Histological Observations of the Reproductive Organs of the Male Dog from Birth to Sexual Maturity

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ABSTRACT. Development of the testis, epididymis and prostate in 53 male beagles was examined histologically with PAS-hematoxylin stain from birth to sexual maturity. The diameter of the seminiferous tubules of the testes was less than 100 μm until 20 weeks of age, however, it increased markedly between 22 and 28 week of age, reaching 180±7 (mean±SD) μm at 28 weeks of age. Only Sertoli cells and gonocytes (or spermatogonia) were detected in the seminiferous tubules until 16 weeks of age. Spermatocytes and spermatids appeared in the tubules at 20 and 22 weeks of age, respectively. Spermatozoa were first observed in the testes of 2 of the 5 dogs at 26 weeks of age and were found in the testes of all the 3 dogs at 28 weeks of age. The diameter of the ducts in the cauda epididymidis was 146±4 μm at 20 weeks of age. Thereafter it increased markedly, reaching 341±14 μm at 28 weeks of age. The height of the epithelium and stereocilia in the ducts of the caput epididymidis increased markedly at about 28 weeks of age. A large number of spermatozoa was seen in the lumens of the ducts of the corpus and cauda epididymidis after 32 weeks of age. The shape of the lumens in the glandular alveoli of the prostate became irregular as a result of projection of the glandular epithelium into the enlarged lumens and the epithelial cells of the alveoli became PAS-positive at 24 weeks of age. The projection of the glandular epithelium into the lumen became more marked after 32 weeks of age. Thus it was demonstrated that histological development of the testis, epididymis and prostate of the dog is most marked between 20 and 32 weeks of age.—KEY WORDS: dog, genital organ, histology, sexual maturity.


The authors [24, 26, 27] have reported that the weight of the testis and accessory reproductive organs increases markedly between 24 and 32 weeks of age and that peripheral and spermatic vein plasma testosterone levels are high from 26 weeks of age on. Studies of sexual maturation of the male dog by other researchers have included examination of changes in plasma androgen levels [14, 22] and semen quality [21, 22, 23]. Few papers, however, have been published reporting histological changes in the canine testis during sexual maturity in detail [29]. In the present study, the development of testicular, epididymal and prostatic function in the beagle was examined histologically from birth to 48 weeks of age in relation to the results of previous studies [24, 26, 27].

MATERIALS AND METHODS

A total of 53 male beagles, animals whose testicular and accessory reproductive organ weights and plasma androgen levels had been measured by the authors previously [24, 26, 27], were used as the subjects of this study. The testes, epididymides and prostates were removed from 3–5 dogs at various week of age after euthanasia with pentobarbital sodium anesthesia at 4 weeks intervals from 0 to 20 and from 32 to 48 weeks of age and at 2 week intervals from 20 to 32 weeks of age. These excised tissues were fixed in Bouin's fluid, embedded in paraffin, cut into 3 μm sections, and stained with PAS-hematoxylin.

Round transverse sections of 10 seminiferous tubules of essentially the same size were examined in each testis. The diameters of these seminiferous tubules were measured with a micrometer. Sertoli cells and germ cells in the seminiferous tubules were counted, and the results were corrected by the Abercrombie method [1] and Clermont and Morgentaler method [3]. In accordance with the classification of Ibach et al. [10], type A spermatogonia, pachytene spermatocytes and round spermatids in the tubules of the seminiferous epithelium at stage 9 of the cycle were counted at the first evidence of spermatogenesis. Leydig cell counts of 20 populations of these cells in the intertubular spaces were performed and the values corrected by the same methods as used in the case of the germ cells in the seminiferous tubules. The diameter and epithelial height of 5 round ducts in the caput, corpus and cauda epididymidis were measured.

The authors [26] have found that the weight of the
right and left testis and epididymis in the dog are almost exactly the same between 0 and 48 weeks of age. Thus, in this study we chose to observe the right testes and epididymides.

RESULTS

Testis: Histological findings in the testes of the dogs are shown in Figs. 1 and 2. There were few seminiferous tubules in the testicular cross sections, and interstitial tissue predominated over tubules at 0 week of age. Only Sertoli cells and gonocytes were observed in the seminiferous tubules at 0 week of age. A large number of undifferentiated Sertoli cells which had dark staining ovoid nuclei were located along the basement membrane. A few gonocytes which had light staining round nuclei and PAS-positive cytoplasm were located along the basement membrane or in the central region of the seminiferous tubules. Two types of gonocytes, large cells (average nuclear diameter, 9 μm) and small cells (average nuclear diameter, 5 μm), were identified. Both types of gonocytes had a large and obvious nucleoli and a few chromatin granules in the nuclei. Meiotic division of large gonocytes was seen. The diameter of the seminiferous tubules was slightly greater at 8 weeks of age. Occupation of the stroma became relatively small because of an increase in the diameter of the seminiferous tubules at 16 weeks of age. A kind of germ cell which were different from gonocytes were also seen along the basement membrane at 16 weeks of age. These cells had light, oval-shaped nuclei (average nuclear diameter, 8 × 5 μm) containing one or two nucleoli, and the chromatin in the nucleus was dust-like. These cells were thus identified as type A spermatogonia. Type B spermatogonia were first observed at 20 weeks of age. These cells had oval-shaped nuclei containing the coarse chromatin clumps along the nuclear membrane. Gonocytes were hardly seen after 20 weeks of age.

Marked changes in the histological findings in the testes were observed between 20 and 28 weeks of age. Though the mean diameter of the seminiferous tubules was less than 100 μm until 20 weeks of age, this value rose to 180±7 (mean±SD) μm at 28

Fig. 1. Diameter of the seminiferous tubules and number of Sertoli cells, germ cells and Leydig cells (mean±SD) per cross section of seminiferous tubule in 3–5 dogs at various weeks of age.

a) The values were corrected by the methods of Abercrombie (1) and Clermont & Morgentaler (3).

b) Number of the cells were counted with 10 seminiferous tubules of stage 9 according to the classification of Ishibashi et al. (10) after onset of spermatogenesis.

c) The values were counted with 20 populations of Leydig cell.

d) --: no sperms, +: small number of sperms, ++: middle number of sperms, +++: large number of sperms
weeks of age as a result of marked increases in tubular diameter between 22 and 28 weeks of age. A few pachytene spermatocytes were seen in the seminiferous tubules of the testes of 2 of the 3 dogs at 20 weeks of age. A lumen was formed in many seminiferous tubules of all 3 dogs, and round spermatids were observed in the tubules of 1 of the 3 dogs at 22 weeks of age. The number of each type of germ cell increased markedly after 26 weeks of age. In addition, a small number of spermatozoa first appeared in the seminiferous tubules of 2 of the 5 dogs at 26 weeks of age and were observed in many tubules of all 3 dogs 28 weeks of age. Active spermatogenesis was recognized in the testes of the
dogs from 32 weeks of age on. The number of Leydig cells in the interstitial tissue tended to increase after 28 weeks of age.

**Epididymis:** Histological findings in the epididymis of the dog are shown in Figs. 3 and 4. Simple and low columnar epithelium was observed in the ductus epididymidis, and the epithelial cells possessed stereocilia in the caput, corpus and cauda epididymidis at 0 week of age. The number of the ductus epididymidis of all 3 divisions and the height of the epithelium increased at 16 weeks of age. The ductus epididymidis was lined with pseudostratified epithelium consisting of tall principal cells and small basal cells, and the epithelial cells were PAS-positive in all 3 divisions at 20 weeks of age. The mean diameter of the ducts in the cauda epididymidis was 146±4 μm at 20 weeks of age. Later on account of a marked increase in the diameter of the ducts of the cauda epididymidis, its diameter was the widest 341±14 μm of the 3 divisions at 28 weeks of age.

Shed spermatoctyes and round spermatids were seen in the lumen of the ductus epididymidis, and PAS-positive secretion was especially present in the ducts of the cauda epididymidis at 24 weeks of age. A small number of spermatozoa appeared in the ducts of the corpus and cauda epididymidis of the dogs in which spermatogenesis was observed at 26 weeks of age. The height of the epithelium and the length of the stereocilia of the ducts in the caput epididymidis increased markedly and reached maximum values in the 3 divisions after 28 weeks of age. Numerous spermatozoa were accumulated in the ductal lumen of the corpus and cauda epididymidis after 32 weeks of age.

**Prostate:** Histological findings in the prostate of the dog are shown in Fig. 5. Small glandular alveoli approximately 50 μm in diameter were scattered in the external portion of the prostate at 0 week of age. Most of the prostatic tissue was occupied by connective tissue stroma and no lumens were seen in the glandular alveoli. An increase in the number of alveoli and the appearance of lumens in the glands were observed at 8 weeks of age. The number and size of the glands had increased, and a great many of the alveoli had formed the glandular lobules which surrounded by connective tissue stroma at 20 weeks of age. The shape of the lumen of the glandular alveoli was irregular as a result of projection of the glandular epithelium into the enlarged lumen at 24 weeks of age. The height of the glandular epithelium also had increased and the epithelial cells were PAS-positive. The glandular epithelium markedly projected into the lumen, and the diameter of the

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**Fig. 3.** Diameter of the ducts and height of ductal epithelium in the caput, corpus and cauda of the epididymidis (mean±SD) of 3-5 dogs at various weeks of age.

- a) The values were counted with 5 ducts.
- b) •: no sperms, +: small number of sperms, ++: middle number of sperms, +++: large number of sperms
glandular alveoli increased to 100–200 μm at 32 weeks of age. Thereafter the epithelial cells of the alveoli had secretory droplets, the inner cell margins broke down, and PAS-positive secretion accumulated in the glandular lumen.

DISCUSSION

The authors [27] and other researchers [7, 14, 22, 25] have reported that peripheral plasma testosterone levels in the male dog increase between 24 and 28 weeks of age and are higher level after 28 weeks of age. Mialot et al. [13] found that the testes of fox terrier dogs developed markedly between 22 and 36 weeks of age. The authors [26] found that the testicular weight of the beagle also increased markedly between 24 and 32 weeks of age. In this study, marked development of the testis, epididymis
and prostate was observed histologically between 20 and 32 weeks of age. It is believed that the increase in number of Leydig cells after 28 weeks of age in this study is related to the increase in plasma testosterone levels [27] and the histological development of the reproductive organs observed at this time in the dog. In addition, it is presumed that the marked increase in testicular weight of the dogs between 24 and 32 weeks of age [26] is caused by the obvious increase in germ cells in the seminiferous tubules observed between 20 and 32 weeks of age in this study.

Yamauchi et al. [29] have reported that Sertoli cells, large spermatogenic cells and spermatogonia were seen in the seminiferous tubules of infant dogs. It is guessed that these large spermatogenic cells and spermatogonia were equal to large gonocytes and small ones in the present study, respectively. Hucksins and Clermont [9] have described that gonocytes in the rat testis between 3 and 7 days after birth divided to produce pre-type A spermatogonia, which were smaller than gonocytes. Hilscher et al. [8] have reported that the neonatal rat has two types of gonocytes, I- and II-gonocytes, and II-gonocytes were the daughter cells of I-gonocytes. In this study meiotic division of large gonocytes was seen. Therefore it was considered that small gonocytes arised by division of large one, i.e. small gonocyte was pre-type A spermatogonium, the same as in the report of Huckins and Clermont [9]. In the future, it will be necessary to determine whether small gonocyte differentiate into type A spermatogonium.

Kiso et al. [12] and Orsi [18] observed histologically that the epithelium of the epididymal ducts of the adult dog had a secretory function. It is known that sperm maturation is induced by the secretion in the epididymal ducts [5, 15, 16, 28, 30]; especially PAS-positive glycoproteins in the secretion, in particular, are related to sperm fertility [15, 17].

In other animals, it has been reported that androgen secreted by the testes influences the secretory function of the epididymis [6, 11, 17, 19]. In this study it was observed that the epithelium of the epididymal ducts was PAS-positive and that PAS-positive secretion was present in the lumen of the ducts of the cauda epididymis after 20 weeks of age. These findings suggest that the epididymal function becomes more active as a result of an increase in androgen concentration secreted by the testes at this time [24].

The prostate of the dog is the only well developed accessory reproductive organ. Thus the majority of the seminal plasma in the dog consists of prostatic

Fig. 5. Histological changes of prostates of the dogs.
PAS-hematoxylin stain. ×100.
A: 0 week of age. B: 8 weeks of age. C: 20 weeks of age. D: 24 weeks of age. Epithelium showed nipple-like projections in the lumen. E: 32 weeks of age. Vacuole (v) was observed in the epithelial cells. Some acini had PAS-positive secretion in the lumen. F: 48 weeks of age.
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fluid. It has been stated that androgen secreted by the testes directly influences the prostate via the deferential vein [20]. Berg [2] reported that the secretory function of the prostate in the dogs began to increase at 9 months of age. It was reported by Creed [4] that the epithelial cells of the glandular alveoli in the adult canine prostate contained secretory droplets and performed apocrine-like secretion by means of breakdown of the interior edge of the cell itself. In this study these histological findings were observed in the prostate of the dog after 32 weeks of age. Hence it is believed that the secretory function of the prostatic epithelium of the dog began to increase at 32 weeks of age as a result of the increase in androgen secreted by the testes after 20 weeks of age [24].

Takeishi et al. [22] and Taha et al. [21] found that spermatozoa appeared first in the ejaculate of young beagles at 6 or 7 months of age and 34 weeks of age, respectively. It has been reported that semen volume, sperm count and motility in the young dog increase gradually from 7–8 months of age [21, 23].

In conclusion, the results of present study show that the histological and functional development of the testis, epididymis and prostate of the dog is most marked between 20 and 32 weeks of age.

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