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Effect of manipulating progesterone before timed artificial insemination on reproductive and endocrine parameters in seasonal-calving, pasture-based Holstein-Friesian cows

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ABSTRACT

Fertility to timed AI (TAI) is profoundly affected by progesterone (P4) levels during hormonal synchronization protocols. Holstein-Friesian dairy cows managed in a seasonal-calving, pasture-based production system were randomly assigned to 2 treatments to manipulate P4 before TAI during growth of the preovulatory follicle. Cows in the first treatment (High P4; n = 30) were submitted to a Double-Ovsynch protocol {Pre-Ovsynch [GnRH; 7 d, $PGF_{2\alpha}$; 3 d, GnRH] followed 7 d later by Breeding-Ovsynch [GnRH (G1); 7 d $PGF_{2\alpha}$; 24 h, $PGF_{2\alpha}$; 32 h, GnRH (G2); 16 h, TAI]}. Cows in the second treatment (n = 30; Low P4) received the same Double-Ovsynch protocol but with an additional $PGF_{2\alpha}$ treatment 24 h after G1. Overall, synchronization rate did not differ between treatments and was 92% (55/60). Unexpectedly, 37% of Low P4 cows were detected in estrus ~24 h before scheduled TAI and were inseminated ~16 h before scheduled TAI. Overall, P4 did not differ between treatments at G1, whereas High P4 cows had greater P4 concentrations at $PGF_{2\alpha}$ and G2 than Low P4 cows. High P4 cows had the smallest mean follicle diameter at G2, whereas Low P4 cows with no estrus before TAI had intermediate mean follicle diameter at G2, and Low P4 cows with estrus before TAI had the largest mean follicle diameter. Low P4 cows with estrus before TAI had larger corpora lutea 15 d after TAI than Low P4 cows without estrus before TAI or High P4 cows. In accordance with corpus luteum size on d 15, High P4 cows and Low P4 cows without estrus before TAI had lower P4 from 4 to 46 d after TAI than Low P4 cows with estrus before TAI. Relative mRNA levels of the interferon-stimulated genes *ISG15*, *MX1*, *MX2*, and *OAS1* were greater for Low P4 than for High P4 cows, whereas relative mRNA levels of *RTP4* were greater for High P4 than for Low P4 cows 18 d after TAI. Treatment did not affect plasma pregnancy-associated glycoprotein concentrations after TAI; however, pregnancy-associated glycoprotein concentrations were affected by pregnancy status and parity. Treatment did not affect pregnancy per artificial insemination at 29, 39, or 60 d after TAI, and no pregnancy losses were observed from 39 to 60 d after TAI. We concluded that (1) Low P4 cows were more likely to express estrus than High P4 cows; (2) the subpopulation of Low P4 cows that expressed estrus had larger preovulatory follicles and greater P4 concentrations after TAI; and (3) regardless of estrus before TAI, all Low P4 cows had greater mRNA expression for 5 of 6 interferon-stimulated genes than High P4 cows 18 d after TAI.

Key words: progesterone, pregnancy-associated glycoprotein, interferon-stimulated genes, dairy cow

INTRODUCTION

Progesterone (**P4**) is the most biologically active progestogen in cattle and is produced and secreted primarily by the corpus luteum (**CL**) during the estrous cycle and the placenta during pregnancy. For cows inseminated to estrus, pregnancy per artificial insemination (**P/AI**) decreased 12.4 percentage points for every 1 ng/mL decrease in P4 concentration during the last half of the estrous cycle preceding the first AI (Fonseca et al., 1983). Fertility to timed artificial insemination (**TAI**) after hormonal synchronization protocols is profoundly affected by cyclicity status, as well as P4 concentration, at the onset of a synchronized breeding protocol. For example, cows with low P4 at the first GnRH treatment or at the $PGF_{2\alpha}$ treatment

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of an Ovsynch protocol have lower fertility than cows with high P4 (Fricke et al., 2003; Bisinotto et al., 2010; Carvalho et al., 2014). Increased P4 concentrations during the period of follicle growth before AI are associated with increased embryo quality (Cerri et al., 2009; Rivera et al., 2011) and increased fertility (Folman et al., 1990; Bisinotto et al., 2010; Dirandeh, et al., 2015b) in lactating dairy cows.

Steady-state P4 concentration in circulation is a balance between P4 production by the CL and P4 catabolism by the liver (Wiltbank et al., 2014). Because milk production is highly correlated (r = 0.88) with feed intake (Harrison et al., 1990), hepatic blood flow increases as milk production and feed intake increase, providing a potential physiological mechanism for decreased circulating P4 concentrations in high-producing lactating dairy cows (Sangsritavong et al., 2002; Vasconcelos et al., 2003). A potential mechanism by which low P4 during follicular growth could adversely affect embryo growth and development is overexposure of the oocyte to LH pulses during the final stages of follicular growth as a result of the low P4 environment (Kinder et al., 1996). Exposure of dominant follicles to increased LH pulses during the final stages of follicular growth before ovulation results in ovulation of a larger follicle (i.e., a persistent follicle; Ahmad et al., 1995), which generates a larger CL after ovulation that produces more P4 (Vasconcelos et al., 2001). Altering P4 before or after AI could in turn alter embryonic growth, thereby affecting maternal recognition of pregnancy or pregnancy loss. It is now possible to indirectly assess conceptus development 15 to 22 d after AI by measuring mRNA expression of interferon-stimulated genes (**ISG**) in circulating blood leukocytes (Stevenson et al., 2007; Gifford et al., 2008; Green et al., 2010) and by measuring pregnancy-associated glycoprotein (**PAG**) levels in blood or milk samples beginning approximately 25 d after AI (Green et al., 2000; Ricci et al., 2015).

The objective of this experiment was to hormonally manipulate Holstein-Friesian dairy cows managed in a seasonal-calving, pasture-based system into a high or a low P4 environment during the growth of a synchronized preovulatory follicle. Our hypotheses were that cows with low P4 would (1) ovulate larger follicles; (2) have more P4 after TAI; and (3) have decreased early embryonic development based on ISG expression and PAG levels than cows with high P4.

MATERIALS AND METHODS

Cows, Housing, and Feeding

This study was conducted from March to June 2014 at the Animal and Grassland Research and Innovation Centre at Teagasc, Moorepark (County Cork, Ireland). Holstein-Friesian dairy cows (n = 60) were managed as a single herd in a spring-calving, intensive rotational grazing system with a mating start date of May 1, 2014. Cows were housed in a freestall barn during the dry period. After parturition, cows were turned out to grass in early February and grazed under a rotational grazing system, as described by Dillon et al. (1995) in a predominantly perennial ryegrass (*Lolium perenne* L.) sward. Fresh pasture was allocated daily after morning milking by moving temporary fencing in the grazing paddocks. Cows were milked twice daily at 0730 and 1630 h throughout the experiment. All experimental procedures involving cows were approved by the Teagasc Animal Ethics Committee and authorized by the Health Products Regulatory Authority, which is the competent authority in Ireland responsible for the implementation of European Union legislation (Directive 2010/63/EU) for the protection of animals used for scientific purposes.

Experimental Design, Synchronization Treatments, and AI

Cows were enrolled in a completely randomized block experimental design and were blocked by lactation number and DIM at TAI (calculated based on the mating start date and calving dates of individual cows) as part of the randomization procedure. Holstein-Friesian cows (n = 60) were randomly assigned to each of 2 hormonal synchronization protocols to receive their first TAI (Figure 1). Synchronization protocols used i.m. treatments with GnRH (gonadorelin diacetate tetrahydrate; Ovarelin; Ceva Santé Animale, Libourne, France) and $PGF_{2\alpha}$ (dinoprost; Enzaprost; Ceva Santé Animale).

Cows (n = 30) randomized to the first treatment (**High P4**) were submitted for first TAI using a Double-Ovsynch protocol (Souza et al., 2008; Herlihy et al., 2012) with several modifications. For the first Ovsynch protocol (i.e., Pre-Ovsynch), cows were treated with 100 μg of GnRH, followed by 25 mg of $PGF_{2\alpha}$ 7 d later and 100 μg of GnRH 72 h after the $PGF_{2\alpha}$ treatment. For the second Ovsynch protocol (i.e., Breeding-Ovsynch), cows were treated with 200 μg of GnRH (i.e., a double dose, **G1**) followed by 25 mg of $PGF_{2\alpha}$ 7 d later, a second 25-mg $PGF_{2\alpha}$ treatment 24 h after the first, and 100 μg of GnRH 32 h later (i.e., 56 h after the first $PGF_{2\alpha}$ treatment, **G2**).

Cows (n = 30) randomized to the second treatment (**Low P4**) were submitted for first TAI using the same modified Double-Ovsynch protocol but with the addition of 25 mg of $PGF_{2\alpha}$ administered 24 h after the first GnRH treatment of the Breeding-Ovsynch portion of the Double-Ovsynch protocol (Figure 1).