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NO EVIDENCE FOR GENES WITH LARGE EFFECT ON TWINNING IN A BEEF HERD WITH UNUSUALLY HIGH FECUNDITY

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SUMMARY

Fertility is a major driver of productivity and profitability in many livestock production systems. This has inspired a search for mutations with large effects on fecundity. In sheep, at least seven such mutations have been reported. In cattle, mutations with large effects on these traits seem much rarer. In this study, the hypothesis that there are mutations of large effect for fecundity (specifically twinning rate) segregating in a population of cattle with an unusually high frequency of twining was tested. Sixty seven cows in the population, with two years of records of number of calves born per pregnancy, were genotyped with 632K genome wide SNP. In a genome wide association study, no evidence was found for mutations of very large effect on twinning rate (the study had 50% power to detect a mutation explaining 30% of the variance explained at $P<5x10^{-8}$). However, the substantial increases in twinning rate over time as a result of selection achieved in the Ivanhoe herd demonstrates that improvement in this trait is possible, and this might be accelerated by genomic selection.

INTRODUCTION

Fertility is a major driver of productivity and profitability in many livestock production systems. This has inspired investigation into the genetic architecture of fertility and it's component traits – if mutations of large effect are found, and these mutations do not have other deleterious effects (such as on survival), these mutations might be increased in frequency to improve fertility of the population. In sheep, at least seven mutations with large effects on fecundity (number of offspring per dam) have been reported (Davis 2004). For example, the high fecundity of Booroola merino sheep, results from a mutation (FecB) in the bone morphogenetic protein receptor 1B (BMPR-1B) gene (Wilson et al., 2001; Souza et al., 2001, Mulsant et al., 2001) (*Bb* versus *bb* effect of +7 lambs, Bindon 1984). The high fecundity of Inverdale Romney sheep is due to a mutation (FecXI) in the bone morphogenetic protein 15 (BMP15) gene (Galloway et al., 2000) (*iI* versus *ii* effect of 0.6 lambs). In cattle, there is only a single report of a mutation with a large effect on ovulation rate, and on the rate of twins and triplets (Kirkpatrick and Morris 2015). Although some QTL regions with small effects were identified in Norwegian Red and Holstein cattle (Meuwissen et al. 2002, Bierman et al. 2010). The paucity of studies reporting mutations of large effect might be a result of the very low frequency of twins in most cattle populations. This in turn may mean mutations of large effects are at very low frequency and therefore hard to identify. For example, the mutation reported by Kirpatrick and Morris (2015) appears to occur only within a single sire family.

In this study, the hypothesis that there are mutations of large effect for fecundity (specifically twinning rate) in a population of cattle with an unusually high frequency of twining was tested.

MATERIALS AND METHODS

The cattle population was located at "Ivanhoe", Cavendish, Victoria. The herd was established by importing US Meat Animal Research Center (USMARC) Nebraska, Twinner genetics in 2004. The USMARC Twinner line has been selected for increased twinning rate for over 30 years and had

a calving rate of 1.56 per parturition in 2004 (Echternkamp et al 2007).

Sixty seven cows from the herd were selected for genotyping, with the criteria of at least two pregnancies in two years and a range of litter sizes from one in both years to twins in both years. The 67 cows were genotyped with the Bovine HD Array (Illumina, San Diego). Quality control included use of the Illumina GenCall score, genotype calls with <0.6 were excluded, and SNP with multiple or missing map positions were excluded. 632003 SNP remained after quality control as described by (Erbe et al. 2012).

Phenotypes were for each cow's number of calves born alive (2014) and number of foetuses in late pregnancy (2015). For each cow, the two numbers were averaged to get the phenotype that was analysed. A previous study demonstrated a broadly similar trait had a repeatability across years of 0.30 (Gregory et al. 1990). The distribution of the phenotype is given in Table 1.

Table 1. Distribution of number of singles, twins and triplets for sixty seven genotyped cows across two years in the Ivanhoe herd

Calvings	Number of cows	Phenotype
Singles both years	26	1
Single one year and twins one year	27	1.5
Twins both years	12	2
Twins one year and triplets one year	1	2.5
Triplets one year*	1	3

*Only calved in 2015

A genome wide association study was conducted fitting each SNP in turn, simultaneously with the genomic relationship matrix among the cows to control for population structure, using EMMAX (Kang et al. 2014), with the model $\boldsymbol{y} = \mu + \boldsymbol{x}b + \boldsymbol{Z}\boldsymbol{u}$, where **y** is a vector of the phenotypes, μ is the mean, **x** is vector of SNP genotypes, with genotypes coded as 0 (homozygote first allele), 1 (heterozygote) or 2 (homozygote alternate allele), -2*p, where p is the frequency of the first allele, b is the effect of the SNP, **Z** is matrix allocating records to animals, and u is a vector of polygenic breeding values, where $\mathbf{u} \sim N(0, \mathbf{G}\sigma_g^2)$, with **G** the genomic relationship matrix among animals constructed as described by VanRaden (2008). The heritability of the phenotype was estimated in the same analysis.

RESULTS AND DISCUSSION

The heritability of the fecundity phenotype was 0.12±0.24. This study is too small to estimate heritability, as evidenced by the large standard error. It is perhaps encouraging that the heritability is not zero, and our estimate was close to other estimates (eg 0.06, Gregory et al. 1990).

There was no evidence for mutations of large effect, Figure 1. No SNP had P-values lower than the significance threshold corrected for multiple testing (of 630K SNP), which was 5×10^{-8}.

The major limitation of this study is clearly the small number of cows genotyped. This means the study only had the power to detect mutations of very large effect. The study had 50% power to detect a mutation explaining 30% of the variance at $P<5 \times 10^{-8}$. There are actually some examples of mutations of large effect detected in even smaller cohorts, including a mutation resulting in 4 horns in sheep (Ockert et al. 2016), and mutations associated with ridge back phenotypes in dogs (Salmon Hillbertz NHC, et al. 2007). Kirkpatrick and Morris (2015) detected a mutation with a large effect on bovine ovulation rate in only 131 animals. It can only concluded, from these results, there is no evidence for mutations of really large effect on twinning segregating in the high twinning rate population studied here. Mutations of more modest effect are not ruled out by this study, though it will require much larger numbers of genotyped and phenotyped cows to detect these.

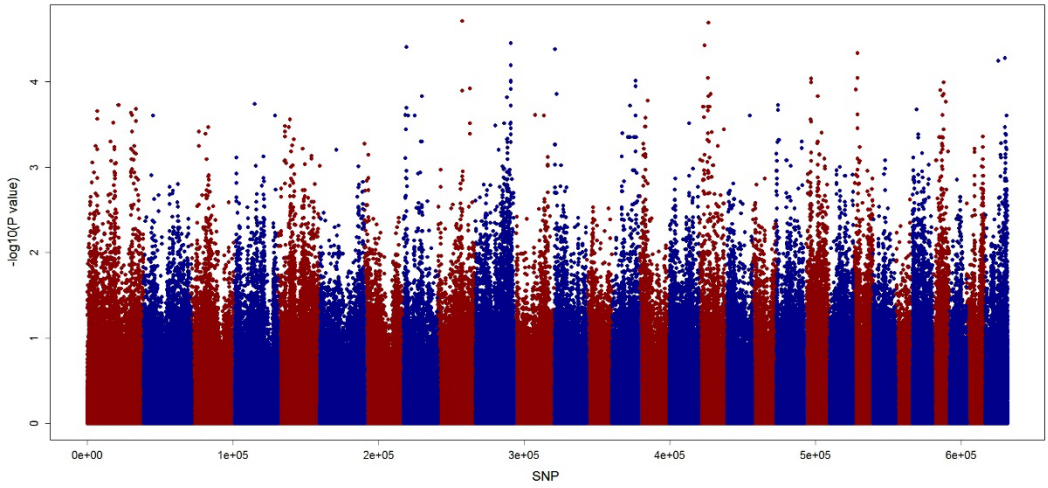

Figure 1. Genome wide association study for number of calves born per calving, with 632003 SNP. SNPs are grouped by chromosome, odd numbered in red and even in blue

There are at least two implications can be drawn from these results. One is that increasing fecundity in cattle is unlikely to be as simple as introgressing a single mutation – the trait appears to be polygenic at least in this population - so improvement will require phenotypic selection, selection with EBVs for twinning rate or genomic selection. Another interesting implication is that these findings do not point to any obvious targets for genome editing (for example with CRISPR/CAS9), if the aim of the editing was to improve fertility.

The USMARC Twinner selection program, from which the population used in this study was derived, made remarkable progress in increasing ovulation rate and twinning rate, demonstrating the traits have a genetic component, and they are now a unique line of cattle. They have also been demonstrated to have suitable growth and carcass characteristics for beef production in American systems (Gregory et al 1996). So the absence of a major effect mutation obviously does not preclude improvement for twinning rate, and outcrosses of USMARC Twinners with other cattle are likely to produce moderate increases in twinning rate, which could be further increased by suitable selection programs. It has been previously demonstrated that twinning could lead to large increases in production and should be manageable in the temperate climatic zones with smaller farms and better pastures (Cummins et al 1992 and Cummins et al 1994). The major issue is the requirement for a moderately increased supervision input at calving time, which can be assisted by ultrasound scanning of foetal numbers. At Ivanhoe, the USMARC Twinners have been managed on a commercial basis since 2004 and over the 10 years, the twinning rate is 24% per cow mated (Cummins et al 2015). The weaning weight (at about 8 months of age) of twin born calves is about 80% of the weaning weight of single born calves. Within this herd, being born a twin did not reduce the pregnancy rate in 15 month old heifers (Cummins and Cummins 2016).

In conclusion, given the polygenic nature of twining rate in this and most other cattle populations, genomic selection or EBV selection is the best strategy, if increased twinning rates are desired and the production system is suitable.

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