

Reproductive Performance in Dairy Cows Synchronized with the Ovsynch Protocol at Different Stages of the Estrus Cycle

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ABSTRACT

The objectives of the study were to evaluate reproductive performance in cows synchronized with the Ovsynch protocol at different stages of the estrus cycle as detected by pedometry, and to compare the reproductive performance of cows inseminated by Ovsynch in different stages of the estrus cycle (no observed estrus, 0-4, 5-12, 13-17 and 18-24 days after estrus) to cows inseminated by pedometry in Israel. Timing the beginning of Ovsynch protocol did not affect first artificial insemination (AI) conception rate, proportion of cows empty at 150 days in milk (DIM) or the time from calving to conception. Cows without observed estrus 25 days or more before initiation of the Ovsynch protocol had rate of cows not pregnant by 150 DIM in comparison to the control group (Odds Ratio (OR) = 1.885, $P=0.013$) and longer calving to conception interval than the control group (155.4 days and 135.7 respectively, $P=0.018$). It was concluded that reproductive performance cannot be improved by combination of pedometry and timed artificial insemination (TAI); Ovsynch has low efficacy as a treatment for anestrus. However, other anestrus treatment options should be compared to Ovsynch.

Keywords: Bovine; Reproduction; Ovsynch; Anestrus; Estrus Detection; Reproduction; Timed Artificial Insemination; Pedometry; Ovsynch; Dairy; Anestrus.

INTRODUCTION

Reproductive efficacy is a key element in dairy farm management. A major component in reproductive management is estrus detection and artificial insemination (AI) of the cow at the correct time in the estrus cycle. In Israel, the vast majority of large dairy farms rely mainly on electronic estrus detection by pedometry or activity meters (1, 2). Recently, automated estrus detection aids have become more available and adopted worldwide (3-5). In farms without automated estrus detection, timed AI (TAI) has become widely accepted as a reproductive management tool. The Ovsynch protocol, first described by Pursley *et al.* in 1995 (6), is most widely used either on its own or in combination with pre-synchronization (7-10) or post insemination protocols (11, 12). The Ovsynch

program consists of 2 injections of Gonadotropin-releasing hormone (GnRH), 7 days before, and 48 hours after an injection of Prostaglandin F₂ alpha (PGF_{2α}). Cows are inseminated 16 to 25 hours after the second injection of GnRH (6). The advantages of Ovsynch include the lack of need for estrus detection by visual or by expensive electronic means, high insemination rates, reduced losses due to trauma or mastitis that are estrus related, and flexibility in the number of cows to be synchronized. Additionally, AI at a fixed times allows for on farm concentrated work effort for hormone injections, for AI in a predicted time, and Ovsynch was also described as a treatment for acyclic cows (13-15). It has been reported that cows inseminated using the Ovsynch protocol conceived in comparable rates to the conception rates (CR) of cows

detected in estrus, but with higher pregnancy rates (PR) due to the higher insemination rates of the Ovsynch treated cows (1, 6, 10, 16-18).. The best conception rates of synchronized insemination are achieved when the Ovsynch protocol is initiated at days 5-12 of the estrus cycle. Pre-synchronization protocols evolved to improve conception rates with the aim of the most commonly used pre-synchronization protocol, Presynch, to achieve higher proportions of cows that are 5-12 days post estrus at the beginning of the Ovsynch protocol. This is done by injecting PGF_{2α} twice 14 days apart and initiation of the Ovsynch protocol 11-14 days after the second PGF_{2α} treatment, thus contributing to higher conception rates at TAI (7, 9).

The extensive use of estrus detection systems in Israeli dairy farms makes the sweeping use of TAI protocols redundant. However, cows not detected in heat or repeat breeders are synchronized in some farms. The intelligent use of TAI in cows that are detected in estrus and are not inseminated due to technical reasons (religious considerations – Saturdays and holidays, or other reasons) according to the known estrus date may yield better reproductive performance. Knowledge of the estrus date (recorded by pedometry) could be used as a substitute to the Presynch protocol in order to time the beginning of the Ovsynch protocol to days 5-12 in the estrus cycle.

The objectives of this study were to compare conception rates between cows treated with Ovsynch protocols initiated at different periods of the estrus cycle detected by pedometry and to compare these to conception rates in cows inseminated by pedometry alone. Finally, we attempted to evaluate the efficacy of Ovsynch as a treatment for anestrus.

MATERIALS AND METHODS

Study design and animals

The study was designed as a retrospective cohort study in a sample of cows that were inseminated for the first time in lactation between January 2006 and December 2007. The study was conducted in two commercial Israeli dairy herds consisting of 450 and 550 Israeli Holstein cows. Cows were housed in loose housing systems in large, completely covered open sheds and fed total mixed ration (TMR) *ad libitum*. In both herds, cows were milked three times daily in computer controlled milking parlors. Mean 305 day milk production was approximately 11,500 kg per cow.

All cows were identified by ear tags, electronic identification tags (SAE, Afikim, Israel) and freeze marking. The herds were within the practice area of the Ambulatory Clinic of The Koret School of Veterinary Medicine, which provided a complete herd-health service. Both herds were visited at least three times weekly during the trial period. Clinical, reproduction, production and management data were recorded to the farm management software (“NOA”, Israeli Cattle Breeders Association). Both farms performed monthly milk recordings through the Central Laboratory for Milk Recording. Reproductive management was solely based on AI performed by highly trained technicians employed by the Artificial Insemination Service of the Israel Cattle Breeders Association (ICBA). In both herds, cows were equipped with computerized pedometry systems (SAE, Afikim, Israel). Pregnancy diagnosis was performed by transrectal palpation of the uterus and its content 45-51 days post insemination.

Clinical Examination

All cows were examined routinely between 5 and 12 days after calving by trained veterinarians, who diagnosed, treated and recorded periparturient diseases as described previously (19).

Body condition scoring (BCS scale: 1 = thin, 5 = obese) was recorded before dry-off, at the routine post-partum examination (5-12 days after calving) and between 40 to 60 days after calving (peak yield BCS).

Ovsynch and treatment groups

The Ovsynch group (OS) included cows treated with the Ovsynch protocol. Hormonal synchronization was used on the farms as part of a study evaluating the efficacy of Ovsynch in comparison to pedometry, or for re-synchronization of animals detected in heat during Saturdays or holidays (the farms did not inseminate cows during Saturdays for religious reasons). OS Cows were treated with IM injection of 150 µg Gonadorelin Acetate (Gonabreed, Parnell Laboratories, New Zealand) followed seven days later by IM injection of 500 µg Cloprostenol Sodium (Estrumate, Schering-Plough, Germany), followed two days later with another IM injection of 150 µg Gonadorelin Acetate. OS cows were insemination 16-24 hours after the last GnRH injection regardless of heat signs.

Cows from the OS group were individually matched to the control cows by farm, calving year and parity. When possible, the calving season, calving diseases and calving to first AI interval (voluntary waiting period) of the matched control cow were also similar.

In the second part of the study the cows were divided into six groups:

1. **Control (CO)** – cows inseminated at the detected heat.

2. **Anestrus (AN)** – cows inseminated after treatment with Ovsynch protocol with no detected estrus in the previous 24 days.

3. **Ovsynch at days 0-4 (OS4)** – cows inseminated after treatment with Ovsynch protocol 0-4 days after detected estrus.

4. **Ovsynch at days 5-12 (OS12)** – cows inseminated after treatment with Ovsynch protocol 5-12 days after detected estrus.

5. **Ovsynch at days 13-17 (OS17)** – cows inseminated after treatment with Ovsynch protocol 13-17 days after detected estrus.

6. **Ovsynch at days 18-24 (OS24)** – cows inseminated after treatment with Ovsynch protocol 18-24 days after detected estrus.

Statistical analysis

Computerized data were retrieved from the farm computers and ICBA central computer and analyzed using Excel (version 2010, Microsoft, Redmond, WA) and SPSS 17.0.1 (SPSS Inc., Chicago IL, USA). Lactation Incidence Risk (LIR) for all recorded diseases and reproductive performance parameters were evaluated for Control and Ovsynch cows. The same evaluation was done for cows in the different Ovsynch timing groups.

Reproductive performance parameters included first artificial insemination conception rate (1st AI CR), the interval from calving to pregnancy (Days Open), time from 1st AI to conception (Waste days) and the proportion of cows not pregnant > 150 days after calving (Empty at 150 DIM ie. Days in Milk = Days from Calving).

The effect of Ovsynch on dichotomous outcome variables was evaluated using conditional multivariable logistic regression modeling.

Separate analysis was done for Ovsynch versus Control cow and for Ovsynch timing groups as an independent vari-

able. Various parameters and disease conditions were tested in the models as possible confounders. These included: farm, calving season (May to September = Summer; October to April = Winter), twin calving, retained placenta (RP, retained fetal membranes \geq 24 hrs. post calving), clinical metritis (CM), ketosis, displaced abomasum, body condition loss \geq 0.5 between calving and peak milk yield, mastitis at calving, mammary edema at calving, stillborn calves and induction of calving. Crude bivariate associations of outcome and potential confounding variables with treatment were initially assessed by use of Pearson χ^2 asymptotic 2-sided tests of significance for reproductive dichotomous parameters and student's T test for waiting period and days empty. To build the models, the Control versus Ovsynch timing group variables were forced into the model and subsequent covariates with significance of $P \leq 0.25$ were included in primary multivariate models. The final models were built with entry criteria set at $P < 0.05$ and exit criteria set at $P > 0.10$. For all analysis, values of $P < 0.05$ were considered significant. The Odds Ratios (OR) were calculated for all variables in the model.

The conditional logistic multivariate analysis was done using the Cox regression procedure for dichotomous outcome variables with pairing as strata, and linear regression procedure was used for scale outcome variables. The effects of Ovsynch treatment and its timing in the estrus cycle on days open were evaluated using Kaplan-Meier survival analysis. As the segmentation of the waste days is not equal, there is an association between specific period of times and elevated risk for conception. Also the risk of conception is higher on the first AI and declining later. To compensate for these facts, the effect of the treatment was analyzed using a Proportional Odds model as proposed by Cox.

For all survival analyses, days open were limited to 200 days, cows not pregnant at 200 DIM were considered as empty.

RESULTS

Descriptive statistics

During the study period 267 cows were treated by Ovsynch in the study farms. Control cows were matched and differed from the Ovsynch cows only in waiting days (Table 1).

Reproductive performance

The Ovsynch protocol and the timing of its beginning in the estrus cycle did not affect significantly the 1st AI CR (Table 2).

Table 1: Descriptive statistic of the calving diseases LIR and waiting period of the Ovsynch and Control cows.

Parameter	Ovsynch	Control	Overall	P value
Farm:				
A	197	197	394 (73.8%)	1
B	70	70	140 (26.2%)	1
Parity:				
1	95	95	130 (35.6%)	1
2	61	61	122 (22.8%)	1
3+	111	111	222 (41.6%)	1
Summer Calving's	37.1%	35.2%	36.1%	0.652
RP ¹	13.9%	17.2%	15.5%	0.282
CM ²	37.1%	36.3%	36.7%	0.857
Ketosis	19.1%	16.5%	17.8%	0.428
Waiting period ³	100.7	95.9	98.3	0.007

- 1 – Retained placenta, retained fetal membranes ≥ 24 hrs.
- 2 – CM – Clinical/Puerperal metritis.
- 3 – Waiting period – calving to 1st service interval.

Table 3: Logistic regression model summaries of the relationship between the cyclic status of the cows when Ovsynch is initiated and the Empty at 150 DIM in cows inseminated by pedometry or by Ovsynch.

Variable	β	S.E.	df	Sig.	O.R.	95% CI for O.R	
						Lower	Higher
Group: Control			2	0.047		Reference	
Anestrus Ovsynch ¹	0.634	0.256	1	0.013	1.885	1.141	3.113
Cyclic Ovsynch ²	0.170	0.209	1	0.416	1.185	.787	1.786
Parity :1			2	<0.001		Reference	
2	-0.045	0.278	1	0.871	1.885	1.141	3.113
3+	0.861	0.232	1	<0.001	1.185	0.787	1.786
Calving Diseases ³	0.353	0.202	1	0.081	1.423	0.957	2.117
Long Waiting period ⁴	1.073	0.222	1	<0.001	2.923	1.890	4.520
Constant	-1.403	0.266	1	<0.001	0.246		

- 1 – Cows inseminated after treatment with Ovsynch protocol with no detected estrus in the previous 24 days.
- 2 – Groups 3-6 combined. The cows had a detected estrus 24 days or less prior to the beginning of the Ovsynch protocol.
- 3 – Retained placenta, clinical metritis and ketosis.
- 4 – Calving to 1st AI interval ≥108 days (higher quartile).

Table 2: Descriptive statistics of different reproductive parameters in the control (Inseminated at estrus) and the different study groups.

	Control	Ovsynch Group*					Over all**
		2	3	4	5	6	
N	267	93	32	98	25	19	267
1 st AI CR ¹	39.7%	35.5%	46.9%	34.7%	40%	31.6%	36.7%
Empty >150 DIM ²	36.7%	54.8%	46.9%	36.7%	48%	31.6%	44.9%
Days empty ³	144.9	169.4	163.1	147.5	161.5	145	158.1
Waste days ⁴	27.1	28.9	33.6	23.5	30.5	24.1	27.3

- * group 2 – Ovsynch protocol started ≥25 days from estrus or no estrus before Ovsynch, group 3 – Ovsynch protocol started 0-4 days after estrus, group 4 – Ovsynch protocol started 5-12 days after estrus, group 5 – Ovsynch protocol started 13-17 days after estrus and group 6 – Ovsynch protocol started – 18-24 days after estrus.
- **Over all – all Ovsynch cows.
- ¹ 1st AI CR – the proportion of cows conceived from 1st artificial insemination.
- ² Empty >150 DIM – the proportion of cows not pregnant > 150 d after calving.
- ³ Days empty – Mean calving to conception interval. Maximum value is 250 days.
- ⁴ Waste days – Mean 1st AI to conception interval.

The control cows Empty at 150 DIM rate was lower than in the Ovsynch cows (36.7% and 44.9 respectively; OR=0.817, P=0.0321, table 2). The timing of the Ovsynch protocol in the estrus cycle affected the probability of groups 1 and 5 to be Empty at 150 DIM.

Only group 1 (Anestrus group) differed from the other Ovsynch groups. For further analysis of cows Empty at 150 DIM, the cyclic Ovsynch groups (3-6) were united. The cyclic status of the cows at the initiation of the Ovsynch protocol affected significantly the Empty at 150 DIM rates. Cows inseminated by Ovsynch protocol without a detected estrous 24 days before the initiation of the protocol had higher Empty at 150 DIM rate in comparison to the control group (OR to be empty at 150 DIM = 1.885, P=0.013, table 3). Cyclic cows inseminated by Ovsynch protocol did not differ from cows inseminated in pedometry detected estrus (Table 3).

Cows inseminated by Ovsynch had longer open periods than control cows (mean days open = 146.1 and 135.7 respectively, P=0.017). This difference was mainly due to the cyclic status of the cows. Cyclic cows inseminated by the Ovsynch protocol did not differ from the control (mean days empty = 141.2, Figure 1), while the anestrus cows had significantly longer empty period (mean days empty = 155.4, Figure 1).

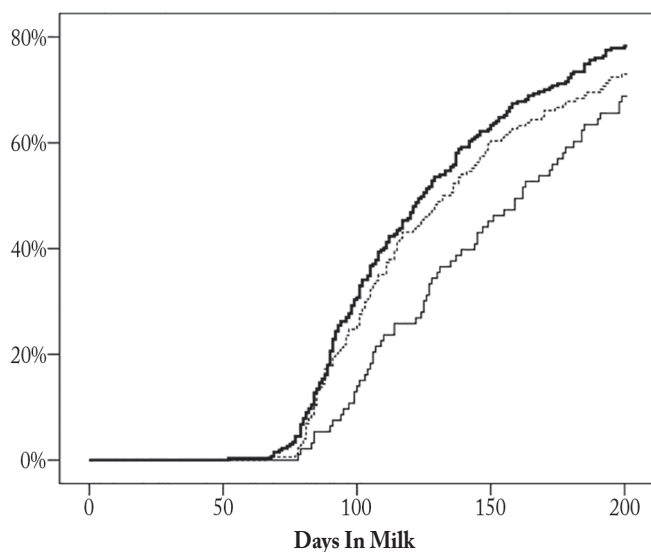


Figure 1: Survival plot of pregnancy rate in Control cows inseminated at estrus detected by pedometry (—), Cyclic cows inseminated by Ovsynch protocol (---) and Anestrus cows inseminated by Ovsynch (—). The mean survival time of the Anestrus cows differed significantly from the Control cows in the Log Rank test ($P=0.018$, mean survival time of 155.4 and 135.7 respectively). The Cyclic cows did not differ from the Control group (mean survival time of 141.2 and 135.7 respectively).

Higher lactation numbers and lower body condition scores at peak yield were associated with reduced pregnancy rates. Post-partum endometritis was associated with reduced pregnancy rates, but the effect declined as DIM increased. Summer calving tended to reduce the pregnancy risk (OR=1.079, $P=0.0607$). The OR to become pregnant of cows inseminated by Ovsynch at 0-4 or 13-17 days after estrus was reduced (OR=0.706, $P<0.0001$ and OR=0.881, $P<0.0001$ respectively).

DISCUSSION

The novelty of this study is in combining the TAI with automated estrus detection systems, and by comparing the conception rate of cows inseminated by Ovsynch protocol started at different times of the estrus cycle. The main finding in this study was that the timing of the beginning of the Ovsynch protocol in the estrus cycle had no effect on 1st AI conception rate. This finding was unexpected as Presynch protocols are widely used for CR improvement (7, 8, 20, 21). The purpose of the Presynch protocols is to lower the proportion of anovular cows (22), and to time the beginning of the Ovsynch protocol to the period of time where maximal reproductive efficacy can be achieved at days 5-12 of the

estrus cycle (20, 21). The start of the Ovsynch protocol at this timing is supposed to ensure the ovulation of smaller follicles at the timed AI, with higher CR (20). Modification of the Ovsynch protocol by adding presynchronization had improved pregnancy rates in previous studies (7, 9, 10, 23). However, the results of this study did not demonstrate advantage (as reflected in the measured reproductive parameter) to the starting of synchronization protocol at days 5-12 of the estrus cycle or to any other different timing. The beneficial effects of presynchronization with prostaglandin might be due to effects on pyometra and subclinical endometritis (24), hence not present in this study.

The anestrus cows that were treated by Ovsynch did not differ significantly from the other study groups in 1st AI CR. The Anestrus cow group was actually composed of two different populations of cows: cyclic cows which the pedometry system failed to detect in estrus and acyclic cows. In some of previous studies, cows with anovular follicles had low CR when inseminated by Ovsynch (13, 25). As the anestrus cows group included the cyclic cows not detected by pedometry system, this sub-population may confound the effect of the acyclic cow population on 1st AI conception.

This confounding effect was limited to the 1st AI, as anestrus cows treated by Ovsynch, had longer calving to conception interval (Figure 1), a fact that can be explained by the continues effect of the acyclic cows sub-population on pregnancy rate of the anestrus cows. The longer calving to conception interval demonstrates a low efficacy of Ovsynch as a treatment for ovarian inactivity as was documented before (13, 26). However, as the control group was composed of cyclic cows detected in estrus by pedometry, the efficacy of Ovsynch as a treatment for ovarian inactivity should be compared to other treatment options.

Over all, cows inseminated based on Ovsynch or by pedometry did not differ in the 1st AI conception rate. This finding is similar to findings in previous studies in Israel (1) and worldwide (5, 6, 10, 17), suggesting that Ovsynch can replace pedometry in farms without pedometers or activity meters, or in farms where management conditions limits the efficacy of estrus detection by electronic aids. Recently, Neves *et al.* (5) found that in north American conditions automated estrus detection systems are at least equal to TAI in the reproduction efficacy, despite the fact that this study was conducted in free stall commercial dairy herds (as opposed to the loose shade Israeli system). They also found

that herd level management may confound the effects of the reproductive management system.

CONCLUSIONS

Timing Ovsynch with estrus detection did not improve the reproductive performance of treated cows. In farms with conditions allowing for high efficacy of estrus detection systems, and where such systems are available, Ovsynch protocols may be redundant as a reproduction management tool for all cows.

The reproductive performance of anestrus cows treated by Ovsynch is not comparable to the cyclic cows; however the efficacy of Ovsynch as a treatment of anestrus should be compared to other treatment options.

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