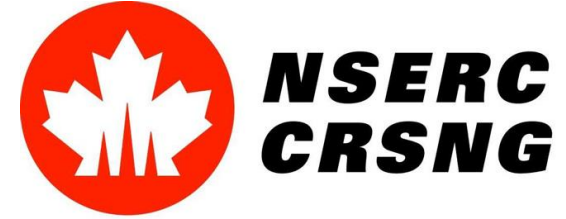


Agriculture and Agri-Food Canada



Identification of Genomic Regions Associated with Bovine Fertility

Hannah Sweett¹, E. Ribeiro², A. Livernois^{1,3}, S. Nayeri¹, M. Romulo Carvalho²,
J.F. Warmling Sprícigo², F. Miglior¹, A. Cánovas¹

¹Centre for Genetic Improvement of Livestock, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada

²Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada

³Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada



Ghent, Belgium
August 28th, 2019



Outline

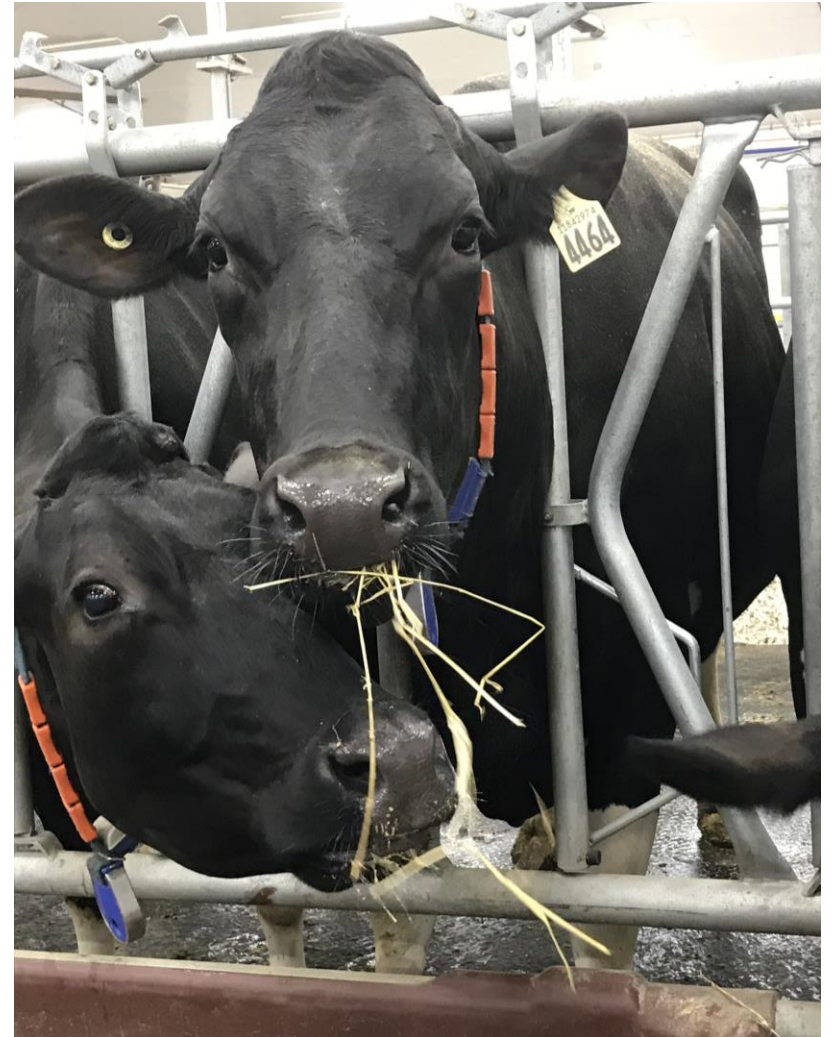
1. Introduction

2. Beef Bull Fertility GWAS

- Scrotal Circumference
- Sperm Motility

3. Future Directions

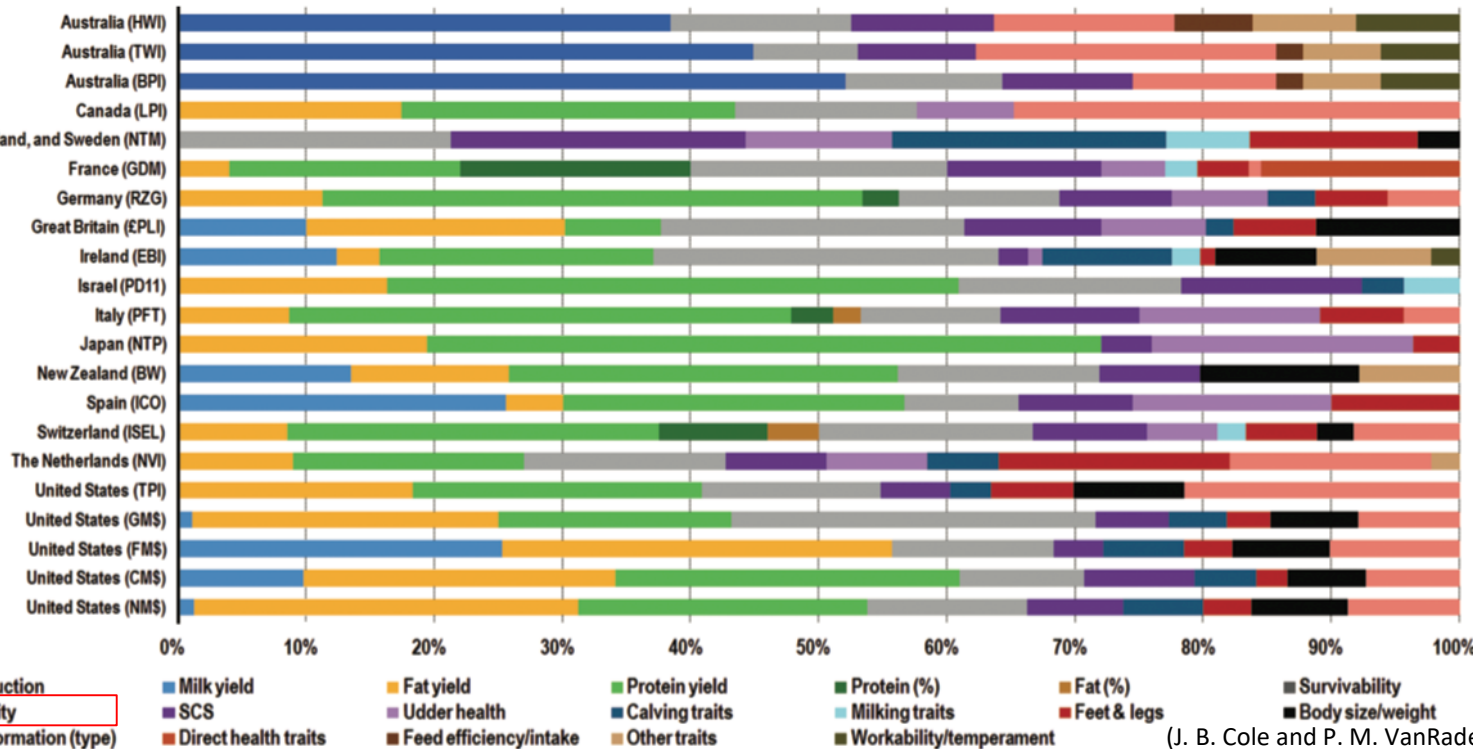
- Transcriptomics



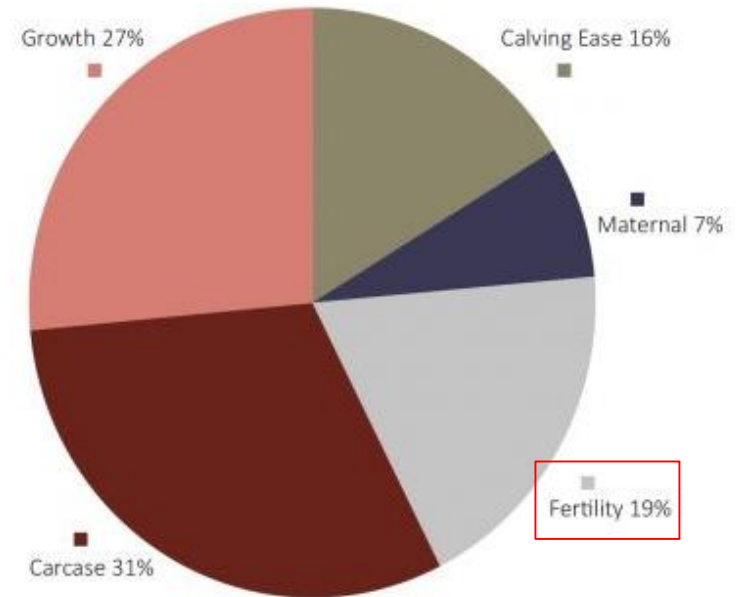
Introduction to Bovine Fertility

- Reproductive efficiency in bovine has **decreased**
 - Early losses after fertilization or;
 - Failure to conceive, due to poor bull and/or cow fertility

Dairy Selection Indexes



Angus Selection Index



(J. B. Cole and P. M. VanRaden, 2018)

(Beef Central , 2016)

The Use of Genetics to Improve Fertility

- Genetics can be used to select for more fertile animals, thereby increasing cow-calf production efficiency
 - **Genome Wide Association Study (GWAS)** scans markers across complete sets of genomes to find genetic variations associated with a particular trait
 - Identify **single nucleotide polymorphisms (SNPs)** involved in bovine fertility
 - Few GWAS studies for bull fertility traits have focused on **crossbred beef cattle**
 - **Transcriptomics** measures gene expression in the entire transcriptome in a specific cell/ tissue at a specific moment
 - Analyze gene expression levels to identify **differentially expressed genes** and isoforms associated with fertility and identify structural variations within these genes

Objective

- To combine structural and functional genomic data to identify candidate regions affecting male fertility in beef cattle

Male traits: Scrotal Circumference and Sperm Motility

Identify SNPs significantly associated with scrotal circumference (SC) and sperm motility (SM) in crossbred beef bulls using a GWA approach

Phenotypic Information

Population

Composite Beef
N=265

Traits

Scrotal
Circumference

N = 265
Mean SC = 36.65 cm
Mean age = 385 days

Sperm Motility

N = 107
Mean SM = 34.54 %
Mean age = 388 days

Collection Methods

Palpating testicles
lower part of the scrotum
looped tape

Semen ejaculates
>70% motility
CASA (IVOS System Software)



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Effects

$y = X\beta + Za + \varepsilon$	SC	SM
Fixed Effects		
Herd Year Season	x	x
Body Weight	x	
Age	x	x
Age ²	x	
Age ³	x	
Breed Composition	x	x
Random Effects		
Additive Genetic	x	x

Breed Composition

- Angus
- Charolais
- Simmental
- Limousin
- Gelbvieh
- Piedmontese

GWAS

Genotype Data
Composite Beef
N = 265

Affymetrix GeneChip
Bovine Genome HD Array
~**640,000 markers**

Quality
Control

Call Rate > 0.95
MAF > 0.05
Autosomal markers
379,591 markers

GWAS
Analysis

BLUPF90
airemlf90 & postGSf90

1 Mb non
overlapping windows
(Wang et al. 2012)

Gene Annotation
Functional Analysis:
-Metabolic Pathways
-Gene Networks

Gene Annotation

Genetic mechanisms underlying spermatic and testicular traits within and among cattle breeds: systematic review and prioritization of GWAS results

Pablo Augusto de Souza Fonseca, Fernanda Caroline dos Santos, Stephanie Lam, Aroa Suárez-Vega, Filippo Miglior, Flavio S Schenkel, Luiza de Almeida Ferreira Diniz, Samir Id-Lahoucine, Maria Raquel Santos Carvalho, Angela Cánovas ✉

Journal of Animal Science, Volume 96, Issue 12, December 2018, Pages 4978–4999,
<https://doi.org/10.1093/jas/sky382>

1Mb windows
>1% of the genetic
variance

QTL annotation

Animal QTLdb

Gene annotation

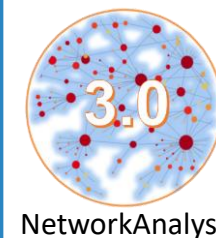
 ToppGene Candidate
Gene Prioritization

Training Gene Set (Fonseca et al., 2018)

Genomic functional annotation in livestock for positional candidate Loci (GALLO) R package

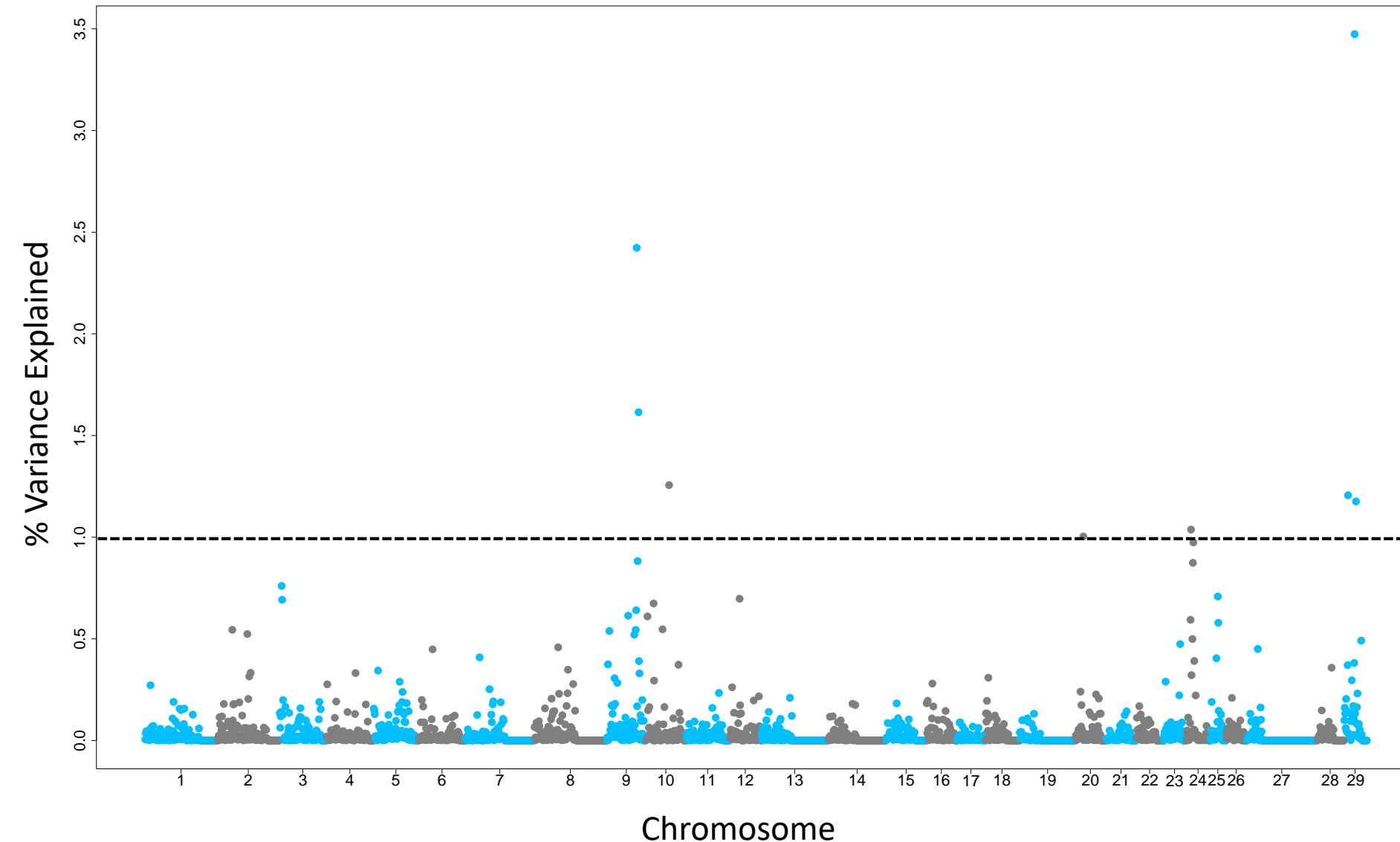
Functional Analysis:

- Metabolic Pathways
- Gene Ontology
- Gene Network



NetworkAnalyst

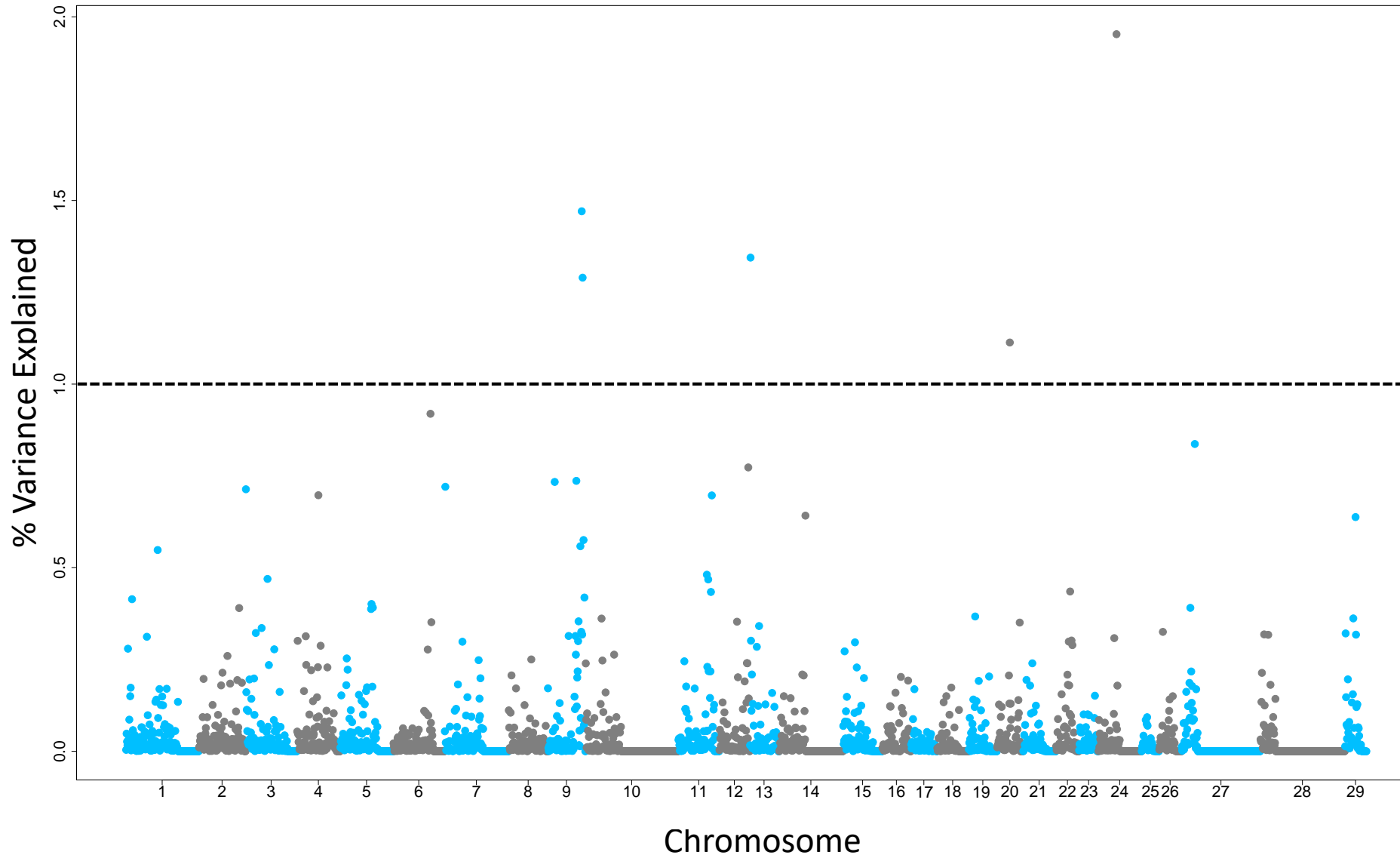
GWAS Results: Scrotal Circumference



8 windows
explaining more
than 1% of the
genetic variance

BTA9, BTA10, BTA20,
BTA24, BTA29

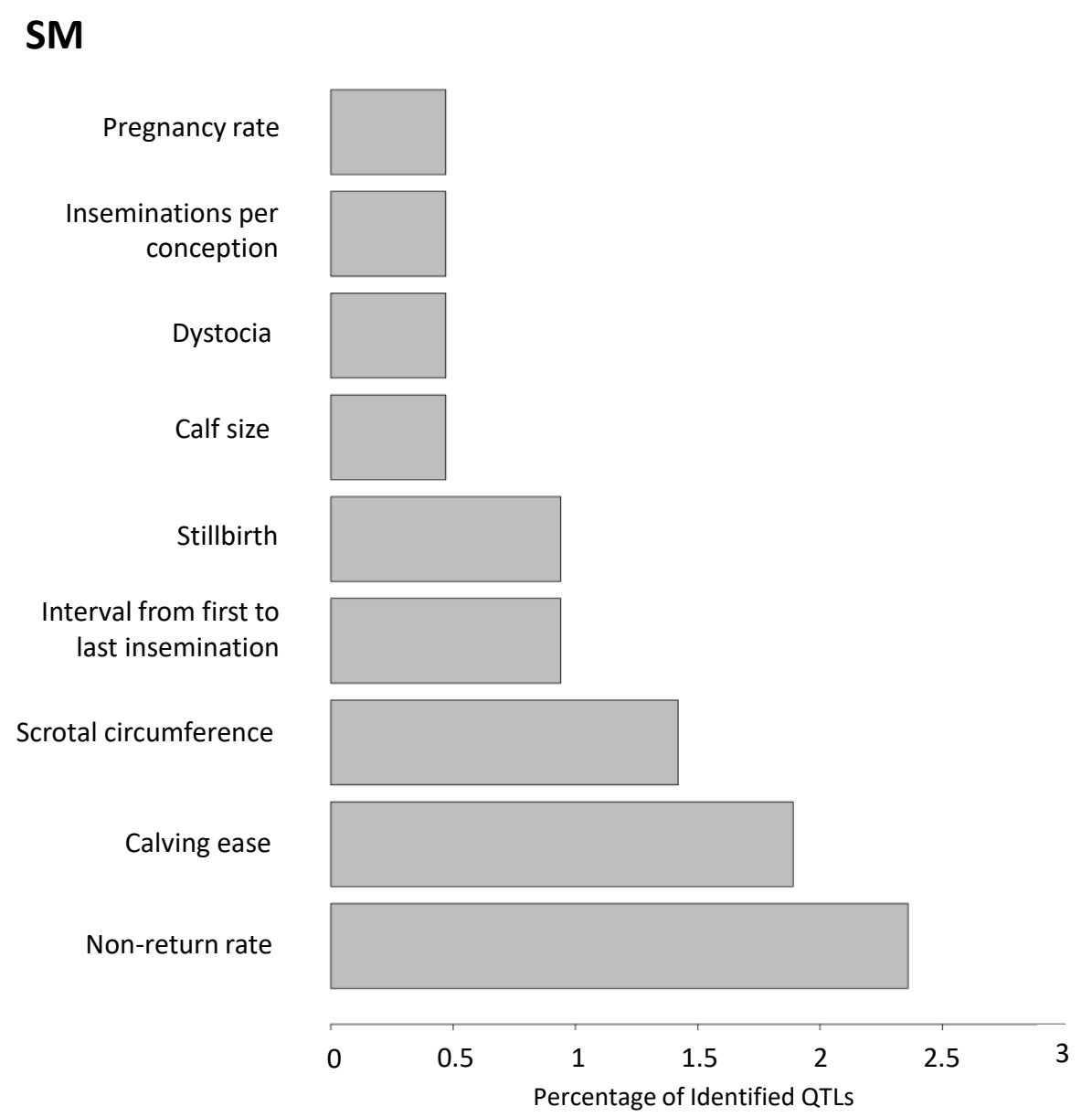
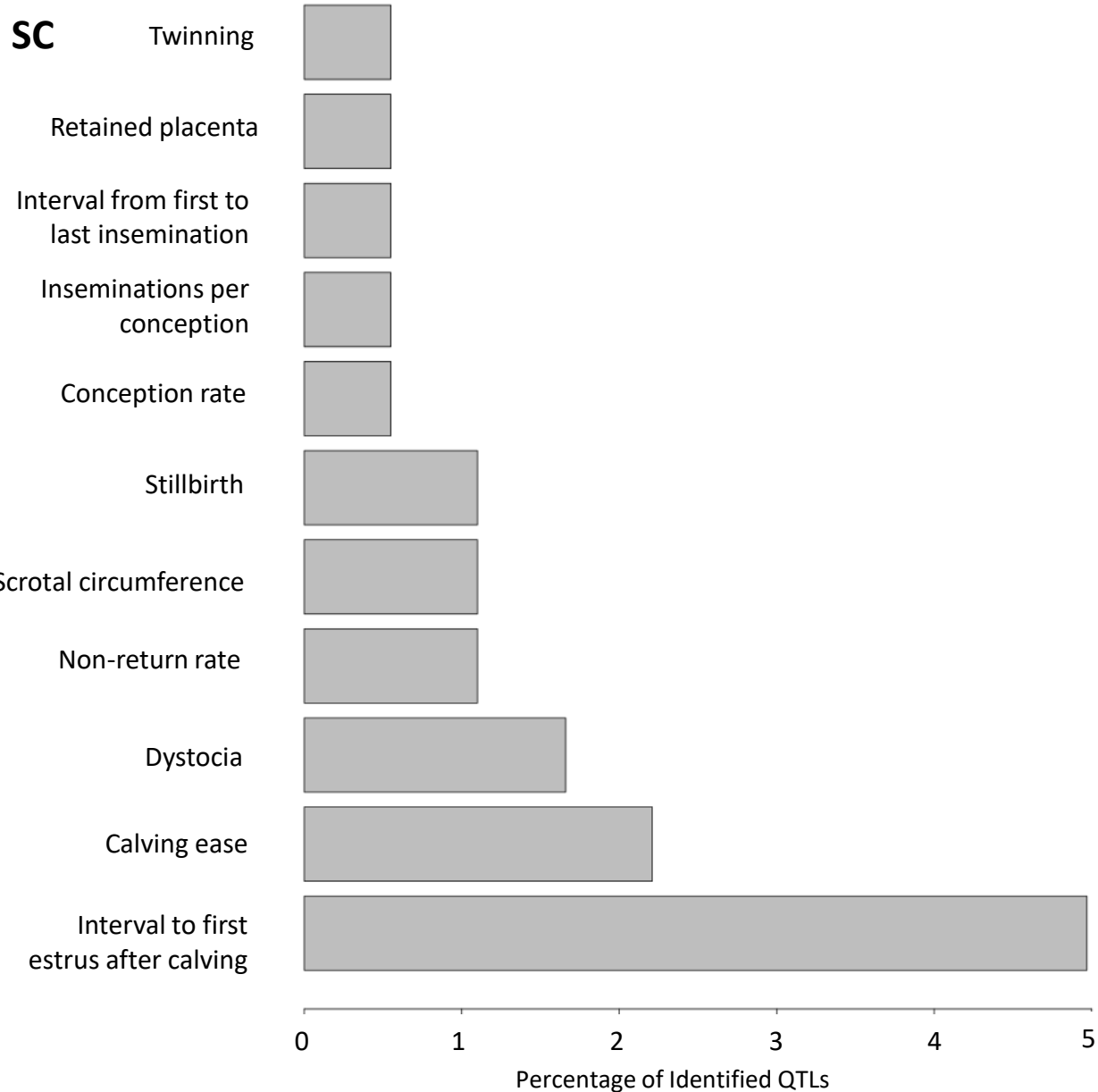
GWAS Results: Sperm Motility



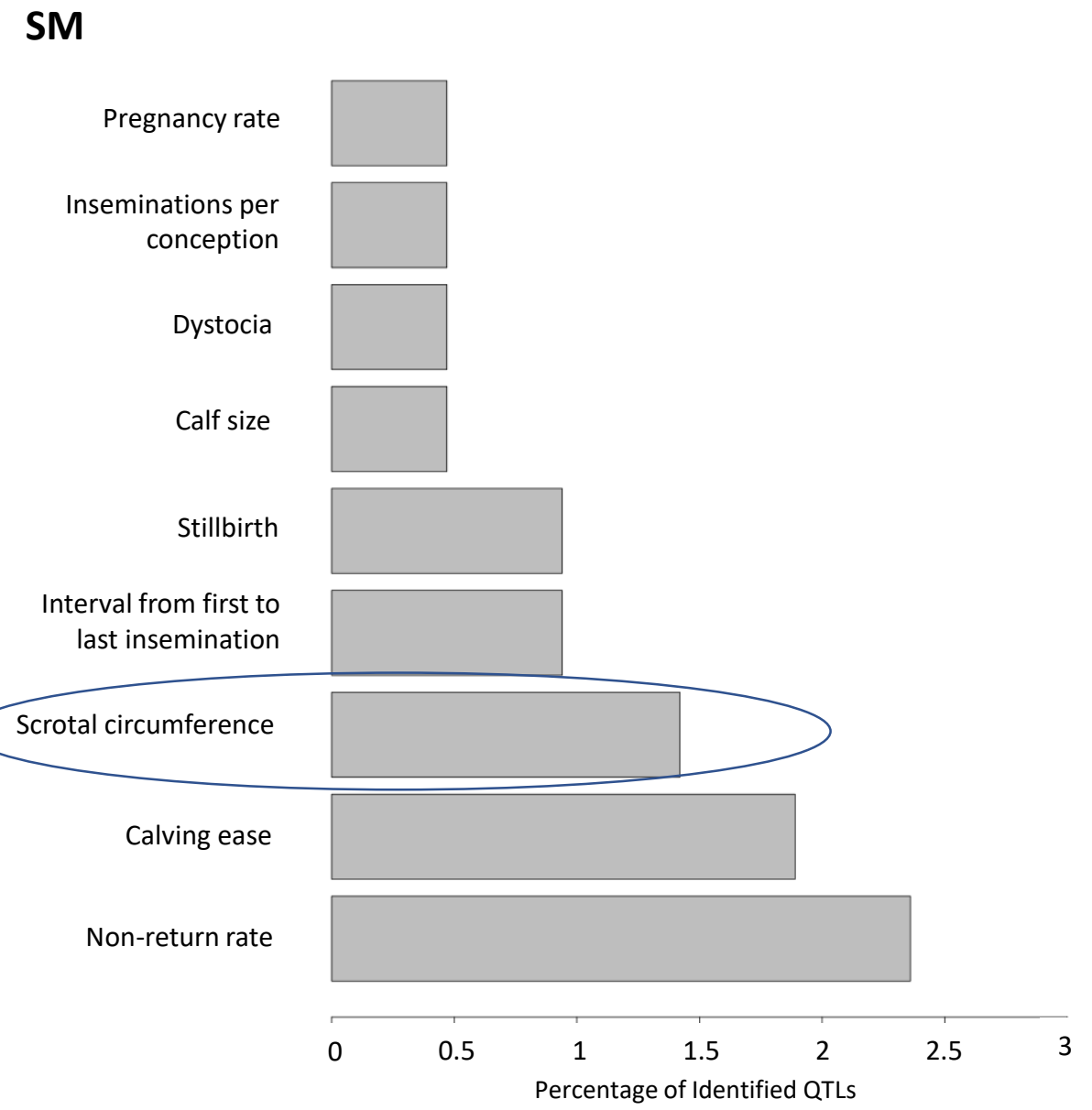
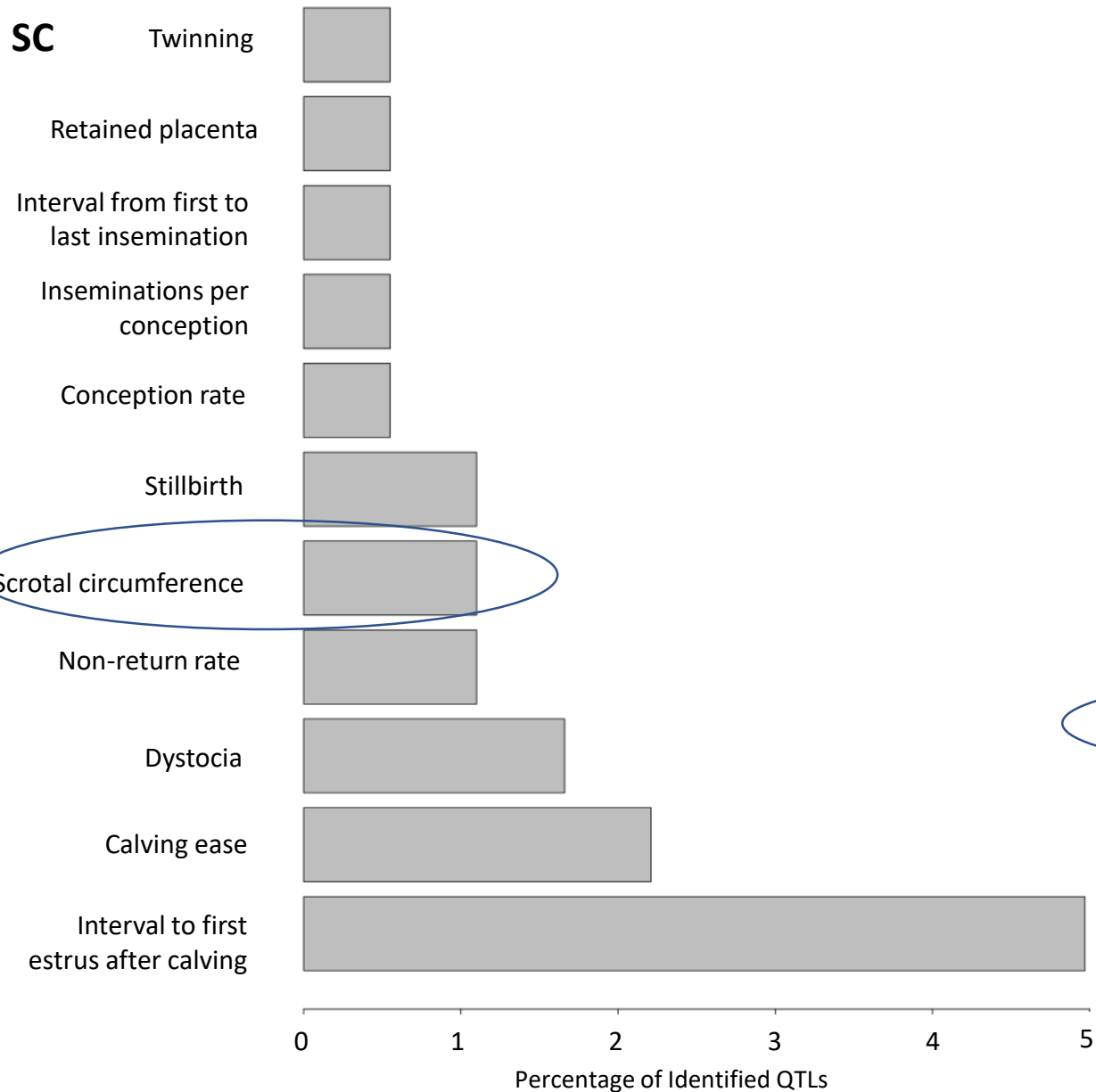
5 windows
explaining more
than 1% of the
genetic variance

BTA9, BTA13, BTA20,
BTA24

QTL Annotation



QTL Annotation



Positional Candidate Genes

SC

32 Positional
Candidate Genes

SM

28 Positional
Candidate Genes

ToppGene candidate gene prioritization



Training Gene Set

Genetic mechanisms underlying spermatic and testicular traits within and among cattle breeds: systematic review and prioritization of GWAS results

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14 Prioritized Candidate
Genes associated with SC

14 Prioritized Candidate
Genes associated with SM

Functional Candidate Genes: Scrotal Circumference

Region*	% variance explained	Prioritized Candidate Genes
BTA9:85069592-86068330	2.42	<i>SASH1</i>
BTA9:89514561-90513278	1.61	<i>VIP, FBXO5, MTRF1L, RGS17</i>
BTA10:62210731-63210223	1.26	<i>SLC24A5, SEMA6D</i>
BTA20:22183762-23180410	1.00	<i>MAP3K1</i>
BTA29:29347332-30347114	3.47	<i>SRPRA, TIRAP, DCPS, ST3GAL4, KIRREL3, CDON</i>

9.76% of the total genetic variance for SC

*Significant associated windows explaining more than 1% of the genetic variance for SC and overlapping within functional candidate genes

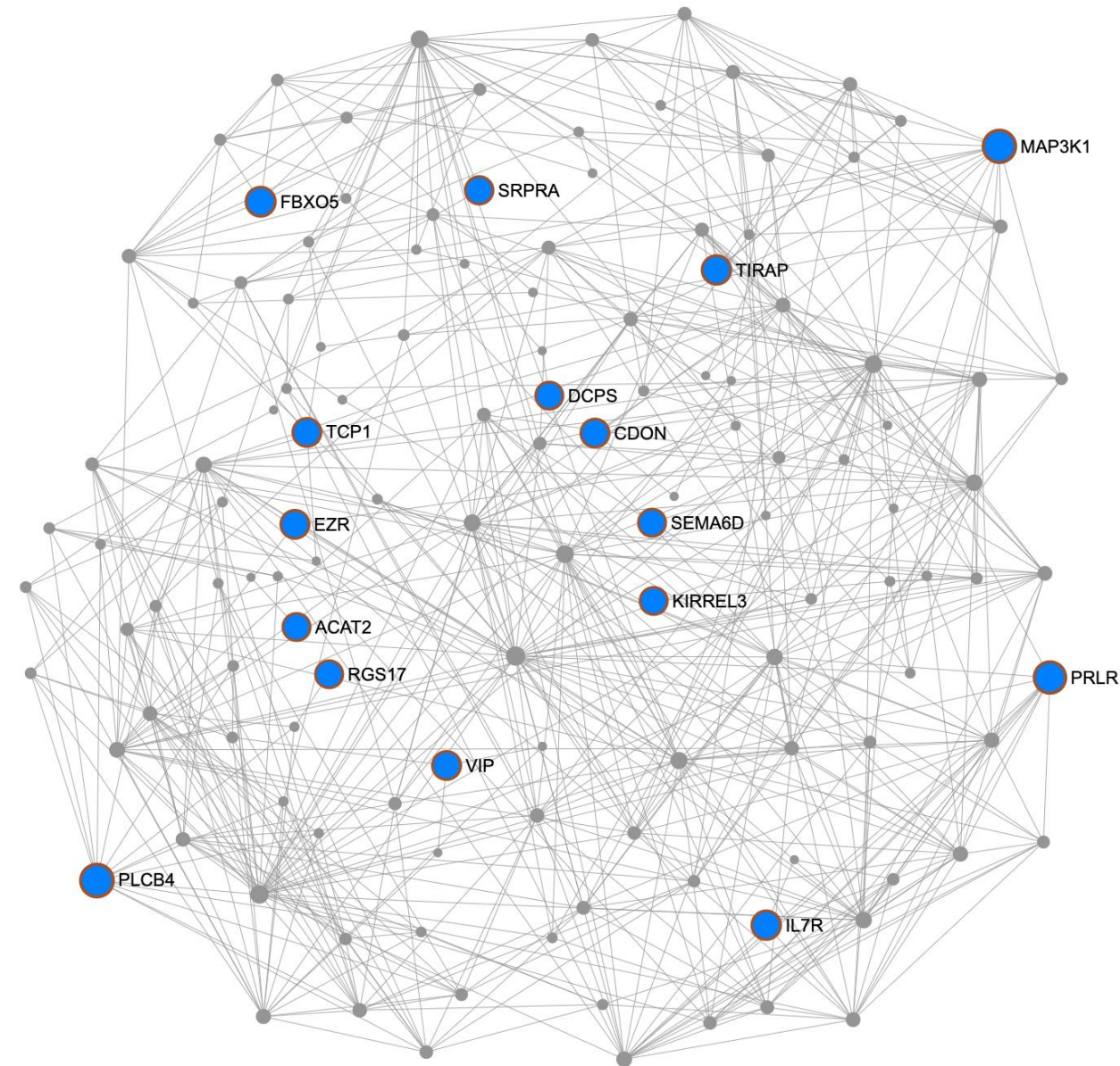
Functional Candidate Genes: Sperm Motility

Region*	% variance explained	Prioritized Candidate Genes
BTA9:95179163-96178394	1.47	<i>SOD2, ACAT2, TCP1, MAS1, EZR</i>
BTA9:97251687-98242399	1.29	<i>PRKN, PACRG</i>
BTA13:1818757-2813363	1.34	<i>PLCB4, PAK5</i>
BTA20:38077292-39076620	1.11	<i>IL7R, SPEF2, SKP2, PRLR</i>
BTA24:51881818-52880825	1.95	<i>DCC</i>

7.16% of the total genetic variance for SM

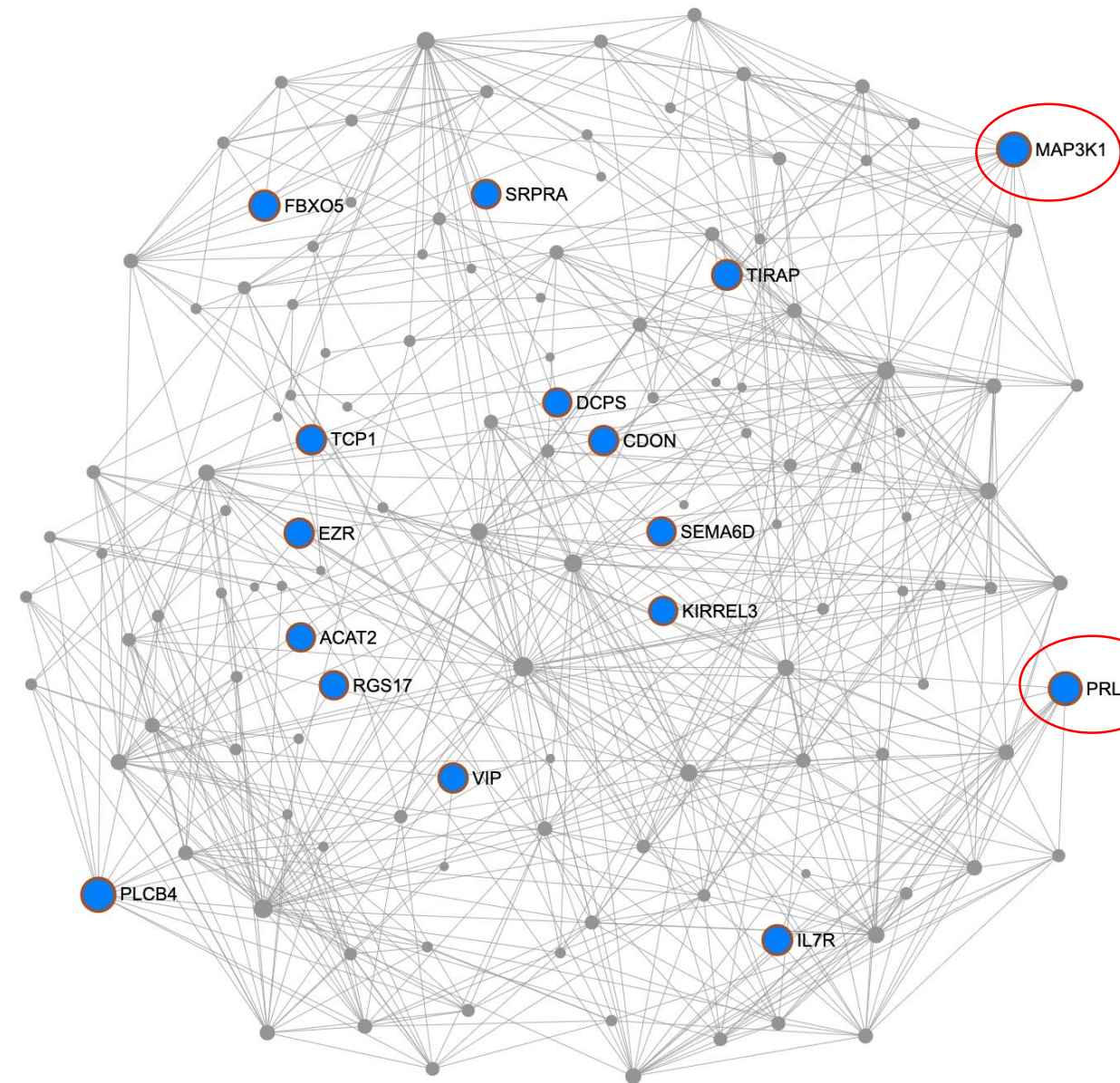
* Significant associated windows explaining more than 1% of the genetic variance for SM and overlapping within functional candidate genes

Functional Analysis



Metabolic Pathway	P-value	Number of Genes
Actin filament bundle assembly	1.49×10^{-17}	11
Actin filament based process	1.10×10^{-9}	14
Hormone secretion	1.46×10^{-7}	9
Actin filament organization	1.30×10^{-5}	5
Spermatid differentiation	2.26×10^{-4}	3
Reproductive process	1.01×10^{-3}	4

Functional Analysis



MAP3K1: BTA20 (1.00% genetic variance SC)

Control testis function and mass
Biomarker of spermatogenesis and apoptosis

(Guan et al., 2017)

PRLR: BTA20 (1.11% genetic variance SM)

Spermatid differentiation
Growth and secretory activity of male accessory organs
Normal levels essential for male reproductive functions

(Mann and Lutwak-Man,
1981)

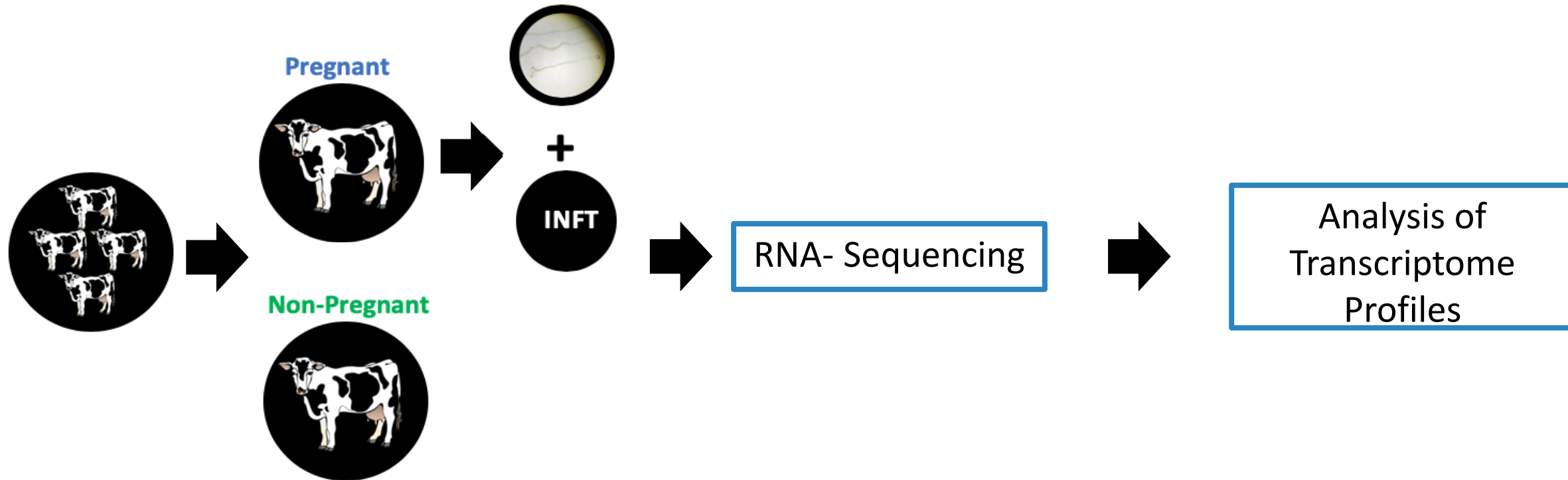
Conclusions

- These results identified important prospective **functional candidate genes** associated with **bull fertility** in composite **beef cattle**
 - 14 functional candidate genes located within significant windows affecting SC
 - 14 functional candidate genes located within significant windows affecting SM
- These preliminary results may help to **better understand the genetic bases** of **SC and SM** and to identify **genetic markers** to ultimately **improve bull genomic prediction** for these traits

Future Directions

- **Female Bovine fertility**

- Provide further insight into the underlying fundamentals of the establishment of pregnancy by comparing the urogenital tract transcriptome profiles of pregnant and non-pregnant cows to identify functional markers associated with pregnancy success



Acknowledgements

Collaborators

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- Dr. Murillo Romulo Carvalho
- Dr. Jose Felipe Warmling Spricigo
- Dr. Aroa Suárez-Vega
- Dr. Pablo Fonseca

UNIVERSITY
of GUELPH



Funding Agencies



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**ANIMAL
BIOSCIENCES**



Thank you!

Hannah Sweett (hsweett@uoguelph.ca)

