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Factors affecting the success of a large embryo transfer program in Holstein cattle in a commercial herd in the southeast region of the United States

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ABSTRACT

The objectives of this study were to evaluate factors affecting *in vivo* embryo production and pregnancy per embryo transfer (P/ET) in Holstein cattle in the southeast region of the United States. Data from a total of 516 embryo collections and 10,297 ETs performed from 2011 to 2014 were available. For embryo production, the effects of donor parity (nulliparous [N], primiparous [P], multiparous [M]), average temperature-humidity index (THI) at embryo collection, days in milk at embryo collection, occurrence of calving problems, and occurrence of metritis postpartum were evaluated. For P/ET, the effects of donor parity (N or parous), recipient parity (N, P, and M), embryo type (fresh, frozen, IVF, and IVF-frozen), embryo developmental stage (4–7), embryo quality (1–3), recipient estrous cycle day at ET (6–9), average THI at ET, days in milk at ET, milk yield at ET, occurrence of calving problems (abortion, dystocia, twins, fetal death, or retained placenta), and occurrence of metritis postpartum were evaluated. Pregnancy was diagnosed at 41 ± 3 days of gestation. Continuous and binary data were analyzed using the MIXED and GLIMMIX procedures of SAS, respectively. Parity affected embryo production; M had greater number and percentage of unfertilized embryos and lesser percentage of viable embryos than P and N. Recipient parity, embryo type, embryo stage, embryo quality, estrous cycle day at ET, and THI at ET affected P/ET. There was an interaction between recipient parity and THI at ET. P/ET was greater for N than P and greater for P than M, greater for fresh embryos than others, greater for stage 7 than others, greater for quality 1 than 2 and greater for quality 2 than 3, and greater for ET on estrous cycle Day 7 and 8 than 6. P/ET was decreased for THI ≥80 in N and THI ≥72 in P and M. Calving problems and metritis also affected P/ET in P and M and was lesser for cows that had calving problems and metritis. In conclusion, embryo production was affected by donor parity, and P/ET was affected by embryo type, embryo stage, embryo quality, recipient estrous cycle day at ET, THI, calving problems, and metritis.

1. Introduction

Embryo transfer (ET) is an important tool to disseminate individual with high genetic merit and potentially improve herd performance [1]. In addition, ET has the potential to

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increase fertility in cows under heat stress [2–4] and in repeat-breeder cows [5,6]. In the past 40 years, there have been improvements in synchrony and hormonal protocols for ovarian superstimulation and synchronization of the estrous cycle and ovulation; however, the mean number of embryos produced *via* superstimulation or pregnancy per ET (P/ET) has not appreciably changed [1].

Reports from large-scale bovine ET programs have greatly improved our understanding of the factors that affect the success of such programs [7–11]. In regard to P/ET, these reports reported that surgical transfers resulted in greater P/ET than nonsurgical transfers, fresh *in vivo* produced embryos resulted in greater P/ET than frozen *in vivo* produced embryos, nulliparous (N) had better P/ET than parous cows, and higher quality embryos resulted in greater P/ET. The effect of stage of embryo development and donor-recipient synchrony were inconsistent. Whereas some studies reported greater P/ET for early blastocysts than other stages of development [7,9], others reported greater P/ET for expanded blastocysts [8] or even no effect of embryo stage of development on P/ET [11]. Whereas some studies reported greater P/ET for more synchronous ET [7,9], others reported greater P/ET when recipients were in estrus 12–48 hours before the donor [8]. Also, although the transfer of *in vitro* produced (IVF) embryos has been increasing worldwide [1], there are scant data comparing *in vitro* with *in vivo* produced embryos fresh and frozen [4,12,13]. Furthermore, with rising global temperature, understanding the effect of heat stress on embryo production and P/ET becomes paramount for the success of an ET program. Whereas most reports show decreased embryo production [11,13] and P/ET in dairy cattle [13–15] under heat stress, one report found an increase in P/ET in dairy cattle as ambient temperature increased [8]. Another important consideration for embryo production and P/ET is postpartum health. Recent reports have pointed out to a large effect of postpartum health, particularly uterine health on pregnancy per artificially inseminated (AI) [16,17] and P/ET [18].

Having a better understanding of the factors that affect embryo production and P/ET could help improve the success of ET programs. The objectives of this study were to evaluate environmental and cow donor factors affecting *in vivo* embryo production and environmental, cow donor, cow recipient, and embryo factors affecting P/ET in Holstein cattle in the southeast region of the United States.

2. Materials and methods

2.1. Location and animals

This a retrospective study with data collected from an ET program between 2011 and 2014 in a commercial dairy farm located in Bell, Florida, USA. The dairy milked approximately 4000 Holstein cows with a rolling herd average of approximately 11,000 kg of milk per cow. Data on embryo production *in vivo* after ovarian superstimulation in 256 lactating cows (186 primiparous [P] and 70 multiparous [M]) and 191 N. The superstimulation treatment was done 1, 2, 3, or 5 times in 385, 57, 4, and 1 cows, respectively, for a total of 516 embryo collections (211 in P, 93 in M, and 212 in N). In donor lactating cows, only embryo collections performed up to 120 days in milk (DIM) were included in the analyses. A total of 10,397 ET in 4112 recipient lactating cows (2235 P and 1877 M) and 6285 recipient N were used in the analyses. In recipient lactating cows, only ET performed up to 120 DIM were included in the analyses.

N was maintained in free-stall barns equipped with fans and sprinklers. Forced evaporative cooling was activated when the environmental temperature surpassed 26 °C. Lactating cows were housed in tunnel-ventilated free-stall barns. Stalls for both N and parous cows were bedded with sand. Both N and parous cows were fed twice daily a totally mixed ration to meet or exceed their requirements for growth and/or lactation according to the nutrient requirement of dairy cattle [19]. Fresh water was available ad libitum. P and M cows were housed separately. Lactating cows were milked 3 times a day, and milk yield was recorded at each milking.

2.2. Production of embryo

2.2.1. In vivo embryo production

The superstimulatory treatment consisted of administration of 400 mg of FSH (Foltropin-V, Bioniche Animal Health, Inc. Athens, GA, USA) given twice daily with intramuscular injections in decreasing doses during 4 days starting between Days 8 and 10 of the estrous cycle. On the fourth day of FSH treatment, cows were given 2 doses of 25 mg $PGF_{2\alpha}$ (Lutalyse, Zoetis, Florham Park, NJ, USA) 12 hours apart. The superstimulated donors were then observed for signs of estrus (standing to be mounted), and they were AI with conventional frozen semen when first detected in estrus, and 12 and 24 hours later. The embryos were recovered by standard nonsurgical uterine flushing 7 days after first AI.

2.2.2. In vitro embryo production

The cumulus-oocyte complexes (COC) were recovered by transvaginal aspiration using an ultrasound unit equipped with a 5-MHz transvaginal convex transducer. Cows at random stages of the estrous cycle were used as donors. The perineal region was cleaned and sterilized with water and 70% ethanol. Epidural anesthesia was applied with 4 mL of 2% lidocaine. The transducer was inserted into the vaginal fundus; ovaries were positioned to obtain a good view of the follicles on the ultrasound screen. Ultrasonographically visible follicles between 2 and 5 mm in diameter were punctured with 19-ga aspiration needle inserted through a guide in the transducer and the COC aspirated.

After recovery, the aspirated material was transferred and filtered through an embryo filter that was washed with the same solution used during aspiration. The recovered material was transferred to Petri dishes and observed under a stereoscope to identify COC. Recovered COC were classified according to morphology, number of cumulus cells layers, and the degree of expansion of the cumulus cells and cytoplasm. The appearance of color, uniformity, and integrity were evaluated as follows: 1, more than 3 layers of compact cumulus cells; 2, at least one layer of