

Genetic improvement of beef bull semen attributes

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Abstract

The genetic basis of fertility of beef bulls collected for artificial insemination (AI) is unclear due to the lack of research and published literature. Researching beef bull fertility traits has the potential to increase the rate of genetic gain, decrease the generation interval, and ultimately allow more genetic improvement in the beef herd not only in the United States, but around the world. In order to drive this genetic gain, quantifying the genetic parameters of beef bull fertility traits is necessary. The objectives of this thesis were to estimate the genetic parameters of beef bull fertility traits and perform a genome-wide association study (GWAS) to identify regions of the genome associated with fertility. Beef bull fertility phenotypes including semen volume (VOL), concentration (CONC), number of spermatozoa (NSP), initial motility (IMOT), post-thaw motility (PTMot), three-hour post-thaw motility (3HrPTMot), percentage of normal spermatozoa (%NORM), primary abnormalities (PRIM), and secondary abnormalities (SEC) on 1,819 Angus bulls were obtained from two bull semen collection facilities. Pedigree and genomic information was provided by the American Angus Association. Variance components were estimated using single-step genomic best linear unbiased prediction (ssGBLUP). All fertility traits were lowly heritable with high repeatabilities. Genetic correlations among the traits varied from low to high. Results from the GWAS indicated there are several regions of the genome associated with previously-reported fertility quantitative trait loci (QTL). While the heritabilities for these traits were low, findings in the current research indicate these traits could be improved by utilizing genetic selection tools for beef bull fertility. Rapidly changing genetic selection tools paired with the ability to obtain thousands of beef bull fertility phenotypes from bull semen collection facilities provide an opportunity to improve beef bull fertility.

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Chapter 1 - Literature Review

Introduction

Whether producers are utilizing conventional breeding methods or artificial insemination (AI), reproductive efficiency is an important area of livestock production. Reproduction is a very complex trait that involves events including gametogenesis, fertilization, implantation, and embryo and fetus development (Abdollahi-Arpanahi et al. 2017). Reproduction has been studied since the time of Aristotle, but it has not been until the last 100 years that the field has begun to experience tremendous growth. In the 1940s and 1950s, AI began to draw the attention of not only beef producers but other livestock producers. The advent of AI caused the area of livestock reproduction to receive more attention (Senger 2012). Artificial insemination is currently one of the main assisted reproductive technologies used for genetic improvement and introducing genetic variation from superior sires into herds (Druet et al. 2009). Currently, cattle producers use various heat detection and fixed time AI (FTAI) protocols in order to synchronize estrus cycles and increase conception rates. By utilizing FTAI, producers can experience an economic benefit of \$49.14 per cow (Rogers et al. 2012). The economic benefits of AI are due to the fact it allows beef producers to increase genetic selection and decrease generation interval (Vishwanath 2003). Improved genetic selection increases the productivity of livestock (Senger 2012).

Despite the importance of AI, advances with estrus synchronization protocols, and an emphasis on conception rate, beef cattle pregnancy rates have remained the same for the past ten years when utilizing assisted reproductive technologies (Oosthuizen et al. 2018). Limited current research does not depict the difference between utilizing assisted reproductive technology and natural service bulls, but Williamson et al. (1978) and Niles et al. (2002) determined pregnancy rates in herds of dairy cattle were not significantly different when comparing AI to natural

service. Furthermore, herds utilizing only natural service experienced more variable conception rates. Within all herds, reproduction is a large contributing factor to obtaining a producer's goal of profitability, regardless of whether it is explicitly included in the breeding objective (Harris 1970). Reproduction, and more definitively, conception rate, can be influenced by a number of environmental factors including nutrition, temperature, and overall animal health. A female's conception rate can be impacted by these common factors and additionally her age and the season of year (Senger 2012). Optimizing environmental conditions of the female can improve conception rates, but producers should also be mindful of the effects of the male (Beef Cattle Handbook). Conception rate is contingent on the quality of the semen, which is dependent on a number of factors such as number of motile spermatozoa, sperm quality, sperm viability, abnormal spermatozooids, and genetic defects associated with the bulls (Druet et al. 2009).

Reproductive technologies such as estrous synchronization and FTAI, have been utilized to optimize female fertility, decrease calving interval, and enhance genetic improvement, but even with the use of assistive reproductive technologies, beef producers are still only experiencing an average single service AI conception rate of 60% (Diskin and Kenny 2016). Improvements to the conception rate of the United States (US) beef cattle herd can be further enhanced by obtaining accurate measures of bull fertility (Berry et al. 2014). Research shows variation in fertility among bulls, which could be the cause of the stagnant conception rate (Flowers 2012). Furthermore, Berry et al. (2014) speculated that semen quality is more heritable than female reproductive traits. Discovering how to improve fertility not only has the potential to increase conception rate but has the capacity to improve other economically relevant production traits. For example, research shows selecting for increased SC size decreases the calving interval

(Meyer et al. 1991), improves daughter pregnancy rates (Evans et al. 1999), and increases average daily gain (Raidan et al. 2016).

In order to improve the productivity of the national herd, advances in bull reproductive performance need to be achieved (Petrunkina and Harrison 2011). A large emphasis has been placed on female fertility, but only focusing on the female reproductive capabilities limits the potential for increased phenotypic performance. Braundmeier and Miller (2001) recognize using subfertile or infertile semen has consequences not only to the companies distributing it but also to the producers using it. Furthermore, a significant percentage of reproductive failure is attributable to bull subfertility, and the fertility of a bull is dependent on the semen quantity, semen quality, and health status of the bull (DeJarnette 2004).

Moreover, the economic impacts of bull fertility have tremendous influence in the AI industry. The National Association of Animal Breeders (NAAB; 2017) reported nearly 2.7 million units of bull semen were collected and over 2.5 million units of bull semen were distributed throughout the US in 2017. Furthermore, the US exported 3.5 million units of bull semen in 2017 totaling over \$12 million. In the last study performed by the National Animal Health Monitoring System, it was reported that only 7.62% of beef cattle producers had adopted AI technology. Along with time and labor, the cost was cited as one of the main reasons for not utilizing the reproductive technology (USDA 2009). The potential cost reduction paired with the ability to increase the quality of a unit of semen should entice more producers to implement AI and cause a more rapid rate of genetic change in the beef industry.

Studying male fertility has many production and economic impacts not only for beef cattle but for the livestock industry. Bull fertility and sperm production need to be optimized for economic reasons, especially because the demand for some AI sires is very high (Ducrocq and

Humblot 1995). Improving fertility in food animals is extremely important in order to produce more food and textile products, and to increase the economic efficiency of livestock production (Senger 2012). Studying fertility in beef bulls may also lead to new diagnostic tools and treatments for idiopathic human male infertility (Abdollahi-Arpanahi et al. 2017), which has caused at least a portion of the 7-year decline in the US fertility rate noted by the Center for Disease Control (Hamilton et al. 2018).

Bull Fertility

Fertility is generally defined as the ability to conceive offspring (Utt 2016). Fertility traits have a large impact on production, and therefore, are of great economic value in the livestock industry (Adbollahi-Arpanahi et al. 2017). For a bull to be considered fertile, he must be able to reproduce normally and meet certain semen quality criteria (Christmas et al. 2001).

Phenotypic Assessment

Williams (1909) was the first to begin outlining traits which would make a bull sterile. Williams (1909) advised that a bull must have normally functioning testicles, excretory ducts, urethra, penis, and accessory gland to be able to reproduce successfully. In addition, the male must also be able to mount the female properly. Carroll et al. (1963) performed a study with beef bulls proving it was possible to test for fertility of bulls on-farm. Carrol et al. (1963) classified semen samples based on motility (MOT), concentration (CONC), morphological characteristics, and percentage of live sperm (%LIV), giving the samples an overall evaluation of satisfactory, questionable, or unsatisfactory based on the semen quality. This method was used by the Rocky Mountain Society for the Study of Fertility in Bulls, which eventually became the American Society for Theriogenology (SFT; Chenoweth 2002). The SFT revised and adopted standardized procedures and assessments from Carrol et al. (1963) for measuring bull fertility (Chenoweth

2002). It became known as the breeding soundness examination (BSE), which is performed by a veterinarian and is still commonly used for measuring bull fertility today (Chenoweth 1980; Hopkins and Spitzer 1997; Fordyce et al. 2006). In 1993, the standards were revised to add minimum acceptable measurements for SC to vary with age, motility, and morphology (Hopkins and Spitzer 1997).

Breeding Soundness Examination

Currently, the BSE involves a physical examination and semen evaluation. A physical examination typically consists of measuring height, weight, SC, and assessing the testicles and penile function. Some advanced facilities have begun performing disease testing and assessing sex drive and mating ability (Chenoweth 2002). Scrotal circumference is measured to estimate the potential number of sperm (NSP) cells that could be produced (Whittier and Bailey 2009). The testicles, penis, and their associated structures are examined visually and physically to check for tumors, warts, frenulum's, or abnormally shaped organs (Whittier and Bailey 2009).

An ejaculate is collected to evaluate the morphology and motility of the sperm (Whittier and Bailey 2009). When assessing the morphology of an ejaculate, technicians identify the percent normal sperm (PNS) cells produced (Whittier and Bailey 2009). This is an important criterion because ejaculates with a high percentage of abnormal sperm cells are associated with poor conception rates (Whittier and Bailey 2009). Prior to 2018, abnormalities were classified as primary (PRIM) or secondary (SEC), identifying a head or tail defect, respectively. Current BSE standards classify abnormalities by location: head, midpiece, or tail (BSE 2018). Motility is estimated by examining the mass movement of a concentrated amount of semen. Semen with very good MOT will present vigorous forward movement (Whittier and Bailey 2009).

To be classified as a Satisfactory Potential Breeder a bull must meet the minimum requirements of BSE put forth by the SFT. The minimum SC size a bull can have ranges from 30 to 34 centimeters, depending on a bulls age. A bull must have at least 30% MOT and 70% PNS. In addition, a bull must have a body condition score of 5 or 6 on the 9-point BCS scale (Farney et al. 2016) and be free of structural issues (Whittier and Bailey 2009).

Artificial Insemination Sires

Artificial insemination collection facilities have varying parameters for their collection protocols, but in general, their standards are the same across collection facilities. Bulls are usually collected two to three times a week (Senger 2012). Due to the damage done to sperm cells during the freezing and thawing process (Senger 2012), collection facilities must carefully inspect and approve each bull's semen to ensure each straw of semen has equivalent viability.

There are two common ways to collect semen from a bull. The most common utilizes an artificial vagina (AV) which simulates vaginal conditions of a female in estrus (Senger 2012). The AV uses temperature and pressure regulated by water. One end of the AV is attached to a cone which funnels into a collection vessel. When using an AV, a surrogate animal must be used for the bull to mount. The second method utilizes electroejaculation to electrically stimulate the accessory sex glands and the pelvic urethra. Electroejaculation is typically performed in bulls that cannot physically mount and ejaculate (Senger 2012).

Once semen is collected, it must be preserved and extended. Before the preservation and extension process, semen is evaluated to determine the ejaculate volume (VOL), CONC, and MOT. These identifications allow the laboratory technician to determine if the semen is freezable and how many potential doses are available in the ejaculate (Senger 2012). Preserving and extending sperm are crucial steps in the semen collection process. If not done correctly, these

steps can cause death and loss of the sperm cells. The process involves slowly cooling the sperm to room temperature, adding the extender, and slowly freezing the packaged straw of semen. Semen is then stored in liquid nitrogen until it is ready for use (Purdy 2004).

Semen Production Cycle

Spermatogenesis

Spermatogenesis is a key process involved in male fertility as it is the production of spermatozoa. Spermatogenesis occurs in the seminiferous tubules and consists of all cell divisions and morphologic changes involved in the process of developing germ cells. The process involves three phases. The first phase is proliferation, which involves several mitotic divisions of spermatogonia. The second phase is meiosis, where primary spermatocytes go through DNA replication and crossing over to produce secondary spermatocytes. After meiosis II, secondary spermatocytes produce haploid spermatids. The final phase of spermatogenesis is differentiation, where spermatozoa are produced which contain a head, flagellum, and principal piece. The process of spermatogenesis for the bull is approximately 60 days. Spermatogenesis is constantly occurring after the bull experiences puberty in order to produce a continual supply of sperm cells. Spermatogenesis takes place within the testis and involves proliferation, meiosis, and differentiation (Senger 2012). Primary abnormalities, such as head or acrosome abnormalities, typically occur during the spermatogenesis cycle. (Whittier and Bailey 2009).

Epididymal Transit Time

Epididymal transit time, or repletion, is the process of moving sperm cells through the epididymis. After spermatogenesis, spermatozoa move from the testis to the head of the epididymis, or the caput. In the caput, the sperm cells acquire fluids necessary for sperm maturation, which takes approximately 2 days. Sperm cells then move to the corpus, or the body

of the epididymis, where the sperm cells complete maturation over the period of 2 days. Finally, the sperm cells arrive in the tail of the epididymis, or the cauda, where the sperm cells are then stored until they are depleted via ejaculation or urination. Sperm cells typically remain in the cauda for ten days. Ejaculation once or twice weekly will deplete the cauda, with no adverse effects to the sperm cells (Schenk 2018). Low or high ejaculation frequency could affect the quality of the sperm cell. The total cycle from caput to the cauda is, on average, 14 days. Environmental factors greatly affect sperm cells in the epididymis. Semen quality can be dependent on the length of time a sperm cell is stored or developed in the epididymis, and furthermore, the epididymis is located outside of the testicles, within the scrotum, which makes the sperm cells more susceptible to environmental stressors such as temperature (Sanger 2012). If sperm cells spend too little or too much time in the epididymis, secondary abnormalities such as distal droplets, detached heads, or bent tails become more prevalent in the ejaculate.

Environmental Factors

Fertility can be highly variable over the life span of the bull and can be influenced by environmental conditions. Natural service bull semen production and quality are influenced by factors such as age, nutrition, social interactions, and environmental temperature. Artificial insemination bulls are also exposed to these environmental factors and are additionally affected by collection intervals and the bull handling procedures.

Age

The average bull reaches puberty at 11 months of age. Once a bull reaches puberty, he will begin expressing important reproductive behavioral traits and producing spermatozoa. In the early stages of puberty, fertility is usually compromised due to the continuing development of important nerves, muscles, and hormones associated with semen production. First ejaculates

generally contain low numbers of viable spermatozoa (Senger 2012). Fuerst-Waltl et al. (2006) found ejaculate volume and total NSP increased with age while CONC decreases with age. Furthermore, differences in the maximum age when a bull can still produce viable spermatozoa has been found for bulls in a natural service scenario versus AI bulls in the stud. Barth and Waldner (2002) found natural service bulls age four to six had the most satisfactory scores on a BSE; whereas, bulls seven to nine years old had the highest percentage of unsatisfactory BSE scores. Unfortunately, Barth and Waldner (2002) had a limited number of bulls over six years of age in the study. Menon et al. (2011) performed a study with relatively few bulls older than 26 months of age and reported the reason for the limited number of bulls over 26 months of age was due to most bulls being culled because they could not physically perform or did not meet BSE requirements. Taylor et al. (1985) found semen production of a bull in stud remained steady for bulls aged 22 months to seven years of age, slightly decreased at seven years and then remained steady until nine years of age and then drastically dropped off after nine years of age. Karoui et al. (2011) analyzed a variety of environmental effects pertaining to AI bulls and found age had the largest impact on semen production traits. Karoui et al. (1989) noted similar results to Taylor et al. (1989), with an increase in production until two years of age, a plateau until five years of age, and then a steady decline until bulls reached ten years of age, after which bulls were typically culled. Age is also associated with more sexual experience and allowing bulls sexual experience increases their sexual activity and number of females serviced (Bertram 1999). For example, Silver and Price (1986) proved young bulls need to learn the correct mounting orientation to be able to effectively service females.

Nutrition

Nutrition has also been shown to affect bull fertility. Pre-pubertal nutrition affects the fertility of bulls later in life, as it affects age at puberty, testicle size at sexual maturity, and the ability to produce sperm (Brito et al. 2006). Bulls fed a higher energy diet have been shown to have increased SC size (Coulter et al. 1997). Young beef bulls fed a moderate energy diet had more morphologically normal spermatozoa and had more progressively motile spermatozoa than bulls fed a high energy diet (Coulter et al. 1997).

Social Interactions

Bull to female ratio has been shown to affect fertility because mating activity tends to increase with the number of females in heat (Pexton et al. 1990). However, there is a limit to the number of cows a single bull can service. Beerwinkle (1974) found that 90% of the females were mounted at a bull to female ratio of 1:30, whereas at a ratio of 1:60 only 65% were mounted. The percentage of females mounted decreased even further when the ratio was increased to 1:100.

Fertility is further impacted by the number of bulls present in a single pasture. Chenoweth (1983) proved sexual activity of bulls increased in the presence of other males. Furthermore, the social order of the herd has been shown to affect servicing rates. Dominant bulls perform more matings than their subordinate contemporaries (Fritz et al. 1999). Dominant bulls affect subordinate bulls' access to females, and although the consequence to differences in fertility may not be great, subordinate bulls may not be able to show their true potential (Fordyce et al. 2002). In a study determining the number of calves sired by a bull in a multi-sire scenario, Van Eenennaam et al. (2007) found 50% of the calf crop in a multi-sire pasture was sired by 5 of the 27 bulls in the study. Furthermore, 10 of the bulls in the study had no progeny, and of those bulls, 9 were yearlings.

Temperature

Bulls are additionally affected by extremes in temperature which could cause either cold or heat stress. Bulls experiencing cold stress are less likely exhibit sexual behavior due to prioritizing maintenance behavior such as finding feed or shelter (Hall 1989). Barth and Waldner (2002) also reported that bulls BSE tested in January, February, and March were less likely to be classified as a satisfactory breeder compared to bulls tested in April through July. Barth and Waldner (2002) accounted for age because the study involved bulls a variety of ages. Barth and Waldner (2002) conducted the study in Canada and attributed the increased classification of unsatisfactory breeders was due to extreme cold stress. On the other hand, Arnold and Dudzinski (1978) determined that bulls experiencing heat stress are less likely to service females. Vogler et al. (1993) reported that elevated temperatures did not affect the total ejaculate concentration but did impact sperm morphology. Holstein bulls were administered a scrotal insulation for 48 hours to simulate a heat stress event. Semen was collected from the bulls every three days for 49 days., and each collection involved two ejaculates collected in succession. Collections were then evaluated to determine the effect of the single heat stress events during spermatogenesis. Morphological changes were first noticed at day 12 of spermatogenesis and progressed through day 30. Morphological abnormalities included tailless, diadem, pyriform and nuclear vacuoles, knobbed acrosome, and Dag defect (Whittier and Bailey 2009).

Environmental Conditions Specific to AI Bulls

Artificial insemination bulls are additionally exposed to certain environmental factors pertaining to the semen collection process that affect semen production and quality. Preparing the bull prior to ejaculation by providing visual, olfactory, or auditory cues linked with sexual

activity are associated with increased semen quality (Amann and Almquist 1976). Furthermore, preparing the bull by prolonging the period of stimulation by using false mounts or restraint (Amann and Almquist 1976) increased MOT, sperm survival rate, and conception rate (Chenoweth 1983). Fuerst-Waltl et al. (2006) tested for the effect of collection interval with intervals varying from one to three days, four to six days, seven to nine days, and more than ten days. Collection intervals of six to nine days yielded increased ejaculate VOL and total NSP. Fuerst-Waltl et al. (2006) also collect, one, two, and three ejaculates, and first ejaculates produced the best ejaculate VOL, CONC, and NSP per ejaculate (Fuerst-Waltl et al. 2006).

Genetic Evaluation of Bull Fertility

Phenotypic semen quality traits are good areas to begin evaluating bull fertility and should be considered when purchasing a bull to ensure that the bull is capable of servicing cows. However, it has been documented that bull fertility is also influenced by genetic factors (Corbet et al. 2013; Huang et al. 2011), so bull fertility could be improved through selection within the context of breeding programs. Semen quality is recognized as a major determinant of bull fertility, and estimates of heritability have been published. Measurements obtained from BSE have been used to determine genetic parameters and heritability of SC, MOT, semen abnormalities (ABNO), and common defects of the penis (Christmas et al. 2001; Smith 1989). Additionally, technologies such as whole genome sequencing and genome-wide association studies (GWAS) have revealed differences between fertile and infertile dairy bulls (Peddinti et al. 2008; Feugang et al. 2010; Gaviraghi et al. 2010).

Semen production traits are relatively easy to measure, and there are often several records available per bull if they have been collected at an bull semen collection facility. Selection for semen production and quality traits is an attractive prospect because heritability estimates for

semen production and quality traits are relatively similar across studies, and there are favorable relationships between semen quality traits.

Many different terminologies are used across the industry to describe semen production and quality traits. Table 1.1 summarizes the different measurements utilized to evaluate bull fertility. Table 1.1 also includes abbreviations, definitions, and units of measurement as a reference.

Genetic Parameters

Tables 1.2 and 1.3 concisely outlines reported heritabilities published within the scientific literature. All reported studies used a best linear unbiased prediction (BLUP) animal model, with the exception of Smith et al. (1989) which used a least square mean method with a sire model. Each reported study had varying fixed effects incorporated in the model.

Scrotal Circumference

Scrotal circumference is often used to predict the number of sperm cells produced and is measured using circular tape around the widest part of the testicles (Whittier and Bailey 2000). Bourdon and Brinks (1985) obtained 4,233 records on Hereford bulls to estimate the heritability of SC and published an estimate of 0.53 utilizing a least square means method. Similarly, Christmas (2001) estimated the heritability of SC to be 0.56 in Angus bulls. In a study of tropical composite bulls, SC heritability was estimated at 6, 12, 18, and 24 months of age and reported estimates of 0.41, 0.46, 0.43, and 0.44, respectively Corbet et al. (2013). Thus, age does not seem to result in changes in the heritability estimate of SC.

Semen Production Measures

Volume

Semen volume is the total amount of the ejaculate, which is expressed in milliliters. Taylor et al. (1985) performed a genetic evaluation on semen production traits in AI dairy bulls and estimated the heritability of VOL to be 0.18. More recently, similar estimates of 0.22 and 0.26 were reported by Druet et al. (2009) and Suchoki and Syzda (2015), respectively, and both studies utilized data from dairy bull semen collection facilities. Berry et al. (2019) estimated the heritability of VOL on a population of AI bulls which contained both dairy and beef bulls and reported an estimate of 0.20. In a study of AI ram fertility, David et al. (2006) estimated the heritability of VOL to be 0.22, which mirrored that of the heritabilities found in the aforementioned studies. All studies used similar types of data including utilizing males in stud from a variety of breeds and ages. These studies also used an animal model to determine heritabilities. On the other hand, Kaps et al. (2000) and Kealy et al. (2006) reported low heritability estimates for VOL in beef bulls (0.04 and 0.09, respectively). Kaps et al. (2000) and Kealy et al. (2006) obtained VOL measurements from yearling Simmental and Hereford bulls, respectively, from ejaculations taken to perform a BSE, which could have caused the difference from the reported heritabilities above because these measurements are taken on young bulls.

Concentration

Concentration is the amount of sperm cells in the ejaculate, expressed as millions per milliliter and is measured with a colorimeter (Suchoki and Syzda 2015). Knights et al. (1984) estimated the heritability of CONC in Angus bulls to be 0.13 ($n = 717$), whereas Mathevon et al. (1998) estimated the heritability of CONC to be 0.52 in a population of Holstein dairy bulls ($n = 198$). Kaps et al. (2000) and Kealy et al. (2006) reported similar heritability estimates for CONC of 0.26 and 0.16, respectively. Kaps et al. (2000) and Kealy et al. (2006) both performed studies

on beef bulls with a similar number of bulls in the study (n= 955 and 841, respectively), but Kaps et al. (2000) data was collected from a herd of Line 1 Herefords from 1967 to 2000. Suchoki and Szyda (2015) found a heritability of CONC to be 0.34 in 1212 AI dairy bulls. Reporting a slightly lower heritability estimate, David et al. (2006) predicted the heritability of CONC to be 0.24 in rams.

Number of Spermatozoa

Number of sperm is a function of both CONC and VOL. It is expressed in millions of sperm cells per ejaculate. Taylor et al. (1985) estimated the heritability of NSP to be 0.03 in a population of 2351 AI dairy bulls. In contrast, Mathevon et al. (1998) estimated the heritability to be 0.38, but only 198 Holstein bulls were included in the study. Taylor et al. (1985) attributed the low heritability estimates partially to the fact that the total NSP is calculated by multiplying CONC and VOL, which would impact the ability to accurately provide genetic estimates pertaining to the trait since the trait is calculated based on the measurements of two other traits. Knights et al. (1984) found a heritability estimate of 0.24 in a population of 717 Angus bulls. In a study of rams, the heritability of NSP was estimated to be 0.18 (David et al. 2006). More recently, Suchoki and Szyda (2015) estimated the heritability to be 0.27 in Holstein-Friesian dairy bulls, and Berry et al. (2019) reported an estimate of 0.38 in a population of 787 beef and dairy AI bulls. Suchoki and Szyda (2015) and Berry et al. (2019) both utilized animal models, but Suchoki and Szyda (2015) only included fixed effects of bull age and season, whereas Berry et al. (2019) additionally included fixed effects of inbreeding, days since last ejaculation, and breed.

Semen Quality Measures

Motility

Motility is a trait which refers to the ability of the sperm to move progressively forward (Sanger 2012). Progressive motility (PROG) is a term often used to characterize whether sperm cells are swimming in mostly straight lines or large circles. Both MOT and PROG define whether or not the sperm is moving forward with a purpose, but PROG is a term which can be used to distinguish between forward movement, circular movement, or no movement in the sample. Motility is scored as a percentage of the ejaculate. Smith et al. (1989) reported a heritability estimate for sperm MOT to be 0.08 in a population of 549 yearling beef bulls receiving a BSE; however, the standard error reported was 0.07. Mathevon et al. (1998) estimated that heritability of MOT was 0.31 in a population of 198 Holstein bulls and did not report standard errors, but the large difference could be attributed to the extremely small population size. Christmas et al. (2001), Gredler et al. (2007), and Garmyn et al. (2011) reported heritabilities ranging from 0.04-0.07 in populations of yearling beef bull with a population size of 1282, 301, and 1281, respectively. Disparately, in a study of 841 Line 1 Hereford bulls, Kealy et al. (2006) estimated heritability to be 0.22. Comparatively, the bulls were the same age, all beef breeds, and the animal models contained similar variables. One potential for the difference in estimates could be due to the increased level of inbreeding in the Line 1 Hereford population. Corbet et al. (2013) estimated heritability for PROG to be 0.15 in both Brahman and tropical composites. The results of Corbet et al. (2013) were interesting because the heritability of MOT decreased as age decreased from 12, 18, and 24 months. In the Brahman population, heritability estimates were 0.44, 0.15. and 0.05, respectively. The decrease in heritability could be due to greater variation in environmental effects as the bulls aged. The bulls were all managed the same throughout the study, so rather than differences in management, differences could be attributed

to puberty or maturity. Druet et al. (2009) estimated the heritability of MOT to be 0.43 in a population of 12 to 18 month old Holstein bulls, and Suchoki and Szyda (2015) reported a heritability estimate of 0.31 in a population of AI dairy bulls with an average age of 32 months. Furthermore, Druet et al. (2009) estimated the heritability of percentage progressive spermatozoa to be 0.13 with a standard error of 0.13, which indicates that the measure may not be heritable.

Motility Score

Motility score (MOSC) is a subjective score based on a scale which varies depending on the individual standards of the collection protocol. A higher MOSC is more desirable. Ducrocq and Humblot (1995) based MOSC from 0 to 4 and estimated the heritability of MOSC in Normande bulls to be 0.23. In a more recent study of dairy bulls, Suchoki and Szyda (2015) estimated the heritability of MOSC to be 0.26. The MOSC used in the Suchoki and Szyda (2015) study ranged from 1 to 3. Mass activity (MASS) is similar to MOSC. It is based on a 0 to 5 scale, with 0 being no activity and 5 being rapid, distinct swirls. Corbet et al. (2013) estimated the heritability of MASS in 18-month-old Brahman bulls to be 0.24.. The studies mentioned utilized an animal model which included different fixed effects. Ducrocq and Humblot (1995) utilized data obtained from bulls in a performance test and included fixed effects of birth year, season of birth, station of performance test, and total number of collections. Suchoki and Szyda (2015) utilized data obtained from an bull semen collection facility in Poland and included fixed effects of bull age at time of collection, season of collection, and insemination center. Corbet et al. (2013) conducted the study at a research station and included the fixed effects of year, birth location, birth month, post-weaning location, dam age, previous lactation status, and dam management groups. A variety of fixed effects were used in the various models and the scoring protocols were not uniform, thus the studies yielded variable heritability results for MOSC.

Percentage of Normal Spermatozoa

Gross percentages of normal sperm measure the percentage of sperm cells with an acceptable morphology. Yilmaz et al. (2004) estimated the heritability of PNS to be 0.47 in yearling Angus bulls. Corbet et al. (2013) estimated the heritability of PNS to be 0.41 in yearling tropical composite bulls, which is consistent with the Yilmaz et al. (2004) estimate, despite differences in species. Kealy et al. (2006) reported a slightly lower estimate of 0.35 for PNS in a population of 14-month old Line 1 Hereford bulls. Smith et al. (1989) and Gredler et al. (2007) found substantially lower heritability estimates (0.07 and 0.10, respectively) in populations of beef bulls. Differences could be caused by population size, Yilmaz et al. (2004), Corbet et al. (2013), and Kealy et al. (2006) had population sizes ranging from 765 to 2424, and Smith et al. (1989) had a population size of 549. Gredler et al. (2007) utilized VCE4 (Groeneveld 1998) to estimate the genetic parameters, whereas Yilmaz et al. (2004), Corbet et al. (2013), and Kealy et al. (2006) heritability estimates were all determined by utilizing a version of REML.

Abnormalities

Abnormalities or percent abnormalities is a measure the sperm cells in an ejaculate that are aberrant or have undesirable characteristics. Percent abnormalities is the inverse of PNS, but generally, studies report either PNS or total ABNO. Ducrocq and Humblot (1995) reported a heritability estimate of 0.19 for ABNO in a population of 2387 Normande dairy bulls. Christmas et al. (2001) reported a heritability estimate of 0.29 for ABNO in a population of 1282 Angus bulls. Differences in breed and population size could account for the difference, but otherwise, the data was collected from bulls to simply evaluate their semen quality for a BSE. Ducrocq and Humblot (1995) accounted for the combination of birth year, season of birth, station of performance test and total number of collections; whereas, Christmas et al. (2001) accounted for

age in days at the time of evaluation and contemporary group effects. Druet et al. (2009) and Silva et al. (2011) reported similar estimates of 0.25 in a population of dairy bulls and 0.15 in a population of Nellore bulls, respectively. Marques et al. (2017) performed a fertility study in swine, reporting a heritability estimate of 0.19 for ABNO.

Abnormalities can be further divided into PRIM or SEC. Smith et al. (1989) reported heritability estimates of PRIM and SEC to be 0.31 and 0.02. The estimate of 0.02 had a standard error of 0.05, and in addition, Smith et al. (1989) utilized a least square means approach to calculating his heritabilities, rather than REML, which was used by the other comparable studies. Christmas (2001) estimated the heritability of abnormalities to be 0.35 and 0.26 for PRIM and SEC, respectively. Garmyn et al. (2011) estimated the heritability of PRIM and SEC to be 0.27 and 0.23, respectively, in a study of Angus bulls. Both of these studies were performed by Kansas State University, utilizing the same type of data and statistical methods. The studies accounted for age of bulls and contemporary group effects as fixed effects. Christmas et al. (2001) utilized bulls ranging from 288 to 455 days with an average of 383, whereas Garmyn et al. (2011) utilized a data set of bulls ranging from 288 to 549 days with an average age of 387 which could be the reason for the slight difference in heritability estimates.

Genetic Correlations

Scrotal Circumference with Semen Quality

Smith et al. (1989) was one of the first to report a correlation estimate between SC and MOT. The low, unfavorable estimate of -0.04 had a standard error of 0.40. Smith et al. (1989) indicated the high standard errors could be attributed to the low estimates of genetic variation in the population of 549 bulls, of which 450 were Herefords. Contradicting Smith's estimate, Christmas (2001) found a moderate, favorable correlation between SC and MOT of 0.56 in a

population of 1282 beef bulls. Corbet et al. (2013) reported 1639 Brahman bulls and 2424 tropical composite bulls had a correlation between PROG and SC of 0.70 and 0.56, respectively. The difference in the correlations between the populations could reflect the breed composition difference. Corbet et al. (2013) estimated the correlation between MASS and SC to be 0.60, which is a moderate, favorable correlation. Although Corbet et al. (2013) was the only study reported to record mass activity, the results correspond to other the above results which reflect the same type of motility measurements. Table 1.4 concisely presents the reported correlations for comparison.

Contradictory genetic relationships have been reported between SC and PNS. Smith et al. (1989) reported a correlation of -0.36, which is unfavorable, but he also reported a standard error of 0.34, which indicates the correlation is not statistically different from zero. Nonetheless, Corbet et al. (2013) reported positive, favorable estimates of 0.50 in Brahman bulls and 0.21 in tropical composite bulls. The differing results between Smith et al. (1989) and Corbet et al. (2013) study could be due to differences in models, breed composition, or even age differences. Smith et al. (1989) utilized a least squares procedure with a sire model, whereas Corbet et al. (2013) used a BLUP animal model. Smith et al. (1989) only accounted for the fixed effects of birth year, age of dam, and breed. In addition to these effects, Corbet et al. (2013) also included the fixed effects of birth location and dam contemporary group. Perhaps a larger contributing factor to the correlation difference could be due to the fact Smith et al. (1989) utilized yearling beef bulls, and Corbet et al. (2013) provided estimates on 18-month old *Bos indicus* cattle.

Smith et al. (1989) reported correlations between SC and PRIM and SEC to be 0.14 and 1.22, respectively. Considering the Smith et al. (1989) heritability estimate for SEC was not statistically different than zero, the lack of genetic diversity in the population could explain the

reason for the anomalous correlation between SC and SEC. Christmas et al. (2001) reported negative, favorable correlations between SC and abnormal sperm traits, which translates to higher SC corresponding to fewer abnormalities. More specifically, Christmas et al. (2001) reported a correlation of -0.32 between SC and ABNO and -0.25 and -0.40 for SC and PRIM and SEC, respectively. Garmyn et al. (2011) also reported negative, favorable correlations between abnormalities and SC, although the correlation estimates were lower than Christmas et al. (2001). Garmyn et al. (2011) estimated the correlation between SC and ABNO, PRIM, and SEC to be -0.23, -0.19, and -0.11, respectively; however, the standard errors are nearly the same as the estimates. Genetic and phenotypic correlations between SC and semen quality traits for all reported studies are typically in the same direction, but phenotypic correlations are generally much lower. Refer to Table 1.4 to compare genetic and phenotypic correlations.

Semen Production Measures

Table 1.4 includes genetic and phenotypic correlations with standard errors between semen production measures. Studies that did not report standard errors are indicated with an * next to the genetic correlation.

Druet et al. (2009) reported a -0.55 correlation with a standard error of 0.18 between VOL and CONC. The negative correlation is unfavorable in this case because as VOL increases, CONC decreases. Ducrocq and Humblot (1995) reported a genetic correlation between VOL and CONC of -0.30, but the phenotypic correlation between the same two traits was -0.15. Ducrocq and Humblot (1995) reported frequencies of inbreeding in the population ranged from 0.008 to 0.022 over the time period of the study (1975-1986), which could impact sperm production traits. Taylor et al. (1985) reported a similar unfavorable correlation estimate between VOL and CONC (-0.10). On the contrary, Berry et al. (2019) estimated that the correlation between VOL and

CONC was 0.40; however, a standard error of 0.20 was reported, and the phenotypic correlation was estimated to be 0.01. Additionally, in a study of ram fertility, David et al. (2006) reported a correlation between VOL and CONC to be 0.24, which is a positive, favorable correlation.

Reported correlations between VOL and NSP are all high, positive, and favorable, which is to be expected as total NSP is impacted by the VOL. Druet et al. (2009) reported a favorable genetic correlation of 0.47 and a phenotypic correlation of 0.71. Similarly, Taylor et al. (1985) reported a correlation estimate of 0.45. Much higher estimates have also been reported, such as Gredler et al. (2007) and Berry et al. (2019), who reported correlations of 0.84 ± 0.13 and 0.66 ± 0.16 , respectively. David et al. (2006) reported a correlation of 0.84 between the same two traits. The correlation between CONC and NSP yielded similar results, as CONC is the additional factor that goes into calculating total NSP. As summarized in Table 1.4, Druet et al. (2009), Taylor et al. (1985), Gredler et al. (2007), Berry et al. (2019), and David et al. (2006) reported positive correlations, which are desirable because as CONC increases, so does the NSP in an ejaculate.

Semen Quality Measures

Relatively few studies have quantified the interrelationship of varying MOT measures. Druet et al. (2009) reported a strong, positive relationship, as would be expected, between MOT and MOSC, with an estimate of 0.88 ± 0.06 . Druet et al. (2009) measured the percentage of progressive spermatozoa (%PROG), and when correlated with MOT reported an estimate of 0.74; however, the standard error was 0.30. A phenotypic correlation of 0.48 was reported by Druet et al. (2009) indicating there is some environmental affect. A strong, favorable correlation is expected, as the %PROG is dependent on the MOT of the ejaculate. Motility is strongly, favorably correlated with %LIV (0.58; Druet et al. 2009). Furthermore, MOSC is favorably

correlated with %PROG and %LIV with estimates of 0.71 ± 0.27 and 0.61 ± 0.15 , respectively (Druet et al. 2009). Corbet et al. (2013) estimated the correlation between PROG and MASS and found high, favorable correlations of 0.99 and 0.98 in Brahman and tropical composite bulls, respectively. In this population, these correlations are high enough to suggest that PROG and MASS are the same traits.

Smith et al. (1989) reported the correlation between MOT and PNS was moderate and favorable (0.43); however, the standard error reported was 0.64. The large standard error could be attributed to a large variability in the results. Kealy et al. (2006) reported a similar correlation estimate between MOT and PNS but did not report standard errors. In a group of tropical composite bulls, Corbet et al. (2013) reported a strong, favorable correlation between PROG and PNS with an estimate of 0.77 ± 0.09 .

Genetic relationships between MOT and traits related to the percentage of abnormal spermatozoa were negative with correlations of -0.55 (total), -0.56 (abnormal head; HEAD), -0.24 (abnormal tail; TAIL), and -0.13 (abnormal cytoplasmic droplet; DROP), indicating that abnormalities were associated with a low MOT (Druet et al. 2009). The results were analogous with the negative, favorable correlation estimates between MOSC and abnormal spermatozoa traits reported by Ducrocq and Humblot (1995) and Druet et al. (2009). Marques et al. (2017) agreed with the negative, favorable correlation between sperm MOT and ABNO and reported an estimated genetic correlation of -0.81 ± 0.03 ; however, this genetic correlation was obtained from a population of boars. Smith et al. (1989) reported similar correlations between MOT and PRIM and SEC at -0.36 and -0.71, respectively. On the other hand, Kealy et al. (2006) reported a positive, unfavorable correlation between MOT and PRIM (0.57). No standard errors were published. Total abnormalities was negatively and favorably correlated to %PROG with an

estimate of -0.38; however, the standard error was 0.38 (Druet et al. 2009). Furthermore, Druet et al. (2009) reported percentages of sperm with abnormal HEAD, TAIL, or DROP that reflected the same negative, favorable correlation with %PROG and %LIV, but these too had large standard errors. Druet et al. (2009) concluded that as the %PROG increased, so did the percentage of living sperm after thawing and reported a strong, favorable genetic and phenotypic correlation between those traits of 0.89 ± 0.17 and 0.70 , respectively.

Corbet et al. (2013) reported a strong, favorable correlation of 0.87 , indicating that as the MASS increased, so did the PNS. The PNS was found to be strongly, favorably correlated to PRIM (Smith et al. 1989). Smith et al. (1989) reported a correlation of -0.85 ± 0.63 ; however, PNS was unfavorably correlated with the percentage of SEC with an estimate of 0.16 ± 1.54 . The reported standard error is outside of the parameter range for a standard error, which diminishes the accuracy of the results.

As expected, Druet et al. (2009) reported strong, favorable correlations between percentage of ABNO and HEAD (0.71) and TAIL (0.78). Unexpectedly, an unfavorable, negative correlation with DROP (-0.15) was reported; however, the standard error was 0.34 . Percentage of spermatozoa with an abnormal head was lowly, positively correlated with TAIL (0.13), but negatively, correlated with DROP (-0.43). Percentage of spermatozoa with an abnormal tail is very lowly correlated with DROP with an estimate of 0.06 . It is difficult to speculate whether or not the correlations could necessarily be considered favorable or not, but certainly, some correlation could be advantageous for selection against abnormalities. Phenotypic correlations between ABNO and HEAD, TAIL, or DROP were 0.61 , 0.77 , and 0.36 . The phenotypic correlation between ABNO and DROP was significantly different from the genetic correlation, which means there was a larger environmental component that affected the

cytoplasmic droplets. In addition, Druet et al. (2009) reported no phenotypic correlation between HEAD and TAIL (0.02).

Semen Production Measures with Semen Quality Measures

Druet et al. (2009) reported that the correlation between VOL and MOT was negative and unfavorable (-0.20) in a population of dairy cattle. Kealy et al. (2008) reported a correlation between VOL and MOT of 0.38 in a population of Line 1 Herefords. Druet et al. (2009) reported an estimate of -0.17 between VOL and MOSC, which is unfavorable and similar to the estimate Druet et al. (2009) reported between VOL and MOT. Ducrocq and Humblot (1995) published an estimate of -0.03 ± 0.21 between VOL and MOSC. Gredler et al. (2007) reported a correlation between VOL and MOSC of 0.21; however, the reported standard error was 0.17, and the phenotypic correlation was -0.12. Volume was unfavorably correlated to %PROG with an estimate of -0.53 (Druet et al. 2009). Volume was also unfavorably genetically correlated with %LIV (-0.47 ± 0.26). The reported phenotypic correlation between VOL and %LIV was -0.10 (Druet et al. 2009). Ducrocq and Humblot (1995) also reported a negative, unfavorable genetic correlation of -0.16 between VOL and %LIV, with a standard error of 0.21. Volume was unfavorably correlated with ABNO, with an estimate of 0.23 ± 0.26 (Druet et al. 2009). On the other hand, Ducrocq and Humblot (1995) reported a correlation between VOL and ABNO of -0.26, but the standard error was 0.24. Druet et al. (2009) further specified the type of abnormalities as HEAD, TAIL, or DROP and found correlations with VOL to be -0.12, 0.43, and 0.32, respectively. All reported correlations had large standard errors, so the correlations were not different from 0.

Druet et al. (2009) found a low, favorable correlation between CONC and MOT with an estimate of 0.12 ± 0.20 . Kealy et al. (2006) also found a positive, favorable correlation between

CONC and MOT, but it was much stronger (0.81). No standard errors were reported. Furthermore, Knights et al. (1984) estimated the correlation between the two traits to be 1.01. Knights et al. (1984) did not report standard errors, but the unusual correlation could be attributed to the low heritability estimates reported which indicate little variance in the population used in the study. Dreut et al. (2009) estimated CONC and MOSC had a slight, negative correlation of -0.01; whereas, Ducrocq and Humblot (1995) and Gredler et al. (2007) reported strong, favorable correlations of 0.67 ± 0.11 and 0.48 ± 0.17 , respectively. Furthermore, as CONC increased, so did the %PROG. Druet et al. (2009) reported a favorable correlation of 0.35; however, the standard error estimate was 0.36, so the correlation was not different from zero. Ducrocq and Humblot (1995) estimated the correlation between CONC and %LIV to be 0.54. The strong, positive correlation is favorable, and the reported standard error was 0.15. The corresponding phenotypic correlation was 0.47. Druet et al. (2009) also reported a favorable correlation of 0.29 between CONC and %LIV, but the standard error was 0.26. More recently, Berry et al. (2019) reported a correlation estimate of 0.37, but the standard error was also large for this estimate (Table 1.4). Concentration was favorably correlated to ABNO and HEAD, TAIL, and DROP, with estimates of -0.34, -0.23, -0.33, and -0.09, respectively (Druet et al. 2009). Furthermore, Kealy et al. (2006) reported correlation estimates between CONC and PRIM to be 0.36. This unfavorable correlation contradicts results Kealy et al. (2006) reported between CONC and SEC of -0.58, which is favorable. Without reported standard errors, it is difficult to evaluate the accuracy of the estimates.

Druet et al. (2009) found the genetic correlation between NSP and MOT to be -0.12 though the reported standard error was 0.25. The corresponding phenotypic correlation between the two traits was -0.03. Berry et al. (2019) reported a positive, favorable correlation between

NSP and MOT with an estimate 0.36 and standard error of 0.08. Furthermore, Druet et al. (2009) reported that the correlation between NSP and MOSC was also unfavorable at -0.24, but this estimate had a large standard error. On the other hand, Gredler et al. (2007) estimated the correlation between NSP and MOSC to be favorable (0.50 ± 0.13). As the NSP increases, the %PROG decreases due to an unfavorable correlation of -0.22 (Druet et al. 2009). A negative, unfavorable correlation was also reported between NSP and %LIV of -0.18 (Druet et al. 2009). Gredler et al. (2007) estimated the correlation between NSP and PNS to be 0.54 with a standard error of 0.11. The favorable correlation found in Gredler et al. (2007) study could be attributed to the fact that the data was collected from an bull semen collection facility on a population of 301 Simmental cattle. Number of sperm was very lowly correlated with ABNO (-0.09 ± 0.38 ; Druet et al. 2009). Furthermore, Druet et al. (2009) reported a negative, unfavorable genetic correlation between NSP and HEAD, although this estimate had a large standard error. On the other hand, NSP had a favorable correlation with TAIL or DROP with estimates of 0.14 ± 0.54 and 0.33 ± 0.43 , respectively (Druet et al. 2009). Corresponding phenotypic correlations between NSP and abnormalities were low (-0.02 to -0.05).

Bull Fertility Index

The sire conception rate (SCR) evaluation encompasses inbreeding of the bull, inbreeding of the embryo from the mating, age of the bull, artificial insemination center the bull was collected at, year of collection, and the effect of the bull itself. The SCR is a selection index which provides genetic prediction for artificial insemination dairy bulls. It has been realized SCR does not account for bulls with poor quality semen attributes as bulls with inferior semen quality are removed from the population because of the strict semen freezing requirements established by artificial insemination centers. While there are concerns pertaining to the variation in SCR

phenotypes, it has been determined there is more than 10% difference in conception rate between high and low fertility bulls measured by SCR (Penagaricano et al. 2012; Rezende et al. 2018)

Determining which bull fertility traits to measure when making selection decisions has proven to be difficult, and it has been suggested the only way to truly measure bull fertility is by directly measuring conception rate (Penagaricano et al 2012). Bull fertility indices have the opportunity to provide beef producers with the ability make selection decisions on traits which are difficult to measure or not observed until later in life, such as conception rate. Additionally, utilizing genomic prediction has the potential to provide more predictive power for estimating genetic merit for bull fertility. Many of the advancements in utilizing genomic prediction pertaining to measuring bull fertility have been led by the dairy industry (Rezende et al., 2018; Nani et al., 2019; Han et al., 2016). While ample beef bull fertility phenotypes do exist from both bull studs and individual producers who use BSEs, the lack of data flow between the phenotype holders and evaluation providers prevents the ability to provide such an index to beef producers using traditional evaluation techniques, let alone genomic prediction.

Genomic Prediction

The genomic prediction of polygenic traits, such as fertility, has revolutionized agriculture and human medicine. Although genomic prediction for improved cow fertility has received a lot of attention, beef bull fertility has largely been ignored (Adbollahi-Arpanahi et al. 2017). With genomic prediction, researchers can utilize information on the bull's entire genome to assist in predicting the fertility of the bull. Incorporation of genome sequence and other biological information with genomic predictions has the potential to make crucial connections between biology and performance that may save producers money as well as improve the accuracy of genomic prediction in the future (Taylor et al. 2018).

Genome-wide association studies have been successful in identifying genomic regions and individual variants associated with numerous complex traits, and some weighted genomic prediction models can incorporate this information by placing more emphasis on these markers in the prediction. The incorporation of these data into predictive models could positively affect both model predictive ability and model robustness (Abdollahi-Arpanahi et al. 2017). While few genomic studies have been performed on beef bulls, analyses including dairy bulls has identified several SNPs within or near genes associated with male fertility traits. Table 5 outlines various genes which have been associated with male fertility traits in cattle.

A few genes pertaining to spermatogenesis have been identified. The gene *PIWIL3* has been identified as associated with spermatozoa development in Holstein cattle (Nicolini et al., 2018). This gene encodes a member of the PIWI family, which is a group of proteins that are essential for spermatogenesis and post-transcriptional events leading to translation (Paronetto and Sette 2010). Another gene found on chromosome 17 that is associated with spermatogenesis is *TMEM119* (Nicolini et al., 2018). *TMEM119* encodes a transmembrane protein actively involved in osteoblast differentiation and is expressed in spermatocytes and spermatids in developing testis (Mizuhashi et al., 2015).

Several genes associated with spermatozoa motility were identified by Ferenčakivoć et al. (2017), when studying inbreeding depression in Austrian Fleckvieh bulls. *SLC25A31* is associated with mediating energy generation and consumption in the distal flagellum; therefore, influencing motility. *CDH18* is a member of the cadherin superfamily which is responsible for mediating calcium dependent cell to cell adhesion and has been found to be associated with spermatozoa motility (Pachecho et al. 2011). *KCNU1* is a gene found on chromosome 27 which is known to encode a testis-specific potassium channel. This channel is involved with

maintaining normal sperm morphology and motility (Schreiber et al. 1998). Rezende et al. (2018) identified a SNP within COX7A2L on chromosome 11. This gene encodes cytochrome c oxidase which is responsible for promoting respiratory supercomplex assembly and regulates energy generation. It has been speculated that this process is involved in sperm motility. Rezende et al. (2018) additionally identified other SNPs within or near genes ZMYND10, SLC25A20, and DNAH3 that were associated with sperm motility traits. ZMYND10 is a gene exclusively expressed in the testis which is involved in cilia integrity and has been implicated in sperm dysmotility, and thus infertility (Moore et al. 2013). SCL25A20 is a gene involved in ATP production and cell energy metabolism (Asgihari et al. 2017). DNAH3 encodes a member of the dynein family and has been proven to cause abnormalities pertaining to the sperm flagella and as a result negatively impact sperm motility (Khelifa et al 2014).

Many genes associated with the percentage of living sperm are noted in the literature. A gene on chromosome X called SPATA16 was found to cause infertility in humans (Dam et al. 2017). The infertility results from spermatogenesis defects which cause malformation of the spermatozoa. Ferenčakivoć et al. (2017) found a SNP associated with this gene in Austrian Fleckvieh cattle that was associated with formation of the spermatozoa. Additionally, Ferenčakivoć et al. (2017) found a SNP on chromosome one associated with the GPX5 gene. This gene encodes epididymis secretory sperm binding protein Li 75p, which is a protein with predicted involvement in protecting the spermatozoa membrane from lipid peroxidation during oxidative stress, and potentially preventing a premature acrosome reaction (Hall et al., 1998). Ferenčakivoć et al. (2017) also identified NYD-SP5 and PIAS1 on chromosome one to be associated with the percentage of living spermatozoa. Yin et al. (2005) found NYD-SP5, which encodes testis development protein, and plays a regulatory role in spermatogenesis. Vernocchi et

al. (2014) noted that PIAS1 is associated with androgen receptor-mediated initiation and the maintenance of spermatogenesis.

Additional genes have been found to be involved in various male fertility processes. For example, SPO11 and RAD21L1 were found to be associated with gametogenesis (Nicolini et al. 2018). SPO11 is a topoisomerase-like protein responsible for the formation of DNA double-strand breaks that occur during meiotic recombination (Keeney et al. 1997). RAD21L1 encodes a protein that is involved in multiple aspects of meiosis (Choi et al. 2008). Genes associated with acrosome reaction and the fertilization process were identified in a study with Jersey bulls (Rezende et al. 2018). These genes are PKDREJ, STX2, and FER1L5. PKDREJ encodes a sperm surface receptor which mediates the sperm-egg interaction (Hamm et al. 2007). STX2 encodes a protein family that controls membrane fusion during the acrosome reaction (Hutt et al. 2005). FER1L5 encodes proteins which are important during fusion events involved in spermatogenesis (Washington and Ward 2006).

Several genomic regions have been associated with sire conception using GWAS. The genes and their chromosome locations are outlined in Table 5. Sire conception rate is currently only predicted in the dairy industry. The function of the genes associated with sire conception rate range from encoding proteins involved in sperm maturation (PARP11) and sperm motility (AKAP3) to encoding proteins which play a role in testis development (IGF1R) and the fertilization process (CCT6A ; Han and Penagaricano 2016).

Beyond standard genomic prediction, there is an additional desire to better understand the underlying biology of male fertility. While utilizing this technology to identify genes in the cattle industry is rare, a few examples include utilizing dual targeted β -defensin and exome sequencing to identify bull fertility genes in the dairy industry (Whiston et al. 2017; Lyons et al., 2018).

Genes identified have been found to be involved with spermatogenesis (Whiston et al. 2017) and sperm binding (Lyons et al. 2018).

Bull fertility has the possibility to be significantly impacted by genomic selection. SNP genotyping continues to gain popularity in the beef industry (Taylor et al 2018). While identifying SNPs that are located within or near certain genes pertaining to male fertility traits helps to better understand the expression of certain traits, this information is not essential for genomic selection. However, providing producers with genomic selection tools could cause a more rapid improvement in fertility traits, but in order to make genomic selection for beef bull fertility a reality, more beef bull fertility trait phenotypes are a necessity (Taylor et al. 2018).

Conclusion

Bull fertility is an economically relevant trait that merits additional research. In order to improve the efficiency of the US beef cattle herd, bull fertility improvement must be made, and change can be furthered accelerated by utilizing genetic selection tools. If beef producers had the capability to make selection decisions for improved fertility, it would save time and resources, as past research shows improvements in reproduction have resulted in more genetic improvement, greater production efficiency and increased profitability (Rogers et al. 2012). Heritability estimates pertaining to semen production traits, which include VOL, CONC, and NSP, are generally low to moderate. Due to the industry-wide implementation of the BSE, several studies have been able to evaluate the semen production traits of beef bulls. The lack of literature for beef bulls becomes more apparent when studying the genetic parameters of semen quality. Semen quality measures, for the purpose of this paper, are related to motility and morphology. Reported genetic correlations could provide a useful candidate for indirect selection to improve

fertility traits. Correlations between semen traits are generally moderate and favorable, indicating selecting for one trait could benefit another.

Genomic selection is another useful tool which can increase genetic prediction capabilities and improve selection. Genomic prediction has an even greater impact on the prediction of polygenic traits, such as fertility, because of the ability to incorporate biological information with genetic prediction. Relatively few studies have been able to identify SNPs related to male fertility and even more; reported studies pertaining to *Bos taurus* bulls do not report SNPs located near the same genes. Additional research is needed in order to be able to utilize genomic prediction for male fertility.

Studying beef bull fertility has the ability to increase beef production and could provide benefits to male fertility in other species. Limited published research and a need for improvement make bull fertility research a necessity.

Table 1.1 Description of various bull traits used to define bull fertility in the literature.

Trait	Abbreviation	Description	Units
<i>Production Traits</i>			
Concentration	CONC	relative amount of sperm cells per ejaculate, measured by a colorimeter	millions/mL
Number of sperm	NSP	calculated by multiplying sperm concentration and semen volume; expressed in millions	millions
Volume	VOL	total amount of the ejaculate	mL
<i>Quality Measures</i>			
<i>Motility</i>			
Mass Activity	MASS	sperm mass activity was score from 0-5 with 0 being no activity and 5 being rapid, distinct swirls/ rapid swirling, slower swirling, generalized oscillation, and sporadic oscillation, called motility on the BSE and rankings are very good, good, fair, poor	score
Motility	MOT	movement and swimming of sperm	%
Motility Score	MOSC	score 1-5, 1-3, 0-4	score
Progressive Motility	PROG	swimming in a mostly straight line or large circles	%
<i>Morphology</i>			
Percent Normal Sperm	PNS	percent morphologically normal sperm	%
Percentage of Living Sperm	%LIV	percent of living sperm with an intact cytoplasmic membrane after thawing	%
<i>Abnormalities</i>			
Percentage of Spermatozoa with an Abnormal Cytoplasmic Droplet	DROP	percentage of sperm cells with a defect to the cytoplasmic droplet	%
Percentage of Spermatozoa with an Abnormal Head	HEAD	percentage of sperm cells with a defect to the head of the sperm cell	%
Percentage of Spermatozoa with an Abnormal Tail	TAIL	percentage of sperm cells with a defect to the tail of the sperm cell	%
Primary Abnormalities	PRIM	generally, defects of the head of the sperm cell	%
Secondary Abnormalities	SEC	Generally. defects of the tail	%
Total Abnormalities	ABNO	percentage of abnormalities in the total ejaculate sample	%
<i>Scrotal Circumference</i>			
Scrotal Circumference	SC	circumference measured at the widest point of the scrotum with both testes fully distended	cm

Table 1.2 Reported heritabilities for commonly measured semen production traits which define bull fertility. * indicates no reported standard error.

Traits	n	Estimate	Standard Error	Breed	Source
Scrotal Circumference, 6 months	2424	0.41	0.08	Tropical Composite	Corbet et al. (2013)
	717	0.36	0.06	Angus	Knights et al. (1984)
	4233	0.53	0.06	Hereford	Bourdon and Brinks (1985)
Scrotal Circumference, 12 months	549	0.40	0.09	Hereford, Angus & Red Angus	Smith et al. (1989)
	1282	0.56	*	Angus	Christmas et al. (2001)
	1281	0.46	0.08	Angus	Garmyn et al. (2011)
	2424	0.46	0.09	Tropical Composite	Corbet et al. (2013)
	--	0.40	0.02	Nellore	Silva et al. (2011)
Scrotal Circumference, 18 months	2424	0.43	0.09	Tropical Composite	Corbet et al. (2013)
	2424	0.44	0.09	Tropical Composite	Corbet et al. (2013)
Scrotal Circumference, 24 months	2351	0.18	*	Holstein	Taylor et al. (1985)
	2387	0.65	0.09	Normande	Ducrocq and Humblot (1995)
	198	0.24	*	Holstein	Mathevon et al. (1998)
	955	0.04	0.54	Simmental	Kaps et al. (2000)
	841	0.09	0.08	Line 1 Hereford	Kealy et al. (2006)
	974	0.22	0.03	Lacaune Rams	David et al. (2006)
	301	0.18	0.02	Beef/ Dairy Cross	Gredler et al. (2007)
	515	0.22	0.05	Holstein	Druet et al. (2009)
	1212	0.26	0.06	Holstein	Suchoki and Syzda 2015
	787	0.20	0.04	Beef & Dairy	Berry et al. (2019)
	717	0.13	0.06	Angus	Knights et al. (1984)
	2387	0.39	0.10	Normande	Ducrocq and Humblot (1995)
Sperm Concentration	198	0.52	*	Holstein	Mathevon et al. (1998)
	955	0.26	0.01	Simmental	Kaps et al. (2000)
	841	0.16	0.08	Line 1 Hereford	Kealy et al. (2006)
	974	0.24	0.04	Lacaune Rams	David et al. (2006)
	301	0.14	0.04	Beef/Dairy Cross	Gredler et al. (2007)
	1212	0.34	0.07	Holstein	Suchoki and Syzda (2015)
	787	0.20	0.07	Beef & Dairy	Berry et al. (2019)
	717	0.24	0.05	Angus	Knights et al. (1984)
	2351	0.03	*	Holstein	Taylor et al. (1985)
	198	0.38	*	Holstein	Mathevon et al. (1998)
Number of Spermatozoa	974	0.18	0.03	Lacaune Rams	David et al. (2006)
	301	0.22	0.02	Beef/Dairy Cross	Gredler et al. (2007)
	1212	0.27	0.06	Holstein	Suchoki and Syzda (2015)
	787	0.38	0.05	Beef & Dairy	Berry et al. (2019)

Table 1.3 Reported heritabilities for commonly measured semen quality traits which define bull fertility. * indicates no reported standard error.

Traits	n	Estimate	Standard Error	Breed	Source
Spermatozoa motility	717	0.13	0.06	Angus	Knights et al. (1984)
	549	0.08	0.07	Hereford, Angus & Red Angus	Smith et al. (1989)
	2387	0.30	0.09	Normande	Ducrocq and Humblot (1995)
	198	0.31	*	Holstein	Mathevon et al. (1998)
	1282	0.07	*	Angus	Christmas et al. (2001)
	974	0.18	0.03	Lacaune Rams	David et al. (2006)
	841	0.22	0.09	Line 1 Hereford	Kealy et al. (2006)
	515	0.43	0.08	Holstein	Druet et al. (2009)
	1281	0.05	0.03	Angus	Garmyn et al. (2011)
	2424	0.15	0.05	Tropical Composite	Corbet et al. (2013)
	1212	0.31	0.06	Holstein	Suchoki and Syzda (2015)
	787	0.37	0.03	Beef & Dairy	Berry et al. (2019)
Progressive motility	515	0.13	0.13	Holstein	Druet et al. (2009)
	2424	0.15	0.05	Tropical Composite	Corbet et al. (2013)
Motility Score	2387	0.23	0.08	Normande	Ducrocq and Humblot (1995)
	301	0.04	0.01	Beef/ Dairy Cross	Gredler et al. (2007)
	1212	0.26	0.05	Holstein	Suchoki and Syzda (2015)
Mass activity	2424	0.13	0.05	Tropical Composite	Corbet et al. (2013)
Percentage of living spermatozoa after thawing	841	0.22	0.09	Line 1 Hereford	Kealy et al. (2006)
	515	0.21	0.08	Holstein	Druet et al. (2009)
	787	0.25	0.08	Beef & Dairy	Berry et al. (2019)
Percent normal spermatozoa	549	0.07	0.06	Hereford, Angus & Red Angus	Smith et al. (1989)
	765	0.47	0.07	Angus	Yilmaz et al. (2004)
	841	0.35	0.10	Line 1 Hereford	Kealy et al. (2006)
	301	0.10	0.03	Beef/Dairy Cross	Gredler et al. (2007)
	2424	0.41	0.10	Tropical Composite	Corbet et al. (2013)
Abnormal spermatozoa percentage/ Total abnormalities	2387	0.19	0.07	Normande	Ducrocq and Humblot (1995)
	1282	0.29	*	Angus	Christmas et al. (2001)
	515	0.25	0.10	Holstein	Druet et al. (2009)
	--	0.15	0.01	Nellore	Silva et al. (2011)
	1281	0.25	0.07	Angus	Garmyn et al. (2011)
Primary Abnormalities	549	0.31	0.09	Hereford, Angus & Red Angus	Smith et al. (1989)
	1282	0.35	*	Angus	Christmas et al. (2001)
	841	0.30	0.10	Line 1 Hereford	Kealy et al. (2006)
	1281	0.27	0.07	Angus	Garmyn et al. (2011)
Secondary Abnormalities	549	0.02	0.05	Hereford, Angus & Red Angus	Smith et al. (1989)
	1282	0.26	*	Angus	Christmas et al. (2001)
	841	0.33	0.09	Line 1 Hereford	Kealy et al. (2006)
	1281	0.23	0.08	Angus	Garmyn et al. (2011)
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)

Table 1.4 Phenotypic (r_P) and genetic (r_G) correlations between semen production and quality traits.

n	r_P	r_G	Breed	Reference
Volume and Concentration				
2351	-0.07	-0.19	Holstein	Taylor et al. 1985
2387	-0.15	-0.42 ± 0.17	Normande	Ducrocq and Humblot 1995
974	0.04	-0.24	Lacaune Rams	David et al. 2006
301	-0.17	0.06 ± 0.13	Beef/Dairy Cross	Gredler et al. 2007
515	0.02	-0.55 ± 0.18	Holstein	Druet et al. 2009
	0.10	0.40 ± 0.20	Beef & Dairy	Berry et al. 2019
Volume and Number of Spermatozoa				
2351	0.55	0.45	Holstein	Taylor et al. 1985
974	0.86	0.84	Lacaune Rams	David et al. 2006
301	0.70	0.83 ± 0.13	Beef/ Dairy Cross	Gredler et al. 2007
515	0.61	0.47 ± 0.18	Holstein	Druet et al. 2009
787	0.63	0.66 ± 0.16	Beef & Dairy	Berry et al. 2019
Volume and Motility				
841	--	-0.38	Line 1 Hereford	Kealy et al. 2006
515	-0.03	-0.20 ± 0.19	Holstein	Druet et al. 2009
787	0.05	0.07 ± 0.06	Beef & Dairy	Berry et al. 2019
974	-0.02	-0.04	Lacaune Rams	David et al. 2006
Volume and Motility Score				
2387	--	-0.03 ± 0.21	Normande	Ducrocq and Humblot 1995
301	-0.12	0.21 ± 0.17	Beef/Dairy Cross	Gredler et al. 2007
515	0.01	-0.17 ± 0.19	Holstein	Druet et al. 2009
Volume and Progressive Motility				
515	-0.05	-0.53 ± 0.45	Holstein	Druet et al. 2009
Volume and Percentage of Living Spermatozoa				
2387	-0.05	-0.16 ± 0.21	Normande	Ducrocq and Humblot 1995
841	--	-0.09	Line 1 Hereford	Kealy et al. 2006
515	-0.10	-0.47 ± 0.26	Holstein	Druet et al. 2009
787	0.01	0.32 ± 0.22	Beef & Dairy	Berry et al. 2019
Volume and Abnormality				
2387	-0.13	-0.26 ± 0.24	Normande	Ducrocq and Humblot 1995
515	0.0	0.23 ± 0.26	Holstein	Druet et al. 2009
Volume and Percentage of Normal Spermatozoa				
301	-0.13	0.31 ± 0.15	Beef/Dairy Cross	Gredler et al. 2007
841	--	0.32	Line 1 Hereford	Kealy et al. 2006
Volume and Percentage of Spermatozoa with an Abnormal Head				
515	0.02	-0.12 ± 0.27	Holstein	Druet et al. 2009
Volume and Percentage of Spermatozoa with an Abnormal Tail				
515	0.0	0.43 ± 0.35	Holstein	Druet et al. 2009
Volume and Percentage of Spermatozoa with an Abnormal Cytoplasmic Droplet				
515	0.01	0.32 ± 0.28	Holstein	Druet et al. 2009
Volume and Primary Abnormalities				
841	--	0.33	Line 1 Hereford	Kealy et al. 2006
Volume and Secondary Abnormalities				
841	--	-0.34	Line 1 Hereford	Kealy et al. 2006
Concentration and Number of Spermatozoa				
717	-0.85	-1.03	Angus	Knights et al. 1984
2351	0.67	0.72	Holstein	Taylor et al. 1985
974	0.51	0.30	Lacaune Rams	David et al. 2006
301	0.52	0.60 ± 0.17	Beef/Dairy Cross	Gredler et al. 2007
515	0.71	0.46 ± 0.18	Holstein	Druet et al. 2009
787	0.68	0.42 ± 0.21	Beef & Dairy	Berry et al. 2019
Concentration and Motility				
717	1.00	1.01	Angus	Knights et al. 1984
974	0.18	0.04	Lacaune Rams	David et al. 2006
841	--	0.81	Line 1 Hereford	Kealy et al. 2006

515	0.01	0.12 ± 0.20	Holstein	Druet et al. 2009	
787	0.20	0.29 ± 0.04	Beef & Dairy	Berry et al. 2019	
Concentration and Motility Score					
2387	--	0.67 ± 0.11	Normande	Ducrocq and Humblot 1995	
301	0.23	0.48 ± 0.17	Beef/Dairy Cross	Gredler et al. 2007	
515	0.02	-0.01 ± 0.20	Holstein	Druet et al. 2009	
Concentration and Progressive Motility					
515	0.06	0.35 ± 0.36	Holstein	Druet et al. 2009	
Concentration and Percentage of Living Spermatozoa					
717	-0.81	0.01	Angus	Knights et al. 1984	
2387	0.47	0.54 ± 0.15	Normande	Ducrocq and Humblot 1995	
515	0.04	0.29 ± 0.26	Holstein	Druet et al. 2009	
787	0.15	0.37 ± 0.23	Beef & Dairy	Berry et al. 2019	
Concentration and Abnormal Spermatozoa					
2387	-0.07	-0.27 ± 0.24	Normande	Ducrocq and Humblot 1995	
515	-0.07	-0.34 ± 0.24	Holstein	Druet et al. 2009	
Concentration and Percentage of Normal Spermatozoa					
841	--	0.36	Line 1 Hereford	Kealy et al. 2006	
301	0.27	0.41 ± 0.17	Beef/Dairy Cross	Gredler et al. 2007	
Concentration and Percentage of Spermatozoa with an Abnormal Head					
515	-0.02	-0.23 ± 0.24	Holstein	Druet et al. 2009	
Concentration and Percentage of Spermatozoa with an Abnormal Tail					
515	-0.06	-0.33 ± 0.30	Holstein	Druet et al. 2009	
Concentration and Percentage of Spermatozoa with an Abnormal Cytoplasmic Droplet					
515	-0.08	-0.09 ± 0.28	Holstein	Druet et al. 2009	
Concentration and Primary Abnormalities					
841	--	0.36	Line 1 Hereford	Kealy et al. 2006	
Concentration and Secondary Abnormalities					
	841	--	-0.58	Line 1 Hereford	Kealy et al. 2006
Number of Spermatozoa and Motility					
974	0.06	-0.01	Lacaune Rams	David et al. 2006	
515	-0.03	-0.12 ± 0.25	Holstein	Druet et al. 2009	
787	0.13	0.36 ± 0.08	Beef & Dairy	Berry et al. 2019	
Number of Spermatozoa and Motility Score					
717	--	-1.04	Angus	Knights et al. 1984	
301	0.06	0.50 ± 0.13	Beef/Dairy Cross	Gredler et al. 2007	
515	0.01	-0.24 ± 0.29	Holstein	Druet et al. 2009	
Number of Spermatozoa and Progressive Motility					
515	0.02	-0.22 ± 0.67	Holstein	Druet et al. 2009	
Number of Spermatozoa and Percentage of Living Spermatozoa					
515	0.03	-0.18 ± 0.38	Holstein	Druet et al. 2009	
Number of Spermatozoa and Abnormal Spermatozoa					
515	-0.05	-0.09 ± 0.38	Holstein	Druet et al. 2009	
Number of Spermatozoa and Percentage of Normal Spermatozoa					
301	0.07	0.54 ± 0.11	Beef/Dairy Cross	Gredler et al. 2007	
Number of Spermatozoa and Percentage of Spermatozoa with an Abnormal Head					
515	-0.02	-0.38 ± 0.36	Holstein	Druet et al. 2009	
Number of Spermatozoa and Percentage of Spermatozoa with an Abnormal Tail					
515	-0.03	0.14 ± 0.54	Holstein	Druet et al. 2009	
Number of Spermatozoa and Percentage of Spermatozoa with an Abnormal Cytoplasmic Droplet					
515	-0.05	0.33 ± 0.43	Holstein	Druet et al. 2009	
Motility and Motility Score					
515	0.66	0.88 ± 0.06	Holstein	Druet et al. 2009	
Motility and Progressive Motility					
515	0.48	0.74 ± 0.30	Holstein	Druet et al. 2009	
Motility and Mass Activity					
2424	0.84	0.98 ± 0.01	Tropical Composite	Corbet et al. 2013	
Motility and Percentage of Living Spermatozoa					
515	0.40	0.58 ± 0.15	Holstein	Druet et al. 2009	
787	0.54	0.89 ± 0.04	Beef & Dairy	Berry et al. 2019	

Motility and Abnormal Spermatozoa				
515	-0.20	-0.55 ± 0.19	Holstein	Druet et al. 2009
Motility and Percentage of Normal Spermatozoa				
549	0.38	0.43 ± 0.64	Hereford, Angus & Red Angus	Smith et al. 1989
841	--	0.51	Line 1 Hereford	Kealy et al. 2006
2424	0.56	0.77 ± 0.09	Tropical Composite	Corbet et al. 2013
Motility and Percentage of Spermatozoa with an Abnormal Head				
515	0.17	-0.56 ± 0.18	Holstein	Druet et al. 2009
Motility and Percentage of Spermatozoa with an Abnormal Tail				
515	-0.11	-0.24 ± 0.24	Holstein	Druet et al. 2009
Motility and Percentage of Spermatozoa with an Abnormal Cytoplasmic Droplet				
515	-0.07	0.13 ± 0.23	Holstein	Druet et al. 2009
Motility and Primary Abnormalities				
549	-0.31	-0.36 ± 0.55	Hereford, Angus & Red Angus	Smith et al. 1989
841	--	0.57	Line 1 Hereford	Kealy et al. 2006
Motility and Secondary Abnormalities				
549	-0.22	0.71 ± 0.89	Hereford, Angus & Red Angus	Smith et al. 1989
841	--	-0.54	Line 1 Hereford	Kealy et al. 2006
Motility and Scrotal Circumference				
549	0.13	-0.04 ± 0.40	Hereford, Angus & Red Angus	Smith et al. 1989
1282	--	0.56	Angus	Christmas et al. 2001
1281	0.11	0.36 ± 0.29	Angus	Garmyn et al. 2011
2424	0.42	0.56 ± 0.10	Tropical Composite	Corbet et al. 2013
Motility Score and Progressive Motility				
515	0.40	0.71 ± 0.27	Holstein	Druet et al. 2009
Motility Score and Percentage of Living Spermatozoa				
2387	0.73	0.83 ± 0.09	Normande	Ducrocq and Humblot 1995
515	0.31	0.61 ± 0.15	Holstein	Druet et al. 2009
Motility Score and Total Abnormalities				
2387	-0.34	-0.68 ± 0.16	Normande	Ducrocq and Humblot 1995
515	-0.16	-0.40 ± 0.18	Holstein	Druet et al. 2009
Motility Score and Percentage of Normal Spermatozoa				
301	0.55	0.90 ± 0.05	Beef/ Dairy Cross	Gredler et al. 2007
Motility Score and Percentage of Spermatozoa with an Abnormal Head				
515	-0.15	-0.54 ± 0.17	Holstein	Druet et al. 2009
Motility Score and Percentage of Spermatozoa with an Abnormal Tail				
515	-0.05	-0.02 ± 0.23	Holstein	Druet et al. 2009
Motility Score and Percentage of Spermatozoa with an Abnormal Cytoplasmic Droplet				
515	-0.14	-0.07 ± 0.21	Holstein	Druet et al. 2009
Progressive Motility and Percentage of Living Spermatozoa				
515	0.70	0.89 ± 0.17	Holstein	Druet et al. 2009
Progress Motility and Total Abnormalities				
515	-0.24	-0.38 ± 0.38	Holstein	Druet et al. 2009
Progressive Motility and Percentage of Spermatozoa with an Abnormal Head				
515	-0.17	-0.39 ± 0.41	Holstein	Druet et al. 2009
Progressive Motility and Percentage of Spermatozoa with an Abnormal Tail				
515	-0.17	-0.15 ± 0.57	Holstein	Druet et al. 2009
Progressive Motility and Percentage of Spermatozoa with an Abnormal Cytoplasmic Droplet				
515	-0.13	-0.08 ± 0.44	Holstein	Druet et al. 2009
Mass Activity and Percentage of Normal Spermatozoa				
2424	0.52	0.87 ± 0.08	Tropical Composite	Corbet et al. 2013
Mass Activity and Scrotal Circumference				
2424	0.43	0.60 ± 0.10	Tropical Composite	Corbet et al. 2013
Percentage of Living Spermatozoa and Total Abnormalities				
2387	-0.37	0.43 ± 0.22	Normande	Ducrocq and Humblot 1995
515	-0.20	0.23 ± 0.29	Holstein	Druet et al. 2009
Percentage of Living Spermatozoa and Percentage of Normal Spermatozoa				
841	--	0.32	Line 1 Hereford	Kealy et al. 2006
Percentage of Living Spermatozoa and Percentage of Spermatozoa with an Abnormal Head				

515	-0.15	-0.44 ± 0.28	Holstein	Druet et al. 2009
Percentage of Living Spermatozoa and Percentage of Spermatozoa with an Abnormal Tail				
515	-0.12	0.10 ± 0.39	Holstein	Druet et al. 2009
Percentage of Living Spermatozoa and Percentage of Spermatozoa with an Abnormal Cytoplasmic Droplet				
515	-0.11	0.05 ± 0.30	Holstein	Druet et al. 2009
Percentage of Living Spermatozoa and Primary Abnormalities				
841	--	0.33	Line 1 Hereford	Kealy et al. 2006
Percentage of Living Spermatozoa and Secondary Abnormalities				
841	--	-0.43	Line 1 Hereford	Kealy et al. 2006
Percentage of Living Spermatozoa and Scrotal Circumference				
--	--	-0.24	Nellore	Silva et al. 2013
Total Abnormalities and Percentage of Spermatozoa with an Abnormal Head				
515	0.61	0.71 ± 0.18	Holstein	Druet et al. 2009
Total Abnormalities and Percentage of Spermatozoa with an Abnormal Tail				
515	0.77	0.78 ± 0.16	Holstein	Druet et al. 2009
Percentage of Spermatozoa with an Abnormal Cytoplasmic Droplet				
515	0.36	0.15 ± 0.34	Holstein	Druet et al. 2009
Total Abnormalities and Scrotal Circumference				
1282	--	-0.32	Angus	Christmas et al. 2001
1281	-0.11	-0.23 ± 0.18	Angus	Garmyn et al. 2011
Percentage of Normal Spermatozoa and Primary Abnormalities				
549	-0.57	-0.85 ± 0.63	Hereford, Angus & Red Angus	Smith et al. 1989
Percentage of Normal Spermatozoa and Secondary Abnormalities				
549	-0.80	0.16 ± 1.54	Hereford, Angus & Red Angus	Smith et al. 1989
Percentage of Normal Spermatozoa and Scrotal Circumference				
549	-0.17	0.36 ± 0.34	Hereford, Angus & Red Angus	Smith et al. 1989
2424	0.31	0.55 ± 0.13	Tropical Composite	Corbet et al. 2013
Percentage of Spermatozoa with an Abnormal Head and Percentage of Spermatozoa with an Abnormal Tail				
515	0.02	0.13 ± 0.39	Holstein	Druet et al. 2009
Percentage of Spermatozoa with an Abnormal Head and Percentage of Spermatozoa with an Abnormal Cytoplasmic Droplet				
515	0.12	-0.43 ± 0.30	Holstein	Druet et al. 2009
Percentage of Spermatozoa with an Abnormal Tail and Percentage of Spermatozoa with an Abnormal Cytoplasmic Droplet				
515	0.15	0.06 ± 0.37		Druet et al. 2009
Primary Abnormalities and Secondary Abnormalities				
549	0.17	0.14 ± 0.64	Hereford, Angus & Red Angus	Smith et al. 1989
841	--	-0.87	Line 1 Hereford	Kealy et al. 2006
Primary Abnormalities and Scrotal Circumference				
549	-0.09	0.14 ± 0.22	Hereford, Angus & Red Angus	Smith et al. 1989
1282	--	-0.25	Angus	Christmas et al. 2001
1281	-0.10	-0.19 ± 0.17	Angus	Garmyn et al. 2011
Secondary Abnormalities and Scrotal Circumference				
549	-0.10	1.22 ± 1.57	Hereford, Angus & Red Angus	Smith et al. 1989
1282	--	-0.40	Angus	Christmas et al. 2001
1281	-0.05	-0.11 ± 0.19	Angus	Garmyn et al. 2011

Table 1.5 Genes associated with male fertility traits.

Associated Sperm Trait	Gene Identified	Chromosome	Breed	Source
Percentage of Living Spermatozoa	SPATA16	1	Fleckvieh	Ferenčakivoč et al., 2017
	GPX5	1	Fleckvieh	Ferenčakivoč et al., 2017
	NYD-SP5	1	Fleckvieh	Ferenčakivoč et al., 2017
	PIAS1	1	Fleckvieh	Ferenčakivoč et al., 2017
	FEM1B	1	Fleckvieh	Ferenčakivoč et al., 2017
	SPESP1	1	Fleckvieh	Ferenčakivoč et al., 2017
	NOX5	1	Fleckvieh	Ferenčakivoč et al., 2017
	FEM1B	10	Fleckvieh	Ferenčakivoč et al., 2017
	SPESP1	10	Fleckvieh	Ferenčakivoč et al., 2017
	NOX5	10	Fleckvieh	Ferenčakivoč et al., 2017
	TSPYL5	14	Fleckvieh	Ferenčakivoč et al., 2017
Total number of spermatozoa	RPL10L	10	Fleckvieh	Ferenčakivoč et al., 2017
	SLC25A31	17	Fleckvieh	Ferenčakivoč et al., 2017
	CDH18	20	Fleckvieh	Ferenčakivoč et al., 2017
	KCNU1	27	Fleckvieh	Ferenčakivoč et al., 2017
Sperm motility	COX7A2L	11	Jersey	Rezende et al., (2018)
	ZMYND10	22	Jersey	Rezende et al., (2018)
	SLC25A20	22	Jersey	Rezende et al., (2018)
	DNAH3	25	Jersey	Rezende et al., (2018)
	PARP11	5	Holstein	Han and Penagaricano (2016)
	AKAP3	5	Holstein	Han and Penagaricano (2016)
	CNNM4	11	Jersey	Han and Penagaricano (2016)
Acrosome reaction/Fertilization	PKDREJ	5	Jersey	Rezende et al., (2018)
	STX2	17	Jersey	Rezende et al., (2018)
	TBC1D20	13	Holstein	Nicolini et al. (2018)
	CCT6A	25	Holstein	Han and Penagaricano (2016)
	CACNA1H	25	Holstein	Penagaricano et al. (2012)
	ITGB5	1	Holstein	Feugang et al. (2009)
	FER1L5	11	Jersey	Rezende et al., (2018)
Spermatogenesis	EPB41L2	9	Jersey	Rezende et al., (2018)
	IP6K1	22	Jersey	Rezende et al., (2018)
	ADAM28	8	Holstein	Nicolini et al. (2018)
	PIWIL3	17	Holstein	Nicolini et al. (2018)
	TMEM119	17	Holstein	Nicolini et al. (2018)
	TDRD9	21	Holstein	Han and Penagaricano (2016)
	CKB	21	Holstein	Han and Penagaricano (2016)
	MGRN1	25	Holstein	Han and Penagaricano (2016)
	SEPT12	25	Holstein	Han and Penagaricano (2016)
	DYNC1I2	2	Holstein	Penagaricano et al. (2012)
	LOC784935	5	Holstein	Penagaricano et al. (2012)
	ZNF541	18	Holstein	Penagaricano et al. (2012)
	LOC617302	25	Holstein	Penagaricano et al. (2012)
Testis development	PDGFD	15	Jersey	Rezende et al., (2018)
	DNAJA1	8	Holstein	Nicolini et al. (2018)
Gametogenesis	ROGDI	25	Holstein	Penagaricano et al. (2012)
	SPO11	13	Holstein	Nicolini et al. (2018)
	RAD21L1	13	Holstein	Nicolini et al. (2018)
	BCL2L1	13	Holstein	Nicolini et al. (2018)
Sire conception rate	PROP1	7	Holstein	Lan et al. (2013)
	FGF2	17	Holstein	Khatib et al (2010)
	STAT5A	19	Holstein	Khatib et al (2010)
	RIMS1	9	Holstein	Han and Penagaricano (2016)
	CTCFL	13	Holstein	Han and Penagaricano (2016)
	SPO11	13	Holstein	Han and Penagaricano (2016)

	CADM1	15	Holstein	Han and Penagaricano (2016)
	IGF1R	21	Holstein	Han and Penagaricano (2016)
	BRF1	21	Holstein	Han and Penagaricano (2016)
	KAT8	25	Holstein	Han and Penagaricano (2016)
	ITGAM	25	Holstein	Han and Penagaricano (2016)
	TYW1	25	Holstein	Han and Penagaricano (2016)
	LOC521021	25	Holstein	Penagaricano et al. (2012)
	COL1A2-AS1	4	Holstein	Feugang et al. (2009)
	RIMS1	9	Holstein	Feugang et al. (2009)
	SFNX1	10	Holstein	Feugang et al. (2009)

Literature Cited

- Abdollahi-Arpanahi, R., G. Morota, and F. Penagaricano. 2017. Predicting bull fertility using genomic data and biological information. *J. Dairy. Sci.* 100: 9656-9666.
- Amann R.P. and J.O. Almquist. 1976. Bull management to maximize sperm output. In: *Proceedings of the Sixth Technical Conference on Artificial Insemination and Reproduction*. Milwaukie, USA, 20-21 February 1976. Pp. 1-10.
- Arnold G.W. and M.L. Dudzinski. 1978. *Ethology of Free-Ranging Domestic Animals*. Elsevier Scientific Publishing Co., Amsterdam, The Netherlands, pp. 51-96 and 125-136.
- Asghari A., S. A. Marashi, and N. Ansari-Pour. 2017. A spermspecific proteome-scale metabolic network model identifies nonglycolytic genes for energy deficiency in asthenozoospermia. *Systems Bio in Repro Med.* 63: 100–12.
- Barth, A. D, and C.L. Waldner. 2002. Factors affecting breeding soundness classification of beef bulls examined at the Western College of Veterinary Medicine. *Can Vet J.* 43: 274-284.
- Beerwinkle, L.G. 1974. Heat detection programs and techniques. In: *Proceedings of the Eighth Conference on Artificial Insemination of Beef Cattle*. Denver, Colorado, National Association of Animal Breeders, Columbia, Missouri, pp. 24-29
- Berry, D.P., B. Eivers, G. Dunne, and S. McParland. 2019. Genetics of bull semen characteristics in a multi-breed cattle population. *Theriogenology*. 123: 202-208.
- Berry, D. P., E. Wall, J.E. Pryce, 2014. Genetics and genomics of reproductive performance in dairy and beef cattle. *Animal*. 8(suppl) 105-121.
- Bertram, J.D. 1999. Structural and behavioral characteristics of reproductive performance in tropically adapted bulls. M. Rur. Sci. thesis. University of New England, Armidale, NSW.
- Bourdon, R.M. and J.S. Brinks. 1985. Scrotal circumference in yearling Hereford bulls: adjustmend factors, heritabilities, and genetic, environmental and phenotypic relationships with growth traits. *J. Anim. Sci.* 62: 958-967.
- Braundmeier, A. G. and D.J. Miller. 2001. The search is on: finding accurate molecular markers of male fertility. *J of Dairy Sci.* 84: 1915-1925.
- Brito, L. C., A.D. Barth, N.C. Rawlings, R.E. Wilde, D.H. Crews Jr., P.S. Mire, J.P. Kastelic. 2006. Effect of improved nutrition during calfhoo on serum metabolic hormones, gonadotropins, and testosterone concentrations, and on testicular development in bulls. *Domestic Anim. Endocrinology*. 33: 460-469.

- Carrol, E.J., L. Ball, and J.A. Scott. 1963. Breeding soundness in bulls-a summary of 10,940 examinations. *J of Am. Vet. Med. Assn.* 142: 1105-1111.
- Charlier, C., W. Li, C. Harland, M. Littlejohn, W. Coppieters, F. Creagh, S. Davis, T. Druet, P. Faux, F. Guillaume, L. Karim, M. Keehan, N. K. Kadri, N. Tamma, R. Spelman, M. Georges. 2016. NGS-based reverse genetic screen for common embryonic lethal mutations compromising fertility in livestock. *Genome Res.* 26: 1333-1341.
- Chenoweth, P.J., 1980. Bull fertility. *Modern veterinary practices.* 61: 987-991.
- Chenoweth, P.J. 1983. Sexual behavior of the bull: a review. *J. of Dairy Sci.* 66:173-179.
- Chenoweth, P.J. 2002. Bull breeding soundness exams and beyond. In: *Proceedings The Applied Reproductive Strategies in Beef Cattle Workshop.* 5-6 Sept 2002. Manhattan, Kansas. pp. 174-181.
- Christmas R. A., D.W. Moser, M.F. Spire, J.M. Sargeant, and S.K. Tucker. Genetic relationships among breeding soundness traits in yearling bulls. In: *Cattlemen's Day.* 2001. Manhattan, KS. Pp. 1-3.
- Choi, E., C. Han, I. Park, B. Lee, S. Jin, H. Choi, D. H. Kim, Z. Y. Park, E. M. Eddy, and C. Cho. 2008. A novel germ cell-specific protein, SHIP1, forms a complex with chromatin remodeling activity during spermatogenesis. *J Bio Chem.* 283: 35283-35294.
- Corah, L and K. Lusby. *Beef Cattle Handbook: Factors Influencing Conception Rates.*
- Corbet, N.J., B.M. Burns, D.J. Johnston, M.L. Wolcott, D.H. Corbet, B.K. Venus, Y. Li, M.R. McGowan, and R.G. Holroyd. 2013. Male traits and herd reproductive capability in tropical beef cattle. 2. Genetic parameters of bull traits. *Anim. Prod. Sci.* 53: 101-113.
- Coulter, G.H., R. B. Cook, and J.P. Kastelic. 1997. Effects of dietary energy on scrotal surface temperature, seminal quality, and sperm production in young beef bulls. *J. Anim. Sci.* 75: 1048-1052.
- Daetwyler, H.D., A. Capitan, H. Pausch, P. Stothard, R. van Binsbergen, R.F. Brondum, X. Liao, A. Djari, S.C. Rodriguez, C. Grohs, D. Esquerre, O. Bouchez, M. Rossignol, C. Klopp, D. Rocha, S. Fritz, A. Eggen, P.J. Bowman, D. Coote, A.J. Chamberlain, C. Anderson, C.P. VanTassell, I. Hilsegge, M.E. Goddard, B. Guldbrandsen, M.S. Lund, R.F. Veerkamp, D.A. Boichard, R. Fries, and B.J. Hayes. 2014. Whole-genome sequencing of 234 bulls facilitates mapping of monogenic and complex traits in cattle. *Nature Genetics.* 48: 858-867.
- Dam, A. H., I. Koscinski, J. A. Kremer, C. Moutou, A. S. Jaeger, A. R. Oudakker, H. Tournaye, N. Charlet, C. Lagier-Tourenne, H. van Bokhoven, and S. Viville. 2007. Homozygous

- mutation in SPATA16 is associated with male infertility in human globozoospermia. *Am J Human Gen.* 81: 813-820.
- David I., L. Bodin, G. Lagriffoul, E. Madfedi, C. Robert-Granie. 2006. Genetic parameters of ram semen traits using an animal model accounting for serial correlation. In: proceedings of the eighth World Congress on Genetics Applied to Livestock Production, Belo Horizonte, Brazil, 13-18 August 2006.
- DeJarnette, J.M., C.E. Marshal, R.W. Lenz, D.R. Monke, W.H. Ayars, and C.G. Sattler. 2004. Sustaining the fertility of artificially inseminated dairy cattle: the role of the artificial insemination industry. *J. Dairy Sci.* 87 (E. Suppl.): E93-E104.
- Diskin, M.G. and D.A. Kenny. 2016. Managing the reproductive performance of beef cows. *Theriogenology.* 86: 379-387.
- Druet, T., S. Fritz, S. Sellem, B. Basso, O. Ge, L. Salas-Cortes, P. Humblot, X. Druart, and A. Eggen. 2009. Estimation of genetic parameters and genome scan for 15 semen characteristics traits of Holstein bulls. *J. Anim. Breed. Genet.* 136: 269-277.
- Ducrocq, V. and P. Humblot. 1995. Genetic characteristics and evolution of semen production of young Normande bulls. *Livest. Prod. Sci.* 41: 1-10.
- Evans, J. L., B.L. Golden, R.M. Bourdon, and K.L. Long. Additive genetic relationships between heifer pregnancy and scrotal circumference in Hereford cattle. *J. Anim. Sci.* 77: 262-268.
- Farney, J.K., D.A. Blasi, S. Johnson, C. Reinhardt, A.J. Tarpoff, J. Waggoner, R. Weaver. 2016. Guide to body condition scoring beef cows and bulls. Kansas State Research and Extension.
- Ferenčaković, M., J. Sölkner, M. Kapš, and I. Curik. 2017. Genome-wide mapping and estimation of inbreeding depression of semen quality traits in a cattle population. *J. Dairy Sci.* 100: 4721-4730. <https://doi.org/10.3168/jds.2016-12164>.
- Feugang J.M., N. Rodriguez-Osorio, A. Kaya, H. Wang, G. Page, G.C. Ostermeier, E.K. Topper, and E. Memili. 2010, Transcriptome analysis of bull spermatozoa: implications for male fertility. *Repro. BioMed.* 21: 312-324.
- Flowers, W. L. 2012 Triennial Reproduction Symposium: Sperm characteristics that limit success of fertilization. *J. Anim. Sci.* 91: 3022-3029.
- Fordyce, G., L.A. Fitzpatrick, N.J. Cooper, V.J. Doogan, J. De Faveri, and R.G. Holroyd. 2002. Bull selection and use in northern Australia. V. Social behavior and management. *Anim. Reprod. Sci.* 71: 81-99.

- Fritz, L., J.M. Stookey, J.L. Clavelle, and C.S. Waltz. 1999. Observations of cattle on extensive range. 1999 Annual Report to the Saskatchewan Agricultural Development Fund: Strategic Program Area X: Food Animal Behaviour and Welfare.
- Fuerst-Waltl, B., H. Schwarzenbacher, C. Perner, and J. Solkner. 2006. Effects of age and environmental factors on semen production and semen quality of Austrian Simmental bulls. *Anim. Repro. Sci.* 95: 27-37.
- Garmyn, A.J., D. W. Moser, R.A Christmas, J.M. Bormann. 2011. Estimate of genetic parameters and effects of cytoplasmic line on scrotal circumference and semen quality traits in Angus bulls. *J. Anim. Sci.* 89: 693-698.
- Gaviraghi, A. F. Deriu, A. Soggiu, A. Galli, C. Bonacina, L. Bonizzi, and P. Roncada. 2010. Proteomics to investigate fertility in bulls. *Vet. Res. Comm.* 34: S33-36.
- Gredler, B. C. Fuerst-Waltl, H. Schwarzenbacher, and J. Solkner. 2007. Genetic parameters for semen production traits in Austrian dual-purpose Simmental bulls. *Repro. Domest. Anim.* 42: 326-328.
- Groeneveld, E. 1998. VCE4: User's guide and reference manual. Version 1.3. Institute of Animal Husbandry and Animal Behavior. Mariensee, Germany.
- Hall, L., K. Williams, A. C. Perry, J. Frayne, and J.A. Jury. 1998. The majority of human glutathione peroxidase type 5 (GPX5) transcripts are incorrectly spliced: implications for the role of GPX5 in the male reproductive tract. *Biochem J.* 333: 5-9.
- Hall S.J.G. 1989. Chillingham cattle: social and maintenance behavior in an ungulate that breeds all year around. *Anim. Behavior.* 38: 215-225.
- Hamm D., B. S. Mautz, M. F. Wolfner, C. F. Aquadro, and W. J. Swanson. 2007. Evidence of amino acid diversity-enhancing selection within humans and among primates at the candidate sperm-receptor gene PKDREJ. *Am J Human Gen.* 81: 44-52.
- Hamilton, B. E., J.A. Martin, M.J.K. Osterman, A.K. Driscoll, and L.M. Rossen. 2018. Births: provisional data for 2017. *Vital Statistics Rapid Release.* 4: 1-5.
- Han, Y., and F. Peñagaricano. 2016. Unravelling the genomic architecture of bull fertility in Holstein cattle. *BMC genetics* 17: 143.
- Hansen, M. 1979. Genetic investigations on male and female fertility in cattle. *Livest. Sci.* 6:325-334.

- Harris, D. L. 1970. Breeding for efficiency in livestock production: defining the economic objectives. *J. Anim. Sci.* 30: 860-865.
- Hopkins, F.M. and J.C. Spitzer. 1997. The new society for theriogenology breeding soundness evaluation system. *Vet. Clinics of North America: Food Animal Practice.* 13: 283-293.
- Huang, J. Z. Ju, Q. Li, Q. Hou, C. Wang, J. Li, R. Li, L. Wang, T. Sun, S. Hang, Y. Gao, M. Hou, and J. Zhong. 2011. Solexa sequencing of novel and differentially expressed microRNAs in testicular and ovarian tissues in Holstein cattle. *Int. J. Biol. Sci.* 7: 1016-1026.
- Hutt D.M., J. M. Baltz, and J. K. Ngsee. 2005. Synaptotagmin VI and VIII and syntaxin 2 are essential for the mouse sperm acrosome reaction. *J Bio Chem.* 280: 20197–203.
- Kaps, M., M. Posavi, N. Stipic, and B. Mikulic. 2000. Genetic evaluation of semen and growth traits of young Simmental bulls in performance test. *Ag Conceptus Scientificus.* 65: 15-20.
- Karoui, S. C. Diaz, M. Serrano, R. Cue. I. Celorrio, M.J. Carabano. 2011. Time trends, environmental factors, and genetic bias of semen traits collected in Holstein bulls under commercial conditions. *Anim. Repro. Sci.* 124: 28-38.
- Kealy, C.G., M.D. MacNeil, M.W. Tess, T.W. Geary, and R.A. Bellows. 2006. Genetic parameter estimates for scrotal circumference and semen characteristics of line 1 Hereford bulls. *J. Anim. Sci.* 84: 283-290.
- Keeney, S., C. N. Giroux, and N. Kleckner. 1997. Meiosis-specific DNA double-strand breaks are catalyzed by Spo11, a member of a widely conserved protein family. *Cell.* 88: 375-384.
- Khatib, H., R. L. Monson, W. Huang, R. Khatib, V. Schutzkus, H. Khateeb, & J.J. Parrish. 2010. Validation of in vitro fertility genes in a Holstein bull population. *J dairy sci.* 93: 2244-2249. doi: [10.3168/jds.2009-2805](https://doi.org/10.3168/jds.2009-2805)
- Khelifa M. B. , C. Coutton, R. Zouari, T. Karaouzene, J. Rendu, M. Bidart, S. Yissine, V. Pierre, J. Delaroche, S. Hennebicq, D. Grunwald, D. Escalier, K. Pernet-Gallay, P.S. Jouk, N. Thierry-Mieg, A. Toure, C. Arnoult, and P. F. Ray. 2014. Mutations in DNAH1, which encodes an inner arm heavy chain dynein, lead to male infertility from multiple morphological abnormalities of the sperm flagella. *Am J Human Gen.* 94: 95–104.
- Knights, S.A., R.L. Baker, D. Gianola, J.B. Gibbs. 1984. Estimates of heritabilities and genetic and phenotypic correlations among growth and reproductive traits in yearling Angus bulls. *J. Anim. Sci.* 58: 887-893.

- Lan, X. Y., F. Peñagaricano, L. DeJung, K.A. Weigel, and H. Khatib. 2013. A missense mutation in the PROP1 (prophet of Pit 1) gene affects male fertility and milk production traits in the US Holstein population. *J. dairy sci.* 96: 1255-1257.
- Lango Allen, H., K. Estrada, G. Lettre, S.I. Berndt, and M.N. Weedon. 2010. Hundreds of variants clustered in genomic loci and biological pathways affect human height. *Nature.* 467: 832-838.
- Luitjens, C., M. Gallegos, B. Kraemer, J. Kimble, and M. Wickens. 2000. CPEB proteins control two key steps in spermatogenesis in *C. elegans*. *Genes and Development.* 14: 2596-2609.
- Lyons, A., F. Narciandi, E. Donnellan, J. Romero-Aguirregomez, C. O'Farrelly, P. Lonergan, K. G. Meade, and S. Fair. 2018. Recombinant β -defensin 126 promotes bull sperm binding to bovine oviductal epithelia. *Reproduction, Fertility and Development*, 30:1472-1481.
- Marques, D.B.D., M.S. Lopes, M.L.W. Brockhuijs, S.E.F. Guimaraes, E.F. Knol, J.W.M. Bastiaansen, F.F. Silva, and P.S. Lopes. 2017. Genetic parameters for semen quality and quantity traits in five pig lines. *J. Anim. Sci.* 95: 4251-4259.
- Mathevon, M. M.M Buhr, and J.C.M. Dekkers. 1998. Environmental, management, and genetic factors affecting semen production in Holstein bulls. *J. Dairy Sci.* 81: 3321-3330.
- Menon, A.G., H.W. Barkema, R. Wilde, J.P. Kastelic, J.C. Thundathil. 2011. Association between sperm abnormalities, breed, age, and scrotal circumference in beef bulls. *Can. J Vet. Research.* 75: 241-247.
- Meyer, K, K Hammond, M.J. Mackinnon, P.F. Parnell. 1991. Estimates of covariances between reproduction and growth in Australian beef cattle. *J. Anim. Sci.* 69: 3533-3543.
- Mizuhashi, K., T. Chaya, T. Kanamoto, Y. Omori, and T. Furukawa. 2015. Obif, a transmembrane protein, is required for bone mineralization and spermatogenesis in mice. *PloS One.* 10: e0133704.
- Moore, D. J., A. Onoufriadis, A. Shoemark, M. A. Simpson, R. I. zur Lage, S. C. de Castro, L. Bartoloni, G. Gallone, S. Petridi, W. J. Woollard, D. Antony, M. Schmidts, T. Didonna, P. Makrythanasis, J. Bevilard, N. P. Mongan, J. Djakow, G. Pals, and H. M. Mitchison. 2013. Mutations in ZMYND10, a gene essential for proper axonemal assembly of inner and outer dynein arms in humans and flies, cause primary ciliary dyskinesia. *Am J Human Gen.* 93: 346–56.
- NAAB, 2017. Semen Production. Available from: National Association of Animal Breeders. (Accessed 6 February 2019).
file:///C:/Users/Madison%20Butler/Downloads/2017_SALES_STATISTICS%20(3).pdf .

- Nicolini, P., R. Amorín, Y. Han, and F. Peñagaricano. 2018. Whole-genome scan reveals significant non-additive effects for sire conception rate in Holstein cattle. *BMC Genomics*. 19: 14.
- Niles, D., C. A. Risco, and M. J. Thatcher. 2002. Seasonal evaluation of artificial insemination and natural service pregnancy rates in dairy herds. *Compend. Contin. Educ. Prac. Vet.* 24:S44–S48.
- Oosthuizen N., P.L.P. Fontest, C.D. Sanford, F.M. Ciriaco, D.D. Henry, L.B. Canal, N. DiLorenzo, and G.C. Lamb. 2018. Estrus synchronization and fixed-time artificial insemination alter calving distribution in *Bos indicus* influenced beef heifers. *Theriogenology*. 106: 210-213.
- Peddinti, D. B. Nanduri, A. Kaya, J.M. Feugang, S.C. Burgess, and E. Memili. 2008. Comprehensive proteomic analysis of bovine spermatozoa of varying fertility rates and identification of biomarkers associated with fertility. *BMC Systems Biology*. 2:19.
- Pacheco, S. E., E. A. Houseman, B. C. Christensen, C. J. Marsit, K. T. Kelsey, M. Sigman, and K. Boekelheide. 2011. Integrative DNA methylation and gene expression analyses identify DNA packaging and epigenetic regulatory genes associated with low motility sperm. *PloS one*. 6: e20280.
- Paronetto, M. P., and C. Sette. 2010. Role of RNA-binding proteins in mammalian spermatogenesis. *Int J Andro*. 33: 2-12.
- Penagaricano, F., K.A. Weigel, and H. Khatib. 2012. Genome wide association study identifies candidate markers for bull fertility in Holstein dairy cattle. *Anim. Genet*. 43: 65-71.
- Petrunkina, A.M. and R.A. Harrison. 2011. Cytometric solutions in veterinary andrology: developments, advantages, and limitations. *Cytometry A* 79: 338-348.
- Pexton, J.E., P.W. Farin, G.P. Rupp, and P.J. Chenoweth. 1990. Factors affecting mating activity and pregnancy rates with beef bulls mated to estrus synchronized females. *Theriogenology*. 34: 1059-1070.
- Purdy. P.H. and J.K. Graham. 2004. Effect of cholesterol-loaded cyclodextrin on the cryosurvival of bull sperm. *Cryobiology*. 48: 36-45.
- Raidan, F.S.S., J.S.A. Tineo, M.M. de Moreaes, T.C. Escarce, A. E.M. de Araujo, M. M. de Carvalho Gomes, H.T. Venture, F.L.B. Toral. 2016. Associations among growth, scrotal circumference, and visual score of beef cattle in performance tests of pasture or in feedlots. *Revista Brasileira de Zootecnia*. 46: 309-316.
- Rezende, F. M., J. P. Nani, and F. Peñagaricano. 2019 Genomic prediction of bull fertility in US Jersey dairy cattle. *J Dairy Sci*. 102: 3230-3240. doi: [10.3168/jds.2018-15810](https://doi.org/10.3168/jds.2018-15810).

- Rezende, F. M., G. O. Dietsch, and F. Peñagaricano. 2018. Genetic dissection of bull fertility in US Jersey dairy cattle. *Animal genetics*. 49: 393-402.
- Rogers, J.C., S.L. Bird, J.E. Larson, N. Dileo, C.R. Dahken, A. Dicostanzo, and G.C. Lamb. 2012. An economic evaluation of estrous synchronization and timed artificial insemination in suckled beef cows. *J. Anim. Sci.* 90: 4055-4062.
- Schenk, J. L. 2018. Review: principles of maximizing bull semen production at genetic centers. *Animal*. 12: 51 pp s142-s147.
- Schreiber, M., A. Wei, A. Yuan, J. Gaut, M. Saito, and L. Salkoff. 1998. Slo3, a novel pH-sensitive K⁺ channel from mammalian spermatocytes. *J Bio Chem*. 273: 3509-3516.
- Senger, P. L. 2012. Pathways to pregnancy and parturition. 3rd ed. Current Conceptions, Inc. Redmon, Oregon.
- Silva, M. R., V. B. Pedrosa, J. C. B. Silva, J. P. Eler, J. D. Guimarães, and L. G Albuquerque. 2011. Testicular traits as selection criteria for young Nelore bulls. *J. Anim. Sci.* 89:2061–2067.
- Silver G.V., and E.O. Price. 1986. Effects of individual vs. group rearing on the sexual behavior of prepuberal beef bulls; mount orientation and sexual responsiveness. *Appl. Anim. Behav. Sci.* 15: 287-294.
- Smith B.A., J.S. Brinks, and G.V. Richardson. 1989. Estimation of genetic parameters among breeding soundness examination components and growth traits in yearling bull. *J. Anim. Sci.* 67: 2892-2896
- Society for Theriogenology Manual for Breeding Soundness Examination. 2nd ed. Society for Theriogenology. Pike Road, AL.
- Suchocki, T., and J. Szyda. 2015. Genome-wide association study for semen production traits in Holstein-Friesian bulls. *J. Dairy Sci.* 98: 5774-5780.
- Syrstad, O. 1981. Selection for fertility in the basis of AI data. *Livest. Prod Sci.* 8: 247-252.
- Taylor, J. F., B. Bean, C.E. Marshall, and J.J. Sullivan. 1985. Genetic and environmental components of semen production traits of artificial insemination Holstein bulls. *J Dairy Sci.* 68: 270.3-2733.
- Taylor, J.F., R.D. Schnabel, and P. Sutovsky. 2018. Identification of genomic variants causing sperm abnormalities and reduced male fertility. *Anim. Repro. Sci.* 194: 57-62.
- Taylor, J.F., R.D. Schnabel, and P. Sutovsky. 2018. Review: genomics of bull fertility. *Animal*. 12: s172-s183.

- USDA, 2009. Part II: Reference of Beef Cow-calf Management Practices in the United States. Available from: United State Department of Agriculture National Animal Health Systems Monitoring System Beef Cow-Calf studies. (Accessed 6 February 2019). https://www.aphis.usda.gov/animal_health/nahms/beefcowcalf/downloads/beef0708/Beef0708_dr_PartII.pdf
- Utt, M.D. 2015. Prediction of bull fertility. *Anim. Repro. Sci.* 169: 37-44.
- Van Eenennaam, A. L., R. L. Weaver, D.J. Drake, M.C. Penedo, R.L. Quaas, D.J. Garrick, and E.J. Pollack. 2007. DNA-based paternity analysis and genetic evaluation in a large, commercial cattle ranch setting. *J. Anim. Sci.* 85: 3159-3169.
- Vernocchi, V., M. G. Morselli, S. Varesi, S. Nonnis, E. Maffioli, A. Negri, G. Tedeschi, and G. C. Luvoni. 2014. Sperm ubiquitination in epididymal feline semen. *Therio.* 82: 636-642.
- Vishwanath, R. 2003. Artificial insemination: the state of the art. *Theriogenology.* 59: 571-584.
- Vogler, C.J., J.H. Bame, J.M. DeJarnette, M.L. McGilliard, and R.G. Saacke. 1993. Effects of elevated testicular temperature of morphology characteristics of ejaculated spermatozoa in the bovine. *Theriogenology.* 40: 1207-1219.
- Washington N.L. and S. Ward. 2006. FER-1 regulates Ca²⁺-mediated membrane fusion during *C. elegans* spermatogenesis. *J Cell Sci.* 119: 2552–62.
- Whiston, R., E. K. Finlay, M. S. McCabe, P. Cormican, P. Flynn, A. Cromie, P. J. Hansen, A. Lyons, S. Fair, P. Lonergan, C. O’Farrelly, and K. G. Meade. 2017. A dual targeted β -defensin and exome sequencing approach to identify, validate and functionally characterise genes associated with bull fertility. *Sci Rep* 7. doi:10.1038/s41598-017-12498-x
- Whittier, W.D., and T. Bailey. 2009. Predicting bull fertility. Virginia Cooperative Extension. 1-3.
- Williams, W.L. 1909. Veterinary obstetrics including the diseases of breeding animals and of the new-born. Ithaca, NY.
- Williamson, N. B., R. S. Morris, and G. A. Anderson. 1978. Pregnancy rates and nonreturn rates following artificial and natural breeding in dairy herds. *Aust. Vet. J.* 54:111–120.
- Yilmaz, A. M.E. Davis, and R.C.M Simmen. 2004. Estimation of (co)variance components for reproductive traits in Angus beef cattle divergently selected for blood serum IGF-I concentration. *J Anim. Sci.* 82: 2285-2292.
- Yin, L. L., J. M. Li, Z. M. Zhou, and J. H. Sha. 2005. Identification of a novel testis-specific gene and its potential roles in testis development/spermatogenesis. *Asian J Andro.* 7: 127-137.

Chapter 2 - Genetic Parameters Estimates for Beef Bull Semen

Attributes

Abstract

Improvements in bull reproductive performance are necessary to optimize the efficiency of cattle production. Female fertility has received much attention and has been enhanced through assisted reproductive technologies, as well as genetic selection; however, improving beef bull fertility has been largely ignored. Phenotypes routinely collected at bull semen collection facilities are believed to affect fertility and provide the phenotypes necessary for a genetic evaluation. The first objective of this study was to determine the significant fixed effects for modeling beef bull fertility using data from bull semen collection facilities. The second objective was to estimate variance components, heritabilities, repeatabilities, and correlations between beef bull semen attributes. Beef bull fertility phenotypes including volume (VOL), concentration (CONC), number of spermatozoa (NSP), initial motility (IMot), post-thaw motility (PTMot), three-hour post-thaw motility (3HRPTMot), percentage of normal spermatozoa (%NORM), primary abnormalities (PRIM), and secondary abnormalities (SEC) were obtained from two bull semen collection facilities. A total of 1,819 Angus bulls with 50,624 collection records were analyzed. A variety of fixed class and covariate effects were tested for significance using backward elimination. The significant class effects were collection location and collection day within year. The significant covariate effects included age at collection, days since previous collection, and cumulative comprehensive climate index (CCI). For this study, the CCI was calculated for a 75 day period including the 61 day spermatogenesis cycle and 14 day epididymal transit time. The 75 days prior to collection accounted for the environmental stress a bull may have experienced over the course of development of the spermatozoa, which was more

significant than CCI calculated for collection day or spermatogenesis start date. Pre-thaw beef bull semen traits had low heritability estimates of 0.11 ± 0.02 , 0.09 ± 0.02 , 0.08 ± 0.02 , and 0.12 ± 0.03 for VOL, CONC, NSP, and IMot, respectively. Heritabilities of post-thaw beef bull semen attributes were more variable at 0.10 ± 0.02 , 0.05 ± 0.04 , 0.10 ± 0.04 , 0.03 ± 0.03 , and 0.18 ± 0.04 for PTMot, 3HRPTMot, %NORM, PRIM, and SEC, respectively. The low to moderate heritability estimates indicate genetic improvement can be made in beef bull semen quality traits through selection.

Introduction

Reproductive efficiency impacts beef producers' profitability and often determines whether beef producers will reach their production goals (Harris, 1970). Reproduction is a dynamic trait, which involves many events including gametogenesis, fertilization, implantation, and embryo and fetal development (Abdollahi-Arpanahi et al., 2017). Though most selection tools for fertility in the beef industry focus on female reproduction, only focusing on female reproductive capabilities limits the potential for increased performance and genetic improvement (Petrunkina and Harrison, 2011). Braundmeier and Miller (2001) acknowledge that using subfertile or infertile bulls affects not only seedstock producers, but also multipliers and commercial producers, because the reproductive success of the herd is contingent on the bull's ability to cause conception. DeJarnette (2004) determined that a significant proportion of reproductive failure is attributable to bull subfertility, and the fertility of a bull is dependent on the semen quantity, semen quality, and health status of the bull.

A number of semen production traits are thought to influence bull fertility. Volume, concentration, and motility are often measured during breeding soundness examinations (BSEs) or semen collection for artificial insemination (AI) to evaluate a bull's breeding fitness. While

fertility traits are generally considered to be lowly heritable, heritability estimates in the literature are, on average, largely moderate (0.20-0.31; Taylor et al., 1985; Mathevon et al., 1994; Kaps et al., 2000; Kealy et al., 2006; Gredler et al., 2007; Druet et al., 2009; Suchoki and Syzda 2015; Berry et al., 2019). Dairy bull fertility phenotypes obtained from bull semen collection facilities have been used to estimate genetic parameters (Taylor et al., 1985; Mathevon et al., 1994; Druet et al., 2009; Suchoki and Syzda 2015; Berry et al., 2019); however, use of phenotypes obtained from beef bull semen collection facilities has been limited (Kaps et al., 2000; Kealy et al., 2006). Most studies of beef bull fertility traits have utilized breeding soundness examinations from yearling bulls (Smith et al., 1989; Christmas et al., 2001; Garmyn et al., 2011).

Limited genetic research utilizing beef bull fertility measures collected at bull semen collection facilities, combined with the need to better understand beef bull fertility to enable genetic progress, necessitates additional research. Developing tools to improve bull fertility in the beef industry would increase beef production, improve producer profitability, improve the efficiency and sustainability of the livestock industry, and could provide benefits to male fertility research in other species. The objective of this study was to determine the effects which contributed significantly to beef bull fertility, with the end goal of estimating variance components for beef bull fertility traits.

Materials and Methods

Population

Bull Stud A

Bull Stud A provided a total of 48,611 ejaculate records collected between 2004 and 2018 on 1,626 bulls. Ejaculate records consisted of information collected prior to freezing including volume (VOL), concentration (CONC), and initial motility (IMot) measurements.

Table 2.1 provides the definitions for each of the pre-freeze traits. Additionally, the records included collection date, collection barn, and whether the semen met quality standards for freezing. The quality threshold for freezing required ejaculates to have greater than 50% progressive motility and less than 30% abnormal sperm cells. Of the initial ejaculate records documented by stud A, 20,366 (42%) met the quality threshold for freezing. When ejaculations passed this threshold, additional semen quality measures were also provided, which included post-thaw motility (PTMot), three-hour post-thaw motility (3HRPTMot), number of straws produced per ejaculate, percentage of normal spermatozoa (%NORM), primary abnormalities (PRIM), and secondary abnormalities (SEC). The definitions and further explanation of when post-thaw semen traits are obtained are outlined in Table 2.1. For the semen to be considered viable for breeding by AI, the sample needed to have greater than 50% motility and no more than 30% abnormalities. Of those abnormal spermatozoa, fewer than 19% could have a primary abnormality.

Information obtained from admission records included registration number, name, breed, birthdate, and owner. Stud A also provided scrotal circumference, weight, and hip height measurements that were collected either when the bull went to the stud to be collected or during a yearly routine examination.

Procedures at stud A data dictated that if more than one ejaculate was collected on a single day, they were combined. Thus, a bull could have more than one ejaculate in a single record. Some of the bulls also had multiple demographic measurements taken on the same day (i.e., scrotal circumference, weight, hip height), so contradictory measurements were treated as missing.

For stud A, straw CONC varied among the collections and was extracted from a comments column. The entry system utilized by stud A auto-filled CONC and motility to receive default values even if a zero VOL measurement was inputted into the database. Thus, for any records with VOL of zero, CONC and motility were treated as missing. In cases where a VOL was entered, but the CONC and motility were the default values, they were also treated as missing to ensure that only real records were utilized. Stud A data additionally included some on-ranch semen collections. All on-ranch collections were excluded from the analysis because the underlying environmental conditions experienced by the bulls could not be determined. Additionally, number of spermatozoa (NSP) was not provided by the stud, so NSP was calculated by multiplying VOL and CONC.

Bull Stud B

Data obtained from stud B included 4,970 total collection records for 296 bulls collected between January 2008 and December 2018. The data included breed, registration number, birth date, collection date, age at collection, ejaculation method, ejaculation number, VOL, IMot, CONC, PTMot, 3HRPTMot, vigor score, morphology, abnormalities, and the number of straws frozen. The abnormality data included percentage of detached heads, proximal droplet, distal droplet, abnormal head, bent tail, midpiece, tail, and distal midpiece reflex. Like stud A, the ejaculate had to meet the same freezing quality in order to be frozen and tested for post-thaw semen quality measures. The freezing requirements were that a sample of the ejaculate had to have a progressive motility of at least 50% and no more than 30% abnormalities. If the pre-thaw standards were met, the post-thaw semen quality standards that were required for the semen to be considered viable were at least 50% progressive motility, no more than 30% abnormalities, and of the abnormalities, there could be no more than 19% of spermatozoa with a primary abnormality.

Stud B data included all ejaculates collected each day for each bull as separate values. As a result of stud B recording all individual ejaculates versus combining ejaculates within a single day like stud A, records from stud B were combined within a single day to make the data consistent between studs. Volume and number of straws were a sum of the respective measurements for all ejaculates in a single day and the remaining traits were an average of the records for each bull per day. Some of the CONC values were inputted as <100 million spermatozoa per milliliter. Values recorded as <100 million spermatozoa per milliliter indicated the values were too low to be measured by the spectrometer and were re-coded as 99 million spermatozoa per milliliter to maintain the variation of the data. If these values were re-coded as zero, they would have been excluded from the analysis and the dataset would have been missing records from bulls with the poorest CONC phenotypes. Like stud A, NSP was not provided for all records, so NSP was calculated by multiplying VOL and CONC.

In an effort to make the more detailed abnormality data from stud B comparable to stud A, the abnormalities were categorized as PRIM and SEC and combined. To find the initial PRIM record for each ejaculate, detached heads, proximal droplets, and abnormal heads were summed. Distal droplets, bent tail, midpiece, abnormal tails, and distal midpiece reflex were summed to obtain SEC. The PRIM and SEC were then averaged across individual ejaculates (only when multiple records existed within a single day for a bull) to obtain the PRIM and SEC measurements. The PRIM and SEC were subtracted from 100 to obtain the average %NORM. After combining the ejaculates collected on the same day, there were 2,828 collections for stud B.

Data Processing

Summary statistics for all phenotypes and potential fixed effects were obtained from SAS 9.4 software (SAS Institute Inc., Cary, NC, USA). Data was edited with R software (R Core Team, Vienna, Austria) using the following criteria: VOL and CONC had to be greater than zero and measurements recorded as percentages must be between 0 and 100. Records with a missing registration number were excluded from further analysis.

A total of 480 records were removed from stud A for having VOL or CONC measures at or below zero. For stud B, a total of 335 records were removed for either having a VOL or CONC phenotype recorded as a zero. No data was removed for any of the percentage traits; which included IMOT, PTMot, 3HRPTMot, %NORM, PRIM, and SEC, from either stud. For stud A, 48,131 collection records from 1,570 bulls were utilized for the analysis. From stud B, 2,493 collection records from 256 bulls were included in the analysis. Cumulatively, 50,624 collection records from 1,819 bulls were used to evaluate beef bull semen attributes. In analyses utilizing data combined across studs, only data recorded at both locations were used, which included VOL, CONC, IMot, NSP, PTMot, 3HRPTMot, %NORM, PRIM, and SEC.

Genetic Data

A five-generation pedigree was obtained from the American Angus Association for 1,819 bulls. The five-generation pedigree consisted of 6,521 males and 17,136 females. The American Angus Association also provided scrotal circumference expected progeny differences (EPDs) for 1,819 bulls. In addition, single nucleotide polymorphism (SNP) data for 1,163 bulls was obtained from the American Angus Association. Imputation was performed by the American Angus Association, so that all genotyped bulls had 54,609 SNP. Data were edited to remove SNPs with a call rate of < 0.90 ($n = 3,921$) and minor allele frequency (MAF) of < 0.05 ($n = 13,478$).

Animals with a call rate of < 0.90 were removed (n = 3). In addition, 620 SNP were removed due to Mendelian conflict. A total of 29.75% of the SNP were removed during quality control, thus, 38,515 SNPs from 1,160 animals were available for analysis after quality control filtering.

Environmental Data

Climatology data was obtained from the National Oceanic and Atmospheric Administration (NOAA; Rossow et al. 2016). The nearest NOAA station to stud A was approximately 20 miles away. The nearest NOAA station from stud B was approximately 10 miles away. Data obtained from NOAA included daily averages for temperature (Fahrenheit), humidity (%), pressure (inches of Mercury), wind speed (miles per hour), and snowfall (inches). All measurements obtained from NOAA were converted from Imperial units to metric units. Temperature humidity index (THI) was calculated for a total of 2,136 and 2,086 days corresponding with the spermatogenesis start date and collection date for each collection, respectively. The spermatogenesis start date was calculated as 75 days prior to the collection date to account for spermatogenesis and epididymal transit time. The numbers vary because NOAA was unable to provide complete weather information for each day. The reason for the incomplete data is unknown. In addition to spermatogenesis start date and collection date, a cumulative THI for the entirety of the spermatogenesis cycle and epididymal transit time (75 days) was also calculated. The THI was calculated for each date using the following formula (Mader et al., 2006):

$$THI = 0.8 \times \text{ambient temperature} + [(\% \text{ relative humidity} \div 100) \times (\text{ambient temperature} - 14.4)] + 46.4.$$

Table 2.2 outlines summary statistics for THI calculated for collection date, spermatogenesis start date, and the cumulative THI. The THI has four stress level categories:

normal, alert, danger, and emergency. Although 5% of the collection dates were categorized as alert or danger levels, most of the THI values calculated were well below the threshold for heat-related stress. The THI values for collection day ranged from 11 to 80. While 95% of the dates fell into a thermoneutral category, the THI may not adequately capture cold stress (Mader et al. 2010), and it has been proven that bull fertility can also be impacted by extremely cold weather conditions (Barth and Waldner 2002). Similarly, for spermatogenesis start date, 89% of dates were within the thermoneutral category. Unlike collection date, 2% of the bulls experienced the most extreme THI stress level on the spermatogenesis start date. The remaining 9% of spermatogenesis start dates were within the alert or danger levels.

The Comprehensive Climate Index (CCI) may be able to capture both heat and cold stress better than THI (Mader et al. 2010). Thus, a CCI for spermatogenesis start date, collection day, and a cumulative CCI was also generated. The CCI was calculated for 2,136 collection days and 2,086 spermatogenesis start days. Again, like THI, weather data was not available for all requested dates, causing the difference in days CCI was calculated. In addition, individual cumulative CCI for the 75-day spermatogenesis cycle were calculated. The following equation was used to calculate CCI:

$$CCI = Ta + Adj.RH + Adj.WS + Adj.RAD$$

Mader et al., 2010

Solar radiation was not available in the NOAA weather data, so yearly average solar radiation measurements (kW/m) for the same location where the NOAA data was obtained were accessed from the National Renewable Energy Laboratory (NREL; DOE 2019). The CCI requires relative humidity, windspeed, and radiation be adjusted to accurately capture environmental conditions. For example, a high wind speed on a cold day could cause the animal

to experience more cold stress than a day at the same temperature, but with less wind. Thus, measurements for relative humidity, windspeed, and solar radiation were adjusted according to the methods outlined by Mader et al. 2010. Summary statistics for calculated CCI are shown in Table 2.2. The CCI for spermatogenesis start day ranged from -18.0 to 40.0, while the CCI for collection day ranged from -19.2 to 40.0. According to Mader et al. (2010), with the dataset utilized to create the CCI, values for CCI ranged from -39.7 to 47.9, so the values calculated in the present study fall within probable CCI ranges and within the data ranges for which the equation was developed. When developing the CCI, Mader et al. (2010) determined arbitrary thermal stress thresholds. As reported by Mader et al. (2010), animals experience little to no stress from heat-related conditions at a CCI less than 25. On the other hand, animals do not experience stress due to cold conditions at CCI greater than 0. Comprehensive climate index stress levels are categorized as no stress (0 to 25), mild (25 to 30 and -10 to 0), moderate (greater than 30 to 35 and less than -10 to -20), severe (greater than 35 to 40 and less than -20 to -30), extreme (greater than 40 to 45 and less than -30 to -40), and extreme danger (greater than 45 and less than -40). Based on the minimum and maximum values from the current study, the highest level of stress the bulls were exposed to was severe stress. On collection day, 59% of the dates were classified as having no stress and 56% of the spermatogenesis start dates were considered to have no stress. Twenty-one percent of the collection dates and 26% of the spermatogenesis start dates had CCI values consistent with animals experiencing mild cold or heat stress. A total of 281 (13%) of collection dates had moderate heat stress and 2% of the collection dates were on moderate cold stress days. Likewise, 251 (12%) of the spermatogenesis start dates were considered moderate heat stress and 2% (37 days) of the spermatogenesis start days were considered moderate cold stress. Of the collection dates, only 4% of the dates were classified as

having severe heat stress, and no dates were classified as having severe cold stress. Similarly, on spermatogenesis start day, only 4% of the dates were considered severe heat stress with none of the spermatogenesis start days falling within the severe cold stress category.

The cumulative CCI was calculated for each bull's spermatogenesis cycle. Summary statistics for the days within the cumulative CCI for the spermatogenesis cycle were obtained and an overall average within each period of cumulative CCI was calculated. The average CCI for the days within the spermatogenesis cycle was 14.4, which is well within the range of no stress; however, the minimum average CCI for the days within the spermatogenesis cycle was -2.8 and the maximum average CCI for the days within the spermatogenesis cycle as 31.8 which falls within the moderate stress level. The minimum and maximum average CCI were within the mild stress level (-4.1 and 29.5, respectively). Altogether, this indicates that the bulls were exposed to minimal levels of stress due to weather events.

Data Processing

Model Selection

There are a variety of environmental factors that affect bull fertility traits including age (Senger 2012; Taylor et al. 1985; Barth and Waldner 2002), social interactions (Beerwinkle 1974; Fritz et al. 1999; Van Ennennaam et al. 2007), temperature (Barth and Waldner 2002; Arnold and Dudzinski 1978), and collection interval (Fuerst-Waltl et al. 2006). All available putative predictor variables, other than climatic conditions, (Table 2.2) were extracted from the dataset to model environmental factors and utilized in a model selection procedure. As CCI was already selected over THI, it was also included, to determine which covariates and fixed effects to include in the genetic prediction model.

For this analysis, the following variables were included in a univariate mixed model with each response variable in SAS 9.4:

$$\begin{aligned}
 y_i = & Own_i + Season_i + Barn_i + Loc_i + DYYR_i + Day_i + Year_i + Age_i + Age_i^2 \\
 & + DaysSince_i + DaysSince_i^2 + Coll_CCI_i + Coll_CCI_i^2 + Sperm_CCI_i \\
 & + Sperm_CCI_i^2 + Cum_CCI_i + Cum_CCI_i^2 + SC_i + SC_i^2 + Weight_i \\
 & + Weight_i^2 + HH_i + Coll_ID_i + Animal_ID
 \end{aligned}$$

where OWN is bull owner, Season is season of collection, Barn is barn, Loc is stud location of bull, DYYR is collection day within year, Day is collection day, Year is collection year, Age is age at collection as a linear effect, DaysSince is days since previous collection, Coll_CCI is CCI on collection day, Sperm_CCI is CCI on spermatogenesis start date, Cum_CCI is cumulative CCI, SC is scrotal circumference, Weight is weight, HH is hip height, Coll_ID is the sequential collection number for each individual bull, and Animal_ID is the random effect of the individual animal as a repeated measure. All variables that are squared are the quadratic effect for the corresponding variable. Backward elimination was used to determine fixed effects contributing significantly to the model for each beef bull semen attribute (Table 2.7).

Genetic Parameter Estimation

First, a univariate animal model with permanent environment effect was utilized to determine starting values for subsequent bivariate analysis. Variance components were estimated utilizing an average information restricted maximum likelihood (AIREML) algorithm (Misztal et al. 2014). Both pedigree and genomic data were included in the single-step GBLUP analysis. The H^{-1} matrix was utilized instead of the traditional numerator relationship matrix, A^{-1} , to incorporate genomic information from genotyped animals. The H^{-1} matrix includes both pedigree and genomic relationship information and is constructed as follows:

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

where $\mathbf{A}^{-1}$ is the inverse of the pedigree-based numerator relationship matrix, $\mathbf{A}_{22}^{-1}$ is a subset of the numerator relationship matrix for the genotyped individuals, and $\mathbf{G}$ is the genomic relationship matrix for the genotyped individuals. Following the univariate analyses, a linear multivariate animal model with repeated records was fitted utilizing the BLUPF90 software package to obtain both variance and covariance parameter estimates. The multivariate models for the various traits are outlined below.

Variance components, heritabilities, repeatabilities, and genetic correlations for VOL, CONC, and NSP were estimated using the following trivariate model:

$$\begin{bmatrix} y_1 \\ y_2 \\ y_3 \end{bmatrix} = \begin{bmatrix} X_1 b_1 \\ X_2 b_2 \\ X_3 b_3 \end{bmatrix} + \begin{bmatrix} Z_{a1} a_1 \\ Z_{a2} a_2 \\ Z_{a3} a_3 \end{bmatrix} + \begin{bmatrix} W_{pe1} p e_1 \\ W_{pe2} p e_2 \\ W_{pe3} p e_3 \end{bmatrix} + \begin{bmatrix} e_1 \\ e_2 \\ e_3 \end{bmatrix}$$

where y_i is a vector of observed phenotypic observations, X_i is an incidence matrix relating the phenotypic observations to the fixed effects in the model, b_i is a vector of the fixed effects, Z_{ai} is an incidence matrix relating the phenotypic observations to the additive direct genetic effects, a_i is a vector of additive direct genetic effects, W_{pei} is an incidence matrix relating the phenotypic observations to the additive permanent environment genetic effects, $p e_i$ is a vector of additive permanent environment genetic effects, and e_i is a vector of random residuals.

Volume, CONC, and NSP were analyzed in a trivariate model because these three traits are measured pre-freezing. In addition, NSP is a function of VOL and CONC.

Initial motility, PTMot, and 3HRPTMot would not converge in a trivariate model. Percentage of normal spermatozoa, PRIM, and SEC also would not converge using a trivariate model. Instead, pairwise combinations of traits in bivariate models were performed within the motility and normality groups of traits. In addition, volume, concentration, and number of

spermatozoa were included in bivariate models with the remaining traits in order to estimate genetic correlations among all the traits. The following model was used to estimate variance components, heritabilities, repeatabilities, and genetic correlations for IMot, PTMot, 3HRPTMot, %NORM, PRIM, and SEC:

$$\begin{bmatrix} y_1 \\ y_2 \end{bmatrix} = \begin{bmatrix} X_1 b_1 \\ X_2 b_2 \end{bmatrix} + \begin{bmatrix} Z_{a1} a_1 \\ Z_{a2} a_2 \end{bmatrix} + \begin{bmatrix} W_{pe1} p e_1 \\ W_{pe2} p e_2 \end{bmatrix} + \begin{bmatrix} e_1 \\ e_2 \end{bmatrix}$$

where y_i is a vector of observed phenotypic observations, X_i is an incidence matrix relating the phenotypic observations to the fixed effects in the model, b_i is a vector of the fixed effects, Z_{ai} is an incidence matrix relating the phenotypic observations to the additive direct genetic effects, a_i is a vector of additive direct genetic effects, W_{pei} is an incidence matrix relating the phenotypic observations to the additive permanent environment genetic effects, $p e_i$ is a vector of additive permanent environment genetic effects, and e_i is a vector of random residuals. The analyses were completed using the BLUPF90 suite of programs (Misztal et al. 2014).

Estimated breeding values (EBV) for each individual, including those in the pedigree, were utilized for further inquiry. For the calculation of EBVs, the following univariate animal model was used:

$$[y_1] = [X_1 b_1] + [Z_{a1} a_1] + [W_{pe1} p e_1] + [e_1]$$

Estimated breeding values (EBV) provide the opportunity for selection decisions to be made using all available information on an animal. There were 8,539 individuals utilized for the estimation of breeding values. A univariate animal model was used for the calculation of EBVs because the univariate results were very similar to the multivariate results. In addition, the correlation between the bivariate EBV results from each of the analyses for a trait were estimated and are reported in Table 2.8. As noted in Table 2.8, only the IMot EBV and SEC EBV results were highly correlated, thus the univariate model was used to calculate the EBV in order to

provide the estimate. In addition to the EBVs, accuracies were calculated. Beef Improvement Federation (BIF) accuracy was calculated utilizing the BLUPF90 suite of programs. The following accuracy equation was used:

$$Accuracy_{BIF} = 1 - \sqrt{\frac{prediction\ error\ variance}{additive\ genetic\ variance}}$$

(BIF Guidelines, 2020)

In addition to BIF accuracy, classical animal breeder accuracy was additionally calculated utilizing the average BIF accuracy obtained from the calculation above. The conversion equation used was:

$$r_{EBV,BV} = \sqrt{1 - (1 - Accuracy_{BIF})^2}$$

(BIF Guidelines, 2020)

Results and Discussion

Summary statistics for the response variables are reported in Table 2.9. Interestingly, mean IMot was 49.18% and the median was 50%, which is just below the 50% minimum a collection must have to be considered for freezing. However, of the 44,431 collection records, 67% of the collections met the initial freezing requirements and had post-thaw quality control records. Comparing the mean to the median, it was determined ejaculates with poor IMot had a very low percentage of motile spermatozoa, which skewed the average IMot down. The average PTMot was 43.54% with a median of 50%. Similar to IMot, very poor PTMot measures were recorded causing the average PTMot to be lower than expected. This was verified by comparing the mean with the median. The average 3HRPTMot was substantially lower, as expected, at 15.73% with a lower median of 15%. It is difficult to speculate whether the three-hour post-thaw measures are good indicators or bad indicators of fertility because of the high standard errors for

the genetic correlations with other beef bull fertility traits. The high standard errors are likely a result of the trait not being recorded for every collection. In addition, collections that did not meet freezing quality standards would not have any post-thaw measures recorded, resulting in biased data.

Model Selection

Table 2.7 outlines the fixed effects that were significant for each beef bull fertility trait. For VOL, collection day within year, age, and days since previous collection were determined to be significant. Statistically significant effects for CONC were collection location and collection day within year. Initial motility and NSP both yielded significant effects of collection location, collection day within year, age, and days since previous collection. Collection location was not significant when modeling the %NORM or PRIM. Days since previous collection was not significant for any of the post-thaw beef bull semen attributes. In addition, cumulative CCI was not significant at a P -value of <0.05 for any of the traits, but when the P -values for CCI on collection day, spermatogenesis start day, and cumulative CCI were compared, cumulative CCI had the most significant P -value.

After examining the significant effects found in the current study and comparing them with previously published studies (Taylor et al., 1985; Berry et al., 2019), the following model was utilized for the genetic evaluation for all beef bull fertility traits:

$$y_i = b_0 + b_1 Location + b_2 DayYear + b_3 Age + b_4 DaysSince + b_5 Cum CCI.$$

The model includes collection location ($n = 2$), collection day within year, age at collection, days since previous collection, and cumulative CCI. Collection location and collection day within year were fit as class variables. There were two different bull semen collection facilities that provided phenotypic records. There were 2,734 classes for collection day

within year. The fixed effects fit as covariates were age at collection, days since previous collection, and cumulative CCI. Age at collection ranged from 296 days to 4,884 days with an average of 1,134 days. The average days since previous collection was 8 with a range of 1 to 2,756 days. The cumulative CCI ranged from -207 to 2,390 with an average of 1,076. Table 2.7 outlines the levels of significance for all traits included in the model.

Variance Components

The additive direct genetic, permanent environment, and residual variances for the ssGBLUP analyses are shown in Table 2.10. The reported variance components, heritabilities, and repeatabilities results for VOL, CONC, and NSP were obtained from the trivariate analysis. For the remainder of the beef bull semen attributes, estimates from all bivariate analyses were averaged and reported. Limited published research makes comparison to previous studies difficult. Taylor et al. (1985) published a direct additive genetic variance for VOL that was slightly lower than the estimate in the current study. However, Taylor et al. (1985) did not account for a permanent environment effect and the environmental variance was much lower than the estimate in the current study. Additionally, Berry et al. (2019) published a similar genetic variance estimate for VOL of 0.72 mL^2 .

All variance estimates in the literature for CONC are much higher than the estimates for this study presented in Table 2.10. Taylor et al. (1985) estimated the genetic and environment variances for CONC were 7.45×10^7 million/ mL^2 and 1.24×10^8 million/ mL^2 , respectively. Kaps et al. (2000) used a population of Simmental bulls with a single breeding soundness examination record to estimate variance components for CONC. The direct additive genetic effect was 4.36×10^6 million/ mL^2 and environmental variance of 1.22×10^7 million/ mL^2 . Mathevon et al. (1998)

estimated variances for the genetic effect, permanent environment effect, and environment effect of 1.86×10^8 million/mL², 2.6×10^4 million/mL², and 3.8×10^4 million/mL², respectively.

Varying motility measures with variance component estimates are presented in the literature (Mathevon et al., 1998; Kealy et al., 2006; Corbet et al., 2012), and most are much higher than the variance components in Table 2.10. The exception are the variances published by Berry et al. (2019), which reported estimates of 30.25%² and 21.72%² for the genetic variance of the percentage of living spermatozoa pre-freezing and the genetic variance post-freezing, respectively. Berry et al. (2019) did not publish variance component estimates for permanent environment or environmental variance.

Variance component estimates in the literature (Kealy et al., 2006; Corbet et al. 2012) for the %NORM are much higher than the estimates in Table 2.10. Kealy et al. (2006) published genetic and environmental variances for PRIM and SEC. While the PRIM variance components are different than those displayed in Table 2.10, the SEC variance components were comparable. Kealy et al. (2006) published a genetic variance estimate of 17.50%² and environmental variance of 36.04%² for SEC; however, there were not repeated records in the study.

Heritability Estimates

Heritability estimates from multivariate ssGBLUP analysis are shown in Table 2.10. The heritability estimate for VOL was low (0.11 ± 0.02), but within the range of published literature estimates. Kaps et al. (2000) reported a heritability for VOL of 0.04 from a study of young Simmental bulls; however, the standard error for the estimate was extremely large (0.54), so the estimate was not different from zero. Kealy et al. (2006) published an estimate of 0.09 ± 0.08 with a population of young Line 1 Hereford bulls. Kaps et al. (2000) obtained VOL measurements from breeding soundness examinations; whereas Kealy et al. (2006) obtained

measurements from semen collected from Line 1 Hereford bulls specifically for the study. Volume estimates in the literature that were obtained from dairy bull semen collection facilities have yielded more moderate heritability estimates of 0.20 (Berry et al. 2019), 0.22 (Druet et al. 2009), and 0.26 (Suchoki and Syzda 2015). It is difficult to speculate the justification for the differences due to the lack information available in the literature. While the sample size and method of phenotype collection in the current study is most similar to the dairy bull studies, the heritabilities are more similar the data obtained from beef bull studies.

The heritability estimate for CONC in this study was lower than published heritability estimates ranging from 0.13 to 0.52. Interestingly, beef bull heritability estimates in the literature for CONC were lower (0.13, Knights et al. 1984; 0.16, Kealy et al. 2006; and 0.26, Kaps 2000) than those estimated using data from dairy bull semen collection facilities (0.34, Suchoki and Syzda 2015; 0.52, Mathevon et al. 1998; 0.39, Ducrocq and Humblot 1995). Thus, the comparably lower heritability estimates from the current study could be attributed to the fact the data were collected from beef bulls. All studies referenced here had a smaller population size than the current study, with the exception of Ducrocq and Humblot (1995) which used a population of 2,387 Normande dairy bulls.

Number of spermatozoa had a low heritability estimate of 0.08 ± 0.02 . Taylor et al. (1985) noted that estimating the heritability of a trait that is a function of two other traits, such as NSP, could impact that ability to accurately provide genetic estimates because the trait that is a function of others is influenced by the phenotypes collected on the original two traits. The heritability estimate for NSP in the current study is slightly higher than the estimate of 0.03 reported by Taylor et al. (1985), which utilized dairy bull phenotypes from an bull semen collection facility. More recently, Suchoki and Syzda (2015) and Berry et al. (2019) published

heritability estimates of 0.27 and 0.38 for NSP with data obtained from dairy bull semen collection facilities. Differing estimates could be attributed to using dairy bulls, smaller population size in the literature, or the model used for genetic evaluation. While Suchoki and Syzda (2015) and Berry et al. (2019) both utilized animal models, the fixed effects included in their models were different than effects included in the current study. Suchoki and Syzda (2015) only included fixed effects of bull age and season, whereas Berry et al. (2019) additionally included fixed effects of inbreeding, days since last ejaculation, and breed.

Initial motility had a heritability estimate of 0.12 ± 0.03 . Initial motility heritabilities published in the literature range from 0.05 to 0.43. The studies that published low heritability estimates (0.13, Knights et al. 1984; 0.08, Smith et al. 1989; 0.07, Christmas et al. 2001; 0.05, Garmyn et al. 2011) come from beef bull motility phenotypes obtained from yearling breeding soundness examinations. Motility phenotypes obtained from beef and dairy bull semen collection facilities had higher heritabilities (0.22-0.43; Ducrocq and Humblot 1995; Mathevon et al. 1998; Kealy et al. 2006; Suchoki and Syzda 2015; Berry et al. 2019).

Post-thaw motility had a heritability estimate of 0.15 ± 0.03 , which was slightly higher than the IMot heritability estimate. The PTMot estimate is lower than those found in the literature, which vary from 0.21 to 0.25 (Kealy et al. 2006; Druet et al. 2009; Berry et al 2019). While there are only three post-thaw estimates found in the literature, the estimates come from both beef and dairy bulls from semen collection facilities. The population size for the current study was larger than the populations used in these studies; therefore, the standard error of the estimate in the current study was smaller than the other studies.

Three-hour post-thaw motility was lowly heritable (0.09 ± 0.04). Three-hour post-thaw motility had the smallest number of phenotypes ($n = 8,299$) with only 561 bulls contributing

phenotypes to the trait. There are no comparable estimates published in the literature; however, if the estimate is compared to the previously discussed published motility estimates, the estimate is much lower. This may be a function of the trait if the variance in the population collapses quickly after thawing, or an artifact of the number of phenotypes available for analysis.

The %NORM yielded one of the more variable heritability estimates from the bivariate analyses (0.09 ± 0.04). The genetic variance in the bivariate model with primary abnormalities was much lower than the bivariate model with secondary abnormalities. In studies using beef bulls, Smith et al. (1989) and Gredler et al. (2007) published similar heritability estimates of 0.07 and 0.10, respectively. Gredler et al., (2007) utilized data obtained from Austrian Simmental bulls collection for AI. In the Kealy et al. (2006) study using semen collected from Line 1 Hereford bulls, an estimate of 0.35 was published. Differences in literature estimates could be attributed to differing population sizes ($n = 301$, Gredler et al. 2007; $n = 841$ Kealy et al., 2006), as both reports used a smaller population size than the current study.

The abnormality traits had varying heritability estimates of 0.03 ± 0.03 for PRIM and 0.18 ± 0.04 SEC in both bivariate models. The PRIM heritability estimates in the literature that were obtained from beef bulls range from 0.27 to 0.35 (Smith et al. 1989; Christmas et al. 2001; Kealy et al. 2006; Garmyn et al. 2011), which are higher than in this study. Comparable studies reporting post-thaw semen attributes from phenotypes obtained from bull semen collection facilities are difficult to find; however, Druet et al. (2009) published moderate heritability estimates of 0.35 for percentage of spermatozoa with an abnormal head, a trait comparable to PRIM. Similar to SEC, Druet et al. (2009) published an estimate for percentage of spermatozoa with an abnormal tail and percentage of spermatozoa with an abnormal cytoplasmic droplet of 0.19 ± 0.12 and 0.19 ± 0.08 , respectively. Smith et al. (1989) published a SEC estimate not

different from zero (0.02 ± 0.05); however other beef bull studies reported moderate heritability estimates of 0.26 (Christmas et al. 2001), 0.33 (Kealy et al. 2006), and 0.23 (Garmyn et al. 2011).

While the reported heritability estimates are low to moderate, the majority of the standard errors are small and thus estimates are different from zero. While fertility traits are generally lowly heritable, it does not mean that these traits should be ignored and genetic improvement cannot be made. An example of another lowly heritable trait which has been well-studied and improved is productive life in Holstein dairy cattle. Productive life is a trait which is indicative of how long a cow stays in the herd after her first calving (Strandberg 1992) and has an average heritability estimate of 0.085 (Bennet 2009). The Council on Dairy Cattle Breeding (CDCB) estimated the average breeding value of productive life was -12.73 in 1960. In 2016, the average estimated breeding value for productive life was 4.53. In 56 years, the dairy cattle industry was able to improve productive life by 17.26 days through recording phenotypes and utilizing selection tools. Similar improvements in male fertility can be made if the necessary genetic selection tools are developed and deployed within the industry.

Repeatability Estimates

The average number of collections per bull was 28 with a range of 1 to 1,181. Repeatability estimates are shown in Table 2.10. All estimates were moderately to highly repeatable with small standard errors, meaning AI beef bull fertility traits are consistent within bull.

Published repeatability estimates for bull semen attributes are similar to the estimates found in the current study. The estimated repeatability for VOL was similar to that published in other studies. Using dairy bull populations, Haque et al. (2001), Gredler et al. (2007), and Druet

et al. (2009) published similar repeatability estimates of 0.34, 0.29, and 0.33, respectively. Taylor et al. (1985) published a slightly lower estimate of 0.23 using dairy bulls. Mathevon et al. (1998) estimated the repeatability of VOL in young and mature dairy bulls, and published estimates were 0.45 and 0.51, respectively. Additionally, Stalhammar et al. (1989) published a repeatability of 0.57 in a population of dairy bulls.

The repeatability of CONC was estimated to be 0.33 with a small standard error in the current study. Taylor et al. (1985), Druet et al. (2009), and Gredler et al. (2007) estimated similar values ranging from 0.32 to 0.37. Haque et al (2001) found a lower repeatability of 0.20 in a population of 30 dairy bulls. On the other hand, Mathevon et al. (1998) found a higher estimate of 0.61 in a population of mature dairy bulls.

Number of spermatozoa had a similar repeatability to VOL and CONC, unsurprisingly, as NSP is a function of the two. The repeatability estimate for NSP are shown in Table 2.10. Gredler et al. (2007) and Druet et al. (2009) found a slightly lower estimate of 0.24. Both studies previously mentioned used dairy bull phenotypes collected from an bull semen collection facility. Mathevon et al. (1998) published a repeatability estimate for total spermatozoa, a phenotype similar to NSP. For mature dairy bulls, Mathevon et al. (1998) reported an estimate of 0.54.

Initial motility, PTMot, and 3HRPTMot had repeatability estimates of 0.40, 0.33, and 0.29, respectively. Literature estimates for motility repeatability range from 0.08 to 0.64. The lowest repeatability was estimated using motility score phenotypes (Gredler et al. 2007) from dairy bulls. The highest estimate was published by Mathevon et al. (1998) in a population of mature dairy bulls. Haque et al. (2001) used motility phenotypes for mass activity, which is a scoring system evaluating the overall motility of the sample. Stalhammar et al. (1989) published

repeatability estimates for motility pre-freezing and post-freezing with estimates of 0.54 and 0.53, respectively.

Repeatability estimates for %NORM, PRIM, and SEC are difficult to find in the literature. Percentage of normal spermatozoa had a repeatability of 0.53 with a standard error of 0.01. The moderate repeatability indicates phenotypes are consistent across collections. Primary abnormalities had the highest repeatability estimate of the study (Table 2.10). Secondary abnormalities had a repeatability estimate of 0.48, suggesting a bull will routinely have a similar PRIM and SEC measures from collection to collection.

Estimated Breeding Values

Estimated breeding values were obtained for each beef bull semen attribute from a univariate analysis. Summary statistics for EBV for the bulls with phenotypes are depicted in Table 2.11. The averages for each beef bull fertility EBV are near zero for all traits, but the minimum and maximum values for the EBV indicate the values have a wide range. Notably, CONC, NSP, and IMot have negative average EBV, which would indicate that bulls with phenotypes are slightly lower than the population as a whole (including animals in the pedigree without phenotypes). In addition, accuracies are in Table 2.11. The average BIF accuracies ranged from 0.05 to 0.24. In addition to BIF accuracy, classical animal breeder accuracy was calculated, and as expected, the results were higher. The classical accuracies were generally moderate, with the exception of PRIM. The accuracy for PRIM was 0.30. This would be expected as PRIM had the lowest heritability with a standard error that made the estimate not different than zero. While the EBV in the current study are preliminary, the moderate accuracies indicate the EBV estimated in the current study are reliable estimates. Reliable estimates indicate producers can confidently use estimates when making selection decisions.

Genetic Trends

Genetic trends are shown in Figures 2.1 through 2.9. As expected, the genetic trends for all traits are variable. There are currently no selection tools for producers to utilize for the improvement of beef bull fertility traits. Volume appears to increase from 1997 to 2004 and then shows a slight decrease. The trend for VOL could be a result of indirect selection, but it is more probable that the trend is due to sample size. Until 2005, there were less than 50 bulls born each year contributing the average; however, in 2005 the number of bulls doubled. In subsequent years after 2005, the population remained above 100 bulls in each birth year. The genetic trend for CONC appears to present a cubic effect where the trait decreased until 2002, increased until 2010, and then experienced a decrease again. Number of spermatozoa appears to be quadratic where the EBV increased until 2009 and then decreased in subsequent birth years. Considering NSP is a function of VOL and CONC, it would be expected the genetic trend would be a blend of the two traits. For IMot the smooth line indicates a decrease in the trait from 1997 to 2004; however, the standard error bars shown in the figure indicate the estimates are very variable and this could be attributed the extremely small sample size in 1997 and 1998 ($n = 3$ and 1 bulls, respectively). After the decrease in IMOT until 2007, the trait remained steady in the following years. Similar to PTMot, 3HRPTMot shows an initial decrease, but then increases until 2017. The trendline for %NORM shows a slight decrease until 2005 and then the trait begins to slightly increase, but again, small population sizes within the early birth years could be attributed to the variable estimates. Primary abnormalities and SEC show inverse genetic trends. When the average EBV for PRIM was low, the average EBV for SEC was high, and vice versa. Providing producers with genetic selection tools for beef bull fertility traits could make the genetic trends more favorable in the future.

While some trends may be speculated, there is not a common trend among all fertility traits. It does not appear the traits evaluated have improved or declined as a whole over the past 20 years. The trends shown in Figures 2.1 through 2.9 generally indicate fertility has remained the same.

Correlations

Genetic and phenotypic correlations between Angus bull semen attributes are shown in Table 2.12. The majority of the genetic and phenotypic correlations were low, with several notable exceptions. Number of spermatozoa was strongly and positively phenotypically correlated with VOL and CONC. This would be expected as NSP is a function of VOL and CONC. The genetic correlation between VOL and NSP mirrored the phenotypic correlation, which was strong and favorable. Literature estimates for phenotypic and genetic correlations between VOL and NSP are varied. Gredler et al. (2007) estimated the genetic correlation to be 0.83 ± 0.13 in a population of dual-purpose Simmental bulls. Similarly, Berry et al. (2019) estimated phenotypic and genetic correlations of 0.63 and 0.66 ± 0.16 , respectively. Taylor et al. (1985) and Druet et al. (2009) published lower genetic correlation estimates (0.45 and 0.47, respectively); however, the phenotypic correlations were more similar to the phenotypic correlation estimates presented in the current study. Genetic correlations between CONC and NSP in the literature are higher than the genetic correlation reported in this study (0.54). Gredler et al. (2007) estimated the genetic correlation was 0.60, and Taylor et al. (1985) published a slightly higher correlation of 0.72. However, estimates from the literature are similar to our estimated phenotypic correlation. All phenotypic correlations in the literature range from 0.51 to 0.71 (Taylor et al. 1985; David et al. 2006; Gredler et al. 2007; Druet et al. 2009; Berry et al. 2019), and our estimate was 0.61.

The phenotypic and genetic correlations between pre-freeze and post-thaw semen characteristics were generally low. Interestingly, CONC was negatively phenotypically and genetically correlated with PTMot, indicating as CONC increases, PTMot decreases; however, the large standard errors make the estimate essentially not different from zero. The phenotypic correlation between CONC and 3HRPTMot mirrored the correlation between CONC and PTMot. Several of the genetic correlation estimates among pre-freeze and post-thaw semen traits had large standard errors and the estimates were not different from zero. The large standard errors could be attributed to the small sample size of the post-thaw semen traits.

Table 2.12 shows that IMot was strongly genetically correlated with all post-thaw semen characteristics. An increase in IMot would be associated with an increase in PTMot, 3HRPTMot, and %NORM. One would expect that a high IMot would lead to more motile spermatozoa after thawing with fewer abnormalities because abnormalities should affect the ability of the spermatozoa to move progressively. Previous literature has reported strong, positive phenotypic and genetic correlations between IMot and PTMot (Druet et al. 2009; Berry et al. 2019). In addition, previous studies have reported strong, positive phenotypic and genetic correlations between motility and %NORM (Druet et al. 2009; Berry et al. 2019). Varying and limited correlation estimates between IMot and spermatozoa abnormalities have been reported in the literature. While Smith et al. (1989) estimated the phenotypic and genetic correlations between motility and PRIM were -0.31 and -0.36, respectively, Kealy et al. (2006) reported a conflicting genetic correlation of 0.57. Literature estimates for correlations between motility and SEC are also contradictory. Smith et al. (1989) estimated a phenotypic correlation of -0.22 and a genetic correlation of 0.71. Conversely, Kealy et al. (2006) estimated a genetic correlation between motility and SEC of -0.54. A negative correlation between motility and PRIM and SEC traits

would be expected as the movement of the sperm cell is dependent upon a normally functioning head and tail, which is consistent with the genetic correlation results presented in Table 2.12.

Three-hour post-thaw motility was strongly and positively phenotypically correlated with PTMot (Table 2.12), implying that as PTMot increased, 3HRPTMot increased. The positive correlation indicated bulls with high PTMot continued to have high motility for an extended period of time after thawing. However, only 28% of the collections had a 3HRPTMot phenotype. There are currently no literature estimates for an extended PTMot measure making it impossible to compare current estimates with previous findings.

Primary abnormalities had a strong, negative phenotypic and a slightly less negative genetic correlation with 3HRPTMot indicating that as 3HRPTMot increased, PRIM decreased. A negative correlation would indicate that as motility increased, fewer abnormalities of the head would be observed. While there are no estimates in the literature pertaining to an extended PTMot measure, estimates from the current study can be compared to other motility estimates in the literature. Druet et al., (2009) reported a strong, negative genetic correlation between motility and percentage of spermatozoa with an abnormal head, but a positive phenotypic correlation, which contradicts the estimates from the current study. As discussed previously, Smith et al., (1989) reported a negative genetic correlation between motility and PRIM, but Kealy et al., (2006) reported a strong, positive genetic correlation. Again, the current study is most comparable to Kealy et al., (2006) because the phenotypes were obtained from frozen semen samples from Hereford bulls.

Secondary abnormalities had a moderate, positive phenotypic correlation with 3HRPTMot, which suggested that as 3HRPTMot increased, SEC increased. However, the genetic correlation was strong and negative. Similarly, Kealy et al., (2006) presented a strong,

negative genetic correlation between motility and SEC. A negative correlation would be expected, as 3HRPTMot would be expected to increase with fewer abnormalities of the tail present in the sample.

As expected, Table 2.12 shows that the %NORM was strongly, negatively phenotypically correlated with both PRIM and SEC. While the genetic correlation between %NORM and SEC was negative, there was a slight, positive genetic correlation between %NORM and PRIM. However, the standard error is extremely high and could be attributed to sample size. Smith et al. (1989) estimated that %NORM was strongly, negatively genetically correlated with PRIM. However, Smith et al. (1989) reported %NORM was positively genetically correlated with SEC (0.16).

Primary and SEC exhibited a negative phenotypic correlation with each other, which indicates that a sample with more PRIM tends to have fewer SEC. The genetic correlation was strong and negative; however, the standard error was greater than one. Kealy et al. (2006) reported a similar genetic correlation of -0.87 from a population of Line 1 Hereford bulls; however, Smith et al. (1989) reported a genetic correlation between PRIM and SEC of 0.14. The corresponding phenotypic correlation estimated by Smith et al. (1989) was 0.17. Smith et al., (1989) utilized breeding soundness examination data from yearling beef bulls for the genetic evaluation; whereas, Kealy et al., (2006) used phenotypes from frozen semen samples collected from Line 1 Hereford bulls. Thus, it would be expected the estimate in the current study would mirror that of Kealy et al., (2006).

Notably, some genetic correlations had exceedingly large standard error estimates. The large standard error estimates indicate the correlation estimate is not reliable. High standard errors were obtained for some of the correlations between post-thaw traits. The post-thaw traits

had fewer observations and were biased because the sample had to meet certain quality standard for post-thaw phenotypes to be recorded. These two reasons could account for the large standard errors.

Genetic Correlation among Scrotal Circumference EPD and Beef Bull Fertility

EBV

Correlations among scrotal circumference EPDs and beef bull semen attribute EBV were low (Table 2.13). The average BIF accuracy for the SC EPD was 0.52 indicating the SC EPD are fairly reliable estimates. Thus, the low correlations are not necessarily the fault of unreliable estimates. Literature estimates for the correlations among SC EPD and beef bull fertility EBV are limited, thus genetic correlations among fertility phenotypes are used for comparison. Generally, literature estimates indicate the correlations among scrotal circumference and fertility phenotypes are stronger than what was determined in the current study. The weakest correlation in the current study was between VOL EBV and scrotal circumference EPD (-0.01 ± 0.02), and the estimate was not different from zero. The strongest correlation found was between scrotal circumference EPD and the EBV for CONC (0.15 ± 0.02). According to the correlation, as scrotal circumference EPD increases, so does CONC EBV. Similarly, the correlation between scrotal circumference and NSP was favorable (0.12 ± 0.02) indicating as scrotal circumference EPD increases, so does NSP EBV, but the relationship was weak. Literature estimates for the correlation of VOL, CONC, and NSP to SC were not found. All correlations between scrotal circumference and motility measures were close to zero, but negative. Smith et al., (1989) estimated the genetic correlation between motility and scrotal circumference was 0.04, which is substantially lower than other literature estimates of 0.56 (Christmas et al., 2001), 0.36 ± 0.29 (Garmyn et al., 2011), and 0.56 ± 0.10 (Corbet et al., 2013). The correlation between scrotal

circumference EPD and %NORM EBV was -0.06 ± 0.02 . This estimate differs from genetic correlations in the literature which are positive, moderate, and favorable (Smith et al., 1989; Corbet et al., 2013). A majority of the literature estimates for the genetic correlation between scrotal circumference and PRIM are strong and negative (Christmas et al., 2001; Garmyn et al., 2011). The estimate in the current study is positive, albeit very low. In contrast, the correlation between scrotal circumference EPD and SEC EBV is negative, but also small. While our estimates are much lower than those in the literature, the direction of the estimates concurs with Christmas et al., (2001) and Garmyn et al., (2011).

Conclusion

Model selection revealed collection location, collection day within year, age at collection, days since previous collection, and cumulative CCI all impact beef bull fertility. Utilizing the determined fixed effects in a genetic model, genetic parameters were determined. Although the reported heritabilities in the current study were low, it should not diminish the importance of studying the genetics of beef bull fertility traits. Similarly to other lowly heritable traits, recording phenotypes and providing producers with genetic selection tools could allow genetic improvement. The nine different fertility phenotypes in the current study had varying correlations. While the majority of traits were highly genetically correlated, traits like concentration and motility or post-thaw motility and primary abnormalities were very lowly genetically correlated. While there did not appear to be significant trends for the EBV of the beef bull fertility phenotypes, the classical animal breeder accuracies for the EBV were generally moderate. In addition, beef bull fertility EBV were correlated with SC EPD to determine if SC could potentially be an indicator of beef bull fertility. However, the low correlations indicate SC EPD did not affect beef bull fertility EBV. Findings in the current research provide evidence beef

bull fertility could be impacted by genetic selection tools and current predictor traits available are not enough. The genetic potential of a bull's fertility is dependent upon fertility phenotypes being collected and evaluated rather than utilizing indicator traits to select for beef bull fertility.

Table 2.1 Beef bull fertility traits, units of measure, and definitions utilized in the genetic evaluation. Abbreviations are utilized in text. All post-thaw motility measures are observed the day after the sample is collected. All traits are measured within one hour with the exception of three-hour post-thaw motility.

Trait	Abbreviation	Units	Definition
Volume	VOL	mL	total amount of the ejaculate, measured by milliliters
Concentration	CONC	Million/mL	relative amount of sperm cells per ejaculate, measured by a colorimeter
Number of spermatozoa	NSP	Million	calculated by multiplying sperm concentration and semen volume; expressed in millions
Initial Motility*	IMot	%	percentage of progressively swimming spermatozoa in the ejaculate immediately after collection
Post-thaw Motility*	PTMot	%	percentage of progressively swimming spermatozoa in the ejaculate, measured within one hour of thawing
Three-hour post-thaw motility*	3HRPTMot	%	percentage of progressively swimming spermatozoa in the ejaculate, measured within three hours after thawing
Percentage of Normal Spermatozoa*	%NORM	%	percent morphologically normal spermatozoa
Primary Abnormalities*	PRIM	%	percentage of spermatozoa with a defect to the head
Secondary Abnormalities*	SEC	%	percentage of spermatozoa with a defect to the tail

*Trait is measured subjectively by a trained laboratory technician.

All post-thaw motility measures are observed the day after the sample is collected. All traits are measured within one hour with the exception of three-hour post-thaw motility.

Table 2.2 Potential fixed effects utilized in model selection with number of records (N), mean, standard deviation (std dev), minimum, and maximum for each potential class and covariate effect.

<i>Effect Type</i>	Fixed Effect	N	Mean	Std Dev	Minimum	Maximum
<i>Class</i>	Bull owner	835				
	Season of Collection	4				
	Barn	7				
	Stud	2				
	Collection Day Within Year	2574				
	Collection Day	363	162.97		2	366
	Collection Year	16	2012		2004	2019
	Age At Collection	50624	1135	757.40	296	4884
	Days Since Previous Collection	48780	7.97	46.96	0	2756
	Daily Average Temperature	49575	13.12	10.32	-17.22	36.67
	Daily Average Humidity	46992	39.35	17.41	1	83
	Daily Average Pressure	50298	56.91	17.96	1	125
	Daily Average Windspeed	50292	120.76	80.07	1	228
	Daily Average Snowfall	47988	3	7.25	1	60
	THI on Collection Day	46301	55.50	12.62	11.44	80.50
<i>Covariate</i>	THI on Spermatogenesis Start	45554	54.63	14.25	11.44	94.35
	CCI on Collection Day	46301	15.71	11.59	-19.27	40.04
	CCI on Spermatogenesis Start	45538	14.59	12.95	-18.04	40.04
	Cumulative CCI	19741	1077	503.37	-207.15	2390
	Cumulative Temperature	23156	884.99	437.44	-309.44	2118
	Cumulative Humidity	42149	2923	591.54	1247	4193
	Cumulative THI	19914	26126	12779	-8012	62537
	Weight	43885	1867	442.16	850	3000
	Scrotal Circumference	46190	40.53	3.64	21	55
	Hip Height	43656	54.75	2.78	45.50	62.50
	Straw Concentration	16557	46.02	10.37	20	125
	Collection ID	50624	70.94	124.41	1	1181

Table 2.3 Phenotypic correlations (standard errors below) among demographic predictor variables to determine collinearity.

	Collection Day Within Year	Collection Day	Collection Year	Age At Collection	Days Since Previous Collection	Weight	Scrotal Circumference	Hip Height	Collection ID
Collection Day Within Year	1.00								
Collection Day	0.07 0.01	1.00							
Collection Year	0.99 0.01	-0.01 0.01	1.00						
Age At Collection	-0.07 0.01	0.07 0.01	-0.07 0.01	1.00					
Days Since Previous Collection	0.03 0.01	0.02 0.01	0.03 0.01	0.04 0.01	1.00				
Weight	-0.07 0.01	0.05 0.01	-0.07 0.01	0.77 0.01	0.02 0.01	1.00			
Scrotal Circumference	-0.09 0.01	0.01 0.01	-0.09 0.01	0.60 0.01	0.02 0.01	0.68 0.01	1.00		
Hip Height	-0.19 0.01	0.06 0.01	-0.19 0.01	0.63 0.01	0.03 0.01	0.80 0.01	0.59 0.01	1.00	
Collection ID	0.06 0.01	0.06 0.01	0.07 0.01	0.59 0.01	-0.03 0.01	0.45 0.01	0.29 0.01	0.36 0.01	1.00

Table 2.4 Phenotypic correlations (standard errors below) between average daily temperature, humidity, pressure, wind speed, and snowfall measures and the temperature-humidity index (THI) predictor variables to determine collinearity.

	Collection Day	Daily Average Temperature	Daily Average Humidity	Daily Average Pressure	Daily Average Windspeed	Daily Average Snowfall	Collection Day THI	Spermatogenesis Start THI	Cumulative Temperature	Cumulative Humidity	Cumulative THI
Collection Day	1.00										
Daily Average Temperature	0.18 0.01	1.00									
Daily Average Humidity	-0.06 0.01	-0.53 0.01	1.00								
Daily Average Pressure	0.09 0.01	-0.11 0.01	0.03 0.01	1.00							
Daily Average Windspeed	0.06 0.01	0.16 0.01	0.08 0.01	0.09 0.01	1.00						
Daily Average Snowfall	-0.05 0.01	-0.32 0.01	0.42 0.01	0.03 0.01	-0.02 0.01	1.00					
Collection Day THI	0.17 0.01	0.99 0.01	-0.54 0.01	-0.11 0.01	0.14 0.01	-0.37 0.01	1.00				
Spermatogenesis Start THI	0.66 0.01	0.17 0.01	-0.10 0.01	0.10 0.01	0.07 0.01	-0.05 0.01	0.16 0.01	1.00			
Cumulative Temperature	0.54 0.01	0.47 0.01	-0.08 0.01	-0.02 0.01	0.09 0.01	-0.12 0.01	0.46 0.01	0.48 0.01	1.00		
Cumulative Humidity	-0.46 0.01	-0.44 0.01	0.32 0.01	-0.10 0.01	-0.13 0.01	0.14 0.01	-0.42 0.01	-0.53 0.01	-0.29 0.01	1.00	
Cumulative THI	0.54 0.01	0.48 0.01	-0.03 0.01	-0.03 0.01	0.10 0.01	-0.12 0.01	0.46 0.01	0.46 0.01	0.96 0.01	-0.04 0.01	1.00

Table 2.5 Phenotypic correlations (standard errors below) between average daily temperature, humidity, pressure, wind speed, and snowfall measures and the comprehensive climate index (CCI) predictor variables tested for significance to determine collinearity.

	Collection Day	Daily Average Temperature	Daily Average Humidity	Daily Average Pressure	Daily Average Windspeed	Daily Average Snowfall	Cumulative Temperature	Cumulative Humidity	Collection Day CCI	Spermatogenesis Start CCI	Cumulative CCI
Collection Day	1.00										
Daily Average Temperature	0.18 0.01	1.00									
Daily Average Humidity	-0.06 0.01	-0.53 0.01	1.00								
Daily Average Pressure	0.09 0.01	-0.11 0.01	0.03 0.01	1.00							
Daily Average Windspeed	0.06 0.01	0.16 0.01	0.08 0.01	0.09 0.01	1.00						
Daily Average Snowfall	-0.05 0.01	-0.32 0.01	0.42 0.01	0.03 0.01	-0.02 0.01	1.00					
Cumulative Temperature	0.54 0.01	0.47 0.01	-0.08 0.01	-0.02 0.01	0.09 0.01	-0.12 0.01	1.00				
Cumulative Humidity	-0.46 0.01	-0.44 0.01	0.32 0.01	-0.10 0.01	-0.13 0.01	0.14 0.01	-0.29 0.01	1.00			
Collection Day CCI	0.19 0.01	1.00 0.01	-0.51 0.01	-0.11 0.01	0.16 0.01	-0.33 0.01	0.48 0.01	-0.43 0.01	1.00		
Spermatogenesis Start CCI	0.68 0.01	0.19 0.01	-0.09 0.01	0.09 0.01	0.07 0.01	-0.05 0.01	0.55 0.01	-0.52 0.01	0.19 0.01	1.00	
Cumulative CCI	0.52 0.01	0.49 0.01	-0.09 0.01	-0.03 0.01	0.11 0.01	-0.13 0.01	1.00 0.01	-0.28 0.01	0.49 0.01	0.55 0.01	1.00

Table 2.6 Phenotypic correlations (standard errors below) between the temperature humidity index (THI) measures and comprehensive climate index (CCI) as potential predictor variables tested for significance to determine collinearity.

	Collection Day THI	Spermatogenesis Start THI	Cumulative Temperature	Cumulative Humidity	Cumulative THI	Collection Day CCI	Spermatogenesis Start CCI	Cumulative CCI
Collection Day THI	1.00							
Spermatogenesis Start THI	0.16 0.01	1.00						
Cumulative Temperature	0.46 0.01	0.48 0.01	1.00					
Cumulative Humidity	-0.42 0.01	-0.53 0.01	-0.29 0.01	1.00				
Cumulative THI	0.46 0.01	0.46 0.01	0.96 0.01	-0.04 0.01	1.00			
Collection Day CCI	1.00 0.01	0.17 0.01	0.48 0.01	-0.43 0.01	0.48 0.01	1.00		
Spermatogenesis Start CCI	0.18 0.01	0.97 0.01	0.55 0.01	-0.52 0.01	0.53 0.01	0.19 0.01	1.00	
Cumulative CCI	0.47 0.01	0.48 0.01	1.00 0.01	-0.28 0.01	0.96 0.01	0.49 0.01	0.55 0.01	1.00

Table 2.7 Levels of significance for the fixed effects used in the analysis of beef bull semen attributes.

Fixed effect	Trait								
	Volume	Concentration	Initial Motility	Number of Spermatozoa	Post-thaw Motility	Three hour PT Motility	Percent Normal	Primary	Secondary
n (50624)	17026	16951	17029	16951	11690	3378	7672	7686	7728
AIC	85270.8	234998.3	126589.1	311531.6	81398.4	19649.9	47311.9	43643.6	44657
Collection Location	0.7701	<0.0001	0.0016	<0.0001	<0.0001	--	0.9817	0.149	<0.0001
Collection Day within Year	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Age	<0.0001	0.5752	0.0004	<0.0001	0.0184	0.0007	0.0023	0.046	0.0771
Days since previous collection	<0.0001	0.4152	0.0022	<0.0001	0.8981	0.7902	0.3268	0.5217	0.7807
Cum CCI	0.2798	0.217	0.6691	0.4196	0.7352	--	0.8383	0.2431	0.9834

Table 2.8 Correlations between the bivariate EBV results for each individual trait.

Trait	Correlation
Initial Motility EBV	0.98
Post-thaw Motility EBV	0.86
Three-hour Post-thaw Motility	0.65
Percentage of Normal Spermatozoa	0.81
Primary Abnormalities	0.79
Secondary Abnormalities	0.99

Table 2.9 Number of records (N), mean, standard deviation (std dev), minimum, and maximum for each Angus bull semen attribute.

	N	Mean	Median	Std Dev	Minimum	Maximum
Volume	44431	7.96	7.10	4.22	0.10	74.00
Concentration	44038	1021.16	972.00	495.02	10.00	3906.00
Initial Motility	44418	49.18	50.00	16.16	0.00	100.00
Number of Spermatozoa	44038	8004.18	6877.50	5519.61	64.50	69795.00
Post-thaw Motility	29877	43.53	50.00	13.78	0.00	75.00
Three Hr Post-thaw Motility	8299	15.72	15.00	12.48	0.00	60.00
Percentage of Normal Spermatozoa	19455	75.18	76.00	8.37	2.00	100.00
Primary Abnormalities	19452	13.00	12.00	7.53	0.00	100.00
Secondary Abnormalities	19521	12.09	11.00	7.70	0.00	100.00

Table 2.10 Variance component estimates for direct additive genetic (σ_a^2), permanent environment (σ_{pe}^2), and environmental (σ_e^2) variance using a multivariate single-step genomic best linear unbiased prediction (ssGBLUP) for semen traits. Volume, concentration, and number of spermatozoa were analyzed with a trivariate approach, and the remainder of the traits were modeled with a bivariate model. If a bivariate model was used the range of the estimates is reported. Heritability (h^2) and repeatability (r^2) estimates and standard errors are also reported.

	σ_a^2	σ_{pe}^2	σ_e^2	h^2	Minimum h^2	Maximum h^2	r^2	Minimum r^2	Maximum r^2
Volume (mL^2)	1.60	2.76	10.22	0.11 ± 0.02	NA	NA	0.30 ± 0.01	NA	NA
Concentration (m/mL^2)	18560.20	52040.82	142806.10	0.09 ± 0.02	NA	NA	0.33 ± 0.01	NA	NA
Number of Spermatozoa	2.05×10^6	5.68×10^6	1.82×10^7	0.08 ± 0.02	NA	NA	0.30 ± 0.01	NA	NA
Initial Motility ($\%^2$)	29.90	74.68	155.55	0.12 ± 0.03^a	0.11 ± 0.03	0.12 ± 0.02	0.40 ± 0.01^g	0.40 ± 0.01	0.40 ± 0.01
Post-thaw Motility ($\%^2$)	28.81	56.44	106.75	0.15 ± 0.03^b	--	--	0.44 ± 0.01^h	--	--
Three-hour Post-thaw Motility ($\%^2$)	7.38	19.16	112.56	0.09 ± 0.04^c	--	--	0.34 ± 0.02^i	--	--
Percentage of Normal Spermatozoa ($\%^2$)	8.61	40.98	44.03	0.09 ± 0.04^d	0.05 ± 0.03	0.14 ± 0.04	0.53 ± 0.01^j	0.52 ± 0.01	0.54 ± 0.01
Primary Abnormalities ($\%^2$)	1.57	34.59	24.22	0.03 ± 0.03^e	0.03 ± 0.03	0.02 ± 0.03	0.60 ± 0.01^k	0.60 ± 0.01	0.60 ± 0.01
Secondary Abnormalities ($\%^2$)	10.29	17.70	29.75	0.18 ± 0.04^f	0.18 ± 0.04	0.18 ± 0.04	0.48 ± 0.01^l	0.48 ± 0.01	0.48 ± 0.01

--Only one estimate reported due to convergence issues.

^a Reported results are the average heritabilities from the bivariate analyses with post-thaw motility and three-hour post-thaw motility

^b Reported result is the heritabilities from the bivariate analyses with initial motility. The bivariate model with three-hour post-thaw motility did not converge.

^c Reported results are the heritabilities from the bivariate analyses with initial motility. The bivariate model with post-thaw motility did not converge.

^d Reported results are the average heritabilities from the bivariate analyses with primary abnormalities and secondary abnormalities

^e Reported results are the average heritabilities from the bivariate analyses with percentage of normal spermatozoa and secondary abnormalities

^f Reported results are the average heritabilities from the bivariate analyses with percentage of normal spermatozoa and primary abnormalities

^g Reported results are the average repeatabilities from the bivariate analyses with post-thaw motility and three-hour post-thaw motility

^h Reported result is the repeatabilities from the bivariate analyses with initial motility. The bivariate model with three-hour post-thaw motility did not converge.

ⁱ Reported results are the average repeatabilities from the bivariate analyses with initial motility. The bivariate model with post-thaw motility did not converge.

^j Reported results are the average repeatabilities from the bivariate analyses with primary abnormalities and secondary abnormalities

^k Reported results are the average repeatabilities from the bivariate analyses with percentage of normal spermatozoa and secondary abnormalities

^l Reported results are the average repeatabilities from the bivariate analyses with percentage of normal spermatozoa and primary abnormalities

Table 2.11 Number of records (N), mean, standard deviation (std dev), minimum, and maximum for estimated breeding values (EBV) for beef bull semen attributes for bulls with phenotypes in the genetic analysis.

	N	Mean	Std Dev	Minimum	Maximum	BIF Accuracy	Classical Animal Breeding Accuracy
Volume	1819	0.04	2.45	-7.10	11.00	0.21	0.61
Concentration	1819	-9.97	104.55	-335.46	428.38	0.16	0.54
Initial Motility	1819	-0.03	4.38	-16.97	11.62	0.18	0.57
Number of Spermatozoa	1819	-1.15	67.47	-208.11	327.73	0.16	0.54
Post-thaw Motility	1819	0.23	3.93	-18.30	11.57	0.18	0.57
Three Hr Post-thaw Motility	1819	0.07	1.00	-4.44	4.54	0.11	0.45
Percentage of Normal Spermatozoa	1819	0.13	2.04	-16.25	7.33	0.16	0.53
Primary Abnormalities	1819	0.19	2.99	-10.39	30.01	0.05	0.30
Secondary Abnormalities	1819	0.38	9.45	-29.20	114.16	0.24	0.64

Table 2.12 Genetic correlations (above the diagonal) and phenotypic correlation (below the diagonal) between beef bull semen attributes traits using a multivariate single-step genomic best liner unbiased prediction (ssGBLUP) model. Standard error estimates are below correlation estimates.

	Volume	Concentration	Number of Spermatozoa	Initial Motility	Post-thaw Motility	3 Hr Post-thaw Motility	Percentage of Normal	Primary Abnormality	Secondary Abnormality
Volume		-0.10 0.17	0.75 0.08	0.23 0.16	0.17 0.16	0.54 0.69	0.18 0.22	0.52 0.61	-0.13 0.17
Concentration	-0.06 0.01		0.55 0.13	0.09 0.19	-0.15 0.19	0.04 0.59	-0.11 0.24	dnc	0.04 0.19
Number of Spermatozoa	0.66 0.01	0.61 0.01		0.23 0.18	0.02 0.19	0.31 0.61	0.13 0.24	0.08 0.73	-0.10 0.20
Initial Motility	0.13 0.01	0.12 0.01	0.16 0.01		0.92 0.04	dnc	0.77 0.09	0.33 0.20	0.63 0.82
Post-thaw Motility	0.16 0.01	-0.03 0.01	0.12 0.01	0.32 0.01		dnc	-0.74 0.30	-0.02 0.61	0.11 1.97
3 Hr Post-thaw Motility	0.22 0.01	-0.24 0.01	-0.01 0.01	0.04 0.01	0.74 0.01		-0.44 0.15	-0.25 0.17	dnc
Percentage of Normal	0.09 0.01	0.12 0.01	0.16 0.01	0.20 0.01	0.03 0.01	0.15 0.02		0.03 2.07	-0.81 0.10
Primary Abnormality	-0.09 0.01	-0.03 0.01	-0.10 0.01	-0.21 0.01	-0.33 0.01	-0.55 0.01	-0.60 0.01		-0.60 1.13
Secondary Abnormality	-0.01 0.01	-0.13 0.01	-0.09 0.01	-0.15 0.01	0.30 0.01	0.54 0.01	-0.58 0.01	-0.31 0.01	

dnc: Model did not converge.

Table 2.13 Correlations between scrotal circumference EPDs obtained from the American Angus Association and beef bull semen attribute estimated breeding values.

Trait	Correlation +/- SE
Volume	-0.01 ± 0.02
Concentration	0.15 ± 0.02
Initial Motility	0.12 ± 0.02
Number of Spermatozoa	-0.06 ± 0.02
Post-thaw Motility	-0.07 ± 0.02
Three-hour Post-thaw Motility	-0.07 ± 0.02
Percentage of Normal Spermatozoa	-0.06 ± 0.02
Primary Abnormalities	0.07 ± 0.02
Secondary Abnormalities	-0.08 ± 0.02

Figure 2.1 The change in genetic merit for volume for the population of Angus bulls from two artificial insemination (AI) centers from 1997 to 2017.

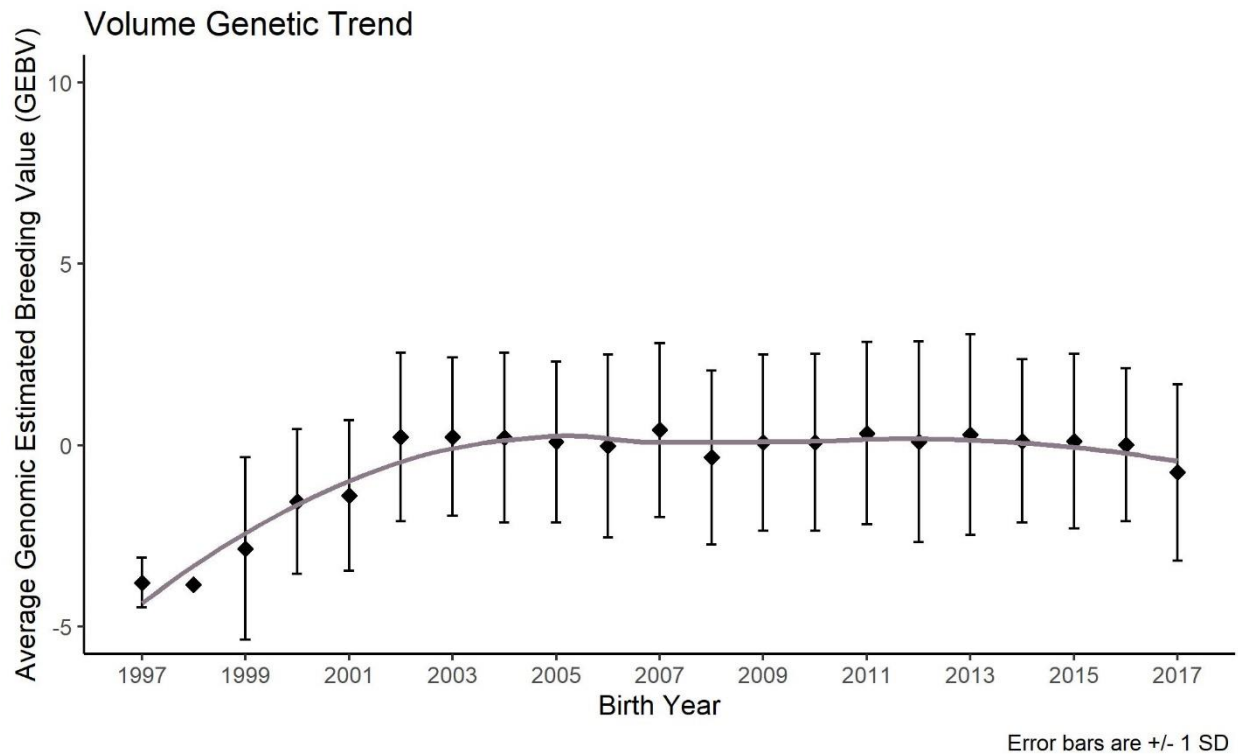


Figure 2.2 The change in genetic merit for concentration for the population of Angus bulls from two artificial insemination (AI) centers from 1997 to 2017.

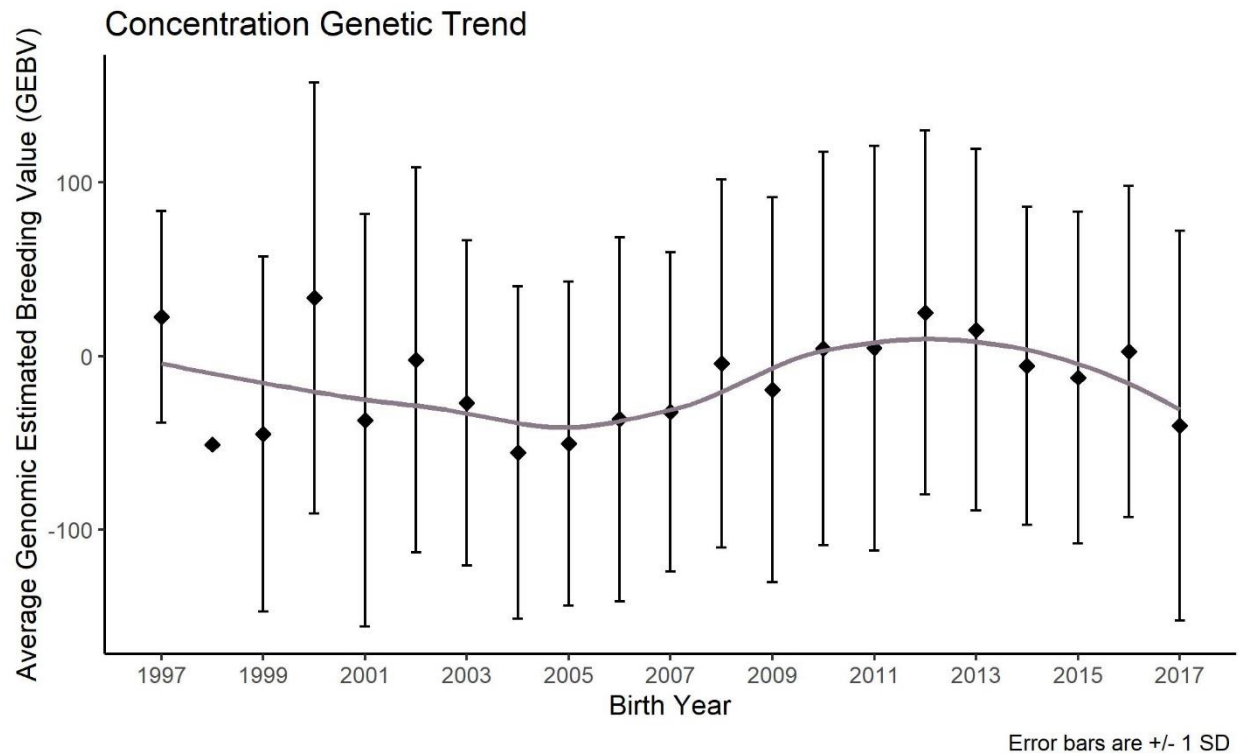


Figure 2.3 The change in genetic merit for number of spermatozoa for the population of Angus bulls from two artificial insemination (AI) centers from 1997 to 2017.

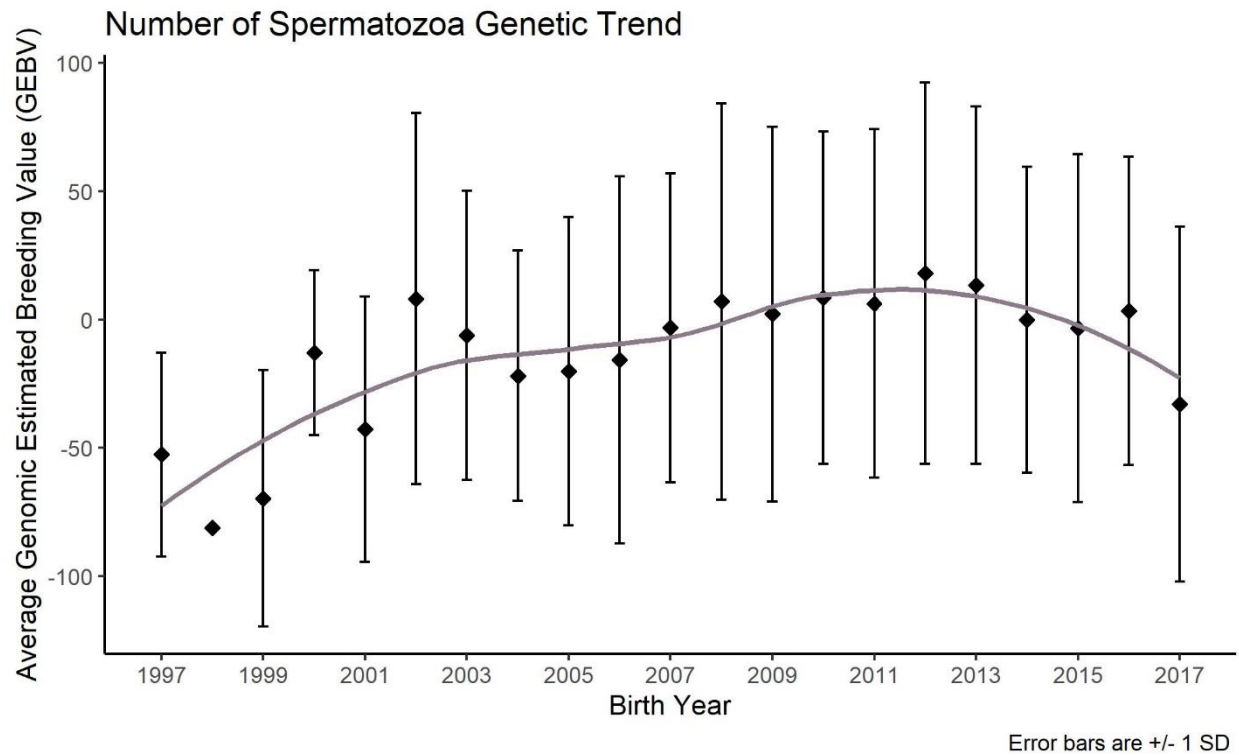


Figure 2.4 The change in genetic merit for initial motility for the population of Angus bulls from two artificial insemination (AI) centers from 1997 to 2017.

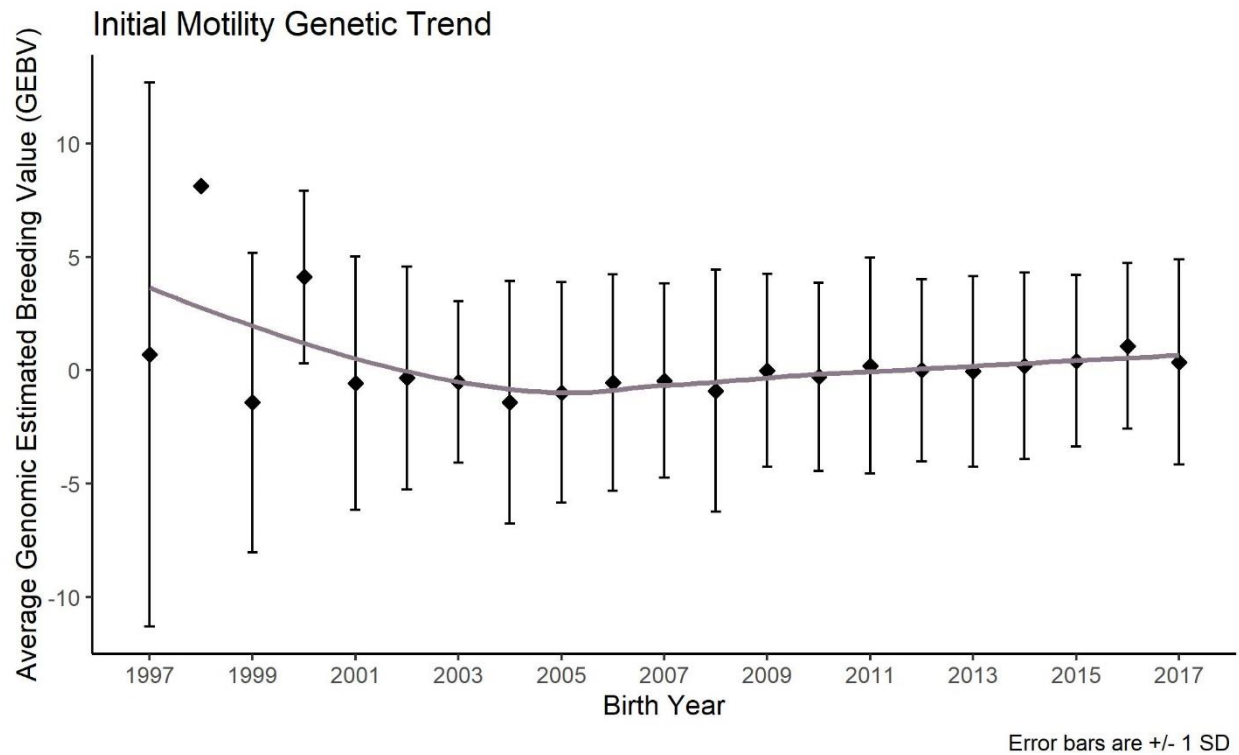


Figure 2.5 The change in genetic merit for post-thaw motility for the population of Angus bulls from two artificial insemination (AI) centers from 1997 to 2017.

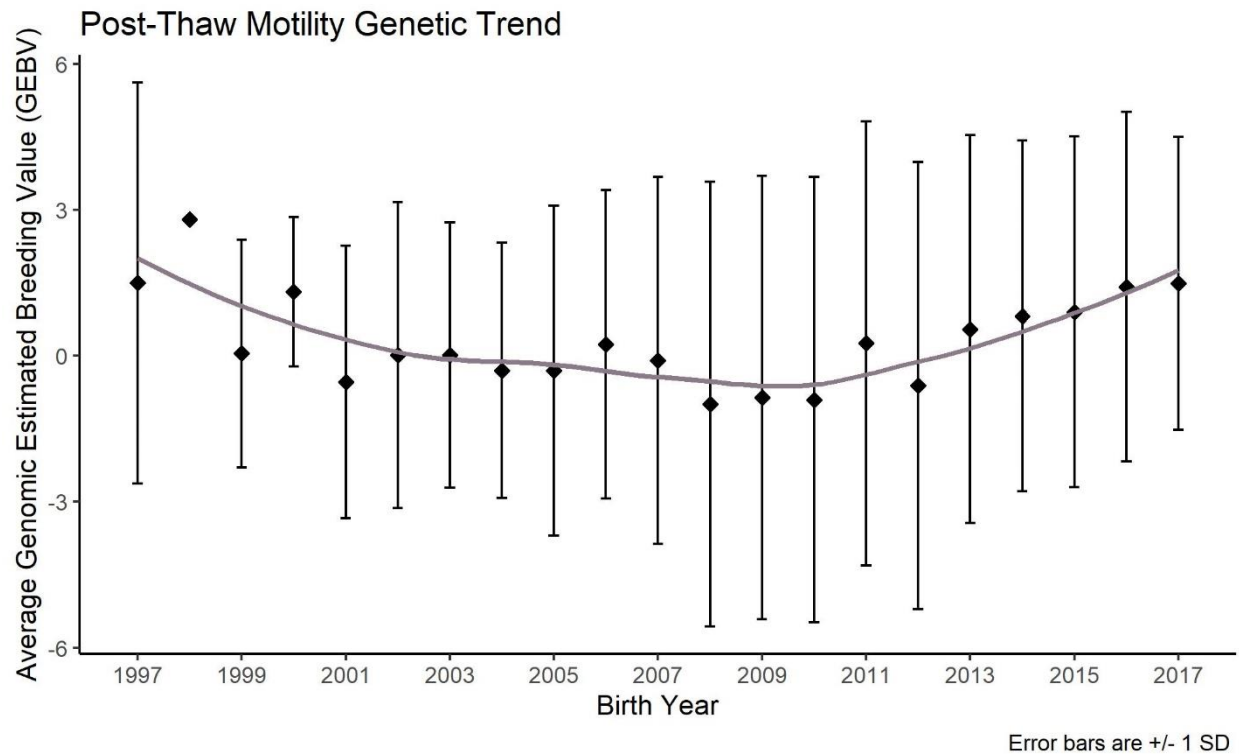


Figure 2.6 The change in genetic merit for three-hour post-thaw motility for the population of Angus bulls from two artificial insemination (AI) centers from 1997 to 2017.

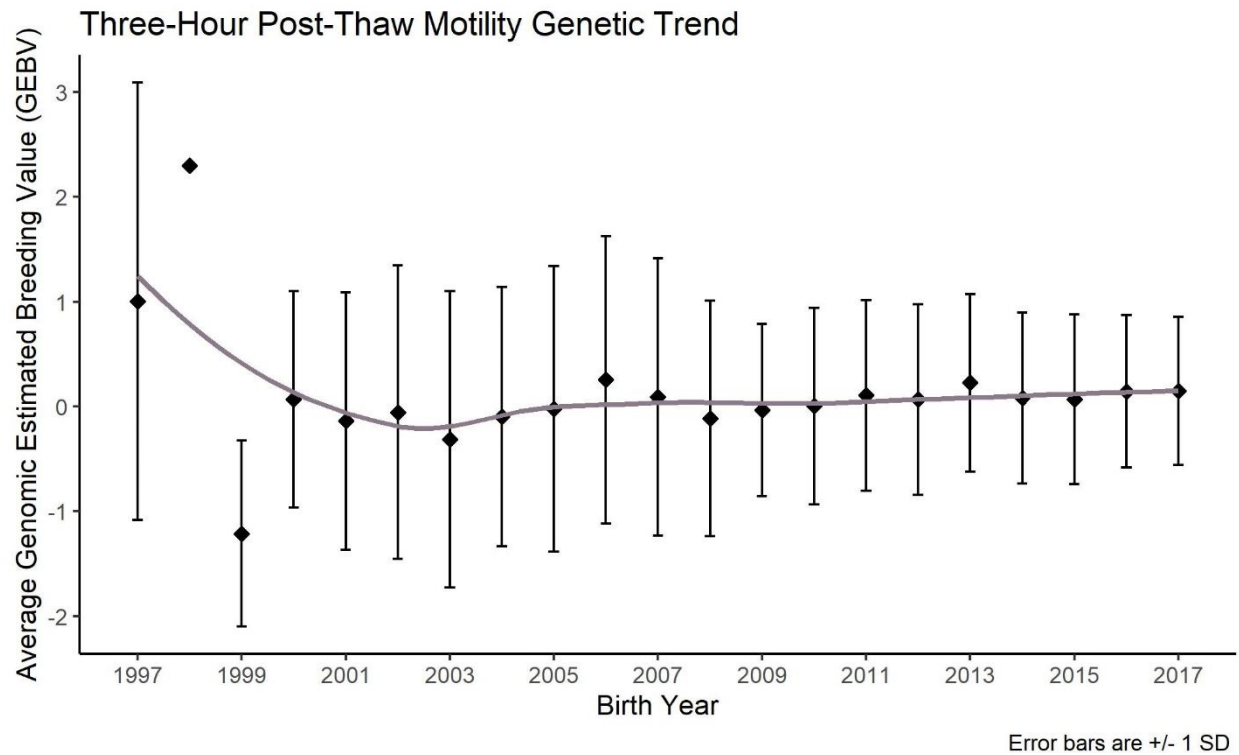


Figure 2.7 The change in genetic merit for percentage of normal spermatozoa for the population of Angus bulls from two artificial insemination (AI) centers from 1997 to 2017.

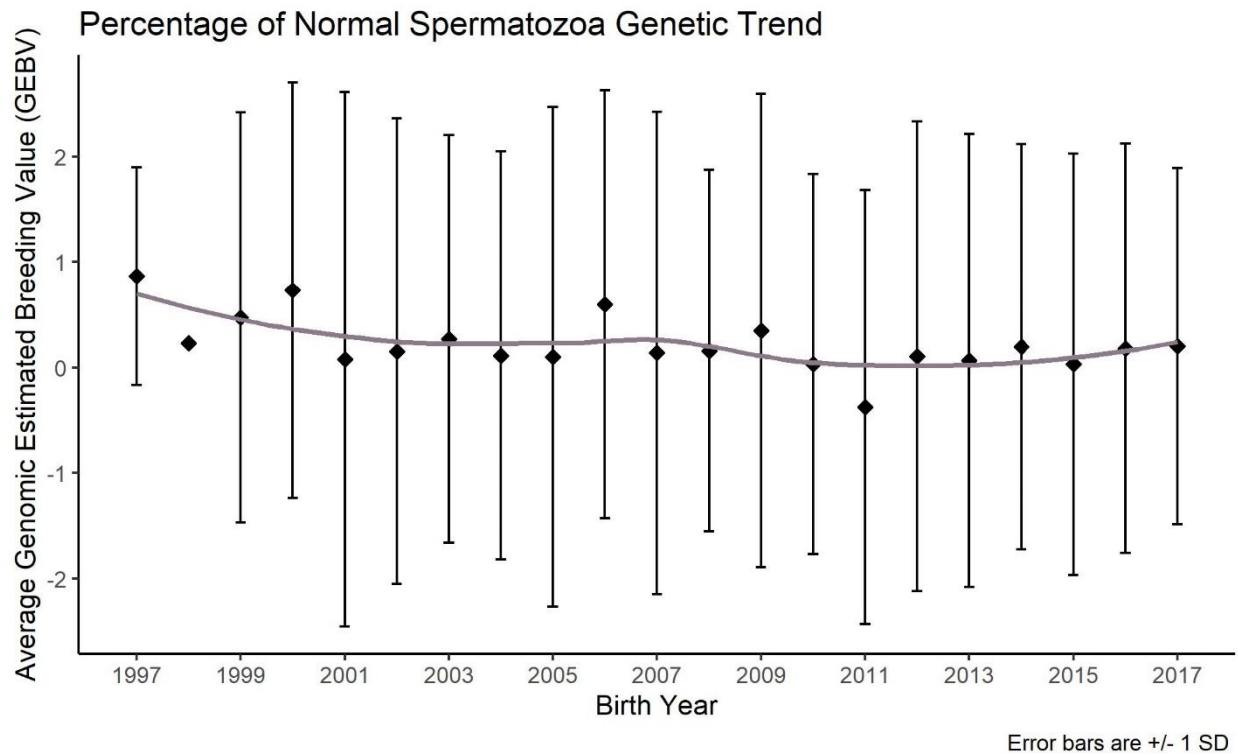


Figure 2.8 The change in genetic merit for primary abnormalities for the population of Angus bulls from two artificial insemination (AI) centers from 1997 to 2017.

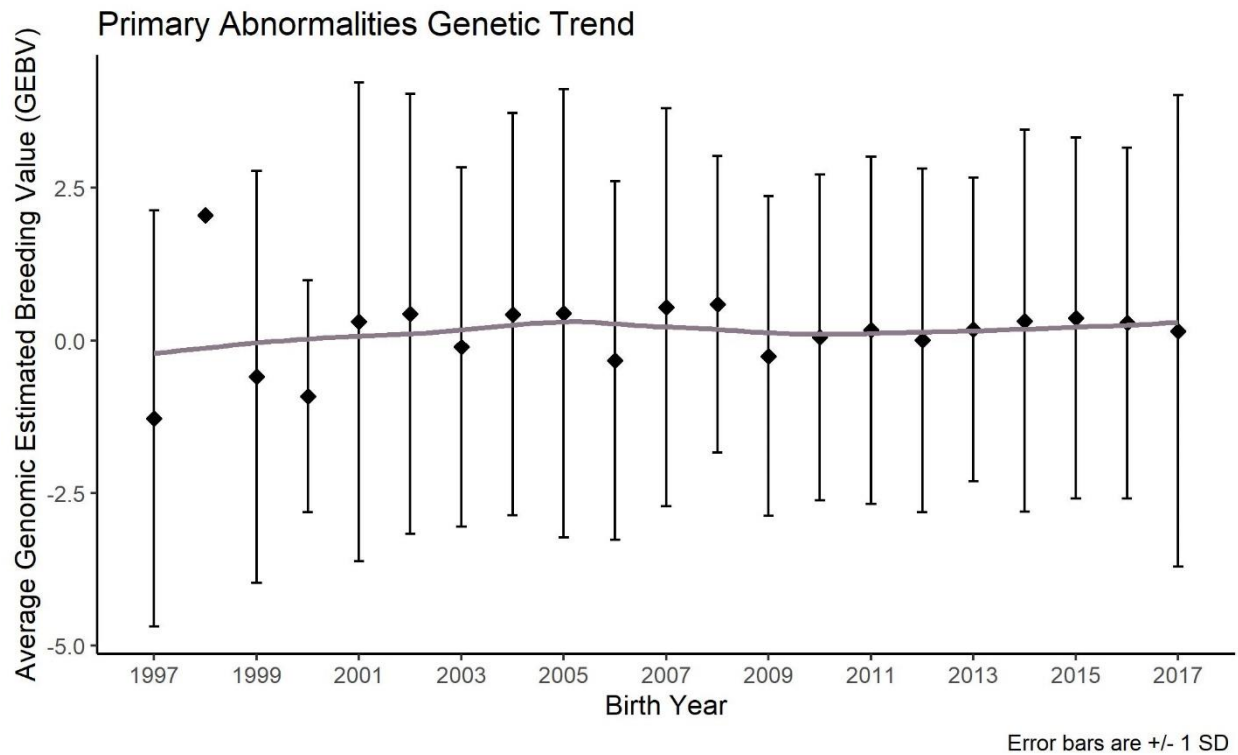
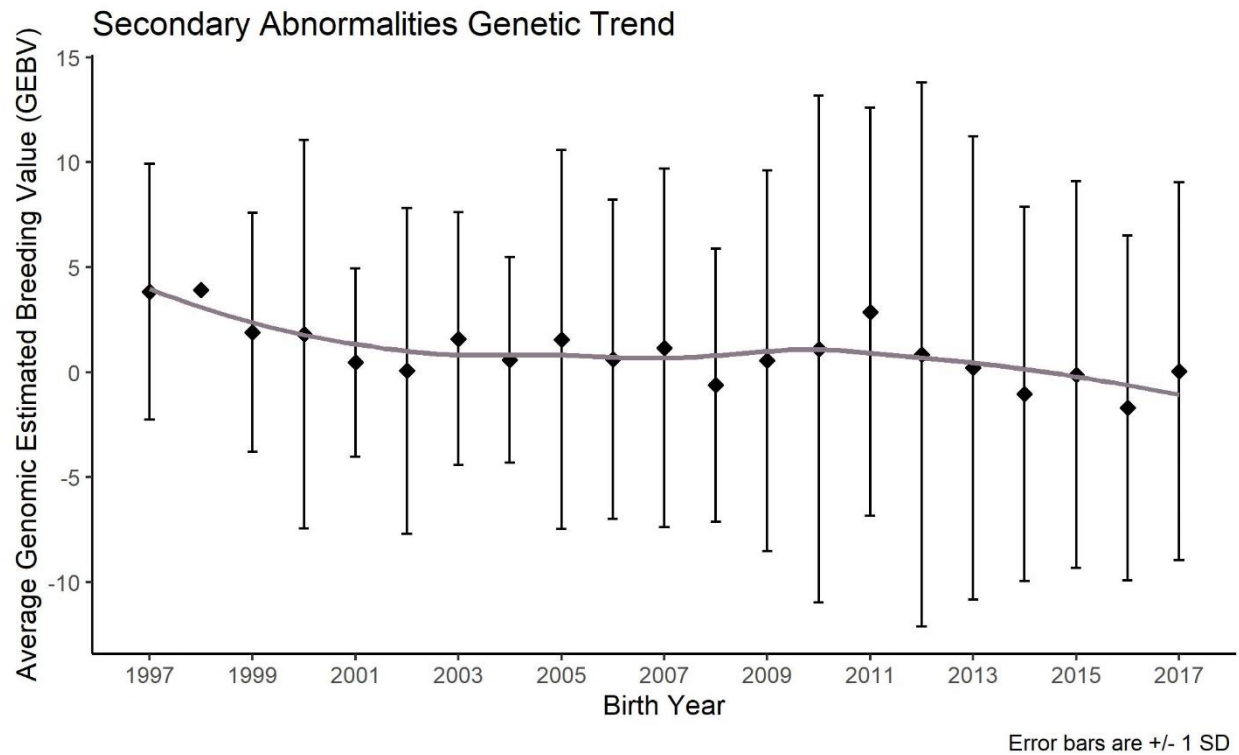


Figure 2.9 The change in genetic merit for secondary abnormalities for the population of Angus bulls from two artificial insemination (AI) centers from 1997 to 2017.



Literature Cited

- Abdollahi-Arpanahi, R., G. Morota, and F. Penagaricano. 2017. Predicting bull fertility using genomic data and biological information. *J. Dairy. Sci.* 100: 9656-9666. doi: 10.3168/jds.2017-13288.
- Arnold G.W. and M.L. Dudzinski. 1978. *Ethology of Free-Ranging Domestic Animals*. Elsevier Scientific Publishing Co., Amsterdam, The Netherlands, pp. 51-96 and 125-136. doi: [10.2307/1380297](https://doi.org/10.2307/1380297).
- Barth, A. D, and C.L. Waldner. 2002. Factors affecting breeding soundness classification of beef bulls examined at the Western College of Veterinary Medicine. *Can Vet J.* 43: 274-284.
- Beerwinkle, L.G. 1974. Heat detection programs and techniques. In: *Proceedings of the Eighth Conference on Artificial Insemination of Beef Cattle*. Denver, Colorado, National Association of Animal Breeders, Columbia, Missouri, pp. 24-29.
- Bennet C. Using Heritability for genetic improvement. College of Agriculture and Life Sciences, Virginia Polytechnic Institute and State University. 2009. <https://goo.gl/EzMWCT>
- Berry, D.P., B. Eivers, G. Dunne, and S. McParland. 2019. Genetics of bull semen characteristics in a multi-breed cattle population. *Theriogenology*. 123: 202-208. doi: 10.1016/j.theriogenology.2018.10.006.
- Braundmeier, A. G. and D.J. Miller. 2001. The search is on: finding accurate molecular markers of male fertility. *J of Dairy Sci.* 84: 1915-1925. doi: [10.3168/jds.S0022-0302\(01\)74633-4](https://doi.org/10.3168/jds.S0022-0302(01)74633-4).
- Christmas R. A., D.W. Moser, M.F. Spire, J.M. Sargeant, and S.K. Tucker. Genetic relationships among breeding soundness traits in yearling bulls. In: *Cattlemen's Day*. 2001. Manhattan, KS. Pp. 1-3. doi: 10.4148/2378-5977.1712.
- Corbet, N.J., B.M. Burns, D.J. Johnston, M.L. Wolcott, D.H. Corbet, B.K. Venus, Y. Li, M.R. McGowan, and R.G. Holroyd. 2013. Male traits and herd reproductive capability in tropical beef cattle. 2. Genetic parameters of bull traits. *Anim. Prod. Sci.* 53: 101-113. doi: 10.1071/AN12163.
- David I., L. Bodin, G. Lagriffoul, E. Madfedi, C. Robert-Granie. 2006. Genetic parameters of ram semen traits using an animal model accounting for serial correlation. In: *proceedings of the eighth World Congress on Genetics Applied to Livestock Production*, Belo Horizonte, Brazil, 13-18 August 2006.
- Department of Energy. National Renewable Energy Laboratory. Accessed 15 August 2019. <https://maps.nrel.gov/nsrdb-viewer/?aL=UdPEX9%255Bv%255D%3Dt%26f69KzE%255Bv%255D%3Dt%26f69Kz>

E%255Bd%255D%3D1&bL=clight&cE=0&lR=0&mC=4.740675384778373%2C22.8515625&zL=2

- DeJarnette, J.M., C.E. Marshal, R.W. Lenz, D.R. Monke, W.H. Ayars, and C.G. Sattler. 2004. Sustaining the fertility of artificially inseminated dairy cattle: the role of the artificial insemination industry. *J. Dairy Sci.* 87 (E. Suppl.): E93-E104. doi: 10.3168/jds.S0022-0302(04)70065-X.
- Druet, T., S. Fritz, S. Sellem, B. Basso, O. Ge, L. Salas-Cortes, P. Humblot, X. Druart, and A. Eggen. 2009. Estimation of genetic parameters and genome scan for 15 semen characteristics traits of Holstein bulls. *J. Anim. Breed. Genet.* 136: 269-277. doi: 10.1111/j.1439-0388.2008.00788.x.
- Ducrocq, V. and P. Humblot. 1995. Genetic characteristics and evolution of semen production of young Normande bulls. *Livest. Prod. Sci.* 41: 1-10. doi:10.1016/0301-6226(94)00029-7.
- Fritz, L., J.M. Stookey, J.L. Clavelle, and C.S. Waltz. 1999. Observations of cattle on extensive range. 1999 Annual Report to the Saskatchewan Agricultural Development Fund: Strategic Program Area X: Food Animal Behaviour and Welfare.
- Fuerst-Waltl, B., H. Schwarzenbacher, C. Perner, and J. Solkner. 2006. Effects of age and environmental factors on semen production and semen quality of Austrian Simmental bulls. *Anim. Repro. Sci.* 95: 27-37. doi: 10.1016/j.anireprosci.2005.09.002.
- Garmyn, A.J., D. W. Moser, R.A Christmas, J.M. Bormann. 2011. Estimate of genetic parameters and effects of cytoplasmic line on scrotal circumference and semen quality traits in Angus bulls. *J. Anim. Sci.* 89: 693-698. doi:10.2527/jas.2010-3534.
- Gredler, B. C. Fuerst-Waltl, H. Schwarzenbacher, and J. Solkner. 2007. Genetic parameters for semen production traits in Austrian dual-purpose Simmental bulls. *Repro. Domest. Anim.* 42: 326-328. doi:10.1111/j.1439-0531.2006.00778.x.
- Haque, M. N., A. B. M. M. Islam, A. K. F. H. Bhuiyan, S. A. Aziz, and M. G. Kibria. 2001. Phenotypic variation and repeatability of semen characteristics of bulls. *Pakistan J Bio Sci.* 4: 1418-1420. doi: 10.3923/pjbs.2001.1418.1420.
- Kaps, M., M. Posavi, N. Stipic, and B. Mikulic. 2000. Genetic evaluation of semen and growth traits of young Simmental bulls in performance test. *Ag Conceptus Scientificus.* 65: 15-20.
- Kealey, C.G., M.D. MacNeil, M.W. Tess, T.W. Geary, and R.A. Bellows. 2006. Genetic parameter estimates for scrotal circumference and semen characteristics of line 1 Hereford bulls. *J. Anim. Sci.* 84: 283-290. doi: [10.2527/2006.842283x](https://doi.org/10.2527/2006.842283x).

- Knights, S.A., R.L. Baker, D. Gianola, J.B. Gibbs. 1984. Estimates of heritabilities and genetic and phenotypic correlations among growth and reproductive traits in yearling Angus bulls. *J. Anim. Sci.* 58: 887-893. doi: 10.2527/jas1984.584887x.
- Mader, T. L., L. J. Johnson, and J. B. Gaughan. 2010. A comprehensive index for assessing environmental stress in animals. *J. Anim. Sci.* 88: 2153-2165. doi: 10.2527/jas.2009-2586.
- Mader, T. L., M. S. Davis, and T. Brown-Brandl. 2006. Environmental factors influencing heat stress in feedlot cattle. *J. Anim. Sci.* 84: 712-719. doi: 10.2527/2006.843712x.
- Mathevon, M. M.M Buhr, and J.C.M. Dekkers. 1998. Environmental, management, and genetic factors affecting semen production in Holstein bulls. *J. Dairy Sci.* 81: 3321-3330. doi: [10.3168/jds.S0022-0302\(98\)75898-9](https://doi.org/10.3168/jds.S0022-0302(98)75898-9).
- Misztal, I., S. Tsuruta, D. A. L. Lourenco, I. Aguilar, A. Lagarra, and Z. Vitezica. 2014. Manual for BLUPF90family of programs. http://nce.ads.uga.edu/wiki/lib/exe/fetch.php?media=blupf90_all1.pdf. (Accessed 1 August 2019.)
- Murphy, E. M., A. K. Kelly, C. O'Meara, B. Eivers, P. Lonergan, and S. Fair. 2018. Influence of bull age, ejaculate number, and season of collection on semen production and sperm motility parameters in Holstein Friesian bulls in a commercial artificial insemination centre. *J Anim Sci* 96: 2408-2418. doi: 10.1093/jas/sky130.
- Nicolazzi E. L., A. Caprera, N. Nazzicari, P. Cozzi, F. Strozzi, C. Lawley, A. Pirani, C. Soans, F. Brew, H. Jorjani, G. Evans, B. Simpson, G. Tosser-Klopp, R. Brauning, J. L. Williams, and A. Stella. 2015. [SNPchiMp v.3: integrating and standardizing single nucleotide polymorphism data for livestock species](https://doi.org/10.1186/s12864-015-1497-1). *BMC genomics*. 16:283. doi: 10.1186/s12864-015-1497-1.
- Nicolazzi E. L., M. Picciolini, F. Strozzi, R. D. Schnabel, C. Lawley, A. Pirani, F. Brew and A. Stella. 2014. [SNPchiMp: A database to disentangle the SNPchip jungle in bovine livestock](https://doi.org/10.1186/s12864-015-1497-1). *BMC genomics*. 15:123
- Petrunkina, A.M. and R.A. Harrison. 2011. Cytometric solutions in veterinary andrology: developments, advantages, and limitations. *Cytometry A* 79: 338-348. doi: 10.1002/cyto.a.21044.
- R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>.
- Rossow, W.B.; Walker, A.; Golea, V.; Knapp, K. R.; Young, A.; Inamdar A.; Hankins, B.; and NOAA's Climate Data Record Program (2016): International Satellite Cloud Climatology Project Climate Data Record, H-Series Daily Summaries. NOAA National Centers for Environmental Information. 15 Feb 2019. doi:10.7289/V5QZ281S

- [Sargolzaei, M., J. P. Chesnais and F. S. Schenkel. 2014. A new approach for efficient genotype imputation using information from relatives. BMC Genomics. 15:478. doi: 10.1186/1471-2164-15-478.](#)
- Senger, P. L. 2012. Pathways to pregnancy and parturition. 3rd ed. Current Conceptions, Inc. Redmon, Oregon.
- Smith B.A., J.S. Brinks, and G.V. Richardson. 1989. Estimation of genetic parameters among breeding soundness examination components and growth traits in yearling bull. J. Anim. Sci. 67: 2892-2896. doi: [10.2527/jas1989.67112892x](#).
- Stålhammar, E. M., L. Janson, and J. Philipsson. 1989. Genetic studies on fertility in AI bulls. I. Age, season and genetic effects on semen characteristics in young bulls. Anim Repro Sci. 19:1-17. doi: 10.1016/0378-4320(89)90042-0.
- Strandberg, E. 1992. Lifetime performance in dairy cattle: definition of traits and influences of systemic environmental factors. Anim. Sci. 42:71-81. doi: [10.1080/09064709209410112](#)
- Suchocki, T., and J. Szyda. 2015. Genome-wide association study for semen production traits in Holstein-Friesian bulls. J. Dairy Sci. 98: 5774-5780. doi:10.3168/jds.2014-8951.
- Taylor, J. F., B. Bean, C.E. Marshall, and J.J. Sullivan. 1985. Genetic and environmental components of semen production traits of artificial insemination Holstein bulls. J Dairy Sci. 68: 270.3-2733. doi: [10.3168/jds.S0022-0302\(85\)81155-3](#).
- Van Eenennaam, A. L., R. L. Weaber, D.J. Drake, M.C. Penedo, R.L. Quaas, D.J. Garrick, and E.J. Pollack. 2007. DNA-based paternity analysis and genetic evaluation in a large, commercial cattle ranch setting. J. Anim. Sci. 85: 3159-3169. doi: 10.2527/jas.2007-0284.

Chapter 3 - Genome-wide association study of beef bull semen

attributes

Abstract

Cattle production is dependent upon fertility because it results in producing offspring to offset production costs. A number of semen attributes are believed to affect fertility and are frequently measured as part of routine breeding soundness exams or semen collection procedures. The objective of this study was to perform a single-step genome-wide association study (ssGWAS) for beef bull semen attributes. Beef bull fertility phenotypes including volume (VOL), concentration (CONC), number of spermatozoa (NSP), initial motility (IMot), post-thaw motility (PTMot), three-hour post-thaw motility (3HRPTMot), percentage of normal spermatozoa (%NORM), primary abnormalities (PRIM), and secondary abnormalities (SEC) were obtained from two artificial insemination (AI) centers. A total of 1,819 Angus bulls with 50,624 collection records were used for ssGWAS. A five-generation pedigree was obtained from the American Angus Association and consisted of 6,521 sires and 17,136 dams. Genotypes on 1,163 bulls were also obtained from the American Angus Association and utilized in ssGWAS. A multi-trait animal model was used for the estimation of SNP effects. Significant SNP were those with a $\log_{10} P$ -value threshold greater than 4.0. VOL, CONC, number of spermatozoa, IMot, PTMot, 3HRPTMot, PNS, PRIM, and SEC have five, three, six, seven, two, six, six, and two genome-wide significant SNP, respectively. Significant quantitative trait loci (QTL) exist for beef bull semen attributes, and inclusion of genomic information into genetic evaluation should be advantageous for genetic improvement of male fertility traits.

Introduction

Fertility is a complex trait which is affected by management, nutrition, and genetics. The complexity of fertility may be one reason for the lack information available to producers for making fertility selection decisions. As genomic testing becomes more affordable and a part of regular management practices for seedstock producers, cattle producers become more willing and able to utilize the technology. Utilizing genomic technology in breeding decisions allows cattle producers to more confidently incorporate young, unproven sires into their breeding programs. While using young breeding stock increases the rate of genetic change, the risk of using these bulls is higher because their EPD accuracies are lower. Producers do not currently have a selection tool to confidently choose more fertile bulls.

However, thousands of semen records from bull semen collection facilities provide an opportunity to identify polymorphisms which may affect spermatogenesis, morphology, and motility of spermatozoa (Taylor et al., 2018). Taylor et al. (2018) concluded the only way to truly increase the efficiency of beef cattle production is to build the capacity to identify young bulls with sperm abnormalities and low semen quality so that the bulls can be eliminated from the breeding population. Combining phenotypes with genomic predictions has the potential to assist in making crucial selection decisions that may save producers money. The objective of this study was to perform a genome-wide association study for beef bull semen attributes and to identify quantitative trait loci (QTL) regions and genes associated with fertility traits in beef bulls.

Materials and Methods

Phenotypic Data

Phenotypic observations for 1,819 Angus bulls were obtained from two bull semen collection facilities. The phenotypic observations for beef bull semen attributes included volume (VOL), concentration (CONC), number of spermatozoa (NSP), initial motility (IMot), post-thaw motility (PTMot), three-hour post-thaw motility (3HRPTMot), percentage of normal spermatozoa (%NORM), primary abnormalities (PRIM), and secondary abnormalities (SEC). Trait definitions are discussed in Table 3.1. Data editing procedures are fully described in chapter two. Briefly, records were edited to ensure no VOL or CONC phenotypes were 0, all phenotypes recorded as a percentage ranged from 0 to 100, and all bulls had a registration number. After data editing procedures described in the previous chapter there were 50,624 total collection records utilized for the ssGWAS. Stud A contributed 48,131 collection records from 1,570 bulls and stud B contributed an additional 2,493 records from 256 bulls. Summary statistics for the Angus bulls are shown in Table 3.1.

Genetic Data

The American Angus Association provided pedigree information for 1,819 Angus bulls and single nucleotide polymorphism data for 1,163 bulls. The pedigree contained 6,521 sires and 17,136 dams. The maximum number of SNP per bull was 54,609. Data were edited to remove SNP with a call rate of <0.90 ($n=3,921$) and minor allele frequency of <0.05 ($n=13,478$). Animals with a call rate of <0.90 were removed ($n=3$). There was no imputation performed. Prior to quality control, 29.75% of the genotypes were missing. A total of 38,515 SNP from 1,160 animals were available for analysis after quality control filtering.

Statistical Analysis

The model used was:

$$y_i = b_0 + b_1 Location + b_2 DayYear + b_3 Age + b_4 DaysSince + b_5 Cum CCI .$$

where y is the phenotype being evaluated, Location is the collection location, DayYear is the class effect of day within year, Age is the age of the bull on the day of collection in days, DaysSince is the covariate effect of days since previous collection, and CumCCI is the cumulative CCI over the spermatogenesis cycle. Further details about the model selection technique can be found in Butler et al. (unpublished). To include both pedigree and genomic information in the genetic evaluation of beef bull fertility, a single-step genomic best linear unbiased prediction (ssGBLUP) model was utilized. In a ssGBLUP model, the numerator relationship matrix (A) is replaced with H, which includes the genotyped individuals. The H matrix is generated as follows:

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

where $\mathbf{A}^{-1}$ is the inverse of the pedigree-based numerator relationship matrix, $\mathbf{A}_{22}^{-1}$ is a subset of the numerator relationship matrix for the genotyped individuals, and G is the genomic relationship matrix for the genotyped individuals (Misztal et al., 2016).

The ssGBLUP model allows genotyped and non-genotyped individuals to be included in the same genetic evaluation. The ssGBLUP mixed model used in the current study is:

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

where X is an incidence matrix relating the phenotypic observations to the fixed effects in the model, Z_a is an incidence matrix relating the phenotypic observations to the additive direct genetic effects, W_{pe} is an incidence matrix relating the phenotypic observations to the additive

permanent environment genetic effects, λ is the ratio of residual and additive direct genetic variance, $\mathbf{I}$ is an identity matrix, and α is the ratio of residual variance and additive permanent environment variance, $\mathbf{b}$ is a vector of the fixed effects, $\mathbf{a}$ is a vector of additive direct genetic effects, $\mathbf{pe}$ is a vector of additive permanent environment genetic effects, and $\mathbf{y}$ is a vector of observed phenotypic observations.

SNP Effect Estimation

The following algorithm was used to predict the animal effects for a single-step genome-wide association study (ssGWAS):

$$\mathbf{a} = \mathbf{Z}\mathbf{u}$$

Wang et al., (2012)

where $\mathbf{a}$ is a vector of breeding values for the genotyped individuals generated from BLUPF90, $\mathbf{Z}$ is a matrix relating individuals to phenotypes, and $\mathbf{u}$ is a vector of SNP marker effects.

The variance for the genotyped animal effects is as follows:

$$\text{var}(\mathbf{a}) = \text{var}(\mathbf{Z}\mathbf{u}) = \mathbf{G}\sigma_a^2 = \mathbf{Z}\mathbf{I}\mathbf{Z}'\lambda$$

BLUPF90 Manual (2019)

where $\mathbf{G}$ is the genomic relationship matrix, $\mathbf{I}$ is an identity matrix, and λ is the ratio of the SNP marker effect variance and the breeding value variance.

The SNP effects were predicted utilizing the following equation:

$$\hat{\mathbf{u}} = \mathbf{I}\mathbf{Z}'(\mathbf{Z}\mathbf{I}\mathbf{Z}')^{-1}\hat{\mathbf{a}}$$

where $\mathbf{u}$ is a vector of SNP marker effects, $\mathbf{I}$ is an identity matrix, $\mathbf{Z}$ is a matrix relating individuals to phenotypes, and $\mathbf{a}$ is a vector of breeding values for the genotyped individuals.

SNP effects were obtained from one trivariate and six bivariate analyses. VOL, CONC, and NSP were included in a trivariate analysis. Due to convergence issues, bivariate analyses were performed among the remaining groups of traits. Initial motility PTMot, and 3HRPTMot SNP effects were generated from three different bivariate analyses, and %NORM, PRIM, and SEC from an additional three bivariate analyses. The SNP effects for all bivariate analyses were utilized in the analysis.

After obtaining the SNP effects ($\hat{u}$), Manhattan plots for all nine traits were generated utilizing the CMplot package in R (Yin et al., 2015). Utilizing the Qvalue R package from Storey et al., (2019) the $-\log_{10} P$ -values were converted to p-values so that the false discovery rate (FDR) could be calculated for each P -value. There were no significant SNP identified at an FDR threshold of <0.0001 , <0.001 , <0.01 , <0.025 , or <0.05 . Thus, $\log_{10} P$ -values with a threshold of 4.0 were utilized to identify significant SNP.

To account for linkage disequilibrium in the *Bos taurus* genome, QTL regions were formed 250 kilobases upstream and downstream from the significant SNP locations to account for the range of linkage disequilibrium that has been previously reported for the bovine genome (McKay et al. 2007). The regions were utilized to identify previously reported QTL which were near the significant SNP ($\pm 250,000$ bases from the SNP). The QTL regions were identified utilizing the cattle QTL database (Hu et al., 2019). For each trait, previously reported QTL were identified close to significant SNP. Where possible, SNP are identified by the corresponding rs number. If no rs number was available, the SNP name was utilized. In addition, the same QTL region boundaries were used to identify genes near the significant SNP using the National Center of Biotechnology Information (NCBI) database. Putative candidate genes were identified located near significant SNP. Single nucleotide polymorphisms were considered near previously

reported QTL or putative genes if the QTL or gene was within the QTL region (250 kilobases upstream and downstream the significant SNP). The genes specifically associated with beef bull semen attributes were identified based on the gene functions outlined by The UniProt Consortium (2019). The gene list was then used to perform a functional annotation analysis utilizing the Database for Annotation, Visualization, and Integrated Discovery (DAVID 6.8; Huang et al., 2009a; Huang et al., 2009b) for each individual beef bull semen attribute.

For traits where a bivariate analysis was performed, significant SNP from both bivariate analyses are reported.

Results and Discussion

Genome-wide Association Analysis

Significant SNP were identified for all beef bull semen attributes. Significant SNP are reported in Table 3.2 and Manhattan plots are provided in Figures 3.1 through 3.9. For each trait, previously reported QTL were identified close to significant SNP (Table 3.3). In addition, putative candidate genes were identified located near significant SNP. Single nucleotide polymorphisms were considered near previously reported QTL or putative genes if the QTL or gene was within the QTL region (250 kilobases upstream and downstream the significant SNP). All putative candidate genes are outlined in Tables 3.4 through 3.12. Putative candidate genes specifically associated with beef bull semen attributes are outlined in Table 3.13. The genes specifically associated with beef bull semen attributes were identified based on the gene functions outlined by The UniProt Consortium (2019).

Volume

For VOL, five strongly associated SNP were identified, including BTB-01549373, rs41666488, rs109736826, rs109268478, and rs41575945 (Table 3.2). Three QTL regions

associated with conception rate or non-return rate (Kiser et al., 2019; Ben Jemaa et al., 2008; Ron et al., 2004) were near BTB-01549373 and rs41575945 (Table 3.3). While conception and non-return rate are generally associated with female fertility, it is interesting that SNP significantly associated with male fertility traits are also near these QTL regions. This supports the conclusions of DeJarnette (2004), which reported that the ability to successfully reproduce was dependent not only on the female, but also on the semen quantity and quality of the bull. This may also indicate that although the few reported genetic correlations between male and female fertility are low (Hansen 1979; Syrstad 1981; Meyer et al., 1991), there may be some pleiotropy whereby mutations control aspects of both male and female fertility.

In addition to fertility-related QTL, two average daily gain QTL regions discovered by Peters et al. (2012) and Lu et al. (2013) were near SNP associated with VOL. Furthermore, SNP significant for VOL also neighbored previously reported QTL regions associated with yearling weight and mature weight (McClure et al., 2010). Environmentally, limiting nutrition prior to puberty has been shown to affect testes size (VanDemark and Mauger, 1964), cause smaller scrotal circumference (Pruitt et al., 1986), lower intratesticular testosterone levels (Mann et al., 1967), and decrease VOL, which leads to a lower NSP (VanDemark et al., 1964; Dance et al., 2015); however, genetically, the relationship between fertility and weight is not well defined. Knight et al. (1984) reported genetic correlations among three different weights and various yearling beef bull fertility traits. Although a correlation with weight and volume was not included in Knight et al. (1984), all reported correlations were low. As reported in Butler et al. (unpublished) when performing model selection, weight did not significantly contribute to the model for beef bull fertility traits. As noted in Table 3.3, rs109268478 was not in the vicinity of

any known QTL associated with fertility, but was near the location of QTL regions associated with milk composition (Bouwman et al. 2012; Ibeagha-Awemu et al., 2016).

While there are 17 genes within the QTL regions for VOL (Table 3.4), only one of those genes has an obvious association with fertility. Epididymis-specific alpha-mannosidase (MAN2B2) was in proximity to a SNP significant for VOL. Westfalewicz et al. (2017) reported that MAN2B2 contributes to carbohydrate metabolism and also found that carbohydrate metabolism was an important part of epididymal physiology. Carbohydrate metabolism is important for capacitation, providing energy for the spermatozoa, spermatozoa maturation, and epididymal transit (Hoskins et al., 1975; Panse et al., 1983; Storey 2008).

Concentration

Three significant SNP were identified for CONC, which included rs43067163, rs41623602, and rs29023737 (Table 3.2). McClure et al. (2010) reported a mature height QTL region that was close to rs41623602. In addition, McClure et al. (2010) reported a QTL region associated with yearling weight and mature weight which was in proximity to rs109876252 (Table 3.3). While there are few studies that directly relate size of the bull to the semen quality, weight and height are generally associated with maturity, and the association could be indicative that more mature, larger bulls have greater semen production. According to model selection in Butler et al. (unpublished), weight and hip height of the bull at the time of collection did not contribute significantly to concentration. In addition to QTL associated with bull size, two previously reported QTL regions associated with carcass fat were near significant SNP for CONC from the current study. Increased fat percentages in the carcass may suggest there may also be an increased amount of fat deposited in the scrotal tissue (Coulter 1994). Increased scrotal fat causes decreased semen quantity and quality, as the fat causes impaired

thermoregulation of the testes (Coulter 1994). Elevated testicular temperature has well-known adverse effects on semen attributes (Waites 1970; Setcehl 1978; Ross and Entwistle 1979; Wildeus and Entwistle 1986). It has been speculated that obese bulls experience more stress, which could cause suppression of gonadotrophin-releasing hormone, leading to a decrease in luteinizing hormone, and subsequently testosterone (Coulter 1994). Lower levels of these hormones lead to a decrease in sperm production (Senger 2012). None of the significant SNP were in the vicinity of fertility-related genes, but all genes identified near significant SNP are shown in Table 3.5.

Number of Spermatozoa

Seven SNP had significant associations with NSP (Table 3.2). Three different previously reported conception rate QTL regions (Boichard et al., 2003; Kiser et al., 2019) neighbored significant SNP for NSP. In addition, Hoglund et al. (2015) and Schrooten et al. (2004) reported QTL regions associated with non-return rate that were in the vicinity of two SNP from the current study. Like conception rate, non-return rate is usually related to female fertility, and this association provides further validation male fertility traits are integral in the reproductive success of the herd (DeJarnette 2004) or that male and female fertility share a genetic correlation in these regions of the genome.

In addition, a QTL region associated with average daily gain (Xu et al., 2019) was found near a SNP for NSP. Olson et al. (2020) evaluated the relationship among VOL, CONC, and daily weight gain in yearling Norwegian Red bulls. The genetic correlation between VOL and daily weight gain was 0.17 ± 0.08 . The genetic correlation between CONC and daily weight gain was -0.25 ± 0.15 . Olson did not report a correlation between NSP and daily weight gain, but NSP is a function of VOL and CONC, so it could be speculated a genetic correlation would have been

detected. In addition, McClure et al. (2010) reported a variety of QTL regions affecting yearling weight, mature weight, and mature height and several SNP significant for NSP were in proximity to these QTL regions (Table 3.3). Knight et al. (1984) reported a phenotypic correlation between NSP and weight of zero, and no genetic correlation due to a null sire variance for the trait.

While there were no genes with known associations to fertility, all the genes neighboring significant SNP are provided in Table 3.6.

Initial Motility

For IMot, 6 SNP on 6 different chromosomes were significant, including rs41623436, rs109798673, rs43526428, rs29003479, rs42861585, and rs109512383. One SNP, rs29003479, on chromosome 11 was significant in both bivariate analyses for initial motility and the other five were only significant in one of the analyses. The SNP significant in both bivariate analyses was near a previously-reported scrotal circumference QTL region (McClure et al., 2010). According to Christmas et al. (2001) and Corbet et al. (2013), scrotal circumference is strongly and positively genetically correlated with motility. Druet et al. (2009) reported a QTL for sperm average path velocity which overlapped the QTL region for rs109512383. Sperm average path velocity is a measure obtained from a computer-assisted semen analysis, and represents the average trajectory of the sperm cell. The trajectory of the spermatozoa is dependent upon the ability of the spermatozoa to move, thus providing further evidence of the relationship between initial motility and sperm average path velocity. A QTL region for sire conception rate (Melo et al., 2018) was in the vicinity of a SNP significant for IMot (table 3.3). In addition, Kiser et al. (2019) and Zhou et al. (2018) reported a conception rate QTL region, and in the current study, a significant SNP for IMot was close to the region. Once the spermatozoa are deposited into the female reproductive tract, it is essential for the sperm cells to progress towards the oocyte for

fertilization and, therefore; conception (Senger 2012). Interestingly, a QTL region found to influence sexual precocity (Melo et al., 2018) is near a significant SNP associated with IMot in this study. This is interesting because the average age of bulls in this study was just over two years old, with the youngest bull in the evaluation just under a year old. Bull studs often collect very young bulls and producers demand the semen because these bulls often have more desirable expected progeny differences (albeit unproven and lower accuracy) than their older stud mates. Young bulls must have the libido and semen quality to produce viable sperm, so the relationship between sexual precocity and initial motility could indicate young bulls are able to produce a viable ejaculate. Quantitative trait loci associated with milk composition (Costa et al., 2019; Bouwman et al., 2012) and interval to first estrus after calving (Zhang et al., 2019) were found neighboring rs41623436. Return to estrus after calving is dependent on the hormones gonadotropin releasing hormone (GnRH), luteinizing hormone (LH), and follicle stimulating hormone (FSH). The production of spermatozoa is also dependent on GnRH, signaling the release of LH to induce the leydig cells to produce testosterone. Testosterone paired with FSH signaling to the Sertoli cells causes the production of male gametes. It could be speculated that females which return to estrus more quickly after calving have increased hormone signaling, which could indicate males which have significant SNP near these QTL additionally have stronger hormone signaling; and therefore; produce more and higher quality gametes.

All genes near SNP significant for IMot are outlined in Table 3.7. A SNP contributing significantly to IMot was within the gene E3 ubiquitin protein ligase 2 (HERC2). Rodriguez and Stewart (2007) reported that mice without HERC4 expression had reduced sperm motility as a result of improper maturation and having a retained cytoplasmic droplet. Furthermore, IMot had a significant SNP that is found in the gene melanosomal transmembrane protein (OCA2). The

gene OCA2 is most commonly associated with albinism; however, it also contributes to spermatid development (The UniProt Consortium 2010). Reported in table 3.7 are additional genes identified within the QTL regions for IMot.

Post-thaw Motility

Seven SNP were identified for PTMot (Table 3.3). A QTL region on chromosome 11 which reportedly influences spermatozoa motility (Hering et al., 2014; Druet et al., 2009) encompassed rs42653645, a SNP significant for PTMot. Kiser et al. (2019) reported two different conception rate QTL regions, and the current study had two SNP significant for PTMot close to the regions. As motile spermatozoa are necessary for conception, this is a logical relationship.

An average daily gain QTL region (Gutierrez-Gil et al., 2009; Alexander et al., 2007) was found near a SNP significant for PTMot. While the effects of early-life nutrition have on bull fertility has been studied, the relationship among bull fertility traits and average daily gain has not been quantified. However, Awda et al., (2012) did look at the relationship between bull fertility traits and residual feed intake and concluded that bulls with low residual feed intake had decreased sperm quality, indicating more feed efficient bulls are less fertile. Olson et al. (2020) reported a low, positive genetic correlation between PTMot and daily weight gain; however the standard error was large. As shown in Table 3.3, rs41623436 on chromosome one was a significant SNP; however, previously reported QTL regions near the SNP were not associated with fertility traits.

A SNP significant for PTMot was in proximity to collagen like tail subunit of asymmetric acetylcholinesterase (COLQ). According to Gaudet et al. (2011), COLQ contributes to the structural integrity of the extracellular matrix and heparin binding. It is important to note heparin

is an important enhancer of capacitation of the bovine spermatozoa (Handrow et al., 1982). Furthermore, heparin-binding proteins allow the spermatozoa to undergo the acrosome reaction (Lane et al., 1999). In addition, a significant SNP for PTMot was in proximity to the heat shock protein family B (small) member 8 (HSPB8) protein. Huszar et al., (1998) speculated that elevated heat shock protein levels are associated with immature spermatozoa. This research addressed human infertility and found that an increase in the 70-kDa heat shock protein HspA2 resulted in spermatozoa not completing the phases of changing the plasma membrane during epididymal maturation and having larger amounts of cytoplasmic proteins in mature spermatozoa (Huszar et al., 1998). As previously discussed, heat shock protein can affect the maturation of the spermatozoa and cause infertility (Huszar et al., 1998). Reported in Table 3.8 are additional genes identified near SNP affecting PTMot.

Three-hour Post-thaw Motility

For 3HRPTMot, two SNP were significant: rs29003479 and rs109719529. McClure et al. (2010) reported a QTL region which affected many growth traits including birth weight, mature weight, body length, and scrotal circumference. The SNP rs29003479 was near the QTL region. While limited research is available pertaining to prolonged motility measures, this may suggest size of the bull and scrotal circumference influences motility of the spermatozoa. Additional QTL are reported in Table 3.3; however, rs109719529 is not near any other previously-reported QTL associated with fertility. The QTL found within the vicinity of the significant SNP were associated with milk composition (Bouwman et al., 2012) and shear force (McClure et al., 2012; Cesar et al., 2014).

Heat shock protein family B (small) member 8 (HSPB8) is near a SNP significant for 3HRPTMot. As previously discussed, heat shock protein can affect the maturation of the

spermatozoa and cause infertility (Huszar et al., 1998). All other reported genes in proximity to significant SNP for 3HRPTMot are outlined in Table 3.9.

Percentage of Normal Spermatozoa

Six significant SNP were identified for %NORM (Table 3.2). The SNP were rs41606310 on chromosome one, rs110964837 on chromosome two, rs41594758 on chromosome three, rs109928164 on chromosome five, rs41591913 on chromosome five, and rs41666416 on chromosome ten. Notably, rs110964837 was significant in both bivariate models where percentage of normal spermatozoa was evaluated with either PRIM or SEC. The SNP rs110964837 was one of the two SNP near conception-rate QTL regions and a non-return rate QTL region (Druet et al., 2009; Kiser et al., 2019; Jiang et al., 2019; Ben Jemaa et al., 2008). McClure et al. (2010) reported scrotal circumference QTL regions, and a significant SNP for %NORM in the current study was near the reported region. Two conception-rate QTL regions and a non-return rate QTL region (Druet et al., 2009; Kiser et al., 2019; Jiang et al., 2019; Ben Jemaa et al., 2008) were close to SNP significantly associated with %NORM. As previously discussed, SNP significantly associated with male fertility traits nearby fertility-related QTL regions is further evidence that the quality of a bull's semen is important for conception or that the same genes underlie attributes affecting conception in both males and females.

A previously-reported QTL which affects yearling weight, mature weight, and mature height was near rs41594758, a SNP significant for %NORM. Smith et al. (1989) reported a moderate, positive correlation between yearling weight and %NORM, indicating that as yearling weight increases, the percentage of normal spermatozoa increases. Limited recent research pertaining to body weight and male fertility traits is lacking. However, Olson et al. (2020)

reported a genetic correlation between birth weight and percentage of spermatozoa defects to be strong and negative, indicating that as the percentage of defects increases, birth weight decreases.

Table 3.10 illustrates all genes which were in proximity of significant SNP for %NORM; however, the most pertinent gene identified was the major facilitator superfamily domain containing 14A (MFSD14A) gene, also known as HIAT1. The MFSD14A gene is important for spermatid nucleus differentiation and sperm mitochondrion organization. In a study performed by Doran et al., (2016), it was determined mice with a disruption to the MFSD14A gene were infertile due to incomplete acrosome formation and round head defects.

Primary Abnormalities

Primary abnormalities had six strongly associated SNP: rs41566683, rs41255529, rs110487590, rs42940192, rs110425782, and rs41646489 (Table 3.2). A previously-reported scrotal circumference QTL region was near rs41255529, a SNP significant for PRIM (McClure et al. 2010). Genetic correlations between scrotal circumference and PRIM indicate increased scrotal circumference results in fewer PRIM (Christmas et al., 2001; Garmyn et al., 2013). Three significant SNP for PRIM were in proximity to a QTL region associated with conception rate and non-return rate (Kiser et al., 2019; Boichard et al., 2003; McClure et al., 2010). Primary abnormalities are abnormalities of the head, and without proper head formation, the sperm cell cannot penetrate the zona pellucida on the oocyte and cause conception (Senger 2012). Furthermore, two of the significant SNP associated with PRIM were near two QTL regions associated with average daily gain (Komatsu et al., 2011; Casas et al., 2001). As previously discussed, average daily gain has been associated with sperm quality (Awda et al., 2012). In addition, many different QTL regions affecting carcass fat, such as dressing percentage, fat thickness at the 12th rib, and marbling score (Hou et al., 2010, Yuan et al., 2011; Yokouchi et al.,

2009; Melucci et al., 2012; Orru et al., 2011) were in proximity of rs42940192, a SNP significant for PRIM. The environmental association between beef bull semen attributes and fat was further validated by Coulter and Kozub (1989) which performed a multi-sire breeding study for three years and determined that bulls with more backfat had decreased fertility. Coulter and Kozub (1989) fed bulls higher energy diets and the bulls had increased increased back fat. Bulls with increased back fat had poorer semen quantity and quality, and therefore, fertility. Limited published research relating production traits genetically to male fertility traits is available; however, Smith et al. (1984) reported that average daily gain was only slightly correlated to PRIM (0.10). The correlation by Smith et al. (1984) indicates a negligible relationship. All SNP associated with beef bull semen attributes near previously reported QTL regions are detailed in Table 3.3.

PRIM had significant SNP in the vicinity of genes in three locations on chromosomes one and four (Table 3.11). One of the notable genes was testis expressed 55 (TEX55), also noted in the literature as testis-specific conserved, cAMP dependent type PK anchoring protein (TSCPA). Yu et al., (2009) found that TSCPA expressed in the human testis contributed to spermatogenesis, and the lack of expression of this the protein was found in cryptorchid men. In addition, PRIM had a significant SNP in sperm mitochondrial-associated cysteine-rich protein-like (LOC101902976). Hawthorne et al., (2006) reported sperm-mitochondrial cysteine-rich protein is located in the mitochondrial capsule and plays a role in sperm motility. While the mitochondria are located in the midpiece of the spermatozoa rather than the head, it is still important to note it is associated with male fertility.

Secondary Abnormalities

One significant SNP on chromosome 22 was identified for SEC (rs41646489). The SNP rs41646489 was significant in both bivariate analyses, and is shown in Figure 3.9. No reported QTL regions for fertility-related traits exist that are near this SNP. However, growth and carcass trait QTL regions influencing yearling weight, mature height, carcass weight, and carcass fat were near rs41646489. It has been previously discussed the affect nutrition and fat coverage has on semen quality.

SEC had one significant SNP in the vicinity of two different genes. Neither of the genes have known contributions to fertility traits and more details about the gene functions are in Table 3.12.

Functional Annotation Analysis

Using DAVID 6.8, enriched biological pathways were attempted to be identified. No enriched pathways were determined for an individual trait. When all the genes identified for all of the traits were combined and included in the analysis, again, no enriched pathways were determined.

Conclusion

Several QTL associated with fertility traits were identified in this study and validated by previously published literature. In addition, genes within the QTL regions for beef bull semen attributes were discussed. While a few gene functions could be associated with beef bull semen attributes, some of the genes found in the current study do not have any known association with fertility traits reported in previous literature and have unknown related physiological association. Results from the current study, paired with findings in previous literature, validate that bull fertility traits are controlled by genetic factors. The increased use of genomic testing in tandem with the number of phenotypes routinely recorded by bull semen collection facilities makes

identification of beef bulls with superior genetic merit for fertility traits feasible. Determining SNPs which control fertility traits and continuing to include the SNPs in genomic tests will improve fertility traits. This advancement would increase profitability of beef producers by improving fertilization rate, increasing overall herd reproductive rate, and providing producers another genetic tool to use in making selection decisions.

Table 3.1 Trait list, definitions, and summary statistics of beef bull fertility traits collected at an artificial insemination. All post-thaw motility measures are observed the day after the sample is collected. All traits are measured within one hour with the exception of three-hour post-thaw motility.

Trait	Units	Definition	N	Mean	Minimum	Maximum
Volume (VOL)	mL	total amount of the ejaculate, measured by milliliters	44431	7.96	0.10	74.00
Concentration (CONC)	Million/mL	relative amount of sperm cells per ejaculate, measured by a colorimeter	44038	1021.16	10.00	3906.00
Number of spermatozoa (NSP)	Million	calculated by multiplying sperm concentration and semen volume; expressed in millions	44418	49.18	0.00	100.00
Initial Motility (IMot)*	%	percentage of progressively swimming spermatozoa in the ejaculate immediately after collection	44038	8004.18	64.50	69795.00
Post-thaw Motility (PTMot)*	%	percentage of progressively swimming spermatozoa in the ejaculate, measured within one hour of thawing	29877	43.53	0.00	75.00
Three-hour post-thaw motility (3HRPTMot)*	%	percentage of progressively swimming spermatozoa in the ejaculate, measured three hours after thawing	8299	15.72	0.00	60.00
Percentage of Normal Spermatozoa (%NORM)*	%	percent morphologically normal spermatozoa	19455	75.18	2.00	100.00
Primary Abnormalities (PRIM)*	%	percentage of spermatozoa with a defect to the head	19452	13.00	0.00	100.00
Secondary Abnormalities (SEC)*	%	percentage of spermatozoa with a defect to the tail	19521	12.09	0.00	100.00

*Trait is measured subjectively by a trained laboratory technician.

Table 3.2 Genomic regions identified by genome-wide association study contributing significantly to beef bull semen attributes. Significant SNP with a p-value of < 0.00001.

Trait	SNP Name	rsID	Chromosome	Position
Volume	BTB-01549373	No rsID	2	81679350
	Hapmap48133-BTA-96243	rs41666488	3	61947686
	ARS-BFGL-NGS-71827	rs109736826	3	112997892
	ARS-BFGL-NGS-25127	rs109268478	6	102859342
	Hapmap49899-BTA-18490	rs41575945	27	3495048
Concentration	BTB-01963898	rs43067163	1	133936071
	BTA-122016-no-rs	rs41623602	3	38811314
	Hapmap55203-rs29023737	rs29023737	5	3645270
Number of Spermatozoa	BTA-110980-no-rs	rs41618035	1	31173269
	ARS-BFGL-NGS-101891	rs109740774	6	75137290
	Hapmap33368-BTA-146079	rs43567728	8	89743053
	Hapmap44146-BTA-83959	rs41661101	9	50922485
	ARS-BFGL-NGS-112914	rs110005257	11	99686154
	ARS-BFGL-NGS-101386	rs110190516	17	67017094
	ARS-BFGL-NGS-85970	rs108993490	24	16118203
Initial Motility	Hapmap48211-BTA-120784	rs41623436	1	152412536
	ARS-BFGL-NGS-74920	rs109798673	2	827626
	BTB-00319289	rs43526428	7	73777772
	Hapmap59148-ss46527122	rs29003479	11	63430172
	BTB-01751684	rs42861585	23	46295105
	Hapmap23061-BTC-074055	rs109512383	25	30964321
Post-thaw Motility	Hapmap48211-BTA-120784	rs41623436	1	152412536
	ARS-BFGL-NGS-74920	rs109798673	2	827626
	Hapmap45703-BTA-105835	rs41611445	2	82083660
	BTB-01323835	rs42446055	4	89708810
	BTB-01537954	rs42653645	11	25036830
	ARS-BFGL-NGS-41002	rs110418161	16	36415574
	ARS-BFGL-NGS-111058	rs109719529	17	56344620
Three Hour Post-thaw Motility	Hapmap59148-ss46527122	rs29003479	11	63430172
	ARS-BFGL-NGS-111058	rs109719529	17	56344620
Percentage of Normal Spermatozoa	BTA-50285-no-rs	rs41606310	1	7669386
	ARS-BFGL-NGS-43775	rs110964837	2	73209337
	BTA-91078-no-rs	rs41594758	3	43031873
	ARS-BFGL-NGS-25359	rs109928164	5	70049126
	BTA-74480-no-rs	rs41591913	5	84578337
	BTA-98744-no-rs	rs41666416	10	68089016
Primary Abnormalities	BTA-115758-no-rs	rs41566683	1	36903496
	Hapmap57078-ss46526391	rs41255529	1	60956897
	ARS-BFGL-NGS-44433	rs110487590	1	63799085
	BTB-01831345	rs42940192	4	10648384
	ARS-BFGL-NGS-80666	rs110425782	20	68324872
	Hapmap42996-BTA-60916	rs41646489	22	33690494
Secondary Abnormalities	Hapmap42996-BTA-60916	rs41646489	22	33690494

Table 3.3 Previously reported selected QTL regions which overlap significant SNP in this study identified for beef bull semen attributes. If no QTL were determined to be associated with fertility, then no QTL were listed in the table.

Trait	SNP Name	rsID	Chr:Position	QTL Trait	Source
Volume	BTB-01549373	No rsID	2: 81679350	Birth weight	McClure et al., 2010; Grosz and MacNeil 2001
				Marbling score	Grosz and MacNeil 2001
				Carcass weight	McClure et al., 2010
				Conception rate	Kiser et al., 2019
				Non-return rate	Ben Jemaa et al., 2008
	Hapmap48133-BTA-96243	rs41666488	3:61947686	Carcass weight	McClure et al., 2010
				Average daily gain	Peters et al., 2012
				Yearling weight	McClure et al., 2010
	ARS-BFGL-NGS-71827	rs109736826	3:112997892	Average daily gain	Lu et al., 2013
	ARS-BFGL-NGS-25127	rs109268478	6:102859342	*	
Concentration	Hapmap49899-BTA-18490	rs41575945	27:3495048	Carcass weight	McClure et al., 2010
				Mature weight	McClure et al., 2010
	BTB-01963898	rs43067163	1:133936071	Non-return rate	Ron et al., 2004
				Metabolic body weight	Seabury et al., 2017
				Retail Product Yield	Mosig et al., 2001, Casas et al., 2003
				Fat thickness at the 12 th rib	Imumorin et al., 2011
				Kidney, pelvic, and heart fat percentage	Casas et al., 2003
				Weaning weight	Saatchi et al., 2014, McClure et al., 2010
	BTA-122016-no-rs	rs41623602	3:38811314	Mature body height	McClure et al., 2010
				Average daily gain	Li et al., 2002
				Tenderness score	Alexander et al., 2007
	Hapmap55203-rs29023737	rs29023737	5: 3645270		
Number of Spermatozoa	BTA-110980-no-rs	rs41618035	1:31173269	Lean meat yield	Doran et al., 2014
				Carcass weight	Doran et al., 2014
				Fat thickness at the 12 th rib	Alexander et al., 2007
				Marbling score	McClure et al., 2010
				Kidney, pelvic, and heart fat percentage	Mateescu et al., 2017

				Subcutaneous fat	Doran et al., 2014
				Body weight	McClure et al., 2010
				Conception rate	Boichard et al., 2003
				Inseminations per conception	Hoglund et al., 2015
				Non-return rate	Hoglund et al., 2015
	ARS-BFGL-NGS-101891	rs109740774	6:75137290	First service conception	Kiser et al., 2019
	Hapmap3336 8-BTA-146079	rs43567728	8: 89743053	Cold tolerance	Cole et al., 2011
				Conception rate	Kiser et al., 2019
	Hapmap4414 6-BTA-83959	rs41661101	9:50922485	Marbling score	McClure et al., 2010
				Average daily gain	Xu et al., 2019
				Mature weight	McClure et al., 2010
				Non-return rate	Schrooten et al., 2004
				Structural soundness of feet, legs, penis, and prepuce	Ashwell et al., 2005
	ARS-BFGL-NGS-112914	rs110005257	11: 99686154	Pelvic area	Michenet et al., 2016
	ARS-BFGL-NGS-101386	rs110190516	17: 67017094	Non-return rate	Frischknecht et al., 2017
				Calving ease	Saatchi et al., 2014
	ARS-BFGL-NGS-85970	rs108993490	24:16118203	First service conception rate	Kiser et al., 2019
				Conception rate	Kiser et al., 2019
Initial Motility	Hapmap4821 1-BTA-120784	rs41623436	1:152412536	*	
	ARS-BFGL-NGS-74920	rs109798673	2: 827626	Trapezius muscle lean area	Abe et al., 2008
				Tenderness score	Casas et al., 1998
				Calving ease	Casas et al., 1998
				Conception rate	Kiser et al., 2019
				Fertilization rate	Huang et al., 2010
	BTB-00319289	rs43526428	7:73777772	Conception rate	Kiser et al., 2019, Zhou et al., 2018
				Daughter pregnancy rate	Zhou et al., 2018, Cole et al., 2011
				Sire conception rate	Melo et al., 2018
				Sexual precocity	Melo et al., 2018
	Hapmap5914 8-ss46527122	rs29003479	11:63430172	Carcass weight	McClure et al., 2010
				Marbling score	McClure et al., 2010

				Body length	Sun et al., 2011
				Body weight	Sun et al., 2011
				Scrotal circumference	McClure et al., 2010
	BTB-01751684	rs42861585	23: 46295105	Weaning weight	Michenet et al., 2016
				Intramuscular fat	Mateescu et al., 2017
	Hapmap2306 1-BTC-074055	rs109512383	25: 30964321	Lean meat yield	Doran et al., 2014
				Subcutaneous fat	Doran et al., 2014
				Carcass weight	McClure et al., 2010
				Foot angle	Ashwell et al., 2005
				Mature height	McClure et al., 2010
				Weaning weight	McCluer et al., 2010
				Rump Angle	Schnabel et al., 2005
				Conception rate	Kiser et al., 2019
				Scrotal circumference	McClure et al., 2010
				Sperm average path velocity	Druet et al., 2009
Post-thaw Motility	Hapmap4821 1-BTA-120784	rs41623436	1: 152412536	*	
	ARS-BFGL-NGS-74920	rs109798673	2:827626	Conception Rate	Kiser et al., 2019
				Fertilization Rate	Huang et al., 2010
	Hapmap4570 3-BTA-105835	rs41611445	2: 82083660	Carcass weight	McClure et al., 2010
				Marbling score	MacNeil and Grosz 2002
				Birth weight	McClure et al., 2010; MacNeil and Grosz 2002
				Conception rate	Kiser et al., 2019
				Non-return rate	Ben Jemaa et al., 2008
	BTB-01323835	rs42446055	4: 89708810	--	
	BTB-01537954	rs42653645	11:25036830	Yield Grade	Casas et al., 2003
				Kidney, pelvic, and heart fat percentage	Imumorin et al., 2011
				Residual feed intake	Sherman et al., 2009
				Birth weight	McClure et al., 2010
				Sperm motility	Hering et al., 2014, Druet et al., 2009
	ARS-BFGL-NGS-41002	rs110418161	16:36415574	Social separation (vocalization)	Gutierrez-Gil et al., 2008

				Carcass weight	Alexander et al., 2007
				Kidney, pelvic, heart fat percentage	Casas et al., 2003
				Dry matter intake	Tetens et al., 2014
				Average daily gain	Gutierrez-Gil et al., 2009, Alexander et al., 2007
				Birth weight	McClure et al., 2010
				Weaning weight	Alexander et al., 2007, McClure et al., 2010
	ARS-BFGL-NGS-111058	rs109719529	17:56344620	--	
Three-hour Post-thaw Motility	Hapmap5914 8-ss46527122	rs29003479	11: 63430172	Carcass weight	McClure et al., 2010
				Kidney, pelvic, heart fat percentage	Imumorin et al., 2011; Stone et al., 1999
				Marbling score	McClure et al., 2010
				Birth weight	Sun et al., 2011
				Body length	Sun et al., 2011
				Mature weight	McClure et al., 2010
				Rump angle	Boichard et al., 2003
				Weaning weight	Imumorin et al., 2011
				Yearling weight	McClure et al., 2010
				Scrotal circumference	McClure et al., 2010
	ARS-BFGL-NGS-111058	rs109719529	17: 56344620	*	
Percentage of Normal Spermatozoa	BTA-50285-no-rs	rs41606310	1: 7669386	Daughter pregnancy rate	Schnabel et al., 2005
	ARS-BFGL-NGS-43775	rs110964837	2:73209337	Carcass weight	McClure et al., 2010
				Birth weight	McClure et al., 2010
				Weaning weight	McClure et al., 2010
				Conception rate	Druet et al., 2009, Kiser et al., 2019, Jiang et al., 2019
				Non-return rate	Ben Jemaa et al., 2008
	BTA-91078-no-rs	rs41594758	3:43031873	Carcass Weight	McClure et al., 2010
				Retail product yield	Casas et al., 2003

				Kidney, pelvic, heart fat percentage	Casas et al., 2003
				Marbling score	Casas et al., 2003
				Birth weight	McClure et al., 2010
				Weaning weight	Saatchi et al., 2014
				Yearling weight	McClure et al., 2010
				Mature weight	McClure et al., 2010
				Mature Height	McClure et al., 2010
	ARS-BFGL-NGS-25359	rs109928164	5: 70049126	Carcass weight	Mullen et al., 2011
				Fat thickness at the 12 th rib	McClure et al., 2010
				Marbling score	McClure et al., 2010
				Birth weight	McClure et al., 2010
				Body condition score	Mullen et al., 2011
				Hip height	Boichard et al., 2003
				Mature weight	McClure et al., 2010
				Rump angle	Mullen et al., 2011
				Rump width	Boichard et al., 2003
				Weaning weight	McClure et al., 2010
				Daughter pregnancy rate	Jiang et al., 2019
	BTA-74480-no-rs	rs41591913	5:84578337	Conception Rate	Jiang et al., 2019
	BTA-98744-no-rs	rs41666416	10: 68089016	Carcass weight	McClure et al., 2010, Mateescu et al., 2017
				Average daily gain	Xu et al., 2019
				Mature weight	McClure et al., 2010
				Fertility index	Cai et al., 2019
				Scrotal circumference	McClure et al., 2010
Primary Abnormalities	BTA-115758-no-rs	rs41566683	1: 36903496	Carcass weight	Kim et al., 2003
				Fat thickness at the 12 th rib	Alexander et al., 2007
				Marbling score	McClure et al., 2010
				Weaning weight	McClure et al., 2010
				Yearling weight	Kim et al., 2003

				Conception rate	Kiser et al., 2019; Boichard et al., 2003
	Hapmap5707 8-ss46526391	rs41255529	1:60956897	Carcass weight	Komatsu et al., 2011
				Feed conversion	Sherman et al., 2009
				Dry matter intake	Nkrumah et al., 2007
				Average daily gain	Komatsu et al., 2011
				Body weight gain	Snelling et al., 2010
				Conception rate	Kiser et al., 2019, Boichard et al., 2003
				First service conception	Kiser et al., 2019
				Non-return rate	Ben Jemaa et al., 2008
				Scrotal circumference	McClure et al., 2010
	ARS-BFGL- NGS-44433	rs110487590	1: 63799085	Conception rate	Jiang et al., 2019; Boichard et al., 2003
				Daughter pregnancy rate	Jiang et al., 2019
				Non-return rate	Ben Jemaa et al., 2008
				Scrotal circumference	McClure et al., 2010
				Testicular hypoplasia	Neves et al., 2019
	BTB- 01831345	rs42940192	4:10648384	Carcass weight	Casas et al., 2001, Hou et al., 2010
				Dressing percentage	Hou et al., 2010, Yuan et al., 2011
				Fat thickness at the 12 th rib	Hou et al., 2010, Yuan et al., 2011
				Intramuscular fat	Bolormaa et al., 2011
				Marbling score	Yokouchi et al., 2009
				Meat fat content	Orru et al., 2011
				Feed conversion ratio	Sherman et al., 2009
				Residual feed intake	Sherman et al., 2009
				Average daily gain	Casas et al., 2001
				Non-return rate	Schulman et al., 2011, Pimentel et al., 2011
				Male fertility	Blaschek et al., 2011

	ARS-BFGL-NGS-80666	rs110425782	20: 68324872	Rump width	Cole et al., 2011
	Hapmap42996-BTA-60916	rs41646489	22: 33690494	Carcass weight	Kim et al., 2003
				Fat percentage	Gutierrez-Gil et al., 2009
				Subcutaneous fat	Li et al., 2017
				Mature height	McClure et al., 2010
				Rump angle	Ashwell et al., 2005
Secondary Abnormalities	Hapmap42996-BTA-60916	rs41646489	22:33690494	Yearling weight	McClure et al., 2010
				Carcass weight	Kim et al., 2003
				Fat percentage	Gutierrez-Gil et al., 2009
				Subcutaneous fat	Li et al., 2017
				Yearling weight	McClure et al., 2010
				Mature Height	McClure et al., 2010

* No known connection to fertility.

-- No QTL were found within linkage disequilibrium regions.

Table 3.4 Genes in the vicinity of significant SNP for volume determined by a genome-wide association study.

Trait	rsID	Chr:Pos	GeneID	Symbol	Description
Volume	rs109736826	3:112997892	112445892	LOC112445892	uncharacterized LOC112445892
			112446116	LOC112446116	small Cajal body-specific RNA 6
			112446117	LOC112446117	small Cajal body-specific RNA 6
			112446126	LOC112446126	small nucleolar RNA SNORA11
			509587	ATG16L1	autophagy related 16 like 1
			513389	DGKD	diacylglycerol kinase delta
			507329	INPP5D	inositol polyphosphate-5-phosphatase D
			280922	SAG	S-antigen visual arrestin
			614542	NEU2	neuraminidase 2
			531139	USP40	ubiquitin specific peptidase 40
	rs109268478	6:102859342	523503	MAN2B2	epididymis-specific alpha-mannosidase
			100298890	LOC100298890	high mobility group protein 20A
			540970	JAKMIP1	janus kinase and microtubule interacting protein 1
			782110	PPP2R2C	protein phosphatase 2 regulatory subunit Bgamma
			100298456	WFS1	wolframin ER transmembrane glycoprotein
	rs41575945	27:3495048	112444700	TRNAC-GCA	transfer RNA cysteine (anticodon GCA)
			526825	CSMD1	CUB and Sushi multiple domains 1

Table 3.5 Genes in the vicinity of significant SNP for concentration determined by a genome-wide association study.

Trait	rsID	Chr:Pos	GeneID	Symbol	description
Concentration	rs43067163	1:133936071	112448276	LOC112448276	U6 spliceosomal RNA
			534731	EPHB1	EPH receptor B1
	rs41623602	3:38811314	782217	LOC782217	renin receptor pseudogene
			787637	LOC787637	60S ribosomal protein L10a pseudogene

Table 3.6 Genes in the vicinity of significant SNP for number of spermatozoa determined by a genome-wide association study.

Trait	rsID	Chr:Pos	GeneID	Symbol	description
Number of Spermatozoa	rs109740774	6:75137290	112447159	LOC112447159	uncharacterized LOC112447159
			100141023	LOC100141023	condensin-2 complex subunit D3-like
	rs43567728	8:89743053	541068	SPIN1	spindlin 1
			530279	NXNL2	nucleoredoxin like 2
			100298873	LOC100298873	PRSS2 protein-like pseudogene
			107132723	LOC107132723	60S ribosomal protein L21-like
			104969476	LOC104969476	40S ribosomal protein S3 pseudogene
			101902749	LOC101902749	microtubule-associated proteins 1A/1B light chain 3B pseudogene
			101902554	LOC101902554	putative ubiquitin-like protein FUBI-like protein ENSP00000310146
			100140258	LOC100140258	vasculin-like protein 1
	rs41661101	9:50922485	112448129	LOC112448129	uncharacterized LOC112448129
			535452	FBXL4	F-box and leucine rich repeat protein 4
			783320	POU3F2	POU class 3 homeobox 2
	rs110005257	11:99686154	512902	CRAT	carnitine O- acetyltransferase
			535315	MIGA2	mitoguardin 2
			526844	DOLK	dolichol kinase
			782019	IER5L	immediate early response 5 like
			540828	PHYHD1	phytanoyl-CoA dioxygenase domain containing 1

			514460	PTPA	protein phosphatase 2 phosphatase activator
			504908	DOLPP1	dolichyldiphosphatase 1
			504750	SH3GLB2	SH3 domain containing GRB2 like, endophilin B2
			504307	NUP188	nucleoporin 188
			112448842	LOC112448842	uncharacterized LOC112448842
			112448841	LOC112448841	uncharacterized LOC112448841
			112448840	LOC112448840	uncharacterized LOC112448840
			107132963	LOC107132963	uncharacterized LOC107132963
			101904916	LOC101904916	uncharacterized LOC101904916
			101904867	LOC101904867	uncharacterized LOC101904867
	rs110190516	17:67017094	786290	MN1	MN1 proto-oncogene, transcriptional regulator
			104974692	LOC104974692	uncharacterized LOC104974692
			100849610	LOC100849610	uncharacterized LOC100849610
	rs108993490	24:16118203	112444224	LOC112444224	U2 spliceosomal RNA

Table 3.7 Genes in the vicinity of significant SNP for initial motility determined by a genome-wide association study.

Trait	rsID	Chr:Pos	GeneID	Symbol	description
Initial Motility	rs41623436	1:152412536	107132229	LOC107132229	uncharacterized LOC107132229
			112448103	LOC112448103	uncharacterized LOC112448103
			112448105	LOC112448105	uncharacterized LOC112448105
			112448120	LOC112448120	uncharacterized LOC112448120
			512676	HACL1	2-hydroxyacyl-CoA lyase 1
			514615	COLQ	collagen like tail subunit of asymmetric acetylcholinesterase
			537669	BTB	biotinidase
			618048	CAPN7	calpain 7
			112448102	LOC112448102	collagen alpha-4(VI) chain-like
			507577	EAF1	ELL associated factor 1
			535332	METTL6	methyltransferase like 6
			618056	SH3BP5	SH3 domain binding protein 5
			107132228	LOC107132228	ubiquitin-like protein 5
			112448117	LOC112448117	uncharacterized LOC112448117
	rs109798673	2:827626	100141021	CYFIP1	cytoplasmic FMR1 interacting protein 1
			507430	NIPA2	NIPA magnesium transporter 2
			539162	NIPA1	NIPA magnesium transporter 1
			535440	HERC2	HECT and RLD domain containing E3 ubiquitin protein ligase 2
			524289	OCA2	OCA2 melanosomal transmembrane protein
			112443737	TRNAE-CUC	transfer RNA glutamic acid (anticodon CUC)
			112443600	LOC112443600	U6 spliceosomal RNA
			107132230	LOC107132230	DNA-directed RNA polymerases I, II, and III subunit RPABC4 pseudogene
			104971093	LOC104971093	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 5 pseudogene

	rs43526428	7:73777772	780973	GABRA1	gamma-aminobutyric acid type A receptor alpha1 subunit
			282240	GABRG2	gamma-aminobutyric acid type A receptor gamma2 subunit
	rs29003479	11:63430172	100295066	LOC100295066	60S ribosomal protein L22-like
			101905979	LOC101905979	thymosin beta-4 pseudogene
			112448803	LOC112448803	uncharacterized LOC112448803
			112448804	LOC112448804	uncharacterized LOC112448804
			538486	ACTR2	actin related protein 2
			540684	CEP68	centrosomal protein 68
			326577	SLC1A4	solute carrier family 1 member 4
			539339	RAB1A	RAB1A, member RAS oncogene family
			519081	SPRED2	sprouty related EVH1 domain containing 2
	rs42861585	23:46295105	784682	OFCC1	orofacial cleft 1 candidate 1
	rs109512383	25:30964321	615936	AUTS2	activator of transcription and developmental regulator AUTS2
			112444379	LOC112444379	U6 spliceosomal RNA
			112444317	LOC112444317	uncharacterized LOC112444317
			107131834	LOC107131834	40S ribosomal protein S6 pseudogene

Table 3.8 Genes in the vicinity of significant SNP for post-thaw motility determined by a genome-wide association study.

Trait	rsID	Chr:Pos	GeneID	Symbol	description
Post-thaw Motility	rs41623436	1:152412536	514615	COLQ	collagen like tail subunit of asymmetric acetylcholinesterase
			618056	SH3BP5	SH3 domain binding protein 5
			618048	CAPN7	calpain 7
			537669	BTD	biotinidase
			535332	METTL6	methyltransferase like 6
			512676	HACL1	2-hydroxyacyl-CoA lyase 1
			507577	EAF1	ELL associated factor 1
			112448120	LOC112448120	uncharacterized LOC112448120
			112448117	LOC112448117	uncharacterized LOC112448117
			112448105	LOC112448105	uncharacterized LOC112448105
			112448103	LOC112448103	uncharacterized LOC112448103
			112448102	LOC112448102	collagen alpha-4(VI) chain-like
			107132229	LOC107132229	uncharacterized LOC107132229
			107132228	LOC107132228	ubiquitin-like protein 5
	rs109798673	2:827626	104971093	LOC104971093	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 5 pseudogene
			107132230	LOC107132230	DNA-directed RNA polymerases I, II, and III subunit RPABC4 pseudogene
			112443600	LOC112443600	U6 spliceosomal RNA
			112443737	TRNAE-CUC	transfer RNA glutamic acid (anticodon CUC)
			100141021	CYFIP1	cytoplasmic FMR1 interacting protein 1
			535440	HERC2	HECT and RLD domain containing E3 ubiquitin protein ligase 2
			539162	NIPA1	NIPA magnesium transporter 1
			507430	NIPA2	NIPA magnesium transporter 2
			524289	OCA2	OCA2 melanosomal transmembrane protein

	rs42446055	4:89708810	782604	LOC782604	elongation factor 1-alpha 1 pseudogene
	rs42653645	11:25036830	101906077	LOC101906077	uncharacterized LOC101906077
			112448764	LOC112448764	uncharacterized LOC112448764
			112448960	LOC112448960	U6 spliceosomal RNA
			112448980	TRNAI-UAU	transfer RNA isoleucine (anticodon UAU)
			510602	HAAO	3-hydroxyanthranilate 3,4- dioxygenase
			512854	MTA3	metastasis associated 1 family member 3
			112448763	KCNG3	potassium voltage-gated channel modifier subfamily G member 3
	rs110418161	16:36415574	104974416	LOC104974416	uncharacterized LOC104974416
			104974419	LOC104974419	uncharacterized LOC104974419
			112441964	TRNAC-ACA	transfer RNA cysteine (anticodon ACA)
			112441949	TRNAC-GCA	transfer RNA cysteine (anticodon GCA)
			504963	DPT	dermatopontin
			319096	XCL1	chemokine (C motif) ligand 1
	rs109719529	17:56344620	112442041	LOC112442041	uncharacterized LOC112442041
			112442119	LOC112442119	U6 spliceosomal RNA
			539524	HSPB8	heat shock protein family B (small) member 8
			614115	SRRM4	serine/arginine repetitive matrix 4

Table 3.9 Genes in the vicinity of significant SNP for three-hour post-thaw motility determined by a genome-wide association study.

Trait	rsID	Chr:Pos	GeneID	Symbol	description
Three-hour Post-thaw Motility	rs29003479	11:63430172	538486	ACTR2	actin related protein 2
			539339	RAB1A	RAB1A, member RAS oncogene family
			540684	CEP68	centrosomal protein 68
			519081	SPRED2	sprouty related EVH1 domain containing 2
			326577	SLC1A4	solute carrier family 1 member 4
			112448804	LOC112448804	uncharacterized LOC112448804
			112448803	LOC112448803	uncharacterized LOC112448803
			101905979	LOC101905979	thymosin beta-4 pseudogene
			100295066	LOC100295066	60S ribosomal protein L22-like
	rs109719529	17:56344620	539524	HSPB8	heat shock protein family B (small) member 8
			614115	SRRM4	serine/arginine repetitive matrix 4
			112442119	LOC112442119	U6 spliceosomal RNA
			112442041	LOC112442041	uncharacterized LOC112442041

Table 3.10 Genes in the vicinity of significant SNP for percentage of normal spermatozoa determined by a genome-wide association study.

Trait	rsID	Chr:Pos	GeneID	Symbol	description
Percentage of Normal Spermatozoa	rs41606310	1:7669386	112448287	LOC112448287	U6 spliceosomal RNA
			104970788	LOC104970788	uncharacterized LOC104970788
	rs110964837	2:73209337	100848336	LOC100848336	HIG1 domain family member 1A, mitochondrial pseudogene
			112442723	LOC112442723	60S ribosomal protein L36 pseudogene
			112443671	LOC112443671	U4atac minor spliceosomal RNA
			112443805	TRNAE-UUC	transfer RNA glutamic acid (anticodon UUC)
			523441	CLASP1	cytoplasmic linker associated protein 1
			509598	NIFK	nucleolar protein interacting with the FHA domain of MKI67
			518436	TFCP2L1	transcription factor CP2 like 1
			509943	TSN	translin
	rs41594758	3:43031873	104971615	LOC104971615	high mobility group protein B1 pseudogene
			107132302	LOC107132302	60S ribosomal protein L23a pseudogene
			112446155	LOC112446155	U5 spliceosomal RNA
			531899	LOC531899	AT-rich interactive domain-containing protein 3A pseudogene
			614777	CDC14A	cell division cycle 14A
			516584	MFSD14A	major facilitator superfamily domain containing 14A
			616488	LRRC39	leucine rich repeat containing 39
			280759	DBT	dihydrolipoamide branched chain transacylase E2
			510469	RTCA	RNA 3'-terminal phosphate cyclase
			504467	SASS6	SAS-6 centriolar assembly protein
			614311	TRMT13	tRNA methyltransferase 13 homolog
			520918	SLC35A3	solute carrier family 35 member A3
	rs109928164	5:70049126	539347	TMEM263	transmembrane protein 263
			782988	MTERF2	mitochondrial transcription termination factor 2
			540839	POLR3B	RNA polymerase III subunit B
			535947	CRY1	cryptochrome circadian regulator 1
			533900	RFX4	regulatory factor X4

			522873	RIC8B	RIC8 guanine nucleotide exchange factor B
			112446970	TRNAY-GUA	transfer RNA tyrosine (anticodon GUA)
			107132502	LOC107132502	40S ribosomal protein S8-like
	rs41591913	5:84578337	104972526	LOC104972526	uncharacterized LOC104972526
			783110	ETFRF1	electron transfer flavoprotein regulatory factor 1
			541140	KRAS	KRAS proto-oncogene, GTPase
			518130	LMNTD1	lamin tail domain containing 1
			526444	CASC1	cancer susceptibility 1
	rs41666416	10:68089016	615096	TBPL2	TATA-box binding protein like 2
			538831	ATG14	autophagy related 14
			537619	KTN1	kinectin 1
			112448700	TRNAS-GCU	transfer RNA serine (anticodon GCU)
			112448699	TRNAE-UUC	transfer RNA glutamic acid (anticodon UUC)
			112448676	TRNAI-UAU	transfer RNA isoleucine (anticodon UAU)
			112448598	LOC112448598	U7 small nuclear RNA
			112448564	LOC112448564	uncharacterized LOC112448564
			101903890	LOC101903890	uncharacterized LOC101903890

Table 3.11 Genes in the vicinity of significant SNP for primary abnormalities determined by a genome-wide association study.

Trait	rsID	Chr:Pos	GeneID	Symbol	description
Primary Abnormalities	rs41566683	1:36903496	537951	EPHA3	EPH receptor A3
	rs41255529	1:60956897	112448483	TRNAK-UUU	transfer RNA lysine (anticodon UUU)
			100336456	LSAMP	limbic system associated membrane protein
	rs110487590	1:63799085	282113	UPK1B	uroplakin 1B
			100139661	TEX55	testis expressed 55
			540003	IGSF11	immunoglobulin superfamily member 11
			511328	B4GALT4	beta-1,4-galactosyltransferase 4
			101901963	LOC101901963	uncharacterized LOC101901963
			101906646	LOC101906646	ubiquitin specific peptidase 40
	rs42940192	4:10648384	112446356	LOC112446356	uncharacterized LOC112446356
			112446530	LOC112446530	U6 spliceosomal RNA
			613317	CALCR	calcitonin receptor
			513430	HEPACAM2	HEPACAM family member 2
			100498808	MIR378-2	microRNA mir-378-2
			100313272	MIR489	microRNA mir-489
			100313075	MIR653	microRNA mir-653
			514205	SAMD9	sterile alpha motif domain containing 9
			535248	VPS50	VPS50 subunit of EARP/GARPII complex
	rs110425782	20:68324872	112443018	LOC112443018	uncharacterized LOC112443018
			112443017	LOC112443017	uncharacterized LOC112443017
			101902976	LOC101902976	sperm mitochondrial-associated cysteine-rich protein-like
	rs41646489	22:33690494	537713	SUCLG2	succinate-CoA ligase GDP-forming beta subunit
			782935	TAFA1	TAFA chemokine like family member 1

Table 3.12 Genes in the vicinity of significant SNP for secondary abnormalities determined by a genome-wide association study.

Trait	rsID	Chr:Pos	GeneID	Symbol	description
Secondary Abnormalities	rs41646489	22:33690494	782935	TAFA1	TAFA chemokine like family member 1
			537713	SUCLG2	succinate-CoA ligase GDP-forming beta subunit

Table 3.13 Significant SNP with putative candidate genes for semen production traits.

Trait	SNP	Chromosome: Pos	Closest gene	Biological Process	Distance from gene (bp)	Relative Location of SNP to gene
Volume	rs109268478	6:102859342	MAN2B2	Contributes to carbohydrates metabolism integral for epididymal physiology	182,601	Downstream
Initial Motility	rs109798673	2:827626	HERC4	Caused reduced spermatozoa motility	0	Within
Initial Motility	rs109798673	2:827626	OCA2	Contributes to spermatid development	122,628	Downstream
Post-thaw Motility	rs41623436	1:152412536	COLQ	Enhancer of capacitation and necessary for acrosome reaction	69,395	Upstream
Post-thaw Motility	rs109798673	2:827626	HERC4	Caused reduced spermatozoa motility	0	Within
Post-thaw Motility	rs109798673	2:827626	OCA2	Contributes to spermatid development	122,628	Downstream
Post-thaw Motility	rs109719529	17:56344620	HSPB8	Affects maturation of spermatozoa	174,652	Downstream

Three-hour post-thaw motility	rs109719529	17:56344620	HSPB8	Affects maturation of spermatozoa	174,652	Downstream
Percentage of Normal Spermatozoa	rs41594758	3:43031873	MFSD14A	Necessity for spermatid nucleus differentiation and sperm mitochondrion organization	150,326	Upstream
Primary Abnormalities	rs110487590	1:63799085	TEX55	Lack of expression found in cryptorchid men	182,394	Upstream
Primary abnormalities	rs110425782	20:68324872	LOC101902976	Necessity for sperm motility	133,920	Downstream

Figure 3.1 Manhattan plot showing the result of genome-wide association mapping for volume with a significance threshold of 4.0.

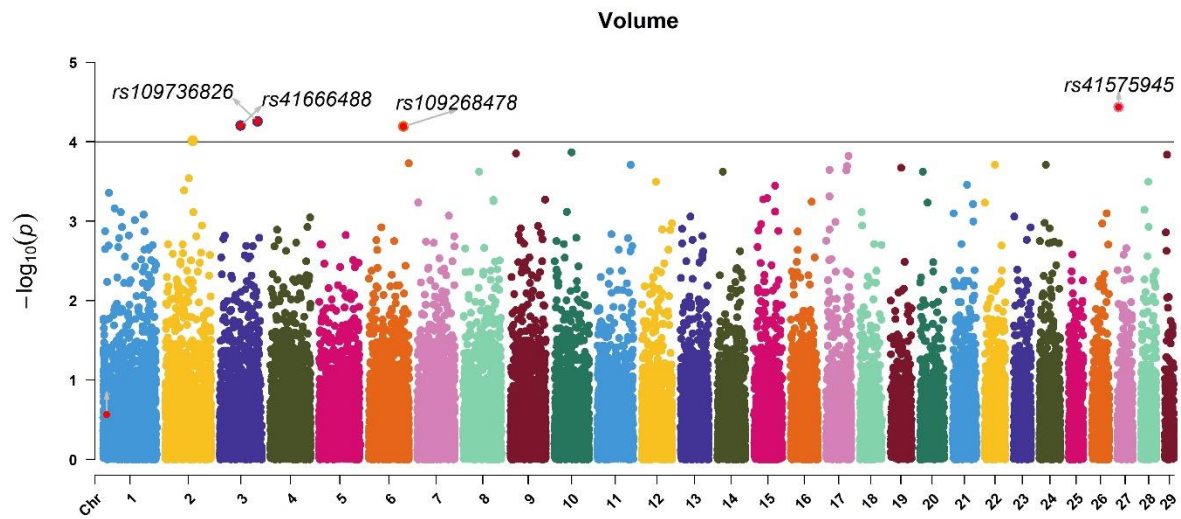


Figure 3.2 Manhattan plot showing the result of genome-wide association mapping for concentration with a significance threshold of 4.0.

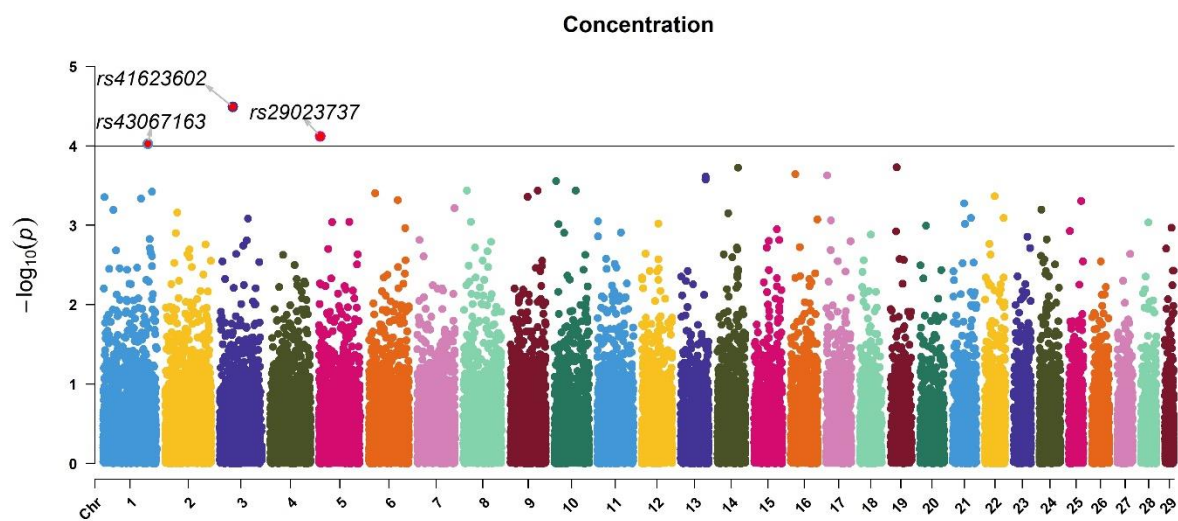


Figure 3.3 Manhattan plot showing the result of genome-wide association mapping for number of spermatozoa with a significance threshold of 4.0.

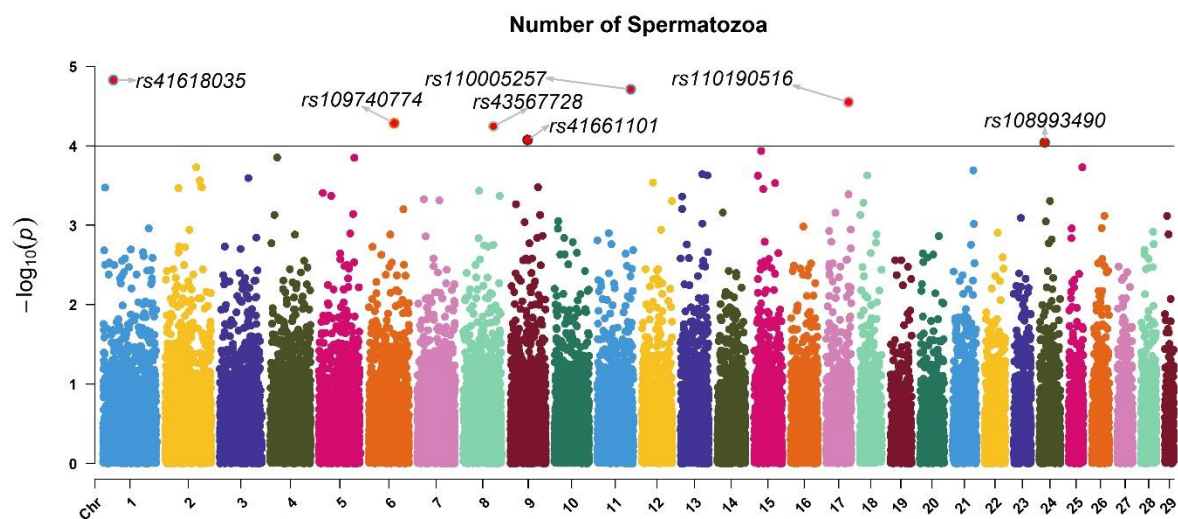


Figure 3.4 Manhattan plot showing the result of genome-wide association mapping for initial motility with a significance threshold of 4.0. A bivariate analysis was performed, thus significant SNP from both bivariate analyses are reported.

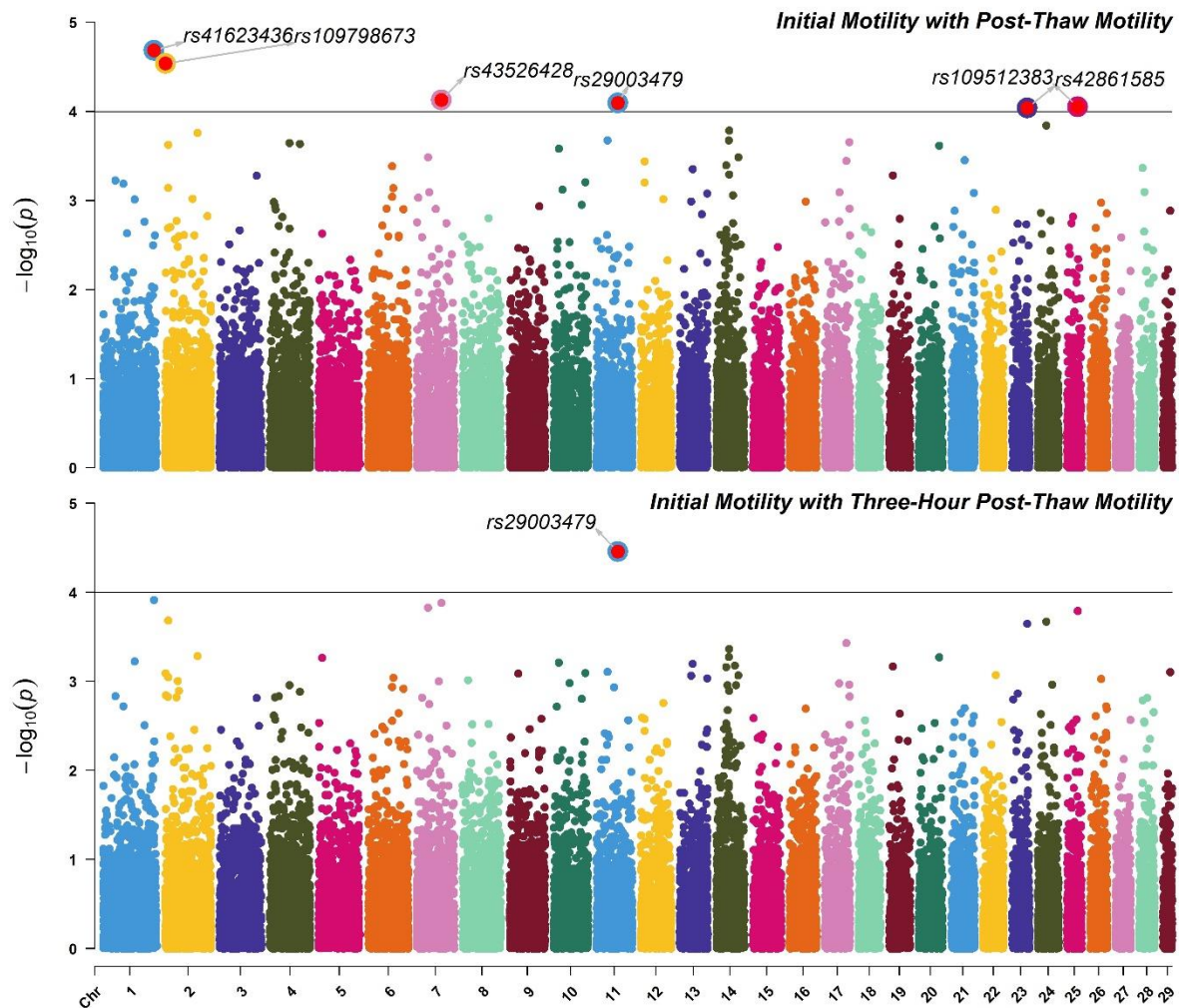


Figure 3.5 Manhattan plot showing the result of genome-wide association mapping for post-thaw motility with a significance threshold of 4.0. A bivariate analysis was performed, thus significant SNP from both bivariate analyses are reported.

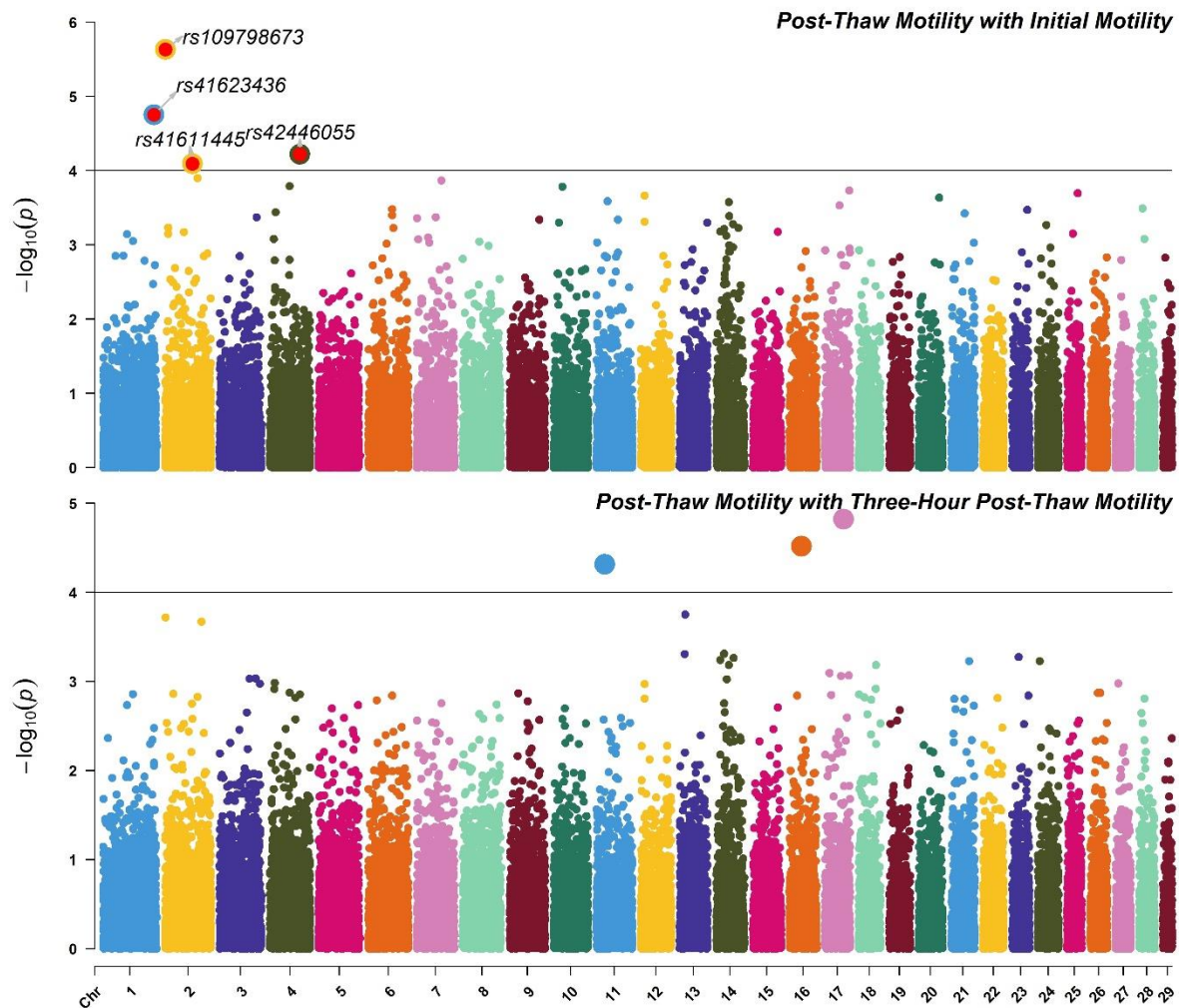


Figure 3.6 Manhattan plot showing the result of genome-wide association mapping for three-hour post-thaw motility with a significance threshold of 4.0. A bivariate analysis was performed, thus significant SNP from both bivariate analyses are reported.

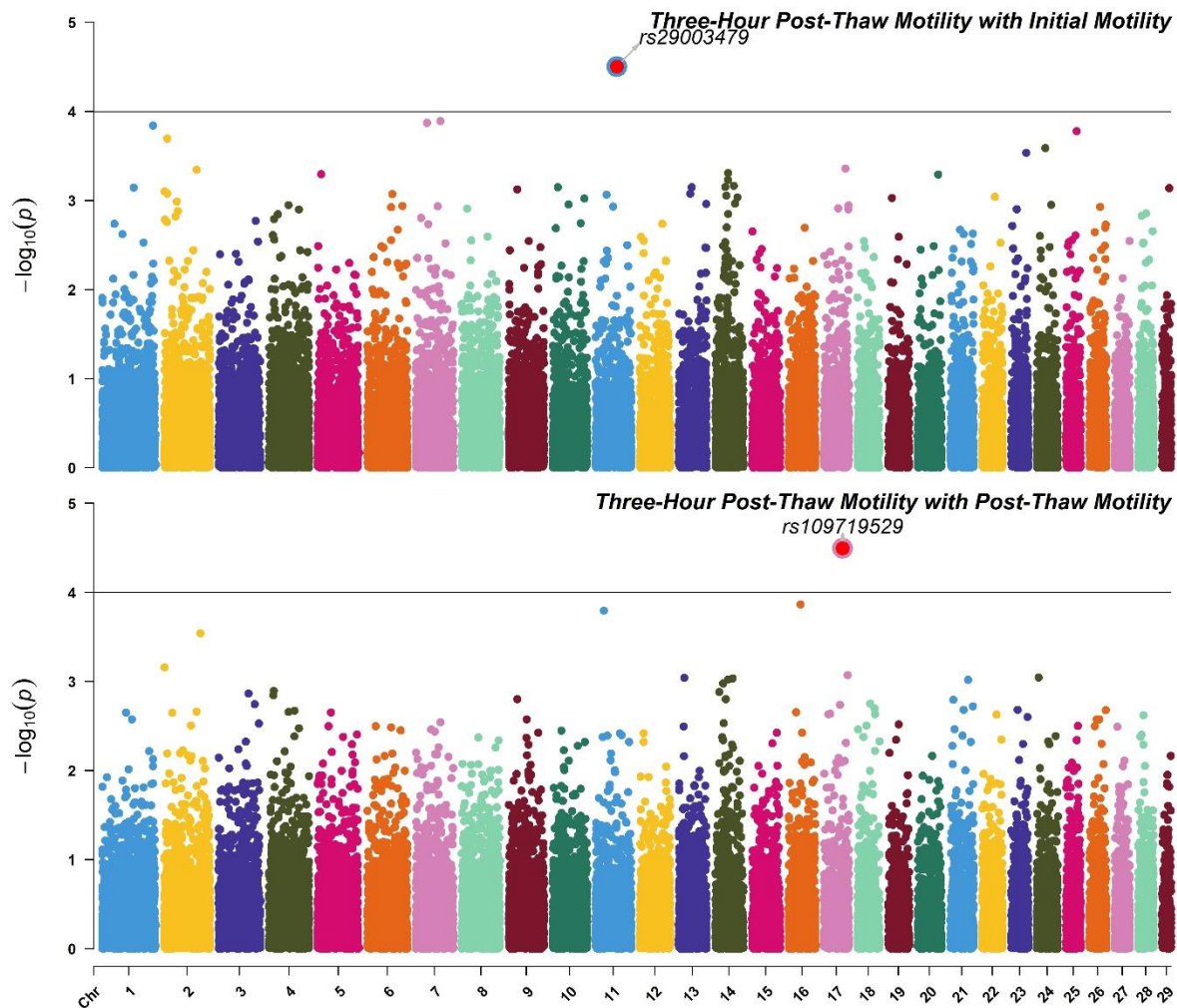


Figure 3.7 Manhattan plot showing the result of genome-wide association mapping for percentage of normal spermatozoa with a significance threshold of 4.0. A bivariate analysis is performed, thus significant SNP from both bivariate analyses are reported.

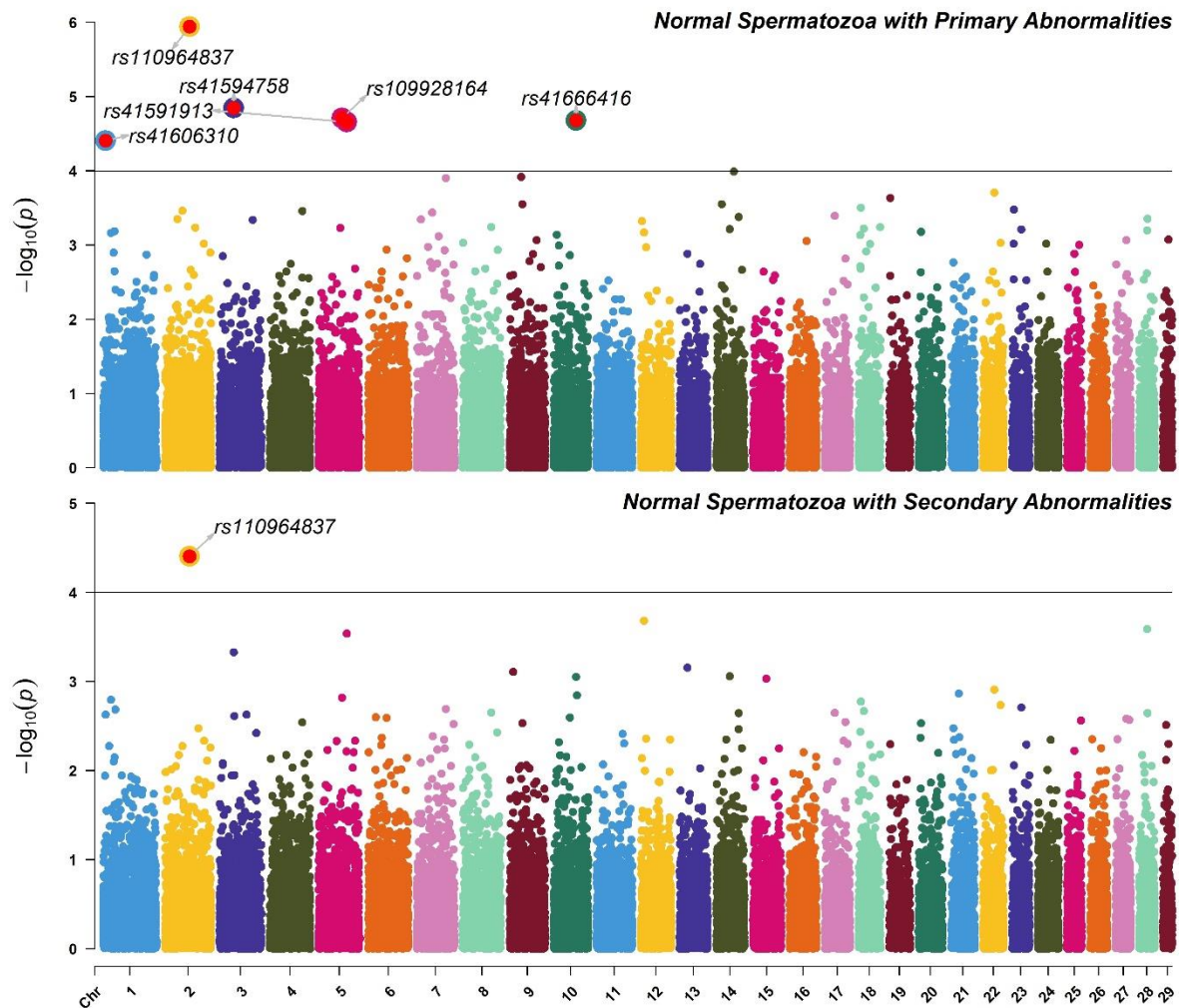


Figure 3.8 Manhattan plot showing the result of genome-wide association mapping for primary abnormalities with a significance threshold of 4.0. A bivariate analysis was performed, thus significant SNP from both bivariate analyses are reported.

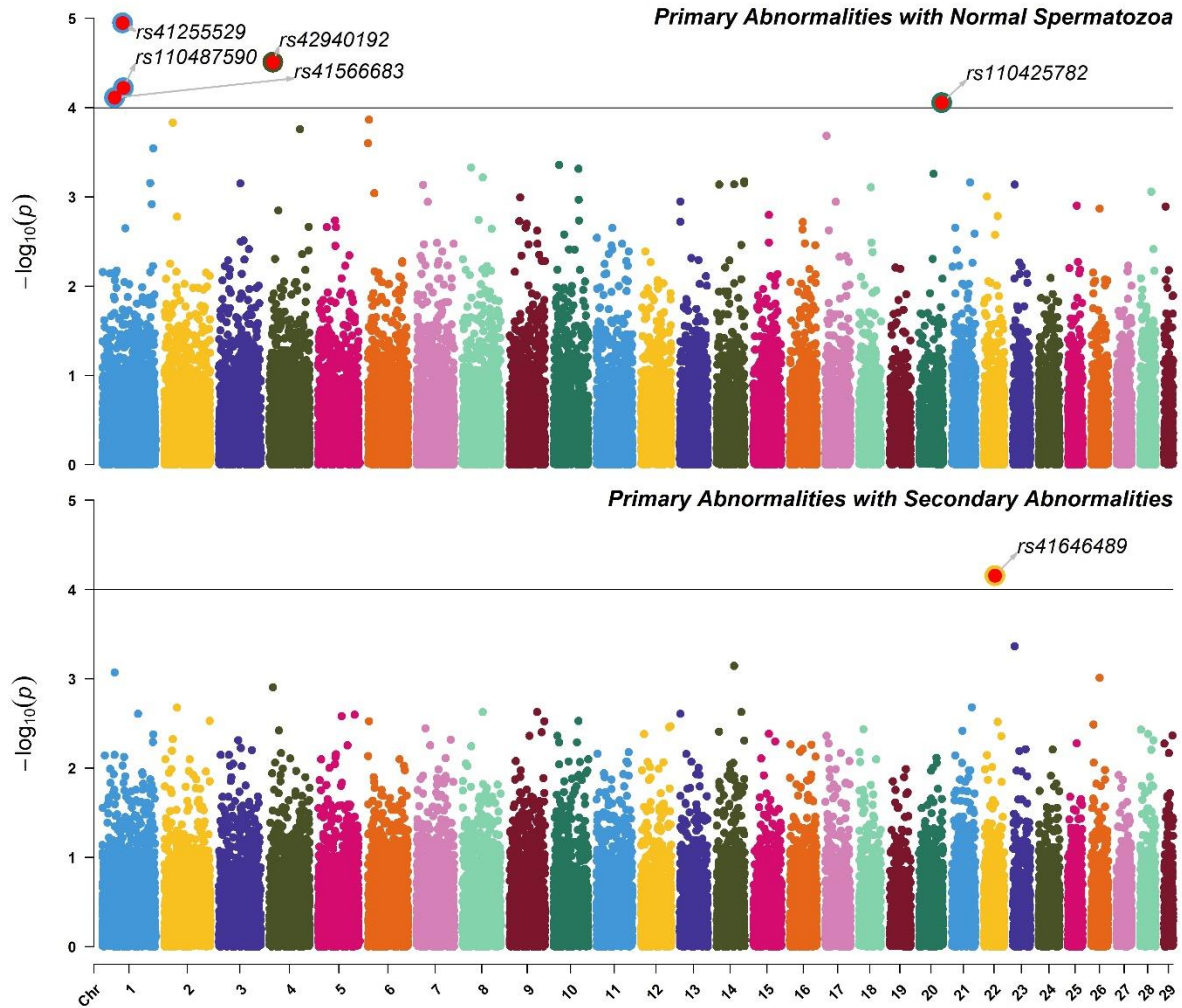
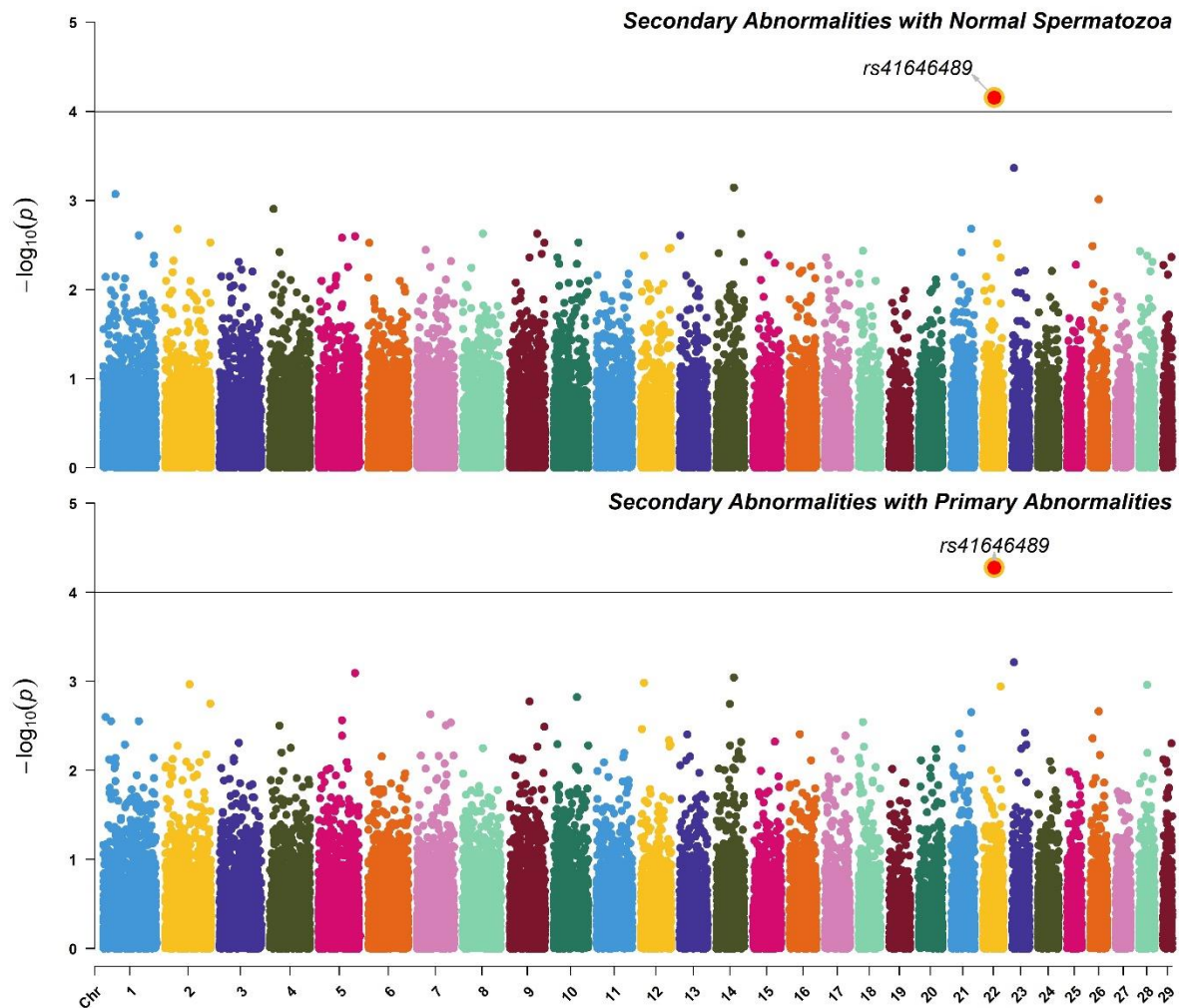


Figure 3.9 Manhattan plot showing the result of genome-wide association mapping for secondary abnormalities with a significance threshold of 4.0. A bivariate analysis was performed, thus significant SNP from both bivariate analyses are reported.



Literature Cited

- Abe T, J. Saburi, H. Hasebe, T. Nakagawa, T. Kawamura, K. Saito, T. Nade, S. Misumi, T. Okumura, K. Kuchida, T. Hayashi, S. Nakane, T. Mitsuhasi, K. Nirasawa, Y. Sugimoto, and E. Kobayashi. 2008. Bovine quantitative trait loci analysis for growth, carcass, and meat quality traits in an F2 population from a cross between Japanese Black and Limousin. *J. Anim. Sci.* 86: 2821-2831. doi: [10.2527/jas.2007-0676](https://doi.org/10.2527/jas.2007-0676)
- Alexander, L. J., T. W. Geary, W. M. Snelling, and M. D. Macneil, 2007. Quantitative trait loci with additive effects on growth and carcass traits in a Wagyu-Limousin F2 population. *Animal Genetics*. 38 (4): 413-416. doi: [10.1111/j.1365-2052.2007.01616.x](https://doi.org/10.1111/j.1365-2052.2007.01616.x)
- Ashwell, M. S., D. W. Heyen, J. I. Weller, M. Ron, T. S. Sonstegard, C. P. Van Tassell, and H. A. Lewin, 2005. Detection of quantitative trait loci influencing conformation traits and calving ease in Holstein-Friesian cattle. *Journal of Dairy Science*. 88 (11):4111-4119. doi: [10.3168/jds.S0022-0302\(05\)73095-2](https://doi.org/10.3168/jds.S0022-0302(05)73095-2).
- Awda, B. J., S. P. Miller, Y. R. Montanholi, G. V. Voort, T. Caldwell, M. M. Buhr, and K. C. Swanson. 2013. The relationship between feed efficiency traits and fertility in young beef bulls. *Can. J. Anim. Sci.* 93:185-192. doi: 10.4141/CJAS2012-092
- Ben Jemaa, S., S. Fritz, F. Guillaume, T. Druet, C. Denis, A. Eggen, M. Gautier, 2008. Detection of quantitative trait loci affecting non-return rate in French dairy cattle. *Journal of Animal Breeding and Genetics*. 125 (4): 280-288. doi: 10.1111/j.1439-0388.2008.00744.x.
- Blaschek, M., A. Kaya, N. Zwald, E. Memili, and B. W. Kirkpatrick, 2011. A whole-genome association analysis of noncompensatory fertility in Holstein bulls. *Journal of Dairy Science*. 94 (9):4695-4699. doi: [10.3168/jds.2010-3728](https://doi.org/10.3168/jds.2010-3728)
- Boichard, D., C. Grohs, F. Bourgeois, F. Cerqueira, R. Faugeras, A. Neau, R. Rupp, Y. Amigues, M. Y. Boscher, H. Leveziel, 2003. Detection of genes influencing economic traits in three French dairy cattle breeds. *Genetics*. 1 (35): 77-101. doi: [10.1186/1297-9686-35-1-77](https://doi.org/10.1186/1297-9686-35-1-77).

- Bolormaa, S., L. R. Neto, Y. D. Zhang, R. J. Bunch, B. E. Harrison, M. E. Goddard, and W. Barendse, 2011. A genome-wide association study of meat and carcass traits in Australian cattle. *Journal of Animal Science*. 89(8):2297-309. doi: [10.2527/jas.2010-3138](https://doi.org/10.2527/jas.2010-3138).
- Bouwman, A. C., M. H. Visker, J. A. van Arendonk, and H. Bovenhuis. 2012. Genomic regions associated with bovine milk fatty acids in both summer and winter milk samples. *BMC genetics*. 13: 93.
- Brito, L. F., A. D. Barth, N. C. Rawlings, R. E. Wilde, D. H. Crews Jr, P. S. Mir, and J. P. Kastelic. 2007. Effect of improved nutrition during calthood on serum metabolic hormones, gonadotropins, and testosterone concentrations, and on testicular development in bulls. *Dom. Amin. Endo*. 33: 460-469. doi: [10.1016/j.domaniend.2006.09.004](https://doi.org/10.1016/j.domaniend.2006.09.004).
- Byrne, C. J., S. Fair, A. M. English, M. Cirot, C. Staub, P. Lonergan, and D. A. Kenny. 2018. Plane of nutrition before and after 6 months of age in Holstein-Friesian bulls: I. Effects on performance, body composition, age at puberty, and postpubertal semen production. *J Dairy. Sci*. 101: 3447-3459. doi: [10.3168/jds.2017-13719](https://doi.org/10.3168/jds.2017-13719).
- Cai, Z., B. Guldbrandtsen, M. S. Lund, and G. Sahana. 2019. Prioritizing candidate genes for fertility in dairy cows using gene-based analysis, functional annotation and differential gene expression. *BMC Genomics*. 20:255. doi: [s12864-019-5638-9](https://doi.org/10.1186/s12864-019-5638-9)
- Casas, E., J. W. Keele, S. D. Shackelford, M. Koohmaraie, and R. T. Stone, 2003. Identification of quantitative trait loci for growth and carcass composition in cattle. *Animal Genetics*. 35: 2-6. doi: [10.1046/j.1365-2052.2003.01067.x](https://doi.org/10.1046/j.1365-2052.2003.01067.x)
- Casas, E., J. W. Keele, S. D. Shackelford, M. Koohmaraie, T. S. Sonstegard, T. P. L. Smith, S. M. Kappes, and R. T. Stone, 1998. Association of the muscle hypertrophy locus with

- carcass traits in beef cattle. *Journal of Animal Science*. 76: 468-473. doi: [10.2527/1998.762468x](https://doi.org/10.2527/1998.762468x).
- Casas, E., R. T. Stone, J. W. Keele, S. D. Shackelford, S. M. Kappes, and M. Koohmaraie, 2001. A comprehensive search for quantitative trait loci affecting growth and carcass composition of cattle segregating alternative forms of the myostatin gene. *Journal of Animal Science*. 79(4):854-860. doi: [10.2527/2001.794854x](https://doi.org/10.2527/2001.794854x).
- Casas, E., S. D. Shackelford, J. W. Keele, M. Koohmaraie, T. P. L. Smith, and R. T. Stone, 2003. Detection of Quantitative Trait Loci for Growth and Carcass Composition in Cattle. *Journal of Animal Science*. 81: 2976-2983. doi: [10.2527/2003.81122976x](https://doi.org/10.2527/2003.81122976x)
- Cesar A. S., L. C. Regitano, G. B. Mourao, R. R. Tullio, D. P. Lanna, R. T. Nassu, M. A. Mudado, P. S. Oliveira, M. L. do Nascimento, A. S. Chaves, M. M. Alencar, T. S. Sonstegard, D. J. Garrick, J. M. Reecy, and L. L. Coutinho. 2014. Genome-wide association study for intramuscular fat deposition and composition in Nellore cattle. *BMC genetics*. 15: 39.
- Christmas R. A., D.W. Moser, M.F. Spire, J.M. Sargeant, and S.K. Tucker. Genetic relationships among breeding soundness traits in yearling bulls. In: *Cattlemen's Day*. 2001. Manhattan, KS. Pp. 1-3.
- Cole, J. B., G. R. Wiggans, L. Ma, T. S. Sonstegard, T. J. Lawlor Jr, B. A. Crooker, C. P. Van Tassell, J. Yang, S. Wang, L. K. Matukumalli, and Y. Da, 2011. Genome-wide association analysis of thirty one production, health, reproduction and body conformation traits in contemporary U.S. Holstein cows. *BMC Genomics*. 12:408. doi: 10.1186/1471-2164-12-408.
- Corbet, N.J., B.M. Burns, D.J. Johnston, M.L. Wolcott, D.H. Corbet, B.K. Venus, Y. Li, M.R. McGowan, and R.G. Holroyd. 2013. Male traits and herd reproductive capability in tropical beef cattle. 2. Genetic parameters of bull traits. *Anim. Prod. Sci*. 53: 101-113.

- Costa A, H. Schwarzenbacher, G. Mészáros, B. Fuerst-Waltl, C. Fuerst, J. Sölkner, M. Penasa. 2019. On the genomic regions associated with milk lactose in Fleckvieh cattle. *J Dairy Sci.* doi: [10.3168/jds.2019-16663](https://doi.org/10.3168/jds.2019-16663).
- Dance, A., J. Thundathil, R. Wilde, P. Blondin, and J. Kastelic. 2015. Enhanced early-life nutrition promotes hormone production and reproductive development in Holstein bulls. *J Dairy Sci.* 98: 987-998. doi: [10.3168/jds.2014-8564](https://doi.org/10.3168/jds.2014-8564).
- Doran, J., C. Walters, V. Kyle, P. Wooding, R. Hammett-Burke, and B. Colledge. 2016. Mfsd14a (Hiat1) gene disruption causes globozoospermia and infertility in male mice. *Repro.* 152: 91-99. doi: 10.1530/REP-15-0557.
- Doran A. G., D. P. Berry, and C. J. Creevey, 2014. Whole genome association study identifies regions of the bovine genome and biological pathways involved in carcass trait performance in Holstein-Friesian cattle. *BMC Genomics.* 15: 837. doi: [10.1186/1471-2164-15-837](https://doi.org/10.1186/1471-2164-15-837)
- Druet, T., S. Fritz, E. Sellem, B. Basso, O. Gerard, L. Salas-Cortes, P. Humblot, X. Druart, and A. Eggen, 2009. Estimation of genetic parameters and genome scan for 15 semen characteristics traits of Holstein bulls. *Journal of Animal Breeding and Genetics.* 126 (4): 269-277. doi: 10.1111/j.1439-0388.2008.00788.x.
- Frischknecht, M., B. Bapst, F. R. Seefried, H. Signer-Hasler, D. Garrick, C. Stricker, Intergenomics Consortium, R. Fries, I. Russ, J. Sölkner, A. Bieber, M. G. Strillacci, B. Gredler-Grandl, and C. Flury, 2017. Genome-wide association studies of fertility and calving traits in Brown Swiss cattle using imputed whole-genome sequences. *BMC Genomics.* 18 (1): 910. doi: 10.1186/s12864-017-4308-z.
- Garmyn, A.J., D. W. Moser, R.A Christmas, J.M. Bormann. 2011. Estimate of genetic parameters and effects of cytoplasmic line on scrotal circumference and semen quality traits in Angus bulls. *J. Anim. Sci.* 89: 693-698.
- Grozs, M. D. and M. D. MacNeil. 2001. Putative quantitative trait locus affecting birth weight on bovine chromosome two. *J. Anim. Sci.* 79: 68-72. doi: 10.2527/2001.79168x

- Gutierrez-Gil, B., J. L. Williams, D. Homer, D. Burton, C. S. Haley, and P. Wiener, 2009. Search for quantitative trait loci affecting growth and carcass traits in a cross population of beef and dairy cattle. *Journal of Animal Science*. 87 (1): 24-36. doi: [10.2527/jas.2008-0922](https://doi.org/10.2527/jas.2008-0922)
- Handrow, R. R., R. W. Lenz, and R. L. Ax. 1982. Structural comparisons among glycosaminoglycans to promote an acrosome reaction in bovine spermatozoa. *Biochem. Biophys. Res. Commun.* 107: 1326. doi: [10.1016/S0006-291X\(82\)80143-5](https://doi.org/10.1016/S0006-291X(82)80143-5)
- Hansen, M. 1979. Genetic investigations on male and female fertility in cattle. *Livest. Sci.* 6:325-334.
- Harstine, B. R., M. Maquivar, L. A. Helser, M. D. Utt, C. Premanandan, J. M. DeJarnette, and M. L. Day. 2015. Effects of dietary energy on sexual maturation and sperm production in Holstein bulls. *J Anim. Sci.* 93: 2759-2766. Doi: [10.2527/jas.2015-8952](https://doi.org/10.2527/jas.2015-8952)
- Hawthorne, S. K., G. Goodarzi, J. Bagarova, K. E. Gallant, R. R. Busanelli, W. J. Olend, and K. C. Kleene. 2006. Comparative genomics of the sperm mitochondria-associated cysteine-rich protein gene. *Genomics*. 87: 382-391. doi: [10.1016/j.ygeno.2005.09.010](https://doi.org/10.1016/j.ygeno.2005.09.010).
- Hering, D. M., K. Olenski, and S. Kaminski, 2014. Genome-wide association study for poor sperm motility in Holstein-Friesian bulls. *Animal Reproduction Science*. 146 (3-4): 89-97. doi: [10.1016/j.anireprosci.2014.01.012](https://doi.org/10.1016/j.anireprosci.2014.01.012)
- Hoglund, J. K., A. J. Buitenhuis, B. Guldbrandtsen, G. Su, B. Thomsen, and M. S. Lund, 2009. Overlapping chromosomal regions for fertility traits and production traits in the Danish Holstein population. *Journal of Dairy Science*. 92 (11): 5712-5719. doi: [10.3168/jds.2008-1964](https://doi.org/10.3168/jds.2008-1964).
- Hoskins, D. D., D. Munsterman, and M. L. Hall. 1975. The control of bovine spermatozoa glycolysis during epididymal transit. *Biol. Reprod.* 12: 566-572. doi: [10.1095/biolreprod12.5.566](https://doi.org/10.1095/biolreprod12.5.566)

- Hou, G.Y., Z. R. Yuan, X. Gao, L. Y. Li, H. J. Gao, J. B. Chen, and S. Z. Xu, 2010. Genetic polymorphisms of the CACNA2D1 gene and their association with carcass and meat quality traits in cattle. *Biochemical Genetics*. 48 (9-10): 751-759. doi: [10.1007/s10528-010-9357-9](https://doi.org/10.1007/s10528-010-9357-9)
- Hu, Z., C. A. Park, and J. M. Reecey. 2019. Building a livestock genetic and genomic information knowledgebase through integrative developments of Animal ATLdb and CorrDB. *Nucleic Acids Res*. 47: D701-D710. doi: doi.org/10.1093/nar/gky1084.
- Huang, D. W., B. T. Sherman, and R. A. Lempicki. 2009. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nature*. 4: 44-57. Doi: 10.1038/nprot.2008.211.
- Huang, D. W., B. T. Sherman, and R. A. Lempicki. 2009. Bioinformatics enrichment tools:paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids. Res*. 37:1-13. Doi: [10.1093/nar/gkn923](https://doi.org/10.1093/nar/gkn923)
- Huang, W., B. W. Kirkpatrick, G. J. Rosa, and H. Khatib, 2010. A genome-wide association study using selective DNA pooling identifies candidate markers for fertility in Holstein cattle. *Animal Genetics*. 41 (6):570-578. doi: 10.1111/j.1365-2052.2010.02046.x.
- Huszar, G., P. Patrizio, L. Vigue, M. Willets, C. Wilker, D. Adhoot, and L. Johnson. 1998. Cytoplasmic extrusion and the switch from creatine kinase B to M isoform are completed by commencement of epididymal transport in human and stallion spermatozoa. *J. Androl*. 19:11-20. doi: [10.1002/j.1939-4640.1998.tb02465.x](https://doi.org/10.1002/j.1939-4640.1998.tb02465.x)
- Ibeagha-Awemu, E. M., S. O. Peters, K. A. Akwanji, I. G. Imumorin, and X. Zhao. 2016. High density genome wide genotyping-by-sequencing and association identifies common and low frequency SNPs, and novel candidate genes influencing cow milk traits. *Scientific reports*. 6:31109.
- Imumorin, I. G., E. H. Kim, Y. M. Lee, D. J. De Koning, J. A. van Arendonk, M. De Donato, J. F. Taylor, and J. J. Kim, 2011. Genome scan for parent-of-origin QTL effects on bovine growth and carcass traits. *Frontiers in Genetics*. 2:44. doi: [10.3389/fgene.2011.00044](https://doi.org/10.3389/fgene.2011.00044)
- Jiang, J., L. Ma, D. Prakapenka, P. M. VanRaden, J. B. Cole, and Y. Da, 2019. A Large-Scale Genome-Wide Association Study in U.S. Holstein Cattle. *Frontiers in Genetics*. 10: 412. doi: [10.3389/fgene.2019.00412](https://doi.org/10.3389/fgene.2019.00412)

- Kim, J. J., F. Farnir, J. Savell, and J. F. Taylor, 2003. Detection of Quantitative Trait Loci for Growth and Beef Carcass Fatness Traits in a Cross Between *Bos taurus* (Angus) and *Bos indicus* (Brahman) Cattle. *Journal of Animal Science*. 81: 1933-1942. doi: [10.2527/2003.8181933x](https://doi.org/10.2527/2003.8181933x)
- Kiser, J. N., E. Clancey, J. G. N. Moraes, J. Dalton, G. W. Burns, T. E. Spencer, and H. L. Neibergs, 2019. Identification of loci associated with conception rate in primiparous Holstein cows. *BMC Genomics*. 20(1): 840. doi: 10.1186/s12864-019-6203-2.
- Kiser, J. N., E. M. Keuter, C. M. Seabury, M. Neupane, J. G. N. Moraes, J. Dalton, G. W. Burns, T. E. Spencer, and H. L. Neibergs, 2019. Validation of 46 loci associated with female fertility traits in cattle. *BMC Genomics*. 20 (1): 576. doi: 10.1186/s12864-019-5935-3.
- Komatsu, M., T. Itoh, Y. Fujimori, M. Satoh, Y. Miyazaki, H. Takahashi, K. Shimizu, A. E. Malau-Aduli, and M. Morita, 2011. Genetic association between GHSR1a 5'UTR-microsatellite and nt-7(C>A) loci and growth and carcass traits in Japanese Black cattle. *Journal of Animal Science*. 82(3):396-405. doi: [10.1111/j.1740-0929.2010.00852.x](https://doi.org/10.1111/j.1740-0929.2010.00852.x)
- Lane, M. E., I. Therien, R. Moreau, and P. Manjunath. 1999. Heparin and high density lipoprotein mediate bovine sperm capacitation by different mechanisms. *Biol. Reprod*. 60:169-175. doi: [10.1095/biolreprod60.1.169](https://doi.org/10.1095/biolreprod60.1.169)
- Li, C. J. Basarb, W. M. Snelling, B. Benkel, B. Murdoch, and S. S. Moore. 2002. The identification of common haplotypes on bovine chromosome 5 withing commercial lines of *Bos Taurus* and their associations with growth trait. *J. Anim Sci*. 5: 1187-1194. doi: 10.2527/2002.8051187x
- Li, Y., Y. Gao, Y. S. Kim, A. Iqbal, and J. J. Kim, 2017. A whole genome association study to detect additive and dominant single nucleotide polymorphisms for growth and carcass traits in Korean native cattle, Hanwoo. *Asian-Australasian Journal of Animal Sciences*. 30(1): 8-19. doi: [10.5713/ajas.16.0170](https://doi.org/10.5713/ajas.16.0170)
- Lu, D., S. Miller, M. Sargolzaei, M. Kelly, G. Vander Voort, T. Caldwell, Z. Wang, G. Plastow, and S. Moore, 2013. Genome-wide association analyses for growth and feed

- efficiency traits in beef cattle. *Journal of Animal Science*. 91 (8): 3612-3633. doi: [10.2527/jas.2012-5716](https://doi.org/10.2527/jas.2012-5716)
- Mann, T., L. E. A. Rowson, R. V. Short, and J. D. Skinner. 1967. The relationship between nutrition and androgenic activity in pubescent twin calves, and the effect of orchitis. *Journal of Endo* 38: 455-NP. doi: 10.1677/joe.0.0380455.
- Mateescu, R.G., D. J. Garrick, and J. M. Reecy, 2017. Network analysis reveals putative genes affecting meat quality in angus cattle. *Frontiers in Genetics*. 8: 171. doi: [10.3389/fgene.2017.00171](https://doi.org/10.3389/fgene.2017.00171)
- McClure M. C., H. R. Ramey, M. M. Rolf, S. D. McKay, J. E. Decker, R. H. Chapple, J. W. Kim, T. M. Taxis, R. L. Weaber, R. D. Schnabel, J. F. Taylor. 2012. Genome-wide association analysis for quantitative trait loci influencing Warner-Bratzler shear force in five taurine cattle breeds. *Animal genetics*. 43: 662-73. doi: [10.1111/j.1365-2052.2012.02323.x](https://doi.org/10.1111/j.1365-2052.2012.02323.x).
- McClure, M. C., N. S. Morsci, R. D. Schnabel, J. W. Kim, P. Yao, M. M. Rolf, S. D. McKay, S. J. Gregg, R. H. Chapple, S. L. Northcutt, and J. F. Taylor. 2010. A genome scan for quantitative trait loci influencing carcass, post-natal growth and reproductive traits in commercial Angus cattle. *Animal Genetics*. 41 (6): 597-607. doi: 10.1111/j.1365-2052.2010.02063.x.
- McKay, S. D., R. D. Schnabel, B. M. Murdoch, L. K. Matukumalli, J. Aerts, W. Coppieters, D. Crews, E. Dias Neto, C. A. Gill, C. Gao, H. Mannen, P. Stothard, Z. Wang, C. P. Van Tassell, J. L. Williams, J. F. Taylor, and S. S. Moore. 2007. Whole genome linkage disequilibrium maps in cattle. *BMC Genetics*. 8(1): 74.
- Melo, T. P., M. R. S. Fortes, T. Bresolin, L. F. M. Mota, L. G. Albuquerque, and R. Carvalheiro, 2018. Multitrait meta-analysis identified genomic regions associated with sexual

- precocity in tropical beef cattle. *Journal of Animal Science*. 96(10): 4087-4099. doi: 10.1093/jas/sky289.
- Meyer, K, K Hammond, M.J. Mackinnon, P.F. Parnell. 1991. Estimates of covariances between reproduction and growth in Australian beef cattle. *J. Anim. Sci.* 69: 3533-3543.
- Michenet, A. M. Barbat, R. Saintilan, E. Venot, and F. Phocas. 2016. Detection of quantitative trait loci for maternal traits using high-density genotypes of Blonde d'Aquitaine beef cattle. *BMC Genomics*. 17:88. Doi: 10.1186/s12863-016-0397-y
- Misztal, I., S. Tsuruta, D. A. L. Laurencio, Y. Masuda, I. Aguilar, A. Legarra, Z. Vitezica. 2016. Manual for BLUPF90 family programs. University of Georgia.
<http://nce.ads.uga.edu/wiki/doku.php?id=documentation>
- Mosig, M. O., E. Lipkin, G. Khutoreskaya, E. Tchourzyna, M. Soller, and A. Friedmann, 2001. A whole genome scan for quantitative trait loci affecting milk protein percentage in Israeli-Holstein cattle, by means of selective milk DNA pooling in a daughter design, using an adjusted false discovery rate criterion. *Genetics*. 157(4):1683-1698.
- Mullen M. P., D. P. Berry, D. J. Howard, M. G. Diskin, C. O. Lynch, L. Giblin, D. A. Kenny, D. A. Magee, K. G. Meade, and S. M. Waters. 2011. Single nucleotide polymorphisms in the insulin-like growth factor 1 (IGF-1) gene are associated with performance in Holstein-Friesian dairy cattle. *Frontiers in Genetics*. 2:3. doi: [10.3389/fgene.2011.00003](https://doi.org/10.3389/fgene.2011.00003)
- Neves H. H.R., G. Vargas, L. F. Brito, F. S. Schenkel, L. G. Albuquerque, and R. Carneiro. 2019. Genetics and genomic analyses of testicular hypoplasia in Nelore cattle. *PloS one*. 14:e0211159. doi: [10.1371/journal.pone.0211159](https://doi.org/10.1371/journal.pone.0211159)
- Nkrumah, J. D., E. L. Sherman, C. Li, E. Marques, D. H. Crews, R. Bartusiak, B. Murdoch, Z. Wang, J. A. Basarab, and S. S. Moore, 2007. Primary genome scan to identify putative quantitative trait loci for feedlot growth rate, feed intake, and feed efficiency of beef cattle. *Journal of Animal Science*. 85 (12): 3170-3181. doi: [10.2527/jas.2007-0234](https://doi.org/10.2527/jas.2007-0234)

- Olsen, H. B., B. Heringstad, and G. Klemetsdal. 2020. Genetic correlations between body weight, daily weight gain, and semen characteristic traits in young Norwegian Red bull. *J Dairy Sci.* In press.
- Orru, L., G. F. Cifuni, E. Piasentier, M. Corazzin, S. Bovolenta, and B. Moioli, 2011. Association analyses of single nucleotide polymorphisms in the LEP and SCD1 genes on the fatty acid profile of muscle fat in Simmental bulls. *Meat Science*. 87(4):344-348. doi: [10.1016/j.meatsci.2010.11.009](https://doi.org/10.1016/j.meatsci.2010.11.009).
- Panse, G. T., S. Jayaramn, and R. A. Sheth. 1983. Shift of glycolysis as a marker of sperm maturation. *Arch. Androl.* 11:137-140. Doi: 10.3109/01485018308987472
- Peters, S. O., K. Kizilkaya, D. J. Garrick, R. L. Fernando, J. M. Reecy, R. L. Weaver, G. A. Silver, M. G. Thomas. 2012. Bayesian genome-wide association analysis of growth and yearling ultrasound measures of carcass traits in Brangus heifers. *J. Anim. Sci.* 90: 3398-3409. doi: 10.2527/jas.2011-4507.
- Pruitt, R. J., L. R. Corah, J. S. Stevenson, and G. H. Kiracofe. 1986. Effect of energy intake after weaning on the sexual development of beef bulls. II. Age at first mating, age at puberty, testosterone and scrotal circumference. *J Anim. Sci.* 63: 579-585. doi: 10.2527/jas1986.632579x.
- Pimentel, E.C., S. Bauersachs, M. Tietze, H. Simianer, J. Tetens, G. Thaller, F. Reinhardt, E. Wolf, and S. Konig, 2011. Exploration of relationships between production and fertility traits in dairy cattle via association studies of SNPs within candidate genes derived by expression profiling. *Animal Genetics*. 42 (3):251-62. doi: [10.1111/j.1365-2052.2010.02148.x](https://doi.org/10.1111/j.1365-2052.2010.02148.x).
- Ron, W., E. Feldmesser, M. Golik, I. Tager-Cohen, D. Kliger, V. Reiss, R. Domochofsky, O. Alus, E. Seroussi, E. Ezra, and J. I. Weller, 2004. A complete genome scan of the Israeli

- Holstein population for quantitative trait loci by a daughter design. *Journal of Dairy Science*. 2 (87): 476-490. doi: [10.3168/jds.S0022-0302\(04\)73187-2](https://doi.org/10.3168/jds.S0022-0302(04)73187-2).
- Saatchi, M., R. D. Schnabel, J. F. Taylor, and D. J. Garrick, 2014. Large-effect pleiotropic or closely linked QTL segregate within and across ten US cattle breeds. *BMC Genomics*. 15: 442. doi: 10.1186/1471-2164-15-442.
- Schnabel, R. D., T. S. Sonstegard, J. F. Taylor, and M. S. Ashwell, 2005. Whole-genome scan to detect QTL for milk production, conformation, fertility and functional traits in two US Holstein families. *Animal Genetics*. 36 (5):408-416. doi: [10.1111/j.1365-2052.2005.01337.x](https://doi.org/10.1111/j.1365-2052.2005.01337.x).
- Schrooten, C., M. C. A. M. Bink, and H. Bovenhuis, 2004. Whole genome scan to detect chromosomal regions affecting multiple traits in dairy cattle. *Journal of Dairy Science*. 87(10):3550-3560. doi: [10.3168/jds.S0022-0302\(04\)73492-X](https://doi.org/10.3168/jds.S0022-0302(04)73492-X).
- Schulman, N. F., G. Sahana, T. Iso-Touru, S. D. McKay, R. D. Schnabel, M. S. Lund, J. F. Taylor, J. Virta, and J. H. Vilkki, 2011. Mapping of fertility traits in Finnish Ayrshire by genome-wide association analysis. *Animal Genetics*. 42 (3): 263-269. doi: [10.1111/j.1365-2052.2010.02149.x](https://doi.org/10.1111/j.1365-2052.2010.02149.x).
- Seabury, C. M., D. L. Oldeschulte, M. Saatchi, J. E. Beever, J. E. Decker, Y. A. Halley, E. K. Bhattarai, M. Molaei, H. C. Freetly, S. L. Hansen, H. Yampara-Iquise, K. A. Johnson, M. S. Kerley, J. Kim, D. D. Loy, E. Marques, H. L. Neibergs, R. D. Schnabel, D. W. Shike, M. L. Spangler, R. L. Weaver, D. J. Garrick, and J. F. Taylor, 2017. Genome-wide association study for feed efficiency and growth traits in U.S. beef cattle. *BMC Genomics*. 18(1): 386. doi: [10.1186/s12864-017-3754-y](https://doi.org/10.1186/s12864-017-3754-y)
- Senger, P. L. 2012. Pathways to pregnancy and parturition. 3rd ed. Current Conceptions, Inc. Redmon, Oregon.
- Sherman, E. L., J. D. Nkrumah, C. Li, R. Bartusiak, B. Murdoch, and S. S. Moore, 2009. Fine mapping quantitative trait loci for feed intake and feed efficiency in beef cattle. *Journal of Animal Science*. 87 (1): 37-45. doi: [10.2527/jas.2008-0876](https://doi.org/10.2527/jas.2008-0876)

- Snelling, W. M., M. F. Allan, J. W. Keele, L. A. Kuehn, T. McDanel, T. P. Smith, T. S. Sonstegard, R. M. Thallman, and G. L. Bennett, 2010. Genome-wide association study of growth in crossbred beef cattle. *Journal of Animal Science*. 88(3):837-848. doi: [10.2527/jas.2009-2257](https://doi.org/10.2527/jas.2009-2257)
- Stone, R. T., J. W. Keele, S. D. Shackelford, S. M. Kappes, and M. Koohmaraie. 1999. A primary screen of the bovine genome for quantitative trait loci. *J. Anim. Sci.* 77: 1379-1384. Doi: [10.2527/1999.7761379x](https://doi.org/10.2527/1999.7761379x)
- Storey, B. T. 2008. Mammalian sperm metabolism: Oxygen and sugar, friend and foe. *Int. J. Dev. Bio.* 52:427-437. Doi: 10.1387/ijdb.072522bs
- Storey J. D., A. J. Bass, A. Dabney, and D. Robinson. 2020. qvalue: Q-value estimation for false discovery rate control. R package version 2.20.0, [http://github.com/jdstorey/qvalue](https://github.com/jdstorey/qvalue).
- Sun, J., Q. Jin, C. Zhang, X. Fang, C. Gu, C. Lei, J. Wang, and H. Chen, 2011. Polymorphisms in the bovine ghrelin precursor (GHRL) and Syndecan-1 (SCD1) genes that are associated with growth traits in cattle. *Molecular Biology Reports*. 38(5): 3153-3160. doi: [10.1007/s11033-010-9986-4](https://doi.org/10.1007/s11033-010-9986-4)
- Syrstad, O. 1981. Selection for fertility in the basis of AI data. *Livest. Prod Sci.* 8: 247-252.
- Taylor, J.F., R.D. Schnabel, and P. Sutovsky. 2018. Identification of genomic variants causing sperm abnormalities and reduced male fertility. *Anim. Repro. Sci.* 194: 57-62.
- Tetens, J., G. Thaller, and N. Krattenmacher, 2014. Genetic and genomic dissection of dry matter intake at different lactation stages in primiparous Holstein cows. *Journal of Dairy Science*. 97(1): 520-531. doi: [10.3168/jds.2013-7301](https://doi.org/10.3168/jds.2013-7301)
- The UniProt Consortium. 2019. **UniProt: a worldwide hub of protein knowledge**. *Nucleic Acids Res.* 47: D506-515.
- VanDemark, N. L., G. R. Fritz and R. E. Mauger. 1964. Effect of energy intake on reproductive performance of dairy bulls. I. Growth, reproductive organs, and puberty. *J. Dairy Sci.* 47:898. doi: 10.3168/jds.S0022-0302(64)88799-3
- VanDemark, N. L. and R. E. Mauger. 1964. Effect of energy intake on reproductive performance of dairy bulls. 1. Growth, reproductive organs and puberty. *J. Dairy Sci.* 47:798. doi: 10.3168/jds.S0022-0302(64)88767-1.
- Wang, H., I. Misztal, I. Aguilar, A. Legarra, and W. M. Muir. 2012. Genome-wide association mapping including phenotypes from relatives without genotypes. *Genetics Research*. 94:73-83. doi: 10.1017/S0016672312000274.

- Westfalewicz, B. M. A. Dietrich, A. Mostek, A. Partyka, W. Bielas, W. Nizanski, and C. Ciereszko. 2017. Identification and functional analysis of bull (*Bos taurus*) cauda epididymal fluid proteome. *J. Dairy. Sci.* 100:6707-6719. doi: [10.3168/jds.2016-12526](https://doi.org/10.3168/jds.2016-12526).
- Yin, L. 2020. Circular manhattan plot. R package version 3.6.0, <https://github.com/YinLiLin/R-CMplot>
- Yokouchi, K., Y. Mizoguchi, T. Watanabe, E. Iwamoto, Y. Sugimoto, and A. Takasuga, 2009. Identification of a 3.7-Mb region for a marbling QTL on bovine chromosome 4 by identical-by-descent and association analysis. *Animal Genetics*. 40(6): 945-951. doi: [10.1111/j.1365-2052.2009.01956.x](https://doi.org/10.1111/j.1365-2052.2009.01956.x)
- Yu, Z., B. Wu, A. Tang, J. Chen, X. Guo, J. Qin, Y. Gui, and Z. Cai. 2009. Expression profile of a novel germ cell-specific gene, TSCPA, in mice and human. *Journal of Huazhong University of Science and Technology [Medical Sciences]*. 29:535. doi: 10.1007/s11596-009-0502-9.
- Yuan, Z. R., and S. Z. Xu, 2011. Novel SNPs of the bovine CACNA2D1 gene and their association with carcass and meat quality traits. *Molecular Biology Reports*. 38(1): 365-370. doi: [10.1007/s11033-010-0117-z](https://doi.org/10.1007/s11033-010-0117-z)
- Xu, L., L. Yang, L. Wang, B. Zhu, Y. Chen, H. Gao, X. Gao, L. Zhang, G. E. Liu, and J. Li, 2019. Probe-based association analysis identifies several deletions associated with average daily gain in beef cattle. *BMC Genomics*. 20(1): 31. doi: [10.1186/s12864-018-5403-5](https://doi.org/10.1186/s12864-018-5403-5)
- Zhang, Z., M. Kargo, A. Liu, J. R. Thomasen, Y. Pan, and G. Su. 2019. Genotype-by-environment interaction of fertility traits in Danish Holstein cattle using a single-step genomic reaction norm model. *Heredity*. 123:202-214. doi: 10.1038/s41437-019-0192-4.
- Zhou, Y., E. E. Connor, G. R. Wiggans, Y. Lu, R. J. Tempelman, S. G. Schroeder, H. Chen, and G. E. Liu, 2018. Genome-wide copy number variant analysis reveals variants associated with 10 diverse production traits in Holstein cattle. *BMC Genomics*. 19(1): 314. doi: 10.1186/s12864-018-4699-5.