

**INITIAL STUDY OF PHILIPPINE NATIVE PIG (*Sus scrofa*) POST MORTEM  
EPIDIDYMAL SPERM MORPHOMETRY IN BANTUG  
SCIENCE CITY OF MUÑOZ NUEVA ECIJA**

**GABRIELLE SIMONNE TEODORO OLIVERIA**

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## **BIOGRAPHICAL SKETCH**

Gabrielle Simonne Teodoro Oliveria was born on September 19, 1996 in Olongapo City, Zambales. She graduated elementary from Olongapo Wesley School in 2009 and she graduated on March 2013 in Juan R. Liwag Memorial High School. Also in the same year she entered college and took her premed course, Bachelor of Science in Animal Husbandry and graduated in the year of 2018 at Central Luzon State University, Science City of Muñoz, Nueva Ecija. Currently she is taking up Doctor of Veterinary Science and Medicine in CLSU. She had her On-the-Job training at Brookdale Farms Corporation, Bamban, Tarlac from June 16, 2015 to July 20, 2015.

Aside from academic involvement, he is also a member of the Junior Clinicians' Club, 2017-2018 and Senior Clinicians' Club, 2018-2019.

She also attended several seminars including Virbac Pet Wellness at CVSM ODQ hall, Central Luzon State University on November 21, 2018. Philippine Gamefowl Industry: "An Overview and Disease Management", held at AMC hall, CLSU on November 14, 2018. The Role of Probiotics in Animal's Health at CVSM ODQ hall, CLSU on October 7, 2018. Philippine Poultry Production: "Status, Trends and Outlook", at AMC hall, CLSU on October 24, 2018. Overview of Poultry Breeding Generations held CVSM ODQ hall, CLSU on October 17, 2018. Swine Industry: "Challenges over the Decades", held at CVSM ODQ hall, CLSU on October 3, 2018. Practical Swine Management held at CVSM ODQ hall, CLSU on September 19, 2018 and Goat and Sheep Industry in the Philippines: "An Update", at CVSM ODQ hall, CLSU on September 12, 2018.

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## ABSTRACT

**OLIVERIA, GABRIELLE SIMONNE T.**, College of Veterinary Science and Medicine, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines, **June 2019, INITIAL STUDY OF PHILIPPINE NATIVE PIG (*Sus scrofa*) POST MORTEM EPIDIDYMAL SPERM MORPHOMETRY IN BANTUG SCIENCE CITY OF MUÑOZ NUEVA ECIIJA**

Adviser: DARLENE FE P. CASTRO, DVM, MSc

Co-adviser: MARLON B. OCAMPO, DVSM, Ph.D.

Sperm morphometry has been extensively studied and proven to be important in assessing reproductive parameters. However, there are no studies yet on Philippine native pig epididymal sperm. This study established data about the morphometry of fresh and refrigerated epididymal spermatozoa of mature native pig using computer assisted measurements.

The recovery of epididymal sperm from the cauda of post-mortem testicles of six (6) Philippine native pigs was elucidated in this study. The effect of handling conditions in ice-cooled temperature of 4°C on epididymal sperm viability was also evaluated. Epididymal sperm from native pigs was collected using Minitube III and BTS extender and was immediately recovered after death ( $\leq$  1hour) in treatment 1 at 30-32°C handling temperature then after 24 hours post-mortem in Treatment 2 maintained at refrigeration temperature (4°C). The different morphometric parameters measured included sperm head length, width, area, perimeter and diameter, tail length, mid piece, flagellum and sperm total length. Results were analyzed using one-way ANOVA with post hoc test of LSD at 5% level of significance.

The results of this study showed slightly lower values on morphometric measurements from refrigerated epididymal sperm compared to ejaculated sperm from hybrid pigs such as Polish Landrace. However, statistical analysis between fresh epid sperm and refrigerated epid sperm of Native pig, and ejaculated sperm from Polish Landrace showed little to no significant difference. This would mean that native pigs aged one-year old may be ready for collection of sperm for artificial insemination.

Keywords: Epididymal sperm; Morphometry; Philippine native pig

## LITERATURE CITED

- Abu, A.H., Kisani, A.I., & Ahemen, T. (2016). Evaluation of sperm recovered after slaughter from cauda epididymides of Red Sokoto bucks. *Veterinary World*, 9(12), 1440-1444.
- Amman, R.P. (2009). Considerations in evaluating human spermatogenesis on the basis of total sperm per ejaculate. *Journal of Andrology*, 30(60), 626-41.
- Armenia, M.R.A.E., Mercado, R.E. & Mercado, J.O. (2016). Production and management practices of backyard swine raisers in the three selected municipalities of Surigao del Sur. *Journal of Scientific Research and Development*, 3(7), 54-58.
- Ayalew, W., Rege, J.E.O., Getahun, E., Tibbo, M., & Mano, Y. (2003). Delivering systematic information on indigenous animal genetic resources-the development and prospects of DAGRIS. University of Gittingen, Deutschar,1.
- Bettencourt, E.M.V., Tilman, M., Narciso, V., Carvalho, M.L.D.S., & Henriques P.D.D.S. (2015). The livestock roles in the wellbeing of rural communities of Timor-Leste. *Revista de Economia e Sociologia Rural*, 53(1), 63-80.
- Bondoc, O.L., Dominguez, M.D., & Peñalba, F.F. (2013). DNA barcoding of domestic swine breeds and crossbreeds (*Sus scrofa*) in the Philippines. *Philippine Journal of Veterinary and Animal Sciences*, 39(1), 31-42.
- Bondoc, O.L. & Ramos, S.M. (1998). Findings of a survey of native pigs in some provinces of the Philippines. *Philippines Journal of Veterinary and Animal Sciences*, 21(1), 1-16.
- Brion, A.C.B. (2017). Raising native animals. Retrieved from: <http://businessdiary.com.ph/6301/raising-native-animals/#ixzz4f2NVWeE6> on April 23, 2017.
- Broekhuijse, M.L.W.J., Feitsma, H. & Gadella, B.M. (2012). Artificial insemination in pigs: predicting male fertility. *Veterinary Quarterly*, 32(3), 151-157.
- Brooks, J.W. (2016). Postmortem changes in animal carcasses and estimation of the postmortem interval, *Veterinary Pathology*, 53(5), 929-940.

- Calamera, J.C., Fernandez, P.J., Buffone, M.G., Acosta, A.A. & Doncel, G.F. (2001). Effects of long-term incubation of human spermatozoa: functional parameters and catalase effect. *Andrologia*, 33(1), 79-86.
- Cerovsky, J., Frydrychova, S., Lustykova, A., & Rozkot, M. (2005). Changes in boar semen with a high and low level of morphologically abnormal spermatozoa. *Czech Journal Animal Science*, 50(7), 289-299.
- Cooper, T.G. (2005). Cytoplasmic droplets: the good, the bad or just confusing? *Human Reproduction*, 20(1), 9-11.
- Cooper, T.G. (2010). The epididymis, cytoplasmic droplets and male infertility. *Asian Journal of Andrology*, 13(1), 130-138.
- Chang, M.C. (1943). Disintegration of epididymal spermatozoa by application of ice to the scrotal testis. School of Agriculture, Cambridge University, 16-22.
- David, I., Kohnke, P., Lagriffoul, G., Pratud. O., Plouarboue, F., Degond, P. & Druart, X. (2015). Mass sperm motility is associated with fertility in sheep. *Animal Reproduction Science*, 161(1), 75-81.
- De Ambrogi, M., Ballester, J., Saravia, F., Caballero, I., Johannisson, A., Wallgren, M., Andersson, M., RodriguezMartinez, H. (2006). Effect of storage in short- and long-term commercial semen extenders on the motility, plasma membrane and chromatin integrity of boar spermatozoa, *International Journal of Andrology*, 29(1), 543-552.
- Donadeu, M. (2004). Advances in male swine artificial insemination (AI) techniques. *The Pig Journal*, 54(1), 110-122.
- Elia, J., Imbrogno, N., Delfino, M., Mazzilli, R., Rossi, T. & Mazzilli, F. (2010). The importance of sperm motility classes- future directions. *The Open Andrology Journal*, 2(1), 42-43.
- Food and Agriculture Organization. (2012). Cryoconservation of animal genetic resources. FAO Animal Production and Health Guidelines, 12.
- Frye, C. A. (2014). Chapter three-endocrine-disrupting chemicals: elucidating our understanding of their role in sex and gender-relevant end points. *Vitamines and Hormones*, 94(1), 41-98.
- Gadea, J. (2002). Sperm under the microscope. *Pig International*, 32(1), 24-27.

- Grant, A., 1982. The use of tooth wear as a guide to the domestic ungulates. BAR British Series, 91-108.
- Guerrero, R.D. (2016). Currents. Retrieved from:  
<http://www.pressreader.com/philippines/agriculture/20160201/282458527993394>  
 on April 23, 2017
- Hori, T., Yoshikuni, R., Kobayashi, M. & Kawakami, E. (2014). Effects of storage temperature and semen extender on stored canine semen. *Journal of Veterinary Medical Science*, 76(2), 259–263.
- Jeong, H.S., Kim, D.W., Chun, S.Y., Sung, S., Kim, H.J., Cho, S., Kim, H. & Oh, S.J. (2014). Native pig and chicken breed database: NPCDB. *Asian-Australian Journal of Animal Sciences*, 27(10), 1394-1398.
- Kaabi, M., Paz, P., Alvarez, M., Anel, E., Boixo, J.C., Rouissi, H., Herraiez, P., Anel, L. (2003). Effects of epididymis handling conditions on the quality of ram spermatozoa recovered post-mortem. *Theriogenology*, 60(7), 1249-1259.
- Karageorgiou, M. A., Tsousis, G., Boscós, C. M., Tzika, E. D., Tassis, P. D., Tsakmakidis, I. A. (2016). A comparative study of boar semen extenders with different proposed preservation times and their effect on semen quality and fertility, *Acta Veterinaria Brunensis*, 85(1), 23-31.
- Knox, R.V. (2011). Semen processing, extending & storage for artificial insemination in swine. Department of Animal Sciences, University of Illinois, USA.
- Kondracki, M., Ballester, J., Saravia, F., Caballero, I., Johannisson, A., Wallgren, M., Andersson, M., RodriguezMartinez, H. (2006). Effect of storage in short- and long-term commercial semen extenders on the motility, plasma membrane and chromatin integrity of boar spermatozoa, *International Journal of Andrology*, 29(1), 543-552.
- Laca, E. A. & Demment M. W. (2011). Livestock production systems.management of agricultural, Forestry Fisheries and Rural Enterprises, 275-307.
- Lachaud, C., Tesarik, J., Canadas, M.L. & Mendoza, C. (2004). Apoptosis and necrosis in human ejaculated spermatozoa. *Human Reproduction*, 19(1), 607-610.

- Lemoine, X., Zeder, M. A., Bishop, K.J., & Rufolo, S.J. (2014). A new system for computing dentition-based age profiles in *Sus scrofa*. *Journal of Archaeological Science*, 47(1), 179-193.
- Maddul, S.B. (1991). Production management and characteristics of native pigs in the Cordillera. Philippines University, Los Banos, College, Laguna, Philippines.
- Mamuad, HV, Venturina, EV, Moruso, RT, Atabay, EC and Kudo, K.(2005). Artificial insemination manual for water buffaloes. A Joint-JICA Assisted Project of the Philippine Carabao Center and the Bureau of Animal Industry Philippines.
- Martin-Rillo, S., Martinez, E., Garcia, C., Artiga C., & De Alba C. (1996). Boar semen evaluation in practise. *Reproductive Domestic Animal*, 31(1), 519-526.
- Martins, C.F., Driessen, K., Melo Costa, P., Carvalho-Neto, J.O., De Sousa, R.V., Rumpf, R., Dodec, M.N. (2009). Recovery, cryopreservation and fertilization potential of bovine spermatozoa obtained from epididymides stored at 5°C by different periods of time. *Animal Reproductive Science*, 116(2), 50-57.
- Martinez-Pastor, F., Guerra, C., Kaabi, M., Diaz, A.R., Anel, E., Herraez, P., Anel, L. (2005). Decay of sperm obtained from epididymides of wild ruminants depending on postmortem time. *Theriogenology*, 63(1), 24-40.
- McLeod, T. (2006). Cryopreservation of sperm physiology of animal production. Retrieved from: <http://classes.uleth.ca/200601/biol3850n/sperm.pdf> on April 23, 2017
- Medina, A.L. (2012). Fresh boar semen: quality control and production. Dissertation, Faculty of Veterinary Medicine, Ghent University, 15-21.
- Neumann, C., Harris, D.M., & Rogers, L.M. (2002). Contribution of animal source foods in improving diet quality and function in children in the developing world. *Nutrition Research*, 22(1), 193-220.
- O'Connell, Mc Clure, N. & Lewis, S.E.M (2002). The effects of cryopreservation n sperm morphology, motility and mitochondrial function. *Human Reproduction*, 17(3), 704-709.
- Paras, E. P. & Cordoves, R.K.S. C. (2014). Estimating liveweight of Philippine native pigs using external body measurements. *Philippine Journal Veterinary Animal Science*, 40(1), 47-52.

- Perera, K. U. E., & Ariyaratne H. B. S. (2013). Cryopreservation of goat sperms collected from different regions of the epididymis. *International Journal of Science and Research*, 2(7), 36-38.
- Petrocelli, H., Bautista, C., & Gosalvez, J. (2015). Seasonal variation in sperm characteristics of boar in Southern Uruguay. *Resvista Brasileira de Zootecnia*, 44(1), 1-7.
- Phillips, R.W. & McKenzie, F.F. (1934). Res. Bull. University of Missouri, 217.
- Rolett, B.V. and Chiu. M. (1994). Age estimation of prehistoric pigs (*Sus scrofa*) by molar eruption and attrition. *Journal of Archaeological Science*, 21(1), 377-386.
- Rodriguez, A.L. (2012). Fresh boar semen: quality control and production. Dissertation, Faculty of Veterinary Medicine, Ghent University, 15-21.
- Rozeboom, K.J. (2000). Evaluating semen boar quality. North Carolina Cooperative Extension Service, 1-7.
- Rurangwa, E., Kime, D.E., Ollevier, F. & Nash, J.P. (2004). The measurement of sperm motility and factors affecting sperm quality in cultured fish. *Aquaculture*, 234(1), 1-28.
- Santiago, K. F. (2016). The importance of boar sperm motility and morphology for sperm fertility. *International Pig Topics*, 27(5), 130-145.
- Shipley, C.F. (1999). Breeding soundness examination in the boar. *Swine Health Production*, 7(3), 117- 120.
- Schmid, S., Henning, H., Oldenhof, H., Wolkers, W.F., Petrunkina, A.M., Waberski, D. (2013). The specific response to capacitating stimulus a sensitive indicator of chilling injury in hypothermally stored boar spermatozoa. *Andrology*, 1(1), 376386.
- Soler, A.J, Estes, M.C., Santos, M.R.F., Garde, J.J. (2005). Characteristics of Iberian Red Deer (*Cervus elaphus hispanicus*) spermatozoa cryopreserved after storage at 5°C in the epididymis for several days. *Theriogenology*, 64(7), 1503-1517.
- Steinfeld, H., Gerber, P., Wassenaar, T., Castel, V., Rosales, M., & De Haan, C. (2006).

Livestock's Long Shadow: Environmental Issues and Options. Food and Agriculture Organization of the United Nations, 271.

- Strand, J., Ragborg, M.M., Pedersen, H.S., Kristensen, T.N., Pertoldi, C. & Callesen, H. (2016). Effects of post-mortem storage conditions of bovine epididymides on sperm characteristics: investigating a tool for preservation of sperm from endangered species. *Conservation Physiology*, 4(1), 69.
- Pittarelli, C., Savignone, C.A., Arnaudín, E., Stornelli, M.C., Stornelli, M.A., De La Sota, R.L. (2006). Effect of storage media and storage time on survival of spermatozoa recovered from canine and feline epididymides. *Theriogenology*, 66(7), 1637-1640.
- Valete, E.J.P., Maylem, E.R.S., Ocampo, M.B., Abella, E.A., & Ocampo, L.C. (2015). Post mortem viability of epididymal sperm from Philippine Native Water Buffalo (*Bubalus bubalis*). *Journal of Agricultural Technology*, 11(8), 2283-2294.
- Weitze, K. F. (2012). The importance of boar sperm motility and morphology for fertility. *International Pig Topics*, 27(5), 13-15.
- Xu, H., Yuan, S.Q., Zheng, Z.H. & Yan, W. (2013). The cytoplasmic droplet may be indicative of sperm motility and normal spermiogenesis. *Asian Journal of Andrology*, 15(6), 799-805.