OPEN ACCESS

ISAR Journal of Applied Medical Sciences
Abbriviate Tittle- ISAR J App Med Sci
ISSN (Online)- XXXX-XXXX
https://isarpublisher.com/journal/isarjams

OPEN ACCESS

ASSESMENT OF SEMEN PARAMETERS IN RELATION TO TOTAL ANTIOXIDANT CAPACITY AMONG INFERTILE MALE PATIENT IN SELECTED STATES OF NORTHWESTERN NIGERIA

Bitet D. E.[1*], Kumurya AS[2], Isa AA[3]

[1]Rural hospital Fadan Kagoma, Kaduna State ministry of health
[2, 3] Department of medical laboratory sciences, Bayero University Kano.

***Corresponding Author**
Bitet D. E.
Rural hospital Fadan Kagoma, Kaduna State ministry of health.

Article History
Received: 15.11.2023
Accepted: 27.11.2023
Published: 20.12.2023

Abstract: Background: Infertility has become a common public health challenge affecting not less than 15% of marriages globally. Out of the 15% of the global infertility, 40 – 50% of the infertility is due to male factor. **Methodology**: This study analyzed routine samples from patients who consented and were attending fertility clinic in six selected health facilities in the selected states. A total of 383 samples were collected and analyzed using basic semen analysis methods and TAC colorimetric method. **Results:** We found a prevalence of 51.7% abnormal semen parameters, age of between 29 – 37yrs form the bulk of the study subjects, predominantly Hausa/Fulani 254(66.3%) with 41.8% farmers, 76.8% are monogamous mostly 6+yrs old marriage. Significant statistics values were observed between types of infertility, pH and viscosity (0.02 and 0.03) respectively, also between diagnosed infection, liquefaction and viscosity (0.006 and 0.004) respectively. No correlation was found between Total Antioxidant Capacity (TAC) and sperm count ($r = 1$, $r = 0$) both were statistically significant 0.007 ($P<0.05$). Sperm motility and TAC shows negative correlation among the control group with no correlation among the test group ($r = -0$, $r = 0$) statistically not significant. Morphology revealed negative correlation with test group and no correlation with the control group ($r = -0$, $r = 0$) and were statistically significant, pH and liquefaction shows no correlation, viscosity shows correlation with TAC. **Conclusion**: Our result failed to established correlation between Total antioxidant and sperm count, but was able show statistically significant negative correlation between TAC and sperm morphology. Significant statistics values were observed between types of infertility, pH and viscosity (0.02 and 0.03) respectively, also between diagnosed infection, liquefaction and viscosity (0.006 and 0.004) respectively.

Keywords: Total antioxidant capacity, infertility, semen and Oligoasthenoteratozoospermia.

INTRODUCTION

The inability of couples to conceive has become a common health problem that affects 15% of couples worldwide (Albert *et al.,* 2014). Male infertility refers to the inability of a male to achieve pregnancy with a fertile female without interrupting regular sexual intercourse for a year. This accounts for 40-50% of infertility. There are shreds of evidence to show that sperm counts have been declining over the last 50 years, with a consequent increase in male infertility (Albert *et al.,* 2014; Udia and Enokpe, 2015).

Sperm DNA integrity is an important parameter of sperm quality in the diagnosis and treatment of male infertility and the outcome of assisted reproductive procedures (Shamsi *et al.,* 2011). The integrity of DNA in the chromosomes of the spermatozoon is a prerequisite to normal fertilization and transmission of paternal genetic information (Collins *et al*., 2008). In a standard andrology laboratory, the assessment of the sperm quality relies on the World Health Organization guidelines, hence, DNA integrity analysis is a better diagnostic tool of sperm reproductive potential (WHO, 2010; Shamsi *et al*., 2011; Rilcheva *et al*., 2016).

Semen analysis is a baseline test in the investigation of male infertility and remains the cornerstone of the investigation of male infertility (Barratt, 2007; WHO, 2010). WHO standard operating procedures (SOP) must be maintained to evaluate the descriptive parameters of the ejaculate (WHO, 2010). The test provides no insights into the functional potential of the spermatozoon to fertilize an ovum or to undergo the subsequent maturation processes required to achieve fertilization. It is important to understand that while the results may correlate with "*fertility*," the assay is not a direct measure of fertility (Brazil, 2010; Rilcheva *et al*., 2016).

Oxygen stress is a potent mechanism that ends up in spermatozoon harm and male physiological state. Normally, the seminal plasma contains a specialized antioxidant system that provides effective protection against OS (Rilcheva *et al*., 2016).

Several studies have shown a correlation between male infertility and OS. However, a fertility parameter that is not commonly evaluated by most andrology laboratory is the molecular

***Corresponding Author:** Bitet D. E.

assessment of DNA integrity. Sperm chromatin structure is highly organized, consisting of DNA and heterogeneous nucleoproteins (Agarwal *et al*., 2018). The dominant nucleoprotein in the sperm cell is 85% protamines, which replace the histones during spermiogenesis retaining only 15% (Agarwal *et al*., 2018).

Normal chromatin is essential for the transmission of paternal genomic information and it is equally studied and documented that fertility is negatively correlated with damage sperm chromatin or DNA Fragmentation. DNA integrity is an important molecular indicator therefore, essential and critical for the assessment of male infertility Complex (Rilcheva *et al*., 2016; Agarwal *et al*., 2018).

Aside from the DNA fragmentation, aneuploidy is another form of abnormality in sperm DNA, it's also not a routine andrology laboratory test (Balkan *et al*., 2008). Aneuploidy is a condition in which the sperm has an extra autosome (disomy) or a missing chromosome (nullisomy). Human spermatozoa are haploid germ cells (n=23) that contain 22 chromosomes and one sex chromosome, either X or Y (Quilter *et al.,* 2003).

A semen analysis can reveal some potential male fertility problems, and provide some important baseline information giving a direction to understanding the male's patient fertility. However, the results are usually not conclusive (Wang and Swerdloff, 2014). Studies have shown that about 1 in 5 couples who seek fertility treatment have unexplained infertility and in many of these cases, normospermic is observed, but most of them lack DNA integrity (Hamada *et al*., 2012; Wang and Swerdloff, 2014; Rilcheva *et al*., 2016).

TAS2R60 as an example could be a sequence that's thought to possess a vital role in serving to spermatozoon "find" the egg and therefore the sequence CATSPER encompasses a well-documented role in helping the sperm penetrate the egg. While ID3 is important for embryo development, by showing where abnormalities are occurring, therefore, DNA integrity is a way to go (Zorrilla and Yatsenko, 2013).

There are few tests available that examine sperm DNA fragmentation; damaged or broken DNA. These pieces of evidence suggest that DNA fragmentation is a marker for infertility to some extent. This damaged DNA is found in 25% of infertile men with abnormal semen analyses and 5% in infertile men with normal semen analyses. DNA fragmentation tests are mainly used to identify and help reduce the burden of IVF/ICSI failure or early pregnancy loss (Honig, 2017; Pandiyan *et al*., 2017).

MATERIALS AND METHODS

STUDY AREA

The study covered selected states of the Northwest geopolitical region of Nigeria. The region covers the entire seven states of Northwestern Nigeria; Kano, Jigawa, Kaduna, Katsina, Kebbi, Sokoto and Zamfara. The weather is usually dry and the temperature drops at night, the region is largely an agricultural region.

The Northwest region has a population of about 35,915,467 million and the total land mass of 216,029 km^2 (Census, 2006; Adisa, 2011).

The region shares boundaries with Bauchi State in the North East from Kano State, in the North with Niger republic and Niger State through Zamfara State, from Sokoto it also borders with Niger republic in the Northern part of the State. From Kebbi State the region borders with Niger and the Benin Republic, through Katsina, Kaduna and Jigawa state the region borders the Niger Republic in the north, FCT Abuja, Nasarawa, Plateau, Bauchi and Yobe states respectively (Adisa, 2011).

The region is multicultural and multi-ethnic but majorly dominated by Hausa Fulanis and many other minority ethnic groups (Northwestern state, 1968).

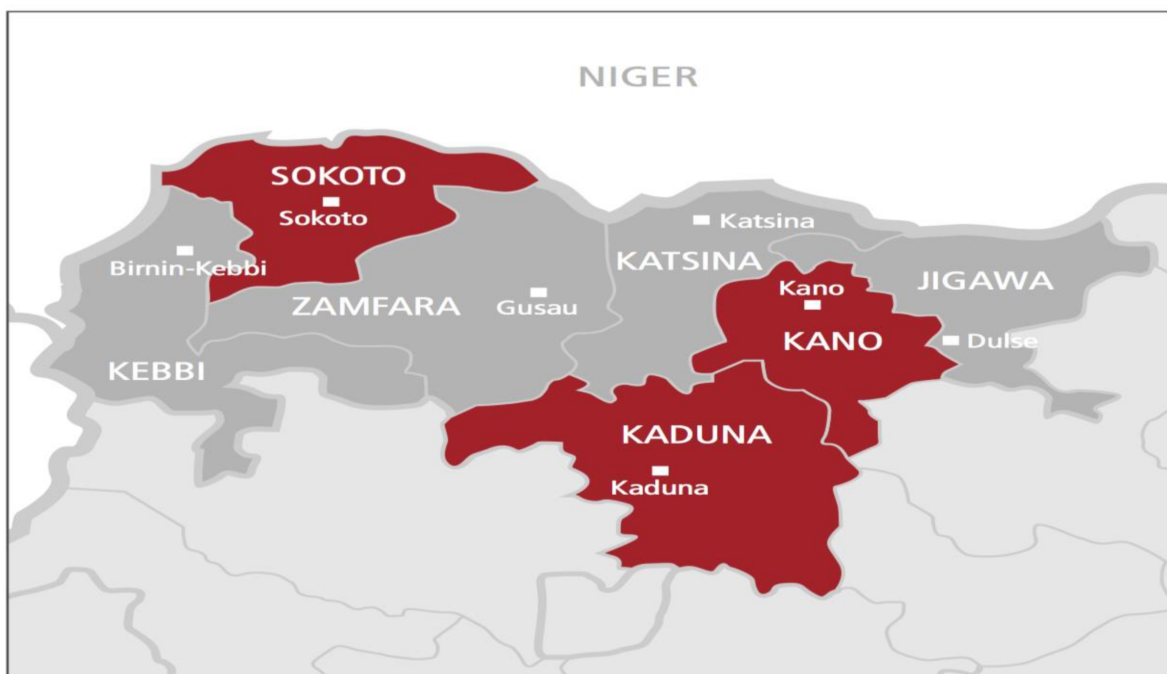

Figure1. Showing states of the Northwest region of Nigeria (Ado 2009).

STUDY POPULATION

The study population consisted of male infertile patients attending any of the health facilities within the selected states in the Northwestern region of Nigeria, who consented and gave their semen sample for the research. The selected states and health facilities are; Kano State: Aminu Kano teaching hospital (AKTH Kano) and Murtala specialist hospital Kano, Kaduna State: Barau Dikko teaching hospital Kaduna and Dr. Gwamna Awan General hospital Kakuri, Kaduna and Jiigawa State: Federal medical Center Dutse and State specialist hospital.

Inclusion criteria

Adult infertile male patients that can produce semen and are attending any of the health facilities within the selected states and are married

Exclusion criteria

Adolescent, singles, fertile males, those that cannot produce sperms (oligospermia) and those who refused to consent are to be excluded.

ETHICAL CONSIDERATION

Ethical approvals were obtained from the ethical committees of the various health institutions. Verbal and written consents were obtained from the volunteers. All clients received an explanation of the study before obtaining informed consent. Well-structured questionnaires were administered to all clients before samples were collected. The entire financial implication of running the tests lies solely on the researcher and any implicating outcome from any participant were appropriately informed and referred for proper counseling and management where necessary.

SAMPLE COLLECTION AND STORAGE

All samples were collected by masturbation into sterile graduated wide mouth containers following 3-4 days of sexual abstinence; equally plain condom were given to those who couldn't restrain self-control during masturbation in order not to compromised the semen volume. After liquidation, a conventional analysis was performed according to the World Health Organization guidelines (WHO Laboratory Manual for the Examination of Human Semen, 2010).

The parameters to be measured are; the volume of the ejaculate (ml), the sperm concentration (x106/ml), the motility (%) and the morphology (% of normal forms). All the semen samples were analyzed with flow cytometry (FCM), a colorimetric assay for total antioxidant capacity (TAC), and PCR for Y-chromosome micro deletion and bacterial presence. Samples from healthy fertile men will be collected and use specifically as controls.

BACTERIOLOGICAL ANALYSIS

Semen analysis

Semen samples of the research participants were collected in sterile wide mouth containers by masturbation and coitus interrupt using plain condoms after 3 – 5 days of sexual fasting. The samples were examined for volume, liquefaction for 20 – 30 minutes at 37^0 C, Ph, sperm count, motility and morphology after staining according to WHO standard guidelines (WHO, 2010).

Semen TAC assay

Sample preparation: The samples were prepared according to the method used by Said *et al*. Following liquefaction, aliquots (300 μl) of each sample was centrifuged at 300 x g for 7 min. The supernatant was separated and re-centrifuge at 300 x g for another 10 min. The seminal plasma will then be frozen at -70˚C until use.

Colorimetric assay for TAC

The colorimetric assay for TAC was performed according to the method of Mahfouz *et al*. frozen seminal plasma was allowed thawing by placing the vials in an incubator at 37˚C for 20 min and then immediately was assess for the TAC. The TAC of the seminal plasma was measured with the colorimetric method using the Cayman's Antioxidant Assay kit (Cayman's Chemicals Company, Ann Arbor, MI, USA). The seminal plasma samples were diluted at 1:10 with the assay buffer before assaying (Omran *et al*., 2013).

All the reagents and samples were allowed to come to room temperature before starting the assay. The samples and Trolox standards were tested twice. The Trolox standards and reagents were prepared according to the manufacturer's instructions at the time of the assay. Trolox standard (10 μl) and the samples were transferred into the corresponding tubes. Then, 10 μl methemoglobin and 150 μl chromogen will be added to all the standard/samples. The reaction was initiated by adding 40 μl hydrogen peroxide immediately. The tubes were incubated for 5 min at room temperature. Absorbance was read at 750 nm using spectrophometer (Thomas Scientific, Swedesboro, NJ, USA). The accuracy and sensitivity of the TAC assay kit was calculated according to the manufacturer's instructions (Omran *et al*., 2013).

STATISTICAL ANALYSIS

Data analysis was performed using SPSS version 22.0 for Windows Software Package (SPSS Inc., Chicago, IL, USA). Data were expressed as percentages and mean ± SD. To identify the independent association between the various seminal parameters and selected variables, independent t test and one way ANOVA was used while Spearman correlation was used to determine the relationship between TAC and semen parameters in conjunction with one way ANOVA.

RESULTS

The distribution of socio-demographic factors among the study population is shown in table1. The age population's shows that those within the ages of 29 – 37 had the highest number of study subjects; 207(54.0%) while ages 58-68 had the least number 3(0.8%). Tribe had the Hausas with the highest population of 187(48.8%) and Igbos with the least number of 25(6.5%). Occupation had farmers with the highest number of 160(41.8%) while others had the least number of 34(8.9%). Type of marriage had monogamy with the highest number of subjects; 294(76.8%) while polygamy had the least number of 89(23.2%). Years in marriage had those who have spent more than six years into their marriages with the highest number of 116(30.3%), while those who are 1 year old into their marriage had the least of 18(4.7%).

Table 2 Show the relationship between risk factors and semen parameters. Independent t test, and one way ANOVA was used to test for significance between the two groups. The mean SD was also determine as seen from the table below. There was no significant relationship recorded among those who smoke, consume alcohol, infection and drug use. ($p > 0.05$). However, significant result was recorded between fertility and PH and viscosity, as well as diagnosed infections and viscosity and liquefaction respectively. ($P < 0.05$).

Relationship between Total Antioxidant Capacity (TAC) and seminal parameters as presented in table 3. Spermans correlation was used to determine the relationship between TAC and sperm count, sperm motility, morphology, PH and viscosity. On the other hand, one way ANOVA was used to determine the relationship between TAC and liquefaction. From the table, sperm count, sperm motility (test group), and PH shows no correlation (0) with Total Antioxidant Capacity (TAC). Sperm morphology (test group), and viscosity suggest a fairly strong negative correlation (-0). There was also no relationship between TAC and liquefaction (p>0.05), while table 4.4 shows the relationship between physical parameters and semen parameters among the study subjects. Normospermia had the highest number of study subjects (184) while Oligoasthenospermia had the least (4).

The mean SD for sperm count (million/L) is 5.54±1.57 and 2.25±1.26; sperm motility had 2.89±0.42 for the control group and 1.35±0.73 for the test group (Normospermia). 2.00±1.16 for the control group and 2.00±1.16 for the test group (Oligoasthenospermia). Sperm morphology had 70.03±12.65 for the control group and 1.30±0.46 for the test group (Normospermia). Then 63.75±7.50 for the control group and 1.25±0.50 for the test group (Oligoasthenospermia). TAC had a mean SD of 4.66±4.40 for Normospermia group and 4.79±3.16 for the Oligoasthenospermia group. The result were all statistically significant (p<0.05) except for TAC which has a p-value greater than 0.05 (p>0.05). Spermans correlation was used to determine the relationship between TAC and sperm count, sperm motility, morphology, PH and viscosity. Sperm count, sperm morphology, suggest a fairly strong negative correlation (-0), while motility (test group), PH.

Table1. Socio-demographic characteristics of participants

VARIABLES	FREQUENCY	PERCENTAGES (%)
AGE		
18 – 28	60	15.7
29 – 37	207	54.0
38 – 48	89	23.2
49 – 57	24	6.3
58 – 68	3	0.8
TRIBE		
Hausa	187	48.8
Fulani	67	17.5
Yoruba	36	9.4
Igbo	25	6.5
Others	68	17.8
OCCUPATION		
Civil servants	110	28.7
Farmers	160	41.8
Artisans	79	20.6
Others	34	8.9
TYPE OF MARRIAGE		
Monogamy	294	76.8
Polygamy	89	23.2
YEARS IN MARRIAGE		
1	18	4.7
2	38	9.9
3	54	14.1
4	68	17.8
5	89	23.2
6+	116	30.3

Table2. Distribution of the risk factors in relation to semen parameters among the study population

		Semen parameters						
Risk factors	N	Sperm count	Vol.	Motility	Morph.	PH	Liq.	Vis.
Smoking								
Yes	38	4.68±2.26	2.66±0.71	1.76±0.94	1.24±0.43	1.13±0.34	1.39±0.68	1.71±1.45
No	345	4.23±2.22	2.76±0.59	1.76±0.93	1.30±0.46	1.18±0.38	1.28±0.53	1.96±1.69
p-value		**0.24**	**0.32**	**0.98**	**0.41**	**0.49**	**0.20**	**0.33**
Alcoholic Consumption								
Yes	79	4.63±2.09	2.81±0.48	1.75±0.94	1.27±0.45	1.10±0.30	1.30±0.63	1.80±1.48
No	304	4.19±2.25	2.73±0.62	1.76±0.92	1.30±0.46	1.19±0.39	1.28±0.53	1.97±1.71
p-value		**0.11**	**0.31**	**0.89**	**0.52**	**0.06**	**0.79**	**0.38**
Drug use								
Normal	369	4.28±2.24	2.75±0.59	1.75±0.92	1.30±0.46	1.18±0.38	1.28±0.54	1.91±1.64
Hard	12	4.08±1.88	2.67±0.78	1.83±1.03	1.33±0.49	1.08±0.29	1.42±0.67	2.25±2.26
Snuff	2	5.50±0.71	3.00±0.00	3.00±0.00	1.00±0.00	1.00±0.00	1.50±0.71	3.50±3.54
p-value		**0.71**	**0.74**	**0.16**	**0.63**	**0.57**	**0.61**	**0.32**
Fertility								
Primary	276	4.28±2.19	2.74±0.60	1.74±0.92	1.31±0.46	1.14±0.35	1.29±0.55	2.05±1.75
Secondary	107	4.28±2.31	2.77±0.58	1.82±0.95	1.25±0.44	1.24±0.43	1.29±0.55	1.64±1.38
p-value		**1.00**	**0.73**	**0.42**	**0.26**	**0.02**	**0.96**	**0.03**
Infection								
Yes	226	4.42±2.17	2.75±0.60	1.79±0.93	1.30±0.46	1.16±0.37	1.31±0.55	1.95±1.66
No	157	4.07±2.28	2.75±0.59	1.71±0.92	1.29±0.46	1.19±0.39	1.26±0.55	1.91±1.67
p-value		**0.13**	**0.95**	**0.41**	**0.94**	**0.43**	**0.44**	**0.84**
Diagnosis								
Yes	4	4.00±2.45	2.50±0.58	1.75±0.96	1.00±0.00	1.00±0.00	1.00±0.00	1.25±0.50
No	303	4.23±2.23	2.75±0.58	1.75±0.92	1.28±0.45	1.18±0.38	1.25±0.53	1.90±1.63
Staph.	53	4.49±2.16	2.72±0.66	1.66±0.94	1.42±0.50	1.13±0.34	1.40±0.63	1.85±1.62
Staph./E.coli	2	4.50±3.54	3.00±0.00	1.00±0.00	1.50±0.71	1.00±0.00	1.00±0.00	1.00±0.00
Chlamydia	6	2.67±2.25	2.33±1.03	2.67±0.82	1.33±0.52	1.50±0.55	1.83±0.41	4.67±1.20
Gonorrhea	2	6.00±1.41	3.00±0.00	2.00±1.41	1.50±0.71	1.50±0.71	2.50±0.71	1.00±0.00
Gonorrhea/Staph.	7	5.29±1.50	3.00±0.00	2.00±1.00	1.00±0.00	1.14±0.39	1.29±0.49	1.57±1.13
Syph. /Staph.	2	7.00±0.00	3.00±0.00	1.50±0.71	1.50±0.71	1.00±0.00	1.50±0.71	1.50±0.71
Chlamydia/Staph.	4	3.75±2.06	3.00±0.00	2.50±1.00	1.50±0.58	1.00±0.00	1.25±0.50	3.50±2.89
p-values		**0.28**	**0.55**	**0.20**	**0.21**	**0.33**	**0.006**	**0.004**

Table3. Correlation between the seminal TAC and semen parameters

	Number (N)	Mean SD	Coefficient of Correlation (r)	**p-Value**
TAC (Mmol/L)	383	4.69±5.05	1	**0.07**
Sperm count (million/ml)	383	4.28±2.22	0	**0.07**
Sperm motility (%)				
Control Group	383	2.70±0.65	-0	**0.27**
Test Group	383	1.76±0.93	0	**0.92**
Sperm morphology (%)				
Control Group	383	65.56±15.74	0	**0.10**
Test Group	383	1.30±0.46	-0	**0.005**
PH	383	1.17±0.38	0	**0.32**

Liquefaction				
Normal	291	4.80±5.02		**0.10**
Abnormal	74	4.86±5.51		
Watery	18	2.18±2.42		
Total	383	4.69±5.05		
Viscosity	383	1.93±1.66	-0	**0.15**

Table4. Different physical parameters and Group of semen

	MEANS SD					
Group (N)	sperm count (million/L)	sperm motility (%)		sperm morphology (%)		TAC (Mmol/L)
		C	T	C	T	
Normospermia (184)	5.54±1.57	2.89±0.42	1.35±0.73	70.03±12.65	1.30±0.46	4.66±4.40
Oligoasthenoter (81)	1.35±0.50	2.49±0.81	2.25±0.92	58.40±20.14	1.41±0.49	4.92±7.13
Asthenospermia (60)	4.13±1.78	2.58±0.74	1.92±0.93	61.33±17.00	1.25±0.44	4.96±5.34
Oligoasthenospe (4)	2.25±1.26	2.00±1.16	2.00±1.16	63.75±7.50	1.25±0.50	4.79±3.16
Asthenospermia (54)	4.69±1.94	2.56±0.69	2.22±0.93	65.87±11.33	1.15±0.36	4.09±2.70
P-value	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.02**	**0.89**

Table5. Distribution of the class of sperm in relation to the locations and among the study population

CLASS (N)	**AKTH** N (%)	**MMSH** N (%)	**BDTH** N (%)	**GAGHK** N (%)	**FMCBKJ** N (%)	**RSSHDJ** N (%)
Normospermia (184)	40(21.7)	36(19.6)	43(23.4)	20(10.9)	28(15.2)	17(9.2)
Oligoasthenoteratospermia (81)	32(39.5)	10(12.3)	9(11.1)	8(9.9)	11(13.6)	11(13.6)
Asthenospermia (60)	17(28.3)	21(35.0)	15(25.0)	1(1.7)	5(8.3)	1(1.7)
Oligoasthenospermia (4)	1(25.0)	0(0.0)	0(0.0)	1(25.0)	2(50.0)	0(0.0)
Asthenoteratospermia (54)	1(1.9)	0(0.0)	0(0.0)	16(29.6)	20(37.0)	17(31.5)
p-value = 0.0001						

AKTH – Aminu Kano Teaching Hospital, MMSH – Murtal Mohammed Specialist Hospital, BDTH – Barau Dikko Teaching Hospital, GAGHK – Gwamna Awan General Hospital Kakuri FMCBKJ – Federal Medical Center Birnin Kudu Jigawa, RSSHDJ – Rasheed Shekoya Specialist Hospital Dutse Jigawa

DISCUSSION

Infertility is a common reproductive public health problem in our country and the world at large. Therefore, the need to find out the male partner's impact in the infertility challenge in our society cannot be over emphasized. The clinical subjective semen analysis still plays a critical role in the diagnosis and management of male infertility. However, précised diagnosis of infertility rarely depends on semen analysis results. There is significance interrelationship between the semen parameters of fertile and infertile men. Hence, there is the need to assess the sperm nuclear integrity in our society.

This study shows that out of 383 infertile males subject recruited for this research work, 199(51.0%) had abnormal semen parameters and 184(49.0%) had normal semen parameters. This finding is similar to some research outcome in Nigeria carried out by Green and Nwachuku; Ugwa *et al*; Chika *et al*; Ikyermum *et al*; Nanna and Unuajowofia; Agu *et al*; Nwafia *et al*. (47.5%, 48.4%, 47.6%, 46.0%, 53.0%, 53.6%, 56.4%) respectively. The outcome

of this research work is however, in contrast with the studies of Ajah *et al*; Owolabi *et al*; Ugwu *et al*. (39.9%, 31.8%, and 74.0%) which are far lower and higher respectively. With abnormal sperm parameters compared to their counterparts with normal sperm parameters, the research outcome among Nigerian men with abnormal sperm parameters corresponds with other findings from other countries such as Norway, Italy, Belgium, Spain, Scotland, France, Tunisia, China, Senegal and Ghana which indicates that sperm parameters of men is on the decline over a period of time (Akang *et al*., 2023; Chukuma, 2022; Keihani *et al*., 2021; Diallo *et al*.,2020; Ajayi *et al*., 2017).

Majority of the subjects fall within the age range of 29 – 37years (54.0%) and the least was within 58 – 68yeas (0.8%), this could be attributed to eagerness, societal factor, active age and dormant or inactive age, this corroborate with the studies of Ogunlaja *et al*., 2022; Selvan *et al*., 2020 and Elbashir *et al.,* 2018. Several findings in Nigeria indicates a steady declining male fertility and this is most likely attributed to nutritional, environmental, socio-economic and demographic factors, this statement correspond with the outcome of this study (Niederger., 2019; Veron *et al*., 2018; Shi *et al*., 2018; Omu, 2013) as shown in table 1 and 2. Globally, the rate of male infertility in North America, Australia, and central and Eastern Europe varied from 4.5 – 6.0%, and 8 – 12% respectively, WHO in her report of 2023 tag the global fertility at 17.5%. But generally speaking infertility rates were highest in Africa and central/Eastern Europe (WHO, 2023; Agarwal *et al*., 2015).

This study reports 72.1% primary infertility and 27.9% secondary infertility. Several studies in Nigeria on male infertility report similar outcomes 83.7% in Lagos (Peter *et al*., 2016), 75.7% in Lagos (Akinola *et al*., 2010), 73.0% in Ibadan (Adeniji.,2003) 62.8% in Ilorin (Omokanye *et al*., 2016), 70.0% Ile Ife (Owolabi *et al*., 2013), 65.0% in Delta (Nanna & Unuajohwofia, 2017), and 54.1% in Abakaliki (Ugwuja *et al*., 2008) which are in conformity with the outcome of this study, this necessitated for DNA assessment to ascertain and specifically identify the cause of the higher rate of primary infertility in the study area. In contrast to this study were reports of primary infertility of 65.0% in Nigeria, 52.0% from Benue and in other developing nations, Sudan (62.4%), Senegal (57.4%) (Ikechibelu *et al.,* 2003, Ogunlaja et al., 2022; Diallo *et al*., 2012; Elussein *et al*., 2008; Al- Turki, 2015).

There was no significant relationship recorded among those who smoke, consume alcohol, infection and drug use. ($P > 0.05$). However, significant result was recorded between fertility, pH and viscosity, as well as diagnosed infections, viscosity and liquefaction respectively with means SD of 1.14±0.35 and 1.25±0.43; P = 0.02 ($P < 0.05$).2.05±1.75 and 1.64±1.38; P= 0.03, 1.10±0.30 and 1.19±0.39; P= 0.06, 1.00±0.00, 1.25±0.53, 1.40±0.63, 1.00±0.00, 1.83±0.41, 1.50±0.71, 1.06±0,00, 1.00±0.00, 1.00±0.00; P=0.006, 1.25±0.50, 1.90±1.63, 1.85±1.65, 1.00±0.00, 4.67±1.20, 1.00±0.00, 1.29±0.49, 1.50±0.71and 3.50±2.89; P=0.004(p > 0.05) respectively. This result is similar to the outcome of Aydos *et al*., 2015 and Akbari *et al*., 2010 and in conflicts with Selvan *et al*., 2020, Luo *et al*., 2020, Al Omrani *et al*., 2018 and Hadwan *et al*., 2013.

There is no association between some of the identified risk factors and semen parameters; including smoking, alcohol consumption and drugs abuse (p>0.05) this finding is in contrast with several other studies that showed a significant relationship between male factor fertility and smoking, alcohol consumption and social life style (Okonofua *et al*., 2022; Kumar *et al*., 2022, Bisconti *et al*., 2021; Boeri *et al*., 2020; Umar *et al*., 2020; Pokhrel *et al.,* 2020; Jurewicz *et al.,* 2014). However, significant association was recorded between fertility, passed infection and PH, liquefaction and viscosity (P>0.004) (Okonofua *et al*., 2022, Kumar *et al.,* 2022; Bisconti *et al.,* 2021; Boeri *et al*., 2020; Umar *et al.,* 2020; Pokhrel *et al*., 2020; Jurewicz *et al*., 2014).

The prevalence of the class of semen parameters based on facility and location, BDTH had the highest number 43(23.4%) and RSSHDJ had the least number of 17(9.2%) among the Normospermia class. AKTH had the highest number of 32(39.5%) while GAGHK had the least number of 8(9.9%) among the Oligoasthenoteratospermia class. AKTH had the highest number of 17(28.3%) and GAGHK and RSSHDJ had the least number of 1(1.7%) among the Asthenospermia class. FMCBKJ had the highest number of 2(50.0%) and MMSH, BDTH, RSSHDJ had none, 0(0.0%) among the Oligoasthenospermia class. FMCBKJ had the highest number of subjects while MMSH, BDTH had none, 0(0.0%) among the Asthenoteratospermia class. The variation in the distribution of result was found to be statistically significant. (p<0.05), this could be as a result of variation in the sample size from each facility, density of environmental factors such as exposure to heavy metals, pesticide and personal lifestyles (WHO, 2023; Kumar and Singh, 2022; Abarikwu, 2013; Swan, 2006 and Hraska *et al*., 2000).

We found a fairly significantly lower seminal fluid total antioxidant capacity (TAC) activity in infertile men. Our finding correspond with Mannucci *et al* and Zunjarrao *et al.,* 2011 who reported that TAC levels is significantly lower in the asthenozoospermic, asthenoteratozoospermic and oligoasthenoteratozoospermic. Mannucci *et al* showed that sseminal plasma TAC in infertile asthenozoospermic and asthenoteratozoospermic male is lower than the fertile men. They also observed a positive correlation between seminal plasma TAC and sperm motility which is in contradiction with the finding of this research which showed a fairly strong negative correlation (-0) between seminal fluid TAC, morphology and viscosity. While on the other hand there was no correlation between seminal plasma fluid TAC and Motility (0). Therefore, low levels of TAC indicate that antioxidants are utilized to detoxify the excessive amount of reactive oxygen species.

Motility and morphology are strong indicators for good or poor sperm function (Agarwal *et al*., 2014). Differences in samples size and method of analysis in other studies may be responsible for the variation in the findings. Ethnic, geographical, environmental, nutritional, endocrine and or life style variation may also contribute to the variation of the results more especially the high temperature of the Northwest region of Nigeria might affect motility of the sperm. The relationship between semen count with morphology and motility indicates a significant association among some semen parameters.

In conclusion, the quality of the spermatozoa is very important in fertilization. Lower levels of TAC are observed among the infertile men. Negative correlation between TAC and sperm count, motility and morphology was observed. Therefore, TAC could be useful as oxidative stress markers and their assays could be used in

identifying sperm quality and can be a guide in antioxidant therapy. Despite the fact that conventional sperm parameters are not all clearly correlated with sperm DNA fragmentation integrity. Hence DNA test should be given a second thought as to be in cooperated in the routine investigation of male fertility test though it might be expensive.

5.6 RECOMMENDATION

1. TAC should be in cooperated into routine infertility investigation as this will help to identify and mitigate the effect of reactive oxygen specie (ROS) and oxygen stress in IVF.

2. in cooperation of DFI in some specific cases where IVF is required to reduce the rate of failure and facilitate prompt achievement of conception.

4.7 LIMITATION OF THE STUDY

The major setback in this study was the inability to obtained enough samples from healthy normal male fertile volunteers to serve as controls but was able to obtain but 20 volunteers, in security and delay in importation of reagents by importers was another setback that delayed the entire research.

CONFLICT ON INTEREST: There is no conflict of interest within the best of my knowledge.

REFERENCES

1. Agarwal A., Mwagund A., Hamada A and Chyatte M.R.(2015). A unique view on male infertility around the globe. *Reproductive Biology and Endocrinology* 13 (37): 15 – 32.
2. World Health Organization (2023). *Global male infertility report*.
3. "Report of Nigeria's National population commission on the 2006 census" (2007). Population and Development Review. *Journal Storage (JSTOR),* **33** (1):206 – 210..
4. Ajayi A.B., Ajayi V.D., Oyetunji I., Biobaku O., Aikhuele H., Adedomilola A., Ayelehin I.I., and Afolabi.(20170). Are semen parameters worsening ? comparing semen parameters 10 years a parts. *Nigeria Medical Journal*. 58 (2): 72 – 75. Doi: 10. 4103/ 0300 – 1652. 219350.PMID. 29269985; PMCID: PMC5726177.
5. Akang E.N., Opuwari C.S., Enyioma – Alozie S., Moungala L.W., Amatu T.E., Wada I., Ogbeche R.O., Ajayi o.o., Aderonmu M.M., Shote O.B., Akinola L.A., Ashiru O. A., and Henkel R. (2023). Male infert5ility in Nigeria and South Africa; A ten years observational study. *Research Square*. 3 (1): 1 – 11. Doi: https//: doi.org/10.21203/rsw-245901/VI
6. Akinyoshi O., Hiroshi O., Sae O., Takashi T., Toshiyaki I., Yoshitomo K., Kazutaka S and Saitawa K. (2021). Evaluation of the sperm DNA fragmentation index in infertile Japanese men by house flow cytometric analysis. *Asian Journal of Andrology* 24(1): 40 – 44. Doi: 10. 4103/aja.aja.49.21.
7. Albert, O., Danie, B., Dan, Y.O., Kweku, B.A. and Frank, A.K. (2014). Semen Characteristics of Male Infertile Couples in the Kumasi Metropolis: A Study of Primary and Secondary Infertile Couples *British Journal of Medicine & Medical Research **4(6)**: 1432-1441.*
8. Andersen A.G.(2000). High frequency of sub-optimal semen quality in an unselected population of young men. *Human Reproduction,* 15: 366b – 372.
9. Balkan M; Tekes S; Gedik A.(2008). Cytogenetic and Y chromosome microdeletion screening studies in infertile males with Oligozoospermia and Azoospermia in Southeast Turkey. *Journal of Assisted Reproductive Genetics* **25**:559–65.
10. Bazil C. (2004a). Standardized methods for semen evaluation in the multicenter & research study. *Journal of Andrology,* **25**:635 – 644.
11. Behre H. M. (2000). Diagnosis of male infertility and hypogonadism. In: NI eschlag E, Behre HM, eds. Andrology, male reproductive health, and dysfunction. Berlin, *Springer*: 92.
12. Bjorndahl l. Barratt C. L. (2000). Semen analysis: Setting standards for the measurement of sperm numbers. *Journal of Andrology,* 26:11.
13. Bjorndahl L.Krist U. (2003). The sequence of ejaculation affects the spermatozoa as a carrier and its message. *Reproduction Biomedicine online*, **7**: 440 – 448.
14. Bonde J.P. (1998). The relation between semen quality and fertility: a population-based study of 430 first pregnancy planners. *Lancet*, **352**: 1172 – 1177.
15. Brazil C. (2004b). Quality control of laboratory methods for semen evaluation in a multicenter research study. *Journal of Andrology,* **25**: 645 – 656.
16. Clinical Laboratory News, 1 January 2019.
17. Chukuma M. (2022). Declining sperm count raises human extinction concern. The Guardian – National 17 November, 2022 at 4.52am https//guardian.ng
18. Chopra S., Varma A., Jain S., Choudhary D. (2021). sperm DNA correlation between fragmentation and conventional semen parameters among different age groups. *Biomedical pharmacology Journal* 14(3): 234 – 241.
19. Cooper, T.G. (2007). Ejaculate volume is seriously underestimated when semen is pipetted or decanted into cylinders from the collection vessel. *Journal of Andrology.* **28**:1 – 4.
20. Esteves S. C and Agarwal A. (2011). Novel Concepts in Male Infertility. *International Brazil Journal of Urology* **37**: 5-15.
21. Festus, A.O., Deji, A., M., Olubunmi, A. O. and Edward, J. (2013).Seminal Fluid Characteristics of Men Attending Infertility Clinic of a Teaching Hospital, *Open Journal of Medical Microbiology*, 3: 1-4
22. Fredlund H; Falk L; Jurstrand M and Unemo M. (2004). Molecular genetics methods for diagnosis and characterization of Chlamydia trachomatis and Neisseria gonorrhoeae: Impact on epidemiological surveillance and interventions. *APMIS*; **112**: 771 – 784.
23. Gosalvez J; Lopez – Fernandez C; Esteves S.C and Johnston S.D. (2015). Unpacking the mysteries of sperm DNA fragmentation: Ten frequently asked questions. *Journal of Reproductive Biotechnology and fertility*; **4**:1 – 16.
24. Hamada A; Sandro C. E; Nizza M, and Agarwal A. (2012). Unexplained Male infertility: Diagnosis and Management. *International Brazilian Journal of Urology*. **38**(5):576-94.
25. Handlesman D. J. (1998). Testicular function in potential sperm donors: Normal ranges and the effects of smoking and varicose. *International Journal of Andrology*, 7:369 -382.
26. Haugen T.B and Grotmol T. (1998). The PH of human semen. *International Journal of Andrology*, 21: 105 – 108.
27. Hofherr S. E; Wiktor A. E; Kipp B. R; Dawson D. B and Van Dyke D. L. (2011). Clinical diagnostic testing for the cytogenetic and molecular causes of male infertility: the Mayo Clinic experience. *Journal of assisted reproductive genetics* **28**: 1091 – 1098.

28. Honig S.C. (2017). Sperm DNA fragmentation testing: A standard semen test or not ready for prime time? *Translational Andrology Urology*. **6**(4):339-340.
29. Hossain A.M. (1998). Time course of hypo-osmotic swellings of human spermatozoa: evidence of the ordered transition between swelling subtypes. *Human Reproduction*, 13:1578 – 1583.
30. Huggin C. (1942). Clinical composition of human semen and the secretion of the prostate and seminal vesicles. *American Journal of Physiology*, 136:467 – 473.
31. Infertile Couples *British Journal of Medicine & Medical Research* ***4(6)**: 1432-1441.*
32. Jouanet P. (1988). Malefactors and the likelihood of pregnancy in infertile couples 1. Studyof sperm characteristics. *International Journal of Andrology,* **11**: 379 - 394 Keihani S., Verilli L. E., Zhang C., Presson A. P., Hanson H. A., Pastuozak A.W., Johnstone E. B., Hotaling J.M. (2021). Semen parameters threshold and time to conception in subfertile couples: how high is high enough? *Human Reproduction*; 36 (8): 121 – 2133. Doi: 10. 1093/humrep/deab133. PMID. 34097024; PMCID: PMC8660554.
33. Kumar M; Selvan P and Agarwal A. (2018). A systemic review on sperm DNA fragmentation in male factor infertility: Laboratory assessment. *Arab Journal of Urology;* **16**: 65 – 76.
34. Larsen L. (2000). Computer-assisted semen analysis parameters as predictors for the fertility of men from the general population. The Danish first pregnancy planner study team. *Human Reproduction,* **15**: 1562 – 1567.
35. Le M.T., Nguyen T.A.T., Nguyen H.T.T., Nguyen T.I.T., Nguyen V.T., Le Dd.d., Nguyen V.O.H., Cao N.T. 920190. Does sperm DNA fragmentation correlate with semen parameters? *Reproductive Medical Biology* 18(4):390–396. Doi.10.1002/rmb2.12297.PMID:31607800.PMCID:PMC6780033.
36. Lu J.C., Jing J., Chea L., Ge Y-F., Feng R. X., Liang Y-J and Yao B. (2018). Analysis of human sperm DNA fragmentation index (DFI) related factors: a report of 1010 subfertile men in China. Reproduction Biological Endocrinology 16.23. https://doi.org/10.1186/s12958 -08 - 0345-y.
37. Macleodd J. Wang Y. (1979). Male fertility potential in terms of semen quality: a review of the past, a study of the present. *Fertility and Sterility*, **31**: 103 – 116.
38. Omran H.M; Bakhhiet M and Dashti M. G. (2013). DNA integrity is a critical molecular indicator for the assessment of male infertility. *Molecular medicine* **7**(5): 1631 – 1635.
39. Majzoub A., Agarwal A. (2021). Antioxidant therapy in idiopathic ologoasthenoteratozoospermia. *Indian Journal of Urology*. 33 (3): 207 – 214. Doi: 10. 4103/iju_15_17. PMID: 25717270: PMCID:PMC5508431
40. Mannucci A., Argento F.R., Fini E., Coccia M.E., Taddei N., Becatti M and Fiorillo C. (2022). The impact of oxidative stress in male infertility. *Frontiers in molecular Biosciences* 8: 799294. Doi: 3389/fmolb.2021.799294.
41. Martins I. (2021). Sperm count declining by 3% among Nigerian males. Thisday 4 february, 2021. https//www.thisdaylive.com.
42. Niederger C. (2019). Re: Impact of age, clinical conditions and life style on routine semen parameters and sperm kinematics. Journal of Urology 201 (4): 652. Doi:10. 1097/01.ju.0000553264.88701.qd.PMID:30653020.
43. North-western State (Nigeria)(1969). Introducing 5the North-western State of Nigeria. Sokoto: Department of information, office of the secretary to the military Governor. Retrieved 6 June, 2023.
44. Omu A.E., (2013). Sperm parameters: paradigmatic index of good health and longevity. Medical principle practice. (Supl 1): 30 – 32. Doi:10. 1159/000354208. Epub PMID: 24051979: PMCID: PMC5586815.
45. Oztekin U., Caniklioglu M., Sani S., Selmi V., Guvel AQ., Isikay L. (2019). Evaluation of male infertility prevalence with clinical outcomes in middle Anatolian region. Cuveus (7): C5122 doi:10. 7759/cureus 5122. PMID: 3152553.PMCID:PMC6741393.
46. Palmer W. J. (2019). Sperm DNA Fragmentation.The New Frontier of Fertility Testing
47. Pandiyan N, Radha Pandiyan R, Raja D.R.(2017). A perspective on sperm DNA fragmentation. *Translational Andrology Urology*; **5**: 935-50.
48. Quilter C. R; Svennevik E. C; Serhal P; Ralph D; Bahadur G; Stanhope R; et al.,(2003). Cytogenetic and Y chromosome microdeletion screening of a random group of infertile males. *Fertility Sterility* **79**:301–7.
49. Rilcheva V.S., Ayvazova N.P., Iieva L.O., Iranova S.P and Konova E.I. (2016). Sperm DNA Integrity test and assisted reproductive technology (ART) outcome. *Journal of biomedical clinical reproduction* **3**: 178 – 181.
50. Rose N.R. (1976). Techniques for the detection of Iso- and antibodies to human spermatozoa. *Clinical and experimental immunology*, **23**:175 – 199.
51. Sellami H, Znazen A, Sellami A, Mnif H, Louati N, et al. (2014) Molecular Detection of Chlamydia trachomatis and Other Sexually Transmitted Bacteria in Semen of Male Partners of Infertile Couples in Tunisia: The Effect on Semen Parameters and Spermatozoa Apoptosis Markers. PLoS ONE 9(7): e98903. doi:10.1371/journal.pone.0098903
52. Shamsi M.B., Imam S.N and Dada R.(2011). Sperm DNA integrity assays: diagnostic challenges and implications in the management of infertility. *Journal of Assisted Reproductive Genetics*; 28:1078 – 1085.
53. Shi X., Chan C.P.S.., Waters I., Chi l., Chan D.Y.L., Li T. C. (2018). Life style and demographic factors associated with human semen quality and sperm function. *Systemic biology reproduction medicine* 64(5): 358 – 367. Doi: 10. 1080/19396368.2018, 149174. PMID: 30033774
54. Simoni M, Bakker E, Eurlings MC, Matthijs G, Moro E, Muller CR, *et al.* (1999). Laboratory guidelines for the molecular diagnosis of Y-chromosomal microdeletions. *International Journal Andrology*; **22**:292–9.
55. Slama R. (2002). Time to pregnancy and semen parameters: a cross-sectional study among fertile couples from four European cities. *Human Reproduction*, 107: 503 – 515.
56. Tandulwadkar S., Babar S.R., Mishra S. and Gupta S. (2022). Prevalence and clinical utility of sperm DNA fragmentation index in couples with un explained infertility. *International Journal of Reproduction, contraception, Obstetrics and Gynaecology* 11(6): 1679 – 1684. Doi: https:// dx.doi.org/10.18203/2320 – 1770. Ijrcog20221439
57. Udia P. O and Enokpe A. M. (2015). Male infertility in Nigeria: A neglected reproductive health issue requiring attention. *Journal of basic and clinical reproductive science* 4(2): 50 – 51.
58. Veron G.L., Tissera A.D., Bello R., Beltramone F., Estofan G., Molina R.I., Vazquel-LEVIN m.h. (2018). Impact of age, clinical conditions and life style on routine semen parameters

and sperm kinematics. *Journal of Fertility Sterility* 110 (1):68 – 75. E4. Doi: 10. E4. Doi: 10. 1016/j.fertstert. 2018.03.016.PMID:29980266.

59. Wang W and Ronald S. Swerdloff R.S. (2014). Limitations of Semen Analysis as a Test of Male Fertility and Anticipated Needs from Newer Tests. *Fertility Sterility*. **102**(6): 1502–1507.
60. Vincenzo D.L., Claudia T., Giuseppe M., Rosetta P., Alice L., Laura G and Paola P. (20220. Positive effect of a New combination of Antioxidants and natural hormone stimulants for the treatment of Oligoasthenoteratozoospermia. *Journal of Clinical Medicine* 1197): 1991. https://doi: org/10.3390/jcm11071991.
61. Westmann A. (20180. Semen analysis: Sperm counting test procedure and results. *Biology, Microbiology*; 124 – 134.
62. WHO (1987). (Prepared by Comhaire F.*)* Towards more objectivity in the diagnosis and management of male infertility. *International Journal of Andrology*,(Suppl.7:22 – 24.
63. WHO (1996). Task force for the regulation of male fertility. Contraceptive efficiency of testosterone – induced azoospermia and oligozoospermia in normal men. *Fertility and Sterility* 65: 821 – 829.
64. WHO (2010). WHO Laboratory manual for the examination of Human Semen and sperm-cervical mouse interaction 5th edition. *Cambridge University Press*.
65. Zheng H-Y., Yan L., Shen F-J and Tong Y-Q(2014). A novel universal multiplex PCR improves detection of AZFc Y-chromosome microdeletions. *Journal of assisted reproduction genetics*, **31**: 613 – 620.
66. Zimanan M.J. (2000). Semen quality and human fertility: a prospective study with healthy couples. *Journal of Andrology*, 21:145 153.
67. Zorrilla M and Yatsenko A. N. (2013). The Genetics of Infertility: Current Status of the Field. *Current Genetic Medicine Reproduction*. **1**(4):13 – 31.
68. Zunjarrao G.B., More K.M., Narshetty J.G., Badade V-Z and Yadav B.K. (2011). Human seminal oxidative stress: correlation with antioxidants and sperm quality parameters. *Annal of Biological Research* 2(5): 351 – 359.

.