Estrous response of Etawah Crossbred does on estrous synchronization using the prostaglandin F2α based protocol

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Abstract. Prostaglandin F2α is one protocol that is widely used in estrous synchronization programs for livestock. The aim of this study was to determine the estrous response of Etawah Crossbred does on estrous synchronization using a 1ml of prostaglandin F2α. 1ml of prostaglandin F2α (Lutalize™) with 11 days interval was injected to each of nine selected Etawah Crossbred does aged above 3-year-old, with parity more than twice, and did not pregnant. The goats were individually housed at Faculty of Animal Science of Universitas Gajah Mada (UGM) with average body weight, BCS, DM intake, and TDN of 41.15±8.31 kg, 3, 685.98±287.52 gr/head/day and 393.33±180.41 gr/head/day, respectively. Estrous responses observed were changes in vulva size (length and width), pH, color, presence of mucus, and behavior which was observed before injection (0h) and after the 2nd injection at 12h, 24h, 36h, 48h. Data were analyzed in descriptive quantitative and one-way ANOVA followed by DMRT. Changes in vulva length did not differ, but the width and pH vulva were significantly different (P <0.05) at 0h, 12h with 24h, 36h and 48 h. The pattern was similar to that which occurred in vulva discoloration, the presence of mucus and behavioral changes whose scoring values tended to increase at 12h, 24h, 36h and 48h after 2nd injection, except for the vulva color which decreased in the 48h. It can be concluded that the injection of 1ml of prostaglandin F2α with 11d interval could cause estrous in Etawah crossbred does after 24h.

Keyword: Estrous synchronization, prostaglandin F2α, superficial cells, vaginal smear, vulva.

1. Introduction
Goats have an important role as livestock in the agricultural business system in Indonesia. In socioeconomic terms, goat livestock is mostly raised by smallholder farmers as producers of meat, milk, fertilizer, skin, usually as savings and insurance as well as government facilities in poverty alleviation in several regions. In socio-culture, goat livestock plays a role in religious and cultural
celebrations that have become a tradition of Indonesian society (1). Low reproductive efficiency of tropical goats is caused by low fertility, unobserved estrous and uncontrolled estrous time, which subsequently, making matting and breeding cannot be estimated (2). Various breeding system improvement programs with hormonal assistance have been developed to overcome this problem. One of the programs is estrous synchronization.

Estrous synchronization is one of the methods used to synchronize estrous in livestock by using hormone preparations. The most common estrous synchronization in Indonesia was by using hormone treatment. There are several types of hormones that can be used to synchronize estrous. In general, there are two hormones that are commonly used for estrous synchronization, namely progesterone and prostaglandins hormones (3). The principle used in the estrous synchronization is to extend or shorten the life span of the corpus luteum (CL) or the luteal phase (2). Prostaglandin compounds have luteolytic properties that function to induce the estrous through the removal of the corpus luteum, which can occur because prostaglandins inhibit blood flow to the corpus luteum. Inhibition of blood flow will occur long enough and cause regression of the corpus luteum. In the reproduction technology, there are several types of prostaglandin hormone preparations that are widely known to be used in estrous synchronization, namely prostaglandin F2α (PGF2α) and prostaglandin E2 (PGE2) (2) and the most widely used today is prostaglandin F2α (PGF2α) with an application via intramuscular injection. Prostaglandin F2α or its synthetic analogs as luteolytic substances can assume great importance in reproductive management of livestock. Either as a single or double injection (9–11d apart), PGF2α provokes a good induction. PGF2α protocol seems to be a promising and more cost-effective for reproductive management in goats farming (4).

Estrous synchronizing with the injection of PGF2α simultaneously in a group of goats will cause estrous simultaneously (5). (6) reported that the PGF2α protocol can be performed with single or double injection at 7-11d intervals. The onset of estrous and ovulation differ according to differences in the intervals between PGF2α treatments. When a single PGF2α is administered between 8-11d after onset of estrous, the proportion of ewes in estrous is 21/25 with duration 46.3±1.32h, similar to that obtained with a double PGF2α injection which given in 10d apart, namely 22/25 and 51.6±2.4h. The single injection of 0.5 ml PGF2α hormone (Lutalize™) is effective in estrous synchronization on Etawah Crossbred does that have the onset of estrous that is equal to 100% after 24h (7). The effectiveness of the use (Lutalize™) of injection (10 mg Dinoprost tromethamine) with an 11d interval as a synchronization protocol on West African Dwarf (WAD) is 42h after the second injection with estrous duration of 41.67±2.22h (8). Synchronization of PGF2α hormone (125 μg cloprostenol) at 11d intervals shows estrous of 100% (9).

Successful estrous synchronization in goats is done by using various parameters to get a good quality of estrous. The parameters are used to observe the clinical signs of estrous. Some parameters to predict the occurrence of estrous are changed in the dimensions of the vulva or vulvar biometry (10). Estrous detection by visual observation changes in behavior, the appearance of mucus, color, and swelling of the vulva (2). Based on this information, this study was conducted in order to observe the response of Etawah Crossbred does through several estrous parameters to the estrous synchronization program by using the 1 ml of Prostaglandin F2α based protocol at 11-day intervals.

2. Methodology
This research was conducted for 1 month in August 2017 in the farm laboratory at the Faculty of Animal Science of Universitas Gadjah Mada. Nine selected Etawah Crossbred were used in this study. Selection criteria include health, aged more than 3-years-old, second or third parturition and the goats did not pregnant. The goats were individually housed in a 1.5x1m pen with a feed and water bank available. Etawah Crossbred does had an average body weight of 41.15±8.31 kg and its body condition score was 3 (1-5 on the scale). Feeding was done twice a day, namely by using commercial concentrate (Nutrivit™) at 08.00 a.m. and forage (peanut straw) at 03.00 p.m. Freshwater was provided ad libitum. Nutrient content and consumption are presented in Table 1.
Table 1. Nutrient content of feed and nutrient intake of Etawah Crossbred does.

| Parameters                  | as feed | DM* | CP* | CF* | EE* | Ash* | NFE* | TDN* |
|-----------------------------|---------|-----|-----|-----|-----|------|------|------|
| Nutrivit™ Peanut straw      | 91.66   | 12.71 | 24.33 | 5.02 | 11.38 | 53.91 | 55.65 |
| Nutrivit™ Peanut straw      | 7.81    | 20.04 | 18.95 | 5.67 | 12.18 | 43.16 | 69.99 |
| Nutrivit™ Total             | 662.10  | 606.88 | 77.13 | 147.65 | 30.47 | 69.06 | 324.17 | 337.97 |
| Nutrivit™ Peanut straw      | 1012.78 | 79.10 | 15.85 | 14.99 | 4.48 | 9.63 | 34.14 | 55.36 |
| Nutrivit™ Total             | 1674.88 | 685.98 | 92.98 | 162.64 | 34.95 | 78.79 | 361.31 | 393.33 |

*DM: Dry Matter, CP: Crude Protein, CF: Crude Fiber, EE: Extract Eter, NFE: Nitrogen-Free Extract, TDN: Total Digestible Nutrient

**Analized by Laboratory of Feed Technology at Faculty of Animal Science, Universitas Gadjah Mada

All goats were injected with the 1 ml/head (5 mg Dinoprost tromethamine) of Prostaglandin F2α (Lutilize™ by Zoetis Ireland Limited: Dublin) at 11d interval. Before and after the 2nd injection, the estrous response was observed. Estrous response parameters observed were changes in the size of the vulva in the form of the length (vertical side) and width (horizontal side) of the vulva, the distance on the outer side of the vulva was measured using digital vernier calipers (10). Vulva pH was measured by using litmus paper (Universal™ indicator pH paper Merck: Germany). Qualitative parameters were observed and converted by a scoring system, including the vulva color (1. pale white, 2. white, 3. pink, 4. red), the presence and appearance of mucus (1. not slimy, 2. slightly slimy i.e. marked by appearing on the surface of the vulva, 3. Slimy with a lot of mucus, which was characterized by dripping and sticking to other body parts or in the cage) and behavior (1. normal and 2. agitated, i.e. making a sigh, climbing the cage, actively moving). Observations were made at 6-hour intervals, namely before injection (0h) and at 12h, 24h, 36h and 48h after injection. Data were analyzed descriptively on qualitative parameters and quantitative data were analyzed for differences at each observation time by using one-way ANOVA followed by Duncan Multiple Range Test.

3. Result and Discussions

The results of observations of the estrous response in Etawah Crossbred does that synchronized with estrous with 1ml of Prostaglandin F2α which included vulva size dynamics and vulva pH is presented in Table 2. The dynamics of changes in the size of the vulva are measured as one of the estrous responses assuming that one of the events of estrous is characterized by a vulva that swells from normal size. (10) states that one parameter to predict the occurrence of estrous are changed in the dimensions of the vulva or vulva biometry. Table 3 shows that changes in vulva length did not differ at each observation hour, but the width and pH vulva were significantly different (P <0.05) at 0h, 12h with 24h, 36h and 48h. Although there was no difference, there was an increase of 16.85% in the length of the vulva under normal and estrous conditions. The length of the vulva under estrous conditions (31.55±6.67 mm) was the same as the report (14) which was 3.06 cm in Bligon goats and higher than (10), which reported that the length of the vulva under estrous conditions was 1.88 ± 1.17 cm in West African Dwarf goats. While the size of the vulva width was significantly different (P <0.05) by 13.28% mm from the normal size of 0h (11.67 ± 0.70 mm) to 24h (13.22 ± 0.83 mm), this value was the same as the reported results (10), there was a change of 0.9 mm width of WAD vulva goat in estrous conditions. Vaginal pH values showed the same pattern with changes in vulva size, which increased significantly (p <0.05) by 20.80% after 24h. Based on (12), vaginal pH in estrous conditions of the Saanen goat was 7.17. The dynamic pattern of vulva size and pH indicated that estrous occurred at 24h after the second injection up to 48h and was likely to continue. The onset of estrous by injection of Lutilize™ was 36h with an estrous duration of 27-37h (8).
**Table 2.** The mean and standard deviations of vulva dimension and pH of Etawah Crossbred does before and after estrous synchronization

| Parameters          | Before estrous synchronization (0h) | After estrous synchronization |
|---------------------|-------------------------------------|------------------------------|
|                     |                                    | 12h  | 24h  | 36h  | 48h  |
| Vulva length (mm)   | 27.00±4.39ª                        | 30.33±6.06ª | 31.55±6.67ª | 31.89±6.09ª | 31.44±7.81ª |
| Vulva wide (mm)     | 11.67±0.70ª                        | 12.78±1.09ª | 13.22±0.83ª | 13.22±1.56ª | 12.89±1.45ª |
| Vulva pH            | 6.44±0.53ª                         | 6.89±0.60ª | 7.78±0.67ª | 7.67±0.70ª | 8.22±0.87ª |

ªª superscripts on the same line show significant differences (P <0.05)

The estrous response is related to changes in the color of the vulva, the presence of mucus and the behavior of the parent or is a clinical symptom of estrous. Determination of these clinical symptoms were conducted by scoring based on criteria, the results are presented in Table 3.

**Table 3.** The mean and standard deviation of scoring the visual and signs observation of Etawah Crossbred does before and after estrous synchronization

| Parameters          | Before estrous synchronization (0h) | After estrous synchronization |
|---------------------|-------------------------------------|------------------------------|
|                     |                                    | 12h  | 24h  | 36h  | 48h  |
| Vulva colors*       | 1.00±0.00                          | 3.30±0.71 | 3.11±0.78 | 3.22±0.44 | 2.89±0.33 |
| Appearance of mucus ** | 1.00±0.00                     | 1.00±0.00 | 1.89±0.93 | 1.89±0.78 | 2.33±0.71 |
| Behavior ***         | 1.00±0.00                          | 1.00±0.00 | 1.56±0.53 | 1.44±0.53 | 1.78±0.44 |

*: 1. pale white, 2. white, 3. pink, 4. red
**: 1. not slimy, 2. slightly slimy, 3. slimy with a lot of mucus
***: 1. normal, 2. agitated

The color of the vulva showed the change from white to red at 12h, while for the presence of mucus began to appear after entering 24h and increased more at 48h. Agitated behavior, like sighing, and climbing the cage also occurred at the same time observation at 24h and the intensity increased at 48h. The pattern occurred in vulva discoloration, the presence of mucus and behavioral changes whose scoring values tended to increase at 12h, 24h, 36h and 48h after 2nd injection, except for the vulva color which decreased in the 48h. Thus, the onset of estrous occurred at 24h with a duration of more than 48h. (1) states that during the estrous phase in general, goats exhibited clinical symptoms of high-intensity estrous as indicated by the presence of clear and viscous vaginal mucus, swollen vulva and reddish color. (13) also reported that the clinical signs of estrous in the form of swelling of the vulva, redness, and appearance of mucus began to appear by 90% at 18h and increased at 24h-36h followed by an increase in the size of large follicles (7-8 mm) and categorized as de graff follicles which will ovulate.

**4. Conclusion**
The conclusion of this study is: the injection of 1ml of prostaglandin F2α with 11d interval could cause estrous in Etawah Crossbred does after 24h.

**References**

[1] Budisatria, IGS, Panjono, Maharani D and Ibrahim A 2018 Kambing Peranakan Etawah Hitam atau Coklat? (Yogyakarta: Gadjah Mada University Press)
[2] Hafez B and Hafez ESE 2000 Reproduction in farm Animal 7th ed (USA: Lippincott Williams and Wilkins)
[3] Inoumu I 2014 Upaya meningkatkan keberhasilan inseminasi buatan pada ternak ruminansia kecil Wartazoa 24 201-209.
[4] Sinoes J 2016 Synchronization of ovulation in goats using prostaglandin F2α based protocols during the breeding season J. Coast Life Med 4 240-243
[5] Lopes-sebastian A, Gonzalez-Bulnes A, Carrizosa JA, Urrutia B, Diaz-Delfa C, Santiago-
Moreno J and Gomez-Burnet 2007 New estrus scyncronation and artificial insemination protocol for goats based on male exposure, progesteron and cloprostenol during the non-breeding season Theriogenology 68 1081-1084

[6] Fierro S, Gil J, Vinoles C and Olivera-Muzantze J 2013 The use of prostaglandins in controlling estrous cycle of the ewe: A review Theriogenology 79 399-408

[7] Atmoko BA, Bintara S, Budisatria IGS, Maharani D, Sumadi, Sidadolog JHP, Yusiat LM and Londra IM 2019 Artificial insemination on the Etawah Grade goats using frozen semen of Gembong goat using Estrous KnE Life Science 21 149-155

[8] Oyeyemi MO, Akusu MO and Adeniji DA 2012 Comparative responses of West African Dwarf goats to three oestrous synchronizing agents Israel J of Vet Sci 67 48-54

[9] Siregar TN, Armnasyah T, Sayuti A and Syafrudinn 2010 Tampilan reproduksi kambing betina lokal yang diinduksi birahinya dilakukan dengan sinkronisasi singkat J. Veteriner 11 30-35

[10] Leigh OO, Raheem AK dan Oluwadamilare OJA 2010 Improving the reproductive efficiency of the goat: vagina cytology and vulvar biometry as predictors of synchronized estrus/breeding time in West African Dwarf Goat Int. J. Morphol 28 923-928

[11] Widiyono I, Putro PP, Sarmin, Astuti P and Airin CM 2011 Kadar estradiol dan progesteron serum, tampilan vulva dan sitologi apus vagina kambing Bligon selam siklus birahi J Veteriner 12 263-268

[12] Sitaresmi PI, Astuti PK, Widyobroto BP, Bintara S and Widayati DT 2018 Exfoliate vaginal cytology and vaginal acidity profile in Etawah-Saanen grade does Int J of Pure and App Math 118 1-16

[13] Famakinde SA, Leigh OO, Odeyemi AJ and Oluwatosin BO 2017 Reproductive parameters of prostaglandin-treated female Kalahari red goats in Abeokuta, Ogun state, Nigeria. Alexandria J of Vet Sci 53 180-186

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