Evaluation of prostaglandin F_{2α} versus prostaglandin F_{2α} plus gonadotropin-releasing hormone as Presynch methods preceding an Ovsynch in lactating dairy cows: 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) at first service. Recently, a presynchronization protocol including GnRH and PGF_{2α} (Double-Ovsynch; GnRH, 7 d, PGF_{2α}, 3 d, GnRH) followed 7 d later by an Ovsynch protocol was introduced to overcome the limitations of PGF_{2α}-based protocols for presynchronization of anovular cows and to precisely set up cows on d 7 of the estrous cycle when the Ovsynch is initiated. A systematic review of the literature and a meta-analytical assessment was performed with the objective to compare the reproductive performance of lactating dairy cows presynchronized with these 2 protocols for the first timed AI (TAI) considering parity-specific effects. A fixed or a random effects meta-analysis was used based on the heterogeneity among the experimental groups. Reproductive outcomes of interest were P/AI measured on d 32 (28–42) and pregnancy loss between d 32 and 60 (42–74) of gestation. A total of 25 articles with 27 experimental groups from 63 herds including 21,046 cows submitted to first TAI using either a Presynch-Ovsynch or a Double-Ovsynch protocol were reviewed. Results for P/AI were then categorized by parity if available. Information was available for P/AI for 7,400 and 10,999 primiparous and multiparous cows, respectively. Information regarding pregnancy loss was available for 7,477 cows. In the random effects model for all cows, the overall proportion of P/AI was 41.7% [95% confidence interval (CI): 39.1–44.3; n = 8,213] and 46.2% (95% CI: 41.9–50.5; n = 12,833) on d 32 after TAI for Presynch-Ovsynch and Double-Ovsynch, respectively. In the

random effects model for primiparous cows, the overall proportion of P/AI was 43.4% (95% CI: 36.2–47.7; n = 2,614) and 51.4% (95% CI: 47.4–55.4; n = 4,786) on d 32 after TAI for Presynch-Ovsynch and Double-Ovsynch, respectively. In the random effects model for multiparous cows, the overall proportion of P/AI was 39.2% (95% CI: 36.2–42.3; n = 3,411) and 41.4% (95% CI: 36.4–46.4; n = 7,588) on d 32 after TAI for Presynch-Ovsynch and Double-Ovsynch, respectively. The overall proportion of pregnancy loss was 11.3% (95% CI: 7.6–15.7; n = 3,247) and 11.7% (95% CI: 9.3–14.3; n = 4,230) on d 60 after AI for Presynch-Ovsynch to and Double-Ovsynch, respectively. Substantial heterogeneity existed among the experimental groups regarding P/AI and pregnancy loss. In summary, a benefit was detected for P/AI in primiparous cows presynchronized with a Double-Ovsynch protocol for the first TAI, but this benefit was not observed in multiparous cows.

Key words: presynchronization, Presynch-Ovsynch, Double-Ovsynch, meta-analysis

INTRODUCTION

The widely adoption of synchronization protocols for timed artificial insemination (TAI) in the dairy industry has led to improved reproductive performance (Caraviello et al., 2006). The most common TAI protocol is a 7-d Ovsynch (Stevenson, 2016a). Primarily, this protocol increases the insemination risk without affecting the risk of conception (Pursley et al., 1995). However, conception risk in an Ovsynch protocol can be improved when cows are at specific stages of the estrous cycle at the beginning of the TAI protocol (Vasconcelos et al., 1999).

Therefore, presynchronization protocols were implemented to increase the proportion of cows in early diestrus at the first GnRH injection of the Ovsynch protocol for achieving maximum conception risk (Wiltbank and Pursley, 2014). Specifically, several studies have shown that compared to no presynchronization, presynchronization with 2 doses of PGF_{2α} administered 14 d apart improves pregnancy per artificial insemination

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(P/AI) after TAI using an Ovsynch protocol (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, PO was implemented in the majority (15/16) for the first service (Ferguson and Skidmore, 2013).

The major limitation of the PO protocol is the inability to improve fertility in anovular cows (Bisinotto et al., 2014) that are prevalent in high-producing herds (7.1–41.7%) at the end of the voluntary waiting period (Bamber et al., 2009).

Recently presynchronization protocols including GnRH and PGF_{2α} (e.g., Double-Ovsynch, PG-3-G, G-6-G) were introduced to overcome the limitations of PGF_{2α}-based protocols in anovular cows (Stevenson, 2016a). These programs improved pregnancy outcomes in anovular cows and ensured that a large proportion of cycling cows were at the most ideal stage of the estrous cycle (d 6–8) when the Ovsynch protocol was initiated.

Among these protocols, the Double-Ovsynch (DO) seems to be the most precise method to presynchronize the estrous cycle (Wiltbank and Pursley, 2014). However, only 2 studies are available that compare pregnancy outcomes between PO and DO protocols using a 7-d Ovsynch for TAI (Souza et al., 2008; Herlihy et al., 2012). Although both studies showed a positive effect on P/AI for cows presynchronized with the DO protocol, particularly for cows in their first lactation, the number of cows and herds was limited.

Individual studies often encompass a limited number of herds with similar management practices, climatic conditions, and genetic background, which might limit the inference observed for a treatment effect (Tempelman, 2009). To address this challenge, a meta-analysis was designed to evaluate the effect of presynchronization using either a PO or a DO protocol for first TAI across different managerial conditions in primiparous and multiparous cows separately. The main hypothesis of the present study was that P/AI for the first TAI increases when cows are presynchronized with DO compared to PO, particularly in primiparous cows.

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” and “dairy cow AND Double-Ovsynch.” Additional articles 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 either with 2 applications of PGF_{2α} 14 d apart or a combination of GnRH and PGF_{2α} (i.e., GnRH, 7 d, PGF_{2α}, 3 d, GnRH) followed by an Ovsynch protocol for timed AI 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 breeding Ovsynch protocol until timed AI had to range from 12 to 20 h. Hence, studies using other protocols for timed AI (e.g., CoSynch72, 5d-CoSynch) were not included. For PO the time interval between the second injection of PGF_{2α} and the first GnRH injection from the breeding Ovsynch protocol was set at 10 to 14 d. Studies with insemination after estrus detection (i.e., cherry picking) within the PO protocol were not included. For DO the time interval between the second injection of GnRH of presynch-Ovsynch and the first injection of the breeding Ovsynch was 7 d. Four randomized controlled studies were available that directly compared the effect of presynchronization using either a PO or a DO protocol (Souza et al., 2008; Herlihy et al., 2012; Ribeiro et al., 2012; Ayres et al., 2013). The study from Ribeiro et al. (2012) was not considered because a 5d-Cosynch was used for TAI. The study from Ayres et al. (2013) did not report information on P/AI.

For articles with a significant ($P < 0.05$) effect of a certain treatment on P/AI or pregnancy loss, we only considered the control group (i.e., without treatment effect) for evaluation. Otherwise we combined the data from the control group and the treatment group regarding P/AI and pregnancy loss.

Based on these criteria, the meta-analysis included a total of 17 articles including 8,213 cows presynchronized using a PO protocol and 10 articles including 12,833 cows presynchronized using a DO protocol for first TAI.

Data Extraction

Data extraction was performed by a single investigator (S. Borchardt) and validated by the coauthors (P. Haimlerl, A. Pohl, W. Heuwieser). For each study,

Table 1. Summary of manuscripts (n = 25) using either a Presynch-Ovsynch (PO) or a Double-Ovsynch (DO) protocol for the first timed AI considering evaluated information criteria

Reference	Protocol	Country	Milk yield, ¹ kg	No. of AI	No. of herds	Sample size calculated ²	Parity considered	Pregnancy loss calculated
Astiz and Fargas, 2013	DO	Spain	10,507	6,783	27	No	Yes	No
Bicalho et al., 2007	PO	USA	NA ³	399	5	No	No	No
Brusveen et al., 2008	PO	USA	NA	115	1	No	No	Yes
Brusveen et al., 2009	DO	USA	NA	453	2	No	No	No
Carvalho et al., 2014a	DO	USA	13,880	306	1	Yes	Yes	Yes
Carvalho et al., 2014b	DO	USA	NA	1,103	4	No	Yes	Yes
Carvalho et al., 2015b	DO	USA	14,338	395	2	No	Yes	Yes
Chebel and Santos, 2010	PO	USA	12,035	639	1	Yes	No	Yes
Colazo et al., 2013	PO	Canada	10,370	241	1	No	Yes	Yes
El-Zarkouny et al., 2004	PO	USA	10,000	310	2	No	No	No
Fricke et al., 2014	PO	USA	44	664	1	No	No	Yes
Giordano et al., 2013	DO	USA	13,463	1,084	1	No	Yes	Yes
Giordano et al., 2015	PO	USA	39	1,210	1	No	Yes	No
Gumen et al., 2012	PO	Turkey	9,800	385	1	No	No	Yes
Herlihy et al., 2012	PO/DO	USA	NA	850/837	3	Yes	Yes	Yes
Lima et al., 2013	DO	USA	45	1,342	1	Yes	Yes	Yes
Moreira et al., 2001	PO	USA	NA	269	1	No	No	Yes
Navanukraw et al., 2004	PO	USA	39	135	1	No	No	No
Pooch et al., 2015	PO	USA	10,925	1,790	1	No	Yes	No
Rivera et al., 2010	PO	USA	41	399	1	No	Yes	Yes
Rutigliano et al., 2008	PO	USA	11,630	260	1	No	Yes	Yes
Souza et al., 2008	PO/DO	USA	NA	180/157	1	No	Yes	No
Sterry et al., 2006	PO	USA	41	383	1	No	Yes	Yes
Stevenson et al., 2012	PO	USA	45	105	1	No	Yes	Yes
Wiltbank et al., 2015	DO	USA	NA	373	1	Yes	Yes	No
Overall				21,046	63	5/25	16/25	16/25

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.

recorded information included authors, year of publication, number of herds, milk yield, bST usage, sample size calculation, stratification of results by parity, GnRH and PGF_{2α} products and doses, time schedule of the Ovsynch protocol, DIM at TAI, and time of pregnancy examination (Tables 1, 2, and 3).

For articles in which results were not categorized by parity the corresponding authors were contacted to acquire the additional information. Experimental groups within the articles were classified regarding their management of first service into PO or DO.

Pregnancy status was evaluated twice after AI in 15 articles. 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 article, respectively. Reproductive 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 (version 15.6.1, MedCalc Software, Mariakerke, Belgium) as described previously (Borchardt et al., 2016).

Briefly, we included 27 experimental groups with information on P/AI for 21,046 TAI and 15 experimental groups with information on pregnancy loss for 7,477 pregnancies from first service. Most of the groups were not designed to evaluate the effect of presynchronization method in a timed AI program. Therefore a direct comparison of P/AI and pregnancy losses in PO and DO cows was not possible. To determine the effect size of a given protocol on P/AI we calculated the pooled proportion from the 17 experimental groups using PO and the 10 experimental groups using DO. For the effect size of pregnancy loss we calculated the pooled proportion from the 10 experimental groups using PO and the 5 experimental groups using DO with information

Table 2. Description of reproductive management in 17 experimental groups using a Presynch-Ovsynch protocol for first service (n = 8,213)

Reference	GnRH ¹	PGF _{2α} ²	DIM at 1st PGF _{2α}	Interval (d) 2nd PGF _{2α} –GnRH	DIM at TAI ³	No. of TAI	PD 1 ⁴	PD 2 ⁵
Bicalho et al., 2007	Cystorelin	Dinoprost	30	14	68	399	37	NA ⁶
Brusveen et al., 2008	Gonadorelin	Dinoprost	33	11	68	115	31	52
Chebel and Santos, 2010	Gonadorelin	Dinoprost	35	13	72	318	32	60
Colazo et al., 2013	Gonadorelin	Dinoprost	33	12	69	122	32	60
El-Zarkouny et al., 2004	Gonadorelin	Dinoprost	23–43	12	59–79	310	29	57
Fricke et al., 2014	Gonadorelin	Dinoprost	39	12	75	331	35	67
Giordano et al., 2015	Gonadorelin	Cloprostenol	29	14	69	1,210	31	NA
Gumen et al., 2012	Buserelin	Cloprostenol	44	11	79	118	31	62
Herlihy et al., 2012	Gonadorelin	Dinoprost	45	12	81	850	39	74
Moreira et al., 2001	Cystorelin	Dinoprost	37	12	73	369	32	74
Navanukraw et al., 2004	Cystorelin	Dinoprost	Variable	14	Variable	135	42	NA
Poock et al., 2015	Cystorelin/ gonadorelin	Dinoprost	32	14	70	1,790	38	NA
Rivera et al., 2010	NA	Dinoprost	37	10	71	399	33	66
Rutigliano et al., 2008	Gonadorelin	Dinoprost	37	12	73	260	28	42
Souza et al., 2008	Gonadorelin	Dinoprost	42	12	78	180	39	NA
Sterry et al., 2006	Gonadorelin	Dinoprost	32	14	69	383	33	61
Stevenson et al., 2012	Gonadorelin	Dinoprost	36	10	70	105	32	60

¹GnRH was either gonadorelin (100 µg), Cystorelin (100 µg; Merial Ltd., Duluth, GA), or buserelin (10 µg).

²PGF_{2α} was either dinoprost (25 mg) or cloprostenol (500 µg).

³TAI = timed AI.

⁴Days after AI for the first pregnancy diagnosis (PD).

⁵Days after AI for the second pregnancy diagnosis.

⁶NA = not available.

on pregnancy loss. The pooled proportion is an overall estimate of the effect of a protocol on 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 significant heterogeneity was present ($P \leq 0.05$). 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 $I^2 = 100\% \times (Q - df)/Q$. Herein, Q is Cochran's heterogeneity statistic, and df is the degrees of freedom. Negative values of I^2 are put equal to zero so that I^2 lies between 0 and

Table 3. Description of reproductive management in 10 experimental groups using a Double-Ovsynch protocol for first service (n = 12,833)

Reference	GnRH ¹	PGF _{2α} ²	DIM at 1st GnRH	Interval (d) 2nd GnRH–GnRH	DIM at TAI ³	No. of TAI	PD 1 ⁴	PD 2 ⁵
Astiz and Fargas, 2013	Gonadorelin/ buserelin	Dinoprost/ cloprostenol	60	7	Avg. 87	6,783	28–45	NA ⁶
Brusveen et al., 2009	Gonadorelin	Dinoprost	48–54	7	75–81	453	39	NA
Carvalho et al., 2014a	Cystorelin	Dinoprost	53	7	80	306	32	70
Carvalho et al., 2014b	Cystorelin	Dinoprost	53	7	80	1,103	32/40	70
Carvalho et al., 2015b	Cystorelin	Dinoprost	51/53	7	78/80	395	32	67
Giordano et al., 2013	Gonadorelin	Cloprostenol	56	7	83	1,084	29	74
Herlihy et al., 2012	Gonadorelin	Dinoprost	54	7	81	837	39	74
Lima et al., 2013	NA	Dinoprost	48	7	75	1,342	32	60
Souza et al., 2008	Gonadorelin	Dinoprost	51	7	78	157	39	NA
Wiltbank et al., 2015	Gonadorelin	Cloprostenol	54	7	81	373	32	NA

¹GnRH was either gonadorelin (100 µg), Cystorelin (100 µg; Merial Ltd., Duluth, GA), or buserelin (10 µg).

²PGF_{2α} was either dinoprost (25 mg) or cloprostenol (500 µg).

³TAI = timed AI.

⁴Days after AI for the first pregnancy diagnosis (PD).

⁵Days after AI for the second pregnancy diagnosis.

⁶NA = not available.

100%. A value of 0% indicates no observed heterogeneity, and larger values show increasing heterogeneity (Higgins et al., 2003).

The overall proportion of P/AI for PO and DO under the fixed and the random effects model were presented separately with the 95% CI for all cows and categorized by parity.

RESULTS

Effect of Presynchronization Protocol on P/AI for All Cows

The PO group consisted of 17 experimental groups with 8,213 TAI using a Presynch-Ovsynch protocol for the first service (Table 4 and Figure 1). The overall proportion of P/AI was 41.7% (95% CI: 39.1–44.3) on d 32 after AI for PO. Substantial heterogeneity existed ($I^2 = 80.2\%$; $P = 0.0001$) among the experimental groups regarding P/AI. The individual proportion of P/AI on d 32 after AI in the experimental groups ranged from 32.7% (95% CI: 27.0–38.8; Rutigliano et al., 2008) to 55.9% (95% CI: 46.5–65.1; Gumen et al., 2012). We could not identify a plausible classification of subgroups in these experimental groups to reduce the amount of heterogeneity considerably, but we observed more extreme results in smaller experimental groups, which indicated no publication bias.

The DO group consisted of 10 experimental groups with 12,833 TAI using a Double-Ovsynch protocol for the first service (Table 5 and Figure 2). The overall proportion of P/AI was 46.2% (95% CI: 41.9–50.5) on

d 32 after TAI for DO. Substantial heterogeneity ($I^2 = 94.5\%$; $P = 0.0001$) existed among the experimental groups regarding P/AI. The individual proportion of P/AI on d 32 after AI in the experimental groups ranged from 36.3% (95% CI: 35.2–37.5; Astiz and Fargas, 2013) to 52.9% (95% CI: 47.9–57.9; Carvalho et al., 2015b). Excluding the study from Astiz and Fargas (2013) improved homogeneity ($I^2 = 79.0\%$; $P = 0.0001$) but did not result in an increased overall proportion of P/AI ($n = 6,050$; P/AI = 47.2%; 95% CI: 44.3–50.1).

Based on the random effects model, the difference in P/AI between PO and DO was 4.5 percentage units on d 32 (41.7 vs. 46.2%) after TAI. The 95% CI for the overall proportion of P/AI for PO and DO were overlapping (Figure 3).

Information on the ovarian response in a 7-d Ovsynch protocol for cows presynchronized either with $F_{2\alpha}$ or prostaglandin $F_{2\alpha}$ plus GnRH for the first TAI are summarized in Table 6.

Effect of Presynchronization Protocol on P/AI for Primiparous Cows

The PO group consisted of 12 experimental groups with 2,614 TAI using a Presynch-Ovsynch protocol for the first service in primiparous cows. The overall proportion of P/AI was 43.4% (95% CI: 36.2–47.7) on d 32 after AI for PO in primiparous cows. Substantial heterogeneity ($I^2 = 73.9\%$; $P = 0.0001$) existed among the experimental groups regarding P/AI. The individual proportion of P/AI on d 32 after AI in the experimental groups ranged from 31.7% (95% CI: 18.1–48.1; Steven-

Table 4. Pregnancy per artificial insemination (P/AI) in 17 experimental groups including 8,213 timed AI using a Presynch-Ovsynch protocol for the first service

Reference	No. pregnant	No. of AI	P/AI of %	95% CI
Bicalho et al., 2007	467	1,318	35.4	32.8–38.1
Brusveen et al., 2008	52	115	45.2	35.9–54.8
Chebel and Santos, 2010	126	318	39.6	34.2–45.2
Colazo et al., 2013	54	122	44.3	35.3–53.5
El Zarkouny et al., 2004	145	310	46.8	41.1–52.5
Fricke et al., 2014	131	331	39.6	34.3–45.1
Giordano et al., 2015	513	1,210	42.4	39.6–45.2
Gumen et al., 2012	66	118	55.9	46.5–65.1
Herlihy et al., 2012	325	850	38.2	34.9–41.6
Moreira et al., 2001	131	269	48.7	42.6–54.8
Navanukraw et al., 2004	67	135	49.6	40.9–58.4
Pooch et al., 2015	680	1,790	38.0	35.7–40.3
Rivera et al., 2010	141	399	35.3	30.6–40.2
Rutigliano et al., 2008	85	260	32.7	27.0–38.8
Souza et al., 2008	75	180	41.7	34.4–49.2
Sterry et al., 2006	193	383	50.4	45.3–55.5
Stevenson et al., 2012	35	105	33.3	24.4–43.2
$I^2 = 80.2\%$ ($P = 0.0001$) ¹				
Total (fixed effects)	3,286	8,213	40.0	38.9–41.1
Total (random effects)	3,286	8,213	41.7	39.1–44.3

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

Table 5. Pregnancy per artificial insemination (P/AI) in 10 experimental groups including 12,833 timed AI using a Double-Ovsynch protocol for the first service

Reference	No. pregnant	No. of AI	P/AI of %	95% CI
Astiz and Fargas, 2013	2,464	6,783	36.3	35.2–37.5
Brusveen et al., 2009	225	453	49.7	45.0–54.4
Carvalho et al., 2014a	161	306	52.6	46.9–58.3
Carvalho et al., 2014b	520	1,103	47.1	44.1–50.1
Carvalho et al., 2015b	209	395	52.9	47.9–57.9
Giordano et al., 2013	509	1,084	46.9	43.9–50.0
Herlihy et al., 2012	383	837	45.8	42.3–49.2
Lima et al., 2013	536	1,342	39.9	37.3–42.6
Souza et al., 2008	78	157	49.7	41.6–57.8
Wiltbank et al., 2015	163	373	43.7	38.6–48.9
$I^2 = 94.5\%$ ($P = 0.0001$) ¹				
Total (fixed effects)	5,248	12,833	40.8	40.0–41.7
Total (random effects)	5,248	12,833	46.2	41.9–50.5

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

son et al., 2012) to 55.8% (95% CI: 41.3–69.5; Colazo et al., 2013).

The DO group consisted of 9 experimental groups with 4,786 TAI using a Double-Ovsynch protocol for the first service in primiparous cows. The overall proportion of P/AI was 51.4% (95% CI: 47.4–55.4) on d 32 after AI for DO in primiparous cows. Substantial heterogeneity ($I^2 = 81.4\%$; $P = 0.0001$) was detected

among the experimental groups regarding P/AI. The individual proportion of P/AI on d 32 after AI in the experimental groups ranged from 44.3% (95% CI: 42.4–46.2; Astiz and Fargas, 2013) to 65.2% (95% CI: 52.8–76.3; Souza et al., 2008).

Based on the random effects model, the difference in P/AI between PO and DO in primiparous cows was 8.0 percentage units on d 32 (43.4 vs. 51.4%) after TAI.

Figure 1. Pregnancy per AI in 17 experimental groups with 8,213 timed AI using a Presynch-Ovsynch protocol for the first service. The weight (black square) and the 95% CI (whiskers) were depicted for each study. Diamonds represent the pooled effect. The location of the diamond represents the estimated effect size and the width of the diamond reflects the precision of the estimate.

Figure 2. Pregnancy per AI in 10 experimental groups with 12,833 timed AI using a Double-Ovsynch protocol for the first service. The weight (black square) and the 95% CI (whiskers) were depicted for each study. Diamonds represent the pooled effect. The location of the diamond represents the estimated effect size and the width of the diamond reflects the precision of the estimate.

The 95% CI for the overall proportion of P/AI for PO and DO overlapped slightly (Figure 4).

Effect of Presynchronization Protocol on P/AI for Multiparous Cows

The PO group consisted of 12 experimental groups with 3,411 TAI using a Presynch-Ovsynch protocol for the first service in multiparous cows. The overall proportion of P/AI was 39.2% (95% CI: 36.2–42.3) on d 32 after AI for PO in multiparous cows. Substantial heterogeneity ($I^2 = 64.8\%$; $P = 0.001$) existed among the experimental groups regarding P/AI. The individual proportion of P/AI on d 32 after AI in the experimental groups ranged from 31.7% (95% CI: 20.3–45.0; Stevenson et al., 2012) to 53.6% (95% CI: 42.3–64.8; Navanukraw et al., 2004). We could not identify a plausible classification of subgroups in these experimental groups to substantially reduce the amount of heterogeneity.

The DO group consisted of 9 experimental groups with 7,588 TAI using a Double-Ovsynch protocol for the first service in multiparous cows. The overall proportion of P/AI was 41.4% (95% CI: 36.4–46.4) on d 32 after AI for DO in multiparous cows. Substantial

heterogeneity ($I^2 = 92.8\%$; $P = 0.0001$) was detected among the experimental groups regarding P/AI. The individual proportion of P/AI on d 32 after AI in the

Figure 3. Overall proportion (95% CI) of pregnancy per artificial insemination (P/AI) in 17 experimental groups with 8,213 timed AI (left side) using a Presynch-Ovsynch protocol and 10 experimental groups with 12,833 timed AI (right side) using a Double-Ovsynch protocol for the first service under the fixed and the random effects model. Color version available online.

Figure 4. Overall proportion (95% CI) of pregnancy per artificial insemination (P/AI) in 12 experimental groups with 2,614 timed AI (left side) using a Presynch-Ovsynch protocol and 9 experimental groups with 4,786 timed AI (right side) using a Double-Ovsynch protocol for the first service under the fixed and the random effects model in primiparous cows. Color version available online.

Figure 5. Overall proportion (95% CI) of pregnancy per artificial insemination (P/AI) in 12 experimental groups with 3,411 timed AI (left side) using a Presynch-Ovsynch protocol and 9 experimental groups with 7,588 timed AI (right side) using a Double-Ovsynch protocol for the first service under the fixed and the random effects model in multiparous cows. Color version available online.

experimental groups ranged from 31.4% (95% CI: 30.0–32.8; Astiz and Fargas, 2013) to 51.6% (95% CI: 45.6–57.7; Carvalho et al., 2015b).

Based on the random effects model, the difference in P/AI between PO and DO in multiparous cows was 2.2 percentage units on d 32 (39.2 vs. 41.4%) after TAI. The 95% CI for the overall proportion of P/AI for PO and DO overlapped (Figure 5).

Effect of Presynchronization Protocol on Pregnancy Loss for All Cows

For the evaluation of pregnancy loss in the PO group 10 experimental groups were included, with 3,247 TAI resulting in 1,332 pregnancies. The overall proportion of pregnancy loss was 11.3% (95% CI: 7.6–15.6) on d 60 after AI for PO. Substantial heterogeneity existed (I^2

Table 6. Ovarian response (% with no./total in parentheses) in a 7-d Ovsynch protocol for cows presynchronized either with PGF_{2α} (Presynch-Ovsynch) or PGF_{2α} plus GnRH (Double-Ovsynch) for the first timed AI

Reference	Presynch-Ovsynch	Double-Ovsynch
Ovulation after GnRH on d 0		
Ayres et al., 2013	69.9 (65/93)	80.0 (80/100)
Carvalho et al., 2015b		60.3 (70/116)
Colazo et al., 2013	61.5 (75/122)	
Giordano et al., 2013		57.5 (187/325)
Gumen et al., 2012	83.1 (98/118)	
Navanukraw et al., 2004	35.8 (19/53)	
Rivera et al., 2010	59.4 (237/399)	
Souza et al., 2008	66.7 (32/48)	71.8 (28/39)
Complete luteolysis after a single PGF _{2α} injection on d 7		
Ayres et al., 2013	93.5 (87/93)	95.0 (95/100)
Baez et al., 2016		92.0 (648/704)
Colazo et al., 2013	89.3 (109/122)	
El-Zarkouny et al., 2004	78.0 (241/309)	
Giordano et al., 2013		84.3 (253/300)
Rivera et al., 2010	90.5 (361/399)	
Stevenson et al., 2012	87.8 (79/90)	
Wiltbank et al., 2015		83.0 (146/176)
Ovulation after GnRH on d 9		
Carvalho et al., 2015b		98.0 (99/101)
Colazo et al., 2013	88.5 (108/122)	
Gumen et al., 2012	89.8 (106/118)	
Navanukraw et al., 2004	81.1 (43/53)	
Rivera et al., 2010	92.0 (367/399)	
Stevenson et al., 2012	96.2 (101/105)	

= 78.8%; $P = 0.0001$) among the experimental groups regarding pregnancy loss.

In the DO group, 5 experimental groups were included, with 4,230 AI resulting in 1,713 pregnancies. The overall proportion of pregnancy loss was 11.7% (95% CI = 9.3–14.3) on d 60 after TAI. Heterogeneity ($I^2 = 60.8\%$; $P = 0.037$) existed among the experimental groups regarding pregnancy loss.

Based on the random effects model, no difference in pregnancy loss on d 60 was detected between PO and DO (11.3 vs. 11.7%). The 95% CI for the overall proportion of pregnancy loss for PO and DO, overlapped, also indicating no difference.

DISCUSSION

The results from the present study indicate that the DO protocol achieves greater P/AI for the first service in primiparous cows compared with a PO protocol. In multiparous cows, however, no difference was detected between the 2 protocols regarding P/AI. In addition, no difference in pregnancy loss was found between the 2 protocols.

The prerequisites for achieving excellent fertility results in Ovsynch-type AI protocols for timed AI in lactating dairy cows are (1) a functional corpus luteum (CL) at the first GnRH injection (GnRH1), (2) ovulation after GnRH1, (3) adequate luteolysis after PGF_{2α}, and (4) timely ovulation after the final GnRH (GnRH2). Therefore, differences in reproductive performance between the 2 protocols for presynchronization of the first TAI might be explained by these 4 factors.

Starting the Ovsynch protocol with a functional CL was shown to have a major effect on P/AI in a 7-d Ovsynch protocol (Bisinotto et al., 2015). Based on the 3 articles (Souza et al., 2008; Herlihy et al., 2012; Ayres et al., 2013) with a direct comparison of DO and PO, the DO protocol is very effective in inducing ovulation in anovular cows before initiation of the breeding Ovsynch compared with a PO protocol [Souza et al., 2008: PO 33.3% (14/42) vs. DO 9.4% (3/32); Herlihy et al., 2012: PO 24.7% vs. DO 6.3%; Ayres et al., 2013: PO 30.1% (28/93) vs. DO 12.0% (12/100)]. This mechanism might also explain the increase in P/AI in a DO protocol, particularly for primiparous cows. In a summary using 13 studies with 5,818 cows, the prevalence of anovulation between 50 and 65 d postpartum was 29.6 and 19.1% in primiparous and multiparous cows, respectively (Bamber et al., 2009). The assumption that primiparous cows have a greater risk for anovulation should not be generalized because this statement is contra-indicated by Herlihy et al. (2012). One of the key aspects for the improvement in P/AI in DO pro-

tol seems to be a reduction in anovular cows at the beginning of the breeding Ovsynch compared with a PO protocol. Cows that initiate the Ovsynch protocol with a functional CL [progesterone (P4) > 0.50 ng/mL] had comparable P/AI when presynchronized either with a PO [44.1% (124/281)] or a DO protocol [47.5% (163/343)] (Herlihy et al., 2012). As shown in other studies, cows presynchronized with a PO protocol and a functional CL at GnRH1 have a greater conception risk compared with cows without a CL [Bicalho et al., 2007: CL+ 46.6% (129/277) vs. CL– 22.1% (27/122); Herlihy et al., 2012: CL+ 44.1% (124/281) vs. CL– 33.3% (31/91); Sterry et al., 2006: CL+ 55.2% (153/277) vs. CL– 37.7% (40/106)]. Therefore, in herds with a large prevalence of anovulation the DO protocol is a better alternative for achieving high P/AI. This protocol, however, only allows TAI and no insemination after detected estrus for the first service. For herds with a low prevalence of anovulation the PO protocol can be considered because it is less complicated and also allows insemination of cows after detected estrus. It is noteworthy, however, that using a 100% TAI approach in a PO protocol improved P/AI by 10.8% compared with incorporation of estrus detection during the protocol as shown in a recent meta-analysis (Borchardt et al., 2016).

Multiple studies showed that ovulation to GnRH1 in an Ovsynch protocol improved P/AI (Galvão et al., 2007; Giordano et al., 2013; Carvalho et al., 2015b). But this effect seems to be limited to cows lacking a functional CL at GnRH1 (Giordano et al., 2012b). This finding is supported by recent evidence indicating that the concentration of P4 during follicular development rather than ovulation to GnRH1 itself seems to be more important for improving P/AI (Stevenson, 2016b).

Ovulatory response to GnRH1 is dependent on the stage of the estrous cycle when GnRH is administered, with cows on d 5 to 9 of the estrous cycle having the greatest ovulation rate (Vasconcelos et al., 1999) and ensuring an elevated P4 milieu during the development of the ovulatory follicle. Cows presynchronized with a DO protocol are more tightly synchronized with the majority of cows being at d 5 to 6 at GnRH1 compared with a PO protocol. Only 2 randomized controlled studies are available that directly compare the ovulatory response in a PO and DO protocol. One study showed a greater ovulation rate after GnRH1 for DO (Ayres et al., 2013), and the other study showed no difference between DO and PO (Souza et al., 2008). The reported ovulation risk after GnRH1 for cows presynchronized with a PO protocol ranges from 35.8 to 83.1% and for cows presynchronized with a DO protocol from 60.3 to 80.0% (Table 6).

Attempts to improve the ovulatory response after GnRH1 in a DO protocol by either increasing the GnRH dose (Giordano et al., 2013) or administering half a dose of PGF_{2α} (12.5 mg) 2 d before GnRH1 (Carvalho et al., 2015b) showed a positive effect on ovulation rate (Carvalho et al., 2015b: +20.8%; Giordano et al., 2013: +9.1%) but failed to improve P/AI. The possibility of a Type II error (declaring no difference between groups when a difference does exist), however, must be considered when interpreting data from these studies. The likelihood of detecting a treatment effect on fertility was limited because of only a modest improvement of P/AI for cows ovulating after GnRH1 compared with nonovulatory cows (Carvalho et al., 2015b: +16.4%; Giordano et al., 2013: +13.7%).

Although ovulation to GnRH1 was shown to improve fertility by creating a more optimum P4 environment during the preovulatory follicle growth phase (Bisinotto et al., 2010), this newly formed CL may be prone to incomplete luteal regression after a single administration of PGF 7 d later in an Ovsynch protocol. Cows that ovulated after GnRH1 had a greater risk of incomplete luteal regression (21.6 vs. 7%) resulting from a lack of luteolytic capacity of the CL (Giordano et al., 2012b). Cows with delayed or incomplete luteolysis resulting in residually elevated concentrations of P4 near the time of TAI have reduced fertility (Carvalho et al., 2015c). Only 1 randomized controlled study (Ayres et al., 2013) compared luteolysis risk between a PO [93.5% (87/93)] and a DO protocol [95.0% (95/100)], and it indicated no difference. The reported risk of complete luteal regression after a single PGF_{2α} injection in a 7-d Ovsynch protocol for cows presynchronized with a PO protocol ranges from 78.0 to 93.5% and for cows presynchronized with a DO protocol from 83.0 to 95.0% (Table 6). Recent studies indicate that administration of a second prostaglandin injection in an Ovsynch protocol improved the rate of cows with complete luteal regression and fertility (Carvalho et al., 2015a; Santos et al., 2016), particularly in multiparous cows (Wiltbank et al., 2015). Adding a second prostaglandin treatment in the breeding Ovsynch protocol seems to be more important than the method of presynchronization. The benefit of the second prostaglandin injection was restricted to cows with only a GnRH1-induced CL at the time of PGF_{2α} injection (Stevenson, 2016b). In that particular study, incomplete luteolysis in response to 1 PGF_{2α} injection was less prevalent when a more mature CL was coexistent with the new GnRH-induced CL. Therefore, it remains speculative if the addition of a second PGF_{2α} treatment is more important for cows presynchronized with PO owing to the possibility for more anovular cows at GnRH1 when presynchronized with PGF_{2α}. However, when this modification

was implemented in a DO protocol, a marked increase occurred in P/AI, especially in multiparous cows [Wiltbank et al., 2015: 1PGF 36.6% (37/101) vs. 2PGF 45.0% (45/100)].

Timely ovulation after the final GnRH in an Ovsynch protocol is required to release an oocyte for fertilization after TAI. None of the randomized controlled studies compared this aspect between a PO and a DO protocol. The reported ovulation rate after GnRH2 in a 7-d Ovsynch protocol for cows presynchronized with a PO protocol ranges from 81.1 to 96.2% (Table 6). For cows presynchronized with a DO protocol only one study was available [Carvalho et al., 2015b: 98.0% (99/101)].

The positive effect of a DO protocol for presynchronization of the first TAI seems to be driven by an improvement of P/AI in primiparous cows. Conflicting reports exist on the effect of parity on P/AI, with the majority of studies showing a greater conception risk in primiparous cows (Galvão et al., 2007; Carvalho et al., 2015a; Fuenzalida et al., 2015), but some others showing no effect of parity (Giordano et al., 2012a; Carvalho et al., 2014a; Carvalho et al., 2015b). The greater fertility response in primiparous cows in a DO protocol has primarily been argued to be a result of a more effective induction of cyclicity in anovular cows (Souza et al., 2008; Herlihy et al., 2012). Recent research indicates that lacking luteolytic response in multiparous cows to a single prostaglandin administration in a 7-d Ovsynch protocol has a detrimental effect on P/AI (Wiltbank et al., 2015). For multiparous cows presynchronized with a DO protocol an additional prostaglandin injection on d 8 of the breeding Ovsynch led to exceptional P/AI (Carvalho et al., 2015b; Wiltbank et al., 2015). No studies are available that have implemented an additional prostaglandin injection on d 8 of the breeding Ovsynch in a PO protocol.

Besides parity-specific differences related to successful synchronization in TAI protocols, other contributors might be responsible for reduced fertility in multiparous cows, such as the greater uterine size (Baez et al., 2016), greater BCS loss in early lactation (Carvalho et al., 2014b), or a greater disease incidence in the transition period, which was associated with reduced fertility later in lactation (Santos et al., 2010).

Pregnancy loss on d 60 was not affected by the method of presynchronization as indicated by the overall proportion of the random effects model. As the major difference between the PO and DO protocol is the reduction of anovular cows at GnRH1, this finding is in agreement with a previous report (Bisinotto et al., 2015). Neither the presence of a functional CL nor supplementation of P4 at GnRH1 for cows lacking a CL had an effect on pregnancy loss compared with cows without a CL.

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). Results of such an approach, however, should be interpreted with caution because they are prone to a wide range of biases (Egger et al., 1998). Consequently, an evaluation of heterogeneity among the studies is important. The proportion of between-study variation accounted for by heterogeneity was substantial for PO ($I^2 = 80.2\%$) and DO ($I^2 = 94.5\%$). The source of this high heterogeneity remains speculative. For studies using the PO protocol the interval between the second PGF_{2α} injection of the presynchronization and the onset of Ovsynch varied among the studies, ranging from 10 to 14 d. This interval was shown to have an effect on P/AI in a PO protocol when 100% TAI was used (Galvão et al., 2007; Colazo et al., 2013). To explain heterogeneity in the 17 experimental groups using the PO protocol, we grouped them according to the interval between the second Presynch PGF and the GnRH1 of the Ovsynch into Group 1 (10–11 d; 4 experimental groups; n = 737), Group 2 (12 d; 7 experimental groups; n = 2,322), and Group 3 (13–14 d; 6 experimental groups; n = 5,154). We could not detect any difference in P/AI [Group 1: 42.2% (95% CI 32.6–52.1); Group 2: 41.5% (37.5–45.6); Group 3: 41.9% (37.7–46.0)] and no improvement in heterogeneity [Group 1: 84.2% (60.4–93.7); Group 2: 72.5% (40.7–87.3); Group 3: 87.3% (74.3–93.6)] among the 3 groups. The exclusion of the study from Astiz and Fargas (2013) substantially improved heterogeneity among studies using the DO protocol but did not result in improved P/AI. This large study (n = 6,783) was conducted in Spain using 27 commercial dairy herds. The authors could also identify a parity-specific response, but they did not report an explanation for the relatively poor reproductive performance in multiparous cows (P/AI: 31.4%). Another source of heterogeneity in the studies using a DO protocol might come from a modification in the breeding Ovsynch. The addition of a second PGF_{2α} application was shown to improve the rate of luteal regression and fertility, particularly in multiparous cows by approximately 3 percentage points (Wiltbank et al., 2015). Three studies used this approach in either all cows (Carvalho et al., 2015b) or in half of the cows (Brusveen et al., 2009; Wiltbank et al., 2015).

Ideally, only randomized controlled studies directly evaluating PO and DO should have been included. Unfortunately, only 2 studies (Souza et al., 2008; Herlihy et al., 2012) were available that matched such inclusion criteria. We therefore encourage researchers to conduct further studies comparing these 2 protocols for pre-

synchronization. Nevertheless, for this meta-analysis a large number of experimental groups (n = 27; n = 15) including 63 Holstein herds, 21,046 cows with information on P/AI, and 7,477 cows with information on pregnancy loss, respectively, were used. This study demonstrates the underlying power of meta-analytic assessments as well as the value of multiple replications of consistent results considering varying factors (e.g., management, climate, genetics).

CONCLUSIONS

Based on a meta-analytical assessment considering 25 articles including 21,046 TAI, we compared 2 different protocols for presynchronization of the first timed AI in lactating dairy cows. Primiparous cows presynchronized with a DO protocol had more P/AI compared with a PO protocol. No difference existed in multiparous cows between the 2 protocols. The results of this meta-analysis are based on 63 herds with different management conditions and can therefore be applied to high-producing dairy herds under confinement housing.

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REFERENCES

- Astiz, S., and O. Fargas. 2013. Pregnancy per AI differences between primiparous and multiparous high-yield dairy cows after using Double Ovsynch or G6G synchronization protocols. *Theriogenology* 79:1065–1070.
- Ayres, H., R. M. Ferreira, A. P. Cunha, R. R. Araujo, and M. C. Wiltbank. 2013. Double-Ovsynch in high-producing dairy cows: Effects on progesterone concentrations and ovulation to GnRH treatments. *Theriogenology* 79:159–164.
- Baez, G. M., R. V. Barletta, J. N. Guenther, J. M. Gaska, and M. C. Wiltbank. 2016. Effect of uterine size on fertility of lactating dairy cows. *Theriogenology* 85:1357–1366.
- Bamber, R. L., G. E. Shook, M. C. Wiltbank, J. E. Santos, and P. M. Fricke. 2009. Genetic parameters for anovulation and pregnancy loss in dairy cattle. *J. Dairy Sci.* 92:5739–5753.
- Bicalho, R. C., S. H. Cheong, L. D. Warnick, and C. L. Guard. 2007. Evaluation of progesterone supplementation in a prostaglandin F2α-based presynchronization protocol before timed insemination. *J. Dairy Sci.* 90:1193–1200.

- Bisinotto, R. S., L. O. Castro, M. B. Pansani, C. D. Narciso, N. Martinez, L. D. Sinedino, T. L. Pinto, N. S. Van de Burgwal, H. M. Bosman, R. S. Surjus, W. W. Thatcher, and J. E. Santos. 2015. Progesterone supplementation to lactating dairy cows without a corpus luteum at initiation of the Ovsynch protocol. *J. Dairy Sci.* 98:2515–2528.
- Bisinotto, R. S., R. C. Chebel, and J. E. Santos. 2010. Follicular wave of the ovulatory follicle and not cyclic status influences fertility of dairy cows. *J. Dairy Sci.* 93:3578–3587.
- Bisinotto, R. S., E. S. Ribeiro, and J. E. Santos. 2014. Synchronisation of ovulation for management of reproduction in dairy cows. *Animal* 8(Suppl. 1):151–159.
- Borchardt, S., P. Haimel, and W. Heuwieser. 2016. Effect of insemination after estrous detection on pregnancy per artificial insemination and pregnancy loss in a Presynch-Ovsynch protocol: A meta-analysis. *J. Dairy Sci.* 99:2248–2256.
- Brusveen, D. J., A. P. Cunha, C. D. Silva, P. M. Cunha, R. A. Sterry, E. P. Silva, J. N. Guenther, and M. C. Wiltbank. 2008. Altering the time of the second gonadotropin-releasing hormone injection and artificial insemination (AI) during Ovsynch affects pregnancies per AI in lactating dairy cows. *J. Dairy Sci.* 91:1044–1052.
- Brusveen, D. J., A. H. Souza, and M. C. Wiltbank. 2009. Effects of additional prostaglandin F₂alpha and estradiol-17beta during Ovsynch in lactating dairy cows. *J. Dairy Sci.* 92:1412–1422.
- Caraviello, D. Z., K. A. Weigel, P. M. Fricke, M. C. Wiltbank, M. J. Florent, N. B. Cook, K. V. Nordlund, N. R. Zwald, and C. L. Rawson. 2006. Survey of management practices on reproductive performance of dairy cattle on large US commercial farms. *J. Dairy Sci.* 89:4723–4735.
- Carvalho, P. D., M. J. Fuenzalida, A. Ricci, A. H. Souza, R. V. Barletta, M. C. Wiltbank, and P. M. Fricke. 2015a. Modifications to Ovsynch improve fertility during resynchronization: Evaluation of presynchronization with gonadotropin-releasing hormone 6 d before initiation of Ovsynch and addition of a second prostaglandin F₂alpha treatment. *J. Dairy Sci.* 98:8741–8752.
- Carvalho, P. D., J. N. Guenther, M. J. Fuenzalida, M. C. Amundson, M. C. Wiltbank, and P. M. Fricke. 2014a. Presynchronization using a modified Ovsynch protocol or a single gonadotropin-releasing hormone injection 7 d before an Ovsynch-56 protocol for submission of lactating dairy cows to first timed artificial insemination. *J. Dairy Sci.* 97:6305–6315.
- Carvalho, P. D., A. H. Souza, M. C. Amundson, K. S. Hackbart, M. J. Fuenzalida, M. M. Herlihy, H. Ayres, A. R. Dresch, L. M. Vieira, J. N. Guenther, R. R. Grummer, P. M. Fricke, R. D. Shaver, and M. C. Wiltbank. 2014b. Relationships between fertility and postpartum changes in body condition and body weight in lactating dairy cows. *J. Dairy Sci.* 97:3666–3683.
- Carvalho, P. D., M. C. Wiltbank, and P. M. Fricke. 2015b. Manipulation of progesterone to increase ovulatory response to the first GnRH treatment of an Ovsynch protocol in lactating dairy cows receiving first timed artificial insemination. *J. Dairy Sci.* 98:8800–8813.
- Carvalho, P. D., M. C. Wiltbank, and P. M. Fricke. 2015c. Progesterone concentration at each treatment during an Ovsynch protocol affects fertility to timed AI in lactating Holstein cows. *J. Dairy Sci.* 98(Suppl. 2):92. (Abstr.)
- Chebel, R. C., and J. E. Santos. 2010. Effect of inseminating cows in estrus following a presynchronization protocol on reproductive and lactation performances. *J. Dairy Sci.* 93:4632–4643.
- Colazo, M. G., P. Ponce-Barajas, and D. J. Ambrose. 2013. Pregnancy per artificial insemination in lactating dairy cows subjected to 2 different intervals from presynchronization to initiation of Ovsynch protocol. *J. Dairy Sci.* 96:7640–7648.
- DerSimonian, R., and N. Laird. 1986. Meta-analysis in clinical trials. *Control. Clin. Trials* 7:177–188.
- Dickersin, K. 2002. Systematic reviews in epidemiology: Why are we so far behind? *Int. J. Epidemiol.* 31:6–12.
- Egger, M., M. Schneider, and G. Davey Smith. 1998. Spurious precision? Meta-analysis of observational studies. *BMJ* 316:140–144.
- Egger, M., G. Smith, and D. G. Altman. 2001. Systematic Reviews in Health Care. Meta-Analysis in Context. *BMJ Books*, London, UK.
- El-Zarkouny, S. Z., J. A. Cartmill, B. A. Hensley, and J. S. Stevenson. 2004. Pregnancy in dairy cows after synchronized ovulation regimens with or without presynchronization and progesterone. *J. Dairy Sci.* 87:1024–1037.
- Ferguson, J. D., and A. Skidmore. 2013. Reproductive performance in a select sample of dairy herds. *J. Dairy Sci.* 96:1269–1289.
- Freeman, M. F., and J. W. Tukey. 1950. Transformation related to the angular and the square root. *Ann. Math. Stat.* 21:607–611.
- Fricke, P. M., J. O. Giordano, A. Valenza, G. Lopes Jr., M. C. Amundson, and P. D. Carvalho. 2014. Reproductive performance of lactating dairy cows managed for first service using timed artificial insemination with or without detection of estrus using an activity-monitoring system. *J. Dairy Sci.* 97:2771–2781.
- Fuenzalida, M. J., P. M. Fricke, and P. L. Ruegg. 2015. The association between occurrence and severity of subclinical and clinical mastitis on pregnancies per artificial insemination at first service of Holstein cows. *J. Dairy Sci.* 98:3791–3805.
- Galvão, K. N., M. F. Sá Filho, and J. E. Santos. 2007. Reducing the interval from presynchronization to initiation of timed artificial insemination improves fertility in dairy cows. *J. Dairy Sci.* 90:4212–4218.
- Giordano, J. O., M. L. Stangaferro, R. Wijma, W. C. Chandler, and R. D. Watters. 2015. Reproductive performance of dairy cows managed with a program aimed at increasing insemination of cows in estrus based on increased physical activity and fertility of timed artificial inseminations. *J. Dairy Sci.* 98:2488–2501.
- Giordano, J. O., M. C. Wiltbank, P. M. Fricke, S. Bas, R. Pawlisch, J. N. Guenther, and A. B. Nascimento. 2013. Effect of increasing GnRH and PGF₂alpha dose during Double-Ovsynch on ovulatory response, luteal regression, and fertility of lactating dairy cows. *Theriogenology* 80:773–783.
- Giordano, J. O., M. C. Wiltbank, J. N. Guenther, M. S. Ares, G. Lopes Jr., M. M. Herlihy, and P. M. Fricke. 2012a. Effect of presynchronization with human chorionic gonadotropin or gonadotropin-releasing hormone 7 days before resynchronization of ovulation on fertility in lactating dairy cows. *J. Dairy Sci.* 95:5612–5625.
- Giordano, J. O., M. C. Wiltbank, J. N. Guenther, R. Pawlisch, S. Bas, A. P. Cunha, and P. M. Fricke. 2012b. Increased fertility in lactating dairy cows resynchronized with Double-Ovsynch compared with Ovsynch initiated 32 d after timed artificial insemination. *J. Dairy Sci.* 95:639–653.
- Gumen, A., A. Keskin, G. Yilmazbas-Mecitoglu, E. Karakaya, A. Alkan, H. Okut, and M. C. Wiltbank. 2012. Effect of presynchronization strategy before Ovsynch on fertility at first service in lactating dairy cows. *Theriogenology* 78:1830–1838.
- Herlihy, M. M., J. O. Giordano, A. H. Souza, H. Ayres, R. M. Ferreira, A. Keskin, A. B. Nascimento, J. N. Guenther, J. M. Gaska, S. J. Kacuba, M. A. Crowe, S. T. Butler, and M. C. Wiltbank. 2012. Presynchronization with Double-Ovsynch improves fertility at first postpartum artificial insemination in lactating dairy cows. *J. Dairy Sci.* 95:7003–7014.
- Higgins, J. P., S. G. Thompson, J. J. Deeks, and D. G. Altman. 2003. Measuring inconsistency in meta-analyses. *BMJ* 327:557–560.
- Lima, F. S., R. S. Bisinotto, E. S. Ribeiro, L. F. Greco, H. Ayres, M. G. Favoreto, M. R. Carvalho, K. N. Galvão, and J. E. Santos. 2013. Effects of 1 or 2 treatments with prostaglandin F₂(2)alpha on subclinical endometritis and fertility in lactating dairy cows inseminated by timed artificial insemination. *J. Dairy Sci.* 96:6480–6488.
- Moreira, F., C. Orlandi, C. A. Risco, R. Mattos, F. Lopes, and W. W. Thatcher. 2001. Effects of presynchronization and bovine somatotropin on pregnancy rates to a timed artificial insemination protocol in lactating dairy cows. *J. Dairy Sci.* 84:1646–1659.
- Navanukraw, C., D. A. Redmer, L. P. Reynolds, J. D. Kirsch, A. T. Grazul-Bilska, and P. M. Fricke. 2004. A modified presynchronization protocol improves fertility to timed artificial insemination in lactating dairy cows. *J. Dairy Sci.* 87:1551–1557.
- Poock, S. E., W. R. Lamberson, and M. C. Lucy. 2015. Effect of different gonadorelin (GnRH) products used for the first or resynchronized timed artificial insemination on pregnancy rates in postpartum dairy cows. *Theriogenology* 84:504–508.

- Pursley, J. R., M. O. Mee, and M. C. Wiltbank. 1995. Synchronization of ovulation in dairy cows using PGF α and GnRH. *Theriogenology* 44:915–923.
- Ribeiro, E. S., A. P. Monteiro, F. S. Lima, H. Ayres, R. S. Bisinotto, M. Favoreto, L. F. Greco, R. S. Marsola, W. W. Thatcher, and J. E. Santos. 2012. Effects of presynchronization and length of proestrus on fertility of grazing dairy cows subjected to a 5-day timed artificial insemination protocol. *J. Dairy Sci.* 95:2513–2522.
- Rivera, F., C. Narciso, R. Oliveira, R. L. Cerri, A. Correa-Calderon, R. C. Chebel, and J. E. Santos. 2010. Effect of bovine somatotropin (500 mg) administered at ten-day intervals on ovulatory responses, expression of estrus, and fertility in dairy cows. *J. Dairy Sci.* 93:1500–1510.
- Rutigliano, H. M., F. S. Lima, R. L. Cerri, L. F. Greco, J. M. Vilela, V. Magalhaes, F. T. Silvestre, W. W. Thatcher, and J. E. Santos. 2008. Effects of method of presynchronization and source of selenium on uterine health and reproduction in dairy cows. *J. Dairy Sci.* 91:3323–3336.
- Santos, J. E., R. S. Bisinotto, E. S. Ribeiro, F. S. Lima, L. F. Greco, C. R. Staples, and W. W. Thatcher. 2010. Applying nutrition and physiology to improve reproduction in dairy cattle. *Soc. Reprod. Fertil. Suppl.* 67:387–403.
- Santos, V. G., P. D. Carvalho, C. Maia, B. Carneiro, A. Valenza, P. M. Crump, and P. M. Fricke. 2016. Adding a second prostaglandin F α treatment to but not reducing the duration of a PRID-Synch protocol increases fertility after resynchronization of ovulation in lactating Holstein cows. *J. Dairy Sci.* 99:3869–3879.
- Souza, A. H., H. Ayres, R. M. Ferreira, and M. C. Wiltbank. 2008. A new presynchronization system (Double-Ovsynch) increases fertility at first postpartum timed AI in lactating dairy cows. *Theriogenology* 70:208–215.
- Sterry, R. A., M. L. Welle, and P. M. Fricke. 2006. Treatment with gonadotropin-releasing hormone after first timed artificial insemination improves fertility in noncycling lactating dairy cows. *J. Dairy Sci.* 89:4237–4245.
- Stevenson, J. S. 2016a. Synchronization and artificial insemination strategies in dairy herds. *Vet. Clin. North Am. Food Anim. Pract.* 32:349–364.
- Stevenson, J. S. 2016b. Physiological predictors of ovulation and pregnancy risk in a fixed-time artificial insemination program. *J. Dairy Sci.* 99:10077–10092.
- Stevenson, J. S., S. L. Pulley, and H. I. Mellieon Jr. 2012. Prostaglandin F α and gonadotropin-releasing hormone administration improve progesterone status, luteal number, and proportion of ovular and anovular dairy cows with corpora lutea before a timed artificial insemination program. *J. Dairy Sci.* 95:1831–1844.
- Tempelman, R. J. 2009. Invited review: Assessing experimental designs for research conducted on commercial dairies. *J. Dairy Sci.* 92:1–15.
- Vasconcelos, J. L., R. W. Silcox, G. J. Rosa, J. R. Pursley, and M. C. Wiltbank. 1999. Synchronization rate, size of the ovulatory follicle, and pregnancy rate after synchronization of ovulation beginning on different days of the estrous cycle in lactating dairy cows. *Theriogenology* 52:1067–1078.
- Wiltbank, M. C., G. M. Baez, F. Cochrane, R. V. Barletta, C. R. Trayford, and R. T. Joseph. 2015. Effect of a second treatment with prostaglandin F α during the Ovsynch protocol on luteolysis and pregnancy in dairy cows. *J. Dairy Sci.* 98:8644–8654.
- Wiltbank, M. C., and J. R. Pursley. 2014. The cow as an induced ovulator: Timed AI after synchronization of ovulation. *Theriogenology* 81:170–185.