CHAPTER I

INTRODUCTION

Importance and Rationale

During the past decade, reproductive biotechnologies such as artificial insemination (fresh, chilled and frozen semen), *in vitro* embryo production and embryo transfer (ET) in equine industry have become increasingly important means of improving reproductive potential of desired horses (Theerawat Tharasanit and Mongkol Techakumphu, 2007). In the first generation of reproductive biotechnology, artificial insemination (AI) is a technique of choice known to maintain and accelerate the genetic potential of valuable male animals. Semen used for artificial insemination can be broadly classified into 3 categories: fresh, cool and frozen semen. The use of fresh semen for artificial insemination is a promising technique in obtaining high pregnancy rates (Beckman et al., 2004). However, sperm rapidly reduces their viability and fertilizability during storage. In order to prolong their longevity, the semen should therefore be extended with a proper semen extender and cooled to approximately 4°C. Although cooled semen maintains sperm viability for up to 48-72 hrs. without affecting pregnancy rate (Theerawat Tharasanit et al., 2007), this technique provides only "short-term" semen preservation. In this regard, "long-term" semen storage, by means of semen cryopreservation has been recently integrated in the equine industry (Vidament et al., 1997; Beckman et al., 2004; Metcalf, 2007) because this technique can preserve genetic potentials, reduce disease transmission, and is logistic acceptable when shipment of the semen is required. Nevertheless, non-optimized freezing techniques of the stallion semen induce irreversible changes within the sperm cells. As a result, freezing and thawing of stallion sperm is often associated with poor pregnancy rates after artificial insemination when compared to those obtained from fresh and cold storage semen (pregnancy rates: 60-100% in fresh and cold storage semen versus less than 56% in frozen thawed semen) (Samper et al., 1991; Jasko et al., 1992).

To date, factors responsible for poor freezability of equine sperm are not entirely clear but a number of factors have been examined, such as freezing techniques

(Vidament et al., 1997; Metcalf, 2007), composition of extenders, type of cryoprotectants (Squires et al., 2004) and individual effect of the stallion.

Indeed, suboptimal cryopreservation technique induces cellular injury, which is associated with a disruption of plasma membrane and cell death. In addition, success of cryopreservation of equine semen has been variable among individual stallions, and our understanding of factors associated with this large inter-male variation is poor (Amann and Pickett 1987; Vidament et al., 1997). A large proportion of stallion (20 to 40%) was reported to have poor freezable semen, while only 14 to 20% had very good semen after thawing (30-40% motility: Muller, 1982). To make a matter worse, post-thawing semen quality is variable among laboratories, such that the percentage of ejaculates selected for insemination after freezing-thawing ranges from 23% to more than 70% (Darenius and Darenius, 1992; Palmer and Magistrini, 1992).

Mammalian spermatozoa require exogenous substrates for energy production. They can obtain energy through mitochondrial oxidative phosphorylation and glycolysis by the consumption of glycolysable sugars, such as glucose, fructose, and sorbitol (Mann, 1975). Sorbitol is a monosaccharide sugar alcohol that also presented in semen (Mann, 1975). This sugar as well as glucose is naturally found in seminal plasma of many mammalian species, including horse. Depending on the species, fructose concentrations in seminal plasma vary from high levels in bull and ram to lower amounts in other species, such as dog and stallion (see table. 1).

Freezing and thawing render the damage of sperm structure resulting in a reduction of viable spermatozoa, as well as sperm functions (Watson, 2000). To date, several causes of cryodamage occurred during cryopreservation has been attributed to suboptimum changes in temperature, intracellular and/or extracellular ice crystal formation, oxidative stress, alterations of sperm membrane, DNA damage, toxicity of cryoprotectants and osmotic stress (Watson, 1995, 2000; Gao et al., 1997). Although a number of strategies aimed at improving the post-thawed semen qualities are being developed, the post-thawed quality remain inconsistent.

Hypothesis

Different freezing techniques and sugars supplemented in freezing extenders had no effect on frozen-thawed sperm quality of stallion semen.

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