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A Study of Endocrinopathies and Some Stress Biomarkers in Infertile Male in Abuja, Nigeria

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Abstract **Original Research Article**

Infertility is a growing gynecological problem in our communities with couples of child bearing age having difficulties bearing children; it has been reported that 40% of infertility cases are attributed to the male. This study is aimed at determining the contributions of endocrinopathies and some stress biomarkers to male infertility. A total of one hundred and thirty two (132) participants were recruited into the study. They consist of the study group (82) male with the condition of infertility attending fertility clinic in Federal Capital Territory (FCT) General Hospitals in Abuja, Nigeria, and the control group (50), male without the condition of infertility. The Prolactin, Testosterone, Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), Salivary Cortisol and Salivary Alpha Amylase were estimated using both competitive and non-competitive Enzyme Linked Immunosorbent Assay (ELISA) techniques; while sperm analysis was estimated using conventional methods. The mean Testosterone, Follicle Stimulating Hormone(FSH), Sperm cells count and Sperm activity (%) were significantly lower ($p<0.05$) in the male study group relative to the control group (3.44±2.35 versus 5.86±1.55; 4.50±2.20 versus 5.91±1.66; 19.42±26.08 versus 53.80±11.74 and 33.99±26.07 versus 49.10.8±14.80 respectively); while Prolactin, salivary Cortisol and Alpha Amylase were significantly higher ($p<0.05$) in the male study group relative to the control group (18.01±11.56 versus 6.98±3.34; 449.75±106.81 versus 340.65±72.53 and 13.12±4.39 versus 8.45±3.01 respectively). There was no significant difference in the value of Luteinizing Hormone (LH) in the study group and control group $p>0.05$. Both conditions of oligospermia and azospermia were observed in the male study group, with associated hormonal abnormalities. Stress in male cause decreased semen quantity and quality.

Keywords: Male Infertility, Salivary Cortisol, Hormonal profile, Sperm cells count, Sperm cells active.

INTRODUCTION

Endocrinopathies, stress and infertility are common all over the world affecting up to 14% of couples of child bearing age [15, 1]. The prevalence of infertility is high in sub-Saharan Africa, ranging between 20-40% [5]. Although the Africa socio-cultural setting has before now focused on the female, fertility problems are obviously shared between both male and female sexes. Male infertility may account for up to 40% of infertility in couples [2]. Approximately one-third of the cases of infertility are equally attributable to the man. Male infertility is established when identifiable female causes of infertility is excluded and when semen quantity and quality fails to meet WHO criteria [22]. Male infertility is referred to as male inability to cause pregnancy in the female who is fertile, male factor infertility accounts for about 45-50% of couple infertility [4]. In Nigeria, infertility is a growing medical conditions, studies has shown that about 25% of couples are affected by infertility. Out of these numbers, the male factor of infertility accounts for 45-50% of the infertility cases [10]. A Center for Disease Control and Prevention (CDC) study analyzes data from the 2002 National Survey of Family Growth, and found that 7.5% of all sexually active men younger than 45 years reported seeing a fertility doctor during their life time. This is equal to 3.3-4.7 million men. Among men who sought for help in infertility, 20% were diagnosed with male related infertility problem, these includes sperm or semen abnormality (14%) and variococele (6%) [6].

The causes of male infertility could be pretesticular, testicular and post testicular [17]. The pretesticular and to some extent the testicular causes are mainly endocrine disorders originating from the hypothalamus- pituitary- gonadal- axis which have adverse effects on spermatogenesis. The main determinant of male potential is the quantity and quality

of spermatozoa ejaculated during sexual intercourse [8]. Male fertility is critically dependent upon normal hormonal parameters. Evaluation of the sub fertile male requires a complete medical history, physical examination and specific laboratory investigation [17]. Several authors have suggested that the increased incidence of infertility in Africa is due to high prevalence of sexually transmitted disease [17]. There are scares literatures on the hormonal abnormalities and stress biomarkers in infertile male in North Central Nigeria.

MATERIALS AND METHODS

Study Area

The study was carried out in Asokoro, Garki, Gwarinpa, Maitama, Wuse General Hospitals and Department of Chemical Pathology Laboratory and Microbiology Department of Alpha Royal Medicals Ltd, in Federal Capital Territory (FCT), Abuja Nigeria.

Subject and Sample

One hundred and thirty two subjects were involved in the study, which consist of Eighty two infertile male and fifty fertile male. Five milliliters of blood sample was drawn from each of the subject from the articular vein on their clinic visit days ; the sample were allowed to clot after which, it was spin at 3000rpm for 5 minutes; serum sample was then extracted from the clotted sample and then refrigerated at the temperature of 4-8 degree centigrade until analysis. Also the Saliva sample was collected into a universal container containing a preservative, Sodium benzoate. The Saliva sample is stable until analysis. The male participants were instructed to collect the semen sample after abstinence for 3 – 5 days, through withdrawal or masturbation into clean universal container, the semen samples were analyzed within 30 – 60 minutes after collection of specimen.

Ethical Approval

Ethical approval was sought and obtained from the Ethical clearance committee of Igbinedion University and Health Research Ethics Committee Abuja with reference number FHREC/2018/01/97/21-08-18 dated August 21, 2018.

Exclusion and Inclusion Criteria

Infertile male due to vasectomy, those who were less than 18 years and above 45 years and with chronic diseases were excluded; while those within 18 – 45years and without any known use of contraceptive were included.

Informed Consent

The purpose and protocol of the study were clearly explained to each patient and all participants were requested to voluntarily sign the consent forms in their own hand writing as proof of willingness to provide samples for the research work.

Data Collection

Prior to specimen collection, demographic information of the participants were obtained through administration of prepared questionnaires. Interpreter was provided for translation where it was necessary. Each questionnaire had a unique participant identification number (PIDN). The first part of the questionnaires contained the bio data of the patients e.g. sex, age etc. The second part consists of duration of the condition of infertility. For reason of privacy, all data were kept confidential in accordance with World Medical Association declaration of Helsinki [21]. For each participant, only the PIDN was recorded on the laboratory forms (no names). All the filled questionnaires were destroyed after data entry had been completed.

Hormonal Assay

The serum LH, FSH, Prolactin, Testosterone, Salivary Cortisol and Salivary Alpha Amylase were measured spectrophotometrically by the microwell enzyme linked immunosorbent assay (ELISA) technique based on the noncompetitive sandwich principle, in accordance with the methods provided by diagnostic reagent kit supplied by Darlez Nig Ltd. The semen analysis was estimated using conventional method.

STATISTICAL ANALYSIS

The demographic characteristics of the participants were expressed as mean values and standard deviation. Differences in serum hormones levels, salivary cortisol levels, salivary alpha amylase levels, sperm cells count and sperm cell activity (%) levels between the study group and the control group were tested by student t- test. P value <0.05 were considered statistically significant. The statistical package for social science (SPSS) window version 20.0 was used for all calculation and data analysis and Pearson's correlation was used for the correlation of the study conditions with biomarkers.

RESULTS

This present study examined the hormonal profile, some stress biomarkers and the semen analysis in infertile male in Abuja, Nigeria. A total of one hundred and thirty two subjects were recruited into the study. The subjects consist of 82 subjects who are known with infertility condition as study group and 50 subjects without the condition of infertility as control group. The mean age of the subject participants are presented in Table-1. The mean Hormones, Biomarkers and Sperm parameters in the male study and control groups are presented in Table-2. The Comparisons of measured Hormones and Biomarkers according to semen quality of the male category are presented in Table-3 The Multiple Comparisons of semen quality of measured variables among male are presented in Table-

4. The correlation of biomarkers with various sperm conditions are presented in Tables 5-8; and Figures 1-4.

Table 1 Characteristics of the Studied Subjects

The mean age of the male subjects of the Study Group was 33.07±4.56 while that of the Control Group was 32.25±4.14 as shown in Table-1. They have been in infertility for a period of 4–5 years.

Table-1: Mean Age of Study Group and the Control Group in the subjects

Parameter	Study Group Mean± S.D	Control Group Mean ± S.D	t-student test	p-value
Age	33.07±4.56	32.25±4.14	1.064	0.201
Duration of infertility	4-5years			

Table 2 Levels of measured hormones, biomarkers and semen quality among study group male participants

The mean of the FSH concentration of the male study group (4.50±2.20 miu/ml l) was statistical significantly lower ($p<0.05$) than that of the male control group (5.91±1.66 miu/ml); mean value of the LH of the male study group (5.64±2.26 miu/ml l) show no statistical significance ($p>0.05$) to that of the male control group (5.43±1.66 miu/ml); mean value of the Prolactin of the male study group (18.01±11.56ng/ml l) was significantly higher ($p<0.05$) than that of the male control group (6.98±3.34 ng/ml). The level of testosterone in the male study group (3.44±2.35 ng/ml) was significantly lower ($p<0.05$) than those of the male control group (5.86±1.55ng/ml); the Salivary cortisol level was equally significantly higher ($p<0.05$) in the male study group (449.75±106.81) compared to the male control group (340.65±72.53). Salivary Alpha Amylase level was significantly higher ($p<0.05$) in the male study group (13.12±4.39) compared with the male control group (8.45±3.01); the Sperm cell count ($x10^6$) was significantly lower ($p<0.05$) in the male study group (19.42±26.08 cells/ml) compared with that of the control group (53.80±11.74 cells/ml); the percentage of active Sperm cells (%) was significantly lower ($p<0.05$) in the male study group (33.99±26.07) compared with the control group (49.10±14.80) as shown in table 2; the sperm cells viability was significantly lower in the study group relative to the control group ($p>0.05$). The mean ejaculate volume was also higher in the control group ($p<0.05$) when compared with the study group, 5.6ml versus 3.3ml.

Table-2: Mean Hormonal, Biomarker and Sperm quality parameters in the study group and the control group in the Males Category

Parameter	Study Group Mean± S.D	Control Group Mean ± S.D	Normal values	p-value
FSH (miu/ml)	4.50±2.20	5.91±1.66	2.0 – 14.0	<0.001
LH (miu/ml)	5.64±2.26	5.43±1.66	2.0 – 14.0	0.560
Prolactin (ng/ml)	18.01±11.56	6.98±3.34	4.0 – 12.0	<0.001
Testosterone(ng/ml)	3.44±2.35	5.86±1.55	2.5 – 10.0	<0.001
S. Cortisol(micromol/L)	449.75±106.81	340.65±72.53	221 - 552	<0.001
S. Amylase (U/L)	13.12±4.39	8.45±3.01	1 - 15	<0.001
Sperm Cells Count ($x10^6$)	19.42±26.08	53.80±11.74	> 20 $x10^6$	<0.001
Sperm Cells Active (%)	33.99±26.07	49.10±14.80	> 50%	<0.001
Viability (%)	60.7±13.12.9	74.8±14.71	-4.712	<0.001
Semen Volume (ml)	3.25±1.97	5.62±2.0	-6.077	<0.001

Keys: LH = Luteinizing Hormone; FSH = Follicle Stimulating Hormone; S = Salivary.

Of the 82 male cases examined, 54(65.9%) were normospermia, 15(18.3%) were Oligospermia while 13(15.8%) were Azoospermia as shown in table 4.11. There was statistical significant difference ($p<0.05$) in the hormones, biomarkers and semen quality measured across control group, normospermia, Oligospermia and Azoospermia group respectively as shown in Table-3.

Table-3: Comparison of Measured Hormones and Biomarkers according to semen quality of the Male Category

Variables	Control Group	Study Group				
	Control Mean± SD n=50	Normospermia Mean± SD n=54(65.9%)	Oligospermia Mean± SD n=15(18.3%)	Azoospermia Mean± SD n=13(15.8%)	P-VALUE	Sig
FSH (miu/ml)	5.91±1.66	5.53±1.96	3.22±0.87	1.80±0.48	0.0001	S
LH (miu/ml)	5.43±1.66	6.46±1.97	5.12±2.03	2.90±1.01	0.0001	S
Prolactin(ng/ml)	6.98±3.34	12.94±6.05	18.82±7.50	37.73±11.45	0.0001	S
Testosterone (ng/ml)	5.86±1.55	5.75±1.65	2.73±0.81	1.04±0.75	0.0001	S
S. Cortisol (micromole/L)	340.65±72.53	397.5±79.95	505.33±73.76	598.46±46.52	0.0001	S
S. Amylase (U/L)	8.45±3.01	11.19±2.94	14.13±3.20	19.85±3.53	0.0001	S
Sperm Cells Count ($x10^6$)	53.80±11.74	46.40±19.43	5.73±3.41	0.00±0.00	0.0001	S
Sperm Cells Active (%)	49.10±14.80	48.92±18.97	10.67±6.78	0.00±0.00	0.0001	S

Keys: LH = Luteinizing Hormone; FSH = Follicle Stimulating Hormone; S = Salivary.

Table-4 shows the post Hoc multiple comparisons of measured parameters of the different semen quality state and the control group. The prolactin, Salivary Alpha Amylase and Salivary Cortisol level in the control were significantly different from those of Normospermia group ($p<0.05$), Oligospermia ($p<0.05$) and Azoospermia group ($p<0.05$), the LH level of the control group show no statistical significant difference from those of Normospermia group($p>0.05$) and Oligospermia ($p>0.05$), but show significant difference ($p<0.05$) in Azoospermia, there was no significant difference ($p>0.05$) in LH level of Normosperima and Azoosperima, it was however significantly different ($p<0.05$) in Normospermia and Azoospermia and also in Oligospermia and Azoospermia respectively. The FSH and testosterone level of the control group show no statistical significant difference from those of Normospermia group ($p>0.05$), but show significant difference ($p<0.05$) in Oligospermia and in Azoospermia respectively; there was significant difference ($p<0.05$) in FSH and testosterone levels of Normospermia, Oligospermia, Normospermia and Azoospermia.

The sperm cells count($x10^6$) and Active sperm cell (%) of the control group show no statistical significant difference from those of Normospermia group ($p>0.05$), but show significant difference ($p<0.05$) in Oligospermia and in Azoospermia respectively, there was significant difference ($p<0.05$) in sperm cells count and Active sperm cell level of Normosperima and Oligospermia, Normospermia and Azoospermia and in Oligospermia and Azoospermia respectively as shown in Table-4.

Table-4: Multiple comparisons of semen quality of measured variables among the male groups

Group	LH (miu/ml)	FSH(miu/ml)	Prolactin (ng/ml)	Testosterone (ng/ml)	S. Cortisol Microm/L	S. Amylase U/L	Sperm Cells Count ($x10^6$)	Sperm Cells Active (%)
AvB	0.38	**0.56**	0.01	0.87	0.02	0.002	0.09	0.17
AvC	0.97	**0.01**	0.01	0.01	**<0.001**	**<0.001**	**<0.001**	**<0.001**
AvD	**0.001**	**0.001**	<0.001	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
BvC	0.35	**0.03**	0.01	0.01	**<0.001**	0.02	**<0.001**	**<0.001**
BvD	**0.003**	**0.001**	<0.001	**<0.001**	**<0.001**	**0.001**	**<0.001**	**<0.001**
CvD	**0.002**	**0.09**	<0.001	**0.26**	**0.03**	**0.02**	**<0.001**	**<0.001**

A – Control, B – Normospermia, C –Oligospermia, D – Azospermia

Correlations of hormones and biomarkers in the male subjects

A Tables 5-8 below highlights multiple comparisons showing correlation between the measured hormones and the biomarkers in the controls and the different categories of the cases (Normospermia, Oligospermia and Azoospermia state).In the control category, there was weak positive significant correlation between FSH and active sperm cells (r=0.304, p<0.05), a weak positive significant correlation was found between salivary cortisol and salivary alpha amylase (r=0.354, p<0.05), a very strong significant positive correlation between sperm cell count and active sperm cells (r=0.825, p<0.05) as shown in Table-5**.**

Table-5: Correlation between hormones, biomarker and semen quality in Control group of the Males Category

Correlation	R-Value	P-Value
LH and FSH(in Control	0.003	0.984
LH and Prolactin in Control	0.135	0.345
LH and Testosterone in Control	0.089	0.536
LH and S. CORTISOL in Control	-0.120	0.403
LH and S. AL. AMYLASE in Control	-0.003	0.981
LH and Sperm Cells Count in Control	-0.112	0.432
LH and Sperm Cells Active in the Control	0.030	0.837
FSH and Prolactin in Control	-0.067	0.641
FSH and Testosterone in Control	-0.062	0.664
FSH and S. CORTISOL in Control	0.126	0.377
FSH and S. AL. AMYLASE in Control	-0.097	0.497
FSH and Sperm Cells Count in Control	0.221	0.120
FSH and Sperm Cells Active in the Control	0.304	0.030
Prolactin and Testosterone in Control	0.058	0.688
Prolactin and S. CORTISOL in Control	-0.060	0.677
Prolactin and S. AL. AMYLASE in Control	-0.213	0.133
Prolactin and Sperm Cells Count in Control	-0.133	0.352
Prolactin and Sperm Cells Active in the Control	-0.050	0.726
Testosterone and S. CORTISOL in Control	0.143	0.318
Testosterone and S. AL. AMYLASE in Control	0.042	0.772
Testosterone and Sperm Cells Count in Control	-.0144	0.314
Testosterone and Sperm Cells Active in the Control	-0.073	0.610
S. CORTISOL and S. AL. AMYLASE in Control	0.354	0.011
S. CORTISOL and Sperm Cells Count in Control	0.151	0.289
S. CORTISOL and Sperm Cells Active in the Control	0.048	0.737
S. AL. AMYLASE and Sperm Cells Count in Control	0.270	0.056
S. AL. AMYLASE and Sperm Cells Active in the Control	0.134	0.348
Sperm Cells Count and Sperm Cells Active in the Control	0.825	0.0001

Keys: LH = Luteinizing Hormone; FSH = Follicle Stimulating Hormone; S = Salivary.

In the Normospermia category, there was a strong significant positive correlation between LH and FSH (r=0.416, p<0.05), there was significant correlation between LH and testosterone(r=0.313, p<0.05), sperm cell count (r=0.471, p<0.05), active sperm cells (r=0.462, p<0.05), There was significant correlation between FSH and all the other parameters including the semen quality, there was weak negative significant correlation between prolactin and sperm cell count (r=-0.351, p<0.05) and active sperm cells (r=-0.426, p<0.05), a very strong significant positive correlation was found between testosterone and sperm cell count (r=0.854, p<0.05) and active sperm cell (r=0.811, p<0.05) a very strong significant positive correlation between sperm cell count and active sperm cells (r=0.913, p<0.05) as shown in Table-6.

Table-6: Correlation between hormones, biomarker and semen quality in Normospermia group of the Males Category

Correlation	R-Value	P-Value
LH and FSH in Normospermia	0.416	0.002
LH and Prolactin in Normospermia	-0.134	0.338
LH and Testosterone in Normospermia	0.313	0.023
LH and S. CORTISOL in Normospermia	-0.222	0.110
LH and S. AL. AMYLASE in Normospermia	-0.249	0.072
LH and Sperm Cells Count in Normospermia	0.471	0.0001
LH and Sperm Cells Active in the Normospermia	0.462	0.0001
FSH and Prolactin in Normospermia	-0.395	0.003
FSH and Testosterone in Normospermia	0.501	0.0001
FSH and S. CORTISOL in Normospermia	-0.430	0.001
FSH and S. AL. AMYLASE in Normospermia	-0.488	0.0001
FSH and Sperm Cells Count in Normospermia	0.550	0.0001
FSH and Sperm Cells Active in the Normospermia	0.580	0.0001
Prolactin and Testosterone in Normospermia	-0.371	0.006
Prolactin and S. CORTISOL in Normospermia	0.445	0.001
Prolactin and S. AL. AMYLASE in Normospermia	0.287	0.037
Prolactin and Sperm Cells Count in Normospermia	-0.351	0.010
Prolactin and Sperm Cells Active in the Normospermia	-0.426	0.001
Testosterone and S. CORTISOL in Normospermia	-0.499	0.0001
Testosterone and S. AL. AMYLASE in Normospermia	-0.568	0.0001
Testosterone and Sperm Cells Count in Normospermia	0.854	0.0001
Testosterone and Sperm Cells Active in the Normospermia	0.811	0.0001
S. CORTISOL and S. AL. AMYLASE in Normospermia	0.552	0.0001
S. CORTISOL and Sperm Cells Count in Normospermia	-0.511	0.0001
S. CORTISOL and Sperm Cells Active in the Normospermia	-0.521	0.0001
S. AL. AMYLASE and Sperm Cells Count in Normospermia	-0.636	0.0001
S. AL. AMYLASE and Sperm Cells Active in the Normospermia	-0.608	0.0001
Sperm Cells Count and Sperm Cells Active in the Normospermia	0.913	0.0001

Keys: LH = Luteinizing Hormone; FSH = Follicle Stimulating Hormone; S = Salivary.

In the Oligospermia category, there was a strong significant positive correlation between LH and FSH (r=0.529, p<0.05), There was significant correlation between FSH and all the other parameters including the semen quality, except in cortisol (r= -0.351, p>0.05) and active sperm cells (r= 0.311, p>0.05), a strong significant positive correlation was found between testosterone and sperm cell count (r=0.552, p<0.05) and active sperm cell (r=0.572, p<0.05) as shown in Table-7.

Table-7: Correlation between hormones, biomarker and semen quality in Oligozoospermia group of the Males Category

Correlation	R-Value	P-Value
LH and FSH in Oligospermia	0.529	0.042
LH and Prolactin in Oligospermia	-0.269	0.332
LH and Testosterone in Oligospermia	0.054	0.848
LH and S. CORTISOL in Oligospermia	0.103	0.716
LH and S. AL. AMYLASE in Oligospermia	-0.080	0.776
LH and Sperm Cells Count in Oligospermia	-0.015	0.957
LH and Sperm Cells Active in Oligospermia	-0.007	0.982
FSH and Prolactin in Oligospermia	-0.542	0.037
FSH and Testosterone in Oligospermia	0.616	0.015
FSH and S. CORTISOL in Oligospermia	-0.351	0.200
FSH and S. AL. AMYLASE in Oligospermia	-0.543	0.037
FSH and Sperm Cells Count in Oligospermia	0.679	0.005
FSH and Sperm Cells Active in Oligospermia	0.311	0.260
Prolactin and Testosterone in Oligospermia	-0.338	0.219
Prolactin and S. CORTISOL in Oligospermia	0.497	0.060
Prolactin and S. AL. AMYLASE in Oligospermia	0.519	0.048
Prolactin and Sperm Cells Count in Oligospermia	-0.476	0.073
Prolactin and Sperm Cells Active in Oligospermia	-0.268	0.335
Testosterone and S. CORTISOL in Oligospermia	-0.241	0.386
Testosterone and S. AL. AMYLASE in Oligospermia	-0.553	0.033
Testosterone and Sperm Cells Count in Oligospermia	0.552	0.033
Testosterone and Sperm Cells Active in Oligospermia	0.572	0.026
S. CORTISOL and S. AL. AMYLASE in Oligospermia	0.492	0.062
S. CORTISOL and Sperm Cells Count in Oligospermia	-0.485	0.067
S. CORTISOL and Sperm Cells Active in Oligospermia	-0.450	0.092
S. AL. AMYLASE and Sperm Cells Count in Oligospermia	-0.585	0.022
S. AL. AMYLASE and Sperm Cells Active in Oligospermia	-0.383	0.159
Sperm Cells Count and Sperm Cells Active in Oligospermia	0.317	0.249

Keys: LH = Luteinizing Hormone; FSH = Follicle Stimulating Hormone; S = Salivary.

In the Azoospermia category, no significant correlation was found among the hormones, except a strong significant negative correlation that was found between salivary cortisol and salivary alpha amylase (r= -0.607, p<0.05) as shown in Table-8.

Table-8: Correlation between in hormones, biomarker and semen quality in Azoospermia group of the Males Category

Correlation	R-Value	P-Value
LH and **FSH** in Azoospermia	0.083	0.788
LH and Prolactin in Azoospermia	-0.244	0.422
LH and Testosterone in Azoospermia	-0.090	0.771
LH and S. CORTISOL in Azoospermia	-0.136	0.657
LH and S. AL. AMYLASE in Azoospermia	-0.533	0.061
FSH and Prolactin in Azoospermia	-0.083	0.788
FSH and Testosterone in Azoospermia	0.024	0.939
FSH and S. CORTISOL in Azoospermia	0.041	0.895
FSH and S. AL. AMYLASE in Azoospermia	-0.014	0.964
Prolactin and Testosterone in Azoospermia	-0.542	0.056
Prolactin and S. CORTISOL in Azoospermia	0.258	0.395
Prolactin and S. AL. AMYLASE in Azoospermia	0.407	0.168
Testosterone and S. CORTISOL in Azoospermia	-0.213	0.486
Testosterone and S. AL. AMYLASE in Azoospermia	-0.348	0.243
S. CORTISOL and S. AL. AMYLASE in Azoospermia	-0.607	0.028

Keys: LH = Luteinizing Hormone; FSH = Follicle Stimulating Hormone; S = Salivary.

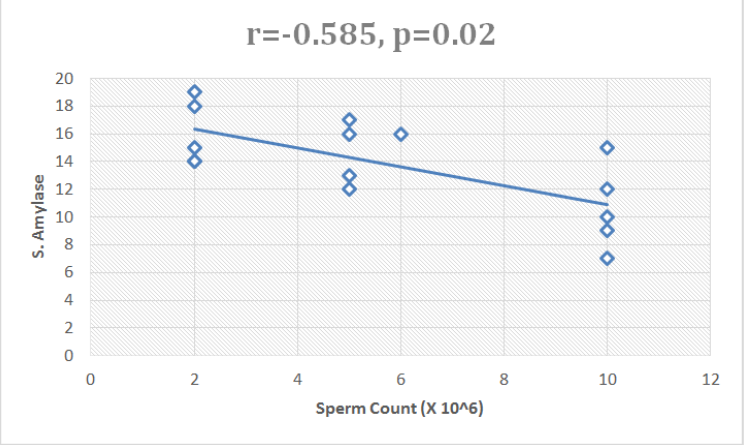

Fig-1: Correlation of S. Amylase and Oligozoospermia

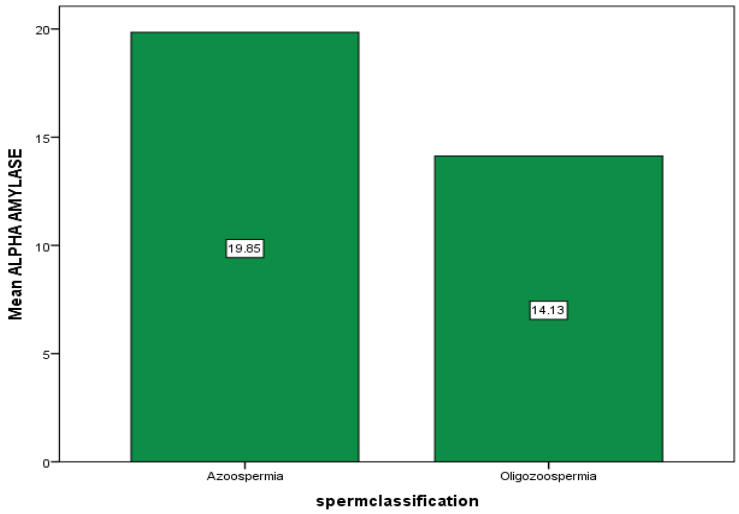

Fig-2: Shows the mean level of S. AL. Amylase between Azoospermia and Oligospermia

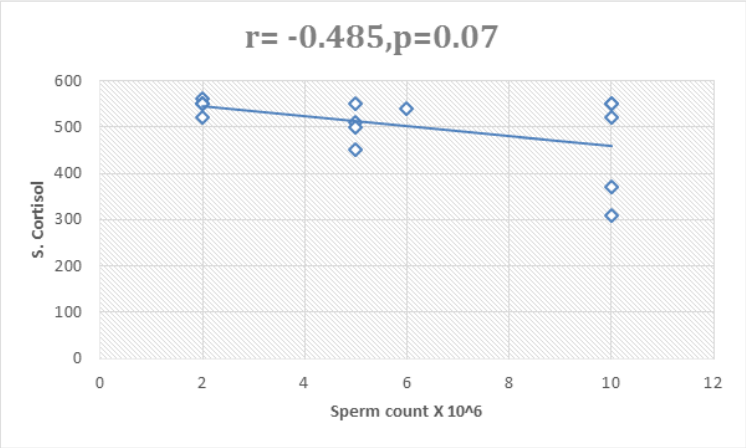

Fig-3: Correlation of S. Cortisol and Oligozoospermia

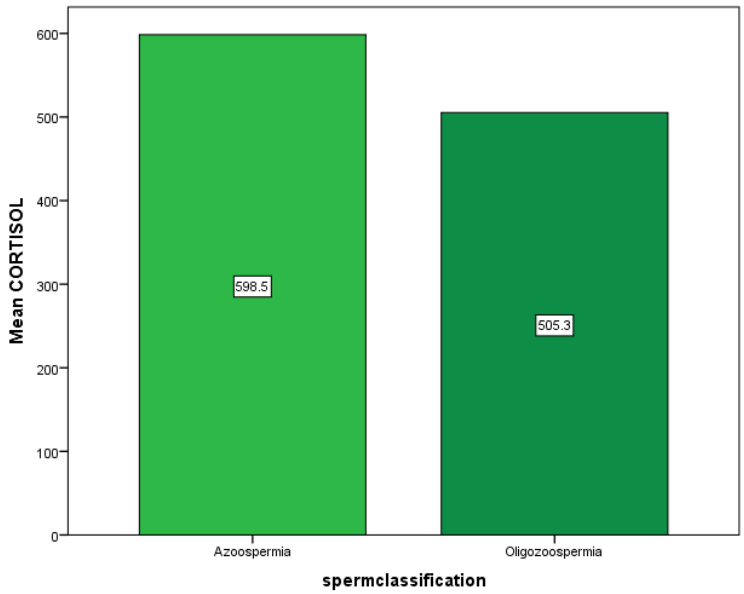

Fig-4: Shows the mean level of S. Cortisol between Azoospermia and Oligospermia

DISCUSSION

It is observed in this research work that the male in the study group presented with significantly low Serum FSH level, Testosterone level, Sperm cells count and Active sperm cells (%), ($p<0.05$) relative to the control group; this findings support the report of [10], who reported low sperm quality, low sperm quantity and hormonal imbalance as causes of infertility in male of child bearing age. The sperm count in this study is categorized into normospermia (normal sperm cell count), oligospermia (low sperm cell count) and azoospermin (no sperm cell); and 65.1% of the male study subjects presents with the condition of normospermia, 18.3% present with oligospermia, while 15.8% presents with azoospermia. In this study the hormonal abnormalities were pronounced in the azoospermic condition, but less in oligospermic condition; also [8] reported hormonal abnormalities in male infertility in Kano, Nigeria in both conditions of oligospermia and azoospermia. Also [11], reported that abnormal Testosterone and FSH levels can impair the mechanisms of spermatogenesis; furthermore, low Testosterone concentration is a marker of HPA activation, one factor that can deregulate Testosterone and FSH secretion is chronic anxiety and depression. The low values of Testosterone and FSH in the male with azoospermia is suggestive that, the cause is secondary to anterior pituitary failure. In men, stress adversely affect semen quality and can inhibit GnRH secretion through H-P-axis activation [18]; stress-induced spermatogenesis impairment is typically manifested in decreased sperm count and motility and increased percentage of morphologically abnormal sperm. An increase in stress hormone levels i.e. cortisol can impair androstenedione to testosterone conversion in the Leydig cells. This disrupts the hormonal transformation cycle required for testosterone secretion, leading to lower average values of semen volume and sperm quality [9].

However, there was no significant difference in the LH values between the male study group and the control group; but there is a statistically significant difference in the values of prolactin ($p<0.05$) in the male study group relative to the control group, this finding agrees with that of [20], who reported high Prolactin concentration in the males with infertility. The findings in this study suggest that most of the infertility cases experienced by couples, about 40% could be attributed to the male subjects; this is due to the fact that without a viable active sperm cells during the process of sexual intercourse, there will be no fertilization of the ovum, resulting in infertility.

In this study, the mean concentration of both Salivary Cortisol and Salivary Alpha Amylase were significantly higher in the study group when compared with the control group ($p<0.05$); although the Values were within the upper limit of normal (220-550 micromol/L) for Salivary Cortisol and (0–15U/L) for Salivary Amylase. However, in the male study subjects 13(15.8%) with Azoospermic condition, the biomarkers were significantly higher ($p<0.05$) when compared with the normospermic and oligospermic conditions. The findings in this study support the reports of [14, 13] who reported high level of Cortisol as the adverse effect of stress on fertility, and opined that 30% cases of infertility are attributed to stress; that when stress reducing measures are applied, those women who could not get pregnant before got pregnant. This result confirms the impacts of stress on sperm quality, in line with those reported by other authors [7, 23, 3]. Although, some researchers have disclaimed the effect of stress on fertility, Lovely *et al*., [12] could not see any obvious link of stress and infertility. However, the elevated values of salivary cortisol and salivary amylase in this study suggest and support the adverse negative impact of stress on fertility in some male of child bearing age, as also reported by [3], that male patients with anxiety and depression were found to have lower testosterone levels and low sperm quality. Thus, stress can compromise every aspect of fertility including libido, sperm quality, ovulatory capacity, and implantation [16]. High cortisol level in this study suggests an indication of chronic stress; where the stress neuroendocrine are stimulated via the hypothalamus-pituitary – adrenal axis, which in turn affects the activities of the gonadotrophic releasing hormones (GnRH). The stress hormones inhibit and decrease the pulsatility of the GnRH which is responsible for the stimulation and production of the gonadotropins (FSH and LH), these suggest the reason for low FSH value obtained in this study. High cortisol level, high alpha amylase and hyperprolactinemia may occur primarily as a result of physiological changes in hypothalamus-pituitary gland due to stress or any disease affecting them. However, currently, the potential for infertility stress induced risk is primarily based on the theoretical side effects of stress on the infertile couples. Salivary cortisol and alpha amylase produced respectively by the hypothalamus –pituitary –adrenal (HPA) axis are still not included in the routine evaluation of causes of infertility and require additional and definitive validation. The results in this study suggest and support the use of salivary cortisol and salivary alpha amylase along with hormonal profile as a potential diagnostic tool for detecting stress induced infertility in infertile male in order to determine their contribution to infertility.

The purpose of this study was to assess the correlation between some stress biomarkers and the secretion of selected hormones and to assess the impact of these on semen quality in men with infertility. This present study demonstrated that correlation between some stress biomarkers and hormone levels and semen characteristics analyzed are only statistically significant in the study group. The negative correlation between some stress biomarkers and Testosterone and FSH levels in the study group can be related to lower sperm quality and quantity resulting from spermatogenesis dysfunction induced by stress which affects the hypothalamus – pituitary – gonadal axis (HPG). This study show higher salivary cortisol and salivary alpha amylase to be correlated with lower Testosterone and FSH levels and increase prolactin level. Based on the correlation between hormonal values and (Some Stress Biomarkers) with sperm indices, the study found that higher values of some stress biomarkers is associated with lower sperm quality, lower Testosterone and FSH values; and higher sperm abnormalities, higher Prolactin, which is corroborated or reported by Bhongade [3].

CONCLUSION

- Male in infertility has higher values of stress biomarkers (Cortisol and Amylase).
- The male suffering infertility show lower levels of Testosterone, FSH and sperm quality and quantity; and higher values of Prolactin.
- Stress in form of depression and anxiety cause decreased semen volume and quality.

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