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AN INVESTIGATION ON THE SEMINAL AND PERIODONTAL BACTERIA DIVERSITY AMONG SUBFERTILE MALES IN LAGOS

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ABSTRACT

Background: *This study was aimed at examining bacteria diversity in the semen and mouth of infertile men.*

Method: *This was a case-control study of male (n=43) participants including infertile men (n=30) as test and fertile men (n=13) as control within 30-50 years of age. Semen, mouth swab and blood samples were collected and processed by standard practice. Semen and mouth swab samples were screened to determine microbial presence. The semen qualitative and quantitative characteristics were determined using standard methods. Blood serum samples were evaluated to determine hormonal and lipid profiles as well as liver and kidney function biomarkers using standard kits.*

Results: *The frequency of bacterial occurrence or growth from the semen and mouth swab of infertile men was higher in comparison to the fertile group. Notably, the frequency of Staphylococcus aureus (36.7%) isolated from infertile men semen was higher as compared to fertile men with Staphylococcus aureus (15.4%). Moraxella catarrhalis (30%), Staphylococcus aureus (36.7%) and Escherichia coli (10.0%) were the major bacteria frequently isolated from infertile men mouth swab in comparison to the fertile group with Moraxella catarrhalis (46.1%) and Staphylococcus aureus (23.3%). There was a significant decrease ($p<0.05$) in the semen volume, concentration and motility of the test group compared to the control group, with a corresponding significant increase ($p<0.05$) in the abnormal semen morphology of the test group compared to the control. Significant ($p<0.05$) elevated levels of FSH and LH with decreased level of testosterone in the infertile men as compared to the control was also observed.*

Conclusion: *These results suggest increased bacteria diversity in relation to infertility. However, a relationship between bacterial isolates in the semen and mouth might exist.*

Keywords: Male infertility, periodontal infection, Staphylococcus aureus, Hormones.

INTRODUCTION

Infertility affects approximately 15% of all couples trying to conceive and male factor infertility is implicated in almost half of these cases (Sharlip *et al*., 2002). Problems with fertility can range from hormonal imbalances, to physical problems, to psychological and/or behavioral problems.

Intact Hypothalamo-pituitary Testicular axis plays a key role in male Fertility. (Jabbar *et al*.,2018). This is made possible by concerted actions of Gonadotropins (Follicle stimulatory Hormones and Luteinizing Hormones) and Testosterone secreted by the axis, which generate appropriate signals that initate and maintain quantitative and qualitative normal spermatogenesis, maintain normal secondary sex gland functions as well as sexual function (Jabbar *et al.*, 2018). However, imbalance in these hormones disrupts the normal regulatory mechanisms underlying fertility in male. Hence, profiling hormones that are of Hypothalamo-pituitary Testicular axis origin is one of the major tools for clinical diagnosis of infertility (Geidam *et al.*, 2008).

Furthermore, Cholesterol, a typical example of lipid is involved in steroid synthesis and plays an essential role in spermatogenesis. Hence, male reproductive system highly relies on its bioavailability for proper functioning. Owing to these facts, there might be relationship between serum lipid and male fertility (Liu *et al*., 2017).

It has long been suggested that at least half of the human male infertility of unknown etiology may be attributed to various environmental exposure and life style. In recent times, there has been an increasing interest in the contribution of other secondary infections such as untreated tooth decay in declining sperm concentration and human male infertility (Rekha and Angadi, 2010).

Many researchers agree that sperm counts have been declining for decades and fertility reflects a man's overall health. Men who live a healthy life style are more likely to produce healthy sperm and increase male fertility (Sofikitis and Miyagawa, 1991). Previous medical reports indicates that periodontal infections may lead to heart diseases, stroke, lower weight babies, premature births and even more. Interestingly, a research carried out by Klinger *et al.*, showed a possible link between male infertility and gum disease, indicating that gum infections may contribute to male infertility (Klinger *et al.*, 2011).

Bieniek *and* Riedel (1993) suspects a direct causal relationship between dental diseases and asymptomatic bacteriosperms, which probably leads to subfertility (Bieniek and Riedel, 1993). Although, scientist have yet to conduct extensive research to prove oral bacteria can create a bacterial infection in the male reproductive system, and lead to male infertility by reducing sperm motility, morphology and density. Thus, this study was aimed at identifying bacteria diversity in the semen and mouth of infertile men.

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MATERIALS AND METHODS

ETHICAL APPROVAL

This study was carried out according to the approval from the Research Grants and Experimentation Ethics Committee (RGEEC), College of Medicine of the University of Lagos Idi-Araba. Meanwhile, informed consent was obtained before participants' recruitment into the study.

STUDY DESIGN

This is a case-control study that involved a total of forty-three (n=43) men, who were recruited. Thirty (n=30) of the study population who were attending Public and Private hospital in Ikeja and Isolo Local government areas in Lagos state and indicated willingness to participate in the study were selected from a population of patients that have been diagnosed with acquired urogenital abnormalities related to obstructions and other testicular disorders (testicular torsion, testicular tumor, orchitis) that have been reportedly suggested to be among the major causes of sub-fertility in male (Sharma, 2017) . These made up the test group referred to as infertile men. In addition, thirteen (n=13) men who have impregnated their female counterparts within the past 12 months as reflected in their completed questionnaire were selected as the control group referred to as fertile men. The study population was grouped into four groups according to fertility status and age:

Group A - infertile men (n=22) of 30-40 years;
Group B - infertile men (n=8) of 41-50 years;
Group C - fertile men (n=10) of 30-40 years; and
Group D - fertile men (n=3) of 41-50 years.

All the forty three (43) men were given questionnaires for documentation of their bio-data, educational status, health seeking behaviour, and other basic information.

SAMPLE COLLECTION

Semen samples were collected according to the method of Sarker and Henry (2001). The samples were collected by masturbation into sterile receptacles, the specimen was collected after 2 or 3 days of abstinence. The specimens were kept at room temperature and examined within 1 hour.

Blood samples were collected through the vein with the use of a 5ml syringe into plain bottles and later centrifuged to obtain the serum. A swab of the mouth was also taken around the gum area with a swab stick.

SEMEN CULTURE AND ANALYSIS

Semen culture and analysis were carried out using the method of McGowan *et al.* (1981) and Alo *et al.* (2013). Briefly, semen samples were allowed to liquefy on the bench for 30 minutes before the samples were processed. The samples were inoculated on the media before semen analysis was performed. Exactly 0.1ml of each of the specimen was inoculated on three media plates consisting of Mueller Hinton Agar, Tripticase Soy Agar and MacConkey Agar. All media were prepared according to manufacturer's instructions. The Tripticase Soy Agar plated inoculum was incubated in anaerobic jar at 37^{o}C while the other media plated inoculums were incubated in aerobic environment at 37^{o}C for 24 hours. The cultured plates were examined and the bacterial isolates were identified and characterized using standard methods. Semen analysis was also performed on each of the samples using standard methods.

MOUTH SWAB CULTURE ANALYSIS

This was carried out using the method of McGowan *et al.,* 1981 and Alo *et al.* (2013). Mouth swabs taken with swab sticks were inoculated on agar medium. The inoculation procedure was similar to the procedure mentioned previously above.

BIOCHEMICAL AND HORMONAL ASSAYS

Aspartate Transaminase (AST) and Alanine Transaminase (ALT) were determined using Reltman and Frankel method (Reitman and Frankel, 1957) using Randox Reagent kit. Triglycerides were determined using GPO-PAP method. Creatinine was determined using Fabiny *and* Ertingshausen (1971). And Labbe *et al*. (1996) methods. Urea was determined using Searcy *et al.* (1967) method. Total cholesterol was determined using enzymatic end point method. Prolactin, Testosterone, Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) assay were determined using the method of Amballi *et al.* (2007).

STATISTICAL ANALYSIS

All data are presented as Mean ± Standard Error of Mean (SEM). Normality of data was tested with D' Agostino and Pearson Omnibus normality test. Independent student's t-test was used to compare the control and test groups with $P<0.05$, $P<0.01$ and $P<0.001$ considered to be statistically significant, statistical analysis was done using Graph Pad Prism 5 statistical software.

RESULTS

BACTERIAL ISOLATES IN SEMEN AND MOUTH SWAB OF INFERTILE AND FERTILE MEN

The frequency of seminal fluid bacterial growth was about 27 (90%) of the total population of the infertile men (n=30) studied while 8 (61.5%) was the frequency of bacterial growth observed among the seminal fluid of the total number of fertile men investigated (**Table 1**). In contrast, the occurrence of no bacterial growth from the semen among the infertile men (10.0%) was lower in comparison to the fertile men (38.4%). The frequently isolated bacteria from the semen of infertile men were major *Staphylococcus aureus* (36.7%) and *Escherichia coli* (13.3%). Although the frequency of bacterial occurrence or growth from the semen of fertile men was lower in comparison to the infertile group, *Staphylococcus aureus* (15.4%) and *Escherichia coli* (15.4%) remained the most frequently observed isolates in the fertile group (**Table 1**). Bacillus was not observed among the isolates from the semen of infertile men. In comparison to the bacterial isolates from the semen of infertile men, mixture of culture including *Streptococcus spp., Proteus spp., Klebsiella,* and *Pseudomonas* were not observed among the bacterial isolates from fertile men semen (**Table 1**).

Likewise, bacterial growth frequency from the mouth swab of infertile men (27 (90%)) was higher in comparison to that of the fertile men (11 (84.6%)). The frequency of growth of *Moraxella catarrhalis* from the mouth swab of infertile men (9 (30%)) was lower in comparison to that of the fertile men (6 (46.1%)) (**Table 2**). The frequently isolated bacteria from the mouth swab of infertile men were majorly *Moraxella catarrhalis* (30%), *Staphylococcus aureus* (36.7%) and *Escherichia coli* (10.0%). Meanwhile, the frequently isolated bacteria from the mouth swab of fertile men were majorly *Moraxella catarrhalis* (46.1%) and *Staphylococcus aureus* (23.3%). In comparison to the bacterial isolates from the mouth swab of infertile men, mixture of culture including *Streptococcus spp.*, *Klebsiella,* and *Pseudomonas* were not observed among the bacterial isolates from fertile men mouth swab (**Table 2**).

Table 1: Frequency comparison of semen microbial diversity in male infertility versus fertility

Organism	**Infertile Frequency (%)**	**Fertile Frequency (%)**
Staphylococcus aureus	11 (36.7)	2 (15.4)
Escherichia coli	4 (13.3)	2 (15.4)
Klebsiella spp.	2 (6.7)	1 (7.7)
Staphylococcus aureus and *Escherichia coli*	2 (6.7)	1 (7.7)
Bacillus	-	1 (7.7)
Staphylococcus aureus and *Candida albicans*	1 (3.3)	-
Proteus spp.	2 (6.7)	-
Pseudomonas and *Proteus*	2 (6.7)	-
Staphylococcus aureus and *Pseudomonas*	1 (3.3)	-
Streptococcus spp.	1 (3.3)	-
Staphylococcus aureus and *Proteus*	1 (3.3)	1 (7.7)
No growth	3 (10.0)	5 (38.4)
Total	**30 (100)**	**13 (100)**

Table 2: Frequency comparison of mouth swab microbial diversity in the infertile versus fertile males

Organism	**Infertile Frequency (%)**	**Fertile Frequency (%)**
Staphylococcus aureus	7 (23.3)	3 (23.1)
Escherichia coli	3 (10.0)	1 (7.7)
Moraxella catarrhalis (normal flora)	9 (30.0)	6 (46.1)
Escherichia coli and *Staphylococcus aureus*	4 (13.3)	1 (7.7)
Escherichia coli and *Bacillus*	1 (3.3)	-
Streptococcus spp.	1 (3.3)	-
Escherichia coli and *Klebseilla*	1 (3.3)	-
Staphylococcus aureus and *Pseudomonas*	1 (3.3)	-
No growth	3 (10.0)	2 (15.4)
Total	**30 (100)**	**13 (100)**

CHARACTERISTICS OF THE SEMEN OF INFERTILE AND FERTILE MEN

Decreased levels of seminal volume, motility and concentration, which were statistically significant (P<0.05), was observed in the infertile men as compared to the fertile men (**Table 3**). However, there was a significant (P<0.05) increase in the percentage of semen with abnormal morphology in the infertile men in comparison to the fertile group. The pH of both fertile and infertile men was within basic range, and there was no statistically significant difference between both groups (**Table 3**).

Table 3: Seminal fluid characteristics of the infertile and fertile male

Semen Prameters	Fertile (control)	Infertile (test)	P value
Age (mean)	36.54 ± 1.43	38.47 ± 0.67	NA
Volume (ml)	3.29 ± 0.22	2.57 ± 0.20 *	0.035
pH	8.15 ± 0.10	8.20 ± 0.07	0.729
Sperm Concentration (10^6/ml)	69.16 ± 12.74	42.42 ± 6.67 *	0.048
Motility (%motile)	65.77 ± 3.75	49.31 ± 3.94 *	0.014
Morphology (% abnormal)	26.15 ± 1.617	34.48 ± 2.20 *	0.021

***shows statistical significance compared to the control**

BIOCHEMICAL AND HORMONAL PROFILE OF THE INFERTILE AND FERTILE MEN

The levels of prolactin, LH and FSH were significantly (P<0.05) higher in the infertile group in comparison to the fertile group. But the observation was on the contrary for testosterone, whose level was significantly (P<0.001) lower in the infertile group in comparison to the fertile group (**Table 4**). There was a significant increase in the levels of AST (P<0.05) and ALT (P<0.001) in the infertile group as compared to the fertile group. There was also a significant increase in the levels of LDL and TG in the Test Group compared to the Control Group; however, HDL level was significantly lower. However, there was no significant (P>0.05) difference in the levels of total cholesterol, creatinine and urea between the infertile and fertile groups (**Table 4**).

Table 4: Hormonal and biochemical profiles of the infertile and fertile male

	Parameters	Fertile (control) (N=13)	Infertile (test) (N=30)	P Value
Hormonal Parameters	Testosterone	6.42 ± 0.22	3.71 ± 0.36***	<0.0001
	Prolactin	126.10 ± 11.32	171.6 ± 11.18*	0.0190
	Luteinizing Hormone (LH)	5.59 ± 0.26	6.72 ± 0.28*	0.0180
	Follicle Stimulating Hormone (FSH)	8.05 ± 0.49	10.26 ± 0.67*	0.0467
Biochemical Parameters	Total Cholesterol (TC)	164.70 ± 10.12	186.80 ± 6.85	0.0813
	Triglceride (TG)	106.70 ± 7.79	135.10 ± 8.32*	0.0445
	High Density Lipoprotein (HDL)	75.32 ± 2.13	59.28 ± 3.99*	0.0142
	Low Density Lipoprotein (LDL)	71.45 ± 9.56	102.60 ± 8.34*	0.0338
	Alanine Amino Transferase (ALT)	8.31 ± 0.48	11.87 ± 0.60***	0,.0007
	Aspartate Amino Transferase (AST)	10.51 ± 0.50	12.94 ± 0.63*	0.0210
	Creatinine	0.82 ± 0.05	0.84 ± 0.03	0.8160
	Urea	29.08 ± 3.13	31.52 ± 2.07	0.5202

***Shows statistical significance compared to the control at P<0.05**
****Shows statistical significance compared to the control at P<0.01**
*****shows statistical significance compared to the control at P<0.001**

BIOCHEMICAL AND HORMONAL PARAMETERS IN THE SERUM OF THE INFERTILE MEN OF DIFFERENT AGE GROUPS

Figures 1 and 2 shows a significant increase (P<0.05) of FSH, ALT and AST in the infertile male serum of the 41-50 years age group as compared to that of the infertile male serum of 30-40 years age group. While the levels of LH, Prolactin, HDL, LDL, TC, TG, Creatinine and urea had no significant difference between the infertile age groups. There was a significant decrease (P<0.05) in the level of testosterone in both the fertile and infertile semen of 41-50 years age group as compared to the 30-40 age group (**Figure 3**).

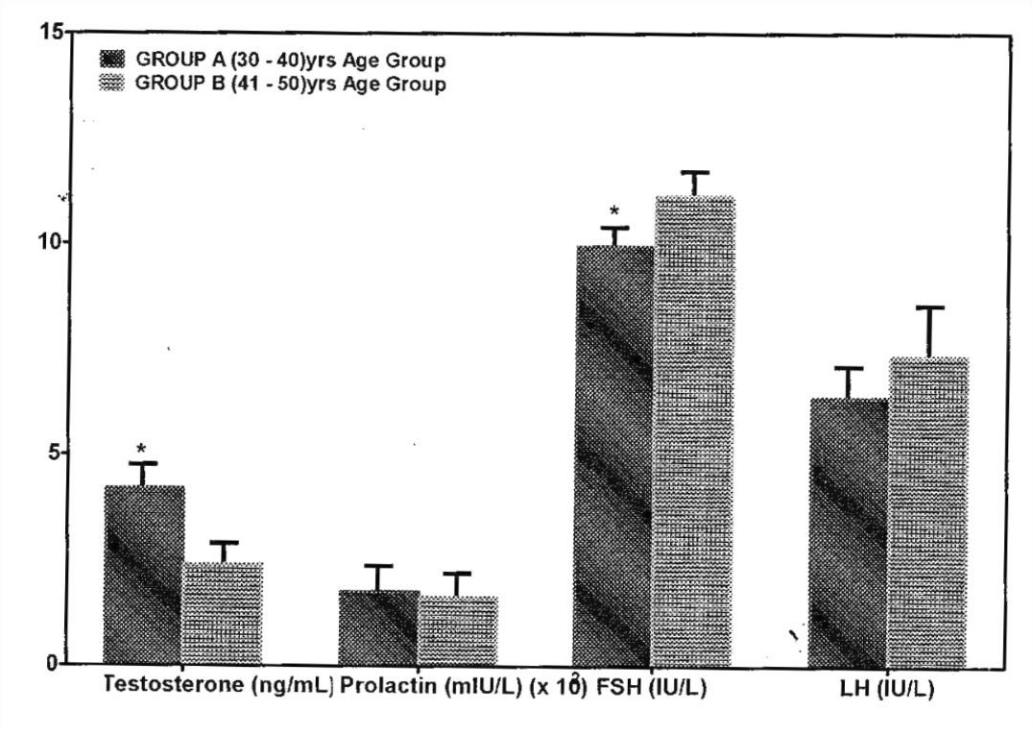

Figure 1: Hormonal parameters evaluation in the serum of infertile men of age groups A and B. Group A (30-40yearsrs, n= 22), Group B (41-50 years, n=8).

Data are presented as Mean ± Standard Error of Mean (SEM). Normality of data was tested with D' Agostino and Pearson Omnibus normality test. Independent student's t-test was used to compare the control and test groups while Pearson Correlation was used to test the relationship among the continuous variables. $P<0.05$ was considered to be statistically significant.

***Shows statistical significance compared to the control at $P<0.05$**

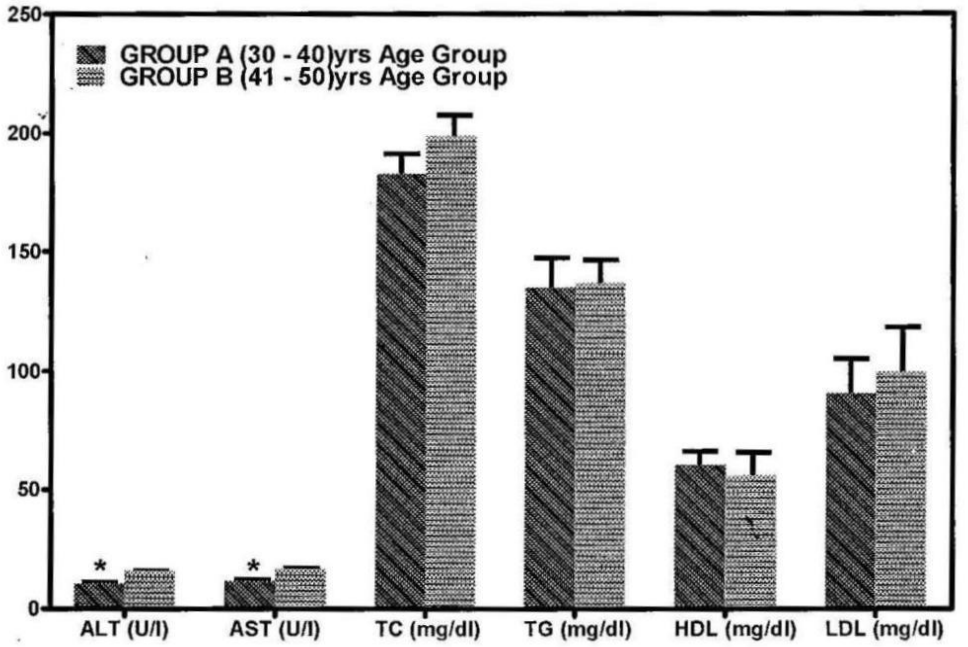

Figure 2: Biochemical parameters and Lipid Profile evaluation in the serum of infertile men of age groups A and B. Group A (30-40yearsrs n= 22), Group B (41-50 years n=8).

Data are presented as Mean ± Standard Error of Mean (SEM). Normality of data was tested with D' Agostino and Pearson Omnibus normality test. Independent student's t-test was used to compare the control and test groups while Pearson Correlation was used to test the relationship among the continuous variables. $P<0.05$ was considered to be statistically significant.

***Shows statistical significance compared to the control at P<0.05**

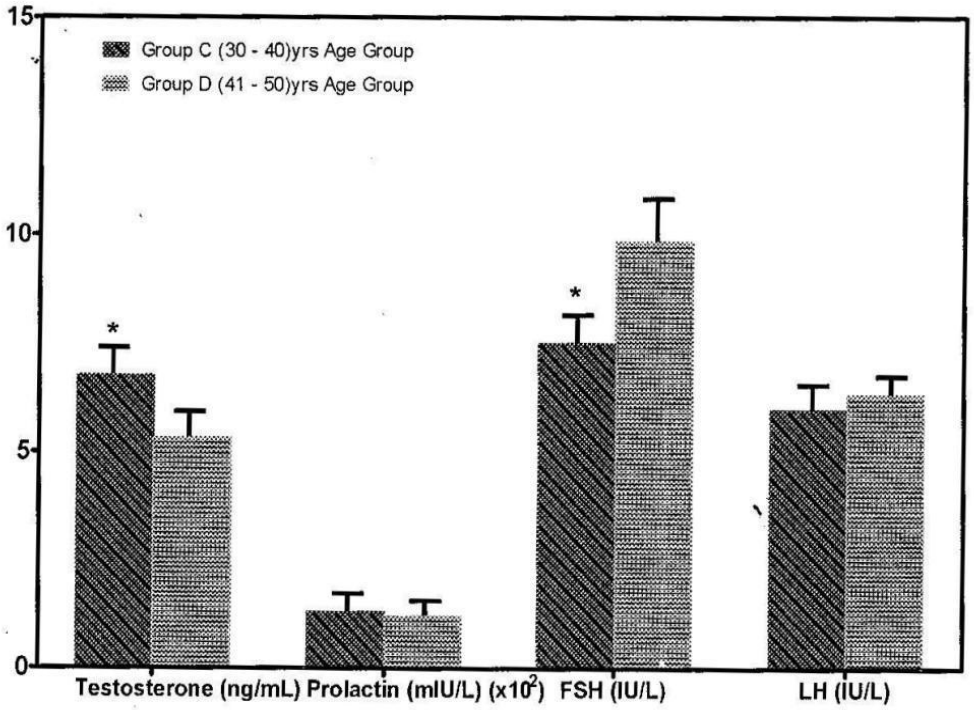

Figure 3: Hormonal parameter evaluation in the serum of fertile men of age groups C and D. Group C (30-40yearsrs n= 10), Group D (41-50 years n=3) Data are presented as Mean ± Standard Error of Mean (SEM). Normality of data was tested with D' Agostino and Pearson Omnibus normality test. Independent student's t-test was used to compare the control and test groups while Pearson Correlation was used to test the relationship among the continuous variables. $P<0.05$ was considered to be statistically significant.

***Shows statistical significance compared to the control at $P<0.05$**

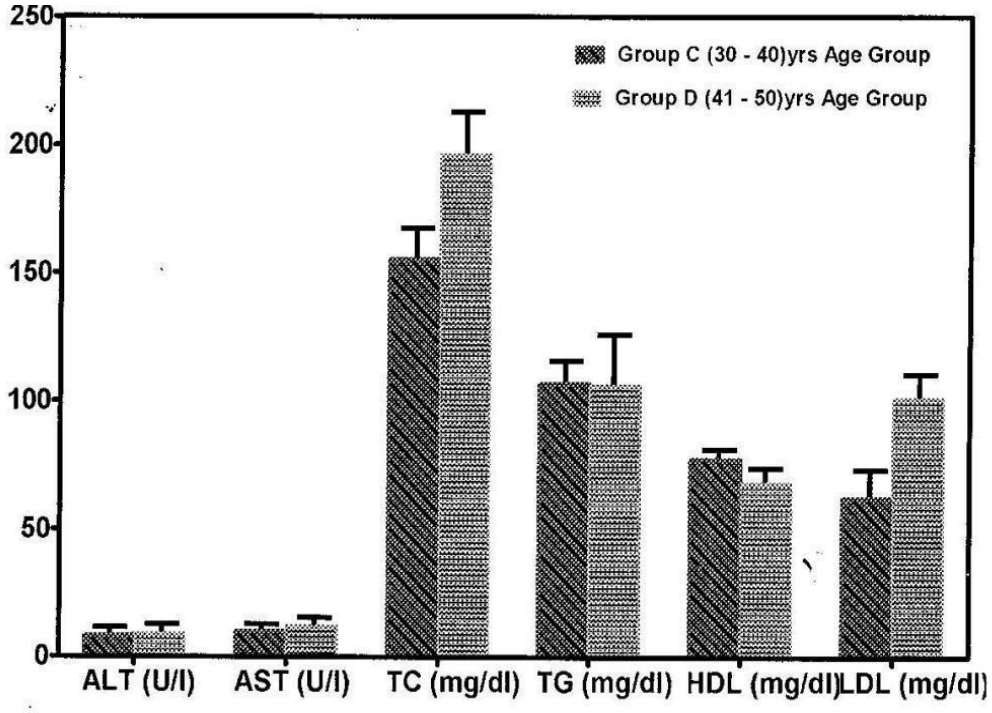

Figure 4: Biochemical parameters and Lipid Profile evaluation in the serum of fertile men (Control) of age groups C and D. Group C (30-40years n=10), Group D (41-50 years n=3)

Data are presented as Mean ± Standard Error of Mean (SEM). Normality of data was tested with D' Agostino and Pearson Omnibus normality test. Independent student's t-test was used to compare the control and test groups while Pearson Correlation was used to test the relationship among the continuous variables. $P<0.05$ was considered to be statistically significant.

***Shows statistical significance compared to the control at $P<0.05$**

DISCUSSION

Many studies examined the impact of male reproductive potentials; however, the effect of bacteria on sperm quality is still controversial (Moretti *et al*., 2009; Li, 2009). The result of this study shows the frequency of the bacterial isolates particularly the genera *Staphylococcus* and *Escherichia coli* as predominant bacterial isolates cultured from the semen of men with clinical conditions that are factorial as causes of infertility. This is consistent with the findings reported by Okon *et al.* in Maiduguri, where *Staphylococcus aureus* was isolated from 62.5%

of the seminal fluid of infertile men (Okon *et al*., 2005). Although, most medical practitioners dismiss this occurrence as mere contamination assumed to be of no significance, Bukharin *et al.* (2000) states that opportunistic microorganisms causes classical infections of urogenital tract and subclinical reproductive tract infections. These infections of the seminal fluid leads to decrease in the number of spermatozoa, the suppression of their motility, changes in their morphology and fertilizing capacity (Bukharin *et al.*, 2000). Treatment of periodontal disease has been shown to improve semen parameters in male patients (Bieniek *et al*., 1993). Linossier *et al.* (1982) were able to establish a link between poor oral hygiene and low sperm motility by studying 56 men aged between 23 and 52 years. It was discovered that about 13 percent of the subjects in the three year pilot study had healthy gums, 50 percent were diagnosed with gingivitis, 32 percent with chronic periodontitis and 5 percent with aggressive periodontitis. They observed that 65 percent of the patients with low sperm count suffered from gingivitis as compared to only 48 percent among those with normal sperm, and half of those with no sperm had chronic periodontitis (Linossier *et al.*, 1982).

Our result suggests a relationship between the bacterial isolates from the semen and mouth swab of the infertile men. Although, there is no substantial evidence to prove that the bacteria of the semen samples and mouth swabs are of the same genetic strain, even though the bacterial isolates from the mouth swab may be as a result of the semen bacteria diversity, further research is needed to ascertain the genetic strain, and the possible pattern of migration of these bacterial isolates. Also, common bacterial isolates responsible for tooth decay such as *Streptococcus mutans* and *Lactobacillus* species should also be looked into in order to establish the association between tooth/gum decay and male infertility.

The significant decrease in the semen concentration, motility and volume coupled with significant increase in the abnormal semen morphology in the infertile men compared to the fertile men is supported by the observation of Shubhada *et al*. (2013). Shubhada and his colleagues concluded that semen volume, motility and concentration in infertile men are significantly decreased compared to fertile men (Shubhada *et al*., 2013). The reason for this decrease might be due to the presence of high percentage of bacterial isolates in the semen. The idea that abnormal seminal fluid parameters had a direct link with male infertility arises from clinical observation of the patients' male reproductive system. Male urogenital tract infections are one of the most important causes of male infertility worldwide. Infection processes may lead to deterioration of spermatogenesis, impairment of

sperm functions, and obstruction of the seminal tract. In-view of the above, there is the need to institute a Microbiological intervention to detect the probable microbial agents. It should be noted that presence of urogenital tract infection and inflammation poses a danger to the fertility profile of male patient and should be eradicated by the use of appropriate prescribed antibiotics and anti-inflammatory treatment. Therefore, because of the important role of bacterospermia in male infertility, more attention should be attached to sexually active men in this regards. Follicle stimulating hormone, luteinizing hormone and testosterone are prime regulators of germ cell development. The production of spermatozoa generally requires of FSH, LH and testosterone. Follicle stimulating hormone acts directly on the seminiferous tubules whereas luteinizing hormone stimulates spermatogenesis indirectly via testosterone. The significant ($P<0.05$) elevated levels of FSH and LH with decreased level of testosterone in the infertile men as compared to the control established in this study are consistent with previous study by Babu *et al.* (2004). Sulthan *et al.* (1985) had illustrated elevated concentration of FSH in infertile male due to seminiferous epithelial destruction. This result suggests that low sperm qualities observed in the infertile group may be as a result of the hormonal imbalance.

The significantly increased triglycerides and low density lipoprotein (LDL) as well as decreased HDL in the infertile group compared with the fertile group ($P<0.05$) suggests high levels of lipids in infertile men. Padron *et al.* (1989) suggests that high lipid levels express direct adverse effects at the testicular level by altering sperm mutation process in male reproductive tract and capacitation modification. Remirez-Torres and colleagues in 2000 studied the incidence of hypercholesterolemia and hypertriglyceridemia among infertile men, and found that 65% of their cases had the aforementioned lipid defects (Remirez-Torres *et al*., 2000). This observation supports the present finding which showed elevated total cholesterol level in the infertile group as compared to the control group. It is also in line with the study of Yamamoto *et al.* in 1999 that stated that only high level of cholesterol affects sperm motility in rabbit (Yamamoto *et al.*, 1999). The observation of inverse proportion between the levels of testosterone and prolactin as well as between testosterone and total cholesterol is consistent with the previous study of Sajeda *et al.* (2010) who concluded that infertile subjects with low level of testosterone also had a corresponding high level of LDL. Meanwhile, the observation of no significant difference in the level of creatinine and urea in the infertile men compared to the fertile men indicates that infertility does not have any

direct relationship with the malfunctioning of the kidneys and creatinine measurement is used almost exclusively in the assessment of kidney function.

The lower level of testosterone in the infertile and fertile male serum of 41-50 years age group as compared to that of 30-40 years age group indicates that testosterone level decreases with older age resulting in the decreases sperm production. This finding, is consistent with the findings of Zahed *et al*.(2012) indicates atrophy of glandular tissue of pituitary or testes resulting in significantly lower level of testosterone in the infertile men's semen of 41-50 years age group. This may also be due to prostatic inflammation and obstruction or blockage of the male reproductive tract. Hence, testes not functioning properly and testosterone production being affected.

CONCLUSION

In conclusion, the results obtained from this study suggests a relationship between bacterial isolates in the semen and mouth, although, further research needs to be carried out to ascertain that the bacterial isolates found are of the same genetic strain. A direct relationship was not established in regards to male infertility; however, the increase in seminal fluid bacterial isolates might have elicited decrease in sperm concentration, volume, motility and morphology. These parameters, happens to be one of the direct indices of assessing male infertility, as well as hormonal imbalance and lipid profile parameters.

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