UNIVERSITY OF THE WITWATERSRAND
27 SEP 2018
FACULTY OF SCIENCE REGISTRAR'S DEPT

Faculty of Science

Division of Postgraduate Academic Management

EXAMINER'S REPORT ON A THESIS SUBMITTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

Name of Candidate:	**Rosaleen Mcneil**
Person Number:	**0602446D**
Title of Thesis:	**Expression of Vascular Endothelial Growth Factor (VEGF) in the Developing Avian Lung; And the Effect of Efavirenz on Embryonic Development and Angiogenesis in Chick Chorioallantoic Membrane (CAM)**

Please read the information sheet before completing this form

	Examiner's Recommendation/s - Please answer all questions by ticking the appropriate box	YES	NO
1	Does the Thesis constitute a definite contribution to the advancement of knowledge in the subject chosen?	Yes√	
2	Does it show originality?	Yes√	
3	Is the substance of the Thesis worthy of publication?	Yes√	
4	Is the literary presentation of the Thesis satisfactory?	Yes√	
5	More than one category of b) may be yes. Only **ONE** of c) should be **YES** a) Do you recommend award of the degree of PhD for the Thesis as it stands?		
	b) Do you recommend the award of the degree subject to: i) correction of minor errors and/or	Yes√	
	ii) revision involving, for example, rewriting of certain sections and/or	Yes√	
	iii) extension, for example, additions of sections and/or provision of additional data?		No√
	c) If the answer to b) ii or b) iii is yes, i) Are you satisfied to leave the checking of the revised Thesis to the Head of School?	Yes√	
	ii) Do you wish to re-examine the revised Thesis?		No√
	d) Do you recommend outright rejection?		No√
6	The names of examiners are confidential, but successful candidates may be told the names of their examiners with their consent. Would you agree to your name being divulged in this case?	Yes	

Examiner's Detailed Report

Please frame your report in the light of the requirements for the degree referred to in the Information Sheet. Please expand your recommendations under item 5 of this form. If you suggested correction, revision and/or extension, please be specific about what the candidate should be required to do to make the Thesis acceptable.

I have examined the thesis of **Rosaleen Mcneil on the: Expression of Vascular Endothelial Growth Factor (VEGF) in the Developing Avian Lung; And the Effect of Efavirenz on Embryonic Development and Angiogenesis in Chick Chorioallantoic Membrane (CAM)** for the award of Doctor of Philosophy and herewith append my report.

The candidate thesis is reported in two parts (1) **Expression of Vascular Endothelial Growth Factor (VEGF) in the Developing Avian Lung, (2) the Effect of Efavirenz on Embryonic Development and Angiogenesis in Chick Chorioallantoic Membrane (CAM).**

General: The First part of the present work was done predominantly on developing lung using the Avian lung as a model and focussed on the role of vascular endothelial growth factor (VEGF) on avian lung development with it specialized complex structural characteristic adaption for functional (gas exchange requirement and flight) efficiency in adult. The study details information on the critical developmental stages for the expression of VEGF for early formation of the structural complexity of Avian lung developmentally married with the energy demand for flight efficiency. The study further informed on the on the effect of VEGF, Efavirenz and Thalidomide on angiogenesis as associated with embryonic viability. VEGF its receptors play essential roles in many different aspects of cardiovascular development, including endothelial cell differentiation, migration and survival as well as heart formation and hematopoiesis.

This study has potential to generate knowledge in the topical field embryogenesis. There is evidence of two published articles, two published abstracts and four conference presentations. I am impressed with these outcomes.

However, there is room for substantial improvements to this submission that would ultimately improve the quality and overall scientific relevance.

Formatting: The candidate need to give very serious attention to proper formatting throughout the thesis especially in relation to legends and footnotes.

Title: require amendment, my suggestion: **Expression of Vascular Endothelial Growth Factor (VEGF) in the Developing Avian Lung and**

the effect of Efavirenz and Thalidomide on Angiogenesis in Chick Chorioallantoic Membrane (CAM).

Abstract: Obviously too long in nature and with references and detailed methodology, structured and failed to present a synthesis. The recommended word number is 350 and unstructured. This should be amended.

Nomenclature: The candidate did not present a list of acronyms and has a lot of acronyms. These should be listed and defined in alphabetical, immediately after the list of tables.

Chapter one: Introduction: Please attend to the numerous suggestions in this section. This section is too long and will require careful streamlining to focus it on the reason for the study. There is no clear and complete statement of the problem investigated, the hypotheses tested or the purpose of the study. It could benefit by addition of an opening paragraph to introduce the reader to importance of the work. There should a problem statement section which will clearly identify the specific gaps in knowledge. This must be separated from the rationale which should also be included.

Subheadings

a) Angiogenesis and lung development
b) Role of VEGF and angiogenesis in cancer, link to the discovery of lung cancer resolution in HIV patients
c) The uncertainty on the role of ARV in HIV and cancer patients- the need for investigative study.

Subheading

The Avian respiratory system, etc.

Aims and Objectives: The candidate did not indicate the aims and objectives for this study. The inclusion will help give this study an organizational form and improve the quality. The objectives of the parts of the study be put together while each speaks to its own focus.

Organizational structure of the thesis: A preview of the organisation of the remainder of the write-up/thesis should be included. This is stipulated in the thesis guidelines.

Footnotes and Legends: The footnotes and Legends to tables and figures should be properly positioned and should follow the standard practice. See corrections in the text. Some of the figures are too blurred. Pages 2, 7, 12 etc. Ensure that the figures are presented separately on different pages with clear legends. The legend should not be part of the text.

Page 6, delete Figure 5, there is no figure 5 in the whole of the thesis. Properly position the legend.

Chapter two: This is the literature review section and would be better if a statement is included to introduce the reader to the content of this chapter which concentrated on developmental genes and their role in morphogenesis. The candidate to attend to the corrections/edits in body of the thesis. Some of the proteins described (e.g. Testis determining factor, SOX, BMP etc) has no link with the topic of the study. It is suggested that the candidate through a sentence for each link up the role of these proteins (either separately or in combination) in the early formation of various structures and organs.

Chapter three: The title of this chapter gives the impression that the facts about this study have already been documented since the similarity between this chapter title and the main title of the thesis is striking. It was painful to note that this chapter that ought to be for "**Materials and methods**" was almost completely a **repetition of chapter two**. It is suggested that this chapter be deleted from page 39 to subsection 3.5 on page 49. The candidate to consider moving subsection 3.5: 'JUSTIFICATION' to the introductory chapter where it belongs. The justification for the study will be improved and add more value to the work if such aspects as –the role of VEGF on lung morphogenesis/angiogenesis, implications of on cancer in general and the use of Avian lung as a model are focussed in the write-up. Materials and Methods is a critical and central component of any study and should be a chapter of its own- and suggested to be the chapter three of this thesis and should incorporate the materials and methods used in both study components. The candidate to take note of suggested corrections on the body of the thesis.

Chapter 4: It is suggested that chapter 4 be the **result section** for the work and should not be mixed with chapter three or sandwiched with materials and methods. It is suggested that this be separated and the result section be organised to include the results of the two studies. Therefore under the result section the candidate should present:

Results;

- Results of the experiment on VEGF
- Results of the experiment on ARV (Efavirenz) and Thalidomide:

Chapter 4 in the present thesis is hence suggested to be under the result section.

Some of the figures are over stretched; do correct this to improve the quality of this study.

Chapter 5: Discussion: The quality of this thesis will improved if the discussion is also summarized to main findings of the present study relative to the focus of the study.

Technical and linguistic aspects of the research thesis

The technical and linguistic aspects of the thesis meet the requirement for the degree. The publication of two articles is a testament for meeting the scientific standard for the award of PhD degree. The publications evidently showed logical reasoning in analysis of the data and conclusions drawn from such.

I recommend that this study be accepted as having met the requirement for PhD degree.

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

UNIVERSITY OF THE WITWATERSRAND
1 SEP 2016
FACULTY OF SCIENCE

Faculty of Science

Division of Postgraduate Academic Management

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	Examiner's Recommendation/s - Please answer all questions by ticking the appropriate box	YES	NO
1	Does the Thesis constitute a definite contribution to the advancement of knowledge in the subject chosen?	✓	
2	Does it show originality?	✓	
3	Is the substance of the Thesis worthy of publication?	✓	
4	Is the literary presentation of the Thesis satisfactory?	✓	
5	More than one category of b) may be yes. Only **ONE** of c) should be **YES** a) Do you recommend award of the degree of PhD for the Thesis as it stands?		
	b) Do you recommend the award of the degree subject to: i) correction of minor errors and/or	✓	
	ii) revision involving, for example, rewriting of certain sections and/or		
	iii) extension, for example, additions of sections and/or provision of additional data?		
	c) If the answer to b) ii or b) iii is yes, i) Are you satisfied to leave the checking of the revised Thesis to the Head of School?		
	ii) Do you wish to re-examine the revised Thesis?		
	d) Do you recommend outright rejection?		
6	The names of examiners are confidential, but successful candidates may be told the names of their examiners with their consent. Would you agree to your name being divulged in this case?	✓	

Examiner's Detailed Report

Please frame your report in the light of the requirements for the degree referred to in the Information Sheet. Please expand your recommendations under item 5 of this form. If you suggested correction, revision and/or extension, please be specific about what the candidate should be required to do to make the Thesis acceptable.

RECOMMENDATION (FOR OFFICE USE):

I have read the thesis by Rosaleen McNeil and testify that work reported therein is of a quality that merits award of the degree of Doctor of Philosophy (PhD). The research had the breadth and depth that will empower the candidate to conceptualize a research question and perform the necessary experiments to achieve her specific aims.

STRENGTHS:

- The literature on embryology of several organ systems is scanty in the literature. Therefore, the area of study is novel albeit difficult one. The choice of angiogenesis during lung development is also a plus because lung function is primarily driven by blood flow and capillary function.
- Literature review and background information by candidate was adequate and relevant to experimental objectives.
- Candidate performed a longitudinal assessment of angiogenesis during lung development (stage 22 to 40, including the time of bifurcation of the trachea, appearance of secondary bronchi and parabronchi and blood vessels.
- Candidate also performed a 'toxicological study' on the effects of angiogenesis blockers on embryonic development. Data were acquired 5- and 15-days post treatment with test chemicals (recombinant VEGF, thalidomide and efavirenz). While this section of the study does not approximate a clinical study, the results suggest that test chemicals will be useful in study of mechanisms

associated with regulation of angiogenesis during development.

- Immuno-histochemical localization of specific proteins in avian tissues is more challenging than in rodents due to lack of specific antibodies for required assays.
- The chapter on general discussion (Chapter 5) is a good closure to the thesis and is succinct while providing more information on her findings than were available in the abstract.
- In spite of the fact that literature on avian biology is not as extensive as for many other species, references were reasonable and covered several years, e.g., from 1951-2015.
- In addition to conference papers, candidate has already published two major publications from the thesis.

MINOR WEAKNESSES:

- Chapter 3 included results of VEGF expression in kidneys (Fig. 3.31) and spinal cord (Figs. 3.32-3.35) which were not reflected in the title.
- Because the dosage of efavirenz used in studies reported in Chapter 4 were very high (200 μg), any extrapolation to the potential use of this agent in clinical trials need to be carefully made in statements in the discussion sections.
- Figures need to have their legends on the same page as the figure and not spill over to a second page (e.g., Fig. 3:38 on page 73).
- If 'Efavirenz' is not a trade name, it needs to be revised to read as 'efavirenz' throughout the text.
- Yellow fonts on some figures were hard to discern, e.g., Fig. 3.3. In the same figure, there is a reference to Fig. 14A and Figure 14B which were confusing.
- Black arrows will seem to be better for Fig. 3:16 than yellow.

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1	Does the Thesis constitute a definite contribution to the advancement of knowledge in the subject chosen?	X	
2	Does it show originality?	X	
3	Is the substance of the Thesis worthy of publication?	X	
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	b) Do you recommend the award of the degree subject to: i) correction of minor errors and/or	X	
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	iii) extension, for example, additions of sections and/or provision of additional data?		
	c) If the answer to b) ii or b) iii is yes, i) Are you satisfied to leave the checking of the revised Thesis to the Head of School?	X	
	ii) Do you wish to re-examine the revised Thesis?		
	d) Do you recommend outright rejection?		
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Examiner's Detailed Report

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RECOMMENDATION (FOR OFFICE USE):

REPORT ATTACHED.

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
FACULTY OF HEALTH SCIENCES

SPECIFIC QUESTIONS TO BE ANSWERED BY THE EXAMINER OF A THESIS SUBMITTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

This sheet should be returned to the Postgraduate Office within **6 weeks** of receipt of the thesis

PLEASE ANSWER "YES" OR "NO"

1.0 Does the thesis reveal a thorough accuaintance with the methods of research and the relevant literature, and a mastery of the necessary techniques? [**Yes**] or [No]

2.0 Does the thesis reveal that original work has been undertaken by the candidate? [**Yes**] or [No]

If your answer to this question is "**yes**", in what direction is the originality displayed?

PUBLICATIONS.....

.....

.....

3.0 Does the thesis constitute a substantial contribution to the advancement of knowledge in the subject? [**Yes**] or [No]

4.0 Is the substance (though not necessarily the whole) of the thesis worthy of publication? [**Yes**] or [No]

5.0 Is the thesis satisfactory as regards literary style and presentation? [**Yes**] or [No]

6.0 Is the thesis adequate as the sole ground for the award of the degree? [**Yes**] or [No]

If **yes** - please specify what you recommend: **Y/N**

- Award of the degree, with no corrections?	
- Award of the degree, subject to minor corrections to the satisfaction of the Head of Department	Y
- Award of the degree, subject to substantial amendments, undertaken to the satisfaction of the Head of Department	

7.0 If your answer to (6) is "no", would you recommend:

- Substantial amendments, and re-examination? .....
- Major revision, extension or elaboration and re-examination? .....
- reject the thesis outright? .....

Please use the box below for your report. Please add additional pages should you require additional space for the report. A **report is mandatory**.

REPORT:

FULL REPORT ATTACHED AS SEPARATE DOCUMENT.

Name of Candidate:.....**R. MACNEIL**.....

Name of Examiner: [redacted] .....

Signature: ....

Date: .....13 .....

Thank you for your help in the evaluation of our postgraduate students.

EX
EX

TITLE: Expression of Va) in the Developing Avian Lung; And th elopment and Angiogene CAM).

1. FIELD OF STUDY

Developmental angiogenesis is the process through which the embryo's complex pattern of blood vessels throughout the forming body, organs and tissues is established and maintained. Blood vessels are essential for the normal development of the embryo and angiogenesis, where new vessels form from existing networks through proliferation and migration of endothelial cells to form new vessels. Vascular malformations or the failure of normal vessel formation or the failure of the transition from the embryonic to the adult vessel networks can result in damage to the embryo and misregulation of signaling molecules important for normal angiogenesis can also result in malformations. The importance of the vascular system in embryonic development is further underlined by the large number of signaling molecules that when mutated to cause a loss-of-function result in embryonic lethality due to failure of vascular development.

During lung development, the conducting airways are formed first, followed by the formation and enlargement of the gas exchange area. In addition, the process of developmental angiogenesis is a complex process that requires a sequential interplay between embryonic cells, growth factors and extracellular matrix (ECM) components. The role of vascular endothelial growth factor (VEGF) which is a specific and potent mitogen for endothelial cells that is expressed in a distinct temporo-spatial pattern during mammalian lung development. However, the role played by VEGF and its isoforms in early processes of development, are not well understood.

Also, since spontaneous resolution of HIV-associated lung cancers has been observed in patients taking highly active antiretroviral therapy, the question arises as to the anti-cancer properties of antiretroviral drugs. Efavirenz, a Non-Nucleoside Reverse Transcriptase Inhibitor is drug used in the management of HIV-1 infection that has not been adequately studied even in patients with advanced HIV disease. The Chick Chorioallantoic Membrane (CAM) of the domestic fowl used as an in vivo vascular test environment is suitable to study the anti-angiogenic property of Efavirenz.

This study focused on evaluating the expression of VEGF in the developing avian lung; and the effect of Efavirenz on embryonic development and angiogenesis in CAM. The field of study is fully justified considering the high and increasing incidence of lung disease worldwide as well as in our country together with the incidence of HIV infection and the possible serendipitous effect that ARDs may affect during treatment.

The study is justified and the importance of the study is described by explaining the underlying elements of the problem. The background, aim and objectives of the study are described allowing the reader an understanding of the importance and sequence of the study.

2. LITERATURE REVIEW

The thesis was divided into several chapters in the literature review covering the avian respiratory system, molecular regulation of lung development, expression pattern of VEGF, and the effect of Efavirenz on angiogenesis in CAM.

The literature review is well written although there is a slight disjunction within the research design as first 2 chapters consist mainly of a literature review while the 3rd and 4th chapters consist of a literature review in addition to the materials and methods, results and discussion.

The main problem that I find in this thesis is to really find a logical research design of the study. It really seems that 2 independent studies were conducted and written up as a single thesis.

3. RESEARCH DESIGN AND METHODOLOGY

Two chapters include methodology used in order to meet the objectives of the study: Chapter 3: EXPRESSION PATTERN OF VEGF IN THE DEVELOPING AVIAN LUNG OF THE DOMESTIC FOWL and Chapter 4: EFFECT OF EFAVIRENZ ON ANGIOGENESIS IN CAM. While each chapter gave a literature review on topics relevant to the issue being investigated followed by the methodology, results and discussion, it is difficult to connect chapter 3 and 4. The only connecting factor is the addition of VEGF in Efavirenz treated samples. VEGF is an important factor for angiogenesis and one would presume since cancerous tissue requires increased vascularization Efavirenz inhibits this process and reduces the expression of VEGF.

A number of different techniques and methods were utilized and mastered in this study although there is a clear indication that not all results were as reliable as it may have been had additional experiments been performed. Suitable statistical analysis was performed in all as was the inclusion of positive and negative controls. The use of individual chapters to discuss the methods used, methodology and results as well as a brief discussion is well done. It made it easy to follow and understand. The selection of methods used is explained and the candidate clearly has a good grasp of the results obtained, explaining and discussion relevant findings.

Results were presented and "discussed" in each chapter followed by a summary and an additional discussion chapter at the end of the thesis. The thesis also contains a concluding chapter with recommendations for future work.

4. RESULTS AND DISCUSSION

One of the most significant findings of this thesis is the fact that ARDs inhibits vascularization and may therefore reduce or play a significant damaging role for embryo development. This is of great significance when considering the use of ARDs by HIV infected women during pregnancy. Although Thalidimide was compared to Efavirenz and concluded to be more safe for use in pregnant women, I believe this conclusion is not evident or supported by scientific evidence from this study.

The results generated in the study are appropriate and it was well integrated within the existing literature. The presentation of the results as well as the discussion of findings indicate that the candidate has a firm understanding of the study and could interpret the findings in context. Chapter 3 results concluded that early VEGF expression in the avian lung, as is the case with the mammalian lung, shows that VEGF plays a normal role in early avian lung endothelial biology. Chapter 4 showed Efavirenz to be a more potent anti-angiogenic agent than thalidomide, possessing an absolute anti-angiogenic effect in the developing chick CAM. I believe that, considering the work performed, results obtained and outcomes of the experiments, this study has made an original contribution to the existing body of literature. The work was disseminated to both national and international peers by presentation at conferences and publications emanated from this study.

5. THE COMPETENCE OF NOVICE SCHOLARSHIP

The candidate has shown competence to graduate at doctorate level. She understands the theory and literature in the specific field of inquiry and has clearly identified the research problem, aim and objectives of the study. She has mastered the skills to practice the required techniques used in the field of investigation, has shown competence in gathering results and analysing it. Suitable conclusions have been drawn after a thorough discussion of the results. With further investigation and development the work may present more information for publication.

6. LANGUAGE AND PRESENTATION

Generally, the thesis is well organised and all necessary introductory sections, chapters and reference list are included. The order of chapters follows the development and use of different assays to investigate and meet the objectives of the study. Each chapter discusses, briefly, the assay used and then methodology and results are given. This made for clear reading and understanding and following the train of thought of the candidate.

Research outputs and additional information including ethics approval letter have been provided in appendices at the end of this thesis. Technical presentation is of adequate quality. The majority of the diagrams, images and figures are clear and well presented. Language and grammar use is acceptable and jargon common to the field of study was used.

7. INTELLECTUAL OUTPUT OF THE THESIS

The candidate has shown competence at the level of emergent scholarship as reflected in this thesis. It is expected that research produced at a doctorate level will be published to the international scientific community. This is reflected in the list of peer reviewed publications and presentations arising from this study. A number of oral presentations seem to have been done at conferences allowing for further dissemination of results. I highly commend the candidate for the outputs produced.

8. OVERALL IMPRESSION

This work contributed to highlighting the importance of developmental angiogenesis. It also eluded to the use of certain ARDs as potential cancer treatment drugs and the role that it may play during embryonic development. The research design, methodology and techniques used showed that the candidate has acquainted herself with the methods and understands the purpose of the study. The candidate has accomplished interrogating the research results and provided sound arguments and explanations for the results shown.

9. RECOMMENDATIONS

I believe that a concluding paragraph clearly describing the connection between the 2 parts of this study would significantly contribute to the thesis overall. It would also be of great benefit if suitable future research and directives can be stated at the end of the thesis.

This study is well designed, performed, written and concluded. Once recommended changes and corrections has been made to the satisfaction of the supervisor, it is my recommendation that this degree be conferred. Well done and congratulations!