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Research Article

Effect of Progestagen Application During Ovsynch Protocol on Pregnancy Rates of Lactating-Grazing Cows

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Abstract

The aim of this study was to test the effect of progesterone supplementation to Ovsynch protocol in lactating and grazing cows on pregnancy rate after fixed time artificial insemination (TAI). Animals, from a total of 158 dairy cows, were randomly assigned to two groups. In Grup 1 (n = 75) Ovsynch protocol was carried out (Group OV), and in Grup 2 (n = 83) Ovsynch + PRID protocol was carried out (Group OV + PRID). Conception rates following timed Artificial Insemination (TAI) were determined as 33.3% for Grup OV and 53.0% for Group OV+PRID. Differences between groups were statistically significant (P<0.05). When Body Condition Score (BCS) of cows considered, pregnancy rates of cows with 2.5 and upper BCS for Group OV and Group OV + PRID were determined as 36.8% and 59.2%, respectively. Differences between groups for BCS were statistically significant according to pregnancy rates (P<0.01). While premature heat rate was considered for TAI, 12.0 and 7.2 % were determined for Group OV and Group OV + PRID, respectively (P>0.05). The findings of the present study suggest that progesterone administration between first GnRH to PGF2α in Ovsynch protocol increases conception rates and numerically decreases premature heat rates, also BCS was a significant factor affecting conception rates in OV or OV + PRID protocols in cows. Moreover, usage of PRID with Ovsynch was more effective to increase conception rates in cows with optimum body condition (2.5-4 BCS) in Kars region.

Keywords: *Cow, Ovsynch, Premature estrus, PRID, TAI*

Ovsynch Protokolü Sırasında Progestagen Uygulamasının Laktasyonda-Otlayan İneklerin Gebelik Oranları Üzerine Etkisi

Özet

Bu çalışmanın amacı laktasyonda ve otlayan ineklerde Ovsynch protokolüne progesterone katkısının sabit zamanlı suni tohumlamada gebelik oranları üzerine etkisini test etmekti. Toplam 158 sütçü inek, rastgele iki gruba ayrıldı: Grup 1 (n = 75)'de bulunan ineklere Ovsynch protokolü uygulandı (Grup OV), Grup 2 (n = 83)'de bulunan ineklere ise Ovsynch + PRID protokolü uygulandı (Grup OV + PRID). Uygulama sonrası sabit zamanlı tohumlama ile gebelik oranları; Grup OV için %33.3, Grup OV + PRID için %53.0 olarak tespit edildi. Gebelik oranları açısından önemli derecede fark (P<0.05) oluştuğu belirlendi. İneklerin Vücut Kondüsyon Skoru (VKS)'na göre sınıflandırılarak incelendiğinde, VKS 2.5 ve üzerinde olan ineklerde gebelik oranı; Grup OV içerisinde %36.8, Grup OV+PRID grubunda ise %59.2 olarak tespit edildi. VKS'na göre gebelik oranları açısından ise önemli derecede (P<0.01) fark olduğu belirlendi. Sabit zamanlı suni tohumlamada, prematüre kızgınlık oranları açısından incelendiğinde, Grup OV için %12.0, Grup OV + PRID için %7.2 olarak saptandı, istatistiksel açıdan bir fark (P>0.05) olmadığı saptnadı. Çalışmadaki bulgular Ovsynch protokolünde, ilk GnRH ile PGF2α uygulaması arasında progesteron uygulamasının gebelik oranlarını yükselttiği ve prematüre kızgınlık oranlarını sayısal olarak aşağıya çekebileceği belirlenmiştir. Buna ek olarak hem Grup OV hem de Grup OV + PRID uygulamalarında VKS'nin gebelik oranlarını etkileyen önemli faktör olduğu tekrar görülmüştür. Ayrıca, bu çalışma ile Kars bölgesinde optimum VKS'ye sahip (2.5-4) ineklerde Ovsynch'le birlikte PRID uygulamasının gebelik oranlarını yükseltmede çok daha etkili olduğu ortaya konulmuştur.

Anahtar sözcükler: *İnek, Ovsynch, Prematüre östrus, PRID, TAI*

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INTRODUCTION

The application of Ovsynch protocol in cattle yields satisfactory conception rates since a report by Twagiramungu et al.[1], working with beef cattle in Canada. They suggested that pretreatment with GnRH, 6 days before the administration of prostaglandin, may eliminate the need for heat detection prior to breeding by AI. The first injection of GnRH was designed to induce ovulation and formation of a new or accessory corpus luteum and a new follicular wave. The corpora lutea was subsequently caused to regress by prostaglandin injection and ovulation occurred about a day after the second administration of GnRH. It is suggested that the protocol could eliminate the need for estrus detection. However, initiation of the protocol in late luteal phase (i.e. after day 13 of the estrous cycle) can induce pre-mature heat then ovulation before the timed insemination [2]. Therefore, reduced conception rates are to be expected when the protocol is initiated after day 13 of the estrous cycle [3]. In an attempt to increase conception rates, pre-synchronization with two luteolytic doses of PGF2a has been tested in Ovsynch by some researchers [3,4].

It has been shown that progesterone concentration during late luteal phase before insemination is positively linked with conception rates in cattle [5]. Adding progesterone to Ovsynch protocol to improve pregnancy rate has been employed with different degree of efficacy application of progesterone with Ovsynch protocol has been shown to improve conception rates. Moreover, duration of progesterone may be important factor affecting premature heat and pregnancy rate. In cyclic heifers that had a progesterone-releasing device (CIDR-B) in their vagina for 7 days and given PGF at CIDR-B removal, estradiol treatment 24-30 h later effectively synchronized estrus with acceptable pregnancy rate (52%) [6]. Similar pregnancy rates (65%) have been achieved after fixed-time AI with exogenous progesterone delivered by intra-vaginal CIDR-B devices in heifers assigned to an Ovsynch protocol [7]. Murugavel et al.[5] attained higher pregnancy rate with 9 days insertion of PRID + Ovsynch protocol (35.5%) compared Ovsynch (26.7%) and PRID (24.4%) protocols in cows. Moreover Ambrose et al.[8,9] determined that CIDR usage with Ovsynch may increase pregnancy rate and ovarian response to GnRH in heifers.

The main objective of this study was to evaluate the efficacy of the Ovsynch protocol with the inclusion of exogenous progesterone delivered by intra-vaginal PRID on pregnancy rate and also effects of BCS on the protocol's efficacy to increase synchronization and pregnancy rates in lactating and grazing cows in Kars region.

MATERIAL and METHODS

Animals

This study was conducted with lactating and grazing Brown-Swiss, Simental and crossbred cows from villages (N40.8055/E42.8917/Alt. approx. 2600 m; N40.2794/E42.9428/Alt. approx. 2200 m) in spring-summer period in Kars/Turkey. All cows (n = 158) were kept under similar management conditions in order to minimize environmental differences: animals were housed indoors, milked twice a day and fed with a total mixed ration ad libitum to meet the nutritional requirements of lactating cows (10-15 L per day) and also two times grass hay feeding. Only cows without a history of reproductive disorder and illness were included in the study. In addition, body condition scoring system from 1 = very thin to 5 = very fat was evaluated for each cows at the time of the first GnRH administration according to Ferguson et al.[10]. Only cows between 1.5 and 4 were included in the study (*Table 1*).

Synchronization Protocol, AI and Pregnancy Determination

One hundred fifty eight cows were randomly divided into two treatments: (a) group OV, standard Ovysnch protocol (n = 75) characterized by the administration of GnRH (gonadorelin diacetate tetrahydrate, 2 mL, 100 µg, i.m., Ovarelin®; CEVA-DİF İlaç A.Ş., İstanbul, Turkey) at days 0 and 56 h after PGF administration, PGF2α (dinoprost, 5 mL, 25 mg, i.m., Enzaprost®T, CEVA-DİF® İlaç A.Ş., İstanbul, Turkey) at day 7 and (b) group OV + PRID7, Ovsynch protocol integrated with the use of progesterone releasing intravaginal device (PRID®, 1.55 g, progesterone, without its estradiol capsule; CEVA-DİF® İlaç A.Ş., İstanbul, Turkey) inserted at the initial GnRH injection and removed at PGF2α administration for seven days (n = 83). PRIDs were used according to EU norms: all capsules (containing estradiol) on PRID were removed before insertion). All cows were inseminated 16-18 h after the second GnRH administration, with frozen-thawed semen with proven fertility sires. Cows detected in heat based on standing estrus were either artificially inseminated based on AM/PM rule or naturally bred by bull, and these cows were accepted as non-pregnant for TAI for analyses.

Pregnancy determinations were performed with trans-rectal B-mode ultrasonography (USG) between days 28-35 following TAI *(Fig. 1)*. Pregnancy diagnosis were performed in 158 cows; therefore, these cows were completed the experiment and used for further analyses.

Table 1. *Primary individual properties of cows in Ovsynch (OV) and Ovsynch + PRID (OV+PRID7) groups for pregnancy rates*

Groups	OV	OV+PRID7
Cows for TAI (n)	75	83
Body Condition Score, n (%)		
<2.5	37 (49.3%)	34 (41.0%)
≥2.5	38 (50.7%)	49 (59.0%)
Premature Estrus	9 (12.0%)	6 (7.2%)
Pregnancy	25 (33.3%)	44 (53%)

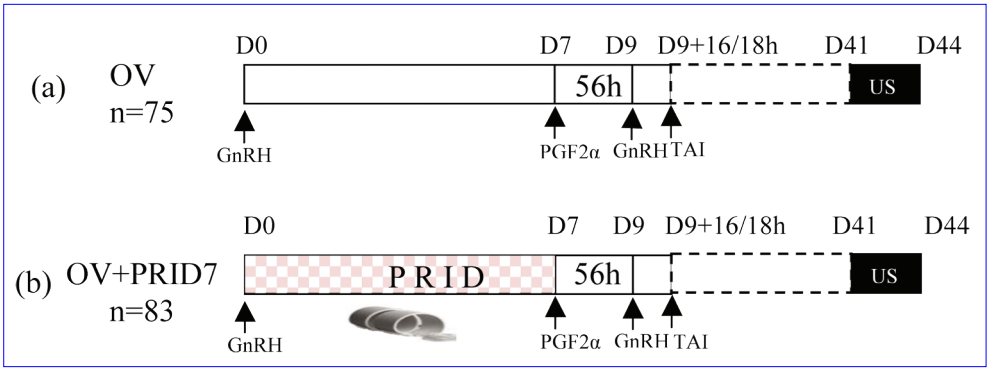

Fig 1. Diagram of treatments during current study. The protocols were **(a)** Ovsynch protocol (OV): 100 µg GnRH i.m. were administered on Day 0 (D0), 25 mg dinoprost (PGF2α) was injected i.m. on Day 7 (D7), second GnRH treatment was administered after 56 h (D9) from PGF2α, and timed artificial insemination n(TAI)s were carried out around 16-18 hours following second GnRH; **(b)** Application of progesterone releasing intravaginal device (PRID, 1.55 g) with Ovsynch (OV + PRID7): 100 µg GnRH i.m., and a PRID containing 1.55 g progesterone for 7 days were applied. At PRID removal 25 mg of dinoprost (PGF2α) was injected i.m. Cows received the second GnRH treatment at 56 h after PRID withdrawal and TAI at 16-18 h later. On 32-35 days (D41-44) after TAI for checking pregnancies, ultrasonographic (USG) examinations were performed following treatments

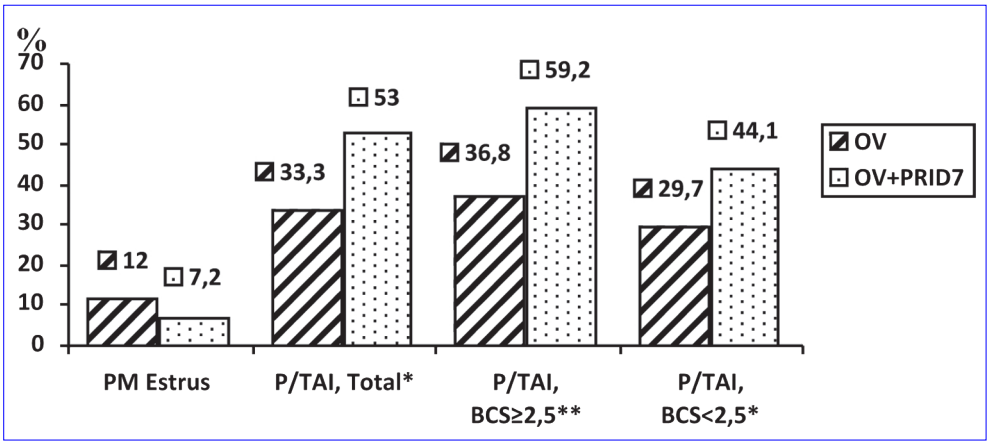

Fig 2. Effect of PRID treatment on Premature Heat **(PM Estrus),** Pregnancy per Timed Artificial Insemination (P/TAI) in whole animals (BCS 1.5-4; **P/TAI, Total**), BCS≥2.5 **(P/TAI, BCS≥2.5)** and BCS<2.5 **(P/TAI, BCS<2.5)** in Ovsynch Protocol. OV= Ovsynch Protocol, OV + PRID7 = Ovsynch Protocol with PRID, P/TAI=Pregnancy rate per timed artificial insemination, PM = Premature, BCS = Body Condition Score (5 point scale). * and ** determine that differences between OV and OV + PRID is statistically significant for parameters: * P<0.05 and ** P<0.01

Statistical Analysis

Binomial dependent variable were analyzed by stepwise selection method in logistic regression procedure (PROC LOGISTIC) of the SAS software (SAS Institute Inc., Cary, NC, USA). The effects of treatments, breed, body condition score (BCS; optimal vs poor), farm (location), replicate (year), parity (primiparous vs multiparous), and interactions of treatment by BCS and treatment by parity on the binominal dependent variables were considered in statistical models. In the final logistic regression models, P<0.05 was considered as a statistically significant. Results were reported as odds ratios along with 95% confidence intervals.

RESULTS

Fig. 2 shows overall reproductive performance for the two treatment groups. Premature heat showed a numerically decrease in OV + PRID7 group (7.2%), compared to the OV group (12.0%) (P>0.05). Pregnancy rate for TAI was higher in OV + PRID group (53.0%), as compared to the OV group (33.3%). Moreover, pregnancy rate for BCS≥2.5 was also higher in OV + PRID group (59.2%), as compared to the OV group (36.8%, P<0.01).

For premature heat expression rate, logistic regression stepwise selection procedure indicated that only main effect of BCS was significant (P<0.01). In this regard, premature heat expression rate did not differ between OV (12.0%; 9/75) and OV + PRID7 (7.2%; 6/83), although it was numerically higher in OV group. However, cows with optimal BCS were significantly (P<0.01) higher premature heat expression rate (14.3%; 14/98) than those with poor BCS (2.6%, 2/76) regardless of treatments.

For TAI pregnancy rate, logistic regression stepwise selection procedure indicated that pregnancy rates for cows in OV + PRID7 (53.0%; 44/83) were higher than those in OV group (33.3%; 25/75). However, logistic regression stepwise selection procedure indicated that only treatment by BCS interaction effect was significant (P<0.01) when interactions were included in the statistical model *(Fig. 2)*. In this regard, pregnancy rates were similar for cows in OV group with poor BCS (29.7%; 11/37) and optimal BCS (36.8%; 14/38). Whereas, pregnancy rates were higher in cows with optimal BCS (59.2%; 29/49) compared to those with poor BCS ((44.1%; 15/34) in OV + PRID7 group.

DISCUSSION

In current study, we found that simultaneous application of PRID with Ovsynch protocol increases pregnancy rates in cows. Especially, this beneficial effect of PRID was more evident in cows with optimum BCS (2.5-4) in our study. Moreover, usage of PRID with Ovsynch numerically decreased premature heat in cows.

Murugavel et al.[5] determined that PRID and PRID + Ovsynch combination provided higher pregnancy rates in cows with low progesterone levels while there was no effect on cows with higher progesterone levels. In other words, usage of PRID with Ovysnch more effectively treated cows in anestrus and may have induced ovulations in these animals. It is well known that anestrus is most common situation attributable to an anovulatory condition in early postpartum period [11]. Progesterone application is one of the treatment options in cows with ovarian cyst syndrome [12]. Similar condition may have occurred in our current study and progesterone supply with PRID in Ovsynch may have treated or beneficially affected to cows with anestrus and anovulatory syndrome.

It was determined that ovulation of an early-stage dominant follicle triggered with exogenous GnRH results in a reduced ovulatory follicle size and also fertility in dairy cattle. Moreover, low progesterone levels may result in the development of large persistent follicles, oocyte quality in such follicles is determined to be compromised resulting in increased embryonic loss found that diameter of follicle in first GnRH directly affects pregnancy rates [13-17]. They hypothesized that large CL producing high P4 are developed from large follicle at the time of first GnRH administration. Pre-ovulatory follicle developing in higher P4 condition could effectively response to second GnRH injection. Similar physiological condition, high P4 levels, may have been artificially created in application of PRID with Ovsynch in our study.

Although presynchronization treatment was not performed in current study, it was determined that PRID administration with Ovsynch increased pregnancy rates in TAI, compared with Ovsynch without any presynch. Similar results had been obtained by Colazo et al.[18]. They used not only Ovsynch but also presynch+ovsynch protocols with PRID administration. It is interesting (or predicted) that PRID did not have any effect on pregnancy/AI in Presynch + Ovsynch groups while PRID increased pregnancy rate in Ovsynch group and pregnancy rates in Ovsynch and presynch groups were similar while PRID application was administered with Ovsynch. So it may be said that PRID usage in Ovsynch increase pregnancy rate, although PRID usage may not be necessary in Ovsynch protocols used with Presynch. In field, PRID + Ovsynch application without Presynch or any other hormonal usage may have some advantages, reducing extra-expenses because of hormonal usage and time consumption. Nevertheless, PRID + Ovsynch or Presynch + Ovsynch applications may be chosen or decided according to expenses and time consuming in field.

It is well known that body condition score (BCS) of cows is an important factor determining success of artificial insemination. It is possible to base nutritional advice on target cow condition scores at critical points in the annual production cycle. It is determined that one critical target is the condition score at the time of mating. In autumn-calving herds the target condition score is set at 2.5 as cows expected to rebreed while mobilizing body reserves on a winter diet which is sufficient to prevent any loss of condition becoming prohibitively expensive. In comparison, the target condition score at mating in spring-calving cows is near 2; the high nutritive value of spring grass permits the cows to be in positive energy balance throughout the mating period [19]. In current study, BCS based artificial insemination had been carried out in cows with Ovsynch and Ovsynch + PRID and it was determined that cows with optimum body condition scores (2.5-4 BCS) had higher possibility to be pregnant after AI in Ovsynch + PRID group, compared with Ovsynch. Clearly, cows with optimum body condition score (BCS≥2.5 in current study) had higher pregnancy rates, compared those with poor conditions (BCS<2.5). However, PRID application increased pregnancy rates in cows with both poor and optimum body conditions. Moreover, supplementation of PRID to Ovsynch was more effective in cows with optimum BCS (≥2.5). It is well known that leptin is an important factor which determines success of reproduction in cattle. Leptin levels in blood are correlated with BCS in ruminants. Animals with low BCS have low leptin levels in blood [20,21].

Another important factor affecting success of timed artificial insemination programs is premature estrus/heat before planned time for AI. In ovulation synchronisation protocols (i.e. Ovsynch, Cosynch, Heatsynch etc.) some percentage of animals show premature estrus in estrus synchronization protocols. In current study, 7.2-12.0% of animals showed premature, undeserved estrus before second GnRH administration. The main reason of this unwanted condition is known that early or premature

increase of estrogens in blood and early ovulation of follicle following PGF2α injection before second GnRH administration. This condition causes uncontrolled, early ovulations. Premature estrus in TAI is undesired situation because these animals cause extra-time consumption and expenses if they are inseminated in unexpected/unplanned time. So a lot of studies have been carried out to prevent early-premature estrus and also ovulations before TAI in Ovsynch or Cosynch protocols with administration of progestagens [14,18,22-26]. In current study, usage of PRID with Ovsynch numerically prevented premature estrus in cows although there was not statistically significant difference. Nevertheless, premature estrus decreased more than 40% in PRID+Ovsynch compared with Ovsynch. In other words, approximately two-fold higher premature estrus had been detected in Ovysnch group. Sample size limitation of current study may have made findings not statistically significant. Colazo et al.[14] had found similar results and percentages in prevention of ovulations before TAI with PRID administration in Ovsynch. Based on our results and that of others, we suggest that it is possible to reduce by half the incidence of premature estrus or ovulation before TAI with addition of PRID to Ovsynch under field conditions.

Colazo and Ambrose [22] compared 5 days and 7 days duration of PRID in Ovsynch protocol. Moreover, they investigated effect of first GnRH injection with PRID application. In cows, it was determined that pregnancy rates in 7 days PRID application with Ovsynch was higher than in 5 days PRID even when no first injection of GnRH was given. First GnRH injection in PRID usage may be important to ovulate follicles with adequate size and develop a mature CL, which will be regressed with PGF2α. In this way, synchronization of ovulations may be more effectively controlled in PRID applications with Ovsynch.

Kars region has hard climate condition during winter and farmers cannot provide enough food and energy intake for their cows during winter period. During winter, cows have to be fed with grass and pasture oaths yielded during summer. Therefore, cows, which have not got enough food intakes, lose weight and BCS. Generally this condition causes “anestrus” at the end of winter in these animals because of low BCS (1.5-2). These animals show estrus again when pasture feeding start at the beginning of spring period. So in this region, treatment of anestrus cows with low BCS (<2.5) is important with usage of Ovsynch + PRID applications at the end of winter period to obtain satisfied pregnancy rates. There are a lot of studies [25,27-33] which have been carried out to deal with reproductive problems and to find good fixed time artificial insemination program using Ovsynch-based for cattle. However, it is certain that a lot of similar and endocrinologic studies are needed to gain good result from timed artificial insemination programs in cattle reared in hard climate condition and also Kars region.

In conclusion, it was determined that PRID application with Ovsynch protocol increased pregnancy rates and prevented premature heat in cows. Moreover, beneficial effect of PRID with Ovsynch is more clear in cows with optimal BCS in Kars region.

Conflict of Interests Statement

The authors declare that there is no conflict of interests regarding the publication of this article.

Animal Rights Statement

The authors declare that the experiments on animals were conducted in accordance with local Ethical Committee laws and regulations as regards care and use of laboratory animals. All procedures on animals were carried out by veterinarians/researchers who are expert and certificated on cattle reproduction.

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