Original Research Article

Analysis of factors affecting the semen pathology in male infertility

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Abstract

Background: Abnormalities in the male are the sole cause of infertility in approximately 20% of infertile couple and important contributing factor in another 20-40% of couple with reproductive failure. Despite our ability to assess sperm quality through a semen analysis methodology harmonized across laboratories, the use of these parameters cannot precisely and accurately predict the fertility of a man presenting to a clinician. **Objectives:** to evaluate various factors for male infertility and correlate various factors which affect the semen quality. **Methodology:** It was a prospective observational study done over a period of two years. Semen was examined for physical appearance, viscosity, volume, pH, sperm motility, biochemical test fructose, sperm count and the morphology for sperm was done. **Results:** 84% patients were presented as primary infertility and 16% presented as secondary infertility. 35% of patients had oligozoospermia, 42.5% patients had oligoazoospermia and 17% of patients had azoospermia. Only 35 out of 118 smoker had >50% motility while 25 had motility of <5%. Also teratozoospermia dominated in alcoholics [65(32.5%)]. 25 out of 60 obese patients had sperm motility < 5%. While 10 out of 70 overweight and 5 out of 70 normal weight patients had sperm motility <5%. **Conclusion:** Smoking and alcohol decrease fertility by decreasing sperm count, motility and also by changing the morphology of sperm. Obesity also directly contributes in the fertility of a person by altering the hormonal status of patients.

Keywords: Semen analysis, Smoking, Alcohol, Obesity, male Infertility

Introduction

Infertility is common problem affecting nearly one out of the six couples. Among them male infertility is a problem of the reproductive system, and the word infertility itself means no fertile, and that would be equivalent to sterility [1]. Sterility means that a man is totally unable to have a child [2].

As the definition given by WHO and the American Society for Reproduction Medicine Practice Committee, infertility means no conception after at least 12 months of unprotected sexual intercourse [3]. Infertility can be permanent or subfertility which means the probability of spontaneous conception may be decreased [1]. Abnormalities in the male are the sole cause of infertility in approximately 20% of infertile couple and important contributing factor in another 20-40% of couple with reproductive failure [4]. Infertility can either be primary or secondary; primary male infertility is when the man has never impregnated a woman, while secondary male infertility is when a man has impregnated a woman irrespective of the outcome of the pregnancy[3]. Men with secondary infertility, in general, have better chance of future fertility [3].

Duration of infertility is defined as the number of months during which the couple has been having sexual intercourse without the use of any contraceptive method [3]. This indicator gives an important information about the couple's future fertility, if the duration of infertility of 3 years or less the couples have a better chance of future pregnancy, but if the duration has been longer, then there is a severe biological problem [1]. Despite our ability to assess sperm quality through a semen analysis methodology harmonized across laboratories, the use of these parameters cannot precisely and accurately predict the fertility of a man presenting to a clinician.

The objective of the study was to evaluate various factors for male infertility and correlate various factors which affect the semen quality.

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Materials and methods

Study design and setting: This is prospective observational study in which total 200 cases of infertility were enrolled during the period of July 2013 to November 2015 B J Medical College.

Inclusion and Exclusion criteria: All patients who came to us in outdoor patient pathology laboratory were included in the study. There were no any exclusion criteria.

Detailed clinical history inclusive of their presenting complaints, type of infertility, age of patient, occupation, education, detailed marital history, relevant family & past history, inclusive of medical, surgical illness if any.

Past consultation, urological history & sexual history were elicited. General physical and local examination of penis, scrotum, testis, vas deference, prostate, seminal vesicle was done. Patients were examined for neurological defects.

After explaining to patient semen was collected after 3 days of abstinence. The sample was received in laboratory within 30 min of collection. Entire sample were obtained by masturbation in clean wide mouth plastic container provided by laboratory. Container was labelled with patient name, registration number, time & date of collection.

Sample was examined by physical appearance, viscosity, volume and pH. After physical examination wet preparation for sperm motility. Biochemical test for fructose is carried out. Sperm count in percentage with the help of neubauer counter chamber and stained with field's stain to check the morphology for sperm.

Method for sperm motility:

1. Place a drop of semen on glass slide & cover with a coverslip.
2. Examine under high power(40x).
3. Count a total 100 spermatozoa, and note out of the hundred how many are motile. Record the percentage that are motile and non motile.

Method of Sperm count:

1. Semen was diluted 1:20 with formalin.(0.1 ml semen and 1.9 ml formalin)
2. Charge the Neubauer++ chamber with diluted semen sample.
3. Sample was allowed to settle for 10 to 15 minutes.
4. The chamber was placed under the microscope and spermatozoa were counted in 4 large corners squares using the 20x or 40x objectives
5. Sperm count per ml was calculated as follows:

$$\frac{Spermscounted\ (N) x correctionfactorfordilution(20)}{Noofsquarescounted(4)\ xvolumeof\ 1\ square(0.1)} \times 1000$$

= N × 50000/ml.

Smear examination for sperm morphology :

1. Place a drop of semen on a glass slide and prepared a smear.
2. Stained the smear with field stain.
3. At least 200 spermatozoa counted under oil immersion.
4. Percentage of normal & abnormal spermatozoa recorded.

Biochemical test

Fructose test:

1. Taken 3 test tubes.
2. In 1st test tube, take 2 ml fructose reagent & 0.1 ml distilled water as negative control.
3. In 2nd test tube, take 2ml fructose reagent & 0.1 ml positive control.
4. In 3rd test tube, take 2 ml fructose reagent & 0.1 ml sample.
5. Boil in hot water bath for 2 minutes, cool and compare the colour with positive and negative controls.
6. Note the reading along with positive and negative control.

Results

The study was done with collaboration with the department of Obstetrics& Gynecology. Total 200 patients of infertility was taken into consideration over a period of 2 years. 160 (80%) of patients were from urban area and 40 (20%) from rural area. As seen in table 1, more than half i.e. 56% of patients were from age group of 25 – 30 years of age with ranges from 21 to 40 years of age.

Occupation wise distribution shows that majority of the patients is from middle and lower middle class who are manual worker by their occupation [Table 2]. As seen in table 3, majority of the patients (~70%) have active marriage life less than 5 years.

Table-1: Age wise distribution of all infertile patients.

Age	No of patients	Percentages
21 – 25	56	28%
26 – 30	112	56%
31 – 35	24	12%
36 – 40	8	4%
Total	**200**	**100%**

Table-2: Occupation wise distribution of all infertile patients.

Occupation	Frequency	Percentage
Laborers	24	12%
Factory workers	16	8%
Vendor	16	8%
Shopkeeper	16	8%
Driver	16	8%
Teacher	16	8%
Other	96	48%
Total	**200**	**100%**

Type of infertility shows, 84% patients were presented as primary infertility and 16 % presented as secondary infertility. Semen analysis report is seen in table 4.

Majority of patients (72%) had semen quantity less than 2 cc and 84% of patients had sperm density of >20 million/ml.

However actively motile sperm was seen in only 20% of patients. Sluggishly motile sperm was seen in 25% of patients and non-motile sperm were seen in 55% of patients.

Frequency of motile sperm shows that 60% of patients had <20% motile sperm. 35% of patients had oligozoospermia, 42.5% patients had oligoazoospermia and 17% of patients had azoospermia. > 50 % of patients had pus cells >10/HPF.

Table-3: duration of active married life.

Duration of active married life in years	No of patients	Percentages
2 – 3	72	36
3.1 – 5	64	32
5.1 – 7	40	20
7.1 – 9	16	8
9.1 – 15	8	4
Total	**200**	**100**

Table-4: Semen analysis parameters.

Semen analysis	No. of patients	Percentages
1. Quantity (c.c.)		
<1	32	16
1.1 – 2	114	57
2.2 – 3	32	16
> 3	24	12
Total	**200**	**100**
2. Sperm density (million/ml)		
>20	168	84
10.1 – 20	24	12
< 10	8	4
Total	**200**	**100**
3. Sperm motility		
Actively motile	40	20
Sluggishly motile	50	25
Non-motile	110	55
4. Frequency of motile sperm (%)		
>50	32	16
20.1 – 50	48	24
5 – 20	120	60
Total	**200**	**100**
5. Abnormality		
Oligozoospermia	70	35
Oligoasthenozoospermia	85	42.5
Azoospermia	34	17
Aspermia	3	1.5
Cryptospermia	5	2.5
Nacrospermia	3	1.5
6. Pus cell/HPF		
>10	104	52
4 – 10	72	36
2 – 3	24	12
Total	**200**	**100**

Table-5: Motility of sperm in relation to smoking

Sperm motility	Smoker				Non-smoker	Total
	Mild	Moderate	Sever	Total		
>50%	17 (8.5%)	13 (6.55%)	5 (2.5%)	35 (17.5%)	42 (21%)	77 (38.5%)
20 – 40%	12 (6%)	22 (11%)	2 (1%)	36 (18%)	12 (6%)	48 (24%)
5 – 19%	3 (1.5%)	8 (4%)	12 (6%)	23 (11.5%)	14 (7%)	37 (18.5%)
<5%	5 (2.5%)	8 (4%)	12 (6%)	25 (12.5%)	13 (6.5%)	38 (19%)
Total	37 (18.5%)	51 (25.5%)	31 (15.5%)	118 (59%)	82 (41%)	200 (100%)

Table-6: Count of morphologically normal sperm in relation to smoking.

Sperm morphology (normal sperm)	Smoker				Non-smoker	Total
	Light	Moderate	Heavy	Total		
> 30%	18 (9%)	13 (6.5%)	2 (1%)	33 (16.5%)	14 (7%)	47 (23.5%)
20 – 30 %	6 (3%)	11 (5.5%)	3 (1.5%)	20 (10%)	25 (12.5%)	45 (22.5%)
10 – 19 %	6 (3%)	9 (4.5%)	1 (0.5%)	16 (8%)	20 (10%)	36 (18%)
3 – 9 %	3 (1.5%)	11 (5.5%)	5 (2.5%)	19 (9.5%)	12 (6%)	31 (15.5%)
< 3 %	8 (4%)	10 (5%)	12 (6%)	30 (15%)	11 (5.5%)	41 (20.5%)
Total	**41 (20.5%)**	**54 (27%)**	**23 (11.5%)**	**118 (59%)**	**82 (41%)**	**200 (100%)**

Light Smokers: 01-20 cigarettes/day;

Moderate Smokers: 21-40 cigarettes/day; and

Heavy Smokers: 41 and more cigarettes/day.

Table 5 and 6 shows the motility and morphology of sperm in relation to smoking habit of the patients. Total 118 (59%) patients were having smoking habit. While 82 (41%) were nonsmokers. 42 out of 82 nonsmoker had good sperm motility i.e. >50% motile sperm. Only 35 out of 118 smoker had >50% motility while 25 had motility of <5%. Also 49 patients of smoking group had <10% of morphologically normal sperm. While only 22 patients of nonsmoker group had <10% of normal sperm.

Table 7 shows the sperm morphology in comparison to alcohol consumption of the patients. Within the alcoholic subgroups, teratozoospermia dominated in alcoholics [65(32.5%)] than the nonalcoholic [31(15.5%)] cases.

Similarly oligozoospermia was present in as high in alcoholics [55 (27.5%)] than in nonalcoholic cases [33(16.5%)]. All three abnormality like teratozoospermia, asthenozoospermia and oligozoospermia seen in 22 patients of alcoholic group while 7 patients of nonalcoholic group.

Table-7: alcohol consumption in relation to sperm morphology.

Sperm morphology (normal sperm)	Alcoholic				Non-alcoholic	Total
	Mild	Moderate	Heavy	Total		
N	10 (5%)	3 (1.5%)	1 (0.5%)	14 (7%)	32 (16%)	46 (23%)
A	6 (3%)	2 (1%)	0 (0%)	8 (4%)	9 (4.5%)	17 (8.5%)
A+O	3 (1.5%)	3 (1.5%)	2 (1%)	8 (4%)	8 (4%)	16 (8%)
A+T	4 (2%)	6 (3%)	4 (2%)	14 (7%)	8 (4%)	22 (11%)
A+O+T	6 (3%)	8 (4%)	8 (4%)	22 (11%)	7 (3.5%)	29 (14.5%)
O	4 (2%)	6 (3%)	5 (2.5%)	15 (7.5%)	10 (5%)	25 (12.5%)
O+T	4 (2%)	4 (2%)	2 (1%)	10 (5%)	8 (4%)	18 (9%)
T	7 (3.5%)	7 (3.5%)	5 (2.5%)	19 (9.5%)	8 (4%)	27 (13.5%)
Total	**44 (22%)**	**39 (19.5%)**	**27 (13.5%)**	**110 (55%)**	**90 (45%)**	**200 (100%)**

N=Normozoospermia, A=Asthenozoospermia,

O=Oligozoospermia, T=Teratozoospermia

Mild alcohol: those consuming 40g or less;

Moderate alcohol: consuming 40-80g; and

Heavy alcohol: consuming more than 80g per day.

Table-8: Correlation of obesity and sperm motility

	Normal (BMI = 20-24 kg/m2)	Overweight (BMI= 25-30 kg/m2)	Obese (>30 kg/m2)
Sperm motility			
>50%	40	30	10
20 – 40%	15	20	12
5 – 19%	10	10	13
<5%	5	10	25
Total	**70**	**70**	**60**

25 out of 60 obese patients had sperm motility < 5%. While 10 out of 70 overweight and 5 out of 70 normal weight patients had sperm motility <5%. Good motility (>50%) seen in normal weight patients [40 out of 70 (57.14%)]. [Table 8]

Table-9: Correlation between cryptorchidism and sperm count and motility

	Unilateral cryptorchidism (n=4)	Bilateral cryptorchidism (n=2)
Sperm count (million/ml)		
Normal	3	1
<10	1	1
Motility		
>50%	1	0
15 -30 %	2	1
<15%	1	1

Table-10: correlation between varicocele and sperm morphology abnormality.

	Patients with Varicocele (n=30)
Asthenozoospermia	14
Oligozoospermia	9
Teratozoospermia	7

In this study 6 patients found with cryptorchidism with 4 patients had unilateral and 2 patients had bilateral cryptorchidism. Total 2 patients one from both unilateral and bilateral cryptorchidism had sperm count <10 million/ml and motility <15% [Table 9]. While total 30 (15%) patients were found varicocele[Table 10] Among them 14 had asthenozoospermia, 9 had oligozoospermia, and 7 had teratozoospermia.

Discussion

Semen analysis is one of the most important investigations and should be done thoroughly in male partner of each infertile couple. In present study total 200 numbers of infertile patients were studied for the full semen analysis and correlation with epidemiological and other factors.

In present study, it was found that 80% of patients were from urban area. It might be because of pollution or other lifestyle related factors like stress and other factors may affect the fertility of male. The age group of presentation was 21 to 40 years with commonest age group was 25 – 30 years (mean age 28.8 years) in this study. Other study by Saxena SC [5] found the similar type of result with mean age of 30.68 years. In present study 84% patients were presented as primary infertility and 16 % presented as secondary infertility. Ring & scragg [3] found 90% 0f men had primary Infertility and 10% cases secondary infertility. Osegbe&amaku[6] found 64 % of men had primary infertility while in

36% cases secondary infertility was noted. Osegbe & amaku [6] reported in their series duration of active married life varying from 1 to 28 years with mean of 5.3 years. In present study 36% had 2 to 3 years of active married life and 32% had 4 to 5 years of active married life with mean of 4.96 years. In patients with 2 years of active married life, this was reduced to 5% after 6 years of active married life. Thus shorter the duration of male related infertility, better the prognosis.

In present study, the mean of semen quantity was 1.8 c.c. Saxena [5] reported a mean of 2.0 c.c. Osegbe and Amaku [6] reported semen volume ranging from 0.4 to 10 ml, with a mean of 2.56 c.c. Mclane[7] suggested homologous insemination be performed if semen volume is 1.5 ml or less. In this series, there were 4 patient (16%) who had semen quantity less than or equal to 1 ml. The sperm density in present study is compared with study by McLeod and Gold et al.[8], Saxena et al. [5], and Zukerman et al. [9].

Authors	**Sperm density(millions/ml)**		
	<10	10.1 – 20	>20
Present study	4%	12%	84%
McLeod and Gold et al (1951) [8]	9%	5%	86%
Saxena et al. (1972) [5]	33.1%	2%	64.9%
Zukerman et al. (1977) [9]	28%	14%	58%

It was found that 34.2% of nonsmokers showed below 5% sperm motility and 65.7% of smokers showed below 5% sperm motility. Among the 3 groups of smokers, sperm motility below 5% was present in 20% of light smokers, 32% of moderate smokers, and 48% of heavy smokers. In similar result were obtained by Zhang et al. in 2000 [10].

The sperm motility was below 5% in 30% of non smoker while 70% of smokers. The sperm morphology was normal below 3 % in 26.8% of non smokers while 73.1% of smokers. Among the 3 groups of smokers, morphology of sperm, less than 3% of normal sperm cells were present in 26.6% of light, 33.3%of moderate, and 40% of heavy smokers.

Thus the highest abnormal sperms are present in heavy smokers and it also suggests that as the amount of smoking increases, it also increases the number of abnormal sperms. This is also supported by Zukerman et al [9]. In 21% non smoker showed below 3% normal sperm morphology and 69% in smoker.

14(30.4%) patients of alcoholics showed normozoospermia, of which 10(71.4%) were mild alcoholics and 1(7.1%) was heavy alcoholics. 32(69.5%) cases of non alcoholic showed normozoospermia. This study was comparable to previous study of VillaltaJ [11] showed in 15% in heavy smoker and 72% in non alcoholic cases.

In present study 70 patients (35%) were normal weight, 70 (35%) overweight and 60 (30%) obese patient. < 5% Sperm motility is noted in 25(41.6%) in obese patient while only 5 (7.1%) Normal weight patient. In the present study > 50% sperm motility was noted in 40(57.1%) obese patient. Korte et al [12] concluded that men with high BMI values (>25) present with only few normal-motile sperm cells.

Pus cells > 4 in number in semen sample are an indicative of infection in the male reproductive tract. Normally up to 1-2 pus cells/HPF are present in semen sample. 9 (36%) patients had pus cells count ranging between 4-10 pus cells/HPF indicating moderate infection. There were 3(12%) patients who had more than 10 pus cells/HPF indicating severe infection. Saxena [5] reported 20% cases with severe infections.

The sperm count was normal in 75% unilateral cryptorchidism patient while 50% of bilateral cryptorchidism. Nistal M et al [13] study 95% normal sperm count in unilateral and 50% in bilateral cryptorchidism. In present study 15% cases of varicocele.

Out of them 46.6% examined asthenozoospermia, 30% oligospermia and 23.3 % teratozoospermia. Ali Ali BM 2010 [14] showed 36% asthenozoospermia, 25% oligospermia and 21% teratozoospermia.

Conclusion

In conclusion smoking and alcohol decrease fertility by decreasing sperm count, motility and also by changing the morphology of sperm. Obesity also directly contributing the fertility by altering the hormonal status of patients. Presence of pus cells suggests infective etiology.

Along with the other etiological factors like smoking, obesity, varicocele and hormonal abnormality, infective etiology is also responsible for infertility.

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