CHAPTER I
INTRODUCTION

1.1 Background and significance

Cloning is a useful technology for basic and applied research in agriculture and biomedicine. Especially, porcine cloning has been studied for xenotransplantation or "pig-to-human organ transplantation" due to the similar physiology, immunology and anatomy of pigs and humans (Prather et al., 2003; Whyte and Prather, 2011). The low numbers of live offspring of cloned animals were observed with many defects, including large offspring syndrome (LOS) as well as placental abnormalities, an enlarged placenta and failure acute respiratory (Loi et al., 2016). Cloning by somatic cell nuclear transfer (SCNT) is a powerful technology that allows the reprograming of terminally differentiated cells into totipotent cells (Gurdon and Wilmut, 2011). To date, a global effort of research has improved the efficiency of cloning, but the efficiency remains extremely low due to incomplete epigenetic reprogramming of donor cells or donor nuclei. (Dean et al., 2001; Matoba and Zhang, 2018; Simmet et al., 2020; Wang et al., 2020).

Small molecules such as Trichostatin A (TSA), Reversine and SAHA have been used to improve epigenetic reprogramming and the developmental capacity of porcine cloned embryos (Jeong et al., 2021; Miyoshi et al., 2010; Whitworth et al., 2015; Sun et al., 2020). The 2-(4-morpholinoanilino)-6-cyclohexylamino-purine analogue known as Reversine was reported to have a concentration of 1–10 μM, which can induce myogenic progenitor cells to become multipotent mesenchymal progenitor cells that can proliferate and re-differentiate into bone cells (osteoblast) and fat cells (adipocytes) (Chen et al., 2004). A previous report found that Reversine can inhibit nonmuscle myosin II (NMMII) and mitogen-activated protein kinase (MEK)1 barriers, enhancing the hyperacetylation that is associated with transcriptional activity and euchromatin maintenance in mammalian cells (Chen et al., 2007). Reversine can modulate the development of cloned embryos in many species, such as miniature pigs (Miyoshi et al., 2010) and cattle (Yoisungnern et al., 2011). Suberoylanilide hydroxamic acid (SAHA) commonly known as histone deactylase inhibitors (HDACi), SAHA could enhance the developmental efficiency of clone embryos in many species, including mice (Ono et al., 2010), pig (Whitworth et al., 2015; Sun et al., 2022) and cattle (Yoisungnern et al., 2012). Several studies have reported positive effects of

Reversine and SAHA promotes histone acetylation on developmental competence in cloned embryos. However, it is necessary to investigate the mechanisms of action of these novel compounds in activation and *in vitro* culture on the improvement of porcine cloned embryos development. In the present study, we investigated the optimal concentration and duration of Reversine and SAHA treatments on development of porcine cloned embryos (cleavage rate, blastocyst formation rate, total cell number) and confirmed the effects of Reversine and SAHA on changing of development and epigenetic reprogramming during porcine SCNT embryos development using quantitative polymerase chain reaction (qPCR) and Immunocytochemistry staining (ICC).

1.2 Research objectives

1.2.1 To investigate the effects of Reversine and SAHA treatments on the development of porcine cloned embryos.

1.2.2 To investigate the effects of Reversine and SAHA treatments on the levels of specific genes related to epigenetic reprogramming of porcine cloned embryos.

1.2.3 To investigate the effects of Reversine and SAHA treatments on the histone acetylation, histone methylation and global DNA methylation of porcine cloned embryos.

1.2 Research hypothesis

1.3.1 Optimal concentration and duration of Reversine and SAHA supplemented in activation and culture medium can enhance cleavage rate, blastocyst formation rate and total cell number in porcine cloned embryos compared to a group without treatments.

1.3.2 Optimal concentration and duration of Reversine and SAHA supplemented in activation and culture medium can enhance the levels of specific genes related to epigenetic reprogramming in porcine cloned embryos compared to a group without treatments.

1.3.3 Optimal concentration and duration of Reversine and SAHA supplemented in activation and culture medium can increase histone acetylation but reduce histone methylation and global DNA methylation of porcine cloned embryos compared to a group without treatments.

1.4 Scope and limitations of the study

To find the optimal concentration and duration of Reversine and SAHA supplemented in activated and IVC medium on *in vitro* development porcine SCNT embryos. Subsequently, the optimal conditions of Reversine and SAHA were chosen and used for further investigations. Then, porcine embryos derived from IVF and SCNT with and without treatment at PN, 2-, 4-, 8-cell and blastocyst stages were evaluated the effects of Reversine and SAHA on development and epigenetic reprogramming using quantitative polymerase chain reaction (qPCR) and Immunocytochemistry staining (ICC).

1.5 Research methodology

1.5.1 Instrumentation

The provider of all the supplies and instruments was Embryo Technology and Stem Cell Research Center, School of Biotechnology, Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima, Thailand.

1.5.2 Location of research

The experiments were conducted at Embryo Technology and Stem Cell Research Center, School of Biotechnology, Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima, Thailand.