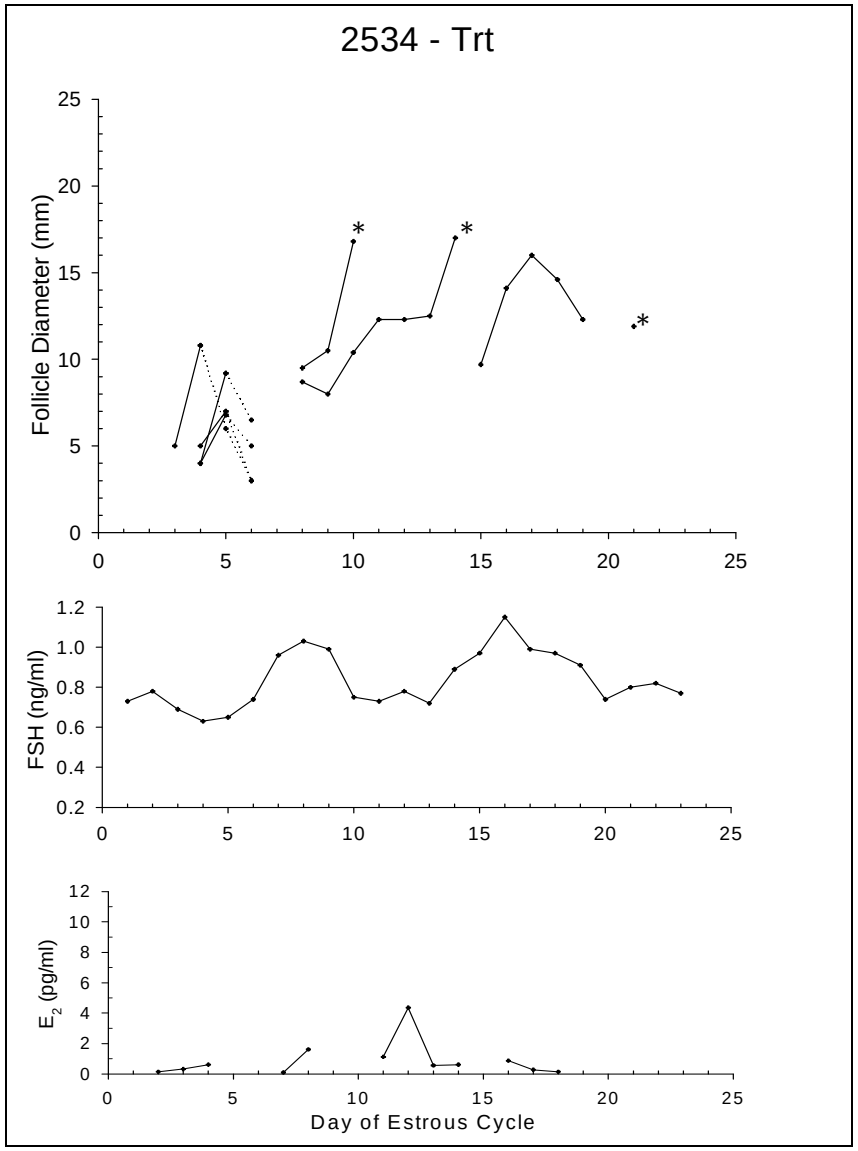
Follicle Diameter (mm)
FSH (ng/ml)
E2 (pg/ml)
Day of Estrous Cycle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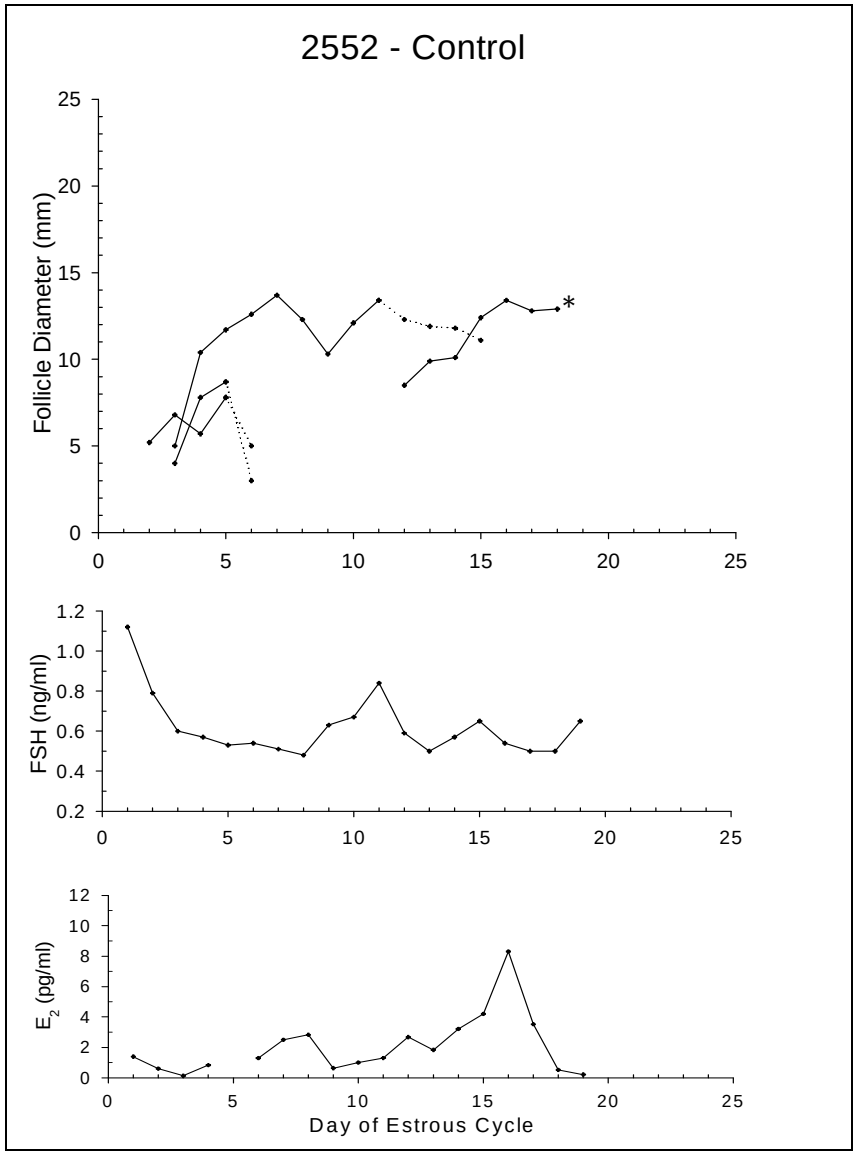
Follicle Diameter (mm)
FSH (ng/ml)
E2 (pg/ml)
Day of Estrous Cycle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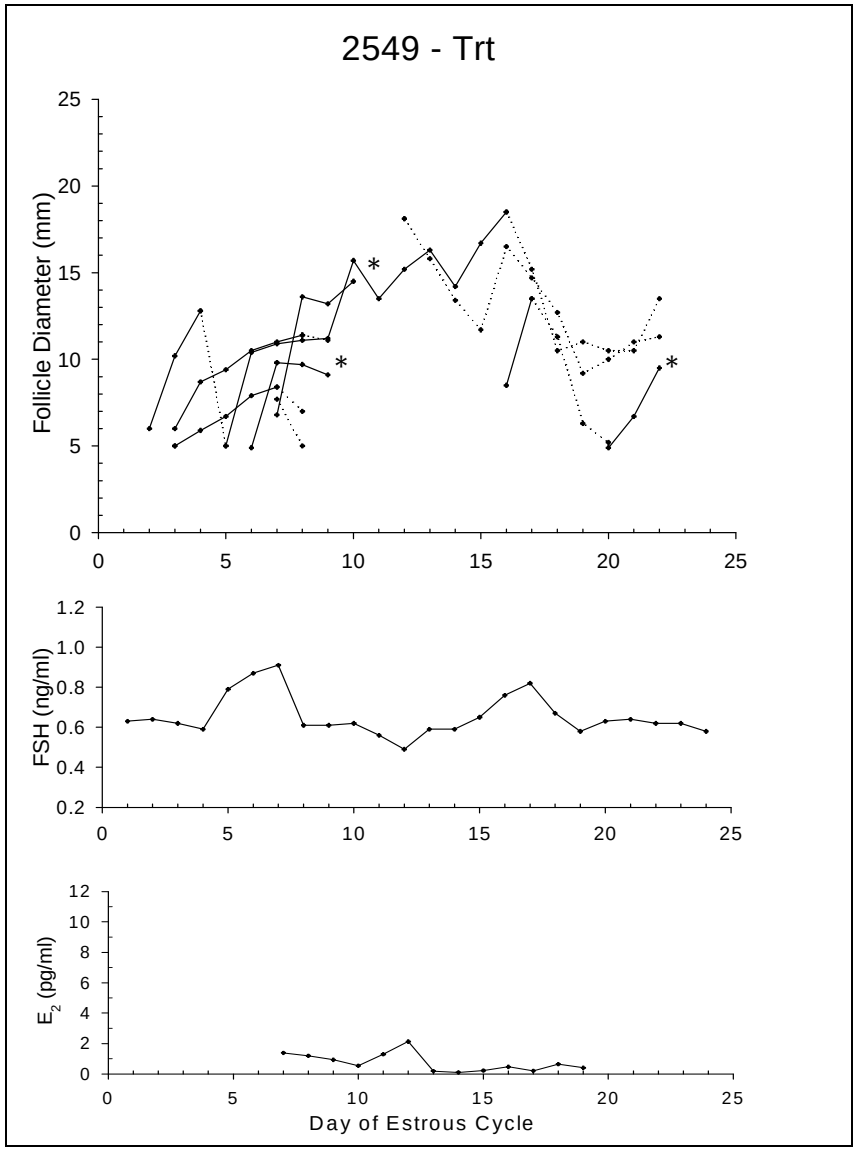
Follicle Diameter (mm)
FSH (ng/ml)
E2 (pg/ml)
Day of Estrous Cycle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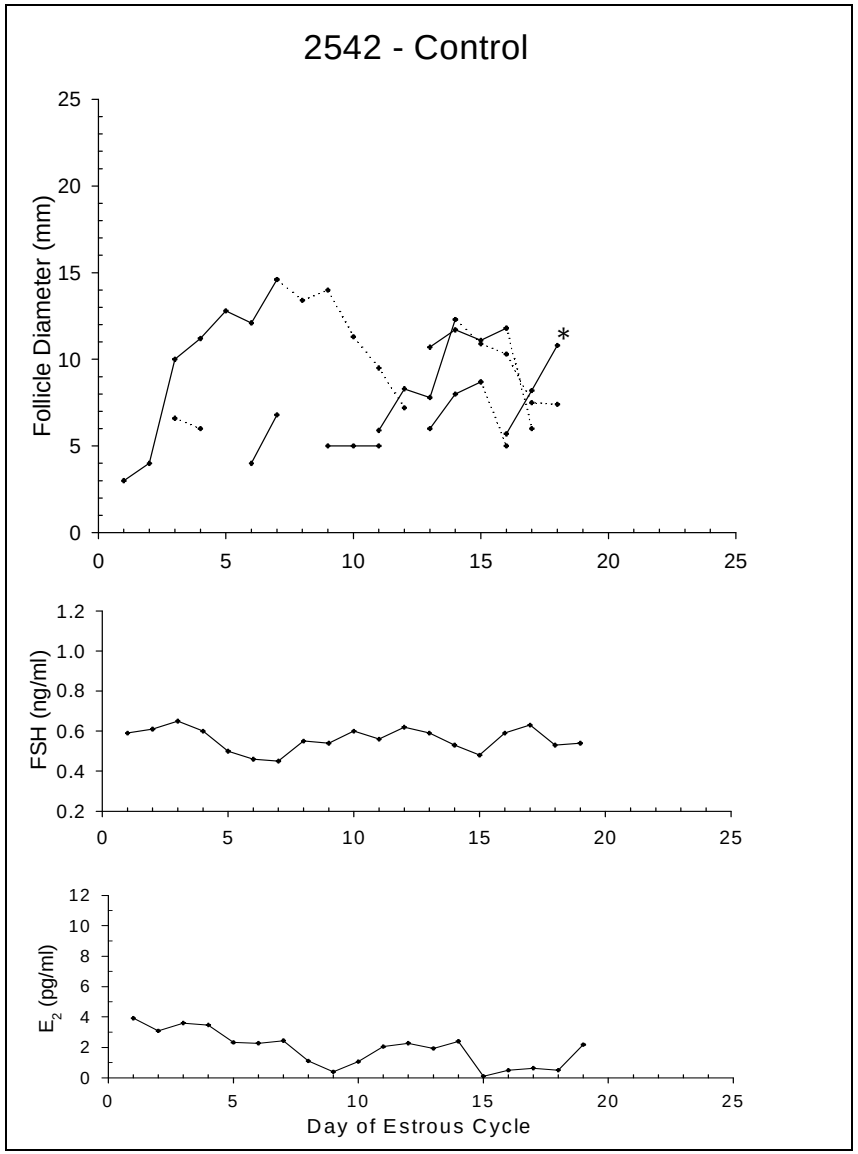
FSH (ng/ml)
E2 (pg/ml)
Day of Estrous Cycle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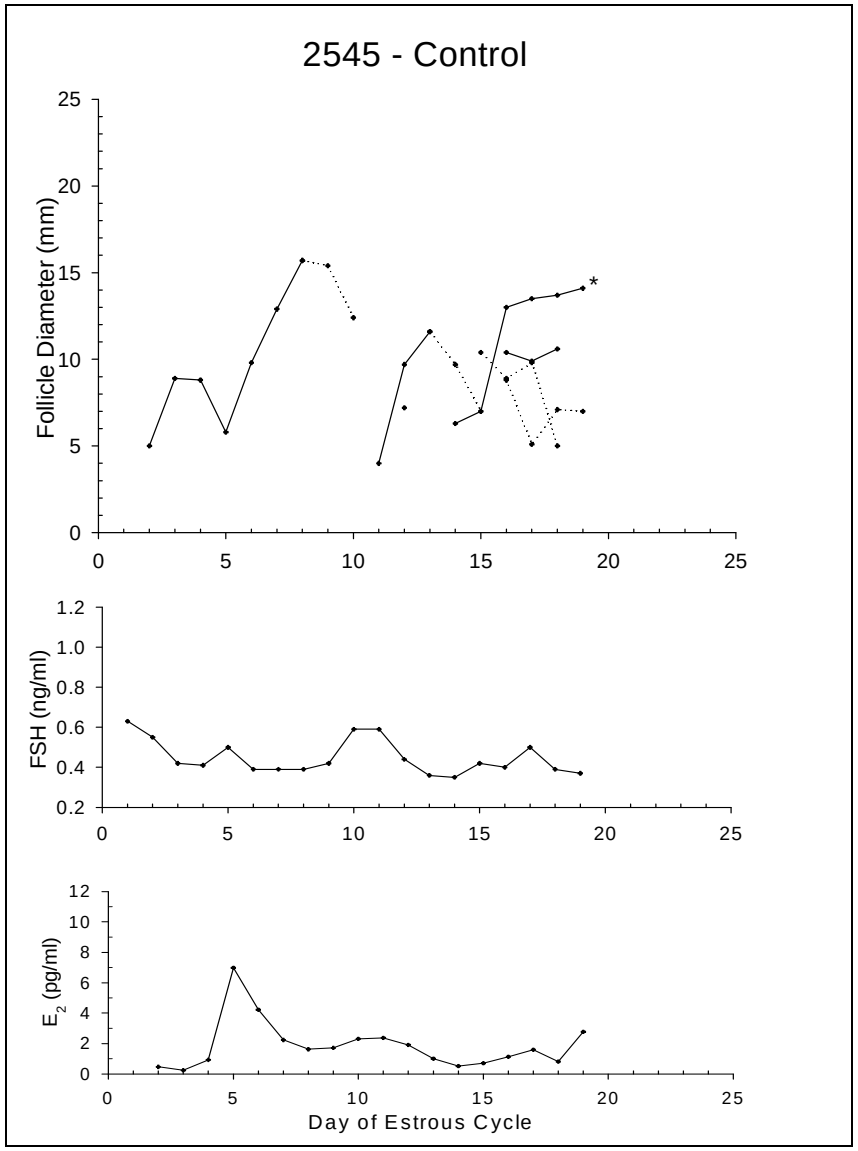
FSH (ng/ml)
E2 (pg/ml)
Day of Estrous Cycle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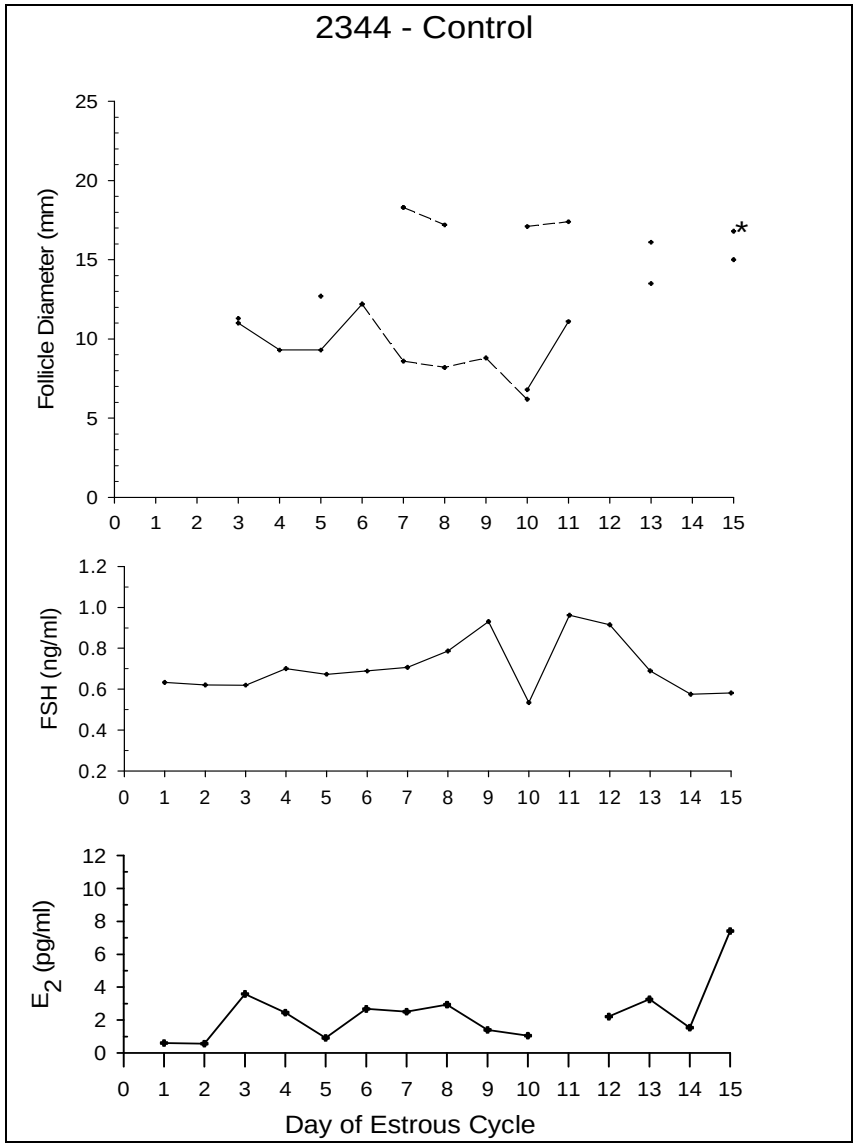
Follicle Diameter (mm)
FSH (ng/ml)
E2 (pg/ml)
Day of Estrous Cycle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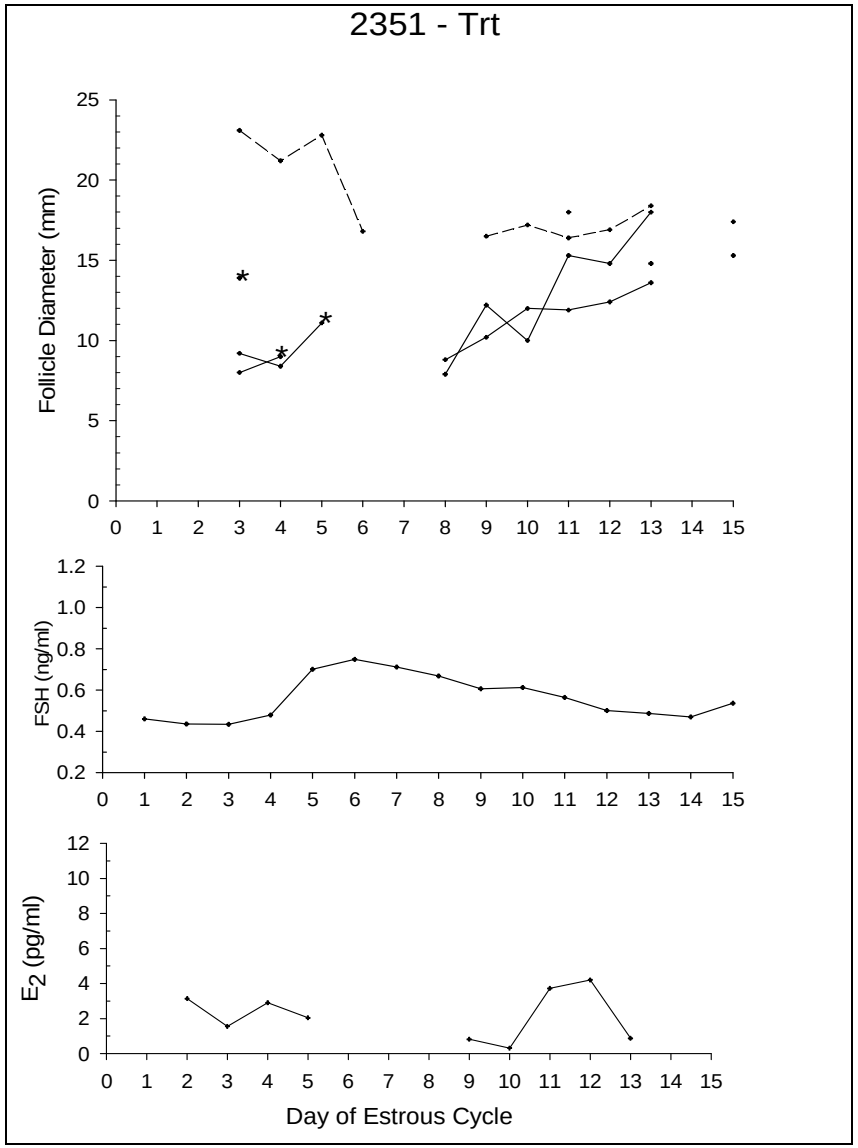
FSH (ng/ml)
E2 (pg/ml)
Day of Estrous Cycle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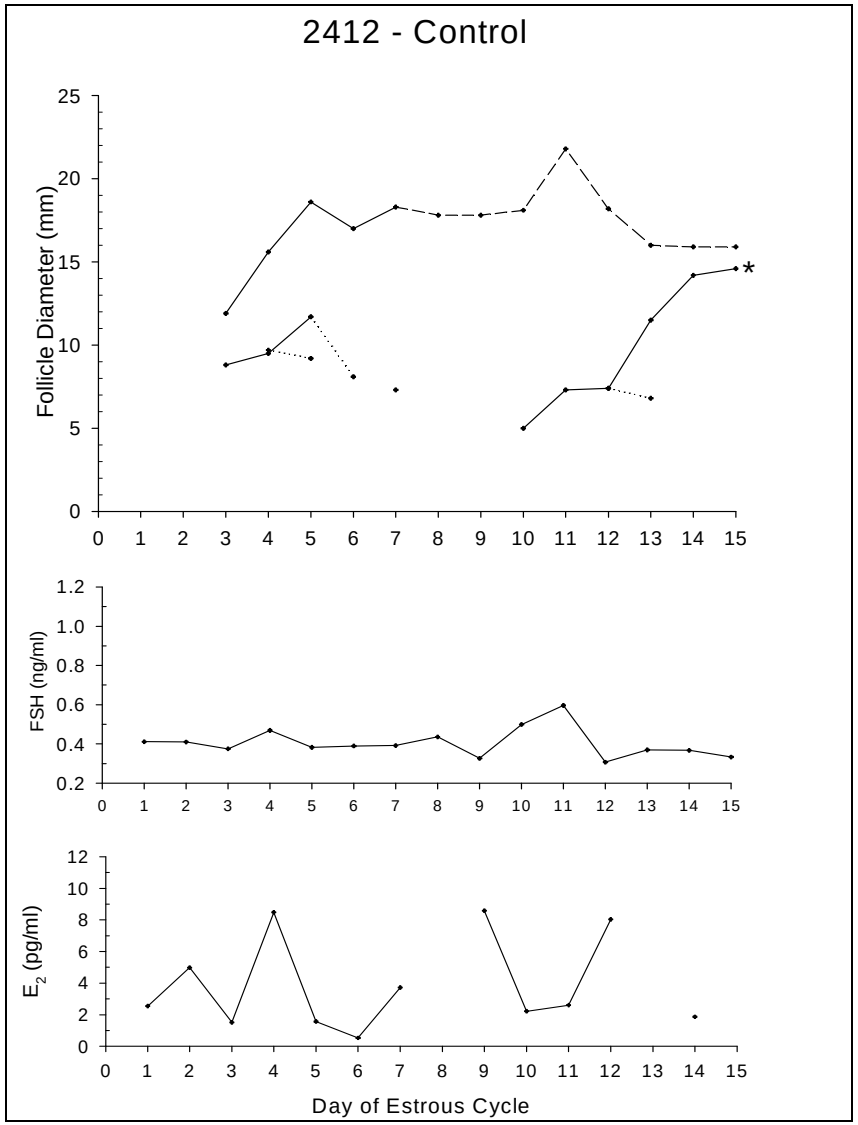
Follicle Diameter (mm)
FSH (ng/ml)
E2 (pg/ml)
Day of Estrous Cycle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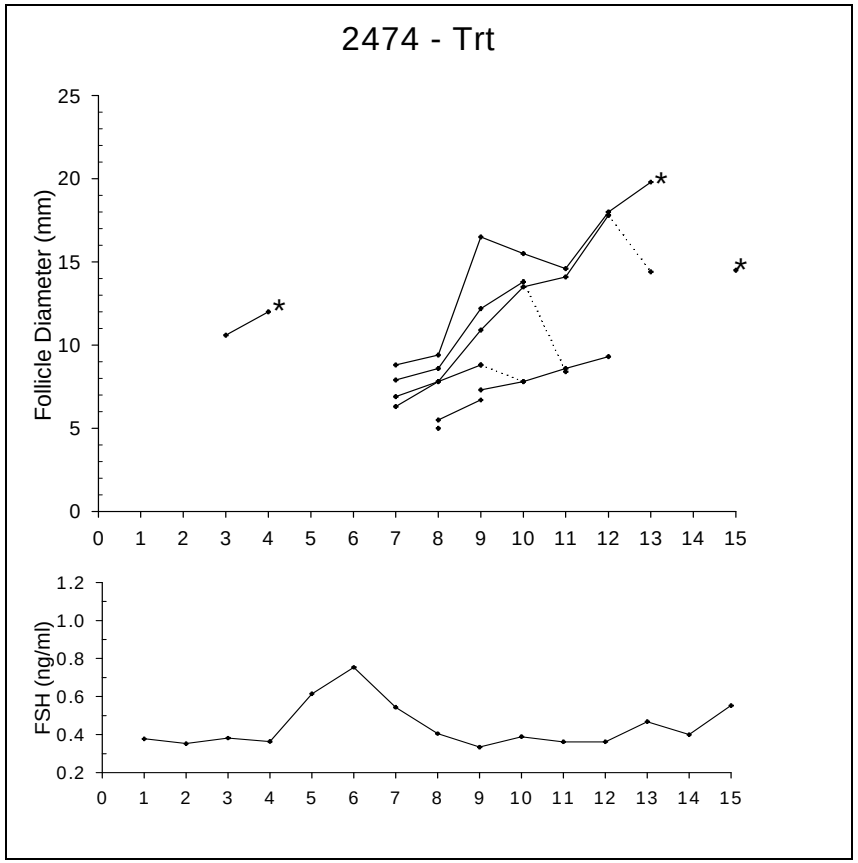
FSH (ng/ml)
E_2 (pg/ml)
Day of Estrous Cycle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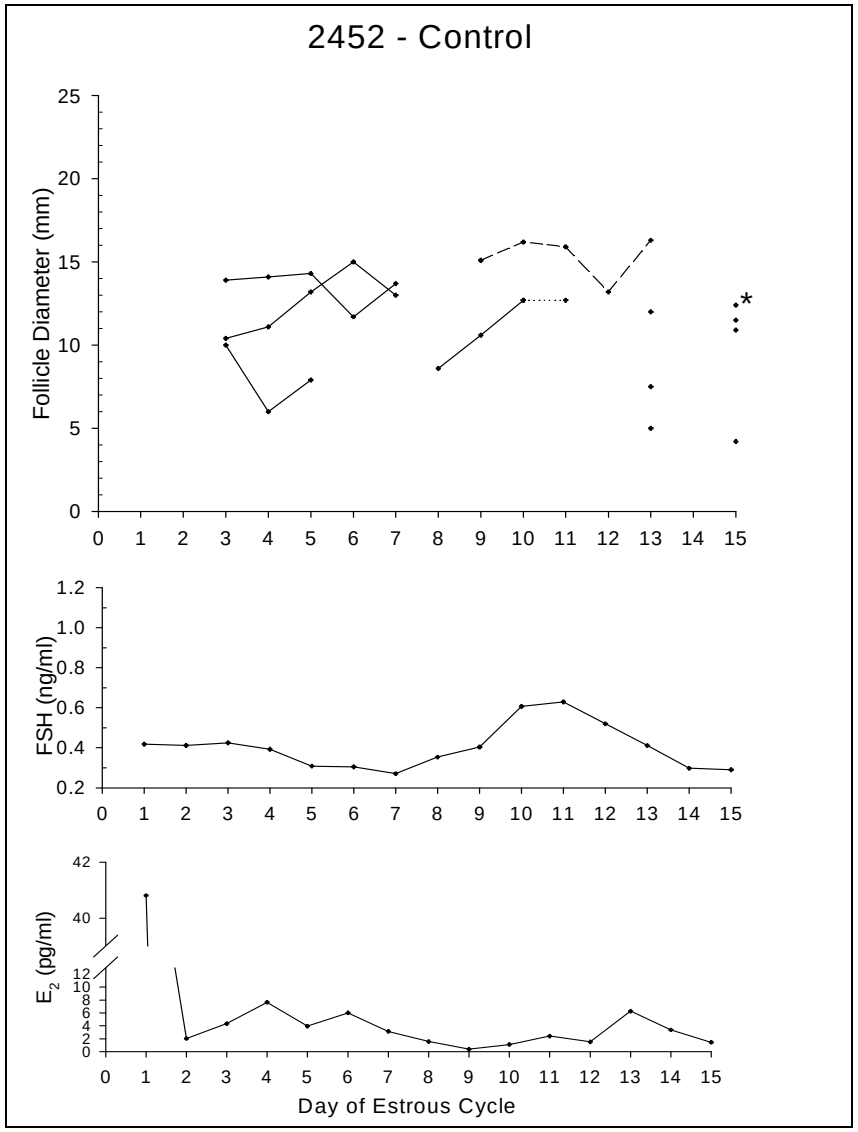
Follicle Diameter (mm)
FSH (ng/ml)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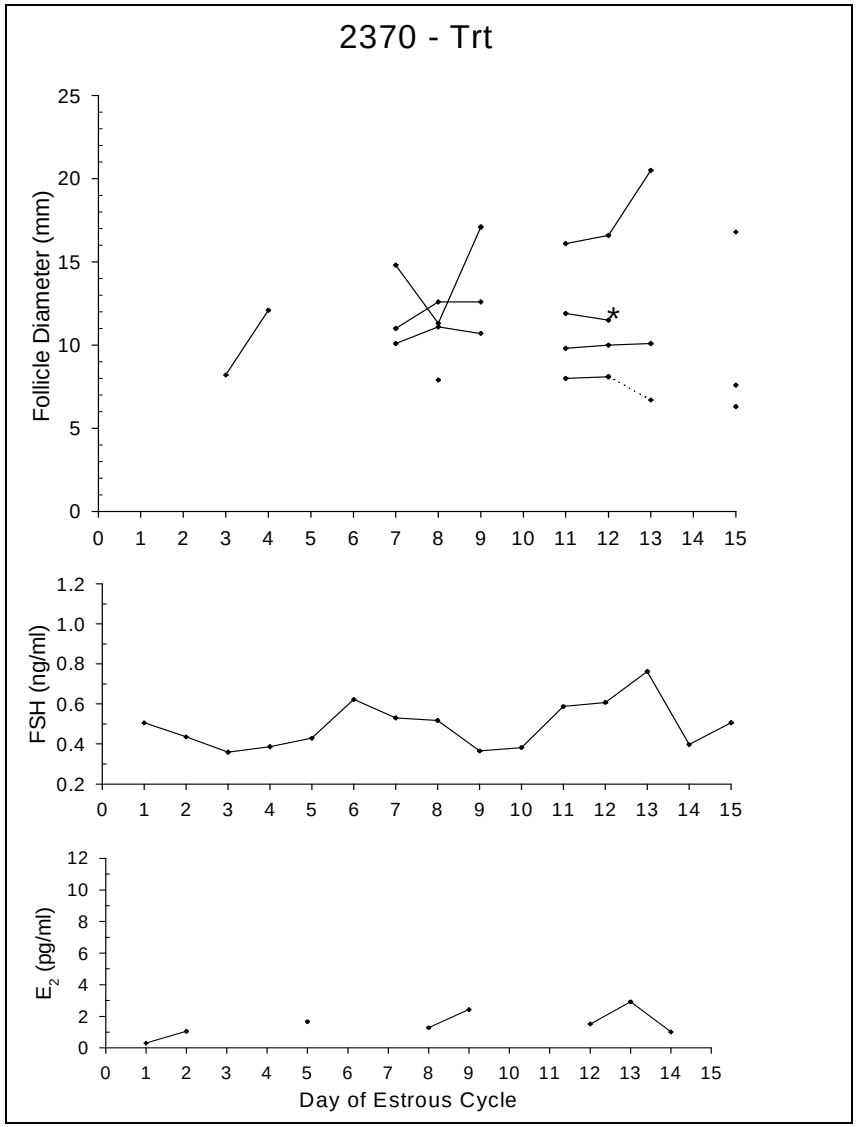
Follicle Diameter (mm)
FSH (ng/ml)
E2 (pg/ml)
Day of Estrous Cycle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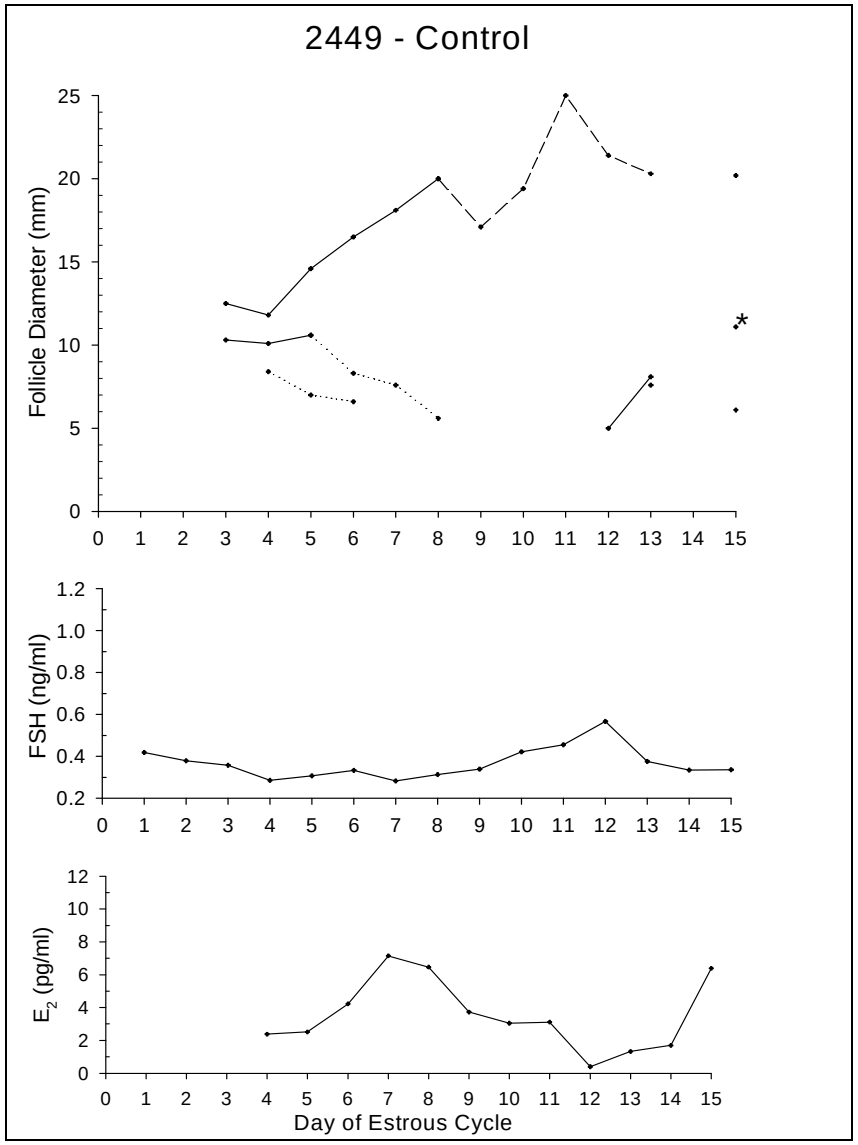
FSH (ng/ml)
E2 (pg/ml)
Day of Estrous Cycle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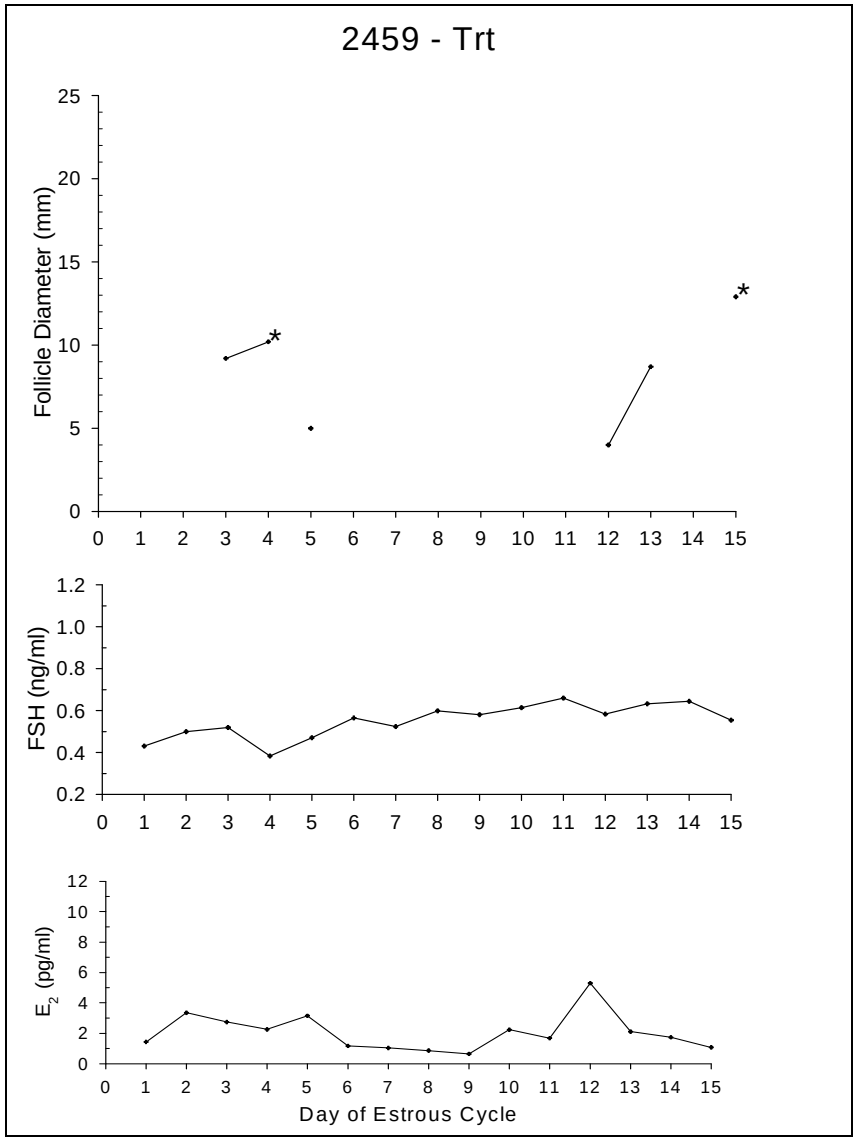
Follicle Diameter (mm)
FSH (ng/ml)
E2 (pg/ml)
Day of Estrous Cycle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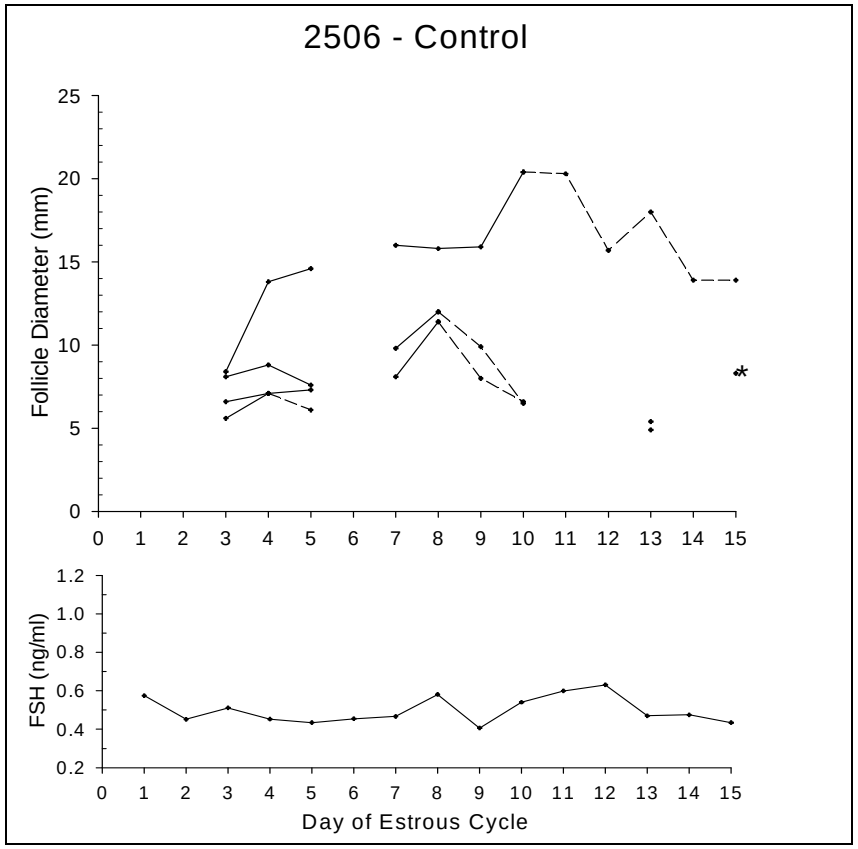
FSH (ng/ml)
E2 (pg/ml)
Day of Estrous Cycle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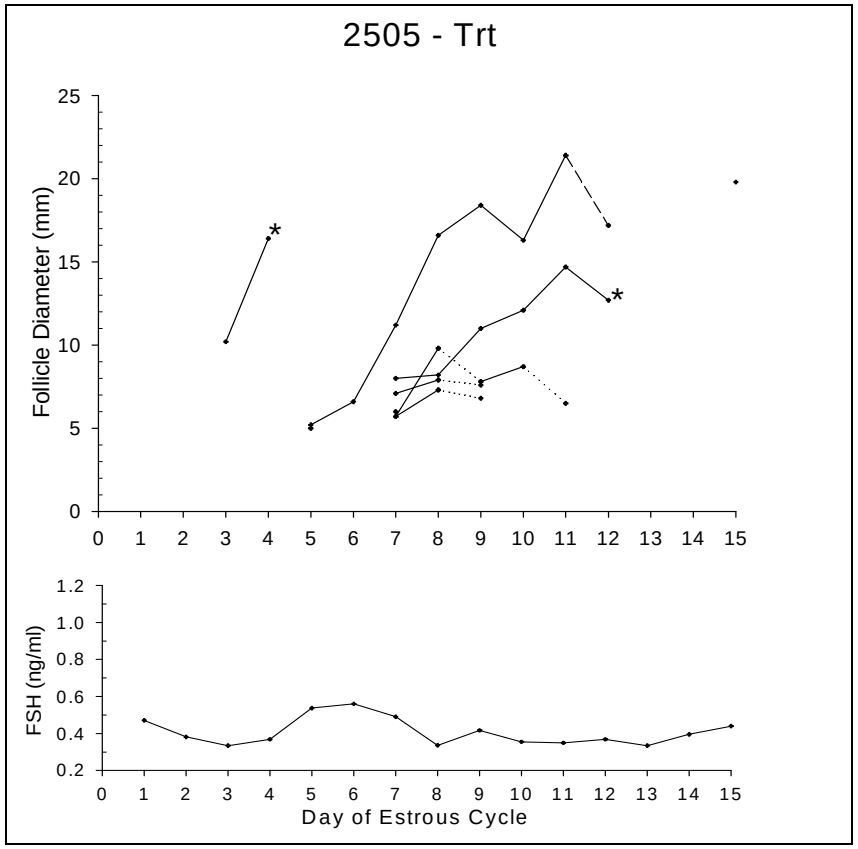
Follicle Diameter (mm)
FSH (ng/ml)
Day of Estrous Cycle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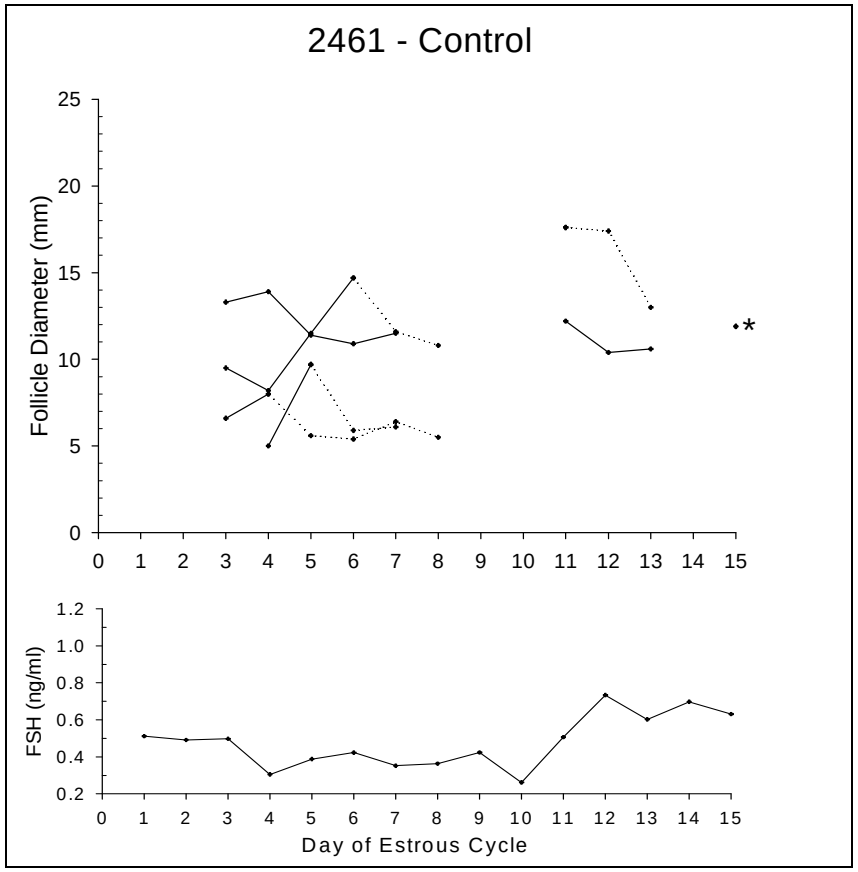
Follicle Diameter (mm)
FSH (ng/ml)
Day of Estrous Cycle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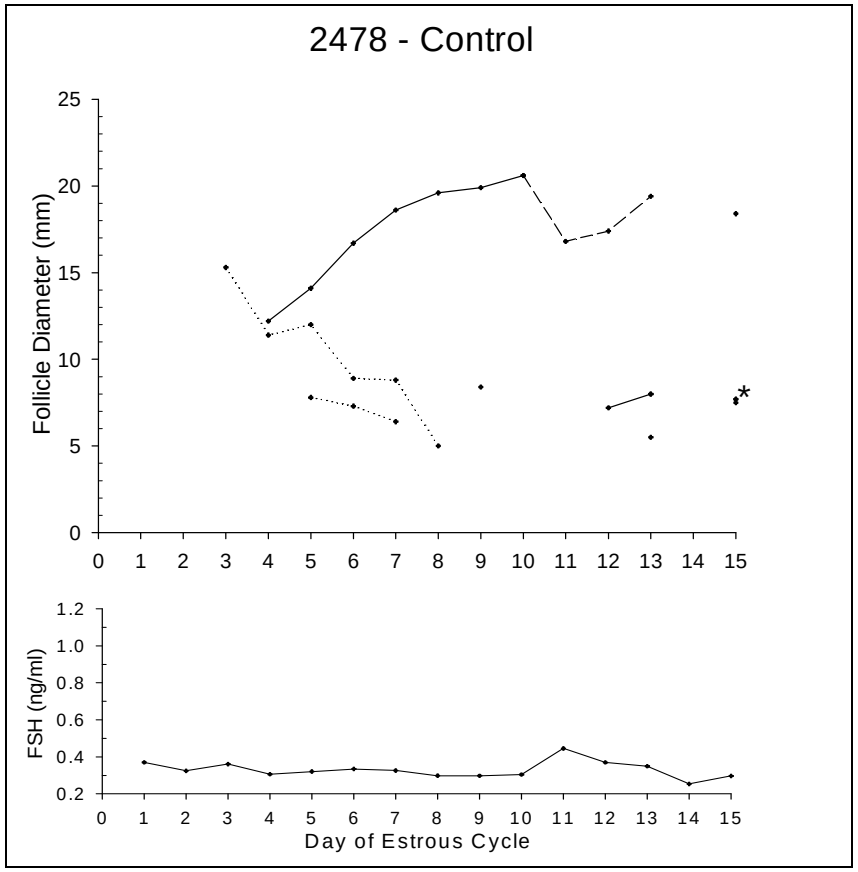
Follicle Diameter (mm)
FSH (ng/ml)
Day of Estrous Cycle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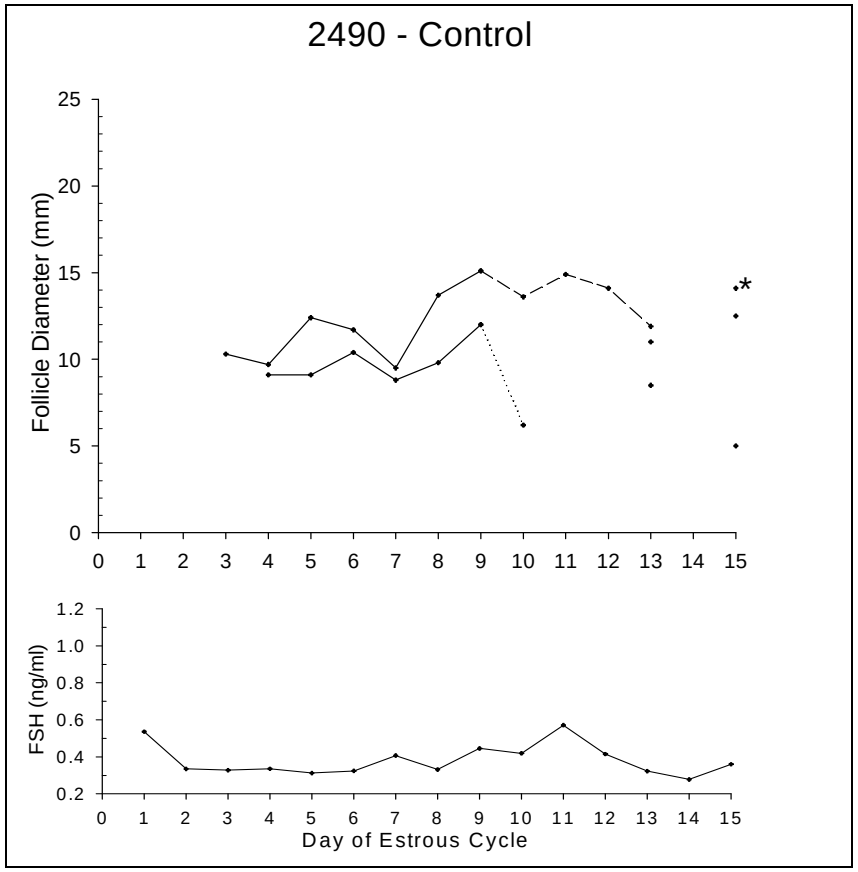
FSH (ng/ml)
Day of Estrous Cycle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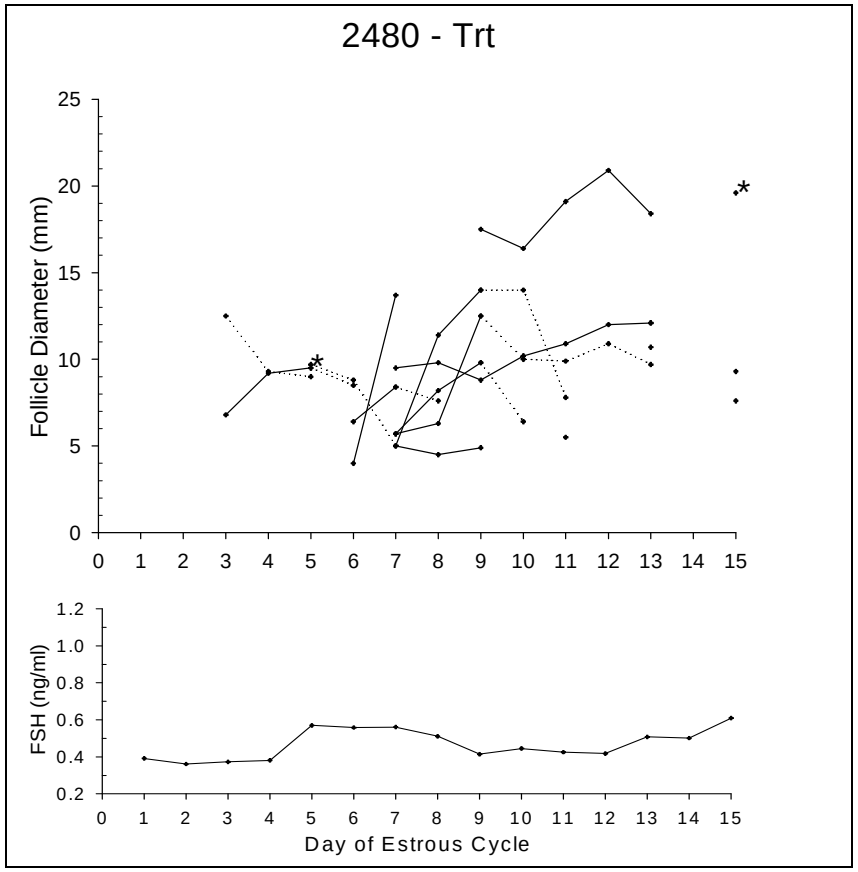
Follicle Diameter (mm)
FSH (ng/ml)
Day of Estrous Cycle
*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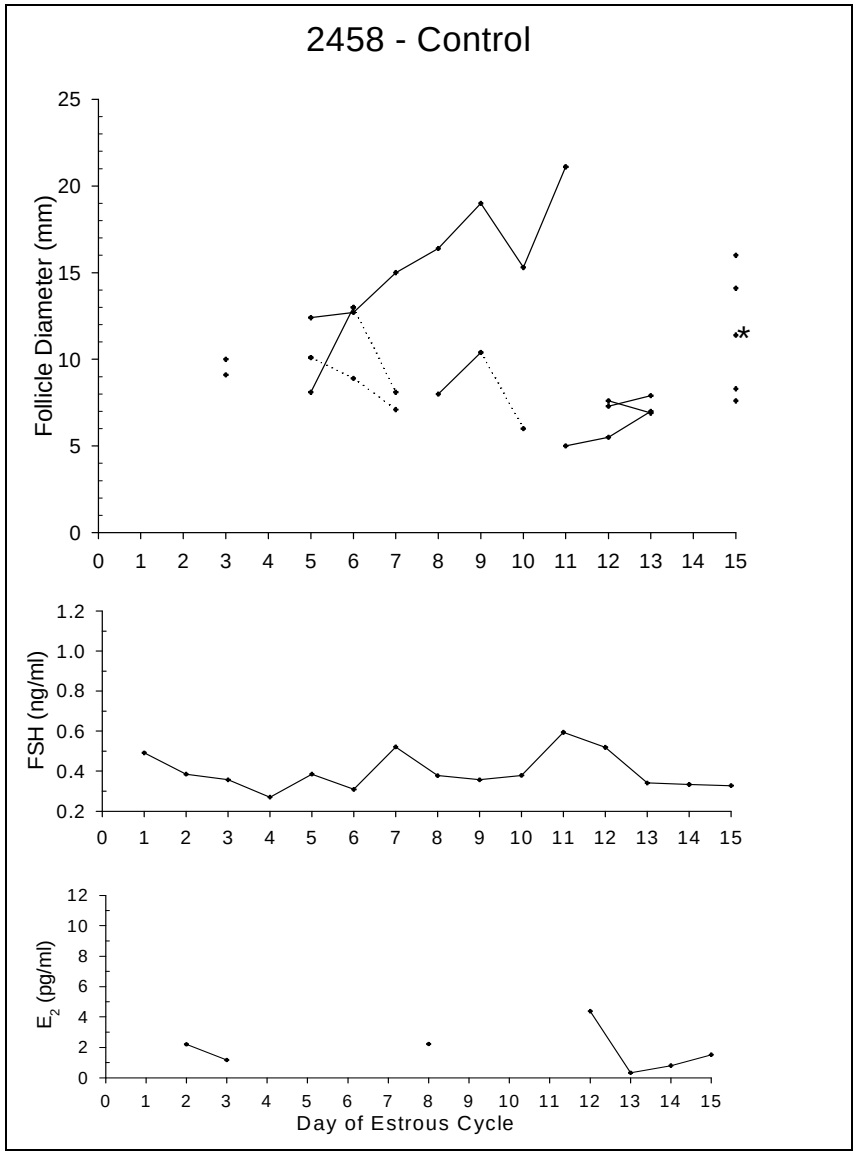
FSH (ng/ml)
Day of Estrous Cycle
*
*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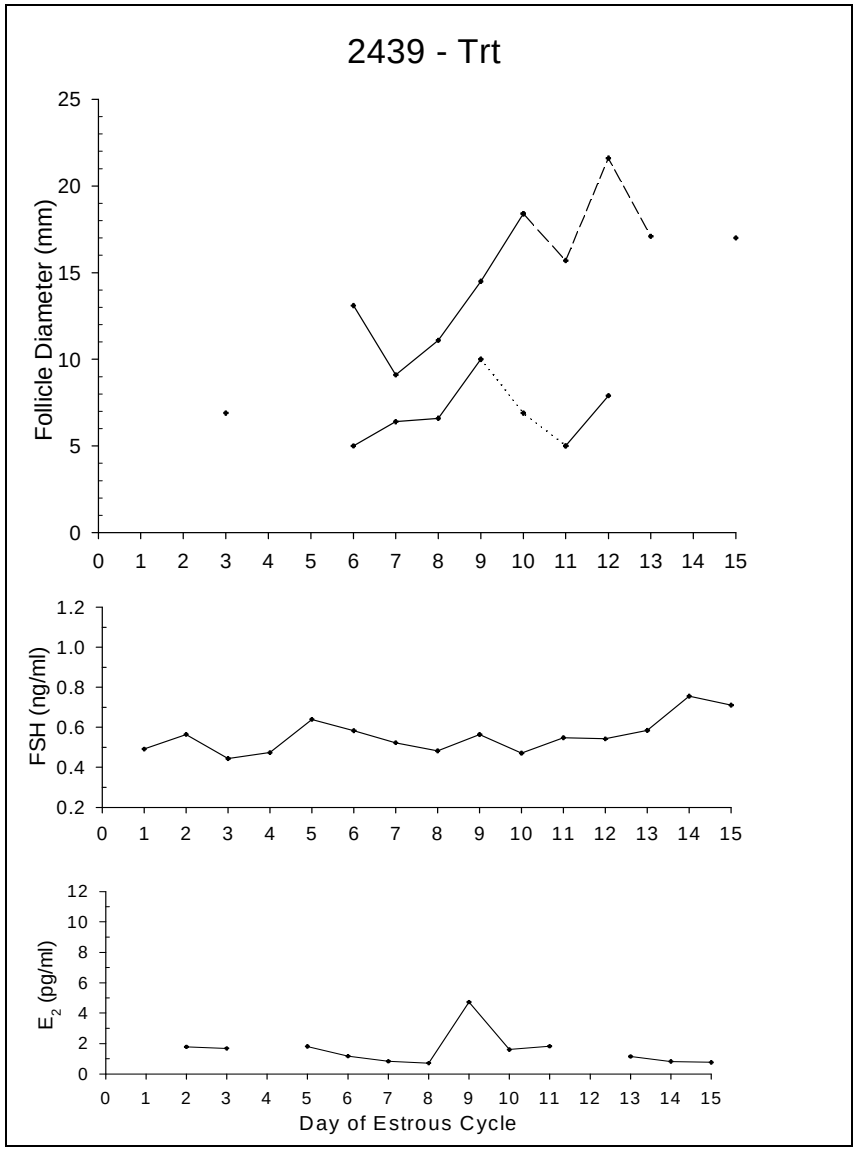
Follicle Diameter (mm)
FSH (ng/ml)
E2 (pg/ml)
Day of Estrous Cycle


2439 - Trt
Follicle Diameter (mm)
FSH (ng/ml)
E2 (pg/ml)
Day of Estrous Cycle

Appendix C: Table A.1 Number of animals in experiments 1 and 2 with 2 or 3 ovarian follicular waves

Table A.1 Number of animals in experiments 1 and 2 with 2 or 3 ovarian follicular waves

Parity	2 Ovarian Follicular Waves	3 Ovarian Follicular Waves
Primiparous		
CONT (n=6)	4	2
TRT (n=4)	2	2
Multiparous		
CONT (n=9)	5	4
TRT (n=7)	4	3

Appendix D: Table A.2 Number of follicles per follicular wave based on size for experiments 1 and 2

Table A.2 Number of follicles per follicular wave based on size for experiments 1 and 2

	1st Follicular Wave			2nd Follicular Wave			3rd Follicular Wave		
Parity	5-9 mm±SEM	10-15 mm±SEM	>15 mm±SEM	5-9 mm±SEM	10-15 mm±SEM	>15 mm±SEM	5-9 mm±SEM	10-15 mm±SEM	>15 mm±SEM
Primiparous									
CONT (n=6)	7	4	3	3	11	3	5	3	1
TRT (n=4)	5	9	0	7	5	4	0	2	1
Multiparous									
CONT (n=9)	8	12	9	8	5	5	7	1	1
TRT (n=7)	18	9	6	22	12	4	3	2	1

Appendix E: Table A.3 Average number of follicles on CL-bearing and non-CL-bearing ovary throughout the estrous cycle

Table A.3 Average number of follicles on CL-bearing and non-CL-bearing ovary throughout the estrous cycle

Parity	CL-bearing ovary Number of follicles ± SEM	Non-CL-bearing ovary Number of follicles ± SEM
Primiparous		
CONT (n=6)	3.3	3.3
TRT (n=4)	4.7	3.5
Multiparous		
CONT (n=9)	3.2	4.2
TRT (n=7)	6.3	4.1

Appendix F: Effects of Chronic Administration of Deslorelin Treatment on Concentrations of P_4 in Milk in Primiparous Cows

Figure A.1

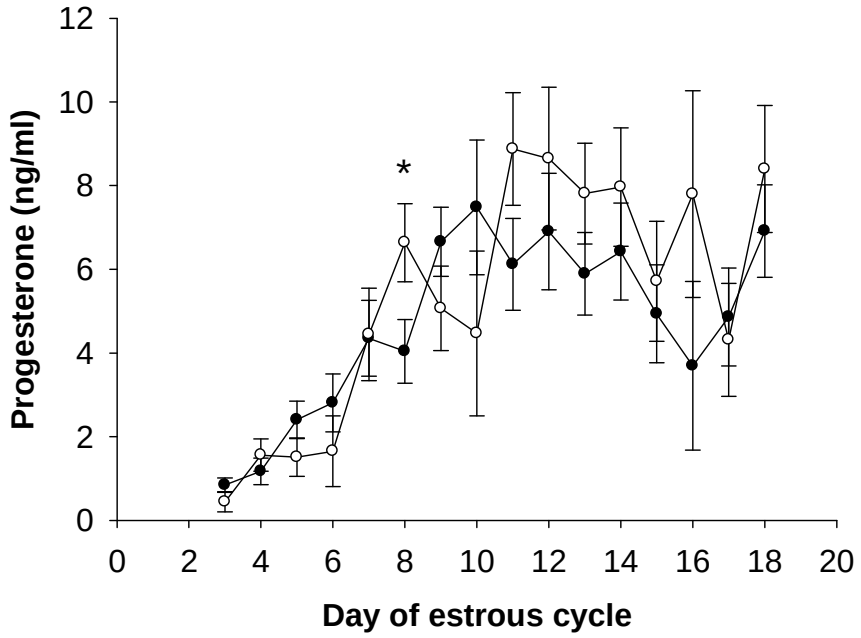


Figure A.1: Effects of chronic administration of Deslorelin treatment on concentrations of P_4 in milk in primiparous cows. Cows were left untreated (n = 6; closed circles) or treated with Deslorelin for 12 days beginning day 3 (n = 4; open circles). Data are represented as mean ± SEM. *P = 0.03

Appendix G: Effects of Chronic Administration of Deslorelin Treatment on Concentrations of E_2 in Milk in Primiparous Cows

Figure A.2

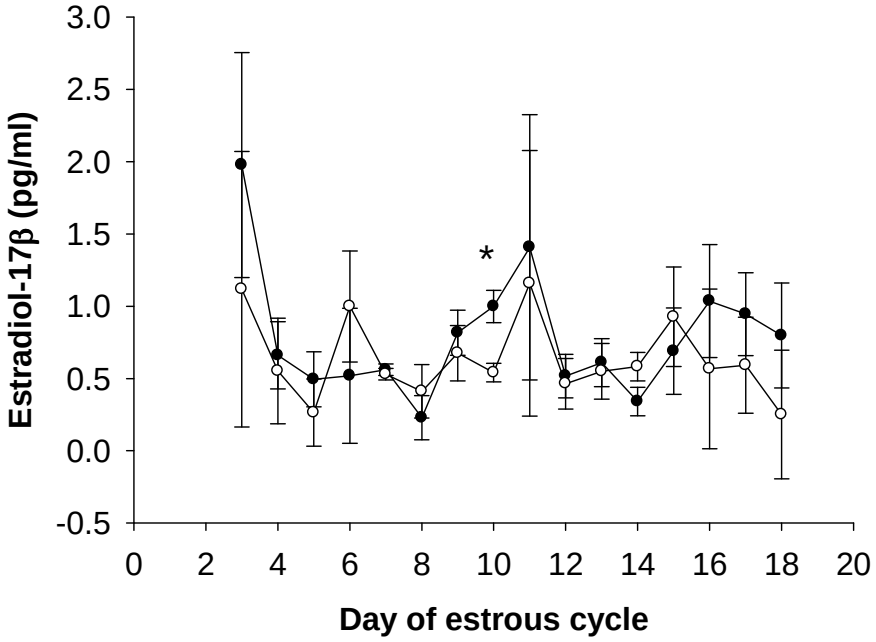


Figure A.2: Effects of chronic administration of Deslorelin treatment on concentrations of E_2 in milk in primiparous cows. Cows were left untreated (n = 6; closed circles) or treated with Deslorelin for 12 days beginning day 3 (n = 4; open circles). Data are represented as mean ± SEM. *P < 0.05