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

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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 ± SEM: 87 ± 3%) and a pregnancy rate of 52% (median; range 28–82%; mean 55 ± 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 ± 0.5 × 10⁶ ml⁻¹) of men (n = 36) who impregnated their partners was significantly higher than that (9.9 ± 0.5 × 10⁶ 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 ± 23 × 10⁶ ml⁻¹ versus 10 ± 9 × 10⁶ ml⁻¹) were significantly different and the sperm count of either treated group was significantly lower than that of the control group (816 ± 79 × 10⁶ 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 (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 ± 13%) of atrophic seminiferous tubules in five testes from five unilaterally vasectomized (for 3 years) guinea-pigs was consistently higher than that (6 ± 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 (Jarrow 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 dissector, which is not only unbiased but also efficient compared with the physical dissector. The optical dissector 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 dissector, 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 hematicastration 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 spermatagonia; type B spermatagonia; 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 × 2677. A set of four rectangular frames (each with area 17 μm × 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 sper-
mats and other non-spermatozoa cells identified accord-
ing 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 $5 \mu m \times 6 \mu m$) was superimposed on
the image. An average of 1290 optical disectors (each with
volume $5 \mu m \times 6 \mu m \times 10 \mu m$) was used for counting
spermatozoa and 257 spermatozoa were counted per epi-
didymis.

*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 testi-
cular 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 sper-
matozoa) 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 $10 \times 10$ 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 $10 \times 10$
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

**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 $6.9 \pm 0.6$ kg, which was significantly
increased by $9.3 \pm 2.5\%$ at 6 months (to $7.4 \pm 0.6$ kg) or by
$12.2 \pm 4.7\%$ at 12 months (to $7.6 \pm 0.6$ kg), without signif-
cant 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
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 spermato- gonia 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 77–191% (n = 8), and the number per unit volume increased significantly by 17–455% (n = 8, decreased by 2% in one animal and increased by 6–455% in the other seven animals), whereas the diameter of the seminiferous tubules decreased significantly 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 (× 10^6 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 1. Age, body weight and blood counts in vasectomized (n = 5) and control (n = 3) adult rhesus monkeys

<table>
<thead>
<tr>
<th></th>
<th>Vasectomized</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) at start of study</td>
<td>5.0 ± 0.3</td>
<td>4.7 ± 0.3</td>
</tr>
<tr>
<td>Body weight (kg) at start of study</td>
<td>7.6 ± 0.8</td>
<td>5.7 ± 0.4</td>
</tr>
<tr>
<td>Body weight (kg) at 6 months after vasectomy</td>
<td>8.1 ± 0.8</td>
<td>6.3 ± 0.6</td>
</tr>
<tr>
<td>Body weight (kg) at 12 months after vasectomy</td>
<td>8.2 ± 0.8</td>
<td>6.6 ± 0.9</td>
</tr>
<tr>
<td>Haemoglobin (g l⁻¹) at 6 months after vasectomy</td>
<td>154 ± 3</td>
<td>150 ± 10</td>
</tr>
<tr>
<td>Erythrocyte count (10¹² l⁻¹) at 6 months after vasectomy</td>
<td>6.52 ± 0.20</td>
<td>6.11 ± 0.39</td>
</tr>
<tr>
<td>Leucocyte count (10⁹ l⁻¹) at 6 months after vasectomy</td>
<td>13.3 ± 2.0</td>
<td>11.6 ± 1.0</td>
</tr>
</tbody>
</table>

Values are mean ± 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 × 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.
Table 2. Quantitative data of the main testicular and epididymal structures in vasectomized (n = 5) and control (n = 3) rhesus monkeys

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Six months after vasectomy</th>
<th>Twelve months after vasectomy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vasectomized</td>
<td>Control</td>
</tr>
<tr>
<td>Volume (cm³)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testis</td>
<td>18.4 ± 3.0</td>
<td>16.8 ± 4.8</td>
</tr>
<tr>
<td>Epididymis</td>
<td>4.31 ± 0.60</td>
<td>3.24 ± 0.55</td>
</tr>
<tr>
<td>Volume per organ (cm³)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seminiferous tubules</td>
<td>14.6 ± 2.2</td>
<td>13.5 ± 3.9</td>
</tr>
<tr>
<td>Epididymal tubules</td>
<td>1.90 ± 0.48</td>
<td>1.52 ± 0.33</td>
</tr>
<tr>
<td>Diameter (μm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seminiferous tubules*</td>
<td>177 ± 8</td>
<td>160 ± 15</td>
</tr>
<tr>
<td>Epididymal tubules</td>
<td>277 ± 11</td>
<td>287 ± 26</td>
</tr>
<tr>
<td>Length per organ (m)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seminiferous tubules</td>
<td>573 ± 38</td>
<td>620 ± 85</td>
</tr>
<tr>
<td>Epididymal tubules</td>
<td>29.6 ± 6.3</td>
<td>21.2 ± 2.2</td>
</tr>
<tr>
<td>Number of cells (× 10⁶ per organ)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type A spermatogonia*</td>
<td>312 ± 28</td>
<td>293 ± 114</td>
</tr>
<tr>
<td>Type B spermatogonia</td>
<td>292 ± 82</td>
<td>419 ± 148</td>
</tr>
<tr>
<td>PI-Z</td>
<td>508 ± 193</td>
<td>750 ± 274</td>
</tr>
<tr>
<td>P</td>
<td>1460 ± 328</td>
<td>1634 ± 557</td>
</tr>
<tr>
<td>Secondary spermatocytes</td>
<td>100 ± 24</td>
<td>110 ± 34</td>
</tr>
<tr>
<td>R-E</td>
<td>6250 ± 1444</td>
<td>5289 ± 2772</td>
</tr>
<tr>
<td>L</td>
<td>2932 ± 526</td>
<td>2688 ± 1225</td>
</tr>
<tr>
<td>Sertoli cells (testis)</td>
<td>1144 ± 89</td>
<td>1391 ± 136</td>
</tr>
<tr>
<td>Spermatocytes (epididymis)</td>
<td>3374 ± 1662</td>
<td>3504 ± 1550</td>
</tr>
</tbody>
</table>

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).

*Significantly 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 ± 2 × 10⁶ and 7 ± 2 × 10⁶ at 6 months and 2 ± 1 × 10⁶ and 4 ± 1 × 10⁶ 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 dissectors 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 ± 2 × 10⁶ and 5 ± 5 × 10⁶ at 6 months, and 22 ± 11 × 10⁶ and 2 ± 2 × 10⁶ at 12 months, respectively; those of other non-sperm cells were 8 ± 5 × 10⁶ and 5 ± 4 × 10⁶ at 6 months, and 148 ± 144 × 10⁶ (the number in one animal was extremely high: 726) and 2 ± 2 × 10⁶ 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-canilization.

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.
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, 20 objective lens on methacrylate-embedded sections, 25 μm in thickness, stained with periodic acid–Schiff (PAS) plus haematoxylin. Scale bar represents 40 μm.

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 × 20 objective lens on methacrylate-embedded sections, 25 μm in thickness, stained with periodic acid–Schiff (PAS) plus haematoxylin. Scale bar represents 40 μm.
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 \(0.65 \pm 0.18\) (\(n = 8\), 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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### 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

<table>
<thead>
<tr>
<th>Organ</th>
<th>Vasectomized Mean ± SEM</th>
<th>Control Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostate gland</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glandular epithelium</td>
<td>2.01 ± 0.27</td>
<td>2.03 ± 0.29</td>
</tr>
<tr>
<td>Glandular cavity</td>
<td>0.404 ± 0.063</td>
<td>0.385 ± 0.031</td>
</tr>
<tr>
<td>Concretion in the glandular cavity</td>
<td>0.247 ± 0.045</td>
<td>0.369 ± 0.096</td>
</tr>
<tr>
<td>Smooth muscle bundles in the stroma</td>
<td>0.071 ± 0.022</td>
<td>0.028 ± 0.006</td>
</tr>
<tr>
<td>Seminal vesicle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Organ</td>
<td>4.45 ± 0.63</td>
<td>4.09 ± 0.18</td>
</tr>
<tr>
<td>Tubular gland</td>
<td>3.03 ± 0.57</td>
<td>3.12 ± 0.16</td>
</tr>
</tbody>
</table>

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).


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