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RESEARCH ARTICLE

Determining The Optimum Voluntary Waiting Period and Synchronization Protocol in Simmental Cows

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ABSTRACT

The objective of the present study was to compare four different synchronization protocols at the end of various voluntary waiting period (VWP) and evaluate the effect of parity and milk yield on pregnancy rates with synchronization in Simmental cows. All animals were randomly allocated into four synchronization groups and one control group. Parity, milk yield (≤23 kg/d; >23 kg/d), and days in milk (DIM; 45-60, 61-90, >90 days) were compared according to synchronization protocols. Ovsynch (Group I; n=81), Ovsynch+Progesterone (Group II; n=30), G6G (Group III; n=66), 2xG6G (Group IV; n=45), and a control group (n=35) were designed for the study. The parity significantly affected the pregnancy rates in the primiparous cows, especially in Group II (P<0.05). The pregnancy rates between synchronization and control groups did not differ significantly (P>0.05). There was no significant difference between milk yield and pregnancy rates (P>0.05). In conclusion, the synchronization of Simmental cows with Ovsynch, Ovsynch+Progesterone, G6G, or 2xG6G did not affect pregnancy rates across various DIM. These data suggest that the suitable VWP could be planned according to the farm's economic and local market targets, with veterinarians able to choose a protocol based on practicality, effectiveness and cost, as well as the specific requirements of the herd.

Keywords: Simmental cow, Synchronization, Voluntary waiting period

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Simmental Irkı İneklerde Optimum Gönüllü Bekleme Süresi ve Senkronizasyon Programının Belirlenmesi

ÖZ

Bu çalışmanın amacı, Simental ırkı ineklerde farklı gönüllü bekleme sürelerinin (GBS) sonunda dört farklı senkronizasyon programının etkinliğinin karşılaştırılması, doğum sayısı ve süt veriminin bu senkronizasyon protokolleri ile gebelik oranlarına etkilerinin değerlendirilmesidir. Hayvanlar rastlantısal olarak dört farklı senkronizasyon ve bir kontrol grubuna ayrıldı. Doğum sayısı, süt verimi (≤23 kg/gün; >23 kg/gün) ve sağılan gün sayısının (SGS; 45-60, 61-90, >90 günler) uygulanan senkronizasyon protokollerine etkileri karşılaştırıldı. Ovsynch (Grup I; n=81), Ovsynch+Progesteron (Grup II; n=30), G6G (Grup III; n=66), 2xG6G (Grup IV; n=45) ve kontrol (n=35) grupları oluşturuldu. Doğum sayısının gebelik oranlarına etkisi karşılaştırıldığında, ilk laktasyondaki primipar ineklerde multipar ineklere göre özellikle Grup II'de istatistiksel önemin (P<0,05) yüksek olduğu tespit edildi. Senkronizasyon ve kontrol grupları arasında gebelik oranları bakımından fark gözlenmezken (P>0,05), süt veriminin de gebelik oranı üzerinde istatistiksel öneme yol açmadığı (P>0,05) belirlendi. Sonuç olarak, Simental ırkı ineklerin Ovsynch, Ovsynch+Progesteron, G6G ve 2xG6G protokolleri ile senkronizasyonunun farklı sağılan gün sayısı (SGS)'na göre gebelik oranlarını etkilemediği belirlendi. Sunulan çalışmadan elde edilen veriler veteriner hekimlerin pratik, etkili, uygun maliyetli ve çiftliğin özel ihtiyaçlarını karşılayabilecek yukarıda belirtilen herhangi bir senkronizasyon protokolünü seçebileceğini, en uygun gönüllü bekleme süresinin ise çiftliğin ekonomik ve bölgesel piyasa hedeflerine göre planlanmasının daha etkili ve verimli olacağını ortaya koymaktadır.

Anahtar Kelimeler: Gönüllü bekleme süresi, Senkronizasyon, Simental inek

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INTRODUCTION

Reproductive efficiency is the main objective in cattle breeding. However, genetic selections for milk production and pressure to increase milk yield have adversely affected reproductive performance in dairy cattle over recent decades (Thatcher et al. 2006, Shahzad et al. 2019). One of the most crucial challenges in reproductive efficiency is managing the VWP and high pregnancy rates in the first service. Fertility parameters such as calving interval, number of days open, average service per conception, and heat detection are in excess in high milk yielding dairy cows in the postpartum period. In dairy herds, insufficient heat detection is the principal management problem affecting reproductive profitability because of poor visual detection of estrous signs, insemination at the wrong time, lack of reproductive records, reproductive health problems, nutrition deficiencies, and housing factors (Ben Salem et al. 2006, Stangaferro et al. 2018, Lindley et al. 2021). Nowadays, different estrus and ovulation synchronization protocols are using to improve pregnancy rates without any estrous detection in dairy cows. Ovulation synchronization protocols allow the insemination of cows in a fixed time, called Fixed Time Artificial Insemination (FTAI); it also helps veterinarians schedule a predefined breeding period and calving time in a suitable environment and season for calves (Pursley et al. 1995, Bo et al. 2016).

Pregnancy rates have decreased from around 65% to 35% in the past 40 years (Fleming et al. 2018). The Ovsynch program was developed to solve this problem and synchronize ovulation time, FTAI and increase AI success but it could not enhance the pregnancy rate per AI (Pursley et al. 1997, Nak et al. 2011, Yılmaz et al. 2011, Fricke et al. 2014). New strategies related to gonadotropin-releasing hormone (GnRH) and prostaglandin F2α (PGF2α) treatments, frequently Ovsynch based developed for ovulation control and increasing pregnancy/AI success. protocols such as G6G, Double Ovsynch (DO), Presynch/Ovsynch, and Progesterone+Ovsynch aim to improve reproductive management success and pregnancy rates at the end of the VWP. However, each protocol has its advantages and disadvantages: herd size, labor, parity, milk yield, breed of animals, drug cost, etc. (Astiz and Fargas 2013, Wiltbank et al. 2015, Heidari et al. 2017, Shahzad et al. 2019). One of these presynchronization protocols is G6G, which involves PGF2α and GnRH two days apart, and then six days later, following the standard Ovsynch protocol. The results showed that the ovulation rates and circulatory progesterone levels were increased with higher synchronization rates following G6G synchronization (Ribeiro et al. 2012). Double Ovsynch is a more extended protocol than Ovsynch and G6G (28 days vs. 10 and 18 days), and uses two GnRH and PGF2α administrations before Ovsynch to achieve pregnancy rates. Studies suggest that the DO protocol gives better results in primiparous cows (Astiz and Fargas 2013). Progesterone integration to the classic Ovsynch protocol hypothesis to improve synchronization prevents premature estrus and ovulation before PGF2α injection (Larson et al. 2006).

Many studies using the modified Ovsynch protocol give different synchronization and pregnancy rates, and it is still unclear which environmental and/or endocrine factors affect the synchronization success. The objective of this study was to compare four different synchronization protocols at the end of various VWPs days and evaluate the effect of parity, milk yield, and the number of services per conception on pregnancy rates with synchronization in Simmental cows.

MATERIAL and METHODS

All procedures performed with cows were approved by the Experimental Animal Ethics Committee of Afyon Kocatepe University (approval number: 167-20).

Animals

This experiment was performed on a commercial farm with 257 healthy, lactating Simmental cows (aged between two and nine years) housed in free-stall barns. Cows were milked twice daily, and TMR was presented twice daily with ad libitum access to water (NRC 2001). All cows were evaluated to body condition score (BCS) on the day of enrollment (one=emaciated, five=obese).

Experimental Design

All animals were randomly allocated into four synchronization groups and one control group. Parity, milk yield (≤23 kg/d; >23 kg/d), and DIM (45-60; 61-90; >90 days) were compared according to synchronization protocols.

Group I (Ovsynch; n=81): Cows in Group I received GnRH analogue (2.5 ml, I.M; 0.004 mg/ml Buserelin acetate, Receptal, Intervet International GmbH, Germany) on day 0; 500 μg PGF2α (2 ml, I.M.; Cloprostenol, Dalmazin, FATRO, Italy) was injected to all cows on day 7, and second GnRH treatment was administered with the same dose on day 9. Cows were FTAI 16 hours after the second GnRH administration (Figure 1a).

Group II (Ovsynch+Progesterone; n=30): Cows in this group were treated the same as with the Ovsynch protocol, but CIDR (1.38 g progesterone, Easy Bred, Zoetis, Turkey) was inserted on day 0 and removed on day 7 (Figure 1b).

***Group III (G6G; n=66):* Animals in this group received** 500 μg PGF2α (2 ml, IM.; Cloprostenol, Dalmazin, FATRO, Italy) on day 0, two days later GnRH (2.5 ml, I.M; 0.004 mg/ml Buserelin acetate, Receptal, Intervet International GmbH, Germany) was injected, 6 days later classic Ovsynch protocol was applied (Figure 1c).

Group IV (2xG6G; n=45): Cows in Group IV received G6G protocol with a modification by two times more GnRH doses (5 ml, I.M; 0.004 mg/ml Buserelin acetate, Receptal, Intervet International GmbH, Germany) (Figure 1d). The effect of GnRH dose on pregnancy rates was studied in this group.

Control Group (n=35): The cows in this group were observed for estrus detection without any hormone administration previously and inseminated.

The animals in the study groups were inseminated at a fixed time, and the animals in the control group were inseminated once at the specified time after the estrus detection.

Pregnancy Diagnosis

Pregnancy status was determined by ultrasonography (HT838, Hasvet, Turkey) on day 45 after FTAI. The presence of amniotic vesicle, positive hearth beat, and intraluminal uterine fluid was used as a determinant of pregnancy.

Statistical Analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software, Version 20.0. The findings are presented as means and percentages, and mean values are expressed with their standard error and standard deviation. The differences in age, milk production, DIM, and parity between groups were determined using ANOVA. The Kruskall-Wallis test was used to determine the impact of synchronization protocols on primiparous and multiparous cows, VWP groups, and milk production

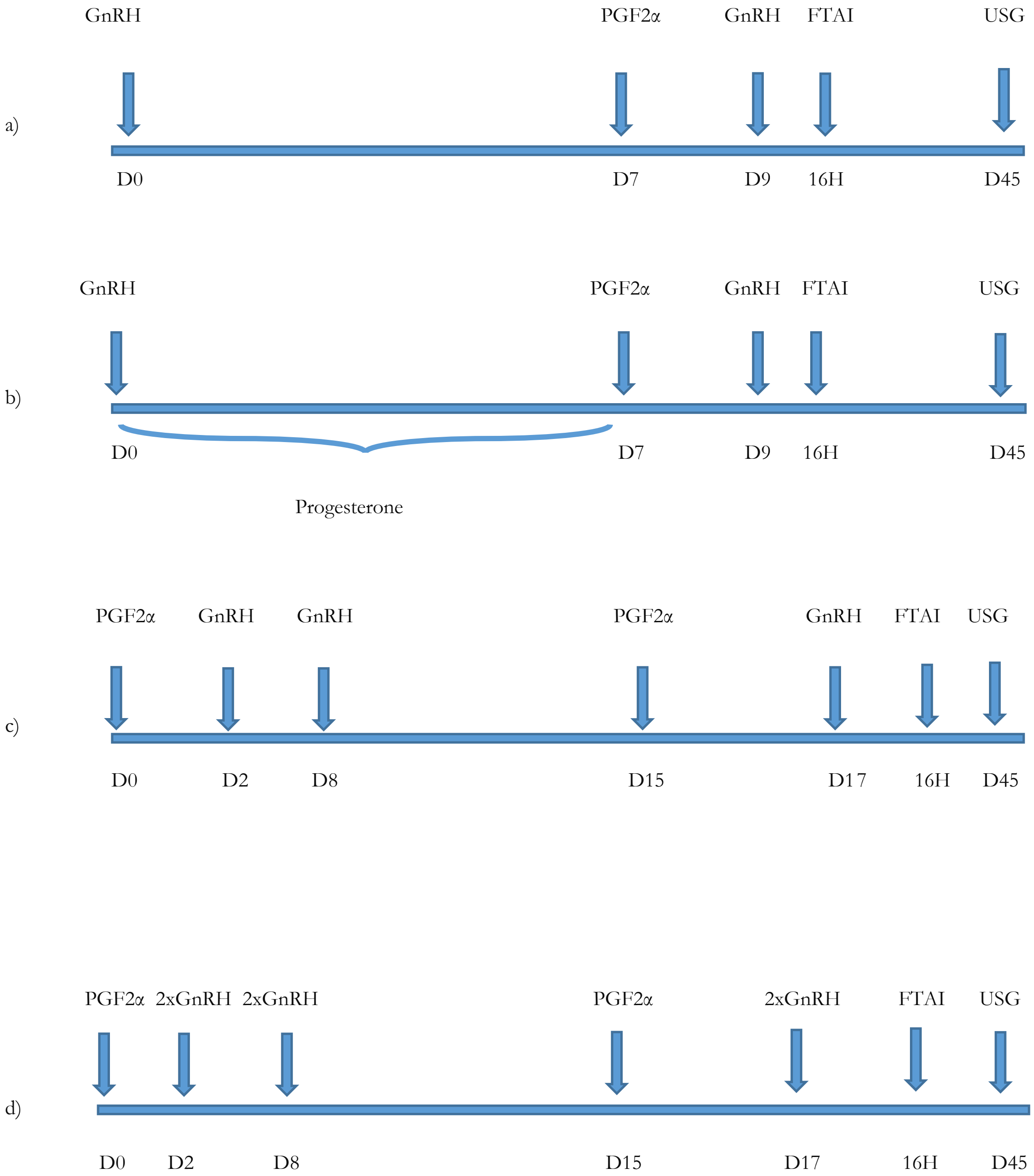

Figure 1. Schematic representation of synchronization protocols during the study in lactating Simmental cows. a) Ovsynch, b) Ovsynch+Progesterone, c) G6G, d) 2xG6G protocols.

RESULTS

The average age of the cows was 4.2±2.0 (2 to 9 years old) in the study. The mean milk production (kg/d), DIM (days), and parity were 23.6±4.1, 106.9±68.0, and 2.6±1.8, respectively (Table 1).

Number and proportions of animals to be first inseminated postpartum in groups; Group I (45/81, 55.6%), Group II (19/30, 63.3%), Group III (37/66, 50.1%), Group IV (22/45, 48.9%) and Control (20/35, 57.1%) respectively. The pregnancy rates on day 45 after FTAI in Group I, Group II, Group III, Group IV, and Control were 55.6%, 53.3%, 54.5%, 51.1%, and 57.1%, respectively. Pregnancy rates following the first AI and two or more AI did not differ significantly between groups (P>0.05). One pregnancy loss was observed in Group I in the fourth month, three aborts in Group III between the second and fifth months, and one abort in Group IV in the fifth month.

Parity significantly affected the pregnancy rates in the primiparous cows, especially in Group II (P<0.05) (Table 2). The pregnancy rates in the other study groups were also higher than in the control group. However, the synchronization protocols did not increase the pregnancy rates compared with the control group in the multiparous cows; the control group rates were significantly higher than those in the study groups (P<0.05).

Pregnancy rates at the end of the VWP of 45-60 DIM were 61.5% in Group I, 60% in Group II, 42.8% in Group III, 50.0% in Group IV, and 53.8% in Control group (Table 3). At the 61-90 days the pregnancy rates were 48.3%, 33.3%, 52.0%, 40.0%, and 33.3%, respectively (Table 3); the rates were 58.9%, 55.5%, 58.8%, 60.9%, and 68.8%, respectively at >91 days at the end of VWP (Table 3). The pregnancy rates between synchronization and control groups did not differ significantly (P>0.05). The relationship between milk production (≤23 kg/d and >23 kg/d) and pregnancy rates is shown in Table 4 and Table 5, and there was no significant difference between milk yield and pregnancy (P>0.05).

Table 1. The descriptive statistics of age, milk production, DIM, and parity of Simmental cows in the study.

Variables	Mean	Standart Deviation
Age	4.2	2.0
Milk production (kg/d)	23.6	4.1
DIM (days)	106.9	68.0
Parity (number)	2.6	1.8

Table 2. The effect of parity on pregnancy numbers according to synchronization protocols

Parity Groups	Synchronization Groups	N	P value
Primiparous	Group I[ab]	39	
	Group II[b]	14	
	Group III[ab]	25	
	Group IV[ab]	13	0.05
	Control[a]	11	
Multiparous	Group I[b]	42	
	Group II[b]	16	
	Group III[b]	41	0.03
	Group IV[b]	32	
	Control[a]	24	

Table 3. The pregnancy rates between synchronization and control groups.

	Postpartum 45-60 days				Postpartum 61-90 days				Postpartum >91 days				P Value
Groups	**n**	**Pregnant**	**Non-pregnant**	**Pregnancy rate (%)**	**n**	**Pregnant**	**Non-pregnant**	**Pregnancy rate (%)**	**n**	**Pregnant**	**Non-pregnant**	**Pregnancy rate (%)**	
Group I	13	8	5	61.5	29	14	15	48.3	39	23	16	58.9	0.612
Group II	15	9	6	60.0	6	2	4	33.3	9	5	4	55.5	0.547
Group III	7	3	4	42.8	25	13	12	52.0	34	20	14	58.8	0.708
Group IV	12	6	6	50.0	10	4	6	40.0	23	13	10	56.5	0.686
Control	13	7	6	53.8	6	2	4	33.3	16	11	5	68.8	0.323

Table 4. The relationship between milk production (≤23 kg/d) and pregnancy rates.

Groups	n	Pregnancy Number	Non-pregnancy Number	Pregnancy Rate (%)	P Value
Group I	45	24	21	53.3	
Group II	11	5	6	45.5	
Group III	39	23	16	59.0	0.885
Group IV	19	12	7	63.2	
Control	21	12	9	57.1	

Table 5. The relationship between milk production (>23 kg/d) and pregnancy rates.

Groups	n	Pregnancy Number	Non-pregnancy Number	Pregnancy Rate (%)	P Value
Group I	36	21	15	58.3	
Group II	19	11	8	57.9	
Group III	27	13	14	48.2	0.840
Group IV	26	11	15	42.3	
Control	14	8	6	57.1	

DISCUSSION

Determining the duration of the VWP is still unclear in dairy cows. Various studies suggest the optimal VWP is between 42 to 150 days for primiparous and multiparous dairy cows (Arbel et al. 2001, Inchaisri et al. 2011, Toledo-Alvarado et al. 2017, Stangaferro etal. 2018). In most dairy farms, reducing the VWP is the

primary purpose of having a calving interval of 12 to 13 months. However, in the early days at the end of

VWP might need more services per conception due to anovulatory cows before 60 days postpartum, and the other disadvantage of early VWP is drying off of cows too early that have still relatively high milk yield. On the other hand, the simulation models and field data showed that every delayed week increases economic losses in dairy farming (Inchaisri et al. 2011). In the present study, we evaluated the effect of different VWP of 45-60, 61-90, and >90 days on pregnancy rates using different synchronization protocols in Simmental cows. No differences between groups were observed in terms of pregnancy rates. Our results showed that the early or late services did not give significant differences in pregnancy rates compared with the control group, and these data suggest the VWP could be planned according to the farm's economic and local market targets.

Ovulation synchronization and FTAI programs can be used for any kind of herd system (pasture/confinement based; seasonal/year-round herds) to eliminate the oestrus detection requirement or to treat reproductive diseases such as anovulatory anoestrus (Lindley et al. 2021a). Ovsynch protocol is one of the earliest ovulation synchronization programs used in cows (Pursley et al. 1997). However, this protocol does not ensure satisfactory pregnancy rates in anoestrous cows with low conception rates and high embryo mortality or heifers due to frequent follicular waves and dampens LH response to GnRH because of circulating progesterone levels (Kaçar et al. 2008, Herlihy et al. 2013, Lindley et al. 2021a). All presynchronization protocols aim to control the estrous cycle stage to improve synchronization success rates when the ovulation synchronization protocol begins (Butler et al. 2019). The present study compared three different presynchronization protocols and classic Ovsynch with the non-synchronized group in a confinement based year-round herd. Pregnancy rates were similar in all groups following the first and second FTAI, and the presynchronization did not affect synchronization success. The results of this study confirm that the selection of ideal protocol should be planned according to individual herd nuances, as stated by Lindley et al. (2021b). The synchronization protocols have their advantages and disadvantages, and the veterinarian should determine the ideal protocol as the most practical, most successful, least cost path, and herd's specific requirement.

Exogenous GnRH is one of the primary hormones used for ovulation synchronization, and it induces LH surge and affects ovarian follicular development during the program (Sartori et al. 2001, Butler et al. 2019). An optimal standard dose of GnRH is 100 μg for the synchronization of cows (Pursley et al. 1997, Souza et al. 2009). However, it was detected that the progesterone environment at GnRH treatment affects GnRH-induced LH secretion, and a 200 μg dose of GnRH increases LH secretion (Giordano et al. 2012). Giordano et al. (2013) studied the higher dose of GnRH (100 μg vs. 200 μg) with the Double Ovsynch protocol to increase ovulatory response in cows. They reported that the higher GnRH dose increased ovulation rates only in cows with elevated progesterone, but no effect of higher dose of GnRH on fertility was detected. In this study, two different GnRH doses (100 μg-Group III vs. 200 μg-Group IV) were compared with the G6G synchronization protocol in Simmental cows. Pregnancy rates did not differ significantly, either with WVPs or parity in either GnRH doses. Studies have reported that G6G protocol increases ovulation rates, corpus luteum formation in anovulator cows, and higher progesterone levels (Ribeiro et al. 2011, Kohsari and Benjamin 2022). Nevertheless, two different G6G protocols in the present study did not increase fertility compared with Ovsynch and Ovsynch+Progesterone protocols. The circulatory progesterone levels or ovulation rates were not evaluated in this study. However, the reports cited above showed that the synchronization protocols could increase ovulation rates and circulatory progesterone, but pregnancy rates were not shown increase satisfactorily. Hence, future researches could determine the factors affecting final pregnancy rates.

Several factors influence fertility and the success of synchronization and presynchronization protocols. The conditions such as breed, nutrition, parity, postpartum diseases, and milk yield may affect the pregnancy rates following synchronization. In primiparous cows, ovarian cyclicity could be delayed, and there could be longer intervals from parturition to the first service and conception; in addition, multiparous cows might tend to have lower luteolysis rates (Astiz and Fargas 2013, Meikle et al. 2004, Toledo-Alvarado et al. 2017, Lindley et al. 2021a). Some studies stated that the Double Ovsynch program gives higher pregnancy results in primiparous cows than in multiparous cows (Herlihy et al. 2012, Astiz and Fargas 2013). Our research showed that parity affects the pregnancy rates, and the Ovsynch+Progesterone protocol was given a statistically higher pregnancy rate in primiparous cows.

It is known that there is a strong interaction between milk yield and fertility, higher milk yielding breeds such as Holstein and Brown Swiss have worsened fertility records than the dual-purpose Simmental and Alpine Grey breeds. Also, milk yield may vary across the individual cows within the same breeds (Toledo-Alvarado et al. 2017, Martinez-Castillero et al. 2020). Toledo-Alvarado et al. (2017) reported that reproductive efficiency and fertility rates increase at least up to a milk yield about 25 kg/d. The present study was conducted on Simmental cows with mean milk production of about 23.6 kg/d (10 to 35 kg/d). In the case of milk yield of ≤23 kg/d and >23 kg/d, there was no significant difference between synchronization and control groups in pregnancy rates in our study.

CONCLUSION

In conclusion, the synchronization of Simmental cows either with Ovsynch, Ovsynch+Progesterone, G6G, or 2xG6G did not affect pregnancy rates across various DIM. These data suggest that the suitable VWP could be planned according to the farm's economic and local market targets, with veterinarians able to choose a protocol based on practicality, effectiveness and cost, as well as the specific requirements of the herd.

Conflict of interest: The authors declare that there is no conflict of interest.

Authors Contribution Rate: UK, MD, and DBA planned the study, designed and performed the experiments, and helped with manuscript writing; HH analyzed the statistics data. All authors read and approved the final manuscript.

Ethical Approval: This study has received permission with Experimental Animal Ethics Committee of Afyon Kocatepe University (Decision number: 167-20).

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