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Original Research Article

Correlation study of stress biomarkers, endocrinopathies, semen quality and quantity in infertile men in Abuja, Nigeria

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ABSTRACT

Background: The quality and quantity of sperm cells in the male determines his fertility potentials. It has been reported that 40-45% cases of infertility is attributable to men, and that stress compromises fertility indices. Therefore, the study was done to assess the correlation between some stress biomarkers, male endocrinopathies, sperm quality, and quantity in infertile men.
Methods: This was a cross sectional case- control study. A total of 154 participants were recruited into the study, which consist of 100 males having the challenge of infertility as study group and 54 fertile male which serves as control group. Serum testosterone, LH, FSH, prolactin, salivary cortisol and amylase were analyzed using ELISA techniques; while the semen was examined after liquefaction according to WHO criteria.
Results: In the study group, higher values of stress biomarkers correlated with significantly decreased testosterone and FSH values (p=0.001), and increased prolactin, salivary cortisol amylase (p<0,001); semen quality and quantity correlate with stress biomarkers (p<0.001). There are both positive and negative correlation between the stress biomarkers, sex hormones, sperm quality and quantity.
Conclusions: Higher values of stress biomarkers in infertile male show both negative and positive correlation with abnormal sex hormones, decreased semen quality and quantity.

Keywords: Correlation, Infertility, Sex Hormones, Sperm quality, Stress

INTRODUCTION

The male potential in fertility is determined by the quality and quantity of sperm cells produced during ejaculation in sexual intercourse.[1] When the quality and quantity of sperm cells are compromised, it would lead to infertility. Infertility affects nearly one in five couples within reproductive age. Men 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 male.[2] Male infertility is established when identifiable female causes of infertility is excluded and when semen quality and quantity fails to meet WHO criteria.[3] Male infertility is referred to as male inability to cause pregnancy in the female who is fertile after above six month of sexual intercourse; and male factor infertility accounts for about 45-50% of couple infertility.[4]

In majority of cases, the main cause of infertility in men is decreased sperm cells quality and quantity in the male partner.[5] Semen quality and quantity depends on factors such as sex hormones, environment, and lifestyle.[6,7] The sex hormones can be affected by the hypothalamic-pituitary-gonadal axis and by cerebral cortex activity, which can be altered by disorders such as depression and anxiety.[8]

Hormone secretion in the testes is controlled by pituitary gonadotropins: the luteinizing hormone (LH) stimulates sex hormone production by Leydig cells, and follicle stimulating hormone (FSH), which together with testosterone activates the seminiferous tubules, through the sertoli cells to activates and maintains spermatogenesis. The release of these two gonadotropins from the pituitary gland is controlled by the hypothalamic decapeptide, gonadotropin releasing hormone (GnRH); FSH plays key role in hormonal regulation of spermatogenesis, but androgen activity independent of FSH is equally recognized.[9]

FSH stimulates division and differentiation of spermatogenesis, inhibits apoptosis of spermatogonia, and stimulates meiosis processes, while testosterone controls the course of meiosis, spermatid transformation and elongation, and spermatid adhesion to Sertoli cells.[3] Normal release of gonadotropins (FSH and LH) occurs with the pulsatile secretion of GnRH. A number of factors, including psychogenic ones such as stress disrupt the pulse activity of the hypothalamus, decreasing gonadotropin levels to a varying extent.[1]

The hypothalamus-pituitary-adrenal axis is much involved in the stimulation and production of stress hormones. The adrenal cortex produces cortisol, which its increases is activated by stress or stressor activities. Prolactin production by the pituitary gland is also stimulated by stress, and can inhibit the production of LH and FSH.[9]

The aim of this current study was to assess the correlation between stress biomarkers, endocrinnopathies, sperm quality, and quantity in infertile men in Abuja Nigeria.

METHODS

Study area

The study was carried out in the following major general hospitals: Asokoro, Garki, Gwarinpa, Maitama, Wuse, and departments of chemical pathology and microbiology laboratory of Alpha Royal Medicals Ltd., in federal capital territory (FCT), Abuja Nigeria.

Subject and Sample

A cross sectional case-control study was carried out between September, 2017 to December, 2018. One hundred and fifty four participants were involved in the study, which consist of hundred infertile men and fifty four fertile men. 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 3000 rpm 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 was stable until analysis. The 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 specimens.

Ethical approval

Ethical approval was sought and obtained from the ethical clearance committee of health research ethics committee Abuja with reference number FHREC/2018/01/97/21-08-18 dated August 21, 2018.

Inclusion and exclusion 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 18-45 years 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 was 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 consisted 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 (WMA, 2008). 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 and semen analysis

The serum LH, FSH, prolactin, testosterone, salivary cortisol and salivary alpha amylase were measured 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.

Semen was obtained by masturbation, and examined directly after liquefaction according to the 2010 World Health Organization criteria. Prior to the semen collection, the patients were asked to maintain a four days abstinence from sex and alcohol.

Statistical analysis

The demographic characteristics of the participants were expressed as mean values and standard deviation (SD). 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.

RESULTS

The age distribution of the participants is presented in Figure 1.

The mean standard deviation (SD) of hormones, stress biomarkers and sperm parameters in the men study and control groups are presented in Table 1. The comparisons of measured hormones and stress biomarkers according to semen quality and quantity of the men category are presented in Table 2. The comparison of correlation between the sex hormones and stress biomarkers and sperm quality and quantity are shown in Table 3 and 4, Figures 1-10 respectively.

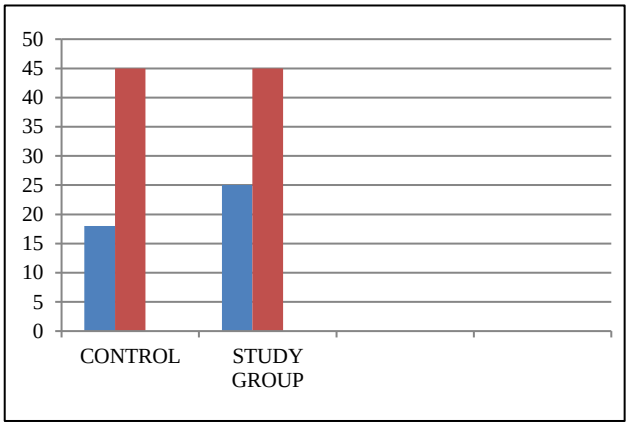

Figure 1: Age distribution of participants.

Table 1: Mean hormones, biomarkers and sperm quality in the study and control group in the males category.

Parameters	Study group mean±SD (100)	Control group mean±SD (52)	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
Serum cortisol (micromole/l)	449.75±106.81	340.65±72.53	221-552	<0.001
Serum 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.

Table 2: Comparison of hormones and biomarkers according to semen quality of the male.

Variables	Control group	Study group			P value	Sig
	Control Mean±SD n=52	Normospermia Mean±SD n=54 (54%)	Oligospermia Mean±SD n=33 (33%)	Azoospermia Mean±SD n=13 (13%)		
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
Serum cortisol (micromole/l)	340.65±72.53	397.5±79.95	505.33±73.76	598.46±46.52	0.0001	S
Serum 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

The mean (SD) of the FSH concentration of the study group (4.50±2.20 MIU/ml) was statistical significantly lower (p<0.05) than that of the control group (5.91±1.66 MIU/ml); mean value of the LH of the study group (5.64±2.26 MIU/ml) show no statistical significance (p>0.05) to that of the control group (5.43±1.66

MIU/ml); mean value of the prolactin of the study group (18.01±11.56ng/ml) was significantly higher ($p<0.05$) than that of the control group (6.98±3.34 ng/ml). The level of testosterone in the study group (3.44±2.35 ng/ml) was significantly lower ($p<0.05$) relative to those of the control group (5.86±1.55ng/ml); the salivary cortisol level was equally significantly higher ($p<0.05$) in the study group (449.75±106.81) compared to the control group (340.65±72.53). Salivary alpha amylase level was significantly higher ($p<0.05$) in the study group (13.12±4.39) compared with the control group (8.45±3.01); the sperm cell count ($x10^6$) was significantly lower ($p<0.05$) in the 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 study group (33.99±26.07) compared with the control group (49.10±14.80); 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, 5.6 ml versus 3.3 ml, as shown in Table 1.

Of the 100 men study group examined, 54 (54%) were normospermia, 33 (33%) were oligospermia, while 13 (13%) were azoospermia. There was statistically 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 2.

Correlations of hormones and biomarkers in the subjects: The Tables 3 to 5 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 conditions). 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 3.

Table 3: Correlation between in hormones, biomarker and semen quality in normospermia group of the male 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 serum cortisol in normospermia	-0.222	0.110
LH and serum alpha 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 serum cortisol in normospermia	-0.430	0.001
FSH and serum alpha 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 serum cortisol in normospermia	0.445	0.001
Prolactin and serum alpha 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 serum cortisol in normospermia	-0.499	0.0001
Testosterone and serum alpha 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
Serum cortisol and serum alpha amylase in normospermia	0.552	0.0001
Serum cortisol and sperm cells count in normospermia	-0.511	0.0001
Serum cortisol and sperm cells active in the normospermia	-0.521	0.0001
Serum alpha amylase and sperm cells count in normospermia	-0.636	0.0001
Serum alpha 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

Table 4: Correlation between hormones, biomarker and semen quality in oligospermia group of the male 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 serum cortisol in oligospermia	0.103	0.716
LH and serum alpha amylase in oligospermia	-0.080	0.776
LH and sperm cells count in oligospermia	-0.015	0.957
LH and sperm cells active in the 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 serum cortisol in oligospermia	-0.351	0.200
FSH and serum alpha amylase in oligospermia	-0.543	0.037
FSH and sperm cells count in oligospermia	0.679	0.005
FSH and sperm cells active in the oligospermia	0.311	0.260
Prolactin and testosterone in oligospermia	-0.338	0.219
Prolactin and serum cortisol in oligospermia	0.497	0.060
Prolactin and serum alpha amylase in oligospermia	0.519	0.048
Prolactin and sperm cells count in oligospermia	-0.476	0.073
Prolactin and sperm cells active in the N oligospermia	-0.268	0.335
Testosterone and serum cortisol in oligospermia	-0.241	0.386
Testosterone and serum alpha amylase in oligospermia	-0.553	0.033
Testosterone and sperm cells count in oligospermia	0.552	0.033
Testosterone and sperm cells active in the oligospermia	0.572	0.026
Serum cortisol and serum alpha amylase in oligospermia	0.492	0.062
Serum cortisol and sperm cells count in n oligospermia	-0.485	0.067
Serum cortisol and sperm cells active in the oligospermia	-0.450	0.092
Serum alpha amylase and sperm cells count in oligospermia	-0.585	0.022
Serum alpha amylase and sperm cells active in the oligospermia	-0.383	0.159
Sperm cells count and sperm cells active in the oligospermia	0.317	0.249

Table 5: Correlation between hormones, biomarker and semen quality in azoospermia group of the male 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 serum cortisol in azoospermia	-0.136	0.657
LH and serum alpha 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 serum cortisol in azoospermia	0.041	0.895
FSH and serum alpha amylase in azoospermia	-0.014	0.964
Prolactin and testosterone in azoospermia	-0.542	0.056
Prolactin and serum cortisol in azoospermia	0.258	0.395
Prolactin and serum alpha amylase in azoospermia	0.407	0.168
Testosterone and serum cortisol in azoospermia	-0.213	0.486
Testosterone and serum alpha amylase in azoospermia	-0.348	0.243
Serum cortisol and serum alpha amylase in azoospermia	-0.607	0.028

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), but negative correlation between the biomarkers as shown in Table 4.

In the azoospermia category, no significant correlation was found among the hormones, except a strong significant negative and positive correlation that was found between salivary cortisol, salivary alpha amylase and sex hormones (r=-0.607, p<0.05) as shown in Table 5.

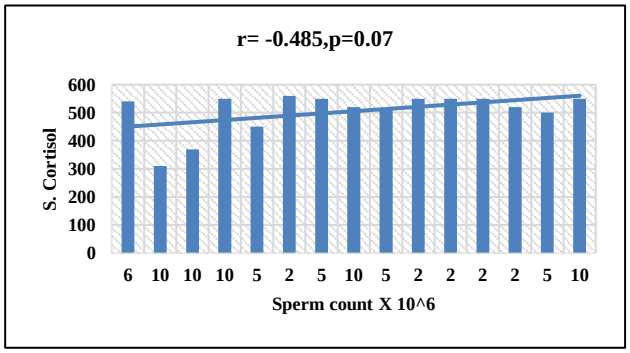

Figure 2: Correlation of serum cortisol and oligozoospermia.

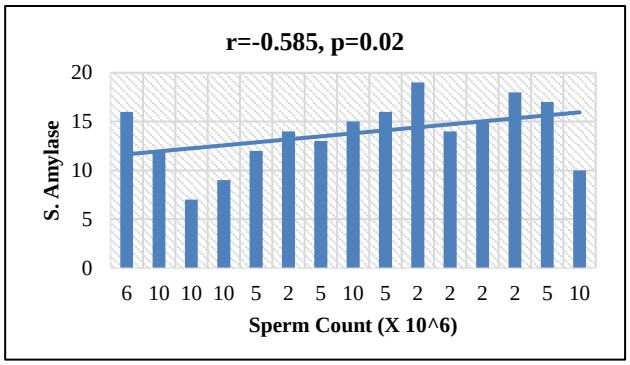

Figure 3: Correlation of serum amylase and oligozoospermia.

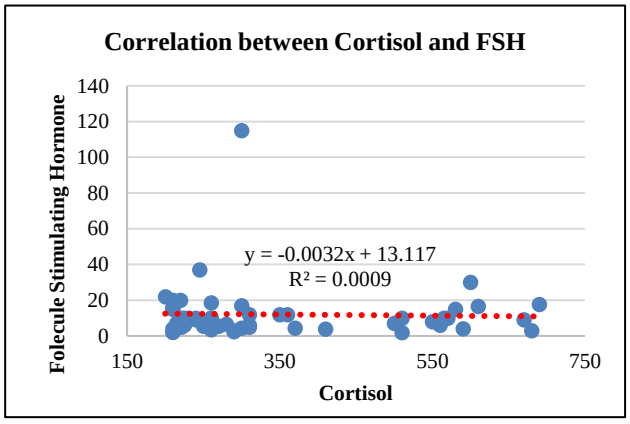

Figure 4: Linear correlation between cortisol and follicle stimulating hormone (FSH).

We found a very weak negative correlation (r=3%) between cortisol and follicle stimulating hormone (FSH) and the linear equation was y = - 0.0032x + 13.117.

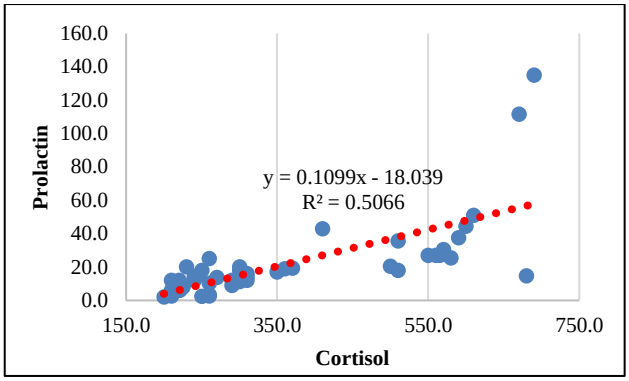

Figure 5: Linear correlation between cortisol and prolactin.

We found a strong positive correlation (r=71.2%) between Cortisol and Prolactin and the linear equation was y = 0.1099x - 18.039.

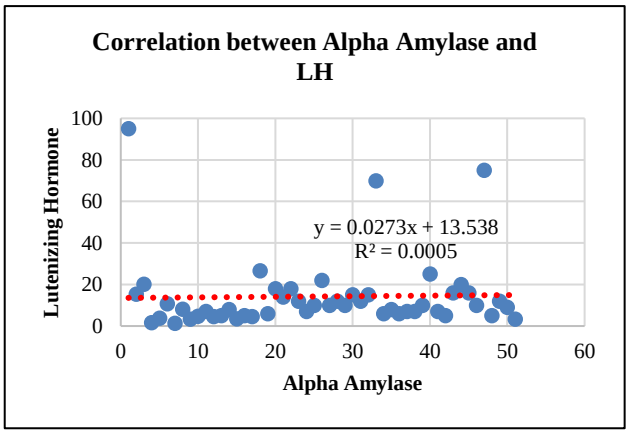

Figure 6: Linear correlation between alpha amylase and luteinizing hormone (LH).

We found a very weak positive correlation (r=2.24%) between alpha amylase and luteinizing hormone (LH) and the linear equation was y = 0.0273x + 13.538.

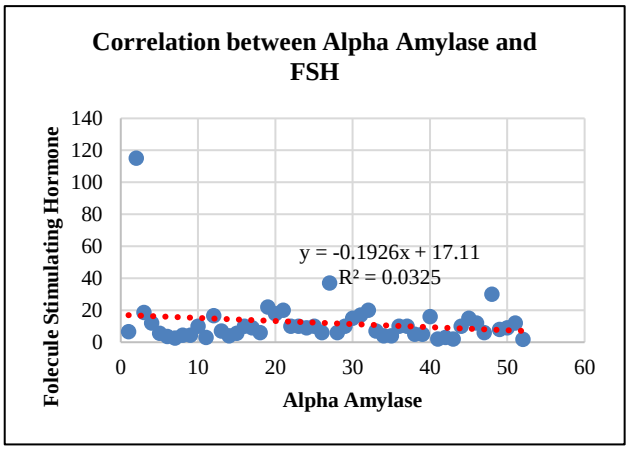

Figure 7: Linear correlation between alpha amylase and follicle stimulating hormone (FSH).

We found a weak negative correlation (r=18.0%) between alpha amylase and follicle stimulating hormone (FSH) and the linear equation was y = -0.1926x + 17.11.

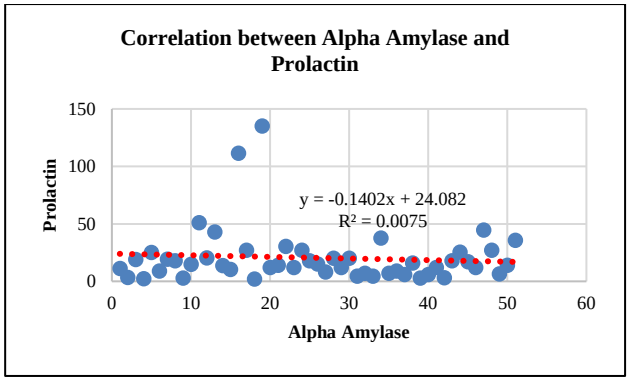

Figure 8: Linear correlation between alpha amylase and prolactin.

We found a weak negative correlation (r=8.7%) between alpha amylase and prolactin and the linear equation was y = - 0.1402x + 24.08.

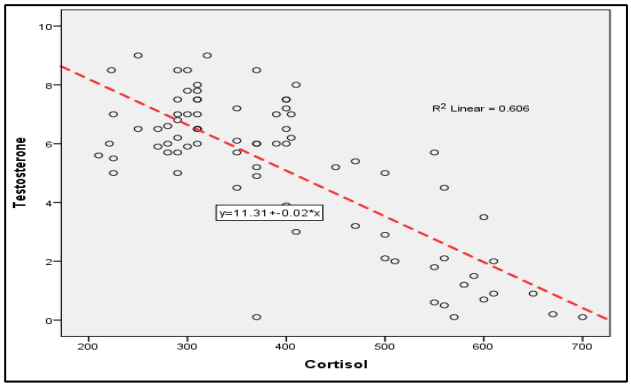

Figure 9: Linear correlation between cortisol and testosterone.

We found a strong negative correlation (r=77.8%) between Cortisol and the linear equation was y = - 0.02x + 11.31.

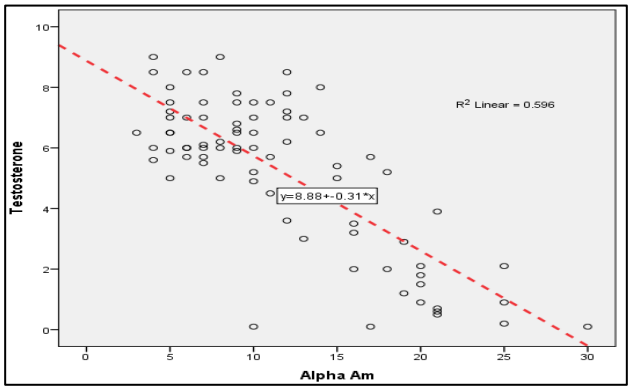

Figure 10: Linear correlation between alpha amylase and testosterone.

We found a strong negative correlation (r=77.2%) between Alpha Amylase and Testosterone and the linear equation was y = - 0.31x + 8.88.

DISCUSSION

This present study demonstrated that correlation between some stress biomarkers, sex hormone levels and semen quality and quantity 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 is corroborated by.[1,10]

This study showed 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 sex hormones values and some stress biomarkers with sperm indices, the study found that higher value of some stress biomarkers is associated with lower sperm quality, lower testosterone and FSH values; and higher sperm abnormalities, higher prolactin, which is also supported by the report of Bhongade.[6]

The present study demonstrated that correlation between stress biomarkers and the sex hormones levels and sperm characteristics analyzed are statistically significant in the study group, and not in the control group; it is likely due to the fact that the control group was less affected by emotional stress, which is also supported by.[1,10] The negative correlation between stress biomarkers and testosterone levels in the study group can be related to the lower sperm quality and quantity as a result of spermatogenesis dysfunction induced by stress, which affects the hypothalamic-pituitary-gonadal axis. The impact of stress biomarkers on sex hormone secretion, sperm cells quality and reproductive dysfunction has been confirmed in number of studies[1,11,12] This present study showed that correlation exists between biomarkers, sperm quality and prolactin.

Abnormal testosterone and FSH levels can impair the mechanism of spermatogenesis. Much more, low testosterone value is a marker of HPA activation. One factor that can deregulate testosterone production is chronic stress.[4] In this study, higher stress biomarkers were associated with lower testosterone and FSH values. This is not however, supported by Ponholzers study, where testosterone levels were not correlated with depression severity.[13]

The present study showed that stress can disrupt sex hormones and adversely affect semen quality and quantity. They can also have a negative impact on infertility care outcomes, thereby requiring the development of measures to manage stress in infertility clinics as reported by Fisher et al.[8] However, the psychological background of infertility is somehow difficult to diagnose by healthcare givers, and as such requires the collaboration of other specialists, as psychiatrists, psychologists and psychotherapists.[14] However, Fisher and Hammerberg reported that men with fertility challenges prefer to receive emotional support from infertility clinicians rather than from mental health professionals, self-help support groups or friends.[8,15] The

study is focused on only stress biomarkers: cortisol and amylase their correlation with sex hormones, sperm quality and quantity; not comorbidities and mortality.

The limitation of the study was that we did not include questionnaires such as Beck depression inventory (BDI), state-trait anxiety inventory (STAI), Beck anxiety inventory (BAI), and infertile men on treatment. However, more research in this area is required in order to validate the correlations of stress biomarkers, endocrinopathies, sperm quality and quantity in infertile male.

CONCLUSION

Some men in infertility have higher values of stress biomarkers (cortisol and amylase). The men suffering infertility show lower levels of testosterone, FSH and sperm quality and quantity; and higher values of prolactin and stress biomarkers, thereby showing both negative and positive correlation. Decreased semen quality and quality is observed in subjects with high stress biomarkers showing negative correlation. Men investigating for infertility were found to have more severe stress biomarkers than those confirmed to be fertile. Men investigating for infertility with increased stress biomarkers show decreased testosterone and FSH levels demonstrating negative correlation.

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Conflict of interest: None declared
Ethical approval: The study was approved by the health research ethics committee Abuja with reference number FHREC/2018/01/97/21-08-18 dated August 21, 2018

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