

DYNAMICS OF LUTEOLYSIS IN LACTATING DAIRY COWS USING TWO
PROSTAGLANDIN F_{2α} ANALOGUES

By

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ABSTRACT

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The probability of a pregnancy decreases substantially in lactating dairy cows treated with Ovsynch if corpus luteum regression is delayed or incomplete. There are two PGF_{2α} products approved in the USA for luteolysis, dinoprost tromethamine and cloprostenol sodium. Cloprostenol has a longer half-life compared to dinoprost because it is more resistant to endogenous metabolism. In this thesis, we hypothesized that cloprostenol would reduce the time to complete luteolysis and increase conception rates compared to dinoprost because of differences in half-life. In the studies presented in Chapter 2, the decline in circulating P₄ was accelerated between 0 and 12 h post-injection for cows treated with cloprostenol vs. dinoprost, but not at 24, 36 and 48 h. Serum concentrations of E₂ were greater in cloprostenol vs. dinoprost treated cows 48 h following treatment. In studies presented in Chapter 3, there was no difference in conception rates, rate of luteolysis or concentrations of estradiol between cows treated with cloprostenol vs. dinoprost. Serum concentrations of progesterone at time of PGF_{2α} was positively related with predicted probability of a pregnancy and with predicted probability of luteolysis. In summary, cloprostenol induced a greater decrease in P₄ for the first 12 h following treatment and subsequently a greater increase in E₂ compared to dinoprost, although there were no differences in these two products in conception rates, % of cows with complete luteolysis, and pregnancy loss.

I dedicate this thesis to Ayra C. K. de Almeida,
for her support, friendship, comprehension and love at all moments.

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LIST OF ABBREVIATIONS

AI	artificial insemination
CL	corpus luteum/corpora lutea
CR	conception rate
CV	coefficient(s) of variation
d	day (s)
DF	dominant follicle(s)
DIM	days in milk
E₂	estradiol
FSH	follicle stimulating hormone
GnRH	gonadotropin releasing hormone
h	hour (s)
LH	luteinizing hormone
ng	nanogram
pg	picogram
P₄	progesterone
PGF_{2α}	prostaglandin F _{2α}
R²	coefficient of determination
RIA	radioimmunoassay
TMR	total mixed ration

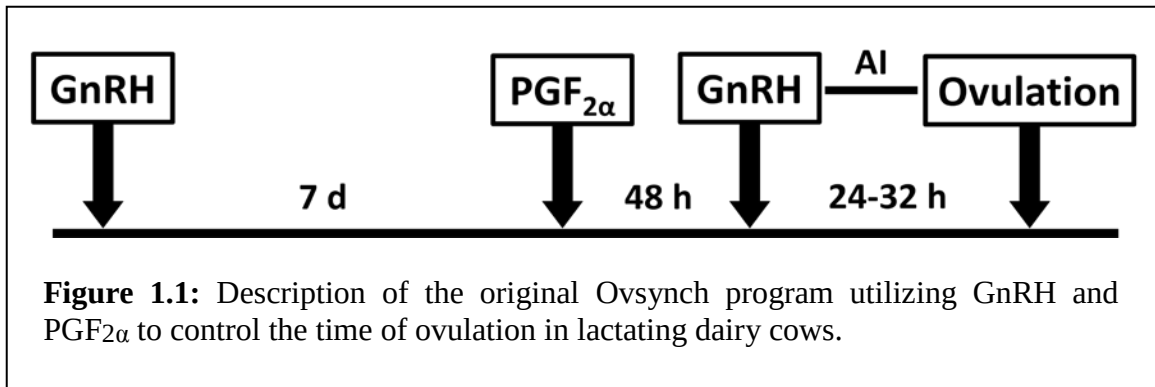
CHAPTER 1

INTRODUCTION

IMPORTANCE OF REPRODUCTIVE MANAGEMENT ON DAIRY FARMS

As milk production per cow continues to increase, infertility of the lactating dairy cow continues to be a critical problem limiting profitability and sustainability of U.S. dairy farms (Lucy, 2001; Washburn et al., 2002). Therefore, new methods to improve reproductive management of dairy cows are key to the optimization of reproductive performance and enhanced profitability of dairy operations (Lucy et al., 2004; Bello et al.; 2006). Reproductive management of dairy cows is a key profit center on dairy farms and is driven by management decisions, compliance of standard operating procedures, and fertility parameters of cows (Stevenson, 2001; Caraviello et al.; 2006; Olynk and Wolf; 2008). Reproductive performance of lactating dairy cows is dependent upon three factors: service rate (or estrus detection rate), fertility of the service sire, and maternal fertility. Service rate can be controlled utilizing Ovsynch technology (Figure 1.1; Pursley et al.; 1995; Pursley et al., 1997a). Ovsynch allows producers to regulate time to 1st and subsequent artificial inseminations (AI). High fertility sires can be chosen utilizing the new USDA-ARS sire conception rate summaries. However, maternal fertility, defined as the mother's ability to ovulate a competent oocyte and provide an oviductal and uterine environment capable of fertilization and complete embryonic and fetal development, continues to be the key limiting factor for profitable reproductive performance in lactating dairy cows (Lucy, 2001; Washburn et al., 2002). While Ovsynch technology allows for greater control of service rate, conception rates of lactating cows are still approximately 35% compared to approximately 60% in virgin dairy heifers (Pursley et al., 1997b; Roth et al., 2008). Increasing conception rates of lactating cows to that of heifers would allow producers to employ the most profitable calving interval strategies

for cows with varying production capabilities, and increase profit. However, previous studies have not reported variations of Ovsynch that can enhance aspects of maternal fertility while continuing to control service rate. Increasing the percentage of cows that have complete luteolysis after $\text{PGF}_{2\alpha}$ of Ovsynch is one of the aspects of this protocol that could be improved in order to increase conception rates of the Ovsynch protocol (Souza et al., 2007; Brusveen et al., 2009). This thesis will explore the importance of induced-luteolysis with two $\text{PGF}_{2\alpha}$ analogues as it relates to Ovsynch technology and how this part of Ovsynch may be improved to enhance reproductive performance of dairy cattle.



DEFINITION OF LUTEOLYSIS IN CATTLE

Luteolysis is defined as loss of function of the corpus luteum (CL) and its regression or involution which terminates the estrous cycle of cows (McCracken et al., 1999; Niswender et al., 2000). Luteolysis is characterized by two events. First, there is a decline of progesterone (P_4) secretion which is known as functional luteolysis. This event is followed by the structural or morphological luteolysis that is the loss of the cells that forms the corpus luteum and its gradual involution to form a corpus albicans (McCracken et al., 1999; Niswender et al., 2000). Natural luteolysis is initiated by intermittent

pulsatile secretion of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) from the uterus (Kindahl et al., 1976b; Mann and Lamming, 2006). Prostaglandin $F_{2\alpha}$ enters the ovarian artery from the utero-ovarian vein by a countercurrent transfer mechanism and appears to act locally on the corpus luteum to initiate luteolysis (McCracken et al., 1999). This mechanism is essential for natural luteolysis because approximately 65% of the endogenous $PGF_{2\alpha}$ is metabolized during one pass through pulmonary circulation (Davis et al., 1985).

When $PGF_{2\alpha}$ is used in cattle to induce luteolysis, a single intramuscular injection needs to have the same effectiveness of the uterine pulses of $PGF_{2\alpha}$, acting locally on the CL to induce functional and structural luteolysis. However, in this case $PGF_{2\alpha}$ will pass through the systemic circulation prior to local action. Therefore, large doses of natural $PGF_{2\alpha}$ or potent analogues resistant to endogenous metabolism are necessary to cause an effective $PGF_{2\alpha}$ –induced luteolysis in cattle. An effective or complete $PGF_{2\alpha}$ –induced luteolysis event has been identified by a drop in serum progesterone concentrations by 2 d after $PGF_{2\alpha}$ injection to basal levels (Brusveen et al., 2009).

OVERVIEW OF OVSYNCH IN LACTATING DAIRY CATTLE

One of the main causes of poor reproductive efficiency in lactating dairy cows has been attributed to low estrus detection rates (or service rate). Washburn et al. (2002) reported a significant reduction from 50.9% in 1985 to 41.5% in 1999 for estrus detection rates in commercial dairy farms in southeastern USA. In order to increase service rate without the necessity of estrus detection, a synchronization of ovulation, also known as Ovsynch, was developed to improve reproductive performance (Pursley et al., 1995). Ovsynch allows AI at a fixed time (timed-AI). This protocol is based on three treatments,

100 µg of GnRH followed 7 d later by an injection of PGF_{2α} (25 mg of dinoprost tromethamine or 0.5 mg of cloprostenol sodium), and a last injection of 100 µg of GnRH 2 d later. Artificial insemination (AI) is performed approximately 16 h following the last treatment of Ovsynch (Figure 1.1).

The 1st treatment of the Ovsynch program, 100 µg gonadotropin releasing hormone (GnRH), in absence of pre-synchrony programs is administered at a random stage of the estrous cycle. The intent of the 1st GnRH-induced luteinizing hormone (LH) surge is to induce ovulation of a mature functional dominant follicle(s) (Pursley et al., 1995). Cows with two follicular waves have approximately a 65% chance that a functional dominant follicle (DF) capable of ovulating is present in the ovaries at a random stage of the estrous cycle (Vasconcelos et al., 1999; Bello et al., 2006). Ovulation of a DF induces the subsequent emergence of a new follicular wave ~1.5 d later (Pursley et al., 1995) followed by the growth and development of both a new dominant follicle and an *accessory corpus luteum* during the next 7 d. The new DF has approximately a 97% chance of remaining functional during the 7 d leading up to the PGF_{2α} then continuing on to ovulation following the final GnRH-induced LH surge, even if luteolysis following the PGF_{2α} is not complete (Pursley et al., 1995). At time of first GnRH injection, cows have approximately a 30% chance to be in early stages of follicular development (1st or 2nd wave) at a time when granulosa cells have not yet acquired sufficient LH receptors to respond to an LH surge (Xu et al., 1995). In this case, the 1st GnRH of Ovsynch does not induce ovulation and the potential DF continues to grow, deviate from subordinates and develop as a DF (Pursley et al., 1995). This DF has approximately an 80% chance of remaining functional prior to the PGF_{2α}-induced

luteolysis and subsequent increase in LH pulsatility that allows further development (Pursley et al., 1995). If this DF remains functional until the time of PGF_{2α}, it will continue to develop into a pre-ovulatory follicle and has a 97% chance of ovulating following the final injection of GnRH (Bello et al., 2006). However, this follicle could be as much as 12 d from emergence. Thus, conception rates following the ovulation of this follicle could be attenuated due to the antral age of the follicle and oocyte (Austin et al., 1999). Approximately 20% of these follicles become atretic prior to the PGF_{2α}. If this happens, a new wave will develop generally just prior to the PGF_{2α} and the new potential DF will emerge, deviate from subordinates and become a DF. However, at time of the second GnRH of Ovsynch this follicle will likely not have deviated from subordinates and acquired LH receptors prior to the final GnRH-induced LH surge because at this time this follicle would not be dominant yet (Xu et al., 1995; Sartori et al., 2001). As a result, it will not ovulate in response to the final GnRH injection of Ovsynch. In this scenario, cows will likely have natural estrus that may or may not be detected several days after the timed-AI as the new DF develops under basal concentrations of progesterone into an ovulatory follicle. Conception rates from the timed-AI in this case would be near 0%. Thus, it is critical to control ovulation of a DF in response to the 1st GnRH of Ovsynch to not only induce the formation of an *accessory CL* to increase P₄ but to control the age of the DF to control ovulation to the final GnRH-induced LH surge and avoid the asynchrony of ovulation just described. To ensure that cows respond to the 1st GnRH-induced LH surge of Ovsynch, cows must be on d 6 or 7 of the estrous cycle (Bello et al., 2006). Thus it is absolutely imperative that cows be pre-synchronized prior

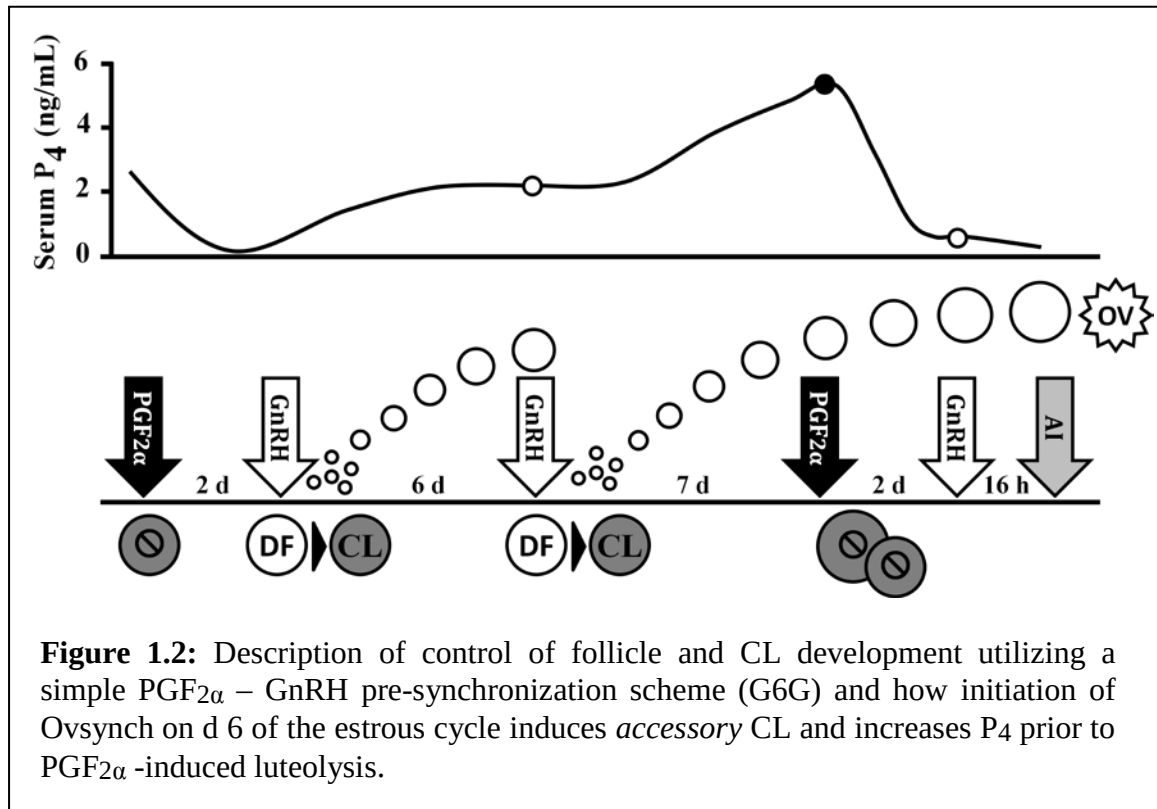
to Ovsynch so that cows are on d 6 or 7 of the estrous cycle at the first GnRH of Ovsynch.

Pre-synchronization protocols are scheduled treatments that have the objective to improve ovulatory response to the first GnRH of Ovsynch by increasing the percentage of cows at approximately d 6 to 8 of the estrous cycle at time of this injection. Most commonly, pre-synchronization programs are based on injection(s) of only PGF_{2α} or in combination with GnRH prior to the initiation of Ovsynch (Moreira et al., 2001; Navanukraw et al., 2004; Bello et al., 2006; Galvão et al., 2007; Souza et al., 2008). Our laboratory developed (Bello et al., 2006) a pre-synchronization protocol called G6G in 2006 (Figure 1.2). This protocol consists of an injection of PGF_{2α} 8 d prior to Ovsynch and administration of GnRH 2 d later. Cows that respond to both injections start a new cycle. This happens in approximately 70% of the cows that receive the G6G protocol (Bello et al., 2006). In this study, cows treated with G6G had a greater response for the 1st GnRH of Ovsynch compared with cows that started in a random stage of the cycle (Bello et al., 2006). This is an example of how pre-synchronization programs may improve the ovulatory response of the first GnRH of Ovsynch.

The 2nd treatment in the Ovsynch regime, PGF_{2α} (25 mg dinoprost tromethamine or 0.5 mg cloprostenol sodium), is administered to induce the regression of corpora lutea (luteolysis), thus enabling the DF of the *new* follicular wave to develop into a pre-ovulatory follicle. If serum concentrations of P₄ do not decline to approximately 0.5 ng/mL in 2 d following treatment the chances for conception after timed-AI of Ovsynch are reduced significantly (Souza et al., 2007; Brusveen et al., 2009). Thus, a fast drop in serum concentrations of P₄ following PGF_{2α}-induced luteolysis injection is vital for the

success of Ovsynch. A drop in serum concentrations to < 0.5 ng/mL 2 d after PGF_{2α} injection characterizes complete luteolysis. When Ovsynch is used with a pre-synchronization protocol such as G6G (Figure 1.2), there are at least two CL to be regressed, a 13 d old CL and a 7 d old CL. It is not known if the number of CL can affect the percentage of cows with complete luteolysis after PGF_{2α} of Ovsynch. However, maturity of these CL might play a role in the effectiveness of luteolysis (Skarzynski et al, 2008). It is known that the CL is refractory to a luteolytic injection of PGF_{2α} during the first 5 d of the estrous cycle (Lauderdale et al., 2009). This resistance during the early stage of CL development might be to the lack of expression of mediators responsible for luteolysis (McCracken et al., 1999). A portion of the 7 d old CL formed during a pre-synchronization + Ovsynch protocol may be less mature. It is not completely clear why but one possible explanation is that some ovulatory follicles that formed those CL may have been smaller than average at time of ovulation. Therefore, we suspect that the 7 d old CL might be the CL with lower probability of complete regression compared to the 13 d old CL. However, this hypothesis needs to be tested.

The high percentage of cows that fail to have complete luteolysis after PGF_{2α} of Ovsynch appears to be a problem in the Ovsynch protocol and varies among studies from 5 to 20% (Pursley et al., 1997b; Moreira et al., 2000; Gümen et al., 2003; Brusveen et al., 2009). Brusveen et al. (2009) used two injections of PGF_{2α} 24 h apart in the Ovsynch protocol with the objective to increase the percent of cows with complete luteolysis. Cows treated with two injections of PGF_{2α} had a significant reduction ($P < 0.001$) in the percentage of cows with incomplete luteolysis compared with cows treated with only one injection (2.5% vs. 14%, respectively).



Also, when Ovsynch is initiated late in the estrous cycle, there is a high likelihood that CL may undergo natural luteolysis prior to the PGF_{2α}. If this happens cows may have a natural estrus and ovulate early. If cows are not detected in estrus at this time, usually around the time of PGF_{2α}, conception rates are significantly less due to the asynchrony of AI and ovulation, i.e., timed-AI may occur well after ovulation. The 3rd treatment of Ovsynch protocol is 100 µg GnRH. This additional GnRH treatment is administered 48 to 60 h after PGF_{2α} to induce a pre-ovulatory LH surge, trigger ovulation of the functional DF 24 to 32 h later (Pursley et al., 1995) and release the oocyte to be fertilized following AI. The chance of ovulation to this treatment is > 95% even if luteolysis is not complete prior to this injection (Pursley et al., 1995). However, cows with a functional CL that do not reduce serum concentrations of P₄ to < 0.5 ng/mL have

significantly reduced chances to become pregnant even if they have an ovulation in response to final GnRH of Ovsynch (Souza et al., 2007; Brusveen et al., 2009).

PHARMACOLOGICAL DESCRIPTIONS OF PGF_{2α} ANALOGUES APPROVED FOR LUTEOLYSIS IN CATTLE

The only PGF_{2α} products currently commercially available in the U.S. are dinoprost tromethamine and cloprostenol sodium. Dinoprost tromethamine was developed in the early 1970's (Lauderdale, 2009). The high cost of this substance, a tromethamine salt, was a reason why analogues of this substance such as cloprostenol sodium were developed (Cooper and Furr, 1974; Dobson, 1975; Lagar, 1977). Cloprostenol sodium (0.5 mg) has an effective dose that was 50 times lower than dinoprost tromethamine (25 mg; Cooper and Furr, 1974). Even though this smaller dose had the same potency, Cooper and Furr, 1974, concluded that cloprostenol sodium had a wide margin of safety in cattle (Cooper and Furr, 1974). An intramuscular injection of 200 times the effective dose produced few side effects (Cooper and Furr, 1974).

One explanation for the extreme difference in effective dose is likely the way these compounds are metabolized once in circulation. The half-life of dinoprost tromethamine in general circulation is approximately 7~8 minutes (Kindahl et al., 1976a). Approximately 65% of the injected dose is metabolized in one passage through the lungs (Davis et al., 1985; McCracken et al., 1999). Smaller doses (5 mg) appear to be as effective as intramuscular injections when dinoprost tromethamine is administered via intrauterine (ipsilateral to the corpus luteum) infusion. On the other hand, this route is

not well suited for treatment in a large number of cattle due to the extra amount of time it would take to perform this task.

On the contrary, cloprostenol sodium has a much longer half-life compared to dinoprost tromethamine (3 h vs. 7~8 min; Kindahl et al., 1976a; Reeves, 1978). The reason for the greater resistance in endogenous metabolism is the presence of a benzyl chlorine ring (oxyaryl moiety structure) that blocks and/or reduces the action of enzymes responsible for the metabolism of $\text{PGF}_{2\alpha}$ (Bourne et al., 1980).

COMPARISON OF CLOPROSTENOL SODIUM VS. DINOPROST TROMETHAMINE IN CATTLE

There have been only a handful of studies (Furr et al., 1981; Sudweeks et al., 1983; Seguin et al., 1985; Turner et al., 1987; Salverson et al., 2002; Hiers et al., 2003; Martineau, 2003; Répási et al., 2005; Esterman et al., 2009; Stevenson and Phatak, 2010) during the past 30 years that have compared cloprostenol sodium and dinoprost tromethamine in both beef and dairy cattle. Taken together, differences in reproductive performance among these two $\text{PGF}_{2\alpha}$ analogues were inconsistent across studies. Results ranged from increases in estrus expression in favor of cloprostenol sodium to no differences in estrus expression or conception rates between products. Recently, two large studies have been reported. One report was published in *Theriogenology* (Stevenson and Phatak, 2010) and the other reported in a pharmaceutical company technical service bulletin. Once again there were different outcomes reported for each study.

Stevenson and Phatak (2010) reported no differences in conception rates but reported that dinoprost tromethamine treatment resulted in a greater % of cows that had luteolysis compared to cloprostenol sodium. Luteolysis in this case was defined as cows with serum progesterone concentrations above 1 ng/mL at time of PGF_{2α} of Ovsynch followed by a drop of serum P₄ concentrations to < 1 ng/mL 3 d later. This cutoff may have been too high and late to interpret these data in this manner. Previous studies have indicated that cows with serum concentrations of P₄ between 0.5 and 1.0 ng/mL 2 d following PGF_{2α} of Ovsynch had a reduction of 50% in predicted probability of pregnancy (Souza et al., 2007; Brusveen et al., 2009). In these studies the cutoff to determine luteolysis was established at 0.5 or 0.4 ng/mL 2 d following the PGF_{2α} of Ovsynch. These studies indicated that a faster reduction in serum concentration of P₄ after PGF_{2α} of Ovsynch resulted in greater fertility. Therefore, in order to test the luteolytic efficiency of cloprostenol sodium and dinoprost tromethamine in the Ovsynch program, the cutoff to determine luteolysis should be likely approximately 0.5 ng/mL after 2 d of PGF_{2α} of Ovsynch.

The other recent study was the largest study on record for this comparison (n=4532). There were no overall differences in conception rates or estrus detection rates, but there was a tendency (P=0.10) for greater conception rates in the cloprostenol sodium group that were inseminated on d 3 and 4 following treatment. Interestingly, 1st lactation cows had a greater % of cows detected in estrus following cloprostenol sodium vs. dinoprost tromethamine. Pregnancy rates (conception rate x estrus detection rate) were greater for cows that were inseminated on d 3 and 4 following treatment and once again

there was an effect of treatment with cloprostenol sodium on 1st lactation cows with more (P = 0.046) becoming pregnant following treatment.

Taken together, it is not clear if there are any differences between cloprostenol sodium and dinoprost tromethamine in luteolytic potency and conception rates when used in the Ovsynch protocol for lactating dairy cows. An improvement of luteolytic potency of the PGF_{2α} of the Ovsynch protocol could potentially increase conception rates after Ovsynch, resulting in a greater reproductive efficiency of lactating dairy cows. Improvements in conception rates for Ovsynch could enhance profitability of dairy farms since the Ovsynch protocol is being used in approximately 60% of commercial dairy farms in the USA (USDA, 2009).

POTENTIAL DIFFERENCES IN THE EFFECT OF CLOPROSTENOL SODIUM VS. DINOPROST TROMETHAMINE IN OVSYNCH

Data from Bello et al., 2006, indicated that inducing *accessory* CL during Ovsynch increases serum concentrations of P₄ from 3.5 to 5.2 ng/ml and increases the likelihood that the ovulatory follicle would emerge, deviate from subordinates, and develop into a DF under greater concentrations of P₄ compared to a normal estrous cycle. These data indicate that the greater serum concentrations of P₄ prior to PGF_{2α}-induced luteolysis the greater the conception rates (Figure 1.2). Thus, as mentioned previously, developing pre-synchronization programs that allow cows to be on d 6 or 7 of the estrous cycle at the 1st GnRH of Ovsynch increases the likelihood that a new *accessory* CL will develop and enhance progesterone. Cows assigned for this program will most likely have at least a CL that is 13 d old and a CL that is 7 d old (*accessory*) at time of PGF_{2α} of

Ovsynch. Therefore, the use of a pre-synchronization protocol that uses PGF_{2α} and GnRH enhanced conception rates of timed-AI of Ovsynch (Bello et al., 2006; Pursley unpublished data) not only by increasing synchronization rate (percentage of cows that respond to the last two injections of the Ovsynch protocol) but also by raising serum concentrations of P₄ at time of PGF_{2α} (Bello et al., 2006).

Although these *accessory* CL increases serum concentrations of P₄, it appears from that study that incomplete or attenuated luteolysis may be a byproduct of a portion of these *accessory* CL that still may be somewhat refractory to PGF_{2α}. Some of these CL may not be sensitivity enough to PGF_{2α} since the sensitivity of the CL to PGF_{2α} appears to gradually increase during the luteal phase (Skarzynski et al., 1997). Also, some follicles that formed some of these *accessory* CL may be small, resulting in a small and less functional (mature) CL (Vasconcelos et al., 2001; Echterkamp et al., 2009), that may contribute to the problem. However, further studies are necessary to investigate if the cause of incomplete or attenuated luteolysis is due to these *accessory* CL as suggested above.

Although is not know which CL is failing to undergo complete luteolysis following PGF_{2α} of Ovsynch, it is imperative for the success of Ovsynch that both CL have complete luteolysis by 56 h after PGF_{2α} of Ovsynch (Souza et al., 2007; Brusveen et al., 2009). Since cloprostenol sodium is a more potent PGF_{2α} analogue and has a greater half life compared to dinoprost, cloprostenol may increase the percentage of cows that undergo complete luteolysis when used as PGF_{2α} of Ovsynch in a situation with 2 or more CL (at least a d 7 CL and a d 13 CL). Also, cloprostenol may cause a faster decrease in serum concentrations of P₄. As a result, cloprostenol sodium may increase

fertility of cows following PGF_{2α} of Ovsynch compared to dinoprost tromethamine since probability of pregnancy is increased if cows have complete luteolysis.

To our knowledge, no studies have compared cloprostenol sodium to dinoprost tromethamine when used in a pre-synchronization protocol to determine if the rate of decrease of P₄ following treatment is different. In Chapter 2, we designed an experiment to test whether cloprostenol sodium may decrease P₄ at a greater rate compared to dinoprost tromethamine in cows with at least one 7 d old CL and a 13 d old. In Chapter 3, we determined if there were any differences in these two luteolytic agents in the percent of cows with complete luteolysis and the percent of cows that became pregnant when a pre-synch/Ovsynch program was administered.

Since cloprostenol has a longer half life, it probably has a longer local action than dinoprost. Based on this pharmacokinetic difference, in Chapter 2, we expected that cows treated with cloprostenol sodium would have a faster rate of decrease in P₄ following PGF_{2α} injection of Ovsynch compared to cows treated with dinoprost tromethamine. In addition, in Chapter 3, we also expected a greater % of cows with complete luteolysis for cows treated with cloprostenol sodium vs. dinoprost tromethamine when used in the Ovsynch protocol. As a result, a greater conception rate for cows treated with cloprostenol sodium is expected also.

CHAPTER 2

LUTEOLYTIC EFFECTS OF CLOPROSTENOL SODIUM VS. DINOPROST TROMETHAMINE ON SUBSEQUENT PROGESTERONE AND ESTRADIOL CONCENTRATIONS IN LACTATING DAIRY COWS TREATED WITH G6G/OVSYNCH

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ABSTRACT

The probability of a pregnancy decreases substantially in lactating dairy cows treated with Ovsynch if luteolysis is delayed or incomplete. There are two PGF_{2α} products approved in the US for luteolysis, dinoprost tromethamine and cloprostenol sodium. Cloprostenol sodium has a longer half-life compared to dinoprost tromethamine, is more resistant to endogenous metabolism, and is thus maintained in circulation for a longer time. We hypothesized that cloprostenol sodium could reduce the time to complete luteolysis at final PGF_{2α} of Ovsynch compared to dinoprost tromethamine because of differences in half-life. Lactating dairy cows received the same pre-synchronization strategy (25 mg PGF_{2α} – 2d - 100 µg GnRH) and initiated Ovsynch protocol without the final administration of GnRH 6 d later (100 µg GnRH – 7 d – final PGF_{2α} treatment). At the time of the final PGF_{2α}, cows were assigned randomly to receive either 500 µg cloprostenol sodium or 25 mg dinoprost tromethamine. Blood samples were collected daily prior to, and serially after, PGF_{2α} treatments to analyze circulating concentrations of progesterone (P₄) and estradiol (E₂). Ultrasound examinations of ovaries were performed daily prior to, and serially after, PGF_{2α} treatment to measure sizes of follicles and CL and determine time of ovulation. Only cows with at least one d 7 and one d 13 CL at time of PGF_{2α} were utilized in the analyses (n=35). The decline in circulating P₄ was accelerated (P = 0.047) for cloprostenol sodium vs. dinoprost tromethamine treated cows with complete luteolysis (P₄ < 0.5 ng/mL until 56 h post-treatment) between 0 and 12 h post-injection, but not at 24, 36 or 48 h. There was a rapid drop in P₄ 1 h after PGF_{2α} then a complete rebound 1 h later followed by a steady decline in both treatment groups. Serum concentrations of E₂ were greater, P < 0.05, in cloprostenol sodium vs.

dinoprost tromethamine groups 48 h following treatment. Cows that did not have complete luteolysis did not ovulate (0/7) during the 6 d period following treatment. Time to complete luteolysis and ovulation was 29.1 ± 1.1 vs. 29.4 ± 1.7 ($P = 0.89$) and 101 vs. 103 h ($P = 0.56$) post-treatment in cloprostenol sodium vs. dinoprost tromethamine. In summary, cloprostenol sodium induced a greater decrease in P4 for the first 12 h following treatment and subsequently a greater increase in E2 compared to dinoprost tromethamine, although there were no differences in these two products in time to complete luteolysis or time to ovulation.

INTRODUCTION

Pharmacological control of luteolysis is a key component of Ovsynch programs in lactating dairy cows (GnRH -7 d- PGF_{2α} -2 d- GnRH -16 h- AI; Pursley et al., 1995; Pursley et al., 1997a; Pursley et al., 1997b). There are two types of PGF_{2α} products commercially available in the USA, dinoprost tromethamine, a tromethamine salt of the natural PGF_{2α}, and cloprostenol sodium, a synthetic analogue. Dinoprost tromethamine is metabolized in a similar mechanism to that of endogenous PGF_{2α} by three major steps. The initial metabolic step is the dehydrogenation of the OH group on C-15 and reduction of the double bond at carbons 13-14 by the enzymes 15-hydroxydehydrogenase and 13,14-reductase. This rapid, initial metabolic step is followed by β -oxidation and ω -oxidation (Oesterling et al., 1972; Bourne and Hathway, 1979; Bourne et al., 1980). On the contrary, cloprostenol sodium has an oxyaryl moiety structure that blocks ω -oxidation and the action of the dehydrogenase and reductase enzymes and reduces β -oxidation (Bourne et al., 1980). Therefore, cloprostenol sodium is more resistant to endogenous

metabolism and as a result has a much longer biological half-life ($t_{1/2} \sim 3$ h; Reeves, 1978) than dinoprost tromethamine ($t_{1/2} \sim 7$ -8 min; Kindahl et al., 1976a). Approximately 65% of dinoprost tromethamine is metabolized in one passage through the lungs in the cow (Davis et al., 1985; McCracken et al., 1999). Hence, cloprostenol sodium is maintained in circulation longer and may be a more potent luteolytic agent.

Several studies using Ovsynch have shown that some cows treated with dinoprost tromethamine have incomplete or delayed luteolysis (Pursley et al., 1997b; Moreira et al., 2000; Souza et al., 2007). At the time of PGF_{2 α} of Ovsynch there could be more than one corpus luteum (CL) depending on the stage of the estrous cycle when Ovsynch is initiated. For example, if Ovsynch is initiated following deviation of the dominant follicle (DF) from subordinates during the first follicular wave, the DF will likely ovulate to the GnRH-induced LH surge and a new CL and follicular wave may develop. Thus, at the time of PGF_{2 α} 7 d later, at least a d 7 and an older CL will be present in the ovaries.

Ovsynch induces ovulation with a final GnRH-induced LH surge 48 h after PGF_{2 α} (Pursley et al., 1995) to allow for timed-artificial insemination (AI) 16 h after final GnRH injection. Serum concentrations of P₄ following PGF_{2 α} must decline to less than approximately 0.5 ng/mL by 2 d after injection or the probability of pregnancy is diminished (Souza et al., 2007; Brusveen et al., 2009). Sub-normal concentrations of P₄ at the time of AI may reduce uterine contractility given that P₄ decreases the number of oxytocin, angiotensin II, and estrogen receptors (ER) in uteri (Graham and Clarke, 1997) and antagonizes estrogen induction of ER in the uterus, especially in the myometrium (Graham and Clarke, 1997). Prolonged P₄ clearance due to delayed regression, or

incomplete luteolysis, may also have a negative indirect effect on estradiol-17 β (E₂) production (Bridges and Fortune, 2003).

The objective of this study was to determine differences in dynamics of luteolysis between dinoprost tromethamine and cloprostenol sodium treated lactating dairy cows at the last PGF_{2 α} injection of the Ovsynch protocol without the final administration of GnRH. We hypothesized that cloprostenol sodium used at the last PGF_{2 α} of Ovsynch would reduce circulating concentrations of P₄ at a greater rate and in turn enhance subsequent concentrations of E₂ compared to dinoprost tromethamine due to differences in pharmacokinetic characteristics.

MATERIALS AND METHODS

Management of Cows

This trial was conducted between May and October of 2007 at the Michigan State University Dairy Teaching and Research facility. Four groups of lactating Holstein dairy cows between 45 and 85 DIM with average milk production during the week of treatment of 45.6 ± 0.32 (mean $\pm$ S.E.M) kg/d were utilized in this experiment. Cows were housed in a tie-stall barn with free access to water and fed twice daily with a TMR consisting primarily of corn and alfalfa silages and ground corn balanced to NRC (2001) recommendations. Cows were milked twice daily. The Institutional Animal Care and Use Committee (IACUC) at Michigan State University approved all animal related procedures.

Calculation of Sample Size and Definition of Complete Luteolysis

Number of cows needed per treatment was calculated utilizing G*Power 3.0 (Faul et al., 2007). The “MANOVA Test for Repeated Measures Between Factors” power analysis indicated that $n = 26$ was needed to detect a 0.5 ng/mL difference in P4 concentrations between $n = 2$ treatments with $\alpha = 0.05$ and $\beta = 0.8$. To determine the effect of treatment on rate of decrease in P4 and subsequent increase in E2, we used only cows that had complete luteolysis as defined by cows with serum P4 concentrations below 0.5 ng/ml 56 h following treatment and continued decrease to or towards basal concentrations for the next 3 d. Effect of treatment on % of cows with complete luteolysis was analyzed in a larger group of cows in a subsequent trial reported in Chapter 3. We expected approximately 15% of cows to not have complete luteolysis, therefore we increased “n” to account for this.

Treatment and Data Collection

Fifty-four cows received pre-Ovsynch treatments, which consisted of an injection of 500 μ g of cloprostenol sodium (P1; Estrumate, Schering-Plough Animal Health, Union, NJ), followed in 2 d with 100 μ g of GnRH (G1; Ovacyst, IVX Animal Health Inc., St. Joseph, MO) then 6 d later the 1st GnRH of Ovsynch was initiated (100 μ g GnRH, G2; Figure 2.1). Only cows that had basal concentrations of P4 after P1 and ovulation characterized by the disappearance of a DF and subsequent formation of a new CL by 48 h after G1 and G2 were included in the experiment. Cows ($n = 35$) that met these criteria were blocked by parity and randomly assigned within block to treatments of either 500 μ g of cloprostenol ($n = 17$) or 25 mg of dinoprost ($n = 18$) 7 d after G2. Retrospectively, cows with decreasing P4 prior to treatment were excluded from analyses.

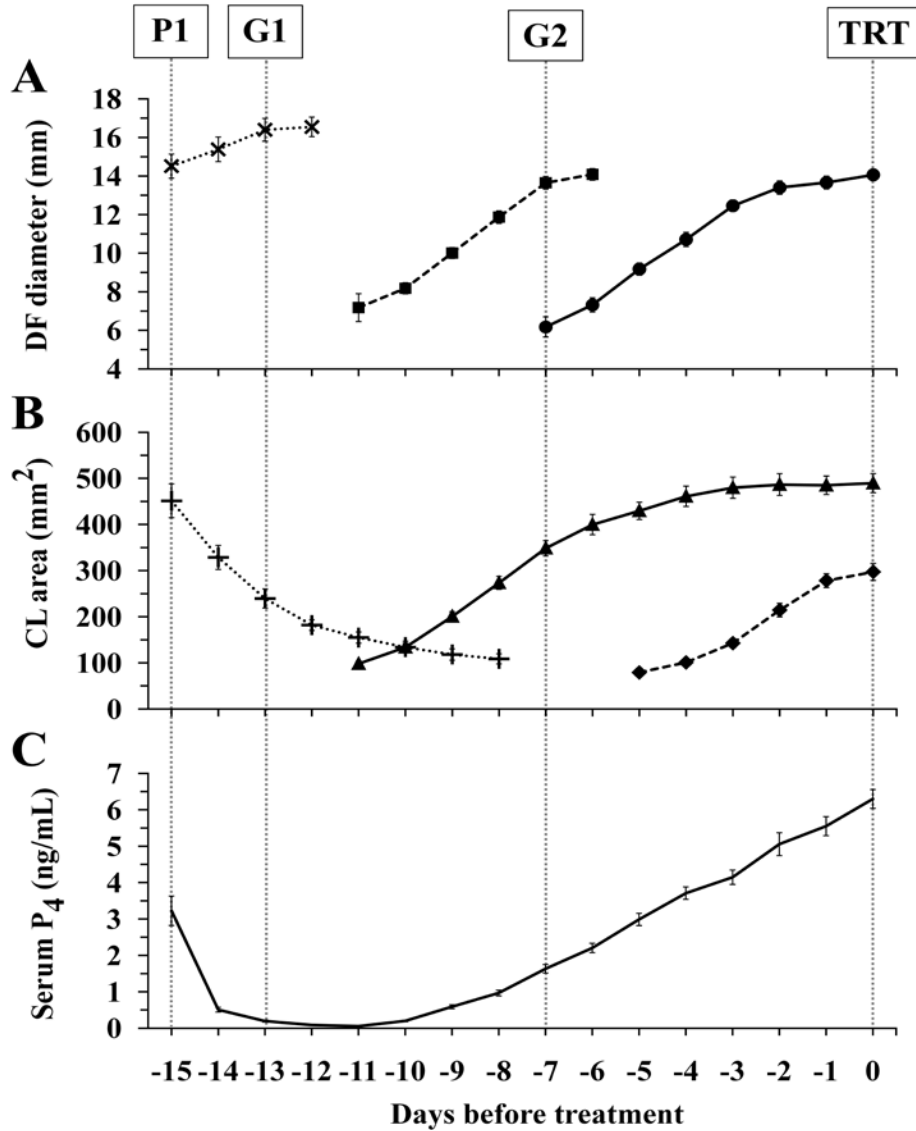


Figure 2.1: Description of follicle and corpora lutea development and P4 concentrations prior to treatment in lactating dairy cows. Mean diameter ($\pm$ SEM) of dominant follicles (DF; mm) in cows ($n = 29$) at 1st PGF_{2 α} (P1; $\cdots\times\cdots$) and in cows that ovulated following the 1st (G1; $\cdots\times\cdots$) and 2nd (G2; $--\blacksquare--$) injections of GnRH, and the final pre-ovulatory follicle that ovulated within 144 h following treatment ($-\bullet-$) are described in Panel A*. Corpora lutea area (mm²) in cows ($n = 26$) with a single functional CL at 1st PGF_{2 α} (P1; $\cdots+\cdots$) and with single ovulations ($n = 29$) to the 1st (G1; $-\blacktriangle-$) and 2nd (G2; $--\blacklozenge--$) injections of GnRH are described in Panel B*. Serum concentrations of P4 (ng/mL) are described in relation to each pre-treatment injection of PGF_{2 α} (P1) and GnRH (G1 and G2) in Panel C. Timing of pre-treatment injections of PGF_{2 α} and GnRH are designated with vertical dotted lines. *Cows ($n = 6$) with multiple ovulations following G1 and/or G2 were not included although growth and development of follicles and CL from these cows had similar patterns to cows with single ovulations.

Although Ovsynch normally includes GnRH approximately 2 d following PGF_{2α}, this experiment did not include this final GnRH in order to assess the effect of treatment on follicular growth and peak concentrations of E₂. Thus, cows were allowed to manifest estrus and ovulation via endogenous mechanisms. All injections were administered intramuscularly with either an 18-gauge (injections of PGF_{2α}) or 20-gauge (injections of GnRH) 3.8 cm needle in the semimembranosus or semitendinosus muscles.

Blood samples were collected daily from P1 until treatment by coccygeal venipuncture before ultrasound examinations and prior to any injection of either GnRH or PGF_{2α}. An indwelling catheter was placed in the jugular vein 2 or 3 d before treatment (0 h) to collect blood samples at frequent intervals. Blood samples were collected from jugular catheters from treatment (0 h) to ovulation or 144 h after treatment if cows did not ovulate. Blood collections were made every h for the first 12 h after treatment, every 2 h between 12 to 24 h, every 4 h between 24 h and 72 h, and every 6 h between 72 h and ovulation or 144 h. Blood samples were collected using vacutainer tubes without anticoagulant (BD Vacutainer, Preanalytical Solutions, Franklin Lakes, NJ) and refrigerated for 6 to 12 h. Serum was separated by centrifugation at 2000 x g for 20 min at 4°C and stored at -20°C for later hormonal analyses.

Transrectal ultrasound examinations were conducted daily from P1 until treatment, every 12 h for 3 d, then every 6 h until detection of ovulation or 144 h post-treatment using a real time, B-mode, Aloka SSD-500V ultrasound machine with a 7.5-MHz linear array probe (Aloka Co. Ltd, Wallingford, CT). Height and width of the maximal size of CL and the antrum of each follicle > 4 mm in diameter were measured with built-in calipers. Follicular and luteal measurements were recorded in an ovarian

map for each cow with date and time of examinations. Mean follicular diameter (d) was calculated by the average of height and width ($d = H+W/2$) of each follicle. If a fluid-filled central cavity was detected within the CL, a cross section area of the cavity was determined the same as a cross section area of the total CL. Total luteal area of each CL was calculated subtracting the cavity area from the total CL area. The following equation was used to calculate CL and cavity area: $0.5 H \times 0.5 W \times \pi$ (Kastelic et al., 1990). Cows at time of treatment (0 h) had a minimum of 2 and maximum of 4 CL.

Hormonal Assays

Concentrations of P₄ were quantified in serum via RIA (Coat-A-Count Progesterone, Siemens Diagnostics, Los Angeles, CA). Inter- and intra-assay CVs were 9.2 and 5.5%, respectively, for high P₄ quality controls and 9.6 and 6.6%, respectively, for low P₄ quality controls. Sensitivity of the assay was 0.02 ng/mL. Estradiol concentrations were quantified in a subset group of serum samples, including samples 0 (time of treatment), 24, and 48 h, and every 12 h between 48 h and ovulation or until 144 h in cows that did not ovulate. Serum samples (500 µl) were ether extracted in duplicate and then measured using a modified version of a commercially available RIA MAIA kit (Polymedco Inc., Cortland Manor, NY) (Prendiville et al., 1995). Inter- and intra-assay CVs were 8.9 and 13.5% respectively. Sensitivity of the assay was 0.5 pg/mL.

Statistical Analyses

Data were tested for normality of residuals with the Shapiro-Wilk test and/or studentized residual plots for each variable. Variables that did not fulfill assumptions for normality were transformed by natural log and re-analyzed. For clarity, actual means of the data are presented. Discrete variables were analyzed by least-squares ANOVA, using

the GLM procedure of SPSS (Statistical Package for Social Sciences, Version 16.0, SPSS Inc., Chicago, IL, USA). Repeated measures variables such as mean concentrations of P4 and E2 over time were analyzed using the MIXED procedure with the REPEATED statement with cows nested in treatment specified in the SUBJECT option of SAS (Version 9.2, SAS Inst. Inc., Cary, NC). For the MIXED procedure, fit statistic parameters for unstructured (UN), compound symmetry (CS), first order autoregressive (AR(1)), heterogeneous compound symmetry (CSH), and heterogeneous first order autoregressive (ARH(1)) covariance structures were tested. The covariance structure with lowest values for the Bayesian information criterion (BIC) was used for the analyses. Only cows that had complete luteolysis and ovulation by 144 h after treatment were analyzed for repeated measures. Serum P4 concentrations over time were analyzed in the periods: -24 h from treatment to 12 h after treatment, 12 to 24 h, 24 to 48 h, and 48 to 90 h after treatment. Parity, treatment, time and their interactions were included in the original model. Interactions that were not significant ($P > 0.2$) were removed from the model. Only time, treatment, parity, treatment x time, and parity x time remained in the final model for effect of treatment on serum P4 concentrations.

Serum concentrations of E2 were analyzed from 0 to 96 h after treatment. Also, parity, treatment, time and their interactions were included in the original model. Only treatment, time and their interactions remained in the final model for serum E2 analyses. Parity, treatment x parity, parity x time, and group x parity x time were not significant ($P > 0.20$) and were removed from the final model.

Effects of ovulatory follicle diameter at time of G1 and G2 on P4, and CL area at time of treatment were tested using regression analyses with the REG procedure of SAS.

Effect of serum P₄ concentrations at 12 h after treatment on serum E₂ concentrations at 24 and 48 h after treatment were analyzed by the REG procedure of SAS. Effect of time from complete luteolysis to ovulation was analyzed by the REG procedure of SAS.

RESULTS

Pre-Treatment Validation

Figure 2.1 describes DF and CL growth prior to treatment. All cows (n=35) had luteolysis following P1 and ovulation and a new follicular wave following G1 and G2. Thus all cows had at least one d 13 and 7 CL and a pre-ovulatory follicle 7 d from induction (G2) of emergence at time of treatment. Three cows had two d 13 and one d 7 CL, one cow had one d 13 and two d 7 CL, and two cows had two d 13 and two d 7 CL (n = 4 were in the cloprostenol sodium group and n = 2 were in the dinoprost tromethamine group). Average diameter of pre-ovulatory follicles at time of treatment for cows with single ovulations (n = 26) were not different (P = 0.12) between treatments or parities (P = 0.15) and averaged 13.3 ± 0.3 mm. Luteal areas of d 13 CL and d 7 CL were not different (P = 0.6 and P = 0.6) between treatments with average sizes of 446.3 ± 17.8 mm² and 273.2 ± 17.1 mm², respectively. Average serum P₄ concentrations 24 h prior to (5.4 ± 0.2 and 5.6 ± 0.3 ng/mL for cloprostenol sodium and dinoprost tromethamine; P = 0.6), and at time of treatment (6.0 ± 0.3 and 6.3 ± 0.4 ng/mL for cloprostenol sodium vs. dinoprost tromethamine; P = 0.5), were not different between treatments. Average serum P₄ concentrations for treatments combined were 6.1 ± 0.2 at time of treatment (Figure 2.1), ranging from 3.6 to 9.0 ng/mL in individual cows.

Follicle size at time of G1 and G2 had a direct impact on size and function of the resulting CL (Figure 2.2). In follicles that ovulated in response to G1 and G2, there were

positive relationships between sizes of follicles at time of each injection of GnRH with luteal area (G1, $P = 0.01$; G2, $P = 0.04$) and P4 concentrations (G1, $P < 0.001$; G2, $P < 0.001$) at time of treatment.

Average milk production from 3 d prior, to 3 d after, treatments was not different ($P = 0.3$) between treatments (44.0 ± 2.9 and 47.2 ± 2.5 Kg/d for cloprostenol sodium and dinoprost tromethamine). However, average milk production was different ($P < 0.0001$) among 1st ($n = 12$), 2nd ($n = 16$) and 3rd + parities ($n = 7$; 35.4 ± 1.5 , 47.0 ± 2.0 , and 60.0 ± 2.5 Kg/d). There was not an effect ($P = 0.9$) of parity on treatment outcomes.

Effect of Treatment on Dynamics of Luteolysis and Ovulation

There was an effect of PGF_{2 α} type on serum concentrations of P4 for the 1st 12 h following treatment ($P = 0.047$; Figure 2.3). Cows treated with cloprostenol sodium had a more rapid drop in P4 during this period compared to dinoprost tromethamine. Specifically, cows treated with cloprostenol sodium had lower serum concentrations of P4 3 ($P = 0.042$), 4 ($P = 0.049$), 8 ($P = 0.029$) and 9 h ($P = 0.017$) after treatment. Mean serum P4 concentrations were not different between types of PGF_{2 α} from 12 to 24 ($P = 0.91$; Figure 2.4). However, cloprostenol sodium treated cows had lower ($P = 0.04$) serum P4 concentrations at 14 h after treatment compared to cows treated with dinoprost tromethamine. There was no effect ($P = 0.31$) of treatment 24 to 48 h post treatment, yet cloprostenol sodium treated cows had a tendency ($P = 0.097$) to have lower serum concentrations of P4 36 h post-treatment compared to cows treated with dinoprost tromethamine. There was no effect of treatment ($P = 0.24$) from 48 to 90 h post-treatment.

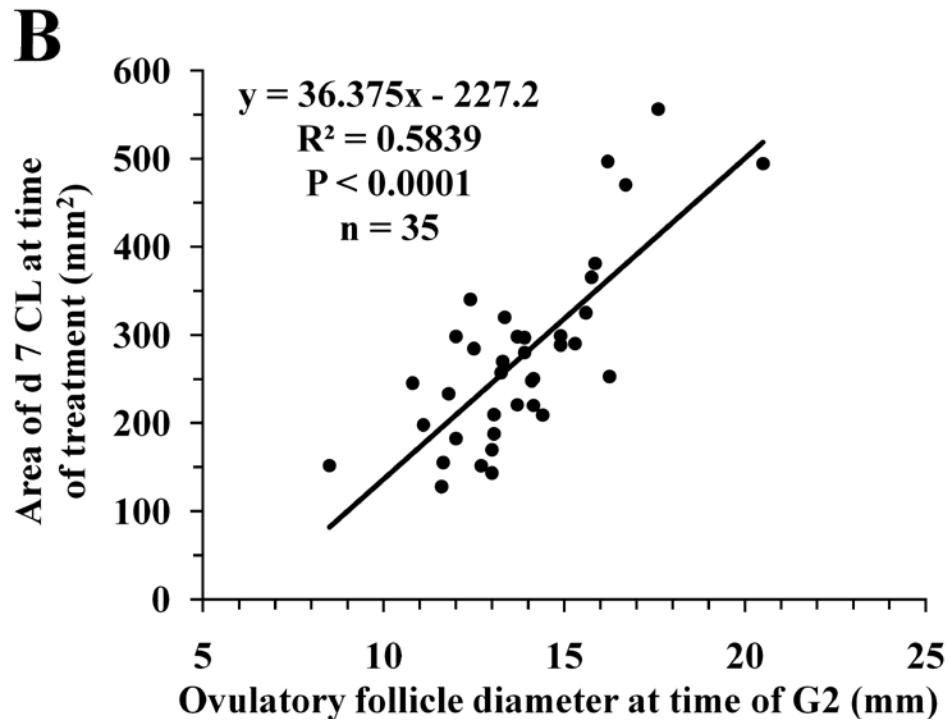
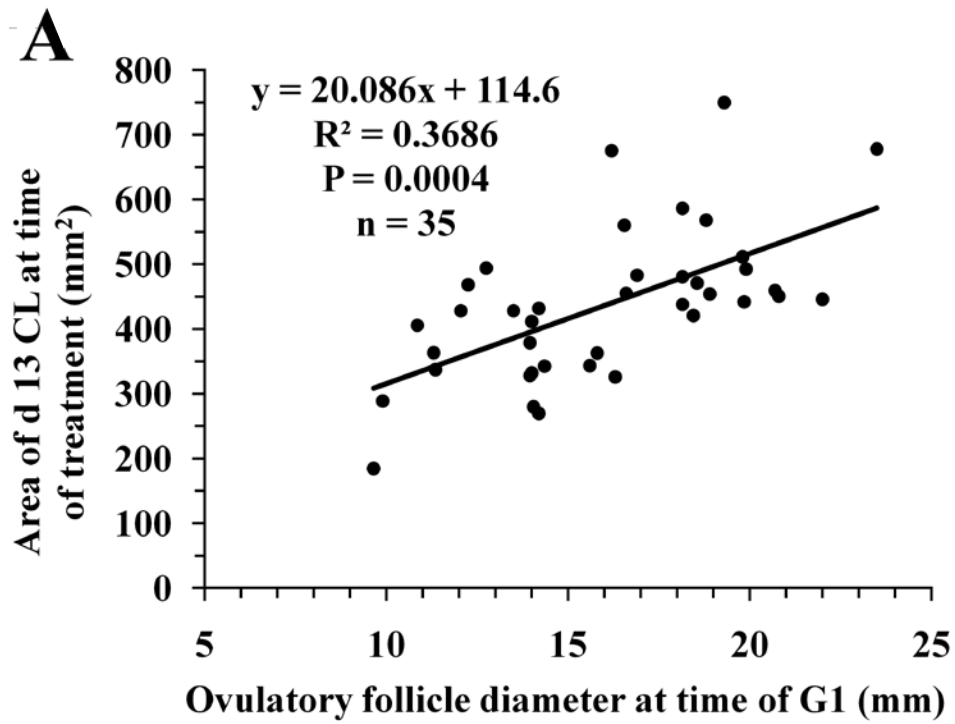


Figure 2.2: Regression analyses of the relationship between ovulatory follicle diameter (mm) at time of the 1st (G1) and 2nd (G2) injections of GnRH and area of CL (mm²) that developed following ovulation of these ovulatory follicles measured at time of treatment with PGF_{2α} (n = 35) in lactating dairy cows.

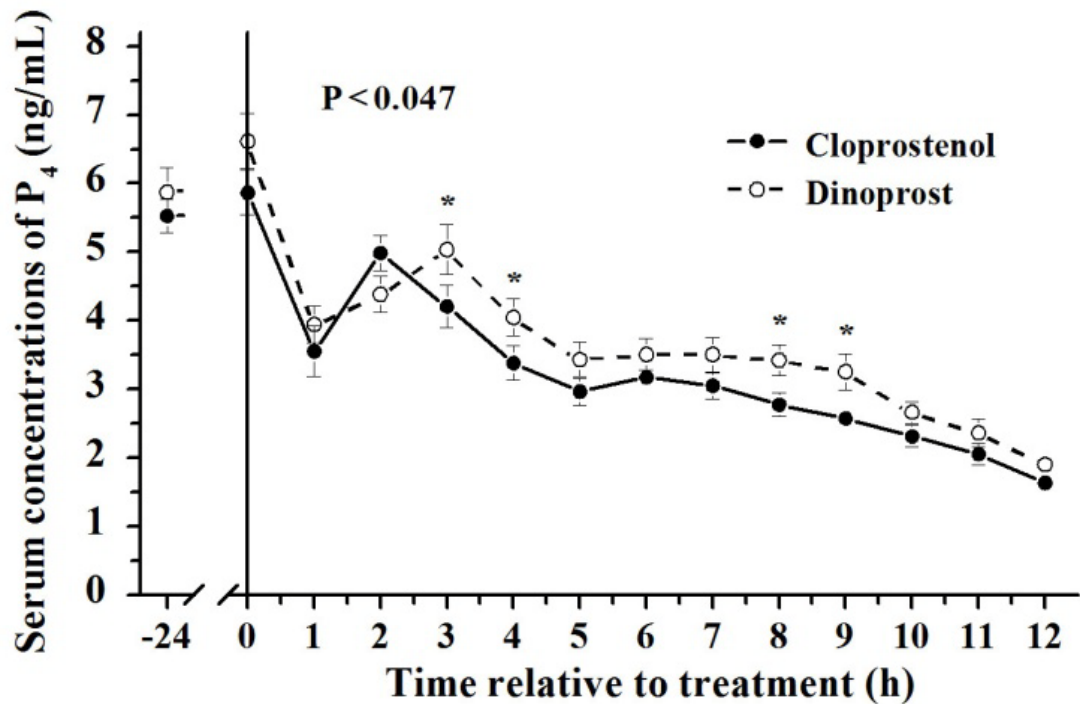


Figure 2.3: Mean ($\pm$ SEM) serum P_4 concentrations (ng/mL) from 1 d prior to 12 h following treatment with cloprostenol sodium vs. dinoprost tromethamine in lactating dairy cows with at least one d 7 and one d 13 CL that had complete luteolysis by 56 h after $PGF_{2\alpha}$ and also ovulated via endogenous LH surge by 144 h after treatment (27/35). Rate of decrease during this 1st 12 h period was different between treatments ($P = 0.047$).

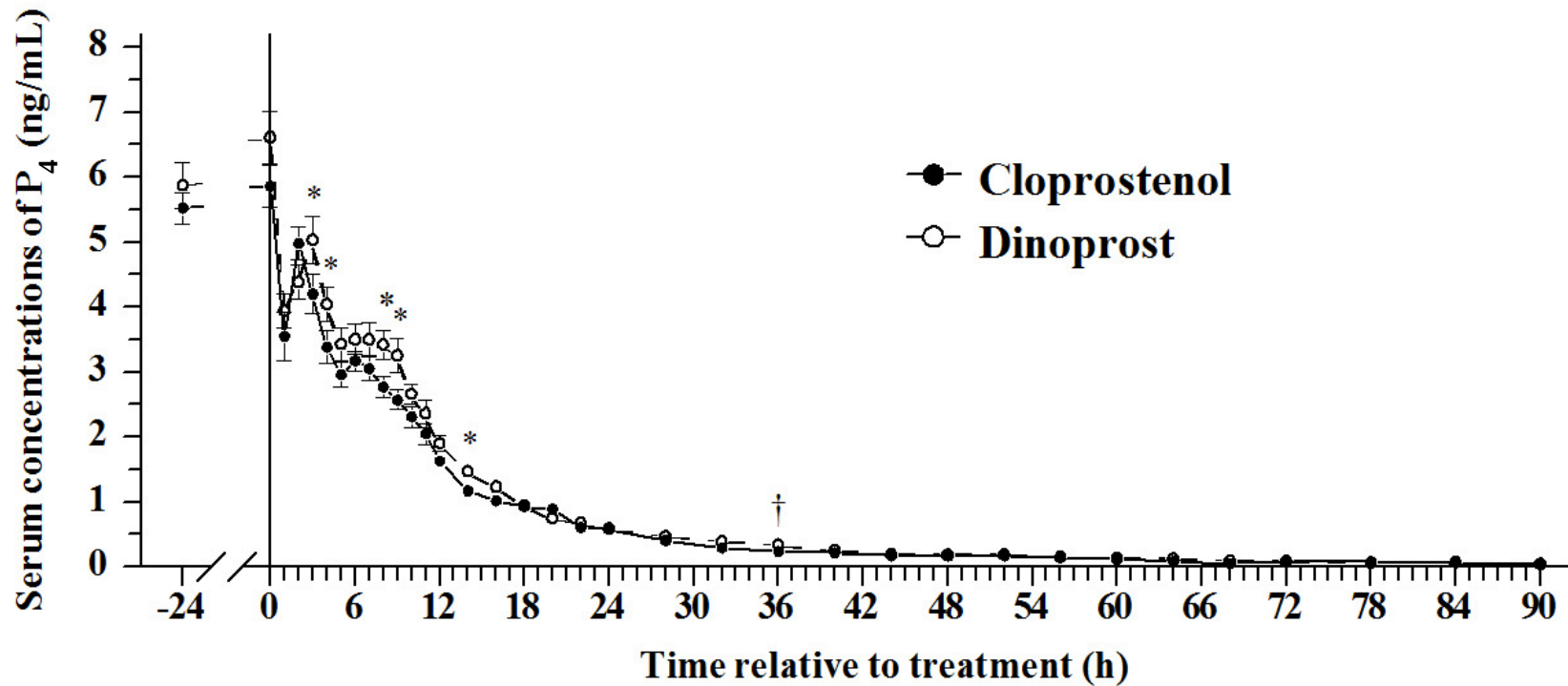
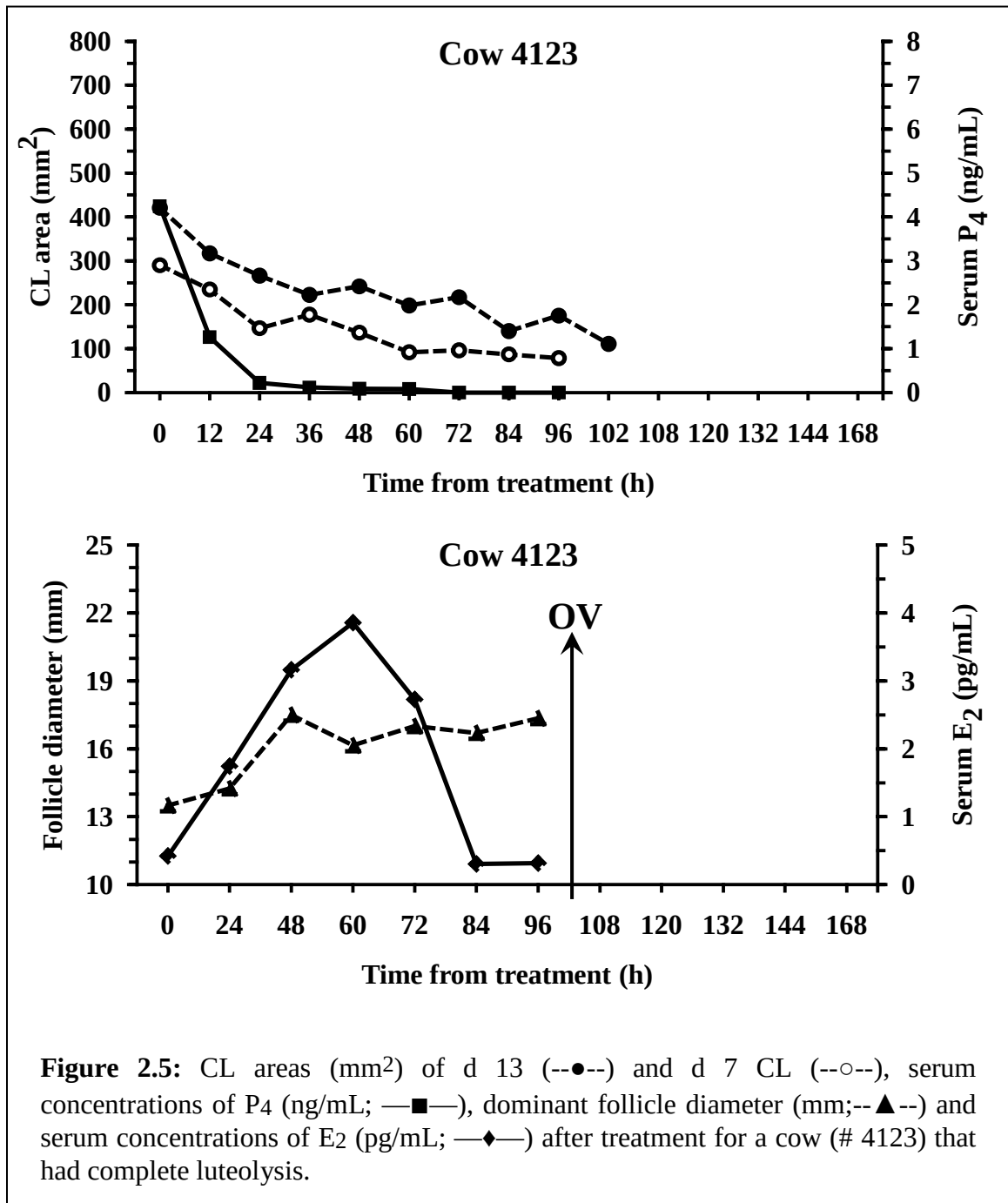
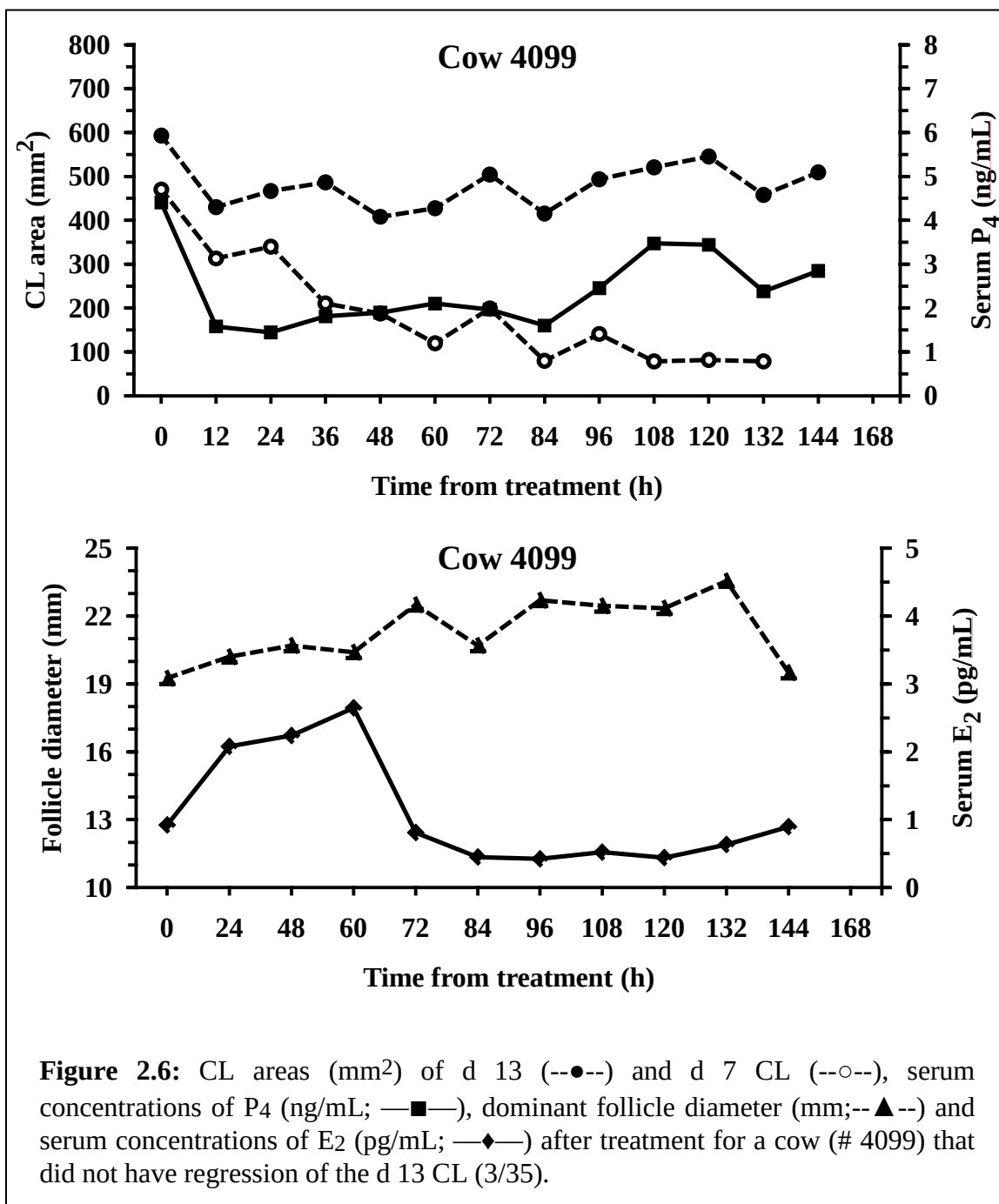


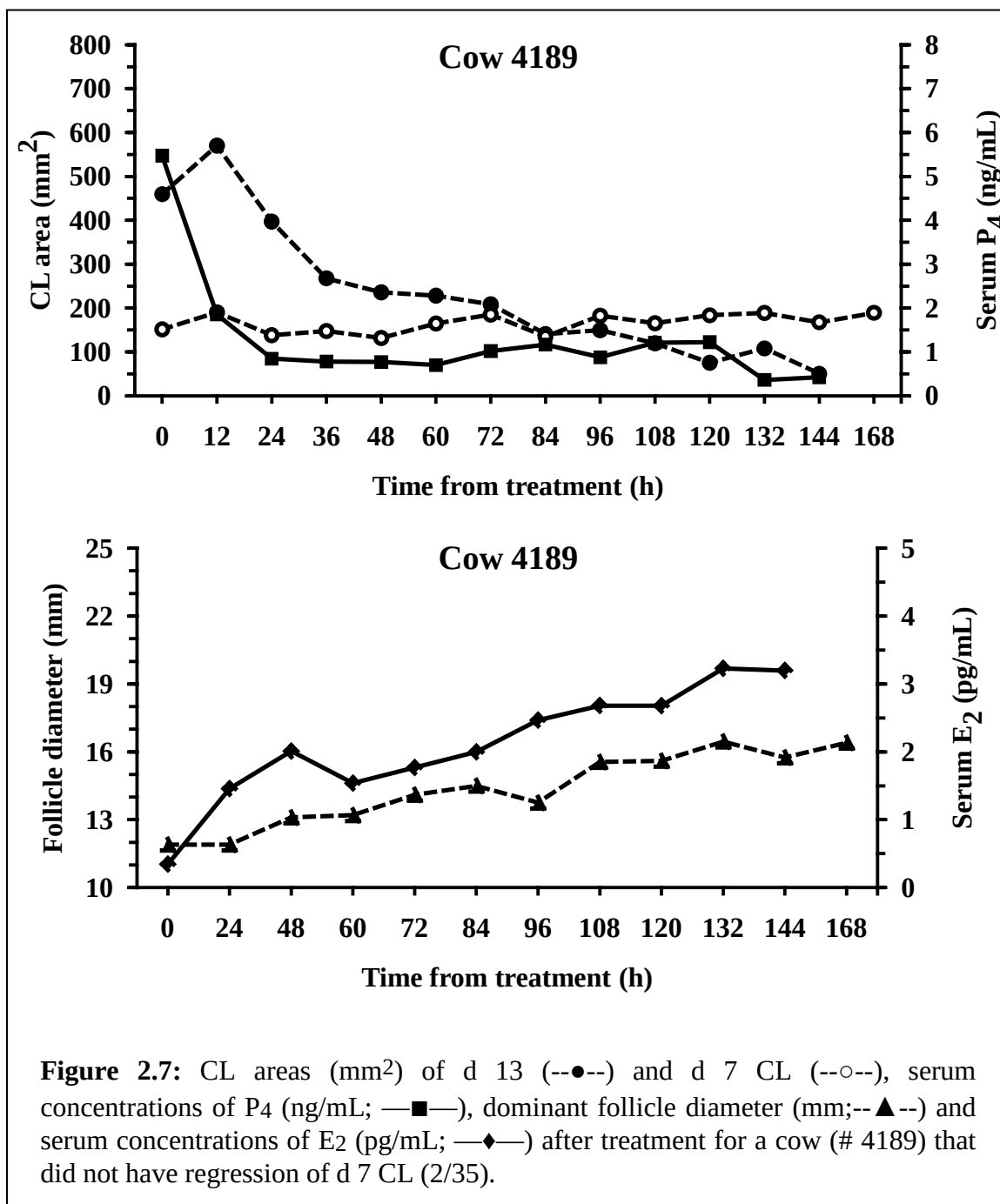
Figure 2.4: Mean ($\pm$ SEM) serum P_4 concentrations (ng/mL) from 1 d prior to 90 h following treatment with cloprostenol sodium vs. dinoprost tromethamine in lactating dairy cows with at least one d 7 CL and one d 13 CL that had complete luteolysis by 56 h after $PGF_{2\alpha}$ and also ovulated via endogenous LH surge by 144 h after treatment (27/35). Rate of decrease during this 1st 12 h period was different between treatments ($P = 0.047$), but not different from 12 to 24 ($P = 0.91$), 24 to 48 ($P = 0.31$), or 48 to 90 h ($P = 0.24$) after treatment.

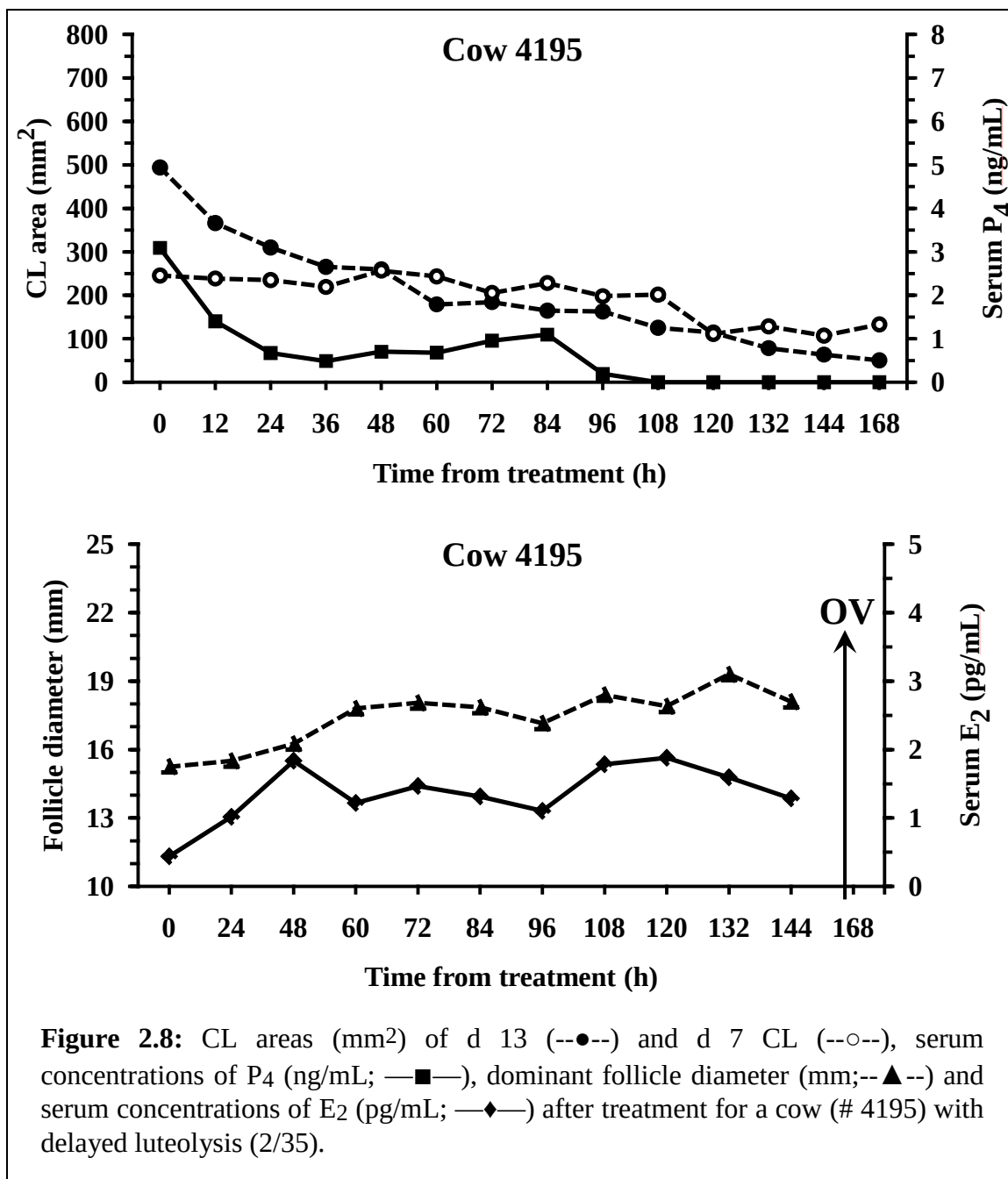
Mean time between treatment and complete luteolysis ranged from 18 to 40 h and was not different ($P = 0.8$) between cloprostenol sodium, 29.1 ± 1.1 h, and dinoprost tromethamine groups, 29.4 ± 1.7 h. Mean interval from treatment to ovulation ranged from 87 to 123 h and was not different ($P = 0.56$) between cloprostenol sodium, 101 ± 2 h, and dinoprost tromethamine, 103 ± 2 h. Interval from complete luteolysis to ovulation ranged from 55 to 93 h with mean interval of 72.5 ± 8.3 h. There was an effect ($P = 0.04$) of parity on time to regression. Second parity cows had a shorter time to complete luteolysis compared to 1st (26.5 vs. 32.0 h; $P = 0.02$) and there was a similar tendency in 3rd parity cows (26.5 vs. 31.6 h; $P = 0.07$). Size of pre-ovulatory follicles at last measurement before ovulation for cows with single ovulations was not different ($P = 0.39$) for cloprostenol sodium (18.0 ± 0.5 mm) vs. dinoprost tromethamine (18.6 ± 0.4 mm), respectively.

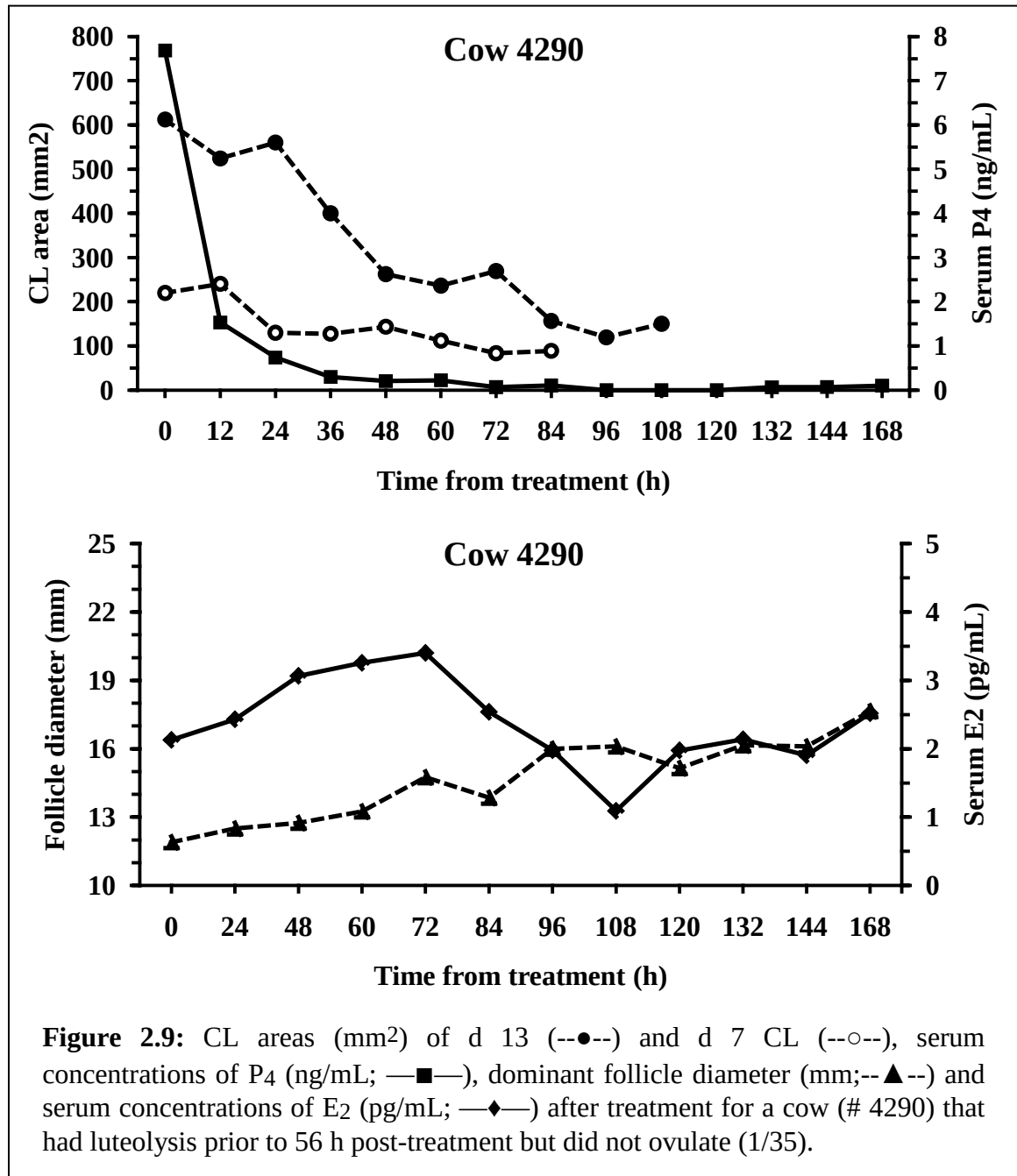
Seven cows did not have complete luteolysis. Two of the seven cows that did not have complete luteolysis had delayed luteolysis at 90 h and 114 h from treatment and ovulated 156 and 180 h after treatment, respectively, with intervals of 66 h between complete luteolysis and ovulation. Five of the seven cows that did not have complete luteolysis had a decrease in serum P₄ concentrations to < 1 ng/ml 36 h post-treatment. However, $n = 4$ of these cows subsequently had an increase in serum P₄ concentrations to over 1 ng/ml from 36 to 84 h after treatment, and $n = 1$ of these cows maintained sub-luteal concentrations (between 0.5 and 1 ng/ml) until 144 h post treatment. Example of a cow that had complete luteolysis is described in Figure 2.5 and examples of cows that did not have complete luteolysis by 56 h post-treatment are described in Figures 2.6, 2.7, 2.8 and 2.9.











Effect of Treatment on Subsequent Concentrations of E₂

Mean serum E₂ concentrations were greater ($P = 0.04$) at 48 h after treatment for cloprostenol sodium compared to dinoprost tromethamine (Figure 2.10). Serum concentrations of E₂ were lower ($P = 0.048$) at 84 h and 96 h ($P = 0.05$) in cloprostenol sodium vs. dinoprost tromethamine groups after treatment.

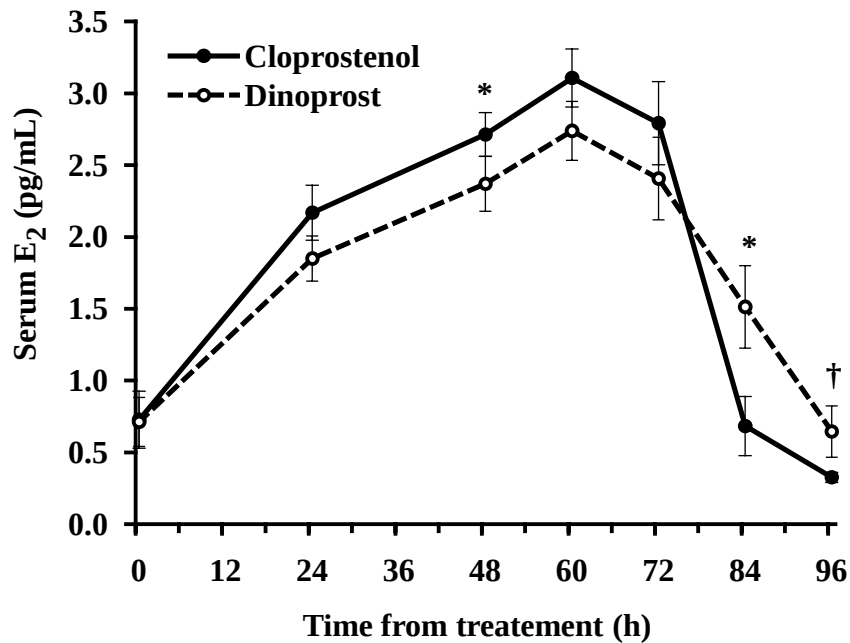
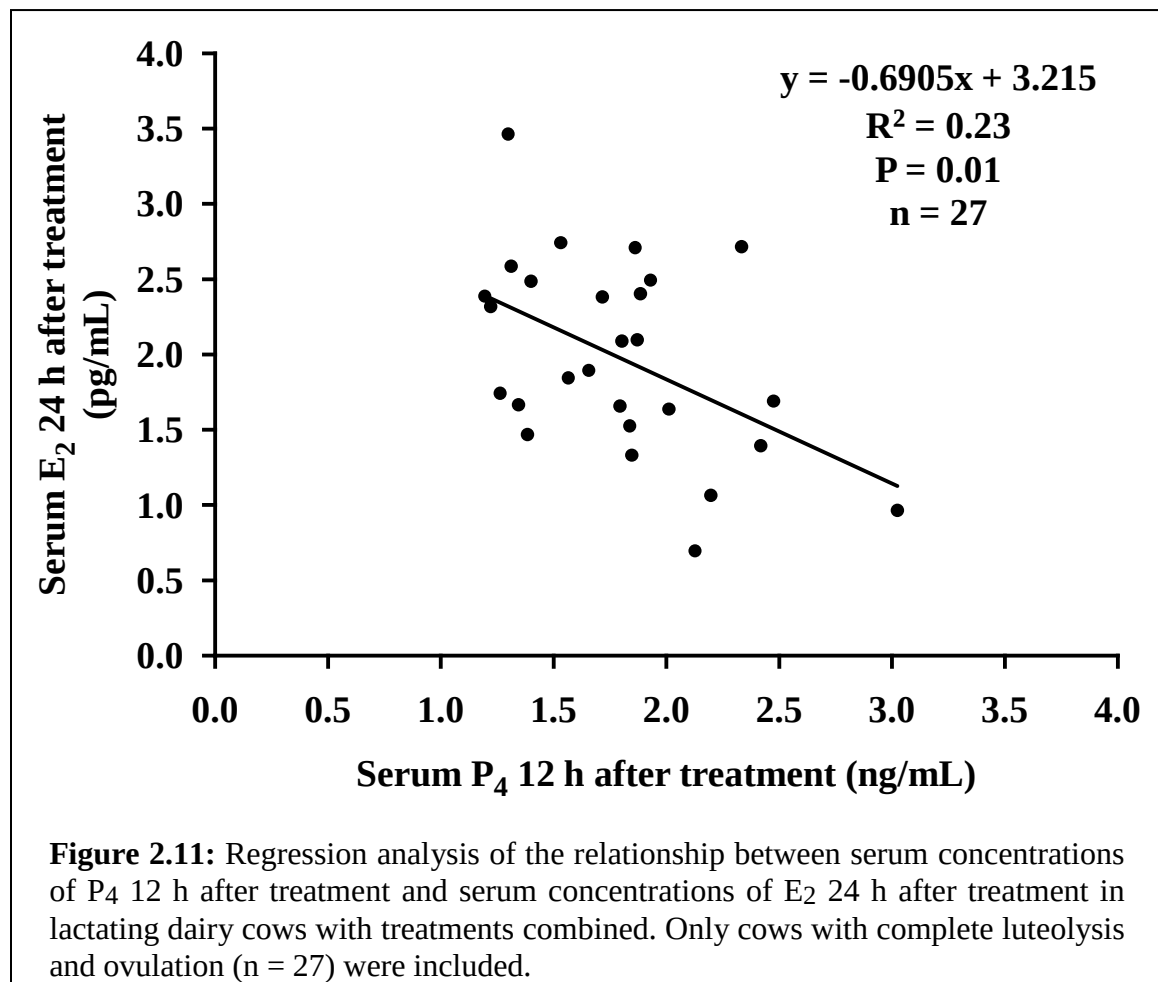


Figure 2.10: Mean ($\pm$ SEM) serum concentrations of E₂ (pg/mL) normalized to PGF_{2 α} treatment in lactating dairy cows with at least one d 7 and one d 13 CL and treated with cloprostenol sodium or dinoprost tromethamine that had complete luteolysis by 56 h after PGF_{2 α} and also ovulated via endogenous LH surge by 144 h after treatment (27/35). Mean serum E₂ concentrations were greater ($P = 0.04$) at 48 h after treatment for cloprostenol sodium compared to dinoprost tromethamine treated animals. Serum concentrations of E₂ were lower at 84 h ($P = 0.048$) and 96 h ($P = 0.05$) after treatment in cloprostenol sodium vs. dinoprost tromethamine groups.

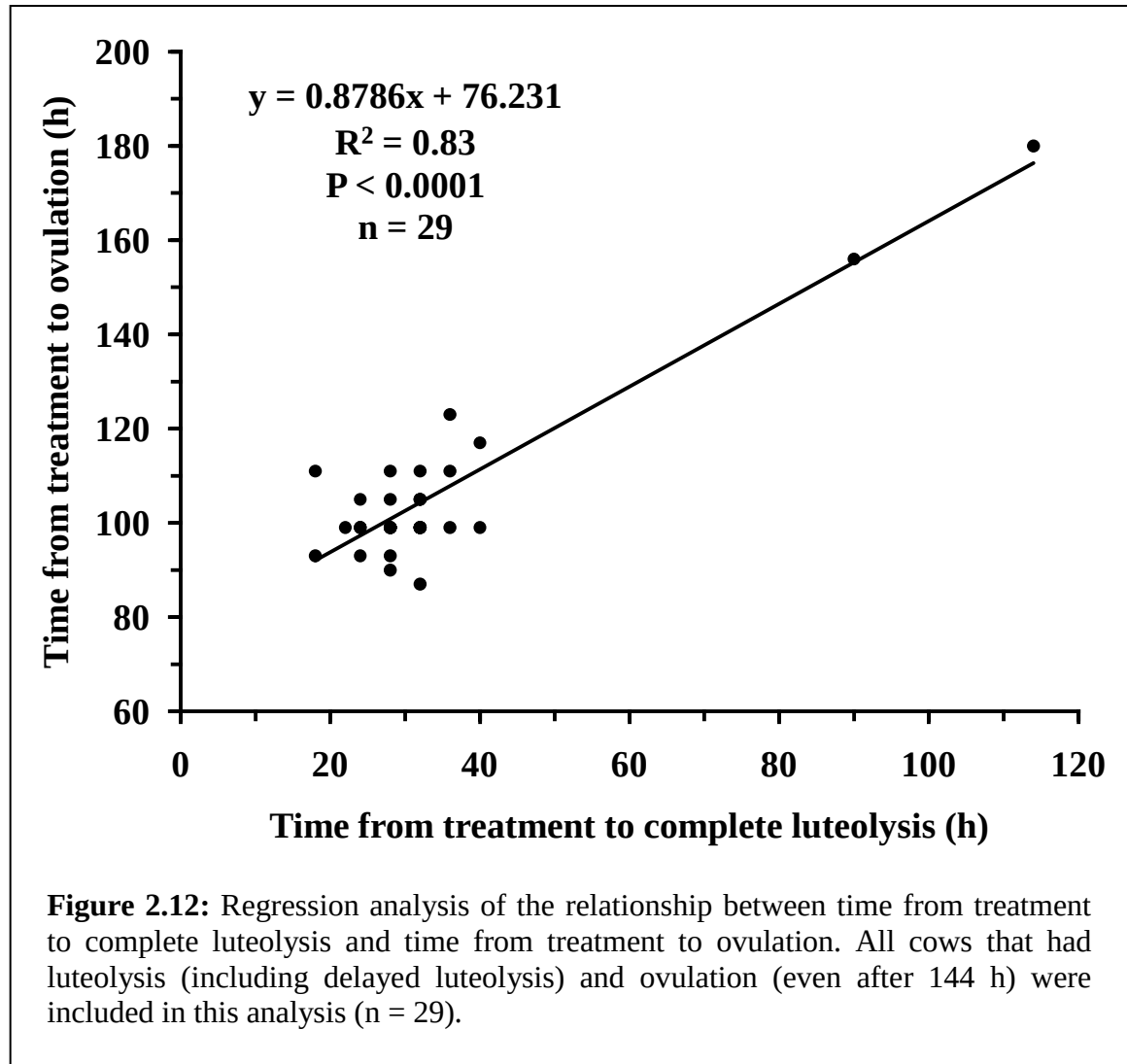
Dynamics of Luteolysis with Treatments Combined

Mean serum concentrations of P₄ decreased ($P < 0.0001$) from 6.54 ± 0.27 to 3.77 ± 0.22 ng/mL in the first hour after treatment. Subsequently, there was a significant

increase ($P < 0.0001$) in serum P₄ in the second hour after treatment from 3.77 ± 0.22 to 5.07 ± 0.31 ng/mL. Mean serum P₄ concentrations were greater ($P = 0.04$) from -24 to 12 h post-treatment for 1st compared to 3rd lactation cows and a tendency ($P = 0.05$) was observed for 1st compared to 2nd lactation cows. Second and 3rd lactation cows did not differ ($P = 0.5$) in mean serum P₄ concentrations during this period. There was also an effect of parity ($P = 0.04$) on mean serum P₄ 12 to 24 h post-treatment. First lactation cows had greater ($P = 0.01$) mean serum concentrations of P₄ compared to 2nd lactation cows. Regression analyses indicated that serum concentrations of E₂ at 24 h after PGF_{2 α} injection were greater in cows when their serum concentrations of P₄ at 12 h after PGF_{2 α} were lower ($P = 0.01$; Figure 2.11).



Time from treatment to ovulation was positively related ($P < 0.0001$) to time from treatment to complete luteolysis (Figure 2.12). The regression equation for prediction of time from treatment to ovulation in this study was $76.2 \text{ h} + (0.88 \times \text{time from treatment to complete luteolysis})$.



DISCUSSION

Results of the present study indicate that cloprostenol sodium enhanced the decrease in P₄ following induced luteolysis for the initial 12 h period compared to dinoprost tromethamine. Cloprostenol sodium's longer half-life is likely responsible for this difference. Cloprostenol sodium has an oxyaryl moiety structure that blocks ω -oxidation and the action of the dehydrogenase and reductase enzymes and reduces β -oxidation (Bourne et al., 1980). Therefore, cloprostenol is more resistant to endogenous metabolism and as a result has a much longer biological half-life ($t_{1/2} \sim 3$ h; Reeves, 1978) than dinoprost tromethamine ($t_{1/2} \sim 7$ -8 min; Kindahl et al., 1976a). Approximately 65% of dinoprost tromethamine is metabolized in one passage through the lungs in the cow (Davis et al., 1985; McCracken et al., 1999). Hence, cloprostenol is maintained in circulation longer and may be a more potent luteolytic agent.

Subsequent concentrations of E₂ were also enhanced following treatment with cloprostenol sodium compared to dinoprost tromethamine. Although cause-effect is difficult to prove without LH pulsatility data in the present study, a study by Bergfeld et al., 1996, indicated that a more rapid decrease in P₄ increased LH pulsatility, which in turn enhanced E₂ production of the dominant follicle. Studies that have compared cloprostenol sodium to dinoprost tromethamine show either no difference in conception rates following treatment or a slight increase in favor of cloprostenol sodium. This may explain the positive effect of cloprostenol sodium on estrus expression in beef heifers (Sudweeks et al., 1983), which could be attributed to greater circulating concentrations of E₂ following treatment. When treatments were combined there was a significant

relationship between the level of P₄ in cows 12 h after treatment and subsequent concentrations of E₂ in the same cows 24 h post-treatment.

The intent of this study was to determine if a PGF_{2α} product with a longer half-life could enhance induced luteolysis of cows treated with Ovsynch. Luteolysis is one of several key steps in Ovsynch that is limiting to fertility in cows that are timed-inseminated following Ovsynch. Previous data from our laboratory indicated that initiating Ovsynch on d 6 of the estrous cycle increased P₄ prior to PGF_{2α} of Ovsynch and more consistently controlled growth of the ovulatory follicle (Bello et al., 2006). In that study, both level of P₄ prior to PGF_{2α} of Ovsynch and size of the ovulatory follicle at time of final GnRH of Ovsynch were key factors in predicting the probability of a pregnancy (Bello et al., 2006). These data also indicated that the greater the ovulation rate to the 1st GnRH of Ovsynch the greater the % of cows with *accessory* CL and increased P₄ (Bello et al., 2006). The three most widely used pre-synchronization strategies on dairy farms today, 14/11 Presynch (Galvão et al., 2007), G6G (Bello et al., 2006), and Double Ovsynch (Souza et al., 2008) all increase the % of cows with 1st wave dominant follicles that will have a greater likelihood of responding to the 1st injection of GnRH of Ovsynch vs. Ovsynch alone. Thus, by the time of the PGF_{2α} of Ovsynch most cows that were pre-synchronized with these strategies would have a mature and an *accessory* CL that would still be functional at time of induced luteolysis, and prior to endogenous luteolysis. In the current study, we chose to ensure that all cows in this study had at least 1 d 13 and 1 d 7 CL at time of treatment to test potential differences in cloprostenol sodium vs. dinoprost tromethamine in the rate of decrease of P₄ in cows in this scenario. Our findings indicate that most cows had complete luteolysis by our

definition with a single dose of cloprostenol sodium or dinoprost tromethamine. The only differences in this experiment between these two luteolytic agents were decreases in P₄ in the initial 12 h and an increase in E₂ 48 h post-treatment. Otherwise there were no differences uncovered in this study. Six cows in the present study had double ovulations either following the pre-synchrony injection of GnRH or the 1st GnRH of Ovsynch, n = 4 were in the cloprostenol sodium group and n = 2 were in the dinoprost tromethamine group. Of the n = 7 cows in this current study that did not have complete CL regression by our definition, only n = 1 cow had > 2 CL. It appeared that n = 4 d 13 CL and n = 3 d 7 CL did not completely regress, or had delayed luteolysis, as interpreted via decrease in luteal areas (mm²). So it does not appear that it was only the potentially immature CL that did not respond to treatment.

There were too few cows in the present study to determine differences in % of cows that did not have complete luteolysis. In Chapter 3 we report the efficacy of induced CL regression with cloprostenol sodium vs. dinoprost tromethamine in a much greater number of cows.

Time to complete functional luteolysis had a tendency to have a positive association with time to ovulation. The majority of cows had approximately a 60 to 80 h interval between the time that they had P₄ concentrations below 0.5 ng/mL and ovulation. Cows that did not drop P₄ concentration to lower than 0.5 ng/mL 56 h post-treatment did not experience ovulation of the dominant follicle. These follicles were most likely functional at time of PGF_{2α} due to an increase in E₂ in each of these cows following treatment and the lack of a new follicular wave in the days that followed. Therefore, subluteal serum concentrations of P₄ (between 0.5 and 1.0 ng/mL) appeared to exert a

negative feedback on the hypothalamus or pituitary, resulting in a suppression of the spontaneous LH surge and ovulation. However, in Ovsynch programs, if luteolysis does not happen in this expected time, as seen in some cows in our experiment, it may negatively influence conception rates even though cows are administered a GnRH-induced LH surge to ovulate this dominant follicle (Souza et al., 2007; Brusveen et al., 2009).

There was a significant decrease in P4 levels in the first hour after treatment then a rebound at the second hour for each cow. Ginther et al. (2007) observed a similar rebound in P4 concentrations in dairy heifers. This may be due to an acute increase in oxytocin. Studies indicated that PGF_{2α} injection acutely stimulated the release of oxytocin in vivo (Flint and Sheldrick, 1982; Ohtani et al., 1998; Shaw and Britt; 2000; and Keator et al., 2008) approximately 10 fold within 15 minutes. Oxytocin has a vasoconstrictive action in high doses in mammary gland capillaries of other species (Cross and Silver, 1962; Davis et al., 1995) and may also contract luteal capillaries during this first hour of acute release inhibiting the liberation of P4 into circulation resulting in lower serum P4 concentrations one hour after treatment. After this first hour, oxytocin concentration likely declined to basal levels allowing P4 to be released into circulation. Prostaglandin F_{2α} is also a vasoconstrictive agent and may also have the same effect on luteal capillaries during the first hour after the injection of PGF_{2α}. In the current study, all cows had a rebound in serum P4 concentrations at the second hour after treatment then all cows had a consistent decrease in P4, with the exception of the few cows that did not have complete CL regression.

CONCLUSION

Cloprostenol sodium treatment resulted in a greater decrease in serum P₄ concentrations during the 1st 12 h following treatment compared to dinoprost tromethamine administration. However, there were no differences in the decrease in P₄ from 12 h until 144 h after treatment. The initial difference in the decrease in P₄ during the 1st 12 h appeared to result in an increase in serum E₂ concentration in cows treated with cloprostenol sodium 48 h after treatment.

CHAPTER 3

EFFECTS OF CLOPROSTENOL SODIUM VS. DINOPROST TROMETHAMINE AT FINAL PGF_{2α} OF OVSYNCH ON COMPLETE LUTEOLYSIS AND CONCEPTION RATES IN LACTATING DAIRY COWS

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ABSTRACT

Luteolysis is a key event in Ovsynch programs of lactating dairy cows. Studies indicate that insufficient luteolysis is a rate-limiting factor for successful conception rates (CR) following Ovsynch. There are two PGF_{2α} products approved in the US for luteolysis, dinoprost tromethamine and cloprostenol sodium. Dinoprost tromethamine has a shorter half-life ($t_{1/2}$) of ~ 7-8 minutes while cloprostenol sodium is more resistant to endogenous metabolism and maintains a higher circulating concentration for a longer time ($t_{1/2}$ = 3 hours). Our objective in this study was to determine the effect of cloprostenol sodium vs. dinoprost tromethamine treatment at PGF_{2α} of Ovsynch on CR (%) and % of cows with complete luteolysis in lactating dairy cows within a presynchronization + Ovsynch program for 1st artificial insemination (AI; n = 651) and an Ovsynch resynchronization program for 2nd + AI (n = 394). Blood samples were collected daily for 5 d beginning at the PGF_{2α} of Ovsynch in a subset of cows for 1st and 2nd + AI to measure circulating concentrations of progesterone (P₄) and estradiol (E₂). Percentage of cows with functional CL that had complete luteolysis after treatment did not differ between cloprostenol sodium and dinoprost tromethamine in 1st (P = 0.42; 79 vs. 80%; respectively) and 2nd + AI (P = 0.39; 70 vs. 72%; respectively). In addition, daily rate of decrease in serum concentrations of P₄ did not differ (P = 0.77) between products. Conception rates of cows treated with cloprostenol sodium or dinoprost tromethamine also did not differ between products for 1st (P = 0.17; 40 vs. 35%; respectively) and 2nd + AI (P = 0.63; 23 vs. 21%; respectively). Cows with greater serum P₄ concentrations at time of PGF_{2α} of Ovsynch had a greater probability of complete luteolysis after PGF_{2α} of Ovsynch (P < 0.0001) and pregnancy at 39 d after timed AI (P <

0.0001). Serum concentrations of E₂ at 56 h after PGF_{2α} of Ovsynch was also a positive predictor of pregnancy at 39 d after timed AI (P = 0.02). In summary, luteolytic response and CR were not different in cows treated with cloprostenol sodium or dinoprost tromethamine when used in the Ovsynch program. Cows with greater serum concentrations of P₄ at time of PGF_{2α} of Ovsynch appeared to have a greater chance for luteolysis and pregnancy. Cows with greater concentration of E₂ at time of final GnRH of Ovsynch also appeared to have a greater chance for pregnancy.

INTRODUCTION

Luteolysis is a key event in Ovsynch programs of lactating dairy cows. Studies indicate that insufficient luteolysis is a rate-limiting factor for successful conception rates (CR) following Ovsynch (Souza et al., 2007; Brusveen et al., 2009). Data from Souza et al. (2007) indicated that cows with progesterone (P₄) concentrations > 0.5 ng/mL 2 d after PGF_{2α} injection of Ovsynch had 50% lower CR compared to cows < 0.5 ng/mL. The percentage of cows that do not have complete luteolysis following PGF_{2α} of Ovsynch varies among studies, ranging from 5 to 20% (Pursley et al., 1997b; Moreira et al., 2000; Gümen et al., 2003; Brusveen et al., 2009). In addition to the importance of complete luteolysis, the timing of P₄ decrease after PGF_{2α} injection may play a critical role in Ovsynch success. Cows with complete luteolysis that had a more rapid drop in circulating P₄ concentrations were more fertile than those with a slower decline in P₄ levels (Brusveen et al., 2009). The mechanism(s) involved in how sub-luteal concentrations of P₄ (between 0.5 and 1.0 ng/mL) 2 d following the PGF_{2α} of Ovsynch

reduce CR are not clear. It is likely not due to lack of ovulation since the final GnRH of Ovsynch causes ovulation 97% of the time (Pursley et al., 1995).

Dinoprost tromethamine, a tromethamine salt of the natural $\text{PGF}_{2\alpha}$, was the luteolytic agent utilized in the above studies. Dinoprost tromethamine has a short half-life ($t_{1/2} \sim 7 - 8$ min; Kindahl et al., 1976a) and is rapidly metabolized similar to endogenous $\text{PGF}_{2\alpha}$ metabolism (Bourne et al., 1980; Davis et al., 1985; McCracken et al., 1999). Approximately 65% of dinoprost tromethamine is metabolized in one passage through the lungs of the cow (Davis et al., 1985). A recent study indicated that a second injection of dinoprost tromethamine 24 h after the first injection improved the percentage of cows that had complete luteolysis (Brusveen et al., 2009). A more potent synthetic analogue of $\text{PGF}_{2\alpha}$, cloprostenol sodium (Cooper, 1974; Cooper and Furr, 1974; Dukes et al., 1974), is also commercially available in the US. Cloprostenol sodium has an oxyaryl moiety function that reduces rate of metabolism (Bourne et al., 1980), and thus it is more resistant to endogenous metabolism and has a much longer half-life ($t_{1/2} \sim 3$ h; Reeves, 1978) compared to dinoprost tromethamine. Studies have compared the differences in luteolysis and fertility between these two drugs in lactating dairy cows with mixed results (Seguin et al., 1985; Martineau, 2003; Répási et al., 2005). Yet, it is not clear whether differences exist between these two luteolytic agents in new Ovsynch programs that intend to initiate 1st GnRH of Ovsynch during the first follicular wave when dominant follicles are responsive to a GnRH-induced LH surge. Cows that were synchronized prior to Ovsynch to allow the first injection of GnRH at d 6 or 7 of the estrous cycle generally have at least two CL at the time of the $\text{PGF}_{2\alpha}$ of Ovsynch if the GnRH induced-LH surge caused ovulation (Bello et al., 2006). It appeared that greater numbers of CL at

time of luteolysis increased P₄ (Bello et al., 2006; Stevenson et al., 2007) and cows with greater P₄ had a greater probability of pregnancy (Bello et al., 2006). Thus, in this scenario, at least one young *accessory* CL (7 d old) and an older CL (d 13 or 14) will require luteolysis (Bello et al., 2006; Stevenson et al., 2007) and more if double ovulations occur (Fricke and Wiltbank, 1999; Wiltbank et al., 2000).

The overall objectives of this study were to: 1) determine the effect of two distinctly different PGF_{2α} products, cloprostenol sodium and dinoprost tromethamine, during Ovsynch on rate of luteolysis and conception rates, and 2) determine the effect of P₄ concentrations at time of, and following, treatment on luteolysis and fertility in lactating dairy cows. We hypothesized that cloprostenol sodium would reduce circulating concentrations of P₄ faster than dinoprost tromethamine, increase the percentage of cows with complete luteolysis due to its pharmacokinetic characteristics, and subsequently improve conception rates of lactating dairy cows.

MATERIALS AND METHODS

This trial was conducted from January to August of 2008 at Green Meadow Farms in Elsie, Michigan. Lactating Holstein cows (n= 862) with milk production between 11.8 and 71.2 kg / d received a total of n = 1,046 artificial inseminations (AI; n = 652 1st AI, n = 394 2nd + AI). Cows were housed in a free-stall barn with free access to water and fed a TMR three times daily. Cows were separated in pens (n = 4) by parity (1st, 2nd and two pens for 3rd + parities). The TMR consisted of corn and alfalfa silages and corn-soybean meal-based concentrates formulated to meet or exceed nutrient recommendations for lactating dairy cows (NRC, 2001). Cows were milked three times

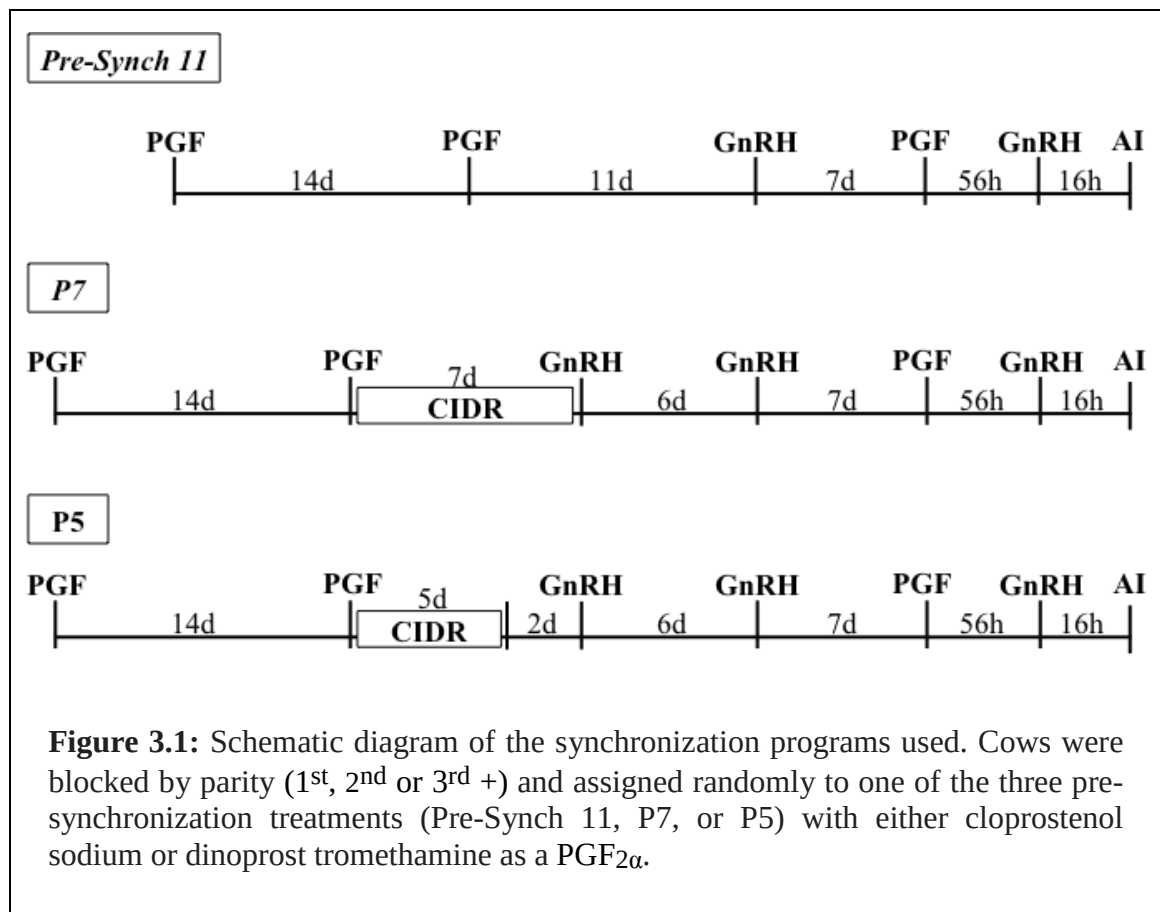
daily. All injections were administered with single dose syringes in semimembranosus or semitendinosus muscles of cows by trained personnel from our laboratory with 18-gauge (injections of PGF_{2α}) or 20-gauge (injections of GnRH) 3.8 cm needles. The Institutional Animal Care and Use Committee (IACUC) at Michigan State University approved all procedures.

Experimental Design

Synchronization for 1st AI

Lactating Holstein cows (n = 652) were blocked by parity (1st, 2nd or 3rd +) then randomly assigned to treatment on a weekly basis to receive either 25 mg of dinoprost tromethamine (Lutalyse, Pfizer Animal Health, Kalamazoo, MI) or 500 µg of cloprostenol sodium (Estrumate, Schering Plough Animal Health, Summit, NJ) for each PGF_{2α} injection of a pre-synchronization and Ovsynch program. Cows received one of three pre-synchronization protocols (Figure 3.1) beginning 33 to 41 DIM: 1) Pre-Synch 11: two injections of PGF_{2α} 14 d apart with the 2nd injection 11 d (Galvão et al., 2007) before the 1st GnRH of Ovsynch (Galvão et al., 2007; Brusveen et al., 2008), 2) P7: two injections of PGF_{2α} 14 d apart and an intravaginal progesterone releasing device (CIDR; Pfizer Animal Health, Kalamazoo, MI) inserted at time of the 2nd PGF_{2α} for 7 d. GnRH (100 µg) was administered upon CIDR removal. The 1st GnRH of Ovsynch was administered 6 d later (Martins et al, 2009), 3) P5: two injections of PGF_{2α} 14 d apart and a CIDR inserted at time of the 2nd PGF_{2α} for 5 d. GnRH (100 µg) was administered 2 d after CIDR removal. The 1st GnRH of Ovsynch was administered 6 d later (Martins et al., 2009). All cows received an Ovsynch program that consisted of 100 µg GnRH followed in 7 d with PGF_{2α} (treatment) then 100 µg GnRH 56 h later (Brusveen et al.,

2008). All cows received AI 16 h following the final GnRH of Ovsynch (Pursley et al., 1998) 70 to 76 DIM. Cows with signs of uterine disorders prior to AI or other signs of acute illness were excluded from the experiment. Five AI technicians performed AI with commercial semen from multiple sires purchased by the farm. Technicians were blind to treatments. Pregnancy diagnoses were performed by transrectal palpation 39 d after AI by farm veterinarians that were also blind to treatments. A second pregnancy diagnosis was performed 99 ± 3 d after AI in cows determined to be pregnant at the initial diagnosis. All cows that were detected in estrus prior to first pregnancy diagnosis were considered to not be pregnant.



Resynchronization of Non-Pregnant Cows

All cows on this farm that received AI (1st and greater) and were not re-inseminated following detected estrus received 100 µg GnRH 32 ± 3 d post AI to initiate an Ovsynch resynchronization program. Cows diagnosed not pregnant 7 d later (n = 394) were randomly assigned by parity and service number to receive 25 mg dinoprost tromethamine or 500 µg cloprostenol sodium. Cows received an injection of 100 µg GnRH 56 h after PGF_{2α} (treatment) of Ovsynch and AI was performed approximately 16 h later. Cows received AI by the same technicians and service sires and were diagnosed for pregnancy as in 1st AI cows.

Analysis of Luteal Function

To determine the effect of PGF_{2α} type on % cows with complete luteolysis and conception rate (%) a subset of cows with functional CL were considered in the analyses. Functional CL were defined as cows with decreasing concentrations of P₄ from treatment to 24 h after treatment but maintained concentrations ≥ 0.24 and 0.09 ng/mL 24 and 56 h after treatment, respectively. These cutoff points were defined based on the study in Chapter 2 that measured P₄ concentrations prior to and after treatment in cows with known functional CL. In that study, cows with functional CL at time of PGF_{2α} (n = 35) did not have P₄ levels below these cutoff points. Cows that had P₄ concentrations below these cutoff points were already undergoing luteolysis at time of treatment based on P₄ prior to and at time of PGF_{2α}.

Cows with increasing concentrations of P₄ from treatment to 24 and 56 h following treatment were defined as cows with early CL (e.g., d 1 – 3) and were not included as functional CL. Regardless of concentrations of P₄, cows that decreased

between d of treatment and 24 h later and did not decrease below the cutoff points were considered to have functional CL. Cows with no P₄ on d of treatment were defined as non-cycling cows (no functional CL). Only cows defined as having functional CL were included in the calculation for the effect of treatment on % of cows with complete luteolysis (n = 490). Complete induced luteolysis was defined as cows with declining P₄ concentrations to lower than 0.5 ng/mL 56 h after PGF_{2α} and a continued decrease to < 0.5 ng/mL 72 and 96 h after PGF_{2α} injection. Previous studies indicated that cows with > 0.5 ng/mL P₄ 2 d after PGF_{2α} had significantly reduced chances of a pregnancy (Souza et al., 2007; Brusveen et al., 2009).

P₄ and E₂ Assays

Blood samples were collected by coccygeal venipuncture in a subset of 1st and 2nd + AI cows (n= 680) at final PGF_{2α} of Ovsynch and daily for 4 d to assess P₄ and E₂ concentrations. Blood samples were taken using vacutainer tubes without anticoagulant (BD Vacutainer, Preanalytical Solutions, Franklin Lakes, NJ) and refrigerated for 6 to 12 h. Serum was then separated by centrifugation at 2000 x g for 20 min at 4 °C and stored at -20 °C for later hormonal analyses.

Concentrations of P₄ were quantified in serum samples by RIA (Count-A-Count Progesterone, Siemens Diagnostics, Los Angeles, CA). Intra- and inter-assay CVs were 4.9 and 3.2% respectively. Sensitivity of the assay was 0.02 ng/mL.

Concentrations of E₂ were quantified from blood collected on the day of PGF_{2α} and the next 3 d in a subset of 1st AI cows that had functional CL and complete luteolysis (n = 192). Serum samples (500 µL) were ether extracted in duplicate and then measured using a modified version (Prendiville et al., 1995) of a commercially available RIA

MAIA kit (Polymedco Inc., Cortland Manor, NY). Intra- and inter-assay CVs were 13.9 and 11.5% respectively. Sensitivity of the assay was 0.5 pg/mL.

Statistical Analyses

Binomial variables were analyzed using the MIXED procedure of SAS (Version 9.2, SAS Inst. Inc., Cary, NC). The final model considered treatment, parity, and their interactions as fixed effects and cows as a random effect. There was no treatment x pre-synchronization program interaction ($P = 0.9$), thus, data from the 3 different pre-synchronization programs were combined.

Repeated measures variables such as concentrations of P4 and E2 over time were analyzed using the MIXED procedure of SAS with the REPEATED statement and cows nested in treatment specified in the SUBJECT option. Fit statistic parameters were tested in the MIXED procedure. The covariance structure with lowest values for the Bayesian information criterion (BIC) was used for the analyses. Predicted probabilities of pregnancy were computed using the LOGISTIC procedure of SAS.

Data were tested for normality of residuals with the Shapiro-Wilk test and/or studentized residual plots for each variable. Variables that did not fulfill assumptions for normality were transformed by natural log and re-analyzed. For clarity, actual means of the data are presented.

RESULTS

Effect of Cloprostenol Sodium vs. Dinoprost Tromethamine on Luteolysis

There were no differences in PGF_{2α} types on the decrease in concentrations of P4 measured daily during the 4 d period following treatment ($P = 0.46$) or the rate of

decrease in cows with complete luteolysis following treatment (Figure 3.2; $P = 0.77$). There were no differences in the % of cows with complete luteolysis overall ($P = 0.42$) or when divided into quartiles based on P_4 at time of treatment for first service (Table 3.1; $P > 0.1$) for cows administered the two $PGF_{2\alpha}$ types. When treatments were combined, only 79% 1st AI cows ($n = 258$) and 71% of 2nd + AI cows ($n = 232$) had complete luteolysis following treatment with a single dose of cloprostenol sodium or dinoprost tromethamine.

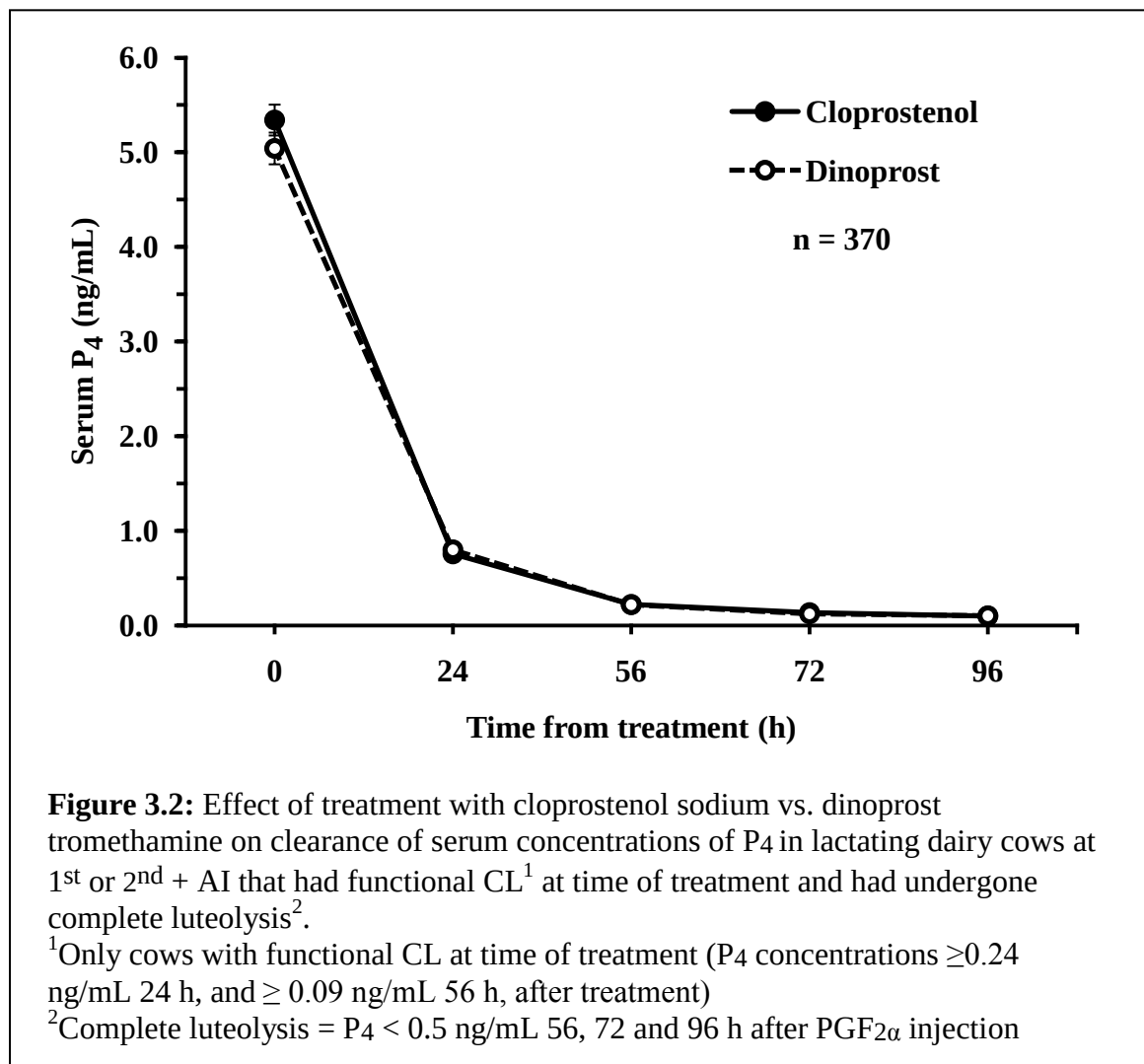


Table 3.1: Effect of treatment with cloprostenol sodium or dinoprost tromethamine on % of cows with complete luteolysis and % pregnant in a subset of lactating dairy cows at 1st AI (n=258) with functional CL¹ at time of treatment within quartiles of concentrations of P₄ on day of treatment.

	Quartile based on P ₄ at day of PGF _{2α} for first service									
	0 – 25%		25 -50%		50 – 75%		75 – 100%		TOTAL	
	CLO	DINO	CLO	DINO	CLO	DINO	CLO	DINO	CLO	DINO
Range P ₄ at day of PGF _{2α} (ng/mL)	0.64 – 3.78	1.00 – 3.59	3.85 – 5.26	3.60 – 5.03	5.31 – 6.51	5.13 – 6.28	6.55 – 13.74	6.38 – 13.23	0.64 – 13.74	1.00 – 13.23
P ₄ mean at day of PGF _{2α} (± SEM ²)	2.54 (0.14)	2.29 (0.15)	4.50 (0.06)	4.51 (0.07)	5.91 (0.07)	5.71 (0.06)	8.09 (0.27)	7.84 (0.28)	5.24 (0.20)	5.06 (0.20)
Cows with complete luteolysis ³ (%)# (n/n)	52 (17/33)	67 (22/33)	91 (29/32)	81 (26/33)	81 (26/32)	84 (26/31)	94 (30/32)	91 (29/32)	79 (102/129)	80 (103/129)
CR 39-42 d (%)# (n/n)	21 (7/33)	24 (8/33)	50 (16/32)	39 (13/33)	31 (10/32)	35 (11/31)	66 (21/32)	56 (18/32)	42 (54/129)	38 (50/129)
CR 39-42 d (%) of cows w/complete luteolysis (%)# (n/n)	41 (7/17)	36 (8/22)	55 (16/29)	50 (13/26)	38 (10/26)	38 (10/26)	70 (21/30)	59 (17/29)	53 (54/102)	48 (48/103) ⁴
Primiparous (%)#	39	30	38	33	41	29	53	62	43	40
¹ P ₄ concentrations ≥ 0.24 ng/mL 24 h, and ≥ 0.09 ng/mL 56 h, after treatment. ² SEM = standard error of the mean. ³ Complete luteolysis = P ₄ < 0.5 ng/mL 56, 72 and 96 h after PGF _{2α} injection #No differences between treatments (P > 0.05) ⁴ Two cows that did not have complete luteolysis by our definition conceived.										

Effect of Cloprostenol Sodium vs. Dinoprost Tromethamine on Conception Rates and Pregnancy Losses

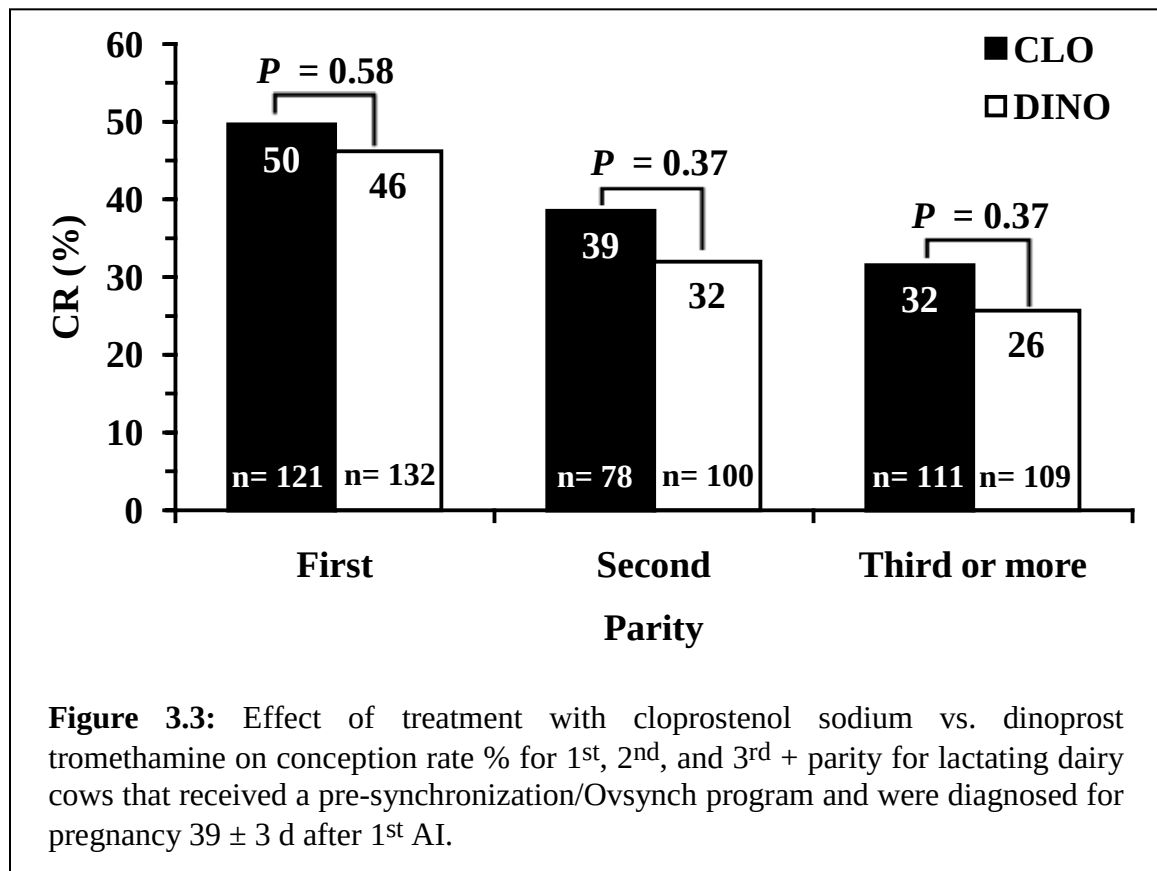
There were no differences in conception rates in cows treated with cloprostenol sodium vs. dinoprost tromethamine at the final PGF_{2α} of Ovsynch that was preceded by a pre-synchronization program for 1st AI or following an Ovsynch resynchronization program for 2nd + AI when diagnosed at 39 or 96 d post-AI (Table 3.2). In addition, there were no differences in % of pregnancies lost between 39 and 96 d post-AI (Table 3.2). There were no differences in conception rates across PGF_{2α} types within 1st, 2nd, or 3rd + parities (Figure 3.3). With treatments combined, 1st parity cows had greater conception rates compared to 2nd and 3rd + parity cows ($P < 0.01$).

Table 3.2: Effect of treatment with cloprostenol sodium vs. dinoprost tromethamine on conception rate (CR) % and pregnancy loss % in lactating dairy cows between first and second pregnancy diagnosis for 1 st and 2 nd + AI.						
	1st AI			2nd + AI		
	CLO	DINO	<i>P</i> -value	CLO	DINO	<i>P</i> -value
CR 39-42 d (%)	40	35	0.17	23	21	0.63
(n/n)	(125/310)	(121/341)		(49/213)	(38/181)	
CR 96-103 d (%)	37	33	0.23	20	20	0.95
(n/n)	(114/307)	(112/340)		(42/212)	(35/179)	
Pregnancy loss between 39-42 and 96-104 (%)	7	7	0.95	13	3	0.11
(n/n)	(8/122)	(8/120)		(6/48)	(1/36)	

Effect of Pre-Synchronization Program on 1st AI Conception Rates

There were no differences ($P = 0.5$) in conception rates 39 d post-AI for cows treated with Pre-Synch 11, P7 and P5 (39%, 37%, and 34%; respectively) for 1st AI. Conception rates 96 d post-AI also did not differ ($P = 0.43$) for cows treated with

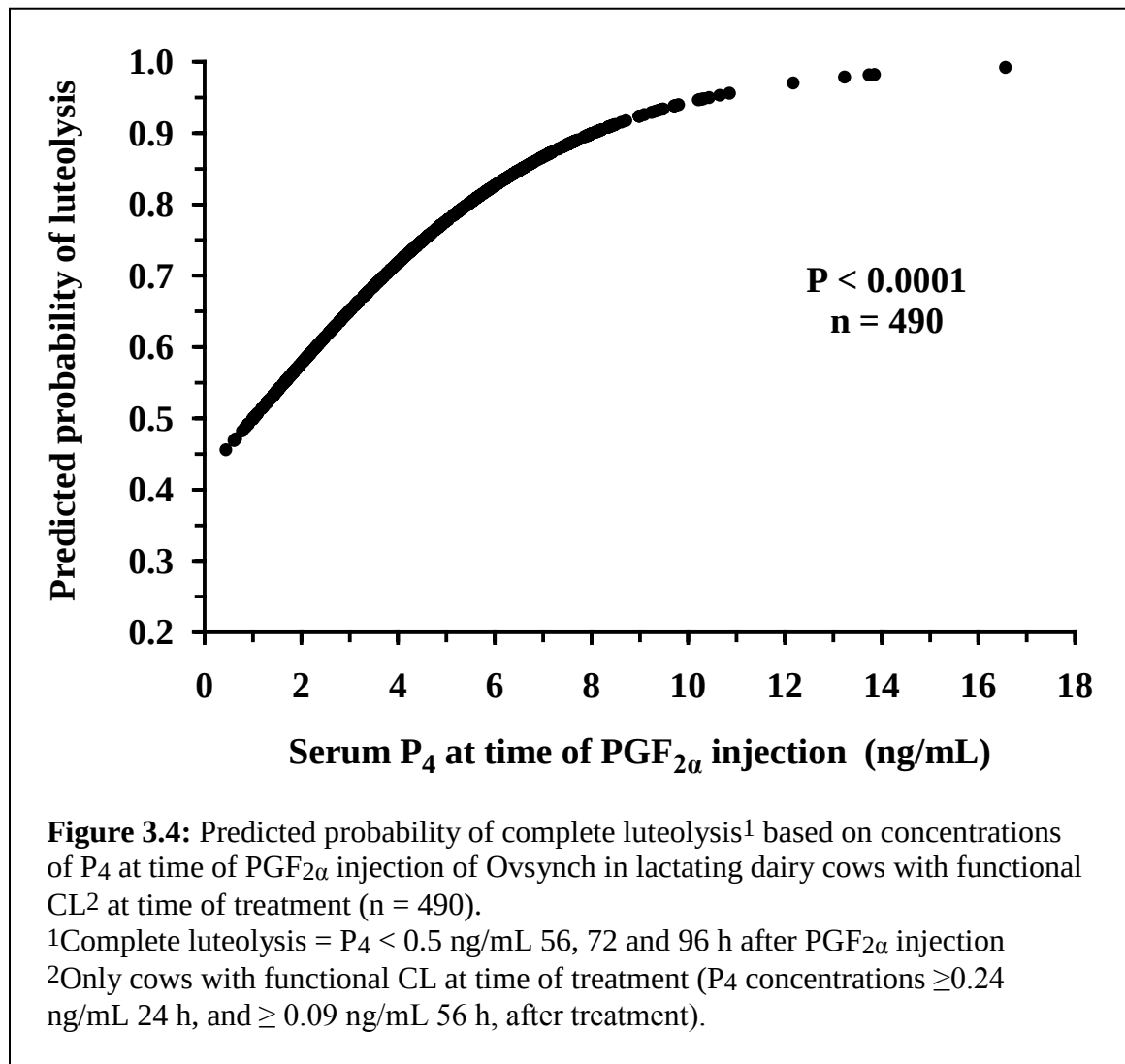
different pre-synchronization programs (37% for Pre-Synch 11; 33% for P7; and 32% for P5).

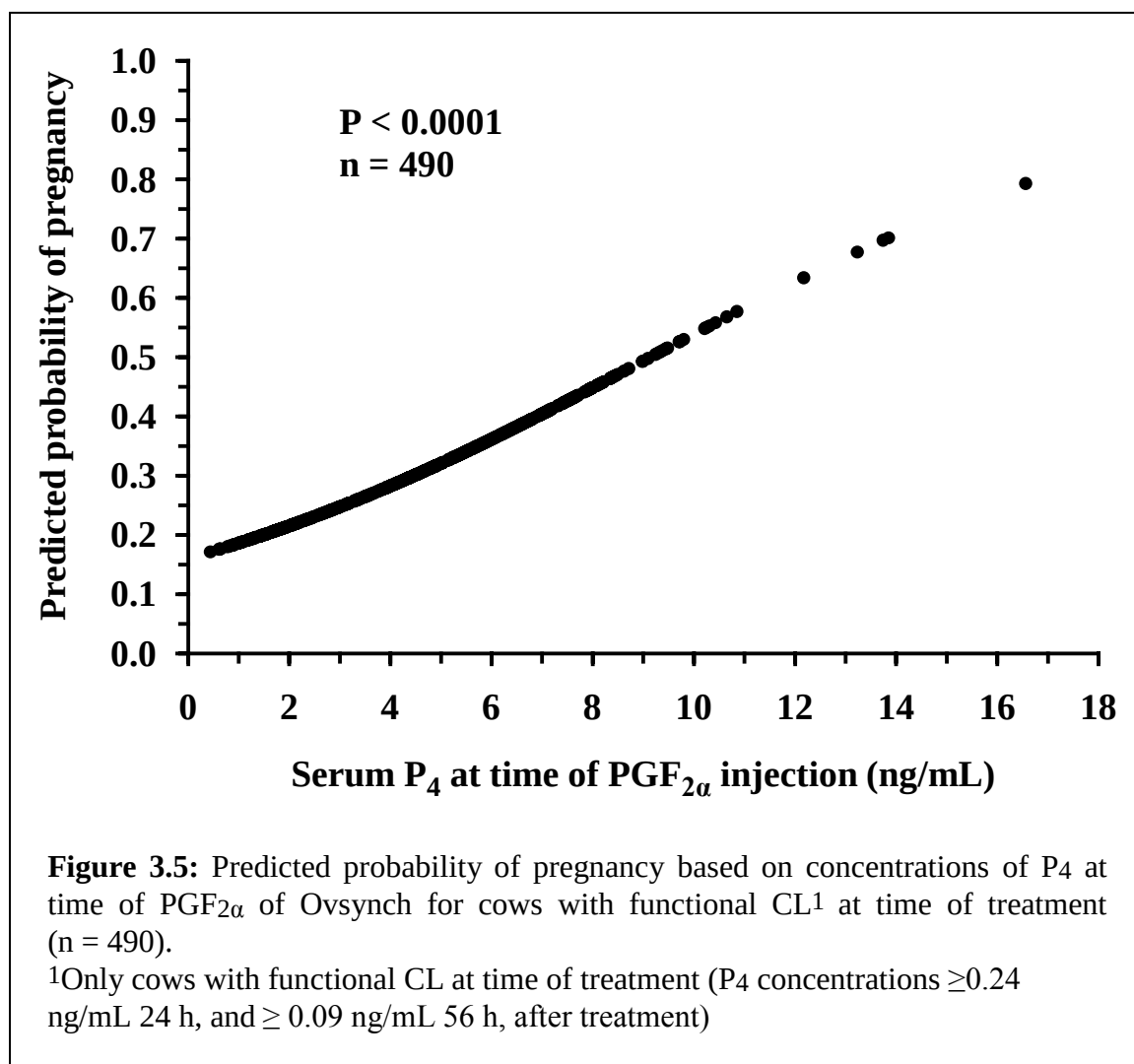


Relationships of Serum Concentrations of P₄ at Treatment with Luteolysis and Pregnancy

There was a highly significant relationship ($P < 0.0001$) between concentrations of P₄ at PGF_{2α} of Ovsynch and the predicted probability of luteolysis (Figure 3.4) in cows with functional CL at time of treatment. The greater the concentrations of P₄ the greater chance for complete luteolysis. There was also a highly significant relationship ($P < 0.0001$) between concentrations of P₄ at time of treatment and the probability of pregnancy in cows with functional CL at time of treatment including the cows that did not have complete luteolysis (Figure 3.5). When only considering cows with complete

luteolysis, there was only a trend ($P = 0.08$) for a positive relationship of P_4 at time of treatment with the probability of a pregnancy (Figure 3.6). There was a greater ($P < 0.001$) % of pregnant cows that were classified as having > 6 ng/mL of P_4 at time of treatment compared to non-pregnant cows (Table 3.3). There was a greater ($P < 0.01$) % of non-pregnant cows that were classed as having between 1 – 2 ng/mL P_4 at time of treatment compared to pregnant cows (Table 3.3). Only cows with functional CL at time of treatment were included in Table 3.3.





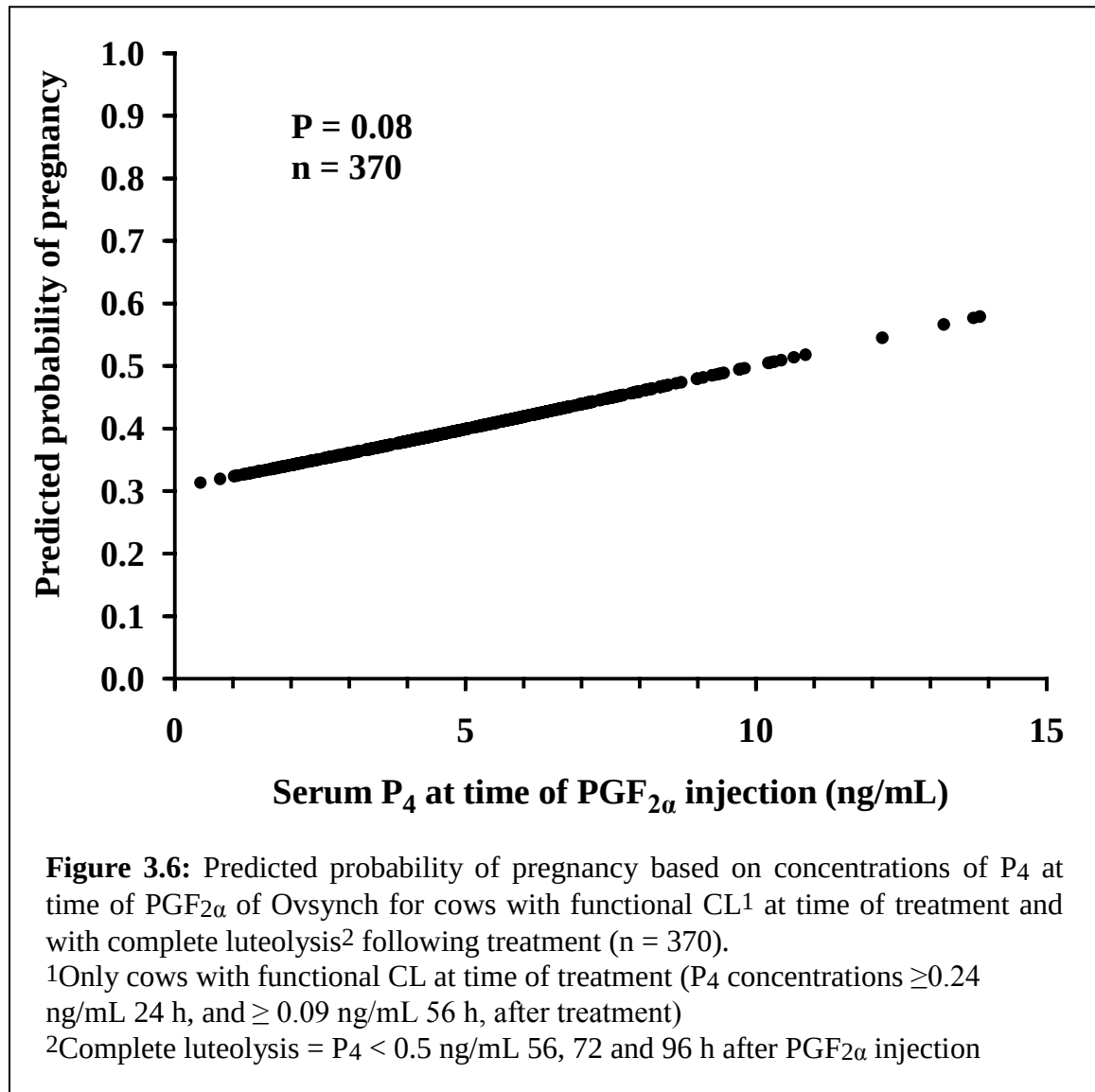


Table 3.3: Distribution (% of total) of pregnant and non-pregnant lactating dairy cows across ranges in P₄ concentrations at time of treatment with cloprostenol sodium vs. dinoprost tromethamine (treatments combined).¹

Time of PGF _{2α} injection (h)	Pregnant status 39 ± 3 d after AI	Progesterone range (ng/mL)							Total	n
		0 - 1	1 - 2	2 - 3	3 - 4	4 - 5	5 - 6	> 6		
0	n	8	56	60	53	90	77	146		490
	Not pregnant (%)	2.1	14.1	13.8	11.7	17.1	16.2	24.9	100	333
	Pregnant (%)	0.6	5.7	8.9	8.9	21.0	14.6	40.1	100	157
	<i>P</i> -value	0.23	<0.01	0.12	0.34	0.31	0.64	<0.001		490

¹Only cows with functional CL at time of treatment (P₄ concentrations ≥0.24 ng/mL 24 h, and ≥ 0.09 ng/mL 56 h, after treatment)

Table 3.4 describes the shift in % of cows that fall into 7 classes of P₄ concentrations following treatment. There were no differences in % of cows within classes 24 h after treatment. However, beginning 56 h after treatment and continuing at 72 and 96 h after treatment there were significant shifts in the % of cows that became pregnant and the cows that were diagnosed not pregnant. At 56 h, there was a greater ($P < 0.001$) % of pregnant cows that fell in the 0.1 – 0.2 ng/mL range compared to non-pregnant cows and a greater % of non-pregnant cows between 0.5 – 1 and > 1.0 ng/mL compared to pregnant cows. Interestingly at 56 h post-treatment, only 8% of cows that fell between 0.5 and 1 ng/mL P₄ became pregnant. At 96 h post-treatment, 94% of pregnant cows were ≤ 0.3 ng/mL compared to 68 % of cows diagnosed not pregnant. Also at 96 h post-treatment 29% of non-pregnant cows were > 0.5 ng/mL compared to 2% of pregnant cows ($P < 0.001$).

Effect of Serum E₂ Concentrations 56 h Post-Treatment on Pregnancy Outcome

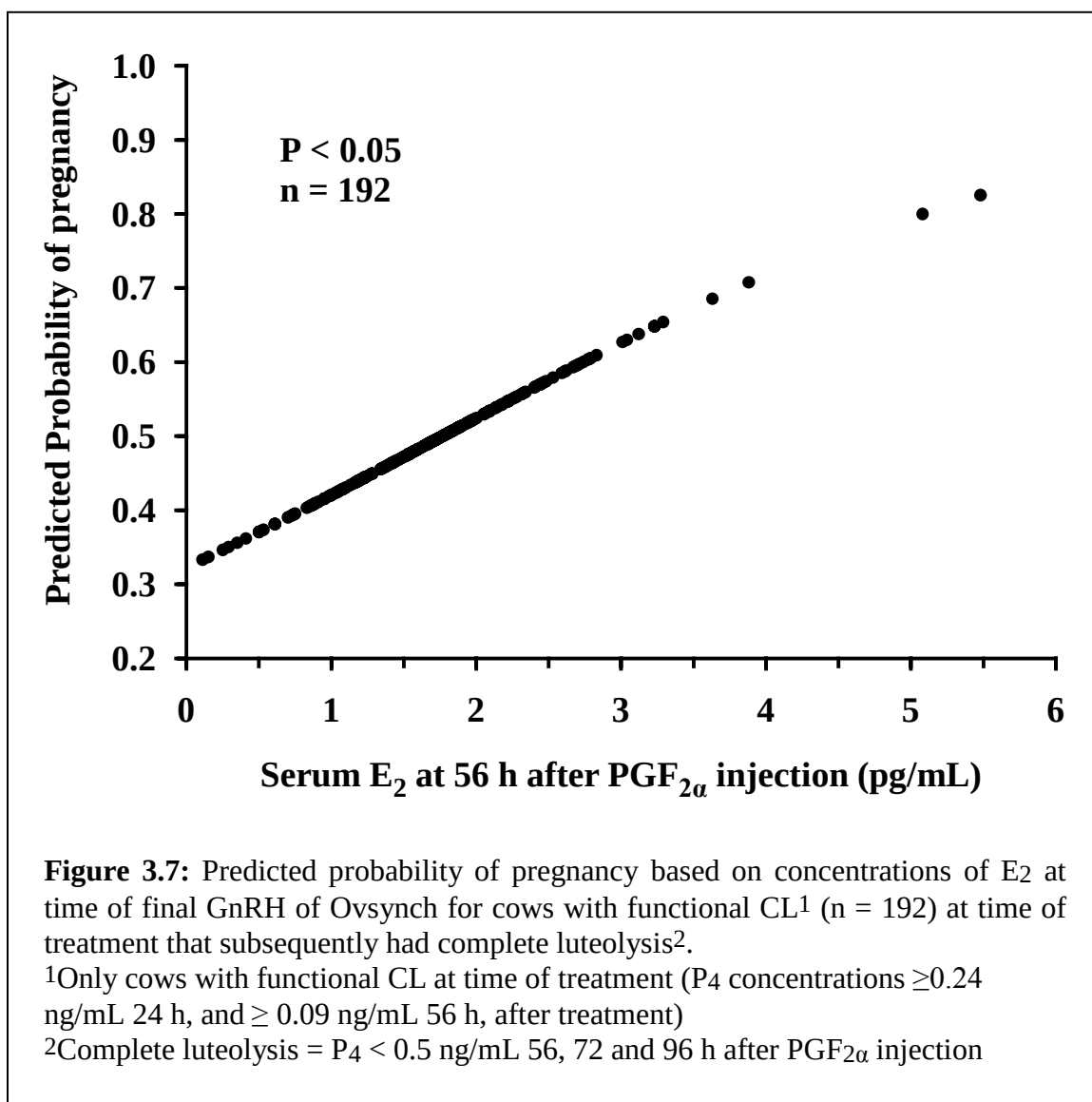
There was a positive relationship between concentrations of E₂ at final GnRH of Ovsynch and the predicted probability of pregnancy (Figure 3.7) among 1st AI cows with functional CL at time of treatment and complete luteolysis following treatment. Pregnant cows had greater concentrations of E₂ 56 h after PGF_{2 α} compared to non-pregnant cows ($n = 192$ total; $P = 0.02$). PGF_{2 α} type did not affect ($P > 0.1$) circulating concentrations of E₂ in cows with functional CL at time of PGF_{2 α} and with complete luteolysis.

Table 3.4: Distribution (% of total) of pregnant and non-pregnant lactating dairy cows across ranges in P₄ concentrations 24, 56, 72, and 96 h following treatment with cloprostenol sodium vs. dinoprost tromethamine (treatments combined).¹

Time after PGF _{2α} injection (h)	Pregnant status 39 ± 3 d after AI	Progesterone range (ng/mL)							Total (%)	n*
		0 - 0.1	0.1 - 0.2	0.2 - 0.3	0.3 - 0.4	0.4 - 0.5	0.5 – 1.0	> 1.0		
24	n	0	0	19	46	63	232	126		486
	Non-pregnant (%)	0.0	0.0	3.6	9.4	11.5	47.4	28.2	100	329
	Pregnant (%)	0.0	0.0	4.5	9.6	15.9	48.4	21.7	100	157
	P-value	-	-	0.66	0.95	0.17	0.84	0.15		
56	n	17	167	111	48	32	48	59		482
	Non-pregnant (%)	3.3	29.5	20.1	9.4	6.4	13.4	17.9	100	328
	Pregnant (%)	3.9	45.5	29.2	11.0	7.1	2.6	0.6	100	154
	P-value	0.76	<0.001	0.03	0.61	0.78	0.0002	<0.0001		
72	n	154	157	54	21	14	26	62		488
	Non-pregnant (%)	27.5	28.4	9.0	5.1	4.2	7.2	18.6	100	333
	Pregnant (%)	40.0	40.0	15.5	2.6	0.0	1.3	0.6	100	155
	P-value	<0.01	0.02	0.04	0.19	<0.01	0.007	<0.0001		
96	n	216	111	26	9	9	28	63		462
	Non-pregnant (%)	39.5	22.5	5.8	2.3	1.3	8.1	20.6	100	310
	Pregnant (%)	61.2	27.0	5.3	1.3	3.3	2.0	0.0	100	152
	P-value	<0.0001	0.22	0.87	0.52	0.13	0.01	<0.0001		

¹Only cows with functional CL at time of treatment (P₄ concentrations ≥0.24 ng/mL 24 h, and ≥ 0.09 ng/mL 56 h, after treatment)

*Different n between times were due to missing samples



Analysis of Cows without Complete Luteolysis

Cows that did not have complete luteolysis (n = 119) were separated into 3 different groups based on concentrations of P4 at 96 h post-treatment: Delayed luteolysis (P4 levels at 96 h after PGF_{2α} injection < 0.5 ng/mL), incomplete luteolysis (P4 levels at 96 h after injection between 0.5 and 1 ng/mL), or no luteolysis (P4 levels at 96 h after injection > 1.0 ng/mL) and were compared to cows with complete luteolysis (Figure 3.8) throughout the collection period. The number of cows that were pregnant at first

pregnancy diagnosis was 150/370 for complete, 4/19 for delayed, 3/32 for incomplete, and 0/68 for the no luteolysis groups.

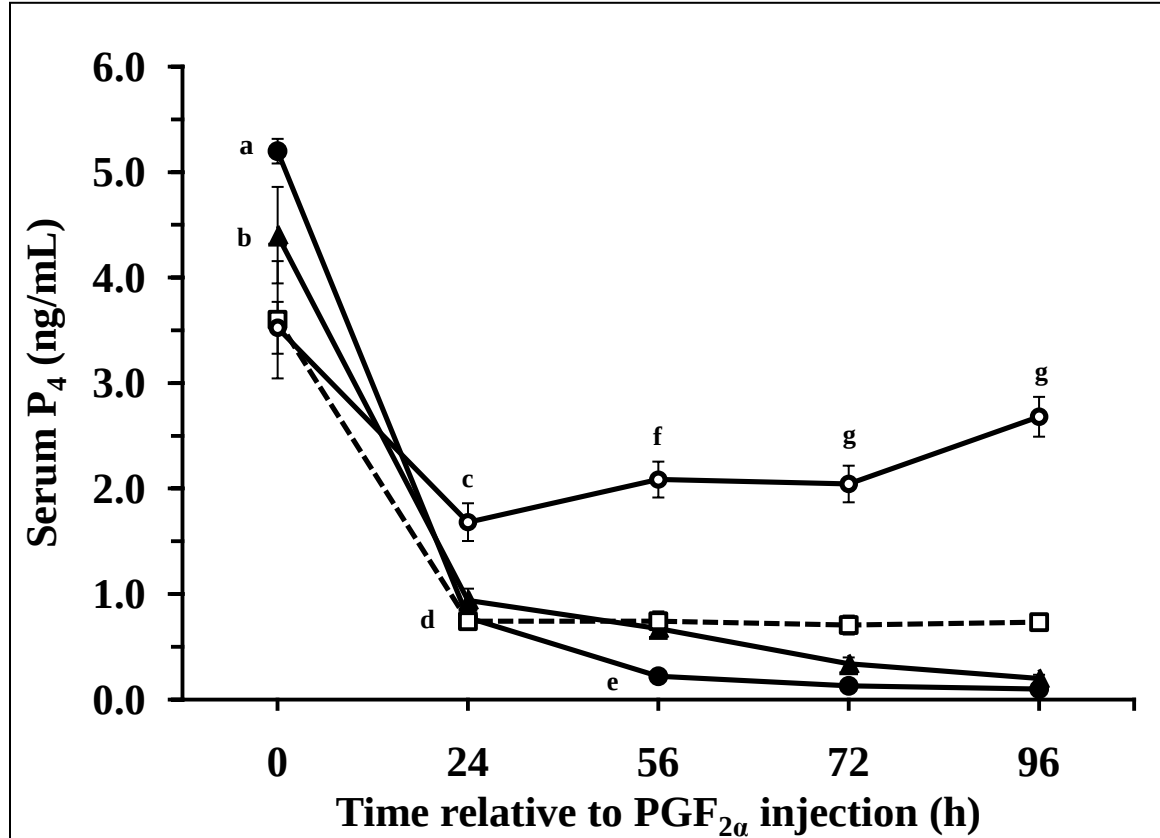


Figure 3.8: Daily concentrations of P₄ from treatments (combined) to 96 h post-treatment in lactating dairy cows with functional CL¹ that had complete luteolysis (C, --●--, P₄ < 0.5 ng/mL 56, 72 and 96 h after PGF_{2α} injection; n = 370), delayed luteolysis (D, --▲--, P₄ < 0.5 ng/mL 96 h after PGF_{2α} injection; n = 19), incomplete luteolysis (I, --□--, P₄ between 0.5 and 1 ng/mL 96 h after injection; n = 32), no luteolysis or recovery (N, --○--, P₄ > 1.0 ng/mL 96 h after injection; n = 68).

¹Only cows with functional CL at time of treatment (P₄ concentrations ≥ 0.24 ng/mL 24 h, and ≥ 0.09 ng/mL 56 h, after treatment)

a C different than I and N (P < 0.0001)

b D different than I and N (P < 0.05)

c N different than C, D, and I (P < 0.0001)

d C different than D (P < 0.05)

e C different than D, I and N (P < 0.0001)

f N different than D and I (P < 0.0001)

g All groups at this time were different from each other (P < 0.02)

DISCUSSION

The main objective of this study was to determine the effects of cloprostenol sodium versus dinoprost tromethamine treatments on complete luteolysis and conception rates in an Ovsynch program preceded by pre-synchronization. Since cloprostenol sodium has a longer half-life than dinoprost tromethamine (3 h vs. ~7-8 min), we hypothesized that cloprostenol sodium would increase the percentage of cows with complete luteolysis compared to dinoprost tromethamine. Subsequently, we expected a greater conception rate for cows treated with cloprostenol sodium rather than dinoprost tromethamine since the probability of pregnancy is greater in cows with complete luteolysis (Souza et al., 2007; Brusveen et al., 2009). Results of the present study indicated that cloprostenol sodium did not increase the % of cows with complete luteolysis or % pregnant, and did not enhance the decrease in serum concentrations of P4 after the injection of PGF_{2α} of Ovsynch, compared to dinoprost tromethamine. However, these results pointed out the significant lack of luteolysis associated with Ovsynch programs in cows that were pre-synchronized to enhance the % of cows that receive the 1st GnRH of Ovsynch at time of follicular dominance of the first follicular wave.

It is clear from data from our laboratory (Bello et al., 2006) that cows must be pre-synchronized to maximize synchronization and conception rates of Ovsynch. Three events must take place in order to maximize synchronization and conception rates of Ovsynch. 1) Cows must ovulate in response to the 1st GnRH of Ovsynch. Our laboratory (Bello et al., 2006) and others (Vasconcelos et al., 1999; Souza et al., 2008) have shown the importance of causing ovulation in response to the 1st injection of GnRH of Ovsynch. This event allows for both a new *accessory* CL and a new follicular wave.

Both of these outcomes are critical to the success of Ovsynch (Pursley et al., 1995). Induction of a new follicular wave places more control of the antral age of the dominant follicle at time of induced ovulation following the final GnRH of Ovsynch. Cows with dominant follicles that start antral growth following the 1st GnRH of Ovsynch and are 16 mm in diameter at time of final GnRH-induced LH surge have greater chances for pregnancy (Bello et al., 2006). Induction of *accessory* CL enhances P₄ concentrations prior to the final PGF_{2α} of Ovsynch (Bello et al., 2006). Increased serum P₄ concentrations prior to luteolysis enhanced the probability of pregnancy in lactating dairy cows (Bello et al., 2006). 2) Seven d following the 1st GnRH of Ovsynch the PGF_{2α} of Ovsynch must control luteolysis. Cows that are not pre-synchronized to the time of follicular dominance during the 1st follicular wave have greater chances of endogenous luteolysis prior to the PGF_{2α} of Ovsynch and asynchrony of AI with time of ovulation. These cows are generally in latter stages of the estrous cycle when the 1st GnRH of Ovsynch is initiated. Control of luteolysis with PGF_{2α} is maximized when cows are presynchronized to the time of follicular dominance of the 1st wave. For example, if cows are d 6 of the estrous cycle when the 1st GnRH of Ovsynch is administered, cows would be d 13 of the estrous cycle at time of the PGF_{2α} and prior to endogenous luteolysis. 3) Cows must ovulate following the final GnRH of Ovsynch in order to have a chance to become pregnant following a timed-AI approximately 16 h following the final GnRH.

The present study focused on luteolysis of Ovsynch and the importance of P₄ at time of induced-luteolysis. These data indicate that the chance of complete luteolysis is ≤ 80% regardless of type of PGF_{2α} product. Our definition of complete luteolysis in this

study was based on previous data from our laboratory (unpublished) and others (Souza et al., 2007; Brusveen et al., 2009) that indicated that cows with > 0.5 ng/mL P4 2 d following PGF_{2α} had very limited chances of pregnancy. Similarly, data from the present study indicated that cows that did not have complete luteolysis by our definition had ~ 5% chance of pregnancy. Ten % of cows on study had between 0.5 and 1 ng/mL P4 56 h following treatment and surprisingly only 8% of these cows became pregnant. Other papers have used 1 ng/mL 2 or 3 d after PGF_{2α} as cutoff for indicating complete luteolysis (Pursley et al., 1995; Moreira et al., 2001; El-Zarkouny et al., 2004; Bello et al., 2006; Stevenson and Phatak, 2010). These data and data from others (Souza et al., 2007; Brusveen et al., 2009) would argue that the cutoff for luteolysis 2 d following treatment should be decreased to < 0.5 ng/mL. Thus, it is critical that luteolysis is maximized during the Ovsynch program and that circulating concentrations of P4 fall below 0.5 ng/mL within 2 d following PGF_{2α}. Only 3% of cows that fell below 0.5 ng/mL P4 2 d following PGF_{2α} increased to above that level 3 or 4 d following PGF_{2α}. Many of the cows that did not fall below the 0.5 ng/mL range of P4 2 d following treatment continued to decrease the next 2 d but as stated above had a very poor chance of pregnancy. Although we did not collect ovulation data on these cows, it is highly likely that the final GnRH-induced LH surge of Ovsynch caused ovulation of a dominant follicle in the majority of cows. Previous data indicated that ~ 90% or more of cows assigned to Ovsynch even in random stages of the cycle have ovulation at the second GnRH of Ovsynch (Pursley et al., 1995; Vasconcelos et al., 1999). In Chapter 2, there was a very high correlation from the time of complete luteolysis to ovulation caused by endogenous mechanisms. It is likely the time needed for cows to be near basal levels of

P₄ to cause an LH surge is also needed for other reasons that may affect fertility in cows that are being induced to ovulate. Thus, it appears very important to cause complete luteolysis in as short of a period as possible even in cows that are induced to ovulate with GnRH.

There have been several studies during the past 30 years that have compared cloprostenol sodium and dinoprost tromethamine in both beef and dairy cattle (Furr et al., 1981; Sudweeks et al., 1983; Seguin et al., 1985; Turner et al., 1987; Salverson et al., 2002; Hiers et al., 2003; Martineau, 2003; Répási et al., 2005; Esterman et al., 2009). In these studies, the effects of these two PGF_{2α} analogues on luteolysis, estrus response, conception rates, and pregnancy rate were investigated. Results ranged from increases in estrus expression in favor of cloprostenol to no differences in estrus expression or conception rates between products. In addition, prior to the start of the current study there were no studies that compared these two PGF_{2α} products in a presynchronization / Ovsynch and resynchronization scheme with timed AI for lactating dairy cows. Since that time one study was published that indicated no difference in conception rates but an advantage in luteolysis rate for dinoprost tromethamine (Stevenson and Phatak, 2010). In most synchronization programs, the majority of cows have multiple CL including *accessory* CL that may only be 7 d old at the time of the PGF_{2α} of Ovsynch (Bello et al., 2006). Studies that have used dinoprost tromethamine in these programs have indicated a lack of complete luteolysis for 5 to 20% of cows after PGF_{2α} of Ovsynch (Pursley et al., 1997b; Moreira et al., 2000; Gümen et al., 2003; Brusveen et al., 2009). The present study would indicate that cloprostenol sodium and dinoprost tromethamine cause luteolysis in only ~75% of 1st and 2nd + AI cows. Conception rates of lactating dairy

cows following timed-AI after Ovsynch could increase significantly if complete luteolysis was achieved after PGF_{2α} for a greater % of cows. Interestingly, conception rates were consistently ~ 5% numerically greater across parities (1st, 2nd, and ≥ 3rd) for 1st AI when cows were treated with cloprostenol sodium compared with dinoprost tromethamine.

A recent study indicated that serum concentrations of P₄ at time of PGF_{2α} of Ovsynch was a predictor for probability of pregnancy ($P < 0.01$; Bello et al., 2006). The present study with much greater numbers also indicates that level of P₄ at time of PGF_{2α} is critical for pregnancy success. Level of P₄ was also predictive of chances of complete luteolysis. This clearly explains part of the reason for the positive relationship between P₄ concentrations at PGF_{2α} and conception rates. The positive relationship between P₄ and luteolysis may be simply because greater serum concentrations of P₄ is reflective of CL maturity. As CL become more mature the likelihood of induced luteolysis may be greater, since sensitivity of CL to the luteolytic action of PGF_{2α} appears to increase progressively as the CL turns more mature (Skarzynski et al, 2008). However, fertility improvement does not appear to be only by greater luteolytic response. When we analyzed this relationship in only cows that had complete luteolysis we still found a trend ($P = 0.08$) for this relationship.

The sum of these results indicates that greater serum concentrations of P₄ before PGF_{2α} of Ovsynch appears to be beneficial for fertility of dairy cows. It is not clear how greater serum concentration of P₄ prior to PGF_{2α} of Ovsynch can affect fertility. However, greater serum concentration of P₄ during follicular development may improve oocyte quality. A positive role of higher serum concentrations of P₄ during growth of pre-

ovulatory follicles on fertility was also reported by Rivera et al. (2009). In this study, they showed that cows exposed to high serum levels of P₄ during superovulation had a greater number and proportion of grade 1 and 2 embryos compared to cows with lower levels of P₄. In this study, it appears that P₄ during superovulation affected fertilization, as cows with lower levels of P₄ had a greater number of unfertilized oocytes and a greater proportion of ova/embryos classified as unfertilized oocytes (Rivera et al., 2009).

In the present study, cows that had lower P₄ secretion were more likely to be refractory to exogenous PGF_{2α}. Previous data from our laboratory (Bello et al., 2006) indicated that cows with both a d 13 and d 7 (*accessory*) CL had greater concentrations of P₄ compared to cows without *accessory* CL. In the current study, the likelihood exists that cows with the greatest concentrations of P₄ would likely have *accessory* CL. Thus it seems illogical that the cows with *accessory* CL would have a greater chance at complete luteolysis. In this study, however, it is likely that most cows would have *accessory* CL. So we speculate that there is significant variation in maturity amongst these *accessory* CL, accounting for both progesterone producing and luteolytic abilities. The variety in the size of ovulatory follicle that formed these CL may be the reason for the range in maturity, since size of ovulatory follicle affects the production of progesterone of the CL that this follicle formed (Vasconcelos et al., 2001).

The relationship between serum P₄ at time of the PGF_{2α} injection and probability of complete luteolysis is also further indicated in the analysis of cows without complete luteolysis. Cows with delayed luteolysis had a tendency to have greater P₄ at time of PGF_{2α} injection than cows with incomplete or no luteolysis. By 24 h after PGF_{2α} injection P₄ decreased in the incomplete, delayed and no luteolysis groups. Cows with no

luteolysis had almost a complete recovery of serum P₄ by 96 h after PGF_{2α}. This response appears to be similar to cows that received a smaller dose of PGF_{2α} analogue than recommended by label in a different study (125 µg of cloprostenol vs. 500 µg of cloprostenol; Colazo et al., 2002).

Average serum concentrations of P₄ did not decrease from 56 to 96 h after injection in cows defined to have “incomplete luteolysis.” It is not clear if these CL would have recovered after 96 h and continued to stay functional. But, Répási et al. (2005) proposed that, in some cases, exogenous PGF_{2α} could affect the size of large luteal cells (LLC) and luteal capillary cells without affecting small luteal cells (SLC). They believed the initial decline in P₄ production may have been due to temporary degenerative changes in the endothelial cells of luteal capillaries which subsequently recovered. Then, SLC would be able to respond to LH with an increase in P₄ secretion (Répási et al., 2005). Since SLC are responsible for approximately 30% of the total P₄ production by the CL (Farin et al., 1989; Weems et al., 2006), in the case of incomplete luteolysis, a small fraction of SLC may still be producing and secreting subluteal P₄ concentrations making complete luteolysis during this time impossible.

Serum E₂ concentration at time of GnRH of Ovsynch was also an indicator of fertility. Probability of pregnancy was greater in cows with higher E₂ at last GnRH of Ovsynch. In addition, pregnant cows diagnosed at 39 d after AI had greater E₂ concentrations at time of GnRH than non-pregnant cows. This finding was similar to previously reported studies (Ireland and Roche, 1982; Bello et al., 2006). However, Bello et al. (2006) reported that the importance of E₂ levels as a predictor of fertility differs with size of preovulatory follicle. In another study, supplementation of 1 mg E₂ 8 h

before the final GnRH of Ovsynch increased fertility in cows that ovulated follicles between 15 and 19 mm and in first service cows (Souza et al., 2007).

CONCLUSION

Luteolytic response and conception rates were not different in cows treated with cloprostenol sodium or dinoprost tromethamine when used in the Ovsynch program. Cows with greater serum concentrations of P4 at time of PGF_{2α} of Ovsynch showed a greater chance for luteolysis and pregnancy. Cows with greater concentration of E2 at time of final GnRH of Ovsynch also exhibited a greater chance for pregnancy.

CHAPTER 4

GENERAL DISCUSSION

ARE THERE DIFFERENCES IN THE EFFICACY OF DINOPROST TROMETHAMINE AND CLOPROSTENOL SODIUM?

Ovsynch technology has been used effectively in the dairy industry for the past 14 years to control time to 1st and subsequent AI, but conception rates still remain very low averaging 30% in many herds. One of the limitations of Ovsynch is that up to 20% of cows do not undergo complete luteolysis thereby decreasing conception rates. Therefore, the focus of this thesis was to improve conception rates of Ovsynch by increasing the % of cows with complete luteolysis and accelerating the rate of decrease of serum concentrations of P4 induced at the PGF_{2α} of Ovsynch.

In order to improve the luteolytic response of PGF_{2α} of Ovsynch, we proposed the use of cloprostenol sodium, a synthetic analogue of the natural PGF_{2α} (dinoprost tromethamine). Cloprostenol sodium has a longer half life compared to dinoprost tromethamine (3 h vs. 7~8 min); therefore, it was expected to have greater luteolytic potency and increase conception rates when used in the Ovsynch strategy. In this thesis we tested the effect of these two products used in the Ovsynch protocol on: (1) rate of decrease of serum concentrations of P4 (Chapter 2); (2) % of cows with complete luteolysis (Chapter 3); and (3) conception rates (Chapter 3).

In Chapter 2, our findings indicated that cows treated with cloprostenol sodium had a faster decrease in serum concentrations of P4 during the first 12 h following treatment compared with cows treated with dinoprost tromethamine. In addition, there was a significant negative relationship between serum concentrations of P4 at 12 h after treatment and serum concentrations of E₂ at 24 h after treatment. These factors might contribute to greater serum concentration of E₂ seen at 48 h after treatment for cows

treated with cloprostenol sodium compared with cows treated with dinoprost tromethamine. The next study presented in Chapter 3 showed that cloprostenol sodium did not increase the % of cows with complete luteolysis or percentage of cows pregnant compared with dinoprost tromethamine.

Taken together, both products had the same luteolytic efficiency when applied in an Ovsynch protocol. Most importantly, there were no statistical differences in conception rates between treatments. Therefore, both products have the same efficacy when used in the Ovsynch protocol. However, in the study present in chapter 2, cows treated with cloprostenol had a greater rate of reduction on serum concentrations of P4 for the first 12 hours which likely resulted in an increase in serum concentrations of E₂ at 48 h post-injection. This very slight difference in these two products gives cloprostenol an edge if all other variables, such as cost, are the same.

RECOMMENDATIONS FOR DAIRY PRODUCERS

Since there were no significant differences in the % of cows with complete luteolysis and conception rates between cloprostenol sodium and dinoprost tromethamine used in the Ovsynch protocol, dairy producers can use either cloprostenol sodium or dinoprost tromethamine in lactating dairy cows in the Ovsynch protocol. However, cost and availability should be evaluated by dairy producers before choosing one of these products. The product with lower cost should be used to decrease the cost of the Ovsynch protocol.

The % of cows that did not have complete luteolysis was a problem in the Ovsynch protocol. The use of a double injection of PGF 24 h apart was shown to increase

the % of cows with complete luteolysis compared with a single injection (96% vs. 85%; Brusveen et al., 2009). Although there was a numerical increase in CR using a double injection, there were no significant differences in CR between double and single injections of PGF in the Ovsynch protocol ($P = 0.34$). However, in a large dairy farm with an intensive use of Ovsynch protocol for a long period, a difference in CR may be detected. Therefore, farms that have good compliance are recommended to use double injection of PGF in the Ovsynch protocol.

Another important recommendation is to use a presynchronization + Ovsynch protocol that increase serum concentrations of P4 to increase fertility and % of cows with complete luteolysis. Double-Ovsynch is a good option since it creates a greater % of cows with serum concentrations of P4 higher than 3 ng/mL at time of PGF of Ovsynch compared with pre-synch 14-12 (Souza et al., 2008). This might be due to a greater % of cows ovulating during the pre-synchronization because of the use of GnRH instead of only PGF. In a previous study, conception rates of Double-Ovsynch were greater than with Pre-synch 14-12 (Souza et al., 2008).

SIGNIFICANT FINDINGS AND FUTURE DIRECTIONS

Data presented in Chapter 3 indicated that cows treated with Ovsynch in the first (~20%) and repeated (~30%) services, have insufficient luteal regression in response to administration of a single dose of either dinoprost (25 mg) or cloprostenol (0.5 mg). Therefore, lack of complete luteolysis still appears to be a big obstacle to improve CR for Ovsynch.

In understanding this problem, one of the most important physiological findings of this thesis was the positive relationship of serum concentration of P₄ at time of treatment and predicted probability of complete luteolysis. It appears that P₄ secretory capacity has a positive relationship with the sensitivity of the CL to PGF_{2α}. This P₄ secretory capacity may be related to the maturity of the CL and its refractory period to PGF_{2α}. In addition to the positive relationship with luteolysis, greater serum concentrations of P₄ at time of treatment resulted in increased fertility.

Another important finding was the negative relationship between serum concentrations of P₄ at 12 h after PGF_{2α} of Ovsynch and serum concentrations of E₂ at 24 h after PGF_{2α} of Ovsynch. Therefore, a faster decrease in serum concentrations of P₄ after PGF_{2α} of Ovsynch may cause a quicker increase in serum concentrations of E₂ 24 h after PGF_{2α} of Ovsynch. This faster elevation in E₂ may contribute to cows having a higher serum concentration of E₂ 48 h after PGF_{2α} of Ovsynch which was positively related with fertility.

Therefore, based on these results, future studies should focus on developing modifications of the Ovsynch procedure that will increase P₄ prior to PGF_{2α}-induced luteolysis and amplify the rate of P₄ decrease following induction of luteolysis. This should result in a greater % of cows with higher P₄ at PGF_{2α} of Ovsynch and faster decrease in P₄ after induced luteolysis which will significantly improve conception rates following Ovsynch. Since traditional Ovsynch is already in place in many dairy management systems across the U.S. and the world, key improvements in Ovsynch have the potential to be implemented within the industry in a straightforward and trouble-free fashion despite the complexity of enhancing this program to improve fertility.

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