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Evaluation of genetic components in traits related to superovulation, in vitro fertilization, and embryo transfer in Holstein cattle

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ABSTRACT

The objectives of this study were to estimate variance components and identify regions of the genome associated with traits related to embryo transfer in Holsteins. Reproductive technologies are used in the dairy industry to increase the reproductive rate of superior females. A drawback of these methods remains the variability of animal responses to the procedures. If some variability can be explained genetically, selection can be used to improve animal response. Data collected from a Holstein dairy farm in Florida from 2008 to 2015 included 926 superovulation records (number of structures recovered and number of good embryos), 628 in vitro fertilization records (number of oocytes collected, number of cleaved embryos, number of high- and low-quality embryos, and number of transferrable embryos), and 12,089 embryo transfer records (pregnancy success). Two methods of transformation (logarithmic and Anscombe) were applied to count variables and results were compared. Univariate animal models were fitted for each trait with the exception of pregnancy success after embryo transfer. Due to the binary nature of the latter trait, a threshold liability model was fitted that accounted for the genetic effect of both the recipient and the embryo. Both transformation methods produced similar results. Single-step genomic BLUP analyses were performed and SNP effects estimated for traits with a significant genetic component. Heritability of number of structures recovered and number of good embryos when log-transformed were 0.27 ± 0.08 and 0.15 ± 0.07, respectively. Heritability estimates from the in vitro fertilization data ranged from 0.01 ± 0.08 to 0.21 ± 0.15, but were not significantly different from zero. Recipient and embryo heritability (standard deviation) of pregnancy success after embryo transfer was 0.03 (0.01) and 0.02 (0.01), respectively. The 10-SNP window explaining the largest proportion of variance (0.37%) for total structures collected was located on chromosome 8 beginning at 55,663,248 bp. Similar regions were identified for number of good embryos, with the largest proportion of variance (0.43%) explained by a 10-SNP window on chromosome 14 beginning at 26,713,734 bp. Results indicate that there is a genetic component for some traits related to superovulation and that selection should be possible. Moreover, the genetic component for superovulation traits involves some genomic regions that are similar to those for other fertility traits currently evaluated.

Key words: embryo transfer, genetic parameter, in vitro fertilization, superovulation

INTRODUCTION

The widespread adoption of AI using frozen semen has allowed bulls of superior genetic merit to produce many more offspring than was possible using natural service. However, female reproductive rates have generally remained limited by the number of pregnancies a cow can carry to term during her life. Introduction of embryo technologies began several decades ago, with the development of protocols for superovulation and embryo transfer (**ET**) beginning in the late 1940s (Hasler, 2014). Reproductive technologies, including superovulation, in vitro fertilization (**IVF**), and ET, allow for higher rates of genetic improvement to be achieved by increasing the reproduction of superior females (Tonhati et al., 1999). Studies conducted in the 1980s and 1990s indicated that reproductive technologies could increase genetic gain by 10 to 20% compared with traditional breeding schemes (e.g., Nicholas and Smith, 1983; Colleau, 1991; Ruane and Thompson, 1991).

Opportunities remain to combine reproductive technologies with selective breeding programs to increase genetic gain (Loi et al., 2016). Improvements in sequencing technologies over the past decade have allowed for dense panels of molecular markers to be

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produced in a cost-effective manner. Single nucleotide polymorphisms are the most commonly used markers for genotyping. Genomic selection methods have been widely investigated and implemented in livestock species due to this increased availability of dense SNP marker panels. In addition to being used to predict genomic values for quantitative traits (Meuwissen et al., 2001), SNP can also be used to identify regions of the genome associated with a trait of interest. Thomasen et al. (2016) concluded that using reproductive technologies in combination with genomic selection methods can increase the annual rate of genetic gain in dairy breeding programs, which may result in greater farm profitability. Limiting factors of reproductive technologies continue to be high cost as well as variability of animal response to procedures (Jaton et al., 2016). Despite this, in 2014 the bovine ET industry reported 614,464 in vitro-derived embryos collected and 464,582 in vitro-derived embryos transferred throughout the world (Perry, 2015).

If these traits have a genetic component, a producer may select animals that respond well to these procedures. Moreover, it is possible that some of the genes controlling response to embryo technologies are also involved in determining reproductive function in females subjected to natural or artificial insemination. Previous research conducted with Holstein-Friesian cows in Brazil estimated the heritability of number of transferable embryos in a superovulation program as 0.03 with the repeatability equal to 0.13 (Tonhati et al., 1999). These estimates are low compared with production traits such as milk yield, which has a heritability of approximately 0.20 (VanRaden and Cole, 2014) and repeatability of 0.55 (Council on Dairy Cattle Breeding, 2014). When studying results from an IVF program, Machado et al. (2006) found significantly less variation in ovum pick-up response and in vitro embryo production among monozygotic twins compared with unrelated animals, indicating that the traits may have a genetic component. In support of these results, a later study estimated genetic components for several traits related to IVF including number of cumulus-oocyte complexes, quality of cumulus-oocyte complexes, number and proportion of cleaved embryos at d 4, and number and proportion of total and transferable embryos at d 7 of culture (Merton et al., 2009). Significant genetic components were estimated for number of cumulus-oocyte complexes as well as for both total and transferable embryos at d 7 of culture (Merton et al., 2009). Heritabilities accounting for covariance between donor, sire, and recipient in superovulation procedures have also been estimated for traits such as number of flushed ova and number of transferable ova (König et al., 2007). Most recently, significant heritability estimates for total number of embryos and number of viable embryos from superovulation have been reported for the Canadian Holstein population (Jaton et al., 2016). In this population, heritability of total number of embryos was 0.15 ± 0.01 and 0.17 ± 0.01 using a logarithmic or Anscombe transformation, respectively. Heritability for number of viable embryos was 0.14 ± 0.01 in the study regardless of transformation method.

The objectives of this research were to estimate genetic parameters for traits related to embryo production technologies, including pregnancy success after ET. For traits with a significant ($P < 0.05$) genetic component, genome-wide association analyses were conducted and genomic regions of interest were further investigated to identify genes that may explain the effects observed.

MATERIALS AND METHODS

Data

Data were collected from a registered Holstein dairy operation located in Bell, Florida (29.75° N, 82.86° W) from 2008 through 2015. Lactating cows were housed in either free-stall barns equipped with fans, sprinklers, and misters or in tunnel ventilation barns. Cows were milked 3 times per day. Selected females (cows and heifers) were used to produce embryos either in vivo by superovulation with FSH or by IVF of oocytes harvested from FSH treated cows using transvaginal, ultrasound-guided follicular aspiration. Production of embryos by IVF was performed by the laboratory of TransOva in Boonsboro, Maryland. In vitro fertilization donors were typically transported to the Transova facility in Maryland for embryo production and embryos were then shipped to the farm for transfer. Both conventional and sexed semen were employed for superovulation, and conventional and reverse-sorted semen were used for IVF. Reverse-sorted semen allows sexed sperm to be obtained from samples that have been previously frozen (Morotti et al., 2014). Embryos produced by superovulation and IVF were transferred to recipient females. Both heifers and cows were used as recipients. Few animals (n = 45) overlapped between the superovulation and IVF data sets. For ET, embryos were transferred either fresh (i.e., without cryopreservation) or after conventional slow freezing with ethylene glycol. Additional details of the ET protocols can be found in Ferraz et al. (2016).

Superovulation data collected (n = 926) included total number of structures recovered (i.e., total number of unfertilized oocytes and embryos) and total number of good embryos [grade 1 embryos using the grading system described by Robertson and Nelson (1998)]. Proportion of good embryos was also calculated as the