pISSN 1598-298X
J Vet Clin 31(5) : 382-388 (2014)

Journal of
Veterinary Clinics
www.ksvc.or.kr

Risk Factors for the Probability of Pregnancy Following Synchronization Protocols in Dairy Cows

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(Accepted: October 11, 2014)

Abstract : The objective of this study was to determine the risk factors associated with pregnancy following 3 synchronization protocols in dairy cows. Data were collected on 1,952 cows from 22 dairy farms, including synchronization protocols ($PGF_{2\alpha}$ + estradiol benzoate [PG+EB], Ovsynch, and CIDR-ovsynch), cow parity, body condition score (BCS), and dates of previous calving, insemination and conception. The odds ratio (OR) for pregnancy were analyzed by logistic regression using the LOGISTIC procedure in SAS. The analysis revealed that farm ($p = 0.005$), cow parity ($p = 0.0001$), BCS ($p < 0.005$), and AI season ($p < 0.05$) significantly affected and calving to AI interval tended to affect ($p < 0.1$) the probability for pregnancy. Although synchronization protocols did not affect the probability for pregnancy ($p > 0.05$), cow parity and synchronization protocols showed a significant interaction ($p < 0.005$); the OR (0.60) was significantly lower ($p < 0.0001$) for multiparous cows compared to primiparous cows using PG+EB, whereas the OR (1.44) tended to be higher ($p < 0.1$) for multiparous cows compared to primiparous cows using the Ovsynch, and the probability for pregnancy did not differ between multiparous and primiparous cows using the CIDR-ovsynch ($p > 0.05$). Cows with BCS $\geq$ 3.00 were more likely pregnant (OR: 1.41) compared with cows having BCS $\leq$ 2.75, whereas cows inseminated during summer had a lower OR (0.73) compared with those inseminated during spring. Cows with a calving to AI interval > 150 days were more likely to be pregnant (OR: 1.20) compared with cows with a calving to AI interval $\leq$ 150 days. In conclusion, the OR for pregnancy following synchronization protocols in dairy cows was affected by farm, parity, BCS, calving to AI interval of the cow, and AI season, and there was a significant interaction between cow parity and synchronization protocols; the OR for pregnancy was lower for multiparous cows compared with primiparous cows using the PG+EB protocol.

***Key words* :** dairy cows, synchronization protocols, probability for pregnancy, risk factors.

Introduction

Genetic selection for higher milk yield in dairy cows has led to a dramatic increase in milk production, with a concomitant inverse decrease in reproductive performance worldwide (12,31,42,55). Likewise, annual milk production per cow in dairy herds of Korea has increased enormously over the last 2 decades, reaching 9,737 kg in 2013 (9). Korea ranked 4th among the 49 member countries of the International Committee for Animal Recording (ICAR), according to the Yearly Milk Enquiry Online Database. However, calving interval, which is one of important parameters for fertility (28), was 462 days. Diverse factors including nutritional and health conditions and/or productivity of individual animals, environment (climate) and herd management practices may affect the reproductive performance in dairy herds (11,22,23,33,50, 51). Additionally, several studies demonstrated that weak estrus or anestrus leads to lost opportunities for inseminations, especially in high milk-yielders, resulting in an extended calving interval (16,30,39,55). One of the potential reasons for weak and shortened estrus, was reportedly that the greater feed consumption by high milk yield cows increased the blood flow to the liver, steroid catabolism, and the progesterone and estrogen clearance rates, thereby leading to lower circulating hormone concentrations (46). Moreover, the estrus periods in nearly half of the normal cycling dairy cows in a herd may not be detected (40,53). The estrous detection rate following synchronization using $PGF_{2\alpha}$ treatment was reported as 52.5% in dairy herds (27). Thus, in modern dairy herds various reproductive hormone treatments including Ovsynch have been developed to timely inseminate dairy cows without estrous detection (13,15,16,25,26,32,43,48,51). The outcomes obtained from synchronization protocols are variable depending on herds, insemination seasons, climates, hormone treatment programs, cow parity, body condition, peak milk yield, average milk protein, and other factors (2,6,24,29,35, 36,47). Determination of potential factors influencing the probability of pregnancy may provide valuable information for veterinary practitioners and farmers to maintain proper reproductive performance in dairy herds. Therefore, the objective of this study was to determine the risk factors for the probability of pregnancy following 3 synchronization protocols in dairy herds.

Materials and Methods

Animals and reproductive management

The study was conducted on 22 Holstein dairy farms (A-

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V) located in Chungcheong Province. All cows were fed a total mixed ration and milked twice daily. They received regular reproductive health checks every 2 to 4 weeks, by veterinarians at the College of Veterinary Medicine, Chungbuk National University. These included examination of ovarian structures and the uterus *via* transrectal palpation and ultrasonography. Three synchronization protocols were employed by 2 personnel: 1) 500 μg of the $PGF_{2\alpha}$ analogue, cloprostenol (Estrumate, MSD Animal Health, Seoul, Korea) followed by 1 mg estradiol benzoate (EB SY Esrone, Samyang, Seoul, Korea) 24 h (PG+EB), and timed AI (TAI) 24 h after the EB injection; 2) 100 μg gonadorelin (a GnRH analogue; Godorel, Uni-Biotech, Yesan, Korea), 500 μg of cloprostenol 7 d after injection of gonadorelin, a 2^{nd} dose of 100 μg gonadorelin 56 h after the injection of cloprostenol, and TAI at 16 h after the 2^{nd} injection of gonadorelin (Ovsynch); 3) the same as Ovsynch, plus an internal drug-release device containing 1.9 g of progesterone (CIDR™, InterAg, Hamilton, New Zealand) between the first GnRH and cloprostenol injections (CIDR-ovsynch). Before initiation of the synchronization protocols, the cows received an ultrasonography to determine the ovarian structures (CL or follicles) (Tringa Linear VET Ultrasound scanner fitted with a 5.0 MHz array transducer, Esaote Pie Medical, Maastricht, the Netherlands, or Sonoace 600 fitted with a 5.0 MHz linear-array transducer, Medison, Seoul, Korea). Cows with a CL (> 20 mm diameter; luteal phase) received 1 of the 3 synchronization protocols (PG+EB, Ovsynch, or CIDR-ovsynch), whereas cows without a CL (presumably, metestrous or the other phases except luteal phase) received either Ovsynch or CIDR-ovsynch. Cows without a CL were initiated on the Ovsynch protocol 5 days after ultrasonography, to prevent possibility of the spontaneous luteolysis and ovulation before the final GnRH injecton (54). However, cows on the CIDR-ovsynch were initiated immediately. Pregnancy was diagnosed rectally by both ultrasonography and manual palpation, 40-50 days after AI.

Data collection and statistical analysis

Data were collected on 1,952 cows (609 primiparous and 1,343 multiparous; mean [± SD] parity = 2.3 ± 1.4) from 22 dairy farms. The data set included detailed information on the farm, synchronization protocols, cow parity, and body condition score (BCS) of the cow at the time of treatment, and dates of previous calving, insemination and conception. Table 1 listed independent variables that described farm, synchronization protocols, parity, BCS, and calving to AI interval of the cow, and TAI season. For statistical analyses, cow parity was categorized as primiparous or multiparous cows, and BCS was grouped as ≤ 2.75 (mean [± SD] 2.78 ± 0.14; ranges of 2.25-2.75) and ≥ 3.0 (3.22 ± 0.14; ranges of 3.00-4.50). Calving to AI interval was categorized as ≤ 150 days (101.2 ± 25.0; ranges of 50-150 days) and > 150 days (228.7 ± 60.3; ranges of 151-364 days), whereas AI season was grouped as spring (March to May), summer (June to August), fall (September to November) and winter (December to February). The probability for pregnancy was analyzed by logistic regression using the LOGISTIC procedure in SAS (version 9.2, SAS Institute Inc., Cary, NC, USA). The logistic regression model included farm, synchronization protocols, cow parity, BCS, calving to AI interval, AI season, and the interactions between these variables. Backward stepwise regression was used in all models, and elimination was performed based on the Wald statistic criterion when $p > 0.11$. The odds ratio

Table 1. Logistic analysis of independent variables to determine the risk factors for the pregnancy following synchronization protocols in dairy cows

Variables	Level	No. cows	
		Pregnancy	Non-pregnancy
Farm	A	27	76
	B	59	87
	C	22	66
	D	36	21
	E	24	62
	F	54	95
	G	34	66
	H	22	29
	I	43	70
	J	24	36
	K	25	36
	L	24	46
	M	20	50
	N	24	52
	O	25	17
	P	27	57
	Q	48	76
	R	37	102
	S	28	55
	T	37	61
	U	32	51
	V	26	43
Synchronization protocols[1]	PG+EB	400	888
	Ovsynch	187	226
	CIDR-ovsynch	111	140
Cow parity	Primiparous	248	361
	Multiparous	450	893
BCS	≤ 2.75	295	646
	≥ 3.00	403	608
Calving to AI interval (days)	≤ 150	403	786
	> 150	295	468
Timed AI season	Spring	215	359
	Summer	140	319
	Autumn	158	292
	Winter	185	284

[1]PG+EB: 500 μg of the $PGF_{2\alpha}$ analogue cloprostenol followed by 1 mg estradiol benzoate 24 h, and timed AI (TAI) 24 h after the EB injection; Ovsynch: 100 μg gonadorelin, 500 μg of cloprostenol 7 d after injection of gonadorelin, a second dose of 100 μg gonadorelin 56 h after the injection of cloprostenol, and TAI at 16 h after the second injection of gonadorelin; CIDR-ovsynch: the same as Ovsynch, plus an internal drug-release device containing 1.9 g of progesterone (CIDR™) between the first GnRH and cloprostenol injections.

(OR) and 95% confidence interval (CI) were computed by logistic regression. Results were presented as proportions and OR with their respective 95% CIs.

Pregnancy rates according to the presence or absence of a CL in each Ovsynch and CIDR-ovsynch protocols were compared by chi-square analysis.

Differences with $p \leq 0.05$ were considered significant, and $0.05 < p \leq 0.1$ were designated as a tendency toward a significant difference.

Results

The final model revealed that farm ($p = 0.005$), cow parity ($p = 0.0001$), BCS ($p < 0.005$), and AI season ($p < 0.05$) significantly affected, and calving to AI interval tended to affect ($p < 0.1$) the probability for pregnancy (Table 2). Although synchronization protocols did not affect the probability for pregnancy ($p > 0.05$), cow parity and synchronization protocols had a significant interaction ($p < 0.005$). The OR for pregnancy (0.60) was significantly lower ($p < 0.0001$) for multiparous cows compared to primiparous cows using PG+EB; whereas the OR (1.44) tended to be higher ($p < 0.1$) for multiparous cows compared to primiparous cows using the Ovsynch; and the probability for pregnancy did not differ between multiparous and primiparous cows using the CIDR-ovsynch ($p > 0.05$). Pregnancy was more likely on 8 farms (B, D, F, H, I, J, K, and O) by 1.73, 4.02, 1.75, 2.13, 1.81, 2.01, 2.29 and 3.48-fold compared with farm A. The OR (0.78) was lower for multiparous cows compared with primiparous cows, whereas cows with BCS ≥ 3.00 were more likely to be pregnant (OR: 1.41) compared with cows having BCS ≤ 2.75. Cows with calving to AI interval > 150 days were more likely to be pregnant (OR: 1.20) compared with cows having calving to AI interval ≤ 150 days, whereas the OR (0.73) was lower for cows inseminated during summer compared with cows inseminated during spring. Pregnancy rates according to the presence or absence in each Ovsynch and CIDR-ovsynch protocols did not differ ($p > 0.05$), respectively (Table 3).

Discussion

The logistic analysis revealed that farm, cow parity, BCS, calving to AI interval, and AI season affected the probability

Table 2. Odds ratio (OR) and variables included in the final logistic regression model for the probability of pregnancy following synchronization protocols in dairy cows

Variable	OR	95% CI	*p*-value	Variable	OR	95% CI	*p*-value
Farm			0.005	Cow parity			
A	Reference			Primiparous	Reference		
B	1.73	0.984-3.030	< 0.1	Multiparous	0.78	0.637-0.965	0.0001
C	0.79	0.041-1.539	> 0.05	BCS			
D	4.02	1.940-8.314	< 0.0005	≤ 2.75	Reference		
E	0.99	0.510-1.917	> 0.05	≥ 3.00	1.41	1.142-1.730	< 0.005
F	1.75	0.992-3.086	< 0.1	Calving to AI interval (days)			
G	1.33	0.715-2.470	> 0.05	≤ 150	Reference		
H	2.13	1.031-4.390	< 0.05	> 150	1.20	0.983-1.468	< 0.1
I	1.81	0.997-3.279	< 0.1	Timed AI season			< 0.05
J	2.01	1.001-4.027	< 0.05	Spring	Reference		
K	2.29	1.146-4.559	< 0.05	Summer	0.73	0.558-0.962	< 0.05
L	1.31	0.662-2.580	> 0.05	Autumn	0.93	0.704-1.217	> 0.05
M	0.97	0.482-1.950	> 0.05	Winter	1.10	0.850-1.435	> 0.05
N	1.07	0.548-2.094	> 0.05	Cow parity*synchronization protocol[1]			< 0.005
O	3.48	1.546-7.817	< 0.005	PG+EB			
P	1.21	0.630-2.309	> 0.05	Primiparous	Reference		
Q	1.42	0.790-2.546	> 0.05	Multiparous	0.60	0.465-0.783	< 0.0001
R	0.97	0.537-1.754	> 0.05	Ovsynch			
S	1.37	0.713-2.622	> 0.05	Primiparous	Reference		
T	1.54	0.835-2.849	> 0.05	Multiparous	1.44	0.945-2.205	< 0.1
U	1.19	0.593-2.394	> 0.05	CIDR-Ovsynch			
V	1.66	0.849-3.260	> 0.05	Primiparous	Reference		
				Multiparous	0.89	0.511-1.562	> 0.05

[1]PG+EB: 500 μg of the $PGF_{2\alpha}$ analogue cloprostenol followed by 1 mg estradiol benzoate 24 h, and timed AI (TAI) 24 h after the EB injection; Ovsynch: 100 μg gonadorelin, 500 μg of cloprostenol 7 d after injection of gonadorelin, a second dose of 100 μg gonadorelin 56 h after the injection of cloprostenol, and TAI at 16 h after the second injection of gonadorelin; CIDR-ovsynch: the same as Ovsynch, plus an internal drug-release device containing 1.9 g of progesterone (CIDR™) between the first GnRH and cloprostenol injections.

Table 3. Pregnancy rates according to the presence or absence of a CL in the Ovsynch and CIDR-ovsynch protocols

Synchronization protocols[1]	CL presence	No. Timed AI	No. pregnant	Pregnancy rate (%)
Ovsynch[2]	Yes	170	75	44.1
	No	243	112	46.1
CIDR-Ovsynch	Yes	98	49	50.0
	No	153	62	40.5

[1]Ovsynch: 100 μg gonadorelin, 500 μg of cloprostenol 7 d after injection of gonadorelin, a second dose of 100 μg gonadorelin 56 h after the injection of cloprostenol, and TAI at 16 h after the second injection of gonadorelin; CIDR-ovsynch: the same as Ovsynch, plus an internal drug-release device containing 1.9 g of progesterone (CIDR[TM]) between the first GnRH and cloprostenol injections.
[2]Cows without a CL, which were presumably regarded as in metestrous or late diestrous phases, received the initial treatment of Ovsynch 5 days later.
* Pregnancy rates according to the presence or absence in each Ovsynch and CIDR-ovsynch protocols did not differ ($p > 0.05$), respectively.

for pregnancy following synchronization protocols in dairy cows. Moreover, although synchronization protocols did not affect the probability for pregnancy, there was a significant interaction between cow parity and synchronization protocols; the OR for pregnancy was lower for multiparous cows compared with primiparous cows using the PG+EB protocol. This might suggest that farm management, environment, weather, and cow nutrition and reproductive physiology might be associated with the reproductive outcomes following synchronization protocols.

High variation in the probability of pregnancy among farms in the present study were consistent with previous publications (18,27,47,56), which may be associated with different farm management practices, environment, and facilities, including nutritional control and cow population, or barn conditions.

BCS has been used worldwide as an efficient tool to measure energy balance and nutritional status in dairy herds (5,52). Our observation that lower BCS (≤ 2.75 *vs.* ≥ 3.00) at TAI decreased the probability for pregnancy was consistent with previous studies (24,37,47). It is assumed that lower BCS reflects poor nutritional status, leading to an increased likelihood of delayed resumption of cyclicity during lactations (8, 44,47), which might result in negative reproductive outcomes. A higher probability for pregnancy in cows with longer calving to AI interval, compared to those with shorter calving to AI interval observed in the present study might be because cows with earlier initiation of a synchronization protocol would have delayed involution of reproductive organs (anestrus) and/or negative energy balance. Our findings were supported by previous studies, in which the conception rate increased as calving to AI interval became longer (21,24).

We showed that cows who received TAI during summer had a lower OR for pregnancy compared to those received TAI during spring, consistent with several previous reports (2,7,17,47). The negative effects of heat stress on reproductive performance in dairy cows might be associated with endocrine imbalance, compromised folliculogenesis, ovulation failure, and damage to oocyte (1,10,20,29,31). The effects of heat stress in dairy cows was *via* not only immediate decreases in plasma inhibin and consequent increases in FSH, but also the delayed effect by marked increase in FSH secretion and a reduction in the number of medium size follicles (45). Another study demonstrated that heat stress resulted in delayed embryo cleavage and lower expression of the POU5F1 gene, which is essential for the maintenance of totipotency/pluripotency in embryonic stem cells and primordial germ cells (19).

The lower OR for pregnancy in multiparous cows compared to primiparous cows was reported in many previous studies (2,18,41,47). We found a significant interaction between cow parity and synchronization protocols; multiparous cows showed a significantly lower OR for pregnancy compared with primiparous cows using PG+EB protocol, which was consistent with a recent study (14). Another study additionally supported our results where primiparous cows who received estradiol had higher pregnancy rates than multiparous cows who received estradiol (49). Moreover, it was found that treatment with estradiol did not improve pregnancy rate in multiparous cows at any follicle size, and even tended to decrease the pregnancy rate in multiparous cows ovulating large follicles, whereas primiparous cows receiving estradiol treatment had high pregnancy rate at all follicular sizes (49). The potential mechanisms of the positive effects of estradiol treatment on fertility in primiparous *vs.* multiparous cows are as yet unclear, and requires further investigation. However, the OR for pregnancy tended to be higher for multiparous cows *vs.* primiparous cows using the Ovsynch, and the probability for pregnancy did not differ between multiparous and primiparous cows using the CIDR-ovsynch in the present study. Our findings corroborated a previous study where the pregnancy rate at 1st service rate was higher for cows with parities ≥ 3, than those with lower parities (24). Another study demonstrated that there was no effect of cow parity on the pregnancy rate following Ovsynch (38), which was inconsistent with our results. We were unable to clarify the reasons for the discrepancy among the studies. However, the proportion of ovulation of follicles and/or progesterone concentrations during synchronization protocols might be associated with the outcomes of reproductive performance. The emergence of new wave using the Ovsynch or CIDR-ovsynch protocols might improve the probability of pregnancy in multiparous cows. Therefore, Ovsynch or CIDR-ovsynch might be preferable for multiparous cows rather than the PG+EB protocol.

Cows enrolled in TAI protocols during late diestrus are likely to undergo spontaneous luteolysis and ovulate before the final GnRH injecton is administered (54). Moreover, the 5th to 9th day of the estrous cycle is the preferable period for the initiation of the Ovsynch regimen (3,54). Likewise, cows with no CL at the onset of synchronization protocol, had higher synchronization rates when treated with CIDR-ovsynch, as compared with Ovsynch, possibly due to the reduced incidence of premature luteal regression (24). Moreover, first-service conception rate was greater in cows having a CL before the synchronization protocol than in those without a CL (34). Cows without a CL were regarded as in metestrous or late diestrous, and were initiated on Ovsynch protocol 5 days after ultrasonography, in the present study. Consequently, the similar pregnancy rates in cows using Ovsynch regardless of the presence or absence of a CL, supports the hypothesis that postponed initiation of Ovsynch in cows without a CL may increase the ovulation rate of a dominant follicle after the 1st GnRH, and prevent premature estrus or ovulation before the final GnRH. The advantage of postponed initiation of Ovsynch for cows without a CL, who are presumably in metestrous or late diestrous, might be effectively applied to field conditions in dairy herds. On the other hand, the non-significant effect on the probability for pregnancy in CIDR-ovsynch treated-cows regardless of the presence or absence of a CL was similar to a previous study (4).

Taken together, our results indicated that careful consideration of farm management including nutrition (BCS), calving to AI interval (not early time point), strategies to reduce heat stress during summer, such as use of fans and sprinklers, might be needed to increase the probability for pregnancy following synchronization protocols in dairy herds. Additionally, the Ovsynch or CIDR-ovsynch protocol is preferable in multiparous cows than the PG+EB protocol.

Acknowledgments

This work was carried out with the support of the "Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ008464)" Rural Development Administration, Republic of Korea.

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젖소에서 배란동기화 프로그램 적용 후 임신율에 영향을 미치는 요인 분석 연구

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요 약 : 본 연구는 젖소에서 배란동기화 프로그램 적용 후 임신율에 영향을 미치는 요인을 분석하기 위하여 실시하였다. 분석에 포함된 자료는 젖소 목장 22개소내 사육 젖소 1,952두에 정시 인공수정을 위하여 실시한 배란동기화 프로그램(PG+EB, Ovsynch, CIDR-ovsynch), 젖소의 산차, 신체상태지수(BCS), 전 산차 분만일, 인공 수정일 및 임신 진단 결과를 포함하였으며, 통계분석은 SAS 로지스틱 회기분석을 이용하였다. 분석 결과, 목장($p = 0.005$), 산차($p = 0.0001$), BCS($p < 0.005$) 및 인공수정 계절($p < 0.05$)이 유의적으로 임신율에 영향을 미쳤으며, 공태기간은 경향성을 나타내었다($p < 0.1$). 배란동기화 프로그램에 따른 임신율의 유의적인 차이는 인정되지 않았으나, 산차와 배란동기화 프로그램 사이에 교호관계가 성립되었다($p < 0.005$). PG+EB 프로그램을 사용 시에는 초산 차에 비해 2산 이상의 경산우에서 임신율의 감소(odds ratio [OR]: 0.60, $p < 0.0001$)가 있었으나, Ovsynch 프로그램을 사용 시에는 초산 차에 비해 2산 이상의 경산우에서 임신율이 증가하는 경향이 있었으며(OR: 1.44, $p < 0.1$), CIDR-ovsynch 프로그램을 사용 시에는 초산차와 2산 이상의 경산우에서 임신율의 차이가 없었다($p > 0.05$). BCS가 3.00을 이상일 때가 2.75 이하일 때 보다 1.41배 임신 가능성이 증가되었으나, 인공수정을 여름에 실시할 때가 봄에 실시할 때에 비해 임신 가능성이 0.73배로 감소되었다. 인공 수정 시 공태기간이 150일을 경과 할 때가 150일 미만일 때 보다 임신 가능성이 1.20배 증가 경향이 있었다. 결론적으로, 젖소에서 배란동기화 프로그램 적용 후, 목장, 젖소의 산차, BCS, 공태기간 및 인공수정 계절이 임신율에 영향을 미치는 중요한 요인이었다. 또한 배란동기화 프로그램과 산차 사이 교호관계가 성립되었으며, PG+EB 프로그램 사용 시 초산 차에 비해 2산 이상의 경산우에서 임신율의 감소가 있었다.

주요어 : 젖소, 배란동기화 프로그램, 임신율, 위험요인