# Nishrin Bohra<sup>1,\*</sup>, Poornima Agrawal<sup>1</sup>, Jishi Joshi<sup>1</sup>, Pooja Gandhi<sup>2</sup>, Kalpesh Gaur<sup>3</sup>, Mahendra Singh Rathore<sup>1</sup>

<sup>1</sup>Department of Pharmacy Practice, Geetanjali Institute of Pharmacy, Geetanjali University, Manvakhera, Udaipur, Rajasthan, INDIA. <sup>2</sup>Department of IVF, Geetanjali Institute of Pharmacy, Geetanjali University, Manvakhera, Udaipur, Rajasthan, INDIA. <sup>3</sup>Department of Pharmacology, Geetanjali Institute of Pharmacy, Geetanjali University, Manvakhera, Udaipur, Rajasthan, INDIA.

### ABSTRACT

Introduction: The study is based on evaluation of impact on sperm quality due to alcohol at tertiary care hospital. There are many risks involved due to addictive substances like alcohol were observed and the data of overall study proves the significant results. Materials and Methods: A non-interventional prospective observational study of six months duration was carried out on outpatients qualifying inclusion criteria. The socio-demographic details, alcohol history, type of beverages and frequency was collected in a specialized data collection. Data collected was analyzed with MS EXCEL sheet and data were represented as mean, standard deviation, p-value, and percentages. Results: Out of 246 majorities of patients 53.25% were in the age group of 28-37 years. The patient who was alcoholic (39.8%) and non-alcoholic (60.1%). The lab parameter for alcoholic were compared to non-alcoholic which elucidates significant difference (p < 0.001) that effect the sperm quality. The type of alcoholic beverages was studied where the increase of types results in decrease of semen parameter the significance (p<0.05). The frequency was measured as mild(monthly), moderate(weekly), and severe(daily) the various parameters show significant difference in value with increased. According to WHO-semen analysis parameters were evaluated. Conclusion: The alcohol consumption was observed as the patterns of types of beverages used and frequency of consumption and that leads to the result that it affects the morphology of sperm and increases the risk of sterility.

Keywords: Alcohol, Male Sterility, Morphology defect, Motility, Semen Parameter.

# INTRODUCTION

Infertility is defined as failure to obtain clinical pregnancy after twelve months or more of frequent unprotected sexual intercourse, is world-wide problem that affects 15% of couples of reproductive ages.<sup>1</sup> An increasing percentage of people suffer from infertility, which is described as the inability to conceive while having regular, minimum yearly sexual relations without the use of contraception. Up to 15% of couples globally, or over 70 million couples who are of reproductive age, are thought to struggle to conceive, with male infertility linked with 50% of cases. Human fertility rates have sharply decreased in industrialized nations during the last few decades. Approximately thirty percent of sub-fertile couples today need artificial fertility treatment to address unsuccessful reproductive attempts due to aberrant semen characteristics. There is no denying the detrimental effects of risk factors like drinking and smoking, and semen is also known to be impacted by obvious malnutrition.<sup>2</sup>



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## Correspondence: Dr. Nishrin Bohra

Department of Pharmacy Practice, Geetanjali Institute of Pharmacy, Geetanjali University, Manvakhera, Udaipur, Rajasthan, INDIA. Email: nisrinhakimji@gmail.com

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Sperm: The majority of animals generate sperm, a male reproductive cell. The testes are where higher vertebrates, particularly mammals, generate sperm. An ovum from the female is fertilized by the sperm to create a new child. The head, neck, and tail are the two distinctive features of mature sperm. An ovum, or egg, that a sperm cell is trying to fertilize via penetrating. Every animal species has a different sperm head shape. It is 4-5 mm long and 2-3 mm wide in humans, flattened and almond-shaped. The head region mostly consists of a nucleus of genetic materials called chromosomes that pass down particular traits to offspring, such as skin, hair, and eye colour.

Only twenty-three chromosomes are present in the sperm cells. The 23-chromosome ovum and 23-chromosome sperm cell combine to form the ensuing 46 chromosomes, which define the traits of the offspring. The X or Y chromosome, which decides the future child's gender, is also transferred by the sperm cells.<sup>3</sup>

# Morphology

The acrosome, a cap-like structure that covers the sperm head, has enzymes that facilitate sperm penetration into an egg. Even though an average ejaculation contains 300 million to 400 million sperm, a single sperm fertilizes each egg. The genetic information in the chromosomes of each egg and sperm generated varies somewhat, which explains the heterogeneity and homogeneity amongst offspring of the same parents.<sup>4</sup>

The mitochondria which provide energy are located in the sperm's tiny central section. Connected to the head and central part of the sperm is a slender, hair-like bundle of filaments known as the flagellum. The tail measures around 50 mm in length, with a thickness of one micrometer at the mitochondria and less than half a micrometer at the end. It waggles and quivers to allow the cell to reach the egg.<sup>5</sup> The women's reproductive tract's sperm deposit causes the activation of tail mobility to be repressed until the sperm are delivered to a short distance from the ovum.

During capacitation, sperm cytoplasm goes through a fundamental shift known as alkalinization, which raises intracellular pH values, particularly in the flagellum. The acrosome response is the process by which sperm can pass through the thick layer that surrounds an egg when enzymes within the acrosome are triggered. The nucleus is subsequently transferred into the ovum when the sperm cells and the egg's membranes merge.<sup>6</sup>

Alcohol consumption has been linked to lower sperm parameters such as volume, quantity, motility, and shape. Excessive alcohol use has been associated to changes in serum hormonal levels, resulting in a reduction in seminal plasma fluid.<sup>7</sup> The relationship between moderate alcohol use and sperm morphology has produced contradictory findings, even though long-term excessive alcohol consumption has been linked to aberrant sperm morphology. Consistent with previous findings, alcohol consumption was linked in the current study to an increase in sperm with aberrant morphology.8 The sperm head is deemed normal if the acrosome, nuclear envelope, chromatin, and plasma membrane are all normal in appearance. The tail is deemed normal if the mitochondria, axonemal components, and plasma membrane are all normal. Infertile men often exhibit axonemal or peri axonemal abnormalities and deficits, which are believed to impair sperm motility. In spermatozoa, DNA damage has been linked to regional nuclear shape distortion.9 The morphologically aberrant nuclei and plasma membranes that were linked to alcohol use varied significantly.

Understanding how these elements as semen volume, total motility, progressive motility, non-progressive motility, immotile sperm, vitality, and normal forms impact sperm quality can help couples undergoing fertility treatments, such as *in vitro* Fertilization (IVF), optimize treatment procedures and raise success rates. Researching how alcohol consumption affect sperm quality and fertility in a tertiary care setting can have a significant positive impact on patient outcomes and treatment planning, as well as public health policy and the promotion of better lives.

# **MATERIALS AND METHODS**

The study included 246 consecutive sperm samples obtained from men who came to IVF Unit for sperm analysis for reproductive objectives. The data was collected in following steps:

Step 1- Primary screening was done based on eligibility criteria. The objective, procedures, and needs of the study were explained to the study participants.

Step 2- If the patients agree to participate, informed consent in the language best understood by the subject was obtained according to the ethical guidelines provided by the institute. [Ethical clearance certificate-Ref: GU/HREC/EC/2023/2374].

Step 3- Prior to semen analysis, a customized data collecting form was completed with information on age, height, weight, medical history, alcohol intake frequency, and type of beverage used.

Step 4- All samples were obtained by masturbation after 3-4 days of sexual abstinence, and semen analysis was carried out following 2021 WHO recommendations.

Step 5- Alcohol assessment was done by questioning about How many drinks were consumed in the last year per month per week or per day was asked and the type of alcoholic beverages consumed such as wine, beer, brandy, sake, whiskey, and champagne.

Step 6- The measurement of alcohol intake was estimated based on.

(1) Frequency: mild(monthly), moderate(weekly), and severe(daily).

(2) type of beverages: one type, two types, three types, and four types of beverages and five types of beverages.

Step 7- Sperm parameter assessment was done by evaluated all variables as potential confounders for the relationship between drinking and IVF results. The WHO-semen analysis scale is instrumental. It consists of 8 parameters: 1. Semen volume (1.3-1.5) ml, 2. Total sperm number (35-40)10<sup>6</sup> / ejaculation; 3. Total motility (42-43) %, 4. Progressive motility (29-31) %; 5. Non-progressive motility (1-1) %; 6. Immotile sperm (19-20) %, 7. Vitality (50-56) %; and 8. Normal forms (3.9-4) %.

Step 8- Finally Statistical analysis was done by compared on a different basis. The first was between types of alcoholic beverages used as 1 type, 2 types, 3 types, 4 types, and 5 types and the second was on the frequency of consumption as mild, moderate, and severe. For which we have used average and standard deviation formulas in Microsoft Excel.

# RESULTS

The total number of patients were 246 in which the majority of patients who visited the IVF department belonged to the 27-38 years of age group which made it above 50%, followed by the 18-27 age (22.6%), the 38-47 age group (21%), the 48-57

years of age group (3%). The maximum patients, 60.1% were non-alcoholic (148 patients) followed by 39.8% with alcohol history (98 patients) average age was 61.3.

The maximum patients, 36.7% consumed one type of beverage (36 patients) followed by 26.48% consuming two types of beverages (30 patients) followed by 23.46% consuming three types of beverages (23 patients) and 13.26% consuming four or five types of beverages (13 patient).

In Table 1, we considered the semen parameter of alcoholic and non-alcoholic whereas the mean (average) and standard deviation was accordingly calculated. The lab parameter for alcoholic was total motility, progressive motility, vitality and normal forms were compared with the lab parameters of non-alcoholic total motility, progressive motility, vitality and normal forms which elucidates significant difference (p<0.001) between the parameters which highly effect the sperm quality.

For the further investigations that how the alcohol exactly being influencing the parameters we studied on types of alcoholic beverages in Table 2, the type of alcoholic beverages consume and their effect are describe where the total motility is seen to reduce with the increase in types of beverages consumption from one type to very less motility in three typer of beverages consumers and the significance (p < 0.05) justify difference in value, the progressive motility compared between one type and three type which has significance of (p < 0.05) in three types of beverages the immotile sperm was high then rest of the categories while it has significance of (p < 0.05) as compared to one type and two type of alcohol consumers, vitality was significantly decreasing with the increase of alcoholic type from one type to five type significant different of (p < 0.05) is observed between one type and three type and normal forms shows significant decline from in one type to in five type while (p < 0.05) signifies between two type and three type of alcoholic beverage consumers.

In Table 3 the frequency: mild(monthly), moderate(weekly), and severe(daily) the various parameters which are studied as comparison which shows different in the frequency association monthly and weekly use of alcohol affects the progressive motility ( $M=28.6\pm13.4$  and  $W=21.5\pm17.3$ ), immotile sperm ( $M=50.2\pm18.9$ 

and W=38.5±27.8) and also vitality of sperms (M=59.1±21.8 and W=44.7±31.9) with the significant difference of (p<0.05) while as the daily consumption significantly increase the non-progressive motility as compared to monthly consumption in sperm and that has a significancy of (p<0.05).

### DISCUSSION

Male factor is common reasons for infertility and evaluating male infertility was crucial for defining infertility treatment techniques and also for men's health may be a predictor of future co-morbidity. Male infertility is caused by a combination of genetic and environmental variables, including smoking, intake of alcohol, psychological and mental stress, substance abuse, exercise and comorbid diseases such as cardiovascular disease, hyperlipidaemia, diabetes, and obesity. Male infertility is the leading health concern, having economic, psychological, and medical consequences. Furthermore, it is characterized by an

Table 1: Alcoholic and non-alcoholic semen parameters.

No. of Man	246	p-value
Age	61.50%	
Semen Volume	2.41±1.4	
Total Motility	37.7±23.7	0.0001
Progressive Motility	24.62±16.5	0.0001
Non-Pro	13.4±7.9	0.0001
Immotile Sperm	44.1±26.1	0.01
Vitality	50.9±29.4	0.0001
Normal Forms	1.5±1.5	0.0001
Non-Alcoholic		
Semen Volume	2.3±1.0	
Total Motility	53.21±18.2	
Progressive Motility	35.8±13.5	
Non-Pro	17.9±6.8	
Immotile Sperm	37.4±14.3	
Vitality	68.2±24.0	
Normal Forms	2.3±1.4	

Table 2: Types of alcoholic beverages comparisons.

Type of Beverages	One	Two	Three	Four	five	<i>p</i> -value
Semen Volume	2.2±1.3	2.4±1.5	2.9±1.7	$1.8 \pm 0.8$	2.2±0.25	NS
Total Motility	42.6±23.7	40.1±25.1	30.6±20.5	35.8±18.9	49.5±4.5	0.04
Progressive Motility	27.4±16.2	25.8±17.1	19±15.6	24.4±14	34.5±1.9	0.04
Non-Progressive Motility	15.4±8.3	14.7±9	11.8±6.2	11.7±5.5	15.5±2.8	NS
Immotile Sperm	41.8±23.6	36.8±23.9	55.2±27.9	53±23.4	50.5±4.5	0.01
Vitality	56.2±28.7	53±32.2	42.2±24.1	53.5±24	66±6	0.04
Normal Form	2.1±1.7	1.6±1.6	$0.8 \pm 0.8$	1.2±0.9	1.5±0.5	0.02
Sample Size	36	26	23	9	4	

Alcohol Status	Mild	Moderate	Severe	<i>p</i> -value
Semen Volume	2.3±1.2	2.6±1.5	2.1±1.5	NS
Total Motility	43.8±17.8	34.2±25.7	33.6±26.5	NS
Progressive Motility	28.6±13.4	21.5±17.3	22.1±18.4	0.04
Non-Progressive Motility	15.2±5.5	12.5±9.1	11.±8.5	0.02
Immotile Sperm	50.2±18.9	38.5±27.8	43.1±30.5	0.03
Vitality	59.1±21.8	44.7±31.9	47.6±33	0.02
Normal Form	$1.7 \pm 1.4$	1.4±1.6	1.4±1.5	NS
Sample Size	38	38	22	

#### Table 3: Frequency of alcohol consumption.

inability to produce enough sperm to fertilize an egg. Dietary nutrients have a significant impact on male reproductive capacity.<sup>1</sup>

In this study the result which is significantly depending on alcohol consumption the factor which we used is different type of beverages consumption where the progressive motility, total motility, immotile sperm, normal forms (morphology) and vitality are defining the deformities caused due to alcohol consumption and a define value which show the decreased motility. All the semen parameter showed declined in valued from one type of alcoholic beverages and five type of alcoholic beverages. The second factor is the frequency of alcohol consumption in which progressive motility, immotile sperm, non-progressive motility, and vitality showed significant difference while the morphological changes are not defined. The mild consumption (monthly) and moderate (weekly) consumption has significant difference in the parameters due to increase in alcohol consumption vitality is highly effected. The decreased in motility was seen in comparison of mild (monthly) and severe (daily) consumption of alcohol. In comparison of total alcoholic and non -alcoholic the semen parameter has significant difference which show the overall deformities caused to the sperm parameters (except semen volume). This shows that alcohol directly affects the morphology of the semen parameters and leads to various structural deformations. Similar results were seen in a study performed by Brooke V. Rossi et al., lower IVF live birth rate was linked to men and women who self-administered alcohol use of as few as four drinks per week in this prospective cohort study,10 and similar results was seen in the study the effect of tobacco smoking and alcohol on semen quality.11-16

# CONCLUSION

The risk factors studied that affect male reproductive health and fertility due to alcohol consumption history. The alcohol consumption was observed as the patterns of types of beverages used and frequency of consumption and that leads to the result that it affects the morphology of sperm and increases the risk of sterility.

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# **CONFLICT OF INTEREST**

The authors declare that there is no conflict of interest.

### ABBREVIATIONS

**IVF:** *In vitro* Fertilization, **ROS:** Reactive Oxygen Species, **ICSI:** Intracytoplasmic Sperm Injection, **SDF:** Sperm DNA Fragmentation, **WHO:** World Health Organization, **BMI:** Body Mass Index.

## ETHICAL CLEARANCE CERTIFICATE

Ref: GU/HREC/EC/2023/2374.

### SUMMARY

The study is based on evaluation of impact on sperm quality due to alcohol at tertiary care hospital. There are many risks involved due to addictive substances like alcohol were observed and the data of overall study proves the significant results. Study of six months duration was carried out on outpatients qualifying inclusion criteria. The socio-demographic details, alcohol history, type of beverages and frequency was collected in a specialized data collection. Data collected was analysed with MS EXCEL sheet and data were represented as mean, standard deviation, *p*-value, and percentages. Results were taken out of 246 majorities of patients the lab parameter for alcoholic were compared to non-alcoholic which elucidates significant difference (p < 0.001) that effect the sperm quality. The type of alcoholic beverages was and the frequency was measured as mild (monthly), moderate (weekly), and severe (daily) according to WHO-semen analysis parameters were evaluated and concluded the alcohol consumption was observed as the patterns of types of beverages used and frequency of consumption and that leads to the result that it affects the morphology of sperm and increases the risk of sterility.

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