

Occurrence of proximal droplets in performance-tested beef bulls

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DEDICATION

This thesis is dedicated to my family as they have continued to support and shape me into who I am today. Thank you to my grandparents, parents, sisters, and my girlfriend. Without your help along the way, I could not have accomplished this endeavor.

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ABSTRACT

To determine the occurrence of proximal droplets in performance-tested bulls, parameters related to breeding soundness examination (BSE) classification were evaluated. Bulls (542 Angus bulls) were classified as satisfactory (BSE guidelines; Society for Theriogenology) or deferred. On-test risk factors were identified, associated with increased probability for bulls to be deferred due to proximal droplets. On-test weight ($P = 0.019$), on-test scrotal circumference ($P = 0.018$) and the covariate of age ($P = 0.007$) were all associated with the probability for a bull to be deferred on test due to proximal droplets. Accounting for the covariate of age and the effect of on-test SC, bulls in weight category 5 (>500 kg on-test weight) had 12.5 times the odds of deferral due to proximal droplets compared to bulls in weight category 2 (350 – 400 kg) (OR: 12.5, 95% adj. CI: 1.47, 111.11; adj. $P = 0.017$). Accounting for the covariate of age and the effect of on-test weight, bulls that were categorized as SC on-test 1 (< 30 cm) had 7.9 times the odds for deferral due to proximal droplets compared to bulls categorized as SC 3 (33 – 36 cm; OR: 7.9, 95% CI: 1.4, 44.4; $P = 0.012$). Additionally, bulls that were categorized as SC 2 (30 – 33 cm) had 4.7 times the odds for deferral due to proximal droplets compared to bulls categorized 3 (33 – 36 cm; 95% CI: 1.1, 20.0; $P = 0.03$). After accounting for age and bull on-test weight, bulls that were categorized as SC growth 3 (> 6.2 cm growth) had 3.7 times the odds for deferral due to proximal droplets compared to bulls categorized 2 (3.4 to 6.2 cm growth; OR: 3.7, 95% CI: 1.69, 8.26; $P = 0.001$). In summary, bull on-test weight, on-test scrotal circumference, scrotal circumference growth and age are important factors associated with unsatisfactory classification due to proximal droplets. Occurrence of proximal droplets have been previously associated with bull maturity; however the cause of its relationship to on-test weight, on-test scrotal circumference and rapid scrotal circumference growth in performance-tested bulls remains unclear.

Keywords: proximal droplet, bulls, fertility, scrotal circumference, breeding soundness examination

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CHAPTER 1. INTRODUCTION

The increasing demand for efficiency continues to build within food animal production systems. In order to achieve the goal of obtaining a live calf every 365 days, bull fertility must continue to be an emphasis considering many are deficient in breeding capacities (Hopkins and Spitzer, 1997). Sometimes, little attention is focused upon the male's contribution (Tan, 2002). With efficiency of production becoming the ultimate goal, closing generation gaps through the use of young sires is essential. However, challenging bulls to maximize their fertility competency at a young age can have severe ramifications.

Over the past few years, an increased incidence of bulls with an acceptable scrotal circumference have been associated with proximal droplets and an increase in the number of bulls failing the breeding soundness examination (BSE; Menon et al., 2011; Strickland, 2016). Although there has been many different suggestions as to what exactly a proximal droplet is, it is thought to be the remnant of a germ cell cytoplasm that is adherent to the neck region of a spermatid (Cooper, 2005). As the newly formed spermatozoon is ready to be released, cytoplasmic swelling takes place, leaving cytoplasm around the neck of the spermatozoa (Barth and Oko, 1989). With maturity, the incident of proximal droplets decreases (Amann et al., 2000). In a study conducted by Thundathil and others (2001), sperm with proximal droplets resulted in reduced cleavage and embryo formation during in vitro production (Thundathil et al., 2001).

Young bulls on a performance test will have high weight gains and high energy diets can create issues come breeding season (Silcox, 2017). In a study conducted by Coulter et al. (1987), Angus and Hereford bulls fed two energy diets (high and medium) were evaluated for effects of diet on testicular development and seminal quality (Coulter et al., 1987). The results demonstrated that a high energy diet can have a harmful impact on sperm by decreasing morphology score (Coulter et

al., 1987; Callaghan et al., 2016). Callaghan et al. (2016) reported a direct effect of feed induced subacute ruminal acidosis on sperm morphology in bulls. Finally, downstream effects of proximal droplets can be detrimental to a herd, especially when thinking about the potential genetic relationship that can occur. In a study by Roberts et al. (2010) with semen collected from 908 bulls, heritability of proximal droplets was reported at 0.37%.

Consequently, the question has been raised if there is a genetic relationship with incident of proximal droplets in bull ejaculates with acceptable scrotal circumferences. Thus, the following study tested the hypothesis that a genetic relationship exists between the occurrence of proximal droplets and young Angus bulls with large scrotal circumferences. The objective was to evaluate if proximal droplets are associated with scrotal size and genetic lines of Angus bulls.

CHAPTER 2. LITERATURE REVIEW

2.1 Spermatogenesis in Bulls

Spermatogenesis is the process of producing spermatozoa. It is a rather complex and lengthy process, requiring 56-63 days in bovine (Bearden et al., 1992). The process takes place within the seminiferous tubules of the testes. Specifically, in association with the seminiferous epithelium (lining of the seminiferous tubules) consisting of two cell types: somatic and germinal (Hess Rex, 1999). Surrounding the germ cell is the cytoplasm of the somatic cell known as Sertoli cells, which act as nurse cells (Hess Rex, 1999). Throughout the seminiferous epithelium, Sertoli cells touch and form what is known as the blood testis barrier. This acts as a filter for blood coming in contact to germinal cells as well as blood borne chemicals (Hess Rex, 1999). Intercellular bridges help to pass germ cells from different phases within the process known as spermatogenesis. Below, the process of spermatogenesis will be discussed within three different phases consisting of: proliferation, meiotic, and differentiation phase. The morphologic changes occurring to these germ cells is completed within the seminiferous tubules (Senger, 1997).

The proliferation phase is the first phase in which all of the mitotic divisions occur. Basic germ cells at the base of seminiferous epithelium proliferate by a series of mitotic divisions. In part of the proliferation phase, stem cell renewal occurs (Meng et al., 2000). Those cells that are not destined to become differentiated into potential spermatozoa are typically either apoptotic or are used as the foundation for the next wave of stem cells of spermatogenesis (Meng et al., 2000). This process allows for new spermatogonia to develop continuously. Throughout this first phase, some cells degenerate (Senger, 1997). Although typically the last to divide, B spermatagonia start the second phase of spermatogenesis (Hess Rex, 1999).

In order for the presumptive spermatid to continue on its journey to becoming a spermatozoon, it must go through the second phase known as meiosis. This reduction phase starts with

spermatocytes undergoing DNA synthesis to then undergo meiotic division and to produce four haploid cells of DNA (Hess Rex, 1999). The first meiotic division (primary spermatocyte) is about 30% of the time required for the entire spermatogenic process (Senger, 1997). The spermatocyte then enters into the second meiotic division which will result in haploid spherical spermatids (Senger, 1997).

The differentiation phase is the final phase in producing a viable spermatozoa. Also known as the spermiogenesis. This third phase takes haploid spermatids to spermatozoa (Johnson, 1995). No more cell division takes place within this phase. However, the undifferentiated spermatid develops a head, flagellum along with a midpiece, and a principal piece all through a highly specialized transformation (Senger, 1997).

From an endocrinology standpoint, it is known that an increase in LH facilitates testosterone needed for spermatogenesis to take place, in species that are non-seasonal breeders (Bearden et al., 1992; Ramaswamy and Weinbauer, 2014). When LH binds to LH receptors on the Leydig cells, testosterone is released and transferred to the Sertoli cells (Senger, 1997; Ramaswamy and Weinbauer, 2014). Within the Sertoli cells, testosterone is converted to dihydrotestosterone along with estradiol (Senger, 1997; Ramaswamy and Weinbauer, 2014). Throughout this process, dihydrotestosterone and estradiol are fed back into the hypothalamus to create a negative feedback loop (Senger, 1997; Ramaswamy and Weinbauer, 2014). Within the male bovine, there are 3 to 7 pulses of LH that initiate testosterone production from that of the Leydig cells (Bearden et al., 1992; Ramaswamy and Weinbauer, 2014).

2.2 Breeding Soundness Exam

A breeding soundness examination is a measurable assessment of a bull's possibility of confirming a pregnancy within a fertile, cycling female (DACT, 2000). A relatively inexpensive procedure that has a significant impact within a cow herd's fertility is a breeding soundness exam (BSE; Hopkins and Spitzer, 1997). Normally, producers find that a BSE should be conducted at one of three different times of the year: pre-purchase sale, pre-breeding season, and post breeding season in order to maximize breeding efficiency (Yelich, 2008). A BSE entails four essential parts including: physical exam, genital exam, scrotal measurement, and semen evaluation (Yelich, 2008).

In order to copulate, a bull must have the ability to see, smell, and move (Parker, 2004). Physical examinations allow for these senses to be checked within the first portion of the exam. Anything that may decrease a bull's chance to mate with a female due to physical traits are reviewed with the client, and could prevent the bull advancing onto the next portion of the exam (Ayars, 2006). A bull should be in good health, have the appropriate body condition score, and have good body conformation/structure (Yelich, 2008).

The genital exam has an obvious role with the male's potential of producing live offspring. Bulls should be examined for genital abnormalities to assess their fertility. The prepuce, penis, scrotum, and accessory sex glands all need to be evaluated (Yelich, 2008). Abnormalities can be found through inflammation, swelling, unevenness, tone, position, and other means (Yelich, 2008).

Scrotal circumference has a direct correlation to sperm production and output (Parker, 2004). Although different breed influences will vary in scrotal circumference, in order to pass a BSE, the average bull at or under 15 months of age should measure at least 30 cm (Ayars, 2006). Scrotal circumference represents 40% of the total BSE score of the bull (Parker, 2004).

Semen is normally collected through the use of electro-ejaculation during the BSE. A bull is not always successful at omitting sperm cells during electro-ejaculation, and this does not automatically place a bull in the unsatisfactory breeder category (Parker, 2004). It is also known that just because a bull is considered normal in physical and genital exams, does not mean he will be normal in terms of semen quality (Parker, 2004). Three different steps are taken when evaluating semen. First the visual exam should present a creamy white color and be free of any blood, urine, dirt or pus (Parker, 2004; Yelich, 2008). Second, motility is evaluated both in terms of gross and individual motility (Parker, 2004; Yelich, 2008). Determination of gross motility evaluates the swirling movement of multiple sperm while individual motility evaluated by determining the forward progression of individual sperm (Parker, 2004; Yelich, 2008). The minimum motility must be equal or greater than 30% to pass a BSE (Hopper, 2015). Finally, morphology must be evaluated. There are two types of abnormalities: major (primary) and minor (secondary). Major abnormalities usually occur in the testes due to faulty spermatogenesis. Minor abnormalities occur during sperm transport through the duct system and outside of the testes (Yelich, 2008). Bulls must pass with at least 70% normal sperm cells in order to be classified as a satisfactory breeder (Hopper, 2015).

2.3 Semen Abnormalities and their Impact in the Female

Sperm abnormalities have been associated with male infertility (Chenoweth, 2005). Abnormalities associated with sperm have a significant economic impact on a producer's calf crop. Two categories are prevalent for sperm abnormalities, major and minor defects. Abnormalities that are within substantial proportion, consistent in occurrence, associated with male infertility, and may be heritable are classified as major defects (Chenoweth, 2005). These defects are categorized as acrosome, sperm head, sperm mid piece, and sperm tail defects (Chenoweth, 2005).

2.3.1 Causes of Semen Abnormalities in Bulls

Abnormalities within semen can be caused in number of different ways. For this review, major and some minor defects will be discussed within two different regions of the sperm: head and tail. Within these two regions, it is realized that there are more specific classifications of each region as it relates to sperm which will be mentioned when applicable.

2.3.1.1 Sperm Head Defects

Acrosome defects, specifically knobbed acrosomes, have a genetic relationship with the Friesian bull as reviewed by Chenoweth (2005). Since the genetic link within Friesian bulls, other breeds have noticed a similar existence (Barth and Oko, 1989; Chenoweth, 2005). Although genetics plays a role in knobbed acrosomes, environment is often associated with heat regulation issues within testes, illness, nutritional deficiencies, and more (Jelinski et al., 2002).

Detached heads are common to find in small amounts within bull semen samples (Barth and Oko, 1989). Hereford bulls, along with certain dairy breeds, are genetically at a higher risk of detached heads (Chenoweth, 2005). Detached heads can be observed with the separation of both the head and tail of sperm. Hypoplasia of the testicles are associated with increased numbers of detached heads (Barth and Oko, 1989). It is also thought bulls with inflammation, heating of testicles and prolonged inactivity can experience an increased amount of detached heads (Barth and Oko, 1989; Jelinski et al., 2002).

2.3.1.2 Tail Defects

Dag defects are signified by extreme coiling and folding of the tail. Named after the Jersey bull in which the defect was first identified, has a strong hereditary basis with a genetic correlation at 0.50% (Chenoweth, 2005; Roberts et al., 2010). It should be noted that environment will play a role with this disorder in the testis or epididymis (Barth and Oko, 1989; Chenoweth, 2005).

Pseudodroplets defect, not to be confused with proximal droplets as they are more dense and irregular in shape, are often rare in finding (Barth and Oko, 1989; Chenoweth, 2005). A hereditary basis is suggested with five Friesian bulls having relation with the defect (Chenoweth, 2005). It is reported that with the added mitochondrial accumulations this defect can be associated with Bovine Ephemeral Fever as well as gossypol spermatotoxicity (Chenoweth, 2005).

Proximal droplets are most often associated with immaturity in young bulls (Barth and Oko, 1989). If seen in excess, especially in older bulls, there might be a link to the developmental process (Jelinski et al., 2002). Proximal droplets are known to have a heritability with a 0.37 genetic correlation (Roberts et al., 2010). More information will be presented below.

Distal midpiece reflex's (bent tails), although classified as a minor defect, are of the most commonly found tail abnormalities in bulls (Barth and Oko, 1989). It is thought that sperm cells are impacted by this defect when they are exposed to abnormal secretions in the epididymis (Barth and Oko, 1989; Jelinski et al., 2002). This secretion can be the influence of several factors mainly from stress induced factors (Barth and Oko, 1989; Jelinski et al., 2002).

2.3.2 Compensable vs. Uncompensable

Not all bulls have the capability to settle cows due to certain sperm morphology issues (Williams and Savage, 1927). However, certain morphology issues can be fixed with the addition of more sperm to a specific dose of semen, being noted as compensable semen traits (Saacke, 2008). Some bulls have morphology issues so severe that additional sperm added to a dose of semen, will not be beneficial in settling cows (Saacke, 2008). This is noted as uncompensable seminal deficiencies (Saacke, 2008). Chenoweth reported that there are two different paths associated with sperm infertility not reaching the location for fertilization, and the inability to fertilize once arriving at the fertilization spot (Chenoweth, 2005). Sperm defects that can be fixed

with added sperm (compensable) are generally a dysfunction of motility (Chenoweth, 2005). However, sperm issues that cannot be corrected with added sperm (uncompensable) are thought to be sperm with defects leading to failed fertilization or pregnancy loss (Chenoweth, 2005).

2.3.3 Sperm Action in Female Tract

When copulation occurs within bovines, semen is deposited within the cranial region of the vagina (Hafez and Hafez, 2013). Upon deposition, three major events must take place in order for fertilization to take place. Transportation, capacitation and the acrosome reaction all must take place for the presumptive spermatozoa to fertilize an oocyte (Senger, 1997).

Within the female bovine, sperm must be present and staged ready for the oocytes to arrive for fertilization (Katz et al., 1989; Senger, 1997). As review by Hawk, contractions of the reproductive tract aid in the rapid transportation of sperm (Hawk, 1987). Sperm can reach the cervix within a female bovine in as little as 90 seconds after insemination (Hafez and Hafez, 2013). This allows for some sperm to reach the site of fertilization in a matter of minutes, while most become trapped within sperm reservoirs throughout the cervix, and it is not thought that these sperm fertilize (Hafez and Hafez, 2013). In part of the sperm reservoirs in the cervix, sperm are “filtered” due to the cervical mucus, sialomucin and sulfomucin (Katz et al., 1989; Senger, 1997). Spermatozoa with abnormalities and non-motility are washed out of the cervix whereas privileged sperm are kept as reservoirs and released slowly to impact fertilization of the egg (Hawk, 1987; Senger, 1997; Hafez and Hafez, 2013).

Sperm must reside in the female tract for some time, before they are able to fertilize an oocyte (Yanagimachi, 1990). One of the physiological changes that must take place for sperm to become fertile is known as capacitation (Yanagimachi, 1990). This process begin soon after spermatozoa enter the female tract, and is not entirely site dependent as it can begin once the removal of

glycoproteins of the spermatozoa (Yanagimachi, 1990). Spermatozoa, once capacitated, becomes hyperactive as it relates to motility (Yanagimachi, 1990; Senger, 1997). Hyperactivity is thought to promote sperm-oocyte contact due to the linear motility pattern of spermatozoa (Senger, 1997). Throughout the capacitation process, the acrosome reaction must take place. It has been noted that the acrosome reaction starts after the spermatozoa comes in direct contact of the zona pellucida (Katz et al., 1989; Florman et al., 2004; Berruti and Paiardi, 2011). With an influx of calcium, the spermatozoa and oocyte can bind at the ZP3 of the oocyte and create penetration (Katz et al., 1989; Florman et al., 2004).

2.4 Proximal Droplets

There is still much mystery that surrounds cytoplasmic droplets, despite being discovered by Retzius in 1909 (Cooper, 2005). Although there has been many different suggestions as to what exactly a proximal droplet is, it is thought to be the remnant of a germ cell cytoplasm that is adherent to the neck region of a spermatid (Cooper, 2005). Proximal droplets, although very distinctive, should not be confused with distal droplets. Distal droplets surround the midpiece, proximal to the annulus (Barth and Oko, 1989). Distal droplets unlike proximal droplets are not viewed as a serious problem. Distal droplets are mostly prevalent in the cauda epididymis while proximal droplets are found in the caput (Barth and Oko, 1989). During the maturation phase, at the point in time that spermatids are entering the lumen, cytoplasm left over from cytoplasmic bridges pushes down onto the proximal piece of the spermatids tail (Keating et al., 1997). In layman terms, during spermatogenesis, cytoplasm builds around the neck region of the spermatid. As the newly formed spermatozoa is ready to be released, cytoplasmic swelling takes place, leaving cytoplasm around the neck of the spermatozoa (Barth and Oko, 1989). This phenomena is

similar to several species from stallions, boars, bulls (beef and dairy) and human males (Althouse, 1998; Cooper, 2005; Cooper, 2011).

There are conflicting theories as to the purpose of proximal droplets. Early literature suggest that the cytoplasmic droplet is utilized to provide nutrition to the spermatozoon prior to being released into seminal plasma (Manns, 1964). Since that time however, there has been no clear explanation and merely thought of as having no true value (Barth and Oko, 1989).

The period as to which a proximal droplet should be shed is thought to be during the time spent within the epididymis (Barth and Oko, 1989; Amann et al., 1993; Amann et al., 2000; Thundathil et al., 2001). Redistribution of the cytoplasmic droplet from the proximal position of the tail to the distal position is thought to happen with different epididymal fluid exposure (Thundathil et al., 2001). Seminal vesicular fluid containing phospholipid binding protein can help with the release of the cytoplasmic droplet (Bialy and Smith, 1958). Physical contact and movement of the spermatozoa are thought to shed the proximal droplet (Cooper, 2011). Within the boar, fructose can help shed the cytoplasmic droplet from the seminal vesicles (Harayama et al., 1996; Althouse, 1998).

Infertility is quickly associated with retained proximal droplets, and for good reasoning as well. Bulls with normal morphology compared to those with 33% proximal droplets in their semen analysis are shown to have drastic differences in their ability to achieve a pregnancy (Nöthling and Arndt, 1995). Bulls with a proximal droplet issue is normally a sign of sexual immaturity, due to abnormal spermatogenesis. With maturity, bulls have a lowered amount of proximal droplets (Amann et al., 2000). Mature bulls that have normal semen, droplets are not associated with spermatozoa (Barth and Oko, 1989). But often many other factors can be overlooked when it comes to the reason behind a retained cytoplasmic droplet.

Weather impacts and specifically temperature has been shown to influence a bulls overall sperm morphology (Gerona and Sikes, 1970; Sekoni and Gustafsson, 1987; Barth and Bowman, 1994). Temperature can be influenced through that of the seasonal effects demonstrated by (Sekoni and Gustafsson, 1987) in which proximal droplets come at a higher occurrence during the summer months compared to that of the colder winter months. This can be further demonstrated in a multitude of studies with scrotal insulation and the detrimental impacts on sperm morphology (Barth and Bowman, 1994; Karabinus et al., 1997; Brito et al., 2003; Wettemann and Boehmer, 2014)

The significance that proximal droplets can have on fertilization are alarming. In a study by Amann and others (2000), observations were evaluated to determine effects of proximal droplets on fertilization rates of beef bulls. Utilizing a Complete Block Design, two experimental groups had 15 bulls in each (droplets vs. control), with two different time periods (beginning vs. 3-4 weeks later). Bulls with smaller scrotal circumferences were more likely to fall into the droplet category (Amann et al., 2000). Additionally, droplet bulls on their second collection (3-4 weeks later) decreased in incidents of proximal droplets (Amann et al., 2000). Cleavage rates for droplet bulls were at 18.0 and 35.5% on the first and second evaluations, respectively; whereas, the control bulls were 46.4 and 38.2%, respectively (Amann et al., 2000). Spermatozoa with a proximal droplet have a low fertilizing potential appears to be the underlying concept of this study (Amann et al., 2000).

In a similar study conducted by Thundathil and others (2001), effects of proximal droplets on sperm-oocyte binding were evaluated along with development of presumptive zygote (Thundathil et al., 2001). Bulls who were classified as PD1, PD2, and PD3 (carrying a percentage of proximal droplets) had less presumptive zygotes make it to the cleavage stage (<20%), and 0 make it to the

blastocyst stage. Although the end result of experimental group (PD1, PD2, and PD3) was substantially different of that of the control bull, there was merely no difference in fertilization rates between the groups. Results suggest that normal sperm along with a high number of sperm with proximal droplets will result in deficient embryo formation (Thundathil et al., 2001). However this finding can be disputed by (Carreira et al., 2012) in which a control group ($\leq 1\%$ Proximal Droplets) and PCD group ($\geq 24\%$ Proximal Droplets) were analyzed for blastocyst embryo formation. Within the in vitro study, there was no statistical difference between the two groups and their ability to form blastocyst stage embryos. However there was difference between specific bulls within the groups (Control vs. PCD). Thus this brings rise to the fact that there may be certain sires with characteristics more prone to proximal droplets (Carreira et al., 2012).

From a nutritional standpoint, it is very common to “push” young bulls to excel on a performance test before sale time, to measure post-weaning grow traits (Silcox, 2017). In a study conducted by Coulter et al. (1987), Angus and Hereford bulls fed two energy diets (high and medium) were evaluated for effects of diet on testicular development and seminal quality (Coulter et al., 1987). The results showcased that a high energy diet can have a harmful impact (lower morphology score) on sperm (Coulter et al., 1987; Callaghan et al., 2016). Callaghan et al. (2016) determined the direct effect of subacute ruminal acidosis and its impact on fertility in bulls. There was multiple significant differences between the control and experimental group when mean FSH levels were evaluated (Callaghan et al., 2016). This presented a cascade of events in which testosterone differed in both groups over multiple periods (Callaghan et al., 2016). With endocrine impacts on spermatogenesis, significant differences were observed on normal sperm. Specifically over the duration of the diet, significant impacts were observed on the percent of proximal droplets in bulls challenged with oligofructose (Callaghan et al., 2016).

Downstream effects of proximal droplets can be detrimental to a herd, especially when thinking about the potential genetic relationship that can occur. In a study by Roberts et al. (2010) with semen collected from 908 bulls, heritability of proximal droplets was reported at 0.37% (Roberts et al., 2010). This is an alarming number given the fact that weaning weight is reported at a 0.28% heritability in Angus cattle (Association, 2018).

2.5 Summary

Efficiency continues to be a key concept for those involved within the agriculture industry. With the added need for efficiency, more specifically faster sexual maturity, proximal droplets continue to bring hardship to both bull sellers and buyers. If a bull cannot ultimately pass a breeding soundness exam, then the question must arise “what is his true purpose for any producer?” With the understanding that bulls who have an increased amount of proximal droplets cannot fertilize an egg, we know this to be a true problem. It comes at a continued concern with recent findings stating a high correlation of heritability as it pertains to proximal droplets (Roberts et al., 2010).

To our knowledge, there is still a gap in knowledge as it relates to possible triggers of proximal droplets. The overall objective of this thesis is to identify possible sources of proximal droplet inducers. For this study, we will be analyzing bulls from two different bull tests and analyzing several variables (pedigrees, growth rate, scrotal circumference and heifer pregnancy rates). If in fact there is a potential correlation between the stated variables and proximal droplets, we feel there is the potential to study downstream effects in daughters from these sires of problem.

CHAPTER 3. OCCURRENCE OF PROXIMAL DROPLETS IN PERFORMANCE-TESTED BEEF BULLS

3.1 Abstract

To determine the occurrence of proximal droplets in performance-tested bulls, parameters related to breeding soundness examination (BSE) classification were evaluated. Bulls (542 Angus bulls) were classified as satisfactory (BSE guidelines; Society for Theriogenology) or deferred. On-test risk factors were identified, associated with increased probability for bulls to be deferred due to proximal droplets. On-test weight ($P = 0.019$), on-test scrotal circumference ($P = 0.018$) and the covariate of age ($P = 0.007$) were all associated with the probability for a bull to be deferred on test due to proximal droplets. Accounting for the covariate of age and the effect of on-test SC, bulls in weight category 5 (>500 kg on-test weight) had 12.5 times the odds of deferral due to proximal droplets compared to bulls in weight category 2 (350 – 400 kg) (OR: 12.5, 95% adj. CI: 1.47, 111.11; adj. $P = 0.017$). Accounting for the covariate of age and the effect of on-test weight, bulls that were categorized as SC on-test 1 (< 30 cm) had 7.9 times the odds for deferral due to proximal droplets compared to bulls categorized as SC 3 (33 – 36 cm; OR: 7.9, 95% CI: 1.4, 44.4; $P = 0.012$). Additionally, bulls that were categorized as SC 2 (30 – 33 cm) had 4.7 times the odds for deferral due to proximal droplets compared to bulls categorized 3 (33 – 36 cm; 95% CI: 1.1, 20.0; $P = 0.03$). After accounting for age and bull on-test weight, bulls that were categorized as SC growth 3 (> 6.2 cm growth) had 3.7 times the odds for deferral due to proximal droplets compared to bulls categorized 2 (3.4 to 6.2 cm growth; OR: 3.7, 95% CI: 1.69, 8.26; $P = 0.001$). In summary, bull on-test weight, on-test scrotal circumference, scrotal circumference growth and age are important factors associated with unsatisfactory classification due to proximal droplets. Occurrence of proximal droplets have been previously associated with bull maturity; however the cause of its relationship to on-test weight, on-test scrotal circumference and rapid scrotal circumference growth in performance-tested bulls remains unclear.

Keywords: proximal droplet, bulls, fertility, scrotal circumference, breeding soundness examination

3.2 Materials and Methods

3.2.1 General

During a four-year period, 542 Angus bulls that completed a performance test were utilized for data analysis. Of these bulls, 322 resulted from information obtained from a centralized testing facility and the remaining bulls (n=220) were acquired from a private testing facility, both in central Tennessee. Requirements for inclusion in the study were similar between the two locations. For participation in the performance bull sale, bulls must have met the birth date requirements (12-16 months of age at time of sale for senior bulls and 12-15 months of age for junior bulls), attain an 84-day test average daily gain (ADG) ratio of 80 within contemporary breed groups and an minimum adjusted 365 day weight ratio of 80 within contemporary breed groups, and a frame score of 5.0 or greater at 365 days of age. Bulls must have also passed a Breeding Soundness Examination (BSE; Hopkins and Spitzer, 1997) with a minimum semen motility of 30% and normal sperm morphology of at least 70%, along with a satisfactory scrotal circumference (≥ 30 cm for 12 months of age and ≥ 31 cm for 15 months of age and greater at the end of the 84 day test). Bulls were then classified as either satisfactory (successfully passed all aspects of the BSE as set forth by the Society for Theriogenology) or deferred (unsuccessfully completed the semen portion of the BSE). Data observations collected during the performance test period included body weight and scrotal circumference (SC) measurements collected at on-test and off-test dates, and evaluation of spermatozoa motility and morphology performed during the first BSE immediately at the end of the testing period. If bulls were deferred due to semen abnormalities, a second breeding soundness examination was conducted approximately 24-30 days after the initial BSE. Abnormal sperm included: tightly coiled tails, bent tails, Dags, detached heads, misshaped heads, and proximal droplets.

Diet consisted of a concentrate-based pellet and mixed grass hay offered as separate ration components. Bulls were provided with *ad libitum* access to feed and water. Minerals met requirements set forth by the NRC. Bulls were vaccinated and tested for any communicable diseases prior to admission. Similar specifications were outlined for bulls from the private testing facility to be eligible for sale. Bulls were combined together to evaluate different variables that impact the central hypothesis and other measurable differences.

3.2.2 Statistical Analysis

To obtain demographics of the bulls included in records and descriptive statistics, frequency procedures of SAS along with Proc Univariate were used to obtain descriptive statistics (SAS 9.4, SAS Inst., Inc., Cary, NC, USA). Tables were used to determine the number of bulls within the study. Additionally, tables described the demographics of bull variables for those that successfully completed their first BSE and those that were deferred on their first BSE. Frequency distributions were determined of sperm defects and BSE deferment due to proximal droplets, as well as the frequency of bulls that were initially deferred to pass subsequent examinations. Simple correlations between continuous variables were observed using PROC CORR.

To determine on-test risk factors associated with deferral due to proximal droplets, GLIMMIX procedure was used to develop generalized linear mixed models with binomial distributions and logit link to test what factors were associated with the probability for bulls to be deferred due to proximal droplets on their first BSE. Final model selection was performed with manual forward selection. Based upon visual examination of distributions of the continuous variables on-test scrotal circumference and on-test body weight, categorical variables were created. For on-test weight, categories 1, 2, 3, 4, and 5 corresponded to bulls that were < 350 kg, 350 to 400 kg, 400 to 450 kg, 450 to 500 kg, and >500 kg, respectively on test day 1. Scrotal circumference on test

day 1 was categorized as 1, 2, 3, and 4 for bulls with SC of < 30 cm, 30 to 33 cm, 33 to 36 cm, and > 36 cm, respectively on test day 1. Age was included in the model as a possible covariate. Tukey's adjustment for multiple comparisons was used for variables with greater than 4 levels.

To determine if scrotal growth during the bull test was associated with increased risk of deferral due to proximal droplets, the difference between on-test SC and SC at the end of the test period was defined as SC growth. This variable was then categorized similarly to the SC on-test and body weight variables. Bull SC growth was categorized as 1, 2, and 3 for growth of < 3.4 cm, 3.4 to 6.2 cm, and > 6.2 cm, respectively. Age and on-test SC were included as potential covariates. Multilevel, multivariable logistic regression was performed for the outcome of deferral due to proximal droplets. Manual forward model selection was utilized. Mean separation was performed using the LSMEANS option, and model adjusted probabilities obtained using the ILINK function. Tukey's adjustment for multiple comparisons was used for variables with greater than 4 levels.

The final analysis investigated ancestry influence on probability for bulls to test positive for proximal droplets. A reverse sort on Angus ancestors was performed to identify possible ancestors of interest. Bulls of interest were identified as those sires with at least 10 offspring included in the bull test. Dichotomous variables were created for each ancestor of interest, where 0 indicated that the ancestor was not included in the bull on test's pedigree, and 1 indicated that the ancestor of interest was found in the test bull's pedigree. The final GLIMMIX model from the prior analysis that included variables associated with proximal droplets was utilized and the bulls of interest were included in model building process. Ancestors remained in the model if their presence was associated at a significance level of $\alpha = 0.05$.

For all logistic regression analyses, means separation were performed using the LSMEANS statement and DIFF option, and model adjusted probabilities were obtained using the ILINK function. Tukey's adjustment for multiple comparisons was used for variables with more than 4 levels. Statistical significance was set at $\alpha = 0.05$.

3.3 RESULTS/DISCUSSION

3.3.1 General Characteristics of Bull Fertility and Performance

Over the entire study, proximal droplets accounted for 91 (62.8%) out of 145 total sperm defects. This is in agreement with Ellis and others (2005) who reported that proximal droplets was the primary reason for BSE retest (Ellis et al., 2005). In the current study, 59 (10.9%) of 542 bulls were deferred upon the first examination due to proximal droplets. Of those deferred bulls, 39 were retested approximately 24-30 days after initial BSE and 28/39 (72%) were able to pass. These data are conclusive with other literature that indicate a reduction in the occurrence of proximal droplets with sexual maturity associated with age (Barth and Oko, 1989; Amann et al., 2000; Thundathil et al., 2001; Ellis et al., 2005). A cross section of the bulls within the current study are listed in tables 1 and 2 to better understand the general demographics.

Table 1. Demographics of bulls that successfully passed the first breeding soundness examination.

Variable	Number	Mean ($\pm$ STD DEV)	Median ($\pm$ IQR)
Birthweight (kg)	422	32.48 ($\pm$ 4.10)	32.65 ($\pm$ 4.54)
Age (days)	482	427.87 ($\pm$ 23.32)	434.00 ($\pm$ 30.00)
HP EPD	451	10.58 ($\pm$ 2.39)	10.50 ($\pm$ 3.40)
On Test Weight (kg)	482	414.55 ($\pm$ 72.21)	414.51 ($\pm$ 121.54)
Off Test Weight (kg)	482	589.09 ($\pm$ 70.25)	585.03 ($\pm$ 99.77)
Daily Gain (kg/day)	482	1.86 ($\pm$ 0.30)	1.84 ($\pm$ 0.39)
On Test SC (cm)	276	32.93 ($\pm$ 2.93)	33.00 ($\pm$ 3.40)
Off Test SC (cm)	481	37.35 ($\pm$ 2.41)	37.40 ($\pm$ 3.50)
SC Growth (cm)	275	4.84 ($\pm$ 2.10)	4.50 ($\pm$ 2.50)

Table 2. Demographics of bulls that were classified as deferred due to proximal droplets at the first breeding soundness examination.

Variable	Number	Mean ($\pm$ STD DEV)	Median ($\pm$ IQR)
Birthweight (kg)	51	32.43 ($\pm$ 3.04)	33.56 ($\pm$ 2.72)
Age (days)	59	414.56 ($\pm$ 29.20)	418.00 ($\pm$ 45.00)
HP EPD	55	10.88 ($\pm$ 2.66)	10.60 ($\pm$ 3.20)
On Test Weight (kg)	59	427.20 ($\pm$ 76.07)	436.73 ($\pm$ 113.83)
Off Test Weight (kg)	59	599.96 ($\pm$ 72.08)	611.34 ($\pm$ 112.47)
Daily Gain (kg/day)	59	1.93 ($\pm$ 0.29)	1.89 ($\pm$ 0.39)
On Test SC (cm)	45	31.40 ($\pm$ 4.02)	31.50 ($\pm$ 3.70)
Off Test SC (cm)	59	37.18 ($\pm$ 3.07)	37.00 ($\pm$ 4.90)
SC Growth (cm)	45	6.11 ($\pm$ 2.85)	5.70 ($\pm$ 4.20)

3.3.2 Preemptive Analysis determining bulls on-test risk factors

Risk factors associated with increased probability for bulls to be deferred due to proximal droplets were identified through various statistical analyses. On-test weight ($P = 0.019$), on-test scrotal circumference ($P = 0.018$) and the covariate of age ($P = 0.007$) were all associated with the probability for a bull to be deferred due to proximal droplets at the first BSE. The model-adjusted probability of proximal droplet-related deferral associated with a bull on-test weight is shown in figure 1. Accounting for the covariate of age and the effect of on-test SC, bulls in weight category 5 (>500 kg on-test weight) had 12.5 times the odds of deferral due to proximal droplets compared to bulls in weight category 2 (350 – 400 kg) (OR: 12.5, 95% adj. CI: 1.47, 111.11; adj. $P = 0.017$). Bulls that arrived to test with a heavier bodyweight are perhaps those that were offered creep feed before arriving to the test station. Another option may include bulls of high growth potential which may also be associated with later maturity. The probability for proximal droplets due to on-test SC is denoted in figure 2. Accounting for the covariate of age and the effect of on-test weight, bulls that were categorized as SC on-test 1 (< 30 cm) had 7.9 times the odds for deferral due to proximal droplets compared to bulls categorized as SC 3 (33 – 36 cm; OR: 7.9, 95% CI: 1.4, 44.4; $P = 0.012$). Additionally, bulls that were categorized as SC 2 (30 – 33 cm) had 4.7 times the odds for

deferral due to proximal droplets compared to bulls categorized 3 (33 – 36 cm; 95% CI: 1.1, 20.0; $P = 0.03$). Although genetic performance of bulls have changed over time, Coulter and Foote (1977) demonstrated the importance of bulls with heavier body weights correlating with larger scrotal circumferences despite age (Coulter and Foote, 1977). Additionally, our data suggest that bulls have an optimum scrotal circumference range that lends itself to less proximal droplets at the time of breeding soundness examinations. There have been studies describe what a bulls growth pattern should be in terms of scrotal circumference (Pratt et al., 1991). However, these studies do not describe the optimum scrotal circumference range to have a decreased probability for proximal droplets.

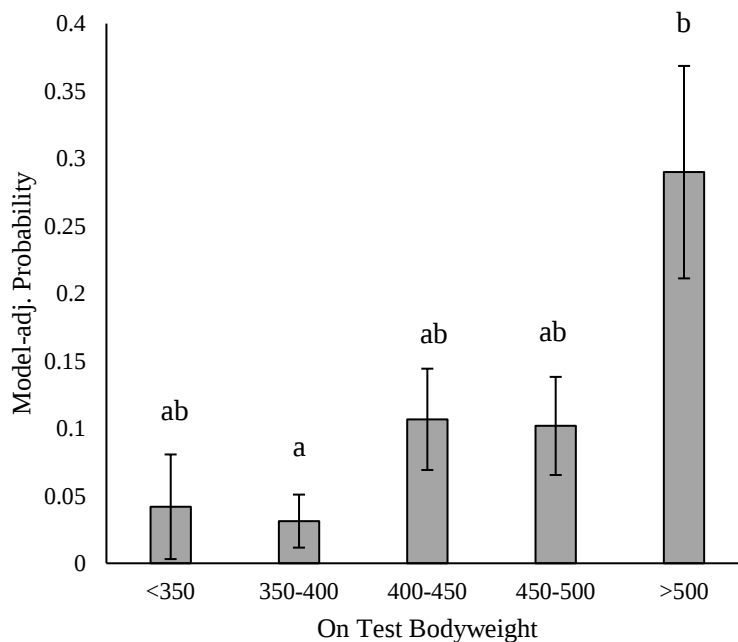


Figure 1: Model-adjusted probability for bulls to be deferred due to proximal droplets by on test body weight at the first BSE.

Like Superscripts are not statistically different at $P \leq 0.05$.

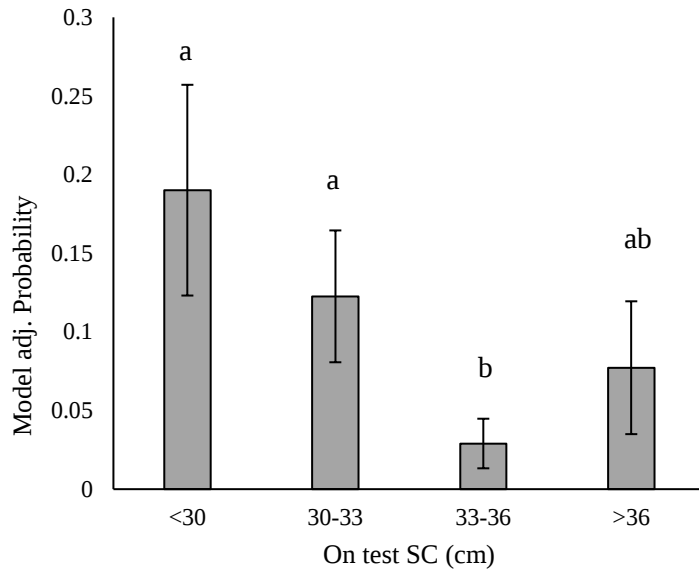


Figure 2: Model-adjusted probability for bulls to be deferred due to proximal droplets by on-test scrotal circumference at the first BSE.

Like Superscripts are not statistically different at $P \leq 0.05$.

3.3.3 Factors Associated with Proximal Droplets Post-Test

It was determined that there was a moderate negative correlation between on test scrotal circumference and scrotal circumference growth over the duration of the test period ($R = -0.57$, $P < 0.0001$). To determine if there were differences in the probability of bulls to be deferred due to proximal droplets based upon the categorical scrotal growth variable, the continuous variable of on-test SC in the model as a possible covariate, but was removed after having no significant effect ($P > 0.05$). The categorical variable of SC growth was associated with the probability for deferral ($P = 0.004$). Age ($P = 0.001$) remained in the model as an important covariate, and on-test weight remained in the model with significance ($P = 0.04$). Accounting for the effect of age and SC growth, bulls that were categorized as weight category 5 (> 500 kg on-test weight) had 9.5 times

the odds for deferral due to proximal droplets compared to bulls categorized as weight 2 (350 - 400 kg; OR: 9.5, 95% adj. CI: 1.14, 76.9; $P = 0.03$).

The probability of proximal droplets due to scrotal circumference growth during the testing period is noted in figure 3. After accounting for age and bull on-test weight, bulls that were categorized as SC growth 3 (> 6.2 cm growth) had 3.7 times the odds for deferral due to proximal droplets compared to bulls categorized 2 (3.4 to 6.2 cm growth; OR: 3.7, 95% CI: 1.69, 8.26; $P = 0.001$).

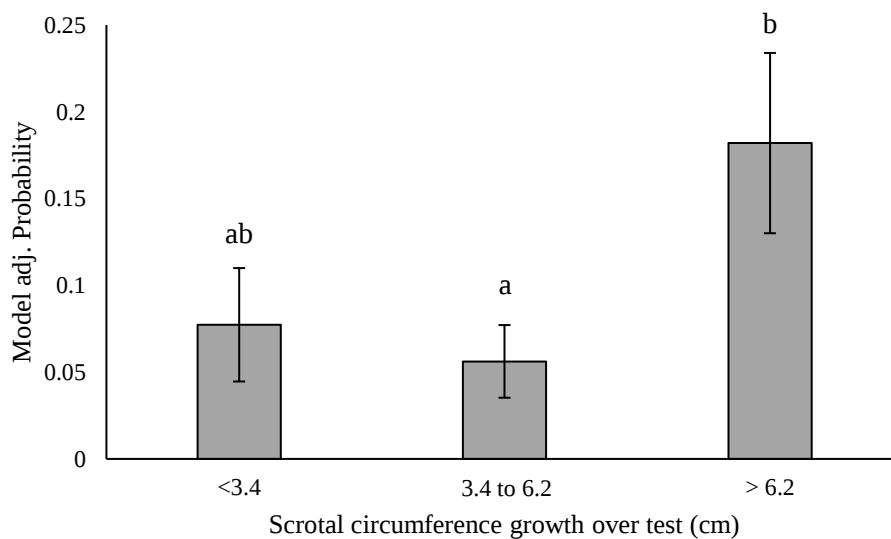


Figure 3: Model-adjusted probability for bulls to be deferred due to proximal droplets by scrotal circumference growth during the test period.

Like Superscripts are not statistically different at $P \leq 0.05$.

Many bull testing sites emphasis high weight gain from energy-rich rations to maximize performance (Silcox, 2017). However, little is known about the true ramifications of this fast

scrotal gain on bulls. We speculate that perhaps bulls with rapid scrotal circumference growth are not sexually mature, even with acceptable scrotal circumferences, to withstand the increased growth. This continues to remain consistent of previous studies describing the importance of sexual maturity in bulls (Barth and Oko, 1989; Johnson, 1997; Amann et al., 2000; Thundathil et al., 2001; Jelinski et al., 2002). Moreover, little is known if rapid scrotal circumference growth is occurring in younger bulls. Conceivably younger bulls must meet the scrotal circumference size of the Society of Theriogenology guidelines to pass this section of the BSE. In order for this to take place, rapid growth must take place. It can be speculated that epididymis function may be compromised in bulls with rapid testicular growth (Amann et al., 1993).

3.3.4 Ancestry Influence

It has been documented that proximal droplets are highly heritability with a 0.37 genetic correlation (Roberts et al., 2010). In the current study, two sires were identified as increasing the odds to be deferred at the first BSE due to proximal droplets. The presence of these bulls within the pedigree significantly increased probability of deferral due to proximal droplets. Within the model, sires #127 ($P = 0.03$) and #205 ($P = 0.04$) were identified as sires of interest. The categorical variables of on-test weight ($P = 0.04$) and SC growth ($P = 0.002$) remained in the model as significant factors, as well as the covariate of age ($P = 0.001$).

After adjusting for ancestors of influence, age, and SC growth, bulls that were classified as weight category 5 (> 500 kg on-test weight) had 11.11 times the odds for deferral due to proximal droplets compared to bulls categorized as weight 2 (350 - 400 kg; 95% CI: 1.21, 100.0; $P = 0.03$). This remains consistent to our findings throughout the analyses that bulls with a heavier bodyweight on test are more likely to be deferred because of proximal droplets. Bulls coming onto test heavier, in theory, should be further along in their body condition score and it has been shown

that excess body condition may be detrimental to fertility in cattle (Whitman, 1975). This same thought could be applied to bull fertility as bulls with increased body condition may deposit fat around the neck of the scrotum and causing over heating within the testis (Barth and Bowman, 1994; Karabinus et al., 1997; Brito et al., 2003; Wettemann and Boehmer, 2014).

After accounting for other variables in the model, bulls that were categorized as SC growth 3 (> 6.2 cm growth) had 4.3 times the odds for deferral due to proximal droplets compared to bulls categorized 2 (3.4 to 6.2 cm growth; 95% CI: 1.87, 9.70; $P = 0.0006$). The impact of scrotal circumference growth rate was noted by (Pratt et al., 1991) as characterizing bulls with 0.06 cm growth per day, completing a BSE. However, to the best of our knowledge, rapid scrotal circumference growth has not been investigated.

Bulls who had Sire #127 within their pedigree had 8.33 times the odds (95% CI: 1.21-57.50; $P = 0.03$) of deferment due to proximal droplets compared to bulls without sire #127 pedigree. Bulls who have Sire #205 within their pedigree had 5.95 times the odds (95% CI: 1.133-31.29; $P = 0.0352$) of deferment due to proximal droplets compared to bulls without sire #205 pedigree. As noted earlier, proximal droplets are known to have relevance as it relates to heritability with a 0.37 genetic correlation (Roberts et al., 2010). This analysis indicates a possible genetic influence of sire having a direct impact on the occurrence of proximal droplets in performance tested bulls. Downstream impacts are possible as well, considering the majority of the bulls within the study reduced the occurrence of proximal droplets upon the second BSE (79%) 24-30 days afterwards. However, there are many factors that are known to directly impact female fertility and specifically the onset of puberty in daughters (Martin et al., 1992). More specifically for the sire's impact, scrotal circumference size is often attributed to daughter's fertility (Moser et al., 1996). Even with fertility often thought of as a lowly heritable trait, it is still of significant selection importance due

to the downstream effects of efficiency within a herd (Moser et al., 1996). Those sires that have proximal droplet occurrences could have daughters (or sons for that matter) with a lengthened time to puberty.

CHAPTER 4. CONCLUSION

Efficiency continues to be a key concept for those involved within the agriculture industry. With the added need for efficiency, more specifically faster sexual maturity, proximal droplets continue to bring hardship to both bull sellers and buyers. If a bull cannot ultimately pass a breeding soundness examination, then the question must arise “what is his true purpose for any producer?” With the understanding that bulls who have an increased amount of proximal droplets cannot fertilize an oocyte, this is an area of concern regarding subsequent fertility of the bull. It comes at a continued concern with recent findings stating a high correlation of heritability as it pertains to proximal droplets (Roberts et al., 2010). Age remains consistent of previous findings to have a direct impact on a bull’s ability to pass a breeding soundness examination as a successful breeder (Bath and Oko, 1989; Johnson 1997; Amann et al., 2000; Thundathil et al., 2001; Jelinski et al., 2002). Rapid scrotal circumference growth can be thought of a detriment to a bull’s fertility specifically for incidents of proximal droplets. Perhaps this can be attributed to a bull’s natural performance requirements and nature of the modern bull testing facility. Bulls coming onto test with a heavier weight could impact their fertility and ability to pass a breeding soundness exam. This could be due to their heavier body condition score and heating effects on the scrotum (Barth and Bowman, 1994; Karabinus et al., 1997; Brito et al., 2003; Wettemann and Boehmer, 2014). Downstream implications could have lasting effects on daughter’s fertility and efficiency within a herd.

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VITA

Zach Bartenslager was born in Roanoke, Virginia to parents Arthur and Ruth Bartenslager. He was raised in Lewisburg, West Virginia where he graduated Greenbrier East High School in 2011. In the fall of 2011, he moved to Hutchinson, Kansas, where he pursued an Associates of Arts Degree at Hutchinson Community College. Upon completion of his two years at Hutchinson Community College, Zach moved to Columbus, Ohio, to pursue a Bachelor of Science Degree in Agriculture at The Ohio State University. Upon completion of his undergraduate career, Zach moved to Knoxville, Tennessee, to work towards obtaining a Master's of Science Degree in Animal Science with a concentration in Reproduction Physiology. Zach plans to conclude his Master's degree in May of 2018. Upon completion of his degree program at the University of Tennessee, Zach will continue to coach the livestock judging team at the University of Tennessee, along with moving into a lecturer position for the department of Animal Science.