Abstract

Klein, Jennifer Nicole. Increasing sperm production in mature boars via manipulation of their neonatal environment. (Under the direction of Dr. William L. Flowers.)

The objective of this study was to determine if litter size during lactation (neonatal environment) influenced semen quality of mature boars. Boars born in October 2003 (n = 18) and in March 2004 (n = 18) were allocated into two treatments by crossfostering piglets one day after birth. Boars were nursed in litters of either six (small litter size) or litters of ten and greater (large litter size) through weaning. Following weaning, boars were raised under identical conditions comparable to those used in commercial production environments.

At 24 weeks of age, boars were trained to mount and collect. Boars were then put on a once per week collection regimen. Data collected and analyzed included body weight, testicular measurements, semen characteristics, and seminal plasma protein profiles. All data was analyzed using SAS and the proc GLM procedure.

While the boars continue to undergo weekly collections, analysis showed that litter size during lactation affected body weight. For boars born in the fall, boars reared in small litters were not significantly different ($p<.05$) than their counterparts reared in large litters except at weaning. In contrast, boars born in the spring and reared in small litters were significantly different ($p<.05$) than their counterparts reared in large litters at all ages except from 20 to 28 weeks of age. Beginning at 13 weeks of age, boars born in the fall also consistently weighed more and maintained larger testicular parameters than boars born in the spring.

There were no significant effects of litter size on semen quality although litter size did have an effect on the amount of seminal plasma proteins. However, results indicated that those boars reared in large litters had higher protein levels than those reared in small litters. At 35 weeks of age, the boars that nursed in large litters had 124.0 ± 7.3 relative units of the two seminal plasma proteins of interest while the boars that nursed in small litters had only 106.9 ± 4.3 relative units. It was also observed that all boars experienced a decrease in the amount of seminal plasma proteins between 35 and 45 weeks of age.

Although the initial results do not strongly support the idea of rearing boars in smaller litters, the possibility that this theory may provide producers with an economical method for increasing on farm production validates continued research. While these results only reflect the performance of the fall replicate through 60 weeks of age and the spring replicate through 38 weeks of age, further analysis of data collected over time will be necessary to make final conclusions on whether the neonatal environment influences the quality and quantity of semen in mature boars.

KEY WORDS: sperm production, litter size, swine

Increasing sperm production in mature boars via manipulation of their neonatal environment

By

Jennifer Nicole Klein

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Approved by:

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Chair of Advisory Committee	
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Biography

Jennifer Klein was born on April 20, 1980 in Sumter, South Carolina to Carolyn and Joseph Klein, Jr. She has one older brother, Jason. Both children attended elementary, middle, and high school in Sumter. Upon graduation from Sumter High School in May 1998, the author chose to pursue her undergraduate degree in Animal Science at North Carolina State University.

Unlike her family, who all attended Furman University in Greenville, South Carolina, Jennifer moved to Raleigh and completed her bachelor's degree from N.C. State in May 2003. In August 2003, she began a graduate program at North Carolina State University and is pursuing her Master of Science degree in Animal Science.

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Table of Contents

List of Tables

List of Figures

Introduction

In commercial production, boar studs are required to produce a specified number of insemination doses each day. Therefore, consistent production of large quantities of fertile semen is a primary concern in boar management for producers. An individual boar greatly impacts the efficiency of the breeding herd. While a sow generally produces two litters per year, a boar can sire thousands of piglets. Therefore, recognizing the importance of the boar and implementing strategies to effectively increase the amount of fertile spermatozoa per ejaculate is essential to maximizing the reproductive efficiency of the breeding herd. Understanding testicular development and the process of spermatogenesis in the boar is critical to designing successful strategies that increase sperm production in an efficient yet economical manner for producers.

Literature Review

Testis Differentiation and Development

The process of sex determination begins at fertilization. The initial step occurs when a sperm delivers either an X or Y chromosome to the oocyte. All other secondary sex characteristics are dependent upon the presence or absence of hormones produced by the testis or ovary (reviewed by Capel, 2000). The undifferentiated gonad is located on the inner surface of the dorsal body wall and is referred to as the genital ridge. Prior to sexual differentiation, primordial germ cells, which originate from the proximal epiblast, migrate from the base of the allantois along the hindgut and finally reach the genital ridge where they undergo mitosis and their number increases significantly (De Rooij et al., 2000). There are three segments that make up the genital ridge. They include, from anterior to posterior: (1) the pronephros; (2) the mesonephros, the central region from which the gonad arises; and (3) the metanephros, the most posterior region in which the kidney forms (reviewed by Capel, 2000).

Once the primordial germ cells populate the undifferentiated gonads that are beginning to form, they become gonocytes. The process of primordial germ cells becoming gonocytes is due to their enclosure by the cords formed by Sertoli precursor cells and then the surrounding of peritubular cells (De Rooij et al., 2000). The pre-Sertoli cell is the first type of somatic cell to differentiate in the testis (Franca et al., 20005). In addition, this cell is thought to originate from the coelomic epithelium and its precursors are believed to express the male sex-determining gene Sry in the short arm of the Y chromosome (Franca et al., 2005). Sertoli cells play a vital role in determining testicular

differentiation. For swine, differentiation occurs at approximately 27 days of gestation (Pelliniemi, 1975). Peritubular myoid cells, endothelial cells, fibroblasts, and Leydig cells are the other somatic cells of the testis (Franca et al., 2005). The morphological interactions seen among germ cells, Sertoli cell precursors, and peritubular myoid cells result in the formation of the seminiferous cords and represent the sequence of events necessary for the normal development of spermatogenesis and for the inhibition of gonocyte progression toward meiosis, as occurs in females (Yao et al., 2002).

Sertoli cells are believed to be the organizing center of the male gonad because of their crucial role in functional testis development (reviewed by Capel, 2000). During early testis development, Sertoli cells secrete anti-Mullerian hormone (AMH), which suppresses the development of Mullerian ducts or the female reproductive tract and causes Leydig cells to differentiate. Regulation of the male reproductive tract and secondary sex characteristics are controlled by steroids secreted by fetal Leydig cells (Merchant-Larios et al., 2001). Leydig cells are situated in the interstitial tissue of the testis and are the steroid hormone producing cells (Van Straaten et al., 1978). These steroid hormones are essential to the regulation and development of the male reproductive tract and secondary sex characteristics. Overall, events that are crucial for male reproductive tract function include the developmental changes that occur within genetically male gonads such as Sertoli and Leydig cell differentiation as well as seminiferous cord formation (Sharpe et al., 2002).

Although the development of the male reproductive tract is a series of highly complex events, Alfred Jost established the basis of mammalian sex determination in 1947. Jost conducted an experiment in which he removed the testes or ovary from

developing rabbit fetuses and discovered that when lacking a testis or ovary, the fetus develops as a female (Jost, 1947). However, despite the seeming simplicity of Jost's findings there are several known molecular players in the testis determination pathway. The male sex-determining gene, Sry, is in the short arm of the Y chromosome (reviewed by Capel, 2000). It operates in a dosage-dependent manner in XY gonad primordia to induce differentiation of a testis among these cells. Other common players in sex determination pathways include Sox9, MIS, Wt1, and Sf1. The gene, Sox9, found to be sexually dimorphic in the gonads of many species, is up regulated in XY gonads and down regulated in XX gonads. Sox9 is considered a critical gene in the early differentiation of Sertoli cells (reviewed by Capel, 2000).

Mullerian inhibiting substance (MIS), which is a TGF-β family member, is one of the earliest known products of Sertoli cells and induces complete Mullerian ductal system regression (reviewed by Capel, 2000). The synthesis and secretion of MIS is also a functional marker of Sertoli cell differentiation (Kaminski et al., 1999). The gene, Sf1, encodes a steroid hormone receptor necessary for the regulation of genes involved in steroid synthesis in both the gonads and the adrenal gland and has been thought to be involved in the regulation of MIS. Finally, Wt1 may be involved in the regulation of Sf1 as suggested in studies by Nachtigal et al. (1998) and is also thought to be up regulated by the expression of Sry.

Factors Affecting Testicular Size: Impact of Sertoli and Leydig cells on Testes Size

Adult testicular size is generally accepted to be correlated with the capacity to produce sperm and total Sertoli cell numbers determine mature testis size in males of various mammalian species (Amann, 1970). The mammalian testes are divided into two compartments. The first compartment consists of vascularized interstitial tissue formed mainly by Leydig cells, macrophages, lymphatic vessels, and connective tissue while the second compartment is made up of the avascular seminiferous tubules that contain Sertoli and germ cells (Saez et al., 1987). The blood-testis barrier separates the compartments. This barrier possesses a three-layer "filter" composed of: (1) the surrounding layer of peritubular myoid cells, (2) the basal lamina, and (3) the Sertoli-Sertoli cell tight junctions (Saez et al., 1987). The primary function of the blood-testis barrier is the exclusion of substances from the seminiferous tubules. However, because the blood-testis barrier appears only at puberty, it seems that another primary role may be to create the correct environment inside the tubules for meiosis to occur (Setchell, 1979). Due to this anatomical arrangement of the testis, the essential communication between different testicular cells is important for normal male reproductive function.

Sertoli and Leydig cells are two of the most prominent cells in the testes and are separated by the blood-testis barrier. Due to their importance in steroidogenesis and spermatogenesis, it is essential to understand when they undergo the largest and most definitive periods of proliferation. In 2000, Franca et al. stated that the Sertoli cell number established during the prepubertal period determines the final testicular size and

the number of sperm produced in sexually mature animals. This occurs because of discontinuous Sertoli cell proliferation before puberty and limits in the ability of Sertoli cells to support germ cells (Russell et al., 1984).

Sertoli cells, discovered in 1865 by Sertoli, have two mitotic phases of proliferation in the pig (Courot, 1970 and Franca et al., 2000). It is generally accepted that proliferation occurs prepubertally and that total Sertoli cell numbers are established prior to the formation of the blood-testis barrier and puberty (Waites et al., 1985). The first phase of proliferation occurs between birth and one month of age while the second occurs just before puberty at three to four months of age (Franca et al., 2000). During each phase, the number of Sertoli cells increase dramatically. However, in contrast, Lunstra et al. (2003) states that one study reports that Sertoli cell numbers per testis decline by 40% between three and five months of age in conventional boars. They state that enumeration and proliferation of Sertoli cells during postnatal development remains to be clarified. Despite this discrepancy, there were no reports found that challenged the theory of Sertoli cell numbers being stable by puberty. Regardless of when proliferation occurs, Sertoli cells play a particular and essential role in the architecture of the seminiferous epithelium, the metabolic exchanges with the germ cells, and the coordination of spermatogenesis (Courot, 1970).

The regulatory mechanisms of Sertoli cell proliferation are not yet established. However, it is well accepted that FSH or follicle stimulating hormone is the major factor responsible for postnatal Sertoli cell proliferation (Heckert et al., 2002). Franca et al. (1995) also believes that thyroid hormone (T3) is responsible for transition of Sertoli cells from the mitotic to non-mitotic status before puberty. In addition, the two phases of

prominent Sertoli cell proliferation coincide with higher FSH plasma levels and a six-fold increase in the length of seminiferous cord/tubules (Franca et al., 2000). Because Sertoli cells per testis ultimately dictates the magnitude of testicular size and sperm production, all conditions that affect their proliferation also influence the number of spermatozoa per testis.

Leydig cells, the steroid hormone producing cells, vary in number from species to species with numerous cells found in the pig and cat (Hooker, 1970). Initially, the cells of Leydig were thought to be simply connective tissue cells containing fat and pigment. This theory was soon challenged. During the early 1900's, a number of French investigators recognized, almost simultaneously, that it was possible that the Leydig cells were the sites of elaboration of the masculinizing hormone of the testis (Hooker, 1970). The credit for originating this idea is usually given to Bouin and Ancel (1903a).

In recent years, more elaborate ideas have surfaced. For example, it has been reported that the amount of Leydig cell smooth endoplasmic reticulum, rather than the quantity and size of Leydig cells, is a better indicator of the capacity of the cell to produce testosterone (Zirkin et al., 1980). Swine Leydig cells, in contrast to other mammals, have three stages of Leydig cell development (Van Straaten et al., 1978; Franca et al., 2000). The three consecutive stages are: (1) a transient development in the fetal period that is related to development of male genital tract, (2) a transient development in the perinatal period, and (3) a final development from puberty to adulthood that is responsible for the development of male secondary sex characteristics and for the functional maintenance of organs and structures that are androgen-dependent such as accessory sexual glands and spermatogenesis (Dierichs et al., 1973; Van Straaten

et al., 1978; Franca et al., 2005). The transient phases involve proliferation and hypertrophy producing a large number of well-differentiated Leydig cells followed by regression and dedifferentiation of the Leydig cells (Lunstra et al., 1986). What occurs in the final phase, with regard to the ultra structural and functional changes that occur in Leydig cells, have not been well defined in the pig (Lunstra et al., 1986).

Leydig cells in pigs are fully differentiated at birth (Franca et al., 2005). This may explain why hypothyroidism does not affect pigs in the same way it does rodents. For rodents, transient neonatal hypothyroidism promotes proliferation of Leydig cell precursors (Mendis-Handagama et al., 2001). Hess et al. (1993) reported that hypothyroidism in mice and rats delays Sertoli cell proliferation and therefore causes increased testis size. This is not the case for swine. Klobucar et al. (2003) reported finding no significant effects on testis development after piglets were treated with 0.1% 4-propyl-2-thiouracil in order to induce hypothyroidism. Because no effect on either Sertoli cell maturation or testicular size was observed in boars as reported in rodents, it is suggested that different mechanisms regulate postnatal development of Sertoli cells in swine (Klobucar et al., 2003). The general regulators of Leydig cell proliferation and differentiation, other than LH, include TGFα, TGFβ, PDGF-A, IGF-1, thyroid hormones, androgens, estrogens, anti-Mullerian hormone, and macrophages (Mendis-Handagama et al., 2001). Luteinizing hormone (LH) is recognized to be the factor that causes Leydig cells to produce testosterone. These factors that regulate Leydig cell proliferation promote the following changes in pigs during different periods of testis development: the volume of the individual Leydig cells increase, the number of LH-receptors per Leydig

cell increase, and the amount plasma testosterone also increases (Van Straaten et al., 1978; Lunstra et al., 1986; Franca et al., 2000).

Unlike Sertoli cells, fetal germ cells undergo continuous proliferation, although at a different growth rate, after birth in pigs and are probably insensitive to gonadotropins (Van Vorstenbosch et al., 1987). Because Sertoli cells do not proliferate after puberty, the proliferation of germ cells is largely responsible for seminiferous tubule growth, both in diameter and length, that occurs after this period (Franca et al., 2000). This study of cell proliferation in the postnatal development of the testis in the pig demonstrated how Sertoli cells, Leydig cells, and germ cells proliferated actively during prepubertal development with their total number per testis being significantly correlated. Because of the dependence upon all of the cell types for normal male development, the balance between Sertoli and Leydig cells, in addition to the communication among different testicular cell types, is critical to allow the testis to fulfill both its endocrine and exocrine functions (Franca et al., 2000). Franca et al. (2005) stated that during testiscular development in pigs, the positive correlation between the total number of Sertoli, Leydig, and germ cells per testis, as well as between plasma FSH and testosterone levels, shows that there is well-balanced paracrine regulation of this reproductive organ.

Spermatogenesis

Spermatogenesis is the culmination of all cellular transformations in developing germ cells that occur in the seminiferous epithelium. The process of spermatogenesis takes place in the seminiferous tubule. The first stage of spermatogenesis is referred to as

spermatocytogenesis and is considered the "multiplication stage" due to the mitotic divisions that occur (Roosen-Runge, 1977). During this stage, the cells are known as spermatogonia. The stage that follows spermatocytogenesis is the "meiotic stage." This stage involves primary and secondary spermatocytes, which give rise to spermatids. The essential event of the "meiotic stage" is the reduction of the diploid set of chromosomes to the haploid complement (Roosen-Runge, 1977). Finally, after meiosis, the last stage of spermatogenesis is spermiogenesis. At this point, spermatids evolve into fully differentiated spermatozoa with species-specific features.

The testis parenchyma in boars is divided into two main compartments known as the tubular compartment and the interstitial compartment. The tubular compartment is composed of the tunica propria, constituted by peritubular myoid cells and basal lamina; the seminiferous epithelium with Sertoli and germ cells at different stages of development; and the tubular lumen. The interstitial or intertubular compartment contains blood vessels, connective tissue cells and fibers, lymphatic vessels, nerves, and a large number of Leydig cells (Franca et al., 2005). The process of spermatogenesis occurs in the seminiferous epithelium. The seminiferous epithelium and the basement membrane make up the seminiferous tubule. Two regions can further detail the seminiferous epithelium and they are called the basal compartment and the adluminal compartment. The names for each compartment are derived from their relative positions. Basal denotes the position of the compartment because it is located just above the basement membrane of the seminiferous tubule. The adluminal compartment lies adjacent to the lumen of the seminiferous tubule. Sertoli cell junctional complexes separate the compartments.

Mammalian spermatozoa undergo morphological, biochemical, and physiological modification first in the testis and later in the epididymis (Toshimori, 2003). The spermatogenic process in swine consists of three distinct phases: spermatogonial (proliferative or mitotic), spermatocytary (meiotic), and spermiogenic (differentiation) (Russell et al., 1990). As previously summarized, the gonocytes multiply and give rise to spermatogonia. These diploid cells are located in the basal compartment of the seminiferous epithelium. During spermatocytogenesis, the spermatogonia undergo several mitotic divisions. Pigs have strikingly similar spermatogenesis characteristics to that of the mouse. Frankenhuis et al. (1982) found, after studying whole mounts of spermatogonia, that four classes of spermatogonia could be distinguished and that boar spermatozoa is quite similar to that of small laboratory rodents. Both rodents and swine have four classes of spermatogonia: undifferentiated type A spermatogonia or stem cells, differentiated type A, intermediate spermatogonia, and type B spermatogonia (Frankenhuis et al., 1982). The A-type spermatogonia are large cells with an ovoid nucleus and, in general, the chromatin is homogenous and dusklike in all mammals (Courot et al., 1970). The nuclei of the intermediate and B-type spermatogonia decrease in size as they undergo divisions, at the same time taking a spherical shape (Courot et al., 1970). Type B spermatogonia display the largest amount of visible chromatin (Frankenhuis et al., 1982). During spermatocytogenesis, mitotic divisions cause the progression of spermatogonia from stem cells, to differentiated type A spermatogonia, to intermediate spermatogonia, and finally to result in primary spermatocytes. A group or pool of stem cells is kept so that the process is continual. The spermatocytary or meiotic phase immediately follows the mitotic divisions and the primary spermatocytes are the

cells that will undergo meiosis. The primary spermatocyte undergoes meiosis, which results in two diploid secondary spermatocytes eventually forming four haploid spermatids (Mann, 1964). Without depicting each detail of the meiotic process, it is important to remember that primary spermatocytes immediately enter the first meiotic prophase. The five stages that make up the meiotic prophase are preleptotene, leptotene, zygotene, pachytene, and diplotene. The chromosomal changes during meiosis are intricate and variable, but they are essential in order to achieve the haploid condition (Roosen-Runge, 1977).

The synthesis of DNA occurs during the preleptotene stage and the beginnning of the leptotene stage. However, the synthesis ends just before spiralization (Courot, 1970). The leptotene stage ends with a phase of contraction and maximum spiralization of the chromosomes. Chromosomes pair off and become thicker during the zygotene stage and this stage generally ends when the meiotic divisions of the previous generation of spermatocytes take place. The pachytene stage is where each chromosome splits lengthwise to form two chromatids, but still adhere to each other. In addition, the process of "crossing over" occurs and, because of this, hereditary material is redistributed in the four chromatids (Roosen-Runge, 1977). Maximum size of the nucleus, dispersion of the chromosomes, and formation of tetrads between analogous chromosomes characterize the diplotene stage (Courot, 1970). The splits between chromosomes become visible during this stage and the chromatids contract and thicken more and thereby become ready for the actual divisions in the stage of diakinesis (Roosen-Runge, 1977).

It is important to note that the prophase of the first meiotic division insures that each spermatid will be genetically different. This is why this part of spermatogenesis is

so crucial. Also, prophase of the first meiotic division is quite long making the lifespan of the primary spermatocyte the longest of all germ cell types found in the seminiferous epithelium (Senger, 1999). The lifespan of the secondary spermatocyte is much shorter. This shortened lifespan is due to the rapid progression of the second meiotic division. The end result is the production of haploid spherical spermatids.

After meiosis, spermiogenesis, the last stage of spermatogenesis, occurs. Spermiogenesis is accepted to be the sub-category during which spermatids undergo species-specific morphological transformations. The spermatids become highly specialized spermatozoa by enduring the Golgi phase, the cap phase, the acrosomal phase, and the maturation phase. These four phases constitute the basis of spermiogenesis.

The Golgi phase essentially equals acrosomic vesicle formation. During this stage, the pro-acrosomic granules arising in the Golgi apparatus, located above the nucleus, fuse to form the pro-acrosome (Mann, 1964). In addition, during the last half of the Golgi phase, the centrioles move to a position opposite the acrosomic vesicle. Later, the proximal centriole will give rise to the attachment point for the tail and the distal centriole will give rise to the developing axoneme, central portion of a flagellum, inside the cytoplasm of the spermatid.

The cap phase is so named because of the acrosome forming a “cap” over the anterior portion of the nucleus. This occurs by the pro-acrosome separating from the Golgi apparatus and assuming the “cap” shape over the nucleus (Mann, 1964). The Golgi apparatus, following separation, migrates toward the opposite pole of the cell and the distal centriole forms the flagellum (Courot et al., 1970 and Senger, 1999).

The next stage of development is the acrosomal phase. This phase involves the transformation of the pro-acrosome into the acrosome proper, with the nucleus becoming condensed and beginning to elongate, and the bulk of the cytoplasm migrating posteriorly towards the region of the future middle-piece and tail (Mann, 1964). The acrosome is increasingly covering the nucleus as it undergoes elongation. Also, the manchette forms around the posterior end of the nucleus and extends towards the developing flagellum. During the acrosomal phase, the nucleus and acrosome become oriented toward the nucleus of the Sertoli cells with tails pointing towards the lumen of the seminiferous tubule (Courot, 1970 and Senger, 1999).

The final transformation of the spermatids is known as the maturation phase. This is the final stop before being released into the lumen of the seminiferous epithelium. Mitochondria form the middle piece; the fibrous sheath is produced; the post nuclear cap is formed; and the annulus forms the juncture between the middle piece and principal piece during this phase. Following these last modifications, the functionally immature spermatozoa are released from the Sertoli cells into the lumen of the seminiferous epithelium (Mann, 1964). This process is also known as spermiation.

Maturational modifications are necessary for spermatozoa to become fertilization-competent cells and to be safely stored in the male (Toshimori, 2003). It is important to remember that spermatozoa acquire species-specific characteristics. The duration of spermatogenesis, based on 4.5 spermatogenic cycles, for mammals is approximately 30-75 days (Russell et al., 1990; Sharpe, 1994). Although breed or strain may have an influence on the length of time it takes to complete spermatogenesis, it is generally accepted that the length of spermatogenesis cycle is constant for a given species. For

boars, the total duration of spermatogenesis is around 40 days with each spermatogenic cycle lasting 8.6-9.0 days (Swierstra, 1968; Franca et al., 1998).

Sperm Maturation

It is obvious that complex biochemical, physiological, and morphological events of cellular differentiation occur in the testis during spermatogenesis, but additional post gonad differentiation is required to be able to fertilize eggs (Dacheux et al., 2003). Some of the events during spermatogenesis in the testis are controlled by the genomic regulation of the gamete. The final stages of sperm differentiation outside the gonad are not under the genomic control of germ cells (Dacheux et al., 2003). This last stage of maturation is thereby largely dependent upon the consequences of the spermatozoa's interaction with the epididymal fluid, especially the proteins present in the lumen of the epididymal tubule (Dacheux et al., 2005). Franca et al. (2005) stated that the final stages of sperm differentiation outside the gonad are believed to require sequential interactions with the medium surrounding the sperm during its transit through the epididymis.

Once spermatogenesis is complete and spermiation has occurred, most of cytoplasm and cellular organelles are removed from the nascent spermatozoa and phagocytosed by Sertoli cells (Toshimori, 2003). From the testis, spermatozoa move into the epididymis. Epididymal maturation occurs as the spermatozoa move through the three functionally distinct regions. The three regions encountered are the caput, corpus, and cauda. As the spermatozoa enter the epididymis, they are considered immature and immobile, but as they exit the distal epididymis, during ejaculation, they are motile and

mature. The spermatozoa defined as motile and mature are said to have forward movement and the ability to fertilize (zona pellucida recognition and acrosome reactivity) (Lakoski et al., 1988). Due to the obvious importance of epididymal maturation, the epididymis can be considered critical for normal reproduction of mammals because sperm leaving the testis, before epididymal maturation, are not capable of fertilizing an oocyte (Franca et al., 2005).

The epididymal duct is derived from the mesonephric duct and is extensive in length and greatly coiled. The epididymal duct of a boar is 50-100m long in contrast to that of a ram, which is approximately 50m in length (Franca et al., 2005). As previously indicated, the organ is divided into three sections known as the caput, corpus, and cauda. Franca et al. (2005) also suggests that these three sections can probably be further sub-divided into more segments based on function and species. He gives the example of the boar. Franca et al. (2005) states that the boar's caput could be subdivided into four segments, the corpus into three, and the cauda into two, but no more elaboration on the segments followed.

The epididymal duct is lined by epithelium that consists of principal and basal cells. Other cells that are present in the duct on a segment-specific manner include apical, narrow, clear, and halo cells. The combination of these cells produces the required functions for epididymal activity and function. The epididymal functions include the following: principal cells are responsible for protein secretion and absorption; the clear cells are responsible for endocytosis; clear cells and narrow cells for secretory activities responsible for acidification of the luminal fluid; and basal cells for immune defense, phagocytosis, and the production of antioxidants (Hermo et al., 1994).

In addition, principal cells present tight junctions that are largely responsible for the epididymal duct barrier. In summary of the basic functions of the epididymis, it provides a specialized microenvironment responsible for spermatozoa transport, maturation, and storage.

Sperm transit time through the epididymis is different in mammals depending on their species. For pigs, transit time for the caput and corpus is around 5.4-7.0 days, for the cauda around 6.4 days, and, therefore, total length of time to move through the entire epididymis is approximately 9.0-11.8 days (Franca et al., 2005). Briz et al. (1999) reported that one study found slightly different approximate time ranges for maturation. Singh (1962) and Swiersta (1968) used autoradiographic methods to measure the length of time necessary for boar sperm to travel through the epidiymis. They reported findings of three days travel time through the caput, two days for the corpus, and four to nine days for the cauda (Briz et al., 1995). The intervals of epididymal maturation are varied depending on species. The mean transit time through the caput and corpus are believed not to be influenced by ejaculation (Amann et al., 1981) or ligation of efferent ductules (Amann et al., 1974). Unlike the cauda, where contractions of smooth muscle cells surrounding the epididymal duct occur only when stimulated, sperm movement through the caput and corpus occurs mainly by continuous peristaltic contractions of these cells (Amann et al., 1983).

During almost the two weeks required for the journey through the epididymis, sperm undergo additional maturation processes in the first two regions and then are stored in the epididymal cauda until ejaculation. Other than motility and the ability to fertilize an egg, epididymal maturations alter the spermatozoon in the following ways: (1)

modifications in patterns of movement; (2) changes in the metabolic pattern and the structural condition of the specific organelles of the tail; (3) changes in the nuclear chromatin and modification in the acrosomal shape; (4) progressive loss of water and increase of specific gravity; (5) migration through the midpiece and then detachment of the cytoplasmic droplet; and (6) modifications of the plasma membrane that determine variations in their biophysical qualities (Briz et al., 1995).

Briz et al. (1995) reported the findings of their study that observed spermatozoa in the caput, corpus, and cauda regions of the epididymis in sexually mature Landrace boars. Using light microscopy, scanning electron, and transmission electron microscopy, morphological and physiological differences were demonstrated among boar sperm isolated from the three epididymal regions. The morphology was distinct depending on the region: (1) the caput has a large percentage of immature spermatozoa with proximal cytoplasmic droplets, (2) the corpus still has immature sperm, but the cytoplasmic droplet is distal, and (3) the cauda has a large percentage of mature spermatozoa (Briz et al., 1995). In this study, Briz et al. reported that the percent of immature boar spermatozoa varied from 49.2 % in the caput to 11.8% in the epididymal cauda. Other modifications listed included the mitochondria of the midpiece joining together to form the mitochondrial sheath, the changing size and shape of the acrosome, and the loss of ability to fold the tail by the midpiece. This study showed that there are characteristically different qualities from the three distinct regions of the epididymis. The authors suggested that the presence of epididymal gamete forms in the ejaculate is a sign of incomplete sperm maturation and allows researchers and workers to better understand the consequences of stress produced by high frequency semen collection (Briz et al., 1995).

Sperm Maturation After Ejaculation and Insemination

Sperm maturation is not halted at ejaculation, but instead continues in the female reproductive tract (Mann, 1964). Upon ejaculation, spermatozoa are essentially "mature," but must undergo several more changes/modifications in order to be able to penetrate an egg. C.R. Austin (1951) reported that when spermatozoa were introduced into the fallopian tube of a rabbit before ovulation, most of the eggs did not become fertilized. During that same year, after several researchers concluded that spermatozoa must remain in the female reproductive tract for a certain amount of time, Austin (1952) introduced the term "capacitation." He further defined this term and theory by stating "the sperm must undergo some form of physiological change or capacitation before it is capable of penetrating the egg."

Whether a sow is bred by natural mating or artificial insemination, the semen is deposited intra-cervically (Langendijk et al., 2005). The boar ejaculates a large (200-250mL) suspension of spermatozoa in a fluid, known as seminal plasma, composed of a mixture of contents from the epididymal ducts and secretions from the accessory sex glands (Einarsson, 1985). The ejaculate is composed of three series of fractions. Rodriguez-Martinez et al. (2005) characterize the three fractions as the following: (1) pre-sperm (mostly consists of secretions of the urethral and bulbourethral glands in addition to the prostate), (2) sperm-rich fraction (largest percentage of spermatozoa present here), and (3) post-sperm-rich fraction (few spermatozoa present and the fluid greatly consists of secretions from the seminal vesicles, prostate, and the bulbourethral

glands. The last fraction produces the floccula that coagulates the seminal plasma in order to somewhat minimize retrograde flow.

Due to the large amount of ejaculate, the bulk of the semen deposited is flushed directly into the lumen of the uterine body. Because of this, semen deposition is often regarded as intrauterine (Langendijk et al., 2005). Sperm cells must then be distributed over both horns and moved to the tubal ends of the horns. Prior to ovulation, the female establishes a functional sperm reservoir, located at the utero-tubal junction and the first part of the oviduct, where a relatively small number of spermatozoa are stored to ensure the availability of viable sperm for fertilization (Langendijk et al., 2005 and Rodriguez-Martinez et al., 2005). The rest of the spermatozoa, which is the majority, are eliminated in utero (Einarsson, 1985).

The spermatozoa that are able to make it to the sperm reservoir are transported by myometrial contractions. Langendijk et al. (2005) reviewed reports that when approaching estrus, the percentage of sows showing myometrial activity increases, and the frequency and amplitude of contractions increases also. However, during estrus, females show maximal myometrial activity. It has been suggested that the tissue and plasma levels of estrogen and progesterone determine the variation of myometrial activity (Langendijk et al., 2005). The amount of sperm transported by this myometrial activity into the sperm reservoir is dependent upon the number of spermatozoa inseminated (Rodriguez-Martinez et al., 2005). In the sperm reservoir, the spermatozoa are immersed in the tubal fluid and can contact the epithelium that covers its lumen. The sperm reservoir maintains sperm viability and fertilizing capacity (Mburu et al., 1997 and

Rodriguez-Martinez et al., 2001) in addition to helping sperm avoid phagocytosis in the uterine lumen.

Yanagimachi et al. (1994) defines sperm capacitation as a gradual, essential event during mammalian reproduction, in preparation for fertilization, which occurs during the time spermaotozoa spend in the reproductive tract of a female. Only then, once capacitated, are spermatozoa functionally ready for fertilization. The capacitated spermatozoa are now capable of the following: (1) release from the sperm reservoir (Fazeli et al., 1999); (2) penetration of the cumulus layers; and (3) binding to the zona pellucida that allows the acrosome reaction to occur (Rodriguez-Martinez et al., 2001). During the time spermatozoa are inside the female reproductive tract, decapacitation factors, such as bound proteins from the cauda epididymis and the sperm reservoir, are removed from the sperm surface, in particular over the acrosomal region. The sperm's exposed surface is then vulnerable and accessible to lipid-binding components of the female luminal fluids.

In the pig, bicarbonate appears to be the effector molecule and is able to trigger the "lipid scrambling" seen in the lipid bilayer of the plasma membrane, and is therefore considered one of the earliest signs of capacitation (Harrison et al., 1996). However, it is clear that complete capacitation does not occur in the sperm reservoir and all spermatozoa do not leave the reservoir at the same time in search of the fertilization site. The continual capacitation and release of a low number of spermatozoa ensure that there are capacitated sperm available for the extended time period that occurs between sperm deposition and ovulation in the pig (Rodriguez-Martinez et al., 2005). Once capacitated and released from the sperm reservoir, the spermatozoa become hyperactive, bind to the

zona pellucida, and undergo the acrosome reaction in order to release enzymes that help hydrolyze the zona pellucida. All of these complex events are necessary for the spermatozoa to be produced, matured, transported, and joined with an egg.

Differentiation of Sexual Behaviors in Swine

The differentiation of sexual behavior in mammals is usually referred to as either processes of defeminization or masculinization. Defeminization refers to the loss of behavioral traits that are characteristic of females. Masculinization is the acquisition of behaviors typical of males. Naftolin et al. (1984) stated that the capacity of mammalian brains is usually thought of as inherently female or undifferentiated, and sexual differentiation involves the action of testicular androgens that are transported to the brains of males and aromatized to oestrogens. The elements and changes involved in the differentiation of sexual behavior are not widely documented. The changes that occur prior to and during puberty for boars are less documented than those of gilts. For this reason, additional published data would be beneficial to the complete understanding of the differentiation of sexual behaviors in swine.

Sexual behavior in farm animals is a result of the additive effects of two actions of gonadal steroids. The two actions are referred to as organizational and activational. The early organizational effects of gonadal steroids are irreversible and cause sexual differentiation of behavior as a result of androgen and estrogen action within the brain during sensitive periods of development (Clarke, 1982; Gorski, 1985). Activation of

behavioral processes that exist within an animal is the second way the gonadal steroids affect dimorphic behavior. The expression of the behavior is dependent upon the presence or recent presence of the gonadal steroids. The activational actions that steroids have in stimulation of sexual behavior after puberty are reversible. While genetic sex is determined at conception, in mammalian species, the development of the masculine phenotype is dependent upon responses to testicular secretions during sensitive periods of development (Ford, 1990).

Again, with swine, the number of studies that pertain to the differentiation of sexual behavior are limited. The gonads of both sexes in domestic pigs are quite similar at 24 days gestation, but by 26 days gestation, the testes and ovaries are morphologically distinct. Testosterone secretion is elevated in male fetuses at 35 days gestation, but by day 40, serum testosterone concentrations decline in male fetuses yet remain greater than the levels present in the female fetuses (Ford, 1990). If the rodent and sheep model could be used and extrapolated to the pig, differentiation of sexual behavior would be expected after 40 days of gestation because defeminization is thought to occur shortly after the external genitalia in males are fully developed.

The rodent model of endocrine differences between the two sexes during late fetal development is considered in regards to swine. Nemeskeri et al. (1983) stated that, in rats, defeminization of sexual behavior occurs shortly after the appearance of immunoreactive luteinizing hormone-releasing hormone (LHRH) in neurons and nerve terminals of the median eminence. For pigs, these changes occur during the second half of gestation (Ford, 1990). Also, it was concluded by Ford (1990) that any sexually dimorphic changes that occur during the second half of gestation are likely caused by

testosterone concentrations in male fetuses and not related to fetal blood estrogen concentrations. This is due to the evidence that the placentae of sows synthesize and secrete considerable quantities of estrogen, but no differences were observed between male and female fetuses (Knight et al., 1977 and Ponzilius et al., 1986). Ford (1990) summarizes that males experience elevated testosterone secretion around day 35 of gestation, during the first month of life, and during and after pubertal development. However, no evidence was found to conclude that the first two periods of androgen exposure have anything to do with differentiation of sexual behaviors.

The behaviors noted in pigs are sexually dimorphic by one month of age. Mounting is greatest at two months of age, but is observed more frequently in males than females (Berry et al., 1984). Most of this mounting behavior in males results from activational actions of testicular secretions. If castrated at birth, mounting behavior is decreased to that similar of a female, but if not castrated until after 30 days of age, the mounting activity will be more comparable to that of an intact boar. Social play is also observed more frequently in two-month-old males than females (Berry et al., 1984). Pushing, biting, or mounting that persists for at least five seconds characterizes this type of social play. In addition, as boars mature during the prepubertal period, three to five months of age, the mounting expands to estrous females and they also allow older, mature boars to mount them (Signoret et al., 1989).

There is evidence that defeminization of sexual behaviors is associated with pubertal development in male pigs. This is due to studies that have shown a lack of defeminization in females that are exposed to exogenous testosterone prenatally (Ford et al., 1987). Proceptivity and receptivity are generally characteristic of females and

lacking in males. Ford et al. (1987) and Berry et al. (1984) conducted research to determine when defeminization of receptivity occurred. Using males castrated at different ages from birth to eight months of age, the proportion that displayed sexual receptivity after acute estrogen treatment decreased as age at castration increased (Ford et al, 1987 and Berry et al., 1984). It was then concluded that the duration of receptivity was shortened for those males castrated between two and four months of age and then, for males castrated between four and six months of age, there was a decrease in the proportion that became receptive. Ford also (1983) conducted an experiment to determine when defeminization of proceptivity occurs. After castrated males were treated with acute estrogen, it was found that the amount of time spent near a mature boar was greater for neonatally castrated males than for males castrated during pubertal development (Ford, 1983). From these findings, it seems that defeminization of proceptivity occurs sooner in development than defeminization of receptivity. It appears that the primary sensitive period for differentiation of proceptivity and receptivity coincides with early pubertal development in boars (Ford, 1990). In addition, there is a pubertal rise in testicular steroid secretion that begins by three months of age and continues through six months of age. This postnatal period of elevated testicular steroid secretions is associated with the defeminization of sexual behavior in boars (Ford and D'occhio, 1989).

The masculinization of sexual behavior, especially in regards to swine, is not clearly documented. However, it is documented that when mature female pigs are given lengthy testosterone treatments, they do develop mounting behaviors similar to males (Scheffrahn et al., 1981). The behaviors documented include side-nudging towards

estrous females, mounting, remaining mounted for extended periods of time, and, many times, pelvic thrusting. Despite these conclusions, the question still remains if both sexes are identically sensitive to testosterone or are males more sensitive to testosterone and will they show sexual behavior more rapidly or at a lower dose of testosterone than females?

Rodents and sheep have the most definitive studies available associated with puberty. However, in pigs, it is not reliable to use these models to explain differentiation of sexual behavior. This unreliability is due to the evidence in swine that shows significant modifications in sexual behavior around the same time as pubertal development. Because development of the masculine phenotype is dependent on the responses to testicular secretions during sensitive periods of development, Goy and McEwen (1980) found that these sensitive periods usually occur during late gestation and early postnatal development in species with short gestations and prenatally in species with longer gestations. As previously discussed, pigs are the exception to these findings and further research is needed.

Semen Production and Associated Factors

Artificial insemination in swine has increased more than three-fold over the past 17 years and in many countries in Europe more than 90% of the females are bred by AI and in North America the figure for AI has already surpasses 70% (Rodriguez-Martinez et al., 2005). Boars that consistently produce large amounts of fertile semen are a primary concern for many producers because an individual boar has a great impact on the

efficiency of the breeding herd. A sow generally produces two litters per year while a boar can sire, literally, thousands of piglets per year. Based on the recognition of boar importance, factors that positively or negatively influence the number of spermatozoa produced and the overall quality of semen need to be considered.

Standard tests currently used to evaluate semen quality in the boar include sperm motility, morphology, and concentration. The ability of such tests to determine actual fertility is limited, but these tests do allow for evaluators to determine if a particular boar has poor quality ejaculates. There are several criteria for fresh boar semen that are generally accepted and implemented when breeding. The criteria include: (1) greater than or equal to 70% gross motility (unextended); (2) total sperm numbers must exceed 15 billion sperm per ejaculate; (3) less than or equal to 20% abnormal morphology; (4) no more than 15% cytoplasmic droplets (proximal and distal); and (5) the ejaculate should be gray-white to white in color and have a milky consistency (Althouse, 1997). In order to optimize the efficiency in the breeding herd, it is necessary to evaluate semen quality. However, as previously mentioned, these tests are very limited in the accuracy of predicting fertility.

There are a number of biological and environmental factors that influence the total number of spermatozoa. Some factors contribute positively while others are detrimental and have a negative impact on the total number of sperm. For example, malnutrition and heat stress are both inhibitory factors for spermatogenesis. A producer's interest lies in wanting to omit all detrimental processes from affecting the breeding herd and implementing all strategies, in an economical way, that are stimulatory in hopes of maximizing spermatogenesis.

There is little doubt that semen quality consists of a genetic component, an environmental component, and a variety of interactions between the two (Foote, 1978). It is thought that the heritability of fertility is low and the environmental components will overshadow any genetic components. There are many conditions linked to inheritance. Cryptorchidism, hermaphroditism, hypoplasia (not always hereditary), variation in size of testes, defective sperm formation, and tumor susceptibility are all thought to be inherited (Foote, 1978).

The size of the testes varies considerably from species to species and even individual to individual. It is one of the most obvious indicators of the amount of spermatozoa that a particular boar will be able to produce. As reviewed by Franca et al. (2005), the size of the testes and the number of spermatozoa are directly dependent on the number of Sertoli cells per testis. In other words, the larger the testes, the greater number of spermatozoa that can be produced. Rathje et al. (1995) reported the results of their study that involved evaluating sperm production in boars after nine generations of selection for increased weight of testes. They found that if one selects for greater predicted weight of paired testes, results will include increased body weight at younger ages, increased daily sperm cell production at all ages, and greater numbers of spermatozoa stored in the cauda epididymis of young boars (Rathje et al., 1995).

Johnson et al. (1994), after experimenting with selection for increased testes weight and its effects on body weight, back fat, age at puberty, and ovulation rate, stated that, indeed, testicular weight/size is highly heritable and is positively correlated with increases in body weight and back fat. However, the correlated responses for ovulation rate and age at puberty were small and insignificant. Therefore, the authors concluded

that while selection for increased testicular size does increase body weight and back fat, it is not recommended to use testis weight as an indicator trait to select for female reproductive traits in swine (Johnson et al., 1994).

Huang and Johnson (1996) also tested the theory that increased testicular size is correlated with daily sperm production and total sperm reserves. Using methods similar to those of previous studies, boars for this experiment were from the 10^{th} and 11^{th} generations of lines that were selected for increased size of testes. The benefits of this particular study were that the researchers looked specifically at the impacts on volume, motility, abnormalities, and concentrations of spermatozoa produced. No effects were observed for volume, motility, and percentage of abnormal spermatozoa between the control and treatment groups. The major findings included that the concentration and number of sperm in the semen were higher for those subjects in the treatment group. Therefore, Huang and Johnson (1996) concluded that selecting for increased testes size is an effective method to increase concentration of sperm in the semen and total number of sperm per ejaculate. This method of selection would be beneficial to producers, especially those implementing the use of artificial insemination.

Schnickel et al. (1984) looked at the testicular development and endocrine characteristics of boars selected for either high or low testiscular size. As with other studies pertaining to the impacts of large testiscular size, this study found that boars with high testiscular weight had a higher percentage of seminiferous tubules in which spermatogenesis was present, a larger percentage of tubules with a lumen, and tubules had a larger mean diameter when compared to boars with low testiscular weight. This study also presented some endocrine findings. The major results included: (1) boars with

high testiscular weight had higher maximum concentrations of LH during the pubertal rise and they tended to reach these maximums at younger ages; (2) concentrations of testosterone tended to be higher for boars with high testiscular weight; and (3) the high testiscular weight boars had a more rapid increase of estradiol-17β than the boars with low testiscular weight (Schinckel et al., 1984). However, when the two groups, high testiscular weight and low testiscular weight, were adjusted to a constant testiscular size, there were no significant differences between the concentrations of testosterone and estradiol-17β for both groups. Furthermore, the authors concluded that genetic changes in the size of the testiscles are associated with changes in LH concentrations.

It is obvious that selecting for certain traits, such as testiscular size, can greatly influence spermatozoa production, but environmental conditions may have a heavier impact on spermatozoa production due to its variability and unpredictability. Impacts from malnutrition and season can be detrimental to sperm production and issues like season are difficult for producers to avoid. However, if the negative factors can be avoided, there may be environmental components that could be implemented to influence better sperm production.

Research has been conducted to observe the effects of nutritional status during the first 28 days postnatally on subsequent growth and reproductive performance of swine (Martin and Crenshaw, 1989). The nutritional status was altered by adjusting litter size at birth to either six or twelve pigs and maintaining lactation length for either 13 or 28 days. Differences in ovulation rate were detected, but due to prenatal losses, the number of offspring produced was similar among treatment groups. Therefore, subsequent litter size was not affected by postnatal litter size, lactation length, or feed restriction (Martin

and Crenshaw, 1989). More research was implied to ultimately determine what effects postnatal nutritional status will have on growth and reproductive performance in gilts. Similar studies in boars would be beneficial to determine if there are any positive correlations between decreased litter size and postnatal nutritional status and their growth and reproductive rates.

Another plausible factor for semen quality is season. Sancho et al. (2004) studied the effects of photoperiod during increasing day length and during decreasing day length on the semen quality of boars. The length of day was independent, in this study, of temperature. Twenty eight month old Landrace boars were randomly distributed into two groups. Once group denoted the "spring boars" were exposed to increasing day length from February 3, 2000 to April 17, 2000 and the other group, the "fall boars," were exposed to decreasing day length from August 14, 2000 to November 1, 2000. The temperature was maintained at an average of 21 ± 1° C and 60 – 75% humidity. Ejaculate volumes remained unaffected by photoperiod. However, sperm concentration, sperm production, and the number of sperm doses per ejaculate were 50% lower in boars under decreasing photoperiod when compared to boars under increasing photoperiods (Sancho et al., 2004). In both photoperiods, sperm motility and vitality maintained normal values. Trudeau and Sanford (1986) further investigated the effects of season on semen quality. They found that, even before extreme temperature fluctuation, photoperiod does affect semen quality. However, it is important to note, that excessively high ambient temperature is followed by decreases in sperm concentration, motility, and increases the number of abnormal sperm in the ejaculate, usually four to six weeks later (McNitt and First, 1970).

Genetic and environmental components both have impacts on the total number of spermatozoa produced and the overall semen quality. It seems a daunting task for producers to effectively eliminate all of the factors that may negatively impact sperm production. However, if strategies are applied that maximize the sperm production potential of an individual boar, then certain factors that are uncontrollable, e.g. season, may not cause severe decrease in the breeding herd's efficiency.

General Conclusions

Testicular size has a positive correlation with the number of spermatozoa produced. Selection for larger testicles has been found to lead to greater spermatozoa concentration and total number. The number of Sertoli cells per testis determines the amount of spermatozoa that can be produced. During the first month following birth, the cells in the testicles and secondary sex glands experience a rapid increase in numbers and size. This may be an impressionable period of time in which management strategies could have a positive result. If litter size were manipulated such that boars are nursed in smaller litters, the additional nutrition received would coincide with this key developmental period. Similar studies for gilts have shown that when nursed in small litters (n=6), the gilts produced 2.4 more ova than gilts reared in larger litters (n=12) (Martin and Crenshaw, 1989). More research is needed to determine if nursing in smaller litters would have positive influences on the reproductive function, in particular semen quality and quantity, of boars.

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INCREASING SPERM PRODUCTION IN MATURE BOARS VIA MANIPULATION OF THEIR NEONATAL ENVIRONMENT

Introduction

Boar studs are required to produce a specified number of insemination doses each day. Therefore, consistent production of large quantities of fertile semen is a primary concern in boar management for producers. Management strategies to increase spermatozoa per ejaculate have been implemented in the past, but have focused on adult boars and have not been very successful (Flowers, 1997). Boars undergo a period of rapid sexual development during the first three weeks after birth. During this time, the cells in the testicles and secondary sex glands experience a rapid increase in numbers and size. Subsequently, if boars are able to receive additional nutrition during lactation, which coincides with this important developmental period, by nursing in smaller litters, then it may be possible to influence their sperm production and stud performance as an adult. Thus, the objective of this study was to determine if litter size is associated with any positive increases in reproductive function, especially semen quality, of adult boars. The assessment of reproductive function included testicular size, semen concentration and quality, and libido.

Materials and Methods

Animals

Boars used in the study were offspring of Yorkshire x Landrace x Large White sows bred to Hampshire x Duroc x Pietran boars such that offspring were expected to exhibit 100% heterosis. Two groups of sows were used to produce the experimental animals. Sows bred in June 2003 and farrowed on October 10, 2003 produced the fall replicate of boars (n=18). Sows bred in November 2003 and farrowed on March 11, 2004 produced the spring replicate of boars (n=18). Four unrelated sires produced boars in the fall replicate. Three different and unrelated sires produced boars in the spring replicate. All animals used tested negative for porcine reproductive and respiratory syndrome, mycoplasma hyopneumonia, parvovirus, leptospirosis, hemophilus parasuis, streptococcus suis, swine influenza, and transmissible gastroenteritis.

Facilities

The study was conducted at the North Carolina State Swine Educational Unit, a farrow to finish operation, located in Raleigh, North Carolina. Sows were moved into a farrowing house with a side-wall baffle ventilation system approximately 4 to 7 days prior to their estimated farrowing date. The farrowing crate was a 1.5 m x 2.5 m bow-bar crate. There was an airplane slat flooring pattern with cement slats underneath the sow; TriBar®, an expanded metal, behind the sows; and Tenderfoot®, a plastic-coated wire, in

the piglet area. Two heat lamps were placed, at varying heights, in each crate above the piglet area as additional heat sources for the piglets. While in the farrowing house the sows were fed a corn and soybean diet ad libitum twice daily.

Experimental Design

Boars in the fall and spring replicates were divided into two treatments. Treatment one was defined as small litter size and consisted of 6. The second treatment was defined to be the large litter size and consisted of 10 or more piglets. One day after birth, boars were randomly crossfostered into either treatment one or treatment two litters. The fall replicate of eighteen boars was crossfostered into five litters, which included three litters of 6 and two litters of 10 or more. Approximately equal numbers of gilts and boars per litter were maintained based on sex ratios at birth. The boars were crossfostered such that each sow was nursing multiple genotypes. For example, if a particular sow's litter contained five boars, then four boars were crossfostered out to other sows and four boars were crossfostered into that sow's litter. Therefore, the sow would be nursing four different genotypes in addition to her own. The spring replicate of eighteen boars was crossfostered into three litters of six and two litters of ten or more. The same method for crossfostering was also applied to the spring replicate. Once the treatments were established, the boars remained in the farrowing house for approximately three weeks. During this time, piglets in the spring replicate became sick with a scours-like illness for approximately 4 to 5 days. All piglets were treated with gentamyacin. Two boars died because of illness related complications and one boar was removed from the study because of falling behind others in terms of body weight and condition. Body

weight and testicle size (height and width) were measured and recorded weekly, while boars were nursing their sows.

All boars were processed within 48 hours of birth. Processing included ear notching for permanent identification, tail docking, and needle teeth clipping, but did not include castration. Boars were also vaccinated for leptospirosis (9-way) and parvovirus. During these three weeks, nursing observations were made twice a week for a two-hour period each time. The boars were marked with different color All-Weather Paintstick® livestock markers for identification and then allowed to nurse freely. When nursing began, teat number, teat location (top or bottom), and nursing duration were recorded for each boar. A standard numbering system was established for all sows. Teat 1 was located most cranial with teat 7 being the most caudal. The sow's position was also recorded in order to differentiate between the right and left side teats (top or bottom).

Both replicates were weaned at 3 weeks of age (October 30, 2003 and April 1, 2004). At the time of weaning, weight and testicle size was recorded. Boars were housed together in a nursery room with a side-wall baffle ventilation system with 6 pens on either side of a central hallway. Each pen was 1.82 x 1.82 m with 0.91 m of feeder space and 4 nipple waterers. Boars were fed a standard 23% protein starter diet consisting of milk by products for 7-10 days. After that, they received corn and soybean meal based diets with the protein level gradually being decreased to 18%. Upon entering the nursery, the boars were divided into two pens. The boars were randomly assigned to the two pens so that individuals from both treatments were housed together. The pigs remained in the nursery for six weeks. During that time, an every third week regimen of weight and testicle measurements was established and carried out through the study.

At 9 weeks of age, the boars were moved to the finishing floor. From 9 to 23 weeks of age, boars were housed in a curtain-sided environmentally controlled building with totally slatted floors and an underslat ventilation system. Misters and cooling fans mounted from the ceiling that were programmed to activate when the ambient temperature in the barn reached 25.5 °C provided supplemental cooling for the boars during the summer months. Boars were kept in 1.84 by 2.84 m pens. The fall replicate was divided into six pens of three and the spring replicate was divided into four pens of three and two pens of two. Once again, the pigs were randomly assigned to ensure that the two treatments were well dispersed. Each pen had a two-hole feeder with a total of 0.91 m of feeder space and 2 nipple waterers. The feeder was mounted on either the east or west side of the pen and the nipple waterers were located in either the southwest or northwest corner of each pen. Boars were fed a corn-soybean meal-based diet ad libitum that was formulated to meet the NRC requirements for growing boars (NRC, 1998). Crude protein in the diet was adjusted according to the age of the boars in the following manner: 9 to 12 weeks – 18%; 12 to 18 weeks – 16%; and 18 to 23 weeks – 14%. Weight and testicle measurements continued.

The boars were moved into individual crates in the breeding barn at 23 weeks of age. The barn was a curtain-sided building with underslat ventilation. The crates were 2.43 x 1 m. Misters and cooling fans mounted from the ceiling were set to activate when the ambient temperature reached 25.5º C. Boars received 2.7 kg of a 14 % corn and soybean meal diet formulated to meet NRC requirements for boars. At this time or approximately 23 weeks of age, the boars were vaccinated for erisyphelas.

At 24 weeks of age boars were trained for semen collection using a dummy sow. The collection pen was 2.43 x 3.65 m. The collection dummy (Minitube of America, Inc., Verona, WI) was 0.3 m wide and 1.21 m long. The height of the collection dummy was adjustable and was kept at 0.76 m during the training period. The collection dummy was located in the center of the pen such that boars could move freely on either side and behind the dummy sow, but not in front of it. All the naïve boars were housed in stalls located across a l m alley from the collection pen. Consequently, all boars could observe other boars being trained. Boars were removed from their stalls each day and moved into the collection pen. Duration of each training period was 5 minutes. There were two exceptions to this strategy. The first occurred when the boar was actively interacting with the dummy after the initial 5-minute period had expired. In this situation, the training period was continued until the boar failed to make contact with the dummy for 1 minute. The second occurred when the boar mounted and was collected before the 5-minute period had expired. In this situation, the boar was removed from the pen after being successfully collected. At the end of each training session, each boar's behavior was given a numerical score according to the following guidelines:

0 - No interest or contact with the dummy during the training session
1 - Sniffs and rubs against the dummy but does not chew on, bite, or charge it
2 - Aggressively interacts with the dummy – bites, chews on, and charges it
3 - Mounts the dummy, but does not extend penis
4 - Mounts the dummy, extends penis, but does not collect
5 - Mounts the dummy, extends penis and is collected

After the first successful collection, boars were collected each day for two additional days. After a three-day rest period, boars were collected on a weekly basis.

After 4 weeks or when boars were 29 weeks of age, individuals not trained to collect were first placed in a pen adjacent to the collection pen and allowed to observe and interact with trained boars during the collection process. Immediately after observing a trained boar, they were moved into the collection pen for their 5 minute training session and managed as described previously. After 2 additional weeks or when the boars were 31 weeks of age, individuals not trained to collect were first placed in a pen adjacent to the collection pen containing a gilt and allowed to interact with the gilt for 2 minutes. After interaction with the gilt, boars were moved to the collection pen for their 5 minute training session and managed as described previously. The gilt remained in the pen adjacent to the collection pen during the training session. After 2 additional weeks or when the boars were 33 weeks of age, individuals not trained to collect were considered to be failures and removed from the study.

At approximately 40 weeks of age, eight boars from the fall replicate were selected based on body weight, testicular size, and testicular size to body weight ratio to continue through the duration of the study. Boars were ranked from highest to lowest in terms of kilograms of body weight, size of testes (height x width), and the ratio of testicular size to body weight. Motility and morphology were also considered in the process. A minimum of 70% normal motility and morphology was used as a baseline. The two boars with the highest and the two boars with the lowest testicular size to body weight ratio were kept for each treatment; provided motility and morphology were acceptable. The ratios for the boars selected were between 6.62 and 10.51 % for the highest testicular size to body weight ratios and between 5.71 and 6.43 % for the lowest testicular size to body weight ratios.

Weight and Testicle Data

Weights were recorded weekly for the first three weeks following birth and every third week thereafter. Each boar was weighed individually and the weights recorded in kilograms. A rolling crate scale was used once the boars entered the finishing floor.

Testicle size was determined using manual calipers until 17 weeks of age. At this point, a standard measuring tape was used for all remaining measurements. The same technician performed all testicle measurements during the study and the testicle measurements were recorded in centimeters. Testicular height was taken by measuring from the top of the right testicle to the bottom. Width was determined by measuring across both testicles from left to right. The product of testicular height and width was divided by the body weight to determine the ratio of testicle size to body weight.

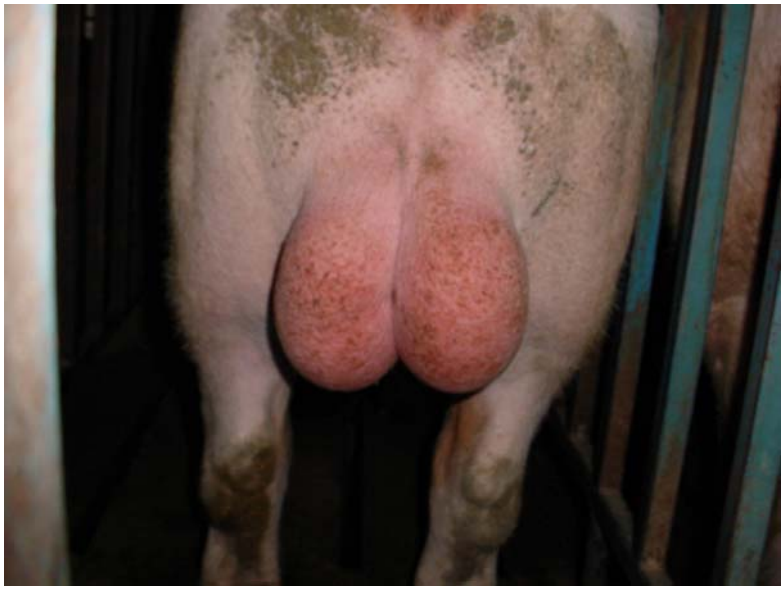

Figure 1. Picture of boar testicles while housed in the breeding barn.

Semen Collection

Semen was collected from each boar weekly by the gloved-hand technique (Almond et al., 1998) into a plastic semen collection bag (Minitube of America, Verona, WI). The collection bag was positioned inside a thermos preincubated at 37ºC. The top of thermos was covered with a piece of cheese cloth. After collection, the cheese cloth was removed and the thermos was weighed. The pre-collection weight was then subtracted. The semen volume was recorded and the collection bag properly identified and then placed into a water bath maintained at 37ºC. Samples remained in the water bath until the collection of all other boars was complete and samples were transported back to the laboratory.

Once all boars were collected, the samples were placed into a Styrofoam cooler for transport back to the laboratory which is approximately 7 miles from the site of collection (North Carolina State University, Raleigh, NC). The semen was placed directly into another 37ºC water bath upon reaching the laboratory. Beltsville Thawing Solution (BTS) was prepared the morning of collection and warmed to 37ºC. The fresh BTS consisted of 37.0 g/l anhydrous glucose, 6.0 g/l sodium citrate, 1.25 g/l sodium bicarbonate, 1.25 g/l EDTA/disodium, and .75 g/l KCl. The pH ranged between 7.2 – 7.4 with the osmolality equal to 330 μosm. Once samples and extender were at 37ºC, the samples were extended. Each sample was extended so that the semen evaluation could be performed as accurately as possible. The samples were generally extended at about a 1:13 dilution in order to be roughly around 3 billion sperm per extended dose. This

dilution was chosen because it is representative of the standard insemination dose used in the industry. As the boars aged, dilutions were adjusted, if necessary, to compensate for the increasing number of spermatozoa per ejaculate. During the extension process, whole semen was first added to a 50 ml conical tube (Port City Diagnostics, Inc., Wilmington, NC) and then the extender was slowly dripped from a serological pipette (Fisher Scientific, Atlanta, GA) into the conical tube. Adding the extender at a very slow rate reduced the amount of shock caused to the spermatozoa.

In addition, a 1 mL sub-sample of whole semen was frozen in a 1.5 ml bullet tube (Fisher Scientific, Atlanta, GA). An additional 1.5 ml sample of whole semen was centrifuged (Fisher Model 235C Micro-Centrifuge, Fisher Scientific) for eight minutes at approximately 12,000 rpm. The sperm pellet was then discarded and the seminal plasma was frozen for later analysis .

Evaluation of Semen Quality

Percentage of motile sperm cells:

Motility of all of the semen samples was determined using a computer assisted semen analysis system (Sperm Vision, Minitube of America, Verona, WI). Prior to assessing motility, 1 ml of extended semen from each boar was placed into a test tube (12 x 75 mm; Port City Diagnostics, Inc., Wilmington, NC). The test tubes were then placed into an incubator (Fisher Isotemp Oven 200 Series; Fisher Scientific, Atlanta, GA) kept at 37ºC for 30 minutes. Each sample was thoroughly mixed before 3 µl were pipetted into a Leja slide (Minitube of America, Verona, WI). The slides were maintained on a 37ºC

slide warmer (Minitube of America, Verona, WI). Each slide was then placed under the microscope and five fields were analyzed for average motility, progressive motility, and an approximate concentration. After data were recorded, the same procedure was repeated. Therefore, each boar's semen was sampled twice with ten fields being analyzed.

Percentage of morphologically normal acrosomes:

The percentage of morphologically normal acrosomes was assessed by using 1 ml of extended semen from each boar. The 1 ml of extended semen was placed into a test tube (12 x 75 mm; Port City Diagnostics, Inc., Wilmington, NC) and then mixed with 100 µl of 10% formalin. Formalin was added to prevent further deterioration of the sperm. Once the sample was thoroughly mixed, approximately 10 µl were placed onto an ethanol cleaned, glass microscope slide (Fisher Scientific, Atlanta, GA). An 18 x 18 mm coverslip (Fisher Scientific, Atlanta, GA) was then carefully placed on top of the sample. The slides were allowed to sit for a few minutes in order to allow the sperm to settle. The slide was then placed on the stage of a phase contrast microscope (Zeiss, West Germany) and sperm were visualized using a 25X objective. Upon visualizing the sperm, the objective was turned away and a drop of immersion oil (Fisher Scientific, Atlanta, GA) was placed on top of the cover slip so that the acrosomes could be better viewed. The oil objective on the Zeiss microscope was then slowly positioned over the sample. Once the sperm cells were in clear view, a random sample of 100 sperm cells was counted to determine the percentage of normal to abnormal acrosomes using the classifications of Pursel et al. (1972). Normal shape and smooth surface of the acrosomal ridge were

recorded as normal acrosomes. Spermatozoa with broken acrosomes or an uncharacteristic or uneven shape were recorded as abnormal. During this procedure, only sperm with normal tails were included in the random sample. Sperm cells positioned on their sides were also avoided during the counting process. The percentage of morphologically normal acrosomes was calculated from the number of normal acrosomes counted divided by the number of total spermatozoa counted.

Percentage of capacitated spermatozoa:

The chlorotetracycline technique (CTC) as modified by Popwell (1999) was used to determine the percentage of capacitated spermatozoa in each sample. Prior to preparing the samples for this assay, the NaCl/Tris buffer, DABCO (working), and CTC solution were mixed. The NaCl/Tris buffer and the DABCO (working) were made weekly, but the CTC solution was prepared the day of the assay. 1.8975g of NaCl and .6055g Tris were mixed together with 250 ml of distilled water to make the NaCl/Tris buffer. The DABCO (working) was prepared by placing 1 ml of stock DABCO into a 15 ml conical tube (Port City Diagnostics, Inc., Wilmington, NC) and adding 9 ml of glycerol. The tube was repeatedly inverted until thoroughly mixed. The .22M DABCO stock consisted of 2.46g of DABCO and 100 ml of PBS. Paraformaldehyde solution was also used, but was not made fresh weekly. The paraformaldehyde solution was prepared with 12.5g paraformaldehyde, 7.88g Tris-HCl, and 100 mls of distilled water. It was mixed over low heat until it became a solution and the pH was adjusted to 7.4. The mixing took place under a hood. Finally, the CTC solution was made using .019g of CTC, .039g of cysteine, and 50 ml of NaCl/Tris buffer. A 100 ml bottle (Pyrex,

Germany) was covered with aluminum foil to keep light out. The solution was mixed well and refrigerated until use.

The CTC method was performed under very low lighting. While preparing samples, ethanol cleaned, glass microscope slides were placed on a slide warmer (Fisher Scientific, Atlanta, GA) set at 37ºC. In addition, a slide box (Fisher Scientific, Atlanta, GA) was lined with aluminum foil and damp gauze. The slide box served to keep slides fresh and free of light while the other slides were prepared or viewed under the scope. 1 ml of each boar's extended semen was placed into a 15 ml conical tube (Port City Diagnostics, Inc., Wilmington, NC). The tubes were then centrifuged (Angle Centrifuge; Hamilton Bell Co, Inc., Montvale, NJ) at 1800 rpm for about four minutes. Following centrifugation, the seminal plasma was removed and discarded. The sperm pellet was kept and 1 ml of .87% saline was added. The .87% saline was made by adding 8.7g of NaCl to 1 liter of sterile water. The saline was then sterilized by autoclaving. Each sample was thoroughly mixed with the saline.

When the samples and materials were ready, the lights were turned off and the CTC slides were prepared. To make the CTC slides, 2.5 µl of the sperm/saline suspension was placed on the warmed microscope slides. 10 µl of the CTC solution was then added and mixed thoroughly for 30 seconds using a disposable, gel loading pipette tip (Fisher Scientific, Atlanta, GA). Next, a very small drop of paraformaldehyde was added and mixed well to fix the CTC pattern. The DABCO (working) helps prevent the fading of fluorescence and a single drop was added and mixed. A coverslip (24 x 50 mm; Port City Diagnostics, Inc., Wilmington, NC) was then placed on top of the slide and

excess solution was wiped off of the edges. Following the coverslip, the slides were stored in the slide box until examination.

The CTC slides were examined under blue-violet illumination (excitation at 400-440 nm, emission at 470 nm) provided by a VANOX microscope (Olympus Optical Co, Ltd., Japan). Once the sperm were visualized, a random sample of 100 cells was classified according to the following CTC staining patterns. Spermatozoa were categorized as capacitated if they exhibited a fluorescence free band in the post-acrosomal region of the head. Uncapacitated spermatozoa were characterized by a uniform fluorescence over the entire head. Sperm displaying a dull fluorescence over the entire head except for a thin, bright band of fluorescence in the equatorial segment of the head were placed in the acrosome reacted category. Any spermatozoa with unusual shaped heads, including broken acrosomal ridges, were categorized as abnormal. The percentage of capacitated spermatozoa was determined by dividing the number of cells found to be capacitated by the total number of spermatozoa counted.

Percentage of Spermatozoa with Acrosin Activity:

Gelatin slides were used to determine the percentage of spermatozoa that had undergone the acrosome reaction. Acrosin activity was determined using the procedures of Penn et al. (1972) slightly modified by Popwell (1999). Gelatin was prepared using 100 ml of distilled water, 3.5g of gelatin, and 30 µl of Tween 80. All materials were added into a glass beaker and stirred on low heat until solution was clear. Glass microscope slides (Fisher Scientific, Atlanta, GA) were cleaned with 80% ethanol and

dryed. Then the slides were labeled with a "G" on the left end of the slide and placed on a slide warmer (Fisher Scientific, Atlanta, GA) set at 37ºC.

To prepare the gelatin slide, 10 µl of gelatin was pipetted vertically near the end of the slide labeled "G." A glass spreader was then used to distribute the 10 µl of gelatin as evenly as possible from left to right. The slides were allowed to air dry and then placed vertically in a slide box (Fisher Scientific, Atlanta, GA) to be refrigerated until use. Gelatin slides were brought to room temperature before using. One ml of extended semen from each boar was placed into a test tube (12 x 75 mm; Port City Diagnostics, Inc., Wilmington, NC) and then allowed to warm in a 37ºC incubator (Fisher Isotemp Oven 200 Series; Fisher Scientific, Atlanta, GA) for at least 40 minutes. Ten µl were then pipetted onto the gelatin coated slides in the same manner that the gelatin had been applied. A glass spreader was then used to distribute the semen across the slide. Slides were then allowed to dry vertically for five minutes. The gelatin slides were then placed on a slide warmer (Fisher Scientific, Atlanta, GA) for 15 minutes. An incubation period of 1.5 hours at 37ºC and 100% humidity was then required in a CO2 incubator (NAPCO Model 6100; NAPCO Scientific Company, Tualatin, Oregon). Once removed from the incubator, the slides were immediately stained.

Toludine blue stain was used in this assay. To prepare the stain, 100 ml of borate buffer (pH 10) was added to .03g of toludine blue in a glass beaker and stirred. Once well mixed, the stain was poured into a slide staining jar. The slides were individually immersed in the toludine blue stain for 15 seconds and then removed. The end of the slide was blotted and then the slide was dipped three times in distilled water. Three different beakers were used. Therefore, after staining and blotting, the slides were rinsed

by dipping each one into three separate beakers and blotting between each. The slides were then allowed to dry vertically for analysis.

Phase contrast microscopy (Zeiss, West Germany) was used to perform the analysis. Using a 25X magnification, the cells were visualized and a random sample of 100 was categorized as either displaying acrosin activity or not. The presence of a glowing "halo" around a sperm cell's head indicated the presence of acrosin activity. Therefore, spermatozoa that lacked a "halo" were categorized as not possessing acrosin activity. The percentage of spermatozoa possessing acrosin activity was calculated by dividing the number of spermatozoa found to have acrosin activity by the total number of spermatozoa counted.

Concentration of Spermatozoa per Ejaculate:

The number of sperm cells per ejaculate for each boar was determined using a Bright-Line hemacytometer (Hausser Scientific, Horsham, PA). Using the original extended semen, another dilution was performed based on the concentration data from Sperm Vision. In order to make counting the sperm cells on the hemacytometer possible, dilutions were necessary. The goal was to dilute the semen such that 30 to 60 sperm cells were present on the counting grids of the hemacytometer. Once diluted properly, a 24 x 40 mm coverslip (Port City Diagnostics, Inc., Wilmington, NC) was placed over the grid on the hemacytometer and 10 µl of extended semen was carefully pipetted into the chambers. The hemacytometer had two separate grids that were each loaded separately

with 10 µl of extended semen. The slides were allowed to sit for several minutes in order to allow the spermatozoa to settle and be accurately assessed.

The hemacytometer was evaluated under bright field microscopy (Zeiss, West Germany) to determine the number of spermatozoa present (Almond et al., 1998). The spermatozoa found within the inner, center 1 mm square of the hemacytometer were counted. Therefore, all 25 boxes in the 1 mm square were counted. The sperm were counted such that all cells, including those without tails, were included as long as they were completely enclosed in the grid or partially on the top or left side borders. If any part of the cell was touching the bottom or right side border of the grid, it was not counted. For each boar, 4 separate, 1 mm center squares were counted and recorded.

In order to determine the total sperm in each boar's ejaculate, the mean of all four counts was determined. That number (n) was then divided by 400 and multiplied by 4000, 1000, and the dilution factor. For example, if the average count was 42.4, the equation to determine the number of spermatozoa would look like the following if the dilution factor is 1500.

$$(42.4/400) \times 4000 \times 1000 \times 1500 = 636 \text{ million / milliliter}$$

This equation finds the number of spermatozoa per milliliter. Therefore, this example found 636 million sperm per milliliter. In order to find the total spermatozoa per ejaculate, that number was multiplied by the volume of the ejaculate. For example: (636 million/ml) (151 ml) = 96.04 billion represents the total number of sperm cells found in that particular boar's ejaculate. Spermatozoa concentration for each boar was calculated weekly.

Protein Gel Procedures and Analysis

A commercially available kit (Invitrogen, Carlsbad, CA) was used for the 2-dimensional gel electrophoresis. The reagents used were as follows:

Isoelectric Focusing Gels – *All gel supplies were purchased from Invitrogen (Carlsbad, CA)*

Product	Catalog #
Novex® pH 3-10 IEF gel 1.0 mm, 10 well	EC6655A
Novex® IEF Cathode Buffer pH 3-10 (10x)	LC5310
Novex® IEF Anode Buffer (50x)	LC5300
Colloidal Blue Staining Kit	LC6025

1-Dimensional Gels – *All gel supplies were purchased from Invitrogen (Carlsbad, CA)*

Novex® 4-20% Tris-Glycine Gel 1.5 mm x 2D well	EC6029
Novex® Tris-Glycine SDS Running Buffer (10x)	LC2675
See Blue® Plus2 Pre-Stained Standard	LC5925
Gel-Dry™ Drying Solution (1x)	LC4025

2-Dimensional gels were used to determine seminal plasma characteristics. Two separate seminal plasma protein profiles were determined for each boar in this study. The first profile was determined using the seminal plasma from the ejaculates collected at the beginning of full semen analysis when the boars were 35 weeks of age. The second profile was determined using the seminal plasma from the ejaculates collected when the boars were 45 weeks of age, prior to culling eight of the boars. The seminal plasma protein profiles were then analyzed and compared for two proteins previously identified and thought to have a positive relationship with fertility (Flowers, in press). The two proteins are thought to have the following molecular weights and p.I.: Protein I – 26 kilodaltons, pI = 6.2 and Protein II – 55 kilodaltons, pI = 4.8.

Novex Pre-cast gels were used for the IEF gels (20% TBE Gel/1.0 mm x 10 wells; Invitrogen, Carlsbad, CA). Cathode buffer, anode buffer, and IEF stain were prepared according to Invitrogen directions. The fix solution was prepared by adding 17.3g of sulphosalicyclic acid and 57.3g of trichloroacetic acid into a flask and then filling the flask to 500 milliliters with distilled water.

Once all materials were ready, the seminal plasma samples were removed from the freezer and allowed to thaw. Once thawed, samples were thoroughly mixed and 100 µl was pipetted into a clean bullet tube and 100 µl of sample buffer was added. The solution was then mixed well.

The IEF Gel run apparatus (Novex E19001- XCELL II Mini Cell; Invitrogen, Carlsbad, CA) was assembled. Each gel was cut out of the plastic bag, rinsed with distilled water, and the tape and sample well comb removed. Once the gels were placed in the apparatus, the IEF cathode buffer was added to the inner chamber until the gels were totally submerged. The outer chamber was checked for any leakage. When no leakage was present, the outer chamber was filled with anode buffer. 25 µl of each sample was then pipetted into each well. Each sample was pipetted into two wells with 5 µl of standard placed into the middle and far right wells. The IEF markers used as standards were amyloglucosidase (pI = 3.5), glucose oxidase (pI = 4.2), trypsin inhibitor (pI = 4.5), β-lactoglobulin (pI = 5.3, 5.2), carbonic anhydrase (pI = 6.0), myoglobin (pI = 7.4, 6.9), and lectin (pI = 8.0). In a 10 well IEF gel, a maximum of four boars could be run. Once all samples were inserted, the top was placed on the apparatus and plugged into the power source. The IEF gels were then run at 100 volts for one hour, 200 volts

for one hour, and finally 500 volts for 45 minutes or until the dye approached the foot of the gel.

The gels were then removed from their plastic casing and "floated" out into water. After prying the plastic casing apart, the back piece of casing was carefully peeled away from the gel. Next the remaining portion of casing and gel were submerged in a water filled glass dish. The foot of the gel was carefully dislodged from the casing and the rest of the gel was gently peeled off into the water. The gel was then trimmed to remove the bottom portion and "fingers" of the wells. The gels were allowed to rinse in water for five minutes and then placed in the fix solution. The gels remained in the fix solution for one hour in a shaker bath set on low. Next, the gels were rinsed in distilled water for an additional five minutes. The gels were then placed in the IEF stain overnight.

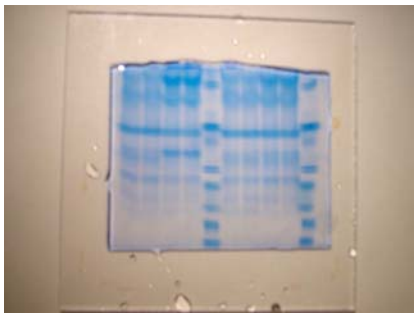

Figure 2. Photograph of an IEF gel after staining, but before the individual lanes were cut.

After the IEF gels were stained enough to distinguish individual lanes, they were placed in water. Each sample's lanes were cut down to fit in the sample slot of the 2D gel. Care was used to be sure no proteins or standards were lost in the trimming process. All cut lanes were placed in distilled water.

The 2D gels used were also Novex Pre-cast gels (4-20 % Tris-Gly gel / 1.5mm x 2D well; Invitrogen, Carlsbad, CA). 2D gels required Tris-Glycine SDS running buffer and Tris-Glycine staining solution. Both were prepared according to Invitrogen directions. The same apparatus used to run the IEF gels was also used to run the 2D gels. The 2D gels were prepared in the same manner as the IEF gels.

Once the IEF gels were run and the lanes cut, the lanes were inserted into the top of the 2D gels with the darker end of the lanes placed away from the standard well. Once the apparatus was assembled and the gels were in position, the SDS running buffer was added; first to the inner chamber and then to the outer chamber. Ten µl of See Blue Standard (Invitrogen, Carlsbad, CA) was pipetted into the standard lane. The molecular weights of the protein bands found in the standard lane were myosin (250 kDa), phosphorylase (148 kDa), BSA (98 kDa), glutamic dehydrogenase (64 kDa), alcohol dehydrogenase (50 kDa), carbonic anhydrase (36 kDa), myoglobin red (22 kDa), lysozyme (16 kDa), aprotinin (6 kDa), and insulin, B chain (4 kDa). The apparatus top was secured and plugs were inserted into the power source. The 2D gels were run at 125 volts for one hour and 45 minutes or until the dye front reached the desired position in the gel. The gels were then removed from the plastic casing with the same technique as the IEF gels. The gels were rinsed in water for five minutes before being placed in the tris-glycine stain on a shaker bath overnight. Once gels were stained so that the proteins were clearly visible, the gels were rinsed in water until the backgrounds were clear.

The gels were dried using cellophane squares, Gel-Dry™ and drying racks. Gel-Dry™ is a drying solution that regulates the rate at which the gels dry in order to minimize cracking. All materials were purchased from Invitrogen (Carlsbad, CA). Each

gel was cycled through different gel dry trays. First, they were placed in gel dry that had been used twice and allowed to sit for about one minute. Next, the gels were placed in once used gel dry for at least two minutes. Finally, the gels were placed in new gel dry for at least ten minutes. Then the gels were carefully, positioned between two pieces of gel dry soaked cellophane on a drying rack. The gels were placed in a cool, dry place away from air currents and allowed to dry. Throughout the entire gel process, the gels were continually marked to ensure proper identification.

After two or more days of drying, the gels were removed from drying racks, cellophane was trimmed, and the gels were placed in individual, folded, precut pieces of blotting paper. The gels were then stored beneath weighted objects to ensure that the gels would not curl and cause difficultly during analysis.

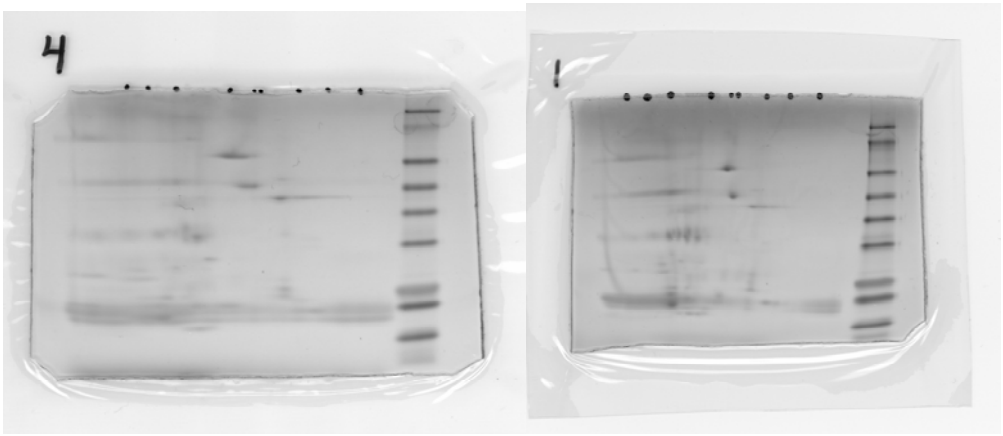

Figure 3. Scanned images of 2D gels from two different boars.

Protein Gel Analysis

After gels were completely dry, they were matched up to the IEF standards and the p.I. range was marked on each gel. The nine standard p.I.'s marked included 3.5, 4.2, 4.5, 5.2, 5.3, 6.0, 6.9, 7.4, and 8.0. Marking the standard p.I.'s on each gel allowed easy reference between p.I. and molecular weight after the gels were scanned in to the computer.

Each gel was then scanned twice using a Hewlett Packard hp scanjet 8200 scanner. The first scan produced a horizontal image used for molecular weight and then a second scan produced a vertical image for p.I. Image Quant TL (Amersham Biosciences, Piscataway, NJ) was used for protein detection. Using the computer software, all proteins present on the gels were marked with two horizontal lines framing the area in which the protein was identifiable. The volumes of each protein were then normalized relative to the protein band myosin. Myosin has an approximate molecular weight of 250 kilodaltons. Therefore, following normalization, the myosin protein band had a normalized volume of 200 μg and all other volumes were normalized with respect to that particular protein band and its volume. After marking and normalizing all proteins present on the gels, the images and their data were printed. Following printing, the relative units or normalized volumes of the two proteins of interest were recorded.

Statistical Analysis:

The effects of season (fall and spring); treatment (small and large lactation litter); and age (1 through 41 weeks of age) on body weight, testicular measurements, and semen quality parameters were analyzed with analysis of variance procedures for repeated measures (Gils and Hafs, 1971) using the general linear models procedure (GLM) of SAS (SAS, 2002, Version 9.1). Main effects of treatment and season, and the treatment by season interaction were tested using boar nested within season and treatment, *ID*(*treatment x season)* as the error term. Effects of age, age by season, age by treatment, and age by season by treatment were tested using the residual error.

When a significant three-way interaction was observed, effects of treatment, age and their interaction within each season were evaluated. Analysis of variance procedures for repeated measures were used for these analyses by using boar nested within treatment to evaluate the main effect of treatment. When significant two-way interactions between age and season or season and treatment were detected, changes over time as the boars matured within each season or treatment, respectively, were analyzed. When a significant interaction between season and treatment was present, the effect of treatment within each season was determined. When a significant effect of age was present, differences among individual ages were determined by examining probability values (PDIFF) for all possible combinations of least-squares estimates of marginal means (LSMEANS).

The effects of season (fall and spring); treatment (small and large lactation litter); and age (1 through 41 weeks of age) on body weight, testicular measurements, and semen

quality parameters were analyzed with analysis of variance procedures for repeated measures using mixed models procedures (PROC MIXED) of SAS (Littell et al., 1996; SAS, 2001). Compound symmetry was determined to be the best estimate of the covariance structure and the Satterthwaite method was used to estimate degrees of freedom. Main effects of treatment and season, and the treatment by season interaction were tested using boar nested with in season and treatment, *ID (treatment x season)* as the error term. Effects of age, age by season, age by treatment, and age by season by treatment were tested using the residual error.

When a significant three-way interaction was observed, effects of treatment, age and their interaction within each season were evaluated. Analysis of variance procedures for repeated measures using mixed models procedures were used for these analyses by using boar nested within treatment to evaluate the main effect of treatment. When significant two-way interactions between age and season or age and treatment were detected, differences between treatments within each season or age, respectively, were analyzed. When a significant interaction between season and treatment was present, the effect of treatment within each season was determined. When a significant effect of age was present, differences among individual ages were determined by examining probability values (PDIFF) for all possible combinations of least-squares estimates of marginal means (LSMEANS).

Analysis of variance procedures for categorical data (Koch et al., 1997) were used to determine the effects of season and treatment on how quickly boars were trained to collect from a dummy sow using the PROC GENMOD procedure of SAS (SAS, 1998). The statistical model for the proportion of boars trained included season, treatment, time

(week after the training session began) and appropriate interactions. A significant interaction between treatment, season, and time was present (p=.007). Consequently, effects of treatment and age within each season were determined. The statistical model for these analyses included treatment, time and the treatment by time interaction.

Significance was determined by the following p-values. Statistical significance was represented by a p-value less than or equal to 0.05. A trend or tendency was noted if the p-value fell into the range of 0.05 to 0.1. A complete listing of SAS analysis program codes used is located in Appendix A.

Data were analyzed in two different manners. The first analysis included all boars involved in the study. The second analysis included only those boars selected, as previously discussed, to remain a part of the study during the breeding phase. Results for this analysis are located in Appendix D. Figure 4 depicts the timeline for the entire study. A second timeline, Figure 5, is pictured to represent the amount of data analyzed for this portion of the study.

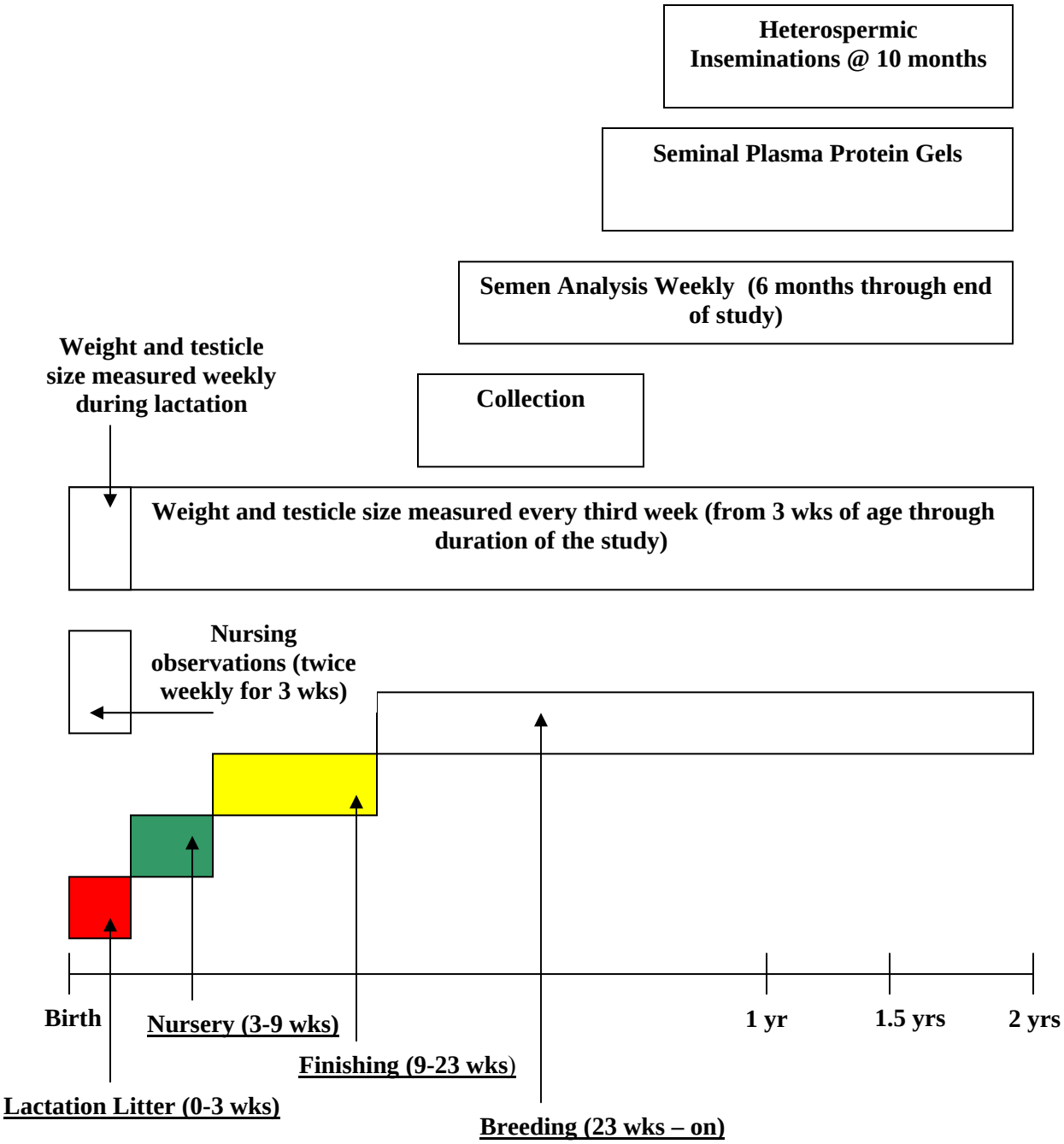

Figure 4. Timeline detailing an overview of the entire study.

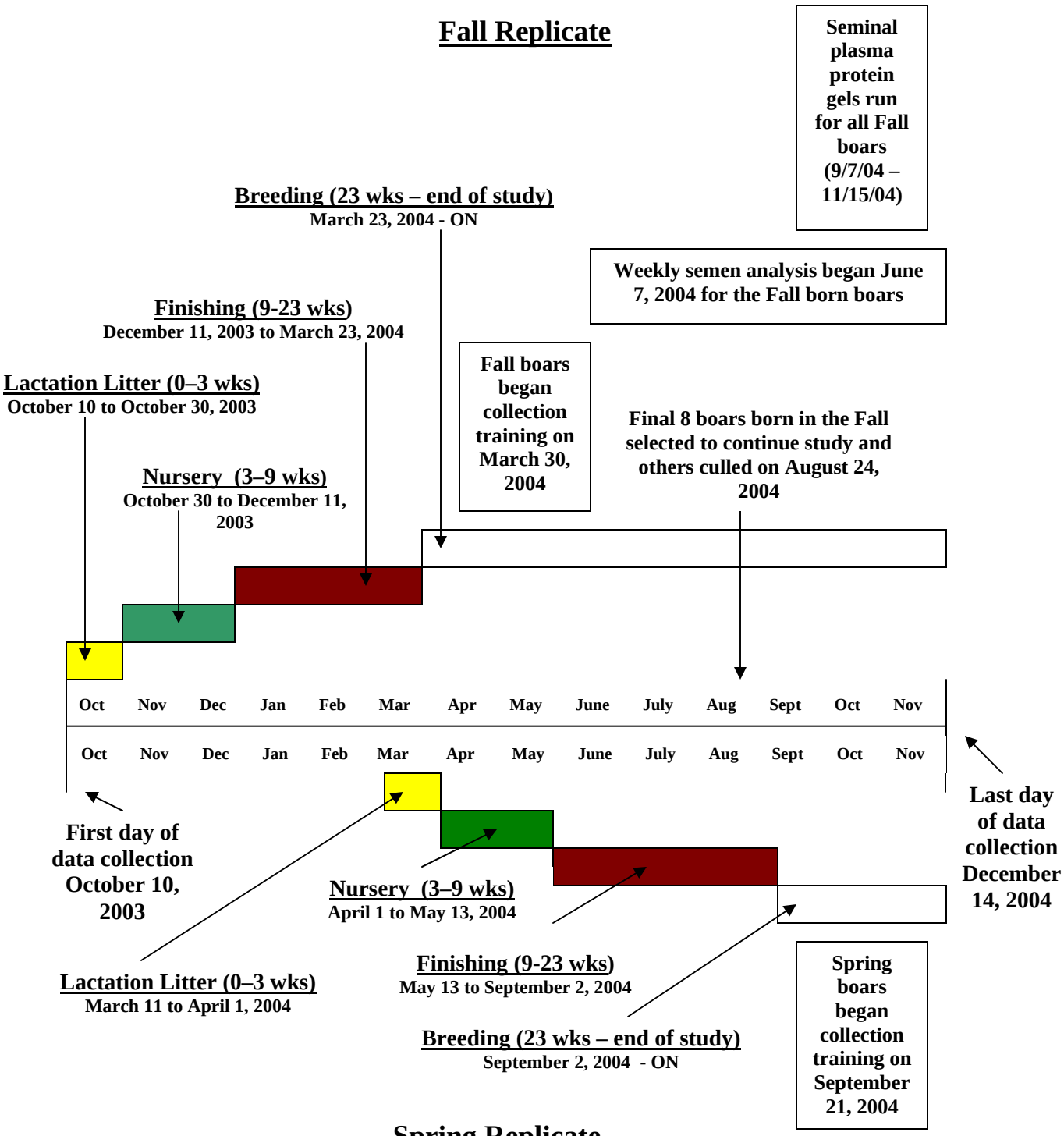

Figure 5. Timeline detailing the portion of study covered in this thesis.

Results:

Weight and testicle measurement results:

A significant interaction ($p<.05$) among treatment, season, and age was observed for body weight (Tables 2 and 3). For boars born in the fall, boars reared in small litters were not significantly different ($p>.05$) than their counterparts reared in large litters except at weaning. At weaning, the boars born in the fall and nursed in small litters were heavier ($p<.05$) than the boars nursed in large lactation litters (Table 2). In contrast, for boars born in the spring, boars reared in small litters were significantly different ($p<.05$) than their counterparts reared in large litters at all ages except from 20 to 28 weeks of age (Table 3). The boars born in the spring and reared in small litters were heavier, at most ages, than the boars reared in large litters. Body weight increased ($p<.05$) for all boars during the first 37 weeks of life. However, boars born in the fall were consistently heavier than boars born in the spring beginning at 13 weeks of age (Table 2 and 3).

A significant interaction ($p<.05$) between season and age was observed for testicular height (Figure 6) and testicular width (Figure 7). For boars born in the fall, testicular height increased ($p<.05$) from 6 weeks of age to approximately 37 weeks of age. Testicular height for the boars born in the spring increased ($p<.05$) from 3 weeks of age through 37 weeks of age (Figure 6). Likewise, testicular width increased ($p<.05$) from 6 weeks of age to 37 weeks of age for all boars except those born in the spring and nursed in large litters. The boars born in the spring and reared in large litters had an extended period, nine weeks, following birth with no increase in testicular width. In addition, at 24 weeks of age, testicular width ceased increasing for the spring born boars

nursed in large litters. Beginning at 6 weeks of age, the boars born in the fall had consistently larger testicular width than those boars born in the spring.

A significant interaction ($p<.05$) among season and age was also observed for testicular area and the percentage of testicular area per body weight (Figures 8 and 9). There was also a trend ($p=.1017$) among season and treatment for testicular area. Boars born in the fall had consistently larger testicular area than those boars born in the spring beginning at approximately 6 weeks of age (Figure 8). However, boars born in the spring and nursed in small litters had consistently larger testicular area than their counterparts nursed in large litters. Testicular area increased ($p<.05$) from 13 weeks of age until 43 weeks of age for the fall born boars and from approximately 9 weeks of age on for the spring born boars. Boars born in the fall had larger ratios of testicular area to body weight for the first several weeks of life in comparison to the boars born in the spring (Figure 9).

Mounting and collecting during training period results:

Significant interactions among age, season and litter size were present ($p<.05$) for the first successful mounting attempt (Figure 10); the cumulative percentage of boars trained to mount (Figure 11); the first successful collection (Figure 12); and the cumulative percentage of boars trained to collect (Figure 13). As a result, the effects of litter size and age within each season were examined. Results from these analyses indicated a significant interaction between litter size and age also was present ($p<.05$) for each of the dependent variables. Thus, the effect of litter size within each age group was determined.

In general, boars raised in small litters mounted the dummy sow earlier ($p<.05$) during the 6-week training period compared with their counterparts that nursed in large litters (Figures 10 and 11). For boars born in the fall, 89 % of the small litter boars mounted at 24 weeks of age (first week of training) compared with only 78 % of those raised in large litters. Similar means for boars born in the spring from small and large litters were 57 % and 28 %, respectively. By 28 weeks of age, there were no differences ($p = 1$) between litter size treatments in the cumulative percentage of boars trained to mount (Figure 11). All the boars born in the fall and 88 % of the boars born in the spring successfully mounted the dummy sow during the training period.

Boars raised in small litters were trained to collect off a dummy sow earlier ($p<.05$) in the training period than boars raised in large litters (Figures 12 and 13). However, the time frame over which this occurred followed a slightly different pattern than the one observed for mounting activity. In the fall replicate, 67 % of the boars raised in small litters were successfully collected at 24 weeks of age (first week of training) compared with only 55 % of those raised in large litters. However, by 29 weeks of age, no effect ($p = 1$) of litter size was present (Figure 13). In contrast, in the spring replicate, it was not until the third week of the training period (26 weeks of age) that a greater number of boars ($p=.05$) raised in small litters were being collected compared with those raised in large litters (Figure 13). By 30 weeks of age, there was no difference ($p=1$) between litter size treatments in the number of boars trained to collect (Figure 13).

Semen analysis results:

There was a tendency ($p=.0641$) observed between season and age for concentration of spermatozoa (Figure 14). The concentrations of boars born in the fall and reared in both small and large litters increased ($p<.05$) as they aged. In contrast, the concentrations of boars born in the spring were unaffected by age ($p>.05$).

No interactions were observed for the total number of spermatozoa (Figure 15). For both fall and spring born boars, only age was significant ($p<.05$) in the total number of spermatozoa per ejaculate. The total number of spermatozoa increased as the age of the boars increased. A significant interaction ($p<.05$) between season and treatment was observed for volume of ejaculates (Figure 16). However, age was not observed to be significant for volume for either season or treatments ($p>.05$). The boars born in the spring and reared in small litters had a significantly lower volume than their counterparts nursed in large litters during the initial analysis (Figure 16). The volumes of spermatozoa from the boars born in the fall were similar to those of the spring born boars reared in large litters. Despite the initial difference in volume for the boars born in the spring and nursed in small litters, their volumes increased quickly to levels similar of those in other boars (Figure 16).

A significant interaction ($p<.05$) between season and age was observed for motility (Figure 17). The fall born boars reared in small litters were not affected by age ($p>.05$), but the boars nursed in large litters had a tendency ($p=.0977$) for motility to be affected by age. Motility for the boars born in the spring and nursed in small litters was significantly ($p<.05$) affected by age, but those reared in large litters only displayed a trend ($p=.0671$). The boars born in the spring experienced a decrease in the percentage of

normal motility around 38 weeks of age. It was observed that the boars born in the spring and reared in small litters then had a sharp increase in the percentage of normal motility while their counterparts reared in large litters displayed a gradual increase.

No interactions were observed for morphology (Figure 18). However, age was noted to be significant ($p<.05$) in affecting morphology. The percentage of normal acrosome morphology slightly increased as the boars aged. A significant interaction ($p<.05$) between season and age was observed for the percentage of spermatozoa that displayed acrosin activity (Figure 19). For boars born in the fall, age was significant ($p<.05$) in affecting acrosin activity. Both treatments of boars born in the fall experienced fluctuations in acrosin activity as they aged (Figure 19). In contrast, for the boars born in the spring age was not significant ($p>.05$) for acrosin activity in either boars reared in small or large litters.

Trends between season and age ($p=.0901$) and between season and treatment ($p=.0841$) were observed for the percentage of uncapacitated spermatozoa (Figure 20). The number of uncapacitated spermatozoa for fall born boars nursed in small and large litters was significantly ($p<.05$) affected by age. The boars born in the fall had significantly higher numbers of uncapacitated spermatozoa during the first week of analysis or at 35 weeks of age. The boars born in the fall experienced a decreasing trend in the number of uncapacitated spermatozoa as they aged. In contrast, the number of uncapacitated spermatozoa was not affected ($p>.05$) for boars born in the spring and reared in small and large litters.

No significant interactions were observed for the number of capacitated spermatozoa (Figure 21). Only age was found to be significant ($p<.05$). The boars born

in the fall experienced lower numbers of capacitated spermatozoa at 35 weeks of age during the first week of analysis. In contrast, the boars born in the spring and reared in large litters were observed to have higher numbers of capacitated spermatozoa at 35 weeks of age and then experienced a decrease in these numbers. The same was observed for the number of spermatozoa that had undergone the acrosome reaction (Figure 22). No interactions were observed and only age was found to be significant ($p<.05$) in influencing the number of acrosome reacted spermatozoa. During the period of time when the fall born boars were ages 45 to 49 weeks, the number of acrosome reacted spermatozoa increased and then a drastic decrease occurred.

A tendency ($p=.0676$) among season and age was observed for the number of abnormal spermatozoa (Figure 23). For fall born boars, age was found to be significant ($p<.05$) for the number of abnormal spermatozoa. Those boars born in the spring, and reared in small and large litters, were not significantly ($p>.05$) affected by age. In contrast to the boars born in the spring, the boars born in the fall experienced a significant decrease in the number of abnormal spermatozoa during the first weeks of analysis or during the time period that they were 35 to 41 weeks of age.

Gel analysis results:

No interactions between treatment and age were observed for concentrations of the two seminal plasma proteins (Table 4). Proteins were normalized as previously mentioned. However, age was observed to be significant ($p<.05$) and treatment displayed a tendency ($p=.1063$) towards influencing normalized volume. Large litter boars were observed to have higher normalized volumes of seminal plasma proteins than the boars nursed in small litters at both 35 and 45 weeks of age (Table 4). In addition to the differences between treatments, the volumes of both small and large litter boars decreased from 35 weeks of age to 45 weeks of age.

Discussion

The amount of fertile semen that an individual boar produces is an essential contribution to a swine production facility. Management strategies for increasing spermatozoa per ejaculate in boars have been implemented in the past, but most have focused on adult boars and have not been very successful (Flowers, 1997). In the present study, manipulation of neonatal litter size of replacement boars was examined to determine what effects, if any, nursing in small litters (n=6) or large litters (n≥10) had on semen production, in the adult animal.

Because the first three weeks following birth is an active period of mitotic activity in the testicles, this may be an impressionable period of time in which strategies could be beneficial to growth rate, testicular size, and semen production. This time period also coincides with lactation. Therefore, if pigs experience less competition for food among littermates and can nurse freely, the added nutrition may have a positive effect upon sperm production. It is reasonable to speculate that due to the added nutrition and less competition, boars nursed in small litters should weigh more, have larger testicles, and thereby produce higher quality and quantity of spermatozoa.

Differences in growth rate were observed in this study. Boars that were born in the fall consistently weighed more than boars born in the spring. This is probably due to the environmental conditions present as the boars grew and matured. The fall born boars matured during the late fall and winter while the boars born in the spring matured during the late spring and summer. Mavrogenis and Robison (1976) reported that season was a major environmental factor that influenced sexual development of gilts. In fact, they

inferred from their results that it seemed reasonable to suggest that gilts born in the fall attain sexual maturity very close to a minimum age in the following breeding season (Mavrogenis and Robison, 1976).

European wild boars are distinctly seasonal breeders, usually producing only one litter a year. Decreased reproductive performance during the late summer and early autumn has been reported (Love et al., 1993). There are many factors that contribute to this period of decreased reproductive performance. They include food availability, temperature and humidity, duration of photoperiod, and social associations (Kunavongkrit et al., 2005). In addition to affecting reproductive performance, these factors can also be associated with decreased weight gain or, if severe, weight loss. In this study, the boars that were born in the fall were able to grow during a period of time when environmental stresses, temperature and humidity, were limited. The boars born in the spring, however, were undergoing rapid growth during the hottest months of the year in North America.

Based on the findings of Mavrogenis and Robison (1976), if the same hypothesis of fall born gilts is applied to these boars, it would seem reasonable to suggest that the boars born in the fall may have matured at a faster rate in order to obtain sexual maturity at a minimum age the following breeding season. Thus, those boars born in the spring may, in fact, be less likely to grow at the same rate as the boars born in the fall due to environmental conditions and possible innate tendencies based on their wild ancestry. Traditionally, the wild boar was reproductively inactive during the summer and early autumn. Breeding for the wild boar occurred during the winter and early spring. While domestic pigs are usually considered non-seasonal breeders and produce two litters a

year, the impact of their ancestors' seasonal breeding is not completely known although seasonal variations have been widely studied.

Early work (Rutledge, 1980) reported that there were no differences in body weight at breeding age due to postnatal litter size. However, in the present study, the boars born in the spring and reared in small litters were heavier than their counterparts reared in large litters. In agreement with these findings, Martin and Crenshaw (1989) reported that gilts reared in a litter size of 6 were heavier ($p<.01$) than gilts reared in a litter size of 12. Therefore, enhanced nutritional status of nursing in a litter of 6, as opposed to a litter of 12, did increase body weight and supports the proposed hypothesis.

The enhanced nutritional status of a smaller litter size is due, primarily, to decreased competition among littermates. With less competition, pigs spend less time fighting or determining social dominance and have free access to teats. Because nursing coincides with important mitotic activity in the testicles, the added nutrition, as hypothesized, could promote and increase testicular growth. Although the boars born in the spring and reared in small litters did weigh more than their counterparts reared in large litters, the same was not observed during the fall.

As previously mentioned, the boars born in the spring came down with a scours-like illness while in the farrowing house. Therefore, it would seem reasonable to suggest that because they were compromised early in life, the added nutrition for the smaller litters could be causing the significant difference in body weight between the boars born in the spring and reared in small and large litters. However, upon further investigation, the weaning weights for both the fall and spring born boars were not significantly

different. Thus, the significant difference in body weight between the small and large litter boars born in the spring was not influenced by the early illness.

While litter size did have an impact on body weight, the same was not observed for testicular size. Only seasonal differences were noted. Fall born boars maintained larger testicular parameters than the boars born in the spring. This is probably due to the same factors discussed for body weight. However, it does not appear that being reared in a smaller litter size impacts the size of the testes. The effects of added nutrition may only serve to increase body weight and not testicular tissue mass. Several studies (Rathje et al., 1995, Johnson et al., 1994, Huang and Johnson, 1996 and Schnickel et al., 1984) examined the results of selecting for increased testicular weight. All found that by selecting for increased testicular weight, beneficial results included increased body weight, increased concentration, and increased total number of spermatozoa produced and stored. Based on previous research and the findings of this study, testicular size is highly heritable and needs to be genetically selected for in order to achieve increased testicular size and sperm production.

After 8 generations of selecting for increased predicted weight of testis, Rathje et al. (1995) observed that for the first 100 days or approximately 14 weeks following birth the differences between the control and treatment groups were limited. However, once the boars reached 130 days or 18.5 weeks of age, the group that had undergone selection for increased testicular weight began to be consistently heavier than the control boars. In the present study, the boars born in the fall, regardless of litter size, weighed more than the boars in both Rathje et al.'s (1995) control and treatment groups. The boars born in the spring and reared in small litters were also heavier than both the control and treatment

groups in Rathje et al.'s (1995) study. However, the boars born in the spring and raised in large litters had a mean weight very similar to that of the selected group from Rathje et al. (1995).

Huang and Johnson (1996) were able to further examine the effects of selecting for increased testicular weight by performing castration. The control group (n=18) and the treatment group (n=24) were castrated after 64 collections. The body weights at castration were not significantly different, but the boars in the selected group had a significantly heavier testicular weight. In addition, the treatment group of boars also had larger amounts of total testicular sperm, greater daily sperm production, and more numerous sperm reserves. No significant treatment or litter size effects were observed for testicular size in the present study, but body weight was affected by the neonatal litter size. It seems that genetic selection for increased testicular weight does increase testicular weight and semen parameters, but does not significantly influence body weight. The current study suggests that manipulation of neonatal litter size may cause significant differences in body weight, but not in testicular weight or size.

Semen characteristics were not observed to be affected by manipulation of postnatal litter size. The evidence presented does not support the original hypothesis of rearing boars in smaller litters to increase sperm production. In contrast, Martin and Crenshaw (1989) observed that gilts, when reared in litters of 6 as opposed to litters of 12, produced 2.4 more ($p<.05$) ova. Therefore, postnatal nutritional status appeared to have a positive impact on reproductive performance in females. However, although differences in ovulation rate were detected, the number of offspring produced was similar among the treatment groups due to differences in prenatal losses. Thus, these data

suggest that prenatal losses, not ovulation rate, are the limiting factors for increased litter size for swine just as the number of Sertoli cells present in the testes are the limiting factor for the total number of spermatozoa produced (Cunningham et al., 1979; Martin et al., 1989).

Although litter size was not found to affect semen parameters, the aging process was identified to be an obvious factor. Generally, for the data analyzed, characteristics such as semen concentration, total number of spermatozoa, and the percentage of normal morphology increased as the boars aged. In addition to age, season of birth was observed to also be a factor in influencing semen characteristics.

The semen characteristics for the boars born in the fall were often found to be significantly different over time while the semen characteristics for the boars born in the spring were not. These observations may be due to the amount of data collected and analyzed for each season of boars. Ejaculates from the boars born in the fall began being analyzed in June 2004 while the ejaculates from the boars born in the spring began being analyzed in mid-September 2004. Data were gathered for both until mid-December 2004. Therefore, the fall born boars had considerably more data than the spring born boars. Perhaps the extended period of data collection allowed for more seasonal effects to occur and be detected. Whether or not the same effects will be seen for the spring born boars is yet to be evaluated.

The data analyzed for the behavior of the boars during the mounting and collecting training clearly shows a litter size treatment effect. Those boars reared in small litters mounted the dummy sow and collected earlier than their counterparts reared in large litters. A definite seasonal effect was also present. The boars born in the fall had

a larger percentage of boars mount and collect than the boars born in the spring. The boars in the fall replicate also achieved this at an earlier age than the boars in the spring replicate. The evidence suggests an increased libido for both boars reared in small litters and boars born in the fall. High heat and humidity can result in increased stress for swine and may contribute to a delayed mounting and collecting behavior in boars born in the spring (McNitt et al., 1972).

The changes that the boars born in the fall experienced may be due to several factors. Previous findings by McNitt et al. (1972) and Wettemann et al. (1979) stated that heat and high humidity can result in stress for swine, especially if the temperatures between night and day consist of a wide-range of temperature fluctuations. This is largely due to the fact that pigs have little capacity for sweating when thermally stressed and, therefore, during or immediately after hot summer months, a decrease in reproductive performance of boars is often observed (McNitt et al., 1972). Kunavongkrit et al. (2005) stated that hot weather, humidity, and photoperiod may all have a negative effect on the reproductive efficiency of boars, but, likewise, poor nutrition and reduced feed intake, which may also be caused by similar factors, can also be important. The effects of elevated ambient temperatures on semen quality have been shown in many experiments. The negative effects widely noted include decreases in sperm motility and percentages of normal sperm with non-aged acrosomes (McNitt and First, 1970 and Wettemann et al., 1976. The percentage of sperm head abnormalities and of sperm with proximal cytoplasmic droplet also increased. Semen volume and the number of sperm in ejaculate are also highest in the fall and winter (Ciereszko et al., 2000).

Despite the limited support of the original hypothesis, it is important to note that results represent approximately 60 weeks of a planned three year study. The collection and analysis of the boars involved in this study is currently continuing and the culmination of all data will be imperative in determining the final conclusions.

The gel analysis of seminal plasma proteins provided interesting results. Flowers (accepted) reported that two seminal plasma proteins may be positively correlated with increased fertility. In addition, it matters not how much each individual protein appears on the gel, but it is the combined amount that coincides with enhanced fertility. As the relative concentration of seminal plasma proteins increase, the farrowing rate and the number of pigs born alive also increase (Flowers, accepted). However, these results were observed using boars that were all mature and much older than the boars involved in the present study. Therefore, little is known about seminal plasma protein concentrations in young boars and how the concentrations may change as the boars age.

However, the results of the present study dictated that boars reared in large litters had higher amounts of seminal plasma proteins present on gels than their counterparts reared in small litters. Regardless of litter size, all of the boars involved in this study had seminal plasma protein concentrations that were equal to or greater than the concentrations of the boars involved in Flowers, earlier retrospective study. The amount of proteins also decreased for all boars between 35 and 45 weeks of age. Based on these results it appears that seminal plasma proteins may peak at an early age and then decrease over time. In addition, the boars reared in large litters experienced a decrease in the concentration of seminal plasma proteins almost twice that of what the boars reared in small litters experienced. Thus, it may be reasonable to speculate that boars raised in

small litters maintain a greater concentration of these proteins over time. Before any speculations can be made, gels need to be performed and analyzed with the seminal plasma from the boars born in the spring. If similar results are found, it would be important to decipher why large litter boars are consistently producing more seminal plasma proteins and what happens to their concentrations over time.

Despite efforts to standardize or control the amount of standard and seminal plasma used for each gel run, variation with the two dimensional gel electrophoresis process is difficult to avoid. Therefore, for the gels performed on the seminal plasma of the boars born in spring, it would be ideal to include an internal standard. By utilizing the same seminal plasma from the same boar for each gel run, any variation attributed to the two dimensional gel electrophoresis process can be determined.

The original hypothesis that manipulation of postnatal litter size may be an effective way to increase sperm production and quality in the mature boar is still inconclusive from the preliminary nature of the results presented here. These early results suggest that this hypothesis may be false. As reviewed by Foote (1978), testicular size is highly heritable and directly affects sperm output potential. In the present study, considerable genetic variation among boars was obvious. Secondary studies may want to further examine genetic variation among boars and their semen quality to perhaps identify superior genetic lines.

For example, in this study, all boars were randomly crossfostered into either large or small litters. Despite randomization, several brothers ended up in the same treatment. Therefore, if two brothers are from genetically superior lines in terms of testicular size and semen quality and are crossfostered into the same litter size, the effects that litter size

may ultimately have on these boars cannot be compared and contrasted. In fact, despite the impact that litter size may have, their genetic superiority may outweigh the environmental effects and ultimately sway the results in favor of that treatment.

In the present study, paternal genetic variation was accounted for among boars. Although four unrelated sires were used to produce the fall replicate and three different and unrelated sires were used to produce the spring replicate, genetic variation was not likely a factor in the significant differences found between seasons. This is because the r-squared for our model was quite high, greater than 0.95, indicating a well fitting model. Therefore, using the same sires for both seasons is not essential because a well balanced model is already present.

Currently, according to Robinson and Buhr (2005), traits that are evaluated when determining replacement and superior genetic potential include traits such as growth rate, back fat, lean yield, feed efficiency and litter size (total number born or total number born alive). Clark et al (1989) disagrees with using litter size as a measure of boar fertility. Instead, conception rate would be expected to be better measure of boar fertility because neither boars nor their semen characteristics have been associated consistently with litter size, where as both have been associated with conception rate. It will, thus, be interesting to compare the results of future heterospermic inseminations involving the boars in this study to the results from semen analysis. While certain boars consistently yield ejaculates with high motility and normal morphology, do they also sire the most piglets in the litter when competing with another boar's spermatozoa?

Other recommendations for future studies to enhance sperm production would include examining the endocrine component of testicular size. Sharpe (1993) reported

that testosterone, gonadotropins, and growth hormone control major testicular functions such as the regulation of spermatogenic waves and the establishment of Sertoli and Leydig cell populations. Perhaps the identified period of extensive mitotic activity that occurs during the first month of life could be a plausible time for manipulation via exogenous hormonal supplementation. One study administered FSH or growth hormone to boars between 8 and 40 days of age and found that FSH increased Sertoli cells while growth hormone promoted tubular and Sertoli cell maturation (Swanlund et al., 1995). If litter size manipulation proves to be completely unsuccessful, administration of exogenous gonadotropins and prostaglandins may be beneficial for increasing sperm production.

Body weight and the amount of seminal plasma proteins were observed to be affected by litter size in this study. Other variables such as testicular size and semen parameters did not prove to be influenced by litter size in the preliminary results. The study is on-going and the longevity of the data collection will supply more evidence to either support or refute the original hypothesis. Although the preliminary data analyzed did not convincingly prove that litter size affects semen quality, it did provide enough evidence, especially with regards to body weight and seminal plasma proteins, to yield continued research. If long-term data unveil more supportive evidence of this hypthesis, then manipulation of litter size would be an inexpensive alternative for swine producers to employ and, therefore, perhaps increase herd efficiency and decrease production costs.

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Table 1. Summary of significant effects for all dependent variables for all boars through approximately 60 weeks of age.

Dependent Variable	3-way	2-way	Main Effects
Body Weight (kg)	p=<.0001	Fall: Trt x Age p=.0116 Spring: Trt x Age p=.0152	Fall: Age 3 wks (p<.05) Spring: Age (p<.05) (for all except ages: 20,24, & 28 wks)
Testicle Height (cm)	No	Season x Age p<.0001	Fall-Small: Age p<.0001 Fall-Large: Age p<.0001 Spring-Small: Age p<.0001 Spring-Large: Age p<.0001
Testicle With (cm)	No	Season x Age p<.0001	Fall-Small: Age p<.0001 Fall-Large: Age p<.0001 Spring-Small: Age p<.0001 Spring-Large: Age p<.0001
Testicular Area (cm^2)	No	Season x Age p<.0001 Season x Trt p=.1017	Fall-Small: Age p<.0001 Fall-Large: Age p<.0001 Spring-Small: Age p<.0001 Spring-Large: Age p<.0001
Ratio of Testicle Area To Body Weight (%)	No	Season x Age p<.0001	Fall-Small: Age p<.0001 Fall-Large: Age p<.0001 Spring-Small: Age p<.0001 Spring-Large: Age p<.0001
Concentration (millions)	No	Season x Age p=.0641	Fall-Small: Age p<.0001 Fall-Large: Age p<.0004 Spring-Small: Age p=.8398 Spring-Large: Age p=.2419

Table 1, continued.

Total # of Sperm (billions)	No	No	Age p<.0001
Volume of Ejaculate (mL)	No	Season x Trt p=.0229	Fall-Small: Age p=.2985 Fall-Large: Age p=.2202 Spring-Small: Age p=.2205 Spring-Large: Age p=.4430
Motility (% Normal)	No	Season x Age p=.0023	Fall-Small: Age p=.1376 Fall-Large: Age p=.0977 Spring-Small: Age p=.0014 Spring-Large: Age p=.0671
Morphology (% Normal)	No	No	Age p<.0001
Acrosin Activity (%)	No	Season x Age p=.0173	Fall-Small: Age p<.0001 Fall-Large: Age p=.0001 Spring-Small: Age p=.8771 Spring-Large: Age p=.1854
Uncapacitated (% Uncapacitated)	No	Season x Age p=.0901 Season x Trt p=.0841	Fall-Small: Age p=.0242 Fall-Large: Age p=.0108 Spring-Small: Age p=.4796 Spring-Large: Age p=.3022
Capacitated (% Capacitated)	No	No	Age p<.0001
Acrosome Reacted (% acrosome reacted)	No	No	Age p=.0002

Table 1, continued.

Abnormal (% abnormal)	No	Season x Age .0676	Fall-Small: Age p<.0001 Fall-Large: Age p<.0001 Spring-Small: Age p=.8910 Spring-Large: Age p=.1857
Seminal Plasma Proteins	No	No	Trt: p=.1063 Age: p=.0012

Table 2. Mean weights (kg) for all boars born in the fall.

Ages (wks) for Fall Born Boars	Small Litter Mean ± S.E.	N	Large Litter Mean ± S.E.	N
1	3.2 ± 0.2	9	3.1 ± 0.2	9
2	5.8 ± 0.2	9	5.3 ± 0.3	9
3	8.3 ± 0.2*	9	7.4 ± 0.4	9
6	14.1 ± 0.6	9	13.8 ± 0.6	9
9	27.1 ± 0.9	9	27.7 ± 0.9	9
13	49.8 ± 1.8	9	50.1 ± 1.8	9
17	79.7 ± 2.4	9	81.5 ± 2.4	9
20	101.6 ± 3.0	9	104.4 ± 3.1	9
24	123.9 ± 3.4	9	129.9 ± 3.9	9
31	135.5 ± 3.5	9	144.6 ± 5.3	9
34	143.6 ± 3.0	8	153.0 ± 5.1	8
37	151.4 ± 2.5	8	162.0 ± 5.7	8
40	157.3 ± 3.6	8	165.3 ± 6.0	8
43	162.5 ± 3.9	8	168.8 ± 6.8	8
47	163.1 ± 4.1	4	173.2 ± 7.4	4
50	164.9 ± 1.6	4	176.7 ± 8.1	4
53	166.4 ± 2.2	4	181.0 ± 9.2	4
57	185.9 ± 3.4	4	189.9 ± 6.8	4
60	188.3 ± 2.5	4	189.3 ± 8.5	3

*** mean weight (kg) for small litter boars is significantly different from large litter boars ($p<.05$)**

Table 3. Mean weights (kg) for all boars born in the spring.

Ages (wks) for Spring Born Boars	Small Litter Mean ± S.E.	N	Large Litter Mean ± S.E.	N
1	3.1 ± 0.2*	4	2.1 ± 0.1	4
2	5.7 ± 0.3*	4	3.8 ± 0.4	4
3	8.7 ± 0.3*	4	5.6 ± 0.5	4
6	14.1 ± 0.6*	4	10.1 ± 0.8	4
9	27.0 ± 0.9*	4	21.4 ± 0.9	4
13	41.4 ± 1.5*	4	33.6 ± 1.8	4
15	59.8 ± 3.0*	4	49.3 ± 2.9	4
17	81.3 ± 3.9*	4	67.1 ± 3.2	4
20	100.5 ± 5.6	4	86.1 ± 4.6	4
24	111.7 ± 5.6	4	97.4 ± 3.3	4
28	108.7 ± 6.5	4	100.6 ± 1.4	4
31	126.7 ± 5.5*	4	109.7 ± 3.0	4
34	139.2 ± 6.2*	4	118.6 ± 4.5	4
37	134.0 ± 4.4*	4	114.0 ± 6.1	4

*** mean weight (kg) for small litter boars is significantly different from large litter boars ($p<.05$)**

Table 4. Seminal plasma protein levels (relative units) for boars born in the fall.

Fall Born Only	**N**	**Mean ± S.E.**
Small Litter Boars / 35 Wks	**16**	**106.9 ± 4.3**
Large Litter Boars / 35 Wks	**16**	**124.0 ± 7.3**
Small Litter Boars / 45 Wks	**16**	**94.9 ± 4.1**
Large Litter Boars / 45 Wks	**16**	**99.6 ± 5.6**

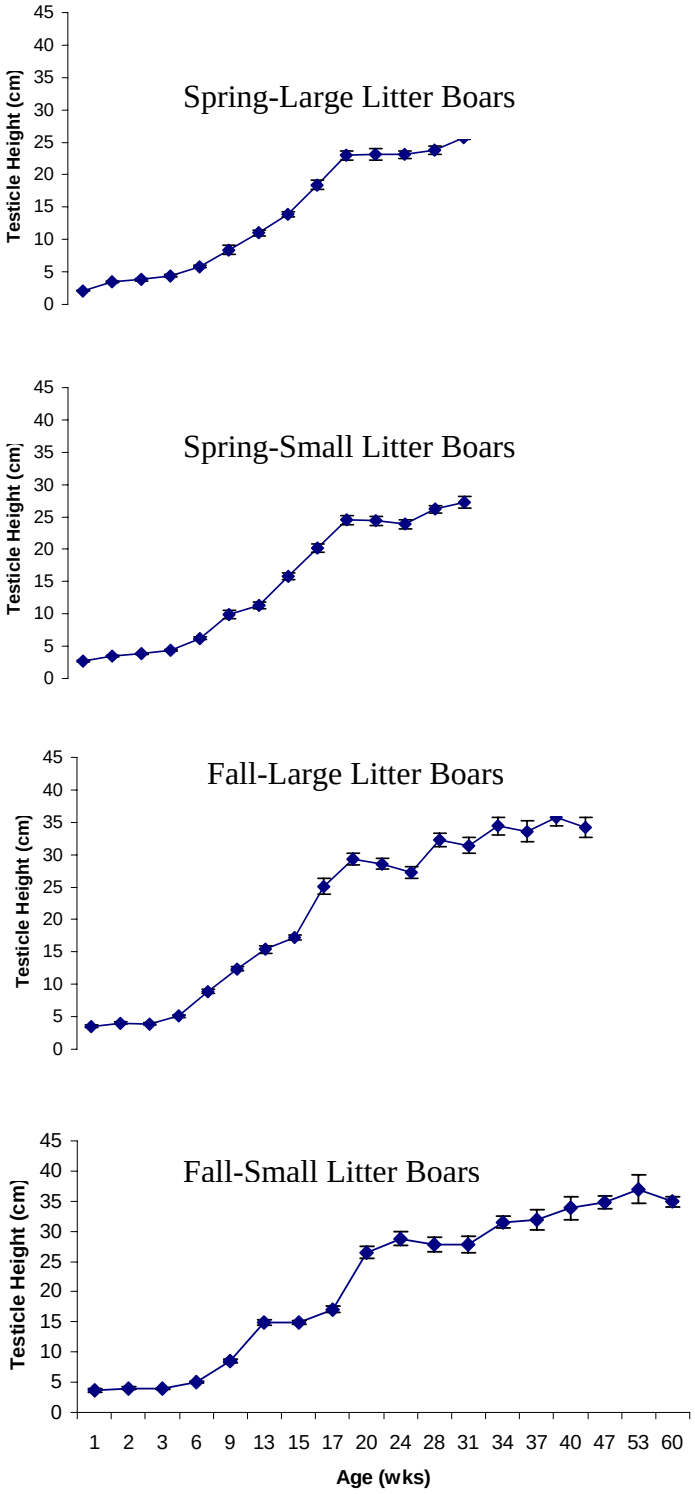


Figure 6. Testicle height (cm) for all boars across both seasons and treatments.

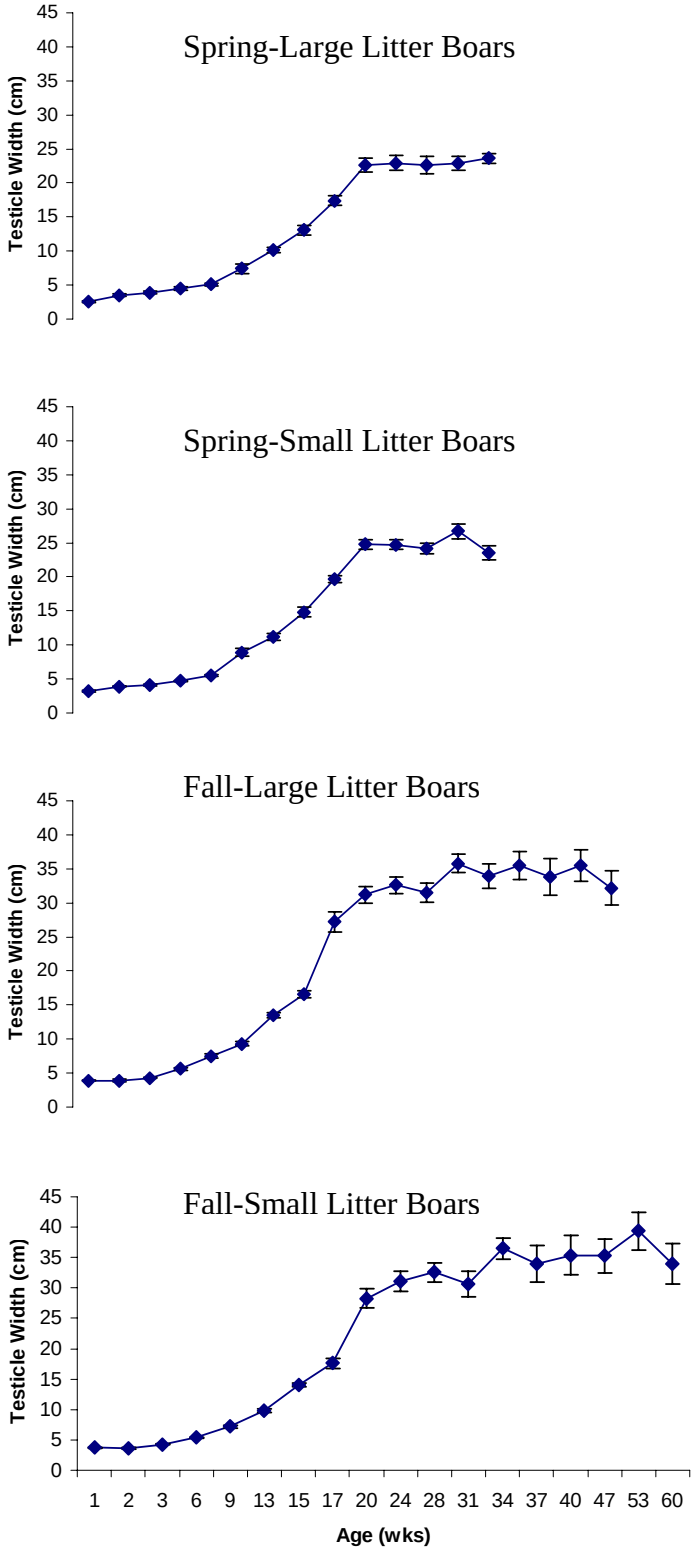


Figure 7. Testicle width (cm) for all boars across both seasons and treatments.

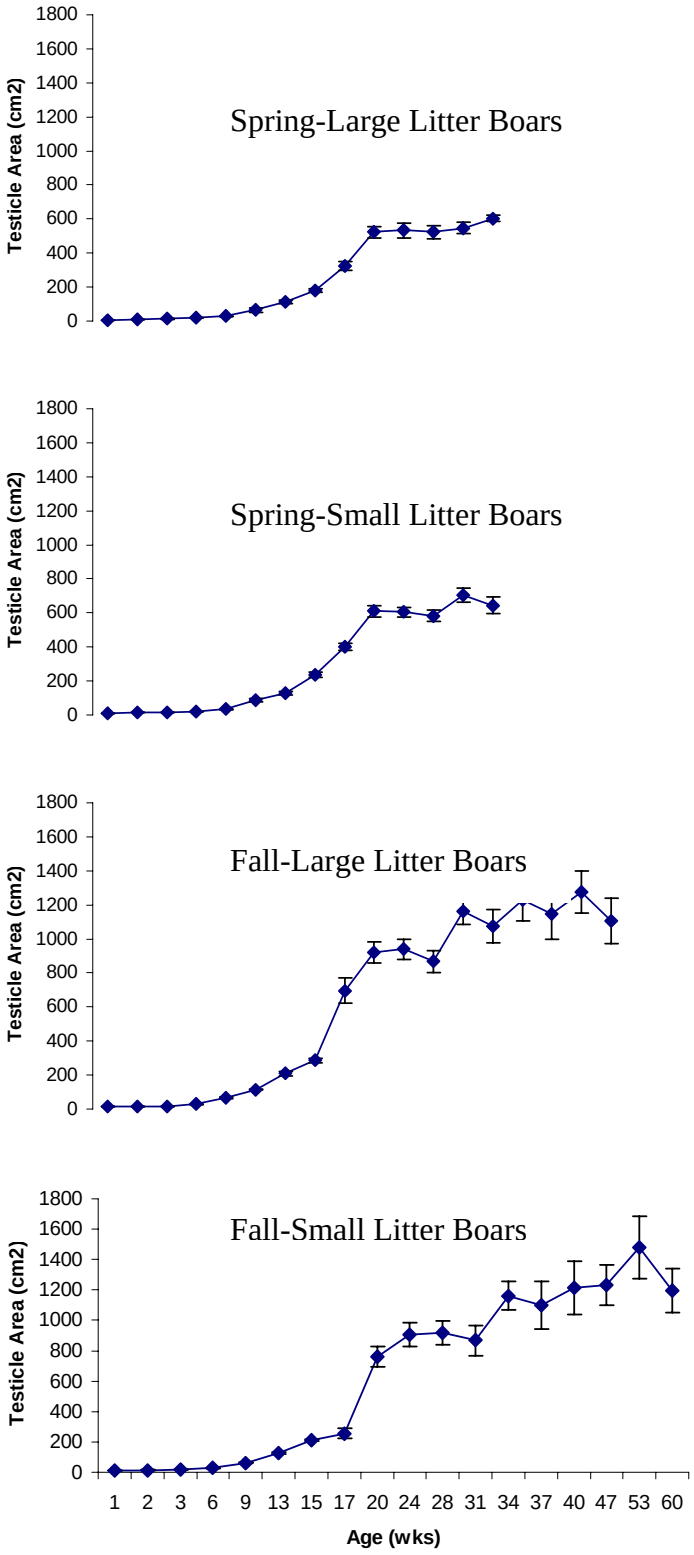


Figure 8. Testicular area (cm²) for all boars across both seasons and treatments.

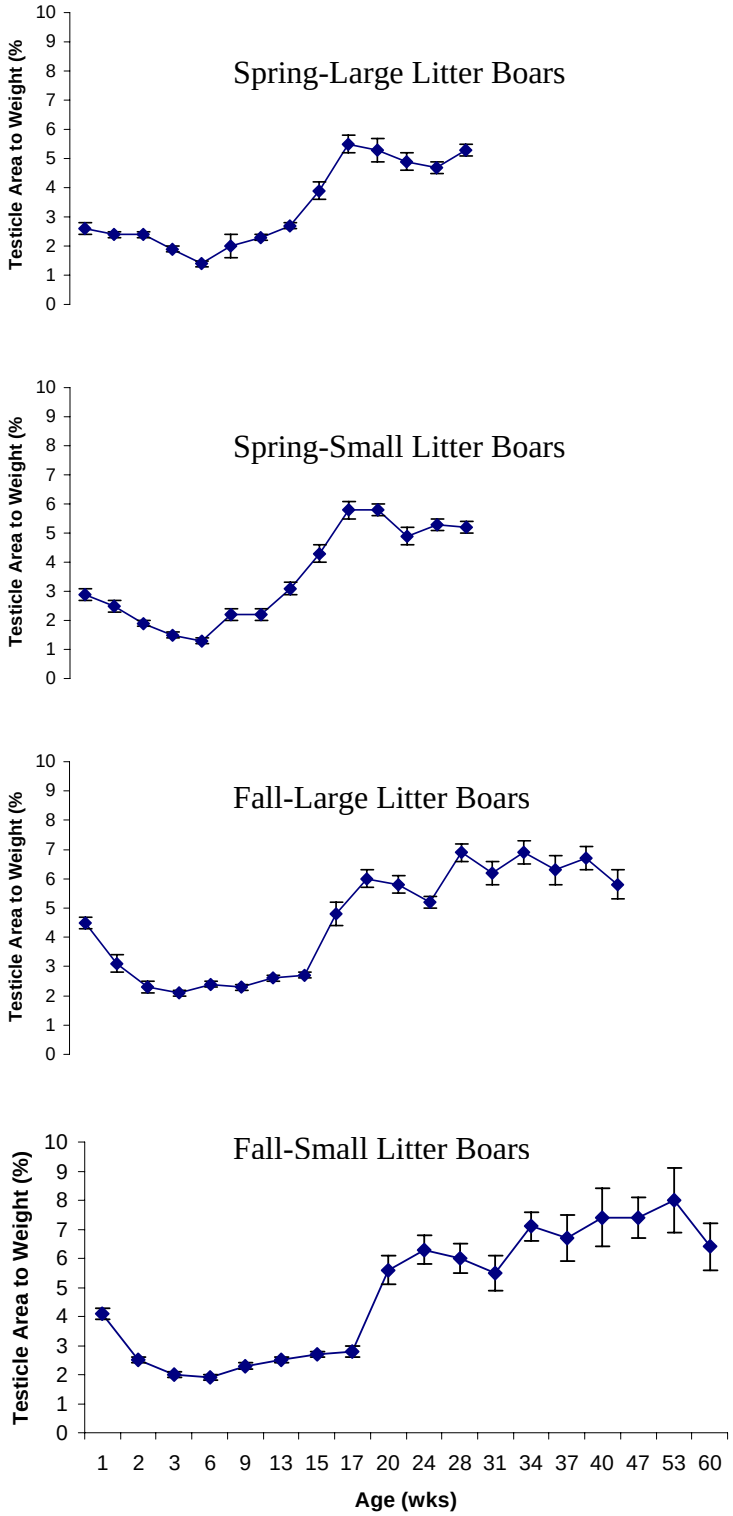


Figure 9. Ratio of testicle area to body weight (%) for all boars across both seasons and treatments.

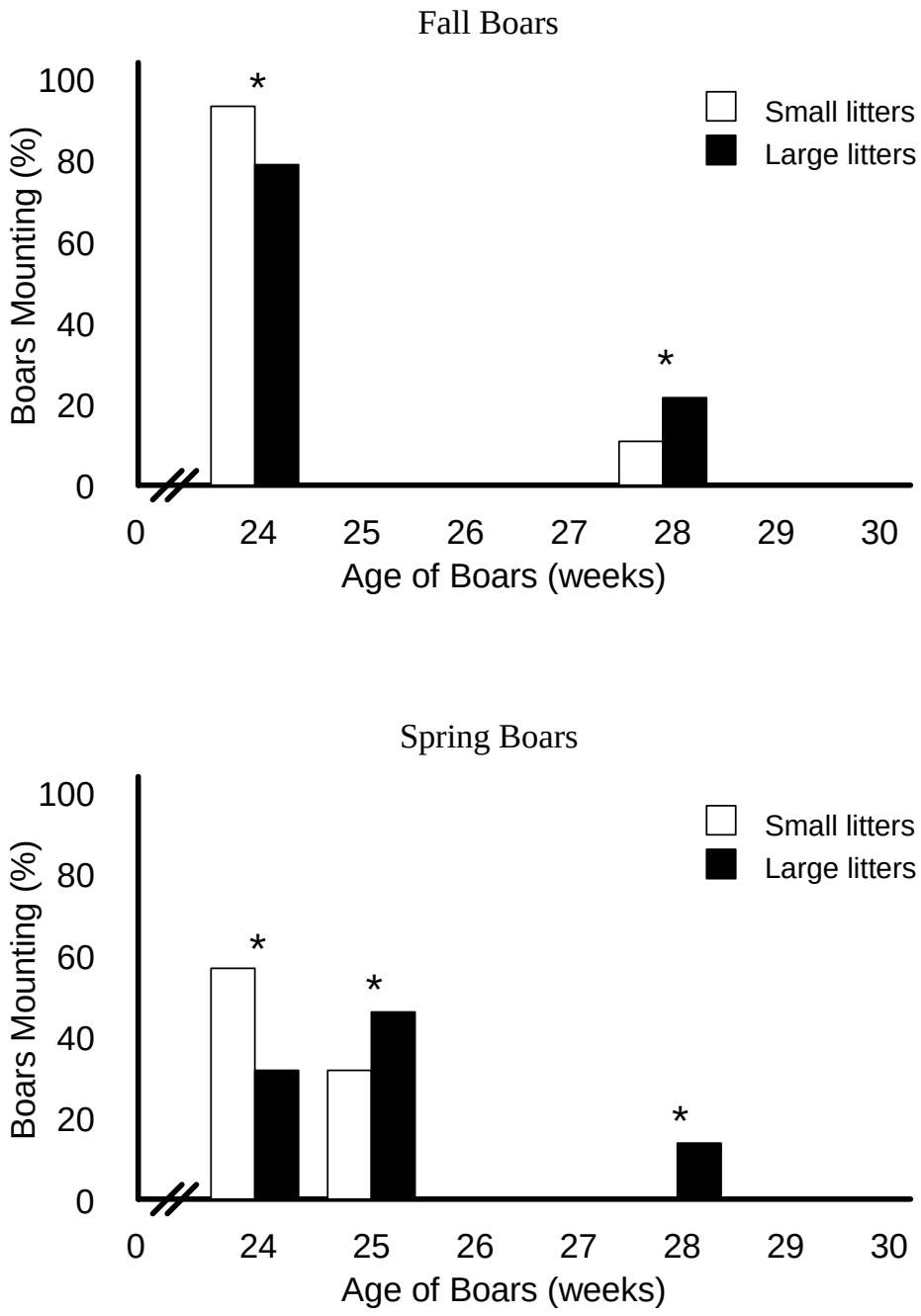


Figure 10. Effect of litter size, age and season on the first successful mounting by boars on the dummy sow during the 6-week training period. A significant litter size by age by season interaction was present (p=.04). Therefore, differences between boars raised in small and large litters within each season and age group were evaluated. An * indicates a significant difference (p<.05).

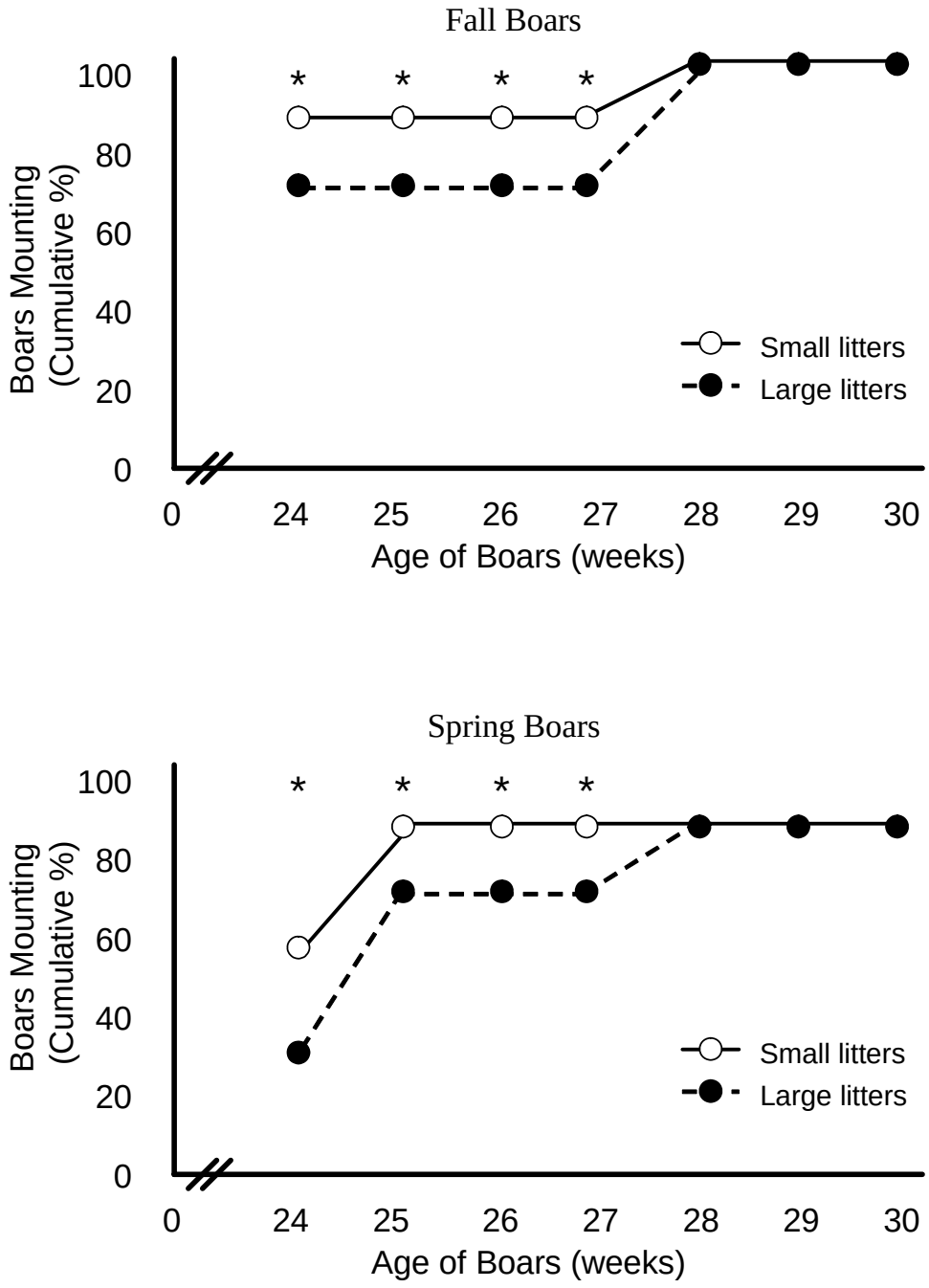


Figure 11. Effect of litter size, age and season on cumulative percentage of boars mounting the dummy sow during the 6-week training period. A significant litter size by age by season interaction was present (p=.05). Therefore, differences between boars raised in small and large litters within each season and age group were evaluated. An * indicates a significant difference (p<.05).

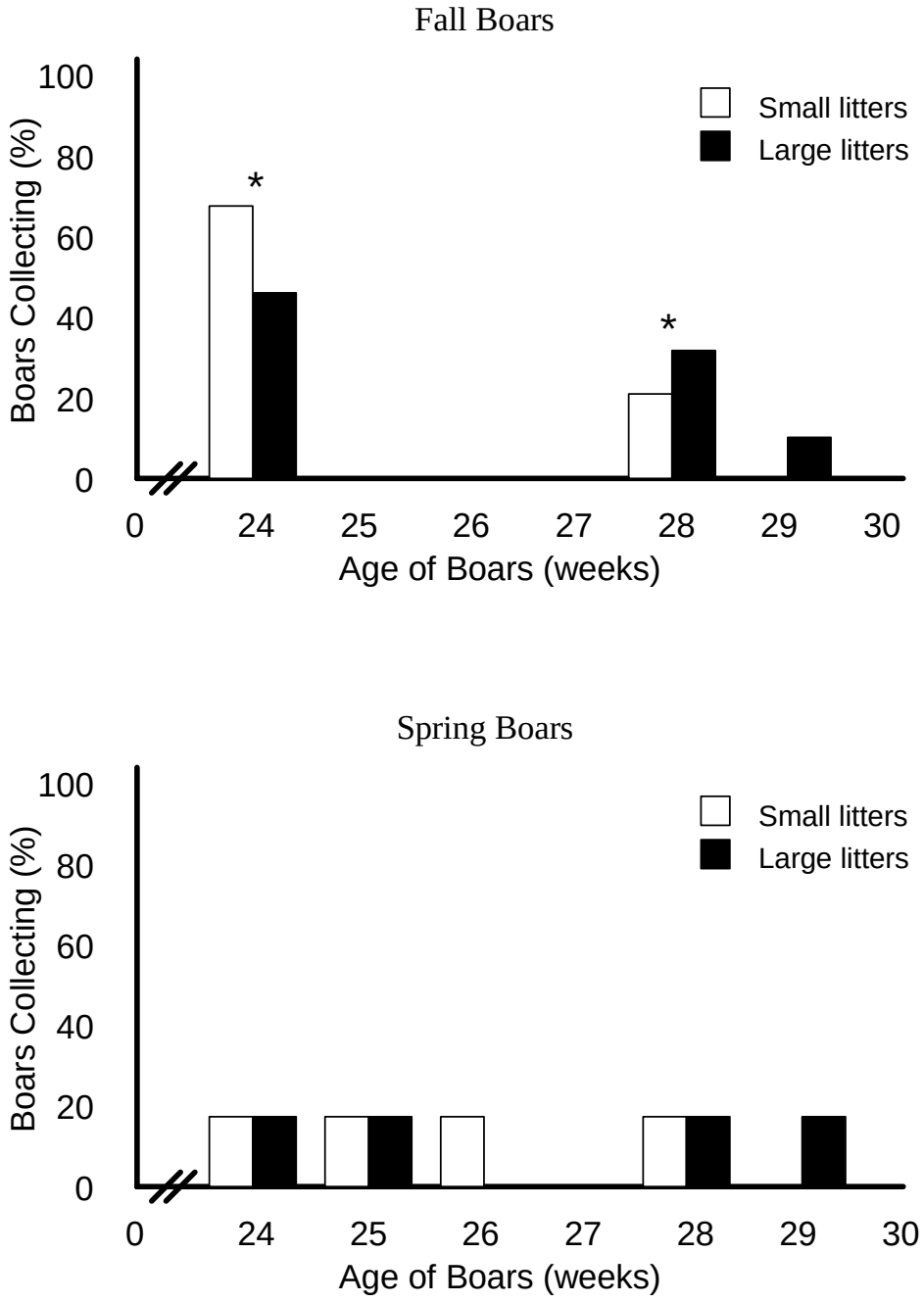


Figure 12. Effect of litter size, age and season on the first successful collection of boars off the dummy sow during the 6-week training period. A significant litter size by age by season interaction was present (p=.03). Therefore, differences between boars raised in small and large litters within each season and age group were evaluated. An * indicates a significant difference (p<.05).

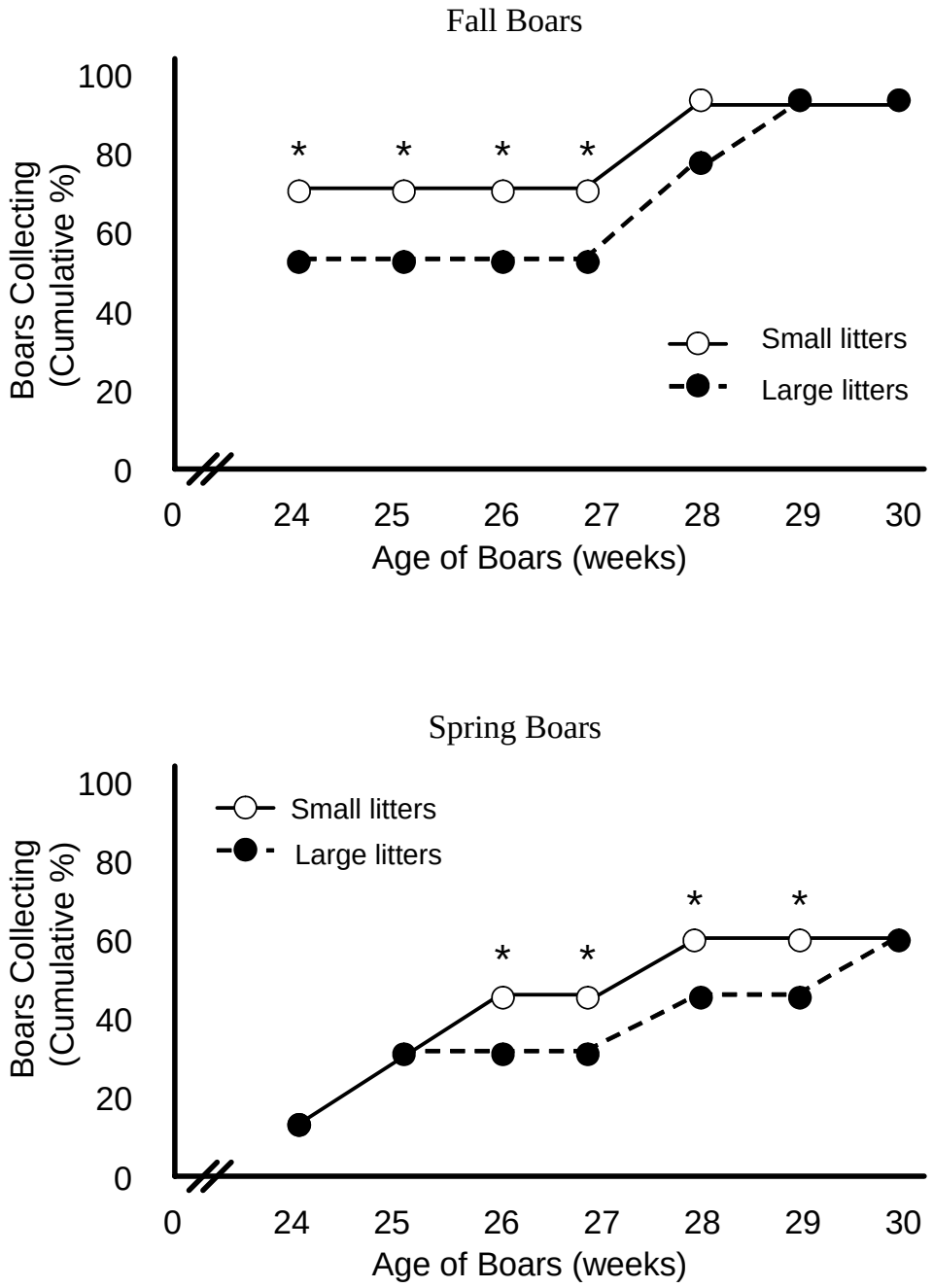


Figure 13. Effect of litter size, age and season on cumulative percentage of boars collected off the dummy sow during the 6-week training period. A significant litter size by age by season interaction was present (p=.01). Therefore, differences between boars raised in small and large litters within each season and age group were evaluated. An * indicates a significant difference (p<.05).

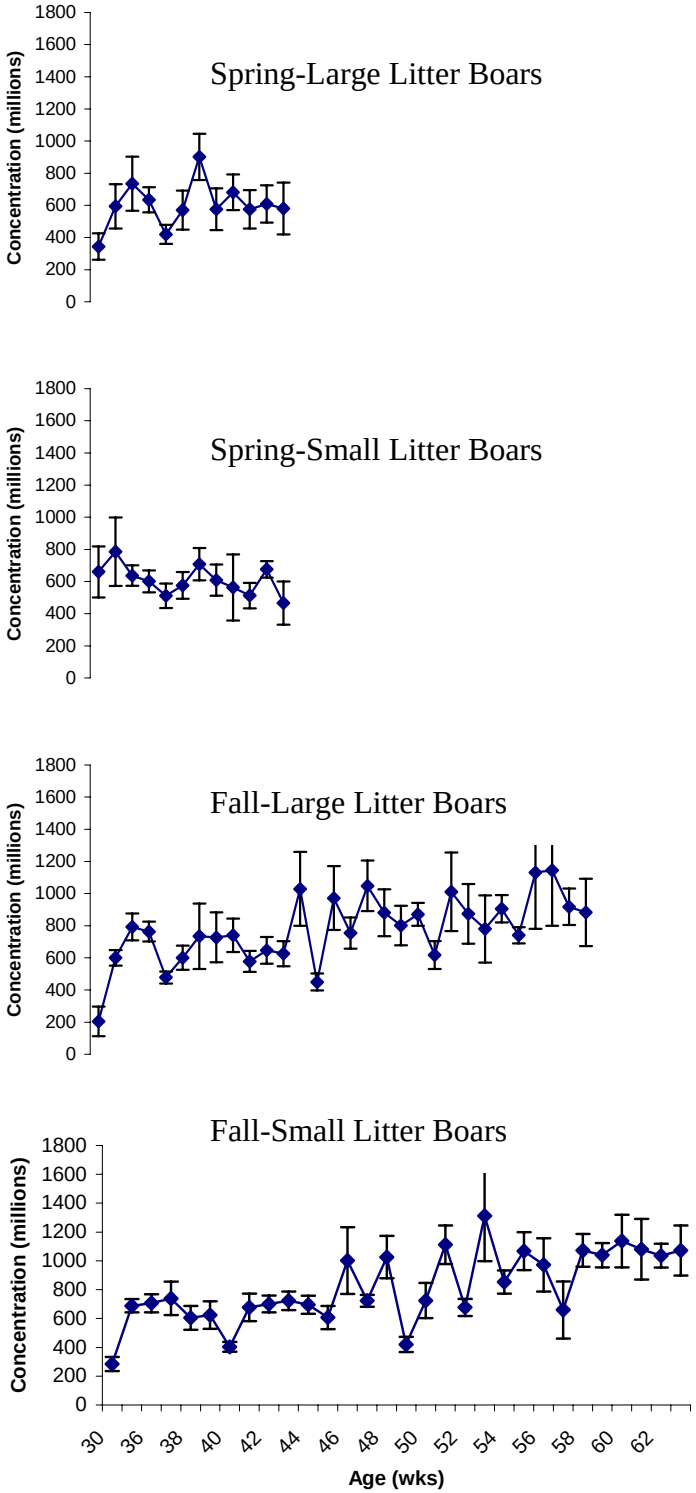


Figure 14. Concentration of spermatozoa (millions) for all boars across both seasons and treatments.

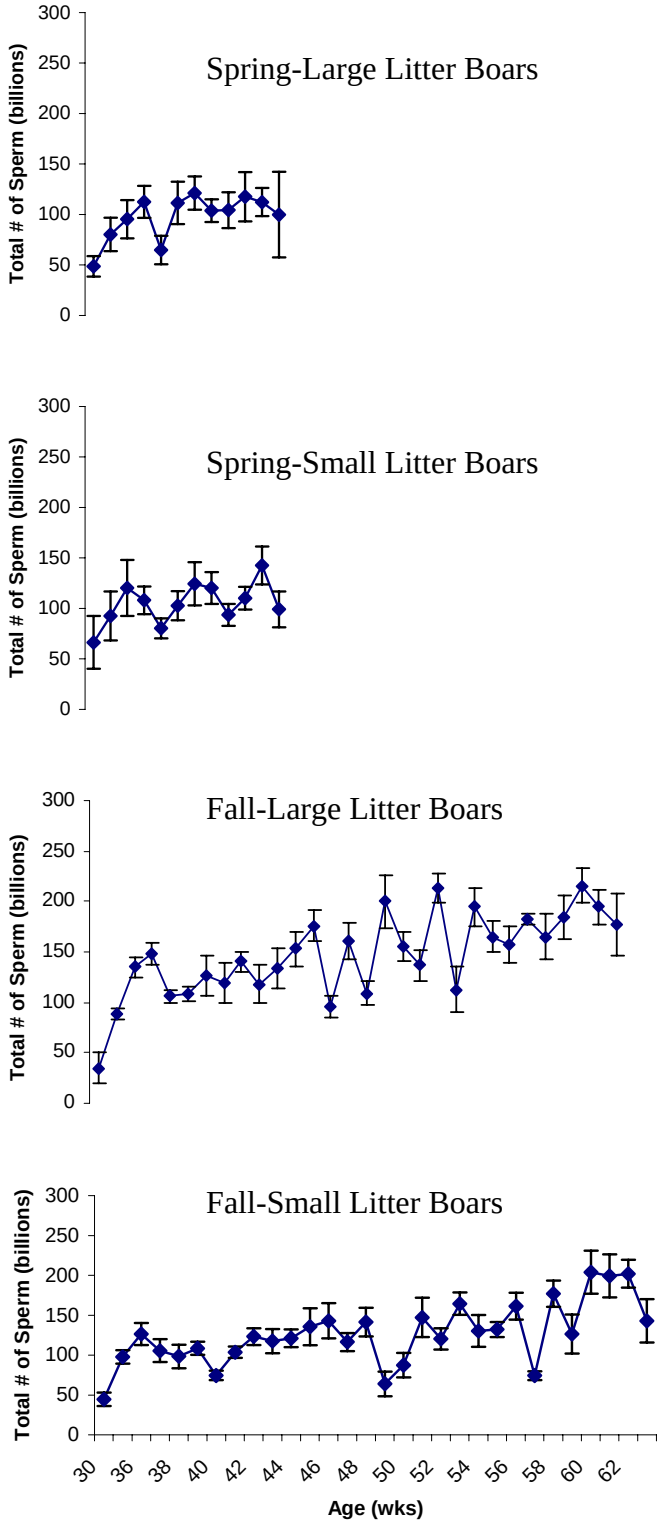


Figure 15. Total number of spermatozoa (billions) per ejaculate for all boars across both seasons and treatments.

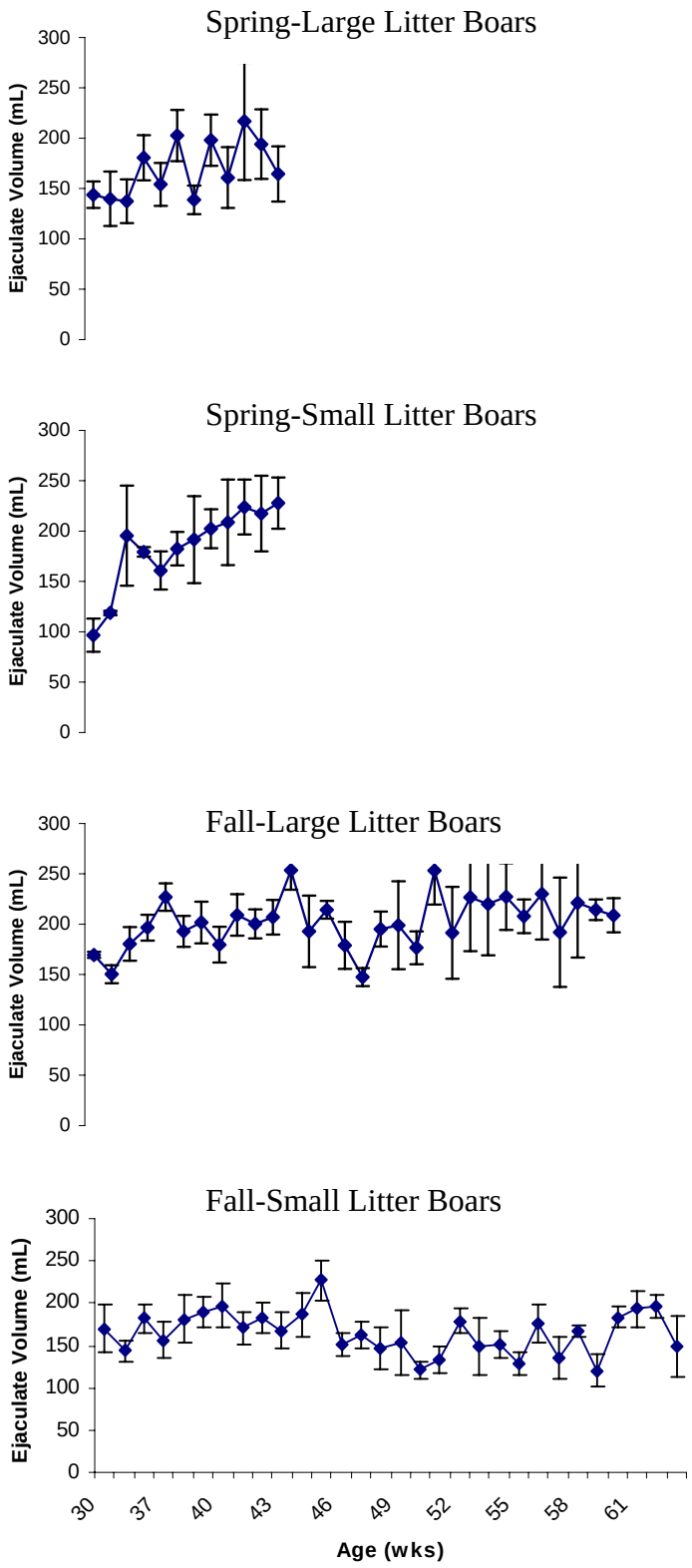


Figure 16. Ejaculate volume (mL) for all boars across both seasons and treatments.

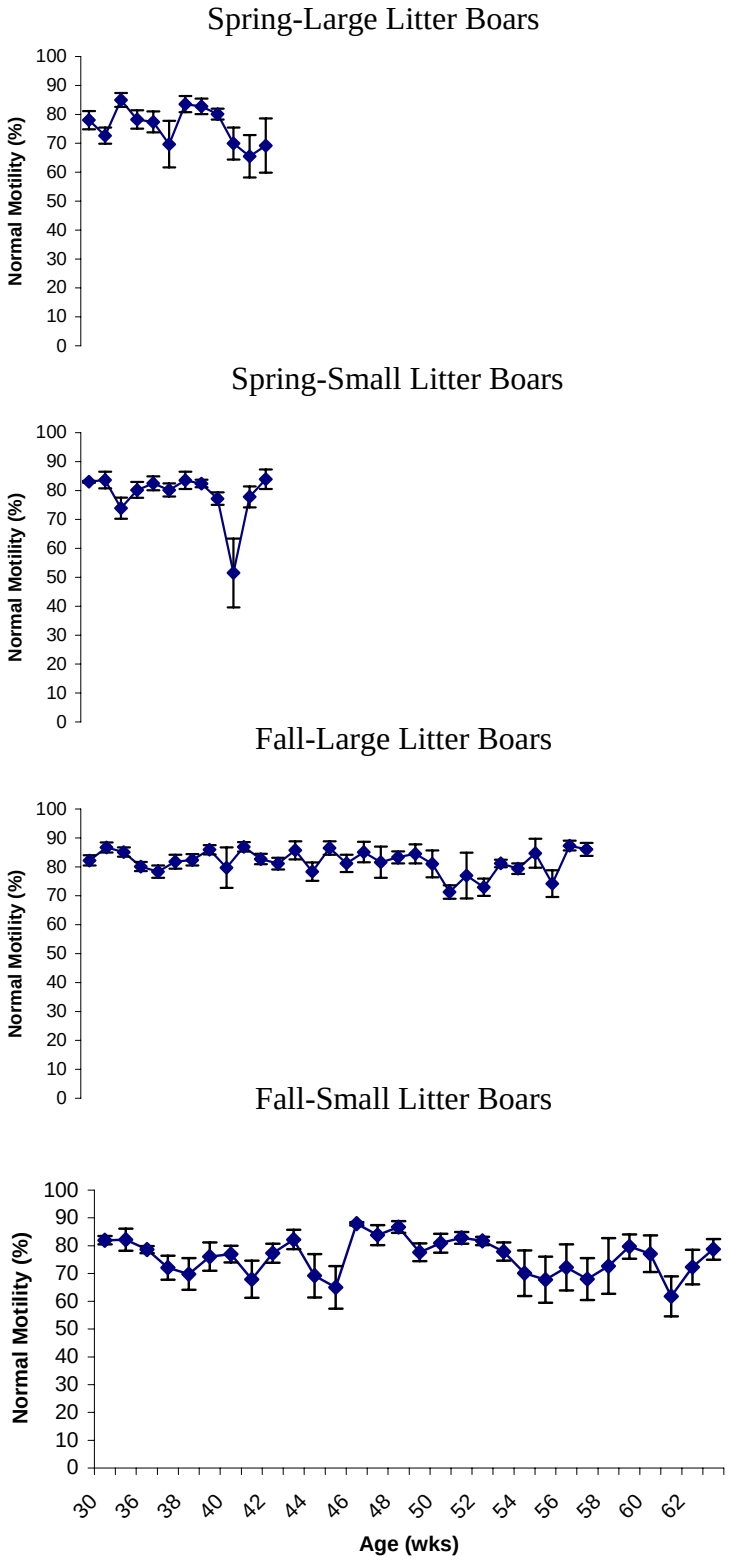


Figure 17. Percentage of motile spermatozoa per ejaculate for all boars across both seasons and treatments.

Figure 18. Percentage of morphologically normal spermatozoa per ejaculate for all boars across both seasons and treatments.

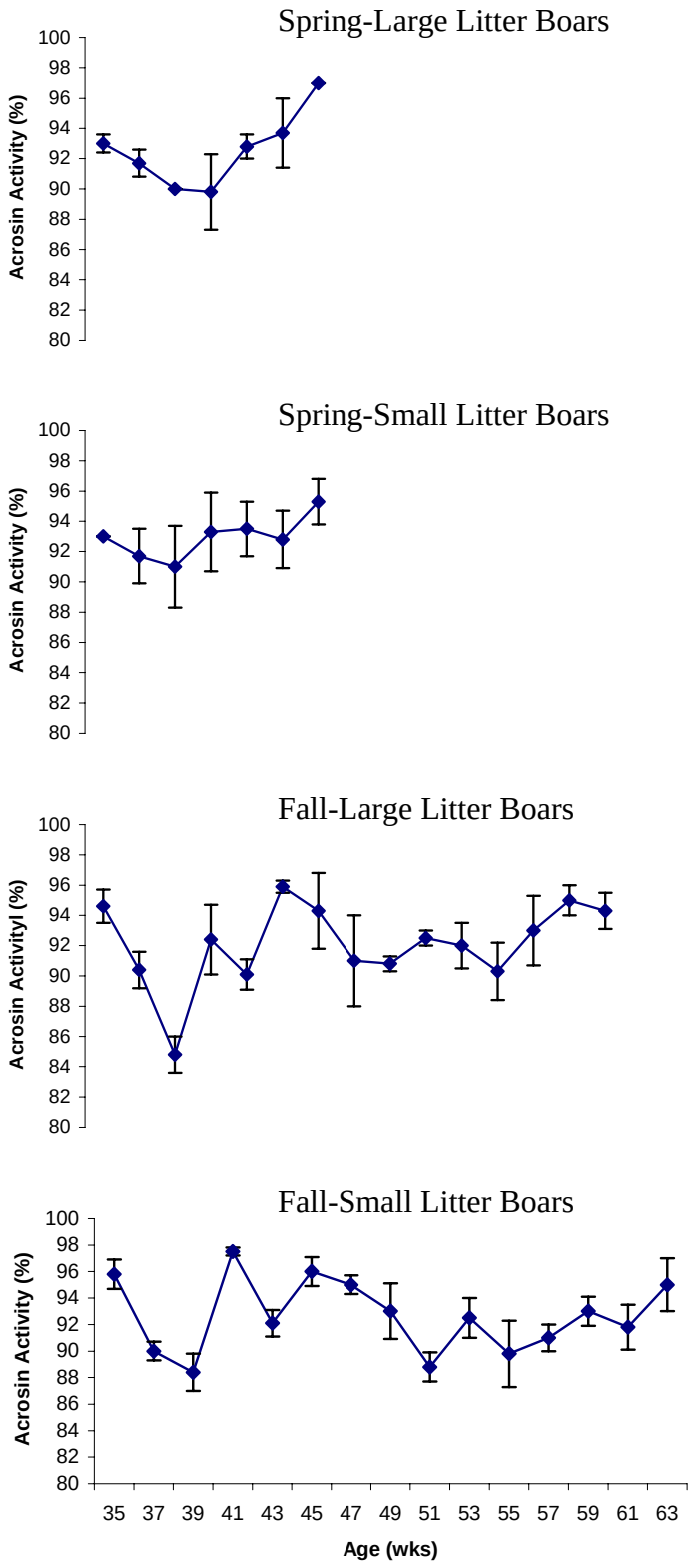


Figure 19. Percentage of spermatozoa with acrosin activity per ejaculate for all boars across both seasons and treatments.

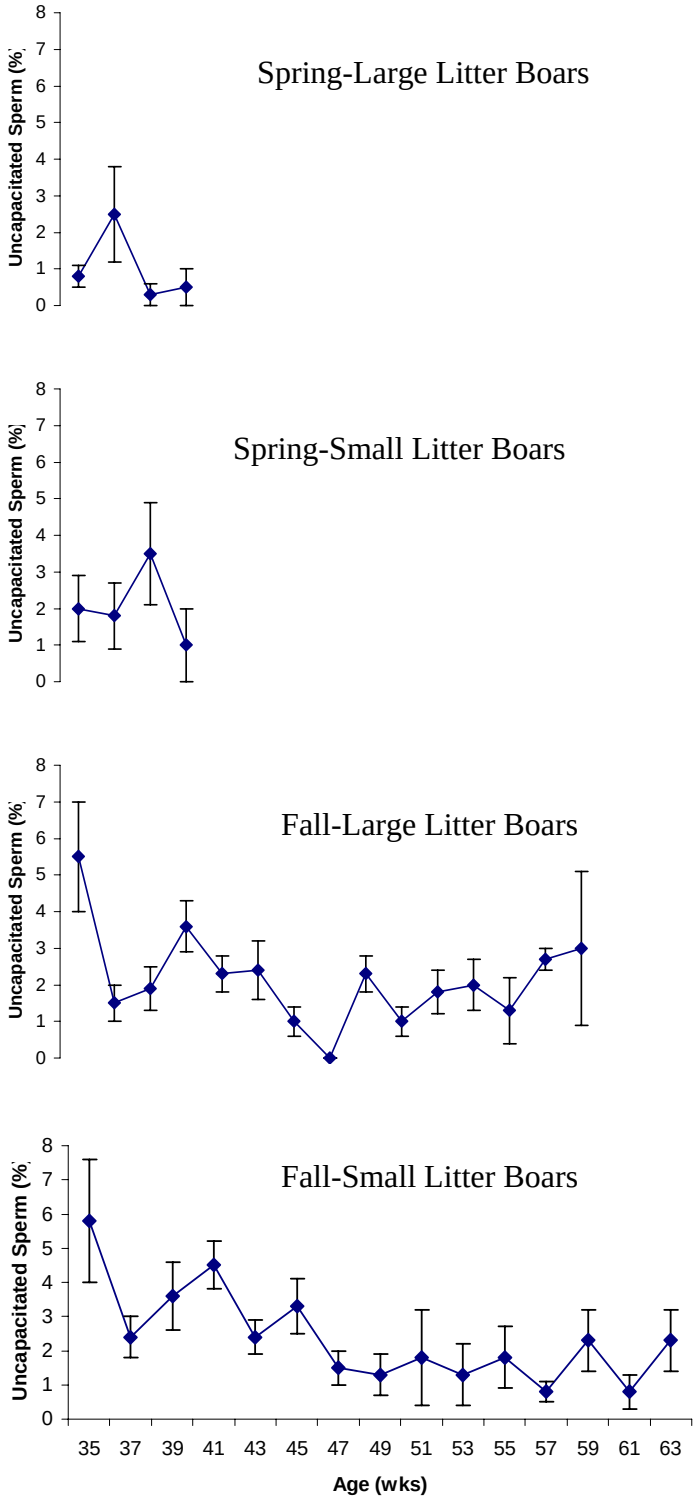


Figure 20. Percentage of uncapacitated spermatozoa per ejaculate for all boars across both seasons and treatments.

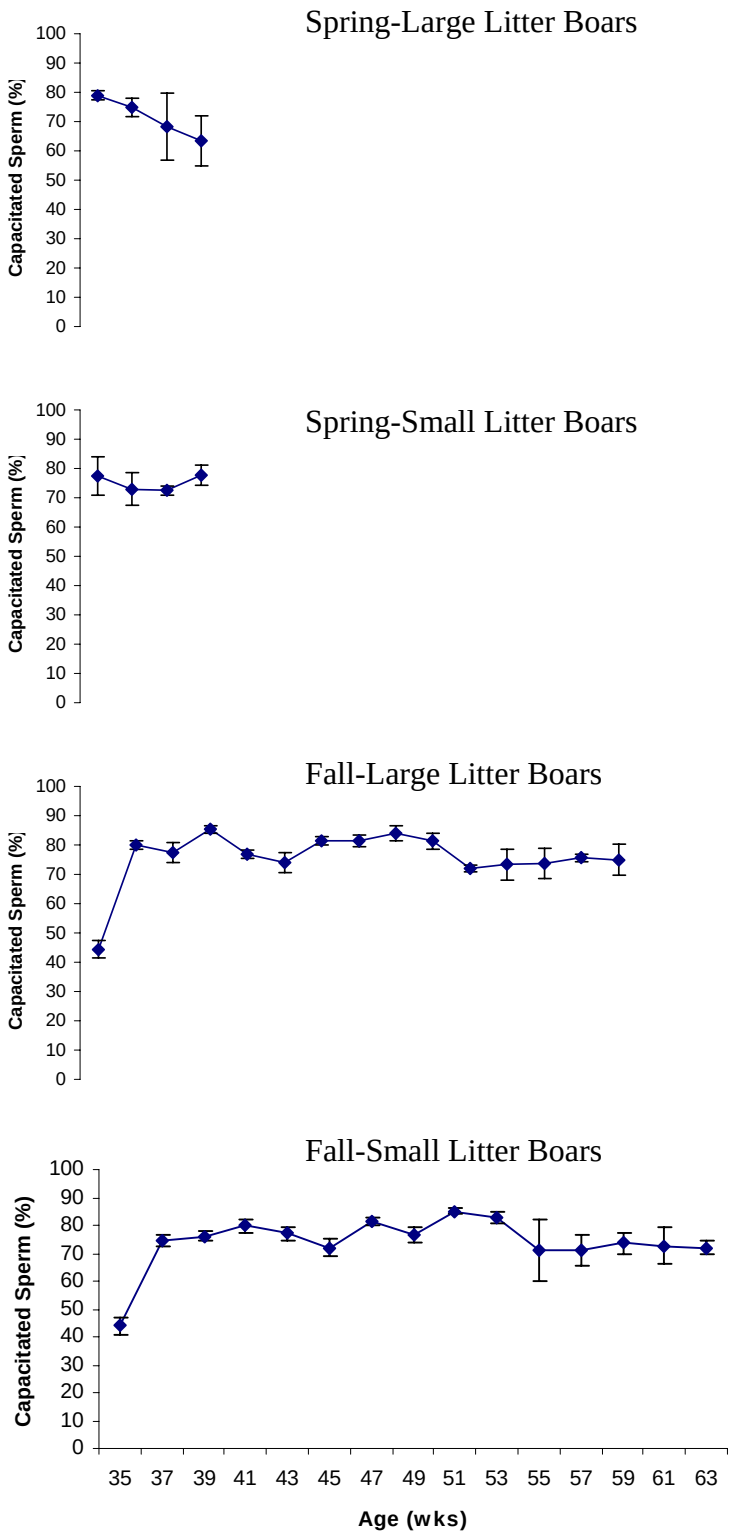


Figure 21. Percentage of capacitated spermatozoa per ejaculate for all boars across both seasons and treatments.

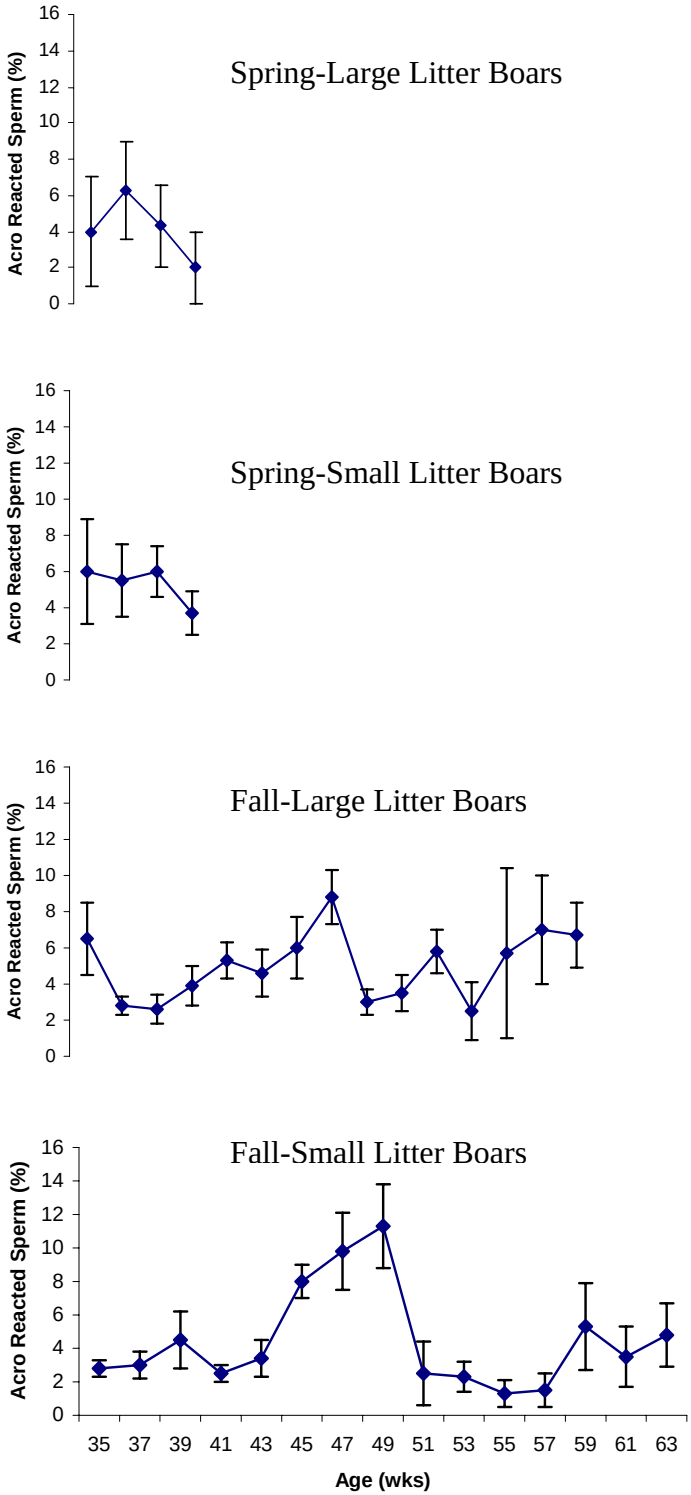


Figure 22. Percentage of spermatozoa that had undergone the acrosome reaction per ejaculate for all boars across both seasons and treatments.

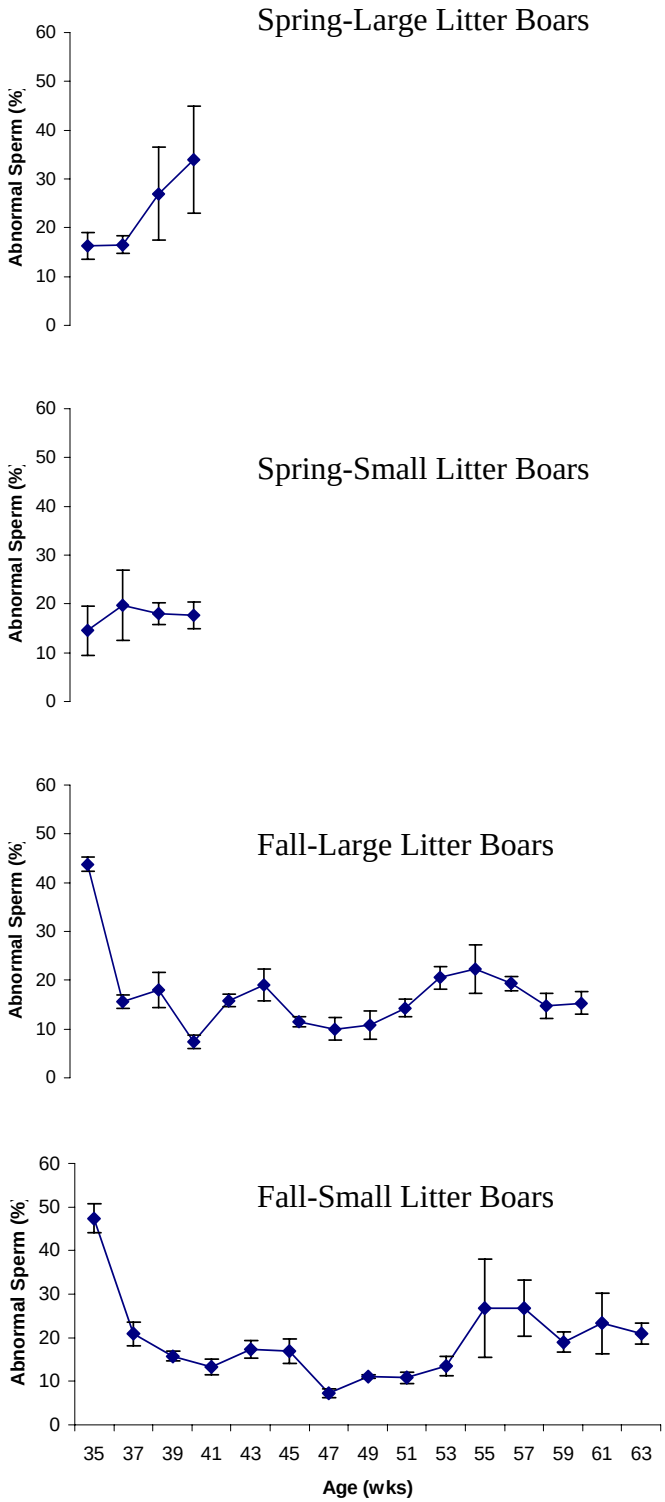


Figure 23. Percentage of abnormal spermatozoa per ejaculate for all boars across both seasons and treatments.

Appendix A: SAS Code

Weight and Testicle Repeated Measures

```
Proc glm;
Classes season aage trt;
Model wt tht twd tarea taperwt=trt season aage trt*aage trt*season season*aage
    trt*season*aage id(trt);
Test h=trt e=id(trt);
Run;
```

Semen Parameters Repeated Measures

```
Proc glm;
Classes season aage trt;
Model con tot vol mot mor gel uncap cap acro abnorm=trt season aage trt*aage
    trt*season season*aage trt*season*aage id(trt);
Test h=trt e=id(trt);
Run;
```

Seminal Plasma Proteins Repeated Meaures

```
Proc glm;
Classes trt aage;
Model norvol= trt aage trt*aage id(trt);
Test h=trt e=id(trt);
Run;
```

Weight and Testicle Probability Values & Least-Squares Means

```
Proc glm; by season trt;
Classes season aage trt;
Model wt tht twd tarea taperwt=aage;
LSMEANS aage / PDIFF;
Run;
```

Semen Analysis Probability Values & Least-Squares Means

```
Proc glm; by season trt;
Classes season aage trt;
Model con tot vol mot mor gel uncap cap acro abnorm=aage;
LSMEANS aage / PDIFF;
Run;
```

Seminal Plasma Proteins Probability Values & Least-Squares Means

```
Proc glm; by trt;
Classes aage trt;
Model norvol=aage;
LSMEANS aage / PDIFF;
Run;
```

Weight and Testicle Means & Stardard Errors

```
Proc Means mean n max min stddev stderr maxdec=1;
Var wt tht twd tarea taperwt;
Run;
```

Semen Parameters Means & Standard Errors

```
Proc Means mean n max min stddev maxdec=1;
Var con tot vol mot mor gel uncap cap acro abnorm;
Run;
```

Seminal Plasma Proteins Means & Standard Errors

```
Proc Means mean n max min stddev maxdec=1;
Var protein norvol;
Run;
```

Appendix B. Boars

Each boar for both replicates is listed below by boar number and status of participation in the study

Fall Replicate:

37206 – *selected to continue through duration of study*

37208 – *selected to continue through duration of study*

37209 – *selected to continue through duration of study*

31208 – *selected to continue through duration of study*, removed from study due to bad attitude (11-22-04)

31209 – *selected to continue through duration of study*

31210 – removed from study after selection of final 8

37711 - removed from study after selection of final 8

37308 – removed from study after selection of final 8

37309 – did not learn to mount and collect from dummy sow, removed from the study

37311 – removed from study after selection of final 8

37904 – *selected to continue through duration of study*

37907 – did not learn to mount and collect from dummy sow, removed from the study

37909 – removed from study after selection of final 8

37911 – *selected to continue through duration of study*

37912 – removed from study after selection of final 8

37604 – removed from study after selection of final 8

37605 – *selected to continue through duration of study*

37607 – removed from study after selection of final 8

Spring Replicate: *All boars in the Spring replicate became sick with scours for about 4-5 days while in the farrowing house. All piglets were treated with gentamyacin.*

08509 – *continued for duration of study*

08511 – *continued for duration of study*

08513 – culled due to bad hind leg 9-2-04

08606 – culled 11-9-04 due to bad legs and feet

08607 – housed in the sick pen during nursery & finishing / died on 5-30-04

08609 – did not mount & collect / removed from the study

08610 – did not mount & collect / removed from the study

08906 – *continued for duration of study*

08907 – did not mount & collect / removed from the study

08910 – *continued for duration of study*

08911 – removed from study on 3-30-04

09006 – *continued for duration of study*

09012 - *continued for duration of study /* died 1-21-05 / did mount and collect

09014 – did not mount & collect / removed from the study

09410 – culled 11-9-04 due to blindness

09609 – *continued for duration of study /* housed in the sick pen during nursery & finishing

09610 – *continued for duration of study*

09711 – removed from study on 3-23-04 because he "fell behind" in terms of growth

Appendix C. Sows

Sows for both replicates are listed below along with general information and the size litter she nursed.

Fall Replicate:

Sow # 0133802 – Nursed small litter (n=6)
Date of Birth: 9-27-01
Parity: 4 (including the present)
Avg. Total Born: 17.3
Avg. Born Alive: 12.5
Avg. Weaning Weight: 6.4 kg
Litter ID # 373

Sow # 0023703 – Nursed small litter (n=6)
Date of Birth: 7-19-00
Parity: 6
Avg. Total Born: 15.4
Avg. Born Alive: 13.2
Avg. Weaning Weight: 6.3 kg
Litter ID # 372

Sow # 0205703 – Nursed large litter (n=10)
Date of Birth: 2-15-02
Parity: 2
Avg. Total Born: 12.0
Avg. Born Alive: 12.0
Avg. Weaning Weight: 5.4 kg
Litter ID # 379

Sow # 022202 – Nursed small litter (n=6)
Date of Birth: 7-4-02
Parity: 2
Avg. Total Born: 17.0
Avg. Born Alive: 14.0
Avg. Weaning Weight: 5.0 kg
Litter ID # 376

Sow # 0124907 – Nursed large litter (n=10)
Date of Birth: 7-19-01
Parity: 4
Avg. Total Born: 8.7
Avg. Born Alive: 8.0
Avg. Weaning Weight: 8.2 kg
Litter ID # 77

Spring Replicate:

Sow # 0133902 – Nursed small litter (n=6)
Date of Birth: 9-27-01
Parity: 4 (including the present)
Avg. Total Born: 12.3
Avg. Born Alive: 12.3
Avg. Weaning Weight: 6.7 kg
Litter ID # 085

Sow # 0132801 – Nursed small litter (n=6)
Date of Birth: 9-27-01
Parity: 5
Avg. Total Born: 12.3
Avg. Born Alive: 8.5
Avg. Weaning Weight: 7.0 kg
Litter ID # 086

Sow # 0240007 – Nursed large litter (n=10)
Date of Birth: 11-21-02
Parity: 2
Avg. Total Born: 12.0
Avg. Born Alive: 12.0
Avg. Weaning Weight: 5.5 kg
Litter ID # 088

Sow # 0133903 – Nursed large litter (n=10)
Date of Birth: 9-27-01
Parity: 4
Avg. Total Born: 9.3
Avg. Born Alive: 9.0
Avg. Weaning Weight: 7.2 kg

Litter ID # 089

Sow # 0133905 – Nursed small litter (n=6)
Date of Birth: 9-27-01
Parity: 5
Avg. Total Born: 12.5
Avg. Born Alive: 11.5
Avg. Weaning Weight: 5.8 kg
Litter ID # 093

Appendix D: Analysis of only the boars kept during the breeding portion of the study

The data collected from the present study was analyzed two separate ways. The first included all of the boars involved in the study and the second only included the boars kept for the breeding portion of the study. The second analysis, including SAS code and results, is in Appendix D.

D.1.: Results for analysis of only the boars kept during the breeding portion of the study

Weight and testicle measurement results for only boars kept for the duration of the study:

A tendency (p=.1055) among treatment, season, and age was observed for body weight (Appendices D.3 and D.4). For the fall born boars, those reared in large litters were slightly heavier, 108.5 ± 8.2 kg, than those boars that nursed in small litters, 106.6 ± 7.5 kg. In contrast, the boars born in the spring and raised in small litters were always heavier than their counterparts that nursed in large litters (Appendix D.4). Between 13 and 17 weeks of age, the fall born boars began consistently weighing more than the boars born in the spring. Age was also observed to be significant in increasing body weight.

A significant interaction ($p<.05$) among season and age was observed for testicular height (Appendix D.9). Age was found to be significant ($p<.05$) in increasing testicular height. For boars born in the fall, their testicular height was larger and increased more rapidly from 6 weeks to 17 weeks of age in comparison to the boars born in the spring. However, the spring born boars had a rapid increase in testicular height from 15 weeks of age to 20 weeks of age. The same was observed for testicular width. A significant interaction ($p>.05$) among season and age was observed for testiclular width (Appendix D.10). As the boars aged, testicle width increased ($p<.05$). The boars born in the fall experienced a rapid period of growth in testicular width from 13 weeks of age to 24 weeks of age. A more gradual increase in testicular width was observed for the boars born in the spring from 13 weeks of age to approximately 24 weeks of age.

A tendency (p=.1137) among season and age was observed for testicular area (Appendix D.11). As the boars aged, testicular area increased significantly ($p<.05$).

Those boars born in the fall were observed to generally have greater testicular area than the boars born in the spring. In addition, the fall born boars had a more rapid increase in testicular area from approximately 17 weeks of age to 30 weeks of age than the boars born in the spring. At 37 weeks of age, the mean testicular area for the fall born boars reared in small litters was 859.9 ± 102.8 cm^2 and in large litters was 959.7 ± 108.9 cm^2. At the same age for the spring born boars, the small litter subjects had a mean testicular area of 708.0 ± 38.2 cm^2 and the large litter subjects had a mean of 619.8 ± 19.4 cm^2. Testicular area increased ($p<.05$) for the boars born in the fall for 40 weeks while the boars in spring only had testicular area increases for 20 weeks.

A significant interaction ($p<.05$) among season and age and a tendency ($p=.0617$) among season and treatment was observed for the ratio of testicular area per body weight (Appendix D.12). Age was also observed to be highly significant ($p<.05$) in affecting the ratio of testicular area to body weight. Both boars born in the fall and spring experienced a higher ratio of testicular area to body weight immediately following birth and then gradually decreasing for several weeks. By 17 weeks of age, the ratio of testicle size to body weight was increasing. For boars born in the fall, beginning at approximately 40 weeks of age, the small litter boars consistently had larger testicles for their body weight than did their counterparts reared in large litters.

Semen analysis results for only boars kept for the duration of the study:

A tendency ($p=.1193$) among season and treatment was observed for concentration (Appendix D.13). For the boars born in the fall and nursed in small litters, their concentrations increased ($p<.05$) as they aged, but their counterparts reared in large litters only displayed a tendency ($p=.0797$) towards concentration increasing as they

aged. In addition, concentration for the boars born in the spring remained unaffected by age ($p>.05$).

No interactions were observed for the total number of spermatozoa (Appendix D.14). Only age was noted to be significant ($p<.05$) in the increase of the total number of spermatozoa. Beginning at 37 weeks of age, boars born in the fall and reared in large litters had consistently higher total numbers of spermatozoa than those boars reared in small litters. At 37 weeks of age, the fall born, small litter boars had a mean total number of 95.9 ± 13.8 billion spermatozoa while the fall born, large litter boars had a mean total number of 137.0 ± 18.1 billion spermatozoa.

A significant interaction ($p<.05$) among season and treatment was observed for ejaculate volume (Appendix D.15). Age was found to not be significant in affecting volume for either season or treatment of boars. The volume of ejaculates for the fall born boars was consistently greater in those reared in large litters. However, in the spring born boars, the boars that nursed in small litters began to have consistently larger ejaculates at 36 weeks of age than those boars born in the spring and reared in large litters.

A tendency ($p=.0988$) among treatment, season, and age was observed for the percentage of normal motility (Appendix D.5). For boars born in the fall, those reared in large litters had a higher percentage of normal motility, 82.3 ± 0.7, than those boars nursed in smaller litters, 78.3 ± 1.0. Boars born in the fall generally had 80% normal motility until they reached 53 weeks of age. At 53 weeks of age, the fall born boars experienced approximately 10 consecutive weeks of less than 80% normal motility (Appendix D.6). The boars born in the spring and reared in small litters generally had

higher percentages of normal motility than their counterparts nursed in large litters (Appendix D.7).

No interactions were observed for the percentage of normal acrosome morphology (Appendix D.16). Only age was found to be significantly ($p<.05$) influential. For the boars born in the fall, the percentage of spermatozoa with normal acrosome morphology was generally higher in the boars reared in large litters. For both seasons and treatments of boars, the percentage of normal acrosome morphology slightly increased as the boars aged.

A significant interaction ($p<.05$) among season and age and a tendency ($p=.0552$) among season and treatment were observed for the percentage of spermatozoa displaying acrosin activity (Appendix D.17). Age was observed to be significant ($p<.05$) in affecting acrosin activity in the fall born boars reared in small and large litters, but not significant ($p>.05$) for the spring born boars reared in small and large litters. The fall born boars experienced fluctuation in the percentage of spermatozoa displaying acrosin activity from 35 weeks of age to 45 weeks of age. In contrast, the spring born boars maintained a percentage of acrosin activity greater than or equal to 90%.

A significant interaction ($p<.05$) among season and age and a tendency ($p=.0585$) among season and treatment were observed for the percentage of uncapacitated spermatozoa (Appendix D.18). Age was noted to be significant ($p<.05$) for the percentage of uncapacitated spermatozoa produced by the boars born in the fall and reared in small litters. However, the boars born in the fall and nursed in large litters and both treatments of spring born boars were not affected by age. The boars born in the fall experienced a much greater fluctuation in the number of uncapacitated spermatozoa than

the boars born in the spring. For the fall born boars, the number of uncapacitated spermatozoa began to remain consistent, around 2%, at 47 weeks of age.

No significant interactions were observed for the percentages of capacitated, acrosome reacted, and abnormal spermatozoa. However, age was observed to be significant for all three. The fall born boars reared in small and large litters and the spring born boars nursed in small litters were observed to have slight fluctuations in the number of capacitated spermatozoa, but the percentages were, on average, above 70% (Appendix D.19). In contrast, the boars born in the spring and reared in large litters had decreasing numbers of capacitated spermatozoa. The number of acrosome reacted spermatozoa fluctuated as the boars aged, but were maintained at less than 10% (Appendix D.20). The boars born in the fall and reared in large litters had a consistently lower percentage of abnormal spermatozoa than their counterparts reared in small litters. In contrast, the boars born in the spring and reared in large litters had higher percentages of abnormal spermatozoa than those boars born in the spring and reared in small litters (Appendix D.21).

Gel analysis results for only boars kept for the duration of the study:

No interactions were observed for normalized volume (Appendix D.8). The large litter boars born in the fall had greater normalized volumes of seminal plasma proteins than those of their counterparts nursed in small litters. At 35 weeks of age, the large litter boars had a mean normalized volume of 119.1 ± 5.8 and the small litter boars had a mean normalized volume of 106.8 ± 6.4. At 45 weeks of age, the large litter boars had a mean normalized volume of 94.5 ± 8.0 and the small litter boars had a mean normalized

volume of 90.1 ± 4.1. As the boars aged, the mean normalized volume of seminal plasma proteins decreased ($p<.05$).

Appendix D.2. Summary of significant effects for all dependent variables for only the boars kept for the duration of the study or 60 weeks of age.

Dependent Variable	3-way	2-way	Main Effects
Body Weight (kg)	p=.1055	Fall: No Age x Trt p=.0155	Fall: Trt=.0161 Age<.0001 Spring-Small: Age p<.0001 Spring-Large: Age p<.0001
Testicle Height (cm)	No	Season x Age p=.0022	Fall-Small: Age p<.0001 Fall-Large: Age p<.0001 Spring-Small: Age p<.0001 Spring-Large: Age p<.0001
Testicle With (cm)	No	Season x Age p=.0062	Fall-Small: Age p<.0001 Fall-Large: Age p<.0001 Spring-Small: Age p<.0001 Spring-Large: Age p<.0001
Testicular Area (cm^2)	No	Season x Age p=.1137	Fall-Small: Age p<.0001 Fall-Large: Age p<.0001 Spring-Small: Age p<.0001 Spring-Large: Age p<.0001
Ratio of Testicle Area To Body Weight (%)	No	Season x Age p=.0030 Season x Trt p=.0617	Fall-Small: Age p<.0001 Fall-Large: Age p<.0001 Spring-Small: Age p<.0001 Spring-Large: Age p<.0001
Concentration (millions)	No	Season x Trt p=.1193	Fall-Small: Age p<.0001 Fall-Large: Age p<.0797 Spring-Small: Age p=.8398 Spring-Large: Age p=.2419
Total # of Sperm (billions)	No	No	Age p<.0001
Volume of Ejaculate (mL)	No	Season x Trt p=.0230	Fall-Small: Age p=.5532 Fall-Large: Age p=.8721 Spring-Small: Age p=.2205 Spring-Large: Age p=.4430

Appendix D.2., continued.

Motility (% Normal)	p=.0988	Fall: No Spring: Age x Trt p=.0398	Fall: Age p=.0002 Spring-Small: Age p=.0014 Spring-Large: Age p=.0671
Morphology (% Normal)	No	No	Age p<.0001
Acrosin Activity (%)	No	Season x Age p=.0501 Season x Trt p=.0552	Fall-Small: Age p<.0004 Fall-Large: Age p=.0079 Spring-Small: Age p=.8771 Spring-Large: Age p=.1854
Uncapacitated (% Uncapacitated)	No	Season x Age p=.0538 Season x Trt p=.0585	Fall-Small: Age p=.0116 Fall-Large: Age p=.2034 Spring-Small: Age p=.4796 Spring-Large: Age p=.3022
Capacitated (% Capacitated)	No	No	Age p<.0001
Acrosome Reacted (% acrosome reacted)	No	No	Age p=.0027
Abnormal (% abnormal)	No	No	Age p<.0001
Seminal Plasma Proteins (Normalized Volume)	No	No	Age: p=.0007

Table D.3. Mean weights (kg) for only the boars kept from the Fall replicate for the duration of the study.

Age (wks) for boars born in the Fall	Small Litter Boars Mean ± S.E.	N	Large Litter Boars Mean ± S.E.	N
1	3.3 ± 0.2	4	2.8 ± 0.2	4
2	5.9 ± 0.2	4	4.9 ± 0.5	4
3	8.3 ± 0.2	4	6.9 ± 0.6	4
6	15.6 ± 0.7	4	12.4 ± 0.7	4
9	28.9 ± 1.1	4	25.8 ± 1.3	4
13	52.6 ± 2.3	4	46.3 ± 2.6	4
17	82.9 ± 3.7	4	77.5 ± 3.2	4
20	106.1 ± 4.7	4	100.3 ± 4.3	4
24	126.5 ± 4.2	4	124.4 ± 6.2	4
31	134.9 ± 4.2	4	136.0 ± 7.3	4
34	140.3 ± 5.4	4	145.2 ± 7.1	4
37	148.0 ± 4.1	4	158.9 ± 9.0	4
40	149.6 ± 3.9	4	162.4 ± 10.4	4
43	153.8 ± 3.5	4	169.1 ± 10.4	4
47	163.1 ± 4.1	4	173.2 ± 7.4	4
50	164.9 ± 1.6	4	176.7 ± 8.1	4
53	166.4 ± 2.2	4	181.0 ± 9.2	4
57	185.9 ± 3.4	4	189.9 ± 6.8	4
60	188.3 ± 2.5	4	189.3 ± 8.5	3

Appendix D.4. Mean weights (kg) for boars born in the Spring and kept for the duration of the study.

Ages (wks) for Spring Born Boars	Small Litter Mean ± S.E.	N	Large Litter Mean ± S.E.	N
1	3.1 ± 0.2*	4	2.1 ± 0.1	4
2	5.7 ± 0.3*	4	3.8 ± 0.4	4
3	8.7 ± 0.3*	4	5.6 ± 0.5	4
6	14.1 ± 0.6*	4	10.1 ± 0.8	4
9	27.0 ± 0.9*	4	21.4 ± 0.9	4
13	41.4 ± 1.5*	4	33.6 ± 1.8	4
15	59.8 ± 3.0*	4	49.3 ± 2.9	4
17	81.3 ± 3.9*	4	67.1 ± 3.2	4
20	100.5 ± 5.6	4	86.1 ± 4.6	4
24	111.7 ± 5.6	4	97.4 ± 3.3	4
28	108.7 ± 6.5	4	100.6 ± 1.4	4
31	126.7 ± 5.5*	4	109.7 ± 3.0	4
34	139.2 ± 6.2*	4	118.6 ± 4.5	4
37	134.0 ± 4.4*	4	114.0 ± 6.1	4

*** mean weight (kg) for small litter boars is significantly different from large litter boars ($p<.05$)**

Appendix D.5. Percentage of motile spermatozoa per ejaculate for only the boars kept for the duration of the study across all ages for the boars born in the Fall.

	N	Mean ± S.E.
Fall-Small Litter Boars	**120**	**78.3 ± 1.0**
Fall-Large Litter Boars	**115**	**82.3 ± 0.7**

Appendix D.6. Percentage of motile spermatozoa per ejaculate for only the boars kept for the duration of the study across both treatments for the Fall born boars.

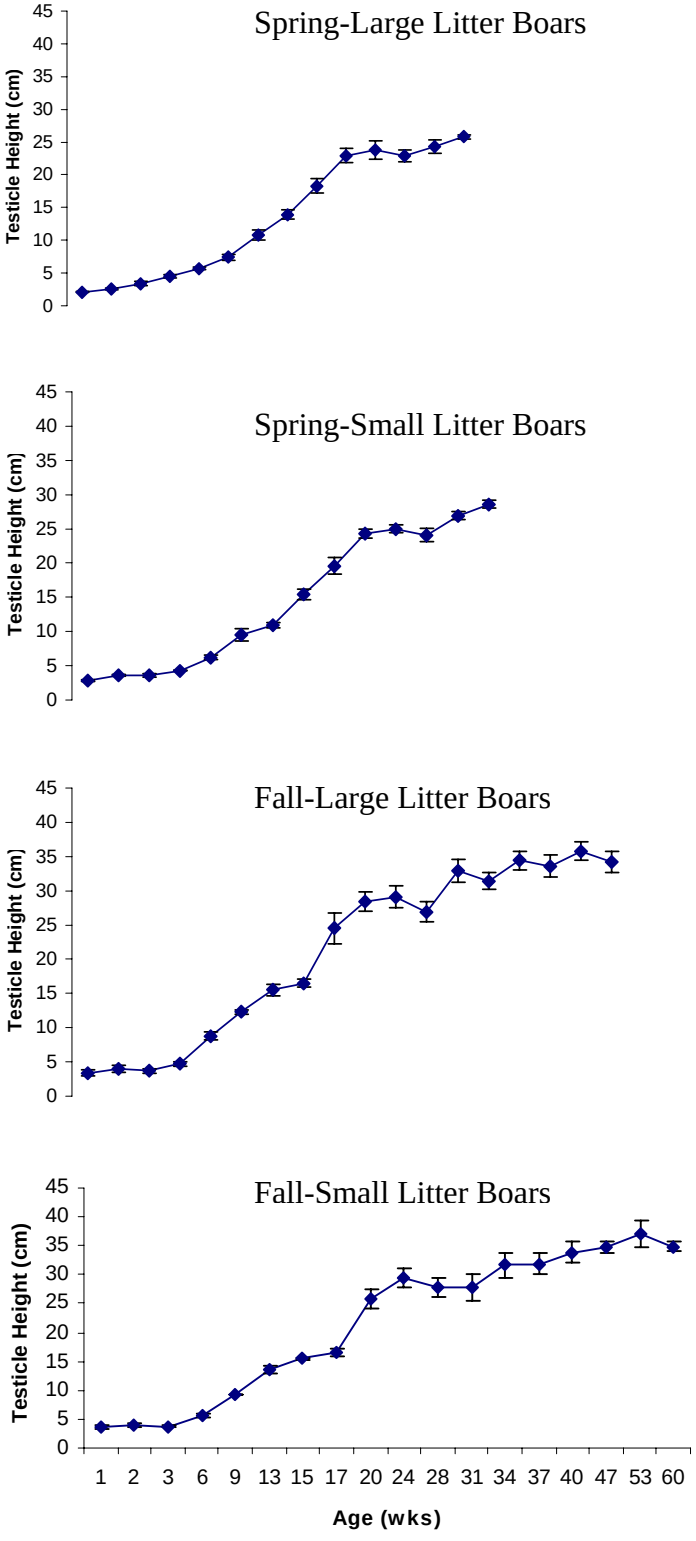
Ages (wks) for Fall Born Boars	N	Mean ± S.E.
30	8	80.3 ± 1.4
35	8	82.1 ± 4.2
36	8	82.2 ± 1.2
37	8	79.5 ± 2.1
38	8	80.0 ± 2.0
39	8	84.8 ± 1.7
40	8	82.9 ± 1.8
41	8	83.5 ± 3.0
42	8	81.6 ± 3.5
43	8	87.2 ± 1.4
44	8	84.7 ± 1.9
45	8	79.6 ± 1.7
46	8	86.8 ± 1.5
47	8	81.0 ± 2.5
48	8	86.6 ± 1.5
49	8	79.4 ± 2.1
50	8	83.0 ± 2.4
51	8	82.2 ± 2.7
52	8	82.5 ± 1.2
53	8	81.2 ± 2.5
54	8	75.5 ± 4.8
55	8	69.6 ± 4.1
56	8	74.6 ± 5.4
57	8	70.4 ± 3.8
58	8	76.9 ± 4.9
59	7	79.5 ± 2.4
60	7	80.3 ± 4.3
61	7	67.1 ± 4.9
62	7	78.7 ± 4.6
63	7	81.8 ± 2.6

Appendix D.7. Percentage of motile spermatozoa per ejaculate for only the boars kept for the duration of the study for the boars born in the Spring.

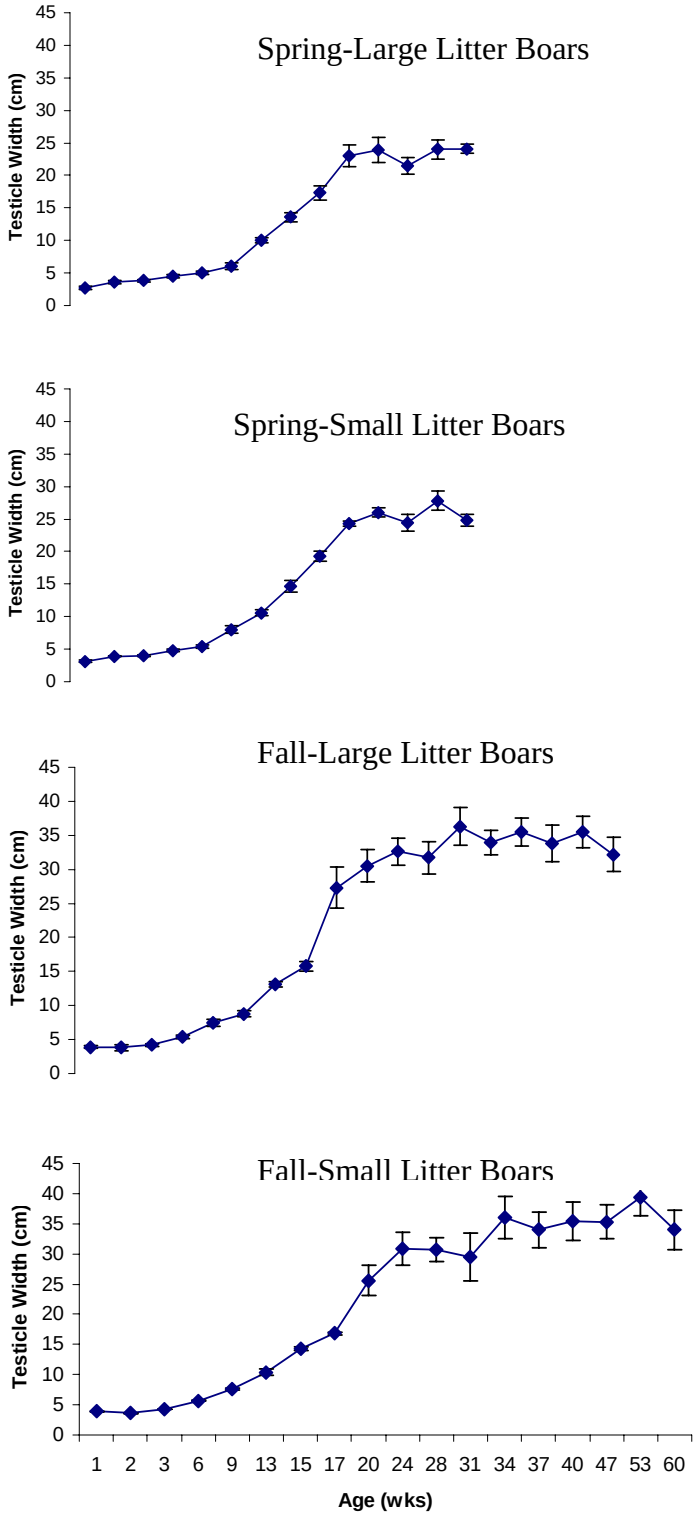
Ages (wks) for Spring Born Boars	Small Litter Mean ± S.E.	N	Large Litter Mean ± S.E.	N
30	83.0 ± 0.3	2	78.0 ± 3.1	3
31	83.6 ± 2.9	3	72.6 ± 2.8	3
32	73.9 ± 3.7	3	85.0 ± 2.4	4
33	80.2 ± 2.7	4	78.2 ± 3.2	4
34	82.5 ± 2.4	4	77.4 ± 3.6	4
35	80.2 ± 2.3	4	69.7 ± 8.0	4
36	83.5 ± 3.0	4	83.5 ± 2.8	4
37	82.4 ± 1.3	4	82.8 ± 2.7	4
38	77.2 ± 2.2	4	80.1 ± 1.9	4
39	51.5 ± 11.9	4	69.9 ± 5.5	3
40	77.8 ± 3.6	4	65.5 ± 7.3	3
41	83.9 ± 3.4	3	69.2 ± 9.4	2

Appendix D.8. Seminal plasma protein levels (relative units) for only the boars born in the Fall and kept for the duration of the study.

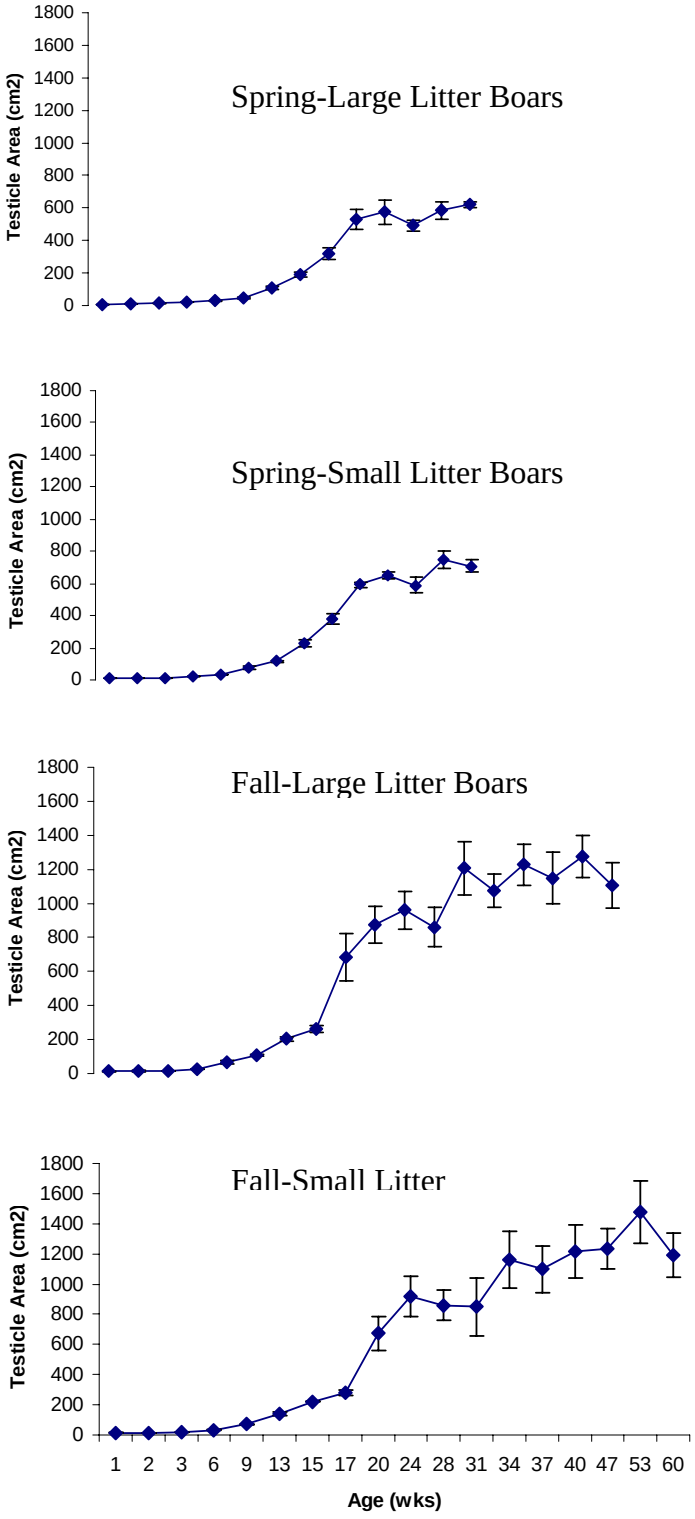
Fall Born Only	N	Mean ± S.E.
Small Litter Boars / 35 Wks	8	106.8 ± 6.4
Large Litter Boars / 35 Wks	8	119.1 ± 5.8
Small Litter Boars / 45 Wks	8	90.1 ± 4.1
Large Litter Boars / 45 Wks	8	94.5 ± 8.0



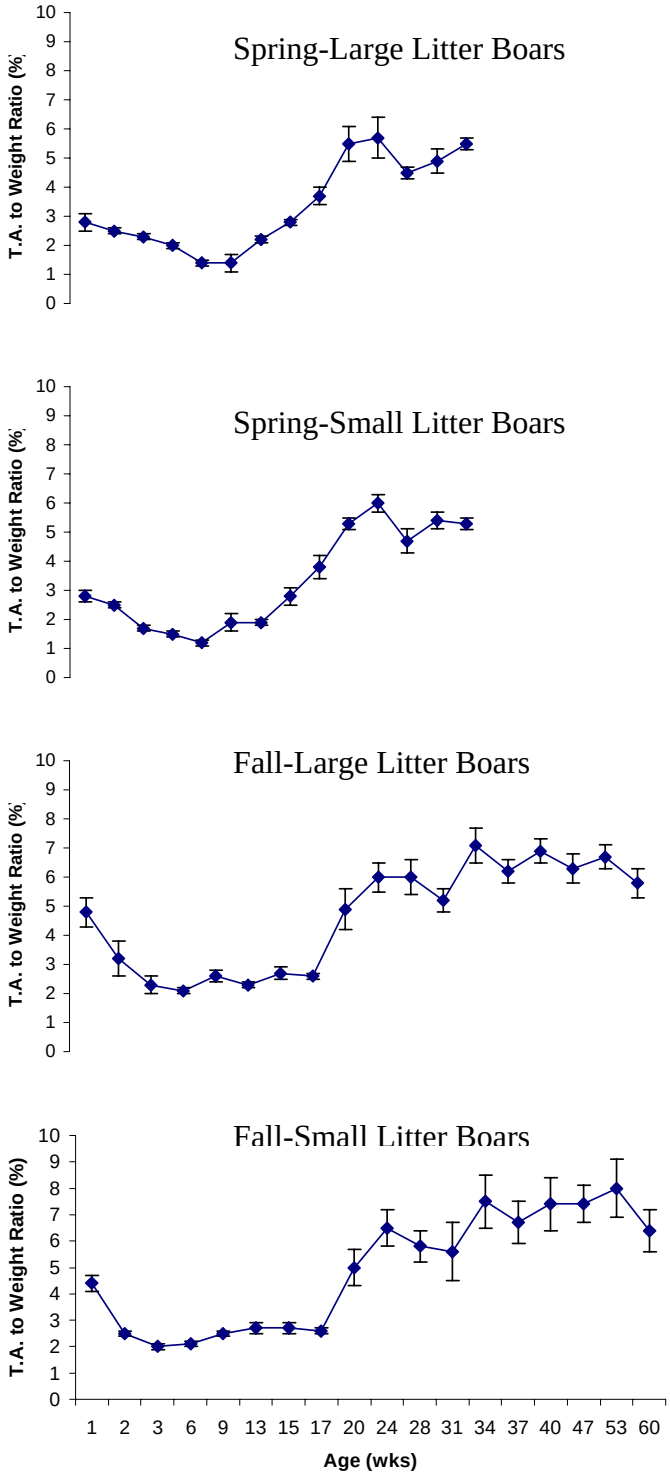
Appendix D.9. Testicular height (cm) for only boars kept for the duration of study across both seasons and treatments.



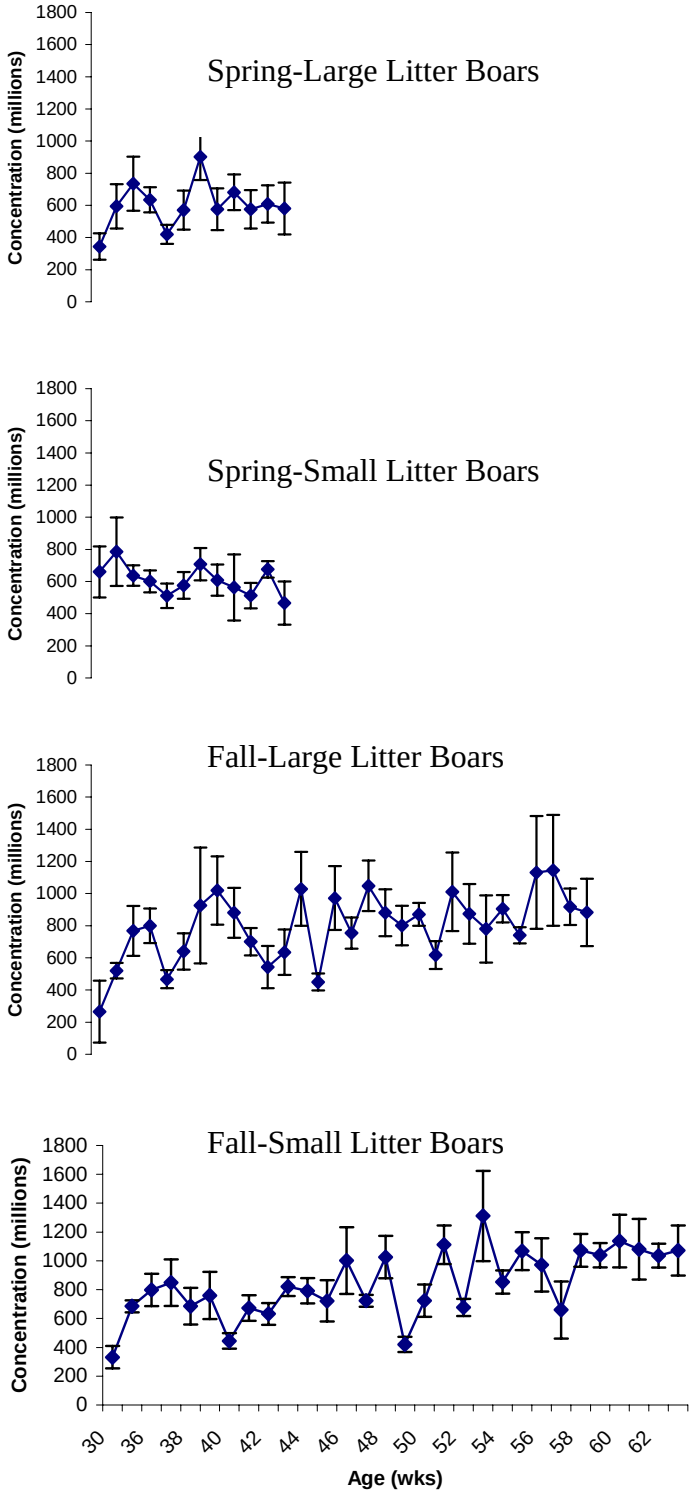
Appendix D.10. Testicular width (cm) for only boars kept for the duration of the study across both seasons and treatments.



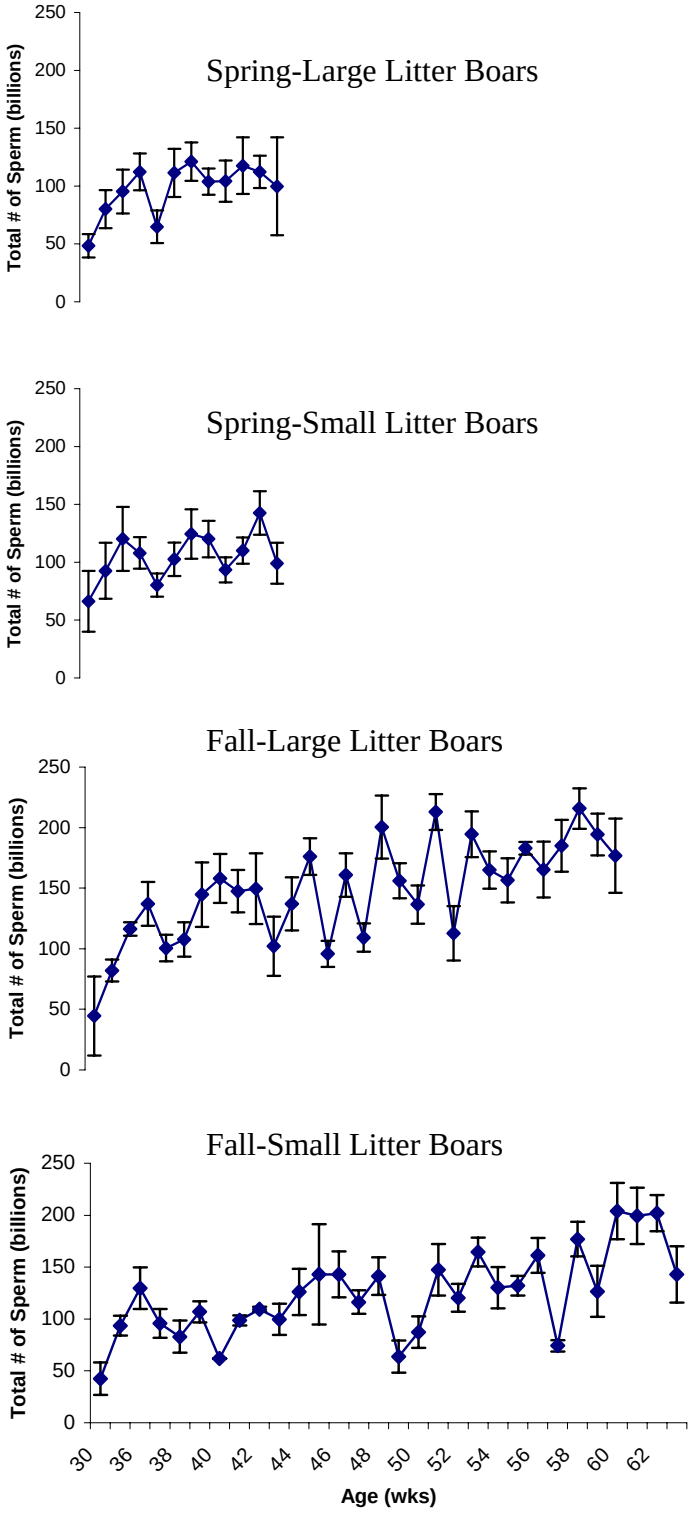
Appendix D.11. Testicular area (cm²) for only the boars kept for the duration of the study across both seasons and treatments.



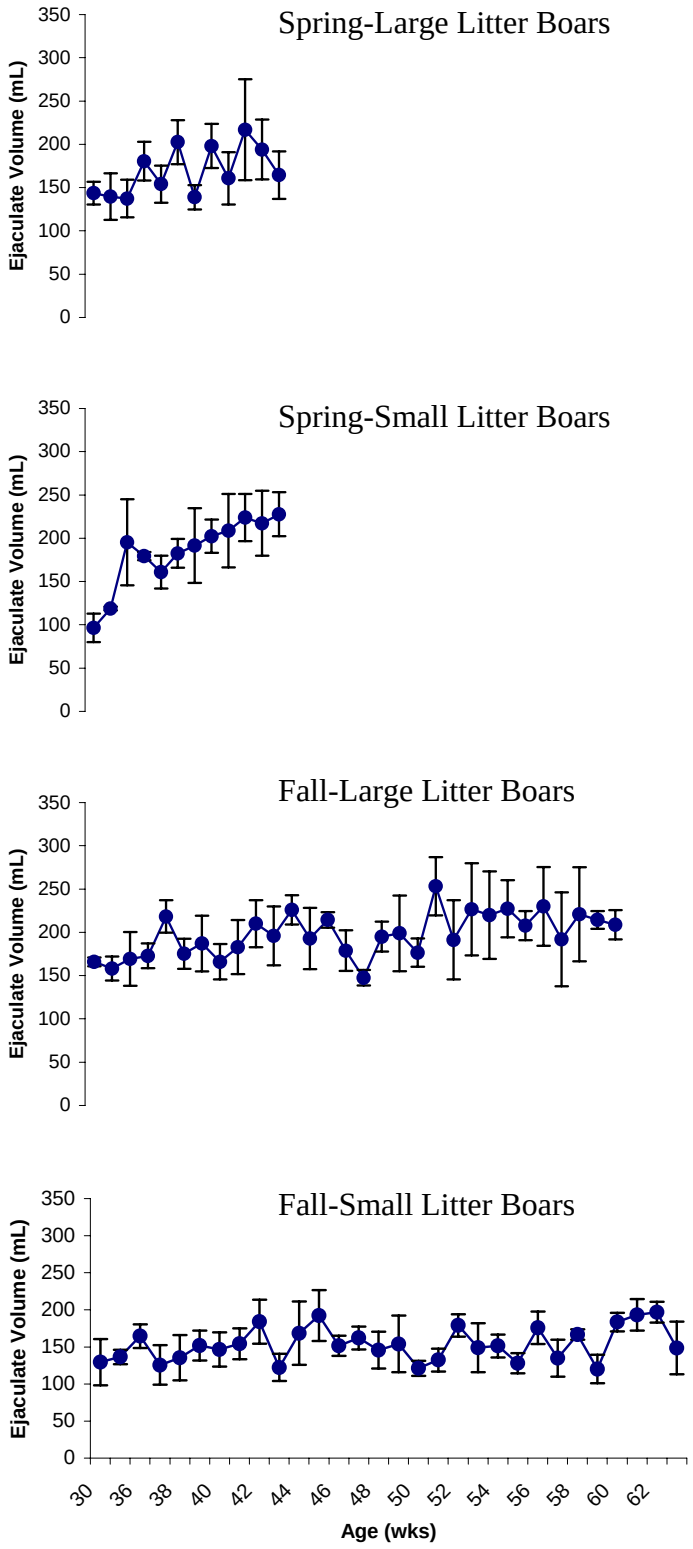
Appendix D.12. The ratio of testicle size to body weight (%) for only boars kept for the duration of the study across both seasons and treatments.



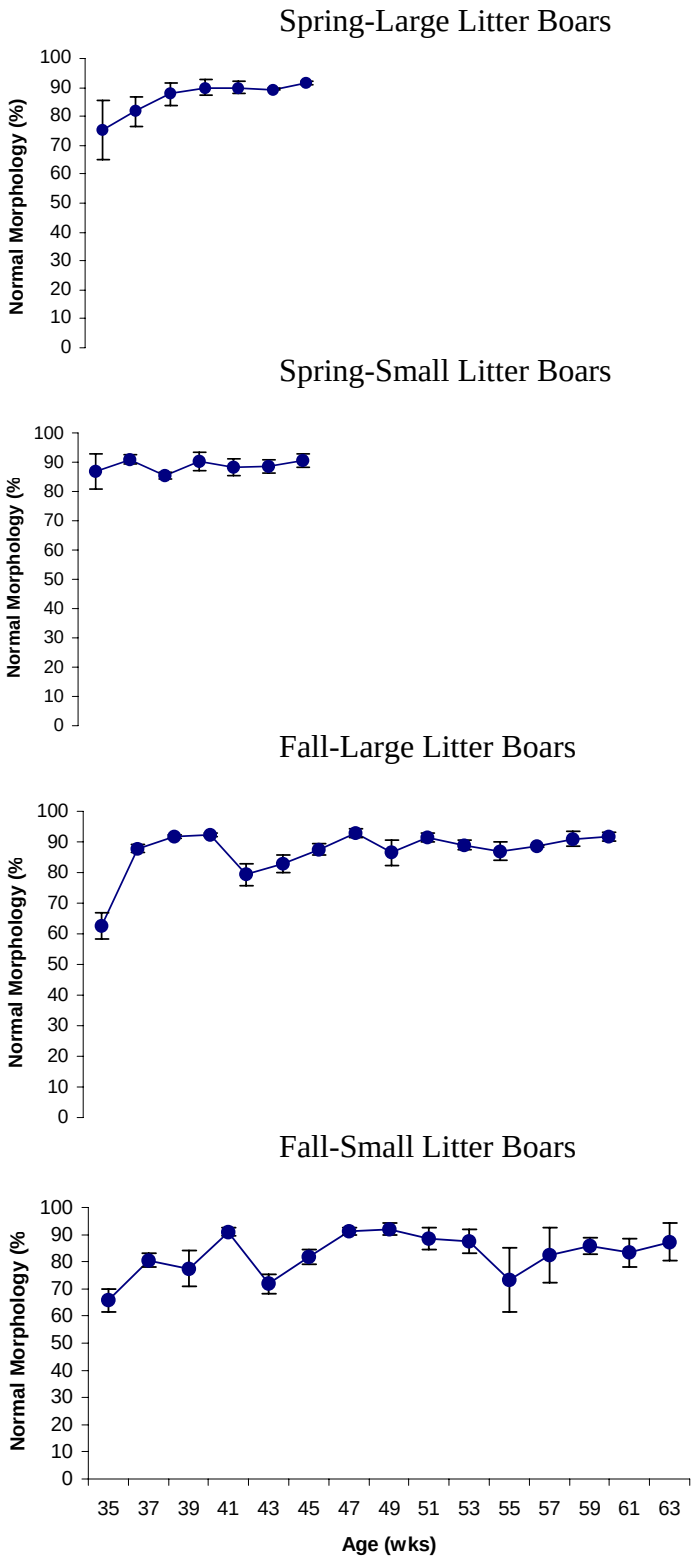
Appendix D.13. Concentration of spermatozoa (millions) per ejaculate for only the boars kept for the duration of the study across both seasons and treatments.



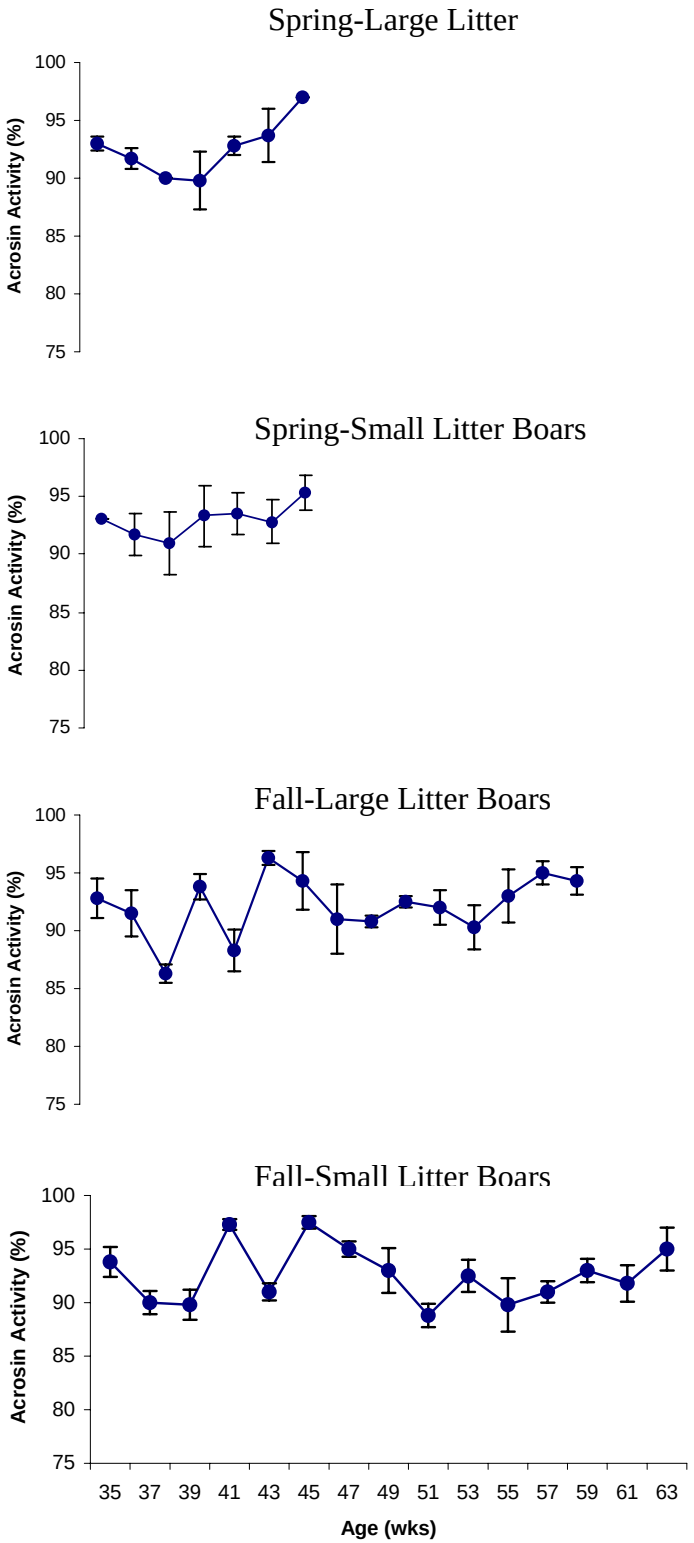
Appendix D.14. Total number of spermatozoa (billions) per ejaculate for only the boars kept for the duration of the study across both seasons and treatments.



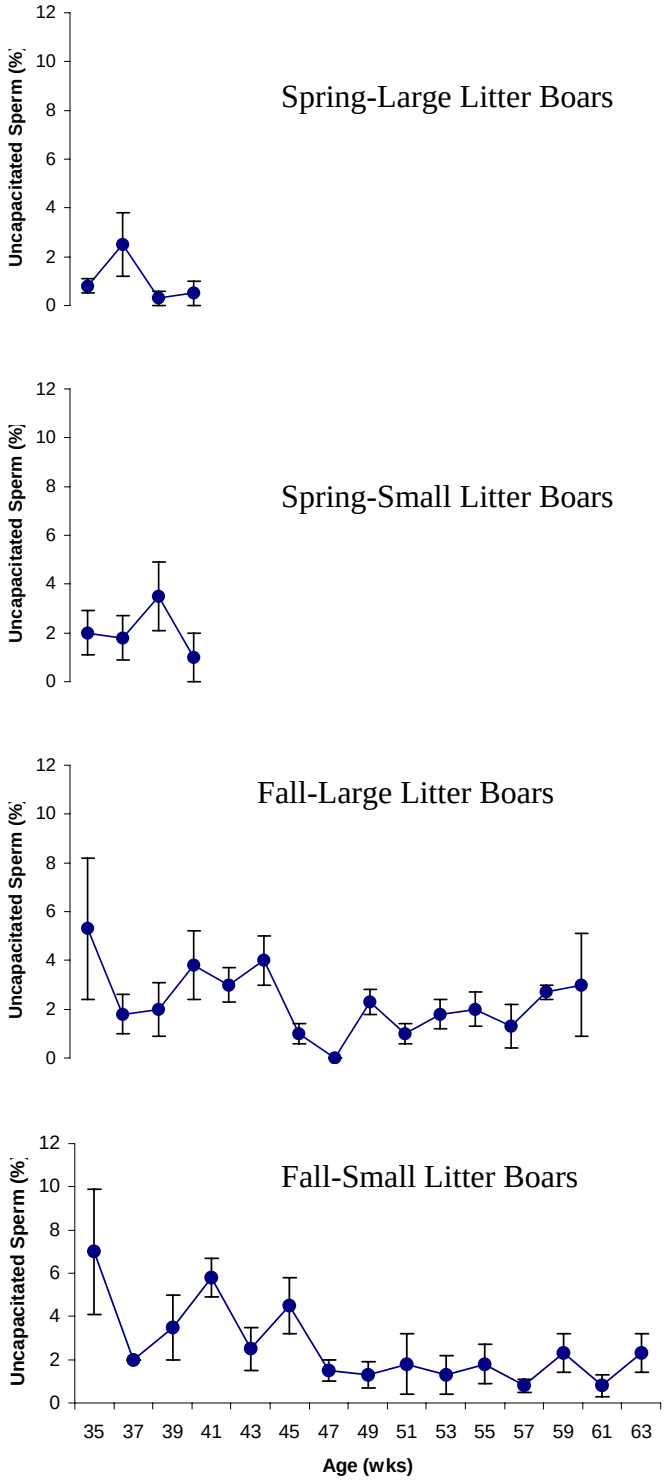
Appendix D.15. Ejaculate volume (mL) for only the boars kept for the duration of the study across both seasons and treatments.



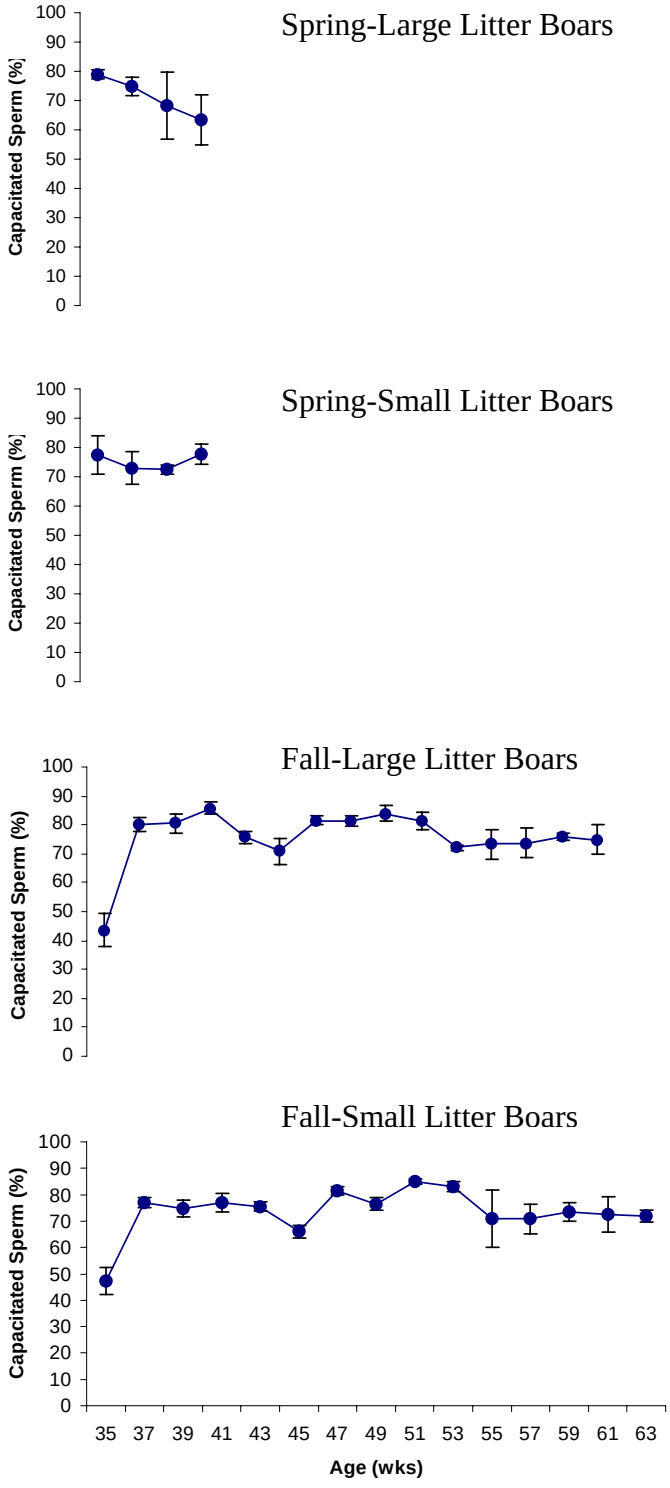
Appendix D.16. Percentage of morphologically normal spermatozoa per ejaculate for only the boars kept for the duration of the study across both seasons and treatments.



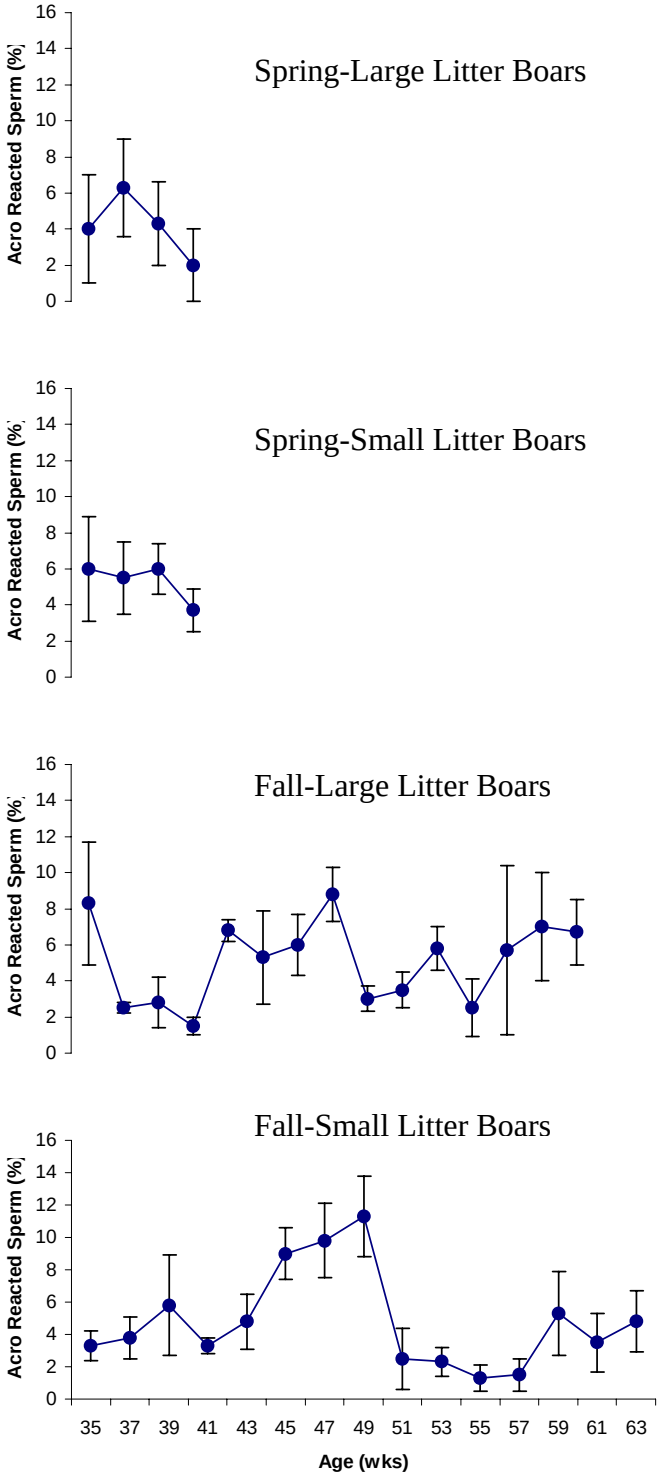
Appendix D.17. Percentage of spermatozoa with acrosin activity per ejaculate for only the boars kept for the duration of the study across both seasons and treatments.



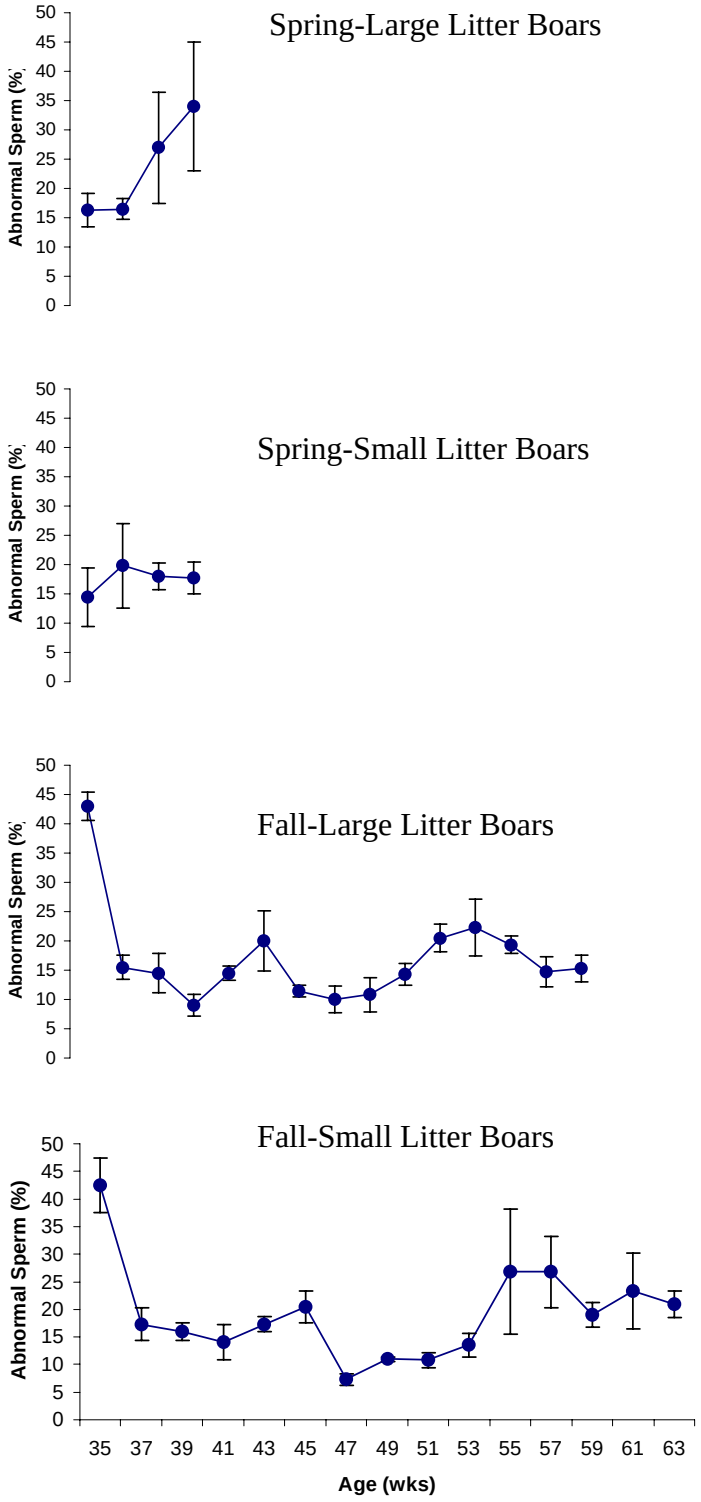
Appendix D.18. Percentage of uncapacitated spermatozoa per ejaculate for only the boars kept for the duration of the study across both seasons and treatments.



Appendix D.19. Percentage of capacitated spermatozoa per ejaculate for only the boars kept for the duration of the study across both seasons and treatments.



Appendix D.20. Percentage of acrosome reacted spermatozoa per ejaculate for only the boars kept for the duration of the study across both seasons and treatments.



Appendix D.21. Percentage of abnormal spermatozoa per ejaculate for only the boars kept for the duration of the study across both seasons and treatments.