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RESEARCH ARTICLE

Effect of PGF2α and GnRH Injections Applied Before Ovsynch on Pregnancy Rates in Cows and Heifers [1]

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[1] *This article is summarized from the MSc thesis of same title*

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Summary

The objective of the study was to investigate the effect of PGF2α and GnRH injections applied before Ovsynch protocol (G6G) on pregnancy rates in cows and heifers. Totally, 196 Holstein cows in postpartum 50-100 days and 169 Holstein heifers were used. Animals in group 1 (Control: 40 cows, 40 heifers) were inseminated artificially in their standing heat without hormonal treatment. In group 2 (Ovsynch: 37 cows, 80 heifers), Ovsynch protocol (day 0, 10 µg buserelin acetate, intramuscular (IM); day 7, 500 µg cloprostenol, IM; day 9, 10 µg buserelin acetate, IM; 16 h later following last injection, artificial insemination) was applied. In group 3 (G6G: 119 cows, 49 heifers), 500 µg cloprostenol on day 0 and 10 µg buserelin acetate on day 2 were injected and Ovsynch protocol was performed 6 days later following last injection. Pregnancy rates in cows and heifers were detected 57.50-60.00%; 37.83-32.50% and 53.78-59.18% in 1st, 2nd and 3rd groups, respectively. The pregnancy rate in G6G protocol was significantly higher than those of Ovsynch protocol, in heifers ($P<0.05$). In conclusion, it is suggested that G6G protocol is an important approach in control of reproduction in heifers and lactating dairy cows instead of Ovsynch protocol which causes low pregnancy rates. Moreover, the present study provides evidence that G6G protocol has higher pregnancy rates in Holstein heifers.

Keywords: *Cow, Heifer, Ovsynch, G6G, Pregnancy rate*

İnek ve Düvelerde Ovsynch Öncesi Uygulanan PGF2α ve GnRH Enjeksiyonlarının Gebelik Oranlarına Etkisi

Özet

Bu çalışmada inek ve düvelerde Ovsynch öncesi PGF2α ve GnRH (G6G) uygulamalarının gebelik oranlarına etkisinin araştırılması amaçlandı. Postpartum 50–100 günler arasında, 196 Holştayn inek ve 169 düve kullanıldı. Grup 1'deki hayvanlara (Kontrol: 40 inek, 40 düve) herhangi bir hormonal girişim uygulanmaksızın sun'i tohumlama yapıldı. Grup 2'deki hayvanlara (Ovsynch: 37 inek, 80 düve) Ovsynch protokolü (0. gün 10 µg buserelin asetat, kas içi (IM); 7. gün 500 µg kloprostenol, IM; 9. gün 10 µg buserelin asetat, IM; 16 saat sonra sun'i tohumlama) uygulandı. Grup 3'e (G6G: 119 inek, 49 düve) 0.günde 500 µg kloprostenol ve iki gün sonra 10 µg buserelin asetat enjeksiyonu yapılmasını takiben 6 gün sonra Ovsynch protokolü uygulandı. İnek ve düvelerde gebelik oranları 1., 2. ve 3. grupta sırasıyla, %57.50-60.00; %37.83-32.50; %53.78-59.18 olarak belirlendi. G6G protokolü uygulanan düvelerdeki gebelik oranlarının Ovsynch grubuna göre daha yüksek olduğu gözlendi ($P<0.05$). Sonuç olarak, inek ve düvelerde düşük gebelik oranları veren Ovsynch protokolü yerine, üremenin denetlenmesinde G6G uygulamalarının önemli bir yaklaşım olacağı düşünülmektedir. Diğer taraftan sunulan çalışma ile G6G protokolünün özellikle düvelerde daha yüksek gebelik oranı sağladığı ortaya konulmuş oldu.

Anahtar sözcükler: *İnek, Düve, Ovsynch, G6G, Gebelik oranı*

INTRODUCTION

Estrous detection failure is the most common problem in dairy farms [1] and the synchronization of estrus and ovulation are taken into consideration in the solution of this problem [2]. Recently, it has been focused on ovulation synchronization methods such as ovsynch protocol since many positive effects of Ovsynch protocol are present [3-8].

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For example it is possible to see 10-30% unsynchronized cows following last GnRH injection [9,10]. However, this management strategy, administration of Ovsynch protocol in heifers, has no satisfactory pregnancy rates. Pursley et al.[5] have reported that pregnant heifer rate was 74.4% after artificial insemination following PGF2α injection and 35.1% following Ovsynch protocol. In addition to this, Williams et al.[11] have shown that pregnancy rate was 39.1%. In consequence of unsynchronized animals as well as low pregnancy rates, it has been assumed that a preparation period was needed before Ovsynch, such as G6G protocol. To start with ovsynch protocol it is recommended to be better to begin between 5-9th days of estrous cycle [9,12]. In G6G protocol, firstly a PGF2α analogue is injected to preclude the possibility of luteal tissue and a GnRH analogue is injected to ovulate the follicle on 48 h following PGF2α injection [13]. Thereafter, Ovsynch protocol is performed at day 6 after GnRH injection. Two GnRH injections initiate a new estrous cycle. Thus, a functional dominant follicle is existed to fully respond by Ovsynch protocol with two GnRH injections 6 days apart from other [13]. Moreover, it was reported that in this protocol the rate of the cows synchronized for Ovsynch was 92% and the pregnancy rate was 50% [13]. It was shown that the pregnancy rate in yaks was higher in G6G than Ovsynch [14]. On the other hand, to the authors' knowledge, there is no report about the pregnancy rates after applying G6G protocol in heifers. Hence, the study was performed to evaluate the effect of G6G protocol on pregnancy rate in Holstein heifers and lactating dairy cows.

MATERIAL and METHODS

The presented study was conducted at commercial dairy farms in Sincan and Ayaş counties in Ankara province between September 2009 and August 2010.

Animals

The study was approved by the animal local ethics committee, Afyon Kocatepe University. A total of 196 Holstein cows being in postpartum period of 50-100 days with 400-500 kg live weight and 169 dairy heifers aged 15-20 months with 300-350 kg live weight were used. These records were co-verified by means of Ankara Holstein-Friesian Stud Association.

The body condition score (BCS) of all animals were 3.0-3.5. All cattle were housed in tie-stall barn, milked twice daily and fed a nutritionally balanced total mixed ration, based on the National Research Council [15].

Experimental Procedure

Lactating Holstein dairy cows (n=196) and Holstein heifers (n=169) were divided into three groups which were mentioned below.

Group 1 (Control, 40 Cows and 40 Heifers)

These animals were inseminated artificially after observing spontaneous estrous symptom which was detected by the presence of a clear mucous vaginal discharge, increasing uterine edema and examining the Graafian follicle by rectal examination.

Group 2 (Ovsynch Protocol, 37 Cows and 80 Heifers)

The animals were treated with the Ovsynch protocol consisting of a 10 µg GnRH-analogue (buserelin acetate, Receptal, Intervet, Istanbul, Turkey) intramuscularly at days 0 and 9, and a 500 µg prostaglandin $F_{2\alpha}$ (PGF2α) analogue (cloprostenole, Juramate, Egevet, Izmir, Turkey) intramuscularly on day 7. Artificial insemination (AI) was carried out by the same veterinarian in all animals 16 h after the last treatment.

Group 3 (G6G Protocol, 119 Cows and 49 Heifers)

The treatment was performed by the injection of 500 µg PGF2α analogue (cloprostenole, Juramate, Egevet, Izmir, Turkey) intramuscularly on day 0, 10 µg GnRH-analogue (buserelin acetate, Receptal, Intervet, Istanbul, Turkey) intramuscularly on day 2 and Ovsynch protocol on day 6 [13].

Pregnancy Diagnosis and Statistical Analysis

Diagnosis for pregnancy was performed by palpation per rectum of uterine contents 60 and 90 days after AI. Statistical analysis of the results was performed by chi square test (SPSS 13.0). All values are presented as mean ±S.D. Group differences were declared significant at $P<0.05$.

RESULTS

The mean weights, ages, postpartum days and the number of calving of the cows (n=196) in groups are illustrated in *Table 1*. The postpartum days of the cows were shorter only in group 1 than other groups (P<0.05)

Table 1. *The mean weights, ages, postpartum days and the number of calving of the cows in groups*

Tablo 1. *İneklerin gruplara göre ortalama canlı ağırlık, yaş, postpartum bulundukları günler ve buzağılama sayıları*

	Groups	Age (year)	Weight (kg)	Postpartum Days	Number of Calving
Cows	1 (n = 40)	5.47±0.27	412.23±8.08	55.08±3.56^{a}	2.78±0.23
	2 (n = 37)	4.99±0.67	432.78±9.10	83.45±2.34^{b}	3.01±0.13
	3 (n = 119)	5.33±0.12	436.27±4.89	88.78±3.45^{b}	2.89±0.11

Letters (a, b) in the same row indicate significant differences between different letters (P<0.05)

while other data were not significant between groups, statistically.

The mean weights and ages of heifers (n=169) in groups are shown in *Table 2*. There was no statistical difference between groups according to these data.

Pregnancy rates in groups of cows and heifers are illustrated in *Table 3*. While there were no differences between groups in cows, the pregnancy rates of heifers in groups 1 and 3 were higher than group 2 and this difference was significant, statistically (P<0.05).

Table 2. *The mean weights and ages of heifers in groups*
Tablo 2. *Düvelerin gruplara göre ortalama canlı ağırlık ve yaşları*

	Groups	Age (Month)	Weight (kg)
Heifers	1 (n = 40)	18.05±0.47	315.50±4.00
	2 (n = 80)	17.80±0.65	323.45±3.56
	2 (n = 80)	17.40±0.14	330.35±4.45

Means within rows followed by different superscripts are not significantly different (P>0.05)

Table 3. *Pregnancy rates in groups of cows and heifers*
Tablo 3. *İnek ve düvelerde gruplara göre gebelik oranları*

	Groups	n	Pregnancy Rates
Cows	1	40	%57.50
	2	37	%37.83
	3	119	%53.78
Heifers	1	40	%60.00[a]
	2	80	%32.50[b]
	3	49	%59.18[a]

Letters (a, b) in the same row indicate significant differences between different letters (P<0.05)

DISCUSSION

This study was performed to find out the efficiency of G6G protocol on pregnancy rate in Holstein heifers and lactating dairy cows. Calving-first AI interval is very important fertility parameter for dairy farms. This interval may change between 73±3 [16] - 83 [5] days in dairy cows. Therefore, the animals in group 2 and 3 were selected from 83.45±2.34 and 88.78±3.45 days in postpartum.

The mean age, weight and number of calving values in cows were not different in groups, significantly. Moreover, there was no significant difference in the values of mean age and weight of heifers in groups. Hereby, it suggested that these parameters did not affect the efficiency of protocol either in lactating cows or heifers.

Pursley et al.[5] have reported that the pregnancy rates were 74.4% in heifers and 38.9% in cows following artificial insemination after spontaneous estrous behavior. There are also some reports that the pregnancy rates of cows are 58.8% [17] and 66.7% [18]. In present study, the pregnancy rates of cows and heifers in control group were observed 57.50% and 60.0%, respectively. It suggests that these discrepancies might be occurred due to climate condition, detection of standing heat, management and breeding factors [19].

Pregnancy rates in Ovsynch protocol in heifers were reported as 49.7% [20] and 35.1% [5]. In our study, the pregnancy rate was 32.50%. This low pregnancy rate may be related to the beginning day of Ovsynch protocol. This suggestion is also confirmed by the earlier finding of Moreira et al.[12] in which Ovsynch protocol used in early luteal phase of estrous cycle has higher rates than the protocol used in 15th day of estrus cycle, due to early ovulation. In many Ovsynch protocol studies, performed in lactating cows, the pregnancy rates were observed in different rates such as 37.8% [5], 55% [21], 32% [22], 42.2% [23], 42.3% [8] and 76.92% [24]. There are also some reports that the pregnancy rates are 25.8% [25] and 33.3% [26] and these are not satisfactory even though it is attempted to increase by additional injections such as β-carotene. In the present study, our pregnancy rate was 37.83% in Ovsynch group. Many factors such as postpartum days of cows, region and climate might be effective on this rate. On the other hand, timing of artificial insemination may also alter the pregnancy success in cows [6].

Bello et al.[13] reported that the pregnancy rates in lactating cows were higher in G6G group (50.0%) than Ovsynch group (27.0%). Our pregnancy rate in G6G group (53.78%) was consistent to those reported by Bello et al.[13]. On the other hand, to our knowledge, there is no report about the pregnancy rates after applying G6G protocol in heifers. In the present study, 59.18% pregnancy rate was determined in heifers administered G6G protocol. Moreover, this rate was higher than those of Ovsynch group (32.50%) and was similar to those of control group (60.00%). This result corroborates the suggestion of Pursley et al.[5] reported that the reason of low pregnancy rate of Ovsynch protocol in heifers due to low synchronization capability of luteal function following first GnRH injection. In addition to this, elimination of potential luteal tissue and initiation of new estrous cycle following G6G protocol may be possible because of the evidence that higher successful results of Ovsynch protocol was noted when Ovsynch starts in early luteal phase [12].

In summary, this study showed the effect of G6G protocol on pregnancy rate in Holstein heifers and lactating dairy cows. It is suggested that G6G protocol is an important approach in control of reproduction in heifers and lactating dairy cows instead of Ovsynch protocol which causes low pregnancy rates. Moreover, the present study provides evidence that G6G protocol has higher pregnancy rates in Holstein heifers.

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