SWING, SHELLEY ELIZABETH. Assessing Boar Reproductive, Physiological, and Behavioral Response in Two Different Housing Environments. (Under the direction of Dr. William L. Flowers.)

The objective of this study was to determine the effect of housing environment on reproductive performance of boars. Mature boars (n=14) were randomly assigned to be housed in crates or pens. After a 5-week acclimation period, semen was collected weekly for 10 weeks. Several other physiological parameters and selected behaviors were also evaluated at the beginning, middle and end of this 10 week period. Semen from weeks 8 and 10 was also used to make heterospermic insemination by mixing spermatozoa from boars housed in crates and pens in equal amounts in order to obtain an estimate of any relative effects of boar housing on boar fertility. After the initial 10-week period, boars in crates were moved to pens and their counterparts in pens were moved to crates. After a 1-week acclimation period, boars were evaluated for a second 10-week period as described previously. Boars housed in crates showed decreased collection times, semen volume, and total sperm per ejaculate (p<.05) when compared to boars in pens. Boars in crates also had a longer average reaction time prior to collection than their counterparts housed in pens. There were statistical tendencies (p<.1) for sperm from boars in crates to have higher overall linear, curvilinear and average path velocities and to travel longer distances compared to boars housed in pen. There were no differences in morphology (p>.05) due to the housing environment. However several significant (p<.05) differences were found
regarding percentages of normal and abnormal spermatozoa related to the age of the boars. In general, increased abnormalities were observed as the boars aged.

There were no differences between treatments in body weight, hair weight, hormone profiles, expression of normal behaviors or behavioral responses to novel objects (p > .05). Paternity tests revealed that boars housed in crates and pens sired a similar percentage of piglets overall (p > .05; crates = 49.3%; pens = 50.3%).

Housing conditions affect libido and sperm production with these being superior for boars housed in pens versus crates. There were no differences in estimates of semen quality, boar fertility, normal behaviors, and physiological parameters normally associated with stress responses in boar. In a commercial setting these differences found may influence production decisions, especially as they relate to labor costs and dosages for insemination.
Assessing Boar Reproductive, Physiological, and Behavioral Response in Two Different Housing Environments

by
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Biography

Shelley Swing was born on May 27, 1987 in Greensboro, North Carolina to Debra and Gary Swing. She has one older brother, Matt. She attended elementary, middle, and high school in McLeansville, North Carolina. She was the valedictorian of her graduating class in 2005. Upon graduation, she was accepted into the Thomas Jefferson Scholars program and pursued undergraduate degrees in Animal Science and Psychology from North Carolina State University.

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Spermatogenesis

Spermatogenesis takes place within the seminiferous tubules of the testes and is described as the development of mature germ cells from undifferentiated cell to spermatozoon which is released into the lumen of the seminiferous tubule (Roosen-Runge 1962). This process lasts anywhere from 30 to 75 days in mammals; this time is dependent upon the individual species (França et al. 2005). In boars, spermatogenesis takes 34.4 days to complete with an additional 9-12 days to complete epididymal passage and maturation before ejaculation (Hafez 1993; Kuster and Althouse 2007). There are three stages of spermatogenesis consisting of the proliferation phase, the meiotic phase, and the differentiation phase. The proliferation phase consists of several mitotic divisions producing spermatogonia. The meiotic phase produces haploid spermatids, and the differentiation phase includes many morphological changes which create a highly specialized spermatozoan. The first phase occurs in the basal compartment, while the last two occur in the adluminal compartment of the seminiferous tubule (Senger 2005).

The environment in which spermatogenesis takes place is important to ensure proper maturation. Adjacent to the seminiferous tubule is the interstitial compartment, which is adjacent to the basal compartment. Within the interstitial compartment are lymphatic channels, blood vessels, Leydig cells, and connective tissue. The lamina propria, or basement membrane, separates the basal compartment of the seminiferous tubule from the surrounding
interstitial compartment. Again, the basal compartment is where the first process of proliferation occurs. The area from the basal compartment to the lumen is the adluminal compartment, so named because of its placement next to the lumen. Together, the basal and adluminal compartments are known as the tubular compartment. Within the tubule are Sertoli cells which nourish the rapidly growing and dividing spermatogonia. Sertoli cells are surrounded by a junctional complex, often called the blood-testis barrier. This precludes other molecules from the body from entering the lumen of the seminiferous tubules, as well as protecting the germ cells from the immune system of the animal. This morphology of the seminiferous tubule is consistent among mammals, although there are some differences in the interstitial compartment (Amann 1983).

The first phase of spermatogenesis is comprised of all mitotic divisions of spermatogonia, and results in B-spermatogonia, which are also known as primary spermatocytes. This process occurs in the basal compartment, which is named for its proximity to the basement membrane of the seminiferous epithelium. As these spermatogonia mature, they move toward the lumen of the seminiferous tubule. The cells that enter the proliferation phase are specialized diploid cells, and go through several divisions that progress from A₁ to A₄, to an intermediate stage, and finally to B-spermatogonia. The number of divisions in the A₁ to intermediate stages are species-dependent (Senger 2005). In boars, there are six spermatogonial generations before meiosis (França et al. 2005). Before the terms "A" and "B" were given to these types of spermatogonia, they were referred to as "dust-like" and "crust-like," respectively. Dust-like cells showed a nucleus with fine, palely
stained chromatin. In contrast, crust-like cells showed coarse flakes of heavily stained chromatin within the nucleus.

The stain used to characterize these two types of cells was the commonly used safranin (Clermont 1972). This staining and type of characterization was first demonstrated in mice, but has also been shown to have striking similarities to spermatogenesis in swine. For example, swine and mice both show four classes of spermatogonia (França et al. 2005). During the mitotic stage, the cells are rounded to ovoid in shape (Cheng and Mruk 2010). Also during this time, a pool of stem cells is maintained so that there is always a source of A-spermatogonia. Many A-spermatogonia do not undergo all divisions and restart the mitotic divisions later to ensure continual production (Senger 2005).

The next phase of spermatogenesis is the meiotic phase, which reduces the cells to a haploid state. Primary spermatocytes enter into meiosis, which transform into secondary spermatocytes after meiosis I, and further develop into spermatids at the end of this phase (Senger 2005). During this meiotic phase, the spermatocytes move into the adluminal compartment of the seminiferous tubule and continue to mature there. More specifically, when still in the basal compartment, the spermatocyte is in the preleptotene stage of prophase of meiosis I. Within prophase of meiosis I consists five stages: preleptotene, leptotene, zygotene, pachytene, and diplotene. By pachytene of meiosis I, the spermatocyte has already moved through the blood-testis barrier into the adluminal compartment (Cheng and Mruk 2010). It is during preleptotene that tetrads form, allowing for crossing over to occur. This
allows for genetic diversity and ensures that each spermatocyte and spermatid will be genetically unique. Additionally, prophase of meiosis I is the longest single stage of spermatogenesis; it occupies approximately 30% of the time spent in spermatogenesis (Senger 2005). During the meiotic phase, there is a large increase in cell size, especially from preleptotene to diplotene (França et al. 2005). The spermatocytes remain rounded in shape during this phase as well (Cheng and Mruk 2010).

The third and final stage of spermatogenesis is that of differentiation, also called spermiogenesis. During this phase, spermatids undergo many morphological and physiological changes leading to a highly differentiated germ cell. Some of the changes include condensation of the nucleus, elongation of the cell, acrosomal formation, and motility (Senger 2005). A spermatid entering the differentiation phase is characterized by a small, spherical nucleus, and the usual cellular organelles, the most prominent of which is the Golgi apparatus. Differentiation can be divided into four phases consisting of the Golgi phase, cap phase, acrosomal phase, and maturation phase.

The Golgi phase is marked by the production of small granules by the Golgi apparatus, which are called proacrosomic granules. After these appear, they begin to fuse into a single larger granule called the acrosomic granule. This spherical granule is closely attached to the surface of one side of the nucleus (Clermont 1972). Smaller vesicles are continuously added to the larger granule, further increasing its size. Centrioles are also
migrating from the cytoplasm to the base of the nucleus, and will form as the base of the flagellum (Senger 2005).

The second phase, or cap phase, involves the flattening of the acrosomic granule, as well as expansion over the nuclear surface to form the "cap" which is beginning to have distinct membranes (Clermont and Leblond 1955). The cap forms over the anterior portion of the nucleus with the aforementioned centrioles at the posterior portion. At this time, the acrosome is being filled with several types of enzymes, which are necessary for penetration into the zona pellucida of the oocyte. The Golgi also begins to migrate toward the posterior end of the spermatid, and the axoneme (or flagellum) begins to form from the distal centriole (Senger 2005).

The acrosomal phase of differentiation is marked by the spread of the acrosome along the side of the nucleus as well as elongation of the nucleus. During this time, the acrosome is oriented toward the basement membrane of the seminiferous tubule, with the developing tails facing toward the lumen (Clermont and Leblond 1955). The acrosome spreads to cover a majority of the anterior portion of the nucleus while the nucleus itself is elongating. The cytoplasm also begins to shift toward the posterior end of the spermatid, which pushes the nucleus toward the cell membrane. As the nucleus elongates, it begins to take a size and shape that is species-specific; in boars this shape is spatulate (Clermont 1972). Toward the tail end of the spermatid, the manchette, or caudal tube, forms. This structure consists of microtubules which form around the posterior base of the nucleus and extend toward the
flagellum. Some of these microtubules also go on to form the postnuclear cap which surrounds the portion of the nucleus which is not already covered by the acrosome.

The last stage of spermiogenesis is the maturation phase. During this phase, mitochondria shift to surround the middle piece of the flagellum in a spiral formation, and a structure called the annulus shifts to separate the midpiece from the principal piece. Within the flagellum, the axoneme of the midpiece and principal piece is comprised of nine pairs of microtubules which all surround two central filaments. This allows for the tail to move side-to-side while the flagellum beats. The entire spermatozoon at this time is still surrounded by a plasma membrane, which serves to protect the integrity and function of the cell (Senger 2005). At the very end of this stage, the cytoplasm which was pushed toward the flagellum is shed off with help from the surrounding Sertoli cells just before spermiation. This cytoplasm becomes a residual body (Clermont 1972). At the end of this process, the spermatozoon looks radically different from the stem cell from which it arose. It is elongated with a beating flagellum, and has gained several enzymes, sugars, and proteins which allow it to function properly.

During the cellular divisions of spermatogenesis, there is some spermatogonial degeneration. In most species, sperm output is not maximized because of this. The loss usually occurs in the seminiferous epithelium so that the number of cells entering meiosis is fewer than those that begin proliferation (Stefanini et al. 1984). Only two to three spermatozoa out of a possible ten are produced from each A¹ spermatogonium in pigs. The
loss ensures that there are not more germ cells than can be supported by accompanying Sertoli cells (França et al. 2005).

Regulation of the number of germ cells may be influenced by a testicular chalone. Chalones are tissue specific but not species-specific, and act as non-cytotoxic inhibitors of cell division. These are thought to be locally synthesized peptides or glycoproteins. This chalone however has not been isolated or characterized, so the specific effect or mechanism is still unknown. Further regulations of germ cell number can be attributed to regulation of meiosis, as shown by studies done in mice. A meiotic inducing substance (MIS) must be present in order for meiosis to begin. There is also evidence of a meiotic preventing substance (MPS) which may serve to act as an inhibitor to the process. Interestingly, lowering the levels of MPS induces meiosis at puberty (Stefanini et al. 1984). Germ cell loss can also be attributed to apoptosis (programmed cell death), which is frequent during meiosis and may be due to chromosomal damage (França et al. 2005).

The final step in spermatogenesis is the release of mature spermatozoa into the lumen of the seminiferous tubule. This process is called spermiation, and is akin to ovulation in the female with the exception that spermiation is a continuous process, while ovulation is an intermittent phenomenon. In order to produce a continual supply of spermatozoa, the boar utilizes a cycle within the seminiferous tubule which allows for multiple generations at multiple developmental stages to exist simultaneously. As a result, deleterious events (such as heat stress, infection, etc.) can produce a delay in observable effects by as much as 4-5
weeks (Senger 2005). Because of this, it is possible for a stressful event to affect germ cells at any stage of maturation, and can thus be observed as soon as the next day, or as long after as seven weeks from the initial date of the stress. The stage of development a germ cell is in when stressed determines what morphological symptoms may arise. For instance, stresses on a spermatocyte undergoing spermiogenesis would show as tail or acrosomal defects rather than chromosomal or nuclear defects (Senger 2005).

In addition to the three phases of spermatogenesis, there is also a cycle within the seminiferous epithelium that ensures constant production of sperm cells. As one wave of spermatozoa matures, a new wave will be started in the same area. This results in a staggered sequence of production. Therefore, if a transverse section of a seminiferous tubule is cut out, many different generations of germ cells can be found (Bearden et al. 2004). These are seen in the seminiferous tubules as concentric “layers” of germ cells (Senger 2005). Due to the organization within the tubule, some cell types are always found together. There are two systems of classification – one that identifies 12 stages of cellular associations, and another that identifies only eight (Bearden et al. 2004). This is due to species differences and individual classification preferences. Boars are characterized under the 8-stage system, based on the histologic features of the cells. The duration of one of these cycles of the seminiferous epithelium, from stage one through stage eight, is 8.6 days in the boar. The duration of the seminiferous epithelial cycle is characterized by the time between two successive appearances of the same cellular associations. The boar’s total spermatogenic cycle which
consists of 34.4 days results in four cycles of the seminiferous epithelium being needed to produce fully differentiated spermatozoa (Hafez 1993).

The eight stages of the seminiferous epithelial cycle are characterized by key changes in cellular association. Stage 1 includes rounded spermatids, two generations of primary spermatocytes (one young and one old), and spermatozoa which have just been released into the lumen. Stage 2 comprises two generations of primary spermatocytes, and the period when spermatid nuclei are elongating. Stage 3 embodies the end of the elongation period to the first meiotic division, and a new generation of spermatogonia. Stage 4 consists of elongated bundles of spermatids within the cytoplasm of Sertoli cells, which are associated with both one generation of secondary and primary spermatocytes. In stage 5, the bundles of spermatids are linked with a new generation of spermatids. Stage 6 inclues changes in chromatin appearance to dust-like in young spermatids, and to network-like in primary spermatocytes which are in pachytene. Stage 7 consists of elongated spermatids which are migrating towards the lumen of the seminiferous tubule. The last stage (stage 8) describes the time in which mature spermatozoa are just next to the lumen and are released. The amount of time a germ cell spends in stages 1-8 are as follows: 0.9, 1.2, 0.3, 1.0, 0.8, 1.7, 1.6, and 1.0 days, respectively (Hafez 1993). Not all stages of spermatogonial development are included in each description of stage because they are not the key changes with which each stage is classified. Once that key change is noted, it is then possible to identify the other cell types and stages of development (Hafez 1993). Additionally, each stage consists of a different microenvironment which is unique to that specific stage (Farin 2011).
Closely related to the stages of the cycle of the seminiferous epithelium is the spermatogenic wave. The wave describes a length of the tubule which includes germ cells from the first to last stage of the seminiferous epithelial cycle. That is, a portion of the tubule containing stage 3, would be followed by stage 4, stage 5, and so on. The length of tubule from stage 1 to stage 8 is a wave of spermatogenesis. This results in several waves in each seminiferous tubule (Perey et al. 1961). This is contrasted with the cycle of the seminiferous epithelium, which is timed at one location of the tubule and considers the time from stage one until stage one is seen again at that same location. The wave has been described by Regaud with the statement “the wave is in space what the cycle is in time” (1901).

**Sperm Maturation**

After spermiation when spermatozoa are released into the lumen of the seminiferous tubule, the cells must undergo further maturation in the epididymis. Spermatozoa are still not able to fertilize a female gamete until further alterations occur. These gradual alterations take place in the epididymis, which rests on the side of the testis. The epididymis is comprised of three main parts: the caput (head), corpus (body) and cauda (tail) each with different functions. The main functions of the epididymis are to serve as a passage for transport of spermatozoa, to provide an environment where they can mature, to concentrate spermatozoa, and to store mature spermatozoa (Orgebin-Crist and Olson 1984; Bearden et al. 2004). The epididymal duct is approximately fifty meters in length, with the epididymis being around
twenty centimeters long (Guyonnet et al. 2009). It is surrounded by smooth muscle which aids in forcing spermatozoa to travel through the duct (Senger 2005).

To reach the epididymis, spermatozoa must travel through the 10-12 small efferent ducts which converge to the epididymal duct at the head of the epididymis (Senger 2005; Bearden et al. 2004). Spermatozoa undergo morphological, biochemical, and physiological changes in the caput and corpus. The cauda region of the epididymis stores sperm until the time of ejaculation. In the boar, spermatozoa are in the caput for three days, the corpus for two days, and in the cauda for four to nine days for a total of nine to fourteen days for passage through the entire epididymis. When ejaculation frequency increases, the period of passage through the cauda epididymis may decrease by as much as twenty percent (Briz et al. 1995). Duration of passage through the caput and corpus is quite consistent (Amann et al. 1993). Spermatozoa which spend an unusual length of time in the cauda may be of poor quality (Senger 2005).

Because sperm gain different capabilities in each region of the epididymis and change morphologically, it is possible to use these changes as a marker to ascertain when a stressful event has occurred. For example, the position of the cytoplasmic droplet can indicate from what portion of the epididymis the sperm cell was ejaculated from in an exhaustive mating scheme (Hunter et al. 1976). In general, sperm in the caput are immature with a proximal cytoplasmic droplet, those in the corpus are immature with a distal cytoplasmic droplet, and those in the cauda are mature with no droplet. Thus, there are more abnormal sperm in the
cauda than in the first two regions (Briz et al. 1995). The final stages of maturation which occur outside the gonad are believed to be dependent upon interactions with the medium in the epididymis, rather than genomic as in spermatogenesis (França et al. 2005). Fluids surrounding the sperm cells are responsible for adding enzymes and lipid exchange proteins which are not available within the cells.

Because pigs may mate early in estrus but before ovulation, all sperm cells will not be at the same stage of maturation. This ensures that if ovulation does occur days after copulation, there will still be sperm in the female tract which are able to fertilize the ovum (Amann et al. 1993).

For sperm to become motile, they must gain intracellular cyclic adenosine monophosphate (cAMP), and be exposed to certain epididymal secretions. There are variations in the level of cAMP between the caput and cauda regions. A forward motility protein which is a glycoprotein binds to spermatozoa during their transport through the epididymis. After this addition of motility to the sperm, it is still unable to fertilize a mature ovum. It must next acquire fertilizing ability (Orgebin-Crist and Olson 1984).

Fertilizing ability of spermatozoa is first observed in the corpus region. Some individual variations occur, but the location is not drastically different. Once sperm have gained this ability, they are able to initiate the first few steps of fertilization, but transit through the female reproductive tract is still required for full competency. Once a
spermatozoan has entered the female tract, it is fully capable of fertilizing an ovum (Orgebin-Crist and Olson 1984).

The sperm plasma membrane also endures some changes during maturation. These changes include changes in distribution of proteins, and change in surface charge (Nikolopoulou et al. 1985). The surface of the plasma membrane is characterized by proteins which have a large mass (75 kDa and above). These proteins tend to be species-specific but the range of 105-115 kDa in swine has some overlap in size with the sheep. There are also several testicular sperm surface proteins which are shed, creating a new epididymal sperm surface. A few proteins which have been tested did not appear in the fluid surrounding the sperm, suggesting that they have been degraded beyond recognition by a probe. New components which are added in place of testicular sperm surface proteins usually have a low molecular mass and high glycosylation (Dacheux et al. 1989). Completion of these membrane changes corresponds with acquisition of fertilizing ability (Orgebin-Crist and Olson 1984).

The charge of the sperm cell becomes more negative as it passes through the epididymis. Thus, cauda spermatozoa will be more negative than those from the caput (Schroter et al. 1999). Studies have supported this observation by showing that spermatozoa show an increase in affinity for positively charged particles during transit through the epididymis. However, the binding was not uniform over the entirety of the surface of the sperm (Orgebin-Crist and Olson 1984). Once these further modifications have taken place in
the epididymis, sperm have been modified as much as possible in the male tract, and will await further changes after ejaculation from the cauda region.

Libido and Sexual Behavior

Libido is very important when assessing the effects of housing on reproduction measures. Lack of libido can indicate problems such as obesity, heat stress, a plane of nutrition which is too high, introduction to gilts at too early an age, or by mismanagement of young boars during previous training or service (Hafez 1993; Houpt 1998). Libido refers to the willingness and eagerness of a male animal to mount and to attempt service of a female or dummy. Mating behavior of the male describes his behavior immediately before, during, and after service which can also be indicative of his overall well-being (Chenoweth 1981).

In most species, it is extremely important that the male is raised around other males. Boars that are raised in isolation from others show abnormal sexual behavior. This effect has been shown to be long lasting without much improvement in subsequent weeks (Kilgour 1984). The age at which a boar is separated from his cohorts also has an effect; those separated earlier in life show poorer performance. Contact with sows later in their lives is also important, as those housed around sows ejaculated longer than those in isolation (Houpt 1998). Once males have reached puberty and have been trained properly, their mating behavior and libido can be used as a measure of comfort and well-being.
In the commercial swine industry, several methods to measure libido have been introduced. All of these measures can vary widely due to individual differences, so the unique boar should be taken into consideration when using these measures. One such measure is that of number of mounts. A higher number of mounts before service may indicate a poor ability to serve for collection. Other widely used measures of libido are reaction times; these include time to first contact, time to first mount, and time to first collection (Kilgour 1984).

Factors affecting libido

There are several factors which may influence libido. Among them are genetic and breed differences, hormonal influences, post-weaning management, nutrition, and season (Chenoweth 1981). Breed has a large impact on a boar’s interest in mating, and several studies have been conducted to describe these effects, with a majority focusing on differences between crossbred and purebred boars. For example, it is known that crossbred males show higher libido than their purebred cohorts. Additionally, Yorkshire males are more interested in mounting and more willing to mate when compared with Durocs (Flowers 2008).

Hormones play a role in sexual behavior and libido of boars. While testosterone is the main influence on sexual activity in males, the mechanism may be explained in two ways: circulating androgens have a positive correlation with sexual performance, and differences
among boars may be due to the responsiveness of tissues to those androgens. These methods are complicated by testosterone’s ability for conversion to estradiol. Estradiol is known to aid in copulatory behavior, and has been postulated to have a strong effect on overall sexual behavior. The role of testosterone is also difficult to characterize due to its conversion to various steroids at the active site (Chenoweth 1981).

Of great importance to a boar’s later sexual performance and libido is post-weaning management. It is well documented that boars should be housed around other boars, because some social learning regarding correct sexual behaviors occurs (Kirkwood and Thacker 1992; Chenoweth 1981). Abnormal sexual behavior thus occurs in boars housed apart from females. Males housed in groups (either male or female groups) showed better performance than those housed in isolation (Chenoweth, 1981).

In regard to nutrition, Louis et al. found that boars fed a low-protein diet (7% vs. 16% crude protein) did not show a decrease in libido when compared to those fed a normal diet (1994). There is only a decrease in libido in boars that are in extremely poor condition, such as those who have been fed 50% of their NRC recommendation for an extended period of time (Kemp and Den Hartog 1989). Also of note is that sexually active boars do not require higher protein levels in their diet than those that are not sexually active (Close 2006). Overweight boars are more likely to have foot, leg, or joint problems which may shorten their reproductive life, or reduce libido due to discomfort (Bearden et al. 2004).
Lastly, libido can also be affected by season. Most notably, temperature and humidity play a role in sexual behavior, with boars that are exposed to higher temperatures showing adverse effects on libido. This is not a factor in boars produced in a temperate climate (Chenoweth 1981). Steinbach noted that there is a positive correlation between refusal to mount a dummy and increasing temperature (1973). Premating and mating behaviors were also greatly reduced in heat-stressed animals (Winfield et al. 1981). Much of the research describing effect of temperature and humidity focuses on sperm abnormalities, which will be discussed later.

*The Stages of Male Reproductive Behavior*

Adult reproductive behavior in males is divided into three main stages consisting of the precopulatory stage, the copulatory stage, and the postcopulatory stage. Within each of these stages are various events that define each stage (Senger 2005). If a female is in estrus or if the male has been trained to be collected at a dummy, all of the events within these stages may take only a few minutes. However, in pigs this series of events is usually much longer, with ejaculation lasting up to ten minutes (Bearden et al. 2004).

The first step in the precopulatory phase is sexual arousal. Once the boar has received the right stimuli, he will achieve an erection, which is under control of the autonomic nervous system. Blood is then pumped into the penis and trapped there
temporarily. During erection boars experience an extension of the penis with little increase in size, unlike the stallion. This is due to boars having a fibroelastic penis, with a small area of cavernous tissue where the blood collects (Bearden et al. 2004). During this time it is common for males to grunt, nuzzle the female or dummy, grind their teeth, and foam at the mouth (Senger 2005).

The next phase of reproductive behavior is the copulatory phase. Boars will mount mainly in response to visual and tactile factors. Mounting dummies have been shown to be the greatest stimulus to a boar (Chenoweth 1981). After mounting, intromission occurs. Intromission is the insertion of the penis into the vagina or other collecting device and is followed by ejaculation. In natural mating, the semen would be deposited into the cervix and uterus.

Ejaculation is initiated by nerves in the glans penis, which triggers a set of contractions involving smooth muscle. Fluids from the accessory sex glands are also pumped into the urethra to combine with spermatozoa at this time (Bearden et al. 2004). Two to three waves of semen may be ejaculated during a single collection (Signoret et al. 1975). Among domestic animals, the boar has the longest ejaculation time. It can last anywhere from three to ten minutes, with an average of four to five (Bearden et al. 2004; Signoret et al. 1975). Due to this, the boar is considered a sustained copulator like the dog, and in contrast to short copulators such as the bull and ram (Senger 2005). Swine also produce the largest amount of semen among farm animals, with an average volume of 200 ml. However, their total
spermatozoa concentration is of an intermediate value (200 million per ml of semen) which is lower when compared to the bull, ram, and buck. Boars also have a segmented ejaculate, which can be divided into three fragments: a spermatozoa-free fraction, a spermatozoa-rich fraction, and a spermatozoa-poor fraction which includes a large volume of gel that is separated out during collection (Bearden et al. 2004).

The postcopulatory phase immediately follows ejaculation. During this time, the boar will dismount and undergo a refractory period, in which a second copulation will not take place. A memory will then be formed regarding the experience which is helpful when training young boars. The refractory period is not to be confused with sexual exhaustion; it is actually a part of satiation. Sexual satiation describes the condition in which further stimuli will not cause a response under normal stimulus conditions, whereas sexual exhaustion describes a time when no further sexual behavior can be induced with added stimuli. The refractory period can vary in length due to many factors including degree of rest before collection, age, breed, and number of previous ejaculations (Senger 2005). After the refractory period is complete, restimulation may occur. The memory which is created immediately following collection or natural service serves an important role in sexual behavior. Positive experiences will serve to promote sexual behavior and negative experiences are likely to inhibit normal sexual behavior (Senger 2005). Some cases of impotence may be attributed to a previous negative experience, but are usually reversible with further conditioning (Wodzicka-Tomaszewska et al. 1981).
Abnormal Sexual Behavior

When a boar has been managed and trained well, he will most likely perform well during collection or natural service. However, when these requirements have not been met or when another factor has contributed, a boar may show low levels of sexual behavior which are not conducive to collection in a large commercial operation. Poor sexual behavior may be attributed to low sexual motivation or poor mating competency, and reports suggest it was a leading reason for culling of commercial boars in the past (Hemsworth and Tilbrook 2007). As discussed earlier, season may also have an adverse effect on sexual behavior or libido; that is, extremely high temperatures can show a change in libido (Okere et al. 2005). These effects can be avoided by collecting early in the day, when boars are most willing to cooperate during a hot season.

Low sexual motivation can be explained in a variety of ways. It is known that both genetic and psychological components play a large role in level of motivation. Injuries to the penis or ones that restrict movement can have a detrimental effect to normal sexual behavior, as well as obesity and any skeletal defects a boar may have (Hemsworth and Tilbrook 2007).

Additionally, poor mating competency can manifest as a problem mounting, achieving erection, or at various other time points during collection. Mating competency refers to a male’s ability to copulate; this includes all the steps starting with mounting which may be the most problematic in terms of collection. Correct mounting from behind is a learned behavior, and is one reason for raising boars around other boars. It may take a long
time to be able to correct, and for some operations this means a loss in revenue or more likely, culling of the animal (Hemsworth and Tilbrook 2007). It is well-known that in some zoo animals, incorrect mating postures or lack of knowledge of correct mating procedures may contribute to an animal’s inability to breed (Sun 2004).

Factors outside of the boar’s control such as season, isolation, and housing can prove to be real indicators of a reproductive problem. This study’s goal is to investigate one of these external factors, housing, and to monitor its effect, if any, on libido, sexual behavior, and sperm viability.

Effects of Stress on Animals

Welfare of production animals is increasingly a debated topic among scientists, the media, and animal welfare activists. Therefore, it is important to be able to assess an animal’s level of comfort within its production system. Stress can be defined as a biological response elicited when an environmental change may affect an animal’s homeostasis (Moberg 2000; Broom and Johnson 1993). These environmental factors are due to natural causes as well as human intervention including genetics, age, nutrition, and experiences (Blecha 2000). Much of the research that has been conducted regarding swine and other livestock has been focused on temperature as a stressor. Even within this limited area, a majority of research has investigated stress effects on sows rather than boars. While temperature is not the focus of
this study, some observations may be similar to those of temperature studies, and of studies done involving sows.

Stress can be divided into two categories: acute and chronic. Acute stress denotes a stressor that is intense and lasts for only a short period of time whereas chronic stress refers to a stressor that is present long-term. When an animal receives a signal that a threat exists, several neurophysiological mechanisms engage in a biological effort to respond to the threat to prevent damage to the animal. Neural signals are sent to the centers of the nervous system to create a combined response. The body then translates these signals to biological responses that can be seen among several body systems. Most notably, the neuroendocrine, immune, and reproductive systems are affected (Moberg 2000). The degree of response of each of these systems depends upon the duration and intensity of the stressor. Response in pigs can also be attributed somewhat to genetics (Murani et al. 2010).

Neuroendocrine responses to stress assess the impact that a stressor may have on the communication that takes place between the central nervous system and endocrine glands. The most well-known stress response is the activation of the hypothalamic-pituitary-adrenal (HPA) axis, as its primary role is to maintain homeostasis of an animal (Matteri et al. 2000; Abel and Majzoub 2005). The HPA axis is mostly regulated by corticotropin-releasing factor (CRF). CRF and vasopressin act together to stimulate production of proopiomelanocortin (POMC), and the release of adrenocorticotropic hormone (ACTH). ACTH is released in a circadian rhythm and acts with its receptor to stimulate release of the adrenal steroids
aldosterone, corticosterone, and cortisol (Abel and Majzoub 2005). Cortisol and corticosterone are glucocorticoids that act to convert glycogen to glucose to use as an energy source during a stressful event. This is necessary to sustain a metabolic response to be able to cope with the stressor. Aldosterone is used to regulate mineral balance, and contributes to maintenance of blood volume, blood pressure, and blood composition (Ewing et al. 1999). Steroids produced by the HPA axis create a cascade of responses by the other body systems.

HPA-axis activation has a genetic component in pigs; domestic pig breeds show lower cortisol levels than pigs in the wild. It is unclear whether this decrease in cortisol is due to selection for leaner body composition or due to domestication, however. Also, species that have been bred for tameness show decreased HPA activity during stress (Murani et al. 2010).

The immune system is also affected during a time of stress in animals. The immune system enters a serious depression in response to a prolonged stressor with a large energy expense. Host immunity also experiences a decrease in the ability to defend off environmental pathogens, creating an opportunity for microbial infections (Amadori et al. 2009). Other effects from stress include changes in lymphocyte number and ratio of helper : suppressor T cells, decreased lymphocyte proliferation and natural killer (NK) cell number and activity, and reactivation of latent viral infections. NK cells kill viral infected cells, bacteria, parasites, and fungi. T cells destroy foreign or non-self cells as well as prompt B lymphocytes to make antibodies. Lymphocytes usually proliferate during infection in order to
bolster defense against infection (Webster Marketon and Glaser 2008). Decreases in all of these cell types due to stress can seriously alter an animal’s health.

A pig’s reproductive capacity can also be seriously altered, as the processes that regulate reproduction are very sensitive to stress (Lovejoy and Barsyte 2011). For example, in sows heat stress decreases reproductive efficiency and produces seasonal infertility (Williams 2009). Sows have also been shown in several studies to have impaired reproduction when housed in groups as compared to those individually housed. The mixing of unfamiliar sows has led to increased levels of cortisol in plasma (Kongsted 2004). Administered cortisol has been shown to impair the pre-ovulatory estrogen surge, pre-ovulatory luteinizing hormone (LH) surge, and timing between estrus behavior and the LH surge (Kongsted 2004). All of these events serve to reduce the reproductive capacity of sows. In boars, however, impaired reproduction may be observed as reduced sperm counts and impotency. Poor husbandry methods can also lead to the loss of an animal’s ability to breed or its libido (Lovejoy and Barsyte 2011). Reproduction is also closely related to energy – animals have only a finite amount of energy at a given time. Therefore, if a stressor is introduced, the animals will opt to use that energy for other more important processes than reproduction. Because stress also decreases the need to eat and digest, energy intake will be inhibited as well (Lovejoy and Barstye 2011). However, domestic animals which have been selected for good reproductive performance may continue to show normal reproductive performance even under difficult conditions (Broom and Johnson 1993).
In sows, stress from relocation or interaction with a male is followed by an increase in plasma cortisol as little as fifteen minutes after the stressful event (von Borell et al. 2007). Other findings have indicated that after a combination of heat, spatial, and mixing stresses, cortisol levels were significantly higher in the stressed groups on days seven and fourteen and during the overall two week period of the study (Sutherland et al. 2006). Effects on reproduction are of great importance in commercial operations, so understanding the mechanisms and stressors is key.

**Environmental Factors Affecting Reproductive Success**

*Temperature*

Temperature is the most documented stressor in pigs. There have been many studies investigating the effects of both hot and cold temperatures on reproduction. Boars housed in a hot environment regularly have lower sperm concentrations, overall motility, total sperm per ejaculate, and morphologically abnormal sperm (McNitt and First 1970). Experiments involving heat stress subjected boars to a temperature greater than 33°C. In a 72 hour heat stress study, semen was collected during the heat stress event and every 4 days for a 64 day period. Boars produced spermatozoa with coiled tails, abnormally shaped heads, and sperm with no tails. These morphological abnormalities as well as decreased motility are due to heat stress during secondary spermatocyte and spermatid stages of spermatogenesis. When heat
stress was observed at a later time (concentration levels and total sperm), sperm were affected during the primary spermatocyte stage. This can be explained by the heat stress disrupting spermatogenesis and sperm maturation at all stages of development at the same time. Then in the following weeks sperm abnormalities in ejaculates changed depending on the location of the sperm in the testis or epididymis at the time of the heat stress (McNitt and First 1970). Exposure to hot temperatures for as little as six hours a day increases abnormal sperm counts (Kunavongkrit 2005). Sperm viability for both fresh and frozen semen is also decreased in response to heat (Sibblies 2006). While semen volume may remain similar between heat-stressed and control boars, the relative makeup is subject to change. Differences in volume between the gel fraction and sperm-rich fraction can be seen, although total volume remains the same (McNitt and First 1970).

Heat stress affects libido, as the vigor of courting and mounting behavior decreases with an increase in temperature. Therefore, collections should be scheduled for the coolest part of summer days. Changes in semen quality have an impact on fertility, and thus, farrowing rate. When sows were inseminated, heat stressed boars only had a 59% pregnancy rate, compared to 82% of control boars (Curtis 1985). Cold temperatures also affect sperm production, although the effects and mechanisms are less well known.

Outward signs of heat stress that indicate a problem for the pig include panting, heavy breathing, a decrease in feed intake, and variations in lying patterns that will increase their surface area with the ground. Likewise, cold stress is identified by huddling (Hoff 2001).
However, because boars cannot lie close to other pigs in normal commercial operations, they are less able to offset extreme cold by huddling (Brent 1986). In order to combat extreme cold, some suggestions for modifications in the pig’s environment are to provide supplemental heat and bedding, and group animals to allow huddling. By huddling, pigs can live in a lower critical temperature than they would be able to if housed individually (Smith and Crabtree 2005). Interventions to cool swine when it is too hot include supplemental cooling, increased ventilation, and reducing the amount of bedding (Ewing et al. 1999).

Photoperiod

Photoperiod only has a slight influence on reproductive success, if any. It is often studied along with temperature, without differentiating results between the two factors. A study investigating the effect of seasonal light on boars showed that steroid synthesis, sperm count, and libido are lower during the long day summer than during the short day winter (Claus et al. 1983). In pre-pubertal boars, the same is true: decreasing plasma testosterone occurs during periods of decreasing day length (Claus and Weiler 1985). Andersson et al. found that when they avoided large changes in photoperiod, a short day photoperiod induced maturation of spermatogenesis (1998). Large changes in photoperiod tend to disrupt testicular steroidogenesis (Andersson et al. 1998).
Another study, however found the opposite – that decreasing day length reduces sperm quality (sperm production and sperm concentration) when compared to boars exposed to increasing day length (Sancho et al. 2004). They report that this is due to impaired testicular function. However, other measures of the study such as sperm vitality and motility did not show a difference between increasing and decreasing photoperiods (Sancho et al. 2004). A previous study by Minton et al. shows similar findings that longer daily photoperiods (16 hours vs. 8 hours) result in higher concentrations of testosterone at six months of age (Minton et al. 1985). However, when varying amounts of light were given each day to prepubertal boars, no difference in testicular function was noted therefore light only needs to be present in a boar’s environment for a short period of time each day (Minton and Wetteman 1987). These conflicting studies highlight the need for further research on the effect of photoperiod on the reproductive capacity of boars.

Housing

Housing can refer to a variety of topics including socialization with other animals, individual space allocation, and degree of grouping. As mentioned previously, socialization with other pigs is vital to the proper development of a good performing artificial insemination (AI) boar. Without this, boars may lack an appropriate sex drive.
The effects of space on reproduction are essential to understand due to increased pressure from both American and European animal welfare agents and governments wishing to impose new regulations on farm animal housing. In a usual 24-hour period, animals spend a majority of their time using their space to sit or lie comfortably. For the other few hours in the day, animals need more space in order to perform particular behaviors such as foraging or exploratory behaviors in swine, or wing-flapping in poultry. It is known that space restriction may cause animals to show frustration behaviors; however if space is too restricted, animals may not have enough room to even perform these necessary behaviors (Nicol 2007). The U.S. Government has set space requirements for all developmental stages of swine. For boars, the recommended amount of floor space for a single animal is between twenty and eighty square feet, depending on the type of barn in which they live and the age of the boar (Fritschen and Muehling 1981). If combined with a collection area, the size should be extended to 108 square feet (Brent 1986). Alternatively, a crate size of 2’4” by 7’ is recommended for confinement systems (Ewing et al. 1999).

Gilts and sows raised in the crate (or confinement) system often show delayed puberty, irregular cycles, more silent heats, and lower conception rates (Esbenshade et al. 1979). Boars showed higher libido the first time placed with a sow than those raised in pasture. Confinement boars also had less firm testes, but no difference was found in the sperm characteristics measured. These included semen volume, total sperm number, gel weight, percent motile sperm, and percent total sperm (Esbenshade et al. 1979).
In female mice, Whittaker et al. found no difference in reproductive measurements between those housed in large versus small individual cages (2012). The only difference found was a slight trend towards higher body weights and shorter inter-birth intervals in those housed in small cages (Whittaker et al. 2012). Much more research is needed to determine the spatial requirements of boars, and their reproductive responses.

The last factor of housing that is of importance to swine reproduction is animal density, or the number of animals per pen. In mice, crowding contributes to shorter seminal vesicle and testis length (Whittaker et al. 2012). In pigs, an unusually large number of animals per pen may increase the chance of aggressiveness between members of the group as they assert their dominance (Sutherland et al. 2006). Optimal stocking density is a small group around five to eight individuals, because pigs in the wild tend to live in small groups (Bracke et al. 2002). Due to potential aggression, care must be taken when introducing new members to the group. In reference to reproduction specifically, sows have been documented with various responses to group housing. One study reported that there was no difference in fertility between individual and group housed sows (Jansen et al. 2007). However, in a different study that investigated early pregnancy effects of group housing, it was shown that there was a detrimental effect when compared with sows kept in stalls through day 28 of pregnancy (Munsterhjelm et al. 2008). This was most likely due to social factors such as aggression and fear, as well as individual differences in food intake. Another outcome of group housing was a high irregular rebreeding rate that approached seventy percent.
(Munsterhjelm et al. 2008). Boars do not have similar group housing information because it is inadvisable to house them together due to increases in aggression among individuals.

Several environmental and genetic factors impact a boar’s sperm production and quality. Importance is placed upon producers increasing reproductive traits and decreasing stress factors which are under their control. Strategies to reduce these negative impacts are vital to maintaining a productive breeding herd.


ASSESSING BOAR REPRODUCTIVE, PHYSIOLOGICAL, AND BEHAVIORAL RESPONSE IN TWO DIFFERENT HOUSING ENVIRONMENTS


**Introduction**

In order to maximize production from each boar, a producer must carefully evaluate the quality of semen at each collection. Any small change during spermatogenesis can severely alter the final spermatozoon. Several environmental factors can impact the quality of semen. Some of these factors include temperature, social interactions, nutrition, photoperiod, age of the boar, schedule of collection, and housing environment (Kunavongkrit, 2005). It is imperative for the producer to provide an ideal environment in order to maximize semen production from each boar. If this is not achieved, stressors applied to the boar can instigate a change in reproductive fitness. Spermatozoa from boars may be subject to morphological abnormalities, decreased concentrations, and decreased volume. The boar may also exhibit changes in libido resulting in poor ejaculates. Several hormones including LH, testosterone, prolactin, glucocorticoids, and heat shock proteins also fluctuate in response to stress (Wingfield and Sapolsky 2009; Weissman et al. 2006). Hormonal changes may then lead to changes in behavior which can be seen more readily as possible indicators of stress. Considering these factors, the objective of this study was to determine the response, if any, to possible stress caused by a change in the housing environment of a boar. Several reproductive, behavioral, and physiological variables were evaluated in order to assess the response of boars.
**Materials and Methods**

**Animals**

There were fourteen crossbred boars (Duroc x Pietran x Yorkshire x Large White) used in the study. Seven sets of littermates were chosen for study animals such that one boar from each pair went to each treatment group. Eight boars selected were young boars, and six boars selected were mature, or older, boars. The young boars weighed $109.7 \pm 1.7$ kg, and were $11.1 \pm 0.1$ months old. The mature boars weighed $183.7 \pm 3.3$ kg, and were $22.2 \pm 0.3$ months of age.

Seventy mature crossbred (Yorkshire x Landrace x Large White) sows were also used in the study. These sows had an average parity of $3.5 \pm 1.1$ litters.

Six hundred eighty-four, 1-day old piglets were also used in the study. These piglets were the result of several mating combinations of the 14 boars to the 70 sows. When the piglets were each 1 day old, skin samples were collected and they were ear notched for identification purposes.

All experimental procedures performed on the boars, sows, and piglets, were approved by the North Carolina State University Institutional Animal Care and Use Committee and were consistent with policies and procedures described for swine in the Guide for the Use and Care of Animals in Agricultural Research (FASS 1999).
Facilities and Housing

The study was conducted at the North Carolina State Swine Educational Unit, an operation which houses animals from farrowing to finishing which is located in Raleigh, North Carolina. Prior to the start of the study, the young boars were all housed in groups of two pens that measured 1.82 x 2.43 meters. The older boars were housed in individual crates, measuring 0.91 x 2.13 meters. All boars were housed in a building that was curtain-sided with an under-slat ventilation system. The building also had misters for supplemental cooling. Pit fans were located at the end of each air plenum on each end of the barn and ran at full speed continuously. Cooling fans were also in the building, and were equally distributed throughout. These fans were set to activate when the ambient temperature reached 23.8°C. The misters in the barn were set to activate when the ambient temperature reached 25.5°C, and stayed on for 7 out of every 10 minutes.

All boars were fed 3 kg of a 15% protein, corn, and soybean-based diet daily. This diet was balanced to meet the maintenance requirement for breeding boars set by the NRC (2005). They also had ad libitum access to water via a nipple type waterer. Waste was removed via an under-slat flush system. This system used recycled water from a second stage lagoon. The system was flushed once every 12 hours during winter months, and once every 6 hours during the summer months.

After breeding, sows were housed in individual gestation stalls, measuring 1.0 x 3.0 meters. These stalls were located in a curtain-sided building with an under-slat ventilation
system. The building also included 18 stirring fans (5000 cfm per fan), which were organized in three rows of six fans each. The gestation barn also included a dripper system that activated when the ambient temperature reached 26°C. These drippers then ran for 8 out of every 10 minutes to provide supplemental cooling for sows during the summer months. During gestation, sows were fed between 2 and 4 kg of a 15% protein, corn, and soybean-based diet that was also formulated to meet all nutrient requirements for pregnant sows expected to gain 30kg during gestation (NRC 2005). Feeding levels were adjusted weekly on an individual basis after a visual estimation of a sow’s body condition.

At 108 days of gestation, sows were moved into farrowing rooms which were solid-walled; these rooms were equipped with a side-wall baffle ventilation system. Evaporative cooling units were the main air inlets, and were used when the ambient temperature reached 24°C. Each farrowing room included 12 farrowing crates which measured 1.5 x 4.0 meters. Sow movement within each crate was restricted to an area which measured 1.0 x 3.5 meters. Piglets were allowed access to the entire crate, allowing some area that the piglets could go to avoid danger from the sow. During lactation, sows were fed according to their appetite. The peak daily lactational intake for all sows during the study averaged 8.3 ± 1.2 kg. The lactation diet was an 18% corn and soybean meal-based diet formulated to provide adequate energy, vitamins, and minerals for sows anticipated to wean litters in which piglets gained 300 g per day (NRC 2005).
Experimental Design

Five weeks before the experiment began, half of the young boars were randomly selected to be moved to 0.91 x 2.13 m crates. The other four young boars were housed individually in 1.82 x 2.43 m pens. During this same time period, the mature boars were distributed in a similar manner. Half of this group (n=3) were randomly chosen to remain in the 0.91 x 2.13 m crate. The other 3 boars in the mature boar group were then moved to the 1.82 x 2.43 pens, to be housed individually. This ensured that there were 7 boars in each housing scenario (4 young and 3 mature), and that littermate pairs were equally distributed across the different treatment groups. Both the pens and crates in which the boars were housed were located across an alley-way from each other in the same barn.

After an initial acclimation period, boars remained in their respective housing treatment (pens versus crates) for 10 weeks. These were weeks 1 through 10 of the study. During week 11, the boars were switched. Therefore, all the boars housed in pens were moved to crates, and all the boars housed in crates were moved to pens. Boars then remained in their new housing treatment for another 10 weeks. This corresponds to weeks 12 through 21 of the study. Semen was collected weekly (weeks 1 through 21) from each boar. Estimates of boar libido, semen quantity, and semen quality were recorded at each collection by an experienced technician. During weeks 1, 6, 11, 17, and 21, body weights, hair growth, hormone levels, and behavioral observations of boars were evaluated. During weeks 8, 10, 19, and 21, heterospermic inseminations were performed. These inseminations used a
combination of semen from boars in different housing treatments, and subsequent paternity
testings were performed. This was done in an attempt to evaluate any potential effects from
housing conditions on relative fertility levels.

The acclimation period for all the boars began on January 24, 2011. Data collection
for the boars began on February 28, 2011 and ran through July 22, 2011. Sows used for
heterospermic insemination and paternity testing were bred during the weeks of April 25,
May 9, July 4, and July 18, 2011.

Table 1. Schedule of Study.

<table>
<thead>
<tr>
<th>Week of Study</th>
<th>Data Collected</th>
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<tbody>
<tr>
<td>Week 1</td>
<td>Semen collected, weight, hair growth, blood samples, behavioral observations</td>
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<td>Week 2</td>
<td>Semen collected</td>
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<td>Week 3</td>
<td>Semen collected</td>
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<td>Week 4</td>
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<td>Week 5</td>
<td>Semen collected</td>
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<tr>
<td>Week 6</td>
<td>Semen collected, weight, hair growth, blood samples, behavioral observations</td>
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<tr>
<td>Week 7</td>
<td>Semen collected</td>
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<tr>
<td>Week 8</td>
<td>Semen collected, heterospermic inseminations</td>
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<tr>
<td>Week 9</td>
<td>Semen collected</td>
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<tr>
<td>Week 10</td>
<td>Semen collected, heterospermic inseminations</td>
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Table 1 Continued

<table>
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<tr>
<th>Week</th>
<th>Activity</th>
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<tbody>
<tr>
<td>11</td>
<td>Semen collected, weight, hair growth, blood samples, behavioral observations; <strong>Housing environment was switched AFTER data collection was finished for the week.</strong></td>
</tr>
<tr>
<td>12</td>
<td>Semen collected</td>
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<td>13</td>
<td>Semen collected</td>
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<td>17</td>
<td>Semen collected, weight, hair growth, blood samples, behavioral observations</td>
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<td>18</td>
<td>Semen collected</td>
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<tr>
<td>19</td>
<td>Semen collected, heterospermic inseminations</td>
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<tr>
<td>20</td>
<td>Semen collected</td>
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<tr>
<td>21</td>
<td>Semen collected, weight, hair growth, blood samples, behavioral observations</td>
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<td>18</td>
<td>Semen collected</td>
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<tr>
<td>19</td>
<td>Semen collected, heterospermic inseminations</td>
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</tbody>
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**Semen Collection, Boar Libido, and Semen Quantity Estimates**

Collections for each boar were done weekly using the double-gloved hand technique (Almond et al. 1998) during exposure to a collection dummy. The gloves used were powder-free, polyvinyl (IMV America, Eden Prairie, MN). The collection dummy, or dummy sow,
(Minitube of America, Verona, WI) was 1.5 m long and adjusted to a height of 0.67 m high during collection periods. The collection vessel was an insulated 1-quart thermos that was heated to 37°C prior to collection. A plastic, 1-quart collection bag (IMV America, Eden Prairie, MN) was placed inside the thermos. The mouth of the thermos was covered with a milk filter (IMV America, Eden Prairie, MN) and secured in place with a rubber band. This filter was used to separate out the gel fraction of the ejaculate. The thermos was then weighed on a programmable scale (Fisher Scientific, St. Louis, MO) to get an initial weight. The entire ejaculate from the boar was then collected.

During the collection procedure, reaction time and collection time were recorded by a second technician as an estimate of boar libido. Reaction time was defined as the amount of time it took for the boar to make contact with the dummy after entering the collection pen. Collection time was defined as the amount of time it took to collect the entire ejaculate. The collection period started when the boar’s penis was fully extended, and was terminated when boars would no longer mount the dummy. Boars were allowed to dismount and remount as many times as they wanted to during the collection. In this case, when boars dismounted the dummy, the timer paused the recording of collection time for as long as the boar was not on the dummy sow. The recording time was then restarted when he remounted the dummy, or stopped completely if he did not remount. All boars were collected by the same experienced technician during the study.
The schedule of collection included collections on Tuesdays and Thursdays each week. Since each boar was only collected once weekly, boars were split into groups so that 7 were collected on Tuesday and the other 7 were collected on Thursday of each week. Each day, the collection order was randomized. There was an exception to the Tuesday-Thursday collection during the four weeks of heterospermic inseminations. In order to have semen available for the course of the scheduled breeding weeks, boars were shifted to collect on Monday and Wednesday. Therefore, boars that were usually collected on Tuesday were shifted to collect on Monday, and those that were usually collected on Thursday were instead collected on Wednesday during these four weeks. This was done to keep up with the daily breeding demands of the overall operation of the swine unit.

Immediately after collection, the volume and concentration of spermatozoa were determined. After these measurements were determined, the ejaculate was placed into a 37°C water bath while the other boars were being collected. The thermos including ejaculate was weighed on a programmable scale (Fisher Scientific, St. Louis, MO) in order to estimate volume. Weight was used to determine volume based on the assumption that 1 mL of semen weighs 1 g. The initial weight of the empty thermos container setup was then subtracted from this final weight to estimate volume. The concentration of the spermatozoa was determined using a self-calibrating spectrophotometer (SpermaCue®, Minitube of America, Verona, WI).
After the initial measurements, a 5 mL sample from each ejaculate was put into a portable incubator also set to 37°C. The samples were all transported back to the laboratory in this portable setup. The remainder of each ejaculate was processed for heterospermic inseminations. The laboratory which was used for further analyses was located in Polk Hall on the main campus of North Carolina State University in Raleigh, NC which is approximately 10 minutes from the research farm. Once the samples were in the laboratory, the temperature of each sample was determined. Then the samples were placed into a preheated water bath set to 37°C.

**Semen Motility Estimates**

A computer-assisted semen analysis system (SpermVision®, Minitube of America, Verona, WI) was used to determine various measures of semen quality. The proportion of motile spermatozoa, proportion of spermatozoa exhibiting progressive forward motility, average curvilinear velocity of motile spermatozoa (VSL), average curvilinear velocity of motile spermatozoa (VCL), average straight line distance traveled by motile spermatozoa (DSL), and average curvilinear distance traveled by motile spermatozoa (DCL) were estimated.

In preparation for these analyses, one mL of semen from each ejaculate was extended in a 1:13 ratio with Androhep Plus® (Minitube of America, Verona, WI) extender. To begin
the process, the neat semen was added to a 50 mL conical tube (Port City Diagnostics, Inc., Wilmington, NC), and then the extender was added. This was done in such a manner so that the extender slowly dribbled down the inside of the conical tube. This process was done slowly to minimize the exposure of spermatozoa to any temperature shock. A one mL sample of this extended semen was put into a test tube (12 x 75 mm; Port City Diagnostics, Inc., Wilmington, NC) and allowed to again equilibrate to 37°C inside an incubator (Fisher Isotemp Oven 200 series; Fisher Scientific, Atlanta, GA) for 30 minutes.

After incubation, the sample of extended semen was removed and swirled gently to thoroughly mix the sample until a uniform suspension of spermatozoa was achieved. Then, three µl of this sample were placed into a Leja slide (Minitube of America, Verona, WI) which was pre-warmed to 37°C, and analyzed on an Olympus microscope (BX41). The microscope was equipped with a heated stage that was maintained to 37°C. After the first field was analyzed, the slide was randomly moved to show a different field. Twenty different fields (consisting of 600 to 800 spermatozoa depending on initial concentration of ejaculate) were analyzed for each boar’s extended semen sample.

**Semen Morphology**

In order to analyze spermatozoa morphology, one mL of extended semen used for motility and mobility analyses was placed into a test tube (12 x 75 mm; Port city Diagnostics,
Inc., Wilmington, NC). One hundred µl of a 10% formalin solution was added to the tube in order to prevent deterioration of the spermatozoa. Each tube was then thoroughly mixed. A 10 µl sample was then pipetted onto an ethanol-cleaned, glass microscope slide (Fisher Scientific, Atlanta, GA). An 18 x 18 mm glass cover slip (Fisher Scientific, Atlanta, GA) was then gently placed on top of the slide with sample. Spermatozoa were allowed to settle in the slide during a period of a few minutes, by simply letting the slide sit untouched.

All morphology analyses were performed using a phase contrast microscope (Zeiss, Olympus B071, Berlin, West Germany). The initial location of the spermatozoa was determined using the 25x objective. Once located, a drop of immersion oil (Fisher Scientific, Atlanta, GA) was added to the slide and the 100x oil immersion objective was used to visualize head, tail, and acrosome morphology. A random sample of 100 spermatozoa was counted, assessing both normal and abnormal features. Detached, small, large, and abnormal shaped heads were grouped together in a category designated as abnormal head morphology. Translocated, curved, and bent tails were grouped together in a category for abnormal tails. Spermatozoa with proximal or distal cytoplasmic droplets were placed in a separate category, however. The classification scheme for acrosome morphology was based on criteria reported by Pursel et al. (1972). According to this classification system, a normal acrosome with a normal shape would have a smooth and unbroken surface. An abnormal acrosome in contrast would appear to have a broken and uneven surface. All spermatozoa with abnormal acrosomes regardless of abnormality were grouped together in a single category.
Body Weights, Hair Growth, and Hormone Profiles

Body weights, hair growth samples, blood samples, and behavioral responses were collected from all boars during the following weeks of the study: February 28 (Week 1); April 11 (Week 6); May 9 (Week 10); June 27 (Week 17); and July 18 (Week 21), 2011. Body weights and hair growth estimates were collected on Wednesdays during each of these weeks. A patch of hair measuring 7.6 x 7.6 cm was shaved from the boar’s rump to estimate hair growth. The hair sample was then removed and weighed. The length and width of the area shaved was measured using digital calipers. Hair growth was expressed as mg/cm².

Blood samples were taken from boars during collection via the marginal ear vein. A rubber band was briefly placed around the base of the ear after the boar had begun to release the sperm-rich fraction of the ejaculate. An infusion set was used to collect from a large marginal ear vein and consisted of a 21 gauge, 2.54 cm needle attached to a 15.24 cm length of plastic tubing. Two 3 mL syringes were used to withdraw 5 mL of blood from the vein. Blood samples were put on ice immediately after removal and transported back to the laboratory. These samples were then allowed to clot overnight at 4°C. Serum was then separated from the cellular components by centrifugation at 1000 rpm for 20 minutes at 4°C and stored at -10°C for later analysis.

Serum concentration of testosterone (Diagnostic Systems Laboratory, Webster, TX), cortisol (Siemans Medical Solutions Diagnostics, Los Angeles, CA), and heat shock protein 70 (Stressgen, Inc., Ann Arbor, MI) were determined using commercially available kits.
Prolactin concentrations were determined using a previously validated radioimmunoassay (Howard and Britt 1990). By examining parallelism and recovery, the assay kits for testosterone, cortisol, and heat shock protein 70 were validated for porcine serum. Each of these showed parallelism with the standard curve over a range of dilutions between 1:2 and 1:32. Recovery of testosterone from serum obtained from barrows and supplemented with 5 to 25 ng/mL averaged 83%. Recoveries of cortisol and heat shock protein 70 from serum spiked with 5 to 200 ng/mL (cortisol) or 50 to 250 pg/mL (heat shock protein 70) were 82% and 71%, respectively. Assay sensitivities were 1.0 ng/mL, 2.0 ng/mL, 0.2 ng/mL, and 5 pg/mL for testosterone, cortisol, prolactin, and heat shock protein, respectively.

**Heterospermic Inseminations**

Semen used for heterospermic inseminations consisted of 6 billion spermatozoa, with 3 billion spermatozoa coming from each of two different boars, and extended in 80 mL of Androhep Plus® semen extender (Minitube of America, Verona, WI). Semen from the two different boars was combined in the following manner: each ejaculate was extended 1:1 by adding 3 equal parts of extender slowly over a 30 minute period, again, to prevent shock. After the 30 minute period which allowed each ejaculate to equilibrate with the extender, the partially extended ejaculates were combined so that there were 3 billion total spermatozoa from each boar. All extensions were performed at 37°C.
Boars used in the study contained 2 pairs of full siblings and 3 boars that were half siblings. Because of the difficulty in using paternity testing to distinguish piglets sired by siblings, heterospermic inseminations were arranged so that siblings and half-siblings wouldn’t be compared against one another in fertility evaluations. This resulted in semen from each of the 14 boars being used to breed between 8 and 10 sows total over the 2 periods of inseminations.

Sows were checked daily for estrus by exposure to mature boars (36 ± 2 months of age and weighing 257 ± 5 kg). Boars were walked down an alley way in front of the crates in which weaned sows were housed, and were confined to this area. While the boar provided face-to-face interactions with sows, two breeding technicians walked behind the sows and applied back pressure to groups of four to five sows. Sows that exhibited a standing reflex were inseminated using Golden Pig® insemination catheters (IMV America, Eden Prairie, MN). Doses for insemination were packaged in insemination cochettes (IMV America, Eden Prairie, MN), making sure they were used within 72 hours of collection. Sows were bred once during each day of estrus by one of two experienced technicians during the study.

Paternity Testing

Collins et al. (2008) provided the procedures to determine the paternity of the piglets born from heterospermic inseminations. For DNA samples from boars, DNA was extracted
from semen obtained during collection using a Puregene® purification kit for 60 to 100 million spermatozoa according to the manufacturer’s instructions (Gentra Systems, Minneapolis, MN). To obtain DNA samples from sows and male piglets, DNA was extracted from skin (tail samples) one day after farrowing using the Puregene® purification kit for 5 to 10 mg of mouse tail tissue according to the manufacturer’s instructions (Gentra Systems, Minneapolis, MN).

Once extracted, DNA samples were sent to GeneSeek, Inc. (Lincoln, NE) for paternity testing. GeneSeek, Inc. examined the genetic heterogeneity of 22 different alleles in their paternity testing procedures. A brief description of the procedures used by GeneSeek is as follows: oligonucleotide primers for paternity identification were selected based on normal variation inherent to the population of boars for DNA microsatellite markers from the X-Y chromosome map published by the USDA (www.marc.usda.gov/genome/swine/ChromosomeX-Y/). PCR reactions were performed for each of the microsatellite markers used by GeneSeek, Inc. Volumes of 20 µl were used and consisted of 0.2 µM of each primer (forward and reverse), 0.5 U of Taq DNA Polymerase, 100 ng DNA, 200 µM dNTPs and PCR buffers supplied by the manufacturer. Mineral oil was then placed on top of the reaction mixtures and the 0.5 mL reaction tubes were placed in a thermocycler. All reagents used were purchased from Qiagen (Valencia, CA) unless otherwise noted. The amplification reactions used the following program: 2 minutes at 94°C, 40 cycles of denaturing (10 sec at 93°C), annealing (20 sec at 58°C), and extension (20 sec at 72°C), ending with a final extension phase for 5 minutes at 72°C. For each reaction, a control
sample in which distilled water was added to the mixture instead of DNA was used. These control samples were processed via the same amplification cycle previously described. Single- and double-stranded DNA fragments from PCR amplification reactions were purified using QIAquick spin columns according to instructions provided by the manufacturer (Qiagen). Alleles were identified by gel electrophoresis (40 V/cm for 2 hours) with 8% denaturing acrylamide gels using sequencing gel apparati.

**Behavioral Observations**

In the barn in which the boars were housed, several video cameras were mounted. They were mounted so that approximately half of the boars housed in either pens or crates could be recorded simultaneously to assess their activity. Each camera could include 7 boars in the frame, and all boars were recorded at the same time. Each recording session was 30 minutes in length and was performed in the latter portion of the day: either from 1900-1930 or from 1930-2000 h. These recordings were conducted on Mondays of the weeks beginning on February 28 (Week 1), April 11 (Week 6), May 9 (Week 11), June 27 (Week 17), and July 18 (Week 21), 2011. Video footage was evaluated by two different technicians once all the recording sessions had been completed. During evaluation of each video session, the amount of time the boars spent standing, moving around, or lying down was recorded. Additionally, the occurrence of stereotypic behaviors such as playing with the waterer, bar biting, or attempts at social interactions with other boars was recorded as a qualitative response.
Each boar was also subjected to a modified version of a novel object test on Fridays during the weeks of February 28 (Week 1), April 11 (Week 6), May 9 (Week 11), June 27 (Week 17), and July 18 (Week 21), 2011. During weeks 1, 11, and 21, the novel object used was a rubber boot. During weeks 6 and 17, a 10 gallon plastic bucket was used as the novel object. In order to perform the novel object test, the following procedure was used. Each boar was walked to the opposite end of the barn in which they were usually housed, and put into a 1.82 x 2.43 side-walled pen. After two minutes of acclimation had passed, the bucket or boot was placed into the center of the pen. The amount of time it took each boar to make contact with the novel object after its placement into the pen was recorded. The length and number of each subsequent contact with the object was also recorded. Each novel object test lasted 10 minutes, and boars were removed from the test area and returned to their respective housing treatment afterwards. The order in which boars were chosen for the novel object test was randomized using the random number generator in Microsoft Excel®.

Statistical Analyses

All semen, hormonal, and behavioral data (both qualitative and quantitative) were analyzed with analyses of variance for repeated measure nested within a cross-over design (Steel et al 1997) using the mixed model procedures of SAS (SAS, Inc., Cary, NC). The statistical model included treatment (crate versus pen), period (weeks 1-10 versus weeks 12-21), time (week of study), and appropriate interactions. Boar nested within treatment and
period was considered to be a random effect and used to test for effects of treatment and its interactions. Initial analyses determined that the treatment by time by period interaction was not significant (p=0.37). For significant two-way interactions, differences either within period, between treatments, or changes over time within treatment were evaluated via the appropriate modifications of the original statistical model.

The proportion of piglets sired by boars housed in different housing scenarios was determined using analysis of variance procedures for categorical data with a model that included treatment and period (Hollander and Wolfe 1999).
Results

Semen Production

There was a significant interaction between time and treatment for four of the semen production traits (Table 2). Reaction time was significantly different (p<.05) between groups (Figure 1). Boars housed in a crate environment showed a longer average reaction time than those housed in a pen. Those that started in a crate and moved to a pen showed more variability in reaction time than those that started in a pen and moved to a crate (Figure 2). Larger variability in reaction time only occurred in the last 3 weeks for the group that started in the pen. A significant difference (p<.001) was also found between length of collection time for each group. Boars housed in a crate had a much shorter collection time than those housed in a pen. This trend was evident during all but one week of the study (Figure 3). Boars that started in crates experienced a marked increase in collection time whereas those that started in pens experienced very little, if any decrease in collection time after the move (Figure 4).

Similarly, there was a significant (p<.05) decrease in semen volume in crated boars as compared to boars in pens (Figure 5). Boars that moved from crates to pens had a dramatic increase in semen production, especially during the last quarter of the study. Boars that moved from pens to crates had relatively consistent semen production profiles (Figure 6). In contrast, concentration of sperm in crated boars did not decrease (Table 2), (p>.05).
Overall, there was a higher total sperm count in boars housed in pens when compared to those housed in crates (p<.05). Those housed in crates first produced more total sperm once moved into pens, and those housed in pens had decreased total sperm production once moved to crates. There were no major differences for individual boars that were not reflected in overall averages of sperm production. Housing affected all boars similarly with regard to production.

**Semen Motility**

There were no significant differences (p>.05) between treatment groups regarding overall motility and progressive motility (Table 3). There were nonetheless some tendencies in types of motility (Table 3). Both motility and progressive motility of sperm showed no differences between crated boars and those kept in pens (p>.05). There was a tendency (p=.07) for the curvilinear distance traveled by a sperm cell to be higher in semen from crated boars. Crated boars also tended (p=.07) to have a longer distance traveled by sperm over an average path. The same is true for distance traveled in a straight line; sperm from boars in crates tended (p=.08) to have a slightly longer distance traveled in a straight line.

There were slight tendencies regarding velocity of sperm as well (Table 3). Curvilinear velocity of sperm had a tendency (p=.09) to be faster in semen from crated boars. Similarly, average path velocity of crated boars also tended (p=.09) to be higher than their
counterparts in pens. Straight line velocity of sperm from crated boars also tended (p=.08) to be higher as well. There were no differences (p>.05) in any motility measurements when compared between periods – measurements taken from the first 10 weeks of the study did not differ from those taken during the second 10 weeks.

When separated by period a couple differences are of note, although none are significant (p>.05). Spermatozoa seemed to be most negatively affected when boars were housed in a pen (Figures 9, 10, and 11). Motility, distances traveled, and velocities all had higher averages for boars that were housed in crates, regardless of order. If boars were housed in pens first, spermatozoa motility increased when moved to a crate. If boars were housed in a crate first, spermatozoa motility decreased when moved to a pen. Although none of these observations show significant differences, they are noteworthy.

Despite no differences being found between those in crates and those in pens, there was an age effect on overall motility. Sperm from mature boars tended (p=.06) to have higher overall motility when compared to the young boars (Figure 12). The progressive motility, distance measurements, and velocity measurements were not different (p>.05) between the mature and young boars (Table 4).
Semen Morphology

Semen morphology was analyzed by grouping abnormalities together in a group based on location: head, tail, acrosome, or the presence of cytoplasmic droplets. There were no differences (p>.05) in the percentage of normal or abnormal tail morphology, as well as the presence of cytoplasmic droplets between treatment groups. There was also no difference (p>.05) in spermatozoa between treatment groups with regard to the percentage of normal heads, abnormal heads, normal acrosomes, abnormal acrosomes, and detached acrosomes.

However, when separated and analyzed by age of the boar, several differences were found in spermatozoa morphology (Figure 13). In mature boars, the percent of spermatozoa with normal tails was significantly (p=.005) higher. Concordantly, the percent with abnormal tails was significantly (p<.0001) lower in mature boars. There was not a difference in percentage of spermatozoa with cytoplasmic droplets between groups. Mature boars also had spermatozoa that showed a significantly (p<.0001) higher percentage of normal heads, as well as a significantly (p<.0001) lower proportion of abnormal heads. There was a significant difference (p<.05) in percent of spermatozoa with normal acrosomes. The percent of normal acrosomes was consistently higher in the mature boars. In contrast, no difference (p>.05) was seen regarding percent of abnormal acrosomes. The last category, percent of detached acrosomes, also showed a significant difference (p=.009) in that mature boars showed lower instances of these. Overall, it appears that mature boars have higher counts of normal spermatozoa than the younger boars.
Body Weights, Hair Growth, and Hormone Profiles

None of the variables categorized as possible indicators of stress showed any significant differences between groups. There was an increase in body weight in both groups throughout the study due to natural growth patterns, but there was no difference in the rate of growth between groups. This produced overall averages that were very similar and not significant (p>.05). Hair growth was also relatively constant throughout, with no real differences (p>.05) in the weight of collected hair between treatment groups.

Four hormones were measured and interestingly none of the four showed significant differences (p>.05). There was some slight variability in levels of each of these throughout the study, but again there was no real significance in these variations as data from both groups varied together. Results from cortisol, testosterone, heat shock protein 70, and prolactin measurements show no major differences between treatment groups. In fact, testosterone, heat shock protein, and prolactin all remained fairly constant throughout. Cortisol was the only hormone which showed a wider range in weekly changes, but these changes were seen in both groups.

Behavioral measures of stress were also not significantly different (p>.05). In observations of normal behavior, those in pens spent more time pacing when compared to those in crates, though these data were not significant (p>.05). There were no differences in the amount of time a boar was standing or lying within his enclosure. Boars were either observed as lying on their belly or lying on their side, and data show that even when given a
large area and the opportunity to lie on their side, some boars still preferred to lie on their belly. Other normal behaviors such as licking the bars and nosing other boars were also measured to be not significantly different (p>.05) between treatment groups.

The novel object test done with either a bucket or a boot introduced into a neutral pen also showed no significant differences (p>.05) between groups. The time it took to make contact, the total contact time, and the number of contacts with the object were all found to be similar for each treatment. Most often the boars were seen trying to mount the object more often than they were seen performing normal non-sexual investigative behaviors with it.

**Heterospermic Inseminations**

There was no significant difference (p>.05) between the percentage of piglets sired between the boars housed in crates and those housed in pens, for either period. Not all piglets were able to have their paternity determined however, due to the relatedness of some of the boars used in the study.
Discussion

It is essential for swine producers to enact management schemes which will contribute to higher production from their breeding boars. Several factors contribute to the overall semen production and breeding quality of a boar. These factors include testicular capacity, libido, and physical soundness which are in turn determined by another set of factors that influence the overall reproductive fitness of a boar. Studies indicate that elements such as heritability, nutrition, age of the boar, photoperiod, housing conditions, social environment, and schedule of collection all contribute to the reproductive performance of a boar (Smital, 2009). In the present study, manipulation of the housing environment was examined to determine what effects, if any, the amount of space, pen or crate, had on semen production, hormone production, and behaviors which may be indicative of stress.

Numerous studies in various fields have indicated that reproduction is inhibited when stressors are applied to an animal. The reproductive success of mammals often becomes a trade-off in order to ensure survival of an individual by moving necessary energy to vital body systems that are crucial for survival during times of stress (Wingfield and Sapolsky 2003). One example is heat stress which damages DNA within sperm cells as well as reduces sperm output, decreases sperm motility, and increases abnormal morphology (Hansen, 2009). Stress can also decrease LH levels, as shown in rats (Chand and Lovejoy 2011) and decrease GnRH levels as well. An increase in the stress-induced secretion of prolactin may be the cause for the reduction in LH (Wingfield and Sapolsky 2003). Testosterone is also often
reported to show a decrease in circulation during periods of stress, including restraint stress (Weissman et al. 2006). Stress may also prohibit erection or cause premature ejaculation (Wingfield and Sapolsky 2003). It is expected due to these findings in other mammals, that housing boars in larger areas may reduce stress responses that may be created by confined living areas. It is expected that boars housed in pens should have a lower incidence of abnormal sperm, produce more sperm, produce low levels of stress-related hormones, and show fewer abnormal behaviors.

Before use as a breeding animal, boars are trained to collect with most completing training within three weeks. Boars also begin training at an average of 7-9 months in age (Knox et al. 2008). This training is vital to ease collection and reduce labor costs associated with time spent collecting animals. Therefore, indicators of sexual libido such as reaction time, along with collection time, can serve as markers of reproductive performance when evaluating a production system.

Differences in reaction time were observed in this study. Boars housed in pens had shorter reaction times than boars housed in crates. Levis and Reicks (2005) reported no correlation between time to first mount a dummy and the total number of sperm per ejaculate, though all boars were housed in the same conditions. They argued that design of the pen may have an impact on the duration of reaction time as well as duration of time before the boar leaves the collection pen (Levis and Reicks 2005). Likewise, Hemsworth and Galloway
(1979) found that reaction time and time to begin collection was shorter in boars that had been sexually stimulated prior to collection.

Perhaps the difference in reaction time in the present study may be explained by a decrease in stressors on the boars housed in pens. Testosterone is the hormone most closely related to sexual behavior (Flowers 2008), and may be responsible for the decrease in reaction time for pen-housed boars. However, as mentioned previously there was no significant difference in testosterone levels between groups. Another explanation may include a boar’s familiarity with the collecting pen. Boars housed in pens are used to being in a larger pen such as the size of the collecting pen. It is possible that boars housed in crates spent more time investigating and exploring the collecting pen before mounting the dummy sow. In a similar study by Esbenshade et al. (1979), pasture raised boars showed the opposite trend; pasture reared boars consistently demonstrated longer recognition times than confinement reared boars. No further explanation was given for this phenomenon.

There was also a difference in collection time and volume during the study. Collection time was longer for boars from pens, and they produced a larger volume as well. It is known that older boars produce a larger quantity of semen than younger boars, and that crossbreds produce more than purebreds (Sonderman and Luebbe 2008). Decreased nutrition and hot environmental conditions also cause a decrease in semen volume (Foote, 1978). The difference in collection time volume may be attributed to a decrease in estradiol-17β levels. Estradiol-17β is involved in maintenance of libido and it has been demonstrated that this
hormone is responsible for maintaining semen volume in castrated boars (Louis et al. 1994; Joshi and Raeside 1973). This hormone was not measured in this study so further investigation into this area is needed in order to establish a correlation with semen volume. Kunavongkrit et al. (2005) also argue that a decrease in collection time often follows a decrease in libido, which can be seen in the results from the boars which were crated. Volume is not dependent upon collection time but results showed that as collection time increased, semen volume increased for boars housed in pens.

Total sperm per ejaculate was also found to be different between groups. If boars truly are more stressed in a smaller housing scenario, then these results support that hypothesis. The boars housed in pens first showed the greatest difference in total sperm per ejaculate earlier in the study. The boars moved from crates to pens also showed a slight increase in total sperm produced per ejaculate, even though their production had been increasing steadily from the beginning. Smital (2009) has shown breed differences can account for differences in total sperm, and Flowers (1997) attributed decreases in total sperm to periods of heat stress.

LH and follicle stimulating hormone (FSH) stimulate spermatogenesis in boars and are possibly the reasons for the lower total number of sperm produced by crated boars. If boars are chronically stressed, LH and FSH could be inhibited greatly due to decreases in GnRH levels. Restraint stress, which has slight similarities to the present study, was shown to have these effects in rams (Tilbrook et al. 2000). In a restraint stress experiment done with
male rats during development, it was found that those that were restrained for six hours a day showed significant reductions in the amount of maturing spermatids and in concentration of spermatozoa (Almeida et al. 1998). Results from the current study are in agreement with those found in mice during restraint stress. It is still unclear as to which mechanism is contributing most toward the increase in total sperm found in boars from pens. Further studies including collection of gonadotropins for analysis could prove useful in the future.

There were no differences seen in various semen quality traits, although some tendencies were found. As discussed previously, morphological changes which affect motility occur during spermiogenesis, which is the last stage in spermatogenesis. Stresses that occur when a spermatid is undergoing differentiation during spermiogenesis can cause defects in the normal functioning of a sperm cell. These defects may show morphologically and in motility measures.

In a heat stress study done by Wettemann et al. (1976), boars exposed to a constant increase in ambient temperature for 90 days presented ejaculations with decreased sperm motility and percent normal cells. Inseminations from heat stressed boars also had much lower fertility rates. Restraint stressed male rats have also been reported to have sperm that show decreased straight-line velocity, curvilinear velocity, and percent motile sperm. The explanation is given that acute restraint stress increased the activity of the HPA axis, and thus interfered with pituitary and gonadal hormone activity (Ren et al. 2010). Results from the present study show the opposite effect; distance and velocity of sperm were increased in
boars housed in crates. This suggests that boars may be more stressed in pens when sperm motility is evaluated. The reason for this is unclear; however moving to a new environment such as a pen from one which is familiar may be stressful for boars.

Various morphological abnormalities may have an effect on a boar’s fertility. These include abnormalities of the tail, head, acrosome, and the presence of cytoplasmic droplets. It was found that type of housing environment did not affect sperm morphology of these boars. However, when the age of the boar was taken into consideration, several differences emerged. There were higher instances of abnormal heads, tails, and detached acrosomes in younger boars. The increase of spermatozoa abnormalities with age has been noted in many species including humans (Chen et al. 2003). Data found in the present study however, contradict this common finding that has been applied to many mammal species. In boars, it has been shown that percentage of live sperm and the overall motility score both decrease as age increases (Kennedy and Wilkins 1984). Presumably, tail abnormalities affect motility of spermatozoa and may serve to decrease a boar’s motility score. Smital et al. (2009) reported in a data survey a strong influence of age on sperm output, but did not assess the effect of age on the degree of abnormalities in sperm.

The increase in abnormal sperm with age is a common reason for culling boars in commercial operations. In a survey by D’Allaire and Leman (1990), reproductive problems were the second leading cause of culling amongst boars, and boars in the survey averaged 20 months of breeding lifetime expectancy. In various surveys and studies, reproductive
problems consistently rate in the top three reasons for culling. Boars in the present study were approaching the average age for culling however, which does explain the correlation found between increased abnormalities and younger age.

No differences were found in morphological abnormalities between treatment groups or between periods of the study. This suggests that effects of housing may be limited to libido and overall semen production measures such as volume of ejaculate. These differences also suggest that housing does not affect the reproductive capacity of boars until after spermatogenesis has been completed and sperm have matured in the epididymis.

The findings from the four sessions of heterospermic inseminations also contribute to the explanation that overall fertility is not affected by a boar’s housing environment. Heterospermic inseminations are often used to assess differences in fertility between boars when no outward signs are detected (Stahlberg et al. 2000). The advantages that a sperm may have in heterospermic inseminations can be associated with the speed of attachment to and penetration of the egg (Dziuk 1996). In the present study, a difference in percentage of piglets sired between boars in different housing types would be expected if there was a difference in fertility. This was not found to be the case however, and fertility may be more dependent upon an individual boar’s reproductive capacity, or any number of other environmental factors that were not evaluated.

The manipulation of feeders or waterers in preparation of feeding is a stereotypic behavior that has been evaluated in swine (Lawrence and Terlouw 1993). Frustration levels
of pigs being deprived of a perceived need can be measured by examining activity levels, oral activity towards other animals, and oral activity towards other objects (Lewis 1999). The present study sought to examine the degree of these behaviors expressed as a response to differing housing environments. Animals showing stress would be expected to engage in pacing, biting bars of the cage, seeking out interaction with other boars, and oral activity towards unfamiliar objects. No differences were seen in any of the behavior parameters evaluated in this study, suggesting that if boars were stressed by changes in their housing environment, then that stress was not shown outwardly though changes in behavior.

A study was conducted in which pigs were not allowed access to food, but a either an empty feeder or a full feeder with the lid bolted shut was introduced during fasting. When regular feeders were removed, both pigs housed individually and in pairs showed increased activity levels, increased oral manipulation of the empty or bolted shut feeder, increased oral contact with other pigs, and increased cortisol levels (Lewis 1999). The absence of similar behaviors in the present study suggest that if boars were stressed, they were not acting out in response, or that boars were equally stressed in both housing environments. It is possible that if boars were allowed outdoor pasture access, a difference in these behaviors would show. Sows allowed access to the outdoors spent more time standing and walking than those confined in crates (Hotzel et al. 2004). It would be difficult to discern if boars would react similarly if allowed outdoor access, but further study into this area would be beneficial.
Results from the novel object test also indicate no outward signs of stress within either of the two treatment groups. Boars were not reluctant to approach the object, and most often preferred to attempt to mount the object instead. This could be due to the fact that the only interaction boars have with a different environment from where they are housed is when taken to the collection pen. The results from this behavioral test indicate that this type of test may not be useful in assessing the stress response of boars.
References


Table 2. Total averages for boar libido and sperm quantity between boars housed in crates and boars housed in pens. Letters indicate significance at the p=.05 level. Asterisks indicate significance at the p=.001 level.

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th>Crate</th>
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<td>Mean</td>
<td>SE</td>
<td>n</td>
<td>Mean</td>
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</tr>
<tr>
<td>Reaction Time (sec)</td>
<td>153</td>
<td>2.5&lt;sup&gt;a&lt;/sup&gt; ± 0.4</td>
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<td>154</td>
<td>3.9&lt;sup&gt;a&lt;/sup&gt; ± 0.5</td>
<td></td>
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<tr>
<td>Collection Time (sec)</td>
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<td>372.3* ± 9.3</td>
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<td>154</td>
<td>319.1* ± 8.3</td>
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<td>Volume (mL)</td>
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<td>230.4&lt;sup&gt;b&lt;/sup&gt; ± 6.5</td>
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<td>154</td>
<td>194.1&lt;sup&gt;b&lt;/sup&gt; ± 4.7</td>
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<tr>
<td>Concentration (million/mL)</td>
<td>154</td>
<td>316.5 ± 7.5</td>
<td></td>
<td>154</td>
<td>335.6 ± 6.9</td>
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<tr>
<td>Total sperm (billion)</td>
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<td>68.1&lt;sup&gt;c&lt;/sup&gt; ± 1.6</td>
<td></td>
<td>154</td>
<td>63.3&lt;sup&gt;c&lt;/sup&gt; ± 1.5</td>
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</table>
Figure 1. Average reaction time (mean ± SE) for boars in pens and boars in crates over time.
Figure 2. Average reaction time (mean ± SE) for each group of boars (n=7) throughout the study.
Figure 3. Average collection time (mean ± SE) for boars in pens and boars in crates over time.
Figure 4. Average collection time (mean ± SE) for each group of boars (n=7) throughout the study.
Figure 5. Average volume of ejaculate (mean ± SE) for boars in pens and boars in crates over time.
Figure 6. Average volume of ejaculate (mean ± SE) for each group of boars (n=7) throughout the study.
Figure 7. Total sperm per ejaculate (mean ± SE) for boars in pens and boars in crates over time.
Figure 8. Total sperm per ejaculate (mean ± SE) for each group of boars (n=7) throughout the study.
Table 3. CASA motility data for boars in pens and boars in crates. A few tendencies were noted, and are denoted by differing letters. a: p = .07, b: p = .08, c: p = .09

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<td></td>
<td>n</td>
<td>Mean</td>
<td></td>
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<tr>
<td>MOT (%)</td>
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<td>88.0 ± 1.6</td>
<td></td>
<td>140</td>
<td>91.4 ± 1.4</td>
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<tr>
<td>PMOT (%)</td>
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<td>76.4 ± 1.9</td>
<td></td>
<td>140</td>
<td>79.7 ± 1.7</td>
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<tr>
<td>DCL (µm)</td>
<td>140</td>
<td>63.6&lt;sup&gt;a&lt;/sup&gt; ± 1.7</td>
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<td>140</td>
<td>65.7&lt;sup&gt;a&lt;/sup&gt; ± 1.5</td>
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<td>DAP (µm)</td>
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<td>34.3&lt;sup&gt;a&lt;/sup&gt; ± 0.8</td>
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<td>140</td>
<td>35.3&lt;sup&gt;a&lt;/sup&gt; ± 0.8</td>
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<td>DSL (µm)</td>
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<td>23.3&lt;sup&gt;b&lt;/sup&gt; ± 0.5</td>
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<td>140</td>
<td>23.5&lt;sup&gt;b&lt;/sup&gt; ± 0.5</td>
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<td>VCL (µm/s)</td>
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<td>145.2&lt;sup&gt;c&lt;/sup&gt; ± 3.9</td>
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<td>140</td>
<td>150.3&lt;sup&gt;c&lt;/sup&gt; ± 3.6</td>
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<td>VAP (µm)</td>
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<td>78.6&lt;sup&gt;c&lt;/sup&gt; ± 2.0</td>
<td></td>
<td>140</td>
<td>81.0&lt;sup&gt;c&lt;/sup&gt; ± 1.9</td>
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<td>VSL (µm/s)</td>
<td>140</td>
<td>53.5&lt;sup&gt;b&lt;/sup&gt; ± 1.3</td>
<td></td>
<td>140</td>
<td>54.1&lt;sup&gt;b&lt;/sup&gt; ± 1.2</td>
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Figure 9. CASA average motility (MOT) and progressive motility (PMOT) (%) for each period of the study. Due to the switchback design, boars in crate period 1 are the same as boars in pen period 2, and boars in pen period 1 are the same as boars in crate period 2. p>.05
Figure 10. CASA motility data of curvilinear distance traveled (DCL), average path distance traveled (DAP), and distance traveled in a straight line (DSL). p > .05
Figure 11. CASA motility data of curvilinear velocity (VCL), average path velocity (VAP), and straight line velocity (VSL). p > 0.05
Figure 12. CASA motility (MOT) and progressive motility (PMOT) of mature and young boars. (MOT, p=.06).
Table 4. CASA motility data for mature and young boars. A tendency (p=.06) is denoted by an asterisk. All other data are not significant.

<table>
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<tr>
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<td></td>
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<td>n</td>
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<tr>
<td>MOT (%)</td>
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<td>PMOT (%)</td>
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<td>79.8</td>
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<td>VCL (µm/s)</td>
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<td>VSL (µm/s)</td>
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<td>±1.0</td>
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Table 5. Percent of spermatozoa from boars in pens and crates showing various morphological traits. p>.05

PNTAIL: normal tail
PABTAIL: abnormal tail
PDROP: cytoplasmic droplets
PNHEAD: normal head
PABHEAD: abnormal head
PNACRO: normal acrosome
PABACRO: abnormal acrosome
PDACRO: detached acrosome

<table>
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<tr>
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<td>SE</td>
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<td>82.5</td>
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<td>PABTAIL</td>
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<td>12.6</td>
<td>± 0.9</td>
<td>140</td>
<td>12.6</td>
<td>± 0.9</td>
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<tr>
<td>PDROP</td>
<td>139</td>
<td>4.8</td>
<td>± 0.4</td>
<td>140</td>
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<td>± 0.4</td>
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<tr>
<td>PNHEAD</td>
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<td>90.8</td>
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<td>90.4</td>
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<td>PABHEAD</td>
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<td>9.1</td>
<td>± 0.7</td>
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<td>± 0.5</td>
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<td>± 0.2</td>
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<td>± 0.2</td>
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<td>PDACRO</td>
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<td>1.4</td>
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<td>140</td>
<td>1.4</td>
<td>± 0.1</td>
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Table 6. Percent of spermatozoa from mature and young boars showing various morphological traits. Different letters correspond to the following levels of significance: a: p=.005, b: p<.0001, c: p=.04, and d: p=.009

<table>
<thead>
<tr>
<th>%</th>
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<td>PNACRO</td>
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<tr>
<td>PDACRO</td>
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<td>0.8a</td>
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</table>
Figure 13. Percent of normal and abnormal spermatozoa morphology. Differences in letter represent significant differences at varying levels: ab: p=.005; cd, gh, ij: p<.0001; ef: p=.04; kl: p=.09
Table 7. Averages between treatment groups for body weight, hair weight, hormone profiles, and behavioral observations.

<table>
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<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
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<td>Mean</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>35</td>
<td>368.6 ± 11.7</td>
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<tr>
<td>Hair weight (mg)</td>
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<td>1.4 ± 0.0</td>
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<tr>
<td>Cortisol (ng/mL)</td>
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<td>91.1 ± 4.4</td>
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<td>Testosterone (ng/mL)</td>
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<td>8.0 ± 0.6</td>
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<td>Heat shock protein (pg/mL)</td>
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<td>Prolactin (ng/mL)</td>
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<td>0.8 ± 0.0</td>
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<tr>
<td>Standing time (s)</td>
<td>35</td>
<td>1156.6 ± 101.9</td>
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<tr>
<td>Licking bars</td>
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<td>0.7 ± 0.0</td>
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<tr>
<td>Nosing boars</td>
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<tr>
<td>Pacing</td>
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<td>Lying time (s)</td>
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<td>Belly</td>
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<td>Side</td>
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<td>Latency time (s)</td>
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<td>Contact time (s)</td>
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<td>Number of contacts</td>
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Figure 14. Percentage of piglets (n=657) sired from boars in each housing type from heterospermic inseminations.