# CHAPTER I
# INTRODUCTION

## 1.1 Background and significance

Cryopreservation is an important technique in assisted reproductive technologies (ARTs). In addition to preserving livestock animals and endangered genotypes, cryopreservation allows the store oocytes or embryos for later use (Woods et al., 2004). In livestock, one cryopreservation benefit is that it allows for oocyte and embryo transport over long distances, which can be more practical and cost-effective than live-animal transportation. Vitrification, a rapid method of cryopreservation associated with creating an amorphous, non-crystalline solid, successfully prevents the formation of intracellular and extracellular ice crystals (Rall & Fahy, 1985). In the fields of oocyte and embryo cryopreservation, the approach of vitrification methods has recently become dominant because the procedure is relatively simple and efficient, with higher post-thaw survival rates for oocytes and embryos compared to slow-freezing methods.

However, cryopreserved *in vitro* produced (IVP) embryos have lower survival rates than *in vivo*-derived embryos, as indicated by post-warming survival in culture or successful pregnancies after embryo transfer (Kaidi et al., 1998; Lonergan et al., 2003; Massip et al., 1995). This is related to the formation of damaging reactive oxygen species (ROS), which causes oxidative stress, mitochondrial damage, a shift in calcium oscillation during fertilization, apoptosis, and embryonic development failure. As a result, cells have evolved adaptations to utilize the reactive nature of ROS for beneficial purposes while avoiding their negative impacts (Morado et al., 2009). The female reproductive system has a variety of oxygen scavengers that shield oocytes and embryos from oxidative damage (Guerin et al., 2001). Nevertheless, *in vitro* systems, oocytes, and embryos lack these natural defenses and are constantly exposed to a

variety of stressors (Guerin et al., 2001; Marques et al., 2007). To optimize embryo development *in vitro*, one option is to supplement the culture media with antioxidant molecules that can reduce ROS formation, neutralize it, and help cells repair ROS-induced damage.

In recent years, there have been reports focused on several substances, such as antioxidants, to prevent cell damage caused by free radicals, such as enzymes, thiol compounds, vitamins, flavonoids, amino acids, and amino acid derivatives. Resveratrol (3,4,5-trihydroxy-trans-stilbene), a well-known antioxidant, has been reported to reduce oocyte damage from vitrification injuries and improve blastocyst formation rate in various species, including mice, goats, pigs, and cattle (Piras et al., 2019; Itami et al., 2015; Chinen et al. 2020). Due to their physical and molecular features, resveratrol is a phytoalexin with intra- and extracellular antioxidant potentials (Gambini et al., 2015). In cattle, resveratrol-supplemented *in vitro* maturation medium (IVM) improved cumulus expansion, polar body formation, blastocyst rate, and the number of cells in blastocysts while reducing ROS levels and increasing glutathione (GSH) levels (Wang et al., 2014). In previous studies in our laboratory, supplementing resveratrol in the culture medium, and vitrification solution improved the cryotolerance of mouse embryos and altered the expression of apoptotic and implantation genes (Puangjit, 2019). Likewise, the addition of resveratrol in short-term recovery culture before *in vitro* fertilization (IVF) resulted in improved blastocyst formation rate and reduced oxidative stress (Chinen et al., 2020). Consistent with these findings, it has also been reported that adding 0.5 μM resveratrol to the embryo culture medium can improve the cryotolerance of embryos. (Salzano et al., 2014). This study aimed to investigate whether 0.5 μM resveratrol supplementation *in vitro* culture (IVC) and post-warming culture media affects the survivability and gene expression of post-warmed vitrified bovine embryos. Additionally, to evaluate the developmental competence (based on blastocyst yield) and quality of blastocysts by counting total cell numbers through IVF and IVC.

## 1.2 Research objective

1.2.1 To evaluate developmental competence and quality of IVF-derived bovine embryos treated with 0.5 μM resveratrol in IVC medium.

1.2.2 To investigate the effects of 0.5 μM resveratrol in IVC and post-warming culture media on the survivability of post-warmed vitrified bovine embryos.

1.2.3 To examine the effects of 0.5 μM resveratrol in IVC and post-warming culture media on gene expression of post-warmed vitrified bovine embryos.

## 1.3 Research hypothesis

1.3.1 Resveratrol in IVC medium could enhance developmental competence after IVF and improve the quality of bovine blastocysts.

1.3.2 Resveratrol in IVC and post-warming culture media could increase the survival rate of post-warmed vitrified blastocysts.

1.3.3 Resveratrol supplementation in both the IVC and post-warming culture media could enhance cell programming, pluripotency, embryonic quality, mitochondrial activity, and the expression of stress response-related genes.

## 1.4 Scope and limitations of the study

1.4.1 The effects of 0.5 μM resveratrol supplementation IVC medium on the developmental competence and quality of bovine embryos through IVF and IVC were examined until the blastocyst stage.

1.4.2 The effects of 0.5 μM resveratrol supplementation in IVC and post-warming culture media on the survivability of post-warmed vitrified bovine embryos were investigated, with survival, re-expansion, and hatching rates assessed at 24 and 48 h post-warming.

1.4.3 The effect of 0.5 μM resveratrol supplementation in IVC and post-warming culture media on the gene expression of post-warmed vitrified bovine embryos was examined using quantitative PCR (qPCR).

## 1.5 Research methodology

### 1.5.1 Instrumentation

The Embryo Technology and Stem Cell Research Center (ESRC) and Laboratory Service Unit, The Center for Scientific and Technological Equipment, Suranaree University of Technology, Nakhon Ratchasima, Thailand, supplied all their instruments and materials.

### 1.5.2 Location of research

The experiments were conducted at ESRC, Suranaree University of Technology, Nakhon Ratchasima, Thailand.