

NEGATIVE EFFECTS OF SMOKING ON SEMEN PARAMETERS AFTER VARICOCELECTOMY

Sigara Kullanımının Varikoselektomi Sonrası Semen Parametreleri Üzerine Olumsuz Etkileri

Muhammet Serdar BUĞDAY¹  Ersoy ÖKSÜZ²  Murat DURSUN³  Serhan ÇİMEN⁴ 
Muhammed SULUKAYA⁵  Battal Selçuk ÇAKMAK⁶ 
^{1,2,3,4,5,6}Malatya Turgut Ozal University, Training and Research Hospital, Malatya,

Geliş Tarihi / Received: 02.11.2022

Kabul Tarihi / Accepted: 08.01.2023

ABSTRACT

The negative effects of varicocele and smoking on infertility are known. In our study, we aimed to evaluate the effect of smoking on the healing process after varicocelectomy. 378 male patients who have undergone varicocelectomy operation were included in the study. Patients were divided into three groups as non-smokers, patients, who smoke less than 10 cigarettes per day, and patients who smoke 10 or more cigarettes per day. Semen analysis of the patients before varicocelectomy operation and three months after varicocelectomy operation were evaluated and semen volume, sperm concentration and sperm motility were evaluated according to WHO (World Health Organizations) guidelines. Patient age changed between 16 and 52 and the mean age was 27.20±6.74. When cigarette usage was evaluated, it was determined that 52.82% of patients (n=220) were non-smokers, 13.8% of patients (n=52) smoke less than 10 cigarettes per day and 28.0% of patients (n=106) smoke 10 or more cigarettes per day. Improvement in sperm concentration and motility rates after varicocelectomy was determined to be lower in 10 or more cigarette smoking group compared to other groups. It is shown that cigarettes play a negative role in recovery process after varicocelectomy operation.

Keywords: Infertility, Smoke, Semen parameters, Varicocelectomy, Varicocele.

ÖZ

Varikozel ve sigaranın fertilité üzerindeki olumsuz etkileri bilinmektedir. Çalışmamızda varikoselektomi sonrası sigaranın iyileşme sürecine etkisini değerlendirmeyi amaçladık. Çalışmaya varikoselektomi operasyonu geçirmiş 378 erkek hasta dâhil edildi. Hastalar, sigara içmeyenler, günde 10 adetten az sigara içenler ve günde 10 adet ve üzeri sigara içenler olmak üzere üç gruba ayrıldı. Hastaların semen analizleri varikoselektomi operasyonu öncesi ve varikoselektomi operasyonundan 3 üç ay sonra değerlendirildi ve semen hacmi, sperm konsantrasyonu ve sperm motilitesi WHO (Dünya Sağlık Örgütü) kılavuzlarına göre değerlendirildi. Hasta yaşı 16 ile 52 arasında değişmekte olup, yaş ortalaması 27.20±6.74'tü. Sigara kullanımı değerlendirildiğinde hastaların %52.82'sinin (n=220) sigara içmediği, hastaların %13.8'inin (n=52) günde 10 adetten az sigara içtiği, %28.0'mın (n=106) günde 10 ve üzeri sigara içtiği belirlendi. Varikoselektomi sonrası sperm konsantrasyonu ve motilite oranlarındaki iyileşme 10 ve üzeri sigara içen grupta diğer gruplara göre daha düşük saptandı. Varikoselektomi ameliyatı sonrası iyileşme sürecinde sigaranın olumsuz rol oynadığı gösterilmiştir.

Anahtar kelimeler: Infertilite, Sigara, Semen parametreleri, Varikoselektomi, Varikozel.

INTRODUCTION

Varicocele, which is known as the most common reason for male infertility, is defined as abnormal dilation of spermatic veins. Clinical varicocele is determined to have 15% prevalence in normal male population and 35% prevalence in infertile male population. It is believed that varicocele may cause sperm DNA damage, high apoptosis rate and highly reactive oxygen types and consequently influence semen parameters negatively (Saleh et al., 2003). In many studies, improvement in semen parameters after varicocelectomy was determined (Argawal et al., 2007).

It is known that 30% of males over 15 years old use cigarettes according to the World Health Organization (WHO) data (Saleh, Agarwal, Sharma, Nelson & Thomas, 2002). In this context, many studies focus on parameters like concentration, morphology and mobility when evaluating the relationship between smoking and semen analysis (Rossi, et al., 2011). Smoking is related to subfertility in males and causes low sperm concentration, low sperm motility and low percentage of morphologically normal sperm (Sofikitis et al., 1995; Zinaman, Brown, Selevan & Clegg, 2000). However, some studies suggest that smoking has no effect on semen parameters, moreover some studies suggest that it has positive effects (Lewin, Gonen, Orvieto & Schenker, 1991; Younnus et al., 1998). It is believed that some of the reasons for these contradictions are alcohol consumption, other existing diseases and medical treatments for these diseases and socioeconomic conditions (Harley, Agarwal, Gunes, Shetty & Plessis, 2015). Some of the chemicals like carbon monoxide, hydrocarbons, hydrogen cyanide, which is exposed to individuals due to smoking, are carcinogenic and some play a role in infertility (Pappas, Fresquez, Martone & Watson, 2014).

In this study, we have aimed to demonstrate the negative effect of smoking on semen parameters after varicocelectomy operation done due to male infertility.

MATERIAL AND METHOD

Ethical Approval

Ethical approval for the study was granted by the Inonu University Clinical Research Ethics Committee (Decision no:2019/08).

Study Population

The study was conducted with 378 male patients who have undergone varicocelectomy operation in Malatya Turgut Ozal University Training Research Hospital Urology Clinic. Patients who were diagnosed with varicocele between January 2016 and August 2019 via

physical examination and imaging methods (scrotal doppler ultrasonography), who have their semen samples analysed and consequently undergone varicocelelectomy operation were retrospectively scanned from the hospital database. The decision to operate was made in case of two abnormal semen analyzes. Follow-up was done with single analysis after three months. Obstructive pathology was investigated in all patients with a semen volume less than 1.5 ml, and patients with pathology were excluded from the study. Patients with additional diseases, alcohol consumption and additional drug usage were excluded from the study in order to maintain the study quality. Patients who were undergone open inguinal varicocelelectomy operation were included in the study. Patients were divided into three groups: non-smokers, patients who smoke less than 10 cigarettes per day and patients who smoke 10 or more cigarettes per day. All participants who smoke in our study stated that they had a smoking habit of 1 year or more. While it was not questioned whether there was someone else smoking in their house in the smoking groups, it was stated that the non-smoker group did not have a habit of smoking in the people they lived with. Semen samples were taken by masturbation after 2-4 days of sexual abstinence. Afterwards, semen volume, sperm concentration and sperm motility were reported according to WHO (World Health Organizations) guidelines in mL, $n \times 10^6$ /ejaculate and %.

Statistical Analysis

NCSS (Number Cruncher Statistical System) 2007 program was used for statistical analysis (Kaysville, Utah, USA). Descriptive statistical methods (mean, standard deviation, median, frequency, ratio, minimum, maximum) were used to evaluate study data. Compatibility of quantitative data to normal distribution was examined via Shapiro-Wilk test and graphical evaluation. Mann Whitney U test was used in dual comparisons of variables without normal distribution. Kruskal Wallis test was used for three or more group comparisons and Bonferroni-Dunn test was used for dual comparisons of variables without normal distribution. Wilcoxon Signed Rans test was used in preop and postop comparison of variables without normal distribution. Significance level was $p < 0.05$ in evaluation.

RESULTS

Patient age changes between 16 and 52 and the mean age was 27.20 ± 6.74 (Table 1). When cigarette usage was evaluated, it was determined that 52.82% of patients ($n=220$) were non-smokers, 13.8% of patients ($n=52$) smoke less than 10 cigarettes per day and 28.0% of patients

(n=106) smoke 10 or more cigarettes per day. Statistically significant difference in age distribution of groups was not found ($p>0.05$).

The increase in postop semen volume compared to preop semen volume in non-smoking group was found to be statistically significant ($p=0.001$; $p<0.01$). The difference in postop semen volume compared to preop semen volume in group smoking more than 10 cigarettes per day was found to be statistically insignificant ($p>0.05$). The difference in postop semen volume compared to preop semen volume in group smoking 10 or more cigarettes per day was found to be statistically insignificant ($p>0.05$) (Table 2).

There was no statistically significant difference in preop sperm concentration measurements in different groups in terms of cigarette usage ($p>0.05$). A statistically significant difference in postop sperm concentration measurements in different groups in terms of cigarette usage was found ($p=0.001$; $p<0.01$). After dual comparisons to find out which group causes the statistically significant difference, the measurements of the group smoking 10 or more cigarette per day was determined to be lower compared to non-smoking group ($p=0.001$) and group smoking 10 or less cigarettes per day ($p=0.037$) ($p>0.05$). The increase in postop sperm concentration compared to preop evaluation was determined to be statistically significant in non-smoking group ($p=0.001$; $p<0.01$). The increase in postop sperm concentration compared to preop evaluation was determined to be statistically significant in the group smoking less than 10 cigarettes per day ($p=0.001$; $p<0.01$). A statistically significant difference was determined between groups in terms of change in postop sperm concentration compared to preop ($p=0.001$; $p<0.01$). It was determined that the increase in group smoking 10 or more cigarettes per day was smaller compared to non-smoking group ($p=0.001$) and group smoking less than 10 cigarettes per day ($p=0.005$) ($p<0.01$) (Table 2).

A statistically significant difference was determined in postop motility rates when cigarette usage was considered ($p=0.001$; $p<0.01$). After dual comparisons to find out which group causes the statistically significant difference, the measurements of group smoking 10 or more cigarettes per day was determined to be lower compared to non-smoking group ($p=0.001$) and the group smoking less than 10 cigarettes per day ($p=0.007$) ($p<0.01$).

The increase in postop motility rates compared to preop evaluation was determined to be statistically significant in non-smoking group ($p=0.001$; $p<0.01$). In evaluations regarding postop motility rate changes compared to preop evaluation, a statistically significant difference was determined between groups ($p=0.001$; $p<0.01$). After dual comparisons to find out which group causes the statistically significant difference, the increase in the group smoking 10 or more cigarettes per day was determined to be lower compared to non-smoking group ($p=0.001$)

and the group smoking 10 or less cigarettes per day ($p=0.034$) ($p<0.05$). The increase in group smoking less than 10 cigarettes per day was determined to be lower than non-smoking group ($p=0.001$; $p<0.01$) (Table 2).

Table 1. Distribution of Descriptive Properties

Smoking condition	n (%)	
	Non-smoker	
	<10 cigarettes/day	220 (58.2)
	≥10 cigarettes/day	
	52 (13.8)	106 (28.0)

Table 2. Evaluations Regarding Smoking Condition

		Smoking condition			p
		Non-smoker (n=220)	<10 cigarettes/day (n=52)	≥10 cigarettes/day (n=106)	
Age (year)	Min-Max (Median)	16-52 (26)	17-47 (24.5)	17-47 (26)	*0.671
	Mean±Sd	27.27±6.52	26.67±7.13	27.32±7.04	
	≤25 years	99 (45.0)	28 (53.8)	52 (49.1)	
	>25 years	121 (55.0)	24 (46.2)	54 (50.9)	
Preop volüme	semen				*0.983
	Min-Max (Median)	0.5-7 (3)	0.5-8 (3)	0-6 (3)	
	Mean±Sd	2.61±1.13	2.64±1.23	2.57±1.05	
Postop volüme	semen				*0.137
	Min-Max (Median)	0.5-8 (3)	2-10 (3)	0-5 (3)	
	Mean±Sd	2.94±0.96	2.96±1.25	2.67±1.04	
	^c p	0.001**	0.101	0.161	
Difference (Postop-Preop)	Min-Max (Median)	-4-3 (0)	-2-6 (0)	-2-2 (0)	*0.067
	Mean±Sd	0.33±1.05	0.32±1.24	0.10±0.81	
Preop concentration/ml	Min-Max (Median)	0-348 (45)	0-316 (43.5)	0-297 (32)	*0.137
	Mean±Sd	69.39±71.14	66.52±76.27	57.57±66.29	
Postop concentration/ml	Min-Max (Median)	0-386 (65)	0-312 (67)	0-316 (36)	*0.001 **
	Mean±Sd	91.71±77.07	94.20±87.05	62.60±69.81	
	^c p	0.001**	0.001**	0.333	
Difference (Postop-Preop)	Min-Max (Median)	-216-284 (11)	-297-230 (13.2)	-174-250 (0)	*0.001 **
	Mean±Sd	22.32±63.85	27.69±74.02	5.03±57.64	
Preop motility%	sperm				*0.104
	Min-Max (Median)	0-85 (35)	0-90 (40)	0-90 (35)	
	Mean±Sd	36.11±17.35	42.00±19.32	38.81±20.47	
Postop motility%	sperm				*0.001 **
	Min-Max (Median)	0-104 (55)	0-88 (52.5)	0-90 (40)	
	Mean±Sd	56.41±19.91	50.96±19.36	40.60±21.19	
	^c p	0.001**	0.001**	0.278	
Difference (Postop-Preop)	Min-Max (Median)	-35-70 (20)	-50-40 (10)	-40-45 (0)	*0.001 **
	Mean±Sd	20.30±18.39	8.96±18.68	1.79±16.67	

^aKruskal Wallis Test^bMann Whitney U Test^cWilcoxon Signed Ranks Test

**p<0.01

DISCUSSION

The relation of vericocele, which is defined as the dilation of pampiniform plexus veins, with infertility depends on many mechanisms like testicular heat increase, hormonal function disorder, venous pressure increase, autoimmunity, acrosomal reaction disorders, increase of oxidative stress (OS) and reactive oxygen species (ROS), and the related DNA damage (Fujisawa, Yoshida, Matsumoto, Kojima & Kamidono, 1988). The most studied and accepted mechanism is testicular heat increase. Additionally, it is known that heat increase induces androgen deprivation and consequently apoptosis. In similar studies, it is shown that hypoxia in testicular tissue due to vericocele induces angiogenesis by causing the secretion of some growth factors like “Hypoxia Inducible Factor-1” (HIF-1), “Vascular endothelial Growth Factor” (VEGF) in testicular tissue (Kılınç et al., 2004). At the same time, it is determined in several studies that vericocele may be progressive and may cause structural and functional damage in testes (Witt & Lipshultz, 1993). In a similar study, Lenzi et al. determined significantly different testes volume and semen quality between an adolescent group with not operated vericocele, an adolescent group who have undergone varicocelectomy and an adolescent control group (Kılınç et al, 1998). In a meta-analysis conducted in recent years it is shown that a recovery in semen parameters is observed after varicocelectomy. In another study, an improvement in sperm DNA damage was determined after varicocelectomy operation (Zini & Dohle, 2011).

The prevalence of infertility is approximately 10% worldwide (Davar, Sekhvat & Naserzadeh, 2012). Many studies determined negative effects of smoking on fertility, especially it is shown that it has a negative effect on sperm production, motility and morphology when the effects on male factors are considered (Mak et al., 2000; Trummer, Habermann, Haas & Pummer, 2002). In some studies, it is shown that cigarettes may decrease sperm mitochondrial activity and damage chromatin structure of sperm with harmful chemical compounds it contains (Calogero et al., 2009). In similar studies, it is shown that cigarette usage decreases sperm creatine kinase activity and consequently causes a disruption in sperm motility (Ghaffari & Rostami, 2013). In another study conducted by Ramlau-hansen et al., it is shown that sperm concentration, total sperm concentration and sperm motility decreases in a significant rate for smokers (Ramlau-Hansen et al., 2007).

In a retrospective study conducted in 2009 on patients with vericocele by Collodel et al., it is reported that smoking more than 10 cigarettes per day has a negative effect on sperm motility (Collodel et al., 2009) In a meta-analysis published by Vine et al., it is shown that sperm

concentration is lower in smokers compared to non-smokers and this impact is especially apparent in individuals smoking 10 or more cigarettes per day (Vine, Margolin, Morrison & Hulka, 1994). However, in a study conducted by Tazarek et al. on 27 smokers, 75 non-smoker infertile patients, it is reported that there is not any significant difference in sperm concentration and morphology and only a statistical difference in sperm motility is existent (Pawelczyk & Jedrzejczak, 1995; Tazarek, Depa- Martynów, Derwich).

In a study conducted in 2012, it is shown that smoking and the existence of vericocele induce DNA damage together via oxidative stress in spermatozoa (Fariello et al., 2012). In a similar study comparing patients with vericocele and patients who smoke, an increase seminal plasma and cadmium rate on testicular level is determined and in experimental studies, it is shown that the accumulated cadmium causes testicular damage and sperm function disorder (Hew, Erickson & Welsh, 1993; Benoff et al., 1997). These studies present an opportunity to better understand the synergistic effect of smoking and vericocele.

In another study, it is determined that although there is an improvement in all values of both groups who have undergone varicocelectomy, compared to preop results, the improvement in smoking group was not statistically significant. This result supports the synergistic effect of smoking and vericocele reported by the study (Fariello et al., 2012).

All of these studies are related to negative effects caused by smoking, vericocele or both smoking and vericocele together. When literature is considered, the effects of smoking on positive effects of varicocelectomy on semen parameters is not researched. We have researched the effects of varicocelectomy on semen parameters and effects of smoking on recovery period after operation by looking at the semen parameters before and after varicocelectomy operation. This study is valuable due to its contribution to the literature.

Even though we have observed an improvement in all parameters for all of the 3 groups after varicocelectomy, which is compatible with literature, this improvement was on a higher level on non-smoking group and the group smoking less than 10 cigarettes per day, especially in sperm concentration and motility.

Dikshit et al. claimed that the number of patients in studies related to the effects of smoking on semen parameters was low and that there is no relation between smoking and sperm quality. When the number of patients in our study is compared to the studies cited by Dikshit et al., it is observed that patient numbers of our study is sufficient (Dikshit, Buch & Mansuri, 1987).

In this study, a statistical difference in preop values between groups was not determined. This fact increases the statistical value of the significant improvement in postop semen analysis of non-smoking group and the group smoking less than 10 cigarettes per day.

Many factors such as the laboratory where semen analysed, the method and time of semen sample taking, existing diseases, used drugs, smoking and alcohol consumption may effect semen volume, concentration, motility and morphology which are the most examined parameters in male infertility. There are many studies showing the negative effects of alcohol and narcotic substances on semen parameters (Baska et al., 2008). The aforementioned factors may create contradictions in many studies on smoking (Harlev et al., 2015). We have done the procedures in the direction of the same standard in order to eliminate these factors, which may cause contradictions. We have excluded patients with additional diseases, drug usage and alcohol etc. usage from the study. Additionally, the fact that there is no significant difference in terms of patient age ensured that the data is more reliable. However, the fact that semen morphology could not be evaluated due to the technical difficulties at the time, may be considered as a weakness in our study.

CONCLUSION

It is determined that groups smoking lightly and heavily is affected negatively in sperm concentration and motility rates in third month control semen analysis of patients who have undergone varicocelectomy and it is shown that smoking plays a negative role in recovery process after varicocelectomy operation. In order to increase operation success of patients with vericocele, smoking situation of patients should be examined and they should be advised to quit smoking.

REFERENCES

- Argawal, A., Deepinder, F., Cocuzza, M., Agarwal, R., Short R. A., Sabanegh, E., ...Sabanegh, E. (2007). Efficacy of varicocelectomy in improving semen parameters: new meta-analytical approach. *Urology*, 70(3), 532-538.
- Baska, K. M., Manandhar, G., Feng, D., Agca, Y., Tengowski, M. W., Sutovsky, M., ...Sutovsky, P. (2008). Mechanism of extracellular ubiquitination in the mammalian epididymis. *J Cell Physiol*, 215(3), 684-696.
- Benoff, S., Hurley, I., Barcia, M., Mandel, F. S., Cooper, G. W. & Hershlag, A.(1997). A potential role for cadmium in the etiology of varicocele-associated infertility. *Fertil Steril*, 67, 336-347.
- Calogero, A., Polosa, R., Perdichizzi, A., Guarino, F., La Vignera, S., Scarfia, A., ...Vicari, E.(2009). Cigarette smoke extract immobilizes human spermatozoa and induces sperm apoptosis. *Reprod Biomed Online*, 19(4), 564-571.

- Collodel, G., Capitani, S., Iacoponi, F., Federico, M.G., Pascarelli, N. A. & Moretti, E. (2009). Retrospective assessment of potential negative synergistic effects of varicocele and tobacco use on ultrastructural sperm morphology. *Urology*, 74(4), 794–799.
- Davar, R., Sekhavat, L. & Naserzadeh, N. (2012). Semen parameters of non-infertile smoker and non-smoker men. *J Med Life*, 5(4), 465-468.
- Dikshit, R. K., Buch, J. G. & Mansuri, S. M. (1987). Effect of tobacco consumption on semen quality of a population of hypofertile males. *Fertil Steril*, 48, 334–336.
- Fariello, R. M., Pariz, J. R., Spaine, D. M., Gozzo, F. C., Pilau, E. J., Fraietta, R., ...Cedenho, A. P. (2012). Effect of smoking on the functional aspects of sperm and seminal plasma protein profiles in patients with varicocele. *Hum Reprod*, 27(11), 3140–3149.
- Fujisawa, M., Yoshida, S., Matsumoto O., Kojima, K. & Kamidono, S. (1988). Deoxyribonucleic acid polymerase activity in the testes of infertile men with varicocele. *Fertil Steril*, 50, 795-800.
- Ghaffari, M. A. & Rostami, M. (2013). The effect of cigarette smoking on human sperm creatine kinase activity: as an ATP buffering system in sperm. *Int J Fertil Steril*, 6(4), 258-265.
- Harlev, A., Agarwal, A., Gunes, S., Shetty, A. & Plessis, S. (2015). Smoking and male infertility: An evidence-based review. *World J Mens Health*, 33(3), 143–160.
- Hew, K. W., Erickson, W. A. & Welsh, M. J. (1993). A single low cadmium dose causes failure of spermatogenesis in the rat. *Toxicol Appl Pharmacol*, 121, 15-21.
- Kılınç, F., Kayaselçuk, F., Aygün, C., Güvel, S., Eğilmez, T. & Özkardes, H. (2004). Experimental varicocele induces hypoxia inducible factor-1, vascular endothelial growth factor expression and angiogenesis in the rat testis. *J Urol*, 172, 1188-1191.
- Lenzi, A., Gandini, L., Bagolan, P., Nahum, A. & Dondero, F. (1998). Sperm parameters after early left varicocele treatment. *Fertil Steril*, 69, 347–349.
- Lewin, A., Gonen, O., Orvieto, R. & Schenker, J. G. (1991). Effect of smoking on concentration, motility and zona-free hamster test on human sperm. *Arch Androl*, 27, 51–54.
- Mak, V., Jarvi K., Buckspan, M., Freeman, M., Hechter, S. & Zini, A. (2000). Smoking is associated with the retention of cytoplasm by human spermatozoa. *Urology*, 56, 463–466.
- Pappas, R. S., Fresquez, M. R., Martone, N. & Watson, C. H. (2014). Toxic metal concentrations in mainstream smoke from cigarettes available in the USA. *J Anal Toxicol*, 38(4), 204-211.
- Ramlau-Hansen, C. H., Thulstrup, A. M., Aggerholm, A. S., Jensen, M. S., Toft, G. & Bonde, J. P. (2007). Is smoking a risk factor for decreased semen quality? A cross-sectional analysis. *Hum Reprod*, 22, 188–196.
- Rossi, B. V., Berry K. F., Hornstein, M. D., Cramer, D. W., Ehrlich, S. & Missmer, S. A. (2011). Effect of alcohol consumption on in vitro fertilization. *Obstet Gynecol*, 117(1), 136-142.
- Saleh R. A., Agarwal A., Sharma R. K., Nelson D. R. & Thomas A. J. (2002). Effect of cigarette smoking on levels of seminal oxidative stress in infertile men: a prospective study. *Fertil Steril*, 78, 491–499.
- Saleh R. A., Agarwal A., Sharma R. K., Said T. M., Sikka S. C. & Thomas A. J. (2003). Evaluation of nuclear DNA damage in spermatozoa from infertile men with varicocele. *Fertil Steril*, 80, 1431-1436.
- Sofikitis, N., Miyagawa, I., Dimitriadis, D., Zavos, P., Sikka, S. & Hellstrom, W. (1995). Effects of smoking on testicular function, semen quality and sperm fertilizing capacity. *J Urol*, 154, 1030–1034.
- Taszarek, H. G., Depa- Martynów, M., Derwich, K., Pawelczyk, L. & Jedrzejczak, P. (2005). The influence of cigarette smoking on sperm quality of male smokers and nonsmokers in infertile couples. *Przegl Lek*, 62(10), 978-981.

Trummer, H., Habermann, H., Haas, J. & Pummer, K. (2002). The impact of cigarette smoking on human semen parameters and hormones. *Hum Reprod*, 17, 1554–1559.

Vine, M. F., Margolin, B. H., Morrison, H. I. & Hulka, B. S. (1994). Cigarette smoking and sperm density: a meta-analysis. *Fertil Steril*, 61, 35–43.

Witt, M. A. & Lipshultz, L. I. (1993) Varicocele: a progressive or static lesion? *Urology*, 42, 541–543.

Zinaman, M. J., Brown, C. C., Selevan, S. G. & Clegg, E. D. (2000). Semen quality and human fertility: a prospective study with healthy couples. *J Androl*, 21, 145–153.

Zini, A. & Dohle, G. (2011). Are varicoceles associated with increased deoxyribonucleic acid fragmentation? *Fertil Steril*, 96(6), 1283-1287.