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Circulating progesterone concentrations in nonlactating Holstein cows during reuse of intravaginal progesterone implants sanitized by autoclave or chemical disinfection

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ABSTRACT

The aim of this study was to compare plasma progesterone (P4) concentrations in nonlactating, multiparous Holstein cows (n = 24) treated with 2 types of intravaginal implants containing either 1.0 or 1.9 g of P4 either at the first use or during reuse of the implants after sanitizing the implant by autoclave or chemical disinfection. In a completely randomized design with a 2 × 3 factorial arrangement and 2 replicates, every cow underwent 2 of 6 treatments. Two sources of P4 [controlled internal drug release (1.9 g of P4) from Zoetis (São Paulo, Brazil), and Sincrogest (1.0 g of P4) from Ourofino (Cravinhos, Brazil)] and 3 types of processing, new (N), reused after autoclave (RA), and reused after chemical disinfection (RC), were used. After inducing luteolysis to avoid endogenous circulating P4, the cows were randomized in 1 of 6 treatments (1.9 g of N, 1.9 g of RA, 1.9 g of RC, 1.0 g of N, 1.0 g of RA, and 1.0 g RC). Cows were treated with the implants for 8 d and during this period blood samples were collected at 0, 2, 12, 24, 48, 72, 96, 120, 144, 168, and 192 h. Statistical analyses were performed using Proc-Mixed and the mean ± standard error of the mean P4 concentrations were calculated using the Proc-Means procedures of SAS 9.4 (SAS Institute Inc., Cary, NC). No interaction between treatments was observed. Comparing types of implant, average P4 concentrations during treatments were greater for 1.9 g than 1.0 g (1.46 vs. 1.14 ± 0.04 ng/mL). When types of processing were compared, average P4 concentrations did not differ between autoclaved and new inserts (1.46 vs. 1.37 ± 0.05 ng/mL; respectively), but both were greater than chemically disinfected implants (1.09 ± 0.04 ng/mL). Within 1.9-g P4 inserts, P4 concentrations from autoclaved implants were greater than new, which were greater than chemically disinfected (1.67 ± 0.06 vs. 1.49 ± 0.07 vs. 1.21 ± 0.05 ng/mL; respectively). For 1.0-g P4 implants, P4 concentrations from autoclaved did not differ from new, but both were greater than chemically disinfected (1.20 ± 0.08 vs. 1.24 ± 0.06 vs. 0.97 ± 0.05 ng/mL; respectively). In conclusion, the mean plasma P4 concentration in nonlactating Holstein cows was greater for 1.9 than 1.0 g of P4 and regardless of the type of implant, the autoclaving process provided greater circulating P4 in relation to chemical disinfection, and similar or greater P4 concentrations compared with a new implant.
Key words: hormone, disinfection, device, *Bos taurus*

INTRODUCTION

Intravaginal progesterone (**P4**) inserts were initially developed to treat anovular heifers and cows in seasonally calving New Zealand herds with smaller cows with much lower milk production, and luteal phase circulating P4 concentrations could be achieved (Macmillan et al., 1991; Macmillan and Peterson, 1993). However, more recent studies have used these intravaginal P4 implants in high-producing dairy cattle and in whole-herd synchronization programs, with much lower circulating P4 concentrations being achieved (Rabiee et al., 2002a; Gümen and Wiltbank, 2005; Zuluaga and Williams, 2008; Bisinotto et al., 2013). In anovular cows, 2 intravaginal implants, rather than only 1, are required to achieve sufficient circulating P4 and normal fertility (Padula and Macmillan, 2006; Bisinotto et al., 2013; Pereira et al., 2017a,b). Several types of intravaginal P4 inserts are commercially available worldwide, with designs that allow retention within the vagina, usually with a T-shape, and prolonged delivery of P4, usually from P4-impregnated silicone molded over a nylon spine. In nonlactating ovariectomized cows, P4 inserts that have a similar surface area but contain 1.34 versus

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1.9 g of P4 release a similar amount of P4, on average, 620 and 610 mg of P4, respectively, over a period of 7 d (Rathbone et al., 2002). These treatments produced circulating P4 of ~4 ng/mL on the day after insertion, with concentrations at ~2.5 ng/mL by 7 d after insertion and few differences due to P4 load (10 to 30% wt/wt; P4:silicone) or presence of additives (liquid paraffin, arachis oil, or polyethylene glycol), as long as surface area was kept constant (Rathbone et al., 2002). However, increasing surface area of silicone available for release of P4 produced a linear increase in circulating P4, indicating the fundamental nature of this aspect of insert design. In anovular high-producing dairy cows, use of a single, new intravaginal P4 insert containing 1.34 g of P4 increased circulating P4 to only 0.8 to 1.0 ng/mL (Cerri et al., 2009; Lima et al., 2009), probably due to the greater P4 metabolism in lactating dairy cows related to elevated liver blood flow (Wiltbank et al., 2006). Thus, surface area for release of P4 and physiology of treated cows seem to be major determinants of the circulating P4 concentrations produced by treatment with P4 inserts.

In many countries, the reuse of intravaginal inserts is a common method to reduce costs of synchronization programs, although not recommended by manufacturers. For example, treatment of cows with a 1.9-g P4 insert for 7 d only removes ~600 mg of P4, leaving ~1.3 g of residual P4 load (Macmillan et al., 1991; Macmillan and Peterson, 1993; Rathbone et al., 2002). However, disinfection of the inserts before reuse is a major consideration, with producers primarily using either chemical disinfection of inserts or high-pressure steam sterilization using an autoclave (Zuluaga and Williams, 2008; Cerri et al., 2009; Long et al., 2009). Oral communication of results with reused P4 implants have been discussed in the scientific community, but publication of these results has generally not occurred due to concern from the manufacturer that off-label use could adversely affect product efficacy, product registrations with governmental agencies, or product sales. Thus, the P4 profiles have not been extensively evaluated in the scientific literature or directly compared following these 2 methods of disinfection before reuse of different intravaginal P4 implants containing different amounts of P4 in cattle.

Therefore, the objective of this experiment was to compare plasma P4 concentrations in cyclic nonlactating Holstein cows during use and reuse of intravaginal P4 inserts that originally contained 1.9 or 1.0 g of P4. Thus, along with evaluating the circulating P4 concentrations during use of implants with different P4 loads, we also evaluated whether circulating P4 would differ during reuse of the implants that were sanitized by 2 very different methods, using a high-pressure and -temperature autoclave or by chemical disinfection. The hypotheses for this experiment were that (1) plasma P4 concentrations during use of a new 1.9-g intravaginal P4 implant would be similar to the profile for a new 1.0-g intravaginal P4 implant; 2) independent of method of disinfection, plasma P4 concentrations during treatment with a reused implant would be greater for a 1.9-g implant compared with a 1.0-g implant; and (3) independent of type of implant, plasma P4 concentrations would be greater for an autoclaved reused implant than for a chemically disinfected reused implant, based on data from other studies (Cerri et al., 2009; Long et al., 2009).

MATERIALS AND METHODS

This experiment was conducted at the Department of Animal Science facilities at Escola Superior de Agricultura "Luiz de Queiroz"/University of São Paulo, located in Piracicaba city, São Paulo, Brazil. The Animal Research Ethics Committee of Escola Superior de Agricultura "Luiz de Queiroz"/University of São Paulo approved all procedures involving cows in this study.

For this study, 24 nonlactating multiparous cycling Holstein cows were used. At the beginning of the experiment, cows averaged 600 kg of BW and a BCS of 3 (Ferguson et al., 1994). Cows were kept in confinement with free access to water and mineral salt, and were fed a TMR maintenance diet (NRC, 2001) based on sugar cane bagasse as forage and concentrate based on corn and soybean meal, minerals, and vitamins.

Cows were randomly assigned to 1 of 6 treatment groups using a completely randomized design with a 2 × 3 factorial arrangement of treatments and 2 replicates, and every cow underwent 2 treatments. We used 2 sources of intravaginal P4 implants [controlled internal drug release (1.9 g) from Zoetis (São Paulo, Brazil), and Sincrogest (1.0 g) from Ourofino (Cravinhos, Brazil)] and 3 types of processing [new (**N**), reused autoclaved (**RA**), and reused chemically disinfected (**RC**)], resulting in the treatments 1.9 g N, 1.9 g RA, 1.9 g RC, 1.0 g N, 1.0 g RA, and 1.0 g RC.

At the beginning of the experiment (d 0), each cow had its estrous cycle synchronized with a new 1.9-g P4 implant that remained for 8 d. At 7 and 8 d after implant insertion, 25 mg of dinoprost tromethamine ($PGF_{2\alpha}$; Lutalyse, Zoetis) was administered, and on d 8, after the withdrawal of the P4 implant, a Norgestomet (Crestar; MSD, São Paulo, Brazil) ear implant was inserted, which was maintained for 48 h to avoid ovulation and allow for a complete drop in circulating P4. On d 10, cows were randomized to 1 of 6 treatments. The implants were left within the vagina for 8 d and during this period blood samples were collected