Effect of Using Progesterone Releasing Intravaginal Device with Ovsynch Program on Reproduction in Dairy Cattle during Summer Season*

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ABSTRACT : Sixty postpartum lactating Friesian cows in 3 treatments at a commercial dairy farm were used to study the effect of using progesterone supplementation with GnRH and PGF2α synchronization with and without timed AI on fertility during summer. Cows in treatment1(Tr_1) and treatment2 (Tr_2) were fitted with progesterone releasing intravaginal device (PRID) device and injected with 10 g GnRH agonist on 51±3 d postpartum (pp). Seven days later, PRID was removed and cows received 25 mg PGF2α. Two days later, Tr_1 cows received another injection of 10 g GnRH and timed AI 16-20 h later. Control cows received only 25 mg PGF2α 58±3 d pp. Tr_2 and control cows were AI at detected estrus. Serum progesterone for all cows was determined on days of injection, AI and 21, 23 and 28 d postinsemination. Pregnancy rates from first AI based on serum P4 concentrations on d 21, 23 and 28 postinsemination (50, 40 and 35%) and that based on rectal palpation 40-45 d postinsemination (30, 15 and 15% for Tr_1, Tr_2 and control cows, respectively) did not differ among the three groups. Whereas, pregnancy rate at 120 d pp for Tr_1 (65%) was higher ($p<0.05$) than that in Tr_2 (30%) or control (30%). The overall pregnancy rate was not significantly different (90, 90 and 75% for Tr_1, Tr_2 and control, respectively). Days open for cows in Tr_1 (100.3±9) was less ($p<0.03$) than that in Tr_2 (130.9±9) or control (135.1±10). Results indicate that using PRID device with Ovsynch program had significantly increased pregnancy rate and decreased days open compared to AI at detected estrus after synchronization with GnRH, PRID and PGF2α or synchronization with one injection of PGF2α. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 9 : 1268-1273)***

Key Words : Dairy Cows, Estrus-synchronization, TAI, Progesterone, Fertility, Summer

INTRODUCTION

Pregnancy rate in dairy cows declines in summer months due to heat stress (Badinga et al., 1985). Ovarian follicles are susceptible to heat stress (Badinga et al., 1993). The preovulatory follicle is a key component of the reproductive system and impairment of its development during thermal stress may affect other reproductive events, such as gonadotrophin secretion (Gilad et al., 1993) and subsequent development of both the corpus luteum (Howell et al., 1994; Wilson et al., 1998) and the embryo (Putney et al., 1989).

Discrepancies in literature exist concerning the effect of heat stress on the concentration of progesterone in blood during the estrous cycle (Wise et al., 1988b; Wolfenson et al., 1995). It appears that high ambient temperature may decrease the plasma LH concentration in cows (Wise et al., 1988a), which is needed for full development of the dominant follicle (Gong et al., 1996). Dominant follicles in heat-stressed cows may develop in lower LH environment in which reduced gonadotrophin support may affect negatively the final development and differentiation of follicles, as well as luteal progesterone secretion, which causes reduced fertility. About 50% of standing heats are undetected during postpartum period (Mialot et al., 1999). Therefore, development of treatment programs to induce estrus and ovulation in noncyclic cows may improve fertility in dairy cows. Previous studies have shown that most progestogen estrus synchronization programs for cows were associated with a reduction in conception rate following synchronized estrus (Larson and Ball, 1992; Ryan et al., 1995). The reduction in fertility after synchronization with progesterone has been attributed to the development of persistant dominant follicles and subsequently the ovulation of aged oocytes that, if fertilized, result in poor quality embryos with decreased developmental capacity (Savio et al., 1993). Therefore, estrus synchronization programs should aim to synchronize follicular wave development as well as onset of estrus and ovulation. The use of timed insemination of the Ovsynch protocol in some studies has shown that conception rates were not different from those cows inseminated after detected estrus (Pursley et al., 1997a and b; Cartmill et al., 2001b) whereas in other studies, conception rates were reduced (Stevenson et al., 1999; Jemmeson, 2000), but pregnancy rates were increased (Pursley et al., 1997a and b; Cartmill et al., 2001b). Previous studies have shown that blood progesterone concentration during the luteal phase before insemination is positively associated with conception rate (Folman et al., 1973; Rosenberg et al., 1990). Progesterone supplementation to cows that were synchronized with PGF2α increased estrus response and conception rate of cows at the time of the second PGF2α treatment (Xu et al., 1997). Therefore, if

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progesterone is administered during the period between the GnRH and PGF2 injection in the Ovsynch program it may improve reproductive performance of lactating dairy cows. The aim of the current study was to compare the effect of using Ovsynch protocol in summer months with progesterone releasing device (PRID) and time insemination 16-20 h after the last GnRH injection or insemination at detected estrus and compare that to the control practice of insemination after detected estrus following synchronization by one injection of PGF2α at 58 ±3 days postpartum.

MATERIALS AND METHODS

This experiment was conducted on a private dairy farm at Dulail area during the summer period from June to October 2001. Sixty postpartum lactating Friesian cows, primiparous (n=29) and multiparous (n=31) were randomly assigned to three groups of 20 cows each. Cows were housed in free-stall barns provided with shade and were milked three times daily at approximately 8 h intervals. Cows were fed three times daily according to NRC recommendations (NRC, 1989) a total mixed ration of 40% forage (corn silage and chopped alfalfa or haylage) and 60% concentrate (whole cottonseed, barely grain, wheat bran, soybean meal, and commercial concentrate for lactation with trace minerals and vitamins). Cows had a free access to fresh water.

About 35 d postpartum (pp), all cows were rectally palpated to ensure that uterine involution and ovaries were normal. At about 51±3 d pp, every cow in the first two groups (Tr_1 and Tr_2) was fitted with an progesterone intravaginal releasing device impregnated with 1.55 g progesterone without the oestradiol capsule (PRID, CEVA, Sanofi, Sante Animale) at the same time the cow was i.m. injected with 10 μg of buserelin, a GnRH agonist (Receptal®, Hoechst Roussel Vet GmbH). Seven days after injection (58±3 d pp), the PRID device was removed and every cow was i.m. injected with 25 mg of PGF2α (Lutalyse, Phamacia & Upjohn S. A. Puurs- Belgium). Every cow in Tr_1 group received a second injection of 10 μg GnRH 48 h after the injection of PGF2α. Cows in this group were inseminated at 16-20 h following the second GnRH injection while cows in the second group (Tr_2) were inseminated at detected estrus after an injection of 25 mg of PGF2α. The third group was used as control (C). Cows in this group, which had not been detected in estrus were given an i.m. injection of 25 mg of PGF2α 58±3 d pp and inseminated at detected estrus. Estrus detection was performed by visual observation of cows throughout the day. Cows in all groups, which returned to heat, were re-inseminated at detected estrus. And those cows, which were diagnosed not pregnant, received another injection of 25 mg PGF2α and re-inseminated at detected estrus. Pregnancy was diagnosed by rectal palpation between 40-45 d post-insemination. For cows in Tr_2 and C, estrus detection rate or AI submission rate was defined as the proportion of cows that responded to synchronization within 5 d after PGF2α injection while conception rate was the percentage of cows that became pregnant divided by the total number of cows inseminated at estrus within 5 d of PGF2α injection. For these groups, pregnancy rate was defined as the product of estrus detection rate or AI submission rate and conception rate. For cows in Tr_1 group, conception rate and pregnancy rate were equivalent. Days open were defined as the number of days from calving to conception for pregnant cows till the fourth insemination.

Blood samples were collected via Jugular venipuncture from all cows at the day of each injection, at the day of insemination, and at the days 21, 23, and 28 post-insemination. Serum was harvested by centrifugation and serum samples were stored at -20°C until assayed for progesterone (P4). Concentrations of serum P4 were determined by RIA (Immunotech a Coulter Company, Marseille, France) employing highly specific polyclonal antibodies in a radioimmunoassay for the quantitative determination of progesterone in animal serum. The standards used for serum were 0.0, 0.35, 1.80, 8.8 and 44 ng/ml. The inter- and intra- assay CV were 7.41 and 5.49%; respectively. The incidence of cyclicity of each cow was determined by the concentrations of P4 in each of three (Tr_1) or two (Tr_2) blood samples at the time of hormonal treatment. A concentration of P4≥1 ng/ml was indicative of a functional CL (cycling). A concentration of P4<1 ng/ml was considered non functional corpus luteum (CL) (non-cycling).

Environmental temperatures and relative humidity were obtained from official national station at Dulail area. The temperature humidity index (THI) was calculated following the formula of Kelly and Bond, reported by Ingraham and his associates (1979).

Data were analyzed using Statistical Analysis System package (SAS, 1998). For all cows, days from PGF2α injection to first insemination, days from calving to first insemination and for pregnant cows, number of insemination per pregnancy, and days open data were analyzed using two-way analysis of variance with group (Tr_1, Tr_2, C) and parity (primiparous and multiparous) and their interaction as factors. Where appropriate, orthogonal contrasts were used to examine differences between groups. When significant group×parity interactions were found, t-test was used to locate differences between means. Data of estrus detection rate or AI submission rate, conception rate and pregnancy rate were analyzed using the logistic regression test (SAS) with group and parity as independent variables. Progesterone data were analyzed using repeated

Table 1. Reproductive performance for time inseminated cows (Tr_1), cows inseminated at detected estrus (Tr_2) and cows in the control group (C)

Reproductive performance	Treatment[1]		
	Tr_1	Tr_2	C
No. of cows	20	20	20
AI submission rate, %	100^{a}	85^{c}	70^{bc}
Conception rate,%	30^{d}	17.6^{d}	21.4^{d}
Days from calving to 1st AI	62.7 ± 1.0^{e}	64.1 ± 1.0^{ef}	65.9 ± 1.0^{f}
Days from PGF2α injection to 1st AI	2.7 ± 0.8^{e}	4.2 ± 0.8^{e}	7.2 ± 1.0^{f}
Days open for all pregnant cows	100.3 ± 9^{e}	130.9 ± 9^{f}	135.1 ± 10^{f}

[1] Tr_1=(Ovsynch+PRID); Tr_2=(GnRH+PGF2α+AI at detected estrus); C=(PGF2α −AI at detected estrus).

[a,b] Percentages within a row different superscripts differ ($p<0.01$).

[a,c] Percentages within a row different superscripts differ ($p<0.05$).

[d] Percentages within a row different superscripts do not differ ($p>0.05$).

measure analysis of variance. The analysis considered parity, pregnancy incidence (pregnant or nonpregnant) as non-repeated factors and day of blood collection as repeated factor.

RESULTS

Mean lactation numbers for cows in Tr_1, Tr_2, and C groups were 1.99±0.22, 2.20±0.22 and 2.10±0.22, respectively. There was no significant difference among the three groups. For the 40 cows studied in Tr_1 and Tr_2 groups, 85% of the cows were considered cycling since they had at least one blood sample with P4 concentration ≥1 ng/ml on either d 51±3 or d 58±3 or they had exhibited estrus. Only 15% (three cows from each group) had P4 concentrations <1 ng/ml on either d 51 or d 58. These cows did not respond to the synchronization treatment because they were not detected in estrus during 5 days after the removal of PRID device and the injection of PGF2α in Tr_2. Meanwhile in Tr_1 cows were inseminated without being detected in estrus. For cows in C group, six cows were not detected in estrus during 5 days after PGF2α injection. They had P4 concentrations <1 ng/ml just prior to the injection of PGF2α which was lower ($p<0.01$) than that for cows in Tr_1 and Tr_2.

Treatment differences ($p<0.01$) were detected for AI submission rates for cows in Tr_1, Tr_2 and C groups. These differences occurred by design, since all cows in Tr_1 group were inseminated, whereas Tr_2 and C, cows were inseminated only after detected estrus (Table 1). Conception rates at first AI among the three groups were not significantly different. Pregnancy rate from first AI estimates based on serum progesterone concentrations >1 ng/ml on d 21, 23 and 28 did not differ among the three groups. The estimated pregnancy rates on these days for Tr_1, Tr_2 and C groups were 50, 40 and 35%, respectively. Similarly pregnancy rate based on palpation per rectum at d 40 to 45 from first insemination did not differ among the three groups and were 30, 15 and 15% for Tr_1, Tr_2 and C groups, respectively. Whereas, pregnancy rate for cows pregnant to 120 d pp in Tr_1 was greater ($p<0.05$) than that for cows in both Tr_2 and C (65 vs. 30 and 30% for the three groups, respectively, Figure1). Similar observation was found for cows pregnant to 150 d (90 vs. 65 and 55% for the three groups, respectively). The overall pregnancy rate up to the fourth AI of cows in the three groups was not significantly different (90 vs. 90 and 75% for the three groups, respectively, Figure 1). Orthogonal contrast for number of AI per pregnancy, mean days from calving to first AI, from PGF2α to first AI and days open for cows in all groups are shown in Table 1. These three variables were unaffected by parity or treatment×parity interaction. The least squares means for days from calving to first AI for cows in Tr_1 group was less ($p<0.03$) than that for cows in Tr_2 or C groups. While days from PGF2α injection to first AI was less ($p<0.03$) for cows in Tr_1 and Tr_2 groups than that for cows in C group. The mean number of days open for cows in Tr_1 group was less ($p<0.03$) than that for cows in Tr_2 and C groups. The injection of GnRH and PRID insertion increased the blood P4 concentrations of cows in Tr_1 and Tr_2 groups from day of insertion up to the day of PGF2α (d of PRID removal). The means for serum P4 concentrations were 1.31±0.34 vs. 1.56±0.34 ng/ml at d 51 ±3 and 2.95±0.36 vs. 2.86±0.36 ng/ml at d 58±3 (PGF2α injection) for cows in Tr_1 and Tr_2, respectively, with no significant differences between the two groups (Figure 2). Meanwhile, the mean serum P4 concentration for cows in C group at d 58±3 (1.27±0.41 ng/ml) was lower ($p<0.004$) than that for cows in both Tr_1 and Tr_2 groups. Means for P4

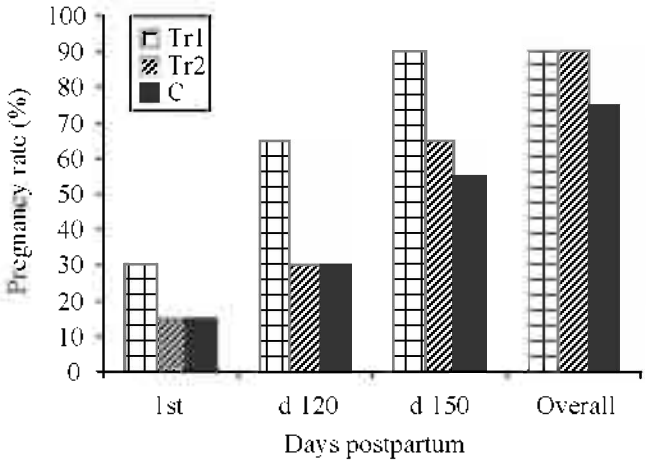

Figure 1. Pregnancy rates to first AI, d 120, d 150 and over all pregnancy for cows in Tr1 (Ovsynch+PRID), Tr2 (GnRH+ PGF2α+AI at detected estrus). [e, f] Means within a row different superscripts differ ($p<0.03$).

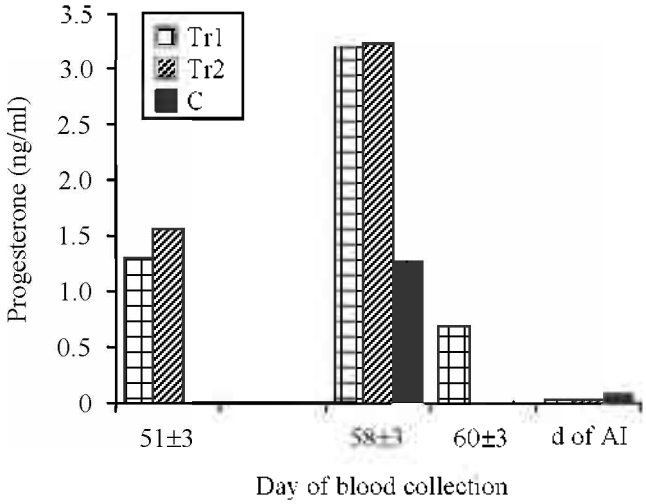

Figure 2. Serum progesterone concentration at the time of bolld collection for cows in Tr1 (Ovsynch+PRID), Tr2 (GnRH+PGF2α +AI at detected estrus) and C (PGF2α+AI at detected estrus).

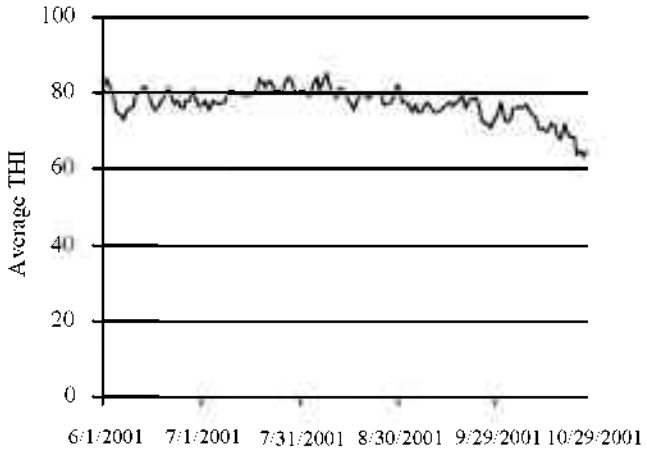

Figure 3. Daily average values for temperature humidity index (THI) during the experimental period.

concentrations at the time of AI did not differ among the three groups and were 0.027±0.03, 0.028±0.03 and 0.07±0.03 (ng/ml) for cows in Tr_1, Tr_2 and C groups, respectively. These results revealed that Ovsynch protocol could eliminate the need for heat detection. Temperature humidity index (THI) during the experimental period is shown in Figure 3. The high THI (≥72) during summer imposed heat stress on cows for various periods either before or after insemination.

DISCUSSION

A greater synchrony of CL function was evident in cows in Tr_1 and Tr_2 since high serum P4 concentrations were detected in the sera of 85% of the cows at the time of PGF2α injection. Pursley et al. (1997b) reported similar observations. Accordingly, Pursley et al. (1995) found that the first injection of GnRH stimulated ovulation of a follicle in 85% of lactating dairy cows. Administration of GnRH at random stages of the estrous cycle caused LH release and ovulation of a dominant ovarian follicle in 66 to 80% of dairy cows (Pursley et al., 1995; Vasconcelos et al., 1997). However, about 15% of synchronized cows (Tr_1 and Tr_2) were not detected in estrus during 5 d after PRID removal. Moreover, Xu and Burton (2000) have shown that using program-involving progesterone, GnRH and PGF2α and AI at detected estrus can synchronize dairy cows. Thus these GnRH programs can be used to synchronize all cows in a herd, irrespective of their cyclic status at the time of initiation of treatment, but the effect of season on synchronization treatment was not assessed during summer. It is likely that those cows did not have a functionally dominant follicle at the time of PRID removal which might be because the dominate follicle had recently turned over. This situation could occur if the dominant follicle was too small at the time of GnRH injection and the less response of the granulosa cells (Twagiramungu et al., 1994). In addition, some anestrus cows might have formed a CL in response to the GnRH injection administered on d 10 before AI (Kesler et al., 1978), which would have elaborated the estimate of cycling cows on d 3 before AI. These results indicate that 5-15% of the cows were not cycling when the insemination period began. This agree with results reported by Cartmill et al. (2001a) in which 83.8% of cows were cycling by 35-54 days in milk based on three estimates of P4 in blood sera collected during 12 d. About 70% of heats for cows in C group were detected. Administration of PGF2α protocol in diestrus, 90 to 95% of the cows should be in estrus by 7 d after injection and 70 to 90% of these estruses should occur on d 3 to 5 after PGF2α as reported by Ferguson and Galligan (1993). Our results indicate that treatment of cows with 10 μg GnRH and PRID 50-60 d pp (Tr_2) followed 7 d later by an injection of 25 mg PGF2α and removal of PRID and then insemination of cows at detected estrus, did not increase pregnancy rate nor decrease days open compared to control cows which were only injected with 25 mg PGF2α at 58±3 d and inseminated at detected estrus. Meanwhile, cows in Tr_1 group, which were treated with GnRH, PRID and PGF2α (like those in Tr_2) then given another injection of 10 μg GnRH 2 d after PRID removed then time inseminated at 16-20 h following the last GnRH injection (Tr_1) had significantly increased pregnancy rate from first insemination, at 120 and 150 d pp and significantly decreased days open for cows pregnant compared to treatment with GnRH, PRID and PGF2α (Tr_2) or treatment with PGF2α alone (C) and insemination at detected estrus. The percentage of cows detected in estrus is very low as a result of absence of heat or missed heats.

Moreover those cows, which are inseminated at detected estrus especially in summer months, have low fertility. Treatment of cows with GnRH, PRID and PGF2α did not improve the situation. Meanwhile, time insemination of cows after treatment with GnRH, PRID and PGF2α did improve fertility and reduced the need for estrus observation. Increased pregnancy rates during summer heat stress (without progesterone supplementation) have been reported (De La Sota et al., 1998) for lactating dairy cattle inseminated after the Ovsynch protocol compared with those inseminated after PGF2α induced estrus. In another study Cartmill et al. (2001b) found that pregnancy rates at d 40 to 50 were not different between Ovsynch protocol (16.4) and GnRH+PGF2α then AI at detected estrus (13.3%) when dairy cows were exposed to high ambient temperature. Those authors explained that low fertility was due to poor embryo survival after d 27 as reported elsewhere (Vasconcelos et al., 1998). Depression of pregnancy rate during the worm or hot period of the year has been well demonstrated (Badinga et al., 1985; Ryan et al., 1993). In addition, a THI value exceeding 72 indicates mild to extreme heat stress conditions for lactating dairy cows (Armstrong, 1994). Improved reproductive performance (days from calving to first AI, days open etc.) has been found (Burke et al., 1996; Pursley et al., 1997a; De La Sota et al., 1998) for cows inseminated after Ovsynch protocol compared with AI after PGF2α treatment. In addition, improved pregnancy rate and reduced intervals from the start of breeding season to conception by AI for lactating cows treated with progesterone, GnRH and PGF2α compared with control group and AI at detected estrus were observed (Xu and Burton, 2000) but the effect of season on synchronization treatment was not assessed during summer. In this study, cows in Tr_1 and Tr_2 groups were also treated with a PRID device (Without oestradiol capsule) to supplement progesterone to those cows that were likely to have a less concentration of progesterone during the treatment period. The measurement of serum progesterone concentrations at the time of PGF2α revealed that CL induced by the GnRH treatment produced sufficient progesterone to increase serum concentrations of cows in the Tr_1 and Tr_2 groups compared with C group (Figure 2). Similar observations were reported (Xu et al., 2000). Previous research reported that progesterone concentration in the late luteal phase before breeding is positively associated with conception rate (Folman et al., 1973; Rosenberg et al., 1990; Xu et al., 1997). Therefore, supplementation of progesterone during the program should improve pregnancy rate for synchronized estrus compared with GnRH and PGF2α alone. Further studies are needed to investigate the effects of pre-synchronization and progesterone supplementation on pregnancy rates with a timed AI protocol in lactating dairy cows.

CONCLUSIONS

This study examined whether fertility of dairy cows could be improved by using progesterone supplementation with timed AI estrus synchronization protocol or insemination at detected estrus with two other synchronization treatments. Using P4 supplementation with timed AI estrus synchronization protocol significantly increased pregnancy rate and decreased days open compared with both synchronization treatments and insemination at detected estrus.

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