Sexual behaviors of Ongole Crossbred bulls and cows with colony housing system

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Abstract. This study was conducted to determine the sexual behaviors of Ongole crossbreed bulls and cows with colony housing systems. Bull sexual behaviors was observed, including Oro-Nasal Contact (ONC), Flehmen, Mounts Orientation Response (MOR), Mounts, and Services. Cows sexual behaviors were: Mounting, Standing-to-be-mounted (STBM), and vulvar appearance (color, swelling, secretion of mucus). Sexual behaviors were observed in 24 hours, with the estrous cycle stage was determined by changes in the character of the vaginal epithelial cells. The data was analyzed by using statistic varian one-way method. The result showed that Ongole crossbreed bulls had sexual behaviors (ONC, Flehmen, MOR, Mounts, and Services) significantly higher than cows with estrous phase (P<0.01). The intensity of bull sexual behaviors had been influenced by the phase of estrous of the cows. At the time of estrus, the cows had the response of mounting (25%), STBM (100%), reddening vulva (75%), swelling vulva (75%), and mucus secretion (100%) that could be a factor of high sexual behavior intensity of the bulls. The conclusion of this study was bulls and cows that kept with the colony housing system had increased sexual behaviors when the cows at the estrous phase.

Key word: Bulls, Colony housing system, Cows, Sexual behaviors

1. Introduction
Increasing beef cattle productivity needs to be done so that it can meet the national meat’s needs. Reproductive parameters that need to be improved to increase parent productivity are calving intervals (1). Long calving intervals have an impact on lower cow productivity. Calving interval is influenced by service per conception (S/C). The accuracy of mating affects S/C that is usually based on estrous behavior. Estrous behavior can be shown with lifting the tail, groaning, mounting other livestock, and being relaxed while mounted by other cattle or bull. Estrous behavior is also usually followed by changes in the characteristics of the vulva such as red color, swollen, and appearance of clear mucous (2; 3).

The weakness of smallholder farmers’ understanding in estrus detection is one of the causes of return matting and increase of S/C. Besides the estrous detection, the feed and nutrient consumption of cows that are still below the nutrient adequacy standard is also the cause of the low incidence of estrous (4). Nutrient deficiencies in the cow will have difficulty expressing sexual behavior due to low secretion of reproductive hormones (5). As a result, the cow that is kept with low feed quality...
and quantity will experience silent heat due to lack of nutrient consumption. So that smallholder farmers often have difficulty in observing estrous behavior.

One effort to prevent silent heat and return mating is the use of males or bull as biostimulation which serves to stimulate the emergence of estrous behavior in the cow. Generally, bull can play a role in stimulating of estrous, detecting the presence of estrous in the cow who has the disorder of silent heat, and naturally, service of estrus cow with higher conception rate. This method is considered the most appropriate to be applied in a colony or group-housed maintenance system (6; 7).

The results of the study (8) showed that the use of a bull can increase conception rate. The bull can minimize the silent heat or estrous behavior caused by a lack of nutrients in the cow. The study of the use of male in the cows’ maintenance system will provide information that can be used as a consideration in the application of appropriate maintenance and mating systems. This study aimed to observe changes in male sexual behavior towards cow that are maintained in a colony. Another objective of this study was to find the sexual behavior expressed by the cow during the estrous period due to the presence of a male in the colony housing system.

2. Materials and Methods

The study was conducted in farm laboratory of Meat, Draught and Companion Animal, Faculty of Animal Science, Universitas Gadjah Mada, Sleman, Special Region of Yogyakarta from February to September 2018. One superior male (bull) of Peranakan Ongole (PO) cattle (500 kg of body weight) and four adult females of Peranakan Ongole (PO) cattle (±300 kg and 3-4yo) were housed and maintained in colony pen (20x10m).

2.1. Determination of estrous phases

Determination of estrous phases was done by analyzing the condition of vaginal epithelial cells. The vaginal smear was taken every 24 hours. Intake of vaginal epithelial cells was conducted by using a cotton bud then it was smeared on a glass object. The glass object was then immersed in an alcohol solution for 10 minutes, then it was soaked in a 3% of Giemsa solution for 40 minutes. Vaginal epithelial cells were observed using a microscope with a 40 times-magnification by 15 points from 3 reviews on the glass object. The cell characteristics that became the reference for determining estrous phases were parabasal cells, intermediate cells, and superficial cells.

2.2. Sexual behavior

The data taken was male sexual behavior and cow sexual behavior. Observation of sexual behavior was carried out as long as the cattle were aired together with the help of a Hikvision ™ CCTV camera with 1080p image quality. Observed male behaviors included the frequency of Oro-Nasal Contact (ONC), Flehmen, Mounts Orientation Response (MOR), Mounts, and Services, which were described in Table 1. Observed parent behavior i.e., Mounting, Standing-to-be-Mounted (STBM), and visual characteristics of the vulva, including vulva reddening, swelling of the vulva, mucous secretion which is described in Table 2.

| Table 1. Description of male sexual behaviors (9) |
|-----------------------------------------------|
| Sexual behavior | Description |                     |
| Oro-nasal contact (ONC) | The stimulus is characterized by sniffing, heading or licking the vulva. |                     |
| Flehmen | Sniff the cows genital area then pull the upper lip or grin |                     |
| Mounts orientation response (MOR) | The sudden movement will climb the cows from the rear position, often beginning by placing the head on the back of the cows |                     |
| Mounts | The position of the bull riding on the cows is characterized by the bull’s two front legs raised completely from the floor |                     |
| Services | Climb the cows followed by copulation |                     |
Table 2. Description of sexual behaviors and vulva characteristics of cows (9)

| Parameters                  | Description                                                                                     |
|-----------------------------|-------------------------------------------------------------------------------------------------|
| sexual behaviors            |                                                                                                 |
| Mounting                    | The response to climbing other cows is shown by the cows both to the bull or other cows          |
| Standing-to-be-Mounted      | When the cows receive being mounted by other cows or bulls, the cows in STBM does not mean silence completely, often moving forward because of the burden received |
| (STBM)                      |                                                                                                 |
| vulva characteristics       |                                                                                                 |
| Reddening vulva,            | The appearance of reddish color changes by comparing when not estrus                           |
| Swelling vulva              | Swelling of the vulva by comparing when not estrus                                              |
| Mucuse secretion            | The presence of clear mucous secretions                                                          |

2.3 Data analysis
The percentage of cell types in vaginal cytology is calculated in percentage to determine the estrous phase. Male sexual behavior in the phases of proestrous, estrus metestrus, and diestrus was statistical analyzed by one-way ANOVA, and the cow sexual behavior was analyzed descriptive quantitatively.

3. Result and Discussion
3.1. Determination of estrous phases
The Study on Vaginal cytology as a useful tool for estrous detection and estrous phase in clinical animals and breeding stations has been described for some species and breeds of animals (10), (11). Vaginal cytology showed variation in percentages of epithelial cells observed during the study to predict estrous. The results of vaginal cytology observations are presented in Table 3. The observations obtained in Table 3 showed differences in each cell type at each estrous phase. In the anestrous phase, the parabasal and intermediate cells were dominated, and no superficial cells were found limitless. Changes began to occur at the estrous phase with superficial cells increased the percentage and were dominated (49.96%). Changes in a composition that occurred in parabal cells and superficial cells can be used as indicators in determining the estrous phase, and superficial cells were the main indicators of estrous phase determination (12). At the estrous time, the superficial cells were dominated; they were 84.3% (4), 63.70% (11), and 90.57% (11).

Table 3. Percentage of vaginal histology cell types in each estrous phase of Ongole Crossbreed cow

| Phase   | Duration (days) | Percentage of vaginal histology cell types (%) | Parabasal | Intermediate | Superficial |
|---------|-----------------|-----------------------------------------------|-----------|--------------|-------------|
| Proestrous | 2-3             | 30.77                                         | 45.42     | 23.82        |
| Estrous  | 1-2             | 15.06                                         | 34.98     | 49.96        |
| Metestrous | 3-4             | 32.46                                         | 41.70     | 25.85        |
| Diestrous | 10-12           | 58.94                                         | 29.30     | 11.76        |

3.2. Males’ sexual behavior
Bulls provided varied sexual responses to the cow in various estrous phases (Table 4). The behavior that occurred was a response to the cow who began to give a signal which was then captured by the bull. In the proestrous phase, bulls exhibited sexual behavior in the form of ONC, Flehmen, and MOR responses. The response of bull sexual behavior was inseparable from attractiveness in the form of cows behavioral and non-behavioral stimulation that affected bull responses (13).
The estrous phase is the phase in which the cows receive matting by the bull. In this phase, the bull sexual behavior response arises as a whole. ONC response was 94.75 ± 24.55 times, flehmen was 31.75 ± 15.80 times, MOR was 138.75 ± 79.73 times and performed service was 2.25 ± 1.26 times. Sexual behaviors response shown by bulls in this study were in accordance with previous studies which stated that when the cows estrous, male responded with ONC and flehmen (14; 15), MOR (16), mounts (2), and services (13). Therefore, one of the male’s functions as a stimulant in a colony or group housing is to be able to service a cow in estrus condition. Sexual behavior of bulls to the cow at the estrous phase showed significantly higher (P < 0.05) compared to when the cow were in the anestrus phase.

The post-estrus period ended in a vulnerable period of 24h or entered the metestrus phase. Male showed that the response of sexual behavior to the cow was ONC and flehmen, which were 1.75 ± 1.71 and 0.75 ± 1.50 times respectively. The response was also raised by male after the end of the cow estrus period, which included ONC (9) and flehmen (15). Sexual behavior responses in the diestrus phase were not found. Male behavior was to find a partner, then approach and investigate (13). Investigation through olfactory molecules that provide clues to the phase of the cows’ estrous cycle and stimulate bull sexual behavior and endocrine function (17). The results showed that there was no sustainability in the bull response, where the bull no longer had attention to the cow in diestrus phase. This is consistent with the opinion (18), who stated that bulls were constantly looking for and identifying the cows to find out whether the cows were sexually accepting or not.

The function of bulls as detection of estrous can also be seen in the proestrus phase, namely the response of ONC and flehmen, which were 12.25 ± 10.87 and 6.50 ± 6.86 times respectively. The behavioral response increased dramatically when the cow entered the estrus phase. The bull naturally exhibited different behavior towards the cow’s estrous cycle. Expression of bull behavior towards estrous cows can be observed visually. (19) stated that, hormones affected livestock behavior. (17) stated that there were chemical compounds or pheromones produced by cows, these compounds became an indication that the cows entered the estrus phase, then the bull was able to capture the specific odor and stimulated the male with the appearance of signs of sexual behavior.

### 3.3. Cows’ sexual behavior

Sexual behavior exhibited by the cows, one of which was mounting (20). The results of the study (3) found 96% of the cows of the population showed that response. In this study, only 1 in 4 cows (25%) found a response to mounting behavior (Table 5). This result can be caused by various factors. Response to other animal was strongly influenced by the number of animals that were in the estrous phase and social hierarchy within the herd. The STBM response was one of the most accurate behaviors to show that cattle were in the estrous phase (21). The cows as a whole (100%) showed STBM. This result showed more STBM response than the research conducted by (3), which was 98% of the total estrus cows population.

Changes in the vulva characteristic during the estrous phase did not appear entirely in this research. (4) reported that signs of estrous of PO cows that were kept in the smallholder farmers group included slimy vulva (100%), red vulva (71.42%), swollen vulva (57.14%) and acted aggressively and agitated (57.14%), and groaning/sounding (42.85%). In another animal, (19)

| Parameters                        | Proestrus | Estrous | Metestrus | Diestrus |
|-----------------------------------|-----------|---------|-----------|----------|
| Oro-nasal contact (times)         | 12.25±10.87 | 94.75±24.55 | 1.75±1.71 | 0^b      |
| Flehmen (times)                   | 6.50±6.86^a | 31.75±15.80^a | 0.75±1.50^a | 0^b      |
| Mounts orientation response (times)| 0^b     | 138.75±79.73^a | 0^b     | 0^b      |
| Mounts (times)                    | 0^b     | 15.25±10.50^a | 0^b     | 0^b      |
| Services (time/phase)             | 0^b     | 2.25±1.26^a   | 0^b     | 0^b      |

^a,b Different superscripts on the same line show significant differences lines (P<0.05)
reported that the goat’s behavior which was estrous was agitated, sounded, while the vulva conditions as the estrous signs that were swelling, reddish colored and slimy (produce the mucus or a clear liquid).

Table 5. Sexual behavior and vulva characteristic of Ongole crossbreed cows at estrous phase

| Parameters                        | % cows |
|-----------------------------------|--------|
| Mounting                          | 25     |
| Standing-to-be-mounted (STBM)      | 100    |
| Reddening vulva                   | 75     |
| Swelling vulva                    | 75     |
| Mucuse secretion (%)              | 100    |

(22) stated that increased estrogen levels were associated with reddening and swelling of the vulva during the estrous phase, which stimulated blood flow to the reproductive tract and related genital organs. (23) stated that increased mucus secretion began with thickening of the vaginal wall and increased vascularity by stimulating the hormone estrogen. These conditions caused changes in the condition of the vulva that was swollen and red in color. The cow’s sexual behavior was influenced by the presence of the male as a stimulator, (7) stated that the cow who showed no signs of estrous, then housed with the bull in the colony will be showing these signs.

Hormone production affected the physiology of the animal. In estrous condition, the cows among others affected the odor and appearance outside the body that has changed (19). The presence of bulls gave biostimulants effects on cows, which can affect sexual maturity, ovulation, and decrease the time needed to restore the function of reproductive organs (24). The presence of males to females was also able to accelerate the appearance of the post-partum estrous in the heifers and shorten the post-partum time (6).

4. Conclusion
Based on the results, it was concluded that PO bull that were colonized with the cows in this research showed higher sexual behavior, especially when the cow was in the estrous phase and followed by the emergence of estrous behavior and characteristics in the cow.

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