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Relationship Between Calving Rate and Concentration of Hormones and Blood Metabolites During Pregnancy in Post-Induction Pasundan Cows GnRH

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Abstract: Twenty Pasundan heifers were used in the study to find out calving rates related to the concentration of hormones and metabolites of the mother's blood during the farm. The experimental mother cow was given a combination of the hormone prostaglandin F2α as much as 5 ml per head and gonadotropin realising hormone (GnRH) of 2.5 ml per head intramuscular to uniformize fertility conditions and improve fertility. The mother cow is immediately carried out artificial insemination 2 times with an interval of 6 hours. All experimental cows were given forage basalt food and adlibitum drinking water. Pregnancy examination is carried out on the 60th and 150th days of post-insemination using the rectal palpation method. The observed variables in the form of calving rate were related to plasma concentrations of the hormones progesterone (P), estrogen (E), and blood metabolites levels β-OH butyric (BHBA), blood urea nitrogen (BUN), and non-esterified fatty acids (NEFA). The results showed that in Garut calving average rate of 90%, the plasma concentration of hormone progesterone 4.73±0.28 ng/ml and estrogen 24.59±1.91 pg/ml higher than Bogor with an average calving rate of 70%, the plasma concentration of progesterone hormone 3.46±0.71ng/ml and estrogen 21.67±1.57 pg/ml. The concentration of BHBA, BUN, and NEFA for Bogor region respectively was 12.33±1.81 mg dl-1; 23.70±2.10 mg dL; 1.85±0.25 mmol L-1) higher than Garut in a row is 10.17±1.25*mg dl-1, 22.70±2.70mg dL; 1.76±0.37mmol L^{-1}). The results of regression analysis showed that the relationship of calving rate (CR) with the concentration of hormones conceptus estrogen (E) and progesterone (P) in garut region has a real form of linear relationship ($P<0.05$) by following the equation Calving rate=4772 +0.232 Estrogen + 0.643 Progesterone - 0.074 BHBA + 0.335 NEFA - 0.082 BUN with a coefficient of determination of 43.65%, while for Bogor region according to Calving regression rate=5,590+ 0.010 Estrogen +0.638 Progesterone - 0.231 BHBA + 1.11NEFA – 0.105 BUN with a coefficient of determination of 16.34%. It was concluded that calving rate is strongly influenced by the concentration of the hormones progesterone and estrogen, as well as having a close relationship with the condition of the mother blood metabolite during the pregnancy.

Keywords: Pasundan Cows, Synchronization, Calving Rate, Hormones, Blood Metabolite

1. Introduction

Pasundan cows as a local genetic resource of West Java, have high fertility but do not yet have high reproductive efficiency. The problem that is still often encountered in pasundan cows with the pattern of people's livestock business until now is the performance of reproduction that is not optimal which is characterized by the presence of service per conception of 1.8±0.2 and calving rate of 65±5.0% [1]. Not optimal reproductive efficiency is thought to be caused by inadequate pregnancy hormones (estrogen, progesterone, and placenta lactogen) that play a role in the treatment and regulation of growth and development of the uterus and placenta [2, 3]. A decrease in the concentration of these

pregnancy hormones will lead to an increase in embryonal death, a decrease in calving rate, and a decrease in birth weight. In the process of pregnancy there is an increase in several conceptual hormones, especially estrogen and progesterone, along with increasing gestational age [4]. Furthermore, in order to maintain the success of the reproductive process, of course, it will be followed by changes in the mother's blood metabolism [5, 6].

Lack of feed, especially for hot tropics including in Indonesia, is one of the causes of decreased reproductive efficiency because it is always followed by reproductive disorders that cause the onset of Infertility in female cattle [7]. That nutritional deficiency will affect the function of the anterior pituitary resulting in the production and secretion of the hormones Follicle Stimulating Hormone (FSH) and low Luteinizing Hormone (LH), which causes the ovaries to not develop or experience hypofunction [8]. In the placenta phase, the growth of the fetus depends largely on the availability of nutrients in the mother's blood. This affects the parent blood metabolites of both β-OH butyric acid (BHBA), blood urea nitrogen (BUN), and non-esterificated fatty acids (NEFA). The concentration of blood metabolites depends largely on the amount of feed consumed and greatly influences embryonic growth during pregnancy [9]. Triglycerides, proteins, and glucose is part of the nutrients needed for the growth and development of fetuses during pregnancy [10, 11] and the potential to improve the appearance of calves at birth [12]. Metabolic profile of the blood (BHBA; NEFA; BUN). very useful as an indicator that the body's homeostasis mechanism serves to keep blood parameters within the physiological range of different feed and maintenance conditions [13].

One of the efforts in improving the ability of the uterus and placenta in facilitating the growth and development of embryos and fetuses during pregnancy can be done through increased secretion and availability of pregnancy hormones. To increase the percentage of calving intervals, the use of Gonadorrophin-releasing hormone (GnRH), which has the power to stimulate growth and development of follicles, causes ovulation and is able to maintain an adequate uterine environment for embryo life [14]. The results of previous researchers reported that the induction of GnRH in the mother will increase the pregnancy hormone progesterone while also increasing the placenta capacity manifested through increased wet and dry weight, active cell mass, cell synthetic activity (DNA and RNA), and placenta nutrient synthesis up to 70 days of gestation [15]. Increased placenta capacity is also influenced by the maintenance of the corpus luteum and the production of progesterone by the placenta [15]. This study aims to find out the relationship between the birth weight of the child and the concentration of hormones and metabolites in the blood plasma of the mother during pregnancy in Pasundan cows.

2. Materials and Method

Twenty (20) pasundan heifer were used in this study, with the same relative weight and age belonging to farmers consisting of 10 heads from Bogor area representing the North Priangan region and 10 heads from Garut area representing the South Coast. The purpose of this study was to find out the calving rate related to the concentration of hormones and metabolites of the mother's blood during pregnancy.

The research was conducted based on experimental methods designed into 2 (two groups of observation variables, namely bound variable (Y) in the form of calving rate, and free variable (X) in the form of hormone concentration and blood metabolites. All experimental cows are adapted to the local environment and given basalt food in the form of field grass while drinking water is given adlibitum. The experimental mother cow was continued using a combination of the hormone prostaglandins (PGF2α, dinoprost tromethamine) at a dose of 5 ml/head intramuscular as much as 2 (two) times with an interval of 11 days, but on the 9th day injected gonadotropin realising hormone (GnRH, gonadorelin) as much as 2.5 ml/head intramuscular. to uniformize fertility conditions and increase fertility. The mother cow is immediately carried out artificial insemination 2 times with an interval of 6 hours. Pregnancy examination is carried out on the 60th and 150th days of post-insemination using the rectal palpation method. The observed modifiers were the concentration of hormones including estrogen and progesterone, while blood metabolites included β-hydroxybutyric acid (BHBA), blood urea nitrogen (BUN), and non-esterificated acid (NEFA).

Blood sampling during pregnancy is done 3 (three) times at the time of estrus, age 60 days of pregnancy, and age 150 days of pregnancy. Blood is taken from the jugular vein of 10 ml using a disposable syringe containing anticoagulant, then inserted into a test tube and placed in an ice-filled flask. Blood is left for 30 minutes then dysentery at a speed of 2500 rpm for 15 minutes. The formed plasma is separated into an evendorf tube that will be used for the analysis of hormones and blood metabolites.

2.1. Hormone Analysis

Estrogen. Estrogen concentrations in plasma are measured by KIT (Sigma Chemical Co., St. Louis, MO) using the Enzyme-Linked Immunosorbent Assay (ELISA) technique. On each plate Elisha inserted 25 µl of standard dissolver, sample, and control, then each mixed with 200 µl estradiol conjugate reagents on each well. Next incubation for 120 minutes at room temperature. The absorbance value is read in the ELISA reader after 10 minutes with a wavelength of 450±10 nm.

Progesterone. Estrogen concentrations in plasma are measured by KIT (Sigma Chemical Co., StLouis, MO) using the Enzyme-Linked Immunosorbent Assay (ELISA) technique. On each plate Elisha inserted 25 µl of standard dissolve, sample, and control, then each mixed with 200 µl of progesterone conjugate reagents on each well. Next

incubation for 120 minutes at room temperature. The absorbance value is read in the ELISA reader after 10 minutes with a wavelength of 450±10nm.

2.2. Blood Metabolite Analysis

B-OH Butyric Acid (BHBA). BHBA concentrations were measured using enzymatic techniques using KIT (Sigma Chemical Co., St. Louis, MO) with a standard range already available by dripping blood of 1 drop of blood each (30 µl) using Precisian XtraTM Blood Test Strips, which took 25 seconds for each sample.

Blood Urea Nitrogen (BUN). BUN concentration was measured using enzymatic techniques using ELISA KIT (Sigma Chemical Co., St. Louis, MO) with the standard range already available by dripping 30 µl of blood on each kit stem. Next put into the auto analyzer Tool Refloton (R) plus and the result will be read automatic tool.

Non-Esterificated Fatty Acid (NEFA). NEFA concentration was measured using enzymatic techniques using Max Discovery NEFA ASSAY KIT (Sigma Chemical Co., St. Louis, MO) with the standard range already available and wavelength used 550 nm.

2.3. Data Analysis

The collected data were analyzed using Correlation Regression analysis using software Minitab version 19, to determine the shape and density of the relationship between calving rate (Y) bound changers and hormone-free modifiers and blood metabolites (X).

3. Results and Discussions

The most realistic assessment of the application of technology is to calculate the calving rate. If the results of the application of reproductive technology have not produced a child standing next to the mother, then the application of synchronization of estrus and artificial insemination can not be said to be successful [16]. Calving rate is related to dynamic changes in hormone parameters (estrogen and progesterone) and blood metabolites (β-OH Butyric Acid, Blood Urea Nitrogen and Non-Esterificated Fatty Acid) during pregnancy. The results showed that the average calving rate, hormone concentration, and blood metabolites of Pasundan cows can be seen in Table 1.

Table 1. *Calving Rate, Hormone Concentration, and Blood Metabolites of Pasundan Cows*

Group (Region)	**Pesisir Selatan / Garut**		**Priangan Utara/ Bogor**	
Sub group (pregnancy rate)	**Mean**	**Correlation Coefficient**	**Mean**	**Correlation Coefficient**
Calving rate (%)	90	-	70	-
Estrogen (pg/ml)	24.59±1.91*	0.010*	21.67±1.57*	0.232*
Progesteron (ng/ml)	4.73±0.28**	0.638**	3.46±0.71**	0.643**
BHBA (mg dl^{-1})	10.17±1.25*	0.231*	12.33±1.81*	-0.074*
NEFA (mmol L^{-1})	1.76±0.37**	1.111**	1.85±0.25*	0.335
BUN (mg dL^{-1})	22.70±2.70**	0.105	23.70±2.40*	0.082

Superscript* indicates real correlation rates (P<0.05) and ** very real (P<0.01) with calving rate.

Based on the results in Table 1. Indicates that the concentration of blood metabolites (beta butyric hydroxy (BHBA); non-esterification fatty acid (NEFA); blood urea nitrogen (BUN) of Pasundan cows when estrus, bunting 60 and 150 days induced (PGF2α + GnRH+ PGF2α) is still in the normal range. This is in accordance with previous researchers stating that the range of BUN levels in normal cows is 6-27 mg / d [17], while the normal range of beta hydroxy butyric acid (BHBA) is <9.00 mg dL-1 [18] and the normal range for NEFA is 1.00 - 2.00 mmol L-1 [19]. Further results in this study showed that the concentration of blood metabolites (beta-hydroxybutyric (BHBA); non-esterification fatty acid (NEFA); blood urea nitrogen (BUN) of Pasundan cows for the North Priangan region is higher than the South Coast region. The condition is suspected in North Priangan there is a lack of feed consumed by cows, both the quality and quantity of forage of animal feed provided, so that negative energy balance (NEB) occurs. The increasing concentration of BHBA, BUN, and NEFA shows indications of mobilization of carbohydrates, proteins, as well as lipids, and oxidation of fatty acids that produce energy [20].

The results showed that Pasundan cows in Garut region have an average Calving rate of 90% higher than the Bogor area with a value of 70%'. The difference in calving rate in the two study areas, it is suspected that Pasundan cows in Bogor region are relatively negative energy balance (NEB), which is shown by a lower average concentration of hormones (estrogen, progesterone), while blood metabolite concentration includes β-hydroxybutyric acid (BHBA), blood urea nitrogen (BUN), and non-esterificated acid (NEFA) relatively higher than the South Coast region. This is in line with previous researchers who stated that an imbalance in protein levels would interfere with the secretion of gonadotropin hormones [21]. The presence of progesterone is a key hormone that plays an important role in regulating the estrous cycle and maintaining pregnancy, and progesterone concentrations are maintained high. until near the end of pregnancy [22]. Progesterone is also very important to support the development of the mammary glands [23]. Progesterone is an important role in preparing the uterine environment for implantation and increases in progesterone during pregnancy and plays a role in maintaining pregnancy [24]. Besides being produced by the corpus luteum at the beginning of pregnancy, progesterone is also produced by the placenta and fetus after placentation [3, 25]. Furthermore, the tendency of the progesterone hormone pattern with estradiol stimulates uterine gland secretion and increases the expression of uterine epithelial growth factors, proliferation, and conceptus differentiation [26]. Furthermore,

high concentrations of blood metabolites include BHBA, BUN, and NEFA during pregnancy, affecting the development of embryos and fetuses in the uterus, so that it can be followed the death of embryos and the absorption of embryos by the uterine wall, abortus, or the birth of weak children and neonatal death [27, 28].

Based on the results of correlation analysis shows that the variable calving rate in Garut region has a very real level of positive relationship density (P<0.01) with progesterone (+64.3) and a real relationship (P<0.05) with estrogen (+64.3) 23.2), while in Bogor calving rate has a real relationship (P<0.05) with estrogen (+1.0) and very real (P<0.01) with progesterone (+63.8.). These results showed that the increase in calving rate was in line with the increased concentration of the hormones progesterone and estrogen during pregnancy.

The results of correlation analysis showed that calving rate in Garut region has a noticeable level of negative relationship density (P<0.05) with BHBA (-7.4) and NEFA (-8.2), very real relationship (P < 0.01) with a BUN (-33.5), while in Bogor calving rate has a very real relationship (P<0.01) with NEFA (-111.1, and the real relationship (P,005) with BHBA (23.1) and BUN (-10.5), these results show that calving rate is inversely proportional to the concentration of blood metabolites during pregnancy. This means that the calving rate will be lower if the concentration of blood metabolites increases due to poor nutrient intake during pregnancy.

The results of regression analysis showed that the relationship of calving rate (CR) with the concentration of estrogen conception hormone (E) and progesterone (P) in the Garut region has a real form of a linear relationship (P<0.05) by following calving rate equation=4,772 +0.232 Estrogen + 0 0.643 Progesterone - 0.074 BHBA + 0.335 NEFA - 0.082 BUN with a coefficient of determination of 0.4365 meaning calving rate is influenced by the concentration of estrogen and progesterone hormones, blood metabolites (BHBA, NEFA, BUN) during pregnancy by 43.65%, while for Bogor region according to regression equation Calving rate=5,590+ 0.010 Estrogen +0.638 Progesterone - 0.231 BHBA + 1.11NEFA – 0.105 BUN with a coefficient of determination of 0.1634 means calving rate is influenced by estrogen hormone concentration, progesterone, and blood metabolites (BHBA, NEFA, BUN) during pregnancy by 16.34%.

Based on regression equations it turns out that BHBA, BUN, and NEFA all have negative regression coefficients, meaning that if the blood metabolite increases it will decrease calving rate as a result of negative nutrient balance. This condition indicates that the high concentration of blood metabolites as a result of poor nutrient intake during pregnancy will lower the calving rate of the Pasundan heifer. This means that if there is a shortage of nutrient substrates, then there will be a mobilization of food reserves such as fats (triglycerides) stockpiled during pregnancy that will cause acetyl coa buildup and cannot enter the citric acid cycle so that it will be converted into ketone objects such as acetone, β-OH butyric as a result of condensation of 2 moles of CoA acetyl. Similarly, if there is a metabolism protein, then livestock are in a negative nitrogen balance that is characterized by an increase in the concentration of blood urea nitrogen. The same in triglyceride fat metabolism products will be transformed into free fatty acids non-esterificated fatty acids (NEFA) [9, 28]. The relationship between reproduction and nutritional status is very closely related to nutrient deficiency as the main factor that inhibits the reproductive system of cows in the tropics [29]. Lack of nutrients or insufficient inputs can have a direct effect on reproductive efficiencies such as low reproductive performance and productivity [30]. Low glucose levels can lead to high levels of non-esterified fatty acids (NEFA) which have toxic effects on follicles, oocytes, embryos, and fetuses, and decreased GnRH secretion by the hypothalamus [31]. The decrease in GnRH inhibits the synthesis of FSH and LH in the anterior pituitary and causes the follicle to not develop and does not appear estrus [32]. Furthermore, low concentrations of blood glucose and total protein disrupting reproductive hormone function and not an optimal function of the reproductive tract failing follicle, oocyte, and embryo development, this condition will lead to premature embryo death and fertilization failure resulting in repeated mating [33].

4. Conclusions

It was concluded that increased concentrations of the hormones estrogen conception and progesterone during pregnancy had a positive impact on calving rates. High blood metabolites due to poor nutrient intake have a negative impact on the calving rate.

It is recommended that the improvement of feed given to the mother who is bunting, in addition, to need to be researched further increased concentration of hormone conception due to the induction of hormones exogenously related to the micro-uterine environment, milk production, and immunologic response.

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