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Follicular dynamics, circulating progesterone, and fertility in Holstein cows synchronized with reused intravaginal progesterone implants that were sanitized by autoclave or chemical disinfection

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ABSTRACT

This experiment aimed to compare circulating progesterone (P4), follicular dynamics, and fertility during reuse of intravaginal P4 implants that were sanitized by autoclave or chemical disinfection in lactating Holstein cows submitted to fixed-time artificial insemination (FTAI). For this, 123 primiparous and 226 multiparous cows from 2 farms, averaging (mean ± standard deviation) 163.9 ± 141.9 d in milk, 35.7 ± 11.3 kg of milk/d, and a body condition score of 2.9 ± 0.5, were enrolled in the study. Cows were randomly assigned to 1 of 2 treatments using a completely randomized design and each cow received a reused implant (1.9 g of P4; previously used for 8 d) that was either autoclaved (AUT; n = 177) or chemically disinfected (CHEM; n = 172) on d −10. Also on d −10, cows received 2 mg of estradiol benzoate and 100 μg of GnRH. On d −3, cows received 25 mg of dinoprost ($PGF_{2\alpha}$). A second $PGF_{2\alpha}$ was given on d −2, along with 1 mg of estradiol cypionate and P4 implant removal. Cows received FTAI on d 0. A subset of cows (n = 143) was evaluated by ultrasound on d −10, −8, −6, −3, −2, 0, and 5 to identify ovarian structures, and blood was sampled on d −10, −3, and −2 for P4 concentrations by RIA. Pregnancy diagnoses were performed at d 32 and 60. Statistical analyses was performed using PROC-MIXED for continuous variables and PROC-GLIMMIX of SAS 9.4 (SAS Institute Inc., Cary, NC) for binomial variables. The treatments did not differ in circulating P4 on d −10 or −3, but P4 was greater on d −2 in CHEM cows. Ovulation to the treatments on d −10 was associated with lower circulating P4 on d −10 (2.0 vs. 3.1 ng/mL) and resulted in greater P4 on d −3 (4.0 vs. 2.4 ng/mL) and more cows with a corpus luteum on d −3 (100 vs. 40%) than nonovulating cows. Cows that ovulated to d −10 treatments were more likely to have a synchronized new follicular wave (97.9 vs. 63.2%) and had an earlier wave emergence (1.9 vs. 2.6 d), resulting in less cows ovulating a persistent follicle (0.0 vs. 35.7%). Type of P4 implant, corpus luteum presence on d −10, and ovulation to d −10 treatments did not affect fertility (pregnancy per AI; P/AI). However, P/AI on farm A was greater than on farm B at 32 (40.8 vs. 27.8%) and 60 d (35.8 vs. 24.3%), independent of treatment. In conclusion, P4 implants with different P4 release patterns did not produce detectable differences in follicular dynamics, synchronization rate, or P/AI. Nevertheless, presence of corpus luteum or ovulation at the beginning of the FTAI protocol affected reproductive variables, such as timing and synchronization of follicular wave emergence, and size of the ovulatory follicle. Beyond that, more overall synchronized cows became pregnant to the FTAI protocol.
Key words: hormone, synchronization, device, *Bos taurus*

INTRODUCTION

Fixed-time artificial insemination (**FTAI**) programs are widely used worldwide and represent an important reproductive management tool to improve reproductive efficiency and profitability of commercial dairy herds (Norman et al., 2009). Although many dairies use AI as a way to improve the genetics of their herds with the use of proven sires (Vishwanath, 2003), challenges exist for maintaining good reproductive performance due to reduced detection of estrus (Washburn et al., 2002; Lopez et al., 2004) and declining pregnancies per AI (**P/AI**; Butler, 2000; Lucy, 2001; Washburn et al., 2002). The use of FTAI programs can reduce labor for managing AI by precisely synchronizing ovulation and have contributed to improvements in reproductive indexes (Wiltbank and Pursley, 2014).

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Since the first reported FTAI protocol was developed (Pursley et al., 1995), several modifications and improvements have been documented (Binelli et al., 2014; Wiltbank and Pursley, 2014); however, the main objectives continue to be the same: (1) synchronization of follicle wave emergence; (2) synchronization of corpus luteum (**CL**) function and circulating progesterone (**P4**); and (3) synchronization of final ovulation with optimally scheduled FTAI. To achieve these objectives, 2 major types of pharmaceutical approaches are available: (1) GnRH-based protocols, which use a combination of GnRH analogs at the beginning and at the end of the protocol, followed by 1 (Pursley et al., 1995; Souza et al., 2008) or 2 (Brusveen et al., 2009; Wiltbank et al., 2015) $PGF_{2\alpha}$ treatments; and (2) estradiol (**E2**)/P4-based protocols, which use a combination of P4/progestin and E2 esters, usually estradiol benzoate (**EB**), at the start of the protocol and 1 (Pereira et al., 2013a,b) or 2 $PGF_{2\alpha}$ treatments (Binelli et al., 2014; Pereira et al., 2015). These protocols also use E2 esters, EB or estradiol cypionate (**EC**), to synchronize ovulation at the end of the protocol.

These 2 types of hormonal protocols have different advantages and disadvantages. Treatment with GnRH at the beginning of the GnRH-based protocols can induce ovulation of the dominant follicle, if present, leading to initiation of a new follicular wave and formation of a new CL, potentially increasing circulating P4 concentrations during development of the preovulatory follicle wave (Pereira et al., 2015). However, many studies have reported that 50% or fewer dairy cows ovulate when GnRH is given at a random stage of the estrous cycle (Giordano et al., 2012b; Bilby et al., 2013; Bisinotto et al., 2013; Lopes et al., 2013, Melo et al., 2016). Lack of ovulation to the initial GnRH treatment leads to less than optimal follicle wave synchronization and fertility. On the other hand, the combination of P4 and E2 at the beginning of the protocol in E2/P4-based protocols leads to a suppression in secretion of gonadotropins (FSH and LH), causing regression of the follicles in the current follicular wave (Burke et al., 1996; Bó et al., 2002; Cavalieri et al., 2003) and initiation of a new follicular wave 3 to 5 d later. Although the protocol can be initiated at any stage of the estrous cycle, almost 30% of the cows do not have emergence of a new follicular wave after the initial E2/P4 treatment, leading to ovulation of a larger persistent follicle at the end of the protocol, which produces lower fertility (Monteiro et al., 2015).

To offset these problems, a combination of GnRH with E2/P4 treatments at the initiation of the FTAI protocol has been evaluated with the encouraging observation of improved fertility in lactating dairy cows submitted to a protocol that lasted 11 d and had 2 treatments with $PGF_{2\alpha}$ at the end of the protocol (Pereira et al., 2015). However, this initial study did not evaluate the follicular dynamics during the protocol and, in particular, whether ovulation of persistent follicles was avoided with this approach. Moreover, the ovarian physiological responses of cows treated with GnRH plus E2/P4 at the beginning of a protocol has not been tested, especially when using intravaginal inserts with distinct P4 release patterns.

Several researchers have reported the importance of adequate concentrations of P4 during preovulatory follicle development, particularly in FTAI programs (Diskin et al., 2006; Stevenson et al., 2006, 2008; Chebel et al., 2010; Cerri et al., 2011a,b; Bilby et al., 2013; Bisinotto et al., 2013). Depending on circulating P4 concentrations, the pattern of follicle development can be modified, and low circulating P4 during the growth of the ovulatory follicle is often associated with lower fertility in lactating dairy cows undergoing a FTAI protocol (Cerri et al., 2011b). Low concentration of P4 allows for increased LH pulse frequency, which could extend follicular dominance (Savio et al., 1993), compromise oocyte quality, possibly due to premature resumption of meiosis (Revah and Butler, 1996; Inskeep, 2004), and, consequently, reduce fertility (Cunha et al., 2008; Bisinotto et al., 2010). Adequate circulating P4 during development of the ovulatory follicle is particularly important in the nearly 30% of dairy cows that are anovular or lack a CL at the beginning of the FTAI protocol (Stevenson et al., 2008; Santos et al., 2009; Melo et al., 2016). In these cows, the risk of becoming pregnant is reduced by 30% (Bisinotto et al., 2010).

For the purposes of our physiological studies, we chose to compare the autoclaved versus chemically disinfected, reused 1.9-g P4 implants because of the dramatic differences in the P4 profile throughout the 8-d treatment period, as shown in our previous study (Melo et al., 2018). In this previous study, the average circulating P4 was greater ($P < 0.05$) for the autoclaved, reused controlled internal drug release (**CIDR**; 1.67 + 0.06 ng/mL) than the new CIDR (1.49 + 0.07), and both were greater than the chemically disinfected CIDR (1.21 + 0.05). Thus, we hypothesized that the greater circulating P4 that results from using autoclaved compared with chemically disinfected P4 implants would alter the patterns of follicle growth and these changes would be associated with an improvement in fertility. Thus, our objectives were to compare circulating P4, ovarian dynamics, and fertility in lactating dairy cows treated with reused 1.9-g intravaginal P4 implants that were previously autoclaved or chemically disinfected as part of an FTAI protocol that combined GnRH and EB treatment at the beginning of the protocol.