Varicoceles affect semen quality of infertile men in Southern China
A cross-sectional study of 5447 cases

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Abstract
The association of varicoceles with infertility is well established, but the exact effect of varicoceles on semen quality among patients with infertility is still poorly known. The study aimed to examine the prevalence of varicoceles among Chinese men with infertility and to examine the factors associated with semen quality.

This was a cross-sectional study of 5447 male patients treated for infertility at the Affiliated Hospital of Guangdong Medical University from October 2012 to December 2015. The patients were divided on the basis of the presence of varicoceles. Examinations of the amount of semen and sperm morphology were performed according to seminal parameter detection methods recommended by the World Health Organization.

Patients with varicoceles (n=1429/5447, 26.2%) were slightly younger (P=.046), and had smaller testes (P=.019), higher frequency of abnormal epididymis (P<.001), slightly shorter infertility duration (P=.046), and lower frequency of smokers (P=.012). There was no difference in the distribution of occupations (P=.777). Using multiple linear regression analysis, varicoceles were shown to be independently associated with semen volume (B=-0.153, 95% confidence interval [95% CI]: -0.245 to -0.062, P=.001), sperm concentration (B=9.633, 95% CI: 7.152–12.114, P<.001), proportion of sperms with normal morphology (B=0.951, 95% CI: 0.623–1.278, P<.001), motility (B=3.835, 95% CI: 2.675, 4.995, P<.001), total sperm count (B=22.481, 95% CI: 13.333–31.629, P<.001), and forward movement sperm count (B=15.553, 95% CI: 9.777–21.329, P<.001). Varicoceles were present in 26% of Chinese male patients with infertility.

Varicoceles were independently associated with sperm volume, sperm concentration, proportion of sperms with normal morphology, motility, total sperm count, and forward movement sperm count.

Abbreviations: B = beta value, CI = confidence interval, SCA = sperm class analyzer, SD = standard deviation.
Keywords: forward motility sperms, male infertility, sperm morphology, varicocele

1. Introduction
Infertility is the inability to conceive after 1 year of unprotected sexual intercourse.[1–4] Male factor infertility alone accounts for about 30% to 50% of the infertile couples.[11] In Beijing (China), the prevalence of couple infertility is around 4.2%.[1] Risk factors for male infertility are many and include obstructive, genetic, endocrine, and occupational risk factors, as well as age, diet, and ejaculatory disorders.[4]

A varicocele is a vascular lesion characterized by dilation of gonadal veins in the scrotum, sometimes described as having a “bag of worms” appearance.[6] Varicoceles are most frequent at the beginning of puberty and are found in 14% to 20% of adolescents and adults.[4,6,6] Varicoceles may lead to elevated scrotal temperature, which impairs spermatogenesis, for which optimal temperature is typically 33°C to 34°C.[7] Varicoceles are easily corrected by surgery, restoring proper scrotal temperature and fertility.[6,8,9]

The prevalence of varicoceles is about 15% to 20% in the general population, but the prevalence is 30% to 40% among patients with infertility.[10] The prevalence of varicoceles increases with age and the risk of varicoceles increases by about 10% for each decade of life.[11,12] Nevertheless, even if the association of varicoceles with infertility is well established, the exact effect of varicoceles on semen quality among patients with infertility is still poorly known and studies are limited by a small sample size.[12,14] In addition, far from all, men with a varicocele are infertile.[14] The incidence of varicocele ranges from 35% to 40% in men with primary infertility, but increases to 80% in men with secondary infertility, suggesting a progressive decline in male fertility.[9,15] A recent European meta-analysis showed that
there is an adverse effect of varicoceles on semen quality among men unselected for fertility status.\textsuperscript{[16]} Nevertheless, data in a Chinese population are lacking.

Therefore, the aim of the present study was to examine the prevalence of varicoceles among Chinese men with infertility and to examine the factors associated with semen quality. The findings of this study provided clear data on the risk factors for poor seminal fluid quality in Chinese males, which could help identify the risk factors that could be modulated to improve semen quality.

2. Methods

2.1. Study design and patients

This was a cross-sectional study of male patients treated for infertility at the Reproductive Medicine Department of the Affiliated Hospital of Guangdong Medical University from October 2012 to December 2015. A total of 8648 patients were initially screened. The inclusion criteria were duration of infertility ≥ 1 year; abstinence of 2 to 7 days; and no fertility drugs in the past 6 months. The exclusion criteria were azoospermia; or incomplete outpatient data. A total of 8648 patients were screened and 5447 were included.

This study was approved by the Reproductive Ethics Committee of the Affiliated Hospital of Guangdong Medical University. All subjects signed the informed consent forms.

2.2. Data collection

A detailed medical history inquiry and physical examination were performed. Semen analysis and microbiological culture of semen were examined. Demographic information and physical examination results of the patients were collected. The patients were divided into 2 groups based on the presence of varicoceles. Occupational hazards were evaluated and classified into 4 groups: high temperature and heat exposure (e.g., cooks and drivers); toxic substance exposure (e.g., building and decoration workers, crews, farmers, hairdressing workers, factory workers, and petroleum workers); computer radiation (e.g., designers and civil service staffs); and no obvious risk factor (e.g., teachers, medical personnel, business personnel, breed personnel, self-employed businessmen, soldiers, and policemen). Abnormal epididymis referred to enlargement of the head or tail of the epididymis.

2.3. Semen collection

The semen was collected after an abstinence of 2 to 7 days. Urine was collected, and the hands and penises were washed using soap. After rinsed off the soap, the hands and penises were dried using new disposable towels. Semen was collected by masturbation and injected into a sterile container. Aseptic incubation was immediately performed for 100 μL of semen in Mycoplasma urealytium and nonspecific bacterial culture. Remaining semen in the container was used to perform semen analysis after liquefaction.

2.4. Seminal parameters

Examinations of the amount of semen and sperm morphology were performed according to seminal parameter detection methods recommended by the World Health Organization.\textsuperscript{[17]} Concentration of sperms (10⁶/mL) and forward sperm motility (%) were measured using a sperm class analyzer (SCA) (Microptic S.L., Barcelona, Spain). Sperm morphology was examined using Papanicolaou staining. Optimized semen (10 μL) was dropped at one end of a slide glass, and the pull-thin technology was used to coat semen on the surface of the slide. After air drying, staining was performed using Papanicolaou staining.\textsuperscript{[17]} Using oil microscopy, 200 sperms were examined repeatedly and the percentage of normal sperm morphology of each semen sample was calculated. The morphological assessment of the sperm was performed strictly according to the WHO Laboratory Manual for the Examination and Processing of Human Semen (5th edition)\textsuperscript{[18]} and 200 sperms were assessed. The parameters of the sperms were determined after 2 examinations.

Sperms included head, neck, connecting piece, tail, and end piece. It is difficult to observe the end piece of sperms through an optical microscope, so it was considered that sperm was constituted by head (and neck) and tail (connecting piece and tail). Only the sperms with normal head and tail were called normal, and all the other sperms were called abnormal. More specifically, according to the classification criteria of abnormal sperm described by the World Health Organization,\textsuperscript{[19]} the abnormalities include head abnormality, including big head, small head, conical head, pear-shaped head, round head, amorphous head, head with vacuole (with 2 or more vacuoles, or the size of unstained vacuole was larger than 20% of the area of the head), vacuole at the postacrosomal region, too big or small acrosome (less than 40% of the head area, or larger than 70% of the head area), 2 heads, or any combination of these abnormalities; neck and middle part abnormality, including asymmetric connecting of the middle part to the head, too thick or irregular, acute-angle bending, abnormally thin middle part, or any combination of these abnormalities; tail abnormality, including short tail, multiple tails, broken tail, hairpin-shaped smooth bending, acute angle bending, irregular thickness, curling, or any combination of these abnormalities; or excessive residual cytoplasm, which is mainly found in sperms from abnormal spermatogenesis; the features of such sperms include containing large volume of irregular stained cytoplasm, of which the volume is over one-third of the head area; defects in the middle part are also very common.

2.5. Statistical analysis

Continuous data were checked using the Kolmogorov–Smirnov test to see if they met the normal distribution. Normally distributed data were presented as mean ± standard deviation (SD) and analyzed with the Student t test. Non-normally distributed data were presented as median (range) and analyzed using the Mann–Whitney U test. Categorical data were presented using frequencies and analyzed with the Chi-square test or the Fisher exact test, as appropriate. Multiple linear regression analyses were used to examine the factors independently associated with the seminal parameters (dependent variables). Results were presented using the B value (the coefficient of the model) and the 95% confidence interval (95% CI) for the B value. Statistical analysis was performed using SPSS 22.0 (IBM, Armonk, NY). Two-sided P < .05 were considered to be statistically significant.

3. Results

3.1. Characteristics of the patients

Table 1 presents the characteristics of the patients. Patients with varicoceles were slightly younger (P = .046), had smaller tests
had a higher frequency of abnormal epididymis $(P < 0.001)$, had a slightly shorter infertility duration $(P = 0.046)$, and had a lower frequency of smokers $(P = 0.012)$. There was no difference in the distribution of the occupations $(P = 0.777)$.

### 3.2. Seminal parameters

Table 2 presents the seminal parameters. The varicocele group showed larger semen volume $(P = 0.01)$, lower sperm concentration $(P < 0.001)$, lower total sperm count $(P < 0.001)$, lower proportion of spermatozoa with normal morphology $(P < 0.001)$, lower total count of normal spermatozoa $(P < 0.001)$, and lower proportion of motile spermatozoa $(P < 0.001)$.

#### 3.3. Multiple linear regression analyses

Table 3 presents the multiple linear regression analyses of factors associated with the seminal parameters. Each semen quality parameter was tested separately with the potential risk factors. Age was independently associated with semen volume $(B = -0.023, 95\% \text{ CI: } -0.031 \text{ to } -0.016, P < 0.001)$, semen concentration $(B = 0.486, 95\% \text{ CI: } 0.277-0.695, P < 0.001)$, proportion of sperms with normal morphology $(B = -0.030, 95\% \text{ CI: } -0.059 \text{ to } -0.002, P = 0.033)$, motility $(B = -0.437, 95\% \text{ CI: } -0.537 \text{ to } -0.338, P < 0.001)$, and forward movement sperm count $(B = -1.064, 95\% \text{ CI: } -1.561 \text{ to } -0.566, P < 0.001)$. Primary/secondary infertility was associated with semen volume $(B = 0.177, 95\% \text{ CI: } 0.089-0.264, P < 0.001)$, proportion

### Table 1

**Characteristics of the patients.**

|                        | All n = 5447 | Varicocele n = 1429 | No varicocele n = 4018 | P          |
|------------------------|--------------|---------------------|------------------------|------------|
| Age, y                 | 31.7 ± 5.4   | 31.4 ± 5.4          | 31.8 ± 5.4             | 0.046      |
| Testis size, mL        |              |                     |                        | 0.19       |
| <3.0                   | 1 (0.0%)     | 0                   | 1 (0.0%)               |            |
| 3.1–8.0                | 372 (6.8%)   | 110 (7.7%)          | 262 (6.5%)             |            |
| 8.1–10.0               | 877 (16.1%)  | 262 (18.3%)         | 615 (15.3%)            |            |
| 10.1–15.0              | 4178 (76.7%) | 1064 (73.8%)        | 3124 (77.8%)           |            |
| >15.0                  | 19 (0.3%)    | 3 (0.2%)            | 16 (0.4%)              |            |
| Epididymis             |              |                     |                        | <0.001     |
| Normal                 | 1574 (28.9%) | 319 (22.3%)         | 1255 (31.2%)           |            |
| Left abnormal          | 1327 (24.4%) | 437 (30.6%)         | 890 (22.2%)            |            |
| Right abnormal         | 444 (8.2%)   | 86 (6.0%)           | 358 (8.9%)             |            |
| Both abnormal          | 2102 (38.6%) | 587 (41.1%)         | 1515 (37.7%)           |            |
| Vas deferens           |              |                     |                        | 0.218      |
| Normal                 | 5437 (99.8%) | 1426 (99.9%)        | 4011 (99.8%)           |            |
| Unilateral impalpable  | 7 (0.1%)     | 1 (0.0%)            | 6 (0.2%)               |            |
| Bilateral impalpable   | 3 (0.1%)     | 2 (0.1%)            | 1 (0.0%)               |            |
| Infertility duration   | 2.33 ± 2.28  | 2.22 ± 2.08         | 2.36 ± 2.35            | 0.046      |
| Etiology               |              |                     |                        | 0.02       |
| Primary                | 3429 (63.0%) | 948 (66.3%)         | 2481 (60.9%)           |            |
| Secondary              | 2018 (37.0%) | 481 (33.7%)         | 1569 (39.1%)           |            |
| Cigarettes             | 3089 (56.7%) | 851 (59.5%)         | 2238 (55.7%)           | 0.12       |
| No                     | 2358 (43.3%) | 578 (40.5%)         | 1780 (44.3%)           |            |
| Smoking duration, y (smokers only) | 231 (9.8%)                         | 63 (9.0%)            | 168 (9.4%) | .434 |
| 5–10                   | 1169 (40.6%) | 290 (50.3%)         | 879 (49.4%)            |            |
| >10                    | 958 (40.6%)  | 224 (38.8%)         | 734 (41.2%)            |            |
| Occupation             |              |                     |                        | 0.738      |
| High temperature and heat exposure | 739 (13.6%)                        | 206 (14.4%)          | 533 (13.3%) |            |
| Toxic substance exposure | 1722 (31.6%)                     | 448 (31.4%)          | 1274 (31.7%) |            |
| Computer radiation exposure | 868 (15.0%)                        | 229 (16.0%)          | 665 (16.6%) |            |
| No obvious risk        | 1637 (30.1%) | 429 (30.0%)         | 1208 (30.1%)           |            |
| Inoccupation           | 481 (8.8%)   | 117 (8.2%)          | 364 (9.1%)             |            |

### Table 2

**Seminal parameters.**

| Parameters               | All, n = 5447 | Varicocele, n = 1429 | No varicocele, n = 4018 | P          |
|-------------------------|--------------|---------------------|------------------------|------------|
| Semen volume, mL        | 3.40 (2.50–4.50) | 3.50 (2.60–3.50)    | 3.30 (2.40–4.40)        | 0.01       |
| Sperm concentration, 10^6/mL| 44.00 (24.20–73.90) | 36.20 (19.70–62.20) | 47.30 (26.18–77.90)     | <0.001     |
| Total sperm count (×10^9) | 147.66 (74.10–253.68) | 127.40 (61.11–219.76) | 156.86 (78.94–264.99)   | <0.001     |
| Normal morphology (%)   | 6.00 (2.00–10.00) | 5.00 (1.50–9.00)    | 6.00 (2.50–10.00)       | <0.001     |
| Forward movement sperm count (×10^9) | 53.77 (20.81–114.81) | 41.68 (15.40–93.12) | 56.98 (23.35–120.42)    | <0.001     |
| Motility (%)            | 38.00 (23.8–52.8) | 32.50 (21.00–49.23) | 39.60 (24.90–53.70)     | <0.001     |
of sperms with normal morphology ($B=0.411$, 95% CI: 0.098–0.752, $P=.010$), motility ($B=2.323$, 95% CI: 1.211–3.434, $P<.001$), total sperm count ($B=17.223$, 95% CI: 8.903–25.543, $P<.001$), and forward movement sperm count ($B=9.488$, 95% CI: 3.971–15.005, $P=.001$).

Occupation was independently associated with sperm concentration ($B=−3.478$, 95% CI: $−5.704$ to $−1.252$, $P=.002$), total sperm count ($B=−10.537$, 95% CI: $−18.748$ to $−2.326$, $P=.012$), and forward movement sperm count ($B=−7.026$, 95% CI: $−12.202$ to $−1.849$, $P=.008$).

Cigarette smoking was independently associated with semen volume ($B=−0.090$, 95% CI: $−0.171$ to $−0.009$, $P=.029$), sperm concentration ($B=−3.294$, 95% CI: $−5.500$ to $−1.088$, $P=.003$), motility ($B=1.384$, 95% CI: 0.355–2.413, $P=.008$), and total sperm count ($B=−13.495$, 95% CI: $−21.597$ to $−5.394$, $P=.001$).

Vas deferens status was independently associated with sperm volume ($B=0.623$, 95% CI: 0.092–1.153, $P=.033$).

Epididymis status was independently associated with sperm concentration ($B=−3.996$, 95% CI: $−6.418$ to $−1.574$, $P=.001$), total sperm count ($B=−12.804$, 95% CI: $−21.676$ to $−3.932$, $P=.005$), and forward movement sperm count ($B=−6.579$, 95% CI: $−12.201$ to $−0.956$, $P=.022$).

Testis size was independently associated with semen volume ($B=0.085$, 95% CI: 0.018–0.153, $P=.013$), sperm concentration ($B=16.380$, 95% CI: 14.749–18.410, $P<.001$), proportion of sperms with normal morphology ($B=0.953$, 95% CI: 0.704–1.202, $P<.001$), motility ($B=4.985$, 95% CI: 4.105–5.866, $P=.008$), total sperm count ($B=58.807$, 95% CI: 52.062–65.553, $P<.001$), and forward movement sperm count ($B=30.686$, 95% CI: 26.322–35.051, $P<.001$).

| Dependent variables | Independent variables | $B$ | Lower limit | Upper limit | $P$ |
|---------------------|-----------------------|-----|-------------|-------------|-----|
| Semen volume        | Age                   | −0.023 | −0.031 | −0.016 | <.001 |
|                     | Primary/secondary      | 0.177 | 0.089 | 0.264 | <.001 |
|                     | Cigarettes             | −0.090 | −0.171 | −0.009 | .029 |
|                     | Testis size            | 0.085 | 0.018 | 0.153 | .013 |
|                     | Vas deferens           | 0.623 | 0.092 | 1.153 | .021 |
|                     | Varicoceles            | −0.153 | −0.245 | −0.062 | .001 |
| Sperm concentration | Age                   | 0.486 | 0.277 | 0.695 | <.001 |
|                     | Infertility years      | −0.543 | −1.036 | −0.050 | .031 |
|                     | Occupation             | −3.478 | −5.704 | −1.252 | .002 |
|                     | Cigarettes             | −3.294 | −5.500 | −1.088 | .003 |
|                     | Testis size            | 16.580 | 14.749 | 18.410 | <.001 |
|                     | Epididymis status      | −5.396 | −6.418 | −1.574 | .001 |
|                     | Varicoceles            | 9.633 | 7.152 | 12.114 | <.001 |
| Normal morphology   | Age                   | −0.030 | −0.059 | −0.002 | .033 |
|                     | Primary/secondary      | 0.411 | 0.098 | 0.725 | .010 |
|                     | Testis size            | 0.053 | 0.704 | 1.202 | <.001 |
|                     | Vas deferens           | 0.951 | 0.623 | 1.278 | <.001 |
| Motility            | Age                   | −0.437 | −0.537 | −0.338 | <.001 |
|                     | Primary/secondary      | 2.323 | 1.211 | 3.434 | <.001 |
|                     | Cigarettes             | 1.384 | 0.355 | 2.413 | .008 |
|                     | Testis size            | 4.985 | 4.105 | 5.866 | <.001 |
|                     | Varicoceles            | 3.835 | 2.675 | 4.995 | <.001 |
| Total sperm count   | Infertility years      | −1.781 | −3.547 | −0.016 | .048 |
|                     | Primary/secondary      | 17.223 | 8.903 | 25.543 | <.001 |
|                     | Occupation             | −10.537 | −18.748 | −2.326 | .012 |
|                     | Cigarettes             | −13.495 | −21.597 | −5.394 | .001 |
|                     | Testis size            | 56.807 | 52.062 | 65.553 | <.001 |
|                     | Epididymis status      | −12.804 | −21.676 | −3.932 | .005 |
|                     | Varicoceles            | 22.481 | 13.333 | 31.629 | <.001 |
| Forward movement sperm count | Age | −1.064 | −1.561 | −0.566 | <.001 |
|                     | Primary/secondary      | 9.488 | 3.971 | 15.005 | .001 |
|                     | Occupation             | −7.026 | −12.202 | −1.849 | .008 |
|                     | Testis size            | 30.686 | 26.322 | 35.051 | <.001 |
|                     | Epididymis status      | −6.579 | −12.201 | −0.956 | .022 |
|                     | Varicoceles            | 15.553 | 9.777 | 21.329 | <.001 |

Age referred to increasing age; testis size referred to larger testis. Primary/secondary, occupation, epididymis status, and varicoceles were categorical variables. 95% CI = 95% confidence interval.
Finally, varicoceles were independently associated with semen volume ($B = -0.153$, $95\% \, CI: -0.245$ to $-0.062$, $P = .001$), sperm concentration ($B = 9.633$, $95\% \, CI: 7.152-12.114$, $P < .001$), proportion of sperms with normal morphology ($B = 0.951$, $95\% \, CI: 0.623-1.278$, $P < .001$), motility ($B = 3.835$, $95\% \, CI: 2.675-4.995$, $P < .001$), total sperm count ($B = 22.481$, $95\% \, CI: 13.333-31.629$, $P < .001$), and forward movement sperm count ($B = 15.553$, $95\% \, CI: 9.777-21.329$, $P < .001$).

4. Discussion

The association of varicoceles with infertility is well established, but the exact effect of varicoceles on semen quality among patients with infertility is still unclear. Therefore, the objective of this study was to examine the prevalence of varicoceles among Chinese men with infertility and to examine the factors associated with semen quality. Results showed that varicoceles were present in 26% of Chinese male patients with infertility. Participants with varicoceles were independently associated with worse sperm concentration, proportion of sperms with normal morphology, motility, total sperm count, and forward movement sperm count. The results of the recent study suggest that varicoceles may have a negative impact on semen quality.

In conclusion, varicoceles were present in 26% of Chinese male patients with infertility. Participants with varicoceles were independently associated with worse sperm concentration, proportion of sperms with normal morphology, motility, total sperm count, and forward movement sperm count. The present study suggests that the prevalence of varicoceles could explain many cases of male infertility, but that other factors such as age and occupational risks have to be taken into account. Nevertheless, many issues remain unanswered and additional studies will have to examine the molecular risk factors (such as oxidative stress) associated with male infertility.

References

[1] Esteves SC, Hamada A, Konday V, et al. What every gynecologist should know about male infertility: an update. Arch Gynecol Obstet 2012;286:217–29.
[2] Hwang K, Walters RC, Lipshultz LI. Contemporary concepts in the evaluation and management of male infertility. Nat Rev Urol 2011;8:86–94.
[3] Practice Committee of the American Society for Reproductive M. Diagnostic evaluation of the infertile male: a committee opinion. Fertil Steril 2015; 103:e18-e23.
[4] Jungwirth A, Fiener T, Fohle GR, et al. Guidelines on Male Infertility. European Association of Urology, Arnhem, The Netherlands 2015.
[5] Zhang H, Wang S, Zhang S, et al. Increasing trend of prevalence of infertility in Beijing. Chin Med J (Engl) 2014;127:691–5.
[6] Tekgul S, Dogan HS, Erdem E, et al. Guidelines on Paediatric Urology. European Association of Urology, Arnhem, The Netherlands: 2015.
[7] Mohammed A, Chinegwundoh F. Testicular varicocele: an overview. J Urol Int 2009;82:373–9.
[8] Practice Committee of the American Society for Reproductive M, Society for Male R, Urology. Report on Varicocele and Infertility: a Committee Opinion. Fertil Steril 2014; 102:1536–1560.
[9] Baazeem A, Belnile E, Ciampi A, et al. Varicocele and male factor infertility treatment: a new meta-analysis and review of the role of varicocele repair. Eur Urol 2011;60:796–808.
[10] Jarow JP. Effects of varicocele on male fertility. Hum Reprod Update 2001;7:59–64.
[11] Leverger U, Gornish M, Gas Y, et al. Is varicocele prevalence increasing with age? Andrologia 2007;39:77–80.
Mori MM, Bertolla RP, Fraietta R, et al. Does varicocele grade determine extent of alteration to spermatogenesis in adolescents? Fertil Steril 2008;90:1769–73.

Lund L, Nielsen KT. Varicocele tests and testicular temperature. Br J Urol 1996;78:113–5.

Marmar JL. The pathophysiology of varicoceles in the light of current molecular and genetic information. Hum Reprod Update 2001;7:461–72.

Sheehan MM, Ramasamy R, Lamb DJ. Molecular mechanisms involved in varicocele-associated infertility. J Assist Reprod Genet 2014;31:521–6.

Damsgaard J, Joensen UN, Carlsen E, et al. Varicocele is associated with impaired semen quality and reproductive hormone levels: a study of 7035 healthy young men from six European countries. Eur Urol 2016;70:1019–29.

Cooper TG, Noonan E, von Eckardstein S, et al. World Health Organization reference values for human semen characteristics. Hum Reprod Update 2010;16:231–45.

World Health Organization. WHO Laboratory Manual for the Examination and Processing of Human Semen. 5th edition. Geneva: World Health Organization; 2010.

Kimura M, Nagao K, Tai T, et al. Age is a significant predictor of early and late improvement in semen parameters after microsurgical varicocele repair. Andrologia 2017;49(3). doi: 10.1111/and.12620. Epub 2016 Jun 1.