

CHAPTER I INTRODUCTION

1.1 Background

Artificial Insemination (AI) can be a means to increase successful copulations. The challenge of assisted copulation through Artificial Insemination (AI) happens in ensuring that only healthy spermatozoa are used in the AI programme. Effective semen storing is a vital step to find out how long it can maintain the longevity of alive, functioning, and progressively motile spermatozoa required for AI (Hallowell, 2019).

Described in the Decision of Minister of Agriculture 2389/KPTS/LB.430/8/2012, *Sapudi* sheep are a breed with unique performance and meat quality which has become a genetic resource known from Indonesia. Not only are the performance and meat quality good, *Sapudi* sheep are well-known for their body immunity against diseases. Its sigmoid tail and thick wool are needed to be taken care of so when copulation occurs, no contaminants will cause infection thus resulting in a failed fertilization which can be avoided by ensuring an effective extender used for storing good quality spermatozoa (Boukhliq *et al.*, 2018).

Reported by Fernandez-Novo (2021), longevity of semen storing can vary and this is influenced by many factors including temperature in which it is stored e.g. cold storage of 5°C. Another factor which affects preservation of semen is the type of semen extender that is used. Egg-yolk skimmed milk (EYSM) extender is a common semen extender. According to Barbas and Mascarenhas (2009), a good extender is one that

fulfils prerequisites such as, able to feed nutrition into spermatozoa, acts as a buffer (isotonic), nontoxic, and contains antibiotics. Skimmed milk has predominant proteins such as casein micelles, alpha-lactalbumin, and beta-lactoglobulin (Manjunath, 2018) able to co-protect ram's spermatozoa even better than just egg-yolk extender by itself (Lusignan *et al.*, 2011). Furthermore, the skimmed milk in this extender is able to prevent intracellular crystallisation and create a preferred osmotic pressure for spermatozoa (Amal *et al.*, 2019) for their progressive movements while egg-yolk acts as a cold shock absorber and a source of energy (Nguyen *et al.*, 2019).

Although egg-yolk skimmed milk extender has been able to maintain fertile spermatozoa upto day 4, there is a study by Bustamante-Filho *et al.* (2009) who found that antioxidant enzymes of the extended semen decreases drastically lower than fresh/raw semen. There was a decrease of 64% for catalase, 43% for glutathione peroxidase and 78% for superoxide dismutase. This means an addition of exogenous antioxidant into the extender can be observed to compensate for the high loss of it.

Moreover, the addition of natural biomolecules (flavonoids) in green tea extract is unlike others. It has been reported to be able to target multiple cellular pathways at once (Mustofa *et al.*, 2021). Green tea extract (GTE) has many properties that can benefit longevity of spermatozoa preservation. Its antioxidant hydroxyl group (both flavonoid and polyphenol properties especially in the form of EGCG) is known to be a powerful antioxidant to combat ROS. It also induces antioxidant enzymes to maintain not only plasma membrane integrity of spermatozoa but also their motility and viability.

Swari *et al.* (2019) found that the higher dose of GTE used in egg-yolk skimmed milk means the more effective it is. Their study concluded a dose of EGCG 0.15%/10 ml has positive effects and the results show a 5-day storage of fertile spermatozoa. Author would like to test if green tea extract together with citrate can even farther prolong the fertile life of spermatozoa as they have chelating agent properties which should increase antioxidant enzymes to combat prooxidant ions thus prolonging storage duration of spermatozoa in the extender even longer.

Supplementation of Green Tea Extract in an Egg-Yolk Citrate Skimmed Milk Extender on *Sapudi* Ram's Spermatozoa Quality at a Cold Temperature (5°C) are hoped to be able to maintain the semen quality as a good alternative for semen storing to complement AI.

1.2 Formulations of Problem

- 1.2.1** How does supplementation of green tea extract in an egg-yolk citrate skimmed milk extender affect motility of *Sapudi* ram's spermatozoa quality at a cold temperature (5°C)?
- 1.2.2** How does supplementation of green tea extract in an egg-yolk citrate skimmed milk extender affect viability of *Sapudi* ram's spermatozoa quality at a cold temperature (5°C)?
- 1.2.3** How does supplementation of green tea extract in an egg-yolk citrate skimmed milk extender affect plasma membrane integrity of *Sapudi* ram's spermatozoa quality at a cold temperature (5°C)?

1.3 Theoretical Base

Skimmed milk has many benefits. Lactose in milk increases efficiency of a semen extender and its protection against excess stressors is available due to whey protein and casein (20% of total protein) (Fu *et al.*, 2018) which suppress stressors, thus, prevent the breakage of spermatozoa membrane, therefore, motility and viability of spermatozoa are maintained from decreasing too rapidly. Gallo *et al.*, (2021) added that spermatozoa's membrane integrity has a linear correlation with spermatozoa motility.

However, it is a challenged process to add citrate solution for it has also many benefits. Citrate solution functions as a heavy metal and calcium binder (Vavrusova, 2017). It also acts as a buffer, and forms emulsion out of egg yolk lipid which is important to make clear the observation of spermatozoa under the microscope.

Alongside with citrate solution, egg-yolk facilitates isotonic environment for spermatozoa in an extender. Lipoprotein of egg-yolk and its lecithin function as a protecting layer for spermatozoa commonly due to its abundant phospholipid (Nguyen *et al.*, 2019). The content of glucose gives energy for spermatozoa to survive. Moreover, lipid of egg-yolk limits access of spermatozoa's unnecessary movements which is an advantage as it minimizes energy breakdown.

The supplementation of green tea extract rich in EGCG is said to have a high antioxidant property. This catechine prevents excess intracellular oxidative stress and ROS so it suppresses cases of disturbed motility and even non-motile cases as well as changes in morphology and functions of spermatozoa (Bernatoniene and Kopustinskiene, 2018).

The effects of DNA fragmentation are even stronger if the level of ROS is high in the cell. This negatively affects ATP (*Adeno-triphosphate*) from being produced during glycolysis. Glycolysis is a process to produce ATP anaerobically which occurs at the head of spermatozoa and axoneme. Spermatozoa's motility is put in danger if the production of ATP decreases due to strong effects of ROS and DNA fragmentation (Susilowati *et al.*, 2020). The addition of nutrient agent in an egg-yolk and citrate skimmed-milk is needed for capacitation so they do not run out of energy in being stored for days.

1.4 Aims of Research

1.4.1 General Aims of Research

1.4.1.1 To be able to lengthen spermatozoa's fertile duration of storage so successful artificial inseminations are more achievable.

1.4.1.2 To be a varying semen extender that can be chosen for storing semen.

1.4.2 Specific Aims of Research

1.4.2.1 To find out how supplementation of green tea extract in an egg-yolk citrate skimmed milk extender affects motility of *Sapudi* ram's spermatozoa quality at a cold temperature (5°C).

1.4.2.2 To find out how supplementation of green tea extract in an egg-yolk citrate skimmed milk extender affects viability of *Sapudi* ram's spermatozoa quality at a cold temperature (5°C).

1.4.2.3 To find out how supplementation of green tea extract in an egg-yolk citrate skimmed milk extender affects plasma membrane integrity of *Sapudi* ram's spermatozoa quality at a cold temperature (5°C).

1.5 Outcomes of Research

1.5.1 Theoretical Outcome

This research is hoped to give an insightful contribution to effective extender literatures which then can be used and modified into a better extender for the sake of Veterinary Medicinal Developments.

1.5.2 Practical Outcome

This research is hoped to motivate other researchers and vets to teach farmers to apply the knowledge in real life during semen collection and storing for a more effective and successful artificial insemination.

1.6 Hypotheses

1.6.1 Supplementation of green tea extract in an egg-yolk citrate skimmed milk extender is able to maintain motility of *Sapudi* ram's spermatozoa at a cold temperature (5°C).

1.6.2 Supplementation of green tea extract in an egg-yolk citrate skimmed milk extender is able to maintain viability of *Sapudi* ram's spermatozoa at a cold temperature (5°C).

1.6.3 Supplementation of green tea extract in an egg-yolk citrate skimmed milk extender is able to maintain plasma membrane integrity of *Sapudi* ram's spermatozoa at a cold temperature (5°C).