Effects of Unilateral Cryptorchidism on Contralateral Epididymal Sperm Quality, Quantity and Fertilizing Capacity

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The effect of unilateral cryptorchidism on ipsilateral testicular function has been well studied both in human and experimental animals. However, the effect of unilateral cryptorchidism on contralateral testicular function remains unclear. To evaluate the effect of left cryptorchidism on right testicular function, 3 groups of immature male rats were used: group A, left cryptorchid rats; group B, sham operated rats and group C, orchidopexed rats. Ten weeks post-cryptorchidism induction, right testicular versus intraabdominal temperature difference (RΔT), bilateral epididymal sperm content and motility, bilateral testicular weight, fertility rate in vivo and in vitro, and serum testosterone responses to human chorionic gonadotropin stimulation were tested. Most of the above parameters were significantly lower in group A than in group B and significantly higher in group C than in group A. The current findings suggest that there is a detrimental effect of unilateral cryptorchidism on sperm fertilizing capacity in vitro not only on the ipsilateral side but also on the contralateral side.

Key words: cryptorchidism; infertility; rat; spermatozoa

Male infertility is both a private and a social problem. Cryptorchidism is a well described clinical condition associated with male infertility (Alpert and Klein, 1983; David et al., 1992; Seppo et al., 1996).

Cryptorchidism is a defect involving maldescent of the testicle. The objectives of therapy for cryptorchidism include preservation of fertility, reduction of the risk of malignancy and alleviation of physiological stress. The incidence of cryptorchidism in the newborn is 2% to 6%; however with spontaneous descent this incidence drops to 1% by the age of 3 months (Alpert and Klein, 1983; David et al., 1992; Seppo et al., 1996). Prematurity, low birth weight and multiple gestations are predisposing factors with the increase in incidence correlating to the degree of prematurity. A family history of cryptorchidism may be present in 5%. Mothers of children with cryptorchidism tend to have mild pituitary impairment manifested by short menses and delayed menarche (Alpert and Klein, 1983; David et al., 1992; Seppo et al., 1996).

By the 12th to 14th week of gestation the testis migrates from the urogenital ridge to the level of the internal inguinal ring. The testis begins its transinguinal descent during the 26 th to 28 th week of gestation with simultaneous gubernaculum swelling and processus vaginalis extension into the scrotum. Testicular descent is believed to depend on an integration of factors: an increase in intraabdominal pressure, gubernaculum tension and the hormonal influence of high local concentrations of dihydrotestosterone.

Cryptorchidism is a preexisting factor in 3% to 8% of infertile men and in 20% of men with azoospermia (Alpert and Klein, 1983; David et al., 1992; Seppo et al., 1996). Animal studies also support the effect of cryptorchidism on fertility. It is generally accepted that a relatively

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low temperature is preferable in spermatogenesis in mammalian species (Harrison and Weiner, 1949). Actually, several studies indicate that the temperature in the scrotum is 4 to 5˚C lower than in the abdomen in most mammals (Harrison and Weiner, 1949). Waites and Moule (1961) measured the temperature of the abdominal aorta and testicular artery at various points and found that the temperature of the testicular artery was significantly smaller. A major deleterious effect of high intratesticular temperature on spermatogenesis has been demonstrated (David et al., 1981; Nagler et al., 1987). It is well known that the function of the cryptorchid testis is impaired by the rise in temperature (Alpert and Klein, 1983; Nagler et al., 1987; David et al., 1992; Seppo et al., 1996). The effect of unilateral cryptorchidism on ipsilateral testicular function has been well studied in humans and experimental animals (Rager et al., 1975; Keel et al., 1980; Jegoue et al., 1983). However, the effect of unilateral cryptorchidism on contralateral testicular function has not been extensively studied. Ono and Sofikitis (1997) have held that the endocrine and exocrine function of the contralateral testis are impaired by a rise in temperature.

The present study focuses on the effects of left cryptorchidism on the right testicular and epididymal function and the overall sperm fertilizing capacity.

Materials and Methods

The Wistar rats used were 2-week-old males obtained from the Shimizu Experimental Material Company (Kyoto, Japan). The rats were divided into 3 groups: A = rats which had undergone cryptorchidism induction; B = sham operated rats; and C = rats which had undergone cryptorchidism induction and subsequently orchidopexy. To create left cryptorchidism (groups A and C) left inguinal incision was performed . The left testis was sutured at the posterior abdominal wall with 6-0 nylon sutures under sodium pentobarbital anesthesia (25 mg/kg; Nembutal, Abott Laboratories, Chicago, IL). The sham operation (group B) included a left inguinal incision, placement of the left testis into the abdomen and replacement of the testis into its normal position. Four weeks after cryptorchidism induction, rats of group C had undergone left orchidopexy. Ten weeks after the initial operation, rats of groups A ($n = 20$), B ($n = 10$) and C ($n = 10$) were processed for evaluation of abdominal temperature, right testicular temperature and fertility potential in vivo. The concentration of testosterone was then determined in blood aspirated from the inferior vena cava by a midline abdominal incision. The incision was closed and all the rats were administered human chorionic gonadotropin (hCG) (1500 units; Teikoku Zoki Co., Tokyo, Japan) intraabdominally. Three hours after hCG stimulation, testosterone responses in the vena cava were evaluated. Then, all the rats were sacrificed by an injection of saturated potassium chloride into the left venticle. Bilateral epididymal caudal sperm content and motility and bilateral testicular weight were measured. Furthermore in vitro fertilization (IVF) trials were performed to appreciate epididymal caudal sperm fertilizing potential both on the right and left side.

Testicular temperature

Right testicular temperature was assessed by percutaneous insertion of a 29-gauge needle probe attached to a digital thermometer (Unique Medical, PTC201 model, Tokyo). Intraabdominal temperature was monitored with a rectal probe and body temperature was maintained between 36.7 and 37.3˚C with radiant heat throughout the procedure. The difference between the intraabdominal and intratesticular temperature on the right side (RΔT) was recorded as in the method described previously (Sofikitis et al., 1992).

Determination of the weight of testes

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

Epididymal caudal sperm content and quantitative sperm motility (% motility)

Each epididymis was separated carefully from its testicle under a magnification of 10 times, provided by a stereo zoom microscope (model TL2, Olympus Corp., Tokyo). The epididymis was divided into 3 segments: the head, the body and the tail. The epididymal tail was trimmed and minced in 5 mL of Biggers-Whitten-Whittingham (BWW) (Sofikitis et al., 1992) medium adjusted to pH 7.4 at 37°C with 6-mol/ L 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 with a Makler counting chamber (Sefi Medical Instruments, Haifa, Israel). The chamber was placed on the slide of an ordinary microscope, and a 20-power objective and 10-power eyepiece were used. Ten droplets of the filtrate were counted to calculate the percentage of motile spermatozoa immediately after its preparation, so that the estimate of motility became more accurate.

Determination of serum testosterone concentration

Testosterone concentration was determined by radioimmunoassay using a kit from the Nihon DPC Corporation (Tokyo) according to the method of Coyotupa and coworkers (1992). 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.

Fertility assessment in vivo

Two female Wistar rats (3 months old) in the first hours of estrus as determined by vaginal smear examination, were placed in a single cage with each male rat. Two hours later, the female rats were checked after mating to detect sper-matozoa in their vagina by microscopic examination of the vaginal fluid. Females were then checked 3 times daily from day 21 for parturition (day 1 was designated as the day of mating). A male rat was considered fertile if its matings resulted in at least one pregnancy.

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 gonadotropin (Sigma Chemical Company, St. Louis, MO). Fifty-four hours later an intraabdominal injection of 20 IU hCG (Teikoku Zoki Company, Tokyo) was done. Rats were killed 19 h after hCG injection. The oviducts were removed and the ampullar portion was put into a plastic dish containing 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

Table 1. Difference (Δ) between intra-abdominal and right intratesticular temperature

Group		ΔTemperature (°C)
A: Cryptorchid	[20]	3.2 ± 0.3
B: Control	[10]	4.3 ± 0.3
C: Orchiopexy	[10]	3.7 ± 0.3

Values are expressed as mean ± SD.
[ ], number of samples.
*Significant differences; $P < 0.05$.

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 the left epididymis of each group. Furthermore, 10 oocytes were inseminated with spermatozoa from the right cauda epididymis of each rat in each group .

Examination of oocytes

Twenty-four hours after insemination, the percentage of oocytes with 2 pronuclei plus a second polar body was evaluated via an inverted microscope (Olympus IX-70, Tokyo).

Statistical analysis

Values were expressed as mean ± SD. Statistical analysis was performed on all data using Fisher's PLSD test to analyze intergroup differences. A probability $P < 0.05$ was considered to be statistically significant. Assessment of the various parameters was performed in a blinded fashion.

Results

Testicular temperature

The cryptorchid group showed a significantly smaller mean RΔT value compared with the

Table 2. Testicular weight (mg) in cryptorchid rats

| | Group | | |
	A Cryptorchid [20]	B Control [10]	C Orchiopexy [10]
Right	814.0 ± 91.7	1341.3 ± 135.4	1060.0 ± 149.7
Left	277.1 ± 70.7	1266.2 ± 123.5	631.2 ± 144.7

Values are expressed as mean ± SD.
[ ], number of samples.
*Significant differences; $P < 0.05$.

control group and the orchidopexy group (Table 1).

Testicular weight

Left testicular weight was significantly lower than right testicular weight in cryptorchid rats (Table 2). Left testicular weight was significantly smaller in cryptorchid rats than in control rats and rats which had undergone orchidopexy. Right testicular weight in cryptorchid rats was significantly lower than in control rats or rats which had undergone orchidopexy.

Epididymal sperm content and motility

The epididymal caudal sperm content was significantly lower bilaterally in the cryptorchid

Table 3. Sperm content and motility in the cauda epididymis

Group	Concentration (× 10^6/mL)		Motility (%)	
	Right	Left	Right	Left
A: Cryptorchid [20]	27.9 ± 5.7	2.5 ± 0.9	29.8 ± 11.7	5.4 ± 2.5
B: Control [10]	83.1 ± 10.3	79.9 ± 12.3	72.7 ± 11.5	73.8 ± 9.6
C: Orchiopexy [10]	57.0 ± 9.5	19.5 ± 6.1	48.3 ± 9.7	40.2 ± 10.1

Values are expressed as mean ± SD.
[ ], number of samples.
*Significant differences; $P < 0.05$.

rats than in the control group (Table 3). The percentage of motile sperm was significantly lower bilaterally in cryptorchids rats than in the control group (Table 3). The epididymal caudal sperm content and motility on the left and right side were significantly higher in rats which had undergone orchidopexy than in the cryptorchid rats.

Leydig cell function

Peripheral serum basal testosterone levels were not significantly different among the 3 groups. Peripheral serum testosterone responses to hCG stimulation were significantly lower in cryptorchid rats than in rats which had undergone orchidopexy or control rats (Table 4). There was no significant difference in testosterone responses between control rats and rats which had undergone orchidopexy.

Fertility potential in vivo and in vitro

The effects on fertility are summarized in Table 5. The proportion of fertile rats in the cryptorchid group was significantly lower than in the control group. The percentage of oocytes with 2 pronuclei plus a second polar body after the IVF technique using left or right caudal sperm was significantly lower in cryptorchid rats than in control rats or rats which had undergone orchidopexy.

Table 4. Testosterone responses to hCG stimulation

Group		Testosterone (ng/dL)	
		Pre-hCG	Post-hCG
A: Cryptorchid	[20]	174 ± 53	488 ± 74
B: Control	[10]	199 ± 47	819 ± 89
C: Orchiopexy	[10]	188 ± 62	781 ± 87

Values are expressed as mean ± SD.
[ ], number of samples.
hCG, human chorionic gonadotropin.
*Significant differences; $P < 0.05$.

Discussion

Cryptorchidism is a preexisting factor in 3% to 8% of infertile men and in 20 % of men with azoospermia (Waites and Moule, 1961; Alpert and Klein, 1983; Tellaioglus et al., 1994). The incidence of infertility ranges from 10% to 20% in patients with unilateral cryptorchidism (Tellaloglus et al., 1994). Animal studies indicate a detrimental effect of unilateral cryptorchidism on fertility potential (Tellaloglus et al., 1994). In congenital unilateral cryptorchid rats the fertility rate is 0% compared with 100% in control rats (Patkowski et al., 1992). In the congenital canine cryptorchid model all bilateral cryptorchid animals were azoospermic, whereas the total sperm count, sperm motility and percentage of normal sperm morphology were

Table 5. Effect of cryptorchidism on fertility in vivo and in vitro

Group		Number of fertile rats	Number of inseminated oocytes†	Number of 2-PN oocytes‡	
				Right caudal epididymis	Left caudal epididymis
A: Cryptorchid	[20]	2 (10)	200	23 (11.5)	0 (0)
B: Control	[10]	10 (100)	100	82 (82)	84 (84)
C: Orchiopexy	[10]	7 (70)	100	68 (68)	47 (47)

( ), percentage; [ ], number of samples of the group.
†Inseminated oocytes by sperms from each side.
‡Oocyte with 2 pronuclei plus second polar body.
*Significant differences; $P < 0.05$.

diminished in the unilateral cryptorchid animals (Kawakami et al., 1995).

A model of cryptorchidism was created in the present study in rats at 2 weeks of age. The results of the present study confirm several previous studies (Patkowski et al., 1992; Tellaloglus et al., 1994; Kawakami et al., 1995) indicating a detrimental effect of unilateral cryptorchidism on ipsilateral testicular function. Furthermore, the present study indicates that the RΔT, the right testicular weight, the right epididymal caudal sperm content, motility and fertilizing capacity are significantly smaller in rats with left cryptorchidism than in control rats. In addition, the current study demonstrates that right orchidopexy partially restores the influence of left cryptorchidism on right epididymal sperm quantitative parameters. Although the effect of unilateral cryptorchidism on ipsilateral testicular function may be attributable to the increase in ipsilateral testicular temperature, there is much controversy concerning the mechanism(s) that mediate the detrimental effect of unilateral cryptorchidism on contralateral testicular function. Ono and Sofikitis (1997) have provided evidence that the consequences of left cryptorchidism on right testicular function may be due to an increase in right testicular temperature affecting adversely the right testicular Leydig and Sertoli cell secretory function. The above increase in right testicular temperature is attributable to an increase in right testicular blood flow (Ono and Sofikitis, 1997). Alterations in contralateral testicular blood flow in animals with unilateral cryptorchidism may be due to an increase in the number of testicular arterial microvessels or an extension of intravascular space (Ono and Sofikitis, 1997). A detrimental effect of left cryptorchidism on right testicular function is additionally indicated by the significantly smaller right testicular weight in animals with left cryptorchidism since it is known that testicular weight is positively and significantly correlated with testicular function (Sofikitis et al., 1992).

The absence of significant difference in serum basal testosterone concentration among the 3 groups does not indicate an absence of differences in total (left and right) Leydig cellular secretory function since basal testosterone concentration in serum is not a reliable marker of the testosterone biosynthesis rate (Steinberger et al., 1973).

Impaired secretory function of Leydig and Sertoli cells on the right side due to a temperature increase in animals with left cryptorchidism will detrimentally affect spermatogenesis and epididymal sperm maturation on the right side. It is known that optimal intratesticular and intraepididymal testosterone (secreted by the Leydig cells) and androgen-binding protein (secreted by the Sertoli cells) concentrations are important for activating and maintaining spermatogenesis and the epididymal sperm maturation process. In fact, a harmful effect of left cryptorchidism on right epididymal caudal sperm content and motility was demonstrated in the current study. Furthermore, the present study reveals a detrimental effect of left cryptorchidism on the epididymal caudal sperm function since the outcome of IVF trials using spermatozoa from the right epididymal cauda was significantly smaller in cryptorchid animals. This decrease in right epididymal caudal sperm function may be attributable to a) the smaller quantitative motility profiles of spermatozoa recovered from the right epididymis and b) the smaller acrosin content of right epididymal caudal spermatozoa in animals with left cryptorchidism as a previous study tends to support (Ono and Sofikitis, 1997). Optimal sperm motility and acrosin content are prerequisites for sperm movement towards the oocyte, acrosomal reaction, and penetration of the zona pellucida by the spermatozoon. Furthermore, considering that there is a defect in the right epididymal function in animals with left cryptorchidism (Ono and Sofikitis, 1997) and that the epididymal sperm maturation process involves a cascade of multiple events influencing several qualitative and quantitative parameters of spermatozoa, the probability that additional sperm characteristics important for the fertilization process are detrimentally affected in the right epididymis in rats with left cryptorchidism can not be excluded.

The detrimental effect of unilateral cryptor-

chidism on contralateral testicular/epididymal function is of great clinical importance. Further studies are necessary to clarify whether this defect in contralateral epididymal sperm fertilizing capacity is progressive. If progressive damage occurs in the normally descended testis/ epididymis of subjects with unilateral cryptorchidism in whom orchidopexy cannot be performed, epididymal sperm aspiration and sperm cryopreservation techniques should be performed on the contralateral side to the cryptorchid testis-side. Such frozen sperm samples may be used in assisted reproduction programs in the future.

The present study additionally indicates that orchidopexy of a cryptorchid testis partially alleviates not only ipsilateral testicular and epididymal function but also contralateral epididymal caudal sperm content, motility and fertilizing capacity. Therefore, orchidopexy of a unilaterally cryptorchid testis should be regarded as a treatment for the contralateral, normally descended testis, as well.

A defect in right epididymal caudal sperm fertilizing capacity is revealed in rats with left cryptorchidism in the current study. This defect may represent a diminished sperm capacity to bind and/or penetrate the zona pellucida. Additional studies are necessary to investigate separately various events after the sperm's entrance into the oocyte (i.e., sperm nucleus decondensation, male pronucleus formation and oocyte activation). Furthermore, the potential for implantation of embryos generated by the fertilization of oocytes with spermatozoa from the right epididymis of rats with left cryptorchidism needs to be evaluated. Such studies are in progress in our laboratory.

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