



RESEARCH ARTICLE

Comparison of the effects of two pre-synchronization protocols (G6G and PG-3-G) on some reproductive performance parameters in Holstein cows

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Received:31.05.2020, Accepted: 03.11.2020
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Holstein ineklerde iki ön senkronizasyon protokolünün (G6G ve PG-3-G) bazı reprodüktif performans parametreleri üzerindeki etkilerinin karşılaştırılması

Eurasian J Vet Sci, 2020, 36, 4, 248-254
DOI: 10.15312/EurasianJVetSci.2020.305

Öz

Amaç: Bu çalışmada postpartum 28-40 günler arasında uygulanan G6G ve PG-3-G ön senkronizasyon protokollerinin Holstein ineklerde bazı üreme parametreleri üzerindeki etkilerinin belirlenmesi amaçlanmıştır.

Gereç ve Yöntem: G6G grubundaki ineklere (n=35) 0. gün PGF2 α , 2. gün GnRH, 8. gün GnRH, 15. gün PGF2 α , ikinci PGF2 α uygulamasından sonraki 56. saatte GnRH uygulaması yapıldı ve bunu takiben 16-18. saatte zamanlı suni tohumlama işlemi uygulandı. PG-3-G grubundaki ineklere (n=35) ise 0. gün PGF2 α , 3. gün GnRH, 10. gün GnRH, 17. gün PGF2 α , ikinci PGF2 α uygulamasından sonraki 56. saatte GnRH uygulaması yapıldı ve bunu takiben 16-18. saatte zamanlı suni tohumlama işlemi uygulandı.

Bulgular: G6G ve PG-3-G gruplarında arz oranı (%100 ve %100), ilk tohumlamada gebe kalma oranları (%42.9 ve %51.4), doğum ilk tohumlama aralığı (53.5 ve 54.0 gün), doğum gebe kalma aralığı (54.1 ve 53.7 gün), buzağılama aralığı (334.1 ve 333.7 gün) parametrelerinde gruplar arasında istatistiksel olarak farklılık bulunmadı. Protokol başlangıcında inekler siklik ve nonsiklik olmalarına göre değerlendirildiğinde ilk tohumlamada gebe kalma oranları nonsiklik ineklerde ve siklik ineklerde sırasıyla %64,7 ve %41,5 olarak tespit edildi. Yapılan istatistiksel analizde gruplar arasındaki farkın önemli olma eğiliminde olduğu görüldü (p=0,098).

Öneri: Sonuç olarak postpartum erken dönemde başlatılan ön senkronizasyon protokolleri ile gönüllü bekleme süresini takip eden erken dönemde %100 arz oranı sağlanmıştır. Ön senkronizasyon protokolleri buzağılama aralığını 400 günden aşağı çekerek etkisini bariz şekilde ortaya koymuştur. Östrüs takibi ne gerek duyulmadan zaman ve iş gücünden tasarruf edilebileceği, nonsiklik ineklerde de uygun gebelik oranına ulaşılabileceği kanısına varılmıştır.

Anahtar kelimeler: Süt ineği, postpartum, ön senkronizasyon, G6G, PG-3-G

Abstract

Aim: The present study was aimed to determine effects of G6G and PG-3-G presynchronization protocols on some reproductive parameters in Holstein cows between postpartum 28-40 days.

Materials and Methods: Cows in the G6G group (n=35) were treated with PGF2 α on day (d) 0, GnRH on days 2 and 8, and PGF2 α on day 15. The injection of last GnRH was applied 56 hours after the second PGF2 α injection and then timed artificial insemination (TAI) was performed 16-18 hours later. Cows in the PG-3-G group (n=35) were treated with PGF2 α on day 0, GnRH on day 3 and 10, and PGF2 α on day 17. The injection of last GnRH and TAI were applied as they were done for G6G group.

Results: There were no statistical differences between the G6G and PG-3-G groups in terms of submission rate (100% and 100%), the first-service pregnancy per artificial insemination (P/AI) (42.9% and 51.4%), calving to first insemination interval (53.5 and 54.0 d), calving to conception interval (54.1 and 53.7 d), calving interval (334.1 and 333.7 d) were determined. When the data analyzed according to the protocol at the beginning of cows to be "cyclic" and "noncyclic", P/AI were found to be 64.7% and 41.5%, respectively. The difference between the groups was tended to be significant (p=0.098).

Conclusion: Start of presynchronization protocols in the early postpartum period, provides 100% SR after the voluntary waiting period. It is clearly shown that the presynchronization protocols reduces the calving interval less than 400 days. Time and labor cost could be saved as there would be no needs for oestrus detection and appropriate P/AI could also be achieved in non-cyclic cows.

Keywords: Dairy cow, postpartum, presynchronization, G6G, PG-3-G





Introduction

In dairy herds, reproductive performance parameters must be carefully considered to prevent economic losses. In fact, the submission rate (SR) of a herd is one of these parameters (Dinç 2013). The way to obtain a good reproductive efficiency in dairy farming is that the cows show oestrus in the early postpartum (PP) period and even this oestrus can be detected. Oestrus detection efficiency can be assessed by calculating the SR in herds (Cafre 2015). Submission rate is the proportion of cows eligible for breeding that are served bred over a specific period of time (18 or 24 days) (Dinç 2013, Cafre 2015, Kutlu and Dinç 2015).

If the SR is calculated, the time to start service period should be determined. The voluntary waiting period (VWP) determines the start of the SR calculated. In farms where the year-round calving policy is applied, the SR target should be equal or more than 75% for every 3 weeks period (Hudson et al 2012, Dinç 2013). Creating more chances for cows to be become pregnant through improved SR is more important to overall fertility performance than the success rate of single service (Mawhinney and Biggadike 1998).

In practise, several methods of oestrus detection can be implemented. These include visual observation, heat mount detectors, tailhead markers, chin-ball markers, detector animals, and electronic heat detection devices. Due to varying degrees of success in the detection, more recently oestrus synchronization protocols have been applied to improve the success. It is reported that oestrus synchronizations protocols increase the SR. In order to increase the submission rate; ovulation synchronization protocols, which do not require oestrus detecting, could be applied to problematic cows whose oestrus cannot be detected. In fact, the aim of the presynchronization; instead of waiting for the cows to show oestrus after VWP, is taking the cows into treatment as early as after calving and served by ovulation synchronization protocols without waiting to observe their oestrus. It should also be noted that hormonal protocols such as Presynch, Double-Ovsynch, Doublesynch, G6G and PG-3-G, which increase the SR, are important in this context (Kutlu and Dinç 2015).

These presynchronization protocols match the onset of the Ovsynch protocol in early luteal phase of the oestrus cycle (in the presence of a dominant follicle - dioestrus, between days 5 to 9 of oestrus cycle) a high first-service pregnancy per artificial insemination (P/AI) is achieved. Cows that began Ovsynch on days 5 to 9 oestrus cycle also had greater circulating progesterone (P4) at the time of the PGF2 α treatment (3.6 ng/ml), probably due to the presence of two corpus luteum (CL), than cows that initiated Ovsynch on days 1 to 4 oestrus cycle (2.5 ng/ml). The best response is reported to be achieved in the presence of a dominant follicle with a diameter of 10 mm (Moreira et al 2000, Souza et al 2008, Wilt

bank and Pursley 2014). When the G6G or PG-3-G protocol is applied, cows will be on days 4-5-6 (Stevenson 2014, Pursley 2015) and 5-6 (Stevenson 2014) of oestrus cycle respectively, at the beginning of the Ovsynch protocol.

Presynchronization protocols including GnRH and PGF2 α have been developed to overcome the limitations of PGF2 α -based Presynch protocols for especially anovular cows (Stevenson 2016). As PGF2 α cannot change the course of follicular development, when applied, the difference in oestrus and ovulation time due to the developmental period of the follicular wave can cause problems. Advantage of PG-3-G protocol over PGF2 α based Presynch protocols; by stimulating oestrus and ovulation in anovular cows, at the beginning of the Ovsynch protocol, create at least 1 functional CL and increase P4 concentration. By this way, the conception rate which is one of the most important reproductive performance parameters in dairy cows will be increased (Bisinotto et al 2015, Borchardt et al 2017).

The aim of the present study was to evaluate the possible effect(s) G6G and PG-3-G presynchronization protocols, which may increase of the SR and the efficiency of ovulation synchronization protocol, on some reproductive performance parameters in cyclic and noncyclic Holstein cows.

Material and Methods

Location, Animals, housing, and milking

This study was carried out with the permission of the Çukurova University Animal Experiments Local Ethics Committee (Decision No: ÇÜ-HADYEK/2015-6-1) report.

The experiment was conducted at the Research Farm of Çukurova University Agriculture Faculty, Sarıçam - Adana province during November to June. In the study seventy multiparous Holstien cows were used. The average milk production was 31.5 \pm 6.81 kg/day during the first 85 days in milk (DIM). Cows were housed in free-stall barns and had free access to fresh and clean water. They were fed a total ration consisting primarily of grain (barley or corn) and silage (corn) balanced to meet nutrients recommended by National Research Council (2001) for lactating dairy cows. The barns, with concrete floor, were equipped with fans and sprinklers to cool cows during periods of heat stress. Cows were and milked twice a day (DeLaval Tumba, Sweden). Individual milking information (milk quantity, flow rate, etc.) and individual animal information were recorded in the herd management program (DeLaval ALPRO). Since the VWP on the farm is 60 days, cows are inseminated in the first natural oestrous after days 60



Body condition score (BCS) evaluation

The body condition score of the cows was evaluated according to the Edmonson et al (1989) that allows visual observation. Scores were given between 1 (very weak) to 5 (very fat) with 0.25-point interval. The cows were scored by the same person and without considering their previous score. BCS's were evaluated and noted at the beginning of the treatment (BCS1) and at the TAI (BCS2).

Ultrasonographic (USG) examinations

Transrectal ovarian scans were conducted in all cows by ultrasonography (6.5-MHz linear-array transducer, KX5200-VET, Xuzhou Kaixin Electronic Instrument Co Ltd, Xuzhou, Jiangsu, China) to any CL present at PP 21 day and at the first day of the presynchronization protocol to determine cyclicity and the presence of CL was assessed as cyclic, non presence of CL was assessed noncyclic. During the ultrasound examination all cows were confirmed having no clinical abnormalities of the uterus or no abnormal vulva discharge (the presence of anechoic fluid in the uterine lumen, bioassay of potential endometritis). Pregnancy diagnosis was conducted by transrectal ultrasonography on day 28-35 after TAI. Pregnancy was confirmed by anechoic uterine fluid and presence of an embryo with a heartbeat.

Treatments and TAI

Since the number of noncyclics was low, the PG-3-G protocol was applied to the noncyclic cows, considering that the protocols were similar, and a single protocol would be obtained. In the G6G group, 34 cyclic, 1 noncyclic and PG-3-G group included 19 cyclic and 16 noncyclic cows. Enrollment in the study began at PP 28-40 days. Cows in the G6G group (n=35) were treated with PGF2 α (Cloprostenol sodium, 10 μ g, Ovi-ren®, TOPKİM, Turkey) on day 0, GnRH (Buserelin acetate, 500 μ g, Lutelen®, TOPKİM, Turkey) on day 2 and day 8, and PGF2 α on day 15, respectively. The injection of last GnRH was applied 56 hours after the second PGF2 α injection and then TAI was performed 16-18 hours later. Cows in the PG-3-G group (n=35) were treated with PGF2 α on day 0, GnRH

on day 3 and on day 10, and PGF2 α on day 17, respectively. The injection of last GnRH and TAI were applied as in G6G group (Figure 1). DUNNS-PRIDE CRIMSON ET (7H010102) (Ege Vet Animal Industry Corporation İzmir, Turkey) semen was used for insemination of all cows in the study group. All injections, inseminations and USG examinations were made by the same professional veterinarian.

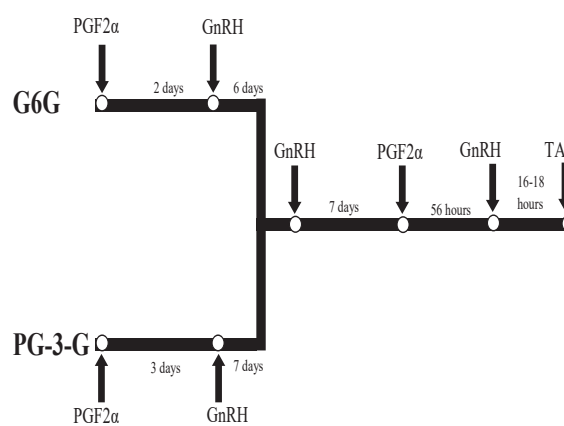


Figure 1. G6G and PG-3-G protocols

Determination of reproductive performance

In the final stage of the study, the data obtained from the G6G group and PG-3-G group were compared based on reproductive performance parameters; submission rate (number of cows served / number of cows eligible for served \times 100), the first-service pregnancy per artificial insemination (P/AI) (number of pregnant cows/number of served cows \times 100), calving to first insemination interval, calving to conception interval and calving interval were determined

Statistical analyses

The first-service pregnancy per artificial insemination (P/AI) at d 28-35 was analyzed by SAS procedure LOGISTIC. In addition to the independent effects of cycling status (cyclic vs noncyclic) and BCS (greater \geq 2.75 or less than \leq 2.5) were included in the model. Other reproductive performance parameters analyzed by chi-square using the Proc GENMOD

Table 1. Results of reproductive performance parameters

	G6G	PG-3-G
Body condition score (BCS)		
BCS1	2.73 \pm 0.25	2.72 \pm 0.23
BCS2	2.71 \pm 0.25	2.65 \pm 0.15
Ovarian status on the 0th day		
Cyclic	34 total	19 total
Noncyclic	1 total	16 total
Days the treatment started (mean, day)	35.5 \pm 3.90	34.0 \pm 3.47
Calving to first insemination interval (day)	53.5 \pm 3.90	54.0 \pm 3.47
Calving to conception interval (first TAI)	54.1 \pm 3.73	53.7 \pm 3.24
Calving interval (day)	334.1 \pm 3.73	333.7 \pm 3.24
Submission rate	100% (35/35)	100% (35/35)
Pregnancy per P/AI	42.9% (15/35)	51.4% (18/35)

*Statistical analysis showed no significant difference between the groups (p>0.05).



Table 2. Factors included in the logistic models that significantly influenced pregnancy per P/AI

		Pregnancy per P/AI	p
Ovarian status on the 0th day	Cyclic	41.5% (22/53)	=0.098
	Noncyclic	64.7% (11/17)*	
Body condition score (BCS)			
BCS1	High (≥ 2.75)	55.6% (25/45)	>0.05
	Low (≤ 2.5)	32% (8/25)	
BCS2	High (≥ 2.75)	50% (21/42)	>0.05
	Low (≤ 2.5)	42.9% (12/28)	

*The statistical analysis revealed that the difference between the groups tended to be significant ($0.05 < p < 0.10$)

function of SAS (2000). A significant difference between the levels of a classification variable was considered when $p < 0.05$, whereas differences between $0.05 < p < 0.10$ were considered a statistical tendency. All results are presented as means with \pm standard error.

Results

As a result of synchronization in the study, there were no statistical differences ($p > 0.05$) between the G6G and PG-3-G groups in terms of submission rate (100% and 100%), the first-service pregnancy per artificial insemination (P/AI) (42.9% and 51.4%), calving to first insemination interval (53.5 and 54.0 d), calving to conception interval (54.1 and 53.7 d), calving interval (334.1 and 333.7 d) were determined. This results with respect to reproductive performance parameters obtained in the trial are summarized in Table 1.

Factors included in the logistic models that significantly influenced pregnancy per P/AI were determined. The data analyzed according to the protocol at the beginning of cows to be "cyclic" and "noncyclic" in all cows, P/AI were found to be 64.7% and 41.5%, respectively. The difference between the groups tended to be significant ($p = 0.098$). All cows were divided into two groups as "Low" for ≤ 2.5 and "High" for ≥ 2.75 according to BCS. The first-service pregnancy per artificial insemination were found to be 55.6% (25/45) in BCS1 high, 32% (8/25) in BCS1 low and 50% (21/42) in BCS2 high, 42.9% (12/28) in BCS2 low. There were no significant differences between the groups (high-low) according to the statistical analysis ($p_1 > 0.05$; $p_2 > 0.05$). Factors included in the logistic models that significantly influenced pregnancy per P/AI are given in Table 2.

Discussion

It is well known that in dairy farms calving interval is one of the most important parameters in the evaluation of reproduction performance. In fact, calving interval is normally

desire to have 365 days. However, this is not an easy task to achieve. For this reason, if it is not more than 400 days, it is considered to be normal. The typical value is expected to be between 355-430 days. If the calving interval exceeds 400 days, there could be a sharp drop in milk production due to the prolongation of the lactation period. Open days must be less than 115 days to reach appropriate calving interval (Dinç and Kutlu 2015). In fact, SR is reported to be one of the performance evaluation parameters for herds having long calving intervals. It is evident from the relevant literature that presynchronization could have a potential to solve such reproduction problems. The SR is one of the best performance evaluation parameters if the calving interval is long. Aköz et al (2008) reported that the calving interval was shorter as it was recorded 430.5 ± 0.9 days in the control group and 386.8 ± 5.7 days in the Presynch-14 group. In the present study, the calving interval in the cows which became pregnant in the postpartum 1st TAI was 334.1 ± 3.73 in the G6G group and 333.7 ± 3.24 in the PG-3G group. It is clearly shown that the presynchronization protocols could have a potential to reduce the calving interval less than 400 days. The results obtained in the experiment suggest that having shorter calving intervals could be attributed to presynchronization protocols and their success.

Despite Ovsynch's overall positive impact, 10 to 30% of Ovsynch-treated cows were reported to fail to synchronize ovulation in response to final GnRH (Navanukraw et al 2004, Bello et al 2006). For this reason, G6G and PG-3G protocols have been tried to achieve the highest success in the Ovsynch protocol. In fact, the G6G protocol was first developed by Bello et al (2006). The results of the study showed that P/AI at d 35 was 50% in the G6G group. Astiz and Fargas (2013) reported that P/AI at d 28-45 was 34.8% in the G6G group. Yılmaz et al (2011) reported that P/AI at d 60-90 was 53.8% in the G6G group. Ribeiro et al (2012a) reported that modified G6G protocol (5-d cosynch72 instead of Ovsynch) P/AI at d 35 were; when last PGF2 α administration is administered in a single dose was 28.7% and last PGF2 α admi-



nistration was performed in 2 divided doses 45.4%. Ribeiro et al (2011) reported that modified G6G protocol P/AI at d 30 was 49.9% in the G6G group. Sonat et al (2014) reported that P/AI at d 35 were 45% in the G6G group and 52.5% G6G + Beta-carotene group. Dirandeh et al (2015) reported that P/AI at d 32 were 39.5% in the G6G group and 38.0% in the G7G group. Yousuf et al (2016) reported that P/AI at d 35 was 57% in the G6G group. Heidari et al (2017) reported that P/AI at d 32 were; the last PGF2 α in a single dose 32.9% and, last PGF2 α in 2 divided doses 37.5% in the G6G protocols. In the present study P/AI in the G6G group was found to be 42.9%. While these results are in agreement with some of the studies, they do not comply with the values transferred from some others. The variation between the results could be attributed to the differences in feeding and housing conditions of the experiments, especially in terms of animal welfare and barn condition.

PG-3-G protocol was first developed by Peters and Pursley (2002). The results of their study showed that P/AI at d 36 was 41.5% in the PG-3-G protocol. Stevenson et al (2012) reported that P/AI at d 32 were 40% including temperature stress, 59.1% in the cold season and 43.3% in ovular/cyclic cows in the PG-3-G protocol. Stevenson and Pulley (2012) reported that P/AI at d 32-38 d was 41.2% in the PG-3-G protocol (46.8% in the cold season). Pulley et al (2015) reported that P/AI at d 31 were 57.1% and 56.3% in the PG-3-G protocol. Stevenson et al (2018) reported that P/AI were; the last PGF2 α in a single dose 52.1%, last PGF2 α in 2 divided doses 44.0%, last PGF2 α in a single dose 25.1% (Ovsynch-5days) and last PGF2 α in 2 divided doses 39.4% in the PG-3-G protocols. In the present study the P/AI in the PG-3-G group was found to be 51.4%. Our results were in accordance with some previous findings reported by Peters and Pursley (2002), Stevenson et al (2012), Stevenson and Pulley (2012) and also Stevenson et al (2018). However, P/AI values in our both groups (42.9% in the G6G group and 51.4% in the PG-3-G group) were found to be only numerically different but not statistically ($p > 0.05$).

Prevalence of noncyclic postpartum in dairy herds is around 30% by PP 50 to 60 days, but varies a lot from herd to herd from 5% to as high as 50% according to management conditions in farms (Opsomer et al 2000, Wiltbank et al 2002, Santos et al 2009). Gümen et al (2003) reported from their study that 20% of lactating Holstein cows were not cyclic by about PP 60 day. Opsomer et al (2000) reported that in their study consistently low milk P4 levels for the first 50 days after calving (delayed cyclicity) was found 21.5% in cows. Chebel et al (2006) reported from their study that 31.5% cows having concentrations of P4 < 1.0 ng/ml in both of the first 2 blood samples (PP 35 and 49 day) were classified as anestrus. Lopez et al (2005) reported that cows were fitted with a transmitter to record standing activity during oestrus, and serum P4 was assessed weekly starting at week 1 postpartum for all

cows in their study. They found 28.5% cows were anovular at PP 71 day. In the present study we found 24.3% cows were noncyclic at PP 28-40 day.

It is reported that in anovular cows, fertility decreases at the first TAI (Santos et al 2009) or the risk of embryo loss increases (Santos et al 2004). In anovular cows, GnRH induces ovulation by 88% (Gümen et al 2003). However, in anovular cows, oestrodial concentration is low during insemination. Therefore, the conception rate decreases (Ribeiro et al 2011) and the number of open days increases. In fact, it has been reported that it is possible to stimulate the formation of CL in anovular cows prior to the Ovsynch protocol to increase fertility in anovular cows (Herlihy et al 2012). Ribeiro et al (2012b) reported that P/AI were 61.7% and 35.1%, ovular and anovular cows regardless of protocol, respectively. In the Double-Ovsynch group, P/AI were 60.1% in ovular and 36.8% in anovular cows. The P/AI were in the Presynch group, 63.3% in ovular and 33.3% anovular cows. Stevenson et al (2012) compared the PG-3-G and Presynch-10 protocols in their study. They reported that P/AI were 39.8% and 20.6%, ovular and anovular cows, regardless of protocol, respectively. The P/AI were in PG-3-G group, 43.3% in ovular and 20% in anovular cows. The P/AI were in Presynch-10 group, 36.0% in ovular cows and 21% in anovular cows. Öztürk et al (2010) compared the Doublesynch and Ovsynch protocols in anoestrus cows. They reported that P/AI were 72.0% in Doublesynch group and 23.1% in Ovsynch group. Ahmed et al (2017) compared Ovsynch, Ovsynch+CIDR and G6G protocols in anoestrus cows. They reported that P/AI were 28.6% in the Ovsynch group, 42.9% in the Ovsynch+CIDR group, and 71.43% in the G6G group. In our study when P/AI were evaluated according to cows' being cyclic - noncyclic; P/AI in all cows were found to be 41.5% and 64.7%, respectively. Statistical analysis of our data revealed that the difference between the groups tended to be significant ($p = 0.098$). This difference could be attributed to some influential factors; firstly, PG-3-G protocol was applied to 16 noncyclic cows, since the number of materials was low and the effect of the protocol was seen to be more significant in noncyclic cows. When the results were evaluated in terms of BCS, 8 of the 11 pregnant cows were found to have a BCS of 2.75 (High) at the time of protocol was started. It could be speculated that the rate of conception is high due to the high number of non-cyclic cows with high BCS. Presynchronization protocols have been proposed for cows without "CL" in their ovaries (Pursley and Martins 2011) or for anovular cows (Ribeiro et al 2011). As a result, 62.5% (10/16) of PG-3-G protocol was found to work well in noncyclic cows. While these results are consistent with the findings reported in Öztürk et al (2010) and Ahmed et al (2017) but not in accordance with the results reported by Ribeiro et al (2012b) and Stevenson et al (2012). The difference is thought to be due to the fact that the protocols applied are not exactly similar and the number of materials is relatively low besides the differences in the



experimental conditions.

Conclusion

In conclusion, start of presynchronization protocols in the early postpartum period, provides 100% SR after VWP. It is clearly shown that the presynchronization protocols reduces the calving interval less than 400 days. Time and labor cost could be saved without the need for oestrus detection and also appropriate P/AI could be achieved leading to an effective increase in reproduction performance of non-cyclic cows under our experimental condition.

Acknowledgement

This article has been prepared by the master thesis of the first author and oral presentation at the 5th National Heard Health & Management Congress and 1st International Heard Health & Management Congress on 14-17 October 2018.

Conflict of Interest

The authors did not report any conflict of interest or financial support.

Funding

During this study, any pharmaceutical company which has a direct connection with the research subject, a company that provides and / or manufactures medical instruments, equipment and materials or any commercial company may have a negative impact on the decision to be made during the evaluation process of the study or no moral support.

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Ethical Approval

This study was carried out with the permission of the Çukurova University Animal Experiments Local Ethics Committee (Decision No: ÇÜ-HADYEK/2015-6-1) report.

CITE THIS ARTICLE: Kutlu M, Dinc DA, 2020. Comparison of the effects of two pre-synchronization protocols (G6G and PG-3-G) on some reproductive performance parameters in Holstein cows. *Eurasian J Vet Sci*, 36, 4, 248-254.

