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Original Article

Relevance between Semen Analysis Parameters and Infertility Risk Factors: A Cross-Sectional Study in Iran

Sana Ahmadi[1], Maryam Shabani Neshati[2], Ali Tavoosian[3*]

[1]*Labbafinezhad Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

[2]*Anatomy Department, Tehran University of Medical Sciences, Tehran, Iran*

[3]*Infertility Department, Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran*

HIGHLIGHTS

- This is a cross-sectional study between 210 infertile men to find out the relationship between male infertility risk factors and sperm parameters.
- There is no significant relationship between sperm parameters and infertility duration, history of miscarriage, smoking history, history of scrotal surgery, urogenital diseases.
- Increasing age has significant correlation with sperm parameters.

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***Corresponding Author**:
Ali Tavoosian
Email: alitavoosian@gmail.com
Address: Infertility Department, Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran.

ABSTRACT

Introduction
Men are involved in infertility in about 30-50% of cases. Many risk factors can cause disorders in male fertility by affecting endocrine, temperature and genetic mechanisms. Therefore, the purpose of this study is to determine the effect of infertility risk factors on changes in semen.

Methods
200 infertile men were included in this study. Patients' initial information including age, sex, weight, height and were collected in data collection forms. Their semen samples were being collected to check sperm parameters according to the WHO protocol and analyzed by SPSS software.

Results
A total of 210 men with a mean age of 31.94±5.66 years. The mean duration of infertility in the participants in this study was 4.29±3.35 years. The mean sperm count of participants in this study was 23.21±15.49. The mean morphology index was 5.03±8.35, the mean activity index B was 10.87±7.39 and the mean activity index A was 17.85±13.86. Increasing age has a significant correlation with decreasing number, morphology index and B movement in semen. There is no significant correlation between the duration of infertility, previous history of abortion, history of scrotal surgery and disturbance of semen parameter. Activity index A was significantly higher in patients who smoked than other patients. There is no significant relationship between the history of genitourinary diseases with the number and morphology and movement indices of A. Activity index B was significantly higher in patients without genitourinary diseases.

Conclusions
There are controllable factors of male infertility have significant effects on semen parameter, that can be enhanced by educating and governmental supporting. On the other hand, factors such as abortion history shouldn't cause despair such as they don't have a significant effect on semen parameters. Further studies on larger population recommended.

Keywords: Infertility Factors; Spermogram; Semen Fluid Analysis

Introduction

Infertility is one of the most important health and social issue in the world. Different sources provide different explanation of Infertility term, which can lead us to different rates of infertility and even different treatment based on different etiologies (1, 2). Mostly it has been defined as an inability to get pregnant after at least 12 months, or 6 months in cases with female partner older than 35 years old, of unprotected and regular intercourse, which can cost a lot for families and health care systems. Based on WHO reports, it has been estimated 8-12 percentage of couples around the world are suffered from

infertility and male factors in primary or contributing causes is the main problem in 50% of couples (3).

Beside the financial costs for infertile couples, the mental health, social stigma, partnership and emotional problems are serious consequence of infertility and for countries the worst burden caused by infertility is to reduce the population which is the only human source for communities (4, 5).

In the other side, high cost and low success rate of assisted reproductive techniques (with approximate rate of 30% in IVF technique) add to the frustration of infertile couples (6).

Analysis of reports from Iran, showed that the primary infertility prevalence in Iran is about 18.3% and secondary infertility rate is 2.5%, And it has been claimed that the causes of infertility in Iran are: 12.5% of both sexes, 32 % male cause, 43.3% female cause, and 13.6% unknown causes (5).

The most important causes of infertility in men are: Congenital or acquired anomalies of the genitourinary system, malignancies, urogenital infections, increased scrotal temperature (e.g., Varicocele, work environment), endocrine disorders, immunological and idiopathic causes (7, 8). Prognostic factors in male infertility include: Duration of infertility, primary or secondary types of infertility, sperm test parameters, age and infertility status of the female partner (9).

For men, semen analysis is the first and the best step in the evaluation of infertility, and is considered an inexpensive, simple, and non-invasive test that can clearly assess infertile men. To find out the sperm function, at least two different semen samples are required in separate times (10). According to the last edition of WHO booklet, minimum numbers for normal sperm analysis factors are; 1.4 ml of volume, 16×106 sperm count per ml, 42% of total motility (A+B+C motility), 30% of progressive motility (A+B motility) and 4% of normal morphology (11).

The main idea of this study is to determine the quality of semen analysis parameters in men referring to the infertility clinic of Arash Hospital, Tehran, Iran and to find out the relationship between these parameter and infertility risk factors which are; age, duration of infertility, history of abortion, female partner medical and infertility history, smoking details and relevant medical history.

Methods

Study data

This is a cross-sectional study, designed in infertility department of Arash hospital in Tehran, Iran. This study conducted on 210 husbands of women referred to infertility clinic in one year. Inclusion criteria are; age over 18 years and whom had failed to conceive after one year or more of trying. Exclusion criteria: Patients with azoospermia were excluded from the study. Sampling was performed using a non-probability and convenience method, and all patients who met the inclusion criteria were selected. Sampling continued until the intended number of samples was completed. This study was conducted after obtaining permission from the hospital and approval from the Ethics Committee of Tehran University of Medical Sciences (Ethic code: IR.TUMS. Medicine.Rec.1399.003).

Sample size

The sample size was calculated using the population proportion estimation formula.

$n=(P(1-P)\ z\hat{}2)/d^2$

To calculate the sample size, the prevalence was assumed to be 0.45 and the error was assumed to be 15% prevalence.

$Z1-\alpha/2=1.96$

Therefore, the sample size was assumed to be 208, considering a 10% dropout, the sample size increased to 231.

Data collection tools

Information on age, gender, duration of infertility, abortion, smoking status and consumption, and history of genitourinary diseases (such as Varicocele, scrotal infection, inguinal surgery) was collected in data collection forms.

Procedure

Semen samples were collected after 3 to 4 days of abstinence from intercourse. Sperm parameters including concentration, motility, and morphology were evaluated according to the World Health Organization (2021) criteria (11). Sperm concentration was measured using a Makler Counting Chamber in millions per liter, sperm motility was measured using CASA (Computer Aided Sperm Analysis) software, and sperm morphology was measured using Diff Quik staining according to the WHO (2021) criteria (11). The data analysis software was SPSS 26.0 IBM Crop.

Data analysis method

All data were analyzed by SPSS 26.0 statistical software and the results were reported. Mean and standard deviation were used to display quantitative variables and frequency and percentage were used to display qualitative variables. T-test was used to compare quantitative variables in two groups and one-way analysis of variance was used for more than two groups, and Chi-square test was used to compare qualitative variables. A significance level (Sig. value or P-value) of <0.05 will be considered. Parametric tests were used to analyze parametric data and non-parametric tests were used to analyze non-parametric data.

Results

A total of 210 men were involved in this study, with a mean age of 31.94±5.66 years. The youngest man was 19 years and the oldest was 49 years. The mean duration of infertility in the participants in this study was 4.29±3.35 years.

Three patients had a history of one miscarriage, two had a history of 2 miscarriages and one case had a history of 3 miscarriages. Fifty-three patients (25.4%) reported smoking, 12 men alcohol, 9 cases of hookah and 11 patients using opium.

Among the diseases of the genitourinary system, one suffered from HIV, one diagnosed with UDT, one had UTI, one patient had epididymitis, one man had diabetes, one had hepatitis B, 3 patients suffered from kidney stones, 6 patients had inguinal hernia, and 4 men diagnosed by Varicocele.

Thirty-nine patients underwent varicocelectomy, a man underwent TUL, one man with history of UDT surgery, and 7 male patients underwent right or left hernioplasty.

The mean sperm count in the participants in this study was 23.21±15.49. The mean morphology index was 8.35±5.03 with a range of 0 to 75, the mean motility index B was 10.87±7.39 with a range of 2 to 56, and the mean motility index A was 17.85±13.86 with a range of 0 to 73 (Table 1).

Referring to the Table 2 data, which shows the parameters of semen analysis in patients in two age groups: less than and more than 30 years, there is no significant relationship between the number and morphology, motility A and B indices in the two groups: less than and more than 30 years.

Table 3 shows the results of the regression test between age and semen analysis parameters. According to the results of this table, increasing age has a significant correlation with a decrease in the number, morphology index, A and B motility in semen. The P-value for this relationship is 0.033, 0.010, 0.251 and 0.041, respectively, and the adjusted ORs are 0.017, 0.027, 0.002 and 0.015.
Table 3. Regression test between parameters of sperm Analysis and age

Table 4 shows the parameters of semen analysis in patients in two groups with infertility duration of less than and more than 3 months. Based on the results of this Table, there is no significant relationship between the number and morphology indices, motility A and B with infertility duration of less than and more than 3 months.

Table 5 shows the results of the regression test between the duration of infertility and semen analysis parameters. According to the results of this Table, there is no significant correlation between the duration of infertility and the decrease in the number, morphology index, and motility of A and B in semen.

Table 6 shows the parameters of semen analysis in patients in two groups with and without a previous history of miscarriage. Based on the results of this table, there is no significant relationship between the number and morphology indices, A and B motility, and a previous history of miscarriage.

Table 7 shows the parameters of semen analysis in patients in two groups with and without smoking and drug use. According to the results of this table, there is no significant relationship between smoking and the number and morphology and motility indices B. The motility index A was significantly higher in patients who smoked than in other patients, 17.87±12.83 compared to 10.50±5.58, P-value is 0.011.

Table 8 shows the parameters of semen analysis in patients in two groups with and without a history of scrotal surgery. Based on the results of this table, there is no significant relationship between the history of scrotal surgery, number, morphology, motility A and B indices.

Table 9 shows the parameters of semen analysis in patients in two groups with and without a history of urogenital diseases. According to the results of this table, there is no significant relationship between the history of urogenital diseases and the number and morphology and motility indices A. The motility index B was significantly higher in patients without urogenital diseases, 11.21±7.62 compared to 7.95±4.25, P-value=0.006.

As a result of all these data we found that:

- No significant relationship between the number, morphology and motilities in the two groups of people in the ages of less than and more than 30 years.
- No significant relationship between the number, morphology, motilities and the duration of infertility.
- No significant relationship between the number, morphology, motilities and the history of miscarriage or abortion.

Table 1. Sperm Analysis

	Minimum	Maximum	Mean	SD
Count	1	106	23.21	15.487
Morphology	0	75	5.03	8.348
Index B	2	56	10.87	7.392
Index A	0	73	17.85	13.864

Table 2. Parameters of Sperm Analysis based on age

Sperm	Age group	Mean	SD	P-value
Count	Less than 30 y	20.96	13.298	0.074
	More than 30 y	24.84	16.768	
Morphology	Less than 30 y	4.17	5.198	0.206
	More than 30 y	5.65	10.014	
Movement B	Less than 30 y	9.90	7.139	0.103
	More than 30 y	11.59	7.520	
Movement A	Less than 30 y	17.42	14.187	0.706
	More than 30 y	18.16	13.675	

Table 3. Regression test between parameters of sperm analysis and age

Variable	Adjusted OR	95% C I	P-value
Morphology	0.027	0.0025-0.297	0.010
Movement B	0.015	0.0056-0.041	0.041
Movement A	0.002	0.0007-0.0054	0.251
Count	0.017	0.0063-0.045	0.033

Table 5. Regression test between parameters of sperm analysis and infertility duration

Variable	Adjusted OR	95% C I	P-value
Morphology	0.014	0.0051-0.038	0.055
Movement B	0.002	0.0007-0.0054	0.428
Movement A	0.005	0.0007-0.0054	0.780
Count	0.009	0.0034-0.0244	0.101

Table 7. Parameters of sperm analysis based on smoking history

Sperm	Smoking history	Mean	SD	P-value
Count	Negative	16.50	7.709	0.208
	Positive	24.36	17.099	
Morphology	Negative	2.50	1.309	0.511
	Positive	4.42	8.120	
Movement B	Negative	8.75	5.175	0.349
	Positive	11.47	7.878	
Movement A	Negative	10.50	5.581	0.011
	Positive	17.87	12.830	

• No significant relationship between the number, morphology, motility-B and smoking.
• No significant relationship between the number, morphology, motilities and the scrotal surgery.
• No significant relationship between the number, morphology, motility-A and the history of urogenital diseases.
• The motility-A index is significantly higher in smoker patients and the motility-B index is significantly higher in patients with no history of urogenital disorders.
• Increasing age has a significant correlation with a decrease in the number, morphology and motilities indexes.

Discussion

The most common clinical definition of infertility is to not be able to get pregnant after 12 months of unprotected, correct and regular intercourse in normal population and 6 months try in the couple with female older than 35 years old. It has been estimated that male factors are contribute on 30-50% of infertility cases. Male factors which had major role in reduced fertility or infertility are; life-style factors (high BMI, alcohol), pathology in testis function and/or anatomy, genitourinary system and endocrine system, other congenital or anatomical factors, aging, genetics, oxidative stress, infection, leukocyte-spermia, febrile illness, elevated testicular temperature, diet, use of

Table 4. Parameters of sperm analysis based on infertility duration

Sperm	Duration of infertility	Mean	SD	P-value
Count	<3 months	24.26	15.571	0.158
	>3 months	21.17	14.842	
Morphology	<3 months	4.27	5.031	0.129
	>3 months	6.13	11.393	
Movement B	<3 months	10.88	7.913	0.736
	>3 months	11.24	7.045	
Movement A	<3 months	17.69	13.583	0.691
	>3 months	18.49	14.519	

Table 6. Parameters of sperm analysis based on abortion history

Sperm	Abortion history	Mean	SD	P-value
Count	Negative	23.18	15.758	0.855
	Positive	22.00	7.294	
Morphology	Negative	5.12	8.520	0.609
	Positive	3.33	1.366	
Movement B	Negative	10.93	7.513	0.846
	Positive	10.33	4.274	
Movement A	Negative	17.87	14.077	0.914
	Positive	18.50	10.015	

medication (especially antibiotics), Varicocele, exposure to ionizing radiation (such as X-rays and gamma rays), and exposure to environmental and occupational pollutants are among the factors that cause sperm DNA fragmentation and put people at risk of unhealthy pregnancy (12, 13).

Steps for examination and evaluation of patients with infertility complaints, start with complete history taking from both partner, whole body physical examination, and the best evaluation system is to analyzing the male patient's semen and sperm (14).

The last version of WHO infertility booklet (6th edition in 2021) described lower fifth percentile of normal semen parameters which is showed in Table 10 (11).

A cross-sectional study in Iran conducted to find out the relationship between obesity and sperm parameters. About 200 male patients were involved in this study over a period of one year (2015-2016). Based on the statistical analysis on the results, they reported that; there was no significant relationship between sperm counts, sperm motility and sperm shape and BMI in men (P-value> 0.05) (15).

A Pakistani researchers designed cross-sectional study between 376 male participates in two groups of smoker and non-smoker. The purpose of the study was to find the association between smoking and reduction of fertility. The study approved the main sperm parameters (total count, motility, and morphology) are decreased in infertile smokers as compared to infertile non-smokers, and it showed that smoking causes a significant decrease in sperm count and morphology but it did not have any

Table 8. Parameters of sperm analysis based on scrotum surgical history

Sperm	History of scrotal surgery	Mean	SD	P-value
Count	Negative	22.40	14.326	0.165
	Positive	25.94	18.788	
Morphology	Negative	4.82	7.541	0.509
	Positive	5.73	10.685	
Movement B	Negative	10.79	7.831	0.756
	Positive	11.17	5.737	
Movement A	Negative	17.88	14.478	0.956
	Positive	17.75	11.707	

Table 9. Parameters of sperm analysis based on Urogenital diseases

Sperm	Urogenital diseases history	Mean	SD	P-value
Count	Negative	23.36	16.089	0.671
	Positive	21.80	9.294	
Morphology	Negative	5.26	8.777	0.274
	Positive	3.10	1.889	
Movement B	Negative	11.21	7.622	0.006
	Positive	7.95	4.249	
Movement A	Negative	18.03	13.924	0.588
	Positive	16.25	14.238	

Table 10. Normal Parameters of Sperm Analysis (WHO 2021)

Parameters	WHO 2021 numbers
Semen volume (mL)	1.4
Total sperm count (106 per ejaculation)	39
Total motility %	42
Progressive motility %	30
Non-progressive motility %	1
Immotile sperm %	20
Vitality %	54
Normal forms %	4

significant effect on motility (16).

A retrospective descriptive study in Chile with 2681 male patients over 3 years between 2014 to 2017, conducted to looking for relevance between age and sperm parameters. This study proved that there are significant differences in sperm quality in older male, so that over the age of 50 it's been more likely to present anomalies in semen volume, sperm concentration, and sperm DNA fragmentation. Men over 40s will present by reduction in sperm concentration levels and at the ages above 31 we expected decreased sperm motility. It been reported that there is negative correlations between age and semen volume, concentration, progressive motility. And it has been claimed that the most affected parameter by aging would be motility and this theory can be explained by the endocrine and anatomical changes by aging (17).

In this study, we investigated the parameters of semen analysis in men referred to the infertility department of Arash Hospital and checked the relationship between changes in these parameters and infertility risk factors.
The mean sperm count in the participants in this project was 23.21±15.49. The mean morphology index was 5.03±8.35 with a range of 0 to 75, the mean motility index B was 10.87±7.39 with a range of 2 to 56, and the mean motility index A was 17.85±13.86 with a range of 0 to 73. Our study results also showed that increasing age was significantly correlated with a decrease in the number, morphology index, and motility of B in semen. That can be explainable by increasing the exposure to environmental factors such as; toxins, pesticides, and radiation, which have harmful effects on male infertility. And it can be relevant to anatomical and hormonal negative changes during aging process.

Also, there was no significant correlation between the duration of infertility and a decrease in the number, morphology index, and motility of A and B in semen. According to our results, there was no significant correlation between a history of scrotal surgery or a history of abortion with the number, morphology index, and motility of A and B.

Alcohol and tobacco use, as well as heavy physical activities and sedentary jobs, can also be associated with male infertility. Varicocele is also an important environmental factor affecting male infertility, but the effect of its treatment on improving infertility is unclear. In the case of celiac disease, the treatment or non-treatment of the disease and its effect on the process of infertility are contradictory. In some studies, increasing body mass index has been reported to be effective on infertility, while in others it has been reported to be ineffective (18). Based on our results, there was no significant association between smoking and the number and morphology and movement indices B. Movement index A was significantly higher in patients who smoked than in other patients and this finding was not consistent with the results of other researchers (19-21).

In our study, there was no significant association between the history of urogenital diseases and the number and morphology and A motility index. The motility index of B was significantly higher in patients who did not have urogenital diseases. In the study of Gharemani (20) and Bonde (22), physical illness had a negative effect on sperm quality. Although in the past, Varicocele was known as the most important cause of infertility in men, based on evidence and studies in recent years, we can't claim certainly about the relationship between Varicocele and infertility. The mechanism of the effect of Varicocele on male infertility is multifactorial, and all of the mentioned factors are involved in the occurrence of this effect (23). In the other hand we found a systematic review and meta-analysis, revealed a relationship between varicocele (as a most common urogenital disorder in male) repair and

infertility based on 2,420 infertile men's data mentioned in sixteen studies (24).

The recent reports for global trends of semen parameters in infertile men showed us significant changes such as sperm count and sperm concentration decrease (25), which can lead us for new studies about this trend in Iran.

Conclusions

There are some controllable factors of male infertility with serious effects on sperm parameters, knowing about them can be the best approach to reduce the infertility rate. In this study we statistically discussed about some of the risk factors for sperm insufficiency, but we recommend more studies with other variable factors, such as different race, wider age spectrum and more environmental factors.

Authors' contributions

All authors contributed equally.

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Conflict of interest

All authors declare that there is no conflict of interest.

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Ethics statement

This study was conducted after obtaining permission from the hospital and approval from the Ethics Committee of Tehran University of Medical Sciences (Ethic code: IR.TUMS.Medicine.Rec.1399.003). All information about the study samples has been and will remain confidential throughout the study, and patient information has been recorded without including first and last names.

Data availability

Data will be provided on request.

Abbreviations

CASA Computer Aided Sperm Analysis

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Author (s) biosketches

Ahmadi S, MD, Labbafinezhad Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
Email: sanaahmadi750525@gmail.com

Shabani Neshati M, MD, Anatomy Department, Tehran University of Medical Sciences, Tehran, Iran.
Email: Maryam.shabani.n@gmail.com

Tavoosian A, Assistant professor, Infertility Department, Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran.
Email: alitavoosian@gmail.com

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