J. Dairy Sci. 101:1–6
https://doi.org/10.3168/jds.2017-14191

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

S. Borchardt,* A. Pohl,* P. D. Carvalho,† P. M. Fricke,† and W. Heuwieser*[1]
*Clinic for Animal Reproduction, Faculty of Veterinary Medicine, Freie Universität Berlin, Koenigsweg 65, 14163 Berlin, Germany
†Department of Dairy Science, University of Wisconsin, Madison 53706

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 ($P = 0.450$) and P/AI ($P = 0.942$). 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 μg instead of 500 μg (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 complete luteolysis of 10 to 15 percentage units. The proof of an effect on P/AI, however, is challenging to

Received November 24, 2017.
Accepted April 25, 2018.
[1] Corresponding author: w.heuwieser@fu-berlin.de

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 $PGF_{2\alpha}$ in an Ovsynch protocol. Therefore, this meta-analysis might be useful to evaluate the effect of adding a second $PGF_{2\alpha}$ treatment during the Ovsynch protocol across different managerial conditions. The main hypothesis of the present study was that additional treatment with $PGF_{2\alpha}$ 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 $PGF_{2\alpha}$ treatment on d 8 during the Ovsynch protocol for TAI. The time interval between the first GnRH treatment and the first $PGF_{2\alpha}$ 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.

Data extraction was performed by a single investigator (S. Borchardt) and validated by the coauthors

Table 1. Summary of manuscripts (n = 6) using either 1 (1PGF) or 2 (2PGF) doses of $PGF_{2\alpha}$ 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

[1]Service number (1st = first AI postpartum; 2+ = resynchronized AI).
[2]Timing of progesterone (P4) measurement relative to the Ovsynch protocol (GnRH d 0 – $PGF_{2\alpha}$ d 7 – GnRH d 9 – timed AI d 10). G2 = d 9. Timed AI (TAI) = d 10.
[3]NA = not available.