Bilateral Testicular Consequences in the Unilateral Vasectomy of Immature Rats

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We evaluated the effects of unilateral obstruction of the vas deferens on testicular endocrine and exocrine function in rats. The left vas deferens was ligated and divided in 1-week-old Wistar rats. Additional rats of the same age underwent a sham operation. Bilateral testicular weight and epididymal weight were compared between the vasectomized and sham-operated animals at 4, 8 and 12 weeks postoperation. Intratesticular versus intraabdominal temperature difference, epididymal caudal sperm content (ECSC), motility (ECSM) and in vitro fertilizing capacity (IVF) were compared between the vasectomized and sham-operated animals at 12 weeks postoperation. Serum testosterone response to human chorionic gonadotropin and bilateral testicular weight were significantly smaller in the vasectomized animals than in the sham-operated animals at 8 and 12 weeks postoperation. Bilateral ECSC, ECSM and IVF outcomes were significantly smaller in vasectomized rats than in sham-treated rats 12 weeks postoperatively. Bilateral intratesticular versus intraabdominal temperature differences were not significantly different between the vasectomized and control rats at 12 weeks postoperation. Unilateral obstruction of the vas deferens results in a bilateral defect in spermatogenesis and in the epididymal sperm maturation process.

Key words: epididymis; spermatogenesis; testicular function; unilateral vasectomy

The epididymis is a complex system whose functions include sperm transit and storage, the maturation of spermatozoa, and the creation of sperm fertilizing potential. The basic mechanism for moving spermatozoa through the epididymis involves spontaneous contractions of the epididymal ducts (Schlegel and Chan, 1998). Sperm epididymal transit time is influenced more by daily testicular sperm production than by age (Johnson and Varner, 1988). In humans, almost half of the total number of epididymal spermatozoa is stored in the caudal region. Although the cauda epididymis plays an important role in sperm storage, long term storage of spermatozoa in the caudal region results in the aging of spermatozoa and impaired motility (Yeung et al., 1993) and lower capacity for fertilization (Zeuzus et al., 1992). Human spermatozoa develop an increased capacity for motility as they migrate through the epididymis. Spermatozoa received from the caput epididymis show weak tail movement. However, more than half of the spermatozoa taken from the cauda epididymis show mature motility patterns (low-amplitude and high-frequency beats) (Bedford et al., 1973). Morphological changes have been observed also in epididymal spermatozoa. Along the epididymal duct, light and scanning electron microscopy reveal that the shape of the sperm cytoskeleton changes and that the cytoplasmic droplet regresses and moves toward the posterior end of the middle piece (Flechon and Hafez 1975). Antypas et al. (1994) demonstrated bilateral epididymal dysfunction following ipsilateral vas deference obstruction.

The vas deferens is a tubular structure that carries sperm from the cauda epididymis to the ejaculatory duct. In a cross section view, it can be seen that the vas deferens consists of 3 mus-

Abbreviations: BWW, Biggers-Whitten-Whittingham; ECSC, epididymal caudal sperm content; ECSM, epididymal caudal sperm motility; IVF, in vitro fertilizing capacity; hCG, human chorionic gonadotropin

cular layers and a mucosal inner layer (Neaves, 1975). Adrenergic nerve fibers are also seen in all 3 layers, with the greatest concentration in the outer layer (McConnel et al., 1982). During sexual rest, epididymal contents are transported into the urethra gradually by spontaneous motility. Once sexual stimulation and ejaculation occur, the contents of the vas deferens are propelled into the urethra by strong contractions that are elicited by adrenergic neurotransmitters (Bruschini et al., 1977). It is also known that the vas deferens secretes glycoproteins into the tubular lumen (Nistal et al., 1992) and absorbs proteins and spermatozoa from the tubular lumen (Murakami and Yokoyama, 1989). These absorptive and secretory functions of the vas deferens may contribute to the generation of a luminal environment capable of supporting the maturation of sperm fertilizing ability (Bedford, 1994).

Damage of the vas deferens during childhood often occurs as a complication of inguinal surgical operations such as inguinal herniorrhaphy. Matsuda et al. (1992) reported that the incidence of vasal obstruction in infertile men with histories of herniorrhaphy was as high as 27.8% when unilateral obstruction was diagnosed. More than half the patients with vasal obstruction caused by infant inguinal herniorrhaphy have serum antisperm antibodies (Matsuda et al., 1993) and oligozoospermia (Parkhouse and Hendry 1991). Several experiments have been reported regarding the influence of vas deferens obstruction on spermatogenesis and the sperm maturation process. Increased diameters of seminiferous tubules have been observed in the testes of animals with vas deference obstruction (Lamano-Carvalho et al., 1984). Additionally, interstitial sperm granuloma surrounding epididymal tubules has been revealed histologically in humans postvasectomy (Silber, 1979). Vasectomy may result in increased pressure within the ipsilateral epididymal tubule leading to the development of granuloma around the tubule. Furthermore, a significant reduction of Sertoli cells and spermatid per tubular cross section and focal interstitial fibrosis have been observed in the human testis postvasectomy (Jarow et al.,

1985). However, the effect of unilateral obstruction on contralateral testicular function has not been adequately studied. Antypas et al. (1994) showed that a bilateral deficiency in both Leydig and Sertoli cell secretory functions occurs in unilaterally vasectomized animals with an overall result of bilaterally impaired spermatogenesis and inhibited sperm maturation process. Furthermore, whether unilateral vasectomy affects bilateral sperm fertilizing capacity is unknown. The present study focuses on the effect of left vasectomy on bilateral testicular and epididymal function and on overall sperm fertilizing capacity.

Materials and Methods

Animals

Five female and 5 male 12-week-old Wistar rats were obtained from Shimizu Experimental Material Company (Kyoto, Japan). Following mating experiments, male pups received either a left vasectomy or a sham operation at the age of 7 days. Vasectomized animals were divided into Groups A ($n = 11$), B ($n = 11$) and C ($n = 11$). Sham-operated animals were divided into Groups A1 ($n = 11$), B1 ($n = 11$) and C1 ($n = 11$). Groups A and A1 were sacrificed at 4 weeks postoperation. Groups B and B1 were killed at 8 weeks postoperation, and Groups C and C1 were killed at 12 weeks postoperation.

Surgical procedure

Pups were anesthetized for surgery by inhalation of diethyl ether. A small vertical midline lower abdominal incision was made. The left vas deferens was ligated twice with absorbable sutures and then transected between the ligatures. The spermatic vessels were carefully dissected. The wound was closed in 2 layers; 4-zero absorbable sutures were used for the musculature and 4-zero absorbable sutures for the skin. Sham operations were performed in the same manner except that the vas deferens was neither ligated nor divided.

At the end of the experimental period, bi-

lateral intratesticular versus intraabdominal temperature differences were evaluated in Groups C and C1 under sodium pentobarbital anesthesia (25 mg/kg; Nembutal, Abott Laboratories, Chicago, IL). One milliliter of blood was aspirated from the left renal vein of all animals in Groups B, B1, C and C1. Then human chorionic gonadotropin (hCG) (1500 units; Teikoku Zoki Co., Tokyo, Japan) was administered intramuscularly and 3 h later, 1 mL of blood was received from the right renal vein. Blood samples were processed for testosterone serum evaluation. Subsequently, testicular weight and epididymal weight were assessed bilaterally. Then each epididymis was divided into a head, body and tail. Epididymal caudal sperm content (ECSC), epididymal caudal sperm motility (ECSM) and in vitro fertilizing capacity (IVF outcome) were evaluated in Groups C and C1.

Testicular temperature

Intraabdominal and bilateral testicular temperature were assessed by percutaneous insertion of a 29-gauge needle probe attached to a digital thermometer (Unique Medical, PTC201 model, Tokyo) in Groups C and C1. Intraabdominal temperature was monitored using a rectal probe and body temperature was maintained between 36.6 and 37.4˚C. The difference between the intraabdominal and intratesticular temperature (ΔT) was recorded (Sofikitis et al., 1992).

Testicular and epididymal weight

In all groups, each testis and epididymis were excised, dissected free of surrounding tissue, and weighed on a Mettler Basbal Scale (Delta Range, Tokyo).

Epididymal caudal sperm content and quantitative sperm motility

Each epididymis was separated carefully from the respective testicle under $10 \times$ magnification provided by a stereo zoom microscope (Model TL2, Olympus Corp., Tokyo) in Groups C and C1. The epididymis was divided into 3 seg-ments: the head, the body and the tail. The epididymal tail was trimmed and minced in 5 mL of Biggers–Whitten–Whittingham (BWW) medium (Sofikitis et al., 1992) adjusted to pH 7.4 at 37˚C with 6 mol/L of sodium hydroxide. The minced epididymal tissue was then separated from the liberated spermatozoa by filtration through a stainless steel wire mesh with a pore size of 60 μm. Six droplets of the filtrate were used for assessing the sperm count (number of spermatozoa/mL BWW medium) and the average number was calculated for each animal. The sperm count was determined using a Makler Counting Chamber (Sefi Medical Instruments, Haifa, Israel). The chamber was placed on the slide of an ordinary microscope, and 20-power objective and 10-power eyepieces were used. Ten droplets of filtrate were counted to calculate the percentage of motile spermatozoa immediately after its preparation, so that the motility estimate became more accurate.

Determination of serum testosterone concentration

Serum testosterone concentration was determined by radioimmunoassay using a kit from the Nihon DPC Corporation (Tokyo) according to the method of Coyotupa et al. (1972). The intra- and inter-assay coefficients of variation were 5.5 and 9.2%, respectively. The sensitivity of the assay was 0.1 ng/mL.

Preparation of sperm suspension for IVF

Epididymal caudal suspensions were centrifuged at $300 \times g$ for 20 min. Sperm pellets were transferred to Toyoda and Chang medium (Toyoda and Chang, 1974). Finally, sperm suspensions containing 2×10^6 spermatozoa/mL were prepared and processed for IVF as previously described (Toyoda and Chang, 1974).

Collection of oocytes and IVF

Immature female rats were injected subcutaneously with 25-IU pregnant mare serum

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Table. 1. Difference between intraabdominal and intratesticular temperature

	C: vasectomy group [11]		C1: sham group [11]	
	Left	Right	Left	Right
ΔT (˚C)	4.2 ± 0.5	4.5 ± 0.7	4.3 ± 0.7	4.7 ± 0.6

Values are expressed as mean ± SD.
[ ], number of animals.
ΔT, Δtemperature: intraabdominal versus intratesticular temperature difference.
There were no significant differences in ΔT value within each group or between groups.

gonadotropin (Sigma Chemical Co., St. Louis, MO). Fifty-four hours later the rats were injected intraabdominally with 20-IU hCG (Teikoku Zoki Co., Tokyo). The rats were killed 19 h after hCG injection. The oviducts were removed and each ampullar portion was placed into a plastic dish containing the Toyoda and Chang medium (Toyoda and Chang, 1974). The oocytes in cumulus masses were removed from the oviducts and introduced into the Toyoda and Chang medium. A volume of 0.1 mL of sperm suspension was introduced into 0.9 mL of the Toyoda and Chang medium containing the oocytes within cumulus masses. The dishes were kept within a CO_2 incubator (5% CO_2 in air). Ten oocytes were inseminated with spermatozoa from each caudal epididymis of each rat in Groups C and C1.

Examination of oocytes

Twenty-four hours after insemination, the percentage of oocytes with 2 pronuclei plus a 2nd polar body was determined microscopically (Olympus IX-70, Tokyo).

Statistical analysis

Intergroup differences were analyzed using the unpaired t-test. A P value less than 0.05 was considered statistically significant. Values were expressed as mean ± SD.

Results

Testicular temperature

No significant differences were observed in mean DT values between the vasectomized rats

Table 2. Testicular weight and epididymal weight

Weight	Vasectomy group [11]		Weight	Sham group [11]	
(mg)	Left	Right	(mg)	Left	Right
Testicular			Testicular		
A	440 ± 48	484 ± 71	A1	490 ± 62	508 ± 51
B	$1156 \pm 89*$	$1280 \pm 80***$	B1	$1465 \pm 98**$	$1518 \pm 90****$
C	$1228 \pm 79*$	$1367 \pm 88***$	C1	$1741 \pm 76**$	$1763 \pm 93****$
Epididymal			Epididymal		
A	79 ± 30	74 ± 28	A1	83 ± 21	77 ± 45
B	$151 \pm 44*$	$302 \pm 57***$	B1	$371 \pm 68**$	$383 \pm 63****$
C	$386 \pm 50*$	$429 \pm 54***$	C1	$487 \pm 50**$	$521 \pm 39****$

Data are expressed as mean ± SD.
[ ], number of animals.
Within each transverse line, * versus **; *** versus ****; * versus ***: $P < 0.05$.
Values of testicular weight and epididymal weight were significantly larger bilaterally in Groups B1 and C1 than in Groups B and C respectively.
Values of testicular weight and epididymal weight of the left side were significantly smaller than those of the right side in Groups B and C.

Table 3. Sperm content and motility in the cauda epididymis

		C: vasectomy group [11]		C1: sham group [11]	
		Left	Right	Left	Right
ESCS	(× 10⁶/mL)	47.2 ± 9.4*	61.5 ± 7.6***	77.0 ± 6.6**	75.8 ± 8.7****
ECSM	(%)	19.1 ± 7.6*	46.8 ± 5.7***	60.0 ± 7.0**	65.6 ± 5.0****

Data are expressed as mean ± SD.
[ ], number of animals.
ECSC, epididymal caudal sperm content; ECSM, epididymal caudal sperm motility.
Within each transverse line, * versus **; *** versus ****; * versus ***: $P < 0.05$.
Values of ECSC and ECSM were significantly larger in Group C1 than in Group C.
Values of ECSC and ECSM of the left side were significantly smaller than these of the right side in Group C.

and sham-operated rats at 12 weeks post-operation (Table 1).

Testicular and epididymal weight

No significant difference was observed in bilateral testicular and epididymal weight between unilaterally vasectomized rats and sham-treated rats at 4 weeks postoperation. However, the weight of each left and right testis and epididymis was significantly lower in the vasectomized rats than in the sham-treated rats at 8 and 12 weeks postoperation. The weights of the left testis and epididymis were significantly smaller than those of the right side in vasectomized rats at 8 and 12 weeks postoperation (Table 2).

Caudal epididymal sperm count and motility

ECSC was significantly lower bilaterally in the unilaterally vasectomized rats than in the respective control group at 12 weeks postoperation (Table 3). ECSM was also significantly lower bilaterally in the unilateral vasectomized rats than in the respective control animals. ECSC and ECSM values of the left side were significantly smaller than those of the right side in the vasectomized rats at 12 week postoperation (Table 3).

Leydig cell function

Serum basal testosterone levels showed no significant difference between the unilaterally vasectomized rats and sham-treated rats at 8

Table 4. Testosterone responses to hCG stimulation

Level (ng/dL)	Vasectomy group [11]	Level (ng/dL)	Sham group [11]
Basal testosterone		Basal testosterone	
B	155.3 ± 22.6	B1	146.8 ± 32.4
C	159.1 ± 31.5	C1	189.9 ± 76.8
Testosterone response		Testosterone response	
B	562.1 ± 82.2*	B1	817.8 ± 140.2**
C	729.8 ± 123.9*	C1	1055.7 ± 162.0**

Data are expressed as mean ± SD.
[ ], number of animals.
Within each transverse line, * versus **: $P < 0.05$.
There were no significant difference in basal testosterone level between Groups B and B1, and between Groups C and C1.
Values of testosterone responses were significantly larger in Group B1 than in Group B, and in Group C1 than in Group C.

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Table 5. **Outcome of in vitro fertilization using cauda epididymal spermatozoa**

Number of oocytes	C: vasectomy group [11]		C1: sham group [11]	
	Left	Right	Left	Right
Inseminated oocytes	110	110	110	110
2-PN oocytes†	34 (31)*	54 (49)***	79 (72)**	84 (76)****

[ ], number of animals; ( ), percentage.
† Oocytes with 2 pronuclei plus a 2nd polar body.
  Within each transverse line, * versus **; *** versus ****: $P < 0.05$
  Percent of fertilized oocytes was significantly larger bilaterally in Group C1 than in Group C.
  Percent of fertilized oocytes of the left side was significantly smaller than that of the right side in Group C.

and 12 weeks postoperation (Table 4). However, peripheral serum testosterone responses to hCG stimulation were significantly lower in the unilaterally vasectomized rats than in the control animals at 8 and 12 weeks postoperation (Table 4).

Fertility potential in vitro

The percentage of oocytes with 2 pronuclei plus a 2nd polar body after undergoing the IVF technique using caudal spermatozoa was significantly lower bilaterally in the unilaterally vasectomized animals than in the control rats. The percent of fertilized oocytes of the left side was significantly smaller than that of the right side in the vasectomized rats at 12 weeks postoperation (Table 5).

Discussion

Inguinal canal reconstruction operations such as varicocelectomy and orchiopexy procedures entail the risk of damaging the ipsilateral vas deferens. Several experimental and clinical studies have demonstrated an adverse effect of vas deferens injury on ipsilateral testicular function. Matsuda et al. (1992) reported that the incidence of unilateral vas deferens obstruction caused by inguinal herniorrhaphy was 26.7% for infertile patients with a history of the operation. They also reported that there were fewer total germ cells and a larger seminiferous tubular diameter in patients with lifelong vasal obstruction caused by childhood inguinal

herniorrhaphy compared to vasectomy with an average obstruction period of 8 years (Matsuda et al., 1996).

All of the above studies examined the effects of unilateral vas deferens damage on the ipsilateral testis. We attempted to evaluate the effects of unilateral vasectomy on bilateral testicular and epididymal functions. The results of the current study support the finding that unilateral vasectomy results in bilateral damage in spermatogenesis. This is indicated by the significantly smaller ECSC in unilaterally vasectomized rats than in control rats bilaterally. In addition, the significantly smaller ECSM in unilaterally vasectomized rats than in control rats bilaterally indicates a deficiency in the epididymal sperm maturation process bilaterally in vasectomized rats. The epididymal sperm maturation process is known to involve a cascade of biophysical and biochemical events that result in progressive sperm motility, the ability for capacitation, and the acquisition of fertilizing potential. Therefore, the significantly smaller bilateral IVF outcome in unilaterally vasectomized rats may be attributed to defects in the epididymal sperm maturation process and subsequently reduced sperm fertilizing capacity. Defects in the epididymal sperm maturation process leading to an impaired progressive sperm motility, the ability to undergo capacitation and to penetrate the zona pellucida of oocytes results in a diminished sperm fertilizing capacity and suboptimal IVF outcome. Defects in bilateral epididymal and testicular function are also supported by the findings of a significantly smaller epididymal and testicular weight

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in vasectomized rats. It is known that strong positive correlations exist significantly between epididymal weight and epididymal function, and between testicular weight and testicular function (Takihara et al., 1987).

Although we did not evaluate Leydig and Sertoli cell function separately on the left and right side, the significantly smaller serum testosterone responses in the vasectomized rats suggests a defect in Leydig cell function in vasectomized animals. A deficiency in Leydig cell secretory function may be responsible for the defects in spermatogenesis and the epididymal sperm maturation process in vasectomized rats because it is known that optimal concentrations of intratesticular testosterone and epididymal testosterone are necessary for the maintenance and activation of spermatogenesis and the epididymal sperm maturation process (Sofikitis et al., 1992).

The defect in ipsilateral testicular function and spermatogenesis in animals with an obstructed left vas deferens can be easily explained. Increased diameters of ipsilateral seminiferous tubules have been observed in the testes of animals with obstructed vas deferens (Lamano et al., 1984). Furthermore, interstitial sperm granuloma surrounding epididymal tubule postvasectomy has been revealed histologically (Silber, 1979; Matsuda et al., 1992) in humans. Also, a significant reduction of Sertoli cells and spermatid count per tubular cross section and focal interstitial fibrosis have also been observed in the human testis postvasectomy (Matsuda et al., 1996). We suggest that high pressure within the epididymal and seminiferous tubules due to vas obstruction leads to extension and dilation of the tubules and the exit of spermatozoa from epididymal and seminiferous tubules which may result in interstitial fibrosis of the testes and secondary epididymal obstruction. In addition, accumulation of toxic substances from disintegrated spermatozoa may occur within the testis and epididymis and detrimentally affect spermatogenesis and the epididymal sperm maturation process. Furthermore, the increased pressure within the testicular seminiferous tubules may detrimentally

affect ipsilateral Leydig cell function either directly (due to mechanical pressure from the expanded seminiferous tubules) or indirectly (due to Sertoli cell secretory dysfunction). It is known that the Sertoli cells affect Leydig cell function via the production of peptides (Sylvester, 1996).

However, it is not easy to explain the defects in contralateral testicular function, spermatogenesis, the epididymal sperm maturation process, and sperm fertilization potential after obstruction of the unilateral vas deferens. Sperm antibodies are found in the serum of approximately 80% of bilaterally vasectomized men and achievement of pregnancy after vasectomy reversal is significantly less when postoperative antisperm antibody titer is high (Parslow et al., 1982). Parkhouse and Hendry (1991) reported that of 17 infertile patients with unilateral vas obstruction following inguinal surgery in childhood, 11 developed oligospermia and all had detectable titers of antisperm antibodies. We speculate that the damage in the unilateral testis blood barrier leads to an autoimmune disorder that results in testicular dysfunction and subsequent damage to the epididymal maturation process in the right side. In addition, antibodies after left vasectomy may not develop only against spermatozoa but also against the components of seminiferous tubules and interstitial testicular tissue in the left side. Left Leydig cells may be damaged due to pressure from the dilated, overexpanded seminiferous tubules. This is consistent with the smaller left testicular weight in left vasectomized rats. Antibodies or substances toxic to components of seminiferous tubules and interstitial testicular tissue (Leydig cell) of the diseased left testis may affect right testicular seminiferous tubule components (spermatozoa or Sertoli cells) or Leydig cells and subsequently may influence right side spermatogenesis, the epididymal sperm maturation process and epididymal caudal sperm fertilizing capacity. The latter thesis is also consistent with a previous study by Antypas et al. (1994) who demonstrated bilateral testicular damage and impaired epididymal sperm maturation

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process in unilaterally vasectomized animals. More studies are required to explain these mechanisms.

It appears that bilateral epididymal caudal sperm fertilizing capacity decreases after unilateral vasectomy at an early age. Therefore, in the case of iatrogenic injury of the vas deferens during childhood, spermatozoa may not be allowed an optimal fertilizing capacity. Subsequently, assisted reproductive techniques in infertile adolescents with a history of unilateral vas deferens damage may not be successful. The latter men require anastomosis of the injured vas deferens which will definitely increase the sperm count in the ejaculate and may improve testicular function. Therefore, assisted reproductive techniques may not be the first choice for the therapeutic management of men with vas deferens obstruction.

The absence of significant differences in testicular versus intraabdominal temperature difference may suggest that the increased intratesticular pressure postvasectomy is not accompanied by a defect in the efficiency of the ipsilateral counter-current heat exchange system that regulates the testicular temperature. The absence of significant differences in testicular weight between vasectomized and control rats at 4 weeks postoperation suggests that a lag time is necessary for the development and manifestation of the detrimental consequences of unilateral vasectomy on bilateral testicular function.

In conclusion, we demonstrated that bilateral testicular and epididymal damage develops after unilateral vas deferens injury. We advocate that unilateral vas deferens injury during inguinal operation in childhood should be diagnosed and repaired as early as possible.

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