Effect of Short Period Vasectomy on FSH, LH, Inhibin and Testosterone Secretions, and Sperm Motility in Adult Male Rats

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Abstract: The present study was undertaken to clarify changes in secretions of FSH, LH, inhibin and testosterone, and sperm motility after bilateral vasectomy in adult male rats. Bilateral vasectomy was created surgically (treated group) and intact rats were used as control (control group). On days 3, 5, 7, 14, 30, 60, and 90 after surgery, plasma concentrations of FSH, LH, inhibin, and testosterone were measured by radioimmunoassay, and sperm motility characteristics were measured by computer-assisted sperm analysis (CASA). The results show that weights of epididymides significantly increased in vasectomized rats as compared to control rats. Histologically, damage to spermatogenesis was observed in vasectomized rats. Multinucleated giant cells were observed in the lumen of some seminiferous tubules, and there were degenerative spermatids in the epididymides of vasectomized rats. Plasma levels of LH, FSH, and testosterone only decreased on day 3 after vasectomy; however, plasma levels of ir-inhibin significantly increased on day 3 after vasectomy. In addition, the sperm motility parameters, straight-line velocity, curvilinear velocity, deviation of the sperm head from the mean trajectory and the maximum amplitude of lateral head displacement were decreased from day 60 after vasectomy. These results suggest that vasectomy reduces sperm motility starting from day 60 after vasectomy, and early bilateral vasectomy does not strongly affect the endocrine function of the testis, though it may result in damage to spermatogenesis in vasectomized rats.

Key words: epididymis, sperm motility, testis, vasectomy

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Introduction

Vasectomy is regarded as the safest method now available for male fertility control. More than 100 million men worldwide have used vasectomy for family planning [3]. Vasectomy is also used in the field for controlling numbers of wild animals. In the field of experimental animals, vasectomy of males is generally used for inducing pseudopregnancy in females. Following vasectomy, however, an obstruction is suddenly imposed on a previously patent duct and an inflammatory reaction frequently ensues as sudden backpressure from accumulating spermatozoa causes the duct to swell and eventually burst, leading to formation of sperm granulomas [23]. Not surprisingly, >80% of vasectomized men have anti-sperm antibodies in the peripheral blood within a year [1]. In a survey on the effects of chronic vasectomy in laboratory animals (hamster, rat, and monkey), Bedford [7] observed rapid formation of sperm granulomas in nearly all cases, either at the site of ligation on the vas deferens or in the cauda epididymis. In the vasectomized rats, leakage and granuloma formation invariably occur about 2 weeks after vasectomy, either at the vasectomy site or in the tail of the epididymis [31, 32, 46].

After leaving the testis, mammalian spermatozoa must transit through the epididymis to reach the vas deferens [26]. The mammalian epididymis has two principal functions. It creates a unique microenvironment within the lumen of the duct that helps transform immotile, immature testicular spermatozoa into fully fertile competent cells, and it also stores fertile spermatozoa in a viable state within the cauda epididymidis/vas deferens regions until they are ejaculated [23]. Although it is known that these functions are altered under vasectomy, the different animal models used to study effects of vasectomy on the male reproductive tract have given varying results between species and even between individuals [7]. Vasectomized males generally show normal mating behavior; however, the consequences of vasectomy on the pattern of reproductive hormones and sperm motility are poorly documented. To develop knowledge for understanding reproductive physiology in vasectomized animals, in the present study, we designed a vasectomized rats model to monitor sperm motility characteristics using a computer-assisted sperm analysis system (CASA). We also elucidated the spermatogenic changes caused by vasectomy in adult male rats. In addition, changes in secretions of follicle-stimulating hormone (FSH), luteinizing hormone (LH), immunoreactive (ir)-inhibin, and testosterone were determined by radioimmunoassay (RIA) after bilateral vasectomy in adult male rats.

Materials and Methods

Animals and treatments

Adult male Wistar-Imamichi strain rats (Iar: Wistar, originated from Wistar Institute Standard Strain Lot No.1359) weighing between 350–450 g, and 3–4 months of age, were used in this study. Animals were anesthetized by ether and subjected to vasectomy by transection of a U-shaped segment from the vas deferens between 2 ligatures. Sham-operated controls underwent the same surgical procedure without vasectomy. Rats were returned to their room, under a light regimen of 14 h light/10 h dark, and were given water and commercial food ad libitum. Care and use of the animals were in accordance with the requirements established under the Guide for the Care and Use of the Laboratory Animals by the Tokyo University of Agriculture and Technology, Japan.

Sample collection

Rats were decapitated 3, 5, 7, 14, 30, 60, and 90 days postoperation. Blood samples were collected and both testes and epididymis were removed. Blood samples were collected from each animal into individual heparinized centrifuge tubes, and plasma samples were immediately obtained by centrifugation at 1,700 × g for 15 min at 4°C. Plasma samples were stored at ~20°C until assayed for FSH, LH, ir-inhibin, and testosterone. Testes and epididymis were fixed in 4% paraformaldehyde in 0.1 M PBS for morphological observation.

Sperm concentration

The concentration of spermatozoa in the testes was examined by a method described previously [34]. In brief, tunica albuginea was removed from the testes, which were then homogenized in 10 ml saline by a homogenizer (Phycotron; Microtech Nition, Chiba, Japan)
for 10 s, followed by sonication using a sonicator (TOMY, SEIKO Co., Ltd., Tokyo, Japan) for 3 min on ice. One drop of extracted emulsion was placed on a hemocytometer after adequate dilution with saline, and sperm heads were then counted under a phase contrast microscope. The remaining extracts were centrifuged at 38,000 \( \times g \) for 30 min at 4°C, and supernatants were stored at -20°C for hormone assays.

**Sperm motility analysis**

Semen from the cauda epididymidis was collected into 1.5 ml tubes containing 1 ml of modified Tyrode's medium (3 \( \mu l \) semen sample from the cauda epididymidis diluted with 1 ml modified Tyrode's medium). The sperm motility was measured by CASA using a C.IMAGING C.MEN system. Briefly, diluted sperm suspensions were placed in prewarmed slide chambers with depths of 20 \( \mu m \). The slides were viewed using an Olympus microscope (Olympus BX50F, Olympus optical Co., Tokyo, Japan) equipped with a 4 \( \times \) dark field optics and a video camera (CCD XC77, Sony Co., Tokyo, Japan) connected to a personal computer. The temperature of the microscope stage was maintained at 37°C throughout the observation by a stage warmer (MP-10DM, Kitazato Supply Co., Shizuoka, Japan). CASA was performed using the C.IMAGING C.MEN system and C.IMAGING software (C.IMAGING systems, Compix Inc., Tualatin, OR, USA). Our CASA system analyzes 15 consecutive, digitalized photographic images obtained from a single field. These 15 consecutive photographs were taken with a time lapse of 0.5 s. Two to three separate fields were taken for each sample. Percentage of motile spermatozoa (%), straight-line velocity (VSL, \( \mu m/s \)), curvilinear velocity (VCL, \( \mu m/s \)), linearity (ratio of the straight line distance to the actual tracked distance), deviation of the sperm head from the mean trajectory (ALH, mean \( \mu m \)) and the maximum amplitude of lateral head displacement (ALH, max \( \mu m \)) and beat frequency of centroids crossing the average trajectory (BCF, Hz) were determined.

**Histology**

Fixed testes and epididymides were dehydrated through a series of graded concentrations of ethanol and xylene, and embedded in paraffin. The paraffin-embedded testes were serially sectioned at 4–6 \( \mu m \) thickness and placed on poly-L-lysine coated slide glasses. The sections were stained with hematoxylin-eosin (HE) for morphological observations.

**RIA for FSH, LH, ir-inhibin, and testosterone**

Concentrations of FSH, LH, ir-inhibin, and testosterone in the plasma were determined by specific RIAs. Iodinated preparations were rat FSH-I-7 and LH-I-7. The antisera used were anti-rat FSH-S-11 and LH-S-10. Results were expressed in terms of NIDDK rat FSH-RP-2 and LH-RP-2. The intra-assay and inter-assay coefficients of variation were 4.3 and 5.3% for FSH and 7.2 and 11.2% for LH, respectively. Plasma concentrations of ir-inhibin were measured as described previously [20]. The iodinated preparation was 32 kDa bovine inhibin and the antisera used was rabbit antisum against bovine inhibin (TNDH-1). Results were expressed in terms of 32 kDa bovine inhibin. The intra- and inter-assay coefficients of variation were 8.8 and 14.4%, respectively. Testicular contents and plasma concentrations of testosterone were determined by a double-antibody RIA system with \(^{125}\)I-labeled radioligands as described previously [49]. The antisera against testosterone (G dN 250) was kindly provided by Dr. G. D. Niswender (Colorado State University, Fort Collins, CO, USA). The intra- and inter-assay coefficients of variation were 6.3 and 7.2%, respectively.

**Statistical analysis**

All data are expressed as means ± SEM of five animals. One-way ANOVA was performed, and the significance between two means was determined by Student’s t-test. A value of \( P<0.05 \) was considered statistically significant.

**Results**

**Weights of testis, epididymis, prostate, and seminal vesicle**

The weights of epididymides in the vasectomy group showed a significant increase until 30 days postoperation compared with control group (Fig. 1B). However, the weights of epididymides decreased from 30 days postoperation compared with the control group (Fig. 1B). In addition, there were no significant changes in the weights
of testes, prostate, and seminal vesicles between the vasectomy and control groups (Fig. 1A, 1C, and 1D).

**Sperm concentrations**

Data from the sperm head count are shown in Fig. 2. There was no significant difference between the vasectomy and control groups in sperm head count.

**Morphology of testes and epididymis**

Morphology of testes and epididymides of the rats after vasectomy are shown in Fig. 3. No changes of morphology of testes or epididymides were observed in the control from 3 to 90 (Fig. 3A and 3E). Only four of 35 vasectomized rats showed connection between the epididymal duct and a sperm granuloma through which spermatozoa appeared to be escaping (Fig. 3B and 3C). These four rats had abnormal testes. Damage to sper-
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Sperm motility

The sperm of the four rats which formed sperm granulomas after vasectomy did not move. Data from CASA are shown in Fig. 4. In the vasectomy group, the sperm motility parameters, straight-line velocity (the average velocity measured in a straight line from the beginning to the end of the track, \( \mu \text{m/s} \)), curvilinear velocity (the average velocity measured over the actual point to point track, \( \mu \text{m/s} \)), ALH mean and ALH max began to decrease 60 days after vasectomy. (Fig. 4A–D). There were no significant changes in three sperm motility parameters, the mean percentage of sperm motility (%), BCF (Hz), and linearity index after vasectomy (Fig. 4E–G).

Plasma concentrations of LH, FSH, testosterone, and ir-inhibin

Plasma concentrations of LH were significantly lower in the vasectomized group on day 3 after the operation than the control group, then increased gradually until day 7, and became significantly higher than that of the control group on day 90 after the operation (Fig. 5A). There were no differences in plasma concentrations of FSH between vasectomized and control rats (Fig. 5B). There was a significant decrease in testosterone concentrations on day 3 postoperation in vasectomized rats, but they increased again to become comparable to control levels (Fig. 5C). Plasma concentrations of ir-inhibin increased remarkably, after day 3 in the vasectomized group compared with the control group; however, inhibin levels decreased in the vasectomized group compared to the control level (Fig. 5D).

Discussion

The present results demonstrate that short period vasectomy in adult rats significantly increased epididymis weights, induced morphological changes in seminiferous tubules and epididymal ducts, and decreased sperm motility parameters. However, apart from day 3, there were no significant changes in plasma concentrations of LH, FSH, testosterone, and ir-inhibin after vasectomy. These results suggest that vasectomy in rats leads to early damage to spermatogenesis and decreased sperm motility; however, vasectomy did not strongly affect the endocrine function of the testis in adult male rats.
Fig. 4. Changes in epididymal sperm motility parameters: straight velocity (A), curvilinear velocity (B), amplitude of lateral displacement (ALH) mean (C), amplitude of lateral displacement (ALH) max (D), percentages of motile spermatozoa (E), linearity index (F), and beat frequency of centroids crossing the average trajectory (BCF, Hz) (G) after vasectomy (solid bar) or control (open bar). Each point represents the mean ± SEM of five animals. *P<0.05 compared to control group.
Common features can be discerned in the response of the epididymis to vasectomy, despite species differences. Increases in size and number of lysosomes are the most frequent changes in the epididymal epithelium [17]. In the present study, the weight of epididymides in the vasectomy group showed a rapid increase starting on day 3, but there were no significant differences in the weights of testes, prostate and seminal vesicles between the vasectomy and control groups. The change of epididymal weight may be due to accumulation of spermatids in the epididymis [22, 41]. Studies of vasectomized rams have shown that the seasonal pattern was ill-defined, and the testicular parameters were not only lower, but the cauda epididymis was larger than in intact rams [41]. In this respect, the rabbit has proved a better experimental model as the vas deferens and cauda epididymis are quite distensible and, following vasectomy, can accommodate considerable backpressure from accumulated fluid and spermatids without rupturing [7, 22]. Also, Moore and Bedford [35] observed a linear increase in the number of spermatids in the epididymides of rabbits vasectomized for up to 6 months that equated closely with estimates of sperm production by the testes.

In the present study, though there was no significant changes in the weights of testes, multinucleated giant cells were found in the lumen of some seminiferous tubules in vasectomized rats. Multinucleated giant cells are common in vasectomy-induced spermatogenic damage, probably as a result of germ cell sloughing, an increase of pressure in the seminiferous tubules and an autoimmune reaction [37, 47, 51]. A histophysiological study of vasectomized rats indicated that a higher frequency of stage VII–VIII of the tubular cycle was observed, showing sperm accumulation [25]. At 5 and 10 weeks after vasectomy, vasectomized left testes were significantly lighter than unvasectomized right testes and sham-operated testes. During that period, the seminiferous tubules of vasectomized testes were highly damaged, presenting narrow tubular diameter, disorder of cellular

![Fig. 5. Changes in plasma concentrations of (A) luteinizing hormone (LH), (B) follicle-stimulating hormone (FSH), (C) testosterone, and (D) immunoreactive (ir-) inhibin in male rats after vasectomy (solid bar) or sham operation (open bar) in rats. Each value represents the mean ± SEM of five animals. *P<0.05 compared to control value.](image-url)
arrangement, depletion of germ cells, and local interstitial fibrosis. Vasectomized testes demonstrated a significantly increased number of apoptotic germ cells per cross-sectional area than sham-operated testes at 5 and 10 weeks after operation [24]. The damage is probably pressure-mediated rather than immunological [30], because dramatic damage occurred in a short period after vasectomy.

There were significant decreases in plasma levels of LH, FSH, and testosterone only on day 3 after vasectomy. In previous studies, surgical [2, 29, 36, 50], physical [11, 13, 28, 38, 39], and social stress [43–45] resulted in decreased plasma levels of gonadotropins, inhibin and/or testosterone in humans, male primates and rats. However, in the present study, plasma levels of inhibin significantly increased on day 3 after vasectomy. The increase in plasma levels of inhibin may be similar to efferent duct ligation indicating that the inhibin secreted into the seminiferous tubule lumen is trapped [4]. However, the accumulated inhibin did not appear to influence FSH secretion significantly, probably because of the integrity of the blood-testis barrier and the failure of the raised intratubular levels to affect an increase in the secretion of inhibin across the base of the Sertoli cells into testicular lymphatics [5]. Nevertheless, others have suggested that the passage of inhibin into the rete testis fluid and its subsequent absorption represents an important pathway by which inhibin reaches the circulation [27].

The percentage of progressively motile spermatozoa and sperm motion parameters were recorded in the present study by CASA, which is more objective than visual assessment. There were significant differences between vasectomized and control rats in the VSL, VCL, ALH mean, and ALH max from 60 days after operation. In many studies [15, 16, 21, 55], a correlation between sperm motility and fertility has been demonstrated. Sperm motion parameters are important for oocyte penetration; progressive sperm motility is essential for efficient penetration. The characterization and regulation of the epididymal protein microenvironment has been of interest for many years [10, 48, 54], and it has been established that some of these proteins are important for sperm maturation and sperm function at the site of fertilization. Vasectomy significantly reduces concentrations of the major protein, cysteine-rich secretory protein (CRISP-1), and the minor proteins, phosphatidyethanolamine-binding protein and prostaglandin D₂ [52, 53]. Furthermore, there is a significant increase in antisperm antibody production after vasectomy [12]. The main risk factor for the development of antisperm antibodies in the male is disruption of the vas deferens, which is achieved during vasoresection for sterilization. Antisperm antibodies in men occur in blood and seminal fluid, and attach to the sperm surface [33]. Antisperm antibodies may have heterogeneous effects, depending on their cognate antigens [40]. They have been found to affect sperm motility [6, 19], the acrosome reaction [8], penetration of cervical mucus [14], sperm binding to the zona pellucida [18], and sperm-egg fusion [9, 42]. In addition, in the present study, sperm granulomas were histologically observed in 4 of 35 rats, and degenerated spermatids were found within the lumen of the cauda epididymis. Taken together, the results of the present study suggest that early bilateral vasectomy in the rats may affect sperm maturation and motility.

In conclusion, the present results demonstrate that early bilateral vasectomy decreased sperm motility, and damaged spermatogenesis in vasectomized testes, as well as inducing formation of sperm granulomas and degenerative spermatozoa in the epididymis. However, the present results also provide new evidence that early bilateral vasectomy does not affect Sertoli cells and the endocrine function of the testis in vasectomized rats.

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