Journal of Anatomical Sciences

Email:anatomicaljournal@gmail.com

J Anat Sci 6 (2)

Histomorphologic and Histomorphormetric Changes of the Fallopian Tube of the Female Wistar Rat Following Induced Physical and Oxidative Stress

Shugaba AI[1], Usman YM[1], Abdullahi YN[2], Oj0 SA[3], Hambolu JO[3], Asala Sa[4]

[1]Department of Anatomy, Faculty of Medical Sciences, University of Jos, Jos
[2]Department of Human Anatomy, Ahmadu Bello University Zaria
[3]Department of Veterinary Anatomy, Ahmadu Bello University, Zaria
[4]Department of Human Anatomy, College of Medicine, University of Abuja, Abuja

Corresponding Author: Shugaba AI.

E-mail: alishugaba@yahoo.com; +2348033454227

ABSTRACT

The hypothalamo-pituitary-adrenal axis exerts profound multilevel inhibitory effects on the reproductive system. But it has remained contentious how these connections influence reproduction, especially if vision is impaired or there is a subsisting stress condition. Many works have been carried out on the male reproductive tract of various rats without corresponding work on the female. Even those carried out on the female are on the Biochemical parameters. The fallopian tube is one of the most important organs of the female reproductive system needed for viable and optimal fertility. This is because it is not only needed for the transportation of the ova and spermatozoa but also the fertilized zygote. This oviduct is common to both the female humans and the female Wistar rats. This work is aimed at studying the microscopic disposition of the fallopian tube in both normal and stressed conditions. Obtained results can be used by future Researchers and various Health Professionals as foundation data. The experiment involved 40 matured female Wistar rats which were grouped into four groups of ten each after acclimatization: Group1 is the control, Group 2 is the monoenucleated, Group 3 is the bilateral enucleation and Group 4 is the alcohol administered rats. After sacrificing the rats and harvesting the fallopian tubes, the following parameters were measured: Diameters of the fallopian tubes, Lengths of the fallopian tubes, Histomorphology of the fallopian tubes, Weights of the rats. The results showed that oxidative stress caused various degrees of nuclear damage in the fallopian mucosa compared to the enucleation and control groups. And there were no changes in the mean weights of the rats and mean lengths of the fallopian tubes in all the groups.

Keywords: Histomorphology, Histomorphometry, Fallopian Tube, Physical stress, Oxidative stress

INTRODUCTION

Reproduction is the sum total of all the processes involved in the production of an offspring involving the reproductive system[1]. 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[1].

The place of reproduction and stress in humans 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[2]. 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 and/or 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[2].

Alcoholic women are known to have a variety of menstrual and reproductive disorders, from irregular menstrual cycles to complete cessation of menses, absence of ovulation and infertility. However, alcoholics often have other health problems such as liver disease and malnutrition, so reproductive deficits may not be directly related to alcohol use[2]. While there are many myths surrounding the causes of infertility, the idea that stress is a factor is true[3].Recent studies have confirmed a link between stress and infertility, both as a cause and as an effect. Stress affects the body in many ways, and one of them is by altering the neurochemical make-up. There is a direct link between the brain and reproductive tract. Nerve fibers connect the brain indirectly to both the Fallopian tubes and the uterus. For example, when a woman is under stress, spasms occur in the Fallopian tubes. These spasms can interfere with the movement of a fertilized egg through the fallopian tube[4]. The reproductive potential of the rat

is also influenced by spectrum of light intensity (from low photoperiod to total blindness)[5]. There is therefore the need to understand the actual microscopic effects on the fallopian tube as a result of stressful conditions. This study looked at the microscopic integrity of the Fallopian tube of the female Wistar Rat in carrying out expected physiologic functions after inducing stressful conditions. Stress is one very important phenomenon that influences the living, both physiologically and anatomically[5].

It is therefore worthwhile to see whether some of the microscopic effects of stress can influence fertility, the use of anti-oxidants in improving reproductive mucosa and also the success rate of in-vitro fertilization (IVF).

MATERIALS AND METHODS

The experimental period of the 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: 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 given intraperitoneally and allowed to take effect while the dose of alcohol administered was 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 having unhindered access to both tap water and continuous availability of feeds. All the rats were weighed twice weekly throughout the period of the experiment and average weight 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 Fallopian tubes harvested. These were measured and put in labeled storage containers with Bouin`s fluid in them. They were allowed to be fixed for a week in 10% formaldehyde before preparing the slides thus: Tissue processing, sectioning, mounting, Staining and microscopic reading (Histomorphological and histomorphometric observations). Histomorphometric observations were done using the light microscope and reading graticle taking into consideration the shrinking factor.

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. ANOVA was used with p value <0.05.

DISCUSSION

The gross Anatomical dispositions of all the studied fallopian tubes were essentially normal. There were no abnormal gross structures found in any of the dissected fallopian tubes. The weights of the rats in the four groups were taken for six weeks. Groups 2 and 3 show appreciable increase in weight as seen from the weekly average, while groups 1 and 4 show fluctuations in their weights. This may be due to acclimatization or accessibility to feeds.

The histomorphometric measurements of the named parts of the Fallopian tube (luminal diameters, Muscularis thickness and thickness from the mucosa to the adventitia) showed changes. The Muscularis thickness (inner circular and outer longitudinal) in all the groups are relatively similar, showing only a small margin of (± 0.67) from the normal. The Fallopian tube thickness in the control group was observed to be higher than those of the other groups. The luminal diameter in the control group was observed to be considerably lower than those of the other three groups (unilaterally enucleated, bilaterally enucleated and alcohol given rats). (Tables II – IV).

Fallopian tube length and weight taken freshly before tissue processing for all groups were observed to have the same values (Table II). For this period of the experiment, this organ did not show significant changes in weight and length.

The mucosa of the normal Fallopian tube of a mammal is composed of a simple columnar epithelium and of a lamina propria that is made up of loose connective tissue. The cells that make this epithelium contain nuclei for proper functioning [6,7].

In the experimental groups, the cells in the mucosae of the control, unilaterally enucleated and the bilaterally enucleated were normal because they all contained nuclei. In fact, other cells, glands and muscles of their walls are essentially normal with normal vascular channels (Plates I and II). While the cells in the mucosae of the alcohol treated group showed marked changes having no nuclei and fewer glands. The muscle fibers are

RESULTS

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

There is an increase in the mean, minimum and maximum weights of the rats across weeks 1 to 6.

Table 2: Showing the Lenghts (in Millimeters) and Weights (in Grammes) of the Fallopian Tubes.

Group one				Group two				Group three				Group four			
Control rats				Unilaterally enucleated rats				Bilateral enucleated rats				Rats given alcohol			
Length		Weight		Length		Weight		Length		weight		Length		Weight	
right	Left	Right	left	Right	Left	Right	Left	Right	Left	right	left	right	left	right	Left
0.4	0.4	0.1	0.1	0.4	0.4	0.1	0.1	0.4	0.4	0.1	0.1	0.4	0.4	0.1	0.1
0.4	0.4	0.1	0.1	0.4	0.4	0.1	0.1	0.4	0.4	0.1	0.1	0.4	0.4	0.1	0.1
0.3	0.3	0.09	0.09	0.4	0.4	0.1	0.1	0.4	0.4	0.1	0.1	0.4	0.4	0.1	0.1
0.4	0.4	0.1	0.1	0.4	0.4	0.1	0.1	0.4	0.4	0.1	0.1	0.4	0.4	0.1	0.1
0.4	0.4	0.1	0.1	0.4	0.4	0.1	0.1	0.3	0.3	0.1	0.1	0.4	0.4	0.1	0.1
				0.4	0.4	0.1	0.1	0.4	0.4	0.1	0.1	0.4	0.4	0.1	0.1
				0.4	0.4	0.1	0.1	0.4	0.4	0.1	0.1	0.3	0.3	0.1	0.1
				0.3	0.3	0.1	0.1	0.4	0.4	0.1	0.1	0.3	0.3	0.1	0.1

There is no variation in the length or weight of the fallopian tubes of the rats on each side neither is there a change in same between the test groups and the control. All weights were taken in grammes and lengths were taken in millimetres.

Table 3: Cross Sectional Measurements of Named Parts of the Fallopian Tube in Micrometers.

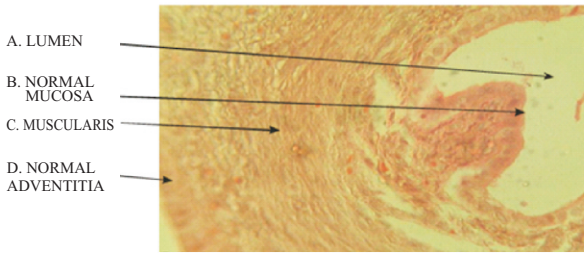
Group	Lumen diameter (μm)	Muscularis thickness (μm)	Fallopian tube thickness (μm)
Control rats			
	10	3	5
	9	3	6
	10	3	5
	11	3	5
	9	3	6
Rats given alcohol			
	29	4	4
	20	3	4
	25	3	4
	28	3	4
	24	3	5
Bilaterally enucleated rats			
	40	3	5
	34	3	5
	36	3	5
	37	3	5
	35	2	4
Unilaterally enucleated rats			
	24	3	4
	27	3	4
	26	3	4
	26	3	4
	25	2	3

The Muscularis thickness remained essentially constant for all the test groups and the control group while variations occurred for the diameter of the lumen.

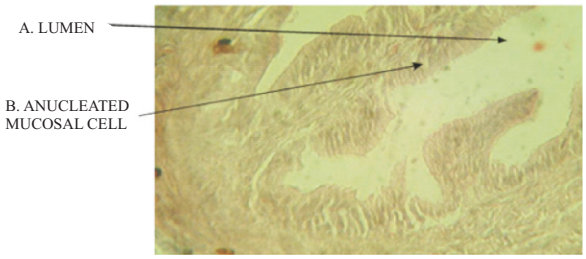
Table 4: Cross Sectional Dimensions of Named Parts of the Fallopian Tube in Microns (Product of the Values in UM and the Callibration Factor

Group	Lumen diameter (μm)	Muscularis thickness (μm)	Fallopian tube thickness (μm)
Control rats			
	33.30	9.99	16.65
	29.97	9.99	19.98
	33.30	9.99	16.65
	36.63	9.99	16.65
	29.97	9.99	19.98
Rats given alcohol			
	96.97	13.32	13.32
	66.60	9.99	13.32
	83.25	9.99	13.32
	93.24	9.99	13.32
	79.92	9.99	16.65
Bilaterally enucleated rats			
	133.20	9.99	16.65
	113.22	9.99	16.65
	119.88	9.99	16.65
	123.21	9.99	16.65
	116.55	6.66	13.32
Unilaterally enucleated rats			
	79.92	9.99	13.32
	89.91	9.99	13.32
	86.58	9.99	13.32
	86.58	9.99	13.32

There is relative in fallopian tube thickness in the test groups as compared to the control resulting to the increase in lumen diameter in the test groups compared to the control.

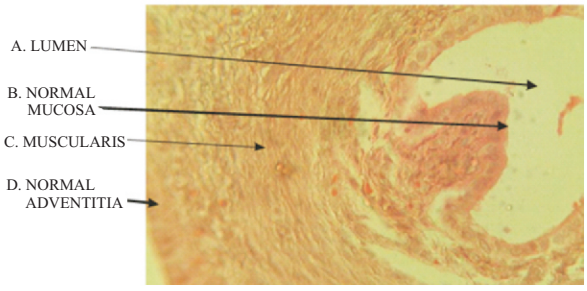


Fallopian tube control stained in H & E x400



Fallopian tube alchohol-treated rat stained with H & E x400

Plate I: Control fallopian tube treated with alcohol showing anucleated mucosal cells (B)



Fallopian tube control stained in H & E x400

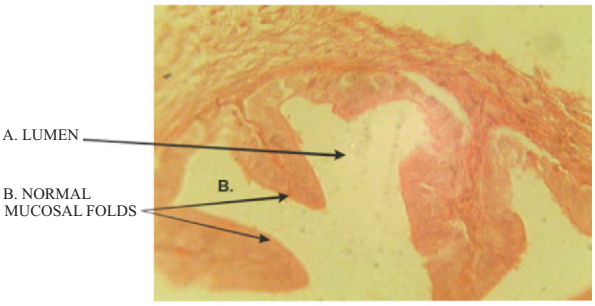
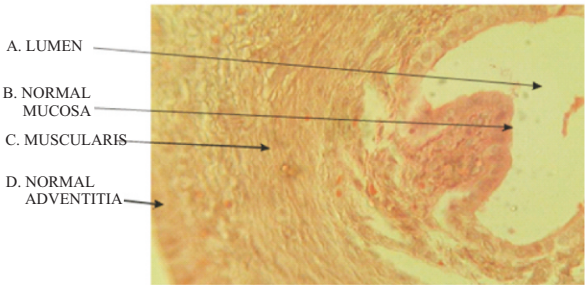
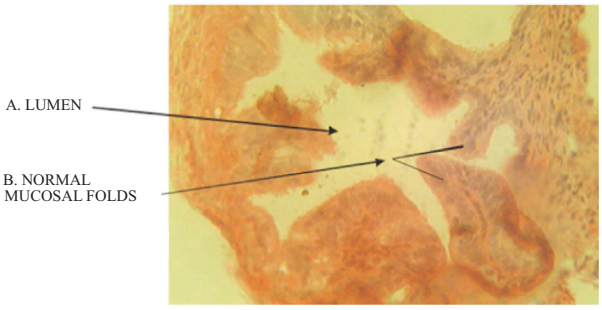


Plate 2: Cross sections of control fallopian tube and bilaterally enucleated showing normal mucosa and mucosal folds



Fallopian tube control stained in H & E x400



Fallopian tube bilateralenucleated in H & E & 400

Plate 3: Cross sections of control fallopian tube and bilaterally enucleated showing normal mucosa and mucosal folds

hypertrophied and the blood vessels dilated (Plate III).

All these changes in the Fallopian tube of the alcohol treated rats will definitely affect the normal functions of their Fallopian tubes. Normally the ciliated cells, through the beating of their cilia help move the ova from the ovaries to the uterus and also in the movement of the fertilized zygote towards the uterus and the goblet or peg cells produce secretions that have protective and nutritional functions for the oocytes. Similarly, the Fallopian tube has to be normal for effective movement of spermatozoa from its cornual part to the Ampulla for fertilization to take place. Without nuclei in the epithelial cells, the cilia on the ciliated cells may not be able to propel oocytes from the ovaries to the uterus and the goblet or peg cells may not be able to produce secretions that are meant to protect and nourish the oocytes. This invariably will damage the oocytes and may reduce their chances of getting fertilized. If this continues, the rat may not be able to conceive and will subsequently become infertile.

On the other hand, a damaged Fallopian mucosa can predispose it to ectopic gestation since the fertilized zygote cannot reach the uterus which is the normal site for implantation. It therefore means that longer duration of exposure to these stressful conditions will show further damaging effects[8,9].

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 measurement 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.

CONCLUSION

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.

The findings of this work have also shown no significant difference in the gross weights of the rats and lengths of the fallopian tubes 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 studies should also look at hormonal and chemical changes especially for comparative purposes between animals' species and sexual differences within same species. Combination of various forms of stress can also be applied.

In this way, reproductive models in future will be useful in studying general fertility trends in the presence of one or more stressful conditions and also the efficacy or otherwise of anti – stress and anti – oxidants like vitamin C can also be found.

REFERENCES

1. Hornby AS. *Oxford Advanced Learners Dictionary of current English*. 6th ed. Blackwell Science Ltd. 2000; Pp 1185.
2. Wilsnack SC, Klassen AD and Wilsnack RW. *Drinking and reproductive dysfunction among women*.1984; http://pubsnaaa.nihgov. Retrieved 27/3/15
3. Pablo AN, Kathy W, Dan M, Beverly IS and Barry GE. *Stress and female reproductive function: a study of daily variations in cortisol, gonadotrophins and gonadal steroids in a rural Mayan population.* Am. J. Hum. Biol. 2004; **16**: 523 – 532.
4. Faster DL, Nagatani S, Bucholtz DB, Tsukamura H, Tanaka T and Ferrin M. *Stress and the reproductive cycle*.1999; www.sciencedirect.compage.17681774.
5. Rechtschaffen A and Bermann BM. *Sleep deprivation in the rat*. 2002; www.sciencedirect.com.
6. Young B and Health JW. *Wheathers' Functional Histology, a text and atlas*. 4th ed. Churchill Livingstone, Harcourts; 2000.
7. Carlos L and Careiro J. *The female reproductive system. Basic Histology text and atlas*. 9th ed. Connecticut. Appleton and Lange. pp 421 – 432; 2003.
8. Reinsenfield A and Oliva H. *The effect of nicotine and alcohol on the fertility and lifespan of rats.* Acta Anat. 1987; **128**: 45 – 50.
9. Van Thiel DH. *Ethanol: its adverse effects upon the hypothalamic – pituitary – gonadal axis*. J. Lab Med. 1983; **101**: 21 – 33.