Seminal Histone Deacetylase, Fructose and Serum Reproductive Hormones as Diagnostic Marker in Sub-Groups of Infertile Males

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Abstract- Accurate diagnosis of the cause of infertility assists in the choice of treatment modalities and amelioration of the associated psychosocial problems. The research was carried out using 75 infertile males and 75 males with proven fertility as controls. The anthropometrics (weight, height) were measured and body mass index (BMI) computed. Venous blood was collected from each participant, allowed to clot, and centrifuged to obtain the serum which was analysed for testosterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH) concentrations. Semen was collected by masturbation and analysed for sperm quality, seminal fructose concentration, and histone deacetylase (HDAC) activities. A non-significant difference (P>0.05) in weight, height, and BMI; a significant increase (P<0.05) in ejaculated volume, serum LH, FSH, seminal fructose concentrations and HDAC activities; and a decrease in sperm count, sperm motility, serum testosterone concentration was observed among subgroups of infertile men. A direct and significant correlation exists between seminal HDAC activities and fructose concentration. Also, an inverse non-significant correlation exists between HDAC activities and spermatozoa motility. Base on the result obtained from this study, it can be concluded that measuring seminal fructose and HDAC activities in addition to routine biochemical and biophysical parameters will assist in diagnostic work up in subgroups of male infertility.

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Introduction

Having regular unprotected sexual intercourse by couples for a period of a year or more without any conception is not without its consequences ranging from psycho-social, marital disharmony, divorce, and multiple marriages to heavy burdens (1). Infertile men may have deficiencies in spermatozoa formation, concentration (azoospermia, oligospermia), or transportation (due to deficiency of macronutrients for energy production). This general division allows for appropriate workup of potential underlying causes of infertility and help to define a course of action for treatment (2).

Efforts to proffer solutions to identified underlying problems are much appreciated in situations of positive outcomes. Increasingly, couples are turning to assisted reproductive technology (ART) which includes *in vitro* fertilisation (IVF), intracytoplasmic sperm injection (ICSI), and intrauterine insemination (3). The choice of assisted reproductive method adopted for the management of infertile men is dependent on the identified underlying cause of the problem as indicated by physiologically or pathologically related biochemical markers.

Biochemical molecules secreted into the semen by prostate, seminal vesicles and epididymis give information about the functional state of these organs and their concentrations can be used as etiologic and diagnostic predictors in male infertility. Commonly used markers include acid phosphatase as prostate marker, fructose as seminal vesicles marker; zinc, and carnitine as epididymis marker (4); and others including plasma

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gonadotropins, testosterone, lipid, and malondialdehyde concentrations; seminal antioxidant levels; spermatozoa creatine kinase activities; and genetic and epigenetic markers.

There are controversies on the plasma concentration of reproductive hormones (FSH, LH and testosterone) and seminal characteristics (semen quality, spermatozoa motility and fructose concentrations) reported by researchers in subgroups of male infertility. While some researchers reported increase in the plasma and seminal concentrations of these indices of fertility, others reported decrease or no change in the indices among infertile male. Though these markers can help in defining some forms of idiopathic infertility in the diagnostic workup of infertile men, other possible marker could still have roles that could be translated into clinical practice in the management of infertile men. Histone deacetylases (HDACs) have recently been discovered to play important roles in metabolism due to their involvement in glucose homeostasis by modifying histones (5,6). The involvement of HDACs in glucose homeostasis will by extension affect seminal fructose concentration, glucose being the precursor of seminal fructose. The semen contains high concentration of alkaline phosphatase which splits a number of phosphorylated derivatives of fructose including 6-phosphofructose, I-phosphofructose and 1,6-diphosphofructose to phosphoric acid and free fructose. Meanwhile, the energy needed for spermatozoa motility is derived from oxidation of seminal fructose through glycolysis and oxidative phosphorylation (7). Hence, this study aimed to use seminal HDAC activities, seminal characteristics, and plasma reproductive hormones as markers of infertility in sub-groups of male infertility.

Materials and Methods

Study design and sample size

The research was a case control cross-sectional study. The sample size was calculated using the formula of Araoye (2004) and prevalence rate of 11.1% (8,9). A total of 150 adult males comprising of 75 infertile men as test and 75 men with proven fertility as control. It involved the use of questionnaire as instrument to obtain some information from the participants.

Inclusion and exclusion criteria

Included in the study are men diagnosed of primary and/or secondary infertility and men with proven fertility that have consented to be part of the study as test and control, respectively. Excluded from the study are infertile and fertile men that are on drugs (such as cimetidine, cocaine); chronic alcoholics; cigarette smokers; obese; that had undergone vasectomy; and who do not consented to participate in the study.

Physical examination

Physical examination was done with interest in hair distribution; gynecomastia; external genitalia for penile curvature, hypospadias, and surgical scars to exclude possible injuries to the testicular blood supply and/or vas deferens. Manual palpation of the scrotum was done to ascertain presence of testes and determine the testicular size, consistency, presence of testicular mass, or asymmetry, and epididymal enlargement.

Anthropometric measurements

The weight of all the participants was measured using bathroom weighing scale and their height was measured using a height measuring tape. The weight measurements were done with participants asked to remove their heavy garments and shoes; empty their pockets and stands in the centre of a bathroom scale. The weights were recorded to the nearest 0.1 kg.

Height was measured using measuring tape with all participants standing steady or straight on a hard flat surface, their shoes, heavy outer garments, and hair ornaments removed. They were asked to stand with the back of head, back, buttocks, calves, and heels touching the upright; and feet placed together. The height measuring tape was vertically fixed to the wall not bent or curved and the height was recorded to the nearest metre with participant looking straight. The body mass index (BMI) was computed using the formula: $BMI=Weight/Height^2$ (kg/m^2).

Sample collection for analysis

Blood sample

Ten millilitres (10 mL) of venous blood sample was aseptically collected from antecubital vein of each participant using pyrogen-free needle and syringe, transferred into a clean plain labelled tube, allowed to clot, and then centrifuged at 5000 rpm for 5 minutes at room temperature. The clear serum was separated and kept at -20° C till assayed.

Semen sample

Semen was collected from the infertile subjects by masturbation, after haven abstained from sexual intercourse for a minimum of five days and a maximum of seven days, in a private room within the laboratory building to prevent exposure of the semen to fluctuations

in temperature and also to shorten the time between collection and analysis of semen. The collection was done into a clean, dry, wide-mouthed glass container that is non-toxic for spermatozoa. The specimen container was kept in an incubator at a temperature of 30° C. The estimation of sperm counting was done using the Neubauer haemocytometer chamber. Sperm analysis was carried out according to the World Health Organization guidelines (10).

Biochemical parameters

Serum testosterone, follicle stimulating hormone (FSH), and luteinizing hormone (LH) were assayed by Immunoenzymatic method using ELISA kits obtained from Abnova (11,12). Seminal histone deacetylase activity was determined by Colorimetric method using EpiQuik™ HDAC Activity/Inhibition Assay Kit while seminal fructose was determined by method described by Mann (1948) (13).

Ethical approval

The ethical approval was obtained from the Research Ethical Committee of State Hospital, Ifako-Ijaye, Lagos State, Nigeria with approval number IBLGH/P/MED/59/30. Informed consent was also obtained from all participants after the procedures; potential benefits/risks had been explained to them.

Statistical analysis

The statistical analysis was done using SPSS software version 21. Descriptive statistics and bar chart representations were used to describe and represent variables. Independent t-test was used to compare differences in mean between the two groups and one way ANOVA was used to compare differences in mean between more than two groups. The level of statistical significance was set at $P<0.05$.

Results

Result of the anthropometric features of the participants revealed a non-significant difference ($P>0.05$) in weight, height and BMI in subgroups of infertile male when compared with the controls. Among the infertile group, 4% were normospermic, 21.3% were azoospermic, and 74.7% were oligospermic (Figure 1).

Result of the spermatozoa characteristics of the participants revealed a significant increase ($P<0.05$) in ejaculated volume among oligospermic and azoospermic infertile men, a significant decrease ($P<0.05$) in sperm count among oligospermic infertile men and a non-significant decrease ($P>0.05$) in sperm motility among oligospermic infertile men when compared with the controls (Table 2).

Result of serum reproductive hormones revealed a significant increase ($P<0.05$) in serum LH and FSH; and a significant decrease $P<0.05$) in serum testosterone among oligospermic and azoospermic infertile men when compared with normospermic infertile men and the controls (Table 3).

Result of seminal biochemical parameters revealed a significant increase ($P<0.05$) in seminal fructose concentration and HDAC activities among oligospermic and azoospermic infertile men when compared with the controls (Table 4).

Discussion

The anthropometric features of the participants revealed no significant difference ($P>0.05$) in weight, height and BMI in subgroups of infertile male when compared with the controls (Table 1). BMI provides an estimate of total body fat and the risk of developing weight-related diseases. The mean values of BMI in this study were 24.5±4.91 kg/m^2, 26.4±7.4 kg/m^2, 24.8±6.8 kg/m^2 and 23.7±5.0 kg/m^2 for control, normospermic, oligospermic, and azoospermic groups, respectively (Table 1). This implies that none of the participants is obese, obesity being described as BMI > 30 kg/m^2 (14). Thus, any abnormality seen in the infertile men are due to some other factors other than obesity.

Table 1. Anthropometric features of the controls and subgroups of infertile male

Parameters	Control n=75	Infertile n=75			F	*P*
		Normospermia n=3	Oligospermia n=56	Azoospermia n=16		
Age (yr)	41.2±8.5	39.1±7.42	40.6±4.9	42.8±9.2	0.473	0.702
Weight (kg)	65.2±13.3	71.3±17.6	67.3±16.1	70.8±16.5	0.397	0.755
Height (m)	1.69±0.10	1.59±0.09	1.68±0.09	1.69±0.09	3.088	0.034
BMI (kg/m^2)	24.5±4.91	26.4±7.4	24.8±6.8	23.7±5.0	0.471	0.704

Values are mean±standard deviation (mean±SD) and statistically significant at $P<0.05$

Result of sperm count revealed that 4% of infertile men (3 out of 75) were normospermic, 21.3% (16 out of 75) were azoospermic, and 74.7% (56 out of 75) were oligospermic (Figure 1). This corroborates similar proportions of 5.9%, 73.5%, and 20.6% for normospermic, oligospermic, and azoospermic infertile men, respectively reported by Olooto *et al.*, (15). This shows that azoospermia is the most prevalent subtype of infertility seen in male, supporting the work of Zainab *et al.*, but contradicts the work of Owolabi *et al.*, (16,17).

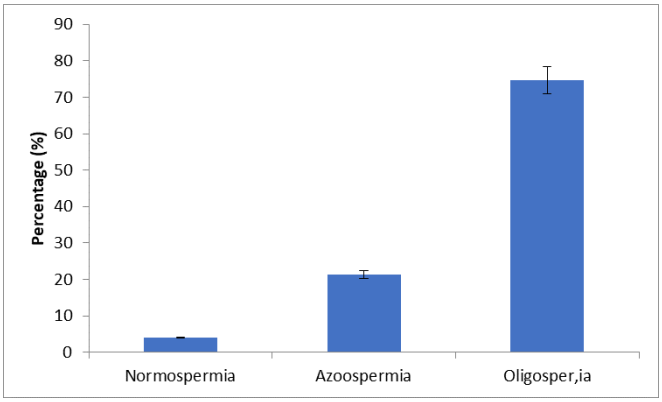

Figure 1. The pattern of infertility subgroups among the studied infertile group. Values are mean ± standard deviation (mean±SD) and statistically significant at $P<0.05$

The normal seminal indices of fertility include sperm concentration of 12-16 million, semen volume 1.4-1.7 mL, seminal fructose ≥13 mmol/ejaculate, progressive motility of 31-34% among others (10). The infertile men were described as normozoospermia (sperm concentration ≥15 billion/mL), oligozoospermia (sperm concentration <15 billion/mL), and azoospermia (no sperm) (10). Result of spermatozoa characteristics showed a significant increase ($P<0.05$) in the ejaculated volume among oligospermic and azoospermic infertile men, a significant decrease ($P<0.05$) in sperm count among oligospermic infertile men and a non-significant decrease ($P>0.05$) in sperm motility among oligospermic infertile men when compared with the controls (Table 2). Azoospermic men with a normal ejaculate volume (1.4-1.7 mL) may have either epididymal/vassal obstruction or an abnormality of spermatogenesis. The observation of semen volume and fructose levels to be within normal ranges indicates a non-obstructive type of azoospermia in the studied infertile men. However, these parameters can also be lower in obstructive azoospermia.

Spermatogenesis is regulated by the hypothalamic-pituitary-testicular axis which produces and secretes gonadotropin releasing hormone, luteinizing hormone (LH), follicle stimulating hormone (FSH) and testosterone (15). These hormones exhibit synergism in the initiation of spermatogenesis; meiotic division and spermatid development; production of inhibin B and spermatozoa; and maintenance of spermatogenesis. Result of gonadotropins obtained in this study revealed a statistically significant increase ($P<0.05$) in the serum mean concentration of LH and FSH and a statistically significant decrease ($P<0.05$) in serum mean concentration of testosterone among oligospermic and azoospermic infertile men when compared with the control (Table 3). These findings are consistent with findings in the previous studies (18-20) but contradicts that of Dhananjay and Roshan (2013) (21). The observed increase in serum mean concentration of LH and FSH is an indication of non-obstructive azoospermic (NOA) infertility in the studied men. However, elevated FSH in azoospermic infertile men does not totally eliminate the possibility of obstruction and the capacity for fertility, as focal normal spermatogenesis has been reported to occur in 50-60% of men with NOA (22,23). The low testosterone concentration positively stimulates the hypothalamic-pituitary-gonadal axis with eventual secretion of gonadotropin releasing hormone from the hypothalamus and gonadotropins (LH and FSH) from the anterior pituitary which stimulate the Sertoli and Leydig cells in the gonads for the production and secretion of testosterone.

The post-coital efficient movement of spermatozoa through the vagina to the fallopian tube to fertilize the ovum has to do with the speed of movement which had been found to be at least 25 μm/s for rapidly progressive sperm movement (24). To achieve this speed, spermatozoa need energy in the form of ATP generated

intracellularly by oxidation of substrates (fructose, glucose, sorbitol, lactate, or pyruvate) through glycolysis and oxidative phosphorylation (7). The vagina is rich in microorganism that conventionally competes with sperm to use glucose. Thus, using fructose removes this competition thereby ensuing availability of ATP, since the bacteria prefer glucose. Seminal fructose was observed to be significantly lower (P<0.05) in the control group when compared with the infertile groups. Among the infertile men, fructose level was noted to be highest in azoospermic group; and higher in oligospermic than the normospermic infertile group (Table 4). This finding corroborates the earlier similar findings by Zahoor *et al.* and Nguyen *et al.*, (25,26). The observed decrease in fructose concentration is due to its use as metabolic fuel to supply required proportionate energy for sperm activities. An inverse relationship thus exists between sperm concentration, sperm motility, and fructose concentration in seminal plasma. The higher the sperm concentration and motility, more fructolysis occurs to generate energy and thus the lower its concentration. Fructose and glucose are convertible metabolites in the generation of energy through glycolytic pathway. The involvement of HDACs in glucose homeostasis predicts its important roles in metabolism. Seminal HDAC activities were observed to be significantly higher (P<0.05) in the control and azoospermic infertile men when compared with other infertile groups. A direct relationship was observed to exist between seminal fructose concentration and HDAC activities.

Correlating spermatozoa characteristics and plasma parameters in the controls and subgroups of infertile male, Pearson correlation revealed a direct and significant correlation (r=0.562, P=0.000) between HDAC activities and fructose concentration implying a strong relationship between HDAC and fructose (Table 5). Also, spermatozoa motility and HDAC activities shows an inverse and non-significant correlation (r= -089, P=0.498) implying a weak relationship between HDAC activities and spermatozoa motility (Table 5).

Infertility in men is not always associated with abnormalities in adiposity indices but other factors which include variations in reproductive hormones (LH, FSH and testosterone), functionality of the hypothalamo-pituitary-gonadal axis, genetic susceptibility and metabolic sufficiency of the seminal fluid. The inclusion of seminal HDAC activities as marker of spermatozoa metabolic sufficiency during infertility workup in men will expand the scope of management in male infertility.

Table 2. Spermatozoa characteristics of the controls and subgroups of infertile male

Parameters	Control n=75	Infertile n=75			F	*P*
		Normospermia n=3	Oligospermia n=56	Azoospermia n=16		
Volume (mL)	1.94±0.46	1.85±0.25*	2.41±0.72*	2.35±0.70*	3.954	0.013
Count x10^6	19.4±3.30	18.0±2.98*	11.0±2.55*	0.0±0.00*	233.94	0.000
Motility (%)	54.7±10.45	53.5±8.31	52.0±6.52	0.0±0.00*	0.247	0.863

Table 2 above shows the spermatozoa characteristics of the participants Values are mean±standard deviation (mean±SD) and statistically significant at P<0.05.

Table 3. Serum reproductive hormones of the controls and subgroups of infertile male

Parameters	Control n=75	Infertile n=75			F	*P*
		Normospermia n=3	Oligospermia n=56	Azoospermia n=16		
LH (IU/L)	6.52±1.15	5.41±1.16	8.64±4.82*	9.25±5.22*	3.143	0.032
FSH (IU/L)	6.52±1.76	5.92±2.27	11.09±2.70*	15.65±4.98*	30.13	0.000
Testosterone (IU/L)	15.35±6.12	8.00±3.74*	5.00±2.24*	6.10±4.10*	18.09	0.000

Values are mean±standard deviation (mean±SD) and statistically significant at P<0.05

Table 4. Seminal biochemical parameters of the controls and subgroups of infertile male

Parameters	Control n=75	Infertile n=75			F	*P*
		Normospermia n=3	Oligospermia n=56	Azoospermia n=16		
Fructose (mg/ml)	3.00±0.82	3.10±0.62	3.26±0.22*	3.91±0.11*	70.66	0.000
HDAC (ng/mL)	3.61±0.49	2.11±0.39*	2.93±0.11*	3.11±0.43*	46.84	0.000

Values are mean±standard deviation (mean±SD) and statistically significant at $P<0.05$

Table 5. Pearson correlation of Spermatozoa characteristics and plasma parameters in the controls and subgroups of infertile male

	BMI	Volume	Count	Motility	LH	FSH	Testosterone	Fructose	HDAC
BMI	1								
Volume	004	1							
P	0.975								
Count	041	-365	1						
P	0.758	0.004							
Motility	067	187	-0737	1					
P	0.613	0.152	0.781						
LH	-032	244	-367	-063	1				
P	0.805	0.061	0.004	0.633					
FSH	-078	390	-778	077	213	1			
P	0.552	0.002	0.000	0.558	0.102				
Testosterone	026	-187	495	-223	-002	-413	1		
P	0.842	0.152	0.000	0.087	0.989	0.001			
Fructose	116	-371	821	032	-173	-654	494	1	
P	0.377	0.003	0.000	0.807	0.187	0.000	0.000		
HDAC	110	-276	805	-089	-187	-632	562	824	1
P	0.405	0.033	0.000	0.498	0.152	0.000	0.000	0.000	

Values are mean±standard deviation (mean±SD) and statistically significant at $P<0.05$

References

1. Rasak B, Oladipo P. Childlessness and Its Socio-Cultural Implication on Married Couples within Some Selected Yoruba Communities in South-West Nigeria. Int J Innov Soc Sci Humanit 2017;5:42-54.
2. Kristen M. Male Infertility. Medspace; 2018 [Online]. (Accessed July 17, 2019, at https://emedicine.medscape.com/article/436829-overview.)
3. Farquhar C, Marjoribanks J. Assisted reproductive technology: an overview of Cochrane Reviews. Cochrane Database Syst Rev 2018;2018:CD010537.
4. de Agostini A, Lucas H. Semen analysis. Geneva, switzerland: Geneva Foundation for Medical Education and Research; 2003.
5. Berger SL. Histone modifications in transcriptional regulation. Curr Opin Genet Dev 2002;12:142-8.
6. Xiaojie W, Xinbing W, Qi P, Fan Y. Histone deacetylases and their inhibitors: molecular mechanisms and therapeutic implications in diabetes mellitus. Acta Pharm Sin B 2012;2:387-95.
7. Misro MM, Ramya T. Fuel/Energy Sources of Spermatozoa. Male Infertil 2012;26:209-23.
8. Araoye OM. Research Methodology with Statistics for Health and Social Sciences. In: Subject selection. Ilorin: Nathadex Publishers; 2004:115-21.
9. Adegbola O, Akindele MO. The Pattern and Challenges of Infertility Management in Lagos Nigeria. Afr Health Sci 2013;13:1126-9.
10. World Health Organization. WHO Laboratory manual for the examination and processing of human semen. 5th ed. Cambridge, Cambridge University Press; 2010.
11. Horton R, Kato T, Sherins R. A rapid method for the estimation of testosterone in male plasma. Steroids 1967;10:245-56.
12. Beastall GH, Ferguson KM, O'Reilly DS, Seth J, Sheridan B. Assays for follicle stimulating hormone and luteinising hormone: guidelines for the provision of a clinical biochemistry service. Ann Clin Biochem 1987;24:246-62.
13. Mann T. Fructose Content and Fructolysis in Semen. Practical Application in the Evaluation of Semen Quality. J Agric Sci 1948;38:323-31.
14. Stefan K. Body Mass Index; 2014. (Accessed September

12, 2019, at https://www.researchgate.net/publication/310447991 Retrieved on 12 September, 2019.)

15. Olooto WE, Amballi AA, Adeleye AO, Mosuro AO. Evaluation of hormonal and physical factors responsible for male infertility in Sagamu South Western Nigeria. Der Pharmacia Lettre 2012;4:1475-9.
16. Zainab KI, Lubna AA, Hayder ALM. Relationship of FSH, LH, DHEA and Testosterone Levels in Serum with Sperm Function Parameters in Infertile Men. J Pharm Sciences 2017;9:2056-61.
17. Owolabi AT, Fasubaa OB, Ogunniyi SO. Semen quality of male partners of infertile couples in Ile-Ife, Niger J Clin Pract 2013;16:37-40.
18. Emokpae MA, Adobor CA. Association of Seminal Plasma Cadmium Levels with Semen Quality in Non Occupationally Exposed Infertile Nigerian Males. J Environ Occup Sci 2015;4:40-3.
19. Ozoemena OE, Mbah J, Esom A, Ayogu E, Ejezie BF. Abnormality of Pituitary Gonadal Axis among Nigerian Males with Infertility: Study of Patterns and Possible Etiologic Interrelationships. Open Access J Urol 2011;3:133-7.
20. Oladosu WO, Biliaminu SA, Abdulazeez IM, Aliyu1 GG, Adelekan A, Okesina AB. Reproductive Hormonal Profile Patterns among Male Partners of Infertile Couples at the University of Ilorin Teaching Hospital. Afr J Infertil Assist Conception 2017;2:6-10.
21. Dhananjay VB, Roshan KM. Evaluation of LH, FSH and Testosterone in Infertile Males. Int J Recent Trends Sci Technol 2013;9:238-40.
22. Hauser R, Temple-Smith PD, Southwick GJ, de Kretser D. Fertility in cases of Hypergonadotropic Azoospermia. Fertil Steril 1995;63:631-6.
23. Esteves SC, Miyaoka RA. Sperm Retrieval Techniques for Assisted Reproduction. Int Braz J Urol 2011;37:570-83.
24. Sikka SC, Ahmet A. Standardized Semen Analysis and Quality Control Management for Multicenter Male Reproductive Toxicology Clinical Trials. In: Sikka SC, Hellstrom WJG, eds. Bioenvironmental Issues Affecting Men's Reproductive and Sexual Health. Massachusetts, United States: Academic Press 2018:571-96.
25. Zahoor A, Muhammad SK, Mudassir AK, Amin H, Jamil R. Seminal fructose in various classes of infertile patients. Pakistan J Physiol 2010;6:36-8.
26. Nguyen TT, Vu TH, Nguyen TTL, Trieu TS. Seminal Fructose Concentration in Man Infertility and the Fructose Test's meaning in Diagnosis Reason of Azoospermia Man. Biomed J Sci Tech Res 2018;8:6270-4.