The aims of this research is to determine of therapeutics effect of red fruit addition in infertility mice models that can increase foliculogenesis and increasing pregnant rate. The experimental animal used are 24 female mice with 25 gram average body weight. The treatment divided into 4 groups and each group gets 6 repetitions. The control group (P0) is given only aquadest without getting treatment. P1 group were given injections of testosterone propionate 1 mg / kg subcutan (sc) for 14 days for the effects of infertility. The treatment group P2 and P3 have an injection of testosterone propionate 1 mg / kg subcutan (sc) for 14 days to effect, infertility and given a red fruit extract therapeutic dose of 0.05 ml and 0.1 ml for 14 days in orally. Testosterone propionate were injected as much as 0.02 ml subcutan conducted for 14 days. The parameters used are estrus cycle between infertility mice that given treated with red fruit and mice infertility without given treated with red fruit and diameters of pre antral and antral follicle. The data were analysed using ANOVA(analysis of variance). The result showed there are significant estrus cycle between infertility mice that given treated with red fruit and mice infertility without given treated with red fruit. However, analysis of data from the ovary preparations showed no significant difference between the number of follicles either primary follicle, and follicle de graaf, but for secondary follicles was significantly different between the K (-) with K(+).

Keywords: Red fruit, Mice, Estrous, Follicle

1. INTRODUCTION

Infertility is a disease defined by the failure to achieve a successful pregnancy after 12 months or more of regular unprotected intercourse (American Society for Reproductive Medicine, 2005) This definition has its basis in the finding that the majority (85%) of couples with no adverse factors are likely to achieve a pregnancy within 12 ovulatory cycles (Dick et al., 2003). Infertility may also refer to the state of woman who is unable to carry a pregnancy to full term (Amutha et al., 2013). Infertility affects 7.3 million people in the United States (Resolve, 2013).

Infertility can occur in either men or women and across all nationalities and socioeconomic levels. The U.S. Supreme Court stated that reproduction is a major life activity and confirmed that conditions that interfere with reproduction should be regarded as disabilities, as defined in the Americans with Disabilities Act (Macaluso et al., 2010). Womens are defined as infertile if they are married or cohabitating (Kelly-Weeder & Cox, 2008). As a result, 15 states have passed laws requiring insurance policies to cover some level of infertility treatment (Resolve, 2013).
The utilization of *Pandanus conoideus* is an alternative to solving such a problem. This plant is mainly found in the Indonesian province of Irian Jaya and the neighboring Papua New Guinea. The fruit contains fatty acids, namely oleic acid which sums up to about 30% of its contents and can improve public nutrition. The fruit also contains a high level of antioxidants - namely carotenoid and tocoferol, and minerals such as Fe, Ca and Zn (Budi, 2003). The fruit’s extract also contains both alpha- and beta-caroten in amounts of 130 μg and 1.980 μg/100 g samples, respectively. Its vitamin E levels are also quite high at 21.2 mg/100 g sample, making the fruit a good source of vitamin E.

The mouse (*Mus musculus*) is a prolific rodent which is easily reared in large quantities, has a wide genetic variety and well-understood anatomical and physiological characteristics. Mice are often used in researches, as they possess several desirable characteristics which include regular and detectable estrus cycle, short gestation period, large number of offspring, and a growth level that is easily relatable to that of humans. The mouse is polyestric, which means multiple reproductive cycle occur within one year. The estrus cycle of this animal is divided into five phases which are Proestrus, Estrus, Metestrus I, Metestrus II and Diestrus. The whole cycle lasts for 5 days. Estrus is a phase in which females are receptive to the male’s attempts to copulate and lasts for 12 hours. In the phase, the de graaf follicle enlarges and matures while the ovum undergoes changes that lead to maturity. Fertilization occurs 7-10 hours post-copulation. The resulting embryo will reach the blastula stage within 3-4.5 days and the gestation period is 21-23 days long.

Despite the information above, the use of *Pandanus* fruit to reduce infertility is still poorly researched, prompting the writer to conduct one that aims to discover the potential of the fruit in decreasing infertility rates and increase conception rates.

### 2. MATERIALS AND METHODS

This research was carried out in the Laboratory Animals Unit of Airlangga University’s Faculty of Veterinary Medicine in Surabaya. It took place within the span of one month (September-November) of 2015.

The materials used in this research are BALB/c mice (*Mus musculus*) obtained from Gadjah Mada University. Other materials included testosterone propionate hormone, *Pandanus conoideus* extract, aquadest, pellets (mice feed), wood shavings, alcohol, cotton buds, Giemsa coloring, alcohol, drinking water, gloves, Hematoxillin Eosine (HE) coloring and formaldehyde buffer.

Equipment used are four rodent enclosures, scales (for weighing mice), water bottles (for mice), modified syringes for oral administration of *Pandanus* fruit extract, tuberculin syringes for testosterone propionate injections, object glasses, minor surgery kit and a microscope.

#### 2.1. Population and Research Sampling

Animals used for this research are 3 month-old female BALB/c mice (*Mus musculus*) weighing an average of 20-30 g, obtained from Gadjah Mada University. The number of samples used in the research was determined based on Federer (1963) and Kusriningrum (2011):

\[
t ( n - 1 ) \geq 15
\]

\[t = \text{treatment groups}
\]

\[n = \text{number of samples}\]
2.2. Laboratory Animals Preparation

Prior to research, mice were given 1 week to adapt living in enclosures made of plastic tubs with a wire mesh lid. The mice were fed with pellets and given drinking water per ad libitum. They were then weighed, numbered and randomly divided into 4 treatment groups (P1, P2, P3, and P0), each using 6 repetitions.

2.3. Vaginal Swab Examination

Vaginal swab examination was done twice: before and after testosterone propionate injection (SC). The purpose was to determine the mice’s estrus cycle condition.

2.4. Treatment Stages

Mice were divided into four different treatment groups (P1, P2, P3 and P0), each consisting of 6 repetitions. The control group (P0) was only given distilled water (aquadest) without any treatment. P1 was given a testosterone propionate injection (1 mg/kg BW) SC for 14 consecutive days to induce infertility. P2 and P3 were subjected to the same treatment but are also given an oral administration of Pandanus fruit extract during the subsequent 14 days.

The regular injection of testosterone propionate for 14 consecutive days would induce a condition similar to Polycystic Ovarian Syndrome (PCOS), with visible signs including absence of corpus luteum, presence of polycystic ovary, hypertechosis on stroma and thinning/atroisia of granular cells (Beloosky et al, 2004). PCOS disrupted the ovulation process, thus was causing disturbances in the menstrual cycle and could cause infertility (Santoso & Irawan, 2007).

2.5. Mice Dissection

After all mice were subjected to the 28-day treatments, dissection was done on the 29th day. After euthanasia used diethyl ether, dissection was performed and the ovaries were recovered. The said ovarium was then suspended in 10% formaldehyde buffer and grouped based on the different treatment groups. They were then made into histological preparations and stained with Hematoxylin Eosine (HE) coloring.

2.6. Ovarium Hystological Slide Preparation

The ovarium preparation was done using paraffin method and HE staining. The ovaries were then fixated in 10% PBS solution, and then proceeded to be dehydrated with varying levels of alcohol and cleared with xylol. After the clearing, paraffin infiltration was conducted and the organs were embedded in paraffin blocks. The blocks were then attached to holders, trimmed, and sliced using a rotary microtome. The resulting preparations were then put onto object glasses. The last process was the hematoxylin eosine staining and mounting of the preparation slides (Handari, 1983).

2.7. Hystopathological Preparations Examination

Observation was done via microscope with x100 magnification. The objective was to measure the average diameter of antral and pre-antral follicles. The obtained data in quantitative in nature.

2.8. Research Methodology Planning
Due to homogenous environmental and age factors, and also due to sampling being randomized, the method used was Complete Randomized Sampling. In this method the only difference between treatment results is caused by difference in treatment and random chances.

2.9. Data Analysis

Data analysis was conducted with ANOVA (Analysis of Variance). The data obtained was processed with an SPSS (Statistical Programs for Social Scientific) software. Had there been any significant difference generated within treatment groups, further testing was conducted with Duncan’s multiple range test (Kusriningrum, 2010).

3. RESULT AND DISCUSSION

Vaginal swabs were done three times: before treatment, after testosterone injection and after administration of Pandanus extract, with the objective of identifying the stages of the mice’s estrus cycles. The possible results were infertile (diestrus) or fertile (estrus). The following were the data obtained from the vaginal swab tests.

Based on the results of the vaginal swab tests, it was known that the beginning of estrus cycle was characterized by the disappearance of leucocytes and nucleated cells, all that were left were keratinized epithelial cells that were irregular and large in shape and size. Identifying the mice’s estrus cycle prior to and after treatment is important to make sure that all animals are indeed fertile before the receiving treatments and ensuring that the therapeutic effects of Pandanus extract match the hypothesis, respectively.

On the other hand, during the diestrous cycle an abundance of leukocyte and nucleated epithelial cells are encountered. These cells were well-distributed and homogenous in appearance. The aim of this test was to determine if the mice treated with testosterone were indeed infertile.

The following pages show estrus cycle graphs for all four treatments. K(-), K(+), P1, and P2. All mice undergo a phase of estrus prior to receiving any treatment. The next showed how all mice undergo a diestrous cycle after subjected to testosterone treatment for K(+), P1, and P2. The final one showed that the effects of Pandanus extract therapy for P1 and P2 were visible and could cause estrus. Some results in P1 and P2 exhibited changes from diestrous to estrus;

![Figure 1: the graphic of estrus cycle from mice that were not treatment of red fruit](image-url)
Figure 2: The graphic of estrus cycle from mice that finish to treatment of red fruit

3.1. Mice Ovary Preparations Examination.

Following the vaginal swabs, the mice would be euthanized and their ovaries recovered. The objective was to observe the process of folliculogenesis post-treatment with Pandanus extract, and the results were as followings:

<table>
<thead>
<tr>
<th>Table 1: Result Specimen Reading From Mice Ovarium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>K(-)</td>
</tr>
<tr>
<td>P :5</td>
</tr>
<tr>
<td>S:10</td>
</tr>
<tr>
<td>D:0</td>
</tr>
<tr>
<td>P :6</td>
</tr>
<tr>
<td>S:7</td>
</tr>
<tr>
<td>D:0</td>
</tr>
<tr>
<td>P :7</td>
</tr>
<tr>
<td>S:8</td>
</tr>
<tr>
<td>D:1</td>
</tr>
<tr>
<td>P :6</td>
</tr>
<tr>
<td>S:9</td>
</tr>
<tr>
<td>D:1</td>
</tr>
<tr>
<td>P :4</td>
</tr>
<tr>
<td>S:5</td>
</tr>
<tr>
<td>D:0</td>
</tr>
<tr>
<td>P :7</td>
</tr>
<tr>
<td>S:15</td>
</tr>
<tr>
<td>D:2</td>
</tr>
</tbody>
</table>

P : primary follicle, S: Secondary follicle, D: De Graaf Follicle

The table of result specimen reading from mice ovarium that finish to treatment of red fruit

4. CONCLUSION

Out of the three variables observed from the administration of the fruit’s extract, one that was apparent was the difference in estrus cycles between mice treated with Pandanus extract and the infertile mice lacking the treatment. In the future, this data can be used as a reference to determine the percentage of conception. However, data obtained from ovary preparations showed no significant difference between primary and de graaf follicles, but was more readily observable difference on secondary follicles of K(-) and K(+).
REFERENCES


12. Departmen of Veteriner Reproduction, Faculty of Veterinary Medicine,