

**MOLECULAR CHARACTERIZATION OF INSULIN-LIKE GROWTH FACTOR 1
(IGF-1) GENE IN CATTLE (*Bos indicus*) AND WATER BUFFALO
(*Bubalus bubalis*) WITH CYSTIC OVARIAN FOLLICLE**

ROMANO MARCO BOGNOT RIVERA

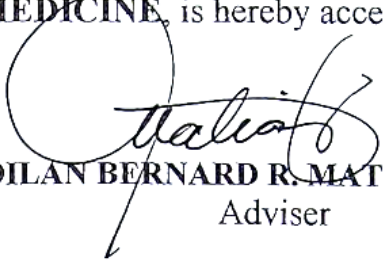
An Undergraduate Thesis Submitted to the Faculty of the College of Veterinary
Science and Medicine, Central Luzon State University,
Science City of Muñoz, Nueva Ecija, Philippines
in Partial Fulfillment of the Requirements
for the Degree of

DOCTOR OF VETERINARY MEDICINE


JUNE 2019

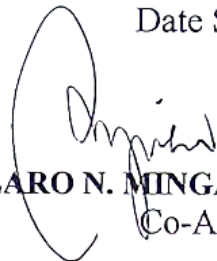
ACCEPTANCE SHEET

This undergraduate thesis entitled **“MOLECULAR CHARACTERIZATION OF INSULIN-LIKE GROWTH FACTOR 1 (IGF-1) GENE IN CATTLE (*Bos indicus*) AND WATER BUFFALO (*Bubalus bubalis*) WITH CYSTIC OVARIAN FOLLICLE”**, prepared and submitted by **ROMANO MARCO BOGNOT RIVERA**, in partial fulfillment of the requirements for the degree of **DOCTOR OF VETERINARY MEDICINE**, is hereby accepted:

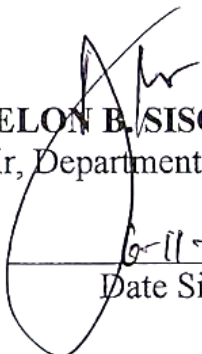

FROILAN BERNARD R. MATIAS, DVM, MSc
Adviser
6-17-19
Date Signed

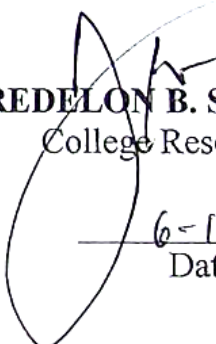

RODOLFO F. MEDRANO JR., DVM, MSc
Critic
6-17-19
Date Signed



JONATHAN V. LAZARO, DVSM, Ph.D.
Co-Adviser
6-17-19
Date Signed


CLARO N. MINGALA, DVSM, Ph.D.
Co-Adviser
6-17-19
Date Signed

Accepted as partial fulfillment of the requirements for the degree of **DOCTOR OF VETERINARY MEDICINE**:


FREDELON B. SISON, DVM, MVPH
Chair, Department of Pathobiology
6-11-19
Date Signed


FREDELON B. SISON, DVM, MVPH
College Research Coordinator
6-11-19
Date Signed


VIRGINIA M. VENTURINA, DVSM, Ph.D.
Dean, College of Veterinary Science and Medicine
6-17-19
Date Signed

BIOGRAPHICAL SKETCH

The author was born on February 7, 1995 in Tarlac City, Tarlac and is now currently residing in Capas, Tarlac. He is the youngest among the three children of Francisco D. Rivera Jr. and Susan B. Rivera.

He took his primary education at Montessori School of Saint Nicholas (A.Y. 2001 – 2007); secondary education at Wellspring High School (A.Y. 2007-2011) and tertiary education at Central Luzon State University (A.Y. 2011-2017).

ACKNOWLEDGMENT

The author would like to express his gratitude by acknowledging the following people.

Francisco D. Rivera Jr. and Susan B. Rivera, his parents, for the gift of love, support, and comfort;

Dr. Froilan Bernard R. Matias, his adviser, for the exemplary supervision in the writing and conducting of the study;

Dr. Jonathan V. Lazaro and Dr. Claro N. Mingala, his co-advisers, for the active participation in the conduct and hands-on mentoring in writing the study;

Dr. Rodolfo F. Medrano Jr, his critic, for giving suggestive remarks for the improvement of this manuscript;

Dr. Fredelon B. Sison, his research coordinator, for making sure that this manuscript is in its best form in terms of content and format;

Dr. Marvin Bryan S. Salinas, CVSM faculty, for the guidance in writing this manuscript;

Dr. Gabriel Alexis SP. Tubalinal, PCC research assistant, for his assistance in the conduct of this research;

Dale S. Pinili, biotechnologist, for his valuable insights;

Above all, Almighty God for creating everything that mattered in this study;

And to all the people who made this manuscript a reality, he is quite grateful.

TABLE OF CONTENTS

	PAGE
LIST OF TABLES	viii
LIST OF FIGURE	ix
LIST OF PLATE	x
LIST OF APPENDICES	xi
LIST OF APPENDIX TABLE	xii
LIST OF APPENDIX PLATES	xiii
ABSTRACT	xiv
INTRODUCTION	1
Background of the Study	1
Objectives of the Study	2
Significance of the Study	3
Scope and Limitation of the Study	3
Time and Place of the Study	3
REVIEW OF RELATED LITERATURE	4
Large Ruminant	4
Large Ruminant Industry	4
Breeds	4
Inventory	5
Type of Production	6
Large Ruminant Reproductive Phenomena	7
Female Reproductive Tract	7
Reproductive Parameters	7
Cystic Ovarian Follicles	8
Causes	8

Mechanism of Occurrence	9
Gross	10
Histopathology	10
Epidemiology	11
Diagnosis	12
Treatment	12
Insulin-Like Growth Factor	13
Functions	13
Mechanism	14
Diseases That Affect IGF Production	15
Reported Polymorphisms	15
Single Nucleotide Polymorphism	16
MATERIALS AND METHODS	17
Sample Collection	17
DNA Extraction	18
Polymerase Chain Reaction	19
Gel Electrophoresis	20
DNA Sequencing	20
RESULTS AND DISCUSSION	21
SUMMARY, CONCLUSION AND RECOMMENDATIONS	34
LITERATURE CITED	36
APPENDICES	39

LIST OF TABLES

TABLE		PAGE
1	Optimized conditions for the PCR amplification of IGF-I gene	19
2	Primer used for DNA amplification of IGF-I gene	19
3	Nucleotide sequence of IGF-I gene in blood of water buffalo with COF	23
4	Nucleotide sequence of IGF-I gene in blood of cattle with COF	23
5	Nucleotide sequence of IGF-I gene in normal ovary of cattle	24
6	Nucleotide sequence of IGF-I gene in normal ovary of water buffalo	24
7	Nucleotide sequence of IGF-I gene in ovary of water buffalo with COF	25
8	GenBank- available sequences homologous to IGF-1 gene	26
9	Nucleotide polymorphism of IGF-I gene in comparison with the sequence of Y18831.1 and FJ196583.1	27

LIST OF FIGURE

FIGURE		PAGE
1	Work flow chart	17

LIST OF PLATE

PLATE		PAGE
1	Amplified PCR products of IGF-1 gene. Lane 1 (blood of cattle with cystic ovarian follicle). Lanes 2 and 3 (normal ovary of cattle). Lanes 4 and 5 (ovary of water buffalo with cystic ovarian follicle). Lane 6 (blood of water buffalo with cystic ovarian follicle). Lane 7 (normal ovary of buffalo)	21

LIST OF APPENDICES

APPENDIX		PAGE
I	Procedures	40
II	Primer	43
III	Raw Data	44
IV	Plates	50
V	Expenses	54

LIST OF APPENDIX TABLE

APPENDIX TABLE

PAGE

1 Primer designed to amplify *Bos taurus* IGF-I gene –
BC126802.1

43

LIST OF APPENDIX PLATES

APPENDIX PLATE		PAGE
1	Cystic ovarian follicle	50
2	Collection of ovaries in abattoirs	51
3	Cutting off of stromal tissues	51
4	DNA extraction	52
5	Manipulation of PCR machine	52
6	Gel electrophoresis of PCR products	53
7	Browsing of gel electrophoresis products	53

ABSTRACT

RIVERA, ROMANO MARCO B., Department of Pathobiology, College of Veterinary Science and Medicine, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines, **June 2019, MOLECULAR CHARACTERIZATION OF INSULIN-LIKE GROWTH FACTOR 1 (IGF-1) GENE IN CATTLE (*Bos indicus*) AND WATER BUFFALO (*Bubalus bubalis*) WITH CYSTIC OVARIAN FOLLICLE**

Adviser: FROILAN BERNARD R. MATIAS, DVM, MSc

Co-advisers: JONATHAN V. LAZARO, DVSM, Ph.D.

CLARO N. MINGALA, DVSM, Ph.D.

Cystic ovarian follicle (COF) is a significant cause of subfertility, especially in dairy cattle. It develops when one or more follicles fail to ovulate and subsequently does not regress but maintains their growth and steroidogenesis. Insulin-like Growth Factor 1 (IGF-1) gene is found in many cell types and is essential for normal fetal and postnatal growth and development. It is also associated with normal follicle and antral follicle development. It also participates in a cascade in the hypothalamo-pituitary-gonadal axis resulting to luteinizing hormone (LH) surge that can affect the presence of COF.

Insulin-Like Growth Factor (IGF-1) gene in cattle and water buffalo with cystic ovarian follicle (COF) was characterized using DNA sequencing. A total of three ovarian samples (two normal one cystic) were collected in abattoirs in Nueva Ecija and Pangasinan while a total of two blood samples (one cattle and one water buffalo) were collected from the Philippine Carabao Center (PCC). Collected samples were processed at Molecular Biology and Biotechnology Laboratory, PCC wherein they were subjected to DNA extraction, conventional Polymerase chain reaction, and Gel electrophoresis. After that, PCR products were sent to First Base Malaysia for DNA sequencing. Sequences derived were compared with DNA sequences in the Gene Bank to detect polymorphisms.

Results showed that in comparison with the IGF-1 gene sequences with the accession numbers; *Y18831.1* and *FJ196583.1*, there were 307 polymorphisms detected. The derived sequences, however, were closely homologous to *Bos mutus yak QHI chromosome 21* and *Ovis canadensis canadensis isolate 43U chromosome 18 sequence* meaning the segment of the sequence derived may not be exclusive to the IGF-1 gene.

Keywords: cattle, cystic ovarian follicle (COF), IGF-1 gene, water buffalo

LITERATURE CITED

- Abraham, F. (2017). An Overview on Functional Causes of Infertility in Cows. *Journal of Fertilization: In vitro - IVF-Worldwide, Reproductive Medicine, Genetics & Stem Cell Biology*, 5 (2): 1-6.
- Abdi, Z., S. A. Rafat, M. Firouzamandi, G. Moghadam, and J. Shojaabraham, F. (2017). Polymorphisms of the Bovine Luteinizing Hormone Choriogonadotropin Receptor (LHCGR) Gene and its Association with Ovarian Follicular Cysts. *Revue de médecine vétérinaire*, 168, 7-9, 143-150.
- Adamek, A and A. Kasprzak. (2018). Insulin-Like Growth Factor (IGF) System in Liver Diseases. *International Journal of Molecular Sciences*, 19 (1308): 1-24.
- Ali, A and D. R. Derar. (2014). Comparative Anatomy of the Female Reproductive Tract in Farm Animals. *Research Gate*. Retrieved on January 19, 2018 from the World Wide Web: <https://www.researchgate.net/publication/264118743>.
- Arcaro, A. (2013). Targeting the Insulin-like Growth Factor-1 Receptor in Human Cancer. *Frontiers in Pharmacology*, 4 (30): 1-8.
- Bondoc, O. L. (2013). Genetic Diversity and Relationship of Domestic Buffalo and Cattle Breeds and Crossbreeds (Subfamily Bovinae) in the Philippines Based on the Cytochrome C Oxidase I (COI) Gene Sequence. *Philipp Agric Scientist*, 96 No. (1): 93-102.
- Brito, L. F. C and C. W. Palmer. (2004). Cystic Ovarian Disease in Cattle. *SNELL Medical Communication Inc*, 4 (10): 1-6.
- Castillo, A. C. Backyard Beef Production in the Philippines. (1996). Retrieved on December 23, 2017 from the World Wide Web: <https://pdfs.semanticscholar.org/25ab/08a37c09df365964f6fdfbe766ce149ddf6a.pdf>.
- Ceranowicz, P., Z. Warzecha, J. Cieszkowski, D. Ceranowicz, B. Ku'Snierz-Cabala, J. Bonior, J. Jaworek, T. Ambrozy, K. Gil, R. Olszanecki, M. Pihut and A. Dembi'Nski. (2018). Insulin-Like Growth Factor (IGF) System in Liver Diseases. *International Journal of Molecular Sciences*, 18 (1118): 1-14.
- Clarke, R. B., A. Howell, and E. Anderson. (1997). Type I Insulin Like Growth Factor Receptor Gene Expression in Normal Human Breast Tissue Treated with Oestrogen and Progesterone. *British Journal of Cancer*, 75 (2): 251-257.

- Deng, C., R. Ma, X. Yue, X. Lan, H. Chen, and C. Lei. (2010). Association of IGF-I Gene Polymorphisms with Milk Yield and Body Size in Chinese Dairy Goats. *Genetics and Molecular Biology*, 33, 2, 266-270.
- Domingo, S. N. and M. D. C. Olaguera. (2017). Review of High-Value Agriculture in the Philippines with Comprehensive Subsectoral Focus: Livestock Industries. *Philippine Institute for Developmental Studies*, 2017-51.
- Fan, C., Y. Huang, Y. Bao, L. Sun, Y. Wu, C. Yu, Y. Zhang, Z. Song, L. Zheng, Y. Sun, G. Wang, and Y. Li. (2012). Virtual Screening of Specific Insulin-Like Growth Factor 1 Receptor (IGF1R) Inhibitors from the National Cancer Institute (NCI) Molecular Database. *International Journal of Molecular Sciences*, 13: 17185-17209.
- Genetics Home Reference. (2019). Help Me Understand Genetics Genomic Research. Retrieved on May 14, 2019 from the World Wide Web: <https://ghr.nlm.nih.gov/>.
- Javic, K. and N. Conroy. (2003). Bovine reproductive: Pathological condition. New Bolton Center Field Service Department. Retrieved on January 19, 2018 from the World Wide Web: <http://cal.vet.upenn.edu/projects/fieldservice/Dairy/REPRO/cowrepro.html>.
- Kassam, S., P. Meyer, A. Corfield, G. Mikuz, and C. Sergi. (2005). Single Nucleotide Polymorphisms (SNPs): History, Biotechnological Outlook and Practical Applications. *Current Pharmacogenomics*, 3, 000-000.
- Li, Y, H. Wang, X. Zhu, L. Guo, and J. Zuo. (2014). The Polymorphism of Insulin-like Growth Factor-I (IGF-I) is Related to Osteoporosis and Bone Mineral Density in Postmenopausal Population. *Pak J Med Sci* 2014 Vol. 30 No. 1.
- Louden, E., M. M. Chi, and K. H. Moley. (2008). Crosstalk Between The AMP-Activated Kinase and Insulin Signaling Pathways Rescues Murine Blastocyst Cells from Insulin Resistance. *Reproduction*, 136: 335–344.
- Merck Animal Health. Reproduction in Buffalo: Physiology. MSD Animal Health. Retrieved on December 23, 2018 from the World Wide Web: <http://www.partners-in-reproduction.com/reproduction-buffalo/buffalo-physiology.asp>.
- Panigrahi, M., S. Kumar, S. M. Deb, A. Mitra, A. Sharma and K. M. Bujarbaruah. (2012). Characterization of Insulin-like Growth Factor-1 (IGF-1) partial gene in mithun. *Indian Journal of Animal Sciences* 82 (1): 89–91.

- Philippine Statistics Authority. (2016). Cattle Industry Performance Report. Quezon City, Philippines. 1-18.
- Philippine Statistics Authority. (2017). Carabao Situation Report. Quezon City, Philippines. 1-12.
- Philippine Statistics Authority. (2017). Cattle Situation Report. Quezon City, Philippines. 1-13.
- Sadeesh, E. M. (2009). Analysis of CYP19 Gene Expression in Buffalo Ovarian Cyst. *Division of Animal Biochemistry National Dairy Research Institute*. p. 1-44.
- Satham J. (2018). Follicular Cystic Ovary Disease in Cattle. MSDVETMANUAL. Retrieved on June 7, 2018 from the World Wide Web: <https://www.msdevetmanual.com/reproductive-system/cystic-ovary-disease/follicular-cystic-ovary-disease-in-cattle>.
- Satham J. (2018). Luteal Cystic Ovary Disease in Cattle. MSDVETMANUAL. Retrieved on June 7, 2018 from the World Wide Web: <https://www.msdevetmanual.com/reproductive-system/cystic-ovary-disease/luteal-cystic-ovary-disease-in-cattle>.
- Stubbs, S. A., Webber, L. J., Stark, J., Rice, S., Margara, R., Lavery, S., Trew, G. H., Hardy, K., and Franks, S. (2013). Role of Insulin-like Growth Factors in Initiation of Follicle Growth in Normal and Polycystic Human Ovaries. *J Clin Endocrinol Metab*, 98 (8): 3298–3305.
- Vanholder, T. (2005). Cystic Ovarian Follicles in the High Yielding Dairy Cow Postpartum Role of Metabolic and Hormonal Adaptations in the Pathogenesis. Unpublished Doctoral Thesis. Faculty of Veterinary Medicine, Ghent University.
- Vanholder, T., Opsomer, G. and A. D. Kruif. (2006). Aetiology and Pathogenesis of Cystic Ovarian Follicles in Dairy Cattle: A review. *Reproduction Nutrition Development*. *EDP Sciences* 46 (2): 105-119.
- Williams, R. C. (1989). Restriction Fragment Length Polymorphism (RFLP). *American Journal of Physical Anthropology*, 32:159-184.