Journal of Andrology, Vol. 18, No. 6, November/December 1997

Quantitative (Stereological) Study of Normal Spermatogenesis in the Adult Monkey (*Macaca fascicularis*)

YANG ZHENGWEI,* ROBERT I. McLACHLAN,* WILLIAM J. BREMNER,† AND NIGEL G. WREFORD‡

*From the *Prince Henry's Institute of Medical Research, Melbourne, Australia; the †Department of Medicine, University of Washington, Veterans Affairs Puget Sound Health Care System, Seattle, Washington; and the ‡Anatomy Department and Institute of Reproduction and Development, Monash University, Melbourne, Australia.*

ABSTRACT: Germ cell and Sertoli cell numbers were estimated in six normal adult monkeys (*Macaca fascicularis*) using a contemporary unbiased and efficient stereological method—the optical disector. The data was used to assess the efficiency of spermatogenesis from type B spermatogonia to elongated spermatids. Animals underwent orchidectomy, and the right testis (volume 17.5 ± 1.7 cm^3 [mean ± SEM], range 13.2–25.1 cm^3) was fixed in Bouin's fluid. Blocks were embedded in methacrylate resin and germ cells were counted in thick (25 μm) sections using the optical disector in conjunction with a systematic uniform random-sampling protocol. The total numbers of Sertoli cells and all germ cells per testis were 566 ± 43 (419–683) million and 12.8 ± 1.6 (9.0–20.2) billion, respectively. On average, one Sertoli cell supported 12.4 ± 1.9 (range 8.2–18.4) step 1–12 spermatids, 3.1 ± 0.4 (2.3–4.5) pachytene spermatocytes, and 23.7 ± 4.1 (15.0–39.0) total germ cells. Sertoli cell number correlated poorly with both testicular size (correlation coefficient $r = -0.12$) and germ cell numbers ($r = -0.35$ with total germ cell number). However, testicular size had a consistent and significant correlation with germ cell numbers ($r = 0.97$ with total germ cell number). The conversion ratio of pachytene spermatocytes to step 1–12 spermatids was 3.94 ± 0.19, which is close to the theoretical maximum of 4. Similarly, the conversion between other cell types was consistently close to the maximum theoretical value. We conclude that the efficiency of spermatogenesis in the adult monkey is high, with stepwise conversion being consistently close to the maximal values. The capacity of Sertoli cells to support a cohort of germ cells varies widely between monkeys. Although absolute number of cells per testis is always the preferred parameter, it cannot always be obtained in an experimental situation where cost and ethical constraints mean that biopsies, rather than whole testes, are collected. Thus, if absolute data on germ cell numbers are not available, experimental outcomes impacting on cells beyond preleptonene spermatocytes may be best expressed in terms of changes in germ cell conversion rather than the traditional germ cell : Sertoli cell ratio.

Key words: Sertoli cell, spermiogenesis, stereology, testis.
J Androl 1997;18:681–687

Clermont and colleagues (Clermont and Leblond, 1959; Clermont, 1969; Clermont and Antar, 1973) used serial sections, whole-mounted seminiferous tubules, and autoradiography to describe a model for spermatogenesis in the monkey. It was proposed that spermatogenesis begins with the division of the renewing stem cell (pale type A spermatogonia), which then undergoes further division with half the progeny becoming type B1 spermatogonia. Type B1 spermatogonia then undergo four further mitotic divisions to yield primary spermatocytes, which then undergo the first of two meiotic divisions to yield secondary spermatocytes and a second division to give round spermatids. Finally, round spermatids proceed through extensive structural modifications (spermiogenesis) to become elongated spermatids.

Supported by the Contraception 21 grant of the Rockefeller Foundation, the Australian NH&MRC grant 943208, NIH grants RP00166 and P50, HD-12926, and Australian Research Council grant 809600657.

Correspondence to: Dr. Nigel G. Wreford, Anatomy Department, Monash University, Clayton, Victoria 3168, Australia.

Received for publication March 24, 1997; accepted for publication July 29, 1997.

No studies have tested these models of primate spermatogenesis using the quantification of total germ cell numbers in the testis. In addition, it has been proposed that the spermatogenic process is inefficient with significant degeneration of spermatogonia, spermatocytes, and spermatids occurring in most mammals (for review see Johnson, 1995). However, the overall efficiency of cell progression through spermatogenesis and the number of germ cells undergoing spontaneous degeneration in the monkey have not been studied. Unbiased stereological estimators of cell number have recently become available (Sterio, 1984; Gundersen et al, 1988) and considered in relation to the testis (Wreford, 1995). The present study employs an unbiased and efficient stereological method—the optical disector approach (Gundersen et al, 1988)—to quantitate germ cell populations. It shows that: 1) germ cell number is strongly correlated with testicular volume but poorly associated with Sertoli cell number; and 2) primate spermatogenesis is a highly efficient process that conforms to the model of spermatogenesis from type B spermatogonia proposed by Clermont (1969).

Table 1. *Age, body weight, testicular volume, and other stereological data describing testicular structure in six individual animals and their means*

	1	2	3	4	5	6	Mean ± SEM
Age (years)	#	#	6.7	5.9	5.6	#	6.1 ± 0.3
Body weight (kg)	5.0	5.2	7.4	6.9	5.1	3.8	5.6 ± 0.5
Testicular volume (cm^3)	17.1	13.2	25.1	18.6	15.9	15.1	17.5 ± 1.7
V_v(st/t) (%)*	83.5	82.8	85.4	78.4	83.8	81.3	82.5 ± 1.0
Tubule diameter (μm)	226	197	233	205	188	211	210 ± 7
Tubule length (m)	359	355	499	438	480	348	413 ± 28
Tubule frequency							
Stage I–VI (%)	43.9	49.1	43.9	45.8	47.4	46.8	46.1 ± 0.8
Stage VII–XII (%)	56.1	50.9	56.1	54.2	52.6	53.2	53.9 ± 0.8

* V_v(st/t), volume fraction of the seminiferous tubules in the testis; #, unknown.

Materials and Methods

Animals

Six normal adult monkeys (*Macaca fascicularis*) were obtained from the Regional Primate Research Center at the University of Washington, Seattle. Three animals were estimated to be between 5.6 and 6.7 years (by breeding activity), while the age of the other three was not known (Table 1). The latter group of animals had been in the colony for 2.1–2.6 years. The average body weight of the six animals was 5.6 ± 0.5 kg (range 3.8–7.4 kg). All animals had an adult pattern of spermatogenesis with all germ cells through step 14 spermatids present. All studies were approved by the Animal Ethics Committee of the University of Washington.

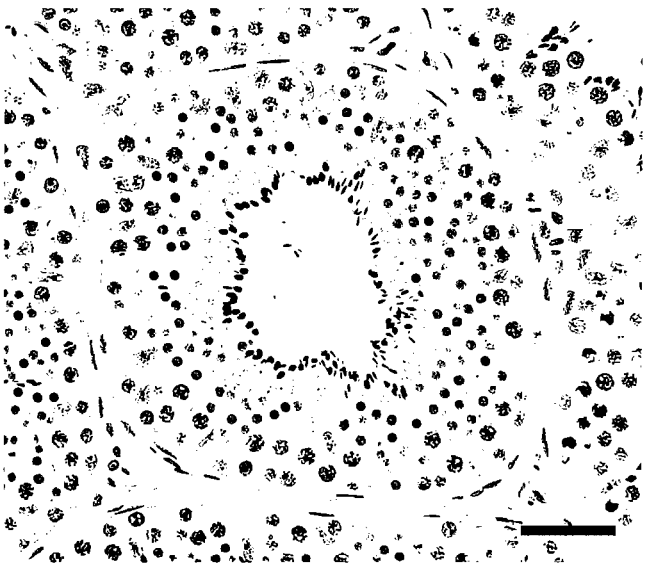

FIG. 1. Photomicrograph of a 3-μm methacrylate section showing a seminiferous tubule in stage VI of the cycle. Sertoli cell nuclei are readily recognized as large and pea-shaped with a prominent nucleolus. The basal layer of germ cells consists of A and B type spermatogonia. A ring of larger pachytene spermatocytes with the typically mottled pattern of heterochromatin is apparent inside of this outer rim of cells. Inside the ring of pachytene spermatocytes, 3 or 4 layers of smaller round spermatids are readily identified. At this stage, the mature spermatozoa form a uniform ring at the luminal aspect of the epithelium. (Bar is 50 μm.)

Tissue Processing

The right testis of all animals was removed under general anesthesia. Three testes were perfusion-fixed following cannulation of the testicular artery on the surface of the testis. The vasculature was first flushed with NaCl (0.154 M), then fixed by perfusion with Bouin's fluid. The testicular artery in the remaining three testes could not be cannulated, and the testes were immersion-fixed overnight in Bouin's fluid after incision of the tunica albuginea. The testes were then stored in 70% ethanol for 12 weeks prior to processing. Subsequent examination showed that tissues were generally well-preserved and stained. There was little qualitative variation between perfusion and immersion-fixed tissue, and there were no statistically significant differences in any of the stereological parameters. Accordingly, the data from both groups of testes have been combined for analysis.

The density of the testis was determined by immersion in a graded-ethanol solution of known density (Hodgman et al, 1958), and the volume was calculated by dividing weight by density. Each testis was cut into a series of six parallel slices orthogonal to its long axis, and three alternate slices were sampled systematically. One wedge-shaped block (one-eighth of the slice, ~80 mm^2) was arbitrarily sampled from each slice, the apex of the wedge being at the center of the slice. After being dehydrated in absolute ethanol followed by butanol, sampled blocks were embedded in hydroxyethyl–methacrylate resin (Technovit 7100, Heraeus Kulzer GmbH, Wehrheim/Ts, Germany). One thick (25 μm) section was cut from each block using a Leica RM 2055 microtome (Nussloch, Germany). The section was stained with the periodic acid–Schiff (PAS), counterstained with hematoxylin, and mounted under DPX (BDH, Poole, England). Additional 3 μm sections were cut for photomicrography (Fig. 1).

During sampling, one additional block of similar size and shape was cut from each testis, and its volume (before processing) was determined from its weight and density. These blocks were then processed as described above and serial 25 μm sections cut. One in every 10–15 sections was sampled depending on the thickness of the block (on average, 13 sections being sampled per block). The total area of the sampled sections from each block was determined by point counting, and the volume of each processed block was calculated using Cavalieri's principle as described previously (Zhengwei et al, 1990). Tissue

shrinkage was determined by comparing the volume of the block before and after processing.

Cell Identification and Grouping

Sertoli and germ cells were readily identified by the appearance of their nuclei in conjunction with the stage of the cycle of the seminiferous epithelium according to the descriptions of Clermont and Leblond (1955, 1959) and Clermont (1969). They were grouped as follows and additional reasons for their identification are stated (Fig. 2).

Sertoli Cells—The nuclei of these cells were differentiated from germ cells by their irregular shape, paler chromatin pattern, and a single nucleolus.

Type A Spermatogonia (A)—These cells were located on the basement membrane and had a nucleus with diffuse, finely granulated chromatin and one or two nucleoli associated with the nuclear membrane. Type A spermatogonia were present through the seminiferous cycle and could normally be divided into two groups, A dark (Ad) and A pale (Ap), on the basis of their cytoplasmic content of PAS-positive material. In the present study they were counted as one group.

Type B Spermatogonia (B)—The nuclei of these cells were identified and differentiated from type A spermatogonia by the presence of a centrally located nucleolus and clumps or granules of darkly stained chromatin that was distributed along the nuclear membrane and free in the nucleoplasm. Type B1 spermatogonia (B1) were present in stages X–XII, while types B2, B3, and B4 spermatogonia were in stages I–VI of the cycle (Clermont, 1969). Some B4 remaining in a stage VII tubule were carefully differentiated from early preleptotene spermatocytes according to the slightly smaller nuclear size of the latter.

Preleptotene, Leptotene, and Zygotene Primary Spermatocytes (Pl + L + Z)—The nuclei of these cells were characterized by threadlike clumps of chromatin and their association with stages VII–XI tubules. They were considered as a single group in the present study. A few early preleptotene cells observed in stage VI (Clermont and Leblond, 1959) were carefully identified.

Pachytene Spermatocytes (P)—The nuclei of these spermatocytes were larger and positioned closer to the tubule lumen than Pl, L, or Z, and their chromatin had a mottled appearance. They developed in stage XII from zygotene spermatocytes (Z) and were present through all the stages of one seminiferous cycle. Pachytene spermatocytes were subdivided according to their stage associations into early (stages I–VI) and late (stages VII–XII) subgroups. Diplotene spermatocytes were counted with the pachytene.

Secondary Spermatocytes (SS)—These were identified in stage XII based on their smaller size and finer chromatin pattern compared to pachytene spermatocytes. The meiotic form of these cells were also included in this group.

Spermatids (St)—They were identified on the basis of their position (close to the tubule lumen), size (smaller than spermatocytes), lack of heterochromatin, presence of an acrosome in more advanced cells, and shape. Two major groupings were defined in this study: 1) steps 1–12 spermatids containing the subgroups of steps 1–6 round and steps 7–12 elongating spermatids and 2) steps 13, 14 elongated spermatids (Fig. 2).

Stereology

Cell number was estimated using the optical disector method as previously described (McLachlan et al, 1995; Wreford, 1995). Nuclear number was assumed to equate to cell number. Briefly, 25 μm methacrylate-embedded sections were optically sectioned using a 100× oil-immersion lens with NA 1.40 (Olympus Splan Apo). The upper surface of the section was brought into focus and the upper 3 μm was then traversed to avoid possible surface imperfections. The next 10 μm of the section was examined by focusing through, and nuclei were counted as they came into focus according to the disector principle (Sterio, 1984). The numerical density (N_v) of each cell type was calculated by dividing the total number of cells counted by the total volume of all disectors counted. In turn, the absolute number of germ cells per testis was calculated using the testicular volume. At least 300 frames (area of each frame 252 μm^2) were used for the counting of spermatids and 1,200 frames for that of other cells per testis.

Tubule-stage frequency was estimated using a 10× objective lens (NA 0.25, DPlan, Olympus) in conjunction with a 10× eyepiece fitted with a graticule on which a frame (area 0.16 mm^2 at this magnification) was marked. Tubule profiles that included a lumen were counted according to the unbiased counting rule described by Gundersen (1977) and grouped into stages I–VI or stages VII–XII. Tubule staging was checked, where necessary, using an objective lens with a higher magnification. When a transition zone between stages VI and VII or stages XII and I was seen, each stage was ascribed half a profile. Such transitional zones were seen in 3 and 2.3% of the tubules in stages VI–VII and XII–I, respectively. The proportion of tubules in stages I–VI and stages VII–XII was used to determine the relative length of the tubules and therefore the relative duration of these stages. Approximately 200 tubule profiles were counted per testis.

Tubule length was estimated from diameter and volume data. Sections were observed using a 10× objective lens (NA 0.25, DPlan, Olympus) at a final (on screen) magnification of 134×. Round or elliptical tubule profiles with a clear lumen were uniformly sampled using a frame (area 0.24 mm^2) according to the uniform-sampling rule described by Gundersen (1977). The diameter or short axis of 50 tubules was measured per testis. Tubule length was then calculated by dividing the total volume of the tubules per testis by the cross-sectional area of the tubule. The total volume of the tubules was determined by multiplying the testicular volume by the volume fraction of the tubules in the testis, which was, in turn, determined by point counting (Zhengwei et al, 1990).

The conversion ratio of germ cells from one type to another was adjusted for the relative duration of the stages in which particular germ cell types were found:

$$\text{conversion ratio} = \frac{N_2}{N_1} \cdot \frac{D_1}{D_2}, \qquad (1)$$

where N_2/N_1 = number of the daughter/progenitor cells per unit volume of testis and D_1/D_2 = absolute or relative duration of the progenitor/daughter cells in the seminiferous cycle.

The method assumes steady-state kinetics and has been previously used to follow the dynamics of spermiogenesis in the rat (O'Donnell et al, 1994). The stage durations used were those

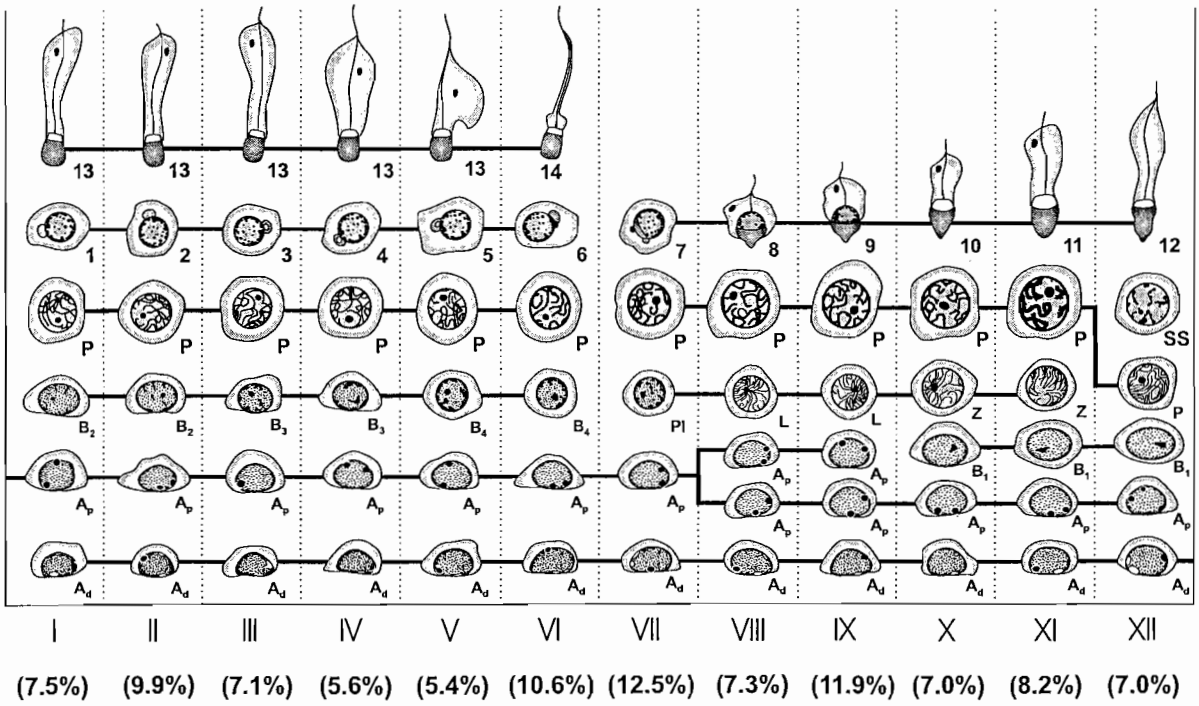

FIG. 2. Stages of the cycle of the primate seminiferous epithelium modified from Clermont (1969) and incorporating the concept of pale type A spermatogonial renewal described by Clermont and Antar (1973) and Fouquet and Dadoune (1986). Each column shows the cell types present in that stage (Roman numerals) of the cycle. Relative duration (%) of each stage (Fouquet and Dadoune, 1986) is in brackets under the stage designation. Cells connected by a solid line were counted as a single group in the present study. Ad, dark type A spermatogonia; Ap, pale type A spermatogonia. B1–B4, successive generations of type B spermatogonia. Pl, preleptotene; L, leptotene; Z, zygotene; P, pachytene primary spermatocytes. SS, secondary spermatocytes. 1–14, steps 1–14 spermatids.

reported by Fouquet and Dadoune (1986) for the spermatogenic model described by Clermont (1969) (Fig. 1).

Statistics

All data in text, figures, and tables are shown as mean ± SEM. Statistical analysis was performed using Sigmastat V1.0 (Jandel Scientific Software, San Rafael, California.). Correlations were determined using the Pearson product moment coefficient.

Results

Qualitative Observations

The tissue was well preserved, and chromatin patterns were clearly apparent, allowing unequivocal identification of the different cell types in thick (25 μm) sections. Figure 1 is indicative of the quality of the histological material.

Testicular Volume and Tissue Shrinkage

The mean testicular volume (Table 1) was 17.5 ± 1.7 cm^3 (range 13.2–25.1 cm^3). Processing was not associated with significant shrinkage (<2%), and no shrinkage corrections were performed.

Tubule Staging

The relative durations of stages I–VI and stages VII–XII tubules in one seminiferous cycle were estimated by profile counting (stage frequency) to be 46.1 ± 0.8% and 53.9 ± 0.8%, respectively (Table 1).

Stereological Data

The volume fraction (Table 1) of the seminiferous tubules in the testis was estimated to be 82.5 ± 1.0% and the mean tubule diameter 210 ± 7 μm. The absolute length of tubules per testis was 413 ± 28 m.

The total numbers of Sertoli cells and total germ cells per testis were 566 ± 43 (range 419–683) million and 12.8 ± 1.6 (9.0–20.2) billion, respectively (Table 2). On average, one Sertoli cell supported 12.4 ± 1.9 (range 8.2–18.4) steps 1–12 spermatids, 3.1 ± 0.4 (2.3–4.5) pachytene spermatocytes, and 23.7 ± 4.1 (15.0–39.0) total germ cells. Sertoli cell number correlated poorly with testicular volume (correlation coefficient $r = -0.12$) and both individual and total germ cell numbers ($r = -0.35$ with total germ cell number) (Table 3). In contrast, testicular volume correlated consistently and significantly with the number of germ cells in each grouping ($r = 0.97$ with total germ cell number) (Table 3).

Table 2. *Germ cell and Sertoli cell numbers (million per testis) in six individual animals and their means**

	1	2	3	4	5	6	Mean ± SEM
A	222	205	392	298	236	213	261 ± 29
B (total)	182	228	362	240	168	270	242 ± 29
B1	9	33	14	15	25	43	23 ± 5
B2–4	173	195	348	225	143	227	219 ± 29
Pl + L + Z	942	665	1,108	821	689	663	815 ± 74
P (total)	1,687	1,369	2,320	1,656	1,602	1,579	1,702 ± 132
P (I–VI)	700	735	849	669	639	658	708 ± 31
P (VII–XII)	987	634	1,471	987	963	921	994 ± 110
SS	187	73	163	94	124	85	121 ± 19
St (1–12)	7,575	4,508	9,487	7,148	6,284	5,613	6,769 ± 704
St (1–6)	3,531	1,971	4,020	3,080	2,931	2,219	2,959 ± 316
St (7–12)	4,044	2,537	5,467	4,068	3,353	3,394	3,811 ± 403
St (13, 14)	3,215	1,989	6,325	1,947	1,938	1,839	2,876 ± 721
Sertoli cells	419	511	517	683	584	683	566 ± 43

* A, type A spermatogonia; B, B1–4; type B, B1–4 spermatogonia; Pl + L + Z, preleptotene, leptotene, and zygotene spermatocytes; P, P (I–VI); P (VII–XII), pachytene spermatocytes in all stages, stages I–VI and VII–XII; SS, secondary spermatocotyes; St (1–12)/(1–6)/(7–12)/(13, 14), steps 1–12, 1–6, 7–12, and 13, 14 spermatids.

Conversion ratios between germ cell types were calculated using equation 1 (Table 4). The conversion rate from Pl + L + Z to pachytene spermatocytes was 0.99 ± 0.04. The conversion ratio of pachytene spermatocytes to spermatids in steps 1–12 was 3.94 ± 0.19. The conversion between round spermatids in steps 1–6 and elongating spermatids in steps 7–12 was 1.11 ± 0.05. The conversion between elongating spermatids in steps 7–12 and late elongated spermatids in steps 13–14 was 0.84 ± 0.12.

Assuming the relative stage durations and the spermatogenic model (Fig. 1) and using equation 1, it is also possible to calculate the number of all type B (B1–B4) spermatogonia needed to yield the observed number of pachytene spermatocytes (1,702 ± 132 million). The observed number of type B spermatogonia was 242 ± 29 million, which corresponds closely to the expected number of 251 ± 19 million per testis predicted by the model.

Table 3. *Pearson product moment correlation coefficients and significance (brackets) between testicular volume, Sertoli cell number, and germ cell numbers*

	Testicular volume	Sertoli cell number
Testicular volume		−0.12 (P = 0.82)
Type A spermatogonia	0.97 (P = 0.002)	0.02 (P = 0.97)
Type B spermatogonia	0.72 (P = 0.11)	0.15 (P = 0.78)
Pl + L + Z spermatocytes*	0.91 (P = 0.01)	−0.47 (P = 0.35)
Pachytene spermatocytes	0.98 (P = 0.001)	−0.19 (P = 0.73)
Steps 1–12 spermatids	0.95 (P = 0.003)	−0.25 (P = 0.63)
Steps 13, 14 spermatids	0.90 (P = 0.02)	−0.45 (P = 0.37)

* Pl + L + Z, preleptotene, leptotene, and zygotene spermatocytes.

Discussion

This is the first study of the monkey testis to report the absolute numbers of Sertoli cells and germ cells and the efficiency of germ cell conversion, which is adjusted for the relative duration of germ cells in the seminiferous cycle. The data show that: 1) germ cell number is strongly correlated with testicular volume but poorly correlated with Sertoli cell number, suggesting that the capacity of Sertoli cells to support a cohort of germ cells varies widely between animals; and 2) spermatogenesis in the normal adult monkey is a highly efficient process involving little cell loss from type B spermatogonia onwards.

The optical disector approach utilized in the present study has been used extensively in the rat testis (McLachlan et al, 1995; Meachem et al, 1996; Wreford, 1995) and provides unbiased estimates of cell (nuclear) numbers that are independent of variation in nuclear shape and size. These methods are also unaffected by factors that alter the length or cross-sectional area of the seminiferous tubules. In the present study, both perfusion and immersion-fixed testes were examined, and no significant differences were observed in either the qualitative or quantitative endpoints assessed.

It has been common practice to report germ cell number per Sertoli cell based on the fact that, even after hypophysectomy, Sertoli cell numbers remain constant (Clermont and Morgentaler, 1955). In studies of different

Table 4. *Germ cell conversion ratios adjusted for duration of cell cycle. Cells in left-hand column are progenitors while those in upper row are daughter cells. Number in brackets is expected if there is 100% survival of daughter cells*

	Pachytene	Steps 1–6 spermatids	Steps 7–12 spermatids	Steps 1–12 spermatids	Steps 13, 14 spermatids
Pl + L + Z* spermatocytes	0.99 ± 0.04 (1)	3.68 ± 0.18 (4)	4.06 ± 0.18 (4)	3.88 ± 0.16 (4)	3.40 ± 0.50 (4)
Pachytene spermatocytes		3.75 ± 0.23 (4)	4.11 ± 0.17 (4)	3.94 ± 0.19 (4)	3.48 ± 0.55 (4)
Steps 1–6 spermatids			1.11 ± 0.05 (1)	1.06 ± 0.03 (1)	0.94 ± 0.14 (1)
Steps 7–12 spermatids				0.96 ± 0.02 (1)	0.84 ± 0.12 (1)
Steps 1–12 spermatids					0.88 ± 0.13 (1)

* Pl + L + Z, preleptotene, leptotene, and zygotene.

species, it is apparent that the Sertoli cell number largely dictates the spermatogenic capacity of the testis, i.e., more Sertoli cells support a larger cohort of germ cells. This quantitative approach is supported by studies in rats and other animals, which indicate that Sertoli cell number is the major factor controlling the spermatogenic output of the testis (for review see Sharpe, 1994). It is therefore interesting that in the present study, there was a poor correlation between Sertoli cell number and both testicular volume and germ cell numbers (Table 3). Similarly, data from Russell et al (1990) showed a poor correlation between Sertoli cell numerical density and daily sperm production per gram of testis ($r = 0.002$). These data make the use of germ cell : Sertoli cell ratios as the endpoint for studies on primate spermatogenesis debatable. In contrast, the total number of all germ cell types showed an excellent correlation with testicular volume (Table 3). Taken together, the data suggest that although the Sertoli cell number might limit the capacity of the testis to produce sperm, there is considerable interanimal variation in the degree to which this capacity is utilized.

Germ cell degeneration during spermatogenesis has been studied in a number of species including mouse, rat, rabbit, horse, sheep, cattle, and human (for review see Johnson, 1995). The critical points at which degeneration occurs appear to be during spermatogonial division and meiosis. However, in the present study, loss during meiosis in the monkey appears to be minimal, as evidenced by the highly efficient interconversion between spermatocytes and spermatids, which closely approximated theoretical (maximal) values (Table 4). In support of the stereological data, no degenerating cells were observed in stage XII. Using the raw data reported by Hadley and Dym (1983) in conjunction with our estimates of the relative diameters of round spermatids and pachytene spermatocytes (not reported), the ratio of round spermatids to pachytene spermatocytes was approximately 3.5, which closely corresponds with this report. Similarly, in the present study, there appears to be little loss during the conversion of type B spermatogonia to pachytene spermatocytes. This high efficiency of germ cell conversion was also consistent with our morphological observation that very few degenerating germ cell nuclei were present. Similarly, van Alphen et al (1988) did not observe any spermatogonial degeneration in normal monkeys.

Thus, in the monkey, if absolute data are not available on germ cell numbers, then experimental outcomes may be best expressed in terms of changes in germ cell conversion rather than germ cell : Sertoli cell ratios. However, this approach demands the use of appropriate stereological methods in conjunction with a uniform random-sampling protocol as employed in the present study.

Acknowledgment

We appreciate the support and help of the staff of the Washington Regional Primate Research Center.

References

Clermont Y. Two classes of spermatogonial stem cells in the monkey (*Cercopithecus aethiops*). *Am J Anat* 1969;126:57–72.

Clermont Y, Antar M. Duration of the cycle of the seminiferous epithelium and the spermatogonial renewal in the monkey *Macaca arctoides*. *Am J Anat* 1973;136:153–166.

Clermont Y, Leblond CP. Spermiogenesis of man, monkey, ram and other mammals as shown by the "periodic acid–Schiff" technique. *Am J Anat* 1955;96:229–253.

Clermont Y, Leblond CP. Differentiation and renewal of spermatogonia in the monkey, *Macacus rhesus*. *Am J Anat* 1959;104:237–273.

Clermont Y, Morgentaler H. Quantitative study of spermatogenesis in the hypophysectomized rat. *Endocrinology* 1955;57:369–382.

Fouquet JP, Dadoune JP. Renewal of spermatogonia in the monkey (*Macaca fascicularis*). *Biol Reprod* 1986;35:199–207.

Gundersen HJG. Notes on the estimation of the numerical density of arbitrary profiles: the edge effect. *J Microsc* 1977;111:219–223.

Gundersen HJG, Bagger P, Bendsten TF, Evans SM, Korbo L, Marcussen N, Møller A, Nielsen K, Nyengaard JR, Pakkenberg B, Sorensen FB,

Versterby A, West MJ. The new stereological tools: dissector, fractionator, nucleator and point sampled intercepts and their use in pathological research and diagnosis. *APMIS* 1988;96:857–881.

Hadley MA, Dym M. Spermatogenesis in the vasectomized monkey: quantitative analysis. *Anat Rec* 1983;205:381–386.

Hodgman CD, Weast RC, Selby SM, eds. *Handbook of Chemistry and Physics.* Cleveland: Chemical Rubber Publishing Co; 1958:2083–2089.

Johnson L. Efficiency of spermatogenesis. *Microsc Res Techn* 1995;32: 423–436.

McLachlan RI, Wreford NG, de Kretser DM, Robertson DM. The effects of recombinant FSH on the restoration of spermatogenesis in the GnRH immunized adult rat. *Endocrinology* 1995;136:4035–4043.

Meachem SJ, McLachlan RI, de Kretser DM, Robertson DM, Wreford NG. Neonatal exposure of rats to recombinant follicle stimulating hormone increases adult Sertoli and spermatogenic cell numbers. *Biol Reprod* 1996;54:36–44.

O'Donnell L, McLachlan RI, Wreford NG, Robertson DM. Testosterone promotes the conversion of round spermatids between stages 7 and 8 of the rat spermatogenic cycle. *Endocrinology* 1994;135:2608–2614.

Russell LD, Ren HP, Sinha-Hikim S, Schulze W, Sinha-Hikim AP. A comparative study in twelve mammalian species with respect to key morphometric parameters related to volume densities and volumes of selected testis components. *Am J Anat* 1990;188:21–30.

Sharpe RM. Regulation of spermatogenesis. In: Knobil E, Neill JD, eds. *The Physiology of Reproduction.* Vol. 1. New York: Raven Press; 1994:1363–1434.

Sterio DC. The unbiased estimation of number and sizes of arbitrary particles using the dissector. *J Microsc* 1984;134:127–136.

van Alphen MAA, van de Kant HJG, de Rooij DG. Follicle-stimulating hormone stimulates spermatogenesis in the adult monkey. *Endocrinology* 1988;123:1449–1455.

Wreford NG. Theory and practice of stereological techniques applied to the estimation of cell number and nuclear volume in the testis. *Microsc Res Techn* 1995;32:423–436.

Zhengwei Y, Wreford NG, de Kretser DM. A quantitative study of spermatogenesis in the developing rat testis. *Biol Reprod* 1990;43:629–635.