

# **The Effect of L-arginine Supplementation on Female Subfertility Caused by Chronic Intermittent Hypoxia**

**Thesis**

*Submitted for Partial Fulfillment of Master Degree  
in Physiology*

**By**

**Nermeen Ehab Selim**

*M.B.B.Ch.*

*Faculty of Medicine - Ain Shams University*

***Under Supervision of***

**Prof. Dr. Amira Metwally Abdel-Rhman**

*Professor of Physiology*

*Faculty of Medicine - Ain Shams University*

**Dr. Enas Abdel-Aziz Mohamed Abdel-Hady**

*Assistant Professor of Physiology*

*Faculty of Medicine - Ain Shams University*

**Dr. Wessam Ezzat Morsy**

*Lecturer of Physiology*

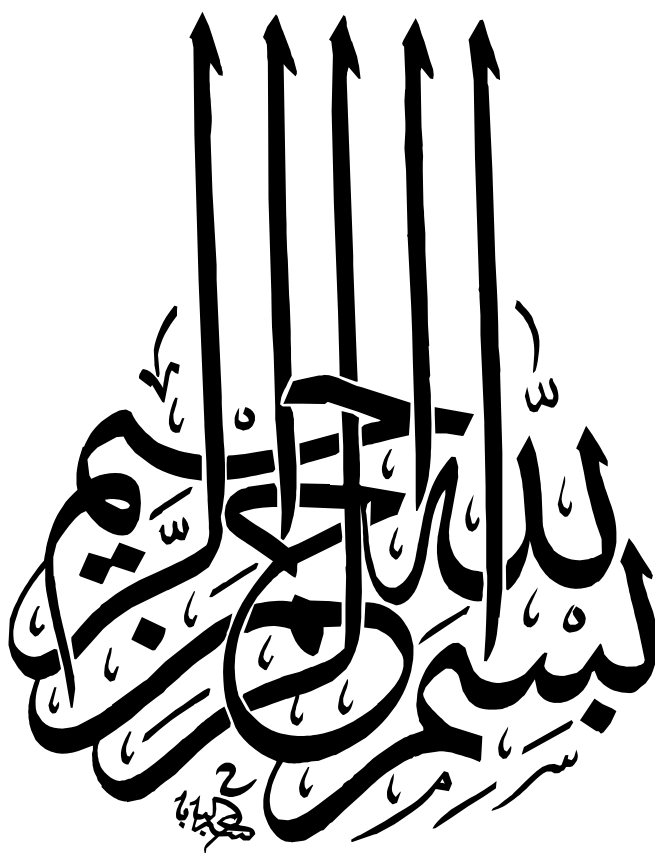
*Faculty of Medicine - Ain Shams University*

**Physiology Department**

**Faculty of Medicine**

**Ain Shams University**

**2015**





## Acknowledgment

*First of all, I thank **ALLAH** for blessing this work as a part of his generous help throughout my life.*

*I would like to acknowledge my deepest gratitude and appreciation to **Prof. Dr. Faten Mahmoud Diah**, the Head of Physiology Department, Faculty of Medicine, Ain Shams University, for her support and encouragement.*

*I would like to express my sincere gratitude and deepest thanks to **Prof. Dr. Amira Metwally Abdel-Rhman**, Professor of Physiology, Faculty of Medicine, Ain Shams University, for suggesting and planning this work, and for her meticulous supervision, scientific support and judicious guidance throughout this work.*

*I would like to display my indebtedness to **Dr. Enas Abdel-Aziz Mohamed Abdel-Hady**, Assistant Professor of Physiology, Faculty of Medicine, Ain Shams University, for her wise council, expert guidance, faithful advice, and keen supervision.*

*I would also like to thank **Dr. Wessam Ezzat Morsy**, Lecturer of Physiology, Faculty of Medicine, Ain Shams University for her support, faithful advice and meticulous supervision.*

*Last but not least, I would like to thank all members of the Physiology Department for their cooperation and support.*



# Contents

List of Tables .....	I
List of Figures .....	III
List of Photos .....	IV
List of Abbreviations .....	V
<b>Protocol .....</b>	
<b>Introduction .....</b>	<b>1</b>
<b>Aim of Work .....</b>	<b>4</b>
<b>Review of Literature .....</b>	
• Chronic Hypoxia .....	5
• Responses to Hypoxia .....	18
• Chronic Hypoxia and Fertility .....	37
<b>Materials and Methods .....</b>	<b>47</b>
<b>Results .....</b>	<b>71</b>
<b>Discussion .....</b>	<b>109</b>
<b>Summary and Conclusion .....</b>	<b>123</b>
<b>References .....</b>	<b>127</b>
<b>Arabic Summary .....</b>	<b>--</b>

## ***List of Tables***

<b>Table</b>	<b>Title</b>	<b>Page</b>
<b>1</b>	Haematocrit value (HV, %) in the control, hypoxic and hypoxic-supplemented groups.	78
<b>2</b>	Body weight (B.W/g) changes in the control group.	79
<b>3</b>	Body weight (B.W/g) changes in the hypoxic group.	80
<b>4</b>	Body weight (B.W/g) changes in the hypoxic-supplemented group.	81
<b>5</b>	Body weight (B.W/g) changes in the control, hypoxic and hypoxic-supplemented groups.	82
<b>6</b>	Ovarian weight (mg) and gonado-somatic index (ovarian weight/B.W, mg/g) in the control, hypoxic and hypoxic-supplemented groups.	83
<b>7</b>	Ovarian tissue levels of malondialdehyde (MDA, nmol/g) in the control, hypoxic and hypoxic-supplemented groups.	84
<b>8</b>	Ovarian tissue levels of catalase enzyme (CAT, U/g) in the control, hypoxic and hypoxic-supplemented groups.	85
<b>9</b>	Ovarian tissue levels of oxidant (MDA) and antioxidant (CAT enzyme) markers in the control, hypoxic and hypoxic-supplemented groups.	86

<b>Table</b>	<b>Title</b>	<b>Page</b>
<b>10</b>	Plasma levels of progesterone hormone (ng/ml) in the control, hypoxic and hypoxic-supplemented groups.	87
<b>11</b>	Plasma levels of luteinizing hormone (LH, mIU/ml) in the control, hypoxic and hypoxic-supplemented groups.	88
<b>12</b>	Plasma hormonal levels in the control, hypoxic and hypoxic-supplemented groups.	89
<b>13</b>	Incidence of mortality in the control, hypoxic and hypoxic-supplemented groups.	90
<b>14</b>	Incidence of rats with irregular cycle in the control, hypoxic and hypoxic-supplemented groups.	91

## ***List of Figures***

<b>Figure</b>	<b>Title</b>	<b>Page</b>
<b>1</b>	Haematocrit value in control, hypoxic and hypoxic-supplemented groups.	92
<b>2</b>	Body weight changes in control hypoxic and hypoxic-supplemented groups.	93
<b>3</b>	Initial and final Body weight in control, hypoxic and hypoxic-supplemented groups.	94
<b>4</b>	Ovarian weight in control, hypoxic and hypoxic-supplemented groups	95
<b>5</b>	Gonado-somatic index in control, hypoxic and hypoxic-supplemented groups.	96
<b>6</b>	Ovarian tissue levels of malondialdehyde in control, hypoxic and hypoxic-supplemented groups	97
<b>7</b>	Ovarian tissue levels of catalase enzyme in control, hypoxic and hypoxic-supplemented groups.	98
<b>8</b>	Plasma levels of progesterone hormone in both rats with regular and irregular cycle in control, hypoxic and hypoxic-supplemented groups.	99
<b>9</b>	Plasma levels of luteinizing hormone in both rats with regular and irregular cycle in control, hypoxic and hypoxic-supplemented groups.	100

## ***List of Photos***

<b>Photo</b>	<b>Title</b>	<b>Page</b>
<b>1</b>	Unstained vaginal smear of the different phases of estrous cycle.	51
<b>2</b>	Histological changes in ovarian tissue of the control group.	101
<b>3</b>	Histological changes in ovarian tissue of the hypoxic group.	102
<b>4</b>	Histological changes in ovarian tissue of the hypoxic-supplemented group.	103
<b>2,3 &amp;4</b>	Histological changes in ovarian tissue of the control, hypoxic and hypoxic-supplemented groups	104
<b>5</b>	Caspase-3 expression in ovarian tissue of the control group.	105
<b>6</b>	Caspase-3 expression in ovarian tissue of the hypoxic group.	106
<b>7</b>	Caspase-3 expression in ovarian tissue of the hypoxic-supplemented group.	107
<b>5,6 &amp;7</b>	Caspase-3 expression in ovarian tissue of the control, hypoxic and hypoxic-supplemented groups	108



## ***List of Abbreviations***

<b>Abb.</b>	<b>Description</b>
<b>BMR</b>	Basal metabolic rate
<b>CAT</b>	Catalase
<b>CIH</b>	Chronic Intermittent Hypoxia
<b>CyIH</b>	Cyclic intermittent hypoxia
<b>DNA</b>	Deoxy ribo nucleic acid
<b>ELISA</b>	Enzyme-linked immunosorbent assay
<b>EPO</b>	Erythropoietin
<b>ETC</b>	Electron transfer chain
<b>FGF</b>	Fibroblast growth factor
<b>FSH</b>	Follicular stimulating hormone
<b>GPX</b>	Glutathione peroxidase
<b>GR</b>	Glutathione reductase
<b>HIF</b>	Hypoxia induced factor
<b>HRE</b>	Hypoxia response elements
<b>HRP</b>	Horseradish peroxidase
<b>IGF 2</b>	Insulin-like growth factor 2
<b>IHT</b>	Intermittent Hypoxic Training
<b>iNOS</b>	isoform of nitric acid synthase
<b>LH</b>	Luteinizing hormone
<b>MDA</b>	Malondialdehyde

<b>Abb.</b>	<b>Description</b>
<b>mRNA</b>	Messenger ribo nucleic acid
<b>NADPH</b>	Nicotinamide adenine dinucleotide phosphate
<b>NO</b>	Nitric oxide
<b>OSA</b>	Obstructive sleep apnea
<b>PCO<sub>2</sub></b>	Pressure of carbon dioxide
<b>PDGF</b>	Platelet-derived growth factor
<b>PO<sub>2</sub></b>	Pressure of oxygen
<b>REM</b>	Rapid eye movement
<b>ROS</b>	Reactive oxygen species
<b>SOD</b>	Superoxide dismutase
<b>TGF- <math>\beta</math>3</b>	Transforming growth factor- $\beta$ 3
<b>VEGF</b>	Vascular endothelial growth factor



---

# Protocol

---





---

# Introduction

---





---

# Aim of Work

---





---

# Review of Literature

---





---

# Materials and Methods

---

