ejpmr, 2020,7(3), 131-146

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

IMPACT OF FUNGAL INFECTIONS AND SIGNIFICANT COUNT STATUSES ON FERTILITY PARAMETERS IN COMMERCIAL MOTORCYCLISTS AND NON-CYCLISTS IN NNEWI, NIGERIA

Ochiabuto O.M.T.B.*[1] and Chukwura E. I.[2]

[1]Department of Medical Laboratory Science, Faculty of Health Sciences, Nnamdi Azikiwe University, Awka, Nnewi Campus.

[2]Department of Applied Microbiology and Brewery, Faculty of Biosciences, Nnamdi Azikiwe University, Awka, Awka Campus.

***Corresponding Author: Ochiabuto O.M.T.B.**

Department of Medical Laboratory Science, Faculty of Health Sciences, Nnamdi Azikiwe University, Awka, Nnewi Campus.

Article Received on 10/01/2020 **Article Revised on 30/01/2020** **Article Accepted on 20/02/2020**

ABSTRACT

Fungal infections cause male assessory gland infections (MAGI) affecting male fertility especially in high - risk occupations. A case- control study on the impact of fungal infection and significant count statuses on fertility parameters was conducted in commercial motorcyclists and non-cyclists in Nnewi, Nigeria. A total of 304 samples consisting of 152 semen and blood samples from 49 commercial motorcyclists and 103 non-cyclists, aged 19-79 years old were analyzed. Subjects were recruited using non-probability sampling technique, data was collected using questionnaire, yeasts isolated using semen-dilution method and identified. Significant counts were calculated, semen quality and biomarkers assessed using WHO methods, serum hormone by ELISA technique. Fertility index was mathematically deducted and statistics analyzed with SPSS version 21. Results identified seven kinds of yeast isolates; *Candida guilliermondii* (47.2%), *C. tropicalis*(30.3%), *C. albicans*(13.2%), *C. glabrata* (2.6%), *C. famata* (2.0%), *C. krunzi* (1.3%) and *Cryptococcus laurentii* (0.7%), with *C. guilliermondii* highest in both groups, more types isolated from controls (7) than test groups (6). Fungal (73.5%) and mixed fungal infections (MFI) (42.9%) were higher in test groups; significant single and mixed fungal count statuses (FCS 1 and FCS 2) were higher in controls (60.9%; 58.6%). Age-wise, 45-64 years old groups had higher (FCS 1) (64.3%), while for (FCS 2), it was 19- 44 years (38.7%). Mean ranks of some serum hormones, semen qualities and biomarkers were affected by fungal infections and their significant count statuses in both groups, $P < 0.05$, and affected fertility index of test groups. Middle-aged and younger males are at risk for fungal infections which in significant count affected fertility parameters, more in semen quality and fertility index in test groups. Prompt treatment and awareness is necessary.

KEYWORDS: Fungi, significant count, fertility parameters, commercial motorcyclists, non-cyclists.

INTRODUCTION

In recent times, certain professional groups have been noted as high risk groups to microbial colonization. These may be come from exposures from the occupation, ergo metric problems, trauma, heat exposure encountered or environmental exposures on the individuals due to the nature of the work. Contributing factors to disease emergence, include climate change, globalization and urbanization, and to some extent human activities (Lindahl and Grace, 2015). The association between transport occupations (unskilled workers), jobs that require frequent travelling and increased risks to genitourinary infections/STDs has been noted by several researchers (Shendre and Tiwari, 2005). This can be due to an interplay of several factors including behavioral and biological factors in the host as well as in the organisms and environmental growth determinants. Dose and concentration of the infecting organism, strain, duration, treatment pattern, underlying diseases and confounding factors etc., can determine the effects on the male reproductive organ/fertility outcome. Poverty, poor governance and underdevelopment in most African countries, especially in Nigeria has made commercial motor-cycling one of the major and most popular unskilled occupation for survival, as well as the cheapest and most common means of transport system afforded by the majority. Infertility is causal to several factors including biological, mechanical and environmental factors. Virulence factors of yeasts have been known to alter semen quality, which in turn affects male fertility or increase the time of conception, but these effects are dependent on concentration (cell count status). Male urogenital tract contains microbial colonizers which can most times interfere with semen functions and cause infertility (Ahmad *et al.*, 2017).

Achkar and Fries(2010), noted that factors like changes in cellular morphology, phenotypic change, extracellular enzymatic activity (proteases and phosphatases) and adhesion factors, can cause sperm agglutination, alter sperm quality, morphology, sperm motility as well as induce reactive oxygen species which ultimately affect fertility potential. Trauma, heat, pollen dusts from air, nasal inhalation of fungal spores, and warmth in groin region, contaminated helmets and motorcycle upholstery, as well as promiscuous sexual behavior and lifestyle in commercial motorcyclists enhances the chances of yeast infections, its survival and persistence in these groups. Seminal fluids constitutes an important medium for the spread of various infective agents and have been noted to hold and transport bacteria, fungi, viruses and parasites, encouraging conditions for microorganism survival in semen plasma (Alsterholm *et al.*, 2008). The hormonal environment of the host may also affect the growth, infectivity, and pathogenicity of fungi. Continuous decline in human fertility worldwide has been attributed to many factors including activities of endocrine-disrupting chemicals (EDCs) such as mycotoxins from fungi and pesticides (Uriah *et al.*, 2001). Infectious etiology involving bacteria, virus, fungi, and protozoa acquired exogenously or endogenously according to Diemer *et al.* (2003) contribute to 15% of male factor infertility. Ibadin and Ibeh (2008) noted that they could cause infertility in several ways including by damaging sperm motility, deterioration of spermatogenesis, altering the chemical composition of the seminal fluid by dysfunction of accessory sex glands, or by inducing autoimmune processes due to inflammation. Adamu *et al.* (2012) isolated (50%) yeasts from fomites like helmets from commercial motorcyclists in Lagos.

Male infertility is associated with uro-pathogenic and commensal colonizers of male and female urogenital tract affecting semen quality with fungal infections being most times asymptomatic, man acting as reservoir and *Candida spp*. the most common cause, with increasing incidence in vaginal candidiasis, candidemia and systemic fungal infections over the past decades (Castrillon-Duque *et al.*, 2018), most times affecting semen quality and fertility. Many studies in the past have been on effects of bacteria and viruses on male fertility, undervaluing the effects of fungi. Tian *et al.* (2007) showed how human spermatozooal motility was interfered by Candida infiltrates and Castrillon-Duque *et al.* (2018) demonstrated how yeasts affects fertility in both sexes. Since previous demonstrations with respect to yeast have proved that it is associated with reduction or failure of fertilizing capability of spermatozoa, it is therefore needful that this study aims to determine the significant impacts of fungal infections and its count statuses on fertility parameters in commercial motorcyclists and non-cyclists in Nnewi, Nigeria.

The results of this project may provide insight into the effects of fungal infections on fertility variables of mobile transport workers such as commercial motor-cyclists. The data collected through this project may be used to raise awareness of the reproductive–risk of fungi to males in the occupation and to provide information for organizations to use in an effort to improve prevention plans and trainings regarding workplace -associated reproductive infections especially in males. All methods in the research was conducted with the new WHO method for semen analysis, which is all about using new improved methods in providing standards for Andrology studies with better accuracy and precision.

The aim of the research is to assess the impact of fungal infections and their significant count statuses on mean rank order values of fertility parameters in commercial motor-cyclist and non -cyclists was conducted in Nnewi, Nigeria.

MATERIAL AND METHODS

Study area

The study area encompasses of Nnewi metropolitan commercial city in Anambra state, Nigeria. Nnewi is located in the south-east zone in Anambra state, Nigeria.

Study design

This is a case-control study.

Sample size

The sample size was calculated using the formula by Naing *et al., (*2006)

$n= Z^2 X P (1-P)/ d,$

where

n= sample size.

P= prevalence percentage rate of male infertility in area of study (42.4%) Ikechebelu et *al., (*2003).

Z=Confidence interval of 95% which is equivalent to confidence coefficient of 1.96.

D=desired level of precision or significance = 0.05, using the formula, a total minimum samples size of 196 is required but a total sample size of 304 of blood and semen samples from 152 volunteers were used in the research.

Study population / controls

A total 152 males participated completely, gave full consent, adhered to all abstinence rules and submitted all the necessary samples and questionnaires including a second semen sample. The volunteers consisted of apparently healthy males residing in Nnewi commercial metropolitan city. All participants (test and control groups) were of age-range of between 19-79 years old and were residing in Nnewi. Out of the 152 men, forty-nine (49) were registered commercial motorcyclists and were considered as the test groups while one hundred and three (103) men who were not in "Okada" business, and do not ride motor-cycles were considered as controls. Fifteen (15) of the men in test groups were recruited from Okada point in Otolo village, fourteen (14) from Umudimkwa (Umudim) point and ten (10) each were from Uruagu and Nnewi-ichi centers each,

representing the four villages in the town. Thirty-eight (38) men from each of the four villages were recruited as controls.

Sampling technique

Consecutive non-probability sampling technique was used to collect samples from volunteers. Samples (blood and semen) were collected from subjects based on being a commercial motorcyclist or not and adult reproductive age irrespective of fertility statuses of the time of study. For the test groups, only registered commercial motorcyclists plying in Nnewi were invited to their headquarters in Okada white-house at Otolo, Nnewi through an oral announcement made by a town announcer through the Chairman of the association. Willing participants were recruited from volunteers ensuring that selection was made from four main commercial motorcyclist's parks from each of the four villages where they habitually ply to ensure equal distribution of men representing each village. Control participants were also selected from each villages excluding any commercial motor-cycle operator and anyone who drives a motor-cycle. All willing participants were recruited after explanations were made on the reason for the research and nature of samples needed. The respondents were carefully selected and educated on the study reasons before questionnaires were distributed.

Inclusion criteria

Selection was based on their being apparently healthy fertile males who fulfilled the requirements listed;

1. Adult males who were between 19 and 79 years of age, sexually active as at the time of study and of different occupational category for controls and only commercial motorcyclists for test groups.
2. They must be living in Nnewi.
3. They must not be on antibiotic or herbal therapy 5- 7 days prior to sample collection or during the study.
4. They must be willing to participate and must submit all necessary required samples and questionnaires.
5. They must have no history of vasectomy or prostate removal.

Exclusion criteria

1. Unwillingness to participate and did not submit all necessary required samples and questionnaires.
2. Males of different profession who were below 18 years.
3. Males with history of vasectomy or prostate removal.
4. Those living outside the vicinity of the study area.
5. Those on antibiotic or herbal therapy as at the time of study.

Ethical clearance: Ethical approval for the study was obtained from the Ethical committee of Department of Medical Laboratory Science, Faculty of Health Sciences and Technology, Nnamdi Azikiwe University, Nnewi, and authorization permit for sample collected from Nnewi Okada amalgamated association. Procedures and methods were followed in accordance with the ethical standards of the Helsinki declaration of 1975, revised in the year 2000 (WHO, 2001). Informed consent was obtained from all study participants.

Data collection

Prior to sample collection, all the volunteers were educated on the reason and nature of the research. Willing participants were given a copy of informed consent to fill and return. Questionnaire was used for data collection. It was completed by each volunteer as he submits his sample. English language as well as vernacular was used for data collection by oral interview. The questionnaire was used to gather data from the respondents.

Samples / sample collection: The samples used in the study include a total of 304 samples consisting of 152 semen and serum each. Semen was collected with sterile pre-weighed and personal identity-labeled wide-necked plastic stericon containers and submitted immediately. Subjects who had to collect their sample at home preserved in anticipated delay, they were kept at near body temperature in their pockets until they arrival in the laboratory within 30 minutes. If any delay in processing was expected, they were kept in the incubator at 37 ^{o} C, otherwise they were cultured first, before microscopic analysis. Samples were coded with a unique identification number as well as with the patient's name. Results were collated using this unique identification number to maintain subject's confidentiality. Semen samples were collected by masturbation method. Prior to that, all protocols regarding sample collection and transportation time adherence was explained to the volunteers. Proper information like name, sex, number, and weight of sample containers, time of collection and arrival, method of semen collection and indication of any spills or contamination noted for other necessary activities were noted. All pre-analytical stages for semen culture were adhered to with respect to 3 and 5 days sexual-chemotherapy abstinence aseptic collection procedures, urination prior to sample collection and aseptic serial- dilution was used for semen preparation as described by (Askienazy- Elbhar, 2005). All precautions were according to WHO (2010) specification. Blood was collected and serum separated into sterile plastic stericon containers (Evepon, Nigeria) as described by (Cheesbrough, 2006).

Sample processing (semen plasma extraction and storage)

Semen plasma was extracted by spinning in a centrifuge at 10 000 g /min for 10 minutes in sterile well -cleaned plain glass tubes immediately after cultures were performed to avoid contamination of samples. It was decanted in aliquots using a sterile disposable plastic pipette and sperm-free plasma stored in sterile Eppendorf plastic tubes in the freezer at -4°C. Samples for zinc analysis was stored in clean polystyrene containers

previously ensured to be zinc free by proper cleaning in cleaning fluids according to methods specified by (WHO, 2010). Semen plasma was analyzed within one week. All semen analysis were carried out in registered laboratories following WHO specification. Serum was stored at -4 ^{0} C and used within a week for hormonal assays.

Fungal culture

Semen plating on Candida chrome selective media (Liophilchem diagnostics, Italy) was done after one (1) ml of each of the semen samples were diluted (1:10) with sterile saline, centrifuged at 1500 r.p.m. for 15 minutes to collect the sediment for culture, supernatant aseptically removed and sediments plated using 10 µl calibrated loops. Dilution was to increases the cultural sensitivity, eliminate antibiotic properties in semen and concentrate the fungi in the cell pellet as described by (Vicari *et al.*, 1986). The surface of the pre-prepared media were dried in the incubator for 3 minutes and a sterile 10 µl wire loop used for surface streaking on the media and incubated at 25ºC for 24 hours as described by (Cheesbrough, 2006).

Confirmation of significant fungal growth: Significant count of $>10^5$ c.f.u/ml was used for yeast isolates according to quantitative semen culture described by (La Vignera *et al.*, 2014). Where - (positive fungal isolate with significant fungal count i.e.$>10^5$ c.f.u/ml for single yeast isolates after serial dilution is fungal count significant 1 (FCS 1) and positive fungal culture growth with 2 or 3 pathogenic yeast isolates at significant count level for both yeasts after serial dilution is taken as fungal count significant 2 (FCS 2) and mixed fungal infected status - (if a culture growth has 2 or 3 pathogenic yeast isolates at significant count level for both yeasts).

Biochemical tests and identification for yeast isolates: The isolates were tentatively identified using visual chromogenic appearance of the yeasts with identification charts provided as instructed by manufacturers, interpreted as described in the manual. Yeasts isolated from candida chrome selective agar were further identified using integral system yeast - plus identification kit (Liophilchem, Italy) based on assimilation reaction of sugars as described by NCCLS(2002), interpreted with a color chart. The results of sugar reactions were interpreted and results written down on the test result forms for each test run with numerical codes formed by obtaining a 4 digit code following the manufacturer's instruction. Further confirmation for suspected *Candida spp*. was carried out with digital – light microscopy (Celestron, USA) with KOH –lacto-phenol cotton blue mounts, morphologyviewed with x10 and x40 objective, and *Candida albicans* confirmed further withgerm-tube test as described by (Reynolds and Braude, 1956).

Semen plasma biomarker tests

Fructose concentration- Photometric quantification of fructose in semen plasma was carried out to assess for the level of fructose in semen plasma as a marker of secretory function of epididymis function using fructose kit (Fertipro N.V, Belgium) ref number; FPO9 129 ROI B.5 according to photometric kit resorcinol method as described in (WHO, 2010). Result was expressed as (mg/ml). Biologically important variables like total semen fructose (mg/ml ejaculate), corrected semen fructose (mg x 10^6/ml), true-corrected semen fructose concentration (mg/ml) were obtained by mathematical-deduction methods (WHO, 2010).

Semen plasma citric acid concentration: Citric acid concentration in semen plasma was assessed to determine the prostate function using spectrophotometric method described in (WHO, 2010) using (Agilent Technologies 200 AA 240fs AA series, USA) expressed as (mg/ml). Total citric acid concentration(mg or more /ml ejaculate) was obtained by mathematical-deduction methods.

Seminal zinc concentration: All glassware and plastic wares were rinsed with 10% nitric acid overnight, thoroughly washed with deionized distilled water before use. Zinc concentration (Zn-C) in seminal fluid plasma was determined with flame atomic absorption spectrophotometric method WHO (2010) with (Agilent Technologies 200 AA 240fs AA series, USA.) and zinc concentration expressed in (µg/ml) and total zinc concentration in (µg/ml) calculated by mathematical-deduction methods.

Alpha (ὰ)-neutral glucosidase enzyme: This was performed to assess the secretion contributory status of the epididymis to the ejaculate, and differentiates the major causes of azoospermia, (obstructive and non-obstructive) types using spectrophotometric kit method of WHO (2010) with Episcreen Plus™ Enzyme ὰ-neutral glucosidase kit, (Fertipro, Belgium). Results were expressed in (mU/ejaculate). Incubation time and dilution factor were corrected as corrected factor.

Seminal acid-phosphatase assay: Seminal plasma acid-phosphatase was determined to assess prostate function using Kind and King (1954) colorimetric method modified by Hillman's colorimetric method (1971) as described in Tietz (1999) to suit sperm. Samples were later tested on the automated biochemical analyzer. Since semen contains a high concentration of acid phosphatase, it was diluted 1:2000-1:4000 to fall within parameter with buffer.

Hormonal assessment assays: Serum FSH, LH, Testosterone, Prolactin, and Progesterone assays for assessing male fertility were carried out using Enzyme linked quantitative imunosorbent assay (ELISA) with kits according to methods described by Tietz (1999) following the manufacturer's instructions with kits from

(Immunodiagnostics, Cortez, Inc., Calabasas, California; Accu diagnostic™ C.A) and expressed in (ng/ml and mg/ml).

Semen analysis: These assays were done;
Initial macroscopy and microscopy, appearance and liquefaction by visual method; pH by paper method; viscosity by gravity-fall method; cell observations, agglutination grading, motility, sperm count, motility, immobility, non-progressive and progressive motility (%), manual motility classification and characterization by manual visual method; all biologically important calculations deducted from standard derived formula, vitality for membrane integrity of the cells by one-step dye-exclusion eosin-nigrosin staining method, Bjorn-Dahl et *al.* (2003), within 30mins-1hr post ejaculation; sperm morphology by feathering method for slide preparation and modified Papanicolaou staining method for staining with total number of morphologically normal sperm mathematically calculated; normal forms using two criteria, Kruger's criteria and WHO criteria; abnormal morphology of all sperm part defect was assessed, all according to methods described in (WHO, 2010).

Fertility index: Fertility index was derived mathematically from semen analysis according to method and formula described by (Harvey, 1953). Influence of yeast infections and their count statuses on semen biomarkers, hormones and semen qualities were determined based on noting when the presence or absence of yeast or their significant count statuses caused a decrease or increase in the reference normal ranges of the analytical test variables stipulated according to WHO (2010).

Statistical analysis
Results obtained were analyzed using statistical package-SPSS version 21 after being subjected to normality test. Non -parametric Mann -Whitney (U) and Kristal –Wallis (K) tests, as well as prevalence percentage and chi-square association used where necessary and P values of < 0.05 were considered significant at 95% confidence interval (CI).

RESULT

Morphological descriptions of yeast isolates from semen samples from both groups
The morphological descriptions of each the seven types of yeast isolates from the entire semen sample are described as they appeared chromo genially on Candida chrome agar in table 1.1. Yeast isolates tentatively identified include; *Candida guilliermondii, Candida albicans, Candida krusei, Candida famata, Candida tropicalis, Candida glabrata and Cryptococcus laurentii,* while table 1.2 is the table of code for identification of each isolated yeast based on sugar reactions.

Table 1: Morphological descriptions of yeast isolates from test and controls' semen samples.

Names of yeast isolates	Descriptions of morphology.
Cryptococcus laurentii	Mucoid or slimy in appearance; non-pigmented and creamy-white on candida-chrome agar, becoming a deeper orange-yellow with age, with a smooth mucoid texture. Encapsulated in Indian ink stain. Spherical and elongated budding yeast-like cells or blastoconidia, No pseudo hyphae present.
Candida famata	Colonies are white to cream colored, butyrous. Budding cells are broadly ellipsoidal. Candida famata does not form pseudo hyphae, but shows blastoconids.
Candida albicans	Colonies are cream colored somewhat waxy soft and usually smooth. Budding cells are spherical. Pseudomycellium present with blastoconidia in grapelike arrangement. Pseudo hyphae with terminal chlamidospores
Candida guilliermondii	White to cream colored colonies, butyrous. Budding cells are spherical to broadly ellipsoidal. Pseudomycellium when present radiates from the center of masses of budding cells
Candida tropicalis	*C. tropicalis* has the shape from round to oval, are turquoise blue on candida chrome agar, smooth and butyrous with a fringed border Blastoconidia all along the pseudo hyphae.
Candida krusei	Colonies are cream colored and soft. Budding cells ellipsoidal to cylindrical in shape. Extremely long pseudo hyphae, rarely ramified, with few blastoconids.
Candida glabrata	The colonies appear as small glossy, convex, and smooth, non-hyphae or pseudo hyphae forming yeast. The color is white to cream on candida chrome agar. Microscopically the cells appear as small sized yeast less than half the size of red blood cell, with single budding cells. The cells are often more spherical rather than elongated as with some other candida species. It also shows little spores without pseudo hyphae.

Table 2: Table of codes for the identified yeast isolates.

Groups	**Group 1**			**Group 2**			**Group 3**			**Group 4**		
Sugar tests	**1—GLU**	**2-Mal**	**3-SAC**	**4-LAC**	**5-GAL**	**6-MEL**	**7-CEL**	**8-INO**	**9-XYL**	**10-RAF**	**11-TRE**	**12-DUL**
Code of positivity	1	2	4	1	2	4	1	2	4	1	2	4
Result	+	+	+	-	+	-	-	-	+	-	+	-
Sum of codes		**7**			**2**			**4**			**2**	
Codes		**7242**						***Candida albicans***				
	+	+	+	+	+	+	+	-	+	+	+	-
		7			**6**			**5**			**3**	
		7653						***Candida famata***				
Result	+	+	+	-	+	-	+	-	+	-	+	-
Sum of codes		**7**			**4**			**5**			**2**	
Codes	**7452**							***Candida tropicalis***				
Result	+	+	+	+	+	+	+	+	+	+	+	-
Sum of codes		**1**			**0**			**0**			**0**	
Codes								***Candida krusei***				
Result	+	-	-	-	-	-	-	-	-	-	+	-
Sum of codes		**1**			**0**			**0**			**1**	
Codes								***Candida glabrata***				
Result	+	+	+	-	+	+	+	-	+	+	+	+
Sum of codes		**7**			**6**			**5**			**7**	
Codes	**7657**							***Candida guilliermondii***				
Result	+	+	+	+	+	+	+	+	+	-	+	+
Sum of codes		**7**			**7**			**7**			**6**	
Result	**7776**							***Candida laurentii***				

Key: += positive =Negative

Glu-glucose; Mal-maltose; Gal-galactose; Sac-sacrose; Lac-lactose; Mel-melboise; Cel-cellulose; Ino=inositol; Xyl-xylose; Raf-raffinose; Tre-trelose; Dul-dultose.

Distribution of the individual yeast pathogens according to occupation

The most frequently isolated yeast organism from semen samples in test groups 22(44.9%), control groups 50(48.5%) and in the total males 72 (47.4%) in table 2a below was *Candida guerillamondii*, higher in control groups than test groups. Lowest yeast isolate was *Cryptococcus laurentii* in total males 1(1.0%). Statistical difference was observed between the groups for *Candida glabrata* only (X^2 = 8.635; P= 0.003).

Table 2a: Distribution of the individual yeast pathogens according to occupation.

Disease name	Status	Occupation		Total	X^2	P value
		Cyclists	Non- cyclists	F %		
C. tropicalis	Present	16(32.7)	30(29.1)	46(30.3)	0.196	0.198
	Absent	33(67.3.)	73(70.9)	106(69.4)	**P > 0.05**	
	Total	49(100)	103(100)	152(100)		
Candida. guerillamondii	Present	22(44.9)	50(48.5)	72(47.4)	0.177	0.674
	Absent	27(55.1)	53(51.3)	80(52.6)	**P > 0.05**	
	Total	49(100)	103(100)	152(100)		
Candida krusei	Present	1(2.0)	1(1.0)	2(1.3)	0.293	0.588
	Absent	48(98.0)	102(94.0)	150(98.0)	**P > 0.05**	
	Total	49(100)	103(100)	152(100)		
C. famata	Present	2(4.1)	1(1.0)	3(2.0)	0.661	0.198
	Absent	47(95.9)	102(99.0)	149(98.0)	**P > 0.05**	
	Total	49(100)	103(100)	152(100)		
Cryptococcus laurentii	Present	0(0.0)	1(1.0)	1(1.0)	0.479	0.489
	Absent	49(100)	102(99.0)	151(99.3)	**P > 0.05**	
	Total	49(100)	103(100)	152(100)		
Candida albicans	Present	8(16.3)	12(11.7)	20(13.2)	0.635	0.425
	Absent	41(83.7)	91(88.3)	132(86.8)	**P > 0.05**	
	Total	49(100)	103(100)	152(100)		
	Present	4(8.2)	0(0.0)	4(2.6)	8.635	0.003
Candida glabrata	Absent	45(91.8)	103(100)	148(97.4)	**P < 0.05**	
	Total	49(100)	103(100)	152(100)		

Key:
F = frequency
%= Percentage

Occupational comparison between the groups showed that semen of test groups had more single and mixed fungal infection statuses than in control groups. No statistical significance was however observed between them, P> 0.05 in table 2b below.

Table 2b: Distribution of yeast infection status in relation to occupation.

Yeast infection status	Status	Occupation		Total	X^2	P- value
		Motorcyclists	Non-cyclists	F %		
Fungal infected groups	Infected	36(73.5)	60(58.3)	96(63.2)	3.304	0.069
	Non- infected	13(26.5)	43(41.7)	56(36.8)	P>0.05	
	Total	49(100)	103(100)	152(100)		
Mixed fungal infected groups	Infected	21(42.9)	36(35.0)	57(37.5)	0.885	0.347
	Non- infected	28(57.1)	67(65.0)	95(62.5)	P>0.05	
	Total	49(100)	103(100)	152(100)		

Key:
%= Percentage
F= Frequency
Fungal infected status- (positive fungal isolate with significant fungal count i.e.>10^5 c.f.u/ml for yeast isolates after serial dilution)
Mixed fungal infected status -(if a culture growth has 2 or 3 pathogenic yeast isolates at significant count level for both yeasts)

Table 3a: Distribution of significant fungal count status in relation to occupation.
In relation to occupation, more significant single and mixed fungal count statuses occurred in semen of control groups than test groups.

Fungal infection	Status	Test(cyclists) F %	Controls(non-cyclists) F %	Total F %	X^2	P-value
FCS 1	Significant	35(38.0)	56(60.9)	92(100.0)		
	Not significant	0(0.0)	1 (100.0)	1 (100.0)	4.695	0.320
	No growth	14 (23.7)	45 (76.3)	59 (100.0)		
	Total	49 (32.2)	102(67.1)	152(100.0)		
FCS 2	Significant	23 (39.7)	34(58.6)	58 (100.0)		
	Not significant	1 (100.0)	0 (0.0)	1 (100.0)	6.657	0.155
	No growth	25 (26.9)	68 (73.1)	93 (100.0)		
	Total	49 (32.2)	102 (67.1)	152 (100.0)		

Key:
FCS 1 = fungal count significance for single fungal infection status.
FCS 2 = fungal count significance for mixed fungal infection status i.e. Single and mixed yeast isolates greater than 10^5 c.f.u/ml yeast isolates after serial dilution)
Not significant = Single and mixed yeast isolates less than 10^5 c.f.u/ml yeast isolates after serial dilution)

Table 3a: Distribution of significant fungal count status in relation to age- range.
Fungal significant count status for single infection was higher in 45-64 years old groups (64.3%) while FCS 2 in 19-44 years old category (38.7%) in all males sampled. Occurrence of FCS1 (60.5%) was higher than FCS 2 (38.2%) in table 3a below without a statistical significance amongst age ranges in both status, P > 0.05.

Fungal infection	Status	19-44 years	45-64 years	65-79 years	Total	X^2 P value
		F %	F %	F %	F %	
	significant	63 (59.4)	27 (64.3)	2 (50.0)	92 (60.5)	
FCS 1	Not significant	1 (0.9)	0 (0.0)	0 (0.0)	1 (0.7%)	0.877 0.928
	No growth	42 (39.6)	15 (35.7)	2 (50.0)	59(38.8)	
	Total	106 (100)	42 (100)	4 (100)	152 (100.0)	
FCS 2	Significant	40 (38.7)	16 (38.1)	1 (25.0)	58 (38.2)	0.763 0.943
	Not significant	1 (0.9)	0 (0.0)	0 (0.0)	1 (0.7)	
	No growth	64 (60.4)	26 (61.9)	3(75.0)	93 (61.2)	
	Total	106 (100)	42 (100)	4 (100)	152 (100.0)	

Key:
FCS 1 = fungal count significance for single fungal infection status.
FCS 2 = fungal count significance for mixed fungal infection status i.e. Single and mixed yeast isolates greater than 10^5 c.f.u/ml yeast isolates after serial dilution)
Not significant = Single and mixed yeast isolates less than 10^5 c.f.u/ml yeast isolates after serial dilution)

Graphical representation for significant fungal count statuses in various age ranges in test groups and controls

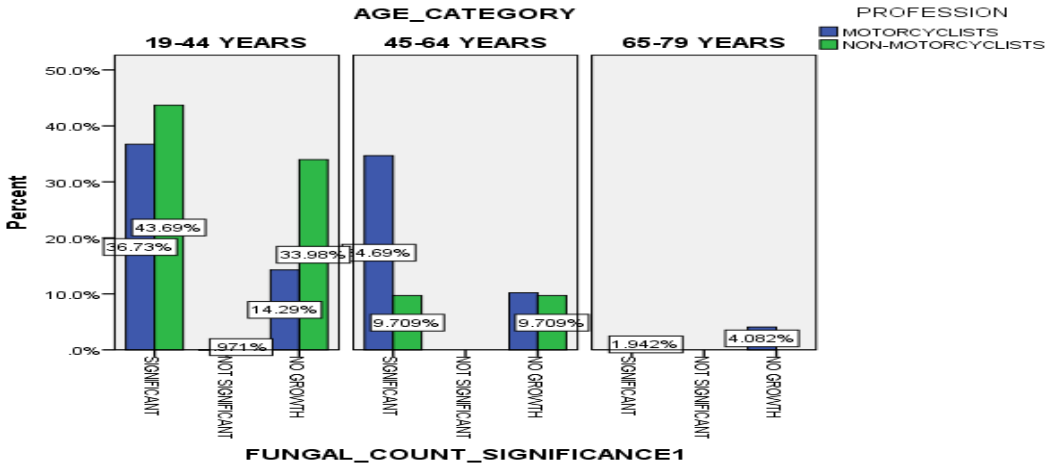

Chart 1a: Comparative bar chart showing the frequency distribution of subjects with significant single fungal count infection and those without growth in the semen of various age category in test*(cyclists) and controls (non-cyclists).

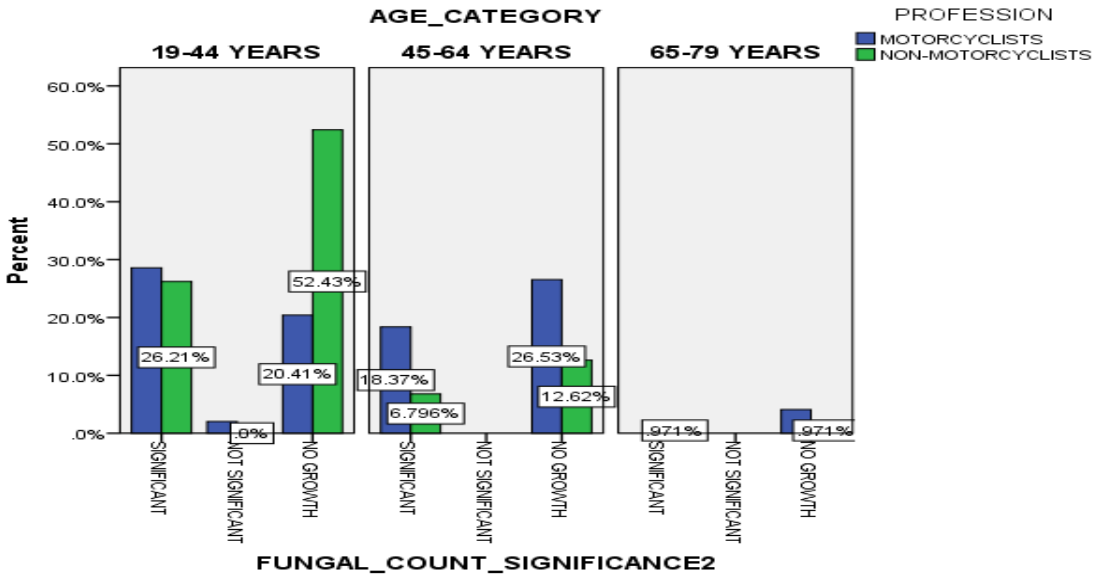

Chart 1b: Comparative bar chart showing the frequency distribution of subjects with Significant mixed fungal count infection (2) and those without growth in the semen of various age groups in test*(commercial motorcyclists) and controls (non-cyclists).

4a: Significant influence of fungal infection on semen biomarkers, hormones, semen quality and fertility index among the participants

Semen biomarkers: In table 4a, Significant difference occurred between mean values of fungal- infected and non-infected groups for total neutral alpha- glycosidase in control groups, (U = - 1.986; P = 0.047) and in citric acid concentration, (U= -3. 533; P= 0.000; U = -2.988; P= 0.003) in total males and control groups. Mean-rank values in the fungal infected males in all groups sampled were normal though infected males had lower normal values of total α-glucosidase and higher values for citric acid concentration.

Hormones: Significant difference was noted between fungal infected (69.60) mean rank of prolactin and non-infected (88.32 $\pm$ 0.00) in the total male participants (P < 0.05) (U= - 2.529; P = 0.011).

Semen qualities: Significant differences were observed between the infected and non- infected groups in the following mean-rank values of semen qualities;

Significant difference was observed in semen volume mean rank values between infected, (68.81) and non-infected (89.69) statuses in total males only, P<0.05 (U = -2.839; P = 0.005), though mean rank values were normal in both tests and controls, lower in infected people. Mean rank values of abnormal forms in fungal–infected categories for all groups were abnormal, mostly in the non- infected tests groups, with a significant difference between the infected (23.31) and non- infected (26.69) categories in the test groups (U= - 2.112; P =0.035) and total males (U = - 2.589; P = 0.010) (P< 0.05) respectively. Significant difference was observed between mean rank values of middle-piece of fungal-infected and non-infected in controls only, (U = - 2.013; P = 0.044), all groups had abnormal results, higher in infected males. Mean values of sperm numbers in those with fungal infected in tests groups were abnormal, higher in the infected (25.64) than non-infected (23.23), with significant difference observed between infected (82.37 X 10^6) and non-infected (66.44) in the total males samples, (U= 2.153; P= 0.031) (P < 0.05). Finally, significant difference was observed between mean rank order values of sperm concentration in fungal infected males; 136(27.35) and non-infected 13(18.50) groups in tests groups (U= -2.005; P = 0.045) and in the total males (infected 96 (53.10) and non-infected 56 (65.1) (U= -2.420; P = 0.016). In all groups, fungal -infected groups had higher sperm concentration.

Fertility index: Fungal infection status did not affect the fertility index in the total population who were all fertile, however in controls, fertility was not defined, while in test groups, with < 30, the husband's sub fertility alone may be enough to account to wives' fate to conceive during the period and no statistical significance was observed between the test (P=0.123) and controls (P=0.138) groups (P > 0.05).

Table 4a: Significant influence of fungal infection on semen biomarkers, hormones, semen quality and fertility index among the participants.

Variables	Categories	Total Mean rank	Test Mean rank	Control Mean rank	Total (2 tailed) UP – value		Test (2 tailed) UP – value		Control (2 tailed) UP – value	
BIOMARKERS										
Citric- acid concentration	Infected	86.11	26.28	58.95	-3.533	**0.000***	-1.043	0.297	-2.988	**0.003***
	Not Infected	60.03	21.46	41.28						
Total alpha-neutral glucosidase	Infected	71.36	24.33	46.53	-1.883	0.060	-0.544	0.587	-1.986	**0.047***
	Not Infected	85.30	26.85	58.31						
HORMONES										
Prolactin	Infected	69.60	24.04	45.36	-2.529	**0.011***	-0.782	0.432	-1.340	0.180
	Not Infected	88.32	27.65	59.92						
SEMEN QUALITIES										
Volume (ml)	Infected	68.81	22.90	46.08	-2.829	**0.005***	-1.537	0.124	-0.764	0.445
	Not Infected	89.69	30.81	58.93						
Abnormal forms	Infected	83.55	23.31	53.55	-2.589	**0.010***	**-2.112**	**0.035***	-1.376	0.169
	Not Infected	64.41	29.69	48.69						
Middle-piece	Infected	76.58	27.58	50.19	-0.217	0.828	-1.021	0.306	-2.013	**0.044***
	Not Infected	75.01	17.85	53.30						
Sperm number	Infected	82.37	25.64	55.40	-2.153	**0.031***	-1.914	0.056	-1.227	0.220
	Not Infected	66.44	23.23	46.15						
Sperm concentration	Infected	83.10	27.35	55.86	-2.420	**0.016***	-2.005	**0.045***	-1.347	0.178
	Not Infected	65.19	18.50	45.52						
Fertility index	Infected	73.05	17.30	52.30	-1.267	0.205	-1.542	0.123	-1.482	0.138
	Not Infected	82.42	21.23	50.41						

Key:
U=Mann-Whitney U test

NB: Fertility index especially in a sub fertile marriage:

- In case of men with high than those with low indices, fertility of wives is probably much lower.
- If F1 is 100, there is no increase in the chances of success of conception rate.
- Fertile men have F1 of > 90, though few fertile men may have F1 of <30.
- A chance of conception depends solely on wives' fertility.
- If husband's F1 is 70 and over, any sub fertility is almost entirely due to the wife and if < 30, the husband's sub fertility alone may be enough to account to wives' fate to conceive during the period.

Table 4b. Significant influence of multiple fungal infection on semen biomarkers, hormones, and semen qualities in test groups and controls

Hormones: Significant difference was observed between mean rank values of prolactin levels of multiple fungal-infected and non- infected total male groups (U = -2.100; P = 0.036) (P<0.05), with lower normal values in test groups than in controls, but normal.

Semen qualities: Significant difference was observed between mean rank value of only middle- piece in multiple- fungal infected control groups (U = - 2.013; P = 0.044) P < 0.05, with abnormal values of middle-piece in all groups, especially in non-infected controls, (55.71%).

Fertility index: No significant influence of multiple fungal infection status on fertility index in both groups sampled (P>0.05), though the mean rank order of those not infected had lower F1 value which means (sub fertile) in total populations (75.20) meaning that subfertility in males is probably due to the wife; while sub fertility was not defined in control (48.37) and in test groups.

Table 4b: Significant influence of multiple fungal infections on hormones and semen qualities in test groups and controls.

Variables	Categories	Total Mean rank	Test Mean rank	Control Mean rank	Total (2 tailed) UP – value		Test (2 tailed) UP – value		Control (2 tailed) UP – value	
Hormones										
Prolactin	Yes	66.82	21.05	46.07	-2.100	**0.036***	-1.678	0.093	-1.340	0.180
	No	82.31	27.96	54.34						
Semen qualities										
Middle piece	Yes	70.82	26.81	43.44	-1.151	0.250	-0.715	0.475	-2.013	**0.044***
	No	79.14	23.64	55.71						
Fertility index	Yes	78.67	56.94	57.50	-0.470	0.638	-0.364	0.716	-1.482	0.138
	No	75.20	44.00	48.37						

Key:
U= Mann-Whitney U test.
Yes= Normal
No= Abnormal

Significant influences of fungal count 1 (single - infection) status on semen biomarkers, serum hormones and semen qualities in test groups and controls

Significant influence of fungal count for single infection (FCS I) status was observed on mean rank values of these biomarkers; citric acid concentration among the various fungal count categories in controls and total males sampled (K = 1.123; P = 0.004) (K= 9.254; P = 0.010); zinc concentration in total males (K = 6.331; P = 0.042). (Sig – 92 (83.17); and corrected α- total neutral – glucosidase values in total males (K = 3.880; P = 0.049) in table 4c below.

For hormones, significant difference was observed among mean of prolactin levels of single fungal-infected categories in total males (K = 6.673; P = 0.036) and controls (K = 7.593; P = 0.022).

Significant difference was observed among semen qualities mean rank values of semen volume in total males (K = 9.396; P =0.009) and in test groups (K = 4.004; P = 0.044) in single fungal infected groups) FCS 1; abnormal forms in total males (K = 8.704; P = 0.013) and in test groups (K = 5.852; P =0.016) and sperm concentration in total males sampled, (K = 7.066; P = 0.029). Fertility index mean rank value was affected in test groups though non-significantly (P > 0.05) and the husband's sub fertility alone may be enough to account to wives' fate to conceive during the period.

Table 4c: Significant influences of fungal count 1 (single -infection) status on semen biomarkers, serum hormones and semen qualities in test groups and controls.

Variables	Categories	Total Mean rank	Test Mean rank	Control Mean rank	Total (2 tailed) KP – value		Test (2 tailed) KP – value		Control (2 tailed) KP – value	
Biomarkers										
Citric acid concentration	Significant	85.1	25.94	58.23	1.123	**0.004***	0.535	0.465	9.254	**0.010***
	Not Significant	127.00	23.98	92.00						
	No Growth	62.13	22.64	42.22						
Zinc concentration	Significant	83.17	26.27	56.88	6.331	**0.042***	0.970	0.325	5.010	0.082
	Not Significant	23.00	27.00	17.00						
	No Growth	67.01	21.82	45.58						
Corrected α glucosidase	Significant	70.39	23.97	45.89	3.880	**0.049***	0.635	0.426	3.819	0.051
	Not Significant	84.75	27.57	57.36						
	No Growth	72.03	23.97	47.69						
Hormones										
Prolactin	Significant	69.11	24.47	44.22	6.673	**0.036***	0.168	0.682	7.593	**0.022***
	Not Significant	101.50	22.65	68.50						
	No Growth	87.60	26.32	60.18						
Semen qualities										
Volume(ml) 1.4-1.7	Significant	67.76	22.41	45.61	9.396	**0.009***	4.044	**0.044***	5.086	0.079
	Not Significant	106.50	15.43	69.00						
	No Growth	89.63	31.46	58.44						
Abnormal forms	Significant	84.36	28.11	53.71	8.704	**0.013***	5.852	**0.016***	2.240	0.326
	Not Significant	15.50	17.96	12.50						
	No Growth	65.28	17.21	49.61						
Sperm concentration	Significant	83.73	27.23	56.76	7.066	**0.029***	2.982	0.084	4.591	0.101
	Not Significant	104.00	29.87	69.00						
	No Growth	64.76	19.43	44.57						
Fertility index	Significant	71.98	24.56	52.09	4.356	0.113	3.455	0.221	2.443	0.295
	Not Significant	143.50	23.54	96.00						
	No Growth	82.41	25.56	49.78						

Table 4d: Significant influences of fungal count 2 (mixed -infection) status on semen biomarkers, serum hormones and semen qualities in test groups and controls

In table 4d below, there was significant difference among the mixed –fungal count statuses mean rank values of the following semen qualities and sperm characteristics in these groups; middle- piece of fungalcount for mixed infection status (FCS 2) and those without growth in control groups, (K = 4.154; P = 0.042); among mean rank values of sperm concentration in controls groups (K = 3.910; P = 0.048); manual motility classification (Grades 0 -3 and class A - D) (K = 4.047; P = 0.044) in control groups, and sperm concentration characterization in total males(K = 6.576; P = 0.037) (sig – (67.79); non sig (72.00); no growth (81.98) respectively.

Fertility index: Fertility index of those in test group with significant and non-significant count status were low (<30) and any infertility observed could be have been caused by the husband's sub fertility which may have been enough to account to wives' fate to conceive during the period, but normal in controls and total males.

Table 4d: Significant influences of fungal count 2 (mixed -infection) status on semen qualities in test groups and controls.

Variables	Categories	Total Mean rank	Test Mean rank	Control Mean rank	General (2 tailed) X^2P – value		Test (2 tailed) X^2P – value		Control (2 tailed) X^2P – value	
Semen qualities										
Middle piece	Significant	71.31	26.13	43.16	3.828	0.148	2.873	0.238	**4.154**	**0.042**
	Not Significant	8.50	3.00	7.60						
	No Growth	79.69	23.83	55.67						
Sperm concentration	Significant	86.74	27.52	59.69	5.177	0.075	1.354	0.508	**3.910**	**0.048**
	Not Significant	84.00	22.00	56.00						
	No Growth	70.03	22.80	47.40						
Fertility index	Significant	78.35	25.48	56.04	0.264	0.876	1.233	0.456	1.206	0.272
	Not Significant	89.00	11.00	49.98						
	No Growth	75.21	24.10	49.23						
Sperm characteristics										
Manual motility classification	Significant	74.78	22.46	52.62						
	Not Significant	106.00	34.00	76.89	0.741	0.690	2.179	0.044	**4.047**	**0.044**
	No Growth	77.25	26.98	50.94						
Sperm concentration characterization	Significant	67.79	23.59	43.75						
	Not Significant	72.00	23.50	49.76	**6.576**	**0.037**	0.783	0.376	0.783	0.376
	No Growth	81.98	26.36	5.38						

DISCUSSION

Seven (7) kinds of yeast isolates were identified from all semen sampled in the research. Their source may be from skin as commensals or from sexually transmitted routes from their partners. Yeasts are human commensal found in female vagina as well as some body parts and is a common human colonizer and microbial fomite found in moist or damp places. They have spores that easily sticking on clothing serving as a source of spread. Factors like having multiple sexual partners, warmth from sitting, air, skin or wet boxer undergarments, contaminants, immunosuppression from diseases and stress could have increased their susceptibility in these subjects. Achkar and Fries (2010) noted that all humans are colonized with *Candida* species, mostly *Candida albicans*, though some develop diseases due to *Candida*, among which genitourinary manifestations are extremely common. Adamu *et al.* (2012) isolated (50%) yeasts from fomites like helmets from commercial motorcyclists in Lagos.

Candida guilliermondii (a non-albican sp.) was the most frequently isolated yeast from male semen in test and control groups (44.9; 48.5%) and in total males (47.4%) while *Cryptococcus laurentii* the least (0.0; 1.0%) in the present research (table 4.2.3 a-b). This may probably be caused by a variety of specific and non-specific factors as well as a result of sexual transmitted sources, bacterial infections or other underlying diseases, or a conducive pH, temperature, sugar level, sexual history and other factors may have been met for the favorable growth of the species most prevalently isolated or not met for the specie least frequently isolated, or because those species are currently emerging in the area, making them more common in study geographical area. Sobel (2006) noted a rising rate of emergence of non-albican yeast infections in humans. *Candida spp*. has exceptional adaptability by rapid alterations in gene expression in response to various environmental stimuli (Vitali *et al.*, 2007).

In relation to occupation, more yeast kinds were isolated from semen of control groups(7) with 45-64 years old groups more affected maybe because exposure –risk factors were more available in control groups that favored the distribution pattern. Poor hygiene, antibiotic use, decline in age, metabolic diseases and weak immune

system may have enhanced their risk factors in age groups more susceptible with *C. glabrata* differing significantly between the two groups amongst all yeasts isolated (X^2= 8.635; P= 0.003). Lowest yeast isolates were *Cryptococcus. laurentii* in test groups and *Candida. glabrata* in controls may be because favorable factors for growth, survival and environmental requirements were not available. Sandhu et *al*. (2017) noted that risk factors for *Candida. krusei* like broad antibiotic usage and long stay in hospitals determines its presence in humans.

Occupational comparison between the groups showed that semen of test groups had more single (73.5%) and mixed fungal infection statuses (42.9%) than in control groups (58.3%; 35.0%). Mobile nature of the occupation, exposure to dust, inhalation, easy skin direct inoculation, warmth, sweat and life-style exposing factors like sexual practices could be encouraging factors for their high presence in the test group. Sipsas and Kontoyiannis (2008) noted that professions involving outdoor activities are associated with increased environmental exposure to pathogenic fungi as well as are at risks of invasive fungal infections (IFIs) On the other hand, higher occurrence of significant single and mixed fungal count statuses in semen of control groups (60.0%; 58.6%) than test groups(38.0%; 39.7%) in table 3a could be related to control group's activity, transmission pathway, distribution levels in the environment, routes of exposure, other matrixes like hormonal and immune status as of the men in the group as of the time of study, bacterial co-infection rate, duration of infection and concentration of exposure. Quantitative Microbial Risk Assessment (QMRA) recommended workers activity, transmission chain and route of exposure as important determinants for microbial counts status in any occupational risk-exposure assessment (Carducci *et al.*, 2016).

Age-wise, higher significant count status for single fungal infection occurred in older adults of 45-64 years old age- range groups (64.3%), while significant fungal count status was found in youngest age-range (19-44 years) (38.7%). This could be associated with variations in contamination and exposure of samples during processing, immunological changes, disease susceptibility, variability in gut micro biota, travelling and relocation, drugs taken, physical activity and initial colonization at infancy. Nagpal *et al.* (2018) and Vemuri *et al.* (2019) noted that pH, temperature, acidity, food source, altered gut morphology, dietary factors, immune status, environmental and life style influences, changes in physiological functions associated with other age-related changes, and others are associated with quantitative microbial risks. Ying *et al*., (2015) noted the association between age and microbial communities. In a human genome project study, Huttenhower *et al.* (2012) showed that there is a great difference in microbiome in different humans at different stages of life and anatomical sites (beta-diversity) Schoenmakers *et al.* (2019) differing in individuals and are influenced by diet, host genetics, body mass, early microbial exposure and antibiotics.

Several significant effects of fungal infection status were observed on mean rank values of fertility variables in the study. Significantly raised the mean values of citric acid in controls and total groups (X^2 = – 2.988; P = 0.003; X^2 = – 3.533; P= 0.000) and lowered mean values of alpha-neutral glucosidase in control groups (X^2 = – 1.986; P= 0.047) could be because infectious processes were present in significant concentration, produced inflammatory cells involving more than one gland, probably altered hormones with pH and assessory gland secretions alterations, creating varying secretory changes depending on duration of infection and strain of the infecting microbes. Differing results in different groups could be due to individual differences, life style, duration and concentration effect as well as other confounding factors. Castrillón-Duque *et al.* (2018) noted that all male associated gland infections (MAGI) have negative impact on the secretory function of the gland and on male fertility with changes in their level indicating a pathological process.

Significant differences observed in mean rank value of prolactin hormone between fungal infected (69.60$\pm$0.00) and non-infected (88.32 $\pm$ 0.00)(U= - 2.529; P = 0.011); multiple fungal infection categories (U = -2.100; P = 0.036) in total males; and fungal count significance for single infection (FCS 1) in controls and total groups (K = 7.593; P = 0.022; K = 6.673; P = 0.036) in tables 4a – 4cis associated with microbial interaction with the endocrinology system that may have produced varying effects because of different sensitivities initiated by hormones on varying tissues and cells. Variability in the actions could depend on concentration, strains and subsets of microbes, virulence of organisms, age, varying pathologies of infectious process, duration as well as state of immunity of the individuals in the groups. Hormonal shifts also affect the growth, metabolism and virulence of microbes. Ricardo (2018) noted that male accessory sex glands display a consistent pattern of differential sensitivity to androgens and estrogens with hormones secreted in these organs exerting their action on different cell types within the organ, agreeing with changes observed in this work. Neuman *et al*. (2015) proved that is the way by which hormonal secretion is modulated in a host is through cross-talk between the microbes and hormones by regulation of host's immunity, metabolism and behavior in a bidirectional manner. Both host hormones and bacterial hormones affect each other through the help of intercellular communication between hormonal receptors on bacteria and host hormones receptors (Hughes and Sperandio, 2008). This effect was noted by Sperandio *et al.* (2003) to occur when host hormones affects the microbial gene expressions and controls its virulence with a consequent effect on the host through the bacterial language-like process of quorum - sensing using auto-inducers. Bacterial attachment to the host has been noted to

enhance and affect its growth and virulence (Freestone and Lyte, 2008).

Fungal infection status (table 4a) and it significant count statuses (FCS 1 and FCS 2) (tables 4b-d) affected semen several qualities and their characteristics more than all the fertility variables in the present study. Significant effects were observed in both groups except for multiple fungal infections which significantly affected semen quality of control groups only. These effects could depend on infectivity of the fungi, duration of in sperm, species involved and the concentration of the fungal organisms. Pathogenic effects of yeasts on semen quality are attributed to be through the interaction of yeast via the mannose sperm receptors, effect relating to incubation period and concentration (Castrillon-Duque *et al.,* 2018). When infections touches two or more male assessory glands according to Ricardo *et al.,* (2018) sperm alterations occurs, while Dohl (2003) noted the deterioration of spermatogenesis with epigenetic changes associated with sperm infectious organisms. Different effects observed in both groups could be as previously explained.

Presence of different fungal status and their count statuses affected fertility index in both groups. Effects depend on count status, species of infecting fungi and concentration. Uropathogenic or commensal yeasts colonizers cause asymptomatic infections that ultimately affect semen quality. Changes in male semen quality with consequent result to male infertility by yeasts occur as previously stated above by (Castrillon-Duque *et al.,* 2018). Mesbah *et al*. (2016) noted the inhibitory effect on human spermatozoa in vitro fertilization by *C. albicans,* associated with impairment in sperm motility and impairment in ultrastructure of spermatozoa (Tian, 2007) leading to male infertility.

Fertility index of test groups was affected negatively by fungal infection status (infected- 17.30; non-infected 21.33), P>0.123, FCS 1 (significant – 24.56; not-significant 23.24), P> 0.221, and FCS 2 (significant – 25.58; not- significant- 11.00), P> 0.456 in the present study. More negative impact observed on fertility potentials of commercial motorcyclists than in non-cyclists despite the fact that significant count statuses for FCS1 and FCS 2 were more in controls could be associated with the fact that these groups also had higher status for single and mixed fungal infection statuses, the environmental impacts of the occupation due to the outdoor nature of the occupation, exposure to heat and promiscuous behaviors, lifestyle factors like age of affected groups, stress exposure, diet, smoking. Others are concentration and specie types of the infecting yeasts, antibiotic exposure and duration of the effects. Environmental and occupational factors are known to affect fertility and IVF success (Younglai *et al.,* 2005). Lifestyle factors according to Homan *et al.* (2007) impacts on general health and capacity to reproduce depending on individual aetiology and circumstances.

CONCLUSION

In conclusion, fungal infection and their count statuses impacted negatively more on fertility variables and potential in commercial motorcyclists more than in non-cyclists especially in 45-64 years groups.

REFERENCES

1. Achkar, A.M and Bettina, C. Fries, B.C. (2010). *Candida* infections of the genitourinary tract. *Clinical Microbiology Reviews,* 23(2): 253–273.
2. Adamu, L., Edeghagba, B., Olatomi, F., Ezeokoli, O., and Elijah, A. (2012). Microorganisms associated with commercial motorcycle helmets in Lagos metropolis. *Journal of Microbiology, Biotechnology and Food Sciences*, 1(5): 1179-1188.
3. Ahmad.G, du Plessis, S.S., and Agarwal. A. (2017). Sexually transmitted infections and impact on male fertility. In: Gunasekaran.K., Pandiyan, N., editors. Male infertility: a clinical approach. New Delhi: Springer India, 167-168.
4. Alsterholm, M., Flystrom I., Leifsdottir, R., Faergemann, J., and Bergbrant, I, M.(2008). Frequency of bacteria, Candida and *Malassezia species* in balanoposthitis. *Acta Derm Venereology*, 88(4): 331-336.
5. Ashkenazy- Elbha, M. (2005). Male genital tract infection: the point of view of the bacteriologist. *Gynécologie Obstétrique & Fertilité*, 33(9): 691-697.
6. Björn-dahl, L., Soderlund, I., Kvist, U. (2003). Evaluation of the one-step eosin-nigrosin staining 8858 technique for human sperm vitality assessment. *Human Reproduction*, 18: 813–816.
7. Carducci, A., Donzelli, G., and Verani. M (2016). Qualitative microbial risk assessment in occupational settings applied to the airborne human adenovirus infection. *International Journal of Research in public health*, 13(7): pii: E733.
8. Castrillon-Duque, E.X., Suárez, J.P., and Maya, W.D.C (2018). Yeast and fertility: effects of in vitro activity of *Candida spp*. on semen quality. *Journal of Reproduction and Infertility*, 19(1): 49 –55.
9. Cheesbrough, (2006). Discrete Laboratory Practice in Tropical Countries, Part Two, 2nd Edition. Tropical Health Technology and Butterworth-Heinemann LTD, Great Britain, 65-70.
10. Diemer, T., Huwe, P., Ludwig, M., Schroeder-Printzen, I., Michelmann, H.W., Schiefer, H.G., and Weidner, W. (2003). Influence of autogenous leucocytes and *Escherichia coli* on sperm motility parameters in vitro. A*ndrologia*, 35(2): 100–105.
11. Dohle, G.R. (2003). Inflammatory – Associated Obstructions of the male Reproductive Tract. *Andrologia*, 35(5): 321 – 324.
12. Freestone, P.P and Lyte, M. (2008). Microbial endocrinology: experimental design issues in the study of inter- kingdom signaling in infectious diseases. *Advance in Applied Microbiology*, 64: 75-105.

13. Google Maps (2018). Navigation and Transit APKs. APKMirror. Retrieved on May 26th, 2018 from www.apkmirror.com
14. *H*arvey, C. (1953). A fertility index derived from semen analysis. *Journal of Clinical Pathology*, 6: 232.
15. Homan, G.F., Davies, M., and Norman, R (2007). The impact of lifestyle factors on reproductive performance in the general population and those undergoing infertility treatment: a review. *Human Reproduction Update*, 13(3): 209-223.
16. Hughes, D.T and Sperandio, V. (2008). Inter-kingdom Signaling: communication between bacteria and their hosts. *National Revolutionary Microbiology*, 6: 11120.
17. Huttenhower, C., Gevers, D., Knight, R., Abubucker, S., Badger, J.H., and Asif, .T (2012). Human Micro biome Project Consortium. Structure, function, and diversity of the healthy human micro biome. *Nature*, 486: 207-2144.
18. Ibadin, O. K. & Ibeh, I. N. (2008). Bacteriospermia and sperm quality in infertile male patient at University of Benin Teaching Hospital, Benin City, Nigeria. *Malaysian Journal of Microbiology*, 4(2): 65-67.
19. Kind, P.R.N., and King E.J. (1954). Estimation of plasma phosphatase by determination of hydrolyzed phenol with amino-antipyrine. *Journal of Clinical Pathology*, 7(4): 322–326.
20. La Vignera, S., Condorelli, R.A., Vicari, E., Salmeri, M., Morgia, G., Favilla, V., Cimino, C., and Calogero, A.E. (2014). Microbiological investigation in male infertility: a practical overview. *Journal of Medical Microbiology*, 63(part 1): 1–14.
21. Lindahl, J.F., and Grace, D. (2015). The consequences of human actions on risks for infectious diseases: a review. *Infection Ecology and Epidemiology*, 5: 10.3402/iee.v5.30048.
22. Mesbah, N., and Salem, H.K (2016). Genital Tract Infection as a cause of Male Infertility. Assessed from intechopen.com./books
23. Nagpal, R., Mainali, R., Ahmadi, S., Wang, S., Singh, R., Kavanagh, K., Kitzman, D.W., Kushugulova, A., Marotta, F., and Yadavm, H (2018). Gut microbiome and aging: physiological and mechanistic insights. *Nutrition and Healthy Aging*, 4(4): 267-285.
24. Naing, L., Winn, T., and Rusli, B.N. (2006). Practical Issues in Calculating the Sample Size for Prevalence Studies. *Archives of Orofacial Sciences*, 1: 9-14.
25. National Committee for Clinical Laboratory Standards(2002). Reference method for broth dilution antifungal susceptibility testing of yeasts. M27-A2.
26. Neuman, H., Debelius, J.W., Knight, R., and Koren, O. (2015). Microbial endocrinology: the interplay between the microbiota and the endocrine system. *FEMS Microbiology Reviews*, 39(4): 509–521.
27. Reynolds, R., and Braude, A.I. (1956). The filament-inducing property of blood for *Candida albicans*: its nature and significance. *Clinical Research and Procedures*, 4: 40.
28. Ricardo, L. H. J (2018). Male Accessory Glands and Sperm Function, Spermatozoa, Rosaria Meccariello and Rosanna Chianese, Intech Open, DOI: 10.5772/intechopen.74321. Retrieved from: https://www.intechopen.com/books/spermatozoa-facts-and-perspectives/male-accessory-glands-and-sperm-function on 5th July 2018.
29. Sandhu, R., Dahiya, S., Sayal, P., and Budhani, .D. (2017). Increased role of non-albicans *Candida*, potential risk factors, and attributable mortality in hospitalized patients. *Journal of Health Research and Reviews*, 4(2): 78-83.
30. Schoenmakers. S., Steegers- Theunissen, R., and Faas, M. (2019). The matter of the reproductive microbiome. *Obstetrics Medicine*, 12(3): 107-115.
31. Shendre, M.C., and Tiwari, R. R. (2005). Role of occupation as a risk factor for sexually transmitted disease: A case control study. *Indian Journal of Occupational and Environmental Medicine*, 9(1): 35-37.
32. Sipsas, N.V., and Kontoyiannis, D.P (2008). Occupation, lifestyle, diet, and invasive fungal infections: Review article. *Infection*, 36(6): 515-525.
33. Sobel, J.D. (2006). The emergence of non-Candida species as causes of invasive candidiasis and candidemia. *Current Infectious Disease Rep.*, 8: 427-433.
34. Sperandio, V., Alfredo, G., Torres, A.G., Jarvis, B., Nataro, J.P., and Kaper, J.B (2003). Bacteria–host communication: The language of hormones. *Proceedings of the National Academy of Science of the United States of America*, 100(15): 8951–8956.
35. Sperandio, V.Torres A.G., Jarvis B. Nataro J.P. and Kaper. J.B. (2003). Bacterial-host communication: the language of hormones. *Proceedings of the National Academy of Science of the United States of America*, 100: 89516.
36. Tian, Y.H., Xiong, J.W., Hu, L., Huang, D.H., and Xiong. C.L (2007). *Candida albicans* and infiltrates interfere with human spermatozooal motility and alter the ultrastructure of spermatozoa; an in vitro study. *International Journal of Andrology*, 30(5): 421-429.
37. Tian, Y.H., Xiong, J.W., Hu, L., Huang, D.H., and Xiong, C.H. (2007). *Candida albicans* and filtrates interfere with human spermatozooal motility and alter the ultrastructure of spermatozoa: an in vitro study. *International Journal of Andrology*, 30: 421-429.
38. Tietz, N.W. ed., (1999). Clinical Guide to Laboratory Tests 3rd Edition, W.B. Saunders, Co., Philadelphia, 509-512.
39. Uriah, N., Ibeh, I.N. and Oluwafemi, F.T. (2001). A study on the impact of Aflatoxins on human reproduction. *African Journal of Reproductive Health*, 5(1): 106-110.

40. Vemuri, R., Gundamaraju, R., Shastri, M.D., Shukla, S.D., Kalpurath, K., Ball, M., Tristram, S., Shankar, E.M., Ahuja, K., and Eri, R (2019). Gut microbial changes, interactions, and their implications on human lifecycle: An ageing perspective. *Bio Med Research International* 2018, Article ID 4178607, 1-13.
41. Vicari, E., Mongioì, A., Speciale, A., Caccamo, F., Calogero, A., Gulizia, S., Pellegrino, M.B., Macchi, M., D'Agata, R. (1986). Enhancing detection of gonococcus in ejaculates of adult males using sperm dilution. *Archives of Andrology*, 16(1): 19–23.
42. Vitali, B., Pugliese, C., Biagi, E., Candela, M., Turroni, S., Bellen, G., G. G., Donders, G., and Brigidi, P. (2007). Dynamics of vaginal bacterial communities in women developing bacterial vaginosis, candidiasis, or no infection, analyzed by PCR-denaturing gradient gel electrophoresis and real-time PCR. *Applied Environmental Microbiology*, 73: 5731-5741.
43. World Health Organization (2001). Declaration of Helsinki world medical association declaration of Helsinki ethical principles for medical research involving human subjects. *Bulletin of the World Health Organization*, 79(4): 1-2.
44. World Health Organization (2010). WHO laboratory manual for the examination and processing of human semen, 5th ed. Geneva, 1-276. Retrieved on 5th April 2017 from http://www.who.int/iris/handle/10665/44261.
45. Ying, S., Zeng, D., Chi, L., Tan, Y., Galzote, C., Cardona, C., Lax, S., Gilbert, J. and Quan, Z. (2015). The influence of age and gender on skin-associated microbial communities in urban and rural human populations. *PLOS ONE*, 10(10): e0141842.
46. Younglai, E.V., Holloway, A.C., and Foster, W.G (2005). Environmental and occupational factors affecting fertility and IVF success. *Human Reproduction Update*, 11(1): 43-57.