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Research Paper

Effects of Zinc and Selenium Coadministration on Semen Parameters and Hormonal Profiles of Male Wistar Rats.

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Abstract
Zinc (Zn) and Selenium (Se) are essential dietary micronutrients or trace elements not produced by the body but required in small amounts for the maintenance of mammalian and human male fertility. The over supplementation of individual and combination of micronutrients in the sick and healthy populations could be the leading cause of prevalence of male factor infertility in the world. This study was designed to evaluate the possible effects of the graded independent doses of oral supplementation of Zn, Se and their co-administration on male reproductive hormones, sperm parameters and histological architecture of male wistar rats.
Fifty (n=50) adult male wistar rats weighing between 150g-250g and randomly grouped into ten groups of five rats each were used for this research that lasted for six weeks. Group I, the control group, received normal rat chow and water ad libitum. The nine test groups (II-X), received graded doses of zinc sulphate ZnSO4 (II-IV: 50mg/kg,150mg/kg,300mg/kg), selenium dioxide SnO2 (V-VII; 100mg/kg,200mg/kg,400mg/kg), co-administration of ZnSO4 and SnO2 (VIII-X:50mg/kg ZnSO4+100mg/kg SeO2, 150mg/kg ZnS04+200mg/kg SeO2, 300mg/kg ZnSO4+400mg/kg SnO2). Serum testosterone (T), follicular stimulating hormone (FSH) and luteinising hormone (LH) was estimated using ELISA Hormone Test kits radio-immuno-assay technique. Semen parameters was analyzed for sperm count, motility, and morphology using the grading system as described by WHO (1999).
Testicular histological architecture was established using animal tissue processing technique. Data obtained were analyzed using one-way Analysis of Variance (ANOVA) from SPSS version 22. Statistical significance was placed at $P<0.05$ and data expressed as mean ± standard error of the mean (SEM).Results obtained from the study showed significant increase in testosterone levels in all the Zn test groups compared to the control and significant decrease in the levels of FSH and LH in higher doses of Zn and Se supplements when compared to the control at $p<0.05$. There was significant reduction in sperm motility, count but increase sluggishness in Zn and co-administration of Zn and Se of all the test groups compared to the control. Photomicrograph of the testes showed tissue inflammation, germinal cells destruction and azospermia. This study showed that chronic single or combined over supplementation of the micronutrients zinc and selenium could cause male factor infertility in wistar rats.

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I. Introduction:

Infertility is a problem of global proportion with male factor now contributing equally as females (WHO,1999). The rate of infertility vary between countries and different regions of the world (Wiersema et al 2006).It is estimated that infertility affects 15% of couples globally amounting to 48.5million couples annually. Males solely account for 20-30% of infertility cases and are responsible for 50% of all infertile cases (Argawal et al, 2015).In South Eastern Nigeria a positive male factor alone was found in 133(42.4%) couples and female

factor alone in 81(25.8%) couples of the 214 couples evaluated for the cause of infertility (Ikechebelu et al 2003).

Infertility is defined as failure to achieve clinical pregnancy after 12 months of regular unprotected sexual intercourse with the same partner (ICMART and WHO, 2009). Infertility is also defined as failure of couple to conceive after 12 months of regular unprotected sexual intercourse without contraception in women less than 35years and after 6 months of regular intercourse without contraception in women 35 years and above, (Fertil Steril,2008). Conception is normally achieved within 12 months in 80%–85% of couples using no contraceptive measures.

One of the commonest causes of male infertility is sperm dysfunction, (Hamada et al, 2013).Oligozoospermia (35.9%) and asthenozoospermia (32.3%) were the most common aetiological factors responsible for male infertility (Ikechebelu et al 2003).Common causes of infertility in the male can be due to pre-testicular, testicular, and post-testicular factors. This categorization allows for a systematic evaluation ranging from simple semen analysis through serum hormonal assays, radiological investigations, and to testicular tissue biopsy for histological analysis (Hjaratu et al, 2017).

Several risk factors are involved in the pathogenesis of male infertility, some of which include: Alterations in spermatogenesis due to testicular cancer, aplasia of the germinal cells, varicocele, defects in the transport of sperm, environmental factors as well as congenital anomalies, infectious diseases, bilateral spermaducts, pregnancy-related infections, alterations in the characteristics of semen such as a decrease in sperm motility and sperm count, presence of antisperm antibodies (ASAs), nutritional deficiency of trace elements such as zinc (Zn) and selenium (Se). (Abariukwu, 2013, Cardona et al, 2009, Mahdi et al, 2011, Wong et al, 2000).

Zinc (Zn) and Selenium (Se) are essential dietary micronutrients required for the maintenance of male fertility (Wu et al, 2015) .However there is increasing frequency of `micronutrient over supplementation (Zinc and Selenium inclusive) in the healthy population suggesting the need for more studies to fully assess their potential side effects.(Wayne et al, 2001).

Zinc is a vital trace element found in small amounts in a variety of cells and tissues of organisms and it is a cofactor of more than 300 enzymes. (Tapiero et all, 2003). Zinc is also involved in several cell functions including signal transduction, transcription and also duplication (Cousins, 2006).

In reproduction, zinc play numerous important functions, and it is essential for conception, implantation, and a favourable pregnancy outcome (Foresta et al,2014: de et al, 2010). For male fertility, Zinc levels are high in the testes and Zinc transporters are expressed in different regions of the epididymis.

Earlier, Favier (1992) reported the diverse effects of zinc that can be elucidated by its multiple actions on the metabolism of androgen hormones, estrogen and progesterone, together with the prostaglandins. Nuclear receptors for steroids are all zinc finger proteins. Zinc supplementation has already demonstrated beneficial in male sterility and has also been implicated in testicular development, testosterone synthesis and sperm maturation. Zinc also plays an important role in sexual development, ovulation and the menstrual cycle in females and has shown in reducing complications in pregnancy. Both folate and zinc have antioxidant properties that neutralize reactive oxygen species (ROS) thereby maintaining antioxidant and oxidant balance (Ebisch et al, 2007). it has been reported that about three to ten percent of all proteins in mammalian genomes are considered to bind zinc for holding activity and conformational changes (Sekler et al, 2007).

It is known that the adult human body has about 1–3g of zinc, and about 0.1% of which is replenished daily (Maret et al,2006). Zinc is recognized as an essential food element required by the body in trace amounts. It is also known that very little zinc in the diet can lead to poor health, reproductive problems, and lowered ability to resist against disease and lots of zinc in diet may also be unfavourable to health (ATSDR,1994). Higher doses of zinc also have the cyto-toxic potential in mice reported by (Gupta et al, 1991). Further, the level of zinc that bring health impairments are much higher than the Recommended Dietary Allowances (RDAs) i.e. 11 and 8 mg/day zinc for men and women respectively. If 10-15 times of RDA of zinc are taken even for a short time, it may lead to stomach cramps, nausea, and vomiting. Further, ingesting high levels of zinc for several months may lead to anaemia, damaged pancreas, and decreased levels of high-density lipoprotein (HDL) cholesterol (ATSDR,2005).

Zinc is a crucial element important for growth, the nervous system, as well as the immune system. Zinc insufficiency as well as its levels well over normal, due to high-dose treatment, demonstrated an impaired immune function (Wellinghausen, (1998).

Zinc is an essential trace element for humans taking role in electron transfer in many enzymatic reactions (Gul et al, 2009).

Zinc is present in high concentrations in the seminal fluid, and it could play a multifaceted role in sperm functional properties. It influences the fluidity of lipids and, thus, the stability of biological membranes (Chvapil,1973). It affects the **stability of sperm chromatin** (Bjorndahl et al, 2010). It is involved in the

formation of free oxygen radicals-Reactive Oxygen Species –ROS, (Gavella et al, 1998). It plays a regulatory role in the **capacitation and the acrosome reaction** (Michailov et al, 2014).

However, little is known concerning the role of zinc in seminal plasma or serum regarding the global functional competence of human spermatozoa, such as the sperm's ability to **penetrate cervical mucus** (CM) or its **fertilizing capacity**. The relationship of zinc to the routinely determined variables of semen quality has been controversial (Nematollahi.Mahani et al,2014, Lewis-Jones et al, 1996, Henkel et al, 1999).

II. MATERIALS AND METHODS

STUDY DESIGN

Fifty (50) male wistar rats, weighing between 150-250g were used for this study procured from the Animal House at Nnamdi Azikiwe University, Nnewi campus. The rats were acclimatized for two (2) weeks during which they were fed normal rat chow and water ad libitum. The rats were housed under ambient temperature and their beddings changed every two (2) days. The experiment lasted for six weeks. The rat feed used for this study was purchsed from Top Feed brand (Topfeed Ltd. Sapele, Delta State Nigeria). Dietary oral Supplemented zinc was provided as zinc sulfate ($ZnSO_4$)(JT Baker, Philipsburg, NJ) and selenium as selenium dioxide (SnO_2). Both Zinc and Selenium supplemented concentrates were converted from milligrams per kg body weight to ml.

The rats were grouped into 10, with 5 rats in each group. Group I served as the control and fed normal rat chow and water ad libitum. Groups II, III, IV, V, VI, VII, VIII, IX and X served as test groups and received (50mg/kg of $ZnSO_4$) , (150mg/kg of $ZnSO_4$), (300mg/kg $ZnSO_4$), (100mg/kg SnO_2),(200mg/kg SnO_2), (400mg/kg SnO_2), (50mg/kg of $ZnSO_4$ and 100mg/kg SnO_2), (150mg/kg of $ZnSO_4$ and 200mg/kgm SnO_2), (300mg/kg $ZnSO_4$ and 400mg/kg SnO_2) respectively. The doses of zinc sulphate and selenium dioxide and combined doses of zinc/selenium were given daily between the hours 8:00-9.00am for six weeks.

The rats were properly taken care of in a healthy environment at the animal house of Nnamdi Azikiwe University, Nnewi campus. Blood samples were collected at the end of the experiment by cardiac puncture after anaesthetising with chloroform. Two (2ml) of blood were collected from rats in each group including the control groups. The samples were carefully introduced into lithium containers free from anticoagulant and labelled properly. The blood samples allowed to clot, retract and then centrifuged for 5minutes at a speed of 5000 revolutions per minute.

Sperm Parameters:

Motility: A 20-µl sample was used to assay sperm motility (% vigorously swimming at 5 and 30mm post collection) transferred to a glass slide that is covered with a cover slip and assessed by visual analysis with a microscope. Progressivity was determined by the grading system as described by WHO (1999).

Sperm count: 0.1 ml sample of semen was placed in 0.9 ml of normal saline for sperm to swim out in a petri-dish. It was well shaken and the sample taken to a counting chamber (haemocytometer). After the sperms have settled on the grid, they were viewed under microscope and counted in five squares used for counting RBCs. Sperm in five squares was multiplied by 10^6 to determine the number of sperm per millimeter.

Sperm viability: Eosin stain was used for sperm viability determination, while Evans and Walls stain was used for viability.

Morphology determination. The morphological characteristics of the sperm cells are important for the complete assessment of the seminal fluid. The differential count of morphologically normal and abnormal cells was done. The presence of abnormal primordial and mature cells above 20% respectively was used as the criteria for measuring abnormal morphology.

III. Results and Discussions:

Acute Toxicity: The median lethal dose (LD50) of zinc sulphate was carried out in the department of physiology, faculty of Basic Medical Sciences. Nnamdi Azikiwe University, NNewi campus. This was determined using the method of Dietrich Lorke 91983). In phase I: 9 rats were used and grouped into three of 3 rats each. In phase II: 4 rats were used in four groups of one rat per group.

Table 4.1: The result of the LD50 study for Zinc Sulphate

Phase	Dose(mg/kg)	Death	Observation
1	10	0/3	The animals were calm
	100	0/3	The animals were calm
	1000	0/3	The animals were calm

2	1200	0/1	The animals were calm
	1600	0/1	The animals were calm
	2900	0/1	The animals were calm
	5000	1/1	Weakness was observed, twisting of neck/panting and died within 12 hours

LD_{50} = axb
a=maximum dose with 0% mortality
b=maximum dose with 100% mortality
LD_{50} =√aXb
LD_{50} = √5000X2900 =3807.80mg/kg
The LD_{50} of Zinc Sulphate is 3807.80mg

Table 4.2: The result of the LD50 study for Selenium Dioxide

Phase	Dose(mg/kg)	Death	Observation
1	10	0/3	The animals were calm
	100	0/3	The animals were calm
	1000	0/3	The animals were calm
2	1200	0/1	The animals were calm
	1600	0/1	The animals were calm
	2900	0/1	The animals were calm
	5000	0/1	Weakness was observed, twisting of neck/panting and died within 12 hours

LD_{50} = axb
a=
b=

EXPERIMENT GROUPS	**FSH**	**P-Value**	**LH**	**P-Value**	**TESTOSTERONE**	**P-Value**
1.00	5.25±0.40		4.80±0.30		4.32±0.19	
2.00	5.10±0.09	0.08	4.40±0.31	0.04*	4.74±0.14	0.03*
3.00	4.80±0.17	0.04*	4.50±0.30	0.07	5.61±0.19	0.00*
4.00	4.26±0.17	0.03*	4.21±0.20	0.03*	5.94±0.14	0.00*

*= Significant when compared to group 1

FSH:

There was significant decrease in the FSH levels of the animals in the test groups 3 and 4 respectively when compared to group 1.

LH:

There was significant decrease in the LH levels of the animals in the test groups 2 and 4 respectively when compared to group 1.

TESTOSTERONE:

There was significant increase in the Testosterone levels of the animals in the test groups 2, 3 and 4 when compared to the group 1.

Table 4.2: The effect of Selenium administration on the reproductive hormones of wistar rats

*=

EXPERIMENT GROUPS	FSH	P-Value	LH	P-Value	TESTOSTERONE	P-Value
1.00	4.32±1.31		4.35±0.33		3.80±0.86	
2.00	1.50±0.25*	0.02*	2.61±0.33*	0.03*	3.45±0.76	0.08
3.00	2.00±0.09*	0.04*	3.14±0.08*	0.04*	2.11±0.14*	0.04*
4.00	2.10±0.17*	0.04*	1.66±0.37*	0.00*	1.40±0.13*	0.03*

Significant when compared to group 1

FSH:
There was significant decrease in the FSH levels of the animals in the test groups 2, 3 and 4 respectively when compared to group 1.
LH:
There was significant decrease in the LH levels of the animals in the test groups 2, 3 and 4 respectively when compared to group 1.
TESTOSTERONE:
There was significant decrease in the Testosterone levels of the animals in the test groups 3 and 4 when compared to the group 1.

Table 4.3: The effect of Zinc and Selenium co- administration on the reproductive hormones of wistar rats

EXPERIMENT	ACTIVE	P-Value	SLUGGISH	P-Value	IMMOTILE	P-Value
GROUPS	Mean±SEM		Mean±SEM		Mean±SEM	
1.00	82.50±2.50		13.25±1.75		4.25±0.75	
2.00	53.75±1.25	0.00*	40.00±0.00	0.13	6.25±1.25	0.60
3.00	39.00±1.00	0.00*	51.00±1.00	0.01*	10.00±0.00	1.00
4.00	51.50±1.50	0.00*	41.25±1.25	0.05*	7.25±0.25	0.44

*= Significant when compared to group 1

FSH:

No effect on FSH levels.

LH:

There was significant decrease in the LH levels of the animals in the test group 4when compared to group 1.

TESTOSTERONE:

There was significant decrease in the Testosterone levels of the animals in the test groups 2, 3 and 4 respectively when compared to the group 1.

Table 4.4: The effect of Zinc administration on the Sperm motility of wistar rats

EXPERIMENT GROUPS	FSH	P-Value	LH	P-Value	TESTOSTERONE	P-Value
1.00	5.28±0.30		2.63±0.05		3.41±0.08	
2.00	5.28±0.66	0.06	2.94±0.47	0.08	2.18±0.79	0.03*
3.00	4.72±0.52	0.07	2.67±0.22	0.07	1.49±0.11	0.00*
4.00	4.60±1.38	0.06	2.13±0.24	0.04*	1.67±0.35	0.00*

*=Significant when compared with control

ACTIVE:

There was significant decrease in the number of active sperms of the animals in the test group 2, 3 and 4 when compared to group 1.

SLUGGISH:

There was significant increase in the number of sluggish sperms of the animals in the test group 2, 3 and 4 when compared to group 1.

Table 4.5: The effect of Selenium administration on the Sperm motility of wistar rats

There was no significant difference in the sperm motility among the experiment group.

EXPERIMENT GROUP	**ACTIVE M** Mean±SEM	**P-Value**	**SLUGISH M** Mean±SEM	**P-Value**	**NON MOTILE** Mean±SEM	**P-Value**
1.00	85.00±5.00		11.50±3.50		3.50±1.50	
2.00	52.50±12.50	0.08	40.00±10.00	0.08	7.50±2.50	0.08
3.00	40.00±20.00	0.06	50.00±15.00	0.06	10.00±5.00	0.06
4.00	50.00±20.00	0.06	42.50±17.50	0.06	7.50±2.50	0.06

Table 4.6: The effect of Zinc and Selenium co- administration on the sperm motility of wistar rats

EXPERIMENT GROUPS	ACTIVE MOTILE	P-VALUE	SLUGISH	P-VALUE	IMMOTILE	P-VALUE
1.00	78.60±0.98		12.60±0.81		8.80±0.37	
2.00	44.75±1.18	0.00*	37.00±0.71	0.00*	18.25±1.75	0.00*
3.00	29.75±2.06	0.00*	47.00±1.22	0.00*	23.25±1.89	0.00*
4.00	46.25±2.39	0.00*	33.75±2.39	0.00*	20.00±0.00	0.00*

*=Significant when compared with control

ACTIVE:

There was significant decrease in the number of active sperms of the animals in the test group 2, 3and4when compared to group1.

SLUGGISH:

There was significant increase in the number of sluggish sperms of the animals in the test group 2, 3and4when compared to group1.

IMMOTILE:

There was significant increase in the number of immotile sperms of the animals in the test group 2, 3 and 4when compared to group1.

Table 4.7: The effect of ZINC administration on the Sperm count of wistar rats

EXPERIMENT GROUPS	COUNT MEAN ±SEM	P-VALLUE
1.00	44.38±2.63	
2.00	12.50±1.00	0.00*
3.00	23.90±1.40	0.00*
4.00	38.00±1.00	0.05*

*=Significant when compared with control at P≤0.05

There was significant decrease in the total number of sperms of the animals in the test group 2, 3and4when compared to group1.

Table 4.8: The effect of Selenium administration on the Sperm count of wistar rats

EXPERIMENT GROUP	TOTAL. COUNT Mean±SEM	p-value
1.00	41.75±0.25	
2.00	11.50±1.30	0.50
3.00	22.50±12.50	0.78
4.00	37.00±1.00	0.88

*=Significant when compared with control at P≤0.05

There was no significant difference in the sperm count among the experiment group.

Table 4.9: The effect of Zinc and Selenium co- administration on the sperm count of wistar rats

EXPERIMENT GROUPS	COUNT Mean±SEM	P-Value
1.00	42.21±0.21	
2.00	12.41±0.61	0.00*
3.00	17.93±0.87	0.00*
4.00	38.03±1.01	0.00*

*=Significant when compared with control at P≤0.05

There was significant decrease in the total number of sperms of the animals in the test group 2, 3 and 4when compared to group1.

Table 4.10: The effect of ZINC administration on the Sperm morphology of wistar ratsrats

EXPERIMENT GROUP	NORMAL	P-Value	ABNORMAL	P-Value
1.00	88.50±1.00		11.50±1.00	
2.00	89.50±0.50	0.60	10.50±0.50	0.60
3.00	88.50±1.00	1.00	11.50±1.00	1.00
4.00	87.00±2.00	0.44	13.00±2.00	0.44

*=Significant when compared with control at P≤0.05
There was no significant difference in the sperm morphology of animals in the test group 2, 3and4when compared to group1.

Table 4.11: The effect of Selenium administration on the Sperm morphology of wistar rats

EXPERIMENT GROUP	Normal .Morphology Mean±SEM	P-Value	Abnormal Morphology Mean±SEM	P-Value
1.00	87.50±2.50		12.50±1.00	
2.00	90.00±0.00	0.09	10.00±1.50	0.09
3.00	87.50±2.50	0.07	12.50±5.00	0.07
4.00	85.00±0.00	0.08	15.00±0.00	0.08

*=Significant when compared with control at P≤0.05

There was no significant difference in the sperm morphology of animals in the test group 2, 3and4when compared to group1.

Table 4.12: The effect of Zinc and Selenium co- administration on the sperm morphology of wistar rats

GROUP	NORMAL	P-VALUE	ABNORMAL	P-VALUE
1.00	85.05±0.84		14.95±0.84	
2.00	88.99±1.00	0.01*	10.89±1.04	0.01*
3.00	88.80±0.99	0.01*	11.14±0.97	0.01*
4.00	86.38±0.88	0.33	13.63±0.88	0.33

*=Significant when compared with control at P≤0.05
There was significant difference in sperm morphology of the animals in the test groups 3 and 4when compared to group1.

PHOTOMICROGRAPHS OF THE TESTES:

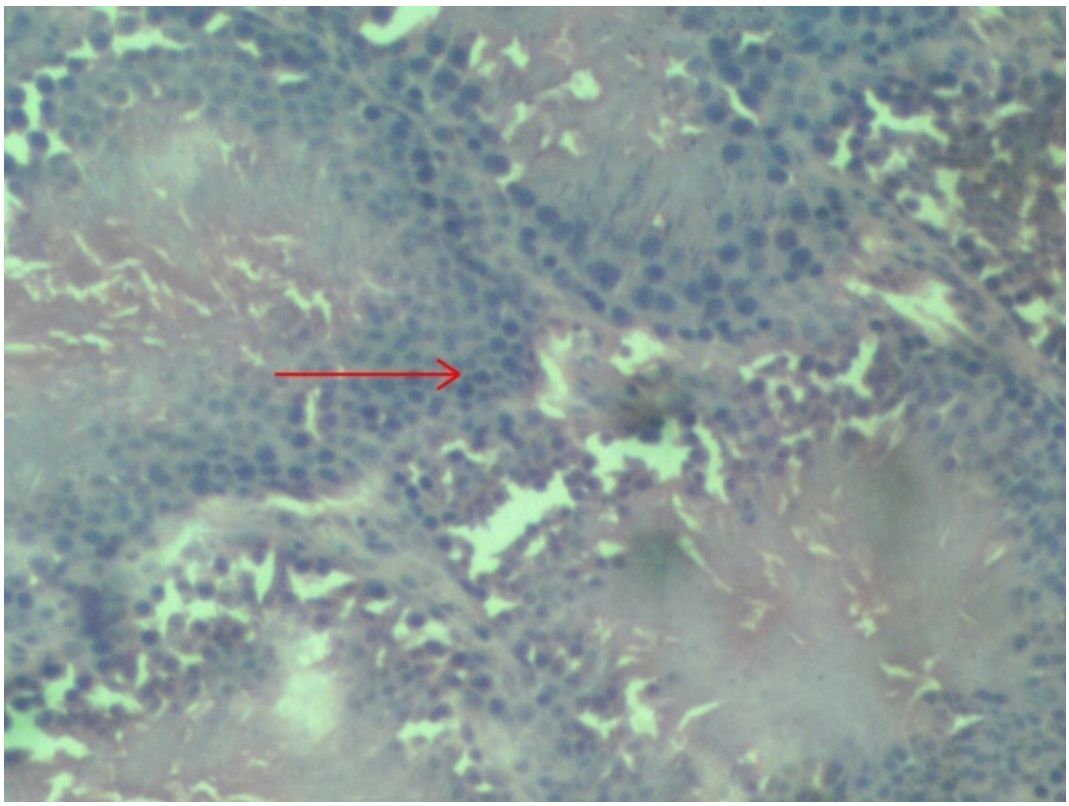

Plate 4.1 CONTROL:
AN H&E STAINED SLIDE OF TESTES SHOWING THE GERMINAL CELLS (RED ARROW) INCLUDING THE SPERMATOZOA. X10
Normal Seminiferous tubules

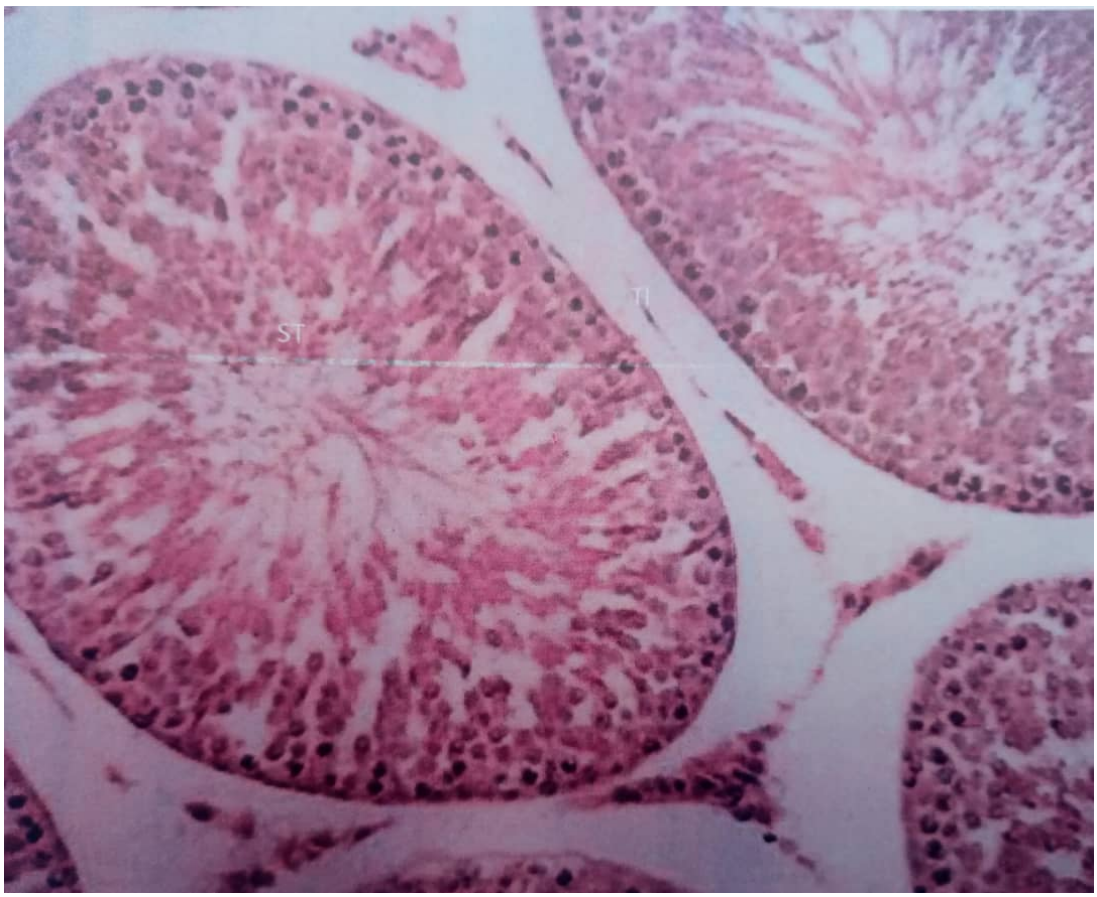

Plate 4.2 Zinc Low:
AN H&E STAINED SLIDE OF TESTES SHOWING GERMINAL CELL DEGENERATION AND INFLAMMATION, EVEN MASKING THE BLOOD VESSELS (CIRCLE) X10

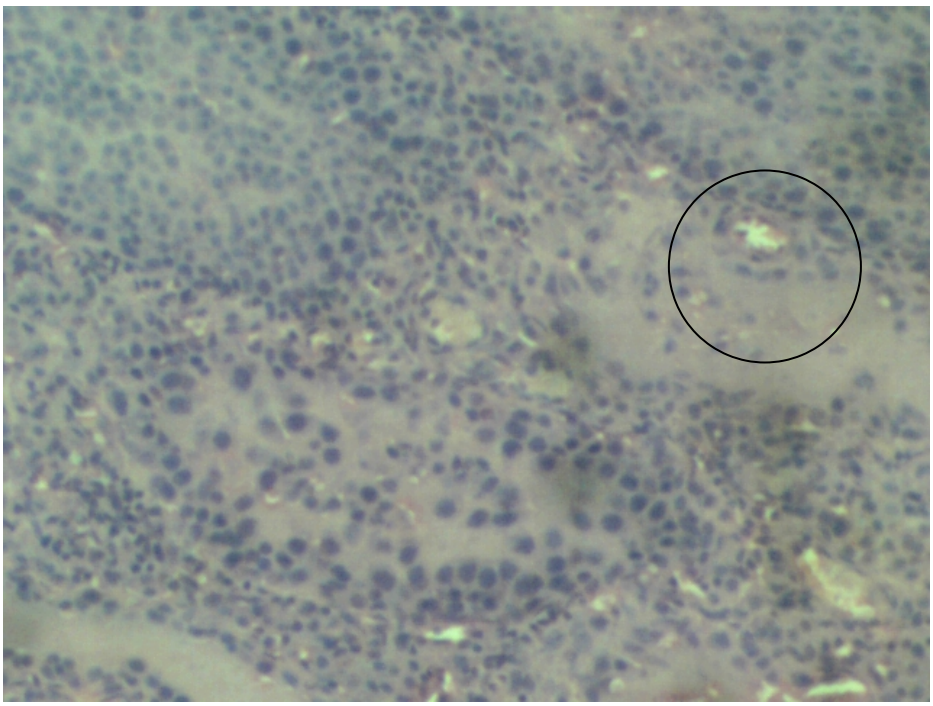

Plate 4.3 Zinc Moderate:
AN H&E STAINED SLIDE OF TESTES SHOWING GERMINAL CELL DEGENERATION AND INFLAMMATION, EVEN MASKING THE BLOOD VESSELS (CIRCLE) X10

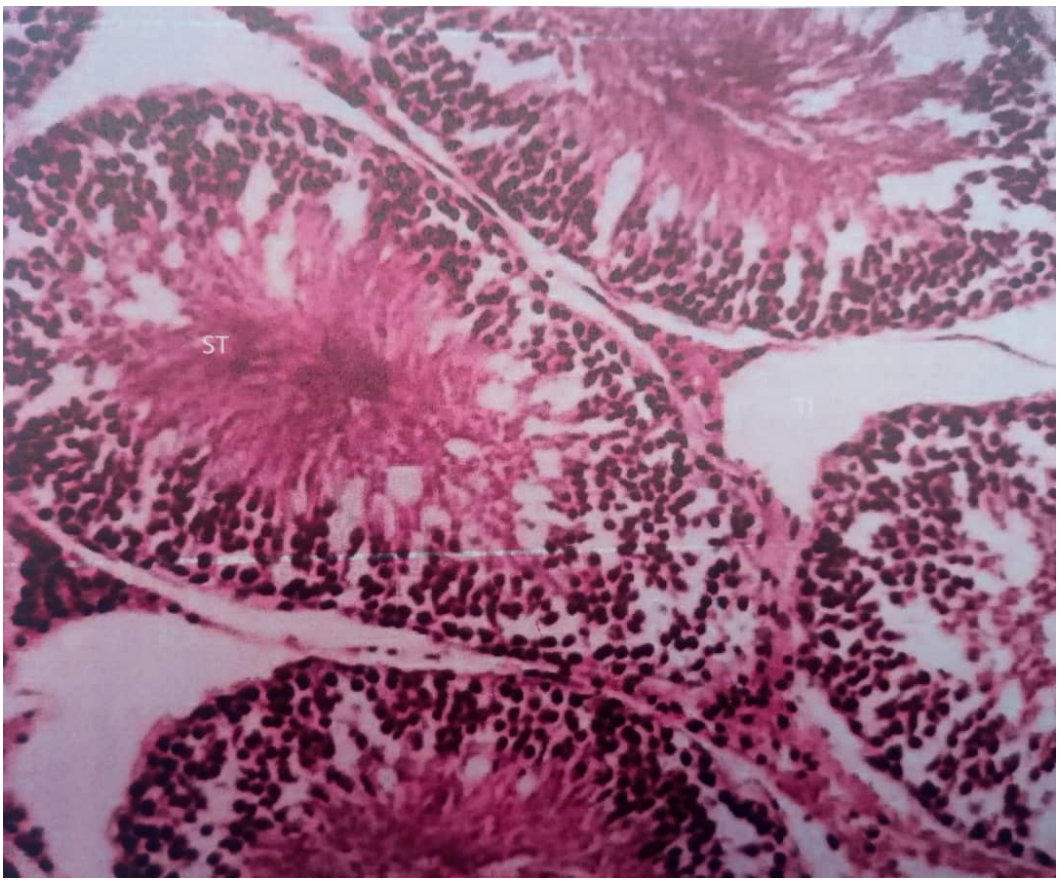

Plate 4.3 Zinc High dose:sX10

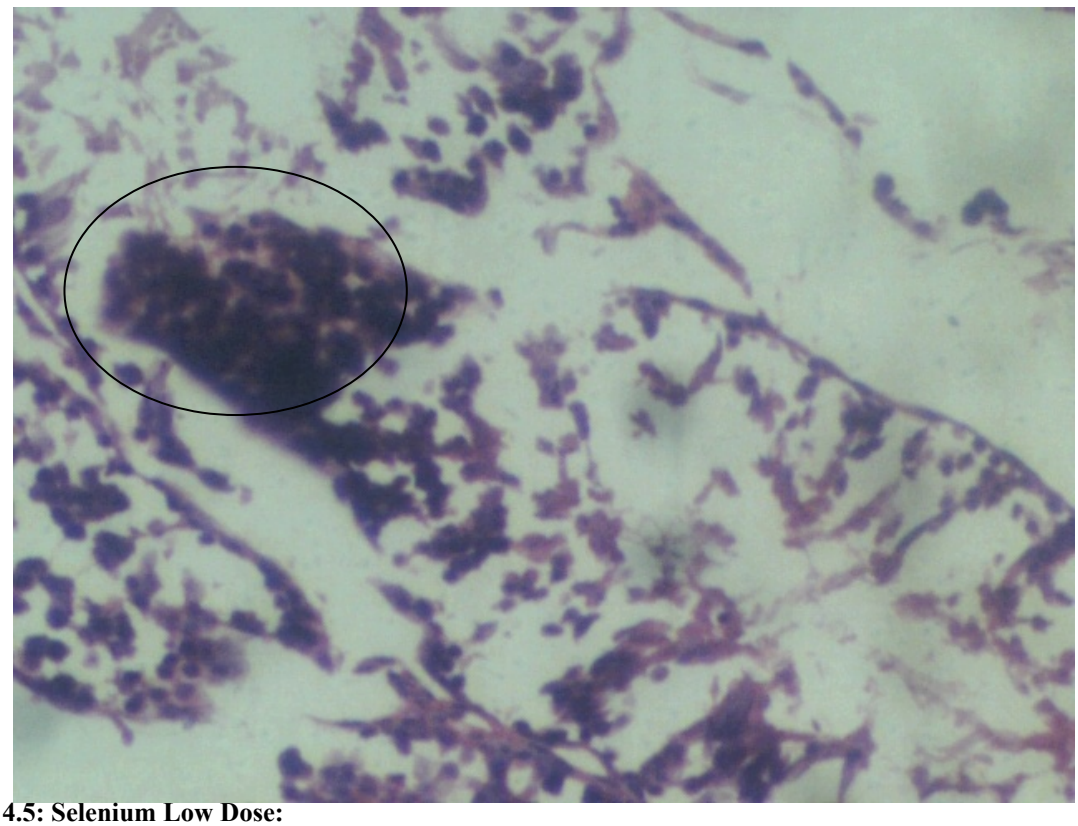

Plate 4.5: Selenium Low Dose:
AN H&E STAINED SLIDE OF TESTES SHOWIN INFLAMMATORY CELLS (CIRCLE)AND GERMINAL CELLS DISARRANGEMENT X10

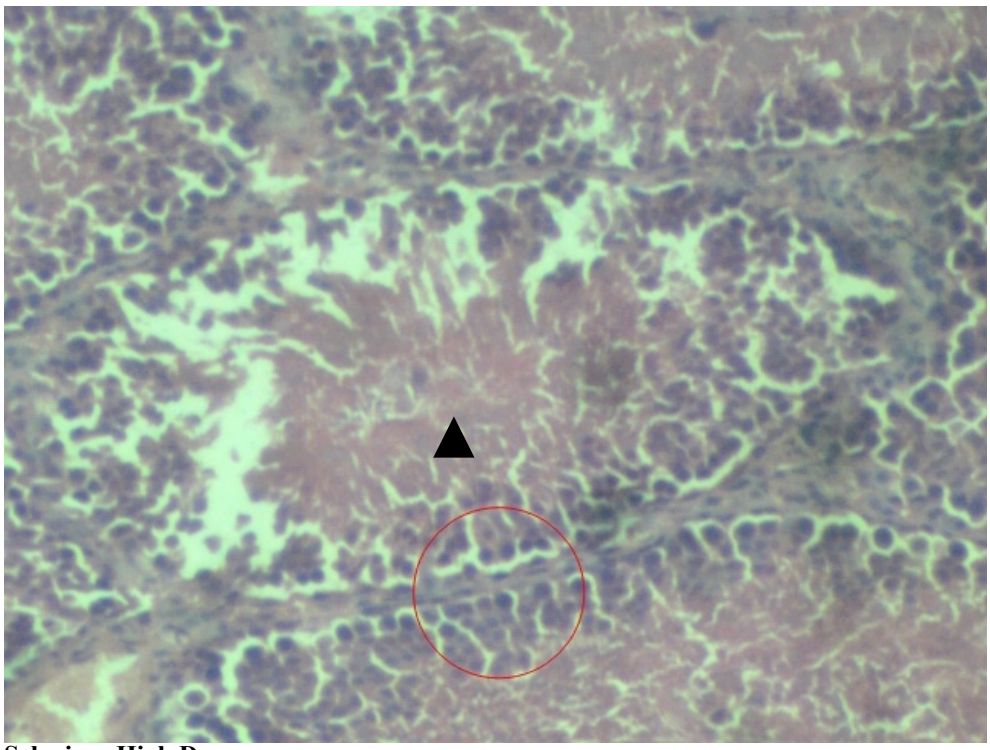

Plate 4.6: Selenium High Dose:
AN H&E STAINED SLIDE OF TESTES SHOWING THE GERMINAL CELS AND PERITUBULAR MYOID CELLS (RED CIRCLE) INCLUDING THE SPERMATOZOA AT THE LUMEN(ARROW HEAD) X10

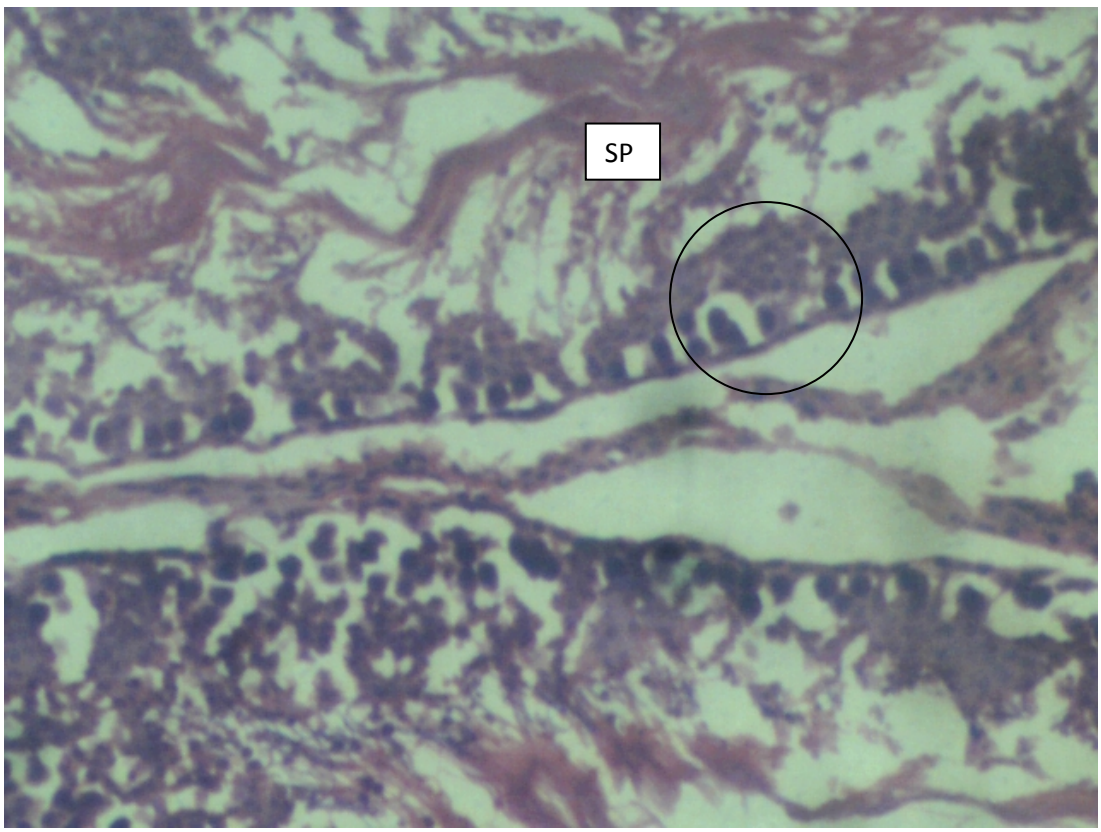

Plate 4.7: Zinc and Selenium Low Dose:
AN H&E STAINED SLIDE OF TESTES SHOWING SPERMATOZOA .....(SP) AND SPERMATOCYTES WITH CLUMPING OF SERTOLI CELLS (CIRCLE)X10

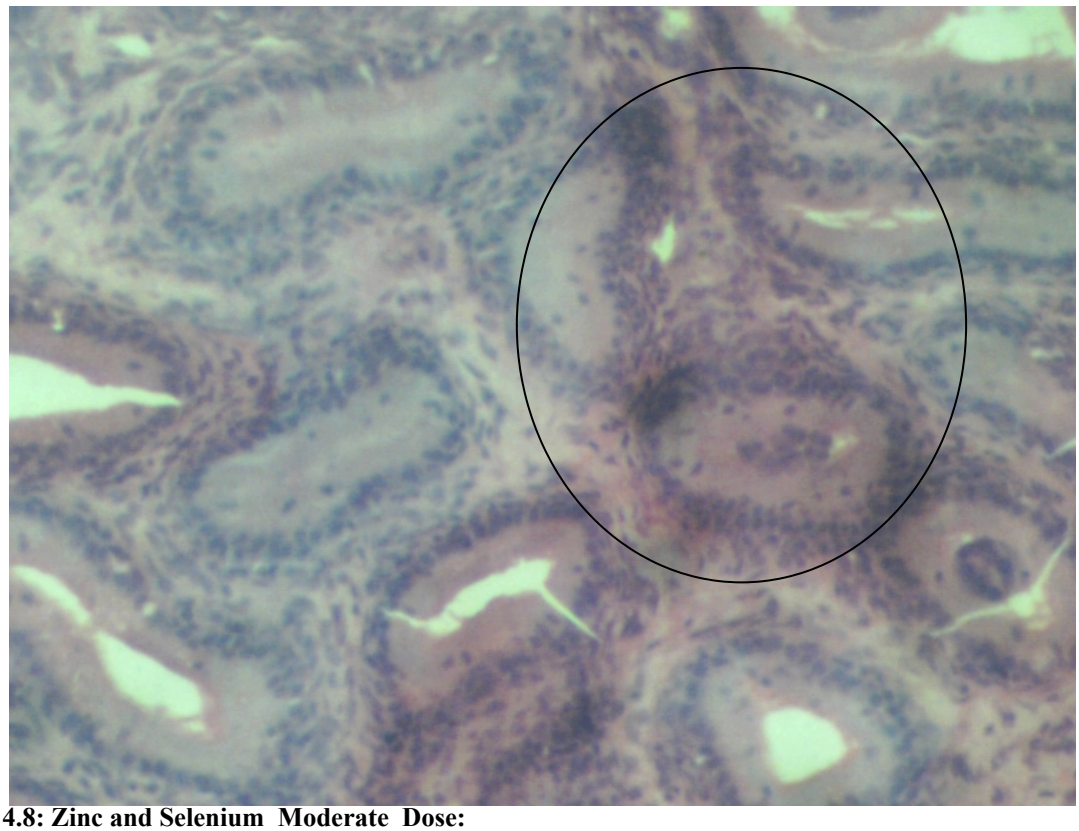

Plate 4.8: Zinc and Selenium Moderate Dose:
AN H&E STAINED SLIDE OF TESTES SHOWING INFLAMMATORY CELLS WITH HAEMORRHAGIC CONGESTION (CIRCLE) X10

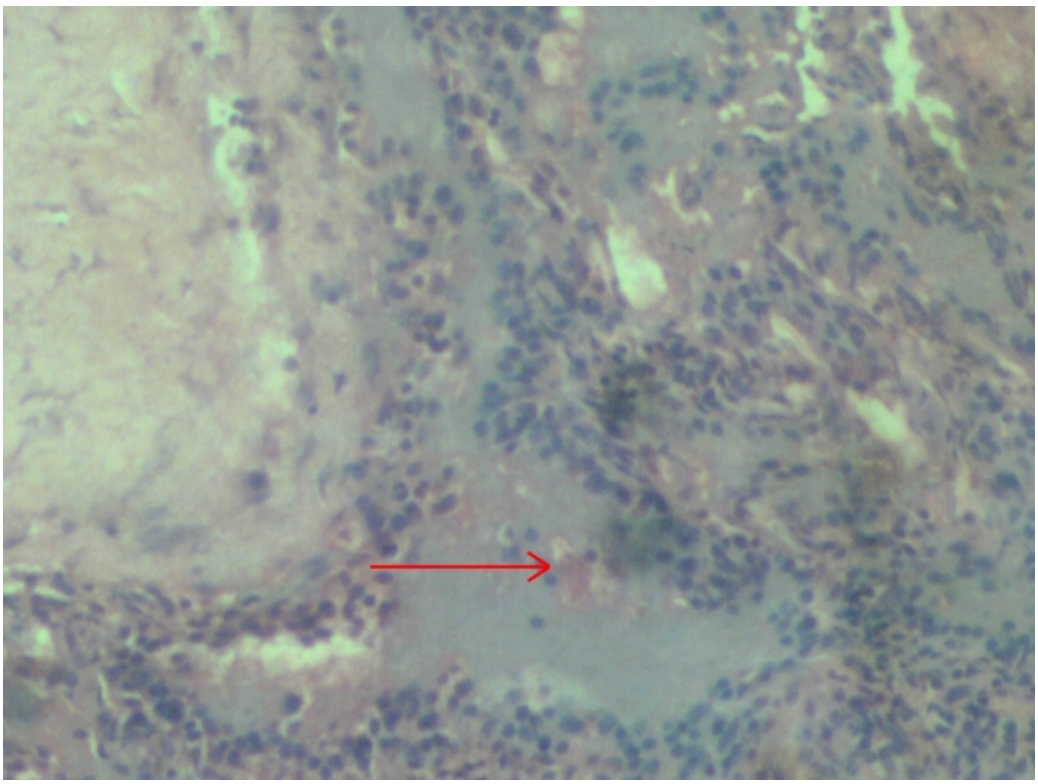

Plate 4.9: Zinc and Selenium High Dose:
AN H&E STAINED SLIDE OF TESTES SHOWING A DISORGANIZED GERMINAL CELLS. SPERMATOZOA NOT SEEN. LUMEN OCCLUDED (RED ARROW) X10

4.2 DISCUSSION OF FINDINDS:

4.21. MALE REPRODUCTIVE HORMONES: Testosterone-T, FSH and LH.

This study was done to determine the effects of oral independent and co-administration of overdose supplementation of Zinc and Selenium compounds on male sex hormones, sperm quality, haematological indices and lipid profiles in male wistar rats over a period of six weeks. The results showed statistically significant increase ($p < 0.05$) in serum testosterone of the test groups II,III and IV that ingested 50mg/kg, 150mg/kg and 300mg/kg body weight of zinc respectively when compared with the control. This result agrees with the works of Ratnasooriya et al (2004), Abdela et al (2011) and Egwurugwu et al (2013). Zinc supplementation activates increased secretion and action of testosterone and can lead to increased spermatogenesis. While Prassad (1996) noted that zinc deficiency lowers plasma testosterone levels but over supplementation has no effect on testosterone levels, Koehler et al (2009). The results also showed statistically significant decrease in the serum values of follicle stimulating hormone (FSH) and luteinizing hormone (LH) when compared with the control in groups supplemented with 50mg, 150mg and 300mg/kg body weight of zinc ($p<0.05$). The significant decrease in serum levels of FSH and LH as testosterone shots up could be as a result of negative feedback system effect of testosterone on the hypothalamus which in response may have caused the decrease in the secretion of FSH and LH by the anterior pituitary gland (Guyton and Hall,2006). Imbalance in their levels could lead to male factor infertility. FSH from sertoli cells regulates spermatogenesis by promoting the growth and maturation of sperm cells. LH from leydig cells stimulates the production of testosterone and also regulates spermiation. These steroid hormones are positively correlated with sperm count, motility and morphology and hence a significant decrease in their values could have limited sperm synthesis resulting in infertility.

This study also revealed that increasing doses of selenium supplementation led to statistically significant decreased levels of serum testosterone, FSH and LH ($p<0.05$) in all the test groups of graded doses of 100mg/kg, 200mg/kg and 400mg/kg body weights compared with the control. This result agrees with the work of (Riaz and Mehmood,2012) on testosterone but differs with the results on FSH and LH whose findings showed significant increase in low doses. The significant low serum levels of T,FSH and LH could be as a result of Selenium supplementation above the tolerable limit that may no longer be beneficial. It has also been observed that selenium is required for normal functioning of the hypothalamo-pituitary-gonadal axis which affects the production of FSH and LH (Gronback and Thorlacius,1992). However, in high doses it is not beneficial.

The results of co-administration supplementation of zinc and selenium showed statistically significant decreased levels of serum testosterone in test groups II,III and IV. Co-administration of Zn and Se seem to eliminate the effect of Zn, which may suggest that these two elements act antagonistically. This agrees with the work done by Darago et al, 2020.It had no effect on serum FSH levels of all the test groups but showed significant increase in serum LH level of the high dose group.

This study also showed that increasing doses of oral supplementation of zinc alone led to statistically significant decreased sperm motility and count and increased morphology ($p<0.05$).This agrees with the works done by Gunfer et al (2003) and Egwurugwu et al (2013). Degenerative changes, spermatic arrest, degeneration of seminiferous tubules and fibrosis in interstitial tissues have been noticed following high doses of zinc supplementation which can significantly alter sperm motility (Turut et al,2003). Also zinc in higher doses has negative effects on sperm motility (Chyb et al, 2000). The observed pathologies could have emanated from zinc high concentration that can impair spermatogenesis and the resultant negative outcome on sperm motility, count and morphology. Zinc ion could have also displaced calcium ions in sperms necessary for the activation of spermatozoa (Chyb et al,2000). Selenium alone supplementation showed decreased sperm parameter values but not significant to affect sperm motility, count and morphology values. The results are not in agreement with the works of two groups that got direct opposing results. Kaur and Kauer(2000): Cabaj et al(2012) reported that selenium cause decrease in sperm motility and count which disagrees with the studies done by Irvine (1996): Keskes-Ammar et al(2003) which reported that selenium cause increase in sperm motility and count. The discrepancies could be as a result of the body's capacity to store selenium as reported from the work done by Richardson et al (2006) which stated that selenium operates three levels of biological activities in animals as follows: (1. Trace concentrations are required for normal growth and development:2. Moderate concentrations can be stored and homeostatic functions are maintained: 3. Elevated concentrations can result in toxic effects.

The results of the co-administration of zinc and selenium supplementation showed statistically significant decrease in sperm motility and count and increased morphology ($p<0.05$).

V. CONCLUSSION AND RECOMMENDATIONS

Conclusion:

The results of this study indicate that these micro elements (Zinc and Selenium) should not be administered in concentrations above the recommended optimal values independently or jointly as dietary supplementation in male only infertility treatment to boost sperm activities..Over supplementation of both

elements independently and in combination created toxic effects that may have eventually compromised the protocols for the biosynthesis of the biological materials including reproductive hormones, enzymes and lipoproteins responsible for the effective production, growth and maturation of sperm cells. These findings should be an important indication for physicians regarding the administration of dietary supplements and also serve a caution to the affluent unmarried adult male members of the society who indulge in self over supplementation of single and combination of fertility enhancing micronutrients like zinc and selenium.

Implications of the Findings:

This study implies that consumers and promoters of health enhancing vitamins and mineral supplements and the society at large should be mindful of over supplementation and abuse of the ubiquitous over the counter dietary supplements containing zinc and selenium compounds. They also should be aware of the activities of dietary supplement producing companies who have taken the space in the mainline and online media to advertise their products containing multiple nutritional fertility micro elements. Research has shown that despite the high cost of dietary supplements, financially affluent strata of the society indulge in self excess consumption of over the counter dietary supplements as a life style status symbol and in the guise of maintaining a balanced diet thereby overshooting the optimal daily recommended values. Many amongst them are prospective bachelors that ends up with the challenge of male factor infertility.

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