Testicular volume, scrotal temperature, and oxidative stress in fertile men with left varicocele

The testicular volume in fertile men with undetermined varicoceles was comparable with men without varicoceles. Elevation of scrotal temperature without an increase of oxidative stress in fertile men with varicoceles indicates a disturbance of the oxidative stress scavenging system in infertile men with varicoceles. (Fertil Steril® 2009;91:1388–91. ©2009 by American Society for Reproductive Medicine.)

Varicoceles are often found in infertile men, but the majority of men with varicoceles do not have significant fertility problems. It is logical to assume that fertile men with varicocele have sufficient fertility potential irrespective of the presence of varicocele. How varicocele has a harmful effect on spermatogenesis in some men is largely unknown. Nagao et al. (1) reported that some fertile men with varicoceles showed disturbed semen parameters and hormonal values, although these were not statistically significant compared with normal fertile men. Pasqualotto et al. (2) found that testicular volume as well as semen parameters and hormone values were similar between fertile men with varicocele and fertile men without varicocele. These results suggest that the clinical parameters in terms of spermatogenesis in fertile men with varicocele are almost normal.

Oxidative stress is one of the factors that cause infertility in men with varicocele (3), and we have shown that the involvement of oxidative stress can impair spermatogenesis in infertile men with varicocele (4, 5). The presence of varicocele in fertile men is not associated with high seminal reactive oxygen species (ROS) levels (6), whereas the fertility potential in infertile patients with varicoceles can decline due to oxidative stress (7). Further investigations of the levels of testicular oxidative stress in fertile men with varicocele are warranted. Our study investigated the pathophysiology of varicocele in fertile men with varicocele.

MATERIALS AND METHODS
The records of consecutive 100 infertile patients with clinical left varicocele (grade 1: 30 men; grade 2: 45 men; and grade 3: 25 men) treated between 1997 and 2004 were evaluated. More than three semen examinations were performed in this study, and all the infertile men with varicocele showed oligozoospermia and/or asthenozoospermia. There were no cases of azoospermia or bilateral varicoceles. We performed endocrinologic examinations and recorded patients’ medical histories to exclude any other causes. This study also involved 100 fertile men with a clinical left varicocele (grade 1: 43 men; grade 2: 35 men; and grade 3: 22 men) who were admitted to our clinic and, after a health screening, required further examination for microscopic hematuria and/or proteinuria, scrotal pain or discomfort, or who had requested a vasectomy.

A consecutive 100 fertile men without varicocele who had visited our multiphasic health screening center or urologic clinic with problems unrelated to the reproductive system or who had requested a vasectomy were included as fertile men without varicoceles. We excluded the fertile men older than 45 years to minimize the age difference with infertile patients.

All the fertile men had had children within 1 to 11 years. Varicocele grading and testicular volume measurements were performed by one of the authors (K.S.). Grade 3 varicoceles were visible to the examiner, grade 2 varicoceles were easily palpable but not visible, and grade 1 varicoceles were palpable with the Valsalva maneuver. Testicular volume measurements were done using a punched-out orchidometer that we had previously developed (8). Scrotal temperature measurements were carried out using Coretemp CTM204 (Terumo, Tokyo, Japan) in the same room, where the ambient temperature was kept constant; the differences in the left scrotum from supine to standing positions were recorded (9). Testicular biopsy specimens were obtained from 30 patients with left varicocele aged 23 to 51 years (range: 30.7 ± 4.5, mean ± standard error of the mean [SE]). Ten biopsy specimens from fertile men without varicocele aged 23 to 40 years (range: 34.5 ± 5.9, mean ± SE) were included as fertile controls (vasectomy: six cases, scrotal hydrocele four cases). After the operations, 12 biopsy specimens had been obtained, with permission, from fertile men with varicocele (five cases of varicocelectomy against pain/discomfort, five cases of vasectomy, and two cases of ureteroscopy). This study was started after approval by the institutional review boards, and all patients provided informed consent before participating.

Western blot analysis for 4-hydroxy-2-nonenal (4-HNE)–modified protein was performed as previously described.
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with (power was found between fertile men with and without varicocele 6.5 years) varicocele. No statistically significant difference a lower mean age (30.7 men with varicocele). Infertile patients with varicocele had right testis (were statistically significantly smaller compared with the men with varicocele, the volumes of the left and right testes

The data were calculated as a percentage of control where the values of the right testis in fertile men without varicocele were assigned a value of 100%. The same control testicular sample was used in every gel to calculate the percentage of control value. All the Western blots were performed twice, and the mean value was calculated for each sample.

The antigens were visualized with an ECL Western blotting detection kit (Amersham Pharmacia Biotech. Piscataway, NJ) and quantified using an image analyzer (Den-sitograph AE-6900M; Atto Co., Tokyo, Japan). The sum of total bands per lane between 247 and 43 kd were calculated. The data were calculated as a percentage of control where the values of the right testis in fertile men without varicocele were assigned a value of 100%. The same control testicular sample was used in every gel to calculate the percentage of control value. All the Western blots were performed twice, and the mean value was calculated for each sample.

The StatMate and Instat 3 programs (GraphPad Software Inc., San Diego, CA) were used for power analysis and statistical analysis, respectively. Data were expressed as mean ± SE. The significance of differences among the groups were evaluated by analysis of variance (ANOVA). Unpaired t and Spearman’s rank tests were performed to examine the difference between right and left testes and the relationship between the generations of 4-HNE–modified proteins and testicular volume, respectively. P < .05 was considered statistically significant. If there was no statistically significant difference between the groups, the power was calculated as 1 – β.

RESULTS

A power analysis indicated that a sample size of 30 patients in each group (90 total) was sufficient to detect a difference or to prove a similarity of 2 mL in testicular volume with an α (significance level) of 0.05. There was no statistically significant difference between the left and right testicular volumes in fertile men without varicocele (21.9 ± 2.9 and 21.5 ± 3.1 mL, respectively; power = 0.99) (Fig. 1A). In fertile men with varicocele, the volumes of the left and right testes were statistically significantly decreased among the groups (P < .01, 16.8 ± 4.0 and 18.4 ± 3.0, respectively). The left testicular volumes with varicocele, irrespective of fertility, were statistically significantly smaller compared with the right testis (P < .05, 20.3 ± 3.1 vs. 21.7 ± 2.9 mL in fertile men with varicocele). Infertile patients with varicocele had a lower mean age (30.7 ± 6.1 years) than the fertile men with (P < .05, 36.1 ± 6.1 years) or without (P < .05, 37.2 ± 6.5 years) varicocele. No statistically significant difference was found between fertile men with and without varicocele (power = 0.99). Fertile men with or without varicocele had the younger children: 5.3 ± 0.6 and 5.9 ± 0.6 years had passed, respectively.

Generation of 4-HNE–modified proteins between the left and right testes in fertile men without varicocele was comparable (100.0 ± 4.9 and 101.2 ± 4.9%, respectively) (see Fig. 1B). Generation in both sides was statistically significantly higher in infertile men with varicocele depending on the grade of varicocele (P < .01). Generation in the left testis was statistically significantly higher than in the right testis in both small varicocele (P = .04, 205.7 ± 12.4 and 170.8 ± 12.1%, respectively) and large varicocele (P = .04, 277.9 ± 15.5 and 212.4 ± 17.1%, respectively). The 4-HNE–modified proteins in the left testis of fertile men with varicocele was statistically significantly increased when compared with the right side (P < .05, 131.2 ± 4.9 and 100.0 ± 4.9%, respectively). There was a statistically significant correlation between the generation of 4-HNE–modified proteins and the testicular volume in both sides, which in turn was statistically significantly correlated in infertile men with varicocele (left: r = −0.45, P = .02; right: r = −0.38; P = .03). However, no statistically significant correlation between testicular volume and the generation of 4-HNE modified proteins was found in fertile men with or without varicocele.

Figure 1C shows the data on scrotal temperature. Scrotal temperature in fertile men without varicocele was −0.2 ± 0.1°C, and statistically significant elevations were observed in both infertile (P < .01, 1.2 ± 0.2°C) and fertile (P < .01, 1.2 ± 0.3°C) men with varicocele, a result we previously reported elsewhere (9).

DISCUSSION

In fertile men with varicocele, the testicular volume and oxidative stress are comparable with those in fertile men without varicocele. On the other hand, the left testicular volume was smaller (see Fig. 1A), and the generation of 4-HNE–modified proteins in the left testis was slightly higher than the right testis (see Fig. 1B) in fertile men with left varicocele, indicating the presence of varicocele-associated effects irrespective of the men’s fertility. As stated in previous reports (1, 2), spermatogenesis in fertile men with left varicocele is not impaired by the varicocele. It is interesting that elevation of scrotal temperature, which many physicians believe is one of the major factors that disturbs spermatogenesis in a testis with varicocele, was obviously observed in fertile men with varicocele. Given that only a small percentage of men with varicocele are infertile, varicocele appears to accelerate spermatogenic injury in combination with other underly- ing factors, which is why we strongly support the idea that varicocele deteriorates the spermatogenesis as a cofactor (10). Excluding the involvement of the other varicocele-associated factor is crucial to confirming this hypothesis.

Another possible explanation for the normal testicular volume, low oxidative stress, and elevation of scrotal
temperature in fertile men with varicocele is the duration that the men have had varicocele, which is difficult to assess. A number of reports support the idea that varicocele exerts a progressive deleterious effect over time, whereas a newly formed varicocele is likely have a minor effect on spermatogenesis. In fact, the incidence of varicocele is found to increase with age (11, 12), resulting in a higher incidence of men with a short period of varicocele exposure. On the other hand, it has been demonstrated that semen quality in men with initially asymptomatic varicocele shows no changes over a long time when compared with controls without varicoceles (13). Although we did not perform semen analyses to confirm the men’s current fertility, an average of 5 to 6 years duration does not seem to cause substantial damage in fertile men with varicocele. The significant alterations in testicular volume and oxidative stress in the left testis (see Fig. 1A and B) may result in a future decline in fertility for the fertile men with varicocele.

Several investigators have substantially disagreed with the efficacy of varicocele treatment in terms of pregnancy rates (14, 15). Based on our results and those of others (3, 10), varicocele may contribute a number of factors that impair the spermatogenesis, and the possible explanations are either that varicocelectomy does not remove the cause of the infertility or that the testicular alterations are irreversible. We assume that success rate of varicocelectomy can be improved in patients with minor testicular damage. In fact, preoperative large testicular volume and nearly normal spermiograms are preferable indicators for varicocelectomy (16, 17). Taken together, the facts that varicocelectomy attenuates the oxidative stress (18) and that presence of oxidative stress is a preferable indicator after varicocelectomy (4) indicate that varicocelectomy will improve the semen parameters of fertile men with varicocele because of the presence of oxidative stress (see Fig. 1B). Predicting the relative contribution of varicocele in each patient will help us to select a preferable candidate for varicocelectomy.

![FIGURE 1](image_url)

**FIGURE 1**
(A) Comparison of testicular volume differential among fertile men without varicocele, infertile patients with varicocele, and fertile patients with varicocele (mean ± SE). Red and blue bars indicate the right and left testes, respectively. *P < .001 among the groups (ANOVA). †P < .01 compared with right testicular volume (paired t-test). (B) Comparison of 4-HNE-modified proteins among fertile men without varicocele, infertile patients with varicocele, and fertile patients with varicocele (mean ± SE). The infertile varicocele group was subdivided by small or large varicocele. *P < .01 compared with 4-HNE-modified proteins in right testis (paired t-test). (C) Elevation of scrotal temperature in fertile men without varicocele, infertile patients with varicocele, and fertile patients with varicocele (mean ± SE). *P < .01 compared with fertile men without varicocele (ANOVA).
There are many hypotheses to account for the observed reduction in left and right testicular volume associated with a left varicocele. Histologic studies from infertile men with left varicocele have shown that both tests were equally injured (19, 20), and the degree of histologic impairment seems to be independent of the clinical stage of the varicocele (21). Using flow cytometric analysis, the percentage of haploid cells in the ipsilateral testes was less than contralateral testes (22). Irrespective of the mechanisms that affect the contralateral testis, our results, for the first time, have shown that left varicocele bilaterally impairs spermatogenesis and that oxidative stress is, at least in part, involved in the process (see Fig. 1B). The presence of left testicular volume loss would have a therapeutic indication, but an unrecognized smaller right testis might lead the physician to erroneously conclude that the volume of the left testis is normal (see Fig. 1A). It is important to note that patients with bilateral testicular volume loss may be at a greater risk of infertility than patients with only left testicular volume loss.

Even if varicocele similarly exists in the infertile and fertile men, we found no statistically significant oxidative stress in the fertile men (see Fig. 1B), which indicates the presence of an increased ROS generating or impaired ROS scavenging system in infertile men with varicocele (23). It is difficult to distinguish which system has a major ROS scavenging system in infertile men with varicocele because 4-HNE–modified proteins as well as thiobarbituric acid-reactive substances and 8-hydroxy-2′-deoxyguanosine are the end products of oxidative stress. Recently, Mostafa et al. (24) indirectly showed decreased levels of antioxidants (i.e., superoxide dismutase, catalase, GPx, and vitamin C) in testes with varicocele using internal spermatic vein blood. We are currently working to elucidate the system against oxidative stress in infertile patients and fertile men with varicocele.

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