J. Dairy Sci. 99:1–11
http://dx.doi.org/10.3168/jds.2015-9903

Expression of estrus improves fertility and decreases pregnancy losses in lactating dairy cows that receive artificial insemination or embryo transfer

M. H. C. Pereira,* M. C. Wiltbank,† and J. L. M. Vasconcelos*[1]
*Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista (UNESP), Botucatu, SP 18618-000, Brazil
†Department of Dairy Science, University of Wisconsin, Madison 53706

ABSTRACT

The objective was to evaluate if expression of estrus by dairy cattle altered fertility in timed artificial insemination (AI; n = 5,430) or timed embryo transfer (ET; n = 2,003) programs that used estradiol and progesterone (P4) to synchronize ovulation. Ovarian ultrasonography was performed on d 0 (time of AI) and 7 to determine ovulatory follicle diameter and ovulation. Only cows with a visible corpus luteum on d 7 were used in this study. At the time of controlled internal drug release removal, all cows received a tail-head device for detection of estrus and were considered in estrus when the paint of the device was completely removed by d 0. Circulating P4 concentrations were evaluated on d 7. Pregnancies per AI (P/AI) or ET (P/ET) were determined by ultrasonography on d 32 and 60. At d-32 pregnancy diagnosis, cows with expression of estrus had increased P/AI [no estrus = 25.5% (222/846) vs. estrus = 38.9% (1,785/4,584)] and P/ET [no estrus = 32.7% (193/606) vs. estrus = 46.2% (645/1,397)]. Similarly, at d-60 pregnancy diagnosis, expression of estrus increased P/AI [no estrus = 20.1% (179/846) vs. estrus = 33.3% (1,530/4,584)] and P/ET [no estrus = 25.1% (150/606) vs. estrus = 37.5% (525/1,397)]. Pregnancy loss was lower in cows that expressed estrus in timed AI [TAI; no estrus = 20.1% (43/222) vs. estrus 14.4% (255/1,785)] and timed ET [TET; no estrus = 22.7% (43/193) vs. estrus = 18.6% (120/645)] compared with cows with no estrus. Independent of expression of estrus cows ovulating either too small or too large of follicles had lower P/AI. No effect of ovulatory follicle diameter on P/ET was noted in cows that expressed estrus; although, cows that did not express estrus tended to have lower P/ET if they ovulated larger follicles. In cows that showed estrus, follicle diameter did not affect pregnancy loss, but cows that did not show estrus and ovulated larger follicles tended to have greater pregnancy loss after TAI and had greater pregnancy loss on TET. A positive effect of d-7 P4 concentrations on P/AI was observed, independent of estrus. In contrast, no effect of P4 was found on d 7 on P/ET. Thus, expression of estrus during protocols for TAI or TET is associated with an increase in fertility and reduction in pregnancy loss. During TAI programs, optimizing follicle diameter and increasing circulating P4 on d 7 after AI were also associated with increased fertility, independent of expression of estrus. However, in cows with TET, the association of fertility with either ovulatory follicle diameter or P4 on d 7 was less dramatic and seemed to be related to whether cows expressed estrus.

Key words: estrus, fertility, pregnancy loss

INTRODUCTION

Protocols for synchronization of the estrous cycle of lactating dairy cows have been used extensively for reproductive management. Classical synchronization protocols attempted to synchronize corpus luteum (**CL**) function to synchronize expression of estrus. More recent protocols are focused on synchronizing time of ovulation using different reproductive hormones that synchronize both CL function and follicular waves to allow AI at a fixed time at the end of the protocol (**FTAI**). In many parts of the world, protocols that synchronize the time of ovulation use estradiol (**E2**), progesterone (**P4**), and $PGF_{2\alpha}$ to synchronize ovarian function and time of ovulation to allow FTAI (Wiltbank and Pursley, 2014). The E2 and P4 protocols generally use E2-benzoate and treatment with a vaginal P4 implant at the start of the protocol to inhibit follicle development, allowing emergence of a synchronized follicular wave about 4 d after initiation of the protocol (Burke et al., 2001; Souza et al., 2009). The preovulatory dominant follicle develops from this synchronized follicular wave and ovulation is generally induced with another E2 treatment near the end of the protocol, such as E2-cypionate (**ECP**). Producers generally breed all cows at the FTAI, regardless of whether the cow expressed estrus, although previous

Received June 2, 2015.
Accepted November 8, 2015.
[1]Corresponding author: vasconcelos@fmvz.unesp.br

studies indicate that expression of estrus near FTAI is associated with greater fertility (Pancarci et al., 2002; Kasimanickam et al., 2005; Souza et al., 2007).

Serum E2 concentrations at FTAI have been positively correlated with ovulatory follicle diameter (Vasconcelos et al., 2001; Perry et al., 2005) and cows ovulating smaller follicles following GnRH injection experienced greater reproductive failure (Perry et al., 2005; Pereira et al., 2013a; Vasconcelos et al., 2013). An interaction between diameter of the preovulatory follicle, optimal hormonal environment (as demonstrated by expression of estrus), and pregnancy establishment and maintenance likely occurs (Jinks et al., 2013; Perry et al., 2014); although studies with large numbers of cows are required to adequately evaluate this intriguing concept. A variety of different strategies have been used to increase circulating E2 and stimulation of expression of estrus in FTAI protocols, such as addition of E2 at the end of the Ovsynch protocol (Souza et al., 2007; Brusveen et al., 2009), use of ECP treatment to induce ovulation (Pancarci et al., 2002; Cerri et al., 2004), increasing the interval between the luteolytic injection of $PGF_{2\alpha}$ and the time of AI (Pereira et al., 2013b), and increasing the length of the protocol (Pereira et al., 2014). In Heatsynch protocols (d-10 GnRH, d-3 $PGF_{2\alpha}$, d-2 ECP, and d-0 FTAI), cows that displayed estrus after the ECP treatment had greater pregnancy per AI (**P/AI**) at FTAI [42.5% (306)] than cows that did not display estrus [21.1% (71)] at the end of the protocol (Cerri et al., 2004). In a different study using the Heatsynch protocol, expression of estrus improved P/AI at the pregnancy diagnosis performed at 27 (43.6 vs. 17.0%; $P < 0.01$) and 41 d (36.6 vs. 12.0%; $P < 0.01$) after FTAI, compared with cows not detected in estrus (Galvão et al., 2004). In E2 and P4 protocols, cows that did not display estrus during the protocol had a reduced percentage of cows with ovulation to the protocol (81.0 vs. 97.4%) and reduced fertility to FTAI (Pereira et al., 2014). Interestingly, even if only cows that ovulated to the protocol are considered in the analysis, P/AI was still reduced at 32 (39.4 vs. 51.2%) and 60 d (31.1 vs. 46.3%) in cows without estrus compared with cows that expressed estrus to the protocol (Pereira et al., 2014). In addition, most of these studies indicate some decrease in pregnancy loss in cows exposed to E2 during the preovulatory period (Madsen et al., 2015) or displaying estrus during FTAI protocols (Cerri et al., 2004; Galvão et al., 2004; Pereira et al., 2014). Consistent with this concept, a reduction in pregnancy loss was observed in cows synchronized with an E2 and P4 protocol [11% (16/135)], which induces elevated expression of estrus, compared with cow synchronized with the 5-d Cosynch protocol that uses GnRH to synchronize ovulation [19.6% (24/119)] and therefore had much less expression of estrus (Pereira et al., 2013a).

Thus, the main hypothesis for our study was that dairy cows that display estrus in response to E2 and P4 protocols would have greater fertility and reduced pregnancy loss compared with cows that did not express estrus. Further, we hypothesized that this fertility-enhancing effect of estrus would be observed in either cows receiving FTAI or cows that received fixed timed embryo transfer (**FTET**) following E2 and P4 protocols. Lastly, we hypothesized that this effect would be related to size of the ovulatory follicle on d 0 and circulating P4 on d 7. To test these hypotheses, we performed a retrospective analysis of past studies in which we had information on size of the ovulatory follicle and expression of estrus during E2 or P4 protocols for FTAI or FTET programs.

MATERIALS AND METHODS

This retrospective study was conducted on 13 different commercial dairy farms in Brazil, from January 2010 to January 2014. These data are combined from several previous experiments with some of the results published in previous scientific manuscripts (Pereira et al., 2013a,b, 2014, 2015). All animal procedures followed the recommendations of the *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (FASS, 1999). During the experimental period, cows were housed in freestall barns with access to an adjoining sod-based paddock. Throughout the experiment cows were milked 3 times daily. All procedures, including injections, ovarian ultrasonography, pregnancy diagnosis, blood collection, and FTAI or FTET, were performed while cows were restrained in self-locking head gates at the feedline. Cows were fed ad libitum a TMR based on corn silage and Tifton hay as forages, with a concentrate mix of corn and soybean meal including minerals and vitamins, which was balanced to meet or exceed the nutritional requirements of lactating dairy cows (NRC, 2001).

Animals and Treatments

A retrospective analysis was performed of past studies using 7,433 [AI = 5,430; embryo transfer (**ET**) = 2,003] lactating dairy cows that had ovulated in response to the following E2 or P4 protocol. The cows first received an intravaginal P4 implant containing 1.9 g of P4 (**CIDR**; Zoetis, São Paulo, SP, Brazil) inserted as the same time as treatment with 2.0 mg (i.m.) of estradiol benzoate (2.0 mL of Estrogin, Farmavet, São