# CHAPTER 1 INTRODUCTION

## 1.1 Research Background

The rate of science development today has increased and this event gives impact to the number of research which using a variety of experimental animals. One of experimental animals which is often used in science research are mice (Kusumawati, 2004). Choosing the right number of experimental animals in research is particularly important because choosing too few or too many experimental animals may result in missing important biological effects (Dell *et al*., 2002).

Livestock productivity improvements can be made through improved feed, livestock management and implementation of breeding programs, but direct research on livestock breeding itself may costs large financing and take relatively long time. To obtain a model that can be applied empirically in livestock animals researcher often used animal experiments. Mice is one of experimental animals that can be used as research material because it has superior properties and meets the requirements of the standard to represent few farm animals condition (Kurnianto *et al*., 2002). Moriwaki in Tahani (2013) mentions that mice are the most common animals used in research as a laboratory animal experiments, which is about 40-80%.

Brandell and Schlegel (2000) stated that fulfillment of the need for mice in large quantities can be done in many ways one of them by accelerate the breeding through increased fertility of male mice. High fertility of males will accelerate the

process of mating and fertilization so that faster gestation in females would be obtained.

Petrelli and Mantovani (2002) and Plas *et al*., (2000) suggested that physiological factors have defective effects on male reproductive performance besides environmental and genetic factors. Reactive Oxygen Species (ROS) are a class of free radicals that produced in various organs including the testis as a physiological event. ROS are highly reactive oxidizing agents that stimulate oxidation and DNA damage in cells (Sikka, 1996). Spermatozoa's plasma membrane contain high concentration of unsaturated fatty acid which can easily damage by oxidative stress, which means ROS production is a potential danger for spermatozoa's activity (Aitken, 2002).

Antioxidants are compounds which suppress the production of ROS (Sikka, 1996; Vernet, 2004). Salman *et al*., (2013) prove that the content of chrysin in honey may improve fertility which can bee seen in the increase of the number of sperm and testosterone hormone levels in male albino rats after oral administration of honey. Chrysin increase sperm concentration, boost testosterone, and attenuate oxidative stress and apoptosis (Darwish *et al*., 2014). The content of vitamins in the aphrodisiac substance also have a positive influence on the increase of fertility (Luthfi, 2008). Propolis is a honeybee product contains chrysin and various vitamin (Krell, 1996).

Indonesia has a large potential of tropical forests and enough production of domestic raw propolis (Harsanto and Mahani, 2011). *Apis mellifera* is one of the species of honey bees which have superior properties to the production excellent

products, one of the flagship products beside honey is propolis (Budiaman and Rachman, 2006; Rinaldhi, 2014). (Hasan, 2007) suggested that propolis is a product that is extracted from the honeycomb sticky proportion, produced by honey bees comes from the mixing of the saliva and the resin obtained from the bark, leaves, branches, and other flowering plants, used as sealant. Bees use it to fill the gap with the goal of resilience as well as keeping water or air to keep it out of the nest. Another function of propolis is as a sterilant, which is a chemical sterilant used with the purpose of sterilization of foreign substances that enter the hive (Suryanto, 2010).

Propolis has been used in folk medicine for centuries. It is known that propolis possesses anti-microbial, antioxidative, anti ulcer and anti tumor activities. Propolis has attracted much attention in recent years as a useful or potential substance used in medicine and cosmetics products. Furthermore, it is now extensively used in foods and beverages with the claim that it can maintain or improve human health (Loutfy, 2006). Propolis has been proved to stimulate various enzyme systems, cell metabolism, circulation and collagen formation ( El Kott and Owayys in Saleh, 2012). People nowadays are not only using materials *Apis mellifera* origin for food additives alone but has expanded into main engredient for medical treatment (Nakajima, 2009).

Krell in Jaya *et al*., (2005) stated that propolis-containing resins such as flavonoids and phenolic acids as much as 45-55%, fatty acids and waxes as much as 25-53%, total protein and the last 5% minerals and other organic compounds such as Zn, iron, vitamin B3 and fructose as much as 5%.

Najafi *et al*., (2007) stated that the major ingredient of propolis is flavonoid. Twelve different flavonoids, pinocembrin, acacetin, chrysin, rutin, catechin, naringenin, galangin, luteolin, kaempferol, apigenin, myricetin, and quercetin, two phenolic acids, cinnamic acid and caffeic acid, and one stilbene derivative, resveratol, in propolis extract were determined by capillary zone electrophoresis (Volpi, 2004). The major active nutraceutical ingredients in plants are flavonoids. As is typical phenolic compounds, they can act as poten antioxidants and metal chelators. They also have long been recognized to possess anti inflammatory, antiallergic, hepatoprotective, antithrombotic, antiviral, and anticarcinogenic activities (Tapas *et al*., 2008). Hozayen (2012), described increased activity of serum testosterone level in doxorubin induced rats which treated with flavonoids hesperidin and rutin.

Propolis could give protection against infertility by improving sperm production, motility, sperm count and quality and increased the process of steroidogenesis and testosterone production (Yousef and Salama, 2009). El Mazoudy *et al*., (2011) and Yousef *et al*., (2010) research showed an increase in testosterone levels for rats and rabbits treated with propolis.

The spermatogenesis is a highly sensitive process toward the physical and chemical agents in which precursor cells from mature haploid spermatozoa within seminiferous tubules and in several studies have been observed that propolis has many potential including improve sperm production and improve steroidogenesis. Based on the explanation above, the author were interested to conduct research on

the spermatogenic cells number and seminiferous tubules diameter of male mice after provision of propolis.

## 1.2 Statements of Problem

Based on the proposed formulation of the problem has been described as follows:

1. Does the provision of propolis may increase the number of spermatogenic cells in male mice?
2. Does the provision of propolis may increase the diameter of seminiferous tubules in male mice?

## 1. 3 Theoritical Base

Propolis is a product that extracted from the beehive (Hasan *et al*., 2007). Bee Propolis is actually generated from resin of variety plants, then this resin mixed with saliva and various enzymes that exist in bees so that it becomes a different resin with resin origin (Winingsih, 2002). Propolis has wide range of biological activities including antibacterial, antiviral, anti-inflammatory, and anti-oxidative properties (Medic-Saric *et al*., 2009) in its composition many phenolic compounds were detected that were associated with its antioxidant activity (Sousa *et al*., 2009).

Propolis was found to significantly increase testosterone level, body weight, relative weight of testis, relative weight of epididymis, semen characteristics and seminal plasma enzymes and decreased the levels of free

radicals and lactate dehydrogenase (Yousef *et al*., 2010). In the previous study, it was described there is increased activity of 17-ketosteroid reductase and testosterone levels in rats treated with 50 mg propolis/kg BW and suggested increased steroidegenesis, improving sperm proliferation and hence fertility (Yousef and Salama, 2009). Propolis also can affect the histological examination of epididymal initial segment revealed increased lumen volume, higher number of tubules, enlargment of epididymal epithelium of initial segment and the stroma layer thickness of treatment group compare to control group (Capucho *et al*., 2012).

Steroidogenic interstitial Leydig cells has a primary function appears to be production of testosterone (Mendis-Handagama, 1997). The myoid cells that surround the seminiferous tubules provide physical support and contractile motion to the structures (Maekawa *et al*., 1996).. The Sertoli cells, who direct contact with proliferating and differentiating germ cells within the seminiferous tubules makes Leydig cells, myoid cells, and Sertoli cells essential for providing both physical and nutritional support for spermatogenesis (Griswold, 1998). Each of these cell types is a dire t target for one or more of the hormons whose actions are esssential for unimpaired male fertility. Testosterone and its metabolites, dihydrotestosterone (DHT) , and estradiol (E2) are collectively reffered to as the sex hormones because of their primary role in the gonadal and germ cell development in male and female as well as to the sexual differentiation of male. Testosterone assumes the lead role in both morphological development and

reproductive function, although E2 and its receptor clearly play some role in the maintenance of male fertility (Krege *et al*., 1998).

## 1.4 Aim of Research

This study aims to

1. Determine the potential of propolis in increasing the number of spermatogenic in male mice.
2. Determine the effect of propolis in increasing the diameter of seminiferous in male mice.

## 1.5 Research Benefits

The results of this study can provide information about the potential in increasing the number of spermatogenic cells and diameter of seminiferous tubules in male mice (*Mus musculus*).

Propolis can be used as a natural remedy for medical purposes in general and particularly in veterinary medical, and expected to be useful for developing research field of veterinary science and also provide input to all aspects of medical science and pure science.

## 1.6 Research Hypotheses

Hypotheses proposed in this study are :

1. Provision of propolis can increase the number of spermatogenic cells in of male mice.

2. Provision of propolis can increase diameter of seminiferous tubules in male mice.