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Level of estrogen and cervical mucus pH as indicator of estrus after calving towards the provision of selenium-vitamin E^{TM} on dairy cow Frisien Holstein (FH)

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Abstract: This research was conducted to assess the effectiveness of selenium-vitamin E^{TM} intramuscularly to the estrus condition after calving. The research conducted in Training Center of Animal Husbandry, Batu, East Java, Indonesia. Assessed indicator was on the level of estrogen in estrus and pH of cervical mucus on 20 dairy cows FH. Four treatments with repetition based on doses of selenium-vitamin E^{TM} for control (P_0), treatment 1 (P_1): 0.5 mg/ml selenium + 50 mg vitamin E^{TM}, treatment 2 (P_2): 1.5 mg/ml selenium + 50 mg vitamin E^{TM} and treatment 3 (P_3): 2 mg/ml selenium + 100 mg vitamin E^{TM}. Results showed that the average time of the returning estrus in control (P_0) is 123.25±49.64 day, treatment 1 (P_1) is 50.8±27.38 day, treatment 2 (P_2) is 53.8±23.95 day and treatment 3 (P_3) is 71.4±13.66 day.The average result of estrogen levels during the first estrus is P_0 7.81±0.95 pg/mL, P_1 for 10.06±0.66 pg/mL, P_2 for 9.63±0.87 pg/mL and P_3 for 10.14±0.84 pg/mL. pH of Cervical mucus in the first estrus after calving is P_0 for 6.6±0.41, P_1 for 8.8±0.83, P_2 for 8.4±0.89 and P_3 for 8.6±0.55. This study concludes that the provision of 0.5-1.5 mg/ml selenium and 50 mg vitamin E^{TM} were significantly influence to the return of estrus after calving.
Keywords: estrus period, level of estrogen, pH level of cervical mucus.

1. Introduction

The efficiency of reproduction success is determined by five factors, i.e. conception rate, calving interval, days open, service per conception and birth rate [1,2]. Supporting factors for the success of dairy cow reproductive efficiency derived from the ability of the dairy farmers on estrus detection, punctuality and technique of artificial insemination (AI), and the accurate diagnosis of pregnancy [3,4].

Roelofs *et al.* [5] and Rao *et al.* [6] stated that the physical signs of estrus include mounting, standing heat, licking and sniffing, as well as the discharge of clear mucus in the vagina and the redness on vaginal labia vulva. The signs should be observed appropriately for the successful insemination. Sign of estrus in cow was a determinant factor in the process of insemination. Failure to detect the estrus is the cause of reproductive problems in the dairy farm group. Accurate and efficient estrus detection is the key to the success of the insemination management.

Sufficient intakes of nutrients during the transition period of periparturient will support the reproduction success [7]. Selenium (Se), zinc (Zn), vitamin A and E as well as several other important minerals are essential source for health and reproductive performance of dairy cows [8]. Kimura [9], Spears & William [10] and Hefnawy & Perez [11] suggested that the adequacy of the selenium and vitamin E in the body of livestock can stimulate the ovary activity and increasing the neutrophils, macrophages and lymphocytes activities to prevent disruption of reproductive health during the period of periparturient. Thus the estrus can occur on 60 to 85 days after calving [12]. The objective of the study was to assess the effectiveness of selenium-vitamin E^{TM} to the return onset time of estrus after calving on dairy cows.

2. Material and methods

2.1 Cows, Feed, Housing

Cow used in this research were 20 females Frisien Holstein (FH) dairy cow at the age of 7 months pregnancy, second lactation and in good health. The feed given to the FH dairy cow in accordance with a formula by Training Center of Animal Husbandry Batu, consisted of forage and concentrate. The nutrient content of this concentrates consist of 17% crude protein (CP), 71% total digestible number (TDN) and 2% of fat, mineral, etc. The housing system used in this study is the type of closure and loose connective system.

2.2 Group of Cows

Each treatment used five cows. Four treatments with repetition based on doses of selenium-vitamin E^{TM} given. Control (P_0) use no selenium-vitamin E^{TM}, treatment 1 (P_1) use 0.5 mg/ml selenium + 50 mg vitamin E^{TM}, treatment 2 (P_2) use 1.5 mg/ml selenium + 50 mg vitamin E^{TM} and treatment 3 (P_3) use 2 mg/ml selenium + 100 mg vitamin E^{TM}.

2.3 Administration of Selenium-Vitamin E^{TM}

A total of 10cc Selenium-vitamin E^{TM} is given for 5 times using disposable syringe Onemead with 18G needle for intramuscular injection in musculus gluteus area. Administration time of selenium-vitamin E^{TM} doses are at 7 and 8 months of pregnancy, two weeks before birth, 7 and 14 days after calving.

2.4 Onset of Estrus

The onset of estrus period calculated from the giving birth day of cow (day 0) until the onset of physical signs during estrus, in units of days. Physical signs that indicate the dairy cow FH is in estrus are mounting, standing heat and mucous discharge of the cervix.

2.5 Levels of Estrogen

The assessment of estrogen levels during the estrus used blood serum taken when FH cows in standing heat condition. Blood serum obtained from 10 cc of blood taken from the left side of jugular vein using Onemead 20cc syringe. The blood collected, left for 2-3 hours at temperature of 15°C then centrifuged with 3000 rpm for 15 minutes to obtain the serum. The serum stored in the refrigerator at the temperature of -20°C. Analysis of the estrogen levels used the method of Bovine Estrogen (EST) Elisa Kit, with the units of pg/mL.

2.6 Cervical Mucus pH

The pH of cervical mucus was measured with pH Merck paper by 0-14 indicators. The pH paper is dipped into the mucus cervix. The changing color of the paper is compared to the attached standard value.

2.7 Data Analysis

The data were analyzed using the F test with the value significance of 95% which were followed by Duncan Multiple Range Test to assess differences between the treatments.

3. Result and Discussion

3.1 Levels of Estrogen and Cervical Mucus pH during Estrus

Levels of Estrogen and cervical mucus pH in estrus period were higher on the treatment compared to the controls (Table 1). This means that the administration of selenium-vitamin E^{TM} provides a real strong influence to the increased levels of estrogen and cervical mucus pH during estrus after calving in dairy cows FH (significance of 95%).

Table 1. Level of estrogen and cervical mucus pH after administration of selenium-vitamin E in dairy cow FH

Treatment	Estrogen level during estrus (pg/mL)	Cervical mucus Ph
P_0 (Control)	7.81 ± 0.95^a	6.6 ± 0.41^a
P_1 (0.5 mg/ml Selenium+50 mg Vit E)	10.06 ± 0.66^b	8.8 ± 0.83^b
P_2 (1.5 mg/ml Selenium+50 mg Vit E)	9.63 ± 0.87^b	8.4 ± 0.89^b
P_3 (2.0 mg/ml Selenium+50 mg Vit E)	10.14 ± 0.84^b	8.6 ± 0.55^b

Notes: different superscript within a column indicates significant difference among mean values ($P<0.05$)

The result regarding the estrogen levels during estrus after calving is similar to the results of Lyimo *et al.* [13], that explained the level of estrogen during estrus are measured on standing heat dairy cows ranged on 7.76±2.39 pg/mL. A similar case was also raised by Lopez *et al.* [14], that the levels of estrogen (estradiol) time of estrus with one ovulation was in the range of 4.1– 9.1 pg/mL with the equivalent of 5.5±0.3 pg/mL. Whereas during estrus and multiple ovulation in a range of 2.2-14.6 pg/mL equivalent to 7.8±0.4 pg/mL. Nelson *et al.* [15] also stated that the cows induced by estradiol cypionate (ECP) resulted the concentration of estrogen (estradiol) in condition of standing heat for 8.3±0.7 pg/mL, whereas without ECP induction resulted 5.2±0.7 pg/mL.

However, this result differs from Mondal *et al.* [16], that estrogen levels at the beginning of estrus have reached 27.29±0.79 pg/ml. Otherwise, Arijie *et al.* [17] states that estrogen levels reached the peak of estrus in two days before estrus for 500 pg/ml whereas Tabataei *et al.* [18] suggest that estrogen levels during proestrus and estrus (18–21 days) in blood plasma is 105.30±22.62 pg/ml. The difference in estrogen levels during estrus assumes the differences in the reproductive physiology of each individual character of the cow.

Obtained data on pH of cervical mucus in estrus period in this study are similar to Bishoni *et al.* [19] ranged from 6.8–8.5. Those results are not much different from Dodamani [20], where cervical mucus pH in estrus ranged 8.83–8.91 (stdev of 0.25). The result of Suharto [21] on goat Etawah breeding mentioned that pH during estrus ranged 7.5 (stdev of 0.67) – 9.8 (stdev of 0.42). However, these result are different from Tsigilliani *et al.* [22] that found mucus pH in estrus for 7.4 (stdev of 0.21) – 7.7 (stdev of 0.42).

The pH of acid or alkaline is often caused by the condition of the biophysics and biochemistry of cervical mucus controlled by hormonal changes during the estrus cycle [23]. This mechanism presented by Noakes *et al*. [24], that explained each of the different stage of estrus cycles produces a different pH value as well. At the beginning of estrus, the pH value is at 6.54 and one day before physical sign of estrus, pH value is in the range of 6.72 – 7.0 [24] and in estrus the pH value is 7.32 [24].

The difference of acid or alkaline pH is also influenced by the site of measurement. As described by Schilling & Zust in Tsiligiani *et al.* [22] that the condition of acid and alkaline of cervical mucus measured in *ex situ* was 0.6 – 0.7, higher than measured *in situ.* The cervical mucus pH is measured while standing heat by *in situ* ranges 6.5 – 6.7. This is also accordance with the statement of Salisbury & Vandenmark [25] explained that the value of cervical mucus pH measured outside tends to be alkali, ranged of 9.0 – 9.2.

The result of research proves that selenium-vitamin E^{TM} acted to fix the function of reproduction after calving [26] to modulate the proliferation of ovary granulose cell to synthesize estradiol 17β. It is similar to the result of Basini & Tamanini [27] *in vitro* granulose cells. The presence of estrogen signals the functioning of ovarian cycle thus capable to stimulate the behavior of estrus [28]. One of the physical signs of estrus time in cows is the discharge of cervical mucus [29]. Estrogen levels during estrus is closely associated with cervical mucus conditions as outlined by Verma *et al*. [30] that cervical mucus pH at the time of estrus is more alkaline, due to the increased levels of estrogen so the levels of sodium chloride and water content on the cervix are also rising.

The pH condition of cervical mucus is important to determine the success of pregnancy on livestock because cervical mucus is the transport medium for sperm [31]. The pH of 7.0 – 8.5 is optimal range which act in support of the sperm viability and motility, whereas pH below 6 lead to the reducing motility of sperm [32,33].

3.2 The return onset time of estrus

The return of estrus after calving on the treatment of selenium-vitamin E^{TM} was faster than the control. This means that the administration of selenium-vitamin E^{TM} affected the return of estrus after delivery in FH dairy cows with significance value 95%.

The result obtained regarding the return of estrus after calving is similar to the study conducted by Opsomer *et al*. [34], that dairy cows under normal conditions would return to estrus within 37 days while dairy cows with *anestrus* – reproductive disorder, back in estrus in 86 days. Some dairy cows with estrus symptom will return to estrus within 30 days and the dairy cow's estrus after calving within 51 days.

Several other studies regard the time of estrus occurrence due to first insemination after calving on dairy cows was performed by Duraisamy [35]. The result showed that the distance from birth to first

insemination is equivalent to 61.7±2.5 days. Similar to Darodjah [36] that found the first insemination after delivery is 32 –188 days with an average estrus events 77.82±29.61 days, whereas Renata & Dejan [37] stated that first insemination after calving is 85–110 days.

The return of estrus in a cow after calving was counted from the day the cows gave birth to the first estrus after calving. Estrus is the period of the cow showing sexual behavior, corpus luteal lysis, very low progesterone production and peaked level of estrogen. This is due to the presence of De Graff follicles stimulating uterine α estrogen receptor (α ER) and progesterone receptor (PR) [17,38].

It proves that the granting of selenium-vitamin E^{TM} on dairy cows before and after calving is very influential to induce estrus and accelerate the time return of estrus in dairy cows. The results is in accordance with El-Shahat & Monem [39] by in vivo in goats, explained the activities of selenium-vitamin E^{TM} stimulated the anterior pituitary gland to secrete the steroid hormones and initiate follicullogenesis in the ovaries. Anterior pituitary gland produces Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH). FSH plays an important role to the mechanism of the selection and the development of dominant follicle and granulose cell, increases the enzymatic activity to catalyze aromatization androgen to produce estrogen [28]. Increased estrogen like estradiol 17α acting against uterine development, influence the behavior of estrus, and increase the secretion of vaginal mucus and induce the release of LH to the onset of ovulation [40].

Table 2. The return onset time of estrus after administration of selenium-vitamin E in dairy cow FH

Treatment	Return onset time of estrus (day)
P_0 (Control)	7.81 ± 0.95^a
P_1(0.5 mg/ml Selenium+50 mg Vit E)	10.06 ± 0.66^b
P_2(1.5 mg/ml Selenium+50 mg Vit E)	9.63 ± 0.87^b
P_3(2.0 mg/ml Selenium+50 mg Vit E)	10.14 ± 0.84^b

Notes: different superscript within a column indicates significant difference among mean values ($P<0.05$)

The difference between the occurrence of the first estrus after calving at P_3, P_2 and P_1 (Table 2) implied the difference in milk production. On P_3, it is assumed that the milk production is higher compared to P_2 and P_1. The high milk production gives negative impacts against the days to inflict estrus. It is because the dairy cow with high milk production would need greater estrogen metabolism, thus needs greater pre-ovulation follicles to produce enough estrogen to induce LH for ovulation. However, the growth and development of the follicle may require a longer time [14]. Noakes *et al*. [24] stated that the mechanism of suckling or milking increases production of oxytocin thus increases the blood flow to the mammae glands, then increases glucocorticoid and inhibit estrus. This fact is supported by Armstrong *et al.* in Spilsbury *et al.* [41] that the mechanism of suckling induces the release of prolactin that inhibits the pituitary and delays the estrus.

The return of estrus is important for determining the time of insemination services. The onset of estrus after calving indicates a dysfunction organ and reproductive tract [36]. Dhali *et al.* [42] suggest that the incidence of estrus by the presence of long standing heat and estrus intensity depends on the presence of estrogen at estrus.

Therefore, we can conclude that the administration of 0.5-1.5 mg/ml selenium and 50 mg vitamin E^{TM} at the time before and after calving accelerate the time return of estrus after calving. However, the return onset time of estrus between the doses in this research were not significantly different, ranged for 9-10 day, thus further research with more interval variation of dose is recommended.

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