

**ISOLATION AND CHARACTERIZATION OF THE GONADAL PRIMORDIAL
GERM CELLS (gPGCs) OF GUINEA FOWL (*Numida meleagris*)
FROM 7-9 DAY OLD EMBRYOS**

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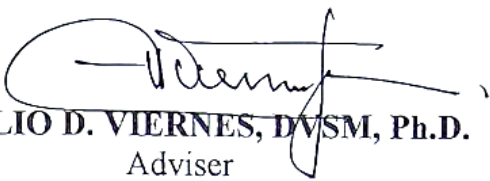
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ACCEPTANCE SHEET

This undergraduate thesis entitled **“ISOLATION AND CHARACTERIZATION OF THE GONADAL PRIMORDIAL GERM CELLS (gPGCs) OF GUINEA FOWL (*Numida meleagris*) FROM 7-9 DAY OLD EMBRYOS,”** prepared and submitted by **NIKKO MELEGRITO PARDUA**, in partial fulfillment of the requirements for the degree of **DOCTOR OF VETERINARY MEDICINE**, is hereby accepted:



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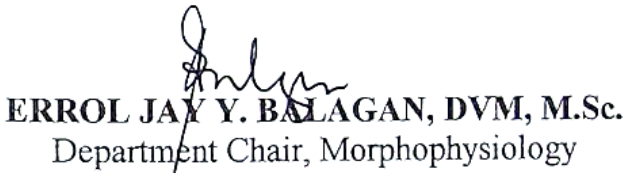
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BIOGRAPHICAL SKETCH

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ABSTRACT

PARDUA, NIKKO M., Doctor of Veterinary Medicine, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines, **June 2019, ISOLATION AND CHARACTERIZATION OF THE GONADAL PRIMORDIAL GERM CELLS (gPGCs) OF GUINEA FOWL (*Numida meleagris*) FROM 7-9 DAY OLD EMBRYOS.**

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The study aimed to isolate and characterize the gonadal primordial germ cells (gPGCs) of guinea fowl. Specifically, the study determined the embryonic age that optimally released gPGCs, the embryonic age that discharged the highest number of gPGCs and morphological characteristics of the gPGCs.

The right and left gonads collected from the 7 and 9 days old guinea fowl embryos were separately placed in a petri dish containing 500 μ l of phosphate buffered saline without Mg²⁺ and Ca²⁺ (PBS-). The petri dishes were placed in an incubator maintained at 38.5 °C and subsequently observed at 3 hours of incubation.

Results showed that the 9- day old guinea fowl embryos released the highest yield of discharged gPGCs and in the 8th day- old has the highest purity percentage of gPGCs collected. It is the optimum incubation age to isolate and characterize the gPGCs of the guinea fowl embryos. It was also found out that the left gonad has more number of released gPGCs than the right. The observed gPGCs were round shaped cells with large central nucleus.

Keywords: embryo; gonadal primordial germ cells; guinea fowl

LITERATURE CITED

- Aoyama, H., Asamoto, K. Nojyo, Y. and Kinutani, M. (1992). Monoclonal Antibodies Specific to Quail Embryo Tissues: Their epitopes in the developing quail embryo and their application to identification of quail cells in quail- chicken chimeras. *J. Histochem. Cytochem* 40, 1769-1777.
- Bachvarova, R. F., Crother, B. I., Manova K., Chatfield, J., Shoemaker C. M., CREWS, D. P. and Johnson, A. D. (2009). Expression of Dazl and Vasa in Turtle Embryos and Ovaries: Evidence for Inductive Specification of Germ cells. *Evol. Dev.* 11, 525-534.
- Bernacki Z., (2012). Guinea Fowls. In: Jankowski, J.(Ed.), Poultry Breeding and Production. First ed.Paristwowe Wydawnictwo Rolnieza I Lesne (PWRiL), Warsaw, Poland, pp. 443- 457.
- Bernie D., and D. E. Wilson. (2012). Editor. Smithsonian Institution Animal. Dorling Kindersley Publishers
- Clawson, R. C., and L. V. Domm, (1963) Developmental changes in glycogen content of primordial germ cells in chick embryos. *Dev. Genet.*, 19,: 290-301
- Choi, J.W., Kim, S., Kim, T.M., Y. M., Seo, H. W., Park, T.S., Jeong, J. W., Song, G. and Han, J.Y. (2010). Basic Fibroblasts Growth Factor Activates MEK/ERK Cell Signaling Pathw
- Chojnacka Puchta., Kito, G., Aramaki, S., Tanaka, K., Soh, T., Yamauchi, N. and Hattori, M., Temporal and Spatial Differential Expression of Chicken Germline- Specific Proteins cDAZL, CDH and CVH Gametogenesis. *J. Reprod. Dev.* 56, 341-346.
- Evans, M. J. and M. H. Kaufman. (1981). Establishment in Culture of Pluripotential Cells from Mouse Embryos. *Nature* 292, 154- 156
- Eyal- Giladi, H. and S. Kochav. (1976). From Cleavage to Primitive Streak Formation: A Complementary Normal and A New Look at the First Stages of the Development of the Chick. I. General Morphology. *Dev. Biol.* 49, 321-337
- Glover, J. D. and M. J. McGrew. (2012). Primordial germ cell technologies for avian germplasm cryopreservation and investigation germ cell development. *Journal of Poultry Sciences*, vol. 49, 2012, p. 155-162.
- Fujimoto, T., A. Ukeshima and R. Kiyofuji. (1976). The Origin, Migration and Morphology of the Primordial Germ Cells in the Chick Embryo. *Anat. Rec.* 185, 139-153.

- Ginsbur, M. and H. Eyal- Giladi. (1987). Primordial Germ Cells of the Young Chick Blastoderm Originate from the Central Zone of the Area Pellucida Irrespective of the Embryo- Forming Process. *Development* 101, 209-219.
- Hughe, G. (1963). The Population of Germ Cells in the Developing Female Chick. *J. Embryol. Exp. Morphol.* 11, 513- 536.
- Illmense, K. and A. P. Mahowld. (1974). Transplantation of Posterior Polar Plasm in *Drosophila*. Induction of Germ Cells at the Anterior Pole of the Egg. *Proc. Natl Acad. Sci. USA* 71, 1016-1020.
- Ishimaru, Y., T. Komatsu, M. Kasahara, Y. Katoh-Fukui, H. Ogawa, Y. Toyama, M. Maekawa, K. Toshimori, R. A. Chandraratna, K. Morohashi, and H. Yoshioka. 2008. Mechanism of Asymmetric Ovarian Development in Chick Embryos. *Development* 135, 677–685.
- Johnson, A. D., R. F. Bachvarova., M. Drum and T. MASI. (2001). Expression of Axolotl DAZL RNA, A Marker of Germplasm: Widespread Maternal RNA and Onset of Expression in Germ Cells Approaching the Gonad. *Dev. Biol.* 234, 402- 415
- Jung, J. G., D.K. Kim, T.S. Park, S.D. Lee, J. M. Lim and J.Y Han. (2005). Development of novel markers for the characterization of chicken primordial germ cells. *Stem Cells*, vol. 23, 2005, p. 689- 698.
- Lawson, K. A., N. R. Dunn, B. A. Roelen, L. M. Zeinstra, A.M. Davis, C. V. Wright, J. P. Korving and B. L. Hogan. (1999). *Bmp4* is required for the Generation of Primordial Germ Cells in the Mouse Embryo. *Genes Dev.* 13, 424- 436.
- Leghari, I.H., D. Zhao, Y. Mi, and C. Zhang, 2015. Isolation and Culture of Chicken Primordial Follicles. Department of Veterinary Medicine, College of Animal Sciences, Zhejiang University, Hangzhou 310058, P. R. China; And Department of Poultry Husbandry, Sindh Agriculture University, Tandijam 760060, Pakistan
- Macdonald, J., J. D. Glover, L. Taylor, H. M. Sang and M. J. McGrew. (2010). Characterisation and Germline Transmission of Cultured Avian Primordial Germ Cells *PLoS ONE* 5, e15518.
- Madge, P., P. McGowan, and G. M. Kirwan. (2002). Pheasants, Partridges and Grouse. Princeton University Press.
- Martin, G.R. (1981). Isolation of a pluripotent Cell Line from Early mouse embryos cultured in Medium Conditioned by Teratocarcinomas Stem Cells. *Proc. Natl Acad. Sci. USA* 78, 7634- 7638

- Matsui, Y., D. Toksoz, S Nishikawa, D. Williams, K Zsebo and B. L. Hogan (1991). Effect of Steel Factor and Leukemia Inhibitory Factor on Murine Primordial Germ Cells in Culture. *Nature* 354, 750- 752.
- Minematsu, T., Tajima, A., and Kanai, Y. (2004). The Migratory Ability of Gonadal Germ Cell in Domestic Chicken. *J. Poult. Sci.* 41: 178-185.
- Naito M., T. Minematsu, T. Harumi, and T. Kuwana, (2009). Preferential migration of transferred PGCs to left germinal ridge of recipient embryos in chickens. *J Poultry Sci.* 46:40- 45
- Nakajima, Y., Minematsu, M. Naito and and A. Tajima. (2011). A New Method for isolating Viable Gonadal Germ Cells from 7- day old Chick Embryos. *J. Poult. Sci.* 48, 106- 111
- Nakamura, Y., H. Kagami and T. Tagami. (2013). Development, differentiation and manipulation of Chicken germ cells. *Development, Growth and Differentiation*, vol. 55 (1), 2013, p. 20-40.
- Ohinata, Y., B. Payer, D. O'Carroll, K. Ancelin, Y. Ono, M. Sano, S.C. Barton, T. Obukhanychy, M. Nussenzweig, A. Tarakhovsky, M. Saitou and M.A Surani. (2005). Blimp1 is a Critical Determinant of the Germ Cell Lineage in Mice. *Nature* 436, 207- 213.
- Ono, T., Yokoi, R. and Aoyama, H. (1996). Transfer of Male or Female Primordial Germ Cells of Quail into Chick Embryonic Gonads. *Exp. Anim.* 45, 347- 352.
- Pain, B., M. E. Clark, M. Shen, H. Nakazawa, M. Sakura, J. Samurat and R. J. Etches. (1996). Long- Term in vitro Culture and Characterisation of Avian Embryonic Stem Cells with Multiple Morphogenetic Potentialities. *Development* 122, 2339- 2348.
- Park, TS. Lee, HC, Rengaraj, D., and Han JY (2014). Germ Cell, Stem Cell, and Genomic Modification in Birds. *J Stem Cell Res Ther* 4: 201.doi:10.4172/2157-7633.1000201
- Park, T. S. and J. Y. Han. (2000). Derivation and Characterization of Pluripotent Embryonic Germ Cells in Chicken. *Mol. Reprod. Dev.* 56, 475- 482
- Park, T. S., D. K. Jeong, J. N. Kim, G. H. Song Y. H. Hong, J. M. Lim and J. Y. Han. (2003). Improved Germline Transmission in Chicken Chimeras Produced by Transplantation of Gonadal Primordial Germ Cells into Recipient Embryos. *Biol. Reprod.* 68, 1657- 1662.

- Petitte, J. N., M. E. Clark and R. J. Etches. (1991). Assessment of Functional Gametes in Chickens after Transfer of Primordial Germ Cells. *J. Reprod. Fertil.* 92, 225- 229.
- Resnick, J. L, L. S. Bixler, L. CHENG and P. J. Donovan. (1992). Long- Term Proliferation of Mouse Primordial Germ Cells in Culture. *Nature* 359, 550-551.
- Stebler, J., D. Spieler, K. Slanchev, K. A. Molyneaux, U. Richter, V. Cojocar, V. Tarabykin, C. Wylie, M. Kessel and E. Raz. (2004). Primordial Germ Cells Migration in the Chicken and Mouse Embryo: The Role of the Chemokine SDF-1/CXCL 12. *Dev. Biol.* 272, 351- 361.
- Swift, C. H. (1915). Origin of the Definitive Sex Cells in the Female Chick and their Relation to the Primordial Germ Cells. *Am. J. Anat.* 18, 441–470.)
- Swift, C. H. (1916). Origin of the Sex- cords and Definitive Spermatogonia in the Male Chick. *Am. J. Anat.*, 20:375- 410.
- Tajima, A., M. Naito, Y. Yasuda, and Kuwana, T. (1993). Production of germ line chimera by transfer of primordial germ cells in the domestic chicken (*Gallus domesticus*). *Theriogenology*, 40: 509–519.
- Thomson, J.A., J. Itskovitz- Eldor, S. S. Shapiro, M. A. Waknitz, J. J. Swiergiel, V. S. Marshall and J. M. Jones. (1998). Embryonic Stem Cell Lines derived from Human Blastocysts. *Science* 282, 1145- 1147.
- Tilgner, K. Stuart, P.A., Golebiewska, A. Stojkovic, M., Lako, M. and Armstrong, L. (2008). Isolation of Primordial Germ Cell from Differentiating Human Embryonic Stem Cell. *STEMCELLS* 2008; 26: 3075- 3085.
- Tsenekawa, N., Naito, M., Sakai, Y., Nishida, T. and Noce, T. (2000). Isolation of Chicken *vasa* Homologue Gene and Tracing the Origin of Primordial Germ Cells. *Development* 127, 2741- 2750.
- Ukeshima, A. (1996). Germ Cell Death in the Degenerating Right Ovary of the Chick Embryo. *Zool. Sci.* 13,559–563.
- Urven, L.E., U. K. Abbott and C. A. Erickson. (1989). Distribution of Extracellular Matrix in the Migratory Pathway of Avian Primordial Germ Cells. *Anat. Rec.* 224, 14-21.
- Van De Lavoie, M. C., J. H. Diamond, P. A. Leighton, C. Mather- Love, B. S. Heyer, R. Bradshaw, A. Kerchner, L. T. Hooi, T. M. Gessaro, S. E. Swanberg, M. E. Delany and R. J. Etches. (2006). Germline Transmission of Genetically Modified Primordial Germ Cells. *Nature* 441, 766- 769.

- Yamaji, M., Y. Seki, K. Kurimoto, Y. Yabuta, M. Yuasa, M. Shigeta, K. Yamanaka, Y. Ohinata and M. Saitou. (2008). Critical Function of *Prdm14* for the Establishment of the Germ Cell Lineage in Mice. *Nat Genet.* 40, 1016- 1022.
- Zaccanti F., M. Vallisneri and A. Quaglia, (1990). Early aspects of sex differentiation in the gonads of chick embryos. *Differentiation*, 43: 71- 80
- Zhao, D.F., and T. Kuwana, (2003). Purification of avian circulating primordial germ cells by Nycodenz density gradient centrifugation. Department of Materials and Life Science, Graduate School of Science and Technology, Kumamoto University, Kumamoto and 2Pathology Section, National Institute for Minamata Disease, Minamata City, Kumamoto, Japan