

ABSTRACT

FALKNER, REBECCA CATHERINE. Social and Environmental Impacts on Sperm Production in Boars. (Under the direction of Dr. William L. Flowers).

The objective of this study was to examine the effect of human socialization prior to sexual maturity on the reproductive performance of adult A.I. boars. Thirty-six low birth weight boars (average birth weight = 1.2 kg) were randomly assigned to treatment groups between weaning and finishing to be socialized or unsocialized. Boars between 4 to 9 weeks of age were randomly assigned to be socialized or unsocialized (nursery) and boars within each nursery socialization group were again randomly assigned to be socialized or unsocialized between 10 to 24 weeks of age (finishing). This arrangement resulted in four different levels of socialization: no socialization; socialization from 4 to 9 weeks of age; socialization from 10 to 24 weeks of age; and socialization from 4 to 24 weeks of age. Boars were trained for semen collection beginning at 160 days of age. Boars that were successfully trained were collected for 68 consecutive weeks. Socialization had a significant ($p < 0.0001$) effect on several measures of collection efficiency. In general, reaction time and mounting time after first entering the collection pen decreased as the amount of socialization increased. Volume of semen and total sperm per ejaculate were both greater in socialized boars compared with their unsocialized counterparts. Several estimates of semen quality were either better or tended to be better in socialized versus unsocialized boars. These included curvilinear distance ($p = 0.03$); curvilinear velocity ($p = 0.01$); cellular beat frequency ($p = 0.0001$); amplitude of lateral head displacement ($p = 0.001$); and proportion of spermatozoa with normal heads ($p < 0.0001$); normal tails ($p < 0.0001$); proximal droplets ($p = 0.0003$); and distal droplets ($p < 0.0001$). The combination of increased sperm production and improved quality resulted in significant improvements in lifetime productivity of useable sperm ($p = 0.01$) and insemination doses ($p = 0.02$) for socialized

compared with unsocialized boars. Competitive fertility results indicated that there were no significant differences ($p=0.39$) between socialized and unsocialized boars. Multiple regression analyses revealed that a boar's total socialization score, birth weight, training age, total socialization score during the nursery, and weaning weight explained between 35 and 40% of the total variation in total sperm per ejaculate and lifetime sperm production traits. Results from this study indicate that socialization with humans after weaning has a positive effect on both the quality and quantity of the lifetime sperm production of adult boars.

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Social and Environmental Impacts on Sperm Production in Boars

by
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BIOGRAPHY

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LITERATURE REVIEW

Introduction

The utilization of artificial insemination in swine breeding facilities has allowed for more advanced selective breeding of future generations. Through the use of natural heats and timed A.I. protocols, producers are able to breed multiple females with semen from a single collection off of a genetically superior boar. This is a more efficient and economic system due to a reduced need to house numerous boars for natural mating, resulting in lower feed and maintenance costs, and lowers the time span needed to breed females. In boar studs, it is imperative to select boars that will possess a greater longevity and excellent sperm quality and quantity. Earlier research has explored multiple factors that impact sperm production once a boar reaches sexual maturity, whereas less focus has been directed towards boar prepubescent life and the impact management factors can affect boars later in their productive lifetime. Human-animal interaction during prepubescent life has been shown to greatly impact the relationship boars have with collection technicians at puberty, which in turn, impacts sperm quality and quantity. Identifying factors of interest and improving management strategies would allow for producers to maximize production. The purpose of this review is to examine factors that most commonly impact boar reproductive performance from prenatal development to sexual maturity, while also discussing factors that impact sperm production in a mature, adult boar.

Spermatogenesis

For any breeding animal, managers often evaluate replacement males or females based off of their physiological structure and genetics in order to maximize longevity. For breeding boars, sperm quality and quantity are important factors. It is essential to understand how sperm is produced within the boar by understanding the complex process of spermatogenesis.

Testicular development and function

Proper testicular development in the immature boar is essential in order to ensure maximum sperm output once sexual maturity is reached. Like most mammals, boar testes are composed of two compartments: interstitial and seminiferous epithelium (Petersen and Soder, 2006). The interstitium is comprised many cells types, where the most important cells in this compartment are Leydig cells. Leydig cells are stimulated by the release of the pituitary gonadotropin, luteinizing hormone (LH) (Ramaswamy and Weinbauer, 2014) and produce the steroid hormone testosterone (Bartlett et al., 1989). Testosterone and other androgens are known to stimulate sexual behaviors in males and are necessary in low levels to maintain spermatogenesis in mammals (Lindner, 1961; Bartlett et al., 1989). Together, appropriate levels of pulsatile release of LH and testosterone stimulate the initial stages of spermatogenesis subsequent to the beginning of puberty (Ramaswamy and Weinbauer, 2014).

The second compartment, the seminiferous epithelium, is responsible for the production of spermatozoa with the aid of Sertoli and germ cells. The seminiferous epithelium is a complex tubule that provides for the progression in growth of spermatozoa from the rete testis to the epididymis (Johnson et al., 2008). Sertoli cells are regulated by the release of follicle-stimulating hormone (FSH) that is produced in the pituitary gland (Petersen and Soder, 2006). Adequate FSH receptors are important to testis development in prepuberal mammals. Research shows that males with reduced FSH receptors develop smaller sized testicles, and in turn, result in lower numbers of Sertoli cells which negatively impact spermatogenesis (Petersen and Soder, 2006). FSH acts through the cyclic AMP second messenger system (Fritz et al., 1976) and is responsible for the production of inhibin, androgen receptors, lactate, and transferrin (Petersen and Soder, 2006). It also increases germ cell numbers to aid in the survival of spermatogonia as

those cells mature into spermatocytes (Ramaswamy and Weinbauer, 2014). However, too much inhibin can impair fertility in both males and females (Petersen and Soder, 2006). The exact role FSH plays in spermatogenesis is still unclear, although it is recognized as being an essential hormone to Sertoli cell development. Sertoli cell development in prepuberal boars is essential for mature sperm production as they only proliferate and differentiate up until the beginning of puberty (Petersen and Soder, 2006; Johnson et al., 2008; Ramaswamy and Weinbauer, 2014).

The blood-testis barrier consists of tight junctions between adjacent Sertoli cells and regulates nutrient intake to germ cells (Petersen and Soder, 2006; Johnson et al., 2008). As a boar reaches puberty, tight junctions appear when Sertoli cells begin to elongate within the testes (Petersen and Soder, 2006). The interaction of multiple junctions and Sertoli cells result in the formation of three compartments: basal, adluminal, and lumen (Dym and Fawcett, 1970; Lie et al., 2013).

The basal compartment contains younger, immature spermatogonia as well as preleptotene spermatocytes, while the adluminal compartments contain more mature spermatocytes (Dym and Fawcett, 1970). Throughout the prepuberal period, molecular mechanisms aid in the disassembly of older pathways to make way for new connections in order for mature spermatocytes and spermatids to be able to migrate into developing germ cells (Lie et al., 2013). The blood-testis barrier is highly specialized to insure forward progress of spermatogenesis and to protect germ cells from stimulating autoimmune reactions (Johnson et al., 2008; Lie et al., 2013). As Sertoli cells elongate, they begin to produce seminiferous fluid, which fills the lumen within the epididymis (Petersen and Soder, 2006).

The epididymis is a semicircular shared reproductive organ that is comprised of three regions: caput, corpus, and cauda. These segments are responsible for the storage, protection,

and transport of maturing spermatozoa (Marengo, 2008). Developing spermatozoa passively travel from the seminiferous tubules and through the rete testis to reach the epididymis.

Adluminal cells possess more cytoplasm than basal cells and communicate directly with lumen in the epididymis (Marengo, 2008). At this stage of development, spermatozoa will not have the ability to fertilize an ovum. However, research proves that spermatozoa taken from the cauda of the epididymis at maturity shows greater motility, membrane fusion, and higher probabilities of zona pellucida penetration within a receptive female (Cooper, 2008).

Sertoli cell mitotic activity

Starting within the seminiferous tubule, germ cells divide and consistently produce spermatozoa. As they are constantly developing, specialized nomenclature was created in order to describe the stage of development of spermatozoa. In order of ascending maturity, the major classes of germ cells within the seminiferous tubule are referred to as: spermatogonia, spermatocyte, spermatid, and spermatozoa. Spermatogonia form within the basal compartment and multiply by mitotic divisions (Johnson et al., 2008). Type A₀ spermatogonia lay adjacent to the wall of the seminiferous tubule and are the only class of germ cell that produce genetically identical daughter cells that will eventually become primary spermatocytes (Woodsdalek, 1913; Hamano et al., 2007). Type A spermatogonia generate multiple generations of intermediate spermatogonia before being classified as Type B spermatogonia (Woodsdalek, 1913; LeBlond and Clermont, 1952). During Type B division, spermatogonia are traveling from the basal to the adluminal compartment via the gap junction between Sertoli cells (LeBlond and Clermont, 1952).

Spermatocytes exhibit the longest lifespan in a primary stage, as they enter meiosis I over 12.3 days in the boar (Swiestra, 1968), and evolve into diploid secondary spermatocytes

(LeBlond and Clermont, 1952). There is research to show primary and secondary spermatocytes in the same field, indicating that there is a very short or non-existing resting period between the two (Woodsdalek, 1913). Secondary spermatocytes enter a short transition period, the shortest lifespan at 0.4 days in the boar (Swiestra, 1968), where they are soon converted into haploid spermatids (Woodsdalek, 1913; LeBlond and Clermont, 1952). Sertoli cells then release spermatids into the lumen of the seminiferous tubule via spermiation (Johnson et al., 2008). During spermiogenesis, spermatids are shaped into spermatozoa during elongation of the cell (Woodsdalek, 1913). In the final storage location in the corpus epididymis, cytoplasmic droplets form on the flagellum of the spermatids, which represent excess cytoplasm that were retained by the sperm cell itself (Johnson et al., 2008). Throughout development, these droplets will fall from a proximal to a distal position on the flagellum and eventually, will disappear from the sperm cell permanently once fully mature as spermatozoa. At the conclusion of the spermatogenic cycle, spermatozoa will remain in the lumen until being released during ejaculation (Woodsdalek, 1913).

Spermatogenesis in the adult boar

After puberty, the reproductive organs of boars continue to develop. The level of secretion of hormones is one factor that is greatly impacted with age. As discussed previously, FSH is an important hormone in spermatogenesis and Sertoli cell development in the prepubertal boar. FSH is responsible for the secretion of additional hormones that aid in the survival of spermatogonia (Petersen and Soder, 2006) and is vital for the maintenance of spermatogenesis in adult mammals (Wagner and Claus, 2009). However, it is interesting to note that the effects of FSH are not facilitated through alterations in androgens, such as testosterone, in an adult animal

(Bartlett et al., 1989). Progesterone receptor expression was found to increase as a boar ages as is determined to have an effect on the maturation of prespermatogonia (Kohler et al., 2007).

In addition to this information, exposure to elevated levels of FSH in the postnatal period of a boar's life does not impact sperm production as it ages (Wagner and Claus, 2009). This is likely due to the cease of Sertoli cell proliferation and differentiation after puberty. Sertoli cell numbers are highest in immature males and decrease with age (Steinberger and Steinberger, 1971). After puberty, the capacity of the testis for sperm production is set in order for Sertoli cells to focus on germ cell maintenance (Petersen and Soder, 2006). The only mammal found to serve as an exception to this occurrence is the stallion (Johnson et al., 2008).

In the young boar, the seminiferous tubule is closed and allows only a small amount of immature spermatozoa to flow within the testes (Tripepi et al., 2000). As a boar ages and matures, the tubules become highly developed where the lumen is expanded and the basal lamina becomes thicker (Tripepi et al., 2000). Likewise, Leydig cells in a boar exhibit an unevenly shaped nucleus and a reduced amount of mitochondria and smooth endoplasmic reticulum as they mature (Tripepi et al., 2000). Spermatogonial stem cells (SCC) are observed in relation to Sertoli cells and determine the supply of spermatozoa throughout the lifetime of the boar. These stem cells reside within a unique area that is comprised of several growth factors within the testis (Hai et al., 2014; van den Driesche et al., 2014). It is proposed that microRNA signaling in both SCC and germ cells could assist in regulating the ideal environment they reside within the adult boar to aid in their self-renewal throughout their productive lifetime (Hai et al., 2014; van den Driesche et al., 2014).

Management factors that affect reproductive function

There are multiple factors than can impact the reproductive performance of an individual boar. Whereas some of these factors can be managed and regulated by producers, others cannot. Boars are generally selected at a young age and are observed for developmental flaws during adolescence in order to select boars that will have higher productivities as adults. Management factors that could damage growth or reproductive function should be avoided to reduce the chance of permanently limiting sperm producing capabilities. As boars mature, other factors can impede normal sperm production such as seasonal temperature and photoperiod and housing environment. These factors can often be reversed, so while it is important to regulate factors that affect mature boars, it is imperative to focus on the factors that affect young boars during their developmental stage of life. The purpose of the following sections is to examine some key developmental influences that are relevant to this particular study.

Testicular growth

Physical traits such as body weight and testicle dimension are two measurements that can impact the amount of viable sperm an individual boar will be able to produce throughout his lifetime. It is easy to assume that boars of a higher weight at birth will possess larger testicles upon maturity. This was found to be true in a studies performed by both Dysart et al. (2015) and Smit et al. (2013), where the testicle dimensions were found to be larger at both birth and at weaning for piglets of a higher birth weight. It is interesting to add that human socialization did not affect testicle size of the boars as adults (Dysart et al., 2015). Owsiany et al. (1998), Coulter and Foote (1977), and Makarechian et al. (1984) found that there were positive associations between mature body weight and testicle size in breeding males, both porcine and

bovine. This suggests that selection for larger boars at birth will result in a higher chance that those same boars will possess larger testes, and in turn, produce greater amounts of viable semen.

Selection for larger testicle dimensions is a common practice in boar stud operations as well as selecting for boars of higher birth weights and average daily gains. Many boars used in today's boar stud operations are selected for multiple generations in which many reproductive factors, including testicle size, are desired. Harder et al. (1995) used boars in their study that were specially chosen for eight generations. It was determined that selection for this trait resulted in boars reaching puberty at a younger age, a positive outcome for boar stud operations (Harder et al., 1995; Huang et al., 1996). It was shown in a recent study by Ford and Wise (2011) that the diameter of seminiferous tubules directly correlate with testicular size when boars were examined between 3 and 5 months of age when testicles were examined through ultrasonography. Boars that were selected for testicle size were shown to be superior to control boars in regards to epididymis and testicle weight, sperm number at collection, location in the cauda epididymis, and in daily sperm volume and concentration (Huang et al., 1996; Ugwu et al., 2009). Once boars exceeded the initial stages of puberty, the seminiferous tubules decrease in diameter (Ford and Wise, 2011).

This is important to note as an indication that not all boars that enter early puberty will have a large testicle size or higher sperm production at maturity (Ford and Wise, 2011). The Meishan boar is an example of a breed that reaches puberty at an earlier age than most other purebreds and crossbreds (Ford and Wise, 2009). Large White boars are shown to have an enhanced development of the length of the testes when compared to Nigerian and F₁ crossbred boars, but they all shared a similar trend in regards to testicle width (Ugwu et al., 2009). Other studies comparing crossbred and purebred boars showed that crossbred boars have a larger

average testicle and cauda epididymal weight (which are highly correlated) and total number of sperm when compared to a purebred (Wilson et al., 1977). This result was similar to earlier studies such as Harder et al. (1995).

Birth weight

In species where a female has multiple offspring per litter, it is well known that the size of each individual newborn varies. Environmental factors are also shown to have an impact on embryonic development on litter bearing species. Adequate feed intake and proper nutrition of the gestating sow or gilt are factors that can impact the fetal development (Foxcroft et al., 2007). It is shown by Rehfeldt and Kuhn (2006) that lower birth weight piglets, in particular, are not supplied with sufficient nutrients in utero due to a display of low blood glucose dilutions.

In terms of feed conversion in the nursery and finishing stages, lower birth weight piglets had a lower average daily gain (ADG) than those of a medium or high birth weight (Smit et al., 2013). However, lower birth weight animals were shown to have greater feed utilization efficiency in both the nursery and finishing stages than their heavier counterparts (Smit et al., 2013). Before determining efficiency in the later stages of life, Smit et al. (2013) observed growth during lactation and determined that higher birth weight piglets also had a higher ADG than their smaller littermates. This was likely due to teat competition. At maturity, protein and energy sources in feed are crucial elements for adequate sperm production (Flowers, 2015).

Uterine capacity and embryo survivability post-implantation are two factors that can impact the size of piglets prior to parturition (Foxcroft et al. 2007). In a study performed by Town et al. (2005), it was shown that sows of higher parities typically ovulated at least 25 ova which was more than immature sows or gilts. Unfortunately, this large number of embryos at Day 30 of gestation will exceed uterine capacity. As a result, the female will have to reduce the

number of fetuses in order to make space for the entire litter prior to parturition, resulting in the largest percentage of losses prenatally for swine (Foxcroft et al., 2007). This observation is the basis for the commonly accepted negative relationship between litter size and litter birth weight.

Blood flow within the uterus during pregnancy was observed to have a medium to high correlation coefficient with total litter weight ($r=0.79$) and litter size ($r=0.56$; Père and Etienne, 2000). Père and Etienne (2000) also discovered that uterine blood flow also increased throughout pregnancy as the fetuses continue to grow. This indicates that an increased blood flow to fetuses may result in higher weights at birth. However, once both uterine horns surpass five fetuses, the uterine blood flow was shown to increase at a lower rate indicating that a larger litter size decreased overall blood flow to each fetus (Père and Etienne, 2000).

In a study performed by Krackow (1995), it was shown that male embryos mature earlier than female embryos resulting in them being more likely to implant first. The article also proposed that a female is more likely to terminate the development of an embryo that she does not feel has the best chance at survival (Krackow, 1995). As it suggests, males are likely to implant before females since they tend to be more developed at the same chronological age. This indicates that more female sexed embryos will be terminated post-implantation than male, making male fetuses more likely to be larger than females at parturition. These conclusions were similar to those of Smit et al. (2013) and Douglas et al. (2013) described at parturition, weaning, and later in life. It is also interesting to note that Gorecki (2003) discovered that when nutritional resources in a wild sow's environment are low, she is more likely to give birth to males due to the fact that they will move to a different location upon maturity. Adversely, if resources were favorable, than the sow is more likely to give birth to more females (Gorecki, 2003). It is also

important to note that Gorecki (2003) observed that litter size, as well as paternal breed, resulted in a lower number of male offspring in a large litter.

The age at which boars reach sexual maturity in relation to birth weight has not been extensively studied. However, there has been some research performed on puberty in gilts, where it is likely that boars follow a very similar outline. Hutchens et al. (1981) examines the relationship between birth weight and puberty in gilts, concluding that there is very little correlation (-0.07) between the two. The study suggests that with the selection for rate of growth, there is likely to be a decrease in the age a gilt reaches puberty (Hutchens et al., 1981). Tummaruk et al. (2007) showed that gilts that were born at a lower birth weight had a longer breeding interval than heavier gilts. This factor also impacted the size of fetuses, number of piglets that were born and the number of piglets born alive in each litter (Tummaruk et al., 2007). It is also interesting to note that primiparous females that possessed a higher backfat thickness and growth rate were able to be bred at a faster rate than gilts that had a lower backfat thickness (Tummaruk et al., 2001). Assuming boars follow a similar pattern as gilts, these studies indicate that lower birth weight boars will reach puberty at a slower rate than medium to high birth weight boars.

As previously stated, litter bearing species will have offspring of different sizes due to the lack of space in the uterus and competition for nutrients from the dam. As a result of this competition, certain fetuses will experience a particular degree of intrauterine growth retardation (IUGR), which results in some piglets being born at a lower birth weight than their littermates (Foxcroft et al., 2007; Flowers, 2015). However, there is an inverse relationship with the litter size and the weight of individual fetuses, resulting in the conclusion that sows that give birth to larger litters are more likely to have “runts” than a sow that gave birth to a smaller litter (Town et

al., 2005). This can impact survivability and viability during the first weeks of life due to competition with littermates for nutrients by nursing, difficulty claiming a teat if the litter exceeds the sow's functional teat number, and an increased probability of becoming mashed.

Smit et al. (2013) concluded a similar impact of litter size on average birth weights, where it was shown that a higher number of piglets born per litter resulted in lower birth weights. In contrast, Klein et al. (2005) observed the rate of growth from birth to puberty and noted that male piglets born in larger litters during the spring had no significant difference in body weight from piglets born in smaller litters. Interestingly, this was the opposite for litters born in the fall as piglets born in larger litters during this season had significant differences in body weight from piglets born in smaller litters, where they weighed more and upheld larger testicle dimensions (Klein et al., 2005). At maturity, ejaculate samples from boars in both sizes showed no significant increase or decrease in semen quality with the exception of an increased amount of seminal plasma proteins for boars reared in larger litters (Klein et al., 2005).

Whereas being born at a lower birth weight can negatively impact piglet survivability in the first days of life, it has been understood that a lower weight postnatal can affect the growth of muscle fibers. In a study performed by Rehfeldt and Kuhn (2006), sows were treated with growth hormones mid-gestation to examine the impact it had on myogenesis in smaller fetuses. In this study, it was found that piglets of lower birth weights grew at a slower rate than their larger littermates as well as had fewer muscle fiber numbers ($P < 0.05$ during development) and at slaughter (Rehfeldt and Kuhn, 2006). Contrasting this finding, larger piglets displayed higher muscle fiber quantities in whole sections of the semitendinosus muscle in relation to small fetuses (Rehfeldt and Kuhn, 2006).

It is important to note that the number of fetus' conceived post-partum impacts the number of muscle fibers as well as semitendinosus muscle mass (Town et al., 2005). Results showing smaller semitendinosus muscle mass in low birth weight piglets were mirrored by Smit et al. (2013). An initial reduction of muscle fibers at birth can be unfavorable to producers because it will take more feed and time to get them to a desirable slaughter weight as opposed to heavier piglets that have a greater muscle fiber number. It was interesting to discover that, where lower birth weight piglets possessed less muscle mass, they were shown to have higher organ weights (Smit et al., 2013; Rehfeldt and Kuhn, 2013).

Effect of human-animal and animal-animal interaction on physiological processes

The impact of human and animal interaction with pigs at various stages of production has been thought to have impacted development for both breeding and market animals. The impact of friendly or unfriendly human interaction could have a positive or negative impact on a pig's stress levels, and in turn, reduce their willingness to consume feed or to breed or collect. Hemsworth and colleagues have dedicated several years of research to examine the implications of multiple forms of interaction and the impact they have on pigs at various ages. All of these studies concentrated on socialization starting at a young age. In order to better determine human-animal interactions, Hemsworth and colleagues developed the human approach test, a test that utilized an arena that was different than the pig's living environment (Hemsworth et al., 1981). Pigs were introduced, either in groups or individually, to the arena and were given 2 minutes to acclimate to the new environment. After this time, a human would enter the arena and stand stationary at one area and another individual would observe and document the pig's reaction to the human based on four criteria. The pigs would be observed for how long it took for them to enter a pre-designed area that was within 0.5 meters from the human; how long they

spent in that space; how many times they interacted with the human (using an interval of 5 seconds per interaction); and the time at which they made initial physical contact (Hemsworth et al., 1981).

In several early experiments, the body position of experimenters and the impact it had on young pigs was examined to determine if human posture impacted the stress response of both male and female pigs. In a series of 3 experiments, Hemsworth et al. (1986b) discovered that pigs were much more willing to approach a human when they remained stationary and displayed hands without gloves. In addition the experimenter's bare hands were held under their armpits before sessions in order to increase their personal odor, so pigs were postulated to have olfactory memory for caretakers (Hemsworth et al., 1986b). In the same year, Gonyou et al. (1986) observed that pigs were less willing to approach experimenters that reached towards them while wearing leather gloves. One interpretation of these responses was that the gloves masked the experimenter's natural odor so the pigs were wary of the approaching action of the human.

Miura et al (1996) mimicked similar body postures as performed in previous experiments, but used a dummy instead of a human. Results from this experiment determined that pigs would approach a figure that was either lying on its face or leaning over in a crouched position much faster than a figure that was standing erect and spent more time in close proximity to a dummy in the positions lower to the ground (Miura et al., 1996). This was similar to the pig's reaction to humans crouching low to the ground while remaining stationary (Hemsworth et al., 1986a). It was also interesting to note that when humans moved throughout the pen, the pigs avoided them if they were approaching much more than if they were walking away. This research indicated that pigs have a flight zone that is 90 to 105 degrees behind them, in which they did not acknowledge a human standing in this area (Miura et al., 1996).

Aside from body position, several studies examined the affects of positive and negative treatments from humans on young pigs and how it impacts growth rates and reproductive performance. In all studies, it was conclusive that pigs treated in positive manners by experimenters, such as gentle, non-aggressive touches, approached humans at a faster rate during the human approach test and stayed closer to the them for longer as opposed to pigs treated in a negative manner, such as with a brief electric shock or slap. Increased adrenal morphology and corticosteroid levels were observed in these types of studies (Hemsworth et al., 1986a; Gonyou et al., 1986; Hemsworth et al., 1981; Hemsworth and Barnett, 1991), which indicated a high stress response in pigs treated unpleasantly. Cortisol concentrations were shown to have a sharp peak immediately after exposure to a human (Hemsworth et al., 1981; Paterson and Pearce, 1992). Growth rate and feed efficiency were evaluated in multiple studies, where studies performed by Gonyou et al. (1986) and Hemsworth et al. (1981) showed an increase in growth for animals treated pleasantly, where as other studies performed by Pearce et al., (1989), and Paterson and Pearce, (1992) showed no impact on growth. A few experiments showed an increase in growth and feed efficiency that was apparently only during the beginning stages of the trial (Gonyou et al., 1986; Hemsworth and Barnett, 1991). This finding implies that the treatments may have been stressful early, but habituation occurred by the end of the trial. Habituation in ease of handling by humans also occurred in trials performed by Hemsworth and Barnett (1991) and Tanida et al. (1995).

Researchers were curious to determine if pigs were able to differentiate a familiar caretaker from a stranger and if any past treatment from any handler would impact the way they reacted to humans in general. Tanida et al. (1995) discovered that piglets that were handled from a young age shown favoritism towards a familiar handler and are more willing to approach a

handler, familiar or not, than a piglet that has not been handled. In fact, both groups of piglets showed avoidance and fear responses when the strange experimenter picked them up. However, the pigs that were handled regularly showed reduced aversion and fear (Tanida et al., 1995). In contrast, Hemsworth et al. (1994) showed that there was no discrimination between familiar and unfamiliar handlers when pigs were unhandled. There was, however, a preference towards the male handler over the female handler when the same 2:3 ratio of positive and negative treatments were performed equally by both handlers (Hemsworth et al., 1994). There is evidence to suggest that pigs do in fact discriminate against humans based on past treatment, especially unpleasant treatment. These pigs will be more difficult to move as a response to fear (Hemsworth et al., 1994).

In boars and breeding females, human-animal socialization has been evaluated to determine whether or not interaction with humans at a young age would affect reproductive performance. Hemsworth and Barnett (1991) tested the effects of estrus intensity when gilts were treated positively or negatively while isolated from other animals and negatively treated in a group with other animals. It was shown that responses to all three treatments were similar; however, gilts that were isolated by themselves and exposed to negative treatments displayed the longest estrus interval and required the highest number of matings compared with the other two treatments (Hemsworth and Barnett, 1991). It is likely that the slightly positive treatment during breeding also allowed gilts to fear humans less. In regards to conception, Hemsworth et al. (1986c) determined that gilts that were treated negatively in either a group or isolated had a reduced pregnancy rate and higher corticosteroid levels, further validating that raised corticosteroid levels negatively impact female reproduction.

A boar that is saved for breeding purposes interacts with humans the most. Hemsworth and colleagues (1986a) examined boars that were socialized with humans in three treatments and observed the amount of times they mounted while in a group setting as well as observed which treatments demonstrated a longer ejaculation when mated with a female. Boars that were reared by a human and handled since birth were shown to a greater interest in mounting behaviors and ejaculated longer than those in other treatments, where there appeared to be no difference in courting behaviors such as nosing or vocalizations (Hemsworth et al., 1986a). It was also shown that boars that were treated in positive manners by humans showed increased sexual behaviors, had larger testicle dimensions at sexual maturity, and completed copulation with a female at an earlier age than a boar treated negatively (Hemsworth et al., 1986c). Dysart et al. (2015) was more specific in explaining that boars that were socialized by humans at a nursery age were easier to train for collections, reacted quicker to a dummy, and had a higher motile sperm count per ejaculate than those that were not socialized with humans.

In addition to human-animal interaction being beneficial for boars in a breeding program, animal-animal interaction is equally as important. Allowing boars to live in groups with other animals preceding puberty has been shown to result in higher sperm counts per collection as opposed to young boars housed individually (Hemsworth et al., 1977; Flowers, 2015; Dysart et al., 2015). Both physical and visual contact with other boars caused them to demonstrate higher courting behaviors and increased the amount of copulations with females, where as physical contact was key for heightened reproductive performance (Hemsworth et al., 1977; Hemsworth et al., 1986c; Levis and Reicks, 2005; Hemsworth and Tilbrook, 2007). Hemsworth and his colleagues (1977) indicated that 70% of reduction in breeding behaviors was due to the lack of animal-animal physical contact between boars. Studies indicated that the age at which boars

were socially restricted from other animals affected their sexual behavior and that the earlier in life a boar was isolated from his peers, then the less libido he has as an adult (Hemsworth and Tilbrook, 2007). Boars that were allowed to witness another boar mating were also quicker to mount and produced a higher number of sperm per ejaculate as a result of increased sexual stimulation (Hemsworth and Galloway, 1979).

Certain trials were interested in discovering if group housing or enrichment items in housing environments during animal-animal interaction experiments would reduce fear in treatments in which humans used negative methods. Pearce et al. (1989) concluded that animals reared and housed in a group setting showed less fear towards humans regardless if they underwent positive or negative treatments by humans. Animals implemented a strategy in which they dealt with unpleasant treatment by seeking comfort from group-mates (Pearce et al., 1989; Hemsworth and Barnett, 1991).

Impact of seasonal changes and housing environment on sperm production

It is well known that temperature can greatly impact swine production performance. When evaluating sperm motility in the wild boar within the four seasons, motility was the lowest during the summer (Kozdrowski and Dubiel, 2004). In fact, this finding was not only apparent in the wild boar, but also in domesticated boars as well as in females. In areas of high ambient temperatures, boars underwent heat stress at temperatures exceeding 31 degrees Celsius (Wettemann et al., 1976).

The longer a boar was exposed to high ambient temperatures resulted in poor spermatozoa qualities such as reduced motility and concentration, increased abnormal sperm morphology, presence of distal cytoplasmic droplets, decreased spermatogenesis, and lower conception rates when bred artificially to a female (Wettemann et al., 1976; Egbunike and Dede,

1980; Hzayama et al., 1992; Huang et al., 2000; Kozdrowski and Dubiel, 2004; Smital, 2009; Zasiadczyk et al., 2015). Exposure to direct sunlight or extreme high temperatures for a few hours a day can cause these effects, but the boar's recovery from them is rapid. As the body temperature increases so does the temperature in the boar's testis, more specifically the cauda epididymis (Stone, 1981). This results in a decrease in spermatogenesis. In addition, elevated humidity can play a role in causing negative effects on sperm morphology (Suriyasomeboon et al., 2005). Higher semen quality and quantity were observed in the end of winter and in the spring when humidities are typically their lowest (Smital, 2009; Zasiadczyk et al., 2015).

During periods of heat exposure, it is apparent that pig body temperature and respiratory rates are increased. Evidence shows, however, that animals are able to adapt to temperatures over a period of time (Wettemann et al., 1976; Cameron and Blackshaw, 1980). Seren et al. (1988) observed cortisol levels increase rapidly when exposed to high ambient temperatures, but return to near normal levels after about 3 days. A wide difference in day and night temperatures as well as high humidity levels can disrupt adaptation, however (Seren et al, 1988). The ability to adapt to extreme temperatures is not uniform for every pig and genetics plays a large role in this trait. Maternal-line purebred boars are the most sensitive to extreme temperatures and do not perform well under these conditions (Sonderman and Luebbe, 2008; Smital, 2009).

Due to wild boars being seasonal breeders, photoperiod is a factor that can impact libido in domesticated boars. When placed in temperature- and light-controlled environments, it was determined that a threshold of 40 lux of photophase light concentration was necessary for pigs to distinguish day from night (Tast et al., 2001). In order for melatonin to be released during scotophase, the photophase light intensity must reach this threshold. Any amount greater than 40 lx was shown to be a surplus of light that had no effect on melatonin levels. It is interesting to

note that photoperiod, specifically shorter day lengths, can stimulate the maturation of spermatogenesis and testosterone production in pubertal boars (Andersson, 2000; Minton et al., 1985).

When analyzing the effect of photoperiod on sperm morphology, Sancho et al. (2004) determined that sperm quality decreased during periods of reduced light exposure. Sperm morphology from boars collected during decreased photoperiods denoted a higher amount of immature sperm cells with distal cytoplasmic droplets as well as a reduced sperm volume and concentration (Sancho et al., 2004). A higher sperm concentration and amount of viable sperm were shown to be significant in boars that were supplied with additional light (Mahone et al., 1979). Under natural light, both wild and domestic boars express a circadian rhythm for melatonin secretion based on the seasons, where scotophase melatonin release was defined when natural light was measured at less than 15 lux within the barn (Tast et al., 2001). It is recommended that breeding animals should be on a regulated lighting sequence in order to maximize productivity.

When analyzing boar success in breeding facilities, it is important to also observe the housing environment in which they are kept. Swing et al. (2012) recorded data on both young and mature boars that were housed individually in pens or crates. Boars that were housed in crates demonstrated a slower reaction time to a collection dummy; collected for a shorter amount of time and had a lower volume and total sperm per ejaculate than boars that were housed in individual pens. Although proven not statistically, it is important to note that boars that were housed in crates showed better motility patterns such as an increased average path velocity and straight line velocity as well as a greater distance travelled in average and straight line paths (Swing et al., 2012). This study indicated that housing environment could affect overall sperm

production and libido levels for boars, suggesting that it is beneficial for boars to be housed in pens over crates.

Although boars are primarily housed in confinement, Esbenshade et al. (1979) examined libido, spermatozoa quality, overall growth, and structural soundness of purebred boars housed on pasture and in confinement. Whereas there was no significant difference for growth or spermatozoa quality, boars raised in confinement demonstrated higher libido levels and better front limb soundness (Esbenshade et al., 1979). In areas with higher ambient temperatures, concrete flooring improved semen quality during the summer while straw bedding improved seminal motility levels (Corcuera et al., 2002). Bedding supplemented during the other seasons, however, had no effect on seminal quality. A change in flooring or bedding additions in a boar's environment could increase his comfort, but may also result in secondary issues such as lameness or increased respiratory distress. Earlier research advocated for straw bedding as its addition resulted in reduced boredom, lower injury rates, and increased weight gain due to increased feed intake (Lyons et al., 1995). Since then, more studies have been performed to evaluate some disadvantages for straw bedding. Respiratory issues and toe erosion was shown more prominent in environments where straw bedding was provided, but fully-slatted concrete floors resulted in more damage to the sole of a pig's foot (Scott et al., 2006).

Genetic selection in boar reproduction

While there has been a heavy concentration on genetic selection for the carcass and meat quality selection associated with terminal sires (Robinson et al., 2005), there has also been expanding research performed to study gene expression in regards to boar reproductive performance and heritability. Traits such as feed efficiency, loin eye area and overall depth, intramuscular marbling, and lean yield have been extensively studied in both male and female

animals. It has been well documented that strategies such as marker assisted selection may result in a loss of selection proficiency for traits of economic importance that were previously selected using Estimated Breeding Values, or EBVs (Robinson et al., 2005). EBVs provide producers with information based on pedigrees. This information can be used in a complementary fashion when selecting sires for females in order to produce offspring that will grow quickly and efficiently during their productive life (Robinson et al., 2005).

When a trait of economic importance is selected for genetic improvement through marker-assisted selection (MAS) technology, too much selection for one trait has been shown to cause a reduction in the intensity of other traits of economic importance (Safranski, 2008). More studies have been conducted on relationship with female traits than male in regards to the connection between reproductive traits and traits related to growth or carcass quality (Safranski, 2008).

It is also important to factor in the breed of a boar when analyzing positive reproductive qualities. Crossbred boars have been proven to produce a higher quality and quantity of semen when compared to purebred boars (Sonderman and Luebbe, 2008). Crossbreds were found to have lower seminal trash rates and respond better during seasonal extremes when compared with purebreds (Sonderman and Luebbe, 2008). The age at which boars produced ejaculates acceptable for processing into commercial insemination doses was younger for crossbred and terminal line purebred boars compared with maternal-line purebreds (Sonderman and Luebbe, 2008; Wolf, 2009). In terms of longevity, Sonderman and Luebbe (2008) showed that the Duroc breed surpassed both maternal line and crossbred boars in productive years; maintained an adequate sperm output; reached puberty at an early age; and had higher trainability. All of these things increased their lifetime productivity.

Genomic selection has been used more recently as a way to select animals very early in their life. This is especially attractive for reproductive traits in boars and sows. Quantitative Trait Loci (QTL) can be identified in select animals using MAS as well as marker-assisted introgression (MAI), where MAI can analyze a span of 20 cM intervals around the QTL in focus (Piyasatian et al., 2006). With the use of genomic advancements, research has been performed to identify several specific genes and locations that play a role in semen quality.

In earlier research performed by Huang et al. (2002), the gene HSP70.2, a heat shock protein, was analyzed for single nucleotide polymorphisms (SNPs) that pertained to sperm quality characteristics. SNPs at sites 44, 232, 250, 345, and 393 were observed and analyzed during warmer and cooler seasons to examine their responses with purebred boars of specific genotypes. During the warmer seasons, SNPs at sites 232 and 250 showed a greater overall sperm number per ejaculate for boars with an AA genotypes and site 345 showed a greater semen volume per collection for boars with TT and TC genotypes where the TT genotype possessed greater averages of normal sperm morphology (Huang et al., 2002). During the cooler seasons, sites 232 had greater sperm motility for boars with an AA genotype while the AC genotype had a greater total sperm number per ejaculation at this site (Huang et al., 2002).

Other QTLs have been discovered on swine genomes that are related to male reproductive traits. Xing et al. (2009) discovered four QTLs on chromosomes, where SSC2 and SSC12 possessed QTLs for semen pH, SSC15 at 16 cM for semen volume per ejaculate, and SSC17 at 86 cM for total ejaculation times. Three locations were also noted to have possible relations to sperm quality, where SSC4 and SSC9 were suggested to impact sperm abnormality and SSC1 was suggested to impact sperm motility (Xing et al., 2009).

Other proteins are involved with heat stress in other mammalian species such as humans, mice, and goats. Heat shock protein-70 (HSP70), for example, in Boer goats affects the quality of both fresh and frozen semen in goats living in warmer regions (Nikbin et al., 2014). Polymerase-chain reaction (PCR) analysis recognized mutations on the HSP70 gene at locations 74A>C and 191C>G, where the A allele on location 74 displayed a significant negative effect for sperm concentration in fresh semen and progressive motility and for normal motility and lateral head displacement once thawed for frozen semen (Nikbin et al., 2014). In regards of recovery from heat shock, HSP105 has been thought to play a part in refolding denatured proteins as a defense against cell death in the mouse (Yamagishi et al., 2011). Identification of this gene, or a similar gene in boars, would be beneficial to localize for the possibility of having a sparing effect on sperm during hot seasons.

Protein ESR1 has been observed to potentially play a role in sperm motility, sperm morphology, and internal organ function by impacting epithelial cells within the cauda and caput regions of the epididymis; smooth muscle in the cauda region of the epididymis; and the cytoplasm of Sertoli cells in the testis (Gunawan et al., 2011). In Large White-based populations, six SNPs were identified on chromosome 1 to be significant in relation to sperm motility while the MTFMT gene was identified as a candidate gene (Diniz, 2014). Another candidate gene that was initially recognized as being essential for sperm-egg synthesis is CD9 (Kaewmala et al., 2011). In a western blot test, this protein was marked in Leydig, epithelial, and Sertoli cells while also appearing within spermatozoa in the epididymis (Kaewmala et al., 2011). The presence of this gene within the male's reproductive system indicates a likely relationship with sperm quality and overall fertility.

In regards to seminal plasma proteins, PSP, AQN, and AWN were found covering the exterior of the sperm during the ejaculation process for protective purposes (Dyck et al., 2011). This coating assists the sperm during fertilization by preserving plasma solidity, facilitating interaction with the female oviduct, and aiding in sperm survivability and endurance by preventing early capacitation and acrosome reaction (Caballero et al., 2008; Dyck et al., 2011). Non-heparin-binding factors, such as PSP-I and PSP-II, are beneficial in the viability of both and thawed semen samples (Caballero et al., 2008). However, boars with high concentrations of these in their seminal plasma have reduced fertility both in conventional (Novak et al., 2010) and competitive fertility tests (Flowers et al., 2016). In contrast, osteoponin and glutathione peroxidase are two seminal plasma proteins that have been consistently shown to be associated with fertility differences among boars that have excellent sperm motility and morphology characteristics (Flowers et al., 2013; Flowers et al., 2016). The presence of ghrelin and the gonadotropin hormone receptor GHS-R1a in the human, rat, and pig testis suggests a role in reproductive function. Fang et al. (2012) observed the direct impact ghrelin has on testosterone production and secretion, while also noting its relationship with the GHS-R1a gene being expressed in porcine testes within boars. This observation lead researchers to conclude that ghrelin and GHS-R1a both play a role in testicular function in mammalian species (Fang et al., 2012).

The protein DAZL has been well studied amongst many researchers since the mid-2000s in both male and female mammals. It is understood to be a good candidate gene for enhancing reproductive traits such as litter size and overall sperm quality (Zhang et al., 2009). Ma et al. (2013) identified DAZL gene in purebred boars and observed its action on spermatogenesis with previous knowledge of two SNPs that were associated with litter size in females. The

researchers concluded that at locus DAZL c.570+385 A>G, GA genotype boars show a significantly higher sperm motility rate than GG genotype boars within the Landrace and Large White breeds (Ma et al., 2013). As locus DAZL c.735+150 C>A, CC genotype Duroc boars had a higher sperm motility rate as well as had a lower abnormal sperm rate than both CA and AA genotyped boars, while AA boars was superior to CA boars in regards to sperm concentration per ejaculate (Ma et al., 2013). DAZL expression has also been examined in the female during oogenesis under in vitro procedures in the pig, human, and mouse (Liu et al., 2009). Liu et al. (2009) concluded that GDNF, EGF, and FSH stimulated DAZL production, which were secreted by antral follicles during in vitro procedures, where DAZL increased in expression throughout oocyte maturation (Liu et al., 2009).

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INTRODUCTION

Artificial insemination usage has been beneficial in the swine industry by allowing for improved sow welfare, enhanced rate of genetic improvement, decreased risk of introducing disease, and economic benefits for producers. As a result there is a renewed interest in the management of boars. There are multiple factors that impact sperm production in boars such as genetic selection at birth and human socialization. Hemsworth and colleagues have analyzed the results of human interaction with pigs and concluded that negative interactions stimulate fear responses that lead to decreased performance (Hemsworth et al., 1981; 1986b; 1987; 1991). More recently, Dysart (2015) demonstrated that positive human interaction with boars during the nursery stage of production resulted in an increase in 10 billion viable sperm per ejaculate over a 1.5 year period and that this effect was present regardless of birth weight. Whether or not this effect of socialization on sperm production is restricted to just the nursery phase when boars typically are between 3 and 9 weeks of age or whether it occurs at other times during sexual maturation is not known. However, if it is, then it would be reasonable to speculate that longer periods of socialization could produce even larger increases in sperm production. Consequently, the objective of this study was to examine the effect of human socialization on the adult reproductive performance of low birth weight boars that were socialized during the nursery phase (4 to 9 weeks of age); finishing phase (10 to 24 weeks of age); or the entire prepubertal period (4 to 24 weeks of age).

MATERIALS AND METHODS

Animals, Facilities, and General Management

The Swine Educational Unit (SEU) at North Carolina State University was the location in which the study was conducted between 7/29/15 and 5/17/17. Boars (n=36) were taken from 17 different litters, where 28 of the 36 boars total entered the study with at least one full sibling. The boars were born between 7/28/15 and 8/3/17 and were selected for the study based on their weight at birth. The smallest boars within their corresponding litters were selected for the study and possessed birth weights ranging from 0.6 kg to 1.5 kg with an overall mean of 1.2 kg. All boars were considered terminal-line genetics in that they were the offspring of Smithfield Premium Genetics (SPG) maternal-line sows bred with semen from SPG terminal-line boars.

Lactation Management

Individual bow-bar crates measuring 1.5 m wide by 2.5 m long were located within 8 separate farrowing rooms at the SEU. The flooring under the farrowing crates consisted of a raised TriBar® expanded metal behind the sows for waste management and a Tenderfoot® plastic-coated wire on each side of the bow-bar crate. Concrete slats were located underneath sows. Temperature and humidity were regulated within the farrowing barns by a side-wall baffle ventilation system equipped with an evaporative cooling cell that served as the air inlet during the summer months. Additional heating was supplemented to piglets through two heat lamps that were suspended over each of the piglet areas in each crate. When sows reached approximately 107 days of gestation, they were moved from gestation stalls into the farrowing crates.

During their lactation, sows were fed a corn and soybean mixed ration twice per day ad libitum. This ration met or exceeded NRC recommendations for lactating sows (NRC, 2012).

After parturition, piglets from each litter were processed within 24 hours. Processing included tail docking, ear notching, weighing, and application of oral spectam, Pen-G and iron injections. A total of 49 boars were pre-selected during this time. At approximately 7 days of age, boars not pre-selected for this study were castrated. Boars within each litter were managed similarly to their littermates during lactation prior to being weaned at approximately 21 days of age. At this stage, all piglets were weighed and transported to the SEU nursery, where they were divided into pens by weight.

Nursery Management

Pens were located in the nursery rooms at the SEU, where 9 boars were housed per pen. The pens within each nursery rooms were organized as follows. Twelve raised pens total, or six pens on each side divided by a walkway in the center, occupied each room and measured 1.82 m by 1.82 m. Each pen had 4 nipple waterers and 0.91 m of feeder space along the front adjacent to the alley way. At the beginning of the nursery stage, all boars were fed a standard 23% protein starter diet ad libitum for 7-10 days. This starter feed consisted of primarily milk by-products to aid in their transition from liquid solid. Boars were then transitioned to a corn and soybean meal diet, which decreased in protein percentage at a gradual rate. Per NRC recommendation for developing pigs between 7 and 35 kg, protein percentages were not reduced below 18% (NRC, 2012). Similar to farrowing, temperature and humidity were regulated within the nursery barns by a side-wall baffle ventilation system. If supplemental heat was necessary, a propane heater was available in the front part of the room. Two boars died during this phase of production for reasons that were not related to the study. Groups were assigned to socialization treatments during the nursery stage which is described subsequently (see Socialization Treatment Allocations).

Finishing Management

Pens within the finishing facilities were 1.84 m wide by 2.84 m long; had one, two-hole feeder; and 2 nipple waterers. Thirty-two of the 34 boars were moved from the nursery to finishing barns at approximately 72 days of age where two of the boars were removed from the study due to structural issues with their feet and legs. Four boars were grouped together in each pen. Boars were fed a corn and soybean ration that was formulated to meet or exceed NRC recommendations for developing boars (NRC, 2012). The temperature and humidity were controlled by a curtain-sided, under-slat ventilation system in which the curtains and exhaust fans were controlled separately by independent thermostats. If ambient temperatures within the barn exceeded 25.5°C, supplemental cooling fans and misters were programmed to turn on.

Management of Adult Boars

Boars were moved to the gestation barn between 157 and 171 days of age where they were housed in individual crates measuring 2.43 m in length and 1.07 m in width. Boars were relocated to the gestation barn one pen at a time due to space availability. All boars in the study were housed in crates adjacent to one another in the northeast corner of the barn. Boars were arranged based on their associated socialization treatment. When space permitted, larger bodied boars were moved across the walkway into adjacent, single occupancy pens. Boars were able to maintain visual contact with one another through bars surrounding their pen or crate. All boars were fed 3 kg of a 14% protein corn and soybean meal diet each morning. This was formulated to meet NRC requirements for mature boars (NRC, 2012). The temperature and humidity were controlled within the gestation barns by a curtain-sided, under-slat ventilation system. If ambient temperatures within the barn exceeded 23.3°C and 25.5°C, supplemental cooling fans and misters were programmed to turn on, respectively.

Socialization Treatments

At weaning, 36 boars were selected for the study; moved into the nursery; and allocated to pens based on weight. There were two pens of 9 boars each in two different nursery rooms. One room was chosen randomly (coin toss) to receive socialization while the other served as the unsocialized controls. Boars that were not being socialized experienced no additional human interaction aside from the minimal contact time associated with routine management practices such as feeding, repairing equipment, and health monitoring. Employees of the SEU were the only humans to enter the non-socialized barn in order to perform daily checks and maintenance, which resulted in approximately 5 to 10 minutes of human interaction per day.

In addition contact with SEU employees performing their routine tasks, socialized boars were exposed to 1 hour of human socialization every other day during weekdays (Monday, Wednesday, Friday) and was performed every other time by either a female or male. Socialization began about 1 week after weaning or when the boars were 4 weeks of age. The two pens in the socialization barn were positioned across the center walkway from each other to allow both groups the ability to interact with the experimenter at the same time. The experimenter divided a one-hour time frame into 30-minute intervals in which they would face/contact one pen for the first 30 minutes and then turn to face/contact the other pen for the last 30 minutes. During both 30-minute intervals, the experimenter would score each individual boar using the following scale that was adopted 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

Boars were introduced to one of the two socializers at a gradual rate. During the first week, either the socializer would enter the room and simply stand in the walkway between pens and attempt to make eye contact with each individual boar, but would not make any type of forward advancements or physical contact with the boars. During the first week of socialization, the highest score a boar could receive would be a 1.

The second week of socialization consisted of the providing the boars the opportunity to make physical contact during socialization. The socializer extended their hands into the front of each pen and allowed the boars to approach and make contact. During this time, boars were encouraged to nudge, nibble, or smell the socializer's hand and the socializer attempted to scratch or pet boars that approached their hands. The highest score a boar received during these two weeks was 4.

The remaining two weeks of socialization consisted of the socializer entering each pen slowly; carefully positioning his- or herself in the middle of the pen; and allowing boars to interact with their boots. The socializer remained in an erect posture throughout the socialization in each pen with the exception of bending over to attempt to make physical contact with their hands. In total, each boar was exposed to 17 hours of socialization over 17 days while in the nursery phase.

At approximately 10 weeks of age, the boars within each room in the nursery, socialized and unsocialized were randomly assigned to one of the two socialization treatments once they were moved to the finishing barns. This resulted in four treatments with 8 boars per treatment as follows: no socialization; socialization during the nursery (4 to 9 weeks of age); socialization during finishing (10 to 24 weeks of age); and socialization during both nursery and finishing (4

to 24 weeks of age). Four boars within the same treatment were assigned to each pen at this phase due to space requirements of a growing boar. All boars were housed in one barn. However, boars that were assigned to no socialization in the finishing phase were housed in pens in the very back of the barn away from the entrance. Boars that were assigned to be socialized were housed in the front of the barn next to the entrance. The distance between both sets of pens was 120 feet. During their time in the finishing phase, SEU employees entered the barn once every 10 days in order to perform feeding and facility maintenance. This resulted in approximately 5 minutes or less of additional human interaction per visit.

Socialization in the finishing phase were similar to those performed in the nursery. Four pens of socialized boars were located in a square with two pens being adjacent to one another and the two other pens, which were also adjacent to one another, were located on the other side of the walkway. This allowed socializers to interact with two pens at the same time by standing in the walkway at the corners of neighboring pens. Similar to the nursery phase, socializers spent 30 minutes of their 1-hour time intervals facing two pens with their back towards the other two pens and then turned around to repeat the procedure with the other two pens.

Socialization assessments occurred three times per week, alternating between the male and female. During the first week, socializers attempted to make eye contact with each boar, but did not make any efforts at physical contact. Attempts at physical contact with boars by extending hands into the pens began during the second week and continued throughout the remainder of the finishing phase. Socializers did not enter into the pens during the finishing phase. Instead, they stood on the lower bars of the gates exposing their boots to the boars for contact purposes. The boars remained in the finishing phase for a total of fourteen weeks resulting in 42 hours of socialization over 42 days. Both male and female socializers were

dressed in similar coveralls and rubber boots, and also reacted with similar mannerisms during socialization sessions.

Hemsworth Testing

Boars were subjected to a Hemsworth test (adapted from Hemsworth et al., 1994) at approximately 90 and 120 days of age over a two-week time frame. During the first test when boars were approximately 90 days of age, either the familiar male or female socializer performed the test. A novel male or female whom had no previous contact with the boars performed the second test at 120 days of age.

A pen within the finishing barn that measured 1.84 m by 2.84 m was used for the test. Before boars were allowed to enter the pen, a 0.5 m by 1.0 m rectangle was drawn with a marking crayon on the ground against one side of the pen indicating the “area of interest”. This is where the human test subject stood stationary while each individual boar performed the test. To start each Hemsworth test, boars entered the pen individually and were allowed one minute to acclimate to their surroundings. After one minute, the human entered the pen and stood within the area of interest. Each boar was then monitored for activity in relation to the area of interest and the human for 10 minutes. The amount of time the boar spent in the area of interest or interacting with the human was recorded. From these, six dependent variables were obtained: number of times in the area of interest; average duration of time spent in area of interest; longest single time event in area of interest; number of times in contact with human; average duration of contact with human; and longest individual contact time with human. In order for a boar to be recorded as entering the area of interest, a minimum of one foot was required to be within the rectangle. Boars that made contact with the human were allowed to continue until he began to

chew forcefully on the human's overalls or boots. At this point, the boar was gently pushed away.

Semen Collection Training

Boars began being trained for semen collection on 2/19/2016 when they are about 160 days of age. The last boar to effectively train was collected to begin semen analysis on 5/11/16. Training was performed in a 2.43 m by 3.65 m pen with horizontal bars on each side that allowed other boars to observe training and collection. During training, the dummy sow (Minitube of America, Verona, WI) was adjusted to a height of 0.67 m and measured 0.30 m in width by 1.21 m in length. Boars had the ability to move beside and behind the dummy sow but were not able to move in front. In order to be considered effectively trained, boars were required to mount the dummy sow and collect for three consecutive days. The individual that trained all the boars to collect was not involved in their earlier socialization. Training was considered to be the first exposure to socialization to humans for the boars that received no socialization during the nursery and finishing phases. Once successfully trained, boars were collected on a weekly basis.

Weekly Collections

Boars within the four treatments were randomly assigned for collection on either Tuesday or Thursday starting from 2/9/2016 until 12/27/2016. From 1/02/2017 until the end of the study, the collection days were shifted to Monday and Wednesday of each week. Semen collections were performed between 0600 and 1200, with the majority of collections being completed between 0630 and 0900. The same individual who trained the boars for collection served as the weekly collection technician throughout the study. Boars were collected in a random order on their assigned collection days.

Reaction time, mounting time, collection duration, and time of collection were recorded for each boar. Reaction time was the time it took for the boar make physical contact with the dummy once he has all four of his feet in the collection pen. If the boar made contact with the dummy while any of his feet were still in the walkway then a time of 2 seconds was recorded. Mounting time was the time from when then boar made contact with the dummy sow to when he attempted to mount the dummy sow. In order to be considered a mount, the boars two front feet had to be completely off the floor of the collection pen and his chest had to be resting on top of the dummy sow. The collection duration was time from when the boar fully extended his penis and began to ejaculate to when ejaculation was completed and the boar dismounted from the dummy sow. The time of collection was the time of day in which the collection began.

Ejaculates were collected using the gloved-hand technique (Almond et al., 1998) with powder-free polyvinyl gloves (IMV America, Eden Prairie, MN). A plastic collection thermos that was pre-warmed to 37°C was used to minimize premature sperm death. A plastic collection bag (Minitube of America, Verona, WI) was placed in the thermos and a milk filter (IMV International, Eden Prairie, MN) was placed on top of the thermos to filter out the gel fraction as well as other contaminants. The filter was fastened to the thermos with a standard rubber band. Once ejaculates were obtained they were taken into the A.I. laboratory at the SEU. The milk filter and rubber band was removed and discarded before measurements were conducted.

In the A.I. laboratory, the ejaculate volume, concentration, and temperature were measured. The ejaculate volume was determined by subtracting the weight of the empty thermos and plastic collection bag from the total weight of the thermos, plastic collection bag, and ejaculate. The assumption made was that 1 mL was equal to 1 gram of semen. Temperature of the ejaculate was recorded using a standard mercury thermometer. The thermometer was

allowed to rest in the sample for approximately 2 minutes. Concentration was measured by taking a small sample from the ejaculate and processing it through a SpermaCue® (Minitube of America, Verona, WI). Sperm concentration measurements were recorded as $\times 10^6$ cells per mL.

Samples were labeled with the boar's ID and plastic collection bags were secured with a rubber band once the measurements were obtained. Samples were placed in a 37°C incubator and remained there until they were transported to the laboratory on campus for additional analysis and further processing. An insulated bag was used to protect ejaculates during transport in order to prevent temperature shock. One individual would take whatever collections had been obtained back to North Carolina State University after the first 90 minutes of the daily collection periods. This typically was between 6 and 9 samples depending on how quickly boars mounted on any given day. The distance from the SEU to the laboratory on campus was approximately 7 miles and it took approximately 15 minutes to transport the samples. Upon arriving to the campus laboratory, temperatures of each collection was once again taken from each individual sample and recorded as the transport temperature. All collection bags were then placed in a 37°C dry air incubator (Ambi Hi/Lo; Lab Line Instruments, Melrose Park, IL) to await further analysis. The remaining collection samples (typically 2-4) were transported to the university in a similar fashion and were processed the same way. The average time period between the first and last collection was approximately 110 minutes throughout the entire experiment. Finally, just prior to the first collection and immediately after the last collection each day, temperature and relative humidity were recorded in the barn where the boars were housed and collected.

Semen Analyses

Motility

Once the transport temperatures were obtained from the samples, ejaculates were analyzed with a computer assisted sperm analysis (CASA) system. One chamber on a Leja slide (Minitube of America, Verona, WI) was loaded with 12.4 μL from the neat ejaculate. The slide was pre-warmed to approximately 36°C and then placed on a heated microscope stage maintained at the same temperature. A phase contract microscope (BMX-41, Olympus, Arlington, VA) attached to a digital video camera (Minitube of America, Verona, WI) was used for all motility analyses. Images from the microscope were transmitted to a computer equipped with SpermVision® software (Minitube of America, Verona, WI). For each ejaculate, data were recorded from five different microscopic fields selected at random by the laboratory technician. Fourteen sperm motility variables were recorded from each field. These included the following: 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 ($\mu\text{m/s}$); average path velocity ($\mu\text{m/s}$); straight line velocity ($\mu\text{m/s}$); 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. Software settings used were those recommended by the manufacturer for analysis of boar semen and were as follows: frames per second=60 Hz; number of frames=45; minimum cell size=7 pixels; cell size=9 pixels; cell intensity=125; minimum VAP=20 $\mu\text{m/s}$; and minimum VSL=5 $\mu\text{m/s}$.

Throughout the 68-week study, the semen dilution protocol was altered and adjusted based on sperm concentration of the ejaculate. Samples collected during the beginning of the study were not diluted due to the low concentrations often observed in young boars. As the boars matured, sperm concentration increased and samples were diluted in order to improve the repeatability and accuracy of CASA measurements. From weeks 18 to 48, samples were diluted 1:1 if their concentration was over 400×10^6 cells/mL. From weeks 49 to 68 of collection, samples were diluted 1:1 if their concentration was below 250×10^6 cells/mL; 1:2 if their concentration was between 251×10^6 cells/mL and 399×10^6 cells/mL; 1:3 if their concentration was between 400×10^6 cells/mL and 699×10^6 cells/mL; and 1:4 if their concentration was above 600×10^6 cells/mL. A BTS extender (Minitube of America, Verona, WI) was used for diluting the samples.

Morphology

Samples from each ejaculate were placed in phosphate buffered saline with 10% formalin for morphological analyses. The ratio of semen to buffer changed throughout the study but was mainly based on the concentration of each ejaculate. Between weeks 1 and 48, the total volume of the diluted sample was 1 mL. However, dilution rates were either 1:1 or 1:3. This was accomplished in the following manner: ejaculates with concentrations less than 400×10^6 cells/mL diluted 1:1 by adding 500 μ L semen to 500 μ L buffer and ejaculates with concentrations greater than or equal to 400×10^6 cells/mL were diluted 1:3 by adding 250 μ L semen to 750 μ L buffer.

As boars matured and sperm concentrations increased, it became necessary to adjust the dilution rates in order to facilitate morphological evaluations. After week 48, samples from ejaculates were diluted according to the following strategy. Ejaculates with concentrations less

than 250×10^6 cells/mL were diluted 1:1 by adding 1000 μ L semen to 1000 μ L buffer for a total volume of 2 mL. Ejaculates with concentrations between 251×10^6 cells/mL and 399×10^6 cells/mL were diluted 1:2 by adding 1000 μ L semen to 2000 μ L of buffer for a total volume of 3 mL. Ejaculates with concentrations between 400×10^6 cells/mL and 599×10^6 cells/mL were diluted 1:3 by adding 1000 μ L semen to 3000 μ L buffer. Lastly, ejaculates with concentrations greater than or equal to 600×10^6 cells/mL were diluted 1:4 by adding 1000 μ L semen to 4000 μ L buffer. Dilution rates would not be expected to affect morphological characteristics of sperm since the buffer contained 10% formalin which preserves sperm in their original state at the moment the neat semen was diluted.

Morphological analyses were performed on each diluted sample by taking approximately 50 μ L; placing it on a glass microscope slide (Fisher Scientific, Atlanta, GA); covering it with an 18 mm x 18 mm glass coverslip (Fisher Scientific, Atlanta, GA) and evaluating it with a phase contrast microscope (Zeiss, Berlin, West Germany).

Morphological analyses were separated into two general categories: head/acrosome morphology and tail morphology. Tail morphology was analyzed first and was examined under the 40x objective. One hundred cells selected randomly from multiple areas of the slide were evaluated and placed into the following categories: normal tail, abnormal tail (detached tails, no tails, coils, sharp bends), proximal cytoplasmic droplets and distal cytoplasmic droplets according to the criteria described by Popwell and Flowers (2004). Translocated cytoplasmic droplets were recorded from weeks 1 to 33. After the morphological evaluation for normal tails was completed head and acrosome morphology assessments were conducted on the same sample with the 100x oil immersion objective. An additional 200 cells selected at random from multiple locations on the slide were evaluated according to the criteria of Pursel et al. (1972) and included

the proportion of sperm with normal or abnormal heads (large or small in shape or distorted shape) and the proportion of sperm with normal or abnormal acrosomes (absent, granular in appearance, or irregular shape). Two separate lab technicians performed analysis of sperm morphology during the study. Technician A analyzed all samples from weeks 1 to 33 and technician B analyzed all samples from week 34 to the conclusion of the study.

Sperm Depletion Assay

During weeks 33, 54 and 64 all boars were subjected to a sperm depletion assay in order to estimate daily sperm production. During each of these weeks, each boar was collected twice per day until his total number of sperm per ejaculate remained similar for at least 3 consecutive collections. This occurred, on average, after 5.3 ± 0.7 collections. After total number of sperm had reached its nadir each boar was given a rest period between 7 and 10 days and then collected again. The total number of sperm in this ejaculate was divided by the number of days in the rest period in order to calculate daily sperm production. The physiological rationale behind this assay is that the initial intensive collection period serves to deplete sperm stored in the cauda epididymi which is thought to occur when sperm numbers remain low over several consecutive ejaculates. During the rest period the cauda epididymi are populated with sperm produced de novo so dividing the total number of sperm in the ejaculate after the rest period by the number of days in the rest period provides an estimate of the daily sperm production rate.

Boar Fertility

The relative fertility of boars in each socialization treatment was determined with the use of heterospermic inseminations and subsequent paternity testing of the resulting litters according to the procedures described by Flowers et al. (2016) using ejaculates from weeks 59, 63, and 67. During each of these weeks heterospermic insemination doses were prepared by pooling 1 billion

viable sperm from one boar in each socialization treatment selected at random provided no full siblings were involved. This resulted in insemination doses with 4 billion viable cells in 60 mL of pooled extended semen. These insemination doses were used to breed at least 8 sows. This resulted in the production of 15 unique hetero-permic doses consisting of different boars from each treatment; 120 sows that were bred with these doses; and 1325 piglets that were born whose paternity was evaluated. For paternity testing, DNA from the boars was obtained from their white blood cells obtained from routine blood sampling during week 59, while DNA from skin and hair samples of sows several days before farrowing. Piglet DNA was obtained from the portions of their tails that were removed when they were processed 24 h after birth. Boar, sow and piglet DNA was extracted from their respective tissues a Purgene® Purification kit (Gentra Systems, Minneapolis, MN). All samples were sent to NeoGen Corporation (GeneSeek, Lincoln, NE) for determination of paternity results.

Statistical Analyses

Average and total socialization scores (sum of all scores for an individual boar) were calculated for the period of time that boars were in the nursery, finishing and over the entire experiment and were analyzed using analysis of variance procedures for categorical data (Koch et al. 1977) using procedures for categorical modeling (PROC CATMOD) in SAS® (SAS Inc., Cary, NC). The statistical model included socialization treatment. Preplanned orthogonal contrasts comparing no socialization with socialization were used to determine differences among individual treatments when appropriate.

Six dependent variables were evaluated from data collected during the Hemsworth tests. These were as follows: number of times boar entered test area; average amount of time boar spent in the test area; longest period of time boar spent in the test area; number of times boar

made contact with human; average amount of time boar made contact with human; and longest period of time boar made contact with human. These variables were analyzed with analysis of variance of procedures for a factorial arrangement of treatments with partial confounding (Snedecor and Cochran, 1998) using the general linear models procedure (PROC GLM) of SAS® (SAS Inc., Cary, NC). The statistical model included socialization treatment, time, test subject and appropriate interactions. The combination of test subject and time were combined into a single independent variable (partial confounding) in the initial analyses and was not significant ($p \geq 0.28$). Therefore, subsequent analyses were performed with a statistical model that only included socialization treatment. Student-Newman-Kuels multiple range test was used to determine differences among means when a significant effect of treatment was observed (Snedecor and Cochran, 1998).

The proportion of boars trained for collection and the age at which boars were trained successfully were analyzed with analysis of variance procedures for categorical (Koch et al., 1977) and continuous (Snedecor and Cochran, 1998) data using procedures for categorical (PROC CATMOD) and general linear (PROC GLM) models, respectively, using SAS® (SAS Inc., Cary, NC). The statistical model included socialization treatment.

Data obtained weekly either during the collection (reaction time, mounting time, and length of collection) or from the resulting ejaculates (total sperm, volume, concentration, CASA variables, and tail and head morphology) were analyzed with analysis of variance procedures for repeated measures using the general linear models procedure (PROC GLM) of SAS® (SAS Inc., Cary, NC). The statistical model consisted of socialization treatment, week and their interaction. Daily average barn temperature and relative humidity calculated from measurements taken before the first and after the last collections were used as covariates. The error term used to test

the main effect of socialization treatment was boar nested within treatment and was treated as a random variable (Littel et al., 1996). When a significant interaction between socialization and week was present, differences over time within each treatment were determined with analysis of variance procedures for repeated measures with week being treated as the repeated measure (Kaps and Lamberson, 2004). Student-Newman-Kuels multiple range test was used to determine differences among individual means when significant effects of socialization treatment or week were present (Snedecor and Cochran, 1998).

Several of the dependent variables were combined across all the collections to estimate the effect of socialization treatments on sperm production efficiency. These included the proportion of ejaculates that were useable; total sperm produced over the boar's lifetime; and total number of insemination doses produced over the boar's lifetime. An ejaculate was considered to be useable if it met the following criteria which are commonly used by commercial boar studs: ≥ 20 billion total sperm; $\geq 70\%$ motile spermatozoa; and $\geq 70\%$ morphological normal spermatozoa. Total lifetime sperm production was the sum of total sperm per ejaculate across all collections. Lifetime production of insemination doses was determined in the following manner. Only ejaculates deemed to be useable as described previously were used in the calculations. For each useable ejaculate, total number of sperm was multiplied by the proportion of motile sperm and this was divided by 3 billion. These calculations are, again, based on methodologies used by commercial boar studs. Finally, total lifetime doses were then calculated adding the number of doses from each ejaculate. These variables as well as daily sperm production and the proportion of piglets in a litter sired by boars from each treatment (competitive fertility) were analyzed with analysis of variance procedures using general linear models procedures (PROC GLM) using SAS® (SAS Inc., Cary, NC). The statistical model

included the main effect of socialization treatment and Student-Newman-Kuels mean separation test was used when socialization treatment was significant.

Previous studies have shown the importance of birth and weaning weights of boars on their adult sperm production (Dysart, 2015). From a chronological perspective these traits were established prior to boars being assigned to socialization treatments and within each treatment there was variation among boars in each of these. There was also variation in how boars among and within treatments responded to socialization, i.e. some socialized more than others based on individual socialization score means among boars. In an attempt to determine the relative importance of these factors on various measures of boar reproductive performance multiple regression analyses (PROC REG in SAS®; SAS Inc., Cary, NC) were used. For these analyses, birth and weaning weight; nursery, finishing, and overall socialization scores; and the age at which boars were trained were considered as independent variables and their contribution to the overall variability observed in daily sperm production; total sperm per ejaculate; the proportion of sperm with normal tail morphology; the proportion of piglets sired within each litter (competitive fertility); and number of lifetime insemination doses was determined. Independent variables were allowed to enter the model when their p value was less than 0.50.

RESULTS

Socialization Scores

Average and total socialization scores during the nursery phase, finishing phase and over the entire study are shown in Tables 1, 2 and 3, respectively. As expected, boars assigned to socialization in the nursery and finishing phases had higher ($p \leq 0.05$) average and total scores compared with those assigned to treatments without socialization. Similarly, boars socialized during both the nursery and finishing phases had the highest total socialization scores and were followed, in order, by those socialized during only the finishing; those socialized during only the nursery; and those not socialized at all ($p \leq 0.01$). In contrast, boars socialized only in finishing had the highest average socialization score and were followed, in order, by those socialized during both nursery and finishing; those socialized only in the nursery; and those not socialized ($p \leq 0.01$)

Hemsworth Test

Effects of socialization on Hemsworth test results are shown in Tables 4 and 5. No differences ($p \geq 0.15$) were observed due to socialization for the total amount of time or the longest time period that boars spent in the test area. In contrast, there was a tendency ($p = 0.06$) for boars socialized in finishing only or in nursery and finishing to enter the test area more frequently than the unsocialized boars and those socialized in the nursery. There was no effect of socialization ($p \geq 0.14$) on any of the Hemsworth test variables associated with contact with humans.

Training and Semen Collection Variables

The effect of socialization on training and semen collection variables are shown in Tables 6, 7 and 8. Socialization during the nursery only resulted in best training results (87.5%) and

was similar ($p>0.05$) to socialization in finishing only (75.0%) but superior ($p\leq 0.05$) to no socialization (50.0%) and socialization during both the nursery and finishing (37.5%) which were similar to each other ($p>0.05$). Age at which boars were trained for collection was not affected by socialization ($p=0.93$) whereas there was a tendency ($p=0.06$) for daily sperm production to increase as the amount of socialization increased. The effect of socialization treatment was highly significant ($p<0.0001$) for reaction time, mounting time and collection time (Table 7). Reaction and mounting times were the shortest and longest for boars socialized in the nursery and boars socialized in both nursery and finishing, respectively, with the unsocialized boars and boars socialized in finishing being intermediate ($p\leq 0.05$). In contrast, collection time for boars socialized only during the nursery or finishing were similar ($p>0.05$) and greater than unsocialized boars which, in turn, was greater than collection time in boars socialized in both nursery and finishing ($p\leq 0.05$).

Reaction time remained similar during the entire collection period ($p=0.11$; Figure 1) whereas there was a highly significant effect of week on mounting and collection times ($p<0.0001$; Figures 2 and 3, respectively). In general, mounting time decreased through week 14 after which it increased through week 64 before decreasing again through week 66 ($p\leq 0.05$). In contrast, collection times increased through week 22 after which they decreased ($p\leq 0.05$).

The effect of socialization treatment was highly significant ($p<0.0001$) for semen volume and total cells per collection (Table 8) whereas sperm concentration was not affected by socialization ($p=0.86$). Semen volume and total sperm per ejaculate for nursery only, finishing only, and both nursery and finishing were similar ($p>0.05$) compared with one another and greater ($p\leq 0.05$) than those observed in unsocialized boars. There was a significant effect of week on semen volume ($p<0.0001$; Figure 4), total sperm per ejaculate ($p<0.0001$; Figure 6) and

sperm concentration ($p=0.0063$; Figure 5). Volume increased through week 42 after which it decreased at week 46, increased until week 54, and then began to decrease again through week 66 ($p\leq 0.05$). Sperm concentration varied substantially where high levels were observed at weeks 6, 34, 46, and 62 along with a nadir at week 54 ($p\leq 0.05$). In contrast, total sperm per ejaculate increased through week 34 after which it decreased until week 58 ($p\leq 0.05$) and then began to increase through week again until week 66 after which it began to increase again ($p\leq 0.05$).

Sperm Motility

The effect of socialization treatment on sperm motility factors from are shown in Tables 9 through 13. The effect of socialization treatment did not affect motility ($p=0.3865$); progressive motility ($p=0.7525$); straight line velocity ($p=0.3641$); straight line distance ($p=0.4833$); linearity ($p=0.1231$); straightness ($p=0.4129$); wobble ($p=0.1160$); and average orientation change ($p=0.1243$). In contrast, the proportion of useable ejaculates ($p=0.0052$); curvilinear velocity ($p=0.0198$); curvilinear distance ($p=0.0330$); average path velocity ($p=0.0093$); average path distance ($p=0.0496$); lateral beat frequency ($p=0.0001$) and amplitude of head displacement ($p=0.0111$) were all influenced by the degree of socialization to which boars were exposed. Boars that were socialized in both nursery and finishing had higher ($p\leq 0.05$) curvilinear velocity, curvilinear distance, average path velocity and amplitude of lateral head displacement compared with the other socialization treatments which, in turn, were similar to each other ($p>0.05$). In contrast, lateral beat frequency for boars socialized during the nursery phase only was higher ($p\leq 0.05$) compared with the other socialization treatments which, in turn, were similar to each other ($p>0.05$). Average path distance increased with the amount of socialization. Boars socialized in both the nursery and finishing or only during finishing were similar ($p>0.05$) but both of these treatments were higher ($p\leq 0.05$) compared with boar

socialized only in the nursery and those without any socialization. Finally, the proportion of useable ejaculates, which is a composite of both sperm quantity and quality, was highest in boars socialized in only the nursery; lowest for unsocialized boars; and intermediate for boars socialized in finishing only and their counterparts socialized in both nursery and finishing ($p < 0.05$).

The effect of week was highly significant ($p < 0.0001$) for all motility variables (Figures 7 through 20). In general, motility and progressive motility increased from week 10 to week 66 with a substantial decrease at week 30 ($p \leq 0.05$). Curvilinear distance, average path distance, straight line distance, straight line velocity, lateral beat frequency, amplitude of lateral head displacement, and average change in orientation of head remained relatively consistent ($p > 0.05$) during the first half of the collection period after which each one increased from weeks 34 to 50 ($p \leq 0.05$) and then remained fairly constant thereafter ($p > 0.05$). Linearity, straightness, and wobble also remained consistent ($p > 0.05$) during nearly the entire collection period with the exception of an increase between week 50 and 58 before decreasing again through week 66 ($p \leq 0.05$).

Morphology

The effect of socialization treatment on sperm morphology are shown in Tables 14 and 15. The effect of socialization treatment was significant for normal head morphology ($p = 0.0055$), normal tail morphology ($p < 0.0001$) and the presence of distal cytoplasmic droplets ($p < 0.0001$). There was a tendency for socialization to influence normal acrosome morphology ($p = 0.0559$), the presence of proximal cytoplasmic droplets ($p = 0.0973$) while there was no effect on the presence of detached acrosomes ($p = 0.9656$). Boars socialized in finishing only had the lowest proportion of normal heads ($p \leq 0.05$) while boars socialized only in the nursery had the

highest proportion of normal tails ($p \leq 0.05$) compared with other treatments, respectively. The presence of distal cytoplasmic droplets was lowest in boars socialized only in the nursery or only during finishing and highest in unsocialized boars with boars socialized during both the nursery and finishing being intermediate ($p \leq 0.05$). Boar socialized in both the nursery and finishing tended ($p = 0.06$) to have more normal acrosomes compared with other treatments which were not different from one another. Finally, boars socialized in only the nursery or only during finishing tended ($p = 0.09$) to have the lowest incidence of proximal cytoplasmic droplets compared with boars socialized in both nursery and finishing. Unsocalized boars tended to have the highest proportion of sperm with cytoplasmic droplets ($p = 0.09$). In general, normal head morphology increased through week 30 after which it decreased through week 38 before increasing again through week 54 at which point it steadily decreased through week 66 ($p \leq 0.05$). Normal acrosome morphology remained consistent during the entire collection period after week 6 ($p > 0.05$). In general, normal tail morphology decreased from week 14 through 38 before increasing again with the exception of a sharp decrease at week 50 ($p \leq 0.05$). Proximal cytoplasmic droplets and distal cytoplasmic droplets decreased from week 2 through 14 after which they increased through week 30 before decreasing through week 38 where they both increased until week 66 ($p \leq 0.05$).

Lifetime Sperm Variables

The effect of socialization treatment for lifetime sperm variables are shown in Table 16 and Table 17. Useable sperm and insemination doses over a boar's lifetime were affected by socialization ($p = 0.0147$ and $p = 0.0201$, respectively) whereas there was a tendency ($p = 0.1000$) for lifetime sperm production to increase as the amount of socialization increased. In general,

any type of socialization increased ($p \leq 0.05$) or tended to increase ($p = 0.10$) lifetime sperm production traits compared with no socialization.

Boar Fertility

The effect of socialization treatment was not significant ($p = 0.3912$) for heterospermic fertility (Table 16). Socialization during both nursery and finishing or during the nursery only resulted in best fertility results arithmetically (29.5% and 27.8%, respectively) and were higher than unsocialized boars (21.1%) and boars socialized in finishing only (20.1%).

Multiple Regression Analysis

Results from multiple regression analyses are shown in Tables (18 through 22). Total socialization score in the nursery ($p = 0.029$); weaning weight ($p = 0.109$); birth weight ($p = 0.191$); and age at first collection ($p = 0.316$) explained 45% of the total variation observed for daily sperm production (Table 18) with total socialization score in the nursery being the most important (partial $R^2 = 0.23$). Total socialization score in nursery and finishing ($p = 0.144$); birth weight ($p = 0.192$); age at first collection ($p = 0.243$); total socialization score in the nursery ($p = 0.340$); and weaning weight ($p = 0.446$) explained 44% of the total variation observed for total sperm per ejaculate (Table 19) with total socialization score in nursery and finishing being the most important (partial $R^2 = 0.11$).

Total socialization score in the nursery ($p = 0.115$); weaning weight ($p = 0.351$); and age at first collection ($p = 0.463$) explained 46% of the total variation observed for heterospermic fertility (Table 20) with total socialization score in the nursery being the most important (partial $R^2 = 0.13$). Birth weight ($p = 0.019$); total socialization score in the nursery ($p = 0.057$); age at first collection ($p = 0.399$); and total socialization score in nursery and finishing ($p = 0.441$) explained 44% of the total variation observed for lifetime production of insemination doses (Table 21) with

birth weight being the most important (partial $R^2 = 0.22$). Total socialization score in the nursery ($p=0.038$); birth weight ($p=0.201$); weaning weight ($p=0.336$); and age at first collection ($p=0.378$) explained 37% of the total variation observed for useable ejaculates (Table 22) with total socialization score in the nursery being the most important (partial $R^2 = 0.21$).

DISCUSSION

The main objective of this study was to determine if socialization during adolescence impacted sperm production and semen quality in the adult boar. One possible explanation for the impact on socialization on semen collection variables is hormonal secretion and stimulation. Early in prepuberal development, Leydig cells secrete testosterone and other androgens in order to aid in the stimulation of sexual behaviors and initiate the beginning stages of spermatogenesis (Ramaswamy and Weinbauer, 2014; Bartlett et al., 1989). As expected, socialization during the nursery phase was favorable in reaction, mounting, and collecting times once boars were trained to collect from a dummy sow. The impact of socialization at this stage indicates that nursery age pigs are at a prime age preceding spermatogenesis in which positive human socialization is beneficial for Leydig development. It appears that boars socialized in finishing also have favorable results behind nursery socialized boars, indicating that cell development is still occurring at this phase.

Training success could also be explained by human posture during the training period. Pigs have been observed to respond more positively to humans if they are in a stationary and crouched position (Hemsworth et al., 1986a). In addition, forward advancement towards pigs, especially from the rear, triggers a flight response (Miura et al., 1996). During collections, the collection technician remained in full view of the boars and collected ejaculates in a crouched position. The posture of the collection technician may have aided in the success of training boars, especially those that were completely unsocialized.

An important factor to analyze prior to socialization is the success of collection training. Boars trained in nursery or finishing only achieved better success than boars that were socialized in both. A possible explanation for this is that boars have the potential to become overly

habituated with humans. As human contact is necessary for collection, boars may become overly distracted by technicians and have a greater desire to play rather than collect.

Conversely, boars that were not socialized at all demonstrated the second lowest success in collection training and collection variables indicating that a fear response may override a sexual response to mount a dummy. This was not surprising to observe, as fear is often associated with human exposure (Hemsworth et al., 1981). Habituation for socialized and unsocialized pigs has been observed in growth and feed efficiency levels by Hemsworth and Barnett (1991) and Gonyou (1986). Piglet growth was only impacted at the beginning of both trials indicating that treatments may have been stressful early, but compensation occurred by the end of the trial. Based on these results, it appears that socializing boars in solely nursery or finishing has a positive influence on collection trainability, however, socialization in both stages leads to unfavorable training success.

Habituation can also serve as an explanation for the Hemsworth results. It is no surprise that boars socialized in both nursery and finishing had the greatest average of times entering the square and interacting with the human experimenter due to double the amount of human exposure in both stages. Boars socialized with humans in only nursery and finishing had intermediate results for this test, indicating that even some exposure in either phase led to higher averages.

Total sperm per ejaculate, a variable calculated using sperm concentration and semen volume, is a factor that is highly significant in both socialization treatment and week of collection whereas the percentage of useable ejaculates is only significant for socialization. This indicates that as a boar ages, sperm numbers increase likely due to an increase in volume. These measures are important in evaluating a male's production proficiency. Boars that were

unsocialized proved to have the lowest total cells per ejaculate whereas cell count increased steadily based on treatment group where boars socialized in both groups produced the most. The highest percentage of the total variation for the proportion of useable ejaculates was explained by the sum of all socialization scores in nursery. This indicates that socialization in general has an effect on semen production. Similar findings were observed by Dysart (2015), where an increase of approximately 10 billion cells/ejaculate were found in sperm cells of boars socialized in the nursery stage.

One possible explanation of this reaction is that socialization with a human at any stage in adolescence reduces corticosteroid stress levels. Cortisol concentrations are shown to increase after exposure to humans (Hemsworth et al., 1981; Paterson and Pearce, 1992). This indicates that any affirmative form of human contact positively impacts the development of sperm. As a result, Sertoli cell mitotic activity may be positively impacted, increasing sperm cell production past sexual maturity. Motility and morphology factors were analyzed to determine the movement and anatomy of sperm cells taken from daily ejaculates. However, these factors would not explain the increase in sperm production in finishing socialized boars due to the decrease in proliferation of mitotic activity after 60 days of age. Some motility and morphology factors were significant while others were not. Variations within results from computer-assisted semen analysis tests reveal that individual boars were inconsistent from week to week in these two measures.

Greater averages for boars experiencing any level of socialization during adolescence may also result in lower stress levels during collections as a mature adult. Based on lifetime averages, unsocialized boars resulted in the lowest scores for lifetime sperm production, useable sperm over lifetime, and doses over lifetime. This indicates that socialization before puberty has

a positive impact on lifetime semen production. Likewise, regression results for lifetime production of insemination doses indicate socialization in nursery only and both nursery and finishing impact total variation. These regression results further indicate that socialization, specifically within the nursery stage was important for enhancing sperm production in boars at and past sexual maturity.

Sperm maturity is indicated by the presence of cytoplasmic droplets. In the corpus epididymis, cytoplasmic droplets form on the flagellum of spermatids, representing excess cytoplasm that was retained by the sperm cell (Johnson et al., 2008). Droplets will fall from a proximal to a distal position on the flagellum and will eventually be removed from the cell once fully mature. Unsocialized boars had the highest presence of cytoplasmic droplets indicating that any form of socialization played a role in sperm maturation. Decreased sperm maturation in unsocialized boars was also observed in weekly collections where both volume and total sperm per ejaculate were lower than boars that were socialized.

Evaluating sperm quality is just as important as analyzing sperm quantity, as the purpose of boar's ejaculate is to successfully breed females. Percentages for heterospermic fertility results from each socialization treatment were not significant. However, boars socialized in both nursery and finishing and nursery only displayed higher percentages of competitive fertilization where a decent percentage of the total variation for heterospermic fertility was explained by the sum of all socialization scores in nursery. This indicates that socialization, especially within the nursery, can impact breeding potential.

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TABLES

Table 1: Effect of socialization on average and total socialization scores during the nursery phase¹ (mean \pm s.e.).

Socialization Treatment	Nursery Socialization Score Average	Nursery Socialization Score Total
None ²	0.0 ± 0.0^x	0.0 ± 0.0^x
Nursery ¹	1.9 ± 0.1^y	32.8 ± 2.4^y
Finishing ²	0.0 ± 0.0^x	0.0 ± 0.0^x
Nursery + Finishing	1.7 ± 0.2^y	28.5 ± 3.5^y
P-value ³	< 0.0001	< 0.0001

¹ socialization in the nursery occurred when pigs were 4 to 9 weeks of age

² no socialization during the nursery occurred for pigs in these two treatments

³ main effect of socialization

^{x,y} Means in the same column with different superscripts are different ($p \leq 0.01$)

Table 2: Effect of socialization on average and total socialization scores during the finishing phase¹ (mean ± s.e.).

Socialization Treatment	Finishing Socialization Score Average	Finishing Socialization Score Total
None ²	0.0 ± 0.0 ^x	0.0 ± 0.0 ^x
Nursery ²	0.0 ± 0.0 ^x	0.0 ± 0.0 ^x
Finishing ¹	3.4 ± 0.1 ^y	93.5 ± 1.4 ^y
Nursery + Finishing	3.4 ± 0.1 ^y	92.1 ± 3.3 ^y
P-value ³	< 0.0001	< 0.0001

¹ socialization in finishing occurred when pigs were 10 to 24 weeks of age

² no socialization during finishing occurred for pigs in these two treatments

³ main effect of socialization

^{x,y} Means in the same column with different superscripts are different ($p \leq 0.05$)

Table 3: Effect of socialization on average and total socialization scores for the entire socialization period¹ (mean \pm s.e.).

Socialization Treatment	Overall Socialization Score Average	Overall Socialization Score Total
None ²	0.0 \pm 0.0 ^w	0.0 \pm 0.0 ^w
Nursery	1.9 \pm 0.1 ^x	32.8 \pm 2.4 ^x
Finishing	3.4 \pm 0.1 ^y	93.5 \pm 1.4 ^y
Nursery + Finishing ¹	2.8 \pm 0.1 ^z	120.6 \pm 6.3 ^z
P-value ³	< 0.0001	< 0.0001

¹ socialization period occurred when pigs were 4 to 24 weeks of age

² no socialization occurred for pigs in this treatment

³ main effect of socialization

^{w,x,y,z} Means in the same column with different superscripts are different ($p \leq 0.05$)

Table 4: Effect of socialization on number of times boars entered Hemsworth square (mean \pm s.e.), total time inside Hemsworth square (seconds \pm s.e.), and longest period inside Hemsworth square (seconds \pm s.e.).

Socialization Treatment	Times Entering Hemsworth Square	Total Time in Hemsworth Square (s)	Longest Period in Hemsworth Square (s)
None	13.0 \pm 1.5 ^x	202.5 \pm 32.3	73.8 \pm 17.3
Nursery ¹	13.2 \pm 1.6 ^x	160.3 \pm 15.7	43.5 \pm 5.5
Finishing ²	16.5 \pm 1.8 ^y	285.7 \pm 76.5	75.3 \pm 20.2
Nursery + Finishing ³	18.3 \pm 1.1 ^y	299.5 \pm 47.4	96.5 \pm 21.7
P-value ⁴	0.0608	0.1536	0.2201

¹ socialization occurred when boars were 4 to 9 weeks of age

² socialization occurred when boars were 10 to 24 weeks of age

³ socialization occurred when boars were 4 to 24 weeks of age

⁴ main effect of socialization

^{x,y} Means in the same column with different superscripts tend to be different ($p \leq 0.06$)

Table 5: Effect of socialization on number of times boars contacted human (mean \pm s.e.), total time in contact with human (seconds \pm s.e.), and longest period in contact with human (seconds \pm s.e.) during the Hemsworth test.

Socialization Treatment	Contacts with Human	Total Time in Contact with Human (s)	Longest Period in Contact with Human (s)
None	7.5 \pm 1.0	16.6 \pm 23.2	42.3 \pm 8.9
Nursery ¹	7.0 \pm 1.3	81.3 \pm 15.1	26.8 \pm 4.2
Finishing ²	9.6 \pm 1.7	161.3 \pm 55.5	26.8 \pm 4.2
Nursery + Finishing ³	11.2 \pm 1.4	139.7 \pm 39.9	47.2 \pm 15.4
P-value ⁴	0.1479	0.4734	0.5640

¹ socialization occurred when boars were 4 to 9 weeks of age

² socialization occurred when boars were 10 to 24 weeks of age

³ socialization occurred when boars were 4 to 24 weeks of age

⁴ main effect of socialization

Table 6: Effect of socialization on training success (%), age of first collection (days \pm s.e.), and daily sperm production ($\times 10^9$ cells/day \pm s.e.).

Socialization Treatment	Training success (%)	Age Trained for Collection (days)	Daily Sperm Production ($\times 10^9$ cells/day)
None	50.0 ^{x,y}	210.5 \pm 15.2	20.0 \pm 2.2 ^m
Nursery ¹	87.5 ^z	214.5 \pm 14.4	23.9 \pm 1.1 ^{m,n}
Finishing ²	75.0 ^{y,z}	210.6 \pm 12.8	23.9 \pm 1.1 ^{m,n}
Nursery + Finishing ³	37.5 ^x	199.3 \pm 21.6	26.5 \pm 1.1 ⁿ
P-value ⁴	0.0310	0.9378	0.0652

¹ socialization occurred when boars were 4 to 9 weeks of age

² socialization occurred when boars were 10 to 24 weeks of age

³ socialization occurred when boars were 4 to 24 weeks of age

⁴ main effect of socialization

^{x,y,z} Means in the same column with different superscripts are different ($p \leq 0.05$)

^{m,n} Means in the same column with different superscripts tend to be different ($p < 0.07$)

Table 7: Effect of socialization on reaction, mounting, and collection times (seconds \pm s.e.). Numbers in parentheses denotes the number of observations used to calculate means.

Socialization Treatment	Reaction Time (s)	Mounting Time (s)	Collection Time (s)
None	5.9 \pm 0.8 ^y (224)	64.3 \pm 5.2 ^y (224)	259.4 \pm 4.5 ^x (224)
Nursery ¹	3.6 \pm 0.2 ^x (410)	41.8 \pm 2.7 ^x (410)	335.5 \pm 4.4 ^z (410)
Finishing ²	4.8 \pm 0.4 ^{x,y} (366)	63.4 \pm 3.5 ^y (366)	326.3 \pm 4.8 ^z (366)
Nursery + Finishing ³	10.5 \pm 1.4 ^z (183)	112.8 \pm 9.9 ^z (183)	292.5 \pm 7.0 ^y (183)
P-value ⁴	< 0.0001	< 0.0001	< 0.0001

¹ socialization occurred when boars were 4 to 9 weeks of age

² socialization occurred when boars were 10 to 24 weeks of age

³ socialization occurred when boars were 4 to 24 weeks of age

⁴ main effect of socialization

^{x,y,z} Means in the same column with different superscripts are different ($p \leq 0.05$)

Table 8: Effect of socialization on semen volume (mL \pm s.e.), sperm concentration ($\times 10^6$ cells/mL \pm s.e) and total sperm ($\times 10^9$ cells/mL \pm s.e.) per ejaculate. Numbers in parentheses denotes the number of observations used to calculate means.

Socialization Treatment	Volume (mL)	Concentration ($\times 10^6$ cells/mL)	Total Sperm ($\times 10^9$ cells/mL)
None	172.7 \pm 3.9 ^y (224)	467.9 \pm 9.4 (224)	78.9 \pm 1.9 ^y (224)
Nursery ¹	219.4 \pm 3.7 ^z (410)	445.8 \pm 8.3 (410)	89.5 \pm 1.3 ^z (410)
Finishing ²	220.4 \pm 3.4 ^z (366)	435.06 \pm 7.8 (366)	90.2 \pm 1.4 ^z (366)
Nursery + Finishing ³	213.2 \pm 4.1 ^z (183)	452.50 \pm 7.7 (183)	93.6 \pm 1.7 ^z (183)
P-value ⁴	< 0.0001	0.8653	< 0.0001

¹ socialization occurred when boars were 4 to 9 weeks of age

² socialization occurred when boars were 10 to 24 weeks of age

³ socialization occurred when boars were 4 to 24 weeks of age

⁴ main effect of socialization

^{y,z} Means in the same column with different superscripts are different ($p \leq 0.05$)

Table 9: Effect of socialization on fresh sperm motility (% \pm s.e.), progressive motility (% \pm s.e.), and lifetime useable ejaculates by industry standards (mean \pm s.e.). Numbers in parentheses denotes the number of observations used to calculate means.

Socialization Treatment	Motility (%)	Progressive motility (%)	Useable Ejaculates (%)
None	91.2 \pm 0.7 (221)	75.9 \pm 1.1 (221)	75.0 \pm 0.03 ^x (220)
Nursery ¹	91.7 \pm 0.4 (403)	76.6 \pm 0.7 (403)	94.0 \pm 0.01 ^z (401)
Finishing ²	90.8 \pm 0.6 (360)	75.8 \pm 0.9 (360)	83.0 \pm 0.02 ^y (356)
Nursery + Finishing ³	90.2 \pm 0.9 (180)	75.5 \pm 1.3 (180)	83.0 \pm 0.03 ^y (179)
P-value ⁴	0.3865	0.7595	0.0052

¹ socialization occurred when boars were 4 to 9 weeks of age

² socialization occurred when boars were 10 to 24 weeks of age

³ socialization occurred when boars were 4 to 24 weeks of age

⁴ main effect of socialization

^{x,y,z} Means in the same column with different superscripts are different ($p \leq 0.05$)

Table 10: Effect of socialization on fresh sperm curvilinear distance (DCL), sperm average path distance (DAP), and straight line distance (DSL) ($\mu\text{m} \pm \text{s.e.}$). Numbers in parentheses denotes the number of observations used to calculate means.

Socialization Treatment	Curvilinear Distance (μm)	Average Path Distance (μm)	Straight Line Distance (μm)
None	58.0 ± 1.0^y (221)	34.2 ± 0.5^y (221)	21.0 ± 0.3 (221)
Nursery ¹	57.9 ± 0.6^y (403)	34.3 ± 0.3^y (403)	21.1 ± 0.1 (403)
Finishing ²	58.6 ± 0.8^y (360)	$34.8 \pm 0.4^{y,z}$ (360)	21.1 ± 0.1 (403)
Nursery + Finishing ³	61.1 ± 1.2^z (180)	35.8 ± 0.6^z (180)	21.4 ± 0.3 (180)
P-value ⁴	0.0330	0.0496	0.4833

¹ socialization occurred when boars were 4 to 9 weeks of age

² socialization occurred when boars were 10 to 24 weeks of age

³ socialization occurred when boars were 4 to 24 weeks of age

⁴ main effect of socialization

^{y,z} Means in the same column with different superscripts are different ($p \leq 0.05$)

Table 11: Effect of Socialization Treatment on fresh sperm curvilinear velocity (VCL), sperm average path velocity (VAP), and straight line velocity (VSL) ($\mu\text{m/s} \pm \text{s.e.}$). Numbers in parentheses denotes the number of observations used to calculate means.

Socialization Treatment	Curvilinear Velocity ($\mu\text{m/s}$)	Average Path Velocity ($\mu\text{m/s}$)	Straight Line Velocity ($\mu\text{m/s}$)
None	136.2 ± 2.4^y (221)	81.0 ± 1.2^y (221)	50.6 ± 0.7 (221)
Nursery ¹	136.6 ± 1.5^y (403)	80.9 ± 0.7^y (403)	50.3 ± 0.3 (403)
Finishing ²	138.4 ± 1.9^y (360)	82.2 ± 0.9^y (360)	50.6 ± 0.5 (360)
Nursery + Finishing ³	144.3 ± 2.8^z (180)	85.3 ± 1.4^z (180)	51.5 ± 0.7 (180)
P-value ⁴	0.0198	0.0093	0.3641

¹ socialization occurred when boars were 4 to 9 weeks of age

² socialization occurred when boars were 10 to 24 weeks of age

³ socialization occurred when boars were 4 to 24 weeks of age

⁴ main effect of socialization

^{y,z} Means in the same column with different superscripts are different ($p \leq 0.05$)

Table 12: Effect of socialization on fresh sperm linearity (LIN), straightness (STR), and wobble (WOB) (mean \pm s.e.). Numbers in parentheses denotes the number of observations used to calculate means.

Socialization Treatment	Linearity	Straightness	Wobble
None	0.38 \pm 0.0 (221)	0.62 \pm 0.0 (221)	0.60 \pm 0.0 (221)
Nursery ¹	0.38 \pm 0.0 (403)	0.63 \pm 0.0 (403)	0.60 \pm 0.0 (403)
Finishing ²	0.38 \pm 0.0 (360)	0.62 \pm 0.0 (360)	0.60 \pm 0.0 (360)
Nursery + Finishing ³	0.37 \pm 0.0 (180)	0.61 \pm 0.0 (180)	0.60 \pm 0.0 (180)
P-value ⁴	0.1231	0.4129	0.1160

¹ socialization occurred when boars were 4 to 9 weeks of age

² socialization occurred when boars were 10 to 24 weeks of age

³ socialization occurred when boars were 4 to 24 weeks of age

⁴ main effect of socialization

Table 13: Effect of socialization on fresh sperm beat lateral frequency (BCF) (Hz \pm s.e.), amplitude of head displacement (ALH) (μm \pm s.e.), and average orientation change (AOC) (mean \pm s.e.). Numbers in parentheses denotes the number of observations used to calculate means.

Socialization Treatment	Beat Lateral Frequency (Hz)	Amplitude of Head Displacement (μm)	Average Orientation Change
None	29.8 \pm 0.2 ^y (221)	5.7 \pm 0.1 ^y (221)	17.7 \pm 0.2 (221)
Nursery ¹	30.7 \pm 0.1 ^z (403)	5.6 \pm 0.1 ^y (403)	17.9 \pm 0.1 (403)
Finishing ²	29.7 \pm 0.1 ^y (360)	5.7 \pm 0.1 ^y (360)	17.7 \pm 0.1 (360)
Nursery + Finishing ³	29.5 \pm 0.2 ^y (180)	6.0 \pm 0.1 ^z (180)	18.2 \pm 0.2 (180)
P-value ⁴	0.0001	0.0111	0.1243

¹ socialization occurred when boars were 4 to 9 weeks of age

² socialization occurred when boars were 10 to 24 weeks of age

³ socialization occurred when boars were 4 to 24 weeks of age

⁴ main effect of socialization

^{y,z} Means in the same column with different superscripts are different ($p \leq 0.05$)

Table 14: Effect of socialization on normal head morphology, normal acrosome, and detached acrosome (% \pm s.e.). Numbers in parentheses denotes the number of observations used to calculate means.

Socialization Treatment	Normal Head Morphology (%)	Normal Acrosome (%)	Detached Acrosome (%)
None	96.0 \pm 0.1 ^z (220)	98.6 \pm 0.1 ^m (220)	0.01 \pm 0.01 (220)
Nursery ¹	96.1 \pm 0.1 ^z (401)	98.6 \pm 0.1 ^m (401)	0.04 \pm 0.01 (401)
Finishing ²	95.6 \pm 0.1 ^y (356)	98.3 \pm 0.2 ^m (356)	0.04 \pm 0.01 (356)
Nursery + Finishing ³	96.1 \pm 0.2 ^z (179)	99.0 \pm 0.1 ⁿ (179)	0.03 \pm 0.01 (179)
P-value ⁴	0.0055	0.0559	0.9656

¹ socialization occurred when boars were 4 to 9 weeks of age

² socialization occurred when boars were 10 to 24 weeks of age

³ socialization occurred when boars were 4 to 24 weeks of age

⁴ main effect of socialization

^{y,z} Means in the same column with different superscripts are different ($p \leq 0.05$)

^{m,n} Means in the same column with different superscript tend to be different ($p=0.055$)

Table 15: Effect of socialization on normal tail morphology, proximal cytoplasmic droplets, and distal cytoplasmic droplets (% \pm s.e.). Numbers in parentheses denotes the number of observations used to calculate means.

Socialization Treatment	Normal Tail Morphology (%)	Proximal Cytoplasmic Droplets (%)	Distal Cytoplasmic Droplets (%)
None	84.7 \pm 1.0 ^y (220)	2.0 \pm 0.2 ^m (220)	5.0 \pm 0.6 ^x (220)
Nursery ¹	89.7 \pm 0.4 ^z (401)	1.3 \pm 0.0 ⁿ (401)	2.2 \pm 0.1 ^y (401)
Finishing ²	86.2 \pm 0.7 ^y (356)	1.3 \pm 0.1 ⁿ (356)	2.1 \pm 0.1 ^y (356)
Nursery + Finishing ³	86.0 \pm 0.9 ^y (179)	1.7 \pm 0.2 ^{m,n} (179)	3.4 \pm 0.5 ^z (179)
P-value ⁴	< 0.0001	0.0973	< 0.0001

¹ socialization occurred when boars were 4 to 9 weeks of age

² socialization occurred when boars were 10 to 24 weeks of age

³ socialization occurred when boars were 4 to 24 weeks of age

⁴ main effect of socialization

^{x,y,z} Means in the same column with different superscripts are different ($p \leq 0.05$)

^{m,n} Means in the same column with different superscript tend to be different ($p=0.09$)

Table 16: Effect of socialization on heterospermic fertility ($\% \pm \text{s.e.}$) and lifetime sperm production ($\times 10^9 \text{ cells/day} \pm \text{s.e.}$).

Socialization Treatment	Heterospermic Fertility (%)	Lifetime Sperm Production ($\times 10^9 \text{ cells}$)
None	21.1 \pm 4.5	4423.6 \pm 546.8 ¹
Nursery ¹	27.8 \pm 2.2	5247.2 \pm 150.2 ^m
Finishing ²	20.1 \pm 5.1	5504.9 \pm 330.2 ^{m,n}
Nursery + Finishing ³	29.5 \pm 2.5	5713.7 \pm 244.4 ⁿ
P-value ⁴	0.3912	0.1000

¹ socialization occurred when boars were 4 to 9 weeks of age

² socialization occurred when boars were 10 to 24 weeks of age

³ socialization occurred when boars were 4 to 24 weeks of age

⁴ main effect of socialization

^{1,m,n} Means in the same column with different superscripts tend to be different ($p=0.10$)

Table 17: Effect of socialization on useable sperm over lifetime ($\times 10^9$ cells/day \pm s.e.) and number of insemination doses over lifetime (mean \pm s.e.).

Socialization Treatment	Useable Sperm over Lifetime ($\times 10^9$ cells)	Doses over Lifetime
None	3418.8 \pm 492.5 ^x	1563.0 \pm 223.0 ^x
Nursery ¹	4957.7 \pm 128.1 ^y	2261.0 \pm 62.0 ^y
Finishing ²	4546.7 \pm 332.1 ^y	2080.0 \pm 162.0 ^y
Nursery + Finishing ³	4658.7 \pm 94.1 ^y	2126.0 \pm 52.0 ^y
P-value ⁴	0.0147	0.0201

¹ socialization occurred when boars were 4 to 9 weeks of age

² socialization occurred when boars were 10 to 24 weeks of age

³ socialization occurred when boars were 4 to 24 weeks of age

⁴ main effect of socialization

^{x,y} Means in the same column with different superscripts are different ($p \leq 0.05$)

Table 18: Multiple regression analysis for Daily Sperm Production.

Independent Variable	Partial R ²	Model R ²	P-Value
Total Socialization Score in Nursery	0.2384	0.2384	0.0290
Weaning Weight	0.1094	0.3478	0.1095
Birth Weight	0.0678	0.4156	0.1914
Age at First Collection	0.0408	0.4564	0.3167

Table 19: Multiple regression analysis for Total Sperm per Ejaculate.

Independent Variable	Partial R ²	Model R ²	P-Value
Nursery + Finishing	0.1146	0.1146	0.1444
Birth Weight	0.0850	0.1996	0.1924
Age at First Collection	0.0701	0.2697	0.2432
Nursery	0.0463	0.3160	0.3409
Weaning Weight	0.0287	0.3347	0.4466

Table 20: Multiple regression analysis for Heterospermic Fertility.

Independent Variable	Partial R ²	Model R ²	P-Value
Nursery	0.1321	0.1321	0.1152
Weaning Weight	0.0445	0.1766	0.3512
Age at First Collection	0.0281	0.2047	0.4634

Table 21: Multiple regression analysis for Lifetime Production of Insemination Doses.

Independent Variable	Partial R ²	Model R ²	P-Value
Birth Weight	0.2296	0.2296	0.0193
Nursery	0.1864	0.4160	0.0573
Age at First Collection	0.0261	0.4421	0.3997
Nursery + Finishing	0.0223	0.4645	0.4412

Table 22: Multiple regression analysis for Proportion of Useable Ejaculates.

Independent Variable	Partial R ²	Model R ²	P-Value
Nursery	0.2163	0.2163	0.0388
Birth Weight	0.0747	0.2910	0.2012
Weaning Weight	0.0416	0.3326	0.3362
Age at First Collection	0.0359	0.3685	0.3788

FIGURES

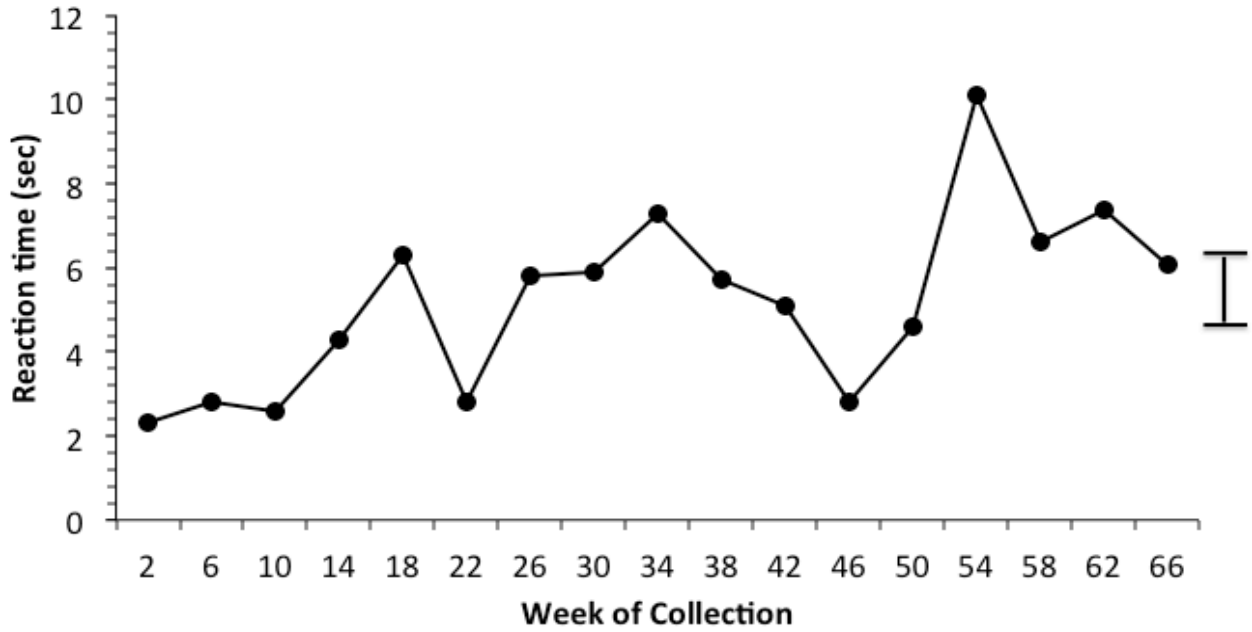


Figure 1: Effect of Week of Collection on reaction time (sec). Pooled standard error of the mean = 1.3 sec. Week 2 represents the week of February 8, 2016 and week 66 represents the week of May 1, 2017.

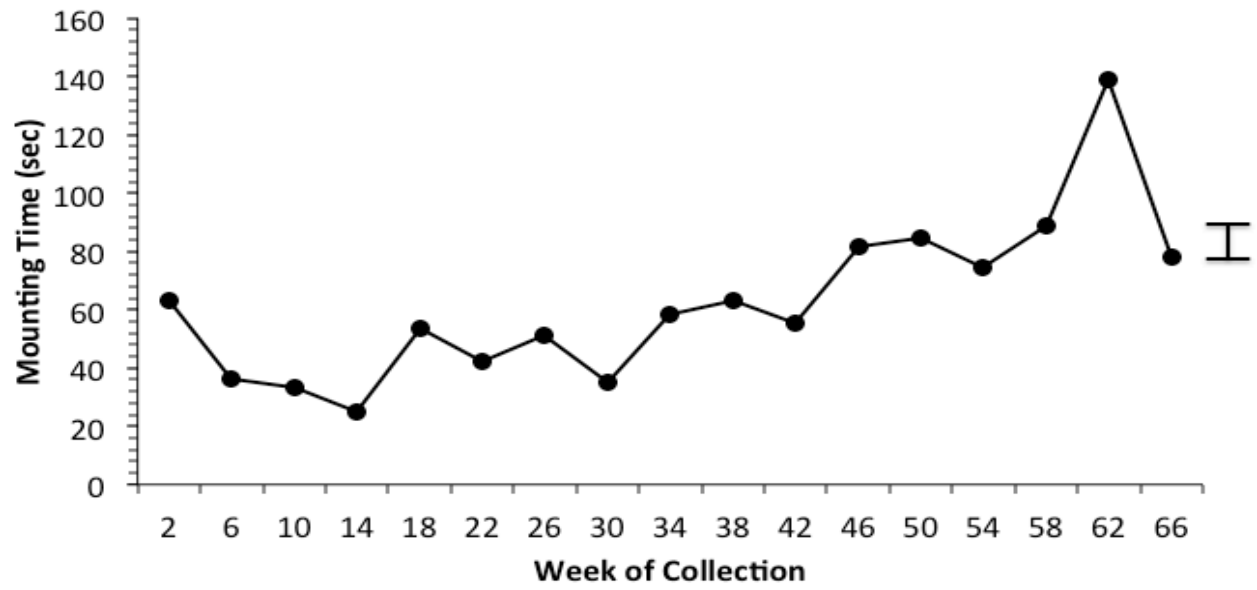


Figure 2: Effect of Week of Collection on mounting time (sec). Pooled standard error of the mean = 9.6 sec. Week 2 represents the week of February 8, 2016 and week 66 represents the week of May 1, 2017.

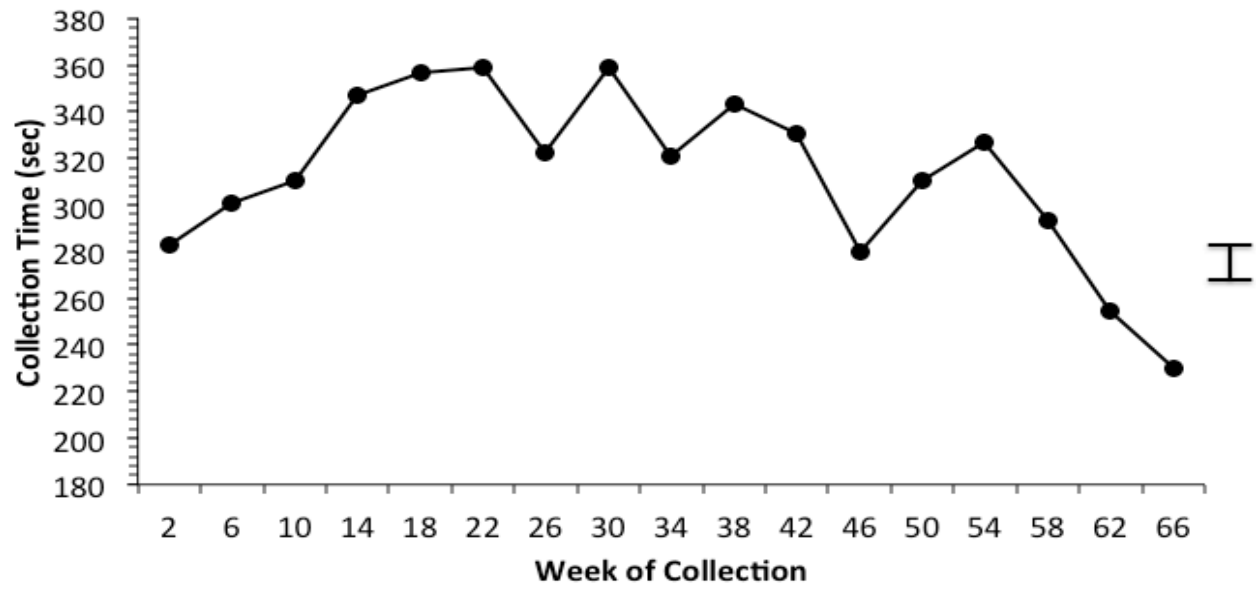


Figure 3: Effect of Week of Collection on collection time (sec). Pooled standard error of the mean = 10.2 sec. Week 2 represents the week of February 8, 2016 and week 66 represents the week of May 1, 2017.

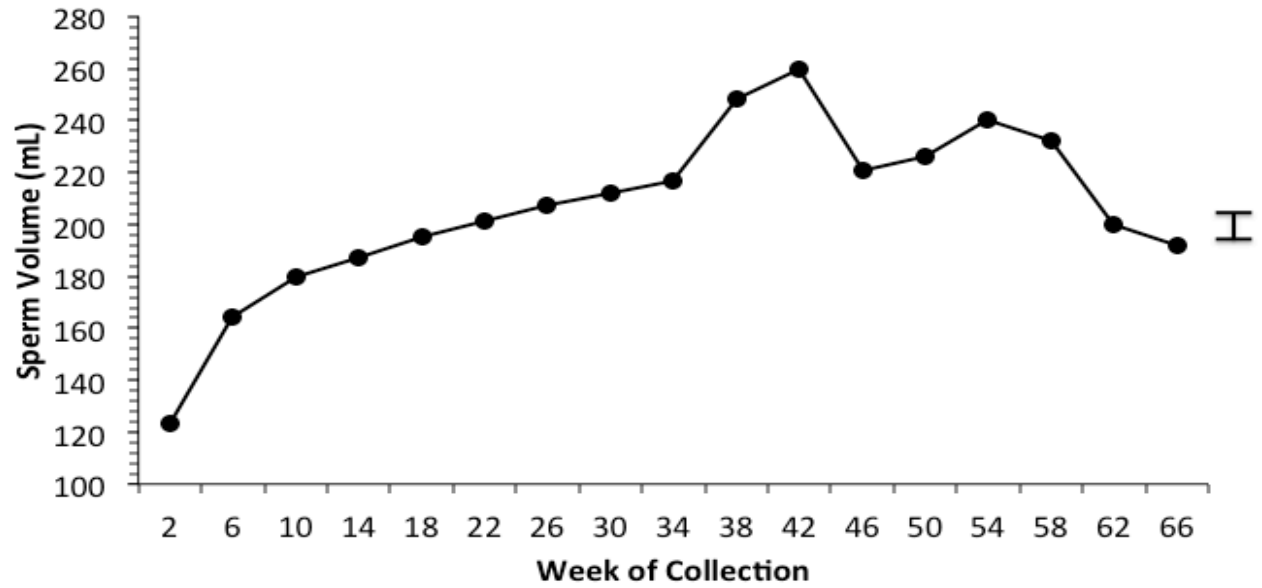


Figure 4: Effect of Week of Collection on sperm volume (mL). Pooled standard error of the mean = 7.9 mL. Week 2 represents the week of February 8, 2016 and week 66 represents the week of May 1, 2017.

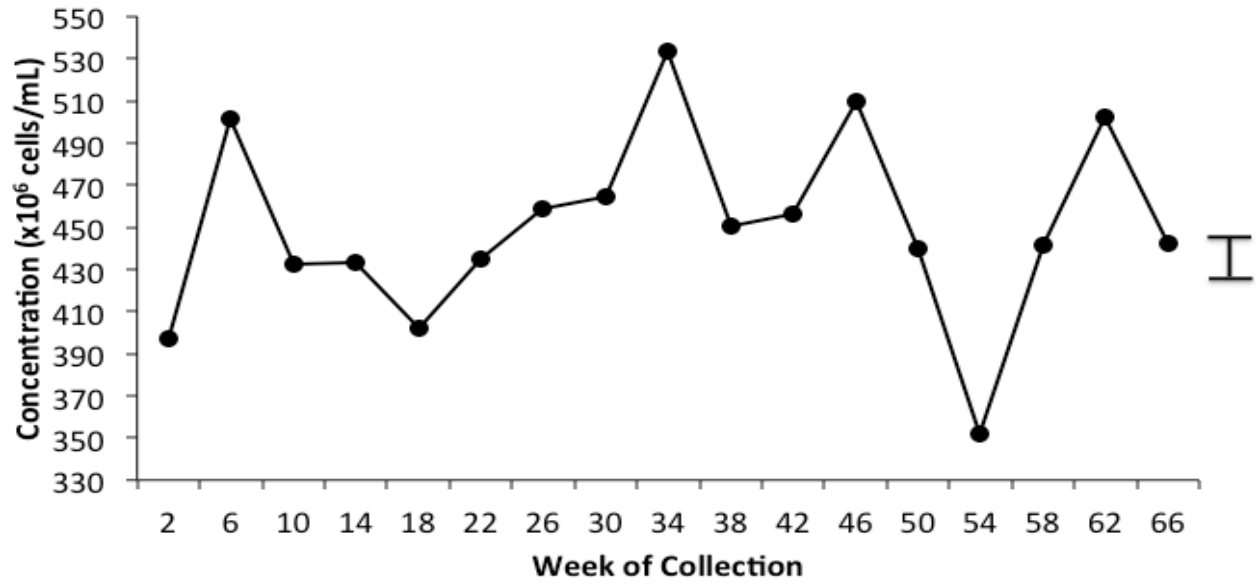


Figure 5: Effect of Week of Collection on sperm concentration (x10⁶ cells/mL). Pooled standard error of the mean = 18.9 x 10⁶ cells/mL. Week 2 represents the week of February 8, 2016 and week 66 represents the week of May 1, 2017.

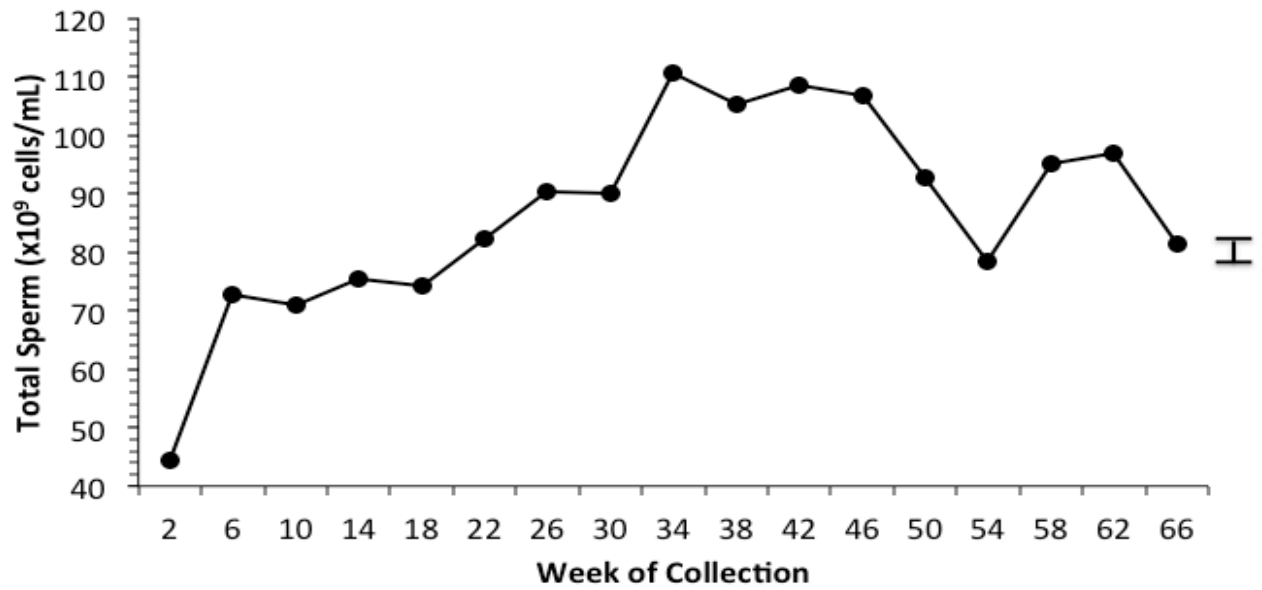


Figure 6: Effect of Week of Collection on total sperm per ejaculate (x10⁹ cells/mL). Pooled standard error of the mean = 2.9 x 10⁹ cells/mL. Week 2 represents the week of February 8, 2016 and week 66 represents the week of May 1, 2017.

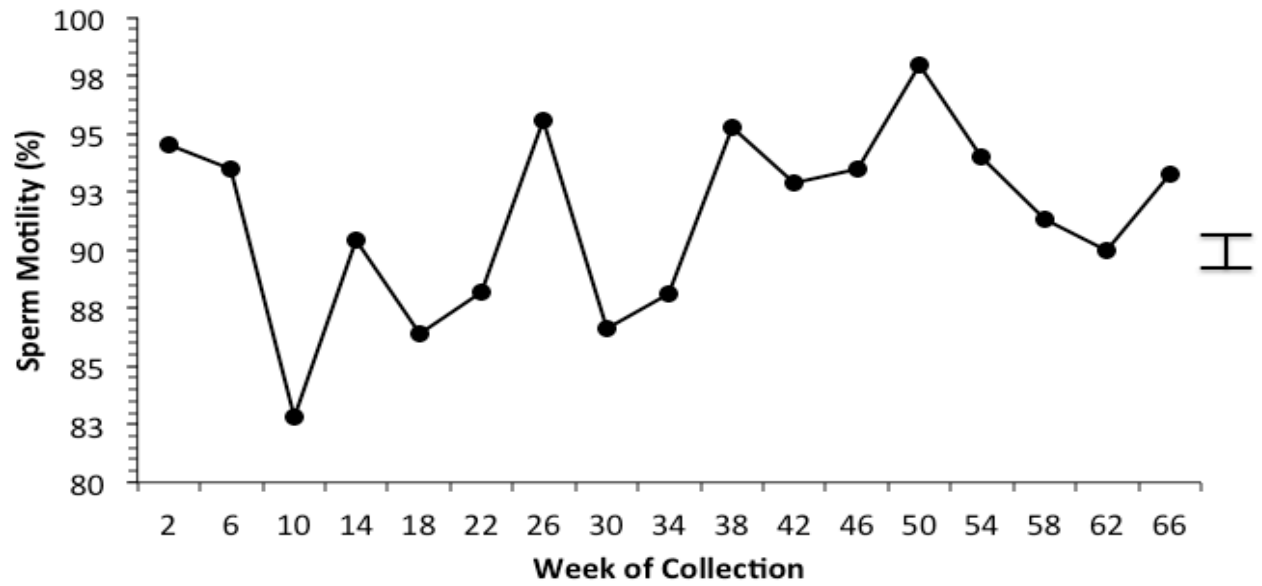


Figure 7: Effect of Week of Collection on sperm motility (%). Pooled standard error of the mean = 1.2%. Week 2 represents the week of February 8, 2016 and week 66 represents the week of May 1, 2017.

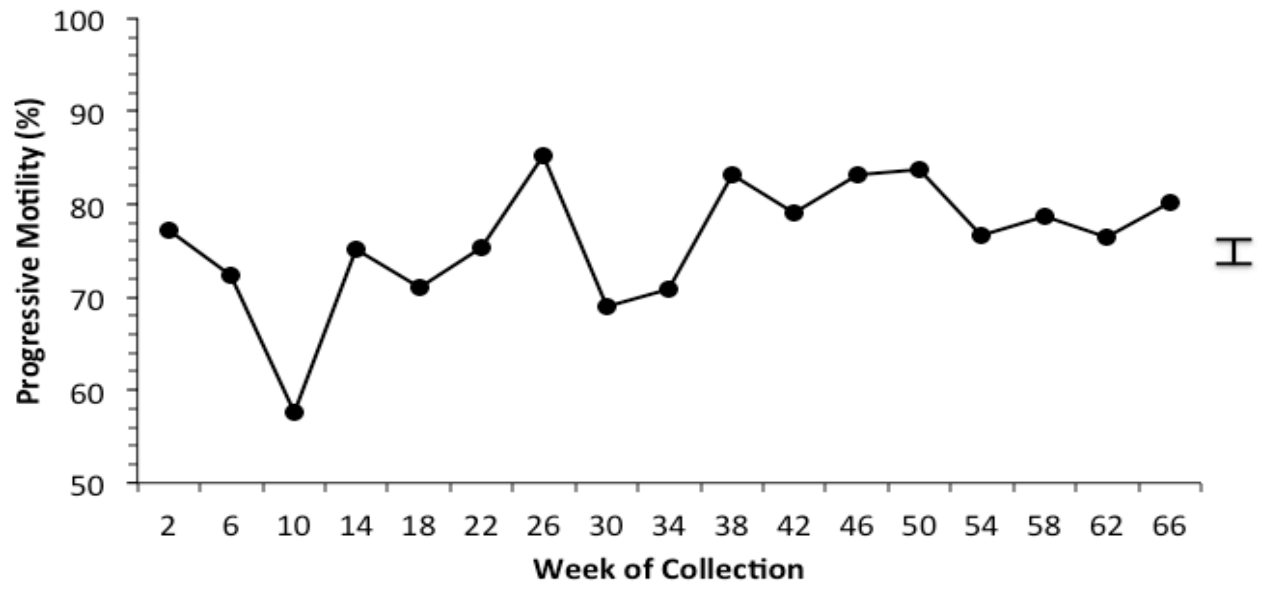


Figure 8: Effect of Week of Collection on sperm progressive motility (%). Pooled standard error of the mean = 1.8%. Week 2 represents the week of February 8, 2016 and week 66 represents the week of May 1, 2017.

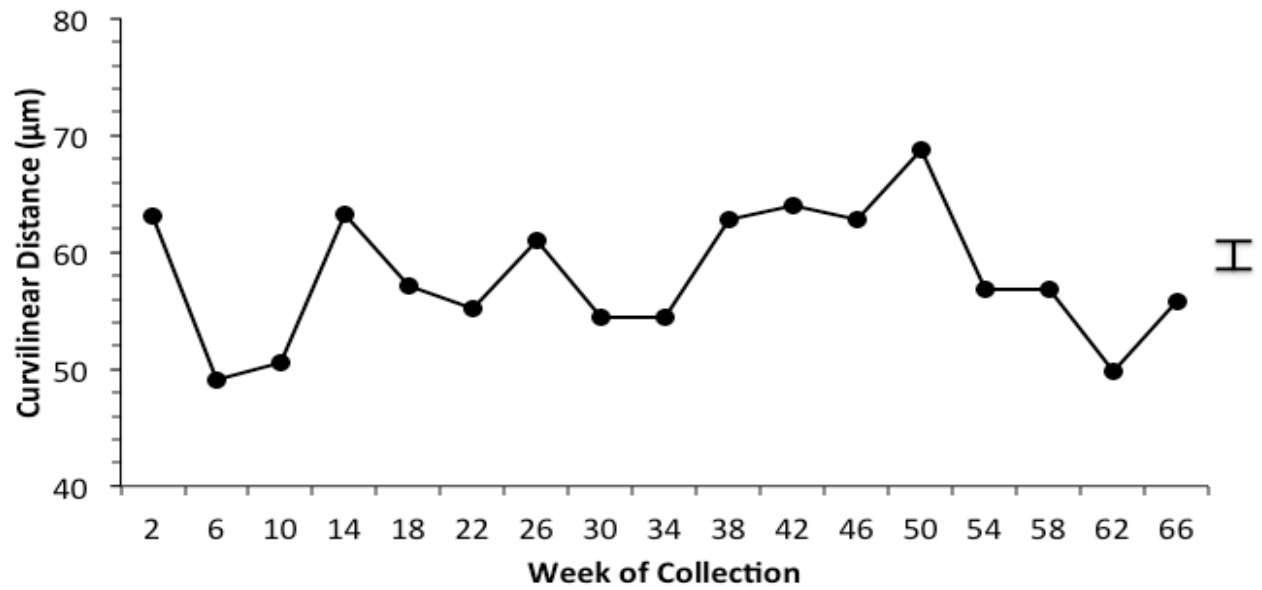


Figure 9: Effect of Week of Collection on curvilinear distance (μm). Pooled standard error of the mean = $1.8 \mu\text{m}$. Week 2 represents the week of February 8, 2016 and week 66 represents the week of May 1, 2017.

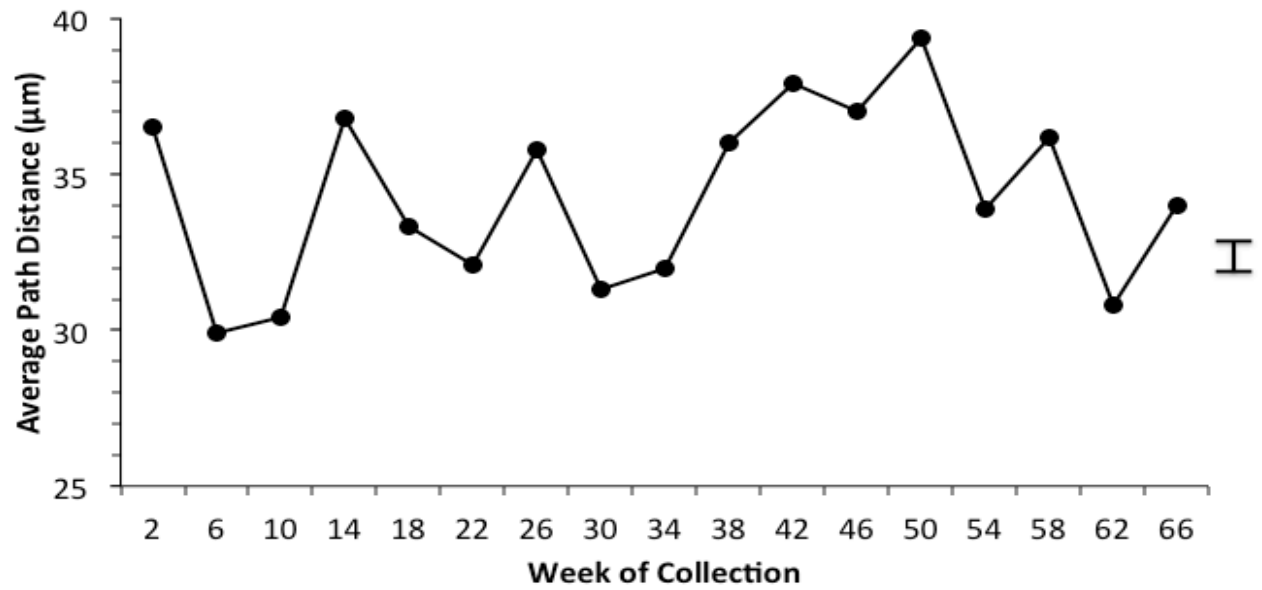


Figure 10: Effect of Week of Collection on average path distance (μm). Pooled standard error of the mean = $0.9 \mu\text{m}$. Week 2 represents the week of February 8, 2016 and week 66 represents the week of May 1, 2017.

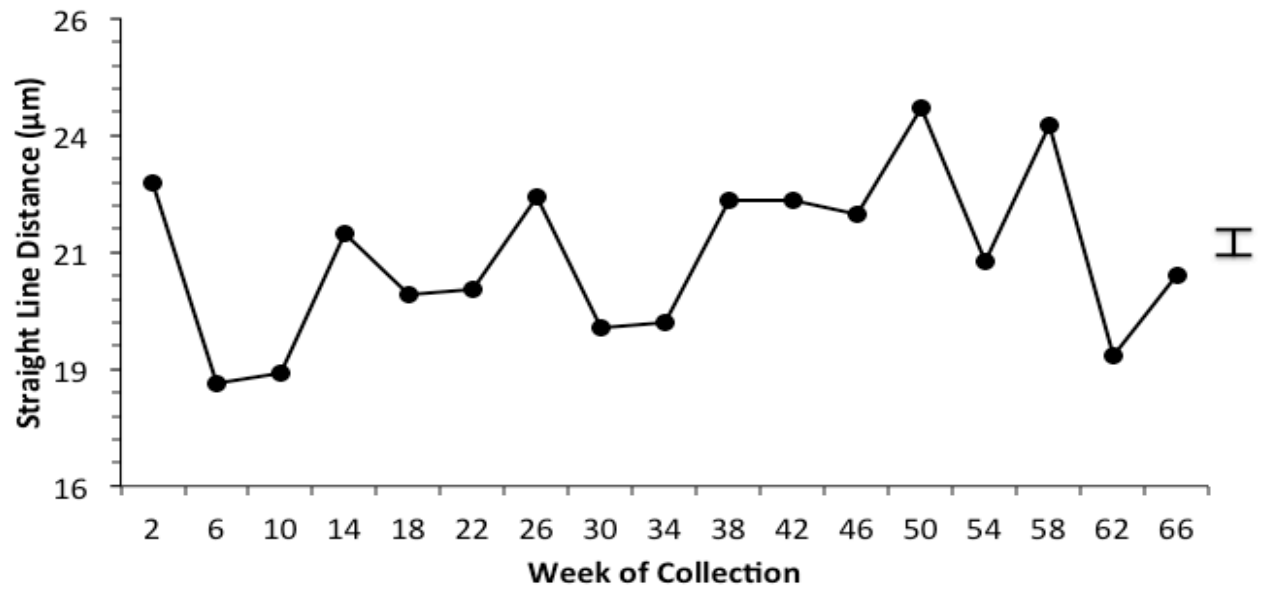


Figure 11: Effect of Week of Collection on straight line distance (μm). Pooled standard error of the mean = $0.5 \mu\text{m}$. Week 2 represents the week of February 8, 2016 and week 66 represents the week of May 1, 2017.

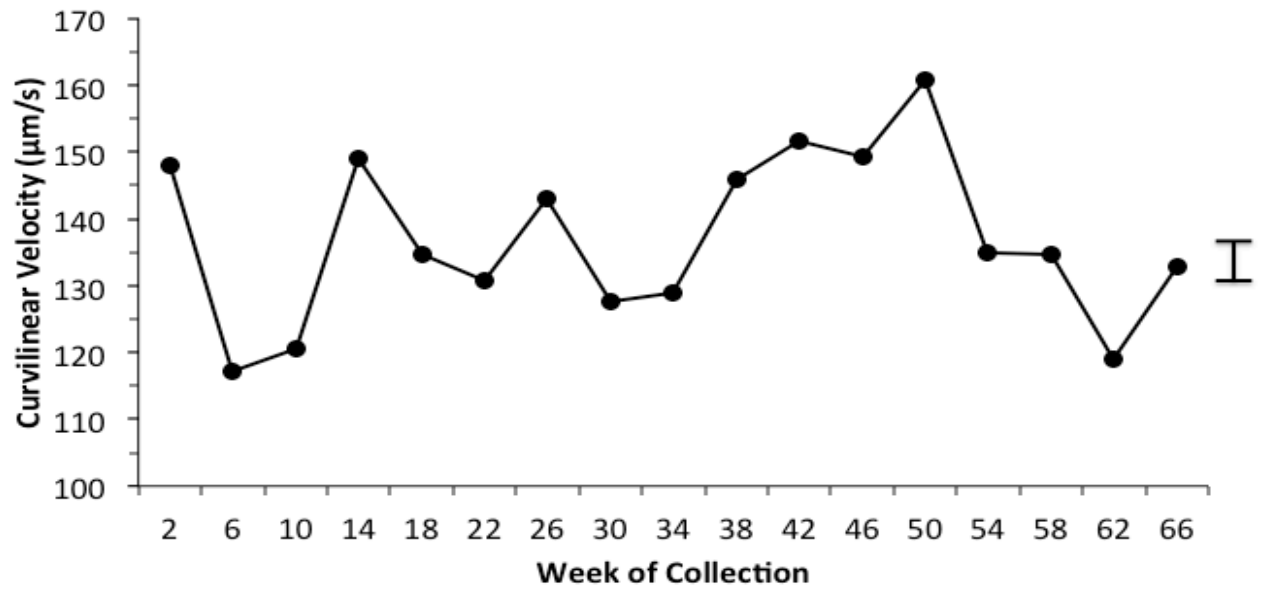


Figure 12: Effect of Week of Collection on curvilinear velocity ($\mu\text{m/s}$). Pooled standard error of the mean = $4.1 \mu\text{m/s}$. Week 2 represents the week of February 8, 2016 and week 66 represents the week of May 1, 2017.

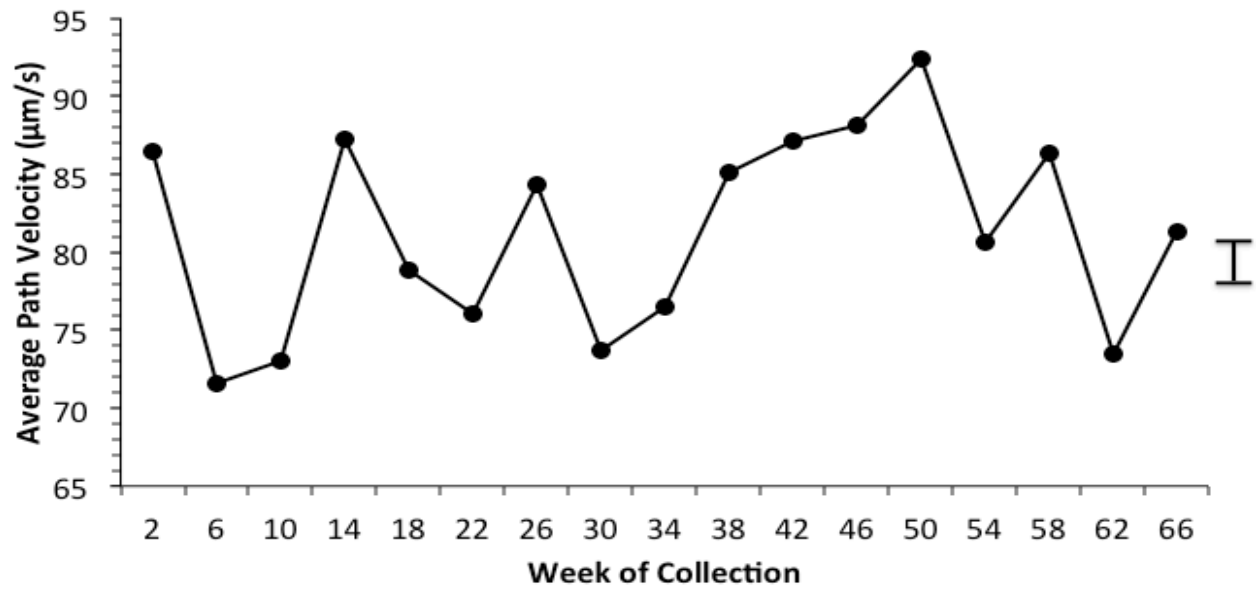


Figure 13: Effect of Week of Collection on average path velocity ($\mu\text{m/s}$). Pooled standard error of the mean = $2.1 \mu\text{m/s}$. Week 2 represents the week of February 8, 2016 and week 66 represents the week of May 1, 2017.

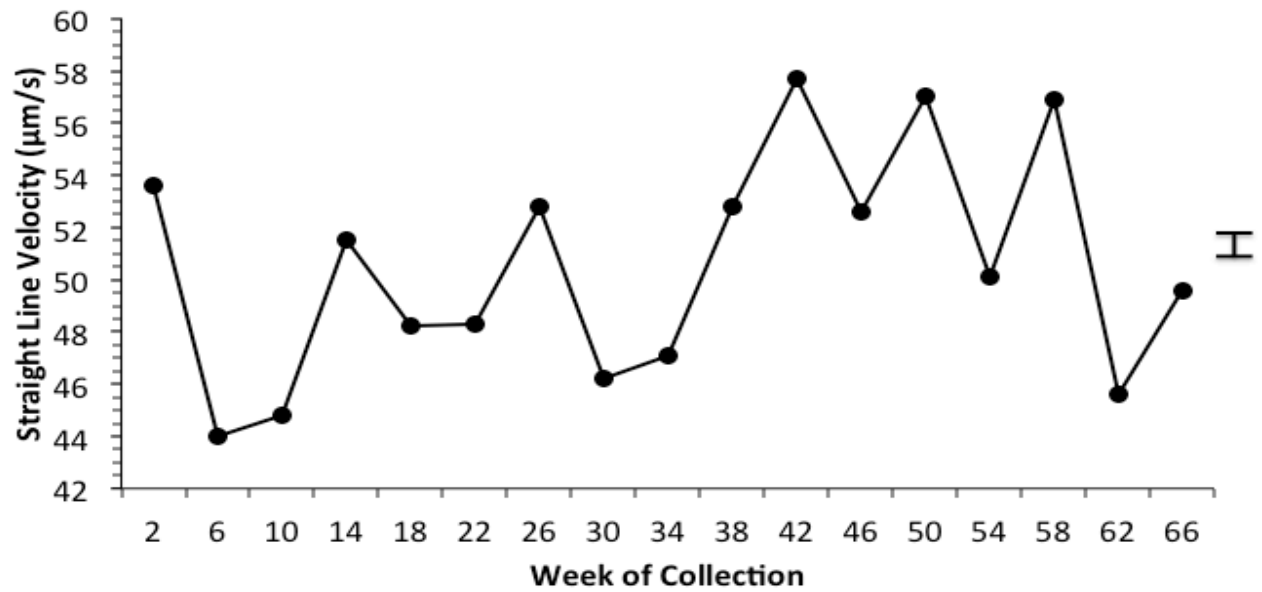


Figure 14: Effect of Week of Collection on straight line velocity ($\mu\text{m/s}$). Pooled standard error of the mean = $1.1 \mu\text{m/s}$. Week 2 represents the week of February 8, 2016 and week 66 represents the week of May 1, 2017.

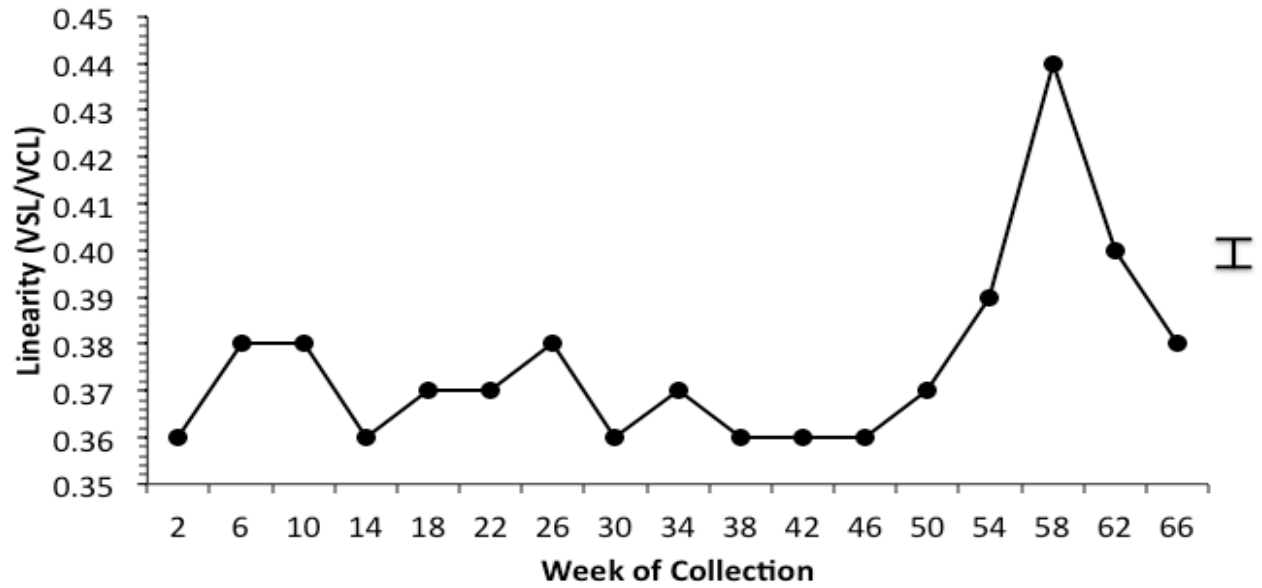


Figure 15: Effect of Week of Collection on linearity (VSL/VCL). Pooled standard error of the mean = 0.007. Week 2 represents the week of February 8, 2016 and week 66 represents the week of May 1, 2017.

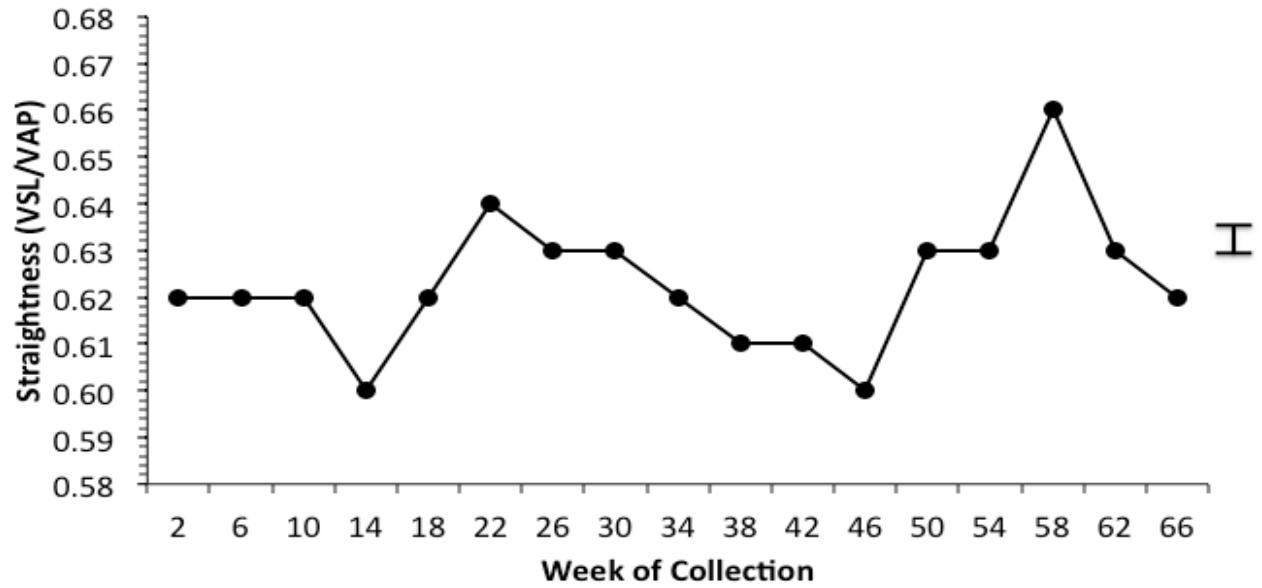


Figure 16: Effect of Week of Collection on straightness (VSL/VAP). Pooled standard error of the mean = 0.002. Week 2 represents the week of February 8, 2016 and week 66 represents the week of May 1, 2017.

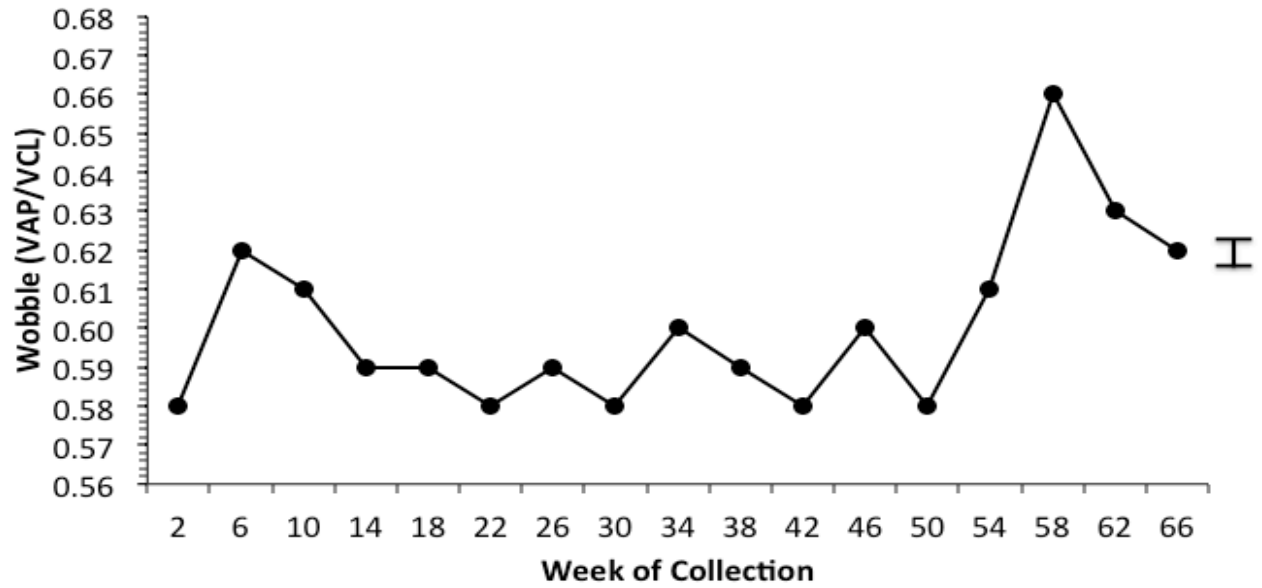


Figure 17: Effect of Week of Collection on wobble (VAP/VCL). Pooled standard error of the mean = 0.005. Week 2 represents the week of February 8, 2016 and week 66 represents the week of May 1, 2017.

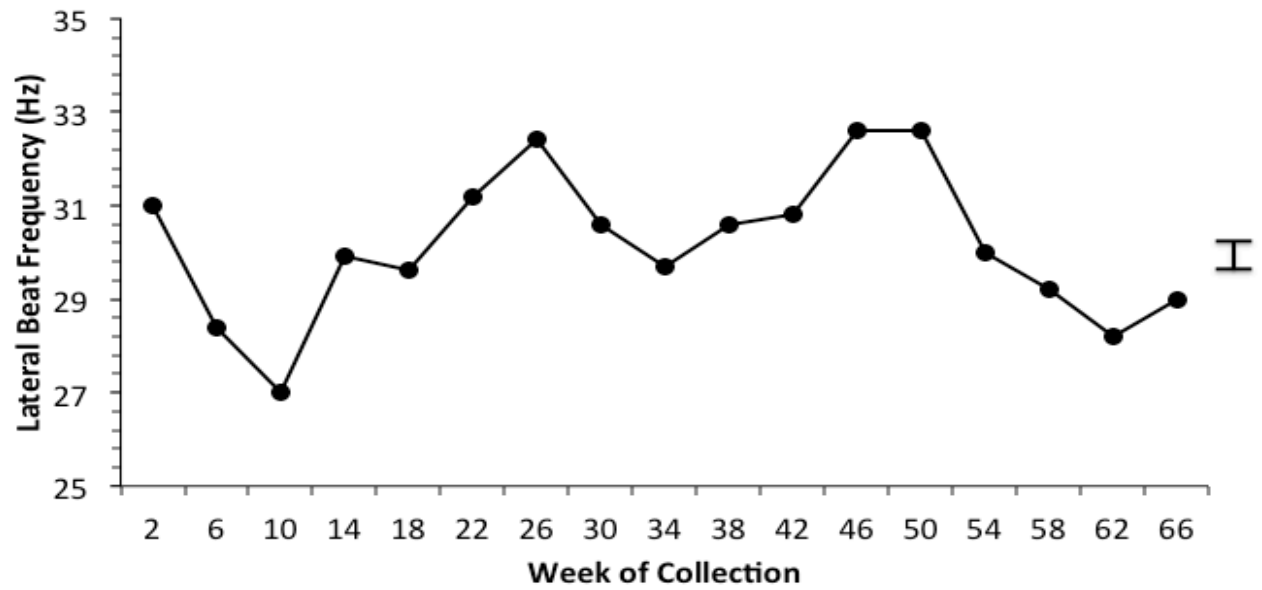


Figure 18: Effect of Week of Collection on lateral beat frequency (Hz). Pooled standard error of the mean = 0.4 Hz. Week 2 represents the week of February 8, 2016 and week 66 represents the week of May 1, 2017.

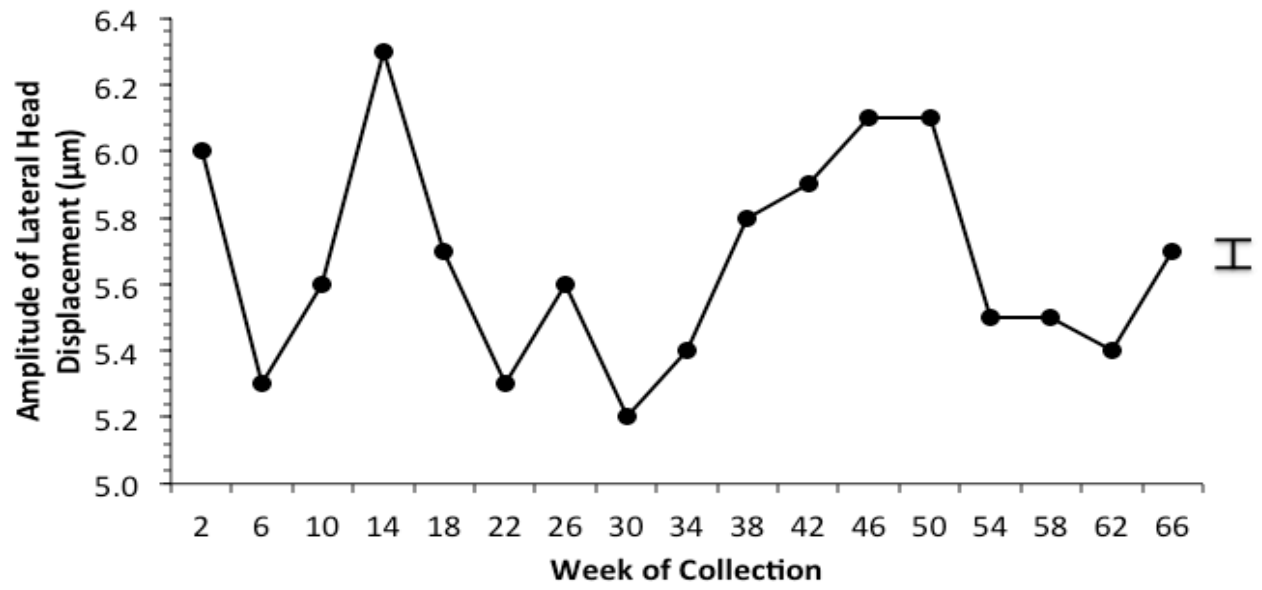


Figure 19: Effect of Week of Collection on amplitude of lateral head displacement (μm). Pooled standard error of the mean = $0.14 \mu\text{m}$. Week 2 represents the week of February 8, 2016 and week 66 represents the week of May 1, 2017.

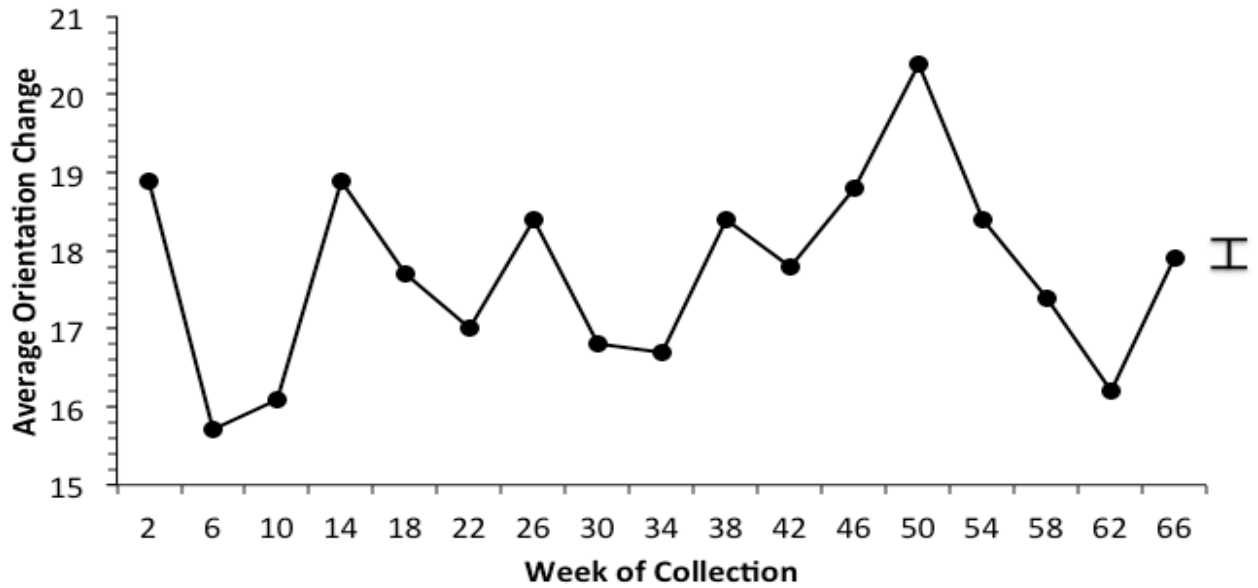


Figure 20: Effect of Week of Collection on average change in orientation of head. Pooled standard error of the mean = 0.4. Week 2 represents the week of February 8, 2016 and week 66 represents the week of May 1, 2017.

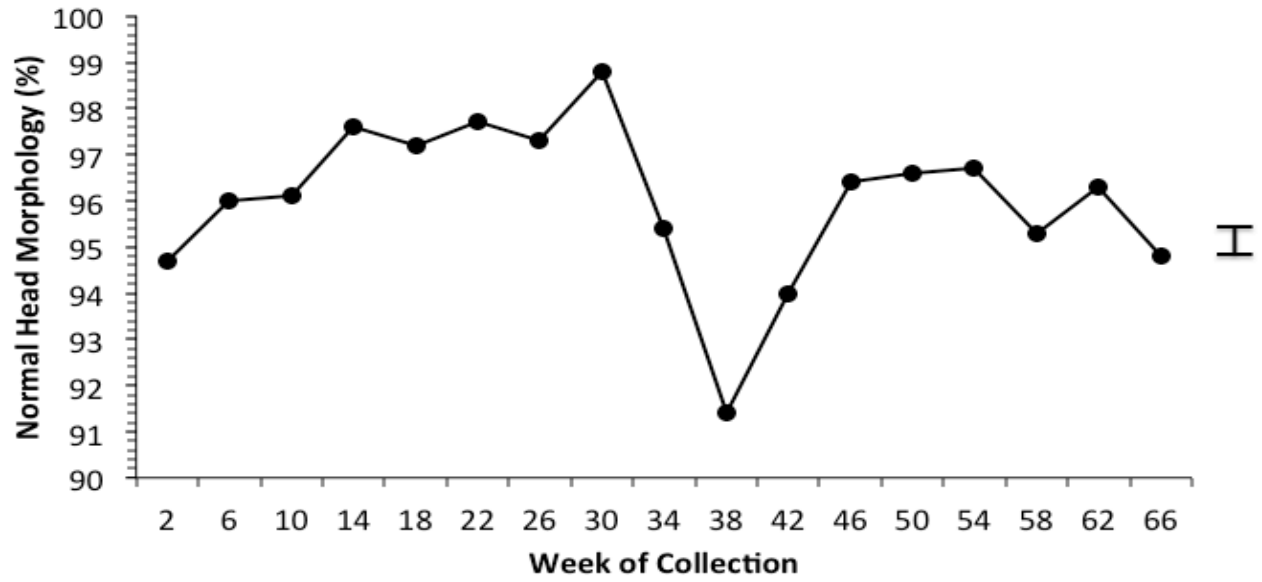


Figure 21: Effect of Week of Collection on normal head morphology (%). Pooled standard error of the mean = 0.3%. Week 2 represents the week of February 8, 2016 and week 66 represents the week of May 1, 2017.

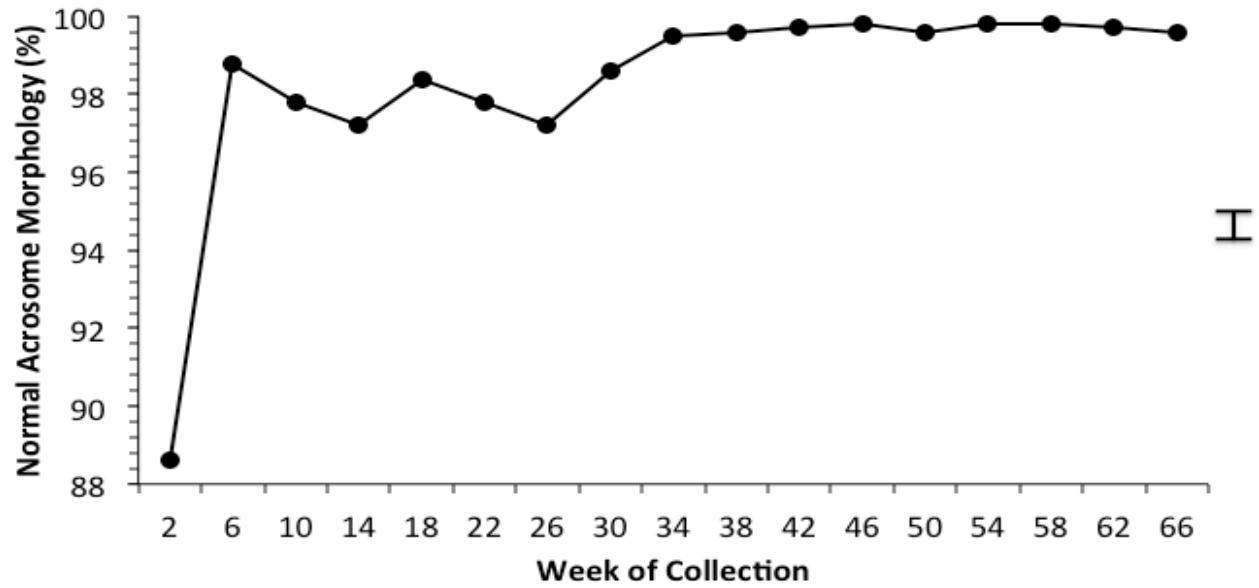


Figure 22: Effect of Week of Collection on normal acrosome morphology (%). Pooled standard error of the mean = 0.4%. Week 2 represents the week of February 8, 2016 and week 66 represents the week of May 1, 2017.

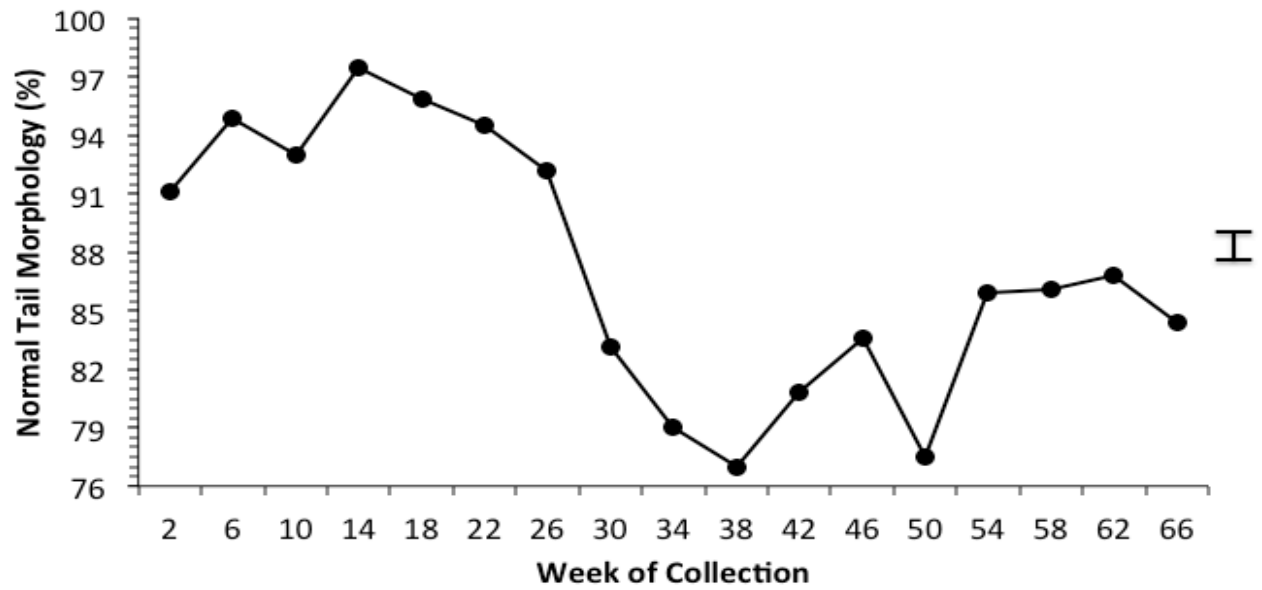


Figure 23: Effect of Week of Collection on normal tail morphology (%). Pooled standard error of the mean = 1.3%. Week 2 represents the week of February 8, 2016 and week 66 represents the week of May 1, 2017.

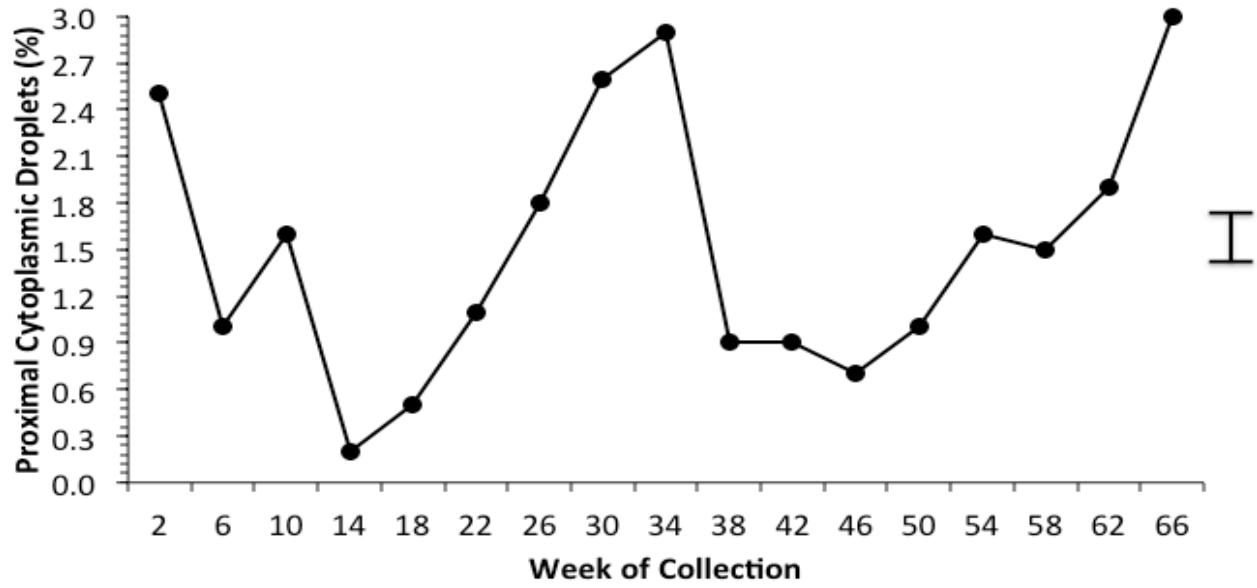


Figure 24: Effect of Week of Collection on sperm with proximal cytoplasmic droplets (%). Pooled standard error of the mean = 0.3%. Week 2 represents the week of February 8, 2016 and week 66 represents the week of May 1, 2017.

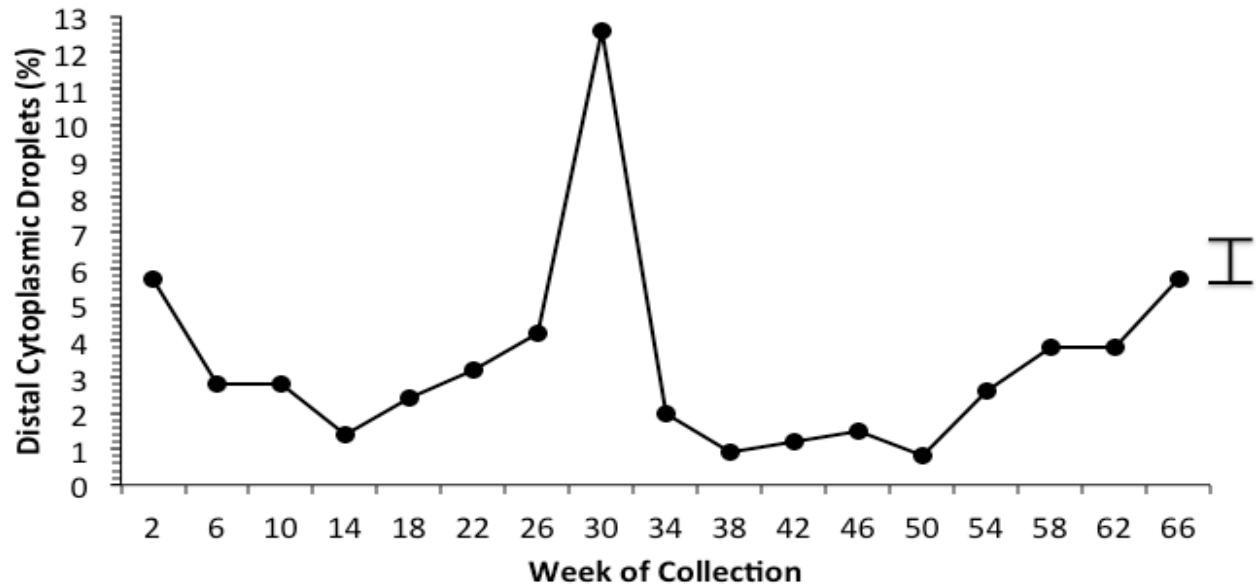


Figure 25: Effect of Week of Collection on sperm with distal cytoplasmic droplets (%). Pooled standard error of the mean = 0.6%. Week 2 represents the week of February 8, 2016 and week 66 represents the week of May 1, 2017.