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By

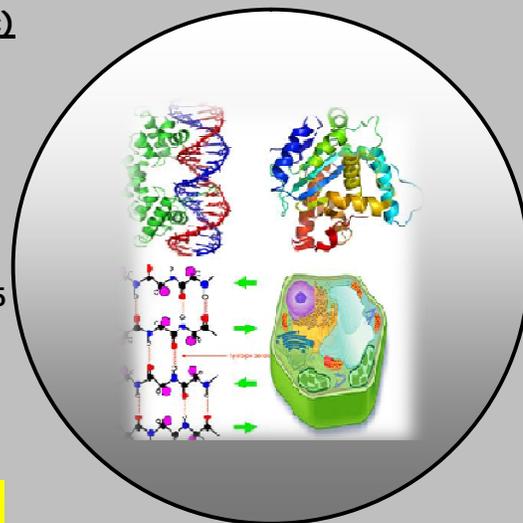
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[info@jbcr.in](mailto:info@jbcr.in)

RESEARCH PAPER

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## The Effects of Physical and Oxidative Stress on the Ovary of the Female Wistar Rat

\*A.I., Shugaba, \*\*A.S., Asala \*\*\*I.M., Gambo, \*B.M., Mohammed, \*F., Shinku, \*\*\*\*A.M., Rabi, \*C.B., Uzokwe, \*\*\*J.O. Hambolu and \*\*\*S.A. Ojo

\*Department Of Anatomy, University Of Ios, Jos.

\*\*Department of Human Anatomy, College of Medicine, University of Abuja, Abuja.

\*\*\*Department of Veterinary Anatomy, Ahmadu Bello University, Zaria.

\*\*\*\*Department of Physiology, University Of Ios, Jos.

### ABSTRACT

*The hallmark of fertility depends on the viability of the reproductive system. The ovary which is responsible for the production of the female gonads is indispensable to the fertile couple. The ovary of the wistar rat and that of the human are very comparable in their anatomy and physiology. Studies on the rat's ovary can therefore be extrapolated to the humans Stress being any mental or/and physical state that can have an adverse affect on one's health. This of course involves ones reproductive system, especially through the hypothalamo-pituitary axis where there are changes in the levels of released cortisol.*

*We used mono and bilateral enucleation as induced physical stress and administration of alcohol as an oxidative stress. These will trigger the stress response of the animal.*

*This study enabled us to find out some of the anatomical changes occurring in the ovaries of the experimental groups as compared to the control.*

*At the end of the experiment, harvested ovaries were weighed and histological slides using haematoxylin and eosin stain were prepared for comparison*

*The Graafian follicles of the enucleated group showed absence of primary oocytes. While the alcohol group showed absence of the oocytes in the follicles and thinning of the germinal epithelium. The alteration in the alcohol group is more marked. There was however no changes in the mean weights of the ovaries.*

*Key Words: Physical Stress, Oxidative Stress, Ovary, Anatomy, Physiology.*

## **INTRODUCTION**

Reproduction is the sum total of all the processes involved in the production of an offspring involving the reproductive system. This system consists of the external genitalia and internal genitalia (Hornby, 2000).

Stress on the other hand is the sum total of all non specific biological phenomena elicited by adverse external influences or can be said to be any situation that upsets homeostasis and threatens one's physical or emotional well being. The Oxford Advanced Learners Dictionary of current English says Stress is any mental and/or physical state which can have an adverse effect on one's health (Hornby, 2000).

The place of reproduction and stress in the human being these days is increasingly receiving both national and international attention especially when considering population studies, maternal and child welfare and the general well being of the human kind. And there are various stresses that can affect this important system of the body.

Any study that will affect these organs especially from direct extrapolation of animal based research for the benefit of mankind will definitely excite any scholar that wants to understand the ultimate effect of the categories of environmental factors on the molecular functions or/and non functions of the cell biology. Before now, most of the studies were on the hormonal, behavioral and chemical changes but not on the histomorphological and histomorphometric parameters.

There is therefore the need to understand the actual microscopic effects on the reproductive system as a result of stressful conditions.

This study is therefore intended to look at the microscopic integrity of the ovary of the female Wistar Rat in carrying out expected physiologic functions after inducing stressful conditions.

Wistar rat has been chosen for this study because of its easy to understand reproductive cycle, early adaptation, having similar food habit with the human and easily reached sexual maturity of eight weeks. The reproductive potential of the rat is also influenced by spectrum of light intensity (from low photoperiod to total blindness) (Rechtschaffen and Bergmann, 2002).

## **MATERIAL AND METHODS**

The period of this research covered six weeks after the initial period of one month used for acclimatization in the animals' house.

A total of 40 matured Wistar rats were used. These were grouped and put into four cages of ten each and allowed to acclimatize for a month in their new environment before the commencement of the experiment. The four groups were labeled thus: Group 1-control Group 2-monoenucleation Group 3-bilateral enucleation Group 4-alcohol

At the end of the one month acclimatization, all the rats were weighed and recorded.

The mono and bilateral enucleation groups were individually anaesthetized with Ketamine hydrochloride at the dose of 30mg/kg body weight and given intraperitoneally and allowed to take effect.

Dosage of Ketamine administered:

30mg /kg body weight

Dosage of Alcohol Administered:

2g/kg body weight

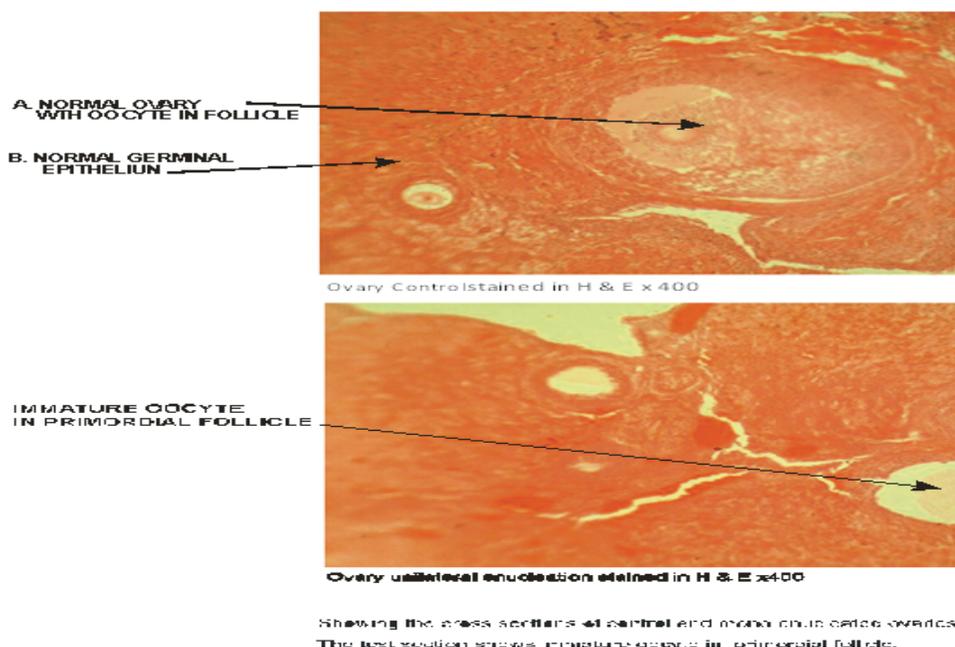
The orbit was exposed by reflecting the eye lids and using sharp dissecting scissors, and going posteriorly the optic nerve was sectioned and the globe removed. Bleeding points from the ophthalmic vessels were controlled by the application of firm pressure with sterile gauge. Care was taken during this procedure not to hold the rats by the neck to avoid strangulation.

They were then returned to their respective cages and having unhindered access to both tap water and continuously available feeds. All the rats were weighed twice weekly throughout the period of the experiment and average of each week recorded. The alcohol group was also weighed twice a week (Wednesdays and Fridays) and on each occasion alcohol was administered using oropharyngeal tube at the dose of 2gm/kg body weight and the calculated amount in milliliter (ml) given. At the end of the sixth week, the rats were sacrificed by gassing in a chloroform chamber, dissected and the ovaries harvested. The harvested ovaries were weighed put in labeled storage containers with Bouin's fluid in them.

The ovaries were allowed to be fixed for a week in the 10% formaldehyde before preparing the slides thus: Tissue processing, sectioning, mounting, Staining and microscopic reading.

The means, standard deviations and differences between the various measured parameters were calculated using appropriate Statistical packages to see whether there is any significance or not.

**PLATE I**



## RESULT AND DISCUSSIONS

Table 1. The mean weights of Wistar rats in the experimental groups during the six weeks of study in grammes.

Weeks	Groups	N	Mean (g)	Minimum (g)	Maximum (g)
Week 1	Group one	9	146.6667	80	200
	Group two	10	133.5	100	175
	Group three	10	136	80	190
	Group four	10	130.5	65	180
	Total	39	136.4103	65	200
Week 2	Group one	9	147.7778	85	210
	Group two	10	144.5	110	185
	Group three	10	150.5	90	210
	Group four	10	132.5	80	185
	Total	39	143.7179	80	210
Week 3	Group one	9	159.4444	90	225
	Group two	10	147.5	120	190
	Group three	10	149.5	90	195
	Group four	10	133	85	180
	Total	39	147.0513	85	225
Week 4	Group one	9	166.6667	90	235
	Group two	10	151	120	190
	Group three	10	156	105	195
	Group four	10	141	95	190
	Total	39	153.3333	90	235
Week 5	Group one	9	169.4444	100	240
	Group two	10	155.5	130	180
	Group three	9	157.2222	110	200
	Group four	10	149	105	205
	Total	38	157.5	100	240
Week 6	Group one	9	162.2222	90	205
	Group two	10	163.5	100	240
	Group three	10	167	125	225
	Group four	10	164	100	225
	Total	39	164.2308	90	240

**Table 2. Number of follicles in both control and test groups.**

GROUPS	Number of Follicles			
	TOTAL	MATURED FOLLICLES	IMMATURED FOLLICLES	ATRETIC FOLLICLES
CONTROL	32	17	13	2
MONONUCLEATED	28	15	10	3
BILATERA ENUCLEATED	27	14	10	3
*ALCOHOL TREATED	28	8	17	3

**\*Note high immature follicles in the alcohol group (at P-Value 0.027 there is significant difference between the groups because P-Value is < 0.05).**

## DISCUSSION

The gross Anatomical dispositions of the ovaries studied were essentially normal. There were no abnormal gross structures found in any of the ovaries. The weights of the rats in the four groups were taken for six weeks. Groups 2 and 3 show appreciable increase as seen from the weekly average. While groups 1 and 4 tend to show fluctuations in their weights.

There is no significant difference in the treatment since the P-value is greater than 0.05. The null hypothesis is accepted in favor of the alternative.(Table 1)It does mean that the effect of mono enucleation, bilateral enucleation and alcohol administration has little or no effect on the weights of the respective rats. There was also no obvious change in the weights of the ovaries in the different groups, the body weight/ organ ratio was not significantly affected.

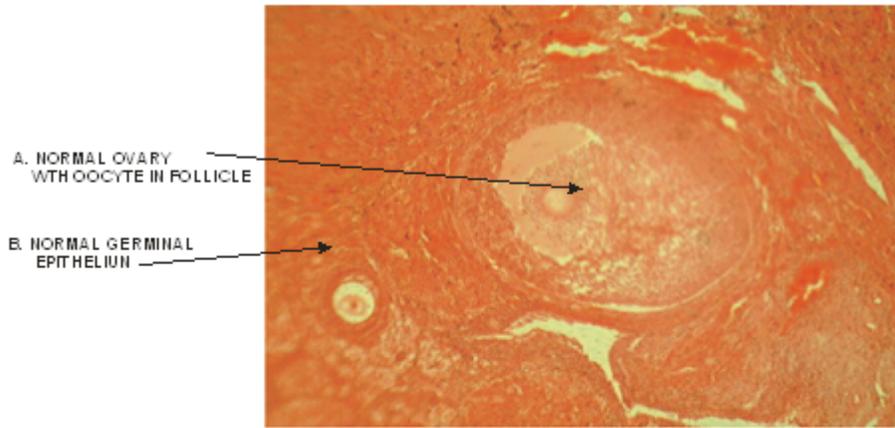
The result of these findings also suggest that induced stress has not in any way altered or reduced the potency of their sense of smell which would ordinarily have affected the unilaterally enucleated and bilaterally enucleated groups. Hence the similarities seen in the fluctuations of the weights in all the groups compared to those of the control group.

Another noticeable feature in all the four groups was that the weights of each rat's ovaries are 0.1gram. This means that the weight or body size has nothing to do with the size of the ovary. What really matter is the sensitivity of the ovary to stress. However the same cannot be said of the ovaries microscopically. The findings in the ovaries of all the groups revealed distinct morphological differences.

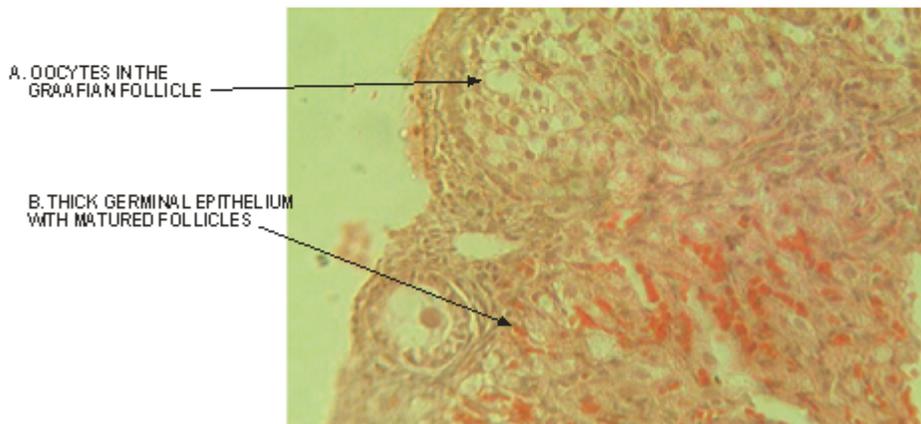
All the cells and histological components of the controlled ovaries are intact. The Graafian follicle contains oocytes; the germinal epithelium is thick and appears cuboidal with the follicles showing regressing corpus Luteum. There are also numerous primordial follicles at various stages of development. The general stroma of the ovarian tissue is highly vascularised.

The mono enucleation group showed some follicles with regressing corpus Luteum and matured Graafian follicles. There is absence of oocytes in the follicular cells. The cortex contains fewer primordial follicles with thick germinal epithelium. These evident alterations in the cells of the ovaries like the absence of oocytes in the follicular cells can lead to irregular ovulation and/ or an ovulation. (Van- Thiel and Cavaler, 1977 and Van- Thiel, 1983).

PLATE II



Ovary Control stained in H & E x 400



Ovary alcohol treated stained in H & E x 400

Shows the control ovary with alcohol treated. The alcohol-treated shows thin germinal epithelium

(A) fewer primordial cells (B) and large vesicular follicles with oocytes (C)

The bilaterally enucleated group has shown oocytes in the Graafian follicle and thick germinal epithelium. There are also matured Graafian follicles. This group shares some similarities with the unilaterally enucleated group since their type of stress is the same except for the different degrees. However, on the whole there are various stages of ovarian tissues' development with general increase in the vascularity of the organ.

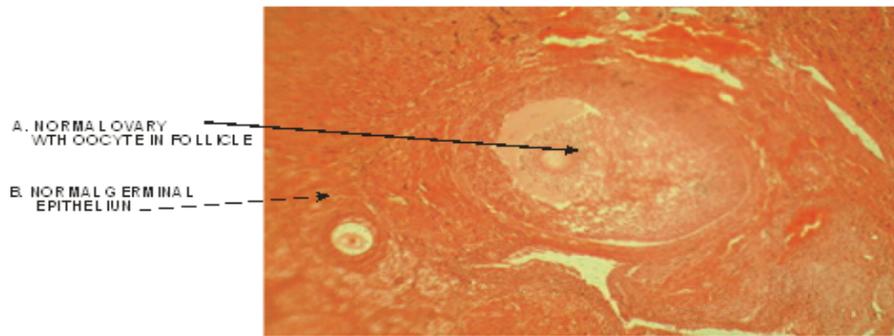
The changes in the alcohol administered group are distinct where there is thinner germinal epithelium, fewer primordial cells with matured but larger vesicular follicles containing no oocytes. So there is obvious degenerative process in this group. This will definitely affect the regularity of the menstrual cycle and where there are no oocytes, such cycles will be anovulatory.

Several studies in both humans and rats have demonstrated these findings. Alcoholic women are known to have a variety of menstrual and reproductive disorders from irregular menstrual cycle to complete cessation of menses, absence of ovulation (an ovulation) and infertility by (Mello et al. 1993).

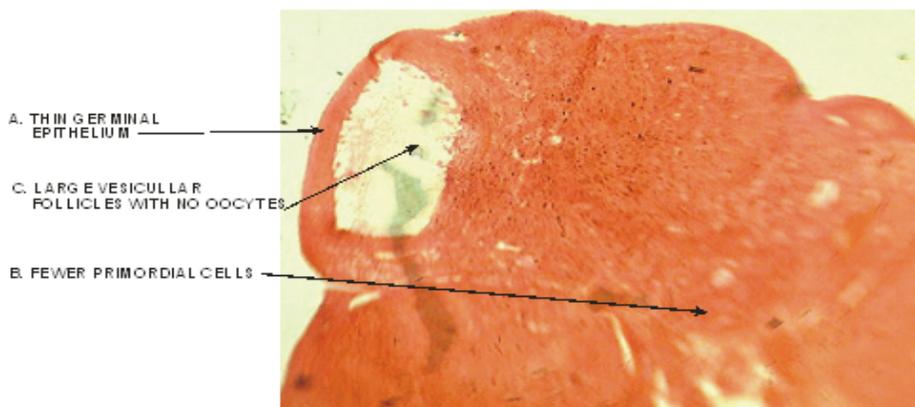
These studies have provided some information on how both acute and chronic alcohol exposure can alter the animals' reproductive systems. For example, acute alcohol exposure in female rats has been found to disrupt female cycling (Van-Thiel, 1983). A study of female rats fed with alcohol for 17 weeks starting at young adulthood (comparable in age to a 21 year old woman) found that alcohol did not lead to anovulation but rather to irregular ovulation (Krueger et al. 1993, Emanuel et al. 2001).

Other investigators, (Gavaler et al. 1980) however have reported that the ovaries of alcohol – exposed female rats were infantile; showing no evidence of ovulation at all and uteri appeared completely estrogen deprived.

PLATE III



Ovary Control stained in H & E x 400



Ovary alcohol stained in H & E x 400

Show the control ovary with alcohol treated. The alcohol treated shows thin germinal epithelium (A), fewer primordial cells (B) and large vesicular follicles with no oocytes (C)

From the various works done in the hormonal and chemical changes following various forms of induced stress (Hornby, 2000 Sies, 1985 and Hans Selye, 1936), there is usually accompanying increases in these parameters that have effects in the hypothalamo-pituitary-gonadal axis. The increased cortisol levels and the oxidative cellular effects of alcohol would most probably be responsible for these changes.

Apart from the obvious and immediate effects on the reproductive cycle and by extension the overall fertility of such animals, cellular and other morphological disruptions will in the long run cause dysplastic and or metaplastic changes responsible for causation of malignancies.(Carlson and Sawada, 1993)

A number of studies where endometrial hyperplasia caused by treatment with pure estrogen or cholesterol which is its precursor have been linked to malignancies extrapolated from rabbits` uterine studies (Batral and Kallstrand, 1997)

In a study by Grady et al., 1995 titled "Hormone replacement therapy and endometrial cancer risk", it was observed that prolonged use of unopposed estrogen was associated with a tenfold increase in the risk of endometrial cancer. Therefore combinations of these stress conditions or indeed the prolonged administration of pure cortisol can greatly cause endometrial mucosal hyperplasia with subsequent undesirable effects.

Unfortunately, there are no reports of such works done on the female reproductive tract to compare with our findings. However these findings can be used for further studies involving not only the combination of stressful conditions but also the measurements of serial levels and changes of cortisol and other reproductive hormones in the presence of such conditions. In this way corrective models can be developed for human clinical benefits.

Interestingly the cortisol levels throughout the period of the experiment continued to rise in the alcohol treated group compared to the control and the enucleated groups. The initial rise in the enucleated groups was due to the initial surgical injuries of enucleation. And at P-Value 0.002, there is significant difference between the groups. Similarly, the neutrophils and eosinophils percentages rose in the alcohol treated group because of the continued effect of alcohol administration with statistically significant difference between the groups. All these have manifested in the high number of immature follicles in the alcohol treated group (Table 2). The immature follicles are higher in the alcohol treated group as compared to the enucleated groups.

## **CONCLUSION**

The nature of the insulting induced stress seems to be the paramount factor in the causation of the cellular, hematological and chemical changes.

The ovaries of unilaterally enucleated and bilaterally enucleated groups showed absence of primary oocyte in the Graafian follicle. On the whole there are various stages of ovarian tissues` development with general increase in the vascularity of these organs which supports Espey`s findings of 1980. This may be as a result of cellular immaturity.

Oxidative stress induced by alcohol consumption, caused some cellular effects on the Fallopian tube mucosa of the female Wistar rat but the physical stress of enucleation did not cause any visible effect.

Statistical analysis of the diameters of the uterus showed significant differences between the groups at 5% and also correlation between the control and the alcohol treated and bilaterally enucleated groups.

The alcohol treated group showed thick endometrial mucosa with some Anucleated cells and evidence of hyperplasia and hypertrophy, glandular cells are quite prominent with some of them showing evidence of loss of nuclei. The myometrial muscular fibres and cells show some degree of hypertrophy compared to the normal. These changes were responsible for the differences noticed in the diameters of uterus (increased mucosal thickness, reduced luminal diameter and increase in the overall thickness of the uterus).

These results are also consistent with corresponding rise in the blood cortisol level and increase in the percentages of neutrophils and eosinophils especially in the alcohol treated group because of the sustained stressful condition.

The various findings of this work have also shown no significant difference in the gross weights and lengths measured of the organs of both the treated and the experimental rats. This may however not be the case in very chronic exposure.

## RECOMMENDATION

It is therefore recommended that further studies involving other forms of stress be carried out for longer periods of time so that more results concerning the effects of stress on these organs can be obtained.

Further hormonal and chemical studies in future will be necessary especially for comparative studies between animals` species and sexual differences within same species.

Combination of various forms of stress can also be applied in future studies.

Reproductive models in future will be useful in studying general fertility trends in the presence of one or more stressful conditions. Anti stress and anti oxidants like vitamin C can also be used in future to see the reversibility of the observed conditions.

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**All correspondences to: Dr. A.I.Shugaba, DEPARTMENT OF ANATOMY, UNIVERSITY OF JOS, JOS.**  
Email: [alishugaba@yahoo.com](mailto:alishugaba@yahoo.com)