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A Novel Mechanism to Explain the Detrimental Effect of Left Cryptorchidism on Right Testicular Functions

Kouji Ono and Nikolaos Sofikitis

Department of Urology, Faculty of Medicine, Tottori University, Yonago 683, Japan

The effect of unilateral cryptorchidism on ipsilateral testicular function has been well studied in human and experimental animals. However the effect of unilateral cryptorchidism on contralateral testicular function has remained unclear. To evaluate the effect of left cryptorchidism on right testicular function, an experimental model was created in 2 groups of immature male rats (group A, 19 rats; and group C, 7 rats). Seven male rats of the same age were sham-operated (groups B and D). Twenty-eight days after the operation, bilateral epididymal caudal sperm content and motility, bilateral testicular weight, bilateral epididymal caudal weight, seminal vesicular weight, right testicular versus intra-abdominal temperature difference (RDT), fertility rate, right epididymal caudal sperm acrosin profile, testosterone response to human chorionic gonadotropin stimulation and mean seminiferous tubular diameters of bilateral testes were tested. They were significantly lower in group A than in group B (Student's *t*-test; $P < 0.05$). In contrast, the concentrations of serum luteinizing hormone and follicle stimulating hormone were significantly higher in group A than in group B (Student's *t*-test; $P < 0.05$). Four months after the operation, right testicular blood flow was significantly higher in group C than in group D (Student's *t*-test; $P < 0.05$). These results indicate a bilateral Leydig cell secretory deficiency in rats with unilateral cryptorchidism results in the impaired spermatogenic process and epididymal sperm maturation process. This effect may be explained by the elevation of the right testicular temperature as a result of an increase in testicular blood flow.

Key words: cryptorchidism; fertility; testicular function; testicular blood flow; testicular temperature

Cryptorchidism, which is the most common disorder of sexual development at full-term birth, is often associated with infertility or subfertility and is the major risk factor for testicular cancer (Lipshultz et al.,1976; Chilvers et al., 1984). Since the work of John Hunter in 1756, both clinical and experimental evidences suggest that fertility is impaired in unilateral cryptorchidism (Alpert et al., 1983; David et al., 1992; Seppo et al., 1996).

It is generally accepted that a relatively low temperature is preferable in spermatogenesis in mammalian species. Actually, it has been reported that temperature in the scrotum was 4–5˚C lower than that in the abdomen (Harrison et al., 1949). Waites (1961) measured the temperature of the abdominal aorta and testicular artery at various points, and found that the temperature of the testicular artery was lower than that in the abdominal cavity by 5.2˚C at several points. Thus, a great effect of the intratesticular temperature on spermatogenesis has been demonstrated (David et al., 1981; Nagler et al., 1987). It is well known that the function

Abbreviations: ABP, androgen binding proteins; BAPNA, *N*-a-benzoyl-DL-arginine-*p*-nitroanilide; BWW, Biggers-Whitten-Whittingham; FSH, follicle stimulating hormone; hCG, human chorionic gonadotropin; LH, luteinizing hormone; RDT, right testicular versus intra-abdominal temperature differences; rFSH, rat FSH; STD, seminiferous tubular diameter; TAA, Total acrosin activity

of the cryptorchid testis is impaired by a temperature rise in unilateral cryptorchidism. The effect of unilateral cryptorchidism on ipsilateral testicular function has been well studied in human and experimental animals. However, the effect on contralateral testicular function has not been studied completely (Dariusz et al., 1992).

We hypothesized that the function of the contralateral testis is impaired by temperature rise in unilateral cryptorchidism, and studied the mechanism on an experimental model of the left cryptorchidism in rats.

Materials and Methods

The experiments were performed on 4-week-old immature male Wister rats weighing 80–100 g. The rats were divided into 4 treatment groups: A and C) cryptorchid; B and D) sham-operated. Rats were made left cryptorchid through a left dorsolumbar incision by suturing the left testis to the lower pole of the ipsilateral kidney with 2 interrupted 6-0 nylon sutures under Nembutol (25 mg per kg body weight) anesthesia. Sham operations consisted of a left dorsolumbar incision, exposure and replacement of the left testis, and closure of the incision using the same anesthesia. Before the surgical procedures we evaluated the difference between the right testicular and the intra-abdominal temperatures. Twenty-eight days after the operation, the groups A (n = 19) and B (n = 7) were examined for abdominal temperature, right testicular temperature and fertility. The concentrations of testosterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH) were determined in blood aspirated from the inferior vena cava by a midline abdominal incision. The incision was closed and all the rats received human chorionic gonadotropin (hCG) (1000 units; Mochida Phamceutical Co., Tokyo, Japan) by intraperitoneal injection. Three hours after hCG stimulation, testosterone response was evaluated for blood aspirated from the same vein. Then, all the rats were sacrificed by an injection of saturated potassium chloride into the left venticle. Bilateral epididymal caudal sperm content and motility, bilateral testicular and epididymal weight, seminal vesicular weight were measured. The right epididymal caudal sperm acrosin profile, the presence of bilateral epididymal caudal cytoplasmic droplets, and anti-sperm antibodies were also evaluated, and the bilateral testes were examined histologically. Four months after the operation, the rats of groups C (n = 7) and D (n = 7) were examined to determine testicular blood flow by using the previously described method (Hurt et al., 1986).

Testicular temperature

Bilateral testicular temperature was assessed by percutaneus insertion of a 29-gauge needle probe attached to a digital thermometer (Unique Medical, PTC201 model, Tokyo). Intra-abdominal 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 intra-abdominal and intratesticular temperature was recorded by the method described previously (Sofikitis et al., 1992).

Fertility Assessment

Two fertile female Wistar rats (2 months old) in the 1st h of estrus as determined by vaginal smear examination were placed in a single cage with each male rat. The female rats were checked after mating to detect spermatozoa in their vaginas by microscopic examination of the vaginal fluid. Females in which spermatozoa were detected 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 mating resulted in at least one pregnancy.

Determination of the weight of the testes, epididymides and seminal vesicles

The testes, epididymides and seminal vesicles were excised, dissected free of surrounding tissue, and weighed on a Mettel Basbal scale (Delta Range, Tokyo).

Epididymal sperm content, quantitative sperm motility (%motility), qualitative sperm motility (motility grade) and bilateral epididymal caudal cytoplasmic droplet presense

Each epididymis was separated carefully from its testicle under a magnification of 10, 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) medium adjusted to pH 7.4 at 37˚C with 6-M 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 80–100 μm. Six droplets of the filtrate was used for the sperm count (number of spermatozoa/mL BWW medium) and the average number was calculated. 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 imediately after its preparation, so that the estimate of motility became more accurate. The proportion of motile spermatozoa was counted for all the cells in the chamber. Motility was also graded microscopically and assigned a value from 0 to 4, with 0 representing no movement and 4 representing the maximum perceived motility. Samples for motility evaluation were stored on a warming tray at 35˚C in the viewings. Bilateral epididymal caudal cytoplasmic droplet rates were counted by laser microscope.

Acrosin activity assay

Total acrosin activity (TAA) of the spermatozoa was measured as described by Zaneveld and colleagues (1972). In brief, the spermatozoa were washed free of seminal plasma by centrifugation at 6,000 × *g* for 20 min over Ficoll (0.12 M NaCl, 0.025 M HEPES) to remove the soluble proteinase inhibitors in semen that interfere with acrosin activity. The sperm pellet was subsequently suspended in buffer that had i) detergent, which facilitated disruption of the acrosomes and released the acrosomal enzymes (Triton X-100, Sigma Chemical Co., St. Louis, MO); ii) basic pH, which allowed activation of proacrosin into enzymatically active acrosin; and iii) synthetic arginine amide substrate [*N*-a-benzoyl-DL-arginine-*p*-nitroanilide (BAPNA); Sigma Chemical Co.], which, when hydrolyzed, released a chromophoric product. The total amount of color developed after 3 h incubation was measured spectrophotometrically.

TAA was expressed as μIU/10^6 sperm: 1 IU of acrosin activity was defined as the amount of enzyme that hydrolyzed 1 μmol of BAPNA per minute at 23˚C. A change in absorbance of 9.9 at 410 nm corresponds to the hydrolysis of 1.0 μmol of BAPNA.

Antisperm antibody assay

The immobilization test was done for the antisperm antibody assay as described by Isojima and colleagues (1968). Small volumes of guinea pig complement were added to epididymal sperm solutions, and the mixture was incubated for 30 min before microscopic evaluation. The percentages of motile and non-motile sperm were determined for each serum of cryptorchidism and a negative control. A rat was considered positive for the immobilizing antibodies if the percentage of motile sperm was less than half that of the negative control.

Determination of serum testosterone concentration

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

Table 1. Difference (Δ) between intra-abdominal and right intra-testicular temperature, presence of anti-sperm antibody, and fertility

Group	*n*	Temperature (˚C)		Number of rats	
		Preoperation	Postoperation	Anti-sperm antibody positive	Fertile
A: Cryptorchid	19	4.1 ± 0.4	3.1 ± 0.6*	9*	2*
B: Control	7	4.2 ± 0.3	4.1 ± 0.4	0	7

Values are expressed as mean ± SD.
*$P < 0.05$ compared with the control group.

Determination of serum LH and FSH concentration

Serum levels of LH and follicle stimulating hormone (FSH) were measured with double antibody radio-immunoassay methods. LH was assayed with a rat LH [^{125}I] assay system from Amersham International plc (Amersham, United Kingdom). The intra- and inter-assay coefficients of variation were 6.5 and 7.2%, respectively. The sensitivity of the assay was 0.8 ng/mL. FSH was assayed with a rat FSH (rFSH) [^{125}I] assay system from Amersham International. The intra- and inter-assay coefficients of variation were 4.2 and 7.0%, respectively. The sensitivity of the assay was 0.9 ng/mL.

Histology

Testicular tissue was fixed in Bouin's solution. The 5-μm thick sections were obtained and stained with hematoxylin and eosin. The diameters of the seminiferous tubules were determined by an ocular micrometer. The 10 most circular tubules were measured in each specimen. The mean seminiferous tubular diameter (STD) was calculated in microns for each testis as described previously (Salman et al., 1988).

Testicular blood flow

Rats were anesthetized with sodium pentobarbital (25 mg per kg body weight) and were maintained at a body temperature of approximately 37˚C. Testicular blood flow was determined using strontium 85-labeled microspheres (15 ± 1.1 μm) with a specific activity of 9.0 mCi/g suspended in 10% Dextran containing 0.05% Tween 80. After 5 min of agitation, 0.15 mL of microsphere suspension (40,000–60,000 microspheres) was drawn into a plastic syringe designed for placement in a gamma radiation counting vial, and analyzed in a gamma counter. This suspension was injected into the left ventricular catheter over 20 s and flushed with 1.0 mL saline (0.9%). Blood was withdrawn from the femoral artery catheter using a constant withdrawal sage pump for 10 s before the injection of microspheres, during the injection, and thereafter for a total withdrawal time of 70 s (to 0.8 mL). The blood sample was placed in a preweighed counting vial and withdrawal rate was determined. The rats were killed using a potassium chloride injection into the left ventricle. The right testis was removed, weighed, and analyzed for radioactivity in the gamma counter along with the blood sample and the empty injection syringe as described previously (David et al., 1981). Blood flow (BF) was calculated using the following formula:

$$BF = (R \times T)/B$$

where BF = blood flow in milliliters per minute; R = rate of withdrawal of blood in milliliters per minute; B = number of counts blood sample; T = number of counts in tissue sample. BF was divided by the weight of the right testis to determine flow per gram of tissue.

Statistical analysis

The data were analyzed with Student's *t*-test at 5% level of significance.

Table 2. Weight of the testis, epididymis and seminal vesicle (mg)

Group	*n*	Testis		Epididymis		Seminal Vesicle
		Right	Left	Right	Left	
A: Cryptorchid	19	1003 ± 163*	263 ± 102*	85.9 ± 27.4*	44.7 ± 24.1*	310 ± 130.9*
B: Control	7	1198 ± 81	1193 ± 87	119.3 ± 16.3	133.1 ± 19.2	477.4 ± 78.4

Values are expressed as mean ± SD.
*$P < 0.05$ compared with the control group.

Results

Testicular temperature

Results were significant between right testicular versus intra-abdominal temperature differences (RDT) within the cryptorchid and the control groups (Table 1). The cryptorchidism group showed a significantly smaller RDT value compared with the control group.

Presence of antisperm antibody

The positive reactions of the antisperm antibody were obtained in 9 sera of 19 cryptorchidism rats, but in no sera of control rats (Table 1).

Fertility

The effects on fertility are summarized in Table 1. All the control rats were proved to be fertile. On the other hand, fertile rats in the cryptorchid group were only 2 out of 19 and significantly lower than the controls.

Testicular weight

Left testicular weight was significantly lower than right testicular weight in cryptorchidism rats (Table 2). The right testicular weights in cryptorchidism rats were also significantly lower than in the control group.

Weights of epididymis and seminal vesicles

Left epididymal caudal weight was significantly lower than right epididymal caudal weight in the cryptorchidism group (Table 2). The right epididymal caudal weights in the cryptorchidism group were significantly lower than in the control group. Seminal vesicular weight in the cryptorchidism group was significantly lower than in the control group.

Epididymal sperm content

The sperm content was significantly lower bilaterally in the cryptorchid rats than in the control group (Table 3).

Quantitative and qualitative assessment of sperm motility

The percentage of motile sperm and the motility grade were significantly lower bilaterally in the cryptorchid rats than in the control group (Table 3).

Table 3. Sperm concentrations, motility and forward progresion score from the cauda epididymis

Group	*n*	Concentration ($\times 10^6$/mL)		Motility (%)		Forward progresion (0–4)	
		Right	Left	Right	Left	Right	Left
A: Cryptorchid	19	25.0 ± 16.5*	6.5 ± 8.5*	17.7 ± 16.4*	3.4 ± 7.3*	1.2 ± 0.4*	0.3 ± 0.2*
B: Control	7	47.8 ± 7.1	43.3 ± 6.2	58.4 ± 10.3	61.2 ± 9.1	1.6 ± 0.4	1.4 ± 0.3

Values are expressed as mean ± SD.
*$P < 0.05$ compared with the control group.

Table 4. Right epididymal caudal total sperm acrosin activity (TAA) and cytoplasmic droplet presence (CDP) in the epididymal caudal sperm

Group	*n*	TAA ($\mu IU/10^6$ sperm cells)	CDP (%)	
			Right	Left
A: Cryptorchid	19	31.1 ± 9.1*	41.8 ± 6.2*	70.3 ± 10.2*
B: Control	7	48.4 ± 10.3	16.3 ± 4.2	15.1 ± 4.8

Values are expressed as mean ± SD.
*$P < 0.05$ compared with the control group.

Table 5. Serum hormone levels and testosterone response to hCG stimulation

Group	*n*	LH (ng/mL)	Testosterone (ng/mL)		FSH (ng/mL)
			Pre-hCG	Post-hCG	
A: Cryptorchid	19	17.8 ± 7.4*	1.63 ± 0.89*	4.39 ± 2.1*	0.7 ± 0.2*
B: Control	7	9.3 ± 1.6	1.72 ± 0.34	7.15 ± 0.81	0.3 ± 0.1

Values are expressed as mean ± SD.
*$P < 0.05$ compared with the control group.

Acrosin activity

TAA was significantly lower in the cryptorchid rats than in the control group (Table 4).

Epididymal caudal cytoplasmic droplet presense

The presence of cytoplasmic droplets shows the immature spermatozoa in cauda epididymis. The percentage of epididymal caudal cytoplasmic droplets were significantly higher bilaterally in the cryptorchid rats than in the control group (Table 4).

Leydig cell functions

Serum testosterone levels were slightly lower in the cryptorchid rats than in the control group (Table 5). Serum LH levels were significantly elevated in the cryptorchid rats compared to the control group. Serum hCG-stimulated testosterone levels were significantly lower in the cryptorchid rats than in the control group.

Sertorli cell function

Serum FSH levels are included in this section as a function of Sertoli cells because it may indirectly reflect inhibin production by the testis, which has been shown to be suppressed in cryptorchidism (Au et al., 1983). The cryptorchid rats showed significantly elevated FSH levels compared to the control group (Table 5).

Testicular blood flow

Right testicular blood flow was significantly higher in the cryptorchid rats than in the control group (Table 6).

Table 6. Right testicular blood flow

Group	*n*	Blood flow (mL/min 100 g testis)
C: Cryptorchid	7	43 ± 8*
D: Control	7	32 ± 7

Values are expressed as mean ± SD.
*$P < 0.05$ compared with the control group.

Histology

Testicular histology was evaluated under light microscopy (Fig. 1). There was no difference in the structures of seminiferous tubules between the descended contralateral testes and the control group (data not shown). The seminiferous tubules were completely and fully differentiated and stratified in a control rat testis (Fig.

1A). Spermatozoa was shown in some of the tubules. However, the number of germinal cells were remarkably decreased in the undescended testes in cryptorchid rats (Fig. 1B). The seminiferous tubules were composed of some Sertoli cells and a few spermatogonia. Numerous processes of Sertoli cells and degenerative cells were shown in the lumen. The mean seminif-

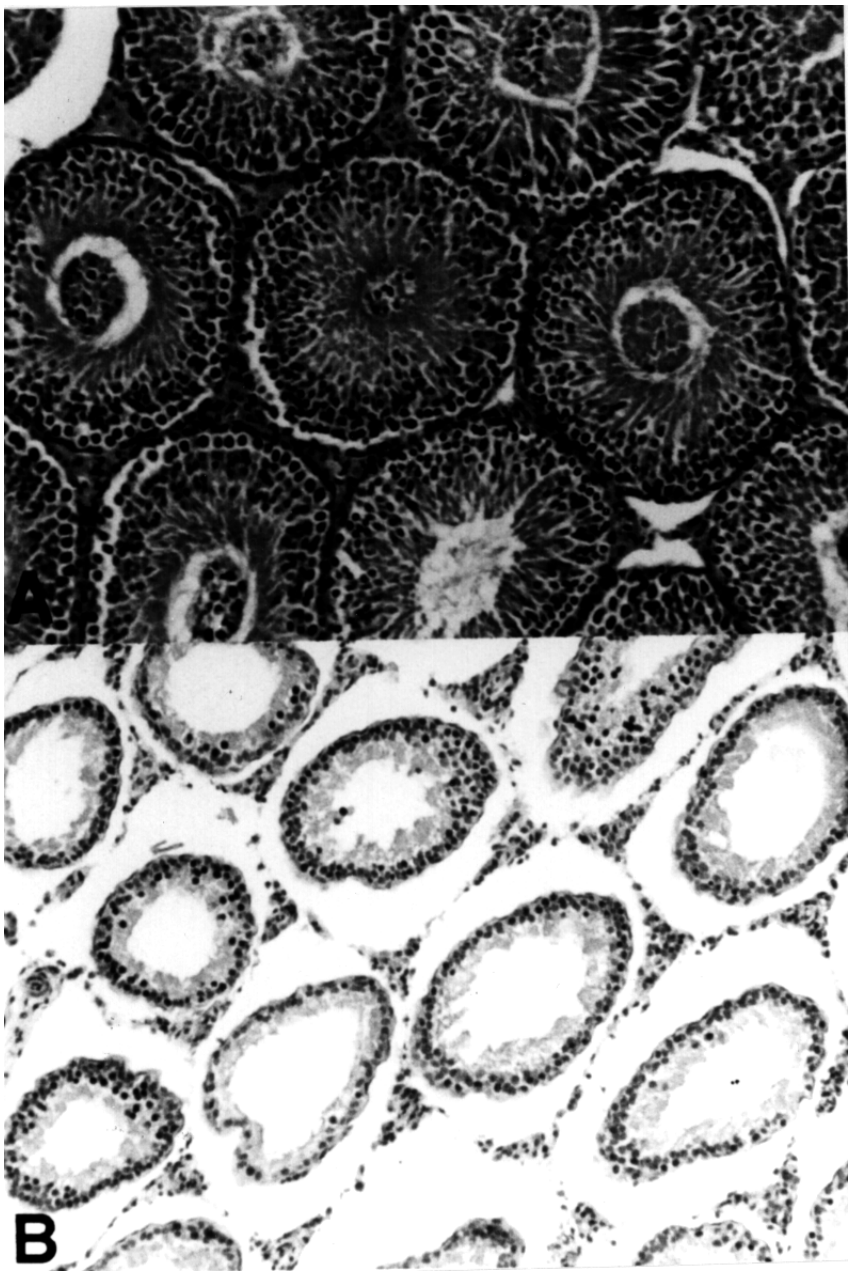

Fig. 1. Light micrograph of an 8-week-old rat testis. **A**: Control. Seminiferous tubule is completely fully differentiated and stratified. Spermatozoa are shown in some of the tubules. ×100. **B**: Left cryptorchid rat left testis. Germinal cells in the undescended testes are remarkably decreased in number. Seminiferous tubules are composed of some Sertoli cells and a few spermatogonia. Numerous processes of Sertoli cells and degenerative cells are shown in the lumen. × 100.

Table 7. Comparative mean seminiferus tubular diameters (μm) of right and left testes

Group	*n*	Right testis	Left testis
A: Cryptorchid	19	230.5 ± 10.5*	120.3 ± 8.9*
B: Control	7	260.4 ± 12.4	258.7 ± 11.5

Values are expressed as mean ± SD.
*$P < 0.05$ compared with the control group.

erous tubular diameters of bilateral testes in the cryptorchid rats were significantly lower than in the control group (Table 7).

Discussion

In many studies on experimental cryptorchidism, 2- or 3-week-old rats have been used (Jegou et al., 1984; David et al., 1992). However, in our preliminary experiments using 3-week-old rats, we found that the testes were movable, endocrine data were not reproducible, and local hemorrhagic necrosis in the testes occurred by injury during the measurement of intra-testicular temperature. Hence, we used 4-week-old rats for our study. Our study demonstrated that cryptorchidism induced in the immature rats before sexual maturation resulted in a significant impairment in all testicular functions, including spermatogenesis, Sertoli cell and Leydig cell functions.

Regarding the seminiferous tubules of the cryptorchid testis, the diameter was significantly shorter than that in the control rats, spermatids were markedly decreased, and complex, thin, long cellular processes of Sertoli cells and a small number of spermatogonia were only observed in the testicular epithelium (Fig. 1); spermatogenesis was also impaired (Table 3). In our present experiment, neither androgen binding proteins (ABP) nor inhibin were measured, but it is well known that Sertoli cells are the target of FSH. Sertoli cells produce ABP and estrogen under the control of FSH, and these products participate in spermatogenesis. At the same time, inhibin which is involved in the feedback mechanism for the regulation of FSH secretion is produced by Sertoli cells. Therefore, the elevation of the blood level of FSH caused by the cryptorchid testis permitted us to speculate on the hypofunction of Sertoli cells (Au et al., 1983). Our data confirms the results of the study of the impairments in spermatogenesis and Sertoli cell function published by Karpe and colleagues (1981). The present data showed that Leydig cell function was impaired by the elevation of serum LH level, the reduction of testosterone level and the much lower testosterone response to hCG stimulation in the immature cryptorchid rats (Table 5). The hyporesponsivity of hCG may be related to a decreased sensibility of the testis to hCG based on the decline of LH receptors expressed on the Leydig cells of cryptorchid rats. The elevated serum LH levels associated with subnormal testosterone levels suggests the existence of a state of compensated Leydig cell failure virtually identical to the adult cryptorchid testis. In this state, elevated levels of LH are required to enable the testicular output of testosterone to be maintained in the normal range (Jegou et al., 1984). And it also suggests that the recovery of serum testosterone levels may be related to the increase of the total number of Leydig cells (Risbringer et al., 1981).

The results in the present study also confirmed that the abdominal versus right intratesticular temperature difference, the testicular weight, the epididymal sperm content, and the motility of epididymal spermatozoa are reduced after 4 weeks of induction of a unilateral left cryptorchidism (Jegou et al., 1984; Hurt et al., 1986). Many investigators have shown that experimental left varicocele leads to a contralateral increase in testicular blood flow (David et al., 1981; Nagler et al., 1987; Turner and Lopez, 1990), increases intratesticular temperature (David et al., 1981; Turner and Lopez, 1990), and decreased spermatogenesis (David et al., 1981; Hurt et al., 1986) or semen quality (Al-Juburi et al., 1979). Increased testicular temperature is probably subsequent to the increase in testicular blood flow (Turner et al., 1993). Increased testicular blood flow is also a possible cause for lower intratesticular testosterone concentration and impairment of spermatogenesis (Nagler et al., 1987). This is the

first report of an increase in contralateral testicular blood flow and temperature on experimental unilateral cryptorchidid rats. The significant decrease in the weights of the epididymis and seminal vesicles in the cryptorchid rats indicates lower levels of androgens in blood, because these glands are androgen dependent (Price and Williams-Ashman, 1961). However, it is compatible with the findings of the Jegou and colleagues (1984) who noted no significant differences in epididymal weight. These differences may be due to the different experimental observation periods or to the difference species of rats used. The significant bilateral decrease in testicular weight in the cryptorchid rats indicates bilateral testicular dysfunction with respect to both spermatogenesis and androgen production, since it has been suggested that testicular size has a direct correlation with the function (Takihara et al., 1987).

The present study strongly supports the hypothesis that the increase in right testicular temperature is associated with the increased testicular blood flow and it may develop the right testicular damage in the rats with left cryptorchidism.

Previous studies have shown significant evidence of a reduction in spermatogenesis in unilateral cryptorchid rats as determined by the caluculation of sperm concentrations in fluid from the tail of the epididymis (Juenemann et al., 1986; David et al., 1992). In the present study, we observed the severe reduction in fertility of unilateral cryptorchid rats. However, the effect of unilateral cryptorchidism on fertility cannot be determined by studying the changes in the standard semen analysis to predict the sperm fertilizing potential (Menkveld et al., 1990). Several assays have gradually been developed to evaluate different functional parameters of the spermatozoa beyond the standard sperm parameters; adenosine triphosphate of spermatozoa (Comhaire et al., 1987), cervical mucus penetration assay (Pretorius et al., 1984), human hemizona attachment assay (Oehninger et al., 1989), zona free hamster egg penetration (Wolf et al., 1983), triple staining (Talbot et al., 1980), spermatozoal acrosin activity and hypoosmotic swelling test (Zaneveld et al., 1990) are the most popular of these tests. Acrosin, a serine proteinase, associated with the sperm acrosome appears to be involved in the acrosome reaction, as well as the ability of spermatozoa to bind to and penetrate the zona pellucida. Acrosin levels in human ejaculates are significantly correlated with their capacity to fertilize oocytes in vitro (Tummon et al., 1991). The present study confirms the earlier finding that there was a positive correlation between acrosin activity and fertility (Felice et al., 1994). Total acrosin activity and fertility were significantly lower in the unilateral cryptorchid rats compared to the controls. It seems that the impairment on fertility is indicative of lower total acrosin activity in the unilateral cryptorchid rats. Antisperm antibody is also known to affect sperm penetration ability and sperm oocyte interaction (Ronald, 1994). It may contribute to reduced fertility in the unilateral cryptorchid rats.

In conclusion, our study suggested that the dysfunction of the Leydig cell was caused by weight loss of the right testis due to temperature rise with the increased blood circulation in the right testis, and the fertility was reduced by impairment of spermatogenesis in the seminiferous tubules and epididymal sperm maturation by the decreased androgen.

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