

EFFICACY OF THE PRESYNCH-OVSYNCH PROGRAM ON SOME REPRODUCTIVE PARAMETERS IN POSTPARTUM DAIRY COWS

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(Received 17. February 2008)

In this study, the aim was to evaluate the presynch-ovsynch protocol on the intervals from calving to first insemination, calving to pregnancy, calving to calving in postpartum lactating cows. Lactating Holstein cows (n=104, 3-5 years old, 450-500 kg BW, 27±3 kg/day average milk yield) were enrolled in the study 26-41 days postpartum. Fifty of them were taken as the control group and 54 of them were the treated experimental group. The control group was not treated and the cows in estrus were inseminated. In the treated group the cows were injected with two PGF_{2α} for presynchronization twice at every 14 days. Fourteen days after the second PGF_{2α}, ovsynch protocol was initiated (GnRH, day 0; PGF_{2α}, day 7; GnRH, day 9) and cows were inseminated 12-16 hours after second GnRH injection. Following AI, cows returning to estrus were inseminated at detected heat. Pregnancy diagnosis was performed by ultrasonography at 33-38 days after AI. Calving to first AI (days), calving to pregnancy (day), the number of AI per pregnancy, calving interval (days) for control cows were 96.08±8.20, 147.58±9.95, 2.19±0.20 and 430.50±10.90 and for the treated group 70.13±1.13, 101.76±5.70, 1.85±0.12 and 386.80±5.70, respectively. Compared to the control group, calving to first AI interval was shorter by 26 days, calving to pregnancy interval by 46 days and the calving interval by 44 days (p<0.001). Number of AI per pregnancy decreased numerically from 2.19 to 1.85. Pregnancy rates for the first AI was 47% for the control and 43% for the treated cows (p>0.05).

As a result, by using the presynch-ovsynch protocol, the need for estrus detection was eliminated and inseminations were performed at a fixed time. Therefore, calving to first AI, calving to pregnancy, calving intervals and the number of AI per pregnancy were improved to the benefit of the farm.

Key words: cow, presynch-ovsynch, reproductive parameters

INTRODUCTION

In recent years, the number of cows in dairy farms has increased, but this increase in herd size has also brought along its problems. Traditional methods of estrus detection are ineffective in farms with large numbers of animals. The rate of the timely and accurate detection of estrus in such farms is below 50 % (Senger, 1994; Lewis and Wulster-Radcliffe, 2001). Detection of estrus and insemination of the animal at the most appropriate time is of critical importance in achieving higher reproductive fertility in cows. Insufficient and inaccurate determination increases the number of inseminations per pregnancy, calving-first insemination and calving interval (Keister *et al.*, 1999). Therefore, synchronization methods have been developed to help determine estrus and inseminate animals at the right time (Nebel and Jobst, 1998).

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) has so far been the most frequently used hormone for estrus synchronization in cows (Patterson *et al.*, 2003). $PGF_{2\alpha}$ controls the life span of the corpus luteum, but cannot alter the course of follicular waves. Estrus will form due to changes at the developmental stage of pre-ovulatory follicles during the post-injection period of $PGF_{2\alpha}$, and the ensuing ovulation may take a week. Therefore, due to the continuation of a need for estrus detection in $PGF_{2\alpha}$ applications, insemination time cannot be controlled (Twagiramungu *et al.*, 1995; Pursley *et al.*, 1997a; Pursley *et al.*, 1997b; Guilbault *et al.*, 1998). Ultimately, reproductive physiologists have developed methods that limit estrus observation where ovulation rather than estrus is synchronized (Pursley *et al.*, 1995).

The method that synchronizes ovulations is named briefly as "ovsynch" (Pursley *et al.*, 1995; Pursley *et al.*, 1997b). The objective in this method is to ensure ovulation at a specified time with consecutive applications of hormones (GnRH, $PGF_{2\alpha}$, GnRH). Synchronization of ovulations is achieved with this protocol in a period of 8 hours by synchronizing the development of a new follicular wave and luteal function (Pursley *et al.*, 1998). Inseminations are performed between the 12th and 24th hours after the 2nd GnRH injection without estrus detection (Fricke *et al.*, 1998; Pursley *et al.*, 1998; Tenhagen *et al.*, 2004; Sarkar and Prakash, 2005). Ovulation synchronization can be achieved at a rate of 80-90 % with ovsynch protocol (Vasconcelos *et al.*, 1999). This protocol is a successful method of synchronization that has been tried intensively in cows at lactation and positive results have been obtained. Particularly, it raises the reproductive performance in cows with high milk yield and makes economic contributions (Keister *et al.*, 1999; Tenhagen *et al.*, 2004; Sarkar and Prakash, 2005).

The success of ovsynch protocol depends on the period of the cycle at the beginning of the application. It has been observed that when it is applied between the 5th and 12th day of the cycle (early luteal phase), fertility increases and the pregnancy rate is higher (Vasconcelos *et al.*, 1999; Moreira *et al.*, 2000b; Moreira *et al.*, 2001). Therefore, application of $PGF_{2\alpha}$ in cows twice with an interval of 14 days prior to ovsynch protocol ensures the application of ovsynch on the days in question at a rate of more than 70 %. This method is called "presynch-ovsynch"

(El-Zarkouny *et al.*, 2004; Navanukraw *et al.*, 2004). It has been reported that calving to pregnancy interval is shortened in cows on which presynch-ovsynch has been applied and pregnancy rates are higher in comparison to only ovsynch treated cows (Thatcher *et al.*, 2001; Moreira *et al.*, 2001; Fricke *et al.*, 2003; Tóth *et al.*, 2006). Moreover, the double PGF_{2α} application affects positively the uterus environment and uterus defense system and ensures the insemination of the animals at the shortest time possible at the end of the voluntary waiting period (Thatcher and Wilcox, 1973; Steffan *et al.*, 1984).

The purpose of the present study was to investigate the effects of presynch-ovsynch protocol applied on cows in early post-partum period on reproductive parameters.

MATERIALS AND METHODS

In the study, 104 Holstein cows (3-5 years old, 26-41 days postpartum, 450 and 500 kg body weight, 27±3 kg daily milk yields) located in Konya city (Turkey) were used. The cows were housed in a semi-open free system, fed with a sufficient and balanced diet (NRC, 2001), and milked twice a day.

Fifty of 104 cows were not taken under treatment and they formed the control group of the study and the other 54 cows formed the treatment group. No application was performed for the synchronization of estrus and ovulations for the control group. Detection of estrus in animals in the control group was performed through the observation method and those on estrus were inseminated according to the morning-evening method.

The cows in the treatment group were twice administered intramuscular (IM) injections of luteolytic doses of PGF_{2α} (D-Cloprestenol 150 µg, Dalmazin^(R) inj., Vetas AS, Istanbul, Turkey) at an interval of 14 days starting on any day (Presynch protocol). Fourteen days after the second injection of PGF_{2α}, ovsynch protocol (GnRH 10 µg, day 0; PGF_{2α} 150 µg, day 7; GnRH 10 µg, day 9) was started. The animals were inseminated at fixed times between the 12th and 16th hour after the second GnRH injection (Receptal^(R) inj., Intervet Tic. Ltd. Sti., Istanbul, Turkey) without observing estrus (Figure 1).

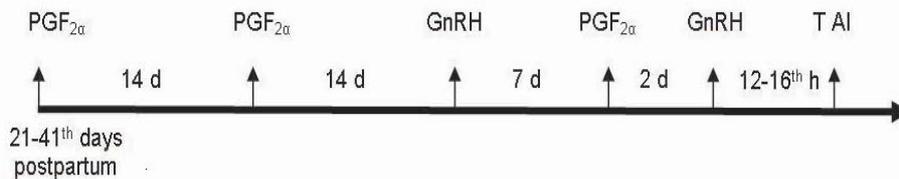


Figure 1. Presynch-Ovsynch Protocol

Estrus was observed without performing any applications on cows which did not get pregnant as a result of the fixed-timed inseminations following presynch-ovsynch protocol and those on estrus were inseminated. Diagnosis of pregnancy was made through an ultrasound performed transrectally between the 33th and 38th days after insemination. Results of pregnancy were re-confirmed through ultrasound or rectal examinations performed between the 75th and 90th day.

In the final stage of the study, the data obtained from the control group and treated group were compared based on reproductive parameters (pregnancy rate in the first insemination, calving-first insemination interval, calving-pregnancy interval, number of inseminations per pregnancy and calving interval) and the efficiency of the presynch-ovsynch protocol was determined.

Student's t test was used in order to establish the significance of differences between the control and treated group. On the other hand, chi-square test was used in the significance control of the difference between pregnancy rates obtained for animals in the study and control groups in the first insemination.

RESULTS

While the pregnancy rate obtained in the first insemination was 47 % for the animals in the control group, it was 43 % for the animals in the treated group. No significant difference was observed between pregnancy rates obtained in the first insemination for the animals in the control and treatment groups ($p > 0.05$). The pregnancy results obtained at the end of the first and second pregnancy examinations were the same. No loss of pregnancy was observed during the period between the pregnancy examinations.

The reproductive parameters obtained from the control group and the treatment group are given in Table 1.

Table 1. Reproductive parameters in animals in the control and treatment groups (mean \pm SD)

The number of cow (n)	Pregnancy rate in the first insemination	Calving-first insemination interval (day)	Calving-pregnancy interval (day)	The number of inseminations per pregnancy	Calving interval (day)
Control group (n=50)	47%	96.08 \pm 8.20 ^a	147.58 \pm 9.95 ^a	2.19 \pm 0.20	430.50 \pm 10.90 ^a
Treatment group (n=54)	43%	70.13 \pm 1.13 ^b	101.76 \pm 5.70 ^b	1.85 \pm 0.12	386.80 \pm 5.70 ^b

a, b: Different letters in the same column are differed statistically $P < 0.001$

In this study, the calving-first insemination interval was reduced by 26 days, the calving-pregnancy interval by 46 days, and calving interval 44 days and the number of inseminations per pregnancy was reduced from 2.19 to 1.85.

The differences found between the animals in the control and treatment groups in terms of calving-first insemination interval, calving-pregnancy interval and calving interval were statistically significant ($p < 0.001$). A decrease was achieved in the number of inseminations per pregnancy for the animals in the treatment group compared with the control group, but this decrease was statistically insignificant ($p > 0.05$).

DISCUSSION

Methods (for example, ovsynch) were developed that synchronize ovulations and ensure insemination at fixed times without a detection of estrus (Pursley *et al.*, 1995). Ovsynch, together with presynchronization, increases the reproductive performance by ensuring the insemination of cows especially on post-partum period at the shortest possible time at the end of the voluntary waiting period and thus makes an economic contribution (Pursley *et al.*, 1997a; Keister *et al.*, 1999; Moreira *et al.*, 2001; Navanukraw *et al.*, 2004; Sarkar and Prakash, 2005; Tóth *et al.*, 2006).

A modified presynch protocol (Pancarci *et al.*, 2002; Fricke *et al.*, 2003; Navanukraw *et al.*, 2004; Kasimanickam *et al.*, 2006) was used in the present study in order to ensure that the first four injections occur on the same day and limit injections to twice a week. Whereas there was an interval of 12 days in the original presynch protocol (Moreira *et al.*, 2001) between the 2nd PGF_{2α} application and the 1st GnRH application of the ovsynch protocol, this period was extended to 14 days in the modified presynch protocol. Thus, the protocol was easily programmed and adopted by the farm as it was more practical.

It has been reported that reproductive performance increases in studies conducted using ovsynch (Burke *et al.*, 1996; Pursley *et al.*, 1997b; Keister *et al.*, 1999; Tenhagen *et al.*, 2004) or presynch-ovsynch (Moreira *et al.*, 2001; Navanukraw *et al.*, 2004; Tóth *et al.*, 2006) protocol. In the present study, it was observed that the presynch-ovsynch protocol increased reproductive performance (Table 1). When a comparison was made with the control group, it was found that calving-first insemination was 26 days, calving-pregnancy interval 46 days and calving interval 44 days shorter for the animals in the treatment group ($p < 0.001$). A decrease was discovered in the number of inseminations per pregnancy although there was not a statistical difference ($p > 0.05$). It has been reported that the rate of accurate determination of estrus in milking cows is below 50 % and accordingly submission rate decreases. Insufficient or inaccurate determination of estrus increases calving-first insemination, calving-pregnancy and calving intervals (Senger, 1994; Lewis and Wulster-Radcliffe, 2001). On the other hand, it has been reported that submission rate is close to 100 % in cows on which ovsynch protocol was applied (Pursley *et al.*, 1995). The better reproductive parameters obtained in animals on which presynch-ovsynch protocol was applied may have resulted from the fact that submission rate was 100 %. In this study, the

animals in the treatment group were inseminated without detecting estrus between the 12th and 16th hour after the last GnRH injection of ovsynch protocol. Thus, it was ensured that all animals were administered their first inseminations on the 71 ± 7th day of post-partum by removing problems concerning detecting estrus.

Pursley *et al.* (1997a; 1997b) report that pregnancy rates obtained through detecting estrus are similar to pregnancy rates obtained through ovsynch protocol. Likewise, no difference was found in the present study between the control group where insemination was performed through observation of estrus and the treatment group where fixed-timed insemination was performed following the presynch-ovsynch protocol ($p > 0.05$).

In the present study, a pregnancy rate of 43 % was obtained in the first insemination through presynch-ovsynch application in cows in the early post-partum period. While pregnancy rates in studies conducted using the ovsynch protocol on milk cows on lactation vary between 29 % and 37.8 % (Burke *et al.*, 1996; Pursley *et al.*, 1997a; Pursley *et al.*, 1997b; Fricke *et al.*, 1998; Navanukraw *et al.*, 2004; Tenhagen *et al.*, 2004; Tóth *et al.*, 2006), they vary between 41.5 % and 49.6 % in studies conducted using presynch-ovsynch protocol (Peters and Pursley, 2002; Moreira *et al.*, 2001; El-Zarkouny *et al.*, 2004; Navanukraw *et al.*, 2004; Toóth *et al.*, 2006). While pregnancy rates obtained after the first insemination in the present study displayed similarities to pregnancy rates obtained through the presynch-ovsynch protocol, they were higher than pregnancy rates obtained through application of ovsynch only. This result is in conformity with the view that a higher pregnancy rate is obtained through presynch-ovsynch protocol in comparison to ovsynch protocol (Moreira *et al.*, 2001; Thatcher *et al.*, 2001; Fricke *et al.*, 2003; El-Zarkouny *et al.*, 2004; Navanukraw *et al.*, 2004). The reason for higher pregnancy rates through the presynch-ovsynch protocol in contrast to the ovsynch protocol only is the PGF_{2α} injections administered prior to ovsynch. It has been reported that PGF_{2α} injections increase pregnancy rates in cows (Roche, 1976; Macmillan and Day, 1982). The PGF_{2α} injections after calving lead to multiple shortened estrus cycles. An increase in the number of estrus that form during the post-partum period in lactating dairy cows increases pregnancy rates (Thatcher and Wilcox, 1973; Moreira *et al.*, 2001). In addition to this, it has been reported that consecutive PGF_{2α} injections positively affect the uterus environment and are an effective treatment for uterus infections and cystic ovaries (Steffan *et al.*, 1984; Chavatte *et al.*, 1993; Kasimanickam *et al.*, 2006). It has been also reported that pregnancy rates increase if ovsynch protocol is started between the 5th and 12th days of the cycle (early luteal phase) (Vasconcelos *et al.*, 1999; Moreira *et al.*, 2001; Thatcher *et al.*, 2001). PGF_{2α} injections administered prior to ovsynch with an interval of 14 days enable the start of ovsynch on the days in question. Thus, cows have a functional corpus luteum prior to the application and are under the influence of high progesterone. The positive effect of high progesterone on implantation and embryo increases fertility (Kesler *et al.*, 1978; Thatcher *et al.*, 1989). More than 70 % of the cows which are treated with presynch prior to ovsynch take place

between the 5th and 12th days of the cycle at the beginning of ovsynch application (El-Zarkouny *et al.*, 2004).

Researchers report that synchronization programs that are implemented using PGF_{2α} and GnRH on early post-partum period (e.g. ovsynch) regulate ovarian functions and hence sexual cycles (Thatcher *et al.*, 2002; Tenhagen *et al.*, 2004; Yàniz *et al.*, 2004) and as a result the number of inseminations per pregnancy decreases (Tóth *et al.*, 2006). The present study has also determined a decrease, though insignificant, in the number of inseminations per pregnancy following presynch-ovsynch protocol.

In the present study, the pregnancy results obtained as a result of the first and second examinations of pregnancy were the same. No loss of pregnancy was recorded during the time that passed between pregnancy examinations. Researchers report a pregnancy loss of 10 to 23 % in cows. It was found that embryonic losses usually occurred more in early stages of pregnancy (before the 28th day) and as pregnancy progressed, embryonic losses decreased (Fricke *et al.*, 1998; Pursley *et al.*, 1998; Moreira *et al.*, 2001; Fricke *et al.*, 2003). The reason for the absence of pregnancy losses in this study may have resulted from the fact that the first pregnancy examination took place at a later stage after insemination (between the 33th and 38th days).

In conclusion, the day of the first insemination in cows was decided post-partum through the presynch-ovsynch protocol, insemination was implemented at the predetermined time by eliminating the visual detection of estrus and significant decreases have been attained in reproductive parameters such as calving-first insemination, calving-pregnancy and calving intervals. Therefore, it has been concluded that presynch-ovsynch protocol may be reproductively beneficial for cows on early post-partum period and make economic contributions.

ACKNOWLEDGMENTS

This study was founded by SUBAPK (No:05401099)

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UTICAJ PRIMENE PROGRAMA "PRESYNCH-OVSYNCH" NA NEKE REPRODUKTIVNE PARAMETRE MLEČNIH KRAVA U POSTPARTALNOM PERIODU

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SADRŽAJ

Cilj ove studije je bio da se proceni uticaj primene "presynch-ovsynch" protokola na reproduktivne pokazatelje mlečnih krava nakon teljenja. U ispitivanja je bilo uključeno 104 jedinke, starosti 3-5 godina, telesne mase 450-500 kg i prosečne dnevne mlečnosti od 27 ± 3 kg. Krave su uključivane u ogled 26-41 dana po teljenju i bile su podeljene u dve grupe. Kontrolnu grupu, koja nije tretirana, sačinjavalo je 50 krava i one su osemenjavane po pojavi spontanog estrusa. Jedinke iz ogledne grupe su tretirane sa dve injekcije PGF_{2 α} u razmaku od dve nedelje a zatim je dve nedelje posle druge injekcije primenjivan "ovsynch" protokol. On se sastajao od sledećih tretmana: 0 dan - GnRH, 7. dan - PGF_{2 α} i 9. dan - GnRH. Plotkinje su zatim osemenjavane 12-16 časova nakon druge injekcije GnRH. U slučaju ponovne pojave estrusa, krave su osemenjavane još jednom. Graviditet je potvrđivan ultrasonografski 33-38 dana posle osemenjavanja. Dobi-

jeni su sledeći rezultati: u kontrolnoj grupi, period od teljenja do prvog osemenjavanja je bio $96,08 \pm 8,20$ dana; od teljenja do utvrđivanja graviditeta $147,58 \pm 9,95$ dana, indeks osemenjavanja $2,19 \pm 0,20$ i međutelidbeni interval $430,50 \pm 10,90$ dana. U ogleđnoj grupi ove vrednosti su bile $70,13 \pm 1,13$, $101,76 \pm 5,70$, $1,85 \pm 0,12$ i $386,80 \pm 5,70$ respektivno. U poređenju sa kontrolnom grupom, u ogleđnoj grupi je period od teljenja do prvog osemenjavanja bio kraći za 26 dana; od teljenja do utvrđivanja graviditeta za 46 dana, a međutelidbeni interval kraći za 44 dana, što je bilo i statistički značajno. Indeks osemenjavanja se smanjio sa 2,19 na 1,85. Procenat steonosti posle prvog osemenjavanja je bio 47 u kontrolnoj i 43 u ogleđnoj grupi plotkinja.

Primenom "presynch-ovsynch" protokola, detekcija estrusa postaje neopotređna jer se osemenjavanje vrši u strogo određenom momentu, a reproduktivni pokazatelji tretiranih plotkinja su značajno bolji.