Estrous Behavior in the Thoroughbred-Indonesian Local Crossbred Mares

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ABSTRACT: The development of horse breeding industry in Indonesia was commenced through horse racing events held all over the country. It were accelerated by the development of Thoroughbred-Indonesian local Crossbred horses. There are many broodmares injured during their racing time and retired from the racetracks. They may still has a reproductive vigor to continue on producing offsprings. A few information has been reported on the monitoring the reproductive capacity of the mares. The objective of this study was to explore the estrus behavior correlated with ultrasonography imaging of the ovarian dynamics of the Thoroughbred-Indonesian local crossbred mares. Three Thoroughbred-Indonesian local crossbred mares with 6.25-12.5% of local genetics aged 12-20 years old were used in this study. Ultrasonography examination was done every morning at approximately at the same time. Estrus behavior was observed by using teaser stallions following a standard method. Results of the experiment indicated that mares show estrous behavior such as winked vulva, squatting, receive the stallion, tail raised, urinating, and mating stand in the ovulation time that occured in the day 25.4±3.78 (estrous cycle) correlated with the diameter of domminant follicle 4.2±1.44 cm. In conclusion, Estrous behavior can be used to monitor the estrous cycle to optimized the mating time in the mares.

Keywords: estrous, behavior, teasing, mares

INTRODUCTION

Indonesian equine industry is being develop in recent years as the effect of horse racing activity around the country. The Up Grading system for Indonesian local mares with Thoroughbred stallions resulted the Crosbred Horses, named G3,G4 and KPI (*Kuda Pacu Indonesia*) that have 6,25 to 25 percent of local genetic. KPI is resulted from G3 x G3, G3 x G4 and G4 x G4 (PP. PORDASI 2000 cit. Berliana 2007)

Reproductive cycles are correlated with many phenomenon including: puberty, sexual matuity, breeding season, estorus cycle, post partum sexual activity, and aging (Hafez, 2000). In the other hand, Donadeu and Ginther (2002) reported that follicular waves are developed in the middle of estrous cycle, and there will be just single domminant follicle will be ovulated by the end of Estrous. Interovulatory interval in mares consist of various minor follicular wave combination that wont ovulated and major waves, that the biggest follicle will be domminant and ovulated.

Interovulatory interval has begun by the time that ovulation occured and will be ended in the next ovulation of the next estrous cycle. The length of interovulatory interval are 21-22 days for horses, and 24 days for ponies (Ginther, 1992). Follicle will grow normally untill the beginning of deviation. After deviation, domminant follicle will continued to grow, and subordinate follicles will be regreted. Deviation is begin when the size of dimminat follicle is 22.5mm (Ginther *et al*, 2004)

Indonesian horse breeding system is traditionally managed. One of the subsystem is the

measurement of the mating time according to the estrus behavior. That caused the result have not optimum yet. The Ultrasoundography (USG) method has begin to used by some practitioners in order to give accurate mating time by combine the traditional system with the USG method to improve pregnancy rate in mares

The aim of this study was to explore the estrous behavior related to ultrasonography imaging of the ovarian dynamics in the estrous cycle of the Thoroughbred-Indonesian local crossbred mares. The result of this study can be reffered as the general information to estimate the optimum mating time based on the estous behavior and ovarian dynamics ultrasonography imaging.

MATERIALS AND METHODS

This study was held at the Reproduction Rehabilitation Unit, Department of Reproduction, Clinic, and Pathology, Faculty of Veterinary Medicine, Bogor Agricultural University, Bogor, Indonesia. Three Thoroughbred-Indonesian local crossbred mares that contains 6.25 until 12.5 percent of local genetic sources, aged 12-20 years old were used in this study. The mares were fed fresh grasses and pellets that contains 12 percent of Proteins.

The equipments that used in this study include: USG (ALOKA SSD-500, Aloka Co. Ltd, Japan), linear probe 5 MHz (ALOKA UST-588U-5, Aloka Co. Ltd. Japan), (SONY, UP-895 MD, Video Graphic Printer, Japan), syringe (One Med, PT. Jaya Mas Medica Industri), plastic gloves (Europlex®, Divasa Farmativa, S.A.), lubrication gel, PGF2α (Dinoprost, Noroprost 0.5% W/V, Norbrook Laboratories Limited, Newry), hCG (Chorulon, Intervet, Cambridge), and 70% alcohol.

Estrous and Ovulation Synchronization

This study was begun by injection of $PGF2\alpha$ (Dinoprost, Noroprost 0.5% W/V, Norbrook Laboratories Limited, Newry) single-dose in the luteal phase to synchronize the estrous cycle. Followed by hCG injection (Chorulon, Intervet, Cambridge) single-dose 1500 I.U. when the domminant follicle obtain 30mm in size.

Estrous Behavior

The observation of estrous behavior done twice, the first, one day after PGF injection until ovulation occured, and the second begun when day 17 until ovulation by teasing methods. Observation based on scoring system according to Coleman dan Powell (2004) below:

 Table 1. Estrous Behavior Scoring System in Mares

Score	Estrous sign
0	Agresive to the stallion, even attacking or kicking
1	Stay when the stallion is approaching
2	Begin to approach the stallion, winked vulva, tail raising
3	More attracted to stallion, tail raising, squatting, urinating
4	Strongly attracted to stallion, winked vulva, and continuous urinating

Source: Coleman dan Powell (2004)

Ultrasonography

USG examination done every day at the same time, begun shortly after the estrous synchronization, and every four hours shortly after the injection of hCG until ovulation occured to observe the ovarian dynamics, include the diameter of the corpus luteum (CL), number and size of the follicles. The diameter of each preovulatory follicle measured by the average value (Shirazi, 2004).

Data Analysis

The collected data presented descriptively by calculating the average and standard deviation. Analysis was used software MS Office Excel 2007.

RESULT AND DISCUSSION

Estrous and Ovulation Syncronization

Tabel 2. Estrous and ovulation Syncronization

Parameter	$Mean \pm SD$	
Largest Follicle diameter (cm)		
Early PGF2α treatment	2.63 ± 0.06	
Early hCG treatment	3.27 ± 0.12	
Maximum	4.50 ± 0.52	
Day before ovulation	4.50 ± 0.52	
CL Diameter (cm)		
Early PGF2α treatment	2.17 ± 0.15	
Early hCG treatment	1.77 ± 0.45	
Day before ovulation	0.83 ± 0.32	
Estrous (days)		
Early PGF2α treatment to estrous onset interval	1.33 ± 0.58	
Estrous duration	4.00 ± 1.00	
Interval to ovulation occured		
Early PGF2α treatment (days)	5.33 ± 1.15	
Early hCG treatment (hours)	66.67 ± 10.07	

Estrous and ovulation synchronization were administered PGF2 α 2ml i.m and hCG 1500 IU i.m obtained the results as shown in table 2. The average diameter of the largest follicles at the beginning of PGF2 α treatment was 2.63±0.06 cm, while in initial hCG treatment amounted to 3.27±0.12 cm. The average maximum diameter of the largest follicle reached on the day before ovulation, that is equal to 4.50 ± 0.52 cm . Bergfelt *et al* (2007) reported that the average diameter of the largest follicles at the beginning of PGF2 α treatment amounted to 2.27±0.19 cm with a range of 1 to 4 cm , whereas at the beginning of hCG treatment amounted to 3.15±0.15 cm in the range of 1.9 up to 4.5 cm. The average diameter of the largest follicle reached a maximum one day before ovulation by 3.65±0.1 cm. The average diameter of the CL at the beginning of PGF2 α treatment obtained at 2.17±0.15 cm, whereas at the beginning of hCG treatmen amounted to 1.77±0.45 cm, and one day before ovulation by 0.83±0.32 cm. Average Interval from early PGF2 α treatment to the onset of estrous throughout 1.33±0.58 days, while the average duration of estrous was 4.00±1.00 days. Interval to achieve ovulation by the initial PGF2 α treatment was 5.33±1.15 days,

while from the beginning of hCG treatment was 66.67±10.07 hours. Conversely, if the follicle has reached its maximum diameter during the luteal phase dominated by progesterone, then the follicle will regress, and there will be recruitment of new follicles, then estrus and ovulation will be delayed (Samper *et al.*, 1993).

Ovarian Dynamics and Estrous Behavior

Based on the research that has been done using ultrasonography every day at the same time on three mares, it's founded the ovarian dynamics include the development and regression of follicles and CL consisting of waves of follicles, and its correlated to estrous behavior scoring during 1 estrous cycle. Based on the data of all the horses in this study can be seen that the average estrous cycle length was 25.4±3.78 days with 2 to 3 follicular waves and estrous duration was 6.8±1.92 days. The average maximum diameter of the largest follicle before ovulation is 4.4±4.2 cm with a range of 3.0 to 5.8 cm. Donadeu and Ginther (2002) reported that the ovulatory follicular waves developed in the mid estrous cycle time and usually one follicle will ovulated at the end of estrous cycle. The interval between ovulation in horses consists of various combinations of follicular minor waves, where the largest follicles do not become dominant, as well as major waves, where the largest follicle becomes dominant. The average length of the interval between ovulation is 21 or 22 days for horses, and 24 days for ponies (Ginther 1992).



Figure 1. Estrous Behavior scoring visualization in mares: a) Score 0, b) Score 1, c) Score 2, d) Score 3, and e) Score 4

Individual behavior during estrous varies among individual horses, but tend to be the same between cycles. Signs of estrous can be seen, among others: the acceptance of the stallion, tail raised, frequent urination, vulva showed rhythmic contractions (winking), and squatting. Signs of estrous is also consistent with the observation that has been done by Coleman and Powell (2004), Waring (2003) and Hafez (2000).

Observation of estrous behavior indicates that the moments before ovulation will be marked by the achievement of the maximum score 3, which is characterized by showed attracted to the stallion, tail raised, winked vulva, squatting and urination and score 4, which is characterized by strong interest to the stallion, thrusting buttock ti the stallion, tail raised, winked vulva and continuous urination. From these results, it is expected in an attempt to increase pregnancy rate in horses, mating should be carried out when estrous behavior score reaches 3 or 4.

CONCLUSION

In conclusion, Synchronization with PGF2 α resulted estrous after 1.33±0.58 days, with estruos duration 4.00±1.00 days, and ovulation occured 66.67±10.07 hours after hCG administration when the follicle diameter reached 4.50±0.52 cm. While estrus cycle was 25.4±3.78 days with 2 to 3 follicular waves , estrous duration was 6.8±1.92 days , and naturally ovulatory follicle diameter was 4.2 cm±1.24. Ovulation occurs in estrus behavior score 3-4. Estrous behavior can be used to monitor the estrous cycle to optimized the mating time in the mares

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