J. Dairy Sci. 97:1–15
http://dx.doi.org/10.3168/jds.2013-7802


# Effects of supplemental progesterone after artificial insemination on expression of interferon-stimulated genes and fertility in dairy cows

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## ABSTRACT

The objectives of the current study were to evaluate the effects of supplemental progesterone after artificial insemination (AI) on expression of IFN-stimulated genes (ISG) in blood leukocytes and fertility in lactating dairy cows. Weekly cohorts of Holstein cows were blocked by parity (575 primiparous and 923 multiparous) and method of insemination (timed AI or AI on estrus) and allocated randomly within each block to untreated controls, a controlled internal drug release (CIDR) containing 1.38 g of progesterone from d 4 to 18 after AI (CIDR4), or a CIDR on d 4 and another on d 7 after AI and both removed on d 18 (CIDR4+7). Blood was sampled to quantify progesterone concentrations in plasma and mRNA expression in leukocytes for the ubiquitin-like IFN-stimulated gene 15-kDa protein (*ISG15*) and receptor transporter protein-4 (*RTP4*) genes. Pregnancy was diagnosed on d 34 ± 3 and 62 ± 3 after AI. Treatment increased progesterone concentrations between d 5 and 18 after AI in a dose-dependent manner (control = 3.42, CIDR4 = 4.97, and CIDR4+7 = 5.46 ng/mL). Cows supplemented with progesterone tended to have increased luteolysis by d 19 after AI (control = 17.2; CIDR4 = 29.1; CIDR4+7 = 30.2%), which resulted in a shorter AI interval for those reinseminated after study d 18. Pregnancy upregulated expression of ISG in leukocytes on d 19 of gestation, but supplementing progesterone did not increase mRNA abundance for *ISG15* and *RTP4* on d 16 after insemination and tended to reduce mRNA expression on d 19 after AI. For *RTP4* on d 19, the negative effect of supplemental progesterone was observed only in the nonpregnant cows. No overall effect of treatment was observed on pregnancy per AI on d 62 after insemination and averaged 28.6, 32.7, and 29.5% for control, CIDR4, and CIDR4+7, respectively. Interestingly, an interaction between level of supplemental progesterone and method of AI was observed for pregnancy per AI. For cows receiving exogenous progesterone, the lower supplementation with CIDR4 increased pregnancy per AI on d 62 in cows inseminated following timed AI (CIDR4 = 39.2; CIDR4+7 = 27.5%); in those inseminated following detection of estrus, however, the use of a second insert on d 7 resulted in greater pregnancy per AI (CIDR4 = 26.9; CIDR4+7 = 31.5%). Pregnancy loss did not differ among treatments. Supplemental progesterone post-AI using a single intravaginal insert on d 4 was beneficial to pregnancy in cows inseminated following timed AI, but incremental progesterone with a second insert on d 7 did not improve fertility of dairy cows.

**Key words:** dairy cow, interferon-stimulated gene, progesterone, reproduction

## INTRODUCTION

Progesterone is pivotal for successful pregnancy in ruminants (Spencer et al., 2007), and lactating dairy cows are known to have reduced systemic concentrations of progesterone during diestrus compared with dairy heifers (Sartori et al., 2004). It is thought that inadequate progesterone concentrations during early development of the conceptus might be one of the reasons for reduced fertility observed in high-producing dairy cows (Wiltbank et al., 2011), in part because catabolism of steroids increases with increased feed intake associated with high production (Parr et al., 1993; Wiltbank et al., 2011). In fact, supplemental progesterone after AI from an exogenous source (Stevenson et al., 2007; Wiltbank et al., 2011) resulted in small increases in pregnancy per AI (**P/AI**). Compared with untreated controls, beef heifers treated with exogenous progesterone starting on d 3 of the estrous cycle had a larger conceptus on d 17 (Carter et al., 2008, Clemente et al., 2009). Supplementing progesterone post-AI increased concentrations of IFN-τ in the uterine lumen (Mann et al., 2006), which reflects the expanded elongation of the trophectoderm from the conceptus of cows with increased concentrations of progesterone (Mann et al., 2006; Clemente et al., 2009).

Received December 4, 2013.
Accepted April 24, 2014.
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Timed AI protocols result in variable sizes of ovulatory follicles in dairy cows (Souza et al., 2007; Santos et al., 2010). Induction of ovulation of small follicles results in reduced P/AI in beef (Perry et al., 2005) and dairy cows (Souza et al., 2007), and increased risk of pregnancy loss (Perry et al., 2005). Embryo quality in cows synchronized for timed AI is of equal or better quality than that of cows inseminated after detected estrus (Cerri et al., 2009b); however, cows subjected to timed AI protocols that are induced to ovulate small follicles have a small resulting corpus luteum (**CL**) with reduced ability to increase peripheral concentrations of progesterone (Vasconcelos et al., 2001), thereby potentially reducing P/AI (Parr et al., 2012). Therefore, benefits from progesterone supplementation might be greater in cows inseminated following timed AI programs.

Interferon-τ secreted by the trophectoderm of the conceptus is the main signal for pregnancy recognition, initiating the process to block the luteolytic cascade and preventing the demise of the CL (Meyer et al., 1995). In the bovine conceptus, mRNA for IFN-τ is detected in trophoblast on d 12 of gestation, with peaks occurring between d 15 and 16 (Farin et al., 1990). Interferon gene mRNA expression in conceptuses is activated with the developmental stage of the blastocyst, and progesterone plays a pivotal role in stimulating conceptus development in utero (Clemente et al., 2009). In general, it is thought that stimulation of embryo development by an early rise in progesterone should benefit fertility (Stronge et al., 2005; Parr et al., 2012), possibly because of advancing conceptus development. Nevertheless, fertility responses to exogenous progesterone seem to be greater when supplementation occurs before (Bisinotto et al., 2013) rather than after (Stevenson et al., 2007) AI. In most studies with post-AI progesterone supplementation to lactating dairy cows, no apparent attempt was made to mimic the normal rise in progesterone observed in heifers, which is greater and faster than that of cows (Sartori et al., 2004). An exception is the recent work by Nascimento et al. (2013), in which lactating dairy cows receiving and injection of 3,300 IU of human chorionic gonadotropin (**hCG**) on d 5, concurrent with insertion of a controlled internal drug release containing progesterone, had progesterone profiles during diestrus similar to those of dairy heifers. The authors speculated that such manipulation mimicking the progesterone concentrations in heifers might benefit fertility of lactating dairy cows. Therefore, it is possible that the limited benefit to post-AI progesterone supplementation on pregnancy might be the result of insufficient supplementation or inability to mimic the continuous rise and incremental difference during diestrus between groups known to have low fertility (lactating cows) and those of high fertility (heifers).

Interferon-τ binds type I IFN receptor (Roberts et al., 1999), which leads to downregulation of oxytocin receptor expression on superficial glandular and luminal epithelia in sheep (Roberts et al., 1999; Spencer et al., 2007), and this mechanism is thought to be similar among all domestic ruminants. The downregulation of oxytocin receptors ultimately inhibits pulsatile release of $PGF_{2\alpha}$ responsible for the demise of the CL (Meyer et al., 1995; Spencer et al., 2007). Production of IFN-τ by the conceptus induces IFN-stimulated genes (**ISG**) in the endometrium, such as myxovirus (influenza virus) resistance 1 (*Mx1*) and receptor transporter protein 4 (*RTP4*; Hicks et al., 2003; Gifford et al., 2008). Blood leukocytes harvested on d 16 or 19 after AI had increased expression of the ISG ubiquitin-like IFN-stimulated gene 15-kDa protein (*IGS15*), *Mx1*, *Mx2*, and *RTP4* (Gifford et al., 2007; Ribeiro et al., 2014), and leukocyte mRNA for ISG was correlated with the amount of IFN-τ in the uterus (Matsuyama et al., 2012). Interestingly, stimulation of conceptus development during pre- and peri-implantation resulted in increased expression of ISG in blood leukocytes and increased P/AI in lactating dairy cows (Ribeiro et al., 2014).

The main hypothesis of the present study was that supplemental progesterone starting during metestrus would improve P/AI in dairy cows, particularly in those synchronized for timed AI. It was thought that supplemental progesterone would increase concentrations of progesterone in plasma in a dose-dependent manner, which would stimulate mRNA abundance for ISG in peripheral blood leukocytes, as a measure of improved embryonic-maternal crosstalk, thereby supporting improved pregnancy. Therefore, the main objective of the present study was to investigate the effects of supplemental progesterone starting during metestrus on P/AI when cows are inseminated following detected estrus or timed AI. Additional objectives were to characterize concentrations of progesterone in plasma, luteal lifespan, and abundance of mRNA for ISG in leukocytes in lactating dairy cows supplemented with progesterone.

## MATERIALS AND METHODS

All procedures involving animals in this study were approved by the University of Florida Non-Regulatory Animal Research Committee.

### *Cows, Housing, and Diets*

Our study was conducted on a dairy farm in central Florida milking 5,400 cows with a yearly rolling herd average milk yield of 10,700 kg during the study period. Weekly cohorts of cows were enrolled during 7 consecu-