

**SEX DETERMINATION OF CATTLE (*Bos taurus*) EMBRYOS
FOLLOWING IN VITRO FERTILIZATION
USING SEMEN SEXING KIT**

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ABSTRACT

GUZMAN, CHENNY DHANRYLL M., Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines, **JUNE 2019, SEX DETERMINATION OF CATTLE (*Bos taurus*) EMBRYOS FOLLOWING IN VITRO FERTILIZATION USING SEMEN SEXING KIT.**

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It has been claimed that frozen-thawed bovine semen treated with commercially produced kits, Heiferfocus™ (in favor of female gender) and Bullfocus™ (in favor of male gender), increases the birth chance of bovine with desired sex by at least 20–25%. Therefore, this study was conducted to investigate on the efficacy of the semen sexing kits Heiferfocus™ and Bullfocus™, through sex determination by Polymerase Chain Reaction (PCR). Two sets of PCR primers were used to determine the sex; Y-chromosome specific primer (BRY1), and bovine specific satellite sequence primer (SAT1). Results showed that unsexed-sorted sperms tend to have greater fertility rate (58.06%) than those treated with semen sex-sorting kits (Heiferfocus™ with 50.87% and Bullfocus™ with 48.21%). In sex determination, the sex determined by BRY1 in blood DNA samples showed a hundred percent agreement with the animals' anatomical sex. On the other hand, bovine autosomal sequences were amplified in all embryo samples using SAT1. The findings revealed that Heiferfocus™ has female sex ratio of 86.20% while Bullfocus™ has male sex ratio of 33.33%. The present results suggest that treatment of semen with Heiferfocus™, but not with Bullfocus™, numerically increases the desired sex ratio at the rates reported by the manufacturer (20-25%).

LITERATURE CITED

- Aasen, E., & Medrano, J. F. (1990). Amplification of the ZFY and ZFX genes for sex identification in human, cattle, sheep and goats. *Journal of Biotechnology*, (8), 1279-1281.
- Ayman, H., Mahrous, U. E., Kamel, S. Z., & A.Sabek, A. (2016). Factors Influencing in vitro Production of Bovine Embryos: A Review. *Asian Journal of Animal and Veterinary Advances*, (11), 737-756.
- Bras, R. (2010). In vitro embryo production in small ruminants (On-line). Accessed April 06, 2019 at http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1516-35982010001300045.
- Bavister, B. D. (1995). Culture of preimplantation embryos: facts and artifacts. *Human Reproduction Update*, (1), 91-148.
- Borghese, A. (2005). Buffalo Production and Research. *Food and agriculture organization edition REU Technical Series*, (67), 1-315.
- Critser, E. S., Leifried-Rutledge, M. L., Eyestone, W. E., Northy, D. L., & First, N. L. (1986). Acquisition of developmental competence during maturation in vitro. *Theriogenology*, (25), 125.
- Curry, E., Pratta, S., Lapin, D. R., & Gibbons, J. R. (2009). Efficacy of a commercially available post-thaw bovine semen sexing kit in both single-ovulating and hyperstimulated cows. *Animal Reproduction Science*, (116), 376-380.
- Dairy Herd Management (2016). Sexed Semen: History and Potential (On-Line), Dairy Herd Management. Accessed November 12, 2018 at <https://www.dairyherd.com/article/sexed-semen-history-and-potential>.
- Duszevska, A. M., Trzeciak, P., & Rapala, L. (2010). Selected issues concerning biotechnology of farm animals breeding - a review. *Animal Science Papers of Reproduction*, (4), 295-306.
- FAO. (2014). Livestock Population (On-line), Food and Agricultural Statistics. Accessed November 08, 2018 at <http://ref.data.fao.org/dataset?entryId=4813cbb2-21f0-4f6c-acbe-8855b340dadd&tab=metadata>.
- First, N. L., & Parrish, J. J. (1987). In vitro fertilization of ruminants. *Journal of Reproduction Fertility*, (34), 151-165.
- Galli, C., Crotti, G., Notari, C., Turini, P., Duchi, R., & Lazzari, G. (2001). Embryo production by ovum pick-up from live donors. *Theriogenology*, (55), 1341-1357.

- Gandhi, A. P., Lane, M., Gardner, D. K., & Krisher, R. L. (2000). A single medium supports development of bovine embryo throughout maturation, fertilization and culture. *Human Reproduction*, (2), 395-401.
- Gerard, O., Salas-Cortes L., Sellem, E., Marquant-Leguienne, B., Clement, L, Humblot, P. (2008). In vitro assessment of the effectiveness of a commercially available post-thaw bovine semen sexing kit on semen quality parameters, in vitro fertilizing ability and sex-ratio deviation. *Association Europeenne de Transfert Embryonnaire*, (1), 153-154.
- Haag, M., & Dorshorst, N. (2013). In-vitro Fertilization: A new tool for the commercial dairyman (On-line), Progressive Dairyman. Accessed November 08, 2018 at <http://www.progressivedairy.com/topics/a-i-breeding/in-vitro-fertilization-a-new-tool-for-the-commercial-dairyman>
- Hayakawa, H. (2012). Sperm Sexing in Cattle Industry. *Journal of Mammalian Ova Research*, 29(3), 119-123.
- Hirst, K. K. (2017). Dairy Farming: The Ancient History of Producing Milk (On-line), Thoughtco. Accessed August 20, 2018 at <http://www.thoughtco.com/dairy-farming-ancient-history-171199>
- Holm, P., Walker, S. K., & Petersen, B. A. (1994). In vitro vs in vivo culture of ovine IVM/IVF ova: effect on lambing. *Theriogenology*, (41), 217.
- Huffman, B. (2000). Artiodactyla (On-line). Accessed May 03, 2019 at <http://www.ultimateungulate.com>.
- Iritani, A., & Niwa K. (1977). Capacitation of bull spermatozoa and fertilization in vitro of cattle follicular oocytes matured in culture. *Journal of Reproduction Fertility*, 50(1), 119-21.
- Izquierdo, D., Villamediana, P., & Lopez-Bejar, M. (2002). Effect of *in vitro* and *in vivo* culture on embryo development from prepubertal goat IVM/IVP oocytes. *Theriogenology*, (57), 1431-1441.
- Kitiyant, Y., Saikhun, J., Siriaronrat, B., & Pavasuthipaisit, K. (2000). Sex Determination by Polymerase Chain Reaction and Karyotyping of Bovine Embryos at First Cleavage in vitro. *Science Asia*, 26(2000), 9-13.
- Lee, E. S., Fukuyui, Y., Lee, B. C., Lim, J. M., & Hwang, W. S. (2004). Promoting effect of amino acids added to a chemically defined medium on blastocyst formation and blastomere proliferation of bovine embryos cultured in vitro. *Animal Reproductive Science*, (84), 257-267.

- Liang, X. W., Lu, Y. Q., Chen, M. T., Zhang, X. F., Lu, S. S., Zhang, M., & Lu, K. H. (2008). In vitro embryo production in uffalo (*Bubalus bubalis*) using sexed sperm and oocytes from ovum pick up. *Theriogenology*, 69(2008), 822-826.
- Lopatarova, M., Krontorad, P., Holy, L., & Zajic, J. (2006). Determining the sex of early bovine embryo in practical conditions. *Veterinarstvi*, (56), 696-699.
- Lu, K.H., Gordon, I., Gallagher, M., & McGovern, H. (1987). Pregnancy established in cattle by transfer of embryos derived from in vitro fertilisation of oocytes matured in vitro. *Veterinary Record*, 121(11), 259-260.
- Mara, L., Pilichi, S., Sanna, A., Accardo, C., Chessa, B., Chessa, F., & Cappai, P. (2004). Sexing of In Vitro Produced Ovine Embryos by Duplex PCR. *Molecular Reproduction and Development*, (69), 35-42.
- Merlo, B., Lacono, E., Zambelli, D., Prati, F., & Belluzzi, S. (2005). Effect of EGF on in Vitro Maturation of Domestic Cat Oocytes. *Theriogenology*, 63(7), 2032-2039.
- Mctavish, E. J., Decker, J. E., Schnabel, R. D., Taylor, J. F., & Hillis, D. M. (2013). New World cattle show ancestry from multiple independent domestication events. *Proceedings of the National Academy of Sciences*, 110(15), 1398-1406.
- Ng, J., & Dewey, T. (2001). *Bos taurus aurochs* (Also: domesticated cattle) (On-line). Accessed May 03, 2019. at <http://userpages.umbc.edu/~dschmi1/cows/facts.html>.
- Nowak, R. 1997. Mammals of the World (On-line). Accessed May 03, 2019 at http://www.press.jhu.edu/books/walkers_mammals_of_the_world/artiodactyla/artiodactyla.bovidae.bos.html.
- Peippo, J., Viitala, S., Virta, J., Raty, M., Tammiranta, N., Lamminen, T., Aro, J., Myllymaki, H., & Vilkki, J. (2007). Birth of correctly genotyped calves after multiplex marker detection from bovine embryo microblade biopsies. *Molecular Reproduction and Development*, (74), 1373-1378.
- Pivko, J., Grafenau, P., & Kopecny, V. (1995). Nuclear fine structure and transcription in early goat embryos. *Theriogenology*, (21), 126-137.
- Plucienniczak, A., Skowronski, J., & Jaworski, J. (1982). Nucleotide sequence of bovine 1.715 satellite DNA and its relation to other bovine satellite sequences. *Journal of Molecular Biology*, 158.
- Pope, C. E., Gomez, M. C., & Dresser, B. L. (2006). In Vitro Embryo Production and Embryo Transfer in Domestic and Non -Domestic Cats. *Theriogenology*, 66(6), 1518-1524.
- Rath, S. (1998). *The Complete Cow*. Vancouver B.C: RAINCOAST BOOK.

- Rao, K., Pawshe, C., & Totey, S. (1993). Sex Determination of In Vitro Developed Buffalo (*Bubalus bubalis*) Embryos by DNA Amplification. *Molecular Reproduction and Development*, (36), 292-296.
- Reprogen, 1997. Domestic Cattle (On-line). Accessed April 06, 2019 at <http://www.hensonrobinsonzoo.org/p001.html>.
- Rhinehart, J. (2016). How it Works: Sex Sorted Semen (On-line). Accessed May, 11, 2019 at <https://www.agweb.com/article/how-it-works-sex-sorted-semen-naa-university-news-release>.
- Selokar, N. L., Saha, A. P., Saini, M., Muzaffar, M., Chauahan, M. S., Manik, R. S., Palta, P., & Singla, S. K. (2012). A protocol for differential staining of inner cell mass and trophectoderm of embryos for evaluation of health status. *Current Science*, 102(9), 1256-1257.
- Seidel, G. E., & Garner, D. I. (2002). Current status of sexing mammalian spermatozoa. *Reproduction*, (124), 733-743.
- Seidel, G. E., & Jr, Johnson, L. A. (1999). Sexing mammalian sperm overview. *Theriogenology*, (52), 1267-1272.
- Shekalogorabi, S. J., Maghsoudi, A., & Mansouria, M. R. (2017). Reproductive performance of sexed versus conventional semen in Holstein heifers in various semiarid regions of Iran. *Italian Journal of Animal Science*, (15), 1-7.
- Sirard, M. A., Lambert, R. D., Menard, D. P., & Bedoya, M. (1985). Pregnancies after in-vitro fertilization of cow follicular oocytes, their incubation in rabbit oviduct and their transfer to the cow uterus. *Journal of Reproduction Fertility*, 75(2), 551-556.
- Souza, A. (2014). In Vitro Embryo Production: Technology that came to Stay. *Journal of Dairy Science*, (5), 12-14.
- Suthar, V. S., & Shah, R. G. (2009). Bovine In Vitro Embryo Production: An Overview. *Veterinary World*, 2(12), 478-479.
- Thibier, M. (2004). Stabilization of numbers of in vivo collected embryos in cattle but significant increases of in vitro bovine produced embryos in some parts of the world. *Data Retrieval Committee Annual Report IETS Newslett*, 22(4), 12-9.
- Turk, G., Yuksel, M., Sonmez, M., Gur, S., Kaya, S. O., & Demirci, E. (2015). Effects of semen sexing kits (HeiferplusTM and BullplusTM) supplemented to frozen-thawed bull semen. *Veterinari Medicina*, (6), 309-313.
- Walker, E. F. (1975). *Mammals of the World*. London: THE JOHNS HOPKINS UNIVERSITY PRESS.

- Waurich, R., Ringleb, J., Braun, B. C., & Jewgenow, K. (2010). Embryonic Gene Activation in in Vitro Produced Embryos of the Domestic Cat (*Felis Catus*). *Reproduction*, 14 (4), 531 –540.
- Williams, T. (2007). The use of HeiferPLUS in superovulated heifers (On-line). Accessed November 14, 2018 at <http://www.emlabgenetics.com/heiferplusdata>.
- Xu, K. P., & King, W. A. (1990). Effects of oviductal cells and heparin on bovine sperm capacitation in vitro. *Biology of Reproduction*, (42), 89-90.