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Investigation of genomic regions associated with embryonic loss in biopsied Holstein Friesian embryos from elite matings

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Introduction

Hereditary defects represent an important challenge in dairy cattle breeding, as they can impair animal welfare and cause economic losses. While some defects are apparent at birth, others affect embryonic or fetal development before term preventing successful pregnancy establishment or maintenance (Brade, 2005; Hoedemaker *et al*., 2007). Genomic approaches have substantially improved the detection and management of deleterious recessive alleles in dairy cattle. In particular, haplotype-based analyses of genotype frequency distributions have enabled the identification of genomic regions associated with fertility traits. VanRaden *et al*. (2011) demonstrated that autosomal recessive defects can be inferred indirectly from population data when homozygous haplotypes are absent or underrepresented relative to Hardy–Weinberg equilibrium (HWE) expectations. This approach has since been applied in several studies, leading to the identification of multiple fertility-associated haplotypes in Holstein-Friesian cattle (Wu *et al*., 2019; Häfliger *et al*., 2022).

However, these studies predominantly rely on population-level observations of adult animals or fertility-related phenotypes and therefore only provide indirect evidence of selection acting during embryonic and/or fetal developmental competence to term. Direct analyses of genotype frequency distributions in embryos remain limited. This is particularly relevant in the context of embryo transfer programs, in which embryos are generated either in vivo or in vitro, and differences in developmental competence between embryo production techniques have been reported (Banliat *et al*., 2022; Gualtieri *et al*., 2024).

The objective of the present study was to investigate fertility-associated genomic regions by comparing genotype frequencies and deviations from HWE between parental and embryonic generations in Holstein Friesian cattle. Based on Single-nucleotide polymorphism (SNP) genotyping data from embryos and their parents, genomic regions selected through a literature-based approach as potentially relevant to fertility and fetal viability were analyzed, with particular emphasis on homozygous Q-genotypes. Differences between embryos in vivo derived (IVD) and in vitro produced (IVP) were examined. Genotype patterns that were over- or under-represented in embryos relative to their parental generation were interpreted as being associated with embryo and/or fetal developmental competence to term.

Materials & Methods

Data set and genotyping. A total of 2,110 Holstein Friesian embryos from elite matings, comprising 911 IVD and 1,199 IVP embryos, provided by SYNETICS Germany GmbH, were analyzed. SNP genotyping data (EuroGenomics MD Chip, ~50K), available for embryos and their parental animals, were used in the analyses. After exclusion of embryos with incomplete data, 1,674 embryos remained for analysis. All subsequent analyses were based on autosomal SNP markers.

Data filtering and marker selection. Data filtering and marker selection followed a stepwise procedure summarized in Figure 1. First, SNP markers were filtered to remove noninformative markers, including unmapped, monomorphic, and sex-chromosomal markers. A literature-based preselection was then applied to restrict the analyses to genomic regions that had previously been reported as potentially relevant to fertility and fetal viability (VanRaden *et al*., 2011; Fritz *et al*., 2013; Sahana *et al*., 2013; Wu *et al*., 2019), resulting in 865 candidate SNP markers. From this candidate set, markers were further filtered based on genotype frequency criteria. SNPs were retained if the frequency of homozygous Q-genotypes (aa) among parental animals was ≤ 10, if homozygous Q-genotypes were present among embryos, and if the minor allele frequency (MAF) among parents exceeded 5%, thereby reducing the risk of false positive signals arising from genotyping errors or rare allele occurrences and ensuring robust detection of genotype frequency distortions. After application of these criteria, 65 autosomal SNP markers were selected for downstream analyses.

Statistical analyses. Genotype frequencies were calculated for parental animals and embryos separately. Deviations from HWE were assessed for each selected SNP marker in both generations. Genotype frequency distributions and deviations from HWE were compared between the two generations in order to identify molecular DNA signatures that were either over- or under-represented in the embryos relative to their parents. In addition, embryos were stratified by embryo production method, and genotype frequencies as well as deviations from HWE were compared between IVD and IVP embryos. All analyses focused on genotype frequency distributions and HWE deviations consistent with an autosomal recessive inheritance model.

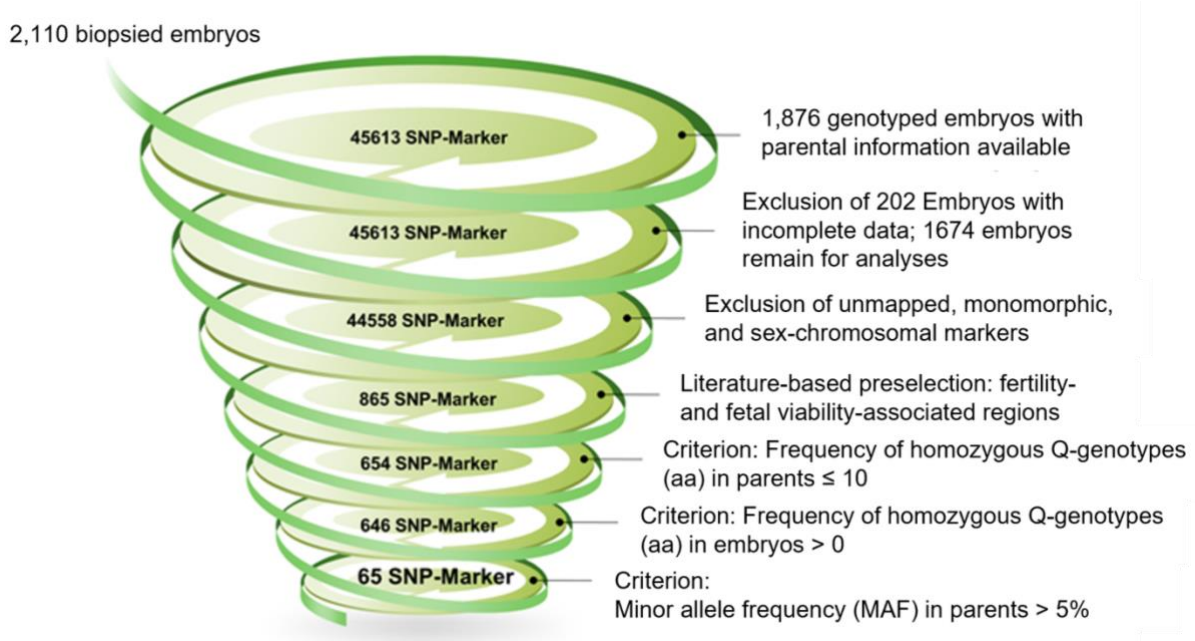

Figure 1. Overview of data filtering, comparing allele frequencies, and literature-based marker selection.

Results & Discussion

Deviations from the Hardy-Weinberg equilibrium. Deviations from HWE were assessed for all 65 selected autosomal SNP markers and compared between parental animals and embryos. In the parental generation, the majority of the SNP markers conformed to the expected frequencies under HWE, with observed genotype frequencies closely matching these. Overall, 95.4% of markers showed no significant deviation from HWE expectations, indicating that

genotype frequencies in the parental population were largely in equilibrium. This finding is consistent with population-based approaches used to infer recessive viability effects, whereby genotype frequencies in adult animals largely align with HWE expectations (VanRaden *et al*., 2011; Sahana *et al*., 2013). In contrast, pronounced deviations from HWE were observed in the embryonic generation. Only 3.1% of SNP markers under study (2 out of 65) showed no significant deviation from HWE, whereas the vast majority of markers (96.9%) deviated significantly ($p \leq 0.05$), with most of these deviations being highly significant ($p \leq 0.001$). These results demonstrate a strong disruption of equilibrium in embryos, suggesting that genotype frequency distortions may already arise at early developmental stages. This phenomenon has previously been inferred indirectly from adult populations but has now been observed directly at the embryo level (VanRaden *et al*., 2011; Fritz *et al*., 2013; Wu *et al*., 2019).

Comparing genotype frequencies in parents and embryos. Genotype frequency distributions were examined to characterize differences between parental and embryonic generations across all 65 selected SNP markers. In the parental generation, observed genotype frequencies closely matched those expected under HWE. In contrast, substantial deviations between expected and observed genotype frequencies were observed in embryos. As illustrated in Fig. 2, homozygous Q-genotypes (aa) were consistently over-represented in embryos relative to expectations based on parental genotype frequencies. In contrast heterozygous (Aa) and homozygous wild-type (AA) genotypes largely followed the expected distribution. This pattern was observed across the majority of analyzed markers as illustrated by the comparison of expected and observed genotype frequencies in parents and embryos (Figure 2).

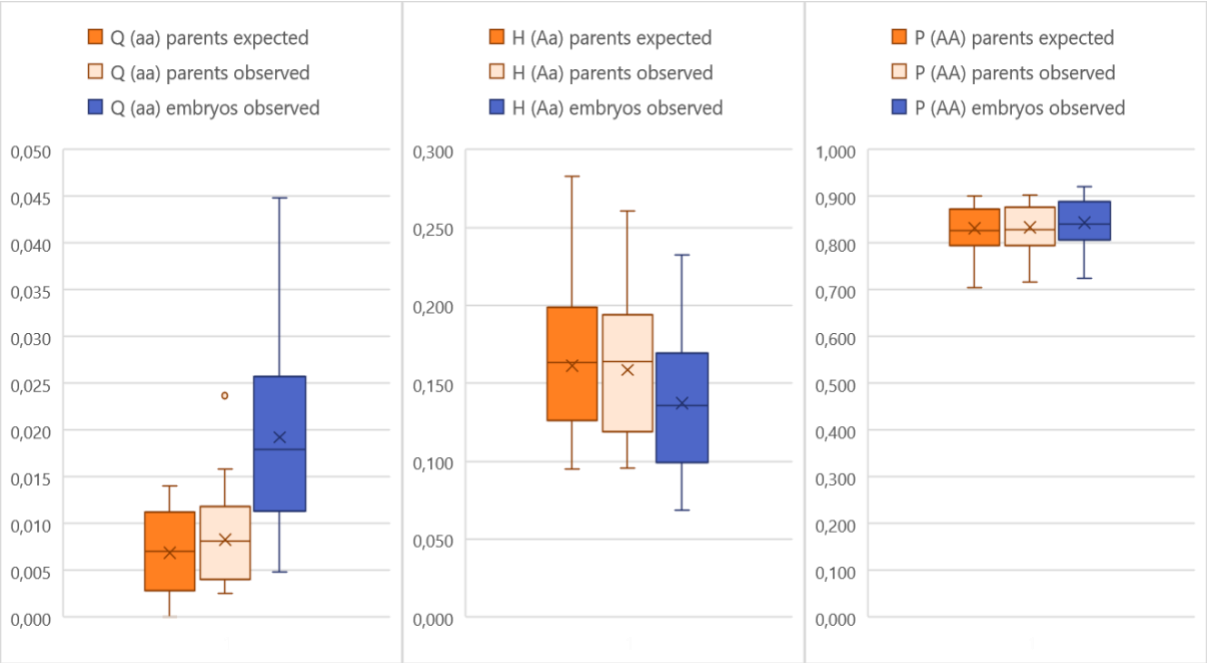

Figure 2. Expected (based on parental generation) and observed genotype frequency distributions for the AA, Aa, and aa genotypes in the parental and embryonic generations across 65 SNP markers.

Overall, these results demonstrate a systematic excess of homozygous Qgenotypes in the embryonic generation compared with the parental generation, which is consistent with the deviations from HWE observed at the embryo level. It can be assumed that the Q-genotypes might provide an developmental advantage during the embryo's initial developmental phases, but may confer a disadvantage at later developmental stages. This interpretation is consistent

with population-based studies in which homozygous recessive haplotypes were inferred to cause early embryonic loss based on their absence among live animals (VanRaden *et al*., 2011).

Comparing different embryo production techniques. Genotype frequency distributions and deviations from HWE were compared between IVD and IVP embryos. IVP embryos showed a higher number of SNP markers deviating from HWE than IVD embryos (38 vs. 23 markers). In addition, homozygous Q-genotypes (aa) were observed more frequently in IVPderived embryos than in IVD embryos (2.00% vs. 1.75%). Overall, genotype frequency patterns differed between the two contrasting techniques, with more pronounced deviations observed in IVP embryos. These findings may reflect differences in oocyst source (in vivo vs. in vitro) and/or environmental (culture-) conditions between the two techniques. This is in agreement with earlier findings demonstrating that bovine embryos produced in vitro and thereby exposed to non-physiological culture environments are associated with reduced developmental competence to term (Wale & Gardner, 2016; Gualtieri *et al*., 2024).

Conclusions

In this study, genotype frequencies and deviations from HWE were compared between parental animals and embryos to investigate fertility-associated genomic regions in Holstein Friesian cattle. Although genotype frequencies in the parental generation largely conformed to expectations based on HWE, a significant imbalance and an over-representation of homozygous Q-genotypes were observed in the embryos. This suggests a potential advantage during early embryonic development, followed by an adverse effect on developmental competence to term, potentially more pronounced in IVP embryos compared to IVD embryos.These findings support and extend the hypothesis proposed by VanRaden *et al*. (2011), demonstrating that recessive deleterious haplotypes may already manifest during early embryonic development rather than being detectable only indirectly from adult populations. Further research should extend these analyses to additional genomic regions and integrate functional and developmental data to improve our understanding of the genetic mechanisms underlying embryonic loss.

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