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A Quantitative Study of Sertoli Cell and Germ Cell Populations as Related to Sexual Development and Aging in the Stallion[1]

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ABSTRACT

Testes from 47 stallions, 1–20 yr of age, were used to examine the influence of age on Sertoli and germ cell populations as well as on functional activity of Sertoli cells. For these stallions, the number of Sertoli cells per paired testes declined linearly with age, and was only 41.7% as great at age 20 as at age 2. However, development of reproductive organs proceeded until age 12–13, as evident from increases in paired testes weight and quantitative rates of spermatozoal production. Although the absolute number of Sertoli cells declined during this period of development, individual Sertoli cells displayed a remarkable capacity to accomodate greater numbers of developing germ cells. Between age 2 and age 12, the mean numbers of developing spermatogonia, young primary spermatocytes, old primary spermatocytes, and round spermatids supported by each Sertoli cell at Stage I of spermatogenesis increased by 49, 176, 153, and 161%, respectively.

INTRODUCTION

Sertoli cells are thought to play a direct role in the production of sperm. The remarkable spatial organization of the seminiferous epithelium is due to the physical structure of the Sertoli cell (Weber et al., 1983; Wong and Russell, 1983). Tight junctional complexes between adjacent Sertoli cells divide seminiferous tubules into basal and adluminal compartments (Dym and Fawcett, 1970), and this selective permeable blood-testis barrier is presumed to maintain an optimal spermatogenic environment as well as to protect germ cells from immonological or environmental hazards (Neaves, 1977). Also, Sertoli cells may be capable of regulating spermatogenesis through their control of the biochemical milieu in which germ cells develop. Androgenic hormones provide a major stimulus for spermatogenesis (Hansson et al., 1976), and the ability of the testis to concentrate androgens is due to the presence of a testis-specific androgen-binding protein (ABP) synthesized by the Sertoli cells (Steinberger and Steinberger, 1977). Sertoli cells also are the source of lactate, a metabolic substrate necessary for survival of germ cells in advanced stages (Mita et al., 1982; Jutte et al., 1982).

The assumption that Sertoli cells form before puberty and then remain as a numerically stable population throughout adult life has constituted the basis for a Sertoli-cell correction factor employed with one widely used histologic method for quantifying mammalian spermatogenesis (Clermont and Morgentaler, 1955; Lino, 1971; Berndtson, 1977). For this correction, it was presumed that any alteration in spermatogenesis in postpubertal animals would necessarily be reflected in a change in the ratio of germ cells to Sertoli cells. However, Johnson and Thompson (1983) reported recently that the population of equine Sertoli cells increased until 4–5 yr of age and fluctuated with season, but that adult equine testes had only a limited capacity to alter the ratio of Sertoli cells to germ cells. Since stability of the Sertoli-cell population constitutes the basis for the Sertoli-cell correction and since the findings of Johnson and Thompson (1983) have important implications for the fundamental mechanisms by which seasonal and age-related changes in equine spermatogenesis might be mediated, a further examination of this issue by means of an alternative approach was undertaken.

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MATERIALS AND METHODS

General

Testes were obtained from 47 stallions of light-horse type at castration or at the time of slaughter at commercial abattoirs during June and July. Age was estimated by examinations of the incisor teeth and was distributed as follows (age, number of stallions): 1,2: 1,5, 6; 2,10; 3,6; 4,3; 5,1; 6,6; 7,5; 9,1; 10,1; 11,2; 13,1; 18,2 and 20,1. The testes from each stallion were weighed immediately. The tunica albuginea was removed and weighed, and the weight of the testicular parenchyma was determined by the difference. One portion of testicular parenchyma from each testis was placed in a tared vial and frozen on dry ice. These samples were stored below −25°C until used for the determination of spermatid reserves. A second portion of tissue was fixed in Zenker-formol solution, dehydrated, embedded in paraffin, sectioned at 5 μm, stained with periodic acid-Schiff's reagent and hematoxylin (Berndtson et al., 1979), and utilized for histometic evaluations.

Histometic Evaluation of Germ Cell: Sertoli-Cell Ratios

The ratio of spermatogonia, young primary spermatocytes, old primary spermatocytes, and round spermatids to Sertoli cells was determined from direct counts of nuclei of germ cells or of Sertoli cells with a visible nucleolus in sections of 20 seminiferous tubules at Stage I (as defined by Swierstra et al., 1974), for each stallion. The mean diameter of the nuclei or nucleoli was also determined for each type of cell from each stallion and used to convert crude cell counts to true counts by application of Abercrombie's procedure (Abercrombie, 1946; Berndtson, 1977). The resulting true counts were used to calculate ratios of germ cells to Sertoli cells.

Determination of Number of Sertoli Cells

The number of Sertoli cells per paired testes was calculated by means of the following equation:

$$\text{Total Sertoli cells} = \text{Total elongated spermatids} \times \frac{\text{Sertoli cells}}{\text{spermatids}} \times 2.033$$

For this equation, the total number of elongated spermatids represented homogenization-resistant elongated spermatids as determined by the procedure of Amann and Almquist (1961). The ratio of Sertoli cells to spermatids was derived from the histometric analyses described previously. The product of the number of homogenization-resistant elongated spermatids times the ratio of Sertoli cells to spermatids represented the number of Sertoli cells associated with this population of elongated spermatids. Since these elongated spermatids are present for only 6 days (Amann et al., 1979) of a 12.2-day cycle of the seminiferous epithelium (Swierstra et al., 1974), an additional factor of 2.033 was applied so that Sertoli cells at all stages of spermatogenesis were included. The number of Sertoli cells per gram was obtained by dividing the total number of Sertoli cells by testicular parenchymal weight.

Quantitative Assessments of Spermatogenesis

The rate of spermatozoal production was determined by each of two different methods. The first was by enumeration of homogenization-resistant spermatids in homogenates of testicular parenchyma (Amann and Almquist, 1961). Previously frozen samples of testicular tissue (1—20 g) were thawed, minced with scissors, and placed in 150 ml of homogenization fluid. The suspension was homogenized for two min in a Waring blender. The blender was rinsed with an additional 300 ml of homogenization fluid that was then added to the homogenate. Samples were stored at 4°C, and quantitative evaluation by hemacytometry was carried out within 48 h. Duplicate evaluations within 10% of each other were made by each of four observers, and the average estimate of the four observers was used to calculate the number of spermatids per gram and per testis.

For the second method, spermatogenesis was assessed by the procedure of Clermont and Morgentaler (1955). This technique was applied as described previously for the histometric evaluation of ratios of germ cells to Sertoli cells, except that the true counts were further adjusted by a Sertoli-cell-correction factor (Clermont and Morgentaler, 1955; Lino, 1971; Berndtson, 1977). The latter eliminates potential biases that can arise from alterations in testicular dimensions as a result of given treatments or by histological processing. The conventional cell adjustment, which enables counts of germ cells to be expressed in relation to a standard number of Sertoli

cells, has been based on the assumption that populations of Sertoli cells are numerically stable (Clermont and Morgentaler, 1955; Lino, 1971; Berndtson, 1977). Since the population of Sertoli cells was found to decline linearly with age in the present study (see Results), it was necessary to modify the standard technique by incorporating an additional adjustment based upon the rate of demise of the Sertoli-cell population. To this end, we first calculated the predicted number of Sertoli cells per paired testes for stallions of each age, based upon the regression equation $Y=2.15-.0654\ X$, where Y equals the number of Sertoli cells in billions and X equals age in years. The predicted average number of Sertoli cells for the 1-yr-old stallions was assigned a value of 1.0, and the relative values for stallions of all other ages were computed and regarded as age-correction factors. The numbers of germ cells obtained by the standard methods (i.e., Abercrombie and Sertoli-corrected true counts for each germ cell type and for each stallion) were then multiplied by the appropriate age-correction factor. The resulting data are designated hereafter as germ-cell counts per tubular cross-section. The precision of estimates of cell counts obtained by the histometric methods used here was quite high. For example, the average coefficient of variation between duplicate estimates of the number of round spermatids per tubular cross-section for each of 10 stallions chosen at random was 11.8%, when each estimate was based on only 10 tubular cross-sections. Stallions means reported here are based on 40 tubules each (i.e., 20 tubules per testis × two testes).

Distribution of Sertoli Cells Per Unit of Seminiferous Tubular Length or Basement Membrane Area

The mean area of the seminiferous tubular basement membrane for each Sertoli cell at Stage I of the cycle of the seminiferous epithelium was calculated as follows. First, the average length of Zenker-formol-fixed Stage I seminiferous tubule associated with one Sertoli cell was computed by dividing 5 μm (i.e, the section thickness) by the number of Sertoli cells per tubular cross section. Data for this calculation were derived from the quantitative histological procedure described previously. Secondly, the average diameter of the seminiferous tubules was determined by measuring—with an ocular micrometer—the diameter of 50 essentially round, randomly selected seminiferous tubular cross sections for each stallion. The repeatability of such measurements was excellent; the average coefficient of variability of duplicate estimates, each based on only 25 seminiferous tubules per estimate (i.e, one half the number used here) for 10 stallions was 2.05%. The seminiferous tubules were assumed to be cylindrical and, thus, the average area of basement membrane in fixed tissue per Sertoli cell was determined by multiplying the average length of tubule per Sertoli cell times the average circumference (i.e., 3.1417 × tubular diameter) of the tubules.

Statistical Analyses

Data for both testes were averaged or pooled within stallions for each parameter and subjected to first- and second-order regression analyses with age as the independent variable. The statistical significance of the linear or quadratic response was assessed by an *F*-test from an analysis of variance and, where this was significant, by a *t*-test for the significance of the individual coefficient(s) of regression (Steel and Torrie, 1960). The lowest level of statistical significance from among all tests (i.e. from the *F*-test or *t*-test(s) for each coefficient of regression) is presented subsequently. All coefficients of determination presented represent values adjusted for degrees of freedom. Spermatogenesis was not sufficiently developed in the two 1-yr-old stallions or in four of the six 1.5-yr-old stallions to permit identification of Stage I seminiferous tubules. Thus, data derived from histometric procedures could not be obtained for these stallions.

RESULTS

The number of Sertoli cells per pair of testes declined linearly with age ($p<0.05$), and the average predicted from the regression equation was only 41.7% as great at age 20 as at age 2 (Fig. 1). Nonetheless, reproductive development proceeded until about 12 yr of age in these stallions. This was evident first from testicular weight (Fig. 2), which more than doubled between 1 and 12 yr of age before declining ($p<0.01$). The change in testicular weight with age was due to similar changes in the weight of both the tunica albuginea and testicular parenchyma (the influence of age on the grams of testicular parenchyma [Y] was characterized by the equation $Y=85.2+37.0x-1.58x^2$, where x=age in years, $p<0.01$). Since the Sertoli population declined during this period of

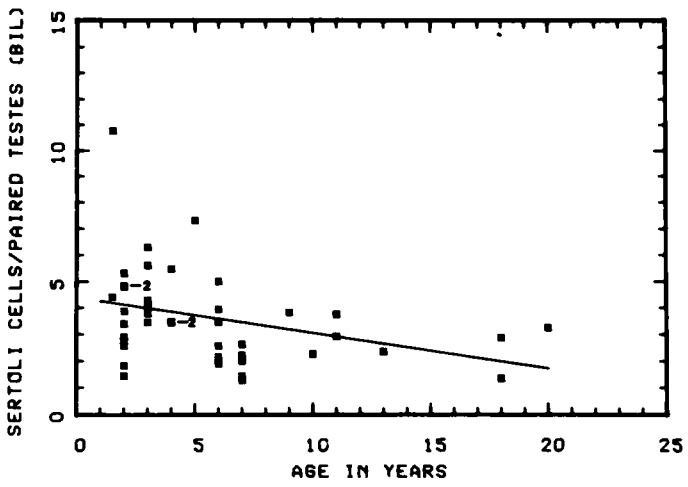

FIG. 1. The relationship between age (X, in yr) and the number of Sertoli cells per pair of testes (Y, in billions) was characterized by the equation $Y = 4.37-0.133X$ (n=41, $p<0.05$). The coefficient of determination (R^2) was 9.6%.

testicular growth, the number of Sertoli cells per gram of testis actually declined until age 13 and then increased slightly (Fig. 3, $p<0.05$).

The number of Sertoli cells per cross-section (Fig. 4) declined linearly with age ($p<0.01$). Since sections were prepared at 5 μm, it was possible to calculate the average distribution of Sertoli cells along the length of these Stage I tubules (Fig. 5). Whereas at 1–2 yr of age there was one Sertoli cell for approximately every 0.6 μm of fixed seminiferous tubule, by age 20 this was reduced to approximately one Sertoli cell for every 1.1 μm of tubule. This change reflected a decline in numbers of Sertoli cells rather than any major change in fixed tubular length. For example, between 2 and 12 yr of age, the average length of seminiferous tubule per Sertoli cell increased by 47.8%, but the 12-yr-old stallions contained only 67.6% as many total Sertoli cells. Thus, the total length of seminiferous tubules was virtually identical at both ages (i.e., 147.8 × 0.676=99.9%). However, the average tubular diameter (Fig. 6) changed over time ($p<0.05$) in a manner essentially resembling that for paired testis weight. From the average length of seminiferous tubule per Sertoli cell and the average tubular diameter, the average area of basement membrane in fixed tissue associated with each Sertoli cell was calculated (Fig. 7). The mean area per Sertoli cell increased throughout life, and was essentially three times as great in the 20-yr-old stallion as in those 1 yr of age ($p<0.05$).

The functional activity of individual Sertoli cells in supporting development of germ cells is depicted in Figure 8. Between 2 and 13 yr of age, the number of spermatogonia per Sertoli cell increased by more than 50% ($p<0.01$). During the same time, the numbers of young primary spermatocytes, old primary spermatocytes, and round spermatids per Sertoli cell increased more than 150% ($p<0.01$). Overall, the average number of germ cells (spermatogonia, spermatocytes, and spermatids) supported by each Sertoli cell at Stage I increased from 9.1 at age 2 to 23.1 at age 13. This represented an average 2.5-fold increase in the number

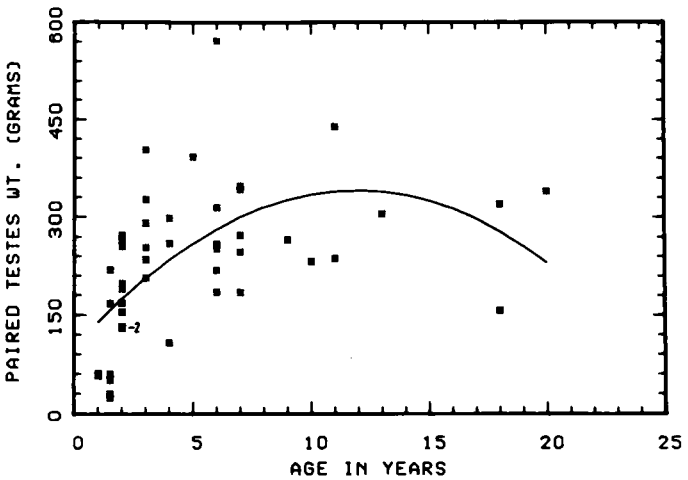

FIG. 2. The relationship between age (X, in yr) and the testis pair weight (Y, in grams) was characterized by the equation $Y = 101.0+40.3X-1.69X^2$ (n=47, $p<0.01$). The coefficient of determination (R^2) was 29.2%.

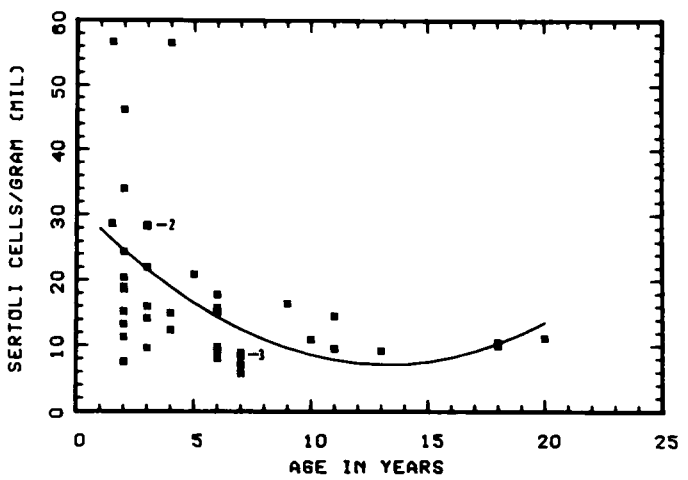

FIG. 3. The change in the number of Sertoli cells per gram of testicular parenchyma (Y, in millions) with age (X, in yr) was described by the equation $Y = 31.4-3.69X+0.14X^2$ (n=41, $p<0.05$). The coefficient of determination (R^2) was 21.7%.

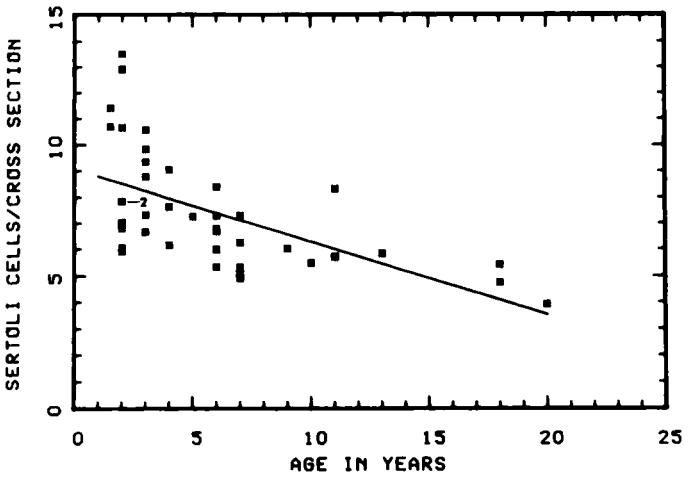

FIG. 4. The decline in the number of Sertoli cells per Stage I seminiferous tubular cross section (Y) prepared at 5 μm due to age (X, in yr) was characterized by the equation Y=9.05−0.276X (n=41, $p<0.005$). The coefficient of determination (R^2) was 32.3%.

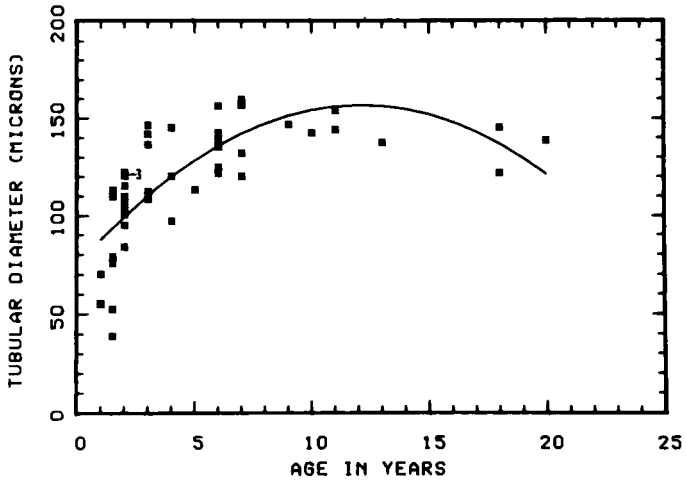

FIG. 6. The change in the mean minor diameter of seminiferous tubules in fixed tissue (Y, in μm) with age (X in yr) was described by the equation Y=74.6+13.6X−0.563X^2 (n=47, $p<0.005$). The coefficient of determination (R^2) was 53.8%.

of developing germ cells supported by each Stage I Sertoli cell.

The number of homogenization-resistant elongated spermatids per gram of parenchyma is depicted in Figure 9. Spermatozoal production was absent in both of the 1-yr-old stallions and also in four of the six 1.5-yr-old stallions in this study, as judged by the absence of elongated spermatids. However, spermatozoal production was well established in all stallions by age 2 (Fig. 9). The number of spermatids per gram increased thereafter from 39.6 million at age 2 to a peak of 69.8 million at age 12. The influence of age on the total number of elongated spermatids per testis pair (Fig. 10) closely resembled that on number of spermatids per gram, with a peak level of spermatozoal production observed at age 12 as judged by this method. Total rates of spermatozoal production varied greatly among stallions of any given age (Fig. 10).

Spermatozoal production was also assessed by determining the numbers of germ cells per Stage-I seminiferous tubular cross-section, as presented in Fig. 11. The mean number of spermatogonia per tubu-

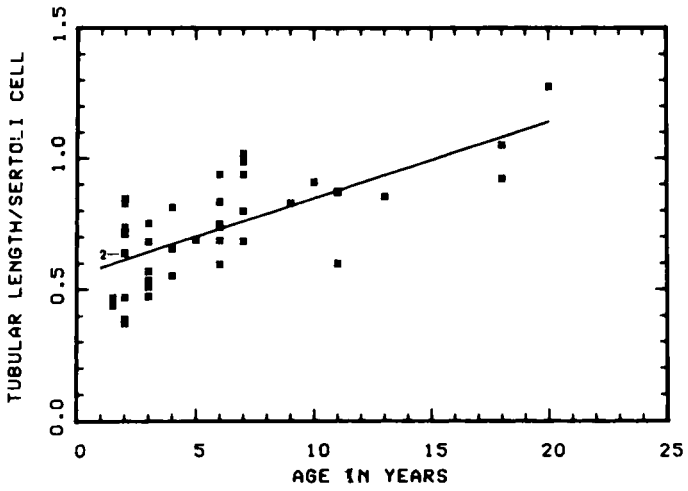

FIG. 5. The relationship between age (X, in yr) and the average length of Stage I seminiferous tubule in fixed tissue per Sertoli cell (Y, in μm) was characterized by the equation Y=0.555+0.0293X (n=41, $p<0.005$). The coefficient of determination (R^2) was 47.8%.

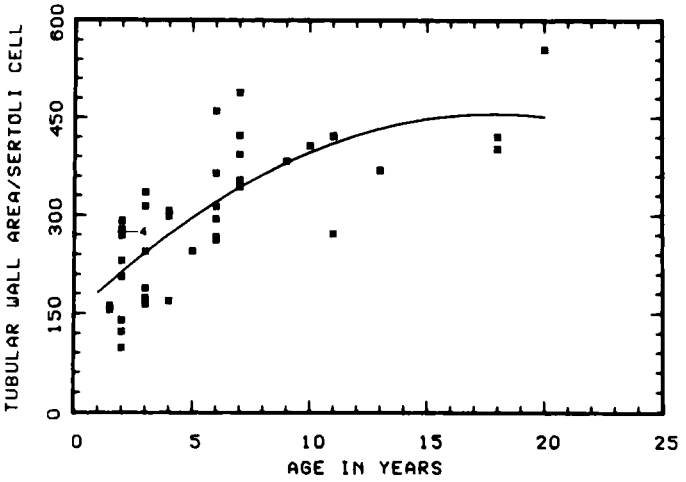

FIG. 7. The relationship between age (X, in yr) and the average area of seminiferous tubular basement membrane in fixed tissue (Y, in μm^2) associated with each Sertoli cell was characterized by the equation Y=148.0+34.5X−0.966X^2 (n=41, $p<0.05$). The coefficient of determination (R^2) was 55.2%.

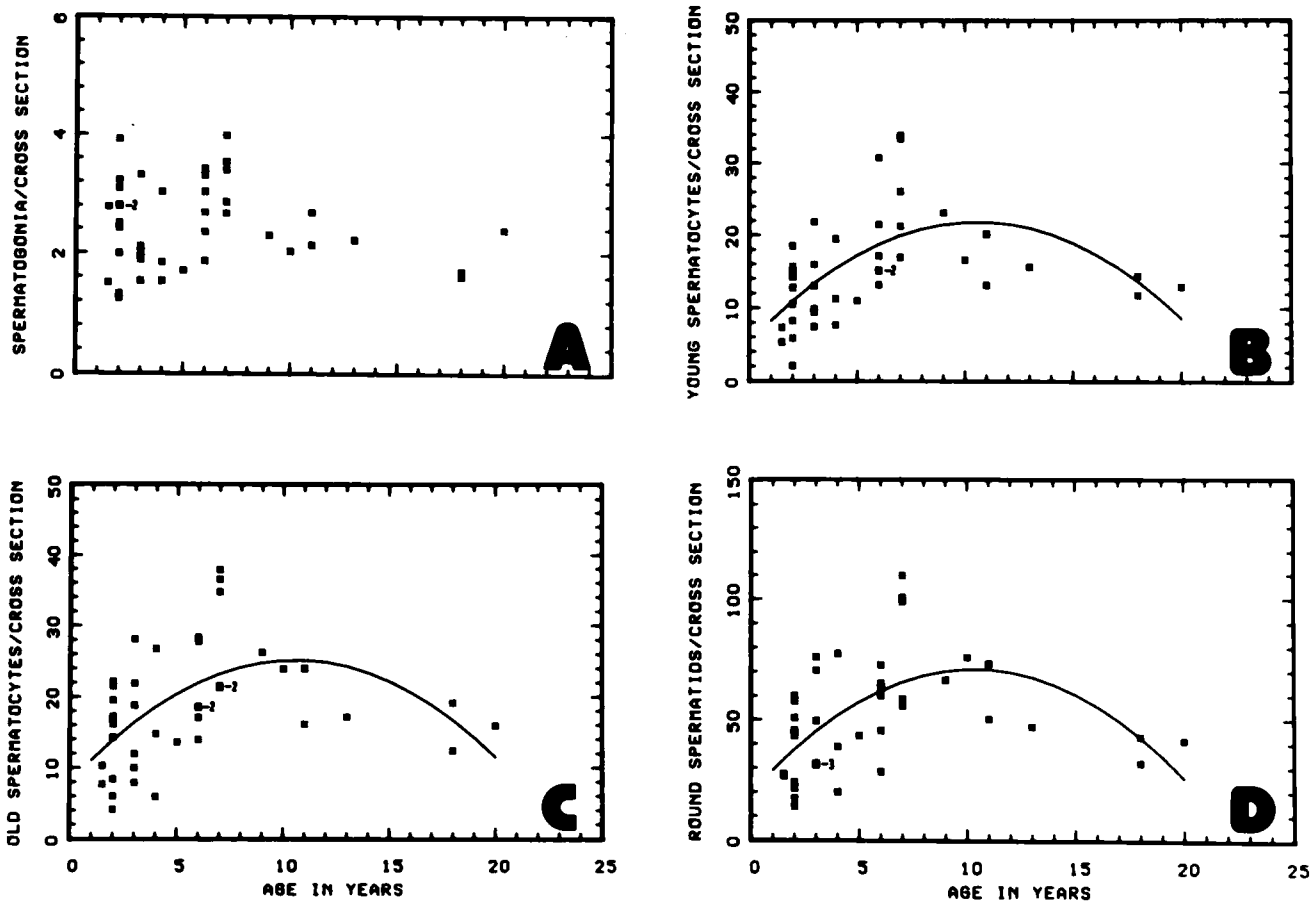

FIG. 8. (A) The relationship between age (X, in yr) and the mean number of spermatogonia per Sertoli cell at Stage I of the cycle of the seminiferous epithelium (Y) was described by the equation $Y=0.302+0.0164X$ (n=41, $p<0.005$). The coefficient of determination (R^2) was 31.5%. (B) The relationship between age (X, in yr) and the mean number of young primary spermatocytes per Sertoli cell in Stage I seminiferous tubules (Y) was characterized by the equation $Y=0.519+0.525X-0.0187X^2$ (n=41, $p<0.01$). The coefficient of determination (R^2) was 50.1%. (C) The relationship between age (X, in yr) and the mean number of old primary spermatocytes per Sertoli cell in Stage I seminiferous tubules (Y) was described by the equation $Y=0.863+0.553X-0.0188X^2$ (n=41, $p<0.01$). The coefficient of determination (R^2) was 46.6%. (D) The relationship between age (X, in yr) and the mean number of round Stage I spermatids per Sertoli cell (Y) was characterized by the equation $Y=1.92+1.73X-0.0646X^2$ (n=41, $p<0.005$). The coefficient of determination (R^2) was 47.4%.

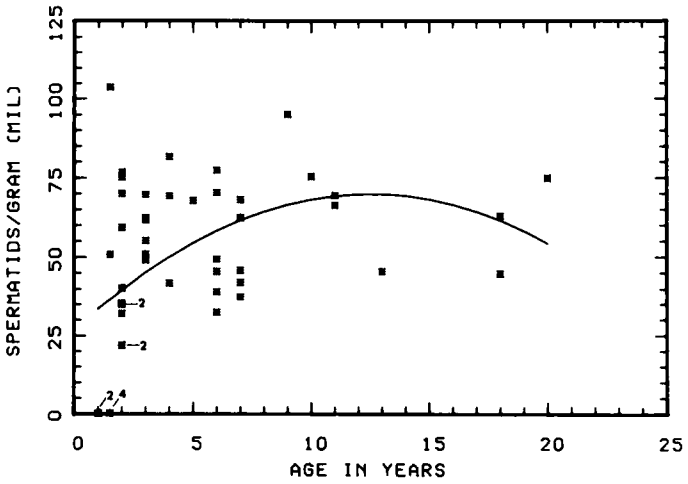

FIG. 9. The change in the number of homogenization-resistant elongated spermatids per gram of testicular parenchyma (Y, in millions) with age (X, in yr) was characterized by the equation $Y=26.9+6.89X-0.276X^2$ (n=47, $p<0.05$). The coefficient of determination (R^2) was 15.5%.

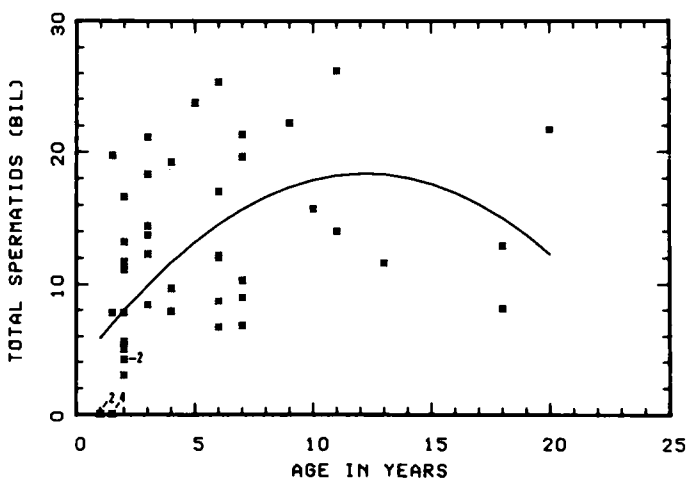

FIG. 10. The relationship between age (X, in years) and the total number of homogenization-resistant elongated spermatids per testis pair (Y, in billions) was described by the equation $Y=3.53+2.43X-0.0996X^2$ (n=47, $p<0.01$). The coefficient of determination (R^2) was 25.5%.

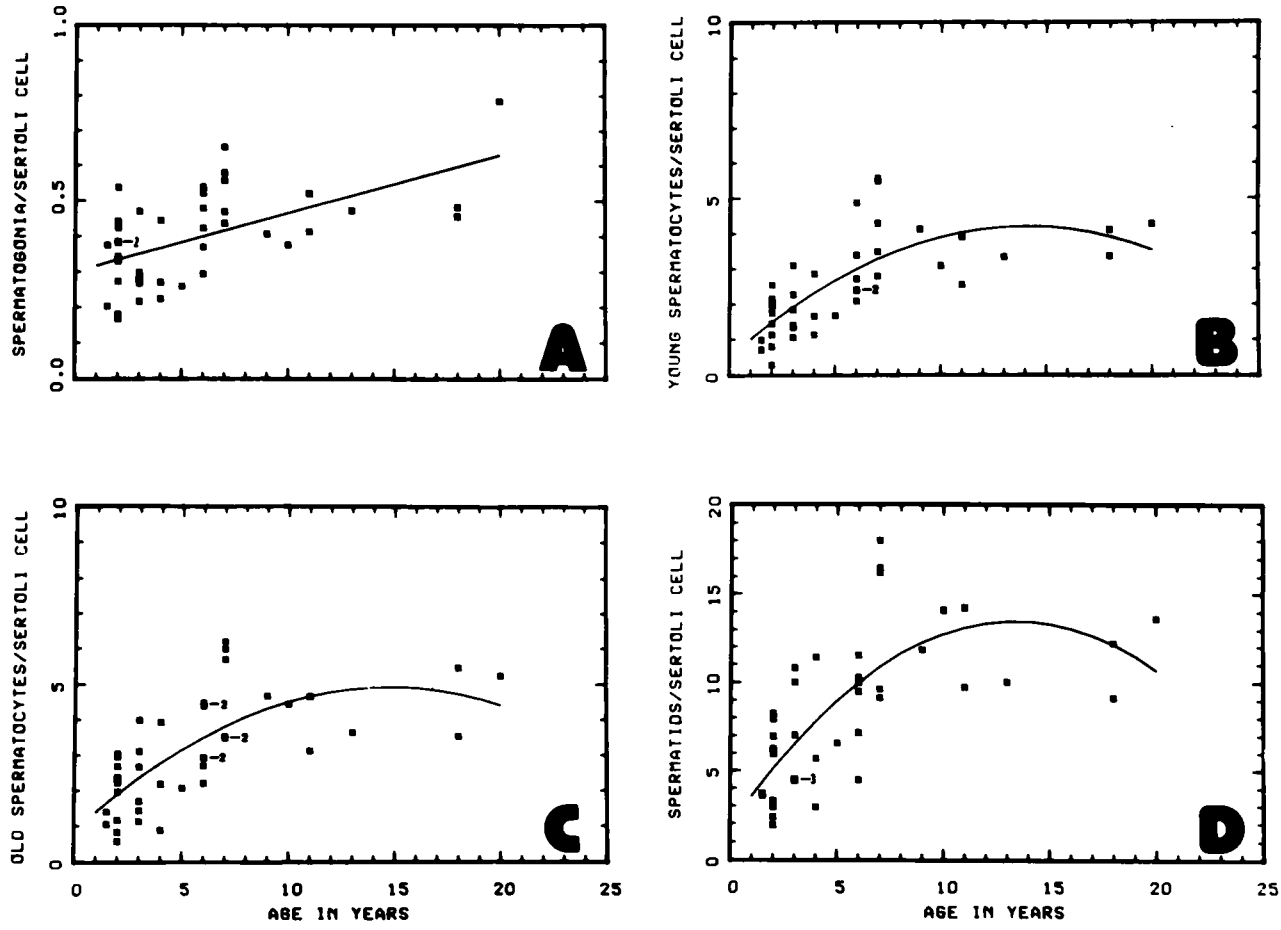

FIG. 11. (A) Mean numbers of spermatogonia per cross section of Stage I seminiferous tubules were similar at each age (n=41, $p>0.10$). Values shown are Abercrombie-corrected true counts adjusted by an age-modified Sertoli cell-correction factor. (B) The relationship between age (X, in yr) and the numbers of young primary spermatocytes per cross section of Stage I seminiferous tubules (Y) was described by the equation $Y=5.27+3.13X-0.148X^2$ (n=41, $p<0.005$). The coefficient of determination (R^2) was 31.3%. Individual observations are Abercrombie-corrected true counts adjusted by an age-modified Sertoli cell-correction factor. (C) The relationship between age (X, in yr) and the numbers of old primary spermatocytes per cross section of Stage I seminiferous tubules (Y) was characterized by the equation $Y=7.95+3.26X-0.154X^2$ (n=41, $p<0.005$). The coefficient of determination (R^2) was 25.0%. Individual observations are Abercrombie-corrected true counts adjusted by an age-modified Sertoli cell-correction factor. (D) The relationship between age (X, in yr) and the numbers of round spermatids per cross section of Stage I seminiferous tubules (Y) was represented by the equation $Y=19.6+9.96X-0.482X^2$ (n=41, $p<0.005$). The coefficient of determination (R^2) was 29.8%. Individual observations are Abercrombie-corrected true counts adjusted by a modified Sertoli cell-correction factor.

lar cross-section was not influenced by age ($p>0.10$). In contrast, the numbers of young primary spermatocytes, old primary spermatocytes, and round spermatids per tubular cross-section were strongly influenced ($p<0.005$), and were greatest at 10–11 yr of age. Thus, estimates of the effects of age on the rates of spermatogenesis were very similar when judged by each of two different methods.

DISCUSSION

The present finding of a decline in the absolute numbers of Sertoli cells with age resembles that cited for the human (Johnson et al., 1984) but differs from that of Johnson and Thompson (1983), who reported proliferation of Sertoli cells in stallions until age 4–5. For both the present study and that of Johnson and Thompson (1983), Sertoli cells were quantified by procedures that were independent of potential influences from alterations in testicular size and tubular or cellular dimensions. However, the methods differed and in each instance were based upon certain assumptions (Berndtson, 1977). The formula we used to compute numbers of Sertoli cells involved an assumption that the ratios of round (Stage I) spermatids to Sertoli cells and of elongated homogenization-resistant spermatids to Sertoli cells were similar. If significant numbers of spermatids had degenerated during spermatogenesis, absolute numbers of Sertoli cells would have been underestimated by our pro-

cedure. More importanly, if rates of such degeneration had been influenced by age, the relative numbers of Sertoli cells at different ages would also have been affected. However, the validity of our assumption was supported in two ways. First, yields of round spermatids from primary spermatocytes remained at a relatively constant ratio at all ages (see Figs. 8C,D). Second, estimates of the effects of age on relative rates of spermatozoal production were similar when judged by the enumeration of round Stage I spermatids in histological sections (Fig. 11D) vs. quantification of the more mature elongated spermatids present in testicular homogenates (Figs. 10 and 11). From such evidence, it is obvious that rates of cellular degeneration during spermiogenesis were not influenced appreciably by age in these stallions; substantial differences would have been necessary to alter our findings materially.

The difference between our findings and those of Johnson and Thompson (1983) is difficult to reconcile at this time. The assignment of stallions of differing ages to discrete groups (e.g., ages 6–12 and 13–20) for statistical analysis in the latter study probably contributed, at least in part, to some discrepancies. In our study, most parameters peaked at 12–13 yr of age. However, these peaks typically would not have been detected had we also assigned stallions to similar broad age categories distributed equally to either side of this peak. Rather, such a categorization would have created the illusion that many parameters stabilized at about 6 years of age while others continued to increase throughout life. Clearly, age is a continuous rather than a discrete variable. More importanly, the presence of stallions of markedly different ages within any given group precludes any possibility of detecting age-related differences that might actually exist among the stallions within that group. It is also important to note that in the study of Johnson and Thompson (1983), the only statistically significant effect of age on the number of Sertoli cells per pair of testes was found between <2-yr-old stallions and all other groups. Since the former included several prepubertal (8- to 12-month) stallions, a subsequent increase in the population of Sertoli cells might be expected.

It is of interest to note that postpubertal mitoses of Sertoli cells have not been demonstrated in any mammal. With the exception of the study by Johnson and Thompson (1983) on the stallion, reports dealing with other species in which the population of Sertoli cells was thought to increase after puberty have not gained acceptance because of the absence of statistical significance, failure to correct for alterations in testicular or cellular dimensions, etc. (see Discussion by Johnson and Thompson, 1983). For the stallion, Johnson and Thompson (1983) reported observing mitotic figures at the base of Stage II and III tubules during the onset of the breeding season. They implied that these might represent mitoses of Sertoli cells, since the mitotic activity of both Type A and Type B spermatogonia was thought to occur between Stages V and VIII (Swierstra et al., 1974). Unfortunately, work on the kinetics of equine spermatogenesis is incomplete (Swierstra et al., 1974). Only two types of spermatogonia were distinguished by Swierstra et al. (1974) during the process of spermatogenesis. Type A spermatogonia reportedly divided to produce Type B spermatogonia, which in turn divided to yield primary spermatocytes. If this model of spermatogenesis were complete, the maximum theoretical yield of spermatocytes from each Type A spermatogonium would be four (This theoretical yield is actually an overestimation since some divisions would be required for stem-cell renewal). However, for 12-yr-old stallions, as an example, we found a ratio of 8.25 young spermatocytes per Type A spermatogonium, which was similar to the ratios ranging from 6.1:1 to 9.7:1 for various populations of stallions examined in other studies (Squires et al., 1981, 1982; Berndtson et al., 1983). Given the fact that spermatogonial divisions are required for stem-cell renewal (Clermont, 1972) and the likelihood of some attrition of germinal cells (Clermont, 1972), it is clear that more divisions occur than were noted by Swierstra et al. (1974). It is quite possible that the mitoses noted by Johnson and Thompson (1983) were of such origin.

In addition to the age-related increase in the population of Sertoli cells cited by Johnson and Thompson (1983), these authors reported that the numerical size of the Sertoli-cell population fluctuated with season. The magnitude of this fluctuation essentially paralleled that associated with the population of germ cells. Thus, the average number of spermatids per Sertoli cell remained relatively constant, while the numbers of both cells increased during the breeding season. The influence of season was not examined in the present study. However, if the ratio of spermatids to Sertoli cells were similar at each season, it would be impossible to detect a seasonal change in spermatozoal production by the histological method involving the Sertoli-cell correction (Berndtson, 1977). Yet, a seasonal influence on

equine spermatogenesis was detected by this approach in another study (Berndtson et al., 1983), as was also observed in other species (Berndtson and Desjardins, 1974; Hochereau-de Reviers and Lincoln, 1978).

Stability of the population of Sertoli cells has been the basis for the Sertoli-cell adjustment included among histological procedures for quantifying spermatogenesis (Clermont and Morgentaler, 1955; Lino, 1971; Berndtson, 1977). In light of the present findings, it is clear that this correction would not be valid for comparisons among stallions of different ages without further adjustment for this age-related decline, as was included here. Fortunately, stallion age has been considered in the assignment of individual stallions to treatment groups in most prior experiments in which the Sertoli-cell adjustment was employed. This undoubtedly contributed to the similarlity of findings based upon this procedure and others used simultaneously to quantify spermatogenesis (Berndtson et al., 1979; Squires et al., 1982).

It should be noted that testicular growth rates of stallions have been examined by in situ measurements (Thompson et al., 1979; Woods et al., 1980) as well as by weighing excised tissues (Pickett and Voss, 1973; Amann et al., 1979; Johnson and Neaves, 1981; ElWishy et al., 1982; Johnson and Thompson, 1983). Although it is clear that marked testicular growth occurs after puberty, a consistent growth pattern has not emerged from these studies. Rather, within individual investigations, growth has either appeared to continue throughout adult life (Pickett and Voss, 1973; Thompson et al., 1979; Johnson and Neaves, 1981; EliWishy et al., 1982) or to have attained a peak size after which it remained relatively stable (Amann et al., 1979; Woods et al., 1980; ElWishy et al., 1982; Johnson and Thompson, 1983).

Several factors have likely contributed to these inconsistencies. First, testicular size is extremely variable, even among stallions of a given age and within a given season of the year (Thompson et al., 1979). Also, the general practice of pooling data from stallions of different ages into rather broadly defined age categories might readily have masked or biased age-related trends, as described previously. Finally, the relatively long life span and high maintenance costs for the stallion have made it virtually impossible to study aging except via the use of tissues obtained through commercial abattoirs. The reasons such animals have been culled are unknown, and such populations of stallions typically contain a preponderance of younger animals. The latter was certainly true in the present study, in which the relatively large sample of 47 stallions contained only three greater than 13 years of age. Given this limited number of senescent stallions, our awareness of marked variability in reproductive characteristics among individual stallions and in the absence of other reliable data on the senescent equine population (data for very old stallions have been pooled with those for much younger stallions in other studies), we are unable to conclude whether or not the very old stallions in the present study are typical of senescent stallions in general. Indeed, the merits of deleting these three stallions from the study were carefully considered. This idea was subsequently rejected because (1) exclusion of these stallions would not alter findings relative to other stallions up to 13 yr of age for which replication was quite extensive; (2) the number of observations for this very old population is clearly evident, as is the resulting need for caution in the generalization of findings from such a population; and (3) their inclusion unveils the potential for the failure to accurately detect or interpret trends with aging caused by pooling of data from such stallions with those from other mature but younger animals. Although reproductive parameters of very old stallions have yet to be well documented, rates of testicular growth and development and quantitative rates of spermatogenesis for the other stallions in the present study are quite consistent with data in earlier reports; the latter constitute evidence that the stallions included in the present study are representative of stallions in the general population.

Although, the population of Sertoli cells declined with age, the physical distribution and functional activity of individual Sertoli cells increased in keeping with testicular growth and the increased rates of spermatogenesis. Between 2 and 12 yr of age, the weight of the testicular parenchyma increased by 97%. This was associated with an average 57% increase in the diameter of fixed seminiferous tubules (Fig. 6) in the absence of a difference in total seminiferous tubule length. Our findings are somewhat in contrast to those of Johnson and Neaves (1981), who observed a 14% increase in tubular diameter and a 37% increase in tubular length between 2–3 vs 13–20 yr of age (testicular parenchymal weight increased by 75%). Despite these differences, which may have resulted because of greater shrinkage of tissue in the present

study, it is clear from both studies that tubular volume does increase with age in the stallion, and in many other species (Steinberger and Steinberger, 1975).

Sertoli cells form a blood-testis barrier, which divides the seminferous tubules into basal and adluminal compartments. It seems reasonable to assume that the increase in the size of both compartments that accompanies testicular growth would likely place additional physical demands on a fixed or declining population of Sertoli cells (i.e. individual Sertoli cells would need to provide the blood-testis barrier over a greater physical area). Furthermore, it is possible that their potential for meeting this demand could limit the maximal rate of spermatogenesis for any given individual. In fact, aging has been associated with both decreases in spermatozoal production and increased permeability of the blood-testis barrier of the mouse (Gosden et al., 1982). If one may assume that the distribution of Sertoli cells per unit of seminiferous tubular length or basement membrane area reflects their relative physical roles, the equine Sertoli cells would appear to have a remarkable capacity to increase their physical role during aging. This is evident from the observation that the basement membrane area per Sertoli cell in fixed tissue increased by 98% between 2 and 12 yr of age (Fig. 7). It is of further interest to note that whereas any given increase in either tubular length or diameter would cause identical increases in total area of basement membrane, influences on the volume contained therein would differ. For example, a doubling of tubular length would cause a doubling of tubular volume, whereas a doubling of tubular diameter would cause a 4-fold increase, since the cross-sectional area of a circle changes exponentially as a function of the radius squared. Thus, increases in tubular diameter would prove effective in increasing tubular volume relative to the area of the baement membrane, whereas increases in tubular length would not. Further studies to assess the relative contributions of tubular diameter vs. length to increases in testicular size and their impact on area of basement membrane would be of interest.

Sexual development was also associated with a marked increase in the number of developing germ cells supported per Sertoli cell. Between 2 and 12 yr of age, the numbers of spermatogonia, young primary spermatocytes, old primary spermatocytes, and round spermatids supported by each Sertoli cell increased by 49, 176, 153, and 161%, respectively. Whereas at age 2 the average Stage I Sertoli cell supported the development of 8.8 germ cells, by age 12 this had increased to 22.8 germ cells, or approximately 2.6 times as many cells. This clearly underscores the remarkable capacity of individual Sertoli cells to assume greater physical and functional roles with aging, thereby permitting testicular development to occur despite a steady decline in their own numbers.

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REFERENCES

Abercrombie M, 1946. Estimation of nuclear population from microtome sections. Anat Rec 94:239–47

Amann RP, Almquist JO, 1961. Reproductive capacity of dairy bulls. I. Technique for direct measurement of gonadal and extragonadal sperm reserves. J Dairy Sci 44:1537–43

Amann RP, Thompson DL Jr, Squires EL, Pickett BW, 1979. Effects of age and frequency of ejaculation on sperm production and extragonadal sperm reserves in stallions. J Reprod Fertil Suppl 27:1–6

Berndtson WE, 1977. Methods for quantifying mammalian spermatogenesis: a review. J Anim Sci 44:818–33

Berndtson WE, Desjardins C, 1974. Circulating LH and FSH levels and testicular function in hamsters during light deprivation and subsequent photoperiodic stimulation. Endocrinology 99:195–205

Berndtson WE, Hoyer JH, Squires EL, Pickett BW, 1979. Influence of exogenous testosterone on spermatozoal production, seminal quality and libido of stallions. J Reprod Fertil Suppl 27:19–23

Berndtson WE, Squires EL, Thomspon DL Jr, 1983. Spermatogenesis, testicular composition and the concentration of testosterone in the equine testis as influenced by season. Theriogenology 20: 449–57

Clermont Y, 1972. Kinetics of spermatogenesis in mammals: seminiferous epithelium cycle and spermatogonial renewal. Physiol Rev 52:198–236

Clermont Y, Morgentaler H, 1955. Quantitative study of spermatogenesis in the hypophysectomized rat. Endocrinology 57:369–82

Dym M, Fawcett DW, 1970. The blood-testis barrier in the rat and the physiological compartmentalization of the seminiferous epithelium. Biol Reprod 3:308–26

ElWishy AB, AbouAhmed MM, Hemeida NA, ElSayed MAI, 1982. Sperm producing capacity of Arab and native horses in Egypt. J Reprod Fertil Suppl 32:27–30

Gosden RG, Richardson DW, Brown N, Davidson DW, 1982. Structure and gametogenic potential of seminiferous tubules in ageing mice. J Reprod Fertil 64:127–33

Hansson V, Calandsa R, Purvis K, Ritzen M, French FS, 1976. Hormonal regulation of spermatogenesis. Vitam Horm 34:187–215

Hochereau-de Reviers, MT, Lincoln GA, 1978. Seasonal variation in the histology of the testis of the red deer, *Cervus elaphus*. J Reprod Fertil 54:209–13

Johnson L, Neaves WB, 1981. Age-related changes in the Leydig cell population, seminiferous tubules, and sperm production in stallions. Biol Reprod 24:703–12

Johnson L, Thompson DL Jr, 1983. Age-related and seasonal variation in the Sertoli cell population, daily sperm production and serum concentrations of follicle-stimulating hormone, luteinizing hormone and testosterone in stallions. Biol Reprod 29:777–89

Johnson L, Zane RS, Petty CS, Neaves WB, 1984. Quantification of the human Sertoli cell population: its distribution, relation to germ

cell numbers, and age-related decline. Biol Reprod 31:785–95
Jutte NHPM, Jansen R, Grootegoed JA, Rommerts FFG, Clausen OPF, van der Molen HJ, 1982. Regulation of survival of rat pachytene spermatocytes by lactate supply from Sertoli cells. J Reprod Fertil 65:431–38
Lino BF, 1971. Cell count correction factors for the quantitative histological analysis of the germinal epithelium of the ram. Anat Rec 170:413–20
Mita M, Price JM, Hall PF, 1982. Stimulation by follicle-stimulating hormone of synthesis of lactate by Sertoli cells from rat testis. Endocrinology 110:1535–41
Neaves WB, 1977. The blood-testis barrier. In: Johnson AD, Gomes WR (eds.), The Testis. Vol. IV. New York:Academic Press, pp. 126–62
Pickett BW, Voss JL, 1973. Reproductive management of the stallion. Ft. Collins: Colorado State Univ Gen Series Bull 934
Squires EL, Berndtson WE, Hoyer JH, Pickett BW, Wallach SJR, 1981. Restoration of reproductive capacity of stallions after suppression with exogenous testosterone. J Anim Sci 53:1351–59
Squires EL, Todter GE, Berndtson WE, Pickett BW, 1982. Effect of anabolic steroids on reproductive function of young stallions. J Anim Sci 54:576–82
Steel RGD, Torrie JH, 1960. Principles and Procedures of Statistics. New York:McGraw-Hill Book Company, Inc.
Steinberger A, Steinberger E, 1977. The Sertoli cells. In: Johnson AD, Gomes WR (eds.). The Testis, Vol. IV. New York: Academic Press, pp. 371–399
Steinberger E, Steinberger A, 1975. Spermatogenic function of the testis. In: Greep RO, Astwood EB, Hamilton DW, Geiger SR (eds.), Handbook of Physiology, Section 7, Endocrinology, Vol. V, Male Reproductive System. Washington, DC:Am, Physiol. Soc., pp. 1–19
Swierstra EE, Gebauer MR, Pickett BW, 1974. Reproductive physiology of the stallion. I. Spermatogenesis and testis composition. J Reprod Fertil 40:113–23
Thompson DL Jr, Pickett BW, Squires EL, Amann RP, 1979. Testicular measurements and reproductive characteristics in stallions. J Reprod Fertil Suppl 27:13–17
Weber JE, Russell LD, Wong V, Peterson RN, 1983. Three-dimensional reconstruction of a rat stage V Sertoli cell: II. Morphometry of Sertoli-Sertoli and Sertoli-germ-cell relationships. Am J Anat 167: 163–79
Wong V, Russell LD, 1983. Three-dimensional reconstruction of a rat stage V Sertoli cell: I. Methods, basic configuration, and dimensions. Am J Anat 167:143–61
Woods GL, Garcia MC, Kenney RM, 1980. Variations in testicular size of Standardbred stallions. Proc 26th Conv Am Assoc Eq Pract: 117–21