

IMPACT OF GLUCOSE LEVEL ON ESTROUS OF DAIRY COW

**A THESIS
BY**

**MD. RAHIBUL ISLAM
STUDENT ID. 1604123
SEMESTER: JAN-JUN, 2024
SESSION: 2022-2023**

**MASTER OF SCIENCE (MS)
IN
PHARMACOLOGY**



**DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY
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**DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY
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JUNE, 2024

Dedicated
To My Beloved
Parents

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ABSTRACT

The present study was conducted to observe the impact of blood glucose level on estrous condition in dairy cow. Blood samples were collected from fifty (50) Holstein Friesian crossbred cows aged 3-5 years with different estrous symptoms for the determination of blood glucose level, calcium level and phosphorus level. Among fifty estrous cows, around forty percentage (40%) had higher glucose level, forty percentage (40%) had lower glucose level and twenty percentage (20%) had normal glucose level and the average blood glucose levels (mmol/L) were 4.68 ± 0.07 , 2.38 ± 0.06 & 3.67 ± 0.04 in high, low and normal blood respectively. These glucose level had significant ($P < 0.05$) impact on the calcium and phosphorus levels. The lower level of glucose markedly decrease the phosphorus level and this lower level also significantly hampered the normal estrous symptoms. About 70% and 80% cows showed estrous condition which had higher and normal blood glucose level and normal calcium and phosphorus levels. But only 20% cow showed estrous condition which had lower blood glucose level and lower phosphorus level. Results showed that blood glucose level significantly affect ($p < 0.05$) the estrous and hamper ovary health. These results of the present study suggest that lower blood glucose level causes adverse effect on reproductive performance in dairy cow.

CONTENTS

CHAPTER	TITLE	PAGE NO.
	ACKNOWLEDGEMENT	i
	ABSTRACT	ii
	CONTENTS	iii
	LIST OF TABLES	iv
	LIST OF FIGURES	v
	LIST OF PHOTOS	vi
	ABBREVIATIONS	vii
I	INTRODUCTION	1-4
II	REVIEW OF LITERATURE	5-12
III	MATERIALS AND METHODS	13-15
	3.1 Study area & duration	13
	3.2 Experimental layout	13
	3.3 Study population	13
	3.4 Blood sample collection	14
	3.5 Estimation of blood glucose level	14
	3.6 Estimation of Ca & P	15
	3.7 Statistical analysis	15
IV	RESULTS	16-20
	4.1 Blood glucose level	16
	4.2 Relation of blood glucose level and calcium	17
	4.3 Relation of blood glucose level and phosphorus	18
	4.4 Effect of blood glucose level on estrus	19
	4.5 Effect of phosphorus level on estrous cow	19
	4.6 Effect of glucose level on calcium & phosphorus	20
	4.7 Association of blood glucose level, calcium level, phosphorus level & estrous	20
V	DISCUSSION	21-22
VI	CONCLUSION	23
	REFERENCES	24-29

LIST OF TABLES

TABLE NO.	TITLE	PAGE NO.
1	Blood glucose level	16
2	Relation of blood glucose level and calcium	17
3	Relation of blood glucose level and phosphorus	18
4	Association of blood glucose level, calcium level, phosphorus level & estrous	20

LIST OF FIGURES

FIGURE NO.	TITLE	PAGE NO.
3.1	Experimental layout	13
4.1	Blood glucose level	16
4.2	Relation of blood glucose level and calcium	17
4.3	Relation of blood glucose level and phosphorus	18
4.4	Effect of blood glucose level on estrus	19
4.5	Effect of phosphorus level on estrous	19
4.6	Effect of glucose level on calcium & phosphorus	20

LIST OF PHOTOS

PHOTO NO.	TITLE	PAGE NO.
3.1	Study farm	14
3.2	Collection of blood sample from animal	14
3.3	Collected blood sample	14
3.4	Estimation of blood glucose level	15

ABBREVIATIONS

Abbreviation	: Full meaning
%	: Percentage
@	: At the rate of
+	: Plus
<	: Less than
>	: Greater than
±	: Plus minus
+ve	: Positive
-ve	: Negative
Av.	: Average
BPDs	: Blood Parasitic Diseases
Contd.	: Continued
dl	: Deciliter
DLS	: Department Of Livestock Services
etc	: More like same category
e.g.	: For example
EDTA	: Ethylenediamine tetraacetic acid
ELISA	: Enzyme-linked immunoassay
Fig.	: Figure
i.e	: That is
HSTU	: Hajee Mohammad Danesh Science and Technology University
gm	: Gram
mg	: Miligram

CHAPTER I

INTRODUCTION

More than half of the people in Bangladesh work in agriculture and livestock farming. People in Bangladesh live on dairy products, which are an important part of farming systems. Dairy products help people get enough protein in their food, which helps the country's economy grow and reduces poverty in both rural and urban areas of the country (Hamid and Hossain *et al.*, 2014). In Bangladesh, about 20 % of the people fully and 75% of the rural people depend on livestock for their livelihood (Uddin *et al.*, 2021). Daily milk consumption rose from 22gm in 1983 to 32gm in 2005, while meat and eggs consumption went up from 10gm to 18g (Jabbar *et al.*, 2017). People of all ages eat a lot of dairy products that are very healthy. Dairy products in our diet give us more nutrients like protein, fat, carbs, vitamins and minerals than any single food (Hossain *et al.*, 2016). People in Bangladesh have traditionally eaten a lot of dairy products in their food. Cream, curd, Chana, butter, and butter oil are all dairy products that aren't the same as other dairy products (ghee). Cream is the part of milk that is rich in fat. It has at least 18 percent milkfat and usually has 18 to 80 percent milk fat. It is called malai or sar by people who live there. It is used to make butter, butter oil (ghee) and ice cream. Milk production in Bangladesh in 2019 was 10.47 million tonnes (IDRN, 2020).

In Bangladesh, there is a lack of accurate data on the prevalence and monetary costs associated with reproductive system disorders. However, the majority of cows are slaughtered due to infertility. Malnutrition, venereal illnesses, parasitic infections, other infectious disorders, hormonal imbalance, and other factors belong to the most often stated reasons of infertility.

Reproductive performance is one of the most essential factors affecting dairy farm profitability and the improvement of national economy, as well as the living standard of rural and urban societies, Because, it directly or indirectly impacts the yield of milk, reproductive culling rate and the cost for breeding and calf sales (Plaizier *et al.*, 1998). To enhance economic efficiency, dairy cows should only calve once per year. Cow fertility has decreased in current decades in cows that have been heavily selected for milk production; however, because cow fertility is a multi-factorial criterion and its degradation has been caused by a network of genetic, environmental, and managerial factors, it is difficult to pinpoint the precise cause of this decline. (Walsh *et al.*, 2011).

Nutrition plays a crucial role in regulating reproductive hormones and follicular development in cattle. This is visible particularly during the time of negative energy balance at the onset of milk production after calving. Here, elongated periods of anovulation have been observed, resulting from alterations in luteinizing hormone concentrations, likely caused by lower glucose and insulin concentrations in the blood. The mechanisms that result in a reduced fertility are not completely understood, although a close relationship to the glucose-insulin metabolism is widely supported (Omari *et al.*, 2020).

For ruminants, the energy content in feed cannot be increased without limits due to the fermentative character of the digestive system (Omari *et al.*, 2020). High energetic feed with little fiber leads to an imbalance of microbes, rumen acidosis, and may even cause severe illness and death. Nevertheless, targeted feeding strategies are able to extenuate the negative energy balance (NEB) and to ensure animal health and welfare (Walsh *et al.*, 2011; Roche *et al.*, 2006 & Janovick *et al.*, 2011).

A number of experimental and clinical studies were performed to examine the relationship between the metabolic status and the fertility of cows, both, in qualitative and in quantitative manners, e.g., (Diskin *et al.*, 2003 & Rawan *et al.*, 2015]. Reduced nutrition intake was observed to delay the onset of puberty in beef heifers (Short *et al.*, 1971; Day *et al.*, 1986 & Yelich *et al.*, 1995), to change the growth pattern of the dominant follicles (maximal diameter, persistence, number of follicular waves) (Murphy *et al.*, 1991) and to increase the period to conception postpartum (Selk *et al.*, 1998). Studies in the postpartum period of dairy cows showed that the NEB is strongly correlated with low concentrations of glucose, insulin and IGF-1 in the blood (Lucy *et al.*, 2008). Changes in the secretion of gonadotropins, caused by low glucose levels, lead to low FSH and LH concentrations (Butler *et al.*, 2003), whereby missing LH peaks cause anovulation (Walsh *et al.*, 2012). Non-regular estrous cycles are often associated with low average concentrations of insulin in the blood (Garnsworthy *et al.*, 2008). On the other hand, it was reported that good feed management, e.g., nutritional manipulation that causes increased insulin, reduces the incidence of non-regular estrous cycles (Gong *et al.*, 2002).

Pregnancy per AI (P/AI) in lactating dairy cows has decreased relative to those recorded more than 50 years ago (Stevenson *et al.*, 2008). Changes in the physiology and

management of dairy cattle seem to contribute to the decrease in fertility (Royal *et al.*, 2002; Chagas *et al.*, 2007). A tremendous demand exists for nutrients during lactation and the associated severity of negative energy balance during early lactation has been one mechanistic explanation for lower fertility in dairy cows (Beam and Butler *et al.*, 1999). Cows with greater NEFA are presumably mobilizing more adipose tissue to support milk production and are losing more body condition compared with cows with lesser NEFA (Beever *et al.*, 2006). Cows with NEFA and BHBA concentrations in blood above a critical threshold can be predicted to have a greater incidence of diseases such as ketosis and displaced abomasum (Ospina *et al.*, 2010a) These diseases were associated with reduced postpartum reproductive performance (Ospina *et al.*, 2010c). In addition to fat mobilization in early postpartum cows, liver gluconeogenesis increases to provide glucose for milk lactose synthesis (Bauman and Currie *et al.*, 1980). The large demand for glucose may decrease the amount of glucose available to other tissues in the body, including those that are involved in postpartum reproduction (Wathes *et al.*, 2011; Green *et al.*, 2012). Follicles that are growing shortly after calving reach ovulatory size when cows are 60 to 90 d postpartum (approximate time of first insemination; Webb *et al.*, 2003). The integrity of follicles and their resident oocytes may be compromised in postpartum cows because extreme concentrations of circulating hormones and metabolites can potentially affect their function (Britt, 1994; Leroy *et al.*, 2008a,b). At the same time, the uterus is undergoing involution and repair, a process that is controlled in part by immune cells that are suppressed in postpartum cows (LeBlanc, 2010). Cows that develop metritis have lesser DMI during the prepartum and postpartum periods, and metritis is a risk factor for infertility in dairy cows (Gumen *et al.*, 2011). Nutritional events that occur during the periparturient and postpartum periods, therefore, can alter ovarian, oviductal, and uterine function and potentially affect subsequent fertility in the postpartum cow. We hypothesized that cows that failed to cycle or failed to conceive to first insemination would have postpartum blood NEFA and glucose concentrations that indicated a greater severity of negative energy balance. The objective of this study, therefore, was to assess blood NEFA and glucose relative to the cycling and first service pregnancy status of the cow.

However, animals require minerals such as calcium (Ca), magnesium (Mg), and phosphorus (P) during late pregnancy and the postpartum period for growth, reproduction, and lactation, which often affect specific requirements and serve as

catalytic components of enzymes or regulate several mechanism involved in pregnancy and lactation (Al-Khayat *et al.*, 2017). For instance, adequate level of glucose during pregnancy in dairy cows is crucial for the growing fetus, reproductive performance, and milk production (Noya *et al.*, 2019). Glucose regulates the concentration in pregnancy causes repeat breeding and reduces the conception rate (CR) (Ahmed *et al.*, 2017). Many protein-related parameters are highly affected around parturition and may significantly impose to further reproductive performance (Kurpińska *et al.*, 2016).

Blood glucose levels can indirectly affect fertility in dairy cattle. Low or high blood glucose levels can be indicative of metabolic imbalances or diseases such as ketosis, which can reduce fertility. Dairy cows with chronic high blood glucose levels, often associated with obesity and insulin resistance, can experience disruptions in their reproductive cycles and decreased fertility. Monitoring blood glucose levels can help identify and manage metabolic disorders that may impact fertility. Heat stress is a major concern in dairy cattle production, especially in regions with hot and humid climates. Heat stress can lead to reduced fertility in several ways like elevated body temperature can lead to reduced estrus expression and decreased conception rates. Heat-stressed cows may have altered hormonal patterns, leading to irregular or delayed estrus cycles. Heat stress can reduce feed intake, leading to energy imbalances, which can affect reproductive performance. Cows under heat stress may also experience decreased embryo survival and increased embryonic mortality.

So, the general objectives of this study are to observe following specific objectives:

- i. Determination of blood glucose, calcium and phosphorus levels from the estrous cows.
- ii. Investigate the co-relation of blood glucose to corresponding blood calcium and phosphorus levels in estrous cows.

CHAPTER II

REVIEW OF LITERATURE

The purpose of this chapter is to provide a selective review of the research works performed in relation to the present study. Literature on analysis of glucose level status in blood reflect on farm diagnosis of disease connected with infertility in dairy cattle on reproductive performance in dairy cows which is related to this study has been reviewed under the following heading

Paula *et al.* (2023) investigated how maternal hyperglycemia and a high-fat diet (HFD) after weaning affected mitochondrial function and ovarian development in the offspring of diabetic rats. Female rats were treated with either a citrate buffer (Control – C) or Streptozotocin (to induce diabetes – D) on postnatal day 5. The adult rats were then mated, and their female offspring (O) were categorized based on maternal condition (control dams – OC, diabetic dams – OD) and fed either a standard diet (SD) or a high-fat diet (HFD) from weaning to adulthood. The groups were OC/SD, OC/HFD, OD/SD, and OD/HFD. Oral Glucose Tolerance Tests (OGTT) and Area Under the Curve (AUC) analyses were performed on these adult offspring, who were later anesthetized and euthanized for sample collection. A significant percentage of rats in the OD/HFD group developed diabetes (OD/HFD 40% vs. OC/SD 0%, $p < 0.05$). Progesterone levels were notably lower in experimental groups (OC/HFD 0.40 ± 0.04 ; OD/SD 0.30 ± 0.03 ; OD/HFD 0.24 ± 0.04 vs. OC/SD 0.45 ± 0.03 , $p < 0.0001$). Mitochondrial fission factor (MFF) expression decreased significantly in OD/SD (0.34 ± 0.33) and OD/HFD (0.29 ± 0.2) groups compared to OC/SD (1.0 ± 0.41 , $p = 0.0015$), as did mitofusin-2 (MFN2) expression (OD/SD 0.41 ± 0.21 ; OD/HFD 0.77 ± 0.18 vs. OC/SD 1.0 ± 0.45 , $p = 0.0037$). Fewer follicles were observed in the OD/SD and OD/HFD groups. Oxidative stress markers showed lower staining for superoxide dismutase (SOD) and catalase, and higher staining for malondialdehyde (MDA) in ovarian cells in the OC/HFD, OD/SD, and OD/HFD groups. Fetal programming caused mitochondrial dysfunction, oxidative stress, and a reduction in ovarian reserve, while maternal diabetes combined with an HFD increased diabetes occurrence in the adult female offspring.

Olawale *et al.* (2020) explored how insulin deficiency in Diabetes Mellitus contributes to reproductive dysfunction, particularly focusing on females. Although much research has examined diabetes-related reproductive issues in men, this study aimed to evaluate long-term hyperglycemia's impact on the ovaries of diabetic rats. Female Wistar rats were divided into control and diabetic groups, each containing five animals, with diabetes induced via Streptozotocin (STZ, 50 mg/kg intraperitoneal injection). After 14 weeks, the animals were sacrificed, and blood glucose levels, body and organ weights, hormone levels, and oxidative stress markers in the ovaries and uterus were measured. Histological and immunohistochemical analysis of the ovaries (using CD79) was also conducted. Diabetic rats showed significantly lower body weight ($p < 0.05$) and reduced levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and estrogen. Antioxidant enzymes, including catalase, SOD, and glutathione-S-transferase (GST), were significantly reduced, while MDA levels were elevated compared to the control group. Histological analysis revealed chronic inflammation, and CD79 expression was observed in the diabetic ovary. The findings suggest that diabetes impairs ovarian health by disrupting hormonal balance and promoting oxidative stress.

Shathili *et al.* (2018) focused on the role of protein glycosylation, a post-translational modification that influences cellular behavior and interactions, in ovarian follicle development and atresia, particularly in diabetic conditions. Despite diabetes' known effects on embryo implantation, little is understood about glycosylation changes in ovarian tissues. Using PGC chromatography and mass spectrometry, the study identified 138 N-glycans and 6 O-glycans in the ovarian tissues of STZ-induced diabetic mice ($n = 3$). Compared to non-diabetic mice, diabetic ovaries showed reduced sialylation and fucosylation, fewer branched N-glycans, and more oligomannose structures. These changes were more pronounced in the ovaries than in the liver of the same diabetic mice, indicating tissue-specific effects of diabetes on protein glycosylation. Although O-GalNAc glycans were present at low levels, there was an increase in the NeuGc ratio between diabetic and control ovarian tissues. Diabetic mice also exhibited increased levels of inflammatory cytokines TNF- α and IL-1 β , which have been linked to changes in protein glycosylation.

Dupont *et al.* (2016) discussed how insulin influences ovarian function by acting through several interconnecting intracellular pathways, including insulin receptor substrates (IRSs), PI3K/Akt, and MAPK, which also interact with follicle-stimulating hormone (FSH) and luteinizing hormone (LH) signaling. These pathways are believed to be active in ovarian cells, allowing insulin to directly regulate ovarian function. The review focused on insulin signaling in follicular cells such as granulosa, theca, and oocytes, and emphasized how glucose uptake, mediated by insulin, plays a critical role in folliculogenesis. The involvement of AMPK and hexosamine in glucose's insulin-mediated signaling within ovarian cells was highlighted, along with the potential interaction of insulin with FSH and LH signaling at key molecular points. The review also examined how insulin-sensitizing drugs, like metformin and rosiglitazone, affect follicular cells.

Tasaki *et al.* (2015) explored the impact of hyperglycemic culture conditions on the development of oocytes obtained from early antral follicles (EAFs). They cultured oocyte-granulosa cell complexes (OGCs) from EAFs for 12 days in media containing either 5.56 mM or 11 mM glucose, and assessed antrum formation, glucose consumption by the OGCs, and oocyte characteristics in vitro. These findings were compared with oocytes naturally grown in vivo, derived from antral follicles (AFs; 3–5 mm in diameter). Additionally, they examined how a high-glucose maturation medium affected the quality of oocytes from AFs. While high glucose conditions led to increased glucose consumption in OGCs, they did not alter antrum formation, oocyte size, chromatin structure, histone 4 K12 acetylation, nuclear maturation, or the developmental potential of oocytes cultured in vitro. However, the high-glucose maturation medium resulted in elevated reactive oxygen species and negatively impacted the developmental capacity of the oocytes. Overall, the study concluded that hyperglycemic culture environments hinder oocyte maturation but do not affect the growth of oocytes from the EAF to AF stage.

Nayki *et al.* (2015) investigated how diabetes mellitus (DM) affects ovarian damage and reserve in a rat model. Sixteen female Sprague-Dawley albino rats, aged 12 weeks and

weighing 220-240 grams, were randomly divided into two groups: a control group of 8 healthy rats and an experimental group of 8 rats where diabetes was induced using streptozotocin (STZ). After diabetes was confirmed (blood glucose > 250 mg/dl), all rats were euthanized, blood samples were collected for biochemical analysis, and their ovaries were removed for histopathological examination. The study measured the expression of nuclear factor-kappa B (NF-kB), caspase-3, and anti-Müllerian hormone (AMH). Results showed significantly higher expressions of NF-kB and caspase-3 in diabetic rats compared to controls ($p = 0.011$ and $p = 0.010$, respectively). AMH levels were also significantly lower in the diabetic group (1.41 ± 0.25 ng/dl) compared to controls (3.22 ± 0.58 ng/ml; $p = 0.024$). The study concluded that hyperglycemia induces severe ovarian damage via the NF-kB and caspase-3 apoptotic pathways, leading to diminished ovarian reserve in diabetic rats.

Isik *et al.* (2012) evaluated the ovarian reserves of women with type 2 diabetes mellitus (T2DM) compared to healthy controls. The study involved 89 women with T2DM and 73 healthy controls, divided into three age groups: group 1 (20–29 years), group 2 (30–39 years), and group 3 (40–49 years). Transvaginal ultrasounds were performed on the third day of their menstrual cycle to assess ovarian volume and total antral follicle count (AFC). Across all age groups, women with T2DM had significantly higher follicle-stimulating hormone (FSH) levels and lower AFCs compared to healthy controls. For example, in group 1, FSH levels were 7.8 ± 0.9 IU/L in diabetic women versus 5.0 ± 1.0 IU/L in controls, and AFC was 21.1 ± 4.8 for diabetics versus 25.0 ± 9.1 for controls ($P < 0.001$). A statistically significant difference in ovarian volume was observed only in group 1. Additionally, AFC was negatively correlated with FSH levels, age, hemoglobin A1C, and fasting blood glucose. This pioneering study demonstrated reduced ovarian reserves in women with T2DM compared to healthy individuals.

Nandi *et al.* (2010) examined how insulin resistance, seen in conditions such as obesity and diabetes, affects female reproductive function. The study aimed to differentiate the direct effects of impaired insulin signaling from those of coexisting conditions like obesity and hyperglycemia. Using lean, normoglycemic mouse lines with varying

degrees of hyperinsulinemia and insulin receptor (Insr) expression, the researchers studied how altered insulin signaling influences reproduction. Although all mouse lines (Ttr-Insr^{-/-}, Insr^{+/-}, and wild-type Insr^{+/+}) maintained fertility, the insulin-resistant and hyperinsulinemic mice exhibited irregular estrous cycles and abnormal ovarian follicle distribution and morphology, independent of hyperandrogenism. Pregnancies in these mice were less successful, with early gestational issues, reduced embryo weights, and increased placental calcification. The findings indicated that altered insulin signaling, irrespective of adiposity or hyperglycemia, can disrupt female reproductive function and pregnancy outcomes.

Chabrolle *et al.* (2008) explored how diabetes affects reproductive dysfunction in female rats, focusing on folliculogenesis and steroidogenesis. Although the molecular mechanisms underlying reduced steroid production in diabetic rats have not been fully elucidated, the study examined the role of adiponectin, an insulin-sensitizing hormone that promotes glucose uptake in muscle and reduces glucose production in the liver. Adiponectin acts via two receptors, AdipoR1 and AdipoR2, which are regulated by hyperglycemia and hyperinsulinemia in liver and muscle tissues. The researchers identified both receptors in rat ovaries and suggested that their regulation in diabetic ovarian tissue warrants further study.

We cultured primary rat granulosa cells in vitro with high glucose concentrations (5 or 10 g/l), both with and without FSH (10^{-8} M) or IGF-1 (10^{-8} M), and examined the ovaries of streptozotocin (STZ)-induced diabetic rats in vivo. Oestradiol and progesterone levels in the culture medium and serum were measured via radioimmunoassay (RIA). Immunoblotting was used to assess key steroidogenesis factors (3 β -HSD, P450_{scc}, P450 aromatase, StAR), adiponectin receptors, and various signaling pathway elements (MAPK ERK1/2, AMPK) both in vitro and in vivo. We also assessed cell proliferation through [³H] thymidine incorporation. In vitro, glucose (5 or 10 g/l) impaired the production of both progesterone and oestradiol in rat granulosa cells under basal conditions and in response to FSH and IGF-1, without affecting cell proliferation or viability. This was linked to a significant reduction in the levels of 3 β -

HSD, P450_{scc}, P450 aromatase, StAR proteins, and MAPK ERK1/2 phosphorylation. However, glucose did not alter the amounts of AdipoR1 or AdipoR2 proteins. In vivo, as expected, STZ treatment caused hyperglycemia and deficiencies in insulin, adiponectin, and resistin in rats. Plasma progesterone and oestradiol levels were reduced in STZ rats, though the levels of 3 β -HSD and P450 aromatase in the ovaries remained comparable to controls, while StAR and P450_{scc} levels were elevated. Streptozotocin did not affect adiponectin receptor levels in rat ovaries but did increase AMPK phosphorylation without altering MAPK ERK1/2 phosphorylation.

Landau *et al.* (2000) reported that insulin and glucose may limit ovarian function in dairy cows selected for high milk production. The study investigated the effects of nutrition on insulin and glucose levels within follicles in Israeli Holstein dairy cows, which were fed a basic total mixed ration and produced 34–39 kg of milk per day. In experiment 1, conducted on 11 oestrus-synchronized cows, little variation in insulin levels was noted in plasma during the luteal phase, but significant variation was observed during the follicular phase. To avoid confounding effects of diet and cycle phase, the following experiments focused on the luteal phase. In experiment 2, designed as a Latin-square trial, six cows were sequentially fed diets with either 17.1% (control) or 19.7% crude protein, using soybean meal (SBM) or corn gluten meal (CGM) as protein sources, which differ in ruminal degradability and leucine content. After adjusting for dry matter intake, plasma insulin was 29.5% and 26.4% higher in cows fed SBM and CGM, respectively, compared to controls ($P < 0.05$). In experiment 3, 17 cows were individually fed the basic diet, then switched to isoenergetic diets containing SBM ($n=5$), CGM ($n=6$), or corn grain (CG, $n=6$) from day 10 to 16 of the synchronized oestrus cycle. On day 16, PGF2 α was administered, and follicular fluid was aspirated from the 26 largest follicles. Follicles were classified as preovulatory or subordinate based on oestradiol concentration and the progesterone ratio. Higher insulin (0.282 vs. 0.127 ng/ml, $P < 0.0001$) and glucose (0.614 vs. 0.386 g/l, $P < 0.002$) concentrations were found in preovulatory follicular fluid. Insulin was 26% higher in cows fed the CG diet compared to those fed CGM ($P < 0.04$), with SBM showing intermediate results. These

findings suggest that insulin plays a role in follicular maturation, and optimizing nutrition could enhance feeding strategies in dairy cows during the oestrus cycle.

Wu *et al.* (2020) found that diabetes negatively impacts ovarian follicular development, leading to infertility. In this study, a type 2 diabetes mouse model was created by feeding the animals a high-fat diet (HFD) for 15 or 20 weeks, and the effects of diabetes on follicular development and ovarian angiogenesis were evaluated. After 15 weeks on the HFD, the mice exhibited characteristics of type 2 diabetes, which worsened after 20 weeks. Following 20 weeks on HFD, the mice demonstrated abnormal ovarian morphology, reduced blood vessel formation, follicular development arrest, and fewer granulosa cells (GCs) in mature follicles—findings that were not present after 15 weeks. Increased markers of DNA damage, endoplasmic reticulum (ER) stress, and GC apoptosis were observed in the ovaries of mice on the HFD for 20 weeks. Additionally, diabetes significantly inhibited ovarian angiogenesis, evidenced by the downregulation of CD31, and suppressed the HIF1 α -VEGF signaling pathway over time. These results suggest that diabetes impairs ovarian function by inducing GC apoptosis and inhibiting ovarian angiogenesis.

Rutter *et al.* (1988) examined the effect of phlorizin-induced hypoglycemia on gonadotropin secretion in mature, lactating Hereford-cross cows following prostaglandin-induced luteolysis. The study involved 20 cows between 43 and 108 days postpartum, all with a functional corpus luteum (CL). The cows were infused with either saline (control) or 3 g/day of phlorizin from the time of prostaglandin injection on day 1 until 0800 on day 5. Blood samples were collected to measure plasma insulin, glucose, and free fatty acids (FFA), as well as serum luteinizing hormone (LH), follicle-stimulating hormone (FSH), and progesterone levels. Phlorizin-treated cows exhibited lower plasma insulin ($P < 0.05$) and glucose ($P < 0.05$) levels, while FFA concentrations increased compared to controls ($P < 0.05$). Serum LH concentrations and LH pulse amplitude were lower in phlorizin-treated cows compared to saline-infused cows ($P < 0.05$), although the frequency of FSH pulses increased in controls and decreased in phlorizin-treated cows. Despite these changes, all but one phlorizin-treated cow ovulated

and developed functional CLs. The study concluded that phlorizin-induced hypoglycemia suppresses the normal increase in LH pulse amplitude during the preovulatory period in postpartum beef cows.

Garris *et al.* (1984) studied the effects of progressive hyperglycemia on ovarian follicular maturation in spontaneously diabetic Chinese hamsters. The hamsters were divided into control, moderate (160–350 mg/dl blood glucose), and overt (≥ 350 mg/dl blood glucose) diabetic groups. Ovaries were collected and analyzed for changes in follicular growth. The total number of primary (100–200 μm) and secondary (200–350 μm) follicles was lower in both moderate and overt diabetic hamsters compared to controls, and the percentage of viable secondary follicles was significantly reduced in overt diabetic animals. No significant differences were observed in the number of viable tertiary follicles among the groups. However, overt diabetic hamsters exhibited a marked increase in atretic secondary follicles compared to controls. These findings suggest that progressive hyperglycemia impairs normal follicular recruitment, leading to reduced reproductive performance in diabetic Chinese hamsters.

CHAPTER III

MATERIALS AND METHODS

3.1 Study area & duration

This study was conducted in collaboration with the Department of Physiology and Pharmacology; and Department of Pathology and Parasitology at Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur from January 2023 to June 2023 to evaluate blood biochemical parameters (calcium, phosphorus, hemoglobin and glucose) and its role for infertility because these periods are very important for the fertility and production in dairy cow. Estrus symptoms were recorded from cyclic animal.

3.2 Experimental layout:

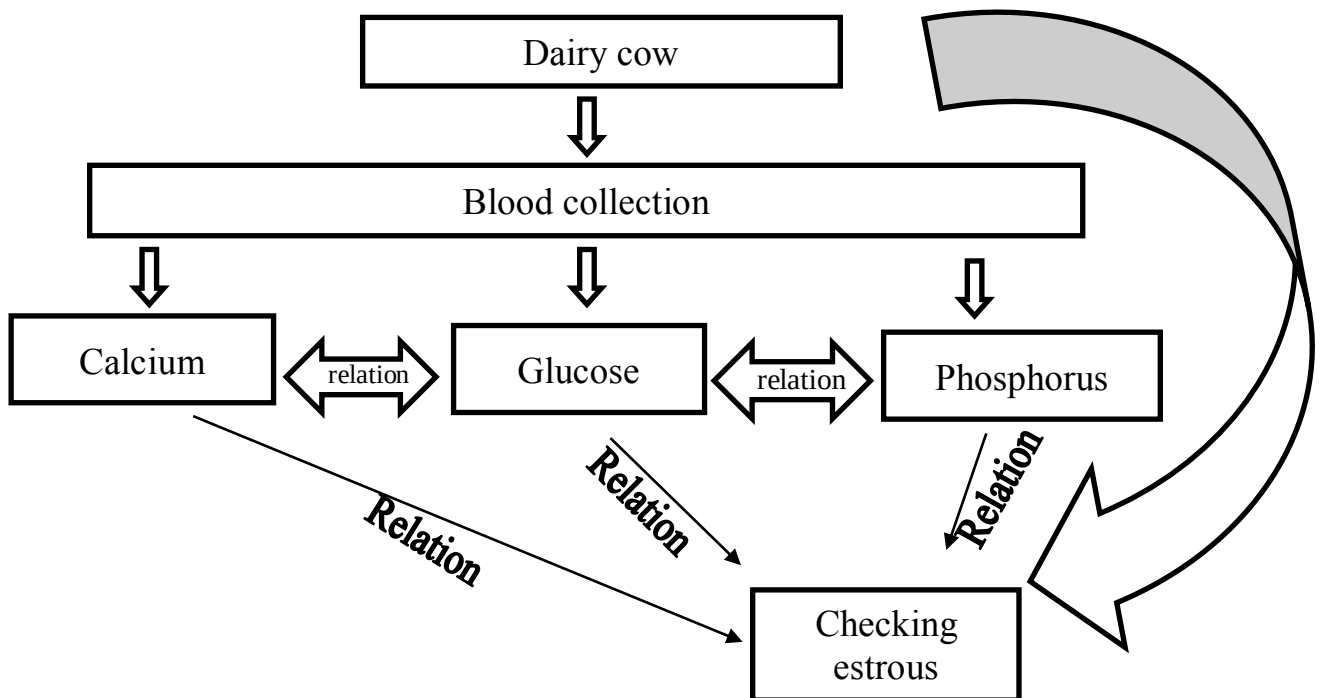


Figure 3.1: Experimental Layout

3.3 Study population : Blood sample was collected from 50 female cattle from voumik dairy; jinnah dairy; and tuhin dairy farm of Dinajpur sadar.



Photo 3.1: Study farm

3.4 Blood sample collection:

About 10 ml of blood sample from each animal was collected from caudal vein of different dairy farm and abattoir of Dinajpur Sadar Upazila, Dinajpur within Ethylene Diamine Tetra Acetate (EDTA) tube and shifted to the Physiology and Pharmacology Department of HSTU.



Photo 3.2: Collection of blood sample from animal



Photo 3.3: Collected blood sample

3.5 Estimation of blood glucose level

Virtually all glucose assays use enzymatic approaches to generate a measurable product proportional to the glucose concentration. The following enzymes can act on glucose and have been utilized to measure glucose levels:

1. Glucose oxidase: β -d-glucose + O₂ → gluconate + H₂O₂

2. Hexokinase: d-glucose (and other hexoses) + ATP $\rightarrow$ glucose (hexose)-6-phosphate; usually coupled with glucose-6-phosphate dehydrogenase:
3. Glucose-6-phosphate + NAD(P)⁺ $\rightarrow$ 6-phosphogluconolactone + NAD(P)H
4. Glucose dehydrogenase: glucose + NAD(P)⁺ $\rightarrow$ gluconolactone + NAD(P)H

Glucose estimation was adjusted by 'Sugar Check' glucometer.



Photo 3.4: Estimation of blood glucose level

3.6 Estimation of Ca & P:

A Semi-auto biochemistry analyzer (MCA-8000A Blood Test Machine Portable Semi-Auto Chemistry Analyzer) is a machine used to do blood tests for calcium & phosphorus. It is determined by using the filter photometry principle. Filter photometry differs from spectrophotometry in a few ways. It is a robot that repeatedly performs a series of steps in carrying out the analysis: taking the sample and reagents, mixing and measuring the changes in the optical density of the specific reaction mixture for the parameter analyzed.

3.7 Statistical analysis

The results of various parameters were expressed as $\pm$ SDM. Statistical data analysis were done by using SPSS version 22 and Microsoft Excel. Statistically significant differences were determined by Analysis of Variance (ANOVA).

CHAPTER IV

RESULTS

4.1 Blood glucose level

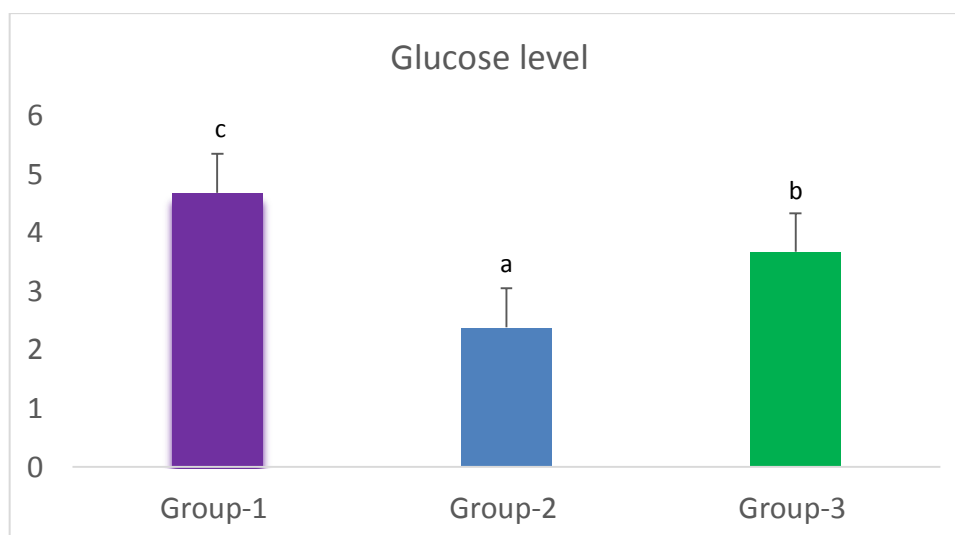
A total of 50 blood samples were collected from voumik dairy; jinnah dairy; and tuhin dairy farm of Dinajpur sadar. Then blood glucose level test was performed by Sugar check glucometer. Percentage of glucose of reproductive problematic animal differs significantly ($p<0.05$). It was significantly different at 5% level.

Table 1: Blood glucose level

Group	Glucose level (mmol/L)		
	Mean $\pm$ SEM	Range	Estrous cow (%)
Group-1 (n=20)	4.68 $\pm$ 0.07 ^c	4.30-5.60	70.0
Group-2 (n=20)	2.38 $\pm$ 0.06 ^a	1.70-2.80	20.0
Group-3 (n=10)	3.67 $\pm$ 0.04 ^b	3.50-3.90	80.0
Level of significance	*		*

* means significant at 5% level of significance ($P<0.05$).

Group-1: high glucose level, Group-2: low glucose level, Group-3: normal glucose level



Group-1: high glucose level, Group-2: low glucose level, Group-3: normal glucose level

Figure 4.1 Blood glucose level : Among which of them, 20 numbers of cows had higher glucose level condition, 20 numbers of cows had lower glucose level condition and 10 numbers of cows had normal glucose level. The average blood glucose level of high, low & normal cows

was 4.68 ± 0.07 , 2.38 ± 0.06 and 3.67 ± 0.04 respectfully. Among of them, about 70% of higher glucose level cows showed estrus, about 20% of lower glucose level cows showed estrus and about 80% of normal cows showed estrus which is significantly high.

4.2 Relation of blood glucose level and Calcium

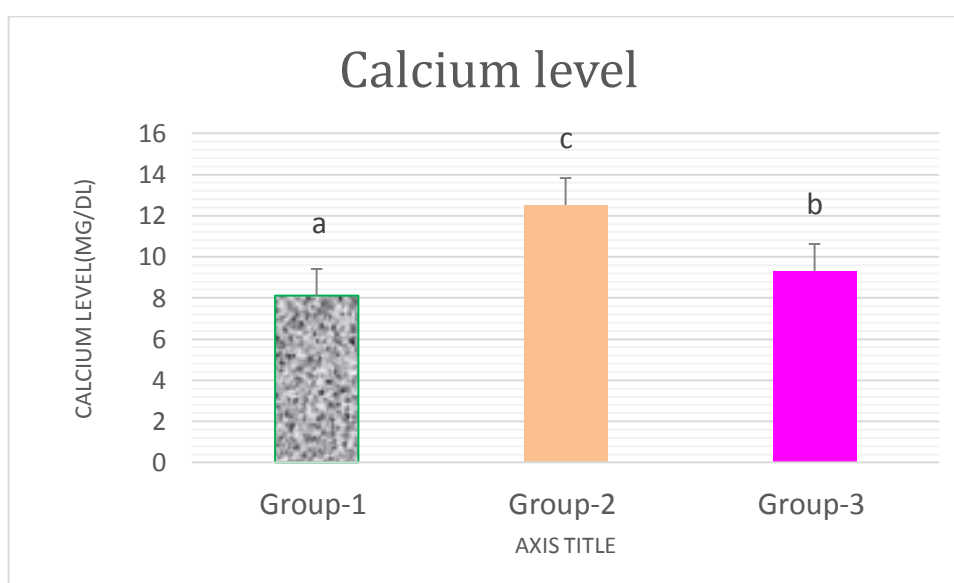
Blood glucose level significantly ($p < 0.05$) decreased the calcium level. It was significantly different at 5% level.

Table 2: Relation of blood glucose level and Calcium

	Calcium level (mg/dl)	Range
Group-1 (n=20)	8.11 ± 0.26^a	5.20-9.10
Group-2 (n=20)	12.51 ± 0.47^c	9.30-16.30
Group-3 (n=10)	9.31 ± 0.54^b	8.90-14.10
Level of significance	*	

* means significant at 5% level of significance ($P < 0.05$).

Group-1: high glucose level, Group-2: low glucose level, Group-3: normal glucose level



Group-1: high glucose level, Group-2: low glucose level, Group-3: normal glucose level

Figure 4.2: Relation of blood glucose level and Calcium: The average calcium level of higher glucose level cows was 8.11 ± 0.26 , lower glucose level cows was 12.51 ± 0.47 & normal cows was 9.31 ± 0.54 . There was significant variation in case of blood calcium level. Different letter denotes statistically significant at 5% ($p < 0.05$).

4.3 Relation of blood glucose level and Phosphorus

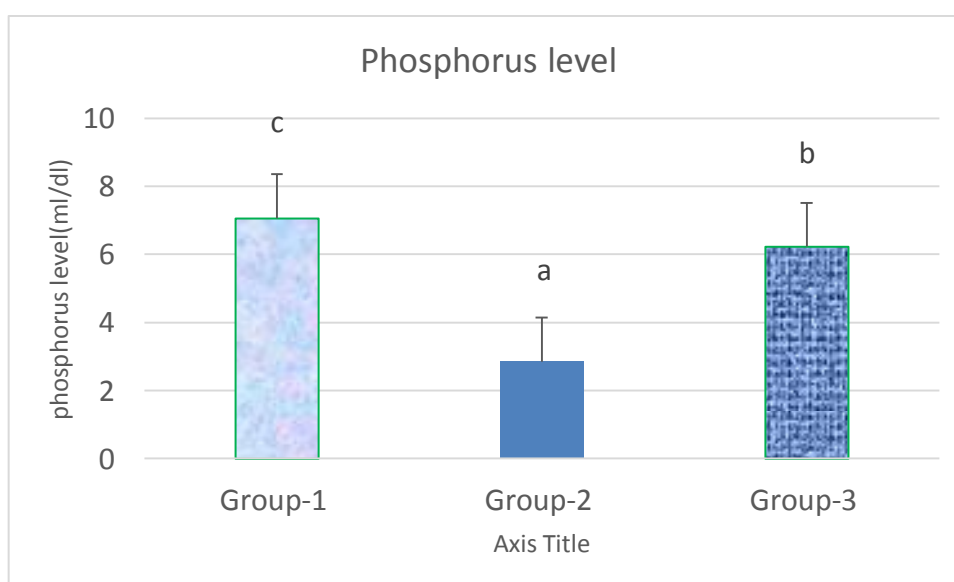
Blood glucose level significantly ($p < 0.05$) decreased the phosphorus level. It was significantly different at 5% level.

Table 3: Relation of blood glucose level and Phosphorus

	Phosphorus level (mg/dl)	Range
Group-1 (n=20)	7.06 ± 0.32^c	5.40-10.50
Group-2 (n=20)	2.85 ± 0.17^a	2.10-5.50
Group-3 (n=10)	6.23 ± 0.64^b	3.60-9.50
Level of significance	*	

* means significant at 5% level of significance ($P < 0.05$).

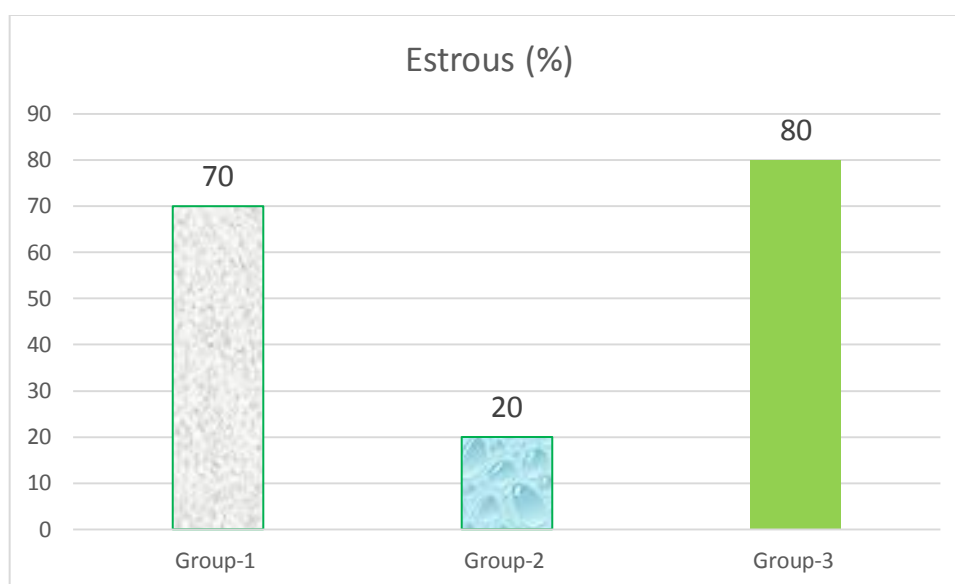
Group-1: high glucose level, Group-2: low glucose level, Group-3: normal glucose level



Group-1: high glucose level, Group-2: low glucose level, Group-3: normal glucose level

Figure 4.3: Relation of blood glucose level and Phosphorus: The average phosphorus level of higher glucose level cows was 7.06 ± 0.32 , lower glucose level cows was 2.85 ± 0.17 & normal cows was 6.23 ± 0.64 . There was significant variation in case of blood phosphorus level. Different letter denotes statistically significant at 5% ($p < 0.05$).

4.4 Effect of blood glucose level on estrus



Group-1: high glucose level, Group-2: low glucose level, Group-3: normal glucose level

Figure 4.4: Effect of blood glucose level on estrus: About 70% of higher glucose level cows showed estrus, about 20% of lower glucose level cows showed estrus and about 80% of normal cows showed estrus which is significantly high. Percentage of glucose of reproductive problematic animal differs significantly ($p < 0.05$). It was significantly different at 5% level.

4.5 Effect of phosphorus level on estrous cow

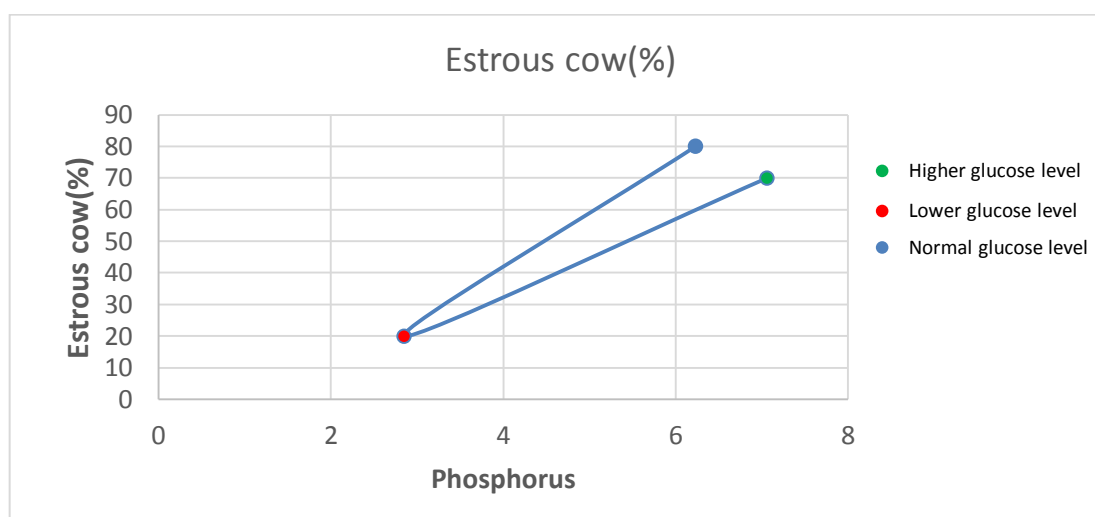


Figure 4.5 Effect of phosphorus level on estrous: About 80% of cow showed normal estrus which have normal phosphorus level and 20% of cow showed estrus which have lower level of phosphorus.

4.6 Effect of glucose level on Calcium & phosphorus

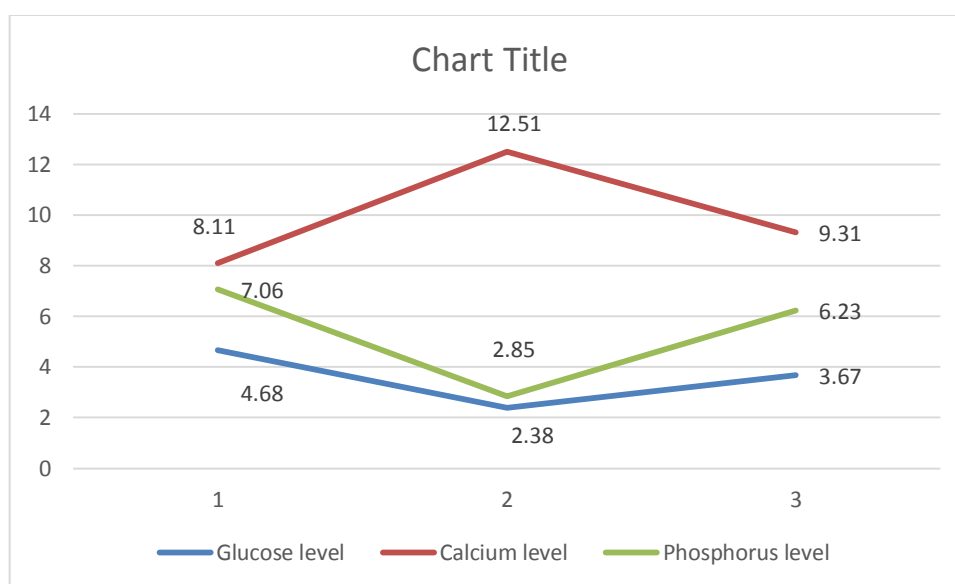


Figure 4.6: Effect of glucose level on Calcium & phosphorus: The average phosphorus level & calcium level of higher glucose level cows was 7.06 ml/dl & 8.11ml/dl, The average phosphorus level & calcium level of lower glucose level cows was 2.85 ml/dl & 12.51 ml/dl and the average phosphorus level & calcium level of normal glucose level cows was 6.23 ml/dl & 9.31 ml/dl.

4.7 Association of blood glucose level, calcium level, phosphorus level & Estrous

Group-1 & group-3 cows having normal calcium & phosphorus level that showed in 70% & 80% estrous cow respectfully and group-2 cows having high calcium & lower phosphorus level that showed in 20% estrous cows

Table 4: Association of blood glucose level, calcium level, phosphorus level & Estrous

Group	Glucose level	Calcium level	Phosphorus level	Estrous cow (%)
Group-1 (n=20)	4.68±0.07 ^c	8.11±0.26 ^a	7.06±0.32 ^c	70.0
Group-2 (n=20)	2.38±0.06 ^a	12.51±0.47 ^c	2.85±0.17 ^a	20.0
Group-3 (n=10)	3.67±0.04 ^b	9.31±0.54 ^b	6.23±0.64 ^b	80.0
Level of significance	*	*	*	*

* means significant at 5% level of significance (P<0.05).

Group-1: high glucose level, Group-2: low glucose level, Group-3: normal glucose level

CHAPTER V

DISCUSSION

The study was performed in HSTU during the year 2023-2024. The experiment was conducted to study the glucose level status in blood reflect on farm diagnosis of disease connected with estrus in dairy cattle.

The relation between blood glucose level, calcium level, phosphorus level & Estrous are presented in table 4. Blood glucose level, calcium level & phosphorus level was significantly ($P<0.05$) related to estrous condition. It was found that 70% high, 20% low & 80% normal glucose level cows showed estrous.

Previous study showed that dietary phosphorus was extremely low, and other dietary deficiencies associated with the low quality diets fed may have contributed to the irregularities in the expression of estrus. It has been reported that dietary phosphorus levels of less than 0.25% can reduce rumen microbial growth (Durand & Kawashima *et al.*, 1980) resulting in less microbial protein, lowered ration digestibility, and decreased energy supply. Additionally, low levels of dietary phosphorus can reduce feed intake, causing coincidental deficiencies of energy, protein, and other nutrients. A decrease in DMI, milk production, and body weight was reported for dairy cows fed a diet containing 0.24% P when compared to cows fed diets ranging from 0.28 to 0.42% (Call *et al.*, 1987 & Valk *et al.*, 1999). It is probably through these mechanisms that low levels of dietary P may have an indirect effect on the expression of estrus in the range cattle utilized in early reports. However, modern dairy diets usually do not approach the low P levels required to impair function of rumen microbes or depress DMI. The P content in current dairy diets usually ranges from 0.33 to 0.40% before P supplementation (Wu, Satter & Sojo *et al.*, 2000).

The relation between phosphorus level and estrous are presented in fig 4.5. Group-1 & group-3 cows contain normal phosphorus level showed estrus 70% & 80% respectively and Group-2 cows contain lower phosphorus level showed estrus 20%. The present result was agree with the findings of (Durand & Kawashima *et al.*, 1980). The average phosphorus level of higher glucose level cows was 7.06 ± 0.32 , lower glucose level cows was 2.85 ± 0.17 & normal cows was 6.23 ± 0.64 . There was significant variation in case of blood phosphorus level (Fig 4.2)

The percentages of glucose of reproductive problematic animal are presented in figure 4.1.

The average blood glucose level of high, low & normal cows was 4.68 ± 0.07 , 2.38 ± 0.06 and 3.67 ± 0.04 respectfully. Among of them, about 70% of high glucose level cows showed estrus, about 20% of low glucose level cows showed estrus and about 80% of normal cows showed estrus which is significantly high. Percentage of condition of reproductive problematic animal differs significantly ($p < 0.05$).

Previous study showed that the liver may ramp up gluconeogenesis to provide more glucose to meet the increased energy needs. Or Heat stress can lead to the release of stress-related hormones, such as cortisol and adrenaline. These hormones can promote the breakdown of glycogen stores in the liver and muscles, releasing glucose into the bloodstream. This process is part of the body's response to stress, but it can also contribute to elevated glucose levels. When the core body temperature of animals exceeds the range specified for normal activities, it provokes the acclamatory response to neutralize the effect of heat stress, resulting in significant changes in the blood metabolites and glucose level (Guo *et al.*, 2019). Therefore, it is evident that the change in the body glucose level depends on the intensity and the duration of heat stress. In dairy cattle, alterations in body glucose during heat stress are due to the reduced feed intake and energy (carbohydrates) balance, pushing the cow to enter an energy de-stabilized condition, and this is dependent of the estrous stage (Baumgard *et al.*, 2013). The present result was similar with the findings of (Guo *et al.*, 2019 & Baumgard *et al.*, 2013).

The effect of glucose reproductive problematic animal with calcium & phosphorus are presented in figure 4.6. The average phosphorus level & calcium level of high glucose level cows was 7.06 ml/dl & 8.11ml/dl, The average phosphorus level & calcium level of low glucose level cows was 2.85 ml/dl & 12.51 ml/dl and The average phosphorus level & calcium level of high glucose level cows was 6.23 ml/dl & 9.31 ml/dl. In animal, there is a specific ratio between phosphorus and calcium level. When calcium level is more than normal level, it will negatively affect on phosphorus. Lower Phosphorus level negatively impact on estrus. According to (Durand & Kawashima *et al.*, 1980) Lower Phosphorus level contributed to the irregularities in the expression of estrus.

CHAPTER VI

CONCLUSION

From the present study it was concluded that the glucose level had significant impact on phosphorus level, calcium level and estrous. Finally, this study revealed that blood glucose level variation plays an important role in the ovary status and estrous condition of dairy cow. Lower glucose level had negative effect on phosphorus and calcium level that impact on estrous condition and decrease production performance.

Further study is recommended to determine the accurate therapy to overcome the effect of lower blood glucose level on reproductive performance in dairy cows.

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