Quantitative (stereological) study of the effects of vasectomy on spermatogenesis in rhesus monkeys (*Macaca mulatta*)

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Vasectomy reversal by vasovasostomy after long-term vasectomy in men results in lower sperm counts and pregnancy rates compared with controls, and severe damage to spermatogenesis has been observed in some animal models such as mice. The primary aim of this study was to evaluate, using sophisticated stereological methods, whether vasectomy of 6 and 12 months in a non-human primate would lead to, among other morphometric changes, reduced numbers of germ cells in testes and spermatozoa in epididymides. Five normal adult male rhesus monkeys (*Macaca mulatta*) underwent bilateral vasectomy, with another three aged-matched normal monkeys not undergoing vasectomy. One testis together with the ipsilateral epididymis was removed from each animal at 6 months, and the other testis and epididymis, the prostate gland and seminal vesicles were removed at 12 months. Various morphometric data were obtained

using stereological methods and an unbiased and efficient stereological tool, the optical disector, was used to estimate nuclear numbers of all types of spermatogenic cells in testes and spermatozoa in epididymides using methacrylate-embedded sections 25 µm in thickness. As shown by a two-way repeated measures analysis of variance, vasectomy or hemicastration (removal of the organs at 6 months) had no significant effects on all quantitative parameters of stereology obtained from the testis, epididymis, prostate gland and seminal vesicle, except that (i) sperm granuloma was observed from three of five vasectomized animals both at 6 and 12 months, and (ii) hemicastration significantly reduced the diameter of the seminiferous tubules and increased the number of type A spermatogonia per testis. In conclusion, vasectomy in the non-human primate is a safe procedure in terms of effects on the structures of the reproductive organs.

Introduction

Vasectomy has been used widely for more than a century as a contraceptive method (especially since the late 1960s and early 1970s), occasionally for compulsory male sterilization for eugenic reasons or as a treatment for prevention of epididymitis in prostatic surgery or for rejuvenation (Drake *et al.*, 1999). It has been estimated that up to 100 million men worldwide might have chosen vasectomy as a means of fertility control (Weiske, 2001).

Vasectomy can be surgically reversed, and a patency (return of spermatozoa to the ejaculate) rate of 86% (median; range: 77–100%; mean $\pm$ SEM: $87 \pm 3\%$) and a pregnancy rate of 52% (median; range 28–82%; mean $55 \pm 6\%$) have been obtained after microsurgical vasovasostomy (Owen and Kapila, 1984; Lee, 1986; Silber, 1989; Belker *et al.*, 1991; Chiang, 1996; Casella *et al.*, 1997; Huang *et al.*, 1997; Heidenreich *et al.*, 2000). Failure to obtain patency was generally ascribed to stricture at the reversal site or secondary

pressure-induced blockage of the epididymis (Silber, 1979, 1989; Matthews *et al.*, 1997); repeat reversal procedures did increase patency to some extent (Belker *et al.*, 1991; Chiang, 1996; Matthews *et al.*, 1997; Donovan *et al.*, 1998; Hernandez and Sabanegh, 1999; Fox, 2000). The pregnancy rate is always lower than the patency rate, which is usually thought to be the result of a decreased sperm count and quality of the semen (Lee, 1986; Huang *et al.*, 1997; Yamamoto *et al.*, 1997). For instance, among the subjects with successful vasectomy reversal, the sperm count ($35.4 \pm 0.5 \times 10^6 \text{ ml}^{-1}$) of men ($n = 36$) who impregnated their partners was significantly higher than that ($9.9 \pm 0.5 \times 10^6 \text{ ml}^{-1}$) of men ($n = 19$) who failed to do so (Huang *et al.*, 1997). Vasovasostomy restored the fertility of seven of 14 vasectomized rabbits; the sperm counts of the two treated groups ($122 \pm 23 \times 10^6 \text{ ml}^{-1}$ versus $10 \pm 9 \times 10^6 \text{ ml}^{-1}$) were significantly different and the sperm count of either treated group was significantly lower than that of the control group ($816 \pm 79 \times 10^6 \text{ ml}^{-1}$) (Wang *et al.*, 1994). Azoospermia or decreased sperm count after vasectomy reversal are usually considered to be attributable to factors such as vas stenosis and epididymal obstruction unrelated to testicular damage. However, the possibility that vasectomy leads to spermatogenic lesions cannot be excluded on the basis of data available so far.

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The effects of vasectomy on spermatogenesis remain controversial (McDonald, 2000). Although many researchers failed to show any effects of vasectomy on spermatogenesis in humans (Silber, 1979), mice (Lohiya *et al.*, 1976), monkeys (Hadley and Dym, 1983), rabbits (Lohiya *et al.*, 1983) or rats (Lohiya *et al.*, 1976; Flickingers *et al.*, 1988), other studies did observe significant effects in dogs (Lohiya *et al.*, 1975; Urry *et al.*, 1976), guinea-pigs (Hutson *et al.*, 1976), hamsters (Urena and Malavasi, 1980), humans (Gupta *et al.*, 1975; Jenkins *et al.*, 1979; Jarow *et al.*, 1985, 1994), mice (Croft and Bartke, 1976; Barratt and Cohen, 1988; Singh and Chakravarty, 2000), monkeys (Chapman *et al.*, 1978; Heidger *et al.*, 1978; Tung and Alexander, 1980; Lohiya *et al.*, 1987), musk shrew (Singh and Dominic, 1981), rabbits (Zhao *et al.*, 1987; Liu *et al.*, 1990; Wang *et al.*, 1992), rams (Perera, 1978) or rats (Neaves, 1978; Dong *et al.*, 1999; Whyte *et al.*, 2000; Kubota *et al.*, 2001). The time course of recovery of spermatogenesis after damage to spermatogenesis by vasectomy was not well demonstrated, but some recovery was reported in dogs (at 3–6 months after surgery: Lohiya *et al.*, 1975; MacDougall *et al.*, 1975; Urry *et al.*, 1976), humans (at 2 years: Gupta *et al.*, 1975), musk shrew (at 6 months: Singh and Dominic, 1981), rabbits (at 12 months: Liu *et al.*, 1990; Wang *et al.*, 1992) and rats (at 3 months: Whyte *et al.*, 1998). Lue *et al.* (1997) showed a transient germ cell apoptosis 3 weeks after surgery in rats, with little or no detrimental effect on the morphological characteristics of spermatogenesis at 12 weeks after surgery. Besides, the pattern and extent of damage are far from clear.

The lack of uniformity in results may be attributable to factors such as species or individual variation, study design, postoperative complications, postoperative time interval and the methodology used to determine the spermatogenic status. In mice, for example, only a slight effect on spermatogenesis (with approximately 30% decrease in number of spermatids at 6 months after vasectomy) was observed by Barratt and Cohen (1988), whereas a marked effect (with much depletion of germ cells 6–12 months after vasectomy) was reported by Singh and Chakravarty (2000). Aitken *et al.* (1999) reported that the percentage ($47 \pm 13\%$) of atrophic seminiferous tubules in five testes from five unilaterally vasectomized (for 3 years) guinea-pigs was consistently higher than that ($6 \pm 2\%$) in the contralateral testis; however, owing to considerable individual variation in the sham-operated animals (62% in one control animal versus 3.5–4.1% in the other three control animals), they were unable to reach a statistical conclusion with one-way analysis of variance that vasectomy led to the tubular atrophy. However, if only the vasectomized testis and the contralateral control testis are compared using the paired *t* test, a significant difference is detected ($P < 0.05$). Heidger *et al.* (1978) and Perera (1978) investigated the effect over a long postoperative period in monkeys (1–66 weeks) and rams (3–45 months), but most testicular samples at each time point were obtained from only one or two animals.

Spermatogenesis involves several types of germ cell

and it seems that the effects of vasectomy on spermatogenesis, if any, are not severe in most cases. Thus the methods used to express the spermatogenic activity are important. Previous studies described spermatogenesis either qualitatively (Gupta *et al.*, 1975; Heidger *et al.*, 1978; Tung and Alexander, 1980; Liu *et al.*, 1990; Wang *et al.*, 1992; Aitken *et al.*, 1999, 2000; Singh and Chakravarty, 2000; Whyte *et al.*, 2000), or with semiquantitative data such as (i) germ cell (two-dimensional nuclear profile) number per tubule (two-dimensional profile) (Zhao *et al.*, 1987; McDonald and Scothorne, 1988), per tubule cross-section (two-dimensional profile) (Barratt and Cohen, 1988; Hirsch and Choi, 1990) or per Sertoli cell (two-dimensional nuclear profile) (Perera, 1978; Hadley and Dym, 1983); (ii) testicular biopsy score count (Flickinger *et al.*, 1988); (iii) number of tubules with spermatids (Jarow *et al.*, 1994) or with fewer germ cells (Aitken *et al.*, 1999); or (iv) the number (percentage) of haploid cells (spermatids and spermatozoa) as determined by flow cytometry (Dong *et al.*, 1999; Shiraishi *et al.*, 2001). The key and directly comparable parameters for the description of spermatogenesis are the numbers of all types of germ cell per unit volume of testis and per testis, yet there is a paucity of such data in vasectomized animals. Lue *et al.* (1997) obtained such numbers of germ cells (not including elongated spermatids) in the hamster testis vasectomized for up to 12 weeks using a standard stereological method based on nuclear profile counts and measurement of nuclear diameters, which is considered to be the best of previous morphometric methods used for quantitative description of spermatogenesis in vasectomized testis. But this method, strictly speaking, is still not unbiased. The best stereological tool for estimation of nuclear number is the optical disector, which is not only unbiased but also efficient compared with the physical disector. The optical disector was first used to estimate the number of germ cells in rats (Wreford, 1995), and we have used it to study spermatogenesis in monkeys (Zhengwei *et al.*, 1997, 1998a; Wen and Yang, 2000), humans (Zhengwei *et al.*, 1998b), rats (Wen and Yang, 2000; Wen *et al.*, 2000) and rabbits (Zhang *et al.*, 2002) under normal, gonadotrophin withdrawal or experimentally cryptorchid conditions.

It was reported that long-term vasectomy in langur monkeys (*Presbytis entellus*) (Lohiya *et al.*, 1987) or men (Jakobsen *et al.*, 1989) affected the secretory function of the prostate gland, thus decreasing the volume of ejaculated semen. The association between vasectomy and prostate cancer as suggested by some epidemiological studies (Weiske, 2001) remains controversial. There is also a paucity of morphometric data on the histological structures of the prostate gland and seminal vesicles after long-term vasectomy.

This study was therefore undertaken using a monkey model to re-evaluate the effects of vasectomy (at 6 and 12 months after surgery) on spermatogenesis by obtaining numbers of germ cells in the testes and spermatozoa in the epididymides using the optical disector, and on the

structures of the prostate gland and seminal vesicles by obtaining morphometric data with other stereological methods.

Materials and Methods

Animals and design

Eight normal mature male rhesus monkeys (*Macaca mulatta*), aged 4–6 (4.9 ± 0.2) years, were included in the experiment. Bilateral vasectomy was performed in June on five randomly chosen animals, with a segment of vas deferens being excised and the cut ends being ligated with silk threads. (All excised parts, which were embedded in paraffin wax for preparation of sections, were histologically confirmed to be vas deferens.) No surgery was performed on the other three animals (control). Six months later, blood was obtained from a subcutaneous vein in one leg to perform blood counts using an automatic haematology analyser (Coulter, Germany) at The First People's Hospital of Yunnan Province in Kunming (Yunnan Province), and hemicastration was performed to remove ipsilateral testis and epididymis of one side (left or right, alternatively chosen) on each animal. After a further 6 months, the contralateral testis and epididymis, together with the prostate gland and seminal vesicles, were removed from all animals after perfusion via the heart with 700 ml of phosphate-buffered saline and then 2 l of Zamboni's fixative.

An experienced surgeon performed all operations and anaesthesia was induced by intra-muscular injection of ketamine hydrochloride. The animals were obtained from the Kunming Institute of Animals Research attached to the Chinese Academy of Science, and were kept there in a large cage (approximately 45 m³) made of metal bars throughout the whole experiment. The study was approved by the Sichuan Committee of Family Planning and North Sichuan Medical College.

Tissue processing

On removal, testes and epididymides were further fixed by immersion in Bouin's fixative for about 48 h. After fixation, testes and epididymides were immersed in 70% ethanol for 2 days and prostate glands and seminal vesicles for 9 months before further processing. Subsequently the whole organs were weighed using an electronic balance (HR-120, A&D Company, Ltd., Tokyo; accuracy 0.1 mg) to calculate organ volumes.

Organs were first cut into parallel thick slices and two to four slices were sampled in a systematic random manner (evenly spaced). The thick slices were cut into thinner slices of about 1–2 mm in thickness, which were further cut into smaller blocks before sampling. Four (at 6 months) or five (at 12 months) testicular blocks per testis and four (at 6 months) or three (at 12 months) epididymal blocks per epididymis were systematically sampled for embedding in methacrylate resin (hydroxyethylmethacrylate, Heraeus Kulzer GmbH, Wehrheim/Ts). Three blocks from each

prostate gland and three from one (random) of the two seminal vesicles of the same animal were sampled for embedding in paraffin wax (JUNG-Histowax, Cambridge Instruments GmbH, Nussloch). As could be seen from the cut surface of organ slices, except for the central area of most testes, all organs were fixed. Therefore testicular blocks were further immersion-fixed in Bouin's solution overnight before dehydration in ethanol and butanol. One methacrylate-embedded section 25 µm in thickness or one paraffin-embedded section 5 µm in thickness was cut from each block on a semi-automatic microtome (RM2145, Leica Instruments GmbH, Nussloch). The average area of sections, as estimated by the simple conventional stereological point counting method, was about 29 mm² for testis, 20 mm² for epididymis and 10 mm² for prostate gland and seminal vesicle.

Methacrylate-embedded testicular sections were stained with PAS (periodic acid–Schiff reagent) plus Harris haematoxylin, but epididymal sections were stained with haematoxylin only. Paraffin-embedded sections from the prostate gland and seminal vesicles were stained using a monoclonal antibody against smooth muscle α -actin and counterstained with Harris haematoxylin. The monoclonal antibody was from Sigma (St Louis, MO). Diaminobenzidine (DAB) was used to develop the immunoreaction product and sections were mounted with a resin mounting medium as described by Zhengwei *et al.* (1998c), Huang *et al.* (2001) and Zhang *et al.* (2002).

Stereology

Number of cells. Germ cells were divided into groups of type A spermatogonia; type B spermatogonia; preleptotene, leptotene and zygotene primary spermatocytes; pachytene primary spermatocytes; secondary spermatocytes; early (steps 1–12) spermatids; and late elongated (steps 13–14) spermatids as described by Zhengwei *et al.* (1997) and Zhang *et al.* (2002). Pyknotic nuclei, the chromatin of which was dark and dense, were smaller and sometimes irregular in shape compared with their adjacent germ cell type and were grouped separately.

Numbers of germ cells and Sertoli cells per testis were estimated using the optical disector as described by Zhengwei *et al.* (1997, 1998a,b), Wen and Yang (2000), Wen *et al.* (2000) and Zhang *et al.* (2002). Briefly, a section was brought into focus using an oil immersion lens and observed on a computer screen at final magnification $\times 2677$. A set of four rectangular frames (each with area 17 µm $\times$ 22 µm) was superimposed on the image and fields were systematically sampled on each section with a motorized stage. In focusing down continually in the tissue section of 10 µm in depth, the nuclei were counted as they came into the frames according to the disector counting rule. On each field all four frames were used for counting spermatogonia and secondary spermatocytes while only one (upper left) frame was used for other types of germ cell and Sertoli cells. The calculation of nuclear numerical density (number per volume of testis) was based on the

number of nuclei counted and the volume of optical disectors used for the counting per testis. The total number of cells (number of nuclei being assumed to be equal to number of cells) per testis was calculated by multiplying the density with the testicular volume. An average of 466 fields per testis was sampled systematically and an average of 1530 nuclei was counted per testis.

The number of spermatozoa and other cells (round spermatids and other non-spermatozoa cells identified according to nuclear morphology) in the lumen of the epididymal tubules per epididymis was estimated in a similar way to that described by Wen and Yang (2000). Epididymal sections were observed at final magnification $\times 4417$ and a set of six frames (each with area $5\ \mu\text{m} \times 6\ \mu\text{m}$) was superimposed on the image. An average of 1290 optical disectors (each with volume $5\ \mu\text{m} \times 6\ \mu\text{m} \times 10\ \mu\text{m}$) was used for counting spermatozoa and 257 spermatozoa were counted per epididymis.

Volume per organ. The upper-left corner of a counting frame, intersection between the upper and left sides of the frame, was regarded as a test point. All four frames for testicular sections and only one upper left frame for epididymal sections were considered, that is four test points for testicular sections and one test point for epididymal sections were superimposed on each field. The numbers of test points hitting different structures such as the seminiferous tubules in testis and the epididymal tubules and sperm agglomerates (epididymal fluid with densely packed spermatozoa) in the epididymal tubules were recorded before nuclear counting on each field as described above.

Sections of the prostate gland and seminal vesicles were observed using a $\times 10$ objective lens at final magnification $\times 268$. Twelve test points (see Huang *et al.*, 2001) were superimposed on each field systematically sampled, and the numbers of test points hitting different structures were recorded (for example the tubuloalveolar glands and the epithelium and concretions in the alveoli, and the smooth muscle bundles in the fibromuscular stroma in the prostate gland, and the tubular glands sharply delineated by the muscular coat in the seminal vesicle). Averages of 335 and 102 test points were counted per epididymis and seminal vesicle, respectively.

The percentage volume (volume fraction) of a structure in an organ was estimated by the percentage of test points hitting the structure (a fraction of the total number of test points, per organ, superimposed on the whole section), and then the total volume of the structure in the organ was calculated by multiplying the percentage by the volume of the organ.

Tubule diameter and length. The same testicular and epididymal sections used for nuclear counting (described above) were observed again on screen using a $\times 10$ objective lens (final magnification $\times 268$). Round or elliptical seminiferous and epididymal tubule profiles (90 per testis and 59 per epididymis on average) were randomly sampled

with a frame to measure their diameters, and then tubule lengths per organ were calculated from diameters and tubule volumes as described by Zhengwei *et al.* (1998a,b), Wen and Yang (2000), Wen *et al.* (2000) and Zhang *et al.* (2002).

Statistical analysis

Data in the text and tables are shown as mean $\pm$ SEM. Two-way repeated measures (RM) analysis of variance (ANOVA) on one factor (general linear model) was used to detect whether vasectomy or hemicastration had an effect on testicular or epididymal structures, and whether there was interaction between vasectomy and hemicastration. When a significant effect ($P < 0.05$) was detected, all-pairwise multiple comparison procedures were performed using the Student–Newman–Keuls method. For the same purpose, the following tests, theoretically inappropriate in this case but more straightforward and easier to understand, were also performed as a double check: unpaired *t* test was used to test the difference between vasectomized and control animals either at 6 or 12 months (to detect any significant effects of vasectomy), and paired *t* test was used to test for any significant differences between 6 and 12 months either in the vasectomized or control animals (to detect any significant effects of hemicastration). Moreover, to check further for the reliability of the statistical inferences reached, the four or five testicular sections obtained from each testis were randomly divided into two subsets (each with two or three sections). Thus two stereological results (results 1 and 2) were obtained from the respective stereological measurements on the sections. Then the same statistical tests were repeated as described above using result 1 and result 2, respectively. Other statistical tests are indicated in the text where they are used. Significance of difference was set at $P < 0.05$.

Results

At the beginning of experiment, the body weight of the eight animals was 6.9 ± 0.6 kg, which was significantly increased by $9.3 \pm 2.5\%$ at 6 months (to 7.4 ± 0.6 kg) or by $12.2 \pm 4.7\%$ at 12 months (to 7.6 ± 0.6 kg), without significant difference between the last two time points (one-way RM ANOVA in conjunction with the Student–Newman–Keuls method). There were no significant differences in age, body weights, haemoglobin concentration, erythrocyte count or leucocyte count between vasectomized and control animals (Table 1).

All sections were observed within a few days and no marked differences were seen in the histology of the testis, epididymis, prostate gland and seminal vesicle between vasectomized and control animals at 6 months (for testis and epididymis) or 12 months of experiment, except that a sperm granuloma was found on three epididymal sections, one from a vasectomized animal at 6 months and the other two from another two different vasectomized animals at 12 months (see Fig. 1). The granuloma on each of the three sections occupied about half of the section in area; since the

Table 1. Age, body weight and blood counts in vasectomized ($n = 5$) and control ($n = 3$) adult rhesus monkeys

	Vasectomized	Control
Age (years) at start of study	5.0 ± 0.3	4.7 ± 0.3
Body weight (kg) at start of study	7.6 ± 0.8	5.7 ± 0.4
Body weight (kg) at 6 months after vasectomy	8.1 ± 0.8	6.3 ± 0.6
Body weight (kg) at 12 months after vasectomy	8.2 ± 0.8	6.6 ± 0.9
Haemoglobin (g l^{-1}) at 6 months after vasectomy	154 ± 3	150 ± 10
Erythrocyte count (10^{12} l^{-1}) at 6 months after vasectomy	6.52 ± 0.20	6.11 ± 0.39
Leucocyte count (10^9 l^{-1}) at 6 months after vasectomy	13.3 ± 2.0	11.6 ± 1.0

Values are mean $\pm$ SEM.

Bilateral vasectomy was performed at the beginning of the experiment. At 6 months, hemicastration was performed on both vasectomized and control animals. There was no significant difference between vasectomized and control animals in all parameters listed in this table (unpaired t test).

Fig. 1. Light micrographs of epididymal sections from two rhesus monkeys (*Macaca mulatta*) undergoing bilateral vasectomy, with one epididymis (together with the ipsilateral testis) being removed at (a) 12 months and the other at (b) 6 months after vasectomy. Micrographs were taken with a $\times 4$ objective lens on methacrylate-embedded sections, 25 μm in thickness, stained with haematoxylin. G: sperm granuloma densely packed with spermatozoa; M: the membrane (consisting mainly of dense connective tissue) of the sperm granuloma; S: sperm agglomerate (densely packed with spermatozoa) in the epididymal tubule. Scale bar represents 300 μm .

total number of epididymal sections from vasectomized animals was 35, the granuloma constituted about 4% of the total section area and therefore about 4% of the total epididymal volume according to the stereological principle.

As indicated by a two-way RM ANOVA, hemicastration had a significant effect on the number of type A spermatogonia per unit volume of testis and per testis and the diameter of the seminiferous tubules (Table 2 and Fig. 2). The same statistical inferences were also reached when the analyses were performed using the two subsets of data obtained by dividing the testicular sections into two subsets as described. Six months after hemicastration, the number of type A spermatogonia per testis increased significantly by $77 \pm 24\%$ (range 1–191%, $n = 8$), and the number per unit volume increased significantly by $176 \pm 56\%$ ($n = 8$, decreased by 2% in one animal and increased by 8–455% in the other seven animals), whereas the diameter of the seminiferous tubules decreased significantly by $17 \pm 6\%$ ($n = 8$, the diameter increased by 12% in one animal and decreased by 9–44% in the other seven animals). After hemicastration the volumes of the remaining testis and epididymis and the numbers of spermatocytes and spermatids per remaining testis and spermatozoa per remaining epididymis appeared to have become smaller but the differences were not significant.

The two-way RM ANOVA revealed that vasectomy did not have any significant effects on all quantitative data obtained in the present study (Table 2), after allowing for effects of hemicastration (described above), except the numerical densities ($\times 10^6 \text{ cm}^{-3}$) of type A spermatogonia, which, in the vasectomized and control groups, were 18.8 ± 3.6 and 16.2 ± 2.2 at 6 months and 50.6 ± 4.9 and 30.1 ± 7.4 at 12 months, respectively. There was no interaction between vasectomy and hemicastration. The statistical conclusions using the two subsets of data (obtained by dividing the testicular sections into two subsets as described) for comparisons were the same for all quantitative parameters obtained in this study except that the significance of vasectomy on the numerical density of type A spermatogonia was detected using one subset of data only.

Table 2. Quantitative data of the main testicular and epididymal structures in vasectomized ($n = 5$) and control ($n = 3$) rhesus monkeys

Parameters	Six months after vasectomy		Twelve months after vasectomy	
	Vasectomized	Control	Vasectomized	Control
Volume (cm ³)				
Testis	18.4 ± 3.0	16.8 ± 4.8	11.0 ± 1.3	13.9 ± 2.1
Epididymis	4.31 ± 0.60	3.24 ± 0.55	3.54 ± 0.53	2.85 ± 0.33
Volume per organ (cm ³)				
Seminiferous tubules	14.6 ± 2.2	13.5 ± 3.9	8.9 ± 1.3	11.6 ± 2.0
Epididymal tubules	1.90 ± 0.48	1.52 ± 0.33	1.46 ± 0.40	1.22 ± 0.28
Diameter (μm)				
Seminiferous tubules*	177 ± 8	160 ± 15	142 ± 6 ^a	154 ± 2
Epididymal tubules	277 ± 11	287 ± 26	272 ± 24	252 ± 23
Length per organ (m)				
Seminiferous tubules	573 ± 38	620 ± 85	539 ± 45	610 ± 99
Epididymal tubules	29.6 ± 6.3	21.2 ± 2.2	21.6 ± 4.4	22.2 ± 1.8
Number of cells (× 10 ⁶ per organ)				
Type A spermatogonia*	312 ± 28	293 ± 114	542 ± 59 ^a	410 ± 117
Type B spermatogonia	292 ± 82	419 ± 148	308 ± 75	173 ± 58
PI-Z	508 ± 193	750 ± 274	372 ± 101	343 ± 119
P	1460 ± 328	1634 ± 557	1084 ± 246	1397 ± 236
Secondary spermatocytes	100 ± 24	110 ± 34	86 ± 27	65 ± 21
R-E	6250 ± 1444	5289 ± 2772	3427 ± 818	4324 ± 1064
L	2932 ± 526	2688 ± 1225	1160 ± 286	1896 ± 911
Sertoli cells (testis)	1144 ± 89	1391 ± 136	1252 ± 159	1391 ± 169
Spermatozoa (epididymis)	3374 ± 1662	3504 ± 1550	2747 ± 1131	1278 ± 993

Values are mean ± SEM.

Ipsilateral testis and epididymis were first removed from bilaterally vasectomized and age-matched control (not undergoing vasectomy) adult animals at 6 months after surgery and then the contralateral organs were removed at 12 months (6 months after hemicastration).

L: late elongated spermatids; P: pachytene primary spermatocytes; PI-Z: preleptotene, leptotene and zygotene primary spermatocytes; R-E: early spermatids (round and elongating spermatids).

*Hemicastration but not vasectomy had a significant effect and there was no significant interaction between hemicastration and vasectomy (two-way repeated measure ANOVA).

^aSignificantly different from the vasectomized in the '6 month' group (paired *t* test).

Only 1–4 pyknotic nuclei were counted per testis; the numbers per testis in the vasectomized and control groups were $8 \pm 2 \times 10^6$ and $7 \pm 2 \times 10^6$ at 6 months and $2 \pm 1 \times 10^6$ and $4 \pm 1 \times 10^6$ at 12 months, respectively. The numbers of round spermatids and other non-sperm cells in the epididymal lumen were very small compared with the numbers of spermatozoa (Table 2). Those non-sperm cells were counted (by the optical disectors used) in half of the epididymides (that is, the numbers counted from the other organs were 0). The numbers of round spermatids per epididymis in the vasectomized and control groups were $3 \pm 2 \times 10^6$ and $5 \pm 5 \times 10^6$ at 6 months, and $22 \pm 11 \times 10^6$ and $2 \pm 2 \times 10^6$ at 12 months, respectively; those of other non-sperm cells were $8 \pm 5 \times 10^6$ and $5 \pm 4 \times 10^6$ at 6 months, and $148 \pm 144 \times 10^6$ (the number in one animal was extremely high: 726) and $2 \pm 2 \times 10^6$ at 12 months, respectively. Owing to the small number of nuclei counted and the large individual variation in the number of cells, statistical tests were not performed.

Compared with controls, the prostate gland and seminal vesicles from vasectomized animals were unchanged in all the quantitative parameters obtained in this study (Table 3).

Discussion

This study, the first to evaluate the spermatogenic effects of vasectomy using the optical disector, demonstrated that vasectomy in the rhesus monkey for 6 or 12 months did not significantly damage spermatogenesis or reduce the number of spermatozoa in the epididymis, although sperm granuloma was formed in the epididymis. Thus, vasectomy is a safe procedure in terms of spermatogenesis, and recovery of sperm concentration following a vasectomy reversal would depend mainly on the quality of the surgery itself in terms of vasal re-canalization.

Semen consists predominantly of secretions from the prostate gland and seminal vesicles. The present study demonstrated that the main histological structures in these organs were unaffected, indicating indirectly that the volume of semen was probably unchanged following vasectomy. The study also showed that prostate cancer was not induced after one year of vasectomy in this model. The conclusion may therefore be that short-term (up to 1 year) vasectomy would not considerably affect the morphology or function of the prostate gland and seminal vesicles.

Fig. 2. Light micrographs of testicular sections of rhesus monkeys (*Macaca mulatta*). (a) and (b) were from the same animal that underwent bilateral vasectomy, with one testis (together with the ipsilateral epididymis) being removed at (a) 6 months and (b) 12 months. (c) Testis, removed at one year, of another rhesus monkey that did not undergo vasectomy but was hemicastrated 6 months after the start of the experiment. Micrographs were taken with a $\times 20$ objective lens on methacrylate-embedded sections, 25 μm in thickness, stained with periodic acid–Schiff (PAS) plus haematoxylin. Scale bar represents 40 μm .

The present study was not originally designed to study the effects of hemicastration on spermatogenesis, but the results did show some effects. Ramaswamy *et al.* (2000) reported that at 44 days after hemicastration in rhesus monkeys the mass (average 37.3 g) of the remaining testis significantly increased by about 36% compared with that (average 27.4 g) of the testis removed at hemicastration. In contrast, a trend of decrease rather than increase was found in the current study: the ratio between the volumes of the remaining and hemicastrated testes of the same animals was 0.76 ± 0.11 ($n = 8$, range 0.40–1.12, five individual ratios < 1 ; paired t test, $P = 0.053$). It remains to be clarified whether the discrepancy was due to a different time interval (6 months in the current study) after hemicastration. Hochereau-de Reviers *et al.* (1976) too reported an increase (by 40%) in testicular size after hemicastration (for 4–6 months) in adult rams. However, most studies showed that hemicastration in adult patients or animals did not induce a change in testicular size, for example in men (Ferreira *et al.*, 1991; Lin *et al.*, 1998), monkeys (Zhengwei *et al.*, 1998a) and rats (Howland and Skinner, 1975; Frankel and Wright, 1982; Bergh and Damber, 1991; Brown and Chakraborty, 1991). Compensatory testicular hypertrophy generally occurred when hemicastration was induced in prepubertal or neonatal animals, for example in boars (Kosco *et al.*, 1987), bulls (Schanbacher *et al.*, 1987), lambs (Waites *et al.*, 1983), rams (Walton *et al.*, 1980; Jenkins and Waites, 1983) and rats (Cunningham *et al.*, 1978; Putra and Blackshaw, 1982; Orth *et al.*, 1984; Ultee-van Gessel *et al.*, 1985; Brown and Chakraborty, 1991; Simorangkir *et al.*, 1995).

After obtaining the number of nuclei per testis indirectly from nuclear counts per tubule cross-section (essentially a nuclear profile counting method) in combination with the length of the seminiferous tubules per testis (although the result may have been affected by bias resulting from the counting method and non-systematic random sampling of cells on sections), Ramaswamy *et al.* (2000) reported that 44 days after hemicastration in monkeys the numbers of spermatogenic cells such as spermatocytes and spermatids increased with increase of testicular sizes, whereas the numbers of Sertoli cells and type A spermatogonia did not increase. Similar changes were observed in the developing rat (aged 1–70 days), in which the numbers of Sertoli cells and spermatogonia per testis remained stable from day 15 to day 30, respectively, whereas there was a trend of continual increase in testicular sizes and numbers of spermatocytes and round spermatids per testis (Wang *et al.*, 1989; Yang *et al.*, 1990). In addition, increases in the testicular size and the number of spermatids (but not the number of spermatogonia) were also reported in adult rabbits that were 7 weeks older (Zhang *et al.*, 2002).

The present study demonstrated that hemicastration resulted in a significant reduction in the diameter of the seminiferous tubules. The number of late elongated spermatids per testis also appeared to be smaller: the ratio between the numbers (per testis) in the remaining and hemicastrated testes of the same animal was 0.66 ± 0.19 ($n = 8$,

Table 3. Volumes (cm³) of the prostate gland and seminal vesicle (average of both sides), and volumes of their main structures per organ in vasectomized ($n = 5$) and control ($n = 3$) adult rhesus monkeys

	Vasectomized	Control
Prostate gland		
Organ	2.01 ± 0.27	2.03 ± 0.29
Glandular epithelium	0.404 ± 0.063	0.385 ± 0.031
Glandular cavity	0.247 ± 0.045	0.369 ± 0.096
Concretion in the glandular cavity	0.071 ± 0.022	0.028 ± 0.006
Smooth muscle bundles in the stroma	0.675 ± 0.120	0.561 ± 0.101
Seminal vesicle		
Organ	4.45 ± 0.63	4.09 ± 0.18
Tubular gland	3.03 ± 0.57	3.12 ± 0.16

Values are mean ± SEM.

Ipsilateral testis and epididymis were removed from bilaterally vasectomized and age-matched control (not undergoing vasectomy) animals 6 months after the start of the experiment, and the prostate gland and the bilateral seminal vesicles were removed at 12 months (6 months after hemicastration). There was no significant difference between vasectomized and control animals in any of the parameters (unpaired t test).

range 0.20–1.50, six individual ratios < 1; paired t test, $P = 0.057$). In accord, the ratio between the numbers (per epididymis) of spermatozoa in the remaining epididymis and the epididymis removed at hemicastration was 0.65 ± 0.18 ($n = 8$, range 0.03–1.65, six individual ratios < 1; paired t test, $P = 0.112$). Thus, it is possible that the number of elongated spermatids per remaining testis, or spermatozoa per remaining epididymis would be significantly reduced in the longer term (more than 6 months after hemicastration). This speculation provides an explanation for the clinical reports that sperm counts significantly decreased after long-term hemicastration due to testicular trauma in men (Ferreira *et al.*, 1991; Lin *et al.*, 1998).

As shown in the present study, hemicastration led to an increase in the number of type A spermatogonia per testis. This finding may be the result of increased FSH concentrations. Although serum FSH concentrations were not measured here, other studies did consistently show that hemicastration brought about sustained high concentrations of FSH after long-term hemicastration in adult men (Lin *et al.*, 1998) and adult monkeys (Ramaswamy *et al.*, 2000). In addition, FSH treatment for 28 days in normal adult cynomolgus monkeys (*Macaca fascicularis*) significantly increased (by 100%) the numbers (probably nuclear profiles per Sertoli cell nuclear profile) of type A pale spermatogonia, type B₄ spermatogonia, PI-Z spermatocytes or round spermatids (van Alphen *et al.*, 1988). The finding of the present study that an increased number of type A spermatogonia in hemicastrated monkeys was not associated with increased numbers of advanced spermatogenic cells indicates that FSH is not the only factor regulating spermatogenesis (Zhengwei *et al.*, 1998a; McLachlan, 2000), and the exact mechanism needs to be studied further.

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