ABSTRACT

This study aims to determine the histology, the number and diameter of follicles, and the corpus albican of the Gayo mare ovary. The micro technical process was applied to 3 pairs of ovarian samples for further hematoxylin-eosin (HE) staining. Observation of ovarian structure was carried out microscopically and the data were analyzed statistically. The results showed that the Gayo mare ovary consists of the medulla on the outside and the cortex on the inside. Medulla consists of small follicles, blood vessels, nerves, and connective tissue. Meanwhile, the cortex consists of de Graff's follicle, corpus luteum, corpus albican, and ovulatory fossa. Follicles are composed of oocytes, granulosa cells, internal and external theca cells, oophorous cumulus, follicular antrum, and follicular fluid. Atretic follicles contain lutein cells that have been damaged. The corpus luteum is composed of granulosa lutein tissue, internal theca and external. The corpus albican is composed of scar tissue and lutein cells. The measurement results showed that the primordial follicle diameter was 26.60±2.37 µm, primary follicle 54.33±6.70 µm, secondary follicle 119.32±25.55 µm, tertiary follicle 250.86±49.46 µm, atretic follicle 49.03±45.47 µm, the corpus albican 511.10±132.41 µm. Thus, it can be concluded that the Gayo mare ovary has a histological structure that is not different from the ovary of other mares. Follicular growth occurs in the medulla of the ovary, and de Graff's follicles are present in the cortex of the ovary.

Key words: Gayo mare, ovary, folliculogenesis, corpus albican

INTRODUCTION

The reproductive system plays an important role in animal conservation efforts. The reproductive system in both male and female animals must be in healthy condition so that it is easy for them to get offspring which will have an impact on increasing the population, including horses. Increasing the number of horse population can be done with the application of reproductive technology which has very developed today. Reproductive technologies that are available and have been used in horses include semen collection techniques in male horses (Falomo et al., 2016), synchronization of heat in female horses (Beest and Schook, 2016), artificial insemination (AI) (Deng et al., 2014; Amrozi et al., 2015), and embryo transfer (Kruamer, 2013).

Knowledge of the status of the reproductive organs can support the success in the application of natural reproductive and mating technologies. The health of the reproductive organs can be determined by examining the anatomy of the reproductive organs using ultrasonography (Lemma et al., 2006), checking hormone levels (Arango and Newcombe, 2009), and histological examination of the reproductive organs themselves (Overbeck et al., 2013).

A study on equine ovarian histology was reported by Markovic et al. (2003), which examined the cycles, folliculogenesis, and luteogenesis in mare ovaries, and Ono et al. (2015), which analyzed of the ovarian structure of mares aged 1-12 months using three-dimensional internal structure microscopic techniques. Currently, research that discusses the histology of the reproductive organs of horses in Indonesia, especially the Gayo horse, is very minimal. Previously, the author had reported on the anatomy and ultrasound images of the reproductive organs during the estrous cycle in Gayo mares (Melia et al., 2016), but to date, reports on the observation of complete histological structures in the ovaries of local horses, especially Gayo mares, have not been found.

This study aims to determine the histology, the number and diameter of follicles and corpus albican in the ovary of Gayo mares. The expected result of this study is that there is information about the histological and...
histomorphometric features of the Gayo mare ovaries, which can be a reference for diagnosing reproductive disorders related to changes in mare ovaries.

MATERIALS AND METHODS

Research Samples

The sample of this study consisted of 3 pairs of ovaries from 3 Gayo mares from Aceh Tengah Regency, each aged 5, 7, and 10 years. Ovarian samples that had been taken were then washed using physiological NaCl 0.9% and put into a 10% PBS fixative solution. Next, the sample was prepared with a thickness of 0.5-1 cm. The prepared sample was then put into a tissue cassette for further micro technical processes. The micro technical process started with dehydration using graded alcohol, namely 70%, 80%, 96%, Absolut I, and Absolut II, followed by the clearing process using xylol I, II, and III. After that, the tissue infiltration process was carried out using liquid paraffin I, II, and III, followed by embedding using an embedding processor. Then cutting was using a microtome with a thickness of 3 µm. The sample slides were then incubated on the warmer slide, followed by HE staining.

HE staining began with deparaffinization using alcohol with a decreased concentration of absolute I, absolut II, 96%, 80%, 70%, xylol I, II, and III. After that another staining was performed using Hematoxylin and Eosin, followed by the rehydration process using 80% alcohol, 95%, absolut I, absolut II, xylol I, II, and III. Next, mounting was done using entellan®. Lastly, histomorphometry observations and measurements of ovarian slides were carried out using a light microscope (Olympus CX31), a Sigma® microscope camera, and the Toupview application.

RESULTS AND DISCUSSION

Based on the results of microscopic observations on the histological preparation, it was found that a Gayo mare ovary is divided into the medulla (outer) and cortex (inner) and is wrapped by tunica albugenia, as reported by Brinsko et al. (2011). The tunica albugenia of the ovary is composed of connective tissue and is bounded by the cuboid germinal epithelium in the medulla. The medulla of the Gayo mare ovary contains small follicles, blood vessels, nerves and connective tissue, while the cortex contains de Graff's follicles, corpus luteum, and connective tissue. In general, the histology of the ovary structure of a Gayo mare is as shown in Figure 1.

The outer part (medulla) of a Gayo mare ovary consists of connective tissue, primordial follicles, primary follicles, secondary follicles, tertiary follicles, atretic follicles, and corpus albican. Apart from the follicles, there are also many blood vessels and nerves in this area. This was found to be different from the histological structure of the ovaries in general, where in other animals the blood vessels are found on the inside of the ovaries, such as in cows (Ihsan, 2010; Akoso, 2012), sheep (Rosadi et al., 2011), deer (Hamny et al., 2010), rabbits (Gabri et al., 2012; Saleh, 2013), and rats (Nurjannah and Widyaningrum, 2016; Mardika et al., 2018). The histological structure of the Gayo mare ovary medulla can be seen in Figure 2.

The inside (cortex) of a Gayo mare ovary consists of de Graff's follicles, hemorrhagic corpus, corpus
luteum, corpus albican, connective tissue and ovulatory fossa. During the growth period of the follicle, the ovulatory fossa consists only of connective tissue (Markovic et al., 2003). When the ovulation period has finished, the ovulatory fossa will close and be filled by the corpus luteum (Brinsko et al., 2011). According to Markovic et al. (2003), in old mares, fossa cysts are often found in the ovulatory fossa and there is ciliated columnar epithelium in the inner layer of the fossa cyst. The histological structure of the Gayo horse ovulatory fossa can be seen in Figure 3.

**Development of Ovarian Follicles in Gayo Mares**

Follicular development (folliculogenesis) in the ovary of a Gayo mare occurs on the outside (medulla). In the medulla, the follicles will develop into de Graff's follicles and lead to the inside (cortex). The development of follicles starts from the primordial follicles, primary follicles, secondary follicles, tertiary follicles, and de Graff's follicles. Growth follicles that do not ovulate will experience death and called atretic follicles (Eroschenko, 2014). Atretic follicles are characterized by granulosa cells undergoing picnosis and the dropping of granulosa cells into the antrum (Hamny et al., 2010). The various levels of follicular growth in a Gayo horse ovary are presented in Figure 4.

The development of the follicle begins with a primordial follicle located in the germinal medulla of the mare's ovary. The primordial follicle consists of an ovum and a layer of squamous follicular cells. The follicular cells in the primary follicles will turn into low columnar or cuboidal cells, while in the secondary follicles, the active follicular cells have mitosis and form a granulosa cell layer in the follicle. The granulosa cell layer surrounding the oocyte is called the corona radiata. Granulosa cells in secondary and tertiary follicles that continue to actively mitigate will cause the follicle wall to push up and enlarge. Granulosa cells will also produce follicular fluid which will fill the space in the follicle. In mature follicles, granulosa cells will form a hill that connects the corona radiata and granulosa cells called the oophorous cumulus (Eroschenko, 2014).

When follicles reach full maturity, the follicle in the cortex leads to the ovulatory fossa to ovulate the oocyte. Melia (2017) categorized the de Graff follicles of a Gayo mare ovary by ultrasound examination into three classes; class 1 with a diameter <2 cm, class 2 with a diameter of 2-4 cm, and class 3 with a diameter of >4 cm. The preovulatory follicles in Gayo horses can reach 5 cm in diameter. After ovulation occurs, the mature follicle (ovulatory follicle) will experience bleeding called the corpus hemorrhage/corpus rubrum. The corpus hemorrhage will then be covered by lutein tissue and become the corpus luteum. A sketch of de Graff's follicle can be seen in Figure 5.

The corpus luteum on a Gayo mare ovary is located in the cortex. Its location in the (deep) cortex makes it

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difficult to identify the corpus luteum by the rectal palpation method. The corpus luteum is composed of theca lutein cells and granulosa lutein cells. These cells will function as producers of the hormone progesterone and less estrogen (Gastal et al., 2005; Eroschenko, 2014; Harisatria et al., 2017). The duration of the existence of the corpus luteum for each individual varies, depending on the type of species and the physiology of the individual reproductive hormone (Ihsan, 2010). The old corpus luteum will turn into a corpus albican. The corpus albican is composed of scar tissue and few lutein cells that make it produce less progesterone than those of the corpus luteum (Eroschenko, 2014). The histology of the corpus albican on the ovary of a Gayo mare can be seen in Figure 6.

Number and Diameter of Follicles on a Gayo Mare Ovary

Based on the results of microscopic observations on the histological preparations of Gayo mare ovaries, the number and diameter of primordial follicles, primary follicles, secondary follicles, tertiary follicles, atretic follicles, and corpus albican are shown in Table 1. The results of the measurement of the average diameter of the Gayo mare follicles are 26.60±2.37 µm on primordial follicles, 54.33±6.70 µm on primary follicles, 119.32±25.55 µm on secondary follicles, 250.86±49.46 µm on tertiary follicles, and 49.03±45.47 µm on atretic follicles. The increase in diameter from primordial follicles to tertiary follicles was due to an increase in follicular fluid produced by granulosa cells whose numbers were also increasing (Eroschenko, 2014).

This study also found a corpus albican with a diameter of 511.10±132.41 µm. The finding of a corpus albican of very small size indicates that it can survive in the ovary until the next estrous cycle. These findings also prove that progesterone levels remain in the individual's body in small amounts even though the individual enters the proestrus phase. In Gayo mares, Melia (2017) reported that progesterone level in the proestrus phase were <1 ng/mL, while in the luteal phase it could reach the highest value of 46.21±8.53 ng/mL.

The follicle that develops but does not reach the size of the dominant follicle will experience lysis during the luteal period and it is called atretic follicle. According to Hamny et al. (2010), the follicle will experience atresia after reaching a certain size, and this size is different for each species. Atretic follicles are characterized by the presence of damaged granulosa cells. The diameter of atretic follicles found in the ovaries of Gayo mares in this study was 49.03±45.47 µm.

Currently, histomorphometric reports of normal equine ovarian follicle diameter are very minimal, while microscopic studies of equine ovarian follicle diameter have led to many pathological cases. Evans (2003) reported the characteristics of follicular development in several domestic animals. In sheep, the maximum diameter of non-ovulatory follicles is 5-7 mm and the maximum diameter of ovulatory follicles is 6-7 mm with 2-4 waves of follicular development in
each cycle; in goats, the maximum diameter of non-ovulatory follicles is 5-9 mm and the maximum diameter of ovulatory follicles is 6-9 mm with 3 or 4 waves of follicular development in each cycle; in horses, the maximum diameter of non-ovulatory follicles is 30-45 mm and the maximum diameter of ovulatory follicles is 40-55 mm with 1 or 2 waves of follicular development in each cycle. According to Melia (2017), the diameter of ovulatory follicles in Gayo mares is 50 mm in size, with 2 waves of follicular development in each cycle.

Griffin et al. (2006) reported a comparative analysis of follicle diameter in 4 types of mammals, namely mice, hamsters, pigs and humans. In mice, the primordial follicle diameter was ±17 µm, the primary follicle diameter ±52.1 µm, the secondary follicle diameter ±104 µm, the tertiary follicle diameter ±248 µm, and the de Graff follicle diameter ±424 µm; in hamsters, the primordial follicle diameter ±26 µm, the primary follicle diameter ±56 µm, the secondary follicle diameter ±125 µm, the tertiary follicle diameter ±402 µm, and the de Graff follicle diameter ±640 µm; in pigs, the primary follicle diameter was ±34 µm, the primary follicle diameter ±64.9 µm, the secondary follicle diameter ±102 µm, the tertiary follicle diameter ±696 µm, and the de Graff follicle diameter ±1780 µm; in humans, the primordial follicle diameter was ±4 µm, the primary follicle diameter ±79 µm, the secondary follicle diameter ±114 µm, the tertiary follicle diameter ±889 µm, and the de Graff follicle diameter ±18,800 µm. Follicle size in goats and sheep has been reported by Mohammadpour (2007). In sheep, the primordial follicle diameter was 25.53±3.71 µm, the primary follicle diameter 39.96±9.49 µm, and the secondary follicle diameter 271.31±147.93 µm; in goats, the primordial follicle diameter was 25.50±4.21 µm, the primary follicle diameter 51.52±11.04 µm, and the secondary follicle diameter 371.3±121.67 µm. Meanwhile, according to Kacinskis et al. (2005), in zebu cattle, the primordial follicle diameter was 36.0±0.9 µm, the primary follicle diameter 48.5±1.4 µm, and the secondary follicle diameter 88.4±2.9 µm. Mondadori et al. (2007) reported measurement of follicles in buffalo: the primordial follicle diameter 35.0±3.11 µm, the primary follicle diameter 41.8±4.83 µm, and the secondary follicle diameter 53.3±12.04 µm.

The number of follicles in a Gayo mare ovary varies at each stage. In this study, the number of follicles in a Gayo mare ovary is presented in Table 2.

Generally, the number of primordial follicles is greater than the number of developing follicles, but in this study, there were fewer primordial follicles. This may be because the ovarian histological preparations were not cut repeatedly. The number of follicles between one ovary and another varies. This can be influenced by several factors, one of which is the status of the individual's estrous cycle when the sample is taken. The number of follicles in each ovary in horses was reported by Driancourt et al. (1982); 10,400-50,400 primordial follicles in ponies and 6,400-72,500 primordial follicles in large horses, and 37-300 developing follicles in ponies and 20-152 developing follicles in large horses. Driancourt and Cardon (1979) reported that the number of atretic follicles in horses averaged 14±10 follicles. Meanwhile, Hamny et al. (2010) calculated the number of luteal phase follicles in mouse deer; 13,600 primordial follicles, 148 primary follicles, 142 secondary follicles, and 13 tertiary follicles. In addition, Kacinskis et al. (2005) reported the number of preantral follicles in Zebu cattle; 80 primordial follicles, 66 primary follicles, and 33 secondary follicles. Mondadori et al. (2007) also reported the number of preantral follicles in buffalo; 17 primordial follicles, 57 primary follicles, and 23 secondary follicles.

CONCLUSION

Gayo mare ovaries have a histological structure that is not different from the ovaries of other types of horses. The growth of follicles in Gayo mares occurs on the outside (medulla) of the ovaries, and de Graff's follicles are found on the inside (cortex) of the ovaries.

ACKNOWLEDGEMENT

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### Table 1. Average diameter of the follicle and corpus albican in a Gayo mare ovary

<table>
<thead>
<tr>
<th>Ovary</th>
<th>Primordial</th>
<th>Primary</th>
<th>Secondary</th>
<th>Tertiary</th>
<th>Atretic</th>
<th>Corpus Albican</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right</td>
<td>32.20</td>
<td>65.26</td>
<td>164.80</td>
<td>330.65</td>
<td>16.88</td>
<td>460.14</td>
</tr>
<tr>
<td>Left</td>
<td>21.00</td>
<td>43.40</td>
<td>73.84</td>
<td>171.07</td>
<td>81.18</td>
<td>624.85</td>
</tr>
<tr>
<td>Average</td>
<td>26.60</td>
<td>54.33</td>
<td>119.32</td>
<td>230.86</td>
<td>49.03</td>
<td>511.10</td>
</tr>
<tr>
<td>SD</td>
<td>2.37</td>
<td>6.70</td>
<td>25.55</td>
<td>49.46</td>
<td>45.47</td>
<td>132.41</td>
</tr>
</tbody>
</table>

### Table 2. Number of follicles and corpus albicans in a Gayo mare (GM) ovary

<table>
<thead>
<tr>
<th>Gayo Mare</th>
<th>Ovary</th>
<th>Follicles (µm)</th>
<th>Corpus Albican</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM 1</td>
<td>Right</td>
<td>15</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>GM 2</td>
<td>Right</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>GM 3</td>
<td>Right</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>6</td>
<td>2</td>
</tr>
</tbody>
</table>
the implementation of funding activities for Basic Research of the Faculty of Veterinary Medicine, Fiscal Year of 2019 Number: 3471/UN11/SKP/PNBP 2019 dated August 9, 2019.

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