

## Heat stress and different timed-AI methods influence on pregnancy rates of dairy cows

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### ABSTRACT

The objective of this study was to evaluate three synchronization fix timed-AI protocols throughout the year on one commercial dairy farm in a semi-arid zone. Mean temperature-humidity indices (THI) were 77.5, 84.9, 70.8 and 58.8 in spring, summer, autumn and winter, respectively. Insemination was synchronized in 3 groups of healthy Holstein Friesian dairy cows (Body Condition Score 2.25-3; scale 1-5) using controlled intra-vaginal drug release (CIDR) +E2 (n = 99), CIDR+GnRH (n = 38), or Select-Ovsynch (n = 181). Pregnancy diagnosis was done by rectal palpation 45-50 days after one AI. Pregnancy rate did not differ between the three methods, but was lower ( $P<0.05$ ) in summer (27.7%) than spring (50.8%), autumn (60.6%) or winter term (68.5%). Pregnancy rates in autumn term did not differ from spring or winter ( $P>0.05$ ), but rates were lower in spring than in winter ( $P<0.05$ ). Thus, a high THI had a negative effect on fertility of dairy cows and none of the three protocols used in this study resulted in good fertility in summer in this semi-arid zone. Therefore, it may be preferable to institute a seasonal calving program to breed in the cooler seasons.

**Key words:** oestrus, dairy cow, controlled intra-vaginal drug release, GnRH, heat stress, synchronization

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### Introduction

Heat stress is a major contributing factor in the low fertility of dairy cows inseminated in the late summer months (RAY et al., 1992; INGRAHAM et al., 1974). Synchronization of oestrus in cattle can facilitate the use of artificial insemination by reducing the time needed for detection of oestrus compared to cattle entering oestrus spontaneously. There are clear seasonal patterns of oestrus detection, day to first service and conception rate in dairy cows (CAVESTANY et al., 1985; DE RENSIS et al., 2002; ALMIER et al., 2002) and lower conception rates are consistently observed in summer months compared to winter months. The effects of heat stress on fertility appear to be observed in the autumn (BADINGA et

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al., 1985; ROTH et al., 1997; DREW, 1999). Heat stress reduces plasma oestradiol during pro-oestrus (GWAZDAUSKAS et al., 1981), and length of oestrus behaviour (ABILAY et al., 1975) and thus a smaller proportion of cows are detected in oestrus (THATCHER and COLLIER, 1986).

Heat stress is the major environmental factor responsible for lower conception rates in the summer (HANSEN, 1997a). Heat stress may cause a mild reduction in LH secretion and luteal progesterone secretion, which result in reduced fertility. The efficiency of follicular selection and dominance are also altered during heat stress, with adverse effects on the quality of ovarian follicles (BADINGA et al., 1993).

Reproductive performance in dairy cows has declined over the last 25 yr in dairy cows, with an increased number of days open and decreased conception rates (SILVIA, 1998). A major problem is lack of accurate oestrus detection. The absence of an efficient and accurate method of oestrus detection is a major factor limiting the reproductive performance of lactating dairy cattle (MAYNE et al., 2002).

Several studies have estimated that 50% of cows are detected in oestrus (BARR, 1975; STEVENSON and BRITT, 1977; HEERSCHKE and NEBEL, 1994). Milk progesterone assays indicate that the proportion of cows not in or near oestrus when inseminated varied from 0 to 60% among herds (REIMERS et al., 1985; NEBEL et al., 1987). Increasing herd size and other factors contribute to the difficulty of accurate oestrus detection.

When progestin-releasing subcutaneous implants or intravaginal progesterone-releasing inserts are used to synchronize oestrus, up to 85% of cattle can be induced to enter oestrus between 36 and 60 h. after the end of treatment (DISKIN et al., 2001). Further improvement in the synchrony of oestrus has been achieved by administering oestradiol benzoate after ending a period of treatment with a progestin (RYAN et al., 1999). Using this method, over 90% of cattle can be induced to enter oestrus within 24 h. (CAVALIERI et al., 2002; CAVALIERI and MACMILLAN, 2002).

Evaluation of three different synchronization and timed AI protocols during all seasons of the year in the semi-arid zone in the Shiraz region was the objective of this research.

## **Materials and methods**

*Farm location.* This study was carried out in 2002 at one dairy farm south-east of Shiraz, located at 29°15'N latitude and 52° 35' E longitude in a hot- and dry-climate area at an elevation of 1500 m ASL. Temperature, humidity and rain readings were taken from the meteorological center of Shiraz. Temperature-humidity index (THI) were calculated by the formula  $[td - (0.55 - 0.55RH)(td - 58)]$  (INGRAHAM et al. 1974). Total rainfall was 323.2 mm during this research.

*Animals.* For this research 318 healthy Holstein Frisian dairy cows were selected at one large commercial dairy farm in the Shiraz region. More than 70 days had elapsed since their normal parturition. Cows with a body condition score of (BCS) 2.25-3 (scale 1-5) were selected. The corpus luteum on the ovary was palpated. Cows without corpus luteum were not included in our study. Therefore, we attempted to select the three groups of cows in the metestrus and diestrus periods. Therefore, cows were not submitted to oestrus and proestrus synchronization at these protocols. Cows were also without clinical signs of endometritis, as noted during routine monitoring of fertility.

*Drugs.* The used drugs included PGF<sub>2</sub>α (Prostaglandin, Pinal Pharma Co., Italy) D-Cloprostenol 75 µg/mL, controlled intra-vaginal drug release (CIDR) (InterAg, Hamilton, New Zealand), GnRH (Gonadorelin, Solarism, Musco) luliberin-A 5 µg/ mL, and estradiol benzoate (E2) (Estradiol Benzoate, Gedeon Richter LTD, Hungary) 2 mg/mL.

*Synchronization of oestrus.* Cows involved in this project were synchronized by three protocols: CIDR+E2, CIDR+GnRH, and Select-Ovsynch. Cows of Select-Ovsynch and CIDR+GnRH groups were injected with 15 µg GnRH at day zero (first day of examination). Cows of CIDR+E2 group were injected with 4 mg estradiol benzoate at day zero. On day (zero) CIDRs were inserted in CIDR+E2 and CIDR+ GnRH groups. The protocols were followed by PGF<sub>2</sub>α (25 mg) injection on day 7 to all cows and CIDRs were removed from the groups in which they were inserted CIDR on day zero. Cows of Select-Ovsynch and CIDR+ GnRH groups were treated with GnRH 48 h. after injection of PGF<sub>2</sub>α. Cows of CIDR+E2 group were injected with 1 mg Estradiol Benzoate 48 h. after injection of PGF<sub>2</sub>α on day 9 of treatment. Cows were AI by different frozen semen at 16-20 h. after GnRH injection, or 24 h. after E2 (Table 1). Pregnancy diagnosis was done by rectal palpation 45-50 days after one AI.

Table 1. Methods of induction of oestrus and ovulation synchronization

Day of protocol				
Synchronization protocols	0 (Thursday)	7 (Thursday)	9 (Saturday)	10 (Sunday)
CIDR+E2	CIDR insert 2 (4 mg)	CIDR removed + PG F <sub>2</sub> α	E2 (1 mg)	AI (24 h)
CIDR+GnRH	CIDR insert +GnRH	CIDR removed + PG F <sub>2</sub> α	GnRH (15 µg)	AI (after 16 h)
Select-Ovsynch	GnRH (15µg)	PGF <sub>2</sub> α	GnRH (15 µg)	AI (after 16 h)

*Statistical analysis.* The conception rates of three synchronization methods were compared with each other (in general and in different seasons) by chi-square and Fisher exact tests using SPSS software (Version 12).

## Results

We found significant differences between means ( $\pm$  SD) of temperature, humidity, rain and temperature-humidity index (THI) in different seasons of the year (Table 2). There was no significant difference between conception rates in the three methods (Table 3). The results of three methods in the present study showed significant difference ( $P < 0.05$ ) between conception rate of summer (27.71%) and other seasons (Table 4). Also, there was a significant difference ( $P < 0.05$ ) between spring and winter conception rates. However, there was no significant difference between spring and autumn conception rates. Additionally, there was no significant difference between autumn and winter conception rates. Table 4 shows the conception rate results of three methods for timed AI. Changes in temperature, humidity and conception rates of four seasons are shown in Fig. 1.

Table 2. Changes of temperature ( $^{\circ}$ C), humidity (%), total measure of rainfall and THI in different seasons of one year in the Shiraz area

Parameters	Spring	Summer	Fall	Winter
Temperature ( $^{\circ}$ C)	20.61 $\pm$ 8.10 <sup>a</sup>	28.89 $\pm$ 7.51 <sup>b</sup>	16.16 $\pm$ 8.50 <sup>ab</sup>	9.32 $\pm$ 5.73 <sup>c</sup>
Humidity (%)	36.11 $\pm$ 7.17 <sup>ab</sup>	23.33 $\pm$ 12.44 <sup>a</sup>	40.00 $\pm$ 12.30 <sup>ab</sup>	54.56 $\pm$ 30.40 <sup>b</sup>
Rainfall (mm)	72.50 <sup>a</sup>	0.00 <sup>b</sup>	34.00 <sup>a</sup>	317.50 <sup>c</sup>
THI	77.47 <sup>a</sup>	84.93 <sup>a</sup>	70.76 <sup>ab</sup>	58.84 <sup>b</sup>

a,b,c Values with different superscripts in each row differ ( $P < 0.05$ ).

Table 3. Methods and pregnancy

Method	Cow number	Non-pregnant	Pregnant	Conception rate %
CIDR+E2	99	42	57	57.58
Select-Ovsynch	181	91	90	49.72
CIDR+ GnRH	38	20	18	47.37
Total	318	153	165	51.89

Table 4. Conception rate percentage (%) of cows at fix time AI groups in different seasons

Season	Total cows	Total (318)	Select-Ovsynch (181)	CIDR+E2 (99)	CIDR+GnRH (38)
Spring	63	50.79 <sup>a</sup>	52.94 <sup>a</sup>	28.57 <sup>a</sup>	60.00 <sup>a</sup>
Summer	83	27.71 <sup>b</sup>	28.81 <sup>b</sup>	28.57 <sup>a</sup>	23.53 <sup>b</sup>
Autumn	99	60.61 <sup>ac</sup>	61.11 <sup>a</sup>	51.14 <sup>b</sup>	70.00 <sup>a</sup>
Winter	73	68.50 <sup>c</sup>	76.47 <sup>a</sup>	66.00 <sup>c</sup>	66.67 <sup>a</sup>
Total	318	51.89	49.72	57.58	47.37

Values with different superscripts in each column are those that differ significantly ( $P < 0.05$ ).

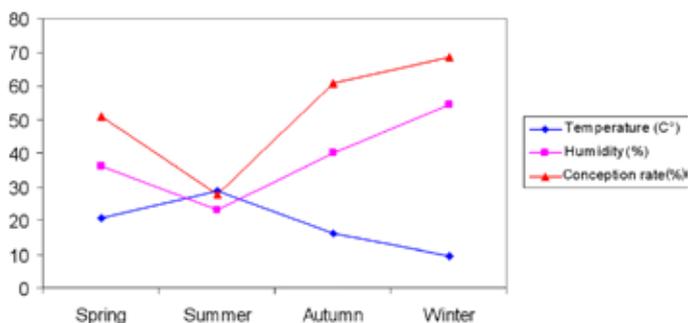


Fig. 1. Comparison between means of temperature, humidity and total conception rates in four seasons.

## Discussion

Results of the present study showed a total conception rate of 51.89% and no significant difference between conception rates of the three methods.

DAY et al. (1997) described a treatment where estradiol benzoate (2 mg) was administered coincident with the insertion of a progesterone-releasing CIDR. After 7 days the CIDR was removed. Each animal received a second injection of estradiol benzoate (1 mg) 48 h. after CIDR removal. Animals were bred after detection of oestrus and the conception rate (62%) of treated animals was similar to that of untreated controls (57%). Timed AI can improve pregnancy rates during periods of heat stress (ARE'CHIGA et al., 1998).

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%). Pregnancy rate to fix time AI was therefore not significantly affected by treatment at CIDR insertion to synchronize follicular wave emergence.

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. In our study there was no significant difference between Select-Ovsynch group and IDR+GnRH.

The present study was performed in the semi-arid zone of the Shiraz region. Therefore, the conception rate at this study was lower than other researchers have reported.

Summer heat stress is a major contributing factor to low fertility among lactating dairy cows. The results of three methods in the present study showed a significantly ( $P < 0.05$ ) lower conception rate in summer (27.71%) than in other seasons. Also, in summer the temperature was higher and humidity percentage was lower than in other seasons. The significant difference ( $P < 0.05$ ) between conception rates during spring and winter corresponded with temperature and humidity percentage. There was a significant difference between conception rates in summer and other seasons in the select-Ovsynch and CIDR+GnRH ( $P < 0.05$ ) methods. There was no significant difference between conception rates in spring and summer in the CIDR+E2 ( $P \geq 0.05$ ) method. However, a significant difference ( $P < 0.05$ ) was found between conception rates in autumn and winter and the two other seasons with CIDR+E2 method.

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 decrease in conception rate during the hot season can range between 20 and 30% compared to the winter season (CAVESTANY et al., 1985; DE RENSIS et al., 2002). The low fertility is generally associated with the warm months of the year (June to September) (HANSEN, 1997b). Heat stress reduces the duration and intensity of behavioural oestrus (ABILAY et al., 1975; GANGWAR et al., 1965), so a smaller proportion of cows are detected in oestrus under heat stress conditions (THATCHER and COLLIER, 1986). Conception Rate is affected by Heat Stress prior to AI (CHEBEL et al., 2004). The various cooling procedures used on farms are not able to improve fertility substantially, and the conception rate of lactating cows in the summer, even in farms equipped with cooling systems, is still pronouncedly below that in the winter (HANSEN, 1997b).

Traditionally, low summer fertility is associated mainly with the warm months of the year (usually June, July, August and September) in the northern hemisphere. Delayed effects of heat stress are expressed in autumn fertility of dairy cows which is lower than in winter, although ambient temperatures drop and cows are no longer exposed to thermal stress. For example, in Florida, the autumn conception rate October-November of Holstein cattle is around 35-40%, compared with more than 50% in winter (January-March) (BADINGA et al., 1985).

We concluded that the conception rate of dairy cows in the hot season was lower than in the cold season in this area. The cooler system is therefore important in dairy farms in the semi-arid zone of the Shiraz region. Three protocols used in this study did not

improve fertility in summer. Therefore, the choice for improving the fertility is evaluation of a seasonal calving program in dairy farms in semi-arid zones.

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M. R. Ahmadi and H. R. Ghaisari: Heat stress and different timed-AI methods influence on pregnancy rates of dairy cows

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M. R. Ahmadi and H. R. Ghaisari: Heat stress and different timed-AI methods influence on pregnancy rates of dairy cows

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## **AHMADI, M. R., H. R. GHAISARI: Utjecaj toplinskog stresa i različita vremena osjemenjivanja na postotak steonosti muznih krava. *Vet. arhiv* 77, 327-335, 2007.**

### **SAŽETAK**

Cilj istraživanja bio je ocijeniti uspješnost triju sinkronizacijskih protokola za umjetno osjemenjivanje u točno određeno vrijeme, tijekom cijele godine na jednoj farmi muznih krava u polusušnom području. Srednja vrijednost indeksa temperature/vlažnosti (THI) iznosila je 77,5 u proljeće, 84,9 ljeti, 70,8 na jesen i 58,8 zimi. U pokusu su bile jednokratno osjemenjene 3 skupine mliječnih krava holštajnsko-frizijske pasmine (ocjene tjelesne kondicije 2,25 do 3; na ljestvici 1-5) sinkroniziranih pomoću intravaginalnih umetaka, na tri načina: kontrolirano intravaginalno otpuštanje lijeka + E2 (n = 99), kontrolirano intravaginalno otpuštanje lijeka + GnRH (n = 38) i Select - Ovsynch (n = 181). Steonost je bila dijagnosticirana rektalnom palpacijom 45 do 50 dana nakon osjemenjivanja. Način sinkronizacije nije utjecao na plodnost pa je postotak gravidnosti u sve tri primijenjene metode bio podjednak. Gravidnost je kolebala ovisno o godišnjem dobu, tako je bila niža (P<0,05) ljeti (27,7%) nego u proljeće (50,8%), jesen (60,6%) ili zimi (68,5%). Postotak gravidnosti krava osjemenjenih u jesen nije se razlikovao od onih osjemenjenih u proljeće ili zimi (P>0,05), ali je u proljeće postotak bio niži nego zimi (P<0,05). U navedenoj polusušnoj zoni visoki THI ima negativan učinak na plodnost muznih krava te se nijedan od triju primijenjenih protokola nije pokazao dobrim za plodnost u ljetnom razdoblju. Prema tome, trebalo bi uvesti sezonsko planiranje teljenja u kojem bi životinje bile osjemenjivane u hladnije doba godine.

**Cljučne riječi:** estrus, muzna krava, intravaginalni umetak, GnRH, toplinski stres, sinkronizacija

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