**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 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 estrus 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 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 ovaries.

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

**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 ovaries.

**Kata kunci:** kuda gayo, ovarium, folliculogenesis, corpus albican
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 (Nurjahmah 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

![Figure 4. Development of follicles on the ovary of a Gayo mare. A. Primordial Follicle. B. Primary Follicle. C. Secondary Follicle. D. Tertier Follicle. E. Atretic Follicle (AF). CT = Connective Tissue, PrF = Primordial Follicle, SC = Squamous Cell, CC = Columnar Cell, PF = Primary Follicle, SF = Secondary Follicle, TF = Tertier Follicle, BV = Blood Vessel, GC = Granulosa Cell, TiC = Theca Interna Cell, TeC = Theca Externa Cell, FA = Follicle Antrum, LGC = Lutein Granulosa Cell. H&E × 100 (C, D) and × 400 (A, B, E).]
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

---

**Figure 5.** Sketch of de Graff's follicle of a Gayo mare ovary with a diameter of 3.8cm and longitudinal cut. TiC = Theca Interna Cell, TeC = Theca Externa Cell, FA = Follicle Antrum, F = Follicle, BV = Blood Vessel, OF = Ova Fossa.

**Figure 6.** Overview of the corpus albican on the ovary of a Gayo mare (CA = Corpus Albican, CT = Connective Tissue, BV = Blood vessel. H&E × 100)
Table 1. Average diameter of the follicle and corpus albican in a Gayo mare ovary

| Ovary   | Primordial | Primary | Secondary | Tertiary | Atretic | Corpus Albican |
|---------|------------|---------|-----------|----------|---------|----------------|
| Right   | 32.20      | 65.26   | 164.80    | 330.65   | 16.88   | 460.14         |
| Left    | 21.00      | 43.40   | 73.84     | 171.07   | 81.18   | 624.85         |
| Average | 26.60      | 55.33   | 119.32    | 270.86   | 49.03   | 511.10         |
| SD      | 2.37       | 6.70    | 25.55     | 49.46    | 45.47   | 132.41         |

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

| Gayo Mare | Ovary | Primordial | Primary | Secondary | Tertiary | Atretic | Corpus Albican |
|-----------|-------|------------|---------|-----------|----------|---------|----------------|
| GM 1      | Right | 15         | 32      | 42        | 23       | 4       | 1              |
|           | Left  | 12         | 10      | 21        | 4        | 1       | 2              |
| GM 2      | Right | 4          | 18      | 11        | 4        | 0       | 1              |
|           | Left  | 3          | 18      | 35        | 12       | 0       | 0              |
| GM 3      | Right | 3          | 18      | 13        | 9        | 0       | 0              |
|           | Left  | 6          | 14      | 14        | 2        | 0       | 3              |

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

This research is part of a research on saving Gayo horses with the title: Study of Ovarian Histology as a Basis for Determining Normal Reproduction in Gayo horses which is funded by Syiah Kuala University, Ministry of Research, Technology and Higher Education, in accordance with the agreement letter for
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.

REFERENCES

Akoso, B.T. 2012. Budi Daya Sapi Perah. Airlangga University Press, Surabaya.

Amrozi, L.I.T.A. Tumbekala, A. Okctaviani, B. Achmadi, and J. Melia (2015). Ovsynch dan inseminasi buatan pada induk kuda warmblood yang diinduksi ovulasi dengan human chorionic gonadotropin dengan jamak. J. Kedokt. Hewan. 9(12):151-154.

Arango, J.C. and J.R. Newcombe. 2009. The effect of hormone treatments (HCG and cloprostenol) and season on the incidence of hemorrhagic anovulatory follicles in the mare: A field study. Theriogenology. 72:1262-1267.

Beest, J.M.T. and M.W. Schook. 2016. Estrus cycle synchronization in theperian onager (Equus hemionus onager). Zoo Biol. 35:87-94.

Brinsko, S.P., T.L. Blanchard, D.D. Varner, J. Schumacher, C.C. Love, K. Hinrichs, and D. Hartman. 2011. Manual of Equine Reproduction. 3rd ed. Mosby Elsevier, Missouri.

Deng, L., H. Duan, X. Zhang, S. Zeng, C. Wu, and G. Han. 2014. Advances in the research and application of artificial insemination to equids in China: 1935-2012. J. Equine Vet. Sci. 34:351-359.

Driancourt, M.A. and M. Cardon. 1979. Follicular kinetics in the mare ovary. Annales de Biol. Anim. Bioch. Biophy. 19(5):1443-1453.

Driancourt, M.A., A. Paris, C. Roux, J.C. Mariana, and E. Palmer. 1982. Ovarian follicular populations in pony and saddle-type mares. Reprod. Nutr. Developm. 22(6):1035-1047.

Eroschenko, V.P. 2014. Atlas Histologi diFiore: Dengan Korelasi Fungsional. Edisi 11. EGC, Jakarta.

Evans, A.C.O. 2003. Caharacteristics of ovarian follicle development in domestic animals. Reprod. Dom. Anim. 38:240-246.

Falcone, M.E., M. Rossi, and R. Mantovani. 2016. Collection, storage and freezability of equine epididymal spermatozoa. Italian J. Anim. Sci. 15(3):386-389.

Gabri, M.S., D.H.A. Kader, M.A. Ibrahim, and B.N. Hassan. 2012. Histological and immunohistochemical changes in rabbit ovary under the effect of two types of contraceptive pills. J. Experiment. Biol. 6(1):1-7.

Gastal, E.L., M.O. Gastal, M.A. Beg, A.P. Neves, B.P.L. Petrucci, R.C. Mattos, and O.J. Ghinter. 2005. Miniature ponies: Similarities from larger breeds in follicles and hormones during the estrous cycle. J. Equine Vet. Sci. 28(9):508-515.

Griffin, J., B.R. Emery, I. Huang, C.M. Peterson, and D.T. Carrell. 2006. Comparative analysis of follicle morphology and oocyte diameter in four mammalian species (mouse, hamster, pig, and human). J. Experiment. Clin. Assist. Reprod. 3(2):1-9.

Hamny, S., Agungpriyono, I. Djuwita, W.E. Prasetyaningtyas, and I. Nasution. 2010. Karakteristik histologi dan perkembangan folikel ovarium fase luteal pada kancil (Tragulus javanicus). Indonesian J. Vet. Sci. Med. 2(1):35-42.

Harissatia, D. Surtina, J. Hendri, and Juwandi. 2017. Respon estrus kuda lokal dengan induksi hormon PGF2α di Kota Payakumbuh. J. Peternakan. 14(2):65-69.

Ihsan, M.N. 2010. Ilmu Reproduksi Ternak Dasar. UB Press, Malang.

Kacinski, M.A., C.M. Lucci, M.C.A. Luque, and S.N. Bao. 2005. Morphometrical and ultrastructural characterization of Bos indicus preantral follicles. Anim. Reprod. Sci. 87:45-47.

Kruamer, D.C. 2013. A history of equine embryo transfer and related technologies. J. Equine Vet. Sci. 33:305-308.

Lemna, A., H.J. Schwartz, and M. Bekana. 2006. Application of ultrasonography in the study of the reproductive system of tropical jennies (Equus asinus). Trop. Anim. Health Product. 38:267-274.

Mardika, K., I. Setyawati, and A.A.K. Darmadi. 2018. Panjang siklus estrus dan struktur histologi ovarium tikus putih setelah pemberian ekstrak etanol daun kailantra merah. J. Vet. 19(3):342-350.

Markovic, D., M. Pavlovic, and V. Pavlopic. 2003. Seasonality, folliculogenesis and luteogenesis in mare ovaries. Facta Universitatis series Medicine an Biology. 10(3):120-126.

Melia, J. 2017. Studi Fisiologi Reproduksi Kuda Gayo Sebagai upaya Penyelamatan Plasma Nuriah Kuda Asli Indonesia: Disertasi. Sekolah Pascasarjana Institut Pertanian Bogor, Bogor.

Melia, J., M. Agil, I. Supriatna, and Amrozi. 2016. Anatomi dan gambaran ultrasound organ reproduksi selama siklus estrus pada kuda Gayo betina. J. Kedokt. Hewan. 10(2):103-108.

Mohammadpour, A.A. 2007. Comparative histomorphological study of ovary and ovarian follicles in Iranian lori-bakhtiari sheep and native sheep. Pakistan J. Biol. Sci. 10(4):673-675.

Mondadori, R.G., M.C.A. Luque, T.R. Santin, and S.N. Bao. 2007. Ultrastructural and morphometric characterization of buffalo (Bubalus bubalis) ovarian preantral follicles. Anim. Reprod. Sci. 97:323-333.

Nurjannah and P. Widyaningrum. 2016. Perkembangan ovarium tikus yang dipapar radiasi sinar x. J. MIPA. 39(2):85-91.

Omo, M., H. Akuzawa, Y. Nambo, Y. Hirano, J. Kiwaki, S. Takemoto, S. Nakamura, H. Yokota, R. Himeno, T. Higuchi, T. Ohtaki, and S. Tsumagari. 2015. Analysis of the equine ovarian structure during the first twelve months of life by three-dimensional internal structure microscopy. J. Vet. Med. Sci. 77(12):1599-1603.

Overbeck, W., K. Jager, H.A. Schoon, and T.S. Witte. 2013. Comparison of cytological and histological examinations in different locations of the equine uterus-an in vitro study. Theriogenology. 79:1262-1268.

Rosadi, B., M.A. Setiadi, D. Sajithi, and A. Boediono. 2011. Preservasi ovarium dan pengaruhnya terhadap morfologi folikel domba. J. Vet. 12(2):91-97.

Saleh, A.M. 2013. Histological study of ovary through last periods (Oryctolagus cuniculus) of pregnancy in domestic rabbit. Kufa J. Vet. Med. Sci. 4(1):11-19.