# AGE-RELATED CHANGES IN THE TESTES AND PROSTATE OF THE BEAGLE DOG

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*Abstract — Age-related changes in the histologic morphology of the Beagle dog prostate and testes must be separated from those changes that may result from the testing of experimental compounds. The prostate and testes of healthy age-matched Beagle dogs 3 to 14 yr of age were obtained. Serum to evaluate testosterone levels was also obtained from each dog at the time of euthanasia. Tissue sections from the prostate and testes were examined by light microscopy for both qualitative and quantitative morphologic assessment. A statistically significant increase in prostatic weight with increased age was noted. Significant morphometric findings in the prostate included a decrease in the relative percent of epithelial cells and an increase in the relative lumen size of glandular acini with increased age. The absolute volume of prostatic interstitial tissue and inflammation showed a statistically significant increase with age. Stereological analysis of the testes showed a decrease in the relative percent epithelium with increasing age. No distinct age-related trend could be detected in serum testosterone levels. Serum testosterone levels did not correlate with the morphologic age-related changes observed in the testes or prostate.*

Benign prostatic hyperplasia (BPH) occurs with high incidence in man and Beagle dogs in association with increasing age. The dog has been used as a model for the development of this disease in man. It has been established that the presence of functioning testes is required for the development of this disease in the dog.[1] Human studies suggest that at least a portion of BPH tissue requires testosterone for its abnormal maintenance.[1]

In the dog, the incidence of BPH increases with age.[1-4] It has been suggested that this incidence approaches 100% of the population by age 7-8 yr.[1] An increase in prostatic weight with age has also been well documented.[1-4] However, only a few reports have addressed the changes that take place with age in the canine testes.[4,5] James and Heywood[4] reported an increased incidence of testicular tumors with age and a low incidence of focal chronic inflammation of the epididymus that had an age-related trend. Ewing *et al.*[6] noted an increase in total Leydig cell numbers and have also shown a correlation between incidence of benign prostatic hypertrophy and testosterone production.

The purpose of this report is to document the morphological changes in the prostate and testes, and changes in serum testosterone levels of closely age-matched healthy Beagle dogs ranging from 3-14 yr of age. These results extend the documented aging changes in the Beagle dog prostate and testes to 14 yr of age, and provide correlation of aging changes in both the testes and prostate with serum testosterone levels.

## MATERIALS AND METHODS

The dogs used in this study were part of an investigation of age-related functional and structural changes in the normal dog lung. Other organ systems were also evaluated for age-related changes. In this report, quantitative and qualitative morphology were used to demonstrate the age-related changes in the Beagle dog testes and prostate.

Twenty-five healthy, male Beagle dogs were selected from the IIRI colony and divided equally into age groups of 3, 6, 9, 12 and 14 yr. Ages of the dogs within each group were within 3 mo of each other, except the 14-yr olds which had a 1 yr range. Prior to inclusion in the study, the health status of the dogs was assessed by a complete physical examination, hematology, serum chemistries, and urinalysis. Only dogs determined to be clinically healthy were included in the study.

The dogs were euthanized with an overdose of pentobarbital given intravenously followed by exsanguination. Prior to euthanasia, a fasting blood sample was obtained and serum was sent to the New Mexico Veterinary Diagnostic Laboratory for determination of serum testosterone concentration. These levels were measured by radioimmunoassay. The prostate and testes were removed, excess tissue trimmed away, and then measured, weighed, and placed in 10% neutral buffered formalin. Fixed volumes of each testicle and the prostate were determined by water displacement. The tissues were slab sectioned at 2-mm intervals, sampled by a stratified random sampling scheme, and embedded in paraffin. Five-micrometer sections were cut and stained with hematoxylin and eosin according to standard histologic techniques. Volume percents of tissue components of each organ were determined by point-counting techniques in a blind fashion. For each testicle, the percent of interstitial tissue, Leydig cells, germ cells (all stages of spermatogenesis were included), lumen, and tumor tissue was determined. For the prostate, the percent of interstitial tissue, epithelial cells, inflammation (aggregates of inflammatory cells including plasma cells, lymphocytes, macrophages, and neutrophils), and lumen was determined. The prostate sections were also examined qualitatively for evidence of benign prostatic hypertrophy (BPH). Diagnoses of BPH were made according to published criteria.[3] Briefly, BPH was characterized by an increase in the amount of glandular epithelium or by areas of glandular hyperplasia with dilated and cystic or atrophic ducts.[3]

A one-way analysis of variance was used to compare the means of the age groups for each parameter measured. The criterion for statistical significance of these analyses was set at $p < 0.05$.

## RESULTS

Stereological analysis of the prostate showed a statistically significant decrease in the relative percent of epithelial tissues with age. Also noted was an age-related increase in the relative lumen size of the glandular acini of the prostate. In addition, the relative percent of inflammatory tissue present in the prostate showed a significant increase with age, especially between the younger ages (3 yr and 6 yr) and the 14-yr group. Significant changes were noted in the absolute volumes of prostatic interstitial tissue and inflammatory tissue, but not in the epithelial tissue or glandular lumen size. A graph of these relationships is presented in Figure 1. The weight of the prostate also increased with age (see Table 1). Morphologically, the prostates of dogs 9, 12, and 14 yr all had evidence of BPH with the majority having a moderate to severe grade (see Table 2).

Qualitative histologic evaluation of both testes from each dog indicated a progressive trend of degenerative changes and incomplete spermatogenesis in seminiferous tubules that was more prominent with increasing age. Multifocal interstitial cell hyperplasia was observed in several dogs greater than 9 yr of age. One animal, in the 14-yr old age group had a grossly observable tumor that was classified as a seminoma. Microscopic intratubular seminomas were also present in one 12-yr old animal.

Morphometry of the testicles showed a significant age-related effect only on the relative percent of germ cell tissue. This decrease was primarily observed in the oldest age group, i.e.,

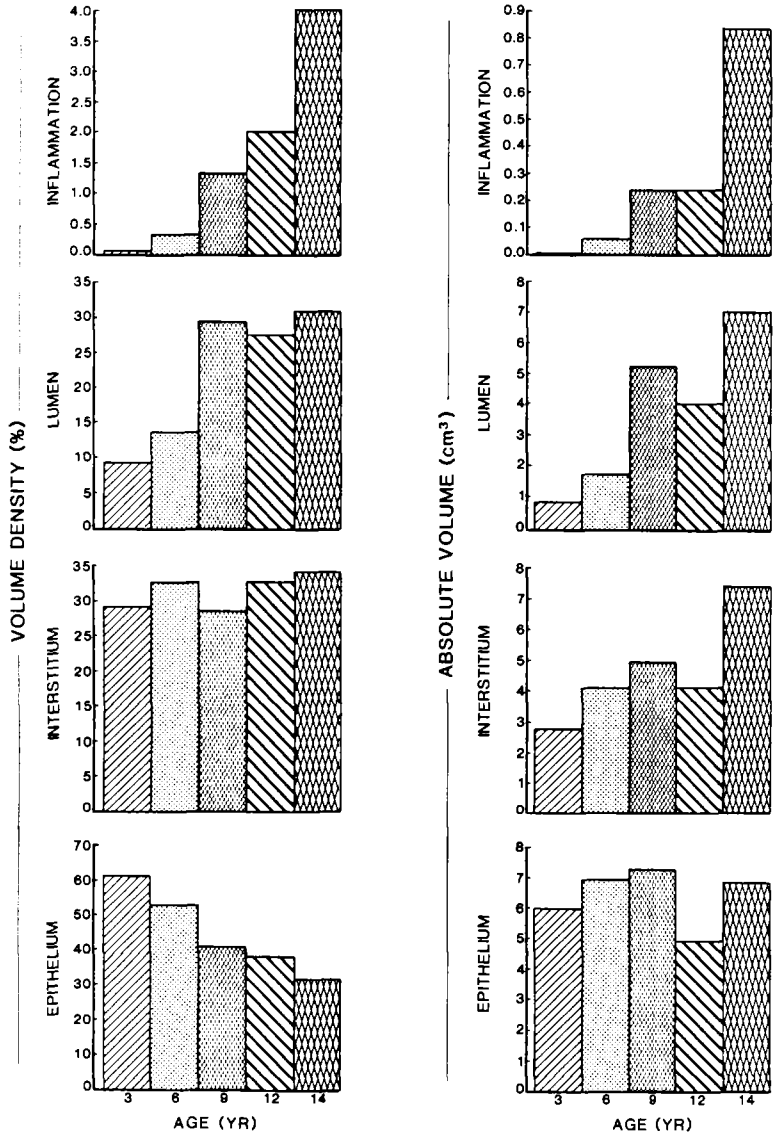


Figure 1. Volume densities of tissue components of the prostate. Densities were determined by point counting techniques. This, along with prostatic volumes, was used to determine the absolute volume for prostatic epithelium, interstitium, lumen, and inflammatory tissue present. Above is the mean for each age group for each tissue component.

Table 1

Average Weights and Volumes for Each Age Group for the Testes and Prostates

| Age (yr) | Average Weight per Testis (g) | Average Volume per Testis ($cm^3$) | Average Volume of Prostate ($cm^3$) | Average Weight of Prostate (g) |
|---|---|---|---|---|
| 3 | 8.90 | 8.50 | 9.64 | 10.79 |
| 6 | 8.88 | 8.26 | 12.89 | 14.00 |
| 9 | 9.41 | 8.78 | 17.74 | 19.10 |
| 12 | 6.23 | 5.83 | 13.30 | 14.20 |
| 14 | 9.23 | 8.70 | 22.00 | 23.99 |

Table 2

Histologic Determination of the Presence of Benign Prostatic Hypertrophy (BPH)

| Age | Dog 1 | Dog 2 | Dog 3 |
|---|---|---|---|
| 3 yr | 1+[a] | 1+[a] | 1+ |
| 6 yr | 2+ | 1+ | 2+ |
| 9 yr | 3+ | 4+ | 3+ |
| 12 yr | 4+ | 4+ | 2+ |
| 14 yr | 4+ | 4+ | 2+ |

[a]Rare cystic gland.

1+ = Normal
2+ = Mild (BPH)
3+ = Moderate BPH
4+ = Severe BPH

14 yr. The actual volume densities (%) of testicular tissues had no statistically significant changes. Figure 2 is a graph of the relative and actual percentages. The weight of the testicles did not change significantly with age. One dog (12-yr old) had a single cryptorchid testicle that was much smaller than the descended one (included in the analyses).

Serum testosterone levels showed no difference with age (data not shown). The presence of a testicular tumor did not effect serum testosterone levels nor did the percent of Leydig cells present.

## DISCUSSION

The stereological results described here of the aging changes of the Beagle dog prostate are in agreement with other authors' findings. It has been shown that the glandular tissue volume (epithelial cells) increases with age.[1,3,4] We saw no difference in the relative interstitial tissue volume, but an increase in the absolute volume, which others have also noted.[1,3,4] The relative lumen volume of the acini increased with age, a finding not previously reported. The weight of the prostate increases with age, which is in agreement with all other authors.[1-4,6] This increase in weight and size of the prostate was due to an increase in the inflammatory and interstitial tissues accompanied by a relative decrease in the epithelium with no absolute change.

As previously reported, an age-related increase in the incidence of BPH in the dog was observed. This increased incidence was used as a model to study the cause of prostatic hyperplasia in man. In the dog, BPH has been shown to require a continuous supply of androgen (testosterone) for its maintenance.[1] Studies done in man have shown that a portion of human BPH tissue requires testosterone for its maintenance.[1] However, there is an important difference in the anatomy and the occurrence of BPH between man and dog. In man, the prostate is anatomically fixed between the symphysis pubis and the rectum. Hyperplastic growth compresses inwardly on the uretha producing urinary obstruction. This hyperplastic growth is thought to be a focal process arising in the periurethral portion of the gland as a strand nodule which is then invaded by glandular elements.[1] In the dog, the prostate is not anatomically fixed. Hyperplastic growth expands in all directions outwardly producing rectal obstruction and constipation. This process in the dog is diffuse, occurring throughout the entire gland primarily involving the glandular epithelial cells with less stromal involvement.[1] In this study, no significance has been detected between the degree of BPH and serum testosterone levels.

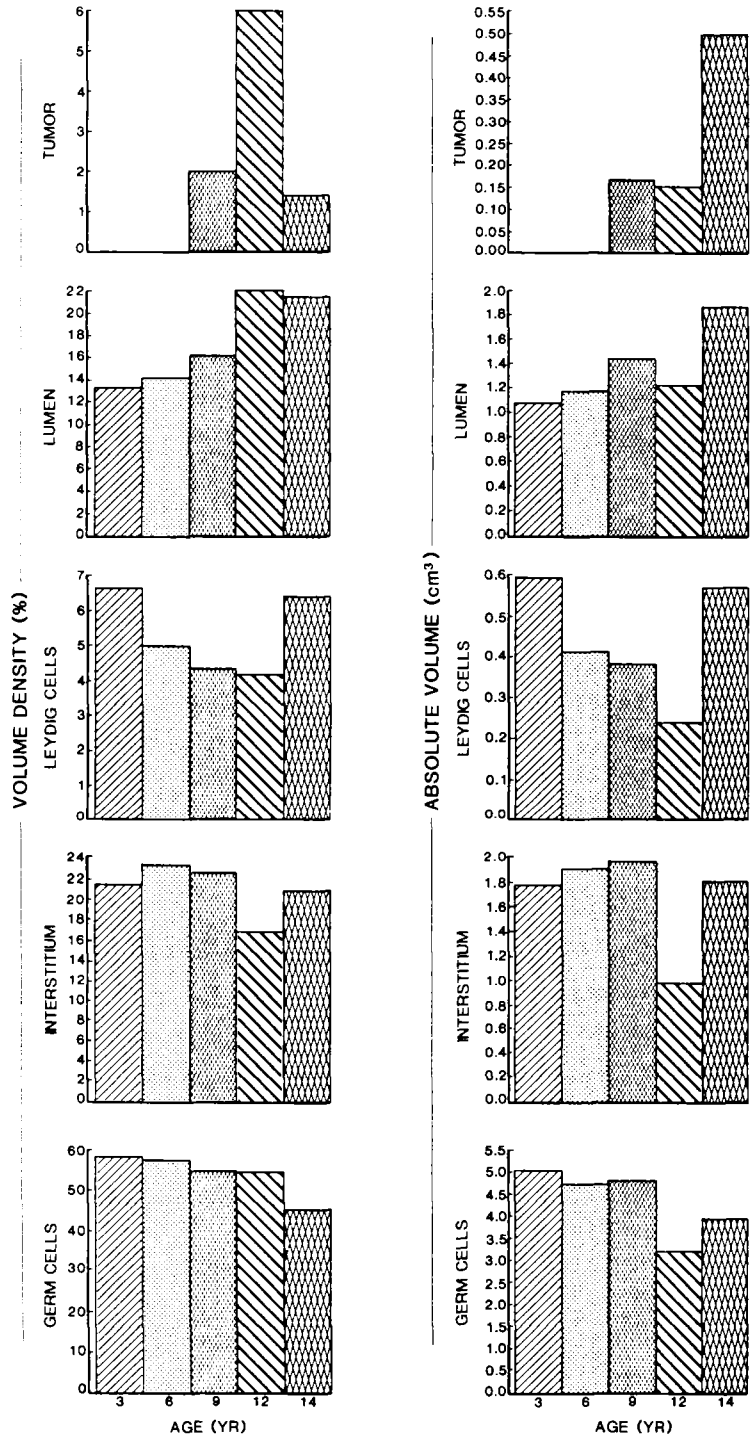


Figure 2. Volume densities of tissue components of the testes. Testicular volumes were used to determine the absolute volumes for testicular epithelium, interstitium, Leydig cells, lumen, and tumor tissue. Above is the mean for each age group for each tissue component.

In the testes, the relative percent of epithelial tissues decreased with age significantly, with the 14-yr olds having the lowest value. Ewing et al.[6] have noted that Leydig cell numbers increased significantly between 3.8 and 6 yr of age. This increase in Leydig cell numbers was thought to compensate for the decreased steroidogenic potential per cell that occurs between 3.8 and 6 yr of age, thus maintaining serum testosterone levels.[6] They also suggested that Leydig cell atrophy occurs between 3.8 and 6 yr of age.[6] We did not note a significant change in the relative volume nor in the absolute volume of Leydig cells, but did not determine actual Leydig cell numbers. No statistical difference in the total testicular weight was noted. James and

Heywood[4] noted a distinct difference between 2- and 6-yr old dogs with the majority of the older dogs having increased total testicular weight. Other authors have also noted no age-related differences in serum testosterone levels.

This study extended the range of the age-related stereological and morphological changes of the Beagle dog prostate and testicles. Some changes noted here were not in agreement with other authors. This could be due to the small number of dogs used in this study, the fact that a single breed was used, or that the dogs came from a closed colony.

## REFERENCES


1. Isaacs, J. T. Common Characteristics of Human and Canine Benign Prostatic Hyperplasia, Prog. Clin. Biol. Res. 145: 217-234, 1984.

2. Berry, S. J. and J. D. Strandberg et al. Development of Canine Benign Prostatic Hyperplasia With Age, Prostate 9: 363-373, 1986.

3. Zirkin, B. R. and J. D. Strandberg. Quantitative Changes in the Morphology of the Aging Canine Prostate, Anat. Rec. 208: 207-214, 1984.

4. James, R. W. and R. Heywood. Age-Related Variations in the Testes and Prostate of Beagle Dogs, Toxicology 12: 273-279, 1979.

5. Heywood, R. and R. W. James. Assessment of Testicular Toxicity in Laboratory Animals, Environ. Health Perspectives. 24: 73-80, 1978.

6. Ewing, L. L. and S. J. Berry et al. Aging, Leydig Cell Structure-Function and Benign Prostatic Hyperplasia in Beagles, in Benign Prostatic Hyperplasia (C. H. Rodgers, D. S. Coffey, G. Cunha, J. T. Grayhack, F. Hinman, Jr., and R. Horton, eds.), Volume II, NIH Publication, U. S. Department of Health and Human Services, pp. 129-137, 1985.