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ESTROGEN HORMONE PROFILE AND ESTRUS RESPONSE OF THIN TAILED EWES SYNCHRONIZED WITH CONTROLLED INTERNAL DRUG RELEASE

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ABSTRACT

This research was conducted to identify the estrogen hormone profile and estrus response in the thin tailed ewes synchronized with controlled internal drug release (CIDR) implant. This research was carried out by employing 8 thin tailed ewes, CIDR, and estrogen kit. The ewes were distributed into: 3 ewes as control group and 5 ewes as treated group (with 12 days-CIDR implantation). Ewes were raised in the Sidomukti farm group, Sleman Regency, Yogyakarta. The data obtained were estrogen hormone profile during estrus examined by using ELISA method and estrus response marked with the reddening and oedema of vulva, mucus vaginal discharge, behavioral changes, vaginal pH, and superficial cells population. The data were analyzed by applying independent sample T-test. The result did not show any significant difference ($P>0.05$) in the normal range between the control and CIDR group in estrogen hormone profile, estrus response, and pH level, but showed significant differences with positive correlation ($P<0.05$) in population of superficial cells. In conclusion, CIDR was an effective tool for estrus synchronization at the farmer group, and gave positive estrus response towards thin tailed ewes with estrogen hormone profile, pH level, and superficial cell percentage on the normal range.

Key words: CIDR, estrogen, estrus, ewe, synchronization

ABSTRAK

Penelitian ini dilakukan untuk mengetahui profil hormon estrogen dan respons estrus pada domba ekor tipis yang disinkronisasi dengan implant controlled internal drug release (CIDR). Penelitian dilakukan dengan 8 ekor domba, CIDR, dan kit estrogen. Domba dibagi menjadi dua kelompok, yaitu 3 ekor sebagai grup kontrol, dan 5 ekor sebagai kelompok perlakuan (implantasi CIDR selama 12 hari). Domba dipelihara di Kelompok Ternak Sidomukti, Sleman, Yogyakarta. Data yang diperoleh meliputi profil hormon estrogen sewaktu estrus yang dianalisis dengan metode ELISA dan respons estrus ditandai dengan kemerahan dan pembengkakan vulva, sekresi mukus vagina, perubahan tingkah laku, pH vagina, dan populasi sel superficial. Data dianalisis dengan independent sample T-test. Hasil penelitian menunjukkan perbedaan yang tidak signifikan ($P>0,05$) dalam kisaran normal antara grup kontrol dan perlakuan pada profil hormon estrogen, respons estrus, dan nilai pH vagina, tetapi menunjukkan perbedaan nyata ($P<0,05$) dengan korelasi positif pada populasi sel superfisial. Disimpulkan bahwa CIDR efektif digunakan sebagai preparat sinkronisasi estrus pada kelompok ternak dan memberikan respons estrus positif dalam kisaran normal terhadap domba ekor tipis betina dengan profil hormon estrogen, nilai pH, dan persentase sel superfisial pada kisaran normal.

Kata kunci: CIDR, estrogen, estrus, domba, sinkronisasi

INTRODUCTION

Thin tailed sheep is the most popular small ruminant raised by the farmer in Indonesia. Smallholder farmers who generally raise only 3-8 sheep per family dominate sheep farming in Indonesia (Sudarman *et al.,* 2017). The thin tailed ewes have good reproduction performance, such as high adaptability and feed conversion ability from low into high quality feed (Rianto *et al.,* 2004). Thin tailed ewes have superiority for its fertile trait had litter size of 1.39 with 8.62 months of lambing interval (Hakim *et al.*, 2019) if it is well maintained and fed (Ngadiyono *et al.*, 2009). Thin tailed ewes tends to have seasonal polyestrus (Hasan *et al.,* 2017), therefore it can reproduce throughout the year. Despite its superiority, the lack of management technique restraints the potency of ewe reproductive ability. This weakness according to Ngadiyono *et al.* (2009) include a lack of local farmer's knowledge that results in many inbreeding cases and also worsened by less attention from the Indonesian government to the livestock development in the village.

The reproduction technology such as a synchronization tool controlled internal drug release (CIDR) can be an alternative to lengthen the luteal phase. After 12 days of CIDR's implantation, the extrication of CIDR will intensify the estrogen level and continued by the entering of the estrus phase at about the same time. Based on this occurrence, this research aimed to investigate estrus response and estrogen hormone profile in the thin tailed ewes synchronized with CIDR. It was presumed that the treated thin tailed ewe would showed better estrus response and higher estrogen hormone profile compared to thin tailed ewes without treatment. This research signified the availability of information regarding estrus response and estrogen hormone profile in synchronized ewes, so the effectiveness of the estrus induction treatment was identified.

MATERIALS AND METHODS

The material used in this study was 8 thin tailed ewes raised at Sidomukti Farm Group at Murangan VIII, Trucuk RT 16 RW 32, Triharjo sub-district, Sleman District, Yogyakarta. Five thin tailed ewes were implanted with CIDR from Eazybred (Zoetis, New Zealand) and three thin tailed ewes as control. The vaginal pH level was measured by using pH-meter

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paper (Merck KGaA, Germany). The superficial cell population was observed by using object-glass, Giemsa stain 3%, alcohol 70%, aquadest, cotton-bud, and counter. The estrogen hormone profile from thin tailed ewe's blood on the estrus phase (both treated and non-treated ewe) was measured by using using an enzyme-linked immunosorbent assay (ELISA method) with a commercial estrogen ELISA kit (DRG, Germany).

Procedure of CIDR Implantation

Controlled internal drug release was implanted intravaginally in 5 thin tailed ewes. First, the ewe's vulva was cleaned by using wet paper sheets. Implantation was carried out by using a sterile lubricated applicator. The next, CIDR which has been installed inside the applicator, was inserted by swivelling it through the vulva slowly. After it got in perfectly, CIDR was placed in by pushing the applicator in and take it off soon after CIDR was placed correctly inside the vagina. Implantation was administered for 12 days with daily monitoring to ensure it will not slip. Then, CIDR was removed and the experiment was conducted consecutively for 5 days.

Estrogen Hormone Profile

Thin tailed ewes blood was obtained during the estrus phase based on identification of vaginal smear and pH value. Thin-tailed ewes blood were obtained from its jugular vein by using a 5 ml syringe and then stored in the vacutainer. The blood was centrifuged for 15 minutes in 15000 rpm to separate the serum. After that, the serum was placed in a microtube and stored inside the freezer. The concentration of estrogen hormone from serum was analyzed using ELISA method with a commercial estrogen ELISA kit (DRG, Germany). Hormone measurements was carried out at Physiology Laboratory, Faculty of Veterinary Medicine, Gadjah Mada University.

Estrus Response

Estrus responses were obtained by daily visual observation during 5 consecutive days (every 7 AM and 4 PM) for 20 minutes. The observation was done by identifying the reddening of vulva, mucus vaginal discharge, swollen of the vulva, and behavior changes such as ewe became vocal and bleat very loudly, and also constant tail wagging. This observation was stated on scoring value from 1-3 referring to Saputra *et al.* (2017), Ridlo *et al.* (2018), and Widayati *et al.* (2013) as shown in Table 1.

Vaginal pH Level

The vaginal pH level was measured by dipping the pH-meter paper (Merck KGaA, Germany) into vaginal mucosa (Sitaresmi *et al.,* 2018). Furthermore, the color formed on the pH-meter paper was compared to the color indicator. The score obtained indicated that in the estrus phase, the vaginal pH tends to be alkaline.

Superficial Cells Percentages

Vaginal cytology was used to identify the estrus phase by using the vaginal smear method referring to Sitaresmi *et al.* (2019). First, the cotton-bud was dipped into aquadest then swapped inside the vaginal mucosa and further smeared on object-glass. The object-glass was soaked into alcohol 70% for 7 minutes. After that, it was stained by using Giemsa 3% for 45 minutes and was rinsed in aquadest. The sample then being observed using a light microscope with 4x magnification. Superficial cells were counted by using a counter and stated in the form of a percentage.

Data Analysis

The data were analyzed by using an independent sample T-test method in SPSS 16.0. Data on estrogen hormone profile, vaginal pH level, and superficial cell percentage were already on the nominal scale. The data of estrus response were converted into a nominal scale

Table 1. Scoring for estrus response by visual observation

Estrus Response	1	2	3
Swollen of vulva	No reddening	Wrinkled texture starting to become unclear	Enlarged vulva, wrinkled become unclear
Mucus discharge	No mucus	Little mucus	Thick mucus until hanging
Reddening of vulva	Pale pink	Pink	Red
Behavioral changes:			
- Ewe bleat very loudly	No bleating	Rare bleating	Often bleating
- Constant tail wagging	No tail wagging	Rare tail wagging	Constant tail wagging

Table 2. Analyses result in control and treatment group

Variabel	Control	Treatment (CIDR)
Estrogen Hormone Profile	42.63±8.17	49.00±9.32
Estrus Response	2.07±0.42	2.32±0.41
Reddening of vulva scores	2.67±0.58	2.40±0.55
Swollen of vulva scores	2.33±0.57	2.60±0.89
Mucus discharge scores	1.00±0.00*	1.40±0.55*
Behavioural changes		
Ewes bleat very loudly scores	2.33±0.58	2.80±0.45
Constant tail wagging scores	2.00±0.00*	2.40±0.55*
pH Level	9.00±0.50	9.50±1.00
Superficial Cell Populations	65.33±25.38*	71.20±4.32*

* = Significant difference (P<0.05)

as classified on Table 1. The significance value (α) for this analysis was 0.05 (5%).

RESULTS AND DISCUSSION

Estrogen Hormone Profile

The result (Table 2) of the estrogen hormone profile between the treated group (49.00±9.32 pg mL^{-1}) and non-treated group (42.63±8.17 pg mL^{-1}) showed no significant difference (P>0.05). Estrogen hormone profile would increase during the estrus phase compared to the luteal phase, especially when it was induced by CIDR removal. This result was still in the normal range according to Widayati *et al.* (2018) who confirm that estrogen hormone profile during the estrus phase on small ruminant (Ettawa Crossbred) is 59.14±13.41 pg mL^{-1}. However, the comparison of estrogen levels between ewes and does is still unknown. According to Pang *et al.* (2010), there is indication of non-identical secretion patterns of pituitary gonadotrophins or ovarian hormones between does and ewes. Wynn (1977) explains that does' estrogen concentration is higher than ewes during pregnancy. Therefore, it needs further study during the estrus phase for both doe and ewes.

In this present study, the insignificant result can be caused by CIDR's function as an estrus synchronization tool, which did not increase the estrogen hormone level. CIDR's function known to induce estrus at same time, which expected not to increase follicular size and estrogen hormone level. The removal of CIDR implantation would stimulate positive feedback from anterior hypophysis, which would increase the level of FSH followed by the increase of estrogen hormone. According to Sitasiwi (2008), fluctuation of estrogen level during the cycle is in line with the development of primary follicles into tertiary follicles during the follicular phase. Bartlewski *et al.* (2011) state that ewes dominant follicular is ≥5 mm before ovulation. Lindsay (1991) states that length of the days, poor nutrition, or other external factors will affect the sensitivity towards estradiol and will enter the anestrus phase. Both treated and non-treated ewes got the same farmer's management style and fed with the same feed which was only fresh grass without the addition of concentrate. The lack of feed management was considered to cause a decrease in the estrogen hormone's sensitivity during the estrus phase. Therefore, it was assumed that whether the doe was treated or not, it would have the same dominant follicle size so that the level of estrogen hormone was almost the same. Still, it needs further study with more samples which are categorized more specifically on body condition score and nutrition quality.

Estrus Response

The percentage of estrus response from treatment group was 100%. This result was in agreement with Swelum et al. (2019) who obtains a 100% result on Awassi ewes that have been implanted with CIDR intravaginally for 12 days. The CIDR were used is the Eazybred type CIDR-G from Zoetis, New Zealand. This CIDR-G contains 0.3 g of natural progesterone (Garcia *et al.*, 2021) and will escalate the progesterone plasma up to 5.5 ng/mL in 2 hours (Wildeus, 2000). The removal of CIDR would induce the increase of the estrogen hormone level which led to estrus behavior.

During estrus small ruminants tend to show changes such as reddening of vulva, mucus discharge, swollen of the vulva, bleating very loudly, constant tail wagging, and mounting or being mounted (Widayati *et al.*, 2013). All results from estrus signs were analyzed and showed no significant result (P>0.05) for both implanted (2.32±0.41) and unimplanted (2.07±0.42) ewes. According to Lindsay (1991), ewes give very little sign of estrus, proestrus, and metestrus which mostly didn't show any sign, but they still accept mating by the male. It is also stated that a certain breed of ewes tends to shorten estrus duration due to the sudden introduction of male sheep. Therefore, it was assumed that some ewes did not show the expected response on some estrus signs.

Reddening of the Vulva

The result of the reddening of the vulva (Table 2) did not show a significant difference (P>0.05) for both implanted (2.40±0.55) and unimplanted (2.67±0.58) ewes. Both implanted and unimplanted ewes exposed pink to red color on their vulva. According to Saputra *et al.* (2017) small ruminant (Ettawa crossbred) shows an average score which is 2 (pink) on the estrus phase. The color change of vulva was related to estrogen hormone as explained by Nurfitriani *et al.* (2015) that estrogen stimulates the thickening of vaginal walls, increasing vascularity so exterior genitals will swell and redden.

Mucus Discharge

The analysis of mucus discharge (Table 2) showed significant difference (P<0.05) between implanted (1.40±0.55) and unimplanted (1.00±0.00) ewes. It was assumed that the significant difference was caused by a large variety of sample values. According to analyses result, unimplanted ewes did not show mucus secretion, while implanted ewes showed a few mucus secretions. During the observation, mucus discharge never hung on the vulva. This result had a lower score compare to Saputra *et al.* (2017) in small ruminants (Ettawa crossbred) with an average score of 1.85 (a few mucus) on the estrus phase which is affected by estrogen hormone. According to Ihsan (2010), ewe did not secrete mucus during the estrus phase. Therefore, it was expected that the result would differ from the previous result.

Swollen of the Vulva

The swollen vulva (Table 2) showed no significant difference (P>0.05) for both implanted (2.6±0.89) and unimplanted (2.33±0.57) ewes. According to analyses result, both implanted and unimplanted ewes showed unclear wrinkled texture and the vulva slightly enlarged. According to Widayati *et al.* (2018), the

changing of estrogen hormone and FSH levels causes physiological changes that affect physical changes such as swollen of the vulva. According to Nurfitriani *et al.* (2015), estrogen stimulates the thickening of vaginal walls and increases vascularity so exterior genitals will swell and redden.

Behavioral Changes

The behavioral changes (Table 2) were observed by monitoring the ewe's bleating and constant tail wagging. The analyses result on ewe's bleat loudly showed no significant difference (P>0.05) between implanted (2.80±0.45) and unimplanted (2.33±0.58) ewes. Based on the analysis result, both implanted and unimplanted ewes rarely bleated. The analyses result for ewe's constant tail wagging showed significant difference (P<0.05) between implanted (2.40±0.55) and unimplanted (2.00±0.00) ewes. The significant difference was considered caused by a large variety of sample values. Based on the analysis result, both implanted and unimplanted ewes rarely wagged the tail. This behavioral change was caused by estrogen hormone, according to Zulkarnin *et al.* (2015), one of the estrus signs is producing a specific voice of bleating. According to Ola and Egbunike (2004), constant tail wagging shows ewe's sexual desire.

Vaginal pH Value

The analyses result of vagina pH level (Table 2) did not show the significant result on the normal range (P>0.05) for both implanted (9.50±1.00) and unimplanted (9.00±0.50) ewes. According to Widayati *et al.* (2018), the vagina pH level on the estrus phase is 10.26±1.36 and will decrease in the next phase (metestrus, diestrus, and proestrus). The result was slightly different because ewes did not secrete much mucus (Ihsan, 2010), therefore it was assumed that the pH level will not be as high as doe's pH level but still show the difference between estrus phase and anestrus phase. Vagina pH level on the estrus phase tends to be more alkaline than other phases. Sitaresmi *et al.* (2018) explain that the decreasing pH level is caused by biophysics and biochemist of cervical mucus which is regulated by hormonal changes during the estrus cycle. According to Rasad and Setiawan (2017), the changing of pH level depended on ions addition such as hydrogen, sodium, chloride, also glycogen, and protein accumulation. Based on Sitaresmi *et al.* (2018), the increase of NaCl and water in the cervical cervix will increase the pH level resulting in more alkaline pH.

Superficial Cells Population

The result of superficial cell percentage (Table 2) showed significant difference with positive correlation (P<0.05) for both implanted (71.20±4.32%) and unimplanted (65.33±25.38%) ewes. This result matched with Leigh *et al.* (2010) which is 77.4±1.05% on small ruminants (West African Dwarf). The significant difference was indicated by the high variance of the sample. The percentage of superficial cells was used to determine the estrus phase so the blood can be obtained in the time of peak estrus. According to Rasad and Setiawan (2017), the estrus phase has a high estrogen level followed by the increase of uterus activity and keratinization of vaginal epithelial cells, the increase of mitotic and also epithelial proliferation to protect vaginal epithelial cells from a pathogenic microorganism.

CONCLUSION

Controlled internal drug release was an effective tool for estrus synchronization at the farmer group. Controlled internal drug release gave a positive estrus response in normal range towards thin tailed ewes on estrogen hormone profile, pH level, and superficial cells percentage also in the normal range.

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