Influence of genetics on fertility traits in Angus bulls

by

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Abstract

Whether a bull is used for natural mating or artificial insemination (AI), a single bull with suboptimal fertility can have a greater impact on herd productivity compared to a single female with reduced fertility. Despite this, extensive research on advanced reproductive technologies and genetic evaluations has focused primarily on female fertility, leaving a gap in the understanding of factors influencing male fertility. Therefore, the objectives of this study were to estimate the genetic parameters for scrotal circumference, sperm motility, and sperm morphology traits, evaluate the utility of combining breeding soundness examination (BSE) and AI bull stud collection data, and perform a genome-wide association study. Breeding soundness examination data were obtained from 4,996 Angus bulls, and stud collection data were obtained from 1,862 Angus stud bulls. The data were analyzed as a BSE dataset, a stud dataset, and a combined dataset with both BSE and stud records. Each of the three datasets were evaluated individually for genetic parameters. A single-step genomic best linear unbiased prediction (ssGBLUP) was used to estimate variance components and heritabilities of bull reproductive traits with the BLUPF90+ suite of programs (Misztal et al., 2014). The heritabilities estimated for scrotal circumference ranged from 0.36 to 0.55, while semen motility and morphology traits had lower heritability estimates of 0.04 to 0.12. Phenotypic and genetic correlations across traits in a single dataset ranged from low to high. Additionally, genetic correlations were estimated within traits to assess the relationship between a measurement taken during a BSE and at a bull stud, which resulted in low to moderate correlations. The significant quantitative trait loci (QTL) regions in the genome were identified at a significance threshold of -log10 *P*-value greater than 4.0 for scrotal circumference (SC), percentage of progressive motility (%MOT), motility score (MOTSC), percentage of secondary abnormalities (%SEC), and percentage of normal

spermatozoa (%NORM). Previously reported QTL and candidate genes associated with fertility traits were identified. Results indicate that bull fertility is influenced by genetics, and selecting bulls with enhanced fertility could improve reproductive efficiency and herd productivity.

Table of Contents

List of Figures viii
List of Tables xi
Acknowledgements xiv
Chapter 1 - Literature Review 1
Introduction 1
Sperm Production Cycle 2
Spermatogenesis 2
Epididymal Transit Time 2
Factors Affecting Sperm Production 3
Age 4
Temperature 5
Nutrition 6
Sexual Behavior and Interactions 7
Health 8
Management Factors for Commercial Semen Collection 9
Breeding Soundness Examination 10
Physical Examination 11
Reproductive Tract Examination 12
Examination of Semen 13
Limitations and Concerns 16
Genetic Evaluation of Bull Fertility 17
Genetic Parameters 17
Scrotal Circumference 18
Semen Production Traits 18
Semen Quality Traits 20
Genetic and Phenotypic Correlations 23
Scrotal Circumference with Semen Production Traits 23
Scrotal Circumference with Semen Quality Traits 23
Scrotal Circumference with Female Reproductive Traits 24

Semen Production Traits 25
Semen Production Traits with Semen Quality 26
Semen Quality Traits 28
Male Fertility Selection Tools 30
Sire Conception Rate 30
Scrotal Circumference Expected Progeny Difference 30
Summary 32
Chapter 2 - Genetic parameter estimates for fertility traits in Angus bulls 50
Abstract 50
Introduction 51
Materials and Methods 52
Study Populations 52
Breeding Soundness Examination Data 52
Bull Stud Collection Data 53
Breeding Soundness Examination and Bull Stud Data Combined 56
Data Filtering 56
Environmental Data 57
Genetic Data 58
Statistical Analysis 59
Model Selection 59
Phenotypic Analysis 60
Genetic Parameter Estimation 60
Results and Discussion 62
Variance Components and Heritability Estimates 64
Genetics Correlations Within Trait 72
Correlations Across Traits 75
Conclusion 80
Chapter 3 - Genome-wide association study of fertility traits in Angus bulls 93
Abstract 93
Introduction 94
Materials and Methods 95

Phenotypic Data 95
Genomic Data 96
Statistical Analysis 97
SNP Effect Estimation 98
Quantitative Trait Loci Analysis 100
Results and Discussion 100
Summary Statistics 100
Quantile-quantile plots 101
Genome-wide Association Analysis 102
Previously Reported QTL Regions 102
Biological Functions 105
Conclusion 107
References 121
Chapter 1 121
Chapter 2 138
Chapter 3 146
Appendix A - Additional Figures and Tables 152

List of Figures

Figure 3.1 Quantile-quantile plots showing the distribution of -log10 p-values for scrotal circumference (SC), percent progressive motility (%MOT), and motility score (MOTSC) before and after genomic control. Panel A: Original -log10 p-values for SC, Panel B: -log10 p-values for SC after genomic control, Panel C: Original -log10 p-values for %MOT, Panel D: -log10 p-values for %MOT after genomic control, Panel E: Original -log10 p-values for MOTSC, Panel F: -log10 p-values for MOTSC after genomic control. 115

Figure 3.2 Quantile-quantile plots showing the distribution of -log10 p-values for percentage of primary abnormalities (%PRIM), percentage of secondary abnormalities (%SEC), and percentage of normal spermatozoa (%NORM) before and after genomic control. Panel A: Original -log10 p-values for %PRIM, Panel B: -log10 p-values for %PRIM after genomic control, Panel C: Original -log10 p-values for %SEC, Panel D: -log10 p-values for %SEC after genomic control, Panel E: Original -log10 p-values for %NORM, Panel F: -log10 p-values for %NORM after genomic control. 116

Figure 3.3 Manhattan plot showing the original results of a genome-wide association study with a significance threshold of 4.0 for scrotal circumference (panel A), percentage of progressive motility (panel B), and motility score (panel C). 117

Figure 3.4 Manhattan plot showing the original results of a genome-wide association study with a significance threshold of 4.0 for percentage of primary abnormalities (panel A), percentage of secondary abnormalities (panel B), and percentage of normal sperm (panel C). 118

Figure 3.5 Manhattan plot showing the results of a genome-wide association study with a significance threshold of 4.0 for scrotal circumference (panel A), percentage of progressive motility (panel B), and motility score (panel C) after genomic control was applied. 119

Appendix Figure A.1 Number of measurements per bull for each trait in the breeding soundness examination dataset. Panel A: Scrotal circumference, Panel B: Percentage of progressive motility, Panel C: Motility score, Panel D: Percentage of primary abnormalities, Panel E: Percentage of secondary abnormalities, Panel F: Percentage of normal spermatozoa. 152

Appendix Figure A.2 Number of records for each trait that passed (Pass) or failed (Fail) the Society for Theriogenology's minimum requirements for an individual bull to pass a breeding soundness examination (BSE) from all bulls in the BSE dataset. 153
Appendix Figure A.3 Number of records for each trait that passed (Pass) or failed (Fail) the Society for Theriogenology's minimum requirements for an individual bull to pass a breeding soundness examination (BSE) from only bulls with two or more records in the BSE dataset. 154
Appendix Figure A.4 Number of measurements per bull for each trait in the stud dataset. Panel A: Scrotal circumference, Panel B: Percentage of progressive motility, Panel C: Motility score, Panel D: Percentage of primary abnormalities, Panel E: Percentage of secondary abnormalities, Panel F: Percentage of normal spermatozoa. 155
Appendix Figure A.5 Number of records for each trait in the bull stud dataset that would have passed (Pass) or failed (Fail) the minimum requirements of semen quality for an ejaculate to undergo cryopreservation at a bull stud and the minimum scrotal circumference measurement a bull should have determined by the Society for Theriogenology's breeding soundness examination guidelines. 156
Appendix Figure A.6 Number of records for each trait in the bull stud dataset that would have passed (Pass) or failed (Fail) the minimum requirements of semen quality for an ejaculate to undergo cryopreservation at a bull stud and the minimum scrotal circumference measurement a bull should have determined by the Society for Theriogenology's breeding soundness examination guidelines from only bulls with two or more records in the stud dataset. 157
Appendix Figure A.7 Number of measurements per bull for each trait in the combined dataset. Panel A: Scrotal circumference, Panel B: Percentage of progressive motility, Panel C: Motility score, Panel D: Percentage of primary abnormalities, Panel E: Percentage of secondary abnormalities, Panel F: Percentage of normal spermatozoa. 158
Appendix Figure A.8 Number of records for each trait that passed (Pass) or failed (Fail) the Society for Theriogenology's minimum requirements for an individual bull to pass a breeding soundness examination (BSE) from all bulls in the combined dataset. 159
Appendix Figure A.9 Number of records for each trait that passed (Pass) or failed (Fail) the Society for Theriogenology's minimum requirements for an individual bull to pass a

breeding soundness examination (BSE) from only bulls with two or more records in the combined dataset. 160

List of Tables

Table 1.1 The minimum thresholds for a bull's scrotal circumference by age to pass a breeding soundness examination. 34

Table 1.2 Previously reported heritability estimates (h^2) and standard error (SE) for semen production traits including the number of bulls (n) in the study and their breed. *Indicates study reported number of semen ejaculate records instead of individual animals. – Indicates no standard error. 35

Table 1.3 Previously reported heritability estimates (h^2) and standard error (SE) for semen quality traits including the number of bulls (n) in the study and their breed. *Indicates study reported number of semen ejaculate records instead of individual animals. – Indicates no standard error. 37

Table 1.4 Previously reported phenotypic (r_P) and genetic (r_G) correlations between semen production and semen quality traits, including the number of bulls (n) in the study and their breed. * Indicates study reported number of semen ejaculate records instead of individual animals. – indicates no standard error. 40

Table 1.5 Genetic (r_G) correlations between scrotal circumference and female reproductive traits. 49

Table 2.1 Abbreviations (Abb), units, and descriptions of bull fertility trait. 83

Table 2.2 Number of breeding soundness examination (BSE) and stud records from the operations including year(s) of examination records, the total number of records, number of bulls, and the number of records for each trait: scrotal circumference (SC), percentage of progressive motility (%MOT), gross motility score (MOTSC), percentage of primary abnormalities (%PRIM), percentage of secondary abnormalities (%SEC), and percentage of normal spermatozoa (%NORM). 84

Table 2.3 Regression coefficient (β) and p-value calculated with the likelihood ratio test for each covariate that was not confounded with contemporary group. Age at collection in days (AgeD) was not tested in stud collection analysis because the average age was 1180 days or 3.25 years old, but age group was tested as a fixed effect. 85

Table 2.4 The effect(s) utilized in the final model for scrotal circumference (SC), percentage of progressive motility (%MOT), gross motility score (MOTSC), percentage of primary

abnormalities (%PRIM), percentage of secondary abnormalities (%SEC), and percentage of normal spermatozoa (%NORM) in the breeding soundness examination (B), stud (S), and combined (C) datasets. 86
Table 2.5 Number of records (N), mean, standard deviation (SD), median, minimum, and maximum for scrotal circumference (SC), percentage of progressive motility (%MOT), gross motility score (MOTSC), percentage of primary abnormalities (%PRIM), percentage of secondary abnormalities (%SEC), and percentage of normal spermatozoa (%NORM) in the breeding soundness examination (B), stud (S), and combined (C) datasets. 87
Table 2.6 Average variance component estimates for direct additive genetic ($\boldsymbol{\sigma a2}$), permanent environmental ($\boldsymbol{\sigma pe2}$), and environmental ($\boldsymbol{\sigma e2}$) variances, heritabilities (h^2) with standard error (SE) and repeatabilities (r^2) calculated from bivariate analyses for scrotal circumference (SC), percentage of progressive motility (%MOT), gross motility score (MOTSC), percentage of primary abnormalities (%PRIM), percentage of secondary abnormalities (%SEC), and percentage of normal spermatozoa (%NORM) in the breeding soundness examination (B), stud (S), and combined (C) datasets. 88
Table 2.7 Genetic correlations (r_G) and standard errors (SE) within each bull reproductive trait using the stud and breeding soundness examination datasets. 89
Table 2.8 Genetic correlations above the diagonal and phenotypic correlations below the diagonal between Angus bull reproductive traits from a breeding soundness examination using a bivariate single-step genomic best linear unbiased prediction. 90
Table 2.9 Genetic correlations above the diagonal and phenotypic correlations below the diagonal between Angus bull reproductive traits from stud collections using a bivariate single-step genomic best linear unbiased prediction. 91
Table 2.10 Genetic correlations above the diagonal and phenotypic correlations below the diagonal between Angus bull reproductive traits from a combination of BSE and stud collection records using a bivariate single-step genomic best linear unbiased prediction.... 92
Table 3.1 Trait list, units, abbreviations (Abbrev), and definitions of beef bull reproductive traits. 109
Table 3.2 Summary statistics for each trait, including the total number of combined records for each trait, number of bulls with phenotypes, number of genotyped bulls with records, minimum trait threshold to pass a breeding soundness examination according to the Society

for Theriogenology guidelines, number of genotypes bulls with observations below the trait threshold, mean, standard deviation (SD), minimum (Min), and maximum (Max) values.110
Table 3.3 Summary of significant genomic regions for beef bull reproductive traits including chromosome (CHR), quantitative trait loci (QTL) region, number of significant single nucleotide polymorphism (N of SNP) identified per QTL region, original -$\log_{10}$ p-value, genomic control (GC) applied -$\log_{10}$ p-value, and the percentage (%) of variance explained by the QTL region. 111
Table 3.4 Previously reported quantitative trait loci (QTL) regions associated with reproduction traits which overlap significant single nucleotide polymorphism (SNP) identified for beef bull reproduction traits in the current study. 112
Table 3.5 Putative candidate genes within close proximity of significant quantitative trait loci (QTL) regions on individual chromosome (Chr:Reg) associated with beef bull fertility traits. 114

Appendix Table A.1 Genes within close proximity of significant quantitative trait loci (QTL) regions on individual chromosome associated with scrotal circumference. 161
Appendix Table A.2 Genes within close proximity of significant quantitative trait loci (QTL) regions on individual chromosome associated with percentage of progressive motility.... 163
Appendix Table A.3 Genes within close proximity of significant quantitative trait loci (QTL) regions on individual chromosome associated with motility score. 164
Appendix Table A.4 Genes within close proximity of significant quantitative trait loci (QTL) regions on individual chromosome associated with percentage of normal spermatozoa. .. 165
Appendix Table A.5 Genes within close proximity of significant quantitative trait loci (QTL) regions on individual chromosome associated with percentage of secondary abnormalities. 166

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Chapter 1 - Literature Review

Introduction

Fertility is broadly defined as the ability to conceive a viable offspring, and both males and females must be sufficiently fertile to produce a viable embryo (Utt, 2016). A beef herd's overall fertility rate is generally measured by its pregnancy rate, or the percentage of cows that successfully became pregnant during their first, second, or third estrous cycle of the breeding season (Hopper, 2014). Conception is impacted by various factors, including age, nutrition, health, and environmental conditions (Chacón et al., 2002; Nichi et al., 2006; Barth, 2007; Koivisto et al., 2009; Amann et al., 2018). Furthermore, genetic factors such as the breed composition (Barth and Waldner, 2002; Koivisto et al., 2009), inbreeding coefficient (Karoui et al., 2011), and long-term selection using tools for reproductive traits (Fleming et al., 2019; Butler et al., 2021) can impact reproductive performance which is critical to the profitability of a beef operation.

Most genetic selection tools available to beef producers focus on female fertility. Expected progeny differences (EPD) have been developed to estimate the probability of pregnancy in first-calf heifers, the probability of calving as a three-year-old given she calved as a first-calf heifer, and the probability that a female will produce a calf every year to at least six years of age (Doyle et al., 2000; RAAA, 2018; AGA, 2022). Several beef breed associations have published a scrotal circumference EPD which predicts an animal's ability to influence the size of scrotal circumference in their offspring (Angus Beef Bulletin, 2015; AGA, 2022). However, it does not account for direct measurement of semen production or semen quality, which can be major determinants of conception (Amann et al., 2018). Heritability estimates and

genetic correlations that have been published for scrotal circumference, semen production measures, and semen quality measures illustrate the potential for improving beef bull fertility.

Sperm Production Cycle

Spermatogenesis

Spermatogenesis is the process where germ cells undergo cell divisions and morphological changes in the seminiferous tubules leading to the production of spermatozoa. Spermatogenesis can be divided into three phases: proliferation, meiosis, and differentiation (Senger, 2012). During the proliferation phase, spermatogonia proceed through all mitotic divisions and the process of stem cell renewal. In the meiotic phase, primary spermatocytes undergo DNA replication and crossing over during meiosis I in the process of developing into secondary spermatocytes. Meiosis II concludes with the production of haploid spherical spermatids. Differentiation or spermiogenesis is the third and final phase of spermatogenesis, where spermatids transform into a spermatozoa containing a head, midpiece, and principal piece (Senger, 2012). The total duration of spermatogenesis in the bull is 61 days (Staub and Johnson, 2018). Spermatogenesis starts in the male once he has reached puberty and uniformly continues throughout his lifetime (Senger, 2012). Approximately 20 weeks after puberty, a bull will reach its maximum daily sperm production per gram of testis (Schenk, 2018).

Epididymal Transit Time

Epididymal transit time (ETT) is the process of transporting spermatozoa from the testis through the epididymis. The epididymis is responsible for sperm concentration, storage, and maturation because spermatozoa cannot fertilize an oocyte immediately following spermatogenesis (Igboeli and Foote, 1968; Caballero et al., 2011). Spermatozoa first enter the head of the epididymis, or the caput, where necessary fluids for maturation are secreted (Cooper,

1986; Amann, 1987). Then in the corpus, or the body of the epididymis, maturation is completed, and spermatozoa acquire their full fertilizing capacity (Amann et al., 1974; Schenk, 2018). Finally, the spermatozoa enter the tail of the epididymis, or the cauda, where they are maintained and stored until they are depleted by urination or ejaculation (Schenk, 2018). The entire process from caput to cauda takes approximately 14 days.

The environment in the cauda prevents premature activation and keeps the spermatozoa in a metabolic quiescence state (Caballero et al., 2011). During the first two years of a bull's life, his cauda's storage capacity, and daily spermatozoa production increases. A sexually rested mature bull has the ability to store five to six days' worth of sperm production (Schenk, 2018), which is sufficient for ten successive ejaculates (Caballero et al., 2011). Semen collection centers can maximize young bulls' weekly sperm production by collecting one to two ejaculates per collection day and then waiting three days before the next collection day (Almquist, 1982). Mature bulls can be collected as often as every other day. However, the frequency of ejaculation can affect semen quantity and quality. When epididymal sperm reserves are consistently depleted the concentration and volume of an ejaculate can decrease, and the transportation time through the cauda can be shortened 1 to 2 days which may reduce secondary abnormalities (Schenk, 2018).

Factors Affecting Sperm Production

The semen production cycle is an extremely sensitive process that changes over a bull's lifetime and can be impacted by many environmental factors. Minor environmental disruptions during spermatogenesis and ETT can negatively affect semen quality. Bulls utilized for natural service can be affected by age, health, climate temperature, nutrition, and social interactions. Bulls collected at artificial insemination centers are exposed to these environmental factors as

well as collection interval and handling procedures, which may affect semen quality (Amann et al., 2018).

Age

Puberty is defined as the age when a bull produces his first ejaculate that contains a minimum of 50 million sperm cells, and of those cells, at least 10% are progressively motile (Wolf et al., 1965; Staub and Johnson, 2018). Most beef bulls reach puberty between 38 to 46 weeks of age, with the average age being approximately 42 weeks (Staub and Johnson, 2018). Lunstra et al. (1978) reported that prepubertal bulls exhibited sexually interested behaviors approximately three weeks before reaching puberty. Six weeks after reaching puberty, bulls attain their ability to successfully deposit semen into a female's reproductive tract (Lunstra et al., 1978).

Bulls' ages at the time of a breeding soundness examination (BSE) or semen collection significantly influences semen characteristics. As bulls get older, their ejaculate volume, concentration, total sperm count, percentage of viable sperm, and sperm motility increases (Mathevon et al., 1998; Fuerst-Waltl et al., 2006; Hartman et al., 2021). Spermatic efficiency tends to progress until a bull reaches full maturity (Staub and Johnson, 2018). Bulls have been observed to remain fertile until 19 years of age (Bishop, 1970). However, many studies have reported the optimum bull ages for semen production and quality traits. Taylor et al. (1985) observed an increase in ejaculate volume until bulls reached seven years of age; volume plateaued and then decreased after nine years of age. Fuerst-Waltl et al. (2006) reported increases in concentration and motility as bull age increased. Conversely, Brito et al. (2002) reported that age did not significantly affect ejaculate concentration or motility in *Bos indicus* cattle, however the study did not state the range of ages of the bulls. Hartman et al. (2021) found that the

optimum bull age for concentration was two to three years old, and the optimum age for motility was four to five years. Primary, secondary, and total abnormalities have been reported to decrease as age increases (Chandler et al., 1985; Söderquist et al., 1996; Hartman et al., 2021).

Young bulls tend to be less reproductively efficient than older bulls. Bulls under 12 months of age at AI centers had lower sperm volume, concentration, motility, and normal spermatozoa when compared to older bulls (Murphy et al., 2018; Hartman et al., 2021). Kennedy et al. (2002) reported that ten and eleven-month-old bulls passed a BSE at a significantly lower rate than their cohorts of older ages due to inadequate semen quality and scrotal circumference (SC). Monday et al. (2018) evaluated factors associated with passing a second or third BSE after failing their initial exam in yearling Angus, Simmental, and Charolais bulls. The proportion of bulls that later passed a BSE after failing the initial exam was 48.9% (Monday et al., 2018). The authors reported that age, breed, spermatozoa morphology, spermatozoa motility, and SC were not significant variables to predict passing a subsequent BSE after failing the initial examination (Monday et al., 2018). However, Monday et al. (2018) and Coe (1999) recommend that producers consider bulls' ages when scheduling their initial BSE.

Temperature

Many studies have shown that bull fertility is affected by season and temperature (Taylor et al., 1985; Mathevon et al., 1998; Barth and Waldner, 2002; Rahman et al., 2018; Sabés-Alsina et al., 2019). The optimal temperature for semen production is approximately 15 to 20°C or 59 to 68°F (Taylor et al., 1985; Morrell, 2020). Heat stress occurs when a bull's physiological mechanisms cannot maintain homeostasis. The testes have a specialized countercurrent heat exchange mechanism known as the pampiniform plexus to regulate temperature (Senger, 2012). Spermatogenesis requires the testes be 4 to 6° C cooler than the body to occur properly (Senger,

2012; Morrell, 2020). Primary spermatocytes are most vulnerable during the meiotic phase of spermatogenesis (Rahman et al., 2018; Morrell, 2020). Kastelic et al. (1996) simulated a heat stress event by raising bulls' testicular temperature to observe their reproductive responses. The authors found that the bulls' ejaculates had more morphologically abnormal spermatozoa, with the greatest increases in head and midpiece defects (Kastelic et al., 1996). Prolonged heat stress reduces spermatozoa concentrations, motility, and viability for several weeks following the heat event (Rahman et al., 2018; Morrell, 2020).

Cold stress occurs when an animal increases heat production to stay warm (Wagner, 1988). Bulls prioritize their maintenance requirements for nutrition and shelter and are less likely to exhibit reproductive behaviors when undergoing cold stress (Silva et al., 2023). In addition, the tunica dartos muscle will contract to hold the testes closer to the body in response to the cold environment (Senger, 2012). Barth and Waldner (2002) studied bulls of various ages and concluded that bulls tested for breeding soundness in January through March were less likely to be classified as satisfactory when compared to bulls tested in April through June. Cold stress has been found to impact older bulls more frequently than younger bulls (Barth and Waldner, 2002). In a Canadian study, bulls with severely frostbitten scrotums needed four to five months to recover before their semen quality returned to satisfactory levels (Barth and Waldner, 2002).

Nutrition

Nutrition is a major limiting factor of male reproductive performance (Short and Adams, 1988). Nutrition can affect bulls' overall fertility and potentially lower conception rates (Singh et al., 2018). Proper nutritional management for prepubertal bulls is crucial because it can affect their age at puberty and mature scrotal circumference (Coulter et al., 1997). Mature bull nutrition should be adjusted depending on the time of year and age of the bull (Leathem, 1975). Greater

intake levels outside the breeding season can result in high body condition scores for yearling and mature bulls, leading to decreased ejaculate volume, concentration, and semen quality (Coulter et al., 1997; Kenny and Byrne, 2018). During the breeding season, bulls can enter a period of nutrient restriction due to reduced feed intake and mating activity. Harrison et al. (2022) reported an increased head and total abnormalities when bulls lost weight from nutrient restriction, but it did not impact progressive motility. Conversely, progressive motility was affected by undernutrition in dairy bulls (Flipse and Almquist, 1961).

Sexual Behavior and Interactions

A bull's sexual and social behaviors can impact his fertility. Rollinson (1955) reviewed literature on bull sexual behavior and demonstrated that genetic factors greatly influence bulls' libido. Monozygous twin bulls were studied by Bane (1954), and he reported that they had similar mating abilities and libido despite different environmental conditions (Bane, 1954). In a study with *Bos indicus* and *Bos taurus* cattle, the crossbred bulls generally had higher libido scores than their parental purebred contemporaries (Chenoweth and Osborne, 1975). Quirino et al. (2004) quantified the libido of 288 Nellore bulls and estimated a heritability of 0.34 ± 0.10. Even though libido is an important component to a bull's fertility, it is rarely evaluated in North American (Koziol and Armstrong, 2018), and is not significantly correlated ($P > 0.05$) to SC or semen quality (Chenoweth et al., 1988).

Pexton et al. (1990) studied how a bull's sexual behavior may be affected by the bull-to-female ratio (BFR). The authors reported that bull mating activity increased with the number of females in estrus (Pexton et al., 1990). However, it is recommended that yearling bulls should be expected to service 15-25 females and mature bulls to service 25-35 females in a single breeding season (USDA, 2009; Chenoweth, 2015). Beerwinkle (1974) compared multiple natural sires'

estrus detection abilities with differing BFRs. The author reported that with a BFR of 1:30, 95% of the females were mounted. At BFRs of 1:60; approximately 65% were mounted, and at 1:100 only 51% of females were mounted (Beerwinkle, 1974).

Numerous studies report that sexual behavior in males can be enhanced when other males are present and sexually active (Chenoweth, 1983; Mader and Price, 1984; Godfrey and Lunstra, 1989). Godfrey and Lunstra (1989) found that bulls with higher libido scores serviced more females than bulls with lower libido scores in the presence of another male. However, Rupp et al. (1977) reported that bulls with more dominance and higher social ranking service more females than lower dominance bulls in multi-sire mating pastures, even if the lower dominance bulls had higher libido. Dominance appears to be related to age, so dominance appears to be more evident in pastures with mixed age bulls (Blockey, 1979). Herd fertility could be affected if the dominant bull is less fertile but controls most of the matings, preventing other bulls from serving females.

Health

A variety of health-related issues such as physical injury, disease, and fever can negatively impact bull fertility. Barth and Waldner (2002) reported that of 17 bulls with lameness issues, 76% did not receive a satisfactory rating for semen quality during a BSE. Pain in the feet and legs would inhibit the section of LH and testosterone, which are critical for spermatogenesis (Barth and Waldner, 2002). Several pathogens are reported to cause infertility in bulls (Givens and Marley, 2008) or can be transmitted via bovine semen and prevent fertilization or full-term pregnancies (Moore et al., 2021; Adnane and Chapwanya, 2022). Bovine herpesvirus 1 (BoHV-1) can cause an infection to a bull's glans penis and prepuce. Although BoHV-1 does not have a direct effect on spermatozoa, it can inhibit sperm binding to the zona-pellucida causing lower conception rates (Givens and Marley, 2008; Senger, 2012).

Brucella abortus has been reported to cause epididymitis, ampullitis, seminal vesiculitis, and decreased libido (Givens and Marley, 2008). Inflammation in any reproductive organs would cause a bull to fail the physical examination portion of a BSE. Fevers can have deleterious effects on semen quality because of the higher-than-normal body temperature. It can take up to three months for semen quality to return to normal morphology and motility levels after the infection (Cumming, 2007).

Management Factors for Commercial Semen Collection

Artificial insemination (AI) is one of the most important reproductive tools for cattle breeders. According to the USDA (2018), 89% of dairy operations utilize AI for mating their cows. However, the beef industry has been much slower in adopting the technology, with only 11.6% of the beef cows in the United States being bred through AI (USDA, 2020). To prevent disease transmission between AI bulls and breeding females, bulls must meet health standards put in place by the Certified Semen Services, Incorporated (CSS), World Organization for Animal Health (OIE), and National Association of Animal Breeders (NAAB) (Barth, 1993; Hopper, 2014). All prospective AI bulls must test negative for bovine tuberculosis, brucellosis, leptospirosis, and viral diarrhea virus (CSS, 2021).

Artificial insemination centers typically collect bulls two to three times per week to maximize their ejaculate volume and the viability of spermatozoa. Mature bulls can produce two usable ejaculates per collection day for cryopreservation (Staub and Johnson, 2018). Sexual stimulation and preparation are essential for optimal sperm harvest. Sexual stimulation evokes a bull's desire to mount and ejaculate quickly. Sexual preparation is designed to help a bull ejaculate the maximum number of spermatozoa possible (reviewed by Chenoweth, 1983). Beef bulls respond differently than dairy bulls to sexual preparation. Almquist (1973) conducted a

study on Angus and Hereford bulls and determined that beef bulls should be given three opportunities to false mount a teaser animal prior to their first ejaculation, and bulls should not be given any false mounts before their second ejaculation.

Breeding Soundness Examination

The BSE is a management tool utilized by producers to provide an assessment of a bull's breeding potential. A BSE aims to identify bulls that cannot breed females successfully. The degrees of fertility are defined by the terms sterile, infertile, subfertile, and fertile (Barth, 2018). Any bull that is permanently unable to reproduce is designated sterile. Infertile is when a bull is temporarily unable to reproduce. Bulls with inadequate or depressed reproductive ability are classified as subfertile. Fertile bulls can reproduce at a normal rate. The incidence of subfertility can range from 20-40% or one in every five bulls (Elmore et al., 1975; Kastelic and Thundathil, 2008). Once identified, inadequately fertile bulls should be removed from the breeding population to not impede pregnancy rates for producers (Hopper, 2014; Barth, 2018). Examinations should be performed on bulls before being sold as breeding animals, before every breeding season for natural service sires, or whenever a bull's fertility is questioned.

The BSE consists of three key components: a physical examination, reproductive tract examination, and semen quality evaluation (Hopper, 2014). The Society for Theriogenology (SFT) established the standards for a BSE with the intention to predict the likelihood that a bull could establish pregnancy in 25 or more healthy, cycling females during a 65-to-70-day breeding season (Koziol and Armstrong, 2018). The requirements of a "satisfactory" BSE include evaluations for functional bone structure, having an adequate scrotal circumference (SC) for his age (Table 1.1), having ≥ 30% progressive sperm motility and ≥ 70% morphologically normal sperm (Barth, 2007; Koziol and Armstrong, 2018). A bull can be classified as "deferred" if the

veterinarian performing the BSE thinks the bull has a temporary condition rendering him to fail but will likely improve in the future (Kastelic and Thundathil, 2008). Bulls are deferred for three main reasons: young or prepubertal bulls, environmental conditions, or transient illness (Hopper, 2014). Bulls are considered prepubertal until they can ejaculate semen with at least 50 million sperm cells, and 10% of those sperm cells must be progressively motile (Wolf et al., 1965). Barth and Waldner (2002) determined that a short-day photoperiod, extreme cold stress, and undernourishment can negatively affect spermatogenesis, causing increased sperm immobility or abnormalities. Deferred bulls should be retested approximately 60 days after the initial test to allow for recovery of normal spermatogenesis (Hopper, 2014). Any bull that does not meet the standards for the BSE and is unlikely to meet them in the future is classified as "unsatisfactory" (Hopper, 2014). Reasons for being considered "unsatisfactory" include permanent physical detriments, lower than recommended SC, or insufficient sperm quality (Barth, 2007).

Physical Examination

The physical examination of the BSE aims to identify any undesirable structural or physical impediments. A bull must be able to walk, mount, and see in order to function as a natural service sire in a pasture or range environment (Hopper, 2014). Bulls should be observed in the holding pen to study conformation and gait to identify soundness issues and sight deficiencies (Hopper, 2014; Vargas et al., 2017). Considering a bull must be able to successfully mount females multiple times per day, feet and leg conformation is an important factor in the physical examination. Feet and leg conformation can also affect his longevity as a service sire within a herd (Hopper, 2014). Chronic laminitis, interdigital fibromas, and screw claw are common hoof issues that the veterinarian should record on the BSE form (Hopper, 2014). An examination of the bull's face should begin once the bull is restrained. The veterinarian should

observe the eyes for corneal damage, squamous cell carcinomas, and lymphomas because bulls rely heavily on their vision to detect cows in estrus (Hopper, 2014; Barth, 2018).

Reproductive Tract Examination

During the reproductive system examination, the scrotum, testicles, and spermatic cord are assessed for size, texture, shape, fluid, and fibrotic tissue (Hopper, 2014). The scrotum shape should ideally be visually appraised in a warmer environment when the bull is relaxed to indicate the testes' size and the scrotum's thermoregulatory abilities (Barth, 2007). The testes should be palpated to determine if there are any testicular abnormalities (Barth, 2007). Palpation of the spermatic cord from the external inguinal ring, distal from the testicle, must be done to detect abnormalities such as hernias, hematomas, fibrosis, or fluid (Hopper, 2014). Finally, the penis should be examined as the bull ejaculates to discover abnormalities such as warts, hair rings, lacerations, or persistent frenulum (Hopper, 2014).

The SC measurement should be taken during the reproductive examination. When taking the measurement, the testes must be gently forced to the bottom of the scrotum and a measuring tape should be placed level with the skin around the widest part of the scrotum (Hopper, 2014). When bovine testicular tissue is functioning normally, a bull can produce approximately 17 million sperm per day per gram of tissue (Koziol and Armstrong, 2018). The SFT recognized the importance of a bull's maximum sperm production for reproductive efficiency, so they set forth minimum SC threshold recommendations for a bull at different ages, in months, which is recorded in Table 1.1 (Koziol and Armstrong, 2018). Even though bulls with less than optimal SC can produce semen with sufficient quality, they are less likely to impregnate a given group of females as quickly as bulls with adequate SC due to their daily sperm production (Elmore et al., 1975; Koziol and Armstrong, 2018). Therefore, if a bull has a significant physical abnormality or

does not meet the minimum threshold for SC, the semen evaluation portion of the exam may be omitted, and the bull can be classified as unsatisfactory or deferred (Hopper, 2014).

Examination of Semen

There are four ways semen can be collected: electroejaculation, manual stimulation, external artificial vagina, or vaginal aspiration. Electroejaculation is the most common collection method during a BSE because of its consistency, reliability, and convenience when collecting samples (Hopper, 2014). Manual stimulation is useful for young bulls that have responded negatively to electroejaculation. However, the quality of the semen sample with the method is not consistent among bulls (Hopper, 2014). Artificial vaginas are usually used in bull studs when collecting semen for AI (Hopper, 2014), but they can be useful for a BSE because they assist in identifying bulls with low libido (Barth et al., 2004). Vaginal aspiration should be used as a last resort to obtain a semen from a bull because this method requires the bull to service a female in estrus. Upon ejaculation, the ejaculate is aspirated from the female's vagina with syringe attached to a mare insemination pipette (Koziol and Armstrong, 2018). Furthermore, this method can lead to difficulties assessing semen quality because the sample can become mixed with vaginal cells and estrual mucous if the vagina wasn't rinsed prior to the service (Koziol and Armstrong, 2018).

Once a semen sample has been collected, it should be grossly observed for color and opacity to establish a rough estimation of sperm concentration. Dilute samples appear more watered down or clearer, while very concentrated samples will appear white, like heavy cream (Hopper, 2014). If a sample is contaminated with urine, it will look yellow-tinted, and the sperm will rapidly decline or have no motility (Hopper, 2014). When a sample is red or brown colored,

it is often due to the presence of blood, and the practicing veterinarian needs to determine the source of the contamination (Hopper, 2014).

After completing the gross semen examination of an ejaculate, the veterinarian should evaluate the semen sample for motility. Prior to 2016, gross motility of an individual ejaculate could be used to classify semen as very good, good, fair, and poor. A sample classified as "very good" has a high concentration of sperm, with a high percentage of those sperm moving progressively (Hopper, 2014). "Very good" motility will appear as dark, thick, rapidly moving swirls. The classification of "good" is given to a sample with slower oscillating swirls. A "fair" sample exhibits significant individual sperm movement but no oscillating swirls. A sample is classified as "poor" when there is very little or no movement within the sample (Hopper, 2014).

In 2016, SFT committee members reviewed the current BSE standards and decided to remove gross motility from the BSE form (Koziol and Armstrong, 2018). The gross motility of an ejaculate was influenced by concentration, percentage of progressively motile cells, and velocity of progressively motile sperm (Koziol and Armstrong, 2018). These three factors, in combination, could have had different appearances and required careful interpretations to be classified correctly.

Individual progressive motility is now the preferred assessment among practicing veterinarians because it is more consistent (Koziol and Armstrong, 2018). Progressive motility of a sample is classed as "very good" if greater than or equal to 70% of the sperm are motile, "good" if 50-69% motile, "fair" if 30-49% motile, and "poor" if less than 30% are motile (Hopper, 2014). The SFT's recommendations state that a bull's sample must have at least 30% progressively motile sperm or "fair" sperm motility to continue to the morphology portion of the BSE (Koziol and Armstrong, 2018).

Evaluating sperm morphology requires the practicing veterinarian to differentiate normal from abnormal sperm and identify the significance of the abnormal cells (Hopper, 2014). For a bull to pass a BSE, Wiltbank and Parish (1982) determined he needs to have 70 percent or greater normal spermatozoa in his ejaculate to successfully impregnate a cow. Sperm abnormalities were classified as either "major/minor," "primary/secondary," or "compensable/uncompensable" prior to 2016. The oldest system classified abnormalities as major/minor because of their suspected implications for fertility (Hopper, 2014). Spermatozoa with major defects decrease fertility when present in an ejaculate (Blom, 1973; Enciso et al., 2011). Minor defects do not significantly impact fertility unless one single abnormality is present at a prevalence of 20-25 percent or greater in an ejaculate (Blom, 1973; Hopper, 2014). Another method of classifying abnormalities is based on the suspected origin of an abnormal sperm cell, and it can be classified as primary or secondary. Primary abnormalities develop during spermatogenesis, while secondary abnormalities occur in the epididymis (Barth and Oko, 1989; Hopper, 2014). Saacke *et al.* (2000) introduced the classification of compensable/uncompensable to reflect defects resulting in reduced pregnancy rates. Compensable defects do not result in lowered fertility rates if an adequate number of normal sperm are also present in the insemination dose (Hopper, 2014). Sperm with this type of defect are not progressively motile, cannot reach the ovum, or they reach the ovum but cannot initiate fertilization in the female reproductive tract (Chenoweth, 2005). Sperm with uncompensable defects can reach the ovum and initiate fertilization, but the viability of the developing embryo is often poor (Chenoweth, 2005). Because these sperm compete with morphologically normal sperm to reach the fertilization site, bull fertility is decreased by the percentage of uncompensable defects in an ejaculate (Hopper, 2014).

In 2016, a differential counting scheme was adopted by the SFT, which groups defects under the classifications of head, midpiece, and principal piece (tail) abnormalities (Koziol and Armstrong, 2018). The new classification scheme is more functional because it does not require a veterinarian to distinguish which abnormalities contribute more to fertility (Koziol and Armstrong, 2018). However, the SFT still requires each sample to be fixed and counted and requires the sample to have a minimum of 70% morphologically normal sperm for a bull to be considered a satisfactory potential breeder (Koziol and Armstrong, 2018).

Limitations and Concerns

Because some components of the BSE are largely subjective, there are concerns with the current format of the BSE. There is a need to improve the consistency of equipment or level of technician skillset, training, and attention to detail for practicing veterinarians who perform BSEs (Hopper, 2014). Another limitation is that the BSE does not include an assessment of libido (Barth, 2007). Because samples for BSEs are largely collected via electroejaculation this removes the opportunity to assess bull libido. In addition to these, there are also concerns about the current standards' minimum "threshold" metrics (Hopper, 2014). Some think the minimum SC of 30 cm is too small for a bull 12-15 months of age and should be increased. However, if increased, it could become a barrier for *Bos indicus*-type cattle that mature more slowly (Brito et al., 2004). Further research into understanding the true role of SC of bull fertility and the development of more affordable and convenient technologies to measure semen quality libido could improve the accuracy of a BSE. Lastly, it is important to mention that BSE only measures a bull's fertility on the day of the exam and does not account for issues that could show up in the future.

Genetic Evaluation of Bull Fertility

Corbet et al. (2013) highlighted the significant influence of genetic factors on bull fertility. They suggested that genetic progress could be made for more fertile animals if a breeding program was genetically selecting for increased bull fertility (Corbet et al., 2013). Many studies have published heritability estimates and genetic correlations for SC, semen production measures, and semen quality measures obtained from BSEs and semen collection facilities (Christmas et al., 2001; Druet et al., 2009; Garmyn et al., 2011; Karoui et al., 2011; Corbet et al., 2013; Atagi et al., 2017; Berry et al., 2019; Burren et al., 2019; Butler et al., 2021; Carvalho et al., 2023; Cesarani et al., 2023).

When creating a genetic prediction model for bull fertility traits, a variety of known environmental factors should be included in the model as covariates or fixed effects to partition the variance between the genetic and residual components better. These environmental factors can consist of the bull's age (Chacón et al., 2002; Fuerst-Waltl et al., 2006; Waldner et al., 2010), nutritional status (Barth and Waldner, 2002; Barth, 2007), and environmental conditions (Nichi et al., 2006; Koivisto et al., 2009). In addition, fitting appropriate contemporary groups and random effects is important due to the subjective nature of measuring bull fertility phenotypes (Butler et al., 2020). Computer-assisted semen analysis systems can provide more objective measurements of semen quantity and quality, but the technology expense is an issue for commercial use by veterinarians for BSEs (Corbet et al., 2013; Onofre et al., 2021).

Genetic Parameters

Tables 1.2 and 1.3 outline the reported heritabilities published within the scientific literature for semen production and quality traits, respectively. All studies from scientific literature utilize a best linear unbiased prediction (BLUP) univariate or multivariate animal

model. The two exceptions were Smith et al. (1989) and Kriese et al. (1991), who both analyzed their data with sire models. Smith et al. (1989) also utilized least squares procedures instead of BLUP methods. Each study's model had varying fixed effects, but most included bull age, season, and year. Studies that utilized information from AI centers generally included fixed effects such as month or year when semen collection occurred, ejaculate number, and semen collector.

Scrotal Circumference

Scrotal circumference is favorably correlated with daily spermatozoa production, semen quality, paired testis weight, and offspring reproductive performance (Lunstra et al., 1978; Coulter and Foote, 1979; Brito et al., 2002; Barth, 2007). Heritability estimates in the literature for SC range from 0.36 to 0.75 and are reported in Table 1.2. In studies examining British-type yearling bulls heritability estimates ranged from 0.36 to 0.56 (Neely et al., 1982; Knights et al., 1984; Kriese et al., 1991; Christmas et al., 2001). Scrotal circumference in *Bos indicus* bulls has been reported as moderate to highly heritable. Carvalho et al. (2023) estimated a SC heritability of 0.75 ± 0.001 in 18-month-old Nellore bulls. Another study in Nellore bulls estimated a moderate heritability for SC (Silva et al., 2013). Corbet et al. (2013) estimated SC heritabilities in 6, 12, 18, and 24-month-old Brahman and Tropical composite bulls and reported similar heritability estimates within each breed.

Semen Production Traits

Volume is the total amount of semen in an ejaculate, measured in millimeters. Most studies reported low heritability estimates for volume. Kealey et al. (2006) and Kapš et al. (2000) reported low heritability estimates for volume in semen collected from Line 1 Hereford and Simmental bulls, respectively, during a BSE. Carvalho et al. (2023) published a similar

heritability estimate for volume in Nellore yearling bulls. Estimates from beef bulls at AI centers range from 0.11 to 0.32 (Butler et al., 2021; Rostellato et al., 2021; Cesarani et al., 2023). Heritability estimates from AI dairy bull semen range from 0.18 to 0.65 (Ducrocq and Humblot, 1995; Atagi et al., 2017), with most between 0.22 and 0.26 (Mathevon et al., 1998; Druet et al., 2009; Karoui et al., 2011; Suchocki and Szyda, 2015). The higher heritabilities reported in the dairy bull studies compared to beef bull studies could be due to breed. It is also a reasonable hypothesis that semen data from AI centers may yield higher heritability estimates compared to BSE studies. This could be due to differences in management conditions, semen collection methods, or the more frequent and accurate recording of subjective phenotypes.

Semen concentration is the density of sperm cells in an ejaculate, measured in millions per milliliter. Most estimates for concentration were lowly or moderately heritable in beef and dairy AI bull populations (Druet et al., 2009; Suchocki and Szyda, 2015; Butler et al., 2021). However, one study reported a highly heritable value for concentration in a population of Holstein bulls (Mathevon et al., 1998). Mathevon et al. (1998) only had 137 bulls in their analysis, which is substantially smaller than other studies in the literature, and the authors did not report a standard error. Interestingly, heritability estimates for concentration from AI dairy bulls tended to be higher than estimates from beef bull semen (Knights et al., 1984; Gredler et al., 2007; Druet et al., 2009; Suchocki and Szyda, 2015; Butler et al., 2021).

The total number of spermatozoa in an ejaculate is calculated by multiplying the volume and concentration values. The number of spermatozoa is expressed in millions. Taylor et al. (1985) noted that the accuracy of genetic component estimates for number of spermatozoa in a multi-trait model could be affected by it being a function of two other traits. Heritability estimates for number of spermatozoa range from 0.03 to 0.38 in beef and dairy studies (Knights

et al., 1984; Taylor et al., 1985; Mathevon et al., 1998; Butler et al., 2021). Estimate differences could be attributed to population size or model effects.

Semen Quality Traits

Semen motility is the percentage of sperm cells progressively moving forward in an ejaculate. Multiple studies have determined that motility is an essential indicator of fertility in beef cattle (Chenoweth and Lorton, 2021), sheep (David et al., 2015), and humans (Nel-Themaat et al., 2006). Heritability estimates for beef bull motility obtained from yearling BSEs were low (Smith et al., 1989; Christmas et al., 2001; Garmyn et al., 2011). Heritability estimates for motility phenotypes obtained at bull semen collection facilities were higher than those recorded from BSEs and ranged from 0.12 to 0.37 (Ducrocq and Humblot, 1995; Mathevon et al., 1998; Kealey et al., 2006; Suchocki and Szyda, 2015; Berry et al., 2019; Butler et al., 2021). The variation in heritability estimates for motility could be due to the subjectivity of the measurement. Other causes could be data collection methods, breed differences, or varying fixed effects in the studies' models.

Heritability estimates for the percentage of morphologically normal spermatozoa in literature have a wide range of values. Smith et al. (1989) reported a low heritability of 0.07 ± 0.06 from their study of BSEs from Hereford, Angus, and Red Angus bulls. More recent studies reported similar estimates in Angus and Italian Simmental AI bull populations (Butler et al., 2021; Cesarani et al., 2023). Conversely, Kealey et al. (2006) and Corbet et al. (2013) published moderate estimates for percentage of normal spermatozoa in Line 1 Hereford and Tropical Composite yearling bulls, respectively.

Heritability estimates for total abnormalities in an ejaculate range from 0.15 to 0.30. Christmas et al. (2001) and Garmyn et al. (2011) reported estimates of 0.29 and 0.25,

respectively, for Angus bulls. Estimates of 0.15 ± 0.001 and 0.30 were reported in Nellore bulls by Carvalho et al. (2023) and Silva et al. (2013), respectively. Ducrocq and Humblot (1995) published a heritability estimate of 0.19 for total abnormalities in dairy bulls.

As previously mentioned, sperm abnormality traits have been reported in various ways. The classification system of major and minor is the oldest system (Hopper, 2014). The classification of a major abnormality includes proximal cytoplasmic droplets, head, and midpiece abnormalities, and any single abnormality with significant presence in the ejaculate (Menon et al., 2011). Looped tails, distal cytoplasmic droplets, distal midpiece reflex, and detached heads would be classified as minor (Menon et al., 2011; Hopper, 2014). Two studies estimated low heritabilities for major and minor abnormalities in Nellore AI bulls. Carvaho et al. (2023) reported an estimate of 0.15 for major abnormalities and 0.04 for minor defects. Silva et al. (2013) reported similar heritability estimates for the percentage of major and minor defects.

The primary and secondary classification system had been widely accepted among examiners. Common primary abnormalities are head defects, Dag defects, mitochondrial sheath, midpieces, and proximal cytoplasmic droplets (Kealey et al., 2006; Koziol and Armstrong, 2018). Heritability estimates for primary abnormalities in the literature range from 0.03 to 0.35 (Smith et al., 1989; Christmas et al., 2001; Garmyn et al., 2011; Butler et al., 2021). Secondary abnormalities are caused by faulty epididymal sperm maturation. Spermatozoa with bent tails, coiled tails, or distal cytoplasmic droplets are secondary abnormalities. Kealey et al. (2006) suggested that genetics could influence secondary abnormalities. Secondary abnormalities had reported heritability estimates between 0.02 and 0.33 (Smith et al., 1989; Christmas et al., 2001; Kealey et al., 2006; Garmyn et al., 2011; Butler et al., 2021). Regardless of data source, all studies estimated low to moderate heritability estimates for primary and secondary abnormalities.

The new differential counting scheme recommended by SFT classifies abnormalities as head, midpiece, and principal piece (tail) (Koziol and Armstrong, 2018). Druet et al. (2009) reported a moderate heritability estimate for head abnormalities and a low estimate for tail abnormalities. Overall, heritability estimates appear to be highest when abnormalities are classified by the new SFT system of head, midpiece, and tail or when analyzed as a total percentage abnormal. However, more records for the different classification systems are needed to determine genetic effects and heritability of sperm abnormalities.

Many of the reported heritability estimates for semen production and quality traits are low to moderate, but most of the standard errors were small and therefore the heritability estimates were different from zero. It is plausible that beef producers could improve bull fertility if they had a genetic selection tool that combined semen quality and production traits. One example of a fertility-related trait with similar heritabilities that has been used successfully is the female-focused daughter fertility (DF) index released by the Canadian Dairy Industry. The DF index indicates a dairy sire's genetic potential for fertility traits based on his daughters' average reproduction performance across all lactations. The following values are included in DF evaluation: records of heifer's age at first service, the interval between calving and first insemination, the interval between first insemination and conception of heifers and lactating cows, 56-day non-return rate for heifers and milking cows (Fleming et al., 2019). The reported heritability estimates for the traits within the DF index ranging from 0.02 to 0.07, with the index itself having an estimated heritability of 0.05 (Fleming et al., 2019). During the first five years of genomic implementation, the national estimated breeding value (EBV) for the DF index increased by 1.78 EBV points per year (Canadian Dairy Network, 2017). It could be

hypothesized that similar genetic gains could be made in the United States beef bull population if genetic selection tools are made available to producers.

Genetic and Phenotypic Correlations

Scrotal Circumference with Semen Production Traits

Table 1.4 presents the reported correlations between SC and sperm production traits, but few estimates are reported in the literature. Scrotal circumference is the easiest measurement for veterinarians to take and is less subjective than other measurements taken during a BSE, which is why understanding genetic and phenotypic correlations between SC and ejaculate characteristics is important for improving bull fertility. Barth (2007) reviewed a variety of papers that showed SC measurements were highly correlated with paired testes' weight and the number of sperm produced per day. Kealey et al. (2006) reported favorable genetic correlations between SC and volume and concentration. The favorable correlations are promising for increasing the number of females a male can successfully service during a breeding season.

Scrotal Circumference with Semen Quality Traits

Many studies published favorable genetic and phenotypic correlations between SC and semen motility which are presented in Table 1.4. Corbet et al. (2013) published a strong genetic correlation and moderate phenotypic correlation in 12-month-old Brahman bulls. In British-type bulls, Christmas et al. (2001) and Kealey et al. (2006) reported favorable, moderate genetic correlations of 0.56 and 0.34, respectively (no standard errors reported). However, Smith et al. (1989) reported a negative genetic correlation of -0.04±0.40 between SC and motility, but the estimate was not different than zero.

Corbet et al. (2013) tested the genetic correlations between SC and the percentage of normal sperm in bulls at 12, 18, and 24 months. The authors reported positive, favorable genetic

correlation estimates in Brahman and Tropical Composite bulls (Corbet et al., 2013). However, Smith et al. (1989) reported an unfavorable, negative genetic correlation in yearling Hereford bulls. The difference in results could be at least partially because of the model type used. Smith et al. (1989) utilized a sire model, and Corbet et al. (2013) utilized an animal model.

Silva et al. (2013) found negative, low genetic correlations between SC and sperm defects in yearling Nellore bulls. Other studies have reported negative genetic associations between SC and sperm traits, with estimates ranging from -0.19 to -0.36, -0.11 to -0.45, and -0.12 to -0.23, for genetic correlations between SC and primary, secondary, and total sperm defects, respectively (Knights et al., 1984; Kealey et al., 2006; Garmyn et al., 2011). These results suggest that direct selection to increase SC could reduce abnormal spermatozoa, which could improve the semen quality of young bulls and subsequently increase the number of males passing a BSE.

Scrotal Circumference with Female Reproductive Traits

In addition to the relationships reported between SC and semen quality and production traits, SC is reported to be favorably associated with female fertility (Brinks et al., 1978; Morris et al., 1992; Vargas et al., 1998; Van Melis et al., 2010). Genetic correlations reported between SC and female reproductive traits can be found in Table 1.5. Favorable negative genetic correlations have been reported between SC and heifer age at puberty (Vargas et al., 1998; Martínez-Velázquez et al., 2003). Toelle and Robison (1985) reported a favorable negative genetic correlation of -0.39 between SC and heifer age at first breeding in Hereford half-siblings. The genetic correlation between SC and age at first calving are varied between North American and South American beef cattle. Martínez-Velázquez et al. (2003) and Toelle and Robison (1985) reported positive genetic correlations of 0.15 and 0.54, respectively, for the relationship

between SC and age at first calving in British-type cattle. Whereas, Santana et al. (2015) reported a negative genetic correlation between the two traits in Nellore cattle. Genetic correlations between SC and heifer pregnancy (HP) range from -0.08 to 0.48 (Toelle and Robison, 1985; Evans et al., 1999; Eler et al., 2004; Van Melis et al., 2010; Santana et al., 2015; Martínez-Velázquez et al., 2020). Toelle and Robison (1985) estimated a genetic correlation between SC and HP in Hereford cattle of 0.15, but no standard error was reported. Another study analyzed Hereford cattle and reported a low genetic correlation with a high standard error (0.002 ± 0.45) between the traits (Evans et al., 1999). Conversely, in more recent studies of Nellore cattle, moderate to strong genetic correlations between SC and HP have been reported (Eler et al., 2004; Van Melis et al., 2010; Santana et al., 2015). Van Melis et al. (2010) estimated the genetic correlation between the two traits to be 0.29 ± 0.05. The result from Van Melis et al. (2010) is promising for the potential of utilizing HP as a predictor in a male fertility index.

Semen Production Traits

Most genetic and phenotypic correlations reported in Table 1.4 between volume and concentration were negative and unfavorable because as volume increases, concentration decreases. Druet et al. (2009) and Burren et al. (2019) estimated moderate negative genetic correlations in dairy AI bulls. Rostellato et al. (2021) estimated a lower moderate genetic correlation in a population of Piemontese bulls, but the estimate did not have a reported standard error. The genetic correlations estimated from studies analyzing British-type beef bulls were not different from zero (Gredler et al., 2007; Butler et al., 2021). Phenotypic correlations between volume and concentration ranged from -0.01 to -0.35 (Berry et al., 2019; Rostellato et al., 2021, respectively).

Literature estimates for genetic and phenotypic correlations show a strong positive relationship between number of spermatozoa and volume. Genetic correlation estimates ranged from 0.47 to 0.83 in both dairy and beef bull populations (Gredler et al., 2007; Druet et al., 2009; Atagi et al., 2017; Butler et al., 2021). Slightly less variation can be seen in the phenotypic correlations between these traits with estimates ranging between 0.53 and 0.7 (Gredler et al., 2007; Druet et al., 2009; Karoui et al., 2011; Atagi et al., 2017; Butler et al., 2021; Rostellato et al., 2021).

The published genetic correlations between concentration and number of spermatozoa are comparable to the correlations between volume and number of spermatozoa. Druet et al. (2009) reported a genetic correlation estimate of 0.46 ± 0.18 between number of spermatozoa and concentration. Higher genetic correlation estimates between these traits were published by Gredler et al. (0.60; 2007), Butler et al. (0.55; 2021), and Rostellato et al. (0.56; 2021). Literature estimates for phenotypic correlations between concentration and number of spermatozoa range from 0.52 to 0.71 (Gredler et al., 2007; Druet et al., 2009; Butler et al., 2021). The strong positive genetic and phenotypic correlations between number of spermatozoa and volume and concentration should be expected as number of spermatozoa is a function of the two traits.

Semen Production Traits with Semen Quality

Genetic correlation estimates between volume and semen quality traits are summarized in Table 1.4. Favorable and positive genetic correlations have been reported between volume and motility (Gredler et al., 2007; Atagi et al., 2017; Burren et al., 2019; Butler et al., 2021). Relatively few studies have quantified the interrelationships between volume and morphology traits. Kealey et al. (2006) reported a positive and unfavorable correlation between volume and primary abnormalities but a negative, favorable correlation between volume and secondary

abnormalities. Butler et al. (2021) reported similar findings between these traits, but their estimates were not different from zero (Butler et al., 2021). Berry et al. (2019) reported an unfavorable, positive genetic correlation estimate of 0.66 ± 0.16 between volume and total abnormalities. Conversely, Ducrocq and Humblot (1995) reported a favorable correlation of -0.26, with no standard error reported, between the two traits.

Table 1.4 presents the genetic correlations between concentration and semen quality traits. Many studies reported moderate and favorable genetic correlations between concentration and motility (Karoui et al., 2011; Berry et al., 2019; Burren et al., 2019). However, Butler et al. (2021) and Druet et al. (2009) reported weaker correlations between the two traits that were not different from zero. Kealey et al. (2006) reported a positive correlation between primary abnormalities and concentration and a negative correlation between secondary abnormalities and concentration. These results are similar to those reported between volume and those two abnormality traits. Druet et al. (2009) reported a favorable, negative genetic correlation of -0.34 ± 0.24 between concentration and percentage of abnormal spermatozoa. Genetic correlations between concentration and percentage of viable spermatozoa have been estimated to be moderate and favorable in several studies (Gredler et al., 2007; Druet et al., 2009; Berry et al., 2019).

Genetic correlations estimated between semen quality traits and number of spermatozoa are reported in Table 1.4. Many positive and favorable genetic correlations have been reported between number of spermatozoa and motility of semen collected at an AI facilities (Gredler et al., 2007; Atagi et al., 2017; Butler et al., 2021). Druet et al. (2009) reported a negative correlation between the two traits, but their estimate was not different from zero. Butler et al. (2021) reported favorable genetic correlations between the number of spermatozoa and two morphology traits, percentage of normal spermatozoa and secondary abnormalities. However,

the authors estimated an unfavorable, positive genetic correlation between the number of spermatozoa and primary abnormalities (Butler et al., 2021). None of the estimates by the authors between the number of spermatozoa and abnormal semen morphology traits were different than zero (Butler et al., 2021). Gredler et al. (2007) reported a favorable and positive genetic correlation between the number of spermatozoa and the percentage of viable spermatozoa. The results indicated that selection to increase the number of spermatozoa could be accompanied by increased sperm quality. Further investigation into using expanded datasets is essential to elucidate the genetic connections between semen production and sperm morphology traits.

Semen Quality Traits

Table 1.4 summarizes the genetic correlations between semen motility and morphology traits. Smith et al. (1989) and Bulter et al. (2021) mainly reported unfavorable genetic correlations between motility and primary and secondary abnormalities, but neither studies' estimates are different than zero. Druet et al. (2009) reported that motility had negative, favorable genetic correlations with head, tail, and percentage of abnormal spermatozoa. However, Berry et al. (2019) reported an unfavorable, moderate genetic correlation between initial motility at an AI facility and total sperm abnormalities. In contrast, several studies have reported moderate to strong favorable correlations between motility and the percentage of normal spermatozoa (Smith et al., 1989; Butler et al., 2021). These results indicate that lower motility could potentially be associated with a higher percentage of abnormal sperm cells.

Literature estimates for genetic correlations between sperm morphology traits are limited but are reported in Table 1.4. Kealey et al. (2006) reported a negative genetic correlation between primary and secondary abnormalities, but their estimate did not include a standard error. A

favorable, negative genetic correlation was reported between primary abnormalities and the percentage of normal spermatozoa (Smith et al., 1989). Butler et al. (2021) reported a similar favorable genetic correlation between secondary abnormalities and the percentage of normal spermatozoa. It is essential to recognize the strong relationship between the percentage of normal spermatozoa and any abnormal spermatozoa trait because as abnormalities decrease, the percentage of normal spermatozoa will increase.

Silva et al. (2013) investigated the genetic relationship between major, minor, and total abnormalities in Nellore bulls. The authors estimated a positive genetic correlation of 0.4 ± 0.03 between major or minor sperm abnormalities (Silva et al., 2013). Major abnormalities had a positive genetic correlation of 0.54 ± 0.03 with total abnormalities. The genetic correlation estimated between minor and total abnormalities was 0.99 ± 0.04 (Silva et al., 2013).

Druet et al. (2009) estimated the genetic correlation between sperm with abnormal heads and tails and the percentage of abnormal spermatozoa. The authors reported positive genetic correlations between the percentage of total abnormal spermatozoa and the percentage of abnormal heads. Similar results were reported for the positive genetic correlation between the percentage of abnormal tails and the percentage of total abnormal spermatozoa (Druet et al., 2009). Druet et al. (2009) reported a positive genetic correlation between the percentage of abnormal heads and the percentage of abnormal tails.

The observed positive correlation between the percentage of total abnormalities and specific abnormalities, including primary, secondary, major, minor, head, or tail abnormalities in a semen ejaculate, is logical because it suggests a relationship where a corresponding increase in total abnormalities accompanies an increase in a specific abnormality. The relationship indicates a consistent pattern of abnormality occurrence in a semen sample. Consequently, the negative

relationship between the percentage of normal spermatozoa and the percentage of a specific abnormality suggests that selecting to increase the percentage of normal sperm would decrease abnormalities, reflecting an improvement in overall semen sample quality.

Male Fertility Selection Tools

Sire Conception Rate

The sire conception rate (SCR) index provides dairy producers with a selection tool for male fertility in AI bulls. Sire conception rate is defined as the expected difference in the conception rate of a given bull when compared to the other bulls in the analysis. The genetic evaluation for SCR includes fixed effects of the bulls' inbreeding coefficient, the inbreeding coefficient of the embryo from the mating, the bull's age, the AI organization where the bull was collected, the year of collection, and the random effect of the bull itself (Kuhn et al., 2008; USDA, 2008). A major downfall of SCR is that it is only reported in AI dairy bulls, and the evaluation does not include bulls with poor semen quality attributes because bulls who produce semen that does not meet the strict freezing requirements for AI are removed from the population (Butler et al., 2020). In addition, SCR has been criticized by some researchers because it based on pregnancy rate at 70 days, but does not account for the female's microenvironment and management factors that influence on oocyte quality and early embryonic death which could affect the pregnancy outcome (Amann et al., 2018).

Scrotal Circumference Expected Progeny Difference

The only available male fertility selection tool for beef producers is the SC EPD. The SC EPD evaluation predicts the difference in an animal's ability to transmit scrotal circumference to its male offspring compared to other animals (AGA, 2022; AAA, 2023). Moser et al. (1996) analyzed relationship between SC EPDs and semen quality traits and reported that Limousin

bulls with higher SC EPDs tended to have fewer abnormalities in their semen. Butler et al. (2021) utilized the BLUPF90 family of programs (Misztal et al., 2014) to correlate SC EPDs, provided by the American Angus Association, with EBVs for beef bull semen quality and production traits to determine if SC could be a potential indicator of beef bull fertility. The authors reported that SC may have limited predictive capability for fertility due to low correlations (Butler et al., 2021).

Limited studies have examined the relationship between SC EPD and female reproductive traits. Most studies have investigated the SC and heifer age at puberty. Moser et al. (1996) studied the relationship between SC EPDs of Limousin bulls and those bulls' daughters age at puberty. The authors reported that bulls with higher SC EPDs had daughters who reached puberty at significantly earlier ages when compared to bulls with lower SC EPDs (Moser et al., 1996). A comparable study was performed with Hereford cattle, and the authors reported similar results with high SC EPD sires' daughters reaching puberty earlier (Hough, 1991). A heifer's age at puberty relative to the beginning of her first breeding season is a primary determinant of pregnancy success (Day and Nogueira, 2013). Furthermore, her ability to rebreed in subsequent years can be affected by age at puberty and pregnancy success (Day and Nogueira, 2013).

The literature provides evidence that genetic selection tools could impact beef fertility. However, there are few male fertility selection tools currently available within the industry. Expected progeny differences and genomics could allow producers to make selection decisions on younger animals for reproduction traits which require an animal to be at least 12 months old or older to record a phenotype. Additionally, utilizing genomic technology could give producers more predictive power and more confidence when incorporating young, unproven sires into their breeding programs. While the dairy industry has led many of the advancements in utilizing

genomic prediction to evaluate bull fertility (Abdollahi-Arpanahi et al., 2017; Rezende et al., 2018), there have been studies performed on beef bulls (Peñagaricano et al., 2012; Sweett et al., 2020; Butler et al., 2022). Although ample bull fertility phenotypic records are taken at AI centers and by individual producers during BSEs, evaluation providers do not currently have a data pipeline for collecting the information from the phenotype holders, which limits the ability to provide male fertility selection tools to beef producers.

Summary

In the United States dairy industry, most cows are bred with AI. However, beef producers utilize a large number of bulls to naturally service their cows because reproductive technologies such as AI or embryo transfer are not as widely adopted practices. A BSE evaluating a natural service sire's scrotal circumference, sperm motility, and sperm morphology should be performed before every breeding season. The BSE allows subfertile bulls to be culled from the breeding population based on their phenotypic information. The wide array of environmental factors that influence semen production accentuates the complexity of bull fertility. However, improvements to bull fertility must be made and could be further expedited with the use of genetic selection tools. Genetic selection tools such as EPDs for individual reproductive traits or a comprehensive male fertility index would allow beef producers to select for increased fertility in their bulls, improving production efficiency, saving on resources, and increasing profitability (Rodgers et al., 2012).

Therefore, a deeper understanding of the genetic influences on male reproductive traits such as scrotal circumference, sperm motility, and morphology is imperative for improving conception and pregnancy rates in beef cattle. Scrotal circumference has been estimated to be moderate to highly heritable. Most heritability estimates for semen production and quality are

generally low to moderate. Genetic correlations between semen traits are generally favorable and moderate in strength. The favorable associations indicate that selecting to improve one fertility trait could indirectly improve other traits.

Investigating beef bull fertility and implementing genetic selection could enhance reproductive performance and ultimately improve the profitability and sustainability of beef cattle operations. The need for improvement and limited published literature makes further research into beef bull fertility necessary.

Table 1.1 The minimum thresholds for a bull's scrotal circumference by age to pass a breeding soundness examination.

Age	Minimum Threshold
< 15 months	30 cm
> 15 months to ≤ 18 months	31 cm
> 18 months to ≤ 21 months	32 cm
> 21 months to ≤ 24 months	33 cm
> 24 months	34 cm

Table 1.2 Previously reported heritability estimates (h^2) and standard error (SE) for semen production traits including the number of bulls (n) in the study and their breed. *Indicates study reported number of semen ejaculate records instead of individual animals. – Indicates no standard error.

Trait	n	h^2	SE	Breed	Reference
Concentration	794	0.20	0.07	Beef and Dairy	Berry et al. (2014)
	2617	0.25	0.03	Swiss Cattle	Burren et al. (2019)
	1819	0.09	0.02	Angus	Butler et al. (2021)
	515	0.19	0.05	Holstein	Druet et al. (2009)
	1644	0.39	--	Normande	Ducrocq and Humblot (1995)
	301	0.14	0.04	Fleckvieh	Gredler et al. (2007)
	955	0.26	--	Simmental	Kapš et al. (2000)
	502	0.19	--	Holstein	Karoui et al. (2011)
	717	0.13	0.06	Angus	Knights et al. (1984)
	137	0.52	--	Holstein	Mathevon et al. (1998)
	693	0.26	--	Piemontese	Rostellato et al. (2021)
	1212	0.34	0.07	Holstein-Friesian	Suchocki and Szyda (2015)
	2351	0.10	--	Holstein	Taylor et al. (1985)
Concentration Score	841	0.16	0.08	Line 1 Hereford	Kealey et al. (2006)
Number of Spermatozoa	2065	0.14	0.02	Holstein	Atagi et al. (2017)
	808	0.18	0.04	Japanese Black	Atagi et al. (2017)
	1819	0.08	0.02	Angus	Butler et al. (2021)
	622	0.11	0.08	Italian Simmental	Cesarani et al. (2022)
	515	0.09	0.04	Holstein	Druet et al. (2009)
	502	0.18	--	Holstein	Karoui et al. (2011)
	717	0.24	0.05	Angus	Knights et al. (1984)
	137	0.38	--	Holstein	Mathevon et al. (1998)
	693	0.23	--	Piemontese	Rostellato et al. (2021)
	2351	0.03	--	Holstein	Taylor et al. (1985)
Scrotal Circumference (6 months)	1608	0.46	0.08	Brahman	Corbet et al. (2013)
	2388	0.41	0.08	Tropical Composite	Corbet et al. (2013)
Scrotal Circumference (12 months)	4233	0.53	0.06	Hereford	Bourdon and Brinks (1986)
	622	0.50	0.14	Italian Simmental	Cesarani et al. (2022)
	1282	0.56	--	Angus	Christmas et al. (2001)
	1447	0.65	0.08	Brahman	Corbet et al. (2013)
	2092	0.46	0.06	Tropical Composite	Corbet et al. (2013)
	1280	0.46	0.08	Angus	Garmyn et al. (2011)
	626	0.57	0.09	Line 1 Hereford	Kealey et al. (2006)
	717	0.36	0.06	Angus	Knights et al. (1984)
	10511	0.53	--	Hereford	Kriese et al. (1991)

	549	0.40	0.09	Hereford, Angus, & Red Angus	Smith et al. (1989)
Scrotal Circumference (18 months)	18435	0.75	0.001	Nellore	Carvalho et al. (2023)*
	1409	0.75	0.09	Brahman	Corbet et al. (2013)
	2081	0.43	0.09	Tropical Composite	Corbet et al. (2013)
	51161	0.40	0.02	Nellore	Silva et al. (2013)*
Scrotal Circumference (24 months)	1403	0.75	0.09	Brahman	Corbet et al. (2013)
	2067	0.44	0.09	Tropical Composite	Corbet et al. (2013)
Volume	2065	0.16	0.02	Holstein	Atagi et al. (2017)
	808	0.18	0.04	Japanese Black	Atagi et al. (2017)
	1819	0.11	0.02	Angus	Butler et al. (2021)
	15882	0.05	0.0001	Nellore	Carvalho et al. (2023)*
	622	0.32	0.11	Italian Simmental	Cesarani et al. (2022)
	515	0.22	0.05	Holstein	Druet et al. (2009)
	1644	0.65	--	Normande	Ducrocq and Humblot (1995)
	301	0.18	0.02	Fleckvieh	Gredler et al. (2007)
	955	0.04	--	Simmental	Kapš et al. (2000)
	502	0.22	--	Holstein	Karoui et al. (2011)
	840	0.09	0.08	Line 1 Hereford	Kealey et al. (2006)
	137	0.24	--	Holstein	Mathevon et al. (1998)
	693	0.22	--	Piemontese	Rostellato et al. (2021)
	1212	0.26	0.06	Holstein-Friesian	Suchocki and Szyda (2015)
	2351	0.18	--	Holstein	Taylor et al. (1985)

Table 1.3 Previously reported heritability estimates (h^2) and standard error (SE) for semen quality traits including the number of bulls (n) in the study and their breed. *Indicates study reported number of semen ejaculate records instead of individual animals. – Indicates no standard error.

Trait	n	h^2	SE	Breed	Reference
Initial Motility	794	0.37	0.03	Beef and Dairy	Berry et al. (2014)
	1819	0.12	0.03	Angus	Butler et al. (2021)
	502	0.09	--	Holstein	Karoui et al. (2011)
Motility	2065	0.09	0.03	Holstein	Atagi et al. (2017)
	808	0.08	0.02	Japanese Black	Atagi et al. (2017)
	2617	0.09	0.03	Swiss Dairy	Burren et al. (2019)
	17225	0.05	0.001	Nellore	Carvalho et al. (2023)*
	622	0.07	0.05	Italian Simmental	Cesarani et al. (2022)
	1282	0.07	--	Angus	Christmas et al. (2001)
	515	0.43	0.08	Holstein	Druet et al. (2009)
	1245	0.05	0.03	Angus	Garmyn et al. (2011)
	301	0.04	0.01	Fleckvieh	Gredler et al. (2007)
	717	0.13	0.06	Angus	Knights et al. (1984)
	137	0.31	--	Holstein	Mathevon et al. (1998)
	423	0.08	0.07	Hereford, Angus, & Red Angus	Smith et al. (1989)
	1212	0.31	0.06	Holstein-Friesian	Suchocki and Szyda (2015)
Motility Score (All ages)	1644	0.35	--	Normande	Ducrocq and Humblot (1995)
	1212	0.26	0.05	Holstein-Friesian	Suchocki and Szyda (2015)
Motility Score (12 months)	1919	0.32	0.06	Tropical Composite	Corbet et al. (2013)
	1333	0.44	0.09	Brahman	Corbet et al. (2013)
	841	0.22	0.09	Line 1 Hereford	Kealey et al. (2006)
Motility Score (18 months)	2080	0.15	0.05	Tropical Composite	Corbet et al. (2013)
	1398	0.15	0.06	Brahman	Corbet et al. (2013)
Motility Score (24 months)	2063	0.10	0.04	Tropical Composite	Corbet et al. (2013)
	1394	0.05	0.04	Brahman	Corbet et al. (2013)
Post-Thaw Motility	2065	0.15	0.03	Holstein	Atagi et al. (2017)
	808	0.12	0.03	Japanese Black	Atagi et al. (2017)
	794	0.13	0.06	Beef and Dairy	Berry et al. (2014)
	1819	0.15	0.03	Angus	Butler et al. (2021)
	1644	0.30	--	Normande	Ducrocq and Humblot (1995)
	502	0.22	--	Holstein	Karoui et al. (2011)
	693	0.08	--	Piemontese	Rostellato et al. (2005)
Three- Hour Post-Thaw Motility	8299	0.09	0.04	Angus	Butler et al. (2021)

Percentage of Normal Spermatozoa (All ages)	1819	0.09	0.04	Angus	Butler et al. (2021)
Percentage of Normal Spermatozoa (12 months)	970	0.41	0.10	Tropical Composite	Corbet et al. (2013)
	103	0.00	0.00	Brahman	Corbet et al. (2013)
	622	0.16	0.10	Italian Simmental	Cesarani et al. (2022)
	837	0.35	0.10	Line 1 Hereford	Kealey et al. (2006)
	549	0.07	0.06	Hereford, Angus, & Red Angus	Smith et al. (1989)
Percentage of Normal Spermatozoa (18 months)	1794	0.20	0.06	Tropical Composite	Corbet et al. (2013)
	826	0.25	0.09	Brahman	Corbet et al. (2013)
Percentage of Normal Spermatozoa (24 months)	1912	0.27	0.06	Tropical Composite	Corbet et al. (2013)
	1234	0.15	0.06	Brahman	Corbet et al. (2013)
Percentage of Abnormal Spermatozoa	515	0.25	0.10	Holstein	Druet et al. (2009)
Primary Abnormalities	1819	0.03	0.03	Angus	Butler et al. (2021)
	1282	0.35	--	Angus	Christmas et al. (2001)
	1238	0.27	0.07	Angus	Garmyn et al. (2011)
	839	0.30	0.10	Line 1 Hereford	Kealey et al. (2006)
	549	0.31	0.09	Hereford, Angus, & Red Angus	Smith et al. (1989)
Secondary Abnormalities	1819	0.18	0.04	Angus	Butler et al. (2021)
	1282	0.26	--	Angus	Christmas et al. (2001)
	1238	0.23	0.08	Angus	Garmyn et al. (2011)
	838	0.33	0.09	Line 1 Hereford	Kealey et al. (2006)
	549	0.02	0.05	Hereford, Angus, & Red Angus	Smith et al. (1989)
Major Sperm Abnormalities	14312	0.15	0.001	Nellore	Carvalho et al. (2023)*
	17648	0.16	0.02	Nellore	Silva et al. (2013)*
Minor Sperm Abnormalities	13743	0.04	0.001	Nellore	Carvalho et al. (2023)*
	17648	0.04	0.01	Nellore	Silva et al. (2013)*
Percentage of Spermatozoa with Abnormal Head	515	0.35	0.12	Holstein	Druet et al. (2009)
Percentage of Spermatozoa with Abnormal Tail	515	0.19	0.12	Holstein	Druet et al. (2009)

Percentage of Spermatozoa with Abnormal Cytoplasmic Droplet	515	0.19	0.08	Holstein	Druet et al. (2009)
Percentage of Viable Spermatozoa	301	0.10	0.03	Fleckvieh	Gredler et al. (2007)
Percentage of Viable Spermatozoa Post-Cryopreservation	794	0.25	0.08	Beef and Dairy	Berry et al. (2014)
	515	0.21	0.08	Holstein	Druet et al. (2009)
	835	0.22	0.09	Line 1 Hereford	Kealey et al. (2006)
Total Abnormalities	14621	0.3	0.001	Nellore	Carvalho et al. (2023)*
	1282	0.29	--	Angus	Christmas et al. (2001)
	1644	0.19	--	Normande	Ducrocq and Humblot (1995)
	1238	0.25	0.07	Angus	Garmyn et al. (2011)
	17648	0.15	0.01	Nellore	Silva et al. (2013)*

Table 1.4 Previously reported phenotypic (r_P) and genetic (r_G) correlations between semen production and semen quality traits, including the number of bulls (n) in the study and their breed. * Indicates study reported number of semen ejaculate records instead of individual animals. – indicates no standard error.

n	r_P	r_G	Breed	Reference
Scrotal Circumference and Volume				
626	--	0.20	Hereford	Kealey et al. (2006)
Scrotal Circumference and Concentration				
717	-0.52	-0.26	Angus	Knights et al. (1984)
Scrotal Circumference and Concentration Score				
626	--	0.77	Hereford	Kealey et al. (2006)
Scrotal Circumference (12 months) and Motility				
1282	--	0.56	Angus	Christmas et al. (2001)
1447	0.47	0.70 ± 0.08	Brahman	Corbet et al. (2013)
2092	0.42	0.56 ± 0.10	Tropical Composite	Corbet et al. (2013)
717	-0.52	-0.25	Angus	Knights et al. (1984)
423	0.13	-0.04 ± 0.40	Hereford, Angus, & Red Angus	Smith et al. (1989)
Scrotal Circumference (12 months) and Motility Score				
626	--	0.34	Line 1 Hereford	Kealey et al. (2006)
Scrotal Circumference (18 months) and Motility				
1409	0.38	0.79 ± 0.10	Brahman	Corbet et al. (2013)
2081	0.24	0.44 ± 0.15	Tropical Composite	Corbet et al. (2013)
Scrotal Circumference (24 months) and Motility				
1403	0.20	0.86 ± 0.31	Brahman	Corbet et al. (2013)
2067	0.17	0.33 ± 0.18	Tropical Composite	Corbet et al. (2013)
Scrotal Circumference (12 month) and Percentage of Normal Spermatozoa				
2092	0.31	0.55 ± 0.13	Tropical Composite	Corbet et al. (2013)
626	--	0.33	Line 1 Hereford	Kealey et al. (2006)
549	0.17	-0.36 ± 0.34	Hereford, Angus, & Red Angus	Smith et al. (1989)
Scrotal Circumference (18 month) and Percentage of Normal Spermatozoa				
1409	0.31	0.50 ± 0.13	Brahman	Corbet et al. (2013)
2081	0.22	0.21 ± 0.16	Tropical Composite	Corbet et al. (2013)
Scrotal Circumference (24 month) and Percentage of Normal Spermatozoa				
1403	0.12	0.22 ± 0.19	Brahman	Corbet et al. (2013)
2067	0.13	0.20 ± 0.14	Tropical Composite	Corbet et al. (2013)
Scrotal Circumference and Percentage of Viable Spermatozoa				
626	--	0.63	Line 1 Hereford	Kealey et al. (2006)
717	0.54	0.11	Angus	Knights et al. (1984)
Scrotal Circumference and Primary Abnormalities				
1282	--	-0.25	Angus	Christmas et al. (2001)
1238	-0.10	-0.19 ± 0.17	Angus	Garmyn et al. (2011)
626	--	-0.36	Line 1 Hereford	Kealey et al. (2006)
549	-0.09	0.14 ± 0.22	Hereford, Angus, & Red Angus	Smith et al. (1989)

Scrotal Circumference and Secondary Abnormalities				
1282	--	-0.32	Angus	Christmas et al. (2001)
1238	-0.11	-0.23 ± 0.18	Angus	Garmyn et al. (2011)
626	--	-0.45	Line 1 Hereford	Kealey et al. (2006)
Scrotal Circumference and Total Abnormalities				
1282	--	-0.32	Angus	Christmas et al. (2001)
1238	-0.11	-0.23 ± 0.18	Angus	Garmyn et al. (2011)
17648	--	-0.24 ± 0.02	Nellore	Silva et al. (2013)
Volume and Concentration				
794	-0.01	-0.40 ± 0.20	Beef and Dairy	Berry et al. (2014)
2617	-0.28 ± 0.01	-0.56 ± 0.05	Swiss Dairy	Burren et al. (2019)
1819	-0.06 ± 0.01	-0.10 ± 0.17	Angus	Butler et al. (2021)
515	-0.02 ± 0.02	-0.55 ± 0.18	Holstein	Druet et al. (2009)
1644	-0.15	-0.42	Normande	Ducrocq and Humblot (1995)
12746	-0.17	0.06 ± 0.13	Fleckvieh	Gredler et al. (2007)
502	-0.06	-0.13	Holstein	Karoui et al. (2011)
693	-0.35	-0.44	Piemontese	Rostellato et al. (2005)
Volume and Motility				
2065	0.05 ± 0.02	0.17 ± 0.15	Holstein	Atagi et al. (2017)
808	0.12 ± 0.03	0.44 ± 0.16	Japanese Black	Atagi et al. (2017)
2617	0.01 ± 0.02	0.19 ± 0.11	Swiss Dairy	Burren et al. (2019)
515	-0.03 ± 0.03	-0.20 ± 0.19	Holstein	Druet et al. (2009)
301	-0.12	0.21 ± 0.17	Fleckvieh	Gredler et al. (2007)
Volume and Initial Motility				
2617	0.01 ± 0.02	0.19 ± 0.11	Swiss Dairy	Burren et al. (2019)
1819	0.13 ± 0.01	0.23 ± 0.16	Angus	Butler et al. (2021)
502	0.04	0.07	Holstein	Karoui et al. (2011)
Volume and Gross Motility Score				
515	0.01 ± 0.03	-0.17 ± 0.19	Holstein	Druet et al. (2009)
1644	0.01	-0.03	Normande	Ducrocq and Humblot (1995)
626	--	-0.04	Line 1 Hereford	Kealey et al. (2006)
Volume and Post-Thaw Motility				
2065	0.01 ± 0.03	0.03 ± 0.12	Holstein	Atagi et al. (2017)
808	0.07 ± 0.03	0.31 ± 0.16	Japanese Black	Atagi et al. (2017)
794	0.02	0.20 ± 0.19	Beef and Dairy	Berry et al. (2014)
1819	0.16 ± 0.01	0.17 ± 0.16	Angus	Butler et al. (2021)
1644	-0.05	-0.16	Normande	Ducrocq and Humblot (1995)
1724	0.11 ± 0.02	-0.26 ± 0.27	Australian Sheep	Hodge et al. (2022)
502	0.02	0.13	Holstein	Karoui et al. (2011)

693	-0.04	0.17	Piemontese	Rostellato et al. (2005)
Volume and Three-Hour Post-Thaw Motility				
1819	0.22 ± 0.01	0.54 ± 0.64	Angus	Butler et al. (2021)
Volume and Percentage of Normal Spermatozoa				
626	--	0.32	Line 1 Hereford	Kealey et al. (2006)
1819	0.09 ± 0.01	0.18 ± 0.22	Angus	Butler et al. (2021)
Volume and Percentage of Abnormal Spermatozoa				
515	0.00 ± 0.03	0.23 ± 0.26	Holstein	Druet et al. (2009)
Volume and Percentage of Viable Spermatozoa				
301	-0.13	0.31 ± 0.15	Fleckvieh	Gredler et al. (2007)
835	--	-0.09	Line 1 Hereford	Kealey et al. (2006)
Volume and Percentage of Viable Spermatozoa Post-Cryopreservation				
794	0.01	-0.32 ± 0.22	Beef and Dairy	Berry et al. (2014)
515	-0.10 ± 0.03	-0.47 ± 0.26	Holstein	Druet et al. (2009)
Volume and Primary Abnormalities				
1819	-0.09 ± 0.01	0.52 ± 0.61	Angus	Butler et al. (2021)
626	--	0.33	Line 1 Hereford	Kealey et al. (2006)
Volume and Secondary Abnormalities				
1819	-0.01 ± 0.01	-0.13 ± 0.17	Angus	Butler et al. (2021)
626	--	-0.34	Line 1 Hereford	Kealey et al. (2006)
Volume and Percentage of Spermatozoa with Abnormal Cytoplasmic Droplet				
515	0.01 ± 0.03	0.32 ± 0.28	Holstein	Druet et al. (2009)
Volume and Percentage of Spermatozoa with Abnormal Head				
515	0.02 ± 0.03	-0.12 ± 0.27	Holstein	Druet et al. (2009)
Volume and Percentage of Spermatozoa with Abnormal Tail				
515	0.00 ± 0.03	0.43 ± 0.35	Holstein	Druet et al. (2009)
Volume and Total Abnormalities				
794	0.63	0.66 ± 0.16	Beef and Dairy	Berry et al. (2014)
1644	-0.13	-0.26	Normande	Ducrocq and Humblot (1995)
Volume and Number of Spermatozoa				
2065	0.56 ± 0.02	0.48 ± 0.08	Holstein	Atagi et al. (2017)
808	0.66 ± 0.02	0.69 ± 0.08	Japanese Black	Atagi et al. (2017)
1819	0.66 ± 0.01	0.75 ± 0.08	Angus	Butler et al. (2021)
515	0.61 ± 0.03	0.47 ± 0.18	Holstein	Druet et al. (2009)
301	0.70	0.83 ± 0.13	Fleckvieh	Gredler et al. (2007)
502	0.63	0.66	Holstein	Karoui et al. (2011)
693	0.53	0.51	Piemontese	Rostellato et al. (2005)
Concentration and Motility				
2617	0.20 ± 0.01	0.36 ± 0.14	Swiss Dairy	Burren et al. (2019)
515	0.01 ± 0.03	0.12 ± 0.20	Holstein	Druet et al. (2009)
301	0.23	0.48 ± 0.17	Fleckvieh	Gredler et al. (2007)
717	1.00	1.01	Angus	Knights et al. (1984)
Concentration and Initial Motility				

794	0.20	0.29 ± 0.04	Beef and Dairy	Berry et al. (2014)
1819	0.12 ± 0.01	0.09 ± 0.19	Angus	Butler et al. (2021)
502	0.33	0.54	Holstein	Karoui et al. (2011)
Concentration and Gross Motility Score				
515	0.02 ± 0.03	-0.01 ± 0.20	Holstein	Druet et al. (2009)
1644	0.60	0.67	Normande	Ducrocq and Humblot (1995)
502	0.52	0.73	Holstein	Karoui et al. (2011)
840	--	0.81	Line 1 Hereford	Kealey et al. (2006)
Concentration and Post-Thaw Motility				
23626	0.13	0.56 ± 0.25	Beef and Dairy	Berry et al. (2014)
1819	-0.03 ± 0.01	-0.15 ± 0.19	Angus	Butler et al. (2021)
1644	0.47	0.54	Normande	Ducrocq and Humblot (1995)
502	0.19	0.38	Holstein	Karoui et al. (2011)
693	0.05	-0.08	Piemontese	Rostellato et al. (2005)
Concentration and Three-Hour Post-Thaw Motility				
1819	-0.24 ± 0.01	0.04 ± 0.59	Angus	Butler et al. (2021)
Concentration and Percentage of Normal Spermatozoa				
1819	0.12 ± 0.01	-0.11 ± 0.24	Angus	Butler et al. (2021)
837	--	0.36	Line 1 Hereford	Kealey et al. (2006)
Concentration and Percentage of Abnormal Spermatozoa				
515	-0.07 ± 0.03	-0.34 ± 0.24	Holstein	Druet et al. (2009)
Concentration and Percentage of Viable Spermatozoa				
794	0.15	0.37 ± 0.23	Beef and Dairy	Berry et al. (2014)
515	0.04 ± 0.03	0.29 ± 0.26	Holstein	Druet et al. (2009)
301	0.27	0.41 ± 0.17	Fleckvieh	Gredler et al. (2007)
717	-0.81	--	Angus	Knights et al. (1984)
Concentration and Primary Abnormalities				
1819	-0.03 ± 0.01	--	Angus	Butler et al. (2021)
626	--	0.36	Line 1 Hereford	Kealey et al. (2006)
Concentration and Secondary Abnormalities				
1819	-0.13 ± 0.01	0.04 ± 0.19	Angus	Butler et al. (2021)
626	--	-0.58	Line 1 Hereford	Kealey et al. (2006)
Concentration and Percentage of Spermatozoa with Abnormal Cytoplasmic Droplet				
515	-0.08 ± 0.03	-0.09 ± 0.28	Holstein	Druet et al. (2009)
Concentration and Percentage of Spermatozoa with Abnormal Head				
515	-0.02 ± 0.03	-0.23 ± 0.24	Holstein	Druet et al. (2009)
Concentration and Percentage of Spermatozoa with Abnormal Tail				
515	-0.06 ± 0.03	0.33 ± 0.30	Holstein	Druet et al. (2009)
Concentration and Total Abnormalities				
794	0.68	0.42 ± 0.21	Beef and Dairy	Berry et al. (2014)
1644	-0.07	-0.27	Normande	Ducrocq and Humblot (1995)
Concentration and Number of Spermatozoa				
1819	0.61 ± 0.01	0.55 ± 0.13	Angus	Butler et al. (2021)
515	0.71 ± 0.04	0.46 ± 0.18	Holstein	Druet et al. (2009)

301	0.52	0.60 ± 0.07	Fleckvieh	Gredler et al. (2007)
502	0.63	0.60	Holstein	Karoui et al. (2011)
717	-0.85	-1.03	Angus	Knights et al. (1984)
693	0.56	0.56	Piemontese	Rostellato et al. (2005)
Number of Spermatozoa and Initial Motility				
1819	0.16 ± 0.01	0.23 ± 0.18	Angus	Butler et al. (2021)
2065	0.23 ± 0.02	0.21 ± 0.15	Holstein	Atagi et al. (2017)
Number of Spermatozoa and Motility				
2065	0.23 ± 0.02	0.21 ± 0.15	Holstein	Atagi et al. (2017)
808	0.27 ± 0.02	0.55 ± 0.18	Japanese Black	Atagi et al. (2017)
717	-0.85	-1.04	Angus	Knights et al. (1984)
515	-0.03 ± 0.02	-0.12 ± 0.25	Holstein	Druet et al. (2009)
301	0.06	0.50 ± 0.13	Fleckvieh	Gredler et al. (2007)
Number of Spermatozoa and Post-Thaw Motility				
2065	0.08 ± 0.02	-0.03 ± 0.13	Holstein	Atagi et al. (2017)
808	0.10 ± 0.03	0.36 ± 0.15	Japanese Black	Atagi et al. (2017)
1819	0.12 ± 0.01	0.02 ± 0.19	Angus	Butler et al. (2021)
502	0.15	0.32	Holstein	Karoui et al. (2011)
693	0.11	-0.20	Piemontese	Rostellato et al. (2021)
Number of Spermatozoa and Three-Hour Post-Thaw Motility				
1819	-0.01 ± 0.01	0.31 ± 0.61	Angus	Butler et al. (2021)
Number of Spermatozoa and Gross Motility Score				
515	0.01 ± 0.03	-0.24 ± 0.29	Holstein	Druet et al. (2009)
502	0.39	0.57	Holstein	Karoui et al. (2011)
Number of Spermatozoa and Percentage of Progressive Motility				
515	0.02 ± 0.03	-0.22 ± 0.67	Holstein	Druet et al. (2009)
Number of Spermatozoa and Percentage of Normal Spermatozoa				
1819	0.16 ± 0.01	0.13 ± 0.24	Angus	Butler et al. (2021)
Number of Spermatozoa and Percentage of Abnormal Spermatozoa				
515	-0.05 ± 0.03	-0.09 ± 0.38	Holstein	Druet et al. (2009)
Number of Spermatozoa and Percentage of Viable Spermatozoa				
301	0.07	0.54 ± 0.11	Fleckvieh	Gredler et al. (2007)
717	0.79	--	Angus	Knights et al. (1984)
Number of Spermatozoa and Primary Abnormalities				
1819	-0.10 ± 0.01	0.08 ± 0.73	Angus	Butler et al. (2021)
Number of Spermatozoa and Secondary Abnormalities				
1819	-0.09 ± 0.01	-0.10 ± 0.20	Angus	Butler et al. (2021)
Number of Spermatozoa and Percentage of Spermatozoa with Abnormal Cytoplasmic Droplet				
515	-0.05 ± 0.02	0.33 ± 0.43	Holstein	Druet et al. (2009)
Number of Spermatozoa and Percentage of Spermatozoa with Abnormal Head				
515	-0.02 ± 0.03	-0.38 ± 0.36	Holstein	Druet et al. (2009)

Number of Spermatozoa and Percentage of Spermatozoa with Abnormal Tail				
515	-0.03 ± 0.03	0.14 ± 0.54	Holstein	Druet et al. (2009)
Number of Spermatozoa and Percentage of Viable Spermatozoa				
717	0.79	--	Angus	Knights et al. (1984)
Number of Spermatozoa and Post-Thawing Percentage of Viable Spermatozoa				
515	-0.03 ± 0.03	-0.18 ± 0.38	Holstein	Druet et al. (2009)
Motility and Gross Motility Score				
515	0.66 ± 0.02	0.88 ± 0.06	Holstein	Druet et al. (2009)
Motility and Post-Thaw Motility				
2065	0.57 ± 0.02	0.93 ± 0.04	Holstein	Atagi et al. (2017)
808	0.28 ± 0.02	0.90 ± 0.07	Japanese Black	Atagi et al. (2017)
Motility and Percentage of Normal Spermatozoa				
423	0.38	0.43 ± 0.64	Hereford, Angus, & Red Angus	Smith et al. (1989)
Motility and Percentage of Abnormal Spermatozoa				
515	-0.20 ± 0.03	-0.55 ± 0.19	Holstein	Druet et al. (2009)
Motility and Percentage of Viable Spermatozoa				
717	-0.81	--	Angus	Knights et al. (1984)
301	0.55	0.90 ± 0.05	Fleckvieh	Gredler et al. (2007)
Motility and Percentage of Viable Spermatozoa Post-Cryopreservation				
515	0.40 ± 0.03	0.58 ± 0.15	Holstein	Druet et al. (2009)
Motility and Primary Abnormalities				
423	-0.31	-0.36 ± 0.55	Hereford, Angus, & Red Angus	Smith et al. (1989)
Motility and Secondary Abnormalities				
423	-0.22	0.71 ± 0.89	Hereford, Angus, & Red Angus	Smith et al. (1989)
Motility and Percentage of Spermatozoa with Abnormal Cytoplasmic Droplet				
515	-0.07 ± 0.03	0.13 ± 0.23	Holstein	Druet et al. (2009)
Motility and Percentage of Spermatozoa with Abnormal Head				
515	0.17 ± 0.04	-0.56 ± 0.18	Holstein	Druet et al. (2009)
Motility and Percentage of Spermatozoa with Abnormal Tail				
515	-0.11 ± 0.03	-0.24 ± 0.24	Holstein	Druet et al. (2009)
Initial Motility and Post-Thaw Motility				
794	0.58	0.92 ± 0.03	Beef and Dairy	Berry et al. (2014)
1819	0.32 ± 0.01	0.92 ± 0.04	Angus	Butler et al. (2021)
502	0.41	0.87	Holstein	Karoui et al. (2011)
Initial Motility and Three-Hour Post-Thaw Motility				
1819	0.04 ± 0.01	--	Angus	Butler et al. (2021)
Initial Motility and Percentage of Normal Spermatozoa				
1819	0.20 ± 0.01	0.77 ± 0.09	Angus	Butler et al. (2021)
Initial Motility and Percentage of Viable Spermatozoa Post-Cryopreservation				
794	0.54	0.89 ± 0.04	Beef and Dairy	Berry et al. (2014)
Initial Motility and Primary Abnormalities				

1819	-0.21 ± 0.01	0.33 ± 0.20	Angus	Butler et al. (2021)
Initial Motility and Secondary Abnormalities				
1819	-0.15 ± 0.01	0.63 ± 0.82	Angus	Butler et al. (2021)
Initial Motility and Total Abnormalities				
794	0.13	0.36 ± 0.08	Beef and Dairy	Berry et al. (2014)
Gross Motility Score and Post-Thaw Motility				
1644	0.73	0.83	Normande	Ducrocq and Humblot (1995)
502	0.43	0.79	Holstein	Karoui et al. (2011)
Gross Motility Score and Percentage of Normal Spermatozoa				
837	--	0.51	Line 1 Hereford	Kealey et al. (2006)
Gross Motility Score and Percentage of Abnormal Spermatozoa				
515	-0.16 ± 0.04	-0.40 ± 0.18	Holstein	Druet et al. (2009)
Gross Motility Score and Percentage of Viable Spermatozoa Post-Cryopreservation				
515	0.31 ± 0.03	0.61 ± 0.15	Holstein	Druet et al. (2009)
Gross Motility Score and Primary Abnormalities				
839	--	0.57	Line 1 Hereford	Kealey et al. (2006)
Gross Motility Score and Secondary Abnormalities				
838	--	-0.54	Line 1 Hereford	Kealey et al. (2006)
Gross Motility Score and Percentage of Spermatozoa with Abnormal Cytoplasmic Droplet				
515	-0.14 ± 0.03	-0.07 ± 0.21	Holstein	Druet et al. (2009)
Gross Motility Score and Percentage of Spermatozoa with Abnormal Head				
515	-0.15 ± 0.04	-0.54 ± 0.17	Holstein	Druet et al. (2009)
Gross Motility Score and Percentage of Spermatozoa with Abnormal Tail				
515	-0.05 ± 0.04	-0.02 ± 0.23	Holstein	Druet et al. (2009)
Gross Motility Score and Total Abnormalities				
1644	-0.34	-0.68	Normande	Ducrocq and Humblot (1995)
Post-Thaw Motility and Percentage of Normal Spermatozoa				
1819	0.03 ± 0.01	-0.74 ± 0.30	Angus	Butler et al. (2021)
Post-Thaw Motility and Percentage of Viable Spermatozoa Post-Cryopreservation				
794	0.84	0.94 ± 0.04	Beef and Dairy	Berry et al. (2014)
Post-Thaw Motility and Primary Abnormalities				
1819	-0.21 ± 0.01	-0.02 ± 0.61	Angus	Butler et al. (2021)
Post-Thaw Motility and Secondary Abnormalities				
1819	0.30 ± 0.01	0.11 ± 1.97	Angus	Butler et al. (2021)
Post-Thaw Motility and Three-Hour Motility				
1819	0.74 ± 0.01	--	Angus	Butler et al. (2021)
Post-Thaw Motility and Total Abnormalities				
794	0.12	0.21 ± 0.33	Beef and Dairy	Berry et al. (2014)
1644	-0.37	-0.43	Normande	Ducrocq and Humblot (1995)
Three-Hour Post-Thaw Motility and Percentage of Normal Spermatozoa				
1819	0.15 ± 0.02	-0.44 ± 0.15	Angus	Butler et al. (2021)
Three-Hour Post-Thaw Motility and Primary Abnormalities				
1819	-0.55 ± 0.01	-0.25 ± 0.17	Angus	Butler et al. (2021)
Three-Hour Post-Thaw Motility and Secondary Abnormalities				

1819	0.54 ± 0.01	--	Angus	Butler et al. (2021)
Percentage of Normal Spermatozoa and Percentage of Viable Spermatozoa				
835	--	0.32	Line 1 Hereford	Kealey et al. (2006)
Percentage of Normal Spermatozoa and Primary Abnormalities				
1819	-0.60 ± 0.01	0.03 ± 2.07	Angus	Butler et al. (2021)
549	-0.57	-0.85 ± 0.63	Hereford, Angus, & Red Angus	Smith et al. (1989)
Percentage of Normal Spermatozoa and Secondary Abnormalities				
1819	-0.58 ± 0.01	-0.81 ± 0.10	Angus	Butler et al. (2021)
549	-0.80	0.16 ± 1.54	Hereford, Angus, & Red Angus	Smith et al. (1989)
Percentage of Abnormal Spermatozoa and Percentage of Spermatozoa with Abnormal Cytoplasmic Droplet				
515	0.36 ± 0.03	-0.15 ± 0.34	Holstein	Druet et al. (2009)
Percentage of Abnormal Spermatozoa and Percentage of Spermatozoa with Abnormal Head				
515	0.61 ± 0.03	0.71 ± 0.18	Holstein	Druet et al. (2009)
Percentage of Abnormal Spermatozoa and Percentage of Spermatozoa with Abnormal Tail				
515	0.77 ± 0.02	0.78 ± 0.16	Holstein	Druet et al. (2009)
Percentage of Viable Spermatozoa and Primary Abnormalities				
835	--	0.33	Line 1 Hereford	Kealey et al. (2006)
Percentage of Viable Spermatozoa and Secondary Abnormalities				
835	--	-0.43	Line 1 Hereford	Kealey et al. (2006)
Percentage of Viable Spermatozoa Post-Cryopreservation and Percentage of Abnormal Spermatozoa				
515	-0.20 ± 0.04	-0.23 ± 0.29	Holstein	Druet et al. (2009)
Percentage of Viable Spermatozoa Post-Cryopreservation and Percentage of Spermatozoa with Abnormal Cytoplasmic Droplet				
515	-0.11 ± 0.03	0.05 ± 0.30	Holstein	Druet et al. (2009)
Percentage of Viable Spermatozoa Post-Cryopreservation and Percentage of Spermatozoa with Abnormal Head				
515	-0.15 ± 0.04	-0.44 ± 0.28	Holstein	Druet et al. (2009)
Percentage of Viable Spermatozoa Post-Cryopreservation and Percentage of Spermatozoa with Abnormal Tail				
515	-0.12 ± 0.04	0.10 ± 0.39	Holstein	Druet et al. (2009)
Percentage of Viable Spermatozoa Post-Cryopreservation and Total Abnormalities				
794	0.12	-0.01 ± 0.31	Beef and Dairy	Berry et al. (2014)
Primary Abnormalities and Secondary Abnormalities				
1819	-0.31 ± 0.01	-0.60 ± 1.13	Angus	Butler et al. (2021)
838	--	-0.87	Line 1 Hereford	Kealey et al. (2006)
549	0.17	0.14 ± 0.64	Hereford, Angus, & Red Angus	Smith et al. (1989)
Major Sperm Abnormalities and Minor Sperm Abnormalities				
17648	--	0.4 ± 0.03	Nellore	Silva et al. (2013)*
Major Sperm Abnormalities and Total Abnormalities				
17648	--	0.54 ± 0.03	Nellore	Silva et al. (2013)*
Minor Sperm Abnormalities and Total Abnormalities				
17648	--	0.99 ± 0.04	Nellore	Silva et al. (2013)*

Percentage of Spermatozoa with Abnormal Head and Percentage of Spermatozoa with Abnormal Cytoplasmic Droplet				
515	0.12 ± 0.04	-0.43 ± 0.30	Holstein	Druet et al. (2009)
Percentage of Spermatozoa with Abnormal Head and Percentage of Spermatozoa with Abnormal Tail				
515	0.02 ± 0.04	0.13 ± 0.39	Holstein	Druet et al. (2009)
Percentage of Spermatozoa with Abnormal Tail and Percentage of Spermatozoa with Abnormal Cytoplasmic Droplet				
515	0.15 ± 0.04	0.06 ± 0.37	Holstein	Druet et al. (2009)

Table 1.5 Genetic (r_G) correlations between scrotal circumference and female reproductive traits.

n_M[1]	n_F[2]	r_G	Breed	Reference
Scrotal Circumference and Age at Puberty				
7580	5292	-0.15	Beef	Martínez-Velázquez et al. (2003)
287	292	-0.32	Brahman	Vargas et al. (1998)
Scrotal Circumference and Age at First Breeding				
528	645	-0.39	Hereford	Toelle and Robison (1985)
Scrotal Circumference and Age at First Calving				
7580	4835	0.15	Beef	Martínez-Velázquez et al. (2003)
49283	14182	-0.70	Nellore	Santana et al. (2015)
528	645	0.54	Hereford	Toelle and Robison (1985)
Scrotal Circumference and Heifer Pregnancy				
25466	18145	0.20 ± 0.12	Nellore	Eler et al. (2004)
927	986	0.00 ± 0.45	Hereford	Evans et al. (1999)
49283	18063	0.48	Nellore	Santana et al. (2015)
528	645	0.15	Hereford	Toelle and Robison (1985)
47605	27924	0.29 ± 0.05	Nellore	Van Melis et al. (2010)

[1]Number of scrotal circumference records.
[2]Number of female trait records.

Chapter 2 - Genetic parameter estimates for fertility traits in Angus bulls

Abstract

Scrotal circumference (SC) and semen quality traits such as motility, motility score, primary abnormalities, secondary abnormalities, and normal sperm are widely used as indicators of bull fertility during a breeding soundness examination (BSE) or semen collection for artificial insemination (AI) at a bull stud, yet their genetic relationships are not fully understood. The objectives of this study were to estimate variance components, heritabilities, and genetic correlations between SC and semen quality traits to better understand their potential application in a genetic evaluation for improved bull fertility. This study utilized 5,877 BSE records from 4,996 Angus bulls and 64,869 semen collection records from 1,862 Angus bulls from two AI organizations. Three different datasets were evaluated: BSE, stud, and combined (BSE and stud data together). Variance components, heritabilities, and repeatabilities were estimated for SC, percentage of progressively motile, motility score, percentage of sperm with primary abnormalities, percentage of sperm with secondary abnormalities, and percentage of normal spermatozoa within each dataset. Heritability estimates ranged from 0.05 to 0.36 for the BSE data, 0.04 to 0.55 for the stud data, and 0.06 to 0.40 for the combined data. Repeatability estimates were moderately to highly repeatable and ranged from 0.22 to 0.93. Genetic correlations across traits in a single dataset were moderate to high between semen quality traits and low to moderate between SC and semen quality. Genetic correlations estimated between the BSE and stud datasets within each reproductive trait were low to moderate. These results indicate that genetic selection for improved bull fertility is feasible, but further research is needed to determine the best methodology for developing a genetic evaluation.

Introduction

A bull is described as fertile if he can produce viable sperm that can achieve fertilization in the female reproductive tract. Improvements to bull fertility could likely be made with the use of genetic selection tools. Currently, most selection tools for reproduction focus on female fertility. Several beef breed associations publish a scrotal circumference (SC) expected progeny difference (EPD); however, the SC EPD published by the American Angus Association does not account for semen quality measures (Tarpoff, 2023), which can be major determinants of embryo fertilization (Amann et al., 2018).

The breeding soundness examination (BSE) is the most common method for assessing bull fertility, but they are often only performed before the breeding season, before the sale of the animal, or when a bull's fertility is in question. Additionally, BSEs only measure the bull's fertility on the examination day, and may not be representative of his average fertility level throughout the breeding season. The subjective nature of the semen quality assessment of the BSE can also introduce biases due to differences in equipment, training, and attention to detail of the practicing veterinarian or technician (Hopper, 2014). A computer-assisted sperm analyzer (CASA) can provide more objective measurements of sperm quality, but they are expensive to purchase and often are only owned by artificial insemination (AI) semen collection facilities (Tanga et al., 2021). Bulls collected for AI tend to have their semen assessed more frequently and thus have more reliable data about their semen quality, but this data is largely proprietary, making it difficult to access to create a large-scale genetic evaluation for the beef industry.

Several studies have reported low to moderate genetic correlations and heritabilities for SC and semen quality measures utilizing either BSE or AI stud collection records (Smith et al., 1989; Garmyn et al., 2011; Butler et al., 2021; Carvalho et al., 2023; Cesarani et al., 2023; Facy

et al., 2024). However, the relationship between BSE and AI stud data in the context of genetic evaluation has not yet been explored.

The first objective of this study was to estimate heritability, repeatability, and variance components for SC and semen quality traits using data measured during both BSE and AI semen collection and examine the relationships between traits. The second objective was to evaluate the utility of combining BSE and AI semen collection records into one dataset to estimate variance components, heritabilities, repeatabilities, and genetic and phenotypic correlations for bull reproductive traits.

Materials and Methods

Animal Care and Use Committee approval was not obtained for this study because the data were pre-existing fertility records obtained from Angus breeders and companies specializing in bull semen collection.

Study Populations

Breeding Soundness Examination Data

Eleven purebred Angus breeders provided 5,877 BSE records on 4,996 bulls. While each bull had one BSE, 651 bulls had at least one additional BSE, with a maximum of five exams. The number of measurements per bull for each trait are depicted in Appendix Figure A.1. The additional measurements allowed for the utilization of a repeated records model. Most of the additional exams were for bulls who had failed their initial BSE, but some bulls had additional records from a subsequent breeding season. Appendix Figure A.2 (all records) and Appendix Figure A.3 (only bulls with repeated records) depict the number of records for each trait that passed or failed the minimum requirement of a satisfactory breeding bull established by the Society for Theriogenology. The BSE records included measurements of scrotal circumference

(SC_B), percentage of progressive motility ($\%MOT_B$), gross motility score ($MOTSC_B$), percentage of primary abnormalities ($\%PRIM_B$), percentage of secondary abnormalities ($\%SEC_B$), percentage of normal spermatozoa ($\%NORM_B$), and percentage of abnormal spermatozoa ($\%ABN_B$). Trait abbreviations, units, and definitions are summarized in Table 2.1. The phenotypic data provided by each producer is outlined in Table 2.2 and includes the number of total BSEs, individual bulls, and the year(s) when the BSEs were performed. Most operations did not report every measurement listed in Table 2.1. Additionally, different veterinarians or technicians conducted the BSEs; however, only a single veterinarian or technician performed all the exams for each operation on a particular day.

Traits that were missing, but could be calculated from the existing data, were added to the dataset. For example, if $\%NORM_B$ was not directly reported, it was calculated by subtracting the $\%PRIM_B$ and $\%SEC_B$ or $\%ABN_B$ from 100. Additionally, for breeders who reported $\%MOT_B$, but not $MOTSC_B$, $MOTSC_B$ was generated based on the guidelines provided by the Society for Theriogenology, with the following classifications (Hopper, 2014): greater than or equal to 70% motility was considered "very good", 50-69% motility was "good", 30-49% motility was "fair", and less than 30% was classified as "poor". The generated $MOTSC_B$ were added to the producer provided $MOTSC_B$ for analysis. To analyze $MOTSC_B$ with the BLUPF90+ suite of programs (Misztal et al., 2014), the categories were assigned arbitrary values of 75 (very good), 55 (good), 35 (fair), and 15 (poor).

Bull Stud Collection Data

Two artificial insemination (AI) organizations provided semen collection records for this study. Bull stud A provided 68,677 collection attempt records from 1,541 Angus bulls. Of those collection attempts, 61,505 resulted in ejaculated semen. The records from stud A were collected

between the years of 2004 and 2022. Bull stud B included data from 2008 to 2022 and included 3,364 collection records from 321 Angus bulls. Therefore, a total of 64,869 stud collection records from 1,862 Angus bull were available for further analysis. The analysis included repeated records because 1,791 stud bulls had at least two collection attempts. The number of records per trait provided by each stud is summarized in Table 2.2. Additional description of the data structure is included in Appendix Figures A.4, A.5, and A.6. Both studs provided animal information including registration number, cane code identifier, bull name, breed, date of birth, and owner name.

Procedures at both studs dictated that bulls were collected twice per week. At stud A, bulls were ejaculated twice per collection day. Stud A combined the two collections from a bull into a single day ejaculate record. Bulls were allowed to mount a teaser steer and they collected semen with an artificial vagina. All collection attempts were recorded at stud A, even if a bull did not ejaculate any semen on the collection day. At stud B, bulls were collected a minimum of two times per collection day and a maximum of four times per day. Each ejaculate was recorded as a separate value. To make the data consistent between locations, all collection records at stud B for a single day from each bull were combined. When records were combined, volume was summed for a total daily volume, and concentration and traits recorded as percentages were averaged for a daily average of each trait. Stud B also utilized a teaser steer and artificial vagina, but if a bull was unwilling or unable to ejaculate into the artificial vagina, he was collected by electroejaculation.

Ejaculate information included date of collection, volume, concentration, and percentage of initial progressive motility (%MOT$_S$) before cryopreservation. Both studs required an ejaculate to have ≥ 50% initial motility to be cryopreserved. Ejaculates meeting the quality

standards for cryopreservation were frozen, thawed, evaluated for post-thawing motility, and stained to assess sperm morphology. Due to morphology measurements being taken after cryopreservation, there are fewer records for primary and secondary abnormalities. There were exceptions for some bulls whose ejaculates did not meet the ≥ 50% initial motility standard, but the bull's owner requested the ejaculates be cryopreserved for their personal use only and not for commercial sale. After cryopreservation, stud A measured semen samples for post-thaw motility, percentage of primary abnormalities ($\%PRIM_S$), percentage of secondary abnormalities ($\%SEC_S$), and percentage of normal spermatozoa ($\%NORM_S$). Stud B provided measurements for post-thaw motility, and percentages of the following abnormalities: detached heads, proximal droplets, distal droplets, abnormal heads, bent tails, distal midpiece reflexes, and midpiece. To make the data consistent between studs, detached heads, proximal droplets, and abnormal heads were added together and classified as primary abnormalities. Bent tails, distal midpiece reflexes, and midpiece were also summed together and classified as secondary abnormalities. Additionally, a gross motility score ($MOTSC_S$) phenotype was created for the stud data using the same methods previously mentioned to create a trait in common for the BSE and stud data. Table 2.1 provides definitions, units, and abbreviations for traits collected at an AI stud.

Stud A supplied records for scrotal circumference (SC_S), weight, hip height, and body condition score, which were taken upon arrival at the stud and during routine health examinations. Ejaculate records for each animal were matched with the most recent measurement records. Contradictory measurements were treated as missing when one trait (i.e. scrotal circumference, hip height, weight, or body condition score) had more than one measurement on a given day and the measurements differed. Measurements that were greater than four standard deviations away from the mean were also treated as missing.

Breeding Soundness Examination and Bull Stud Data Combined

The BSE data and bull stud data were merged by putting the same trait from each dataset into one column, resulting in 70,746 fertility assessments from 6,845 bulls. Data for SC_B and SC_S were consolidated to form scrotal circumference combined (SC_C). The same method was used for progressive motility ($\%MOT_C$), gross motility score ($MOTSC_C$), percentage of primary abnormalities ($\%PRIM_C$), percentage of secondary abnormalities ($\%SEC_C$), and percentage of normal spermatozoa ($\%NORM_C$). The number of measurements per bull in the combined dataset is depicted in Appendix Figure A.7. Additionally, the number of records for each trait in the combined dataset that would have passed or failed a BSE based on the guidelines of the Society for Theriogenology are show in Appendix Figure A.8 for all bulls and Appendix Figure A.9 for bulls with at least a second measurement.

There were 14 bulls that had both BSE and AI collection records. Three bulls were collected at stud A. Each of these bulls had one BSE record, and between 10 and 34 AI collection records. Eleven bulls were collected at stud B. The number of AI collections for each bull ranged from 5 to 61, with most having between 5 and 20 collection records. Of the 11 bulls collected at Stud B, ten bulls had one BSE record. The other bull had two satisfactory BSE records from two separate breeding seasons provided for the analysis.

Data Filtering

Data were filtered with R software (R Core Team, 2013, Vienna, Austria). All measurements recorded as percentages were treated as missing if they were not between 0 and 100. Scrotal circumference measurements needed to be larger than zero or they were treated as missing. The American Angus Association provided adjusted yearling weight and adjusted

scrotal circumference measurements for the bulls. Any measurements that were reported as zero were treated as missing.

The American Angus Association provided the date of birth for all bulls in the dataset, which was utilized to calculate the bull's age on the day of phenotype collection. Bulls with a BSE record ranged in age from 225 days to 3,009 days and had an average age of 532 days old. Bulls collected at AI facilities ranged in age from 296 days to 4,884 days, with an average age of 1180 days. Age in days was assigned to one of six categories for the stud and combined analyses: < 12 months, 12 – 15 months, 15 – 18 months, 18 – 24 months, 24 – 36 months, and > 36 months of age.

Contemporary group (CG) was generated by concatenating the operation (each producer or stud) and date of phenotype collection. Additionally, days since previous collection was calculated for the AI collection records by determining the difference in days since a bull's last collection. A bull's first collection at the stud was given a missing value for days since previous collection.

Environmental Data

Environmental effects were accounted for by assigning a season of collection based on the date of collection or BSE. The categories for season were as follows: Spring (March, April, May), Summer (June, July, August), Fall (September, October, November) and Winter (December, January, February).

Climatology data was obtained from the National Renewable Energy Laboratory's (NREL) National Solar Radiation Database. The closest latitudinal and longitudinal locations were selected for each AI stud and BSE operation. Data obtained from NREL included hourly temperature (°C), hourly relative humidity (%), hourly average wind speed (m/s), and direct solar

radiation (w/m^2). The hourly data were averaged to obtain a daily value for each weather characteristic. A comprehensive climate index (CCI) was generated because it is able to capture the effect of extreme cold or heat stress that the bulls may have been experiencing (Mader et al., 2010). The following formula was used to calculate CCI for each collection date (Mader et al., 2010):

$$CCI = ambient\ temperature\ (°C) + adjusted\ relative\ humidity\ (\%) + adjusted\ wind\ speed\ (w/s) + adjusted\ radiation\ (w/s^2)$$

Three CCI time periods were created: CCI75 (75 days of spermatogenesis and epididymal transit time), CCI61 (only the 61 days of spermatogenesis), and CCI14 (only the 14 days of epididymal transit time; Senger, 2012). Spermatogenesis start date was assigned 75 days before the AI collection or BSE date. The epididymal transit time start date was assigned 14 days before the collection date. For each time period, the daily CCI values were averaged during the period. The NREL was not able to provide weather information for all dates. Any dates that daily CCI could not be calculated were treated as missing and not included in the average.

Genetic Data

The American Angus Association provided a three-generation pedigree for 6,845 bulls. The pedigree data included 3,676 sires and 15,615 dams. Single nucleotide polymorphism (SNP) data were also provided on 9,589 animals. Of these genotyped animals, 3,215 bulls had BSE records, 1,254 bulls had stud records, and 5,133 genotypes were from animals in the pedigree. There were 13 genotyped bulls that had records in both the BSE and stud datasets. The American Angus Association ensured all genotyped animals had 54,609 SNPs. Quality control filtering was performed with the BLUPF90+ suite of programs (Misztal et al., 2014) to remove animals with possible duplicate samples (11), parent-progeny Mendelian conflicts (27), relationship matrix

blending conflicts (25), and call rates of less than 0.90 (42). Additionally, markers with call rates of less than 0.90 (3,311), minor allele frequency of less than 0.05 (9,074), on sex and mitochondrional chromosomes (1,749), and monomorphic SNP (874) were also removed. After quality control filtering, 9,484 animals with 38,601 SNP loci were available for analysis.

The American Angus Association also provided several expected progeny differences (EPDs) and economic selection indexes ($values) from their genetic evaluation (run on August 18, 2023) for this analysis. The EPDs included SC, heifer pregnancy (HP), birth weight (BW), weaning weight (WW), yearling weight (YW), mature weight (MW), and mature hip height (MH). The $values obtained were dollar maternal weaned calf value ($M), dollar beef value ($B), and dollar combined value ($C).

Statistical Analysis

Model Selection

Many factors affect bull fertility traits including age (Fuerst-Waltl et al., 2006), health (Barth and Waldner, 2002), nutrition (Short and Adams, 1988), environmental conditions (Kastelic et al., 1996), collection interval (Fuerst-Waltl et al., 2006), and handling procedures (Almquist, 1973). All available information describing these factors were extracted from the data (contemporary group, season, days since previous collection, CCI75, CCI61, and CCI14) or provided by the American Angus Association (age and adjusted yearling weight).

Variables included in the final analysis were identified using a forward selection procedure performed in the BLUPF90+ suite (Misztal et al., 2014) and model fit was evaluated using the likelihood ratio test. Animal was treated as a random variable. Fixed effects (CG, season, and age group) and covariates (adjusted yearling weight, days since previous collection, age in days, CCI75, CCI61, and CCI14) were tested for model fit via forward selection, starting

with fitting one variable at a time and continuing to add the most significant variable that improves the model (likelihood ratio test resulted in a p-value of < 0.05 and the Akaike information criteria (AIC) improved by three or more) at each step. This procedure was repeated until no more variables improved model fit.

After model testing it was determined that season and CCI were confounded with contemporary group, because each contemporary group experienced only one season and CCI value. Thus, these values were not included in the final models as those effects were included in the contemporary group estimates. Adjusted yearling weight was not statistically significant for any of the BSE response variables with the likelihood ratio test. Days since the previous collection was not statistically significant for SC_S, $\%PRIM_S$, $\%SEC_S$, and $\%NORM_S$, but it was significant for $\%MOT_S$. Adjusted yearling weight was observed to be statistically significant for each stud collection response trait with the likelihood ratio test. However, it was determined that a one-unit increase in days since previous collection and adjusted yearling weight did not result in biologically meaningful improvement in the response traits, based on the effect size (Table 2.3). Therefore, we chose to exclude both from the final models. The effects that were included in the final model for each trait are summarized in Table 2.4.

Phenotypic Analysis

All phenotypic analyses were performed with R software (R Core Team, 2013, Vienna, Austria). Summary statistics including the number of records, mean, median, standard deviation, maximum and minimum were calculated for each trait and reported in Table 2.5. Pearson correlations were also estimated between SC and all semen quality phenotypes for each dataset.

Genetic Parameter Estimation

Genetic parameters were estimated for male reproductive traits using single-step genomic best linear unbiased prediction (ssGBLUP) methodologies (Christensen and Lund, 2010). The relationship matrix was constructed in the BLUPF90 suite (Misztal et al., 2014) as described by Legarra et al. (2009). In the ssGBLUP analysis, the H matrix augments the pedigree-based numerator relationship matrix with SNP markers from the genotyped animals, and SNP information is projected to non-genotyped animals. The inverse of the H matrix is constructed as follows (Christensen and Lund, 2010):

$$\boldsymbol{H^{-1}} = \boldsymbol{A^{-1}} + \begin{bmatrix} \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \boldsymbol{G^{-1}} - \boldsymbol{A_{22}^{-1}} \end{bmatrix}$$

where A^{-1} is a matrix that captures pedigree relationships, G^{-1} is a genomic relationship matrix for genotyped animals, and A_{22}^{-1} is the pedigree information from genotyped animals. Initially, a univariate animal model with a permanent environmental effect was fit for each trait to account for repeated records. The covariates and fixed effects included in each model are outlined in Table 2.4. These models were utilized to determine the starting values for subsequent bivariate analyses. Variance components, heritabilities, repeatabilities, and genetic correlations were estimated using the following bivariate model in the BLUPF90 suite of programs (Misztal et al., 2014):

$$\begin{bmatrix} \boldsymbol{X'X} & \boldsymbol{X'Z_a} & \boldsymbol{X'W_{pe}} \\ \boldsymbol{X'Z_a} & \boldsymbol{Z_a'Z_a + \lambda H^{-1}} & \boldsymbol{Z_a'W_{pe}} \\ \boldsymbol{X'W_{pe}} & \boldsymbol{Z_a'W_{pe}} & \boldsymbol{W_{pe}'W_{pe} + I\rho_{pe}} \end{bmatrix} \begin{bmatrix} \widehat{\boldsymbol{b}} \\ \widehat{\boldsymbol{a}} \\ \widehat{\boldsymbol{pe}} \end{bmatrix} = \begin{bmatrix} \boldsymbol{X'y} \\ \boldsymbol{Z'y} \\ \boldsymbol{W'y} \end{bmatrix}$$

where y is a vector of phenotypic observations for each bull reproductive trait, b is a vector of fixed effects (contemporary group and age group) and covariates (age in days), X is an incidence matrix relating the observations in y to the contemporary group and age group or age in days, a is a vector of random additive direct genetic effects, Z_a is an incidence matrix relating y to random additive direct genetic effects, pe is a vector of random permanent environmental genetic effects,

W_{pe} is an incidence matrix relating phenotypic observations to pe, and e is a vector of residual error.

Results and Discussion

Summary statistics for BSE, stud collection, and combined records are reported in Table 2.5. The mean for SC_B and SC_s measurements were 36.45 and 40.80 cm, respectively. The difference in average SC may be due to the average age of the stud being approximately 1.75 years older than the average age of the BSE bulls, and thus, the stud bulls could be more mature in their scrotal development. Both average measurements are greater than the minimum SC required by the Society for Theriogenology (SFT) BSE standards (Koziol and Armstrong, 2018). However, the minimum SC measurements in the BSE and stud datasets would not have passed the minimum requirement of 30 cm.

The average $\%MOT_B$ was 55.08% and median was 50%, which surpasses the minimum 30% requirement for BSE standards. The difference in the mean and median values were attributed to a large number of ejaculates having 80-99% progressive motility. The mean $\%MOT_S$ was 49.50%, which is above the requirement to pass a BSE, but is slightly lower than the 50% minimum requirement for cryopreservation. The average $\%MOT_S$ was also lower than the mean $\%MOT_B$. The increased average $\%MOT_B$ compared to $\%MOT_S$ could be due to how precise of a measurement is recorded by the practicing technician. Cryopreservation is a taxing process that can negatively impact sperm motility. Therefore, it is important that the trained technician ensures that sperm motility is greater than or equal to 50% prior to freezing, so there are enough motile sperm after rethawing. In comparison, the motility requirement to pass a BSE is lower ($\geq$ 30%), and BSE are often performed by veterinarians who may not specialize in the service, so their measurements are less precise.

The summary statistics for $MOTSC_B$, $MOTSC_S$, and $MOTSC_C$ were calculated based on the numerical value given to each category of motility score. The mean was slightly higher for $MOTSC_B$ (54.66) compared to $MOTSC_S$ (49.43) and $MOTSC_C$ (50.17), but the median value of 55.00% was the same for all three datasets. The standard deviation was also higher for $MOTSC_B$ (17.49) compared to $MOTSC_S$ (15.34) and $MOTSC_C$ (15.49).

When comparing the summary statistics of the semen morphology traits, it is important to recognize how the mean, median, and minimum values are similar to each other across datasets. Across the three datasets, the average for primary abnormalities ranged from 11.22% to 11.86%, and secondary abnormalities averaged slightly higher with 11.73% to 12.19%. The average percentage of normal sperm ranged from 75.75% to 76.41% across the three datasets. These averages were expected because a bull's ejaculate needed to have at least 70% normal morphology to pass a BSE or for the semen to be cryopreserved. The standard deviations for $\%SEC_B$, $\%SEC_S$, and $\%SEC_C$ were similar. However, the standard deviation for $\%PRIM_B$ (14.60%) was twice as large as the standard deviation for $\%PRIM_S$ (7.17%), and the standard deviation for $\%NORM_B$ (22.74%) is almost three times larger than the standard deviation for $\%NORM_S$ (8.66%). The standard deviations for $\%PRIM_C$ and $\%NORM_C$ were slightly larger than $\%PRIM_S$ and $\%NORM_S$, despite most of the combined records being from the stud data. The higher standard deviations observed for $\%PRIM_B$ and $\%NORM_B$ may be due to age and stage of maturity. Younger bulls tend to produce sperm with more abnormalities when they initially start producing semen, that will eventually reduce to an acceptable percentage after they have time to mature and develop (Mandal et al., 2010).

Variance Components and Heritability Estimates

The additive direct genetic, permanent environment, residual variances for the multivariate ssGBLUP analyses, heritability estimates, and standard errors are reported in Table 2.6. The reported variance components for male fertility traits were obtained from each bivariate analysis and then averaged. It is difficult to make comparisons to previous literature due to the limited number of published studies examining variance components for the same traits. Furthermore, very few studies utilized a repeated records model with a permanent environmental effect.

The direct additive genetic and permanent environmental variance components for SC_B were both slightly smaller than those estimated for SC_S in the current study. Interestingly, the additive genetic and permanent environmental variance estimates for SC_C were between those estimated for SC_B and SC_S. The residual variance estimates for SC_B, SC_S, and SC_C were comparable to each other and ranged from 0.95 cm^2 to 1.51 cm^2. The current study's variance component estimates for SC_B were similar to those reported by Russell et al. (2021) for additive direct genetic (1.98 cm^2) and residual variance (2.38 cm^2) in a population of composite bulls. To our knowledge, no other studies have reported variance component estimates for SC_S or SC_C, but the estimates in the current study are comparable to other studies investigating SC measurements taken during a BSE (Kealey et al., 2006; Russell et al., 2021; Cesarani et al., 2023).

The heritability estimates for SC_B, SC_S, and SC_C were 0.36 ± 0.03, 0.53 ± 0.04, and 0.42 ± 0.02, respectively. The SC_B estimate in the current study is similar to the estimate of 0.36 ± 0.06 reported by Knights et al. (1984) in yearling Angus bulls. However, most heritability estimates from BSE studies are more comparable to the SC_S and SC_C in the current study (Christmas et al., 2001; Kealey et al., 2006; Garmyn et al., 2011; Cesarani et al., 2023). Garmyn

et al. (2011) reported a heritability estimate of 0.46 ± 0.06 for SC_B in yearling Angus bulls, while Kealey et al. (2006) published a highly heritable estimate of 0.57 ± 0.09 SC_B in a population of Line 1 Hereford bulls. The lower heritability estimate for SC_B in the current study could be because the records were provided by several different producers with different practicing veterinarians, which introduced additional environmental variation, instead of a controlled research setting with one technician as in previous literature (Kealey et al., 2006; Cesarani et al., 2023). In the current study, the SC_S phenotypes were reported only from one stud location with the same practicing veterinarian, so even though it was not a controlled research setting there, may have been fewer environmental differences between measurements. In another study with SC measurements from Holstein bulls at seven different AI stud locations, the heritability of SC_S was estimated as 0.67 ± 0.10 (Coulter et al., 1976), which is higher than the estimate reported in Table 2.6. However, the authors utilized a sire model instead of an animal model like the current study (Coulter et al., 1976).

The heritability of SC taken during a breeding soundness exam on yearling to two-year-old bulls has been extensively studied, but there is limited research utilizing bulls three years of age and older. This gap may partly be due to only 15.6% of beef operations in the United States measuring the scrotal circumference of their mature bulls annually (USDA, 2009). Additionally, the lack of producers reporting scrotal circumference measurements to breed associations or researchers for genetic prediction further contributes to the scarcity of mature bull BSE data. Scrotal circumference measurements from stud bulls are also limited, likely because stud data is proprietary. Nevertheless, these results underscore the need for additional research to understand the heritability of SC from bulls in different environments and stages of maturity.

Across the three datasets, the variance component estimates for SC were numerically more similar to each other than the estimates for %MOT. However, the heritability estimates for %MOT were more consistent between the BSE, stud, and combined dataset than the SC heritability estimates. The current study's estimate for direct additive genetic variance ($16.13\%^2$) was similar to the $21.22\%^2$ variance estimate reported by Cesarani et al. (2023) for a population of Italian Simmental bulls. However, estimates from Tropical Composite bulls for additive genetic and residual variance in literature (Corbet et al., 2013; Facy et al., 2024)were higher than the estimates in Table 2.6. The additive genetic, permanent environmental, and residual variance components for $\%MOT_S$ were similar to those reported by Butler et al. (2021), who used a subset of stud bulls from the current study. Interestingly, the additive genetic variance for $\%MOT_C$ was estimated to be $23.82\%^2$, which was between the estimates for $\%MOT_B$ ($16.13\%^2$) and $\%MOT_S$ ($33.0\%^2$). However, the permanent environmental variance estimate for $\%MOT_C$ was similar to $\%MOT_S$, and the residual variance estimate was more comparable to $\%MOT_B$.

The heritability estimates for $\%MOT_B$ (0.10 ± 0.03), $\%MOT_S$ (0.12 ± 0.02), and $\%MOT_C$ (0.10 ± 0.01) in the current study were very similar to each other. Using a subset of the AI stud bulls from the current study, Butler et al. (2021) reported a comparable heritability estimate of 0.12 ± 0.03. These estimates are also consistent with the range of heritability estimates (0.04 to 0.13) for sperm motility reported in other beef BSE studies (Knights et al., 1984; Gredler et al., 2007; Carvalho et al., 2023; Cesarani et al., 2023). However, studies utilizing Holstein stud bulls have reported higher heritability estimates. Suchocki and Szyda (2015) reported a heritability estimate of 0.31 ± 0.06, and Druet et al. (2009) estimated the heritability of motility to be 0.43 ± 0.08. The differences between heritability estimates for beef and dairy bulls could be attributed to breed management differences. Most dairy bulls are housed at bull studs because 89.3% of

North American dairies utilize AI for breeding their females (USDA, 2018). Bull studs usually have one or more trained technicians who specialize in semen collection and computer-assisted semen analysis (CASA) systems to help provide more objective measurements of sperm motility (Onofre et al., 2021). In contrast, most beef females are bred through natural service with a beef bull. Only 11.6% of beef females are bred using AI (USDA, 2020), which is why there are fewer beef bulls at AI studs than dairy bulls. The beef studs utilized in the current study provided housing for stud bulls but also collected bulls who traveled to the stud facility and were taken home on the same day because they were not routinely collected. The higher heritability estimates for the dairy bulls could be because genetic predictions are better able to capture the additive genetic component from the environmental component due to more similar management practices throughout their reproductive lifetime.

A limited number of studies have published variance components and heritability estimates for motility score, possibly because the Society for Theriogenology removed a gross motility score from the BSE form (Koziol and Armstrong, 2018). Nevertheless, in the current study, the variance component estimates for $MOTSC_S$ and $MOTSC_C$ were nearly identical, but the estimates for $MOTSC_B$ were much smaller. These variances also resulted in a similar pattern for heritability estimates of 0.05 ± 0.02 for $MOTSC_B$, 0.11 ± 0.07 for $MOTSC_S$, and 0.11 ± 0.07 for $MOTSC_C$. Corbet et al. (2013) estimated the heritability of $MOTSC_B$ in Tropical Composite bulls at three different ages, reporting estimates for 12 months (0.32 ± 0.06), 18 months (0.15 ± 0.05), and 24 months of age (0.10 ± 0.04). In another study with Line 1 Hereford yearling bulls, the estimated heritability for MOTSCB was 0.22 ± 0.09 (Kealey et al., 2006). The 12-month estimate reported by Corbet et al. (2013) is similar to the findings of Kealey et al. (2006). However, Corbet et al. (2013) heritability estimates for 18- and 24-month-old motility scores are

more similar to those reported in the current study. The higher heritability estimates reported by Kealey et al. (2006) and Corbet et al. (2013) could be because they accounted for maternal effects in their models, which weren't included in the current study. A negative maternal environment has been reported to cause differences in testicular development and semen quality due to the long-term effect of fetal programming (Zambrano et al., 2014; Polizel et al., 2021).

In the current study, it was not unexpected that the $MOTSC_C$ estimates were similar to the $MOTSC_S$ estimates, because motility score only had four possible phenotypes, and most of the combined phenotypes were from the stud data. However, the lower heritability estimate for $MOTSC_B$ could be due to the collection method. The primary collection method for the stud data was an artificial vagina, but all the BSE records were collected with electroejaculation. Although electroejaculation is the most common collection method for BSE because of its convenience, consistency, and reliability in collecting a semen sample (Koziol and Armstrong, 2018), several studies have reported significantly lower motility in ejaculates collected with electroejaculation compared to those collected with an artificial vagina when measured with a CASA (Jiménez-Rabadán et al., 2012; Nadaf et al., 2022). Electroejaculation has also been reported to negatively affect acrosome integrity, DNA fragmentation, and sperm mitochondria function (Jiménez-Rabadán et al., 2012). Despite the negative impacts to semen quality, electroejaculation will most likely continue to be the preferred method for BSE semen collection because it takes appropriate facilities and patience to train a bull to service an artificial vagina (Koziol and Armstrong, 2018). Nevertheless, accounting for different collection methods may improve genetic prediction of motility score.

Most bulls had both a percentage of progressive motility (%MOT) and motility score measurement because if a bull's motility was recorded as the %MOT and not a motility score,

then a motility score was generated based on the Society for Theriogenology guidelines (Hopper, 2014). The %MOT was recorded as a value between 0% and 100%, reflecting the technician's estimate of the percentage of sperm progressively moving forward in an ejaculate. Meanwhile, motility score is an assessment of mass motion in an ejaculate and was recorded as one of four categories: very good, good, fair, and poor. An ejaculate's mass motion is influenced by concentration, sperm moving in a linear fashion, and the velocity of the sperms' movement (Koziol and Armstrong, 2018). The Society for Theriogenology removed gross motility score from the BSE form in 2018 because it needed to be carefully interpreted, and the society's preferred assessment of motility is %MOT. The heritability estimates in the current study for %MOT were similar to those estimated for motility score, but the %MOT estimates had slightly smaller standard errors. Even though both traits for motility are subjective measurements, the smaller standard errors for %MOT may be due to the data's structure. There was more variation between phenotypic measurements for %MOT, creating a more normal distribution between %MOT observations when compared to the four categories of motility score. The BLUPF90+ suite expects that the response variable is normally distributed (Misztal et al., 2014). Additionally, the %MOT models estimated smaller residual variance components than motility score models, which could have impacted the standard errors of the heritability estimates in the ratio calculation between additive and phenotypic variance (Misztal et al., 2014).

In the current study, technicians recorded sperm abnormalities as primary and secondary. Abnormalities thought to originate during spermatogenesis in the testes were called primary. Secondary abnormalities were defects thought to have originated during epididymal transit time. The additive genetic, permanent environmental, and residual variance component estimates were higher for $\%PRIM_B$ than $\%PRIM_{S \text{ and }} \%PRIM_C$. Interestingly, the permanent environmental

variance for %PRIM$_C$ was more comparable to %PRIM$_B$ despite having more primary records from the stud data. The heritability estimates were 0.09 ± 0.03, 0.04 ± 0.02, and 0.06 ± 0.01 for %PRIM$_B$, %PRIM$_S$, and %PRIM$_C$, respectively. Most heritability estimates for %PRIM in literature were more moderate than those estimated in this study (0.31 ± 0.08, Smith et al., 1989; 0.35, Christmas et al., 2001; 0.30 ± 0.09, Kealey et al., 2006; 0.23 ± 0.08, Garmyn et al., 2011). The one exception was reported by Bulter et al. (2021) using a subset of the stud bull population in the current study, and their estimate was not different than zero (0.03 ± 0.03).

The additive genetic variance estimates were comparable between %SEC$_B$, %SEC$_S$, and %SEC$_C$. However, the permanent environmental variance was higher for %SEC$_B$ and %SEC$_C$ compared to %SEC$_S$, which was similar to %PRIM variance for permanent environmental. The heritability estimates for %SEC$_B$, %SEC$_S$, and %SEC$_C$ were 0.09 ± 0.03, 0.08 ± 0.02, and 0.07 ± 0.01, respectively. These estimates were slightly higher than the estimates for primary abnormalities in the current study, but they were smaller than other estimates in literature for secondary abnormalities (Christmas et al., 2001; Kealey et al., 2006; Garmyn et al., 2011; Butler et al., 2021). Studies utilizing BSE reported moderate heritability estimates ranging from 0.23 to 0.33 for %SEC$_B$ (0.26, Christmas et al., 2001; 0.33 ± 0.08, Kealey et al., 2006; 0.23 ± 0.08, Garmyn et al., 2011). These studies did not include repeated records for %PRIM$_B$ or %SEC$_B$ measurements, which may have caused an overestimation of the direct additive genetic component, which was partitioned to the permanent environment variance in the current study (Smith et al., 1989; Christmas et al., 2001; Kealey et al., 2006; Garmyn et al., 2011). Furthermore, these studies only utilized pedigree information to account for genetic relationships, but the current study included both pedigree and genomic data to improve the prediction accuracy (Christensen and Lund, 2010). Butler et al. (2021) also used pedigree and

genomic data to account for genetic relationships. Using a subset of the stud bulls if the current study, they estimated a heritability of 0.18 ± 0.04 for $\%SEC_S$. The lower $\%SEC_S$ estimate reported in the current study could have been because contemporary group was defined differently in our study, which may have changed the partitioning of additive genetic variance.

When comparing the BSE and stud heritability estimates for sperm abnormalities in the current study, slightly smaller estimates were observed for $\%PRIM_S$ and $\%SEC_S$ than $\%PRIM_B$ and $\%SEC_B$. A potential reason for the lower estimates is the possible bias of the stud data. Ejaculates collected at the stud needed greater than or equal to 50% motility before cryopreservation, and morphology measurements were taken after cryopreservation during the post-thawing assessment (Butler et al., 2021). For an ejaculate to have 50% motility, the ejaculate should have approximately 50% normal sperm cells. However, most $\%PRIM_B$ and $\%SEC_B$ measurements were recorded shortly after semen collection and were assessed independently from $\%MOT_B$ (Koziol and Armstrong, 2018). Another potential difference between the $\%PRIM_B$ and $\%PRIM_S$ estimates is the effect of bull age. Age in days was not selected as a significant effect during model testing for $\%PRIM_B$, but other studies have reported a reduction in primary abnormalities as bulls mature (Mandal et al., 2010). Thus, it could be possible that age had an underlying effect.

Unlike the other traits in this study, the variance components for $\%NORM_B$, $\%NORM_S$, and $\%NORM_C$ varied greatly. However, similar heritability estimates for $\%NORM_B$ (0.06 ± 0.01), $\%NORM_S$ (0.05 ± 0.02), $\%NORM_C$ (0.11 ± 0.05) were reported in Table 2.6. The slightly larger heritability estimate for $\%NORM_C$ is due to the uncharacteristic maximum heritability estimate of 0.27 ± 0.20 for $\%NORM_C$ from the $\%NORM_C$ and $MOTSC_C$ bivariate. In the current study, $MOTSC_C$ was a threshold trait that was transformed into numerical data, and $MOTSC_C$

fitted with an ordinal model, because it was less computationally challenging. Therefore, the bivariate model between $\%NORM_C$ and $MOTSC_C$ may have overestimated the heritability of $\%NORM_C$. The heritability estimates for $\%NORM_B$ and $\%NORM_S$ are slightly outside the 0.07 to 0.35 range reported by other British-type bull studies (Smith et al., 1989; Kealey et al., 2006; Butler et al., 2021). Although the heritability estimates for %NORM are low, they are different from zero, so it could be possible to make slow genetic progress to improve sperm cell shape if selection pressure was placed on the trait.

Genetics Correlations Within Trait

The genetic correlations within reproductive traits measured during both a BSE and semen collection for AI are reported in Table 2.7. Only 14 bulls had fertility data recorded in both the BSE and stud datasets. Therefore, the genetic relationships used to estimate these genetic correlations were derived from the H matrix, which incorporated pedigree and genomic information. The limited genetic relationships between the BSE and stud datasets may have contributed to the lower-than-expected correlation estimates.

The genetic correlation between SC_B and SC_S was estimated as 0.49 ± 0.08, which was lower than expected. Scrotal circumference is the least subjective measurement of all the reproductive traits in the current study, so it was expected that SC would result in the highest genetic correlation. These findings may suggest differences in SC measurements taken during a BSE and health examination at a bull stud. Age of bull was accounted for in both the SC_B and SC_S models, but there could be nutritional management differences between BSE and stud bulls in relation to their stage of maturity and scrotal development might not have been accounted for. The BSE bulls in this study were tested prior to each operations' seedstock production sale. Generally, bulls in these types of sale are fed high-energy diets to meet a desired body condition

score (BSC), which some may consider overweight (Walker et al., 2020). The increased energy from a bull's diet can increase both testicular development and fat deposition in the scrotum, leading to increased scrotal circumference (Walker et al., 2020). However, the stud bulls were fed to their maintenance requirements and may not have had additional fat deposition.

The bivariate model estimating the genetic correlation between $\%MOT_B$ and $\%MOT_S$ failed to converge. This may have been due to the limited number of $\%MOT_B$ phenotypic records (2,736) available for analysis compared to the 61,943 $\%MOT_S$ records. A similar issue occurred when attempting to compare $MOTSC_B$ to $MOTSC_S$. Despite having slightly more phenotypic records to compare $MOTSC_B$ (5,323 records) and $MOTSC_S$ (61,943 records), the bivariate model again did not converge. The lack of convergence in the current study may indicate the models were overparameterized and did not have sufficient information to estimate the variances (Misztal et al., 2014).

The estimated genetic correlation between $\%PRIM_B$ and $\%PRIM_S$ was -0.21 ± 0.59. Although the estimate was not different than zero, it is interesting that the correlation indicates an antagonistic relationship. We accounted for bull age in the $\%PRIM_S$ model with bulls averaging 3.25-years-old, but not in the $\%PRIM_B$ model with bulls averaging 18-months-old. Thus, the antagonist relationship might be caused by increased primary abnormalities in ejaculates from younger bulls, which tend to reduce as a bull matures (Mandal et al., 2010). A moderate genetic correlation of 0.61 ± 0.28 was estimated between $\%SEC_B$ and $\%SEC_S$. This favorable correlation was the highest correlation estimated between a fertility trait measured during both a BSE and semen collection. In contrast, the genetic correlation estimated between $\%NORM_B$ and $\%NORM_S$ (0.32 ± 0.59) was not different from zero. The large standard errors for the genetic correlation estimates of %PRIM, %SEC, and %NORM may suggest more

phenotypic data is needed to make comparisons between the BSE and stud measurements for morphology traits.

To our knowledge, there are no other genetic correlations in the published literature comparing the relationships between a single reproductive trait measured during a BSE and semen collection at a bull stud. Further investigation is warranted into the relationships between data collected during a BSE and semen collections for AI, given of the convergence issues and high standard errors reported for some traits. The weak to moderate genetic correlations estimated in the current study suggest that BSE and stud data may need to be treated differently. This could pose a challenge for breed associations aiming to develop a bull fertility genetic selection tool for their producers, as it may be difficult to source sufficient phenotypes to accurately predict an animal's genetic merit for fertility (Berry et al., 2016). If the breed association elected to utilize only BSE data, they could potentially receive fertility records from their breeders on a larger population of bulls. However, a BSE is usually only performed on each animal once per breeding season or year if he is satisfactory and only assesses his fertility on the examination day (Barth, 2018). Additionally, the limited number of beef operations (26.8%) that evaluate the semen quality of their mature bulls (USDA, 2009), may further constraint the number of continued fertility records per BSE bull.

In contrast, fertility phenotypes from stud records may provide a more accurate depiction of a bull's fertility level, as the bull's semen is collected and evaluated more regularly. However, this approach for developing a fertility selection tool may be hindered by potential bias, because only a small percentage of genetically superior beef bulls are collected at bull studs. Furthermore, the proprietary nature of stud data could make it difficult to access data for continued validation of the genetic evaluation (Berry et al., 2016).

Correlations Across Traits

Genetic correlations between bull reproductive traits are reported in three tables. Table 2.8 summarizes the genetic and phenotypic correlations between SC and semen quality traits measured during a BSE. The relationships between traits measured at a bull stud are reported in Table 2.9. Lastly, Table 2.10 reports the genetic and phenotypic correlations between the traits in a combined BSE and stud analysis.

Antagonistic genetic correlations were estimated between SC and progressive motility. A weak unfavorable relationship (-0.16 ± 0.13) was observed between SC_B and $\%MOT_B$, while even stronger unfavorable, moderate genetic correlations were observed between those traits in the stud (-0.66 ± 0.08) and combined datasets (-0.46 ± 0.07). The negative genetic correlations observed in this study could be showing that although SC has been positively correlated to daily sperm production (Coulter and Foote, 1979; Palasz et al., 1994), the percentage of motile sperm in the ejaculate may not necessarily increase, and may remain relatively the same percentage motility or slightly decrease. A similar negative genetic correlation (-0.25) between SC_B and $\%MOT_B$ was reported for in a population of Angus yearling bulls, but it did not include a standard error (Knights et al., 1984). Conversely, most genetic correlation estimates between SC_B and $\%MOT_B$ in the literature are positive. Christmas et al. (2023) reported a genetic correlation of 0.56 with a population of Angus yearling bulls. Corbet et al. (2013) also reported positive genetic correlations between SC_B and $\%MOT_B$ for populations of Brahman and Tropical Composite bulls, which range from 0.33 to 0.86. Interestingly, the current study's phenotype correlations between SC and %MOT for the BSE (-0.21), stud (0.05), and combined datasets (0.02) were more comparable to literature estimates (Smith et al., 1989; Corbet et al., 2013).

Therefore, genetically selecting to increase SC may not be the best approach for improving bull reproduction.

The phenotypic correlation estimates between SC and MOTSC in the stud (0.05) and combined (0.15) datasets were favorable and statistically significant ($P < 0.001$). However, the genetic correlations between SC and MOTSC were negative and not different from zero for all three datasets: BSE, stud, and combined. Previous studies have reported positive and favorable genetic correlations between SC and MOTSC. Corbet et al. (2013) estimated several genetic correlations between SC_B and $MOTSC_B$ at three different age points in Tropical Composite and Brahman bulls. The author's estimated correlations ranged from 0.55 to 0.82 (Corbet et al., 2013). Kealey et al. (2006) reported a genetic correlation of 0.34 (no standard error) between SC_B and $MOTSC_B$ in a population of Line 1 Hereford bulls. One major difference between the current study and other estimates in literature was how MOTSC was categorized. The current study utilized the four motility score categories defined by the Society for Theriogenology, but both Kealey et al. (2006) and Corbet et al. (2013) utilized a different system with five categories for motility score. Less variation in the current study might explain the high standard errors for the correlations observed between SC and MOTSC. However, it is important to mention that the Society for Theriogenology removed an assessment for MOTSC from the BSE form and guidelines in 2018, because of the subjective nature of the gross measurement and %MOT was the more preferred and consistent quantification system between technicians (Koziol and Armstrong, 2018).

Weak and unfavorable genetic correlations were estimated between SC and sperm abnormalities. The genetic correlation between SC_C and $\%PRIM_C$ was 0.18 ± 0.14. A similar correlation was observed between SC_S and $\%SEC_S$ (0.15 ± 0.13). The four remaining correlation

estimates between SC and primary and secondary abnormalities were not different zero. These results suggest that selected for larger SC is resulting in more abnormalities per ejaculate which would be detrimental for improving bull reproduction. Unlike the current study, favorable genetic correlation estimates have been reported between SC and abnormalities in literature (Christmas et al., 2001; Kealey et al., 2006; Garmyn et al. 2011; Silva et al., 2013). Kealey et al. (2006) reported that SC_B had a negative, moderate genetic correlation with $\%PRIM_B$ and $\%SEC_B$ (-0.36 and -0.45, respectively). These results were similar to correlations estimated by Garmyn et al. (2011) between SC_B and $\%PRIM_B$, $\%SEC_B$, and total abnormalities (-0.19 ± 0.17, -0.23 ± 0.18, and -0.23 ± 0.18, respectively). The favorable associations between SC and sperm abnormalities are advantageous for indirect selection to improve sperm morphology by selecting for increase SC, especially because SC has a higher heritability than morphology traits.

The phenotypic correlations between SC and sperm morphology traits were consistent across the different datasets. Notably, the correlations between SC and %PRIM were zero for all three analyses. This may suggest that while primary abnormalities originate during spermatogenesis in the testes (Barth and Oko, 1989), which contributed substantially to scrotal size (Kastelic, 2014), primary abnormalities may be more influenced by external factors such as bull nutrition and environmental temperature. Favorable phenotypic corrections were estimated between SC and secondary abnormalities for the BSE (-0.03), stud (-0.04), and combined (-0.04) analyses. Scrotal circumference also had favorable phenotypic correlations with normal sperm, which was expected due to sperm abnormalities being a function of normal sperm. These results suggest that phenotypic selection for larger scrotal circumference may increase correct sperm cell morphology, which would be advantageous for improving bull reproduction, because SC is

easy to measure, cost effective, and there is already a selection tool for SC provided to American Angus Association members.

Contradictory genetic correlations were observed between SC and normal sperm cells. A favorable genetic correlation was observed SC_B and $\%NORM_B$ (0.21 ± 0.12), but an unfavorable, moderate relationship between these traits was observed if the data was recorded at the bull stud (-0.38 ± 0.28). The relationship between SC_C and $\%NORM_C$ was numerically positive, but not different from zero (0.10 ± 0.11). Kealey et al. (2006) and Corbet et al. (2013) both reported favorable genetic correlations between SC_B and $\%NORM_B$ that were similar to results in Table 2.8 for SC_B and $\%NORM_B$. However, Smith et al. (1989) observed a negative, unfavorable correlation between SC_B and $\%NORM_B$ (Table 2.9), which is more comparable to the correlation reported between SC_S and $\%NORM_S$ in the current study. The contrasting results for SC and normal sperm morphology may have been due to the stage of scrotal development. The BSE bulls' average SC was four centimeters smaller than the stud bulls' average SC, and it may be possible that there is a maximum SC measurement where past that size has negative impacts to sperm production.

Many researchers have identified SC as the best indicator for improving reproduction. Scrotal circumference is easy to measure with less subjectivity, has high repeatability, moderate heritability, and favorable genetic correlations with other reproductive traits (Coulter et al., 1976; Palasz et al., 1994; Menegassi et al., 2019). Multiple studies have reported positive associations between SC and paired testis weight, daily sperm production, semen quality, and daughters' ages at puberty (Lunstra et al., 1978; Coulter and Foote, 1979; Kealey et al., 2006; Barth, 2007). As a results, recommendations for the minimum SC for each breed by age were developed to maximize a bull's sperm-producing capability and his ability breed in more than 24 cycling

females in a given breeding season (Coulter et al., 1987; Koziol and Armstrong, 2018). However, the genetic correlations observed in the current study contradict the existing literature. One potential explanation is that SC measurements should not continue to increase indefinitely, but rather, there may be an optimal SC range that produces the highest quality semen. If further research supports this hypothesis, beef producers may have to modify their management practices to improve bull fertility. One potential adaptation could be developing future herd sires to two years of age instead of yearling. Although this may increase a herd's generational interval, potentially slowing genetic progression, the slower development could help with scrotal fat deposition (Barber and Almquist, 1975), ideal nutritional management to maintain a BCS of 6 (Harrison et al., 2022), and allowing for identification of potential hoof issues that take longer to manifest (Barth and Waldner, 2002), potentially resulting in a longer productive life for the bull.

The current study found that progressive motility had favorable, moderate genetic correlations with primary, secondary, and normal morphology for all three datasets. These relationships could suggest that selecting to increase percent motility could indirectly select for an increase of normal sperm morphology and a decrease in primary and secondary abnormalities. This relationship is biologically plausible, as sperm need to have correct the cell shape and size to efficiently navigate through the female reproductive tract to reach and fertilize the oocyte. Analogous genetic correlations between motility and morphology traits have also been observed in previous research (Smith et al., 1989; Gredler et al., 2007; Druet et al., 2009; Butler et al., 2021). While favorable associations were also observed between motility score and sperm morphology traits in the current study, these correlations were not different from zero. Comparing the correlations for percent motility versus motility score may indicate that recording motility as a percentage may be more useful for genetic evaluation, which is advantageous given

the recent removal of motility score from the Society for Theriogenology's guidelines for breeding soundness exams, prompting veterinarians to solely record motility as a percentage.

Positive genetic and phenotypic correlations were estimated between $\%PRIM_B$ and $\%SEC_B$ (Table 2.8). Conversely, negative relationships were observed between $\%PRIM_S$ and $\%SEC_S$ for the genetic and phenotypic correlations. Interestingly, the genetic correlation estimated between $\%PRIM_C$ and $\%SEC_C$ was positive (0.37 ± 0.25), despite more stud records than BSE records in the combined dataset, which resembles the relationship observed in BSE correlation. However, the phenotypic correlation between $\%PRIM_C$ and $\%SEC_C$ was negative, similar to the stud analysis phenotypic result. Previous studies reported genetic correlations estimated between primary and secondary abnormalities that were not different from zero or did not report a standard error. Nevertheless, Smith et al. (1989) reported a positive genetic correlation estimate (0.14 ± 0.64), consistent with the relationships observed in BSE and combined analyses. In contrast, Kealey et al. (2006) and Butler et al. (2021) reported strong, negative genetic correlation estimates of -0.87 (no standard error) and -0.60 ± 1.13, respectively, between $\%PRIM_S$ and $\%SEC_S$, aligning with the stud analysis. Given the conflicting findings, further research is necessary to clarify the genetic and phenotypic relationships between primary and secondary abnormalities.

Conclusion

Although many researchers have explored the heritabilities and genetic relationships of beef bull fertility traits, this study was unique in analyzing six different reproductive traits data collected during a BSE and stud collection records for AI. There is a gap in knowledge as to whether reproductive traits measured during a BSE and semen collection for AI can be utilized in the same genetic evaluation. To test if BSE and stud data could be combined into one bull

fertility dataset, genetic correlations were estimated within SC, percent motility, motility score, primary abnormalities, secondary abnormalities, and percent normal sperm. Moderate genetic correlations were observed between SC_B and SC_S and $\%SEC_B$ and $\%SEC_S$. The correlation estimates for $\%PRIM_B$ and $\%PRIM_S$ and $\%NORM_B$ and $\%NORM_S$ were not different from zero. The bivariate models comparing the genetic relationships between $\%MOT_B$ and %MOTS and MOTSCB and MOTSCS were not able to converge. Further investigation is needed to determine the relationship between BSE and stud data, but the findings in the current study suggest that the data may describe different variation and may need to be analyzed separately.

The BSE and stud data were then combined into one dataset. The BSE, stud, and combined datasets were all analyzed as individual datasets to estimate heritabilities, repeatabilities, and genetic correlations across reproductive traits. Scrotal circumference is moderately heritable, while semen quality traits such as motility and morphology are lowly heritable. Although the quality traits had low heritability, the estimates largely differed from zero, meaning that genetic progress could still be made to improve bull fertility. All repeatability estimates were moderately to highly repeatable, which suggests that individual beef bull reproductive traits are consistent with animal.

When examining the genetic correlations across SC, semen motility, and morphology traits, many conflicting relationships were observed. Most genetic correlations between SC and semen quality traits were weak and unfavorable, indicating that SC may not be useful as an indicator trait for fertility or that an optimum SC range may exist for semen quality. Favorable genetic correlations were observed between percent motility and semen morphology traits, but the correlations estimated between motility score and semen morphology were not different from zero. The findings may indicate that percent motility would be more useful to create a genetic

evaluation than motility score and indirect selection for motility might be possible if selecting for improved morphology. These findings highlight the need for further research to determine the best methodology for obtaining fertility phenotypes and the feasibility of putting selection pressure on motility and morphology traits to enhance genetic selection for improved bull fertility.

Table 2.1 Abbreviations (Abb), units, and descriptions of bull fertility trait.

Population	Trait	Abb	Units	Description
Breeding soundness examination data	[1]Scrotal circumference	SC_B	cm	Scrotal circumference is measured by gently forcing the testes to the bottom of the scrotum and a measuring tape should be placed level with the skin around the widest part of the scrotum
	[2]Progressive motility	$\%MOT_B$	%	Percentage of sperm moving linearly in a semen ejaculate
	[3]Motility score	$MOTSC_B$	Score	Subjective measurement of motility that analyzes the mass motion of a semen sample
	[4]Primary abnormalities	$\%PRIM_B$	%	Percentage of spermatozoa with an abnormality that originated during spermatogenesis
	[5]Secondary abnormalities	$\%SEC_B$	%	Percentage of spermatozoa with an abnormality that originated during epididymal transit time
	[6]Normal spermatozoa	$\%NORM_B$	%	Percentage of spermatozoa that are morphologically normal
Bull stud data	Scrotal circumference	SC_S	cm	Same definition as SC_B
	Initial motility	$\%MOT_S$	%	The percentage of sperm moving linearly in a semen ejaculate before cryopreservation
	Motility score	$MOTSC_S$	Score	Same definition as $MOTSC_B$
	Primary abnormalities	$\%PRIM_S$	%	Percentage of spermatozoa with an abnormality that originated during spermatogenesis measured after cryopreservation
	Secondary abnormalities	$\%SEC_S$	%	Percentage of spermatozoa with an abnormality that originated during epididymal transit time measured after cryopreservation
	Normal spermatozoa	$\%NORM_S$	%	Percentage of spermatozoa that are morphologically normal measured after cryopreservation

[1]Same information for scrotal circumference in the combined dataset, except the abbreviation is SC_C.
[2]Same information for progressive motility in the combined dataset, except the abbreviation is $\%MOT_C$.
[3]Same information for motility score in the combined dataset, except the abbreviation is $MOTSC_C$.
[4]Same information for primary abnormalities in the combined dataset, except the abbreviation is $\%PRIM_C$.
[5]Same information for secondary abnormalities in the combined dataset, except the abbreviation is $\%SEC_C$.
[6]Same information for normal spermatozoa in the combined dataset, except the abbreviation is $\%NORM_C$.

Table 2.2 Number of breeding soundness examination (BSE) and stud records from the operations including year(s) of examination records, the total number of records, number of bulls, and the number of records for each trait: scrotal circumference (SC), percentage of progressive motility (%MOT), gross motility score (MOTSC), percentage of primary abnormalities (%PRIM), percentage of secondary abnormalities (%SEC), and percentage of normal spermatozoa (%NORM).

Dataset	Operation	Year(s)	Total Records	Bulls	SC	%MOT	MOTSC	%PRIM	%SEC	%NORM
BSE	1	2006-2019	2,756	2,237	2,703	0	2,586	0	0	2,767
	2	2022	217	217	217	213	213	213	213	214
	3	2017-2018	53	44	52	51	51	28	28	51
	4	2015-2019	950	805	944	603	603	579	579	873
	5	2016-2019	330	184	234	328	328	319	319	323
	6	2017-2019	144	117	142	139	139	93	93	138
	7	2015-2019	782	774	782	771	772	770	707	771
	8	2017, 2020, 2021	29	29	29	27	27	15	14	28
	9	2017-2019	342	327	343	341	341	320	320	339
	10	2018-2019	58	57	58	48	48	0	0	46
	11	2017-2019	216	205	219	215	215	204	204	214
Stud	1	2004-2022	58,586	1,545	56,069	58,582	58,582	27,228	27,223	27,223
	2	2008-2022	3,364	315	0	3,361	3,361	1,496	1,493	1,493

Table 2.3 Regression coefficient (β) and p-value calculated with the likelihood ratio test for each covariate that was not confounded with contemporary group. Age at collection in days (AgeD) was not tested in stud collection analysis because the average age was 1180 days or 3.25 years old, but age group was tested as a fixed effect.

Response Trait	AgeD β	AgeD β p-value	Adj. YW β	Adj. YW β p-value	DaysSince β	DaysSince β p-value
SC_B	0.0059	<0.0001	0.0003	1	--	--
SC_S	--	--	0.0007	<0.0001	0.0000	1
$\%MOT_B$	0.0149	1	-0.0002	1	--	--
$\%MOT_S$	--	--	-0.0009	<0.0001	0.0020	<0.0001
$\%PRIM_B$	-0.0060	1	-0.0007	1	--	--
$\%PRIM_S$	--	--	0.0001	<0.0001	-0.0006	1
$\%SEC_B$	-0.0145	<0.0001	-0.0001	1	--	--
$\%SEC_S$	--	--	-0.0001	<0.0001	-0.0002	1
$\%NORM_B$	0.0021	1	0.0008	1	--	--
$\%NORM_S$	--	--	-0.0001	<0.0001	0.0008	1

-- Effect was not tested
Adj. YW: Adjusted yearling weight
DaysSince: Days since previous collection
SC: Scrotal circumference
%MOT: Percentage of progressive motility
%PRIM: Percentage of primary abnormalities
%SEC: Percentage of secondary abnormalities
%NORM: Percentage of normal spermatozoa
B: Breeding soundness examination dataset
S: Stud dataset

Table 2.4 The effect(s) utilized in the final model for scrotal circumference (SC), percentage of progressive motility (%MOT), gross motility score (MOTSC), percentage of primary abnormalities (%PRIM), percentage of secondary abnormalities (%SEC), and percentage of normal spermatozoa (%NORM) in the breeding soundness examination (B), stud (S), and combined (C) datasets.

Trait	Contemporary Group	Age in Days	Age Group
SC_B	X	X	
$\%MOT_B$	X		
$MOTSC_B$	X		
$\%PRIM_B$	X		
$\%SEC_B$	X	X	
$\%NORM_B$	X		
SC_S	X		X
$\%MOT_S$	X		X
$MOTSC_S$	X		X
$\%PRIM_S$	X		X
$\%SEC_S$	X		X
$\%NORM_S$	X		X
SC_C	X		X
$\%MOT_C$	X		X
$MOTSC_C$	X		X
$\%PRIM_C$	X		X
$\%SEC_C$	X		X
$\%NORM_C$	X		X

Table 2.5 Number of records (N), mean, standard deviation (SD), median, minimum, and maximum for scrotal circumference (SC), percentage of progressive motility (%MOT), gross motility score (MOTSC), percentage of primary abnormalities (%PRIM), percentage of secondary abnormalities (%SEC), and percentage of normal spermatozoa (%NORM) in the breeding soundness examination (B), stud (S), and combined (C) datasets.

Trait	N	Mean	Median	SD	Minimum	Maximum
SC_B	5,723	36.40	36.50	3.31	15.00	49.00
SC_S	56,069	40.70	40.50	3.59	21.00	60.00
SC_C	61,762	40.40	40.20	3.75	15.00	60.00
$\%MOT_B$	2,736	55.74	50.00	20.35	0.00	99.00
$\%MOT_S$	61,943	49.09	50.00	15.94	0.00	100.00
$\%MOT_C$	64,679	49.77	50.00	16.08	0.00	100.00
$MOTSC_B$	5,323	54.66	55.00	17.49	15.00	75.00
$MOTSC_S$	61,943	49.43	55.00	15.34	15.00	75.00
$MOTSC_C$	67,266	50.17	55.00	15.49	15.00	75.00
$\%PRIM_B$	2,541	11.22	10.00	14.60	0.00	100.00
$\%PRIM_S$	28,724	11.86	11.00	7.17	0.00	87.00
$\%PRIM_C$	31,265	11.81	11.00	8.01	0.00	100.00
$\%SEC_B$	2,477	12.19	10.00	8.31	0.00	90.00
$\%SEC_S$	28,716	11.73	11.00	6.74	0.00	78.00
$\%SEC_C$	31,193	11.76	11.00	6.87	0.00	90.00
$\%NORM_B$	5,764	75.75	80.00	22.74	0.00	100.00
$\%NORM_S$	28,716	76.41	77.00	8.66	2.00	100.00
$\%NORM_C$	34,480	76.30	78.00	12.12	0.00	100.00

Table 2.6 Average variance component estimates for direct additive genetic (σ_a^2), permanent environmental (σ_{pe}^2), and environmental (σ_e^2) variances, heritabilities (h^2) with standard error (SE) and repeatabilities (r^2) calculated from bivariate analyses for scrotal circumference (SC), percentage of progressive motility (%MOT), gross motility score (MOTSC), percentage of primary abnormalities (%PRIM), percentage of secondary abnormalities (%SEC), and percentage of normal spermatozoa (%NORM) in the breeding soundness examination (B), stud (S), and combined (C) datasets.

Trait	σ_a^2	σ_{pe}^2	σ_e^2	$h^2 + SE$	r^2
SC_B	1.64	1.41	1.46	0.36 ± 0.03	0.68
SC_S	7.09	4.89	0.95	0.55 ± 0.04	0.93
SC_C	3.15	3.63	1.51	0.40 ± 0.05	0.82
$\%MOT_B$	16.13	36.75	116.19	0.10 ± 0.03	0.31
$\%MOT_S$	33.00	76.94	152.61	0.12 ± 0.02	0.42
$\%MOT_C$	23.82	86.61	111.08	0.10 ± 0.01	0.50
$MOTSC_B$	9.36	47.56	134.62	0.05 ± 0.02	0.30
$MOTSC_S$	34.11	74.27	205.43	0.11 ± 0.07	0.35
$MOTSC_C$	34.38	74.43	198.52	0.11 ± 0.07	0.35
$\%PRIM_B$	13.02	116.50	54.36	0.09 ± 0.03	0.70
$\%PRIM_S$	1.45	19.53	18.81	0.04 ± 0.02	0.53
$\%PRIM_C$	7.55	103.44	23.64	0.06 ± 0.01	0.82
$\%SEC_B$	4.89	19.63	31.15	0.09 ± 0.03	0.44
$\%SEC_S$	2.49	7.36	24.50	0.08 ± 0.02	0.29
$\%SEC_C$	3.18	21.82	22.36	0.07 ± 0.01	0.53
$\%NORM_B$	18.63	56.04	261.43	0.06 ± 0.01	0.22
$\%NORM_S$	2.99	28.84	36.30	0.05 ± 0.02	0.47
$\%NORM_C$	15.45	172.30	44.11	0.11 ± 0.05	0.81

Table 2.7 Genetic correlations (r_G) and standard errors (SE) within each bull reproductive trait using the stud and breeding soundness examination datasets.

Trait	$r_G \pm SE$
Scrotal Circumference	0.49 ± 0.08
Percentage of Progressive Motility	dnc
Motility Score	dnc
Percentage of Primary Abnormalities	-0.21 ± 0.59
Percentage of Secondary Abnormalities	0.61 ± 0.28
Percentage of Normal Spermatozoa	0.32 ± 0.59

dnc: Model did not converge

Table 2.8 Genetic correlations above the diagonal and phenotypic correlations below the diagonal between Angus bull reproductive traits from a breeding soundness examination using a bivariate single-step genomic best linear unbiased prediction.

	SC_B	$\%MOT_B$	$MOTSC_B$	$\%PRIM_B$	$\%SEC_B$	$\%NORM_B$
SC_B		-0.16 ± 0.13	-0.02 ± 0.14	0.00 ± 0.15	-0.03 ± 0.16	0.21± 0.12
$\%MOT_B$	-0.21*		--	-0.73 ± 0.33	dnc	dnc
$MOTSC_B$	0.00	0.94*		-0.98 ± 0.50	dnc	dnc
$\%PRIM_B$	0.00	-0.45*	-0.47*		0.25 ± 0.29	dnc
$\%SEC_B$	-0.03	-0.40*	-0.40*	0.01		dnc
$\%NORM_B$	0.18*	0.56*	0.72*	-0.85*	-0.73*	

* Correlation is significantly different from zero at P <0.0001
-- Correlation was not estimated
dnc: Model did not converge
SC: Scrotal circumference
%MOT: Percentage of progressive motility
MOTSC: Motility score
%PRIM: Percentage of primary abnormalities
%SEC: Percentage of secondary abnormalities
%NORM: Percentage of normal spermatozoa
B: Breeding soundness examination dataset

Table 2.9 Genetic correlations above the diagonal and phenotypic correlations below the diagonal between Angus bull reproductive traits from stud collections using a bivariate single-step genomic best linear unbiased prediction.

	SC_S	$\%MOT_S$	$MOTSC_S$	$\%PRIM_S$	$\%SEC_S$	$\%NORM_S$
SC_S		-0.66 ± 0.08	-0.45 ± 1.14	0.35 ± 0.38	0.15 ± 0.13	-0.38 ± 0.28
$\%MOT_S$	0.05*		--	-0.58 ± 0.35	-0.53 ± 0.13	0.78 ± 0.15
$MOTSC_S$	0.05*	0.17*		-0.53 ± 1.40	-0.69 ± 1.44	0.73 ± 1.10
$\%PRIM_S$	0.00	-0.11*	0.23*		-0.42 ± 0.06	-0.21 ± 8.30
$\%SEC_S$	-0.04*	-0.03*	0.94*	-0.23*		-0.83 ± 0.02
$\%NORM_S$	0.02*	0.12*	0.22*	-0.65*	-0.59*	

* Correlation is significantly different from zero at $P < 0.0001$

-- Correlation was not estimated

SC: Scrotal circumference

%MOT: Percentage of progressive motility

MOTSC: Motility score

%PRIM: Percentage of primary abnormalities

%SEC: Percentage of secondary abnormalities

%NORM: Percentage of normal spermatozoa

S: Stud dataset

Table 2.10 Genetic correlations above the diagonal and phenotypic correlations below the diagonal between Angus bull reproductive traits from a combination of BSE and stud collection records using a bivariate single-step genomic best linear unbiased prediction.

	SC_C	$\%MOT_C$	$MOTSC_C$	$\%PRIM_C$	$\%SEC_C$	$\%NORM_C$
SC_C		-0.46 ± 0.07	-0.44 ± 1.04	0.18 ± 0.14	0.00 ± 0.00	0.10 ± 0.11
$\%MOT_C$	0.02*		--	-0.79 ± 0.09	-0.74 ± 0.08	0.90 ± 0.05
$MOTSC_C$	0.15*	-0.02*		-0.54 ± 1.25	-0.85 ± 2.47	0.73 ± 0.84
$\%PRIM_C$	0.00	-0.22*	0.43*		0.37 ± 0.25	-0.91 ± 0.22
$\%SEC_C$	-0.04*	-0.10*	0.43*	-0.20*		-0.94 ± 0.06
$\%NORM_C$	0.06*	0.26*	0.45*	-0.70*	-0.60*	

* Correlation is significantly different from zero at $P < 0.0001$
-- Correlation was not estimated
SC: Scrotal circumference
%MOT: Percentage of progressive motility
MOTSC: Motility score
%PRIM: Percentage of primary abnormalities
%SEC: Percentage of secondary abnormalities
%NORM: Percentage of normal spermatozoa
C: Combined dataset

Chapter 3 - Genome-wide association study of fertility traits in Angus bulls

Abstract

A bull's reproductive efficiency is one of the most important factors contributing to a cow-calf operation success by directly influencing herd productivity and overall performance. The objective of this study was to conduct a single-step genome-wise association study (ssGWAS) to identified quantitative trait loci (QTL) regions associated with beef bull fertility traits including scrotal circumference (SC), percentage of progressive motility (%MOT), gross motility score (MOTSC), percentage of primary abnormalities (%PRIM), percentage of secondary abnormalities (%SEC), and percentage of normal spermatozoa (%NORM). Phenotypic data were obtained from breeding soundness examinations (BSEs) of 11 registered Angus operations (n = 5,877 observations from 4,996 bulls) and two bull stud collection facilities (n=64,869 observations from 1,862 bulls). The American Angus Association provided a three-generation pedigree for 6,845 bulls, and single nucleotide polymorphism (SNP) data for 9,589 animals, 4,255 of which had phenotypic records. A significance threshold of -log10 *P*-value greater than 4.0 was used to identify significant SNPs for each trait. The study identified eight, two, one, zero, twenty-six, and three QTL regions associated with SC, %MOT, MOTSC, %PRIM, %SEC, and %NORM, respectively. Several QTL regions were located near previously reported QTL linked to bull reproductive traits and genes with biological functions related to reproduction. These results indicate there are regions of the genome that do impact bull fertility, and incorporating genomic information into genetic evaluations for bull fertility could lead to genetic improvement.

Introduction

In today's beef industry, fertility assessments of beef bulls primarily rely on phenotypic measurements. These metrics are obtained either through semen collection for artificial insemination (AI) or during routine breeding soundness examinations (BSEs). Bulls selected for AI tend to have more frequent fertility records over the course of a year, providing a more comprehensive representation of their average fertility status. However, the AI data is often proprietary, limiting its accessibility for beef breed associations to develop genetic evaluations, and only includes a small number of bulls. In contrast, BSEs are recommended to be performed on every natural service sire before each breeding season, resulting in the information on a larger population of breeding bulls. Breeding soundness exam data tends to be less proprietary and could be more accessible to breed association, but the measurements are far more subjective and if the bull passes his initial BSE, he might only have one fertility assessment performed per year on a smaller number of traits.

Despite advancements in selection tools for other economically relevant traits, beef cattle producers currently lack a reliable selection tool to select for more fertile bulls. Incorporating genomic information into a genetic evaluation for bull fertility should improve the accuracy of the prediction. However, the existing single nucleotide polymorphisms (SNP) chips used for genomic testing are not enriched in regions where important variants for male fertility could exist, because these regions remain largely underexplored and uncharacterized in beef bulls (Sweett et al., 2020; Butler et al., 2022). Furthermore, genomic data would also allow breeders to more confidently identify superior animals for fertility traits at an earlier age, thereby reducing the generation interval and accelerating the rate of genetic improvement. The objective of this study was to perform a single-step genome-wise association study (ssGWAS) for bull fertility

traits using data from AI collections and BSEs jointly to identify genomic regions important in prediction of genetic merit for scrotal circumference and semen quality traits.

Materials and Methods

Animal Care and Use Committee approval was not obtained for this study because the data were pre-existing records provided from Angus breeders and certified bull semen collection companies.

Phenotypic Data

Phenotypic observations from BSEs were obtained from 11 registered Angus operations (n = 5,877 observations from 4,996 bulls) and two bull stud collection facilities (n=64,869 observations from 1,862 bulls). The bulls were evaluated for their fertility during either routine BSE or semen collection for artificial insemination. There were 14 bulls with phenotypic observations from both a BSE and semen collection at a bull stud. The common fertility traits that were measured in both datasets included scrotal circumference (SC), percentage of progressive motility (%MOT), gross motility score (MOTSC), percentage of primary abnormalities (%PRIM), percentage of secondary abnormalities (%SEC), and percentage of normal spermatozoa (%NORM). The fertility records were combined in one dataset for analysis. Trait definitions, units, and abbreviations are reported in Table 3. 1, and additional data description is depicted in Appendix Figures A.7, A.8, A.9.

Data filtering procedures were thoroughly described in Chapter 2, and implemented using R software (R Core Team, 2013, Vienna, Austria). Briefly, records from bulls not registered with the American Angus Association were excluded from the analysis. Scrotal circumference measurements of zero or less were treated as missing observations. Percentage trait observations outside the range of 0 and 100 were also considered missing. Additionally, some of the BSE

records were reported as MOTSC measurements instead of %MOT. To utilize the reported MOTSC measurements, additional MOTSC measurements were generated from the %MOT phenotypes. The %MOT phenotypes were separated into the same four categories recognized by the Society for Theriogenology guidelines (Hopper, 2014): less than 30% motility was classified as “poor”, 30-49% motility was "fair", 50-69% motility was "good", and greater than or equal to 70% motility was considered "very good.” After the data were filtered, a total of 67,936 records were utilized for the ssGWAS. Table 3.2 summarizes the descriptive statistics for each trait.

R software (R Core Team, 2013, Vienna, Austria) was also used to delineate fixed effects for the statistical model, utilizing data provided from the phenotypic records and American Angus Association. A contemporary group effect was generated by concatenating the location (BSE operation or bull stud) and date of examination or collection record. The American Angus Association provided the date of birth for all bulls in the analysis, which was then used to create age groups. Bulls were assigned to an age group based on their age in months on the date of semen collection or BSE. The age categories were as follows: less than 12 months, 12 – 15 months, 15 – 18 months, 18 – 24 months, 24 – 36 months, and greater than 36 months of age. Other fixed effects available for testing included season of collection, adjusted yearling weight, and comprehensive climate index, but these effects did not improve model fit and were not included in the final analyses.

Genomic Data

A three-generation pedigree for 6,845 bulls was obtained from the American Angus Association. The pedigree information included 3,676 sires and 15,615 dams. Additionally, the American Angus Association provided SNP data for 9,589 animals (4,456 with phenotypes and 5,133 from the pedigree) and performed imputation to ensure all genotyped animals had 54,609

SNPs. There were 4,456 bulls with phenotypes in the combined dataset (3,215 with BSE data; 1,254 with stud data; 13 bulls had both BSE and stud data), and the remaining genotyped animals (n = 5,133) were animals in the pedigree. Quality control filtering removed 104 animals' genotypes because they had parent-progeny conflicts (27), relationship matrix blending conflicts (25), were possible duplicate samples (11), or their call rate was less than 0.90 (42). Furthermore, total of 15,008 individual loci were removed for a minor allele frequency of less than 0.05 (9,074), a SNP call rate of less than 0.90 (3,311), being monomorphic (874), or being on a sex chromosome (1,749). After quality control filtering, 38,601 SNP loci from 9,484 animals were available for further analysis.

Statistical Analysis

Model selection and variance component estimation procedures were comprehensively explained in Chapter 2. A Figure A.1. forward selection approach was used within the BLUF90+ software suite of programs (Misztal et al., 2014) to select effects to include in the final model. Model fit was evaluated using Akaike information criteria (AIC), where the most significant variable was added to the model, provided the AIC improved by three or more. The following statistical model was utilized for all analyses:

$$\boldsymbol{y_{ijkl} = ContemporaryGroup_i + AgeGroup_j + P_k + S_k + e_{ijkl}}$$

where y_{ijkl} is the phenotype for the kth animal in the ith contemporary group and the jth age group, P_k is the random permanent environmental effect for kth animal to account for repeated records, S_k is the random additive direct genetic effect for the kth animal, and e_{ijkl} is the residual.

A single-step genomic best linear unbiased prediction (ssGBLUP) model was utilized for the genetic evaluation of bull fertility traits, because it incorporates both pedigree and genotypic

data from American Angus Association to account for genetic relationships. The following mixed model equation was used:

$$\begin{bmatrix} \boldsymbol{X'X} & \boldsymbol{X'Z_a} & \boldsymbol{X'W_{pe}} \\ \boldsymbol{X'Z_a} & \boldsymbol{Z_a'Z_a + \lambda H^{-1}} & \boldsymbol{Z_a'W_{pe}} \\ \boldsymbol{X'W_{pe}} & \boldsymbol{Z_a'W_{pe}} & \boldsymbol{W_{pe}'W_{pe} + I\rho_{pe}} \end{bmatrix} \begin{bmatrix} \boldsymbol{\hat{b}} \\ \boldsymbol{\hat{a}} \\ \boldsymbol{\widehat{pe}} \end{bmatrix} = \begin{bmatrix} \boldsymbol{X'y} \\ \boldsymbol{Z'y} \\ \boldsymbol{W'y} \end{bmatrix}$$

where X is an incidence matrix relating phenotypes to contemporary group and age group, Z_a is an incidence matrix relating phenotypes to the random additive genetic effects, W_{pe} is an incidence matrix relating phenotypes to the random permanent environmental genetic effects, λ is the ratio of residual error and direct additive genetic variance, H^{-1} is the relationship matrix, I is an identity matrix, ρ is the ratio of residual error and additive permanent environmental variance, $\hat{b}$ is a vector for the solutions of contemporary group and age group, $\hat{a}$ is a vector of additive direct genetic effects, $\widehat{pe}$ is a vector of additive permanent environment effects, and y is a vector of phenotypes.

SNP Effect Estimation

A single-step genome-wide association study (ssGWAS) was conducted using the postGSf90 program from the BLUPF90 suite of programs (Misztal et al., 2014). PostGSf90 generated SNP effects, prediction error variance, and p-values by back solving the estimated breeding values from BLUPF90+. Animal effects were decomposed into effects for genotyped (a_g) and ungenotyped (a_n) animals. The SNP effects are a function of the animal effects of genotyped animals. The following methodology from Wang et al. (2012) was utilized to derive SNP effects:

$$\boldsymbol{a_g = Zu}$$

where a_g is a vector of estimated breeding values for genotyped individuals, Z is a matrix relating genotypes of each locus, and u is a vector of SNP marker effects. The variance for the genotyped animal effects can be written as follows:

$$\boldsymbol{var}(\boldsymbol{a_g}) = \boldsymbol{var}(\boldsymbol{Zu}) = \boldsymbol{G\sigma_a^2} = \boldsymbol{ZIZ'\lambda}$$

where G is the genomic relationship matrix, I is an identity matrix, λ is the ratio of the SNP marker effect variance and the breeding value variance (Masuda, 2019). The SNP effects were estimated with the following equation:

$$\hat{\boldsymbol{u}} = \boldsymbol{IZ'}(\boldsymbol{ZIZ'})^{(-1)}\hat{\boldsymbol{a}}_{\boldsymbol{g}}$$

where u is a vector of breeding values for genotyped individuals, I is an identity matrix, Z is a matrix relating individuals to phenotypes, and a_g is a vector of breeding values for the genotyped individuals (Masuda, 2019). An unweighted ssGBLUP was performed because equal allele substitution effect variance was assumed for each SNP.

Single nucleotide polymorphism effects were obtained from each univariate analysis for SC, %MOT, MOTSC, %PRIM, %SEC, and %NORM. Quantile-quantile (QQ) plots were created for each trait to compare the expected $-\log_{10}$ p-values to the observed $-\log_{10}$ p-values to determine if the SNP effect estimates were normally distributed. To account for violations of the normal distribution assumption, a genomic control correction was applied to the estimated SNP effect p-values for each trait (Devlin and Roeder, 1999; Devlin et al., 2004). This methodology uses Bayesian outlier methods to determine which markers indicate significant linkage disequilibrium and control for population heterogeneity, hidden relationships, and the risk of false positives at the expense of predictive power (Devlin and Roeder, 1999). Genomic control circumvents the need for multiple testing correction with false discovery rate or a Bonferroni correction (Devlin and Roeder, 1999). Thus, the significance threshold was set at $-\log_{10} > 4$.

Finally, Manhattan plots were generated for all six reproductive traits after obtaining the SNP effects and their p-values.

Quantitative Trait Loci Analysis

Quantitative trait loci (QTL) regions were defined as extending 250 kilobases upstream and downstream of the significant SNPs to account for moderate linkage disequilibrium. Due to a data disclosure agreement, we could not provide the exact position of the significant SNPs identified in this study. However, we were able to report chromosome location of each significant SNP and indicate if multiple QTL regions were identified on a single chromosome. The GALLO package in R (R Core Team, 2013), cattle QTL database (Hu et al., 2019), and the NCBI bovine GFF annotation file for the USDA ARS-UCD 1.2 genome assembly (Rosen et al., 2020; accession GCF_002263795.1) were used to identify previously reported QTL and putative candidate genes which overlap the QTL regions for significant SNPs. Biological function of putative candidate genes were identified with the UniProt Consortium database (2019).

Results and Discussion

Summary Statistics

Descriptive statistics of the dataset are reported in Table 3.2. The study included scrotal circumference measurements from 6,485 bulls, with 65% of them having genotypes available from the American Angus Association. Among the genotyped bulls, only 23 had SC measurements below the minimum threshold set by the Society for Theriogenology guidelines for passing a BSE. These guidelines ensure that bulls have sufficient daily sperm production to meet the reproductive demands of approximately 25 cycling females (Koziol and Armstrong, 2018). The small percentage of bulls not meeting the minimum threshold may suggest that producers have actively selected for bulls with SC larger than 30 cm. The number of genotyped

bulls with motility and morphology measurements ranged from 3,035 to 4,233 bulls. A bull's semen must have at least 30% %MOT or a fair MOTSC to be considered satisfactory during a BSE (Koziol and Armstrong, 2018), and semen collected for AI must have at least 50% %MOT to be cryopreserved. Twenty-one percent of the genotyped bulls in this study exhibited suboptimal semen motility by producing an ejaculate with less than 30% progressive motility, and 18% of the bulls were classified as having poor motility based on the MOTSC system (Hopper, 2014). When comparing the morphology, there were more bulls with phenotypes and genotypes for %NORM than %PRIM or %SEC. This may be because many of the morphology measurements for the BSE data only included %NORM and did not include counts for individual abnormalities. Of the genotyped bulls, 19% had a measurement for %NORM below the minimum threshold of 70%, which is required by both a BSE and stud collection criteria. The summary statistics in the current study may indicate that while SC has been a focus for selection, other reproductive traits such as motility and morphology may require further attention to create tools and methodologies that would allow producers to select for improved semen quality in their breeding programs.

Quantile-quantile plots

Two QQ plots were generated for each trait to evaluate the relationship between the observed –log10 p-values, estimated with BLUPF90+ (Misztal et al., 2014), and the expected –log10 p-values from the null hypothesis. The null hypothesis for a GWAS assumes that there are no causal SNP, and therefore, all observed –log10 p-values should be plotted along or near the diagonal line representing the normal distribution between the x and y axes (Wilk and Gnanadesikan, 1968). Deviations from the null hypothesis at the higher p-value range indicate the presence of causal SNPs (Wilk and Gnanadesikan, 1968). The first plot for each trait shows

the original observed –log10 p-values values reported in the POSTF90 output, while the second presents the observed –log10 p-values after applying genomic control. The original QQ plots suggests that the SNP effects as being overestimated for %SEC, and underestimated MOTSC, %PRIM, and %NORM compared to the expected null hypothesis. The QQ plots for SC, %MOT, and MOTSC are displayed in Figure 3.1, and those for %PRIM, %SEC, and %NORM are shown in Figure 3.2.

Genome-wide Association Analysis

Two Manhattan plots were included for each trait. The Manhattan plots with original -$\log_{10}$ p-values for SC, %MOT, and MOTSC are shown in Figure 3.3 and Figure 3.4 for %PRIM, %SEC, and %NORM. Figures 3.5 and 3.6 present the Manhattan plots with the -$\log_{10}$ p-values generated from the genomic control correction. Single nucleotide polymorphisms with a -$\log_{10}$ p-value > 4 after genomic control was applied were identified for each trait. Significant SNPs were identified for SC, %MOT, MOTSC, %SEC, and %NORM. No significant SNPs were found for %PRIM.

Due to restrictions on the use of the proprietary genomic data being utilized in this study, specific SNP names and base pair positions are not reported. However, the chromosome each significant SNP was located on, the percentage of variance explained by the SNP, and the original and genomic control -$\log_{10}$ p-values for each SNP are reported in Table 3.3. All previously reported QTL that overlap a region within 250 kilobases upstream or downstream of the significant SNPs for each trait are summarized in Table 3.4. All genes identified overlapped the QTL regions outlined in Table A.1 through Table A.6. Those genes discussed as potential candidate genes for bull fertility are reported in Table 3.5.

Previously Reported QTL Regions

Two QTL regions were found on chromosome 5 (Table 3.3). The first QTL region was not within close proximity to other QTL regions previously associated with fertility traits. However, the second QTL region identified on chromosome 5 had previously been associated with inhibin level (Fortes et al., 2013), maturity rate (Crispim et al., 2015), heifer pregnancy (Oliveira et al., 2012), calving ease, and stillbirth (Müller et al., 2017). Inhibin is a glycoprotein produced in the testes, and its target cell is the anterior pituitary, where it inhibits follicle stimulating hormone (FSH) secretion (Senger, 2012). Inhibin production is highest right before or at puberty, making it a usable biomarker for early sexual development (Phillips, 2005). After reaching puberty, the negative feedback provided by inhibin reduces, allowing increased secretions of luteinizing hormone (LH) and FSH to stimulate spermatogenesis (Evans et al., 1995) and testicular growth (McCarthy et al., 1979). Bulls with a larger SC at an earlier age generally have an increased maturity rate because reach puberty earlier than bulls with less testicular mass, and they also produce ejaculates with satisfactory semen quality earlier (Koziol and Armstrong, 2018).

Age at puberty was previously associated with a QTL region that overlapped the region identified on chromosome 18 for SC (Hawken et al., 2012). Several studies have demonstrated favorable relationships between sire SC and their daughters' age at puberty (Brinks et al., 1978), as well as negative genetic correlations have also been reported between yearling bull SC and their female counterparts age at puberty (Vargas et al., 2017; Martínez-Velázquez et al., 2020). Heifer age at puberty can significantly influence her pregnancy success during her first and subsequent breeding seasons, and may impact her lifetime production (Day and Nogueira, 2013). Ideally heifers would reach puberty at 12 to 13 months of age, allowing sufficient time for a couple estrous cycles before breeding at 15 months of age (Perry and Cushman, 2013). However,

premature puberty in heifer calves can be problematic for beef producers. Precocious heifers exposed to a mature bull can become pregnant, usually resulting in calving late in or after the calving season, and having suboptimal body size with more nutritional requirements than a two-year old female calving for the first time (Day and Nogueira, 2013). While the relationship between SC and heifer age at puberty is generally favorable, beef producers should consider how a bull's SC may impact his replacement heifers.

Porto-Neto et al. (2015) previously reported a QTL region associated with fixed-time AI pregnancy rate that overlapped with the 12:25 QTL region for %SEC. Secondary abnormalities are suspected of developing in the epididymis, and include abnormalities impacting the tail and motility (Barth and Oko, 1989; Hopper, 2014). The epididymis is responsible for sperm cell maturation, transportation, storage, and exit from the bull reproductive tract (Caballero et al., 2011; Senger, 2012). Sperm cells undergo maturation to acquire progressive motility, and their ability to bind to the oocyte's zona pellucida and plasma membrane (Caballero et al., 2011). All of which are essential for a sperm cell to navigate the female reproductive tract to fertilize the oocyte. If a sperm cell has a secondary abnormality, it is very unlikely that it could reach the oocyte and result in a sustained pregnancy.

Four different QTL regions were identified on chromosome 23 (Table 3.3), suggesting that the genes on this chromosome may play a significant role in bull fertility. The QTL region identified for MOTSC was specific to that trait. However, the 23:8 QTL region for SC overlapped the 23:9 QTL region for %MOT. Additionally, the 23:10 QTL region for %MOT overlapped with the 23:13 and 23:34 QTL regions for %NORM and %SEC, respectively. The 23:14 and 23:35 QTL regions for both %NORM and %SEC, respectively, were also found to overlap. While many previously reported QTL regions for female fertility traits overlapped the

QTL regions identified in this study (Table 3.4), one notable QTL region was associated with sire conception rate (SCR), a predicted transmitting abilities metric for a dairy bull fertility evaluation (Kuhn et al., 2008; Kuhn and Hutchison, 2008). Sire conception rate predicts the likelihood that a unit of conventional semen from a particular bull will result in a 70-day pregnancy, compared to the average of other bulls in the analysis (Kuhn and Hutchison, 2008). Bulls with higher SCR values are expected to achieve more successful pregnancies than bulls with lower SCR values. For a successful pregnancy to occur, sperm cells must have proper morphology and viable progressive motility to navigate the female reproduction tract and locate the ovulated oocyte. Therefore, it should not be surprising that the QTL for SCR overlapped a QTL region associated with %MOT, %SEC, and %NORM in the current study.

Biological Functions

The 4:20 QTL region for %SEC was near the *ADCY1* (Adenylate cyclase type 1) and the *CAMK2B* (Calcium/calmodulin-dependent protein kinase type II subunit beta) genes. According to the UnitProt Consortium (2019), these genes share similar molecular functions, including ATP binding, metal ion binding, and calmodulin binding. Calmodulin is an acidic protein that has been demonstrated to play a critical role in spermatozoa function by regulating calcium-dependent processes that influence sperm motility and fertility (Leclerc et al., 2020). These calmodulin-binding proteins are present in the epididymis (Leclerc and Goupil, 2000), which is also the suspected origin of secondary abnormalities (Barth and Oko, 1989). Previous research has shown that a calmodulin inhibitor can prevent mouse sperm cells from undergoing capacitation and the acrosome reaction, both of which are essential for fertilization (Zeng and Tulsiani, 2003). While the full significance of calmodulin for bull spermatozoa is not yet fully understood, a recent study has identified proteins with functions related to the regulation of

capacitation, sperm motility, sperm-egg recognition, and sperm binding to the zona pellucida (Leclerc et al., 2020).

Five glutathione S-transferase genes were found to overlap the QTL regions associated with both SC (23:8) and %MOT (23:9) on chromosome 23 (Table 3.5). Glutathione S-transferases play three crucial roles in male fertility: cellular detoxification, regulation of cell signaling pathways, and facilitation of fertilization (Llavanera et al., 2020b). These enzymes protect the sperm cells against oxidative stress, which is caused by an imbalance between the production of reactive oxygen species (ROS) and antioxidants (Llavanera et al., 2020b; Llavanera et al., 2020a). Sufficient ROS levels are necessary for sperm cells to undergo several essential processes like capacitation, acrosome reaction, and fusion with the oocyte. However, excessive ROS may be detrimental to sperm cells during spermatogenesis and epididymal transit time because high levels of ROS have been shown to cause increased lipid peroxidation, DNA damage, and reduced sperm motility (Hemachand and Shaha, 2003; Llavanera et al., 2020b). Spermatogenesis occurs in the seminiferous tubules of the testes, while sperm cell maturation and motility acquisition happen during epididymal transit time in the epididymis (Senger, 2012). Therefore, the association between SC and glutathione S-transferase genes is particularly relevant, as both the testes and epididymis are both encased within the scrotum.

Seventeen genes related to olfactory receptors were identified within QTL regions for %MOT, %SEC, and %NORM on chromosome 23. These genes included *OR108, OR10C1, OR11W1, OR12D2, OR12D3, OR1O8, OR1O9, OR2B2, OR2B2D, OR2B6, OR2H1, OR2H1D, OR2I1P, OR2W2, OR2W4, OR2W6,* and *OR5V1*. The relationship between olfactory receptor genes and sperm motility and morphology can be attributed to their roles in chemotaxis and chemokinesis (Flegel et al., 2016). Sperm motility is essential for movement and fertilization.

Chemotaxis influences the direction of motile sperm toward the oocyte in response to the stimulus of the chemical gradient (Eisenbach, 1999). While chemokinesis regulates the speed of sperm movement in response to the concentration of chemical stimulus, it is not impacted by the direction of the stimuli (Eisenbach, 1999). Together, these processes optimize both the speed and direction of sperm movement throughout the female reproductive tract (Flegel et al., 2016). Therefore, normal sperm morphology is crucial, as abnormal sperm may impair the effectiveness of the chemotactic and chemokinetic processes, resulting in decreased conception rates.

Additional genes were identified within the significant QTL regions identified for SC (Table A.1), %MOT (Table A.2), MOTSC (Table A.3), %SEC (Table A.4), and %NORM (Table A.5) in the current study. However, their direct roles in reproductive processes remain unclear. While some of these genes are known to be involved in general cellular processes such as DNA binding, protein ubiquitination, or metabolism, there is limited evidence directly linking them to male fertility traits like SC and semen quality. Their presence within QTL regions suggests potential involvement in reproductive traits, but further functional studies are needed to elucidate how these genes contribute to fertility outcomes.

Conclusion

No significant quantitative trait loci regions were identified for the %PRIM, but several QTL regions were detected SC, %MOT, MOTSC, %SEC, and %NORM across 18 chromosomes. Seventeen significant QTL regions in the current study were validated by previously reported QTL regions that were also associated with fertility traits. Interestingly, chromosome 23 harbored a significant QTL region for every trait studied, except for %PRIM. Additionally, genes within the QTL regions for bull reproductive traits were identified. While several putative candidate genes were discussed, many of the genes identified did not have a

direct association with the reproductive traits in this study. Thus, additional research is needed to better understand how these genes affect reproduction.

Table 3.1 Trait list, units, abbreviations (Abbrev), and definitions of beef bull reproductive traits.

Trait	Units	Abbrev	Definition
Scrotal Circumference	cm	SC	Measured by gently forcing the testes to the bottom of the scrotum and placing the tape level with the skin around the widest part of the scrotum
Percentage of Progressive Motility	%	%MOT	Percentage of sperm moving linearly in a semen ejaculate
Motility Score	Score	MOTSC	Subjective categorical measurement of motility that analyzes the mass motion of a semen sample
Percentage of Primary Abnormalities	%	%PRIM	Percentage of spermatozoa with an abnormality that originated during spermatogenesis
Percentage of Secondary Abnormalities	%	%SEC	Percentage of spermatozoa with an abnormality that originated during epididymal transit time
Percentage of Normal Spermatozoa	%	%NORM	Percentage of spermatozoa that are morphologically normal

Table 3.2 Summary statistics for each trait, including the total number of combined records for each trait, number of bulls with phenotypes, number of genotyped bulls with records, minimum trait threshold to pass a breeding soundness examination according to the Society for Theriogenology guidelines, number of genotypes bulls with observations below the trait threshold, mean, standard deviation (SD), minimum (Min), and maximum (Max) values.

Trait	Total Records	Bulls	Genotyped Bulls	Trait Threshold	Genotyped Bulls below threshold	Mean	SD	Min	Max
SC	65,236	6,485	4,207	≥ 30.0	23	40.40	3.75	15.00	60.00
%MOT	64,679	4,290	3,356	≥ 30.0	690	49.77	16.08	0.00	100.00
MOTSC	67,266	6,477	4,192	Fair	740	50.17	15.49	15.00	75.00
%PRIM	33,699	3,792	3,097	< 25.0	291	11.81	8.01	0.00	100.00
%SEC	33,607	3,728	3,035	< 30.0	205	11.76	6.87	0.00	90.00
%NORM	37,093	6,399	4,233	≥ 70.0	825	76.30	12.12	0.00	100.00

SC: Scrotal Circumference
%MOT: Percentage of Progressive Motility
MOTSC: Motility Score
%PRIM: Percentage of Primary Abnormalities
%SEC: Percentage of Secondary Abnormalities
%NORM: Percentage of Normal Spermatozoa

Table 3.3 Summary of significant genomic regions for beef bull reproductive traits including chromosome (CHR), quantitative trait loci (QTL) region, number of significant single nucleotide polymorphism (N of SNP) identified per QTL region, original -log$_{10}$ p-value, genomic control (GC) applied -log$_{10}$ p-value, and the percentage (%) of variance explained by the QTL region.

Trait	CHR	QTL Region	N of SNP	-log$_{10}$ p-value	GC -log$_{10}$ p-value	% variance explained
Scrotal Circumference	2	1	1	5.24	5.37	7.25e-06
	5	2	1	4.07	4.17	6.41e-06
	5	3	1	4.49	4.60	4.60e-06
	11	4	1	4.09	4.20	6.87e-06
	18	5	2	4.26	4.37	7.17e-06
	19	6	1	3.93	4.03	6.49e-06
	21	7	1	3.91	4.01	5.10e-06
	23	8	1	4.94	5.06	4.20e-06
Percentage of Progressive Motility	23	9	2	3.99	4.18	7.71e-06
	23	10	1	3.98	4.17	1.85e-06
Motility Score	23	11	1	3.49	4.22	2.46e-05
Percentage of Normal Spermatozoa	12	12	1	3.84	4.13	1.20e-06
	23	13	2	4.15	4.46	1.26e-06
	23	14	2	4.41	4.73	1.49e-06
Percentage of Secondary Abnormalities	1	15	1	12.99	4.39	1.66e-06
	1	16	1	12.53	4.23	1.08e-06
	1	17	1	15.62	5.28	1.71e-06
	2	18	1	12.33	4.16	8.73e-07
	4	19	1	14.07	4.75	1.82e-06
	4	20	1	14.20	4.80	1.64e-06
	4	21	3	15.87	5.36	3.49e-07
	7	22	1	13.06	4.41	3.95e-07
	7	23	2	13.25	4.48	7.01e-07
	12	24	1	12.37	4.18	1.64e-06
	12	25	1	16.54	5.58	1.36e-06
	12	26	1	13.97	4.72	1.66e-06
	13	27	1	11.91	4.02	8.01e-07
	16	28	1	13.99	4.73	1.79e-06
	17	29	1	12.46	4.21	1.41e-06
	17	30	1	13.21	4.46	1.65e-06
	19	31	1	15.37	5.19	1.57e-06
	19	32	1	13.85	4.68	1.41e-06
	22	33	1	12.35	4.17	1.16e-06
	23	34	2	14.46	4.88	1.46e-06
	23	35	2	15.85	5.35	1.65e-06
	24	36	1	12.34	4.17	1.46e-06
	25	37	1	12.19	4.12	1.28e-06
	27	38	1	11.93	4.03	1.76e-06
	29	39	1	12.43	4.20	5.55e-07

Table 3.4 Previously reported quantitative trait loci (QTL) regions associated with reproduction traits which overlap significant single nucleotide polymorphism (SNP) identified for beef bull reproduction traits in the current study.

Trait	Chr:Region	QTL Trait	Source
SC	5:2	Maturity rate	Crispim et al., 2015
		Inhibin level	Fortes et al., 2013
		Calving ease	Müller et al., 2017
		Stillbirth	Müller et al., 2017
		Heifer pregnancy	Oliveira Júnior et al., 2017
	11:4	Calving ease (maternal)	Cole et al., 2011
	18:5	Age at puberty	Hawken et al., 2012
		Birth index	Höglund et al., 2012
	19:6	Calving interval	Moore et al., 2016
		Interval to first estrus after calving	Hawken et al., 2012
%MOT	23:10	Interval to first estrus after calving	Tenghe et al., 2016
		Calving to conception interval	Müller et al., 2017
		Sire conception rate	Li et al., 2012
		Fertility treatments	Sahana et al., 2010
MOTSC	23:11	Interval from first to last insemination	Liu et al., 2017
%NORM	12:12	Twinning	Kim et al., 2009
	23:13	Calving to conception interval	Müller et al., 2017
		Sire conception rate	Li et al., 2012
		Fertility treatments	Sahana et al., 2010
		Interval to first estrus after calving	Tenghe et al., 2016
		Reproductive efficiency	Costa et al., 2015
		Age at first calving	Costa et al., 2015
	23:14	Conception rate	Parker Gaddis et al., 2016
		Daughter pregnancy rate	Cole et al., 2011
		Calving ease	Cole et al., 2011
		Stillbirth	Cole et al., 2011
%SEC	1:15	Stillbirth (maternal)	Frischknecht et al., 2017
	1:16	Birth index	Sahana et al., 2011
		Calving ease	Sahana et al., 2011
		Calf size	Sahana et al., 2011
	12:24	Twinning	Kim et al., 2009
		Calving ease (maternal)	Cole et al., 2011
		Stillbirth	Cole et al., 2011
	12:25	Pregnancy rate	Porto-Neto et al., 2015
	16:28	Conception rate	Parker Gaddis et al., 2016
		Interval to first estrus after calving	Liu et al., 2017
	23:34	Calving to conception interval	Müller et al., 2017
		Body weight (birth)	Saatchi et al., 2014
		Sire conception rate	Li et al., 2012
		Fertility treatments	Sahana et al., 2010
		Reproductive efficiency	Costa et al., 2015
		Age at first calving	Costa et al., 2015

23:5	Daughter pregnancy rate	Cole et al., 2011
25:37	Calving index	Sahana et al., 2011

Chr:Reg: Chromosome and Region
SC: Scrotal Circumference
%MOT: Percentage of Progressive Motility
MOTSC: Motility Score
%NORM: Percentage of Normal Spermatozoa
%SEC: Percentage of Secondary Abnormalities

Table 3.5 Putative candidate genes within close proximity of significant quantitative trait loci (QTL) regions on individual chromosome (Chr:Reg) associated with beef bull fertility traits.

Trait	Chr:Reg	Protein Name	Biological function	Gene Name(s)
Scrotal Circumference	23:8	Glutathione S-transferase	Xenobiotic metabolic process	*GSTA1, GSTA2, GSTA5*
Percentage of Progressive Motility	23:9	Glutathione S-transferase	Xenobiotic metabolic process	*GSTA1, GSTA2, GSTA3, GSTA4, GSTA5*
	23:10	Olfactory receptor	Sperm cell receptor	*OR10C1, OR11W1, OR1O8, OR1O9, OR2H1, OR2H1D, OR2I1P*
Percentage of Secondary Abnormalities	4:20	Adenylate cyclase type 1	Calmodulin binding	*ADCY1*
	4:20	Calcium/calmodulin-dependent protein kinase type II subunit beta	Calmodulin binding	*CAMK2B*
	23:34	Olfactory receptor	Sperm cell receptor	*OR108, OR10C1, OR11W1, OR12D2, OR12D3, OR2B2, OR2B2D, OR2B6, OR2H1, OR2H1D, OR2I1P, OR2W2, OR2W4, OR2W6,* and *OR5V1*
Percentage of Normal Spermatozoa	23:13	Olfactory receptor	Sperm cell receptor	*OR10C1, OR11W1, OR12D2, OR12D3, OR1O8, OR1O9, OR2B2, OR2B2D, OR2B6, OR2H1, OR2H1D, OR2I1P, OR2W2, OR2W4, OR2W6,* and *OR5V1*

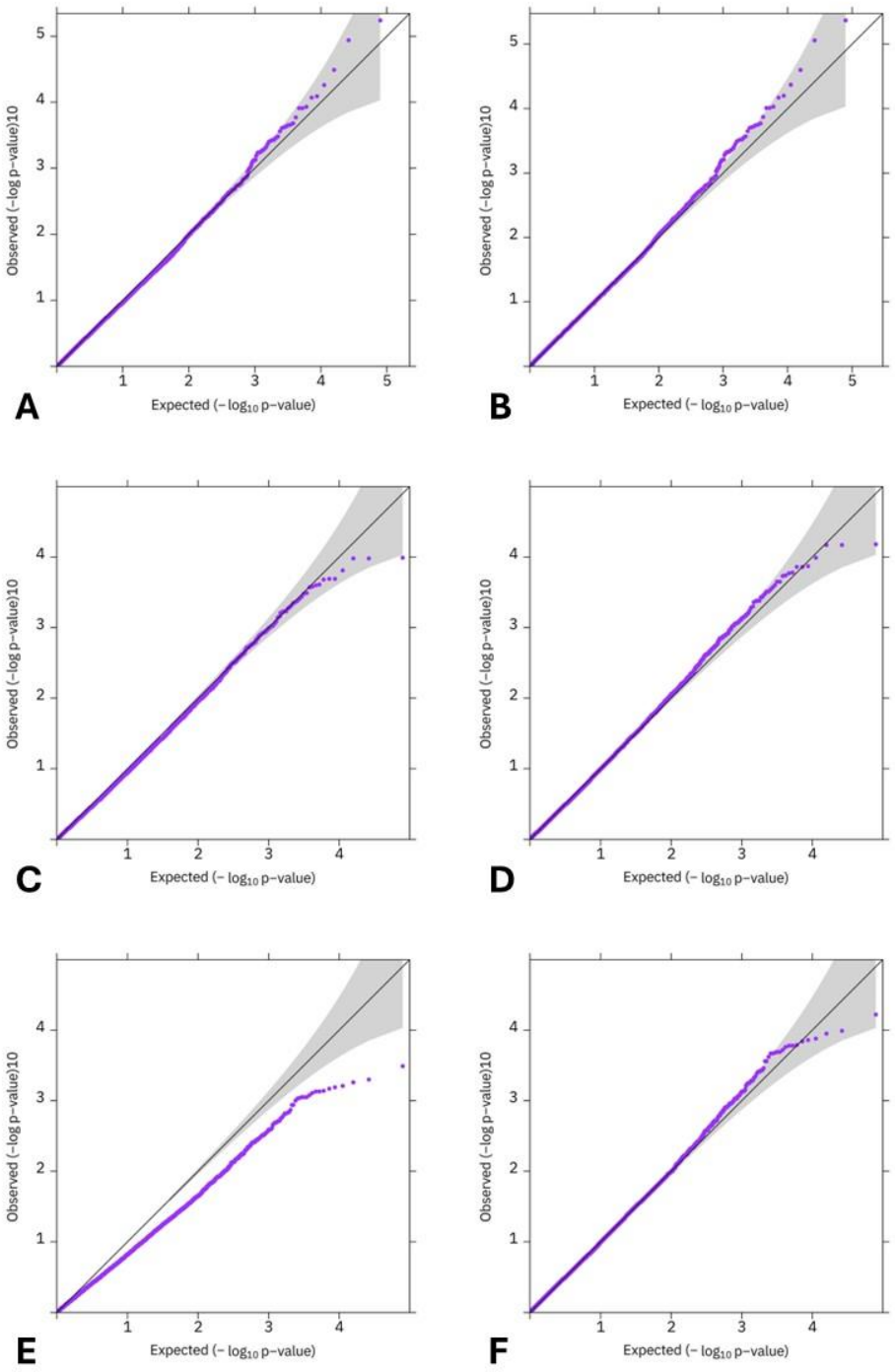

Figure 3.1 Quantile-quantile plots showing the distribution of -log10 p-values for scrotal circumference (SC), percent progressive motility (%MOT), and motility score (MOTSC) before and after genomic control. Panel A: Original -log10 p-values for SC, Panel B: -log10 p-values for SC after genomic control, Panel C: Original -log10 p-values for %MOT, Panel D: -log10 p-values for %MOT after genomic control, Panel E: Original -log10 p-values for MOTSC, Panel F: -log10 p-values for MOTSC after genomic control.

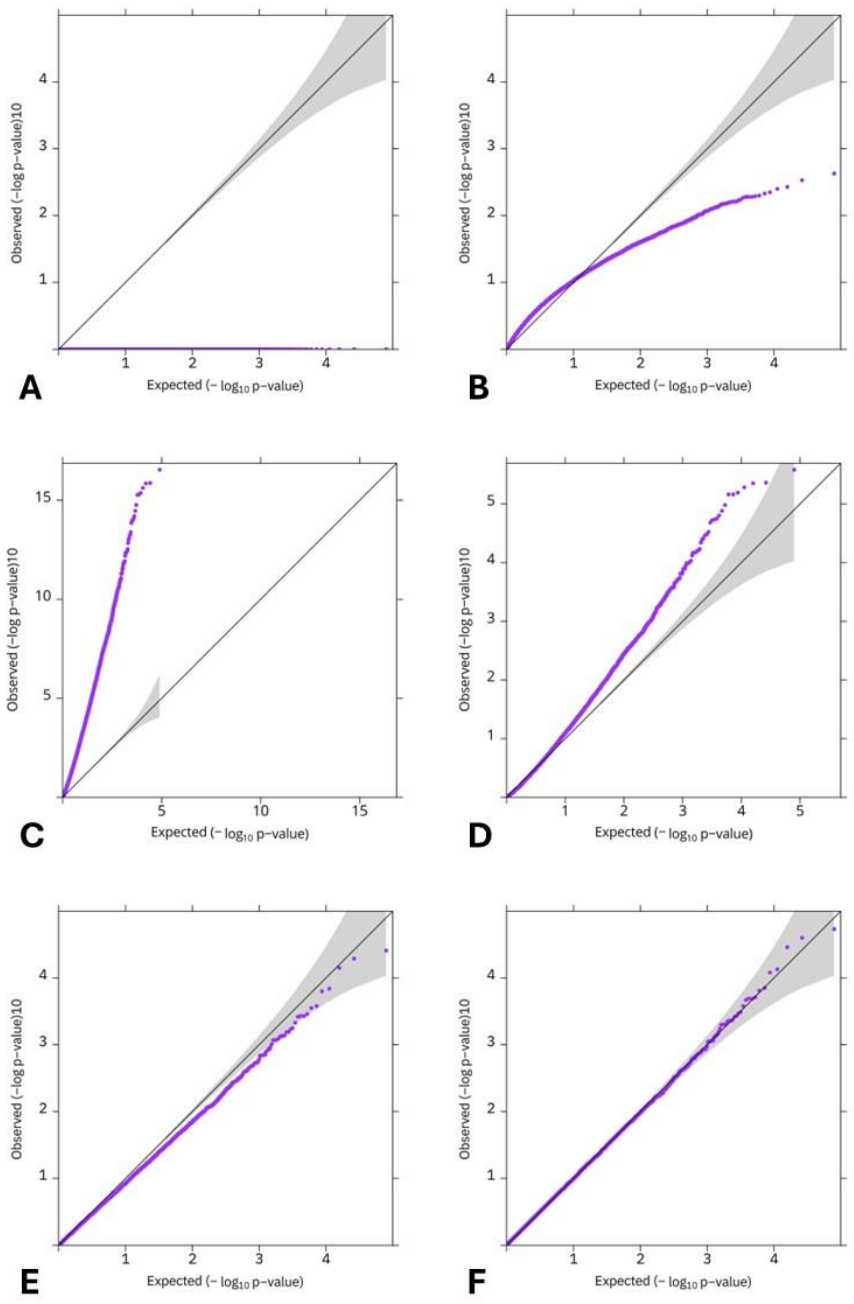

Figure 3.2 Quantile-quantile plots showing the distribution of -log10 p-values for percentage of primary abnormalities (%PRIM), percentage of secondary abnormalities (%SEC), and percentage of normal spermatozoa (%NORM) before and after genomic control. Panel A: Original -log10 p-values for %PRIM, Panel B: -log10 p-values for %PRIM after genomic control, Panel C: Original -log10 p-values for %SEC, Panel D: -log10 p-values for %SEC after genomic control, Panel E: Original -log10 p-values for %NORM, Panel F: -log10 p-values for %NORM after genomic control.

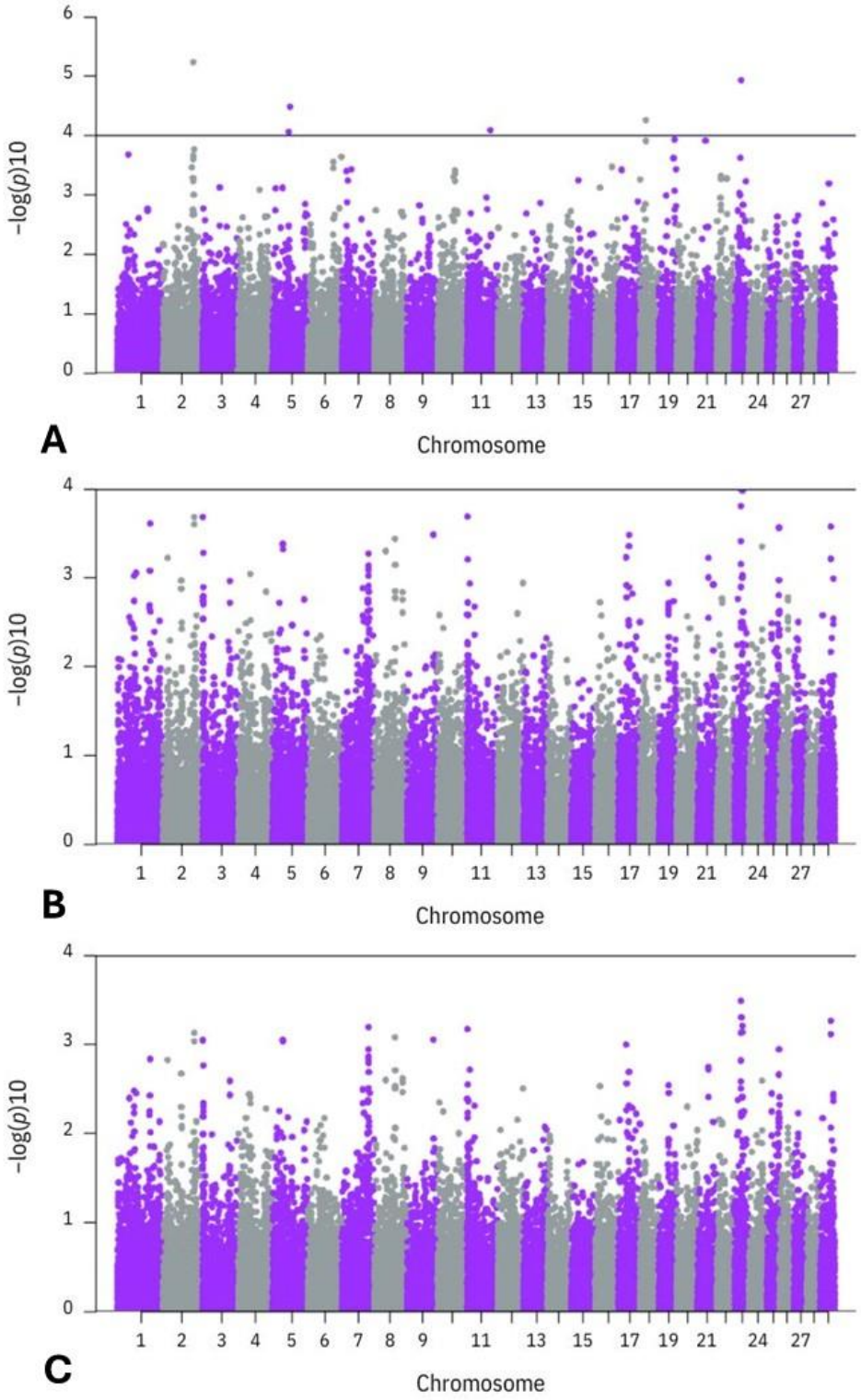

Figure 3.3 Manhattan plot showing the original results of a genome-wide association study with a significance threshold of 4.0 for scrotal circumference (panel A), percentage of progressive motility (panel B), and motility score (panel C).

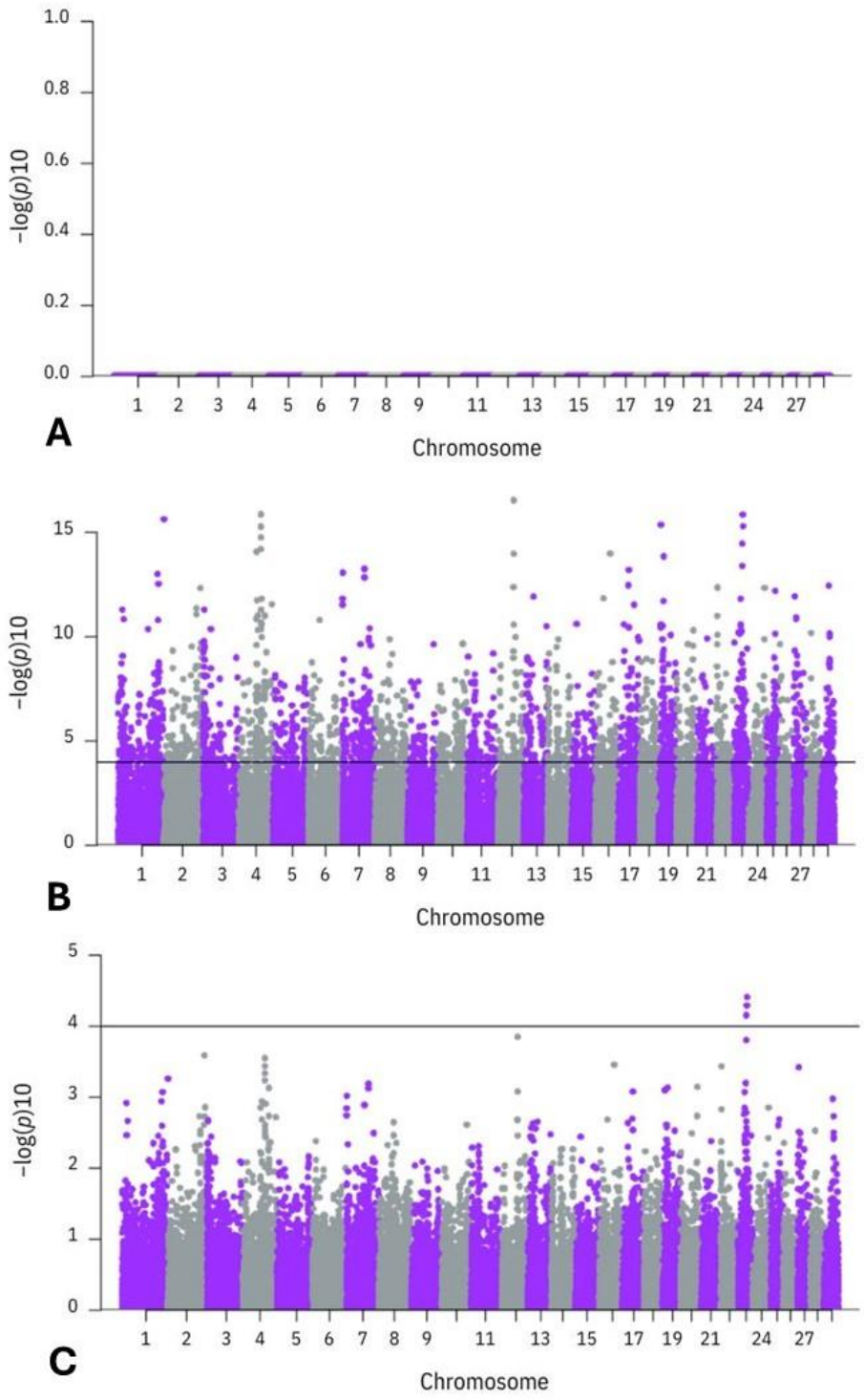

Figure 3.4 Manhattan plot showing the original results of a genome-wide association study with a significance threshold of 4.0 for percentage of primary abnormalities (panel A), percentage of secondary abnormalities (panel B), and percentage of normal sperm (panel C).

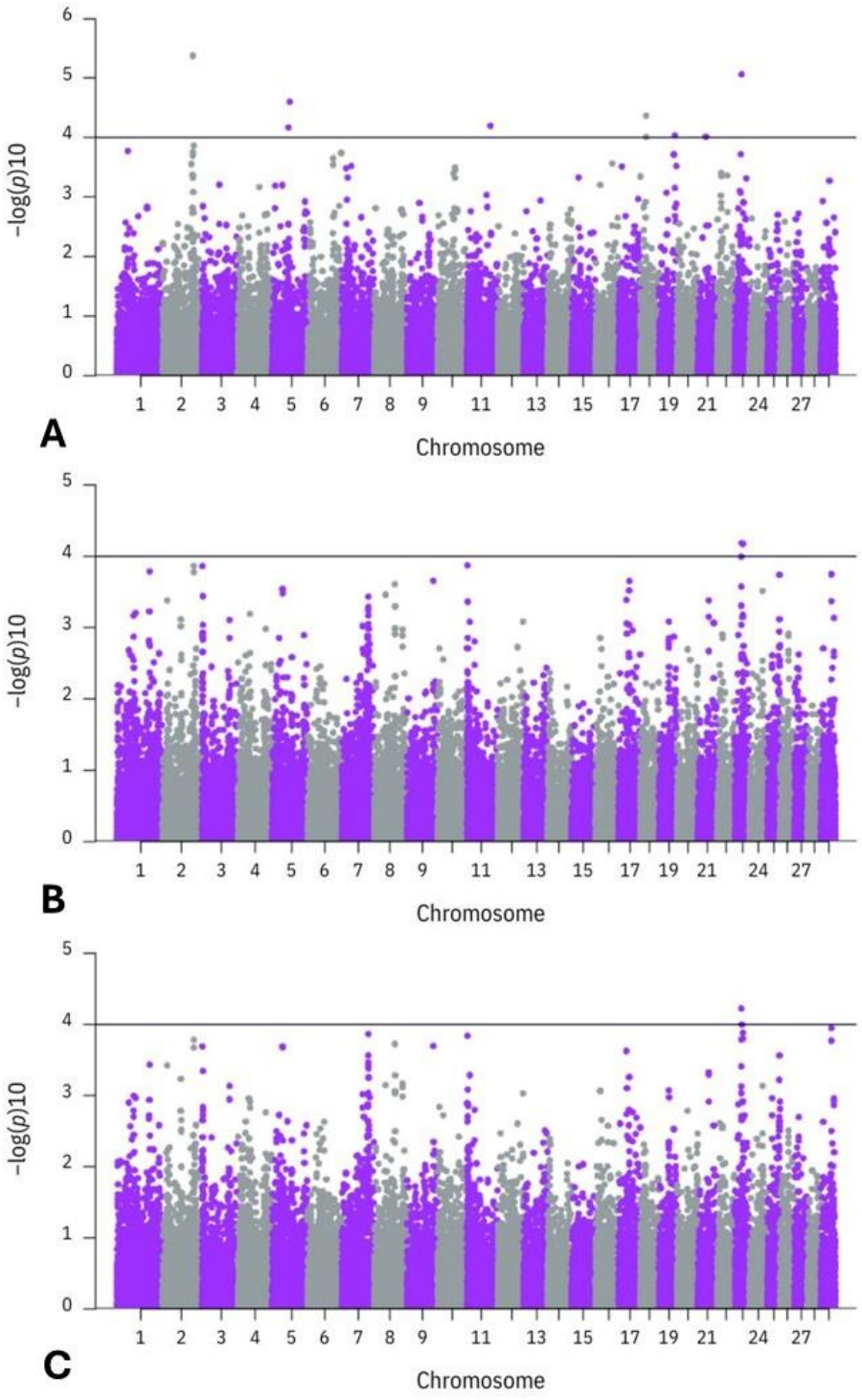

Figure 3.5 Manhattan plot showing the results of a genome-wide association study with a significance threshold of 4.0 for scrotal circumference (panel A), percentage of progressive motility (panel B), and motility score (panel C) after genomic control was applied.

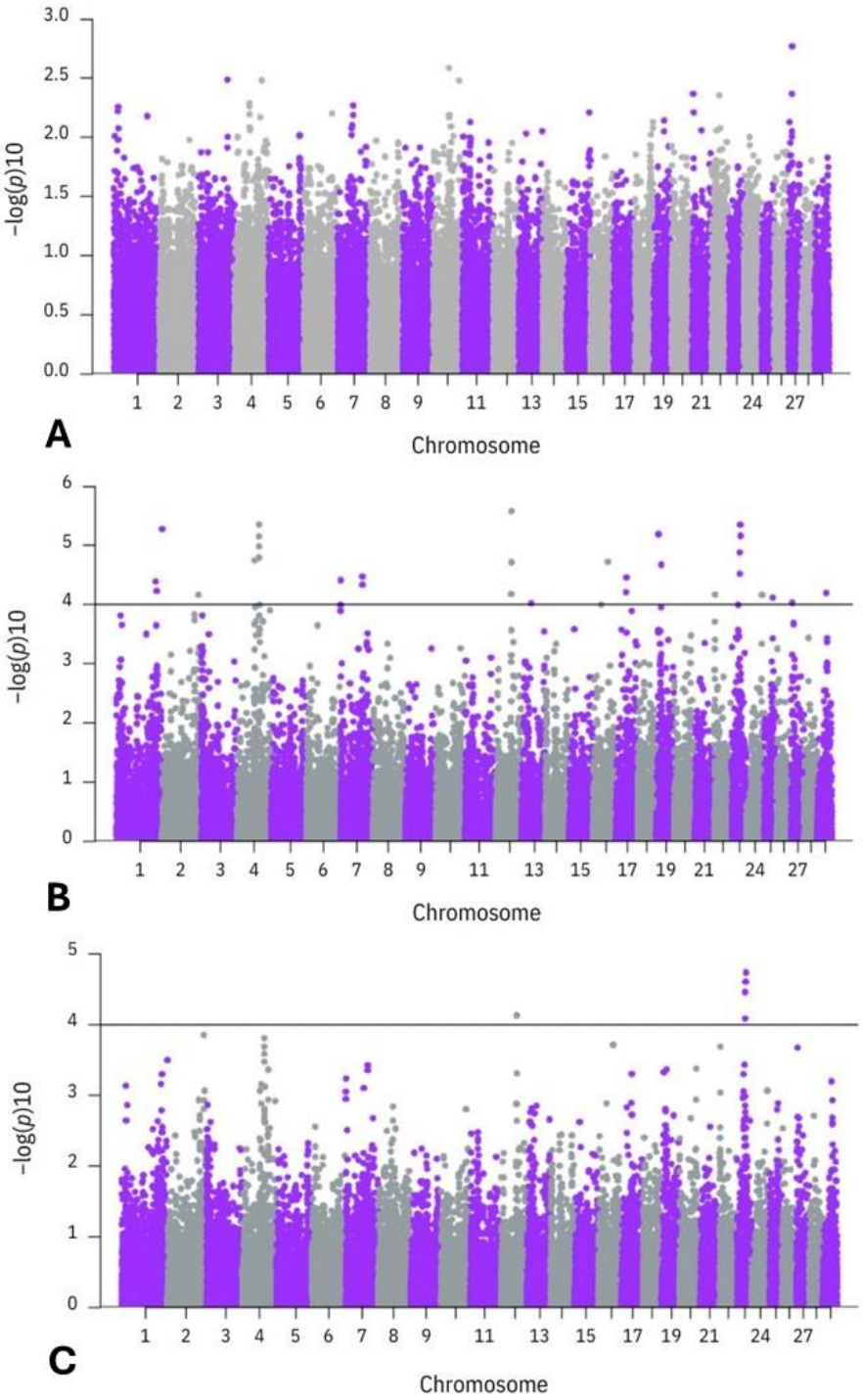

Figure 3.6 Manhattan plot showing the results of a genome-wide association study with a significance threshold of 4.0 for percentage of primary abnormalities (panel A), percentage of secondary abnormalities (panel B), and percentage of normal sperm (panel C) after genomic control was applied.

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Chapter 1

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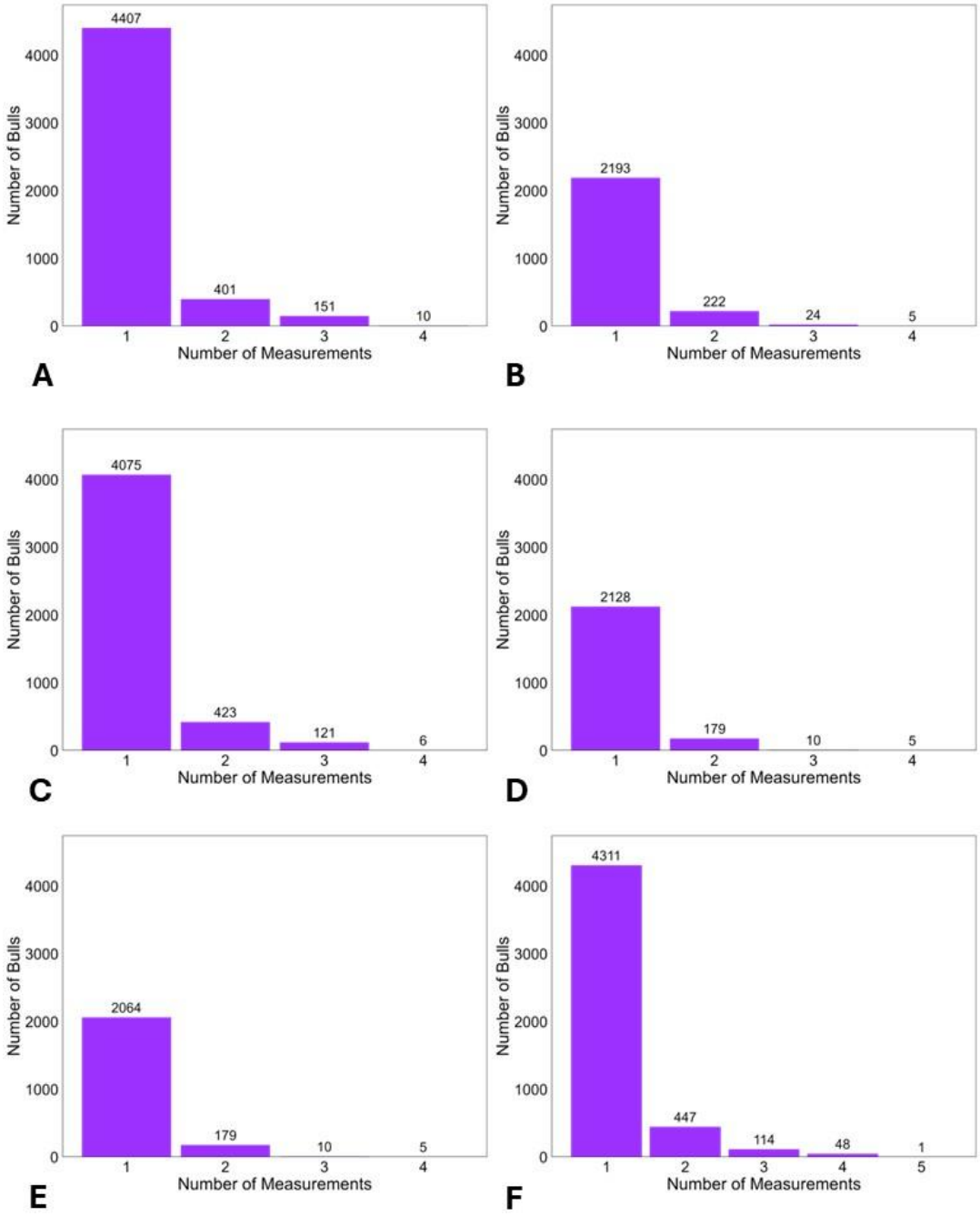
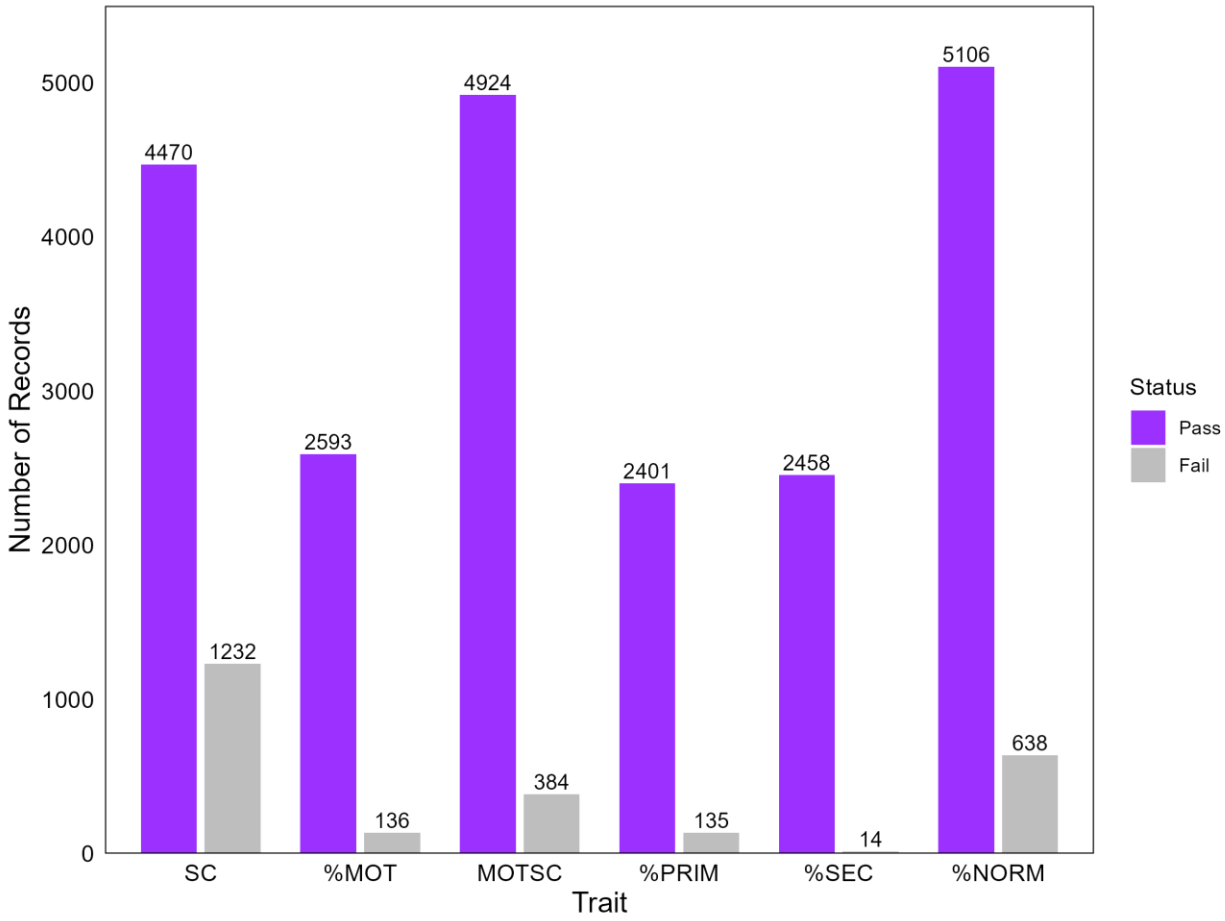
Appendix A - Additional Figures and Tables

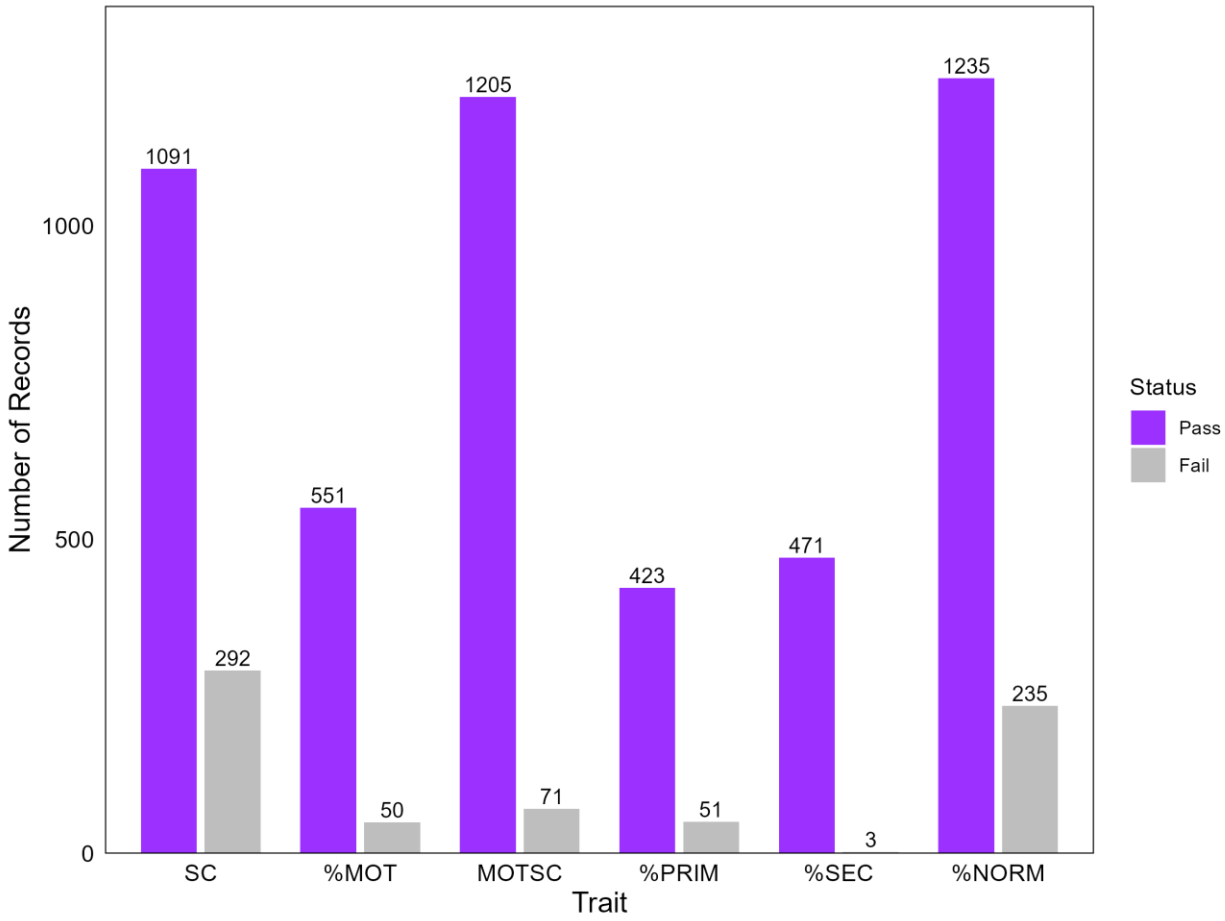


Appendix Figure A.1 Number of measurements per bull for each trait in the breeding soundness examination dataset. Panel A: Scrotal circumference, Panel B: Percentage of progressive motility, Panel C: Motility score, Panel D: Percentage of primary abnormalities, Panel E: Percentage of secondary abnormalities, Panel F: Percentage of normal spermatozoa.



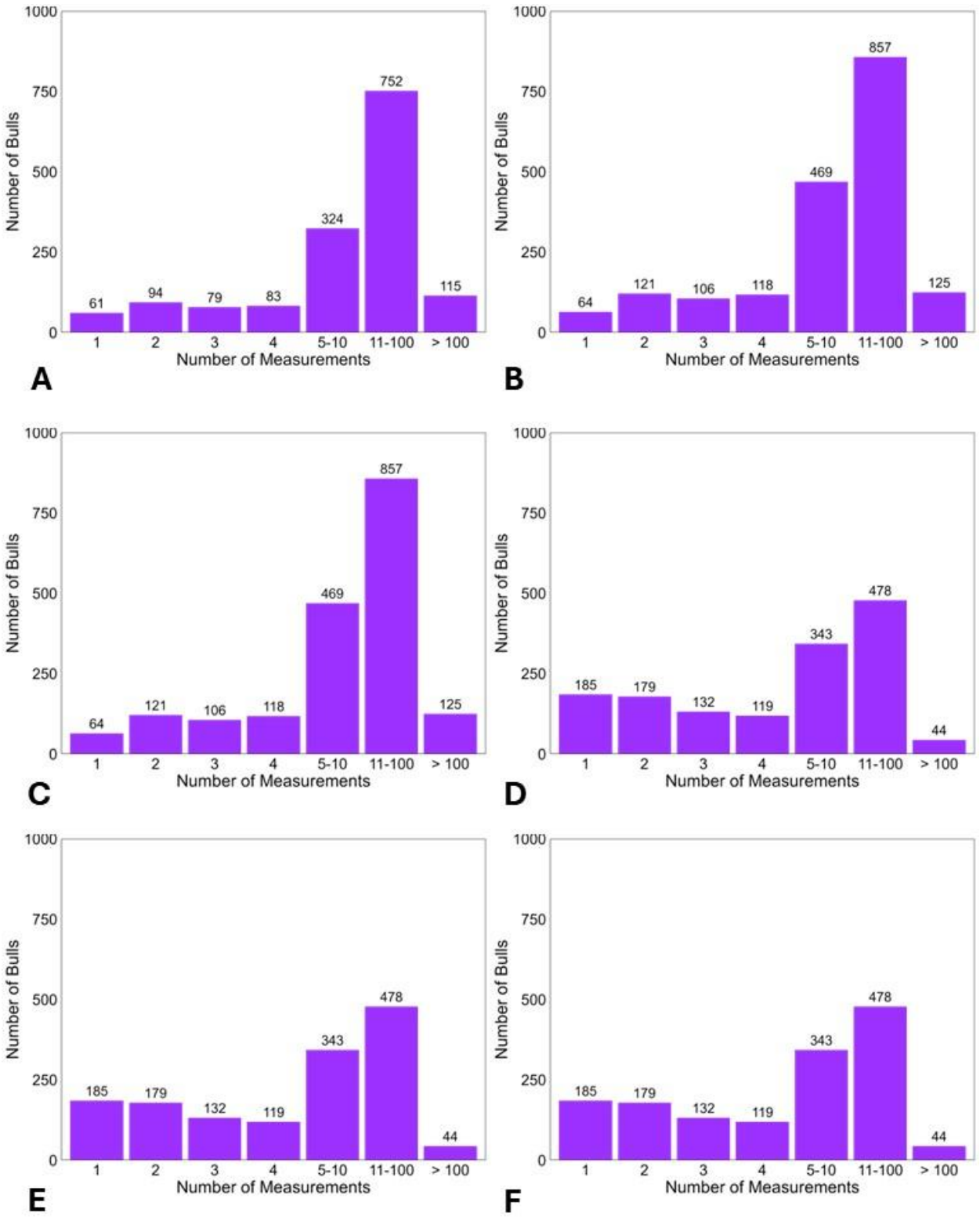
Appendix Figure A.2 Number of records for each trait that passed (Pass) or failed (Fail) the Society for Theriogenology's minimum requirements for an individual bull to pass a breeding soundness examination (BSE) from all bulls in the BSE dataset.

SC: Scrotal circumference
%MOT: Percentage of progressive motility
MOTSC: Motility score
%PRIM: Percentage of primary abnormalities
%SEC: Percentage of secondary abnormalities
%NORM: Percentage of normal spermatozoa

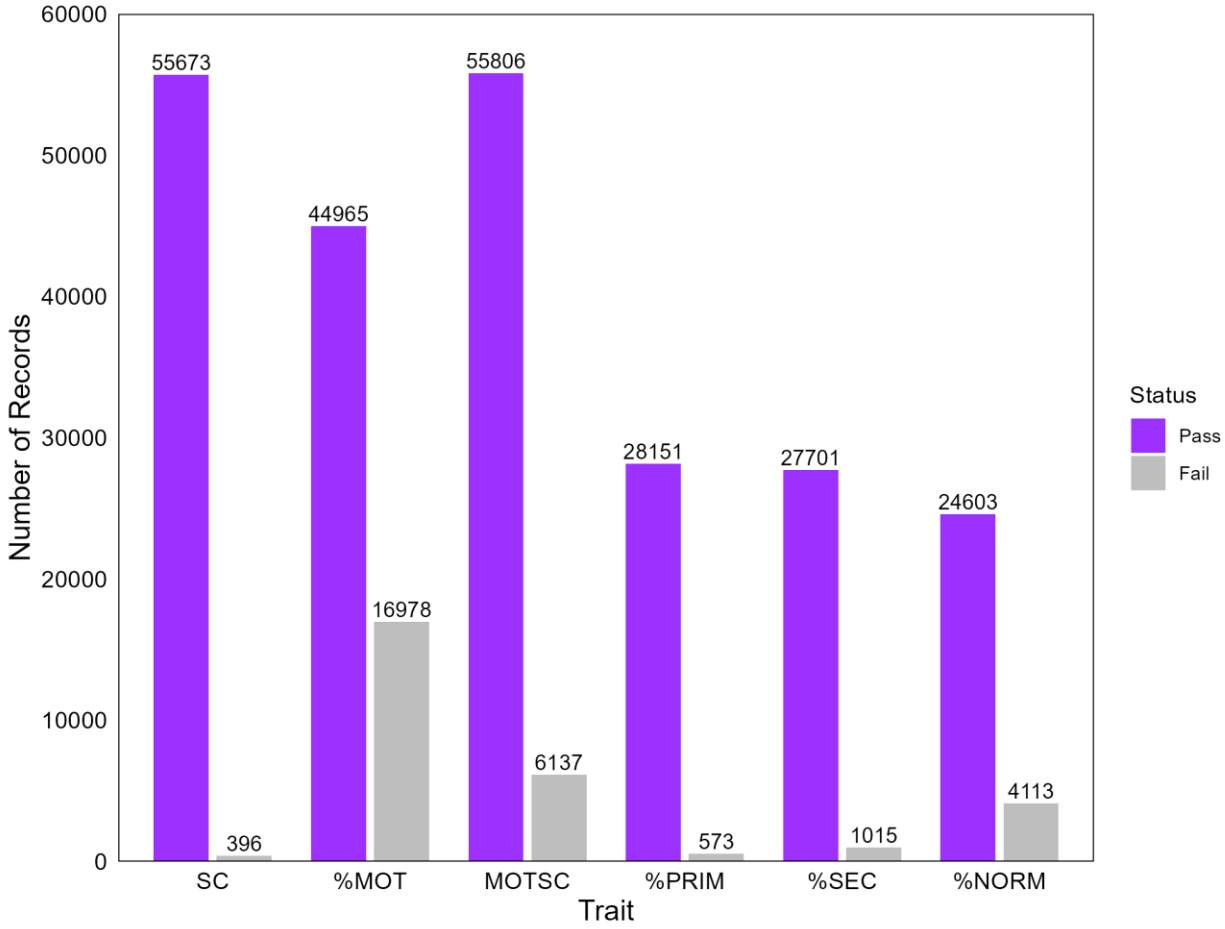


Appendix Figure A.3 Number of records for each trait that passed (Pass) or failed (Fail) the Society for Theriogenology's minimum requirements for an individual bull to pass a breeding soundness examination (BSE) from only bulls with two or more records in the BSE dataset.

SC: Scrotal circumference
%MOT: Percentage of progressive motility
MOTSC: Motility score
%PRIM: Percentage of primary abnormalities
%SEC: Percentage of secondary abnormalities
%NORM: Percentage of normal spermatozoa

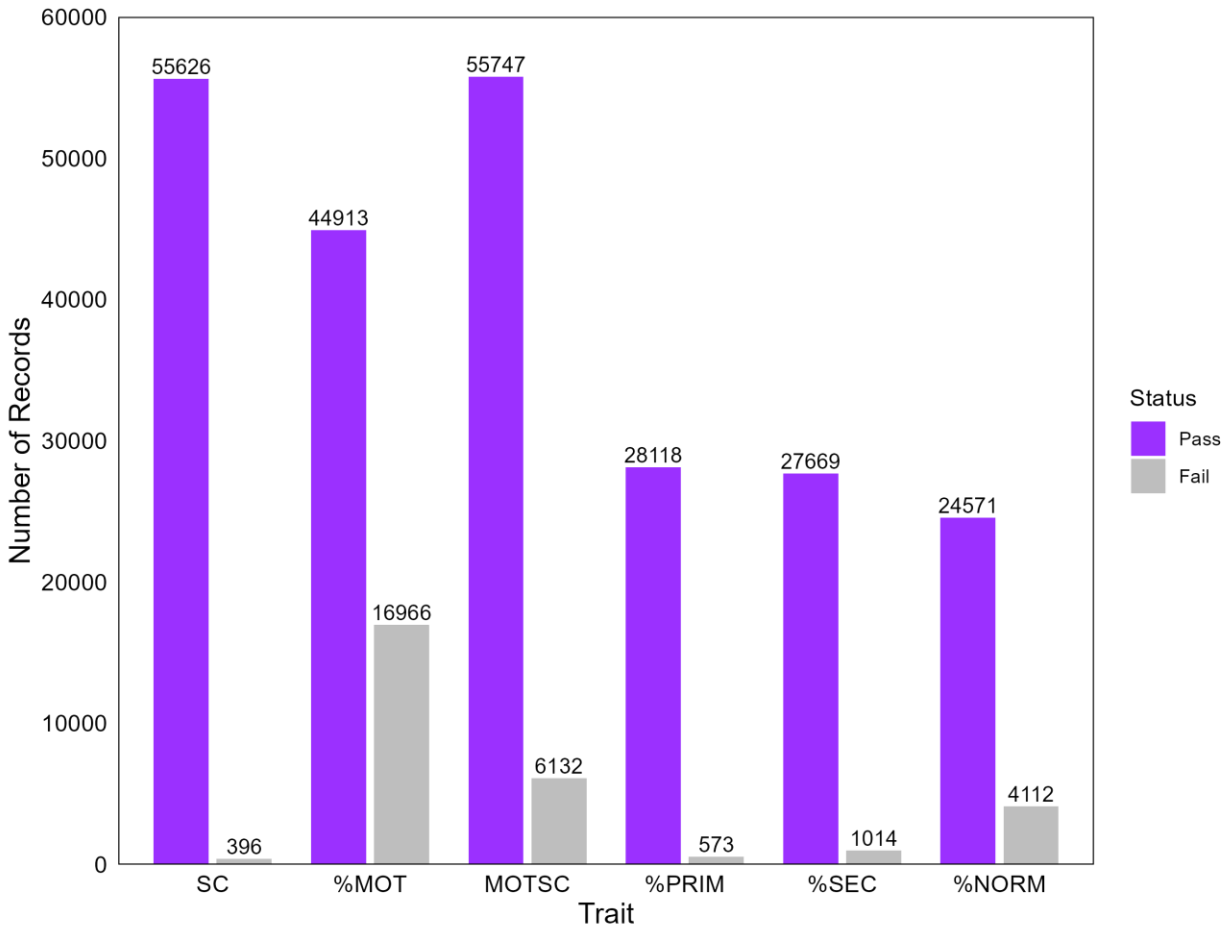


Appendix Figure A.4 Number of measurements per bull for each trait in the stud dataset. Panel A: Scrotal circumference, Panel B: Percentage of progressive motility, Panel C: Motility score, Panel D: Percentage of primary abnormalities, Panel E: Percentage of secondary abnormalities, Panel F: Percentage of normal spermatozoa.



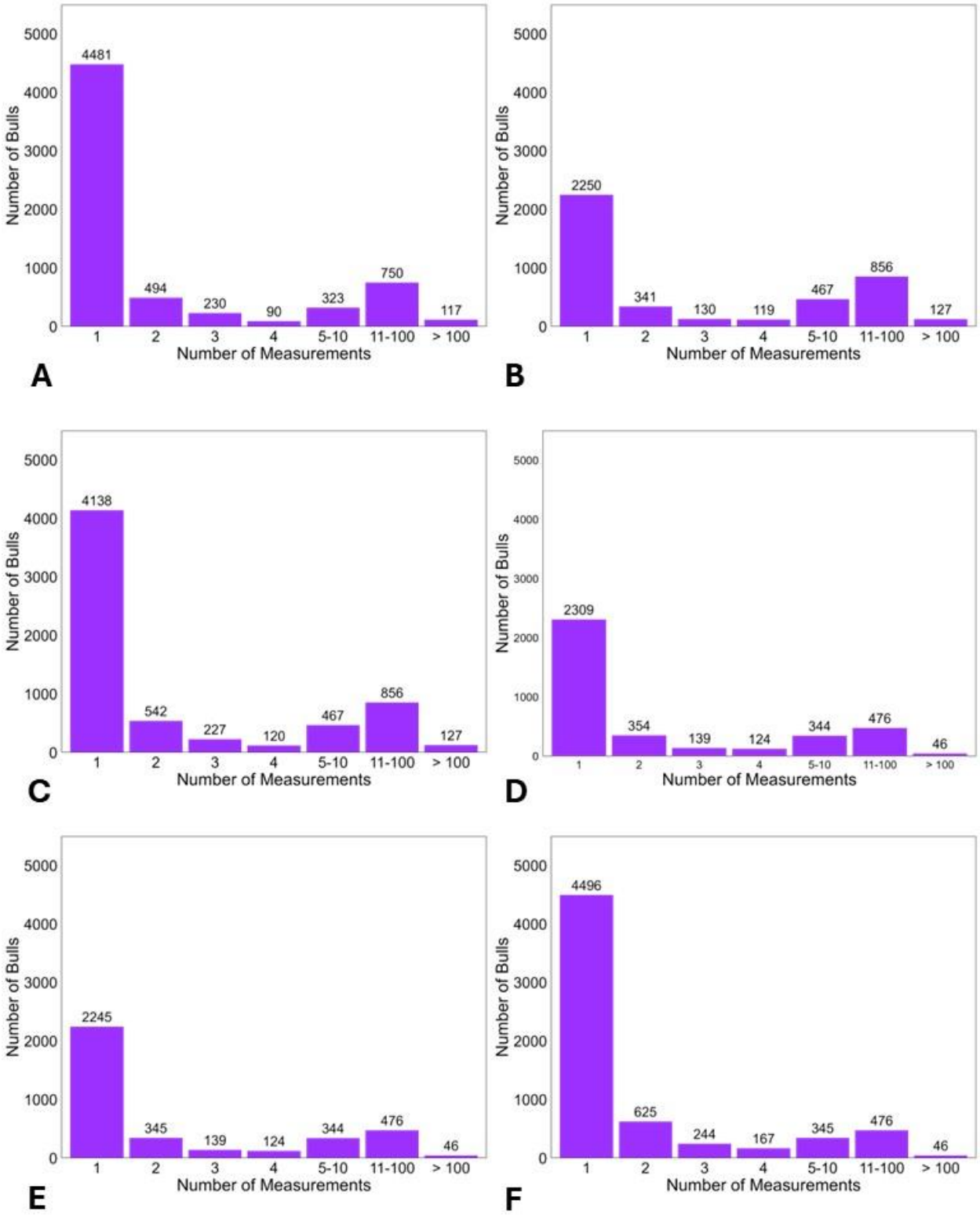
Appendix Figure A.5 Number of records for each trait in the bull stud dataset that would have passed (Pass) or failed (Fail) the minimum requirements of semen quality for an ejaculate to undergo cryopreservation at a bull stud and the minimum scrotal circumference measurement a bull should have determined by the Society for Theriogenology's breeding soundness examination guidelines.

SC: Scrotal circumference
%MOT: Percentage of progressive motility
MOTSC: Motility score
%PRIM: Percentage of primary abnormalities
%SEC: Percentage of secondary abnormalities
%NORM: Percentage of normal spermatozoa

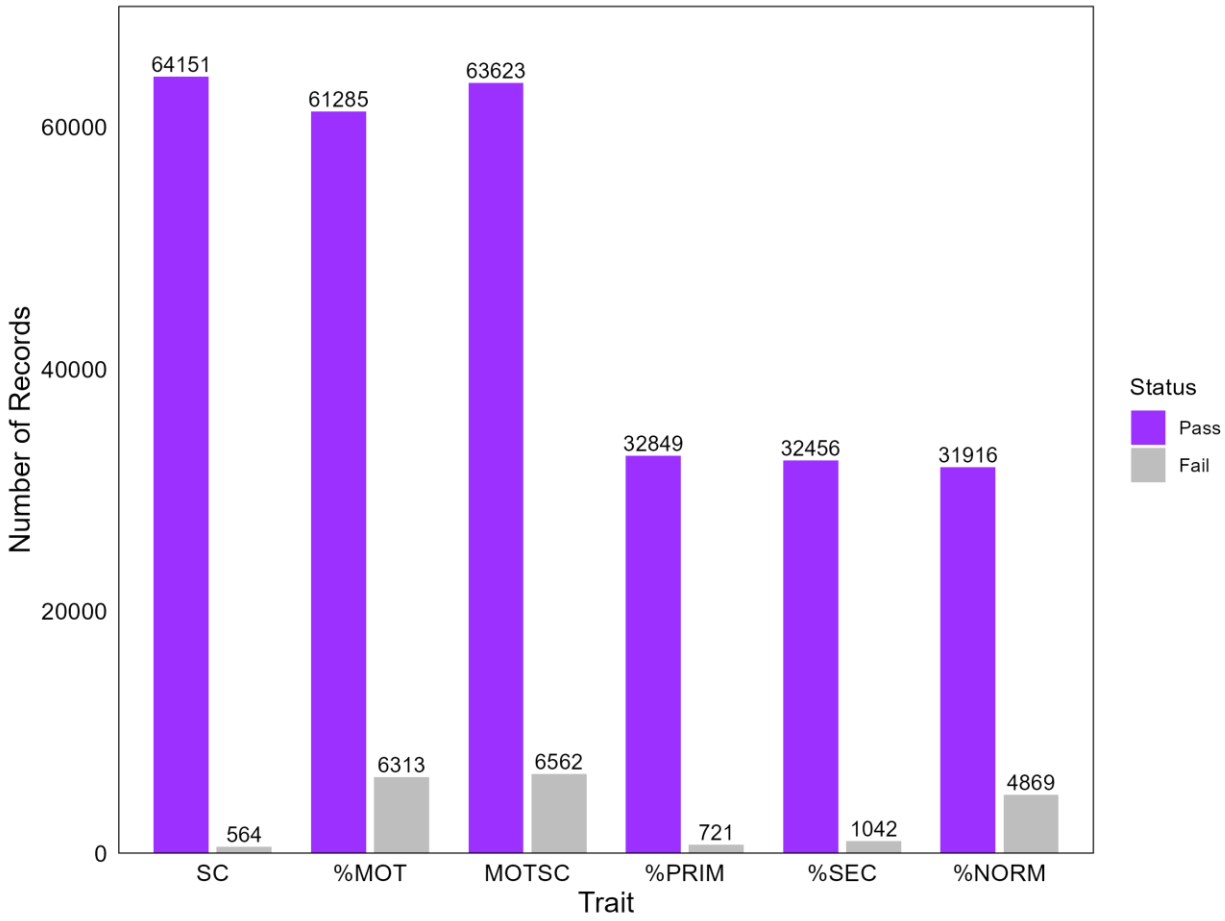


Appendix Figure A.6 Number of records for each trait in the bull stud dataset that would have passed (Pass) or failed (Fail) the minimum requirements of semen quality for an ejaculate to undergo cryopreservation at a bull stud and the minimum scrotal circumference measurement a bull should have determined by the Society for Theriogenology's breeding soundness examination guidelines from only bulls with two or more records in the stud dataset.

SC: Scrotal circumference
%MOT: Percentage of progressive motility
MOTSC: Motility score
%PRIM: Percentage of primary abnormalities
%SEC: Percentage of secondary abnormalities
%NORM: Percentage of normal spermatozoa

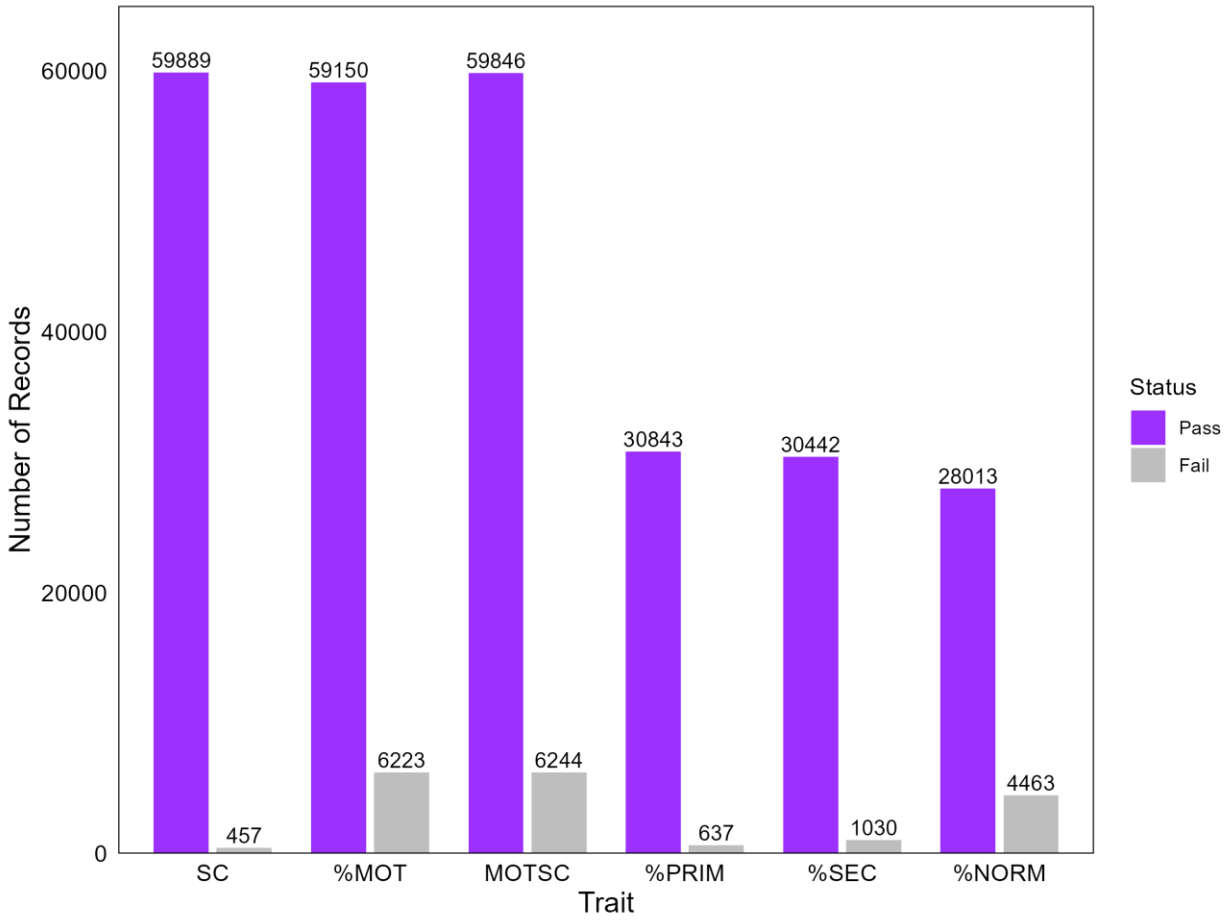


Appendix Figure A.7 Number of measurements per bull for each trait in the combined dataset. Panel A: Scrotal circumference, Panel B: Percentage of progressive motility, Panel C: Motility score, Panel D: Percentage of primary abnormalities, Panel E: Percentage of secondary abnormalities, Panel F: Percentage of normal spermatozoa.



Appendix Figure A.8 Number of records for each trait that passed (Pass) or failed (Fail) the Society for Theriogenology's minimum requirements for an individual bull to pass a breeding soundness examination (BSE) from all bulls in the combined dataset.

SC: Scrotal circumference
%MOT: Percentage of progressive motility
MOTSC: Motility score
%PRIM: Percentage of primary abnormalities
%SEC: Percentage of secondary abnormalities
%NORM: Percentage of normal spermatozoa



Appendix Figure A.9 Number of records for each trait that passed (Pass) or failed (Fail) the Society for Theriogenology's minimum requirements for an individual bull to pass a breeding soundness examination (BSE) from only bulls with two or more records in the combined dataset.

SC: Scrotal circumference
%MOT: Percentage of progressive motility
MOTSC: Motility score
%PRIM: Percentage of primary abnormalities
%SEC: Percentage of secondary abnormalities
%NORM: Percentage of normal spermatozoa

Appendix Table A.1 Genes within close proximity of significant quantitative trait loci (QTL) regions on individual chromosome associated with scrotal circumference.

Chromosome	Gene Name	Description
2	MARCH4	Membrane associated ring-CH-type finger 4
	RPL37A	
	SHOX	Short stature homeobox
	SMARCAL1	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A-like protein 1
2	RPL37A	Large ribosomal subunit protein eL43
5	ELK3	ETS transcription factor ELK3
	CDK17	Cyclin dependent kinase 17
11	FAM49A	CYFIP-related Rac1 interactor A
18	TOX3	TOX high mobility group box family member 3
19	TEN1	TEN1 subunit of CST complex
	ACOX1	Peroxisomal acyl-coenzyme A oxidase 1
	FBF1	Fas binding factor 1
	MRPL38	Large ribosomal subunit protein mL38
	TRIM65	Tripartite motif containing 65
	TRIM47	Tripartite motif containing 47
	WBP2	WW domain binding protein 2
	UNC13D	Unc-13 homolog D
	UNK	Unk zinc finger
	H3F3B	Histone H3.3
	GALK1	Galactokinase
	ITGB4	Integrin beta
	SAP30BP	SAP30 binding protein
	RECQL5	ATP-dependent DNA helicase
	SMIM6	Small integral membrane protein 6
	SMIM5	Small integral membrane protein 5
	LLGL2	LLGL scribble cell polarity complex component 2
	TSEN54	tRNA splicing endonuclease subunit 54
	CASKIN2	CASK interacting protein 2
	TMEM94	Transmembrane protein 94
	GRB2	Growth factor receptor-bound protein 2
21	OTUD7A	ubiquitinyl hydrolase 1
	ADAMTS7	ADAM metallopeptidase with thrombospondin type 1 motif 7
	TBC1D2B	TBC1 domain family member 2B
	SH2D7	SH2 domain containing 7
	CIB2	Calcium and integrin binding family member 2
	IDH3A	Isocitrate dehydrogenase subunit alpha, mitochondrial
	ACSBG1	Acyl-CoA synthetase bubblegum family member 1
23	MCM3	DNA replication licensing factor MCM3
	PAQR8	PAQR8 protein
	EFHC1	EF-hand domain-containing protein 1

TRAM2	Translocating chain-associated membrane protein
TMEM14A	Transmembrane protein 14A
GSTA2	Glutathione S-transferase A2
GSTA1	Glutathione S-transferase
GSTA1	Glutathione S-transferase
GSTA5	Glutathione S-transferase

Appendix Table A.2 Genes within close proximity of significant quantitative trait loci (QTL) regions on individual chromosome associated with percentage of progressive motility.

Chromosome	Gene Name	Description
23	EFHC1	EF-hand domain-containing protein 1
	TRAM2	Translocating chain-associated membrane protein
	TMEM14A	Transmembrane protein 14A
	GSTA2	Glutathione S-transferase A2
	GSTA1	Glutathione S-transferase
	GSTA1	Glutathione S-transferase
	GSTA5	Glutathione S-transferase
	GSTA3	Glutathione S-transferase
	GSTA4	Glutathione S-transferase A4
	ICK	Ciliosis associated kinase 1
	FBXO9	F-box only protein 9
	GCM1	Glial cells missing transcription factor 1
23	ZFP57	ZFP57 zinc finger protein
	ZFP57	ZFP57 zinc finger protein
	OR2H1D	Olfactory receptor
	OR2H1	Olfactory receptor
	UBD	Ubiquitin D
	OR2I1P	Olfactory receptor family 2 subfamily I member 1 pseudogene
	OR10C1	Olfactory receptor
	OR11W1	Olfactory receptor
	OR1O8	Olfactory receptor
	OR1O9	Olfactory receptor
23	BOLA-NC1	Ig-like domain-containing protein
	BOLA	Ig-like domain-containing protein
	TRIM26	Tripartite motif-containing protein 26
	TRIM15	Tripartite motif containing 15
	TRIM10	Tripartite motif-containing protein 10
	TRIM40	Tripartite motif containing 40
	TRIM31	Tripartite motif containing 31
	PPP1R11	E3 ubiquitin-protein ligase PPP1R11
	ZNRD1	DNA-directed RNA polymerase I subunit RPA12
	ZFP57	ZFP57 zinc finger protein
	MOG	Myelin-oligodendrocyte glycoprotein
	GABBR1	Gamma-aminobutyric acid type B receptor subunit 1
	LOC786987	Ubiquitin-like domain-containing protein

Appendix Table A.3 Genes within close proximity of significant quantitative trait loci (QTL) regions on individual chromosome associated with motility score.

Chromosome	Gene Name	Description
23	TFAP2B	Transcription Factor AP-2 Beta

Appendix Table A.4 Genes within close proximity of significant quantitative trait loci (QTL) regions on individual chromosome associated with percentage of normal spermatozoa.

Chromosome	Gene Name	Description
12	RBM26	RNA binding motif protein 26
	NDFIP2	Nedd4 family interacting protein 2
23	TRIM26	Tripartite motif-containing protein 27
	TRIM15	Tripartite motif containing 16
	TRIM10	Tripartite motif-containing protein 11
	TRIM40	Tripartite motif containing 41
	TRIM31	Tripartite motif containing 32
	PPP1R11	E3 ubiquitin-protein ligase PPP1R12
	ZNRD1	DNA-directed RNA polymerase I subunit RPA12
	MOG	Myelin-oligodendrocyte glycoprotein
	GABBR1	Gamma-aminobutyric acid type B receptor subunit 2
	LOC786987	Ubiquitin-like domain-containing protein
	OR2H1D	Olfactory receptor
	OR2H1	Olfactory receptor
	UBD	Ubiquitin D
	OR2I1P	Olfactory receptor family 2 subfamily I member 1 pseudogene
	OR10C1	Olfactory receptor
	OR11W1	Olfactory receptor
	OR1O8	Olfactory receptor
	OR1O9	Olfactory receptor
	OR2W2	Olfactory receptor
	OR2B2	Olfactory receptor
	OR2W6	Olfactory receptor
	OR2B6	Olfactory receptor
	OR2W4	Olfactory receptor
	OR2B2D	Olfactory receptor
	HIST1H2BB	Histone H2B
	ZNF184	Zinc finger protein 184
	ZNF391	Zinc finger protein 391
	POM121L2	POM121 transmembrane nucleoporin like 2
	PRSS16	Serine protease 16
	OR12D2	Olfactory receptor
	OR12D3	Olfactory receptor
	OR5V1	Olfactory receptor

Appendix Table A.5 Genes within close proximity of significant quantitative trait loci (QTL) regions on individual chromosome associated with percentage of secondary abnormalities.

Chromosome	Gene Name	Description
1	KCNH8	Potassium voltage-gated channel subfamily H member 8
1	DSCAM	DS cell adhesion molecule
2	SLC30A2	Solute carrier family 30 member 2
	EXTL1	Exostosin like glycosyltransferase 1
	PAFAH2	Platelet-activating factor acetylhydrolase 2, cytoplasmic
	STMN1	Stathmin
	PAQR7	Progestin and adipoQ receptor family member 7
	AUNIP	Aurora kinase A- and ninein-interacting protein
	MTFR1L	Mitochondrial fission regulator 1-like
	SELENON	Selenoprotein N
	MAN1C1	alpha-1,2-Mannosidase
	LDLRAP1	LDLRAP1 protein
4	DPY19L1	Dpy-19 like C-mannosyltransferase 1
	NPSR1	Neuropeptide S receptor 1
4	ADCY1	Adenylate cyclase type 1
	RAMP3	Receptor activity-modifying protein 3
	TBRG4	FAST kinase domain-containing protein 4
	NACAD	NAC-A/B domain-containing protein
	CCM2	CCM2 scaffold protein
	MYO1G	Myosin IG
	PURB	Purine rich element binding protein B
	H2AFV	Histone H2A.V
4	PPIA	Peptidyl-prolyl cis-trans isomerase A
	ZMIZ2	Zinc finger MIZ-type containing 2
	OGDH	2-oxoglutarate dehydrogenase complex component E1
	TMED4	Transmembrane p24 trafficking protein 4
	DDX56	Probable ATP-dependent RNA helicase DDX56
	NPC1L1	NPC1 like intracellular cholesterol transporter 1
	NUDCD3	NudC domain containing 3
	CAMK2B	Calcium/calmodulin-dependent protein kinase type II subunit beta
	YKT6	Synaptobrevin homolog YKT6
4	GCK	Phosphotransferase
	MYL7	Myosin light chain 7
	POLD2	DNA polymerase delta subunit 2
	AEBP1	AE binding protein 1
4	POLM	DNA-directed DNA/RNA polymerase mu
12	RNF219	ORC ubiquitin ligase 1
	RBM26	RNA binding motif protein 27

	NDFIP2	Nedd4 family interacting protein 3
12	SPRY2	Protein sprouty homolog 2
16	TTC34	Tetratricopeptide repeat domain 34
	MMEL1	Membrane metalloendopeptidase like 1
	PRXL2B	Prostamide/prostaglandin F synthase
	HES5	HES5 protein
	PANK4	4'-phosphopantetheine phosphatase
	PLCH2	Phosphoinositide phospholipase C
	PEX10	RING-type E3 ubiquitin transferase
	RER1	Protein RER1
	MORN1	MORN repeat containing 1
	SKI	Ski oncogene
	FAAP20	Fanconi anemia core complex associated protein 20
	PRKCZ	Protein kinase C
19	RAB11FIP4	RAB11 family interacting protein 4
	NF1	Neurofibromin 1
	EVI2A	Ecotropic viral integration site 2A
	EVI2B	Ecotropic viral integration site 2B
	OMG	Oligodendrocyte myelin glycoprotein
	MSI2	Musashi RNA binding protein 2
22	CMC1	COX assembly mitochondrial protein homolog
	AZI2	5-azacytidine-induced protein 2
	ZCWPW2	Zinc finger CW-type and PWWP domain containing 2
23	TRIM26	Tripartite motif-containing protein 28
	TRIM15	Tripartite motif containing 17
	TRIM10	Tripartite motif-containing protein 12
	TRIM40	Tripartite motif containing 42
	TRIM31	Tripartite motif containing 33
	PPP1R11	E3 ubiquitin-protein ligase PPP1R13
	ZNRD1	DNA-directed RNA polymerase I subunit RPA12
	MOG	Myelin-oligodendrocyte glycoprotein
	GABBR1	Gamma-aminobutyric acid type B receptor subunit 3
	LOC786987	Ubiquitin-like domain-containing protein
	OR2H1D	Olfactory receptor
	OR2H1	Olfactory receptor
	UBD	Ubiquitin D
	OR2I1P	Olfactory receptor family 2 subfamily I member 1 pseudogene
	OR10C1	Olfactory receptor
	OR11W1	Olfactory receptor
	OR108	Olfactory receptor
	OR108	Olfactory receptor
23	OR12D2	Olfactory receptor
	OR12D3	Olfactory receptor
	OR5V1	Olfactory receptor
23	OR2W2	Olfactory receptor

	OR2B2	Olfactory receptor
	OR2W6	Olfactory receptor
	OR2B6	Olfactory receptor
	OR2W4	Olfactory receptor
	OR2B2D	Olfactory receptor
	HIST1H2BB	Histone H2B
23	ZNF184	Zinc finger protein 184
	ZNF391	Zinc finger protein 391
	POM121L2	POM121 transmembrane nucleoporin like 3
	PRSS16	Serine protease 17
25	GALNT17	Polypeptide N-acetylgalactosaminyltransferase