Factors influencing quality of semen : a two year prospective study

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Abstract: In the recent years, male infertility and subfertility has increased, which is attributed to many factors. So our study focuses the effects of age, occupation, smoking, alcohol and varicocele on the semen quality. Detailed history and examination of 93 cases (fulfilled inclusion criterion) was done. Semen analysis of these cases were compared with above parameters using statistical tools like mean, standard deviation, standard error of mean and significance was tested by student's 't' test. The mean sperm density, total motility and rapid progressive motility in control group were 68.95 x10^6/ml, 59.9% and 30.5% respectively. Reduction of sperm density was statistically significant (p value < 0.05) in both tobacco users+ alcoholics and in varicocele patients in comparison with controls. Age and occupation did not alter semen quality significantly. Our study concluded that semen quality is decreasing in the past few decades and combined tobacco+ alcohol use, and varicocele have more detrimental effect on semen quality.

Key Words: semen parameters, age, tobacco use, alcohol, occupation

Indian J Pathol Microbiol 2007; 50 (4):890-895

Introduction

Reproductive medicine has undergone tremendous advances like blastocyst transfer, embryo biopsy and intracytoplasmic sperm injection (ICSI). Semen analysis still remains as a simple, basic, cost effective screening test for evaluation of male in infertility clinic, which provides information on quantitative and qualitative aspects of testicular function. In recent years, infertility and subfertility in men has increased, which may be associated with their advancing age, habits like tobacco use and alcoholism, working environment (occupation), varicocele and other factors. Semen parameters might be sensitive markers for these influencing factors. Hence, our study focuses on the effects of factors like advancing age, working environment, smoking, alcohol, varicocele on the semen parameters.

Materials and Methods

This study was conducted in all patients referred to Pathology department for semen analysis from Obstetrics and Urology department in Sri Devaraj Urs Medical College and Research Centre, Kolar and SNR hospital Kolar from January 2004 to January 2006. Ninety three cases were included in this study, after excluding the patients with history of genitourinary tract infection, systemic illness, tuberculosis, cryptorchidism, mumps, testicular injury, obstructive azoospermia, testicular atrophy (proved by biopsy) and diabetes mellitus. Information was collected as per the proforma by questionnaire from the patients about their occupation, smoking habits, and tobacco chewing and alcohol intake. On physical examination and by ultrasonography of scrotum, varicocele was detected. Semen examination was done by single experienced specialist as per the WHO standard protocol procedures.[1]

Patients were classified into tobacco consumers (a person was considered as a tobacco user, if he chewed tobacco or smoked more than 1 cigarette per day for more than 6 months), alcoholics (alcoholic was considered even if he is a social drinker), combined smokers+alcoholics, varicocele patients (irrespective of their habits) and controls (Table 1). The control groups were non-alcoholics, non-tobacco consumers and non-varicocele subjects and had normozoospermia on semen examination. All these cases were again stratified according to age (20-25 years, 26-30 years, 31-35 years and 36-40 years) and occupation as sedentary workers (clerks, office workers, engineers, lawyers etc, those who sit for more than 8 hours/day and work), professional drivers, business people (shopkeepers, salesmen, automobile workers etc) and agriculturist.

Among 93 cases, nine cases were azoospermic who did not come for further investigations like biopsy and

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Submitted : 17 October 2006 Accepted : 16 April 2007

Table 1

Comparison of mean values of semen parameters in controls and test groups

Parameters	Controls	Tobacco users	Alcoholics	Tobacco users +alcoholic	Varicocele cases
n	27	30	6	14	7
Age in years	30.29	32.06	32.17	33.8	25.85
Volume(ml)	3.21	2.92 †	3.25 †	2.5 †	2.64 †
pH	7.94	7.6	7.73	7.4	7.85
Liquefaction time(min)	35.7	37.3	40.0	43.9	36.42
Sperm density(10^6/ml)	68.95	35.12 †	62.8 †	24.97 ††	27.64 ††
Total motility (%)	59.9	40.76 †	60.0 †	57.85 †	39.28 †
a%	30.5	14.9 †	20.16 †	12.5 †	15.14 †
Morphologically normal sperms (%)	45.6	35.4 †	42.66 †	25.57 †	41.7 †
Morphologically abnormal sperms (%)	54.3	64.6 †	57.3 †	73.0 †	58.28 †
TZI	1.14	1.3 †	1.1 †	1.58 †	1.44 †
SDI	0.65	0.87	0.51	1.22	0.9
Immature germ cells(10^6/ml)	0.82	0.67	0.59	1.08	0.77
WBC(10^6/ml)	0.55	0.32	0.28	0.32	0.21

(†- indicates p value not significant i.e. >0.05, ††- indicates p value < 0.05 i.e. significant when compared with controls)

ultrasonography. Hence, these cases were separately analyzed. Remaining 84 cases were analysed in association with variables like control group, tobacco usage, alcohol intake, varicocele and occupation.

Morphologically abnormal spermatozoa often have multiple defects, which are recorded by following indices.[9]

a) *Teratozoospermia index (TZI):* It is the number of defects divided by number of the defective spermatozoa.

b) *Sperm deformity index (SDI):* It is the number of defects divided by the total number of spermatozoa.

Semen parameters were correlated between control & test groups and analyzed by descriptive statistical tools like mean, standard deviation and standard error of mean etc. Test of significance like independent Student 't' test was also followed.

Observations

The mean age of the patients was 31.37 years (Table 2) Control group constituted 32 .14% (27/84) of the 84 cases. Maximum number of cases i.e. 48% (13/27) of these patients belonged to age group 26-30 years. Semen volume was increasing as the age advances. Sperm density was highest in the age group of 20-25 years. Total motility, rapidly progressive motility (a %) and morphologically normal spermatozoa was maximum in the age group of 20-25 years whereas TZI and SDI were more in age group of 31-35 years. 48.15% (13/27) of the control group were sedentary workers, 37% (10/27) were business people and 14.25% (4/27) were agriculturists and none of the controls were drivers. Sedentary workers had comparatively less sperm density and motility (Table 3).

Tobacco users constituted 35.71% (30/84) of the 84 cases. Common (7/30) abnormality observed was oligoasthenozoospermia (oaz). Rapid progressive motility (a %) was comparatively decreased and TZI was increased. Forty percent (12/30) belonged to the age group 31-35 years (Table 1). Fifty percent (15/30)

Table 2

Distribution of semen quality among age groups

Semen quality	Age groups (in years)				Total
	20-25	26-30	31-35	36-40	
nz	5	21	17	5	48
az	2	1	1	2	6
azoo	0	3	6	0	9
atz	0	1	1	3	5
oatz	1	2	2	4	9
oaz	0	5	2	2	9
otz	0	1	0	0	1
oz	0	2	0	1	3
tz	0	1	2	0	3
Total	8	37	31	17	93

nz-normozoospermia; az-asthenozoospermia; azoo-azoospermia; atz-asthenoteratozoospermia; oatz-oligoasthenoteratozoospermia; oaz-oligoasthenozoospermia; otz-oligoteratozoospermia; oz-oligozoospermia; tz-teratozoospermia.

Table 3

Distribution of control groups according to their occupation

Parameters	Agriculture	Sedentary	Business
n	4	13	10
Volume(ml)	3.0 †	3.38 †	3.07 †
pH	8.0	8.0	7.86
Liquefaction time(min)	37.5	34.23	37
Sperm density(10^6/ml)	72.75 †	67.15 †	69.8 †
Total motility (%)	68.75 †	56.9 †	60.3 †
a%	27.5 †	30.92 †	27.5 †
Morphologically normal sperms (%)	41.5 †	40.85 †	53.6 †
Morphologically abnormal sperms (%)	58.5	59.15	46.4
TZI	1.18 †	1.16 †	1.18 †
SDI	0.7	0.69	0.57
Immature germ cells(10^6/ml)	0.3	0.88	0.96
WBC10^6/ml)	0.35	0.63	0.52

(Results are expressed as mean, †p value is not significant i.e. >0.05)

were farmers, among them 53.3% (8/15) were having normozoospermia (nz) and 26.6% (4/15) cases had oligoasthenozoospermia (oaz). Common abnormality observed in business people, was oligoasthenozoospermia (oaz). 25% sedentary workers (2/8) had oligoasthenoteratozoospermia (oatz).

Alcoholics constituted 7.14% (6/84) of the 84 cases. 83.3% (5/6) of these alcoholics had normozoospermia (nz) and 16.7% (1/6) had asthenozoospermia (az). The asthenozoospermic was agriculturist of 36-40 year age group (Table 1).

Combined alcoholics and tobacco users constituted 16.6 % (14/84) of the 84 cases. Sperm density, rapid progressive motility and morphologically normal sperms were reduced, whereas TZI, SDI along with immature germ cells were increased. 28.57% (4/14) of these cases had normozoospermia(nz) and common abnormality observed was oligoasthenoteratozoospermia(oatz) constituting 28.57% (4/14) and asthenoteratozoospermia (atz) constituted 21.4% (3/14). Overall asthenoteratozoospermia was common. Oligoasthenoteratozoospermia(oatz) was common (3/5) among age group 36-40 years and asthenoteratozoospermia(atz) was seen among both 31-35 years and 36-40 years. 50% (7/14) of these persons were business people, among them common (42.8%) abnormality observed was oligoasthenoteratozoospermia(oatz) and next common (28.8%) was asthenoteratozoospermia (atz). 21.4% (3/14) were drivers among them them, one was normozoospermic(nz) and other two were oligozoospermic (oz) and asthenozoospermic (az). 28.6% (4/14) persons were sedentary workers and 50% (2/4) of them had normozoospermia(nz) and 25% (1/4) had oligoasthenozoospermia(oaz) and asthenozoospermia (az). Overall, business people were more affected (Table 1).

Varicocele patients constituted 8.34% (7/84) of the 84 cases. In these cases sperm density, total motility and rapid progressive motility(a%) was decreased, and TZI was increased. Out of them 3 cases were grade II bilateral, 2 cases- grade II unilateral, one case-grade I unilateral and 1 case- grade I bilateral. None of these were grade III. All unilateral cases were on left sided. Common (42.8%) abnormality observed was asthenozoospermia(az), whereas, oligozoospermia(oz), oligoasthenozoospermia(oaz) and oligoasthenoteratozoospermia(oatz) constituted 14.3% each. Varicocele was common in age group 26-30 years. 42.85% (3/7) cases were seen among business people and all cases had abnormal semen quality. 57% (4/7) of varicocele patients were smokers (Table 1). Oligoasthenoteratozoospermia(oatz) was seen in varicocele patient who was both smoker and alcoholic.

Comparable improvement in semen quality was seen in two patients who underwent varicocelectomy.

Azoospermic patients constituted 9.6% (9/93) of 93 cases. 33.3% (3/9) of azoospermic patients were combined tobacco users and alcoholics, 22.2% (2/9) - alcoholics and 22.2% (2/9) - smokers. 66.6% (6/9) cases were of 31-35 year group and 33.4% (3/9) cases belonged to 26-30 year group. Business people constituted 44.4 %(4/9), drivers-11.1 %(1/9), sedentary and agriculturist -22.2 %(2/9) each. The volume wss more, pH was

alkaline and fructose was positive, thus indicating non obstructive azoospermia.[2]

The comparison of semen parameters of controls and test groups is shown in Table 1. Sperm density was significantly (p value <0.05) reduced in combined tobacco users+ alcoholics and varicocele patients in comparison with controls. Total motility was reduced in varicocele patients, combined tobacco users+ alcoholics, and smokers in comparison with controls. Rapid progressive motility was also reduced in all test groups including alcoholics. Morphologically normal spermatozoa were least among tobacco users+ alcoholics in comparison with controls. Immature germ cells were increased among tobacco users+ alcoholics in comparison with controls. TZI was more among varicocele patients and in both tobacco users+ alcoholics. SDI was more among varicocele patients.

Discussion

The comparison of sperm density of various studies is shown in Table 4.

Table 4

Comparison of sperm density of various studies in general population and controls of present study

Studies[12]	Year	Sperm density (10^6/ml)	Number of cases
Hotchkiss et al	1938	120.6	200
Rutherford et al	1963	110	100
Welch et al	1988	78.6	40
Pol et al	1989	77.7	1222
Ana Carolina M et al[34]	2004	50.9	3194
Present study	**2006**	**68.95**	**27**

(Results are expressed in mean)

The mean sperm density in control group was 68.95x 10^6/ml. Carlson E et al[3] stated that sperm density is declining in general population during the past few decades.

The decline in semen quality may be influenced by pollutant or stressful environment. No significant change in semen quality was observed in association with increasing age in the present study. Henkel R et al[4] have demonstrated significant age dependent decrease in ejaculate volume, normal sperm morphology, total and rapid progressive motility. Ng KK et al[5] have observed increase in abnormal sperm morphology among the population of healthy, non-infertile older men.

The present study and Ana Carolina M et al[6] indicate the decline in volume, sperm density, total motility and rapid progressive motility along with morphologically normal spermatozoa in tobacco users when compared with their respective control groups (Table 5). Decline in sperm density is also observed in Dikshit et al[7], Marshburn PB et al[8]. The TZI observed in tobacco users was 1.3, indicating each spermatozoa have got 1.3 defects. The study by Evan HJ et al[9] had shown increased sperm abnormalities among smokers compared to non smokers, which was hypothesized due to influence of mutagens, aneugens of tobacco on sperm DNA.[10] Zitzmann M et al [11] quoted the findings of Sun JG et al that, the smokers had higher percentage of sperms with fragmented DNA. Thus smoking in males decreases the success rates of assisted reproductive procedures, not only IVF but also in ICSI.

Marshburn PB et al[8] could not find the negative association of alcohol on semen quality. Similar findings were observed in Ana Carolina M et al[6] except for decrease in morphologically normal spermatozoa in comparison with respective control groups. In the present study rapid progressive motility was

Table 5

Comparison of mean values of present study with other studies in tobacco users

Parameters	Present study	Dikshit et al[26]	Ana Carolina M et al[34]	Marshburn PB et al[35]
n	30	338	372	108
Age	32.06	26.5	34.3	nm
Volume(ml)	2.92	2.71	2.9	2.6
Sperm density(106/ml)	35.12	49.75	48.1	79.7
Motility (%)	40.76	60.8	45.7	58.0
a%	14.9	nm	31.1	nm
Morphplogically normal sperms%	35.4	80.06	11.9	nm
Morphplogically abnormal sperms%	64.6	nm	nm	29.0
TZI	1.3	nm	nm	nm
SDI	0.87	nm	nm	nm

(nm= not mentioned.)

Table 6

Comparison of mean values of semen parameters of present study and other studies in alcoholics

Parameters	Present study	Ana Carolina M et al[34] -	Marshburn PB et al[35]
n	6	236	108
Age	32.17	34	nm
Volume(ml)	3.25	2.9	2.9
Sperm density(106/ml)	62.8	52.5	83.5
Motility(%)	60.0	43.5	62
a%	20.16	30.4	nm
Morphplogically normal sperms%	42.66	11.7	nm
Morphplogically abnormal sperms%	57.3	nm	28
TZI	1.1	nm	nm
SDI	0.51	nm	nm

(nm=not mentioned)

comparatively reduced in alcoholics compared to controls (Table 6).

In the present study group, reduction of sperm density was significant (p value was <0.05) in both alcoholics+ tobacco users in comparison with controls, where as, the semen volume, rapid progressive motility, percentage of morphologically normal spermatozoa was comparatively reduced (Table 1). Total motility was less affected. In study by Ana Carolina M et al[6], volume, sperm density, total motility and morphologically normal spermatozoa were affected more than rapid progressive motility (Table 7).

TZI in the present study was 1.58, indicating each spermatozoa have got 1.58 defects. This value is near the threshold value 1.6 for failure of fertilization in vitro.[1] Semen quality was altered by alcohol+ tobacco consumption; hence there exists some significant modifications that suggest a synergic or additive effect of both these toxic habits on male reproductive function. Men who wish to procreate should be specially warned of this matter.

In the present study, sperm density was significantly (p value was < 0.05) reduced in varicocele patients in comparison with controls, whereas, volume, total motility and rapid progressive motility were comparatively reduced, and TZI was increased compared to our control group (Table 1). Similar decrease in sperm density, motility and morphologically normal spermatozoa seen in Chirpriya B et al[12] and Michael JC et al[13] (Table 8).

Table 7

Comparison of mean values of semen parameters of present study and Ana Carolina M et al in alcoholics + tobacco users

Parameters	Present study	Ana Carolina M et al[34]
n	14	174
Age	33.8	33
Volume(ml)	2.5	2.7
Sperm density(106/ml)	24.97	45.3
Motility (%)	57.85	39.6
a%	12.5	27.2
Morphplogically normal sperms%	25.57	11.7
Morphplogically abnormal sperms%	73.0	nm
TZI	1.58	nm
SDI	1.22	nm

(nm=not mentioned.)

Table 8

Comparison of mean values of semen parameters of varicocele patients of present study and other studies

Parameters	Present study	Chirpriya B et al[47]	Michael JC et al[48]
n	7	38	13
Age(years)	25.85	32.84	30
Volume(ml)	2.64	nm	3.5
Sperm density(106/ml)	27.64	33.8	14.9
Motility (%)	39.28	24.9	36.1
a%	15.14	nm	nm
Morphplogically normal sperms%	41.7	36.1	nm
Morphplogically abnormal sperms%	58.28	nm	nm
TZI	1.44	nm	nm
SDI	0.9	nm	nm

(nm = not mentioned)