The Effect of Synchronizing with the PGF2α hormone on the Estrus Activity of Madura Cows

Muchamad Luthfi1,2*, Lukman Affandhy S1, Sri Wahjuningsih3, Gatot Ciptadi3, and Trinil Susilawati3

1 National Research and Innovation Agency, Indonesia
2 Doctoral Program Animal Science, University of Brawijaya, Malang, East Java, Indonesia
3 Faculty of Animal Husbandry, University of Brawijaya, Malang, East Java, Indonesia

Abstract. This research aims to determine the biological response of administering the PGF2α hormone to the estrous activity of Madurese cows. This research method uses two observation techniques, namely estrus synchronization by administering the PGF2α hormone "Alfaglandin® C (Cloprostenol)" and observing natural estrus. The data were analyzed by using the t test using the Software for Research statistix 10. The results showed that the frequency of estrus at the same time of observation was not significantly different from the two treatments (p>0.05), the highest frequency of estrus occurred at 18.00-20.00 in synchronized cows or not. The results of visualization of estrus showed cervical mucus, swollen vulva, and vaginal pH had the same percentage, while the vaginal temperature and duration of estrus had different percentages. The conclusion of this study is the frequency of estrous events, estrus visualization (cervical mucus discharge, swollen vulva, vaginal pH, and length of estrus), and the appearance of cervical ferning has the same response, while estrus temperature has a variable response.

1 Introduction

Madura cattle is a type of dual-function (for draught and meat purposes) that has a significant contribution to the supply of meat. As a genetic resource asset (germplasm) of native Indonesian cattle [1], besides people on the Madura Island make Madura cattle a special culture, namely racing bull (Karapan) and the animal contest-entertainment for cows (Sonok) [2, 3]. This tradition plays an important role in the preservation of Madura cattle as local Indonesian breeds, therefore the existence of Madura cattle must be maintained both in terms of population and genetic purity [4].

The advantages of Madura cattle are that they have good growth in poor quality forage, a high percentage of the carcass with good quality meat, and good adaptability to tropical environments [5]. Madura cattle also have a good reproductive performance with short calving intervals and low service per conception (S/C) [6].

* Corresponding author: muchamad.luthfi@brin.go.id

© The Authors, published by EDP Sciences. This is an open access article distributed under the terms of the Creative Commons Attribution License 4.0 (https://creativecommons.org/licenses/by/4.0/).
One of the efforts to support the development of the Madura cattle population can run well if reproductive aspects are well regulated, such as the ability to detect estrus, the calving interval, and the period of anestrus postpartum at a favorable level. However, the low knowledge of breeders in observing estrus symptoms, especially in cows that are mated by Artificial Insemination (AI) causes mating failure which results in low pregnancy of cows. Visually observing estrus is a common method commonly used by breeders. The problem in this process is determining the optimal time for insemination, which is based on estrus behavior. The estrus expression displayed by a cow that did not appear resulted in a lower detection rate of estrus and a longer calving interval [7].

PGF\textsubscript{2α} hormone is a luteolytic agent that has been widely used to induce estrus in the cow. The emergence of estrus due to the administration of PGF\textsubscript{2α} is due to the lysis of the corpus luteum (CL) by the action of the PGF\textsubscript{2α} vasoconstriction so that blood flow to CL decreases drastically as a result, the levels of progesterone produced by CL in the blood decrease, this decrease in progesterone levels will stimulate the anterior pituitary to release FSH and LH, these two hormones are responsible for folliculogenesis and ovulation, resulting in growth and maturation of follicles. These follicles eventually produce the hormone estrogen which can manifest estrous symptoms. The work of the hormone estrogen is to increase the sensitivity of the female genital organs which is characterized by changes in the vulva and transparent mucus discharge [8].

Accuracy of estrus identification is very important for the efficiency of livestock reproduction time, the more precise the detection of estrus time, the faster the conception rate and birth rate will be known [9]. Failure in the detection of estrus can lead to failure of the marriage which affects the incidence of pregnancy. The main problem in the detection of estrus is generally found in cows that experience silent heat (hidden estrus) because not all breeders can detect it. Field estrus detection is usually carried out at least twice a day, morning and evening/night. Therefore, this study aims to determine the effect estrus synchronization with the PGF2α hormone on the estrus activity of Madura cows

2 Materials and methods

2.1 Location and animals:

The research was carried out at the experimental housing and Animal Reproduction Laboratory, Beef Cattle Research Institute, Pasuruan Regency, East Java Province for 3 months. The materials used were 10 Madura cows aged 3 - 4 years after calving over 60 days with normal reproductive organs.

2.2 The tools used:

The tools used in the research include: individual stalls, tools for sampling cervical mucus, namely glass objects, osse, micropipet and Computer Assisted Sperm Analysis (CASA) microscope.

2.3 Research methodology

The research method used was field and laboratory experiments. Estrus observations were carried out on Madura cows using two techniques, namely by synchronizing estrus and naturally (Figure 1).
Fig. 1. Synchronization stages and estrus observations

a. Estrus Synchronization Method

The method of synchronizing estrus by administering the PGF$_{2\alpha}$ hormone "Alfaglandin® C (Cloprostenol)" twice as much as 2 ml/head intramuscularly (IM), each on day 0 and the second on day 11. Observation of estrus was started after the second injection of the PGF2 alpha hormone which was carried out for 5 days.

b. Natural estrus observation method

Observation of natural estrus in Madura cows was started on the 15th day after the appearance of synchronized Madura cattle estrus was carried out for 10 days.

c. Observation for signs of estrus

The method of observing the signs of estrus is done visually to ensure the occurrence of estrus which includes conditions of vulva temperature, vulva swelling, and vaginal $pH$ as well as cervical mucus discharge, which is carried out every 2 hours, namely 06.00, 08.00, 10.00, 12.00, 14.00, 16.00, 18.00, 20.00, 22.00, 24.00, 02.00 and 04.00. Furthermore, the initial determination of estrus is based on the appearance of cervical mucus. Several techniques for measuring the signs of estrus, namely:

- Vaginal temperature
  The digital thermometer is on and the numeric indicator shows the number 0 (zero), then inserts the digital thermometer "Omron" in the cow's vagina with a depth of ± 5 cm then waits for a notification sound indicating that vaginal temperature data has been obtained.

- Vaginal $pH$
  Prepare the Merck $pH$ 6 - 10 $pH$ paper then insert the $pH$ paper into the vagina until the color changes (± 15 seconds), then compare it with the color of the $pH$ indicator value.

- The vulva is swollen
  Assessment of vulva swelling is based on the change in the surface size of the vulva when compared with the condition of the normal size of the vulva.

- Cervical mucus
  Cervical mucus observation is measured based on the time and frequency of cervical mucus appearance. The technique of making cervical mucus fern is that the samples were smeared on a glass slide and air dried at room temperature. The samples were microscopically assessed at magnification × 400.

The parameters observed included the frequency of estrus events, the visual appearance of estrus cows (cervical mucus discharge, swollen vulva, vaginal $pH$, and vulva temperature), and the appearance of cervical ferning. Data were analyzed by t-test using the Software for Research statistix 10 to distinguish each observed parameter from estrus Madura cow.

3 Results and discussion

3.1 Time for Estrus Madura Cow

The ability to detect estrus is an important factor in achieving reproductive efficiency in beef cattle, especially on farms that use artificial insemination (AI) methods. Reproductive
management directly affects the calving interval which has an impact on farmer profit. Observations on the frequency of occurrence of estrus in synchronized cows (Table 1).

Table 1. Frequency of estrus events based on the timing of cervical mucus discharge in Madura cow

<table>
<thead>
<tr>
<th>No</th>
<th>Clock Observation</th>
<th>Synchronization (%) (10 cows)</th>
<th>Natural (%) (10 cows)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>06.00 – 08.00 am</td>
<td>1.20±0.13</td>
<td>1.54±0.13</td>
</tr>
<tr>
<td>2</td>
<td>10.00 – 12.00 am</td>
<td>7.23±0.49</td>
<td>3.08±0.16</td>
</tr>
<tr>
<td>3</td>
<td>14.00 – 16.00 pm</td>
<td>19.28±0.82</td>
<td>21.54±0.75</td>
</tr>
<tr>
<td>4</td>
<td>18.00 – 20.00 pm</td>
<td>40.96±0.49</td>
<td>44.62±0.50</td>
</tr>
<tr>
<td>5</td>
<td>22.00 – 24.00 pm</td>
<td>18.07±0.61</td>
<td>15.38±0.37</td>
</tr>
<tr>
<td>6</td>
<td>02.00 – 04.00 am</td>
<td>13.25±0.32</td>
<td>13.85±0.35</td>
</tr>
</tbody>
</table>

Information : Value = Average ± SEM

The results of the observations in Table 1, shown that the frequency of estrus events at the same observation hour was not significantly different from the two treatments ($p > 0.05$), the highest frequency of estrus events was at the observation hour 18.00 - 20.00 in cows that were synchronized or not, which showed that the occurrence of estrus in Madura cow is more common at night. Estrous behavior will be manifested through the intensity of estrus and this condition will depend on the concentration of estrogen secreted by the de Graaf follicles at the time of estrus [10]. Some research results state that estrous intensity is influenced by management and environmental factors as well as physiological stress on cows which deals with body temperature regulation, energy balance, and hormonal changes. This disturbs the balance of estrogen so that it affects the performance of estrous intensity [11]. Besides, the incidence of heat in synchronous cows is influenced by many factors, one of which is age [12].

3.2 The visual appearance on the estrus Madura cow

Table 2. Results of visual observation Madura cattle estrus

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Synchronization (n=10 cows)</th>
<th>Natural (n=10 cows)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>heads</td>
<td>%</td>
</tr>
<tr>
<td>Cervical mucus (heads)</td>
<td>8</td>
<td>80</td>
</tr>
<tr>
<td>Swollen vulva (head)</td>
<td>6</td>
<td>60</td>
</tr>
<tr>
<td>Vaginal pH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Acid (6.5-6.9)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>- Base (7.1-8)</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>Vaginal temperature</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Low (37.5-38 °C)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>- Medium (38.1-38.5 °C)</td>
<td>8</td>
<td>80</td>
</tr>
<tr>
<td>- High (38.6-39 °C)</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>Long estrus (hours)</td>
<td>21.20±7.93*</td>
<td></td>
</tr>
</tbody>
</table>

Information : Value = Average ± SEM

The visual observation results in Table 2, show that synchronized and natural estrus Madura cows in the observations of cervical mucus discharge, swollen vulva, and vaginal pH have the same values, while vaginal temperature and estrus duration vary in value. Based on
Table 2. Cervical mucus appeared from both treatments. The higher the estrogen hormone in the blood during estrus, the greater the volume of cervical mucus with a more alkaline pH [13]. The degree of acidity (pH) is an important parameter of cervical mucus in the process of transferring sperm to the female reproductive tract; pH affects the quality of sperm viability in the cervix [14].

The vaginal temperature display of the two treatments of Madurese cows showed a range of 38.1–39°C, this is in accordance with the results of previous research which stated that the vaginal temperature during estrus was 38.80 ± 0.07°C in winter while in summer it was 38.73 ± 0.09°C [15]. The estimated duration of estrus in Table 2 shows that the duration of estrus for Madurese cows was 21 hours in both treatments, this shows that this condition is not much different from previous research, which ranged from 12-30 hours [8]. The inaccuracy of data in estimating the duration of estrus may be due to the fact that doses of nutrients containing high protein cause estrogen levels in the blood to persist for a long time, thereby prolonging the appearance of signs of estrus [16]. However, the range of duration of estrus also depends on each individual cow [8].

3.3 Cervical Mucus Ferning On The Estrus Madura Cow

Cervical mucus appears during estrus and is crystalline like a fern tree when it has dried and is placed on an object glass [17].

Table 3. The Appearance of Cervical Mucus Ferning of Estrus Madura Cattle at Microscopically with a Magnification of × 400.

<table>
<thead>
<tr>
<th>Phase</th>
<th>Synchronization</th>
<th>Natural</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>- at 20.00 pm</td>
<td>- at 22.10 pm</td>
</tr>
<tr>
<td></td>
<td>- watery cervical mucus</td>
<td>- watery cervical mucus</td>
</tr>
<tr>
<td>II</td>
<td>- at 07.50 am</td>
<td>- at 10.00 am</td>
</tr>
<tr>
<td></td>
<td>- slightly thick cervical mucus</td>
<td>- slightly thick cervical mucus</td>
</tr>
<tr>
<td>III</td>
<td>- peak of estrus</td>
<td>- peak of estrus</td>
</tr>
<tr>
<td></td>
<td>- at 09.10 am</td>
<td>- at 13.00 am</td>
</tr>
<tr>
<td></td>
<td>- thick hanging cervical mucus</td>
<td>- thick hanging cervical mucus</td>
</tr>
</tbody>
</table>

Cervical mucus can be used to detect estrus, the typology of cervical mucus ferning will give a different picture according to the period of the estrus cycle [18]. The results of the
ferning display in the two treatments (Table 3) showed almost the same pattern at the point of cervical mucus discharge. Cervical mucus will experience changes during the estrus phase, namely clear at the beginning of estrus, gradually turning cloudy in the middle of estrus and shiny clear at the end of estrus [19]. One of the signs of estrus cattle is cervical mucus, especially when it is nearing the peak of the estrus.

Bovine cervical mucus changes its biochemical composition and biophysical properties due to variations in sex steroid levels during the estrus cycle. As a consequence of increased estrus, cervical mucus is produced in greater quantities during marked estrus characterized by increased mucus crystallization [20]. The cervical mucus of estrus bovine contains a lot of sodium chloride (NaCl) in the form of a fern leaf called ferning. The specific appearance such as fern leaves will be obvious when viewed under a microscope and ferning will be seen when the cow is approaching estrus and at the peak of estrus [21].

The crystallization process does not only occur in cervical mucus; but it also occurs in other biological materials [22]. Organic compounds and electrolytes, which are contained in cervical mucus, are mainly salts, such as NaCl, KCl, and CaCl2 [23]. The condition of thick cervical mucus has a conception rate (CR) value of 68.00% in cows and 50.00% in composite heifers because in this condition the estrogen hormone is at its peak point. reaches its peak and brings the time of ovulation closer so that when AI is carried out there will be high fertility [24].

4 Conclusion

The synchronized appearance of Madura cow with PGF2α and not which includes the frequency of estrous events, estrus visualization (cervical mucus discharge, swollen vulva, vaginal pH, and length of estrus), and the appearance of cervical ferning has the same response, while estrus temperature has a variable response.

The authors would like to thank Indonesian Beef Cattle Research Station for supporting the research and National Research and Innovation Institute (PRN).

References