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硕士学位论文

论文题目　常规与近端开放输精管结扎对兔睾丸和附睾结构的影响

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申请学位级别　硕士　学科、专业名称　人体解剖与组织胚胎学

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重庆医科大学
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日　　期：2006.5

目 录

符号说明

英文缩写	英文全称	中文全称
BCV	Bilateral Conventional Vasectomy	双侧常规输精管结扎
BOEV	Bilateral Open-ended Vasectomy	双侧近端开放输精管结扎
BCO	Bilateral Control Operation	双侧对照手术
BSO	Bilateral Sham Operation	双侧假手术
PAS	Periodic Acid-Schiff's Reagent	过碘酸- Schiff 试剂
Pl-Z	Preleptotene, Leptotene and Zygotene Primary Spermatocytes.	细线前期、细线期和偶线期的初级精母细胞
P	Pachytene Primary Spermatocytes	粗线期的初级精母细胞
SS	Secondary Spermatocytes	次级精母细胞
St	Spermatids	精子细胞

常规与近端开放输精管结扎对兔睾丸和附睾结构的影响*

摘　要

研究背景与目的：

输精管结扎术是目前最有效和广泛开展的男性节育方法，常规输精管结扎和近端开放输精管结扎是其两种主要的手术方式。输精管结扎对睾丸精子发生和附睾结构的影响仍不完全清楚，而它可能影响输精管吻合术后生育能力的恢复或输精管结扎后进行人工受精的成功率。我们前期的研究显示，兔输精管结扎损害了精子发生，最严重的损害发生在术后 10 天所有输精管结扎侧的睾丸，术后 6 月和 12 月对约一半动物的精子发生有显著影响。但这种损害是由于输精管的阻塞还是手术创伤引起的并不清楚。本实验对比研究双侧常规输精管结扎和双侧近端开放输精管结扎 10 天和 3 月对睾丸和附睾的组织学影响，以进一步探讨手术创伤是否影响结扎术后并发症以及近端开放输精管结扎是否可缓解结扎术后并发症。

方　法：

1. 正常雄性新西兰大耳白兔（4～5 月龄）50 只，随机分为 4 组：双侧常规输精管结扎组（BCV）——暴露双侧睾丸和输精管，切除一段输精管并结扎两侧断端；双侧近端开放输精管结扎组（BOEV）——

*本课题由四川省青年科技基金资助（04ZQ026-025）

暴露和切除输精管同 BCV，附睾侧输精管断端保持开放，只结扎另一侧输精管；双侧对照手术组（BCO）——同 BCV 那样暴露输精管后，不切断也不结扎输精管；双侧假手术（BSO）——只作一皮下切口。BCO 组 6 只动物，其余每组 13～15 只动物。

2. 手术后 10 天 4 个组的动物（每组 6 只）以及手术后 3 月 BCV 组、BOEV 组和 BSO 组的剩余动物，经 Bouin 液灌注固定后取材。BSO 组随机取单侧睾丸和附睾，其他组取双侧睾丸和附睾。
3. 用羟乙基甲基丙烯酸树脂包埋睾丸及附睾的组织块，切 10 μm 厚的树脂切片，用 PAS 和苏木精（睾丸）或单独用苏木精（附睾）染色。
4. 光镜下定性观察睾丸和附睾的结构。
5. 用体视学方法测量生精小管和附睾管的体积以及生精小管的直径和长度。

结　果：

1. BSO 组睾丸和附睾形态正常，与周围组织无粘连。BCV、BOEV 和 BCO 组的睾丸和附睾均与周围组织有不同程度的粘连，常扭曲变形，有的明显变小。
2. 术后 10 天，观察到精子发生被明显损害的睾丸数在各组分别为 2/11（BCO 组，即该组观察的 11 个睾丸中有 2 个出现了精子发生的明显损害）、4/12（BCV）、12/12（BOEV）和 0/6（BSO），其中 BOEV 组的损害最严重。术后 3 月，观察到精子发生被明显损害的睾丸数在各组分别为 9/14（BCV）、9/13（BOEV）和 1/9（BSO）。3 月 BCV 组比 10 天 BCV 组有更多的睾丸有精子发生损害，且损害更严重。

与 10 天 BOEV 组相比，3 月 BOEV 组精子发生的损害在 4/13 的睾丸有所恢复，而在 9/13 的睾丸没有改善或有更严重的损害。

3. 精子发生损害的主要特征是：生精细胞（尤其是生精上皮浅层或腔面的生精细胞）脱落、疏松，数量减少；生精小管直径显著变小；生精细胞退化和多核细胞形成。在术后 10 天，BSO、BCO、BCV 和 BOEV 组的睾丸体积（cm^3，mean±SEM）和生精小管直径（μm，mean±SEM）分别是 2.14±0.23、1.74±0.09、1.57±0.14、1.15±0.07 和 181±10、186±6、169±8、130±4。在术后 3 月，BSO、BCV 和 BOEV 组的睾丸体积和生精小管直径分别是 2.45±0.10、1.67±0.24、1.33±0.18 和 199±6、158±10、151±11。
4. 术后 10 天，BCV 组的附睾形态定量参数无显著性改变。但 BOEV 组的附睾头部萎缩，附睾头部体积和头部小管体积显著减少到 BSO 组的 57.4%和 36.6%。
5. 术后 3 月，BCV 组和 BOEV 组均出现附睾尾部精子郁积和附睾管扩张。BSO、BCV 和 BOEV 组的附睾尾部体积（mm^3，mean±SEM）和尾部附睾管体积（mm^3，mean±SEM）分别是 557±30、832±96、936±65 和 259±36、566±82、561±68。BCV 组和 BOEV 组附睾头部萎缩，附睾头部和头部小管体积显著减少到 BSO 组的 63.9%和 67.9%。两组均没有附睾精子肉芽肿形成。

结　论：

1. 输精管结扎后短期内对精子发生的损害可能主要是手术创伤和炎性刺激所致，而长期结扎对精子发生的影响可能主要是输精管结扎

本身的作用。输精管结扎对附睾的主要影响是附睾尾部精子郁积，附睾管扩张。

2. 开放性结扎并未减轻输精管结扎对精子发生的损害，也未减轻附睾的精子郁积，事实上开放性结扎术后 10 天对精子发生的损害更大。这提示，开放性结扎术后短期内可能由于精子溢出引起的刺激——炎性刺激或免疫反应而加重了对精子发生的影响，而以后可能由于输精管开放端封闭（由于周围组织粘连或断端自行愈合），其对精子发生的损害有所缓解。

关键词： 输精管结扎，精子发生，睾丸，附睾， 兔

EFFECTS OF CONVENTIONAL AND OPEN-ENDED VASECTOMIES ON THE TESTICULAR AND EPIDIDYMAL STRUCTURES IN RABBITS

ABSTRACT

Background and Objective:

Vasectomy is an effective and one of the most widely used methods of male contraception; the conventional and open-ended vasectomies are two major surgical techniques. It is still unclear what effects vasectomy has on the spermatogenesis in the testis and on the epididymal structures. It has potential influence on the fertility after reversal of the operation and on the success rate of artificial fertilization. We previously observed that vasectomy induced damage to spermatogenesis in rabbits. The damage was most severe at day 10 after operation, occurring in all the vasectomized testes, but the damage was less severe 6 or 12 months after operation, occuring in half of the vasectomized testes. But it was unclear whether the damage was related to operational trauma or occlusion of the vas deferens. Comparing the effects of bilateral conventional and open-ended vasectomies on the testicular and epididymal structures in rabbits, this study was therefore further undertaken to determine whether the operational trauma had an impact on the post-operational complications

and whether the open-ended vasectomy would reduce complications after vasectomy.

Methods:

1. Fifty normal male New Zealand white rabbits, aged 4~5 months, were randomly divided into four groups: bilateral conventional vasectomy (BCV), in which the testis and vas deferens on each side was exposed and a segment of the vas was excised with both vasal ends being ligated; bilateral open-ended vasectomy (BOEV), in which, after exposure and transection of the vas as in BCV, the epididymal end of the vas was left open while the other end ligated; bilateral control operation (BCO), in which the vas was exposed as in BCV but not cut or ligated; bilateral sham operation (BSO), in which only a hypodermic cut was performed. 6 animals were included in the BCO group and 13~15 animals in each of other groups.
2. Ten days after the four operations (6 animals each group), and 3 months after BCV, BOEV and BCO (remaining animals), the testis and epididymis on a randomly chosen side from each animal in the BCO group and bilateral testes and epididymes from each animal in the other groups were removed after perfusion with Bouin's solution.
3. Hydroxyethyl methacrylate resin-embeded testicular and epididymal sections (10 μm in thickness) were obtained and stained with periodic acid-Schiff's reagent and haematoxylin (testis) or haematoxylin alone

(epididymis).

4. Observe the histology of the testis and epididymis by light microscopy.
5. Measure the volumes of the seminiferous tubules and the epididymal duct, and the diameter and length of the seminiferous tubules, using stereological methods.

Results:

1. The testis and epididymis in the BSO group had normal morphology, without adhesion with surrounding tissues. In the BCV, BOEV and BCO groups, however, the testis and epididymis had adhesion with surrounding tissues to some degree, with some organs being distorted in shape or becoming smaller in size.
2. Ten days after operation, marked damages to spermatogenesis were observed in 2/11 (2 out of 11 testes subjected to the operation in the BCO group)，4/12 (BCV)，12/12 (BOEV) and 0/6 (BSO) testes. The damage in the BOEV group was more severe than in other groups. Three months after operation, marked damages to spermatogenesis were observed in 9/14 (BCV)，9/13 (BOEV) and 1/9 (BSO) testes. The spermatogenic damage was seen in more testes and was more severe in the BCV group at month 3 compared to that at day 10. Three months after BOEV, the spermatogenic damage recovered in 4/13 testes, but failed to recover or became more severe in 9/13 testes, in comparison with the BOEV group at day 10.

3. The primary characteristics of spermatogenic damage were sloughing and looser arrangement of spermatogenic cells, especially the advanced (adluminal) cells, reduction in the numbers of spermatogenic cells and in the diameters of the tubules, and presence of multi-nucleated and degenerating spermatogenic cells. Ten days after BSO, BCO, BCV and BOEV, the results (mean±SEM) of the testicular volume (cm^3) and the tubule diameter (μm) were: 2.14±0.23, 1.74±0.09, 1.57±0.14, 1.15±0.07 and 181±10, 186±6, 169±8, 130±4, respectively. Three months after BSO, BCV and BOEV, the results of the testicular volume and the tubule diameter were: 2.45±0.10, 1.67±0.24, 1.33±0.18 and 199±6, 158±10, 151±11, respectively.
4. Ten days after operation, BCV had no significant effects on all quantitative parameters obtained from the epididymis. But the caput epididymis was atrophied; the volume of the caput and the volume of the tubules and duct in the caput in the BOEV group were significant decreased to 57.4% and 36.6% of the BSO group average.
5. Three months after operation, the cauda epididymis showed spermatozoal stasis and tubular ectasia in BCV and BOEV groups. In BSO, BCV and BOEV groups, the results (mean±SEM) of the cauda epididymis volume (mm^3) and the cauda tubule volume of (mm^3) were: 557±30, 832±96, 936±65 and 259±36, 566±82, 561±68, respectively. The caput epididymis was atrophied, and the volumes of caput tubules

was significant reduced to 63.9% (BCV) and 67.9% (BOEV) of the BSO group average. No sperm granuloma was seen on the epididymal sections.

Conclusion:

1. Spermatogenic damage induced shortly after vasectomy might be mainly a result of the operational trauma or post-operational inflammatory irritation, but the long-term effect of vasectomy on spermatogenesis might be largely a result of vasectomy per se. The effects of vasectomy on the epididymis included spermatozoal stasis and duct ectasia in the cauda.
2. BOEV was unable to alleviate the spermatogenic damage (in the testis) or spermatozoal stasis (in the epididymis) induced by vasectomy. And the damage to spermatogenesis was in fact more severe 10 days after BOEV. This suggested that BOEV aggravated the spermatogenic damage due to inflammatory irritation or immunereaction resulting from spermatozoal extravasation shortly after BOEV, but the damage recovered to a degree later on with closure of the open end of the vas due to adhesion with surrounding tissue or self-healing.

Key Words: vasectomy, spermatogenesis, testis, epididymis, rabbit

常规与近端开放输精管结扎对兔睾丸和附睾结构的影响

前　言

输精管结扎术是目前最有效的男性节育方法，常规输精管结扎（切断输精管并结扎其双侧断端）和近端开放输精管结扎（切断输精管后，只结扎近精囊侧，让其附睾侧保持开放）是其两种主要的手术方式。从上世纪 70 年代广泛开展至今，输精管结扎术在全球已进行达 1 亿例以上[1]，在中国至少已进行 2300 万例[2]。输精管结扎术普遍被认为是非常安全的避孕方法，人们主要关注的是输精管结扎后的临床并发症。结扎后并发症的发生率在国内的报告中多比较低。李顺强等[3]调查 2373 例使用不同残端处理术式的输精管结扎案例，总的并发症发生率仅 0.8%。采取直视钳穿法输精管结扎者的并发症发生率与之相近：唐文豪等[4]调查 1240 例受术者在术后 24 个月总并发症发生率为 1.21%，聂洪平和崔亚琼[5]报道为 1.08%（94/8688）。但在国外的报告中差异较大[1,6]，不同结扎术式的总并发症发生率可达 0.4%～39%，术后血肿的发生率 0.3%～18%，附睾炎 0.1%～3%，术后感染 0.7%～18%，精子肉芽肿 0.05%～18%。由于一般有症状的并发症少见，人们很少关注输精管结扎对人睾丸和附睾的组织学影响，尤其是缺乏定量的实验研究，输精管结扎对睾丸精子发生和附睾结构的影响仍不很清楚[7]。

显微输精管吻合术后的再通率可达 86%，但再授孕率仅有 52%[8]。吻合术后的再授孕率一直远远低于再通率，原因仍不完全清楚，常被认为是输精管狭窄或附睾的堵塞，但也可能是由于输精管阻塞损害了精子发生或附睾的结构而导致精子质量的低下。调查显示输精管结扎后有 2%～6%（在 30 岁以下甚至高达 11%）的人需要进行输精管吻合术[9]，加之试管婴儿技术的发展，使了解输精管结扎对睾丸和附睾的影响变得重要，因为它可能影响吻合术后生育能力的恢复或输精管结扎后进行人工受精的成功率。

输精管结扎对附睾的影响主要是附睾精子郁积、附睾管扩张和精子肉芽肿形

成。虽然一些临床调查[10-13]认为近端开放输精管结扎可以缓解输精管结扎引起的附睾压力增高而减少并发症（主要是充血性附睾炎和痛性精子肉芽肿）的发生，但很少有近端开放性输精管结扎术对睾丸和附睾结构影响的组织学或病理学研究报道。我们推测，开放端溢出的精子势必引起较强的局部刺激和变态反应，有可能更严重地损害睾丸的精子发生；而由于粘连等原因开放端最终可能还是会阻塞，所以从长远看可能仍然不能缓解附睾郁积。

我们前期的研究显示，日本大耳白兔单侧常规输精管结扎术后早期严重损害了精子发生，以后有所恢复：最严重的损害发生在术后 10 天，见于所有输精管结扎侧的睾丸，结扎 6 或 12 个月后有约一半动物的精子发生有一定程度的损害[14]。但这种早期的损害是由于输精管的阻塞还是手术创伤引起并不清楚。

本实验通过光镜观察和形态定量方法，对比研究双侧常规输精管结扎和双侧近端开放输精管结扎10天和3月对新西兰大耳白兔的睾丸和附睾的组织学影响，以进一步探讨手术创伤是否影响结扎术后的并发症，近端开放输精管结扎是否可以缓解结扎术后的并发症。