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AGE-RELATED CHANGES IN THE CAT TESTIS AND EPIDIDYMISS

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INTRODUCTION

Reproductive pathology has been extensively studied in most domestic animals, apparently dictated by the species' economic importance; a large amount of information has been learned about reproductive failure in the bull, boar, ram, stallion, and to a lesser extent, the dog.

It was found that testicular degeneration is the most common lesion associated with infertility and lowered seminal quality. A variety of agents are capable of inducing degenerative lesions in the seminiferous epithelium, including nutrition, temperature, season, inflammation due to trauma/infections, noxious agents, immobilization, and age. Despite the generally low heritability estimates of most fertility components, genetic factors also have an adverse effect on fertility, for inbreeding in the bovine decreases fertility and heterosis is associated with increased fertility. Congenital anomalies account for a small percentage of infertility; examples are testicular hypoplasia and segmental aplasia of the mesonephric duct.

The literature contains little information on feline infertility. However, there is a growing need for such information, for cats are increasing in economic importance as companion, showing, and breeding animals. The purpose of this study is to provide a foundation for research in feline reproduction, and specifically to define age-related changes in the feline testis and epididymis.

I. REVIEW OF LITERATURE

TESTIS AND EPIDIDYMIS

A. DEVELOPMENT

1. Gonad

Gonadal differentiation occurs from a bipotential structure, the indifferent gonad. Proliferation of the cortex results in development of an ovary, while proliferation of the medulla gives rise to the testis.³³ The gonad develops from the genital ridge and is covered by germinal epithelium originating from the mesonephros (an embryological kidney system) and primordial germ cells (PCG) migrating from the yolk sac.³³

The primordial germ cells can be identified by their high content of glycogen and alkaline phosphatase. They migrate to the genital ridges by ameboid movement along the dorsal mesentery of the hindgut. The gonads do not develop if the PCG's fail to reach the ridge, as they have an inductive influence on gonadal development.²⁸

As the PCG's are migrating, the germinal epithelium proliferates inwards to form an internal epithelial mass from which the primary sex cords are formed. As the primordial germ cells arrive, they are gradually surrounded by the cords. The sex cords anastomose with one another to form the testis cords which constitute the primitive seminiferous tubules. Towards the hilus of the gland the cords break up to form a network of tiny cell strands which eventually form the rete testis.²⁸

During further development, the testis cords are separated from the peripheral coelomic epithelium by a dense fibrous connective tissue layer, the tunica albuginea.²⁸ Septa evolve from the mesenchymal tissue to divide the gonad into lobules composed of three to four primitive seminiferous tubules.³³

2. Genital Ducts

Regardless of genital sex, embryos develop paired genital ducts (Wolffian and Mullerian). The Wolffian duct (mesonephric duct) forms the epididymal duct, vas deferens and seminal vesicles. The Mullerian duct (paramesonephric duct) degenerates in the male under the influence of testicular secretions.³³

3. Endocrine Functions of the Fetal Testis

Development of accessory sex organs is controlled by the fetal testis which causes masculinization and defeminization. Female external and internal genitalia are formed in the absence of testes. One testis is sufficient to stimulate development of male external genitalia.³³

4. Descent of Testis and Epididymis

Early internal descent stems from differential growth rate of various embryonic structures, whereas subsequent external descent (testicular descent to the scrotum via the inguinal ring) is an

active process.²⁸ As external descent begins, a pouch of peritoneum evaginates through the inguinal canal and into the scrotum, the processus vaginalis, which eventually forms the tunica vaginalis.^{28,33} The gubernaculum testis, a mesenchymatous column extending from the caudal pole of the testis to the genital swelling (scrotum), functions to pull the testis through the inguinal canal into the processus vaginalis and scrotum. The connection between the tunica vaginalis and intraabdominal peritoneum is obliterated by the spermatic cord.^{28,33} Factors regulating intrascrotal descent of the testis and epididymis are unknown, but testosterone has been implicated.³³

5. Prepuberal Testis

At birth, many mammals including the cat exhibit a loss of interstitial Leydig cells. They are replaced by fibroblast proliferation and do not reappear until puberty approaches.^{19,33} Neonatal sex cords consist of solid cords of precursor Sertoli cells and primordial germ cells.³³ As the animal approaches puberty a spurt of tubular growth occurs, associated with appearance of interstitial cells. Tubules become vacuolated and central lumina appear. Shortly after, spermatogonia and Sertoli cells become distinguishable.¹⁹ Subsequently the spermatogenic cycle begins.

B. STRUCTURE

1. Testis

a. Gross Anatomy

The cat testis is round and its average weight is 2.016 grams.⁸ The testis is enclosed in coelomic epithelium, the tunica vaginalis, underneath which is the tough fibrous testicular capsule, the tunica albuginea.^{3,13} When the testis is removed from the scrotum, the parietal layer of the tunica vaginalis remains closely adhered to the scrotum. The visceral tunica vaginalis remains intimately associated with the tunica albuginea.¹³

b. Histology

The testicular capsule, the tunica albuginea, is composed of dense irregular connective tissue consisting mostly of collagen fibers and a few elastic fibers.¹³ In the cat there is no well-defined vascular layer.⁸ In the horse this capsule contains smooth muscle fibers.¹³ The tunica albuginea sends out connective tissue trabeculae, the septula testis, which divide the testis into lobules.^{3,13} The septa converge toward the centrally located loose connective tissue of the mediastinum testis. The septa are thick in the boar and dog, and thin, often inconspicuous, in the cat.¹³

The testicle consists of lobules of convoluted seminiferous tubules with intertubular connective tissue, vasculature, lymphatics, nerves, and interstitial cells. The rete testis and mediastinum testis are centrally located.^{8,13}

Interstitial or Leydig cells vary largely in number based on species and age. Cat interstitial cells are considerably more abundant than that of the dog, almost completely filling the intertubular spaces.⁸ Morphologic species variation also occurs. Cytoplasmic inclusions unique to the canine Leydig cell have been widely documented. They may represent an androgen dependent storage product involved in sperm maturation.³⁶ Regardless of species, Leydig cells are polyhedral shaped, have a spherical, vesicular nucleus and contain a high amount of lipid. In addition, interstitial cell glycogen is present in the bull, stallion, and cat.¹³ Interstitial cells function to produce testosterone.³

The convoluted seminiferous tubules are tortuous and consist of a surrounding basement membrane and inner germinal epithelium. The epithelium is made up of two cell types: spermatogenic and supporting.³ The supporting cells or Sertoli cells are irregularly shaped, often elongated pyramidal cells whose broad bases lie on the basement membrane. They are rather evenly spaced and have a spherical, oval or pear-shaped nucleus which is usually located in the broad part of the cell. The nucleus contains a prominent nucleolus. The cytoplasm contains varying amounts of lipid inclusions and glycogen in all species.¹³

Sertoli cells are reported to be nutritive, protective, and supportive for spermatogenic cells. In addition, they phagocytize products from regressive spermatozoa and detached residual bodies of spermatids, and participate in synchronization of spermatogenic events.^{13,27}

Sertoli cells also produce estrogens, for Sertoli cell tumors often lead to feminization in the dog.^{13,45} Unique intercellular junctions between Sertoli cells are morphologically equivalent to a physiologic diffusion barrier within tubules, blood-testis barrier. This guarantees a relatively constant environment for spermatogenesis to occur.¹³ Fully differentiated Sertoli cells are reportably unable to divide.¹³

Spermatogenic cells are located between and above the Sertoli cells. The sequence of events in development of spermatozoa from spermatogonia is called spermatogenesis. This is divided into three phases.¹³

1. Spermatocytogenesis: The process during which spermatogonia develop into primary spermatocytes.
2. Meiosis: The maturation division of spermatocytes which produces spermatids with a haploid number of chromosomes.
3. Spermiogenesis: The process of spermatid transformation into spermatozoa.

Spermatogenesis proceeds in an irregular spiral, progressive sequence in the cat. Therefore histologically, tubular stages are not uniform.⁸ The stem cells responsible for initiating the spermatogenic sequence are termed spermatogonia. These cells line the seminiferous tubule. They are small cells with rounded nuclei rich in chromatin.³

Spermatogonia are very resistant to deleterious agents and thus provide the germinal epithelium with an extraordinary ability to recover.²⁷

Mitotic division of spermatogonia results in primary spermatocytes which constitute the largest germ cells. They are located in the middle zone of the germinal epithelium. Primary spermatocytes are frequently observed in a stage of division.³ Primary spermatocytes undergo the initial meiotic division resulting in secondary spermatocytes. These cells are rarely seen as they quickly undergo a second maturation division resulting in spermatids.^{3,13}

Spermatids possess a haploid number of chromosomes. They lie close to the tubular lumen and have spherical nuclei and scanty cytoplasm. Spermatids ultimately give rise to spermatozoa by spermiogenesis.¹³

The convoluted seminiferous tubules eventually connect with the tubuli seminiferi recti, or straight seminiferous tubules. These tubules are lined with simple cuboidal or squamous epithelium. They are responsible for connecting the convoluted seminiferous tubules with the rete testis.³

The rete testis consists of irregularly anastomosing canals surrounded by the mediastinum testis, and is lined by simple cuboidal or simple squamous epithelium. These tubules ultimately connect the testis to the epididymis.¹³

2. Epididymis

a. Gross Anatomy

Grossly, the epididymis is divided into a head, body, and tail and is covered by a thick capsule, the tunica albuginea, which is intimately surrounded by the visceral layer of the tunica vaginalis.¹³

b. Histology

The epididymis consists of a long, tortuous duct, the ductus epididymidis, and the ductuli efferentes.¹³ The ductuli efferentes, located in the epididymal head, are tubules which connect the rete testis to the ductus epididymidis. They are lined with ciliated pseudostratified columnar epithelium and are surrounded by loose connective tissue and smooth muscle cells. Nonciliated cells with microvilli and basal cells also are present. The nonciliated cells are probably involved in resorptive processes.¹³

The ductus epididymidis is lined by pseudostratified columnar epithelium with ovoid basal and columnar cells. Long, branching, cytoplasmic microvilli termed stereocilia are located on the apical surface of the columnar cells, and are indicative of resorptive activity. The duct is surrounded by loose connective tissue and smooth muscle cells. This layer in turn is surrounded by loose connective tissue.¹³ Focal interstitial collections of lymphoid cells can occur in the dog and cat which resemble lymphatic follicles without secondary nodules.⁸

The number of periductal smooth muscle fibers increases towards the epididymal tail, while height of the pseudostratified epithelium and length of microvilli decrease towards the tail of the epididymis.^{13,46,47}

The epididymis functions as the place of storage and maturation of spermatozoa. Spermatozoa taken from the efferent ducts only perform weak, vibratory motions without motility, but from the tail they have progressive, forward movements. The epididymal tail is the primary storage site for spermatozoa.¹³

Histological species variation commonly occurs in the epididymis. In the dog, intranuclear and intracytoplasmic crystalloid inclusions are found normally in caudo-epididymal epithelial cells. They are reported to be proteinaceous in nature.²¹ Cells stated to be lymphocytes are noted in normal fowl, rat, and monkey epididymal epithelium. They are often close to the basal lamina. Their function is uncertain.¹ Ectopic nests of adrenal cortical tissue may be found in the epididymis, testis, and spermatic cord. This is reported primarily in horses.²⁷

C. AGE-RELATED CHANGES

1. Testis

a. Gross Changes

Gross changes in an aged or atrophied testicle primarily involve size and consistency. It may be normal or reduced in size. In both cases, testicular consistency may be soft and flabby, which indicates

progressive degeneration.²⁷ Alternatively, a small testis may be firm if fibrosis is extensive.^{17,27,34,44} This is known as "fibrosis testis".^{8,34} In such instances, the testis may be reduced to half its normal size, and does not bulge on sectioning.^{17,25}

Calcium deposition, tunic adhesions, and a thickened, condensed, wrinkled tunica albuginea are other gross changes occasionally observed in atrophic testicles. On cut surface, calcium deposits appear as yellowish-white flecks in the parenchyma.^{25,27}

b. Microscopic Changes

Aged testicles may have a variety of microscopic changes: tubular degeneration, fibrosis, cellular hyperplasia, cellular pigment deposition, alteration in lipid content, vascular lesions, spermatocetes, spermatoc granulomas, and neoplasia.^{2,4,7,8,9,11,12,15,24,25,27,31,34,35,38,40,44,45}

Tubular degeneration is a common finding with age and involves both germ cell degeneration and changes in tubular diameter. Several sources report a uniform decrease in tubular diameter,^{8,9} but another states that tubular shape merely changes from round to polygonal with age.²

Degeneration of germinal epithelium initially occurs in the more mature stages of spermatogenesis and ultimately progresses to include the stem cells. In advanced cases, the seminiferous tubules are lined only with Sertoli cells. Common degenerative manifestations include cytoplasmic vacuolation, germinal cell desquamation, pyknosis, karyorrhexis, spermatid giant cell formation, spermiostasis, calcification, and occasionally osseous metaplasia of inspissated tubular

contents.^{4,8,9,10,15,25,27,31,34,38,44} Calcification is common in the bull, ram and rat testis but is rare in the dog and has not been reported in the cat.^{8,9}

Fibrosis is found in the aged testicle. The tubular basement membranes may undergo thickening and hyalinization.^{2,4,7,11,25,27,34} This is associated with an increase in elastic tissue and a concurrent decrease in collagenous tissue. A similar thickening and change may occur in the tunica albuginea.² Increase fibrous tissue deposition may also occur in the interstitium, and, with concurrent tubular degeneration and collapse, may completely replace seminiferous tubules.^{2,4,7,8,11,15,24,25,27,34}

Cellular hyperplasia is considered to be an age change in testicles. It affects the interstitial or Leydig cells.^{7,9,11,15,25,31,34,38} Hyperplastic Leydig cells usually stain deeper with eosin, have uniform nuclear size and staining properties, and lack mitotic figures.² Some believe that cellular hyperplasia may represent an early stage of neoplasia.^{11,45} A decrease in Leydig cell number also has been reported as a testicular age change.^{7,8,45}

Cellular pigment deposition, lipofuscin, is a common age-related finding in the testis.²⁴ Leydig cells and Sertoli cells may accumulate intracytoplasmic golden-brown spheroidal granules.^{2,7,8,24,25,38} Germinal epithelial cells also have been reported to accumulate lipofuscin.²⁴

Change in cellular lipid content also occurs with age. The lipid content in Sertoli cells gradually increases with age in man, but, declines in Leydig cells.^{2,40}

Vascular lesions are reported in the aged bull, ram, dog, and rat testis.^{9,27} In the bull, ventral wedge shaped fibrotic areas are observed in association with hyaline degeneration of arteriole walls. The ventral distribution suggests that the lesion may stem from vascular degeneration.^{15,27,31}

Hyaline degeneration occurs first in the arterioles of the testicular tunic and parenchyma and effects the larger vessels of the spermatic cord in advanced cases. Hyaline degeneration affects all vessel layers. The evolution of the lesion is not clear, but the hyaline substances give some of the staining reactions of amyloid.²⁷

Senile degenerative changes also may result in spermatic cyst formation. The term encompasses both spermatocele (tubule or duct dilatation stemming from spermatozoa accumulation or spermiostasis) and spermatic granuloma (a granulomatous response caused by extravasation of spermatozoa).⁴⁴ Spermatic cysts are commonly found in the ductus efferentes and/or rete testis. It is thought that the blood-testis barrier is most vulnerable to damage in these areas.³⁵ The pathogenesis of spermatic cyst formation begins with inspissated, calcified, stagnant tubular contents. Tubular dilatation results with subsequent epithelial desquamation and basement membrane breakdown, and extravasation of spermatozoa occurs and incites a granulomatous reaction.²⁷ Such obstruction in the epididymal head results in back pressure, which causes degeneration of seminiferous tubular epithelium, fluid accumulation, tubule distention and interstitial edema. Grossly the testis may be enlarged.²⁷

Neoplasia is another common change in the aged testis, but species differences are marked. Primary testicular tumors frequently are found in old dogs and bulls, but are rarely reported in other species including the cat.^{8,27} The three primary testicular tumors are derived from the three specialized testicular elements.

The interstitial cell tumor is very common in the dog²⁷ and rare in the horse, mule, and ox.⁴⁵ It grows slowly and causes pressure atrophy of the surrounding tubules.¹⁵ Controversy exists as to whether this tumor secretes androgenic hormones. Evidence of frequent accompanying testicular degeneration and prostatic hyperplasia suggests it may. These conditions can be corrected by castration.²⁷ Another source reports that interstitial cell tumors have no hormonal effect on the opposite testis or other organs and tissues, and libido is not increased. Rather, clinical changes such as prostatic disease, perianal gland tumors, and alopecia are common in old dogs with or without interstitial cell tumors.⁴⁵

The Sertoli cell tumor is fairly common in the dog. It is seen less frequently than the interstitial cell adenoma, and about as often as the seminoma. It is rare in the cat and horse.⁴⁵ Testicular enlargement frequently occurs.²⁷ Feminization may also occur from the high amounts of estrogen secretion.^{27,45}

The seminoma is a fairly common canine testicular tumor and rare in other species. This tumor presumably arises from spermatogenic epithelium. It does not produce hormones.^{27,45}

2. Epididymis

Little information has been cited on epididymal changes associated with age. Microscopic changes include lipofuscin deposition in ductuli efferent cells, interstitial fibrosis, tubule dilatation accompanied by flattened epithelium and loss of stereocilia, epithelial hyperplasia, spermatocytic granulomas, and regenerative attempts characterized by intraepithelial cyst formation.^{7,8,25,26,34}

D. FACTORS INFLUENCING AGE-RELATED CHANGES

A variety of external and internal stimuli affect the testis and epididymis throughout life as they are extremely sensitive to environmental changes, and are capable of adaptation. In order to demonstrate the morphologic changes associated with age, other factors influencing testicular and epididymal morphology must be considered.

1. Congenital Defects

a. Cryptorchidism

Cryptorchidism is defined as retention of the testis at some point during its normal descent to the scrotum. The two most common retention sites in the dog and cat are the abdominal cavity and inguinal canal.⁸

It may be unilateral or bilateral.^{8,34,39} In the cat it is most often unilateral and more commonly involves the left testicle. Incidence of cryptorchidism in the cat is approximately 0.75 percent.⁸

The reported causes of cryptorchidism are multiple. Improper development of the route along which the testis descends and malformation of the gubernaculum are examples of mechanical or anatomical causes.¹⁶ Endocrine factors such as pituitary gonadotropin deficiency can indirectly cause cryptorchidism by producing hypoplasia of the scrotum and deficient contraction of the gubernaculum.⁸ Cryptorchidism also can be inherited by a simple recessive gene.^{6,8,27,39} Biotin deficiency during fetal development has been reported as another cause.²⁹

Morphologic characteristics of the prepuberal retained testis are normal. But after puberty the cryptorchid testis becomes small, flabby, and the tunica albuginea is often thickened and wrinkled. Microscopic changes consist of aspermatogenesis, thickened tubular basement membranes and tunica albuginea, an increase or decrease in Leydig cells, and interstitial fibrosis. Interstitial fibrosis may be extensive in older animals, with almost complete disappearance of seminiferous tubules.^{8,27,34,39} Degenerative testicular changes stem from high abdominal temperature in relation to scrotal temperature.^{8,27,39} Interstitial cells are functional in the cryptorchid testis. Development of accessory sex characteristics occur and libido is normal.⁸

b. Monorchidism

Monorchidism is defined as failure of a testis to develop; it is rare in the cat.⁸

c. Synorchidism

Synorchidism is defined as fusion of the testes. It has not been reported in the dog or cat.⁸

d. Ectopia

Displacement of a testis from its normal course of descent is a frequent occurrence in the dog, but is rare in the cat. Common locations include the perineal region, crural region, abdominal cavity, and along side the prepuce.⁸ The ectopic testis does not manifest normal spermatogenic activity due to thermal degeneration.²⁷

e. Testicular Hypoplasia

Testicular hypoplasia is a congenital condition.¹⁵ The testis descends normally into the scrotum, but mature development is never attained. It is often difficult to differentiate it from a degenerative testis which developed normally and subsequently deteriorated.⁷

Testicular hypoplasia commonly occurs in bulls, rams, boars, and horses, but is rare in the cat.⁸ The condition may be unilateral or bilateral.^{7,8}

Testicular hypoplasia may be caused by several factors, ranging from an autosomal recessive gene with extremely variable expressivity to exogenous factors during gestation. Examples are hormonal disturbances, vitamin deficiencies and certain poisons.^{7,16,25,34} Affected organs are smaller than normal and do not lie in the base of the scrotum. Consistency may be almost normal to flabby.^{25,34}

Microscopically, various degrees of hypoplasia may be observed, from complete aspermatogenesis in which tubules are lined only with Sertoli cells to cases in which spermatogenesis is merely retarded or incomplete.^{16,25,34} Various degrees of interstitial fibrosis and basement membrane thickening are also present. Interstitial cells are usually increased in number.²⁷

Hypoplasia of the epididymis accompanies testicular hypoplasia.²⁷

f. Segmental Aplasia of the Mesonephric Duct

Konig et al. (1972) considered segmental aplasia of the mesonephric duct to be hereditary due to an autosomal recessive gene.⁴⁸ The majority of cases are unilateral and the body, tail, entire epididymis, and even parts or all of the vas deferens may be missing. There is no regular pattern of lesions. Spermatocetes and spermatic granulomas occur as a result of spermiostasis and increased tubular pressure.^{15,25} Tissues adjacent to the aplastic segment are the most severely affected.²⁵ This condition is rare in the cat.⁸

g. Congenital Retention Cysts

Congenital retention cysts originate from embryological aberrations of mesonephric tubules which comprise the Wolffian body.^{8,43} They are commonly found between the epididymal head and the testis and may be unilateral or bilateral. The cysts are slightly raised, focal, circular structures containing clear watery fluid.⁴³ They may reach several centimeters in the bull.²⁷ Histologically, the cysts are lined with low columnar epithelium and surrounded by a fibrous tissue capsule. No inflammatory response or sperm accumulation is evident.⁴³

2. Nutritional Factors

Reproductive organs are very sensitive to nutritional changes. Their growth and function is dependent on hormonal stimulation, and endocrine activity is susceptible to both undernutrition and malnutrition.^{32,44}

a. Undernutrition

Undernutrition can cause development of small testes which are qualitatively adequate but are unable to reach their quantitative potential.²⁷

Undernutrition affects the hypothalamus, hypophysis, and the reproductive organs resulting in a decreased response to hormonal stimulation. It is hypothesized that undernutrition causes inadequate protein levels necessary for hypothalamus releasing factor action. Undernutrition also can affect synthesis and release of hypophyseal hormones. Morphologic pituitary changes such as gland atrophy with cellular vacuolization and multiple cyst formation also are noted. Decreased testosterone levels are the end result of inadequate hypothalamic and hypophyseal output.²⁹

In a rat study, the testis responded to gonadotropin despite inanition with stimulation of interstitial cells, an increase in gonadal size, and a return of spermatozoa. In underfed young bulls, however the accessory organs exhibited a decreased responsiveness to testosterone, therefore a direct nutritional effect on the reproductive organs also can occur.²⁹

b. Protein

Adequate protein levels are important for functional integrity of the male reproductive system. A major portion of gonadal dry weight is protein.²⁹ Not only is the level of dietary protein important, but its nutritional value must also be considered. Variations in protein quality reflect amino acid patterns. Certain amino acid deficiencies can interfere with testis maturation and function in a method not entirely due to inanition. For example, deficiencies in arginine, lysine, and tryptophane disrupted spermatogenesis in the rat.²⁹

Excess amino acid levels can be toxic. High levels of tyrosine in an adequate diet can result in testicular degeneration.²⁹ Degenerative changes in the rat testis occurred after administration of excessive amounts of methionine; spermatid granulomas, atrophic tubules and spermatid giant cells resulted.⁵ In another study in cats, a high protein diet consisting solely of raw heart resulted in epithelial hyperplasia and microvesicles in the epididymis. It was speculated that the lesions stemmed from pituitary hyperactivity.²³

Age has a major influence on the degree of sensitivity to dietary protein imbalances. Protein anabolic levels are higher in tissues of young growing animals, therefore they are more sensitive to changes in daily dietary protein level and quality. Investigations with mature rats revealed that prolonged protein depletion was necessary before testes exhibited a loss in size.²⁹

c. Carbohydrates

Hypoglycemia influences the male reproductive organs. In rats, insulin administration produced germinal epithelial lesions which were prevented by simultaneous administration of glucose.²⁹ Hyperglycemia also affects the testis. In man, urinary androgen levels were low in individuals affected with diabetes mellitus. Many diabetics exhibited low sperm counts and abnormal spermatogenesis. Severe diabetes in young rats prevented testis tubular maturation and Leydig cell function.²⁹

d. Fatty Acids

Fatty acids play an important role in testicular health. The mating potential of male rats decreased when fats were excluded from the diet. Histologically, seminiferous tubule degeneration was noted. Addition of 15 percent erucic acid, a fatty acid antagonist, caused sterility in rats after three months. Another study revealed that bird testis weight was reduced by linoleic acid deficiency.²⁹ Essential fatty acid deficiency may invoke some testicular changes by influencing the pituitary. Morphological changes in the anterior pituitary have been observed.²⁹

e. Vitamins

Both an excess and deficiency of vitamin E produces testicular damage. Vitamin E deficiency in the rat, guinea pig, hamster, and bird caused severe seminiferous tubule damage with little effect on the interstitial cells. Little or no effect was noted in the rabbit, mouse and bull. Excess dietary vitamin E in the hamster produced acute testicular damage.²⁹

Vitamin A levels also influence testicular structure and function. Young bulls fed vitamin A deficient rations developed seminiferous tubule degeneration with little change in the spermatogonia. Marked hypertrophy of interstitial cells also occurred.¹⁸ In the ram and cat, vitamin A deficiency delayed testicular development.⁴¹ In most animals the common clinical signs of vitamin A deficiency, such as lacrimation, night blindness, and ophthalmia preceded infertility.^{18,27}

Vitamin A deficiency indirectly causes gonadal degeneration by suppressing the pituitary gonadotropic hormones. This was confirmed by injecting infertile, vitamin A deficient animals with gonadotropic hormones, spermatogenesis being restored.²⁷

Hypervitaminosis A also is degenerative to the male reproductive system. Cats fed rations containing excess vitamin A developed tubular degeneration and aspermatogenesis. The testes became soft and flabby. When the cats were returned to a diet free of vitamin A, fertility returned.³⁷ Hypervitaminosis A also produced degenerative seminiferous tubule lesions in the rat testis. Changes were proportional to the duration and degree of inanition. When the rats were returned to a ration free of vitamin A, however, normal functional fertility did not return.^{30,37}

A lack of vitamin B₁, or thiamine, has little effect on the testicle but a marked influence on accessory organs. Rats fed a thiamine deficient diet for a number of weeks developed rapid involution of the accessory organs.³² Prior studies revealed that vitamin B₁ deficient diets decreased pituitary gonadotropin in male rats. Accessory organ damage was effectively repaired by administering androgen hormone or anterior pituitary extract.^{29,32}

Biotin deficiency may retard testicular development and damage the adult testis. Severe biotin deficiency in the rat caused degenerative changes in the spermatocytes after four weeks.²⁹

f. Minerals

Reproductive health is dependent on actual calcium and phosphorus levels as well as the Ca:P ratio. Reproductive capacity is poor when levels of either element are high or low, regardless of the ratio.²⁹

Manganese deficiency in rats and rabbits caused testicular damage and infertility. Tubular degeneration occurred after 90 to 100 days and desquamated cells were evident in the lumen of the ductus epididymidis.²⁹

Little is known about the effects of decreased molybdenum on testicular function. An excess of molybdenum caused lack of libido and testicular damage in young bulls, seminiferous tubules and Leydig cells being affected.²⁹

Zinc is normally found in high concentrations in the gonads and accessory organs. Deficiency causes degenerative testicular changes that are more severe in prepuberal animals. Rats fed zinc-deficient diets exhibited severe atrophy of germinal epithelium with reduction of testicular zinc content.^{10,29} Changes were not reversible when zinc was returned to the diet.²⁹ Testicular damage primarily resulted from a pituitary hormone deficiency. Urinary gonadotropin levels in zinc deficient boys (aged 12 to 20) were low and hypogonadism was evident. Administration of gonadotropin to immature rats stimulated testis development despite a zinc-deficient diet.²⁹

The need for iodine for preventing goiter and hypothyroidism is well known. Hypothyroid animals suffer from reduced conversion of carotene to vitamin A, increased requirements for B vitamins and reduced protein synthesis. All of these factors can have a pronounced

effect on reproductive health.²⁹ The influence of hypothyroidism on the testicular structure and function is more pronounced in prepuberal animals. In one study, testicular development was markedly retarded in cretin offspring. Seminiferous tubules contained only spermatogonia and a few spermatocytes, the Leydig cells were atrophic.²⁹ In the adult, decreased libido and reduced semen quantity were noted in iodine deficient bulls and stallions.²⁹ Administration of thyroid hormone is corrective.^{29,39}

3. Temperature Effects

Changes in scrotal temperature (hence gonadal temperature) can result in degenerative testicular changes. A variety of conditions influence gonadal temperature including systemic or local infections, testicular displacement, excessive or insufficient scrotal hair or wool, heavy deposits of scrotal fat, and high or low ambient temperatures.^{15,27,42,44}

The reproductive system is more resistant to cold than it is to hyperthermia.¹⁵ Extensive, short term hypothermia can be tolerated without serious effects. but continuous cold and accompanying scrotal frostbite can cause spermatogenic arrest.⁴²

4. Seasonal Effects

Although spermatogenesis is continuous in most domestic males, the level and quality vary with climatic influences. The extent of seasonal variation is related to species, breed, and latitude.¹⁵ Photoperiodism,

temperature, and humidity exert direct effects on the neuroendocrine system and testes.⁴³ In general, reproductive function is depressed during seasons of peak temperatures, especially when accompanied by high humidity.¹⁵

5. Orchitis and Epididymitis

There are few reports of orchitis and epididymitis in the cat. They may result from cat bite wounds. Systemic infections rarely localize in the testes.^{8,39} Orchitis caused by Brucella sp. has been reported once in the cat.³⁹ Some believe the cat is resistant to natural Brucella infections.²⁷ Occasionally, genital manifestations occur with feline infectious peritonitis spreading from the peritoneal cavity infection.³⁹

Acute orchitis results in a tense, swollen testis⁸ and the scrotum may be thickened and edematous.³⁹ A suppurative inflammatory response is common. Microscopically, tubular lumens and the interstitium may contain numerous neutrophils. The stroma is often edematous. Tubular epithelium may degenerate and desquamate. Resolution is accompanied by fibrous tissue formation and the resulting fibrosis may cause tubular occlusion and lead to spermatic cyst formation.⁸

Testes with chronic orchitis are smaller, firmer, and sometimes irregularly shaped.^{8,39} Fibrous adhesions may occur between the tunica vaginalis and tunica albuginea. Microscopical changes include interstitial fibrosis, dilated or atrophic tubules, thickened basement

membranes, and foci of lymphocytes and plasma cells. A chronic orchitic testis resembles a fibrotic testis except inflammatory cells are absent in the latter.⁸

6. Spermatic Cysts

Aside from senile changes, trauma and chronic inflammation can cause spermatic cyst formation.^{6,8} Trauma to the testis may result in sperm extravasation and a subsequent immune response. Autoimmune damage to the uninjured contralateral testis also can occur.⁶

Cysts arising from chronic inflammation primarily stem from connective tissue proliferation and subsequently result in ductal occlusion.⁸ They are usually located in the epididymal tail, the predilection site for most bacterial infections. According to Jubb and Kennedy, the obstructive lesions do not cause testicular degeneration because fluids and debris are absorbed in the head of the epididymis.²⁷

7. Immobilization

Healthy tom cats confined to the laboratory frequently fail to become sexually active when exposed to females in heat. No microscopic testicular studies have been reported. The condition is readily reversible when more freedom is provided.^{8,39}

8. Postmortem Changes

Postmortem changes may mask age related changes in the testis and epididymis. Autolysis leads to swelling and hydrolysis of Sertoli cells and spermatogenic cells. Pyknosis, desquamation and disintegration of germinal epithelium occur. Cell debris, pyknotic nuclei, and spermatozoa often fill the lumens of the ductus epididymidis and seminiferous tubules.³⁴

9. Noxious Agents

Several chemicals, metals and rare earth salts, and ionizing radiation are capable of inducing testicular degeneration.

a. Ionizing Radiation

The main testicular effect of ionizing radiation is germinal epithelium degeneration, Leydig and Sertoli cells remain relatively resistant.^{14,27}

b. Amphotericin B

Rabbits injected intravenously with Amphotericin B, exhibited decreased spermatozoal migration rate towards the seminiferous tubule lumen. Spermatozoan release from Sertoli cells also was inhibited.²⁷

c. Chlorinated Naphthalenes

Bulls orally exposed to chlorinated naphthalenes developed testicular degeneration, but the changes were reversible when treatment was stopped.²⁷

d. Alkalating Agents

Dogs and rats treated with massive doses of tretamine (triethylenelamine) exhibited spermatogenic arrest and testicular destruction. Rat studies gave similar results.^{20,27}

Busulphan, (dimethanesulphonoxybutane) and isopropyl methanesulphonate also have deleterious effects on spermatogenesis. Fertility usually returns when treatment is stopped.^{20,27}

e. Metallic Salts

Daily subcutaneous injections with certain metallic salts (iron, molybdenum, thallium, lead salts) in rodents caused spermatogenic arrest and spermatozoal destruction in the epididymis. Fertility returned to normal after treatment was stopped.²⁷

f. Cadmium Chloride

Rats, rabbits, and guinea pigs injected with cadmium chloride developed thrombosis and necrosis of the testes and reparative processes consisted of fibrous tissue.²⁷ Interstitial cell hyperplasia also

resulted, with occasional interstitial cell tumor formation.^{22,27}

Another report noted epididymal hyperplasia and microvesicle formation in rats treated with cadmium.²³

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II. AGE-RELATED CHANGES IN THE CAT TESTIS AND EPIDIDYMS

INTRODUCTION

Age-related testicular and epididymal changes have been studied in most domestic animals and include the following: increased testicular and epididymal tunica albuginea thickness in the bull,^{1,2} dog,^{3,4} man,^{1,5} rat,⁶ and all domestic animals;⁷ interstitial cell hyperplasia in the bull,^{1,2,8,9} dog,^{3,4,10} and all domestic animals;¹¹ interstitial cell lipofuscin in the bull,¹² dog,¹⁰ man,^{1,5} and all domestic animals;⁷ Sertoli cell lipofuscin in the bull² and dog;³ ductus efferent lipofuscin in the bull² and man;¹ increased seminiferous tubular basement membrane thickness in the bull,^{1,2} rat,⁶ dog,⁴ and man,^{1,5} seminiferous tubular degeneration in the bull,^{4,8,9} dog,^{3,4,10} cat,³ rat,^{6,13} ram,¹⁴ and all domestic animals;^{7,11} decreased seminiferous tubular diameter in the dog,⁷ rat,¹³ and man;⁵ hyaline degeneration of testicular and epididymal arterioles and spermatic artery in the bull,⁷⁻⁹ ram,⁷ dog,⁷ and rat;¹³ testicular calcification in the bull,³ ram,³ rat,^{6,13} and rarely the dog;³ testicular neoplasia in the bull,^{6,7,15} dog,^{3,7,15} and rarely the cat;¹⁵ epididymal intraepithelial cysts and hyperplasia in the dog,^{3,16} and all domestic animals;¹¹ and spermatic cysts in the bull,¹⁷ dog,^{3,16} and all domestic animals.^{7,11}

Little work has been done on age-related changes in the feline testis and epididymis. The purpose of this paper is to provide information on the feline testis and epididymis, and specifically to define age-related changes.

MATERIALS AND METHODS

Forty-two pairs of testes and epididymes were obtained from clinically healthy cats castrated at surrounding veterinary clinics. The age groups and number of cats in each were:

0.5 to 1 year	10 cats
1 to 3 years	10 cats
3 to 5 years	10 cats
5 to 7 years	5 cats
7 years and older	7 cats

Testes were sectioned longitudinally half way through and visceral tunica vaginales were removed. Testes were immediately placed in Bouin's fluid and fixed for a minimum of 48 hours. They were then washed with lithium carbonate to remove the picric acid. This was done until no yellow was evident in the washing. Tissues were rinsed with tap water to remove any lithium carbonate precipitate and stored in 10 percent buffered neutral formalin.

Dorsal, middle, and ventral sections were taken from each testis and epididymis and one section from the spermatic cord. The tissues were trimmed, embedded in paraffin, cut at 6um, and hand-stained with hematoxylin and eosin (H&E) and periodic acid schiff reaction (PAS).

Tissues were microscopically interpreted both quantitatively and qualitatively. Five randomly selected fields per tissue section were analyzed. Only round tubules and ducts were interpreted. Evaluations of the interstitium were performed on a random sample area between 3 seminiferous tubules. For quantitative purposes, only interstitial cell nuclei were counted.

Quantitative measurements consisted of counting cell numbers and measuring structural thicknesses and diameters with an eyepiece micrometer disc^a calibrated with a stage micrometer^b. The following variables were evaluated quantitatively:

Width: Testicular tunica albuginea, epididymal tunica albuginea

Diameter: Seminiferous tubules, ductus epididymis

Cell Number: Sertoli cells, spermatogonia A, spermatogonia B, primary spermatocytes, secondary spermatocytes, spermatids, spermatozoa, interstitial cells, mononuclear giant cells, multinuclear giant cells

Qualitative measurements were based on a scale of 0 to 5:

0 = normal

1 = mildly affected

2 = mild to moderately affected

3 = moderately affected

4 = moderate to severely affected

5 = severely affected

^aMicrometer Disc, Bausch and Lomb, U.S.A.

^bA. O. Stone Micrometer, Arthur H. Thomas Company,
Philadelphia, Pennsylvania

The following variables were evaluated qualitatively:

Seminiferous Tubules: Tubular degeneration; tubular basement membrane thickness; Sertoli cell karyorrhexis, lipofuscin, cytoplasmic vacuolation, pyknosis; spermatogonia, primary spermatocyte, and secondary spermatocyte karyorrhexis, pyknosis, cytoplasmic vacuolation; proteinaceous tubular luminal/debris; sloughed germinal cells; spermatocele; spermatoc granuloma

Testicular Interstitium: Cytoplasmic vacuolation, lipofuscin, cytoplasmic bodies; interstitial fibrosis

Vasculature: Hyaline degeneration of testicular arterioles, epididymal arterioles, spermatoc artery

Ductus Epididymis: Intraepithelial cysts; epithelial hyperplasia; spermatozoa; sloughed cells; spermatocele; spermatoc granuloma

Epididymal Interstitium: Fibrosis

Regression analysis was initially done to determine if a linear relationship existed between each variable and cat age. Testicular and epididymal means and dorsal, ventral and middle section means were evaluated. Data was then divided into five age groups and analysis of variance was performed to check for significant differences between means of each age group. Fisher's LSD test was used to further clarify these differences.¹⁸

RESULTS

Analysis of variance (Tables 1 and 2), regression analysis (Tables 3 and 4), and original data (Table 5) are located in the Appendix.

Significant quantitative testicular and epididymal changes are shown in Fig 1 to 6. Both testicular and epididymal tunica albuginea thicknesses increased with age (Fig 1 and 2). Spermatogonia A (Fig 3), spermatogonia B (Fig 4), and secondary spermatocyte numbers (Fig 5) decreased significantly, while interstitial cell numbers increased significantly with age (Fig 6). Significant quantitative changes were not noted in seminiferous tubular or epididymal ductal diameter, Sertoli cells, primary spermatocytes, spermatids, spermatozoa, and mononuclear giant cells and multinuclear giant cells.

Significant qualitative testicular and epididymal changes are shown in Fig 7 to 13. Seminiferous tubular degeneration (Fig 7) was most abundant in young and old groups and was reflected by a variety of changes in the germinal epithelium and Sertoli cells (Fig 14 to 19). Significantly increased amounts of cytoplasmic vacuolation were noted in Sertoli cells (Fig 8 and 15). Varying degrees of vacuolation were also seen in spermatocytes (Fig 16). Karyorrhexis and pyknosis of Sertoli cells, spermatogonia, and spermatocytes were occasionally observed; sloughed germinal cells were common within tubular lumens. Mononuclear (Fig 17) and multinuclear (Fig 18) spermatid giant cells were early degenerative findings. Seminiferous tubular lumens in young cats frequently contained large luminal aggregates with vesicular, oval to round nuclei and undiscernible cytoplasmic borders (Fig 19).

Seminiferous tubular basement membranes were significantly thickened and wavy in older cats (Fig 9 and 20). Interstitial cell lipofuscin was also significantly increased (Fig 10). Interstitial fibrosis was occasionally seen. Epithelial hyperplasia and intra-epithelial cysts in the epididymal duct (Fig 21) were evident in 70 percent of cats 0.5 to 1 year old and 20 percent of cats 5 years and older.

Spermatoceles (Fig 22) were seen in 2 cats. The oldest cat (9 years) contained 3 spermatoceles; two were in the testis and one in the head of the epididymal duct. The testicular spermatoceles were characterized by dilated tubules with no observable germinal epithelium or Sertoli cells and tightly packed luminal spermatozoa. The epididymal spermatocele consisted of a dilated duct with numerous tightly packed spermatozoa and spermatid giant cells. A testicular spermatocele was also observed in a 2.8 year old cat.

Significant increases were not noted in spermatogonia and primary and secondary spermatocyte karyorrhexis, pyknosis, cytoplasmic vacuolation; Sertoli cell lipofuscin, pyknosis and karyorrhexis; interstitial cell cytoplasmic vacuolation; sloughed germinal cells; testicular and epididymal spermatoceles and spermatocytic granulomas; testicular and epididymal interstitial fibrosis; hyaline degeneration of testicular and epididymal arterioles, spermatic artery; and epididymal intraepithelial cysts and hyperplasia.

The middle age group (3 to 6.9 years) had several significant qualitative changes. Eosinophilic to amphophilic round cytoplasmic bodies were frequent within interstitial cells (Fig 11 and 23). Spermatozoa in the epididymal duct (Fig 12) and proteinaceous tubular luminal debris (Fig 13) were most abundant in this age group.

Lymphocytic foci were observed in the testis and epididymis of 8 cats aged 0.5, 0.7, 1.4, 2, 4, 5, 8, and 9 years. Most aggregates were in the epididymal interstitium and tunica albuginea with no preference for dorsal, middle or ventral sections. Of the 2 foci located in other areas, one was in the spermatic cord connective tissue; the other in the dorsal testicular tunica albuginea. Most lymphocytic foci were small with a few uniform mature lymphocytes and rare plasma cells, macrophages, and mitotic figures (Fig 24). Aggregates found in the 8 and 9 year old cats were large and densely populated with immature lymphocytes, numerous plasma cells, macrophages, and mitotic figures (Fig 25).

DISCUSSION

Results from this study indicate that age changes in the feline testis are similar to those found in other domestic animals.¹⁻¹⁴ There were significant age-related increases in testicular tunica albuginea thickness, interstitial cell lipofuscin, interstitial cell number, seminiferous tubular basement membrane thickness, and seminiferous tubular degeneration.

Seminiferous tubular degeneration was characterized by significantly decreased numbers of germinal epithelial cells and significantly increased Sertoli cell cytoplasmic vacuolation. Degeneration of the tubular epithelium began centrally and later extended towards the basal layer. Germinal cell pyknosis, karyorrhexis, and cytoplasmic vacuolation, spermatid giant cells, and desquamated cells were commonly observed in degenerative tubules but were not found in significant amounts. This may be due to the small sample size of older cats.

The young group (0.5 to 1 year) had significantly increased amounts of seminiferous tubular degeneration and Sertoli cell cytoplasmic vacuolation. Degenerative tubules often contained large luminal aggregates that may stem from tightly adhered sloughed cells or multinuclear giant cell formation. Individual cell borders were undiscernible. These changes could stem from spermatogenesis onset, but epididymal intraepithelial cysts and hyperplasia were also found in 70 percent of the 0.5 to 1 year old cats. The epididymal changes reportedly result from regenerative attempts by injured epididymal epithelium.¹¹ They have been observed in a variety of species including cat, bull, hamster and man, but the specific underlying cause is unknown.¹⁹ Causes incriminated included toxic,¹⁴ hormonal,^{19,20} and nutritional.^{19,20} Two reports^{6,21} cited epididymal intraepithelial cysts and hyperplasia in cats fed solely raw heart. Changes were more severe in young growing cats possibly indicating increased sensitivity to dietary protein imbalance. Testicular degeneration can also result from dietary protein imbalance such as high levels of tyrosine and methionine.²¹ Future studies are needed to determine the cause of these lesions in young cats and subsequent effects on puberty onset.

The following age-related testicular changes reported in other species were not found in this feline study: hyaline degeneration of testicular arterioles and spermatic artery, testicular calcification, Sertoli cell lipofuscin, decreased seminiferous tubular diameter, and testicular neoplasia. This may be due to the number of cats in the study or there may be a true species difference in testicular aging. The last hypothesis is supported by infrequent literature citations of feline neoplasia¹⁵ and lack of reported feline testicular calcification.

Age changes in the feline epididymis were uncommon;^{1,3-5,7,17} epididymal tunica albuginea thickness significantly increased with age, only a small percentage of aged cats had intraepithelial cysts and hyperplasia, and one cat (9 years old) had a spermatocele in the epididymal head. Increased interstitial fibrosis and ductus efferent lipofuscin were not noted.

The middle age group (3 to 6.9 years) had highest levels of epididymal duct spermatozoa and proteinaceous tubular luminal debris, and minimal seminiferous tubular degeneration suggesting an optimum level of spermatogenesis. Proteinaceous tubular luminal debris may be a biproduct of spermatogenesis such as detached residual bodies, and might eventually be phagocytized by Sertoli cells.²² Cats in this age group also contained significantly increased numbers of interstitial cell cytoplasmic bodies. Their origin is not known, but they do not resemble crystalloid inclusions seen in the dog,²³ rat,²⁴ and human.²⁵ They may represent an androgen-dependent storage product involved in sperm maturation, but electron microscopy and cytochemistry would be needed to substantiate this.

Lymphocytic foci were commonly seen in cats of all ages but aggregates in the 8 and 9 year old cats were reactive as evidenced by frequent mitoses, numerous plasma cells, macrophages, and lymphoblasts. The 9 year old cat with 3 spermatoceles could have an ideal environment for antigenic exposure. The findings suggest a possible role of autoimmunization in feline age-related testicular and epididymal lesions. Similar increases in immunocyte populations have been observed in the aged bull¹² and man.²⁶

SUMMARY

Forty-two pairs of testes and epididymes were obtained from clinically healthy cats and examined histologically for age-related changes. The following age-related testicular changes were noted: increased testicular tunica albuginea thickness, interstitial cell lipofuscin, interstitial cell hyperplasia, Sertoli cell cytoplasmic vacuolation, seminiferous tubular degeneration, seminiferous tubular basement membrane thickness, and 2 spermatocytes in the oldest cat. Age changes in the feline epididymis included increased epididymal tunica albuginea thickness, occasional intraepithelial cysts hyperplasia, and one spermatocyte in the head of the epididymal duct. Lymphocytic foci were common in testicular and epididymal connective tissue of cats of all ages and were reactive in the 2 oldest cats, suggesting an autoimmune mechanism in age-related testicular and epididymal changes. Several changes were most frequent in the middle age group: eosinophilic to amphophilic round cytoplasmic bodies in interstitial cells, proteinaceous tubular luminal debris, and spermatozoa in the epididymal duct. Cats less than a year old often contained seminiferous tubular degeneration, and epididymal intraepithelial cysts and hyperplasia.

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Fig 1 - Regression plot between feline age and testicular tunica
albuginea thickness: $Y = 0.169 + 0.001X$, $N = 42$, $R^2 = 0.20$, $P < 0.01$.

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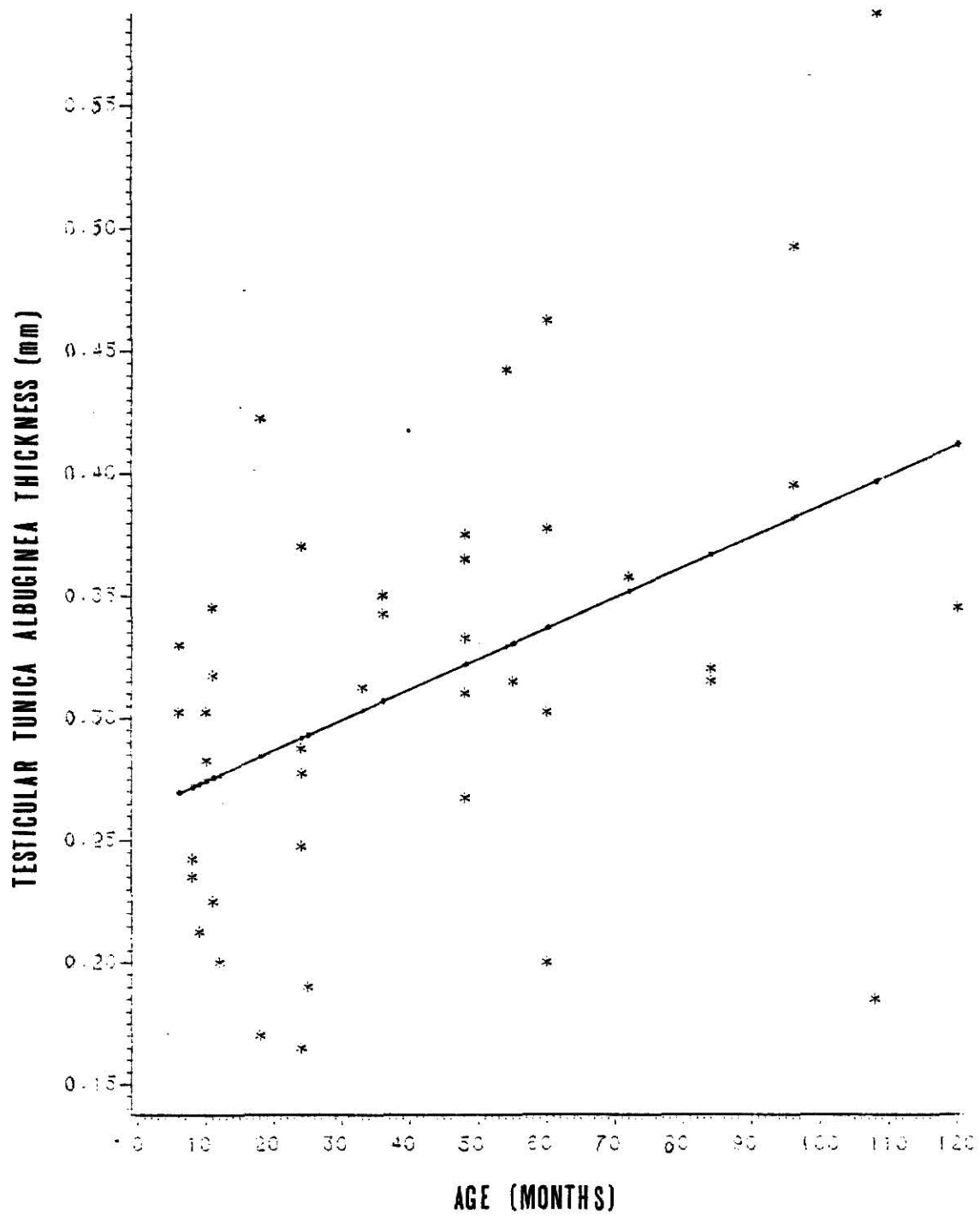


Fig 2 - Regression plot between feline age and epididymal tunica
albuginea thickness: $Y = 0.249 + 0.0006X$, $N = 42$, $R^2 = 0.15$, $P < 0.05$.

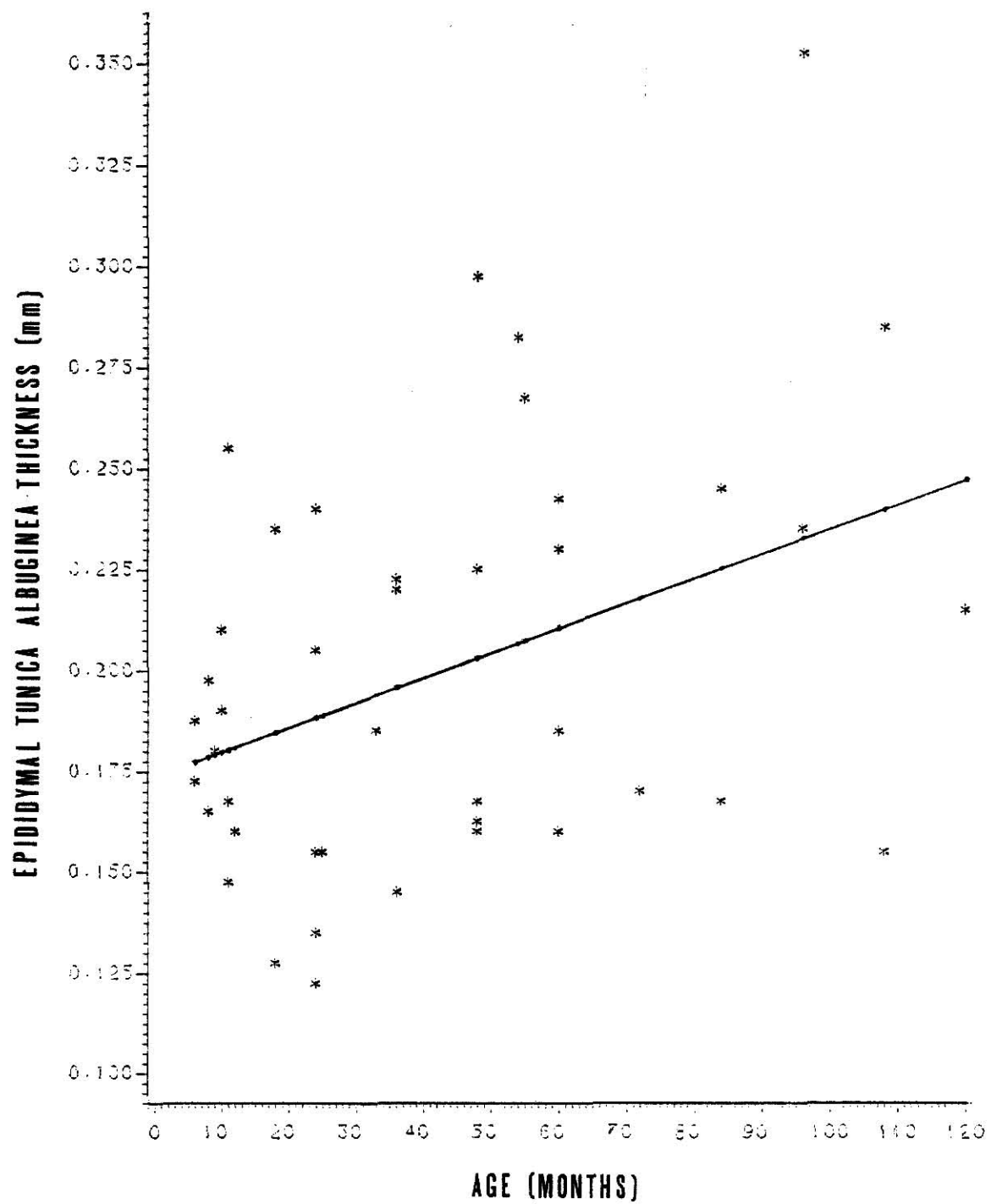


Fig 3 - Regression plot between feline age and spermatogonia A cell
number: $Y = 0.732 - 0.002X$, $N = 42$, $R^2 = 0.09$, $P < 0.05$.

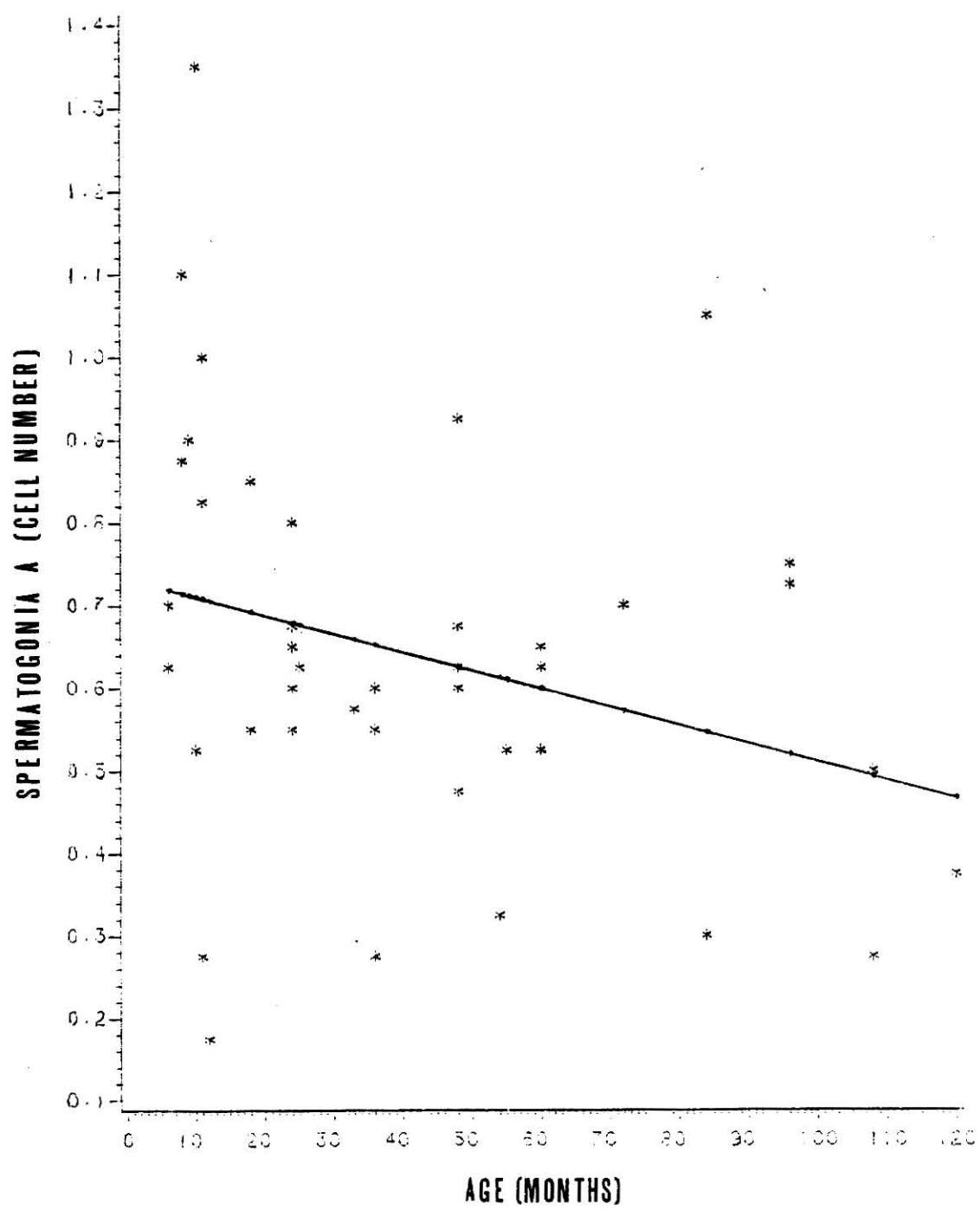


Fig 4 - Regression plot between feline age and spermatogonia B cell
number: $Y = 0.498 - 0.003X$, $N = 42$, $R^2 = 0.26$, $P < 0.01$

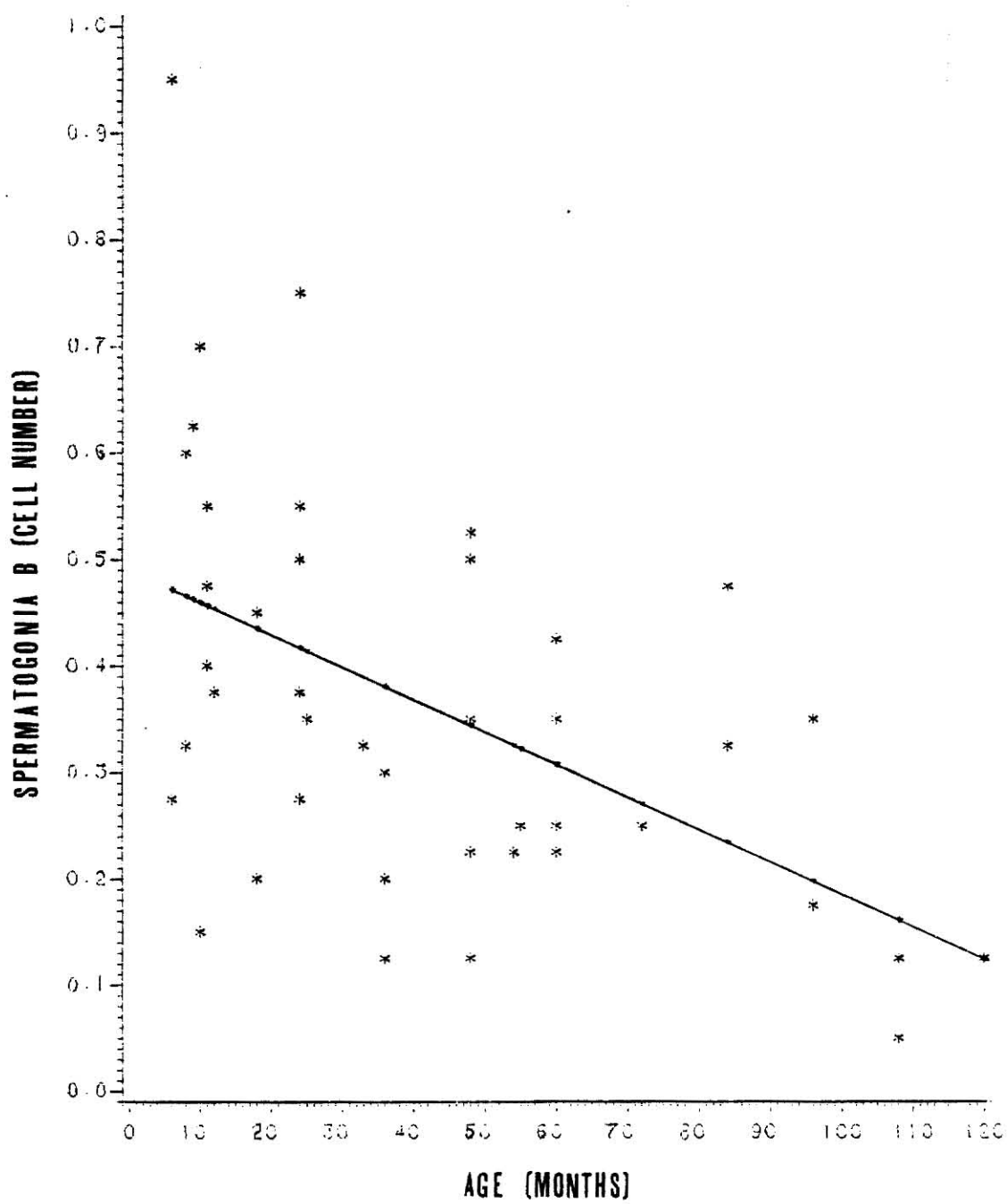


Fig 5 - Regression plot between feline age and secondary spermatocyte cell number: $Y = 0.954 - 0.009X$, $N = 42$, $R^2 = 0.24$, $P < 0.01$.

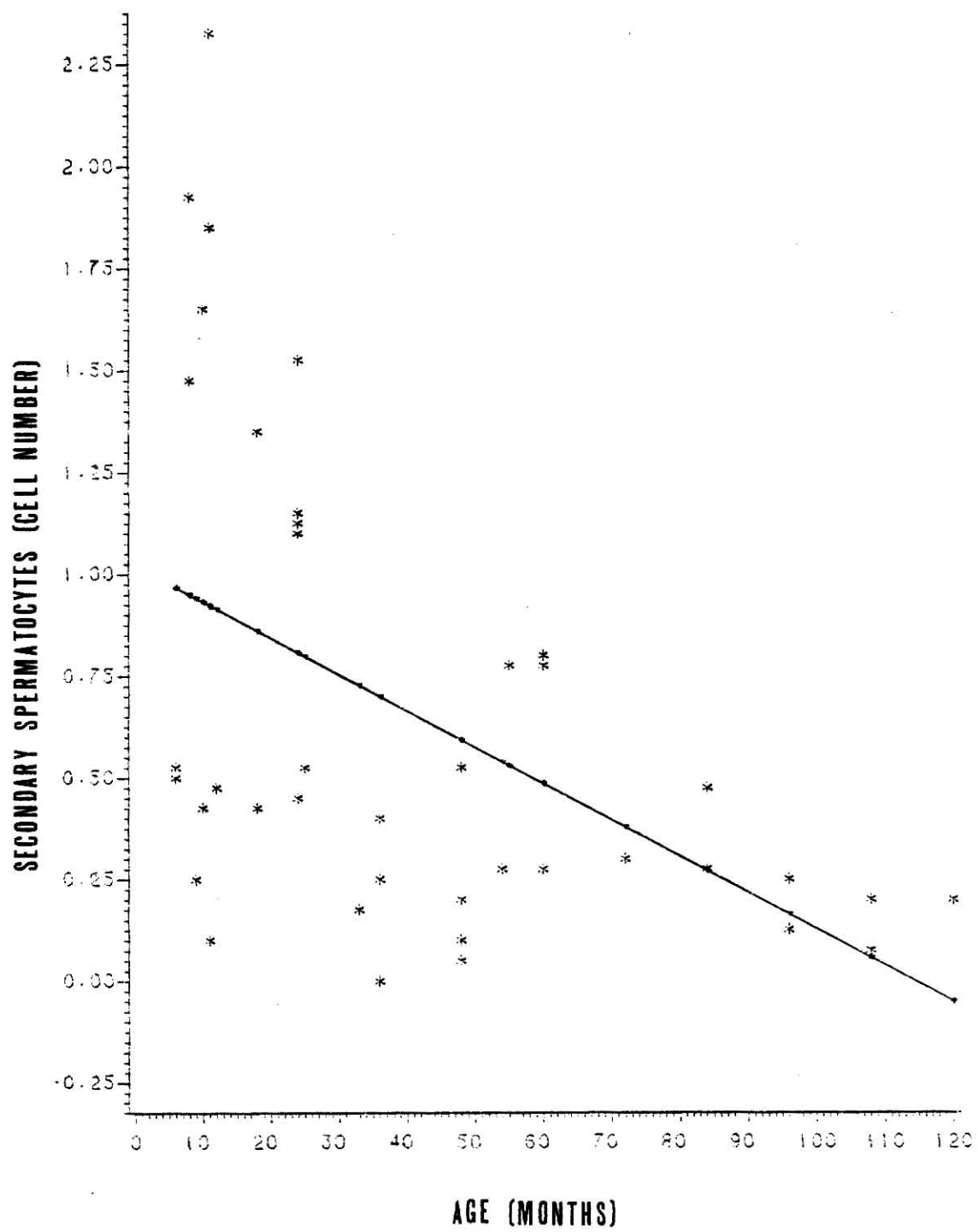


Fig 6 - Regression plot between feline age and interstitial cell number:

$$Y = 5.04 + 0.06X, N = 42, R^2 = 0.35, P < 0.01.$$

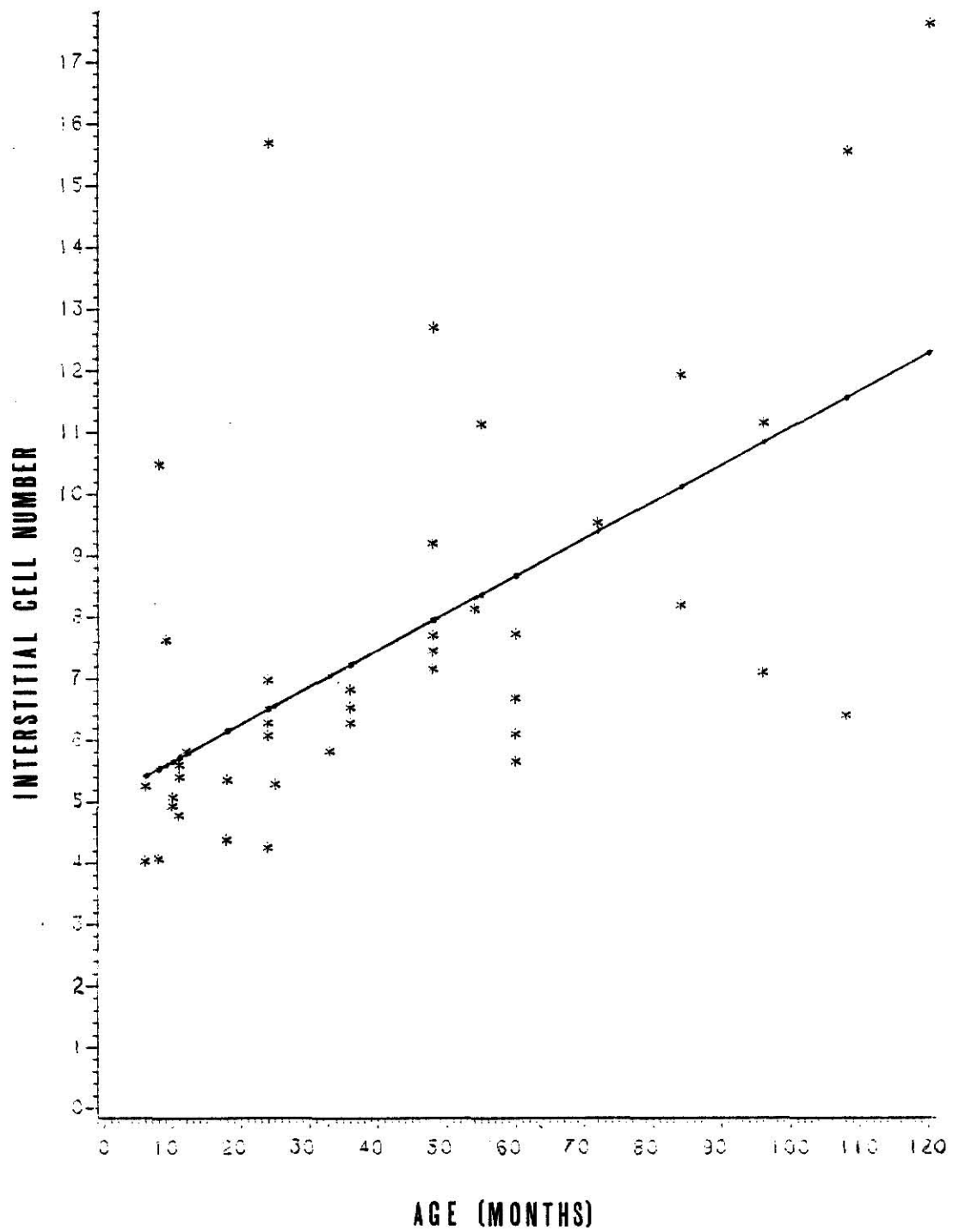


Fig 7 - Regression plot between feline age and seminiferous tubular degeneration: $Y = 1.43 - 0.04X + 0.004X^2$, $N = 42$, $R^2 = 0.25$, $P < 0.01$.
Data were graded on a scale of 0 to 5.

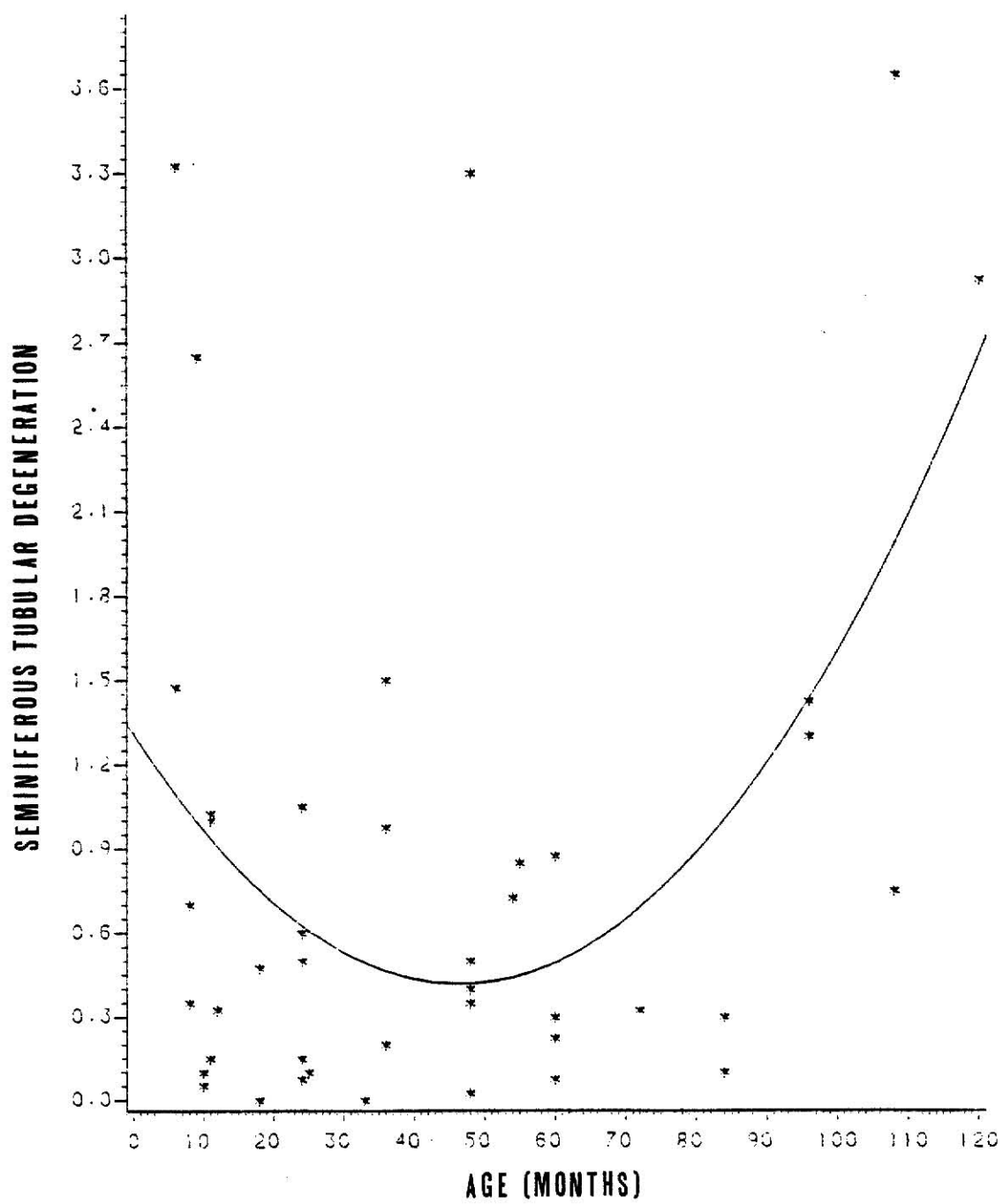


Fig 8 - Regression plot between feline age and Sertoli cell cytoplasmic vacuolation: $Y = 0.94 - 0.01X + 0.0001X^2$, $N = 42$, $R^2 = 0.18$, $P < 0.05$.

Data were graded on a scale of 0 to 5.

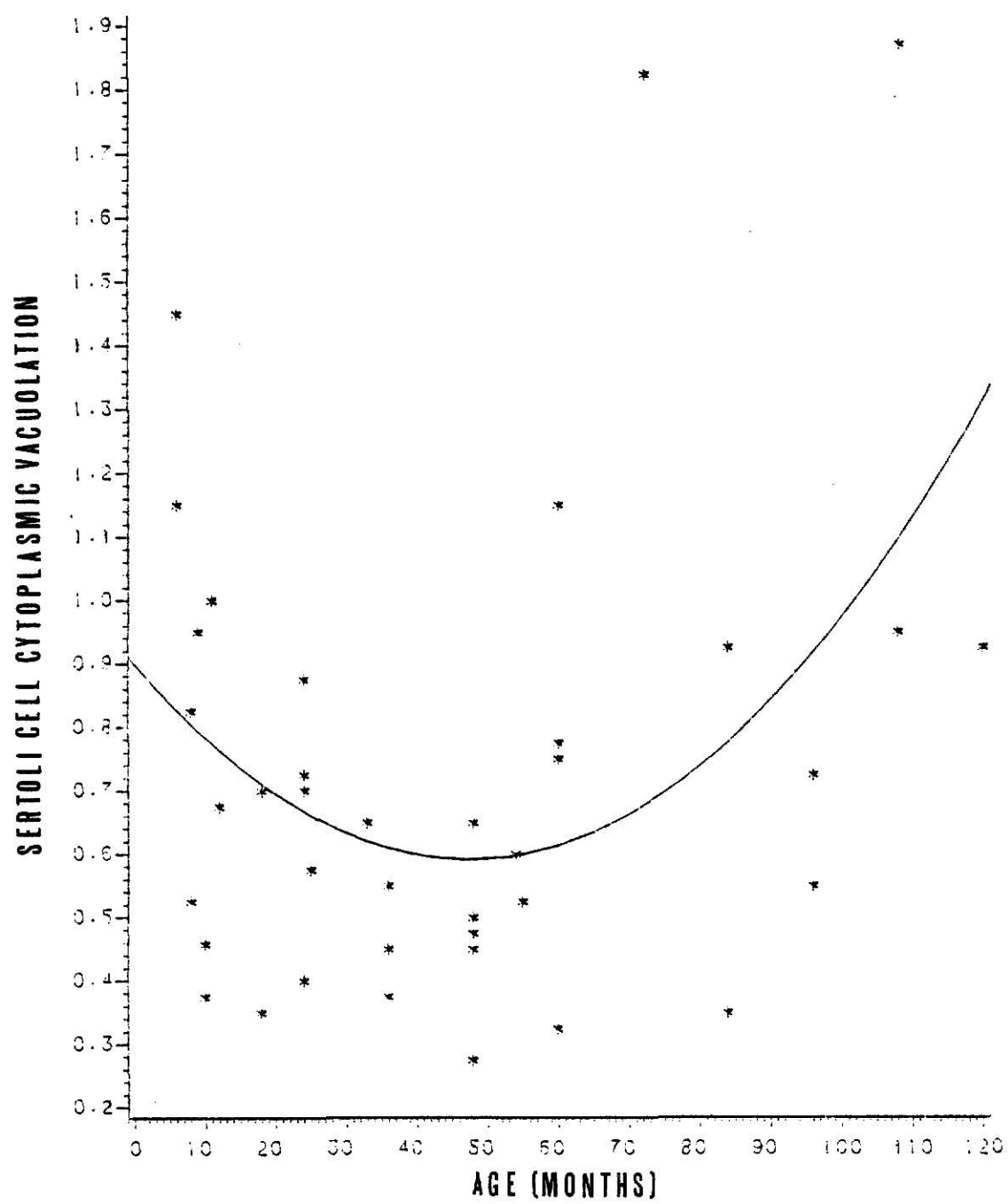


Fig 9 - Regression plot between feline age and seminiferous tubular basement membrane thickness: $Y = 0.46 - 0.02X + 0.0004X^2$, $N = 42$, $R^2 = 0.41$, $P < 0.01$. Data were graded on a scale of 0 to 5.

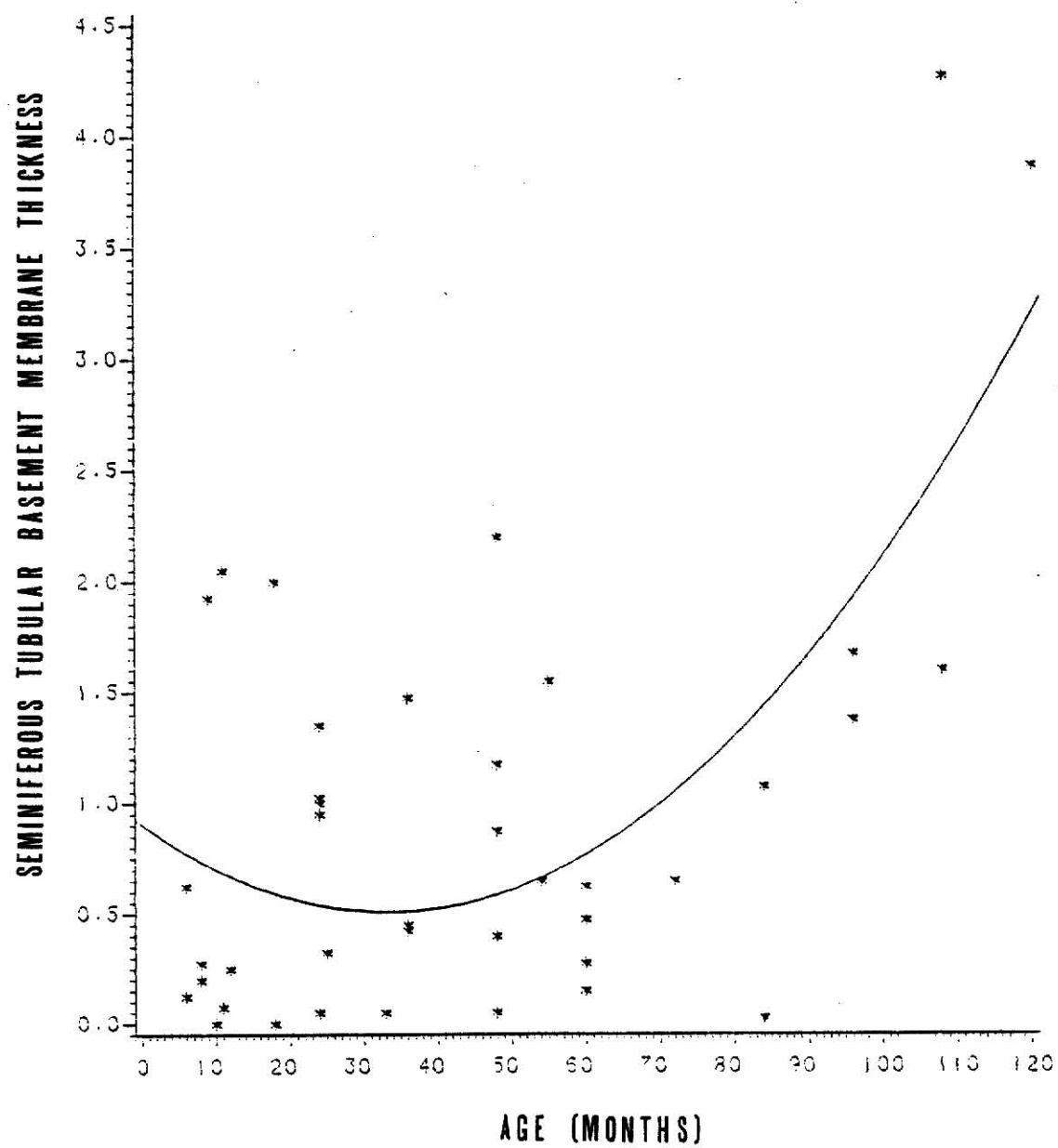


Fig 10 - Regression plot between feline age and interstitial cell
lipofuscin: $Y = 0.13 - 0.006X + 0.0002X^2$, $N = 42$, $R^2 = 0.48$, $P < 0.05$.
Data were graded on a scale of 0 to 5.

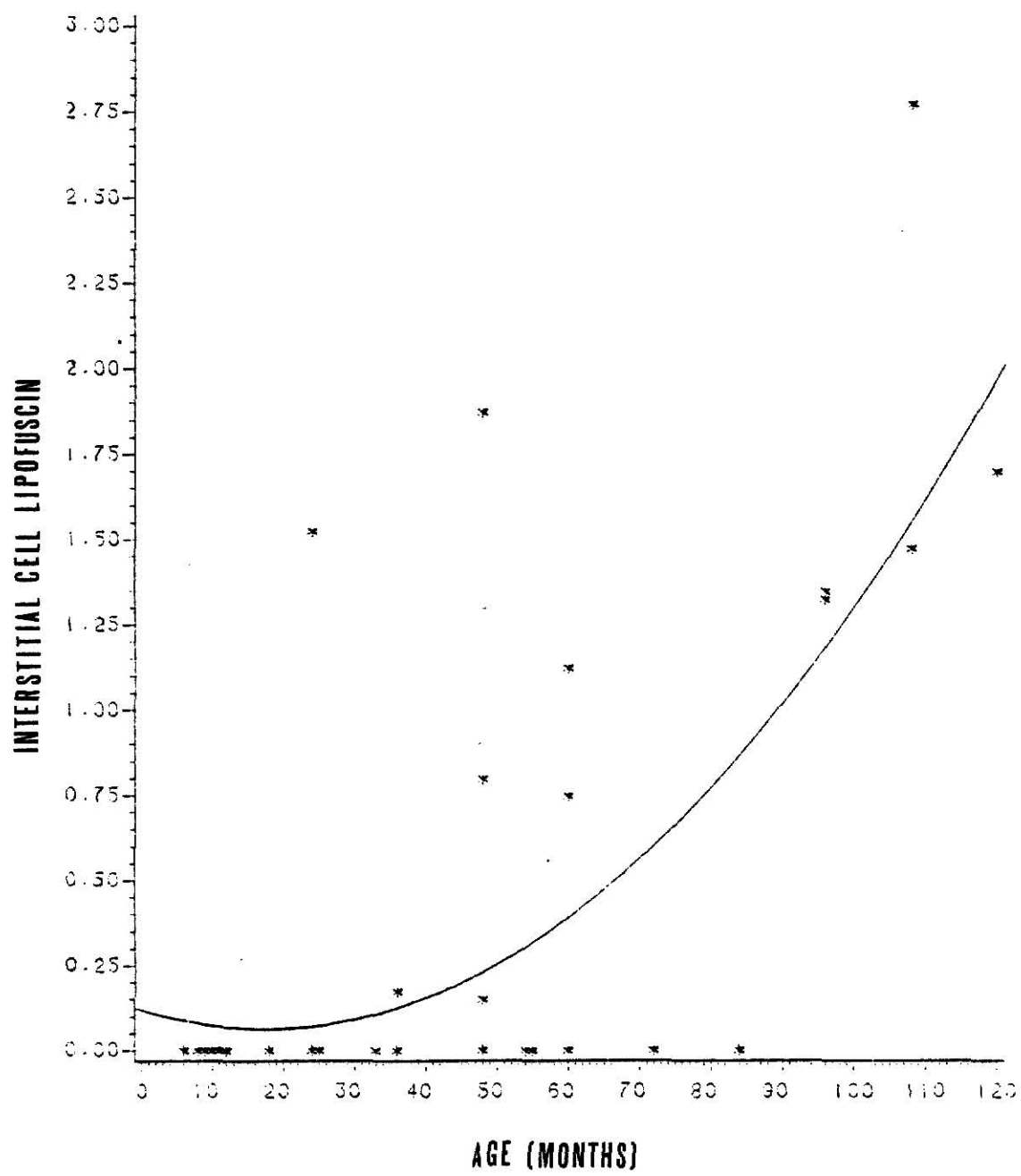


Fig 11 - Regression plot between feline age and interstitial cell cytoplasmic bodies: $Y = -0.15 + 0.027X - 0.0002X^2$, $N = 42$, $R^2 = 0.16$, $P < 0.05$. Data were graded on a scale of 0 to 5.

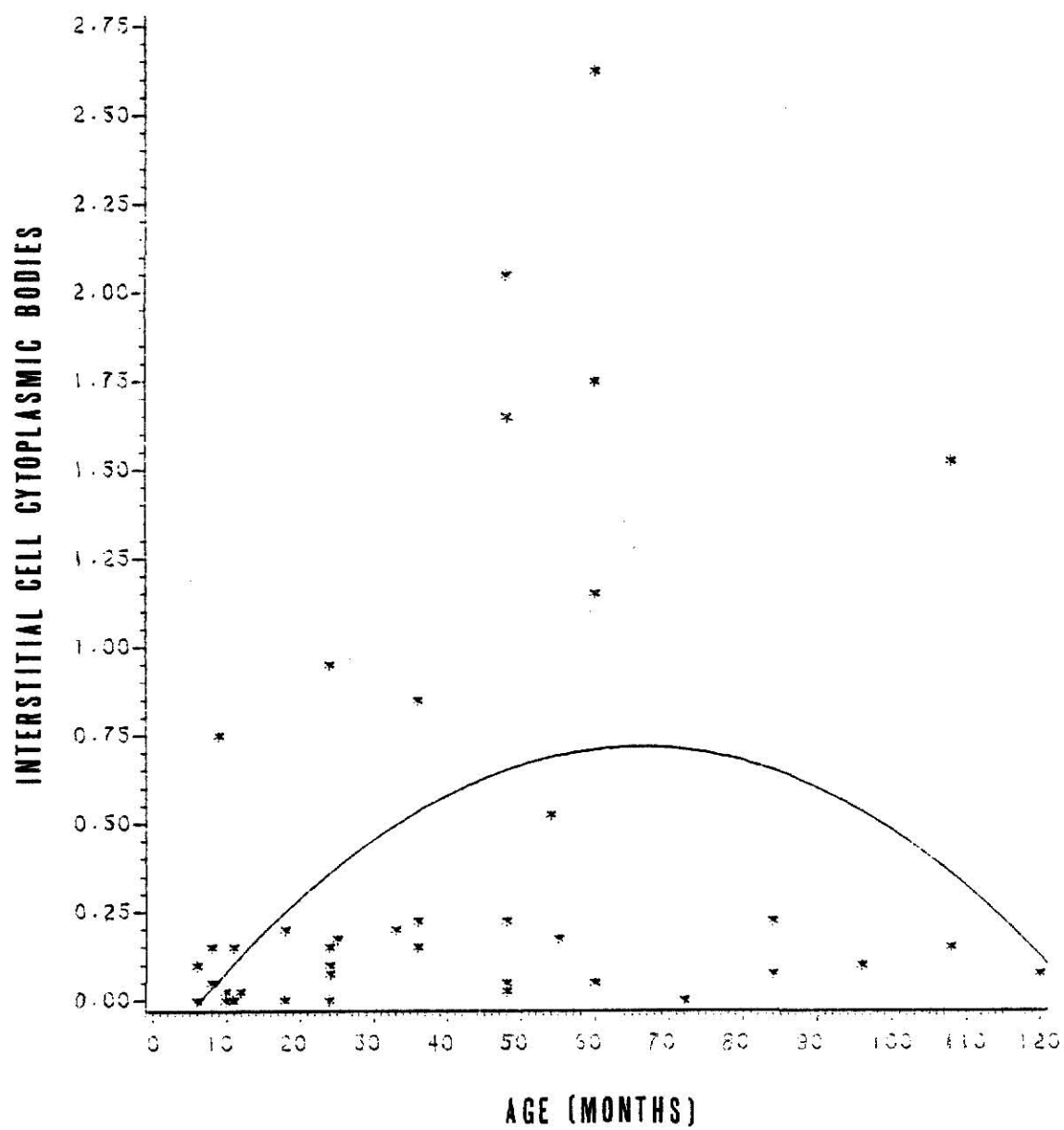


Fig 12 - Regression plot between feline age and epididymal ductal spermatozoa number: $Y = 0.45 + 0.04X - 0.0003X^2$, $N = 42$, $R^2 = 0.23$, $P < 0.05$. Data were graded on a scale of 0 to 5.

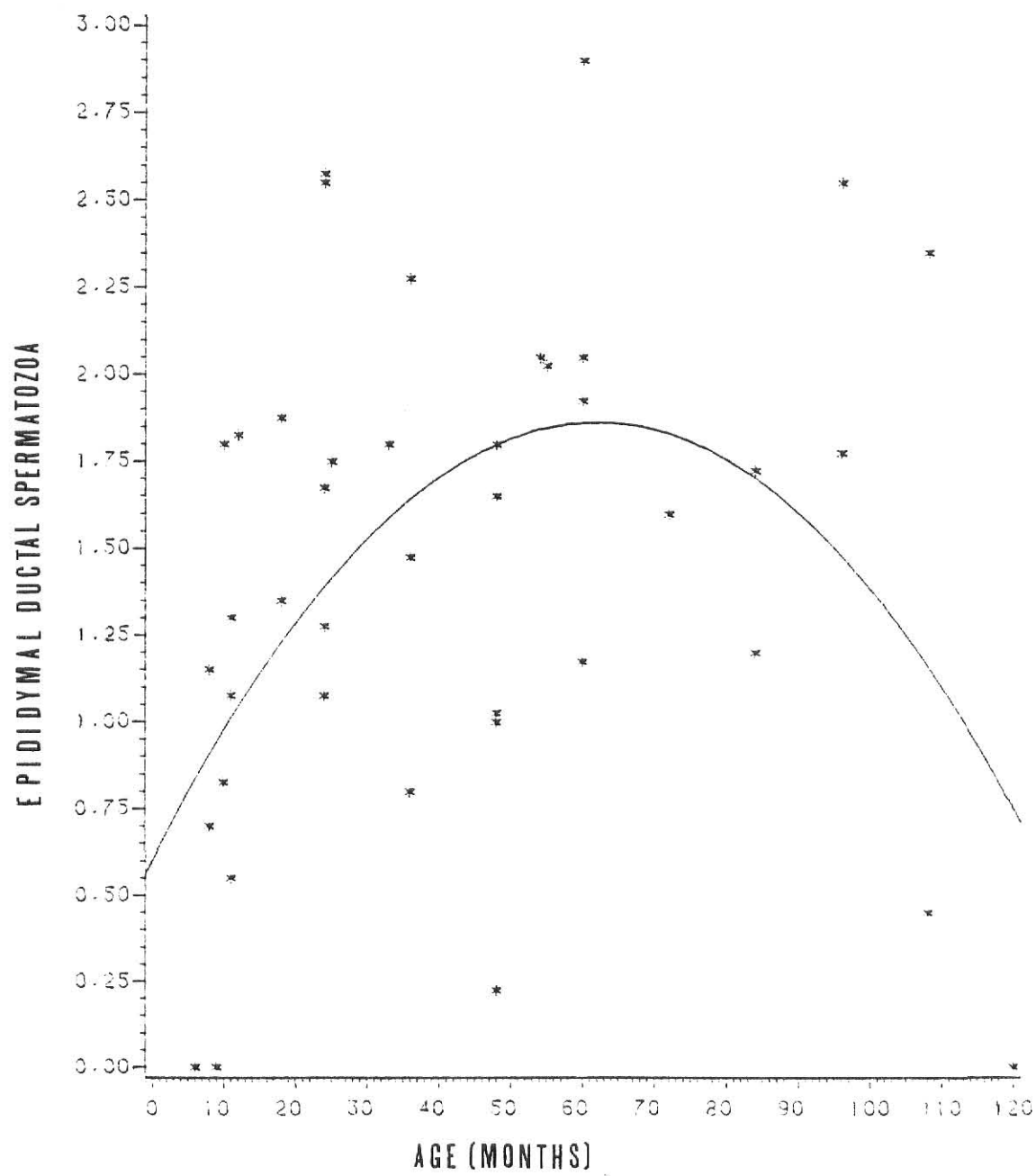
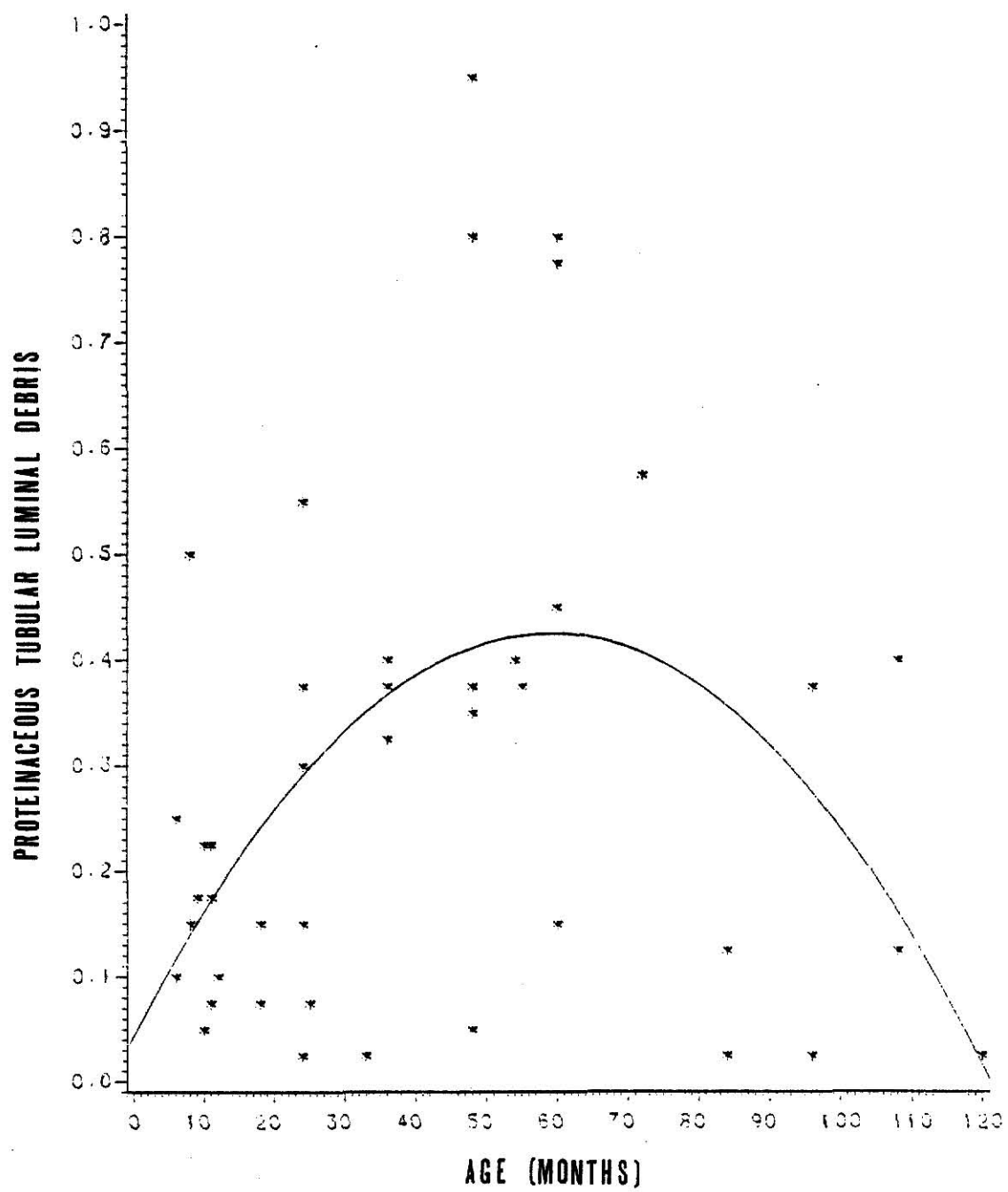


Fig 13 - Regression plot between feline age and proteinaceous tubular luminal debris: $Y = 0.04 + 0.01X - 0.0001X^2$, $N = 42$, $R^2 = 0.24$, $P < 0.01$. Data were graded on a scale of 0 to 5.



14	15
16	17
18	19

Fig 14 - Normal seminiferous tubules in a 2 year old cat. H&E stain;
x 230.

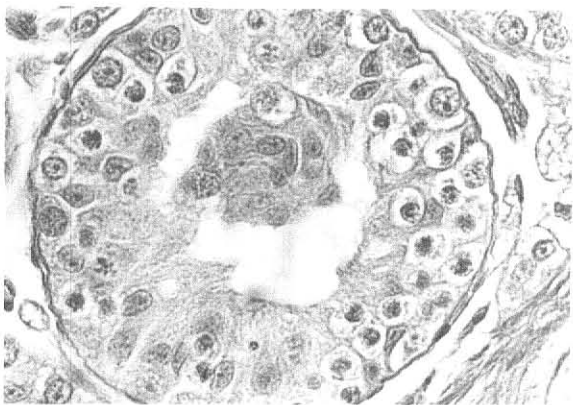
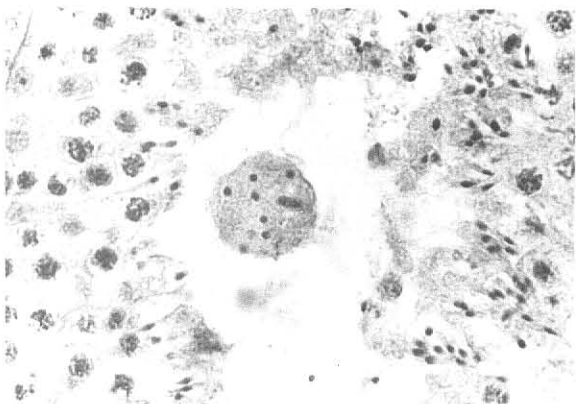
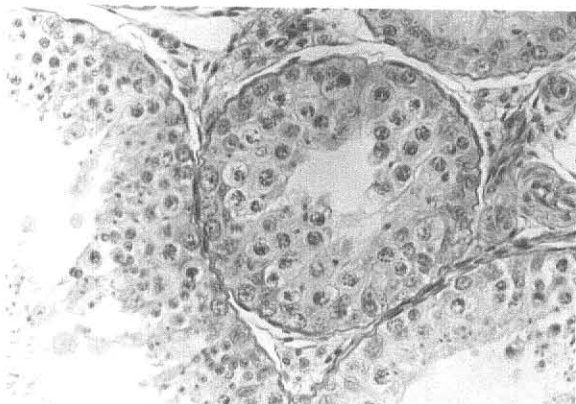
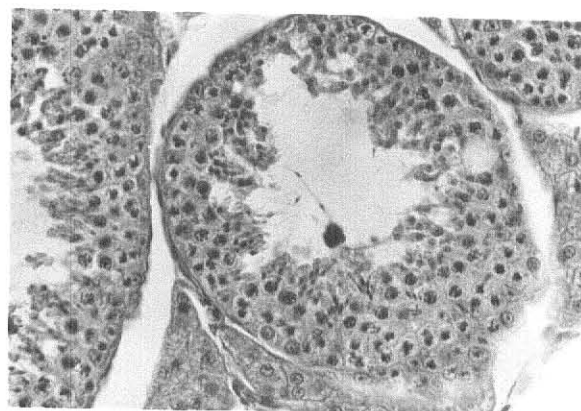
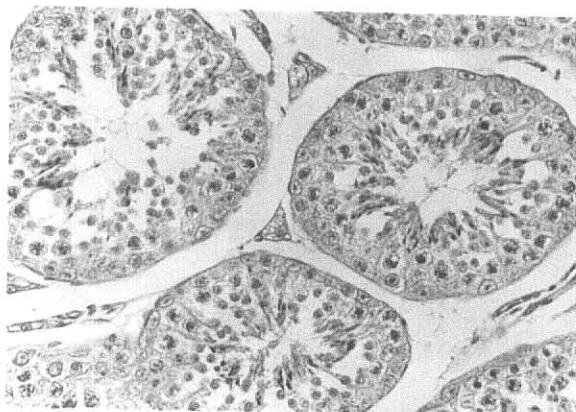
Fig 15 - Mild seminiferous tubular degeneration in a 4.5 year old cat.
There are normal proportionate amounts of germinal epithelium, mild
spermatocyte cytoplasmic vacuolation, one large Sertoli cell cyto-
plasmic vacuole, and one spermatid giant cell. H&E stain; x 250.

Fig 16 - Mild to moderate seminiferous tubular degeneration in a 6 year
old cat. There is mild reduction of spermatids and spermatocytes
with moderate spermatocyte cytoplasmic vacuolation. H&E stain;
x 230.

Fig 17 - Moderate seminiferous tubular degeneration in a 6.1 year old
cat. A mononuclear spermatid giant cell is in the tubular lumen.
H&E stain; x 478.

Fig 18 - A multinuclear spermatid giant cell is in the tubular lumen of
a 4.5 year old cat. H&E stain; x 533.

Fig 19 - Mild to moderate seminiferous tubular degeneration in a 0.5
year old cat. A large luminal aggregate fills much of the tubular
lumen. H&E stain; x 475.



20	21
22	23
24	25

Fig 20 - Severe seminiferous tubular degeneration and basement membrane thickening in a 9 year old cat. H&E stain; x 528.

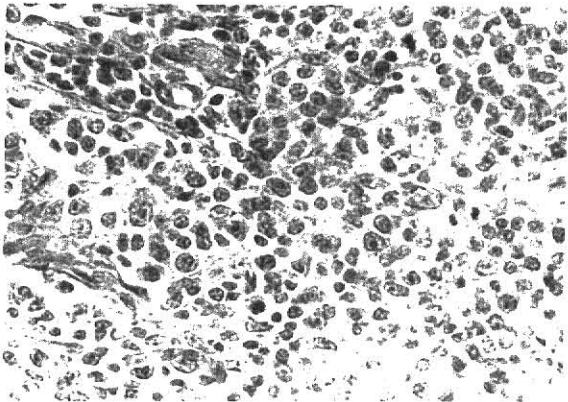
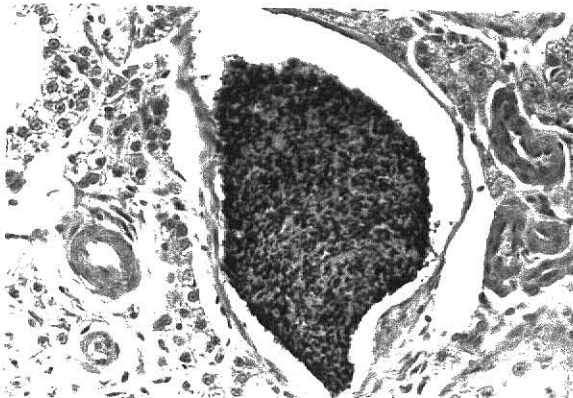
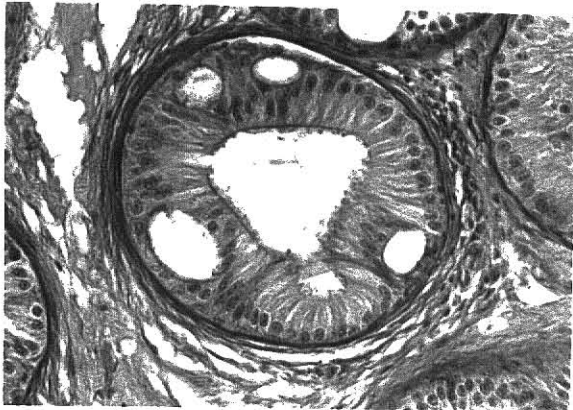
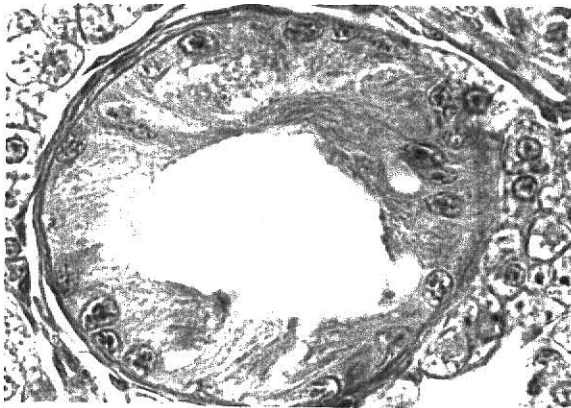
Fig 21 - Epithelial hyperplasia and intraepithelial cysts in the epididymal duct of a 0.6 year old cat. H&E stain; x 247.

Fig 22 - Testicular spermatocoele in a 9 year old cat. H&E stain; x 184.

Fig 23 - Interstitial cell cytoplasmic bodies in a 5 year old cat. H&E stain; x 477.

Fig 24 - Sparsely populated lymphocytic focus in the epididymal interstitium of a 1.5 year old cat. H&E stain; x 230.

Fig 25 - Reactive lymphocytic focus in the epididymal tunica albuginea of a 9 year old cat. H&E stain; x 400.



III. APPENDIX

TABLE 1. Quantitative Measurements of the Cat Testis and Epididymis

Variable	Age							
	6-11 months		12-35 months		36-59 months		60-83 months	
	Mean	S.D.*	Mean	S.D.	Mean	S.D.	Mean	S.D.
Testicular tunica albuginea thickness	0.279 ^{a**}	0.06	0.264 ^a	0.09	0.345 ^b	0.07	0.340 ^b	0.12
Epididymal tunica albuginea thickness	0.199 ^a	0.04	0.172 ^a	0.05	0.290 ^a	0.05	0.198 ^a	0.06
Spermatogonia A	0.818 ^a	0.40	0.605 ^a	0.22	0.558 ^a	0.28	0.605 ^a	0.22
Spermatogonia B	0.505 ^a	0.35	0.415 ^a	0.25	0.283 ^b	0.19	0.300 ^b	0.20
Secondary Spermatocytes	1.103 ^a	0.97	0.830 ^b	0.62	0.310 ^d	0.29	0.590 ^c	0.37
Interstitial Cells	5.723 ^a	2.33	6.590 ^b	3.54	8.308 ^c	2.74	7.135 ^b	1.86
							11.136 ^d	5.17

* Standard deviation

** Means with different superscripts are significantly different (P<0.05)

TABLE 2. Qualitative Measurements of the Cat Testis and Epididymis

Variable	Age									
	6-11 months		12-35 months		36-59 months		60-83 months		84 months	
	Mean	S.D.*	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Tubular atrophy	1.083 ^{a**}	1.18	0.328 ^b	0.38	0.883 ^c	0.98	0.360 ^b	0.35	1.490 ^d	1.34
Tubular basement membrane thickness	0.530 ^a	0.78	0.695 ^b	0.72	0.925 ^c	0.71	0.435 ^a	0.31	1.986 ^d	1.50
Sertoli cell cytoplasmic vacuolation	0.873 ^a	0.44	0.605 ^b	0.25	0.485 ^b	0.25	0.965 ^a	0.60	0.899 ^a	0.52
Proteinaceous tubular lumen debris	0.192 ^a	0.22	0.183 ^a	0.22	0.440 ^b	0.33	0.550 ^b	0.36	0.156 ^a	0.24
Interstitial cell hyperplasia	0.125 ^a	0.17	0.390 ^b	0.27	0.778 ^c	0.21	0.240 ^d	0.45	1.950 ^e	1.87
Interstitial cell lipofuscin	0 ^a	0	0.152 ^b	0.48	0.300 ^c	0.66	0.375 ^c	0.55	1.232 ^d	1.03
Interstitial cell cytoplasmic bodies	0.123 ^a	0.23	0.188 ^a	0.31	0.593 ^c	0.81	1.115 ^d	1.19	0.322 ^b	0.61
Ductus epididymis spermatozoa	0.740 ^a	0.67	1.775 ^b	0.71	1.433 ^c	0.81	1.930 ^b	1.02	1.433 ^c	1.10

* Standard deviation

** Means with different superscripts are significantly different (P<0.05)

Intensity of changes were graded on a scale of 0 to 5.

TABLE 3. Quantitative Measurements: Regression Analysis on Age-Related Feline Testicular and Epididymal Means

Variable	N	Slope	± Standard Error	R ²
Testicular tunica albuginea thickness	42	0.00125	0.00039	0.201 ^{**}
Epididymal tunica albuginea thickness	42	0.00061	0.00023	0.150 [*]
Spermatogonia A	42	-0.00220	0.00114	0.086 [*]
Spermatogonia B	42	-0.00300	0.00081	0.262 ^{**}
Secondary spermatocytes	42	-0.00890	0.00249	0.243 ^{**}
Interstitial cells	42	0.06024	0.01288	0.354 ^{**}

^{*}P<0.05

^{**}P<0.01

TABLE 4. Qualitative Measurements: Regression Analysis on Age-Related Feline Testicular and Epididymal Means

Variable	N	Slope			\pm standard error b_2	R^2
		b_1	b_2	\pm standard error b_1		
Tubular atrophy	42	-0.03838	0.00041	0.01514	0.00013	0.246**
Tubular basement membrane thickness	42	-0.02376	0.00036	0.01345	0.00012	0.408**
Sertoli cell cytoplasmic vacuolation	42	-0.01298	0.00014	0.00593	0.00005	0.183*
Proteinaceous tubular lumen debris	42	0.01293	-0.00011	0.00374	0.00003	0.235**
Interstitial cell hyperplasia	42	-0.01378	0.00032	0.01744	0.00015	0.386*
Interstitial cell lipofuscin	42	-0.00638	0.00018	0.00888	0.00008	0.480*
Interstitial cell cytoplasmic bodies	42	0.02675	-0.00020	0.01053	0.00009	0.155*
Ductus epididymis spermatozoa	42	0.04083	-0.00033	0.01191	0.00010	0.234*

* $P < 0.05$

** $P < 0.01$

A quadratic model, $Y = b_0 + b_1x_1 + b_2x_1^2$, best fits the qualitative data. Intensity of changes were graded on a scale of 0 to 5.

Table 5 - Original Data.

A. Qualitative Data

O B S	I D E S	S L I D E	B R E E D E	A I G E	S E C T I O N	I D N	A T F	B M T F	K F	N S F	L I P O F	C Y T O V F	P Y M K F	P R C Y T F	P R P Y K F	P R K Y F	S E C Y T F
1	3210	1	5	48	1	2	3.6	2.2	0	0.1	0	0.4	0.0	1.700	0.10	0.1	1.60000
2	3210	2	5	48	1	2	3.2	2.0	0	0.0	0	0.6	0.0	1.300	0.20	0.0	0.60000
3	3212	1	1	8	1	2	0.1	0.1	0	0.0	0	0.7	0.0	1.200	0.30	0.0	1.00000
4	3212	2	1	8	1	2	0.1	0.3	0	0.0	0	0.6	0.0	0.700	0.10	0.0	0.20000
5	3214	1	3	9	1	2	1.7	2.3	0	0.1	0	0.9	0.1	2.400	0.80	0.0	0.77778
6	3214	2	3	9	1	2	3.9	1.8	0	0.0	0	0.5	0.0	2.625	1.25	0.0	1.00000
7	3215	1	4	10	1	2	0.2	0.0	0	0.0	0	0.6	0.0	0.900	0.30	0.0	0.50000
8	3215	2	4	10	1	2	0.0	0.0	0	0.0	0	0.1	0.0	0.500	0.10	0.0	0.40000
9	3217	1	4	6	1	2	1.7	0.2	0	0.0	0	1.6	0.0	1.800	0.10	0.0	0.37500
10	3217	2	4	6	1	2	1.4	0.3	0	0.0	0	1.2	0.0	1.600	0.30	0.0	0.44444
11	3219	1	4	11	1	2	1.6	0.0	0	0.0	0	1.0	0.0	0.800	0.30	0.0	0.25000
12	3219	2	4	11	1	2	1.2	0.3	0	0.0	0	1.6	0.0	0.800	0.30	0.0	0.33333
13	3220	1	5	11	1	2	0.8	2.3	0	0.1	0	0.3	0.0	2.600	0.80	0.0	1.12500
14	3220	2	5	11	1	2	0.7	1.7	0	0.0	0	1.4	0.0	2.200	1.50	0.0	0.60000
15	3224	1	4	48	1	2	0.0	0.3	0	0.0	0	0.4	0.0	0.200	0.30	0.0	0.10000
16	3224	2	4	48	1	2	0.1	0.5	0	0.1	0	0.6	0.0	0.400	0.30	0.0	0.20000
17	3226	1	4	18	1	2	0.0	0.0	0	0.0	0	1.1	0.0	0.900	0.30	0.0	0.44444
18	3226	2	4	18	1	2	0.0	0.0	0	0.0	0	0.7	0.0	0.500	0.30	0.0	0.30000
19	3227	1	4	48	1	2	0.4	3.0	0	0.3	0	0.2	0.0	2.100	0.60	0.0	0.22222

O B S	S E P Y K F	S E K F	L P M F	L D S G F	I C H Y F	I C L C F	I C L F F	I C D P F	I C F B F	V A A T S A A F	S P C V F	S P P Y F	S C P K F	G L A Y F	D E N F	D E H F
1	0.000000	0	0.8	0.4	0.6	4.9	0.0	0.1	1.1	0	0.10000	0.0	0	0	0.0	0.0
2	0.800000	0	1.0	0.1	0.1	5.0	0.0	0.1	0.8	0	0.40000	0.0	0	0	0.0	0.0
3	0.000000	0	0.1	0.1	0.0	4.1	0.0	0.1	0.0	0	1.00000	0.0	0	0	0.8	0.4
4	0.000000	0	0.1	0.0	0.0	3.2	0.0	0.1	0.0	0	0.20000	0.0	0	0	0.2	0.1
5	0.000000	0	0.1	1.4	0.0	4.4	0.0	0.7	0.1	0	1.80000	0.2	0	0	0.3	0.2
6	0.250000	0	0.0	2.1	0.0	3.8	0.0	0.7	0.3	0	2.70000	1.7	0	0	0.1	0.1
7	0.000000	0	0.4	1.1	0.0	3.1	0.0	0.0	0.0	0	0.50000	0.0	0	0	0.6	0.3
8	0.000000	0	0.1	0.2	0.0	3.2	0.0	0.0	0.0	0	0.30000	0.0	0	0	0.2	0.3
9	0.000000	0	0.2	0.6	0.0	4.0	0.0	0.0	0.0	0	1.00000	0.0	0	0	0.1	0.2
10	0.000000	0	0.4	1.1	0.0	4.0	0.0	0.2	0.0	0	0.40000	0.0	0	0	0.3	0.3
11	0.000000	0	0.0	0.1	0.0	3.8	0.0	0.0	0.0	0	0.30000	0.0	0	0	0.3	0.3
12	0.000000	0	0.1	0.2	0.0	3.0	0.0	0.0	0.0	0	0.50000	0.0	0	0	0.2	0.2
13	0.000000	0	0.6	1.3	0.0	4.2	0.0	0.0	0.0	0	1.50000	0.1	0	0	0.1	0.1
14	0.000000	0	0.1	0.3	0.1	4.7	0.0	0.2	0.0	0	0.50000	0.0	0	0	0.0	0.0
15	0.300000	0	0.0	0.0	0.0	3.7	1.2	0.7	0.0	0	0.20000	0.0	0	0	0.2	0.0
16	0.000000	0	0.0	0.0	0.0	2.0	0.0	2.7	0.0	0	0.10000	0.0	0	0	0.1	0.0
17	0.000000	0	0.1	0.3	0.0	1.1	0.0	0.1	0.0	0	0.20000	0.0	0	0	0.0	0.0
18	0.000000	0	0.1	0.3	0.0	2.1	0.0	0.1	0.0	0	0.20000	0.0	0	0	0.4	0.1
19	0.000000	0	1.2	0.0	0.1	2.7	0.0	2.3	0.0	0	0.32333	0.0	0	0	0.2	0.0

	D	E	I	S	S						C	P	P	P	S	S	
	S	E	S	I	C		B		N	L	Y	P	P	P	S	S	
	P	E	S	I	I	A	M		S	I	T	Y	P	P	S	S	
	S	F	F	F	F	S	S	S	S	S	V	K	Y	K	S	S	S
1	0.1	0.8	1.8	0	0	3.4	2.4	0	0.0	0	0.8	0	0.80	0.0	0.0	0.50000	0.00
2	0.6	0.0	0.8	0	0	2.0	2.2	0	0.0	0	0.8	0	1.80	0.2	0.0	1.00000	0.00
3	1.7	0.2	0.0	0	0	0.2	0.2	0	0.0	0	0.4	0	0.60	0.2	0.0	0.40000	0.00
4	1.3	0.2	0.0	0	0	1.0	0.2	0	0.0	0	0.4	0	0.00	0.0	0.0	0.00000	0.00
5	0.0	0.4	0.0	0	0	1.6	1.6	0	0.0	0	1.2	0	2.25	1.0	0.0	0.50000	0.00
6	0.0	0.2	0.0	0	0	2.4	2.0	0	0.0	0	1.2	0	2.40	1.0	0.0	1.50000	0.00
7	0.9	0.1	0.0	0	0	0.2	0.0	0	0.0	0	0.6	0	0.20	0.0	0.0	0.00000	0.00
8	0.8	0.0	0.0	0	0	0.0	0.0	0	0.0	0	0.2	0	0.80	0.2	0.0	0.20000	0.00
9	0.0	0.0	0.0	0	0	2.2	0.0	0	0.0	0	1.8	0	2.40	0.2	0.0	0.00000	0.00
10	0.0	0.0	0.0	0	0	0.6	0.0	0	0.0	0	1.2	0	0.80	0.0	0.0	0.20000	0.00
11	0.2	0.0	0.0	0	0	0.2	0.0	0	0.0	0	0.0	0	1.20	1.2	0.0	0.40000	0.00
12	0.8	0.1	0.0	0	0	1.0	0.0	0	0.0	0	1.4	0	1.40	0.0	0.0	0.50000	0.00
13	0.8	0.0	0.2	0	0	1.2	2.4	0	0.0	0	1.0	0	0.80	0.4	0.0	0.00000	0.00
14	0.3	0.6	0.6	0	0	1.4	1.8	0	0.0	0	0.8	0	2.20	1.2	0.0	0.40000	0.00
15	0.2	0.0	0.0	0	0	0.0	0.4	0	0.0	0	0.6	0	0.60	0.0	0.0	0.00000	0.00
16	1.0	0.0	0.0	0	0	0.0	0.4	0	0.0	0	0.4	0	0.40	0.0	0.0	0.00000	0.00
17	1.7	0.0	0.0	0	0	0.0	0.0	0	0.0	0	0.6	0	0.00	0.0	0.0	0.00000	0.00
18	1.1	0.0	0.0	0	0	0.0	0.0	0	0.0	0	0.4	0	0.60	0.0	0.0	0.20000	0.00
19	1.4	0.0	0.4	0	0	0.2	0.0	0	0.0	0	0.6	0	1.00	0.6	0.4	0.00000	0.25

	L	L	I	I	I	I	I	V	S	S	S	C	G	D	D	D	S	S
	D	P	C	C	C	C	F	A	P	P	P	P	E	E	E	E	S	S
	P	S	H	L	L	F	I	T	C	P	K	L	A	E	E	S	F	I
	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
1	0.2	0.6	0.8	5.0	0	0.0	1.4	0	0.6	0.0	0	0	0.0	0.0	0.2	0.0	1.2	0.0
2	1.2	0.8	0.0	5.0	0	0.0	1.6	0	0.4	0.0	0	0	0.0	0.0	0.0	0.0	0.4	0.0
3	0.2	0.0	0.0	3.6	0	0.0	0.0	0	0.0	0.0	0	0	1.0	0.4	0.8	0.0	0.0	0.0
4	0.2	0.4	0.0	2.6	0	0.0	0.0	0	0.0	0.0	0	0	0.2	0.2	0.8	0.0	0.0	0.0
5	0.2	0.2	0.0	4.2	0	0.8	0.0	0	1.2	0.2	0	0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.4	2.6	0.0	4.0	0	0.8	0.4	0	1.4	0.4	0	0	0.0	0.0	0.0	0.0	0.0	0.0
7	0.4	0.0	0.0	3.4	0	0.0	0.0	0	0.6	0.0	0	0	0.0	0.6	0.6	1.2	0.0	0.0
8	0.0	0.0	0.0	3.2	0	0.0	0.0	0	0.0	0.0	0	0	0.0	0.2	0.4	0.2	0.0	0.0
9	0.0	0.6	0.0	2.6	0	0.0	0.0	0	0.8	0.0	0	0	0.2	0.2	0.0	0.0	0.0	0.0
10	0.4	0.0	0.0	4.0	0	0.2	0.0	0	0.8	0.0	0	0	0.2	0.2	0.0	0.0	0.0	0.0
11	0.0	0.0	0.0	4.0	0	0.0	0.0	0	0.2	0.0	0	0	0.0	0.0	0.4	0.4	0.0	0.0
12	0.2	0.0	0.0	2.6	0	0.0	0.0	0	0.6	0.0	0	0	0.2	0.2	0.8	0.0	0.0	0.0
13	0.0	0.2	0.0	4.8	0	0.0	0.0	0	0.4	0.0	0	0	0.0	0.0	1.4	0.2	0.0	0.0
14	0.2	0.2	0.0	4.6	0	0.4	0.0	0	1.0	0.2	0	0	0.2	0.0	1.8	0.2	0.0	0.0
15	0.2	0.0	0.0	2.2	2	1.4	0.0	0	0.2	0.0	0	0	0.2	0.0	1.0	0.0	0.0	0.0
16	0.0	0.0	0.0	2.2	0	3.4	0.0	0	0.2	0.0	0	0	0.0	0.0	1.8	0.2	0.0	0.0
17	0.2	0.0	0.0	1.2	0	0.6	0.0	0	0.0	0.0	0	0	0.0	0.0	1.6	0.0	0.0	0.0
18	0.2	0.0	0.0	2.2	0	0.0	0.0	0	0.2	0.0	0	0	0.2	0.2	1.0	0.0	0.0	0.0
19	1.2	0.0	0.0	3.2	0	1.8	0.0	0	0.2	0.0	0	0	0.2	0.0	0.8	0.0	0.0	0.0

C E S	I O	S R L P I E D	A G E N 2	S E C T I O N 2		A T F	B M F	K F	N S F	P O F	V K F	L Y P I T Y O N K F	P P O Y T F	P P O Y K F	P P O Y K F	S E C Y T F
20	3227	2 4	48	1 2	0.4	0.0	0	0.4	0	0.1	0	0.50000	0.5	0.0	0.00000	
21	3232	1 1	36	1 2	1.1	0.9	0	0.2	0	0.9	0	2.00000	1.7	0.0	0.12500	
22	3232	2 1	36	1 2	0.8	0.3	0	0.0	0	0.7	0	1.90000	0.7	0.0	0.33333	
23	3235	1 4	55	1 2	0.6	1.6	0	0.0	0	3.9	0	1.40000	0.1	0.0	0.33333	
24	3235	2 4	55	1 2	1.0	1.6	0	0.0	0	0.8	0	1.50000	0.5	0.0	1.00000	
25	3237	1 4	36	1 2	0.1	0.6	0	0.0	0	0.4	0	0.80000	0.2	0.0	0.40000	
26	3237	2 4	36	1 2	0.1	0.3	0	0.0	0	0.8	0	0.90000	0.1	0.0	0.30000	
27	3239	1 5	25	1 2	0.0	0.1	0	0.0	0	0.8	0	1.50000	0.2	0.0	0.10000	
28	3239	2 5	25	1 2	0.0	0.4	0	0.0	0	0.5	0	1.00000	0.6	0.0	0.10000	
29	3241	1 7	60	1 2	0.3	0.4	0	0.0	0	0.6	0	1.20000	0.6	0.0	0.60000	
30	3241	2 7	60	1 2	0.0	0.5	0	0.0	0	0.5	0	0.40000	0.0	0.0	0.60000	
31	3242	1 4	6	1 2	3.6	0.8	0	0.2	0	1.0	0	0.85714	0.0	0.0	0.00000	
32	3242	2 4	6	1 2	2.9	0.3	0	0.0	0	0.8	0	1.37500	0.5	0.0	0.40000	
33	3246	1 4	60	1 2	0.0	0.0	0	0.0	0	1.1	0	0.60000	0.0	0.0	0.00000	
34	3246	2 4	60	1 2	0.3	0.4	0	0.0	0	0.4	0	0.80000	0.1	0.1	0.00000	
35	3247	1 4	54	1 2	0.5	0.4	0	0.0	0	0.4	0	1.50000	0.2	0.0	0.11111	
36	3247	2 4	54	1 2	1.2	0.6	0	0.0	0	0.8	0	1.90000	0.9	0.0	0.00000	
37	3248	1 6	108	1 2	0.3	1.3	0	0.1	0	1.0	0	1.30000	0.5	0.0	0.20000	
38	3248	2 6	108	1 2	0.9	1.7	0	0.0	0	1.0	0	1.60000	0.3	0.1	0.42857	

I B S	S E P Y K F	S E K Y F	L D P M F	L D S G F	I C H Y F	I C L C F	J C L F F	I C D R P F	I C F I P F	V A T A F	V A S A F	S P C V F	S P P Y F	S P K Y F	S C P L E F	G E A N F	D E E M F	D E E H F
20	0.222222	0	0.4	0.0	0.0	1.2	0.6	1.1	0.0	0	0	0.100	0.2	0	0	0	0.0	0.0
21	0.125000	0	0.4	2.0	1.2	1.2	0.0	0.2	0.0	0	0	0.700	0.2	0	0	0	0.1	0.0
22	0.000000	0	0.2	1.0	0.4	2.5	0.0	0.3	0.0	0	0	0.400	0.0	0	0	0	0.0	0.0
23	0.111111	0	0.3	1.3	1.3	2.8	0.0	0.3	0.2	0	0	1.100	0.0	0	0	0	0.1	0.0
24	0.000000	0	0.4	0.8	0.5	2.9	0.0	0.0	0.0	0	0	1.000	0.1	0	0	0	0.1	0.0
25	0.000000	0	0.0	1.4	0.0	4.4	0.0	0.7	0.0	0	0	0.200	0.0	0	0	0	0.0	0.0
26	0.000000	0	0.7	1.0	0.0	4.3	0.0	0.5	0.0	0	0	0.300	0.1	0	0	0	0.1	0.0
27	0.000000	0	0.2	0.0	0.0	3.2	0.0	0.2	0.0	0	0	0.300	0.0	0	0	0	0.0	0.0
28	0.000000	0	0.1	0.0	0.0	3.2	0.0	0.1	0.0	0	0	0.200	0.1	0	0	0	0.0	0.0
29	0.400000	0	1.0	1.6	0.0	3.0	0.0	1.2	0.0	0	0	0.300	0.0	0	0	0	0.0	0.0
30	0.000000	0	0.9	0.7	0.0	3.1	0.0	1.0	0.0	0	0	0.300	0.0	0	0	0	0.0	0.0
31	0.000000	0	0.0	0.2	0.0	1.0	0.0	0.0	0.0	0	0	0.200	0.0	0	0	0	0.7	0.5
32	0.000000	0	0.2	0.2	0.0	1.7	0.0	0.0	0.0	0	0	0.700	0.1	0	0	0	0.7	0.7
33	0.142857	0	1.1	0.5	0.1	2.1	0.0	4.1	0.1	0	0	0.200	0.0	0	0	0	0.0	0.0
34	0.000000	0	0.5	1.0	0.0	3.0	0.0	1.4	0.3	0	0	0.300	0.0	0	0	0	0.0	0.0
35	0.111111	0	0.3	0.0	3.4	2.1	0.0	0.6	0.0	0	0	0.300	0.0	0	0	0	0.1	0.0
36	0.000000	0	0.7	0.2	3.7	1.7	0.0	0.5	0.1	0	0	0.375	0.0	0	0	0	0.2	0.0
37	0.100000	0	0.1	0.2	1.4	3.6	1.8	1.2	0.0	0	0	0.600	0.0	0	0	0	0.1	0.1
38	0.000000	0	0.0	0.2	0.3	3.6	1.3	2.1	0.0	0	0	0.500	0.0	0	0	0	0.0	0.0

	D E S P F	D E S G F	I S F F	S G C I I I F	A T S	P M T S	K S	L I P S	C Y T S	P Y N S	P R C T S	P P Y K S	P R K Y S	S E C Y T S	S E P Y K S	S E K S	
20	0.9	0.0	0.1	0.0	0.6	0.2	0.0	0.2	0.0	0.8	0.0	2.4	0.20	0.0	0.00000	0.00	0
21	2.6	0.4	0.3	0.0	2.0	0.4	0.0	0.6	0.0	0.0	0.0	1.5	0.50	0.0	0.25000	0.00	0
22	2.7	0.5	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.2	0.0	1.4	0.60	0.0	0.20000	0.00	0
23	1.2	0.1	0.1	0.0	0.8	1.4	0.0	0.0	0.0	0.2	0.0	0.6	0.00	0.0	0.66667	0.00	0
24	1.7	0.2	0.2	0.0	1.0	1.6	0.0	0.0	0.0	0.2	0.0	1.4	0.60	0.0	0.25000	0.00	0
25	1.7	0.7	0.1	0.0	0.4	0.6	0.0	0.0	0.0	0.4	0.0	0.8	0.80	0.0	0.00000	0.20	0
26	2.0	0.0	0.2	0.0	0.2	0.2	0.0	0.0	0.0	0.6	0.0	2.4	2.00	0.0	1.20000	0.40	0
27	2.1	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.4	0.0	1.2	0.40	0.0	0.50000	0.00	0
28	1.5	0.1	0.0	0.0	0.4	0.6	0.0	0.0	0.0	0.6	0.0	0.4	0.60	0.2	0.00000	0.25	0
29	2.7	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.2	0.00	0.0	0.00000	0.00	0
30	1.4	0.3	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.2	0.0	0.4	0.00	0.0	0.00000	0.00	0
31	0.0	0.0	0.0	0.0	3.0	0.6	0.0	0.0	0.0	1.4	0.0	0.4	0.00	0.0	0.00000	0.00	0
32	0.0	0.1	0.0	0.0	3.8	0.8	0.0	0.2	0.0	1.4	0.0	1.0	0.75	0.0	2.00000	0.00	0
32	3.8	0.7	0.0	0.0	0.6	0.0	0.0	0.0	0.0	0.8	0.0	1.2	0.20	0.2	0.60000	0.00	0
34	1.8	0.9	0.3	0.0	0.0	0.2	0.0	0.2	0.0	0.8	0.0	0.6	0.00	0.0	0.00000	0.00	0
35	2.3	0.4	1.0	0.0	0.4	0.6	0.0	0.0	0.0	0.4	0.0	0.8	0.00	0.0	0.20000	0.20	0
36	2.5	0.3	0.7	0.0	0.8	1.0	0.0	0.0	0.0	0.8	0.0	0.4	0.20	0.0	0.23333	0.00	0
37	3.2	0.4	0.2	0.0	1.2	2.0	0.0	0.0	0.0	1.0	0.0	1.5	1.25	0.0	0.00000	0.00	0
38	2.8	0.4	0.2	0.0	0.6	1.4	0.2	0.0	0.0	0.8	0.0	1.4	0.60	0.0	0.50000	0.00	0

	L	L	I	I	I	I	I	V	S	S	S	C	G	D	D	D	I	S	S
D	D	D	C	C	C	C	C	A	P	P	P	E	P	E	E	E	I	G	C
B	P	S	H	L	L	R	F	T	C	P	K	L	A	F	S	S	S	I	I
S	S	S	S	S	S	P	B	A	V	Y	Y	N	N	M	H	P	D	I	I
20	1.0	0.0	0.0	2.4	0.0	1.4	0.0	0	0.2	0.00	0	0	0	0.2	0.0	1.0	0.0	0.4	0.0
21	0.4	0.0	0.6	1.2	0.0	0.0	0.0	0	1.0	0.20	0	0	0	0.0	0.0	2.2	0.4	0.0	0.0
22	0.2	1.0	0.0	1.8	0.0	0.4	0.0	0	0.6	0.00	0	0	0	0.0	0.0	1.6	1.0	0.0	0.0
23	0.6	0.2	1.2	2.0	0.0	0.2	0.0	0	0.4	0.00	0	0	0	0.0	0.0	3.0	0.2	0.6	0.0
24	0.2	1.4	1.0	2.4	0.0	0.2	0.2	0	0.8	0.20	0	0	0	0.0	0.0	2.2	0.0	0.0	0.0
25	0.6	0.4	0.0	3.8	0.0	1.8	0.0	0	0.0	3.20	0	0	0	0.0	0.0	1.2	0.4	0.0	0.0
26	0.2	0.2	0.0	4.4	0.0	0.4	0.0	0	0.4	0.00	0	0	0	0.0	0.0	1.0	0.0	0.2	0.0
27	0.0	0.0	0.0	3.2	0.0	0.2	0.0	0	0.6	0.00	0	0	0	0.0	0.0	2.2	0.4	0.0	0.0
28	0.0	0.0	0.0	3.0	0.0	0.2	0.0	0	0.2	0.00	0	0	0	0.0	0.0	1.2	0.0	0.0	0.0
29	1.0	1.2	0.0	3.0	0.0	1.6	0.0	0	0.0	0.00	0	0	0	0.2	0.0	2.2	0.6	0.0	0.0
30	0.2	0.4	0.0	3.4	0.0	0.8	0.0	0	0.0	0.00	0	0	0	0.0	0.0	1.4	0.2	0.0	0.0
31	0.2	0.2	0.0	1.4	0.0	0.0	0.0	0	0.4	0.00	0	0	0	1.0	0.8	0.0	0.0	0.0	0.0
32	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0	0.8	0.00	0	0	0	0.2	0.2	0.0	0.0	0.0	0.0
33	1.2	0.8	0.2	2.6	0.0	3.0	0.2	0	0.4	0.00	0	0	0	0.2	0.0	3.6	0.2	0.0	0.0
34	0.4	0.8	0.0	2.8	0.0	2.0	0.6	0	0.4	0.00	0	0	0	0.0	0.0	2.4	0.2	0.0	0.0
35	0.0	0.0	2.8	1.6	0.0	0.4	0.0	0	0.0	0.00	0	0	0	0.4	0.2	1.4	0.0	0.4	0.0
36	0.6	0.0	3.2	1.2	0.0	0.6	0.0	0	0.4	0.00	0	0	0	0.4	0.2	2.0	0.0	0.4	0.0
37	0.2	0.0	1.6	4.2	1.6	0.4	0.0	0	0.0	0.25	0	0	0	0.0	0.0	1.4	0.2	0.0	0.0
38	0.2	0.0	0.4	2.8	1.2	2.4	0.0	0	0.2	0.00	0	0	0	0.4	0.4	2.0	0.0	0.2	0.0

O B S	I D	S L I D E D		A G E	S E C T I O N		A T F	B M T F	K F	N S F	L I P D F	C Y T O V F		P Y M K F	P F C Y T F
		1	2		1	2						0	1		
39	3250	1	1	12	1	2	0.50000	0.7	0.0	0.0	0.0	0.50000	0	1.000	
40	3250	2	1	12	1	2	0.60000	0.1	0.0	0.0	0.0	0.80000	0	0.900	
41	3251	1	1	60	1	2	0.10000	0.1	0.0	0.1	0.0	0.70000	0	1.000	
42	3251	2	1	60	1	2	0.30000	0.4	0.0	0.2	0.0	0.70000	0	1.000	
43	3252	1	4	72	1	2	0.10000	1.0	0.0	0.0	0.0	1.40000	0	1.600	
44	3252	2	4	72	1	2	0.00000	0.8	0.0	0.3	0.0	1.70000	0	1.800	
45	3253	1	4	84	1	2	0.20000	0.1	0.0	0.0	0.0	1.00000	0	0.000	
46	3253	2	4	84	1	2	0.00000	0.0	0.0	0.0	0.0	0.90000	0	0.300	
47	3260	1	4	24	1	2	0.30000	0.4	0.0	0.0	0.0	0.90000	0	0.400	

O B S	P R P Y K F		P R K Y F	S E C Y T F	S E P Y K F	S E K Y F	L D P M F	L D S G F	I C H Y F	I C L C F	I C L F	I C F	I C F	I C F	V A T A F
	0	1													
39	0.400	0.0	0.100000	0.100000	0	0.4	0.7	0.0	4.0	0.0	0.1	0.0	0		0
40	0.300	0.0	0.000000	0.000000	0	0.0	0.1	0.0	3.3	0.0	0.0	0.0	0		0
41	0.200	0.0	0.000000	0.222222	0	0.3	0.4	0.0	2.1	2.0	1.2	0.1	0		0
42	0.300	0.0	0.000000	0.111111	0	0.1	0.5	0.4	1.1	1.1	1.5	0.0	0		0
43	0.000	0.0	0.333333	0.000000	0	0.6	0.2	0.2	4.3	0.0	0.0	0.3	0		0
44	0.100	0.0	0.125000	0.000000	0	0.3	0.0	1.8	4.1	0.0	0.0	0.8	0		0
45	0.000	0.0	0.000000	0.000000	0	0.1	0.2	0.1	2.4	0.0	0.2	0.0	0		0
46	0.200	0.0	0.000000	0.000000	0	0.2	0.1	0.0	2.2	0.0	0.1	0.0	0		0
47	0.000	0.2	0.000000	0.000000	0	0.2	0.3	0.0	2.8	0.0	1.4	0.0	0		0

O B S	V A S F	S P C V F	S P Y F	S P Y F	S P Y F	S P Y F	S P Y F	S P Y F	S P Y F	S P Y F	S P Y F	S P Y F	S P Y F	S P Y F	S P Y F	S P Y F
39	0	0.10000	0.000000	0	0	0	0.4	0.1	2.4	0.8	0.0	0	0.0	0.0	0.0	0.0
40	0	0.60000	0.000000	0	0	0	1.1	0.4	2.3	0.2	0.1	0	0.2	0.2	0.0	0.0
41	0	0.50000	0.100000	0	0	0	0.9	0.3	2.1	0.2	0.0	0	0.2	0.6	0.0	0.0
42	0	0.30000	0.100000	0	0	0	0.6	0.2	1.3	0.1	0.2	0	0.6	0.8	0.0	0.0
43	0	1.30000	0.300000	0	0	0	0.1	0.1	0.3	0.0	0.4	0	0.0	0.4	0.0	0.0
44	0	1.20000	0.000000	0	0	0	0.1	0.0	3.0	0.4	1.5	0	0.4	0.4	0.0	0.0
45	0	0.20000	0.000000	0	0	0	0.0	0.0	1.5	0.2	0.0	0	0.2	0.0	0.0	0.0
46	0	0.10000	0.000000	0	0	0	0.0	0.0	1.1	0.0	0.1	0	0.0	0.0	0.0	0.0
47	0	0.33333	0.000000	0	0	0	0.0	0.0	1.9	0.2	0.2	0	0.6	1.2	0.0	0.0

	V	S	S	S	C	G	D	D	D	D	I	S	S			
	A	P	P	P	E	R	E	E	E	E	S	I	I		B	
D	S	C	P	K	L	A	E	E	S	S	F	I	I	A	M	
B	A	V	Y	Y	E	N	M	H	P	G	B	I	I	T	T	K
S	F	F	F	F	F	F	F	F	F	F	F	F	F	S	S	S
48	0	0.70000	0.000000	0	0	0	0.1	0.1	0.6	0.1	0.0	0	0	0.2	1.0	0.0
49	0	0.50000	0.000000	0	0	0	0.6	0.4	1.4	0.1	0.0	0	0	0.0	0.0	0.0
50	0	0.10000	0.100000	0	0	0	0.0	0.0	2.4	0.2	0.0	0	0	0.0	0.0	0.0
51	0	0.20000	0.100000	0	0	0	0.4	0.2	1.8	0.0	0.0	0	0	0.0	0.0	0.0
52	0	0.10000	0.000000	0	0	0	0.1	0.1	1.8	0.1	0.0	0	0	0.0	0.2	0.0
53	0	0.30000	0.000000	0	0	0	0.0	0.0	3.1	0.2	0.0	0	0	0.0	0.2	0.0
54	0	0.50000	0.100000	0	0	0	0.0	0.0	1.6	0.1	0.0	0	0	0.0	0.0	0.0
55	0	0.00000	0.000000	0	0	0	1.4	0.6	0.8	0.1	0.7	0	0	0.4	0.6	0.0
56	0	0.66667	0.000000	0	0	0	0.9	0.3	0.8	0.2	0.9	0	0	0.6	0.0	0.0

			C	P	P		S		S							
		L	Y	P	P		E		E		L	L	I	I		
	N	P	D	N	Y		C		P		D	D	C	C		
B	S	O	V	K	T		Y		K		P	S	H	L		
S	S	S	S	S	S		T		S		K	S	Y	C		
48	0.0	0	0.6	0	0.8	0.0	0.0	0.000000	0.000000	0	0.6	0.0	0.6	3.2		
49	0.0	0	0.3	0	0.0	0.0	0.0	0.000000	0.000000	0	0.0	0.0	0.0	3.4		
50	0.0	0	0.6	0	0.0	0.0	0.0	0.200000	0.000000	0	0.0	0.0	0.0	3.0		
51	0.0	0	0.6	0	0.6	0.6	0.0	0.200000	0.000000	0	0.0	0.0	0.0	2.0		
52	0.0	0	0.6	0	0.6	0.0	0.0	0.000000	0.000000	0	0.0	0.2	0.0	2.0		
53	0.0	0	0.2	0	0.4	0.2	0.0	0.000000	0.000000	0	0.6	0.0	0.0	3.4		
54	0.2	0	0.6	0	0.8	0.2	0.0	0.000000	0.333333	0	0.4	0.0	0.0	4.4		
55	0.0	0	0.6	0	0.4	0.6	0.0	0.000000	0.000000	0	0.2	0.0	0.0	1.8		
56	0.2	0	1.2	0	0.0	0.0	0.0	0.000000	0.000000	0	0.4	0.0	0.0	2.8		

	I	I	I	V	S	S	S	C	G	D	D	D	D	I	S	S
	C	D	F	A	P	P	P	E	R	E	E	E	E	S	I	G
D	L	P	I	T	C	P	K	L	A	E	E	S	S	F	I	I
B	F	P	P	A	V	Y	Y	E	N	M	H	P	G	B	I	I
S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
48	0.0	0.6	0.2	0	0.50	0.00	0.0	0.0	0	0.0	0.0	2.0	0.0	0.0	0	0
49	0.0	0.0	0.0	0	0.00	0.00	0.0	0.0	0	0.2	0.0	1.6	0.0	0.0	0	0
50	0.0	0.0	0.0	0	0.40	0.00	0.0	0.0	0	0.0	0.0	1.8	0.8	0.0	0	0
51	0.0	0.4	0.0	0	0.00	0.00	0.0	0.4	0	0.0	0.0	2.6	0.0	0.0	0	0
52	0.0	0.2	0.0	0	0.20	0.00	0.0	0.0	0	0.0	0.0	1.0	0.0	0.0	0	0
53	0.0	0.0	0.0	0	0.00	0.20	0.0	0.0	0	0.0	0.0	3.0	0.4	0.2	0	0
54	0.0	0.0	0.2	0	0.20	0.00	0.0	0.0	0	0.0	0.0	2.6	0.2	0.0	0	0
55	0.0	0.0	0.0	0	0.25	0.00	0.0	0.0	0	1.0	0.4	1.2	0.6	1.0	0	0
56	0.0	0.4	0.2	0	0.20	0.00	0.0	0.0	0	2.0	1.2	0.0	0.0	0.2	0	0

P B S	I D	S R L I E D E N D	S E C T I O N 2	A I O N T F	P M T F	K F	N S F	L I P C F	C Y T O V F	P Y N K F	P F C Y T F	P F P Y K F
57	3271	1 4	96 1	2 1.7	1.6	0.0	0.2	0.0	0.7	0.0	0.90000	0.10000
58	3271	2 4	96 1	2 2.2	1.1	0.0	0.1	0.0	1.4	0.0	1.12500	0.12500
59	3272	1 4	120 1	2 2.4	3.7	0.1	0.2	0.0	1.1	0.0	1.30000	0.50000
60	3272	2 4	120 1	2 3.1	3.8	0.0	0.1	0.0	1.2	0.0	2.50000	1.10000
61	3275	1 8	24 1	2 1.0	0.8	0.0	0.0	0.0	0.6	0.0	1.60000	0.40000
62	3275	2 8	24 1	2 0.6	1.5	0.0	0.0	0.0	0.2	0.0	0.30000	0.20000
63	3276	1 4	60 1	2 0.6	0.2	0.0	0.4	0.0	0.8	0.0	1.20000	1.00000
64	3276	2 4	60 1	2 0.7	0.7	0.0	0.3	0.0	1.0	0.0	0.90000	0.40000
65	3277	1 5	24 1	2 0.4	0.6	0.0	0.0	0.0	0.8	0.0	1.00000	0.30000

P B S	P F K Y F	S E C T Y F	S E P Y K F	S E K Y F	L D P M F	L D S G F	I C H Y F	I C L C F	I C L F	I C R P F	I C F F	V A T A F	V A S A F
57	0.0	0.000000	0.000000	0	0.1	3.1	1.4	4.6	2.0	0.0	0.1	0	0
58	0.0	0.000000	0.200000	0	0.0	0.5	0.8	4.4	0.8	0.0	0.1	0	0
59	0.0	0.250000	0.500000	0	0.1	1.5	4.4	2.6	2.2	0.0	0.3	0	0
60	0.1	0.000000	0.000000	0	0.0	1.2	4.8	3.4	1.8	0.3	0.5	0	0
61	0.0	0.428571	0.000000	0	0.0	0.6	0.1	3.1	0.0	0.0	0.1	0	0
62	0.0	0.000000	0.000000	0	0.2	0.6	0.0	2.2	0.0	0.4	0.0	0	0
63	0.0	0.000000	0.333333	0	0.4	0.1	0.1	1.9	0.9	0.0	0.8	0	0
64	0.0	0.000000	0.666667	0	0.8	0.1	0.0	2.7	0.7	0.0	0.5	0	0
65	0.0	0.111111	0.000000	0	0.1	0.7	0.0	3.0	0.0	0.3	0.0	0	0

P B S	S P C V F	S P P Y F	S P K Y F	C E L E N F	G E A M F	D E E H F	D E S P F	D E S G F	I S F B F	S G I I I F	S I I I F	B M T S
57	0.50000	0.100000	0.000000	0	0.0	0.0	2.7	0.4	0.2	0	1.0	1.6
58	0.14286	0.000000	0.000000	0	0.0	0.3	0.2	3.3	0.4	0.7	0	0.8
59	1.00000	0.100000	0.000000	0	0.0	0.0	0.0	0.0	0.9	0	2.2	4.2
60	0.90000	0.100000	0.000000	0	0.0	0.0	0.0	0.0	1.9	0	4.0	3.8
61	0.60000	0.100000	0.000000	0	0.0	0.0	1.8	0.3	0.0	0	0.4	0.2
62	0.00000	0.200000	0.000000	0	0.1	0.0	2.6	0.0	0.0	0	0.4	1.6
63	1.11111	0.444444	0.000000	0	0.0	0.0	3.0	0.1	0.7	0	1.0	0.8
64	0.10000	0.000000	0.000000	0	0.0	0.0	1.3	0.1	0.5	0	1.2	0.8
65	0.50000	0.300000	0.000000	0	0.0	0.0	1.7	0.1	0.0	0	0.2	0.6

P B S	K S	N S	L P O S	C Y T O V S	P V K S	P R C T S	P R P Y K S	P P K Y S	S F C Y T S	S E P Y K S	S E P Y K S	L D P M S	L D S G S	I C H Y S
57	0.0	0.0	0	0.8	0	2.2	0.4	0.0	0.333333	0.00000	0	0.0	0.8	0.6
58	0.0	0.0	0	0.0	0	0.2	0.0	0.2	0.000000	0.00000	0	0.0	0.0	1.2
59	0.0	0.0	0	1.0	0	1.6	0.4	0.2	0.333333	0.00000	0	0.0	0.8	3.6
60	0.0	0.2	0	0.4	0	2.6	0.4	0.0	0.000000	1.00000	0	0.0	0.8	4.8
61	0.0	0.0	0	0.2	0	1.6	0.6	0.0	0.200000	0.20000	0	0.4	0.0	0.0
62	0.0	0.2	0	0.6	0	0.4	0.2	0.0	0.000000	0.00000	0	0.0	0.8	0.0
63	0.6	0.8	0	1.4	0	1.0	0.6	0.0	0.333333	0.33333	0	0.2	0.0	0.2
64	0.6	0.6	0	1.4	0	1.4	0.6	0.0	0.250000	1.00000	0	0.4	0.2	0.0
65	0.0	0.0	0	0.8	0	0.6	0.2	0.0	0.000000	0.00000	0	0.0	0.0	0.0

	I	I	I	I	V	S	S	S	C	G	D	D	D	D	I	S	S
	C	C	C	C	A	P	P	P	E	R	E	E	E	E	S	I	G
	L	L	L	L	T	C	P	K	L	A	E	E	S	S	S	I	C
	S	S	S	S	V	S	Y	Y	E	H	M	H	P	G	B	I	I
	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
57	4.6	1.2	0.4	0.6	0	0.50	0.00	0.0	0.0	0	0.0	0.0	3.2	0.2	0.2	0	0
58	3.8	1.4	0.0	0.2	0	0.40	0.20	0.0	0.0	0	0.0	0.0	1.0	0.2	1.0	0	0
59	3.0	1.6	0.0	1.0	0	0.75	0.00	0.0	0.0	0	0.0	0.0	0.0	0.0	1.2	0	0
60	4.0	1.2	0.0	1.2	0	0.40	0.00	0.2	0.0	0	0.2	0.2	0.0	0.0	1.4	0	0
61	2.8	0.0	0.2	0.0	0	0.20	0.00	0.0	0.0	0	0.0	0.0	2.0	0.0	0.0	0	0
62	3.0	0.0	0.0	0.0	0	0.00	0.20	0.0	0.0	0	0.0	0.0	3.8	0.0	0.0	0	0
63	2.6	0.6	0.0	0.8	0	0.75	0.25	0.0	0.0	0	0.0	0.0	0.4	0.0	0.8	0	0
64	2.8	0.8	0.2	0.6	0	0.00	0.00	0.0	0.0	0	0.2	0.4	0.0	0.0	1.6	0	0
65	2.6	0.0	0.0	0.0	0	0.80	0.00	0.0	0.0	0	0.0	0.0	0.8	0.0	0.0	0	0

		S	R		S	E											
		L	E		C	T		A	M		N	P	C	P		P	
	I	D	E	A	I	O	A	T	T	K	S	O	V	N	K	O	
	S	D	E	E	N	2	F	F	F	F	F	F	F	F	F	F	F
66	3277	2	5	24	1	2	0.0	1.2	0.0	0.0	0.0	0.0	0.7	0.0	1.000000		
67	3278	1	6	24	1	2	1.7	2.0	0.0	0.0	0.0	0.0	0.6	0.0	1.444444		
68	3279	2	6	24	1	2	1.1	1.4	0.0	0.4	0.0	0.0	0.7	0.0	1.500000		
69	3279	1	4	108	1	2	3.9	4.3	0.0	1.3	0.0	2.2	0.0	1.000000			
70	3279	2	4	108	1	2	4.3	4.4	0.0	0.3	0.0	1.7	0.7	1.500000			
71	3281	1	4	84	1	2	0.2	0.7	0.0	0.0	0.0	0.5	0.0	0.800000			
72	3281	2	4	84	1	2	0.6	1.6	0.0	0.1	0.0	0.3	0.0	0.777778			
73	3282	1	4	18	1	2	0.7	2.3	0.0	0.0	0.0	0.4	0.0	0.700000			
74	3282	2	4	18	1	2	0.4	1.9	0.0	0.0	0.0	0.4	0.0	0.400000			

	P		S	S													
	F		E	E													
	P	F	C	P													
	Y	K	Y	Y	K	Y	K	Y	M	G	Y	C	F	F	F	F	F
	S	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
66	0.200000	0	0.200000	0.000000	0	0.0	0.2	0.0	1.8	0.0	0.1	0.0					
67	0.111111	0	0.142857	0.142857	0	0.4	0.9	0.5	2.8	1.2	0.0	0.3					
68	0.800000	0	0.444444	0.000000	0	0.1	0.1	3.9	2.9	1.2	0.0	0.3					
69	0.000000	0	0.500000	0.000000	0	0.0	0.0	4.1	4.1	3.2	0.0	2.0					
70	0.000000	0	.	.	.	0.2	0.3	4.9	4.4	1.5	0.4	2.5					
71	0.400000	0	0.100000	0.000000	0	0.1	0.0	0.2	1.5	0.0	0.2	0.0					
72	0.333333	0	0.111111	0.000000	0	0.0	0.0	0.5	2.8	0.0	0.1	0.0					
73	0.400000	0	0.000000	0.000000	0	0.1	0.0	0.0	3.4	0.0	0.0	0.0					
74	0.100000	0	0.000000	0.000000	0	0.2	0.0	0.0	2.4	0.0	0.0	0.0					

	V	V	S	S	S	C	G	D	D	D	D	I	S	S	S	S
	A	A	P	P	P	E	R	E	E	E	E	S	F	I	I	I
	T	S	C	P	K	L	A	E	E	E	E	S	S	I	I	I
	A	A	V	Y	Y	E	N	M	H	P	F	G	F	I	I	I
	S	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
66	0	0	0.700000	0.000000	0.000000	0	0	0.0	0.0	1.6	0.0	0.2	0	0	0	0
67	0	0	0.500000	0.000000	0.000000	0	0	0.0	0.0	1.7	0.0	0.1	0	0	0	0
68	0	0	0.500000	0.100000	0.000000	0	0	0.0	0.0	0.8	0.0	0.1	0	0	0	0
69	0	0	0.000000	0.000000	0.000000	0	0	1.0	1.1	0.7	0.2	3.1	0	0	0	0
70	0	0	0.166667	0.166667	0.000000	0	0	0.4	0.3	1.1	0.2	3.5	0	0	0	0
71	0	0	0.400000	0.200000	0.000000	0	0	0.0	0.3	1.5	0.2	0.0	0	0	0	0
72	0	0	0.666667	0.222222	0.000000	0	0	0.0	0.0	2.4	0.4	0.2	0	0	0	0
73	0	0	0.500000	0.000000	0.000000	0	0	0.2	0.0	2.3	0.1	0.1	0	0	0	0
74	0	0	0.700000	0.000000	0.000000	0	0	0.2	0.3	1.4	0.1	0.0	0	0	0	0

	S	L	L	I	I	I	I	J	V	S	S	S	G	D
	F	D	D	C	C	C	C	C	A	P	P	P	C	C
	K	M	S	H	L	L	P	I	T	C	P	K	E	E
	Y	F	G	Y	C	F	P	B	A	V	Y	Y	L	E
	F	F	F	F	F	F	F	F	F	F	F	F	F	F
75	0	0.0	0.8	2.3	5.0	1.1	0.2	0.6	0	0	0.333333	0.111111	0.111111	0
76	0	0.3	0.9	2.8	4.1	1.6	0.0	0.4	0	0	0.600000	0.000000	0.000000	0
77	0	0.2	0.9	0.0	4.1	0.0	0.0	0.0	0	0	0.200000	0.200000	0.000000	0
78	0	0.1	0.5	0.0	4.2	0.0	0.0	0.0	0	0	0.100000	0.100000	0.000000	0
79	0	0.2	0.4	0.2	3.9	0.0	0.1	0.0	0	0	0.000000	0.100000	0.000000	0
80	0	0.3	1.0	0.2	3.8	0.0	0.4	0.4	0	0	0.333333	0.000000	0.000000	0
81	0	0.2	1.3	2.5	2.4	1.5	0.0	0.6	0	0	0.500000	0.200000	0.000000	0
82	0	0.4	2.8	1.9	3.3	2.2	0.1	0.5	0	0	0.400000	0.100000	0.000000	0
83	0	0.7	0.6	0.0	4.0	0.2	0.2	0.2	0	0	0.100000	0.000000	0.000000	0
84	0	0.1	0.4	0.0	4.2	0.3	0.2	0.0	0	0	0.200000	0.000000	0.000000	0

	D	D	D	I	S	S					C	P	P	S	S	L
	E	E	E	S	I	I	A	B			I	T	P	E	E	D
	H	P	G	P	I	I	T	T	K	S	C	V	K	Y	K	P
	F	F	F	F	F	F	S	S	S	S	S	S	S	S	S	S
75	0.0	1.9	0.0	1.0	0	0	1.0	1.2	0.0	0.0	0	0.4	0	1.6	1.2	0
76	0.0	2.8	0.8	0.3	0	0	1.6	1.4	0.0	0.0	0	0.4	0	0.8	1.2	0
77	0.0	1.3	0.4	0.0	0	0	0.0	0.0	0.0	0.0	0	1.4	0	0.2	0.0	0
78	0.0	1.3	0.1	0.0	0	0	0.0	0.0	0.0	0.0	0	0.8	0	0.6	0.4	0
79	0.0	2.4	0.1	0.0	0	0	0.2	0.2	0.0	0.0	0	0.0	0	0.8	0.6	0
80	0.3	0.8	0.1	0.0	0	0	1.0	1.6	0.2	0.4	0	0.8	0	1.0	0.4	0
81	0.2	1.2	0.2	1.1	0	0	0.2	1.8	0.0	0.0	0	0.2	0	0.2	0.4	0
82	0.7	0.6	0.3	1.2	0	0	0.4	1.0	0.0	0.0	0	0.2	0	0.4	0.0	0
83	0.0	0.8	0.4	0.2	0	0	2.2	2.0	0.0	0.4	0	0.2	0	0.6	0.0	0
84	0.0	0.6	0.3	0.0	0	0	1.4	1.2	0.0	0.0	0	0.4	0	0.2	0.0	0

	L	I	I	I	I	I	V	S	S	S	C	G	D	D	D	D	S	S
	D	C	C	C	C	C	F	A	P	P	P	E	R	E	E	E	S	I
	S	H	L	L	R	I	T	C	P	K	L	A	E	E	S	S	F	I
	G	Y	C	F	P	B	A	V	Y	Y	E	N	M	H	P	G	B	I
	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
75	0.2	2.0	4.6	1.6	0.0	0.4	0	0.0	0.0	0	0	0.0	0.0	1.4	0.2	0.4	0	0
76	1.4	1.8	4.6	1.0	0.2	0.6	0	0.0	0.0	0	0	0.0	0.0	1.0	0.0	0.0	0	0
77	0.4	0.0	4.0	0.0	0.0	0.0	0	0.2	0.0	0	0	0.0	0.0	1.4	0.6	0.2	0	0
78	0.0	0.0	4.0	0.0	0.0	0.0	0	0.6	0.0	0	0	0.0	0.0	1.2	0.4	0.0	0	0
79	0.0	0.4	3.4	0.0	0.4	0.0	0	0.0	0.0	0	0	0.0	0.0	1.6	0.2	0.0	0	0
80	0.8	0.8	3.2	0.0	0.0	0.8	0	0.4	0.0	0	0	0.6	0.2	2.4	0.2	0.0	0	0
81	2.4	1.2	2.4	2.0	0.0	0.6	0	0.2	0.0	0	0	0.6	2.2	2.4	0.2	0.8	0	0
82	0.6	1.8	3.0	1.8	0.0	0.6	0	0.2	0.2	0	0	0.8	1.6	2.4	0.0	0.4	0	0
83	0.8	0.0	4.6	0.2	0.2	0.0	0	0.4	0.0	0	0	0.0	0.0	1.0	0.0	0.0	0	0
84	0.8	0.2	4.6	0.0	0.0	0.0	0	0.2	0.0	0	0	0.0	0.0	0.8	0.0	0.0	0	0

B. Quantitative Data

OBS	ID	SLIDE	BREED	AGE	SECTION	TUNALB	TUBOIAM	SERTOLI	SPERMATA	SPERMATB
1	3210	1	5	48	1	0.41	0.15	0.3	1.0	0.5
2	3210	1	5	48	2	0.25	0.14	0.4	0.8	0.6
3	3210	2	5	48	1	0.44	0.14	0.2	1.1	0.4
4	3210	2	5	48	2	0.40	0.14	0.2	0.8	0.6
5	3212	1	1	8	1	0.37	0.20	0.4	1.3	0.2
6	3212	1	1	8	2	0.19	0.19	0.4	1.0	0.7
7	3212	2	1	8	1	0.23	0.17	0.4	0.9	0.1
8	3212	2	0	8	1	0.18	0.15	0.6	1.2	0.8
9	3214	1	3	9	1	0.22	0.17	0.4	1.1	0.7
10	3214	1	3	9	2	0.16	0.16	0.0	0.6	0.8
11	3214	2	3	9	1	0.17	0.16	0.6	0.5	0.4
12	3214	2	3	9	2	0.30	0.16	0.6	1.4	0.6
13	3215	1	4	10	1	0.21	0.17	0.4	1.1	0.3
14	3215	1	4	10	2	0.29	0.16	0.0	1.8	1.4
15	3215	2	4	10	1	0.27	0.18	0.1	1.3	0.5
16	3215	2	4	10	2	0.36	0.17	0.4	1.2	0.4
17	3217	1	4	6	1	0.28	0.18	0.5	0.4	0.2
18	3217	1	4	6	2	0.38	0.15	0.6	0.6	0.0
19	3217	2	4	6	1	0.25	0.16	0.6	0.8	0.3
20	3217	2	4	6	2	0.20	0.19	0.4	1.0	0.6
21	3219	1	4	11	1	0.30	0.17	1.4	1.4	0.4
22	3219	1	4	11	2	0.16	0.18	0.3	1.4	1.3
23	3219	2	4	11	1	0.21	0.18	0.4	0.8	0.2
24	3219	2	4	11	2	0.23	0.18	0.8	0.4	0.3
25	3220	1	5	11	1	0.34	0.21	0.7	0.5	0.2
26	3220	1	5	11	2	0.36	0.21	0.6	0.8	0.6

OBS	PRIMSP	SECONDSP	TIDS	TOZA	GIANTS	MONO	INTERST	EPITHICK	DUCTEPI
1	2.3	0.7	2.8	0.0	0.0	0.2	17.6	0.46	0.12
2	1.2	0.8	1.2	0.0	0.0	0.2	8.8	0.21	0.13
3	2.2	0.4	2.2	0.0	0.0	0.2	10.0	0.28	0.14
4	1.6	0.2	0.8	0.0	0.0	0.0	14.4	0.24	0.13
5	2.2	2.0	6.9	1.2	0.0	0.0	10.6	0.15	0.17
6	2.4	2.0	3.6	2.0	0.0	0.0	9.6	0.29	0.17
7	1.3	2.1	4.2	0.5	0.0	0.0	11.9	0.14	0.14
8	2.0	1.6	4.4	2.2	0.0	0.0	9.8	0.08	0.17
9	2.7	0.2	4.2	0.6	0.0	0.3	6.4	0.17	0.14
10	2.2	0.8	6.6	0.0	0.0	0.0	10.0	0.14	0.16
11	2.4	0.0	1.0	0.0	0.0	0.0	7.1	0.23	0.17
12	3.2	0.0	0.0	0.0	0.0	0.0	7.0	0.18	0.14
13	2.3	1.3	4.8	3.1	0.0	0.0	4.9	0.29	0.19
14	1.4	1.2	7.0	1.0	0.0	0.0	6.4	0.18	0.16
15	2.5	1.9	6.6	3.2	0.0	0.0	5.0	0.18	0.15
16	1.8	2.2	3.8	4.0	0.0	0.0	4.0	0.19	0.15
17	2.6	1.1	1.7	0.0	0.1	0.0	8.8	0.28	0.15
18	2.2	0.2	1.6	0.0	0.0	0.0	4.8	0.18	0.16
19	1.7	0.4	1.9	2.6	0.0	0.0	5.3	0.14	0.16
20	2.4	0.4	3.0	2.0	0.0	0.0	2.1	0.15	0.16
21	3.4	4.6	5.0	1.8	0.0	0.0	4.9	0.14	0.14
22	3.4	1.6	9.0	0.4	0.0	0.0	7.4	0.15	0.15
23	2.1	1.1	5.6	6.7	0.1	0.2	4.2	0.18	0.16
24	3.0	2.0	5.6	4.4	0.0	0.0	2.6	0.12	0.12
25	2.6	2.5	6.0	2.4	0.1	0.0	4.8	0.16	0.14
26	2.2	1.0	6.2	4.0	0.0	0.0	6.8	0.16	0.14

OBS ID SLIDE PREED AGE SECTION TJNALR TURDIAM SEPTOLI SPERMAYA SPERMATR

27	3220	2	5	11	1	0.32	0.22	0.5	1.0	0.6
28	3220	2	5	11	2	0.36	0.24	0.2	1.0	0.6
29	3224	1	4	48	1	0.35	0.17	0.9	0.1	0.5
30	3224	1	4	48	2	0.37	0.17	0.8	0.8	0.6
31	3224	2	4	48	1	0.26	0.17	0.9	0.3	0.3
32	3224	2	4	48	2	0.26	0.20	0.6	1.2	0.6
33	3226	1	4	18	1	0.29	0.19	0.7	0.7	0.1
34	3226	1	4	18	2	0.45	0.21	1.0	0.4	0.4
35	3226	2	4	18	1	0.53	0.19	1.2	0.5	0.3
36	3226	2	4	18	2	0.32	0.20	1.2	0.6	0.0
37	3227	1	4	48	1	0.25	0.19	0.6	0.9	0.1
38	3227	1	4	48	2	0.26	0.18	1.4	0.4	0.2
39	3227	2	4	48	1	0.26	0.17	0.9	0.8	0.0
40	3227	2	4	48	2	0.30	0.19	1.2	0.6	0.2
41	3232	1	1	36	1	0.42	0.21	1.1	0.5	0.2
42	3232	1	1	36	2	0.36	0.21	0.8	0.6	0.4
43	3232	2	1	36	1	0.30	0.21	1.1	0.5	0.2
44	3232	2	1	36	2	0.32	0.21	0.8	0.8	0.0
45	3235	1	4	55	1	0.38	0.22	0.7	0.4	0.5
46	3235	1	4	55	2	0.19	0.20	0.4	0.8	0.0
47	3235	2	4	55	1	0.33	0.21	0.7	0.5	0.5
48	3235	2	4	55	2	0.36	0.21	0.8	0.4	0.0
49	3237	1	4	36	1	0.32	0.20	1.3	0.2	0.5
50	3237	1	4	36	2	0.31	0.17	0.6	0.8	0.2
51	3237	2	4	36	1	0.38	0.20	1.0	0.3	0.3
52	3237	2	4	36	2	0.36	0.18	1.0	0.8	0.2

OBS PFIMSP SECONDSP TIDS TOZA GIANTS MONO INTERST EPITHICK DUCTEP!

27	2.9	1.3	5.80	2.7	0.0	0.0	5.8	0.18	0.14
28	2.0	2.6	4.20	5.2	0.0	0.4	5.0	0.17	0.14
29	3.0	0.1	6.80	0.9	0.0	0.0	5.1	0.20	0.17
30	3.6	0.0	6.00	0.4	0.0	0.0	7.2	0.10	0.15
31	3.3	0.1	7.50	1.9	0.0	0.0	10.7	0.23	0.19
32	2.4	0.0	9.20	1.2	0.0	0.0	5.6	0.14	0.15
33	3.4	0.5	9.40	13.4	0.0	0.0	3.1	0.21	0.17
34	3.6	0.8	11.80	1.8	0.0	0.0	7.6	0.16	0.20
35	2.0	0.1	7.20	5.3	0.0	0.1	5.7	0.46	0.17
36	3.2	0.2	4.60	0.0	0.0	0.0	5.0	0.11	0.16
37	1.5	0.0	7.00	1.7	0.0	0.0	8.1	0.19	0.16
38	2.0	0.2	8.80	1.2	0.0	0.0	7.0	0.13	0.18
39	2.6	0.2	8.00	5.7	0.0	0.0	6.9	0.15	0.18
40	1.0	0.0	7.00	5.0	0.0	0.0	7.8	0.18	0.16
41	2.5	0.3	6.20	2.1	0.0	0.0	4.8	0.16	0.13
42	3.4	0.6	7.40	3.4	0.0	0.0	7.8	0.17	0.15
43	1.4	0.3	5.80	10.7	0.0	0.0	8.3	0.14	0.16
44	2.8	0.4	7.40	5.0	0.0	0.0	6.4	0.11	0.16
45	2.8	0.9	8.50	5.3	0.0	0.0	11.7	0.24	0.19
46	2.8	0.4	12.40	3.8	0.0	0.0	8.8	0.19	0.17
47	3.1	0.8	8.50	4.2	0.0	0.0	9.8	0.28	0.16
48	2.8	1.0	9.80	3.4	0.0	0.0	14.2	0.36	0.15
49	3.1	0.3	7.50	5.8	0.0	0.0	7.1	0.19	0.15
50	1.6	0.6	8.00	3.2	0.2	0.0	6.2	0.18	0.17
51	2.6	0.1	7.50	5.4	0.0	0.0	6.8	0.34	0.17
52	3.0	0.0	9.20	0.6	0.0	0.0	5.0	0.18	0.18

OBS ID SLIDE BREED AGE SECTION TUNALB TUBDIAM SEPTOLI SPERMATA SPERMATE

53	3239	1	5	25	1	0.25	0.19	0.6	0.7	0.3
54	3239	1	5	25	2	0.18	0.17	0.6	0.6	1.0
55	3239	2	5	25	1	0.15	0.20	0.8	0.6	0.1
56	3239	2	5	25	2	0.18	0.18	0.8	0.6	0.0
57	3241	1	7	60	1	0.35	0.19	1.0	0.7	0.2
58	3241	1	7	60	2	0.17	0.20	0.6	0.8	0.0
59	3241	2	7	60	1	0.50	0.18	0.6	0.7	0.2
60	3241	2	7	60	2	0.19	0.19	1.0	0.4	0.6
61	3242	1	4	6	1	0.35	0.16	0.7	1.1	0.7
62	3242	1	4	6	2	0.24	0.14	0.6	0.6	1.2
63	3242	2	4	6	1	0.34	0.15	0.5	0.4	0.8
64	3242	2	4	6	2	0.39	0.15	0.4	0.4	1.0
65	3246	1	4	60	1	0.16	0.20	0.4	0.7	0.4
66	3246	1	4	60	2	0.19	0.20	0.4	0.8	0.6
67	3246	2	4	60	1	0.26	0.21	0.6	0.4	0.2
68	3246	2	4	60	2	0.19	0.20	0.6	0.6	0.2
69	3247	1	4	54	1	0.47	0.20	1.0	0.3	0.3
70	3247	1	4	54	2	0.29	0.18	0.8	0.2	0.2
71	3247	2	4	54	1	0.50	0.17	0.8	0.4	0.2
72	3247	2	4	54	2	0.51	0.18	0.8	0.4	0.2
73	3248	1	6	108	1	0.35	0.21	1.1	0.5	0.3
74	3248	1	6	108	2	0.10	0.17	0.7	0.8	0.2
75	3248	2	6	108	1	0.16	0.19	1.4	0.7	0.0
76	3248	2	6	108	2	0.13	0.19	1.2	0.0	0.0
77	3250	1	1	12	1	0.28	0.24	1.3	0.3	0.4
78	3250	1	1	12	2	0.15	0.22	1.0	0.4	0.4

OBS PRIMSP SECONDSP TIDS TOZA GIANTS MOND INTERST EPITHICK DUCTEP1

53	1.7	0.5	10.60	4.2	0.0	0.0	7.1	0.14	0.15
54	1.2	0.2	10.00	2.0	0.0	0.0	3.6	0.10	0.15
55	2.3	0.4	13.60	4.7	0.0	0.1	4.2	0.19	0.16
56	2.1	1.0	9.80	2.1	0.0	0.0	6.2	0.19	0.16
57	3.0	0.0	7.40	6.3	0.0	0.0	8.5	0.22	0.16
58	1.8	0.2	7.00	8.8	0.0	0.0	8.6	0.10	0.15
59	2.5	0.3	4.90	2.1	0.0	0.0	6.6	0.27	0.16
60	2.8	0.6	8.40	2.4	0.0	0.0	7.2	0.33	0.16
61	2.0	0.5	0.80	0.0	0.0	0.0	3.9	0.21	0.14
62	1.2	0.5	0.60	0.0	0.0	0.4	5.8	0.14	0.14
63	2.3	0.3	0.80	0.0	0.0	0.1	3.6	0.18	0.14
64	2.2	0.6	0.40	0.0	0.2	0.0	2.8	0.16	0.13
65	2.4	0.9	8.50	9.9	0.0	0.0	4.2	0.15	0.18
66	2.6	0.2	0.74	10.2	0.0	0.4	5.0	0.11	0.18
67	3.4	1.1	8.00	6.6	0.0	0.0	6.6	0.23	0.18
68	2.8	1.0	9.00	12.4	0.0	0.0	6.6	0.15	0.18
69	2.5	0.2	8.90	0.3	0.3	0.0	6.5	0.36	0.19
70	5.2	0.2	9.40	3.4	0.2	0.0	10.8	0.28	0.17
71	4.1	0.1	9.20	0.6	0.0	0.1	9.0	0.23	0.18
72	3.0	0.6	7.00	2.6	0.0	0.2	6.2	0.26	0.15
73	2.4	0.2	7.90	5.6	0.0	0.0	6.1	0.13	0.20
74	2.6	0.2	6.40	1.6	0.0	0.0	8.2	0.15	0.14
75	2.3	0.4	6.20	3.5	0.1	0.0	7.5	0.17	0.18
76	3.0	0.0	8.20	0.8	0.0	0.0	3.8	0.17	0.12
77	2.9	0.2	7.20	2.4	0.0	0.2	5.7	0.14	0.17
78	4.0	0.2	10.60	4.8	0.0	0.0	7.0	0.10	0.18

OBS ID SLIDE BREED AGE SECTION TUNALB TUBDIAM SERTOLI SPERMATA SPERMATR

79	3250	2	1	12	1	0.22	0.21	1.4	0.0	0.5
80	3250	2	1	12	2	0.15	0.21	1.6	0.0	0.2
81	3251	1	1	60	1	0.42	0.21	0.9	0.2	0.2
82	3251	1	1	60	2	0.36	0.20	0.4	0.6	0.4
83	3251	2	1	60	1	0.40	0.19	0.5	0.9	0.3
84	3251	2	1	60	2	0.33	0.18	0.5	0.4	0.0
85	3252	1	4	72	1	0.45	0.20	0.5	1.1	0.7
86	3252	1	4	72	2	0.29	0.20	1.0	0.4	0.2
87	3252	2	4	72	1	0.39	0.20	0.8	0.7	0.1
88	3252	2	4	72	2	0.30	0.17	1.0	0.6	0.0
89	3253	1	4	84	1	0.41	0.18	1.1	0.4	0.5
90	3253	1	4	84	2	0.35	0.19	1.3	0.4	0.4
91	3253	2	4	84	1	0.26	0.20	1.5	0.4	0.2
92	3253	2	4	84	2	0.26	0.18	1.2	0.0	0.2
93	3260	1	4	24	1	0.42	0.20	0.4	0.5	0.5
94	3260	1	4	24	2	0.33	0.19	0.4	0.6	0.4
95	3260	2	4	24	1	0.31	0.20	0.4	0.5	0.7
96	3260	2	4	24	2	0.42	0.20	0.2	1.0	0.6
97	3263	1	4	10	1	0.31	0.16	1.0	0.6	0.0
98	3263	1	4	10	2	0.26	0.17	1.4	0.2	0.2
99	3263	2	4	10	1	0.36	0.16	1.0	0.9	0.2
100	3263	2	4	10	2	0.28	0.17	1.2	0.4	0.2
101	3265	1	5	33	1	0.32	0.21	1.0	0.7	0.4
102	3265	1	5	33	2	0.31	0.20	1.0	0.8	0.0
103	3265	2	5	33	1	0.32	0.22	0.9	0.4	0.3
104	3265	2	5	33	2	0.30	0.23	0.6	0.4	0.6

OBS PRIMSP SECONDSP TIDS TOZA GIANTS MONO INTERST EPITHICK DUCTEPI

79	1.7	1.5	10.1	2.2	0.2	0.0	6.0	0.24	0.17
80	1.6	0.0	11.2	0.6	0.2	0.0	4.6	0.16	0.17
81	2.7	0.6	9.6	4.1	0.0	0.0	6.3	0.20	0.20
82	2.4	1.0	13.0	3.0	0.0	0.0	4.0	0.17	0.16
83	2.8	0.3	10.0	3.2	0.0	0.0	7.5	0.20	0.18
84	2.8	1.2	8.4	1.8	0.0	0.4	6.6	0.17	0.15
85	2.0	0.4	8.9	1.1	0.0	0.0	6.9	0.21	0.16
86	3.2	0.2	11.2	1.4	0.0	0.0	10.8	0.12	0.15
87	2.7	0.4	11.1	1.7	0.0	0.0	10.8	0.21	0.18
88	2.8	0.2	11.2	0.4	0.0	0.0	9.6	0.13	0.15
89	1.6	0.1	5.0	1.0	0.0	0.0	6.4	0.28	0.17
90	1.8	0.4	5.2	1.0	0.0	0.0	6.6	0.30	0.17
91	2.2	0.2	5.7	2.0	0.0	0.0	8.8	0.17	0.17
92	0.8	0.4	6.6	0.4	0.0	0.0	11.0	0.23	0.19
93	1.9	1.6	5.2	1.3	0.0	0.0	7.2	0.26	0.14
94	3.0	1.4	6.4	1.0	0.0	0.0	5.0	0.22	0.14
95	1.9	1.1	6.2	0.1	0.0	0.0	5.7	0.19	0.15
96	0.4	0.4	5.2	0.0	0.0	0.0	6.4	0.29	0.17
97	2.1	0.3	7.9	2.4	0.1	0.0	4.4	0.15	0.16
98	1.6	0.8	5.8	0.8	0.0	0.0	4.6	0.16	0.15
99	2.2	0.2	4.9	4.8	0.0	0.1	6.7	0.21	0.18
100	1.8	0.4	6.2	1.2	0.0	0.0	4.0	0.24	0.18
101	1.9	0.1	7.2	2.7	0.0	0.0	6.9	0.16	0.17
102	2.6	0.0	14.6	16.0	0.0	0.0	4.0	0.19	0.19
103	2.2	0.2	5.7	3.2	0.0	0.0	8.0	0.15	0.18
104	0.8	0.4	8.4	6.2	0.0	0.0	4.4	0.24	0.18

OBS ID SLIDE BREED AGE SECTION TUNALB TUBDIAM SERTOLI SPERMATA SPERMATB

105	3266	1	4	24	1	0.19	0.20	0.7	0.8	0.4
106	3266	1	4	24	2	0.13	0.20	0.6	0.6	0.2
107	3266	2	4	24	1	0.18	0.18	1.0	0.5	0.3
108	3266	2	4	24	2	0.16	0.18	0.2	0.8	0.2
109	3268	1	4	8	1	0.23	0.18	0.6	0.6	0.7
110	3268	1	4	8	2	0.20	0.21	0.4	0.8	0.4
111	3268	2	4	8	1	0.26	0.18	0.3	0.9	0.5
112	3268	2	4	8	2	0.25	0.21	0.3	1.2	0.8
113	3271	1	4	96	1	0.76	0.17	0.8	0.4	0.0
114	3271	1	4	96	2	0.55	0.16	1.0	0.6	0.2
115	3271	2	4	96	1	0.35	0.18	0.6	0.9	0.3
116	3271	2	4	96	2	0.31	0.18	0.2	1.0	0.2
117	3272	1	4	120	1	0.45	0.17	1.4	0.3	0.0
118	3272	1	4	120	2	0.28	0.16	1.2	0.6	0.4
119	3272	2	4	120	1	0.40	0.19	1.1	0.2	0.1
120	3272	2	4	120	2	0.25	0.20	0.8	0.4	0.0
121	3275	1	8	24	1	0.38	0.16	0.5	0.5	0.5
122	3275	1	8	24	2	0.34	0.19	0.6	0.4	0.8
123	3275	2	8	24	1	0.26	0.18	0.2	0.7	0.5
124	3275	2	8	24	2	0.17	0.18	0.6	0.6	1.2
125	3276	1	4	60	1	0.52	0.20	0.7	0.3	0.4
126	3276	1	4	60	2	0.34	0.18	0.4	0.4	0.6
127	3276	2	4	60	1	0.58	0.17	0.9	0.6	0.3
128	3276	2	4	60	2	0.41	0.14	0.6	0.8	0.4
129	3277	1	5	24	1	0.22	0.20	0.5	0.9	0.2
130	3277	1	5	24	2	0.29	0.18	0.6	0.8	0.6

OBS PP1MSP SECONDSP TIDS TOZA GIANTS MONO INTERST EPITHICK DUCTEPI

105	2.6	0.4	10.8	7.7	0.0	0.0	7.5	0.09	0.15
106	2.2	0.6	13.6	5.6	0.0	0.0	7.8	0.08	0.16
107	3.4	0.2	5.2	3.4	0.0	0.0	7.0	0.13	0.14
108	2.6	0.6	8.2	2.4	0.0	0.0	5.6	0.19	0.14
109	2.6	1.3	5.0	1.2	0.0	0.0	3.3	0.23	0.17
110	3.0	1.6	5.2	0.4	0.0	0.0	2.1	0.25	0.19
111	4.0	0.6	3.9	1.8	0.0	0.2	5.4	0.14	0.17
112	2.6	2.4	2.0	7.0	0.0	0.2	5.0	0.17	0.17
113	2.5	0.2	6.1	3.6	0.0	0.0	7.6	0.75	0.17
114	2.0	0.2	8.2	0.6	0.0	0.0	6.4	0.34	0.18
115	2.1	0.4	9.8	8.6	0.0	0.0	8.6	0.16	0.17
116	3.2	0.2	7.2	0.6	0.0	0.0	5.8	0.16	0.17
117	2.3	0.1	0.7	0.0	0.0	0.3	10.9	0.17	0.17
118	3.8	0.6	0.6	0.0	0.0	0.0	28.2	0.20	0.16
119	3.4	0.1	0.5	0.0	0.0	0.0	16.4	0.25	0.16
120	3.0	0.0	0.4	0.0	0.0	0.0	15.0	0.24	0.16
121	1.9	0.5	6.4	9.1	0.0	0.0	3.7	0.20	0.18
122	4.2	1.0	6.2	2.0	0.0	0.0	5.4	0.13	0.16
123	3.0	0.9	5.4	5.8	0.0	0.0	3.9	0.16	0.16
124	2.2	2.2	8.0	11.4	0.0	0.0	4.0	0.13	0.17
125	2.8	1.2	5.5	1.0	0.0	0.0	7.4	0.21	0.15
126	2.4	0.6	5.6	0.0	0.0	0.0	7.8	0.19	0.16
127	4.2	0.6	5.9	2.8	0.0	0.1	5.9	0.29	0.16
128	3.4	0.8	4.6	0.6	0.0	0.0	5.6	0.28	0.15
129	2.5	0.9	5.8	2.2	0.1	0.0	6.9	0.16	0.14
130	2.0	1.2	6.0	4.2	0.0	0.0	6.2	0.10	0.14

OBS ID SLIDE BREED AGE SECTION TUNALB TUBDIAM SEPTOLI SPERMATA SPERMATB

131	3277	2	5	24	1	0.23	0.22	0.2	0.9	0.6
132	3277	2	5	24	2	0.25	0.20	0.4	0.6	0.6
133	3278	1	6	24	1	0.25	0.20	0.6	0.8	0.2
134	3278	1	6	24	2	0.20	0.21	0.6	0.6	0.4
135	3278	2	6	24	1	0.27	0.20	0.7	0.4	0.6
136	3278	2	6	24	2	0.39	0.20	0.4	0.6	0.2
137	3279	1	4	108	1	0.54	0.15	0.9	0.3	0.0
138	3279	1	4	108	2	0.57	0.14	0.8	0.4	0.0
139	3279	2	4	108	1	0.79	0.13	1.0	0.2	0.2
140	3279	2	4	108	2	0.45	0.11	0.8	0.2	0.0
141	3281	1	4	84	1	0.33	0.21	0.6	0.9	0.2
142	3281	1	4	84	2	0.27	0.20	0.8	1.2	0.4
143	3281	2	4	84	1	0.23	0.22	0.5	1.3	0.5
144	3281	2	4	84	2	0.33	0.21	1.0	0.8	0.8
145	3282	1	4	18	1	0.20	0.20	0.3	0.8	0.4
146	3282	1	4	18	2	0.20	0.20	0.6	1.0	0.2
147	3282	2	4	18	1	0.16	0.21	0.4	0.6	0.6
148	3282	2	4	18	2	0.12	0.20	0.2	1.0	0.6
149	2309	1	9	96	1	0.43	0.20	1.2	0.4	0.3
150	2309	1	9	96	2	0.28	0.17	0.8	0.6	0.4
151	3309	2	9	96	1	0.44	0.17	1.3	0.6	0.7
152	2309	2	9	96	2	0.43	0.20	0.4	1.4	0.4
153	3312	1	4	11	1	0.35	0.20	1.3	0.3	0.1
154	3312	1	4	11	2	0.30	0.21	0.6	0.6	0.0
155	3312	2	4	11	1	0.34	0.21	1.5	0.2	0.3
156	3312	2	4	11	2	0.28	0.19	1.2	0.0	1.0

OBS PRIMSP SECONDSP TIDS TOZA GIANTS MOND INTERST EPITHICK DUCTEPI

131	3.3	0.9	7.5	0.6	0.0	0.0	6.2	0.14	0.15
132	2.4	1.4	7.0	1.6	0.0	0.0	5.8	0.14	0.15
133	2.9	2.9	6.3	2.8	0.0	0.2	13.4	0.25	0.15
134	2.8	1.2	10.4	5.6	0.0	0.0	21.6	0.17	0.14
135	4.2	1.0	8.3	1.8	0.0	0.0	15.7	0.24	0.17
136	2.4	1.0	7.6	2.4	0.0	0.0	12.0	0.16	0.14
137	0.9	0.0	2.3	1.4	0.0	0.0	13.7	0.26	0.13
138	0.6	0.2	0.8	0.2	0.0	0.0	11.6	0.34	0.11
139	0.9	0.1	1.4	3.7	0.0	0.1	20.3	0.28	0.14
140	0.2	0.0	1.0	0.8	0.0	0.1	16.6	0.26	0.11
141	3.1	1.1	13.4	0.6	0.0	0.0	10.2	0.13	0.17
142	4.4	0.0	10.8	5.5	0.0	0.0	13.8	0.19	0.17
143	3.2	0.4	13.1	4.3	0.0	0.0	13.5	0.19	0.19
144	3.0	0.4	12.4	0.0	0.0	0.0	10.2	0.16	0.16
145	1.9	1.5	5.3	2.6	0.0	0.0	5.0	0.20	0.15
146	2.2	1.4	5.4	0.0	0.0	0.0	3.0	0.10	0.13
147	2.0	1.1	5.5	2.2	0.0	0.0	3.7	0.11	0.14
148	3.0	1.4	6.4	3.4	0.0	0.2	5.8	0.10	0.14
149	2.8	0.2	7.2	2.5	0.0	0.0	9.2	0.26	0.19
150	2.1	0.2	6.0	2.0	0.0	0.0	8.0	0.19	0.18
151	3.0	0.1	6.2	2.8	0.0	0.0	11.6	0.26	0.16
152	3.8	0.0	7.2	3.0	0.0	0.0	15.8	0.23	0.16
153	2.8	0.1	9.2	5.9	0.0	0.0	7.0	0.27	0.15
154	3.2	0.2	7.6	1.2	0.0	0.0	5.6	0.20	0.15
155	3.0	0.1	8.3	1.4	0.0	0.0	6.1	0.29	0.14
156	2.8	0.0	4.0	1.6	0.0	0.0	2.9	0.26	0.15

OBS ID SLIDE BREED AGE SECTION TUNALB TURDIAM SERTOLI SPERMATA SPERMATE

157	3313	1	6	48	1	0.35	0.18	1.2	0.9	0.1
158	3313	1	6	48	2	0.40	0.87	1.0	0.2	0.2
159	3313	2	6	48	1	0.39	0.18	1.2	0.4	0.4
160	3313	2	6	48	2	0.19	0.17	1.0	0.4	0.2
161	3314	1	6	48	1	0.42	0.21	1.0	0.7	0.2
162	3314	1	6	48	2	0.24	0.22	0.8	0.8	0.8
163	3314	2	6	48	1	0.45	0.27	0.9	0.6	0.2
164	3314	2	6	48	2	0.35	0.22	1.0	0.4	0.2
165	3315	1	5	36	1	0.31	0.16	1.4	0.5	0.1
166	3315	1	5	36	2	0.36	0.16	1.2	0.0	0.0
167	3315	2	5	36	1	0.43	0.14	1.4	0.2	0.2
168	3315	2	5	36	2	0.30	0.16	1.0	0.4	0.2

OBS PRIMSP SECONDSP TIDS TOZA GIANTS MONO INTERST EPITHICK DUCTEPI

157	3.5	0.2	7.8	0.4	0.0	0	8.3	0.17	0.21
158	2.0	0.8	8.6	1.6	0.0	0	7.4	0.09	0.16
159	2.1	0.5	6.2	0.4	0.0	0	8.9	0.16	0.25
160	2.2	0.6	6.8	2.8	0.0	0	12.2	0.22	0.16
161	2.6	0.2	8.2	11.7	0.0	0	8.1	0.25	0.19
162	2.4	0.2	8.8	7.0	0.0	0	9.6	0.16	0.19
163	3.5	0.0	8.0	10.4	0.0	0	7.3	0.37	0.19
164	3.4	0.4	7.0	5.6	0.0	0	5.8	0.12	0.16
165	2.8	0.0	4.7	0.9	0.4	0	6.9	0.20	0.12
166	2.4	0.0	5.8	5.4	0.4	0	5.5	0.26	0.14
167	2.3	0.0	6.6	1.0	0.4	0	6.2	0.23	0.16
168	5.6	0.0	4.8	0.4	0.2	0	7.4	0.19	0.12

AGE-RELATED CHANGES IN THE CAT TESTIS AND EPIDIDYMIS

by

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AN ABSTRACT OF A THESIS

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Manhattan, Kansas

1983

Forty-two pairs of grossly normal testes and epididymes were obtained from clinically healthy cats and examined histologically for age-related changes. Tissues were interpreted quantitatively by counting cell numbers and measuring structural thicknesses and diameters, and qualitatively by grading changes on a scale of 0 to 5; 0 representing normal or no change, and 5 being most severe. Data were divided into 5 age groups and interpreted statistically and descriptively.

Results indicate that the feline testis responds to age in a similar manner as other domestic animal species. Testicular tunica albuginea thickness, interstitial cell number, seminiferous tubule basement membrane thickness, tubular atrophy, interstitial cell lipofuscin, and Sertoli cell cytoplasmic vacuolation increased significantly with age. Degeneration of tubular epithelium initially involved the most mature cell types and subsequently extended towards the basal layer. Significant decreases in spermatogonia A, spermatogonia B, and secondary spermatocytes were noted. Mononuclear and multinuclear spermatid giant cells were early degenerative findings.

Several age-related changes noted in other species were not observed in cats: hyaline degeneration of arteries/arterioles, testicular calcification, diminished tubular diameter, and neoplasia. This may indicate species predisposition for certain age-related changes.

Changes in the feline epididymis were uncommon, consisting of occasional intraepithelial cysts and hyperplasia, one spermatocele in the ductus epididymal head, and a significant increase in epididymal tunica albuginea thickness. Interspecies comparison was not possible due to paucity of available literature.

Lymphocytic foci were occasionally observed in testicular and epididymal connective tissue of cats ranging from 6 months to 9 years old. Most foci were sparsely populated with lymphocytes and few plasma cells and macrophages. Foci were reactive in the 2 oldest cats, consisting of numerous immature lymphocytes, plasma cells, macrophages, and mitotic figures. This indicated a possible role of autoimmunization in age-related testicular and epididymal changes.

Proteinaceous tubular luminal debris was most common in the middle age group ranging from 36 to 83 months old. It was probably a biproduct of spermatogenesis, for cats exhibited minimal seminiferous tubular degeneration and numerous epididymal ductal spermatozoa. Eosinophilic to amphophilic round, variably sized interstitial cell cytoplasmic bodies were also commonly found in these cats. They may represent an androgen-dependent storage product, but electron microscopy and cytochemical tests are needed to substantiate this.

Cats in the young age group frequently contained seminiferous tubular degeneration characterized by mononuclear and multinuclear spermatid giant cells, cytoplasmic vacuolation of germinal cells and Sertoli cells, and large luminal aggregates containing multiple oval, vesicular nuclei and undiscernible cell borders. These changes may be a function of spermatogenesis onset, the cycle not yet working efficiently or normally. Seventy percent of 6 to 11 month old cats also contained epididymal intraepithelial cysts and hyperplasia, suggesting that other factors may play a role in the pathogenesis of these lesions. More studies are needed to determine their cause.