Short communication: Effect of adding a second prostaglandin $F_{2\alpha}$ injection during the Ovsynch protocol on luteal regression and fertility in lactating dairy cows: A meta-analysis

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ABSTRACT

Incomplete luteal regression after treatment with a single dose of prostaglandin $F_{2\alpha}$ during an Ovsynch protocol decreases fertility to timed artificial insemination (TAI). To increase the proportion of cows with complete luteal regression and subsequently pregnancy per artificial insemination (P/AI), an additional treatment with $PGF_{2\alpha}$ 24 h after the first has been recommended. A systematic review of the literature and meta-analytical assessment were performed with the objective of evaluating the effects of adding a second $PGF_{2\alpha}$ treatment during the Ovsynch protocol on luteal regression and reproductive performance in lactating dairy cows. Based on the heterogeneity among the experimental treatments, a fixed or a random effects meta-analysis was conducted. Reproductive outcomes of interest were luteal regression at the end of the Ovsynch protocol, and P/AI measured 32 to 39 d after TAI. Seven randomized controlled experiments from 6 published manuscripts including 5,356 cows with the primary objective to evaluate the effect of an additional treatment with $PGF_{2\alpha}$ during the Ovsynch protocol on P/AI were used. Information regarding luteal regression at the end of the Ovsynch protocol was available for 1,856 cows. Adding a second $PGF_{2\alpha}$ treatment on d 8 during the Ovsynch protocol increased the relative risk (RR) of complete luteal regression at the end of the Ovsynch protocol (RR = 1.14; 95% confidence interval = 1.10 to 1.17) using a fixed effects model and the RR for pregnancy (RR = 1.14; 95% confidence interval = 1.06 to 1.22) 32 d after TAI using a fixed effects model. No heterogeneity was observed among the 6 manuscripts regarding complete luteal regression and P/AI. In summary, there was a clear benefit of an additional $PGF_{2\alpha}$ treatment during the Ovsynch protocol

on luteal regression (+11.6 percentage units) and on P/AI (+4.6 percentage units).

Key words: dairy cow, meta-analysis, prostaglandin, timed artificial insemination

Short Communication

Synchronization protocols for timed artificial insemination (**TAI**) are widely adopted in the dairy industry (Caraviello et al., 2006). The most common TAI protocol is a 7-d Ovsynch protocol (Stevenson, 2016a). Initially, the Ovsynch protocol increased the insemination risk without affecting the risk of pregnancy (Pursley et al., 1995). Different strategies [e.g., manipulation of progesterone (**P4**), presynchronization, timing of AI] were developed to increase pregnancy per AI (**P/AI**; Wiltbank and Pursley, 2014). Progesterone concentration at each treatment during an Ovsynch protocol affects P/AI to TAI (Carvalho et al., 2015b). At the end of the protocol, near TAI, cows with increased circulating P4 concentrations had decreased P/AI as a result of incomplete luteal regression. In a large data set compiled by Carvalho et al. (2015b), there was a 66% relative decrease in P/AI for cows with $P4 \geq 0.4$ ng/mL (14%; 161/435) than for cows with $P4 < 0.4$ ng/mL (41%; 1,125/2,713) at the time of the second GnRH treatment (**G2**) during the Ovsynch protocol. Lack of complete regression of the corpus luteum (**CL**) to a single $PGF_{2\alpha}$ treatment has been observed in 12 to 21% of cows treated with Ovsynch (Brusveen et al., 2009; Carvalho et al., 2015a; Wiltbank et al., 2015; Heidari et al., 2017; Barletta et al., 2018). Two strategies have been used to overcome the problem of incomplete CL regression. Cows were either given an increased dose of $PGF_{2\alpha}$, 750 μ g instead of 500 μ g (Giordano et al., 2013), or a second treatment of $PGF_{2\alpha}$ was given on the subsequent day after the first $PGF_{2\alpha}$ treatment (Brusveen et al., 2009; Carvalho et al., 2015a; Wiltbank et al., 2015; Santos et al., 2016; Heidari et al., 2017; Barletta et al., 2018). Most studies showed a significant treatment effect on the proportion of cows with

Received November 24, 2017.

Accepted April 25, 2018.

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complete luteolysis of 10 to 15 percentage units. The proof of an effect on P/AI, however, is challenging to demonstrate because of the required sample size for individual experiments to detect a significant difference of relatively small magnitude (i.e., 3 to 5 percentage units) based on a binary outcome.

Furthermore, individual studies often encompass a limited number of herds with similar management practices, climatic conditions, and genetic background. This might limit the inference observed for a treatment effect (Tempelman, 2009). With the exception of one study (Wiltbank et al., 2015) where numerous herds were included, all other studies used 1 to 3 herds to analyze the effect of an additional treatment with PGF_{2α} in an Ovsynch protocol. Therefore, this meta-analysis might be useful to evaluate the effect of adding a second PGF_{2α} treatment during the Ovsynch protocol across different managerial conditions. The main hypothesis of the present study was that additional treatment with PGF_{2α} on d 8 of the Ovsynch protocol would increase the proportion of cows with complete luteal regression at the end of the Ovsynch protocol thereby increasing P/AI.

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 Ovsynch” and “dairy cow AND second prostaglandin.” Additional manuscripts were obtained directly from researchers in the field of reproductive biology. Results from the literature search were assessed individually for the initial screening to be considered for the meta-analysis.

We considered only randomized controlled studies directly evaluating the effect of an additional PGF_{2α} treatment on d 8 during the Ovsynch protocol for TAI. The time interval between the first GnRH treatment and the first PGF_{2α} treatment in the Ovsynch protocol had to be 7 d. The time interval from the last GnRH treatment in the Ovsynch protocol until TAI had to range from 12 to 20 h. Hence, studies or experimental treatments using other protocols for timed AI (e.g., 5d-Cosynch) were not included in this analysis. In manuscripts with a significant ($P < 0.05$) effect of a certain treatment on P/AI, we only considered the control treatment (i.e., without treatment effect) for evaluation. Otherwise we combined the data from control and treated cows regarding P/AI.

Based on these criteria, the meta-analysis included a total of 6 manuscripts including 5,356 cows using an Ovsynch protocol for TAI (Table 1). Of these, 4 manuscripts were conducted using high-producing cows under confinement housing in the United States, 1 in Portugal, and 1 in Iran.

Table 1. Summary of manuscripts (n = 6) using either 1 (1PGF) or 2 (2PGF) doses of PGF_{2α} during Ovsynch protocol considering evaluated information criteria

Reference	Country	No. of AI	No. of herds	AI ¹	Timing of P4 measurement ²	No. of cows sampled for P4 measurement	P4 threshold (ng/mL) to define luteal regression
Barletta et al. (2018)	United States	736	1	2+	G2	270	0.4
Brusveen et al. (2009)	United States	379	2	1st	G2	340	0.4
Carvalho et al. (2015a)	United States	897	2	1st and 2+	G2	600	0.4
Heidari et al. (2017)	Iran	293	1	1st	TAI	100	0.4
Santos et al. (2016)	Portugal	534	3	2+	G2	202	0.4
Wiltbank et al. (2015), experiment 1	United States	373	1	1st	G2	344	0.5
Wiltbank et al. (2015), experiment 2	United States	2,148	11	1st and 2+	NA ³	NA	NA

¹Service number (1st = first AI postpartum; 2+ = resynchronized AI).

²Timing of progesterone (P4) measurement relative to the Ovsynch protocol (GnRH d 0 – PGF_{2α} d 7 – GnRH d 9 – timed AI d 10). G2 = d 9. Timed AI (TAI) = d 10.

³NA = not available.

Data extraction was performed by a single investigator (S. Borchardt) and validated by the coauthors (A. Pohl and W. Heuwieser). For each study, recorded information included authors, year of publication, number of herds, sample size calculation, stratification of results by parity and AI number, GnRH product and dose, PGF_{2α} product and dose, time schedule of the breeding Ovsynch protocol, DIM at TAI, method and timing of P4 analysis, and threshold to define incomplete luteal regression. Relevant information is summarized in Table 1.

Experimental groups were classified according to the number of PGF_{2α} treatments in the Ovsynch protocol into **1PGF** (GnRH d 0 – PGF_{2α} d 7 – GnRH d 9) and **2PGF** (GnRH d 0 – PGF_{2α} d 7 – PGF_{2α} d 8 – GnRH d 9).

Luteal regression after PGF_{2α} treatment was assessed within the 6 manuscripts using blood P4 concentration at G2 (5/6 manuscripts) or at the time of TAI (1/6 manuscripts). Different thresholds ranging from 0.4 to 0.5 ng/mL were used to define complete luteal regression.

The range for the first pregnancy diagnosis was 32 to 39 d after TAI. For simplicity, the time at which pregnancy was evaluated for the first time will be referred to as 32 d after TAI throughout the manuscript.

Reproductive outcomes of interest were luteal regression at the end of the Ovsynch protocol and P/AI. Within the study, complete luteal regression was calculated as the number of cows with blood P4 below the threshold divided by the total number of cows sampled. Pregnancies per AI were calculated as the number of cows diagnosed pregnant after TAI divided by the total number of cows inseminated.

The meta-analysis was conducted using MedCalc (version 15.6.1, MedCalc Software, Mariakerke, Belgium) as described elsewhere (Borchardt et al., 2016).

Briefly, we included 6 manuscripts with information on luteal regression at the end of the Ovsynch protocol from 1,856 cows and information on P/AI for TAI from 5,356 cows. Cows in the 1PGF treatment served as the control group. We calculated the relative risk (**RR**) for having a complete luteal regression and diagnosed pregnant 32 d after TAI using a fixed effects and a random effects model, respectively. Under the fixed effects model, we 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). If heterogeneity is significant, however, 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 RR under the fixed effects model. The heterogeneity statistic is incorporated to calculate the summary RR under the random effects model (DerSimonian and Laird, 1986).

Heterogeneity 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. 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).

A second treatment with PGF_{2α} on d 8 during the Ovsynch protocol increased luteal regression by 11.6 percentage units (RR = 1.14; 95% CI = 1.10 to 1.17) at the end of the Ovsynch protocol using the fixed effects model (Table 2). No heterogeneity ($I^2 = 0.00\%$; $P = 0.450$) was observed among the 6 manuscripts regarding luteal regression.

Adding a second PGF_{2α} treatment during the Ovsynch protocol increased the RR for pregnancy (RR = 1.14; 95% CI = 1.06 to 1.22) in lactating dairy cows 32 d after TAI using the fixed effects model (Table 3). The increment in P/AI was 4.6 percentage units. No heterogeneity ($I^2 = 0.00\%$; $P = 0.942$) was observed among the 6 manuscripts regarding P/AI. The 95% confidence intervals for all of the individual studies varied moderately with each of the 6 studies indicating no significant effect of an additional PGF_{2α} treatment during the Ovsynch protocol on P/AI.

The results from the present study support the concept that incorporating a second PGF_{2α} treatment on d 8 during an Ovsynch protocol increased luteolysis at the end of the Ovsynch protocol, thereby increasing P/AI in lactating dairy cows.

Understanding the limitations of the standard Ovsynch protocol is critical to maximize its fertility (Stevenson, 2016b). Requirements for maximum P/AI in the standard Ovsynch protocol include (1) growth of the follicle during an adequate P4 environment either by inducing ovulation of a dominant follicle after the first GnRH (**G1**) treatment (Galvão et al., 2007; Giordano et al., 2013; Carvalho et al., 2015c) or by having a functional CL present at G1 (Stevenson, 2016b); (2) inducing complete luteolysis in response to PGF_{2α} treatment as evidenced by P4 concentrations <0.4 ng/mL (Carvalho et al., 2015b); and (3) timely ovulation of the preovulatory follicle in response to G2 (Pursely et al., 1998; Stevenson, 2016b).

Ovulatory response to G1 is dependent on the stage of the estrous cycle at which GnRH is administered

Table 2. Effect of an additional treatment with PGF_{2α} during the Ovsynch protocol (n = 1,856) on luteal regression at the end of the protocol considering 6 manuscripts with a randomized controlled study design

Manuscript ¹	Luteal regression		Relative risk ²	95% CI	P-value
	1PGF	2PGF			
Barletta et al. (2018)	112/128	133/142	1.07	0.99–1.16	
Brusveen et al. (2009)	151/178	155/162	1.13	1.05–1.21	
Carvalho et al. (2015a)	262/312	281/288	1.16	1.10–1.22	
Heidari et al. (2017)	28/50	35/50	1.25	0.92–1.70	
Santos et al. (2016)	89/100	100/102	1.10	1.02–1.19	
Wiltbank et al. (2015)	146/176	163/168	1.17	1.09–1.26	
$I^2 = 0.00\%$ ($P = 0.450$)					
Total (fixed effects)	788/944	867/912	1.14	1.10–1.17	0.001
Total (random effects)	788/944	867/912	1.13	1.10–1.17	0.001
Pooled proportion (%)	83.5	95.1			

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

²Relative risk for having a blood progesterone concentration below a certain threshold at the end of the Ovsynch protocol using a single PGF_{2α} treatment (1PGF) compared with 2 PGF_{2α} treatments (2PGF) during the Ovsynch protocol.

with cows on d 5 to 9 of the estrous cycle having the greatest ovulatory response (Vasconcelos et al., 1999). Therefore, presynchronization protocols (i.e., Presynch-Ovsynch, G6G, Double-Ovsynch) have been implemented to increase the proportion of cows in early diestrus at G1 and thereby increase the risk of ovulation and ultimately P/AI (Wiltbank and Pursley, 2014). Multiple studies showed that ovulation to G1 in an Ovsynch protocol improved P/AI (Galvão et al., 2007; Giordano et al., 2013; Carvalho et al., 2015c). The effect of ovulation at G1, however, seems to be limited to cows lacking a functional CL at G1 (Giordano et al., 2012). This finding is supported by recent evidence indicating that not the lack of ovulation to G1, but the concentration of P4 during follicular development seems to be more important for improving P/AI (Stevenson, 2016b). Although ovulation to G1

increased P/AI by creating a more adequate 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_{2α} 7 d later in an Ovsynch protocol. About 60% of cows ovulate a follicle after G1 and form a new CL (Stevenson, 2016b). Cows that ovulated after G1 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., 2012). The lack of luteolytic response from that new CL, however, seems to be conditional on the co-existence of an older CL already present at G1 that is mature enough to undergo luteolysis after treatment with exogenous PGF_{2α}. The benefit of a second PGF_{2α} treatment was restricted to cows with only a G1-induced CL at the time of PGF_{2α} injection (Stevenson, 2016b). In that particular study,

Table 3. Effect of an additional treatment with PGF_{2α} during the Ovsynch protocol (n = 5,356) on pregnancy per AI considering 6 manuscripts with a randomized controlled study design

Manuscript ¹	Pregnancy per AI		Relative risk ²	95% CI	P-value
	1PGF	2PGF			
Barletta et al. (2018)	107/349	137/387	1.15	0.94–1.42	
Brusveen et al. (2009)	78/197	88/182	1.22	0.97–1.53	
Carvalho et al. (2015a)	150/462	168/435	1.19	1.00–1.42	
Heidari et al. (2017)	49/149	54/144	1.14	0.83–1.56	
Santos et al. (2016)	95/266	111/268	1.16	0.93–1.44	
Wiltbank et al. (2015)	436/1,266	471/1,251	1.09	0.98–1.21	
$I^2 = 0.00\%$ ($P = 0.942$)					
Total (fixed effects)	915/2,689	1,029/2,667	1.14	1.06–1.22	0.001
Total (random effects)	915/2,689	1,029/2,667	1.14	1.06–1.22	0.001
Pooled proportion (%)	34.0	38.6			

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

²Relative risk for conceiving at timed AI using a single PGF_{2α} treatment (1PGF) compared with 2 PGF_{2α} treatments (2PGF) during the Ovsynch protocol.

incomplete luteolysis in response to 1 PGF_{2α} treatment was less prevalent when a more mature CL was co-existent with the new G1-induced CL (old CL + new CL 9.9% vs. only new CL 15.7%).

Low circulating P4 concentration after PGF_{2α} treatment is critically important for P/AI in Ovsynch-type protocols (Carvalho et al., 2015b; Stevenson, 2016b). After ovulation in response to G2, complete luteal regression was highly predictive of pregnancy in an Ovsynch protocol (Stevenson, 2016b). Even a modest increase in P4 at G2 resulted in a dramatic decrease in P/AI. Therefore, several recent studies have evaluated the addition of a second PGF_{2α} treatment 24 h after the first PGF_{2α} treatment during an Ovsynch protocol to increase luteolysis and P/AI (Brusveen et al., 2009; Carvalho et al., 2015a; Wiltbank et al., 2015; Barletta et al., 2018; Santos et al., 2016; Heidari et al., 2017). Although most of the studies reported a significant ($P < 0.05$) increase in the proportion of cows with complete luteolysis ranging from 6 to 14 percentage units after addition of a second PGF_{2α} treatment on d 8, most of them failed to detect a significant effect of treatment on 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. Although a clear increase occurred in the proportion of cows with complete luteal regression after the additional treatment with PGF_{2α} on d 8, the resulting increase in P/AI in all treated cows was rather small. The likelihood of detecting a treatment effect on P/AI was limited because most of the individual studies were statistically underpowered to detect a 3 to 5 percentage point increase in P/AI. Combining the results of multiple studies using a meta-analytical assessment can help to prove biologically important treatment effects of a relatively small magnitude. This seems even more important when the outcome of interest is a binomial outcome (i.e., P/AI). Adding a second PGF_{2α} treatment on d 8 during the Ovsynch protocol increased P/AI by 4.6 percentage points. No heterogeneity was observed among the studies regarding the effect of 2 PGF_{2α} treatments on P/AI, although different presynchronization methods were used within the experimental groups.

Based on a meta-analytical assessment considering 6 manuscripts with a randomized controlled study design including 5,356 TAI, we evaluated the effect of an additional treatment with PGF_{2α} on d 8 during the Ovsynch protocol on luteolysis and P/AI in lactating dairy cows. An additional PGF_{2α} treatment during the Ovsynch protocol had a benefit on luteal regression and P/AI.

ACKNOWLEDGMENTS

This study was funded in part by Tiergyn e.V. (Berlin, Germany).

REFERENCES

- Barletta, R. V., P. D. Carvalho, V. G. Santos, L. F. Melo, C. E. Consentini, A. S. Netto, and P. M. Fricke. 2018. Effect of dose and timing of prostaglandin F2alpha treatments during a Resynch protocol on luteal regression and fertility to timed artificial insemination in lactating Holstein cows. *J. Dairy Sci.* 101:1730–1736.
- 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.
- Borchardt, S., P. Haimerl, 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.
- Borenstein, M., L. V. Hedges, J. P. Higgins, and H. R. Rothstein. 2009. *Introduction to Meta-Analysis*. Wiley, Chichester, UK.
- 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., A. H. Souza, M. C. Wiltbank, and P. M. Fricke. 2015b. Progesterone concentration at each treatment during an Ovsynch protocol affects fertility to timed AI in lactating Holstein cows. *J. Dairy Sci.* 98:(Suppl.):M236.
- Carvalho, P. D., M. C. Wiltbank, and P. M. Fricke. 2015c. 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.
- DerSimonian, R., and N. Laird. 1986. Meta-analysis in clinical trials. *Control. Clin. Trials* 7:177–188.
- Galvão, K. N., M. F. Sa 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. 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, R. Pawlisch, S. Bas, A. P. Cunha, and P. M. Fricke. 2012. 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.
- Heidari, F., E. Dirandeh, Z. Ansari Pirsaraei, and M. G. Colazo. 2017. Modifications of the G6G timed-AI protocol improved pregnancy per AI and reduced pregnancy loss in lactating dairy cows. *Animal* 11:2002–2009.
- Higgins, J. P., S. G. Thompson, J. J. Deeks, and D. G. Altman. 2003. Measuring inconsistency in meta-analyses. *BMJ* 327:557–560.

- Pursley, J. R., M. O. Mee, and M. C. Wiltbank. 1995. Synchronization of ovulation in dairy cows using PGF $_{2\alpha}$ and GnRH. *Theriogenology* 44:915–923.
- Pursley, J. R., R. W. Silcox, and M. C. Wiltbank. 1998. Effect of time of artificial insemination on pregnancy rates, calving rates, pregnancy loss, and gender ratio after synchronization of ovulation in lactating dairy cows. *J. Dairy Sci.* 81:2139–2144.
- 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 $_{2\alpha}$ 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.
- 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.
- 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 $_{2\alpha}$ 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.