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Effects of Early Postpartum GnRH and Prostaglandin $F_{2\alpha}$ Administration on Reproductive Activity and Ovulation Synchronization in Lactating Dairy Cows

[1]A.L. Tucker, [1]H.L. Sanchez, [1]W.B. Tucker, [1]A. Williams, [1]J.W. Fuquay,
[1]S.T. Willard and [1,2]P.L. Ryan
[1]Department of Animal and Dairy Sciences,
[2]Department of Pathobiology and Population Medicine,
Mississippi State University, Mississippi State, 39762 Mississippi, USA

Abstract: The objectives of this study were to determine the efficacy of GnRH and $PGF_{2\alpha}$ for inducing early Postpartum (PP) estrual activity, hastening uterine involution and enhancing ovulation synchronization in lactating dairy cows. About 40 Holstein and Jersey cows were assigned at calving to 1 of 3 Treatments (Trt) and balanced by breed: Trt 1 (Control; n = 13) received an injection of saline on days 15 and 25 PP; Trt 2 (n = 14) received saline and $PGF_{2\alpha}$ injections on days 15 and 25 PP, respectively; Trt 3 (n = 13) received GnRH and $PGF_{2\alpha}$ injections on days 15 and 25 PP, respectively. Reproductive function was monitored through day 58 PP via electronic heat detection, rectal palpation for uterine tone, ultrasonography of ovarian structures and blood samples obtained 3 times week^{-1} for serum Progesterone (P_4) analysis. On day 58 PP, an ovulation synchronization protocol was initiated as follows: GnRH given on day 0, $PGF_{2\alpha}$ administered on day 7, GnRH given again on day 9 and artificial insemination conducted 16 h after the 2nd GnRH injection. In both Trt 2 and Trt 3, serum P_4 fell sharply after the $PGF_{2\alpha}$ injection at 25 days PP. An interaction of treatment by sample time ($p<0.05$) associated with treatment effects ($PGF_{2\alpha}$ vs. saline) on P_4 concentrations was observed. Postpartum estrual activity ($p>0.10$), rate of uterine involution ($p>0.05$), follicular dynamics ($p>0.10$) and P_4 concentrations ($p<0.10$) did not differ between treatment groups prior to ovulation synchronization. Pregnancy rate following artificial insemination also did not differ between breeds ($p>0.05$) or among treatment groups ($p>0.05$) and for all cows was 50% (20/40). In summary, early postpartum hormonal treatments did not influence uterine involution, follicular development or estrus activity compared to non-Treated controls (Trt 1). Moreover, early postpartum (days 15-25 PP) treatment with GnRH and/or $PGF_{2\alpha}$ did not enhance subsequent ovulation synchronization nor conception rates for lactating dairy cows in this study.

Key words: Postpartum estrual activity, ovulation synchronization, uterine involution, ovulation synchronization, treatment, concentration

INTRODUCTION

Pregnancy is the most economical approach to initiation of lactation and resulting milk production in the dairy cow (Momcilovic *et al.*, 1998) with profitability being dependent upon the number of days to conception (Pursley *et al.*, 1997a; De Vries, 2006). Calving intervals have been increasing from 0.90-1.07 days each year for Ayrshire, Brown Swiss, Guernsey and Holstein and by 0.49 days year^{-1} for Jersey cows since 1980 (Hare *et al.*, 2006). Reproductive inefficiency can cost dairy producers between \$0.25 and 4.68 day^{-1} open/cow during the first 85 days postpartum (Pankowski *et al.*, 1995; Olynk and Wolf, 2009). To acquire normal fertility and acceptable calving intervals in dairy cattle, it is important that cyclic ovarian activity is resumed early in the PP period (Stevenson and Pursley, 1994). Uterine involution is the return after calving of the uterus to its non-gravid state and it is necessary for achieving normal reproductive function. Macroscopic involution can be characterized by an increase in uterine tone and a decrease in length and weight of the uterus and in the diameter of the previously gravid horn that occurs at 3-5 weeks PP and a decrease in the diameter of the cervix at 4-6 weeks PP (Slama *et al.*, 1991). A good uterine involution status at time of insemination is essential for achieving normal reproductive rates. Gonadotropin Releasing Hormone (GnRH) has been administered during the early PP period in an attempt to reduce the incidence of infertility (Kaim *et al.*, 2003).

Corresponding Author: Peter L. Ryan, Department of Animal and Dairy Sciences, Mississippi State University, 4025 Wise Center Road, P.O. Box 9815, Mississippi State, 39762 Mississippi, USA

Researchers have found that an injection of GnRH on day 14 PP improved conception rates (Youngquist and Bierschwal, 1985) by stimulation of uterine involution and turnover of the first wave of follicular development (Risco *et al.*, 1995).

Frequencies of estrus and ovulations before 60 days PP have been increased by improving uterine involution after administration of GnRH (Benmrad and Stevenson, 1986; Kaim *et al.*, 2003). Gonadotropin releasing hormone and Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) affected PP ovarian function by causing precocious ovulation and premature regression of the 1st luteal structure (Benmrad and Stevenson, 1986).

Manipulation of PP ovarian function enhances fertility by increasing the ovulation frequency and occurrence of estrus before insemination or 1st service. Myometrial contractions of the uterus caused by GnRH and $PGF_{2\alpha}$ induced estrus or increased frequency of estrous cycles, improved uterine involution and cleansed residual materials associated with parturition, i.e., lochia (Benmrad and Stevenson, 1986). Administration of $PGF_{2\alpha}$ in the early PP period stimulates an increase in the myoelectrical activity in the myometrium of both uterine horns and in the uterine body (Gajewski *et al.*, 1999). Cleansing of the uterine environment occurs when $PGF_{2\alpha}$ is injected at 20-33 days PP (Pankowski *et al.*, 1995; Kasimanickam *et al.*, 2005). Prostaglandin $F_{2\alpha}$ is luteolytic (Arosh *et al.*, 2004) and has been used to treat ovarian luteal cysts, pyometra (Whittier *et al.*, 1989) and endometritis (Benmrad and Stevenson, 1986; Melendez *et al.*, 2004; Kasimanickam *et al.*, 2005). Progesterone (P_4) produced by the Corpus Luteum (CL) suppresses the immune system (Lewis, 1997). Prostaglandin $F_{2\alpha}$ induces luteolysis causing a decrease in the P_4 levels and a subsequent up-regulation of the immune function making the uterus able to clear infections (Lewis, 1997).

Follicular wave and therefore ovulation must be synchronized in order to obtain higher conception rates. Because every cow will ovulate at different intervals of time after they have displayed behavioral estrus, it would be more efficient to synchronize ovulations rather than estrus.

Follicular waves can be synchronized by physically or hormonally removing the suppressive effect of the dominant follicle, allowing for the emergence of a new follicular wave at a certain time following treatment. human Chorionic Gonadotropin (hCG) and GnRH analogs have been used to induce luteinization of the follicle or ovulation (Stevenson *et al.*, 2007b) and progestogens and estradiol have been administered to cause atresia of the dominant follicle (Bo *et al.*, 1995). Synchronization of ovulation removes the necessity of estrus detection before artificial insemination (Pursley *et al.*, 1997a, b; Rabiee *et al.*, 2005) which is <50% in most herds (Pursley *et al.*, 1997a; Washburn *et al.*, 2002). Pregnancy Rates (PR) have been increased by the use of ovulation synchronization protocols in comparison with estrus detection (Pursley *et al.*, 1997a; Gümen *et al.*, 2003). The objectives of this study were two fold: to determine if GnRH and $PGF_{2\alpha}$ treatment in early lactation would induce early cycling by enhancing ovarian activity therefore, increasing uterine tone and to assess the influence of this treatment on subsequent fertility response to a synchronized ovulation protocol.

MATERIALS AND METHODS

Animal handling and protocol: About 30 Holstein and 10 Jersey lactating cows that calved during fall (September) at the Bearden Dairy Research Center, Mississippi State University (MSU) were utilized in this study in compliance with the Institutional Animal Care and Use Committee (IACUC) of MSU. Cows were randomly assigned (balanced by breed) to 1 of 3 treatment groups (Fig. 1).

Treatment 1 served as the control group (Trt 1, n = 13). Control cows received a physiological saline (0.9% NaCl) injection (2 mL, i.m.) on days 15 and 25 PP. Treatment 2 cows (Trt 2, n = 14) were treated with an injection of physiological saline on day 15 PP and 25 mg (5 mL, i.m.) of $PGF_{2\alpha}$ (Lutalyse, Pharmacia and Upjohn Co. Kalamazoo, Michigan, USA) on day 25 PP. Treatment 3 cows (Trt 3, n = 13) were treated with 100 μg (2 mL, i.m.) of GnRH (Cystorelin, Abbott Laboratories, North Chicago, IL, USA) on day 15 PP and 25 mg $PGF_{2\alpha}$ on day 25 PP in an attempt to influence the rate of uterine

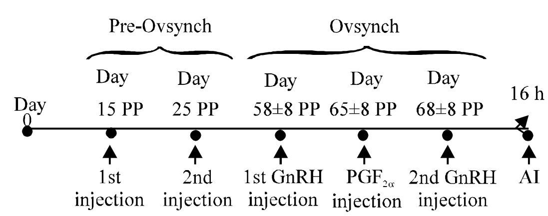

Fig. 1: Timeline for hormonal treatments pre and during ovulation synchronization. Day 0: Day of parturition. The 1st pre-Ovsynch injection was given on day 15 Post Partum (PP) (NaCl, NaCl and GnRH for Trt 1-3, respectively) and the 2nd pre-Ovsynch injection was given on day 25 PP (NaCl, $PGF_{2\alpha}$ and $PGF_{2\alpha}$ for Trt 1-3, respectively). The Ovsynch protocol was initiated on day 58±8 PP and finished on day 68±8 PP. Artificial inseminations were performed 16 h after the 2nd GnRH injection

involution and increase uterine tone. Approximately 3 weeks before the expected calving date most cows were cooled using a sprinkler and fan system in the free stall barn and the holding pens of the milking parlor.

Beginning at the same time, diets were supplemented once daily with 1 L b head^{-1} of dried propylene glycol in an attempt to decrease ketosis incidence. During the last 2 weeks before the expected calving date, diets were also supplemented with anionic salts to promote mild acidosis and mobilization of calcium. For the 1st 10 days PP, the cows were placed in a fresh cow health program adopted from a modified UpJohn protocol. As part of the PP management routine, every cow received 100 USP of oxytocin (Phoenix Pharmaceutical, Inc., St. Joseph, MO, USA) at the first milking. Also during the 1st 10 days PP, cows were fed a transition diet (17% crude protein) twice daily including dried propylene glycol. From day 11 PP, cows were fed a high-cow (18% crude protein) total mixed ration. Cows were housed in a free stall barn with grooved concrete flooring and released into a small paddock for 1 h daily exercise. It is important to note that this study was designed and carried out prior to the 2002 food and drug administration approved introduction and commercial availability of Controlled Intravaginal Drug Release (CIDRS) devices in the United States for use in dairy cattle.

Blood sampling: Blood samples (jugular vein) were collected 3 times weekly from day 15-68±8 PP for P_4 analysis. Samples were allowed to clot in an ice bath for at least 4 h and centrifuged at 1400 x g for 20 min to facilitate serum harvest. Pending P_4 analysis, serum was frozen at -20°C.

Determination of uterine involution: Uteri of the first 25 cows that calved (n = 9, 8 and 8 cows for Trt 1-3, respectively) were palpated weekly from day 15-68±8 PP to determine the state of uterine involution. The rate of uterine involution for each cow received a score of 1-5. A score of 1-2 identified a uterus in poor condition that was soft and doughy with enlarged caruncles. A score of 2-3 identified, a uterus in fair condition showing some improvement but was still doughy and may have had detectable caruncles. A good uterine involution rating categorized a pliable uterus with the previously pregnant horn still larger than the nonpregnant horn and was given a score of 3-4. A uterus in excellent condition was characterized by pliable and springy horns of equal size and was given a score of 4-5.

Ultrasonography: Ovarian activity was identified in the first 25 cows that calved by ultrasonography using an Aloka 500 V system with a 5.0-MHz linear-array transrectal probe. From day 15-68±8 PP, the left and right ovaries were scanned once per week and the diameter of the largest follicle (in either ovary) was recorded to determine follicular dynamics.

In order to determine the time of ovulation, the diameter of the largest follicle was monitored every 12 h during the 36 h after the 2nd GnRH injection of the Ovsynch protocol. Pregnancy rates were determined by ultrasound at days 30 and 51 following AI and were confirmed later by P_4 concentrations, palpation and calving.

Progesterone assay: Progesterone radioimmunoassay kits (Catalog No. DSL-3900) from Diagnostic Systems Laboratories (DSL; Webster, TX) were used to measure P_4 concentration in the serum of all cows. The intra and interassay coefficients of variation for P_4 were 7.78 and 14.77%, respectively. The sensitivity of the assay was 0.075 ng mL^{-1}.

Ovulation synchronization: On day 58±8 PP, all cows were placed on the Ovsynch protocol. Gonadotropin releasing hormone (100 μg; Cystorelin, Merial Ltd.) was injected i.m. on day 58±8 PP. On day 65±8 PP, a 25 mg injection of $PGF_{2\alpha}$ (Lutalyse, Pfizer Animal Health) was administered followed 48 h later by a second GnRH injection. Cows were inseminated 16 h after the 2nd GnRH injection by 3 artificial inseminators.

Statistical analysis: Serum concentrations of P_4 relative to treatment by sample time interaction were analyzed using the Least Squares procedure of Statistical Analysis System. The serum concentrations of P_4 were also analyzed within and between sample times by treatment using the Unpaired t test of StatView and within treatment by using the Paired t test of StatView. The Analysis of Variance (ANOVA) procedure of StatView was used to test follicle and uterine involution data according to treatment. Parameters for analysis included follicular size and uterine involution scores normalized relative to both calving and breeding.

RESULTS AND DISCUSSION

Progesterone concentrations: According to the P_4 serum concentrations, all treatment groups were exhibiting luteal activity by day 25 PP (Fig. 2). Progesterone concentrations, >1 ng mL^{-1} has been reported to correspond with the presence of a functional CL (Stevenson *et al*., 2007b; Stevenson, 2008; Carriquiry *et al*., 2009). A treatment by sample time

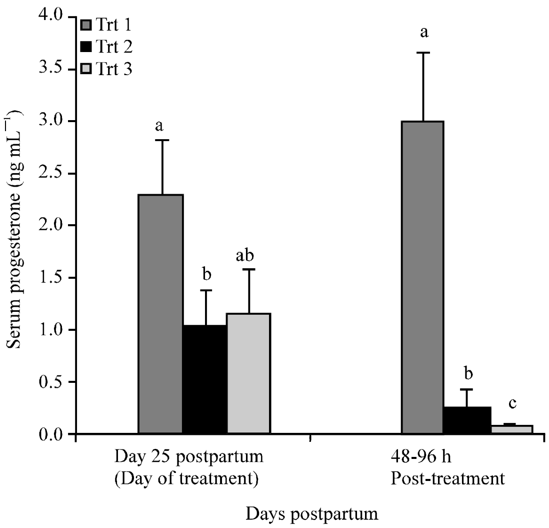

Fig. 2: Progesterone serum concentrations relative to Treatment (Trt) at day 25 Postpartum (PP) (pre-Ovsynch). On day 25 PP: NaCl, $PGF_{2\alpha}$ and $PGF_{2\alpha}$ were injected to Trt 1-3 cows, respectively. Data are means±SEM. Different letters indicate significant differences ($p<0.05$)

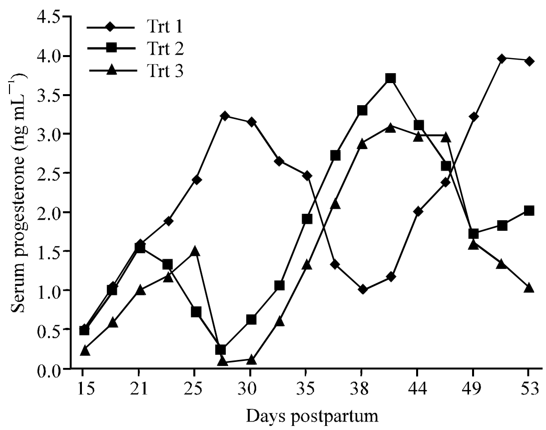

Fig. 3: Progesterone serum concentrations by Treatment (Trt) and day Postpartum (PP). Days 15 and 25 PP correspond to the first (NaCl, NaCl and GnRH for Trt 1-3, respectively) and second (NaCl, $PGF_{2\alpha}$ and $PGF_{2\alpha}$ for Trt 1-3, respectively) pre-Ovsynch injections, respectively. Decline in P_4 concentrations in Trt 2-3 after $PGF_{2\alpha}$ injection on day 25 PP

interaction was significant ($p<0.05$; Fig. 3) due to the injection of $PGF_{2\alpha}$ at day 25 PP to Trt 2-3. As expected, the $PGF_{2\alpha}$ lowered the P_4 concentrations by lysing the CL of the cows in these treatment groups. Stevenson *et al.* (2007a) found a treatment by day

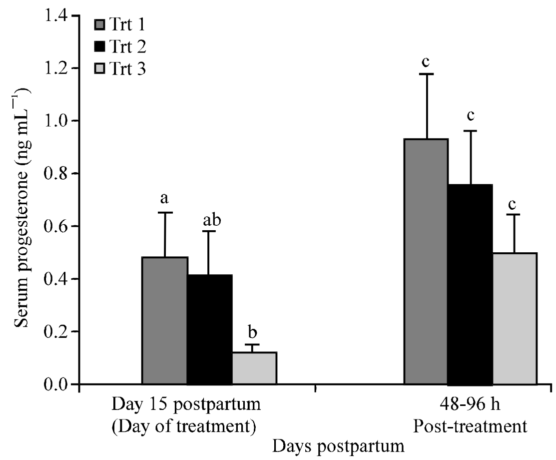

Fig. 4: Progesterone serum concentrations relative to Treatment (Trt) at day 15 Postpartum (PP) (pre-Ovsynch) and 48-96 h later. On day 15 PP: NaCl, NaCl and GnRH were injected to Trt 1-3 cows, respectively. Data are means±SEM. Different letters indicate significant differences ($p<0.05$)

interaction ($p<0.001$) for serum concentrations of P_4 when $PGF_{2\alpha}$ was administered at different days after the 1st GnRH injection in the Ovsynch protocol. Pre-Ovsynch GnRH injections at day 15 PP induced ovulation and CL formation as evidenced by the increase in serum P_4 levels observed in Trt 3 (Fig. 4). Kacar *et al.* (2006) found serum progesterone levels >1 ng mL^{-1} (presence of a functional CL) in 83.3% of the anestrus cows treated with a GnRH 7 days prior to Ovsynch program.

Serum P_4 concentrations in Trt 1 were higher than in Trt 3 ($p<0.05$) on day 15 PP, the day of the 1st pre-Ovsynch injection (Fig. 4). About 48-96 h after the day 15 injections, P_4 concentrations did not differ ($p<0.10$) between treatment groups but were higher ($p<0.05$) than during day 15 PP (Fig. 4).

Ullah *et al.* (1996) and Stevenson *et al.* (2007a) reported that GnRH administration resulted in a predictable release of Luteinizing Hormone (LH) and a significant increase in serum P_4. Pituitary responsiveness to GnRH as evidenced by plasma LH concentrations, appears to be regained by 7 or 8 days PP (Kesler *et al.*, 1977; Fajersson *et al.*, 1999).

Therefore, the GnRH injection for Trt 3 at day 15 PP should result in an increase in LH levels that can cause the ovulation and a subsequent rise in P_4 concentration. Progesterone levels in Trt 3 began to increase sharply until a peak was observed at approximately day 25 PP when $PGF_{2\alpha}$ injection was administered (Fig. 3). The serum

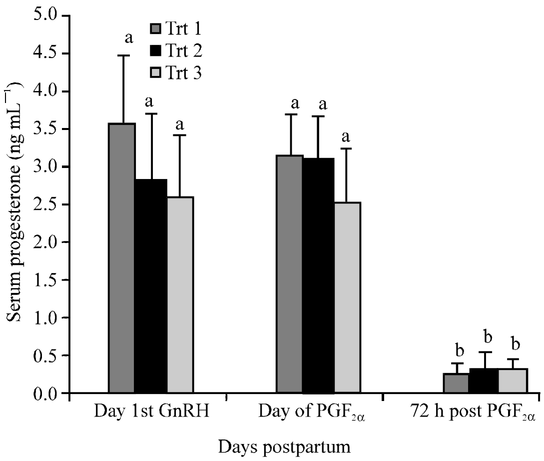

Fig. 5: Progesterone serum concentrations relative to treatment during the Ovsynch protocol. Pre-Ovsynch treatments for Trt 1-3 were NaCl and NaCl; NaCl and PGF$_{2\alpha}$ and GnRH and PGF$_{2\alpha}$ on days 15 and 25 postpartum, respectively. Data are means±SEM. Different letters within treatment and between days with respect to treatment indicate significant differences ($p<0.05$)

concentrations of P_4 on day 25 PP, the day of the 2nd pre-Ovsynch injection were higher ($p<0.10$) for Trt 1 than for Trt 2 (Fig. 2) probably due to differences in occurrence of cyclicity between treatments at this stage. At 48-96 h after day 25 PP injections, serum P_4 concentrations were lower ($p<0.05$) for Trt 2 and Trt 3 than for Trt 1 (Fig. 2) as a result of the luteolytic effect of PGF$_{2\alpha}$. Progesterone serum concentrations decrease as a result of luteolysis can be detected in blood samples taken at 24 h after a PGF$_{2\alpha}$ injection (Stevenson *et al.*, 1999).

The serum concentrations of P_4 of each treatment were also compared for the days of the injections of the Ovsynch protocol (Fig. 5). On the day of the 1st GnRH injection of the Ovsynch protocol, P_4 concentrations were 3.58±0.92, 2.83±0.87 and 2.61±0.83 ng mL^{-1} for Trt 1-3, respectively ($p>0.10$) (Fig. 5). Pursley *et al.* (1997b) found that serum concentrations of P_4 are indicative of synchronization of the luteal function in lactating dairy cows caused by the 1st injection of GnRH in the Ovsynch protocol.

In another study, mean serum concentrations of P_4 were higher for both pregnant and non-pregnant cows treated with GnRH before insemination than for cows not treated (Ullah *et al.*, 1996). Higher plasma P_4 concentrations were observed in the subsequent estrous cycle in GnRH treated cows compared to control cows in a study of Kaim *et al.* (2003). On the day of the PGF$_{2\alpha}$ injection of the Ovsynch protocol, P_4 concentrations were not different ($p>0.10$) between groups (3.15±0.56, 3.11±0.56 and 2.53±0.72 ng mL^{-1} for Trt 1-3, respectively). About 72 h after the PGF$_{2\alpha}$ administration (Fig. 5), P_4 concentrations were 0.26±0.14, 0 .33±0.22 and 0.32±0.13 ng mL^{-1} for Trt 1-3, respectively ($p>0.10$). However, P_4 serum concentrations on the day of PGF$_{2\alpha}$ administration were higher ($p<0.05$) than 72 h later in all treatment groups because of the luteolytic effect of this hormone (Fig. 5).

Postpartum follicular dynamics: Beginning at week 3 PP, the largest follicle of each cow in the subset of 25 animals that were scanned by ultrasound was measured weekly to monitor ovarian activity. Follicle sizes were analyzed by treatment relative to both calving date and day of breeding. There were no differences ($p>0.10$) in follicle size between treatment groups (Fig. 6) or across time (Fig. 7) when analyzed relative to calving or breeding. Stevenson *et al.* (2007a) observed no differences ($p>0.05$) in follicle size when using variations in the Ovsynch protocol according to time of PGF$_{2\alpha}$ administration. The data suggests that the cows in all 3 treatment groups had large follicles present by the 3rd week PP if not before and the presence of these large follicles continued throughout the PP period.

Several studies have reported that follicular activity in dairy cows reassumed before or within the 3rd to 5th week in the PP period (Tallam *et al.*, 2003; Garbarino *et al.*, 2004; Carriquiry *et al.*, 2009).

Uterine involution: A subset of 25 cows was palpated weekly beginning on average at day 19 PP. Uterine involution scores of 1-5 were assigned to these cows each week. These scores were analyzed by treatment relative to day of breeding (Fig. 8). All 3 treatment groups progressed in uterine involution scores at the same rate ($p>0.05$). At day 19 PP, the average score for all 3 treatment groups was 2.45. By the day of insemination (68±8 days PP), uterine involution scores had reached an average of 4.35 for all 3 treatment groups.

Therefore, uteri had returned to excellent tone by the time the cows were inseminated. Uterine involution has been reported to be completed by 27-56 days PP (Kiracofe, 1980; Risco *et al.*, 1994) when the uterus has resumed its nongravid position in the pelvis and the uterine horns have recovered their symmetry in diameter (Risco *et al.*, 1994). Slama *et al.* (1991) reported that at 3-5 weeks PP macroscopic involution occurs, characterized by an increase in the uterine tone and a decrease in the length and weight of uterus and previously gravid horn. The diameter of the cervix is decreased by 4-6 weeks PP (Slama *et al.*, 1991). More

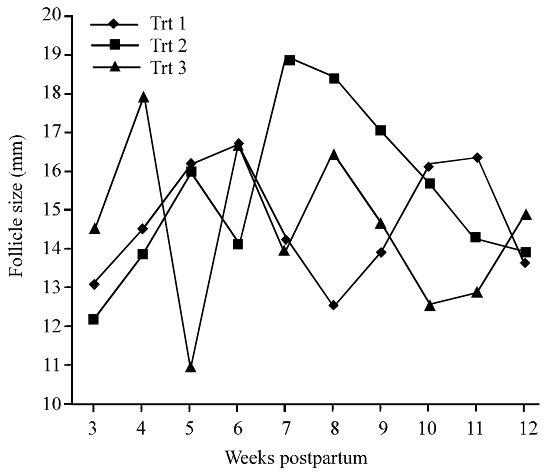

Fig. 6: Largest follicle size (means) by Treatment (Trt) relative to calving date. Animal numbers varied after week 8 due to calving to breeding intervals. Pre-Ovsynch treatments for Trt 1-3 were NaCl and NaCl; NaCl and $PGF_{2\alpha}$ and GnRH and $PGF_{2\alpha}$ on days 15 and 25 postpartum, respectively. There were no significant differences (p>0.10) between groups (treatments) or across time in follicle sizes. All 3 groups had resumed ovarian activity by 3 weeks postpartum

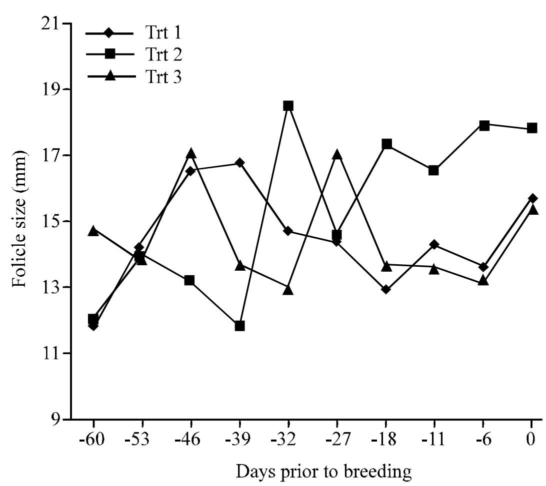

Fig. 7: Follicle sizes (means) by Treatment (Trt) relative to day of breeding. Pre-Ovsynch treatments for Trt 1-3 were NaCl and NaCl; NaCl and $PGF_{2\alpha}$ and GnRH and $PGF_{2\alpha}$ on days 15 and 25 postpartum, respectively. Animal numbers varied between days -39 to -60 due to calving to breeding intervals. There were no significant differences (p>0.10) between follicle sizes between groups or across time

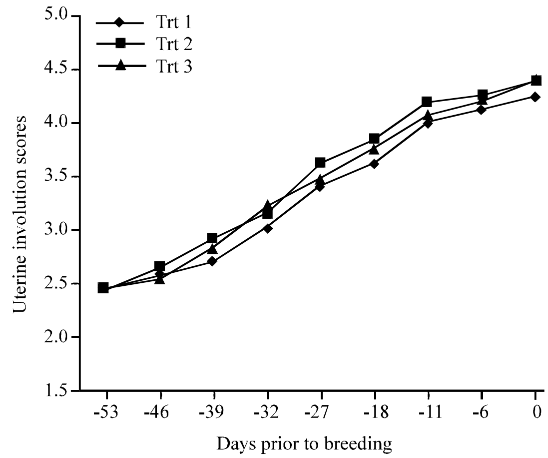

Fig. 8: Uterine involution scores (means) by Treatment (Trt) relative to day of breeding. Pre-Ovsynch treatments for Trt 1-3 were NaCl and NaCl; NaCl and $PGF_{2\alpha}$ and GnRH and $PGF_{2\alpha}$ on days 15 and 25 Postpartum (PP), respectively. At day -53 (day 49 PP), the average score for all treatment groups was 2.45. By insemination time all 3 treatment groups had reached an average score 4.35, meaning that the uteri had reached excellent tone

recently, Sakaguchi *et al*. (2004) found that the uterine involution, defined as <30 mm in diameter of each horn and a difference in size between the previous gravid and non gravid horns = 5 mm was completed at 18 days PP.

Post-Ovsynch follicular dynamics: The size of the follicles destined to ovulate after the Ovsynch protocol were measured in the 25 cows that were scanned by ultrasound. No differences (p>0.05) in average follicle size were found between groups (18.19±1.36, 14.78±1.0 and 15.97±1.2 mm for Trt 1-3, respectively). Stevenson *et al.* (2007a) did not found differences (p>0.05) in the diameter or volume of the ovulatory follicles when administering $PGF_{2\alpha}$ injection at 7, 8 or 9 days after the 1st GnRH injection in the Ovsynch protocol. They found ovulatory follicle diameters of 14.3±0.6, 14.1±0.8 and 15.3±0.9 mm for $PGF_{2\alpha}$ administration at 7-9 days after the 1st GnRH injection of the Ovsynch protocol, respectively (p>0.05). The ovulatory follicle produces a large amount of estrogen which induces the onset of estrus and triggers the release of GnRH causing ovulation by signaling the release of LH (Kinder *et al*., 1996; Gümen and Wiltbank, 2002; Stevenson *et al*., 2007a). Since, ultrasonography was performed on these 25 cows every 12 h beginning at the time of the second GnRH injection of the Ovsynch protocol and continuing for 36 h, the time by which each cow ovulated were analyzed. Of the 25 cows monitored only 2 did not ovulate. Average ovulation times were 34.5±1.5, 36±0 and 36±0 h after the 2nd GnRH injection for Trt 1-3, respectively. There were no differences (p>0.10) in ovulation times between groups. Ovulation has been reported to occur between 24 and 32 h after the 2nd GnRH injection in the Ovsynch protocol (Pursley *et al*., 1998; Sellars *et al*., 2006).

Pregnancy rates: The pregnancy status of each cow was determined at days 30 and 51 post-insemination. Pregnancy rates for Trt 1-3 were 61.5, 50 and 38.5%, respectively and were not different (p>0.05) between groups. The overall PR was 50% which is considerably higher than most of the rates previously reported this early in the PP period.

Stevenson *et al.* (1999) reported a conception rate of 35.7% for cows that were subject to the same Ovsynch protocol and were first inseminated from 58-78 days PP. Sellars *et al.* (2006) artificially inseminated at 22-24 h after the second GnRH injection of the Ovsynch protocol and found a conception rate of 36.6% in PP lactating Holstein cows. Another study indicated that to achieve the maximal PR using AI, cows should not be inseminated until after 75 days PP (Pursley *et al*., 1997b).

Stevenson *et al.* (2007a) obtained a CR of 55.6% after AI at 91±2 days PP at the time of the 2nd GnRH injection in an Ovsynch protocol in lactating dairy cows. Those results are more close to the study even when we artificially inseminated at approximately 68 days PP but those values are conception rates and a variety of factors can interfere with pregnancy, especially in the early stages, decreasing the pregnancy rate values. In a study of Pursley *et al.* (1997a), the Ovsynch protocol caused a reduction in the median days to 1st AI and days open in the treated cows in comparison with the control group. Those results conflict with those of Gümen *et al.* (2003) who did not find differences (p>0.05) in days open between cows inseminated after Ovsynch or estrus detection. Also (Pursley *et al*., 1997a; Gümen *et al*., 2003) reported PR values for the first AI of 29-37 and 32-39% for treated (Ovsynch) and control (estrus detection) cows, respectively (p>0.05).

The higher than average PR in the early PP period obtained in the current study may be due to management practices such as appropriate feed rations, oxytocin administration at 1st milking, the use of a sprinklers and fans system to help alleviate heat stress and timely health care. Most cows in this study were provided with a sprinklers and fans system to help ease heat stress 3 weeks before their expected calving date. Cows exposed to heat stress were 7.42 times more likely to lose their pregnancies than cows not exposed to heat stress at AI after the Ovsynch protocol (Rutigliano *et al*., 2008). Heat stress has been associated with suppressed: dominance of the large follicle, steroidogenic capacity of the theca and granulose cells, P_4 secretion by luteal cells, plasma LH and inhibin, oocyte quality, embryo development and an increase in embryo mortality (Wolfenson *et al*., 2000).

Pregnancy rates by treatment were not influenced (p<0.10) by the occurrence of retained placentas (35 and 45% for pregnant and non-pregnant cows, respectively). Holt *et al*. (1989) did not observe an effect (p>0.05) of GnRH over the conception rates during the first 100 days PP of Holstein cows treated at day 15 PP.

They found conception rates of 35±0.1 and 44.4±0.1% (p>0.05) in the retained placenta and control groups, respectively. In the study, there were also no differences (p<0.10) in PR by treatment between the number of lactations (an average of 2.9±0.31 and 3.25±0.52 lactations for pregnant and non-pregnant cows, respectively). Other important management factors such as nutrition, housing of cows, timing of insemination, handling of frozen semen and the skill of the inseminators has been reported to influence herd fertility (Momcilovic *et al*., 1998; DeJarnette *et al*., 2004) and therefore could affect the results obtained in this study.

CONCLUSION

The purpose of this study was to determine the effect that GnRH and $PGF_{2\alpha}$ have on the early PP period estrual activity, uterine involution, PR and ovulation synchronization in lactating dairy cows. Treatment with GnRH and $PGF_{2\alpha}$ or $PGF_{2\alpha}$ alone did not induce early PP estrual activity affect follicle sizes nor did it hasten uterine involution. Progesterone concentrations differed by sample time between groups only when $PGF_{2\alpha}$ was administered to Trt 2-3 at day 25 PP. Pregnancy rates were not affected by pre-ovulation synchronization hormonal treatment and the overall PR of 50% is much higher than what has previously been observed when cows were bred this early in the PP period. The current study suggests that good management practices such as appropriate feed rations, oxytocin administration at 1st milking, timely health care and the use of sprinklers and fans to help alleviate heat stress may negate the use of early PP hormone therapy and permit the earlier use of ovulation synchronization protocols. Therefore, management regimens that counteract the demands of lactation by providing sufficient energy and reducing stress could increase PR in the early PP period. However, further studies are needed to determine precisely what factors contributed to the high PR that resulted from this study.

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