ABSTRACT

Walker, Sara Elizabeth. Effect of selection for testosterone production on testicular morphology and daily sperm production in pigs. (Dr. Joseph Cassady)

The objective of this study was to determine effects of divergent selection for testosterone on testicular morphology and daily sperm production. Duroc boars from lines divergently selected for testosterone production in response to GnRH challenge for 10 generations followed by random selection were used. In generation 21 endogenous testosterone in the high testosterone line (H, n=54) and low testosterone line (L, n=44) averaged 490 ng/ml and 278 ng/ml ($P < 0.01$), respectively. Plasma FSH concentrations did not differ between lines ($P < 0.3$). Body weight, testicular weight, and epididymal weight were recorded for boars from H (n=82) and L (n=44) castrated at an average age of 211 d and 97 kg. Testicular tissues were sampled from animals castrated in generation 20 (H, n=46 and L, n=13). Volume densities for Leydig cells, seminiferous tubules, and Sertoli cells were estimated along with sperm production. After adjustment for body weight, average paired testicular weights for H and L were 417 g and 457 g ($P < 0.01$), respectively. Adjusted epididymal weights also differed between lines ($P < 0.02$), with H having larger epididymal weights. Line H (n=46) had greater volume densities of Leydig cells than L (n=13) ($P < 0.02$). Volume density of seminiferous tubules tended to differ between lines ($P < 0.07$), and Sertoli cell volume densities did not differ ($P < 0.27$). Sperm production traits, adjusted for age, did not significantly differ between lines. Selection for testosterone production in response to a GnRH challenge was an effective method of changing testosterone levels, testicular size, epididymal weight, and volume density of Leydig cells and seminiferous tubules. However, daily sperm production per

gram of testes was unchanged. At this time, selection for testosterone in order to increase sperm production is not recommended.

Effect of selection for testosterone production on testicular morphology and daily sperm production in pigs.

by

Sara Elizabeth Walker

A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Animal Science

Raleigh

2003

Approved By:

_____	_____
Dr. Charlotte Farin	Dr. William Flowers
_____	_____
Dr. Odis Robison	Dr. Joseph Cassady Chair of Advisory Committee

Personal Biography

Sara Elizabeth Walker graduated from North Carolina State University in 2001 with a Bachelor of Science in Zoology and a minor in Genetics. She entered the Animal Science department in the fall of 2001 to begin a Master's of Science degree in Animal Breeding and Genetics with an emphasis in reproductive physiology. She graduated with a Master's degree from North Carolina State University in the spring of 2003. In the fall she will pursue a Ph.D. degree in Molecular & Cellular Pathology at Wake Forest University.

TABLE OF CONTENTS

Page

List of Tables iv

Literature Review 1

I. Physiology of Male Reproduction 1

A. Differentiation and Development of the Testis 1

B. Testis Function 6

C. Function of the Epididymis 8

D. Spermatogenesis 8

E. Endocrine Control of Testis Function 13

II. Responses to Selection 14

A. Selection for Reproductive Characteristics 14

B. Selection for Hormone Concentration 16

III. Relevance to Human Cancer Research 18

Introduction 22

Materials and Methods 23

Results 27

Discussion 28

Implications 30

Appendix A: Fixation, Dehydration and Embedding Protocol 64

Appendix B: Rehydration and Staining Protocol 66

Appendix C: Daily Sperm Production Protocol 68

Literature Cited 69

LIST OF TABLES

Table		Page
1	Least squares means for TOTWT, EPIWT, TEST, and FSH	31
2	Least squares means for volume density of Leydig cells, seminiferous tubules, and Sertoli Cells	32
3	Least squares means for DSP/g, TDSP, SPM/g, and TTS	33
4	Average animal weight at castration	34
5	Average age of animals at castration	34
6	Least squares means for D140WT, SHBF, LNBF, AVGBF	35
7	ANOVA table, fixed effects, and regression coefficients of TOTWT for LTL	36
8	ANOVA table, fixed effects, and regression coefficients of TOTWT for HTL	37
9	ANOVA table, fixed effects, and regression coefficients of EPIWT for LTL	38
10	ANOVA table, fixed effects, and regression coefficients of EPIWT for HTL	39
11	ANOVA table, fixed effects, and regression coefficients of TEST for LTL	40
12	ANOVA table, fixed effects, and regression coefficients of TEST for HTL	41

13 ANOVA table, fixed effects, and regression coefficients of FSH for LTL 42

14 ANOVA table, fixed effects, and regression coefficients of FSH for HTL 43

15 ANOVA table, fixed effects, and regression coefficients of Leydig cell PVD for LTL 44

16 ANOVA table, fixed effects, and regression coefficients of Leydig cell PVD for HTL 45

17 ANOVA table, fixed effects, and regression coefficients of Leydig cell TVD for LTL 46

18 ANOVA table, fixed effects, and regression coefficients of Leydig cell TVD for HTL 47

19 ANOVA table, fixed effects, and regression coefficients of Seminiferous Tubule PVD for LTL 48

20 ANOVA table, fixed effects, and regression coefficients of Seminiferous Tubule PVD for HTL 49

21 ANOVA table, fixed effects, and regression coefficients of Seminiferous Tubule TVD for LTL 50

22 ANOVA table, fixed effects, and regression coefficients of Seminiferous Tubule TVD for HTL 51

23 ANOVA table, fixed effects, and regression coefficients of Sertoli cell PVD for LTL 52

24 ANOVA table, fixed effects, and regression coefficients of Sertoli cell PVD for HTL 53

25 ANOVA table, fixed effects, and regression coefficients of Sertoli cell TVD for LTL 54

26 ANOVA table, fixed effects, and regression coefficients of Sertoli cell TVD for HTL 55

27 ANOVA table, fixed effects, and regression coefficients of SPM/g for LTL. 56

28 ANOVA table, fixed effects, and regression coefficients of SPM/g for HTL. 57

29 ANOVA table, fixed effects, and regression coefficients of TTS for LTL. 58

30 ANOVA table, fixed effects, and regression coefficients of TTS for HTL. 59

31 ANOVA table, fixed effects, and regression coefficients of DSP/g for LTL. 60

32 ANOVA table, fixed effects, and regression coefficients of DSP/g for HTL. 61

33 ANOVA table, fixed effects, and regression coefficients of TDSP for LTL. 62

34 ANOVA table, fixed effects, and regression coefficients of TDSP for HTL. 63

LITERATURE REVIEW

I. Physiology of Male Reproduction

A. Differentiation and Development of the Testis

Testicular development is important, as proper development is necessary for future reproductive success. It has been established that testicular size is a major factor in establishing sperm production capacity (McCoard et al., 2000). Testicular development occurs in three different phases in males, prenatal development, postnatal development, and puberty. It is important to understand the morphological and histological changes that occur with each phase, in order to select for animals with superior reproductive capabilities.

Testicular differentiation begins in newly developed embryos with sexual differentiation. Primordial germ cells in early male embryos migrate from the yolk sac to the genital ridge, and settle. The genital ridge is visible at around 26 days of age in pigs. Once germ cells settle they cause a cellular proliferation that develops into primitive sex cords. These cords continue to develop into the renal system as well as Wolffian and Müllerian ducts. The Y chromosome of male embryos contains a gene that codes for testis determining factor. Secretion of this protein from sex cords stimulates testicular development (Senger, 1999; Gier and Marion, 1970).

The testes are formed by 35 days of age, and the sex cords are now characterized as seminiferous cords. A thin layer of mesenchyme forms around the differentiating testis to form the tunica albuginea. At this time, large clear cells form and surround the germ cells in the seminiferous cords. These cells are recognized as developing sertoli cells due to their ability to secrete mullerian-inhibiting hormone (MIH), which acts to stimulate müllerian duct

regression (Gier and Marion, 1970). Also, groups of cells left in spaces between developing cords begin to differentiate into interstitial and leydig cells. These leydig cells are derived from the mesenchyme of the gonadal blastema. Formation of the seminiferous cords stimulates differentiation of leydig cells, which begin to secrete testosterone (Orth, 1993). Fetal leydig cell proliferation may be stimulated by LH secretion as well (Tripepi et al., 2000)

The immature testis experiences most of its growth during prenatal and early postnatal phases, with maximal growth occurring after birth. This growth is associated with periods of rapid sertoli cell proliferation that occurs mostly during the first three weeks of postnatal development. Postnatal development coincides with an increase in plasma FSH levels (Ford et al., 2001; Orth, 1993). Plasma FSH acts on sertoli cells to cause basic fibroblast growth factor (bFGF) expression, which further stimulates cell proliferation (Krantic and Benahmed, 2000). This postnatal period of development is very important in determining mature sertoli cell population size, which in turn determines mature testis size (Lunstra et al., 2002; McCoard et al., 2000).

Both the interstitium and seminiferous cords continue to develop and proliferate postnatally. The interstitium, composed of peritubular myoid cells, blood vessels, collagen fibrils and leydig cells, while continuing to increase in size, decreases in total testicular volume. Leydig cells show their greatest decrease in volume between 100 and 190 days postnally (Allrich et al., 1983). This decrease in volume is associated with rapid growth and lengthening of seminiferous cords, which are largely responsible for increased testicular size (Tripepi et al., 2000; Harder et al., 1995).

Two separate generations of leydig cells arise during development. The first occurs shortly after testicular differentiation and is responsible for androgen synthesis and secretion to further stimulate testis growth and differentiation. This population of leydig cells develops independently of FSH. However, shortly after birth these cells do become dependent on gonadotropin secretions for survival (Baker et al., 2003). It is unclear whether the first population of leydig cells regress and the adult population gradually takes their place, or if the first population matures into the adult population (Orth, 1993).

Important morphological changes occur within seminiferous cords shortly after birth. These changes help to alter cord structure into seminiferous tubules, and to assist in germ cell development at maturity. Prior to birth, germ cells are usually found in the center of the seminiferous cords. Shortly after birth, these germ cells, which are usually surrounded by developing sertoli cells, migrate toward the periphery of the seminiferous cords. Here they project cytoplasmic processes which contact the extracellular matrix and interact with the developing basement membrane. The basement membrane is formed from both sertoli cells and myoid cells found in interstitium. This migration and extracellular matrix contact is required for germ cell survival. Cells failing to complete this process degenerate shortly after birth. Migration is initiated by enlargement of golgi complexes within germ cells. Complexes reorient themselves toward the developing basement membrane causing subsequent cellular movement in that direction (Orth, 1993).

During this same time period, adjacent sertoli cells are beginning to form junctional complexes. These complexes begin blood-testis barrier formation. In most species, formation of junctional complexes also coincides with completion of sertoli cell proliferation. Shortly after complex formation, lumens begin to form in the center of the seminiferous

cords. Formation of lumens marks differentiation of seminiferous cords into seminiferous tubules. Also at this time, sertoli cells and seminiferous epithelium begin to secrete fluid into the lumen of the seminiferous cords, and germ cells begin meiosis (Orth, 1993; Neaves, 1977).

In swine, puberty occurs around four to five months of age, with sexual maturity being reached around seven to eight months (França et al., 2000). Puberty is said to have been attained once free spermatozoa are seen in seminiferous tubules and cauda epididymis (Hughes and Varley, 1980). While sertoli cell proliferation ceases near puberty, seminiferous tubules continue to grow in length and diameter. This growth is attributed to germ cell proliferation that continues well after puberty (França et al., 2000). A large increase in leydig cell size, testosterone secretion, and smooth endoplasmic reticulum volume per leydig cell is seen at puberty (França et al., 2000).

Adult testes can be characterized by their histological appearance. Seminiferous tubules are highly developed with wide lumens and a thickened basal lamina. The interstitium is enlarged and contains a large number of leydig cells that are arranged in clusters surrounding blood vessels (Tripepi et al., 2000). Sertoli cells at this stage are fully mature, and capable of producing mature spermatozoa. Mature testis size is positively correlated with sperm production in mature boars, and both of these traits are established by early postnatal sertoli cell proliferation (Ford et al., 2001). Sire can influence both mature testis and epididymal weights of their progeny; however, body weight is not correlated with testis growth or mature testis weight (Allrich et al., 1983).

Mature leydig cells can be recognized by their characteristic cellular structure. Cells are generally round to oval in shape, with a large round nucleus that is displaced to one side.

The nucleus contains abundant heterochromatin and at least one nucleoli. Mitochondria are very abundant and contain a matrix of high electron density. Smooth endoplasmic reticulum is the most abundant organelle and appears in clumps near the cell periphery. Rough endoplasmic reticulum appears in limited numbers near the nucleus and grouped near mitochondria. Ribosomes are found along the plasma membrane, outside of the nuclear envelope, and near mitochondria. Lipid droplets and large vesicles are present, along with lysosome-like bodies, near the cell membrane (Lunstra et al., 1986; Burgos et al., 1970).

Mature sertoli cell function is both structural and secretory in nature. Both the nucleus and golgi apparatus are involved in protein secretion; however, secretory granules for storage of these proteins are not present. These cells possess numerous primary lysosomes and autophagic vacuoles that are used for phagocytosis. Smooth endoplasmic reticulum is also present for purposes of steroid synthesis and metabolism. Intracellular junctions are found joining adjacent sertoli cells. These junctions form part of the blood-testis barrier and serve to maintain structural integrity of seminiferous tubules. Junctions separate the seminiferous epithelium into basal and adluminal compartments. The basal compartment contains spermatogonia and spermatocytes in preleptotene phase. The adluminal compartment contains maturing spermatocytes and spermatids (Bardin et al., 1993). Due to their close relationship with germ cells and developing spermatocytes, accurate sertoli cell enumeration is very difficult.

There are two types of sertoli cells that have been identified within the testis, Type A and Type B. Type A cells possess characteristics described above. They can be differentiated from Type B cells because of their light staining nucleus, large number of ribosomes and smooth endoplasmic reticulum, and intracellular junctions. Type A cells

extend from the basement membrane to the lumen of seminiferous tubule. Type B cells are much fewer in number and only found in close association with the seminiferous epithelium basement membrane. These cells are small in size with dark staining nuclei, and have only limited numbers of smooth endoplasmic reticulum. Type B cells are very difficult to differentiate from developing spermatogonia and are often overlooked during sertoli cell enumeration (McCoard et al., 2000).

B. Testis Function

Main testes functions are production and maturation of spermatozoa and steroidogenesis. Each function is performed within different cells found in different areas. Steroidogenesis occurs within leydig cells found in testicular interstitium surrounding seminiferous tubules (Orth, 1993). Spermatogenesis occurs within sertoli cells located in seminiferous tubules. Tubules can be separated into two structural compartments, the basal and adluminal compartments. The basal compartment is located next to the basement membrane on the periphery of seminiferous tubules. The adluminal compartment begins at the blood-testis barrier, includes the tubule lumen, and is the site of sperm maturation (Bardin et al., 1993).

While sertoli cells are mostly known for their importance in spermatogenesis, they also perform many other useful functions such as phagocytosis and fluid secretion. Sertoli cells are able to ingest degenerating germ cells, residual bodies and particulate matter found in seminiferous epithelium. While degenerating germ cells and particulate matter are mostly ingested after tissue damage, ingestion of residual bodies may serve a function in steroid synthesis (Steinberger and Steinberger, 1977). Sertoli cells also secrete proteins essential for germ cell maturation in development in the adluminal compartment. Sertoli cell secretions

can be separated into those that maintain the tubule environment during spermatogenesis and those that signal other cells. Androgen-binding protein (ABP), cortisol binding globulin, and transferrin are a few examples of environmental secretions (Bardin et al., 1993). Of special significance is ABP, which was one of the first sertoli cell secretions found. It is also secreted into both the tubule lumen and bloodstream, demonstrating that sertoli cells are able to secrete bi-directionally (Bardin et al., 1993; Steinberger and Steinberger, 1977). Inhibins, activins, MIH, IGF-I, and TGF-α and β are a few of the cellular signaling proteins that are secreted by sertoli cells. These proteins can be either stimulatory or inhibitory in nature, and act on a variety of different cell types within the body (Bardin et al., 1993).

Interstitial cells carry out a variety of functions within the testis. Leydig cells are primarily responsible for steroidogenesis, while the rest of the interstitium is mostly made up of loose connective tissue. Within connective tissue, blood vessels, capillaries, and lymphatic vessels are found. These function to nourish testicular tissues and aid in transport of testicular secretions such as testosterone and ABP (Hooker, 1970). Leydig cells, in addition to androgen secretion and synthesis, also synthesize and secrete a number of different peptides. Leydig cells synthesize the polyprotein proopiomelanocortin (POMC). The POMC is broken down into ACTH, MSH, β-endorphin, and corticotropin-like intermediate lobe peptide. While their testicular functions are not yet certain, it is thought they may have a function in testosterone secretion. There is also evidence that oxytocin, enkephalins, dynorphins, and renin-angiotensin are secreted from leydig cells as well, however their function within the testis is still unclear (Ewing and Keeney, 1993).

Seminiferous tubules are composed of both sertoli cells and spermatogonia in different stages of maturation. Primary function of the tubule is sperm maturation and sperm

transport to the epididymus for functional maturation and storage (Senger, 1999). Sperm maturation occurs in epithelial waves within the seminiferous epithelium. Each wave contains cells in different stages within the tubule that correspond to the developmental stage of the spermatozoa (Wright, 1993). The seminiferous epithelium also functions to secrete proteins that promote sperm maturation and are specific to the epithelial wave stage (Wright, 1993; Burgos et al., 1970).

C. Function of the Epididymus

The epididymus is connected to the testis by efferent ducts. These ducts come together to form a single epididymal duct where spermatozoa and rete fluid are transported from the testis to the head of the epididymus (Fisher et al., 2002). Final sperm maturation and storage occurs in the epididymus. Epididymal function is androgen dependant, and sperm maturation in the epididymus is required for sperm fertility and motility. When immature sperm enter the caput or head of the epididymus, sperm concentration is low due to the presence of rete fluid. This fluid is absorbed in efferent ducts and the epididymal proximal head causes sperm to become concentrated. As sperm move from the caput through the corpus, proteins are secreted that cause sperm to enter final maturation. It is during this process that they gain fertility and motility. By the time sperm enter the cauda they are motile and capable of binding to oocytes. Sperm are stored in the cauda region until ejaculation (Senger, 1999).

C. Spermatogenesis

While spermatogenesis usually begins at four to six months of age, sperm quality and production do not reach their peak until maturity at around six to eight months of age (Rothschild and Ruvinsky, 1998). Spermatogenesis is the process by which a germ cell

becomes a mature spermatozoa. The majority of this process occurs within the testis, and is controlled by FSH, LH, and androgens (Guillaumot et al., 1996). It takes around 34 days for a primitive germ cell to mature into a fertile spermatozoa (Hughes and Varley, 1980).

Spermatogenesis occurs in three different phases: proliferation and regeneration of spermatogonia, meiosis, and maturation of spermatids (Zirkin, 1993). It begins with development of gonocytes into prespermatogonia, or A_0 spermatogonia. Prespermatogonia are described as gonocytes where nuclear fusion has occurred, therefore prespermatogonia can produce more prespermatogonia or continue to differentiate into A-type spermatogonia (Courot et al., 1970). Due to their ability to regenerate, A-spermatogonia can also be classified as stem cells (Meistrich and van Beek, 1993). A-spermatogonia must go through several mitotic divisions in which they are classified as A_1, A_2, A_3, or A_4 spermatogonia before moving to the next stage of development (Zirkin, 1993; Courot et al., 1970).

Each of these maturative stages occurs in the basal compartment of seminiferous tubule (Senger, 1999; Courot et al., 1970). The first division of A-type spermatogonia occurs simultaneously with release of spermatozoa into the tubule lumen (Courot et al., 1970). Developing spermatogonia within a section of tubule matures at a constant rate and in synchrony with each other. This is accomplished by formation of intercellular bridges that allow cytoplasm to be shared across developing cells. It is through these bridges, that development is synchronized across cohort cells (Senger, 1999). As cells develop and mature, they move from the basal compartment next to the basement membrane, toward the lumen (Senger, 1999; Zirkin, 1993).

Once A-type spermatogonia have reached the A_4 stage, they then move into the intermediate stage. Cells in this stage are identified by their round nuclei that progressively

decrease in size (Courot et al., 1970). Spermatogonia go through three mitotic divisions in which they are classified as intermediate spermatogonia before they mature to B-type spermatogonia. Once designated as B-type spermatogonia, cells perform two more mitotic divisions before becoming primary spermatocytes, and entering meiosis (Senger, 1999; Zirkin, 1993). The B-spermatogonia move from the basal compartment past the blood-testis barrier to the adluminal compartment during the last mitotic division (Senger, 1999).

Immediately after being formed, primary spermatocytes immediately enter meiotic prophase I. Prophase I can be divided into preleptotene, leptotene, zygotene, pachytene, and diplotene stages (Courot et al., 1970). Throughout each of these stages DNA within the nucleus condenses and replicates. Homologous chromosomes then pair up and join at centromeres to form chromatids (Fox, 1999). Meiotic prophase I is the longest phase during spermatogenesis, occupying 30% of the total process (Senger, 1999). Next, spermatocytes enter metaphase I. During this phase, homologous chromosome pairs migrate and line up on the cell equator. While this is occurring, spindle fibers begin forming at opposite poles and attach themselves to centromeres of paired chromosomes. During anaphase I, attached spindle fibers begin to move toward the pole they originated from, separating paired chromosomes as they move. They continue to move until chromosomes are no longer paired, and there are two complete sets of chromosomes, each at a different pole. Telophase I involves dividing the cellular cytoplasm to form two separate haploid cells (Fox, 1999). Following completion of the first stage of meiosis, cells are classified as secondary spermatocytes (Courot et al., 1970).

Secondary spermatocytes must enter the second meiotic division in order to develop into spermatids. The same stages outlined above are repeated; however, following telophase

II, two more haploid cells are formed from each secondary spermatocyte. For each primary spermatocyte that entered meiotic division, four spermatids are formed (Fox, 1999; Senger, 1999). Each spermatid must undergo spermiogenesis, which involves morphological modifications and transformations, before maturing into spermatozoa (Courot et al., 1970).

Before describing spermiogenesis, it is important to understand basic sperm anatomy. Mammalian sperm are made up of head and tail regions. The head region can be divided into the condensed nucleus, perinuclear theca which is divided into the subacrosomal region and postacrosomal sheath, and the acrosomic system. The acrosomic system is further divided into the acrosome proper and head cap. The perifossal zone is an area of uncovered nucleus where tail attachment occurs. Tail regions are variable in length depending on species, and can be divided into four regions: neck piece, middle, principle, and end piece. The neck piece contains electron-dense material with a hollow center and is short in length. The middle piece is composed of the axoneme made of microtubules, nine coarse outer dense fibers (ODFs), mitochondria, and a small amount of cytoplasm surrounded by a plasma membrane. This region is mainly responsible for tail movement once spermatids have fully matured. The principle segment is made up of the axoneme, seven ODFs, two columns of fibrous sheath, and a small amount of cytoplasm surrounded by a plasma membrane. The end piece is composed of unorganized axonemal elements, a small amount of cytoplasm, and a plasma membrane (Clermont et al., 1993).

Spermiogenesis is a continuous process that can be divided into four distinct periods of development, golgi, cap, acrosome, and maturation (Clermont et al., 1993). Spermatids that enter spermiogenesis are mostly spherical in shape with a central, round nucleus. This shape is maintained through both golgi and cap phases. Axenome growth occurs during both

of these phases and is initiated by migration of the distal centriole to the nucleus base (Clermont et al., 1993). The golgi phase begins acrosome formation. The golgi apparatus, found above the nucleus, goes through a series of transformations in the acrosome beginning with fusion of proacrosomic vesicles to form a large acrosomic vesicle. Cap phase is characterized by the acrosome forming a cap over the anterior nuclear area. The golgi apparatus begins to move toward the caudal end of the spermatid, and the forming flagellum begins to extend toward the seminiferous tubule lumen (Senger, 1999). Acrosomal phase is denoted by nuclear and cytoplasmic elongation and spreading of the acrosome to cover the anterior nucleus. A system of microtubules, the manchette, begins to form and attach posterior to the nucleus, and a few of the microtubules will form the postnuclear cap. Also during this phase, spermatids embed themselves within sertoli cells with their developing tails extending into the tubule lumen (Senger, 1999). During maturation, the manchette continues to develop into the postnuclear cap with the remaining tubules moving toward the tail and eventually disappearing. Mitochondria group in the flagellum behind the nucleus and extend well into the anterior tail region, forming the middle piece in mature sperm. The flagellum finishes development, and a fibrous sheath is produced. Spermiation, the release of spermatozoa into the tubule lumen, marks the completion of spermatogenesis (Senger, 1999).

In order for males to constantly have a supply of mature spermatozoa, the seminiferous epithelium must continually produce sperm. This is possible due to cycles or waves within the seminiferous epithelium. Within any section of tubule, there are always four or five generations of germ cells developing in synchrony. Each of these sections possesses cells moving through differing stages of development. While some sections are releasing spermatozoa into the lumen, other sections in close proximity may be in early

stages of spermatogenesis. These cycles or waves allow males to have a constant production of mature sperm at all times (Senger, 1999).

D. Endocrine Control of Testicular Function

Testosterone, FSH, and LH are all required for normal testicular function; however, while testosterone is produced within testes, FSH and LH are not. Release of FSH and LH is controlled through release of GnRH from the ventromedial nucleus (VMN) and arcuate nucleus (Arc) of the hypothalamus (Senger, 1999). Hypothalamic release of GnRH occurs in frequent, short bursts which act on the anterior pituitary to cause release of FSH and LH. The pulsatile secretion of FSH and LH mimics GnRH secretion. Due to inhibin secretion from the testis, FSH secretion is lower in concentration, and pulses last longer (Senger, 1999). Both LH and FSH travel through the blood stream to the testis. Luteinizing hormone acts on leydig cells found in the interstitium to stimulate synthesis of testosterone, while FSH stimulates formation of LH receptors on the plasma membrane of leydig cells so they can better respond to LH stimulation (Hughes and Varley, 1980; Ewing and Brown, 1977). The pulsatile nature of LH secretion is important, as continued high concentrations of LH cause leydig cells to become refractory and decreases testosterone secretion. While a basal concentration of testosterone is required within the testis for spermatogenesis, consistently high circulating levels will suppress LH and affect leydig cell function (Senger, 1999). This is due to a negative feedback loop that affects hypothalamic secretion of GnRH as well as anterior pituitary secretion of LH (Hughes and Varley, 1980).

Follicle stimulating hormone acts to stimulate testis growth during development, but also stimulates uptake of testosterone into sertoli cells and spermatogenesis (Hughes and Varley, 1980). Testosterone enters sertoli cells through the action of FSH and is converted

into estradiol. Estradiol, much like testosterone, in high amounts exerts negative feedback on GnRH secretion (Senger, 1999). Sertoli cells also secrete inhibin and activin. Inhibin specifically acts on the anterior pituitary to reduce FSH secretion, while activin increases FSH secretion (Fox, 1999; Wright, 1993; Setchell et al., 1977). Sertoli cells also synthesize ABP, which is secreted into both the bloodstream and tubule lumen. When secreted into the tubule lumen, it is transported to the caput section of the epididymus through efferent ducts (Means, 1977). Androgen binding protein acts within the testis and epididymus to bind testosterone and increase its concentration (Fox, 1999).

II. Response to Selection

A. Selection for Reproductive Characteristics

Selection studies are important for studying interrelationships associated with reproduction. By applying selection to a species with higher reproduction rates, selection intensity and rate of response to selection are increased (Land, 1978). Early selection studies have shown that the same genes control reproductive traits for both males and females, and reproduction is controlled by the same hormones in both sexes (McNeilly et al., 1986; Land, 1978). Reproductive traits in one sex are correlated with traits in the other sex; therefore, selection for a male trait should cause a correlated response in females (Land, 1978).

Male reproductive traits are often used as an indicator of female reproductive efficiency, as they can be measured at an earlier age (Schinckel et al., 1984). Some easily measured male reproductive traits are testis weight and size. Several studies have shown that a positive correlation exists between testis size and ovulation rate in mice and swine (McNeilly et al., 1986; Schinckel et al., 1984; Toelle et at., 1984). In mice selected for high

induced ovulation rate, gonadal sensitivity to FSH increased (Schinckel et al., 1984). Testis size has been positively correlated with increased sperm production and semen quality in bulls and boars (Harder et al., 1995; Rathje et al., 1995; Toelle et al., 1984). Testis weight and body weight are uncorrelated, and testis weight and epididymal weights are positively correlated (Harder et al., 1995; Schinkel et al., 1984). McNeilly et al. (1986) concluded that selection for increased testis size in young rams increased growth rate. Though these animals matured faster than animals selected for low testis weight, they had lower mature body weight. Selection for increased testis size decreases age of puberty in boars, while increasing daily sperm production (Harder et al., 1995; Rathje et al., 1995).

Selection for female reproductive traits has been successful. Traits such as litter size, ovulation rate, embryonal survival, and uterine capacity can be measured accurately; however, animals must first reach puberty. In most cases, surgical procedures such as laparotomy or laparoscopy must be used for measurement (Cassady et al., 2000). These practices are costly, potentially detrimental to future reproductive success, and not readily available for most producers. Selection based on ovulation and embryonal survival has resulted in increased litter sizes. While the actual mechanism for this increase is unclear, altered prepubertal secretion of estrogen has been detected (Mariscal et al., 1996). Cardenas et al. (1994) looked at effects of selection for ovulation rate on male rams. Selection for ovulation rate did not alter testis weight, and it was determined that scrotal circumference and testis weight were not correlated with ovulation rate or litter size in selected females. Leydig cell response to gonadotropin secretion was altered in males from these lines (Cardenas et al., 1994). While direct selection for female reproductive characteristics is a

successful means of increasing litter size, indirect selection for these traits may be more efficient.

B. Selection for Hormone Concentration

Selection studies can also be performed by selecting animals based on hormone levels. Divergent selection for a particular hormone allows interactions and effects of that hormone to be described. Hormone selection also allows for manipulation of growth traits, which could lead to more efficient and profitable production practices. Hormone concentrations can still be measured earlier in both males and females than most reproductive traits.

Testosterone has many actions. Not only is it responsible for spermatogenesis, but it also promotes muscle growth and increase ADG in pigs (Lubritz et al., 1991). Males can be selected based on endogenous testosterone secretion anytime after puberty, endogenous testosterone level is a moderate to highly heritable trait. Also, synthesis of both testosterone and estrogen follow the same steroidogenic pathway regardless of sex (Robison et al., 1993). By selecting for testosterone, one could possibly increase several economically important production traits in addition to affecting reproductive traits in both sexes.

Selection based on endogenous testosterone levels has been shown to positively affect both male and female traits in swine. Lubritz et al. (1991) found that testosterone concentration is positively correlated with testis weight, volume, and ADG, and negatively correlated with backfat. Thus, selection for increased endogenous testosterone may positively affect production traits and promote lean growth in pigs. These results are supported by Robison et al. (1994), who reported that boars from lines with increased testosterone levels had increased testis weight, litter size, and ADG. Selection for increased

testosterone positively influenced production and allowed for indirect selection of litter size in females. Thus, testosterone may be an indicator of female reproductive performance.

Follicle stimulating hormone is important for testicular development and sertoli cell proliferation in males. Testosterone production in leydig cells is stimulated by LH. Both LH and FSH are released simultaneously from the anterior pituitary following GnRH secretion in the hypothalamus in males and females. Plasma FSH concentrations are positively correlated with litter size and ovulation rate in female sheep and pigs (Cassady et al., 2000; Haley et al., 1989). Plasma FSH in males is associated with increased growth of seminiferous tubules; however, testis weight is reduced (Zanella et al., 1999). Though LH has obvious effects within the testis, its association with testosterone secretion should directly influence reproductive traits in males and females.

Selection for plasma FSH concentrations can be performed in either sex though, faster genetic progress can be achieved if males are used (Haley et al., 1989). Wheaton et al. (1998) found that increasing plasma FSH by immunizing both passively and actively against inhibin, to be successful in increasing ovulation rates in swine and ewes. However, passive immunization was also able to increase number of embryos recovered, where active immunization had no effect. Increasing plasma FSH concentration in males is associated with lower testis and epididymal weights. In western breeds of pigs, this is also associated with lower seminiferous tubule volume density, and lower sperm producing capacity. For most other species, the opposite is true, and seminiferous tubules are increased along with sperm production in the presence of high FSH concentrations (Zanella et al., 1999; Ford et al., 1997).

III. Relevance to Human Cancer Research

Prostate cancer has become a major health concern among males, with an expected 180,000 new cases being diagnosed each year in the US (Mydlo et al., 2001). Middle age men are at risk of developing this type of cancer, with African Americans showing the highest incidence. Caucasian and Asian males are also afflicted, but Asians at a much lower rate. This trend mimics endogenous testosterone levels found in these races, with African Americans having the highest concentration and Asians the lowest (Mydlo et al., 2000). Increased incidence of prostatic cancer in industrialized countries is attributed to better methods of detection having been developed (Kaaks et al., 2001).

While exact cause of prostate cancer has yet to be determined, it is known that environmental factors related to changes in lifestyle such as diet and activity level are involved. This has been expanded to include energy metabolism and balance, with height and weight being possible risk factors in contributing to tumor development (Kaaks et al., 2000). Mydlo et al. (2001) suggested that childhood obesity could even play an important role. Testosterone has been implicated in predisposition to tumor development; however, this has not been statistically proven. Testosterone has been found to be increased in higher grade tumors when compared to low grade tumors and is a determining factor in metastatic relapse (Mydlo et al., 2001).

Several different hypotheses have been developed to explain the biological cause of prostatic cancer. The androgen hypothesis was one of the first to be developed and is based on knowledge that surgical castration assists in treatment of advanced metastatic cancer. Studies performed during the 1970's showed an increased risk of tumor development after prolonged androgen administration, which further supports the hypothesis that risk is

increased in males with elevated androgen concentrations. Researchers have begun to look at androgens other than testosterone as possible causes of prostatic tumor development. Dihydrotestosterone (DHT) is a more potent variant of testosterone that is produced by the 5-α-reductase enzyme. Both 5-α-reductase type-2 (SRD5A2) and androgen receptor (AR) have been localized within prostate tissue, with polymorphisms within these two genes being positively correlated with prostate cancer risk (Kaaks et al., 2000).

The androgen hypothesis is based on theory that increased unbound testosterone, coupled with an increase in SRD5A2 activity leads to an increase in DHT concentrations within the prostate. Since DHT binds to AR with a much greater affinity than testosterone, it is thought that the AR-DHT complex leads to development of prostatic cancer. Thus, tumor development could be directly related to increased concentrations of unbound testosterone. To this point, studies examining this relationship in humans have not been definitive. However, this is most likely due to biases within studies such as low subject numbers, and selection of subjects from different source populations (Kaaks et al., 2000).

Another hormone implicated as a possible cause of prostate cancer development is IGF-I. Insulin like growth factor has strong mitogenic and anti-apoptotic effects. It is believed that increased levels within the prostate could lead to increased risk of tumor development. Activity of IGF-I is regulated by its interaction with several different binding proteins (IGFBPs) and interaction of these complexes with cellular receptors. The majority of IGF-I and IGFBPs found in circulation are produced within the liver, with IGF-I having different affinities for different binding proteins that are produced. Complexes formed between IGF-I and IGFBPs differ in their activity, with some complexes being stimulatory and others inhibitory to actions of IGF-I. Since IGF-I is involved with insulin regulation, a

positive correlation exists between IGF-I, obesity, and plasma insulin levels (Kaaks et al., 2000).

Elevated concentrations of plasma IGF-I are associated with an increased risk of prostate cancer. It is thought that increased IGF-I levels could be due to an increase in growth hormone (GH) secretion from the pituitary. In conditions caused by increased GH secretion such as acromegaly, an associated increase in IGF-I/IGFBP-3 complexes are also seen. This increase in IGF complex has also been noted in patients with prostate cancer. It is hypothesized that increased GH secretion could be associated with an increased risk of tumor development.

While the exact cause of prostatic tumor development has yet to be determined, several hypotheses have been suggested that appear to be plausible. Each of the hypotheses have been studied with varying results being reported for all. Further investigation of each of these theories is needed before a definitive cause can be determined. While human studies are important, it is difficult to establish similar treatment and control groups to be compared, and it is also difficult to recruit subjects for studies prior to tumor diagnosis. For these reasons, using an animal model to study different theories seems warranted.

The Duroc selection lines could possibly be used as an animal model to study biological causes of human prostate cancer. Since lines have already been selected for high and low endogenous testosterone concentrations, they could be valuable in determination of increased prostatic levels of testosterone and DHT as a cause for tumor development. However, due to occurrence of tumor development later in life, adult males would need to be maintained for several years. During this time, regular blood samples would need to be taken measuring plasma levels of free testosterone and if possible, prostate DHT levels. While this

study would be more conclusive as to the role of testosterone and DHT levels as possible risk factors for prostatic cancer due to lack of bias among subjects, it would be a very expensive and time consuming study.

INTRODUCTION

Use of AI in the pork industry has increased. Nearly 60% of sows mated in 2000 were bred using AI, compared to less than 5% in 1990 (Singleton, 2001). Artificial insemination allows producers to decrease the number of boars, and allows for greater use of superior sires (Estienne, 1999). Selection of boars that consistently produce greater quantity and quality of sperm is of great economic importance. This would allow for fewer boars to be kept and greater selection intensity to be placed on sires.

Testosterone production is positively correlated with growth, and testis size is positively correlated with sperm production in boars (Rathje et al., 1995; Lubritz et al., 1991). In addition, pituitary hormones affect reproduction in both sexes, with hormone production being controlled by the same genes (Rathje et al., 1995; Robison et al., 1994). Both LH and FSH are required for testicular development, spermatogenesis, ovulation, and androgen synthesis (Schinkel et al., 1984). In females, increased FSH concentrations are associated with increased ovulation rate (Cassady et al., 2000); however, increased FSH concentrations are negatively correlated with testis size and sperm production in males (Ford et al., 1997).

Selection based on hormone production may allow for reproductive traits to be altered in both sexes. Male selection allows for higher selection intensity, with indirect responses to selection seen in females (Robison et al., 1994). Testosterone secretion is required for testes development and spermatogenesis, and is associated with increased ADG in intact males (Lubritz et al., 1991). Boars selected for increased testosterone concentrations showed an increase in testis size, litter size and ADG in their offspring (Robison et al., 1994). The

purpose of this study was to determine effects of divergent selection for testosterone on testicular morphology, and sperm production traits.

MATERIALS AND METHODS

Duroc boars from lines divergently selected for testosterone concentration were used. These animals were from lines selected for 10 generations based on testosterone concentration in response to a GnRH challenge (Robison et al., 1993). Data were obtained from boars of the high (HTL) and low (LTL) testosterone lines in generations 20 (HTL, n=46 and LTL, n=13) and 21 (HTL, n=54 and LTL, n=44). Boars were castrated at an average age of 211 d and weight of 97 kg.

Castration

Animals were first weighed and then castrated. The epididymus was removed, and both left and right testes and epididymal weights were recorded. The left testis was immediately sampled. Three randomly selected one-centimeter cubes and one thirty-gram filet was taken from the left testis.

Histological Evaluation

The one-centimeter cubes taken from the left testis of each boar were placed in 15 ml conical tubes filled with 4% paraformaldehyde and immediately placed on ice. Samples were then dehydrated and placed in 70% ethanol for storage at 4°C. Ethanol in each tube was changed weekly until samples were rehydrated. Samples were embedded in parafilm wax blocks and stored at -20°C. A complete description of dehydration and embedding procedures may be found in Appendix A. Tissues were cut into 6 μm thick slices using a microtome, placed on microscope slides, and allowed to dry at room temperature for 8 to 12

hrs, with one slide being made for each tissue block. Two slides were selected and stained for histological evaluation. One set of slides was stained with hematoxylin using eosin as a counter stain for determining leydig cell and seminiferous tubule volume density, and another set was stained using only hematoxylin for determining sertoli cell volume density. See Appendix B for a complete description of the staining process. Slides were allowed to dry completely at room temperature, and were permanently mounted using permount. Tissue sections were imaged using a light microscope and video camera connected to a personal computer. Three randomly selected images were taken at 4x magnification for determination of seminiferous tubule volume density, and three randomly selected images were taken at 20x magnification for leydig cell volume density determination. Seminiferous tubules of similar size and stage of development were selected at 20x magnification from each hematoxylin stained slide for determination of sertoli cell volume density. Volume densities were determined for each picture, with three pictures per animal being counted for Leydig cell and seminiferous tubule volume density, and two pictures being counted for Sertoli cell volume density. Each volume density estimate was treated as a repeated measure within the cell type of interest and the animal. Volume density of each picture was estimated by using a coherent test system as described by Weibel (1979). Pictures were counted independently by two technicians. Each cell that was at least half filled with the structure in question was considered a "positive" cell. Ratio of "positive" cells to total number of cells containing testicular tissue was determined to calculate volume density of the structure within the picture (PVD). The PVD was multiplied by left testis weight to determine total volume density per gram of testis (TVD).

Sperm Production Evaluation

The 30 g filet sampled from the left testis was placed in a 50 mL conical tube. Tubes were immediately placed on ice and stored at -20°C. Each fillet was divided into three equal sections. Sections were homogenized for 30 s in 200 mL 0.15% PBS with 0.1% Triton-X-100. Three 0.5 mL samples were removed and vortexed with 0.5 mL trypan blue stain. A drop of the solution was then placed on a hemacytometer and number of intact mature spermatids was recorded. Estimates of sperm per gram of testis (SPM/g), daily sperm production per gram of testis (DSP/g), total daily sperm production (TDSP), and total testicular sperm (TTS) were calculated according to the procedure outlined by Rathje et al. (1995). For a detailed description of the procedure, see Appendix C.

Testosterone and FSH Assays

Blood samples were taken from all boars in generation 21 at an average of 190 d of age in order to determine mean testosterone and FSH levels. Blood collection was performed using 10 mL EDTA vacutainer tubes, and 20 g vacutainer needles. One vacutainer tube of blood was taken from every available boar and immediately placed on ice. Once all animals had been sampled, the process was repeated so that each animal was sampled twice. Samples were taken approximately 90 minutes apart. Vacutainer tubes were centrifuged at 4°C for 25 min. Plasma was removed by pipette and split between two tubes. Samples were assayed for testosterone and FSH. Plasma from each blood draw was kept separate so variance in concentration due to time could be estimated. Testosterone RIAs were performed, with samples being run in duplicate using the procedure described by McKinnie et al. (1988). A RIA to determine FSH concentration was performed also with samples run in duplicate at the

USDA-ARS Meat Animal Research Center in Clay Center, Nebraska using methods described by Borg et al. (1993).

Statistics

Least square means were obtained for each trait. Total paired testes weight (TOTWT) and left epididymal weight (EPIWT) were estimated by adjusting for the animal's BW. Volume density measurements and hormone concentration calculations were adjusted for age of animal at time of sample collection. Mean differences between lines for total paired testes weight and left epididymal weight was determined according to the model:

$$Y_{hij} = \alpha_j + \beta_h + b(x_i - x) + \gamma_{i(j)} + \epsilon_{hij}$$

where Y_{hij} = weight of the i^{th} animal of the j^{th} line in the h^{th} year, α_j = effect of the j^{th} line, β_h = effect of the h^{th} year, b = regression coefficient, x_i = body weight of the i^{th} animal in kilograms, $\gamma_{i(j)}$ = random effect of the i^{th} animal in the j^{th} line, ϵ_{hij} = random error.

Differences between high and low line means were determined for both PVD and TVD using the model:

$$Y_{hij} = \alpha_j + \beta_h + \alpha_j\beta_h + \gamma_{i(j)} + \epsilon_{hij}$$

where Y_{hij} = volume density % of the picture of the i^{th} animal in the j^{th} line that was counted by the h^{th} technician, α_j = effect due to j^{th} line, β_h = effect due to h^{th} technician, $\alpha_j\beta_h$ = effect due to line by technician interaction, $\gamma_{i(j)}$ = random effect of the i^{th} animal within the j^{th} line, ϵ_{hij} = random error.

Sperm production trait differences were calculated using the model:

$$Y_{hij} = \alpha_j + b(x_i - x) + \beta_{i(j)} + \gamma_{h(ij)} + \epsilon_{hij}$$

where Y_{hij} = sperm production in the h^{th} sample of the i^{th} animal in the j^{th} line, α_j = effect due to j^{th} line, b = regression coefficient, x_i = age of the i^{th} animal at castration, $\beta_{i(j)}$ = random

effect of the i^{th} animal in the j^{th} line, $\gamma_{h(ij)}$ = random effect of the h^{th} tissue within the i^{th} animal and j^{th} line, ϵ_{hij} = random error.

Least square means for testosterone and FSH concentrations were calculated using similar models, however effect due to assay was not determined for FSH. Differences in hormone concentrations were determined using the model:

$$Y_{hijk} = \alpha_j + \beta_h + b(x_i - x) + \gamma_k + \epsilon_{hijk}$$

where Y_{hijk} = testosterone concentration in ng/dl for the k^{th} sample of the h^{th} assay of the i^{th} animal in the j^{th} line, α_j = effect due to the j^{th} line, β_h = effect due to the h^{th} assay, b= regression coefficient, x_i = age of the i^{th} animal when blood samples were taken, γ_k = effect due to the time when the k^{th} sample was drawn, ϵ_{hijk} = random error.

RESULTS

Low testosterone line boars had a greater average TOTWT than HTL boars at 457 g and 417 g ($P < 0.01$; Table 1), respectively. However, LTL boars had a lower mean EPIWT than HTL boars ($P < 0.02$; Table 1). Effects of generation and age were estimated for both TOTWT and EPIWT, and were significant for both traits.

Boars from generation 21 were assayed at an average age of 190 d for testosterone (TEST) and FSH concentrations. The LTL had lower TEST (278 ng/mL) than HTL (490 ng/mL) ($P < 0.01$; Table 1). Fixed effect of assay was significant ($P < 0.01$) as was covariate of age ($P < 0.1$). Concentration of FSH did not differ between lines (Table 1). Covariate effect of age at which samples were drawn was significant ($P < 0.05$).

Morphological characteristics of the testes were measured in boars from generation 20. Picture volume density (PVD) and total volume density of the left testis (TVD) were

determined for leydig cells, seminiferous tubules, and sertoli cells. Effects of technician and line x technician were determined for each estimate. Leydig cell PVD was lower in LTL boars than HTL boars ($P < 0.02$). Also, TVD differed between the lines with LTL again having a lower average per gram of testis than HTL ($P < 0.01$). The LTL had a greater PVD for seminiferous tubules than HTL ($P < 0.07$), with a similar trend observed for TVD ($P < 0.1$; Table 2). Sertoli cell volume density did not differ between LTL and HTL for PVD or TVD (Table 2).

Sperm production traits were determined for generation 20, and all estimates were adjusted for age. Least square means estimates for SPM/g, DSP/g, TDSP, and TTS did not differ between the lines (Table 3).

DISCUSSION

In this study, HTL boars had significantly smaller paired testis weights and larger epididymal weights, than LTL boars. Increased epididymal weights found in HTL are likely due to increased testosterone levels. While the specific action of testosterone with respect to development of the epididymus is not yet known, testosterone acts on the epididymus via androgen receptors. It is thought that a metabolite of testosterone acts through this pathway to cause epididymal differentiation and maturation (Fisher et al., 2002). A decrease in testosterone has also been shown to cause epididymal degeneration (Nair et al., 2002). It is likely that lower testosterone levels in LTL boars during development had a negative influence on epididymal growth and ultimately weight.

Leydig cell volume density was significantly higher in HTL, which is directly related to testosterone levels. Leydig cells are the main source of steroids within the male body

(Tripepi et al., 2000). Thus, more leydig cells would be expected to result in greater testosterone concentrations. Seminiferous tubule volume density tended to differ between lines with LTL having a greater volume density than HTL. It is unknown as to whether the volume density of the tubules differ due to a decrease in tubule diameter or because they are shorter than those of the LTL.

Sertoli cell proliferation is a primary factor in determining testis size and sperm production capacity (Ford et al., 2001; França et al., 2000; Zanella et al., 1999). Since sertoli cells do not continue to proliferate after puberty, prepubertal plasma FSH levels are important for sertoli cell proliferation, and in determining sperm producing capacity of the testis at maturity (França et al., 2000; Krantic and Benahmed, 2000). The small increase seen in the LTL for sperm per gram of testis, daily sperm per gram of testis, total daily sperm production, and total testicular sperm is directly related to the increase in sertoli cell volume density. Sertoli cells play a critical role in maturation of germ cells and sperm production (Ford et al., 2001; Krantic and Benahmed, 2000; McCoard et al., 2000).

IMPLICATIONS

Though divergent selection for testosterone production was successful, traits associated with sperm production were unaffected. At this time, selection for testosterone is not suggested as a means for increasing sperm production for use with artificial insemination practices.

Table 1. Least squares means for TOTWT, EPIWT, TEST, and FSH[a]

	LTL	SE	HTL	SE	P-Value
TOTWT, g	457.33	± 12.06	417.54	± 8.65**	0.008
EPIWT, g	93.15	± 12.65	126.32	± 12.42*	0.013
TEST, ng/mL	278.08	± 43.94	490.09	± 41.50**	0.001
FSH, ng/mL	0.19	± 0.01	0.20	± 0.01	0.236

[a]TOTWT = total paired testicular weight after removal of epididymus, EPIWT = weight of the left epididymus, TEST = concentration of testosterone, FSH = concentration of FSH. LTL = low testosterone line, HTL = high testosterone line.

* $P < 0.05$.

** $P < 0.01$.

Table 2. Least squares means for volume density of Leydig cells, seminiferous tubules, and Sertoli Cells[a]

	LTL	SE	HTL	SE	P-Value
Leydig Cells					
PVD	14.17	± 1.24	17.94	± 0.75**	0.012
TVD	40.43	± 3.59	52.19	± 3.02*	0.010
Seminiferous Tubules					
PVD	51.89	± 2.06	47.36	± 1.25†	0.065
TVD	141.13	± 5.89	129.00	± 4.95†	0.095
Sertoli Cells					
PVD	56.62	± 2.59	53.15	± 2.18	0.273
TVD	153.01	± 6.72	143.77	± 5.66	0.263

[a]PVD = volume density of the structure in the picture, TVD = volume density of the structure in the left testis. LTL = low testosterone line, HTL = high testosterone line.

† $P < 0.1$.

* $P < 0.05$.

** $P < 0.01$.

Table 3. Least squares means for DSP/g, TDSP, SPM/g, and TTS[a]

	LTL	SE	HTL	SE	P-Value
DSP/g	2.89×10^6	± 2.47	2.72×10^6	± 1.50	0.545
TDSP	1.62×10^9	± 0.17	1.42×10^9	± 0.11	0.341
SPM/g	12.65×10^6	± 1.08	11.87×10^6	± 0.65	0.544
TTS	7.08×10^9	± 0.76	6.23×10^9	± 0.46	0.342

[a]SPM/g = sperm per gram of testis, DSP/g = daily sperm production per gram of testis, TDSP = total daily sperm production, TTS = total testicular sperm. LTL = low testosterone line, HTL = high testosterone line.

Table 4. Average animal weight at castration

	Weight (kg)		Weight (kg)	
Year	LTL	SD	HTL	SD
2001	106.64	12.40	109.49	12.75
2002	90.74	10.63	86.73	13.22
Total	96.16	13.48	98.11	17.26

Table 5. Average age of animals at castration

	Age (d)		Age (d)	
Year	LTL	SD	HTL	SD
2001	209.62	6.15	212.39	8.09
2002	197.55	5.94	194.05	7.02
Total	202.00	8.34	203.00	11.61

Table 6. Least square means for D140WT, SHBF, LNBF, AVGBF[a]

	LTL	SE	HTL	SE	P-Value
D140WT	148.34	± 1.06	143.68	± 1.03**	0.001
SHBF	0.77	± 0.005	0.78	± 0.005	0.114
LNBF	0.57	± 0.005	0.58	± 0.005*	0.034
AVGBF	0.67	± 0.005	0.68	± 0.005†	0.054

[a]D140WT = average BW at 140 d of age, SHBF = shoulder BF, LNBF = loin BF, AVGBF = average of SHBF and LNBF. LTL = low testosterone line, HTL = high testosterone line.

† $P < 0.1$.

* $P < 0.05$.

** $P < 0.01$.

Table 7. ANOVA table, fixed effects, and regression coefficients of TOTWT for LTL[a]

ANOVA

Source	DF	Sum of Squares	Mean Square	F-Value	P-Value
Model	2	600756.31	300378.16	50.86	0.0001
Error	41	242158.88	5906.31		
Corrected Total	43	842915.19			

Fixed Effects

Source	Regression Coefficient	P-Value
Year	-----	0.6250
Weight[a]	8.46	0.0001

[a] TOTWT = total paired testicular weight, Weight = animal weight at castration (kg). LTL = low testosterone line.

Table 8. ANOVA table, fixed effects, and regression coefficients of TOTWT for HTL[a]

ANOVA

Source	DF	Sum of Squares	Mean Square	F-Value	P-Value
Model	2	807620.91	403810.46	79.90	0.0001
Error	79	399244.57	5053.73		
Corrected Total	81	1206865.48			

Fixed Effects

Source	Regression Coefficient	P-Value
Year	-----	0.0001
Weight[a]	3.20	0.0001

[a] TOTWT = total paired testicular weight, Weight = animal weight at castration (kg). HTL = high testosterone line.

Table 9. ANOVA table, fixed effects, and regression coefficients of EPIWT for LTL[a]

ANOVA

Source	DF	Sum of Squares	Mean Square	F-Value	P-Value
Model	2	18359.59	9179.79	22.12	0.0001
Error	41	17018.29	415.08		
Corrected Total	43	35377.87			

Fixed Effects

Source	Regression Coefficient	P-Value
Year	-----	0.0786
Weight[a]	1.19	0.0001

[a] EPIWT = left epididymal weight, Weight = animal weight at castration (kg). LTL = low testosterone line.

Table 10. ANOVA table, fixed effects, and regression coefficients of EPIWT for HTL[a]

ANOVA

Source	DF	Sum of Squares	Mean Square	F-Value	P-Value
Model	2	33072.94	16536.47	56.15	0.0001
Error	79	23267.88	294.53		
Corrected Total	81	56340.82			

Fixed Effects

Source	Regression Coefficient	P-Value
Year	-----	0.1265
Weight[a]	1.01	0.0001

[a] EPIWT = left epididymal weight, Weight = animal weight at castration (kg). HTL = High testosterone line.

Table 11. ANOVA table, fixed effects, and regression coefficients of TEST for LTL[a]

ANOVA

Source	DF	Sum of Squares	Mean Square	F-Value	P-Value
Model	3	273352.53	91117.51	2.43	0.0713
Error	84	3156080.91	37572.39		
Corrected Total	87	3429433.44			

Fixed Effects

Source	Regression Coefficient	P-Value
Assay	-----	0.0113
Sample	-----	0.4739
Age	9.71	0.0694

[a] TEST = testosterone concentration. LTL = low testosterone line.

Table 12. ANOVA table, fixed effects, and regression coefficients of TEST for HTL[a]

ANOVA

Source	DF	Sum of Squares	Mean Square	F-Value	P-Value
Model	3	2489672.57	829890.86	6.86	0.0003
Error	104	12574841.42	120911.94		
Corrected Total	107	15064513.98			

Fixed Effects

Source	Regression Coefficient	P-Value
Assay	-----	0.0001
Sample	-----	0.8799
Age	18.27	0.0354

[a] TEST = testosterone concentration. HTL = high testosterone line.

Table 13. ANOVA table, fixed effects, and regression coefficients of FSH for LTL[a]

ANOVA

Source	DF	Sum of Squares	Mean Square	F-Value	P-Value
Model	2	0.1188	0.0594	8.02	0.0011
Error	42	0.3111	0.0074		
Corrected Total	44	0.4299			

Fixed Effects

Source	Regression Coefficient	P-Value
Sample	-----	0.2472
Age	-0.08	0.0003

[a] FSH = FSH concentration. LTL = low testosterone line.

Table 14. ANOVA table, fixed effects, and regression coefficients of FSH for HTL[a]

ANOVA

Source	DF	Sum of Squares	Mean Square	F-Value	P-Value
Model	2	0.0392	0.0196	3.33	0.0416
Error	70	0.4120	0.0059		
Corrected Total	72	0.4512			

Fixed Effects

Source	Regression Coefficient	P-Value
Sample	-----	0.3135
Age	-0.003	0.0319

[a] FSH = FSH concentration. HTL = high testosterone line.

Table 15. ANOVA table, fixed effects, and regression coefficients of Leydig cell PVD for LTL[a]

ANOVA

Source	DF	Sum of Squares	Mean Square	F-Value	P-Value
Model	2	22404.18	11202.09	62.21	0.0001
Error	87	15666.99	180.08		
Corrected Total	89	38071.17			

Fixed Effects

Source	Regression Coefficient	P-Value
Technician	-----	0.8992

[a] PVD = picture volume density. LTL = low testosterone line.

Table 16. ANOVA table, fixed effects, and regression coefficients of Leydig cell PVD for HTL[a]

ANOVA

Source	DF	Sum of Squares	Mean Square	F-Value	P-Value
Model	2	293.31	146.65	3.85	0.0226
Error	243	9256.21	38.09		
Corrected Total	245	9549.52			

Fixed Effects

Source	Regression Coefficient	P-Value
Technician	-----	0.4824

[a] PVD = picture volume density. HTL = high testosterone line.

Table 17. ANOVA table, fixed effects, and regression coefficients of Leydig cell TVD for LTL[a]

ANOVA

Source	DF	Sum of Squares	Mean Square	F-Value	P-Value
Model	2	22404.18	11202.09	62.21	0.0001
Error	243	15666.99	180.08		
Corrected Total	245	38071.17			

Fixed Effects

Source	Regression Coefficient	P-Value
Technician	-----	0.9531
LT Weight[a]	0.27	0.0001

[a] TVD = total volume density, LT Weight = Left testis weight. LTL = low testosterone line.

Table 18. ANOVA table, fixed effects, and regression coefficients of Leydig cell TVD for HTL

ANOVA

Source	DF	Sum of Squares	Mean Square	F-Value	P-Value
Model	2	31632.99	15816.50	58.87	0.0001
Error	243	65285.48	268.66		
Corrected Total	245	96918.47			

Fixed Effects

Source	Regression Coefficient	P-Value
Technician	-----	0.5284
LT Weight[a]	0.24	0.0001

[a] LT Weight = Left testis weight.

Table 19. ANOVA table, fixed effects, and regression coefficients of Seminiferous Tubule PVD for LTL[a]

ANOVA

Source	DF	Sum of Squares	Mean Square	F-Value	P-Value
Model	2	221.14	110.57	1.14	0.3244
Error	87	8435.28	96.96		
Corrected Total	89	8656.43			

Fixed Effects

Source	Regression Coefficient	P-Value
Technician	-----	0.6794

[a] PVD = picture volume density. LTL = low testosterone line.

Table 20. ANOVA table, fixed effects, and regression coefficients of Seminiferous Tubule PVD for HTL[a]

ANOVA

Source	DF	Sum of Squares	Mean Square	F-Value	P-Value
Model	2	51.12	25.56	0.26	0.7682
Error	243	23528.96	96.83		
Corrected Total	245	23580.08			

Fixed Effects

Source	Regression Coefficient	P-Value
Technician	-----	0.9758

[a] PVD = picture volume density. HTL = high testosterone line.

Table 21. ANOVA table, fixed effects, and regression coefficients of Seminiferous Tubule TVD for LTL[a]

ANOVA

Source	DF	Sum of Squares	Mean Square	F-Value	P-Value
Model	2	57345.94	28672.97	44.80	0.0001
Error	87	55686.99	640.08		
Corrected Total	89	113032.93			

Fixed Effects

Source	Regression Coefficient	P-Value
Technician	-----	0.6876
LT Weight[a]	0.432	0.0001

[a] TVD = total volume density, LT Weight = left testis weight. LTL = low testosterone line.

Table 22. ANOVA table, fixed effects, and regression coefficients of Seminiferous Tubule TVD for HTL[a]

ANOVA

Source	DF	Sum of Squares	Mean Square	F-Value	P-Value
Model	2	112401.31	56200.65	87.43	0.0001
Error	243	156199.60	642.80		
Corrected Total	245	268600.91			

Fixed Effects

Source	Regression Coefficient	P-Value
Technician	-----	0.9732
LT Weight[a]	0.447	0.0001

[a] TVD = total volume density, LT Weight = Left testis weight. HTL = high testosterone line.

Table 23. ANOVA table, fixed effects, and regression coefficients of Sertoli cell PVD for LTL[a]

ANOVA

Source	DF	Sum of Squares	Mean Square	F-Value	P-Value
Model	2	27.89	27.89	0.22	0.6412
Error	55	7115.25	127.06		
Corrected Total	57	7143.14			

Fixed Effects

Source	Regression Coefficient	P-Value
Technician	-----	0.6412

[a] PVD = picture volume density. LTL = low testosterone line.

Table 24. ANOVA table, fixed effects, and regression coefficients of Sertoli cell PVD for HTL[a]

ANOVA

Source	DF	Sum of Squares	Mean Square	F-Value	P-Value
Model	2	74.00	74.00	0.78	0.3800
Error	151	14509.67	95.46		
Corrected Total	153	14583.66			

Fixed Effects

Source	Regression Coefficient	P-Value
Technician	-----	0.3800

[a] PVD = picture volume density. HTL = high testosterone line.

Table 25. ANOVA table, fixed effects, and regression coefficients of Sertoli Cell TVD for LTL[a]

ANOVA

Source	DF	Sum of Squares	Mean Square	F-Value	P-Value
Model	2	51531.50	25765.75	26.36	0.0001
Error	55	53752.59	977.32		
Corrected Total	57	105284.09			

Fixed Effects

Source	Regression Coefficient	P-Value
Technician	-----	0.5957
LT Weight[a]	0.501	0.0001

[a] TVD = total volume density, LT Weight = Left testis weight. LTL = low testosterone line.

Table 26. ANOVA table, fixed effects, and regression coefficients of Sertoli cell TVD for HTL[a]

ANOVA

Source	DF	Sum of Squares	Mean Square	F-Value	P-Value
Model	2	82706.90	41353.45	67.27	0.0001
Error	151	92824.26	614.73		
Corrected Total	153	175531.15			

Fixed Effects

Source	Regression Coefficient	P-Value
Technician	-----	0.2992
LT Weight[a]	0.484	0.0001

[a] TVD = total volume density, LT Weight = Left testis weight. HTL = high testosterone line.

Table 27. ANOVA table, fixed effects, and regression coefficients of SPM/g for LTL[a]

ANOVA

Source	DF	Sum of Squares	Mean Square	F-Value	P-Value
Model	1	1.16E15	1.16E15	23.59	0.0001
Error	672	3.31E16	4.92E13		
Corrected Total	673	3.42E16			

Fixed Effects

Source	Regression Coefficient	P-Value
Age	213481.29	0.0001

[a] SPM/g = sperm per gram of testis. LTL = low testosterone line.

Table 28. ANOVA table, fixed effects, and regression coefficients of SPM/g for HTL[a]

ANOVA

Source	DF	Sum of Squares	Mean Square	F-Value	P-Value
Model	1	4.35E14	4.35E14	8.95	0.0028
Error	1799	8.74E16	4.86E13		
Corrected Total	1800	8.79E16			

Fixed Effects

Source	Regression Coefficient	P-Value
Age	-60794.87	0.0028

[a] SPM/g = sperm per gram of testis. HTL = high testosterone line.

Table 29. ANOVA table, fixed effects, and regression coefficients of TTS for LTL[a]

ANOVA

Source	DF	Sum of Squares	Mean Square	F-Value	P-Value
Model	1	1.36E21	1.36E21	69.92	0.0001
Error	672	1.30E22	1.94E19		
Corrected Total	673	1.44E22			

Fixed Effects

Source	Regression Coefficient	P-Value
Age	230825194	0.0001

[a] TTS = total testicular sperm. LTL = low testosterone line.

Table 30. ANOVA table, fixed effects, and regression coefficients of TTS for HTL[a]

ANOVA

Source	DF	Sum of Squares	Mean Square	F-Value	P-Value
Model	1	3.22E18	3.22E18	0.20	0.6549
Error	1799	2.90E22	1.61E19		
Corrected Total	1800	2.90E22			

Fixed Effects

Source	Regression Coefficient	P-Value
Age	5230974	0.6549

[a a] TTS = total testicular sperm. HTL = high testosterone line.

Table 31. ANOVA table, fixed effects, and regression coefficients of DSP/g for LTL[a]

ANOVA

Source	DF	Sum of Squares	Mean Square	F-Value	P-Value
Model	1	6.06E13	6.06E13	23.53	0.0001
Error	672	1.73E15	2.58E12		
Corrected Total	673	1.79E15			

Fixed Effects

Source	Regression Coefficient	P-Value
Age	48790.980	0.0001

[a] DSP/g = daily sperm production per gram of testis. LTL = low testosterone line.

Table 32. ANOVA table, fixed effects, and regression coefficients of DSP/g for HTL[a]

ANOVA

Source	DF	Sum of Squares	Mean Square	F-Value	P-Value
Model	1	2.28E13	2.28E13	8.97	0.0028
Error	1799	4.58E15	2.54E12		
Corrected Total	1800	4.60E15			

Fixed Effects

Source	Regression Coefficient	P-Value
Age	-13927.22	0.0028

[a] DSP/g = daily sperm production per gram of testis. HTL = high testosterone line.

Table 33. ANOVA table, fixed effects, and regression coefficients of TDSP for LTL[a]

ANOVA

Source	DF	Sum of Squares	Mean Square	F-Value	P-Value
Model	1	7.12E19	7.12E19	69.97	0.0001
Error	672	6.84E20	1.02E18		
Corrected Total	673	7.55E20			

Fixed Effects

Source	Regression Coefficient	P-Value
Age	52870299	0.0001

[a] TDSP = total daily sperm production. LTL = low testosterone line.

Table 34. ANOVA table, fixed effects, and regression coefficients of TDSP for HTL[a]

ANOVA

Source	DF	Sum of Squares	Mean Square	F-Value	P-Value
Model	1	1.71E17	1.71E17	0.20	0.6523
Error	1799	1.52E21	8.44E17		
Corrected Total	1800	1.52E21			

Fixed Effects

Source	Regression Coefficient	P-Value
Age	1206857	0.6523

[a] TDSP = total daily sperm production. HTL = high testosterone line.

Appendix A: Fixation, Dehydration and Embedding Protocol

Tissue Fixation:

1. Place newly collected tissue samples in 4% Paraformaldehyde and immediately place on ice.
2. When you return to the lab, pour off the 4% Paraformaldehyde and add fresh to the container.
3. Place tubes on a slow rocker in a refrigerator overnight.
4. Pour off 4% Paraformaldehyde, and add 1xPBS. Place tube back on rocker in refrigerator for 30 minutes.
5. Pour off 1xPBS, and add fresh 1xPBS. Place tube back on rocker in refrigerator for 30 minutes.
6. Pour off 1xPBS, and add 50% Ethanol. Place tube back on rocker in refrigerator for 30 minutes.
7. Pour off 50% Ethanol, and add fresh 50% Ethanol. Place tube back on rocker in refrigerator for 30 minutes.
8. Pour off 50% Ethanol, and add 70% Ethanol. Place tube back on rocker in refrigerator for 30 minutes.
9. Pour off 70% Ethanol, and fresh 70% Ethanol. Place tube back on rocker in refrigerator for 30 minutes.
10. Pour off 70% Ethanol, and transfer tissue to clean tube with fresh 70% Ethanol. Tissue can be stored in refrigerator until embedded. Replace solutions with clean 70% Ethanol at least once a week.

Tissue Dehydration and Embedding:

1. Submerge tissue in 85% Ethanol for 45 minutes.
2. Remove the tissue and place it in 95% Ethanol for 45 minutes.
3. Remove the tissue and place it in 100% Ethanol for 45 minutes.
4. Remove the tissue and place it in a 1:1 solution of 100% Ethanol and Xylene for 45 minutes.
5. Place the tissue in a clean container filled with Xylene for 45 minutes.
6. Remove the tissue and place it in clean Xylene for 45 minutes.
7. Remove the tissue and place it in a 1:1 solution of Xylene and Paraffin for 45 minutes.
8. Place the tissue in a clean container filled with Paraffin for 45 minutes.
9. Remove the tissue and place it in fresh Paraffin for 45 minutes.
10. Remove the tissue and place on the cassette. When the wax is hardened, remove from the mold and store at -20ºC.

Appendix B: Rehydration and Staining Protocol

1. Place slide with attached tissue sample into slide tray, and submerge tray in Xylene for 10 minutes.
2. Remove the tray, and place it in clean Xylene for 10 minutes.
3. Remove the tray, and place it in 100% Ethanol for 5 minutes.
4. Remove the tray, and place it in 95% Ethanol for 5 minutes.
5. Remove the tray, and place it in 70% Ethanol for 5 minutes.
6. Remove the tray, and place it in 50% Ethanol for 5 minutes.
7. Remove the tray, and rinse it well in DI H_2O.
8. Place the tray in Hematoxylin stain solution for 1 minute
9. Place the tray in clean tap H_2O for 2 minutes until the stain blues
10. Dip the tray once in 0.1% HCL in 70% Ethanol solution.
11. Immediately place the tray into tap H_2O for 20 minutes.
12. Place the tray in Eosin stain solution for 45 seconds.
13. Place the tray into clean tap H_2O for 4 minutes.
14. Place the tray into 70% Ethanol for 3 minutes.
15. Place the tray into clean 70% Ethanol for 3 minutes.
16. Place the tray into 95% Ethanol for 3 minutes.
17. Place the tray into clean 95% Ethanol for 3 minutes.
18. Place the tray in 100% Ethanol for 3 minutes.
19. Place the tray into clean 100% Ethanol for 3 minutes.
20. Place the tray into CitriSolv solution for 10 minutes.
21. Allow slides to dry at room temperature.

22. Place 1 drop of Permount on the sample and mount the cover slip.

If using Hematoxylin stain only stop after step 11.

Appendix C: Daily Sperm Production Protocol

1. Obtain a ~10 g piece of testis, being careful to avoid the mediastinum area. Record the weight of the sample and place it in a 50 mL conical tube. Tissue can be held at -20°C until it is ready to be processed.
2. Add tissue to 200 mL ice-cold Phosphate Buffered Saline (0.15M, pH 7.4) containing 0.1% Triton-X-100 and homogenize for 30 seconds at maximum speed.
3. Immediately remove 0.5 mL of the solution and mix it thoroughly with 0.5 mL of Trypan Blue stain solution (0.4% in normal saline) to make a 400-fold dilution.
4. Place a small drop of solution onto each side of a hemacytometer, and count and record the number of intact elongated spermatid nuclei in five of the major squares.
5. Multiply each count by 20×10^6 to obtain the number of total intact spermatids per hemacytometer square. Then divide this number by the weight of the homogenized tissue to obtain Sperm/Gm for that sample.
6. Multiply Sperm/Gm by the total paired parenchymal weight (g) to derive the estimate for total testicular sperm.
7. Daily Sperm Production per Gram of testis is obtained by dividing the Sperm/Gm estimate by the "species-specific DSP Time-Divisor Constant" number of days for boars, which is 4.37 d.
8. Total DSP for the animal is obtained by multiplying the DSP/Gm estimate by the total paired testicular parenchymal weight (g).

Procedure obtained from Donald D. Lunstra, Reproductive Research Unit, RLHUSMARC, Clay Center, NE – May 1997

LITERATURE CITED

Allrich, R.D., R.K. Christenson, J.J. Ford, and D.R. Zimmerman. 1983. Pubertal development of the boar: Age-related changes in testicular morphology and in vitro production of testosterone and estradiol-17β. Biol. Reprod. 28:902-909.

Baker, P.J., P. Pakarinen, I.T. Huhtaniemi, M.H. Abel, H.M. Charlton, T.R. Kumar, and P.J. O'Shaughnessy. 2003. Failure of normal leydig cell development in follicle-stimulating hormone (FSH) receptor-deficient mice, but not FSHβ-deficient mice: Role for constitutive FSH receptor activity. Endocrinology. 144:138-145.

Bardin, C.W., G.L. Gunslus, and C.Y. Cheng. 1993. The cell biology of the sertoli cell. Page 189 in Cell and Molecular Biology of the Testis. C. Desjardins and L. Ewing, eds. Oxford University Press, New York, NY.

Borg, K.D., D.D. Lunstra, and R.K. Christenson. 1993. Semen characteristics, testicular size, and reproductive hormone concentrations in mature Duroc, Meishan, Fengjing, and Minzhu boars. Biol. Reprod. 49:515-521.

Burgos, M.H., R. Vitale-Calpe and A. Aoki. 1970. Fine structure of the testis and its functional significance. Page 551 in The Testis, Vol. 1. A.D. Johnson, W.R. Gomes, and N.L. Vendemark, eds. Academic Press, New York, NY.

Cardenas, H., J.G. Berardinelli, P.J. Burfening, and R. Adair. 1994. Histomorphology, oLH and hCG receptors, and testosterone secretion *in vitro* in rambouillet rams from lines in which females had been selected for low or high reproductive rate. J. Reprod. Fertil. 102:201-207.

Cassady, J.P., R.K. Johnson, and J.J. Ford. 2000. Comparison of plasma FSH concentrations in boars and gilts from lines selected for ovulation rate and

embryonal survival, and litter size and estimation of (co)variance components for FSH and ovulation rate. J. An. Sci. 78:1430-1435.

Clermont, Y., R. Oko, and L. Hermo. 1993. Cell biology of mammalian spermiogenesis. Page 332 in Cell and Molecular Biology of the Testis. C. Desjardins and L. Ewing, eds. Oxford University Press, New York, NY.

Courot, M., M.T. Hochereau-de Reviers, and R. Ortavant. Spermatogenesis. Page 339 in The Testis, Vol. 1. A.D. Johnson, W.R. Gomes, and N.L. Vendemark, eds. Academic Press, New York, NY.

Estienne, M. 1999. Advantages to using artificial insemination in swine breeding programs. Available: http://www.ext.vt.edu/news/periodicals/livestock/aps-99_09/aps-0123.html. Accessed Jan. 22, 2003.

Ewing, L. and B.L. Brown. 1977. Testicular Steroidogenesis. Page 239 in The Testis, Vol. 4. A.D. Johnson, W.R. Gomes, and N.L. Vendemark, eds. Academic Press, New York, NY.

Ewing, L.L. and D.S. Keeney. 1993. Leydig cells: Structure and function. Page 137 in Cell and Molecular Biology of the Testis. C. Desjardins and L. Ewing, eds. Oxford University Press, New York, NY.

Fisher, J.S., N. Pastor-Soler, R.M. Sharpe, and S. Breton. 2002. Modulation on the onset of postnatal development of H^+-ATPase-rich cells by steroid hormones in rat epididymis. Biol. Reprod. 67:1106-1114.

Ford, J.J., T.H. Wise, D.D Lunstra, and G.A. Rohrer. 2001. Interrelationships of porcine X and Y chromosomes with pituitary gonadotropins and testicular size. Biol. Reprod. 65:906-912.

Ford, J.J., T.H. Wise, and D.D. Lunstra. 1997. Negative relationship between blood concentrations of follicle-stimulating hormone and testicular size in mature boars. J. Anim. Sci. 75:790-795.

França, L.R., V.A. Silva Jr., H. Chiarini-Garcia, S.K. Garcia, and L. Debeljuk. 2000. Cell proliferation and hormonal changes during postnatal development of the testis in the pig. Biol. Reprod. 63:1629-1636.

Fox, S.I. 1999. Human Physiology. 6th ed. McGraw-Hill. Boston, MA.

Gier, H.T. and G.B. Marion. 1970. Development of the mammalian testis. Page 2 in The Testis, Vol. 1. A.D. Johnson, W.R. Gomes, and N.L. Vendemark, eds. Academic Press, New York, NY.

Guillaumot, P., E. Tabone, and M. Benahmed. 1996. Sertoli cells as potential targets of prolactin action in the testis. Mol. Cell. Endocrinol. 122:199-206.

Haley, C.S., G.J. Lee, M. Fordyce, G. Baxter, R.B. Land, and R. Webb. 1989. Study of LH response to GnRH in the young male as a criterion of genetic merit for female reproduction in sheep. J. Reprod. Fertil. 86:119-133.

Harder, R.R., D.D. Lunstra, and R.K. Johnson. 1995. Growth of testes and testicular morphology after eight generations of selection for increased predicted weight of testes at 150 days of age in boars. J. Anim. Sci. 73:2186-2192.

Hooker, C.W. 1970. The intertubular tissue of the testis. Page 483 in The Testis, Vol. 1. A.D. Johnson, W.R. Gomes, and N.L. Vendemark, eds. Academic Press, New York, NY.

Hughes, P., and M. Varley. 1980. Reproduction in Pigs. 1st ed. Butterworth, Inc. Boston, MA.

Kaaks, R., A. Lukanova, and B. Sommersberg. 2000. Plasma androgens, IGF-I, body size, and prostate cancer risk: a synthetic review. Prostate Cancer Prostatic Dis. 3:157-172.

Krantic, S. and M. Benahmed. 2000. Somatostatin inhibits follicle-stimulating hormone-induced adenylyl cyclase activity and proliferation in immature porcine sertoli cell via sst2 receptor. Biol. Reprod. 62:1835-1843.

Land, R.B. 1978. Selection among males for the genetic improvement of female fertility. J. Anim. Sci. 47:48-55.

Lubritz, D., B. Johnson, and O.W. Robison. 1991. Genetic parameters for testosterone production in boars. J. Anim. Sci. 69:3220-3224.

Lunstra, D.D., J.J. Ford, R.K. Christenson and R.D. Allrich. 1986. Changes in leydig cell ultrastructure and function during pubertal development in the boar. Biol. Reprod. 34:145-158.

Lunstra, D.D., T.H. Wise, and J.J. Ford. 2002. Sertoli cells in the boar testis: Changes during development and compensatory hypertrophy after hemicastration at different ages. Biol. Reprod. 68:140-150.

Mariscal, D.V., P.L. Wolfe, E.G. Bergfeld, A.S. Cupp, F.N. Kojima, K.E Fike, T. Sánchez, M.E. Wehrman, R.K. Johnson, R.J. Kittok, J.J. Ford, and J.E. Kinder. 1996. Comparison of circulating concentrations of reproductive hormones in boars of lines selected for size of testes or number of ovulations and embryonal survival to concentrations in respective control lines. J. An. Sci. 74:1905-1914.

McCoard, S.A., D.D. Lunstra, T.H. Wise, and J.J. Ford. 2000. Specific staining of sertoli cell nuclei and evaluation of sertoli cell number and proliferative activity in

meishan and white composite boars during the neonatal period. Biol. Reprod. 64:689-695.

McKinnie, M.R., J.H. Britt, and J.L. Esbenshade. 1988. Ovarian function and hormone secretion of gilts actively immunized against androstenedione. J. Anim. Sci. 66:3131-3143.

McNeilly, J.R., M. Fordyce, R.B. Land, G.J. Lee, and R. Webb. 1986. Endocrine differences in rams after genetic selection for testis size. J. Reprod. Fertil. 76:131-140.

Means, A.R. 1977. Mechanisms of action of FSH. Page 163 in The Testis, Vol. 4. A.D. Johnson, W.R. Gomes, and N.L. Vendemark, eds. Academic Press, New York, NY.

Meistrich, M.L. and M. van Beek. 1993. Spermatogonial stem cells. Page 266 in Cell and Molecular Biology of the Testis. C. Desjardins and L. Ewing, eds. Oxford University Press, New York, NY.

Mydlo, J.H., N.L. Tieng, M.A. Volpe, R. Chaiken, and J. G. Kral. 2001. A pilot study analyzing PSA, serum testosterone, lipid profile, body mass index and race in a small sample of patients with and without carcinoma of the prostate. Prostate Cancer Prostatic Dis. 4:101-105.

Nair, N., R.S. Bedwal, and R.S. Mathur. 2002. Effect of adrenalectomy on rat epididymidis. Asian J. Androl. 4:273-279.

Neaves, W.B. 1977. The blood-testis barrier. Page 125 in The Testis, Vol. 4. A.D. Johnson, W.R. Gomes, and N.L. Vendemark, eds. Academic Press, New York, NY.

Orth, J.M. 1993. Cell biology of testicular development in the fetus and neonate. Page 3 in Cell and Molecular Biology of the Testis. C. Desjardins and L. Ewing, eds. Oxford University Press, New York, NY.

Rathje, T.A., R.K. Johnson, and D.D. Lunstra. 1995. Sperm production in boars after nine generations of selection for increased weight of testis. J. Anim. Sci. 73:2177-2185.

Robison, O.W., D. Lubritz, and B. Johnson. 1994. Realized heritability estimates in boars divergently selected for testosterone levels. J. Anim. Breed. Genet. 111:35-42.

Rothschild, M.F. and A. Ruvinsky. 1998. The genetics of the pig. 1st ed. CAB International. New York, NY.

Schinckel, A.P., R.K. Johnson and R.J. Kittok. 1984. Testicular development and endocrine characteristics of boars selected for either high or low testis size. J. Anim. Sci. 58:675-685.

Senger, P.L. 1999. Pathways to pregnancy and parturition. 1st ed. Current Conceptions, Inc. Pullman, WA.

Setchell, B.P., R.V. Davies, and S.J. Main. 1977. Inhibin. Page 189 in The Testis, Vol. 4. A.D. Johnson, W.R. Gomes, and N.L. Vendemark, eds. Academic Press, New York, NY.

Singleton, W.L. 2001. State of the art in artificial insemination of pigs in the United States. Theriogenology. 56:1305-1310.

Steinberger, A. and E. Steinberger. 1977. The sertoli cells. Page 371 in The Testis, Vol. 4. A.D. Johnson, W.R. Gomes, and N.L. Vendemark, eds. Academic Press, New York, NY.

Toelle, V.D., B.H. Johnson, and O.W. Robison. 1984. Genetic parameters for testes traits in swine. J. Anim. Sci. 59:967-973.

Tripepi, S., A. Carelli, E. Perrotta, E. Brunelli, R. Tavolaro, R.M. Facciolo and M. Canonaco. 2000. Morphological and functional variations of Leydig cells in testis of the domestic pig during the different biological stages of development. J. Exp. Zool. 287:167-175.

Weibel, E.R. 1979. Stereological Methods: Practical Methods for Biological Morphometry, Vol. 1. 1st ed. Academic Press, Orlando, FL.

Wheaton, J.E., R.L. Meyer, R.H. Jones, and A.J. Kramer. 1998. Effects of passive immunization using antibody against an α-inhibin peptide on follicle-stimulating hormone concentrations and litter size in sows. Theriogenology. 49:813-822.

Wright, W.W. 1993. Cellular interactions in the seminiferous epithelium. Page 377 in Cell and Molecular Biology of the Testis. C. Desjardins and L. Ewing, eds. Oxford University Press, New York, NY.

Zanella, E., D. Lunstra, T. Wise, J. Kinder, and J. Ford. 1999. Testicular morphology and function in boars differing in concentrations of plasma follicle-stimulating hormone. Biol. Reprod. 60:115-118.

Zirkin, B.R. 1993. Regulation of spermatogenesis in the adult mammal: Gonadotropins and androgens. Page 166 in Cell and Molecular Biology of the Testis. C. Desjardins and L. Ewing, eds. Oxford University Press, New York, NY.