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Conceptus development and transcriptome at preimplantation stages in lactating dairy cows of distinct genetic groups and estrous cyclic statuses

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ABSTRACT

The objectives were to compare development and transcriptome of preimplantation conceptuses 15 d after synchronized ovulation and artificial insemination (AI) according to the genetic background of the cow and estrous cyclicity at the initiation of the synchronization program. On d 39 ± 3 postpartum, Holstein cows that were anovular (HA; $n = 10$), Holstein cows that were estrous cyclic (HC; $n = 25$), and Jersey/Holstein crossbred cows that were estrous cyclic (CC; $n = 25$) were randomly selected in a grazing herd and subjected to the Ovsynch protocol. All cows were inseminated on d 49 ± 3 postpartum, which was considered study d 0. Blood was sampled and analyzed for concentrations of progesterone, estradiol, insulin, and insulin-like growth factor 1 (IGF-1) on study d -10 , -3 , -1 , 7, and 15 relative to AI. On study d 15, uteri were flushed and recovered fluid had IFN- τ concentrations measured and subjected to metabolomic analysis. Morphology of the recovered conceptuses was evaluated, and mRNA was extracted and subjected to transcriptome microarray analysis. Compared with HC, CC presented greater concentrations of progesterone and estradiol in plasma, with corpora lutea and preovulatory follicles of similar size. Conceptuses from CC were larger, tended to secrete greater amounts of IFN- τ , and had greater transcript expression of peroxisome proliferator-activated receptor gamma (PPAR γ), an important transcription factor that coordinates lipid metabolism and elongation at preimplantation development. In addition, pregnant

CC had greater concentrations of anandamide in the uterine flush, which might be important for elongation of the conceptus and early implantation. Conceptuses from HA were also longer and secreted greater amounts of IFN- τ than conceptuses from HC, likely because of the distinct progesterone profiles before and after AI. Nonetheless, anovular cows had reduced concentrations of IGF-1 in plasma, and their conceptuses presented remarkable transcriptomic differences. Some of the altered transcripts suggest that conceptus cells from anovular cows might be under greater cellular stress and presented markers suggesting increased apoptosis and autophagy, which could lead to increased mortality after d 15 of development. Estrous cyclicity had more impact on transcriptome of bovine conceptus than genetic background, and the developmental changes observed during the preimplantation period might be linked to differences in fertility among groups.

Key words: conceptus development, crossbreeding, dairy cow, estrous cyclicity

INTRODUCTION

Anovulation is a normal and temporary physiological condition of most mammals including dairy cows during early postpartum. It is characterized by lack of regular estrous cycles and ovulation, although follicle growth is still present (Wiltbank et al., 2002). Time for resumption of estrous cyclicity postpartum varies among cows and is directly associated with nutritional balance in the first weeks of lactation (Butler, 2003). As consequence, 18 to 43% of dairy cows remain anovular at the end of the voluntary waiting period, constituting an important problem in achieving adequate reproductive performance in dairy herds (Rhodes et al., 2003; Santos et al., 2009).

Adoption of timed AI programs maximizes submission to AI and lessens the problem of anovular cows reducing reproductive performance. Nevertheless, pregnancy per AI (P/AI) of anovular cows after synchro-

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nized estrus or ovulation is reduced compared with that of estrous cyclic herd mates (McDougall et al., 2005; Santos et al., 2009). Thus, anovulation impairs pregnancy rate and reproductive performance even when timed AI programs are implemented. It has been shown that reduced fertility results from impaired capability to establish or maintain pregnancy (Bisinotto et al., 2010). Nonetheless, timing and biology of the events leading to impaired zygote development are unknown.

Genetics is an important component influencing several traits related to production, health, and reproduction in dairy cows (Egger-Danner et al., 2015). One strategy that has been used in commercial herds is crossbreeding of complementary pure breeds not only to obtain a desired phenotype, but also to reduce inbreeding and maximize heterosis (Weigel and Barlass, 2003). The crossbreeding between Holstein and Jersey cattle, for instance, is common and aims to combine the high milk volume yield of Holsteins with the high solids content in milk of Jerseys. In addition, improvements in reproduction have been reported with this crossbreeding strategy (Heins et al., 2008; Ribeiro et al., 2011), and might be a result of the heterosis generated by the combination of the 2 distinct genetic backgrounds. Nonetheless, the effect of crossbreeding or heterosis on early developmental biology remains largely unknown.

We hypothesized that both anovulation at the onset of synchronization of ovulation protocol and crossbreeding affect conceptus development in dairy cows and would reflect in differences in fertility. The first study aimed to evaluate fertility data to further document the effect of estrous cyclicity status at the beginning of the synchronization program and genetic background of the cow on reproductive performance at first AI postpartum. The second study aimed to compare preimplantation conceptus development to the initial elongation stages and their transcriptome in lactating cows with distinct combinations of genetic background and estrous cyclicity to elucidate some of the biology involved in their fertility differences.

MATERIALS AND METHODS

All procedures involving cows were approved by the animal care and use committee of the University of Florida (Gainesville).

Study 1: Fertility Data

This study followed a prospective cohort design. Data comprising breed of cow, estrous cyclicity at the initiation of the synchronization program, and P/AI at the first AI postpartum were collected in 3 seasonally calving farms. Data were collected in 2 breeding seasons on

farms A and B, and in 1 breeding season on farm C. Dairies were located in north central Florida and used similar cow genetics and management practices. The herds were composed of Holstein (**H**), Jersey (**J**), and Holstein/Jersey crossbreds, mostly F₁ (50H:50J) and F₂ (25H:75J or 75H:25J) generations of crossbreeding between Holstein and Jersey genetics. A total of 3,196 lactating cows (737 Holsteins, 1,825 Holstein/Jersey crossbreds, and 634 Jerseys) were enrolled in the study. Primiparous cows represented 31% and multiparous cows represented 69% of the study animals. The mean (\pm SD) lactation number was 2.2 ± 1.1 . The different genetic groups were represented in all herds and breeding seasons, managed in a pasture-based system, and supplemented with concentrates (Ribeiro et al., 2011). Cows were milked twice a day and the average milk production per cow across the different herds and breeds of the cows was approximately 6,000 kg/lactation.

Cows in all herds were enrolled in a preplanned breeding season and received timed AI on the first day of breeding (study d 0). For that, the estrous cycle was presynchronized and then cows were subjected to synchronization of ovulation programs. The presynchronization consisted of 2 injections of PGF_{2 α} (Lutalyse sterile solution; 5 mg/mL of dinoprost tromethamine, Zoetis, Florham Park, NJ) administered 14 d apart. The synchronization protocol was initiated 10 to 12 d after the second PGF_{2 α} of the presynchronization and consisted of GnRH (Cystorelin; 50 μ g/mL of gonadorelin diacetate tetrahydrate, Merial Ltd., Iselin, NJ) on d -8, PGF_{2 α} on d -3 and -2, and GnRH + AI on d 0. Cows were artificially inseminated using sires with North American genetics, 20 Jerseys, 9 Holsteins, and 4 Swedish Red sires. On d -3, tailheads were painted using oil-based tail paint for detection of estrus based on rubbed off tails on the day of AI. On the day of the first GnRH injection of the timed AI program, blood was sampled and analyzed for concentration of progesterone. Cows with progesterone concentration ≥ 1.0 ng/mL were considered estrous cyclic and those with progesterone concentration < 1.0 ng/mL were considered anovular. Mean and median DIM at first AI were 117 and 102, respectively.

Pregnancy was diagnosed in all cows on d 30 via ultrasonography of the uterus and its contents, and was characterized by visualization of an embryo with heartbeat. Cows diagnosed as pregnant on d 30 were reexamined by transrectal palpation 35 d later. Pregnancy per AI was calculated as the number of pregnant cows on d 30 and 65 after an insemination divided by the total number of cows inseminated. Pregnancy loss was calculated as the number of cows that lost pregnancy between gestation d 30 and 65 divided by the number of pregnant cows on d 30.

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