

Male livestock fertility: boar & bull management considerations

by

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A.S., Northwest Community College, 2014
B.S., Kansas State University, 2018
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Abstract

Male livestock fertility is a vital component of efficient reproduction and significantly impacts the profitability of production systems. Over the last several decades, substantial strides have been made regarding the understanding of female fertility, but relative to females, research in male reproductive management is sparse. In swine and cattle production systems, the male can impact thousands of offspring and significantly impact future generations, drawing attention to the value of understanding if and how development and management of male breeding stock affects fertility. The studies within this dissertation contribute to the body of knowledge on bull and boar management practices and subsequent reproductive outcomes.

In the first study, we aimed to assess the effects of percentage body weight change in developing Duroc boars on semen collection training, semen production parameters, and longevity in a commercial boar stud. Retrospectively, 164 boars were divided into one of three groups based on their percentage body weight change from arrival to the boar stud until the end of the 42-day isolation period. The one-third of boars that had the greatest percentage of body weight change during the isolation period gained 36.1% to 10.1% (TOP). The middle one-third of boars that were intermediate in percentage body weight change during isolation gained 9.7% to 2.6% (MIDDLE). The final group consisted of one-third of boars that either minimally gained or lost weight (2.5% to -9.5% change in body weight; BOTTOM). Boars were observed for six months to evaluate the impact of percent body weight change during the 42-day isolation period on semen parameters and longevity. Boars in the TOP group lost the least amount of backfat (indicated by ultrasound or caliper score) ($P < 0.05$), while boars in the BOTTOM group lost the most backfat of the three groups. Average boar age at the time of successfully becoming a working boar (successfully mounting the collection dummy and producing an ejaculate with a

motility $\geq 70\%$ and percent normal morphology $\geq 65\%$) and the proportion of boars that were successful at become working boars did not differ between groups ($P > 0.05$). Among working boars, the concentration of sperm in ejaculates tended to differ ($P = 0.0740$) between groups. Boars in the TOP group had a greater percentage of sperm with normal morphology ($P = 0.0337$) than boars in the BOTTOM group. There was a group by production week interaction ($P < 0.0001$) for semen ejaculate volume, total number of sperm, and total number of sperm with normal morphology. Boars in the TOP and MIDDLE groups produced more total sperm and normal sperm than the BOTTOM group from weeks 8 to 20 of being working boars. During their time as working boars, an average of 26.8% of boars were either culled from the stud or died, and there was no difference ($P > 0.05$) among groups. While there were no differences in semen collection training and longevity in the stud, the increase in production of total normal sperm in the TOP and MIDDLE groups compared with the BOTTOM group could have substantial economic benefits for boar studs.

In the second study our objective was to determine the relative percentages of calves sired by either natural service sires or fixed time artificial insemination (FTAI) sires within the same estrous period. During 2 consecutive years heifers and cows (heifers: $n = 141$; cows: $n = 191$) had estrous cycles synchronized and were inseminated following the 7-day CO-Synch + CIDR FTAI protocol. Females were inseminated by one AI technician using a single sire for heifers and a different single sire for cows. All females were exposed to natural service bulls immediately following AI. After calving, DNA was collected from a random subset of calves (Calves born from heifers in Year 1: $n = 59$ and Year 2: $n = 82$; Calves born from cows in Year 1: $n = 89$ and Year 2: $n = 102$) born in the first 21 day of the calving season to determine sire parentage. In Year 1 among calves born from heifers, the percentage sired by natural service was

5.1% (n = 3/59). Among calves born from cows, the percentage sired by natural service was 14.6% (n = 13/89). In Year 2 among calves born from heifers, the percentage sired by natural service was 9.8% (n = 8/82). Among calves born from cows, the percentage sired by natural service was 20.6% (n = 21/102). If commercial producers use FTAI followed by immediate bull exposure in cows, natural service bulls may sire more calves early in the calving season than expected. When using these practices in heifers, natural service bulls sired a lesser proportion of the calves than observed in cows.

The objective of the third study was to compare breeds and evaluate correlations of sperm quality assessments observed during yearling beef bull breeding soundness exams (BSE). Ejaculates were collected via electroejaculation from yearling Charolais (n=23) and Angus (n=23) bulls as part of BSEs. One veterinarian conducted BSEs, and one technician conducted sperm quality assessments. Additional sperm motility analysis was conducted with the iSperm. Ejaculates meeting minimum thresholds for passing a BSE were subjected to flow cytometry to measure sperm functional traits. Pearson's correlation coefficients were determined, and breed comparisons were made using GLIMMIX in SAS. The iSperm analyzer gross and progressive motilities were ($r = 0.30; 0.38; P < 0.001$) correlated with technician progressive motility. Neither iSperm ($P = 0.26$) nor visual assessment ($P = 0.66$) of sperm motility differed among breeds. Bull breed did not influence ($P = 0.83$) total percentage of viable cells, percentage of viable cells with intact acrosomes ($P = 0.83$), or percentage of live sperm cells ($P = 0.92$) with positive reactive oxygen species (ROS) status. There was a tendency ($P = 0.10$) for greater percentage of sperm from Charolais bulls ($31.1\% \pm 3.35$) to have positive mitochondrial energy potential as compared with Angus bulls ($17.6\% \pm 3.35$). The percentage of live spermatozoa with negative ROS status was moderately correlated with the percentage of spermatozoa exhibiting

secondary abnormalities ($r = 0.33$; $P = 0.02$). Percentage of live spermatozoa with disrupted acrosomes was strongly correlated ($r = 0.66$; $P < 0.001$) with percentage of live spermatozoa with negative ROS. Percentage of live spermatozoa with positive ROS status was correlated ($r = 0.58$; $P < 0.001$) with percentage of spermatozoa with active mitochondrial membranes.

Technician and iSperm sperm motility are positively correlated, offering producers an on-farm evaluation tool. Though bull breed had little influence on sperm quality assessments in this experiment, ROS in sperm appeared to impair sperm health and function.

The fourth study investigated the potential impacts of concurrent enrollment of undergraduate students in lecture and laboratory animal reproduction courses on final course percentages. Student learning outcomes and structure of the laboratory course were designed to provide hands-on learning opportunities, which coincided with concepts discussed in lecture. A total of 307 students were included in the analysis. Students concurrently enrolled in laboratory and lecture had a greater ($P < 0.001$) final course percentage in the lecture compared with those enrolled in lecture alone. Students in the science degree option had a greater ($P < 0.03$) final lecture course percentage compared with those in the production degree option, and juniors had a greater ($P = 0.05$) final course percentage when compared with sophomores. At the end of the semester, students were surveyed about the perceived value of the laboratory course on their learning. Among students enrolled in laboratory sections, 98.4% indicated the hands-on activities improved their knowledge of course concepts in lecture. These student beliefs are supported by our results, which suggest that taking the laboratory and lecture together improves student final course percentages and that students value the hands-on learning opportunities provided in laboratory sections.

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Co-Major Professor
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Abstract

Male livestock fertility is a vital component of efficient reproduction and significantly impacts the profitability of production systems. Over the last several decades, substantial strides have been made regarding the understanding of female fertility, but relative to females, research in male reproductive management is sparse. In swine and cattle production systems, the male can impact thousands of offspring and significantly impact future generations, drawing attention to the value of understanding if and how development and management of male breeding stock affects fertility. The studies within this dissertation contribute to the body of knowledge on bull and boar management practices and subsequent reproductive outcomes.

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graduate program, and I know we have made lifelong friendships. My Hillcrest girls (Mikayla, Allison, Abigail, and Tamra), I am so thankful that life brought me such unique and random people! We have shared some of the most amazing memories, and I can't wait to see where life takes you all. Most of all, I can't wait to be a part of it! Kolton, thank you for your friendship and support over the years. I am so thankful to have been able to finish this chapter of life with you and start the next.

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Dedication

Similar to my thesis, this dissertation is dedicated to my Grandma Rita and Grandpa Joe. My Hartman grandparents played such a large role in molding me into the person I am and giving me my love of animals. I can only hope that they are looking down on me with pride.

Preface

This dissertation is original work completed by the author, A. R. Hartman. Each chapter was formatted according to the required standards of the corresponding journal.

Chapter 1 - Literature Review

Introduction of Boar Management

In the last 60 years, the United States swine industry has experienced drastic changes. Individual farms are now larger, more specialized, and more efficient. The current organizational structure of the majority of the United States swine industry consists of highly specialized individual operations that focus on one phase of the production system (Key and McBride, 2007). The specialized operations of today's United States swine industry include farrow to finish, breed to wean, gilt development units, and boar studs (Knox, 2014). Notably, the advancements in reproductive technologies, genetic flow, and concentrated sperm production have brought about the development of boar studs (Knox et al., 2008; Knox, 2014). The specialization of production has allowed for substantial increases in productivity in each segment and rapid improvement of genetics. The swine industry's genetic structure resembles a pyramid, where superior genetics flow from the top of the pyramid to the bottom (Knox, 2014).

Figure 1.1 Structure of swine genetic pyramid and flow of genetics from the nucleus level of the pyramid to lower levels to create crossbred offspring for market animals

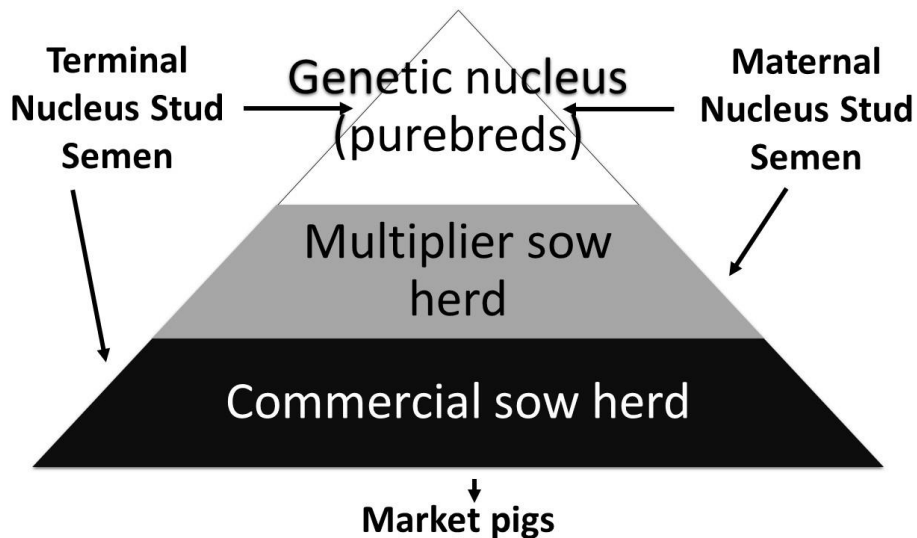


Figure 1.1: Adapted from Knox (2014).

The genetic nucleus is comprised of purebred animals that are used to make either more purebreds, or F1 crosses used at the multiplier sow herd level. The multiplier level then produces commercial females for increasing the sow herd, and those commercial females are mated to terminal sires to produce market pigs. This flow of genetics allows for widespread use of certain breed lines, and for more efficient use of high-indexing boars (more genetically desirable) to produce insemination doses.

With the changes in industry structure, one of the most dramatic differences between 60 years ago and now is in the use of artificial insemination in sows and, consequently, alterations in boar management. Historically, pen mating was the breeding method used for most females where the male and female were physically brought together to mate, but in the 1980s, the industry shifted to use of artificial insemination (AI) (Bortolozzo et al., 2015). Boar studs began appearing in the 1990s as farms transitioned from largely farrow-to-finish operations housing both boars and sows to individualized management facilities (Key and McBride, 2007; Knox, 2014).

With the shift to AI, boars are now housed in semen production centers, or boar studs, managed and developed separately from females. Based on the most recent estimates from 2008, the United States had ~130 boar studs housing nearly 27,000 working boars (Knox, 2016). As of 2012, 91.1% of sows in the United States were inseminated via AI (USDA, 2017). This shift in sow management has consequently changed the demand and management of boars. Swine farms previously required one boar for every 20 sows, and now one boar is required for every 250 sows (Tokach et al., 2016). These changes have allowed farms to better utilize genetically superior boars, advance semen collection methods, improve semen quality control, and enhance biosecurity measures. However, a significant gap has been created in understanding the

development, management, and ways to improve fertility in these boars compared to other swine industry sectors because little research is conducted in this area. A timeline of important development milestones for boars is shown in Figure 1.2.

Figure 1.2 Timeline of significant events for a boar’s productive development and lifetime

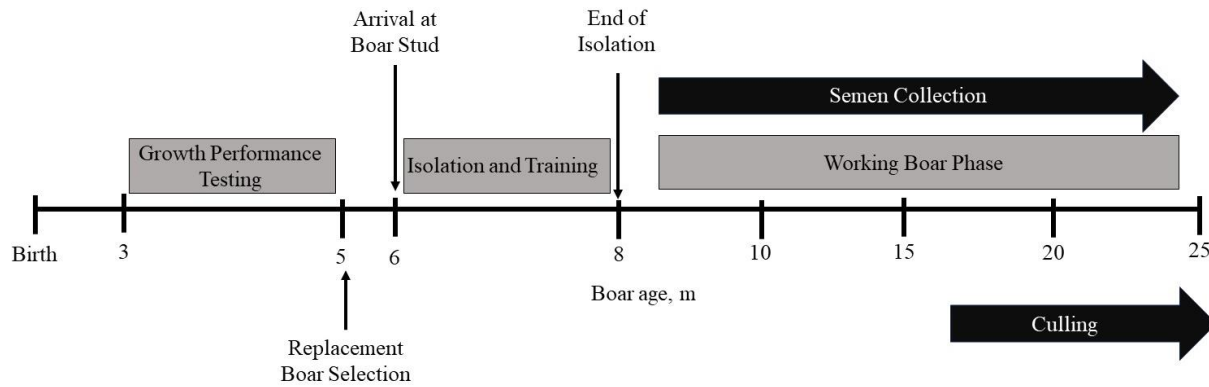


Figure 1.2: Adapted from Flowers (2015).

In the late 1990s and early 2000s, increased use of terminal crossbreeding (Key and McBride, 2007) was facilitated by increased use of AI. Terminal crossbreeding programs allow producers to take advantage of heterosis and capitalize on breed strengths, allowing maternal females to be bred to terminal boars (Key and McBride, 2007). As a result of these programs, the United States boar herd primarily consists of terminal sires selected exclusively for their growth and carcass characteristics (Robinson and Buhr, 2005; Flowers, 2008). One of the most significant challenges in this population is managing their rapid growth rate and the potential stress it places on the boar's skeletal system (Robinson and Buhr, 2005). It is possible that the stress placed on the boar’s skeletal system will lead to lameness later in life. Another challenge of this population is maintaining them at an ideal body condition for semen collection with their rapid growth rates (Robinson and Buhr, 2005). Ensuring that boars do not grow too quickly or

become too thin is a challenge for boar studs. Despite the challenges in these terminal lines, there are no clear recommendations about management of these boars for future semen production.

Boars start producing semen for use in artificial insemination around 9 months of age and spend approximately 9-18 months in the stud with weekly collections averaging 80-100 billion sperm produced each week. During this time boars have the potential to sire thousands of offspring, and boars removed earlier during this time are unable to produce as many sperm. Despite the impact an individual boar has on the production system, literature evaluating how to improve their longevity is scarce. The economic impact of longevity and its reduction has yet to be emphasized in boars, creating many research opportunities (Robinson and Buhr, 2005; Knox et al., 2008). High culling rates because of lameness and poor reproductive performance in boar studs suggest an opportunity to start emphasizing longevity in the boar. Estimates suggest it costs \$1,500 for every boar that enters isolation (PIC, 2022). These costs highlight the significant investment for getting a boar to the stud. Early removal of boars from the herd means they are less productive and less likely to return their development and care costs; this is also a loss in desirable genetics.

Many factors influence boar semen production: pen space while growing, air quality, temperature, warm-up pen stimulation, and exposure to pathogens (Knox et al., 2008). A boar's productive life is relatively short, as culling begins between 18 and 24 months of age (Flowers, 2009). Culling during this time is often because of poor semen quality, structural issues, low libido, and failure to meet genetic performance thresholds (Robinson and Buhr, 2005; Knox, 2016; Kondracki et al., 2021). This short life span makes the quality of boar development and management vital to minimize culling and maximize semen output during this time.

Despite these advancements, little is known about critical factors impacting the collection quality and quantity of boar semen. Futuristically, improving boar development and management could decrease culling frequency and increase semen production. With replacement rates up to 21% because of poor libido and as high as 60% for lameness, a real opportunity exists to reduce culling and decrease its economic losses (Robinson and Buhr, 2005; Knox et al., 2008; Kondracki et al., 2021). Many facets need to be explored to improve boar management and semen collection.

Developmental Management

Pre and Postnatal

Factors affecting a boar's productive lifetime begin prenatally. The primary physiological factors regulating a boar's ability to produce large numbers of quality spermatozoa are the size and quantity of Sertoli cells (Flowers, 2021). Sertoli cell mitotic activity begins mid to late gestation and is the most prolific developmental period and continues until several weeks after birth (Sharpe, 1994). Sertoli cell development during gestation is also when the fetus is undergoing the most rapid growth. Thus, factors that affect fetal development can affect future reproductive functions (Flowers, 2021). Boars originating from litters classified as "intra-uterine growth-restricted" had decreased numbers of Leydig and Sertoli cells as compared with boars from normal birth weight litters (Lin et al., 2017). Lin et al. (2017) also observed that the boars from intra-uterine growth-restricted litters had fewer spermatozoa and lower circulating testosterone concentrations. Similar results are observed when comparing heavy and light birthweight piglets. Heavy birthweight piglets have been shown to consistently have increased testicular size compared to light birthweight piglets (Almeida et al., 2013; Dysart, 2014; Auler et al., 2017). Higher-birthweight piglets also have been shown to have more sperm per ejaculate

and greater lifetime productivity when compared with low-birthweight piglets (Dysart, 2014; Auler et al., 2017). It has been estimated that for every one kilogram increase in birth weight a 35% sperm production increase over their lifetime can be expected (Flowers, 2015).

With continued Sertoli cell proliferation during the first few weeks after birth, it is hypothesized that adequate colostrum intake and milk access play essential roles in boar reproductive development (Flowers, 2021). Adequate colostrum intake has been shown to increase mitosis and differential gene expression in Sertoli cells and the testicular function of boars after sexual maturity (Rahman et al., 2014; Flowers, 2021). Early developmental intervention often includes cross-fostering. When cross-fostering is done strategically so that boars of similar birthweight are reared in litters of six compared to litters of ten, weaning weights are increased, boars have larger testicles, and lifetime semen productivity is increased by 27% (Griffin et al., 2006). Ensuring piglets have equal access to colostrum and milk may increase productivity of replacement boars.

Weaning to Selection

After weaning, boars are moved to developmental facilities where they undergo growth performance testing and are managed similar to market hogs. During development there is an overlap of performance testing and a secondary wave of Sertoli cell mitotic activity. The secondary wave is longer than the first and begins at about three weeks of age and ends between six and ten weeks of age (França et al., 2000). Despite the important and continued Sertoli cell proliferation during the boar's developmental phase, it is the least studied management area of boars. What we do know about boar development may not be exact when applied to today's genetics, as age at puberty has decreased, and Sertoli cell populations become fixed at puberty (França et al., 2000). Increased socialization of boars after weaning and before growth

performance testing have been shown to improve semen collection training success, longevity in the stud, and total sperm per ejaculation. The physiological mechanisms that underlie the improvements have not been fully described (Dysart, 2014).

The positive relationship between size of the testes and body weight has been described by Flowers (2021). When the effect of growth rate post-weaning on reproduction was evaluated, moderate-growth rate boars produced more spermatozoa and had higher conception rates than either low or high-growth rate boars (Knecht et al., 2017a). Despite the evidence for the impact of growth on sperm production and conception rates, an area still needing more understanding is the influence of postweaning nutrition and management strategies for the boar and how they might affect reproductive development (NRC, 2012; Flowers, 2021). Investigation of the developmental period of boars is vital, especially since Sertoli cell populations become relatively fixed just before puberty or approximately 6-7 months of age with current genetics (Flowers, 2021).

The growth period from weaning to puberty has received little attention regarding nutritional development strategies in the boar (NRC, 2012). Boars are often developed like finishing pigs, with a strong emphasis on maximizing average daily gain, with faster-growing boars being more desirable (Flowers, 2020). Unfortunately, these developmental strategies come with negative impacts. Genetic improvement within breeding lines has resulted in rapid change of growth indices. It is required for boars to be classified within the top deciles of these indices to remain in the boar stud. Removal from the boar stud because of decreased standing in the indices is the most common reason for removal from the boar stud (Knox et al., 2008). The intense selection pressure for superior growth traits and not selecting for skeletal structure or semen quality are causing boar longevity issues (Robinson and Buhr, 2005; Flowers, 2008).

It can be hypothesized that the prevalence of lameness in today's population of boars is likely influenced by intense selection pressure on growth rate during the development phase based on observations in other species. This hypothesis is supported by a similar phenomenon observed in poultry, where rapid growth significantly contributes to skeletal abnormalities that lead to lameness (Barbato, 1999; Bradshaw et al., 2002). In beef bulls fed excessive energy during prepubertal development they suffered an increased incidence of lameness compared to bulls on lower energy diets (Barth et al., 2008). In gilts, restricted diets to slow growth from ~130 days of age to ~212 days of age, reduced the incidence of lameness (Quinn et al., 2015). While not yet evaluated in boars, slowing growth prepubertally has helped alleviate lameness issues in poultry and gilts (Bradshaw et al., 2002; Quinn et al., 2015).

With little available research-based guidelines regarding nutritional development of boars, they are often managed similarly to replacement gilts or growing pigs. Gilts provided ad libitum access to feed causes them to become too large at the time of breeding, and often finishing diets lack the nutrients needed for successful reproduction, suggesting a similar strategy might also be valid for the boar (Boyd et al., 2002; Safranski, 2016). Gilts are often fed diets with an amino acid content normally found in finishing diets until selection for breeding. After selection, gilts are transitioned to diets with amino acid contents normally found in gestating sow diets (Boyd et al., 2002; Safranski, 2016). General recommendations are that gilts transition to a diet that meets their reproductive needs at 68.0-81.7 kilograms or 120 days of age (Boyd et al., 2002). Rapid growth in gilts is also causing concerns about feet and leg issues and drawing attention to ways to slow their growth (Gregory et al., 2023).

Premature culling of boars because of the unsoundness of feet and legs is often a problem that begins during development (Knox et al., 2008). Reports of boar removal in North America

for physical unsoundness account for as much as 60% of annual replacement rates (Robinson and Buhr, 2005; Knox et al., 2008). Other studies from Europe and China have reported culling rates for lameness at 14.9% and 27.2% in boar studs (Knecht et al., 2017; Li et al., 2017). It is important to recognize that boar housing and developmental strategies differ across countries and may explain the differences in culling percentages. In Europe, among Pietrain boars heavily selected for feet and leg structure and controlled growth, culling for lameness was reduced to 5.5% (Henneberg et al., 2023). Adverse consequences have occurred in other species due to intense selection pressure for production. Intensive selection for growth rates in broilers and roosters resulted in negative reproductive and skeletal developmental repercussions (Barbato, 1999). In dairy cattle, intense selection pressure for milk production resulted in decreased pregnancy rates and longevity and increased leg problems (Oltenuacu and Broom, 2010). The occurrences of lameness and skeletal developmental issues in other species and culling rates in boars due to lameness suggest it is possible that similar skeletal developmental issues are occurring in the swine industry.

An intervention method shown to reduce locomotion difficulties and improve sexual behavior is group penning from approximately 30 kg body weight to approximately 110 kg body weight (Hacker et al., 1994). However, recent studies using today's swine genetic lines have not been performed. One way to slow growth is by reducing feed intake instead of allowing ad libitum consumption. Restricting feed intake after weaning and during growth performance testing comes with challenges. If feed is restricted to the entire pen, then more dominant boars may push others off feed, increasing variation in feed intake within the pen. Thus, restricting feed intake after weaning or during growth performance testing requires either expensive feed intake systems designed to offer feed on an individual animal basis or individual housing;

however, individual housing of boars during development negatively impacts age at puberty, sexual behavior, and soundness of feet and legs (Levis et al., 2005). In gilts, restricting feed intake to 20-30% of ad libitum estimates during the developmental period slows growth, decreases mobility issues, and prevents over-conditioning (Levis et al., 2005). Restricting feed during development comes with challenges. This practice in gilts can delay puberty when a severe restriction is applied, causing additional labor, management, and increased facility costs (Levis et al., 2005). The challenge with this method may be similar in the boars, as boars whose feed intake was restricted 17-30% experienced delayed puberty of 30-47 days (Levis et al., 2005). Restrictions of feed intake to 85% of ad libitum in Yorkshire boars during development did not impact sexual behavior, reproductive performance, or structural soundness after boars reached puberty (Hacker et al., 1994).

When diet manipulation was performed in bulls, a reduction of crude protein by a third of the NRC requirement was needed to impact reproductive traits, and energy supplied in more than a third of the NRC recommendations resulted in negative consequences to reproduction (Barth et al., 2008). Gilts offered ad libitum access to high-fiber diets from selection to farrowing containing 0.45% lysine and 2954.19 kcal M.E./kg displayed improved reproductive characteristics and longevity compared with lower fiber diets (Levis et al., 2005). Gilts developed on low energy and low lysine did not differ from controls in the percentage that reached puberty before breeding, but they consumed more feed than control gilts (Lents et al., 2018). Gilts fed lower energy diets had delayed mammary gland development compared to those fed more energy (Lents et al., 2018). Researchers feeding high, medium, and low levels of lysine based on NRC (2012) recommendations to gilts found that growth rate slowed without adverse effects on age at puberty and the number of gilts reaching puberty (Lents et al., 2018). Feeding

gilts moderate energy and restricted protein during development successfully regulated body weight and backfat without negative reproductive implications (Gregory et al., 2023).

The challenge in diet manipulation during this time is boars typically undergo growth performance testing from approximately 60 days of age to 150 days of age. One issue during this time is that boars are still developing Sertoli and Leydig cells. As discussed previously, it is hypothesized that increased growth is highly correlated with increased numbers of these cells. A second problem when manipulating diets during performance testing is growth and feed consumption data are used to select boars for semen production, and diet manipulation will change growth outcomes. The selection of boars with greater average daily gains on limited nutrients may translate to a hardier pig that can remain functional and grow rapidly. It must be acknowledged that intentionally slowing growth or manipulating diets during this time does create an added challenge for geneticists when analyzing performance data in selection models. However, with the issues surrounding rapid growth, feet and leg structure, and boar longevity, an intervention is needed.

Stud Management

Based on genetic evaluations and growth performance, potential breeding boars are typically selected by five months of age. The genetic selection of boars is based on their individual estimated breeding values for characteristics such as growth rate and litter size (Safranski, 2008). Growth performance phenotypes such as feed efficiency, days to 100 kg, and ultrasound data for loin depth and backfat are some of the traits of most importance when selecting replacement boars. Other traits assessed include analyzing foot and leg structure or any physical abnormalities a boar may have (Safranski, 2008). After boars are selected or identified as potential replacement boars, they are moved to boar studs or nucleus locations. Based on most

recent estimates, the United States has approximately 130 boar studs housing nearly 27,000 commercial working boars (Knox, 2016). Boars in these studs are typically housed in pens or stalls, with 25 to 2,000 boars at each stud and most studs housing 100-500 boars (Knox, 2016). In the boar studs, approximately 91% of boars are housed in confinement stalls (Knox et al., 2008). Most confinement stalls are on fully slatted floors, utilize nipple waterers or trough waterers, and are environmentally controlled for high temperatures with mechanical cooling, air conditioning, and evaporative cooling systems (Knox et al., 2008). With advancements in genetic technologies, genetic companies are able to identify genetically superior boars and place them in boar studs to be used for AI. These boar studs are clustered near sow farms, use advanced semen collection methods, practice intense semen quality control, and implement enhanced biosecurity measures (Knox et al., 2008; Knox, 2016).

Biosecurity is intense at boar studs to protect the nucleus-level genetics (Knox, 2014). These nucleus-level sites often maintain multiple purebred breeds to create terminal crossbreeding programs. New animal entry is one of the greatest risks for disease entry into a boar stud. In order to maintain a herd large enough to meet semen demands, new boars regularly arrive at the boar stud (Knox, 2014). The best practices to manage risk include source herd health verification, isolation, acclimation, and regular disease testing. Humans also pose a considerable threat to boar stud disease exposure. Personnel in boar studs must have no contact with pigs at other locations, incur ample downtime from other pigs before entrance to the stud, and change clothes and shower at entrance. Other biosecurity management tools include perimeter fencing, sterilization and disinfection of equipment before entry, and air filtration systems (Knox, 2014).

Boars begin their semen production life at approximately seven months of age with training to mount a collection dummy and ejaculate. Often, boars are given an injection of

prostaglandin-F2 α during the training process to stimulate sexual behavior (Estienne, 2014). Estienne (2014) states that prostaglandin administration enhances libido in sexually inexperienced boars. In most instances, the training process takes approximately two weeks, after which time, if a boar passes his semen evaluation tests, he will be assigned to a regular semen collection schedule (Flowers, 2009). In 2008, failure to successfully become trained to become a working boar was the fourth highest reason for removal of boars in studs (Knox et al., 2008). Once boars are on a collection schedule, they are collected every four to seven days, resulting in an average of 72.8 ejaculates per year (Kemp et al., 1988; Knox et al., 2008).

Boar semen collection requires a technician with a gloved hand and manual stimulation of the boar's glans penis (Knox et al., 2008). The challenges with manual collection are that it is physically demanding (~15 minutes per collection) for the technician, involves many critical control points to prevent contamination and ensure semen quality, and requires a large labor force (Aneas et al., 2008). With increasing difficulty in finding adequate labor, automation of semen collection steps is one way to help solve this issue. The Collectis® automated boar collection system is a tool that allows a single technician to collect multiple boars at a time (Aneas et al., 2008). Use of the Collectis® has been shown to increase technician efficiency, decrease labor needed, reduce bacteria in ejaculates, and have no impact on semen quality and quantity (Aneas et al., 2008). More automated technologies like these exist and may become vital to the swine industry with labor shortages.

General semen evaluation in the boar stud involves evaluating sperm motility and morphology and semen volume and concentration. Historically, these analyses were performed via microscopic evaluation and were highly subjective. Currently, semen volume is determined using a calibrated gram scale, concentration is evaluated using a spectrophotometer or a

Computer-Assisted Semen Analysis (CASA), a more objective technology, which can also evaluate motility and morphology of the semen sample. Once ejaculates are identified as meeting minimum quality standards, they are diluted in a media, pooled from 2-6 boars, and packed into individual doses with sperm concentrations of 1.5-2.8 billion total sperm per dose (Knox et al., 2008; Flowers et al., 2016; Knox, 2016). Boar studs also utilize routine third-party evaluations of the consistency of semen doses produced in the stud. These evaluations look at concentration, motility, and viability of the semen doses as well as evaluate for bacterial contamination. Bacterial contamination can have detrimental effects on sperm motility and membrane integrity (Lopez Rodriguez et al., 2017).

The success of boars in the boar stud depends on their ability and willingness to mount the collection dummy, ejaculate, produce adequate numbers of healthy spermatozoa, and maintain genetic indices in the top deciles of their population. A boar's physical ability to complete collection depends on feet and leg soundness. In boar studs, as high as 60% of removals can be because of physical unsoundness (Robinson and Buhr, 2005; Knox et al., 2008). The premature culling of boars because of lameness rather than genetic indices generates losses from developmental investment and genetic benefits. In boar studs housing between 164 and 2,000 boars, they experience culling rates per year from 50% to 145%, which is a massive insult to the economics of a stud and opens them to greater biosecurity threats (Robinson and Buhr, 2005). Other incidences contributing to premature culling include semen quality (10-30%) and low libido (1-21%) (Robinson and Buhr, 2005). The high culling rates due to issues of lameness or poor semen quality that could be reduced, have created many research opportunities, which have not been capitalized on in boars.

The concerns surrounding foot and leg soundness are not exclusive to boars but rather experienced by the entire swine industry. Significant culling, morbidity, and mortality rates occur because of lameness, which causes substantial economic impacts and welfare concerns (MetaFarms, 2021). The economic impacts of lameness cost United States pork producers \$23 million a year (Supakorn et al., 2018). Of equal concern are the growing percentages of death and euthanasia of pigs because of lameness and the resulting welfare issues (Supakorn et al., 2018; MetaFarms, 2021). In sows, lameness is a leading contributor to early culling and, thus, decreased piglet production (Supakorn et al., 2018). In finishing pigs, abnormal gait is reported in 19.7% of pigs, and their removal rates are of similar concern to those in sows (KilBride et al., 2009).

It has been extensively documented that season impacts semen quality, especially in the summer months (Knox et al., 2008; Flowers, 2015; Flowers, 2021). Heat stress can cause reduced sperm motility and increased sperm abnormalities, and depending on the level of heat stress, these detrimental effects can be observed over a lengthy period of time. When boars experience chronic heat stress, the detrimental effects may occur for as long as 14 weeks (Flowers, 1997). However, during severe heat stress, the impacts in the semen can be observed relatively quickly. Spermatogenesis is approximately 5 weeks in the boar, of which more immature sperm are developing in the testicles and final maturation is occurring in the epididymis. The cells most sensitive to heat stress are the most immature cells developing in the testicles. These cells can be negatively impacted by short-term stresses and when these cells are impacted, the changes seen in ejaculated semen may take several weeks following the heat stress. More mature cells in the testicle and the epididymis are more resistant to heat stress and typically are only impacted by severe stresses, resulting in negative impacts to ejaculated semen occurring

immediately following the heat stress. Boars exposed to temperatures above their thermoneutral zone for 72 hours produced ejaculates with reduced spermatozoa and decreased quality for 32 days after insult (McNitt and First, 1970). Boars experiencing acute stress over long periods will experience a decrease in their semen production until the stress is gone. At that point boars do not return to normal production for another 5-7 weeks (Flowers, 2020). The summer months have both a chronic stress, as temperatures and humidity are elevated during the summer months in the U.S., and periods of acute stress as we see increasing days with extreme temperatures with global warming. During summer, greater percentages of ejaculates are discarded due to abnormalities (Knox et al., 2008). As a result of increased discarded samples, regular collection schedules may not be possible, which also increases the number of ejaculates with poor sperm quality, further compounding the heat stress issues (Flowers, 2015). The long-term effects of heat stress make it a critical factor to control regarding its economic impact on production.

The estimates set forth by the NRC (2012) and the basis for boar nutrition is from studies primarily conducted in the 1990s. While these experiments are still highly valuable, the swine industry has undergone drastic changes in management, genetics, and structure, so application of those research outcomes to present day management decisions is questionable. Literature investigating amino acid requirements in the boar is limited, and thus, most levels of amino acids for boars' diets are based on sow requirements (Louis et al., 1994a; NRC, 2012). It does appear that protein restriction causes greater insult to semen production than energy restriction (Kemp et al., 1988; Kemp et al., 1989). Of concern for boar management is that it can take 2-3 months before the effects of nutrient restrictions are observed in semen production (Flowers, 2021). More recent research has focused on macronutrients and their impact on semen quality and quantity. The results for many macronutrients are varied, but consistent improvements in sperm

production have been shown when boars are supplemented with vitamins A and E, arginine, and selenium (Audet et al., 2009; Chen et al., 2018; Lugar et al., 2019; Flowers, 2021).

In sexually mature boars, deficiencies in crude protein resulted in decreased estradiol-17 β levels, increased time to mount and start ejaculating, less ejaculate volume, and reduced quantity of semen ejaculated when compared with controls (Louis et al., 1994a). Boars fed low-protein diets also had less backfat and testis volume than control boars (Louis et al., 1994a). Boars fed both low-energy and low-protein diets had decreased weight gains, libido, and sperm output compared to controls (Louis et al., 1994b). Boars fed low-energy, low-protein diets were more likely to refuse to mount the collection dummy. In contrast, boars fed high-protein, low-energy dietary treatments were intermediate in their amount of refusal, and none of the high-protein, high-energy treatment boars refused to mount as compared with controls (Louis et al., 1994b). Generally, boars with decreased libido tend to have lower levels of estradiol-17 β , demonstrating the relationship between the hormone level and reproduction (Louis et al., 1994b). More recent studies on the effect of protein content have produced less consistent results. Boars fed crude protein meeting recommended requirements produced ejaculates with greater sperm motility, greater total sperm, and less abnormal spermatozoa compared to high crude protein content (Ren et al., 2015).

Conversely, when lysine content was increased above NRC (2012) recommendations for mature boars, authors saw improvements in sperm quality and the number of piglets born alive (Dong et al., 2016). Investigations of energy intake found that mature boars fed a high-energy diet had an increased incidence of lameness and took longer to mount and ejaculate than other boars (Wang et al., 2016). While the effect of protein levels in mature boar diets has been investigated, the impact during boar development is unknown.

Similarities and Differences Between the Bull and Boar

Similar to the swine industry, the use of AI in dairy cattle is dominantly the only method of breeding. Recently semen sales of beef semen have exponentially increased, largely driven by the use of beef semen on dairy cows. The use of AI in beef cattle has grown less than in the dairy sector, mainly due to differences in facilities and industry structure. The dairy cattle industry is more comparable to the swine industry in that cows are managed in large, specialized groups, males are housed in bull studs, and semen is shipped to dairy farms for AI use. Many technological advances have been developed to allow cryopreservation of bull semen in liquid nitrogen (Foote, 2002). In swine, however, 99% of all ejaculates used for AI are freshly extended ejaculates because the freezing process significantly decreases the fertility of boar ejaculates (Foote, 2002; Flowers, 2020). Another semen processing difference between the two species is sperm cell sorting based upon X- or Y-bearing chromosomes. While the technology of sex-sorting semen can be done in both species, it is currently only commercially used in cattle (Foote, 2002; Garner and Seidel, 2008; Rath and Johnson, 2008). Several limitations exist for the use of this technology in swine. The sex sorting process is too slow to meet the daily demands of semen production in a commercial facility, resulting in smaller litter size and decreased farrowing rates compared with conventional semen processing (Foote, 2002; Vazquez et al., 2003).

Compared to dairy and swine, beef cattle are managed in a much less convenient way for producers to perform AI (Foote, 2002). Females are often managed on large ranges with limited access to facilities for estrous detection and AI (Foote, 2002). Generally, bulls are exposed to cows during the breeding season and then managed separately for the remainder of the year. Unlike swine or dairy, beef bulls are not generally housed in a stud year-round. Instead, they are

managed by breeders until identified as possessing ideal genetics (Harstine, 2018). Dairy bulls are often identified at a much younger age than beef cattle as they utilize genomic testing more heavily than beef cattle (Harstine, 2018). Other differences between beef cattle and swine are the ownership of males in the studs and the distribution of genetics. Boars are owned and managed by individual companies that allow for commercially produced boar semen to be rapidly distributed. The genetic companies that own boars also manage and distribute several different genetic lines (maternal and terminal) (Foote, 2002; Knox, 2014). Among beef bulls, while an individual company may own bulls, it is common that they are owned by individual producers. AI dose demands are much more driven by individual bull performance in beef bulls, whereas in swine, doses are ordered from genetic lines not an individual male (Harstine, 2018). More concentrated genetic ownership, and distribution of those genetics as well as shorter generation intervals have enabled the genetic advancement of the swine industry to occur much more rapidly as compared with the beef cattle industry.

With more control over which boars will become replacements, boar studs can schedule and control semen collection training. Dairy bull collection training management is like swine, but unlike beef bulls. Dairy bulls are often reared like boars, as they are housed and fed specifically for their future reproductive uses, with the largest difference being the lack of specific semen collection training for bulls as compared to boars (Harstine, 2018). Dairy bulls may be exposed to semen collection at a young age, but they do not go through specific processes for collection training like boars do. Beef bulls, however, are often not exposed to semen collection until after they are sold in production sales. Boars are trained to mount a collection dummy, and using prostaglandin-F₂ α as a stimulant is a routine protocol, but bulls do not undergo the same processes (Estienne, 2014). Instead of the use of a dummy, bulls are

collected with the use of a live teaser animal (Schenk, 2018). Bulls are also collected by utilizing false mounting, a technique used to elicit sexual preparation and increase the concentration of spermatozoa per ejaculate (Schenk, 2018). Bulls unable or unwilling to mount teaser animals may be collected via electroejaculation. Electroejaculation methods are not used for the boar, which makes libido even more vital for the boar. Semen collection intervals for bulls differ from those of boars. It is ideal for bulls to be collected two to three times per day at three to four-day intervals (Bratton and Foote, 1954; Hafs et al., 1959). The collection frequency of bulls is more frequent than the schedule of boars. In boars, a single ejaculate is collected per collection day, and boars are collected generally once per week (Kemp et al., 1988; Knox, 2014). In both species ejaculate evaluation is relatively similar, with minimums set for sperm motility and morphology. The disadvantage to both species is that while minimum sperm motility and morphology standards help to remove some sub-fertile males, there are still fertility differences between males that meet these standards. Technologies are still not available for identifying sub-fertile males, but many advancements in identification of sperm biomarkers have shown promise in correlation to field fertility of males.

Using live teaser animals adds another layer of complication in controlling the biosecurity of bull studs. As previously stated, the biosecurity of boar studs is highly regulated, which is different than bull studs (Knox, 2014). While bull studs are the most regulated sector of biosecurity in the cattle industry, they practice much less strict biosecurity compared to boar studs. Areas of possible disease exposure in bull studs include using mount animals, regular movement of bulls arriving and leaving from collection, and outdoor facilities exposing bulls to insect vectors (Sanderson and Gnad, 2002). Bull studs combat these exposures by practicing

quarantine when new bulls arrive, regular vaccination, and strenuous disease testing (Sanderson and Gnad, 2002).

No different than boars, bulls also suffer from many different factors that impact semen production. Understood more thoroughly in the bull are the effects of nutrition before puberty on reproduction. Limiting dietary energy and protein in bulls, delays age at puberty (Flipse and Almquist, 1961; Pruitt et al., 1986; Dance et al., 2015), leads to smaller testicular development (Van Demark 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 to achieve a higher average daily gain before puberty typically display increased LH levels, testicular testosterone, and scrotal circumference (Mann et al., 1967; Harstine et al., 2015; Byrne et al., 2017). However, it has been demonstrated that issues arise when bulls are over-conditioned and suffer from increased scrotal fat as they cannot appropriately thermoregulate their testes (Coulter et al., 1997). Coulter et al. (1997) demonstrated that moderate-energy diets improved fertility without accumulating excessive scrotal fat. Developing moderate energy diets for nutritional management of boars may help lessen negative impacts of rapid growth on reproductive and skeletal development.

In both species, season has a detrimental impact on semen production and of greatest insult are the summer months (Everett and Bean, 1982; Mathevon et al., 1998; Knox et al., 2008; Snoj et al., 2013; Flowers, 2015; Flowers, 2021). Like the boar, negative consequences of heat stress in bulls can be observed for several weeks after insult (Rahman et al., 2018). Heat stress occurs more quickly in the bull than in the boar and can be seen as quickly 12 hours after exposure, whereas the boar can tolerate more prolonged heat exposure (Flowers, 1997; Rahman et al., 2018). More clearly documented in bulls is the impact season has on younger males. Bulls

under 25 months of age had the lowest semen volume, sperm concentration, sperm motility, and total sperm output compared to other ages in the summer (Mathevon et al., 1998). A better understanding of how heat stress impacts boars of different ages may help boar studs better manage young boars during times of stress.

Conclusion

Many areas of boar management are highly underserved regarding science-based recommendations with boar development from weaning to puberty, an area of need. Furthermore, a better understanding of nutritional requirements in growing boars is desperately needed. With increases in premature culling because of lameness or poor semen quality, and the economic impact the losses create, there is a need to lessen the amount of desirable genetics being prematurely lost. There are many similar factors that insult semen production in the bull and boar such as season and diet. Other similarities between the two species are current methods of semen evaluation. While the current methods of semen evaluation can remove large portion of unfertile males, they leave room for improvement when identify sub fertile males. As novel semen technologies continue to advance, they leave promise for improvement of male fertility in both species.

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Chapter 2 - Effects of body weight change in pre-production Duroc boars on semen collection training, semen production parameters, and longevity in a commercial boar stud

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Abstract

Objective: Assess the effects of body weight change in developing Duroc boars on semen collection training, semen production parameters, and longevity in a commercial boar stud.

Materials and Methods: Retrospectively, 164 boars were divided into one of three groups based on their percentage body weight change from arrival to the boar stud until the end of a 42-day isolation period. The one-third of boars that had the greatest percentage of body weight change during the isolation period gained 10.1% to 36.1% (TOP). The middle one-third of boars that were intermediate in percentage body weight change during isolation gained 2.6% to 9.7% (MIDDLE). The final group consisted of one-third of boars that either minimally gained or lost weight (-9.5% to 2.5% change in body weight; BOTTOM). Semen collection training data were collected during the 42-day isolation period while working boar data were collected for 25 weeks post-isolation as working boars in the stud.

Results and Discussion: Boars in the TOP group lost the least amount of backfat during isolation (indicated by ultrasound or caliper score) ($P < 0.05$), while boars in the BOTTOM group lost the most backfat of the three groups. Average boar age at the time of successfully becoming a working boar (successfully mounting the collection dummy and producing an ejaculate with a motility $\geq 70\%$ and percent normal morphology $\geq 65\%$) and the proportion of boars that were successful at become working boars did not differ between groups ($P > 0.05$). There was a group by production week interaction ($P < 0.0001$) for semen ejaculate volume, total number of sperm, and total number of sperm with normal morphology. Boars in the TOP and MIDDLE groups produced more total sperm and normal sperm than the BOTTOM group from weeks 8 to 20 of being working boars. During their time as working boars, an average of

26.8% of boars were either culled from the stud or died, but there was no difference ($P > 0.05$) among groups.

Implications and Applications: While there were no differences in semen collection training and longevity in the stud, the increase in production of total normal sperm in the TOP and MIDDLE groups compared with the BOTTOM group could have substantial economic benefits for boar studs.

Introduction

An individual boar requires substantial economic input for development and maintenance. Individual boars can sire over 10,000 offspring per year in swine production systems, with potential significant genetic impact on the swine industry, highlighting the importance of their development and management. Despite their considerable role in the production system, little research has been conducted to identify the best management practices for developing boars. Historically, with rapid genetic turnover, there has been little demand to invest in boar research related to longevity. However, boar studs are experiencing increasing incidences of lameness, and rising development costs (Torrison, 2022). Further, premature removal of boars from the population due to lameness and poor semen quality, while their genetic indices are still among the top percentiles of the population leads to a loss of desirable genetics, development investment, and future income from semen collections.

Prior to reaching the boar stud, boars are currently managed like finishing hogs to quantify growth performance for selection criteria, with the faster-growing boars being more desirable (Flowers, 2020). This developmental method contributes to one of the most significant challenges in this population, their rapid growth rate. Terminal sire lines are selected on growth traits, rather than reproductive performance (Robinson and Buhr, 2005; Safranski, 2008), which

places added stress on the boar's skeletal system. Typically, boars in the top percentile for production traits tend to only be average in reproductive traits (Robinson and Buhr, 2005). Not being selected for their performance in reproductive traits creates issues when these boars' productive lifetimes are dependent on their reproductive performance (Robinson and Buhr, 2005). Yearly replacement rates due to semen quality can account for 10-30% of boar replacement, which is a significant loss to boar studs (Robinson and Buhr, 2005; Knox et al., 2008).

Developing boars like finishing hogs also creates issues when these boars are transferred to the boar stud. At the boar stud, boars undergo diet changes and transition from ad libitum feed intake to daily feed allotments. Further complicating this issue is that boars are still growing and maturing as they reach the boar stud, and weight gain is inevitable (Sulabo et al., 2006). Nutrient recommendations and management strategies for developing boars are sparse (NRC, 2012). Currently, research-based recommendations with contemporary swine industry genetics on developing boar nutritional management and subsequent influence on reproductive function are limited (NRC, 2012; Flowers, 2021). In working boars over a year of age, limiting feed intake did not impact sperm quality or quantity (Kemp et al., 1991; Sulabo et al., 2008). However, it is not known how applying the same strategy to boars between weaning and a year of age will impact their future reproductive performance. In gilts, providing ad libitum access to feed causes them to become too large at the time of breeding, and often finishing diets lack the nutrients needed for successful reproduction, suggesting this may be a similar challenge for the boar (Boyd et al., 2002; Safranski, 2016). There are also weight recommendations for gilts at the time of first breeding, which is not the case for boars, as the ideal weight at first semen collection training is not understood (Patterson and Foxcroft, 2019). A positive relationship between

growth and reproduction has been demonstrated in prepubertal boars (Knecht et al., 2017). Boars with a greater average daily gain also produced a greater total number of insemination doses and increased conception rate in females mated than boars with lower average daily gains (Knecht et al., 2017). Despite what is known about growth rate in mature boars, there is sparse published research on how the growth rate of developing boars impacts their future reproductive success.

Our study aimed to assess effects of percentage body weight change in developing Duroc boars on semen collection training, semen production parameters and longevity in a commercial boar stud.

Materials and Methods

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. The day after arrival at a commercial boar stud, 169 Duroc boars (Line 600; DNA, Columbus, NE; average 190.2 d of age) were enrolled in the experiment across four admission groups (August 2022 to February 2023). A timeline of events that occurred is shown in Figure 1.1. Boars originated from one of five developer barns before being selected to become replacement boars. Boars were initially housed in a separate portion of the boar stud for isolation and after the isolation period all boars were transferred to the production barn. In isolation and the stud, boars were housed in individual crates with nipple waterers, drop feeders (Econo), and concrete floors. Before coming to the stud boars were fed a growing diet ad libitum, and after arriving at the stud moved to the stud diet and feed intake reduced. During their time in the isolation barn, boars were fed between 1.81 – 2.27 kg daily of a diet formulated to meet or exceed NRC (2012) nutrient requirements (Table 2.1). On arrival, all boars underwent disease testing and were given two disease booster vaccinations. Half of the boars were

randomly selected to receive tulathromycin (Draxxin, Zoetis Animal Health, Parsippany, NJ, USA) as a preventative health treatment.

Retrospective Comparison Group Assignment

To meet our objective of assessing the effects of percentage body weight change in developing Duroc boars on semen collection training, semen production parameters and longevity, boars were retrospectively assigned boars to one of three groups. After the isolation period, boars were retrospectively divided into one of three groups based on their percentage body weight change during the 42-d isolation period (Figure 2.1). The 1/3 of boars that had the greatest percentage of body weight change during the 42-d period gained 10.1% to 36.1% (TOP). The middle 1/3 of boars that were intermediate in percentage body weight change during isolation gained 2.6% to 9.7% (MIDDLE). The final group consisted of 1/3 of boars that either minimally gained or lost weight (-9.5% to 2.5% change in body weight; BOTTOM). To account for potential differences in initial body weight it was included as a covariate in all statistical models, and age did not differ between the three groups ($P = 0.8171$).

Boar Management and Semen Collection Training Procedures During Isolation

Five days after arriving at the boar stud and after confirmation of negative disease test status, the following data were collected on boars: body weight, visual body condition score, last rib backfat thickness, and last rib body condition caliper score. Visual body condition scores were assigned on a scale of one to five, with one being very thin, and five being very fat (Stalder et al., 2010). Backfat thickness was measured using the Renco Lean Meater (S.E.C. Repro Inc., Quebec, Canada) at the P2 position (last rib, 6 to 8 cm from the midline) on each side of the boar and averaging the two measurements. The same person performed all measurements and assigned visual body condition scores. Boars remained in the isolation barn for 42 d, or until they

were considered a working boar (successfully mounting the collection dummy and producing an ejaculate that met minimum semen quality standards) and moved to the production barn (Figure 2.1). After the 42-d phase was complete, all boars were weighed, visual body condition assessed, backfat thickness measured, and caliper scores were taken. Boars were observed for six months to evaluate the impact of percent body weight change during isolation on semen production and longevity.

Boars were subjected to semen collection training (training to mount a collection dummy and successfully ejaculate) beginning at an average of 214 (\pm 6.16) d of age using the hand glove technique (Figure 2.2). Each boar was given an injection of prostaglandin-F2 α (5 mg Dinoprost, Lutalyse, Zoetis Animal Health, Parsippany, NJ, USA) within 10 minutes of their first training attempt. During training, boars were given at least 30 minutes to mount the collection dummy and ejaculate successfully. If boars failed to mount the collection dummy, another attempt was made a week later. Boars continued to receive an injection of prostaglandin-F2 α before training until successful mounting and ejaculation occurred. When a boar successfully mounted the collection dummy and ejaculated, the ejaculate was immediately assessed for semen quality by an IVOS 2 CASA (Hamilton Thorne). To become a working boar and considered successfully trained, a boar's ejaculate was required to meet minimum quality standards (Progressive sperm motility \geq 70%; Normal sperm morphology \geq 65%). If an ejaculate failed to meet semen quality standards, boars were given a week of rest before they were collected again. After boars successfully mounted the collection dummy and met semen quality standards, they were moved into the production barn of the stud and transitioned to working boar status. Boars failing to become working boars before the end of the isolation period were transitioned to the production barn and attempts to train continued until they either met minimum requirements or were culled

due to failure to train. During the isolation period, five boars were removed due to health concerns or death.

Working Boar Management

Semen was collected from working boars using the semi-automatic BoarMatic (Minitube USA, Inc., Verona, WI, USA) semen collection system. The gel fraction of the ejaculate was removed at time of collection, and thus not a component of semen volume measurements. Among working boars, routine management decisions of the boar stud managers dictated semen collection frequency and feed amounts. Feed amounts were adjusted for each individual boar to maintain a visual body condition score between 2.5 and 3. Computer-assisted semen analysis previously mentioned was used to evaluate semen characteristics, and ejaculates were required to meet minimum thresholds of 70% sperm motility and 70% normal sperm morphology for further processing and distribution. Any adverse health events or treatments for illness and lameness were documented, and reasons for death or culling were recorded. The culling and death reasons for boars were grouped into one of two categories for analysis (1 = Health-related issues; 2 = Genetics or semen quality issues). Additionally, semen collection records were provided, including days rest between ejaculates, age at collection of each ejaculate, semen ejaculate volume, sperm concentration, and reason for discard. The reasons for ejaculates being discarded were divided into one of three categories: semen quality (proximal droplets, distal droplets, head abnormalities, tail abnormalities, and poor motility), physical failure (presence of urine or blood in the ejaculate, contamination in the ejaculate, or failure to ejaculate while mounted on the dummy) and other (low semen volume, low sperm concentration, or no sperm cells in ejaculate).

Statistical Analysis

All statistical analyses were performed using SAS (version 9.4; SAS Institute., Cary, NC 27513). For all models, admission groups were considered replicates, and farm of origin as a covariate. The MIXED procedure was used to analyze differences between the three groups (TOP, MIDDLE, and BOTTOM in percentage body weight change during isolation) for body composition changes (backfat and caliper score), where the percentage body weight change group was a fixed effect, and boar age at arrival to the stud was a covariate. To evaluate the effect of percent body weight change on body composition measurements from the beginning of isolation to the end, and the ending body composition measurements (after 42 d), the starting (arrival at the boar stud) measurements, such as body weight, were used as covariates. In analyses of the impact of percentage body weight change on visual body condition score differences and changes, boars from the three groups were classified as either a visual body condition score of 3 and greater, or less than 3. Similarly, when evaluating changes in visual body condition score, boars were either classified as maintaining their condition or as having lost visual body condition. For analysis of visual body condition score classification, the GLIMMIX procedure was utilized where group was a fixed effect, and visual body condition score groups were treated as a binary response. Covariates of farm of origin, age at arrival and body weight at the start of isolation were used for all models assessing visual body condition score group.

The MIXED procedure was used in analyses of variables related to time to training and age at training time points, where the percentage body weight change group was a fixed effect, and weight at the start of isolation was used as a covariate. Interactions between replicate and group were tested and removed when nonsignificant. When analyzing the number of attempts to train, age at first attempt was used as the covariate. The GLIMMIX procedure was used to

analyze the proportion of boars that become working boars, where group was a fixed effect, age at becoming a working boar or deemed untrainable were covariates, and a binary distribution was utilized.

Data for semen quality parameters were analyzed using the MIXED procedure, where the percentage body weight change group and week of semen production were fixed effects, week of semen production for working boars was a repeated measure with covariance structure, and boar was nested within group. For analysis of semen quality parameters, farm of origin, body weight at the start of isolation, and age at time of collection were used as covariates. The Slice option was used for LS mean separation tests for interaction effects between group and wk of semen production. When interactions were found to be nonsignificant ($P > 0.10$) they were removed from the model. Binomial data for survival and cause of death were analyzed using the GLIMMIX procedure of SAS where boar was the experimental unit and age at time of death was a covariate. Statistical significance was defined as $P \leq 0.05$ and a tendency was defined as $0.05 > P \geq 0.10$.

Results and Discussion

Training and Isolation

The average body weight of boars at the start and end of isolation are shown in Table 2.2. At the beginning of isolation, boars in the MIDDLE group had a higher ($P = 0.0253$) body weight than boars in the TOP group. After isolation, boars in the TOP group had an average body weight of 14.07 kg more ($P < 0.0001$) than the BOTTOM group. Literature reporting the body weight of boars is rare, and what is available documents the body weights of mature boars. The ideal weight of boars when they enter the boar stud is unknown. In a study using Polish Landrace boars, young boars were classified as 414 d of age or younger had an average body

weight of 237 kg (Falkowski et al., 2014). The youngest boar in that study at 279 d of age, was older than the boars in the present study. Estimates have been made for predicted growth rates of working boars (Sulabo et al., 2006). Those estimates started at 150 kg or 220 d of age, which are similar to the weights and ages found in our study (Sulabo et al., 2006). However, it must be acknowledged that Sulabo et al. (2006) based these estimates on adult working boars selected to provide a wide range of ages and weights, and context was not provided as to how this may have impacted semen production.

Comparison of backfat, caliper measurements, and visual body condition score classification of percentage weight change group are shown in Table 2.3. At arrival at the boar stud, backfat and caliper scores, did not differ ($P > 0.01$) between the groups. Regardless of the percentage weight change group, all boars experienced a decrease in backfat during the isolation period. Boars in the BOTTOM group experienced the most significant loss (-1.42 mm; $P < 0.001$) of backfat during the isolation period and had the lowest (6.58 mm; $P < 0.001$) total backfat at the end of the isolation period compared with the other groups. Boars in the TOP group experienced the least (-0.66 mm) amount of backfat loss, while boars in the MIDDLE group had intermediate (-1.08 mm) backfat loss. The boars in the BOTTOM group lost an average of -0.65 units of caliper measurement during the isolation period and experienced the greatest change ($P < 0.05$) in caliper units when compared with the other groups. After arrival at the boar study, there was tendency ($P = 0.0929$) for a difference between groups regarding the proportion of boars with a visual body condition score greater than 3. After the 42-d isolation period the boars in the BOTTOM group had significantly fewer ($P = 0.0008$) boars with a visual body condition score of 3 or greater. The boars in the BOTTOM group had a greater proportion ($P = 0.0607$) of boars that experienced a loss in visual body condition score than the boars in the

TOP group. There is no comparable literature evaluating visual body condition of boars, but sows experiencing lactation weight loss caused decreased farrowing rates and total born when sows lost more than 10% of their body weight (Thaker and Bilkei, 2005). It is essential to recognize that at arrival to the stud, many boars are still in a prepubertal state. Thus, the impact of a negative metabolic state in the BOTTOM group may be similar to reproductive effects to that of gilts or sows. Given that all boars lost backfat during this time it is possible that there is an effect of transitioning these boars to a different diet at the boar stud caused them to become leaner.

The effects of percentage body weight change on age at semen collection training are shown in Table 2.4, and the time required for boars to become working boars is shown in Table 2.5. There were no differences among groups in mean age ($P = 0.3014$) or the amount of time it took after arrival at the boar stud for boars to become working boars ($P = 0.2860$). There were also no differences among groups in the number of training attempts required to become working boars ($P = 0.2668$) nor proportion of boars that became working boars ($P = 0.4245$). To our knowledge, no previous research has evaluated the impacts of weight changes of developing boars on semen collection training characteristics. Sulabo et al. (2008) did observe that in boars that gained more body weight after 14 months of age, there was a greater percentage of boars removed because of low libido. In gilts with lower average daily gains, they have been shown to have an increased age at puberty when compared with gilt that had higher average daily gains (Roongsitthichai et al., 2013). Although we didn't see the same effects in our study on the time it took boars to become working boars, it is possible that the boars in our study were old enough when transitioned to the boar stud when average daily gain slowed, that it did not impact time to becoming a working boar. Even though we did not observe differences in the time it took to

complete semen collection training, reducing this time could have significant economic benefits for boar studs. The sooner boars train and produce ejaculates acceptable for insemination doses, the fewer days they are housed and on feed before boar studs begin seeing a return on their investment. Of importance to recognize is that, on average, it took boars longer than the standard isolation time to become working boars. Surveys of boar studs stated that the most frequent duration of training took between 1-3 wk, however, the majority of these boars were older than those of the present study, which may contribute to the quicker training time (Knox et al., 2008). The longer time to train compared to other studs may suggest that adjusting expectations or management of these younger boars through the training process may be required for this stud. The time that boars are first trained for semen collection is similar to the time when gilts are first exposed to boars, and they both have the goal of achieving a reproductive milestone (Flowers, 2020). It is well known in gilts at what age to begin boar exposure and that those gilts who reach puberty at a younger age are more productive in their lifetime (Flowers, 2020). However, it is less clear in boars at what age it is ideal to begin semen collection training in order to maximize lifetime productivity and thus semen collection training generally starts between 7 and 9 months of age (Flowers, 2020).

On average, 9.8% of boars failed to become classified as working boars and this metric did not differ among groups. In our study, reasons for not becoming a working boar included failure to produce enough normal spermatozoa, low sperm motility, and failure to mount and ejaculate on the collection dummy. Estimates of percentages of boars failing to train and reasons boars are classified as unable to train vary in the literature. From surveys of U.S. boar studs, the estimates of failure to train because they failure to mount were reported as the fourth highest reason for removal across all breeds and boar studs (Knox et al., 2008). Others in the U.S. have

reported failure rates at individual studs as high as 15% in terminal sire lines, similar to the 9.8% observed in our study (Flowers, 2008). Flowers (2008) also reported minimal phenotypic variation among terminal sire line boars that fail to train to collect, which may explain some of the lack of differences in our study as they are all from similar genetic lines. A study in Holland reported failure to train (inability to mount or semen quality) was as high as 45% in Yorkshire boars and 16% in crossbred Yorkshire x Duroc boars (Colenbrander et al., 1993). In Yorkshire boars in the U.S., developed and trained similarly to the boars in our study, 4.7% of boars failed to collect semen (Hacker et al., 1994). However, when boars in the previously mentioned study were housed in individual pens after 30 kg of body weight, 22.6% of boars failed to collect semen (Hacker et al., 1994).

The inconsistency and high percentages of boars that are unable to train to collect semen suggest there are opportunities for improvement. Possibilities for intervention include the management of boars to begin training at a more consistent age. Some of the differences observed in the literature may be due to inconsistent definitions of training regarding boars that fail to become trained. In some cases, boars are classified as a failure to train for not mounting; in other cases, it's a failure for semen to meet quality standards. High failure rates to train also provide opportunities for reproductive performance evaluations. In other species, selection for production-type traits has negatively affected reproductive performance. In dairy cattle, intense selection pressure for milk production resulted in decreased pregnancy rates and longevity (Oltenucu and Broom, 2010). With selection pressure in the current population of boars being based on growth traits, it is plausible that this has a negative impact on the libido and reproduction of boars. For future improvement of these reproductive traits, including them in genetic analyses may lead to improved semen collection training and semen quality outcomes.

Working Boar

The effect of percent body weight change during isolation on semen quality and quantity of adult working boars are summarized in Table 2.6. There was a significant group by production wk interaction for semen volume (Figure 2.3; $P = 0.0028$), total sperm per ejaculate (Figure 2.4; $P < 0.0001$), and total normal sperm per ejaculate (Figure 2.5; $P < 0.0001$). From wks 6 to 23, the boars from the TOP group produced a greater ($P \leq 0.05$) ejaculate volume than the boars in the BOTTOM group. The MIDDLE group was intermediate to the TOP and BOTTOM groups, and differences varied based on the wk. The increase in volume over time is likely because the boars in our study were still maturing at this time and more mature boars produce a higher volume ejaculate, suggesting that the TOP boars were at an advanced maturity compared to BOTTOM.

Other literature shows that older boars produce more insemination doses per ejaculate than younger boars (Falkowski et al., 2014). The differences between the groups are similar to those seen in mature boars, where boars fed lower crude protein, and thus had decreased body weight, also had a reduction in semen volume (Louis et al., 1994). In our study, boars in the BOTTOM group produced a lower volume of semen from wk 6 to wk 23 than boars in the TOP group. Total sperm per ejaculate followed a similar pattern to volume regarding group differences. From wk 8 to wk 23, the TOP group produced more total sperm than the BOTTOM group. Unlike volume, the total sperm per ejaculation did not increase as drastically over the 25-wk production period. The more subtle changes in total sperm per ejaculation could be because changes in concentration were less over time than for volume. Total normal sperm also slightly increased across the 25-wk production period. Similar to total sperm, the total normal sperm produced from the boars in the TOP group was greater than those in the BOTTOM group from

production wks 8 to 24. The boars in the MIDDLE group also produced more sperm than the BOTTOM group from production wks 8 to 20. The production differences in total normal sperm between the groups significantly impact the production of insemination doses. The boars in the TOP and MIDDLE groups produced more total normal sperm, directly related to more insemination doses and a substantial economic impact on the stud. With the boars in the TOP and MIDDLE groups gaining weight during the isolation period, it is evident that boars remained in a positive metabolic state during this time. The impact of those boars remaining in a positive metabolic state significantly impacts semen production once those boars become working boars. Boars with an average daily gain of 800 to 850 g/d during development had increased total insemination doses and increased conception rates compared with boars with a lower average daily gain (Knecht et al., 2017). While we did not evaluate the conception rate in our study, we did see similar effects of boars with a higher average daily gain of having increased total normal sperm, which leads to more insemination doses. Individually, boars in the TOP group (943.51×10^9 sperm per ejaculate) produced more total normal sperm from wks 8 to 23 than the boars in the BOTTOM group (644.91×10^9 sperm per ejaculate). If the total normal sperm production is translated into AI doses with a concentration of 2.8 billion sperm per dose, the boars in the TOP group produced 106 more AI doses than the BOTTOM group. This substantial increase in doses produced is a significant economic benefit for boar studs.

Even though there were interactions between the group and production wk for total sperm per ejaculate and total normal sperm, there was no evidence of an interaction ($P = 0.2982$) for sperm concentration but there was a tendency for an effect of group ($P = 0.0740$) and a significant effect of production wk ($P = 0.0332$). Knecht et al. (2017) evaluated the impact of weight gain from 170 to 210 d of age on semen quality. They observed that moderate-growth

rate boars (800 to 850 g/d) produced more spermatozoa and had greater conception rates than lower or higher-growth-rate boars (Knecht et al., 2017). We did see improvements in our study of total sperm when comparing boars in the TOP group with the BOTTOM group from wks 9 to 24, and we observed similar results for concentration. One possible reason for these differences is that even the boars in our TOP group had average daily gains of 410 g/d, less than what was required to see the significant impacts by Knecht et al. (2017).

There was no evidence of a group and production wk interaction for percent normal sperm ($P = 0.9790$). However, percent normal sperm was affected by group ($P = 0.0337$) and increased over production wk (Figure 2.6; $P < 0.0001$). From wk 1 to 25, the percentage of normal sperm produced per ejaculate linearly increased, which coincides with previous literature where increases in normal sperm are observed as boars age (Falkowski et al., 2014).

For progressive motility, there was no significant interaction between groups and production wk ($P = 0.5043$) and no effect of production wk ($P = 0.3104$). Group also did not impact progressively motility ($P = 0.1152$). When evaluating the number of collection failures for either semen quality, physical failure, or other reasons, group and production wk did not interact ($P = 0.3958$), but there were effects of production wk ($P = 0.0006$) and group ($P < 0.0001$). Boars in the BOTTOM group experienced the greatest amount of failed collections ($n=137$), while boars in the TOP group experienced the lowest ($n=93$). Hacker et al. (1994) observed decreased libido with no effects on spermatogenesis in boars with protein and energy restriction from 1 to 8 months of age. It is possible that differences in genetic lines contributed to the contrasting results from the present study with Duroc boars Hacker et al. (1994) used Yorkshire boars. The dietary basis of many of the published NRC (2012) requirements in boars are based on studies from genetics from 30 years ago and others from what is known in sows.

There needs to be more information about the actual nutrient requirements of both the growing and mature boar; thus, with today's genetics selected for rapid growth, the recommendations may not meet the boar's needs. In bulls, enhanced nutrition at 10 to 30 wks of age resulted in earlier onset of puberty and increased testicular size (Barth et al., 2008). Barth et al. (2008) summarized that providing bulls with diets containing more concentrates during calthood and adequate nutrition post-weaning maximizes reproductive function. Providing adequate nutrition during the prepubertal period appears to have positive impacts on both bull and boar development. In gilts, it is recommended that they be bred between 135 to 150 kg for optimal reproductive performance, however, standards such as these have not been developed for boars (Williams et al., 2005; Kim et al., 2016). The impact of gilts remaining in a positive metabolic state is summarized by (Patterson and Foxcroft, 2019). In prepubertal gilts, transitioning them from ad libitum feed intake to maintenance feed allotment can have a negative impact on reproductive development; a similar impact to what was observed for the boars in our BOTTOM group in the current study (Patterson and Foxcroft, 2019). Keeping gilts in a positive metabolic state pre-breeding is critical to improving ovulation rate, embryonic survival, and litter size (Patterson and Foxcroft, 2019). The benefits of boars remaining in a positive metabolic state appear to be no different than for gilts. When evaluating visual body condition loss in sows, it appears to have a greater impact on feed intake and lactation output than it does on reproductive performance, suggesting that if the same is true for boars, it may be more beneficial to monitor boar weight than visual body condition as changes would be noticed more quickly (Lavery et al., 2019). In mature boars, protein restriction has been shown to cause greater insult to semen production than energy restriction (Kemp et al., 1988; Kemp et al., 1989). It has also been shown that it can take 2-3 months before the effects of nutrient restrictions are observed in semen production, which

would match the time in our study when we observed separation between the groups (Flowers, 2021). Given that restricted protein can cause greater insult than energy, it is likely that estimates of boar protein requirements are inadequate.

Boars in the BOTTOM group produced the greatest number (137 ejaculates) of ejaculates incompatible with requirements, and the MIDDLE group produced an intermediate amount (110 ejaculates). In comparison, the TOP group produced the least (93 ejaculates). When evaluating the reason that ejaculates were not compatible with quality requirements, there was no difference ($P = 0.08309$) between the group and the reason for not being compatible. Failure to meet semen quality standards was the more frequent reason for an ejaculate not being compatible with requirements (Figure 2.7). In mature boars, those with a greater average daily gain produced fewer discarded ejaculates than those in a lower gain group (Sulabo et al., 2008). Failure to meet semen quality standards as the most frequent reason for ejaculates not meeting standards is consistent with reports from other boar studs (Knox et al., 2008). Unlike the reports from Knox et al. (2008), who found bacterial contamination to be the second most common reason for ejaculates not being compatible with standards, we observed physical failures as the second most common reason for failure. Cleanliness practices in today's boar studs have likely helped reduce the bacterial load as compared to earlier studies.

The effect of percentage body weight changes in isolation on the survivability of boars is shown in Table 2.7. From the end of isolation until the end of the 6-month trial, 37 boars died or were culled for various reasons. There were no differences ($P = 0.9996$) between groups in the percentage of boars that survived even though numerically the TOP group had the highest percentage of boars that produced sperm through wk 25. There were also no differences ($P = 0.1593$) between groups in reasons for boar removal. The lack of differences between weight

change groups differs from those observed in previous literature on working boars. After a 16-month study, more boars that gained more body weight were still active in the boar stud than those who gained less (Sulabo et al., 2008). The percentage of boars removed from each group either due to health-related causes or culling due to decreased genetic value or poor semen production was 25.7% on average. There was also no difference ($P = 0.9649$) in the wk of removal or wk of death between treatments, with the average wk of removal being production wk 22 for all groups. Unlike what we observed for boars, lighter weight gilts and with lesser average daily gain were removed from the herd earlier than gilts with a heavier body weight and greater average daily gain (Roongsitthichai et al., 2013). We likely did not monitor groups long enough to detect differences between groups, as the gilts in the previous study were monitored until their third parity and studies in mature boars were for 16-months.

Applications

Based on our results, boars not remaining in a positive metabolic state significantly impacts their later reproductive performance. While we did not find differences in variables related to age or time to becoming a working boar, survivability, or reason for death, there were apparent differences in semen production. The profitability of a boar stud is directly related to the number of insemination doses they can produce. Boars that remained in a positive metabolic status produced the greatest amount of total normal sperm, which conveys more total insemination doses. Given that the requirements for boars are primarily based on what is known in gilts and sows, we may not be meeting the needs of terminal genetics. Our study suggests that managing boars individually to ensure they stay in a positive metabolic state and their growth requirements are met is ideal for optimal semen production at a boar stud.

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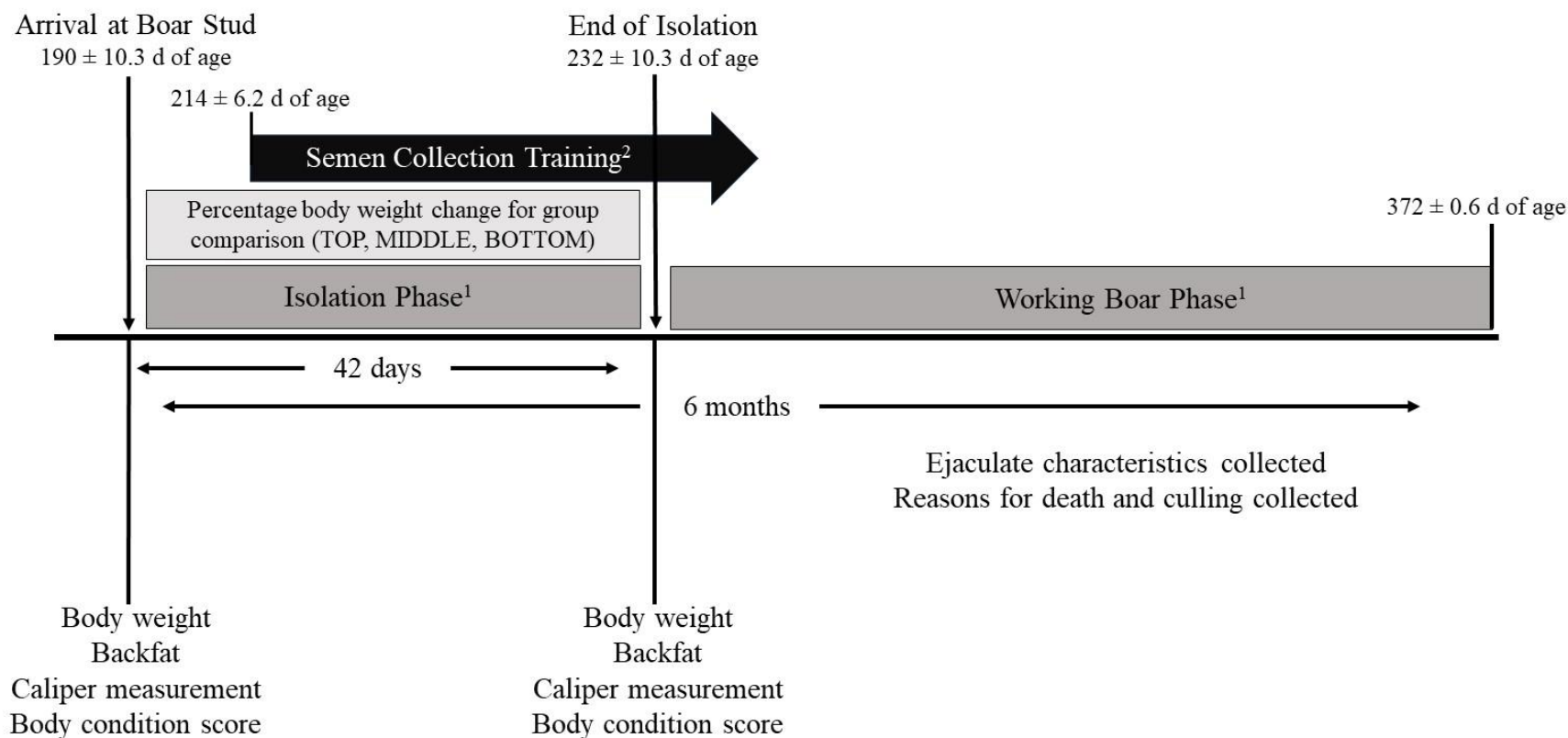
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Figure 2.1 Timeline of significant events for boars after arrival at a commercial boar stud



¹While in the isolation phase boars were housed in the isolation barn until they successfully completed semen collection training. Boars were then moved to the production barn to begin the working boar phase.

²Boars were subjected to semen collection training until successfully mounting the collection dummy and ejaculates met semen quality standards.

Figure 2.2 Timeline of semen collection training events and sequence for boars after arrival at a commercial boar stud

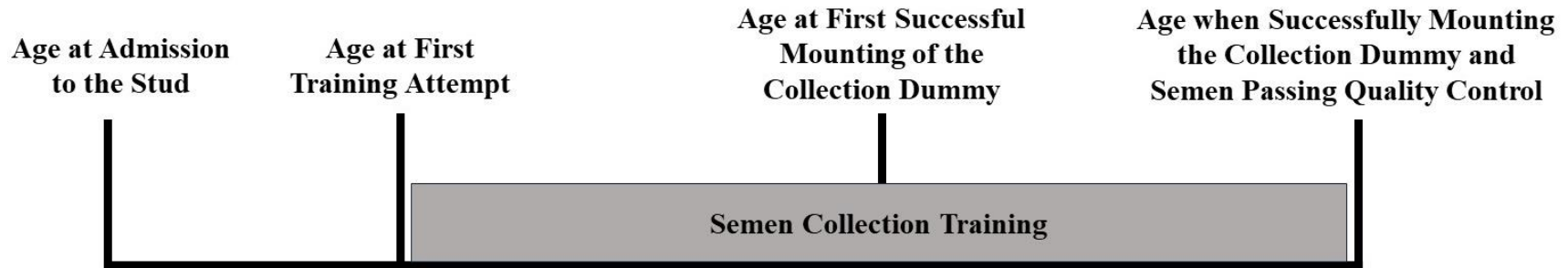


Table 2.1 Dietary Composition as-fed¹

Ingredient	%
Ground corn	65.73
Soybean meal	14.68
Soybean hulls	11.83
Vitamin, mineral, enzyme premix	2.89
Dicalcium phosphate	1.85
Fat source	1.11
Limestone	0.73
Salt	0.50
Lysine, 60%	0.26
L-Threonine	0.16
Choline Chloride, 60%	0.13
L-Methionine	0.12
L-Tryptophan	0.01
Calculated analysis	
ME, kcal/kg	3,078
CP, %	14.50
Lysine (SID), %	0.71
Ca, %	0.88
Total P, %	0.63

¹Boar diets were fed upon entry to the boar stud. After entry, boars were fed 1.81 – 2.27 kg per d for the first 42 days, once they became working boars daily feed allotment was adjusted based on individual visual body condition score.

Table 2.2 The effect of percentage body weight change during the isolation phase on average body weights of boars upon entry to isolation and 42-d later at the exit of isolation (lsmeans)

	Group ¹			SEM	P-value
	TOP	MIDDLE	BOTTOM		
Number of boars, n	54	56	54	--	--
Average Body Weight at the Start of Isolation, kg	135.07 ^a	142.11 ^b	139.38 ^{ab}	7.53	0.081
Average Body Weight at the End of Isolation, kg	151.28 ^a	149.01 ^a	137.66 ^b	7.57	0.0001
Average of Body Weight Change During Isolation, kg	16.44 ^a	6.85 ^b	-1.34 ^c	1.25	< 0.0001

¹After the isolation period, boars were retrospectively divided into one of three groups based on their percentage body weight change during the 42-d isolation period. The 1/3 of boars that had the greatest percentage of body weight change during the 42-d period gained 10.1% to 36.1% (TOP). The middle 1/3 of boars that were intermediate in percentage body weight change during isolation gained 2.6% to 9.7% (MIDDLE). The final group consisted of 1/3 of boars that either minimally gained or lost weight (-9.5% to 2.5% change in body weight; BOTTOM).

^{a,b,c} Means within a row with different superscripts differ ($P \leq 0.05$).

Table 2.3 The effect of percentage body weight change during the isolation phase on changes in backfat, caliper measurement, and visual body condition score in a commercial AI stud¹

	Group ²			SEM	P-value
	TOP	MIDDLE	BOTTOM		
Number of boars, n	54	56	54	--	--
Response					
Boar backfat, mm ³					
Start of isolation	8.06	8.02	7.83	0.253	0.7989
End of isolation	7.35 ^a	6.93 ^b	6.58 ^c	0.080	< 0.0001
Change during isolation	-0.66 ^a	-1.08 ^b	-1.42 ^c	0.080	< 0.0001
Caliper score, units ³					
Start of isolation	6.42	6.59	6.39	0.137	0.5090
End of isolation	6.81 ^a	6.29 ^b	5.82 ^c	0.088	< 0.0001
Change during isolation	0.34 ^a	-0.18 ^b	-0.65 ^c	0.089	< 0.0001
Visual body condition score (BCS) ⁴					
Start of isolation					
≥ 3 BCS, n	53 ^{a, x}	50 ^{b, xy}	49 ^{ab, y}	1.27	0.0929
End of isolation					
≥ 3 BCS, n	54 ^a	55 ^a	43 ^b	1.29	0.0008
Change in isolation					
Lost BCS, n	4 ^{a, x}	12 ^{ab, y}	14 ^{b, xy}	1.15	0.0607

¹A total of 164 boars (Line 600; DNA, Columbus, NE) were used over a 42-d period after arrival at a commercial boar stud.

² After the isolation period, boars were retrospectively divided into one of three groups based on their percentage body weight change during the 42-d isolation period. The 1/3 of boars that had the greatest percentage of body weight change during the 42-d period gained 10.1% to 36.1% (TOP). The middle 1/3 of boars that were intermediate in percentage body weight change during isolation gained 2.6% to 9.7% (MIDDLE). The final group consisted of 1/3 of boars that either minimally gained or lost weight (-9.5% to 2.5% change in body weight; BOTTOM).

³LSmeans

⁴Visual body condition scores were assigned on a scale of 1 to 5, with 1 being thin and 5 obese. When evaluating changes in body condition score, boars were either classified as maintaining their condition or as having lost visual body condition score.

^{a,b,c} Means within a row with different superscripts differ ($P \leq 0.05$).

^{x,y} Means within a row with different superscripts differ ($0.05 \leq P \leq 0.10$).

Table 2.4 The effect of percentage body weight change in boars during the isolation phase on boar age at semen collection training achievements (lsmeans)¹

	Group ²			SEM	P-value
	TOP	MIDDLE	BOTTOM		
Number of boars, n	54	56	54	--	--
Response					
Age at admission to the stud, d	190.1	189.1	189.6	1.05	0.7761
Age at first training attempt, d	214.6	214.4	214.4	0.57	0.9547
Age at first successful mounting of the collection dummy, d	221.2	224.7	220.7	2.38	0.3919
Age when successfully mounting the collection dummy and semen passing quality control (become a working boar), d	233.1	239.5	236.3	3.06	0.3041
Age at failure to train, d	291.7	293.3	300.0	11.21	0.8543
Failing to become a working boar, %	9.3	8.9	11.1	1.253	0.4245
Number of training attempts to become a working boar, number of attempts	4.4	5.7	5.6	0.59	0.2668

¹A total of 164 boars (Line 600; DNA, Columbus, NE) were used after arrival at a commercial boar stud.

² After the isolation period, boars were retrospectively divided into one of three groups based on their percentage body weight change during the 42-d isolation period. The 1/3 of boars that had the greatest percentage of body weight change during the 42-d period gained 10.1% to 36.1% (TOP). The middle 1/3 of boars that were intermediate in percentage body weight change during isolation gained 2.6% to 9.7% (MIDDLE). The final group consisted of 1/3 of boars that either minimally gained or lost weight (-9.5% to 2.5% change in body weight; BOTTOM).

Table 2.5 The effect of percentage body weight change in boars during the isolation phase on time required to successfully complete each training timepoint

	Group ²			SEM	P-value
	TOP	MIDDLE	BOTTOM		
Number of boars, n	54	56	54	--	--
Response					
Time at the boar stud before beginning training, d	24.5	25.3	24.7	0.95	0.7880
Time from arrival at the boar stud until successfully mounting the collection dummy, d	31.7	35.5	31.7	2.46	0.2860
Time from first training to successfully mounting the collection dummy, d	6.4	10.1	6.0	2.37	0.3458
Time from first training to successfully mounting and ejaculate meeting semen quality standards (become a working boar), d	18.5	27.0	20.0	3.09	0.1090
Time from the first time successfully mounting the dummy to ejaculate meeting semen quality standards, d	15.1	19.6	23.7	3.44	0.2287
Time from arrival at the boar stud until becoming a working boar, d	46.8	56.0	55.5	4.05	0.1904

¹A total of 164 boars (Line 600; DNA, Columbus, NE) were used after arrival at a commercial boar stud.

² After the isolation period, boars were retrospectively divided into one of three groups based on their percentage body weight change during the 42-d isolation period. The 1/3 of boars that had the greatest percentage of body weight change during the 42-d period gained 10.1% to 36.1% (TOP). The middle 1/3 of boars that were intermediate in percentage body weight change during isolation gained 2.6% to 9.7% (MIDDLE). The final group consisted of 1/3 of boars that either minimally gained or lost weight (-9.5% to 2.5% change in body weight; BOTTOM).

Table 2.6 The effect of percentage bodyweight changes during the isolation period on semen parameters of adult working boars in a commercial AI stud (lsmean)¹

	Group ²			SEM	P-value		
	Top	Middle	Bottom		Group	Production wk	Group x Production wk
N, boars	48	51	48	--	--	--	--
N, ejaculates	873	852	802				
Semen characteristics (per ejaculate)							
Volume, mL	116.13 ^a	99.19 ^b	92.70 ^b	5.02	0.0040	0.0133	0.0028
Sperm concentration, x 10 ⁹ sperm/mL	0.65 ^{xy}	0.70 ^y	0.61 ^x	0.03	0.0740	0.0332	0.2982
Total sperm, x 10 ⁹ sperm	68.98 ^a	65.70 ^a	53.82 ^b	2.61	0.0002	0.0021	< 0.0001
Progressive Motility, %	90.67	89.35	89.00	0.59	0.1152	0.3104	0.5043
Normal morphology, %	83.06 ^{a, xy}	82.43 ^{ab, x}	78.91 ^{b, y}	1.21	0.0337	< 0.0001	0.9790
Total normal x 10 ⁹ sperm	58.23 ^a	55.50 ^a	44.08 ^b	2.57	0.0003	0.0001	< 0.0001
Ejaculates not meeting minimum requirements	93 ^a	110 ^b	137 ^c	0.17	< 0.0001	0.0006	0.3958

¹A total of 148 boars (Line 600; DNA, Columbus, NE) were used over a 42-d period after arrival at a commercial boar stud.

² After the isolation period, boars were retrospectively divided into one of three groups based on their percentage body weight change during the 42-d isolation period. The 1/3 of boars that had the greatest percentage of body weight change during the 42-d period gained 10.1% to 36.1% (TOP). The middle 1/3 of boars that were intermediate in percentage body weight change during isolation gained 2.6% to 9.7% (MIDDLE). The final group consisted of 1/3 of boars that either minimally gained or lost weight (-9.5% to 2.5% change in body weight; BOTTOM).

^{a,b,c} Means within a row with different superscripts differ ($P \leq 0.05$).

^{x,y} Means within a row with different superscripts differ ($0.05 \leq P \leq 0.10$).

Table 2.7 The effect of percentage bodyweight changes during the isolation period on survivability of adult working boars in a commercial AI stud¹

	Treatment ²			SE	P-value
	Top	Middle	Bottom		Group
Number of boars, n	49	50	49	--	--
Response					
Survival Rate ³ , %	83.3	72.5	70.8	2.8	0.9996
Reason for removal or death, n				1.20	0.1593
Health related ⁴	6	7	6		
Other ⁴	3	7	8		
Wk of removal or death	22.0	22.0	22.0	0.21	0.9649

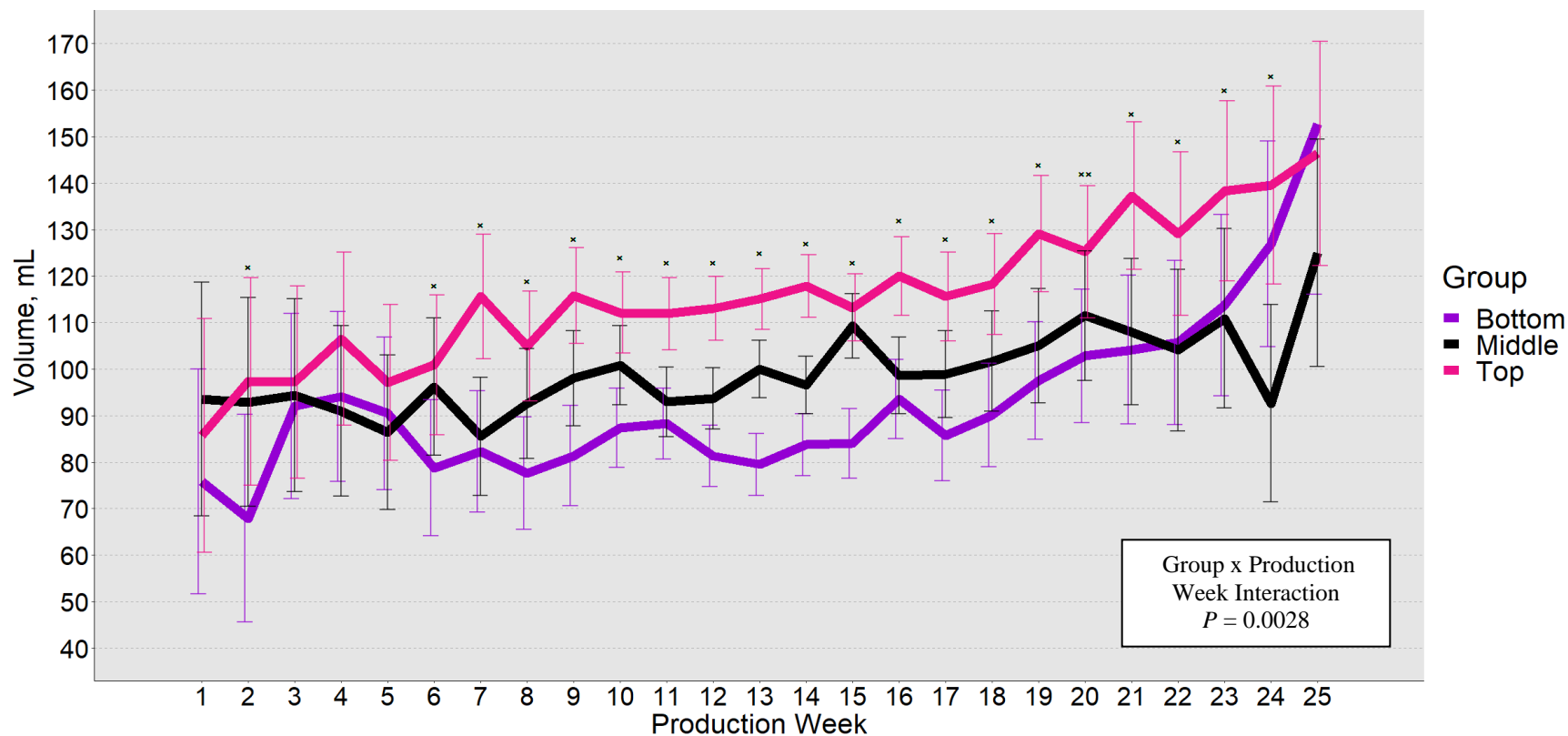
¹A total of 148 boars (Line 600; DNA, Columbus, NE) were used over a 42-d period after arrival at a commercial boar stud.

² After the isolation period, boars were retrospectively divided into one of three groups based on their percentage body weight change during the 42-d isolation period. The 1/3 of boars that had the greatest percentage of body weight change during the 42-d period gained 10.1% to 36.1% (TOP). The middle 1/3 of boars that were intermediate in percentage body weight change during isolation gained 2.6% to 9.7% (MIDDLE). The final group consisted of 1/3 of boars that either minimally gained or lost weight (-9.5% to 2.5% change in body weight; BOTTOM).

³Boars that had not been culled, euthanized, or died at the end of the 25-week production period.

⁴Boars categorized as removed for health-related reasons included lameness, respiratory, or acute deaths. Boars categorized as removed for other included boars culled due to low indexes, and poor semen quality.

Figure 2.3 Effect of Percentage Body Weight Change During Isolation on Volume of Ejaculates by Production Week¹

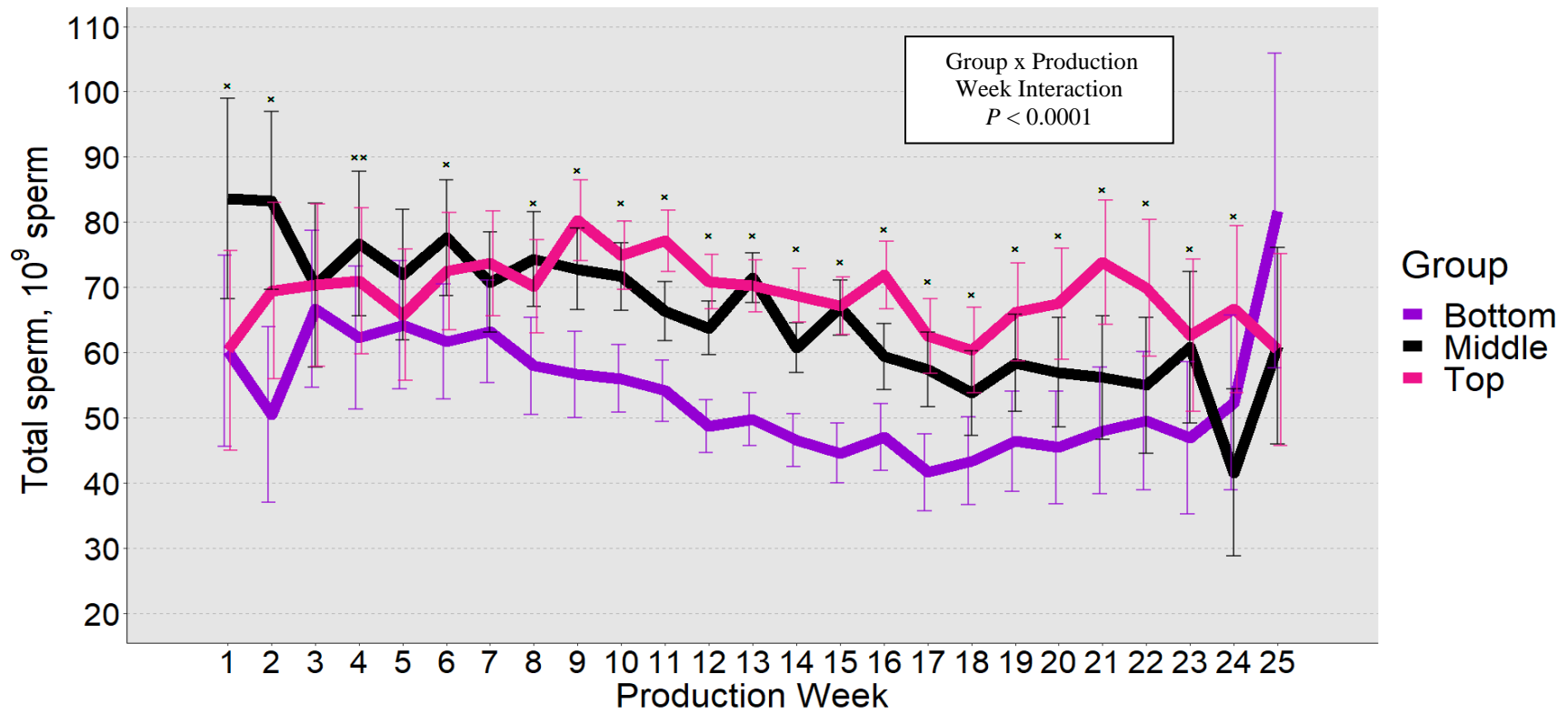


xIndicates at least two groups differ at respective production wk ($P \leq 0.05$).

xx Indicates a tendency for least two groups to differ at respective production wk ($0.05 \leq P \leq 0.10$).

¹After the isolation period, boars were retrospectively divided into one of three groups based on their percentage body weight change during the 42-d isolation period. The 1/3 of boars that had the greatest percentage of body weight change during the 42-d period gained 10.1% to 36.1% (TOP). The middle 1/3 of boars that were intermediate in percentage body weight change during isolation gained 2.6% to 9.7% (MIDDLE). The final group consisted of 1/3 of boars that either minimally gained or lost weight (-9.5% to 2.5% change in body weight; BOTTOM).

Figure 2.4 Effect of Percentage Body Weight Change During Isolation on Total Sperm per Ejaculate by Production Week¹

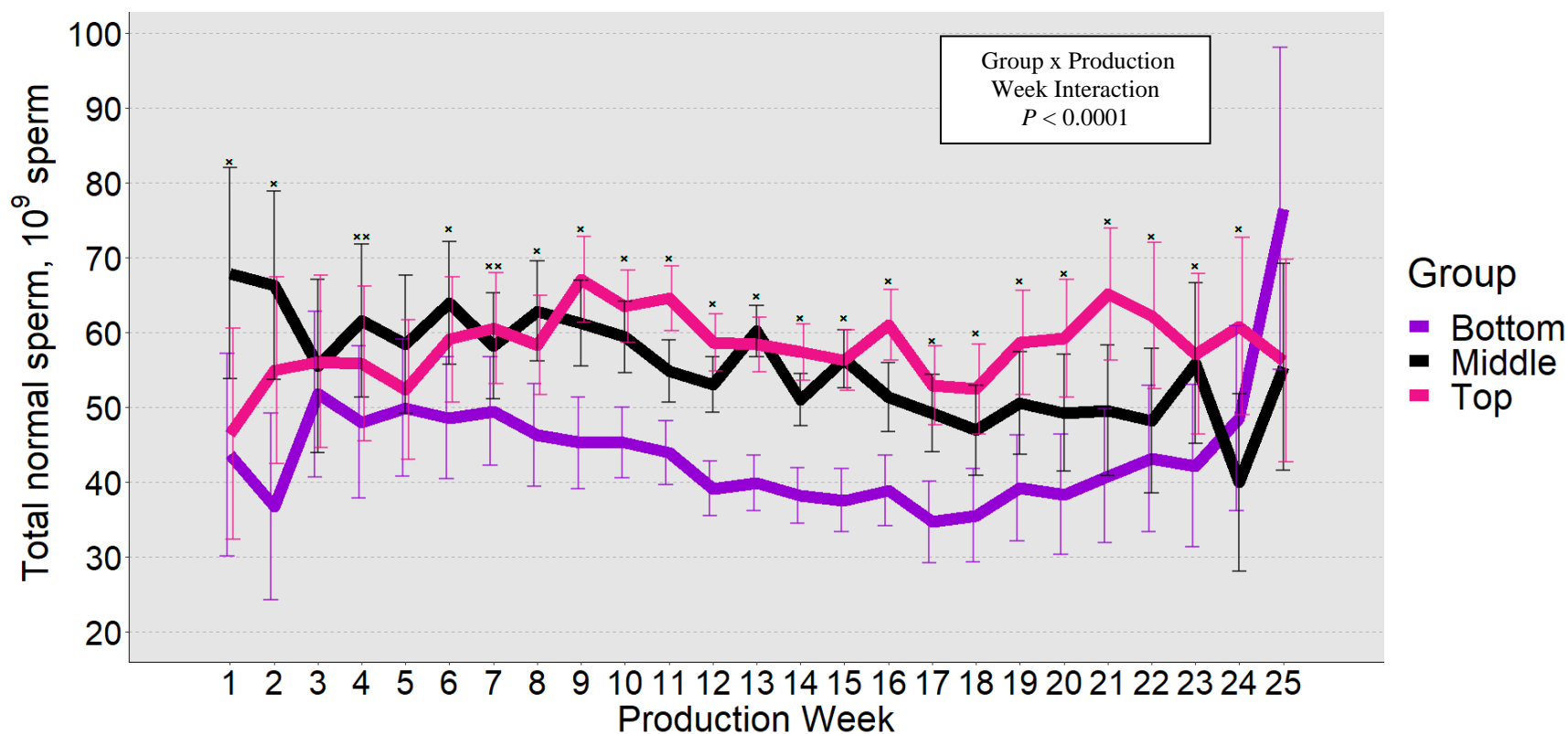


*Indicates at least two groups differ at respective production wk ($P \leq 0.05$).

** Indicates a tendency for least two groups to differ at respective production wk ($0.05 \leq P \leq 0.10$).

¹After the isolation period, boars were retrospectively divided into one of three groups based on their percentage body weight change during the 42-d isolation period. The 1/3 of boars that had the greatest percentage of body weight change during the 42-d period gained 10.1% to 36.1% (TOP). The middle 1/3 of boars that were intermediate in percentage body weight change during isolation gained 2.6% to 9.7% (MIDDLE). The final group consisted of 1/3 of boars that either minimally gained or lost weight (-9.5% to 2.5% change in body weight; BOTTOM).

Figure 2.5 Effect of Percentage Body Weight Change During Isolation on Total Normal Sperm per Ejaculate by Production Week¹



xIndicates at least two groups differ at respective production wk ($P \leq 0.05$).

xx Indicates a tendency for least two groups to differ at respective production wk ($0.05 \leq P \leq 0.10$).

¹After the isolation period, boars were retrospectively divided into one of three groups based on their percentage body weight change during the 42-d isolation period. The 1/3 of boars that had the greatest percentage of body weight change during the 42-d period gained 10.1% to 36.1% (TOP). The middle 1/3 of boars that were intermediate in percentage body weight change during isolation gained 2.6% to 9.7% (MIDDLE). The final group consisted of 1/3 of boars that either minimally gained or lost weight (-9.5% to 2.5% change in body weight; BOTTOM).

Figure 2.6 Change in Percentage of Normal Sperm per Ejaculate by Production Week at a Commercial Boar Stud

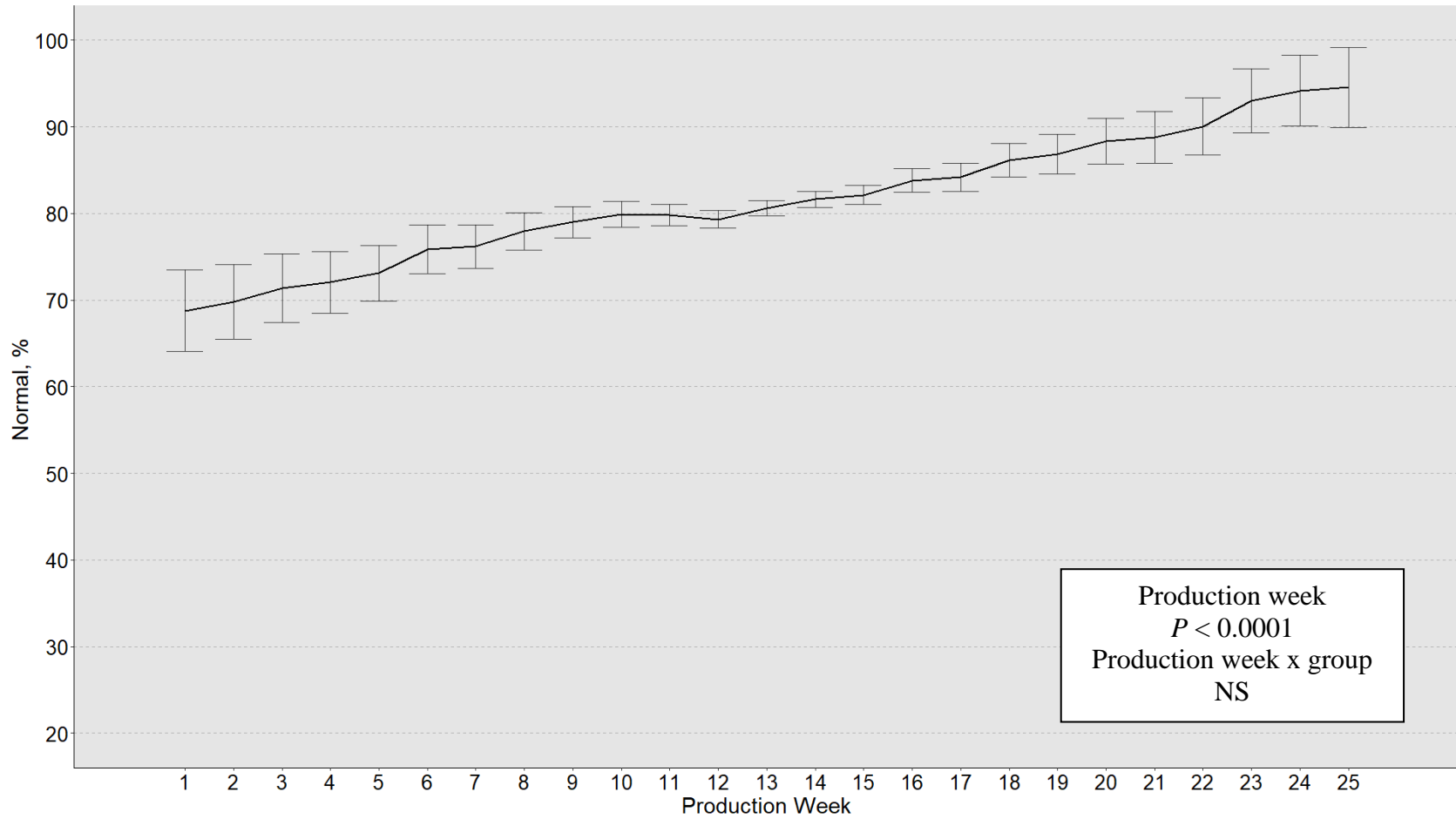
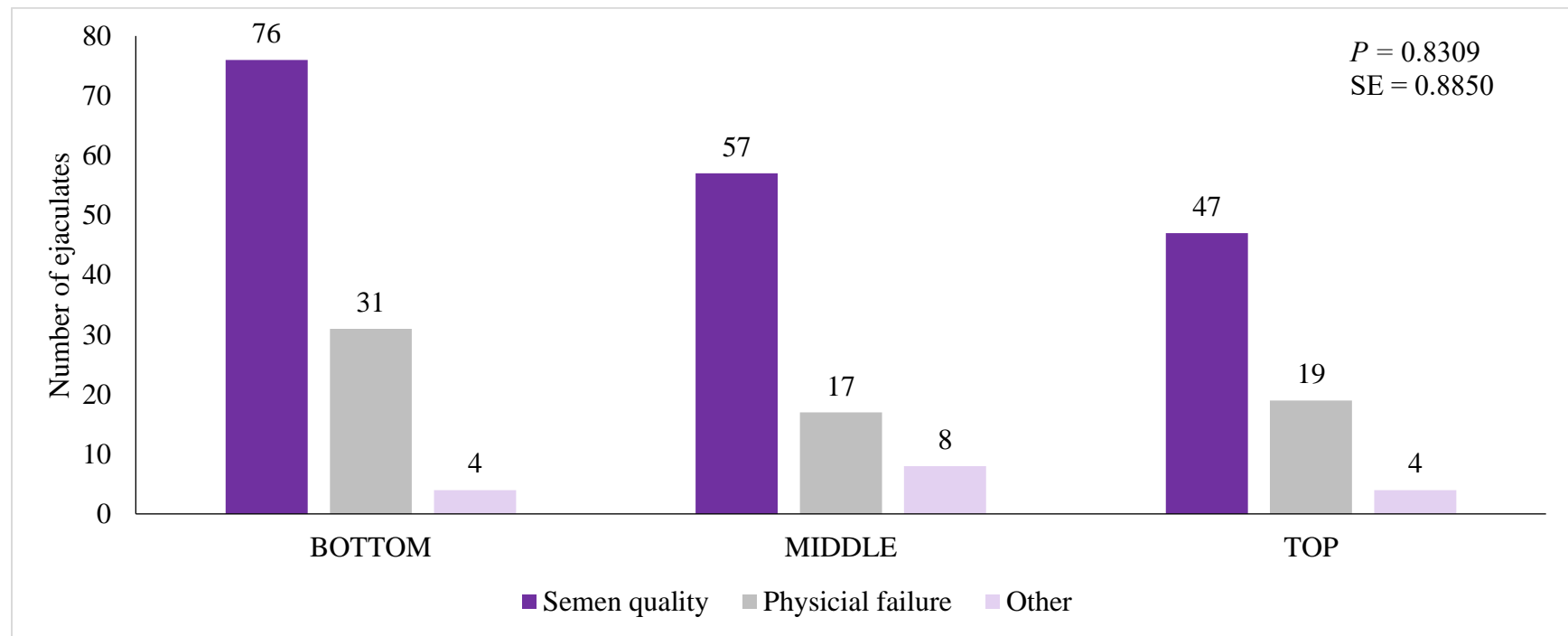


Figure 2.7 Frequency of classification of ejaculates not meeting minimum quality standards¹



¹The reasons for ejaculates being discarded were divided into one of three categories: semen quality (proximal droplets, distal droplets, head abnormalities, tail abnormalities, and poor motility), physical failure (presence of urine or blood in the ejaculate, contamination in the ejaculate, or failure to ejaculate while mounted on the dummy) and other (low semen volume, low sperm concentration, and no sperm cells in ejaculate).

²After the isolation period, boars were retrospectively divided into one of three groups based on their percentage body weight change during the 42-d isolation period. The 1/3 of boars that had the greatest percentage of body weight change during the 42-d period gained 10.1% to 36.1% (TOP). The middle 1/3 of boars that were intermediate in percentage body weight change during isolation gained 2.6% to 9.7% (MIDDLE). The final group consisted of 1/3 of boars that either minimally gained or lost weight (-9.5% to 2.5% change in body weight; BOTTOM).

**Chapter 3 - Sire distribution of calves in a beef herd with use of
fixed time artificial insemination followed by immediate bull
exposure for natural service**

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Abstract

Objective: Our objective was to determine the relative percentages of calves sired by either natural service sires or fixed time artificial insemination (FTAI) sires within the same estrous period.

Materials and Methods: During 2 consecutive yrs heifers and cows had estrous cycles synchronized and were inseminated following the 7-d CO-Synch + CIDR FTAI protocol. Females were inseminated by 1 AI technician using a single sire for heifers and a different single sire for cows. All females were exposed to natural service bulls immediately following AI. After calving, DNA was collected from a random subset of calves (Calves born from heifers in Yr 1: n = 59 and Yr 2: n = 82; Calves born from cows in Yr 1: n = 89 and Yr 2: n = 102) born in the first 21 d of the calving season to determine sire parentage.

Results and Discussion: In Yr 1 among calves born from heifers, the percentage sired by natural service was 5.1% (n=3/59). Among calves born from cows, the percentage sired by natural service was 14.6% (n=13/89). In Yr 2 among calves born from heifers, the percentage sired by natural service was 9.8% (n=8/82). Among calves born from cows, the percentage sired by natural service was 20.6% (n=21/102).

Implications and Applications: If commercial producers use FTAI followed by immediate bull exposure in cows, natural service bulls may sire more calves early in the calving season than expected. When using these practices in heifers, natural service bulls sired a lesser proportion of the calves than observed in cows.

Introduction

The use of AI provides producers with access to more sires to facilitate genetic improvements in traits of interest. Development of estrous synchronization protocols such fixed-time AI (FTAI) have enabled producers to take advantage of AI and improve production efficiencies while limiting cattle handling and eliminating the need for estrus detection (Lamb et al., 2016; Lamb and Mercadante, 2016). However, when using AI or estrous synchronization programs, added labor is still a concern for many producers. Other advantages of synchronizing estrus include improved calving distribution, maintaining a short calving interval, enhanced pregnancy rates earlier in the breeding season, heavier weaning weights, and increased calf value (Odde, 1990; Larson et al., 2006; Rodgers et al., 2012; Lamb and Mercadante, 2016; Lamb et al., 2016). Similar to cows, AI and estrous synchronization in heifers has many benefits. Heifers bred with AI and estrus synchronization calve sooner in the calving season, wean bigger calves, breed back sooner, and remain in the herd longer (Marshall et al., 1990; Cushman et al., 2013).

Using FTAI followed by immediate exposure of females to bulls for natural service can be a beneficial management strategy for commercial cow-calf producers. This management strategy has the potential to limit labor and time related to bull turnout, as well as increase pregnancies established early in the breeding season. If and how factors such as variation in time to estrus onset, length of estrus, and bull fertility influence parentage outcomes among natural service sires versus AI sires are relatively unknown. Our objective was to determine the relative percentages of calves sired by either natural service sires or AI sires within the same estrous period of beef females in a FTAI protocol.

Materials and Methods

Animal breeding and blood collection procedures were approved by Kansas State University Institutional Animal Care and Use Committee (#IACUC-4687).

Breeding and Female Selection

During the spring breeding seasons in 2 consecutive years at a ranch in Kansas, commercial Angus cows and heifers were enrolled in a FTAI program. In Yr 1, cow ages ranged from 2 to 5 yrs of age and averaged 2.6 yrs. In Yr 2, cow ages ranged from 2 to 6 yrs of age and averaged 3.2 yrs of age. Heifers were approximately 15 mo of age at time of AI in both yrs.

In both yrs, cows and heifers were synchronized using the 7-d CO-Synch + controlled internal drug release insert (CIDR, Zoetis) FTAI protocol. Heifers were inseminated 52 to 56 h, and cows 60 to 66 h following removal of the CIDR and prostaglandin F_{2α} (25 mg as 5 mL Lutalyse i.m.; Zoetis). The same AI technician inseminated all females in both years. At time of AI, gonadotropin releasing hormone (100 ug as 2 mL of Cystorelin i.m.; Boehringer Ingelheim) was administered. Heifers were inseminated with semen from a single Angus sire and a different single Angus sire was used for cows with different sires used across the 2 yrs. Immediately following AI, bulls and females were commingled with opportunity for natural mating to occur until conclusion of the 90-d breeding season.

All natural service sires passed a breeding soundness exam before exposure to females and an approximate 1:30 bull to female ratio was maintained for the breeding season. In Yr 1, 6 Hereford bulls were used for natural service on cows while 3 Angus bulls were used for natural service of heifers. In Yr 2, a combination of 6 Hereford and Angus bulls were used on cows and 5 bulls on heifers. Females remained with the natural service bulls for a 90-d breeding season.

Only calves born in the first 21 d of the subsequent calving season were identified for this project and considered to have been conceived during the synchronized estrous period.

Parentage Verification

When calves were approximately 2 mo of age, blood was collected from a random subset of the calves that were born in the first 21 d of the calving season constituting 82 to 99% of all calves born in the first 21 d (Yr 1: Calves born from heifers n=59; Calves born from cows n = 89; Yr 2: Calves born from heifers n = 82; Calves born from cows n = 102). A sample of whole blood from each calf was collected by jugular venipuncture using a 10 mL glass tube (Yr 1: BD Vacutainer Serum Tube; Yr 2: BD Vacutainer Whole Blood Tube, ACD Solution A). Blood samples were centrifuged, at 2,400 x g at 4° C for 20 min. The buffy coat was removed and DNA was isolated from white blood cells using the phenol-chloroform extraction process (Chomczynski and Sacchi, 1987). Samples were washed twice with phenol-chloroform isoamyl alcohol and once with chloroform-isoamyl alcohol and then resuspended in absolute ethanol.

A 500 ng sample of DNA from each calf was dried and used for sire parentage analysis (SeekSire Parentage, Neogen). SeekSire Parentage analyses compared DNA information of calves to AI sires which were on file within the company. In Yr 1, blood was also collected from the 9 natural service sires and processed in the same manner as the calves to enable determination of specific natural service sire parentage. In Yr 2, it was determined whether calves were sired by bulls from AI or natural service, but not which individual bull among the natural service sires used.

Results and Discussion

In Yr 1, among calves born from heifers, the actual percentage sired by natural service bulls was 5.1% (n = 3/59) while among calves born from cows, natural service bulls sired 14.6%

(n = 13/89). In Yr 2, among calves born from heifers, the actual percentage sired by natural service was 9.8% (n = 8/82). Among calves born from cows, the actual percentage sired by natural service was 20.6% (n = 21/102). It is unclear whether calves sired by natural service bulls constitute additional pregnancies established during the same estrous period that would have otherwise not occurred without presence of bulls for natural service or if in vivo sperm competition resulted in fewer AI-sired calves.

Researchers have demonstrated increased pregnancy rates with timed AI and immediate short-term (7 d) exposure to natural service sires in heifers as compared with heifers receiving timed AI only (Kasimanickam et al., 2021). Torell et al. (2007) exposed beef heifers that had been subjected to CO-Synch + CIDR timed AI protocols to natural service sires for a 48 h after prostaglandin injection but preceding timed AI as well as for a 48 h period following timed AI in an effort to maximize first-service conception. Bulls were removed from 48 to 84 h post-prostaglandin injection when timed AI occurred. Torell et al. (2007) found that the combination of exposure to bulls for natural service and timed AI in heifers increased first-service conception by as much as 20%. Similarly, Gutierrez et al. (2014) demonstrated that heifers exposed only to natural service sires required more time to become pregnant as compared with heifers subjected to timed AI and exposed to natural service sires. Sá Filho et al. (2013) demonstrated this same concept in cows. While determination of parentage to AI sires versus natural service sires was not assessed in these aforementioned studies, it is an important factor when considering application to seedstock versus commercial producers. Among seedstock operations, specific sire-dam matings are more likely to contribute to offspring economic value. Commercial beef operations benefit greatly when females conceive early in the breeding season and have a high

percentage of calves born in the first 21 d of the calving season with specific sire parentage being of lesser importance (Rodgers et al. 2012).

It is interesting to note that cows had numerically greater percentages of calves sired by natural service bulls each year as compared with heifers. It is unclear as to physiological factors that may be contributing to these differences, however, potential variation in fertility of AI sires and natural service sires among those mated to cows versus heifers and potential differences in responsiveness to the 7-d Co-Synch + CIDR FTAI protocols among heifers versus cows are potential contributing factors.

Though not a primary research objective, in Yr 1, specific natural service sire parentage of calves was determined. Of the 6 natural service sires used on cows, 2 bulls sired 4 and 5 calves each while the remaining 4 bulls used for natural mating sired 1 calf each. Among heifers, 1 of the 3 natural service bulls sired calves born in the first 21 days. Of the calves born in the first 21 days in Yr 1, just 3 were sired by a single natural service bull while the other 2 bulls used for natural service on heifers did not sire any calves. These results of natural service sire parentage are similar to Mills et al. (2019), who observed that 3 of 24 bulls used sired 30% of the total calf crop. Because a bull's ability to sire calves is directly related to libido, ability to detect cows in estrus, and completion of copulation and fertilization, bulls siring more calves may display more behavioral dominance and (or) have greater semen fertility (Chenoweth, 1997; Abell et al., 2017).

Applications

If commercial producers use fixed time AI followed by immediate bull exposure in females, natural service sires may sire 5 to 10% of calves for heifers and 15 to 20% for cows. It is essential to recognize that this practice may not be practical for operations where the known

parentage of offspring is essential, such as for seedstock producers. Regardless, for commercial producers where parentage is likely to be of lesser concern, this strategy has the potential to reduce labor related to bull turnout and increase pregnancies earlier in the breeding season. Further research should investigate if the calves sired by natural service and conceived during the same estrous synchronization period as AI are added pregnancies that would have not otherwise occurred or if in vivo semen competition is displacing conception to AI sires.

Acknowledgements

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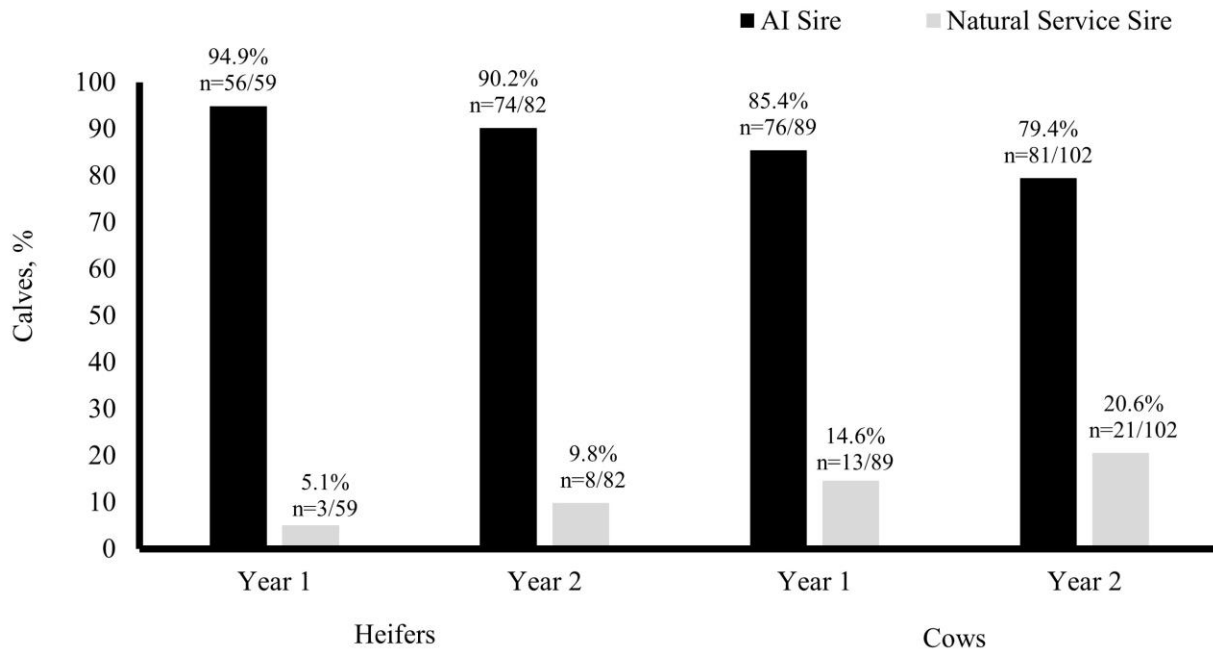
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Figure 3.1 Percentage of calves born to cows and heifers in the first 21 d of the calving season that were sired by AI sires as compared to natural service sires conceived following a 7-d CO-Synch + CIDR FTAI protocol



Chapter 4 - Assessment of novel semen evaluation technologies and breed comparisons in yearling beef bulls

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Abstract

The objective of this study was to evaluate correlations of sperm quality assessments and breed comparisons as observed during yearling beef bull breeding soundness exams (BSE). Ejaculates were collected via electroejaculation from yearling Charolais (n=23) and Angus (n=23) bulls as part of BSEs. One veterinarian conducted BSEs, and one technician conducted sperm quality assessments. Additional sperm motility analysis was conducted with the iSperm. Ejaculates meeting minimum thresholds for passing a BSE were subjected to flow cytometry. Pearson's correlation coefficients were determined, and breed comparisons were made using GLIMMIX in SAS. The iSperm analyzer gross and progressive motilities were ($r = 0.30$; 0.38 ; $P < 0.001$) correlated with technician progressive motility. Neither iSperm ($P = 0.26$) nor visual assessment ($P = 0.66$) of sperm motility differed among breeds. Bull breed did not influence ($P = 0.83$) total percentage of viable cells, percentage of viable cells with intact acrosomes ($P = 0.83$), or percentage of live sperm cells ($P = 0.92$) with positive reactive oxygen species (ROS) status. There was a tendency ($P = 0.10$) for greater percentage of sperm from Charolais bulls ($31.1\% \pm 3.35$) to have positive mitochondrial energy potential as compared with Angus bulls ($17.6\% \pm 3.35$). The percentage of live spermatozoa with negative ROS status was moderately correlated with the percentage of spermatozoa exhibiting secondary abnormalities ($r=0.33$; $P = 0.02$). Percentage of live spermatozoa with disrupted acrosomes was strongly correlated ($r=0.66$; $P < 0.001$) with percentage of live spermatozoa with negative ROS. Percentage of live spermatozoa with positive ROS status was correlated ($r=0.58$; $P < 0.001$) with percentage of spermatozoa with active mitochondrial membranes. Technician and iSperm sperm motility are positively correlated, offering producers an on-farm evaluation tool. Though bull

breed had little influence on sperm quality assessments in this experiment, ROS in sperm appeared to impair sperm health and function.

Introduction

There is currently no definitive test that evaluates a bull's fertility. Current semen evaluation techniques include evaluation of motility and morphology, and while these are insightful tools for bull fertility, they are not definitive fertility tests and are highly subjective. In recent years many new fertility markers have been identified that provide an objective analysis of spermatozoa and an insight into identifying sub-fertile bulls. The use of flow cytometry is a method of analysis that works through excitation and emissions spectra and aids in the detection of fertility markers. Specific colors bind to sperm based on the functional status of the individual cells. In recent years development of assays that target components known to be essential to bull fertility has advanced our knowledge of sperm functional statuses (Bucher et al., 2019). Reactive oxygen species (ROS) are endogenous and highly reactive oxygen (and nitrogen) -bearing molecules that can be found throughout the body (Krumova and Gonzalo, 2016). It is known that ROS can cause oxidative damage to DNA, proteins, fatty acids, and cellular components severely impacting cell function (Krumova and Gonzalo, 2016). In semen, it is hypothesized that the presence of ROS affects spermatozoa characteristics, including mitochondrial membrane potential, acrosomal integrity, and structural abnormalities that can influence spermatozoa function. In bulls, the presence of ROS in semen has been shown to have a direct impact on the function of spermatozoa as well as a relationship to bull fertility (Kumaresan et al., 2017; Leite et al., 2022).

Use of on-farm technologies for semen evaluation in cattle is limited, however, a product called the iSperm offers hope that these technologies may become more accessible as iSperm

works through the camera on an iPad Mini. The iSperm is a relatively easy-to-use, affordable, and portable semen analysis device. This device has been validated in equines and canines but not in the bovine (Moraes et al., 2019; Dini et al., 2019; Domain et al., 2022).

The bull breed has been shown to influence bull fertility. Barth and Waldner (2002) found that Angus bulls were more likely to pass a BSE than Charolais bulls, and Brito et al. (2002) found differences in motility and ejaculate concentrations among *Bos taurus* bulls when compared to *Bos Indicus* but proposed no suggested explanation for these differences. Others have shown that breed influences motility, morphology, concentration, and volume of ejaculates (Hartman, 2021). As the need for a better understanding of bull fertility grows, the influence of breed has been largely understudied.

Our objective was to evaluate the iSperm, when conducting BSEs on bulls by comparing sperm motility to a technician's assessment, investigate breed comparisons between Angus and Charolais bulls, and evaluate correlations between sperm response to ROS and functional sperm measurements.

Materials and Methods

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. This experiment was conducted at a seedstock producers' facility in central Kansas.

Ejaculates were collected via electroejaculation on one of three consecutive days from Angus and Charolais yearling bulls (403 ± 11 d of age; $n=46$) as part of a BSE. One veterinarian conducted all BSEs and ejaculates were evaluated by the same technician. Ejaculates were diluted in BoviFree to accomplish a 1:5 dilution based on manufacturer recommendations, and an additional sperm motility and concentration analysis was conducted with the iSperm analyzer.

Ejaculates meeting minimum thresholds for passing a BSE were diluted to 70 million cells/mL using BoviFree and sent overnight for flow cytometry evaluation. Flow cytometry assays included acrosome and cell membrane integrity, mitochondrial energy potential, and oxidation status. The specific flow cytometry assays included: SYBR-14 sperm viability kit from Lifetech, PNA-FITC/PI from IMV for acrosome and sperm membrane integrity, Easy kit 3: Oxidation molecule D from IMV to assess oxidation status, and Easy kit 2: mitochondrial activity from IMV to assess membrane potential.

All statistical analyses were performed using SAS (version 9.4; SAS Institute., Cary, NC 27513). To evaluate functional sperm attributes, and the relationship between the iSperm and technician, data were assessed using Pearson's correlation coefficients in SAS with the PROC CORR procedure. The GLIMMIX procedure of SAS with bull as experimental unit, bull breed as the main effect, and collection date as a random variable was used to assess potential differences in sperm quality variables between breeds. Statistical significance was defined as $P < 0.05$ and a tendency was defined as $0.05 \leq P \leq 0.10$.

Results and Discussion

The percent of live spermatozoa with positive ROS status was correlated ($r = 0.53$; $P < 0.001$) with percentage progressive motility. The percent of live spermatozoa with negative ROS status was moderately correlated with the percent of spermatozoa exhibiting secondary abnormalities ($r = 0.33$; $P = 0.02$) and tended to be lowly correlated ($r = 0.28$; $P = 0.06$) with the percent of spermatozoa exhibiting primary abnormalities. The percent of live spermatozoa that had disrupted acrosomes was strongly correlated ($r = 0.66$; $P < 0.001$) with the percent of live spermatozoa with negative ROS and moderately negatively correlated ($r = -0.31$; $P = 0.04$) with the percent of live spermatozoa with positive ROS. Given that sperm cells with a positive ROS

status are better able to handle oxidative stress, that could be why there is a positive correlation between functional sperm attributes and a positive ROS status. These results for the relationship between ROS and acrosome integrity are similar to those of Kumaresan et al. (2017). The percentage of live spermatozoa with positive ROS status was correlated ($r = 0.58$; $P < 0.001$) with the percentage of spermatozoa with active mitochondrial membranes. Leite et al. (2022) found that when there were increased levels of ROS and impaired mitochondrial membranes, this often resulted in lower fertility bulls, which supports our findings of this relationship. The percent of live spermatozoa with positive ROS were strongly correlated ($P < 0.001$) with the percentage of live spermatozoa ($r = 0.94$) and live spermatozoa with intact acrosome ($r = 0.92$). Bucher et al. (2019) found that evaluation of the viability, acrosomal status, and mitochondrial function of cryopreserved bovine sperm could be predictive of sperm functional status. Thus, our data agrees with previous research showing ROS's detrimental effects on spermatozoa function.

Both gross and progressive motilities were significantly ($r = 0.30$; 0.38 ; $P < 0.001$) correlated to the technician's progressive motility. These results are similar to previous research comparing technicians to the iSperm when evaluating stallion semen (Moraes et al., 2019; Dini et al., 2019). Our results compare to previous results when validating the iSperm for canine use (Domain et al., 2022).

Neither iSperm ($P = 0.26$) nor visual assessment ($P = 0.66$) of sperm motility differed among breeds. Bull breed did not influence ($P = 0.83$) the total percentage of viable cells or viable cells with intact acrosomes ($P = 0.83$). When evaluating oxidation status by measuring reactive oxygen species, the bull breed did not influence ($P = 0.92$) the percentage of live sperm cells with positive reactive oxygen species status. There was a tendency ($P = 0.10$) for a greater percentage of sperm from Charolais bulls ($31.1\% \pm 3.35$) to have positive mitochondrial energy

potential as compared with Angus bulls ($17.6\% \pm 3.35$). These results differ from those found by Barth and Waldner (2002), who saw differences in motility in relation to BSEs between Angus and Charolais bulls. In our study, the bull breed appears to have little influence on sperm quality assessments among yearling bulls meeting threshold requirements for passing BSEs.

In conclusion, technician and iSperm sperm motility data are positively correlated, offering producers an on-farm evaluation tool. The positive correlation between the two evaluation methods suggests that the iSperm may be a good chute-side tool for conducting BSEs. Though the bull breed had little influence on sperm quality assessments, ROS in sperm appeared to impair sperm health and function.

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Table 4.1 Sperm quality assessments using visual analysis and flow cytometry on ejaculates from Angus and Charolais breeds of yearling bulls meeting breeding soundness exam threshold requirements

Factor	Least squares mean \pm SEM		P-value
	Angus n=23	Charolais n=23	
Bull age, days	402.9 \pm 2.36	403.3 \pm 2.36	0.90
Semen Characteristic			
Technician Progressive Motility ¹ , %	43.7% \pm 1.69	47.39 \pm 1.69	0.26
iSperm Progressive Motility ² , %	50.1 \pm 2.26	47.8 \pm 3.18	0.66
iSperm Gross Motility ² , %	71.6 \pm 2.78	70.5 \pm 2.78	0.82
Cells live and viable ³ , %	42.3 \pm 3.95	43.6 \pm 3.95	0.83
Cells live with intact acrosome ⁴ , %	41.5 \pm 3.40	42.6 \pm 3.40	0.83
Cells viable with positive reactive oxygen species ⁵ , %	29.1 \pm 3.52	28.5 \pm 3.52	0.92
Active mitochondrial potential ⁶ , %	17.6 \pm 3.35	31.1 \pm 3.35	0.10

¹Ejaculate gross motility was analyzed by a single veterinarian as a part of a breeding soundness exam.

²Progressive and gross motility of each ejaculate were analyzed using the iSperm software and manufacturer recommendations.

³Percentage of live and viable cells were determined by flow cytometry using the Invitrogen Live/Dead sperm viability kit.

⁴Percentage of live cells with intact acrosomes and sperm membrane integrity were determined by flow cytometry using the IMV Technologies acrosome and sperm membrane integrity assay.

⁵Percentage of viable cells with a positive reactive oxygen species were determined by flow cytometry using the IMV Technologies Easy Kit 3: Oxidation molecule D assay.

⁶Percentage of spermatozoa with active mitochondrial potential were determined by flow cytometry using IMV Technologies Easy Kit 2: Mitochondrial activity assay.

Table 4.2 Pearson's correlation coefficients of sperm attributes from ejaculates collected following breeding soundness exams

	% Live Negative ROS Spermatozoa ¹	% Live Positive ROS Spermatozoa ²
% Primary Abnormalities ³ r (P-value)	0.28 (0.06)	-0.15 (0.33)
% Secondary Abnormalities ⁴ r (P-value)	0.33 (0.02)	-0.23 (0.12)
% Progressive Motility ⁵ r (P-value)	-0.27 (0.10)	0.53 (<0.001)
% Live with Intact Acrosome ⁶ r (P-value)	-0.16 (0.29)	0.92 (<0.001)
% Live with Disrupted Acrosome ⁷ r (P-value)	0.66 (<0.001)	-0.31 (0.04)
% Live ⁸ r (P-value)	-0.19 (0.22)	0.94 (<0.001)
% Polarized ⁹ r (P-value)	0.03 (0.84)	0.58 (<0.001)

¹Percentage of spermatozoa from ejaculate with an intact cell membrane that have Negative ROS

²Percentage of spermatozoa from ejaculate with an intact cell membrane that have Positive ROS

³Percentage of spermatozoa from ejaculate exhibiting primary abnormalities

⁴Percentage of spermatozoa from ejaculate exhibiting secondary abnormalities

⁵Percentage of spermatozoa from ejaculate that are progressively motile

⁶Percentage of spermatozoa from ejaculate with an intact cell membrane and acrosome

⁷Percentage of spermatozoa from ejaculate with an intact cell membrane and disrupted acrosome

⁸Percentage of spermatozoa from ejaculate with an intact cell membrane

⁹Percentage of spermatozoa from ejaculate with polarized mitochondrial membranes

Chapter 5 - Impact of concurrent enrollment in animal reproduction laboratory and lecture courses

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Abstract

Our study investigated the potential impacts of concurrent enrollment of undergraduate students in lecture and laboratory animal reproduction courses on final course percentages. Student learning outcomes and structure of the laboratory course were designed to provide hands-on learning opportunities, which coincided with concepts discussed in lecture. A total of 307 students were included in the analysis. Students concurrently enrolled in laboratory and lecture had a greater ($P < 0.001$) final course percentage in the lecture compared with those enrolled in lecture alone. Students in the science degree option had a greater ($P < 0.03$) final lecture course percentage compared with those in the production degree option, and juniors had a greater ($P = 0.05$) final course percentage when compared with sophomores. At the end of the semester, students were surveyed about the perceived value of the laboratory course on their learning. Among students enrolled in laboratory sections, 98.4% indicated the hands-on activities improved their knowledge of course concepts in lecture. These student beliefs are supported by our results, which suggest that taking the laboratory and lecture together improves student final course percentages and that students value the hands-on learning opportunities provided in laboratory sections.

Introduction

An animal reproduction course is a common requirement of students in animal science majors across many universities. While providing animal reproduction lecture courses, many curricula do not require or offer a laboratory section with the lecture. As undergraduate animal science student populations are from diverse backgrounds and with varied animal species experience and career interests there is need to provide students with practical experiences beyond lectures to prepare them for their futures (Buchanan, 2008; Ragland et al., 2023). Use of

techniques such as field trips and hands-on activities found in laboratory courses increase classroom engagement and are among the most important teaching tools for animal science instructors (Buchanan, 2008; Ragland et al., 2023). The increasing variation in prior experiences with livestock handling has emphasized the need to teach practical application-type skills often learned in laboratories (Buchanan, 2008). When students are asked about their beliefs toward courses with livestock handling students responded that the experience was invaluable in their education (Woiwode, 2016).

Historically, some universities have used laboratory courses to provide students the opportunity to conduct scientific research, review peer writing, and develop skills for basic care of animals (Horvath & Inskip, 1968). However, as students' needs change, altering course designs to provide more individualized hands-on learning may be more beneficial for the changing student demographics. Hands-on, or experiential, learning, is the act of performing a skillset or learning by "doing" (Roberts, 2006). Examples of hands-on learning activities in animal science include any acts in which the student is able to execute or assist in a skill such as assessing vital signs, or assisting in parturition (Wells et al., 2019). Hands-on learning activities increase student engagement and enable them to reinforce course topics communicated in lectures (Ragland et al., 2023).

Factors previously shown to influence animal science course learning outcomes include gender, major, and college standing (Bormann et al., 2013; Burk et al., 2013; Lancaster & Robinson, 2011; McMillan et al., 2009; Peffer, 2011; Soberon et al., 2012). Yet to be investigated, are if an interaction exists amongst these factors and if concurrent enrollment in a laboratory and lecture course affect learning as indicated by final course percentages. It has been demonstrated that hands-on learning improves student engagement and aids student learning

(Handur et al., 2016; Haury & Rillero, 1994; Wells et al., 2019), however this effect on animal reproduction courses is yet to be investigated (Handur et al., 2016; Haury & Rillero, 1994), and warrants evaluation. Understanding if and how hands-on learning activities increase student interest and motivation may lead to improved awareness of animal care and career preparedness (Ragland et al., 2023). Our study aimed to investigate the potential impact of concurrent enrollment in lecture and laboratory sections on student learning (as indicated by final course percentages) in an animal reproduction course.

Methods

This study was deemed exempt by the Kansas State University Institutional Review Board. Data were collected from participating students enrolled in the animal reproduction lecture and (or) laboratory courses at Kansas State University in the Department of Animal Sciences and Industry during the spring semesters of 2021, 2022, and 2023. The animal reproduction lecture is a 3-credit course required for the Animal Science major. The lecture course meets three times per week for 50 minutes. Student learning is assessed via performance on exams and quizzes.

The animal reproduction laboratory is offered as a 1-credit course only in spring semesters. It is an elective course for most students, except for those completing the Bioscience option within the Animal Science major, who are required to take the animal reproduction laboratory course. At Kansas State University, the Animal Science and Industry undergraduate program offers students five different degree options that require the animal reproduction lecture course. Of those five degree options, two are science-focused (Pre-veterinary Medicine and Biosciences), and the remaining three are production- focused (Production Management, Communication, and Business). The laboratory course meets once per week for 110 minutes.

Students learning is evaluated via their performance on exams, quizzes, and laboratory exercises. The learning outcomes and structure of the laboratory course were designed to provide hands-on learning opportunities that coincided with concepts discussed in lecture. Examples of these learning opportunities include evaluation and dissection of non-pregnant female reproductive tracts, identification of male reproductive tract anatomy, semen evaluation, male and female reproductive tract histology, embryo evaluation, building diagrams to demonstrate hormone feedback, and pregnant tract anatomy and dissection. The species used in the laboratory for dissections were bovine and porcine. When available, preserved specimens from other species were provided to students to learn about anatomy differences.

Data collected included individual students' final course percentages in lecture and laboratory, college standing (sophomore, junior, senior), gender, and degree option. Students' degree options were categorized into either a science (Pre-veterinary Medicine and Biosciences) or production (Production Management, Communication, and Business) focus. After agreeing to participate in the study, 307 students were included in the analysis, of which 95 were concurrently enrolled in the laboratory and lecture, and 212 were enrolled in lecture only. Students who agreed to participate in 2022 were also asked to complete a survey summarizing their perceptions of the courses.

The GLIMMIX procedure of SAS (version 9.4), was used to assess potential effects of factors on students' final lecture course percentage. Fixed effects included concurrent enrollment in laboratory and lecture, college standing, gender, degree option, and two-way interactions between concurrent laboratory enrollment and degree option, concurrent laboratory enrollment and college standing, degree option and college standing, gender and degree option, gender and college standing, and concurrent laboratory enrollment and gender. Utilizing

backward selection, any non-significant ($P>0.05$) factors were removed from the final model. Frequencies were used to evaluate the proportion of student survey responses.

Results and Discussion

Potential two-way interactions (concurrent laboratory enrollment and degree option, concurrent laboratory enrollment and college standing, degree option and college standing, gender and degree option, gender and college standing, and concurrent laboratory enrollment and gender) were non-significant ($P>0.05$). Students concurrently enrolled in animal reproduction laboratory and lecture were associated with having a greater ($P<0.001$) final course percentage in the animal reproduction lecture course (82.88%) than those enrolled in lecture alone (77.66%) (Table 5.1). Other literature shows students who were given reading assignments in an animal physiology lecture course before quizzes had increased grade percentages (Horvath & Inskip, 1968). Perhaps the learning material in the laboratory course had a similar effect to pre-quiz reading assignments by reinforcing concepts from lecture (Horvath & Inskip, 1968). When hands-on activities were incorporated into a computer programming course, authors observed increased student final course percentages (Handur et al., 2016). One possible reason for the differences in students concurrently enrolled and those not, is that students enrolled in the laboratory may inherently have more motivation to learn than those not enrolled, as they may have more interest in the subject area. It is also possible that the improvements in learning from students participating in laboratory courses are due to increased engagement with the subject material, longer retention of course concepts, improvement in students' problem-solving skills, and that the laboratory allowed students more time to process material (Gucwa & Cheng, 2014; Handur et al., 2016; Haury & Rillero, 1994). Increased student engagement in reproduction courses has been shown to increase student grade percentage (Maiga & Bauer, 2013; Poole &

Moore, 2016). Students given the opportunity to participate in review sessions and interactive flash games had an increase in exam grade percentages (Maiga & Bauer, 2013; Poole & Moore, 2016). Perhaps the activities and review sessions are similar to our laboratories where students can interact with material then engage by asking questions of the instructor and peers (Maiga & Bauer, 2013; Poole & Moore, 2016). Other animal reproduction courses have provided students with online laboratory material when it was not available in person, and observed that as students increased their interactions with the material their exam percentages increased (Grizzle et al., 2008). However, the authors noted that it was difficult to recreate the hands-on laboratory sessions in an online format, perhaps emphasizing value of providing students with hands-on laboratory material with which they can physically interact.

Students in the science-focused degree options of the animal science curriculum in the present study were associated with having a greater ($P < 0.03$) final lecture course percentage (81.88%) when compared with those in the production-focused options (78.66%). The association of greater course percentage for students in the science-focused options is similar to the results of a previous study conducted at Kansas State University (Bormann et al., 2013). Bormann et al. (2013) classified student degree options as either pre-veterinary or non-pre-veterinary and found that students in the pre-veterinary options had greater final course percentages in a genetics course, similar to our results where students in the science-focused options had a greater final course percentage. Our results differed from previous literature evaluating agriculture courses, in which there was no association between major and course grade. Reasons for these varied conclusions may include that previous studies were focused on introductory courses, and the authors evaluated majors compared with non-majors, whereas we investigated specifically degree options within the animal science major (Lancaster & Robinson,

2011; McMillan et al., 2009; Pratt-Phillips & Schmitt, 2009; Soberon et al., 2012). Our findings of increased final course percentages by 3.2% for students enrolled in the science focused degree options may be because curricula include more biology, chemistry, and upper-level science courses than students completing the production-focused degree options. Additional science courses may provide students with a stronger foundational knowledge and references points for understanding core concepts in animal reproduction. Martin et al. (2006) found that students in an animal behavior course performed better after taking more than two science courses. Similar findings have been observed for anatomy and physiology courses, in which students who completed more mathematics and science courses performed better than those who did not (Harris et al., 2004). For instructors, realizing potential differences in preparedness and understanding of students based on their degree options may create a need to incorporate more background information, such as explanation of the chemical structure of hormones, for those students who may not have received it in previous courses. Another explanation for the students in the science focused option having a greater final course percentage is that most of those students are striving for acceptance into professional schools such as veterinary medicine and thus are more motivated in academic achievement.

College standing was another factor that significantly affected ($P < 0.03$) final course percentage. Students classified as juniors were associated with having a greater ($P < 0.05$) final course percentage (82.42%) than students classified as sophomores (78.53%) but did not differ ($P = 0.28$) from senior students (79.87%). Juniors would have completed more foundational courses than sophomores, perhaps providing them with a more robust science foundation. No difference ($P = 0.76$) was found in final course percentages when comparing seniors with sophomores. One explanation for not observing a difference between sophomore and senior

standing is that this course is taught in the spring semester before graduation for most of these students, it is possible that this impacted their intrinsic motivation. Our results differ from Bormann et al. (2013) conducted in our department who found that seniors had the greatest course percentage in genetics, with no difference between juniors and sophomores (Bormann et al., 2013). Others have found no association between college classification and final course grades in plant science or other animal science courses (Lancaster & Robinson, 2011; McMillan et al., 2009).

Student gender did not affect ($P=0.83$) final lecture course percentage. Other universities have found no associations between final course percentages in animal science courses and gender (Bormann et al., 2013; Burk et al., 2013; Peffer, 2011). Still other researchers, have found that female students have better grade outcomes than males in agriculture-related courses (Lancaster & Robinson, 2011; McMillan et al., 2009; Soberon et al., 2012) indicating the role of gender in learning course concepts is equivocal.

Students were surveyed at the end of the semester in 2022 about the perceived value of the laboratory course on their learning. Among students enrolled in laboratory sections, 97.4% indicated the hands-on activities improved their understanding of course concepts discussed in lecture. Of the students enrolled in the laboratory whom were enrolled in the lecture during a previous semester, 100% indicated the laboratory would have been helpful for their learning when they were enrolled in the lecture. Of students enrolled in the lecture alone, 88.24% indicated they wished they had enrolled in a laboratory section because they believed it would have improved their understanding of course concepts. Maiga and Bauer (2013) noted that when flash identification games were implemented as a learning tool in an animal reproduction course students felt the tool helped them study for exams, and a majority of students believed flash

identification games helped them learn the course content. Student perceptions of their improved understanding of course concepts when concurrently enrolled in lecture and laboratory sections in the present study are supported by the objective results of improved final lecture course percentages. Not only did taking the laboratory and lecture together empirically improve student final course percentages, but students also expressed the value they found in the hands-on learning opportunities provided in laboratory sections - an important consideration in evaluation of student satisfaction of their education.

Summary

Concurrent enrollment in laboratory and lecture showed a significant association with improved final course percentages compared with students enrolled in lecture alone; the increase in course percentage for those enrolled in the laboratory demonstrates the benefits of hands-on learning. Students validated these results by indicating in the survey that they perceived added benefits from the laboratory course. Students in the science option of the animal science major had a greater mean final course percentage than those in the production option. Students in the science option of the animal science major earning greater a final course percentage is not surprising, as they are required to take more science courses such as multiple semesters of chemistry and microbiology and thus likely develop a more robust foundational science knowledge. Students classified as juniors were associated with performing better than sophomores, suggesting that courses taken in their sophomore year prepare them for the animal reproduction course. In our study, gender did not significantly impact final student course percentages. The benefits of hands-on learning activities warrant providing students access to these activities and incorporating them in lecture courses when laboratory classes are unavailable

to improve student performance and experiences. Our study highlighted the benefits of providing students with a laboratory course concurrently with an animal reproduction lecture.

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Table 5.1 Least square means (LSMEANS) for final lecture course percentages of students in animal reproduction

Fixed Effects	<i>n</i>	LSMEANS Course percentage (%)	SE	<i>P</i> -value
Concurrent enrollment in laboratory				
Yes	95	82.88 ^a	1.31	0.001
No	212	77.66 ^b	0.84	
Animal science degree option ¹				
Production	123	78.66 ^a	1.20	0.028
Science	184	81.88 ^b	0.93	
Standing				
Sophomore	86	78.53 ^b	1.32	0.049
Junior	144	82.42 ^a	1.06	
Senior	77	79.87 ^{ab}	1.43	
Gender				
Female	231	80.36	0.90	0.830
Male	76	80.01	1.46	

^{a,b}Within a column and fixed effect, means without a common superscript differ ($P < 0.05$).

¹At Kansas State University, the Animal Science and Industry undergraduate program offers students five different degree options. Of the five degree options the science focused group included Pre-veterinary Medicine and Biosciences, while the production focused included Production Management, Communication, and Business.

Table 5.2 Summative perception of students who were surveyed in 2022 concurrently enrolled in the animal reproduction laboratory and lecture course about their beliefs towards the lecture and laboratory courses

Question	Response % ¹	
If you were concurrently enrolled in the laboratory and lecture was this course helpful in concurrently learning the content from lecture?	The lab has been helpful with the lecture. 97.4 (n=37)	The lab has not been helpful with the lecture. 2.6 (n=1)
If you were not currently enrolled in the lecture would this course have been helpful in concurrently learning the content from lecture when you had been enrolled?	Yes 100 (n=23)	No 0 (n=0)

¹Surveys were administered online after student consent was given and students were not required to answer every question.

Table 5.3 Summative perception of students surveyed in 2022 in the animal reproduction lecture course about their beliefs towards the lecture and laboratory courses

Question	Response % ¹	
If you were not enrolled in the laboratory, would it have been helpful to have enrolled?	<p style="text-align: center;">Yes</p> <p style="text-align: center;">97.06 (n=66)</p>	<p style="text-align: center;">No</p> <p style="text-align: center;">2.94 (n=2)</p>
Why did you decide to take the laboratory?	<p style="text-align: center;">I took it because it sounded interesting, but I was not required to take it.</p> <p style="text-align: center;">71.05 (n=27)</p>	<p style="text-align: center;">I took it because I was required to.²</p> <p style="text-align: center;">28.95 (n=11)</p>
If you did not take the laboratory which statement best matches your feelings?	<p style="text-align: center;">I did not take it and had no interest in doing so.</p> <p style="text-align: center;">11.76 (n=8)</p>	<p style="text-align: center;">I did not take it but wish that I would have.</p> <p style="text-align: center;">88.24 (n=60)</p>

¹Surveys were administered online after student consent was given and students were not required to answer every question.

²Students at Kansas State University in the Animal Science and Industry undergraduate program who are the Biosciences degree option are required to take the animal reproduction laboratory.

Appendix A - Descriptive Information from Duroc Boars in a Commercial Boar Stud

Source of data

Data from boars included in this appendix were part of a study conducted on Duroc boars at a commercial boar stud. Data was collected from August 2022 to August 2023. The day after arrival at a commercial boar stud, 169 Duroc boars (Line 600; DNA, Columbus, NE; average 190.2 d of age) were enrolled in an experiment across four admission groups (August to February 2022). Boars originated from one of five developer barns before being selected to become replacement boars. Boars were initially housed in a separate portion of the boar stud for isolation and after the isolation period all boars were transferred to the production barn. Data in this appendix provides supplemental information for data collected in this study.

Appendix A Table A.1 Overall population averages of semen characteristic collected from adult working boars in a commercial AI stud

Variable	N (ejaculates)	Mean	Std Dev	Median	Minimum	Maximum
Volume, mL	2389	104.86	44.89	98.6	14	329
Concentration, x 10 ⁹ sperm/mL	2389	0.66	0.26	0.63	0.03	2.46
Total sperm, x 10 ⁹ sperm	2389	64.85	26.94	63.72	1.94	171.4
Progressive Motility, %	2389	90.20	5.50	91.3	26.5	98.6
Normal, %	2389	83.09	9.35	85.9	32.7	97.2
Total normal x 10 ⁹ sperm	2389	54.72	24.44	53.43	0.65	149.83

A total of 148 boars (Line 600; DNA, Columbus, NE) successfully became working boars at a commercial boar stud and their ejaculates analyzed.

Appendix A Table A.2 The effect of percentage bodyweight changes during the isolation period on volume (ml) of adult working boars by production week in a commercial AI stud (lsmean)

Week of production	Group ²			SEM	P-value
	Top	Middle	Bottom		Group x Production wk
Week 1	85.76	93.51	75.82	15.67	0.4765
Week 2	97.33 ^a	92.88 ^a	67.88 ^b	12.44	0.0384
Week 3	97.18	94.32	92.05	11.55	0.8968
Week 4	106.49	90.95	94.12	10.38	0.2789
Week 5	97.10	86.37	90.49	9.83	0.5261
Week 6	100.92 ^{a, xy}	96.17 ^{ab, x}	78.76 ^{b, y}	9.51	0.0447
Week 7	115.63 ^a	85.50 ^b	82.22 ^b	9.48	0.0005
Week 8	104.94 ^a	92.57 ^{ab}	77.62 ^b	10.39	0.0317
Week 9	115.83 ^{a, y}	98.04 ^{ab, x}	81.34 ^{b, y}	10.33	0.0036
Week 10	112.15 ^a	100.79 ^{ab}	87.37 ^b	9.22	0.0268
Week 11	111.93 ^a	92.95 ^b	88.25 ^b	9.57	0.0303
Week 12	113.07 ^a	93.65 ^b	81.33 ^b	9.32	0.0028
Week 13	115.07 ^{a, x}	99.97 ^{a, y}	79.48 ^{b, xy}	9.55	0.0009
Week 14	117.81 ^a	96.56 ^b	83.77 ^b	9.44	0.0013
Week 15	113.21 ^a	109.29 ^a	84.03 ^b	9.47	0.0034
Week 16	120.04 ^a	98.62 ^b	93.56 ^b	9.66	0.0145
Week 17	115.60 ^{a, x}	98.85 ^{b, y}	85.65 ^{b, xy}	9.52	0.0070
Week 18	118.26 ^{a, x}	101.67 ^{b, y}	90.11 ^{b, xy}	9.49	0.0118
Week 19	129.16 ^a	105.05 ^b	97.52 ^b	9.61	0.0026
Week 20	125.21 ^a	111.47 ^{ab}	102.84 ^b	9.68	0.0660
Week 21	137.27 ^a	107.99 ^b	104.18 ^b	9.82	0.0010
Week 22	129.12 ^a	104.08 ^b	105.73 ^b	9.86	0.0153
Week 23	138.32 ^a	110.88 ^b	113.77 ^b	10.21	0.0119
Week 24	139.58 ^a	92.64 ^b	126.94 ^a	12.22	<0.0001
Week 25	146.39	125.01	152.76	31.70	0.3554

¹A total of 148 boars (Line 600; DNA, Columbus, NE) were used over a 42-d period after arrival at a commercial boar stud.

² After the isolation period, boars were retrospectively divided into one of three groups based on their percentage body weight change during the 42-d isolation period. The 1/3 of boars that had the greatest percentage of body weight change during the 42-d period gained 10.1% to 36.1% (TOP). The middle 1/3 of boars that were intermediate in percentage body weight change during isolation gained 2.6% to 9.7% (MIDDLE). The final group consisted of 1/3 of boars that either minimally gained or lost weight (-9.5% to 2.5% change in body weight; BOTTOM).

^{a,b,c} Means within a row with different superscripts differ ($P \leq 0.05$).

^{x,y} Means within a row with different superscripts differ ($0.05 \leq P \leq 0.10$).

Appendix A Table A.3 The effect of percentage bodyweight changes during the isolation period on total sperm per ejaculate (x 10⁹ sperm) of adult working boars by production week in a commercial AI stud (lsmean)

Week of production	Group ²			SEM	P-value
	Top	Middle	Bottom		Group x Production wk
Week 1	60.35 ^b	83.58 ^a	60.24 ^b	10.43	0.0315
Week 2	69.45 ^{a, x}	83.29 ^{a, y}	50.50 ^{b, xy}	8.14	0.0003
Week 3	70.33	70.30	66.70	7.45	0.8342
Week 4	70.97 ^{xy}	76.70 ^x	62.25 ^y	6.48	0.0630
Week 5	65.79	71.91	64.22	6.05	0.3792
Week 6	72.49 ^{ab, x}	77.62 ^{b, xy}	61.67 ^{a, y}	5.80	0.0143
Week 7	73.70	70.77	63.26	5.77	0.1736
Week 8	70.18 ^{ab, x}	74.31 ^{a, xy}	57.98 ^{b, y}	6.60	0.0397
Week 9	80.26 ^a	72.81 ^a	56.62 ^b	6.46	0.0011
Week 10	74.86 ^a	71.63 ^a	56.00 ^b	5.60	0.0013
Week 11	77.15 ^{a, x}	66.33 ^{a, y}	54.14 ^{b, xy}	5.85	0.0004
Week 12	70.87 ^a	63.76 ^a	48.72 ^b	5.64	0.0003
Week 13	70.24 ^a	71.40 ^a	49.77 ^b	5.83	0.0001
Week 14	68.75 ^a	60.70 ^a	46.53 ^b	5.74	0.0004
Week 15	67.16 ^a	66.90 ^a	44.56 ^b	5.76	< 0.0001
Week 16	71.88 ^a	59.39 ^b	47.02 ^c	5.95	0.0002
Week 17	62.51 ^a	57.38 ^a	41.62 ^b	5.80	0.0009
Week 18	60.34 ^{a, xy}	53.78 ^{ab, x}	43.38 ^{b, y}	5.78	0.0126
Week 19	66.22 ^a	58.39 ^a	46.37 ^b	5.86	0.0031
Week 20	67.46 ^{a, x}	56.96 ^{a, y}	45.47 ^{b, xy}	5.93	0.0010
Week 21	73.88 ^a	56.16 ^b	48.03 ^b	6.04	< 0.0001
Week 22	69.91 ^a	54.97 ^b	49.56 ^b	6.02	0.0023
Week 23	62.62 ^a	60.81 ^a	46.88 ^b	6.35	0.0227
Week 24	66.67 ^{a, x}	41.59 ^{b, xy}	52.29 ^{ab, y}	8.02	0.0016
Week 25	60.48	60.98	81.74	22.32	0.6032

¹A total of 148 boars (Line 600; DNA, Columbus, NE) were used over a 42-d period after arrival at a commercial boar stud.

² After the isolation period, boars were retrospectively divided into one of three groups based on their percentage body weight change during the 42-d isolation period. The 1/3 of boars that had the greatest percentage of body weight change during the 42-d period gained 10.1% to 36.1% (TOP). The middle 1/3 of boars that were intermediate in percentage body weight change during isolation gained 2.6% to 9.7% (MIDDLE). The final group consisted of 1/3 of boars that either minimally gained or lost weight (-9.5% to 2.5% change in body weight; BOTTOM).

^{a,b,c} Means within a row with different superscripts differ ($P \leq 0.05$).

^{x,y} Means within a row with different superscripts differ ($0.05 \leq P \leq 0.10$).

Appendix A Table A.4 The effect of percentage bodyweight changes during the isolation period on total normal sperm per ejaculate ($\times 10^9$ sperm) of adult working boars by production week in a commercial AI stud (lsmean)

Week of production	Group ²			SEM	P-value
	Top	Middle	Bottom		Group x Production wk
Week 1	46.47 ^a	67.94 ^b	43.68 ^a	9.25	0.0126
Week 2	54.98 ^a	66.28 ^a	36.77 ^b	7.32	0.0002
Week 3	56.09	55.49	51.78	6.67	0.7550
Week 4	55.89 ^{ab}	61.55 ^b	48.02 ^a	5.87	0.0519
Week 5	52.37	58.43	49.95	5.51	0.2557
Week 6	59.09 ^a	63.97 ^a	48.59 ^{ab}	5.30	0.0085
Week 7	60.55 ^{a, xy}	58.19 ^{ab, x}	49.47 ^{b, y}	5.28	0.0842
Week 8	58.31 ^a	62.87 ^a	46.29 ^b	5.94	0.0174
Week 9	67.13 ^a	61.25 ^a	45.24 ^b	5.83	0.0007
Week 10	63.55 ^a	59.41 ^a	45.30 ^b	5.11	0.0008
Week 11	64.59 ^{a, x}	54.85 ^{a, y}	43.92 ^{b, xy}	5.34	0.0006
Week 12	58.66 ^a	53.07 ^a	39.15 ^b	5.17	0.0005
Week 13	58.41 ^a	60.19 ^a	39.87 ^b	5.33	0.0001
Week 14	57.39 ^a	51.04 ^a	38.21 ^b	5.25	0.0009
Week 15	56.29 ^a	56.46 ^a	37.59 ^b	5.27	0.0002
Week 16	61.00 ^{a, x}	51.32 ^{a, y}	38.91 ^{b, xy}	5.40	0.0002
Week 17	52.91 ^a	49.22 ^a	34.69 ^b	5.34	0.0013
Week 18	52.43 ^a	46.97 ^a	35.56 ^b	5.29	0.0049
Week 19	58.70 ^a	50.60 ^a	39.24 ^b	5.36	0.0013
Week 20	59.23 ^{a, x}	49.27 ^{a, y}	38.38 ^{b, xy}	5.41	0.0006
Week 21	65.14 ^{a, xy}	49.60 ^{b, x}	40.81 ^{b, y}	5.50	< 0.0001
Week 22	62.25 ^a	48.21 ^b	43.16 ^b	5.53	0.0016
Week 23	57.14 ^a	55.89 ^a	42.18 ^b	5.76	0.0138
Week 24	60.83 ^a	40.00 ^b	48.55 ^b	7.05	0.0040
Week 25	56.21	55.37	76.56	19.56	0.5281

¹A total of 148 boars (Line 600; DNA, Columbus, NE) were used over a 42-d period after arrival at a commercial boar stud.

² After the isolation period, boars were retrospectively divided into one of three groups based on their percentage body weight change during the 42-d isolation period. The 1/3 of boars that had the greatest percentage of body weight change during the 42-d period gained 10.1% to 36.1% (TOP). The middle 1/3 of boars that were intermediate in percentage body weight change during isolation gained 2.6% to 9.7% (MIDDLE). The final group consisted of 1/3 of boars that either minimally gained or lost weight (-9.5% to 2.5% change in body weight; BOTTOM).

^{a,b,c} Means within a row with different superscripts differ ($P \leq 0.05$).

^{x,y} Means within a row with different superscripts differ ($0.05 \leq P \leq 0.10$).

Appendix A Table A.5 Summary statistics of flank-to-flank measurements, testicular area, and anal genital measurements

	Group ¹		
	TOP	MIDDLE	BOTTOM
Number of boars, n	54	56	54
Flank-to-flank, cm			
Start of isolation (SD)	88.68 (± 4.58)	90.57 (± 4.62)	90.58 (± 4.44)
End of isolation (SD)	92.53 (± 3.87)	92.02 (± 4.48)	91.24 (± 4.45)
Testicular area, cm ²			
Start of isolation (SD)	658.96 (± 156.11)	651.33 (± 158.36)	687.80 (± 148.98)
End of isolation (SD)	700.13 (± 148.24)	668.62 (± 187.44)	586.49 (± 106.78)
Anal genital measurements			
Number of boars, n ²	37	33	21
Anus to the top of the scrotum, cm	8.41 (± 1.86)	9.00 (± 1.61)	7.98 (± 2.24)
Anus to the end of penile shaft, cm	70.04 (± 6.55)	71.58 (± 5.36)	69.72 (± 4.44)

¹After the isolation period, boars were retrospectively divided into one of three groups based on their percentage body weight change during the 42-d isolation period. The 1/3 of boars that had the greatest percentage of body weight change during the 42-d period gained 10.1% to 36.1% (TOP). The middle 1/3 of boars that were intermediate in percentage body weight change during isolation gained 2.6% to 9.7% (MIDDLE). The final group consisted of 1/3 of boars that either minimally gained or lost weight (-9.5% to 2.5% change in body weight; BOTTOM).

²Anal genital measurements were collected from the first and the last groups of boars to enter isolation and technicians were unable to collect measurements from all boars.

Appendix A Table A.6 Summary statistics of growth characteristics of boars after performance testing

	Group ¹		
	TOP	MIDDLE	BOTTOM
Body weight, kg (SD)	114.62 (\pm 14.64)	118.53 (\pm 11.71)	120.72 (\pm 11.15)
Backfat, mm (SD)	9.10 (\pm 1.97)	9.15 (\pm 2.20)	8.99 (\pm 1.90)
Loin depth, mm (SD)	62.32 (\pm 7.19)	62.87 (\pm 6.44)	61.95 (\pm 6.04)

¹After the isolation period, boars were retrospectively divided into one of three groups based on their percentage body weight change during the 42-d isolation period. The 1/3 of boars that had the greatest percentage of body weight change during the 42-d period gained 10.1% to 36.1% (TOP). The middle 1/3 of boars that were intermediate in percentage body weight change during isolation gained 2.6% to 9.7% (MIDDLE). The final group consisted of 1/3 of boars that either minimally gained or lost weight (-9.5% to 2.5% change in body weight; BOTTOM).

Appendix B - Impact of structural traits on semen collection training and lesion characteristics in Duroc boars

This project was conducted as a part of undergraduate research. Macie Weigand developed the scoring system used in this study for her undergraduate research project and authorized the illustrations.

Lameness is defined as the condition of being disabled by injury or defect in a limb. Rising incidence of lameness in boar studs contribute to increased morbidity and mortality, affecting longevity and likely semen output. The United States boar herd primarily consists of terminal sires selected exclusively for their growth and carcass characteristics a potential contributor to lameness (Robinson and Buhr, 2005; Flowers, 2008). Lameness is not unique to boars as it is one of the leading management concerns in the swine industry and is estimated to cost United States swine producers over \$23 million a year (Supakorn et al., 2018). Rising incidence of lameness in boar studs contribute to increased morbidity and mortality, affecting longevity and likely semen output. Hypothesized contributors to lameness are body, feet, and leg structure. To better understand causes and effects of lameness, our objectives were to evaluate the relationship between boar structural traits and semen collection training traits and determine if structural traits influence presence and severity of lesions. Structural traits of Duroc boars (n = 82) were assessed at either arrival to the stud or six weeks later. Structural traits assessed included front and rear leg set, foot shape, chest width, and hip width (1 = poor; 5 = ideal). An overall structure score for each boar was determined by adding all structural trait scores. Lesions were assessed on a zero to three scale (0 = no lesions; 3 = severe lesions). Based on overall structure score, boars were categorized into one of three groups (Poor; Intermediate; Ideal) to

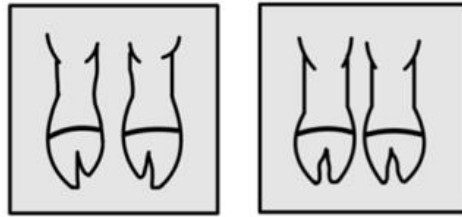
determine if structural traits affected the presence and severity of lesions. All boars were subjected to semen collection training one week after arriving at the stud and considered trained when successful mounting and ejaculation occurred. Statistical analyses included Pearson's correlations and the GLIMMIX procedure of SAS. Neither structure score nor lesion severity was correlated ($P > 0.10$) with number of attempts to train, time to mount, or time to collection of ejaculates. Upon arrival at the stud, boars in the poor structure group displayed a greater incidence of lesions ($P < 0.01$) than other structure groups but did not have a greater level of lesion severity ($P = 0.74$). After six weeks in the boar stud, there were no differences ($P = 0.8$) in lesion presence or severity between structure groups, suggesting the lameness issues are more likely caused by housing environment or other factors than structural traits.

Literature Cited

- Flowers, W. L. 2008. Genetic and phenotypic variation in reproductive traits of AI boars. *Theriogenology*. 70(8):1297-1303.
- Robinson, J. A. B., and M. M. Buhr. 2005. Impact of genetic selection on management of boar replacement. *Theriogenology*. 63(2):668-678.
- Supakorn, C., J. D. Stock, E. Garay, A. K. Johnson, and K. J. Stalder. 2018. Lameness: a principal problem to sow longevity in breeding herds. *CABI Reviews*. 13:1-14.

Appendix B Figure B.1 Boar feet and leg scoring system used to determine total score for structural groups. Foot shape (1 extremely uneven toes – 5 square and even toe length), chest and hip width (1-narrow and 5-wide), front and rear leg set (1-extremely straight, 2 extreme set, 3-moderately straight, 4-moderate set, 5-ideal set).

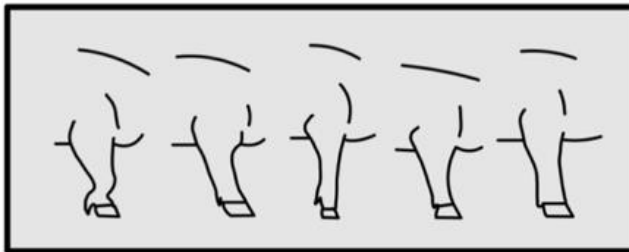
Foot Shape



1

5

Front Leg Set



1

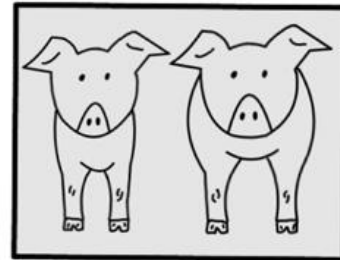
2

3

4

5

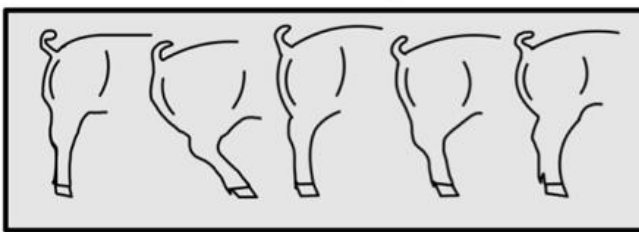
Chest Width



1

5

Rear Leg Set



1

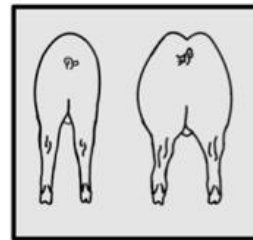
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Hip Width

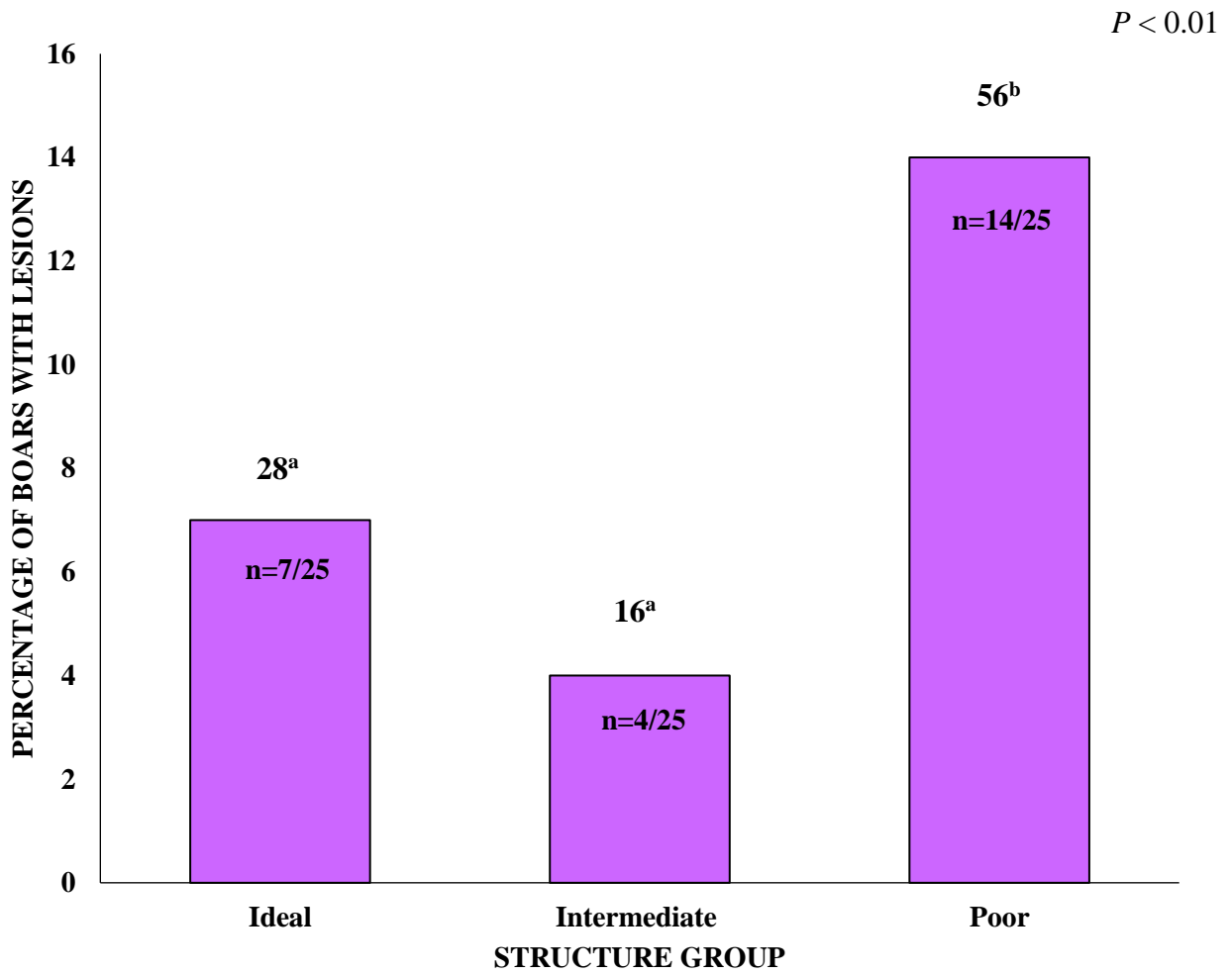


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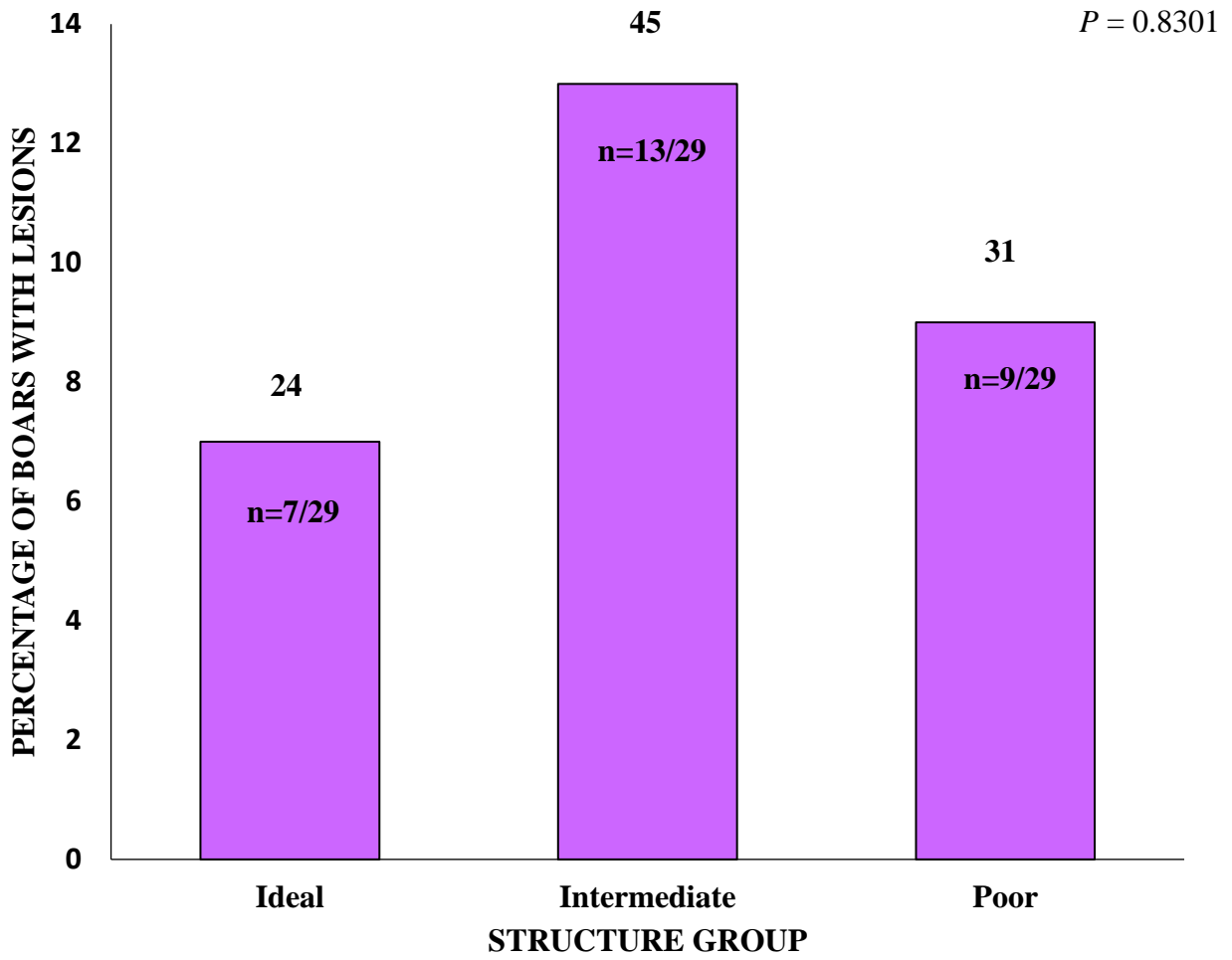
5

Illustration credit must be given to Macie Weigand.

Appendix B Figure B.2 Percentage of lesions present at entry time to the boar stud



Appendix B Figure B.3 Percentage of lesions present 6 weeks after arrival to the boar stud



Appendix B Table B.1 Pearson's correlation coefficients for total structure score and training characteristics

	Total Score
	r (P-value)
Lesion Severity	-0.017 (0.91)
Number of Attempts to Train	-0.007 (0.95)
Time to Mount	0.039 (0.74)
Time to Ejaculate Collection	0.003 (0.98)

Appendix B Table B.2 Summary statistics of structure scores assessed after six weeks in the boar stud

	Foot Shape	Chest Width	Hip Width	Front Leg Set	Rear Leg Set	Total Score
N	82	82	82	82	82	82
Mean	3.7	2.9	2.8	3.2	3.5	16.1
Std Dev	1.10	1.15	1.13	1.20	.77	2.59
Median	4	3	3	3	3	16
Minimum	1	1	1	1	1	9
Maximum	5	5	5	5	5	23