Turk J Vet Anim Sci
30 (2006) 213-217

Research Article

The Effectiveness of Cosynch Protocol in Dairy Heifers and Multiparous Cows

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Received: 26.05.2005

Abstract: The aim of the present study was to evaluate the effects of Cosynch protocol on some reproductive parameters in Holstein multiparous cows (MC) (n = 54) and heifers (n = 53). Follicular development, presence of corpus luteum (CL) at different stages of Cosynch protocol, ovulation time and pregnancy rates were evaluated by ultrasonography in MCs and heifers.

Pregnancy rates (PR) in MCs and heifers were found as 41% and 51% (P: 0.29, Z: -1.06). At the time of first Gonadotropinreleasing hormone (GnRH) (GnRH1), Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and final GnRH (GnRH2) injections, PRs in heifers and MCs which had CL on their ovaries were detected as 47.4%-48.7% (P: 0.91, Z: 0.12), 60.5%-48.7% (P: 0. 30, Z: -1.4) and 62.1%-36.4% (P: 0.031, Z: -2.15) respectively. Ovulations occurred in 24-30 h interval after artificial insemination (AI) in MCs (50%) and heifers (35%) (P: 0.13, Z: 1.51).

In conclusion, Cosynch protocol was found more effective and practical in heifers than MCs. In addition, ovulations that occurred before AI could be the reason of low conception rate for Ovsynch procedure in heifers.

Key Words: Multiparous cows, heifer, pregnancy rates, corpus luteum, ovulation synchronization

Sütçü Düve ve Multipar İneklerde Cosynch Poresedürünün Etkinliği

Özet: Bu çalışmanın amacı, Holstein düve ve multipar ineklerde Cosynch prosedürünün bazı reprodüktif parametreler üzerine etkilerinin değerlendirilmesidir. Düve ve ineklerde Cosynch prosedürünün farklı aşamalarında; folliküler gelişim, korpus luteum varlığı, ovulasyon zamanları ve gebelik oranları ultrasonografik olarak incelenmiştir.

Multipar inek ve düvelerde gebelik oranları % 41 ve % 51 olarak bulunmuştur (P:0,29, Z: -1,06). İlk GnRH (GnRH1), $PGF_{2\alpha}$ ve son GnRH (GnRH2) enjeksiyonu anında, ovaryumlarında korpus luteum bulunan düve ve multipar ineklerde gebelik oranları sırasıyla, % 47,4 - % 48,7 (P: 0,91, Z: 0,12), % 60,5 - % 48, 7 (P: 0,30, Z: -1,4) ve % 62,1 - % 36, 4 (P: 0,031, Z: -2,15) olarak belirlenmiştir. Ovulasyonlar, multipar inek (% 50) ve düvelerde (% 35) çoğunlukla suni tohumlamadan (ST) 24-30 saat sonraki zaman aralığında tespit edilmiştir (P: 0,13, Z: 1,51).

Sonuç olarak, Cosynch prosedürü, düvelerde ineklerden daha etkin ve kullanışlı bulunmuştur. Ayrıca, ST öncesi meydana gelen ovulasyonlar düvelerde Ovsynch prosedüründe elde edilen düşük gebelik oranlarının nedeni olabilir.

Anahtar Sözcükler: Multipar inek, düve, gebelik oranları, korpus luteum, ovulasyon senkronizasyonu

Introduction

Synchronization of ovulation in cows has been extensively investigated and many protocols have been developed for timed artificial insemination (TAI). Protocols such as Cosynch, Ovsynch and Heatsynch had remarkable advantage over oestrus synchronization that all cows could be inseminated (1).

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These protocols are based on the initiation of follicular wave (by GnRH) before inducing regression of luteal tissue (by $PGF_{2\alpha}$). After the $PGF_{2\alpha}$treatment the emerging dominant follicle is forced to ovulate by second GnRH treatment and cows are either inseminated at the same time (Cosynch) or 16 – 24 hour after (Ovsynch) (1). If the second GnRH treatment is replaced by estrodiol cypionate this protocol is called as Heatsynch (1,2).

Low conception rate was shown as the major drawback of TAI which is mostly related to the response of ovary to the first GnRH injection, diverse developmental status of dominant follicle at second GnRH and CL size after ovulation (1). Also such protocols are practical in farms which have undetection or slient heat, ovulation problems and reoccurring luteal phases without oestrus expression (2).

This study was carried out to investigate the effectiveness of Cosynch protocol on Holstein heifers and MCs.

Materials and Methods

The present study was conducted with 54 lactating Holstein MCs and 53 heifers on a commercial dairy farm. Cows were milked twice daily and average milk production was about 7000 kg. Multiparous cows (45-120 days postpartum) were having a normal body condition score. Heifers were minimum 15 months old and 350 kg.

All animals received the same Cosynch program which was 20µg GnRH1 (Busereline acetate-Receptal®) on day 0, 0.150 mg $PGF_{2\alpha}$ (Kloprostenol-Dalmazin®) on day 7 and 20µg GnRH on day 9). Artificial inseminations (AIs) were applied at the same time with GnRH2 on the day 9.

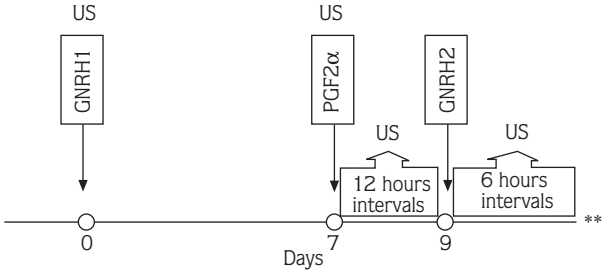

Figure 1. Schematic diagram of treatment protocols in experiment.
+ US: ultrasonography
+** 42 hour after GnRH2

In order to examine the follicles, CL and pregnancy Honda Ultrasound machine with a 5 MHz transducer was used. Ultrasonography was performed on days 0 and 7. Then, it was performed at 12 h intervals between days 7 and 9. Ovaries were examined within 6 h intervals after GnRH2 (day 9) till 42 hour (Figure 1). Pregnancy diagnosis was conducted on 27-33 days post AIs. Statistical analysis was carried out by using SPSS (version 12.0). The relation between presence of CL and PRs were analysed by using Chi-square test. The ovulation rates and PRs of the heifers and MCs with CL were compared by two proportions Z test. Mann Whitney-U test (MWU) was used to compare the avarages of CL diameters in pregnant MCs and heifers, whereas, Kolmogornov-Smirnov test was used to compare ovulation rates distributed in different time intervals. The level of significance was set at 0.05.

Results

In this study, PRs were detected as 41% and 51% in MCs and heifers (P: 29; Z: -1.06). Pregnancy rates in heifers and MCs which had CL (CL+) at GnRH1 were 47.4% and 48.7%, respectively (P: 0.91, Z: 0.12). However, PRs in animals, which had no CL (CL-) were 60% in heifers and 20% in MCs (P: 0.025, Z: -2.24). At the time of $PGF_{2\alpha}$ injection, PRs in CL+ heifers and MCs were 60.5%, 48.7% (P: 0.30, Z: -1.4) while in CL- ones were 26.7% and 20%, respectively (P: 0.67, Z: -0.43). At GnRH2, PRs were 62.1% and 36.4% in CL+ heifers and MCs (P: 0.031, Z: -2.15), while 37.5% and 60% in CL- animals (P: 0.22, Z: 1.22) (Tables 1 and 2).

The avarage diameters of CLs in pregnant MCs and heifers at GnRH1, $PGF_{2\alpha}$ and GnRH2 injections are summarized in Table 3.

Table 1. Pregnancy rates in heifers and MCs which had CL on their ovaries at the time of first GnRH (GnRH1), $PGF_{2\alpha}$ and final GnRH (GnRH2) injections.

	Pregnancy rates (%)			
	Heifers	Multiparous cows	Z	P
GnRH1	47.40	48.70	0.12	0.91
$PGF_{2\alpha}$	60.50	48.70	-1.40	0.30
GnRH2	62.10	36.40	-2.15	0.031

Table 2. Pregnancy rates in heifers and MCs which had no CL on their ovaries at the time of first GnRH (GnRH1), $PGF_{2\alpha}$ and final GnRH (GnRH2) injections.

	Pregnancy rates (%)			
	Heifers	Multiparous cows	Z	P
GnRH1	60	20	-2.24	0.025
$PGF_{2\alpha}$	26.7	20	-0.43	0.67
GnRH2	37.5	60	1.22	0.22

Ovulations were mostly detected between 24-30 h interval after AI in MCs (50%) and heifers (35%). Surprisingly, while ovulations were detected between 0-6 h interval none were detected at 6-12, 12-18, 18-24 h intervals in MCs. On the other hand, ovulations were distributed into all time intervals in heifers. No ovulations were detected in 4 MC (7.4%) and 2 heifers (3.7%) in 42 h period after second GnRH. Ovulation rates are summarized in Table 4.

Discussion

In previous studies, the PRs obtained by Cosynch TAI protocol in MCs (3,4) and beef heifers (5) were found lower than the PRs obtained in our study. Furthermore, DeJarnette et al. (6) inseminated dairy cows with Cosynch-like procedure, with a concurrent GnRH at 72nd h after $PGF_{2\alpha}$ injection and reported the a 32% of PR. Similarly, Colazo et al. (7) applied a Cosynch-like procedure to heifers and reported a 48.2% PR. The differences between former studies or ours could be due to average milk production differences, the usage of different GnRH analogues and AI times. Our results are in an agreement with Colazo et al.'s (7). On the other hand, before Cosynch like procedure selection of healthy heifers in their study has to be considered as a positive effect on their results.

The fertility differences among heifers, primiparous and MCs have been tried to be explained by different

Table 3. Corpus luteum diameters (mm) at different stages of Cosynch protocol in pregnant multiparous cows and heifers.

Ultrasonographic Examination days		Multiparous cows	Heifers	Mann-Whitney	P value U test value
GnRH1	N	19	18		
(Day 0)	Median	25	22.5	99.5	0.029
	Min-Max	15-35	10-30		
$PGF_{2\alpha}$	N	19	23		
(Day 7)	Median	20	20	166	0.142
	Min-Max	20-25	10-30		
GnRH2	N	16	18		
(Day 9)	Median	15	10	65.5	0.006
	Min-Max	10-20	5-20		

Table 4. Ovulation ratios at different time intervals.

Time interval	Heifer (N: (%))	Multiparous Cows (N: (%))	Z value	P value
0-6	8 (15.7)	5(10)	-0.86	0.39
6-12	5 (9.8)	NO*	-	-
12-18	3 (5.9)	NO	-	-
18-24	8 (15.7)	NO	-	-
24-30	18 (35)	25 (50)	1.51	0.13
30-36	5 (9.8)	10 (20)	1.45	0.15
36-42	4 (7.8)	10 (20)	1.79	0.074

*NO : No ovulations were detected.

hypothesis such as duration of dominant follicle, preovulator follicle size, the induction time of ovulation, differences of hormone concentration and age (8-14). As a comparison of heifers and MCs responses to Cosynch protocol, Martinez et al. (15) found lower PR in beef heifers than beef cows. This result is found to be inconsistent with ours in the case that, PRs of heifers were higher than MCs in this study. This could be due to the race and average milk production differences.

In a previous study the PRs of heifers were found lower than MCs in Ovsynch procedure but higher in oestrus synchronization (8). In spite of this, some scientists still suggest oestrus synchronization instead of ovulation synchronization for heifers. However, PRs obtained in this study do not compatible with the data reported by these scientists (13,16). In this study, the PRs obtained in heifers with Cosynch procedure were found higher than MCs, as oestrus synchronization results reported before (8,13,16).

Some scientists (9,11) indicate that, the presence of CL and dominant follicle or progesterone concentrations at beginning and during the ovulation synchronization could be used as contributor determinant factors, in order to evaluate the success of ovulation induction. According to Pursley et al. (8), the presence of functional CL at $PGF_{2\alpha}$ injection time could be considered as a critical stage for success. Our data is compatible with this result for heifers that, at the time of $PGF_{2\alpha}$ injection, the PR of CL+ heifers were significantly higher than the CL-ones (P: 0.026, χ^2: 4.9). In addition, the avarage diameter of CLs were larger in pregnant MCs than heifers at the first (P: 0.029, MWU: 99.5) and second (P: 0.006, MWU: 65.5) GnRH injection times.

Timed artificial insemination protocols have been improved to synchronize the ovulation within distinct intervals. Some scientists suggested that ovulation in cows occurs within 24-48 h after the second GnRH injection in Ovsynch procedure (9,14,17,18). Furthermore, the highest proportions were obtained at between 24 to 32 h (4,19). Similarly, in this study, the highest proportion of ovulations was observed within 24-30 h in MCs (50%) and heifers (35%). However, while the ovulations in heifers were distributed in any time intervals (0-42 h), a great proportion was observed after the 24th hour in MCs as in Tenhagen et al.'s (12) results. According to us, the low PR in heifers could be due to the late application of AI in Ovsynch procedure, generally applied 16-20 h after second GnRH (8,12). As we detected the 47.1% of ovulations in this interval (second GnRH application-AI), aging of oocytes before AI would reduce the PR in heifers.

As a conclusion, Cosynch protocol was found more effective in heifers than MCs. In addition, ovulations that occurred before AI (because of aged oocystes) could be the reason of low conception rate for Ovsynch procedure in heifers.

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