Introduction

Ageing has marked effects on testicular function and morphology in men. In aged men, daily sperm production (Johnson et al., 1984; Matoska and Talerman, 1989), sperm quality (Schwarz et al., 1983), and serum concentrations of testosterone (Zumoff et al., 1982; Bremner et al., 1983; Gray et al., 1991) are lower than in young men, although some reports found unaltered testosterone concentrations (Harman and Tsitouras, 1980; Sparrow et al., 1980; Neaves et al., 1984). Ageing of the human testis is characterized histologically by decreased seminiferous tubular length, diameter and volume (Johnson et al., 1986; Johnson, 1989; Paniagua et al., 1991), by thickening of the basement membrane of the seminiferous tubules (Finch and Schneider, 1985), and by decreased numbers of Leydig cells, Sertoli cells, and germ cells (Kothari and Gupta, 1974; Kaler and Neaves, 1978; Neaves et al., 1984; Paniagua et al., 1991). Some of these changes have also been described in laboratory animals, such as rats and cats (Elcock and Schoning, 1984; Wang et al., 1993; Wright et al., 1993; Chen et al., 1994).

Spermatogenesis was examined in testes from 74 dogs of various breeds without clinically detected testicular disease. A modified Johnsen score system was used to determine whether spermatogenesis deteriorates with ageing. The diameter of seminiferous tubules was measured in dogs without testicular disease to examine other possible effects of ageing on tubular performance. There appeared to be no relation between age and these variables. The influence of testicular tumours on spermatogenesis was also investigated in both affected and unaffected testes. The testes of 28 dogs with clinically palpable tumours and 21 dogs with clinically non-palpable tumours were investigated. In cases of unilateral occurrence of a tumour, impairment of spermatogenesis was observed only in the affected testis of dogs with clinically detected tumours. Bilateral occurrence of tumours, whether detected clinically or non-clinically, was associated with severe impairment of spermatogenesis. The prevalence of tumours increased during ageing. Eighty-six per cent of the clinically detected and 57% of the non-clinically detected tumours were found in old dogs. Multiple types of tumour and bilateral occurrence were very common. Seminomas and Leydig cell tumours were more frequent than Sertoli cell tumours. It was concluded that spermatogenesis per se did not decrease during ageing in dogs but the occurrence of testicular tumours increased with ageing and affected spermatogenesis significantly, as reflected by a lower Johnsen score.

Nevertheless, in some other aspects, laboratory animals differ from men; for example, the number of Leydig cells does not decrease in ageing rats (Wang et al., 1993; Chen et al., 1994) or may even increase with age (Ichihara et al., 1993). The dog has been proposed as a model for studies of the male reproductive system, especially for investigation of the development of prostatic hyperplasia (Lowseth et al., 1990). There have only been a few reports on the effects of ageing on testicular function and morphology in this species, although there are indications that incomplete spermatogenesis is more prominent in aged dogs (James and Heywood, 1979; Lowseth et al., 1990). A reduction in tubular diameter has also been observed in dogs over 6 years of age when compared with younger dogs of the same breed, although the thickness of the germinal epithelium was found not to change during ageing (Taha and Noakes, 1982). A unique aspect of ageing in dogs is the high prevalence of testicular neoplasms and Leydig cell hyperplasia, although one study also reported peak occurrence at 1 year of age (Looijenga et al., 1994). In five different studies using large numbers of dogs, the prevalence of testicular tumours in adult male dogs was shown to vary from 0.068 to 4.6% (Machado et al., 1963; Dorn et al., 1968; Bastianello, 1983; Reifinger, 1988; Hahn et al., 1992). However, in studies that included a greater proportion of old dogs, the prevalence...
was much higher (60–65%) (Guimaraens Chiquiloff and Nascimento, 1977; Mattheeuws and Comhaire, 1977; Mosier, 1989; Weller et al., 1995). The occurrence of microscopic, clinically non-palpable, tumours has also been reported in dogs (Dow, 1962).

In dogs, three types of testicular tumours have been shown to occur with equal frequency: Sertoli cell tumours, seminomas and Leydig cell tumours (Nielsen and Kennedy, 1990). In contrast, in men, in which the occurrence of seminomas and Leydig cell tumours (Nielsen and Kennedy, 1990) has been shown to occur with equal frequency: Sertoli cell tumours, was much higher (60–65%) (Guimaraens Chiquiloff and Nascimento, 1977; Mattheeuws and Comhaire, 1977; Mosier, 1989; Weller et al., 1995). The occurrence of microscopic, clinically non-palpable, tumours has also been reported in dogs (Dow, 1962).

In dogs, the effects of age on testicular function have only been investigated in a small number of animals and possible changes in spermatogenesis have never been quantified. The aims of this investigation were to quantify changes in canine spermatogenesis during ageing, to study the prevalence of non-clinically detected neoplasms, and to study the effects of testicular tumours on spermatogenesis in both the affected and the unaffected testis.

### Materials and Methods

#### Animals and treatment

Testes were collected from 74 dogs that were castrated for reasons other than testicular disease, such as benign prostatic hyperplasia (BPH), perineal hernia (HPR), balanoposthitis, or unwanted behaviour. The testes were collected from several veterinary clinics and a large number of different breeds were included in the study. Material was collected from dogs of 33 different pure breeds and 13 dogs of mixed breeding of various sizes (3–54 kg). Dogs with cryptorchid testes and dogs that were castrated unilaterally were not included in the study.

In addition, testes were collected from 28 dogs with clinically manifest tumours but without signs of feminization. Here, 17 pure breeds and six dogs of mixed breeding were included.

After dissection, the entire testis was cut into pieces of approximately 1 cm \(\times\) 1 cm \(\times\) 0.5 cm and these were fixed by immersion in Bouin’s solution for at least 24 h. From the blocks of each testis, five were selected at random for further processing. From each block, one section of 5 \(\mu\)m was taken for further analysis. Sections were stained by the periodic acid–Schiff (PAS) reaction and Mayer’s haematoxylin.

Initially, Technovit (Heraeus Kulzer, Germany) was used as the embedding material but later paraffin wax was used so that immunohistochemistry could also be performed.

### Histological analysis

In each of the five tissue blocks from each testis, a section was traversed randomly using a \(\times 40\) objective. One hundred round tubular cross-sections were studied per section with regard to the quality of the seminiferous epithelium. The Johnsen method (1970), which gives a score of 1–10 according to the presence or absence of the spermatogenic cell types (Table 1), was used to assess the quality of seminiferous epithelium.

Since the Johnsen score was developed to quantify human spermatogenesis, a minor modification was introduced to account for the difference in seminiferous tubular architecture between humans and dogs. In men, several epithelial stages are present in each tubular cross-section and thus elongated spermatids should be present in each tubular cross-section. In other mammals, each tubular cross-section normally contains only one epithelial stage and, in certain stages of the epithelial cycle, elongated spermatids will already have been released (stage V in dogs) (Russell et al., 1990). In this case, the Johnsen score would only be 7 for a normal tubule. A cross-section in stage V was given a score of 10 if enough normal, round spermatids, characteristic of stage V, were present to overcome this problem. All other stages were classified according to the regular Johnsen score. Subsequently, the average mean score per dog was calculated.

In dogs with clinically diagnosed testicular tumours, only areas not containing tumour tissue were studied. If only tumour tissue was found in the affected testis, while the unaffected testis still contained tubules, a score of 0 was applied. The presence of non-clinically detected tumours and Leydig cell hyperplasia was recorded separately.

<table>
<thead>
<tr>
<th>Johnsen score</th>
<th>Criteria to quantify spermatogenesis (Johnsen, 1970) modified for use in dogs</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>Complete spermatogenesis with many spermatozoa. Germinal epithelium organized in a</td>
</tr>
<tr>
<td></td>
<td>regular thickness leaving an open lumen, or stage 5 of the seminiferous cycle, with sufficient</td>
</tr>
<tr>
<td></td>
<td>round spermatids.</td>
</tr>
<tr>
<td>9</td>
<td>Many spermatozoa present but germinal epithelium disorganized, with marked sloughing or</td>
</tr>
<tr>
<td></td>
<td>obliteration of the lumen.</td>
</tr>
<tr>
<td>8</td>
<td>Only few spermatozoa (&lt;5–10) present in a tubular cross-section.</td>
</tr>
<tr>
<td>7</td>
<td>No spermatozoa but many spermatids present.</td>
</tr>
<tr>
<td>6</td>
<td>No spermatozoa and only few spermatids (&lt;5–10) present.</td>
</tr>
<tr>
<td>5</td>
<td>No spermatozoa, no spermatids but several or many spermatocytes present.</td>
</tr>
<tr>
<td>4</td>
<td>Only few spermatocytes (&lt;5) and no spermatids or spermatozoa present.</td>
</tr>
<tr>
<td>3</td>
<td>Spermatogonia are the only germ cells present.</td>
</tr>
<tr>
<td>2</td>
<td>No germ cells but only Sertoli cells present.</td>
</tr>
<tr>
<td>1</td>
<td>No cells in tubular cross-section.</td>
</tr>
</tbody>
</table>
Effect of ageing on dog testis morphology

The tubular diameter of 15 randomly chosen seminiferous tubules was measured using an ocular micrometer in each of five tissue sections of 74 dogs to obtain information concerning changes in tubular diameter during ageing. Elliptical profiles were not taken into account. The testicular tissue embedded in Technovit \((n=21)\) was analysed separately from material embedded in paraffin wax, because of a difference in tissue shrinking during histochemical processing. The mean diameter of the seminiferous tubules for each dog was calculated.

Classification of dogs

In comparing dogs of different breeds, a correction factor was applied, based on the fact that dogs of small breeds live longer than those of large breeds. The age of a dog at the time of castration was expressed as a percentage of its geriatric age using the method of Goldston et al. (1989) (Table 2). This method divides dogs into categories according to their body weight and the age at which they become geriatric or start having problems associated with ageing. Hence, in the present study, dogs were considered to be old (geriatric) when this age percentage was equal to or higher than 100%.

Characterization of tumours

Tumours were classified both histologically and using immunohistochemistry. Histologically, the criteria of Nielsen and Kennedy (1990) were used. Tumour sections were stained with Leydig-cell specific antibodies against the LH receptor (LH-R) and an antibody against 3β-hydroxy steroid dehydrogenase (3β-HSD) to confirm the suspected diagnosis (M. A. J. Peters, K. J. Teerds, I van der Gaag, D. G. de Rooij and F. J. van Sluijs, unpublished). Tubules that were overgrown with Sertoli cells or germ cells were classified as intratubular Sertoli cell tumours or intratubular seminomas. Tumours were considered to be local when a discrete nodule or group of Sertoli or seminoma cells was visible outside the seminiferous tubules. The size of the tumour and the appearance of more than one tumour in a testis were not quantified.

Leydig cell hyperplasia was defined according to McEntee (1990) as an increase in the numbers of Leydig cells between tubules without destruction or displacement of surrounding tubules. A lesion was scored as a tumour when a discrete nodule or group of interstitial cells locally replaced or displaced the seminiferous tubules.

Tumours were categorized as clinically detected and non-clinically detected. Clinically detected tumours were found by physical examination (palpation of the testis) and confirmed by histology. Non-clinically detected tumours were not palpable at physical examination and were discovered by histology.

Statistical analysis

The testes were categorized according to age and the presence of tumours. Age was expressed as percentage of geriatric age, as described above. Since normal distribution of values could not be assumed, the mean Johnsen scores were categorized as shown (Table 3) to make statistical analysis of the data possible. The influence of ageing on spermatogenesis was calculated using a log-linear model with the Johnsen score (in five groups) as the dependent variable, using S-plus 4.5 statistical software (Mathsoft Inc., Cambridge, MA).

Tissue was categorized as non-tumorous (no neoplastic tissue present), non-clinically detected tumorous (neoplastic tissue found by histological examination but not by physical examination), or clinically detected tumorous. A log-linear model was used to evaluate differences in the Johnsen score (in five groups) between testes of dogs without tumours and the unaffected testes of dogs with non-clinically or clinically detected tumours in the contralateral testes. Differences between the affected and unaffected testes and differences between bilaterally and unilaterally affected dogs were analysed by the same method also using S-plus 4.5 software.

Analysis of the tubular diameter data was performed using SSPS software with the weight of the dogs as a covariate, tubular diameter as the variable and the percentage of geriatric age at time of castration treated as a fixed factor. Paraffin wax-embedded and Technovit-embedded material were analysed separately.

Table 2. Age at which different sized dogs are considered to become geriatric (Goldston, 1989)

<table>
<thead>
<tr>
<th>Weight category (kg)</th>
<th>Geriatric age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small dogs (0–9)</td>
<td>11.5 years</td>
</tr>
<tr>
<td>Medium dogs (10–23)</td>
<td>10.9 years</td>
</tr>
<tr>
<td>Large dogs (24–40)</td>
<td>8.9 years</td>
</tr>
<tr>
<td>Very large dogs (&gt;40)</td>
<td>7.5 years</td>
</tr>
</tbody>
</table>

Results

Seminiferous epithelium in normal dogs of various ages

During the preparation of the testes for histochemical processing, small tumours were found in some of the tissue blocks. These small, sometimes only microscopically recognizable, neoplasms were found in 21 of 74 dogs. In the remaining 53 dogs (45 pure bred dogs from 24 different breeds and eight dogs of mixed breeding), there were no microscopic neoplastic lesions, implying that spermatogenesis would be expected to be normal (Fig. 1a,b). Nevertheless, in only 12 of these 53 dogs, including ten pure bred dogs from six different breeds and two dogs of mixed breeding of 2–10 years of age equalling 17–115% of their geriatric age, spermatogenesis appeared to be completely normal, resulting in a mean Johnsen score of 10 (Fig. 2). In the remaining 41 dogs, most sections of testes contained some tubules in which spermatogenesis was abnormal (Fig. 1c),...
between dogs of different ages within these two groups.

resulting in a mean Johnsen score of slightly less than 10. In such cases, areas of seminiferous tubules of normal appearance were found either intermingled or directly adjacent to groups of tubules with poor spermatogenesis and germ cell loss. In the seminiferous tubules with poor spermatogenesis, there were no germ cells (Fig. 1d) or there was a partial or complete arrest at the spermatocyte (Fig. 1e) or spermaticid stage (Fig. 1f), or complete but reduced spermatogenesis (Fig. 1g). There was no apparent relation between the nature of the abnormalities and the age of the dog. In six dogs of five different breeds in which there was no evidence of testicular tumours or Leydig cell hyperplasia, spermatogenesis was generally poor, evidenced by a Johnsen score of < 9 (Fig. 2). Among this group of six, in two young dogs (a beagle and a dog of mixed breeding), spermatogenesis was completely arrested at the spermatocyte stage, implying that these dogs were not fertile. In three other of these six dogs, there was only a partial arrest, characterized by groups of tubules containing only Sertoli cells or cells up to the spermatocyte stage. In the sixth dog, there were tubules with poor spermatogenesis scattered throughout the testicular sections.

The mean Johnsen score for the entire group of 51 healthy and fertile dogs (two infertile dogs were excluded) was 9.6 (SD = 0.8). In general, there was no difference in the quality and quantity of spermatogenesis between the two testes of individual dogs, as evidenced by the mean Johnsen scores. A relationship between ageing and the quality of spermatogenesis using the modified Johnsen score was not detected.

The mean tubular diameter was determined in the 51 reproductively healthy normal dogs that had no evidence of microscopic or macroscopic tumours. The relation between the mean tubular diameter and the percentage of geriatric age at the time of castration was analysed separately for material embedded in paraffin wax (n = 40) and that embedded in Technovit (n = 11). The mean tubular diameter of testes material embedded in paraffin wax was 201 µm (SD = 25.3) and that of material embedded in Technovit was 217 µm (SD = 22.4) but no significant differences were found between dogs of different ages within these two groups.

### Table 3. Grouping of Johnsen scores for statistic calculations, as applied in dogs

<table>
<thead>
<tr>
<th>Johnsen score</th>
<th>Category used for SPSS</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–3</td>
<td>1</td>
<td>No cells to only spermatogonia</td>
</tr>
<tr>
<td>4–5</td>
<td>2</td>
<td>Only spermatocytes</td>
</tr>
<tr>
<td>6–7</td>
<td>3</td>
<td>Only spermatids</td>
</tr>
<tr>
<td>8–9</td>
<td>4</td>
<td>Complete spermatogenesis with fewer spermatozoa</td>
</tr>
<tr>
<td>10</td>
<td>5</td>
<td>Complete spermatogenesis</td>
</tr>
</tbody>
</table>

Seminiferous epithelium in dogs with non-clinically detected testicular tumours

Histological examination revealed neoplastic changes in 21 of the 74 dogs in which no abnormalities were detected by physical examination. These 21 dogs included nine different pure breeds and five dogs of mixed breeding, and their ages ranged from 1 to 15 years, equivalent to 13–142% of their geriatric age. In five dogs of five different breeds ranging from 2 to 12 years of age (21–142% of their geriatric age), one or both testes were macroscopically abnormal, being flaccid and reduced in size. All five dogs had tumours of different types in both testes that were not detectable by palpation. Microscopic examination of the flaccid testes revealed areas of sclerotic tubules, peritubular and interstitial fibrosis, and thick-walled blood vessels. In four dogs, a Leydig cell tumour and, in one dog, a Sertoli cell tumour was the largest type of tumour present in a solid form. In these five dogs, there was always more than one type of tumour per testis.

In the other 16 dogs with non-clinically detected tumours, the testes were palpably normal but macroscopic and microscopic examination after dissection revealed testicular tumours. In nine of 21 dogs, the tumours were bilateral and, in nine dogs, more than one type of tumour was detected per testis. The tumours included 14 intratubular and four solid seminomas, seven intratubular and three solid Sertoli cell tumours, five cases of Leydig cell hyperplasia, and seven Leydig cell tumours. Hence, 40 non-clinically detected tumours were present in 21 dogs, the majority (57%) being found in the geriatric dogs.

Both the occurrence of solid tumours and the occurrence of two or more different types of tumour in one testis were more frequent in dogs with bilateral tumours. The mean Johnsen score for testes of dogs with bilateral testicular tumours was 5.5 (n = 9, SD = 2.0). In dogs with unilateral tumours, the mean Johnsen score was 9.3 (n = 12, SD = 0.4) for the affected testis and 9.6 (n = 12, SD = 0.4) for the unaffected testis.

Differences in the type of spermatogenic abnormalities between old and young dogs and among tumour types were not significant. The differences in Johnsen scores were related to the number of seminiferous tubules in which there were abnormalities. When a non-clinically detected tumour was present in one testis, there appeared to be no difference in the quality and quantity of spermatogenesis between the two testes, as reflected by the mean Johnsen scores. In dogs affected bilaterally, there were sometimes considerable differences in the Johnsen score between the two testes (Fig. 3).

Seminiferous epithelium in dogs with clinically detected testicular tumours

The ages of dogs with clinically detected testicular tumours varied from 6 to 16 years, equivalent to 62–215% of
their geriatric ages. This group of 28 dogs included 17 different pure breeds and six dogs of mixed breeding, and body weights ranged from 5 to 50 kg.

There was great local variation in spermatogenesis in dogs with clinically detected tumours. Normal spermatogenesis was found in tubules adjacent to tubules overgrown with tumour cells or tubules that were sclerotic. Clusters of tubules containing only Sertoli cells were found adjacent to tubules with normal spermatogenesis or tubules adjacent to a solid tumour (Fig. 4a–c). Abnormalities in the process of spermatogenesis were found at the spermatogonia, spermatocyte and spermatid stages, and tubules devoid of germ cells were also common. The mean Johnsen score was 4.4 in dogs with clinically detected tumours in both testes ($n = 17$, $SD = 2.4$). In dogs with unilateral tumours, spermatogenesis in the unaffected testis resembled that of testes of normal dogs. The mean Johnsen score was 9.4 ($n = 11$, $SD = 0.5$) in the unaffected and 5.6 ($n = 11$, $SD = 2.4$) in the affected testis. A relation between the Johnsen score and the type of tumour could not be demonstrated.

Fig. 1. Histological sections of adult dog testes. (a) Normal spermatogenesis with elongated spermatids (arrowheads). (b) Normal spermatogenesis stage V with released spermatozoa. (c) Tubules with normal (asterisk) and abnormal (O) spermatogenesis. (d) No germ cells present, only Sertoli cells (arrowheads). (e) Tubules with an arrest at the spermatocyte (arrowheads) stage. (f) Tubules with an arrest at the spermatid (arrowheads) stage. (g) Tubules with a reduced number of elongate spermatids (arrowheads). Scale bars represent (a, e, f, g) 45 μm, (b, d) 36 μm, (c) 90 μm.
differences in the mean Johnsen scores were found between the two testes in both unilaterally and bilaterally affected dogs (Fig. 5).

Bilateral tumours were found in 61% of the dogs with clinically detected testicular tumours, and multiple tumours within one testis were found in 46% of the animals. There were 18 solid seminomas (Fig. 6a) and four tubular seminomas, five solid Sertoli cell tumours (Fig. 6b) and four tubular Sertoli cell tumours, and 24 solid Leydig cell tumours (Fig. 6c) and two cases of Leydig cell hyperplasia. Most (86%) of the clinically detected tumours were found in the 24 old dogs, characterized by a relative age higher than 100% of the geriatric age (Table 2).

Fig. 2. The relation between geriatric age (Table 2) and modified Johnsen score (Table 1) in dogs (n = 53) without testicular tumours. In six dogs, spermatogenesis was generally poor, evidenced by a Johnsen score of < 9. In about 60% of the young dogs, the Johnsen score appeared to be less than the maximum value of 10.

Fig. 3. Johnsen score in 21 dogs bearing a non-clinically detected testicular tumour. Each symbol represents one dog. The Johnsen score of the tumour-bearing testis (unilaterally affected animals: □) or the left testis (bilaterally affected animals: △) is indicated on the x-axis. The Johnsen score of the unaffected (unilaterally affected animals) or right (bilaterally affected animals) testis is indicated on the y-axis or, if the origin was not known, randomly assigned to be the left or right testis. Five bilaterally affected dogs were observed, with an abnormal testis that was flaccid and reduced in size (▲).

Fig. 4. Histological sections of early testicular tumours in dogs. (a) Intratubular seminoma (asterisk) and normal tubule (○). (b) Intratubular Sertoli cell tumour. (c) Leydig cell hyperplasia indicated by arrowheads. Scale bar represents 45 μm.
Comparison of normal dogs with dogs bearing non-clinically detected or clinically detected tumours

In unilaterally affected dogs with non-clinically detected tumours, the appearance of the seminiferous tubules in both the affected and unaffected testes was similar to that in normal dogs. The mean Johnsen scores were not significantly different (Fig. 7).

In unilaterally affected dogs with clinically detected tumours, the mean Johnsen score of the affected testes was significantly lower than that of the unaffected testes \((P < 0.001)\) and was also much lower than that of testes bearing non-clinically detected tumours \((P < 0.001)\) (Fig. 7). The appearance of the unaffected testis was quite similar to that of normal testes and, in agreement with this finding, the difference in the mean Johnsen score was not significant.

In bilaterally affected dogs with non-clinically detected tumours, the mean Johnsen score in both testes was significantly lower than in the affected testis of unilaterally tumour bearing dogs \((P < 0.001)\) (Fig. 7). The appearance of the tubules was similar to that of dogs with clinically detected tumours.

The appearance of the clinically detected tumours of bilaterally affected dogs resembled that of dogs in which only one testis was affected, and the mean Johnsen score between these groups of animals was not significantly different (Fig. 7).

Discussion

The results of the present study indicate that spermatogenesis in reproductively healthy dogs does not deteriorate significantly with increasing age. This is the first reported study of the effect of non-clinically detected tumours and of Leydig cell hyperplasia on spermatogenesis in dogs and of
the prevalence of these tumours. In a surprisingly large number of dogs, one or more tumours were found. Moreover, independent of age or neoplasia, in about 10% of the dogs, abnormalities were found in the process of spermatogenesis, and two young dogs out of 53 dogs were azoospermic. These findings indicate that the dog testis is prone to abnormalities.

In dogs, it is generally believed that a breed predisposition for development of testicular neoplasms or abnormalities of the spermatogenic epithelium does not play a role (Nielsen and Kennedy, 1990), except possibly in the boxer breed, in the development of seminomas (Howard and Nielsen, 1965). In the present study, many breeds (including two boxers without clinically detected testis tumours) were examined, and most breeds were represented by only one or two individuals. This is an indication that a breed predisposition is not very likely and, at least in the present study, did not influence the results. In addition, it is difficult to equate the frequency of neoplasia with a particular breed of dog because of changes in popularity of those breeds (Nielsen and Kennedy, 1990).

In dogs of all ages, there were occasional tubules with poor spermatogenesis, resulting in a Johnsen score of < 10. This was even the case in 60% of the non-tumour-bearing young dogs. In comparison, 85% of young men appeared to have normal spermatogenesis (Johnsen score 10) and 14% had incomplete spermatogenesis with a reduced number of germ cells (Johnsen score between 8 and 9) (Paniagua et al., 1991). Johnsen (1970) reported that 73% of men between 23 and 34 years of age had normal spermatogenesis, while 20% had complete spermatogenesis with minor defects, resulting in a Johnsen score of < 10. Hence, the qualitative aspects of spermatogenesis in dogs appear to be inferior to those in men.

No differences were observed between young and old dogs in the nature of the spermatogenic defects in the abnormal tubules. Tubules without germ cells were observed, as were deficiencies at the spermatocyte and spermatid stages. The observation that spermatogenesis in dogs without testicular tumours or hyperplasia did not decrease with increasing age differs from observations in men and rats (Paniagua et al., 1987, 1991; Johnson, 1989; Wright et al., 1993). In these species, age-related testicular atrophy owing to a loss of germ cells has been described. In their study of spermatogenesis during ageing, James and Heywood (1979) observed a lack of mature spermatids in old dogs. In another study, incomplete spermatogenesis in the seminiferous tubules was also found to be more prominent with increasing age (Lowseth et al., 1990) but, as is the case in the study of James and Heywood (1979), these findings were not quantified. In contrast, in the present study, spermatogenesis did not decrease with increasing age in normal dogs and there was no relation between ageing and changes in tubular diameter, as had been reported by Taha and Noakes (1982), who found significant differences in spermatogenesis between dogs over 6 years of age and some groups of younger dogs, although not among all age groups. An explanation for the difference between the present findings and those of Taha and Noakes (1982) might be that Taha and Noakes (1982) did not distinguish between dogs with and without clinically detected, or bilateral non-clinically detected tumours, which, in the present study were found mostly in dogs of advanced age. The impaired spermatogenesis in older dogs with tumours might have been the basis for the conclusion of Taha and Noakes (1982) that spermatogenesis deteriorates during ageing in dogs.

In the present study, 30% of the dogs in which there were no macroscopic abnormalities at the time of castration, there were unilateral or bilateral non-palpable testicular tumours. Dow (1962) reported the occurrence of intratubular seminomas in only 3%, and intratubular Sertoli cell tumours in only 5% of a group of 580 dogs, but did not record the number of dogs with Leydig cell hyperplasia. These proportions are considerably less than those observed in the present study, and may be due to the larger numbers of seminiferous tubules examined here. A thousand tubules per dog in ten different pieces of tissue were studied in the present study, while Dow (1962) studied only one piece of tissue per testis, and did not report the number of tubules examined.

In normal dogs and dogs with non-clinically or with clinically detected testicular tumours, there were seminiferous tubules that lacked germ cells or in which spermatogenesis was arrested at the spermatogonium, spermatocyte, or spermatid stages. No apparent differences were observed in the nature of these spermatogenic abnormalities between young and old dogs or among dogs with different types of tumour or with tumours of different sizes. Only quantitative differences in the number of abnormal tubular cross-sections were found.

In dogs with unilateral non-clinically detected or with clinically detected testicular tumours, spermatogenesis in the unaffected testis was undisturbed. Apparently, changes in hormone concentrations caused by the production of endocrine or paracrine factors by the tumours or the atrophied tubules were not large enough to disturb the
spermatogenic process in the unaffected contralateral testis. In contrast, clinically detected tumours profoundly affected spermatogenesis, reducing the average Johnsen score to 5.6, in affected testes. This effect was probably caused by physical disturbance of spermatogenesis or by paracrine factors produced by the tumour. Owing to the small numbers involved, we did not analyse the effects on spermatogenesis per tumour type, since this would have compromised statistical analysis too much. Spermatogenesis was more severely impaired by bilateral than by unilateral non-clinically detected tumours. In most cases, a solid tumour was present and such tumours were probably close to the threshold of clinical detection. In dogs with clinically detected-tumours, bilateral involvement had marked effects on spermatogenesis. In several testes, only a small band of compressed atrophic tubules remained, resulting in a very low Johnsen score.

The prevalence of clinical testicular tumours reported in other studies has varied from 2 to 60%, depending on the number of old dogs included in the study, since the incidence of testicular tumours increases with age (Reifinger, 1988; Mosier, 1989). This finding is in contrast to the situation in men, in which testicular cancer is most common between the ages of 20 and 34 (Swerdlow, 1993). It is also remarkable that, in dogs, testicular disorders originating from different cell lines – Sertoli cells of epithelial origin, Leydig cells of mesenchymal origin, and spermatogenic cells of germ cell origin – are often encountered in the same testis. Species susceptibility may play a role since dogs have the highest prevalence of primary tumours of the testis among domestic animals (Bloom, 1954). Furthermore, in dogs, nodular hyperplasia similar to that in the testis is also often found in the spleen, pancreas, liver, prostate gland and adrenals (Bloom, 1954), indicating that dogs are generally prone to the formation of neoplasms.

In this study, equal numbers of Leydig cell tumours and seminomas, both clinically detected and non-clinically detected, were found, while the number of Sertoli cell tumours, especially clinically detected, was much lower. In several other studies, nearly equal numbers of Sertoli cell tumours, Leydig cell tumours, and seminomas were observed in dogs (Nielsen and Lein, 1974; Feldman and Nelson, 1987), but Dow (1962) also found Leydig cell tumours and seminomas to be more numerous than Sertoli cell tumours.

In conclusion, age did not affect the quality of spermatogenesis in dogs but definite effects on spermatogenesis were found in the presence of clinically detected tumours and non-clinically detected tumours when they occurred bilaterally. Thirty per cent of aged normal dogs had testicular neoplasms that were not detectable on physical examination. The high prevalence of testicular tumours of different types indicates that the dog testis is a favourable environment for the development of these neoplasms.

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