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RESEARCH ARTICLE

Effect of Presence of Corpus Luteum at the Beginning of Ovsynch Protocol on Pregnancy Rates in Lactating Dairy Cows [1]

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Summary

The aim of the study was to investigate the effect of presence of CL at the beginning of Ovsynch protocol on pregnancy rates in lactating dairy cows. A total of 218 lactating Holstein cows that were at least 45 days postpartum with no reproductive disorders were used in the present study. Cows were divided into two experimental groups according to the presence and absence of corpus luteum (CL) on the first day of transrectal ultrasonography (day 0). Cows having CL (CL (+)) were randomly allocated to two subgroups as CL (+)/A and CL (+)/B. Cows in CL (+)/A group (n = 74) were treated with Ovsynch protocol, while cows in CL (+)/B group (n = 78) were treated with $PGF_{2\alpha}$ on the first day of examination and seven days later Ovsynch protocol was started. Cows having no CL (CL (-)) (n = 66) were left untreated on the first day of examination and Ovsynch protocol was started seven days later. Pregnancy rates were found 23%, 25.6% and 40.9% in CL (+)/A, CL (+)/B and CL (-) groups, respectively. The pregnancy rate in CL (-) group was significantly higher than those of CL (+)/A, and CL (+)/B groups ($P<0.05$). It is suggested that the success of Ovsynch synchronization protocol may be related to starting time of the protocol and to ovarian physiology in cyclic dairy cows.

Keywords: *Cow, Ovsynch, Corpus Luteum, Pregnancy rate*

Laktasyondaki Süt İneklerinde Ovsynch Protokolüne Başlarken Korpus Luteum Varlığının Gebelik Oranları Üzerine Etkisi

Özet

Bu çalışmada, laktasyondaki süt ineklerinde Ovsynch protokolüne başlarken CL varlığının gebelik oranları üzerine etkisinin araştırılması amaçlandı. Çalışmada en az 45 gün önce doğum yapmış ve reprodüktif sorunu bulunmayan 218 baş Holstein ırkı inek kullanıldı. İnekler, transrektal ultrasonografik muayenin ilk gününde (0. gün) korpus luteum (CL)'un varlığı ve yokluğuna göre iki gruba ayrıldı. CL'a sahip olan inekler (CL (+)) rastgele iki alt gruba ayrıldı. CL(+)/A grubundaki (n = 74) ineklere CL belirlenmesinin hemen ardından Ovsynch protokolüne başlanılırken, CL(+)/B grubundaki (n = 78) ineklere CL belirlenmesinin ardından $PGF_{2\alpha}$ enjeksiyonu uygulanarak 7 gün sonra Ovsynch protokolüne başlanıldı. CL (-) grubundaki (n = 66) ineklere ise transrektal muayene gününden sonraki 7. günde Ovsynch protokolü başlatıldı. Gebelik oranları CL(+)/A, CL(+)/B ve CL (-) gruplarında sırasıyla %23.0, %25.6, %40.9 olarak elde edildi. CL (-) grubundaki gebelik oranının CL(+)/A ve CL(+)/B gruplarına göre önemli derecede yüksek olduğu belirlendi ($P<0.05$). Siklik ineklerde uygulanan Ovsynch senkronizasyon protokolünün başarısında, protokolün başlatılma zamanının ve ovaryum fizyolojisinin etkili olduğu kanısına varıldı.

Anahtar sözcükler: *İnek, Ovsynch, Korpus Luteum, Gebelik oranı*

INTRODUCTION

One of the most important reasons not to have optimal reproductive efficiency in dairy farms is having problems in farm management. Estrus detection plays a key role in having optimum fertility rate in a dairy farm. Even though standing heat can be detected in cows by visual inspection three times in a day, accurate estrus detection rate is about 35-51% [1]. Problems in detection of standing heat and insemination at the wrong time cause high economic loses

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such as reduced pregnancy rates, extended calving intervals and decreased in milk production [2,3].

Ovsynch synchronization protocol is a practical method which provides timed artificial insemination without estrous detection. Dairy cows are commonly treated by Ovsynch protocol to synchronize estrus and ovulation [4]. This protocol consists of two GnRH injections nine days apart, one $PGF_{2\alpha}$ injection 48 h before the second GnRH injection and timed artificial insemination 16-20 h later following last GnRH injection [5].

Ovsynch protocol can be started in any phase of the sexual cycle, however ovulation rate decreases when the protocol was started at proestrous, metestrous and late diestrous of the cycle [6,7]. It was reported that ovulation rate was higher (70%) in early diestrous (5-12 d.) than other days (53%) of cycle [8]. Conception rates were the greatest in dairy cows that began the Ovsynch protocol between 5 and 12 days of the estrous cycle [7,9,10].

There are different reports about conception rate and/or pregnancy rate of Ovsynch protocol. The conception rates are not different from those of cows inseminated after a detected estrus [10,11] whereas other studies reported that conception rates were reduced [12,13] but pregnancy rate were increased [11].

Although the protocol has high fertilization rates [14,15], it has been reported that the protocol is needed to be modified due to low pregnancy rates [11,16]. Yilmaz et al.[17] reported that G6G protocol was an important approach in control of reproduction instead of Ovsynch protocol which caused low pregnancy rates in cows and heifers. In another study Nak et al.[18] reported that norgestomet applications did not increase pregnancy rates in cyclic and noncyclic cows and heifers.

In the present study, the aim was to investigate the effect of presence of CL at the beginning of Ovsynch protocol on pregnancy rates in lactating dairy cows.

MATERIAL and METHODS

Animals

The study was approved by the Kocas-TIM in Aksaray, Turkey. The experiment was conducted at between March and July 2008. A total of 218 multiparous Holstein Friesian cows that were at least 45 days postpartum (average live weight: 500-600 kg, milk yield: 25-30 kg/d) with no reproductive disorders were used in the present study. Cows with BCS <2.5 or > 4 were not used in this experiment. The animals were housed in a free-stall barn, milked twice daily and fed diets as total mixed ration (TMR), and based on the NRC [19]. The components of the TMR were maize silage, alfalfa hay, and home blend concentrate.

Experimental Procedure

Cows were divided into two experimental groups based on the presence or absence of CL on ovaries at transrectal ultrasonography. Immediately after transrectal ultrasonography, cows having CL (CL (+) were randomly allocated to two subgroups as CL (+)/A and CL (+)/B. Synchronization and timed artificial insemination protocol schemes in groups are given in *Fig, 1, 2,* and *3*.

Immediately after detection of CL, cows in CL (+)/A group (n = 74) were treated with the Ovsynch protocol consisting of administrations of GnRH-analogue (buserelin acetate, 10 µg, IM, Receptal, Intervet, Istanbul, Turkey) at days 0 and 9, and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) analogue (d-cloprostenole, 150 µg, IM, Dalmazin, Vetaş, Istanbul, Turkey) on day 7. Timed artificial insemination (TAI) was carried out by the same veterinarian 16-18 h after the second GnRH *(Fig. 1)*.

Immediately after detection of CL, cows in CL (+)/B group (n=78) were treated with $PGF_{2\alpha}$ analogue (d-cloprostenole, 150 µg, IM, Dalmazin, Vetaş, Istanbul, Turkey) and seven days later Ovsynch protocol was started as described above *(Fig. 2)*.

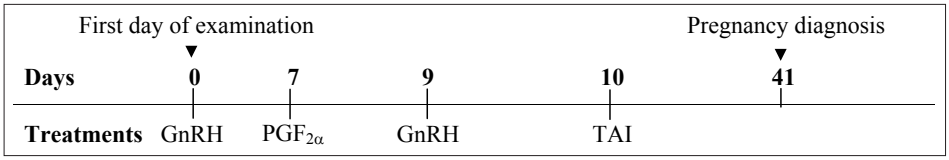
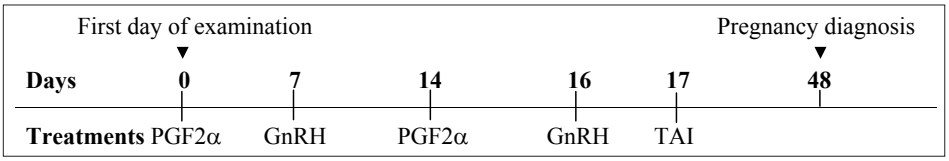


Fig1. The treatments of synchronization and TAI protocol in CL (+)/A group

Şekil 1. CL (+)/A grubuna uygulanan senkronizasyon ve sabit zamanlı tohumlama protokolü

Fig 2. The treatments of synchronization and TAI protocol in CL (+)/B group

Şekil 2. CL (+)/B grubuna uygulanan senkronizasyon ve sabit zamanlı tohumlama protokolü



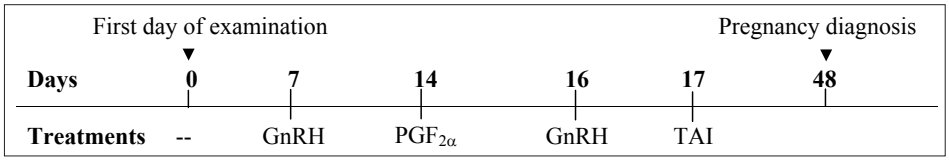


Fig 3. The treatments of synchronization and TAI protocol in CL (-) group

Şekil 3. CL (-) grubuna uygulanan senkronizasyon ve sabit zamanlı tohumlama protokolü

In CL (-) Group (n = 66), Ovsynch protocol was initiated seven days later from the day of transrectal ultrasonography in cows having no CL *(Fig. 3)*.

Pregnancy Diagnosis and Statistical Analysis

Diagnosis for pregnancy was performed by transrectal ultrasonography (Pie Medical 100 Falco Vet Model 7.5 MHz probe, The Netherlands) from uterine contents at 31th day after TAI. Statistical analysis of the results was performed by Chi-Square test (SPSS 13.0).

RESULTS

Pregnancy rates were found 23% (17/74), 25.6% (20/78) and 40.9% (27/66) in CL (+)/A, CL (+)/B and CL (-) groups, respectively. The pregnancy rate in CL (-) group was significantly higher than those of CL (+)/A, and CL (+)/B groups (P<0.05). Overall pregnancy rate in 218 cows were 29.4% *(Table 1)*.

Table 1. *Pregnancy numbers and rates in groups*
Tablo 1. *Gruplarda elde edilen gebelik sayı ve oranları*

Groups	n	Pregnancy (n, %)
CL(+)/A	74	17 (23.0)[a]
CL(+)/B	78	20 (25.6)[a]
CL (-)	66	27 (40.9)[b]
Total	218	64 (29.4)

Letters (a, b) in the same column indicate significant differences (P<0.05)

DISCUSSION

The aim of this study was to investigate the effect of starting day strategy of Ovsynch protocol on pregnancy rate in lactating dairy cows. The pregnancy success is related to individual properties of cows (i.e. age, lactation number, milking yield, etc.) and environmental factors such as season and feeding conditions [20-22].

In this study of the pregnancy rates in CL (+)/A, CL (+)/B and CL (-) groups were found 23% (17/74), 25.6% (20/78) and 40.9% (27/66), respectively. The pregnancy rate of Ovsynch protocol was between 27-37.9% as reported by earlier studies [23-26], and in agreement with our results.

The reason of low pregnancy rate (23%) presented in the study in CL (+)/A group might be related to timed artificial insemination because the exact day of luteal phase of the estrous cycle at the time of beginning day of first GnRH injection was not known. The low pregnancy rate (25.6%) was also observed in CL (+)/B group. It is suggested that the failure in synchronization of follicular wave at the time of the first GnRH injection coinciding with metestrous phase of the estrous cycle may have caused the development of follicles at different stages and sizes. On the other hand, luteal activity at the beginning of Ovsynch protocol for ovulation synchronization did not make any difference on pregnancy rates in cows and heifers [27].

The pregnancy rates were almost similar in CL(+)/A and CL(+)/B groups. It has been observed that this modification added to Ovsynch protocol *(Fig. 2)* was not effective to increase pregnancy rate. Therefore, it may be suggested that there is no need to change the beginning time of protocol in cows having CL. It was reported that presynchronisation treatment with PGF injection, 7 d before the Ovsynch protocol did not have any positive effect on either the ovulation synchronisation or the pregnancy [28]. Also, Cartmill et al.[10] found that multiple-lactation cows given the presynchronizing injection of $PGF_{2\alpha}$ had greater pregnancy rates at d 28 than Ovsynch cows after TAI, with similar nonsignificant trends at d 38 to 58.

The pregnancy rate in CL (-) group was significantly higher than those of CL (+)/A, and CL (+)/B groups (P<0.05). Ovsynch protocol started 7 days later in cows having no CL increased somewhat pregnancy rate. It is suggested that determination of cows having no CL on the first day of transrectal ultrasonography may be an important stage. Because the present study indicated that stage of estrous cycle on the beginning day of the Ovsynch protocol was more important than only the modification of the Ovsynch protocol itself to increase pregnancy rate. The high pregnancy rates in Ovsynch protocol are related to not only ovarian activity but also the period of estrous cycle on the first GnRH injection day. Besides, researchers reported that to increase pregnancy rate in Ovsynch protocol, blood progesterone levels are needed to be high at the time between the first GnRH and $PGF_{2\alpha}$ injections, therefore the protocol is suggested to start on days 5-12 of estrous cycle [9,10,25,29,30].

It has been reported that pregnancy rates were higher in cyclic cows (49.4%) than acyclic cows (19.4%) in Ovsynch protocol [14,31]. Cyclic activity in cows commonly is determined according to the plasma progesterone concentrations. Silva et al.[14] reported that detection of cows having CL as cyclic or having no CL as anovulatory on the first injection day of GnRH in Ovsynch protocol by using ultrasonography were enough, practical and a reliable method for evaluating the cyclic activity in cows. Higher pregnancy rate in CL (-) group than CL (+) group was observed in presented study. This finding supports that to evaluate a cow having no CL on the examination day as anovulatory is not a suitable approach because a second examination 7-10 days later is needed to determinate an inactive ovary. Higher pregnancy rate in CL (-) group presented in the study shows that the cows were not inactive and most of them were in periovulatory period (2-3 days before or after estrous). Low pregnancy rate in CL (+) group indicates to poor cyclic activity as well as cows having disorders related to CL presence (luteal cycst).

It is concluded that in Ovsynch and/or Ovsynch protocols

supported by $PGF_{2\alpha}$; starting Ovsynch protocol 7 days later in CL (-) cows somewhat increased pregnancy rate, however, starting Ovsynch protocol 7 days following $PGF_{2\alpha}$ injection in CL (+) cows did not increase pregnancy rate. Besides, it is suggested that the success of Ovsynch protocol may be related to starting time of the protocol and ovarian physiology in cyclic dairy cows, therefore $PGF_{2\alpha}$-GnRH injection intervals may be 10-12 days as in presynchronization in CL (+) cows.

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