ABSTRACT

BROWN, VANCE DAVID. Effect of Human Socialization on the Reproductive Performance of Low Birth Weight Adult A.I. Boars. (Under the direction of Dr. William L. Flowers).

The objective of this study was to examine the effect of human socialization prior to puberty on the reproductive performance of A.I. boars. Thirty-six boars (average birth weight = 1.2 kg) were randomly assigned to be socialized or unsocialized from 4 to 9 weeks of age (nursery). Boars within each of the nursery socialization treatments were again randomly assigned to be socialized or unsocialized from 9 to 22 weeks of age (finishing). This resulted in four different levels of socialization; no socialization; socialization from 4 to 9 weeks of age; socialization from 9 to 22 weeks of age; and socialization from 4 to 22 weeks of age. Boars were trained over a 120-day period for semen collection beginning when the boars were 160 days of age. Boars that were successfully trained were collected weekly for 29 consecutive weeks. Boars socialized from 4 to 22 weeks of age had the lowest success rates in terms of being trained for semen collection compared with the other treatments. There was a tendency ($p=0.15$) for total sperm to increase with the degree of socialization. Boars that were socialized from 4 to 22 weeks of age averaged 81.8 ± 1.9 billion cells per collection compared to 66.7 ± 2.5 billion cells for their unsocialized counterparts. Sperm quality parameters including motility (CASA analyses) and morphology were not affected ($p > 0.05$) by socialization treatment. Similar to sperm quality parameters, libido was not affected ($p > 0.05$) by socialization treatment. Results from Hemsworth tests indicate boars socialized to humans tend ($p \leq 0.10$) to react more positively around humans. Results from this study indicate that sperm production of low birth weight boars may be increased by socialization with humans in either the nursery or finishing phase of production without any detrimental

effects on sperm quality. However, it appears that there is an inverse relationship between the length of socialization and the effectiveness of training boars for semen collection as adults.

Effect of Human Socialization on the Reproductive Performance of Low Birth Weight Adult A.I. Boars

by
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BIOGRAPHY

Vance Brown was born on January 26, 1992 in Stormstown, Pennsylvania to Ted and Vicki Brown. He has a younger sister Elaine, and they grew up on a small family hobby farm. Vance graduated from State College Area High School in State College, Pennsylvania in 2010.

Vance attended Pennsylvania State University where he received a Bachelor of Science with dual majors in Animal Sciences and Agricultural Systems Management in December, 2014. While at Penn State, Vance was enrolled in the Schreyer Honors College which included some research responsibilities. In January 2015, he began to pursue a Master of Science in Animal Science under the direction of Dr. William L. Flowers at North Carolina State University.

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LITERATURE REVIEW

Introduction

The prevalent use of artificial insemination in the swine industry has allowed producers to take advantage of enhanced paternal genetics without the added expense and labor of managing natural service boars. Artificial insemination allows breeding companies to manage valuable paternal genetics through boar studs designed specifically for the management and collection of boars. However, artificial insemination has placed increased demands upon the boar to produce large amounts of high quality spermatozoa on a regular basis. Anything which reduces the sperm production of a specific boar reduces the number of doses that can he can produce and decreases revenue for the boar stud and the entire production system. Both quantity and quality of sperm are important, as a boar used exclusively for A.I. has the potential to impact the productivity of thousands of sows over his lifetime. Previous research has described many of the management factors for boars once they enter the boar stud that can impact their sperm production (Flowers, 1997). However, less attention has been paid to the adolescent life of the boar between birth and puberty, where boars are typically managed less intensively. It is clear that prenatal, neonatal, and pre-pubertal factors affect the sperm production of adult boars. The purpose of this review is to discuss some of the most commonly studied factors that can affect semen quantity and quality in the adult boar. However, to fully understand these factors, it is important to first review the development of spermatogenesis and how it functions in the adult boar.

Spermatogenesis

In adult boars used for semen collection, semen quality and quantity are biologically and economically important. To best understand the factors which can impact both, it is necessary to understand how sperm is produced in the boar. Spermatogenesis is a complex process by which the testes produce spermatozoa, and is the first step in understanding the lifetime productivity of a boar.

Internal structure of the testes

Similar to most mammalian species, boar testes have two basic compartments: interstitial and seminiferous epithelium. The interstitium is inhabited by several cell types most importantly of which are Leydig cells. Leydig cells produce testosterone (Zirkin et al., 1980) in response to gonadotropins and other hormones (Lindner, 1961). Testosterone and other androgens are well known drivers of male sexual behaviors in many species and boars are no exception. The second compartment of the testes, the seminiferous epithelium, is responsible for spermatozoa production. The seminiferous epithelium is a long, continuous and convoluted tubule in the testes; the ends of which connect to the rete testis to allow spermatozoa to migrate to the epididymis (Curtis, 1918). Within the seminiferous epithelium, two basic cell types are observed; Sertoli and germ cells (Brown, 1885). Sertoli cells are somatic cells which act as “nurse” or support cells for the developing germ cells within the epithelium and stretch from the basement membrane towards the center of the tubule (Brown, 1885; Dym and Fawcett, 1970). Many studies have investigated the structure of the seminiferous tubule and found that Sertoli cells divide the tubule into three compartments; basal, adluminal, and the lumen (Brown, 1885; LeBlond and Clermont, 1952b; Dym and Fawcett, 1970). The basal compartment is found along the basement membrane of the

seminiferous tubule. The separation between the basal compartment and the adluminal compartment is made up by specialized junctions between adjacent Sertoli cells and constitutes the blood-testis barrier (Dym and Fawcett, 1970). These junctions act as a barrier between molecules in the blood and the developing germ cells in the adluminal compartment and lumen of the seminiferous tubules (Dym and Fawcett, 1970). The lumen of the seminiferous tubule is formed by the absence of Sertoli cells in the center of the tubule and is where mature spermatozoa are released and travel to the epididymis (Wodsedalek, 1913). Within the different compartments formed by the supporting Sertoli cells, germ cells in various stages can be found, with the most immature cells found near the basement membrane and the most mature near the lumen (Brown, 1885).

Development of germ cells into spermatozoa

Sertoli cells are crucial to the development of spermatozoa from primitive germ cells. An intimate relationship between germ cells and Sertoli cells is present throughout spermatogenesis. Early work by Brown (1885) and Wodsedalek (1913) as well as later work by LeBlond and Clermont (1952a; 1952b) revealed that germ cells divide and develop in a predictable pattern which is consistent throughout the seminiferous tubule and follows the same steps repeatedly to continuously produce spermatozoa. Hence, spermatogenesis is regarded as a cycle of multiple stages or cellular associations which repeats itself in adult male mammals. Because the germ cell changes dramatically throughout the cycle, nomenclature was developed to differentiate germ cells of varying degree of development. The major types of germ cells found in the seminiferous tubule in order of increasing maturity are: spermatogonia, spermatocyte, spermatid, and spermatozoa. Further divisions

within these major cell types based on maturity were developed to refine distinct stages of cells within each of the major classes. The development of these specific cell types is very well known, and is conserved across most mammalian species. Type A_0 spermatogonia are the most primitive germ cells, and are found flattened against the basement membrane (Brown, 1885; Wodsedalek, 1913). Through mitosis of the Type A_0 spermatogonia, diploid Type A spermatogonia daughter cells are formed. During this first division, one of the type A spermatogonia continues to undergo mitosis to produce more developed spermatogonia, while the other ceases to divide, acting as a new stem cell and allowing for infinite renewal of germ cells in the testis (LeBlond and Clermont 1952b; Hochereau-de-Reviers, 1981). Type A spermatogonia continue to undergo mitosis over multiple generations, generating intermediate spermatogonia, and finally Type B spermatogonia (LeBlond and Clermont, 1952b). During these divisions, the spermatogonia migrate towards the adluminal compartment formed by the Sertoli cell junctions (Dym and Fawcett, 1970). Type B spermatogonia are only found as one generation of spermatogonia and have a finite life, as they subsequently divide by mitosis to form primary spermatocytes (LeBlond and Clermont, 1952b). At this step, Sertoli cells form junctions underneath the primary spermatocyte which pulls the germ cell up into the adluminal compartment where it is now separated from blood circulation (Dym and Fawcett, 1970). Primary spermatocytes enter meiosis I, the end of which generates diploid secondary spermatocytes (LeBlond and Clermont, 1952b). Secondary spermatocytes are transformed into haploid, genetically unique daughter cells called spermatids as meiosis II progresses (Wodsedalek, 1913; LeBlond and Clermont, 1952b). These spermatids are round cells when first formed, but after a complex series of

events collectively referred to as spermiogenesis, they elongate and form an acrosome and flagellum to obtain the characteristics of mature spermatozoa. Spermiogenesis was observed initially by Brown (1885) and Wodsedalek (1913) but was best defined by LeBlond and Clermont (1952a;1952b). In fact, the stages of spermiogenesis defined by LeBlond and Clermont (1952a; 1952b) form the basis of the current classification scheme for Type A_0 spermatogonia to spermatozoa. According to LeBlond and Clermont (1952a), there are four major phases of spermiogenesis: Golgi, cap, acrosome, and maturation. Characterization of these four stages is based on the formation of the acrosome over the nucleus which is important for fertilization of the oocyte. Through the progression of these four phases, the acrosome is formed from many small granules produced by the Golgi apparatus in the spermatid which migrate over the nucleus, while the spermatid itself elongates, loses cytoplasm, and develops a tail. At the end of the spermatogenic cycle, fully formed spermatozoa are released from the seminiferous epithelium into the lumen.

It is well known that across the length of the seminiferous tubule, the various stages of the cycle are staggered so that part of the seminiferous tubule is releasing spermatozoa at all times, ensuring a constant supply of spermatozoa in a wave-like fashion. Overall, 14 stages of spermatogenesis have been defined (LeBlond and Clermont, 1952b). Swiestra (1968) condensed these 14 stages into eight, and calculated the lifespan of some of the major germ cell types as well as the time it takes for a cross section of the seminiferous tubule to exhibit all eight stages in the adult boar. Primary spermatocytes exhibit the longest lifespan at 12.3 days, while secondary spermatocytes exhibit the shortest lifespan at 0.4 days because they almost immediately enter meiosis II to form round spermatids (Swiestra, 1968). The

duration of one cycle of the seminiferous epithelium was estimated to be 8.6 days (Swiestra, 1968).

Hormonal control of spermatogenesis

While the structure of the mammalian testicle has been known since before the turn of the 20th century, the regulation of the spermatogenic process is a more recent discovery. In studies, it was discovered that the pituitary gland has control over the reproductive system (Smith, 1927; Smith, 1930). Gonadotropin-releasing hormone (GnRH) released from the hypothalamus stimulates the release of two gonadotropins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary. FSH and LH have many effects on the function of the testicle. LH exerts an effect on the Leydig cells causing them to produce and release testosterone (Lincoln, 1979; Zirkin et al., 1980). Testosterone drives sexual behavior and development of the male reproductive tract. There is also evidence that testosterone is required for spermatogenesis (Bartlett et al., 1989; Sharpe et al., 1992; Singh et al., 1995). However, minimum levels of testosterone required for qualitatively and quantitatively normal spermatogenesis are unclear (Sharpe, 1994). Testosterone is highest near the Leydig cells in the interstitial fluid of the testes and lower in the seminiferous tubules (Turner et al., 1984). Regardless, testosterone plays an important role in spermatogenesis, possibly during specific stages (Sharpe et al., 1992).

The other gonadotropin important to spermatogenesis, FSH, acts directly on the Sertoli cells in the seminiferous tubule by binding to membrane receptors (Means et al., 1976). FSH then acts through the cyclic AMP second messenger system to invoke a response in the Sertoli cell (Means et al., 1976; Fritz et al., 1976). The Sertoli cell responds by

producing androgen binding protein (ABP) (Fritz et al., 1976) and inhibin (Steinberger and Steinberger, 1976). There is evidence that Sertoli cells also produce estrogen (Huggins and Moulder, 1945). Inhibin produced by the Sertoli cells provides negative feedback on the anterior pituitary to suppress FSH secretion much like it does in the female reproductive system (Steinberger and Steinberger, 1976). In addition, inhibin feeds back on the cells of the testes to reduce the action of FSH by increasing phosphodiesterase activity in the cell (Sheth et al., 1982). Similar to the role of testosterone in spermatogenesis, whether FSH is required for quantitatively normal spermatogenesis is unclear. Bartlett et al. (1989) found that combining androgens and FSH restored spermatogenesis in hypophysectomized rats better than either treatment alone, suggesting FSH does play an active role in quantitatively normal spermatogenesis. Regardless of role, FSH is known to be essential to Sertoli cell development before puberty and may dictate the number of Sertoli cells in the mature testicle (Heckert and Griswold, 2002).

Maturation of Spermatozoa

After successful spermatogenesis in the seminiferous epithelium, spermatozoa are released into the lumen of the seminiferous epithelium and travel through the rete testis and into the epididymis. At this point, spermatozoa do not have the ability to fertilize. In a study by Young (1931), it was found that when sperm were taken from the proximal end of the epididymis, they impregnated less females compared with sperm taken from the distal end of the epididymis. It was concluded that sperm must undergo a process of maturation in the epididymis which increases their fertility. However, Young believed the process of maturation was simply a function of time, and not presence in the epididymis (Young, 1931).

This was based on the observation that when spermatozoa were retained in the testes of guinea pigs, portions of the testicle begun to look like the epididymis. In 1963, Fawcett and Hollenberg observed spermatozoa in the epididymis of guinea pigs as well, and noted morphological changes in the acrosome, but not the nucleus, as the sperm cells moved into the epididymis. Regardless of the physical changes which occur, spermatozoa become concentrated in the epididymis. Turner et al. (1984) found that spermatozoa concentrations increased 800% between the rete testis and the caput epididymis and 2000% between the rete testis and cauda epididymis. In fact, they estimated that 96% of the fluid secreted by the rete testis had been reabsorbed by the time the sperm reached the end of the cauda epididymis. The length of time that spermatozoa spend in the epididymis appears to vary from species to species. In 1968, Swiestra attempted to quantify this duration for boars, and found it to be somewhere around 10.25 days based on observation of four mature boars.

Seminal plasma proteins

While spermatogenesis and maturation is complete by the time the spermatozoa reach the cauda epididymis, secretions from the accessory sex glands as well as the testes and epididymis can have a major impact on the fertilizing capability of sperm in the female reproductive tract. In fact the correlation coefficients between specific seminal plasma proteins and measures of fertility tend to be higher than those of more traditional sperm measures of motility and morphology (Flowers, 2010). Seminal plasma proteins impact the behavior of spermatozoa, behavior of the female tract, and the biological processes associated with fertilization of the oocyte. The most relevant of these are the effects on sperm motility and morphology. Protein analyses have determined that there are hundreds of

different proteins in seminal plasma, which act on sperm in a variety of ways, including protection, zona and oocyte binding, and membrane characteristics. A few proteins which have been identified as potentially impacting sperm motility and morphology are spermadhesins AWN-1, AQN-3, PSP-I, and PSP-II as well as lactadherin just to name a few (Petrunkina et al., 2003; Caballero et al., 2006; Gonzalez-Cadavid et al., 2014). AWN-1 and AQN-3 are implicated in stabilizing the acrosome, preventing premature acrosome reactions and maintaining normal sperm morphology (Gonzalez-Cadavid et al., 2014). PSP-I and PSP-II have been shown to bind together as a heterodimer which can bind to the membranes of the spermatozoa and help preserve membrane integrity, mitochondrial activity, and motility in boar semen (Caballero et al., 2006). Lactadherin may have a similar role, in that it has been shown to be positively correlated with sperm motility and may be involved in preventing premature capacitation and acrosome reactions (Petrunkina et al., 2003; Gonzalez-Cadavid et al., 2014). From a more general viewpoint, seminal plasma proteins as a whole have been implicated in many functions, including maintenance of morphologically normal spermatozoa in Brahman bulls (Boe-Hansen et al., 2015), stabilization of the sperm plasma membrane and delay of the acrosome reaction (Luna et al., 2015a), and induction of hyperactivation (Luna et al., 2015b). Regardless of the specific biological function, seminal plasma proteins certainly have an impact on boar fertility. Fertility is just as important as sperm production to the commercial boar stud, as evidence from the swine industry suggests that boars have a greater potential to cause reproductive failures due to infertility when compared to sows (Flowers, 2013). This high degree in variation of boar fertility due to seminal plasma is evident when heterospermic inseminations are used to breed sows and

paternity tests are performed (Flowers, 1997). Some boars, termed dominant, tend to sire a greater percentage of pigs in a litter than non-dominant boars, even though both boars have adequate sperm motility and normal morphology (Flowers, 1997). Within a single boar, the seminal plasma protein profile is different between the various sperm fractions and even between subsequent sperm rich fractions (Novak et al., 2010), indicating that the sperm rich fractions collected as ejaculates in commercial boar studs may not contain the full complement of seminal plasma proteins that would be present in the female reproductive tract during natural service. While protein analysis was not included in the current study, it is important to remember that it is likely these proteins have an impact on our results.

Pubertal Development of the Boar

Pubertal development in the male pig is essential to sperm production later in his life. However, puberty is not as well defined in boars as it is in gilts. In gilts, the presence of estrus is a clear indication of sexual maturation. The lack of a definitive external cue in boars can make defining puberty challenging. Previous studies have defined puberty in multiple ways; however, the most prevalent definition relates to the presence of spermatozoa in the lumen of seminiferous tubules (Phillips and Andrews, 1936; McFee and Eblen, 1967; van Straaten and Wensing, 1977; Allrich et al., 1983). Many studies investigating pubertal development in the boar often unilaterally or bilaterally castrate boars at various ages, and observe testicular sections histologically. While these studies do not provide a way to estimate sperm production in adults, they are useful results for approximating the age at which boars attain puberty, or when spermatozoa first appear in the lumen of seminiferous tubules. Without subsequent data on the fertility of these early sperm cells, it is difficult to

determine whether their presence in the testicles is sufficient to definitively conclude that they have reached puberty. McFee and Eblen (1967) noted that in the first few weeks after sexual maturity in miniature swine, the organization of maturing sperm cells was abnormal, and that loss of spermatids was high. In addition, the process of spermatogenesis was very susceptible to disruptions during this time. Based on these observations, it can be interpreted that additional criteria may be needed to determine age of puberty in boars in addition to the mere presence of sperm in the seminiferous tubules.

The age at which spermatozoa first appear in the lumen in typical crossbred boars appears to be between 130-150 days (Phillips and Andrews, 1936; van Straaten and Wensing, 1977; Allrich et al., 1983). Similarly, McFee and Eblen (1967) noted spermatozoa in the lumen of seminiferous tubules of miniature swine by 22 weeks of age. However, there appears to be breed-related variation in pubertal development. A classic example of the breed effect is the Meishan. Spermatozoa have been found in the seminiferous tubule lumen of the Meishan boars as early as eight weeks of age and they achieve puberty much earlier than conventional swine breeds at approximately 80 days of age (Lunstra et al., 1997). Increases in testicular weight and seminiferous tubule diameter also occur more rapidly in the Meishan breed due to their earlier onset of puberty (Ford and Wise, 2009). Interestingly, the Piau breed, like the Meishan, is considerably smaller than conventional breeds, but does not show the same pattern of accelerated pubertal development. This observation indicates that the effect of breed on puberty is not necessarily a function of mature body size (Franca et al., 2000; Ford and Wise, 2009). Piau pigs reach puberty around five months of age, similar to conventional breeds (Allrich et al., 1983, Franca et al., 2000).

In addition to genetics, nutrition can have an impact on pubertal development in the boar. In a classic study by Phillips and Andrews (1936), boars fed a low plane of nutrition after weaning at six weeks of age experienced slower growth of the testes and seminiferous tubule diameter when compared to boars fed at an adequate plane of nutrition. Histologically, the epithelium of the seminiferous tubule was less developed in the underfed boar. In another similar experiment by Prunier et al. (1987), testis weight of boars fed at a level 70% of the control group was significantly reduced. However, relative to body weight, the testes were similar in size, approximately 4.5% of body weight. Likewise, the weights of the epididymi and accessory sex glands were also reduced in the boars on the reduced plane of nutrition compared to the control boars at the same age. Overall, it can be deduced that typical (conventional breed) boars will reach puberty at an age of approximately 150 days assuming adequate nutrition is provided.

During pubertal development, the size of the testes increases. After birth, male pigs exhibit a period of accelerated testis growth (van Straaten and Wensing, 1977; Franca et al., 2000). This period is approximately four weeks in length, and is attributable to a growth in volume and number of Leydig cells of the testes. In a study by van Straaten and Wensing (1977), the proportion of testicular volume occupied by Leydig cells reached a peak of 64% at 3 weeks of age. However, these results do not agree with those of Franca et al. (2000) which suggest a peak of approximately 42% at 30 days of age. While the magnitude of the peak may be unclear, changes in the proportion of the testicular volume occupied by Leydig cells is better characterized. Multiple studies have found the highest values in the first two months of age, followed by a steady decline up until pubertal development (van Straaten and

Wensing, 1977; Lunstra et al., 1986; Franca et al., 2000). As boars reach puberty, Leydig cells once again begin to grow in size and number (Lunstra et al., 1986; Franca et al., 2000). This begins around four months of age and continues until they are around six months of age.

In addition to Leydig cells, the development of the seminiferous tubules within the testicular parenchyma has also been well characterized. Several studies have found evidence suggesting growth in seminiferous tubule diameter occurs closer to puberty than Leydig cell growth (McFee and Eblen, 1967; van Straaten and Wensing, 1977; Franca et al., 2000; Ford and Wise, 2011). McFee and Eblen (1967) noted a gradual increase in seminiferous tubule diameter and testis weight up to 18 weeks of age followed by a sharp increase in both up to 22 weeks of age. During this period, the seminiferous tubules begin to occupy a greater percentage of the testicular volume (Franca et al., 2000; Ford and Wise, 2011). The percentage of testis occupied by the seminiferous tubules appears to reach a maximum around 5-6 months of age, achieving nearly 90% of testicular volume in Piau pigs (Franca et al., 2000). In contrast, Lunstra et al.. (1986) and Ford and Wise (2011) found that seminiferous tubule diameter continues to increase past 7 months of age.

During the pre-pubertal and pubertal periods, changes in various hormones in the boar have been implicated in determining when they reach puberty. Some of the early studies on male maturation were done using the male rat as a model, and serve as the basis for understanding puberty in other species. Two studies in the mid-1970s (Odell et al., 1974; Odell and Swerdloff, 1975) determined that changing testicular sensitivity to luteinizing hormone (LH) may be an important factor in pubertal development in males. Evidence suggests that the testes may become more sensitive to LH as the male reaches puberty (Odell

et al., 1974; Odell and Swerdloff, 1975). Subsequently, increased amounts of testosterone is produced as LH sensitivity increases (Odell et al., 1974). Subsequent studies in boars showed a similar pattern in that levels of testosterone increased before puberty while LH did not change (Colenbrander et al., 1978; Tan and Raeside, 1980; Allrich et al., 1982; Allrich et al., 1983; Lunstra et al., 1986). At approximately 130 days of age, LH sensitivity appears to increase substantially; several studies have noted significant increases in serum testosterone levels at this time (Tan and Raeside, 1980; Allrich et al., 1983; Lunstra et al., 1986). Kattesh et al. (1982) found that boars at 150, 200, and 250 days of age had similar mean serum testosterone levels, indicating that testosterone appears to stabilize just prior to and after puberty. While this general pattern is well characterized, considerable variation among individual boars are apparent even when body and testis size are similar (Booth, 1975; Allrich et al., 1982). Therefore, caution should be taken when concluding boars must reach a given level of testosterone production in order to reach puberty.

Perhaps the most relevant and important aspect of pubertal development of the boar is the proliferation and maturation of the Sertoli cell. Research with rat Sertoli cells determined that under the stimulation of FSH, Sertoli cells could produce lactate, a possible essential energy source for spermatids developing in the seminiferous tubules (Mita et al., 1982). In addition, Tres and Kierszenbaum (1983) found that spermatogenic cells were associated intimately with rat Sertoli cells, but not other types of cells indicating that Sertoli cells are "nurse cells" which are essential for their proper growth and development. Consequently, identifying the pattern of Sertoli cell growth has been the focus of several studies. Steinberger and Steinberger (1971) noticed a time dependent pattern of Sertoli cell

proliferation in rats. By using thymidine incorporation as a marker for mitosis, they found that 33% of Sertoli cells showed evidence of mitosis at birth, which dropped to 23% at 7 days of age. By 16 days of age, no evidence of mitosis was seen. While evidence from Steinberger and Steinberger (1971) and Orth et al. (1988) in rats suggests that Sertoli cells only proliferate early in life, Johnson and Thompson (1983) observed a significant difference between the breeding and non-breeding season in horses, indicating that these cells may have the capability to proliferate during adulthood, at least in equine. In contrast, it is generally accepted in swine that Sertoli cells do not proliferate after puberty (Franca et al., 2000; Ford and Wise 2009; Ford and Wise, 2011). Franca et al. (2000) measured Sertoli cell numbers in swine and found a sixfold increase in Sertoli cell number between birth and one month of age, while only small differences were seen between four and sixteen months of age. Because these cells are directly involved in the development of spermatozoa, the number of Sertoli cells in the testis dictates the sperm production capability of an individual (Orth et al., 1988; Johnson et al., 1994; Okwun et al., 1996). In fact, reduced proliferation of Sertoli cells early in life leads to a reduction in sperm production after puberty (Orth et al., 1988). What is somewhat unclear and relevant to the current study is when Sertoli cells are proliferating in swine. Franca et al. (2000) conducted a comprehensive study of Sertoli cell proliferation in swine and observed significant increases in Sertoli cells numbers between birth and four months of age. However, the two most active periods of proliferation were during the first thirty days after birth and between three and four months of age. As expected, numbers of Sertoli cells were also highly correlated with the number of germ cells in the testis ($r=0.92$).

As is the case with testicular growth and development, variation in Sertoli cells is evident across breeds of swine. In an interesting investigation comparing Meishan, West African, and traditional maternal-line boars, Okwun et al. (1996) found similar values for daily sperm production (DSP) per gram of testicular parenchyma. However, numbers of germ cells associated with one Sertoli cell in Meishan boars were double those in the maternal-line boars (39.01 versus 19.1) even though the maternal-line boars had higher daily sperm production (Okwun et al., 1996). One interpretation of these observations is that Meishan Sertoli cells are more efficient at "nursing" spermatogenic cells than the other breeds. It was also found that the number of Sertoli cells was highly correlated with parenchymal mass and DSP; in fact it explained 76% of the variation in DSP over the three breeds studied (Okwun et al., 1996).

Because of their importance to sperm production, research has been aimed at the factors which influence Sertoli cell proliferation. While Orth (1984) found that follicle stimulating hormone (FSH) plays an important role in Sertoli cell proliferation in rats, more recent research has focused on estrogen. Interestingly, reducing serum estrogen early in life increases the number of Sertoli cells in the testis (At-Taras et al., 2006; Berger et al., 2008). This was accomplished by using an aromatase inhibitor to reduce serum estrogen levels (At-Taras et al., 2006). Similarly, Berger et al. (2008) found that treatment with an aromatase inhibitor increased Sertoli cell density and testis weight. In both studies, the aromatase inhibitor was administered beginning at 1 week of age and was continued for several weeks thereafter. Further research is still needed to determine how Sertoli cell proliferation is controlled by reduction in estrogen or other hormones affected by aromatase.

Factors Affecting Adult Reproductive Function

There are many factors that play a role in determining the sperm production of a specific boar. For the most part, these factors can be broken into two broad categories, those that affect the developmental period of sperm production, and those that affect the functional period of sperm production in adult boars. The developmental period can be considered as the period from the development of the testicles *in utero* to when the boar reaches puberty. During this period, the boar is not actively producing sperm, however, he is developing the capability to do so, such as the proliferation of Sertoli cells. Any factor which affects this development permanently affects the sperm producing capacity of the boar. Examples include birth weight, litter size, pre- and post-weaning growth rate, and human interaction. The functional period describes the mature life of the boar as he produces sperm regularly. Factors which affect sperm production in the functional period are typically reversible, and include temperature, humidity, housing, and nutrition. The intention of the proceeding sections is to discuss some of the most influential developmental and functional factors relevant to the current study.

Birth Weight

It is well known that in litter bearing species, there is variation in size of the offspring. It is thought that genetic selection for higher prolificacy in swine has not been accompanied by an equal increase in uterine capacity (Foxcroft et al., 2007). Therefore, fetuses in the gestating sow uterus experience some degree of intrauterine growth retardation (IUGR) with some experiencing more than others, resulting in some littermates that may be deemed low birth weight pigs or even “runts” (Foxcroft et al., 2007). While “normal” size

pigs can be born from a large litter, typically the weight of the fetus (and accompanying placenta in gestation) is inversely proportional to the number of fetuses in gestation (Town et al., 2004). When sows with large litters reach parturition, the resulting offspring typically have lower individual birth weights than offspring born in a small litter (Quiniou et al., 2002; Beaulieu et al., 2010). It is reasonable to assume the physical limitations of uterine size and neighboring littermates can place pressure on growing fetuses, stunting their growth. However, it is also possible that blood flow to fetuses may be impaired in large litters. Pere and Etienne (2000) measured blood flow in first term gilts and compared flow rates to litter sizes. As might be expected, blood flow did increase with litter size, however not at rate high enough to sustain similar blood flow to each developing fetus. Gilts with a litter of only 2-3 pigs provided an average of 0.72 Liters of blood per minute per fetus, while gilts with a litter of 6-8 pigs only provided an average of 0.43 Liters of blood per minute per fetus. The reduced blood flow likely means that less nutrients reach each fetus and therefore growth is slower. It is of particular interest in this study to investigate the effects of a low birth weight. While reduced birth weight can be associated with many traits during the life of a pig, the most relevant to this study are the effects of low birth weight on lifetime growth, age of puberty, and reproductive performance.

The most direct and obvious result of low birth weight is the impact on growth. It is well documented that pigs born small grow slower than their larger littermates (Powell and Aberle, 1980; Rehfeldt and Kuhn, 2006; Berard et al., 2008; Beaulieu et al., 2010; Smit et al., 2013). However, when a small littermate is compared to a large littermate, their ability to grow is confounded with their inability to compete for adequate milk during lactation, which

means the results are not a true indication of the small pig's capacity to grow (Powell and Aberle, 1980). When runts are removed from their birth litter and fostered into a small hybrid litter entirely made up of runts, performance improves considerably (Powell and Aberle, 1980). In fact, Powell and Aberle (1980) found that fostered runts only required two extra days to reach 26 kg than their large littermates compared to runts left in their birth litter during lactation who required 16-18 more days to reach 26 kg body weight. This significant improvement in performance explains why cross-fostering is a tool used to improve performance of small pigs in the commercial swine industry.

It is thought that one of the consequences of this stunted growth relates to myogenesis during gestation. It appears that numbers of muscle fibers in a pig are fixed after birth and that hypertrophy, rather than hyperplasia, of muscle fibers constitutes the increase in body mass as the animal grows (Rehfeldt and Kuhn, 2006). Consequently, a small pig at birth has fewer muscle fibers, and therefore to achieve the same body size of a large littermate (for example at slaughter), the muscle fibers must grow to a larger size than those of the large littermate. Rehfeldt and Kuhn (2006) found that low birth weight pigs grew the slowest, had the fewest number of muscle fibers at slaughter and had the largest muscle fibers at slaughter when compared to medium and high birth weight pigs. The lower growth rate of small pigs is mirrored in other studies by Berard et al. (2008); Beaulieu et al. (2010); and Smit et al. (2013). It also appears that low birth weight pigs are less efficient at converting feed to gain. Powell and Aberle (1980) noted a tendency for decreased feed efficiency in runt pigs, while Berard et al. (2008) found that low birth weight pigs were significantly less efficient in converting feed to gain in the grow/finish period than medium and high birth weight pigs.

The effect of birth weight on the age at which pigs reach puberty is not well studied. However, it appears that there is little correlation between the two (Young et al., 1978; Hutchens et al., 1981). While the literature has only investigated this relationship in gilts, boars likely follow the same pattern. In two studies which calculate a genetic correlation between birth weight and age at puberty, both used first estrus after exposure to a mature boar to define the age at which the gilts had reached puberty. Young et al. (1978) calculated a correlation of -0.14, although it was not statistically significant. Likewise, Hutchens et al. (1981) calculated a low genetic correlation between birth weight and age of puberty of -0.07. While the correlation is low at birth, the correlation increases as the age at which the weight is taken is increased (Hutchens et al., 1981). For example, the genetic correlation between weaning weight and age at puberty was much stronger (-0.25) than the correlation when birth weight is used (-0.07) (Hutchens et al., 1981). It is worth noting these older studies were experimenting with genetics very different from those used today in the commercial industry. Modern genetic lines are often selected for accelerated pubertal development, and so new investigations into the relationship between birth weight and pubertal development is warranted. Based on these older studies however, there may be a very small correlation between birth weight and the age at which a boar reaches puberty, but indirect evidence from gilts suggests it may not be significant.

While specific evidence for the effect of birth weight on puberty in boars is lacking, there is some evidence that birth weight may have an effect on the reproductive performance of the boar in adult life (Dysart, 2015). It is possible that the effect of birth weight on growth discussed earlier may play a role in the sperm producing capability of a boar after puberty. In

a study comparing ten high birth weight boars, which had an average birth weight of 2.0 kg, with ten low birth weight boars, which averaged just 1.1 kg at birth, there was a clear difference in total sperm production per ejaculate after the boars were trained for semen collection (Dysart, 2015). The high birth weight boars produced on average 10.4 billion more sperm per collection over a 27-week collection period than the low birth weight boars (Dysart, 2015).To put this difference into perspective, based on the industry standard of 3 billion sperm cells per semen dose, this difference in total sperm output equates to over three additional insemination doses at each collection. Over a year's time, the high birth weight boars were capable of producing over 200 more insemination doses than their low birth weight counterparts. Based on the results of this study, birth weight can certainly affect (directly or indirectly) the developmental period of sperm production of boars and result in differences in sperm production later in life (Dysart, 2015). Additional research specific to birth weight is needed to validate these findings.

As discussed previously, the effects of birth weight could potentially be confounded with the effects of the neonatal environment, specifically the size of the litter the boar is reared in. Litter size can clearly impact the growth rate of boars during lactation, but it is unclear whether this effect carries into adult reproductive performance. Data from a previous study which examined the effect of litter size on reproductive performance of boars suggests litter size has little influence over sperm production in the mature boar (Klein, 2005). Boars reared in litters of six did exhibit higher body weights from lactation to puberty compared to boars raised in litters of ten or more, however, the difference was not enough to observe a difference in sperm production later in life (Klein, 2005). The small litter size boars also had

increased testicular size, which would implicate greater sperm production potential as adults (Klein, 2005). It is plausible that the large litter size used in the study was not large enough to induce inhibitory effects on the developmental period of the boars. It appears that birth weight is a more reliable predictor of sperm production potential than litter size.

Housing and Environment

The environment within the barn, including temperature, humidity, and even facility design can all effect functional sperm production in adult boars. Heat stress is the most economically important housing issue facing the swine industry in many parts of the world. For boars, the thermoregulation of the testes plays a role in susceptibility to heat stress. As is well known, boars cannot usually adapt well to high ambient temperatures, especially in terms of regulating the temperature of the testes. Boars, like many mammals, have testes outside of the body to facilitate separate temperature regulation of the tissues within and directly around the testicles. In most cases, spermatogenesis is carried out at a temperature lower than that of the rest of the body, and so thermoregulation of the testes is crucial to proper spermatogenesis. While boars do have the ability to use evaporative cooling (sweating) on the surface of the scrotum, they do not appear to actually modulate the level of evaporative cooling based on ambient temperature (McNitt et al., 1972). It is also worth noting that a high degree of variation exists between boars in terms of evaporative cooling capacity, and therefore ability to handle high ambient temperatures (McNitt et al., 1972; Flowers, 2008). Stone (1981) surgically implanted thermocouples into different tissues surrounding the testes to determine how they respond to increased air temperatures. One of the boars was then subjected to a short term exposure to air temperatures of 38°C (Stone,

1981). It was determined that the caput epididymis was the most responsive to the increased outside temperature, however all implanted tissues, including the testicle, experienced increases in temperature (from 35.7°C to 36.4°C) during the short term exposure to 38°C (Stone, 1981). Scrotal and testicular temperatures were closely related during the heat exposure, and so it was concluded that the evaporative cooling of the scrotum was not very effective (Stone, 1981). Because the boar, specifically the testicular region, has a limited ability to adapt to high ambient temperatures, it is important to understand how heat stress impacts spermatogenesis.

The effect of temperature on ejaculate quality in boars has been studied very thoroughly over several decades, and so it is widely accepted how detrimental heat stress can be to boar sperm quality. A study by Thibault et al. (1966) compared the farrowing rates of sows serviced by boars kept outside and boars kept inside and found that there was a significant decrease in fertility from June to September in the boars kept outside where they experienced exposure to summer temperatures of up to 35°C. However, farrowing rates are dependent on a multitude of factors independent of the boar. In the 1970s, researchers began subjecting boars to controlled heat stress and determining the impact on semen quality rather than farrowing outcomes. Short-term (72 hours) exposure to high ambient temperatures (33°C) is enough to observe significant decreases in sperm concentration, motility, total sperm per ejaculate, and the percentage of morphologically normal sperm (McNitt and First, 1970). These detrimental effects were not observed until 3-4 weeks after the heat treatment, suggesting there is a lag in response to heat stress and that sperm already formed in the seminiferous tubule lumen and mature sperm in the epididymis are not effected by heat stress

(McNitt and First, 1970). Interestingly, it appears as if the accessory sex glands are not affected by short term heat stress as seminal volume did not change significantly (McNitt and First, 1970). The results were mirrored by two studies performed by Wettemann et al. (1976; 1979) in which boars were housed at temperatures of 31°C for 16 hours per day and the remaining 8 hours the ambient temperature was increased to 34.5°C. Heat stress became apparent in these boars through increased rectal temperatures and respiratory rates, however after four weeks of continuous treatment, rectal temperatures decreased, indicating that the boars could in fact partially adapt to the chronically high ambient temperatures (Wettemann et al., 1976; 1979). Again, this level of heat stress did not significantly alter semen volume despite the more chronic nature of the heat treatment (Wettemann et al., 1976; 1979). After two weeks of treatment, sperm output per ejaculate decreased to 70% of control values, which was followed by a decrease in motility and percentage of normal acrosomes (Wettemann, 1976). The second study subjected boars to heat treatment for 11 weeks, which resulted in a gradual decrease in sperm motility beginning two weeks after starting treatment to a minimum value of 21% motile sperm at the end of the treatment period (Wettemann et al., 1979). In addition, it took five weeks after cessation of the heat treatment for the boars to restore motility levels similar to control boars, indicating that heat stress can have effects lasting weeks after heat stress disappears (Wetteman et al., 1979; Flowers, 1997). Again, there exists a great deal of variation between boars in the response to heat stress on semen quality measures based on the literature reviewed and some of this variation may be due to genetics (Flowers, 2008). It also appears that the degree of detriment to semen quality is dependent upon the length and intensity of the heat stress event. After a short term (6 hour)

exposure to ambient temperatures above 30°C every day for 4,5, or 7 days, motility only dropped below 80% in two of twelve boars studied, however all but one boar had reduced morphologically normal cells (Cameron and Blackshaw, 1980). This indicates that the effect of heat stress varied considerably between the boars and for some, the exposure duration and/or intensity was not high enough to cause significant decreases in sperm motility. On the contrary, Egbunike and Dede (1980) found that only 45 minutes of outside sunlight exposure coupled with a high summer temperature each day for three days was enough to see significant changes in sperm motility and concentration. Total sperm per ejaculate dropped from 58.22 to 28.42 billion sperm in the week following the sunlight treatment, but once again, no change in semen volume was observed (Egbunike and Dede, 1980). An interesting study was conducted by Stone in 1982 in which ambient temperature around boars was increased 1°C each day beginning at 20°C and ending when boars had experienced 24 hours of ambient temperatures of 40°C. It was found that scrotal temperature increased at an ambient temperature of 30°C, while respiratory rate and rectal temperatures did not increase until 33°C and 35°C respectively, indicating that the scrotum and testicular region of the body is more susceptible to heat stress than the rest of the body (Stone, 1982). Again, detrimental effects on sperm motility lingered for several weeks after the end of the heat treatment, and it was concluded that for Large White boars, 29°C appears to be the critical point above which sperm quality deteriorates (Stone, 1982). Considering the effectiveness of most modern ventilation systems, boar studs are less likely to experience high-intensity acute heat stresses like those induced in the laboratory environment. However, the effects of more common chronic, low-intensity heat stresses (ambient temperatures of 26-29°C) can still be

significant (Flowers, 1997; Flowers 2008). In comparison to acute heat stresses, the effects of chronic heat stress take 2-3 more weeks to become apparent (Flowers, 1997). Some degree of chronic heat stress likely impacts most boar studs, especially those in warmer climates including eastern North Carolina. This chronic heat stress appears as a reduction in total sperm per ejaculate or an increase in the number of ejaculates rejected for quality reasons (Flowers, 1997). Furthermore, additional studies that include month and/or season in the statistical model predicting sperm production or quality find these terms to be significant, indicating that even though domestic swine are not seasonal breeders, boars do show periods of reduced fertility, likely related to environmental temperature (Kennedy and Wilkins, 1984; Cameron, 1985). This environmental factor will likely impact the results of the current study.

While not much research has investigated how facilities play a role in boar reproductive performance, there may be an effect of the type of housing system on sperm production (Swing, 2012). Evidence from Swing (2012) suggests that boars housed in pens may produce more sperm per ejaculate and have higher libido than those raised in crates. Interestingly, when boars were moved from crates to pens, sperm production increased, but the reverse was not true. However, sperm quality did not appear to be affected by housing system, evidenced by similar motility, normal morphology, and paternity rates in heterospermic inseminations (Swing, 2012). As far as facility materials, paneling and flooring may have an effect on sperm quality, especially when boars are challenged with heat stress (Corcuera et al., 2002). In the summer season, boars housed in pens with partial wall and partial gate dividers showed improved motility and normal morphology over boars housed in pens with full gate dividers. Similarly, boars housed on solid concrete floors had

improved sperm motility and morphology compared to those housed on partially slatted floors. These effects were only seen in the summer and not in other seasons, meaning that the increased shading and greater conductive heat loss capacity of solid concrete floors and walls may have allowed the boars to stay cooler and minimize the effects of heat stress (Corcuera et al., 2002). Overall, temperature and its interaction with humidity play a major role in affecting sperm quality in boars, and so facility design is important to maintain proper conditions and minimize the effects of heat stress.

Testicle and Body Size

In addition to developmental traits, certain physical traits in boars may dictate their capability to produce high numbers of quality sperm. Two obvious physical characteristics that may be taken into account when evaluating a boar are body weight and size of the testicles. As discussed previously, the birth weight of a boar can have a significant impact on sperm production in adult life. However, it is easy to conclude that the confounded effect of body weight would be involved in differentiating between low and high birth weight boars, as boars with low birth weights are often smaller throughout life than their normal birth weight counterpart. Regardless of body weight, testicle size is more likely the most influential physical characteristic on sperm production. In 1985, Cameron conducted a simple experiment with 35 boars attempting to correlate various factors with sperm production in boars. Both testis width and length were measured in the study, and the results suggested a correlation of 0.42 between estimated daily sperm output and testis width. The estimated correlation between testis length and daily sperm output was lower at 0.30. It is worth noting however that significant correlations were found between all semen parameters

measured and body weight, including sperm concentration and total sperm, except for daily sperm output. Testicular width was significantly correlated with daily sperm production, but body weight was not.

Along the same lines, a study conducted in 1996 by Huang and Johnson found significant differences in sperm production between a genetic line of boars that had been selected for increased testicle size and a control line of boars, despite the fact that body weights did not differ significantly between the two boar lines. However, the two lines differed considerably in testis and epididymis size, indicating that testicle size is much better predictor of sperm production than the body weight of a boar. The results of Cameron (1985) suggest that testicle width is a better predictor than testicle length, which were mirrored by results from a recent study by Jacyno et al. (2015), who found significant correlations between sperm concentration, total sperm per ejaculate and progressive motility with both testicle volume and testicle width, but not with testicle length. Testicular volume takes into account the length and width of the testicles and has been used in other studies relating testicle size and sperm parameters rather than length or width. Owsianny et al. (1998) used testicular volume at 180 days of age as the measure of testicle size and found significant correlations with sperm progressive motility ($r = 0.18$), sperm concentration (0.22), total sperm number (0.17) and the number of acrosome defects (-0.08). These correlations are low however when compared to the correlations estimated by Ugwu et al. (2009) which were in the range of 0.79 to 0.91. While there is great variation in correlation estimates, the results all indicate that testicular size is a good predictor of sperm production.

Human and Animal Contact

Perhaps more abstract than many of the factors influencing spermatogenesis in the boar, contact with humans and other animals can have an impact on the behavior and consequently the performance of the animal. While this certainly has important implications for growing market hogs for best performance and management, animal behavior is central to successful management of breeding animals. Early work by Hemsworth and colleagues paved the path to understanding how human-animal interactions can affect animal performance. Several studies in the 1970s and 1980s from this laboratory in Australia investigated various human-animal interaction patterns and how these treatments affected growth and feed efficiency of the animal. In addition to the effects of human contact on pig performance, contact with other pigs, especially early in life, may play a role in reproductive performance in adult life (Hemsworth et al., 1977). In a simple investigation of sexual behavior in the boar, Hemsworth et al. (1977) allotted young boars to different levels of exposure to other boars of the same age. It was found that boars reared in an all-male group from three weeks to seven months of age allowing physical and visual contact between boars exhibited greater reproductive behavior (Hemsworth et al., 1977). This was evidenced by increased number of copulations with receptive females and courting behaviors during a 15 minute test period compared to boars that had limited or no visual and physical contact with other boars during rearing (Hemsworth et al., 1977). In studying interactions with humans, Hemsworth and colleagues pioneered a system for evaluating the level of fear a pig has for humans known as the human approach test (Hemsworth et al., 1981). Many of the pig behavioral studies utilized a similar treatment regime which was established by Hemsworth

et al. (1981). One treatment consisted of pleasant human-animal interactions done several times a week for short durations, which required squatting in the pen and gently petting any animals which approach. An unpleasant treatment consisted of the same schedule of interactions except the experimenter maintained an erect posture and delivered a hard slap or small electric shock to any approaching animal. After treatments have ceased, the level of fear was tested using the human approach test. The human approach test consists of introducing an animal into a novel pen and after a short familiarization period, the experimenter is introduced into the pen. The experimenter remains erect and stationary, and the reactions of the animal over a short period are recorded, including the time required to initiate first contact with the experimenter, number of times entering the area within 0.5 meters of the experimenter, total amount of time spent in the 0.5 meter radius, and the total number of times physical contact is made with the experimenter. In one of the early studies using this test, it was found that gilts subjected to the pleasant interaction treatment spent more time within the 0.5 meter radius of the experimenter and had a higher number of contacts with the experimenter. It was postulated that the gilts subjected to the unpleasant treatment developed a "learned fear response" and therefore had a much higher level of fear or distrust of humans based on their past experiences which affected their behavior during the test. Interestingly, Hemsworth et al. (1981) also discovered a significant difference in growth rate between the pleasant and unpleasant treatment gilts, with the favorable effect occurring in the pleasant treatment females. They also noted a higher level of corticosteroids in the unpleasant treatment gilts independent of exposure to humans as well as a greater increase in corticosteroids when a human was introduced into the pen, leading them to conclude these

gilts were under chronic stress, and suffered more acute stress than their pleasant treatment counterparts when a human was introduced. These results were mirrored by a study conducted in 1986 by Gonyou et al.. Gilts that were subjected to aversive or negative (mild electric shock or forced human physical contact) showed decreased growth rates and feed efficiencies during the first six weeks of treatment compared to gilts exposed to positive human interactions. These results were again replicated by Hemsworth et al. (1987). In addition to pleasant and unpleasant treatments, some gilts were subjected to inconsistent and minimal contact treatments. The inconsistent treatment consisted of a mixture of pleasant and unpleasant interactions throughout the study, at a ratio of 5:1 respectively, while the minimal treatment simply minimized the amount of human interaction the gilts experienced. Interestingly, even with the low level of unpleasant interactions in the inconsistent treatment group, gilts still suffered from higher corticosteroid levels as well as reduced growth in the first two weeks of treatment. Gilts in the minimal and pleasant treatments performed significantly better in the approach test by approaching sooner and interacting more with the experimenter. It was concluded that even with a majority of the human exposures being positive, gilts still suffered when subjected to a few negative human experiences. Despite the multiple studies producing evidence of decreased growth performance from Hemsworth and colleagues (1981; 1986; 1987; 1991), others have refuted the claim that negative interactions with humans during the growing phase can reduce growth (Pearce et al., 1989; Paterson and Pearce, 1992). Both of these studies exposed male pigs to interaction treatments similar to those used by Hemsworth and colleagues before 100 days of age for several weeks and noted no change in growth rates or feed efficiencies between treatment groups and argued pigs are

not necessarily under chronic stress when exposed to negative human experiences. Regardless of the effect of human interaction on growth, it is well accepted that pigs exposed to negative interactions with caretakers have a higher level of fear evidenced by various behavior tests like the human approach test. Even if negative human experience does cause decreased growth performance, one could conclude based on the previous discussion about body weight that the decreased growth rate may not even impact sperm production later in life. There are likely more direct relationships between human experiences and spermatogenesis in boars rather than through growth or body size.

An additional question relevant to the current study is whether pigs can discriminate between people. According to data from Hemsworth et al. (1994), it appears as if gilts cannot discriminate between a familiar handler and a novel handler when the handlers act similarly. In addition, evidence from this study also suggested that the gilts could not discriminate between handlers who treat pigs differently (e.g mostly positively or mostly negatively), leading to believe pigs form a single impression of humans as a whole based on the human they interact with the most and will react similarly to any human (Hemsworth et al., 1994). However, evidence from Tanida et al. (1995) disagrees with this interpretation. While pigs that were handled daily responded to both strangers and handlers in behavior tests more positively than those who had not been handled daily, they interacted more quickly with a familiar handler versus a stranger, indicating they could discriminate between people when they had positive experiences with humans.

While all of this evidence regarding socialization has important implications for raising breeding animals, the effect of socialization on sperm production in the boar is not

well studied. While some of the previous conclusions regarding the human interaction effect on growth and behavior may indirectly affect sperm production or trainability of boars, direct effects are unclear. Perhaps the most relevant study in the literature is one by Hemsworth et al. (1986). In this study, boars were allotted to treatments similar to other interaction studies of the time; pleasant, unpleasant and minimal. As expected, boars subjected to unpleasant human experiences beginning at 11 weeks of age had higher corticosteroid levels and performed worse in the human approach tests. Most interestingly, there were significant differences in days to achieve a fully coordinated mating response and testicle size at 23 weeks of age between all three treatment groups. The former measure could be viewed as a measure of attaining puberty, and boars who had positive experiences with humans at a younger age were able to achieve a fully coordinated mating response 14 days sooner than those exposed to minimal human interaction, and 31 days sooner than those who were exposed to negative human interactions. A similar pattern was seen in testicle size (length x width) at 23 weeks of age, with boars that had positive human interactions having testicles nearly 19% larger than those who had negative human interactions (63.3 cm^2 versus 53.2 cm^2, respectively) despite having similar body weights. However, the significant difference in testicle size had disappeared by 39 weeks of age. These results suggest that boars exposed to positive human contact before puberty may attain puberty earlier and have larger testicles at the same age, resulting in earlier sperm production and a shifted sperm production pattern throughout their adult lives.

Recent research has begun to examine how exposing boars to humans early in life in a positive manner may impact their actual semen production. For boars that are regularly

collected for artificial insemination, sperm quality and quantity are extremely economically important. Dysart (2015) "socialized" a group of young boars by exposing them to positive human contact one hour each day, three days a week, for five weeks beginning when the boars were 36 days of age. These socialized boars were then reared in finishing with limited human exposure in a manner similar to most boars or market hogs. The boars were then trained for collection beginning at 173 days of age to begin semen analysis. The boars were then collected weekly for six months with data on reaction time to the dummy, mounting time, and typical semen analysis parameters collected on each ejaculate. Evidence from this study suggests socialization can certainly affect collection performance directly or through an interaction with birth weight. Socialization had a significant effect on total sperm per ejaculate, with boars that had been socialized producing 10.2 billion additional sperm compared to non-socialized control boars (Dysart, 2015). Overall, socialization had no effect on sperm motility, mobility, or morphology, indicating that socialization had an impact on the sperm producing capacity of the boar without any detrimental effects to qualitative spermatogenesis (Dysart, 2015). The results from this study have prompted more investigation into the effect of socialization of boars with a low birth weight.

The experiences that a young boar has with humans certainly has an effect on their performance later in life. Based on the previous discussion, efforts should be made to maintain positive interactions with breeding animals at all times to ensure these animals develop a positive attitude and subsequent behavior response to human interaction. This will ensure easier handling and better performance.

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INTRODUCTION

The use of artificial insemination in the swine industry has no doubt allowed for more rapid genetic improvement, increased sow welfare, and simplified breeding management. Boars have left the sow farm, where they had to be managed individually, to specially designed boar studs which provide greater control over the management of boars as well as the means to collect ejaculates and produce semen doses. However, the boar stud now represents a business enterprise which must be profitable for the genetic companies that manage them. Because of this, management places a high priority on the consistent production of large amounts of high quality sperm.

There are many factors that affect sperm production in boars. From the perspective of swine farm employees, many factors are outside of their control such as housing, genetics, nutrition, and birth weight. Of these, birth weight has been shown to affect lifetime productivity of boars. Recent research from Dysart (2015) has shown a significant decrease in average sperm production per ejaculate in boars that weighed 1 kg at birth compared with their counterparts that weighed 2 kg. However, it appears that interactions with humans later in life can partially reverse or correct the disadvantage that small boars are born with in terms of their sperm production potential. Dysart (2015) also showed a significant increase in sperm per ejaculate in boars that experienced increased (positive) human interaction during the nursery phase compared with boars exposed to limited human contact. This difference was 10.2 billion sperm cells per ejaculate. This observation is not without precedent. Hemsworth and colleagues have shown that negative interactions between humans and pigs induce a fear response in the animal resulting in decreased performance (Hemsworth et al.,

1981; 1986; 1987; 1991) including delayed testicle growth around the expected time of puberty.

Human socialization could possibly provide a new management tool used to ensure adequate sperm production from boars that were born smaller than their littermates based on the results of Dysart (2015). However, there are several important questions with regards to the interaction of boars with humans during pubertal development that remain unresolved. Two of these are related to the length and timing of the socialization period. Consequently, the objective of this study was to determine the relative effectiveness of continuous socialization from 4 to 24 weeks of age; socialization from 4 to 9 weeks of age; and socialization from 10 to 24 weeks of age on sperm production and semen quality of adult boars that were born with low birth weights.

MATERIALS AND METHODS

Animals, Facilities, and General Management

The study was conducted at the Swine Educational Unit (SEU) at North Carolina State University between 7/29/15 and 9/1/16. The SEU is a closed herd, farrow-to-finish operation with 250 sows. All boars used in the study (n=36) were born between 7/28/15 and 8/3/15 and resulted from mating Smithfield Premium Genetics (SPG) maternal-line sows with SPG terminal-line boars. Boars selected for this study were the smallest boars in their respective litters based on birth weight. Boars (n=36) had an average birth weight of 1.2 kg with a range of 0.6 kg to 1.5 kg. The boars came from 17 different litters, and 28 of the 36 boars had at least one full sibling in the study.

Lactation Management

Sows were moved to farrowing crates at approximately day 107 of gestation. Each farrowing room consisted of 12 individual, bow-bar crates that measured 1.5 m wide by 2.5 m long. The raised flooring under the farrowing crates consisted of cement slats underneath sows, TriBar®, an expanded metal, behind sows and Tenderfoot®, a plastic coated wire, in the piglet creep areas. Supplemental heat was provided by two heat lamps in each crate. Each farrowing room was equipped with a side-wall baffle ventilation system with an evaporative cooling cell. During lactation, sows were fed a corn and soybean formulated ration ad libitum twice per day that met or exceeded NRC recommendations for lactating sows (NRC, 2012).

Piglets were processed within 48 hours of birth, which included weighing, ear notching, tail docking, oral scours medication and iron injections. At this time, a total of 49 boars were pre-selected for the study and were not castrated. The boars were managed

similarly during lactation and nursed with their respective littermates until weaning at approximately 21 days of age. At weaning, boars were weighed and moved to the nursery at the SEU. At the time of weaning, a total of 36 boars were assigned to nursery socialization treatments such that the weaning weights of boars in each pen was similar (see Socialization Treatment Allocations).

Nursery Management

The nursery rooms at the SEU consisted of twelve elevated pens measuring 1.82 m x 1.82 m. Six pens were located on each side of the room and divided by a central walkway. Each pen housed 9 boars and provided 0.91 m of feeder space and 4 nipple waterers. The nursery room was equipped with a side-wall baffle ventilation system with supplemental heat provided by a propane heater suspended from the ceiling near the front of the room. All boars in the nursery were fed a standard 23% protein starter diet consisting predominantly of milk by-products for 7-10 days. The diet was then transitioned to a corn and soybean meal based diet in which the protein percentage gradually decreased to 18% which met or exceeded the NRC recommendations for growing pigs between 7 and 35 kg (NRC, 2012) over the next four weeks. Over the course of the nursery phase two boars died for reasons unrelated to the study.

Finishing Management

At approximately 72 days of age, 32 of the 34 boars were moved to the finishing barn. Two boars were removed from the study due to anatomical problems in either front or rear legs. The remaining 32 boars were equally distributed across the four treatment combinations. Boars were housed 4 per pen so that there were two pens of boars for each

treatment combination. Boars within each treatment combination were randomly placed into finishing pens. The finishing barn at the SEU was a curtain-sided, under-slat barn with the curtains and exhaust fans controlled by separate thermostats. The building was equipped with misters and cooling fans that were programmed to provide supplemental cooling if ambient temperature in the barn exceeded 25.5°C. The pens in the finishing barn measured 1.84 m wide x 2.84 m long. Each pen provided 1.1 m of feeder space from a two-hole feeder (four boars per pen) and 2 nipple waterers. During the finishing phase, the boars were fed a corn and soybean formulated ration which met or exceeded NRC recommendations for growing boars (NRC, 2012).

Socialization Treatment Allocations

At the time of weaning, 36 boars were selected for the study and randomly allocated to one of two nursery socialization treatments; socialized or non-socialized. This resulted in two pens of boars in one room being socialized and two pens of boars in a second room exposed to no socialization. Non-socialized boars experienced minimal contact time with humans typical of traditional husbandry practices during the nursery phase. A SEU employee checked the health of the animals and filled feeders as necessary, resulting in less than 5 minutes of human contact per day.

Beginning 10 days after being moved into the nursery, socialized boars were subjected to 1 hour of human contact three times per week (Monday, Wednesday, Friday). The two pens were directly across the central aisle from each other which allowed socialization for both pens to occur simultaneously. Thirty minutes of the one hour socialization period was spent facing/working with one of the two pens with the remaining

30 minutes spent facing/working with the second pen. During each half hour period, the socializer would score each boar using the following scale adapted and modified from Dysart (2015):

0 – Does not approach front of pen/socializer

1 – Approaches front of pen/socializer

2 – Approaches front of pen/socializer with hand extended

3 – Licks/nibbles extended hand

4 – Allows scratching/petting

During the first week of socialization, the socializer simply stood in front of the pen and attempted to make eye contact with each boar in the pen, but the socializer made no attempt to make physical contact with the boar. Therefore, during the first week a boar could only achieve a maximum score of 1. During the next two weeks, the socializer stood in the aisle, but attempted to make physical contact with the boars by extending hands through the front of the pen. A boar could then achieve a full maximum score of 4. During the last two weeks of nursery socialization, the socializer would enter the pen slowly; stand in the center of the pen; allow the boars to make contact with their boots, and then attempt to make contact by reaching down with their hands without crouching. There were two socializers – a male and a female – that provided the human contact on alternate days. Consequently, during the nursery phase, there was a total of 17 hours (17 days) of socialization provided for each individual boar assigned to nursery socialization.

At the end of the nursery phase (approximately 10 weeks of age) boars within each nursery pen were randomly assigned to receive socialization during the grower/development

phase in the finishing barn. This completed the assignment of boars to their respective socialization treatments which was a factorial design with socialization occurring in either the nursery or finishing barns. The end results were four treatment combinations (n=8 boars each) as follows: socialization in both the nursery and finishing barns (4 to 24 weeks of age); socialization during only the nursery phase (4 to 9 weeks of age); socialization during only the finishing phase (10 to 24 weeks of age); and no additional socialization or human contact. Boars were housed in the same finishing barn. However, socialized pens were located in the front of the barn near the entrance of the barn while unsocialized pens were located in the opposite corner in the rear of the barn away from any entrances. In the finishing phase, a SEU worker again checked the health of the animals daily. However, feeders were filled by the study socializers when necessary which usually was once every 10 days. Again, this resulted in less than five minutes of human contact per day.

Socialization in finishing was similar to the nursery. Because of the space requirements of the growing boars, only four boars were placed in each pen in the finishing barn. Socialization was also done three times per week. Because four pens were used to house the socialized boars, the socializer would stand at the front corner of two adjacent pens at the same time and directly "socialize" those two pens. This was done for 30 minutes for each pair of pens, yielding a total of one hour of socialization (indirect and direct) for each boar each day (See Figure 1).

A similar scale was used for the finishing phase. Again, the first week of socialization consisted of no attempts to make physical contact, followed by reaching out towards the boars in the following weeks. In contrast to the nursery phase, socializers did not enter the

pens during socialization periods. The socializer stood on the lower bars of the front gates of the finishing pens which allowed boars to make contact with the socializers' boots. Socialization was done three times per week for nine weeks, meaning each boar received 27 hours (27 days) of socialization.

Throughout the entirety of the nursery and finishing socialization, two socializers (one male and one female) alternated each day (Monday, Wednesday, Friday) and wore similar coveralls and boots, and behaved in a similar manner during socialization.

Hemsworth Testing

At approximately 90 days of age and again at 120 days of age, boars were individually subjected to a Hemsworth test (adapted from Hemsworth, 1994) over a two-week period. A traditional finishing pen (1.84 m x 2.84 m) was used for the testing procedure and was located in the same barn where the boars were housed. Along one side of the testing pen, a 0.5m by 1.0m area, the "area of interest", was drawn with a marking crayon. The tester stood inside the area of interest against the side panel during the Hemsworth test without moving. Each Hemsworth test began by bringing an individual boar into the testing pen and allowing approximately one minute for the boar to get acclimated to the new surroundings. Next, the tester carefully entered the pen and stood in the area of interest as described. For the next ten minutes, a second person standing outside of the testing pen recorded the boar's activity with regards to the area of interest and the human test subject. Four dependent variables were recorded which included the number of times the boar entered the area of interest and the duration of each of these events as well as the number of times the boar made contact with the human subject and the duration of each of these interactions. A boar had to

have at least one front foot within the marked area in order for this to be considered as having entered the area of interest. Conversely, he was considered out of the area of interest if no front feet were in the marked area. If a boar made physical contact with the tester, he was allowed to continue to make contact as long as desired unless he began to chew vigorously on the human's boots or clothes, in which case the boar was gently nudged away.

The Hemsworth test was completed over a two-week period and each boar was tested twice during the finishing phase when the boars were close to 90 days of age and again when they were about 120 days of age. One test for each boar was done with one of the two individuals that were socializers (male or female), while the second test was done with a human with no prior exposure to the boars.

Management of Adult Boars

One pen of boars for each treatment combination was moved to the gestation barn approximately two weeks apart – when boars were approximately 157 and 171 days of age. This was necessary due to space limitations in the gestation barn. The gestation barn was a curtain-sided building with underslat ventilation. Cooling fans and drippers in the barn were programmed to activate when ambient temperature in the barn reached 74 °F and 78°F, respectively, to provide additional cooling for the boars during the summer months. Each boar was housed individually in a crate measuring 2.43 m long and 1.07 m wide and fed 4 kg per day of a 14% corn and soybean meal diet which was formulated to meet NRC requirements (NRC, 2012). All study boars were housed next to each other along the northeast corner of the barn in a random order with respect to their socialization treatment.

Semen Collection Training

Immediately after being moved to gestation, training for semen collection began. To be considered successfully trained, a boar had to mount a dummy sow and successfully be collected for three consecutive days using the gloved-hand technique (Almond et al., 1998). The training and collection pen was a 2.43 m x 3.65 m pen with open sides and a dummy sow located on one end of the pen. Boars could freely move behind and beside the dummy sow without moving in front of it. The dummy sow measured 0.30 m by 1.21 m and was adjusted to a height of 0.67 m for training (Minitube of America, Verona, WI). The person that trained and collected the boars each week thereafter, was not involved in the socialization of the boars. After a boar successfully trained, weekly collection and semen analyses began. Collection for the first boars to successfully train began on 2/9/16. The training period continued for 90 days or until the boars were 10 months of age. The last boar to successfully train was collected for analysis beginning on 5/11/16.

Weekly Ejaculate Collection

Boars were randomly assigned within treatments to be collected on either Tuesday or Thursday of each week. The collection technician was the same individual that trained the boars and was not aware of the respective treatments of each boar. Collections were done between 0600 and 1200 every Tuesday and Thursday, with a majority of the collections occurring between 0630 and 0900. The order of collection each day was randomized.

During collection, reaction time, mounting time, collection duration and time of collection were measured. Reaction time was defined as the time interval between when the boar entered the collection pen with all four feet and when he made physical contact with the

dummy sow. Mounting time was defined as the time interval between when the boar made physical contact with the dummy sow and when they first mounted the dummy sow. Collection duration was defined as the time between when the boar first began ejaculating after full extension of the penis and when collection was terminated by the boar. Time of collection was simply the time of day that the collection began.

Ejaculates were collected using the gloved-hand technique (Almond et al., 1998) using powder-free polyvinyl gloves (IMV America, Eden Prairie, MN). Ejaculates were collected into a plastic collection bag (Minitube of America, Verona, WI) fitted into a plastic thermos pre-warmed to 37°C. The gel fraction and other contaminants were filtered out of the sample using a milk filter (IMV International, Eden Prairie, MN) placed on top of the thermos and secured with a rubber band. The weight of an empty collection bag and thermos was used as the pre-sample weight for determining semen volume. Immediately after collection, the ejaculate was taken to the on-farm A.I. laboratory where the milk filter with gel fraction and the rubber band were removed. The pre-sample weight for that specific thermos and bag was subtracted from the weight measured after collection in order to determine the weight of the ejaculate. It was then assumed 1 mL of semen weighed 1 gram. Immediately after semen volume calculation, the temperature of the sample (°C) was taken and recorded as the collection temperature. Concentration of sperm in the sample was measured using a SpermaCue® (Minitube of America, Verona, WI) and was recorded as the sperm concentration ($x10^6$ cells per mL).

Once the on-farm measurements above were recorded, the ejaculates were labelled with boar ID and date and tied with a rubber band. The samples were kept in a 37°C

incubator until they were taken to the campus laboratory for further analysis. After approximately 90 minutes of collection (typically 6-9 collections), samples were transported to North Carolina State University. The distance of transport was approximately 7 miles and took between 10 and 20 minutes based on traffic conditions. During transport, samples were kept in a closed, insulated bag to help maintain temperature. Once samples arrived at the laboratory, the temperature was measured and recorded as transport temperature, and each bag was placed in a 37°C dry air incubator (Ambi Hi/Lo; Lab Line Instruments, Melrose Park, IL). The last 2-5 collections were brought to campus in a similar manner and treated the same when they arrived on campus. The average time interval between the first and last collection was 110 minutes.

Semen Analyses

Motility

Shortly after the first samples arrived at the laboratory, semen was analyzed using a computer assisted sperm analysis (CASA) system. The average time between ejaculate collection at the farm and motility analysis was 141 minutes with a standard deviation of 32 minutes. Motility analysis was performed by taking 12.4 μL from the sample using a 200 μL Fisherbrand Redi-tip (Fisher Scientific, Atlanta, GA) and a 200 μL Eppendorf pipette and loading it into a Leja slide (Minitube of America, Verona, WI) pre-warmed to approximately 36°C. The loaded slide was placed on the temperature controlled stage of a phase contrast microscope (BMX-41, Olympus, Arlington, VA). The microscope was fitted with a digital video camera (Minitube of America, Verona, WI) connected to computer outfitted with the CASA software (SpermVision®; Minitube of America, Verona, WI). Five separate

microscopic fields per sample were used for analysis and were randomly chosen by the laboratory technician. The SpermVision® software analyzed the five fields and generated fourteen estimates of sperm motility for each field as well as the average of all estimates for the five fields together. The number of cells in each field ranged from 24 to 501 cells and the total number of cells for all five fields ranged from 219 to 2,142 cells. The following fourteen motility estimates were then recorded: proportion of sperm cells exhibiting motility (%); proportion of cells exhibiting progressive forward motility (%); curvilinear distance (μm); average path distance (μm); straight line distance (μm); curvilinear velocity (μm/s); average path velocity (μm/s); straight line velocity (μm/s); linearity (straight line velocity / curvilinear velocity); straightness (straight line velocity / average path velocity); wobble (average path velocity / curvilinear velocity); amplitude of lateral head displacement (μm); lateral beat frequency (Hz); and average change in orientation of head.

Over the course of the study, the protocol for diluting semen samples before motility analysis changed. At the beginning of the study, when boar sperm production was low, all samples were analyzed without dilution. As sperm production increased as the study progressed, it was found that the CASA software was unable to accurately analyze samples with high concentrations. From week 18 of collection to the end of the study, all semen collected with a concentration greater than 400×10^6 cells/mL was diluted at a ratio of 1:1 with BTS extender (Minitube of America, Verona, WI).

Morphology

To prepare samples for morphological analysis, a dilution of semen and phosphate buffered saline with 10% formalin was prepared in a 12 x 75 mm test tube (Port City Diagnostics, Wilmington, NC). The total volume of the sample was 1 mL, and the semen:buffered formalin ratio used was either 1:1 or 1:3 depending on the concentration of cells in the ejaculate measured by the SpermaCue®. Ejaculates with concentrations greater than 400 x 10^6 cells/mL were diluted 1:3 for analysis (250 µL semen, 750 µL saline) while those with lower concentrations were diluted 1:1 for analysis (500 µL semen, 500 µL saline). For analysis, approximately 50 µL was pulled from the diluted sample using a 200 µL tip and 200 µL pipette and placed on a glass microscope slide (Fisher Scientific, Atlanta, GA) and covered with a 18 mm x 18 mm glass coverslip (Fisher Scientific, Atlanta, GA). The slide was then placed onto the stage of a phase contrast microscope (Zeiss, Berlin, West Germany).

The morphological analysis was broken up into two categories; head/acrosome morphology and tail morphology. Tail morphological analysis was done first under the 40x objective by counting the number of cells with the following tail characteristics until 100 random cells had been counted; normal tail, abnormal tail (coiling, sharp bends, missing tail), proximal cytoplasmic droplet (droplet touching the sperm head), and distal cytoplasmic droplet (droplet not touching the sperm head). Head and acrosome morphology analysis was done next using a drop of low viscosity immersion oil and the 100x oil immersion objective. The head was classified as either normal or abnormal (based on size/shape) and the acrosome was classified as either normal (uniform color and smooth outline) or abnormal (acrosome

breaking down, grainy appearance, or missing acrosome). The head and acrosome were analyzed together on each cell, so counting continued until the laboratory counter reached 200 (100 cells counted and classified for both head and acrosome quality).

Analysis of Collection Technician Effect

The socialization of the boars by the two socializers provided an opportunity to test the effect of the familiarity of the collection technician on collection performance and semen quality. To test this effect, some of the boars were collected by one of the two socializers over a two week period during the normal collection period. In addition, two novel collection technicians were used to see if the boars could discriminate between the collection technician that trained them, collection technicians that socialized them at a younger age, and collection technicians that were completely unfamiliar to them but were skilled in collection of semen from boars. This was done twice during the study, 6/13 - 6/22/16 when the boars were approximately 320 days of age and 8/30 – 9/1/16, when the boars were approximately 398 days of age. Other than the collection technician, collection was performed exactly the same as described previously. During these particular collections, if the novel or socializer technician was not able to collect a boar after a reasonable amount of time, the normal collection technician (the trainer) would assist in completing the collection process.

Semen Analyses of Extended, Stored Insemination Doses

Because A.I. boars are commonly housed in boar studs that service many sow farms, semen from boars is rarely used fresh and is typically extended to maintain sperm quality for a longer duration. To analyze whether the socialization treatments had any effect on sperm quality during storage, extended doses were prepared from the weekly ejaculate collections at

the end of the study (8/23/16 and 8/25/16) when the boars were approximately 393 days of age. The doses were extended to industry standards, 3 billion (3×10^9) total sperm cells in a 60 mL semen dose. A total of 15 mL was prepared for each boar, therefore 750 million (750×10^6) total sperm cells were included in each extended sample. The extender used was the same BTS extender (Minitube of America, Verona, WI) used in dilution of motility samples. The extended samples were analyzed using the CASA system described previously on the same day they were prepared (Day 0) and again on days 2, 4, and 6 after collection. The extended samples were stored in a semen storage unit (Reproquest, Fitchburg, WI) at 17°C.

Body Measurements

When the boars were approximately 390 days of age, body and testicle measurements were taken on each boar. Measurements were taken during collection after each boar had successfully mounted and began ejaculating. Body length, flank girth, and hip height were measured using a typical hog measuring tape. Body length was taken along the backbone from the front shoulder blades to the attachment of the tail. Flank girth was measured around the boar just in front of the rear legs and behind the sheath. Hip height was taken in a straight line from the point of hip to the tip of the toes. Testicle width and length were measured using a flexible measuring tape. Testicle width was measured at approximately the midpoint of the length of the testicles and included both testicles.

Statistical Analyses

All dependent variables of interest were analyzed with analysis of variance procedures for repeated measures, where appropriate, using the general linear models procedure (PROC GLM) of SAS® (SAS Inc., Cary, NC). Appropriate two-way interactions

were tested, and when significant ($p \leq 0.05$), the data were analyzed using a one-way analysis of variance testing the effect of one socialization treatment for each level of the other socialization treatment (Snedecor and Cochran, 1989). The Hemsworth test data were summarized from each boar over both tests and analyzed using a statistical model which included nursery treatment, finishing treatment, and their interaction. Body dimensions were analyzed similarly with three model terms.

The effect of collection technician was analyzed using a statistical model which included both socialization treatments, their interaction, and the specific code indicating the type of collection technician performing the collection (novel, socializer, or trainer).

Ejaculate collection data (semen volume and concentration) were used to generate a total cells variable which was the product of semen volume and sperm concentration. These data, along with the morphology and motility (CASA) data were analyzed in the same manner; using a statistical model that included both treatments as main effects, their interaction, as well as week of collection. For analyses on semen volume, sperm concentration, and total cells, additional effects of reaction time, mounting time, and collection time were added. Individual boar within each treatment combination was included as a random effect. When this independent variable was significant ($p \leq 0.05$), it was then used as the error term to test the main effects of socialization treatments and their interaction. When it was not significant, it was assumed all data behaved as independent variables although they were collected from the same boars over time, and the overall experimental error was used to test all main effects and interactions.

The training success data were analyzed with analyses of variance procedures for categorical data (PROC GLMIX) in SAS® (SAS Inc., Cary, NC). The model included nursery socialization treatment, finishing socialization treatment, boar age (23-41 weeks), and appropriate interactions. Interactions and separation of significant main effects were handled as described previously.

The extended and stored semen motility data were analyzed by a statistical model which included both socialization treatments, analysis technician, day of analysis (0,2,4,6), and appropriate interactions. Again, individual boar within each treatment combination was treated as a random effect and was handled as described previously.

Although only 20 boars remained on the study for its entirety, partial data from the remainder of the boars was kept for analysis. Socialization score means were based on 34 boars, Hemsworth test data were based on 32 boars, training success data were based on 27 boars, and all collection data as well as body dimensions were based on 20 boars.

RESULTS

Hemsworth Test

The effect of socialization treatment on the Hemsworth test results are presented in Tables 2 through 7. There were no significant ($p > 0.05$) effects for nursery treatment for any of the Hemsworth results. However, significant ($p \leq 0.05$) effects of finishing treatment were present in several variables. Boars socialized in finishing entered the test area more times than those that were not socialized in finishing (9.2 versus 6.5, respectively, Table 2). Boars socialized in finishing spent more time in the test area than those that were not socialized in finishing (153.6 seconds versus 86.5 seconds, respectively, Table 3). Similarly, boars socialized in finishing made human contact more times than those not socialized in finishing (5.2 versus 3.7 respectively, Table 5). Boars socialized in finishing tended ($p \leq 0.10$) to have an increased maximum time spent in the test area compared with those that were not socialized in finishing (87.8 seconds versus 54.6 seconds, respectively, Table 4). Lastly, boars socialized in finishing tended ($p \leq 0.10$) to maintain more human contact than those that were not socialized in the nursery (82.9 seconds versus 46.8 seconds, respectively, Table 6).

Collection Training

Figure 2 and Table 8 presents the effect of socialization treatment on training success. Seven boars (n = 28 total) from each treatment combination began training, and a total of 20 were successfully trained. One boar from the nursery socialized, finishing unsocialized treatment died for reasons unrelated to the study before he could be trained. The double socialized treatment group had the lowest training success with three of seven boars

successfully training. This was followed by the double unsocialized treatment group with five of seven boars successfully training. The single socialization in finishing treatment had six of seven boars train, and all six of the single socialization in nursery boars successfully trained.

Collection Behavior

The effect of socialization on reaction time, mounting time, and collection time is presented in Tables 9 through 11. No significant ($p > 0.05$) socialization effects were observed. However, the week of collection was significant ($p \leq 0.05$) for collection time. The effect of collection week on collection time over the entire study is presented in Figure 8. Overall, collection time increased with week of collection.

Semen Volume, Concentration, and Total Cells

The effect of socialization treatment on semen volume, concentration, and total cells is presented in Tables 12 through 14. There was no effect ($p > 0.05$) of socialization treatment on any of these variables. Other independent variables not shown in the table had significant effects in the statistical model for these three dependent responses. The week of collection had a significant ($p < 0.0001$) effect on both semen volume and total cells per ejaculate. The effect of week of collection on semen volume is presented in Figure 3 while the effect of collection week on total cells per ejaculate is presented in Figure 5. Both semen volume and total cells per ejaculate increased with collection week. Reaction time was not significant, however, mounting time was significant ($p \leq 0.05$) for all three responses. Collection time was significant ($p < 0.0001$) for both semen volume and concentration, but

not for total cells. The individual within treatment combination was significant ($p < 0.0001$) for semen volume and sperm concentration, but not total cells per ejaculate.

Fresh Semen Sperm Motility

The effect of socialization treatment on sperm motility measures is presented in Tables 15 through 28. No significant main effects were found for any motility variables measured ($p > 0.05$). Additional independent variables not shown in the table had significant effects on the motility variables. The week of collection was significant ($p \leq 0.01$) for all motility measures. The effect of collection week on the motility measures is presented in Figures 9 through 22. Individual within treatment combination was significant ($p \leq 0.05$) for all motility variables except for progressive motility.

Morphology

Socialization treatment effects on the recorded morphology variables are presented in Tables 29 through 32. No significant treatment effects were found for any of the morphology measures ($p > 0.05$). The percentage of normal heads tended ($p \leq 0.10$) to be higher in boars un-socialized in finishing compared to those socialized in finishing (97.4% versus 97.1%, respectively, Table 29). The percentage of normal acrosomes tended ($p \leq 0.10$) to be higher in boars socialized in the nursery versus those that were not socialized in the nursery (97.6% versus 97.2%, respectively, Table 30). Other independent variables not shown in the table had significant effects on the morphology responses. The week of collection was significant ($p \leq 0.001$) for all of the morphology variables. The effect of collection week on the morphology measures is presented in Figures 23 through 26. The percentage of normal heads appeared to increase with week of collection (Figure 23), while the percentage of normal

acrosomes quickly improved at the beginning of the study, and slowly degraded towards the end of the study (Figure 24). The percentage of normal tails appeared to decrease with week of collection (Figure 25) while cytoplasmic droplets increased with week of collection (Figure 26). Individual within treatment combination was significant ($p \leq 0.05$) for cytoplasmic droplets and normal tails, but not for the other morphology variables.

Collection Technician

Tables 33 through 38 present the effect of collection technician on six semen variables. To understand the effects of different technicians, the data from the three weeks prior to each round of technician testing were used to generate a basis for comparison. Only data from the boars successfully collected by the novel and socializer technicians were included in the comparison. There was a tendency ($p \leq 0.10$) that a difference between collection technicians (trainer, socializer, and novel) exists for semen volume with the socializer collecting the highest volume, novel the second highest, followed by the trainer (245.7 mL versus 209.7 mL versus 192.3 mL, respectively, Table 36). There was a significant ($p \leq 0.05$) difference in semen volume between the socializer collection technician and the baseline results with the socializer collecting a greater volume than the previous collections on the same boars (245.7 mL versus 212.8 mL, respectively, Table 36). No other effects were seen for semen volume. No effects ($p > 0.05$) were found for collection technician or between collection technician and baseline results for sperm concentration (Table 37) or cells per ejaculate (Table 38). Boars tended ($p \leq 0.10$) to react slower to the dummy sow with the novel technician compared to the previous collections from the same boars (15.3 seconds versus 5.6 seconds, respectively, Table 33). No significant ($p > 0.05$)

effects were found for boar mounting time (Table 34). Collection time was significantly ($p \leq 0.05$) lower during the technician testing period compared to the previous collections from the same boars (314.8 seconds versus 347.8 seconds, respectively, Table 35).

Body Dimensions

The effect of socialization treatments on measured body dimensions are presented in Tables 39 through 42. Testes length and paired width were measured, and then multiplied to generate the testicular area measure (cm^2) which can be seen in Table 39. There were no significant ($p > 0.05$) main effects of nursery or finishing treatment for testicular area, however a significant interaction ($p \leq 0.05$) existed between the two treatments. Boars that were socialized only in the finishing phase had smaller testicles (1003.8 ± 63.0 cm^2) than the other three treatment combinations (1463.8 ± 147.3 cm^2, 1145.5 ± 97.2 cm^2, 1543.7 ± 267.2 cm^2). For body length (Table 40), flank girth (Table 41), and hip height (Table 42), no significant effects or interactions existed for either treatment.

Extended, Stored Insemination Doses

Table 43 presents the effect of days in storage on sperm motility and progressive motility. Motility was significantly ($p \leq 0.05$) lower at day 6 when compared to day 0 (75.47% versus 89.03%, respectively). Progressive motility was also significantly ($p \leq 0.05$) lower at day 6 when compared to day 0 (57.66% versus 76.44%, respectively).

DISCUSSION

The most important observation from this study is the differences in trainability of boars due to socialization treatment. Based on the results of the present study (Figure 2 and Table 8), it is possible that boars can become too accustomed to human interaction. The double socialized group had the lowest percentage of successfully trained boars at the end of the training period. One possible explanation is a reduction in sexual behavior. Sexual behavior is a hormonally controlled phenomenon; however, it is unlikely that the boars on the study were hormonally deficient enough to significantly reduce libido, as major decreases in testosterone production are required (Hemsworth and Tilbrook, 2007). It can be reasoned that a decrease in libido is not the most probable cause of the failed collection training because boars which did successfully train did not show significant differences in libido (Tables 9 through 11) between socialization treatments. One possible explanation is that the boars which were double socialized had become so accustomed to humans that they struggled to focus in the collection pen, and instead preferred playing with the human in the pen. The other three treatments achieved similar training success rates by the end of the training period, suggesting a single socialization treatment either in the nursery or finishing does not negatively impact the trainability of boars. Studying the cumulative training success (Figure 2) based on treatment reveals another layer of depth to the analysis. Boars socialized only in the nursery trained the quickest, and had significantly more boars trained compared to the double-socialized group through a majority of the training period. This suggests that boars did in fact remember the treatment they received in the nursery or that the nursery socialization had a lasting effect on their behavior. Interestingly, the other single socialization

treatment performed the second best, having significantly more boars trained in the last third of the training period compared to the double-socialized group, again indicating the socialization treatment had a lasting effect on their behavior. It appears that socializing boars strictly in the nursery or finishing phase alone has a positive effect on trainability. The double-unsocialized group and the double-socialized group performed similarly, with the double-socialized group performing the worst. Again, this indicates that the boars can be over socialized to the point they train worse than boars not socialized at all.

Another important observation of this study is the difference in total sperm per ejaculate due to the socialization treatment. Calculated from semen volume and sperm concentration, these measures are typically used to determine whether a boar can successfully breed sows (Wodsedalek, 1913) and are economically important to the boar stud. Total cells per ejaculate generates a more complete estimate of the reproductive performance of a boar, and is the most important measure of a boar's potential profitability. While no significant ($p > 0.05$) differences were observed between socialization treatments for total cells per ejaculate, a developing trend ($p=0.15$) for an interaction between socialization treatments is worth discussing (Table 14). Total cells per ejaculate was lowest in the double un-socialized group (66.7 billion cells/ejaculate). There appears to be an increase in sperm production when moving to the single-socialized treatment combinations (un-socialized in nursery, socialized in finishing and vice versa) with the two groups yielding 75.8 and 78.0 billion cells per ejaculate. Finally, the double-socialized treatment combination yielded the highest average total cells per ejaculate (81.8 billion cells per ejaculate), indicating that the socialization treatments may be partially additive in their effect on sperm

production. These results suggest that socialization in the nursery or the finishing phase is sufficient to boost the sperm production of low birth weight boars compared to boars that receive no socialization at all. The observation that socialization in finishing produced an equivalent response in total cells as socialization in nursery is a novel observation. Dysart (2015) observed a similar increase in total cells per ejaculate (~ 10 billion cells) when boars were socialized in the nursery. It was postulated that this increase in total sperm due to socialization was a result of reduced stress in the nursery and subsequent increased Sertoli cell mitosis. In this way, human socialization affects the developmental production of sperm. However, as discussed previously, Sertoli cell mitosis does not occur after 60 days of age, and so the increase in sperm production due to socialization in finishing is not likely due to an increase in Sertoli cell numbers. Some other mechanism is responsible for the increase. It is possible that a chronic reduction in stress over the entire developmental period of boars had a stimulatory effect on the testes in some way. Another possible explanation is that the positive human experiences before puberty invoked a lower level of stress during the mature life of the boar during collection. The biological reason is unknown, and further research, possibly with the addition of hormone profiling, may shed more light on this mechanism. It is possible that boars under the lower chronic stress level due to socialization harvested a greater percentage of available sperm reserves in the epididymis than boars not socialized at all. The difference of 9-11 billion cells between socialized groups and the un-socialized group can be economically significant. At the industry standard of 3 billion cells per semen dose, these socialized boars can produce three to four more doses per collection, while the

double-socialized group could generate five more semen doses per collection over the completely un-socialized boars.

Semen volume did exhibit an increase in the socialized treatments over the double un-socialized treatment (193.0 mL, 197.0 mL, and 181.2 mL versus 154.5 mL, Table 12). Sperm concentrations were similar between treatment combinations. While socialization treatment was not significant for semen volume, sperm concentration, or sperm cells per ejaculate, some other model terms were significant which are worth discussing. As expected, the week of collection certainly impacts these measures, as this variable includes the effect of the boar's age as well as the environmental effects such as temperature and humidity. A portion of the collection period in this study overlapped the hottest months of the year in North Carolina. Despite the measures taken to keep the boars cool, it is likely that the boars experienced some degree of chronic heat stress. This stress can impact sperm quantity and quality for several weeks (Flowers, 2015). Interestingly, the variation present between weeks for a single boar in semen volume and concentration balanced out to reduce the variation in total cells in a single boar to make it no longer a significant model term. This indicates the boars are more consistent in the number of cells they produce per ejaculate than they are in the volume of ejaculate they produce or the concentration of sperm in their ejaculate. This observation can be reasoned with the fact that semen volume and sperm concentration have an inverse relationship and are affected by the volume of seminal fluids the boar produces during an ejaculation. Total sperm per ejaculate is not affected by the secretions of the accessory sex glands, and therefore is not subject to the same variation inherent in semen volume and sperm concentration.

An interesting observation is the effect of collection technician on the characteristics of the ejaculate. The reason this was included in the present study was to investigate whether boars would respond any differently when the person who socialized them also collected them despite being trained and collected by a different person previously. This was essentially a test of the boars' memories. To further determine whether a boar could distinguish between people, a person unfamiliar to the boars was introduced as a collection technician. In general, the collection technician did not have an effect on the ejaculate characteristics, including semen volume, sperm concentration, total cells per ejaculate, as well as behavioral responses of reaction, mounting, and collection times. This is opposed to the results of Dysart (2015) where significant differences were found in ejaculate volume, with the trainer and novel technicians producing the highest volumes. Tanida et al. (1995) also found evidence suggesting pigs could discriminate between different humans in terms of handling responses. However, Hemsworth et al. (1994) concluded pigs could not discriminate between humans even if they treated pigs differently. In the present study, a tendency to higher ejaculate volumes was observed for the socializer collector. The increase in ejaculate volume due to the socializer collection technician compared to the typical trainer collector is difficult to reason. It is possible that the socializer collectors terminated collection differently than the trainer. The lack of collection experience of the socializers may have certainly played a role in the inability to determine when the boar has finished collecting. This led to a higher collection time average for the socializer technicians, greater ejaculate volumes produced, and higher total cells per ejaculate. However, these differences were not statistically significant, only numerically different. In terms of behavioral responses, the

boars reacted similarly for all three types of technicians. However, a tendency for a difference was observed in reaction time when the collector was unfamiliar to the boar, which caused to the boars to take longer to react to the dummy. It is important to note however, that similar to the results of Dysart (2015), the socializer and novel collection technicians were not able to collect all of the boars attempted. The socializer technicians were able to successfully collect 13 of the 18 boars attempted (72%) and the novel technicians were able to successfully collect 11 of the 17 boars attempted (65%). Even though the boars did not show any significant changes in ejaculate characteristics in response to different collection technicians, the collection technician can influence whether a boar collects or not.

While there is some evidence that socialization may increase sperm production, it is important to ensure this increase is not accompanied by a decreased in sperm quality. For this reason, motility of all collections was evaluated using a computer-assisted semen analysis system as well as morphology using traditional microscope methods. There were no significant treatment effects in any of the morphology variables. Significant individual variation existed for cytoplasmic droplets and normal tails, revealing that some of the boars varied considerably from week to week in these two measures. The effects of socialization treatment on motility variables are recorded Tables 15-28. Once again, no significant socialization effects were found in any of the motility variables. The significance of the collection week in all motility and morphology variables reflects the fact that the sperm quality of boars changes over time, especially when the boars are young, and is subject to environmental effects such as temperature and humidity.

The Hemsworth tests presented interesting results. Because of the tendencies and significant effects of the finishing treatment on several of the Hemsworth results, evidence suggests that socializing the boars in finishing tended to make them more social with humans. The positive experiences with humans during finishing reduced the fear they had for humans. However, there was no effect of the nursery treatment. Numerically, the nursery socialization actually decreased Hemsworth test performance in five out of the six Hemsworth measures. Why this occurred is not known. It can be speculated that the boars did not receive enough positive interaction in the nursery or they simply did not have capacity to remember these past experiences when the Hemsworth tests were conducted. The first explanation appears unlikely since the boars were socialized for five weeks, and as little as three weeks of socialization is enough to differentiate responses in handling (Tanida et al., 1995). The latter explanation is more likely, as evidence from Hemsworth et al. (1981; 1992) shows that pigs under behavior testing many weeks after socialization has ended perform similarly to pigs never socialized, indicating that pigs may not remember past experiences or more recent handling overrides past experience. This is in contrast to the training success results, which suggested boars remembered the nursery socialization treatment. It is possible that boars did remember nursery socialization, but it only affected their willingness to work with humans (collection training) and not their willingness to interact with humans (Hemsworth test).

Similar to other responses, the body dimensions of the boars were not affected by socialization treatment. No significant effects were found between socialization and any of the measured dimensions (Tables 39-42). This suggests the socialization treatments do not

alter the growth pattern of growing boars, at least after reaching one year of age. The interaction between socialization treatments observed in testicular area is likely from chance, and does not suggest any cause and effect.

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TABLES

Table 1: Mean (mean ± s.e.) Socialization Scores for Nursery and Finishing treatments. Un-socialized treatments received a score of "0". Numbers in parentheses indicate the number of scores used to generate the mean.

	Socialization Score Means	
Treatment combination	Nursery	Finishing
Nursery- Un-socialized Finishing- Un-socialized	0	0
Nursery- Socialized Finishing- Un-socialized	1.91 ± 0.15 (136)	0
Nursery- Un-socialized Finishing- Socialized	0	3.51 ± 0.08 (213)
Nursery- Socialized Finishing- Socialized	1.68 ± 0.15 (135)	3.42 ± 0.09 (216)

Table 2: Effect of Socialization Treatment on the Number of times a boar entered the test area during the 10-minute Hemsworth test (mean ± s.e.). Numbers in parentheses indicate number of observations used to calculate mean.

Nursery Treatments	Finishing Treatments		Nursery Means
	Un-socialized	Socialized	
Un-socialized	5.8±0.7 (8)	9.2 ± 1.2 (8)	7.5 ± 0.8 (16)
Socialized	7.1 ± 0.7 (8)	9.1 ± 0.6 (8)	8.1 ± 0.5 (16)
Finishing Means	6.5 ± 0.5[a] (16)	9.2 ± 0.6[b] (16)	

[a,b] means in the same row with different superscripts are different ($p \leq 0.01$)

Table 3: Effect of Socialization Treatment on the total time a boar spent in the test area during the 10-minute Hemsworth test (sec ± s.e.). Numbers in parentheses indicate number of observations used to calculate mean.

Nursery Treatments	Finishing Treatments		Nursery Means
	Un-socialized	Socialized	
Un-socialized	95.8 ± 15.8 (8)	156.6 ± 36.5 (8)	126.2 ± 20.7 (16)
Socialized	77.3 ± 8.2 (8)	150.6 ± 23.7 (8)	114.0 ± 15.4 (16)
Finishing Means	86.5 ± 8.9[a] (16)	153.6 ± 21.0[b] (16)	

[a,b] means in the same row with different superscripts are different ($p \leq 0.01$)

Table 4: Effect of Socialization Treatment on the longest single time a boar spent in the test area during the 10-minute Hemsworth test (sec ± s.e.). Numbers in parentheses indicate number of observations used to calculate mean.

Nursery Treatments	Finishing Treatments		Nursery Means
	Un-socialized	Socialized	
Un-socialized	69.9 ± 17.3 (8)	77.9 ± 19.5 (8)	73.9 ± 12.6 (16)
Socialized	39.4 ± 5.6 (8)	97.8 ± 21.6 (8)	68.6 ± 13.2 (16)
Finishing Means	54.6 ± 9.6[a] (16)	87.8 ± 14.3[b] (16)	

[a,b] means in the same row with different superscripts tended to be different ($p \leq 0.10$)

Table 5: Effect of Socialization Treatment on the number of times a boar made human contact during the 10-minute Hemsworth test (mean ± s.e.). Numbers in parentheses indicate number of observations used to calculate mean.

Nursery Treatments	Finishing Treatments		Nursery Means
	Un-socialized	Socialized	
Un-socialized	3.8 ± 0.6 (8)	5.4 ± 1.0 (8)	4.6 ± 0.6 (16)
Socialized	3.6 ± 0.7 (8)	5.0 ± 0.6 (8)	4.3 ± 0.5 (16)
Finishing Means	3.7 ± 0.4[a] (16)	5.2 ± 0.5[b] (16)	

[a,b] means in the same row with different superscripts are different ($p \leq 0.05$)

Table 6: Effect of Socialization Treatment on the total time a boar maintained human contact during the 10-minute Hemsworth test (sec ± s.e.). Numbers in parentheses indicate number of observations used to calculate mean.

Nursery Treatments	Finishing Treatments		Nursery Means
	Un-socialized	Socialized	
Un-socialized	56.4 ± 11.2 (8)	89.4 ± 27.2 (8)	72.9 ± 14.8 (16)
Socialized	37.1 ± 6.7 (8)	76.4 ± 18.9 (8)	56.7 ± 10.9 (16)
Finishing Means	46.8 ± 6.8[a] (16)	82.9 ± 16.1[b] (16)	

[a,b] means in the same row with different superscripts tended to be different ($p \leq 0.10$)

Table 7: Effect of Socialization Treatment on the longest single time a boar maintained human contact during the 10-minute Hemsworth test (sec ± s.e.). Numbers in parentheses indicate number of observations used to calculate mean.

Nursery Treatments	Finishing Treatments		Nursery Means
	Un-socialized	Socialized	
Un-socialized	43.0 ± 9.0 (8)	53.6 ± 16.6 (8)	48.3 ± 9.2 (16)
Socialized	22.6 ± 2.8 (8)	52.8 ± 14.9 (8)	37.7 ± 8.3 (16)
Finishing Means	32.8 ± 5.2 (16)	53.2 ± 10.8 (16)	

Table 8: Effect of Socialization Treatment on training success (%). Numbers in parentheses indicate the fraction of boars which successfully trained to collect.

Nursery Treatments	Finishing Treatments		Nursery Means
	Un-socialized	Socialized	
Un-socialized	71.4 (5/7)	85.7 (6/7)	78.6 (11/14)
Socialized	100.0 (6/6)	42.9 (3/7)	69.2 (9/13)
Finishing Means	78.6 (11/14)	69.2 (9/13)	

Table 9: Effect of Socialization Treatment on reaction time to the dummy over the entire study with the trainer as the collection technician (sec ± s.e.). Numbers in parentheses indicate the number of observations used to calculate the mean.

Nursery Treatments	Finishing Treatments		Nursery Means
	Un-socialized	Socialized	
Un-socialized	7.2±1.4 (107)	3.1 ± 0.6 (153)	4.8 ± 0.7 (260)
Socialized	3.4 ± 0.4 (159)	8.1 ± 1.8 (75)	4.9 ± 0.7 (234)
Finishing Means	4.9 ± 0.6 (266)	4.8 ± 0.7 (228)	

Table 10: Effect of Socialization Treatment on mounting time over the entire study with the trainer as the collection technician (sec ± s.e.). Numbers in parentheses indicate the number of observations used to calculate the mean.

Nursery Treatments	Finishing Treatments		Nursery Means
	Un-socialized	Socialized	
Un-socialized	38.5 ± 6.6 (107)	30.4 ± 2.5 (153)	33.7 ± 3.1 (260)
Socialized	28.2 ± 3.6 (159)	69.2 ± 9.1 (75)	41.3 ± 4.0 (234)
Finishing Means	32.3 ± 3.4 (266)	43.2 ± 3.6 (228)	

Table 11: Effect of Socialization Treatment on collection time over the entire study with the trainer as the collection technician (sec ± s.e.). Numbers in parentheses indicate the number of observations used to calculate the mean.

Nursery Treatments	Finishing Treatments		Nursery Means
	Un-socialized	Socialized	
Un-socialized	280.7 ± 7.2 (107)	344.5 ± 7.8 (153)	318.2 ± 5.8 (260)
Socialized	348.2 ± 7.4 (159)	278.6 ± 9.0 (75)	325.9 ± 6.2 (234)
Finishing Means	321.0 ± 5.7 (266)	322.8 ± 6.3 (228)	

Table 12: Effect of Socialization Treatment on semen volume over the entire study with the trainer as the collection technician (mL ± s.e.). Numbers in parentheses indicate the number of observations used to calculate the mean.

Nursery Treatments	Finishing Treatments		Nursery Means
	Un-socialized	Socialized	
Un-socialized	154.5 ± 4.6 (107)	197.0 ± 5.2 (153)	179.5 ± 3.8 (260)
Socialized	193.0 ± 5.2 (160)	181.2 ± 4.7 (75)	189.2 ± 3.9 (235)
Finishing Means	177.6 ± 3.8 (267)	191.8 ± 3.8 (228)	

Table 13: Effect of Socialization Treatment on sperm concentration over the entire study with the trainer as the collection technician ($x10^6$ cells/mL ± s.e.). Numbers in parentheses indicate the number of observations used to calculate the mean.

Nursery Treatments	Finishing Treatments: Un-socialized	Finishing Treatments: Socialized	Nursery Means
Un-socialized	434.6 ± 13.7 (107)	430.8 ± 13.3 (153)	432.4 ± 9.6 (260)
Socialized	432.6 ± 13.9 (160)	465.9 ± 12.4 (75)	443.2 ± 10.3 (235)
Finishing Means	433.4 ± 10.0 (267)	442.3 ± 9.8 (228)	

Table 14: Effect of Socialization Treatment on sperm cells per collection over the entire study with the trainer as the collection technician ($x10^9$ cells ± s.e.). Numbers in parentheses indicate the number of observations used to calculate the mean.

Nursery Treatments	Finishing Treatments		Nursery Means
	Un-socialized	Socialized	
Un-socialized	66.7 ± 2.5 (107)	78.0 ± 1.7 (153)	73.3 ± 1.5 (260)
Socialized	75.8 ± 1.7 (160)	81.8 ± 1.9 (75)	77.7 ± 1.3 (235)
Finishing Means	72.1 ± 1.5 (267)	79.2 ± 1.3 (228)	

Table 15: Effect of Socialization Treatment on sperm motility of fresh ejaculates collected by the boar trainer (% ± s.e.). Numbers in parentheses indicate the number of observations used to calculate the mean.

Nursery Treatments	Finishing Treatments		Nursery Means
	Un-socialized	Socialized	
Un-socialized	89.53 ± 1.15 (107)	87.82 ± 1.09 (153)	88.52 ± 0.80 (260)
Socialized	89.45 ± 0.82 (159)	85.86 ± 1.71 (75)	88.29 ± 0.79 (234)
Finishing Means	89.48 ± 0.67 (266)	87.17 ± 0.93 (228)	

Table 16: Effect of Socialization Treatment on sperm progressive motility of fresh ejaculates collected by the boar trainer (% ± s.e.). Numbers in parentheses indicate the number of observations used to calculate the mean.

Nursery Treatments	Finishing Treatments		Nursery Means
	Un-socialized	Socialized	
Un-socialized	72.74 ± 1.85 (107)	71.09 ± 1.60 (153)	71.77 ± 1.21[1] (260)
Socialized	73.07 ± 1.24 (159)	68.64 ± 2.22 (75)	71.65 ± 1.11[2] (234)
Finishing Means	72.93 ± 1.05 (266)	70.29 ± 1.30 (228)	

[1,2] means in the same column with different superscripts tended to be different ($p \leq 0.10$)

Table 17: Effect of Socialization Treatment on sperm curvilinear distance (DCL) of fresh ejaculates collected by the boar trainer (μm ± s.e.). Numbers in parentheses indicate the number of observations used to calculate the mean.

Nursery Treatments	Finishing Treatments		Nursery Means
	Un-socialized	Socialized	
Un-socialized	56.91 ± 1.44 (107)	56.73 ± 1.35 (153)	56.80 ± 0.99 (260)
Socialized	55.99 ± 0.90 (159)	54.92 ± 1.70 (75)	55.65 ± 0.82 (234)
Finishing Means	56.36 ± 0.79 (266)	56.13 ± 1.07 (228)	

Table 18: Effect of Socialization Treatment on sperm average path distance (DAP) of fresh ejaculates collected by the boar trainer (μm ± s.e.). Numbers in parentheses indicate the number of observations used to calculate the mean.

Nursery Treatments	Finishing Treatments		Nursery Means
	Un-socialized	Socialized	
Un-socialized	33.47 ± 0.75 (107)	33.33 ± 0.68 (153)	33.39 ± 0.51 (260)
Socialized	32.82 ± 0.47 (159)	32.70 ± 0.87 (75)	32.78 ± 0.42 (234)
Finishing Means	33.08 ± 0.41 (266)	33.12 ± 0.54 (228)	

Table 19: Effect of Socialization Treatment on sperm straight line distance (DSL) of fresh ejaculates collected by the boar trainer (μm ± s.e.). Numbers in parentheses indicate the number of observations used to calculate the mean.

Nursery Treatments	Finishing Treatments		Nursery Means
	Un-socialized	Socialized	
Un-socialized	20.47 ± 0.39 (107)	20.33 ± 0.34 (153)	20.38 ± 0.26 (260)
Socialized	20.31 ± 0.24 (159)	19.75 ± 0.42 (75)	20.13 ± 0.21 (234)
Finishing Means	20.38 ± 0.21 (266)	20.14 ± 0.27 (228)	

Table 20: Effect of Socialization Treatment on sperm curvilinear velocity (VCL) of fresh ejaculates collected by the boar trainer (μm/sec ± s.e.). Numbers in parentheses indicate the number of observations used to calculate the mean.

Nursery Treatments	Finishing Treatments		Nursery Means
	Un-socialized	Socialized	
Un-socialized	134.32 ± 3.30 (107)	133.65 ± 3.09 (153)	133.93 ± 2.27 (260)
Socialized	131.75 ± 2.09 (159)	130.06 ± 3.93 (75)	131.21 ± 1.90 (234)
Finishing Means	132.78 ± 1.82 (266)	132.47 ± 2.44 (228)	

Table 21: Effect of Socialization Treatment on sperm average path velocity (VAP) of fresh ejaculates collected by the boar trainer (μm/sec ± s.e.). Numbers in parentheses indicate the number of observations used to calculate the mean.

Nursery Treatments	Finishing Treatments		Nursery Means
	Un-socialized	Socialized	
Un-socialized	79.41 ± 1.74 (107)	79.22 ± 1.56 (153)	79.30 ± 1.16 (260)
Socialized	77.56 ± 1.10 (159)	77.79 ± 1.99 (75)	77.63 ± 0.98 (234)
Finishing Means	78.31 ± 0.96 (266)	78.75 ± 1.23 (228)	

Table 22: Effect of Socialization Treatment on sperm straight line velocity (VSL) of fresh ejaculates collected by the boar trainer (μm/sec ± s.e.). Numbers in parentheses indicate the number of observations used to calculate the mean.

Nursery Treatments	Finishing Treatments		Nursery Means
	Un-socialized	Socialized	
Un-socialized	49.07 ± 0.88 (107)	48.83 ± 0.78 (153)	48.93 ± 0.59 (260)
Socialized	48.42 ± 0.54 (159)	47.49 ± 0.97 (75)	48.12 ± 0.48 (234)
Finishing Means	48.68 ± 0.48 (266)	48.39 ± 0.62 (228)	

Table 23: Effect of Socialization Treatment on sperm linearity (LIN) of fresh ejaculates collected by the boar trainer (mean ± s.e.). Numbers in parentheses indicate the number of observations used to calculate the mean.

Nursery Treatments	Finishing Treatments		Nursery Means
	Un-socialized	Socialized	
Un-socialized	0.376 ± 0.004 (107)	0.378 ± 0.005 (153)	0.377 ± 0.003 (260)
Socialized	0.373 ± 0.003 (159)	0.375 ± 0.005 (75)	0.374 ± 0.003 (234)
Finishing Means	0.374 ± 0.002 (266)	0.377 ± 0.004 (228)	

Table 24: Effect of Socialization Treatment on sperm straightness (STR) of fresh ejaculates collected by the boar trainer (mean ± s.e.). Numbers in parentheses indicate the number of observations used to calculate the mean.

Nursery Treatments	Finishing Treatments		Nursery Means
	Un-socialized	Socialized	
Un-socialized	0.626 ± 0.005 (107)	0.624 ± 0.004 (153)	0.625 ± 0.003 (260)
Socialized	0.629 ± 0.003 (159)	0.617 ± 0.005 (75)	0.626 ± 0.003 (234)
Finishing Means	0.628 ± 0.003 (266)	0.622 ± 0.003 (228)	

Table 25: Effect of Socialization Treatment on sperm wobble (WOB) of fresh ejaculates collected by the boar trainer (mean ± s.e.). Numbers in parentheses indicate the number of observations used to calculate the mean.

Nursery Treatments	Finishing Treatments		Nursery Means
	Un-socialized	Socialized	
Un-socialized	0.597 ± 0.003 (107)	0.603 ± 0.004 (153)	0.600 ± 0.003 (260)
Socialized	0.592 ± 0.002 (159)	0.606 ± 0.005 (75)	0.596 ± 0.002 (234)
Finishing Means	0.594 ± 0.002 (266)	0.604 ± 0.003 (228)	

Table 26: Effect of Socialization Treatment on sperm beat lateral frequency (BCF) of fresh ejaculates collected by the boar trainer (Hz ± s.e.). Numbers in parentheses indicate the number of observations used to calculate the mean.

Nursery Treatments	Finishing Treatments		Nursery Means
	Un-socialized	Socialized	
Un-socialized	29.75 ± 0.35 (107)	29.25 ± 0.33 (153)	29.46 ± 0.24 (260)
Socialized	30.73 ± 0.23 (159)	28.84 ± 0.41 (75)	30.12 ± 0.21 (234)
Finishing Means	30.34 ± 0.20 (266)	29.18 ± 0.26 (228)	

Table 27: Effect of Socialization Treatment on sperm amplitude of head displacement (ALH) of fresh ejaculates collected by the boar trainer (μm ± s.e.). Numbers in parentheses indicate the number of observations used to calculate the mean.

Nursery Treatments	Finishing Treatments		Nursery Means
	Un-socialized	Socialized	
Un-socialized	5.71 ± 0.10 (107)	5.73 ± 0.09 (153)	5.73 ± 0.07 (260)
Socialized	5.52 ± 0.07 (159)	5.72 ± 0.11 (75)	5.59 ± 0.06 (234)
Finishing Means	5.60 ± 0.06 (266)	5.73 ± 0.07 (228)	

Table 28: Effect of Socialization Treatment on sperm average orientation change (AOC) of fresh ejaculates collected by the boar trainer (mean ± s.e.). Numbers in parentheses indicate the number of observations used to calculate the mean.

Nursery Treatments	Finishing Treatments		Nursery Means
	Un-socialized	Socialized	
Un-socialized	17.45 ± 0.30 (107)	17.21 ± 0.29 (153)	17.31 ± 0.21 (260)
Socialized	17.66 ± 0.22 (159)	17.12 ± 0.37 (75)	17.49 ± 0.19 (234)
Finishing Means	17.58 ± 0.18 (266)	17.18 ± 0.23 (228)	

Table 29: Effect of Socialization Treatment on normal heads (% ± s.e.). Numbers in parentheses indicate number of observations used to calculate mean.

Nursery Treatments	Finishing Treatments		Nursery Means
	Un-socialized	Socialized	
Un-socialized	97.4 ± 0.2 (116)	96.9 ± 0.2 (160)	97.2 ± 0.1 (276)
Socialized	97.3 ± 0.2 (166)	97.6 ± 0.3 (83)	97.4 ± 0.2 (249)
Finishing Means	97.4 ± 0.1[a] (282)	97.1 ± 0.2[b] (243)	

[a,b] means in the same row with different superscripts tended to be different ($p \leq 0.10$)

Table 30: Effect of Socialization Treatment on normal acrosomes (% ± s.e.). Numbers in parentheses indicate number of observations used to calculate mean.

Nursery Treatments	Finishing Treatments: Un-socialized	Finishing Treatments: Socialized	Nursery Means
Un-socialized	97.2 ± 0.3 (116)	97.3 ± 0.2 (160)	97.2 ± 0.2[1] (276)
Socialized	97.3 ± 0.2 (166)	98.2 ± 0.2 (83)	97.6 ± 0.1[2] (249)
Finishing Means	97.3 ± 0.2 (282)	97.6 ± 0.2 (243)	

[1,2] means in the same column with different superscripts tended to be different ($p \leq 0.10$)

Table 31: Effect of Socialization Treatment on normal tails (% ± s.e.). Numbers in parentheses indicate number of observations used to calculate mean.

Nursery Treatments	Finishing Treatments		Nursery Means
	Un-socialized	Socialized	
Un-socialized	89.1 ± 1.5 (116)	95.3 ± 0.3 (160)	92.7 ± 0.7 (276)
Socialized	94.9 ± 0.3 (166)	91.0 ± 1.3 (83)	93.6 ± 0.5 (249)
Finishing Means	92.5 ± 0.7 (282)	93.9 ± 0.5 (243)	

Table 32: Effect of Socialization Treatment on cytoplasmic droplets (% ± s.e.). Numbers in parentheses indicate number of observations used to calculate mean.

Nursery Treatments	Finishing Treatments		Nursery Means
	Un-socialized	Socialized	
Un-socialized	9.3 ± 1.4 (116)	3.1 ± 0.3 (160)	5.7 ± 0.6 (276)
Socialized	4.2 ± 0.3 (166)	6.9 ± 1.3 (83)	5.1 ± 0.5 (249)
Finishing Means	6.3 ± 0.6 (282)	4.4 ± 0.5 (243)	

Table 33: Effect of Collection Technician on reaction time to the dummy (sec ± s.e.). Weeks 16-18 and 27-29 of collection are represented as baseline performance for the specific boars collected by each technician. Numbers in parentheses indicate the number of observations used to calculate the mean.

Collection Technician	Technician Weeks 19, 20, 30	Trainer Technician Weeks 16-18, 27-29
Trainer	6.4 ± 1.7 (38)	7.4 ± 1.3 (119)
Socializer	6.3 ± 2.1 (13)	4.6 ± 1.5 (54)
Novel	15.3 ± 8.0[a] (11)	5.6 ± 1.7[b] (48)

[a,b] means in the same row with different superscripts tended to be different ($p \leq 0.10$)

Table 34:Effect of Collection Technician on time to mount the dummy sow (sec ± s.e.). Weeks 16-18 and 27-29 of collection are represented as baseline performance for the specific boars collected by each technician. Numbers in parentheses indicate the number of observations used to calculate the mean.

Collection Technician	Technician Weeks 19, 20, 30	Trainer Technician Weeks 16-18, 27-29
Trainer	42.5 ± 7.4 (38)	37.8 ± 4.1 (119)
Socializer	28.7 ± 8.7 (13)	26.1 ± 3.8 (54)
Novel	66.5 ± 23.0 (11)	39.8 ± 7.8 (48)

Table 35: Effect of Collection Technician on collection time (sec ± s.e.). Weeks 16-18 and 27-29 of collection are represented as baseline performance for the specific boars collected by each technician. Numbers in parentheses indicate the number of observations used to calculate the mean.

Collection Technician	Technician Weeks 19, 20, 30	Trainer Technician Weeks 16-18, 27-29
Trainer	314.8 ± 14.5[a] (38)	347.8 ± 8.8[b] (119)
Socializer	342.6 ± 34.7 (13)	352.5 ± 12.5 (54)
Novel	309.6 ± 19.8 (11)	349.8 ± 12.6 (48)

[a,b] means in the same row with different superscripts are different ($p \leq 0.05$)

Table 36: Effect of Collection Technician on semen volume (mL ± s.e.). Weeks 16-18 and 27-29 of collection are represented as baseline performance for the specific boars collected by each technician. Numbers in parentheses indicate the number of observations used to calculate the mean.

Collection Technician	Technician Weeks 19, 20, 30	Trainer Technician Weeks 16-18, 27-29
Trainer	192.3 ± 9.8[1] (38)	197.4 ± 5.6 (119)
Socializer	245.7 ± 16.6[2a] (13)	212.8 ± 7.2[b] (54)
Novel	209.7 ± 22.5 (11)	207.8 ± 8.1 (48)

[1,2] means in the same column with different superscripts tended to be different ($p \leq 0.10$)
[a,b] means in the same row with different superscripts are different ($p \leq 0.05$)

Table 37: Effect of Collection Technician on sperm concentration ($x10^6$ cells/mL ± s.e.). Weeks 16-18 and 27-29 of collection are represented as baseline performance for the specific boars collected by each technician. Numbers in parentheses indicate the number of observations used to calculate the mean.

Collection Technician	Technician Weeks 19, 20, 30	Trainer Technician Weeks 16-18, 27-29
Trainer	437.2 ± 22.2 (38)	436.3 ± 13.9[2] (119)
Socializer	378.3 ± 28.3 (13)	420.2 ± 17.5 (54)
Novel	398.0 ± 44.8 (11)	430.0 ± 19.2 (48)

Table 38: Effect of Collection Technician on cells per ejaculate ($x10^9$ cells ± s.e.). Weeks 16-18 and 27-29 of collection are represented as baseline performance for the specific boars collected by each technician. Numbers in parentheses indicate the number of observations used to calculate the mean.

Collection Technician	Technician Weeks 19, 20, 30	Trainer Technician Weeks 16-18, 27-29
Trainer	78.2 ± 2.6 (38)	81.2 ± 2.1 (119)
Socializer	90.8 ± 6.7 (13)	85.9 ± 3.2 (54)
Novel	80.9 ± 9.7 (11)	84.6 ± 3.1 (48)

Table 39: Effect of Socialization Treatment on Testicular Area at 391 days of age (cm^2 ± s.e.). Numbers in parentheses indicate number of observations used to calculate mean.

Nursery Treatments[1]	Finishing Treatments: Un-socialized	Finishing Treatments: Socialized	Nursery Means
Un-socialized	1463.8 ± 147.3[a] (5)	1003.8 ± 63.0[b] (6)	1212.9 ± 101.4 (11)
Socialized	1145.5 ± 97.2[a] (6)	1543.7 ± 267.2[a] (3)	1278.2 ± 119.5 (9)
Finishing Means	1290.2 ± 95.0 (11)	1183.8 ± 125.3 (9)	

[1] a significant ($p \leq 0.05$) interaction between nursery and finishing socialization treatments was present so the effect of socialization within phase of production was evaluated

[a,b] means with different superscripts are different ($p \leq 0.05$)

Table 40: Effect of Socialization Treatment on Body Length at 391 days of age (in ± s.e.). Numbers in parentheses indicate number of observations used to calculate mean.

Nursery Treatments	Finishing Treatments		Nursery Means
	Un-socialized	Socialized	
Un-socialized	48.4 ± 0.8 (5)	46.8 ± 0.9 (6)	47.5 ± 0.6 (11)
Socialized	48.5 ± 0.3 (6)	49.5 ± 0.3 (3)	48.8 ± 0.3 (9)
Finishing Means	48.5 ± 0.4 (11)	47.7 ± 0.8 (9)	

Table 41: Effect of Socialization Treatment on Flank Girth at 391 days of age (in ± s.e.). Numbers in parentheses indicate number of observations used to calculate mean.

Nursery Treatments	Finishing Treatments		Nursery Means
	Un-socialized	Socialized	
Un-socialized	54.1 ± 1.7 (5)	56.3 ± 0.6 (6)	55.3 ± 0.9 (11)
Socialized	57.1 ± 0.6 (6)	57.3 ± 1.3 (3)	57.2 ± 0.6 (9)
Finishing Means	55.7 ± 0.9 (11)	56.7 ± 0.6 (9)	

Table 42: Effect of Socialization Treatment on Hip Height at 391 days of age (in ± s.e.). Numbers in parentheses indicate number of observations used to calculate mean.

Nursery Treatments	Finishing Treatments		Nursery Means
	Un-socialized	Socialized	
Un-socialized	31.1 ± 0.6 (5)	30.6 ± 0.6 (6)	30.8 ± 0.4 (11)
Socialized	32.1 ± 0.5 (6)	31.3 ± 1.2 (3)	31.8 ± 0.5 (9)
Finishing Means	31.6 ± 0.4 (11)	30.8 ± 0.5 (9)	

Table 43: Effect of day of analysis on motility of extended, stored insemination semen doses. Numbers in parentheses indicate number of observations used to calculate the mean.

Semen Quality Measure	Day 0	Day 6
Motility (%)	89.03 ± 2.01^{a} (19)	75.47 ± 3.19^{b} (19)
Progressive Motility (%)	76.44 ± 2.44^{c} (19)	57.66 ± 3.56^{d} (19)

[a,b] means in the same row with different superscripts are different ($p \leq 0.01$)
[c,d] means in the same row with different superscripts are different ($p \leq 0.01$)

FIGURES

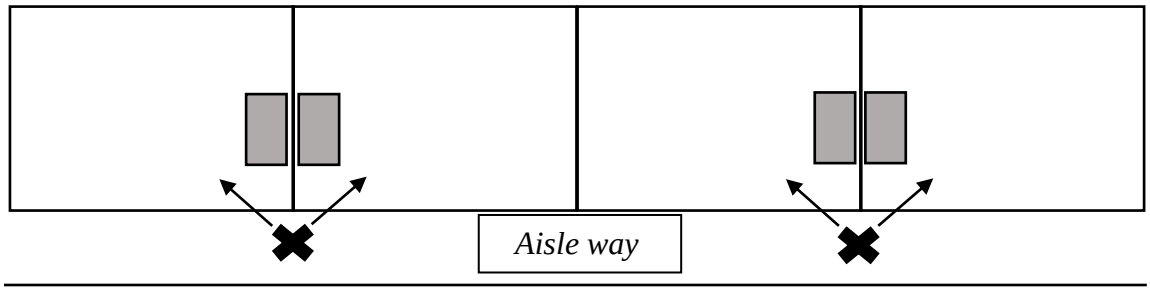

Figure 1: Socializer Location in Finishing. The "X" designates the socializer location.

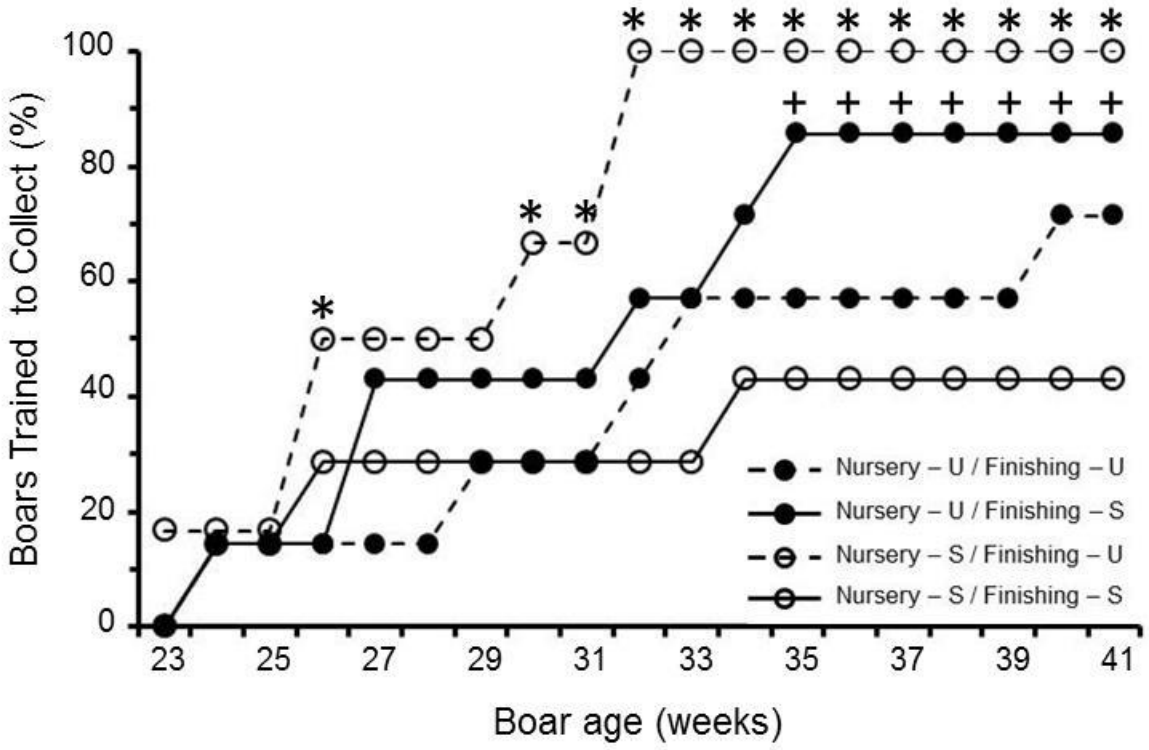

Figure 2: Effect of socialization treatment on cumulative training success of boars.* indicates that nursery socialization alone is significantly higher than socialization during nursery and finishing. + indicates that finishing socialization alone is significantly higher than socialization during nursery and finishing.

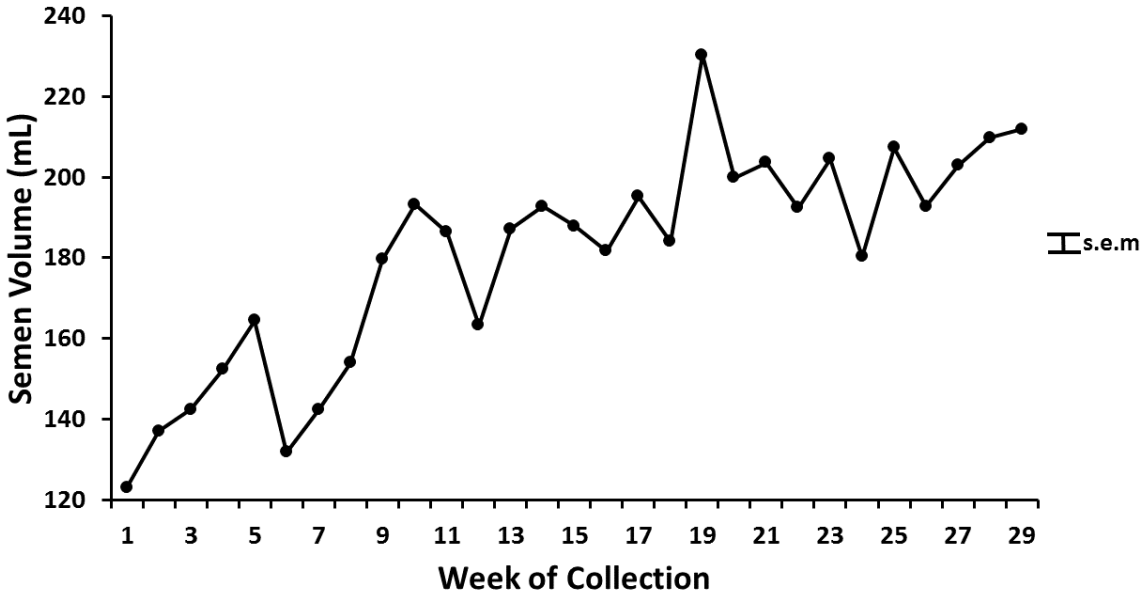

Figure 3: Effect of week of collection on semen volume (mL). Standard error of the mean = 2.7 mL. Week 1 represents the week of February 8, 2016 and week 29 represents the week of August 22, 2016.

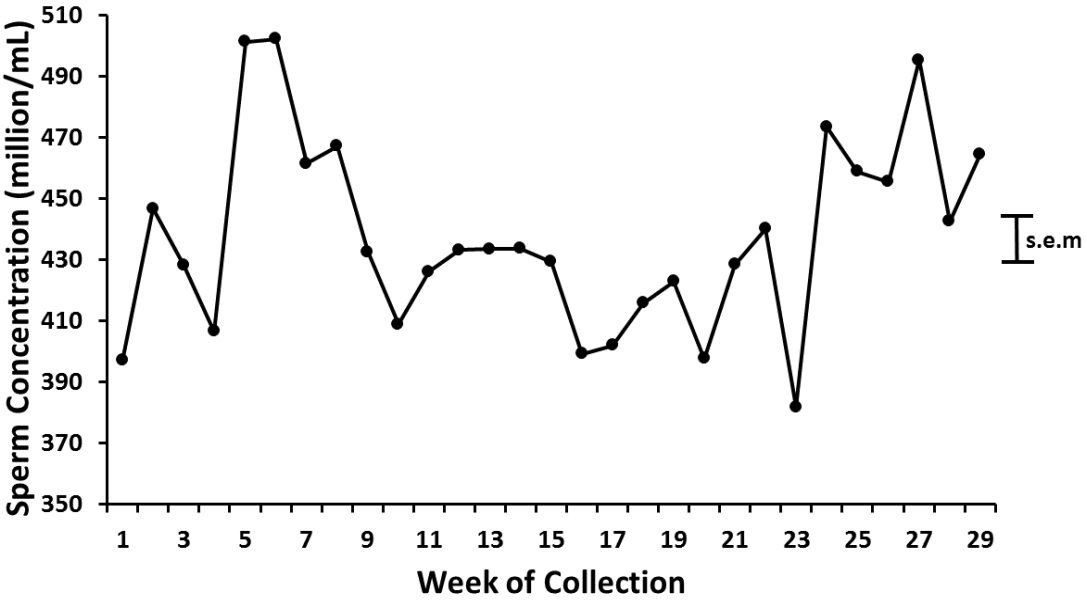

Figure 4: Effect of week of collection on sperm concentration (million cells/mL). Standard error of the mean = 7.0 million cells/mL. Week 1 represents the week of February 8, 2016 and week 29 represents the week of August 22, 2016.

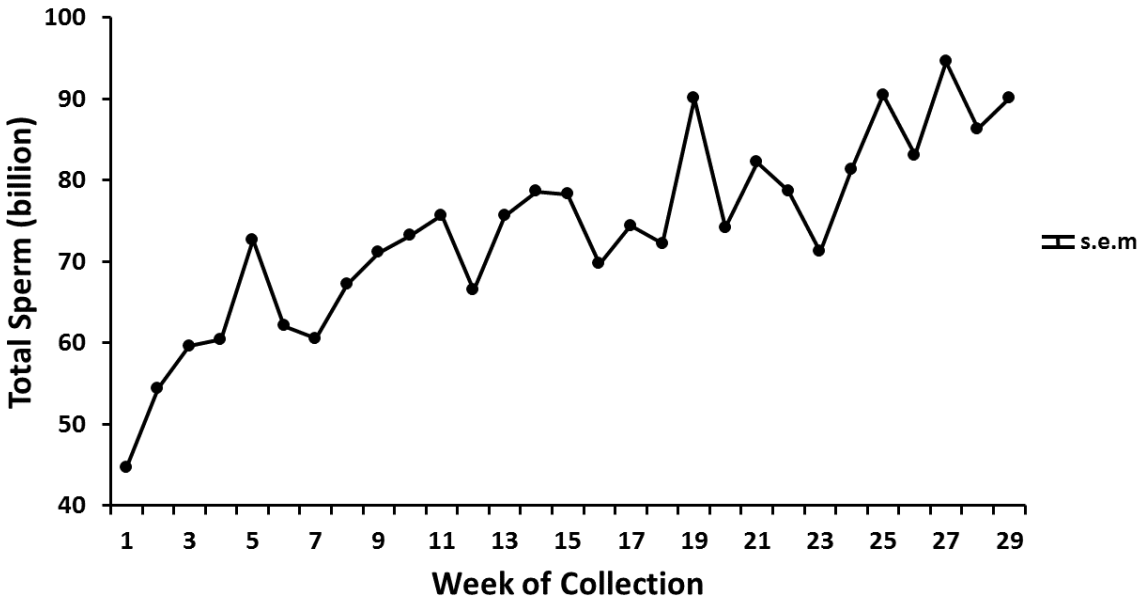

Figure 5: Effect of week of collection on total sperm per ejaculate (billion cells). Standard error of the mean = 1.0 billion cells. Week 1 represents the week of February 8, 2016 and week 29 represents the week of August 22, 2016.

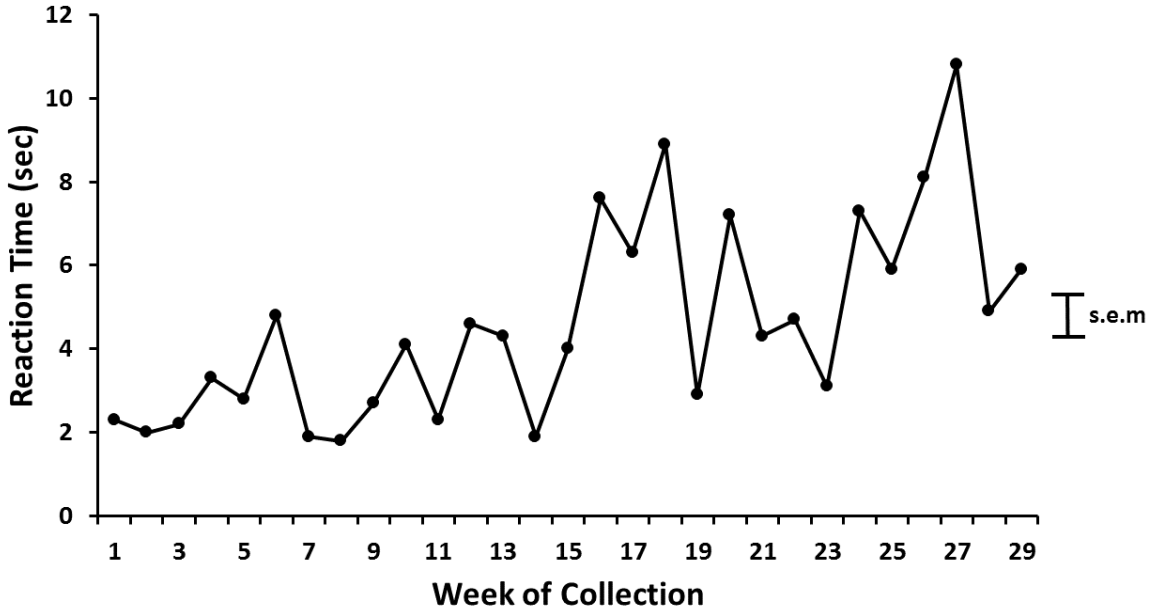

Figure 6: Effect of week of collection on reaction time (sec). Standard error of the mean = 0.5 sec. Week 1 represents the week of February 8, 2016 and week 29 represents the week of August 22, 2016.

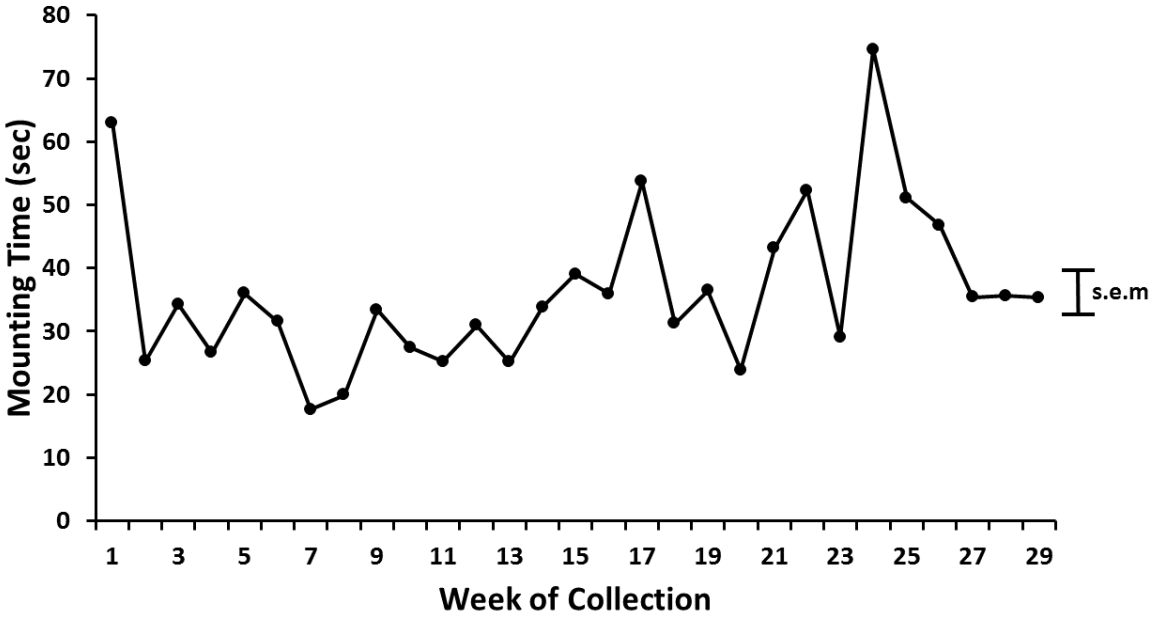

Figure 7: Effect of week of collection on mounting time (sec). Standard error of the mean = 2.5 sec. Week 1 represents the week of February 8, 2016 and week 29 represents the week of August 22, 2016.

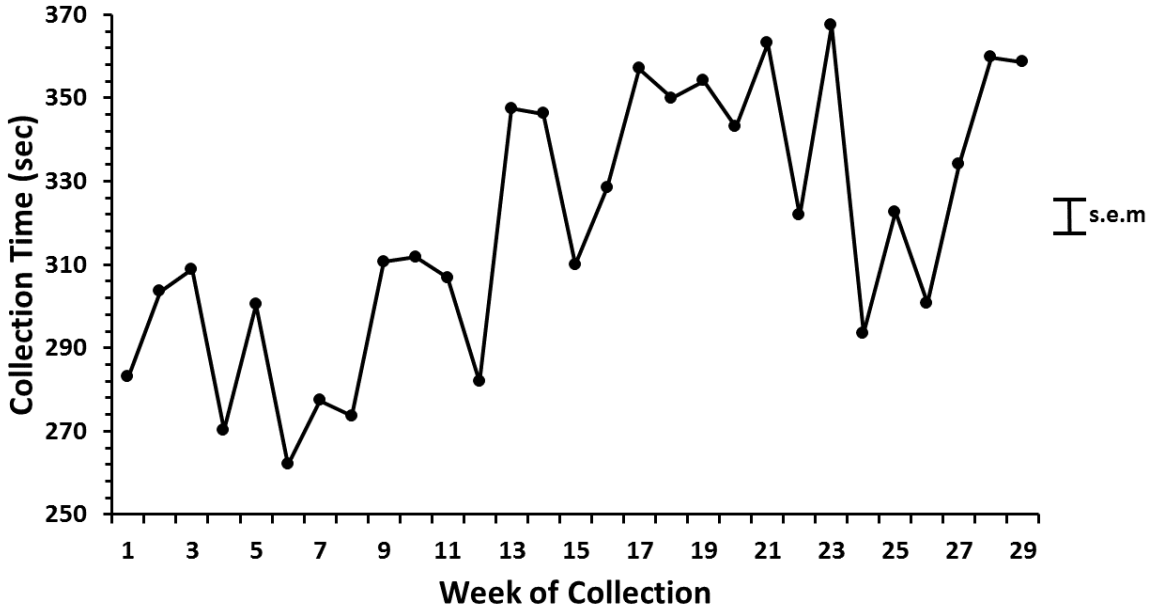

Figure 8: Effect of week of collection on collection time (sec). Standard error of the mean = 4.2 sec. Week 1 represents the week of February 8, 2016 and week 29 represents the week of August 22, 2016.

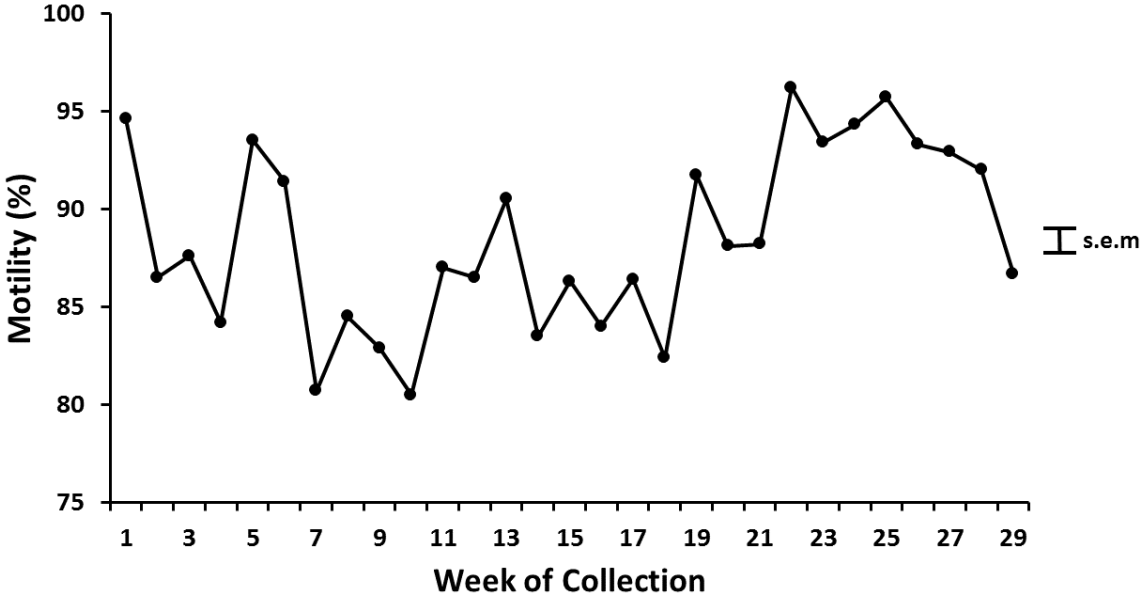

Figure 9: Effect of week of collection on sperm motility (%). Standard error of the mean = 0.6%. Week 1 represents the week of February 8, 2016 and week 29 represents the week of August 22, 2016.

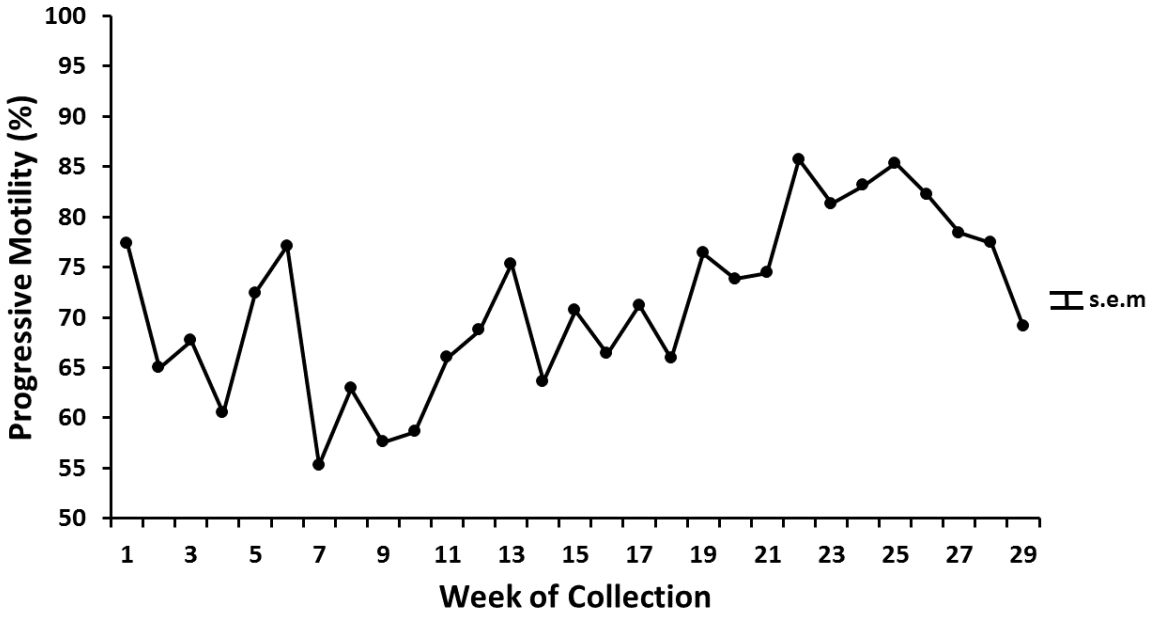

Figure 10: Effect of week of collection on sperm progressive motility (%). Standard error of the mean = 0.8%. Week 1 represents the week of February 8, 2016 and week 29 represents the week of August 22, 2016.

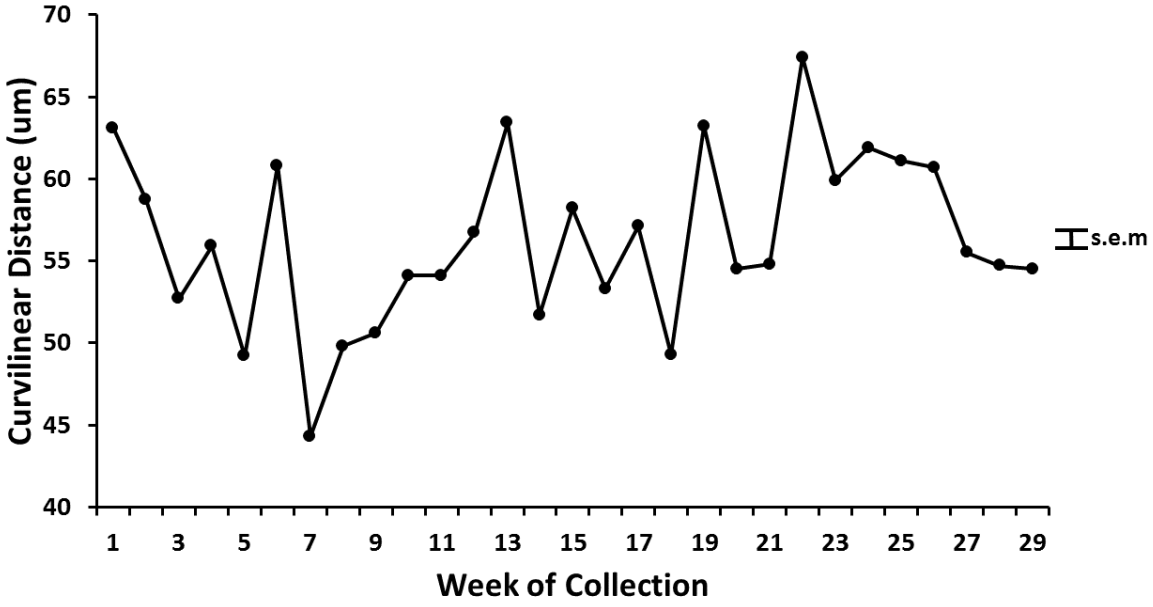

Figure 11: Effect of week of collection on sperm curvilinear distance (μm). Standard error of the mean = 0.6 μm. Week 1 represents the week of February 8, 2016 and week 29 represents the week of August 22, 2016.

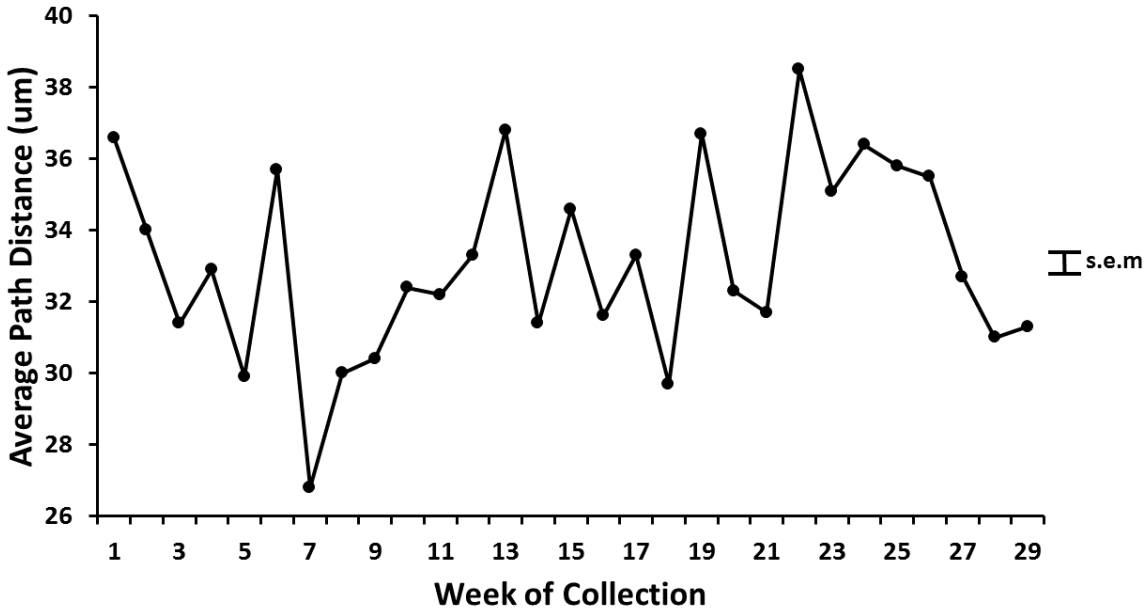

Figure 12: Effect of week of collection on sperm average path distance (μm). Standard error of the mean = 0.3 μm. Week 1 represents the week of February 8, 2016 and week 29 represents the week of August 22, 2016.

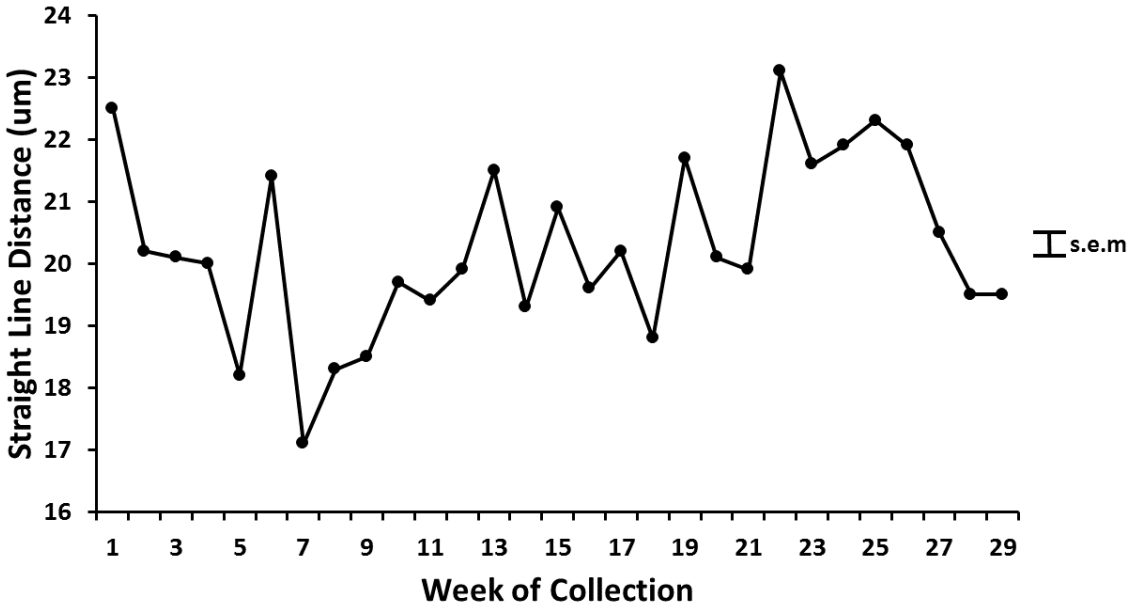

Figure 13: Effect of week of collection on sperm straight line distance (μm). Standard error of the mean = 0.2 μm. Week 1 represents the week of February 8, 2016 and week 29 represents the week of August 22, 2016.

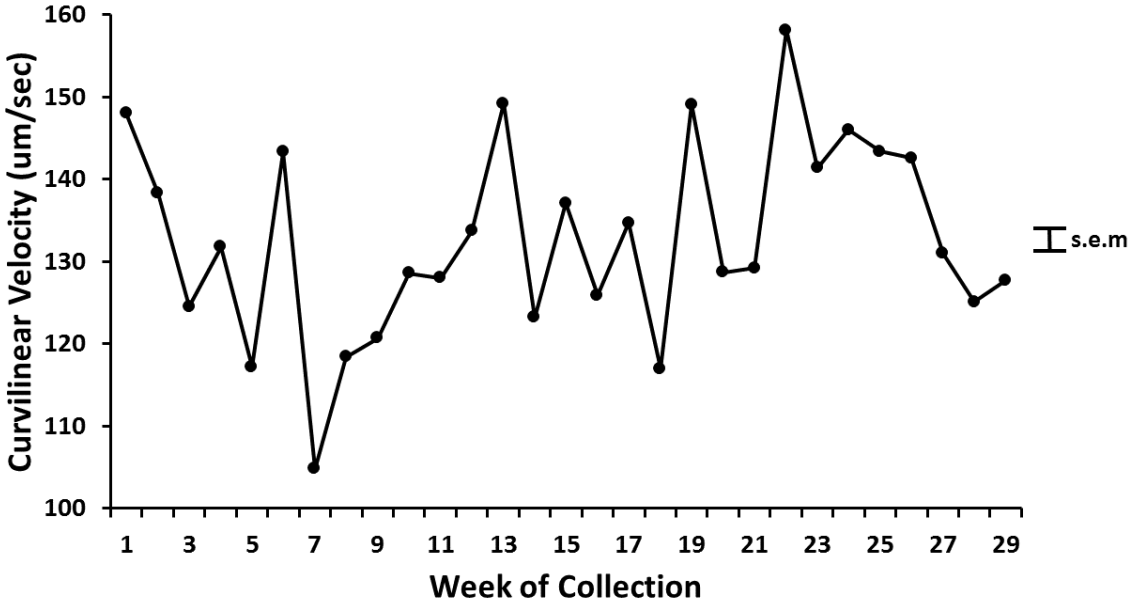

Figure 14: Effect of week of collection on sperm curvilinear velocity (μm/sec). Standard error of the mean = 1.5 μm/sec. Week 1 represents the week of February 8, 2016 and week 29 represents the week of August 22, 2016.

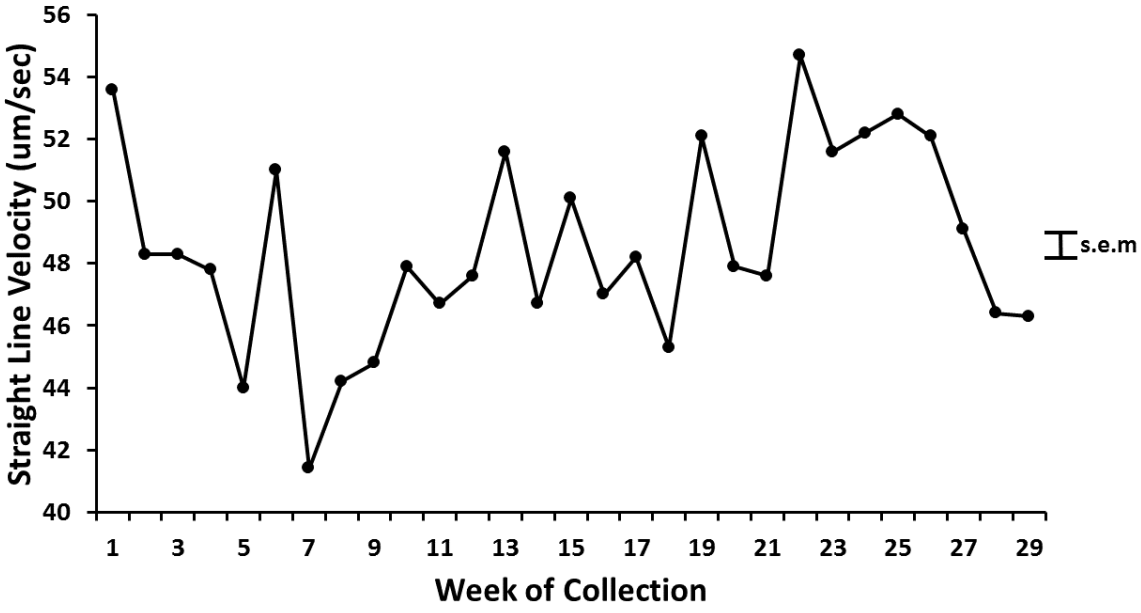

Figure 15: Effect of week of collection on sperm straight line velocity (μm/sec). Standard error of the mean = 0.4 μm/sec. Week 1 represents the week of February 8, 2016 and week 29 represents the week of August 22, 2016.

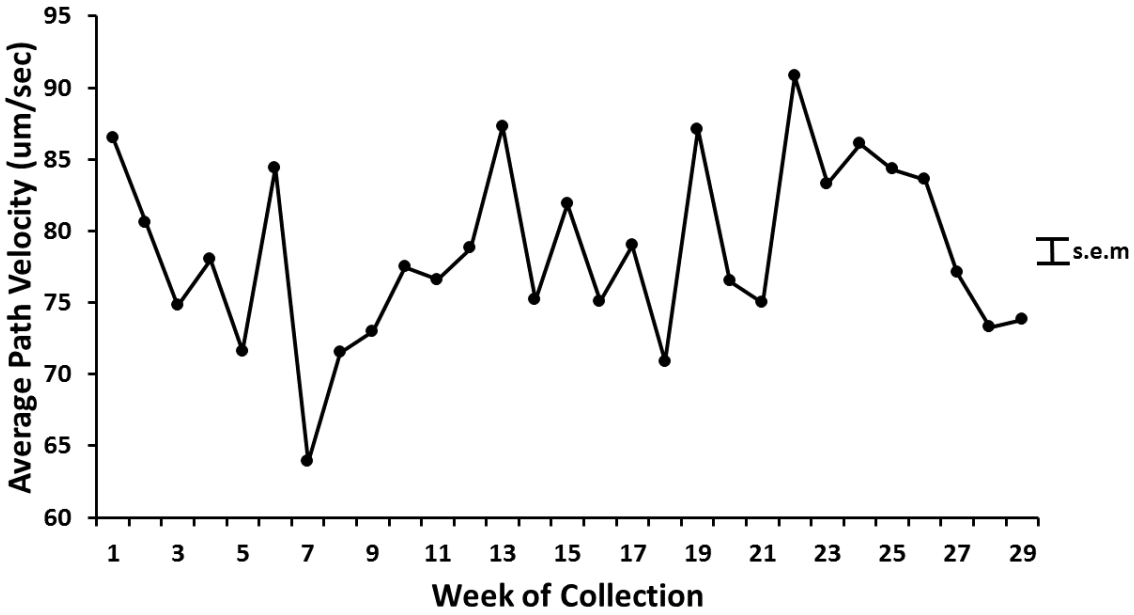

Figure 16: Effect of week of collection on sperm average path velocity (μm/sec). Standard error of the mean = 0.8 μm/sec. Week 1 represents the week of February 8, 2016 and week 29 represents the week of August 22, 2016.

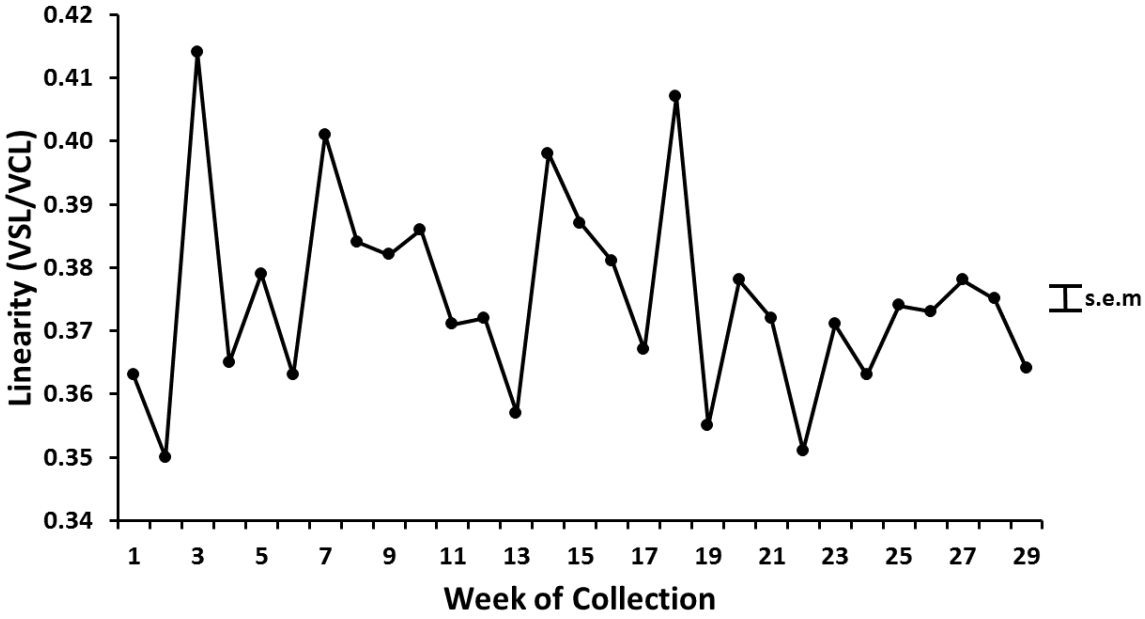

Figure 17: Effect of week of collection on sperm linearity (VSL/VCL). Standard error of the mean = 0.002. Week 1 represents the week of February 8, 2016 and week 29 represents the week of August 22, 2016.

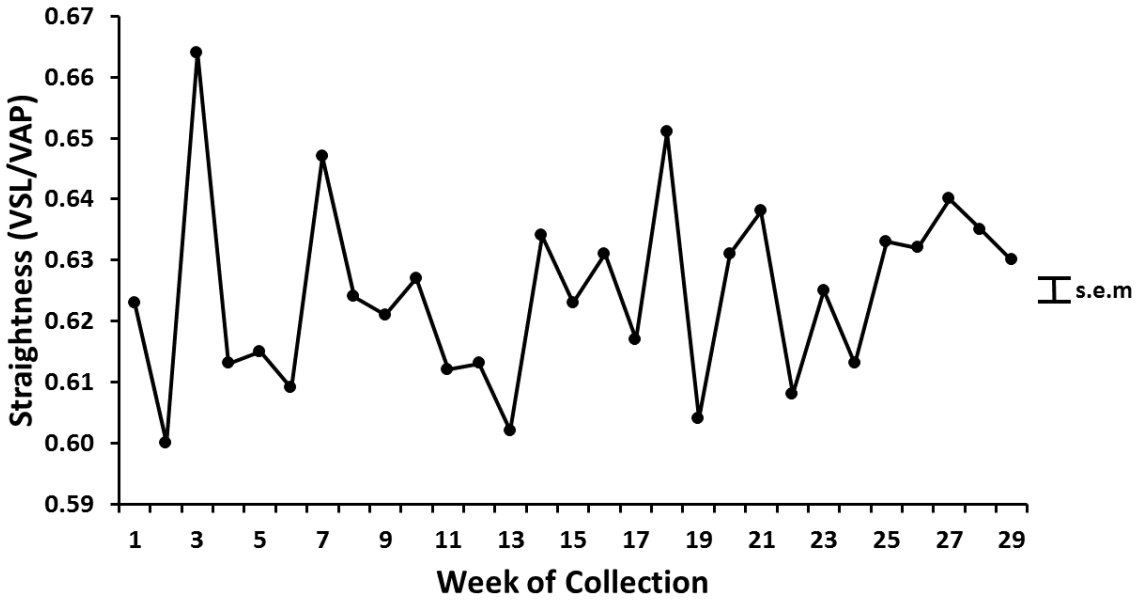

Figure 18: Effect of week of collection on sperm straightness (VSL/VAP). Standard error of the mean = 0.002. Week 1 represents the week of February 8, 2016 and week 29 represents the week of August 22, 2016.

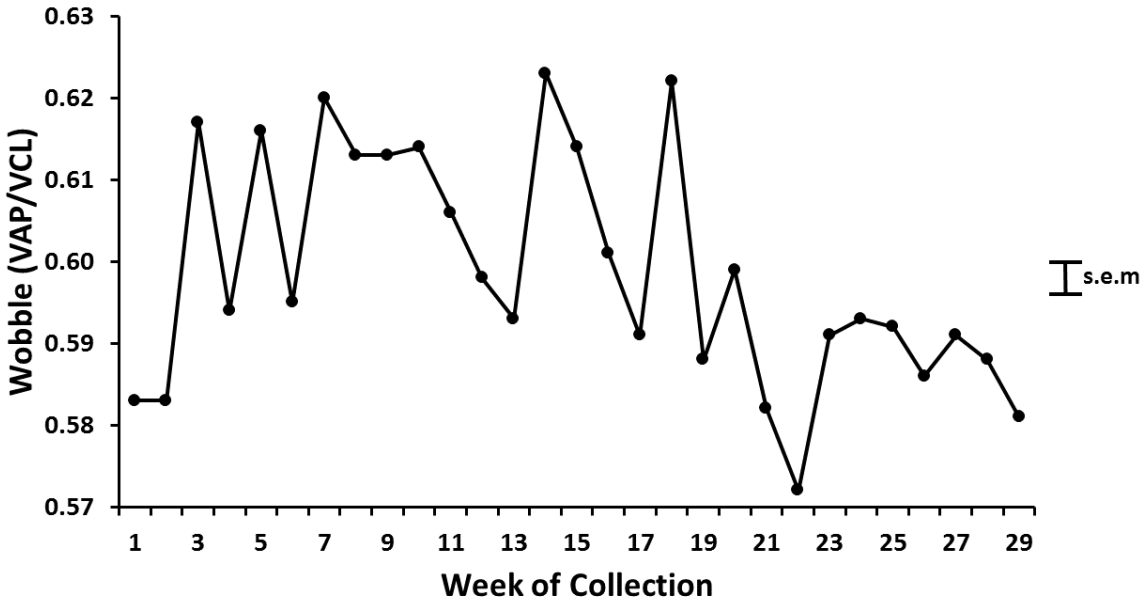

Figure 19: Effect of week of collection on sperm wobble (VAP/VCL). Standard error of the mean = 0.002. Week 1 represents the week of February 8, 2016 and week 29 represents the week of August 22, 2016.

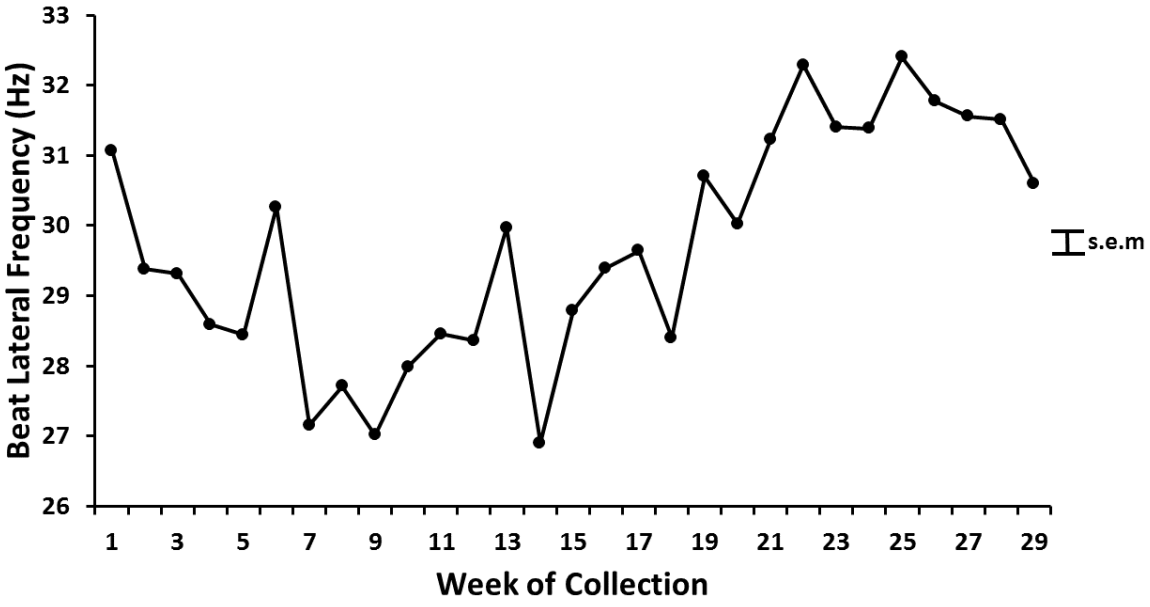

Figure 20: Effect of week of collection on sperm beat lateral frequency (Hz). Standard error of the mean = 0.16 Hz. Week 1 represents the week of February 8, 2016 and week 29 represents the week of August 22, 2016.

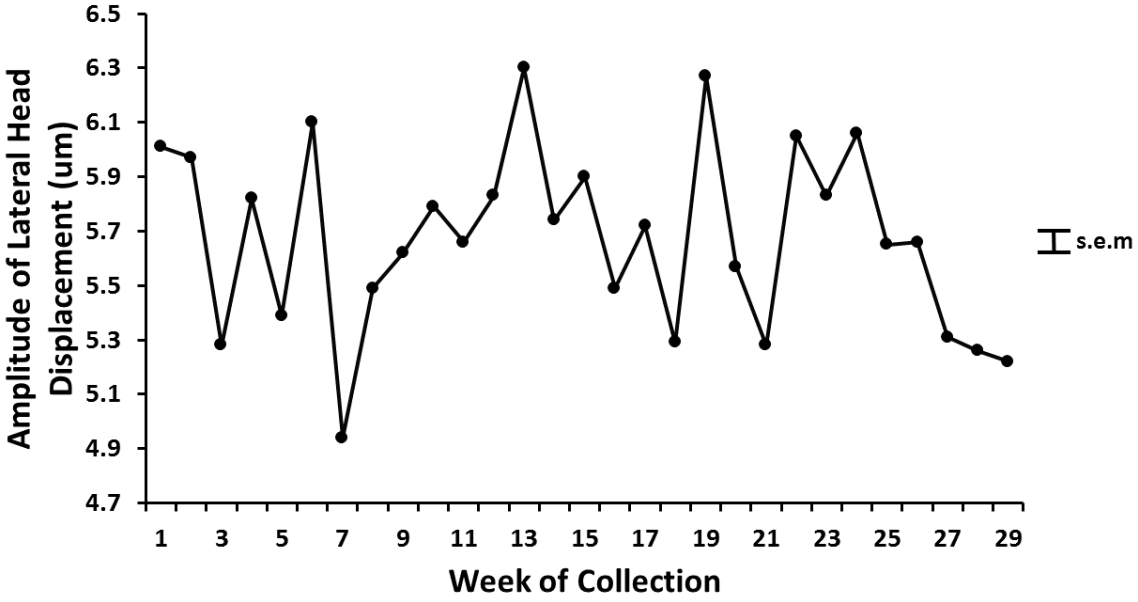

Figure 21: Effect of week of collection on sperm amplitude of head displacement (μm). Standard error of the mean = 0.04 μm. Week 1 represents the week of February 8, 2016 and week 29 represents the week of August 22, 2016.

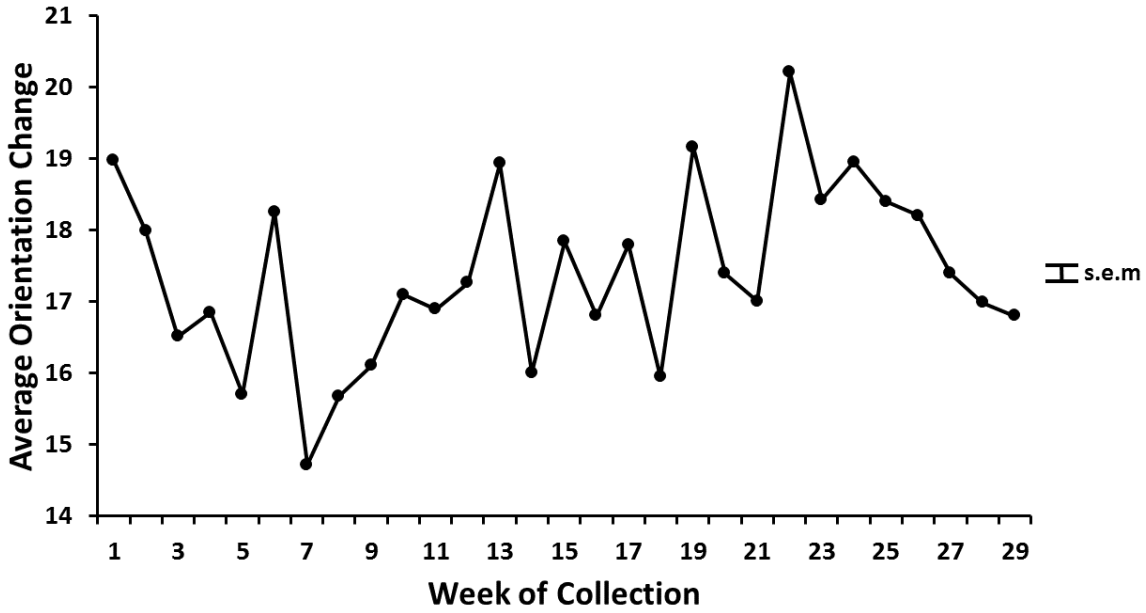

Figure 22: Effect of week of collection on sperm average orientation change. Standard error of the mean = 0.14. Week 1 represents the week of February 8, 2016 and week 29 represents the week of August 22, 2016.

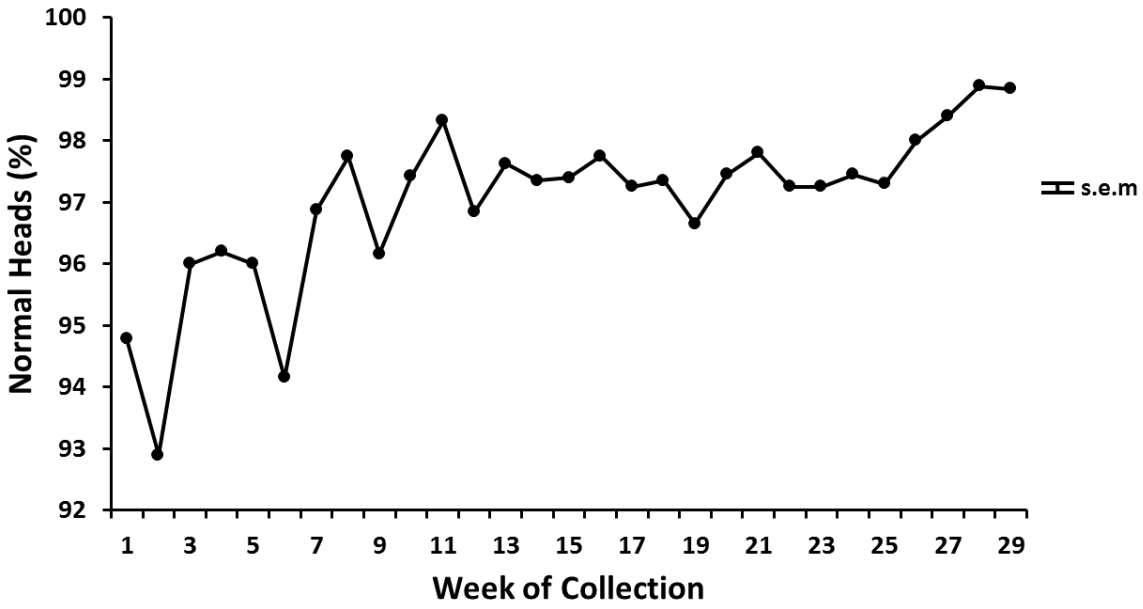

Figure 23: Effect of week of collection on normal heads (%). Standard error of the mean = 0.1%. Week 1 represents the week of February 8, 2016 and week 29 represents the week of August 22, 2016.

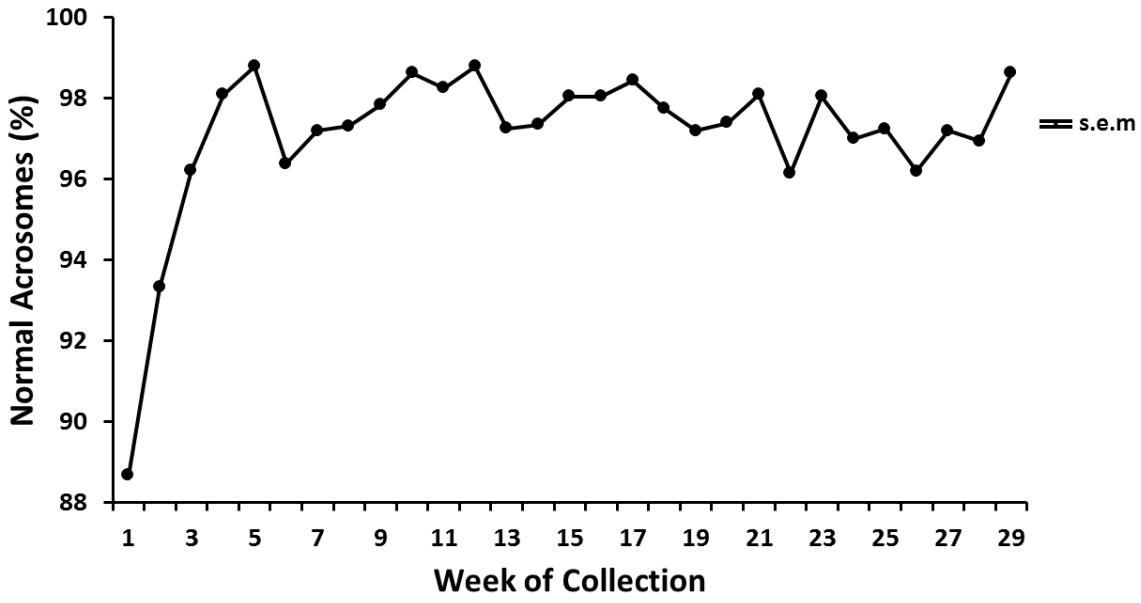

Figure 24: Effect of week of collection on normal acrosomes (%). Standard error of the mean = 0.11%. Week 1 represents the week of February 8, 2016 and week 29 represents the week of August 22, 2016.

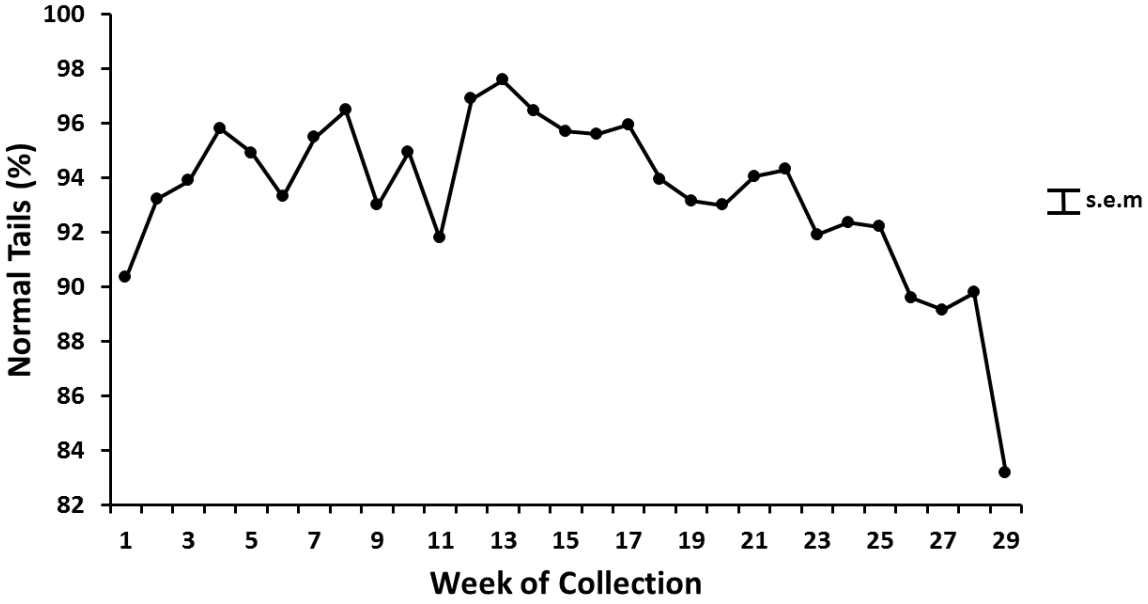

Figure 25: Effect of week of collection on normal tails (%). Standard error of the mean = 0.43%. Week 1 represents the week of February 8, 2016 and week 29 represents the week of August 22, 2016.

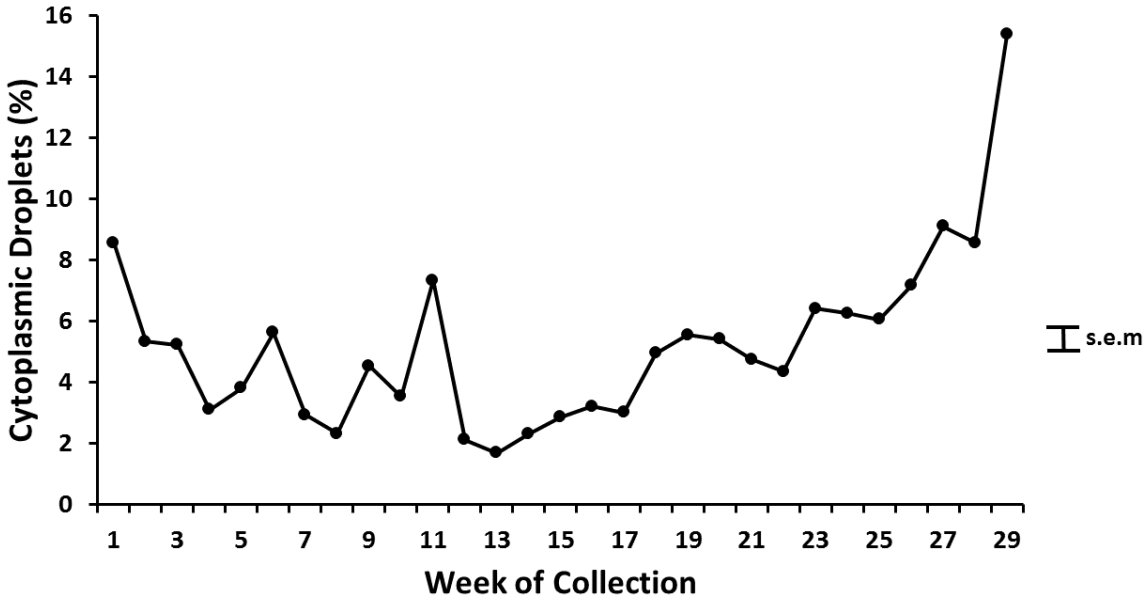

Figure 26: Effect of week of collection on cytoplasmic droplets (%). Standard error of the mean = 0.4%. Week 1 represents the week of February 8, 2016 and week 29 represents the week of August 22, 2016.