CHAPTER I

INTRODUCTION

Rationale

Mammals can be cloned following the transfer of somatic nuclei into enucleated oocytes. This suggests that genome of many types of differentiated somatic cells can be reset to a pluripotent state so far as it can direct development of embryos containing many types of differentiated cells. However, as nuclear functions become specialized in the differentiated cells used as donor nuclei, successful cloning becomes more difficult. In the domestic species, successfully cloned animals such as sheep, cattle, goat, pig or rabbit, the overall efficiency does not exceed 3% (Wilmut et al, 1997; Kato et al., 1998; Baguisi et al., 1999; Polejaeva et al., 2000; Chesne et al., 2002).

The occurrence of low implantation rate, associated with persistent high rates of fetal and perinatal mortality which are specific traits of somatic cloning (Heyman et al., 2002), have considerably stimulated interest in the mechanisms involved in reprogramming and the developmental rate of nuclei introduced into oocytes. It is known that the use of metaphase II oocytes as recipient cytoplast generally results in a higher *in vitro* development of the reconstructed embryos than when using older stages of oocytes. In contrast, the use of older stages of oocytes as recipient cytoplast frequently results in a higher *in vitro* development of the reconstructed embryos than when using metaphase II oocytes reconstructed with progressive stages of donor nuclei (Du et al., 2002; Kurosaka et al., 2002). A better understanding of cell cycle compatibility between the recipient cytoplasm and the donor cell already allowed

some improvements in terms of *in vitro* development of embryos. In the case of nuclear transferred embryos derived from somatic cells, the properties of donor nuclei have to become like those of the normal zygotic nuclei. The replication phase may be of importance in this process, since it corresponds to as phase where the chromatin structure becomes opened, allowing the access to replication.

Bovine and goats are interesting models because of kinetics of development of early embryos and are interesting species for establishing nuclear transfer (NT) technique in Thailand. In this dissertation, in cattle, the influence of the different state of recipient cytoplasts on the onset, the duration of the first DNA replication and *in vitro* developmental potential in bovine species were investigated. In goats, immature and matured oocytes were collected by laparotomy from the donor goats to determine the efficiencies of collection methods on the numbers and quality of oocytes recovered, in order to further use in NT program. Ear skin fibroblasts derived from a female Native goat were used as donor cells to determine whether they could be reprogrammed. According to activation that is an important step of NT procedure, different activation protocols were used to derive a reliable activation protocol for producing goat NT embryos, which have a normal development. Reconstructed embryos were transferred into recipients to determine whether the embryos produced by NT were able to develop to term.

Objectives

The first phase in cattle:

1. To investigate the onset and the length of DNA replication during the first cell cycle in 1-cell stage of somatic NT embryos produced from the different state of recipient cytoplasts.

2. To evaluate the developmental potential of somatic NT embryos produced from the different state of recipient cytoplasts.

The second phase in goats:

3. To establish somatic nuclear transfer technology in goats in Thailand by applying what has been developed in cattle.

4. To evaluate *in vitro* and/or *in vivo* developmental potential of somatic NT embryos produced from the different state of recipient cytoplasts.

5. To determine the post-effect of repeated surgical oocyte collection on pregnancy rate of donor goats.

Hypothesis

The kinetics of DNA replication during the 1-cell stage correlates to the developmental potential of bovine somatic NT embryos. It can be affected by the state of recipient cytoplasts. The development of goat NT embryos can be affected by activation protocols. There is no effect of repeated oocyte collection on subsequent pregnancy.

Key words: activation, cattle, DNA replication, embryo, goats, somatic nuclear transfer, repeated oocyte collection, pregnancy

Research Merit:

1. The information would provide insight into the epigenetic and reprogramming events affecting the developmental potential of NT embryos.

2. The results may suggest new approaches for improving the efficiency and success of nuclear transfer procedure, which becomes a necessity when considering the economic and medical application of widespread cloning of domestic animals by nuclear transfer from differentiated donor cells.

3. The results would provide basic information on oocyte collection and somatic nuclear transfer in goats in Thailand.