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Genomic regions associated with reproductive traits and uterine health in dairy cows under smallholder production systems in Mexico

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Introduction

Reproductive efficiency is a key determinant of profitability and sustainability in dairy production systems because it directly affects milk yield, herd longevity, replacement rates, and calving intervals. Poor reproductive performance increases production costs and culling rates while reducing lifetime productivity of dairy cows (Sheldon and Owens, 2017).

Among the most used indicators of reproductive performance are days open (DO) and number of services per conception (NSC). DO represents the interval between calving and successful conception, whereas NSC reflects the number of inseminations required for pregnancy. Both traits integrate multiple physiological, environmental, and management factors affecting fertility (Lombebo *et al*., 2026). In addition to fertility traits, uterine health plays a crucial role in reproductive success. Subclinical endometritis (SE) is a postpartum uterine disorder characterized by inflammation of the endometrium without visible clinical signs. It is typically diagnosed based on the proportion of polymorphonuclear cells (PMN) in uterine cytology samples and has been associated with reduced conception rates and prolonged calving intervals (Barański *et al*., 2024).

Advances in genomic technologies have improved the understanding of genetic architecture underlying complex traits in livestock. Genome-wide association studies (GWAS) allow the identification of single nucleotide polymorphisms (SNPs) associated with phenotypic variation and facilitate the detection of candidate genes affecting economically important traits (May *et al*., 2022). Although several GWAS have investigated fertility traits in dairy cattle, most studies have focused on large commercial populations managed under intensive production systems.

In contrast, little genomic information is available for dairy cattle raised under smallholder production systems, which are characterized by small herd sizes, heterogeneous management practices, and variable environmental conditions. Understanding the genomic basis of reproductive traits in these systems may contribute to the development of breeding strategies better adapted to local production environments. Therefore, the objective of this study was to identify genomic regions associated with reproductive traits (DO and NSC) and uterine health (SE) in dairy cows managed under smallholder production systems in Mexico.

Materials & Methods

Animals and phenotypic data. The study population consisted of 60 dairy cows raised under smallholder production systems in central Mexico. These production systems typically involve small herd sizes, heterogeneous management conditions, with feeding strategies that combine grazing with supplemental feeding. Phenotypic records were collected for two reproductive traits and one indicator of uterine health. DO was defined as the interval between calving and successful conception, whereas NSC represented the number of inseminations required for a

cow to become pregnant. Uterine health status was evaluated using the PMN percentage obtained from uterine cytology samples as an indicator of SE. To improve normality of the data distribution, a natural logarithm transformation was applied to DO and NSC. For PMN percentage, a log(x+1) transformation was used to account for potential zero values (Diaz-Lundahl *et al*., 2022.

Genotyping and quality control. Genomic DNA was extracted from hair samples and genotyped using the Axiom® Bovine 100K Array. Quality control was performed using PLINK v1.9 (Purcell *et al*., 2007), excluding SNPs and individuals with call rates below 0.95, minor allele frequency < 0.05 and deviation from Hardy-Weinberg equilibrium ($p < 1 \times 10^{-4}$). After quality control, 77,766 SNPs and 60 animals remained for downstream analyses. SNP coordinates were based on the Bos taurus reference genome ARS-UCD1.2.

Statistical analysis. GWAS were performed using linear regression implemented in PLINK v1.9, assuming an additive SNP effect model. Herd-year-season was included as a fixed effect covariate to account for environmental and management-related variation among farms. Genome-wide significance was evaluated using the Bonferroni correction at 5 %. Considering the small sample size of the population, a suggestive significance threshold of $p < 1 \times 10^{-4}$ was also applied to identify biologically relevant genomic regions, as commonly used in exploration GWAS analyses in dairy cattle populations (May *et al*., 2022).

Gene and QTL annotation. Significant SNPs were annotated using Rstudio v4.5.1 (Posit team, 2025) and the Gallo package (Fonseca *et al*., 2020). Candidate genes were identified within ± 10 kb windows around significant SNPs. Overlapping quantitative trait loci (QTL) were retrieved from the Animal QTLdb (Hu et *al*., 2022).

Results

GWAS identified 20 SNP markers associated with the evaluated traits at the suggestive significance threshold ($P < 1 \times 10^{-4}$). Among these, eleven were associated with DO, four with NSC, and five with SE.

Days open

Significant SNPs associated with DO were detected across chromosomes 1, 5, 6, 8, 9, 10, 14, 24, and 29, suggesting a polygenic architecture for this fertility trait. A SNP located on BTA1:139937600 was detected within the PCP4 gene region, which encodes a calmodulin-binding protein involved in calcium-mediated signaling pathways. Another significant region on BTA6:19455356 overlapped the GSTCD and INTS12 genes and coincided with previously reported QTL associated with body weight and weight gain. A SNP on BTA8:7577203 was located within CTSB, which encodes cathepsin B, a lysosomal protease involved in protein degradation and cellular remodeling processes related to reproductive physiology. Another region on BTA9:102689188 overlapped the SMOC2 gene and a previously reported QTL associated with insulin levels, suggesting a potential relationship between metabolic regulation and fertility. A SNP on BTA10:46444896 was located within the USP3 gene region and overlapped a QTL associated with milk protein percentage.

Additional markers were detected on BTA24:53106593 and, overlapping QTL previously associated with teat placement and udder cleft, traits related to udder conformation. Another SNP on BTA29:20449498 was located within the LUZP2 gene.

Number of services per conception

For NSC, significant SNPs were detected on chromosomes 2, 3, 8, and 24. A SNP located on BTA2:88925620 was identified within the SPATS2L gene, which has been associated with cellular regulatory processes. On BTA3:120020055, a SNP overlapping the GPR35 and AQP12 genes was detected within a region previously associated with age at first calving. Interestingly, the SNP located within CTSB on BTA8:7577203 was associated with both DO and NSC, suggesting a potential role of this gene in reproductive physiology. Another SNP on BTA24:346745 was located within PARD6G, a gene involved in cell polarity and epithelial organization.

Subclinical endometritis

For SE, significant SNPs were identified on chromosomes 1, 14, 16, and 24 (Figure 1). Two SNPs located on chromosome 16 were detected within genomic regions containing the C4BPB and C4BPA genes (BTA16:4932597). These genes encode complement regulatory proteins involved in innate immunity and inflammatory responses. Another SNP on BTA16:70393850 was located near RPS6KC1, a gene associated with intracellular signaling and cell growth regulation. Additional markers were identified on chromosomes 1, 14 and 24, although no annotated genes were detected within the ±10 kb window surrounding these SNPs.

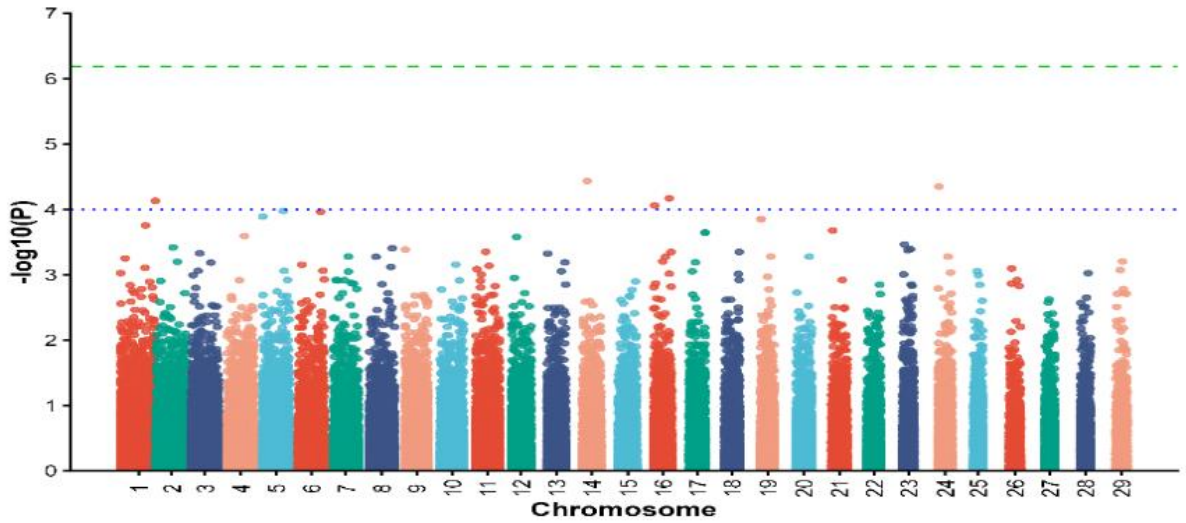

Figure 1. Subclinical endometritis Manhattan plot.

Discussion

The results of this study highlight several genomic regions potentially involved in reproductive performance and uterine health in dairy cows managed under smallholder production systems. Fertility traits such as DO and NSC are influenced by complex interactions among endocrine, metabolic, and immune pathways. One notable result was the identification of CTSB, which was associated with both DO and NSC. Cathepsin B is involved in lysosomal protein degradation and extracellular matrix remodeling, processes that are relevant for ovarian follicular dynamics, endometrial remodeling, and embryo implantation (Mohanty *et al*., 2025). The association detected near SMOC2, which overlapped a QTL related to insulin levels, suggests a potential link between metabolic regulation and fertility. Energy balance and metabolic hormones such as insulin are key determinants of reproductive performance in dairy cows (Butler *et al*., 2003), particularly during early lactation when animals often experience negative energy balance.

The association detected near GPR35 is also noteworthy. This region overlaps a previously reported QTL associated with age at first calving. G-protein-coupled receptors play key roles

in hormonal signaling pathways, including those involved in reproductive endocrine regulation (Friedrichs *et al*., 2014). These suggest a potential role in reproductive development and endocrine regulation. For uterine health, a relevant finding was the identification of C4BPA and C4BPB, which encode complement regulatory proteins involved in innate immunity (Chen *et al*., 2024). The complement pathway plays a key role in the uterine immune response during the postpartum period, when the reproductive tract is highly susceptible to bacterial contamination. Genetic variation affecting these genes may influence susceptibility to inflammatory uterine disorders such as SE. Some significant SNPs were in genomic regions without previously reported genes or QTL, suggesting the presence of potential novel loci influencing fertility and uterine health in dairy cattle raised under smallholder production systems. However, due to the small sample size of this study, these associations should be interpreted as exploratory signals that require validation in larger populations, particularly in populations raised under similar production conditions.

Conclusions

This exploratory GWAS identified several genomic regions associated with reproductive performance and uterine health in dairy cows raised under smallholder production systems in Mexico. Candidate genes such as C4BPA, C4BPB, CTSB, SMOC2 and GPR35 were identified in proximity to significant SNP markers, highlighting biological pathways related to immune response, metabolic regulation, and tissue remodeling.

These findings contribute to a better understanding of the genetic architecture underlying fertility and uterine health traits in dairy cattle and provide a basis for future studies aimed at validating candidate genes and developing genomic selection strategies adapted to smallholder production systems.

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