

DETERMINATION OF OVULATION TIME IN GAYO MARES BASED ON IMAGES OF PREOVULATORY FOLLICLE GROWTH

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ABSTRACT

This study aimed to obtain the ultrasound image of preovulatory follicle growth in Gayo mares to predict the ovulation time. This study used three Gayo mares and one Gayo stallion as teasers, aged 5-10 years and weighed 200-250 kg. Preovulatory follicle growth was observed using transrectal ultrasonography that performed on the dominant follicle with a diameter of ≥ 3.5 cm. Observations were carried out during three consecutive estrous periods at relatively the same time. Occurrence of ovulation was determined based on the absence of previous follicular appearance and the presence of a hypoechoic area in the same ovary. The result revealed that the diameter of the preovulatory follicle of Gayo mares ranged from 4.02 ± 0.32 to 5.05 ± 0.04 cm. There was no significant follicle growth in the last 48 hours before ovulation and the preovulatory follicle shape altered from spherical to pear-shaped. Furthermore, many small follicles (2 cm in diameter) were observed in the peripheral area of the ovary indicating the recruitment of follicles that formed the first follicular wave. It can be concluded that the alteration in size and the pear shape appearance of preovulatory follicles and the recruitment of small follicles can be used as a marker to determine the ovulation time of Gayo mares.

Key words: Gayo mares, ovulation time, preovulatory follicle, ultrasonography

ABSTRAK

Tujuan penelitian ini adalah mengetahui gambaran ultrasonografi pertumbuhan folikel preovulasi untuk memprediksi waktu ovulasi pada kuda gayo betina. Penelitian ini menggunakan 3 ekor kuda gayo betina dan 1 ekor kuda gayo pejantan sebagai teaser dengan kisaran umur 5-10 tahun dan berat badan 200-250 kg. Pertumbuhan folikel preovulasi diamati menggunakan ultrasonografi transrektal yang dilakukan terhadap folikel dominan berukuran $\geq 3,5$ cm. Pengamatan terhadap pertumbuhan folikel dilakukan selama tiga periode estrus secara berurutan dalam waktu yang relatif sama. Terjadinya ovulasi ditentukan berdasarkan tidak ada penampakan folikel sebelumnya dan kehadiran area hypoechoic pada ovarium yang sama. Hasil penelitian menunjukkan bahwa diameter folikel praovulasi kuda gayo adalah $4,02 \pm 0,32$ sampai $5,05 \pm 0,04$ cm. Tidak ada pertumbuhan folikel yang signifikan dalam 48 jam terakhir sebelum ovulasi dan bentuk folikel praovulasi berubah dari spherical menjadi pearshape. Banyaknya folikel kecil (berdiameter 2 cm) yang diamati di daerah perifer ovarium menunjukkan perekrutan folikel yang membentuk gelombang folikel pertama. Kesimpulannya, perubahan ukuran dan adanya bentuk seperti buah pear dari folikel preovulasi dan perekrutan folikel kecil dapat digunakan sebagai penanda untuk menentukan waktu ovulasi pada kuda gayo.

Kata kunci: kuda gayo betina, waktu ovulasi, folikel preovulasi, ultrasonografi

INTRODUCTION

Gayo horse is one of the germplasms of native Indonesian horses in addition to local horses. According to the Decree of the Minister of Agriculture of the Republic of Indonesia No. 1054/Kpts/SR.120/10/2014, the Gayo horse has been designated as one of the horses belonging to the native Indonesian horse family. As a native horse in Indonesia, Gayo horse lives and breed in the Gayo Highlands, Central Aceh District of Aceh Province. Additionally, this horse also found in two other districts in Aceh Province, namely Gayo Lues and Bener Meriah. Currently, the Gayo horse population is declining and if this problem is not taken seriously, it is feared that this horse will become extinct. Factors causing the decline of Gayo horse population are the occurrence of cross-breeding with other horse species, uncontrolled slaughtering, and the shift in the use of Gayo horses which was previously used as working animal, thereby reducing the local community interest in preserving it (Melia *et al.* 2022).

Several efforts can be made to increase the Gayo horse population; one of which is through the application of Artificial Insemination (AI). Artificial insemination has been widely applied in maintaining

the horse population in both developed and developing countries. However, there are still many obstacles in finding the right time for the implementation of AI, such as the long estrus period which make it difficult to predict the time of ovulation. One of the factors causing the success of AI in horses is the timeliness in placing semen in the uterus (Samper 2001). If the timing of AI is not accurate, then natural mating or AI in horses must be repeated (Amrozi *et al.* 2015), thus requiring greater costs. Therefore, veterinarians need to improve their knowledge and skills in detecting estrus and ovulation time in order to achieve good and satisfactory AI results.

Ultrasound is the most precise and appropriate method to observing the growth of the preovulatory follicles in horses. According to McCue (2008), ultrasound can better display the condition of reproductive tract and ovarian potential. Furthermore, according to Ginther and Utt (2004), ultrasound has been widely used in modern horse breeding to guide the AI process to increase pregnancy rates. In addition to ultrasound, the use of transrectal color doppler sonography can increase the success rate of breeding programs in horses, because by using this the ovarian blood flow can be observed clearly (Bollwein *et al.* 2002; Romano *et al.* 2015). Although ultrasonography

has been widely use in breeding program of other horse breed, but its use in research investigating the reproductive aspects of Gayo horses especially in observing the development of preovulatory follicles, has not been carried out. Therefore, the study aims to identify the preovulatory follicle growth as an indicator for determining the timing of ovulation time in Gayo horses. The data obtained can be used to support the successful the AI programs in the Gayo horse.

MATERIALS AND METHOD

This study used three Gayo mares and one Gayo teaser stallion to help estrus detection, aged 5 to 10 years old and weighed 200-250 kg. All horses were adapted for one month and given concentrate (X-Tra Pro®, Putri Gunung Farm, Bogor) with a protein content of 14% twice a day and green grass during the day and evening, vitamin (Multivitamin injection®, Norbrook Laboratories Limited, Newry, 5 mL/horse), and anthelmintic (Panacur 10% suspension® Intervet International GmbH Unterschleissheim, Alemania, 3 mL/40 kg BW). Furthermore, the horses were released to the ranch for exercise every morning until near noon. Preovulatory follicle growth was monitored using transrectal ultrasound (MINDRAY DP20 VET, equipped with 5 MHz transrectal linear probe, 50L60EAV, Shenzhen Mindray Bio-Medical Electronic Co. Ltd., China). The observed parameters include the number and size of the follicles as well as the diameter of the corpus rubrum. Follicles were classified into class 1 follicles with < 2cm in diameter, class 2 with 2-4cm in diameter and class 3 with a diameter of > 4cm. The monitoring was performed when the dominant follicle was ≥ 3.5 cm in size. Observations were made on three consecutive estrous periods in Gayo mares at relatively the same time. To minimize errors in measurement, the preovulatory follicular diameter was obtained from the mean diameter of each follicle in the last three examinations before ovulation (Cuervo-Arango and Newcombe 2008). It was found that the diameter of the follicles remained constant for the last 36-24 hours before ovulation (Koskinen *et al.* 1989). The absence of

previous follicular appearance and the presence of a hypoechoic area in the same ovary indicated that ovulation had occurred. The corpus rubrum only begins to form shortly after the evacuation process of follicular fluid and oocytes ends. Determination of the daily growth diameter of the follicle was carried out based on the difference in value of the diameter of follicles growth, then reduced by the value of the growth diameter on the day of observation. The data of preovulatory follicle diameter during three consecutive estrous periods were presented descriptively.

RESULTS AND DISCUSSION

The range of preovulatory follicle diameter of Gayo mares during the estrous period was 3.70-5.10 cm (mean of 4.02 ± 0.32 to 5.01 ± 0.04 cm). In 48-24 hours before ovulation, the preovulatory follicle growth rate was 0.13 ± 0.08 to 0.14 ± 0.1 cm in size. The increase in preovulatory follicle diameter in 48-24 hours before ovulation was not significantly different. Moreover, in another study, it was found that estrogen levels clearly increased 3-1 days before ovulation. At the same time, the progesterone levels dropped, which indicate that the mares had enough Luteinizing Hormone (LH), allowing for ovulation to be induced. LH concentration increased sharply 18 hours before ovulation (Ginther *et al.* 2010). New follicular ovulation occurred when the preovulatory follicle diameter reached a range of 5 cm, and this occurred repeatedly (in each estrous cycle) in all Gayo mares (Table 1).

The absence of a significant increase in preovulatory follicular diameter at 48-24 hours prior to ovulation in Gayo mares supports the previous finding that follicular diameter remained constant before ovulation (Koskinen *et al.* 1989). According to Cuervo-Arango and Newcombe (2008), the preovulatory follicle diameter in one broodmare was relatively the same. The average maximum diameter of the ovulatory follicles is usually 40-45 mm in riding-type of horses (quarter horse, Arabian, and thoroughbred), but the range can be larger. The variation in preovulatory follicle diameter in 24 hours before ovulation ranged from 34-70 mm (Ginther 1995) or 22-65 mm (Cuervo-

Table 1. Preovulatory diameter of follicles in Gayo mares during the estrous period

Estrous day-	Preovulatory follicle diameter (cm)	Daily follicle growth (cm)	Corpus rubrum diameter (cm)
1	4.02 ± 0.32	0.32 ± 0.18	-
2	4.34 ± 0.34	0.40 ± 0.25	-
3	4.74 ± 0.16	0.13 ± 0.08	-
4	4.87 ± 0.14	0.14 ± 0.13	-
5	5.01 ± 0.40	-	-
ovulation	-	-	-
shortly after ovulation	-	-	3.55 ± 0.31

Table 2. The number of follicles two days before ovulation in Gayo mares

Days before ovulation	Number of follicle		
	Class 1 ($\varnothing < 2$ cm)	Class 2 ($\varnothing 2-4$ cm)	Class 3 ($\varnothing > 4$ cm)
2 (48 h)	20.13 ± 4.24	2.13 ± 1.46	0.88 ± 0.35
1 (24 h)	21.38 ± 3.46	2.25 ± 1.16	1.00 ± 0.00

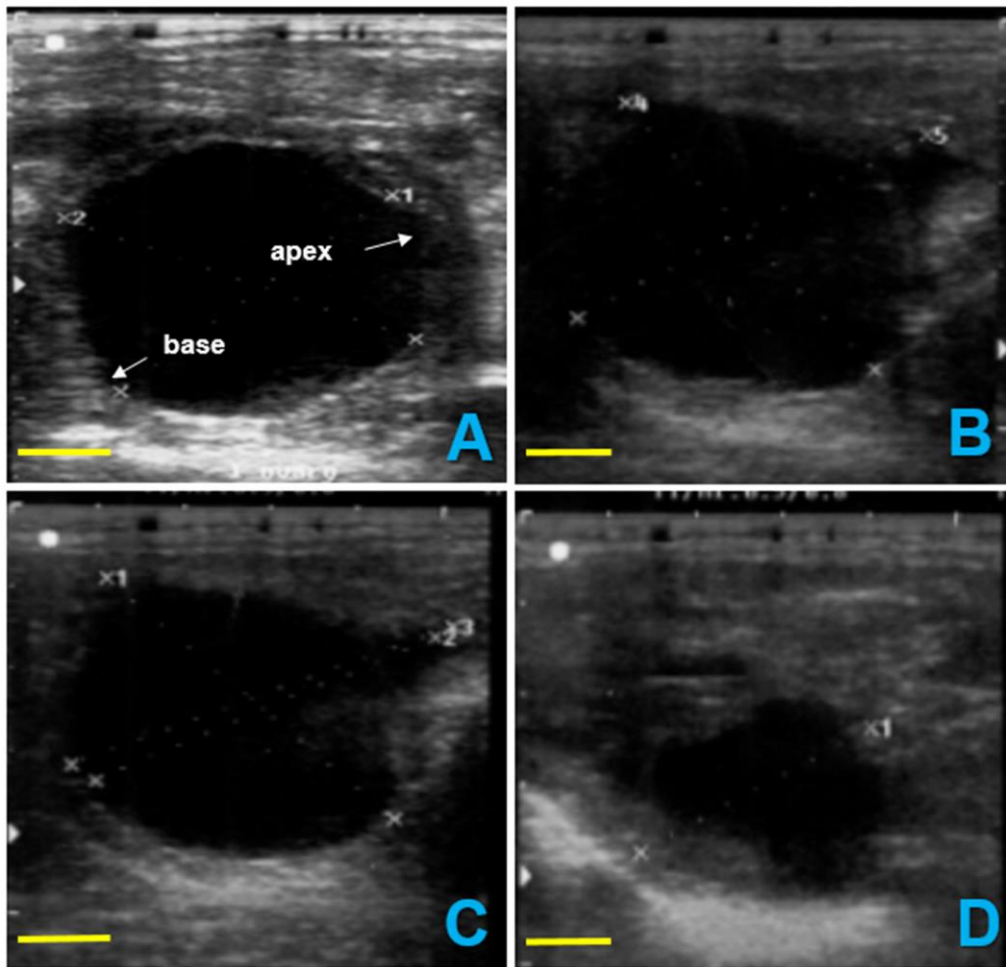


Figure 1. Ultrasonic imaging of Gayo mare's ovary during ovulation. A= Preovulatory follicle 1 hour before starting evacuation, B= 5 minutes before follicular wall rupture, C= Evacuation of the follicle shortly after the onset of follicular wall rupture, D= Immediately after ovulation. Bar scale: 1 cm

Arango and Newcombe 2008), and the current findings in Gayo mares were 4.98-5.10 cm.

A Gayo horse is a large pony that commonly lives in the two-season countries; however, it has the same preovulatory follicle diameter as any other horse in the four-season countries. Meanwhile, a miniature pony has smaller preovulatory follicle diameter (Gastal *et al.* 2008). Many factors influence the diameter of ovulatory follicles, such as breed, age, geographic location (Ginther 1995), body condition, nutrition, climate, stress, and photoperiod (Bossis *et al.* 1999; Tilbrook *et al.* 2000).

The preovulatory follicle of Gayo mares is pear-shaped with a sonographic appearance, such as shadowing on the follicular fluid and the apex leading to the ovulatory fossa (Figure 1). According to Gastal *et al.* (1998), changes in the shape of the follicle from spherical to non-spherical appeared two days before ovulation, with the highest frequency occurring 24-2 hours before ovulation. Currently, ultrasound studies of multiple preovulatory follicles have been carried out with special designs to validate the methodology for predicting ovulation time in mares (Gastal and Gastal 2011). In Gayo mares, class 1 follicles (with < 2 cm in diameter) were found 2-1 days before ovulation (Table 2).

The ultrasonic imaging of Gayo mare's ovary during ovulation is presented in Figure 1. This image can be a guide for sonographers to predict the timing of ovulation in mares other than the pear shape of the preovulatory follicle. After ovulation, the preovulatory follicle will become the corpus rubrum. The average diameter of corpus rubrum was 3.55 ± 0.31 cm, lasting 1-4 days after ovulation.

CONCLUSION

The alteration in the size and the pear-shaped appearance of the preovulatory follicle and one-sided thinning of the follicle wall and the recruitment of small follicles can be used as an indicator to determine the time of ovulation in Gayo mares.

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