

**EVALUATION OF HEMATOLOGICAL PROFILE IN EARLY
PREGNANT COW**

A THESIS

BY

IMRAN NAZIR

STUDENT ID: 1504103

SEMESTER: JANUARY - JUNE/2024

SESSION: 2022-2023

MASTERS OF SCIENCE (MS)

IN

PHARMACOLOGY



**DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY
HAJEE MOHAMMED DANESH SCIENCE AND TECHNOLOGY UNIVERSITY,
DINAJPUR-5200**

JUNE 2024

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**Dedicated To
My Beloved Parents**

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The Author

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ABSTRACT

The present study was conducted to evaluate the changes in hematological biomarkers in early pregnant cows. Fifteen multiparous cows were divided into three groups - Group1= 0 day; Group 2= 11th day and Group 3= 21st day after Artificial Insemination (AI). Blood samples were collected from all the pregnant cows at 0 day, 11th day and 21st day after AI and assayed for hematological biomarkers. The outcome showed that the Erythrocyte Sedimentation Rate (mm/1st hr), Neutrophil (%), Eosinophils (%) and Mean Corpuscular Volume (fl) were significantly increased at 21st day after AI. The Lymphocytes (%) and Mean Platelet Volume (fl) were particularly increased at 11th day and Monocytes (%) increased at 0 day just after AI. In contrast, Hemoglobin (g/dl), Total WBC count (/cmm), Procalcitonin test (%) were significantly decreased at 21st day as compared with 0 and 11th days. Packed cell volume (%) decreased significantly at 11th day after AI. Some blood parameters change in relation to pregnancy.

Keywords: Cattle, Hematological parameters, AI, Conception, Pregnancy

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LIST OF ABBREVIATION AND ACRONYMS

TRUS	Transrectal ultrasonography
ANOVA	Analysis of variance
MPT	Metabolic profile testing
SEM	Standard error mean
%	Percentage
g/dl	Gram per deciliter
TG	Triglyceride
RBC	Red blood cell
WBC	White blood cell
Hb	Hemoglobin
TCE	Trichloroethylene
PCV	Packed cell volume
MCV	Mean capsuler volume
MCH	Mean capsuler hemoglobin
MCHC	Mean capsuler hemoglobin concentration
RDW-SD	Red cell Distribution Width - Standard Deviation
RDW-CV	Red Cell Distribution Width–Coefficient of Variation
PVC	Polyvinylchloride

CHAPTER 1

INTRODUCTION

The main goal of raising dairy herds is to maintain a high level of milk production that can be achieved by a high level of reproduction (Hasan *et al.*, 2021, Hufana-Duran and Duran, 2020). Reproduction is an essential factor in the profitability of dairy cattle breeding because it affects the continuity of milk production and the length of calving intervals (Lonergan and Sánchez, 2020). To achieve sustainable animal production, the reproductive potential must be increased without compromising animal welfare. The most frequently used resource to diagnose and evaluate the health of dairy cattle is the examination of the hematological profile (Hasan *et al.*, 2021, Mekroud *et al.*, 2021). This examination facilitates the detection of productive and reproductive disorders occurring in the cows, as well as the identification of factors that change biomarkers, such as disease, pregnancy or stress (Abramowicz *et al.*, 2019, Loi *et al.*, 2021, Noya *et al.*, 2019, Purwar *et al.*, 2019). The haematological profile of cows varies mainly with the at the total blood leukocyte climate, season, age, body condition, and nutrition received (Hasan *et al.*, 2021, Noya *et al.*, 2019). It is well known that changes in the profile of cattle blood are also observed during pregnancy and lactation Oliveira *et al.*, 2019, Patel *et al.*, 2016). This is due to the adaptation of cows to pregnancy and the increased demand for nutrients and energy, which is associated with an increase in the number of oxygen-transporting erythrocytes and the content of haemoglobin and haematocrit (Oliveira *et al.*, 2019, Setiawati *et al.*, 2021, Sofyan *et al.*, 2020).

Under diagnosis is very crucial to evaluate the health of dairy cattle. This examination facilitates the detection of productive and reproductive disorders occurring in the cows, as well as the identification of factors that change biomarkers, such as disease, pregnancy

or stress. There are almost few reports regarding hematological changes in early pregnant dairy cows.

Therefore, the study was designed to meet the following research objectives:

1. To observe the blood parameters at different days after artificial insemination.
2. To compare and analyse the blood parameters in relation to pregnancy on 0, 11 and 21 days.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Hematological parameters and dairy production

The health of the cows can be evaluated and depends on haematologic and biochemical profile of blood. Hematology is highly informative as a diagnostic tool in bovine medicine, not solely, but in combination with clinical examination or other diagnostic procedures (Roland *et al.*, 2014). Nutrition is the main technological factor that can produce profound changes in the metabolic profile in animals (Dhiman *et al.*, 1991; Khaled *et al.*, 1999; Ingvarsen, 2006). The deficiency of minerals, proteins and vitamins in the diet of lactating cows causes profound changes in metabolism (Pârvu, 1992). Haematological parameters reflect the adaptability of cows to adverse environmental conditions, as well as other stressors. Haematological and biochemical profile within normal physiological limits reflects a good health status and is highly correlated with milk production (Coroian *et al.*, 2011). Blood parameters analysis can identify if there are errors in nutrition in lactating cows (Payne *et al.*, 1970). Biochemical and hematological parameters of cattle blood were analyzed based on several factors and by many authors over time: the influence of blood biochemical profile in cows with reproductive disorders and clinically healthy cows (Ruginosu *et al.*, 2011); influence of lactation on biochemical profile in cows (Filipejová and Kovacik, 2009); biochemical variation within the transition period in dairy cows (Seifi *et al.*, 2007; Quiroz-Rocha *et al.*, 2009; Imhasly *et al.*, 2014); Sabasthin *et al.* (2012) studied blood haematological and biochemical parameters in normal cycling, pregnant and repeat breeding buffaloes (*Bubalus bubalis*) maintained in isothermic and isonutritional conditions. Metabolic profile in buffaloes has been also conducted for pre- and post-partum period (Coroian *et al.*, 2011).

2.2 Increased cattle/buffalo numbers and reasons

Between 2000 and 2005 the number of cows in Bangladesh increased by about 1 percent, while the buffalo population increased by about 4 percent (DLS, 2002). This increase in the number of cattle was due to the success of artificial insemination, and also the importation of animals from India. The main reasons for the increase in buffaloes were the establishment of the Rampal Artificial Insemination Centre in Bagarhat District and a loan programme for buffalo rearing.

2.2.1 Cattle Breeding

Bangladesh has a long history of dairy cattle breeding. For the improvement of indigenous zebu cattle, the tropical breeds Red Sindhi, Sahiwal and Hariana, were introduced in 1937, and the temperate breeds, Holstein-Friesian and Jersey, were introduced in 1974 (Ali, 1985). Australian-Friesian-Sahiwal (AFS) were introduced from Australia in 1983. Frozen semen of Holstein-Friesian and Jersey breeds from Australia, Germany, New Zealand and Kenya were introduced in 1987 and frozen semen of different genotypes such as Holstein-Friesian and Jersey were introduced from 1990 onwards coming from Australia, New Zealand, France and USA (Bhuiyan, 1997). A breeding programme was established by the MOFL in 1982 to firstly breed cows for urban, semi-urban and milk pocket areas of 50% Friesian and 50% Sahiwal / indigenous composition, and secondly to breed cows for rural areas, of 50% Friesian and 50% indigenous breed composition. Some commercial farms also used 100% Friesian. However, this breeding policy was not satisfactory due to improper management and the low adaptability of Friesian crossbreeds to local conditions (Bhuiyan, 1997). Therefore, the breeding policy was revised in late 1999.

The main change to the MOFL breeding programme was to emphasise traditional

farming and the use of semen to improve the germplasm of indigenous cattle. In view of the national demand for milk, a new breeding policy is under formulation by the Ministry of Fisheries and Livestock through the Central Cattle Breeding Station (CCBS). The objectives of CCBS are to produce high quality semen from stud bulls and to disseminate this semen throughout the country via 22 AI centres, 423 AI sub- centres and 554 AI points (DLS, 2002). In addition, BMPCUL recently started their own breeding policy to improve the genetic merit of their members' cattle.

2.2.2 Production and demand for milk throughout the year

There is a great shortage of milk and meat production in Bangladesh (Figure 3.9); this shortage is primarily due to the low production of cows. Based on the estimate that the 141 million people in Bangladesh should each consume at least 120g of milk per day (as fluid or processed in any form), the annual milk demand should be about 6.13 million tons (FAO, 2004). This estimate of the national milk demand is over 25% of FAO's recorded national milk production for the country (FAO, 2004). To meet the shortfall, Bangladesh imports milk from developed countries at the expense of hard-earned foreign currency. Therefore, meeting Bangladesh's demand for milk is a huge national task. The main reason for the failure to meet national milk demands is that the average milk yield per cow reported for Bangladesh is extremely low. On average, a Deshi cow produces around 206 kg/year (Ali and Ali, 2003), which is less than 30 percent of the production of Indian cows. The main reasons for low cow milk productivity are low genetic potential, feed shortage and high incidence of disease. This low productivity could be improved with proper and efficient planning of the whole industry, and would require consistent and objective breeding decision. The yearly milk production (million metric tonnes) of Bangladesh from 1994 to 2003 is presented in Figure 3.8. Bangladesh has seen a slight improvement in milk yields over the period from 2000 to 2005 (FAO,

2006). This increase is due mainly to the importation of temperate germplasm and keeping them for 2 to 3 lactations and also to the importation of live animals from India. The majority of animals, which are local cattle breeds, increased their milk yield by around 5 percent over this period, while the milk yield of crossbred cows increased by 4 to 8 percent (DLS, 2002).

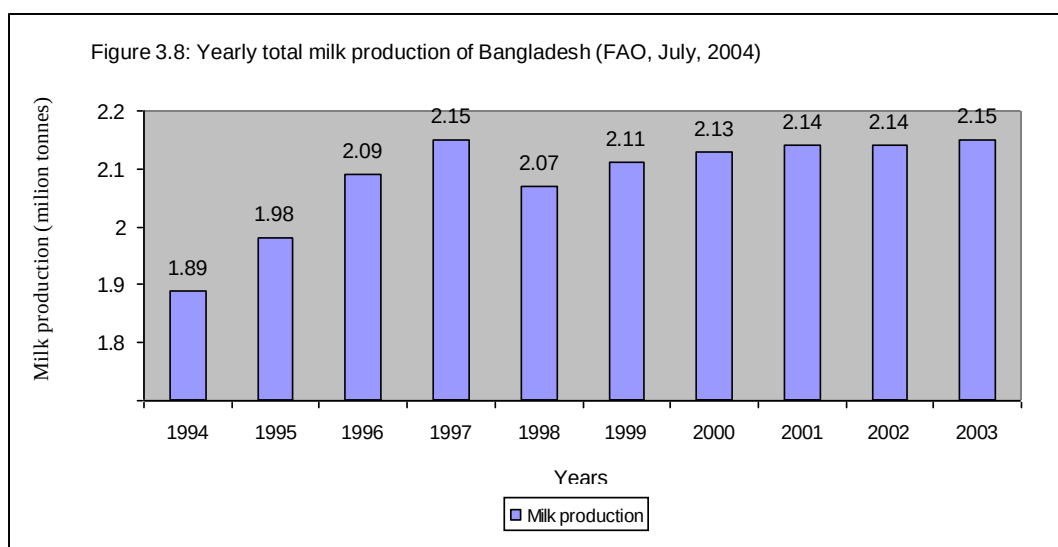


Figure 1: Yearly milk production in Bangladesh

About 64% of the country's milk is cattle milk, with goats contributing 35% and buffalo 1% to national milk production (Hemme *et al.* 2004) Bangladesh produces 0.35% of the total world milk production. This represents about 8% and 2% of the milk production of Pakistan and India respectively, or 1.75% of the milk production of South Asia.

2.3 Crossbreeding

Crossbreeding experiments have been conducted on government dairy farms in Bangladesh since 1970 and consequently, both purebreeding and crossbreeding systems are being used by dairy farmers (Ahmed, 1985). The majority of cattle in Bangladesh are zebu type (*Bos indicus*), but the present cattle improvement programme seeks to improve indigenous cattle by utilising both tropical and temperate breeds.

2.3.1 Productive and reproductive performances of crossbreds

The productive and reproductive performance of different genotypes of cattle in Bangladesh, has been studied by a number of researchers (Ahmed and Islam, 1987; Nahar *et al.*, 1992; Hirooka and Bhuiyan, 1995; Islam and Bhuiyan, 1997; Bhuiyan *et al.*, 1998; Majid *et al.*, 1998; Khan and Khatun, 1998; Khan *et al.*, 2000; Hossain *et al.*, 2002; and Islam *et al.*, 2004). These authors collected productive and reproductive performance records from governmental, cooperative and private dairy farms in Bangladesh. Data was analysed using statistical models suggested by Steel and Torrie, (1980) and Steel *et al.* (1997) to compare the performances of various genetic groups of animals. A summary of the results is presented in Table 3.1. Local crossbred cows were reported to have lower milk production, longer calving intervals, and later sexual maturity compared to Holstein-Friesian (HF) and HF crosses. However, low survivability of temperate breeds (e.g. Ayrshire) in tropical environments has been reported relative to crosses of tropical breeds like Sahiwal and Red-Sindhi with local cattle (McDowell, 1985; Cunningham and Syrstad, 1987; Rege *et al.*, 1998; and Kahi *et al.*, 2000).

2.4 Sign symptom of heat in cattle

More than 90 percent of cows should show heat by 50 days postpartum. Cows should cycle every 21 days by that time. The most reliable sign a cow is in heat is standing to be mounted by a herd mate. Each stand lasts only 4 to 6 seconds. Cows average about 1½ mounts per hour and are in heat 6-8 hours. Therefore, cows are only in heat a little more than a third of a day and only spend a total of 3 to 5 minutes actually standing to be mounted. It is easy to understand why cows must be observed for heat several times daily. Also, producers should monitor secondary signs of heat. These include: * mounting other cows * clear mucous discharge * chin resting and rubbing * swollen red

vulva, frequent urination muddy flanks and ruffled tailhead bawling, restlessness, sniffing behavior decreased milk production and off feed these indicators may signal that a cow is in heat, coming into heat or going out of heat. However, base the decision to inseminate on standing heat, not on secondary signs of heat.

2.5 Technique of heat detection

There are several methods for estrous detection that are given below

2.5.1 Visual observation and tail-painting

The most common used method of oestrus detection is visual (personal) observation. The highest detection rates can be achieved with between two (Xu *et al.*, 1997) and five (Mee, 2004) 20–30 minutes observation sessions per day. One of most important factors which affect the heat detection efficiency is by Diskin and Sreenan (2000) that a head manager responsible for heat determination should be maximally familiar with all the signs of heat detection and be fully prepared as long time as it is possible. But this method incurs high labor costs and is tedious. Several methods have been used to facilitate detection of estrus; these methods have focused on physiological (Carter and Duffy, 1980), behavioral (Gwazdauskas *et al.*, 1990), and physical (Kiddy, 1977) signs that are associated with oestrus. Xu *et al.* (1997) stated that is possible using tail painting, where a strip of paint is applied over the tail head. The paint strip disruption shows possible mounting activity. Foote (1975) and O’Farrell (1980) contended that the paint or chalk application to the tailhead of cows is effective in satisfactorily indicating cows in estrus. In situations that cows are mounting, it is common that the strip of paint is either partially or totally removed. According to this finding Diskin and Sreenan (2000) recommend a combination of early morning and late evening observations and checks for paint loss during milking times, what should result in 90% success of heat detection.

2.5.2 Progesterone in milk and blood

Progesterone is the hormone of the corpus luteum and if it occurs in a mating season, that the insemination shouldn't be considered (Strapák *et al.*, 2013). Changes in hormone levels especially in progesterone content have a very strong correlation with oestrus. The progesterone level can be easily measured from milk or blood plasma. The content of progesterone in milk depends highly on the level of milk fat. Based on study of Döcke (1994) a sharp decline of progesterone from > 10 to < 3 ng.ml⁻¹ in milk indicates when pro-estrus starts. Changes in progesterone levels don't occur at first oestrus after calving. In 1984, Boyd reported that collection and labelling of progesterone samples is difficult to perform. Progesterone measurements were in the past mainly used with various oestrus detection methods (Schofield *et al.*, 1991; Stevenson and Phatak, 1999). But recently, different biochemical methods with sensors have been developed and tested in practice (Koelsch *et al.*, 1994; Delwiche *et al.*, 2001a, 2001b; Mottram *et al.*, 2001). The estrus detection ability of the biosensor and pregnancy in cows was proved by Mottram *et al.* (2001). Delwiche *et al.* (2001a) were able to detect 100% of oestrus by use of a biosensor, while of course some error was found with false detections of oestrus on level of 26.3%.

2.5.3 Ultrasonography

Braun (1997) described, that the practical methods for searching the mating season is also include ultrasonography, which are characterized as a modern noninvasive method using the principle of pulse-echo ultrasound waves. Sonography provides a very objective and immediate information. Ultrasound is used in cows to detect ovarian follicle maturation, for monitoring the physiological changes in the uterine endometrium and the whole cycle and during sexual puerperium, in the diagnosis of early-estate, and the like.

2.5.4 Vaginal (cervical) secretions

Next method of detection of estrus is the measurement of resistance of vaginal or cervical secretions. Most of the hormonal changes directly affect the electrical resistance of vaginal mucus during oestrus. The lowest level of vaginal mucus resistance is 25 hours before ovulation (Boyd, 1984). Vaginal mucus resistance is a parameter well suited for oestrus detection (Erasmus *et al.*, 1992) because it indicates strong correlation with the whole oestrus cycle (Boyd, 1984). Leidl and Stolla (1976) stated, that the intravaginal measurement of vaginal resistance and the cervical secretions looks like a useful tool to determine oestrus objectively. The authors reached pregnancy of 82% in 874 dairy cows that were inseminated by the application of a vaginal probe and detection of resistance mucus below 30 ohms. On contrary Brehme *et al.* (2001) and Brehme and Brush (2002) found this device unsatisfactory. Practical application is complicated because of difficult, time-consuming manual measurements with the risk of inflammation (Roth *et al.*, 1987; Erasmus *et al.*, 1992). Further reasons for the reduction of electrical resistance measurements are cysts or ulcerous inflammations (Boyd, 1984).

2.5.5 Locomotion activity during estrus

The most typical sign for an oestrus is the toleration reflex. Brehme *et al.* (2008) reported that the period of time between first and last on-jump is on an average of 7.1 hours. During this time period on an average 8.5 on-jumps in duration of about 4 seconds are tolerated. The altered behavior patterns of cows during estrus indicate an increased physiological activity as an expression of searching for a compatible mating partner (Kerbrat and Disenhaus, 2004). The movement activity increased and an amplified restlessness resulting from period of estrus are the essential symptoms of the mating season. The average activity rising at the time of the mating season varies from 30 to

93% (Kiddy, 1977; Eradus *et al.*, 1992; Kerbrat and Diesenhaus, 2004).

2.5.6 Heat mount detectors

As the primary and most obvious sign of heat is standing to be mounted. A lot of research laboratories and centers tried to develop pressure sensitive devices that measure standing (mounting) activity. These devices are mostly connected on the top of tail part of the cows and if pressure is applied by the weight of the mounting animal the indicators as intensity or color of the device is changed. The system mingles the location of a pressure sensitive battery powered transmitter on the cows tail head. The device is activated by mounting cow and emits a radio signal which is picked up by either a repeater or receiver and relayed to a personal computer where the information is stored and digitized for use. The time, date and duration of each mount along with the identity of each cows is recorded. From this data the exact time of heat onset is calculated (Diskin and Sreenan, 2000). HeatWatch is another small device which use digital radio transmitters incorporating a pressure switch which are glued on the tailheads of cows. These transmitters continuously monitor all mounting activity of cattle and transmit the mounting data to computer where advanced software algorithms examine the mounting profile of each animal. The software is looking for mounting criteria known to be indicative of true oestrus (HeatWatch, 1992). ShowHeat is also a simple but effective mount monitor. The device has only a single light that is activated when the cow receives at least three mounts. The number of light flashes in a can be counted to calculate when the onset of estrus occurred and insemination can be performed accordingly. The light will continue to flash for up to 18 hours after the onset of heat. Rorie *et al.* (2002) stated, that there mount monitors were found to be equally effective in detecting estrus. According to Stevenson and Phatak (1999) the efficiency of heat detection using these heat mount detectors vary from 56 to 94%. Also the accuracy of estrus detection vary

from 36 to 80%.

2.6 Artificial insemination

Artificial insemination was the first reproductive technology applied to cattle, initially in Russia and Denmark during the early 1900s (Ivanoff, 1922; Perry, 1945). The primary driving force behind AI was its potential to increase the rate of genetic gain in livestock populations by widespread use of sires with elite genetic merit. Cooperative AI centers began in Denmark in 1936 and were replicated internationally (Perry, 1945). Not since the invention of the milking machine has a technology had such an effect. For farmers, transition away from natural service breeding required changes in herd reproductive management. In addition to genetic gains, AI breeding avoided the need to have bulls on each farm and contributed to improved safety for farm employees. Today, use of AI has grown to the extent that internationally approximately 130 million cattle are submitted for AI annually (Vishwanath, 2003).

2.7 Developments in AI

Before the development of AI, cows were bred by natural service and bulls were often shared among farms. It was in Denmark in 1936 that the first large-scale bovine AI organization was established before similar organizations were established in the United States and worldwide (Perry, 1945). Some producers and breeders initially opposed the use of AI because early procedures for collection, handling, and insemination were cumbersome (Polge, 2007; Wilmot, 2007). The benefits of AI over natural service, however, soon became obvious. Artificial insemination was critical to increasing the reproductive potential of sires with elite genetic merit. The reduction in disease transmission between animals and the opportunity to evaluate sperm production and characteristics provided additional important benefits (Ivanoff, 1922; Perry, 1945).

Most important, AI enabled precise genetic evaluation of bulls via hundreds of thousands of daughters. Its growth during the past 80 yr to approximately 130 million inseminations internationally per year (Vishwanath, 2003) was driven by some key advancements, primarily in the collection, extension, and preservation of sperm.

2.8 Blood parameters in cattle

The prepartum period is regarded as important in the reproductive life of the cow because of its important effect upon future fertility (Fonesca *et al.* 1983; Oltenacu *et al.* 1983). It has been reported that the total blood leukocyte counts increase markedly on the day preceding the calving (Merrill and Smith 1954; Paterson 1957). Merrill and Smith (1954) found that lymphocyte and eosinophil numbers increased and neutrophils decreased while monocytes did not show any definite change up to 10 days postpartum. The mean blood total leukocyte and absolute blood lymphocyte counts as well as the neutrophil counts dropped sharply after parturition and then increased gradually toward the 20 days after parturition (Hussain and Danial 1992; Guidry *et al.* 1976). Saad *et al.* (1989) found that the number of blood lymphocytes decreased before and at parturition, returning to a higher level during the second week postpartum. Similar findings were made by the other investigators in cows. Regarding the mammary gland, extensive influx of neutrophils into the colostrums and milk occurs around calving and during the first week after calving (Guidry *et al.* 1976; Straub *et al.* 1959). Peripheral leukocyte count was not influenced by the reproductive state of cow; however, the mean neutrophil concentration was significantly different between the reproductive states. We could not find any significant effect of estrous cycle phases on heifers' blood erythrocytes, hemoglobin, hematocrit, leukocytes, neutrophils, lymphocytes, monocytes, and eosinophils in our previous work (Ahmadi *et al.* 2006). Johnson *et al.* (1990) reported that, during the estrous cycle in dairy cows, red blood cell count, hematocrit, and

hemoglobin levels did not show significant differences between different phases of the estrous cycle. While statistically significant differences were observed in white blood cell count, none of these were sustained over a period longer than 2 days. The increased neutrophil response induced by progesterone, both in normal cyclic and ovariectomized cows after progesterone treatment, may be compensated by the reduced neutrophil phagocytosis and bactericidal activity or the suppression of other uterine defense mechanisms. This response is inconsistent with the long-recognized observation that the uterus of the cow is more susceptible to infection in diestrus than in estrus (Subandrio and Noakes 1997).

CHAPTER 3

MATERIAL AND METHODS

3.1 Study Area

This study was conducted at different dairy farm in Gopalganj, Sadar, Dinajpur.

3.2 Study Period

From 15 January 2024 to 15 June 2024

3.3 Study Collection

Total 15 cows were collected from dairy farm in Gopalganj, Sadar, Dinajpur.

3.4 Experimental Layout

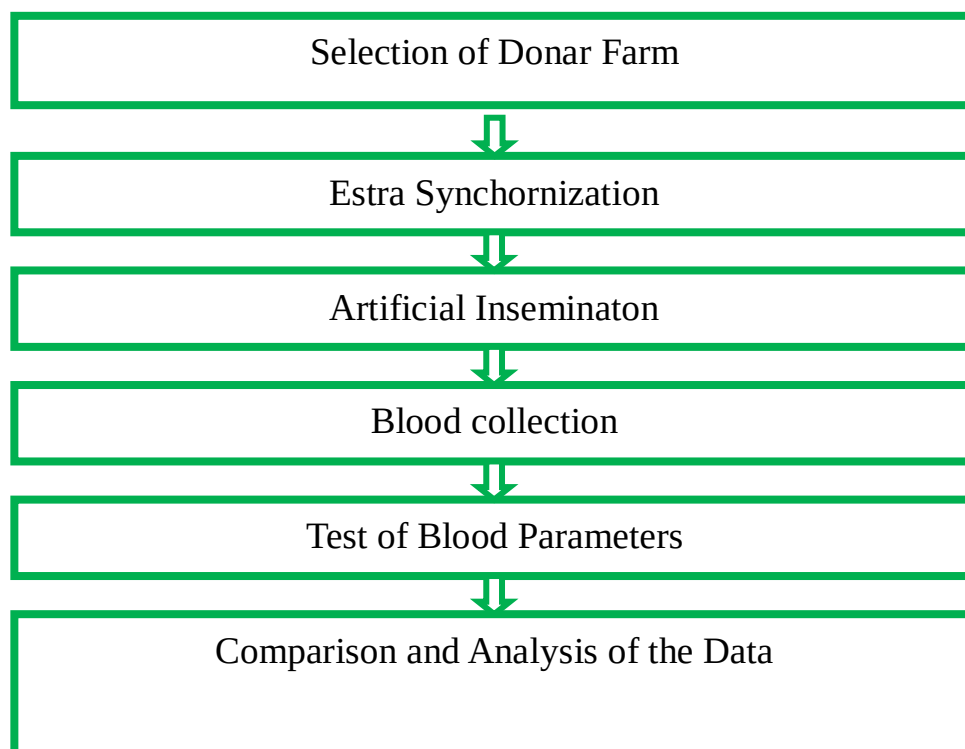


Figure 2: Experimental Layout

3.5 Selection of Donar Farm



Figure 3: Dairy Farm

3.6 Estrous Synchronization

Cows assigned for this study received the Ovsynch protocol. Two administrations (im) of 10 mg of a GnRH analogue (2.5 mL Gonavet, Chemist Laboratories)) were given 7 days before and 48 hours after a 25-mg (im) PGF2a administration (2 mL Prostenol; Techno Drugs LTD.) followed by AI 16-24 hours after second GnRH administration (Azevedo *et. al.*, 2014).

3.7 Artificial Insemination

- Preparing the semen straw
- Preparing the breeding gun
- Inserting the loaded AI gun
- Depositing the semen

3.8 Artificial Insemination

3.8.1 Preparing the semen straw

- Using forceps, quickly removed the desired straw of semen from the goblet below the frost line.
- The straw shook well after removal from the semen tank and eliminated any liquid nitrogen at the end of the cotton plug.
- Thawing of semen straw.

3.8.2 Preparing the breeding gun

- The breeding gun was warmed by stroking vigorously with hand five or six times.
- The plunger pulled back about six inches to prepare for the semen straw. Placed the end with the cotton plug in the gun.
- The sealed end cut at a 90° angle about one-fourth inch from the end.
- The gun kept between body and shirt to maintain optimal temperature until AI.

3.8.3 Inserting the loaded AI gun

- Wiped the vulva and the underside of palpating arm clean of any manure with a paper towel.
- With left hand make a fist and press down directly on top of the vulva. This spread the vulva lips allowing easy access to insert the gun.
- The insemination gun inserted into the vulva upwards at a 30° angle.
- When the gun is 6 to 8 inches inside the vagina, the rear of the gun raised to level position and slide forward until it contacts the beginning of the cervix.

3.8.4 Depositing the semen

- After passing all the cervical rings, the gun slide forward freely with little resistance.

- Slowly deposited semen.
- Gently removed the gun and checked for any abnormal discharge.

3.9 Ultrasound examination of bovine pregnancy

During the research, an ultrasound examination was performed to detect and confirm pregnancy in cattle. For this purpose, transrectal ultrasonography (TRUS) was used. The examination was performed using a realtime B-mode ultrasound device (Dramiński USG iScan 2 MULTI) equipped with a 6–8 MHz linear endo-rectal transducer.

3.10 Blood sample collection at different day

About 10 ml of blood sample from each animal was collected at 0, 11th and 21st day from caudal vein of different dairy farm, Gopalganj, Sada, Dinajpur within EDTA tube and shifted to the Checkup Diagnostic Laboratory, Sadar, Dinajpur.



Figure 4: Blood collection

3.11 Estimation of Blood parameters

A Semi-auto biochemistry analyzer (Mindray, BC-5150) is a machine used to do blood parameters.

3.12 Statistical analysis

The means of hematological parameters in the different groups of studied cows were compared with each other by using the one-way analysis of variance (ANOVA). Repeated measure ANOVA was used to determine the statistical significance of the rising or falling pattern of means ($\pm$ SD) values of the consecutive days. Data were analyzed using the computer software SAS (Version. 9.1). Duncan's multiple range tests were used for detecting differences among means. The statistical significance level was regarded as $P < 0.05$.

CHAPTER 4

RESULTS

4.1 RBC Count (Million/cmm)

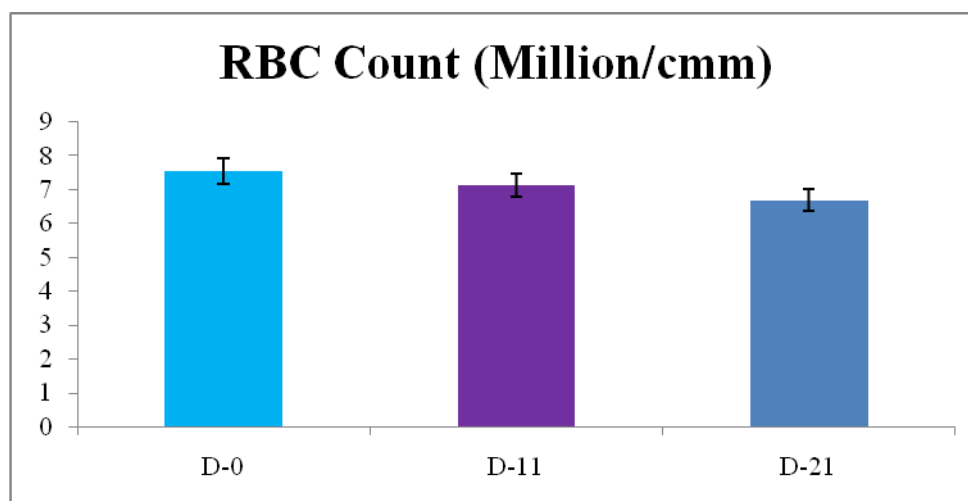


Figure 5: Changes in RBC Count (Million/cmm)

At 0 day the RBC count was higher than the values at 11th and 21st day. Data are presented mean $\pm$ SEM. Different letters denoted non-significant at 5% level.

4.2 Haemoglobin Test

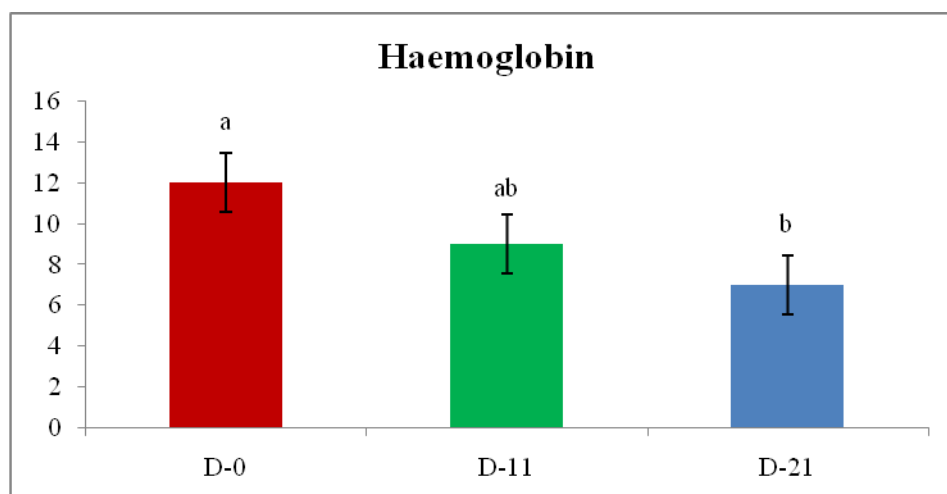


Figure 6: Changes in Haemoglobin (g/dl)

Blood haemoglobin were tested at 0, 11th & 21st days after AI. It showed that haemoglobin level were decreased significantly ($P < 0.05$). Data are presented mean $\pm$ SEM. Different letters denoted significantly at 5% level.

4.3 Packed Cell Volume (%)

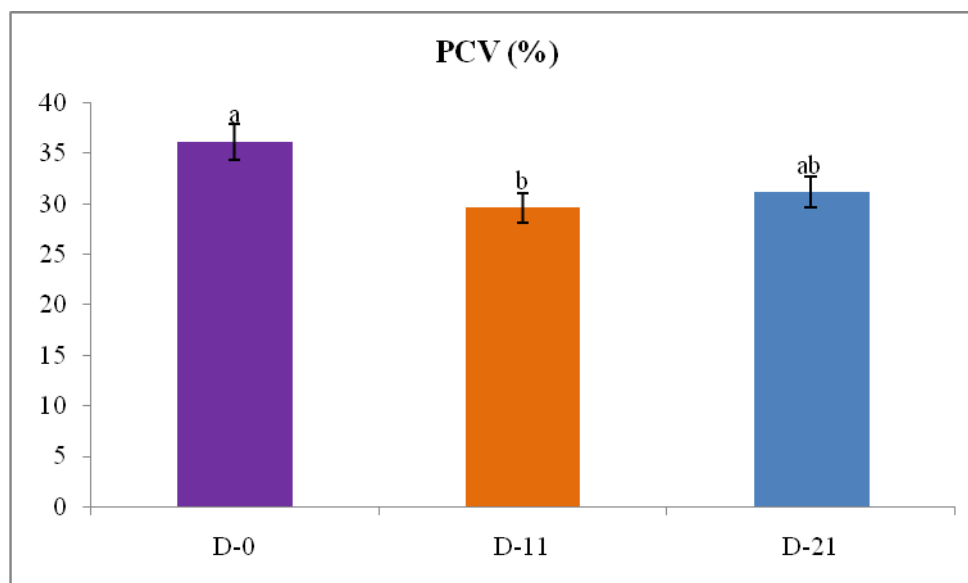


Figure 7: Changes in Packed Cell Volume (%)

The Packed Cell Volume (%) were significantly ($p < 0.05$) increased showed in the figure no 11. Data are presented mean $\pm$ SEM. Different letters denoted significantly at 5% level.

4.4 Erythrocyte Sedimentation Rate (mm/1st hr)

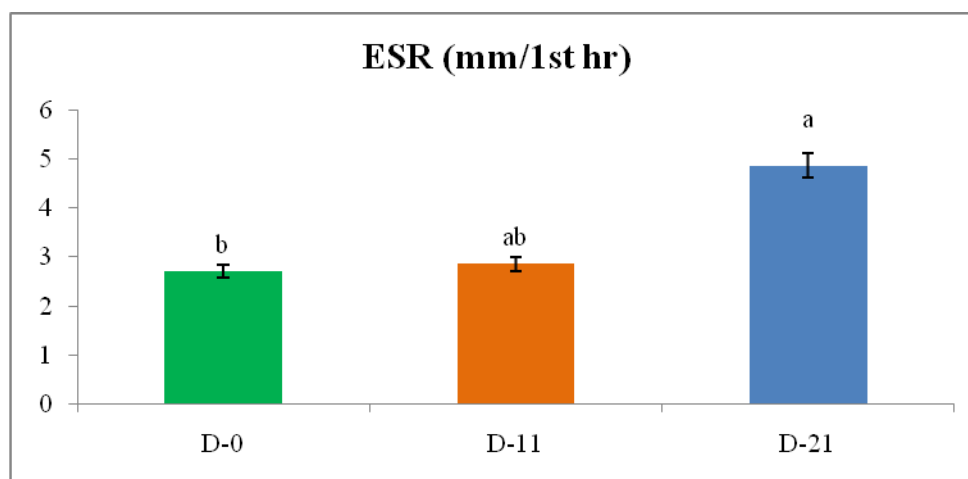


Figure 8: Changes in Erythrocyte Sedimentation Rate (mm/1st hr)

The Erythrocyte Sedimentation Rate (mm/1st hr) were significantly ($p < 0.05$) increased showed in the figure no 05. Data are presented mean $\pm$ SEM. Different letters denoted significantly at 5% level.

4.5 Mean Corpuscular Volume (fl)

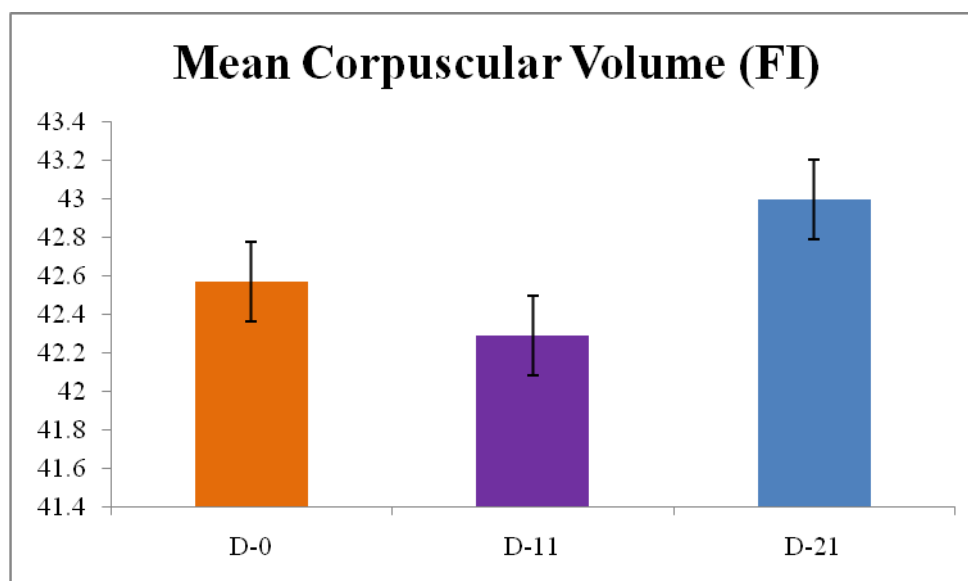


Figure 9: Changes in Mean Corpuscular Volume (fl)

At early pregnancy the Mean Corpuscular Volume (fl) was very close at 0, 11th and 21st day. Data are presented mean $\pm$ SEM. Different letters denoted non-significant at 5% level.

4.6 Mean Platelet Volume (fl)

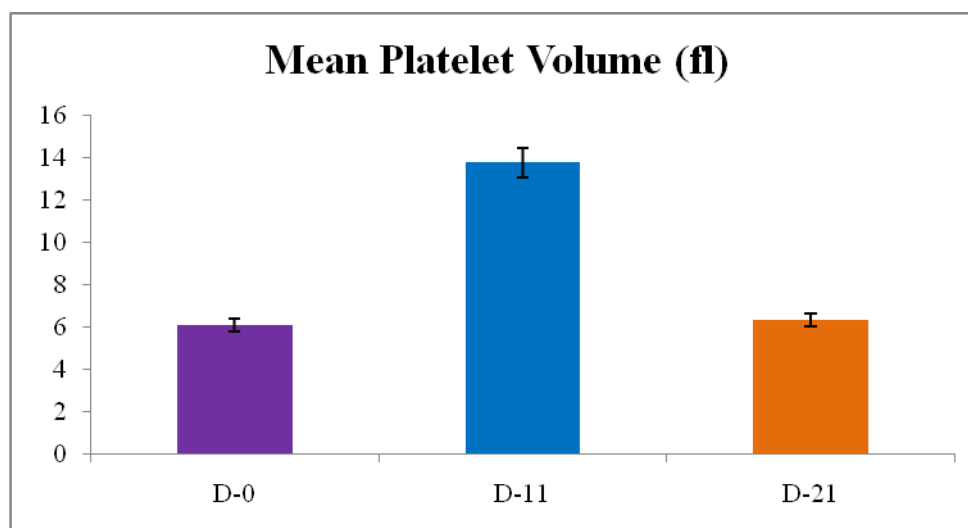


Figure 10: Changes in Mean Platelet Volume (Fl)

This study showed that PCV (fl) were increased non – significantly at 11th day after insemination. Data are presented mean $\pm$ SEM. Different letters denoted non-significant at 5% level.

4.7 Mean Corpuscular Hemoglobin Concentration (g/dl)

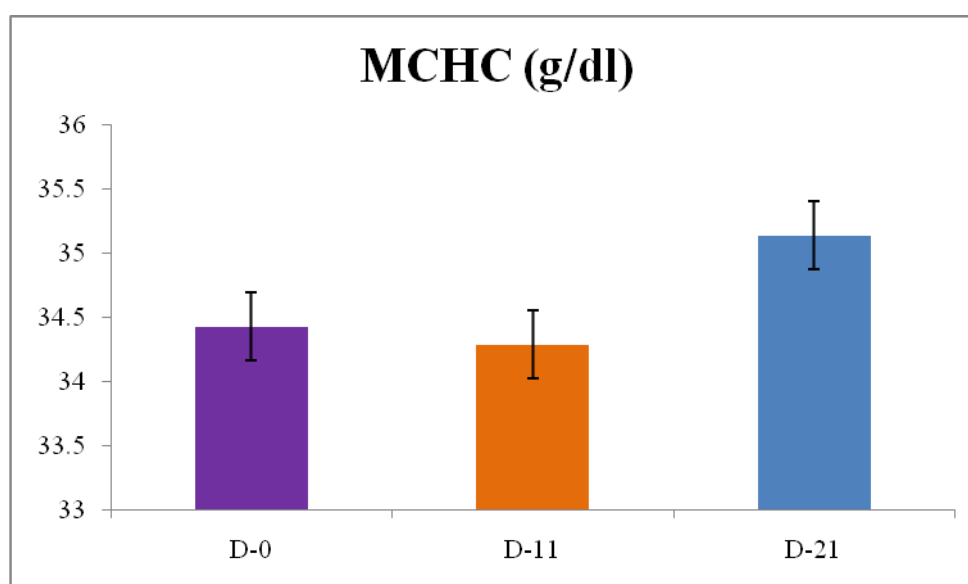


Figure 11: Changes in Mean Corpuscular Hemoglobin Concentration (g/dl)

Mean Corpuscular Hemoglobin Concentration (g/dl) was elevated at 21st day but the result was not significant. Data are presented mean $\pm$ SEM. Different letters denoted non-significant at 5% level.

4.8 Total WBC Count (/cmm)

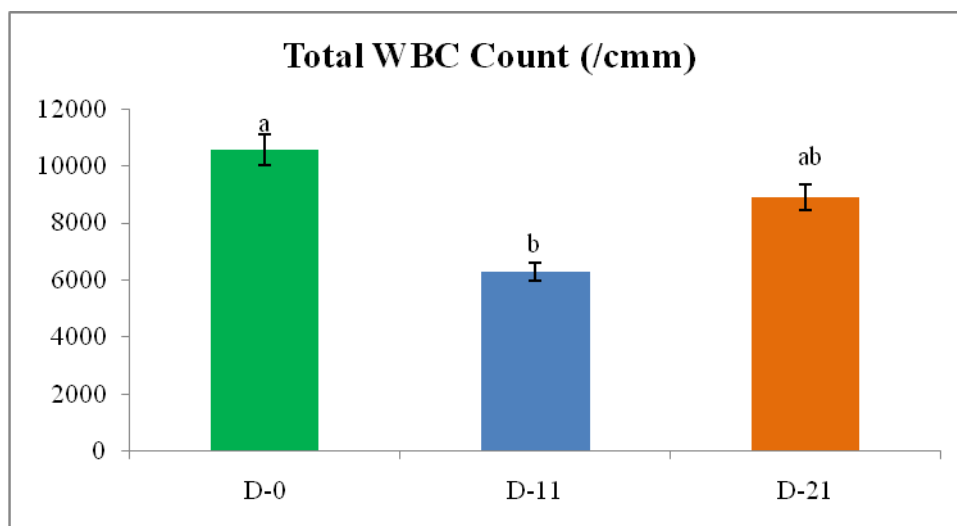


Figure 12: Changes in Total WBC Count (/cmm)

The recorded Total WBC Count of this study clearly showed that Total WBC Count (/cmm) were decreased at 11th day significantly ($p < 0.05$). Data are presented mean $\pm$ SEM. Different letters denoted significantly at 5% level.

4.9 Differential Leukocyte Count

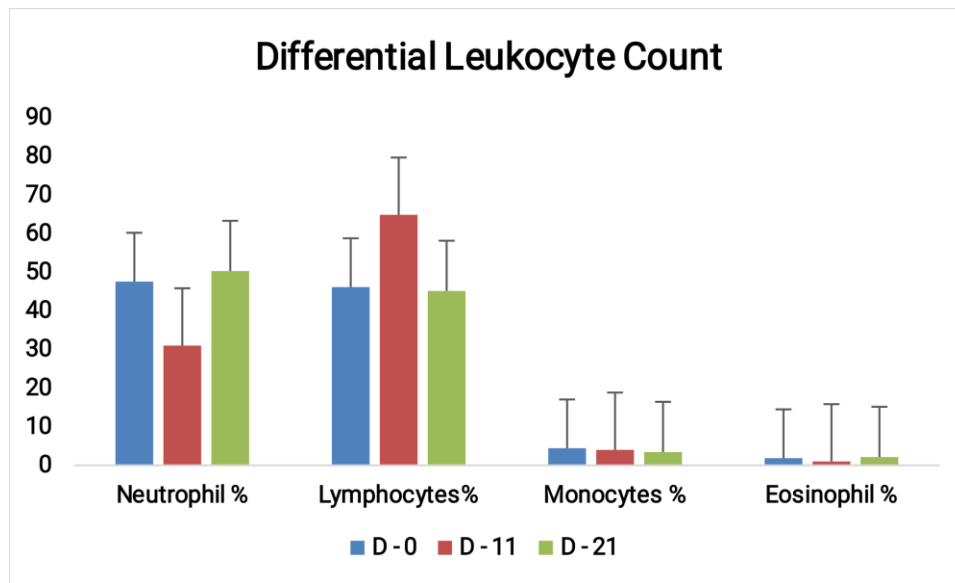


Figure 13: Changes in Differential Leukocyte Count

In this study we found that Neutrophil (%) and Eosinophil (%) significantly increased at 21st day. On the other hand, Lymphocyte significantly increased at 11th day. Monocyte (%) decreased slightly at 21st day than 0 and 11th day.

4.10 Platelet Count (/cmm)

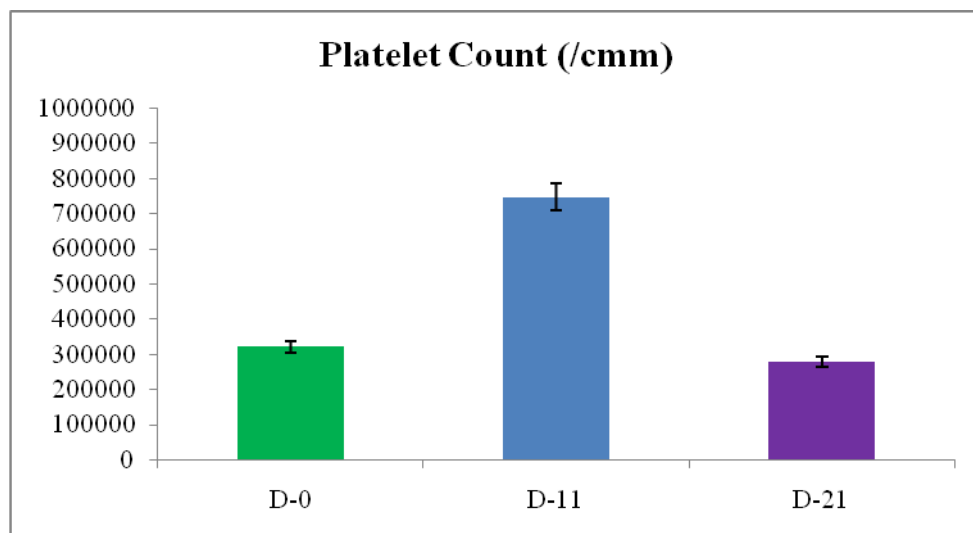


Figure 14: Changes in Platelet Count (/cmm)

From the hematological analysis, platelet count were increased at 11th day but the result was non-significant. Data are presented mean $\pm$ SEM. Different letters denoted no-significant at 5% level.

CHAPTER 5

DISCUSSION

The research aimed to determine the hematological profile of dairy cows at times up to the 0, 11th and 21th day of pregnancy. In the current literature, the hematological profile of cows up to this stage of pregnancy has not been studied. The conducted research showed differences in the hematological indices depending on the physiological condition of cows. Significant differences were found mainly in the content of Total WBC, ESR, Lymphocyte, Neutrophil, Monocyte, and PCV in pregnant cows. Examination of haematological parameters is the basis for monitoring the health and welfare of animals and is of great practical importance in the selection of animals and the assessment of their health condition, adaptation and resistance to stress, and their production and reproductive potential (Barkakati *et al.*, 2020, Coroian *et al.*, 2017). Blood, a connective tissue responsible for transporting nutrients, minerals, vitamins, enzymes and hormones, is involved in maintaining the physiological state, and its composition reflects the physiological state (Coroian *et al.*, 2017). The values of the typically investigated parameters are influenced by various factors, including age, nutrition, quality of conditions in which an animal is kept, lactation and pregnancy, which commonly lead to changes in these values (Oliveira *et al.*, 2019, Saeed *et al.*, 2021). According to Saeed *et al.* (2021) heat stress also affects blood parameters in animals. The optimum ambient temperature for cows is 5 to 25°C. Above this range, it is more difficult for cows to thermoregulate (Bragança *et al.*, 2018). During heat stress, animals consume less feed to maintain optimal body temperature, and stress of this kind can have a negative effect on the success of fertilization and embryo viability (Abud *et al.*, 2016). Changes in the content of white blood cells may result from the migration of lymphocytes to the uterine membrane to protect against the penetration of pathogenic

microorganisms and the development of infections. Moreover, it has been reported that such migration of blood morphotic elements allows for tolerance of the foetus by the mother (Velázquez *et al.*, 2019). In determining pregnancy and foeto placental development, steroid hormones, interferon-tau (IFNT), cytokines from endometrial tissues, and immune cells perform the primary functions (Ott *et al.*, 2020). Fluctuations and changes in mechanisms cause pregnancy loss at up to 30 days of gestation in dairy cattle. The secretion of interferon-tau between days 15 and 18 of gestation in ruminants is vital for maintaining the corpus luteum, progesterone production, and supporting embryo growth, therefore disturbances in IFNT secretion can lead to miscarriage. In addition, when pregnancy is established, the content of monocytes and dendritic cells within the endometrial stroma increases as early as around the 14th day of pregnancy (Ott *et al.*, 2020). The increase in monocyte content occurs in early pregnancy and may be associated with a response to increased expression of monocyte chemoattractant (CCL2 and CCL8) and migration to the uterine endometrium in early pregnancy (Yang *et al.*, 2017). In the endometrium, monocytes differentiate into dendritic cells that present the antigen to T-lymphocytes (Ott *et al.*, 2020). This activation is involved in the formation of the placenta in cattle. In ruminants, modulation of the immune system is noted in the uterus and throughout the body during the peri-implantation period (Toji *et al.*, 2022). The contribution of T-lymphocytes during embryo implantation has been described, and the total leukocyte content has been investigated between days 33 and 34 of gestation in cattle. An increase in the content of lymphocytes during pregnancy was found and was associated with the T-helper anti-inflammatory response (Yang *et al.*, 2017). Granulocytes are presumed to be possible mediators of embryonic signals and are related to the level of expression of their genes as biomarkers for early pregnancy (Nazifi *et al.*, 2008). Increased granulocyte content may be induced by increasing levels of

progesterone (Koreyba *et al.*, 2019, Ott *et al.*, 2020). A decrease in the leukocyte content in the blood (leukopenia) is observed in animals that experience a reduction in production or supply of corticosteroids (Setiawati *et al.*, 2021). In turn, the increase in the number of leukocytes and neutrophils is caused by inflammatory reactions in the early stages of pregnancy that occur during the implantation of the embryo in the uterus (Abramowicz *et al.*, 2019, Oliveira *et al.*, 2019). In addition, an increase in leukocyte content can also be caused by summer parasitic infections and chronic viral infections (Setiawati *et al.*, 2021). Also, these changes may be caused by disturbances in well-being, i.e. stress and pain, which favour more leukocytes in the bloodstream (Oliveira *et al.*, 2019). White blood cells (lymphocytes, monocytes, neutrophils, eosinophils and basophils) may be higher in summer because high temperatures stimulate the release of epinephrine and corticosteroids which increase the number of white blood cells (Mekroud *et al.*, 2021). The authors' research showed statistically significant differences at the level of $P \leq 0.01$ in the leukocyte content between pregnant cows up to the 45th day and those between the 45th and 50th days and differences at the level of in heifers and in cows at different stages of pregnancy. However, there were no higher values of WBC than the reference values for blood parameters of dairy cows (Astuti *et al.*, 2021, Oparina *et al.*, 2021).

The PCV was decreased significantly on 3 day before calving which was increased non-significantly on the day of calving. After calving PCV reduced significantly on 15 days post-partum as compared to the day of calving. Reynolds, (1953) reported that PCV remained stable until the last week of pregnancy when there was a marked increase and then returned to normal soon after parturition. During this study PCV had stable result observed. In the present study, the higher PCV was observed on 7 day before and on the day of parturition. It might be because of the prolonged refusal to drink water and loss of

fetal fluids due to parturition. Higher PCV around immediately before calving may indicate the requirement of higher red cell volume to carry more oxygen to meet the energy requirement of tissues at the time of calving (Jain, 1986). Nazifi *et al.*, (2008) also reported significantly higher PCV in pregnant cows just before calving as compared to postpartum period. Lymphocyte (%) of cattle lowest level of Lymphocyte was observed on 3 day before calving as compared to other pre calving days which was increased non-significantly on the day of calving. After parturition non-significant increase in lymphocytes was observed on 3, 7, 15 and 30 days after calving as compared to the day of calving. During present study, Lower Lymphocyte count was observed on 3 day before calving and on the day of calving. Klinkon and Zadnik (1999) observed the lower number of lymphocytes on before parturition; they were non-significantly lower than values obtained after parturition. Pomsel, (1980) recorded significantly lower mean absolute values of lymphocytes on the day of parturition in comparison to the first seven months of pregnancy and on the second week after parturition. The reduction in lymphocyte per cent at calving may be due to parturition stress and due to other stress hormone. Increasing level of stress hormone may be the reason for reducing the number of lymphocyte at calving (Kehrli *et al.*, 1989). Monocyte (%) of crossbred cattle ranged from. Monocyte percentage was increased non-significantly till 7 day before calving which was reduced significantly on the day of calving as compared to 7 day before calving. After parturition, significantly higher monocyte per cent was observed on 15 and 30 days of calving as compared with the day of calving. Klinkon and Zadnik (1999) reported that the number of monocytes was constant during pregnancy in healthy dairy cows, and decreased slightly in the last month of pregnancy and during parturition. In the present study, lower number of monocyte was observed on the day of calving. It might be due to migration of monocyte from circulation to tissues and undergoes to converting

macrophage to providing immunity at parturition stress. Neutrophil (%) of crossbred cattle which was significant Neutrophil per cent was increased significantly from 30 day before calving to 3 day before calving which is the highest level of neutrophil per cent observed during the study. A slight non-significant reduction in neutrophil per cent was observed on the day of calving as compared to 3 day before calving. After parturition sudden significant reduction in neutrophil per cent was found on 3rd day after calving. Eosinophil (%) of crossbred cattle. Highest level of eosinophil was observed on the day of calving which is significantly of calving which is significantly higher as compared to other pre-partum days. After calving percentage of eosinophil was decreased significantly on 3rd day of calving. Thereafter level of eosinophil continuously decreased till the end of study. Schalm and Jain, (1986) reported that Pregnancy did not affect the number of eosinophils. Whereas, Pomsel, (1980) reported a decrease in the mean absolute value of eosinophils on the day of parturition and a continued decrease through the first two weeks after parturition. Eosinophil had nonsignificant changes during study entire period except on the day of calving, which increased significantly Eosinophil had also important role to provide immunity. Due to the stress factor to provide immunity the percentage of eosinophils might be higher at the calving time. Basophil (%) of crossbred cattle. Basophil decreased significantly on 15th day after calving as compared to prepartum day. Thereafter significantly increased on 30th day after calving. The periparturient period did not affect the number of basophils. This finding supports work by Schalm and Jain, (1986). The present study did not reveal any significant effect of pregnancy on basophil number. uring pregnancy, the body undergoes several physiological changes to support the growing fetus. One such change involves an increase in plasma volume, which dilutes the blood and can lead to a rise in the erythrocyte sedimentation rate (ESR). Additionally, pregnant individuals often

experience elevated levels of fibrinogen, a protein involved in blood clotting, which can further contribute to an increase in ESR. These alterations in blood composition are normal during pregnancy and help ensure adequate oxygen and nutrient delivery to the developing fetus. In the context of blood, PLCC can refer to "Platelet Leukocyte Cell Count," which is a laboratory test used to measure the number of platelets and white blood cells that are closely associated or aggregated together. This test helps in the diagnosis and monitoring of certain medical conditions, such as infections, inflammatory disorders, and some forms of leukemia. An increase in Platelet Leukocyte Cell Count (PLCC) in the blood can occur due to various reasons, including infection: During an infection, white blood cells (leukocytes) can increase in number as the body's immune system responds to the invading pathogens. Platelets may also become activated and aggregate with leukocytes to help combat the infection, leading to an elevated PLCC. Inflammatory conditions: Inflammation triggered by conditions like arthritis, autoimmune diseases, or tissue injury can cause an increase in both platelet and leukocyte counts, resulting in a higher PLCC. Leukemia: Certain types of leukemia, such as chronic myeloid leukemia (CML), can lead to an increased PLCC due to abnormal proliferation of white blood cells and platelets in the bone marrow. Stress or trauma: Physical or emotional stress, as well as trauma or surgery, can stimulate the release of inflammatory mediators and activate platelets and leukocytes, causing an elevation in PLCC. Medications: Certain medications, such as corticosteroids, can stimulate the production or activation of platelets and leukocytes, leading to an increase in PLCC as a side effect. It's essential to consult with a healthcare professional for proper evaluation and diagnosis if there is a persistent increase in PLCC, as it could indicate an underlying medical condition that requires further investigation and management.

CHAPTER 6

CONCLUSIONS

The present study can be concluded that the Erythrocyte Sedimentation Rate (mm/1st hr), Packed cell Volume (%), Neutrophil (%), Eosinophils (%) and Mean Corpuscular Volume (fl) were significantly increased at 21st day after AI. The Lymphocytes (%) and Mean Platelet Volume (fl) were particularly increased at 11th day and Monocytes (%) increased at 0 day just after AI. In contrast, Hemoglobin (g/dl), Total WBC count (/cmm), Procalcitonin test (%), RBC count (Million/cmm) were significantly decreased at 21st day as compared with 0 and 11th days. Some blood parameters change in relation to pregnancy.

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