

The effect of maternal nicotine exposure on cell proliferation on the lungs of the offspring

By

Keitumetse Mothibeli

2769501



UNIVERSITY of the
WESTERN CAPE

Thesis presented in partial fulfillment of the requirements for the degree of Magister Scientiae in the Department of Medical Biosciences, Faculty of Natural Sciences, University of the Western Cape.

November 2013

Supervisor: Professor G.S. Maritz

KEYWORDS

Key words: tobacco; smoking; maternal; nicotine; cell proliferation; tomato juice; antioxidant; lung development; alveoli ; fetal programming.



ABSTRACT

Tobacco consumption and exposure to tobacco smoke is one of the biggest contributing factors to a growing epidemic of non-communicable diseases (NCDs), primarily cancers, diabetes, cardiovascular and chronic lung diseases which account for 63% of all deaths worldwide (WHO, 2011). An increased concern is in pregnant women who smoke. They not only expose themselves to nicotine, but also their unborn child. Cigarette smoking during pregnancy is associated with many developmental and growth complications. There are critical periods within the “program” that directs normal growth and development, during which the fetus is vulnerable to the effects of external factors. During these critical periods of development the program can be changed to increase the susceptibility of the fetal organs to disease and increased risk of adverse health consequences in adulthood. Health care professionals have tried to reduce the consumption of tobacco smoke by prescribing nicotine replacement therapy (NRT) to pregnant females as an alternative to smoking, without considering the effects of nicotine on the developing embryo and the health risk that might arise after birth. It is known that nicotine induces oxidant formation with resulting oxidative effects. This induces an overload of oxidants in the fetus and a decrease in the antioxidant capacity thereof. This may interfere with normal lung development.

Studies conducted in the past have shown that maternal nicotine exposure during **gestation and lactation** interfere with parenchyma development in the lungs of the offspring and also changes the “program” that controls the maintenance of lung structure in the long term. It was suggested that this could be due to the oxidant/antioxidant ratio imbalance created by nicotine exposure.

The present study addresses two questions. Firstly; does maternal nicotine exposure during **gestation**, affect lung development and function in the offspring postnatally? Secondly, will supplementing the diets of the rats with tomato juice, rich in antioxidants such as lycopene and vitamin C during gestation, prevent the adverse effects of maternal nicotine exposure on the developing lung of the offspring.

Wistar rats were used in the study. After mating, the rats were divided into 4 groups. One group received nicotine (1mg/kg body weight/day) only; a second group received tomato juice supplementation only, while the third group received both nicotine and tomato juice. The control rats were exposed to the same environmental conditions as the experimental groups. Morphological and morphometric techniques were used to evaluate the changes in the lung structure of the offspring at postnatal days 14, 21, 42 and 84.

The study showed that maternal nicotine exposure during gestation resulted in an accelerated increase in the body weight (BW) and lung volume (Lv) of nicotine exposed animals as they aged. The number of proliferating cells in the alveolar wall of these animals decreased with age, and maternal nicotine exposure during gestation resulted in a faster decrease in cell proliferation. A consequence of this is a faster deterioration of lung structure of the offspring later in life which is associated with a decrease in lung function. This change in the proliferating cells of the alveolar wall of nicotine exposed offspring was prevented by supplementing the nicotine exposed mothers diet with tomato juice. This means supplementing the diet of the mother with tomato juice prevented the adverse effects of the nicotine on the proliferating cells in the alveoli of the offspring.

DECLARATION

I declare that “*The effect of maternal nicotine exposure on cell proliferation on the lungs of the offspring*” is my own work, that it has not been submitted for any degree or examination in any other university and that all resources I have or quoted have been indicated and acknowledged by complete references.

Keitumetse Mothibeli



November 2013

Signed:

A handwritten signature in black ink, appearing to read "Keitumetse Mothibeli".

DEDICATION

This thesis is dedicated to my family and friends for their unceasing prayers and continuous encouragement. Thank you for your unwavering support. A very special thanks goes to my supervisor, Prof. G.S. Maritz, for mentoring me, always encouraging me, and most of all for believing in me. Most importantly I am grateful to God, El Shaddai for being my pillar of strength through all the challenges and lessons.



AKNOWLEDGEMENT

1. 'To God be the Glory'. I would like to give thanks to God for giving me the power, wisdom and most of all the strength to commit myself into completing this project.
2. I would also like to thank my supervisor, my mentor Professor G. S. Maritz for all the guidance, calm wisdom, motivation and willingness to mentor me through this period.
3. To my loving immediate family, Mr. A.M. Mothibeli (dad), Mrs. T. I. Mothibeli (mom), Mrs. G. Webb (grandmother), Mr. I.U. Mothibeli (brother) and Mrs. S. Mothibeli (sister in law). I would like to thank you for the love, steadfast support and encouragement. I love you too much.
4. To Mr. C. J. Mohlaba thank you for your love, support, encouragement, reassurance and unfailing words of wisdom.
5. To the President of Conquerors Covenant Church J.T. Ledwaba and to the First Lady L. Ledwaba thank you for your supporting prayers.
6. To all my friends, especially Ms. Z. Tomeli, Ms. N. Mngoma and Ms. S. Kamfer thanks for the love, support and understanding.
7. The National Research Foundation and Medical Research council for funding me throughout postgraduate studies.

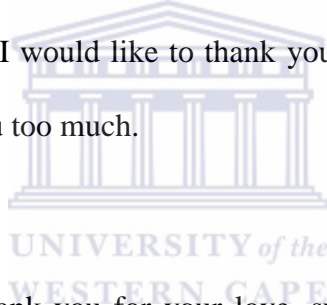


TABLE OF CONTENTS

KEYWORDS.....	I
ABSTRACT.....	II
DECLARATION.....	IV
DEDICATION.....	V
AKNOWLEDGEMENT.....	VI
TABLE OF CONTENTS	VII
LIST OF FIGURES	XI
LIST OF TABLES	XVII
LIST OF ABBREVIATION.....	XXI
CHAPTER 1	1
INTRODUCTION.....	1
CHAPTER 2	5
LITERATURE REVIEW	5
<i>2.1 Lung development.....</i>	<i>6</i>
2.1.1 Embryonic phase	7
2.1.2 Pseudoglandular phase	9
2.1.3 Canalicular phase	10
2.1.4 Saccular phase.....	11
2.1.5 Alveolar phase.....	13
<i>2.2 Cell Proliferation.....</i>	<i>14</i>
2.2.1 The role of PCNA	15
2.2.2 Factors affecting Lung Morphogenesis	16
2.2.3 Nicotine and Lung morphogenesis	19
<i>2.3 Sensitivity of cells to changes in the environment</i>	<i>19</i>
2.3.1 Ability of fetal cells to protect themselves	19
2.3.2 Fetal onset of adult disease.....	20
<i>2.4 The Lung and the natural anti-oxidant pool.....</i>	<i>21</i>
2.4.1 Lycopene	24
2.4.2 Lycopene and the lung	26

CHAPTER 3.....	27
NICOTINE	27
3.1 <i>Introduction</i>	27
3.2 <i>Nicotine Metabolism during Pregnancy</i>	28
3.3 <i>Nicotine and the lung</i>	29
3.3.1 Nicotine and ROS.....	30
3.3.2 Oxidative stress and the lungs.....	31
3.3.3 Role of glucose metabolism in lung development and maintenance.....	33
3.3.4 Effects of maternal nicotine exposure on lung structure: A summary	34
3.3.5 Effects of maternal nicotine exposure on lung function	36
3.4 <i>Motivation for the study</i>	37
3.5 <i>Aims and objectives</i>	37
 CHAPTER 4.....	 39
MATERIALS AND METHODS	39
4.1 <i>Animal preparation</i>	39
4.2 <i>Ethical clearance</i>	40
4.3 <i>Excision of lung tissue</i>	41
4.3.1 Intratracheal instillation	42
4.4 <i>Measurement of lung volume</i>	43
4.5 <i>Processing and embedding of the lung tissue samples</i>	44
4.6 <i>Microtomy</i>	46
4.7 <i>Microscope slide preparation</i>	46
4.8 <i>Staining techniques</i>	47
4.8.1 Mayer’s haematoxylin and eosin staining (H&E) preparation.....	47
4.8.2 Procedure: Proliferating cell nuclear antigen (PCNA) stain used to measure cell proliferation.....	49
4.9 <i>Morphology and Morphometric methods</i>	52
4.9.1 Volume density of the airspaces (Va) and the volume density of the tissue of the lung parenchyma (Vt).....	53
4.9.2 Determination of mean linear intercept (Lm)	55
4.9.3 Cell proliferation.....	57
4.10 <i>Lung compliance</i>	59
4.11 <i>Average amount of fluids consumed a week</i>	59
4.11.1 Lycopene intake is calculated as follows:	60

4.11.2 Energy intake was calculated as follows:	61
4.12 Statistical analysis	61

CHAPTER 5.....62

RESULTS.....	62
5. A weekly average amount of liquids consumed by pregnant female rats during gestation only.	62
5.1. The influence of maternal nicotine exposure during gestation, or receiving tomato juice supplementation only, or nicotine + tomato juice supplementation on growth parameters of the offspring.	64
5.1.1 Body Weight (BW)	64
5.1.2 Lung volume (Lv)	67
5.1.3 Lung volume to body weight ratio (Lv/BW).....	69
5.2 The effect of maternal exposure to nicotine during gestation and supplementing the diet with tomato juice on chest circumference (CC) and crown-rump length (CRL) of the offspring.....	71
5.3 The effect of maternal exposure to nicotine during gestation and supplementing the diet with tomato juice on the chest circumference to lung volume (CC/Lv), crown-rump length to chest circumference (CRL/CC) and chest circumference to body weight (CC/BW) ratios of the offspring.....	75
5.3.1 Chest circumference to lung volume (CC/Lv)	75
5.3.2 Crown rump length to chest circumference ratio (CRL/CC)	76
5.3.3 Chest circumference to body weight ratio (CC/BW)	76
5.4 The effect of maternal exposure to nicotine during gestation on the volume densities of the airspaces (Va) and parenchymal tissue (Vt) of the offspring.	81
5.5 The effect of maternal nicotine exposure during gestation, and supplementation of the diet with tomato juice on lung morphology and the alveolar linear intercepts (Lm) of the lungs of the offspring.	85
5.5.1 Lung morphology.....	85
5.5.2 Mean linear intercept (Lm).....	85

5.6 <i>The effect of maternal nicotine exposure during gestation, and supplementation of the diet with tomato juice on the number of proliferating cells in the alveolar walls of the lungs of the offspring.</i>	88
5.6.1 Effects on aging.	88
5.6.2 The effects of maternal exposure to nicotine during gestation, and supplementing the diet with tomato juice on cell proliferation in the alveolar walls.	90
5.7 <i>The effect of maternal nicotine exposure during gestation, and supplementation of the diet of the mother during gestation with tomato juice on static lung compliance (Cst) of the offspring.</i>	92
CHAPTER 6	95
DISCUSSION.....	95
6.1 <i>Introduction</i>	95
6.2 <i>Body Weight (BW)</i>	97
6.2.1 Male and Female differences:	98
6.2.2 Effect of energy intake	99
6.2.3 Role of hypothalamus	99
6.2.4 Effects of nicotine.....	100
6.3 <i>The effects of Maternal Nicotine exposure and Tomato Juice supplementation during gestation on the CC and CRL of the offspring.</i>	102
6.4 <i>The effect of Maternal Nicotine Exposure on Lung development in the offspring.</i>	103
6.4.1 Lung volume (Lv)	103
6.4.2 Mean linear intercept (Lm), volume densities of the airspaces (Va) and parenchymal tissue (Vt).	104
6.5 <i>The effect of Maternal Nicotine Exposure during gestation on cell proliferation in the lungs of the offspring.</i>	107
6.5.1 Cell proliferation during normal lung development.....	107
6.5.2 The effect of maternal exposure to nicotine on cell proliferation in developing lung	110
6.6 <i>Lung Function.</i>	112
REFERENCES	115

LIST OF FIGURES

FIGURE 2.1. STAGES OF LUNG DEVELOPMENT IN HUMANS: DIAGRAMMATIC REPRESENTATIONS OF THE TIMELINE AND DEVELOPMENTAL ORGANIZATION OF TRACHEA, PRIMARY BRONCHI, INTRAPULMONARY BRONCHI, AND ACINUS IN THE MAMMALIAN RESPIRATORY SYSTEM. REPRINTED FROM (KAJEKAR, 2007).....7

FIGURE 2.2. LATE PSEUDOGLANDULAR STAGE OF THE HUMAN AND MOUSE LUNG (RUTTER, 2008).10

FIGURE 2.3. LUNG TISSUE IN THE PSEUDOGLANDULAR PHASE: LUNG HAS GLANDULAR APPEARANCE; AIRWAYS ARE LINED WITH COLUMNAR EPITHELIUM AND SEPARATED BY POORLY DIFFERENTIATED MESENCHYME. 1) LUNG MESENCHYMA; 2) TYPE II PNEUMOCYTES; 3) CAPILLARIES (VOIGT ET AL. 1999).....10

FIGURE 2.4. LUNG TISSUE IN THE CANALICULAR PHASE: CHARACTERIZED BY PROLIFERATION OF THE MESENCHYME, DEVELOPMENT OF RICH BLOOD SUPPLY, RAPID FORMATION OF CAPILLARIES. 1) TYPE I PNEUMOCYTES; 2) TYPE II PNEUMOCYTES; 3) CAPILLARIES (VOIGT ET AL. 1999).....11

FIGURE 2.5. LUNG TISSUE IN THE SACCULAR PHASE. 1) TYPE I PNEUMOCYTES; 2) SACCULAR SPACE; 3) TYPE II PNEUMOCYTE; 4) BASAL MEMBRANE OF THE AIR PASSAGE; 5) BASAL MEMBRANE OF THE CAPILLARIES; 6) ENDOTHELIUM OF THE CAPILLARIES (VOIGT ET AL. 1999)..12

FIGURE 2.6. THE EFFECTS OF FETAL BREATHING ON LUNG GROWTH AND LUNG DEVELOPMENT (COPLAND AND POST, 2004).....13

FIGURE 2.7. INTERACTION OF FGF, SHH AND SPRY 2 DURING LUNG BUD OUTGROWTH AND LUNG BUD ARREST. (WARBURTON ET AL. 2003).....17

FIGURE 2.8. REGULATORY FACTORS OF LUNG DEVELOPMENT (COPLAND AND POST, 2004).....18

FIGURE 4.1. TREATMENT SCHEDULE FOLLOWED -DAY 1-7: MATING; DAYS 7-21: NICOTINE OR TOMATO JUICE OR BOTH N+T, POSTNATAL DAY 1-21: NO NICOTINE OR TOMATO JUICE.....40

FIGURE 4.2. ILLUSTRATION OF CROWN-RUMP LENGTH.41

FIGURE 4.3. CROSS-SECTION OF A RAT.....42

FIGURE 4.4. PREPARATION OF MOUSE FOR COLLECTION OF LUNG TISSUE SAMPLES. (A) A MIDLINE INCISION IS MADE TO EXPOSE THE DIAPHRAGM AND TRACHEOSTOMIZE THE ANIMAL. (B) THE DIAPHRAGM IS THEN PUNCTURED, AND LUNGS FILLED WITH A FIXATIVE THROUGH THE TRACHEA. THE CHEST PLATE IS THEN REMOVED BY CUTTING ALONG THE DEFLECTION POINT OF THE RIBS ON BOTH SIDES OF THE STERNUM. THE LUNGS ARE THEN REMOVED BY CAREFUL DISSECTION AND CUTTING OF THE TRACHEA DORSAL TO THE SECURE LIGATURE.43



FIGURE 4.5. PROCESS OF STAINING FOR PCNA50

FIGURE 4.6. PCNA STAIN ILLUSTRATION OF THE NUMBER OF PROLIFERATING CELLS PER100 μ M LENGTH OF ALVEOLAR WALL.ARROWS SHOW PROLIFERATING CELLS STAINED DARK BROWN (SEE ARROWS).....51

FIGURE 5 (i). THE AVERAGE VOLUME OF FLUID BY PREGNANT FEMALE RATS FROM WEEK 1 TO WEEK 3 OF PREGNANCY (ML/KG/ WEEK).....63

FIGURE 5.1(A).THE EFFECT OF NICOTINE, OR NICOTINE + TOMATO JUICE, OR TOMATO JUICE ONLY ON BODY WEIGHT OF 42-DAY OLD FEMALE AND MALE RATS66

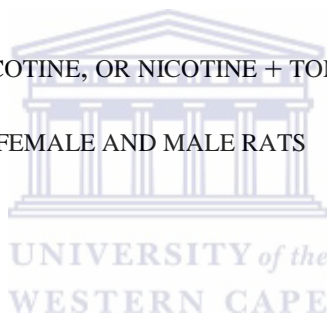


FIGURE 5.1(B). THE EFFECT OF NICOTINE, OR NICOTINE +TOMATO JUICE OR TOMATO JUICE ONLY ON BODY WEIGHT OF 84 DAY-OLD FEMALE AND MALE RATS.66

FIGURE 5.2(A). THE EFFECT OF NICOTINE, OR NICOTINE + TOMATO JUICE, OR TOMATO JUICE ONLY ON LUNG VOLUME OF 42-DAY OLD FEMALE AND MALE RATS.68

FIGURE 5.2(B). THE EFFECT OF NICOTINE, OR NICOTINE +TOMATO JUICE OR TOMATO JUICE ONLY ON LUNG VOLUME OF 84 DAY-OLD FEMALE AND MALE RATS.68

FIGURE 5.3(A). THE EFFECT OF NICOTINE, OR NICOTINE + TOMATO JUICE OR TOMATO JUICE ONLY ON THE LV/BW OF 42-DAY OLD FEMALE AND MALE RATS. 70

FIGURE 5.3(B). THE EFFECT OF NICOTINE, OR NICOTINE + TOMATO JUICE OR TOMATO JUICE ONLY ON THE LV/BW OF 84-DAY OLD FEMALE AND MALE RATS.70

FIGURE 5.4(A). THE EFFECT OF NICOTINE, OR NICOTINE + TOMATO JUICE OR TOMATO JUICE ONLY ON THE CC OF 42-DAY OLD FEMALE AND MALE RATS73

FIGURE 5.4(B). THE EFFECT OF NICOTINE, OR NICOTINE + TOMATO JUICE OR TOMATO JUICE ONLY ON THE CC OF 84-DAY OLD FEMALE AND MALE RATS73

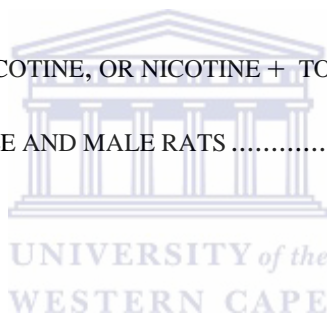


FIGURE 5.5(A). THE EFFECT OF NICOTINE, OR NICOTINE + TOMATO JUICE OR TOMATO JUICE ONLY ON THE CRL OF 42-DAY OLD FEMALE AND MALE RATS.....74

FIGURE 5.5(B). THE EFFECT OF NICOTINE, OR NICOTINE + TOMATO JUICE, OR TOMATO JUICE ONLY ON THE CRL OF 84-DAY OLD FEMALE AND MALE RATS74

FIGURE 5.6(A). THE EFFECT OF NICOTINE, OR NICOTINE + TOMATO JUICE OR TOMATO JUICE ONLY ON THE CC/LV OF 42-DAY OLD FEMALE AND MALE RATS.78

FIGURE 5.6(B). THE EFFECT OF NICOTINE, OR NICOTINE + TOMATO JUICE, OR TOMATO JUICE ONLY ON THE CC/LV OF 84-DAY OLD FEMALE AND MALE RATS.78

FIGURE 5.7(A). THE EFFECT OF NICOTINE, OR NICOTINE + TOMATO JUICE OR TOMATO JUICE ONLY ON THE CRL/CC OF 42-DAY OLD FEMALE AND MALE RATS79

FIGURE 5.7(B). THE EFFECT OF NICOTINE, OR NICOTINE + TOMATO JUICE, OR TOMATO JUICE ONLY ON THE CRL/CC OF 84-DAY OLD FEMALE AND MALE RATS.79

FIGURE 5.8(A). THE EFFECT OF NICOTINE, OR NICOTINE + TOMATO JUICE OR TOMATO JUICE ONLY ON THE CC/BW OF 42-DAY OLD FEMALE AND MALE RATS80

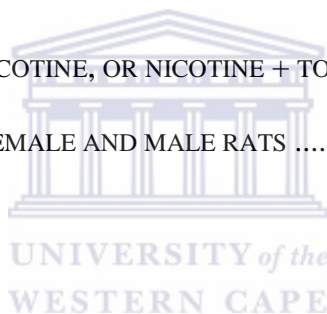


FIGURE 5.8(B). THE EFFECT OF NICOTINE, OR NICOTINE + TOMATO JUICE, OR TOMATO JUICE ONLY ON THE CC/BW OF 84-DAY OLD FEMALE AND MALE RATS.80

FIGURE 5.9. THE EFFECTS OF MATERNAL EXPOSURE TO NICOTINE DURING GESTATION, AND SUPPLEMENTING THE DIET WITH TOMATO JUICE ON AIR SPACE VOLUME DENSITY (V_A) (%) OF THE LUNGS OF THE OFFSPRING.83

FIGURE 5.10. THE EFFECTS OF MATERNAL EXPOSURE TO NICOTINE DURING GESTATION, AND SUPPLEMENTING THE DIET WITH TOMATO JUICE ON PARENCHYMAL TISSUE VOLUME DENSITY (V_T) (%) OF THE LUNGS OF THE OFFSPRING.....84

FIGURE 5.11. THE EFFECT OF MATERNAL NICOTINE EXPOSURE DURING GESTATION AS WELL AS THE EFFECT OF SUPPLEMENTING THE MOTHER’S DIET WITH TOMATO JUICE ON THE STRUCTURE OF THE LUNGS OF THE 84-DAY-OLD OFFSPRING. A) CONTROL, B) TOMATO JUICE ONLY, C) NICOTINE ONLY. D) TOMATO JUICE + NICOTINE. ARROWS INDICATE ENLARGED ALVEOLI.(H&E, 10x).....86

FIGURE 5.12. THE EFFECTS OF MATERNAL EXPOSURE TO NICOTINE DURING GESTATION, AND SUPPLEMENTING THE DIET WITH TOMATO JUICE ON ALVEOLAR LINEAR INTERCEPT (LM) OF THE LUNGS OF THE OFFSPRING.87

FIGURE 5.13. THE EFFECTS OF AGING ON THE NUMBER OF PROLIFERATING CELLS/100 μ M ALVEOLAR WALL.....89

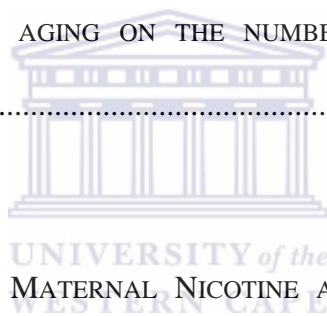


FIGURE 5.14. THE EFFECTS OF MATERNAL NICOTINE AND TOMATO JUICE EXPOSURE ON PROLIFERATING CELLS/100 μ M ALVEOLAR WALL.91

FIGURE 5.15(A). THE EFFECTS OF MATERNAL NICOTINE AND TOMATO JUICE EXPOSURE ON FEMALE LUNG COMPLIANCE.....93

FIGURE 5.15(B). THE EFFECTS OF MATERNAL NICOTINE AND TOMATO JUICE EXPOSURE ON MALE LUNG COMPLIANCE.94

LIST OF TABLES

TABLE 2.1. PHASES OF LUNG DEVELOPMENT BETWEEN HUMAN AND MOUSE (RUTTER, 2008; SCHITTNY & BURRI, 2007).	8
TABLE 2.2. LYCOPENE IN FRUITS AND VEGETABLES (POHAR ET AL. 2003).....	24
TABLE 2.3. CONCENTRATIONS OF LYCOPENE IN HUMAN TISSUE (AGARWAL & RAO 2000, STAHL & SIES, 1992; KAPLAN ET AL. 1990; SCHMITZ ET AL. 1991; NIERENBERG AND NANN, 1992) AND RAT TISSUE (JAIN ET AL. 1999).NOTE: NA=NOT AVAILABLE, ND=NOT DETECTABLE SD=STANDARD DEVIATION.....	25
TABLE 4.1. FORMULA FOR 10% BUFFERED FORMALDEHYDE SOLUTION.	44
TABLE 4.2. HISTOLOGICAL TISSUE PROCESSING OF RAT LUNG TISSUE.	45
TABLE 4.3. PROCEDURE OF HAEMATOXYLIN AND EOSIN STAIN.	48
TABLE 4.4. PROCEDURE FOR PCNA STAIN AS INDICATED BY THE KIT.	52
TABLE 5(i). THE VOLUME OF LIQUIDS CONSUMED BY PREGNANT FEMALE RATS DURING GESTATIONAL PHASE, WHERE THE CONTROL AND NICOTINE GROUPS CONSUMED WATER ONLY. ...	63

TABLE 5(II). A WEEKLY AVERAGE INTAKE OF LYCOPENE IN A FORM OF TOMATO JUICE BY PREGNANT FEMALE RATS BETWEEN WEEK 1 AND WEEK 3.63

TABLE 5(III). A WEEKLY AVERAGE ENERGY INTAKE IN A FORM OF TOMATO JUICE BY PREGNANT FEMALE RATS BETWEEN WEEK 1 AND WEEK 3.63

TABLE 5.1. THE EFFECTS OF MATERNAL NICOTINE EXPOSURE, OR TOMATO JUICE ONLY, OR BOTH NICOTINE AND TOMATO JUICE ON THE BODY WEIGHT (BW) OF THE MALE AND FEMALE OFFSPRING.66

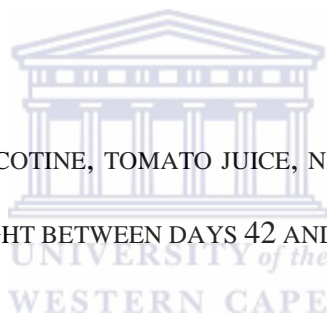


TABLE 5.1.1. THE EFFECTS OF NICOTINE, TOMATO JUICE, NICOTINE + TOMATO JUICE EXPOSURE ON DAILY INCREASE IN BODY WEIGHT BETWEEN DAYS 42 AND 84.66

TABLE 5.2. THE EFFECTS OF MATERNAL NICOTINE EXPOSURE OR TOMATO JUICE SUPPLEMENTATION, OR BOTH NICOTINE EXPOSURE AND TOMATO JUICE SUPPLEMENTATION ON THE LUNG VOLUME (LV) OF THE MALE AND FEMALE OFFSPRING.68

TABLE 5.2.1. THE EFFECTS OF NICOTINE, TOMATO JUICE, NICOTINE + TOMATO JUICE EXPOSURE ON DAILY INCREASE IN LUNG VOLUME BETWEEN DAYS 42 AND 84.68

TABLE 5.3. THE EFFECTS OF MATERNAL NICOTINE EXPOSURE, OR TOMATO JUICE ONLY, OR BOTH NICOTINE AND TOMATO JUICE ON THE ON LUNG VOLUME TO BODY WEIGHT RATIO (LV/BW) OF THE MALE AND FEMALE OFFSPRING.....70

TABLE 5.4. THE EFFECT OF MATERNAL NICOTINE EXPOSURE DURING PREGNANCY, OR RECEIVING TOMATO JUICE SUPPLEMENTATION ONLY, OR NICOTINE + TOMATO JUICE ON THE CHEST CIRCUMFERENCE (CC) OF THE OFFSPRING.....73

TABLE 5.5. THE EFFECT OF MATERNAL NICOTINE EXPOSURE DURING PREGNANCY, OR RECEIVING TOMATO JUICE SUPPLEMENTATION ONLY, OR NICOTINE + TOMATO JUICE ON THE CROWN-RUMP DISTANCE (CRL) OF THE OFFSPRING.....74

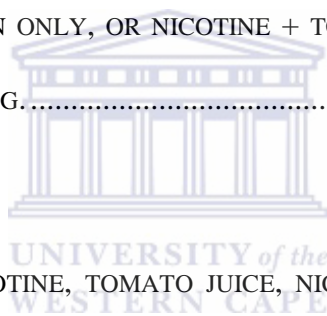


TABLE 5.6. THE EFFECT OF NICOTINE, TOMATO JUICE, NICOTINE + TOMATO JUICE EXPOSURE DURING DIFFERENT LUNG DEVELOPMENT PHASES ON NEONATAL RATIO OF CC/LV (MM/ML).78

TABLE 5.7. THE EFFECT OF NICOTINE, TOMATO JUICE, NICOTINE + TOMATO JUICE EXPOSURE DURING DIFFERENT LUNG DEVELOPMENT PHASES ON NEONATAL RATIO OF CRL/CC (MM/MM). .79

TABLE 5.8. THE EFFECT OF NICOTINE, TOMATO JUICE, NICOTINE + TOMATO JUICE EXPOSURE DURING DIFFERENT LUNG DEVELOPMENT PHASES ON NEONATAL RATIO OF CC/BW (MM/G).80

TABLE 5.9. THE EFFECT OF MATERNAL NICOTINE EXPOSURE DURING PREGNANCY, OR RECEIVING TOMATO JUICE SUPPLEMENTATION ONLY, OR NICOTINE + TOMATO JUICE ON THE VOLUME DENSITY (V_A) % OF THE OFFSPRING.....83

TABLE 5.10. THE EFFECT OF MATERNAL NICOTINE EXPOSURE DURING PREGNANCY, OR RECEIVING TOMATO JUICE SUPPLEMENTATION ONLY, OR NICOTINE + TOMATO JUICE ON THE VOLUME DENSITY (V_T) % OF THE OFFSPRING.84

TABLE 5.11. THE EFFECTS OF MATERNAL NICOTINE EXPOSURE DURING GESTATION AND TOMATO JUICE SUPPLEMENTATION DURING PREGNANCY ON ALVEOLAR LINEAR INTERCEPT (L_M) OF THE OFFSPRING.87

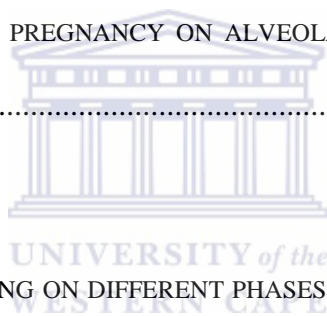


TABLE 5.12. THE EFFECTS OF AGING ON DIFFERENT PHASES OF MATERNAL LUNG DEVELOPMENT ON THE NUMBER OF PROLIFERATING CELLS/ $100\mu\text{M}$89

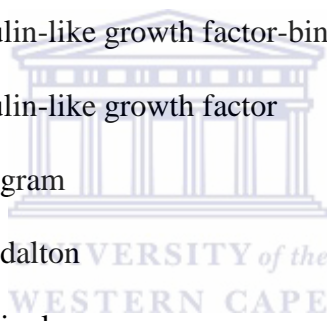
TABLE 5.13. THE EFFECTS OF MATERNAL NICOTINE EXPOSURE DURING GESTATION AND TOMATO JUICE SUPPLEMENTATION DURING PREGNANCY ON PROLIFERATING CELLS/ $100\mu\text{M}$ OF THE ALVEOLAR WALL OF THE OFFSPRING.....91

TABLE 5.14. THE EFFECTS OF MATERNAL NICOTINE EXPOSURE DURING GESTATION AND TOMATO JUICE SUPPLEMENTATION DURING PREGNANCY ON LUNG COMPLIANCE OF THE OFFSPRING.....94

LIST OF ABBREVIATION

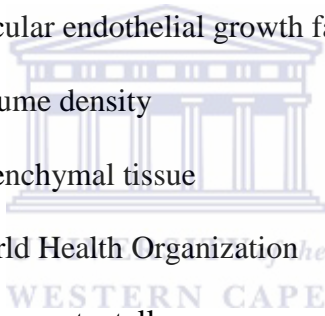
AC	abdominal circumference
AMP	adenosine monophosphate
ATP	adenosine triphosphate
α	alpha
β	beta
bFGF	basic fibroblast growth factor
BMP	bone morphogenetic protein
BPD	biparietal diameter
BW	body weight
CC	chest circumference
CC/Lv	chest circumference/ lung volume
C	control
COPD	chronic obstructive pulmonary disease
CRL	crown rump length
CRL/CC	crown rump length to chest circumference
Cst	static lung compliance
$^{\circ}\text{C}$	degrees Celsius
DNA	deoxyribonucleic acid
Dnp	days postnatally
ECM	extra cellular matrix molecules
EGF	epidermal growth factor
FGF	fibroblast growth factor
FGFR	fibroblast growth factor receptor
Fig.	figure

Foxf1	forkhead box protein F1
Foxa2	forkhead box protein A
GSH	glutathione
g	gram
HC	head circumference
H ₂ O ₂	hydrogen peroxide
H&E	haematoxylin and eosin
HNF	hepatocyt nuclear factor
HIER	heat induced epitope retrieval
hrs	hours
IGFBP	insulin-like growth factor-binding protein
IGF	insulin-like growth factor
kg	kilogram
kDa	kilodalton
kJ	kilojoules
Lm	alveolar linear intercepts
Lv	lung volume
Lv/BW	lung volume/body weight
mRNA	messenger ribonucleic acid
M	meters
μL	microliter
μm	micrometer
MTC	mid-thigh circumference
mg	milligram
ml	milliliter



mm	millimeter
min	minutes
N	number of fields counted
N	nicotine
N+T	nicotine + tomato juice
NA	not available
ND	not detectable
nAChR	nicotinic acetylcholine receptors
ng	nanogram
ng/ml	nanogram/millilitres
nmol/l	nanomole/litre
NBF	neutral buffered formalin
NCD	Non Communicable Diseases
No.	Number
NO	nitric oxide
NRT	Nicotine Replacement Therapy
O ₂ ⁻	oxygen free radicle
OH ⁻	hydroxide
ONOO ⁻	peroxynitrite
PBS	phosphate buffered saline
PCNA	proliferating cell nuclear antigen
PDGF	platelet derived growth factor
PBS	phosphate buffered saline
RTLFL	respiratory tract lining fluid
ROS	reactive oxygen species

RNA	Ribonucleic acid
SD	standard deviation
Shh	Sonic hedgehog
SOD	superoxide dismutase
Spry2	Sprouty homolog
T+N	tomato juice and nicotine
TGF α	transforming growth factor alpha
TGF β	transforming growth factor beta
T	tomato
UL	upper tolerable intake level
VEGF	vascular endothelial growth factor
V _a	Volume density
V _t	Parenchymal tissue
WHO	World Health Organization
Y _{pn}	Years postnatally



CHAPTER 1

Introduction

The Fetal origin of adult disease theory was first introduced by Barker (1995). It is a theory used to understand that non-communicable diseases originate through nutritional deprivation of the fetus during “critical periods” of development that forces the fetus to adopt permanent adaptational changes in the physiology, structure and function of organs. It proposes that alterations in the environment within which the fetus develops, such as fetal nutrition and foreign substances, may change the structure, physiology and metabolism of the individual in such a manner that it predisposes the offspring to cardiovascular, metabolic and endocrine disease in adult life (Godfrey & Barker, 2000). Changes in the environment within which the fetus and neonate develops may therefore, have a profound effect on the future health of the individual.

Tobacco smoke contains more than 4000 different chemicals. Approximately 60 of these are tumor initiating or carcinogenic. Approximately 6 million people die from tobacco use and exposure to tobacco smoke worldwide (WHO, 2011). The World Health Organization (WHO) forecasts that 8 million people a year will die of smoking-related illness by the year 2030, making it the single biggest cause of death worldwide, with the largest increase to be amongst the women. The problem of tobacco smoking arises more with pregnant women, as it causes additional health problems. Pregnant women do not only expose themselves to the effects of tobacco smoke but also expose the unborn baby to the serious effects of tobacco smoke. Tobacco use during pregnancy is associated with a wide range of complications such as pre-term labour, reduced placental blood flow and complications to the fetus (Salihu et al.

2003; Wisborg et al. 2001). It has also been linked to increased incidences of abortions and sudden infant death syndrome (Di Franza & Lew, 1995). Studies have shown an increase in the use of NRT as it is believed to be a safer smoking cessation aid than the direct action of tobacco smoking. Yet, NRT depends on nicotine that is found in tobacco smoke and can have toxic effects on various organ systems in the fetus (Bruin et al. 2010; Ginzler et al. 2007). Nicotine is a habit forming substance that is able to cross the placenta from the maternal circulation (Matta et al. 2007) to the fetal circulation. It binds to nicotinic acetylcholine receptors (nAChR) on target cells in the airway, blood vessels, and alveolar cell wall in the fetal lung (Sekhon et al. 1999).

Nicotine found in tobacco is one of the compounds that cause point mutations in the DNA molecule and, therefore, changing the program that controls development and growth of the cells leading to disease in adulthood (Hecht, 1999). Nicotine can damage the fetal lung, heart and central nervous system (Argentin & Cicchetti, 2004). It also passes to the baby through breast milk (Dahlstrom et al. 1990). Consequently, nicotine replacement therapy NRT is highly recommended by many health professionals as safe to assist with the quitting of smoking (Zwar et al. 2006), the effects that may come with nicotine exposure via NRT on the unborn baby are often overlooked.

The effects of nicotine on the offspring will be addressed, because nicotine induces changes in the *in utero* and external environment of the fetus and neonate respectively. During early phases of lung development, the fetal lung experiences rapid cell proliferation (Burri, 1997; Kaplan, 2000). During these phases of rapid cell proliferation, the cells are susceptible to changes in the environment (Barker, 2004). It is therefore important that the mother's diet, contain all the nutrients required for normal growth and development of the neonate.

Secondly, the exposure to foreign substances must be limited. Intake of high levels of oxidants, for example, may result in an imbalance in the oxidant/antioxidant environment into which the fetus develops and in this way interfere with normal development (Fardy & Silverman, 1995). Smoking contributes to the changes in the *in utero* environment (Hanrahan et al. 1992) within which the fetus develops. These changes may result in irreversible changes in the organ structure, function and metabolism during the critical time window that determines normal growth and development of the organs (Barker, 2001; Curhan et al.1996). Studies by Maritz & Thomas (1994) and Maritz & Windvogel (2003) showed that the lungs of rats that were exposed to nicotine via the placenta and the mother's milk were unable to maintain the structural and functional integrity of the lungs in the long term. Maternal nicotine exposure induces oxidative stress (Halima et al. 2010; Helen et al. 2000), and lowers the antioxidant capacity of the lungs of the offspring (Maritz & Wyk, 1997; Windvogel et al. 2008). Studies by Sekhon et al. 1999 have also demonstrated that maternal nicotine predominantly result in the decrease in the volume density of the airspaces of the alveolar region of the lungs of the offspring and in this way increased alveolar size.() This is followed by a decrease in the internal surface area available for gas exchange (Maritz & Dennis, 1998; Maritz & Windvogel, 2003; Sekhon et al. 2001). It has been suggested that these adverse effects of nicotine are due to its genotoxic effects (Argentin & Cicchetti, 2004), its oxidant properties (Hussain et al. 2001), and its capacity to induce oxidant formation in tissue (Sener et al. 2005; Suzuki & Ohshima, 2002). It is therefore conceivable that supplementing the mother's diet with antioxidant rich tomato juice will prevent the adverse effects of maternal nicotine exposure during gestation and therefore maintain of the lung parenchyma of the offspring in the long term.

This study serves to raise awareness on the effects of maternal smoking and NRT during pregnancy on the developing lung. The aim of this study is to determine whether supplementation of the diet of pregnant rats with lycopene rich tomato juice will protect the lungs of the offspring during gestation against the adverse effects of maternal nicotine exposure on the structure of the lungs of the offspring in the long term.



CHAPTER 2

Literature Review

The pulmonary system ensures optimal gas exchange between the atmospheric air and the circulating blood. Therefore, the lung tissue must undergo a series of complex processes of cell proliferation, and branching morphogenesis steps working interchangeably in the developing lung to ensure the development of a functional lung (Stenmark & Abman, 2005).

The development of the airways and vasculature is carefully and precisely controlled through molecular and physical factors. Some of the molecular factors include transcriptional regulators, growth factors, morphogens, and extra cellular matrix molecules (ECM), all of which must be carefully controlled at the right time and place to produce a properly mature and functional lung. The lung is structurally complex, and its growth and development depends on the integration of a multitude of signaling pathways that guide its development during embryogenesis. The master regulator of one of these signaling pathways is Sonic hedgehog (Shh) morphogen. The Shh signaling plays a crucial role in lung organogenesis by guiding the activity of downstream Gli transcription factor. Gli2 and Gli3 are thought to be the primary transcriptional mediators of Shh signaling. Rutter (2008) showed that Gli2 is the primary mediator of Shh signaling influencing embryonic lung growth and proliferation through cyclic regulation.

Much of what is known about lung development has arisen from studies of rodents. The major difference between the mouse and human lung arises from the asymmetric layout to accommodate the heart, with three lobes on the right and two lobes derived from the left primary bronchus, whereas mice have five secondary bronchi, they have only one lobe off the left primary bronchus and four derived from the right (Fig. 2.2). Rats and mice are born with

saccular lungs and they have to function at a more primitive stage than the human lung (Rutter, 2008; Schittny & Burri, 2007). Lung development of humans is almost complete at birth with just part of alveolarization and microvascular maturation occurring up to 2 years postnatally. Other scientist may argue that alveolarization continues well after birth, possibly being completed by +-3 years of age. The total number of alveoli in the fully develop human lung ranges from 300-600 million (Thurlbeck, 1982; Hislop, 2002; Hyde et al. 2004; Ochs et al. 2004).

2.1 Lung development

To understand the influence of various factors on the development of the lung into a mature organ capable of supplying the body with sufficient quantities of oxygen and to remove carbon dioxide from the blood, it is important to discuss the normal development of the lung. The intrauterine stages of lung development can be divided into five phases, namely, embryonic, pseudoglandular, canalicular, saccular and alveolar phase (Pringle, 1986) (Fig. 2.1, table 2.1). The first phase is the specification of the lung primordium, followed by morphogenesis and cellular differentiation along the trachea. This phase is followed by branching morphogenesis and development of the lung parenchyma and finally, alveologensis and differentiation of distal epithelial cell types into alveolar type I and type II cells (Minoo, 2000).

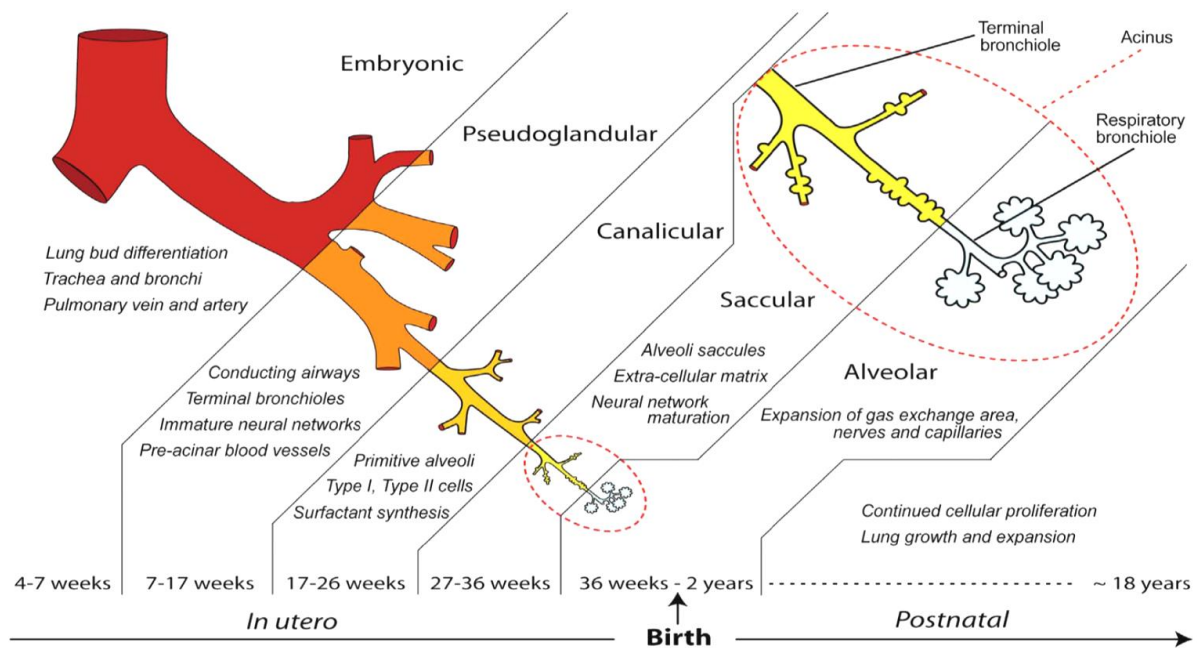
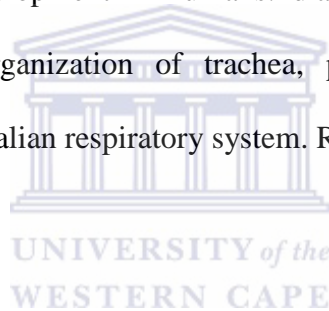


Figure 2.1. Stages of lung development in humans: diagrammatic representations of the timeline and developmental organization of trachea, primary bronchi, intrapulmonary bronchi, and acinus in the mammalian respiratory system. Reprinted from (Kajekar, 2007).



2.1.1 Embryonic phase

The human lung originates as a ventral endodermal pouch from the primitive foregut during the 4th or 5th week of embryonic life, and ends between the 7th (Rutter, 2008) or 8th week of gestation (Boyden, 1977). Lung development begins with the formation of the sulcus laryngotrachealis, which is the formation of a groove in the ventral lower pharynx. A bud forms at different times of development between humans, rats and mice (table 2.1). At this stage it is referred to as a true lung primordium (Kitaoka et al. 1996). After the respiratory primordial pouch is formed, it produces the two bronchial rudiments. The endodermal bud elongates, growing caudally and establishing the asymmetry of the main bronchi, where it will divide into the primary left and right lung buds which develop into the airways of a mature lung.

<i>Stage</i>	<i>Gestational age</i>			<i>Main events</i>
	<i>Human (weeks)</i>	<i>Rats (days)</i>	<i>Mouse(days)</i>	
Embryonic	3.5 -7	11-13	9.5-14.2	Start of Organogenesis. Formation of lung Bud, trachea, left and right bronchus.
Pseudoglandular	5-17	13-18.5	14.2 -16.6	Establishment of a bronchial tree, preacinar bronchi formed
Canalicular	16-26	18.5-20	16.6-17.4	Formation of the prospective pulmonary acinus, increase of
Saccular	24-38	21-4dnp	17.4-5dnp	Capillary bed. Formation of saccules, alveolar ducts and sacs.
Alveolar	36-2 ypn	4-14dnp	4-14dnp	Formation of alveoli thinning of interalveolar septa
Micovascular maturation	Birth -3ypn	14-21dnp	14-21dnp	Fusion of capillary bed to Single layered network.

Ypn: Years postnatally Dnp: days postnatally

Table 2.1. Phases of lung development between human and mouse (Rutter, 2008; Schittny & Burri, 2007).

By the end of the 7-8th weeks of gestation in humans the developing embryo already has two recognizable left segments (upper and lower lung segments) and three upper right segments (middle and lower right lung segments).

The embryonic phase (table 2.1) of rats start on day 11 and of mice on day 9.5 after mating from two endodermal buds sprouting from the ventral foregut. A trachea (containing two primary lung buds) and esophagus will then form at the same location from a single foregut tube. The two primary mouse lung bronchi will then grow out further into the splanchnic mesenchyme, the right primary bronchi will create four secondary bronchi and the left will

not branch therefore will create one lung lobe, shown in the late pseudoglandular phase (Fig. 2.2). All the bronchi will undergo further branching forming a mature airway tree (Rutter, 2008). The mechanisms and control of this stage is unclear.

2.1.2 Pseudoglandular phase

During the pseudoglandular phase (Fig. 2.3) in humans, bronchial development is complete and the lung has a glandular appearance. The pseudoglandular phase is from the 5th to the 17th week of gestation in humans, and from gestational days 13-18.5 in rats and 14.2 to 16.6 in mice (table 2.1). Airways are lined by columnar epithelium and separated by a poorly differentiated mesenchyme. The mesenchyme plays an important role in the development of the lung into a fully functional organ. The Mesenchyme directs the growth and cytoarchitecture of the lung and regulates the growth and differentiation of the lung epithelium at the cellular-molecular level via soluble growth and differentiation factors that are hormonally regulated. During this stage the rate of cell proliferation is at its highest. Large quantities of glycogen occur in respiratory epithelial cells.

During this branching stage the terminal buds contain a population of multipotent epithelial progenitors (Okubo et al. 2005). As the tubes extend, descendants of these cells give rise to the progenitors of the major cell types of the conducting airways (Perl et al. 2002). The appearance of morphologically differentiated epithelial cells begins proximally and proceeds distally (Perl et al. 1999). By the end of this phase, acinar outlines begin to appear as tubes and they continue to further grow and branch (Boyden, 1977; Crapo et al. 1980).

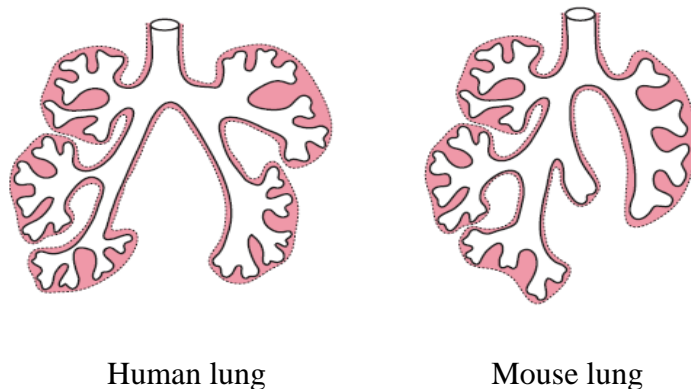


Figure 2.2. Late pseudoglandular stage of the human and mouse lung (Rutter, 2008).

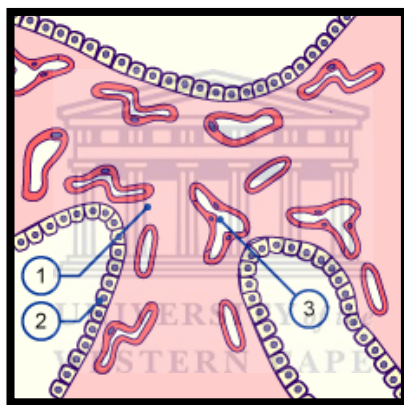


Figure 2.3. Lung tissue in the pseudoglandular phase: Lung has glandular appearance; Airways are lined with columnar epithelium and separated by poorly differentiated mesenchyme. 1) Lung mesenchyma; 2) Type II pneumocytes; 3) capillaries (Voigt et al. 1999).

2.1.3 Canalicular phase

At the 16th week of gestation in human life, and approximately 18 days in rats and 16 days in mice (table 2.1), the fetal pulmonary system enters the canalicular phase (Rutter, 2008). This stage is characterized by the proliferation of the mesenchyme and the development of a rich blood supply within the mesenchyme, and also by a flattening of the epithelium that lines the

5.6.2(a) Number of proliferating cell/100µm

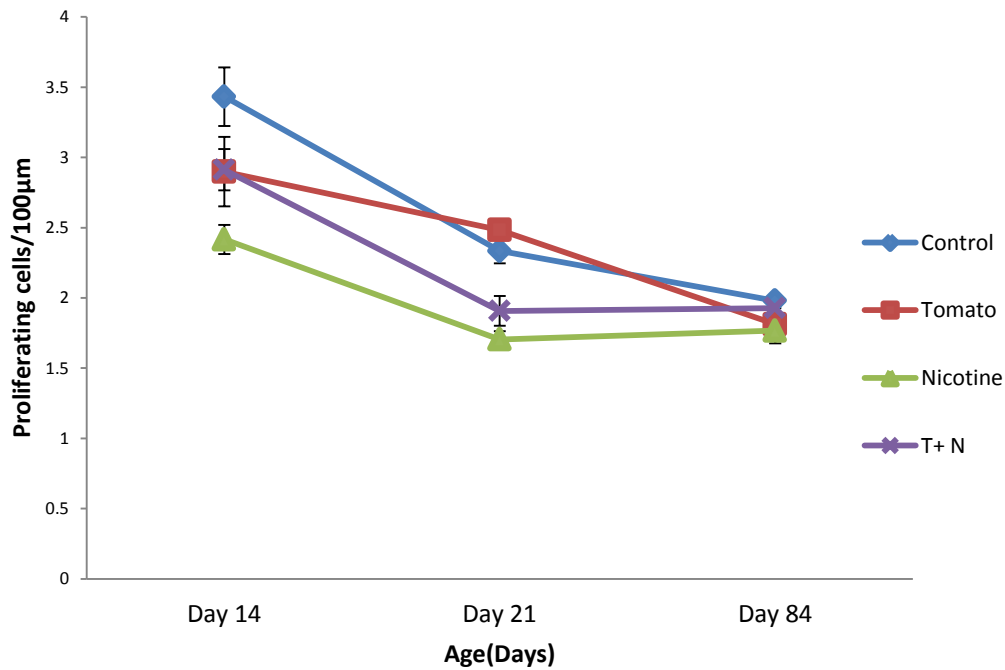


Figure 5.14. The effects of Maternal Nicotine and Tomato Juice exposure on Proliferating cells/100µm alveolar wall.

Proliferating cells/100µm alveolar wall							
Age in days	Control	Nicotine	T+N	Tomato juice	Control vs. Nicotine	Control vs. T+N	Control vs. Tomato juice
14	3.43±0.21	2.42±0.10	2.91±0.15	2.90±0.25	<i>P</i> <0.01	<i>P</i> >0.05	<i>P</i> >0.05
21	2.33±0.09	1.70±0.06	1.90±0.11	2.48±0.07	<i>P</i> <0.001	<i>P</i> <0.05	<i>P</i> >0.05
14 vs.21	<i>P</i> <0.005	<i>P</i> <0.001	<i>P</i> <0.001	<i>P</i> >0.05			
84	1.98±0.03	1.77±0.03	1.93±0.05	1.81±0.14	<i>P</i> <0.05	<i>P</i> >0.05	<i>P</i> >0.05
84 vs.14	<i>P</i> <0.05	<i>P</i> <0.05	<i>P</i> >0.05	<i>P</i> <0.05			
84 vs.21	<i>P</i> >0.05	<i>P</i> >0.05	<i>P</i> >0.05	<i>P</i> <0.005			

Table 5.13. The effects of maternal nicotine exposure during gestation and tomato juice supplementation during pregnancy on proliferating cells/100µm of the alveolar wall of the offspring.

5.7.2(a) Male static lung compliance (ml/cmH₂O/kg)

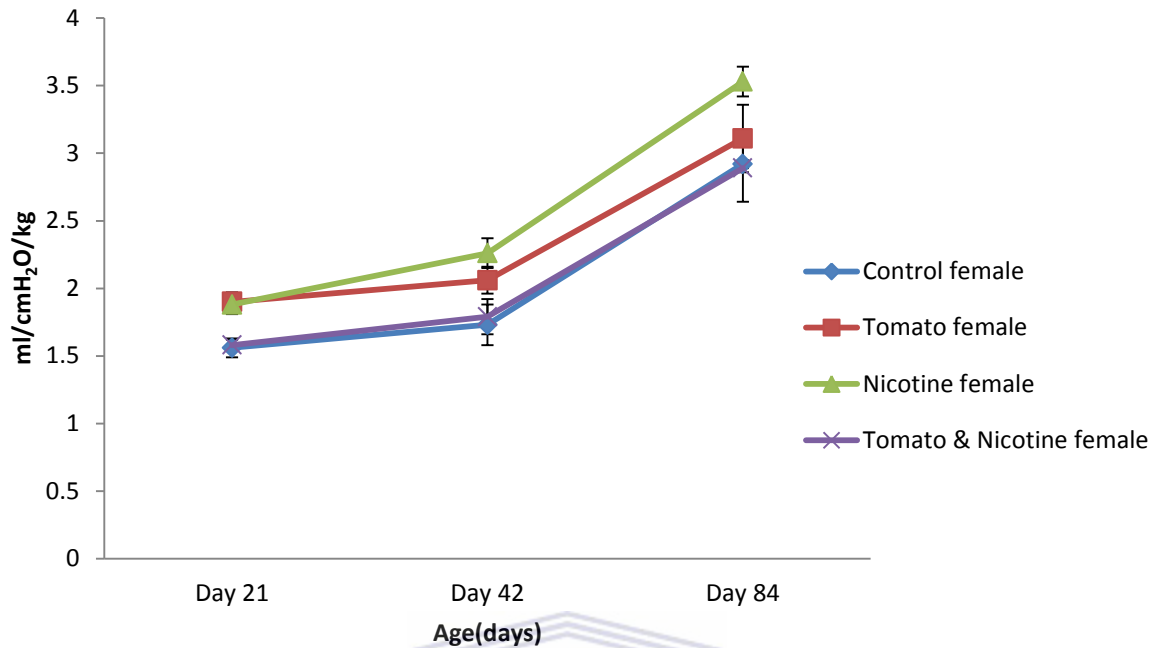


Figure 5.15(b). The effects of Maternal Nicotine and Tomato Juice exposure on male lung compliance.

Age in days	ml/cmH ₂ O/kg	ml/cmH ₂ O/kg				Control vs. Nicotine	Control vs. Nicotine + Tomato	Control vs. Tomato
		Control	Nicotine	Tomato + Nicotine	Tomato			
21	Female	1.56±0.07	1.88±0.07	1.58±0.04	1.71±0.07	<i>P</i> >0.05	<i>P</i> >0.05	<i>P</i> >0.05
	Male	1.43±0.08	1.79±0.09	1.47±0.04	1.70±0.07	<i>P</i> >0.05	<i>P</i> >0.05	<i>P</i> >0.05
F21 vs. M21		<i>P</i> >0.05	<i>P</i> >0.05	<i>P</i> >0.05	<i>P</i> >0.05			
42	Female	1.73±0.15	2.26±0.11	1.79±0.13	2.04±0.10	<i>P</i> <0.05	<i>P</i> >0.05	<i>P</i> <0.05
	Male	1.61±0.09	2.05±0.14	1.77±0.09	2.06±0.12	<i>P</i> <0.05	<i>P</i> >0.05	<i>P</i> <0.05
F42 vs. M42		<i>P</i> >0.05	<i>P</i> >0.05	<i>P</i> >0.05	<i>P</i> >0.05			
84	Female	2.92±0.03	3.53±0.11	2.89±0.25	3.11±0.25	<i>P</i> <0.001	<i>P</i> >0.05	<i>P</i> >0.05
	Male	2.73±0.03	3.12±0.11	2.76±0.16	2.94±0.21	<i>P</i> <0.001	<i>P</i> >0.05	<i>P</i> >0.05
F84 vs. M84		<i>P</i> <0.05	<i>P</i> <0.05	<i>P</i> >0.05	<i>P</i> >0.05			

Table 5.14. The effects of maternal nicotine exposure during gestation and tomato juice supplementation during pregnancy on lung compliance of the offspring.

