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Original Research Article

Improving Conception Rate in Lactating Dairy Cows by Using Modified Ovsynch Protocol during Summer

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ABSTRACT

The aim of this study was to test two different timed artificial insemination (AI) protocols in order to improve the percentage of pregnant cows per AI in lactating dairy cows during the summer. Cows (n=140) were randomly divided into two groups; cows in the OVS group (n=75) received the Ovsynch protocol (GnRH–7d–PGF$_{2\alpha}$–2d–GnRH–16h–AI), and cows in the MOVS group (n=65) received the Ovsynch protocol with exogenous progesterone (P4) which administrated between GnRH and PGF$_{2\alpha}$. In addition, GnRH was injected 7 d after AI in the MOVS group (GnRH–P4–7d –PGF$_{2\alpha}$–2d–GnRH–16h–AI–7d–GnRH). Response to the second GnRH treatment of Ovsynch (GnRH-2) was greater (*P*<0.03) in the MOVS (98.5%) than the OVS (89.3%) group. Although P/AI at 31 d was numerically greater in the MOVS (46.2%, 30/65) than the OVS (37.3%, 28/75) group, this difference was statistically insignificant. Thus, because of the greater response to GnRH-2 and P/AI in MOVS compared with the OVS group, the MOVS protocol can be used to improve P/AI in lactating dairy cows during the summer, but further studies are needed to be evaluated the effects of the MOVS protocol on P/AI during the summer.

Key Words: Cow, Ovsynch, summer, pregnancy

Sağmal Süt sığırlarında Yaz Döneminde Modifiye Ovsynch Protokolü ile Gebelik Oranının Arttırılması

ÖZET

Bu çalışmanın amacı sağmal süt sığırlarında yaz dönemindeki suni tohumlama başına düşen gebelik oranını arttırmak amacıyla iki farklı zaman ayarlı suni tohumlama (ST) protokolünü değerlendirmekti. İnekler (n=140) tesadüfi olarak iki gruba ayrıldı; OVS grubundaki ineklere (n=75) Ovsynch protokolü uygulandı (GnRH–7g–PGF2α–2g–GnRH–16s–ST), MOVS grubundaki ineklere (n=65) ise GnRH ile PGF2α uygulamaları arasına ekzojen progesteronun (P4) eklendiği bir Ovsynch protokolü uygulandı. Bu gruptaki ineklere ayrıca bir de ST'dan 7 gün sonra GnRH uygulaması yapıldı (GnRH–P4–7g –PGF2α–2d–GnRH–16s–ST–7g–GnRH). Ovsynch'in ikinci GnRH'sına (GnRH-2) yanıt MOVS grubunda (98.5%) OVS grubuna (89.3%) gore yüksek (P<0.03) bulundu. MOVS grubunda 31. Gündeki gebelik oranı (46.2%, 30/65) sayısal olarak OVS grubuna (37.3%, 28/75) göre yüksek bulunmasına karşın bu fark istatistiki olarak önemli değildi. Sonuç olarak, MOVS grubunda GnRH-2'ye alınan yanıtın ve gebeliğin OVS grubuna gore yüksek olduğu için MOVS protokolünün sağmal süt sığırlarında yaz döneminde gebelik oranını arttırmak amacıyla kullanılabileceği, ancak yine de MOVS protokolünün yaz dönemindeki gebelik oranları üzerine etkisinin daha iyi değerlendirilebilmesi için daha ileri çalışmaların yapılması gerektiğine karar verildi.

Anahtar Kelimeler: Sığır, ovsynch, yaz dönemi, gebelik

INTRODUCTION

Hot weather leading to heat stress is a major contributing factor to the low fertility of dairy cows during summer. The decrease in the percentage of pregnant cows per artificial insemination (P/AI) during the hot season can range between 20% and 30% in lactating dairy cows. The P/AI is observed consistently lower in summer months compared with winter (De Rensis and Scaramuzzi 2003, Hansen and Aréchiga 1999). There are numerous negative effects of hot weather on reproductive traits in lactating dairy cows such as decreasing embryo quality,

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changing follicular development, and decreasing serum progesterone, estradiol and LH pulses (Lopez-Gatius et al. 2006, De Rensis and Scaramuzzi 2003, Hansen and Aréchiga 1999). Several hormonal treatments or timed AI protocols such as Ovsynch have been used to improve or protect fertility during the summer in lactating dairy cows, but results among these studies have been inconsistent (Lopez-Gatius et al. 2006, De Rensis and Scaramuzzi 2003).

Synchronization protocols that allow to timed artificial insemination (TAI) have been widely used (Pursley et al. 1997, Rabiee et al. 2005) because estrus detection efficiency is a major factor limiting reproductive performance of large dairy farms (Lopez-Gatius 2003, Pursley et al. 1997). The real estrus detection rate is very low in large dairy herds in which the number of cows managed per worker is high, resulting in decreased accuracy and effectiveness of the detection (Lopez-Gatius 2003, Rabiee et al. 2005). In addition, a lower duration of estrus and silent heat are seen in high-producing lactating dairy cows (Wiltbank et al. 2008). An application of the Ovsynch protocol (injection of GnRH 7 d before and 48 h after $PGF_{2\alpha}$, and timed AI 16-18 h after the second GnRH injection) allows for the synchronization of follicular development, luteal regression, and time of ovulation, thereby allowing for timed artificial insemination in dairy cows (Pursley et al. 1997).

The response to each hormone administration during the Ovsynch protocol can dramatically alter success of the program. Greater fertility is found in cows that ovulate to the first GnRH treatment of Ovsynch (GnRH-1) compared with cows that do not ovulate to GnRH-1 (Bello et al. 2006, Galvao and Santos 2010, Keskin et al. 2010, Moreira et al. 2001, Vasconcelos et al. 1999). Other critical factors for successful synchronization during the Ovsynch are responses to $PGF_{2\alpha}$ and second GnRH treatment of Ovsynch (GnRH-2). High progesterone levels at the time of $PGF_{2\alpha}$ and luteal regression shortly after $PGF_{2\alpha}$ treatment induces greater response to GnRH-2 and better fertility in lactating dairy cows (Galvao and Santos 2010, Moreira et al. 2001). However, response to GnRH-1 (45-75%) and GnRH-2 (75-95%) and the subsequent conception rate (35-45%) was found to be inconsistent among previous studies (Bello et al. 2006, Galvao and Santos 2010, Moreira et al. 2001). Adding hormones into the Ovsynch protocol such as exogenous progesterone, estradiol or hCG can improve the Ovsynch outcome (Galvao and Santos 2010, Gaja et al. 2008, Galvao et al. 2004, Stevenson et al. 2006). For instance, exogenous progesterone administration between the first GnRH and $PGF_{2\alpha}$ treatment was previously shown to improve the conception rate (Galvao and Santos 2010, Stevenson et al. 2006).

In addition, the serum progesterone level is the most important factor that affects early embryonic loss in lactating dairy cows. Previous studies have shown that embryonic loss is high in cows with low progesterone levels compared with cows with high progesterone levels during the first week after AI (Lopez-Gatius et al. 2006, Stevenson et al. 1993). Accordingly, many researchers have been focused on increasing progesterone levels after AI between 5 and 15 days by the administration of GnRH/hCG (Stevenson et al. 1993, Beltran and Vasconcellos 2008, Paksoy and Kalkan 2010). However, response to GnRH/hCG treatment after AI and its effects on pregnancy remain unclear (Lopez-Gatius et al. 2006, Stevenson et al. 1993, Beltran and Vasconcellos 2008, Paksoy and Kalkan 2010, Howard et al. 2006).

Thus, the aim of this study was to compare Ovsynch protocols with Modified Ovsynch protocols to improve P/AI at 31 d and 62 d in lactating dairy cows during summer.

MATERIALS AND METHODS

Cows, Housing and Management

Lactating dairy cows on a commercial dairy farm located in the South Marmara region, Bursa, Turkey were enrolled during the 2009 summer season (Jun, July, and August). The farm is located in 40.10°North – 28.21°East zone. Cows were housed in free stall barns with self-catching head-locks, and all barns had fans and sprinklers that were activated during the hotter months of the year. All cows were grouped according to their milk production and were milked three times daily at approximately 8 h intervals. The mean milk production of the herd was 9.880±69.7 kg (305 d) per cow. Cows had free access to water and were fed complete mixed rations according to National Research Council recommendations (NRC 2001). Daily milk yield, reproductive health, and management records for each cow were collected from Alpro 2000 (DeLaval, Sweden). Average milk production for each cow was recorded from the 7 d before to the 7 d after AI. Body condition score (BCS) was determined for all cows at the time of the first GnRH treatment of Ovsynch using a 5-point (1= thin to 5 = fat) scoring system (Ferguson et al. 1994). All protocols involving cows used in this research were approved by the Lalahan Livestock Central Research Institute Animal Care Committee.

Experimental design

Only cyclic cows (n=140) with less than three inseminations and no clinical abnormalities of the reproductive tract were included in this study. Cows were randomly divided into two groups: Ovsynch (OVS) and Modified Ovsynch (MOVS). Cows in the OVS group (n=75) received the Ovsynch protocol: GnRH (Buserelin acetate, i.m., 10 µg, Receptal®, Intervet, Turkey) followed by GnRH and $PGF_{2\alpha}$ (Cloprostenol, 500 µg, i.m., Estrumate®, CEVA-DIF, Turkey) 7 d later. A second GnRH treatment (Buserelin acetate, 10 µg, Receptal®) was administered 56 h after $PGF_{2\alpha}$, and all cows were inseminated at a fixed time 16 h to 18 h after the final GnRH treatment. The MOVS group (n=65) received the Ovsynch protocol as mentioned above. Additionally, cows in the MOVS group also received an ear implant containing progesterone (3 mg norgestomet; Crestar®, Intervet, Turkey) at the first GnRH treatment of the Ovsynch protocol, which was removed upon treatment with $PGF_{2\alpha}$. In addition, a third GnRH (Busereline acetate, 10 µg, i.m., Receptal®, Intervet) was injected into cows in the MOVs group 7 days after TAI.

Ultrasonographic examinations

To determine the cyclicity status of each cow before the first GnRH treatment, ultrasonographic evaluations of their ovaries were performed 7 d apart. The ultrasonographic examinations were performed with a Honda HS 2000 equipped with a 7.5 MHz transducer (Honda, Japan). Cows' ovaries were examined on the day of first GnRH and ear implant administrations and 7 days later to determine ovulation response to the first GnRH treatment. Ovulation to the first GnRH treatment was characterized by appearance of a new corpus luteum (CL) on the ovaries. The maximum follicular size was measured at the time of AI. Cows were examined 7 days after TAI to determine ovulation by disappearance of the dominant follicle at time of AI and appearance of new CL. Cows in the MOVS group were also examined to measure follicle size before the third GnRH treatment 7 days after TAI. Response to the third GnRH administration was evaluated 7 days after the third GnRH application. Pregnancy diagnosis was performed 31 d and 62 d after AI using ultrasonography.

Statistical analysis

Statistical analyses were conducted by using SAS (Version 9.2; SAS Institute, 2010). Data were evaluated using PROC LOGISTIC, PROC GLM and PROC FREQ in SAS. The statistical model included the effects of treatment, parity, Days in Milk (DIM), Body Condition Score (BCS), number of services, average milk production, response to first, second, and third GnRH, and follicle size at the time of AI. PROC GLM was used to compare the differences between groups for milk production, DIM, BCS and follicle size at the time of AI between groups. Chi-square results were obtained by using the PROC FREQ procedure for independence tests between response to GnRH administrations and P/AI (31 and 62 days) between groups. The LOGISTIC procedure was performed to determine the effect of covariant factors on P/AI (31 d and 62 d).

RESULTS

General results

Milk production (38.7 ± 0.9 kg/d in MOVS and 38.1 ± 0.8 kg/d in OVS), service number (1.1±0.10 in MOVS and 1.0±0.10 in OVS), DIM (131.8 ± 6.4 in MOVS and 127.2± 7.0 in OVS), BCS (2.83±0.04 in MOVS and 2.86±0.04 in OVS), and mean lactation number of cows (2.2± 0.12 in MOVS and 2.0± 0.11 in OVS) was similar between MOVS and OVS groups.

Responses to GnRH treatment of TAI protocols

The percentage of cows that ovulated to GnRH-1 did not differ in MOVS (49.2%, 32/65) and OVS (62.7%, 47/75) groups (Table 1); however, response to GnRH-2 was found to be different (P<0.03) between groups (98.5%, 64/65 in MOVS and 89.3%, 67/75 in OVS). In addition, response to the third GnRH treatment was 87.7% (57/65) in MOVS group. Although 87.7% of cows ovulated after the third GnRH treatment, only 49.1% (28/57) of cows had an accessory CL at 31 d and only 40.4% (23/57) were pregnant at 62 d.

Table 1. Ovulatory response to GnRH treatments, P/AI at 31 and 62 d, and embryonic loss in dairy cows.

	MOVS	**OVS**	***P* value**
Ovulation to first GnRH of Ovsynch	49.2% (32/65)	62.7% (47/75)	0.10
Ovulation to second GnRH of Ovsynch	98.5% (64/65)	89.3% (67/75)	0.03
P/AI at 31 d	46.2% (30/65)	37.3% (28/75)	0.29
P/AI at 62 d	41.5% (27/65)	34.7% (26/75)	0.40
Embryonic loss	10.0% (3/30)	7.0% (2/28)	0.69

Effect of TAI protocols on P/AI

Although P/AI at 31 d and 62 d were numerically greater in the MOVS (46.2%; 30/65, 41.5%; 27/65) than the OVS group (37.3%; 28/75, 34.7%; 26/75), these differences were statistically insignificant (Table 1). Pregnancy loss between the 31 d and 62 d pregnancy diagnoses was not different between groups (10.0%; 3/30 in MOVS and 7.0%; 2/28 in OVS). When all cows were evaluated, P/AI at 31 d did not differ between those cows that responded to GnRH-1 (36.7%; 29/79) and those that did not respond (47.5%; 29/61). In addition, when P/AI at 31 d was evaluated in both groups, P/AI was similar between responsive and non-responsive cows to GnRH-1 in the OVS and MOVS groups (Table 2). Although response to GnRH-2 was higher in MOVS than OVS, P/AI did not differ in the cows' responses to GnRH-2 in MOVS (46.8%; 30/64) and OVS (41.8%; 28/67) groups. Milk production, DIM, BCS, parity, response to second GnRH of Ovsynch, and follicle size at the time of AI did not affect 31 d and 62 d P/AI. In addition, maximum follicle size at the time of AI was lower (P<0.02) in OVS (15.1±0.31 mm) than MOVS (16.1±0.32 mm).

Table 2. P/AI in responsive and nonresponsive cows to first GnRH of Ovsynch in MOVS and OVS groups.

	Ovulation to first GnRH of Ovsynch		
	Yes	No	*P* value
MOVS	40.6% (13/32)	51.5% (17/33)	0.37
OVS	34.0% (16/47)	42.9% (12/28)	0.44
Total	36.7% (29/79)	47.5% (29/61)	0.55

DISCUSSION

In this study, we aimed to develop a TAI protocol to improve fertility during the summer by increasing response to each hormonal treatment of the TAI protocol and to compare this new protocol with Ovsynch. In recent studies, when the Ovsynch protocol was applied to normal lactating dairy cows, the ovulatory response to GnRH-1 was between 45% and 75% (Bello et al. 2006, Galvao and Santos 2010, Keskin et al. 2010). Similarly, our results showed that response to GnRH-1 was 49% in MOVS and 62% in OVS. Although the percentage of cows that responded to GnRH-1 tended to be lower (13%) in the MOVS group than the OVS group (P<0.10). Exogenous progesterone administration shortly after GnRH-1 in the MOVS group decreased response to GnRH-1. Similarly, another study showed progesterone concentration after CIDR insertion decreased ovulation to GnRH administration, and this result was attributed to a negative feedback of progesterone on LH secretion

(Galvao et al. 2004). Additionally, when ovariectomized cows receive exogenous progesterone, LH concentrations and pulse frequency decrease in the first 8 h of insert administration (Burke et al. 1996).

In addition, previous studies indicate higher ovulation rates to GnRH-1 increases the conception rate in lactating dairy cows (Bello et al. 2006, Keskin et al. 2010, Moreira et al. 2001, Vasconcelos et al. 1999). In these studies, increased first ovulatory response positively affected luteal and follicular responses to subsequent $PGF_{2\alpha}$ and the final GnRH treatment of Ovsynch. The positive effects were shown to induce higher fertility in lactating dairy cows (Bello et al. 2006, Galvao et al. 2010, Keskin et al. 2010, Moreira et al. 2001). Controversial with previous studies, in our study, the P/AI did not differ between cows that responded and those that did not respond to GnRH-1. However, further studies are needed to determine the effect of response to GnRH-1 on P/AI.

In earlier studies, response to GnRH-2 was determined to be between 75% and 95% (Bello et al. 2006, Galvao et al. 2010, Keskin et al. 2010). Similarly, the present study observed an ovulation rate to GnRH-2 of 89-98%; however, the response to GnRH-2 was statistically different between groups ($P<0.03$: 98% in MOVS and 89% in OVS). This difference may originate from the addition of exogenous progesterone in the MOVS group. Previous studies have indicated cows with high progesterone levels at the time of $PGF_{2\alpha}$ have a higher response to GnRH-2 than cows with low progesterone levels (Bello et al. 2006, Galvao et al. 2010, Stevenson et al. 2006). For instance, Galvao and Santos (2010) showed response to GnRH-2 is greater in cows receiving exogenous progesterone (87%) than cows that did not (80%). In addition, some researchers have indicated that increasing ovulation rates to GnRH-2 and high progesterone levels at $PGF_{2\alpha}$ improve pregnancy in lactating dairy cows (Bello et al. 2006, Galvao et al. 2010, Moreira et al. 2001). However, in the present study, a high ovulation rate to GnRH-2 did not increase P/AI in the MOVS group (46% in MOVS vs. 37% in OVS). Moreover, P/AI did not differ in cows' responses to GnRH-2 in MOVS (46.8%; 30/64) and OVS (41.8%; 28/67) groups. We expected high progesterone at the time of $PGF_{2\alpha}$, by providing exogenous progesterone and high ovulation rates to GnRH-2, should have increased the P/AI in the MOVS group. Consistent with our results, Gaja et al. (2008) used a TAI protocol similar with our MOVS protocol (Ovsynch + CIDR + GnRH) and indicated it did not increase P/AI in dairy cows when compared with the Ovsynch + GnRH protocol.

Progesterone is a vital hormone during the early stages of pregnancy that promotes embryonic development and controls the luteolysis. Thus, many researchers have attempted to increase progesterone levels by using GnRH after AI between 5 and 14 days (Lopez-Gatius et al. 2006, Beltran and Vasconcellos 2008, Paksoy and Kalkan 2010). In some studies, the administration of GnRH after AI increases the concentration of progesterone (Gaja et al. 2008, Stevenson et al. 2006, Beltran and Vasconsellos 2008, Howard et al. 2006), but not all studies have observed this effect (Paksoy and Kalkan 2010). Elevated serum progesterone after GnRH treatment has been attributed to the formation of accessory CLs (Gaja et al. 2008, Howard et al. 2006). Our results show that response to third GnRH treatment was 87.7% (57/65) in the MOVS group, indicating approximately 90 percent of cows had an accessory CL 9-10 days after AI. Some studies have indicated that elevated serum progesterone levels after GnRH treatment improve P/AI in lactating dairy cows (Lopez-Gatius et al. 2006, Beltran and Vasconcellos 2008). Our results showed that P/AI was similar between groups (46%, 30/65 and 37%, 17/45 MOVS and OVS group, respectively). Therefore, GnRH treatment 7 d after AI did not affect P/AI in lactating dairy cows.

Furthermore, previous studies have indicated that P/AI is detected in between 30% and 45% of normal dairy cows after the Ovysnch protocol (Bello et al. 2006, Gumen et al. 2003, Galvao and Satos. 2008, Pursley et al. 1997). In this study, P/AI was detected in 46% of the cows in the MOVS group and 37% of the cows in the OVS group. However, it is well known that hot weather decreases the P/AI. The decrease in P/AI during the hot season can range between 20% and 30% in lactating dairy cows (Lopes-Gatius 2003, De Rensis and Scaramuzzi 2003, Hansen and Aréchiga 1999). Therefore, P/AI in OVS and MOVS groups were in an acceptable range. Particularly, P/AI in MOVS (46%) was similar with P/AI achieved during cold season (45-50% in our other studies). In conclusion, although larger numbers of animals are needed to evaluate the effect of the MOVS protocol on P/AI, our data indicate the MOVS protocol can be utilized to improve decreased fertility in lactating dairy cows during the summer.

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