DOI: https://doi.org/10.53555/nnmhs.v8i4.1209 Publication URL: https://nnpub.org/index.php/MHS/article/view/1209

SEMINAL FRUCTOSE AND CITRIC ACID CONCENTRATIONS RELATIVE TO VIRAL INFECTIONS AMONG MEN FOR FERTILITY EVALUATION IN YAOUNDE, CAMEROON

Esther Voundi Voundi[1,2*], Tanni Bih[3], Serge Nyada[1,2], Eric Nseme[1], Raissa Kamga Nogue[2], Louise Nyimbe Mviena[1,2], Celestin Roger Ayangma[4], Akinyinka Omigbodun[5], Michel Toukam[2,3], Christopher Aimakhu[5]

*[*1] Faculty of Medicine and Biomedical Sciences, University of Yaoundé I, Cameroon*

*[*2] Gynaecological Endoscopic Surgery and Human Reproductive Teaching Hospital, Cameroon*

[3] Pan African University of Life and Earth Sciences Including health and agriculture, Nigeria

[4] Higher Institute of Medical Technology

[5] University College Hospital, University of Ibadan, Nigeria

Corresponding Author: -

vvesther@yahoo.fr

Abstract: -

Introduction: *Nearly 50% of infertile couples' cases are due to male factors. Male infertility is pluri-etiological and as such, it is important to understand the role of seminal plasma novel biomarkers and viral infection in male infertility. The main objective of this study was to compare the fructose and citric acid levels in men with HBV, HCV, HIV amongst those seeking fertility evaluation.* ***Methods:*** *A prospective cross-sectional study was performed on consenting male participants. The semen samples from patients were collected properly and analyzed according to the World Health Organization-2010 manual. Later samples were assayed for biochemical markers and viral antigens and antibodies following their standard protocols. Statistical analysis of the findings was performed using IBM SPSS-24.0 software. Significant statistical difference between median sperm parameters of both groups of men and biomarker levels were considered at $p < 0.05$.* ***Results:*** *the prevalence of hepatitis B, C and Human Immune deficiency Virus were 6.3% (5), 2.5% (2) and 3.8% (3) respectively. The study highlighted that there is no significant difference in the fructose and citric acid levels amongst HBV, HCV, and HIV patients ($p > 0.05$).* ***Conclusion:*** *The assessment of biochemical markers of seminal fluid for fertility evaluation didn't give a clear understanding of the effect of viral infection on the accessory glands. It important to investigate in a larger population to have more pertinent information.*

Keywords: - *fructose, citric acid, viral infections, male infertility*

INTRODUCTION

Infertility is characterized by the failure to establish a clinical pregnancy after 12 months of regular and unprotected sexual intercourse [1]. Recent studies have highlighted that 14%–30% of couples of reproductive age suffer from infertility and nearly 50% of the cases are due to male factors [2]. Male infertility is considered complicated due to its pluri-etiological nature like testicular cancer, smoking, advanced age, abnormal functioning of accessory sex organs, infections. The physiology of the reproductive organs of affected men leads to altered sperm parameters and biomarkers which are key attributes for the evaluation of male infertility [3-5]. Determination of the seminal biomarkers such as fructose and citric acid serve in the evaluation of male infertility [6-8]. Fructose is the principal source of energy for spermatozoa and is essential for spermatozoa metabolism, progressive spermatozoa motility and viscosity [4]. Evaluating the fructose levels in seminal plasma, therefore, is an indicator of the status of seminal vesicles, endocrine anomalies, and ejaculatory duct obstruction. Citric acid, on the other hand, is an organic substance secreted by the prostate primarily to maintain pH and to convert protein, fat, and sugar into carbon dioxide. Determination of the levels of this vital biochemical constituent provides information on the condition of the prostate, coagulation and liquefaction of semen [2]. Toragall highlighted that the most elevated fructose concentrations were in men with azoospermia, oligoasthenozoospermia, asthenoteratozoospermia, oligospermia and severe oligoasthenoteratozoospermia [2]. Idrees' findings showed a positive correlation between sperm count and motility with citric acid concentrations [5]. Viruses such Hepatitis B Virus (HBV), Hepatitis C Virus (HCV) and Human Immunodeficiency Virus (HIV) have been associated to male infertility as they impair the physiological characters of the sperm and the reproductive organs [4, 5]. They have been detected in the semen of men with testicular, accessory gland and urethral infections [9]. Their presence in semen has been associated with poor sperm quality and decreased sperm concentration, motility and vitality [10]. People living with HIV suffer from severe orchitis, low serum testosterone levels, high levels of LH and FSH, suggesting testicular impairment [9, 11]. HBV infection also alters sperm concentration, motility, morphology, and viability, thus reducing male fertility [11]. However, their effect on seminal biomarker concentrations remains unclear. The main objective of this study was to compare the fructose and citric acid levels in men with HBV, HCV, HIV amongst those seeking fertility evaluation.

Methods

Study design and location

A prospective cross-sectional study was performed on consenting male participants who met the inclusion criteria. This study was conducted in the semiology service at Laboratoire d'Excellence (LABEX) of Yaounde, Cameroon. LABEX is a privately owned laboratory, certified, accredited and equipped receiving many referrals from district hospitals, health centers, other laboratories, or voluntary patients.

Study population

Study participants were recruited among male adult attendants (21 years and above) presented for Semen Fluid Analysis at LABEX, Yaoundé. Most of these men were referred from different laboratories, hospitals, Urology and Gynecology clinics within and outside Yaoundé. Were excluded men non-compliance to sample collection conditions and those with hypospermia (<1.5ml).

Semen collection and analysis

The fifth edition of the 2010 World Health Organization (WHO) laboratory manual for the examination and processing of Human Semen, stipulates a minimum of 2 days and a maximum of 7 days of sexual abstinence prior to semen collection. Additionally, subjects were instructed to wash their hands with soap, urinate and wash the glans penis and coronal sulcus with soap and water. Samples were collected in a private room near the laboratory, by masturbation directly into a 100ml sterile container, conveyed to the laboratory and analysed within one hour after ejaculation. The samples were labelled, precising the date, time of collection and patient code. Analysis was done immediately after semen liquefaction (37°C or room temperature) and following WHO recommendations. Samples were assessed for several parameters such as volume, pH, sperm motility (progressive and non-progressive), vitality, morphology, concentration, and count. Samples were later stored at -20°C not more than two hours later for subsequent assay of fructose and citric acid. These parameters were assessed using their specific procedures. For the measurement of total semen sample, the sample was aspirated into a graduated cylinder and total volume read off. The motility and vitality were later assessed under a light microscope. 20 µl of well-mixed semen was placed on a clean glass slide and covered with a coverslip. The preparation was immediately examined at a magnification of X10 and X40 during which the motile (PR, NR) and immotile sperms were counted systematically in different fields and expressed in percentages. Sperm vitality was assessed by adding 1 drop of Eosin and 2 drops of Nigrosine to 50 µl of semen in an Eppendorf tube, mixing thoroughly, then pipetting 20 µl onto a clean slide. It is covered with a coverslip and all live sperm (pink) and dead sperm (white) in five random fields are counted. The vitality was expressed as a percentage of the total number of spermatozoa. Sperm morphology was assessed by obtaining a thin smear of 50µl of well-mixed semen on a clean glass slide. The sperm smear is stained according to the guidelines of the sperm differentiation reagents and observed under a microscope at 100x. Abnormal sperms are counted and classified according to WHO manual recommendation and an abnormality index is calculated. During the assessment for sperm concentration, 50µl of well-mixed semen was diluted in 2450µl of semen diluent solution. Using the improved Neubauer hemacytometer chamber, an aliquot of the mixed diluted sample was loaded into the chambers, and full sperms (with tails) counted to determine the sperm concentration.

The total concentration shall be calculated by multiplication of counted sperm to the dilution factors. Total sperm count is a product of sperm concentration and total semen volume. Not more than two hours after analysis of parameters, about 0.5ml of seminal fluid or plasma was transferred into cryotubes, labelled with codes and dates, and stored at -20°C. This single volume of seminal fluid or plasma was used later for determination of fructose and citric acid levels. They were assayed in batches using microplates.

Classification of normal and abnormal sperm parameters

After determination of parameters as described above, the participants were divided into two groups following the fifth edition of the WHO manual, published in 2010 [9] The reference values for each parameter are clear stated and were diligently followed for the classification of participants as normospermia men and men with abnormal parameters (Table 1). Six classes of abnormal sperm parameters were used following the WHO manual as illustrated in the table below (Table 2).

Table I: 2010 WHO reference values for normal sperm parameters

Parameter	1992 WHO reference values	Lower Reference Limit 2010
Seme volume	2 ml	1.5 ml
Sperm concentration	20 M	15 x 10^6 / ml
Total sperm number		39 x 10^6 / ejaculate
Progressive motility	> 50%	32 % A
Total motility		40 % A+B
Vitality (live sperms)		4 5
Sperm morphology	>15 %	4 %
pH	≥ 7.2	≥7.2
Leucocyte	<1 M	< 1x 10^6 / ml
MAR/ Immunobead test	< 10%	< 50%

Table II: Classification of abnormal sperm conditions

Abnormal sperm conditions	Lower reference limit
Asthenozoospermia	PR < 32%, total motility < 40%
Azoospermia	0 spermatozoa / ml after centrifuging sample
Cryptozoospermia	≤ 1 x 10^5 spermatozoa / ml
Oligospermia	< 15 x 10^6 spermatozoa / ml
Oligoasthenozoospermia	< 15 x 10^6 spermatozoa / ml and PR < 32%, total motility < 40%
Cryptoasthenozoospermia	≤ 1 x 10^5 spermatozoa / ml and PR < 32%, total motility < 40%

Determination of seminal fructose and citric acid levels

Seminal fructose levels were detected using the Fructose-Sperm 360® kit and a spectrophotometer. The principle of the fructose test was that fructose reacts, in the presence of HCl under heat, with indole and produces a colored complex which can be measured at a wavelength of 450-492nm. All samples were left to defrost, reach room temperature, and were centrifuged at 3000rpm for 15minutes to obtain seminal plasma. Then, 100µl of seminal plasma was mixed thoroughly with 500µl fructose TCA reagent and centrifuged at 3500rpm for 10minutes. 20µl of the clear deproteinized supernatant was mixed with 200µl of 32% HCl and 20µl of indole under a fume hood. After 60 minutes of dry incubation, 200µl of NaOH stop solution was added, then 200µl of the mixture was distributed into microwells and OD values read at 450nm from the absorbance microwell reader. The measured value (OD) for the sample was plotted against the standard curve with the OD on the Y axes and the concentration of fructose on the X axes. To obtain total fructose levels, the result is multiplied with the total volume of the semen sample or seminal plasma. Normal values according to the WHO manual are 2.4mg/ejaculate or more and 13µmol/ejaculate or more. Low fructose in semen is characteristic of ejaculatory duct obstruction, bilateral congenital absence of the vas deference, partial retrograde ejaculation and androgen deficiency [12].

Seminal citric acid levels will be detected using the Citric Screen® kit and a spectrophotometer. The Citric Acid test works in two steps: Firstly, spermatozoa and particles are removed by addition of isopropanol. Next, after centrifugation, ferric chloride is added to the supernatant. The Fe3+-ions and citrate form a complex that turns the solution to a pale green color. The intensity of the color is directly related to the amount of citrate and can be measured in a photometer or plate reader. All samples were left to defrost, reach room temperature, and were centrifuged at 3000rpm for 15 minutes to obtain seminal plasma. Then, 100µl of seminal plasma and 100 µl of isopropanol were mixed, centrifuged at 4200 rpm for 15 minutes. Exactly 5 drops of ferric chloride reagent were added to 100 µl of supernatant distributed in the microwells and absorbance was measured at 400 nm. The measured value (OD) for the sample was divided by the OD from the standard and multiplied by the concentration of the standard (4mg/ml). To obtain total citric acid amounts, multiply the result with the total volume of the semen sample or seminal plasma. Normal citric acid value is 10mg or more per ejaculate.

Determination of HBV, HCV, HIV

HBV, HCV, and HIV were tested using Lab Rapid HBsAg kit®, Lab Rapid AbHCV®, Determine HIV Rapid test® respectively. Samples and reagents were kept at room temperature at the time of testing. The reaction plate was removed from the protective envelope, identified properly, and placed on a horizontal surface. 3 drops (0.075 mL) of serum or plasma, with the aid of a pipette were added to the sample well (S) and the results were read between 15 and 30 minutes. Positive (reactive) tests results were marked after formation of a colored line in the control region (C) and in the test region (T). Negative (non-reactive) tests results were after formation of a colored line in the control region (C) and absence of line in the test region (T). Invalid results were marked in the absence of a line in the control region (C). Such tests were repeated with a new plate and reaction strip.

Study variables

Information on sociodemographic factors, HIV, HBV, HCV infections, spermogram results and seminal fluid biomarker concentrations were collected using an approved and verified information form.

Statistical analysis

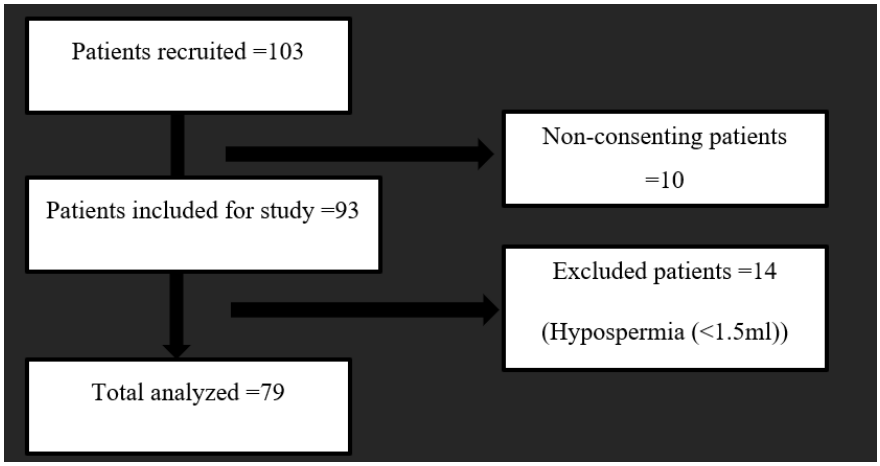
Statistical analysis of the findings was performed using IBM SPSS-24.0 software. Data was expressed in mean (SD), median (IQ), and frequency. Descriptive statistics was used; comparison between variables with the Mann Whitney test, and relation test was done using the Spearman correlation. Significant statistical difference between median sperm parameters of both groups of men and biomarker levels were considered at $p < 0.05$.

Ethical considerations

Ethical approval was obtained from the local institutional Review Board: University of Ibadan/University College Hospital Ethical Committee (UI/UCH EC) and Cameroon National Ethical Committee of Research for Human Health (NECRHH). Signed consent forms were collected upon acceptance to participate in the study. Access to any data was restricted only to the members of the study team who stored the data in a secure database. Confidentiality was respected. The results of the study have been used for scientific purposes only.

Results

During this study period, a total of 103 patients visited the laboratory for SFA. Of all these 103 patients, 24 were excluded; 10 refused to participate in the study and 14 had hypospermia (see figure1).



Profile of our study population

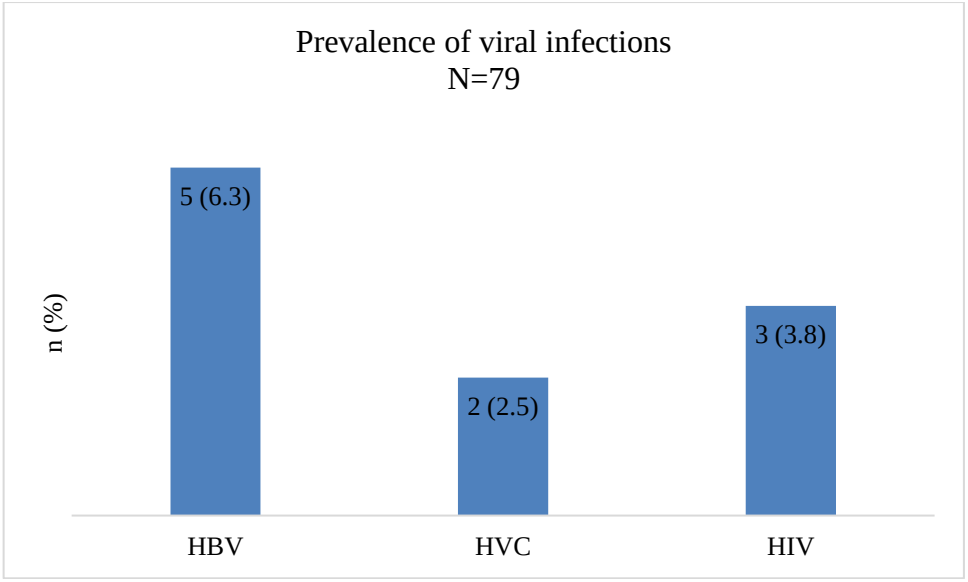
The study revealed that the ages of the participants varied from 26-71 years old with the average age being 42.50 ± 9.44 years representing 43.0% (34) of the sample. The tertiary educational level (66; 83.5%) and the professional occupation (51; 64.6%) were the most represented. Most participants were married (40; 50.6%) or cohabiting (34; 43.0%) with only (5; 6.4%) being single. Nearly half of the study population had an infertility (35; 44.3%); amongst these were primary and secondary infertile men with prevalence of 40% (14) and 60% (21) respectively. Concerning spermatic abnormalities, severe oligospermia, asthenospermia and necrozoospermia were seen in different proportions in our study population, 22.8% (18), 27.8% (22) and 32.9% (26) respectively (see table III).

Table III: distribution of characteristics in our study population

	Frequency (n)	Percentage (%)
Age (years)		
[25 – 35[	13	16.5
[35 –45[	34	**43.0**
[45 – 55[	24	30.4
[55 – 65[	7	8.9
≥75	1	1.3
Educational Level		
Primary	3	3.8
Secondary	10	12.7
Tertiary	66	**83.5**
Occupation		
Professional	51	**64.6**
Unskilled	20	25.3
Unemployed	8	10.1
Marital Status		
Single	5	6.4
Married	40	**50.6**
Cohabitation	34	43.0
Infertility		
Yes	35	44.3
No	44	**55.7**
Type of infertility (N=35)		
Primary	14	40.0
Secondary	21	**60.0**
Oligospermia		
Severe	18	22.8
Moderate	6	7.6
Mild	7	8.9
No	48	**60.8**
Asthenospermia		
Yes	22	27.8
No	57	**72.1**
Necrozoospermia		
Yes	26	32.9
No	53	**67.1**

Viral infections

Concerning viral infections, the prevalence of hepatitis B, C and Human Immune deficiency Virus were 6.3% (5), 2.5% (2) and 3.8% (3) respectively (see figure 2).



HBV= Hepatitis B virus, HVC= Hepatitis C virus, HIV= Human Immune deficiency Virus

Figure 2: repartition of viral infections in our study population

Seminal fructose and citric acid levels

The study highlighted that there is no significant difference in the fructose and citric acid levels amongst HBV, HCV, and HIV patients (p >0.05) (see table IV).

Table IV: evaluation of seminal fructose and citric acid levels relative to viral infections

		Median	Minimum	Maximum	F test	p value
Seminal fructose (mg/mL)						
HBV						
	Positif	10,27	3,28	21,67	0,406	0,526
	Negatif	11,71	2,01	49,63		
HCV						
	Positif	15,46	13,74	17,19	0,080	0,778
	Negatif	10,96	2,01	49,63		
HIV						
	Positif	10,25	3,91	38,41	0,572	0,452
	Negatif	11,71	2,01	49,63		
Seminal citric acid (mg/ml)						
HBV						
	Positif	16,20	6,33	36,11	3,795	0,055
	Negatif	11,25	2,53	33,11		
HCV						
	Positif	8,86	6,33	11,40	0,555	0,459
	Negatif	11,46	2,53	36,11		
HIV						
	Positif	8,33	3,90	15,25	0,702	0,405
	Negatif	11,43	2,53	36,11		

HBV= Hepatitis B Virus, HCV= Hepatitis C Virus, HIV= Human Immune deficiency Virus

Discussion

The issue of male infertility is gaining more attention, as it should, especially in Africa where it is assumed that the men are 'powerful'. However, this global public health problem affects about 48.5 million couples, with the percentage of infertile men ranging between 2·5% to 12% [13]. In addition to this, the prevalence of HIV infection in sub-Saharan Africa is 39% while that of hepatitis B is between 5-8% and mainly in West and Central Africa [14-16]. Many animal and human viruses are disseminated via semen, but there is insufficient knowledge as to how exactly these microorganisms affect sperm composition and functioning, and hence their role in male infertility. As such, a comprehensive evaluation of male infertility could uncover serious and potentially lethal underlying medical conditions. The main objective of this study was to compare the fructose and citric acid levels in men with HBV, HCV, HIV amongst those seeking fertility evaluation. It was observed that the prevalence of men with abnormal sperm parameters amongst the study population was 44%. Amongst these were primary and secondary infertile men with prevalence of 40% and 60% respectively. Benksim in 2018 conducted a study in Morocco and had prevalence values for primary and secondary infertility to be 32.63% and 67.37% respectively [17] which are like the results from this study**.** However, Kbirou and al in 2021 concluded that primary infertility was the most common type among all infertile male patients in his study region; they have noted that infertility was primary in 88 % of cases [18]. These discrepancies could be due to the different study locations. Our findings show that there is no significant difference in the median fructose and citric acid levels in men with HBV, HCV, and HIV except for citric acid levels amongst HBV patients. This highlights the role viral infections play on accessory organs and sperm parameters as seen in the studies conducted by Al-Khazi and Kang [5, 11]. The insignificant differences could be due to assay method used given that Jacklyn's study in 2020 could only detect fructose levels with the resorcinol assay [19]. Unexpected findings were also the non-significant difference between median fructose and citric acid levels among different abnormal sperm conditions such as oligospermia which contrasts with the results from Toragall's study [2]. This study however has highlighted the role of viral infections in male infertility and further research would help highlight any possible relation between seminal biomarker levels and viral infections. The main limit to consider in our study was the nature of the study population. We included men with and without sperm abnormalities due to the low population size.

Conclusion

The presence of viral infection among men for fertility evaluation should not be overlooked. Some viral infection prevalence exceeds those of the general population. Assessment of biochemical markers of seminal fluid for fertility evaluation didn't give a clarify understanding of the effect of viral infection on the accessory glands. It appears to be important to investigate in a largest population to conclude.

Acknowledgements

The research team sincerely appreciate all participants to the study.

Conflicts of interest

The authors declare no conflicts of interests.

Author's contribution

The authors designed the study. Carried out data collection. The statistical analysis wrote the manuscript and objectively proofread it. All authors have given their approval for publication.

REFERENCES

[1] Vander Borght M, Wyns C. Fertility and infertility: Definition and epidemiology. *Clin Biochem*. 2018;62:2-10. doi:10.1016/j.clinbiochem.2018.03.012

[2] Toragall M, Satapathy S, Kadadevaru G, Hiremath M. Evaluation of seminal fructose and citric acid levels in men with fertility problem. *J Hum Reprod Sci*. 2019;12(3):199. doi:10.4103/jhrs.JHRS_155_18

[3] Rollet, J. Biochimie du liquide séminal: Intérêt pour le clinicien. Androl. 9, 468–471 (1999). https://doi.org/10.1007/BF03034663

[4] Winters BR, Walsh TJ. The epidemiology of male infertility. Urol Clin North Am. 2014;41(1):195-204. doi:10.1016/j.ucl.2013.08.006

[5] Abogale Al-Khazali IH, Al-Fartosy AJM, Al-Sawaad HZ. Al-Khazali et al (2020): Effect of seminal fructose and citric acid in infertile men Study the effect of Seminal Fructose and Citric Acid Levels in Men with Infertility. 2020;23. doi:10.36295/ASRO.2020.231375

[6] Sennana Sendi H, Ajina M, Lahouel M A, Khairi H, Saad A. Etats épididymaire, prostatique et vésiculaire en relation avec les paramètres du spermogramme chez les hommes consultant pour infertilité. Andrologie. 1994, (4) : 451-458

[7] Sakandé J, Kabré E, Ekue-Ligan A, Ouédraogo H A, Sawadogo M. Relation entre les anomalies du spermogramme et les constituants biochimiques du liquide séminal de sujets consultant pour hypofertilité masculine à Ouagadougou. Int. J. Biol. Chem. Sci. 2012, 6(3) :1167-1178.

[8] Shemshaki G, Murphy A S, Malini S S. Assessment and establishment of correlation between reactive axidation species, citric acid, and fructose level in infertile male individuals: A machine-learning approach. J Hum Reprod Sci 2021; 14:129-136.

[9] Liu W, Han R, Wu H, Han D. Viral threat to male fertility. *Andrologia*. 2018;50(11). doi:10.1111/and.13140

[10] Gimenes F, Souza RP, Bento JC, et al. Male infertility: A public health issue caused by sexually transmitted pathogens. *Nat Rev Urol*. 2014;11(12):672-687. doi:10.1038/NRUROL.2014.285

[11] Kang XJ, Xie QD, Zhou XL, et al. Effects of Hepatitis B Virus S Protein Exposure on Sperm Membrane Integrity and Functions. *PLoS One*. 2012;7(3):e33471. doi:10.1371/JOURNAL.PONE.0033471

[12] World Health Organization. Laboratory manual for the examination and processing of human semen. *Cambridge Cambridge Univ Press*. Published online 2010:32-99. http://whqlibdoc.who.int/publications/2010/9789241547789_eng.pdf

[13] Agarwal, A., Mulgund, A., Hamada, A. et al. A unique view on male infertility around the globe. Reprod Biol Endocrinol 13, 37 (2015). https://doi.org/10.1186/s12958-015-0032-1

[14] Barth RE, Huijgen Q, Taljaard J, Hoepelman AIM. Hepatitis B/C and HIV in sub-Saharan Africa: an association between highly prevalent infectious diseases. A systematic review and meta-analysis. *Int J Infect Dis*. 2010;14(12):e1024-e1031. doi:10.1016/J.IJID.2010.06.013

[15] Marcellin P. Hepatitis B and hepatitis C in 2009. *Liver Int*. 2009;29(SUPPL. 1):1-8. doi:10.1111/J.147-3231.2008.01947.X

[16] The burden of viral hepatitis in the WHO Region of Africa. Accessed March 22, 2022. https://www.openaccessgovernment.org/viral-hepatitis/67856/

[17] Benksim A, Elkhoudri N, Addi RA, Baali A, Cherkaoui M. Difference between Primary and Secondary Infertility in Morocco: Frequencies and Associated Factors. *Int J Fertil Steril*. 2018;12(2):142-146. doi:10.22074/ijfs.2018.5188

[18] Kbirou A, Jandou I, Adnane E, Mohammed E, Moataz A, Mohammed D, Debbagh A, Aboutaieb R, Profil épidémiologique et clinique de l'infertilité masculineÂ : Ã©tude observationnelle transversale descriptive et analytique, Sexologies,2021,ISSN 1158-1360, https://doi.org/10.1016/j.sexol.2021.05.004.

[19] Johnson J, Flores MG, Rosa J, et al. The High Content of Fructose in Human Semen Competitively Inhibits Broad and Potent Antivirals That Target High-Mannose Glycans. *J Virol*. 2020;94(9). doi:10.1128/JVI.01749-19/SUPPL_FILE/JVI.01749-19-SD001.XLSX