Effect of insemination after estrous detection on pregnancy per artificial insemination and pregnancy loss in a Presynch-Ovsynch protocol: A meta-analysis

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ABSTRACT

Presynchronization of cows with 2 injections of prostaglandin administered 14 d apart (Presynch-Ovsynch) is a widely adopted procedure to increase pregnancy per artificial insemination (P/AI) for the first service. In a Presynch-Ovsynch protocol, 2 different management strategies can be observed. Either all cows receive timed artificial insemination (onlyTAI) or cows detected in estrus after the second PGF_{2α} injection are inseminated and the remaining cows without signs of estrus will be subject to timed AI (EDAI+TAI). A systematic review of the literature was performed with the objective to evaluate the effect of insemination after estrous detection during a Presynch-Ovsynch protocol for the first service on fertility in lactating dairy cows. Two statistical approaches were conducted using either a fixed or a random effects meta-analysis based on the heterogeneity among the experimental groups. Reproductive outcomes of interest were P/AI measured on d 32 (d 28 to 42) and pregnancy loss between d 32 and 60 (d 42 to 74) of gestation. In approach 1, 3 randomized controlled studies including 1,689 cows with the primary objective to evaluate the effect of insemination after estrous detection in a Presynch-Ovsynch protocol were used. The incorporation of insemination after estrous detection decreased the odds of pregnancy by 35% [odds ratio = 0.65; 95% confidence interval (CI) = 0.53–0.80] on d 32 after AI using a fixed effects model. We detected no effect on pregnancy loss on d 60 of gestation (odds ratio = 0.88; 95% CI = 0.55–1.43). There was no heterogeneity among the 3 studies regarding P/AI and pregnancy loss. In approach 2, cows from approach 1 and cohorts from another 17 experimental groups including 8,124 cows submitted to first AI using a Presynch-Ovsynch protocol were used. Information regarding pregnancy loss was available for 5,200 cows. In the random effects model, the overall proportion of P/AI was 30.9% (95% CI = 26.71–35.28; n = 2,400)

and 41.7% (95% CI = 39.76–42.01; n = 7,413) on d 32 after AI for EDAI+TAI and onlyTAI, respectively. The overall proportion of pregnancy loss was 11.7% (95% CI = 6.11–18.8; n = 1,811) and 9.6% (95% CI = 6.37–13.33; n = 3,389) on d 60 after AI for EDAI+TAI and onlyTAI, respectively. We observed substantial heterogeneity among the experimental groups regarding P/AI and pregnancy loss. In summary, there was a benefit on P/AI for cows with 100% TAI after completing a Presynch-Ovsynch protocol.

Key words: presynchronization, estrous detection, meta-analysis

INTRODUCTION

Synchronization protocols for timed artificial insemination (TAI) have been widely adopted in the dairy industry to improve reproductive performance (Caraviello et al., 2006). Initially, these programs consisted of systematic applications of GnRH and PGF_{2α} with the aim of increasing primarily the insemination risk and not necessarily the risk of conception (Pursley et al., 1995). Therefore, several methods have been evaluated to improve fertility to TAI (Wiltbank and Pursley, 2014). Among them, presynchronization with 2 doses of PGF_{2α} administered 14 d apart has been shown to improve pregnancy per artificial insemination (P/AI) after TAI in several studies (Moreira et al., 2001; El-Zarkouny et al., 2004; Navanukraw et al., 2004). This program, known as “Presynch-Ovsynch” (PO), has become popular. In a survey of reproductive elite herds in the United States, the majority (15/16) implemented PO for the first service (Ferguson and Skidmore, 2013). 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 GnRH treatment for the TAI by ovulating a follicle after GnRH treatment (El-Zarkouny et al., 2004). This was shown to be positively associated with conception rate after TAI (Vasconcelos et al., 1999). While improving P/AI after TAI, the PO protocol also introduces some flexibility in allowing insemination on estrus for herd managers that decide not to time-inseminate every cow (Chebel and Santos,

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2010). Therefore, in a PO protocol, 2 different management strategies can be observed. Either all cows are time-inseminated (**onlyTAI**) or cows detected in estrus after the second PGF_{2α} injection are inseminated and the remaining cows without signs of estrus will be subject to TAI (**EDAI+TAI**; Fricke et al., 2014). Recent studies have shown that approximately 50 to 70% of cows presynchronized with 2 injections of PGF_{2α} were inseminated based on estrous detection before the completion of the TAI protocol (Stevenson and Phatak, 2005; Chebel and Santos, 2010; Fricke et al., 2014). There are conflicting reports on the effects of estrous detection in TAI protocols on P/AI. Some authors showed a positive effect on P/AI for cows with onlyTAI compared with EDAI+TAI (Gumen et al., 2012; Fricke et al., 2014), whereas others did not (Stevenson and Phatak, 2005; Chebel and Santos, 2010). Additionally, there was no economic difference between onlyTAI and EDAI+TAI (Chebel and Santos, 2010; Fricke et al., 2014). The latter was due to a tradeoff between DIM at first service and P/AI. It was assumed, however, that maximum P/AI can be achieved with onlyTAI in a PO protocol (Fricke et al., 2014).

Individual studies often encompass a limited number of herds with similar management practices. Therefore, this meta-analysis was designed to evaluate the effects of insemination after estrous detection during a PO protocol across different managerial conditions. The main hypothesis of the present study is that the maximum effect on P/AI in a PO protocol for the first service would be achieved by neglecting estrous detection after the second PGF injection.

MATERIALS AND METHODS

Literature Search

The literature search was conducted in PubMed (<http://www.ncbi.nlm.nih.gov/pubmed>), ScienceDirect (<http://www.sciencedirect.com>), and Google Scholar (<http://scholar.google.com>) using the search terms “dairy cow AND presynchronization.” A total of 59, 230, and 1,580 results were obtained in each search engine, respectively. Additional manuscripts were obtained directly from researchers in the field of reproductive biology. Results from the online search and personal communications were assessed individually for the initial screening to be considered for the meta-analysis.

Inclusion and Exclusion Criteria

We considered only randomized studies using a presynchronization for the first service with 2 applications of PGF_{2α} 14 d apart followed by an Ovsynch protocol

for TAI as described elsewhere (Pursley et al., 1995) with minor modifications. The time interval between the PGF_{2α} injection and the GnRH injection in the Ovsynch protocol had to range from 48 to 56 h. The time interval from the last GnRH injection in the Ovsynch protocol until timed AI had to range from 12 to 20 h. Hence, studies using another protocol for timed AI (e.g., CoSynch72, 5d-CoSynch) were not included. The time interval between the second injection of PGF_{2α} and the first GnRH injection from the Ovsynch protocol ranged from 9 to 14 d. We identified 3 randomized controlled studies directly evaluating the effect of AI following estrous detection in a PO protocol (Chebel and Santos, 2010; Gumen et al., 2012; Fricke et al., 2014).

Based on these criteria, the meta-analysis included a total of 20 articles including 9,813 cows presynchronized using a PO protocol for first AI. Of these, 17 manuscripts described studies conducted with high-producing cows under confinement housing in the United States, 1 in Canada, 1 in Turkey, and 1 in Mexico.

Data Extraction

Data extraction was performed by a single investigator (S. Borchardt) and validated by a coauthor (W. Heuwieser). For each study, recorded information included authors, year of publication, number of herds, sample size calculation, stratification of results by parity, PGF_{2α} product and dose, DIM at first PGF_{2α} injection, time interval between second PGF_{2α} injection and first GnRH injection of the Ovsynch protocol, time schedule of the Ovsynch protocol, DIM at TAI, insemination of cows in estrus during the timed AI protocol, and time of pregnancy examination (Tables 1 and 2).

Experimental groups were classified regarding their management of first service. The EDAI+TAI group consisted of experimental groups, where cows with estrus symptoms after the second PGF injection were inseminated and the remaining cows showing no estrus symptoms were time-inseminated. For these experimental groups, the voluntary waiting period was set at the same day as the second PGF_{2α} injection. The onlyTAI group consisted of experimental groups, where cows were only inseminated on the day of timed AI. The voluntary waiting period ended at the same day when timed AI was performed.

Pregnancy status was evaluated twice after AI, except for 7 manuscripts. The ranges for the first (PD1) and second pregnancy diagnoses (PD2) were 28 to 42 and 42 to 74 d after AI, respectively. For simplicity, the time at which pregnancy was evaluated for the first and second time will be referred to as d 32 and d 60 after AI throughout the manuscript, respectively. Reproductive

Table 1. Summary of manuscripts (n = 20) using a Presynch-Ovsynch protocol for the first AI considering evaluated information criteria

Reference	Country	Milk yield, kg ¹	No. of AI	No. of herds	Random	Sample size calculated ²	Parity considered	Pregnancy loss calculated
Bicalho et al., 2007	USA	NA ³	399	5	Yes	No	No	No
Bruno et al., 2013	USA	38	552	1	Yes	Yes	No	Yes
Brusveen et al., 2008	USA	NA	115	1	Yes	No	No	Yes
Chebel and Santos, 2010	USA	12,035	639	1	Yes	Yes	No	Yes
Chebel et al., 2006	USA	35/48	338	1	Yes	Yes	No	Yes
Colazo et al., 2013	Canada	10,370	241	1	Yes	No	Yes	Yes
El-Zarkouny et al., 2004	USA	10,000	310	2	Yes	No	No	No
Fricke et al., 2014	USA	44	664	1	Yes	No	No	Yes
Giordano et al., 2015	USA	39	1,210	1	Yes	No	No	No
Gumen et al., 2012	Turkey	9,800	385	1	Yes	No	No	Yes
Herlihy et al., 2012	USA	NA	850	3	Yes	Yes	Yes	Yes
Melendez et al., 2006	Mexico	13,790	589	1	Yes	Yes	No	No
Moreira et al., 2001	USA	NA	269	1	Yes	No	No	Yes
Navanukraw et al., 2004	USA	39	135	1	Yes	No	No	No
Poock et al., 2015	USA	10,925	1,790	1	Yes	No	Yes	No
Rivera et al., 2010	USA	41	399	1	Yes	No	No	Yes
Rutigliano et al., 2008	USA	11,630	260	1	Yes	No	No	Yes
Souza et al., 2008	USA	NA	180	1	Yes	No	Yes	No
Sterry et al., 2006	USA	41	383	1	Yes	No	No	Yes
Stevenson et al., 2012	USA	45	105	1	Yes	No	No	Yes
Overall			9,813	27	20/20	5/20	4/20	13/20

¹Milk yield of the study population either presented as rolling herd average or average daily milk yield for the study period.²An a priori sample size calculation was performed.³NA = not available.**Table 2.** Description of reproductive management in 24 experimental groups using a Presynch-Ovsynch protocol for first service (n = 9,813)

Reference	ED ¹	PGF _{2α} ²	DIM at 1st PGF _{2α}	Interval 2nd PGF _{2α} -GnRH	DIM at timed AI	No. of timed AI	No. of ED-AI	PD 1 ³	PD 2 ⁴
Bicalho et al., 2007	No	Dinoprost	30	14	68	399	0	37	NA ⁵
Bruno et al., 2013	Yes	Dinoprost	36	14	74	272	280	36	66
Brusveen et al., 2008	No	Dinoprost	33	11	68	115	0	31	52
Chebel and Santos, 2010	No	Dinoprost	35	13	72	318	0	32	60
Chebel and Santos, 2010	Yes	Dinoprost	35	13	72	132	189	32	60
Chebel et al., 2006	Yes	Dinoprost	35	13	72	172	166	31	60
Colazo et al., 2013	No	Dinoprost	36	9	69	119	0	32	60
Colazo et al., 2013	No	Dinoprost	33	12	69	122	0	32	60
El-Zarkouny et al., 2004	No	Dinoprost	23-43	12	59-79	310	0	29	57
Fricke et al., 2014	No	Dinoprost	39	12	75	331	0	35	67
Fricke et al., 2014	Yes	Dinoprost	39	12	75	104	230	35	67
Giordano et al., 2015	No	Cloprostenol	29	14	69	1,210	0	31	NA
Gumen et al., 2012	No	Cloprostenol	44	11	79	118	0	31	62
Gumen et al., 2012	Yes	Cloprostenol	44	11	79	135	132	31	62
Herlihy et al., 2012	No	Dinoprost	45	12	81	850	0	39	74
Melendez et al., 2006	Yes	Dinoprost	33	12	69	NA	NA	42	NA
Moreira et al., 2001	No	Dinoprost	37	12	73	369	0	32	74
Navanukraw et al., 2004	No	Dinoprost	Variable	14	Variable	135	0	42	NA
Poock et al., 2015	No	Dinoprost	32	14	70	1,790	0	38	NA
Rivera et al., 2010	No	Dinoprost	37	10	71	399	0	33	66
Rutigliano et al., 2008	No	Dinoprost	37	12	73	260	0	28	42
Souza et al., 2008	No	Dinoprost	42	12	78	180	0	39	NA
Sterry et al., 2006	No	Dinoprost	32	14	69	383	0	33	61
Stevenson et al., 2012	No	Dinoprost	36	10	70	105	0	32	60

¹ED = estrous detection and AI after presynchronization.²PGF_{2α} was either dinoprost (25 mg) or cloprostenol (500 µg).³Days after AI for the first pregnancy diagnosis (PD).⁴Days after AI for the second pregnancy diagnosis.⁵NA = not available.

outcomes were P/AI on d 32 and pregnancy loss on d 60. Within the study, P/AI was calculated as the number of cows diagnosed pregnant on d 32 after AI divided by the total number of cows inseminated. Pregnancy loss was calculated as the number of cows diagnosed pregnant on d 32 that lost the pregnancy before d 60 divided by the number of cows diagnosed pregnant on d 32. Experimental groups in which a single pregnancy diagnosis was performed were not included in the analysis of pregnancy loss.

Statistical Analyses

The meta-analysis was conducted using MedCalc software (version 15.6.1, MedCalc, Mariakerke, Belgium). We conducted 2 different approaches for evaluating the effect of insemination after estrous detection in a timed AI program.

Approach 1: Three Manuscripts with a Randomized Controlled Study Design. There were 3 manuscripts with 1,689 AI directly evaluating the effect of estrous detection on P/AI and pregnancy loss in a PO protocol using a randomized controlled study design (Chebel and Santos, 2010; Gumen et al., 2012; Fricke et al., 2014). Cows that were only allowed to be time-inseminated without estrous detection (onlyTAI) served as the control group. We calculated an odds ratio (OR) for conceiving at first AI and losing pregnancy on d 60 using a fixed effects and a random effects model. Under the fixed effects model, it is assumed that all experimental groups come from a common population, and that the effect size is not significantly different among the different trials as described by the heterogeneity (I^2). However, if there is a significant heterogeneity, the random effects model may be more appropriate, in which both the random variation within the experimental group and the variation between the different experimental groups is incorporated (Borenstein et al., 2009). MedCalc uses the Mantel-Haenszel method for calculating the weighted summary OR under the fixed effects model. The heterogeneity statistic is incorporated to calculate the summary OR under the random effects model (DerSimonian and Laird, 1986).

Heterogeneity (I^2) is the percentage of observed total variation across studies that is due to real heterogeneity rather than chance. It is calculated as follows: $I^2 = 100\% \times (Q - df)/Q$, where Q is Cochran's heterogeneity statistic and df is the degrees of freedom. Negative values of I^2 are made equal to zero so that I^2 lies between 0 and 100%. A value of 0% indicates no observed heterogeneity, and larger values show increasing heterogeneity (Higgins et al., 2003).

Approach 2: All 24 Experimental Groups. For the second approach, we included 24 experimental

groups with information on P/AI for 9,813 AI and 17 experimental groups with information on pregnancy loss for 1,986 pregnancies from first service. Most of them were not designed to evaluate the effect of estrous detection in a timed AI program. Therefore, a direct comparison of P/AI and pregnancy losses in onlyTAI and EDAI+TAI cows, as in approach 1, was not possible. However, to determine the effect size of P/AI, we calculated the pooled proportion from the 18 experimental groups using onlyTAI and the 6 experimental groups using EDAI+TAI. For the effect size of pregnancy loss, we calculated the pooled proportion from the 12 experimental groups using onlyTAI and the 5 experimental groups using EDAI+TAI with information on pregnancy loss. The pooled proportion is an overall estimate of the effect for P/AI or pregnancy loss that takes into account both the individual estimate and its precision for the experimental group.

The pooled proportions with their 95% CI under the fixed and the random effects model were compared. MedCalc uses a Freeman-Tukey transformation (Freeman and Tukey, 1950) to calculate the weighted summary proportion under the fixed and random effects model (DerSimonian and Laird, 1986). We reported the pooled proportion from the random effects model when I^2 was significant ($P \leq 0.05$).

The overall proportion of P/AI for onlyTAI and EDAI+TAI under the fixed and the random effects model for EDAI+TAI and onlyTAI were presented separately with the 95% CI.

RESULTS

Approach 1: Manuscripts with a Randomized Controlled Study Design

Three manuscripts had a randomized controlled study design, with a total of 1,689 AI evaluating the effect of estrous detection in a PO protocol on P/AI (Table 3) and pregnancy loss. The incorporation of estrous detection into a PO protocol decreased ($P < 0.01$) the odds of pregnancy (OR = 0.65; 95% CI = 0.53–0.80) in lactating dairy cows on d 32 after AI using the fixed effects model. Based on the raw data, the difference in P/AI between onlyTAI and EDAI+TAI was 8.8 percentage units on d 32 (42.1 vs. 33.3%) after AI. The pooled estimate for P/AI was 32.5% (179/551) and 34.5% (128/371) for cows inseminated after detected estrus and cows receiving TAI that were not seen in estrus after the second PGF injection in the EDAI+TAI group, respectively. There was no heterogeneity ($I^2 = 48.7\%$; $P = 0.1423$) among the 3 studies regarding P/AI. However, the 95% CI for the individual studies varied noticeably, with one study (Chebel and Santos,

Table 3. Effect of estrous detection on pregnancy per AI (P/AI) in a Presynch-Ovsynch protocol for the first service (n = 1,689) considering 3 manuscripts with a controlled study design

Reference	EDAI+TAI P/AI	OnlyTAI P/AI	Odds ratio ¹	95% CI	P-value
Chebel and Santos, 2010	106/321	126/318	0.751	0.544–1.038	
Fricke et al., 2014	105/334	131/331	0.700	0.509–0.963	
Gumen et al., 2012	96/267	66/118	0.442	0.285–0.688	
$I^2 = 48.7\%^2$ ($P = 0.1423$)					
Total (fixed effects)	307/922	323/767	0.654	0.535–0.800	0.001
Total (random effects)	307/922	323/767	0.637	0.478–0.849	0.002

¹Odds ratio for conceiving at first service using only timed AI (onlyTAI) compared with estrous detection (ED) and TAI (EDAI+TAI).

² I^2 = proportion of total variation of effect size estimates that is due to heterogeneity.

2010) indicating no effect of estrous detection in a PO protocol on P/AI.

The incorporation of estrous detection in a PO protocol had no effect on pregnancy loss (OR = 0.88; 95% CI = 0.55–1.43; $P = 0.619$) in lactating dairy cows on d 60 after AI using the fixed effects model. There was no heterogeneity ($I^2 = 50.9\%$; $P = 0.1302$) among the 3 studies regarding pregnancy loss.

Approach 2: Effect of AI After Estrous Detection in a PO Protocol on P/AI Including all Experimental Groups

The EDAI+TAI group consisted of 6 experimental groups with 2,400 AI using estrous detection in a PO protocol for the first service (Table 4 and Figure 1). The overall proportion of P/AI was 30.9% (95% CI = 26.71–35.28) on d 32 after AI for EDAI+TAI. Substantial heterogeneity ($I^2 = 80.9\%$; $P = 0.0001$) was found among the experimental groups regarding P/AI. Excluding the study of Melendez et al. (2006) improved homogeneity ($I^2 = 0.0\%$; $P = 0.4404$) and resulted in an increased overall proportion of P/AI (n = 1,811; P/AI = 32.8%; 95% CI = 30.67–35.04).

The onlyTAI group consisted of 18 experimental groups with 7,413 AI not using estrous detection in a PO protocol for the first service (Table 5). The overall

proportion of P/AI was 41.7% (95% CI = 39.76–42.01) on d 32 after AI for onlyTAI. There was substantial heterogeneity ($I^2 = 76.1\%$; $P = 0.0001$) among the experimental groups regarding P/AI. The individual proportion of P/AI on d 32 after AI in the experimental groups ranged from 31.9% (95% CI = 23.68–41.10; Colazo et al., 2013) to 55.9% (95% CI = 46.50–65.06; Gumen et al., 2012). We could not identify a plausible classification of subgroups in these experimental groups to reduce the amount of heterogeneity considerably. We observed, however, more extreme results in smaller experimental groups, indicating no publication bias.

Based on the random effects model, the difference in P/AI between onlyTAI and EDAI+TAI was 10.8 percentage units on d 32 (41.7 vs. 30.9%) after AI. The 95% CI for the overall proportion of P/AI for EDAI+TAI and onlyTAI did not overlap (Figure 1).

Approach 2: Effect of AI After Estrous Detection in a PO Protocol on Pregnancy Loss Including all Experimental Groups

For the evaluation of pregnancy loss in the EDAI+TAI group, 5 experimental groups were included, with 1,811 AI resulting in 593 pregnancies (Table 6). The overall proportion of pregnancy loss was 11.7% (95% CI = 6.11–18.8) on d 60 after AI for EDAI+TAI. There was

Table 4. Pregnancy per AI (P/AI) in 6 experimental groups including 2,400 AI using estrous detection in a Presynch-Ovsynch protocol for the first service (EDAI+TAI)

Reference	No. pregnant	No. of AI	P/AI (%)	95% CI
Bruno et al., 2013	188	552	34.058	30.108–38.179
Chebel and Santos, 2010	106	321	33.022	27.899–38.461
Chebel et al., 2006	99	338	29.290	24.491–34.456
Fricke et al., 2014	105	333	31.532	26.575–36.820
Gumen et al., 2012	96	267	35.955	30.197–42.029
Melendez et al., 2006	134	589	22.750	19.424–26.353
$I^2 = 80.9\%^1$ ($P = 0.0001$)				
Total (fixed effects)		2,400	30.269	28.437–32.148
Total (random effects)		2,400	30.916	26.712–35.284

¹ I^2 = proportion of total variation of effect size estimates that is due to heterogeneity.

Table 5. Pregnancy per AI (P/AI) in 18 experimental groups including 7,413 AI using only timed AI (onlyTAI) in a Presynch-Ovsynch protocol for the first service

Reference	No. pregnant	No. of AI	P/AI (%)	95% CI
Bicalho et al., 2007	154	399	38.596	33.795–43.569
Brusveen et al., 2008	52	115	45.217	35.918–54.769
Chebel and Santos, 2010	126	318	39.623	34.209–45.232
Colazo et al., 2013	38	119	31.933	23.686–41.103
Colazo et al., 2013	52	122	42.623	33.716–51.896
El-Zarkouny et al., 2004	145	310	46.774	41.113–52.498
Fricke et al., 2014	131	331	39.577	34.272–45.070
Giordano et al., 2015	513	1,210	42.397	39.592–45.238
Gumen et al., 2012	66	118	55.932	46.499–65.060
Herlihy et al., 2012	345	850	40.588	37.266–43.976
Moreira et al., 2001	134	269	49.814	43.684–55.948
Navanukraw et al., 2004	67	135	49.630	40.916–58.359
Pooock et al., 2015	680	1,790	37.989	35.734–40.283
Rivera et al., 2010	141	399	35.338	30.647–40.249
Rutigliano et al., 2008	85	260	32.692	27.025–38.760
Souza et al., 2008	75	180	41.667	34.378–49.234
Sterry et al., 2006	193	383	50.392	45.268–55.510
Stevenson et al., 2012	35	105	33.333	24.433–43.201
$I^2 = 76.1\%^1$ ($P = 0.0001$)				
Total (fixed effects)		7,413	40.886	39.765–42.014
Total (random effects)		7,413	41.690	39.185–44.217

¹ I^2 = proportion of total variation of effect size estimates that is due to heterogeneity.

substantial heterogeneity ($I^2 = 83.3\%$; $P = 0.0001$) among the experimental groups regarding pregnancy loss.

In the onlyTAI group, 12 experimental groups were included, with 3,389 AI resulting in 1,393 pregnancies

(Table 7). The overall proportion of pregnancy loss was 9.6% (95% CI = 6.37–13.33) on d 60 after AI for EDAI+TAI. There was substantial heterogeneity ($I^2 = 78.2\%$; $P = 0.0001$) among the experimental groups regarding pregnancy loss.

Figure 1. Overall proportion (95% CI) of pregnancy per artificial insemination (P/AI) in 6 experimental groups with 2,400 AI using estrous detection and timed AI (left side; EDAI+TAI) and 18 experimental groups with 7,413 AI using only timed AI (right side; onlyTAI) during a Presynch-Ovsynch protocol for the first service under the fixed and the random effects models. Color version available online.

Table 6. Proportion of pregnancy loss in 5 experimental groups including 1,811 AI and 593 pregnancies using estrous detection and timed AI (EDAI+TAI) in a Presynch-Ovsynch protocol for the first service

Reference	No. of lost pregnancies	No. of pregnancies	Pregnancy loss (%)	95% CI
Bruno et al., 2013	10	188	5.319	2.580–9.564
Chebel and Santos, 2010	24	106	22.642	15.078–31.790
Chebel et al., 2006	17	98	17.347	10.442–26.315
Fricke et al., 2014	9	105	8.571	3.995–15.648
Gumen et al., 2012	7	96	7.292	2.982–14.448
$I^2 = 83.3\%$ ¹ ($P = 0.0001$)				
Total (fixed effects)		593	10.861	8.482–13.635
Total (random effects)		593	11.719	6.114–18.836

¹ I^2 = proportion of total variation of effect size estimates that is due to heterogeneity.

Based on the random effects model, we detected a numerical difference in pregnancy loss on d 60 between onlyTAI and EDAI+TAI of 2.1 percentage units (9.6 vs. 11.7%). However, the 95% CI for the overall proportion of pregnancy loss for onlyTAI and EDAI+TAI were overlapping, indicating no difference.

DISCUSSION

The results from the present study indicate that incorporating estrous detection in a PO protocol for the first service impaired P/AI and had no effect on pregnancy loss in lactating dairy cows.

Considering the 3 manuscripts (Chebel and Santos, 2010; Gumen et al., 2012; Fricke et al., 2014) with a randomized controlled study design, the odds of pregnancy evaluated on d 32 after AI in a PO protocol were 35% lower when cows were inseminated based on estrous detection and the remaining cows were timed AI (EDAI+TAI) compared with onlyTAI cows where cows were not observed for signs of estrus and only timed AI was performed. The proportion of cows inseminated

after detected estrus ranged from 49.4 to 68.6% [58.9% (189/321) in Chebel and Santos, 2010; 49.4% (132/267) in Gumen et al., 2012; 68.6% (230/335) in Fricke et al., 2014] in the 3 studies. Pregnancy per AI for cows inseminated after detected estrus ranged from 30.0 to 37.9% [32.3% (61/189) in Chebel and Santos, 2010; 37.9% (50/132) in Gumen et al., 2012; 30.0% (68/230) in Fricke et al., 2014] and was numerically lower compared with cows receiving onlyTAI. In the studies, however, 3 different methods for estrous detection were used (i.e., tail painting, Kamar device, and automatic activity monitoring system, respectively). Fricke et al. (2014) also evaluated the effect of activity before the timed AI in the onlyTAI group. Cows with increased activity before timed AI were more likely to have increased progesterone concentration at the first GnRH injection (65 DIM) of the Ovsynch protocol compared with cows without increased activity (75 vs. 55%). These cows also had numerically higher P/AI (41.4 vs. 35.3%). This is in agreement with Chebel and Santos (2010), who observed that cyclic cows as determined by blood progesterone at 35, 49, and 62 DIM were more likely

Table 7. Proportion of pregnancy loss in 12 experimental groups including 3,389 AI and 1,393 pregnancies using only timed AI (onlyTAI) in a Presynch-Ovsynch protocol for the first service

Reference	No. of lost pregnancies	No. of pregnancies	Pregnancy loss (%)	95% CI
Brusveen et al., 2008	1	50	2.000	0.506–10.647
Chebel and Santos, 2010	27	126	21.429	14.622–29.625
Colazo et al., 2013	2	38	5.263	0.644–17.749
Colazo et al., 2013	2	52	3.846	0.469–13.213
Fricke et al., 2014	4	131	3.053	0.838–7.633
Gumen et al., 2012	8	66	12.121	5.381–22.494
Herlihy et al., 2012	19	345	5.507	3.348–8.467
Moreira et al., 2001	15	134	11.194	6.402–17.790
Rivera et al., 2010	20	141	14.184	8.885–21.053
Rutigliano et al., 2008	17	85	20.000	12.104–30.079
Serry et al., 2006	16	190	8.421	4.890–13.315
Stevenson et al., 2012	3	35	8.571	1.804–23.058
$I^2 = 78.2\%$ ¹ ($P = 0.0001$)				
Total (fixed effects)		1,393	9.243	7.779–10.879
Total (random effects)		1,393	9.567	6.372–13.329

¹ I^2 = proportion of total variation of effect size estimates that is due to heterogeneity.

to conceive after first AI. Therefore, the mechanism by which presynchronization with PGF may improve P/AI seems to be limited to cycling cows. It is intended to manipulate the estrous cycle so that cows begin the Ovsynch protocol on d 5 to 9 resulting in a higher ovulation rate to the first GnRH and ultimately a higher P/AI after timed AI (Vasconcelos et al., 1999).

For the second approach, we included 24 experimental groups (total of 9,813 AI) that used a PO protocol with 2 injections of PGF_{2α} 14 d apart and an Ovsynch protocol for the timed AI. Although all of the included experimental groups in the second approach were from randomized clinical trials, the majority of them were not designed to compare the different approaches during a PO protocol (i.e., EDAI+TAI vs. onlyTAI) in a controlled manner. The populations included were rather cohorts from different manuscripts using the same protocol for presynchronization and were adequate to be included in our analysis. We labeled these studies observational because the allocation to EDAI+TAI or onlyTAI was not randomly controlled (Dohoo et al., 2009). Although meta-analyses have more commonly been used for combining results from randomized clinical trials, meta-analyses of observational studies are becoming more common and equally important (Egger et al., 2001; Dickersin, 2002). However, results of a meta-analysis with observational studies need to be interpreted with caution because they are prone to a wide range of biases (Egger et al., 1998). Consequently, the focus should be an evaluation of heterogeneity among the studies. To improve homogeneity among the studies, we excluded studies using a PO protocol with timed AI protocols other than Ovsynch (e.g., Galvao et al., 2007; Chebel et al., 2010; Ribeiro et al., 2012). However, the proportion of between-study variation accounted for by heterogeneity was substantial for onlyTAI ($I^2 = 78.2\%$) and EDAI+TAI ($I^2 = 80.9\%$). The source of this high heterogeneity remains speculative. For example, the schedule of the Ovsynch protocol itself varied among the studies regarding the intervals between the PGF_{2α} and the second GnRH injection and between the second GnRH injection and the timed AI. Additionally, the interval between the second PGF_{2α} injection of the presynchronization and the onset of Ovsynch varied among the studies, ranging from 9 to 14 d. This interval was shown to have an effect on P/AI in a PO protocol (Galvao et al., 2007; Colazo et al., 2013). The exclusion of the study of Melendez et al. (2006) substantially improved homogeneity in the EDAI+TAI group. That trial was conducted in Mexico, with the majority of cows being not detected in estrus after a PO protocol receiving a timed AI. As speculated by Melendez et al. (2006), this population may have consisted predominantly of anovulatory cows, which

were shown to have decreased P/AI in a PO protocol (Chebel and Santos, 2010).

Nevertheless, the comparison of the overall proportion of P/AI for onlyTAI and EDAI+TAI in approach 2 confirmed the results of approach 1 and our initial hypothesis. There was a difference of 10.8 percentage units in P/AI favoring onlyTAI in a PO protocol.

Pregnancy loss on d 60 was not affected by the incorporation of insemination at detected estrus in a PO protocol. Although the statistical power in approach 1 was not adequate for detecting a considerable difference in pregnancy loss on d 60 between onlyTAI (39/323) and EDAI+TAI (40/307), we also did not observe a fundamental difference in the overall proportion of pregnancy loss on d 60 when considering all the studies in approach 2 comparing onlyTAI and EDAI+TAI (134/1,393 vs. 67/593).

CONCLUSIONS

Based on a meta-analysis considering 20 manuscripts including 27 herds and 9,813 AI, we compared 2 management strategies for first service using a PO protocol. Cows with 100% TAI after completing a PO protocol had more P/AI compared with cows that were inseminated after estrous detection or received TAI for cows showing no signs of estrus. The results of this meta-analysis are based on a large number of herds with different managerial conditions and seem to be applicable to high-producing dairy herds under confinement housing.

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