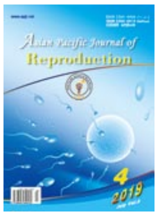

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Pregnancy outcome of lactating dairy cows assigned for Presynch-Ovsynch synchronization program and inseminated either at detected standing heat or at scheduled fixed time

Abdel-Tawab Abdel-Razek Yassyn Khalil✉, Mahmoud Mohamed Hussein

Theriogenology Department, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef, Egypt

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ABSTRACT

Objective: To determine if insemination at standing heat results in a similar or higher pregnancy rate compared with fixed time artificial insemination, and to study some factors affecting the pregnancy rate.

Methods: A total of 8 944 inseminations were included in this study, from which 6 823 inseminations were done in Holstein cows and 2 121 inseminations were performed in Simmental cows. All cows were subjected to a Presynch-Ovsynch protocol. Cows detected in estrus (n=7 424) were artificially inseminated, whereas cows not observed in estrus (n=1 520) were submitted to fixed time artificial insemination.

Results: The overall pregnancy rate of cows inseminated on the basis of the detected standing heat was comparable to that recorded for cows receiving fixed time artificial insemination. A higher pregnancy rate was recorded for cows during cold months than that recorded during hot months (P=0.000). A higher pregnancy rate was recorded for Simmental compared with that recorded for Holstein cows (P=0.001). Regarding parity, significant differences in the pregnancy rate were detected between primiparous and multiparous cows (P=0.040). In addition, artificial insemination technicians had no sigificant effect on pregnancy rate (P>0.05). Meanwhile, the used artificial insemination sires significantly (P=0.000) impacted the pregnancy rate.

Conclusions: Insemination of cows detected in standing heat prior to predetermined fixed time results in similar pregnancy outcome and decreases days to the first service compared with insemination at the scheduled fixed time at the end of the Presynch-Ovsynch synchronization program. The overall pregnancy outcome is not affected by the breeding program, but it is highly impacted by cow's breed, parity, artificial insemination sire and breeding season.

1. Introduction

The reproductive performance exerts a highly significant influence on the dairy herd profitability[1]. In commercial dairy industries, selection of high-yield cows mostly results in diminished fertility[2]. Decline in conception rate and increase in calving interval over the last decades have been assured[3]. Synchronization protocols have been well developed and widely adopted in the dairy industry[4] to eliminate the need for estrus detection and to improve fertility[5]. The Ovsynch protocol has been developed primarily to increase submission rate, not pregnancy rate[6], as the conception rate of artificial insemination (AI) after Ovsynch is usually within the range recorded for cows inseminated after an observed heat[7].

✉Corresponding author: Abdel-Tawab Abdel-Razek Yassyn Khalil, Theriogenology Department, Faculty of Veterinary Medicine, Beni-Suef University, 62511 Beni-Suef, Egypt.
E-mail: yassyn999@yahoo.com; abdeltawab.yaseeen@vet.bsu.edu.eg

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Therefore, Presynch-Ovsynch (PO) was developed[8] to increase the fertility rate[9]. The PO program is intended to ensure that cows are at the most appropriate stage of the estrous cycle at the time of the first gonadotropin-releasing hormone (GnRH) of Ovsynch[4]. The PO protocol introduces some flexibility in allowing insemination on estrus detection besides fixed time artificial insemination (FTAI)[10]. Because of the wide variations in factors that affect reproductive efficiency of a given program such as the stage of lactation[11], season[12], body condition score[13], parity[14], cyclicity status[15] and stage of estrous cycle[16], treatment comparisons are needed to determine the efficacy of the insemination strategies that combine detection of estrus and FTAI. Therefore, the objectives of this study were to determine the pregnancy rate following FTAI compared with cows inseminated on the basis of detected standing heat and to investigate the influence of some factors (breeding system, breeding season, cow's breed, parity, AI technician and AI sire) on the pregnancy outcome.

2. Materials and methods

2.1. Cows, housing and feeding

This retrospective study was performed at a commercial dairy farm in Southwestern Egyptian desert area. Cows were reared up in an open partially shaded yard system with 11 meters shaded area of totally 55 square meters per cow. Coral cooling and evaporating system was applied during the hot season which extended from May to October. The cows had free access to fresh water and were fed a balanced totally mixed ration 4-5 times daily to meet or exceed the minimum nutrient recommendations for lactating dairy cows[17]. The medications used in this study were not harmful to the cows. Moreover, the handling of cows was performed by well-trained persons under close veterinary supervision in accordance with the guidelines of the Animal Care and Use of the Committee of Research Institute for Faculty of Veterinary Medicine, Cairo University, Egypt (CU-II-F-16-18). This study was also additionally approved by the Ethical Committee for Veterinary Medical Research at the College of Veterinary Medicine, Beni-Suef University.

2.2. Breeding strategy

Cows were automatically enrolled in this study by the on-farm dairy management software (Dairy COMP 305®, Valley Ag, and Tulare, CA). All eligible cows (*n*=8 944) were obligatory subjected to a PO14–12 synchronization protocol. Cows detected in estrus (*n*=7 424) based on combined pedometer activity measure and visual observation were inseminated by professional AI technicians and classed as SHAI group and did not receive any further treatment, whereas cows not observed in heat (*n*=1 520) till the predetermined date of AI were received fixed time artificial insemination and classed as FTAI group.

2.3. PO14–12 synchronization program

All cows that reach to (43±3) days postpartum were obligatory enrolled in the protocol as previously described[8]. Briefly, the cows received two doses of synthetic prostaglandin F2 α (PGF2 α) (500 μg Cloprostenol sodium; 2 mL Estrumate®, MSD; USA) administered 14 days apart. The Ovsynch was initiated 12 days later by an injection of 12 μg GnRH (Busrelin; 3 mL; Receptal®, MSD; USA) followed by injection of synthetic PGF2 α (500 μg Cloprostenol sodium; 2 mL Estrumate®, MSD; USA) 7 days later. After 56 h, cows received the 2nd GnRH dose. After that, cows received FTAI services 16 to 18 h post the 2nd GnRH injection.

2.4. Insemination system and pregnancy diagnosis

Semen from 5 proven AI sires were used randomly during the current study to avoid possible AI sire effects. All AI services were performed by 4 well-trained and experienced AI technicians throughout the study. Pregnancy determination was accomplished on (35±3) days post-insemination for all cows using portable ultrasound provided with a 7.5 MHz trans-rectal linear transducer (Easi-Scan; BCF Technology, Rochester, MN, USA).

2.5. Statistical analyses

The means and standard errors of means (mean±SEM), as well as treatment interactions among breeding system, season, breed, and parity and their effects on pregnancy rate, were analyzed by one-way analysis of covariance through the general linear model, univariate analysis using IBM SPSS statistical software (IBM, SPSS v25). $P<0.05$ was considered significantly different.

3. Results

A total of 8 944 first inseminations were included in the statistical analysis of this retrospective study, from which 7 424 (83.00%) were performed on cows observed in standing estrus prior to the scheduled FTAI and only 1 520 (16.99%) of inseminations were performed at a predetermined fixed time after completion of a PO14–12 breeding program. 6 159 (68.86%) of inseminations were achieved during cold months, while only 2 785 (31.14%) of inseminations were carried out in hot months.

As shown in Table 1, no significant (P=0.622) effects of the breeding system on the pregnancy rate were found, as the overall pregnancy rate for cows inseminated on the basis of SHAI (35.98%) breeding system was comparable to that (36.64%) recorded for cows received FTAI.

Season of insemination exerted a highly significant (P=0.000) effect on the pregnancy rate, as higher pregnancy rate (39.11%) was recorded for cows during cold months than that during hot months (29.41%) as shown in Table 1. Moreover, it was interesting to note that during hot months, the effects of both breed and breeding system on the pregnancy rate were faded, as there were no significant differences (P>0.05) in the pregnancy rate between both Holstein and Simmental cows either assigned to SHAI (28.45% *vs.* 32.43%) or to FTAI (28.77% *vs.* 31.34%) breeding system as depicted in Table 2.

Regarding the cow's breed, 6 823 (76.3%) of the inseminations were performed on Holstein cows and 2 121 (23.7%) of inseminations were conducted on Simmental cows. As depicted in Table 1, the breed of the cow exerted a highly significant (P=0.001) influence on the pregnancy outcome, as a higher pregnancy rate was recorded for Simmental (39.70%) compared with Holstein (34.97%) cows.

Regarding parity of the cows, about 27.40% (2 451 / 8 944) primiprous and 72.60% (6 493 / 8 944) multiparous cows were enrolled in this study. The parity exerted a highly significant (P<0.000 1) influence on the estrus expression rate, as a higher proportion (73.80%; 5 479 / 7 424) of multiparous cows were expressed estrus in response to the presynch double PGF2 α injections compared with primiparous cows (26.20%; 1 945 / 7 424). The data of the current study showed a significant (P=0.040) effect of cow's parity on the pregnancy rate, as a higher pregnancy rate was recorded for primiparous (37.90%) compared with multiparous (35.95%) cows as stated in Table 1. Interestingly, both primiparous Holstein and multiparous Simmental cows become acclimatized to the Coral cooling and evaporating system during hot summer months as they showed pregnancy rates (32.14% & 35.39%) comparable to that noted for multiparous Holstein cows (36.66%) during cold months as depicted in Table 3.

The AI technician had no significant (P>0.05) effect on the pregnancy rate, as no significant differences were detected among the four AI technicians (A: 36.30%, B: 35.90%, C: 37.18% and D: 34.84%; F=2.220 P=0.084). Meanwhile, the used AI sires were highly (P<0.000 1) impacted the pregnancy rate, as significant differences were observed among the five used AI sires (A: 35.80%, B: 45.47%, C: 39.56%, D: 28.29 % and E: 33.63%; F=8.640, P=0.000).

Table 1. Factors affecting pregnancy rate of lactating dairy cows.

Variables		*n*	Pregnancy rate	*P* value	95% confidence interval	
					Lower bound	Upper bound
Breeding system	SHAI	7 424	35.98 ± 0.56	0.622	34.88	37.07
	FTAI	1 520	36.64 ± 1.23		34.23	39.06
Season	Cold	6 159	39.11 ± 0.62	0.000	37.92	40.31
	Hot	2 785	29.41 ± 0.86		27.63	31.18
Breed	Holstein	6 823	34.97 ± 0.58	0.001	33.83	36.11
	Simmental	2 121	39.70 ± 1.04		37.66	41.74
Parity	Primiparous	2 451	37.90 ± 0.99	0.040	35.75	39.63
	Multiparous	6 493	35.95 ± 0.60		34.12	36.49

Data are expresed as mean±SEM. SHAI: Cows detected in standing heat and artificially inseminated; FTAI: Fixed time artificially inseminated cows.

Table 2. Effect of breeding system on pregnancy rate in dairy cows with possible breed-season interactions.

Breeding system	Breed	Season	*n*	Pregnancy rate	95% confidence interval	
					Lower bound	Upper bound
SHAI	Holstein	Cold	3 783	38.01 ± 0.79^{c}	36.49	39.53
		Hot	1 768	28.45 ± 1.07^{a}	26.22	30.68
	Simmental	Cold	1 281	42.00 ± 1.38^{b}	39.38	44.61
		Hot	592	32.43 ± 1.93^{a}	28.58	36.28
FTAI	Holstein	Cold	914	37.42 ± 1.60^{c}	34.32	40.52
		Hot	358	28.77 ± 2.40^{a}	23.82	33.72
	Simmental	Cold	181	50.28 ± 3.73^{d}	43.31	57.24
		Hot	67	31.34 ± 5.71^{a}	19.90	42.79

Data are expresed as mean±SEM. Values with different superscript letters in the same column are significantly (P<0.05) different.
SHAI: Cows detected in standing heat and artificially inseminated; FTAI: Fixed time artificially inseminated cows.

Table 3. Effect of parity on pregnancy rate in lactating dairy cows with possible breed-season interactions.

Parity	Breed	Season	*n*	Pregnancy rate	95% confidence interval	
					Lower bound	Upper bound
Primiparous	Holstein	Cold	1 554	41.12 ± 1.25^{d}	38.75	43.49
		Hot	585	32.14 ± 1.93^{b}	28.27	36.00
	Simmental	Cold	156	42.86 ± 3.67^{d}	30.37	55.35
		Hot	156	22.44 ± 3.35^{a}	14.95	29.92
Multiparous	Holstein	Cold	3 143	36.66 ± 0.82^{c}	35.06	38.26
		Hot	1 541	27.06 ± 1.13^{a}	24.68	29.44
	Simmental	Cold	1 306	44.91 ± 1.32^{d}	41.68	48.14
		Hot	503	35.39 ± 2.13^{bc}	31.22	39.55

Data are expresed as mean±SEM. Values with superscript letters in the same column are significantly (P<0.05) different.

4. Discussion

Insemination based on heat detection remains to be an important part of most reproductive management programs on many dairy farms[4]. In proficiently managed dairy herds with efficient estrus detection strategy, the proportion of cows inseminated at detected estrus after Presynch is ~50%; however, it could range between 20% to 70%[10]. The results of the present investigation showed a higher proportion (83%) of cows that received AI on the basis of standing heat, while only 17% of the cows received FTAI services. Nearly the same pattern was reported by Mendonça *et al*[18] who stated that approximately 70% of cows were inseminated before initiating the FTAI synchronization program. Cows in the current study were housed in the free yard, which may have a positive impact on estrus expression. Method of estrus detection is another important factor to be considered when comparing results from studies that evaluated insemination strategies focusing on estrus detection.

It has been reported that induction of ovulation without expression of heat signs may lead to reduced fertility[19] as a result of insufficient estrogen concentrations that desired to accurately create an ideal uterine environment necessary for both gametes and concepts transport and survival[20]. Moreover, it has been mentioned that cows observed in estrus at timed artificial insemination have higher pregnancy rate than cows that did not show heat signs[21]. The high proportion of cyclic cows responded to the Presynch in the current study were supported by previous conclusions that approximately 10%-25% of dairy cows are anovulatory and have lower conception rate than cyclic cows[8].

The results of the current study revealed no significant (P=0.622) difference between cows submitted to SHAI breeding system and cows received FTAI. There are contradictory reports on the effects of insemination at detected estrus on the pregnancy rate. Some researchers[22] supported the results of this study, as they have found no significant variations in the pregnancy rate between cows receiving FTAI and cows inseminated on the basis of the detected estrus breeding system. Other researchers[19] have stated higher pregnancy rate following inseminations based on detected heat compared with that following FTAI. However, others reported more pregnancy rate for cows assigned for FTAI[23,10].

A significant (P=0.040) difference in the pregnancy rate between primiparous and multiparous cows was observed. These results came in agreement with others[24,10] who stated that primiparous cows recorded greater pregnancy rate (P<0.001) than multiparous cows. In the same trend, Giordano *et al*[25] reported that pregnancy rate was greater for primiparous (41.5%) than multiparous cows (33.6%). In contrast to our results, Cerri *et al*[21] reported that pregnancy rate was higher for multiparous (42.2%) than for primiparous (34.4%) cows receiving FTAI. Another researcher[23] reported no effect of parity on pregnancy rate using the same PO14-12 with inseminations performed at detected estrus. The lower pregnancy rate in multiparous cows may be attributed to the fact that older cows produce more milk, which may give an explanation of some reduction in pregnancy rate. An inverse relationship between milk yield and reproductive performance has been documented[26].

The season of insemination exerted a highly significant (P=0.000) effect on the pregnancy rate, as a higher pregnancy rate was recorded during cold months than that recorded during hot months. The obtained pregnancy rate during hot months in the current study is comparable to that recorded by Ahmadi and Ghaisari[27] for dairy cows in Iran (27.70%), and more than that obtained by Ahuja *et al*[28] for lactating anoestrus crossbred cows (20.83%), but less than that recorded by Alnimer *et al*[29] for Friesian cows (56.30%). It has been stated that cows exposed to heat stress recorded significantly (P<0.001) lower conception rate (23%) compared with that recorded for cows (31.3%) not exposed to heat stress[30]. The decreased pregnancy rates during hot months may be ascribed to inadequate dry matter consumption and poor body condition score and consequently negative energy balance that may lead to disturbances in the secretion of hormones needed for sound reproduction[31].

The harmful effect of heat stress is concomitant with a small diameter preovulatory follicles and inconstant biochemistry of the follicular fluid[32], which may lead to ovulation of poor quality oocytes[33]. Hyperthermia of fertilized oocytes may inhibit embryonic growth[34]. Moreover, heat stress maybe resulted in delayed ovulation, anovulation, ovulation of a poor quality oocyte, leading to diminished fertilization rate and high embryonic loss[35]. Furthermore, hyperthermia after breeding increased uterine temperature and diminished the uterine blood flow which may lead to failure of implantation and consequently increased early embryonic death[36].

The breed of the cow exerted a highly significant (P=0.001) impact on the pregnancy rate, as a higher pregnancy rate was recorded for Simmental cows compared with that recorded for Holstein cows. The high pregnancy rate recorded for Simmental cows might be attributed to both the lower milk yield and better body condition score compared with Holstein cows. Moreira *et al*[13] reported that conception rate was lower for cows with lower body condition score. Moreover, Santos *et al*[37] reported that cows with moderate body condition score at the time of AI had greater (P<0.001) conception rate rates than cows with low body condition (49% *vs.* 34%). Cows in a state of negative energy balance often mobilize body fat, resulting in high concentrations of non-esterified fatty acids in circulation[38].

The results of the current study concurred well with Piccand *et al*[39] who stated that reproductive efficiency of Simmental cows was the best among the 4 breeds and attributed this good result to the good body condition score of this breed. Moreover, Toledo-Alvarado *et al*[40] concluded that Simmental cows have a greater reproductive potential than Holstein cows. In addition, the crossbreed between Simmental and Holstein cows improved fertility traits[41]. It has been well documented that there is a close relationship among body condition score, negative energy balance, and fertility[42]. Negative energy balance is recognized by the destruction of body fat reserve and higher levels of ketone bodies and non-esterified fatty acids that exert deleterious effects on both oocytes and corpus luteum functions. These results in low uterine progesterone concentration and hostile uterine conditions are unsuitable for the embryonic development[38]. Furthermore, it has been mentioned that lactating cows entering a state of negative energy balance had more reproductive problems[43].

Utmost reproductive research has been dedicated to cow fertility, while sire fertility has been mostly ignored. However, it was recognized that bull directly impacts fertility[44]. A significant proportion of reproductive disappointment was found to be attributable to service sire subfertility[45]. Hence, the fertility of AI bulls should not be ignored in breeding programs aimed to improve the reproductive efficiency of dairy cattle. The results of the current study showed a highly significant (P=0.000) impact of AI sire on the pregnancy rate, as specified by the significant differences among the used AI sires. These results came in agreement with previous studies that had shown differences in conception rates among AI sires[46], but disagree with that reported by Rabaglino *et al*[47]. These differences may be attributed to the variations in the semen characteristics, especially those are related to sperm survivability and withstand both freezing and thawing stressors and/or sperm capacitation process.

The results of the present study showed the AI technicians had no effect on the pregnancy rate (P=0.084), as there were no significant differences observed among the AI technicians. This was anticipated because the technicians performing the AI process were good selected and well trained and had highly experience. Our results are consistent with those of Rabaglino *et al*[47] and Giordano *et al*[24], but inconsistent with that of Anzar *et al*[48] who reported conception rates varied significantly (P<0.011) due to AI technician. The differences in study design and relatively low number of inseminations for some inseminators may reflect a greater variability among AI technicians.

In conclusion, insemination of cows observed in standing heat prior to predetermined fixed time results in good pregnancy outcome and decreases interval to the first service, saving efforts and medications compared with insemination at the scheduled FTAI at the end of the PO14–12. Moreover, the overall mean pregnancy outcome was not affected by the breeding program and AI technicians; meanwhile, it is highly impacted by cow's breed, parity, AI sire and breeding season.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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