CHAPTER I
INTRODUCTION

1.1 Background

In the past, cattle production industry was highly popular due to the commercial value of cattle as a source of meat and milk. Assisted reproductive technologies (ART) have played a crucial role in enhancing the efficiency of bovine embryo production, particularly in processes of *in vitro* embryo production (IVP) and cryopreservation by vitrification technique. Several researches have effectively enhanced the efficiencies of ART, increasing the number of viability oocytes and transfer embryos (Milachich and Shterev, 2016). However, the efficiency of ART is influenced by the environmental conditions during *in vitro* embryo development, which could potentially impact the health of the next generation (Duranthon and Palmer, 2018). Especially in case of IVP, embryos are particularly affected by external factors from the culture conditions (Rizos et al., 2008; Rizos et al., 2002). Numerous studies have indicated that handling gametes and growing embryos in vitro can cause significant oxidative damage due to the excessive production of reactive oxygen species (ROS). This phenomenon can result in abnormal embryo development and may trigger apoptosis responses (Agarwal and Majzoub, 2017).

Vitrification has proven to be a method that significantly improves the survival rates of oocytes and embryos after thawing (Cao et al., 2009). Moreover, this could lead to an increased chance of pregnancy (Hayashi et al., 2019). Cryotop vitrification has been widely used to freeze oocytes and embryos in various species, particularly cattle (Punyawai et al., 2015). However, a challenge arises because bovine embryos are susceptible to ROS-induced stress, which can adversely affect their development (Prentice and Anzar, 2011). The imbalance between ROS production and cellular antioxidant defenses can damage cytoskeleton structure, membrane lipids, proteins, and DNA. These effects could lead to apoptotic responses and a decrease in embryo viability (Gaviria et al., 2019). The thawing of bovine embryos and the use of culture media for their recovery are crucial in embryo cryopreservation, as they impact the survival rate, quality, and long-term development of

the embryos. Appropriate culture media enhance pregnancy rates and result in healthier calves (Galli et al., 2003).

Resveratrol (3,5,40-trihydroxytrans-stilbene) is a natural antioxidant polyphenol found in various plants and foods, including peanuts, mulberries, cocoa, grapes, and red wine. Previous research has demonstrated that resveratrol has the potential to reduce ROS-induced stress in cat oocytes (Piras et al., 2020). When added to the *in vitro* culture (IVC) medium of pig embryos, resveratrol acts as an antioxidant, which help reduce ROS levels, and increase glutathione (GSH) levels (Wang et al., 2014). Furthermore, resveratrol supplementation in pig IVC medium has been shown to promote embryo development and enhance the expression of genes involved in antioxidant mechanism (Iwata, 2021). Notably, there are reports indicating that resveratrol supplementation in the IVC medium of bovine embryos before vitrification can enhance the expression levels of growth factor genes (Gaviria et al., 2019, 2019; Gaviria et al., 2019).

1.2 Research objectives

1.2.1 To examine the effect of resveratrol in IVC medium on developmental rate of the bovine embryos.

1.2.2 To examine the effect of resveratrol in IVC medium and warming solution on survival rate and gene expression of vitrified bovine blastocysts.

1.3 Hypothesis

1.3.1 The resveratrol in the IVC medium will positively impact the developmental rate of bovine embryos.

1.3.2 The resveratrol in both the IVC medium and warming solution will enhance the survival rate of bovine blastocysts after vitrification, and it will also influence the gene expression profile quality.