

## The Comparison of Two Different Seasons Oestrus Synchronization Results with Fixed-Time AI Protocols

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**Abstract:** The aim of this study, was to compare two different oestrus synchronization methods with fixed-time Artificial Insemination (AI) protocols for Holstein Friesian cow during winter and summer seasons in the Mediterranean region. For this purpose, oestrus was induced using prostaglandins in 110 Holstein Friesian cows kept on an intensive dairy farm. Oestrus was prolonged using progesterone and fixed-time AI applied. Cows were randomly allocated to two groups: The prostaglandin F2 $\alpha$  (Lutealyse) group (n = 60), in which cows received 2 injections of prostaglandin 11 days apart; the progesterone group (n = 50), in which cows received an implant at the base of the ear. When the treatments were applied to animals during the summer months the conception rate decreased from 64.81-54.16%. The efficiency of oestrus synchronization with treatments was satisfactory in the winter season, but in the summer months both treatments will require improvements to achieve better results.

**Key words:** Oestrus synchronization, fixed time, artificial insemination, season, protocols, Holstein cow

### INTRODUCTION

Animals living at temperate latitudes have to face seasonal climatic changes in temperature and food availability. These seasonal climatic changes have led to the development of seasonal reproduction in many species, which ensures that they give birth at the optimal time of year (Hahn, 1999; Silanikove, 2000; Kadzere *et al.*, 2002; De Rensis and Scaramuzzi, 2003; Brown-Brandl *et al.*, 2006). There is a widely observed, decrease in the fertility of postpartum dairy cows that are inseminated in the summer compared with cows inseminated in winter (Silanikove, 2000; Brown-Brandl *et al.*, 2006). Significant seasonality, in reproductive performance has been reported in the Mediterranean region. Studies indicate that, on average, 50% of all heats are missed (Heersche and Nebel, 1994). This is because the most reliable sign of oestrus, standing to be mounted, often goes unnoticed due to difficulties in the timing of heat (during the night) and the short duration of the stand of a cow in heat. These problems are more prominent for high yielding dairy cows because they tend to be in a state of negative energy balance (Maatje *et al.*, 1997; Silanikove, 2000; Lopez *et al.*, 2004; Flores *et al.*, 2006; Brown-Brandl *et al.*, 2006).

Oestrus detection aids used in dairy herds include heat expectancy charts, pressure-sensitive mount detectors, tail chalk, detector animals and electronic aids to improve conception rates at the herd level (Flores *et al.*, 2006). An alternative approach to oestrus detection, namely the control of oestrus by use of hormone administration, is also available (Flores *et al.*, 2006). In this way, oestrus synchronization can be obtained and insemination performed at a fixed time without visual confirmation of oestrus. This technique is commonly used by veterinary surgeons for the treatment of individual cows or groups not observed in oestrus by the required time. Where cows are bred by Artificial Insemination (AI), accurate heat detection is essential. Effective fixed-time AI protocols represent an important advance in making AI a feasible management tool for intensive dairy producers to compensate for the negative effect of heat stress and produce an ideal calving interval (Stegner *et al.*, 2004). These types of protocol eliminate the need for time consuming periods of heat detection and the handling of animals on an individual basis for breeding. The use of fixed-time AI has the distinct advantage of not requiring the detection of oestrus and various effective synchronization methods for fixed time AI have been developed (Patterson *et al.*, 2003). These

programme do not increase the number of cows pregnant compared with the use of fixed time insemination, but they may increase the number of cows pregnant by 120 days postpartum and reduce the number of days open (Ullah *et al.*, 1996; Schmitt *et al.*, 1996; Stegner *et al.*, 2004). Application of controlled breeding under conditions of heat stress could improve whole farm income, depending on the achievement of increased reproductive performance (Boichard, 1990; Stegner *et al.*, 2004).

The present study, was aimed to compare 2 different methods of oestrus synchronization with fixed-time AI protocols in winter and summer on an intensive dairy farm in a subtropical area.

### MATERIALS AND METHODS

Adana is in the subtropical climate zone at a latitude of 36°59" and longitude of 35°18". It has a mild climate with moderate temperatures in winter and relatively high temperatures in summer. Hot and cool seasons extend from mid-November to mid-April and mid-May to mid-October, respectively. According to long-term data, the annual average temperature, relative humidity and precipitation are 18.7°C, 60-90% and 344 mm, respectively. Months of the year were grouped into seasons by similar daily mean temperatures and categorized as follows: cool season = October, November, December, January, February, March, April; hot season = May, June July, August, September. One hundred and ten Holstein Friesian cows (2-4 years old), which were kept at Intensive dairy farm, were utilized. In total 640 Holstein cattle were maintained at the dairy, with 200 cows milked twice daily. The rolling herd average was approximately 5000 kg for a whole lactation. The average daily milk production for the first 90 days (kg), average post calving interval and Body Condition Score (BCS) of the groups are given in Table 1.

Cows were maintained in loose housing systems along a flat concrete feed line. Shade was provided along the feed line. Cows were fed ad libitum with a total mixed ration formulated to meet NRC (2001) recommendations and they had free access to fresh water. All animals were palpated by a veterinary surgeon to determine reproductive tract status prior to treatment. The cows were randomly divided in two groups: The prostaglandin F2 $\alpha$  (Receptal; A ready to use colourless, aqueous solution for parenteral administration, containing synthetic releasing hormone analogue for both the luteinising and follicle stimulating hormones (LH-RH, GnRH, LH/FSH-RH). Each ml contains 0.004 mg Buserelin (Pyr-His-Trp-Ser-Tyr-D-Ser(Bu<sup>+</sup>)-Leu-Arg-ProEthylamide) and 10 mg benzyl alcohol, PhEur as an anti-microbial

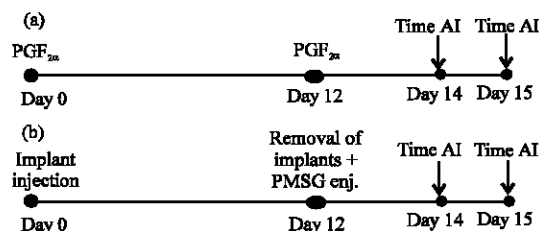


Fig. 1: Oestrus synchronization programs: (A) the prostaglandin F2 $\alpha$  (Lutealyse) group and (B) the progesterone (Crestar) group

preservative. Buserelin is equivalent to the natural LH/FSH releasing hormone produced in the hypothalamus. It causes simultaneous release of Luteinising Hormone (LH) and Follicle Stimulating Hormone (FSH) from the pituitary) group (n = 60), in which cows received two injections of PGF $_{2\alpha}$  11 days apart, the progesterone (Crestar, Intervet, Turkey) group (n = 50), in which cows were fitted with an implant at the base of the ear. Ear implants (Crestar) were inserted subcutaneously on the posterior aspect and towards the base of the ear, midway between the dorsal and ventral edges. The Crestar implants were removed after 9 days and all cows received 300 IU Pregnant Mare's Serum Gonadotropin (PMSG) (PMSG-Intervet is presented as a white, freeze-dried crystalline plug containing 5000 i.u. Serum Gonadotrophin PhEur (PMSG) supplied together with solvent, which when reconstituted gives a solution containing 200 i.u. PMSG mL<sup>-1</sup>) intramuscularly on the day of implant removal (Fig. 1). All cows were inseminated twice, at 48 and 72 h, by Artificial Insemination (AI) without detection of oestrus (Higgins *et al.*, 1986).

Cows were inseminated using high quality frozen-thawed semen. The same controlled breeding protocol was applied to all females in each seasonal group. Pregnancy to AI service was determined by transrectal palpation by a veterinary surgeon approximately 50 days after AI service. Economic analyses were performed including cost of drugs, semen cost, inseminator fee and the cost of each farm visit.

Logistic regression analyses were performed on data from each insemination, using pregnancy detection as the dependent variable (0 or 1) and treatment (prostaglandin versus progesterone) and season (winter versus summer period) variables as independent factors. Parameter estimates from the logistic regression model were used to calculate odds ratios, which are a measure of the strength of association between explanatory and response variables (Kleinbaum *et al.*, 1982). Odds ratios were interpreted as the odds of pregnancy occurring for a particular explanatory variable category relative to the

Table 1: Treatment groups and characteristics

Season	Treatments			
	Progesterone group		Prostaglandin F <sub>2α</sub> group	
	Summer	Winter	Summer	Winter
N	23	27	22	38
Average daily milk production (kg) in first 90 days	29 <sup>a</sup>	33 <sup>a</sup>	28 <sup>a</sup>	35 <sup>a</sup>
BCS (1-5)	2.6 <sup>a</sup>	2.7 <sup>a</sup>	2.7 <sup>a</sup>	2.8 <sup>a</sup>
Post-calving interval (days)	107.26 <sup>a</sup>	95.72 <sup>a</sup>	97.34 <sup>a</sup>	102.53 <sup>a</sup>

<sup>a,b</sup>Values within a row that do not share a common superscript are different at the  $p \leq 0.05$  level of significance

baseline category for that variable when the other explanatory factors were controlled. The 95% Confidence Intervals (CI) were calculated to show the precision of the odds ratio estimates. A CI that contained the numerical value of 1.0 suggests no significant difference between the category and the baseline category for that variable. The conception rates of the groups were analyzed using the logistic regression package of the SPSS statistical program. A value of  $p = 0.05$  was considered significant.

## RESULTS AND DISCUSSION

The pregnancy rates for each treatment group are compared in Table 2. Logistic regression analysis indicated no significant effect of treatment on conception rate and no significant interactions between treatments. Because of this, treatment results were not given at Table 2. However, season had an important effect on conception rate: the conception rate in the hot season was lower than that in the cool season (54.16 vs. 64.81%,  $p < 0.05$ ). Based on the odds ratios (1.046 for winter vs. 0.413 for summer) the likelihood of conception was also affected significantly by season.

Similar results have been achieved after treatment with progesterone-releasing intravaginal devices such as PRID and CIDR (MacMillan and Peterson, 1993) and using PGF<sub>2α</sub> and fixed-time AI (Perry *et al.*, 2002; Stegner *et al.*, 2004; Aygun and Yildirim, 2006; Ahmadi and Ghaisari, 2007; Busch *et al.*, 2007). Colazo *et al.* (2004), reported that the Pregnancy rate to Fix Time AI (overall, 56.2%) was not affected by treatment at CIDR insertion ( $p = 0.96$ ) but was higher ( $p < 0.005$ ) in heifers given Estradiol Cipionate 24 h. after CIDR removal (216/330, 65.4%) than in those given either Estradiol Cipionate at CIDR removal (168/322, 52.1%) or GnRH at AI (169/331, 51.1%). Kawate *et al.* (2004) reported that the addition of a CIDR to the Ovsynch protocol significantly increased conception rates in post-partum suckled Japanese Black beef cows. Conception Rate is affected by Heat Stress prior to AI. Many studies have shown synchronization with fixed-time AI to be a highly effective and economical strategy for improving reproductive performance in high-producing lactating dairy cows

(Burke *et al.*, 1996; Pursley *et al.*, 1997; Chebel *et al.*, 2004; Ahmadi and Ghaisari, 2007; Busch *et al.*, 2007). Researchers have reported that the decrease in conception rate during the hot season can range between 12 and 30% when compared with the results obtained in the winter months (Cavestany *et al.*, 1985; Dransfield *et al.*, 1998; Morton *et al.*, 2007). The main reasons for the low conception rate during the hot season may be a decrease in feed intake, changes in hormone patterns (Schmitt *et al.*, 1996; Ullah *et al.*, 1996; Guzeloglu *et al.*, 2001; Mader *et al.*, 2002; De Rensis and Scaramuzzi, 2003), problems with fertilization (Rekwot *et al.*, 1999), embryonic loss (Dransfield *et al.*, 1998), or failure of heat detection (De la Sota, 1998; Jordan *et al.*, 2002). Implementation of a fixed-time AI programme to ensure that all cows are inseminated within a predefined period after the voluntary waiting period may dramatically improve first-cycle pregnancy rates (Jordan *et al.*, 2002). The treatment must also effectively synchronize ovulation so that fertile oocytes ovulate at a definable time.

**Application cost:** The application cost of the treatments ranged between USD 3.5 and 4.5 and did not differ statistically (Table 3). The pharmaceutical cost of these systems has been moderated in the past few years because several companies now produce these products and veterinary surgeons are willing to promote these systems as a reproductive management tool. However, the cost of the pharmaceuticals will vary with the number of cows synchronized, as larger herds or groups of producers may be able to receive a volume discount. Other costs for the fixed-time AI programme, including semen, AI supplies, inseminator fees and work, were the same for each group.

As can be seen in Table 3, group size affected the total cost of application, although the application cost for each cow did not differ. These costs will vary considerably from farm to farm depending on facilities, number of cows, location and semen selected. This application improved herd reproductive performance via a decreased number of days open and thus decreased the calving interval. Average fertility traits and milk yield of the intensive dairy farm (1973-2003) are given in Table 4.

Table 2: Odds ratios of the variables affecting conception rate

Factor	Class	n	Conception rate (%)	Odds	Odds ratio	Confidence interval 95%	p-value
Seasons	Winter	65	64.81	1.046	0.3951	0.1789-0.8723	0.020
	Summer	45	54.16	0.413	Reference		

Table 3: Application cost of treatments (USD)

Cow number	Treatment			
	Prostaglandin 60		Progesterone 50	
	USD 3.5	USD 4.5	USD 3.5	USD 4.5
Semen cost	15	30	15	30
Inseminator fee	65	130	65	130
Farm visit cost	23	46	23	46
Prostaglandin cost	4.90	9.80		
Progesterone cost			7.20	7.20
PMSG			4.5±0	4.50
Application cost for 1 cow		3.59		4.43
Application cost for group		215.80		217.70

Table 4: Average fertility traits and milk yield of the intensive dairy farm (1973-2003)

Production traits	Average	Minimum	Maximum
Lactation period	299±2	158	450
Lactation yield	491.7±50	974	11779
305-day milk production	4885±46	974	10388
Dry period	77±2	61	96
First calving age	895±6	667	1418
Calving interval	404±3	383	417
Days open	135±3	40	453

This is a worldwide problem which inflicts heavy economic losses and affects about 60% of the world's cattle population. Fertility is consistently lower under temperature changes where maximum temperatures on the day of insemination were greater or equal to 33°C (Cavestany *et al.*, 1985). The average calving interval of the herd is 404±3 (minimum 383, maximum 417 days) and the number of days open is 135±3 (minimum 40, maximum 453 days). The average days open of the treatment groups decreased from 135.4-100.7 days (Table 4). The calving interval was also affected by the treatment via decreased days open. Many studies have reported that poor reproductive efficiency (manifest by long calving intervals) negatively affects milk revenue through reduced life-time milk production (Hohnann *et al.*, 1984). Also, long calving intervals simply translate to having fewer calves born during the productive lifetime of the cow. Several researchers have calculated the costs of additional days open that were associated with production losses and increases in breeding, veterinary and replacement costs, implying marginal benefits for decreased days open (Boichard, 1990).

The reduction in days in open and calving interval for the treatment groups compared to with the farm averages may represent a marked benefit to the profitability of the farm. In general, it can be said that losses ranged from USD 0.18-4.74/cow per added day

open cow/year (Hohnann *et al.*, 1984; Boichard, 1990). For dairy farmers, cost is a very important parameter for any application. This application decreased days open on average from 135-100.7 days. Differences between the herd averages and application groups were between 40 and 28 days. The improvement in days open and calving interval for treatment groups compared with farm averages may be calculated to represent USD 11-12/cow per decreased day open per year improvement in the farm profit.

### CONCLUSION

Synchronization programme are a management tool developed to improve the number of cows pregnant within an acceptable period postpartum. For dairy producers these must be cost effective, user friendly and successful. Using these programme can overcome at least some of the seasonal reductions in fertility normally experienced in areas with high ambient temperatures during the summer, because cows are inseminated whether or not they express oestrus. The results of this experiment indicate that these programme may be a useful tool for improvement of fertility traits in herds during the summer. Further research, is needed to develop successful resynchronization strategies for managing reproduction in lactating dairy cows.

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