A Study of The Impact of Age, Cigarette Smoking and Alcohol Consumption on Sperm Parameters Among Men Presenting for Infertility Screening at Jos University Teaching Hospital

ODEY VE¹, SHINKU F², MOHAMMED MB³, WAZHI MA⁴, NYANGO P⁵, NDEN ZN⁶, IMASA E U⁷, MCNEIL RT⁸, EKWERE E O⁹, NAIDOO V.¹⁰

^{1, 2, 3, 4, 5, 6, 8, 9}Department of Anatomy, Faculty of Basic Medical Science, University of Jos, Jos. Nigeria.

⁷Department of Anatomy and Forensic Anthropology, University of Cross River

¹⁰Department of Family Medicine and Primary Care, University of Witswatersrand, Johannesburg 2050, South Africa.

Abstract- Background

Infertility is a growing concern worldwide, with male factor contributing significantly to couple infertility. The impact of cigarette smoking and alcohol on male infertility is inconclusive, but male age is increasingly recognized as a significant factor affecting sperm parameters and fertility outcomes. The study aimed to investigate the impact of age, cigarette smoking and alcohol consumption on sperm parameters among men who presented for infertility screening at Jos University Teaching Hospital.

Methods

A retrospective study was conducted on 440 folders of men who presented for infertility screening at Jos University teaching hospital for the past 10 years, that is from 2013 to 2022. The objective of the study was to evaluate the relationship between alcohol and cigarette consumption on sperm parameters, and the impact of age on sperm parameters. Data on age. cigarette smoking and alcohol consumption were retrieved from patients' case notes at medical records unit by simple random sampling techniques and analyzed using SPSS software version 23.

Results

Result did not show any statistically significant relationship between alcohol and cigarette consumption on sperm Parameters, meanwhile there was a statistically significant relationship between increase age and sperm parameters.

Conclusion

In conclusion, sperm parameters like count, motility, and morphology did not seem to be substantially influenced by cigarette smoking or alcohol consumption, meanwhile age had effects on sperm parameters.

Indexed Terms- Age, Alcohol, Cigarette, Infertility, Sperm parameters

I. INTRODUCTION

Infertility refers to the inability of a couple to conceive a child despite regular, unprotected sex over a period of one year, there are two types: Primary infertility, this is where couple has never achieved a pregnancy and secondary infertility where a couple has had a previous pregnancy but still struggling to conceive again. [1] Globally, 48 million couples and 186 million individuals are affected by infertility, according to the world health organization [2].

In approximately 30-40% of male infertility cases, the underlying cause remains unidentified, despite semen analysis revealing suboptimal sperm quality. This is often referred to as Idiopathic male infertility [1]. Certain lifestyle and work-related factors, such as poor diet, obesity, substance abuse, and exposure to environmental toxins, can increase a man's risk of experiencing infertility. [3].

While men can still conceive children in their 40s and beyond [4], advancing age can affect their reproductive well-being. As men get older, their testicles undergo degenerative changes, resulting in decreased function of Leydig cells and reduced production of testosterone. This decline in testicular function and testosterone levels can negatively impact sperm production and quality, increasing the difficulty

of conception. [5,6]. Reproductive changes can start in a man's 30s [7], but the specific age threshold for sperm production decline is undefined [8,9], Semen quality, a key measure of male fertility, can be impacted by age and environmental factors. [10,11]. Researchers have found that until the age of 40, the man's age did not seem to have significant defect. After 40, the quality of the semen diminishes, possibly to an extent that leads to in vitro fertilization (IVF) treatment failure. A study by Kidd et al (2001), on "effects of male age on semen quality" revealed that among the methodologically stronger studies, decreases in semen volume of 3%-22%, decreases in sperm motility of 3%-37%, and decrease in percent normal sperm of 4%-18% were likely when comparing 30-year old Men to 50-year-old Men [12]. Most studies examining fertility status suggest a relationship between male age and fertility, but the results are most likely confounded by female partner's age. Among studies that did control for female age, comparisons between Men under 30 and Men over 50 found relative decreases in pregnancy rates between 23% and 38%. A comparison of the various age categories showed that the increased risk for sub fecundity ranged from 11% to 25%. The study concluded that the weight of the evidence suggest that increase male age is associated with decline semen volume, sperm motility, and morphology but not with sperm concentration.

Also, research has consistently shown that cigarette smoking harms the reproductive health and fertility of both men and women [13]. Approximately 37% of men of reproductive age are smokers [14]. According to these studies, tobacco smoking is associated with decreased sperm count, impaired motility and reduced normal sperm morphology. [15,16]. However, some research found no notable difference in standard sperm parameters between smokers and non -smokers. There's still discussion and disagreement about how alcohol and cigarette consumption affect male fertility, sperm parameters and reproductive outcome. However, there is no conclusive agreement about the effects of cigarette smoking and alcohol use on these outcomes and thus no generally accepted guidelines [17] therefore, the aim of these study was to evaluate the relationship between cigarette smoking and alcohol consumption on sperm anomalies.

Relationship Between Cigarette Smoking on Sperm Abnormality

A retrospective study by Martini et al, (2004) on the effects of cigarette smoking on human seminal quality, showed no statistical difference in seminal parameter between the degrees of alcohol consumption, The study concluded that cigarette consumption did not alter the seminal parameters, nevertheless, when patients with this habit were compared to those without these habits, a significant reduction in seminal volume, sperm concentration, percentage of motile spermatozoa and a significant increase of the non-motile viable gametes were detected [18].

Not many studies have been done to implicate cigarette in infertility. However, Augood et al (1998) [19] reported that subjects who smoke before or during an attempt to conceive risk decreasing their fertility when compared to nonsmokers. Men who smoke tend to have decrease sperm parameters since smoking could reduce mitochondrial activity in spermatozoa and decrease fertilizing capacity [20]. Other authors observed that about 6% of moderate and heavy smokers who were evaluated had decreased sperm indices [21].

Alcohol Consumption and Sperm Abnormalities.

Although heavy alcohol consumption has been associated with abnormal sperm morphology, studies regarding the relationship between moderate alcohol consumption and sperm morphology has yielded conflicting results [22,23]. In a similar study on the effects of alcohol intake on sperm quality, it was also the proportion of morphologically revealed that normal sperm cells was significantly lower in those with high alcohol consumption compared with those with moderate alcohol consumption [24]. Moderate and high alcohol consumption were associated with significantly fewer normally shaped nuclei than very low alcohol consumption. Also, significantly few sperm cells in the alcohol consumption were found to have normal plasma membranes than in the very low alcohol group. In addition, alcohol consumption was associated with increased numbers of morphologically abnormal sperm.

A retrospective study on the effects of alcohol consumption on human seminal quality showed no statistical difference in seminal parameter between the degrees of alcohol consumption, The study concluded that alcohol consumption did not alter the seminal parameters, nevertheless, when patients with this habit were compared to those without these habit, a significant reduction in seminal volume, sperm concentration, percentage of motile spermatozoa and a significant increase of the non-motile viable gametes were detected[22].

Sharma et al. in 2001 [24] posited that there is a controversy regarding the effect of alcohol on infertility; whereas some studies have demonstrated an association between alcohol and infertility. It is not clear what amount of alcohol intake relates to an increased risk of infertility. Alcohol consumption has been associated with many negative effects in Men including decreased libido, testicular atrophy and decreased sperm count [25, 26].

Another study by Teijon et al., (2007) on semen quality in a population of volunteers where the effect of smoking, and alcohol consumption were evaluated revealed that there was no statistically significant difference found between smokers and non-smokers or between males who consumed alcohol versus those that did not [27].

II. MATERIALS AND METHODS

Research Design

This is a retrospective cohort study of infertility cases presented at Jos University Teaching Hospital, Jos North Local Government Area, Plateau State, Nigeria over a period of ten years from 2013-2022.

Study Population and Sampling Strategy

The study population consisted of male adults who presented for infertility investigation at Jos University Teaching Hospital. The participants underwent seminal fluid analysis as part of their evaluation. The population was comprised of males aged 18-65 years. All participants were experiencing male factor infertility, defined as the inability to get a fertile female pregnant after one year of unprotected, regular sexual intercourse. All subjects within the age range of 18-65 years who had regular sexual intercourse were

included in the study while those with incomplete results were not included. Seminal Fluid Analyses were done at the microbiology laboratory of the teaching hospital under standard laboratory techniques using WHO criteria which include oligospermia (spermatozoa count below the reference limit of 16 million/ml, azoospermia (absence of spermatocytes), asthenozoospermia (sperm motility below reference limit of 32%) and teratozoospermia (normal forms below the reference value of 4%).

Sample Size

The sample size was calculated using the formula of Dean and colleagues (2013)

$$n = (Za)2 \times P (1-P)/Precision2$$

Where; n = sample size

Z value = 1.96

Precision = 0.05

P = expected prevalence of the condition under study (Obtained from previous research).

50% = 0.5

(1.96)2x 0.5 (1-0.5)

0.052

= 384.16

n = 384.

However, the sample size was increased to 440 to enhance statistical power

Sampling Technique

A simple random sampling technique was used for this study, computer-generated random numbers were used to generate folders of infertile males who presented for seminal fluid analysis at Jos University Teaching Hospital between 2013 to 2022.

Data collection.

A study proforma designed with Microsoft Excel was used to record data. We developed the study proforma through a thorough literature review and expert input

to ensure it accurately captured relevant information. Pilot testing confirmed its face validity and helped refine it. The proforma effectively assessed key variables, including sperm anomalies, infertility duration, lifestyle factors, and demographics. To maintain data accuracy and consistency, we implemented quality control measures, including double data entry and verification.

Data Were Collected as Follows:

Over the 10-year study period, a total of 1017 folders were identified. The annual distribution of folders was as follows:

- 2013: 83 folders (8.2% of total; 83/1017)
- 2014: 81 folders (8.0% of total; 81/1017)
- 2015: 95 folders (9.3% of total; 95/1017)
- 2016: 107 folders (10.5% of total; 107/1017)
- 2017: 105 folders (10.3% of total; 105/1017)
- 2018: 78 folders (7.7% of total; 78/1017)
- 2019: 95 folders (9.3% of total; 95/1017)
- 2020: 82 folders (8.1% of total; 82/1017)
- 2021: 190 folders (18.7% of total; 190/1017)
- 2022: 101 folders (9.9% of total; 101/1017)

The percentage contribution of each year to the total population was calculated using the formula: (Number of folders in a given year / Total number of folders) x 100.

Secondly, to determine the number of folders to select per year, we applied a proportionate sampling approach. The calculated sample size of 440 was allocated to each year based on its percentage contribution to the total number of folders (n=1017). The formula used was:

Number of folders to select per year = (Percentage contribution of year / 100) × Total sample size (440)

The number of folders selected per year was:

- 2013: 35/83 (8% of 440)

- 2014: 35/81 (8% of 440)
- 2015: 40/95 (9% of 440)
- 2016: 48/107 (11% of 440)
- 2017: 44/105 (10% of 440)
- 2018: 35/78 (8% of 440)
- 2019: 40/95 (9% of 440)
- 2020: 36/82 (8% of 440)
- 2021: 83/190 (18.7% of 440)
- 2022: 44/101 (9.9% of 440)

Folders were ranked, labeled, and selected using computer-generated random numbers. This ensured a representative sample for data collection.

Data Analysis

Statistical analysis was conducted with SPSS software, version 23 (IBM Inc., Chicago, IL U.S.A). Analysis of variables was summarized using means and Chi-square (X2) With the level of significance set at less than 0.05 (p<0.05). Frequencies and proportions were used for qualitative variables.

Ethical Considerations

This study was done with ethics approval no. /DCS/IRE/127/XXX1/554 at the Jos University Teaching Hospital. and the research was conducted by management's permission and clearance committee on Research ethics.

Results

Table 1: Age of study population and semen anomalies

	(Age Range in percent)					
SFA Abnormality	25 - 34	35 - 44	45 - 54	55 - 64	χ 2	P

		22/2	- (0			-
Asthenozoo	4(6.	23(3	5(8.	0	47	0.
spermia	80%	9.10	50			0
)	%)	%)			0
						3
Azoospermi	7(11	13(2	5(8.	0		
a	.90	2.10	50			
	%)	%)	%)			
Oligoasthen	7(11	4(6.8	1	1(1		
oteratozoosp	.90	0%)		.70		
ermia	%)			%)		
				·		
Oligoasthen	12(2	12(2	3(5.	0		
ozoospermia	0.40	0.40	10			
	%)	%)	%)			
	ĺ					
Oligospermi	35(5	31(5	8(1	0		
a	9.50	2.70	3.60			
	%)	%)	%)			
	ĺ					
Teratozoosp	2.(3.	0	1	0		
ermia	40%					
)					
Asthenoterat	1.(1.	0	0	0		
ozoospermia	70%					
•)					
		83(1	21(1(1		
TOTAL		41.1	35.7	.70		
	68(1	0%)	%))		
	15.6					
	0)					
·						

Table 1 shows a significant relationship between age and seminal fluid abnormalities with a p value = 0.003. People within the range of 25-34 years had oligospermia as the most common abnormality, followed by Oligoasthenozoospermia. In men within the age range of 35-44 years, asthenozoospermia became more prevalent and oligospermia remained a significant issue, same for age range of 45-54 years where oligospermia was the most common abnormality followed by azoospermia and asthenozoospermia.

Table 2: Effect of alcohol on Cigarette smoking

Alcohol				
Consumption in				
(%)				
(70)				
	YES		2	
CE A A l 114	NO		\mathbf{p}^{χ^2}	
SFA Abnormality	1,0	10/22	-	0.2
Asthenozoospermi	13(22.	19(32.	8.4	0.3
a	10%)	30%)	49	91
Azoospermia	10(17.	15(25.		
	00%)	50%)		
Oligoasthenoterat		3(5.10		
ozoospermia	6(10.2	%)		
	0%)			
Oligoasthenozoos	15(25.	9(15.3		
permia	50%)	0%)		
Oligospermia	40(68.	34(57.		
	00%)	80%)		
Teratozoospermia	0	2(3.40		
		%)		
Asthenoteratozoos		0		
permia	1(1.70			
	%)			
		82(48.		
TOTAL		23%)		
	85(50.			
	00%)			
	,		l	

Table 3: Effect of cigarette on seminal fluid abnormality

Cigarette Smoking in (%) SFA Abnormality	YES	NO	χ	
Asthenozoosperm ia	7(11.9 0%)	25(32.3 0%)	ç	0.2 91
Azoospermia	3(5.10 %)	22(37.4 0%)		

Oligoasthenoterat	5(8.50	4(6.80	
ozoospermia	%)	%)	
(OAT).			
, ,			
Oligoasthenozoos	5(8.50	9(32.3	
permia (OA)	%)	0%)	
Oligospermia	13(22.	61(103	
	10%)	%)	
Teratozoospermia	0	2(3.40	
		%)	
Asthenoteratozoo	0	1(1.70	
spermia		%)	
	33(47.		
TOTAL	60%)	124(21	
		6.90%)	

Tables 2 and 3 present the effects of alcohol consumption and cigarette smoking on seminal fluid abnormality. Despite the fact that more people consumed alcohol and fewer people smoked cigarette compared to those that did not in this study population, there is however no statistically significant difference among them as P>0.05 indicates that alcohol and cigarette did not contribute to their seminal fluid abnormality.

III. DISCUSSIONS

In table 1, The result showed an age-related decline particularly after 35 years, this could be attributed to a number of factors such as; Obesity, sedentary lifestyle, poor diet, exposure to environmental toxins, prolonged exposure to high temperatures, tight inner wears, chronic diseases, and sexually transmitted infections. Meanwhile, in Men within the age range of 55-64 years, most sperm abnormalities were absent or rare, this could imply that; fewer Men within this age range go for seminal fluid screening leading to fewer observed abnormalities, Men in this age bracket might experience a decline in reproductive health, leading to reduced sperm production or quality, making abnormalities less common. Men in this age group might be less likely to attempt fatherhood, potentially due to completed family planning or declining fertility

also, hormonal changes, lifestyle factors, or other agerelated changes might influence sperm quality and abnormalities in this age group, this is in accordance with age-dependent decline in sperm qualities reported by Jung et al, [28] Kidd et al, [29] and Huang et al (2015) [30]. Also, Pasqualotto et al [32] identified an age threshold of > 45 years for sperm concentration and motility reduction. Stone et al [33] reported that sperm concentration declines after 43 years of age and in addition, a decrease in total sperm count and sperm progressive motility were associated with advancing age. In a meta-analysis review, Johnson et al observed age -associated declines in semen volume, percentage motility, normal morphology and unfragmented cells but not in sperm concentration. Many studies have reported a positive correlation between increasing male age and sperm DNA damage doubling from 25 to 55 years of age, Karouch et al [34] studied spermatozoa of a group of aged men and found, despite normal sperm parameters, a significant increase in sperm DNA fragmentation, chromatin decondensation and sperm aneuploidy percentages compared to those detected in a group of young men. These alterations may suggest a link between male aging and changes in the testicular environment, particularly with the increase of reactive oxygen species production by mitochondria, However, the findings of this study contrasted that of Teijon et al, (2007) & Onyebuchi et al (2018) [27,31] which did not show evidence of sperm deterioration with aging. It is surprising that higher percentages 59.5% and 52.7% of semen abnormalities were found in the age groups of 25-34 and 35-44 years, because this represents the active reproductive age group who may be harboring residual infections acquired prior to marital life, it could also be related to the fact that the age of the male alone is not the only determinant of abnormal sperm parameters, exposure to environmental toxins can also result to abnormal seminal fluid analysis. [27,28, 29, 30, 31].

In the current study, cigarette smoking did not contribute significantly to the seminal fluid abnormality of the patients, this findings is similar to that of Martini et al [18]who reported that the effect of cigarette smoking on human seminal fluid quality showed no statistical difference in seminal parameters between the degrees of consumption, also, Joo et al., (2012) [23] reported that semen volume, sperm

count and sperm motility were lower in smokers than in nonsmokers but these difference was not statistically significant. However, some other studies contrasted the current study, Augood et al., (1998) reported that subjects who smoke before or during an attempt to conceive risk decreasing their fertility when compared to nonsmokers, Calgero et al (2003) also reported that men who smoke tend to have decrease sperm parameters since smoking could reduce mitochondrial activity in spermatozoa. Kunzle et al., (2003) [35] reported also that cigarette smoking was associated with significant decrease in sperm density, total sperm count, total number of motile sperms, citrate concentration, the percentage of normal forms was reduced in smokers. The reason there is no statistically significant relationship between cigarette consumption and seminal fluid abnormality in this study could be attributed to the fact that differences in frequency, or amount of alcohol duration. consumption and cigarette smoking might have affected results. Also, multiple factors contribute to seminal fluid abnormalities, making it challenging to isolate the effects of cigarette smoking alone, it is also possible that the study population might have unique characteristics that differ from other populations, limiting its generalizability.

In like manner, the current study did not establish any statistically significant correlation between alcohol consumption and seminal fluid abnormality as men who did not take alcohol also had seminal fluid abnormality, this also corroborates the report of Martini et al., (2004) [18] that alcohol consumption on human seminal quality showed no statistical difference in seminal parameter between the degrees of alcohol consumption. Sharma et al., (2021) [24] posited that there is a controversy regarding the effect of alcohol on infertility, whereas some studies have demonstrated an association between alcohol and infertility. It is not clear what amount of alcohol intake relates to an increased risk of infertility. This is further buttressed by the findings of Joo et al., (2012) [23] that the proportion of morphologically normal sperm cells was significantly lower in those with high alcohol consumption compared with those with moderate alcohol consumption. Meanwhile its possible that genetic traits of the study population may limit the generalizability of the results to other groups or populations.

CONCLUSION

In our study population, sperm parameters like count, motility, and morphology did not seem to be substantially influenced by cigarette smoking or alcohol consumption meanwhile the study established a significant relationship between age and seminal fluid abnormalities.

RECOMMENDATION

Longitudinal studies will be needed to investigate changes in sperm paramters over time within individuals and to examine the impact of lifestyle factors (e.g. diet, exercise) on sperm quality across different age groups. Also, Further studies should be carried out to ascertain what quantity of alcohol and cigarette is sufficient enough to cause abnormality in sperm parameters, and to also know if they are genetic implications making some individuals more vulnerable to effects of alcohol consumption and cigarette smoking than others.

Acknowledgment

We sincerely acknowledge the entire staff of the Department of Medical Microbiology at Jos University Teaching hospital for providing accurate results of seminal fluid analysis, we also appreciate the staff of the records unit of Jos University teaching Hospital who were hospitable to us and helped out in the sorting of patient folders where data were extracted.

REFERENCES

- [1] Bundhun PK, Janoo G, Bhurtu A, Teeluck AR, Soogund M Z S, Pursun M F, Huang. Tobacco smoking and semen quality in infertile males: A systematic review and meta -analysis. BMC 2019; 19(1): 36.
- [2] World Health Organization. Global Status report on alcohol and health. Geneva; 2011.
- [3] Merie Z B, Irshid M, Migdad M, Irshid A B, Mhanna S A. Does cigarette smoking affect seminal fluid parameters? A comparative study. Oman medical Journal. 2013; 28(1): 12.
- [4] Sharma R, Agarwal A, Vikram K, Assidi M, Muhammad A, Turki R F. Effects of increased paternal age on sperm quality, reproductive

- outcome and associated epigenetic risks to offspring. Reproductive biology and Endocrinology. 2015; 19: 13-35.
- [5] Wang C, Leung A, Amiya P. Reproductive aging in the male brown-Norway rat: a model for human. Endocrinology 1993; 133(6): 2773-2781.
- [6] Harman SM, Metter E J, Tobin JD, Pearson J, Blackman M R. Baltimore longitudinal study of Aging. Longitudinal effects of aging on serum and total free testosterone levels in healthy men. J. Clin Endocrinol Metab. 2001; 86: 724-731.
- [7] Amaral S, Ramalho-Santos J. Aging, mitochondria and male reproductive function. Curr Aging Sci. 2009; 2: 165-173.
- [8] Mahmoud AM, Geomaere S, EL-Garem Y, Van Pottelbergh I, Comhaire FH, Kaufman JM. Testicular volume in relation to hormonal indices of gonadal function in community dwelling elderly men. J. Clin Endocrinol Metab. 2003; 88:179-184.
- [9] Sampson N, Untergasser G, Plas E, Berger P. The aging male reproductive tract. J Pathol. 2007; 211: 206-218.
- [10] Cooper TG, Noonan E, Von Eckardstein S, Auger J, Baker H W. World health organization reference value for Human semen characteristics. Human repro update. 2010;16: 231-245.
- [11] Vigeh M, Smith DR, Hsu PC. How does lead induce male infertility? Iran J Reprod med. 2011; 9: 1-8.
- [12] Kidd SA, Eskenazi B, Wyrobek A.1 (2001). Effects of male age on semen quality and Fertility: A review of the literature. Fertility and Sterility, 2001;75(2): 237-248.
- [13] Ramon R, Warli S M, Rasyid N, Atmoko W. Effects of tobacco smoking and alcohol consumption on semen quality and hormone reproductive levels in infertile males: A systematic review and meta-analysis. Pharmacia 2024;71: 1-14.
- [14] Tang SH, Huang WM, Li J J. Effects of cigarette smoking on male fertility: A systematic review. Journal of Men's health. 2019;15(2): e113-e123.doi:10.22374/1875-6859.12.2.4
- [15] Rehman R, Zahid N, Amjad S, Baig M, Gazzaz Z J. Relationship between smoking habits and sperm parameters among patients attending an infertility clinic. Frontiers in physiology. 2019; 10: 1356.

- [16] Mokhtari F, Vahid Dastjerdi M, Ahmadi A. The effects of cigarette smoke exposure on male fertility: A review Journal of Reproductive health. 2020;17(1): 1-11.
- [17] De Jong AM, Menkveld R, Lens J W, Neinhuis S E, Rhemrev J.P. Effect of Alcohol intake and cigarette smoking on sperm parameters and pregnancy. Andrologia. 2014; 46(2):112-117.
- [18] Martini A C, Molina R I, Estofan D, Senestrari D, Ruiz D B. (). Effects of alcohol and cigarette consumption on Human seminal quality. Fertility and Sterilit. 2004; 83:374-377.
- [19] Augood C, Duckit K, Templeton A. Smoking and female infertility: a systematic review and meta-analysis. Human reproduction. 1998; 16(6): 1532-1539.
- [20] Calgero A, Polosa R, Perdichizzi A. Cigarette smoke extract immobilizes human spermatozoa and induces sperm apoptosis. Reprod Biomed Online 2009; 19: 564-5671.
- [21] Gaur D S, Talekar M S, Pathak VP. Alcohol intake and cigarette smoking: impact of two major lifestyle factors on male fertility. Indian Journal of Pathology and Microbiology Mutagenesis. 2010; 53(1): 35-40. https://doi.org/10.4103/0377-4929.59180
- [22] Li Y, Lin H, Li Y. Association between sociopyscho behavioural factors and male Semen quality; Systematic review and meta-analysis. Fertility and sterility, 2011; 95: 116-123.
- [23] Joo K, Kwon Y, Myung S, Kim T. The effects of smoking and alcohol intake on Sperm quality: light and transmission electron microscopy findings. Journal of International Medical Research 2012; 40(6): 2327-2335.
- [24] Sharma A, Minhas S, Dhillo S W, Channa N J. Male infertility due to testicular Disorders. Journal of Clinical Endocrinology Metabolism. 2021;106(2): 442-459.
- [25] Muthusami K R, Chinnaswamy P. Effects of Chronic alcoholism on male fertility Hormones and semen quality. Fertility and sterility. 2005; 79(84):914-924.
- [26] El-Melegy NT, Ali ME. Apoptotic markers in semen of infertile men: association with cigarette smoking. Int Braz J Urol. 2011; 37: 495-506.
- [27] Teijong L M, Garcia F, Serra O, Moragas, M, Rabanal A, Olivares R, Alvarez, JG. Semen quality in a population of volunteers from the

- province of Barcelona. Reproductive biomedicine, 2007;15(4): 434-444.
- [28] Jung A, Schuppe H C, and Schill WB. Comparison of semen quality in older and Younger men attending andrology clinic. Andrologia. 2000; 34(2):116-122.
- [29] Kidd SA, Eskenazi B, Wyrobek AJ. Effects of male age on semen quality and fertility: a review of literature. Fertil Steril. 2001; 75: 237-248.
- [30] Huang C, Baishun Li, Kongrong Xu, Dan L, Jing H, Yang Y, Hongchuang N, Liqing F, Wenbing Z. Decline in semen quality among 30,636 young Chinese men from 2001 to 2015. Fertility and sterility. 2016;107(1): doi: 10.1016/J.fertnstert.2016.09.035
- [31] Onyebuchi A K, Ekwunife CI, Mamah J, Iwe B, Afogu E, Obi V O. Seminal fluid Analysis of male partners of infertile couples in Abakiliki, Ebonyi State, Nigeria. Tropical Journal of Obstetrics and Gynaecology. 2018; 35: 261-265.
- [32] Pasqualotto F F, Sobreiro B P, Hallak J, Pasqualotto E B, Lucon A M. Sperm Concentration and normal sperm morphology decrease and follicle stimulating hormone Level increase with age. British Journal of Urology International. 2005; 96: 1087-1109.
- [33] Stone BA, Alex A, Werlin LB, Mars RP. Age thresholds for changes in Semen parameters in men. Fertil steril. 2013; 100: 952-958.
- [34] Karouch I. Negative impact on sperm genome decays and IVF outcomes after 40 years. Molecular Reproductive Development. 2018; 85: 271-280.
- [35] Kunzle R, Mueller MD, Hangyi, W. Semen quality of male smokers and non-smokers Infertile couples, Fertility and Sterility. 2003; 79:287-291.