Epigenetic changes during male embryonic development and the spermatogenic process of the porcine germline cells *in vivo*

N.C.G. Pieri[1], M.H. Glória[1], R.V.G. Castro[2], A.F. Souza[3], D.S. Martins[3], F.V. Meirelles[3], F.F. Bressan[3], A.F.C. de Andrade[1]

[1]Swine Research Center, Faculty of Veterinary Medicine and Animal Sciences; São Paulo, SP, Brazil; [2]Department of Preventive Veterinary Medicine and Animal Reproduction, Faculty of Agricultural and Animal Science, State University of Sao Paulo, Jaboticabal/SP, Brazil; [3]Department of Veterinary Medicine, Faculty of Animal Sciences and Food Engineering, University of São Paulo, Pirassununga, SP, Brazil.

Primordial germ cells (PGCs) are the precursors of gametes, these cells approach the epiblast region and migrate to the mesenchymal gonads, forming the genital ridges. PGCs undergo several essential epigenetic, genetic and morphological changes during embryonic development. For this reason, these cells have been studied in several species, including pigs, in order to identify, correct and understand the changes they undergo during the process of forming a new individual and to improve their development and reproductive performance. The objective of the present study is to analyze the dynamics of epigenetic markers and the factors associated to the development of Primordial Germline Cells (PGCs) in male porcine, from the beginning of embryonic development until the spermatogenic process. Different gestational age (24, 26, 29, 35 e 40 days) porcine embryos were collected from pregnant sows artificially inseminated, furthermore, testis tissues were obtained from neonate and adult pigs. Sex identification was performed through Polymerase Chain Reaction (PCR) and electrophoresis in agarose gel using the Sry and Zfy-Zfx genes. The samples identified as male were collected and analyzed for the expression of epigenetic, pluripotent and germline markers (POU5F1, DDX4, DAZL, STRA8, 5mC, 5hC, H3K9me2 and H3K27me3) by microscopy and immunofluorescence techniques. These results showed, there are evident morphological differences among the pig embryonic ages, occurring the initiation of the genital ridge development about day 24 after fertilization (AF) until day 29 AF, when differentiation of the primitive gonads takes place and the sexual dimorphism is noticed by 35 days AF. 40 days AF seminiferous cords are formed and give rise to the seminiferous tubules after birth. Between day 24 – 29 the PCGs arrive to the genital ridge and a quick loss of the histone methylation (H9K3me2/me3, H3K27me3) occurs, although this event is followed by a global methylation and demethylation. After birth a new epigenetic modification occurs in the testicles with the activation of histone H3K9me2, a repressor marker with high stability. Later on, in the adult testis H3K9me2 repression is detected while the histone H3K27me3, a repressive marker with apparent plasticity, increases. Based histological analysis and immunofluorescence of the pig embryos at different gestational ages analysis in this study, it was observed that although the majority of migration patterns of CGPs in mice are found in pigs, there are some differences about the embryonic development, emphasizing the importance of further studies on the particularities of each specie so it will be possible to improve the cell culture techniques and to enable the prevention and treatment of reproductive problems and support studies involving gametogenesis of those species *in vitro*.

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E-mail: nairagodoy@gmail.com