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·*Original Article*·

Effect of vasectomy via inguinal canal on spermatogenesis in rabbits

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Abstract

Aim: To determine whether vasectomy away from the epididymal tail (via the inguinal canal) in rabbits can reduce the early postoperative effects on spermatogenesis. **Methods:** Twenty-nine normal male Japanese white rabbits (aged 4–6 months) were subjected to unilateral close-ended (conventional) or open-ended (the cut end of the juxta-epididymal vas deferens not ligated) vasectomy via the inguinal canal. Ten days and 3 months after operation, testes, epididymides and vasa deferentia were removed and methacrylate resin-embedded sections prepared. The histology of the testis, epididymis and vas deferens was examined under light microscope, and the volume and diameter of the seminiferous tubules were quantitatively studied using stereological methods. **Results:** Neither of the methods of vasectomy led to apparent damage to spermatogenesis on the vasectomized side in comparison with the contralateral sham-operated side, but the juxta-epididymal vas deferens on the vasectomized side was highly distended and contained numerous sperm 3 months after operation. **Conclusion:** Vasectomy away from the cauda epididymis has no significant early postoperative effects on spermatogenesis in rabbits. *(Asian J Androl 2008 May; 10: 486–493)*

Keywords: vasectomy; inguinal canal; spermatogenesis; testis; rabbits

1 Introduction

Vasectomy, the most reliable method of male contraception, has a major drawback: the irreversibility of sterilization [1]. The problem, however, has become less worrying with the development of microsurgical vasovasostomy and *in vitro* fertilization, and attention has been paid to studying the effects of vasectomy on the spermatogenesis and the fertility outcome with sperm extraction or after vasectomy reversal [2–6].

Our previous studies in rabbits showed that vasectomy (via the scrotum) resulted in severe spermatogenic damage, which was evident 10 days, 3, 6 or 12 months after operation [7, 8]. An earlier study also observed spermatogenic damage 3–28 months after vasectomy in rabbits [9]. The damage was likely related to the increase of intra-testicular pressure induced by the vasal blockage of sperm transportation [8, 10]. We specu-

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lated that open-ended vasectomy (with the juxta-epididymal vas deferens not ligated) might alleviate the damage. Hence, we carried out a study using open-ended vasectomy (via the scrotum) in rabbits; unexpectedly, the results demonstrated more severe spermatogenic damage 10 days and 3 months after operation [11, 12]. In an earlier study, in which vasectomy was performed away from the cauda of epididymis (immediately before the vasal entry into the inguinal canal) in rabbits, only a small effect on spermatogenesis was seen, with 1/10 (1 out of 10) and 5/13 testes showing histological changes at weeks 26 and 32–48 after vasectomy, respectively [13]. However, the spermatogenic status was not clearly described and essentially no control groups were designed in the study. Therefore, the present study aims to determine whether vasectomy away from the cauda epididymis (via the inguinal canal) in rabbits can reduce the early postoperative effects on spermatogenesis.

2 Materials and methods

2.1 Animals and design

Animals were obtained from the Animal Center sponsored by the Sichuan Administrative Committee of Experimental Animals (Chengdu, China). Experiment protocols were approved by the research section of the college and ethical guidelines constituted by the college were followed during the experiment.

2.1.1 Experiment 1

Seven normal male Japanese white rabbits (age: approximately 4 months; body weight: 1.9–2.4 kg) underwent unilateral vasectomy via the inguinal canal. Testes, epididymides and vasa deferentia were removed 10 days after operation. In our previous study, in which greater damage to spermatogenesis was induced by open-ended vasectomy via the scrotum, pubertal rabbits aged 4–5 months were used [11, 12]. To test whether spermatids sloughing, which is liable to occur in pubertal rabbits [14], contributed to the greater damage, we chose to use animals aged approximately 4 months for this experiment.

2.1.2 Experiment 2

Twenty-four normal male Japanese white rabbits (age: approximately 6 months; body weight: 2.3–3.0 kg) were randomly divided into two groups (12 animals each), undergoing: (i) unilateral close-ended vasectomy via the inguinal canal (UC); and (ii) unilateral open-ended vasectomy via the inguinal canal (UO), respectively. To test whether spermatids sloughing that might occur in younger pubertal rabbits contributed to the greater spermatogenic damage previously observed, we chose to use in this experiment older animals that were less likely to have spermatids sloughing [14], for comparison with Experiment 1. Testes, epididymides and vasa deferentia were removed: (i) from 5 animals subjected to UO at day 10 after operation; and (ii) from the remaining animals 3 months after operation.

2.2 Surgery and treatment

UC and UO were alternately performed on all animals, all by YS and BP. Anesthesia was induced by injection of sodium pentobarbital (3 mg/kg body weight) into the marginal ear vein after fur was cut with scissors and skin disinfected with iodine. The operation on each animal lasted an average of 20 min (range 12–33, duration from incision to closing of the skin). Immediately after operation an intramuscular injection of penicillin *G. sodium* (8×10^6 units) was given, which continued once a day for 5 consecutive days for all animals.

2.2.1 Unilateral close-ended vasectomy via inguinal canal

Above the pubic symphysis was made one longitudinal ventral midline dermal incision (approximately 1 cm in length), through which a longitudinal lateral incision (approximately 0.5 cm in length) was made on the wall of the inguinal canal on either side to expose the vas deferens (Figure 1). First, the vas deferens on one side (left or right, alternately chosen) was ligated with two ligatures (silk thread) approximately 1 cm apart and a segment (approximately 0.5 cm in length) of the vas was excised between the two ligatures before the inguinal canal was closed with 1–2 sutures. Then, on the contralateral side, a sham operation was performed in the same way except that the vas deferens was not severed or ligated. Finally, skin was closed with 2–3 sutures.

2.2.2 Unilateral open-ended vasectomy via inguinal canal

The operation was performed exactly in the same way as described above except that, on the vasectomized side, the juxta-epididymal cut end of the vas was left open (not ligated).

2.3 Tissue processing and section preparation

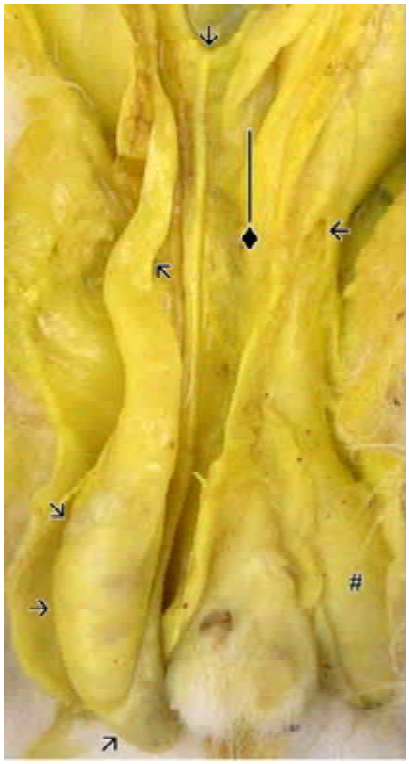

Figure 1. Dissection of the testis, epididymis and vas deferens in a Japanese white rabbit (body weight 2.5 kg) after perfusion fixation with Bouin's solution (for contrasting view of internal structures). The organs are exposed on the right side after opening the tunica vaginalis, while those on the left side are not. ↖, spermatic cord; ↘, caput of epididymis; →, anterior edge of testis; ↗, cauda of epididymis; ↓, vas deferens turning into the pelvic cavity at the internal ring of inguinal canal; ♦, position of the superior border of pubic symphysis;←, external ring of inguinal canal; #, left testis (with intact tunica vaginalis); | , a vertical scale bar (approximately 1 cm) showing the position of the dermal incision for the vasectomy via inguinal canal performed in the present study. Scale bar = 1 cm.

Fresh organs were all immersion-fixed in Bouin's solution for 2 days and then dehydrated in 70% ethanol before weight and density were measured to calculate the volumes of testis and epididymis [14, 15].

As we previously described, two to three tissue blocks were randomly obtained from each testis, and two tissue blocks from the caput and cauda of each epididymis, respectively [14, 15].

For the vasectomized side, the juxta-epididymal segment of vas deferens was removed and divided into four sub-segments (approximately equally spaced). One sub-segment was sampled in an alternate (systematic random) manner: one sub-segment was randomly sampled from the vasectomized side in the first animal, the next (in terms of position along the vas deferens) sub-segment was then sampled from the vasectomized side in the second animal. From the middle of each sub-segment sampled a tissue block was obtained for cutting a cross-sectional vasal section. For the contralateral sham-operated side, one tissue block was obtained from a position corresponding to that for the vasectomized side. The diameter of the vasal section (average of the long and short axes) was measured and regarded as the diameter of the juxta-epididymal vas deferens.

Tissue blocks were embedded in methacrylate resin (hydroxyethyl methacrylate; Historesin, Leica Microsystems Nussloch GmbH, Nussloch, Germany) and one 25 μm thick section was cut from each block and stained with periodic acid-Schiff's reagent plus hematoxylin (testis) or hematoxylin alone (epididymis and vas deferens), the average area per section being approximately 24 mm^2 (testicular sections), approximately 27 mm^2 (epididymal sections), approximately 19 mm^2 (sections of vas deferens on the vasectomized side 3 months after vasectomy) and approximately 3 mm^2 (sections of vas deferens in other groups).

2.4 Morphology

Under a light microscope, the types, numbers, degeneration and sloughing of germ cells in testicular seminiferous tubules were carefully observed; the epididymal duct and vas deferens were observed to determine whether they were distended and whether there was sperm stasis or granuloma.

2.5 Morphometry

Morphometric parameters, the volume and diameter of the seminiferous tubules in the testis, were obtained using stereological methods, as previously described [14, 15]. Briefly, testicular sections were observed on a computer screen (final magnification × 267) and fields were sampled in a systematic (equally spaced) random manner with a motorized stage; the tubular volume was estimated using the stereological point counting method, and round or elliptical seminiferous tubular profiles were sampled for estimation of the tubular diameter.

2.6 Statistics

Comparison of morphometric parameters between the vasectomized side and the contralateral sham-operated side in the same group was performed using the

paired *t*-test. The significance of difference was set at $P \leq 0.05$.

3 Results

In Experiment 2, one animal subjected to UO had a cryptorchid testis on the vasectomized side 10 days after operation; and, 3 months after operation, one animal subjected to UC had a smaller testis with: (i) a swollen scrotum; (ii) adhesion with surrounding tissue; and (iii) non-distended vas deferens on the vasectomized side. Data obtained from these 2 animals were not included in the following results.

Ten days or 3 months after UC or UO (Experiments 1 and 2): (i) the vas deferens in the inguinal canal had no marked adhesion with surrounding tissue; (ii) the scrotum was not swollen; (iii) the testis in the scrotum had no adhesion with surrounding tissue; and (iv) no sperm granuloma was observed in the epididymis or around the vas deferens. The distance from the cauda of epididymis to the juxta-epididymal ligature on the vas deferens or to the juxta-epididymal cut end of the vas deferens on the vasectomized side was approximately 5–8 cm (measured after removal and fixation).

Ten days after operation, the UC or UO (Experiments 1 and 2) had no significant impact on the shape of the testis, epididymis and vas deferens, or on the morphometric parameters (Tables 1 and 2).

Three months after operation, the vasectomies still had no significant impact on the testicular shape or on the volume/diameter of the seminiferous tubules, but the epididymis and, in particular, vas deferens on the vasectomized side were highly distended in comparison with those on the contralateral sham-operated side (Table 2, Figure 2). The distended vasal part appeared milky white and curvy and accounted for approximately 3/5 (in length) of the segment of vas deferens between the cauda of epididymis and the juxta-epididymal vasal ligature or cut end (Figure 2). Observed under a light microscope, the distended epididymal duct or vas deferens was filled with densely packed spermatozoa (Figure 3).

Both qualitative observation and quantitative study on the testicular histology demonstrated that: (i) 10 days after UC (Experiment 1), 6 (out of 7) testes on the va-

Table 1. Results (n = 7, mean ± SE) obtained 10 days after unilateral close-ended vasectomy via inguinal canal (Experiment 1). Vasectomized/sham-operated: results from the vasectomized/the contralateral sham-operated side. Comparison of all parameters between the two sides: $P > 0.05$ (paired *t*-test).

	Vasectomized	Sham-operated
Volume of testis (cm^3)	1.60 ± 0.18	1.60 ± 0.19
Total volume of semeniferous tubules per testis (cm^3)	1.21 ± 0.15	1.21 ± 0.18
Mean diameter of semeniferous tubules (μm)	174 ± 11	173 ± 11
Volume of epididymis (cm^3)	0.63 ± 0.07	0.65 ± 0.05
Mean diameter of juxta-epididymal vas deferens (mm)	2.18 ± 0.12	1.79 ± 0.16

Table 2. Results (mean ± SE) obtained 10 days and 3 months after unilateral close-ended (UC) and unilateral open-ended (UO) vasectomy via inguinal canal (Experiment 2). Vasectomized/sham-operated: results from the vasectomized/the contra-lateral sham-operated side. $^*P < 0.05$ (compared to the sham-operated side in the same group, paired *t*-test).

	10 days, UO (n = 4)		3 months, UO (n = 7)		3 months, UC (n = 11)	
	Vasectomized	Sham-operated	Vasectomized	Sham-operated	Vasectomized	Sham-operated
Volume of testis (cm^3)	2.33 ± 0.30	2.42 ± 0.32	2.59 ± 0.20	2.56 ± 0.15	2.74 ± 0.14	2.69 ± 0.13
Total volume of semeniferous tubules per testis (cm^3)	1.64 ± 0.19	1.71 ± 0.25	1.95 ± 0.17	1.99 ± 0.14	2.08 ± 0.11	2.02 ± 0.10
Mean diameter of semeniferous tubules (μm)	194 ± 12	198 ± 9	193 ± 8	203 ± 7	196 ± 6	196 ± 5
Volume of epididymis (cm^3)	0.80 ± 0.13	0.80 ± 0.11	1.59 ± 0.14*	1.10 ± 0.10	1.83 ± 0.12*	1.15 ± 0.07
Mean diameter of juxta-epididymal vas deferens (mm)	2.26 ± 0.13	2.32 ± 0.21	6.04 ± 0.75*	2.50 ± 0.26	5.39 ± 0.27*	2.46 ± 0.16

sectomized side and 6 testes on the sham-operated side showed normal spermatogenesis, whereas the testes on both sides in one rabbit showed similar spermatogenic damage: atrophy of the seminiferous tubules (average tubular diameter < 140 μm), thinner seminiferous epithelium, and reduction in the number of spermatogenic cells. (ii) Ten days or 3 months after UC or UO (Experimental 2), no apparent damage to spermatogenesis was observed on either the vasectomized or the contralateral sham-operated side.

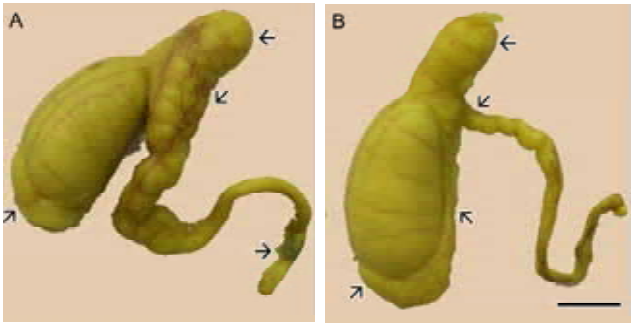

Figure 2. Appearance of the testis, epididymis and vas deferens (after immersion fixation with Bouin's solution) from the same rabbit 3 months after unilateral close-ended vasectomy via inguinal canal. (A): Vasectomized side. (B): Sham-operated side. ↗, caput; ↖, corpus; ←, cauda of epididymis; ↙, vas deferens; →, thread of ligature. Scale bar = 1 cm.

4 Discussion

In the present study, the close-ended or open-ended vasectomy via the inguinal canal did not result in apparent spermatogenic damage 3 months after operation in rabbits. However, the juxta-epididymal vas deferens on the vasectomized side was highly distended and contained numerous sperm. This suggests that sperm were continuously produced by the testis and that transportation of sperm out of the testis still continued after operation. As 2/5 (in length) of the juxta-epididymal vas deferens on the vasectomized side was not yet distended and could still store a lot of sperm, significant increase of intratesticular pressure, if possibly induced by obstruction of sperm transportation out of the testis, did not appear to have occurred. This might well explain why spermatogenic damage was not induced by the operations in the present study.

Our previous studies show that vasectomy via the scrotum (the distance from the cauda of epididymis to the juxta-epididymal vasal ligature was approximately 1 cm) in rabbits leads to marked damage to spermatogenesis in the testis, without marked distention of the epididymis 3 months after operation [7, 11]. The scenario might be that the vasectomy (via the scrotum) first led to an increase in the intra-epididymal pressure (due to sperm stasis) and then the intra-testicular pressure (due to continual production of sperm and testicular fluid by the tes-

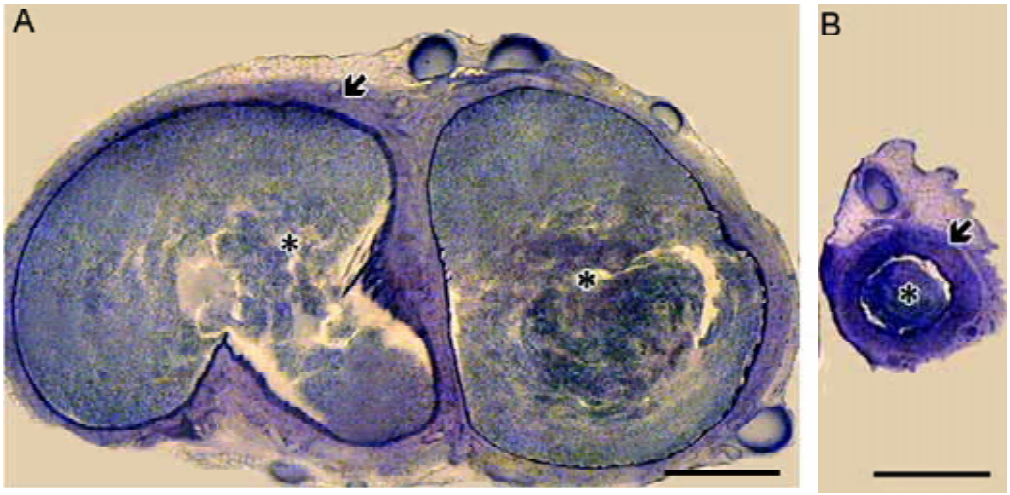

Figure 3. Cross-sectional micrograph of the vas deferens on the vasectomized (A) and the contralateral sham-operated (B) sides of the same rabbit 3 months after unilateral open-ended vasectomy via inguinal canal. ↙, muscular coat of the vas; *, densely parked sperm mass. Scale bar = 1 mm.

tis and the intra-epididymal pressure), which in turn led to spermatogenic damage and, consequently, a decrease in the intra-testicular and intra-epididymal pressure.

Even with vasectomy away from the cauda epididymis, spermatogenic damage would eventually occur if the postoperative time interval is long enough, because the storage capacity of the epididymis and vas deferens is limited. This speculation is supported by Bedford's study in rabbits [13]: spermatogenic damage was observed in 1/10 testes 26 weeks after operation (ligation of vas deferens immediately before its entry into the inguinal canal), and in 5/13 testes 32–48 weeks after operation.

In summary, the long-term vasectomy-induced spermatogenic damage is primarily pressure-mediated [8, 10]. Because vasectomy-induced spermatogenic damage is primarily sloughing of spermatogenic cells in the later stages of spermatogenesis [8], without reduction in the numbers of spermatogonia [5, 8], the testis maintains the potential to restore spermatogenesis. A balance, therefore, could be established after vasectomy between the production of sperm by the testis and the storage of sperm in the epididymis and vas deferens. Variation in the balance might just determine the within-individual, between-individual or between-species variation in the vasectomy-induced effects.

In comparison with the long-term (chronic) effect, the acute spermatogenic effect of vasectomy seems to be more complex and variable. We first observed severe spermatogenic damage in all 5 rabbits (aged 4–5 months) 10 days after unilateral vasectomy via the scrotum [8]. Then we repeated the study with bilateral vasectomy via the scrotum and observed apparent spermatogenic damage in only 4/12 testes [11]. We continued the study with bilateral open-ended vasectomy via the scrotum and observed, to our surprise, even more severe (than previously observed [8]) spermatogenic damage in all 12 testes [11]. In contrast, as shown in the current study, the close-ended (Experiment 1) or open-ended (Experiment 2) vasectomy away from the cauda epididymis (or the scrotum) did not induce damage to spermatogenesis 10 days after operation in younger (Experiment 1) or older (Experiment 2) rabbits. Taken together, these studies seem to suggest that the increase, if any, of intra-testicular pressure after vasectomy was not a key factor in the spermatogenic damage induced 10 days after vasectomy via the scrotum. In addition, the current study also suggests that the age of animals at vasectomy is not a key factor in the acute damage, either, although spermatids sloughing is more liable to occur in younger animals [14]. Therefore, the acute effect might be more likely the result of iatrogenic effects of the operation *per se*, such as: (i) adhesion of the testis with surrounding tissue [8, 11, 12, 14]; (ii) local inflammatory irritation; and/or (iii) effects on the testicular blood supply. To perform vasectomy via the scrotum in rabbits, as we experienced, an incision into the tunica vaginalis had to be made and the testis, epididymis and vas deferens had to be exposed, and then, after vasectomy, the organs had to be returned into the cavity of tunica vaginalis. These procedures would induce marked operative trauma and even distortion of the spermatic cord, thus resulting in adhesion of the testis with the surrounding tissue and even testicular blood supply problems. For example, not only adhesion of the testis with the surrounding tissue but also distortion of the testicular shape were induced 10 days or 3 months after open-ended vasectomy near the cauda epididymis [11].

Similar spermatogenic damage was observed on both the vasectomized and sham-operated sides in 1 rabbit (aged approximately 4 months) in Experiment 1. This should be related to the unstable status of spermatogenesis in pubertal rabbits [14] rather than the specific effect of the operation *per se*.

Somewhat unexpectedly, in spite of distension (sperm accumulation) of the juxta-epididymal vas deferens 3 months after open-ended vasectomy in the present study, outflow of sperm from the open-end of the vas did not appear to be noticeable, because: (i) the vas deferens in the inguinal canal had no marked adhesion with the surrounding tissue, and (ii) there was no significant difference in the mean diameter of the juxta-epididymal vas deferens between the open-ended and close-ended vasectomy groups at 3 months after operation (Table 2). The reason for this might be that the vasal distension had not reached the open-end yet, so the outflow of sperm was still not severe enough, or, perhaps more likely, that the vasal open-end might have been closed one way or another soon after operation.

The effect of vasectomy on spermatogenesis in mammals has been controversial, and factors influencing the effect might include species or individual variation, study design, postoperative complications, postoperative time interval, the methodology used to determine the spermatogenic status [4, 5], and distensibility of the epididymis or vas deferens [10, 13]. In mice, for example, only

a slight effect on spermatogenesis was observed by Barratt and Cohen [16], whereas a marked effect (with much depletion of germ cells 6–12 months after vasectomy) was reported by Singh and Chakravarty [17]. Importance is seldom attached to describing the surgical procedures or postoperative complications in detail in most published papers, including ours [4, 8]. Our previous [11, 12] and present studies have underlined the very importance of including such detail in future published studies. Future studies should not only pay attention to operative procedures and postoperative complications, but also to the sites of vasectomy.

Some studies in men indicate the occurrence of some spermatogenic damage after vasectomy [5, 18]. In particular, a recent study using quantitative (stereological) methods showed a significant reduction (23%–40%) in the numbers of spermatids 1–20 years after vasectomy (details of the vasectomy procedures unavailable) [5]. However, we have not seen reports about the effects of open-ended or close-ended vasectomy via the inguinal canal (or away from the cauda of epididymis) on spermatogenesis in men. An inevitable long-term consequence of vasectomy is the epididymal stasis [19], which might be relieved by vasectomy via the inguinal canal in the short term. For example, Zheng and Zhang [20] found no epididymal stasis 2 years after vasectomy via the inguinal canal in men. In the long term, however, we speculate that such vasectomy would also eventually result in epididymal stasis and, therefore, spermatogenic damage according to the pressure-mediated mechanism described above. Vasectomy via the inguinal canal was used in the present rabbit study to evaluate the effect of vasectomy on spermatogenesis. Such vasectomy (via the middle part of the inguinal canal, for example) in men, however, would not necessarily be more acceptable clinically if epididymal stasis and/or distention of the epididymis and vas deferens would be associated with the operation sooner or later.

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References

1 Lohiya NK, Manivannan B, Mishra PK, Pathak N. Vas deferens, a site of male contraception: an overview. Asian J Androl 2001; 3: 87–95.
2 McVicar CM, O'Neill DA, McClure N, Clements B, McCullough S, Lewis SE. Effects of vasectomy on spermatogenesis and fertility outcome after testicular sperm extraction combined with ICSI. Hum Reprod 2005; 20: 2795–800.
3 Steger K, Slavov M, Failing K, Weidner W, Bergmann M. Effect of vasectomy on sperm nuclear chromatin condensation in the rabbit. J Androl 2005; 26: 289–95.
4 Peng B, Zhang RD, Dai XS, Deng XZ, Wan Y, Yang ZW. Quantitative (stereological) study of the effects of vasectomy on spermatogenesis in rhesus monkeys (*Macaca mulatta*). Reproduction 2002; 124: 847–56.
5 Raleigh D, Hons BS, O'Donnell L, Southwick GJ, Kretser DM, McLachlan RI. Stereological analysis of the human after vasectomy indicates impairment spermatogenic efficiency with increasing obstructive interval. Fertil Steril 2004; 81: 1595–603.
6 Yang G, Walsh TJ, Shefi S, Turek PJ. The kinetics of the return of motile sperm to the ejaculate after vasectomy reversal. J Urol 2007; 177: 2272–6.
7 Wen XH, Deng XZ, Wan Y, Huang AP, Yang ZW. [The effects of vasectomy on spermatogenesis 3 months after operation in rabbits]. Sichuan J Anat 2001; 9: 44. Chinese.
8 Kong LS, Huang AP, Deng XZ, Yang ZW. Quantitative (stereological) study of the effects of vasectomy on spermatogenesis in rabbits. J Anat 2004; 205: 147–56.
9 Liu FX, Zhao XJ, Wang ZS, Lin H, Zhao D, ZhaoYL, *et al*. [Observation of histological changes of testes in vasectomized rabbits with PAP staining.] Journal of Norman Bethune University of Medical Science 1990; 16: 37–9. Chinese.
10 McDonald SW. Cellular responses to vasectomy. Int Rev Cytol 2000; 199: 295–339.
11 Zhang RD. Effects of conventional and open-ended vasectomy on the testicular and epididymal structures in rabbits [dissertation]. Chongqing: Chongqing Medical University, 2006.
12 Zhang RD, Deng XZ, Dai XS, Zhao YY, Guo Y, Huang AP, *et al*. Spermatogenic damage induced shortly after vasectomy was related to operational trauma? Proceedings of the Second Asia-Pacific Forum on Andrology; 2006 Oct 17–21; Shanghai, China. Asian J Androl 2006; 8 (5 Suppl): p36–7.
13 Bedford JM. Adaptations of the male reproductive tract and the fate of spermatozoa following vasectomy in the rabbit, rhesus monkey, hamster and rat. Biol Reprod 1976; 14: 118–42.
14 Zhang RD, Wen XH, Kong LS, Deng XZ, Peng B, Huang AP, *et al*. A quantitative (stereological) study of the effects of experimental unilateral cryptorchidism and subsequent orchiopexy on spermatogenesis in adult rabbit testis. Reproduction 2002; 124: 95–105.
15 Yang ZW, Kong LS, Guo Y, Yin JQ, Mills N. Histological

changes of the testis and epididymis in adult rats as a result of Leydig cell destruction after ethane dimethane sulfonate treatment: a morphometric study. Asian J Androl 2006; 8: 289–99.
16 Barratt CL, Cohen J. Quantitative effects of short- and long-term vasectomy on mouse spermatogenesis and sperm transport. Contraception 1988; 37: 415–24.
17 Singh SK, Chakravarty S. Histologic changes in the mouse testis after bilateral vasectomy. Asian J Androl 2000; 2: 115–20.
18 Gupta AS, Kothari LK, Bapna RB. Surgical sterilization by vasectomy and its effects on the structure and function of the testis in man. Br J Surg 1975; 62: 59–63.
19 Cao LT, Yang ZW, Gu P, Zhang MH, Zhang Q, Deng XZ, *et al*. [Ultrasonographic changes in the epididymis after long-term vasectomy]. Zhonghua Nan Ke Xue. 2007;13: 212–5. Chinese.
20 Zheng TG, Zhang YL. [Clinical observation in 186 cases with vasectomy via the inguinal canal]. Chinese Journal of Andrology 2006; 20: 58–9. Chinese.

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