The Effect of Prepubertal Spermatic Cord Torsion on Subsequent Fertility in Rats

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This study was undertaken to determine the effects of various durations of testicular torsion in prepubertal rats on their subsequent fertility, and to determine whether these effects could be altered by removal of the torsioned testis. Sixty rats (35 days old) were subjected to 720° unilateral spermatic cord torsion for 0, 1, 3, 5, 9, or 12 hours. The torsioned testis was then either detorsioned or removed. At 65 days of age each male was housed with two females for three weeks. Rats undergoing detorsion of the spermatic cord demonstrated a linear decrease in fertility with respect to the duration of torsion ($r = -0.904$). However, all of the animals undergoing unilateral torsion with subsequent orchiectomy were fertile, regardless of the duration of torsion. In addition, the percentage of females impregnated, the number of embryos produced, and the mean embryo size decreased with increasing intervals of torsion ($r = -0.834$ to $r = -0.979$); the sharpest decline occurred between 5 and 9 hours of torsion. All of these parameters were significantly lower ($P < 0.001$ to $P < 0.05$) in the detorsioned group as compared to the orchiectomized group. There was a decrease in seminiferous tubule diameter in the contralateral testis with respect to the duration of torsion ($P < 0.01$). These data indicate that unilateral spermatic cord torsion in young rats significantly reduced their subsequent fertility with respect to duration of the torsion, and that this detrimental effect may be minimized if the damaged testis is removed rather than untwisted and replaced into the scrotum.

Key words: spermatic cord torsion, fertility, rat, testis.

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Spermatic cord torsion presents itself as acute scrotal swelling associated with intense pain. The incidence of spermatic cord torsion in a busy general hospital is about three or four patients per year, with most being adolescent or prepubertal children (Skoglund et al, 1970). Our experience in a large medical center indicates an incidence of 15 to 20 cases per year. Most of the patients were under 16 years of age. The current accepted treatment is to untwist the affected organ in the hope that any damaged testicular tissue will regenerate and contribute to subsequent fertility or at least help to maintain a normal hormonal balance (Moyad et al, 1975; Jhunghunwala et al, 1976). The acute salvage rate of these testes presently stands at approximately 50% (Krarup, 1978). However, 68% of these "saved" testes showed subsequent atrophy at follow-up, even though they were thought viable at the time of surgery. In recent years, many laboratories have shown that unilateral damage adversely affects the contralateral organ (Lipshultz et al, 1976; Krarup, 1978; Bartsch et al, 1980; Urry, 1980; Chakraborty and Jhunjhunwala, 1982; Nagler and deVere White, 1982). Specifically, Chakraborty and Jhunjhunwala (1982) showed spermatid and spermatocyte degeneration in the contralateral testis. Degeneration in the contralateral testis may be due to a substance produced by or released from the damaged organ (Lipshultz et al, 1976). Recently, evidence has been presented indicating that this effect is mediated by an immune response to the damaged testis which, in turn, causes subsequent degeneration of the contralateral testis (Harrison et al, 1981; Lewis Jones et al, 1982). The above studies all indicate subsequent damage to the contralateral testis following prolonged (ie, more than 24 hours) adverse

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conditions (eg, spermatic cord torsion or cryptorchidism) to one testis.

However, in the clinical situation, the patient usually presents with an acute condition, the onset of which has occurred between 1 and 24 hours before being seen by the physician. It is not yet known what duration of spermatic cord torsion warrants surgical detorsion of the affected testis or its removal in order to best preserve the fertility of the patient. Furthermore, this condition often occurs in prepubertal boys, and little information is available on the subsequent fertility of these patients. The purpose of this study was to examine the effects of prepubertal spermatic cord torsion of various durations on the subsequent fertility of male rats.

MATERIALS AND METHODS

Animals

Sixty prepubertal (35-day-old) male and 120 adult female Sprague-Dawley rats were used. The males were obtained at 25 days of age and allowed to acclimatize for ten days. All animals were housed at 21 C with a 12 hour light: 12 hour dark ratio and fed standard pellets (Purina, St. Louis, MO.) and water *ad libitum*.

Spermatic Cord Torsion

The male rats were randomly separated into six groups, each of which was subjected to unilateral spermatic cord torsion for the following periods: 0 (control), 1, 3, 5, 9, or 12 hours. The animals were anesthetized with sodium pentobarbitol (2.4 mg/100 g body weight) and subjected to spermatic cord torsion of 720 degrees on either the left or right side. The testis was replaced into the scrotum and orchiopexed in place both medially and laterally using 6-0 silk suture. Control animals (0 hours) had one testis exposed for 15 minutes and then similarly replaced back into the scrotum with orchiopexy. At the end of the appropriate interval, five animals from each group underwent detorsion (DETOR) of the ipsilateral testes, while the remaining five animals had the ipsilateral testes removed (ORCHI). As the animals at this time were prepubertal rats, they were maintained in our vivarium for 30 days after the surgery until they were 65 days old, so as to attain puberty before the 21-day period of fertility testing.

Fertility and Fecundity Tests

At 65 days of age, each male was housed with two adult female rats for three weeks, thus exposing each male rat to approximately nine female reproductive cycles. At the end of this period, the female rates were sacrificed by ether overdose and examined for gravidity. The number and mean length of the embryos was determined for each female rat. If either mate of the male rat became pregnant, the male rat was considered fertile.

Histological Procedures

Following the mating period the contralateral testis of each male rat was removed, immediately placed in Bouin's fixative, and embedded in paraffin. The sections were stained with hematoxylin and eosin and examined under 100 × magnification. The seminiferous tubular diameter was calculated to within 5 μ for each testis by averaging the diameters of ten round ($\bar{X}$ of height and width ± 20 μ) seminiferous tubules randomly chosen.

Statistical Analyses

Data were analyzed for differences among groups using binomial Chi-square or Student's *t* test. Differences between treatment after spermatic cord torsion (ORCHI vs. DETOR) were analyzed using paired *t* tests, linear regression analysis, and Pearson's product moment correlation.

Results

Ipsilateral Testes

The gross appearance of the testes undergoing spermatic cord torsion was dependent on the period of torsion. After one hour of torsion, the testes showed mild hemorrhagic infarction, while after 12 hours the damage was severe. Intermediate degrees of damage were noted in those testes whose spermatic cords were in torsion for 3 to 9 hours. All contralateral testes appeared grossly normal at the end of the torsion period.

Fertility of Male Rats

A male rat was considered fertile if he impregnated either or both of his mates during the breeding period. The percentage of fertile male rats after unilateral spermatic cord torsion and subsequent detorsion was inversely proportional ($P < 0.01$) to the duration of torsion ($r = -0.992$) (Fig. 1). Torsion for 9- and 12-hour durations significantly depressed ($P < 0.01$) fertility, when compared to the control animals. In contrast, the animals underoing ipsilateral orchiectomy at the end of torsion retained their fertility, irrespective of the duration of spermatic cord torsion. With respect to the percentage of fertile male rats, a paired *t* test showed a significant difference ($P < 0.05$) between the ORCHI and DETOR groups.

Pregnancies

The percentage of female rats impregnated by male rats undergoing unilateral spermatic cord torsion was inversely proportional ($P < 0.01$) to the duration of torsion (ORCHI $r = -0.969$; DETOR

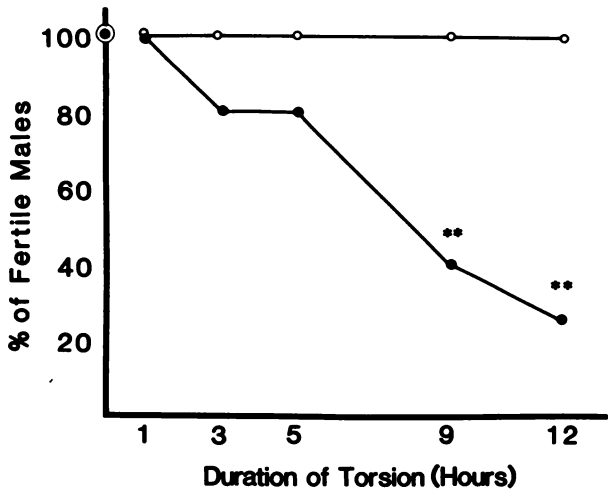

Fig. 1. Percentage of fertile male rats after various durations of unilateral spermatic cord torsion induced before puberty (35 days). The animals underwent ipsilateral orchiectomy ○ or detorsion ● subsequent to torsion for the indicated time period. ⊙ = control animals; ★★ = significantly decreased from control value $P < 0.01$.

r = −0.985) (Fig. 2). Female rats housed with DETOR males undergoing torsion for 9 or 12 hours had a significantly ($P < 0.01$ and $P < 0.001$, respectively) lower percentage of pregnancies when compared to the control animals. The female rats mated with animals of the ORCHI groups, however, had a reduced ($P < 0.01$) pregnancy rate only when mated with animals undergoing 12 hours of torsion. A paired *t* test indicated that significantly ($P < 0.01$) more female rats became impregnated when mated with animals of the ORCHI group.

Fecundity

The mean number of embryos per impregnated female (total of 860 embryos from 84 females) was inversely proportional ($P < 0.01$) to the duration of unilateral testicular torsion to which their mate had been subjected, but only when the damaged testis was untwisted and replaced into the scrotum (r = −0.907) (Fig. 3). The number of embryos per pregnant female of the ORCHI group was independent of the torsion duration (r = −0.060). Using a paired *t* test, the numbers of embryos per pregnant female was significantly ($P < 0.05$) greater when fathered by rats of the ORCHI groups as compared to the DETOR group.

The mean embryo size was determined for those animals mated with rats of the ORCHI and DETOR groups (Fig. 4). Both groups demonstrated a subsequent mean embryo size that was inversely dependent ($P < 0.05$) on the duration of spermatic cord torsion (ORCHI r = −0.823; DETOR r = −0.806). The embryos tended ($P < 0.077$) to be

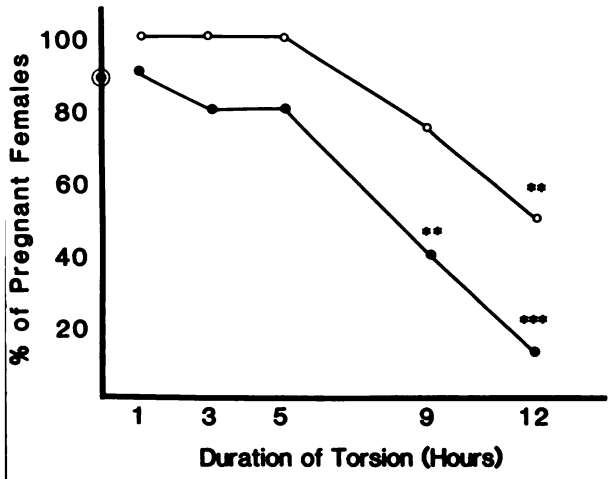

Fig. 2. Percentage of pregnant female rats mated with male animals after various durations of unilateral spermatic cord torsion induced before puberty (35 days). The male rats causing the pregnancies underwent ipsilateral orchiectomy ○ or detorsion ● subsequent to torsion for the indicated time period. ⊙ = control animals; ** = significantly decreased from control value, $P < 0.01$; *** = significantly decreased from control value, $P < 0.001$.

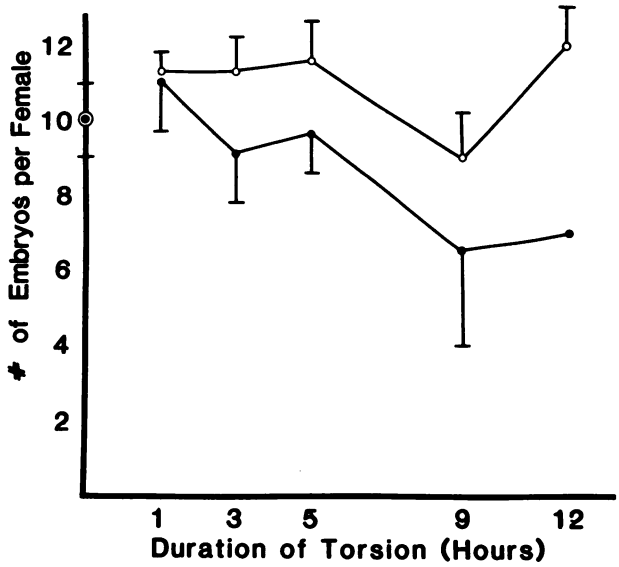

Fig. 3. The number of embryos per pregnant female in rats mated with male animals after various durations of unilateral spermatic cord torsion induced before puberty (35 days). The male rats causing the pregnancies underwent ipsilateral orchiectomy ○ or detorsion ● subsequent to torsion for the indicated time period. ⊙ = control animals; Mean ± SEM. Only one pregnant female resulted from the fertility trials.

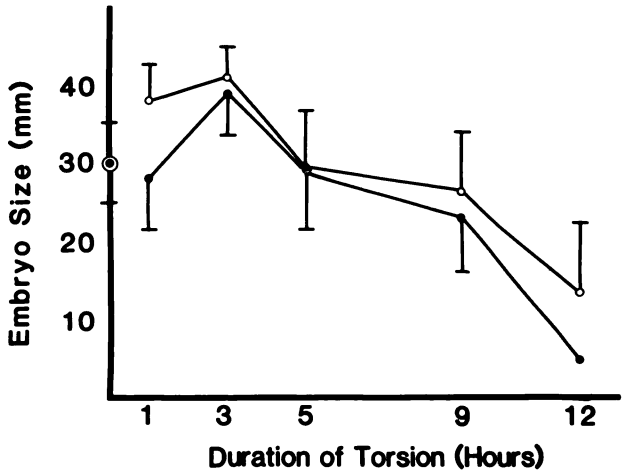

Fig. 4. The mean size of embryos per pregnant female rat mated with male animals after various durations of unilateral spermatic cord torsion induced before puberty (35 days). The male rats causing the pregnancies underwent ipsilateral orchiectomy ○ or detorsion ● subsequent to torsion for the indicated time period. ⊙ = control animals; Mean ± SEM. Only one pregnant female resulted from the fertility trials.

smaller when fathered by male rats of the DETOR group than by males of the ORCHI group.

An overall index of fecundity was obtained by multiplying the number of embryos by their size for each impregnated female and expressing the results as an embryo score (Fig. 5). There was a decreasing embryo score ($P < 0.01$) with respect to the period of torsion to which the male rats were subjected (ORCHI r = −0.871; DETOR r = −0.949). In addition, there was a significantly ($P < 0.01$) lower embryo score for the embryos fathered by animals of the DETOR group as compared to those of the ORCHI group throughout the periods of torsion studied.

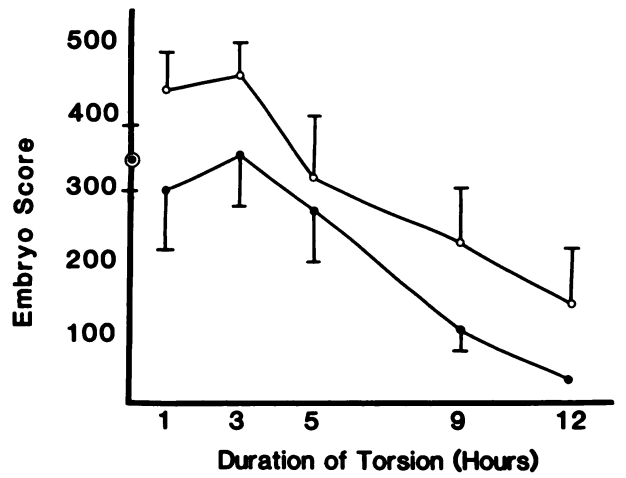

Fig. 5. The embryo score (mean embryo size times number of embryos) of pregnant female rats mated with male animals after various durations of unilateral spermatic cord torsion induced before puberty (35 days). The male rats causing the pregnancies underwent ipsilateral orchiectomy ○ or detorsion ● subsequent to torsion for the indicated time period. ⊙ = control animals; Mean ± SEM. * = significantly decreased from control value, $P < 0.05$. Only one pregnant female resulted from the fertility trials.

Contralateral Testis

Histologic examinations of the contralateral testis of each male rat at the end of the experiment revealed an inverse linear relationship ($P < 0.01$) between the seminiferous tubular diameter and the duration of spermatic cord torsion (r = −0.993), with those of 9 and 12 hours being significantly smaller ($P < 0.05$) than the controls (Fig. 6). However, there was no significant overall difference between the mean seminiferous tubule diameters of the ORCHI and DETOR groups. This analysis was done using a paired *t* test of mean data obtained for each period of torsion. However, the mean seminiferous tubule diameter for the DETOR group undergoing a 12-hour torsion was significantly ($P < 0.01$) smaller when compared to

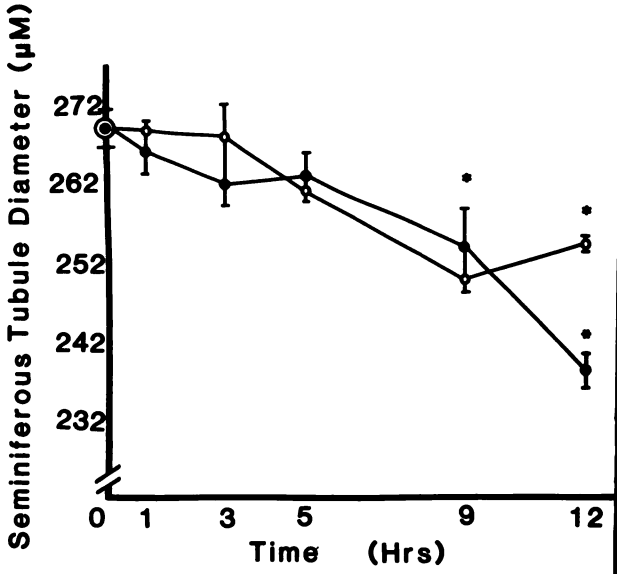

Fig. 6. The seminiferous tubule diameters of the contralateral testes in 86-day-old rats subjected to various durations of unilateral spermatic cord torsion induced before puberty (at 35 days). The animals underwent ipsilateral orchiectomy ○ or detorsion ● subsequent to torsion for the indicated time period. ○ = control animals; Mean ± SEM. * = significantly decreased from control value, $P < 0.05$.

that of the ORCHI group (Student's t test). That is, spermatic cord torsion treatment by orchiectomy preserved the contralateral seminiferous tubular diameter better than detorsion, but only in those animals undergoing torsion for at least 12 hours.

Discussion

Spermatic cord torsion initially occludes the veins but not the arteries, thus resulting in testicular edema and eventual hemorrhagic infarction (Skoglund et al, 1970). The surgical management of spermatic cord torsion is primarily aimed at saving the damaged testis.

Clinically, only the untwisted testis that is grossly nonviable and infarcted is removed. All the rest, and even some that are obviously not viable, are orchiopexed in the hope that the resulting damage may be regenerated or, at least, that a normal hormonal balance can be maintained. However, this conservative management of testicular damage may have very detrimental effects on the long term reproductive capacity of the patient, as evidenced by animal studies indicating that the presence of a chronically twisted spermatic cord (24 hours to six months) adversely affects the contralateral testis (Chakraborty et al, 1980; Chakraborty and Jhunjhunwala, 1982; Nagler and deVere White, 1982). Since this condition is commonly seen in prepubertal and adolescent boys, the present study was done on prepubertal animals before spermatozoa appear in the seminiferous tubules (45 days) (Clermont and Perey, 1957) to assess the development of fertility subsequent to puberty.

Our results indicate the fertility of the males subjected to unilateral spermatic cord torsion was dependent upon the duration of that torsion only if the damaged testis was replaced into the scrotum. The relationship between fertility and duration of torsion did not hold if the damaged testis was removed (Fig. 1). Thus, to best maintain subsequent fertility in a prepubertal rat undergoing unilateral spermatic cord torsion, the affected testis should be removed. Actual mating behavior of the male rats was not recorded; however, reduced copulatory behavior in the animals undergoing torsion for an extended time ($\geq$9 hours), due to a painfully damaged testis, is not likely, since the experimental design of this study allowed 30 days post-torsion recovery. After that, the affected testis was almost completely reabsorbed in every animal undergoing torsion for the longer time periods.

Since the percentage of female rats becoming pregnant was higher in those animals housed with male rats of the ORCHI group as compared to the DETOR animals, it is of interest to note that every male rat in the ORCHI group caused a pregnancy in at least one female. However, even though every male rat of the ORCHI group caused a pregnancy (Fig. 1), those rats having undergone torsion for 12 hours impregnated significantly fewer females ($P < 0.01$) (Fig. 2). Furthermore, since the number of pregnancies occurring was inversely correlated with the duration of spermatic cord torsion to which the male rat was subjected, it is suggested that, at least under the conditions of the present study, the male is not merely fertile or infertile but may demonstrate definite degrees of fertility.

It was assumed that relatively large embryos were indicative of a pregnancy occurring early in the mating periods, while smaller embryos were due to later impregnation. The product of the number and size of embryos per pregnant female rat (ie, embryo score) (Fig. 5) was used as an overall index of fecundity. These data indicate that the male rat appears to be responsible not only for the degree of fertility in the mated pairs (ie, number of pregnant females) but also for the quality of the ensuing pregnancies in terms of the number and size of the embryos produced. Thus, it may be prudent in the field of andrology to be aware of some aspect of male fertility that may affect the quality of the fetus.

Both the ORCHI and DETOR groups underwent spermatic cord torsion for the same durations. The only difference in the two groups during the fertility trials was the presence of a damaged, although untwisted, testicle in the DETOR animals. Thus, it was the presence of the damaged testicle that significantly impeded the function of the contralateral organ. This is consistent with other studies that show that one damaged testis adversely affects the contralateral organ (see introduction). However, it was previously not known how much time was required for this condition to cause a subsequent reduction of fertility in prepubertal animals. The present study indicates that the critical duration of torsion was between 5 and 9 hours. After this time the fertility parameters ex-

amined were found to be significantly lower than the control group.

In assessing the contralateral testis damage histologically, we found that the mean seminiferous tubule diameter was also dependent on the duration of testicular torsion (Fig. 6). However, that there was no difference overall in this parameter between ORCHI and DETOR animals is in sharp contrast to the fertility parameters that showed that unilateral orchiectomy improved fertility. Thus, we conclude that mean seminiferous tubule diameter is, at best, only a crude index of fertility. A recent study by Nagler and deVere White (1982) indicated that contralateral seminiferous tubule diameter was preserved in animals undergoing ipsilateral orchiectomy subsequent to spermatic cord torsion. However, their animals were adults and all underwent torsion for 24 hours. Indeed, after 12 hours, our data (Fig. 6) indicates larger contralateral tubule diameters in the ORCHI animals when compared to the DETOR animals. Currently, a detailed histological examination of both the contralateral and ipsilateral testes after various durations of spermatic cord torsion is underway in our laboratory. The data presented here indicate that the fertility and fecundity of the male rats is dependent upon the duration of unilateral spermatic cord torsion (Fig. 5) and that removal of the affected organ best preserves the fertility and fecundity of the organism when compared to the procedure of untwisting it and placing it back into the scrotum.

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The International Symposium on LHRH and its Analogues: Basic and Clinical Aspects will be held on June 28–30, 1984 at the Holiday Inn Hotel, Ste. Foy, Quebec, Canada. The program will include 11 symposia (from two to seven speakers each) as well as posters. The main topics are the following: Chemistry of LHRH Agonist and Antagonists, Delivery Systems for Controlled Release, Preclinical Studies: Animal Models, Clinical Studies, Male and Female Contraception, Endometriosis, Breast Cancer, Prostate Cancer, Precocious Puberty, Stimulation of Gonadal Functions, and Drug Regulatory Aspects of LHRH Agonists and Antagonists. For further information, contact:

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