

Bilateral Effect of Unilateral Vasectomy on Testicular Testosterone Biosynthesis

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● Twelve immature male dogs underwent a left vasectomy (group A). An additional five underwent a sham operation (group B). Sixteen weeks after the surgery, the bilateral mean values for caudal epididymal sperm content, the percentage of motile spermatozoa, intratesticular testosterone concentration, and testicular secretion of androgen-binding protein (*in vitro*) were significantly lower in group A. The mean peripheral serum testosterone responses 3 hours after human chorionic gonadotropin stimulation (3,000 IU) were significantly lower in group A than in group B (6.3 ng/mL v 9.5 ng/mL). These findings indicate a bilateral deficiency in both Leydig and Sertoli cell secretory function in unilaterally vasectomized dogs, resulting in impaired bilateral spermatogenesis and sperm maturation. The authors suggest that unilateral injuries of the vas deferens during hernia operations in children may result in bilateral testicular dysfunction. Copyright © 1994 by W.B. Saunders Company

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INJURY OF the vas deferens is an uncommon complication of surgical procedures in the inguinal canal in children and adults. Vasectomy is a popular contraceptive method. Prevention of sperm transfer from the ipsilateral cauda epididymis to the ejaculatory duct is not the only harmful effect of vasectomy on potential fertility; it has been proven that vasectomy in humans and experimental animals is followed by the development of antisperm antibodies in a high percentage of cases.¹ Many vasectomized rhesus monkeys have orchitis and/or depressed spermatogenesis.² These changes are associated with evidence of immune complex formation.² It appears that when injury of the vas deferens occurs in a child or an adult, fertility is severely threatened.

Although there are many studies evaluating the

development of antisperm antibodies and their effects on spermatogenesis in vasectomized adults or experimental mature animals,^{1,2} there are no reports concerning the Leydig and Sertoli cell function in subjects having undergone vasectomy before puberty. The aim of the present study is to illustrate the consequences of unilateral vasectomy during childhood on the Leydig and Sertoli cell function of both testes. Such a study is clinically important because, during a variety of operations in children, surgical manipulations of the vas deferens are required, which have a risk of injury. Furthermore, the testes of children are much more susceptible to detrimental anatomic/physiological factors than are those of adults. Because it is clearly impossible to perform the relevant tests in humans, an experimental vasectomy model was created using immature dogs.

MATERIALS AND METHODS

Twelve male 3-month-old dogs were anesthetized with Nembutal (30 mg/kg; Abbott Laboratories, Chicago, IL) and they underwent left vasectomy at the point where the epididymal tail is connected with the vas deferens. The vas deferens was doubly ligated with a 000 stitch, and the intervening part was resected (group A). Another five dogs underwent a sham operation (group B). Postoperatively, ampicillin (250 mg per animal) was administered to all dogs. Sixteen weeks after the surgical procedure, peripheral serum concentrations of testosterone, luteinizing hormone (LH), and follicle stimulating hormone (FSH) were assessed. Peripheral serum testosterone levels were also evaluated 3 hours after intraperitoneal administration of human chorionic gonadotropin (HCG; 3,000 IU; Teikoku Zohki Co, Tokyo, Japan). Four days later, blood was aspirated from a peripheral vein, and the serum was examined for cytotoxic antisperm antibodies. Body weight was measured, and all the dogs were anesthetized by Nembutal. Fluid from the cauda epididymis was collected using a micromanipulator, as previously described by Hurt et al.³ Epididymal sperm content and motility were assessed. Thereafter, both testes were removed and weighed. Each was divided into two equal portions. The first portion was used for estimation of the level of intratesticular testosterone; the second was tested *in vitro* for the secretion of androgen-binding protein.

LH/FSH Assay

LH and FSH were measured in duplicate, in each sample, by means of specific homologous double-antibody radioimmunoassays. Purified LH and FSH were used both as standard and for iodination purposes. Antibodies against dog LH or FSH were raised in guinea pigs.

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Testosterone Assay

Serum testosterone concentration was determined by radioimmunoassay using kits from Nihon DPC Corporation (Tokyo, Japan), according to the method of Coyotupa et al.⁴ Intratesticular testosterone concentrations were measured using a radioimmunoassay procedure, according to the method of Abraham et al.,⁵ as modified by Rajfer et al.⁶

Testicular Secretion of Androgen-Binding Protein In Vitro

Minced decapsulated testicular tissue (100 mg) was incubated in 2 mL of Eagle's tissue culture medium based on Earle's salts,⁷ to which NaHCO₃ (2 g/L), L-glutamine (2 mmol/L), penicillin (100 IU/mL), and streptomycin (50 µg/mL) were added. Incubations were performed at 32°C in a 5% CO₂ environment, with constant shaking. After 4 hours' incubation, the tissue fragments were sedimented by centrifugation at 700 × g for 5 minutes. The tissue fragments or sections were resuspended in fresh medium and incubated for another 16 hours. Preformed androgen-binding protein in the testicular tissue was released into the medium during the initial preparation and the first 4 hours of incubation. The androgen-binding protein secretion rate was obtained by measuring the amount of androgen-binding protein accumulating in the medium during the 16-hour (4 to 20 hours) incubation period. After incubation, the tubes were centrifuged for 1 hour, and the supernatants were assayed for androgen-binding protein as previously described by Ritzen et al.⁸

Testicular Weight

The testes were excised, dissected free of surrounding tissue, and weighed on a Mettler Basbal scale (Delta Range, Tokyo, Japan).

Caudal Epididymal Sperm Content and Motility

One hundred nanoliters of a caudal fluid sample was diluted in 25 µL of 1% hyaluronidase in saline. Sperm samples were counted in triplicate on a Makler Counting Chamber (Sefi Medical Instruments, Haifa, Israel), and sperm concentrations were calculated using appropriate dilution ratios. For motility assay, a 500-nL aliquot of a fresh sample from caudal fluid was transferred under oil and diluted with 5 µL of saline. The percentage of motile spermatozoa was evaluated microscopically 5 minutes later.

Cytotoxic Sperm Antibody Assay

Serum titers of cytotoxic antisperm antibodies were determined by modification of the double fluorochrome cytotoxicity assay of Mathur et al.,⁹ as described by Shook et al.¹⁰ Epididymal spermatozoa were obtained from additional dogs as described previously.

Viable spermatozoa were labelled by diacetyl fluorescein and mixed with serial twofold dilutions of the study serum, in 5 µL aliquots, in the presence of diluted guinea pig complement (GIBCO, Grant Island, NY). The percentage of cells killed per dilution was determined as described previously.¹⁰ Log₂ values were used to express the results (because the dilutions were twofold) and to ensure accurate statistical evaluation.¹⁰

Statistical Analysis

Statistical analysis was performed on all data using the paired *t* test to compare parameters referring to paired organs within a group. Parametric (*t* test for unpaired observations) or nonparametric tests (Wilcoxon's test for unpaired observations) were used to analyze intergroup differences when the data were distributed in a normal or abnormal pattern, respectively. A *P* value of less than .05 was considered statistically significant. Data are expressed as mean ± standard deviation.

RESULTS

Caudal Epididymal Sperm Content and Motility

Caudal epididymal sperm content and motility were significantly lower, bilaterally, in vasectomized animals than in controls (Table 1). There were no significant differences between left and right caudal epididymal sperm content and motility between groups A and B.

Testicular Weight

There were no significant differences in left and right testicular weight between groups A and B. Bilateral testicular weight was significantly lower in vasectomized dogs than in control dogs (Table 1).

Androgen-Binding Protein Assay

Testicular secretion of androgen-binding protein in vitro was significantly lower, bilaterally, in vasectomized dogs than in controls (Table 1). There were no significant differences in testicular secretion of androgen-binding protein on the left or the right side between groups A and B.

Testosterone/LH/FSH Assay

There were no significant differences in peripheral serum testosterone concentrations between vasecto-

Table 1. Effects of Vasectomy on Bilateral Caudal Epididymal Sperm Content (ESC) and Motility (ESM), Testicular Weight (TW), Testicular Secretion of Androgen-Binding Protein (ABP) In Vitro, and Intratesticular Testosterone Concentration

	Vasectomized Animals		Control Animals	
	Left	Right	Left	Right
ESC (×10 ⁹ sperm cells)	234 ± 31 ^a	243 ± 28 ^b	342 ± 44 ^c	339 ± 33 ^d
ESM (%)	34 ± 7 ^a	35 ± 8 ^b	53 ± 9 ^c	55 ± 6 ^d
TW (mg)	4456 ± 54 ^a	4487 ± 71 ^b	5217 ± 61 ^c	5249 ± 49 ^d
ABP (pmol/testis/h)	0.11 ± 0.05 ^a	0.15 ± 0.05 ^b	0.31 ± 0.07 ^c	0.35 ± 0.06 ^d
Testosterone (ng/g testis)	83 ± 22 ^a	95 ± 17 ^b	128 ± 21 ^c	123 ± 14 ^d

NOTE. Data are expressed as mean ± SD. Within each transverse line: a v c, *P* < .05; b v d, *P* < .05; a v b, *P* > .05; c v d, *P* > .05.

Table 2. Effects of Vasectomy on Peripheral Serum Basal Hormonal Profiles, Testosterone (T) Responses to HCG Stimulation, and Titers of Cytotoxic Antisperm Antibodies (CASA)

	Vasectomized Animals	Control Animals
Basal T (ng/mL)	2.0 ± 0.3 ^a	1.9 ± 0.3 ^b
FSH (ng/mL)	3.2 ± 0.5 ^a	2.9 ± 0.5 ^b
LH (ng/mL)	0.7 ± 0.2 ^a	0.8 ± 0.2 ^b
T responses (ng/mL)	6.3 ± 2.0 ^a	9.5 ± 1.1 ^c
Titers of CASA*	7.4 ± 1.1 ^a	0.4 ± 0.1 ^c

NOTE. Data are expressed as mean ± SD. Within each transverse line: a v b, $P > .05$; a v c, $P < .05$.

*Log₂ values were used.

mized and control animals (Table 2). In contrast, the testosterone responses to HCG stimulation and bilateral intratesticular testosterone concentration were significantly lower in vasectomized dogs than in control dogs (Table 2). There were no significant differences in intratesticular testosterone concentration on the left or the right side between groups A and B (Table 1). The differences in peripheral LH and FSH levels between the vasectomized and control dogs were not significant (Table 2).

Titers of Cytotoxic Antisperm Antibodies; Body Weight

The titers of cytotoxic sperm antibodies were significantly higher in vasectomized dogs than in controls (Table 2). There were no significant differences in body weight between these two groups.

DISCUSSION

The significantly lower caudal epididymal sperm concentration and motility in the vasectomized dogs, noted bilaterally, suggest a bilateral defect in spermatogenesis and epididymal sperm maturation in unilaterally vasectomized subjects. These findings are consistent with those obtained by microscopic examination of biopsy material taken from the ipsilateral testis to the vasectomized side in experimental animals.¹ However, the present study is the first to provide clear evidence of a defect in spermatogenesis and sperm maturation in the contralateral testis and epididymis, respectively.

The absence of significant differences in peripheral serum LH and FSH levels between the vasectomized and the control population suggests that pituitary function is intact in vasectomized animals. The absence of significant differences in peripheral serum testosterone levels between control and vasectomized animals does not exclude the probability that Leydig cell dysfunction may have occurred in the vasectomized dogs, because changes in peripheral serum testosterone levels did not show delicate changes in

Leydig cell function.¹¹ The significantly lower testosterone responses obtained after HCG stimulation, and the bilateral significant decreases in intratesticular testosterone concentration and testicular secretion of androgen-binding protein in vitro in the vasectomized dogs clearly indicate bilateral dysfunction in Leydig and Sertoli cells, respectively, because androgen-binding protein secretion is considered as a marker of Sertoli cell function.⁷ The bilateral effects of unilateral vasectomy on spermatogenesis and sperm maturation may be attributed to the bilateral dysfunction of Leydig and Sertoli cells because Leydig cells play an important role in spermatogenesis by producing and secreting testosterone, which activates the spermatogenic process¹² and spermatid maturation.¹³ Sertoli cells regulate the testosterone concentration in the testis and epididymis through the production of androgen-binding protein.¹³ The significantly lower bilateral testicular weight noted in the vasectomized dogs confirms the existence of bilateral testicular dysfunction, ie, it is well known that testicular weight has a direct correlation with testicular function.¹⁴ Alternatively, the bilateral detrimental effects of unilateral vasectomy on spermatogenesis and the epididymal sperm maturation process may be attributable to the development of antisperm antibodies in the vasectomized animals. We speculate that the intraluminal pressure in the left testis increased after left vasectomy, causing damage to the left blood-testis (Sertoli cell) barrier, leading to the development of autoimmune disease.

Although the consequences of left vasectomy in the ipsilateral Leydig and Sertoli cell function may be attributable to an increase in testicular intraluminal pressure affecting the function of the Sertoli and Leydig cells, it is difficult to explain the effects of left vasectomy on right Leydig and Sertoli cell function. Additional studies are necessary to further delineate the possible causes of unilateral vasectomy on overall testicular function.

The present study shows that unilateral vasectomy in immature dogs causes bilateral testicular damage. Although it is not easy to extrapolate conclusions drawn from animal studies to humans, we suggest that unilateral injuries of the vas deferens may result in bilateral testicular dysfunction, emphasizing the need for urgent microsurgical repair of damage to the vas deferens during operations in the inguinal canal in children. More studies are needed to evaluate whether microsurgical vasectomy reversal is efficient in improving testicular dysfunction and epididymal sperm maturation.

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