The influence of environmental and managerial factors on the characteristics of beef bull semen

by

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Abstract

To increase the profitability and productivity of bovine semen collection facilities, a better understanding of factors positively and negatively influencing beef bull collection is needed. The objective of the current study was to evaluate various environmental and managerial factors for their impact on beef bull semen characteristics at two different semen collection facilities. From 2008 to 2018, data on ejaculates were analyzed from two facilities located in different geographical regions of the United States. Stud A, located in Montana, contributed 56,811 ejaculates from 1,715 bulls, and Stud B, located in Kansas, contributed 14,885 ejaculates from 775 bulls. Breed data from both bull studs in the analysis included, seven different breeds were included in the analysis: Angus, Red Angus, Charolais, Gelbvieh, Horned Hereford, Polled Hereford, and Simmental. At both studs, semen quality, age at time of collection, and days between ejaculates were recorded. At Stud A only, barn location, weight, and scrotal circumference at time of collection were recorded, while at Stud B only, collection method and the number of sequential ejaculates per day were recorded. For each stage of sperm development, the average cumulative climate index (CCI) was calculated in order to account for environmental impact. The individual ejaculate characteristics recorded included volume, concentration, motility, and sperm abnormalities. Multiple regression models using the GLIMMIX procedure of SAS were used to determine factors affecting collection characteristics. Individual models were used for each individual ejaculate characteristic. Backwards selection was used until all variables in each model were significant at $P < 0.05$. Bull age influenced ($P < 0.001$) all semen characteristics at both studs. Ejaculate volume increased ($P < 0.001$) with age, then plateaued at approximately 60 months of age. Sperm concentration was highest ($P < 0.001$) from bulls collected between 24 and 60 months of age. Pre-freeze motility was lowest ($P < 0.001$) for bulls

under 12 months of age at Stud A, and lowest ($P < 0.001$) for bulls under 12 months of age and greater than 48 months of age at Stud B. Primary sperm abnormalities were greatest ($P < 0.001$) for bulls less than 15 months of age at Stud A, and less than 12 months of age at Stud B. Breed affected ($P < 0.001$) all semen characteristics but varied in characteristic and breed in its overall influence. Season of ejaculate collection was defined as either Winter (December to February), Spring (March to May), Summer (June to August), or Fall (September to November). At both studs, collection during Spring and Summer resulted in the highest ($P < 0.001$) volume and concentration. Volume of ejaculate generally increased as days between ejaculates increased. Pre-freeze and post-thaw motilities were generally higher for less days between ejaculates. The effect of average CCI during each stage of spermatogenesis, as well as epididymal transit, varied between studs. One consistent finding was that CCI during the meiotic phase did not affect volume but did influence concentration. The average CCI during epididymal transit was associated with the occurrence of both primary and secondary sperm abnormalities. Bulls located in Barn 1 produced the lowest ($P < 0.001$) concentrations, the lowest pre-freeze motility, and the greatest ($P < 0.001$) primary abnormalities compared to bulls in the other barn locations. Pre-freeze motility was greatest ($P < 0.001$) for Barns 3 and 5, and post-thaw motility was greatest ($P < 0.001$) for Barn 5. Bulls weighing greater than 907 kg at time of collection produced the greatest ($P < 0.001$) concentrations compared to other age groups except for bulls weighing 454 – 680 kg. Bulls weighing greater than 1134 kg produced the greatest ($P < 0.001$) post-thaw motilities. Bulls with a scrotal circumference of greater than 40 cm produced the greatest ($P < 0.001$) sperm concentration. Bulls with a scrotal greater than 44 cm produced the lowest ($P < 0.001$) post-thaw motilities. Bulls collected with an artificial vagina produced less ($P < 0.001$) volume and secondary abnormalities, but higher ($P < 0.001$) sperm concentrations compared to

electroejaculation collections. The initial ejaculate of the day had the highest ($P < 0.001$) concentration, but the lowest ($P < 0.001$) pre-freeze motility. Identifying and understanding these influences may lead to ways to minimize the factors that negatively affect production and profitability of beef bull semen collection facilities. This may lead to improved overall efficiency of semen collection and adjust producer expectations of collection.

Table of Contents

List of Figures

List of Tables

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Dedication

This thesis is dedicated to my Grandma Rita and Grandpa Joe, without their guidance in my early life I would not be where I am today. I hope they are watching from above with approval of my continued love for working with bulls.

Chapter 1 - Literature Review

Introduction

As reproductive technologies advance and the use of artificial insemination (AI) of cattle grows, the demand to collect semen from young genetically superior beef bulls increases. The use of genomics has decreased the age at the time of collection for genetically superior bulls. Collecting high-quality semen while optimizing the number of straws produced from these bulls is vital for the economic success of collection facilities and bull studs (Harstine, 2018). Collection facilities must ensure that semen is of high quality to optimize conception. Understanding the factors that affect semen production and collection presents an opportunity to increase efficiency of collection facilities. Semen production in bulls is affected by environment, management, physiological status, and genetics (Mathevon et al., 1998). By better understanding these influences, collection facilities may adjust their management style to minimize environmental impact and keep bulls in an ideal physiological status to maximize profitability.

Spermatogenesis and Epididymal Transit

The process of semen collection begins approximately 75 days before, with spermatozoa production and maturation. Spermatogenesis is the formation of haploid spermatozoa from diploid progenitor cells (Salisbury and VanDemark, 1961; Sharma and Agarwal, 2011). In bulls spermatogenesis takes approximately 61 days (Salisbury and VanDemark 1961; Senger, 2012; Staub and Johnson 2018), while maturation through epididymal transit lasts 14 days (Senger, 2012). Spermatogenesis goals are to provide a continual supply of male gametes, genetic diversity, and immunological protection of germ cells (Senger, 2012). Spermatogenesis can be divided into three phases. The first two phases are proliferation and meiosis, often referred to as spermatocytogenesis (Sharma and Agarwal, 2011; Senger, 2012). The third phase,

spermiogenesis, also known as differentiation, is the transformation from round spermatids into a complex structure called the spermatozoon. All spermatogenesis phases occur within the seminiferous tubules, where spermatozoa develop within the nurse cells or Sertoli cells (Salisbury and VanDemark, 1961; Sharma and Agarwal, 2011; Senger, 2012).

Spermatocytogenesis is defined as the growth of spermatogenic tissue through cell division and a reduction in chromosome numbers. During this time, there is an increase in germ cells (Salisbury and VanDemark, 1961). The initial stage of spermatocytogenesis, or proliferation, begins with germ cells or spermatogonia cells, which undergo mitosis and chromosome duplication (Sharma and Agarwal, 2011; Senger, 2012). The spermatogonia begin development in the seminiferous tubules, where they are housed within the Sertoli cells along a basement membrane. There are two types of spermatogonia, A-type and B-type. A-type spermatogonia divide by mitotic division to produce other spermatogonia. The B-type divides to form two primary spermatocytes (Salisbury and VanDemark, 1961). A-type spermatogonia have intercellular bridges for synchronized mitotic division to form B-type spermatogonia. When the intercellular bridge breaks in A-type spermatogonia, they revert to stem cells, and more spermatogonia are produced (Senger, 2012). The process of proliferation is 21 days in the bull (Staub and Johnson, 2018).

The primary spermatocytes remain connected through intercellular bridges during their mitotic divisions (Sharma and Agarwal, 2011). Post-development of the primary spermatocyte, they begin to undergo meiosis. The meiosis phase consists of two divisions, ultimately producing four secondary spermatocytes (Perry, 1968; Sharma and Agarwal, 2011The meiosis phase takes 23 days for cells to complete their divisions (Staub and Johnson, 2018).

During spermatocytogenesis, Sertoli cells possess a specialized form of protection, called tight junctions. These tight junctions protect spermatogonia and spermatocytes during mitotic division from immunological attack (Sharma and Agarwal, 2011; Senger, 2012). Once spermatocytes form through both mitotic and meiotic divisions, spermatocytogenesis is complete. Germ cells containing half the normal chromosomes then move from the basal compartment's basement membrane to the adluminal compartment (Perry, 1968; Sharma and Agarwal, 2011; Senger, 2012). Meiosis ends when secondary spermatocytes migrate to the adluminal compartment to become round spermatids. (Salisbury and VanDemark, 1961).

Round spermatids cease division during spermiogenesis (differentiation) and begin metamorphosis to reach their final shape as a spermatozoon (Senger, 2012; Perry, 1968). During spermiogenesis, changes include the development of the acrosome, head, midpiece, tail, and other cellular components. Spermiogenesis consists of four phases. The Golgi phase consists of acrosome granule formation in the vesicle of the nucleus. The cap phase entails the spreading of the newly formed vesicle to cover the pole of the nucleus. Golgi bodies begin to develop at the anterior nuclear pole and flatten to form a cap over the nucleus. The acrosomal phase is characterized by complete acrosomal formation through nuclear and cytoplasmic elongation. The previously developed vacuole between the pole and Golgi now contains the acrosome of the cell. The Golgi then extends to the cytoplasm, where some will migrate to the posterior pole to form the cell's neck.

Lastly, during the maturation phase, the spermatozoon undergoes final formation and is freed from the Sertoli cell. The spermatid elongates and forms the axial filament and, ultimately, the tail. The Golgi body develops along with the head until all excess cytoplasm remaining is a protoplasmic droplet (Perry, 1968; Gresson and Zlotnik, 1948; Cole and Coups, 1959). The final

changes in the differentiation phase take 17 days (Staub and Johnson, 2018). This phase ends with releasing the developed spermatozoa from the Sertoli cell into the seminiferous tubule or spermiation (Salisbury and VanDemark, 1961; Senger, 2012; Sharma and Agarwal, 2011). During spermiation, spermatids disconnect their interstitial bridges, releasing their contact from the Sertoli cell's epithelium. Sertoli cells then assist in spermiation through contraction to push the spermatozoon outward into the tubule (Russell and Griswold, 1993).

Following release from the seminiferous epithelium, spermatozoa begin the final process of maturation. From the seminiferous tubules, spermatozoa are pushed to the tubulus contortus and the rectus, which joins with the rete tubules. They are carried to the efferent ducts from the rete tubules, where they converge into a single tube, the epididymal duct. In the epididymal duct, spermatozoa combine with tubular fluid at the proximal epididymis head (Senger, 2012).

Spermatozoa that are stored in the distal tail of the epididymis will either be ejaculated or voided in the urine. Spermatozoa are removed regularly from the distal tail of the epididymis by periodic contractions, which push them into the ductus deferens, the pelvic urethra, and ultimately out through the urine. Spermatozoa not removed in this fashion are eligible for ejaculation. Although the distal tail is where spermatozoa are the most concentrated, if subjected to repeated ejaculation this reserve can be quickly depleted (Senger, 2012).

The epididymis provides an environment of maturation where spermatozoa will gain motility, fertility and are housed in the storage reservoir. The epididymis is composed of three parts: the head, body, and tail. The duct through these sections is surrounded by muscle, which helps to push the spermatozoa forward as they mature. It takes spermatozoa two days to travel through the head of the epididymis; they are not motile or fertile and become more concentrated during this time. While in the head of the epididymis, they possess a proximal cytoplasmic

droplet; a remnant of development. Transition through the body of the epididymis takes two days. In the epididymis body, spermatozoa become even more concentrated, begin to exhibit motility, acquire fertilizing ability, and the cytoplasmic droplet moves midway down the tail to become a translocating droplet. Lastly, spermatozoa reach the distal tail of the epididymis in 10 days. Once they get to the distal tail of the epididymis, they are fully mature. Spermatozoa now have normal motility, fertilization potential, the ability to bind an oocyte, a distal droplet, and are eligible for ejaculation (Senger, 2012).

Spermatogenesis is governed by the endocrine system (Perry, 1968; Salisbury and VanDemark, 1961). Spermatogenesis is dependent primarily on the two gonadotropin hormones, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), both produced in the anterior pituitary gland (Perry, 1968). LH is responsible for the stimulation of Leydig cells and testosterone production, while FSH is responsible for stimulating Sertoli cells and spermatozoa production.

Leydig cells primarily produce androgens and ultimately testosterone. Testosterone released from the Leydig cells feed back to the hypothalamus and stimulate gonadotropin-releasing hormone (GnRH) neurons. These neurons release GnRH into the pituitary stalk, where it is transported through portal vessels of the anterior pituitary. Once in the anterior pituitary, GnRH triggers both FSH and LH synthesis and release. Thus, testosterone levels feeding back onto the hypothalamus regulate the release of the gonadotropins, creating a negative feedback loop (Salisbury and VanDemark, 1961; Perry, 1968). Sertoli cells are self-regulated through their inhibin production, which controls the amount of FSH released from the anterior pituitary (Senger, 2012).

Factors affecting sperm production

Spermatogenesis is an extremely sensitive process, and minor disruptions can cause abnormal spermatocytes (Johnson, 1997). These abnormal spermatocytes can result from either genetic or environmental insult and do not possess fertilizing capabilities. Environmental insults include stress, collection method, management of bulls, weather, and many other factors discussed below. Understanding the effect of these insults is vital for management of these insults.

Age

The increased emphasis on genomic selection has driven the decrease in generation intervals of bulls, significantly influencing bull age at collection facilities (Hayes et al., 2009; Seidel, 2010; Harstine, 2018). Unproven sires now account for nearly half of an AI organizations' semen units sold (Harstine, 2018). Due to different management practices, dairy bulls are often purchased at a younger age and managed more strictly through puberty than beef sires (Harstine, 2018). The use of these young, unproven sires has drawn much attention to decreasing age at puberty to begin collection more quickly.

Puberty in bulls is most defined as the ability of the bull to ejaculate at least 50 million sperm with a motility of 10% or greater (Wolf et al., 1965). Bulls must reach puberty as quickly as possible to begin sperm production and capitalize on superior genetics. Puberty is governed by the hypothalamic-pituitary-gonadal axis triggering the beginning of spermatogenesis (Evans et al., 1996). The axis relies on adequate amounts of GnRH, FSH, and LH to be released into circulation, making these hormones the center of much research to decrease age at puberty. Most importantly, presynaptic neurons within the hypothalamus must transmit information to the GnRH neurons for secretion (Senger, 2012). The month before puberty, the GnRH neurons

become less sensitive to negative feedback from testosterone and begin to increase GnRH production and stimulation of LH and FSH in a pulsatile fashion (Senger, 2012). This changed production of LH and FSH is needed to stimulate the testis and induce sperm production. Testicular growth in bulls is slow until 25 weeks of age, at which time Leydig and Sertoli cells proliferate and become established. Once cells become established, rapid testicular growth occurs until puberty. Puberty in bulls is reached from 35-50 weeks, depending on the breed (Rawlings et al., 2008). This rapid growth is characterized by the development of seminiferous tubules and germ cell division. Leydig cell populations during pubertal establishment begin testosterone production and the negative feedback loop of the hypothalamic-pituitary-gonadal axis (Rawlings et al., 2008). Age affects many collection characteristics well beyond puberty and sexual maturity.

The effects of nutrition before puberty have lifelong impacts. Feeding bulls on a limited nutritional plane delays age at puberty (Flipse and Almquist, 1961; Pruitt et al., 1986; Dance et al., 2015), leads to smaller testicular development (VanDemark and Mauger, 1964; Pruitt et al., 1986), and decreased ejaculate volume and sperm concentrations (VanDemark et al., 1964; Dance et al., 2015). Bulls fed on a higher plane of nutrition before puberty typically display increased LH levels, increased testicular testosterone, and increased scrotal circumference (Mann et al., 1967; Harstine et al., 2015; Byrne et al., 2017).

Bull age at the time of collection influences many semen characteristics. As bull age increases, volume, concentration, total sperm, percent viable sperm per ejaculate, and motility also increase (Everett and Bean, 1982; Mathevon et al., 1998; Fuerst-Waltl et al., 2006; Snoj et al., 2012; Murphy et al., 2018). The increase in sperm characteristics suggests a positive correlation between age, body weight, testicular weight, and semen characteristics (Amann,

1970). Total abnormal spermatozoa decrease with age, and secondary abnormalities can be influenced by age, although those results are inconsistent (Chandler et al., 1985; Soderquist et al., 1996). Conversely, Brito et al. (2002) found that in Brazilian bulls (Bos *indicus* and Bos *taurus*), as age increased, sperm motility decreased, and minor sperm defects increased. Snoj et al. (2012) observed an increase in volume until 84 months of age, after which volume decreased.

Bulls under one year of age tend to have lower semen quality. Bulls that are 10 to 11 months of age passed a breeding soundness exam at a significantly lower rate than older age groups, primarily due to inadequate scrotal circumference and low semen quality (Kennedy et al., 2002). Bulls under one year of age at collection also had lower sperm motility compared to older bulls; however, this was only two percentage points lower than other groups suggesting little biological importance (Murphy et al., 2018).

Reports on the optimum age for peak sperm concentrations has varied, and quadratic effects have been suggested. Ages of peak sperm concentration include 18-20 months of age (Fuerst-Waltl et al., 2006), 20-22 months of age, (Taylor et al., 1985), 12-24 months of age (Murphy et al., 2018), 22 months of age (Mathevon et al., 1998), and four years of age (Everett and Bean, 1981). Holstein bulls have been documented to not reach maximum sperm production until seven years of age; this increase in production is associated with the increase in testicular weight (Amann, 1970). The tail of the epididymis' storage capacity has been shown to increase with age (Macmillan, 1967; Almquist and Amann, 1961). Storage capacity in the tail of the epididymis of yearling bulls was equal to 1.6 days of sperm production, while 3 to 5-year-old bulls store for 3.3 days, and bulls older than seven years store for 3.6 days (Amman, 1970).

Season

Abnormal testicular temperature can cause severe insult to spermatogenesis. Temperature regulation in the testis in part is accomplished through a counter-current cooling mechanism known as the pampiniform plexus and is essential for spermatogenesis (Senger, 2012; Hopper, 2015). Improper testicular temperature regulation due to environmental influence results in negative consequences to spermatogenesis (Johnson, 1997; Rahman et al., 2018). Although not typically categorized as a seasonal breeder, cattle have been shown to have an annual cycle of testis activity (Amann, 1970). This annual cycle may be driven by the environmental influence on the testis' ability to regulate heat or age and environment interactions. When evaluating ejaculates, it is crucial to keep in mind that morphological issues maybe a result of the testes' exposure to above-average heat (Rahman et al., 2018).

Heat stress can have multiple effects on the testis. The primary area of damage is in the Sertoli cells, where the developing germ cells are housed(Setchell, 1998). Spermatozoa under heat stress may respond by undergoing loss of DNA integrity and apoptosis (Banks et al., 2005; Sinha et al., 2003). Bulls exposed to increased heat and humidity for 12 hours display a 30-40% increase in coiled tails and detached heads (Casady et al., 1953). Following scrotal insulation, the abnormalities present are positively correlated to the testis's developmental point when insulation occurred (Rahman et al., 2018). Forty-eight-hour insulation resulted in increased decapacitated sperm, abnormal acrosomes, abnormal tails, detached heads, nuclear vacuoles, knobbed acrosomes, and drag defects (Wildeus and Entwishtle, 1986; Vogler et al., 1991 and 1993). Periods of scrotal insulation greater than 96 hours causes pyriform heads, nuclear vacuoles, abnormal DNA condensation, and large heads (Barth and Bowman, 1994; Fernandes et al., 2008; Newton et al., 2009). Prolonged heat stress leads to increased heat shock proteins,

leading to greater production of reactive oxygen species, which reduced viability of spermatozoa (Rahman et al., 2018). Exposure to heat needs only to be 12 hours long to see effects on concentration and motility (Rahman et al., 2018).

Bulls evaluated for breeding soundness were less likely to be classified as satisfactory when evaluated in January, February, August, or September when compared to bulls tested in April, May, or June (Barth and Waldner, 2002). Prevalence of sperm abnormalities varied by season; in January, February, and March, bulls had more significant midpiece defects and coiled principle pieces than in May or June (Barth and Waldner, 2002). The percentage of normal sperm was lower in January, February, March, and September (Barth and Waldner, 2002). Midpiece defects declined, and head abnormalities increased during spring and summer in bulls not classified as satisfactory (Barth and Waldner, 2002). The authors suggest these results may be from cold stress and decreased feed quality during the winter (Barth and Waldner, 2002). Motility decreased from breeding soundness exams conducted during the summer compared to winter (Malama et al., 2016).

When evaluating bulls from collection facilities, seasonal effects vary. Daily sperm output and motility have been reported as most significant from mid-May to August and the lowest sperm output from January through March (Amann et al., 1966; Everett et al., 1978; Chandler et al., 1985; Murphy et al., 2018). Conversely, the highest average total sperm output was during the winter and spring and lowest in the summer and fall (Everett and Bean, 1982; Methevon et al., 1998; Snoj et al., 2012). A study conducted in Brazil found that ambient temperature and humidity did not affect sperm quality or production (Brito et al., 2002). These authors concluded no effect due to little variation in the semen quality and production variables

(Brito et al., 2002). However, the higher ambient temperature on the day of collection significantly decreased the motility of the ejaculate in Australian bulls (Fuerst-Waltl et al., 2006).

In young bulls, the season most significantly affected volume, concentration, motility, and total sperm output (Mathevon et al., 1998). Bulls collected between 25 to 84 months had the lowest ejaculate volume during the winter; however, regardless of age, the highest ejaculate volumes were observed in all breeds in the summer and the lowest in the winter (Everett and Bean, 1982; Snoj et al., 2012).

Snoj et al. (2012) observed the lowest semen concentration in Charolais bulls when collected in the autumn compared to other breeds. In Holstein Friesian bulls, there was a season by bull age interaction for ejaculate volume, sperm concentration, total sperm, post-thaw gross and total motility, and pre-freeze gross motility (Murphy et al., 2018). Rahman et al. (2018) suggest the severity of heat effects is breed-dependent between *Bos Taurus* and *Box Indicus* bulls. Heating or cooling of stalls may be a way to help sperm quality remain consistent. The extent to which bulls need protection from the environment may vary based on age and breed.

Breed

Breed influenced the percentage of bulls classified as satisfactory breeders during breeding soundness exams. During evaluations, Polled Herefords were classified as satisfactory less often Gelbvieh and Simmental (Kennedy et al., 2002). Simmental and Aberdeen Angus bulls were more likely to be classified as satisfactory than Hereford, Limousin, or Charolais bulls (Barth and Waldner, 2002). Simmental bulls were the least likely to be classified as unsatisfactory (Barth and Waldner, 2002).

Brito et al. (2002) performed a two-year evaluation of *Bos Taurus* verse *Bos Indicus* bulls. Authors concluded that *Bos Indicus* bulls had greater sperm concentrations than *Bos Taurus* (Brito et al., 2002). *Bos taurus* bulls had significantly fewer morphology defects than *Bos indicus* (Brito et al., 2002). Despite the difference in concentration and morphology, the authors did not find consistent results for effects on volume, total spermatozoa, and the number of viable spermatozoa.

A study comparing 16 to 20-month-old Angus, Hereford, Santa Gertrudis, and Hereford bulls found Angus bulls to have the most significant semen volumes and quality (Fields et al., 1979). Hereford bulls had a smaller testicular size and lower semen quality than other breeds. When comparing sperm concentrations of the breeds, the Hereford bulls produced the greatest concentrations. Despite the greatest concentration coming from these Hereford bulls, the Santa Gertrudis bulls had the largest testicular volume. Brahman bulls, generally considered slower maturing, had the smallest testicular volume, lowest sperm concentration, and lowest motility (Fields et al., 1979).

Management Factors

Management of bulls and personnel is a vital component for consistent ejaculation in a semen collection facility. It is fundamental that semen collection personnel identify bull sexual behavior and libido differences to maximize sperm concentration per collection (Shenk, 2018). Bulls vary greatly in their libido level and there may be distractions in collection areas. Key components of collection areas must include excellent footing, safety precautions for bulls and personnel, and be void of distractions (Shenk, 2018). Distractions can range from tours of the facility, daily feeding, and other bulls in watch stalls during collection (Shenk, 2018).

Facilities that house bulls have been changing to accommodate younger, smaller bulls at the time of acquisition (Harstine, 2018). Bulls are housed in smaller individual pens, and smaller collection arenas are needed for young bulls as soon as puberty is reached. More intense collection schedules are also driven by the need to collect semen from high demand bulls (Amann and DeJarnette, 2012).

The collection team significantly affected volume and the total number of sperm but did not affect concentration and motility (Mathevon et al., 1998). The individual bull handler has been shown to affect volume, concentration, and percent viable spermatozoa, but not motility (Fuerst-Waltl et al., 2006). Fuerst-Waltl et al. (2006) observed the personnel collecting semen influences on ejaculate volume in both collection centers in their study; however, they only observed a significant influence on concentration and motility in center two. This suggests personnel within collection facilities can play a significant role in success of collections.

Bull Sexual Behavior, and Intensity of Semen Collection

A bull's libido can greatly affect the quality of the ejaculate. Factors related to libido that affect ejaculate quality include interval of time since the last collection, a bull's willingness to mount, sexual preparation, the semen collection equipment used, handling of collection equipment, and false mounting (Foote, 1978; Chenoweth, 1983). Libido or mating ability is also learned over time, as yearling bulls must develop the desire and ability to mate (Lunstra, 1982). Sexual behavior first became a problem with the libido maintenance of dairy bulls in AI centers, as bulls became less interested over time (Chenoweth, 1983). Bulls that spend time getting ready to mount, or are restrained from mounting, demonstrated an increase in quality and quantity of ejaculate (Kirillov and Morozov, 1934; Anderson, 1945, Collins et al., 1951). Specifically, bulls' restraint from mounting after sexual excitement increased ejaculate volume, sperm motility, and

concentration of spermatozoa (Collins et al., 1951). The restraint of bulls from mounting the teaser animal until sexual excitement is called sexual preparation (Chenoweth, 1983). The second type of sexual preparation is false mounting, where bulls mount the teaser (a steer lead by a collection worker) but cannot ejaculate and the bull is removed from the teaser (Almquist, 1973). False mounting is the most successful technique of sexual preparation. False mounting allows collection personnel direct evaluation of a bull's sexual arousal (Schenk, 2018).

Hafs et al. (1959) found that when collecting bulls daily, the time required for successful ejaculates was longer than those collected once weekly. These authors also found that more false mounts were required for successful ejaculate in bulls collected daily (Hafs et al., 1959). When bulls were collected twice weekly, the use of three false mounts before the first ejaculate was shown to increase sperm concentration, while a false mount before the second ejaculate was not required for increased concentration (Almquist, 1973). Use of three false mounts prior to the first ejaculate and zero after the second, decreased collection time, from 46.0 minutes to 29.8 minutes (Foster et al., 1970). Almquist (1973) concluded for beef bulls; it is practical to use a combination of three false mounts before the first ejaculate and zero false mounts before the second in an AI facility to optimize ejaculate concentrations.

Studies have demonstrated that it was ideal for semen to be collected two to three times per day at three to four-day intervals (Bratton and Foote, 1954; Hafs et al., 1959). A 60% increase in the number of motile spermatozoa collected per week resulted when semen collection was increased from once to twice per day over eight days (Bratton and Foote, 1954). Extending the days between collections up to a six-day interval resulted in increased volume, concentration, and total sperm in the ejaculate (Seidel and Foote 1969, Everett et al., 1978). Depending on the ejaculate characteristic, optimum intervals may vary. The greatest ejaculate volumes came from

an interval of six to nine days, while the interval for concentration was ideal between four to nine days, but motility was not significantly affected by the collection interval (Fuerst-Waltl et al., 2006).

The variation in days between collection was investigated by Mathevon et al. (1998), who concluded the ideal interval is age-dependent. Young bulls had the greatest volume and concentration when collected every four to seven days, and mature bulls every five days. On average, the greatest concentrations were collected per month when bulls were collected after two-day intervals (Mathevon et al., 1998). This suggests that for high demand bulls arranging the collection schedules for a two day collection interval may be beneficial to maximize collection.

Increased ejaculation frequency did not influence sperm production rates but did result in greater sperm output over time (Amann, 1962; Everett et al., 1978). Despite no influence on sperm production, frequent ejaculation can decrease the time spermatozoa travel through the epididymis by depleting the spermatozoa reserves in the distal tail of the epididymis (Almquist and Amann, 1961).

Collection Method

The influence of collection equipment used can affect ejaculate quality. While the artificial vagina (AV) is the most common tool used for collection, some bulls may not be receptive, and the use of electroejaculation (EE) is required. An AV is typically a long-ridged cylinder, with a rubber-lined tube in the middle and a semen receptacle attached to the opposite end of the opening. The area between the cylinder and rubber lining is filled with hot water near body temperature, and the rubber lining is coated with lubricant (Salisbury and VanDemark, 1961). The effect of tactile stimulation, temperature and AV length is optimized for individual bulls (Seidel and Foote, 1969). As bulls mount the teaser animal, and the penis is deflected into

AV until ejaculation. With this method, it is important to keep the AV sanitary, warm, and away from direct sunlight to prevent damage to the ejaculate (Salisbury and VanDemark, 1961).

The EE method involves inserting an electrode probe into the rectum of the bull. Voltage is gradually applied in a pulsatile fashion until the bull begins to extend the penis and ejaculate. The ejaculate is then collected into a receptacle, kept clean, warm, and out of direct sunlight (Salisbury and VanDemark, 1961). Electro-ejaculation is often used when bulls are no longer physically able to mount a teaser animal and service an AV.

Dziuk et al. (1954) were among the first to conclude that semen could be collected with EE without negative consequences. They did conclude the ejaculates were generally of higher volume but lower concentration (Dziuk et al., 1954; Austin et al., 1961; Foster et al., 1970). Austin et al. (1961) found that motility from ejaculates collected with EE was higher than AV, both initially and after four days of storage. Martig et al. (1970) found no difference in the freezing quality of ejaculates collected by EE compared to AV. In a two-year study comparing EE and internal AV differences for breeding soundness exams, differences were observed for volume and motility; however, these results were opposite between years 1 and 2 and authors did not conclude an overall impact. Collection with a vaginally inserted collection device did allow for researchers to identify 4.8% of bulls with copulatory deficiencies that would have otherwise been satisfactory (Barth et al., 2004). It was concluded that EE can be used successfully for bulls that were not able, or lacked the libido, to serve an AV.

Semen Collection for AI

The process of collecting semen began in the 1600s when sperm were first identified by Leeuwenhoek and Hamm (Leeuwenhoek, 1678). Nearly one hundred years later, the first successful artificial insemination was performed by Spallanzani when he inseminated a dog in

1784 (Foote, 2002). Russian scientist Ivanoff, starting in 1899, developed many of today's semen collection technologies for domestic animals. Ivanoff first collected bulls by allowing semen to collect in a sponge within the cow's vagina (Ivanoff, 1922). An alternative method of collection commonly used during this time was stimulating the ampullae and sex glands by massage. The problem with the vaginal sponge and massage of sex glands was collecting a clean sample without waste (Moore and Hasler, 2017). Ivanoff was followed by Milovanov, who developed artificial vaginas for cattle, which replaced the earlier method of the sponge (Foote, 2002). Electro-ejaculators were first introduced for use in bulls in the 1950s (Dziuk et al., 1954). This provided an opportunity to collect bulls that were not able or willing to serve an AV.

The first dairy AI organization began in Denmark in 1936. When this Denmark dairy herd experienced greater conception rates compared with natural service, the use of AI began to spread. The growth of AI was made possible by advances in semen collection, extension, and preservation of sperm (Moore and Hasler, 2017). In 1938, the first United States AI cooperation was formed (Perry, 1968). The use of AI in the United States dairy industry began to grow in the 1940s. Today, many beef and dairy AI cooperatives are in operation, and over 25 million units of semen are sold domestically, with another 30 million units exported from the United States (NAAB, 2019).

Semen Evaluation

There is currently no definitive test that evaluates a bull's fertility or success as an AI sire. There are multiple laboratory methods to evaluate semen and select ejaculates that meet AI facility standards. (Linford, Glover, Bishop, & Stewart, 1976). An initial analysis of progressive motility, concentration, and morphology is often performed to discard poor quality ejaculates

before freezing. This initial analysis can be performed by evaluating an unstained subsample of an ejaculate microscopically for motility and morphology (Graham and Moce, 2005).

A quality control (QC) or frozen semen evaluation is a post-thaw evaluation of semen used to determine if an ejaculate meets a minimum requirement of various traits. The use of QCs provides a level of control within a collection facility (Vincent et al., 2012). For an ejaculate to meet a QC's standards, it must have an acceptable number of motile spermatozoa surviving the thawing procedure; these sperm must then meet the minimum percentage of normal morphology (Vincent et al., 2012; Thundathil et al., 2016). Spermatozoa must also have an intact acrosome, DNA integrity, active mitochondria pathway, and meet an acceptable level of forward progressive motility (Vincent et al., 2012; Thundathil et al., 2016). Quality checks are performed on semen post-thaw in most AI facilities on each batch of semen.

Motility

Analysis of motility is often the first step performed microscopically in a collection facility. A technician performs visual analysis to estimate the percentage of motile spermatozoa in an ejaculate (Graham and Moce, 2005). Higher initial motility is correlated with higher motility after freezing (Salisbury and VanDemark, 1961). For this reason, low motility samples are often culled before the freezing process. Due to this evaluation's subjectivity, the repeatability of technicians should be assessed regularly (Salisbury and VanDemark, 1961). Evaluation of motility is often utilized to detect gross differences between ejaculates but cannot detect subtle differences that may affect fertility (Maule, 1962).

Concentration and Volume

Spermatozoa concentration varies greatly from ejaculate to ejaculate within the same bull and from bull to bull (Salisbury et al., 1942). For an AI facility, determining the concentration of

the ejaculate is essential for proper dilution and to determine the total number of sperm collected. An ejaculate volume is the other vital component for calculating total sperm or dilution (Salisbury and VanDemark, 1961). Volume should be measured directly after collection before any dilutions occur. No method evaluates ejaculate concentration as a whole; thus, it is important to obtain a small yet accurate sample from the ejaculate for analysis.

The hemocytometer method uses a small sample from the ejaculate using a dilution pipette (Salisbury et al., 1942). Since bull semen can be thick, it may need to be diluted and counted twice with this method. The hemocytometer contains a 25-grid block of which the technician counts 5 of the squares. To ensure repeatability in a sample, multiple chambers may be loaded and counted for consistency (Salisbury et al., 1942). The hemocytometer is a difficult and very time-consuming method and should not be used in collection laboratories regularly (Cenariu et al., 2018). Another method that can be used is the photoelectric colorimeter. Where a small sample is taken from the ejaculate, diluted, and measured for turbidity. Proper measurement of concentration is one of the most important parameters for properly calculating dilutions for AI (Evenson et al., 1993; Cenariu et al., 2018).

Morphology

The identification of spermatozoa damaged during development and incapable of fertilization is vital in maintaining semen quality. Fixed subsamples are counted for the percent of abnormal cells, and this evaluation must be performed by highly trained personnel. As the percentage of abnormal cells increases, fertility decreases, making proper identification vital (Andersson, et al., 1990; Graham and Moce, 2005). Decreases in fertility are more likely when greater than 17 percent of the ejaculate has abnormalities (Maule, 1962). During analysis, abnormalities are classified as either primary or secondary; in some systems, they can also be

classified as major and minor (Graham and Moce, 2005; Chenoweth, 2005; Society of Theriogenology, 2018).

Primary abnormalities are associated with spermatogenesis, and secondary abnormalities are associated with damage that occurred after the cells leave the testis (Graham and Moce, 2005; Chenoweth, 2005). Major defects impair fertilization, while minor defects have little effect on fertility and can be overcome with additional spermatozoa (Graham and Moce, 2005; Chenoweth, 2005). Major sperm defects are also defined as primary defects. They occur in a substantial population of the ejaculation (>10%), are consistent in occurrence, associated with infertility, and potentially heritable (Chenoweth, 2005). It has been documented that defects that occur regularly in the ejaculate without environmental influence are of genetic origin (Chenoweth, 2005). For an overview of all types of defects, see Table 1.

CASA

While the classical analysis method is with a light microscope by a technician, new technologies are becoming available. The most recent change to collection facilities is using an objective tool, computer-assisted sperm analysis (CASA) (Vincent et al., 2012). CASA eliminates human bias during analysis by using automated technology (Graham and Moce, 2005). The CASA programs measure motility, sperm concentration, morphology, and viability, and index of DNA fragmentation of frozen-thawed sperm (Vincent et al., 2012). The CASA system may not be the best fit for all situations; debris from milk and egg yolk extenders are similar in size to the head of spermatozoa and may be counted in some analyses (Vincent et al., 2012).

Limitations of Semen Evaluation

Using laboratory results to predict fertility is often inconsistent. Classic evaluation of ejaculates requires highly trained personal and is highly subjective (Cenariu et al., 2018). Cenariu et al. (2018) found that the CASA system was more precisely able to differentiate between progressive and non-progressive motility. Over ten spermatozoa characteristics play a role in predicting a bull's fertility, yet these are not easily or accurately measured (Amann, 1989). Ejaculates are often not evaluated post-thaw to examine the reduced fertility due to pre-capacitation during the freezing process, a major insult to an ejaculate's success (Graham and Moce, 2005). In the future, evaluation methods that isolate highly viable spermatozoa to determine those best suited to respond to capacitation, acrosome reactions, ability to penetrate the zona pellucida, and fertility markers in seminal plasma will be best predictors of fertility (Rodriguez-Martinez and Barth, 2007).

Extension and storage

To maximize breeding potential, ejaculates are diluted or extended. The term extender was given because the use of mediums enhances and extends the usefulness of semen in an ejaculate, increasing the usefulness by as much as 25-fold (Foote and Bratton, 1950). To maximize the use of an ejaculate, it must be extended and stored while maintaining sperm viability.

Initially, a medium of glucose, phosphate-buffered saline, and sodium sulfate was used to extend semen for use without storing (Moore and Hasler 2017). A yolk-phosphate extender was developed, which prolonged the life of semen when stored at 5 degrees C (Phillips and Lardy, 1940). Salisbury et al. (1941) expanded on the extender by buffering the egg yolk with sodium

citrate instead of phosphate buffer, which allowed for the fat globules from the egg yolk to be dispersed. Boiled milk has been demonstrated to be equivalent in effectiveness to egg-based extenders (Thacker and Almquist, 1953). The disadvantage of the glucose and yolk-phosphate extenders were they lacked mechanisms to be used as a cryoprotectant during the freeze-thaw process (Moore and Hasler, 2017).

The growth of AI in the 1940s brought attention to the need to control the spread of venereal diseases and minimize microorganism growth in extenders (Moore and Hasler 2017). The use of streptomycin, penicillin, and the combination of the two became the best way to reduce the bacterial content in bovine semen (Almquist et al., 1949; Almquist et al., 1948a; Almquist et al., 1948b). Later Foote and Bratton (1950) evaluated the use of sulfanilamide, penicillin, streptomycin, and polymyxin, which became the standard antibiotic mix in extenders used today.

Demand to store semen by freezing brought about the use of glycerol for its cryopreservation benefits for semen (Polge et al., 1939; Polge and Rowson, 1952). Foote and Bratton (1949) evaluated the difference between pre-extending or post-extending semen when cooling to 5 degrees C. They discovered that post-thaw motility was greater when semen was cooled and stored in an extender (Foote and Bratton, 1949). Other constituents used for cryopreservation include citric acid, sodium phosphate, buffering system, fructose, and glucose, as metabolizable substrates (Layek et al., 2016). Popular extenders currently include yolk-based, milk-glycerol extenders, and soybean-based extenders (Foote, 2002; Layek et al., 2016).

Cryopreservation is the most popular method of semen storage to extend the life of semen and allow for distribution (Medeiros et al., 2002). Cryopreservation includes temperature

reduction, cellular dehydration, freezing, and thawing. Sperm is not suited for low temperatures, and thus the cryopreservation process causes varying degrees of cellular damage. The cold shock of sperm by rapid cooling causes irreversible loss of motility, membrane permeability, and acrosomal membrane disruption. Cold shock can be prevented by slow-cooling the ejaculate to 4 degrees C in protective solutions. By cooling to 4 degrees C, the cellular metabolic activity is slowed, and the life span is extended when then cooled to below -130 degrees C. During this time, the rate of freezing is vital to prevent intracellular water from freezing or from ice formation around the cells (Medeiros et al., 2002).

Cryopreservation was originally accomplished in two ways: solid carbon dioxide and liquid nitrogen. In the 1950s, storage moved from both methods to liquid nitrogen as a better tool for preservation. It was demonstrated that keeping semen below -196 degrees C prevented any biological change compared to the -79 degrees C of solid carbon dioxide. After the move to liquid nitrogen tanks with improved insulation was developed to provide the storage systems that we use today (Foote, 2002).

Semen was originally preserved in glass ampules that varied in size but were most commonly one ml (Pickett and Berndtson, 1974). Sorensen (1940) developed the first straw for semen packaging, Cassou developed the modern straw that is now used worldwide (Foote, 2002). The industry moved from ampules to straws when automated filling systems made straws more convenient for packaging and storage (Pickett and Berndtson, 1974). French straws that are used today are 0.5 ml and 0.25 ml. Straws contain a seal of polyvinyl alcohol powder between two cotton plugs. When the polyvinyl alcohol powder contacts liquid, it forms a seal, the other end of the straw is closed with an ultrasonic seal (Pickett and Berndtson, 1974).

Cells per Straw

To maximize the production of ejaculates, determining the optimal concentration in AI doses is vital. Due to the reduced viability in the freeze-thaw process, low sperm doses are a limitation of successful collection and extension (Mohanty et al., 2018). Further, the limitation of determining the optimum concentration for doses, is large between bull variations of quality (Salisbury and VanDemark, 1961; Mohanty et al., 2018). Salisbury and VanDemark (1961) implied that increasing the concentration of spermatozoa per dose was beneficial to a certain point, after which the fertility was dependent on the female. Due to between bull variations, the minimum number of motile sperm needed for maximum fertility varies, and the rate at which bulls approach the optimal fertility is unknown (Sullivan and Elliot, 1968; den Daas et al., 1998). Adjusting sperm concentrations per dose on a per bull basis may overcome limiting factors but requires extensive documentation (Mohanty et al., 2018).

Ejaculates from five Angus bulls, when extended to a concentration of 10, 20, and 40 x 10^6 spermatozoa, did not differ in pregnancy rate to fixed-time AI (Zoca et al., 2017). However, pregnancy rates did vary between bulls 48.1 and 40.7%, and within bull concentrations from 0.5 to 4.9% (Zoca et al., 2017). Zoca et al. (2018) further investigated these bulls with the assistance of CASA and flow cytometry for ejaculate characteristics. They found that the highest fertility bulls did not have the greatest values for motility scores, intact plasma membranes, and intact acrosomes, suggesting that CASA and flow cytometry are not a viable option in determining fertility (Zoca et al., 2018). The ideal concentration of spermatozoa per straw has yet to be determined and varies by collection facility and bull.

Bull Breeding Soundness Exams

Physiological factors that affect semen production include the bull's health and physical being or breeding soundness (Carroll et al., 1963). Breeding soundness is typically determined by a Bull Breeding Soundness Exam (BSE), an exam performed by a veterinarian to identify infertile or sub-fertile bulls (Hopper, 2015). BSE is a management tool utilized by producers to provide an assessment of a bull's breeding ability. Exams should be performed on bulls before purchase, annually for natural service herd sires, and whenever concerns of a bull's fertility may arise.

Bull breeding soundness exams consist of three components: physical examination, scrotal circumference, and semen collection and evaluation (Dalton, 2018). The current standards for BSE were last edited in 2016 (Society of Theriogenology, 2018). Bulls are scored in the three areas and classified in one of three categories. Bulls that meet the minimum requirement of all three categories are classified as satisfactory. Bulls that are prepubertal, or predicted to meet the standards later in time, often due to stressors, are classified as deferred and are tested again later. Lastly, bulls that do not meet the minimum requirements are unsatisfactory (Hopper, 2015).

The physical examination consists of an exam at a distance, where a bull's confirmation and overall appearance are observed. Foot and leg confirmation are important to evaluate during this time as it is a trait that can be detrimental to bulls in a range situation. Eyes, nasal passage, and oral cavity should be evaluated for abnormalities. Once physical fitness has been evaluated, the reproductive system is evaluated next. The external indicators which are a part of the exam include the scrotum and testicles. During the scrotal examination, a scrotal circumference

measurement is taken. A transrectal examination is then performed to evaluate the sex glands for abnormalities, and finally, the penis and prepuce are examined for abnormalities (Hopper, 2015).

When the physical examination is complete, bulls are then subjected to semen evaluation. Semen is collected in four ways: electroejaculation (EE), manual stimulation, internal artificial vagina, or an external artificial vagina. The most used method of collection for BSE is EE (Hopper, 2015). Collection with internal artificial vagina or external allows for identifying bulls with low libido (Barth et al., 2004). Once a sample has been collected, a small portion of the sample is evaluated for gross motility. The ejaculate is then diluted and evaluated for progressive motility. Progressive motility is classified into one of four categories. The categories are "very good" for greater than 70%, "good" for 50-69%, "fair" for 30-49%, and "poor" for less than 30% (Society for Theriogenology, 2018). Morphology is then counted and categorized; at least 100 cells are counted and classified as normal, primary abnormalities, or secondary abnormalities (Table 1.1 Society for Theriogenology, 2018). Cells can also be classified as major or minor abnormalities and compensable or uncompensable (Enciso et al., 2011; Hopper, 2015). For the semen sample to be classified as satisfactory, it must meet the minimum requirement of 30% motility and 70% normal sperm (Society for Theriogenology, 2018; Parkinson, 2004).

While a BBSE can provide an estimate of a bull's fertility, it is not a guarantee. A BBSE only provides information about a bull's breeding ability on the day the exam is performed. BBSE results vary between veterinarians; variations are due to differences in equipment and skill set. The minimum threshold parameters also raise concerns from BBSEs. Fertility characteristics vary between age and breed, suggesting that the minimum thresholds may not suit every situation (Hopper, 2015).

A BBSE also provides no evaluation of a bull's libido or their willingness to mount an attempt to service a female (Chenoweth, 1981). Lastly, BBSEs provide no insight into a bull's ability to produce semen of freezing quality. Due to the extensive freezing processes, the semen parameter minimum thresholds are great. These increased thresholds are largely due to the loss of motility during the cryopreservation process. While BBSEs are a useful tool for producers regarding natural service, they provide minimum insight into a bull's ability to collect and freeze AI quality semen.

Table 1.1 Types of spermatozoa abnormalities, their classifications, and causes[*]

Type of Abnormality	Primary or Secondary Abnormality	Major or Minor Abnormality	Genetic, Environmental, Both
Underdeveloped	Primary	Major	Environmental
Double Forms	Primary	Major	Environmental
Acrosome defect (knobbed acrosome)	Primary	Major	Both, most related to Genetics
Narrow head	Primary	Minor	Environmental
Crater/diadem defect	Primary	Major	Genetic
Pear-shaped defect	Primary	Major	Environmental
Abnormal contour	Primary	Major	Environmental
Small abnormal head	Primary	Major	Environmental
Free abnormal head	Primary	Major	Environmental
Proximal droplet	Primary	Major	Environmental
Strongly folded or coiled tail (Dag)	Primary	Major	Both
Accessory tail	Primary	Major	Environmental
Small normal heads	Secondary	Minor	Environmental
Giant and short broad heads	Secondary	Minor	Environmental
Free normal heads	Secondary	Minor	Environmental
Detached, folded, loose acrosomal membranes	Secondary	Minor	Environmental
Abaxial implantation	Secondary	Minor	Environmental
Distal droplet	Secondary	Minor	Environmental
Simple bent tail	Secondary	Minor	Environmental
Terminally coiled tail	Secondary	Minor	Environmental
Tail Stump	Primary	Major	Genetic

[*]Adapted from Society of Theriogenology; Chenoweth 2005; Enciso et al., 2011

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Chapter 2 - The influence of environmental and managerial factors on the characteristics of beef bull semen

Abstract

To increase the profitability and productivity of bovine semen collection facilities, a better understanding of factors positively and negatively influencing beef bull collection is needed. The objective of the current study was to evaluate various environmental and managerial factors for their impact on beef bull semen characteristics at two different semen collection facilities. From 2008 to 2018, data on ejaculates were analyzed from two facilities located in different geographical regions of the United States. Stud A, located in Montana, contributed 56,811 ejaculates from 1,715 bulls, and Stud B, located in Kansas, contributed 14,885 ejaculates from 775 bulls. Breed data from both bull studs in the analysis included, seven different breeds were included in the analysis: Angus, Red Angus, Charolais, Gelbvieh, Horned Hereford, Polled Hereford, and Simmental. At both studs, semen quality, age at time of collection, and days between ejaculates were recorded. At Stud A only, barn location, weight, and scrotal circumference at time of collection were recorded, while at Stud B only, collection method and the number of sequential ejaculates per day were recorded. For each stage of sperm development, the average cumulative climate index (CCI) was calculated in order to account for environmental impact. The individual ejaculate characteristics recorded included volume, concentration, motility, and sperm abnormalities. Multiple regression models using the GLIMMIX procedure of SAS were used to determine factors affecting collection characteristics. Individual models were used for each individual ejaculate characteristic. Backwards selection was used until all variables in each model were significant at $P < 0.05$. Bull age influenced ($P < 0.001$) all semen characteristics at both studs. Ejaculate volume increased ($P < 0.001$) with age, then plateaued at

approximately 60 months of age. Sperm concentration was highest ($P < 0.001$) from bulls collected between 24 and 60 months of age. Pre-freeze motility was lowest ($P < 0.001$) for bulls under 12 months of age at Stud A, and lowest ($P < 0.001$) for bulls under 12 months of age and greater than 48 months of age at Stud B. Primary sperm abnormalities were greatest ($P < 0.001$) for bulls less than 15 months of age at Stud A, and less than 12 months of age at Stud B. Breed affected ($P < 0.001$) all semen characteristics but varied in characteristic and breed in its overall influence. Season of ejaculate collection was defined as either Winter (December to February), Spring (March to May), Summer (June to August), or Fall (September to November). At both studs, collection during Spring and Summer resulted in the highest ($P < 0.001$) volume and concentration. Volume of ejaculate generally increased as days between ejaculates increased. Pre-freeze and post-thaw motilities were generally higher for less days between ejaculates. The effect of average CCI during each stage of spermatogenesis, as well as epididymal transit, varied between studs. One consistent finding was that CCI during the meiotic phase did not affect volume but did influence concentration. The average CCI during epididymal transit was associated with the occurrence of both primary and secondary sperm abnormalities. Bulls located in Barn 1 produced the lowest ($P < 0.001$) concentrations, the lowest pre-freeze motility, and the greatest ($P < 0.001$) primary abnormalities compared to bulls in the other barn locations. Pre-freeze motility was greatest ($P < 0.001$) for Barns 3 and 5, and post-thaw motility was greatest ($P < 0.001$) for Barn 5. Bulls weighing greater than 907 kg at time of collection produced the greatest ($P < 0.001$) concentrations compared to other age groups except for bulls weighing 454 – 680 kg. Bulls weighing greater than 1134 kg produced the greatest ($P < 0.001$) post-thaw motilities. Bulls with a scrotal circumference of greater than 40 cm produced the greatest ($P < 0.001$) sperm concentration. Bulls with a scrotal greater than 44 cm produced the lowest ($P <$

0.001) post-thaw motilities. Bulls collected with an artificial vagina produced less ($P < 0.001$) volume and secondary abnormalities, but higher ($P < 0.001$) sperm concentrations compared to electroejaculation collections. The initial ejaculate of the day had the highest ($P < 0.001$) concentration, but the lowest ($P < 0.001$) pre-freeze motility. Identifying and understanding these influences may lead to ways to minimize the factors that negatively affect production and profitability of beef bull semen collection facilities. This may lead to improved overall efficiency of semen collection and adjust producer expectations of collection.

Introduction

As the use of beef semen expands through artificial insemination, added pressure is applied to collection facilities to increase semen production of sires while maintaining semen quality (NAAB, 2019). In 2019, the United States produced 5,829,266 units of beef semen, up 44 % from 2018 (NAAB, 2019). This substantial increase has been largely due to the increased use of beef semen on dairy cows (Berry, 2021). The expansion of genomics has also contributed to these pressures by increasing the demand to collect young, sexually inexperienced bulls (Harstine, 2018). This is compounded by the fact that reproductive inefficiency of sperm production in bulls is still a major concern for the industry, and accounts for substantial economic losses to seedstock and commercial producers (Braundmeier and Miller, 2001). There are many factors known to influence semen quality and quantity including environmental, physiological, management, and genetic influences (Mathevon, et al., 1998). While semen collection in dairy bulls has been extensively studied, literature on semen collection of beef bulls is less readily available (Butler et al., 2019).

Beef semen in the United States is collected in hundreds of facilities under many different climatic conditions. Understanding the effect of different environments and managerial factors on semen quality and quantity is one way to improve production. In dairy bulls, season significantly affects collection characteristics, but these results were inconsistent (Mathevon et al., 1998; Stalhammer et al., 1989). In Brazil, when evaluating season by monthly increments, there was no effect on semen quality (Brito et al., 2002). These seasonal effects can be attributed to weather and temperature changes during spermatogenesis and epididymal transit (Meyerhoeffer et al., 1985; Taylor et al., 1985; Parkinson, 1987). Managerial factors such as bull handling, semen collection, collection intervals (days between ejaculates), and frequency of collection have been shown to affect semen volume and total spermatozoa production (Mathevon et al., 1998, Everett et al., 1978; Everett and Bean, 1982; Taylor et al., 1985). In most semen collection facilities, the use of artificial vaginas (AV) is the preferred method of collection, but when bulls are unwilling or unable to serve the AV, electro-ejaculation (EE) may be used. It has been reported that EE can be used without negative consequences to semen quality, but is detrimental to quantity (Dziuk et al., 1954; Foster et al., 1970). In contrast, others have found sperm motility is decreased when ejaculates are collected with EE compared to AV (Austin et al., 1961).

The age of bulls at collection has consistently been shown to influence semen quality and quantity (Stalhammer et al., 1989; Mathevon et al., 1998; Brito et al., 2002; Fuerst-Waltl et al., 2006), although the optimum age for semen collection varied between reports. A biological factor influencing collection and fertility is scrotal circumference, however, most studies evaluating scrotal circumference were in young bulls or during breeding soundness exams (Smith et al., 1981; Gipson et al., 1985; Parkinson, 2004; Menon et al., 2011). Breed has been

shown to influence semen quality, although these results were also inconsistent and when compared during breeding soundness exams (Fields et al., 1979; Brito et al., 2002; Kennedy et al., 2002; Barth and Waldner, 2002; Menon et al., 2011).

A better understanding of breed, age, season, weather, and managerial factors on beef bull semen production in the United States is needed. Therefore, the objective of this study was to evaluate environmental and managerial variables at two semen collection facilities to identify factors that influence collection.

Materials and Methods

Data Collection Locations

From 2008 to 2018, data on ejaculates were analyzed from two facilities located in different geographical regions of the United States. Stud A, located in Montana, contributed 71,081 ejaculates, and Stud B, located in Kansas, contributed 16,513 ejaculates. Ejaculate characteristics recorded at both studs included volume, concentration, pre-freeze motility, post-thaw motility, primary abnormalities, secondary abnormalities, and at Stud B only two-hour post-thaw motility. Details of each ejaculate were also submitted by the collection facilities. Those details included bull cane code, bull breed, date of collection, and bull birthdate. At Stud A only bull weight, scrotal circumference, and barn location at time of collection were provided. At Stud B method of collection, and ejaculates collected per day were provided.

Ejaculates from the studs represented thirty-five different bull breeds. Breeds were determined by National Association of Animals Breeds uniform breed codes system. Individual breeds were included in subsequent analyses as a fixed effect if, 1) there were at least 10 different bulls of that breed represented, 2) there were at least 100 ejaculates, and 3) the breed

was represented at both studs. The seven breeds in the analysis that satisfied those criteria were: Angus, Red Angus, Charolais, Gelbvieh, Horned Hereford, Polled Hereford, and Simmental.

Data was edited using R software (R Core Team, Vienna, Austria) to remove data entry errors, such as duplicate entries, or percentages that were greater than 100, these entries were then treated as missing. All non-numeric entries were removed and treated as miss. Ejaculates with a volume and(or) concentration of zero, were treated as missing. Bull age was calculated as months of age from birthdates provided. Once bull age was determined, bulls were assigned to one of 10 age groups: (1) ≤ 12 months, (2) 13 - 15 months, (3) 16 - 18 months, (4) 19 - 21 months, (5) 22 - 24 months, (6) 25 - 30 months, (7) 31 - 36 months, (8) 37 - 48 months, (9) 49 - 60 months, and (10) > 60 months. Based on the date of collection, each ejaculate was assigned to a season of collection: Winter (December, January, February), Spring (March, April, May), Summer (June, July, August) and Fall (September, October, November). Days since previous ejaculate was assigned to one of nine categories: ≤ 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 - 30 days and > 30 days). Number of total ejaculates for each bull was then assigned for each bull, for example a bull who provided 20 ejaculates they were labeled as 1 through 20.

Barn location at time of collection, body weight, and scrotal circumference were additional data available from Stud A. Bulls were housed in one of five barns. Bulls were assigned to a barn based primarily on health testing status for certified semen services and, secondarily, the producer-desired outcome for the semen. Bulls housed in Barn 1 had the lowest level of health testing. Bulls housed in Barn 1 were subjected to initial health testing and remained here if only being collected for custom collection. Bulls in Barn 2 were in the next step of health testing and would remain in Barn 2 until completing the required Certified Semen Services isolation period. Barns 3, 4 and 5 housed bulls that had completed all health testing

standards set by Certified Semen Services and were housed in their respective barns by testing outcomes of disease titers. Weight at time of collection ranged from 390.0 kg – 1360.7 kg. Bull weights were allocated to one of six groups: 390.0 - 430.9 kg, 431 - 453.6 kg, 453.7 - 680.4 kg, 680.5 - 907.2 kg, 907.3 - 1134.0 kg, or > 1134.0 kg. Similarly, scrotal circumference at time of collection was allocated to one of seven groups: 21 - 30.5 cm, 31 - 33.5 cm, 34 - 37 cm, 37.5 - 40 cm, 40.25 - 43.5 cm, 44 - 47 cm, or 47.5 cm.

Collection method and number of sequential ejaculates per day was recorded by Stud B only. Collection methods included artificial vagina (AV) or electroejaculation (EE). Sequential ejaculate within day ranged from 1 to 4 collections per day at Stud B. At Stud A protocol for ejaculate collection was to collect and combine two ejaculates per day, whereas the ejaculates were kept separate for evaluation at Stud B and ejaculate number per day recorded.

After data editing was complete, Stud A included 56,811 collections from 1,715 bulls; Stud B included 14,885 ejaculates from 775 bulls.

Semen Collection Procedures and Semen Quality Measures

Bulls at both studs were collected twice per week. At Stud A bulls were ejaculated twice per collection day, at Stud B bulls ejaculated a minimum of twice per collection day and a maximum of four times. At Stud A bulls housed in Barns 1 and 2 were collected on Monday and Thursday, while bulls housed in Barns 3, 4, and 5 were collected on Wednesday and Friday. At Stud B bulls were all collected on Monday and Thursday. Semen was collected via AV by allowing bulls to mount a teaser steer. Bulls were false-mounted once before the initial collection and again before the second ejaculate at both studs. At Stud B bulls unwilling or unable to service the AV were collected via EE. At Stud A ejaculates were pooled together each collection

day and provided as one observation. At Stud B bulls were collected up to four times per day, and ejaculates were kept separate and labeled with sequential ejaculate numbers.

After collection ejaculates were analyzed for pre-freeze progressive motility, volume (mL), and concentration (spermatozoa x 10^6 per ml) by a single technician at both locations. Total number of spermatozoa per ejaculate was calculated by multiplying volume and concentration. Motility needed to be ≥ 50%, and gross spermatozoa abnormalities less than 30% to meet freezing quality standards. Concentration of spermatozoa were calculated using a spectrophotometer (NucleoCounter SP-100). Ejaculates passing these initial evaluations by the respective technicians were extended with media, packaged in 0.5 cc straws, and frozen in liquid nitrogen. At Stud A, 69% of collections passed the initial evaluation while at Stud B, 30% of ejaculates passed the initial evaluation. After freezing, one straw from each collection was thawed at 37 degrees Celsius (C) in a water bath and evaluated for initial post-thaw progressive motility. At Stud B the samples were stored at 37 degrees C and post-thaw motility was evaluated again two hours later. At Stud A initial post-thaw motility needed to be ≥ 50%, while at Stud B both initial and two-hour post-thaw motility needed to be ≥ 30%. A sample from each collection was fixed and evaluated for primary and secondary sperm abnormalities, which together were required to be ≤ 30%. Primary abnormalities were defined as those abnormalities of the head, while secondary abnormalities affected the tail. Of the ejaculates that passed the initial evaluation, 62% at Stud A and 69% at Stud B passed the post-thaw standards and were approved for distribution.

Environmental Information

Climatology data was obtained from National Renewable Energy Laboratory (NREL; DOE 2020). For each stud, the closest latitudinal and longitudinal locations were selected. Data

from the NREL included daily average wind speed (m/s), daily average temperature (C^{o}), daily average relative humidity (%), and direct solar radiation (w/m^{2}). To calculate true climatic effects of both heat and cold stress, the use of cumulative climate index (CCI) is more inclusive than the temperature-humidity index (THI; Mader et al., 2010). To capture the effect of extreme cold, as well as extreme heat, daily CCI was used instead of THI for our analysis (Butler et al., 2021). Daily CCI was calculated based on the equation from Mader et al., (2010). The CCI calculation considers both hot and cold stress. The following equation was used to calculate daily CCI (Mader et al., 2010):

Cumulative Climate Index = Ambient temperature (Celsius) + Adjusted relative humidity (%) + Adjusted wind speed (m/s) + Adjusted radiation (w/m^{2})

The spermatogenesis start date was calculated as 75 days before the day of collection and spermatogenesis was then divided into three phases. Proliferation was calculated as the first 22 days of spermatogenesis, meiosis as the next 22, and differentiation as the next 17 days (Senger, 2012). The remaining 14 days were defined as epididymal transit time (Figure 2.1). For each time frame, the daily CCI was summed then averaged over the phase. For all dates daily CCI was not calculated as NREL could not provide weather data for all dates, and those were treated as missing during the average calculations. At Stud A bulls experienced a range of CCI from -18.27 to 27.94 (Table 2.2). As defined by Mader et al., (2010) these bulls were experiencing a range of severe cold stress, to mild heat stress. At Stud B bulls experienced a range of CCI from -19.66 to 35.90 (Table 2.2). Bulls at Stud B were classified as experiencing both severe cold and severe heat (Mader et al., 2010).

Statistical Analyses

All statistical analyses were performed using SAS Version 9.4 (SAS Institute Inc., Cary, NC, USA). Due to different fixed effects potentially effecting ejaculate characteristics at each bull stud, they were analyzed separately. Backwards selection was performed utilizing the GLIMMIX procedure of SAS to determine significant effects at both studs. Fixed effects for both studs included year, age in months, breed, days since previous ejaculate, season. Average CCI during proliferation, meiosis, differentiation or ETT were included in the model as covariates For Stud A, barn location, bull body weight, and scrotal circumference were included additionally as fixed effects. For Stud B, collection method and sequential ejaculate were included as additional fixed effects. Count of ejaculates for individual bull was nested within bull and included in the model as repeated and random effects. A value of $P < 0.05$ was considered significant and required for a fixed effect to remain in the model. For each semen characteristic response variables volume, concentration, pre-freeze motility, initial post-thaw motility, primary abnormalities, secondary abnormalities, and total spermatozoa all fixed effects were removed until all remaining in the model were significant. For Stud B, two-hour post-thaw motility was also evaluated. All those effects that were left in each final model are show in Table 2.17 and Table 2.18.

Results and Discussion

In Table 2.3, levels of significance for fixed effects and covariates are shown. Data were analyzed separately for the two studs due to recording differences of fixed effects, and thus displayed separately. Descriptive statistics for each bull stud can be viewed in Tables 2.1 and 2.2. Ejaculates from bulls collected at Stud A, on average, had a pre-freeze motility greater than

the minimum required for freezing. Whereas at Stud B, pre-freeze motility on average was lower than the standards for freezing the ejaculate. Initial post-thaw sperm motility at Stud A was less on average than what was required for post-thaw distribution parameters. At Stud B, both initial-post thaw-motility and two-hour post-thaw motility were greater than the minimum requirement needed for the distribution of semen at that stud.

Age

Age in months significantly affected all semen characteristics at both studs ($P < 0.001$, Table 2.3). Ejaculate volume generally increased with bull age (Table 2.4). The lowest ejaculate volumes were from bulls less than 24 months of age at Stud A and less than 15 months of age at Stud B ($P < 0.01$). The highest ejaculate volume at Stud A were from bulls 48-60 months of age, then decreased ($P < 0.01$) at greater than 60 months of age. At Stud B, bulls greater than 48 months of age produced the greatest volume ($P < 0.01$).

Our finding of increased volume associated with increased age agrees with Everett and Bean (1982), Mathevon et al. (1998), Fuerst-Waltl et al. (2006), Snoj, et al. (2013), and Murphy et al. (2018), who all reported an increase in semen volume as bulls increased in age. Mathevon et al. (1998) and Snoj et al. (2013) reported an interaction between season of collection and bull age, which was not investigated in our study. Snoj et al., (2013) observed a decrease in semen volume for bulls 73 to 84 months of age. Taylor et al., (1985) observed similar effects where volume increased until 7 years of age and then decreased after 9 years of age. The decreases in older bulls seen by Snoj et al., (2013) and Taylor et al., (1985) were similar to our findings from Stud A.

Sperm concentration plateaued for bulls who were 30-36 months of age at Stud A, and 24-30 months of age at Stud B (Table 2.4). Bulls 12 months of age or younger had the lowest (*P*

< 0.01) concentration at both studs. At Stud A concentration steadily increased until 18-21 months of age, then peaked at 30-36. The results for Stud B were similar, although bulls reached their peak concentrations at a younger age than Stud A. At Stud B concentrations were less consistent in bulls older than 30 months as they did not decrease steadily ($P < 0.01$). Total spermatozoa per ejaculate increased with age until decreasing after 60 months at Stud A. Total spermatozoa from bulls at Stud B increased ($P<0.01$) until approximately 36 months of age.

Everett and Bean (1982), Mathevon et al. (1998), Fuerst-Waltl et al. (2006), and Murphy et al. (2018) reported increases in concentration and total spermatozoa as bull age increased. Snoj et al. (2013) reported a consistent increase in total spermatozoa with age. The optimum age to collect the highest concentration has been reported to be two years of age (Fuerst-Waltl et al., 2006; Taylor et al., 1985; Murphy et al., 2018; Mathevon et al., 1998), four years of age (Everett and Bean, 1981), and seven years of age (Amann, 1970). In our study we found the optimum age for concentration to be two to three years of age. Taylor et al., (1985) observed an increase in total spermatozoa until seven years of age and then a decrease after nine years of age. In our study we saw that bulls after 60 months of age had a decrease in total spermatozoa at Stud A, while at Stud B we did not observe this same pattern. The increase in volume, concentration, and total spermatozoa with increased age can be attributed to testicular growth which can continue up to five years after puberty (Amann and Almquist, 1976). Increases in concentration can also be attributed to the increased storage capacity within the tail of the epididymis, which increases in size and capacity until seven years of age (Amman, 1970).

Pre-freeze motility at Stud A was lowest for bulls less than 12 months of age and greatest for bulls 48 – 60 months of age (Table 2.4). At Stud B pre-freeze motility was lowest at 12 months of age and highest from bulls from 18 – 48 months of age.

At Stud A, with the greatest post-thaw motility was from bulls 60 months of age and greater, and in general, it increased with age. However, at Stud B post-thaw motility did not increase with age (Table 2.5) and two-hour post-thaw results were similar to those of initial post-thaw motility. Two-hour post-thaw motility was lowest in bulls 48-60 months, and greatest at age 12-30 months, 36-48 months of age and greater than 60 months (Figure 2.2).

Similar to our results for Stud A, others have reported findings that pre-freeze and post-thaw motility was lowest in bulls less than a year of age, but by small percentages (Murphy et al., 2018). Increased motility as bull age increased was also reported by Fuerst-Waltl et al., (2006). Conversely, motility decreased with age for bulls in Brazil (Britto et al., 2002).

Primary abnormalities were greatest ($P < 0.05$) for bulls less than 12 months at both studs (Table 2.5). At Stud A, bulls 24-60 months of age produced the least percentage of primary abnormalities; at Stud B bulls greater than 24 months of age produced the least primary sperm abnormalities. At Stud A the greatest ($P < 0.05$) percentage of secondary sperm abnormalities were produced by bulls less than 12 months of age, and greater ($P < 0.05$) than 48 months of age. Bulls at Stud B less than 12 months did not differ ($P > 0.05$) from other ages in percentage of secondary abnormalities. Bulls between 21-36 months of age and or greater than 48 months of age produced a higher ($P < 0.05$) percentage of secondary abnormalities than other age groups.

Primary and secondary abnormalities have been documented to decrease with age in dairy bulls, similar to our finding in Stud A (Chandler et al., 1985; Soderquist, et al., 1996). Results for the influence of age on secondary abnormalities drastically differed between the two bull studs, suggesting there may be an environmental cause. Brito et al., (2002) showed an increase in minor defects with age in beef bulls, which is similar to our findings for Stud B. Bulls under 12 months of age are known to have a decreased chance of passing breeding soundness

exams due to abnormal spermatozoa, thus producing lower quality semen not suitable for AI (Kennedy et al., 2002). Bulls younger than 20 months of age in Australia are less likely to pass a breeding soundness exam due to an increase in abnormal spermatozoa (Felton-Taylor et al., 2020). In dairy bulls, semen production has been shown to increase until approximately 60 months of age, after which a decrease in production was seen (Everett and Bean, 1982).

Breed

Breed significantly affected ($P < 0.001$) all semen characteristics (Table 2.6 and Table 2.7). At Stud A, Angus and Gelbvieh bulls produced greater ($P < 0.0001$) semen volume than the other breeds. Red Angus bulls produced a greater ($P < 0.0001$) volume than Horned Hereford while Simmental bulls produced greater ($P < 0.001$) volume than Charolais, Horned Hereford, and Polled Hereford. At Stud B Simmental produced a greater ($P < 0.0001$) volume than all other breeds. Angus bulls produced a greater ($P < 0.001$) volume than all other breeds, with the exception of Simmental. Polled Herefords produced the lowest ($P < 0.001$) volume when compared to all other breeds.

Ejaculates from Angus bulls at Stud A produced the lowest ($P < 0.0001$) concentration of spermatozoa when compared to Red Angus, Charolais, Horned Hereford, Polled Hereford, and Simmental. Red Angus bulls produced lower ($P < 0.001$) concentrations than Charolais, Horned Hereford, Polled Hereford, and Simmental. Polled Hereford bulls produced lower ($P < 0.001$) concentration of spermatozoa than Charolais and Horned Hereford at Stud A. At Stud B, the only difference in concentration was Gelbvieh having a lower ($P < 0.001$) concentration than the other breeds.

At Stud A, Angus and Horned Hereford bulls produced less ($P < 0.001$) total spermatozoa than Red Angus and Simmental. Simmental bulls produced more ($P < 0.001$) total

spermatozoa than Polled Hereford and Red Angus at Stud A. At Stud B Angus and Simmental bulls produced more ($P < 0.001$) total spermatozoa than Red Angus, Gelbvieh, Horned Hereford, and Polled Hereford.

At Stud A pre-freeze motility was greater ($P < 0.001$) in Charolais bulls than in all other breeds. Simmental bulls at Stud A produced greater ($P < 0.001$) pre-freeze motility than Angus, Red Angus, Horned Hereford, and Polled Hereford. Angus bulls pre-freeze motility was greater ($P < 0.001$) than Red Angus, Horned Hereford, Polled Hereford. Polled Hereford produced a higher ($P < 0.001$) percentage of pre-freeze motility than Red Angus and Horned Hereford. At Stud B, Angus, Gelbvieh, and Simmental had a higher ($P < 0.001$) pre-freeze motility than Red Angus, Horned Hereford, and Polled Hereford. Polled Hereford bulls produced a lower ($P < 0.001$) pre-freeze motility than Charolais and Horned Hereford.

Initial post-thaw motility resulted in similar breed differences to pre-freeze motility (Table 2.7). Initial post-thaw motility was greater ($P < 0.001$) for Angus compared to Red Angus, Horned Hereford, and Simmental at Stud A. Red Angus had greater post-thaw motility than Horned Hereford, but lower ($P < 0.001$) than Charolais, Gelbvieh, Polled Hereford, and Simmental. At Stud B Angus had greater ($P < 0.001$) initial post-thaw motility than Red Angus, Gelbvieh, and Horned Hereford. Red Angus, Gelbvieh, and Horned Hereford had lower ($P < 0.001$) initial post-thaw motility than Simmental.

At Stud B, similar to the initial post-thaw, Angus had greater ($P < 0.001$) two-hour post-thaw motility than Red Angus, Gelbvieh, and Horned Hereford (Figure 2.3). Simmental had greater ($P < 0.001$) two-hour post-thaw motility than Red Angus, Gelbvieh, Horned Hereford. Horned Hereford had the lowest ($P < 0.001$) two-hour post-thaw motility when compared with Charolais, Gelbvieh, and Polled Hereford.

Average abnormal spermatozoa within breeds varied greatly between Stud A and B (Table 2.7). At Stud A, Charolais and Polled Hereford bulls produced more ($P < 0.001$) primary abnormalities than Angus, Red Angus, Horned Hereford, and Simmental. At Stud B, Red Angus, Gelbvieh, Horned Hereford, and Polled Hereford produced more ($P < 0.001$) primary abnormalities than all other breeds.

At Stud A, Angus and Red Angus produced more secondary abnormalities than Charolais, Horned Hereford, Polled Hereford, and Simmental ($P < 0.001$). Polled Hereford bulls produced more ($P < 0.001$) secondary abnormalities than Charolais and Simmental. At Stud B Charolais produced fewer ($P < 0.001$) secondary abnormalities than all other breeds. Gelbvieh bulls produced fewer ($P < 0.001$) secondaries than Angus and Red Angus bulls.

Information regarding semen quality difference between breeds is limited. Semen volume from Hereford bulls was less than Angus bulls (Fields et al., 1979). Fields et al. (1979), also concluded that Hereford bulls had a higher percentage of motile spermatozoa compared to Angus bulls. Hereford bulls from Montana genetic lines were the most affected by the adverse environmental conditions; authors suggested this may be explained by genotype and environmental interactions (Fields et al., 1979). This may explain some differences we observed for Hereford bulls in our study. Gelbvieh and Simmental bulls have been classified as satisfactory for breeding soundness exams more often than Polled Herefords due to semen quality (Kennedy et al., 2002). Simmental and Aberdeen Angus bulls were more likely to pass a BSE than Hereford or Charolais bulls due to semen quality (Barth and Waldner, 2002). Gelbvieh bulls produced more sperm head abnormalities than Angus, Charolais, Hereford and Simmental (Menon, et al., 2011). Tail defects were greater in Angus and Hereford bulls compared to Charolais, Gelbvieh, and Simmental (Menon et al., 2011).

Season

Season significantly affected all characteristics except for primary abnormalities in Stud A ($P < 0.05$). The least square means for all characteristics are shown in Table 2.8 and Table 2.9.

Volume at Stud A was the greatest ($P < 0.001$) for bulls collected in the spring compared to those collected in the fall and winter, and at Stud B was highest ($P < 0.05$) in the summer compared to fall and winter. At Stud A bulls collected in the summer had a greater ($P < 0.001$) semen volume than those collected in fall. At Stud B bulls collected in the spring had a greater ($P < 0.05$) volume than those collected in the fall.

The sperm concentration of ejaculates collected in the spring and summer were greater ($P < 0.01$) than those collected in the fall and winter at Stud A. At Stud B bulls collected in summer had a greater ($P < 0.05$) concentration compared to collections in the fall, spring, and winter.

Total spermatozoa per ejaculate at Stud A was greatest ($P < 0.001$) in the spring, next highest in the summer, which was the same as winter, and lowest in the fall. At Stud B bulls collected in the summer produced the greatest ($P < 0.001$) total spermatozoa compared to fall, spring, and winter.

In a previous study, June, July and August were best for quantitative semen traits (Stalhammar et al., 1989). Bulls collected in spring and summer months (April – August) have been shown to have a decrease in testicular size and a reduction in semen production in Hereford and Holstein bulls (Fields et al., 1979; Coulter and Foote, 1976). These authors suggested these breeds maybe more susceptible to heat and humidity (Fields et al., 1979; Coulter and Foote, 1976). Bulls collected from April to August had increased ejaculate concentrations (Fields et al., 1979). In agreement, the highest volumes and total spermatozoa were recorded in the summer, and lowest in the winter (Snoj et al, 2013). Snoj et al., 2013 concluded strong seasonal effects on

volume and total spermatozoa in European cattle breeds, like those in our study. Summer and fall ejaculates produced greater concentrations and total spermatozoa than spring and winter (Murphy et al., 2018). Bulls collected in winter tended to produce a lower volume than spring (Murphy et al., 2018). In Brazil, season did not impact production or quality of beef semen (Brito et al., 2002).

Pre-freeze motility at Stud A in the fall was greater ($P < 0.001$) than in the spring and winter. At Stud B pre-freeze motility was greater ($P < 0.01$) in the spring than in fall, summer, and winter. Pre-freeze motility in fall was greater ($P < 0.001$) than in winter at Stud B. Initial post-thaw motility at Stud A in the fall was greater ($P < 0.001$) than in the spring, summer, and winter. At Stud B bulls collected in spring had greater ($P < 0.05$) motility than those collected in winter. Two-hour post-thaw motility at Stud B was greater ($P < 0.05$) in the spring and summer compared to fall (Figure 2.4).

Literature on seasonal effect on sperm motility was limited. One study saw that semen collected in the winter had a greater post-thaw motility than spring (Murphy et al., 2018).

Primary abnormalities were not significantly affected ($P > 0.05$) by the season at Stud A. At Stud B bulls collected in the summer and winter had a greater ($P < 0.001$) percentage of primary abnormalities than those collected in spring and fall. Secondary abnormalities at Stud A were lower ($P < 0.05$) in the spring compared to fall, summer, and winter. At Stud A secondary abnormalities were fewer ($P < 0.05$) in the summer than in the fall. At Stud B bulls in the spring produced fewer ($P < 0.001$) secondaries than those collected in the winter.

Total sperm abnormalities in dairy bulls are greater in the spring and summer compared to fall and winter (Soderquist et al., 1996). In Australia, the effect of season did not significantly impact semen characteristics of bulls during breeding soundness exams, they did, however, find

an impact on individual abnormalities, (Felton-Taylor et al., 2020). In agreement with our findings for primary abnormalities, percentages of vacuoles and teratoids were increased in the summer compared to spring, fall, and winter (Felton-Taylor et al., 2020). However, our differences were numerically small and may not be biologically relevant. Bulls collected in the winter, spring, and fall and greater midpiece abnormalities than summer, which is similar to our findings for secondary abnormalities (Felton-Taylor et al., 2020).

Days since previous ejaculate

Days since previous ejaculate affected ($P < 0.0001$) all semen characteristics. The least square means for all characteristics are shown in Table 2.10 and Table 2.11.

Volume steadily increased ($P <0.0001$) with each day between collections at Stud A until days six and seven which were the same, after which volume continued to increase. Days between collections from less than 1 day to 3 days produced less ($P < 0.001$) volume than 6 days between and greater.

Ejaculates collected 5 or more days between ejaculates had the highest ($P < 0.001$) sperm concentrations at Stud A. At Stud B, 3 days, 4 days, 5 days, and greater than 30 days between ejaculates resulted in the highest ($P < 0.001$) concentrations. One day or less ($P < 0.001$) between collections resulted in a lower sperm concentration than 2 days between ejaculates.

Similar to the pattern for volume, total spermatozoa at Stud A increased ($P < 0.05$) with each day from the previous ejaculate until 4 days between ejaculates, which was not different than 6 and 7 days between ejaculates. At Stud B 5 days, 6 days, and greater than 30 days between collections resulted in more ($P < 0.001$) total spermatozoa than one day or less, 2 days, 3 days, 4 days, and 7 to 30 days between ejaculates. Less than 2 days between ejaculates,

produced lower ($P < 0.001$) total spermatozoa per ejaculate than 3 days, 4 days, and 7 days between ejaculates.

Similar to our findings, Everett and Bean (1982) saw that as days between collections increased, so did total spermatozoa and concentration. Others have also shown that extending the days between collections up to a 5-day interval results in an increase in volume, concentration, and total sperm in the ejaculate (Seidel and Foote 1969, Everett et al., 1978). Fuerst-Waltl et al., (2006) reported the greatest ejaculate volumes came from an interval of 6-9 days, while the interval for concentration was ideal between 4-9 days. Methevon et al., (1998) concluded that the ideal days between collections were age-dependent, with young bulls having the greatest volume and concentration when collected every 4 to 7 days, and mature bulls every 5 days. We did not investigate the interaction of collection interval and age in our study as it was not practical in these collection facilities to adjust schedules for individual bulls. Mathevon et al., (1998) did find that the greatest overall total spermatozoa were collected when bulls were on a 2-day interval.

Pre-freeze motility was greater ($P < 0.001$) for 1 day or less between ejaculates than for all other collection intervals at Stud A. Stud A pre-freeze motility was higher ($P < 0.001$) for collections 3 days apart when compared to 7 and greater than 30-day intervals. At Stud B collection intervals of 1 day or less resulted in the lowest ($P < 0.001$) pre-freeze motility when compared to all other intervals except for 7 days and greater. Six days since the previous collection at Stud B resulted in greater ($P < 0.05$) pre-freeze motility than 7 days between collections and greater.

Initial post-thaw motility at Stud A was lower ($P < 0.001$) for bulls collected after 1 day or less between collections than after 2 days between collections (Table 2.11). At Stud B initial post-thaw motility was highest ($P < 0.001$) for bulls collected 1 day or less since the last

ejaculate compared to all other groups. At Stud B two-hour post-thaw motility was greater ($P < 0.001$) for collection intervals of 1 day or less than 3 or 4 days between collections and 7 to thirty days between collections (Figure 2.5).

Some studies have shown that motility is not impacted by the length of time between collections, and that increasing collection intervals from 2 to 5 days does not make a difference (Fuerst-Waltl et al., 2006; Seidel and Foote, 1969). At both bull studs in our study, the best collection intervals were from 3-4 days. While we did observe other significant differences in motility in our study, they are small and of little biological value.

Primary abnormalities at Stud A were lower ($P < 0.001$) for an interval of 2 days between collection when compared to 3 through 7 days between collections. At Stud B 1 day or less between collections produced more ($P < 0.001$) primary abnormalities than 4 days between and 7 days between collections.

Secondary abnormalities were highest ($P < 0.001$) for bulls collected 1 day or less since the previous ejaculate when compared to 2 through 5 days and greater than 7 days at Stud A. At Stud B 1 day or less since the previous ejaculate resulted in greater ($P < 0.001$) secondary abnormalities compared to 3 and 4 days between collection intervals. At Stud B an interval of 7 through thirty days between collection intervals resulted in greater ($P < 0.001$) secondary abnormalities than 2 through 5 days, and seven days between collection intervals.

Literature on the effect of collection interval on spermatozoa abnormalities was not found as most published worked focused on the depletion of sperm. The pressure to produce semen within a short timeline limits the number of days that can pass between collections. Intense collection schedules are driven by the need to collect and distribute semen from high demand bulls (Amann and DeJarnette, 2012). Our findings agree with previous literature justified the

current collection pattern of most facilities; with bulls being collected 2 to 3 times per day at 3- to 4-d intervals (Bratton and Foote, 1954; Hafs et al., 1959).

Environmental Influence on Spermatogenesis

Average CCI during Proliferation

The average CCI during proliferation did not affect semen volume at Stud A ($P = 0.07$) but did at Stud B ($P < 0.05$). The effect solution for CCI during proliferation at Stud B was -0.0063, suggesting a decrease in volume for every unit increase in average CCI during proliferation. Sperm concentration was affected ($P < 0.001$) by CCI during proliferation at Stud A but not ($P = 0.50$) Stud B. The effect solution for the impact on sperm concentration at Stud A for CCI during proliferation was 3.97, suggesting for every unit change in average CCI during proliferation the concentration will change 3.97×10^6 spermatozoa per ml. Likewise, total spermatozoa was affected by CCI during proliferation at Stud A ($P < 0.05$), but not at Stud B ($P = 0.31$). The effect solution for average CCI during proliferation at Stud A was 10.71. Although pre-freeze motility was not affected by the average CCI during proliferation, post-thaw motility was influenced ($P < 0.05$) by the CCI at both studs. The effect solution for average CCI during proliferation at Stud A was -0.04, and 0.07 at Stud B. Two-hour post-thaw motility at Stud B was influenced ($P < 0.05$) by the CCI during proliferation, and its effect solution was 0.11. Primary abnormalities were influenced at Stud A ($P < 0.0001$), but not at Stud B ($P > 0.05$). Primary abnormality effect solution for CCI during proliferation at Stud A was -0.04. Secondary abnormalities were not affected ($P = 0.47$) at Stud A but were affected ($P < 0.05$) at Stud B. The effect solution for CCI during proliferation at Stud B were -0.10.

Average CCI during Meiosis

The average CCI during meiosis did not affect ejaculate volume but did affect ($P < 0.05$) sperm concentration (Table 2.3) at both studs. Effect solutions for average CCI during meiosis on concentration was -1.58 at Stud A, and -2.78 at Stud B. Total spermatozoa was affected ($P < 0.0001$) by CCI at Stud B and tended ($P < 0.09$) to be affected at Stud A. For total spermatozoa, the effect solution for CCI during meiosis at Stud B was -21.20. Pre-freeze motility and initial post-thaw motility were affected ($P < 0.05$) by CCI at Stud A, but not ($P > 0.05$) at Stud B. Two-hour post-thaw motility was also not ($P > 0.05$) affected at Stud B. Primary abnormalities tended to be affected ($P < 0.07$) at Stud A, and not ($P > 0.05$) at Stud B. Secondary abnormalities were not ($P > 0.05$) affected at either stud by CCI during meiosis.

Average CCI during Differentiation

Average CCI during differentiation significantly affected ($P < 0.001$) volume and concentration of ejaculates at Stud A, but not ($P > 0.05$) at Stud B. For volume, average CCI during differentiation effect solutions were 0.02, and -2.16 for concentration. Average CCI during differentiation did not ($P > 0.05$) affect total spermatozoa produced at either stud. Pre-freeze motility was not ($P > 0.05$) affected by CCI during differentiation at Stud A but was affected ($P < 0.01$) at Stud B. Average CCI during differentiation effect solution was -0.09 for pre-freeze motility. Initial post-thaw motility was not affected ($P > 0.05$) at either stud, at Stud B two-hour post-thaw motility was also not affected ($P > 0.05$). Primary and secondary abnormalities were not affected ($P > 0.05$) by average CCI during differentiation at Stud A. At Stud B primary and secondary abnormalities were affected ($P < 0.05$) and effect solutions were 0.12 for primary abnormalities and 0.16 for secondary abnormalities.

Average CCI during Epididymal Transit

The average CCI during epididymal transit significantly affected ($P < 0.001$) volume, and total spermatozoa of ejaculates at Stud A, but not at Stud B. Effect solutions for average CCI during epididymal transit and volume were 0.02, and for total spermatozoa were 27.16. The average CCI during epididymal transit tended ($P = 0.06$) to affect concentration at Stud A, but was not affected at Stud B. At both bull studs, pre-freeze motility and initial post-thaw motility were affected ($P < 0.05$) by the average CCI during epididymal transit. For pre-freeze effect solutions for CCI during epididymal transit were 0.13 at Stud A, at Stud B the effect solution was -0.06. For initial post-thaw motility effect solutions for CCI during epididymal transit were 0.06 at Stud A, and -0.11 at Stud B. At Stud B two-hour post-thaw motility was also influenced ($P < 0.05$) by CCI during epididymal transit and had an effect solution of -0.14. At both bull studs, both primary and secondary abnormalities were affected ($P < 0.05$) by average CCI during epididymal transit. Effect solutions for the effect of CCI during epididymal transit on primary abnormalities was 0.03 at Stud A and 0.15 at Stud B. Effect solutions of CCI during epididymal transit on secondary abnormalities was -0.02 at Stud A and 0.14 at Stud B.

Previous literature has focused on the effects of ambient temperature and humidity. Everett and Bean (1982) found that a change in ambient temperature on the day of the collection did not affect semen production. A change in humidity on the day of the collection, however, did affect total spermatozoa output and the volume of semen (Everett and Bean, 1982). Everett and Bean (1982) concluded that their environmental results were inconsistent. Taylor et al., (1985) concluded extreme ambient temperature had only minor effects on semen on Holstein bulls.

Barn Location at Stud A

Bulls were moved between barns based on health testing results, and producer-desired outcomes. Barn location at time of collection significantly affected all semen characteristics (Table 2.12). Bulls in Barn 5 produced the greatest ($P < 0.001$) ejaculate volume (8.2 $\pm$ 0.15 ml) among the five barns at Stud A. Barns 1, 2 and 3 were similar in volume, while bulls in Barn 4 produced the lowest amount of volume (7.5 $\pm$ 0.13 ml) among the five barns. Bulls in Barn 1 produced the greatest ($P < 0.001$) ejaculate concentrations (1053.44 $\pm$ 17.03 x 10^6 per ml) among the five barns at Stud A. Bulls in Barn 2 had the lowest ($P < 0.001$) ejaculate concentrations compared to all other barns (929.03 $\pm$ 17.01 x 10^6 per ml). Barns 3 and 5 were similar in concentration. Barns 2 and 5 produced greater ($P < 0.001$) total spermatozoa than Barns 1 and 4. Total spermatozoa was lowest ($P < 0.001$) at Barns 1 and 4 compared to Barns 2, 3, and 5. Ejaculates collected in Barn 5 had greater ($P < 0.05$) post-thaw motility than Barn 2 and 3. Pre-freeze motility at Barn 1 was lowest ($P < 0.001$) when compared to all other barns. Bull collected in Barns 2 and 4 had ejaculate pre-freeze motilities lower ($P < 0.001$) than Barns 3 and 5. Post-thaw motility from ejaculates collected in Barns 1 and 4 was lower ($P < 0.001$) than Barns 3 and 5. Primary sperm abnormalities in Barn 1 were greater ($P < 0.001$) than Barns 2, 3, and 4. Ejaculates from Barn 2 and 5 produced more ($P < 0.001$) primaries than Barn 3. Ejaculates collected in Barn 2 had fewer ($P < 0.05$) secondary abnormalities than Barns 1, 3, and 4.

It has been reported that bulls collected on certain days of the week had an increase in semen production (Everett and Bean, 1982). In our analysis the barn locations determined the day of the week they were collected, demonstrating a similar pattern to Everett and Bean (1982). They found that bulls collected on Tuesdays and Fridays had the greatest semen production compared to other days of the week (Everett and Bean, 1982). Ejaculate volume, concentration,

and total spermatozoa vary greatly based on the barn of the collection (Taylor et al., 1985). Taylor et al., (1985) concluded that some barn differences may be confounded with age, as bulls in some barns may still need to progeny tested. This is similar to our study where the bulls progressed through barns as they completed health testing; however, bulls of all ages were collected in each barn.

In one study, collection team personnel influenced volume and the total number of sperm but did not affect concentration and motility (Mathevon et al., 1998). Individual bull handlers have been shown to have significant effects on volume, concentration, and percent viable spermatozoa, but not on motility (Fuerst-Waltl et al., 2006). Fuerst-Waltl et al., (2006) observed semen collector influences on ejaculate volume in their study, however; they only observed significant influence on concentration and motility in one of their locations. These data suggest that personnel within collection facilities can play a role in the success of a collection.

Weight at Stud A

The weight of bulls at the time of collection affected all semen characteristics. The least-square means for weight and its association to ejaculate characteristics are shown in Table 2.13. Interestingly, bulls in the heaviest weight group (>1134kg) produced the greatest volume of semen (8.9 $\pm$ 0.13 ml) than all other weight groups except for the lightest group (< 430 kg). Concentration of ejaculates from bulls weighing 385.6 – 430.9 kg was greater ($P < 0.05$) than bulls weighing > 907.2 kg. Bull's weighing 608.4 – 907.2 kg also produced greater ($P < 0.001$) sperm concentrations than bulls weighing over 907.2 kg. Total spermatozoa produced only differed ($P < 0.001$) when bulls weighing over 1134.0 kg were compared to bulls weighing 453.6 – 1134.0 kg. Pre-freeze motility from bulls weighing 385.6 – 430.9 kg was greater ($P < 0.001$) than bulls weighing 430.9 – 1134.0 kg. Post-thaw motility was greater ($P < 0.05$) in bulls

weighing more than 680.4 kg compared to bulls weighing 430.9 - 680.4 kg. Bulls weighing over 1134.0 kg produced a greater ($P < 0.001$) post-thaw motility than bulls weighing between 680.4 and 1134.0 kg. Primary spermatozoa abnormalities were lower ($P < 0.05$) in bulls weighing over 1134.0 kg when compared to bulls weighing 680.4 – 1134.0 kg. Secondary abnormalities tended to be greater ($P < 0.1$) in bulls weighing 453.6 to 907.2 kg and bulls weighing more than 1134.0 kg. For all semen characteristics there was no clear association between weight and semen outcomes.

Scrotal Circumference at Stud A

The scrotal circumference of bulls at the time of collection affected all semen characteristics. The least-square means for scrotal circumference are shown in Table 2.14. There was a strong association of increased scrotal circumference (SC) with increased semen volume. Bulls with a SC greater than 44 cm produced more ($P < 0.5$) ejaculate volume (8.6 – 8.8 ml) than bulls with a smaller SC. The lowest volumes were from bulls in the lowest 3 classes of SC, while bulls between 37.5 and 43.5 produced an intermediate volume (7.8 and 8.3 ml, respectively).

The concentration of ejaculates did not differ ($P > 0.05$) between 21 - 30.5 cm and 31 – 33.5 cm, or between 44 – 47 cm and > 47.5. The concentration of ejaculates increased ($P < 0.001$) as SC increased until circumference reached 40.25 cm. After which 40.25 – 43.5 cm tended to produce a lower concentration than greater than 47.5 cm, and there was no difference between 44 – 47 and greater than 47.5 cm ($P > 0.05$). Total spermatozoa followed a similar pattern to that of concentration. There was no difference between 21 – 30.5 cm SC and 31 – 33.5 cm SC. After which there was a significant increase ($P < 0.001$) in total spermatozoa produced for all categories of scrotal size.

Scrotal circumference affected all pre-freeze motility except for 31-33.5 cm compared to 44 cm and greater, and 44 – 47 cm compared to greater than 47.5 cm ($P < 0.001$). Post-thaw motility did not differ between 21 -30.5 cm ($P > 0.05$) and 31 – 33.5 cm and tended to differ 21 – 30.5 cm and 34 – 37 cm ($P < 0.1$). Post-thaw motilities decreased as scrotal circumference increased in most cases. It was not different between 31 – 33.5 cm and 34 – 37 cm scrotal circumferences. It did not differ when 37.5 - 40 cm and 40.25 – 43.5 cm SC were compared. It did not differ when 44 – 47 cm and greater than 47 cm SC were compared.

In bulls with a SC of greater than 47.5 cm, primary abnormalities were decreased ($P < 0.001$) compared to 31 – 40 cm and 44 – 47 cm. Bulls with a SC of 40.25 – 43.5 cm had fewer ($P < 0.05$) primaries than bulls with 31 – 40 cm and 44 – 47 cm. While SC was significant in the overall model, for secondary abnormalities there was no difference in the least squared means between SC sizes.

Bulls included in our analysis did have SC smaller than those required for breeding soundness examinations. While it was not required that bulls pass a breeding soundness exam at this facility, our data shows that those bulls had decreased semen quality and quantity when compared to other SC groups. SC below minimum scrotal requirements had decreased sperm motility and greater sperm abnormalities than bulls of satisfactory scrotal size during breeding soundness exams (Barth and Waldner, 2002). Menon et al., (2011) showed that sperm abnormalities decreased with increasing scrotal circumference, but there was no overall significant effect. In our study we saw consistent quantity increases, but less consistent results for quality.

Collection Method at Stud B

The collection method did not affect total spermatozoa, post-thaw motility, or two-hour post-thaw motility (Table 2.15). Ejaculates that were collected via EE had greater ($P < 0.001$) ejaculate volume than those that were collected with AV. The concentration of spermatozoa collected with EE was less ($P < 0.001$) than those collected an AV. Pre-freeze motility was greater ($P < 0.001$) for ejaculates collected with EE compared to AV. Primary abnormalities were greater ($P < 0.001$) for AV collections than those collected with EE, conversely AV ejaculates had a lower ($P < 0.001$) percentage of secondary abnormalities than EE.

An AV is the preferred method of collection and the frequent use of EE at Stud B is not common among most collection facilities. It was first reported in the 1950s that EE could be used for regular collection without detrimental impacts on semen quality (Dziuk et al., 1954). In agreement with our results, several studies have shown the use of an EE to result in greater volumes and lower concentrations than AVs (Dziuk et al., 1954; Austin et al., 1961; Foster et al., 1970). Similar to Austin et al., (1961) we also found that bulls collected with EE had higher initial pre-freeze motilities. Barth et al., (2004) found when collecting bulls for breeding soundness exams results were inconsistent in their study as in some years ejaculate volume and motility were greatest with EE over AV collections, but in other years this result was reversed.

Sequential ejaculate within a day at Stud B

For semen traits that were affected by sequential ejaculates per day, the least squared means are displayed in Table 2.16.

The volume of the second ejaculate of the day was greater ($P < 0.001$) than ejaculates 1 and 3 but similar to the ejaculate 4. It has been shown that the first ejaculate of the day had the greatest volume in some studies (Seidel and Foote, 1969; Everett et al., 1978; Fuerst-Waltl et al.,

2006), but was different for other studies (Foster et al., 1970). Ejaculate 1 had the greatest ($P < 0.001$) concentration of sperm (690 million/ml) compared to ejaculates 2, 3 and 4 (490 – 598 million/ml). The decrease after the first ejaculate agrees with other findings (Seidel and Foote, 1969; Everett et al., 1978; Fuerst-Waltl et al., 2006). Total spermatozoa were greatest ($P < 0.001$) for the first collection of the day when compared with ejaculate 2, 3, and 4. This finding is consistent with reports in the literature (Seidel and Foote, 1969; Everett and Bean, 1982). Ejaculate 2 of the day produced more ($P < 0.001$) total spermatozoa than ejaculate 3. Pre-freeze motility in ejaculate 1 was lower ($P < 0.001$) than ejaculates 2, 3, and 4. The second ejaculate of the day resulted in a lower ($P < 0.001$) pre-freeze motility than the ejaculate 3. Initial post-thaw and two-hour post-thaw motility were lower ($P < 0.001$) than for the first ejaculate of the day when compared to ejaculate 2 and 3. The effect on motility in our study is not consistent with other reports of no effect on motility due to ejaculate sequence. (Seidel and Foote, 1969; Foster et al., 1970; Everett et al., 1978; Fuerst-Waltl et al., 2006).

The second ejaculate of the day produce greater volume, concentration, total spermatozoa output, and pre-freeze motility compared to the other ejaculates on that day (Murphy et al., 2018). This same report did not find an effect of sequential ejaculate number on post-thaw motility.

Sequential ejaculate collections within a day did not affect primary abnormalities ($P > 0.05$). For secondary abnormalities, the first ejaculate of the day produced the greatest ($P < 0.01$) percentage of abnormalities compared to the third.

Conclusion

Multiple variables affected semen quality and quantity, and they were not consistent at both facilities. The most unmanageable traits that affected semen characteristic outcomes were

bull age and breed. Understanding the impact of these variables at each of these studs will help them communicate those differences with producers and adjust collection expectations. In general, as days since previous ejaculate increased, so did semen quality and quantity. However, extending the length of this interval may not be realistic in all scenarios, such as when collecting high demand bulls. Season and CCI both impacted semen. While it may not be possible to mitigate all environmental effects, different types of housing may provide a way for these studs to increase semen quality and quantity. Other management factors such as barn locations and collection method may also improve semen collection. Understanding these different biological and management factors may help producers and collectors adjust semen output expectations.

Figure 2.1 Timeline of spermatogenesis used to calculate average cumulative climate index for each ejaculate

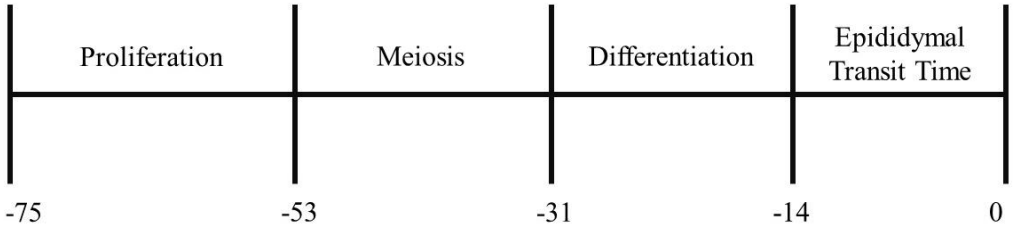


Table 2.1 Non-adjusted means, medians, and ranges for variables describing response criteria of ejaculates at two different semen collection facilities collected from 2008 to 2018

Variable	N	Mean ± SD	Median	Range
	Stud A			
Volume (mL)	50571	8.0 ± 4.19	7.3	0.2 to 56
Concentration x 10^6 per ml	50324	1015.40 ± 504.64	957	10 to 3906
Pre-Freeze Motility, %	50568	50 ± 15.75	50	0 to 100
Primary Abnormalities, %	23897	12 ± 5.60	11	0 to 77
Secondary Abnormalities, %	23893	12 ± 6.31	11	0 to 65
Initial Post-Thaw Motility, %	34645	46 ± 13.35	50	0 to 75
Total Spermatozoa per ejaculate	50324	8082.73 ± 5703.39	6864.00	56 to 69795
	Stud B			
Volume (mL)	14709	4.6 ± 2.24	4.1	0.5 to 30
Concentration x 10^6 per ml	14856	641.25 ± 396.99	600	6 to 2561
Pre-Freeze Motility, %	13460	40 ± 13.09	40	0 to 80
Initial Post-Thaw Motility, %	4577	37 ± 7.70	35	0 to 60
Two-hour Post-Thaw Motility, %	4567	33 ± 8.88	35	0 to 60
Primary Abnormalities, %	4569	14 ± 13.89	10	1 to 100
Secondary Abnormalities, %	3156	16 ± 13.28	13	1 to 100
Total Spermatozoa per ejaculate	4782	3035.79 ± 2609.05	2386.30	30 to 28000

Table 2.2 Non-adjusted means, medians, and ranges for variables influencing ejaculates at two different semen collection facilities collected from 2008 to 2018

Variable	N	Mean ± SD	Median	Range
	Stud A			
Age in months	56811	36 ± 23.85	26	10 to 120
Days Since Previous Ejaculate	55340	7 ± 37.05	3	0 to 1073
Body Weight (kg)	54072	839 ± 197.83	825.5	390.1 to 1360.8
Scrotal Circumference (cm)	54397	40 ± 3.74	40	21 to 55
Average CCI Proliferation (CCI)[1]	57388	1.85 ± 11.93	-2.03	-16.71 to 27.48
Average CCI Meiosis (CCI)[1]	57388	2.12 ± 11.49	-0.96	-16.93 to 27.48
Average CCI Differentiation (CCI)[1]	57388	2.64 ± 11.16	0.21	-17.72 to 27.78
Average CCI Epididymal Transit (CCI)[1]	57388	3.21 ± 10.96	1.69	-18.27 to 27.94
	Stud B			
Age in months	14859	35 ± 22.10	26	10 to 141
Days Since Previous Ejaculate	14093	9 ± 53.89	2	0 to 1094
Average CCI Proliferation (CCI)[1]	14365	8.44 ± 3.89	3.89	-19.66 to 35.38
Average CCI Meiosis (CCI)[1]	14340	8.06 ± 5.74	5.74	-19.89 to 35.51
Average CCI Differentiation (CCI)[1]	14323	8.03 ± 7.28	7.28	-18.78 to 35.90
Average CCI Epididymal Transit (CCI)[1]	14306	8.28 ± 7.75	7.75	-13.43 to 35.90
[1]Cummulative climate index (CCI)				

Table 2.3 Levels of significance for fixed effects used to analyze ejaculate characteristics within each stud

	Ejaculate Characteristic															
Fixed effect	Volume (ml)		Concentration x 10^6 per ml		Pre-Freeze Motility (%)		Total Spermatozoa per ejaculate		Post-thaw Motility (%)		Two-hour post-thaw Motility (%)		Primary Sperm Abnormalities (%)		Secondary Sperm Abnormalities (%)	
Stud	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
n	45211	12735	45001	12158	45208	12849	45001	12149	31222	3873	--	3865	21953	3980	21949	4100
Breed	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	--	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Age	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0002	--	0.0002	<0.0001	<0.0001	<0.0001	<0.0001
Season	<0.001	<0.0001	<0.0001	<0.0001	0.0007	<0.0001	<0.0001	<0.0001	<0.0001	0.0345	--	0.0050	0.4551	<0.0001	<0.0001	0.0014
Days since previous ejaculate	<0.001	<0.0001	<0.001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0021	0.0349	--	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
CCI Proliferation	0.0736	0.0372	<0.001	0.4983	0.6100	0.8811	0.0269	0.3101	0.0444	0.0016	--	<0.0001	<0.0001	0.4473	0.4671	0.0225
CCI Meiosis	0.1841	0.9481	0.0207	<0.0001	0.0002	0.7725	0.0956	<0.0001	0.0453	0.6220	--	0.8452	0.0664	0.6931	0.8777	0.4021
CCI Differentiation	<0.001	0.9702	0.0002	0.5576	0.1798	0.0020	0.6011	0.4672	0.8713	0.1069	--	0.5944	0.0542	0.0266	0.8463	0.0114
CCI ETT	<0.001	0.4685	0.0687	0.9662	<0.0001	0.0283	<0.0001	0.9226	0.0004	<0.0001	--	<0.0001	<0.0001	0.0056	0.0475	0.0144
Barn	<0.001	--	<0.001	--	<0.0001	--	<0.0001	--	<0.0001	--	--	--	<0.0001	--	<0.0001	--
Weight	<0.001	--	<0.001	--	<0.0001	--	<0.0001	--	<0.0001	--	--	--	<0.0001	--	0.0428	--
Scrotal	<0.001	--	<0.001	--	<0.0001	--	<0.0001	--	<0.0001	--	--	--	<0.0001	--	0.0297	--
Collection Method	--	<0.0001	--	<0.0001	--	0.0015	--	0.1970	--	0.3560	--	0.5672	--	0.0125	--	<0.0001
Sequential ejaculates in one day	--	<0.0001	--	<0.0001	--	<0.0001	--	<0.0001	--	<0.0001	--	<0.0001	--	0.4512	--	0.0122

Table 2.4 Effect of bull age on pre-freeze characteristics (volume, concentration, total spermatozoa, and pre-freeze motility) at Studs A and B from 2008 through 2018[*]

	Least squares mean ± SE							
Age in months	Volume (ml)		Concentration x 10^6 per ml		Total Spermatozoa per ejaculate[1] x 10^6		Pre-freeze Motility (%)	
	Stud A	Stud B	Stud A	Stud B	Stud A	Stud B	Stud A	Stud B
N	45,211	12,735	45,001	12,158	45,001	12,149	45,208	12,849
≤ 12 months	7.1^{feg} ± 0.22	3.1^{a} ± 0.17	635.09^{a} ± 28.12	351.54^{a} ± 33.65	4895.90^{a} ± 302.80	1042.67^{a} ± 214.61	43^{a} ± 0.91	41^{a} ± 1.09
13-15 months	7.1^{f} ± 0.14	3.6^{a} ± 0.10	811.31^{b} ± 18.60	513.87^{b} ± 18.68	5831.86^{b} ± 200.23	1862.20^{b} ± 119.22	48^{b} ± 0.60	45^{b} ± 0.63
16-18 months	7.0^{fg} ± 0.14	3.9^{b} ± 0.11	926.36^{c} ± 18.58	553.51^{bc} ± 20.40	6333.71^{c} ± 200.15	2223.44^{c} ± 130.2	50^{cd} ± 0.60	45^{bc} ± 0.69
19-21 months	6.8^{g} ± 0.14	4.2^{b} ± 0.10	1040.33^{d} ± 18.40	589.75^{cd} ± 18.30	6975.51^{d} ± 197.98	2563.71^{d} ± 116.72	50^{cd} ± 0.59	44^{c} ± 0.62
22-24 months	7.5^{e} ± 0.14	4.6^{c} ± 0.11	1044.05^{d} ± 18.28	604.12^{cd} ± 20.28	7793.66^{e} ± 196.88	2865.39^{d} ± 129.16	51^{cd} ± 0.59	45^{bc} ± 0.68
25-30 months	7.8^{d} ± 0.14	4.6^{c} ± 0.10	1075.66^{e} ± 18.06	674.95^{f} ± 18.19	8208.53^{f} ± 194.44	3261.79^{e} ± 115.91	51^{cd} ± 0.58	44^{bc} ±0.62
31-36 months	8.0^{d} ± 0.15	4.9^{d} ± 0.11	1113.44^{f} ± 19.16	640.73^{ef} ± 19.84	8866.94^{g} ± 206.30	3244.69^{e} ± 126.53	50^{cd} ± 0.62	44^{ac} ± 0.62
37-48 months	8.6^{c} ± 0.14	5.3^{e} ± 0.09	1109.33^{f} ± 18.56	645.51^{ef} ± 17.95	9379.56^{h} ± 199.85	3647.49^{f} ± 114.46	50^{bc} ± 0.60	43^{ac} ± 0.60
49-60 months	9.4^{a} ± 0.15	5.5^{ef} ± 0.10	1107.42^{ef} ± 18.97	663.86^{f} ± 19.84	10122.00^{i} ± 204.22	3872.53^{f} ± 126.93	52^{e} ± 0.61	41^{a} ± 0.66
> 60 months	9.1^{b} ± 0.14	5.7^{f} ± 0.10	1027.59^{d} ± 18.43	610.99^{de} ± 18.30	9215.67^{gh} ± 198.44	3639.94^{f} ± 116.87	51^{d} ± 0.59	42^{a} ± 0.62

[a,b,c,d,e,f,g,h,i]Values within a column and within stud without a common superscript differ ($P < 0.05$)

[1]Total spermatozoa calculated by multiplying ejaculate volume by concentration

[*]Ejaculates from bulls at Stud A when more than one ejaculate in a day were collected were pooled together for evaluation, while at Stud B ejaculates were evaluated separately.

Table 2.5 Effect of bull age on post freezing characteristics (initial post thaw motility, primary abnormalities, and secondary abnormalities) at Studs A and B from 2008 through 2018*

Least square means ± SE

Age in months	Initial Post-thaw Motility (%)		Primary Abnormalities (%)		Secondary Abnormalities (%)	
	Stud A	Stud B	Stud A	Stud B	Stud A	Stud B
N	31,222	3,873	21,953	3,980	21,949	4,100
≤ 12 months	$45^{abcd} \pm 1.18$	$39^{a} \pm 1.19$	$16.3^{a} \pm 0.62$	$24.8^{a} \pm 1.93$	$13.7^{a} \pm 0.72$	$15.2^{abc} \pm 2.22$
13-15 months	$45^{a} \pm 0.68$	$37^{a} \pm 0.65$	$14.5^{ab} \pm 0.38$	$17.9^{b} \pm 0.90$	$11.4^{b} \pm 0.44$	$14.4^{a} \pm 1.17$
16-18 months	$46^{b} \pm 0.67$	$37^{abc} \pm 0.72$	$14.0^{bc} \pm 0.37$	$16.6^{bc} \pm 1.04$	$11.3^{b} \pm 0.43$	$15.7^{ab} \pm 1.29$
19-21 months	$46^{ab} \pm 0.67$	$37^{ab} \pm 0.66$	$13.6^{cde} \pm 0.37$	$16.1^{bc} \pm 0.93$	$11.1^{b} \pm 0.43$	$15.5^{ab} \pm 1.18$
22-24 months	$46^{abc} \pm 0.67$	$37^{abc} \pm 0.71$	$13.9^{bc} \pm 0.37$	$15.2^{bcd} \pm 1.06$	$11.1^{b} \pm 0.43$	$18.3^{bc} \pm 1.27$
25-30 months	$47^{c} \pm 0.66$	$37^{ab} \pm 0.66$	$13.2^{def} \pm 0.37$	$13.9^{cd} \pm 0.92$	$10.9^{b} \pm 0.43$	$17.4^{bc} \pm 1.17$
31-36 months	$47^{cd} \pm 0.69$	$36^{bc} \pm 0.70$	$12.9^{f} \pm 0.38$	$15.9^{bc} \pm 1.02$	$11.4^{b} \pm 0.44$	$17.9^{bc} \pm 1.25$
37-48 months	$47^{c} \pm 0.67$	$37^{abc} \pm 0.63$	$13.1^{fe} \pm 0.37$	$14.2^{cd} \pm 0.89$	$11.4^{b} \pm 0.43$	$15.9^{ab} \pm 1.13$
49-60 months	$48^{d} \pm 0.68$	$35^{c} \pm 0.76$	$13.4^{cdef} \pm 0.38$	$15.3^{bcd} \pm 1.12$	$10.2^{c} \pm 0.44$	$18.3^{bc} \pm 1.34$
> 60 months	$50^{e} \pm 0.67$	$37^{abc} \pm 0.66$	$13.7^{cd} \pm 0.37$	$12.8^{d} \pm 0.93$	$10.2^{c} \pm 0.43$	$20.0^{c} \pm 1.18$

^{a,b,c,d,e,f}Values within a factor column and within stud without a common superscript differ ($P < 0.05$)

*Ejaculates from bulls at Stud A when more than one ejaculate in a day were collected were pooled together for evaluation, while at Stud B ejaculates were evaluated separately.

Figure 2.2 Effect of bull age on two-hour post-thaw motility at Stud B from 2008 through 2018

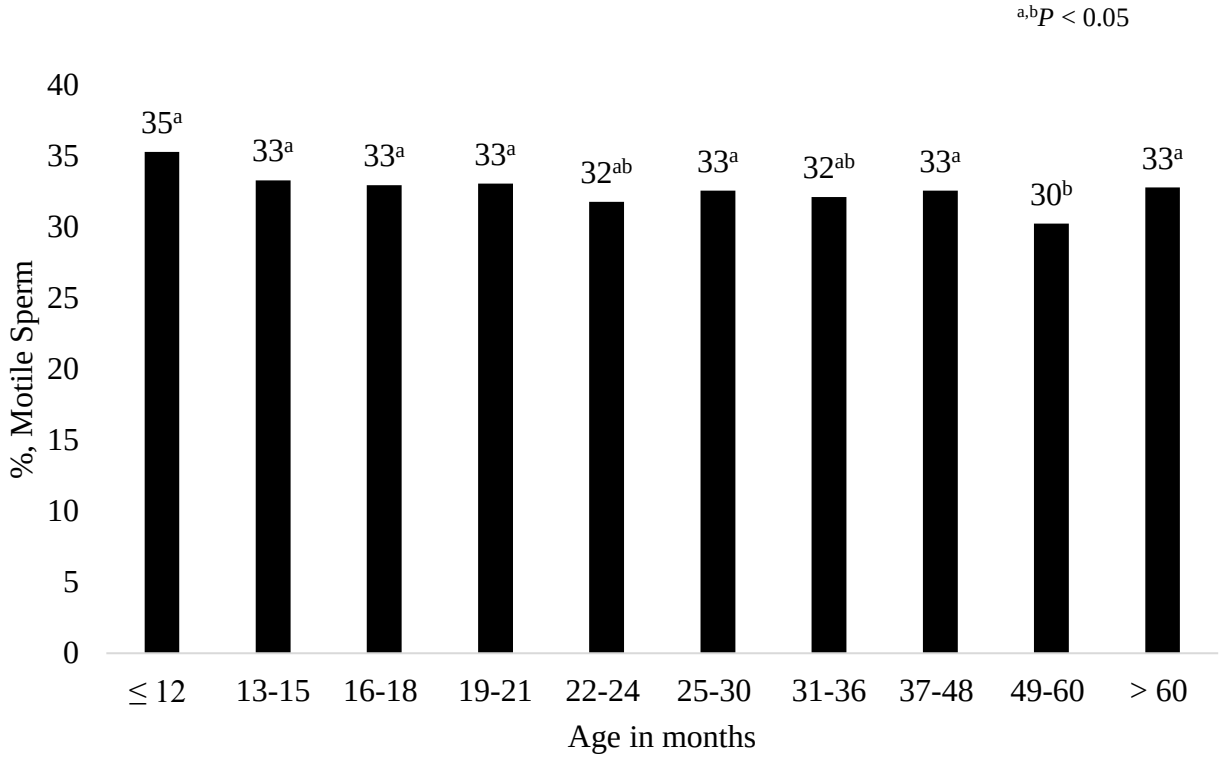


Table 2.6 Effect of bull breed on pre-freeze ejaculate characteristics (volume, concentration, total spermatozoa, and pre-freeze motility) at Studs A and B from 2008 through 2018*

	Least squares mean ± SE							
Breed	Volume (ml)		Concentration x 10^6 per ml		Total Spermatozoa per ejaculate[1] x 10^6		Pre-freeze Motility (%)	
	Stud A	Stud B	Stud A	Stud B	Stud A	Stud B	Stud A	Stud B
N	45,211	12,735	45,001	12,158	45,001	12,149	45,208	12,849
Angus	8.4^{a} ± 0.12	4.8^{b} ± 0.09	883.91^{c} ± 15.35	591.02^{a} ± 16.68	7685.83^{a} ± 165.19	3067.59^{a} ± 106.54	50^{e} ± 0.49	45^{a} ± 0.57
Red Angus	7.7^{bc} ± 0.13	4.5^{cd} ± 0.09	924.20^{b} ± 16.97	589.31^{a} ± 17.66	7144.68^{b} ± 182.69	2816.69^{b} ± 112.88	47^{f} ± 0.55	42^{bc} ± 0.61
Charolais	7.3^{cd} ± 0.22	4.2^{ed} ± 0.13	1072.31^{a} ± 28.34	611.73^{a} ± 24.62	7630.12^{abc} ± 305.15	2872.12^{ab} ± 157.34	54^{a} ± 0.92	43^{ab} ± 0.80
Gelbvieh	9.0^{a} ± 0.29	4.7^{cd} ± 0.09	966.92^{abcd} ± 37.53	525.45^{b} ± 17.53	8470.39^{ac} ± 404.04	2662.8^{b} ± 111.17	50^{bcdef} ± 1.21	45^{a} ± 0.60
Horned Hereford	7.1^{d} ±0.14	4.4^{d} ± 0.10	1062.49^{a} ± 18.35	594.54^{a} ± 19.49	7648.7^{a} ± 197.53	2671.48^{b} ± 124.25	47^{f} ± 0.59	43^{b} ± 0.64
Polled Hereford	7.5^{cd} ± 0.16	4.0^{e} ± 0.12	984.85^{d} ± 20.49	598.91^{a} ± 22.33	7430.26^{ab} ± 220.61	2526.36^{b} ± 142.05	49^{cde} ± 0.66	41^{c} ± 0.73
Simmental	7.9^{b} ± 0.13	5.1^{a} ± 0.10	1028.72^{ad} ± 16.59	583.22^{a} ± 19.16	8326.42^{c} ± 178.65	3139.58^{a} ± 122.08	52^{b} ± 0.53	45^{a} ± 0.65

[a,b,c,d,e,f]Values within a factor column and within stud without a common superscript differ ($P < 0.05$)

[1]Total spermatozoa calculated by multiplying ejaculate volume by concentration

*Ejaculates from bulls at Stud A when more than one ejaculate in a day were collected were pooled together for evaluation, while at Stud B ejaculates were evaluated separately.

Table 2.7 Effect of bull breed on post-thaw ejaculate characteristics (initial post thaw motility, primary abnormalities, and secondary abnormalities) at Studs A and B from 2008 through 2018[*]

	Least square mean ± SE					
Breed	Initial Post-thaw Motility (%)		Primary Abnormalities (%)		Secondary Abnormalities (%)	
	Stud A	Stud B	Stud A	Stud B	Stud A	Stud B
N	31,222	3,873	21,953	3,980	21,949	4,100
Angus	$47^{a} \pm 0.58$	$39^{a} \pm 0.60$	$13.3^{bc} \pm 0.33$	$14.3^{b} \pm 0.78$	$12.5^{a} \pm 0.39$	$18.6^{a} \pm 1.07$
Red Angus	$45^{b} \pm 0.63$	$36^{bcd} \pm 0.65$	$13.1^{bc} \pm 0.36$	$18.6^{a} \pm 0.88$	$12.4^{a} \pm 0.41$	$18.7^{a} \pm 1.15$
Charolais	$48^{ad} \pm 0.92$	$37^{abd} \pm 0.94$	$15.1^{a} \pm 0.49$	$11.7^{b} \pm 1.53$	$10.6^{b} \pm 0.56$	$11.6^{c} \pm 1.71$
Gelbvieh	$50^{ad} \pm 1.25$	$37^{d} \pm 0.62$	$13.6^{abc} \pm 0.59$	$16.8^{a} \pm 0.83$	$11.1^{abc} \pm 0.68$	$16.2^{b} \pm 1.13$
Horned Hereford	$42^{c} \pm 0.68$	$35^{c} \pm 0.70$	$13.7^{c} \pm 0.39$	$19.3^{a} \pm 0.99$	$10.5^{bc} \pm 0.45$	$17.1^{ab} \pm 1.25$
Polled Hereford	$47^{ad} \pm 0.74$	$37^{abcd} \pm 0.89$	$14.8^{a} \pm 0.40$	$19.0^{a} \pm 1.45$	$11.1^{c} \pm 0.46$	$18.4^{ab} \pm 1.58$
Simmental	$47^{d} \pm 0.61$	$38^{a} \pm 0.67$	$13.4^{bc} \pm 0.35$	$14.3^{b} \pm 0.96$	$10.7^{b} \pm 0.40$	$17.4^{ab} \pm 1.21$

[a,b,c,d]Values within a factor column and within stud without a common superscript differ ($P < 0.05$)

[*]Ejaculates from bulls at Stud A when more than one ejaculate in a day were collected were pooled together for evaluation, while at Stud B ejaculates were evaluated separately.

Figure 2.3 Effect of bull breed on two-hour post-thaw motility at Stud B from 2008 through 2018

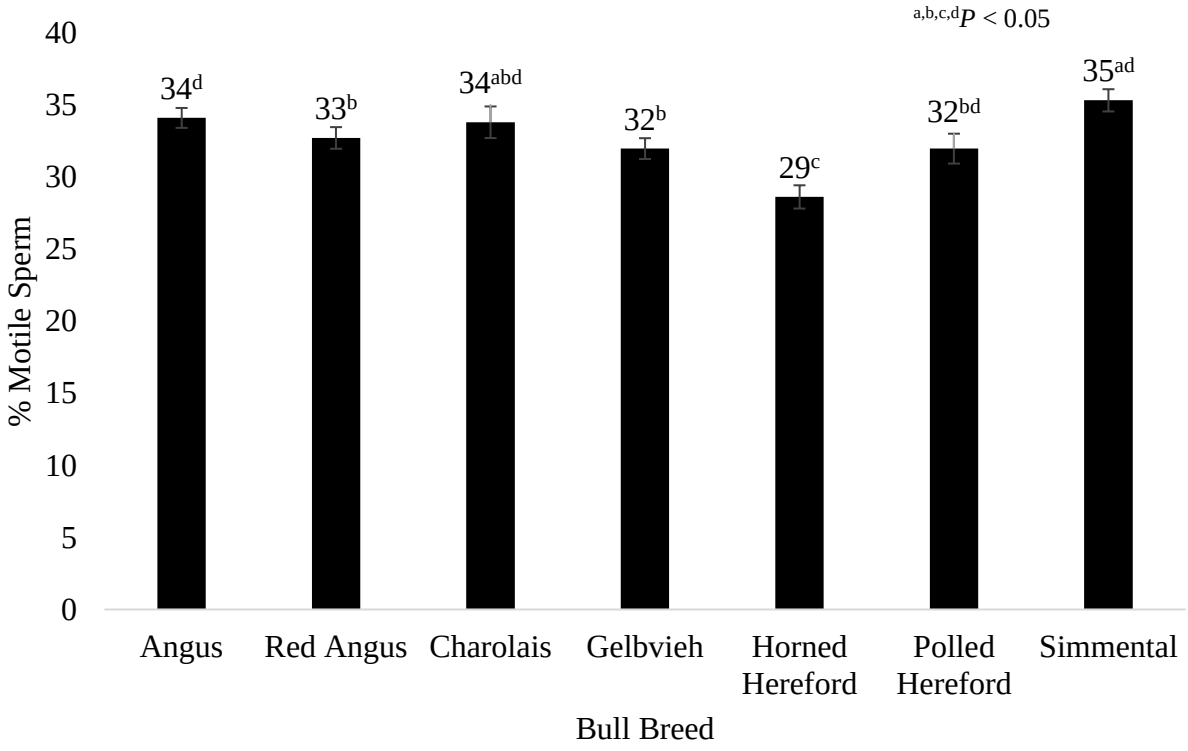


Table 2.8 Effect of season of collection on pre-freeze ejaculate characteristics (volume, concentration, total spermatozoa, and pre-freeze motility) at Studs A and B from 2008 through 2018*

	Least squares mean ± SE							
Season[2]	Volume (ml)		Concentration x 10^6 per ml		Total Spermatozoa per ejaculate[1] x 10^6		Pre-freeze Motility (%)	
	Stud A	Stud B	Stud A	Stud B	Stud A	Stud B	Stud A	Stud B
N	45,211	12,735	45,001	12,158	45,001	12,149	45,208	12,849
Winter	7.8^{bc} ± 0.14	4.5^{bc} ± 0.09	976.59^{b} ± 17.57	560.59^{b} ± 17.93	7642.51^{bc} ± 189.92	2665.28^{b} ± 114.39	49^{b} ± 0.57	42^{c} ± 0.62
Spring	8.1^{a} ± 0.13	4.6^{ab} ± 0.10	1015.83^{a} ± 17.09	580.65^{b} ± 17.26	8205.58^{a} ± 184.16	2802.78^{b} ± 110.02	49^{b} ± 0.54	45^{a} ± 0.59
Summer	7.9^{ab} ± 0.14	4.7^{a} ± 0.11	1015.00^{a} ± 18.28	627.13^{a} ± 20.95	7810.93^{b} ± 198.40	3190.88^{a} ± 133.89	50^{ab} ± 0.60	43^{bc} ± 0.74
Fall	7.7^{c} ± 0.14	4.3^{c} ± 0.10	948.82^{b} ± 18.44	571.16^{b} ± 18.80	7390.35^{c} ± 199.19	2630.55^{b} ± 119.76	51^{a} ± 0.58	43^{b} ± 0.61

[a,b,c]Values within a factor column and within stud without a common superscript differ ($P < 0.05$)

[1]Total spermatozoa calculated by multiplying ejaculate volume by concentration

[*]Ejaculates from bulls at Stud A when more than one ejaculate in a day were collected were pooled together for evaluation, while at Stud B ejaculates were evaluated separately.

[2]Winter (December, January, February), Spring (March, April, May), Summer (June, July, August), Fall (September, October, November)

Table 2.9 Effect of season of collection on post-thaw ejaculate characteristics (initial post thaw motility, primary abnormalities, and secondary abnormalities) at Studs A and B from 2008 through 2018*

Least square mean ± SE

Season[2]	Initial Post-thaw Motility (%)		Primary Abnormalities[1](%)		Secondary Abnormalities (%)	
	Stud A	Stud B	Stud A	Stud B	Stud A	Stud B
N	31,222	3,873	--	3,980	21,949	4,100
Winter	$46^{b} \pm 0.65$	$36^{b} \pm 0.64$	--	$18.4^{a} \pm 0.92$	$11.5^{ab} \pm 0.42$	$18.2^{a} \pm 1.16$
Spring	$46^{b} \pm 0.63$	$38^{a} \pm 0.66$	--	$13.8^{b} \pm 0.83$	$10.7^{c} \pm 0.40$	$15.1^{b} \pm 1.20$
Summer	$46^{b} \pm 0.67$	$37^{ab} \pm 0.83$	--	$19.4^{a} \pm 1.25$	$11.2^{b} \pm 0.42$	$17.0^{ab} \pm 1.44$
Fall	$48^{a} \pm 0.67$	$37^{ab} \pm 0.74$	--	$13.5^{b} \pm 0.89$	$11.7^{a} \pm 0.41$	$17.2^{ab} \pm 1.29$

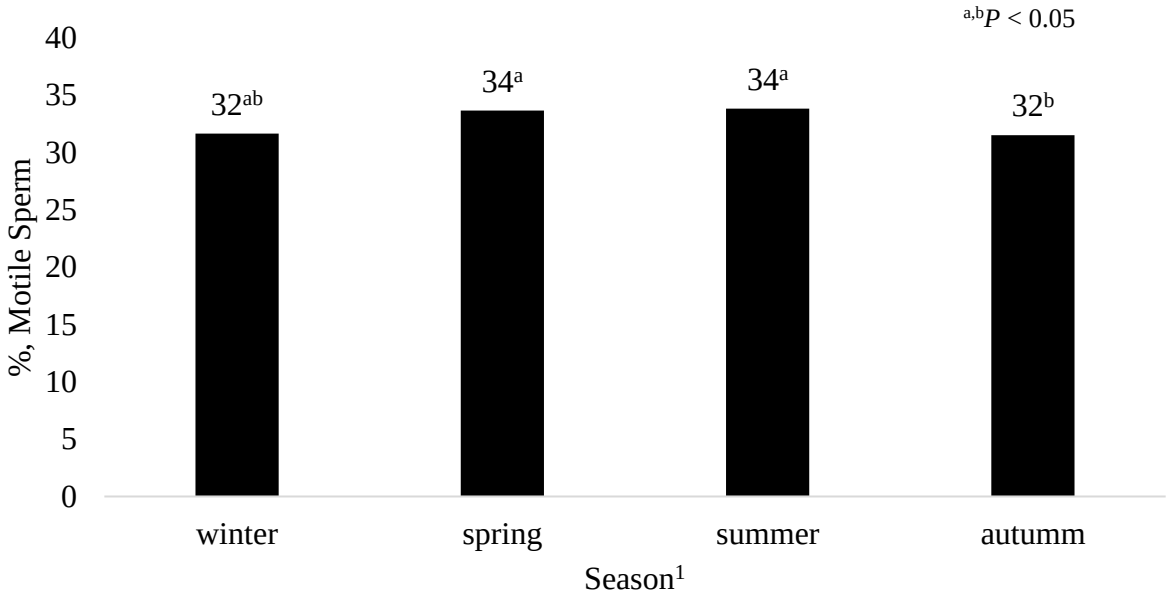
[a,b,c]Values within a factor column without a common superscript differ ($P < 0.05$)

[1]Season of collection did not affect primary abnormalities at Stud A.

[*]Ejaculates from bulls at Stud A when more than one ejaculate in a day were collected were pooled together for evaluation, while at Stud B ejaculates were evaluated separately.

[2]Winter (December, January, February), Spring (March, April, May), Summer (June, July, August), Fall (September, October, November)

Figure 2.4 Effect of bull season on two-hour post-thaw motility at Stud B from 2008 through 2018



[1]Winter (December, January, February), Spring (March, April, May), Summer (June, July, August), Fall (September, October, November)

Table 2.10 Effect of days since previous ejaculate on pre-freeze ejaculate characteristics (volume, concentration, total spermatozoa, and pre-freeze motility) at Studs A and B from 2008 through 2018*

Least squares mean ± SE

Days since previous ejaculate	Volume (ml)		Concentration x 10^6 per ml		Total Spermatozoa per ejaculate[1] x 10^6		Pre-freeze Motility (%)	
	Stud A	Stud B	Stud A	Stud B	Stud A	Stud B	Stud A	Stud B
N	45,211	12,735	45,001	12,158	45,001	12,149	45,208	12,849
≤ 1 day	$4.6^{a} \pm 0.15$	$4.2^{ab} \pm 0.08$	$921.79^{d} \pm 19.33$	$526.47^{c} \pm 15.67$	$3993.46^{f} \pm 208.02$	$2387.55^{d} \pm 99.28$	$53^{a} \pm 0.62$	$40^{d} \pm 0.54$
2 days	$6.9^{b} \pm 0.13$	$3.9^{a} \pm 0.12$	$966.59^{ce} \pm 16.48$	$586.51^{ab} \pm 23.10$	$6471.60^{e} \pm 177.46$	$2360.60^{cd} \pm 147.51$	$50^{cb} \pm 0.53$	$44^{abce} \pm 0.75$
3 days	$7.1^{c} \pm 0.13$	$4.2^{ab} \pm 0.09$	$1036.14^{b} \pm 16.68$	$604.04^{a} \pm 17.28$	$7279.03^{d} \pm 179.51$	$2621.37^{bc} \pm 110.29$	$50^{be} \pm 0.54$	$45^{abe} \pm 0.60$
4 days	$7.4^{d} \pm 0.13$	$4.2^{abc} \pm 0.09$	$1044.90^{b} \pm 16.95$	$639.08^{a} \pm 17.27$	$7700.63^{c} \pm 182.43$	$2771.49^{ab} \pm 110.21$	$51^{e} \pm 0.55$	$45^{a} \pm 0.59$
5 days	$7.9^{e} \pm 0.13$	$4.6^{bc} \pm 0.13$	$1078.14^{a} \pm 17.25$	$644.36^{a} \pm 25.91$	$8540.82^{g} \pm 185.70$	$3204.76^{a} \pm 165.72$	$50^{bce} \pm 0.56$	$44^{abce} \pm 0.83$
6 days	$8.4^{ef} \pm 0.24$	$4.9^{c} \pm 0.16$	$980.88^{bcde} \pm 31.55$	$592.08^{abc} \pm 31.61$	$8277.89^{bcg} \pm 339.65$	$3246.25^{a} \pm 201.91$	$49^{bcde} \pm 1.02$	$45^{ab} \pm 1.02$
7 days	$8.5^{f} \pm 0.13$	$4.8^{c} \pm 0.12$	$925.48^{d} \pm 17.29$	$571.07^{abc} \pm 22.42$	$7982.10^{c} \pm 186.16$	$2855.10^{ab} \pm 143.18$	$49^{cd} \pm 0.56$	$44^{abce} \pm 0.73$
> 7 – 30 days	$9.5^{g} \pm 0.18$	$5.0^{c} \pm 0.14$	$954.26^{cde} \pm 22.80$	$499.60^{bc} \pm 27.46$	$9263.64^{b} \pm 245.46$	$2713.44^{abcd} \pm 175.28$	$49^{bcde} \pm 0.74$	$42^{cde} \pm 0.90$
> 30 days	$10.3^{h} \pm 0.18$	$4.9^{c} \pm 0.14$	$993.35^{cb} \pm 22.96$	$600.74^{a} \pm 26.85$	$10352.00^{a} \pm 247.16$	$3240.81^{a} \pm 171.37$	$48^{d} \pm 0.74$	$42^{cde} \pm 0.88$

[a,b,c,d,e]Values within a factor column and within stud without a common superscript differ ($P < 0.05$)

[1]Total spermatozoa calculated by multiplying ejaculate volume by concentration

*Ejaculates from bulls at Stud A when more than one ejaculate in a day were collected were pooled together for evaluation, while at Stud B ejaculates were evaluated separately.

Table 2.11 Effect of days since previous ejaculate on post-thaw ejaculate characteristics (initial post thaw motility, primary abnormalities, and secondary abnormalities) at Studs A and B from 2008 through 2018*

	Least squares mean ± SE					
Days since previous ejaculate	Initial Post-thaw Motility (%)		Primary Abnormalities (%)		Secondary Abnormalities (%)	
	Stud A	Stud B	Stud A	Stud B	Stud A	Stud B
N	31,222	3,873	21,953	3,980	21,949	4,100
≤ 1 day	$46^{a} \pm 0.68$	$38^{a} \pm 0.61$	$13.5^{abd} \pm 0.38$	$18.1^{a} \pm 0.79$	$12.2^{a} \pm 0.44$	$17.6^{ac} \pm 1.08$
2 days	$47^{b} \pm 0.61$	$36^{ab} \pm 0.76$	$13.4^{d} \pm 0.34$	$16.8^{abc} \pm 1.14$	$11.5^{bc} \pm 0.40$	$15.3^{bc} \pm 1.36$
3 days	$47^{ab} \pm 0.62$	$37^{ab} \pm 0.61$	$13.5^{ab} \pm 0.35$	$17.0^{ac} \pm 0.80$	$11.2^{ce} \pm 0.40$	$15.5^{b} \pm 1.09$
4 days	$47^{ab} \pm 0.63$	$37^{b} \pm 0.61$	$13.9^{a} \pm 0.35$	$15.8^{bc} \pm 0.80$	$11.4^{bce} \pm 0.41$	$15.7^{b} \pm 1.09$
5 days	$47^{ab} \pm 0.63$	$37^{ab} \pm 0.82$	$13.9^{a} \pm 0.35$	$15.9^{abc} \pm 1.30$	$11.0^{de} \pm 0.41$	$15.1^{bc} \pm 1.47$
6 days	$46^{ab} \pm 1.08$	$37^{ab} \pm 1.00$	$14.8^{ac} \pm 0.55$	$14.9^{abc} \pm 1.69$	$11.6^{abcde} \pm 0.64$	$17.8^{abc} \pm 1.79$
7 days	$47^{ab} \pm 0.64$	$37^{ab} \pm 0.75$	$14.5^{c} \pm 0.36$	$13.5^{bc} \pm 1.12$	$11.8^{ab} \pm 0.41$	$15.8^{bc} \pm 1.35$
> 7 – 30 days	$46^{ab} \pm 0.81$	$36^{ab} \pm 0.98$	$13.8^{abcd} \pm 0.43$	$19.2^{ac} \pm 1.55$	$10.2^{de} \pm 0.50$	$21.7^{a} \pm 1.72$
> 30 days	$48^{ab} \pm 0.81$	$37^{ab} \pm 0.89$	$13.6^{abcd} \pm 0.43$	$15.2^{abc} \pm 1.42$	$10.6^{cde} \pm 0.50$	$17.2^{abc} \pm 1.59$

[a,b,c,d,e]Values within a factor column without a common superscript differ ($P < 0.05$)

*Ejaculates from bulls at Stud A when more than one ejaculate in a day were collected were pooled together for evaluation, while at Stud B ejaculates were evaluated separately.

Figure 2.5 Effect of days since previous ejaculate on two-hour post-thaw sperm motility at Stud B from 2008 through 2018

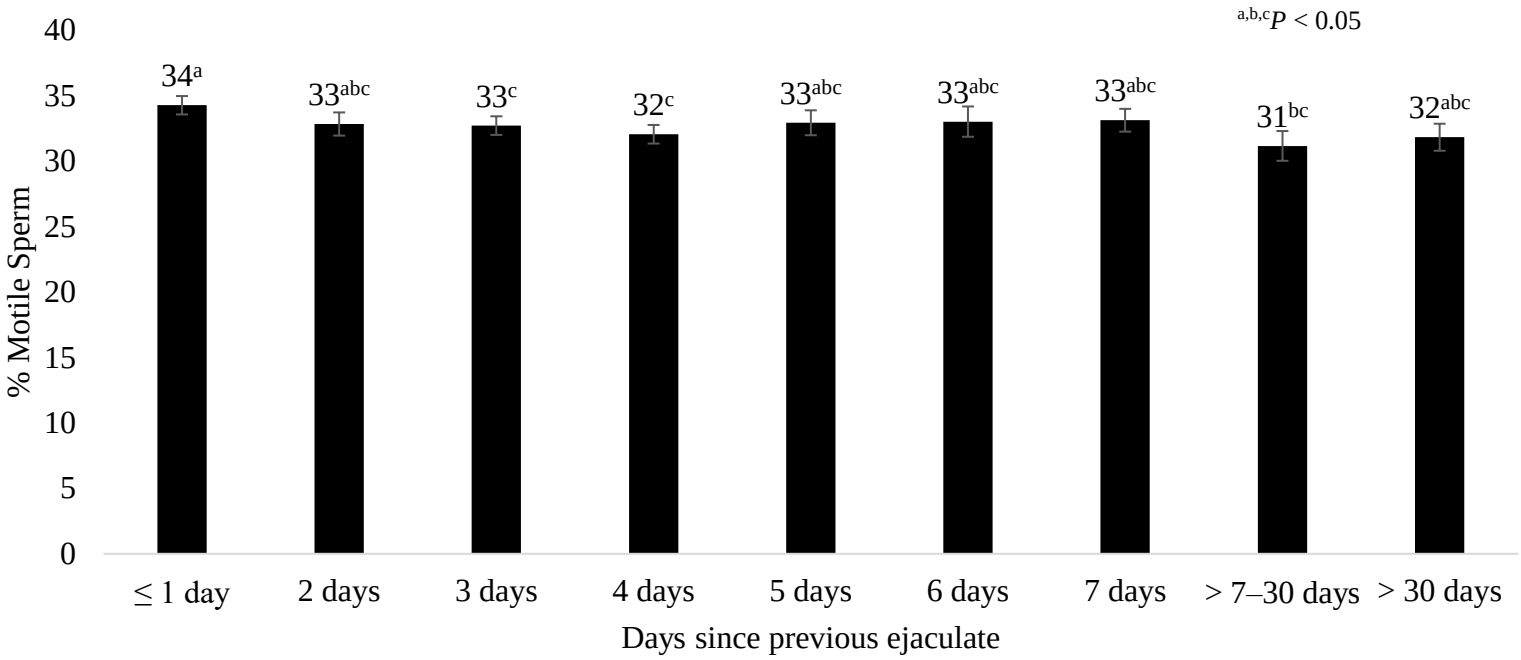


Table 2.12 Effect of barn location on ejaculate characteristics at Stud A from 2008 through 2018

Least squares mean ± SE

Barn	Volume (ml)	Concentration x 10^6 per ml	Total Spermatozoa per ejaculate[1] x 10^6	Pre-freeze Motility (%)	Primary Abnormalities (%)	Secondary Abnormalities (%)	Initial Post-thaw Motility (%)
N	45,211	45,001	45,001	45,208	21,953	21,949	31,222
Barn 1	7.8^{b} ± 0.13	929.03^{d} ± 17.01	7310.22^{c} ± 183.08	47^{c} ± 0.55	14.3^{a} ± 0.35	11.5^{a} ± 0.41	46^{cd} ± 0.63
Barn 2	7.9^{b} ± 0.13	1053.44^{a} ± 17.03	8271.29^{a} ± 183.33	50^{b} ± 0.55	13.9^{b} ± 0.35	10.9^{b} ± 0.41	47^{bc} ± 0.63
Barn 3	7.8^{b} ± 0.13	1013.96^{b} ± 16.72	7917.00^{b} ± 180.02	51^{a} ± 0.54	13.4^{c} ± 0.35	11.3^{a} ± 0.40	47^{b} ± 0.62
Barn 4	7.5^{c} ± 0.13	958.00^{c} ± 17.18	7109.58^{c} ± 184.93	50^{b} ± 0.55	13.7^{bc} ± 0.35	11.5^{a} ± 0.41	46^{d} ± 0.63
Barn 5	8.2^{a} ± 0.15	990.87^{b} ± 19.18	8203.61^{ab} ± 206.45	51^{a} ± 0.62	14.0^{ab} ± 0.38	11.1^{ab} ± 0.43	48^{a} ± 0.69

[a,b,c,d]Values within a factor column without a common superscript differ ($P < 0.05$)

[1]Total spermatozoa calculated by multiplying ejaculate volume by concentration

Table 2.13 Effect of bull weight (kg) on ejaculate characteristics at Stud A from 2008 through 2018

	Least squares mean ± SE						
Weight	Volume (ml)	Concentration x 10^6 per ejaculate	Total Spermatozoa per ejaculate[1] x 10^6	Pre-freeze Motility (%)	Primary Abnormalities (%)	Secondary Abnormalities (%)	Initial Post-thaw Motility (%)
N	45,211	45,001	45,001	45,208	21,953	21,949	31,222
> 385.6 – 430.9 kg	8.2^{abcd} ± 0.54	1144.20^{ac} ± 70.85	8171.91^{ab} ± 762.83	57^{ac} ± 2.27	15.2^{ab} ± 1.38	7.7^{x} ± 1.59	47^{abc} ± 2.58
> 430.9 – 453.6 kg	7.0^{bd} ± 0.43	940.15^{ab} ± 55.06	6936.75^{ab} ± 592.80	47^{bcd} ± 1.78	14.0^{ab} ± 1.38	12.0^{xy} ± 1.59	42^{bc} ± 2.00
> 453.6 – 680.4 kg	7.0^{b} ± 0.10	1050.49^{ac} ± 12.90	7546.98^{a} ± 138.82	47^{b} ± 0.41	13.6^{ab} ± 0.21	12.1^{y} ± 0.24	46^{bc} ± 0.44
> 680.4 – 907.2 kg	7.6^{d} ± 0.09	988.35^{c} ± 12.44	7699.04^{a} ± 133.90	48^{d} ± 0.39	13.6^{a} ± 0.20	12.0^{y} ± 0.23	48^{a} ± 0.42
> 907.2 – 1134.0 kg	8.3^{a} ± 0.10	898.43^{b} ± 13.28	7754.78^{a} ± 142.92	48^{bd} ± 0.42	13.8^{a} ± 0.21	11.8^{xy} ± 0.25	48^{a} ± 0.45
> 1134.0 kg	8.9^{c} ± 0.13	912.72^{b} ± 16.37	8464.6^{b} ± 176.17	52^{ac} ± 0.52	13.0^{b} ± 0.26	12.1^{y} ± 0.30	50^{c} ± 0.54

^{a,b,c,d}Values within a factor column without a common superscript differ ($P < 0.05$)

^{x,y}Values within a factor column without a common superscript tend to differ ($P < 0.1$)

[1]Total spermatozoa calculated by multiplying ejaculate volume by concentration

Table 2.14 Effect of scrotal circumference (cm) on ejaculate characteristics at Stud A from 2008 through 2018

Least squares mean ± SE

Scrotal	Volume (ml)	Concentration x 10^6 per ml	Total Spermatozoa per ejaculate[1] x 10^6	Pre-freeze Motility (%)	Primary Abnormalities (%)	Secondary Abnormalities[2] (%)	Initial Post-thaw Motility (%)
N	45,211	45,001	45,001	45,208	21,953	21,949	31,222
21 – 30.5 cm	6.6^{d} ± 0.38	736.86^{d} ± 51.69	5074.51^{f} ± 556.43	43^{e} ± 1.58	14.5^{ab} ± 0.80	12.5 ± 0.93	52^{a} ± 1.84
31 – 33.5 cm	7.2^{d} ± 0.16	795.80^{d} ± 21.38	5748.96^{f} ± 230.14	50^{d} ± 0.69	14.2^{a} ± 0.43	11.5 ± 0.49	48^{a} ± 0.78
34 – 37 cm	7.5^{d} ± 0.14	990.08^{c} ± 17.59	7242.59^{e} ± 189.36	54^{a} ± 0.57	13.9^{a} ± 0.35	10.9 ± 0.40	47^{a} ± 0.61
37.5 – 40 cm	7.8^{c} ± 0.13	1031.16^{b} ± 17.41	7690.69^{d} ± 187.44	51^{c} ± 0.56	14.0^{a} ± 0.35	11.2 ± 0.40	46^{b} ± 0.61
40.25 – 43.5 cm	8.3^{b} ± 0.14	1095.73^{a} ± 17.53	8870.35^{c} ± 188.72	53^{b} ± 0.56	13.4^{b} ± 035	11.2 ± 0.40	46^{b} ± 0.61
44 – 47 cm	8.6^{a} ± 0.14	1129.39^{a} ± 18.06	9589.05^{b} ± 194.48	50^{d} ± 0.58	14.1^{a} ± 0.36	10.9 ± 0.41	44^{c} ± 0.63
> 47.5 cm	8.8^{a} ± 0.18	1144.39^{a} ± 23.37	10120.00^{a} ± 251.65	49^{d} ± 0.75	13.0^{b} ± 0.45	10.7 ± 0.51	43^{c} ± 0.80

^{a,b,c,d,e,f}Values within a factor column without a common superscript differ ($P < 0.05$)

[1]Total spermatozoa calculated by multiplying ejaculate volume by concentration

[2]Scrotal circumference groups did not significantly differ from each other.

Table 2.15 Effect of collection method (artificial vagina or electroejaculation) on ejaculate characteristics at Stud B from 2008 through 2018*

Characteristic	Number of ejaculates	Least squares mean ± SE		*P* value
		Artificial Vagina	Electroejaculation	
Volume (mL)	12,735	4.2 ± 0.09	4.9 ± 0.09	< 0.0001
Concentration x 10^6	12,158	625.78 ± 16.36	543.99 ± 16.90	< 0.0001
Pre-freeze Motility (%)	12,849	43 ± 0.56	44 ± 0.58	0.0015
Primary Abnormalities (%)	3,980	16.8 ± 0.78	15.7 ± 0.81	0.0125
Secondary Abnormalities (%)	4,100	15.2 ± 1.1	18.5 ± 1.1	< 0.0001

*Effect of collection did not impact total spermatozoa produced, initial post-thaw, and two-hour post-thaw ($P > 0.05$).

Table 2.16 Effect of sequential ejaculates in one day on ejaculate characteristics at Stud B from 2008 through 2018*

		Least squares mean ± SE	P value
Ejaculate number	N	Volume (ml)	< 0.0001
1	8,611	4.5 [b] ± 0.04	
2	5,356	4.8 [a] ± 0.05	
3	674	4.4 [b] ± 0.10	
4	58	4.4 [ab] ± 0.29	
		Concentration x 10^6 per ml	< 0.0001
1	7,878	689.5 [a] ± 8.51	
2	4,884	597.8 [b] ± 10.10	
3	673	565.5 [b] ± 18.00	
4	55	486.7 [b] ± 54.37	
		Total Spermatozoa per ejaculate[1] x 10^6	< 0.0001
1	7,872	3408.4[a] ± 54.38	
2	4,882	2987.8[b] ± 64.30	
3	634	2593.9[c] ± 114.17	
4	55	2299.4[bc] ± 347.29	
		Pre-freeze Motility (%)	< 0.0001
1	8,693	38[a] ± 0.34	
2	5,416	44[b] ± 0.38	
3	679	47[c] ± 0.61	
4	58	45[bc] ± 1.74	
		Initial Post-thaw Motility (%)	< 0.0001
1	2,152	35[a] ± 0.45	
2	2,097	37[b] ± 0.44	
3	305	38[b] ± 0.60	
4	21	38[ab] ± 1.70	

Table 2.16 Continued

		Two-hour Post-thaw Motility (%)	< 0.0001
1	2,146	$31^{a} \pm 0.51$	
2	2,093	$34^{b} \pm 0.51$	
3	305	$34^{b} \pm 0.69$	
4	21	$32^{ab} \pm 1.97$	
		Secondary Abnormalities (%)	< 0.01
1	2,302	$17.8^{a} \pm 0.80$	
2	2,142	$17.1^{ab} \pm 0.81$	
3	313	$14.9^{b} \pm 1.10$	
4	23	$17.7^{ab} \pm 2.97$	

[a,b,c]Values within a factor column without a common superscript differ (P < 0.05)

[*] Effect of sequential ejaculate in one day did not impact primary abnormalities (*P* > 0.05).

[1]Total spermatozoa calculated by multiplying ejaculate volume by concentration

Table 2.17 Significant effects remaining for each ejaculate characteristic model at Stud A

	Effects in model										
Ejaculate Characteristic	Breed	Age in months	Season	Days since previous ejaculate	CCI Proliferation	CCI Meiosis	CCI Differentiation	CCI ETT	Barn Location	Weight	Scrotal
Volume (ml)	X	X	X	X			X	X	X	X	X
Concentration x 10^6 per ml	X	X	X	X	X	X	X		X	X	X
Pre-freeze Motility (%)	X	X	X	X			X	X	X	X	X
Total Spermatozoa per ejaculate x 10^6	X	X	X	X	X			X	X	X	X
Post-thaw Motility (%)	X	X	X	X	X	X		X	X	X	X
Primary Sperm Abnormalities (%)	X	X		X	X			X	X	X	X
Secondary Sperm Abnormalities (%)	X	X	X	X				X	X	X	X

Table 2.18 Significant effects remaining for each ejaculate characteristic model at Stud B

	Effects in model									
Ejaculate Characteristic	Breed	Age in months	Season	Days since previous ejaculate	CCI Proliferation	CCI Meiosis	CCI Differentiation	CCI ETT	Collection Method	Sequential ejaculates in one day
Volume (ml)	X	X	X	X	X				X	X
Concentration x 10^6 per ml	X	X	X	X		X			X	X
Pre-freeze Motility (%)	X	X	X	X			X	X	X	X
Total Spermatozoa per ejaculate x 10^6	X	X	X	X		X				X
Post-thaw Motility (%)	X	X	X	X	X			X		X
Two-Hour Post-thaw Motility (%)	X	X	X	X	X			X		X
Primary Sperm Abnormalities (%)	X	X	X	X			X	X	X	
Secondary Sperm Abnormalities (%)	X	X	X	X	X		X	X	X	X

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