Effect of Estrus Synchronization with Prostaglandins (PGF2A) and Gonadothropin Realising Hormone (GNRH) on the Hematological Profile of Pasundan Heifers during Pregnancy

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International Journal of Environment, Agriculture and Biotechnology
Vol-6, Issue-2; Mar-Apr, 2021

Journal Home Page Available: https://ijeab.com/
Journal DOI: 10.22161/ijeab

Effect of Estrus Synchronization with Prostaglandins (PGF2A) and Gonadothropin Realising Hormone (GNRH) on the Hematological Profile of Pasundan Heifers during Pregnancy

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Received: 20 Dec 2020; Received in revised form: 10 Feb 2021; Accepted: 01 Mar 2021; Available online: 16 Mar 2021

***Abstract*— Twenty Pasundan cows were used in this study to know the effect of synchronization using prostaglandins and hormone gonadotropins on the picture of erythrocytes, leukocytes, and pasundan cow hemoglobin levels during pregnancy. The Pasudan heifers experimented with estrus using a combination of prostaglandin hormone (PGF2α) as much as 5 ml per head and gonadotropin realising hormone (GnRH) as much as 2.5 ml per head intramuscular to homogenize fertility conditions and improve fertility. The estrus mother cow is immediately carried out artificial insemination as much as 2 times with an interval of 6 hours. All test cows were given forage basalt food and adlibitum drinking water. Pregnancy examination is carried out on the 60th day and the 150th day of post-insemination using the rectal palpation method. Changes observed in the form of hematological concentrations include erythrocytes, leukocytes, and hemoglobin. The results showed that the concentration erythrocytes, leucocyte, and hemoglobin for Garut region respectively was 6.24±0.61 (million/μl); 11.54±0.25 (thousand/(μl); 11.54±0.61 (g/dl) higher than Bogor in a row was 5.99 ± 0.64 (million/μl); 11.46± 1.41 (thousand/(μl); 11.13 ± 0.60 (g/dl)). The results of the variance analysis showed that the synchronization of estrus with prostaglandins (PGF2α) and the gonadotropin releasing hormones (GnRH) did not differ markedly (P>0.05), between the concentrations of erythrocytes, leucocytes, and hemoglobin during the gestation period with cows that were not pregnant. It concluded that the synchronization treatment of estrus with prostaglandins (PGF2α) and gonadotropin releasing hormones (GnRH) had no effect on the profile of hematological concentrations during the gestational period (60 days and 150 days) and was no different from Pasundan heifers that are not pregnant.*

***Keywords*— Pasudan heifers, synchronization estrus, haematological Pregnancy Phase.**

I. INTRODUCTION

Cultivation of Pasudan cattle as germplasm cattle is widely carried out by communities in the southern Coastal (Garut) and northern Priangan regions (Bogor), both as a primary livelihood and as an additional income. Pasudan heifers have high fertility but do not have high reproductive efficiency due to slow puberty of 22± 0.2 months, and repeated mating characterized by preconception service of 1.8±0.2 and conception rate of 60 ± 5.0% (Setiawati et al., 2018). A common reproductive problem in cows in Indonesia is low reproductive efficiency indicating reproductive disorders.

One of the symptoms of reproductive disorders is the occurrence of recurrent mating (Båge et al., 2002).

One of the efforts to obtain reproductive efficiency is through the application of synchronization of estrus in pasundan heifers that can manipulate the reproductive system to increase the potential of one head each year. Estrus synchronization is a technique of manipulation of the estrus cycle to present symptoms of estrus and ovulation in a group of cattle simultaneously. This technique proved effective for improving the efficiency of the use of artificial insemination (Patterson et al., 2005). Administration of PGF2α will cause regression of the corpus luteum followed by a decrease in plasma progesterone levels. Regression of the corpus luteum is followed by the rapid development of dominant follicles and ovulation. Additional GnRH administration before PGF2α will increase the size of the corpus luteum and maximize plasma progesterone levels during PGF2α injection, thus increasing the rate of regression of the corpus luteum and increasing the growth of dominant follicles. Synchronization of ovulation with GnRH can increase the number of follicles and corpus luteum to increase the secretion of pregnancy hormones and mammogenic hormones such as estradiol and progesterone during pregnancy that plays an important role in the maintenance of pregnancy until entering the postpartum period of cows (Rasby, 2005).

Blood is a metabolic component of living things that acts as a medium of transportation of oxygen and food juice into the tissues and transports the rest of the tissue metabolism and carbon dioxide for further excretion. On the other hand, the blood circulation system serves also as a means of channeling the secretion of endocrine glands to the target organ. Pregnancy in general causes dynamic changes in hematological parameters such as red blood cell count, hematocrit, and hemoglobin in cows (Sudjatmogo et al., 2001). The main and important thing to note in pregnant cows is their food rations and health care. Various types of diseases can interfere with the health of pregnant cows and the fetus they contain, the transmission of some types of diseases can cause infections in the placenta and fetuses. It can result in pedet being born in a weak condition or it can also result in death. Therefore it is necessary to anticipate by monitoring the hematological profile as an indicator of the level of health of the cow early before the cow shows clinical signs of pain. Hematological examinations that are often used to measure the degree of animal health are the number of red blood cells (erythrocytes), white dark cells (leucocyte), and hemoglobin (Hb) levels (Schalm, 2010). Total erythrocytes may indicate the occurrence of anemia or not, while hematocrit indicates that the cow is dehydrated or not, its total leukocytes and differentials may signal the occurrence or absence of infection (Amulic et al., 2012). There have been no reports of pasundan cow blood in pregnancy.

Based on the background description and problems in pasundan cows, this study aims to determine the effect of synchronization using prostaglandins and hormone gonadotropins on the picture of erythrocytes, leucocyte, and hemoglobin levels of Pasundan cows in the gestation period.

II. RESEARCH METHODS

This research sample uses 20 Pasundan cows, 10 each from Pasundan cattle farms in Pameungpeuk Subdistrict, Garut Regency, and 10 heads from Cariu – Jonggol Subdistrict, Bogor Regency. All test cows were adapted to the local environment and given basalt food in the form of field grass while drinking water was given adlibitum. The mother cow experimented with the hormone prostaglandins (PGF2α, dinoprost tromethamine) at a dose of 5 ml/tail intramuscular 2 (two) times with an interval of 11 days, but on the 9th day was injected with gonadotropin realising hormone (GnRH, gonadorelin) by 2.5 ml/tail intramuscular. to homogenize fertility conditions and improve fertility. The estrus mother cow is immediately carried out artificial insemination as much as 2 times with an interval of 6 hours. Pregnancy examination is carried out on the 60th day of post-insemination using the rectal palpation method. The observed changes are hematological concentrations including erythrocytes, leukocytes, and hemoglobin.

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

Hematology examination

Eritosit. Blood samples are sucked up to the limit of 0.5 using a thinning pipette. The tip is dipped in a diluting liquid (Turk) and the liquid is sucked to the limit of 101. The pipette is lifted, then closed the tip with the thumb and the base is closed with the middle finger with a flat pipette condition. The solution with blood is flattened and mixed by making movements such as the number 8. After a partial homogeneous solution is discarded approximately 3-5 drops. The Counting Room is taken from the glass cover, the glass cover is placed on the embankment of the counting room. The solution is filled into the counting chamber by touching the tip of the pipette on the edge between the glass plains of the cover so that the surface of the plain is filled evenly. After that, it is read under a microscope with a magnification of 40x. The cells that touch the second boundary line are calculated, the other side (right and bottom) do not enter the calculation. The five boxes that are usually counted are four corner boxes and one middlebox. The final calculation result (total number of erythrocytes), total erythrocytes = n x 10,000, where n is the sum of all cells from five squares.

Leucosit. White blood cells (leucocyte) are measured based on the number of white blood cells calculated based on the Turk method in units per mm3 of blood. According to Nugroho (2013) to calculate leukocytes, blood is diluted in leukocyte pipes and then put in the counting room. The diluent used is a solution of Turk. The test measures applied are a) Capillary blood suction, EDTA blood, or oxalate blood up to the 0.5 marks; b) Remove excess blood at the end of the pipette; c) Insert the tip into the Turk solution at a 45o angle, holding it at the 0.5 marks. suction the Turk solution until it reaches the 11 marks. Do not allow air bubbles; d) close the tip with the fingertip and remove the suction rubber; e) Shake for 15-30 seconds; f) place the counting room with the cover attached horizontally on the table; g) Shake the pipette for 3 minutes, keeping the liquid from being wasted from the pipette; h) Remove all liquid in the capillary stem (3-4 drops) and quickly touch the tip of the pipette to the counting room by offending the edge of the glass cover with a 30o angle. Let the counting room be filled with liquid with capillary power; i) Allow 2-3 minutes for leukocytes to settle; j) Using a microscope objective lens with a magnification of 10 times, the focus is placed on the divider lines; k) calculate leukocytes in four large areas from top left to right, down then left, down then left and so on. For the cells on the line, the calculated ones are on the left and top lines; l) The number of leukocytes per µL of blood is: the number of cells x 50.

Hemoglobin. The calculation of hemoglobin levels is done by the Sahli method. Sahli tubes are filled with HCl 0.1 N to the bottom line. Blood is sucked with pipette hemoglobin up to the number 20. The sucked blood is inserted in HCl 0.1 N by blowing slowly. Blood and HCl 0.1 N are mixed by blowing and sucking slowly. The formation of hematin acid is characterized by a change in color to brown or blackish brown. Aquades are dripped using a drip pipette while shaken, the addition of aquades is done until the color is the same as the comparison color. Hemoglobin levels are read by looking at the miniscus of liquid on the Sahli tube. Hemoglobin units are expressed by grams%.

Data analysis

The data obtained was analyzed using Anova's One Way analysis method with Duncan's follow-up test to see how the treatment affects variables.

III. RESULTS AND DISCUSSION

The results of the study in the form of total erythrocyte levels, total leukocytes, total hemoglobin (Hb), Pasundan heifers at the time of estrus, and period of pregnancy observed were the first quarter (gestational age 2 months), the second quarter (gestational age 5 months). The data is analyzed and presented in Table 1.

Table 1. Average Hemotological Concentration When Estrus, Pregnant 60 and 150 Days

Group (Region)	**South Pesisir (Garut)**		**North Priangan (Bogor)**	
Subgroup (Hormone)	PGF2α	PGF2α + GnRH	PGF2α	PGF2 α+ GnRH
Erythrocyte (million/µl)				
Estrus	5.75±0.25	5.72±0.86	5.15±0.43	5.42±0.29
60 days pregnant	5.63±0.35	6.61±0.19[a]	5.35±0.39	6.12±0.67[b]
150 days pregnant	5.78±0.29	6.95±0.28	5.54±0.49	6.94±1.06
Leucocyte (Thousand /(µl)				
Saat estrus	12.74±1.96	11.58±0.55	12.66±1.67	10.15±0.67
Hamil 60 hari	11.29±0.3	11.30±0.35[a]	12.09±0.88	10.25±0.61[b]
Hamil 150 hari	12.02±0.62	11.56±0.54	12.80±1.50	11.50±0.52
Hemoglobin (g/dl)				
Estrus	10.64±0.59	9.56±0.62	10.27±1.22	9.77±0.47

Hamil 60 hari	10.93±0.81	11.63±0.35[a]	11.03±0.86	10.53± 0.99[b]
Hamil 150 hari	11.66±0.61	11.95±1.15	11.57±0.57	11.39±0.51

a, b The average of each modifier on the same line with different superscripts shows a real difference (P< 0.01)

From the results of an examination of leukocytes, erythrocytes, and hemoglobin levels, Pasundan heifers in the gestation period (in Table 1) there was no difference with cows that were not pregnant (P>0.05). The data in Table 1 shows that the hematological average profile of Pasundan heifers in the South Coast and North Piangan regions is still within the normal limit of erythrocytes between 5-7 million/μl; leucocyte 10-12 thousand/(μl) and hemoglobin 9-12 g/dl. The normal range of total bovine erythrocytes is 4.9-10 μl, leucocytes 5.0 – 16 (thousand/μl), hemoglobin levels 8.4-14 g/dL (Roland et al. 2014). The concentration of hematological Pasudan heifers is quite varied, allegedly related to the management of feeding by farmers is quite varied according to the conditions of the existing grazing field location. The more adequate the nutrients in the feed will show normal total erythrocytes and are at a normal high range of cow blood (Adam et al., 2015). Furthermore, the total leukocyte plays a role in the defense of the body and its value will increase in cases of disease infection, food poisoning, anaphylactic shock, and central nervous disorders. The total decrease in leukocytes in cows can be caused when there is a decrease in leukocyte production, viral infection, shrinkage inflammation, the presence of cytotoxic substances, bone marrow disorders, and others (Roland et al., 2014). Hemoglobin functions to transport most of the oxygen and a small fraction of carbon dioxide, as well as maintain a normal blood pH (Baldy., 2003). The condition of leukocytosis is generally a physiological response to protect the body from attacks of microorganisms. In contrast, the condition of leukopenia that indicates a decreased total leukocytes can be caused by its ineffective formation process (Baldy, 2003).

Furthermore, disruption of the formation of blood cells can be caused by the administration of cytotoxic drugs, toxic substances, viral infections, starvation, normal replacement of bone marrow by malignant cells, such as in leukemia (Baldy, 2003). A significant increase in leucocyte percentage can be caused by chronic viral inflammation, adrenal cortex insufficiency disorders, and physiologically (fear, anxiety, and pain) (Vasconcelos and Galyean, 2014). Increased leucocytes can occur due to chronic diseases and increased steroids due to stress.

From the results of the examination of the concentration of leukocytes, erythrocytes, and hemoglobin, in the period of pregnancy (in Table 1) there was no difference with cows that were not pregnant (P>0.05). Similarly, after further analysis with a variety analysis in each period of pregnancy the levels of the elements studied did not show a noticeable difference with cows that were not pregnant. The concentration of erythrocytes, leucocyte, and hemoglobin in the South Coast region (Garut) is noticeably higher (P<0.01) compared to in the Northern Priangan region (Bogor). Important factors that affect the status of hematology are age, gender, status, the altitude of the region or place, feed and water balance of the body (Kadarsih, 2004). Low concentrations of erythrocytes in the North Priangan region (Bogor) are suspected to be related to inadequate feed consumed so that there are negative energy balance and environmental-climate conditions due to differences in place that can affect air oxygen pressure. One of the factors affecting the number of erythrocytes is a mineral deficiency (Njidda et al., 2014). The low concentration of erythrocytes contained in hemoglobin can be caused by the availability of feed source mineral content in a low maintenance environment (Schalm, 2010). The process of erythrocyte formation requires precursors, precursors needed i.e. supply of proteins, iron, copper, cobalt, amino acids, and hormones. The lack of precursors such as iron and amino acids that aid the process of erythrocyte formation will lead to a decrease in the number of erythrocytes (Besung et al., (2019).

Erythrocyte concentrations in pregnant Pasundan cows showed an increase as the gestational age increased from 60 days to 150 days. The increase in the number of erythrocytes occurs as compensation for changes and adaptation of the mother to the condition of pregnancy. The vascularity system and red blood cells serve to regulate the regulation of oxygen, carbon dioxide, nutrition, and circulation of important metabolites such as hormones to all tissues of the body including reproductive organs. The increased concentration of hormones is thought to trigger an increase in the number of red blood cells that are higher to supply the developmental needs of pregnancy (Kadarsih, 2004). Erythrocytes also play a role in the immune system, where when red blood cells are lysis processed by pathogens or bacteria, the hemoglobin in red blood cells will release free radicals that will destroy the walls and membranes of pathogenic cells, as well as kill them (Polizopoulou, 2010). Factors of nutritional status, blood volume, species, and altitude of the premises also affect the number of erythrocytes as well as hemoglobin. The number of erythrocytes and hemoglobin will increase at low environmental temperatures (highlands) and will decrease at high environmental temperatures (lowlands) (Gerardo et al., 2009) Meanwhile, relatively high humidity can result in O2 levels in the air relatively lower, which can lead to hypoxia conditions. Hypoxia may result in increased erythrocyte production (Ganong, 2008).

The concentration of leucocyte and hemoglobin in the North Priangan (Bogor) region during pregnancy is relatively lower than in the South Coast(Garut). It is suspected that Pasundan cows in the North Priangan region (Bogor) experience a lack of feed balance and the unavailability of adlibitum drinking water that can have an impact on environmental stress, while in the South Coast region around (Garut) the grazing field there is a waterhole so that when the cow feels thirsty can immediately drink. Decreased leucocytes can be caused by spinal cord abnormalities and severe kaheksia due to nutritional deficiencies (Dharmawan, 2002). Deficiency of vitamin-B12, vitamin-A, vitamin-C, vitamin-E, folic acid, and riboflavin is associated with the incidence of anemia caused by nutritional factors (Jilani and Iqbal, 2011). The physiological condition of livestock is an indicator of livestock health, which has a positive implication on livestock production. Temperature and humidity are external factors that can affect comfort and productivity. Pasundan cows that experience heat insecurity due to environmental stress can result in the physiological body is not optimal. In this condition, the release of body heat energy is greater to the environment than the energy needs for optimization of production and reproduction. In addition to these conditions, cows will easily experience the stress of temperature insecurity, especially heat. Environmental heat insecurity results in the body no longer being able to expend the heat received from the environment so the body is forced to increase the metabolic rate in the heat release process. Such circumstances will increase the energy needs of metabolism and have an impact on the physiological decline of the body. Leukocyte profile may reflect increased cortisol caused by stress (Widhyari et al., 2010).

The number of leucocytes in the blood circulation can be affected by the level of production, recirculation, and the use or destruction of leukocytes. A decrease in the number of leukocytes (leucopenia) can occur due to the use of corticosteroids, thymectomy, radiation, chemotherapy, decreased production, and acute viral infections. The low value of hemoglobin (Hb) in Pasundan cattle in the northern region, is thought to be due to insufficient nutrition for basic living and reproductive needs derived from the amount of feed consumed and the difference in the height of the place. Factors of nutritional status, blood volume, species, and altitude of the premises also affect hemoglobin (Gerardo et al., 2009), hemoglobin value (Hb) is strongly influenced by the adequacy of nutrition in the body of livestock especially proteins used for the synthesis of hemoglobin (Bashar et al., 2010. Nutritional factors affect the hemoglobin concentration of cows, the more adequate nutrients in the feed will show normal total hemoglobin and is in the normal high range of cow's blood (Adam et al., 2015). The need for O2 increases in areas with low temperatures (highlands) as well as in cattle experiencing stress that has an impact on the increase in hemoglobin content (Santosa et al., 2012). Increased O2 needs at a time when livestock are stressed are necessary for the continuity of energy-intensive metabolic processes at that time. The synthesis of hemoglobin is influenced by the presence of nutrients in feed, such as protein and iron (Mohri et al., 2007). Iron deficiency causes lower levels of hemoglobin in the blood than normal, this state is called anemia, 99% of anemia is caused by iron deficiency, which will lower the body's immunity so that it is very sensitive to attacks of disease seedlings (Găvan, 2010).

IV. CONCLUSION

Synchronization of estrus with prostaglandins (PGF2α) and gonadotropin releasing hormones (GnRH) has no effect on levels of erythrocytes, leucocyte, and hemoglobin during the gestational period and is no different from that of Pasudan heifers estrus / non-pregnant cows, Thus synchronization using prostaglandins and gonadotropin hormones(GnRH), effective to be applied to Pasundan heifers.

ACKNOWLEDGMENT

Thanks to the farmers and the Chairman of the Rundayan Sawargi Group addressed in Kampung Cihurang, Karya Mukti Village, Cibalong District, Garut Regency, and Mekar Tani Livestock Group 2, located in Singasari Village, Jonggol Subdistrict, Bogor Regency, which has helped the facilities in the implementation of research. Thanks were also conveyed to the Head of the Agriculture and Livestock Office of Garut Regency and its ranks, Bogor Regency and its ranks who together have helped research activities for the application of hormone induction and artificial insemination in Pasundan heifers.

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