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Some studies on post-partum period in farm animals by using diagnostic ultrasound

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بسم الله الرحمن الرحيم

قَالُوا سُبْحَانَكَ لاَ عِلْمَ لَنَا إِلاَّ مَا عَلَّمْتَنَا إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ

صدق الله العظيم

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Vita

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LIST OF ABBREVIATIONS

AI	ARTIFICIAL INSEMINATION
BHBA	BETA-HYDROXY BUTYRIC ACID
BCS	BODY CONDITION SCORE
BUN	BLOOD UREA NITROGEN
CA	CALCIUM
CU	COPPER
GNRH	GONADOTROPIN RELEASING HORMONE
HCG	HUMAN CHORIONIC GONADOTROPEIN
IGF1	INSULIN GROWTH FACTOR 1
K	POTASSIUM
MA	MILLIAMPER
MG	MAGNESIUM
MG	MILLIGRAM
MHZ	MEGAHERTZ
ML	MILLILITER

N=	NUMBER
NA	SODIUM
NEFA	NON ESTERFIED FATTY ACID
NPN	NON PROTEIN NITROGEN
NRC	NATIONAL RESEARCH COUNCIL
P	PHOSPHORUS
PMN	POLYMORPHONUCLEAR CELLS
PPM	PARTS PER MILLION
PROMT	PREDICASTS OVERVIEW OF MARKETS AND TECHNOLOGY
R.F.	RIGHT FOLLICLE
RBCPI	RED BLOOD CELLS PHOSPHORUS
RCL	RIGHT CORPUS LUTEUM
RFM	RETAINED FETAL MEMBRANE
SE	SELENIUM
SIPS	THE SAMPLE INTRODUCTION PUMP SYSTEM OF SPECTROPHOTOMETER
SPI	SERUM PHOSPHORUS

TMR	TOTAL MIXED RATION
US	ULTRASOUND
WBPI	WHOLE BLOOD PHOSPHORUS
ZN	ZINC

1. INTRODUCTION

Cattle are the most common type of large domesticated ungulates. They are a prominent modern member of the subfamily Bovinae, are the most widespread species of the genus *Bos*, and are most commonly classified collectively as *Bos Taurus* **(Brown, 2009).**

Cattle are commonly raised as livestock; for meat (beef or veal), for milk (dairy cattle), and for hides, which are used to make leather. They are used as riding animals and draft animals (oxen or bullocks, which pull carts, plows and other implements). Another product of cattle is dung, which can be used to create manure or fuel. Mostly small breeds such as the Miniature Zebu, are kept as pets in some regions, such as parts of India, cattle have significant religious meaning **(Wilson, 2005)**.

Around 10,500 years ago, cattle were domesticated from as few as 80 progenitors in southeast Turkey. According to an estimate from 2011, there are 1.4 billion cattle in the world **(Bollongino et.al, 2012).** In 2009; cattle became one of the first livestock animals to have a fully mapped genome. Cattle population in Egypt was 4,198,658 heads, while cattle population all over the world was count 1,489,744,504 heads **(FAO, 2018).**

The nutritional quality of feeds and forage can have a tremendous influence on the reproductive performance of cattle. Although reproductive failure may occur for several reasons, management and the environment are often important contributing factors. Part of the environment and management of any animal is nutrition (**Boudjellaba et al., 2018)**

During the last decade, reproductive performance declined dramatically worldwide, but little is known concerning the involvement of oxidative stress as a causative factor. Oxidative stress may act at different levels, with negative impacts on cell membrane integrity and other active molecules with potential subsequent effects on reproduction, thus the adjustment of trace elements and hematological parameters in ration play a big important antioxidant role to overcome the effect of oxidative stressors (**Boudjellaba et al., 2018)**

Minerals are loosely classified as macro and micro minerals depending on the relative amounts needed or present in the body. Macro minerals include *calcium, phosphorus, magnesium, potassium, sulphur, sodium* and *chloride. Cobalt, copper, iodine, iron, manganese, molybdenum, selenium* and *zinc* are considered micro or trace minerals. Rations that contain a high percentage of forage usually supply adequate amounts of calcium but may be low in phosphorus. However, rations high in grain contain adequate phosphorus but may be deficient in calcium and other minerals. Micro or trace mineral deficiencies are associated with soil type and are usually geographically related. Abnormal levels of some minerals such as iron and cobalt do not usually cause a problem with reproduction. Other minerals, including those that follow, can significantly affect reproduction (**Yaremcio, 2000**).

Reproductive efficiency of a female is assessed by its ability to produce a viable offspring at the expected intervals. Pregnancy from first breeding is a critical component of cow/calf production profitability. Cows that calve early in the calving season have more time for the resumption of estrus cycles before breeding and their calves are generally heavier at weaning (**Houghton et.al., 1990**).There is a relationship between some minerals and other hematochemical parameters and conception in heifers and cows. Diagnoses of mineral deficiencies or toxicities are difficult and generally require knowledge of the nutrient composition of the diet, as well as at least one measure from body tissue. Concentrations of minerals in blood serum or plasma are generally related to intake, but are influenced by sex, breed, age, and reproductive status e.g. pregnancy or lactation (**Underwood, 1981**).

Small et al., 1997 stated that cattle consumed typically high potassium and Phosphorus forage and coincided with serum K and P concentrations were indicative of excess. Serum Mg levels were low for all heifers and cows and were not likely a cause of failed first service conception. Serum Boron and Phosphorus showed a consistent relationship with first service conception, being higher on day 21 in those animals that conceived. The study has shown a relationship between B and P and conception in cattle. High K and P concentrations are consumed in high quality forages and B supplementation could have a significant role in the nutrition of peri- and post parturient dairy and beef cattle. In conclusion, P and B may be factors limiting first service conception in beef cattle fed conserved forage.

Molefe and Mwanza, (2019) proved that concentrations of calcium, urea or blood urea nitrogen (BUN), magnesium and cholesterol are significantly altered in incidences of reproductive conditions in cows of different breeds. It is also shown that serum biochemistry is affected by reproductive conditions in cows of different ages and parity.

For matching changes in the reproductive tract, Ultrasonography is particularly useful. Early pregnancy diagnoses, assessing fetal health, and fetal sex diagnosis, are among the US applications. Ultrasonography is not associated with higher embryonic loss. Imaging the ovaries in cattle has led to an understanding of follicular protocols for ovarian synchronization and superstimulation. Three-dimensional ultrasonography, ultrasound biomicroscopy, and computer-assisted analysis of ultrasound images will ultimately enable us to determine the precise stage of the estrous cycle based on a single examination, and the health status of individual follicles and their contained egg.

In routine clinical practice of commercial dairy farms, ultrasound scanning (US) is one of the useful tools to monitor reproductive organs since the US monitoring technique has been reported to be an accurate and reliable method of observing dynamic changes in ovarian structures **(Pierson and Ginther, 1984)** and determining the patterns of postpartum resumption of ovarian activity **(Rajamahendran and Taylor, 1990; Savio et al., 1990).** Moreover, the US technique can be very efficient and reliable method for early pregnancy diagnosis.

The period following parturition is known as the puerperium or simply the post partum period. During this time the uterus will undergo a reduction in size and empty itself of bacteria, a process known as the involution of the uterus. The uterus has a powerful self-healing capacity and should preferably be supported instead of treated. It is therefore important to understand the normal pathophysiology of the post partum uterus in order to include a prophylactic perspective so that excessive antibiotic treatments can be avoided. In addition to this the ovaries must resume normal cyclical activity. As it is customary to inseminate or mate a cow shortly after giving birth, the aim being one calf born per cow and year, it is important that the puerperium proceeds normally.

Numerous studies have been carried out to review the postpartum period in high-producing cows (**Rasbech, 1950; Morrow et al., 1969; Kindahl et al., 1992 and Sheldon, 2004**).

The present study aimed to:

1- Estimation of some hematological parameters in nulliparous Holestein heifers on the day of insemination and on 28 days post AI and comparing including (BUN ,blood glucose, P, K , Na, Mg, Zn, Se, and Cu)

2- Estimation of blood parameters in postpartum cows along 4 successive examinations with 10 days interval.

3- Relation between these hematological parameters and conception rate in nulliparous Holestein heifers.

4- Relation between these hematological parameters and PPP in cows with applying early pregnancy diagnosis on 28^{th} day of pregnancy by US examination.

5- US examination of pregnancy in heifers and different stages of postpartum period in multiparous Holestein cows.

6- Detection of the hematochemical parameters that could be used for early pregnancy diagnosis confirmed by US results.

2. REVIEW OF LITERATURE

2.1. Serum hematochemical parameters related to conception in heifers :

2.1.1. Blood urea nitrogen (BUN) :

Blood urea nitrogen (BUN) or serum urea nitrogen is the end product of the hepatic detoxification of ammonia. It is this parameter that is sometimes also used to assess liver function. Urea nitrogen concentration in blood may decrease with impaired conversion of ammonia to urea by the liver. Low serum urea concentrations are, however, not specific for liver disease. Low urea nitrogen concentration is also seen in anorectic patients consuming less protein. In ruminants that are anorectic or on a low-protein diet, rumen microbes recur to BUN as a nitrogen source for their own protein synthesis, decreasing the BUN concentration.

Digestible protein in the diet of ruminants is either degraded in the rumen or escapes to the abomasum and small intestine where it is degraded to amino acids and small peptides then absorbed into the portal blood system. Nitrogen from protein that is degraded in the rumen is used for microbial protein synthesis either by incorporation of free amino acids or small peptides liberated by the process of proteolysis or by incorporation of ammonia nitrogen that arises from deamination of amino acids. Non protein nitrogen (NPN) such as urea also can be made into ruminal microbial protein following enzymatic conversion or breakdown of the NPN to ammonia in the rumen. Yield of microbial protein produced in the rumen is maximized when the ratio of available energy (fermentable organic matter) to protein (nitrogen) is optimized. When there is an excess of nitrogen relative to energy in the rumen, ruminal ammonia concentration increases. Unused ruminal ammonia enters the portal blood through the rumen wall and is transported to the liver where it is detoxified by conversion to urea. The liver also produces urea from ammonia derived from deamination of amino acids arising from postruminal digestion and systemic protein turnover. Urea then circulates in the blood to the kidneys and is excreted with the urine or it can diffuse from the blood back into the rumen, into saliva and back into the rumen, or diffuse from the blood into milk in the case of lactating females. When there is a deficiency of dietary protein, ruminal ammonia concentrations are relatively low and the proportion of nitrogen recycled back to the rumen as urea is increased **(Hammond, 1992)**.

2.1.1.1. Relationship between BUN and conception in heifers and cows :

Blood urea nitrogen is synthesized in the liver, and can have variable concentrations without causing any adverse effects to the fertility of cattle. However, High dietary protein (nitrogen) intake resulting in BUN of greater than 17 to 18 mg/dl has been associated with an altered uterine environment and decreased fertility (reduce the conception rate, and pregnancy rate) **(Elrod and Butler,1993; Elrod et al., 1993; Ferguson et al., 1993; Butler et al., 1996).**

Elevated BUN concentration decreases uterine pH, which is thought to have a negative effect on embryo development and implantation **(Elrod and Butler, 1993).** While **Tshuma et, al., (2010)** find that Bonsmara heifers that has BUN about 11.59 mg/dl or 4.14 mmol/l on mean days to pregnancy about 24 days reach to 100% pregnancy. Blood urea nitrogen concentrations below 7 mg/dl at breeding have been reported to also be associated with reduced reproductive performance due to protein deficiency **(Carlsson and Pehrson, 1993).**

In dairy cows, high blood urea during the first 7 weeks postpartum, and low blood urea during the 10th and 13th weeks postpartum were associated with good fertility in dairy cows **(Sulieman et al., 2017).**

2.1.1.2. BUN during early pregnancy:

Abdullah et. al. (2017) stated that; the plasma BUN concentration remained at a significantly higher level during day 0 to day 20 post-AI in pregnant crossbred cows than in the non pregnant group, and maximum concentration was observed on day 28 post-AI (38.34 ± 2.70 mg/dl).Thus may indicate that the source of BUN is fetal kidney.

Veena et. al. (2015) showed that animals with lesser BUN (14.74 mg/dl) came to heat early compared to those with more amount of BUN (15.69 mg/dl).

2.1.2. Blood glucose :

Blood glucose is the main indicator of energy. Carbohydrates in the form of glucose are the principal source of energy for the life processes of the mammalian cell. All cells require a constant supply of this indispensable nutrient, and only relatively small changes may be tolerated without adverse effects upon the health of the animal.

Energy is the first limiting dietary factor for cows in early lactation and heifers' fertility. Energy demands in the form of milk output and body maintenance exceeds energy inputs in early postpartum and also somewhat significantly exceed in ovulation, conception and early pregnancy in heifers.

2.1.2.1 Relationship between blood glucose and conception in heifers and cows :

Kappel et al., (1994) was stated that, Average plasma glucose was within the normal range (62 ± 8 mg/dl). It increased before calving and then declined to a minimum value between 11 and 25 days postpartum. Glucose then increased after 25 days for the summer-calving group and remained relatively stable for the winter-calving cows. Blood glucose concentrations were inversely related to milk production. Negative correlations existed between milk production and plasma glucose at days 4, 11, 18, 25, and 39 postpartum. First-lactation heifers had higher blood glucose levels than cows in their second or later lactation. Blood glucose concentrations were not related to days-to-conception over both seasons.

Animals with more amount of blood glucose (55.49 ± 0.59 mg/dl) came to heat within 2 months compared to those with lesser blood glucose (50.20 ± 0.43 mg/dl). So there was significant correlation between blood glucose and first postpartum heat (**Veena et. al, 2015)**.

Pandey et al, (2015) concluded that, exogenous supplementation of different hormones like GnRH, hCG and progesterone influenced the levels of glucose and cholesterol in blood, which possibly helped in the biosynthesis of progesterone by the luteal cells during early phases of embryonic development and improved conception rate in crossbred cattle. In which there were significantly higher plasma glucose and cholesterol level in pregnant animals as compared to non-pregnant animals. Plasma glucose level had significantly increased between day 0 and day 5 of estrous cycle compared to control group of pregnant animals.

2.1.2.2 Blood glucose during early pregnancy:

Ali et al., (2014) work on: The influence of blood glucose level on conception rate. It is evident that the correlation coefficient between blood glucose level and conception rate was statistically non–significant on day 1(of insemination) and day 21(after AI). Pregnant cows exhibited relatively lower blood glucose concentration in contrast to non–pregnant cows on day–1 (44.22±0.79 mg/dl and 40.88±1.07 mg/dl respectively). Similarly the blood glucose content measured on day 21 (post–insemination) was higher in non–pregnant group (44.86±0.97 mg/dl) as compared to pregnant group (40.15±1.24 mg/dl).

Blood glucose (mg/dl) levels decreased significantly as pregnancy advanced in both heifers and cows. Decrease in blood glucose as gestation advances may be due to utilization of glucose for the development and growth of the fetus **(Padodar et al, 2014)**. **Green et al, (2012),** illustrated that Fetal growth within cows conceiving at first insemination compared to second or third insemination was more rapid and was associated with greater blood glucose and IGF1 early *postpartum* (before day 30).

Forshell et al, (1991), concluded that there was a significant difference for plasma glucose between cows that became pregnant and those that did not. Cows with low plasma glucose concentrations at first insemination also had low values four and seven weeks after calving, indicating that it is primarily cows with chronically low blood glucose which are likely to have reduced fertility.

3.1.3. Serum minerals:

3.1.3.1. Phosphorus:

Phosphorus has more known functions in the animal body than any other mineral. It is required for bone and tissue development, energy utilization and milk production. Phosphorus is commonly referred to as the “fertility” mineral and its deficiency can severely affect reproductive performance and may be expressed as delayed puberty (associated with poor appetite and growth rate) and increased number of services per conception. Insufficient amounts of phosphorus in the ration results in reduced milk production and consequently lower calf weaning weight.

3.1.3.1.1. Relationship between phosphorus and conception in heifers and cows :

Phosphorus has been most commonly associated with decreased reproductive performance in dairy cows. Inactive ovaries delayed sexual maturity and low conception rates have been reported when phosphorus intakes are low. In a field study when heifers received only 70-80% of their phosphorus requirements and serum phosphorus levels were low, fertility was impaired (3.7 services per conception). Services per conception were reduced to 1.3 after adequate phosphorus was supplemented. Other studies indicated that the **NRC (2006)** recommendations are adequate to maintain growth and reproductive function in heifers and cows. In another experiment, increasing phosphorus supplementation from 0.4% to 0.6% of the ration had no effect on days to first estrus or services per conception.However, in some instances, responses have been reported in the field when phosphorus supplementation was increased to 0.5% or 0.6%. The reason for these differences in response is unclear, but may be related to the availability of the phosphorus that is added to the ration or the actual amount of phosphorus consumed.

Phosphorus is stated to be one of important element for normal sexual behavior **(Kumar, 2003).** Delayed onset of puberty and silent or irregular estrus in heifers, failure of estrus and long inter calving period in cows and still born or weakly expelled calves or even embryonic death due to lack of uterine muscle tone are reported to be some of important clinical manifestation exhibited by the animals from phosphorus deficient areas **(Chaudhary and Singh, 2004).** On the contrary the excess of phosphorus renders the endometrium susceptible for infection **(Chaudhary and Singh, 2004).** Reduced fertility and reduced or delayed conceptions are the prime signs of phosphorus deficiency and this can be overcome with proper phosphorus supplementation. Whereas moderate deficiency may lead to repeat breeding condition and poor conception rate **(Kumar, 2003).**

In many areas of the USA, phosphorus will influence the reproductive rate in cows. This is especially true in the Southwestern part of the USA, where phosphorus deficiencies may reduce conception rates dramatically. In other areas of the United States, possibly associated with the fact that harvested forage adequate in phosphorus is fed, lower incidence of phosphorus deficiency may occur **(Corah, and Lusby, 2002).**

2.1.4.1.2. Phosphorus during early pregnancy :

Macro and microelements play an important role in providing reproductive functions in animals and humans (**Nezhdanov et al, 2014, Nezhdanov et al, 2015, Yanchukov et al, 2011 and Wu et al, 2003**). Calcium, phosphorus, and magnesium together provide the course of energetic and plastic processes in the organs of the reproductive system, activity of uteroplacental and fetoplacental blood flows, and development of the fetus's bone tissue.

Shabunin et al., (2017) stated that definite differences could be revealed in calcium-phosphorus metabolism in cows with physiological embryo development and death. There is no definite proof of a specific effect of calcium and phosphorus deficit on animals' reproductive system. Disorder of calcium-phosphorus metabolism in cows has a negative effect on embryo formation, probably as a consequence of a negative effect of a general metabolic and energy disorder on the balance of other trace elements. Cows had normal physiological gestation course under the increase of gestation periods demonstrated an increase of phosphorus serum level by 7.3%–8.5%. On the contrary, phosphorus serum level decreased by 9.1%– 9.8% in cows with fetal growth restriction and by 4.7%–9.8% in cows with early fetal death in comparison with the initial level.

Sharifi, et al., (2007), had been measured the different blood indicators of phosphorus red blood cells phosphorus (RBCPi), Whole blood phosphorus (WBPi), Plasma phosphorus (PPi), and Serum phosphorus (SPi) in calves, heifers, and pregnant cows. They estimated that Heifers and pregnant cows had significantly higher RBCPi and SPi concentrations, and weaned calves and dry cows had significantly higher WBPi concentrations, compared with other groups. In all groups, WBPi was significantly associated with other variables, including PCV.

2.1.4.2. Sodium and potassium :

Both of these elements are indirectly related to reproduction in animals as the deficiency of sodium can affect the normal reproductive physiology by preventing the utilization of protein and energy where as deficiency of potassium is well known to cause muscular weakness and thereby affect the musculature of female genital tract causing impairment in the normal reproductive process **(Yasothai, 2014).**

Research suggests that feeding high levels of potassium (5% DM basis) may delay the onset of puberty, delay ovulation, impair corpus luteum (yellow body) development and increase the incidence of anestrus in heifers. Lower fertility was noticed in cows fed high levels of potassium or diets in which potassium-sodium ratio was too wide.

Small, et al., (1996) found that Serum Na was higher at estrus (day of insemination) than on day 21 especially in nulliparous heifers and cows. In nulliparous heifers, serum S was also higher at estrus than on day 21 especially in animals that conceived.

Serum K concentrations in heifers and cows were similar to dairy cattle given high K diets containing from 1.6 to 4.1% K kg–1 DM **(Fisher et al. 1994).** Since blood K has been shown to be related to dietary K, it would seem that all groups consumed excess K. However, a relationship with FSC was only found in nulliparous heifers such that those that conceived had the lowest concentrations of serum K at estrus, but there was no difference on day 21. Nulliparous heifers were likely more sensitive to excess K because suckled heifers and cows would have secreted K in milk **(Fisher et al. 1994).**

2.1.4.3. Magnesium :

The fact that magnesium (Mg) is an essential mineral for life was it is the second most prevalent intracellular cation after potassium and the fourth most abundant cation in the body. Magnesium fulfills many physiological functions, with one of the best studied being the activation of enzymes. Intracellular magnesium is important for several enzymes that regulate the metabolism, and although the extracellular concentration is only 1% of the total magnesium in the body, magnesium plays a key role as an extracellular ion for nerve transmission. Plasma magnesium, just like calcium, is also found in ionized form, protein-bound and in complexes, with the ionized magnesium being the most active form. It has ability to form chelates with important intracellular anionic-ligands, especially ATP, and its ability to compete with calcium for binding sites on proteins and membranes **Ryan, (1991)**.

Mg is a constituent of bones (approximately 60 to 70% of the total body magnesium is present in the skeleton), 30-40% is distributed in the soft tissues and only about 1% can be found in the extracellular space **(Martens et al, 2000 and Blaxter et al, 2001)** have reported that the total content of Mg within the body calves: Mg (g) =0.655x-3.5g: x=body weight in kg.

The normal level of magnesium in plasma for cows is in the range of 0.75-1.00 mmol/l or 1.8-2.4 mg/dl **(NRC, 2001)**. The cow is almost solely dependent on a constant dietary uptake of the mineral since magnesium metabolism is not regulated by specific hormones **Martens et al, (2000)**. In a 500 kg db ry cows weighing 500 kg have a daily dietary requirement of 1.2 g magnesium/kg DM. For cows in lactation with a milk production of ~30 kg/day the requirement increases to 2.0 g/kg DM **Spörndly, (2003)**.

Milk contains about 0.12-0.15 g magnesium /kg and a high yielding cow may lose around 3-4 g through milk per day. Colostrum contains about 0.4 g magnesium/kg **(NRC, 2001)**.

The level of minerals in forages varies according to properties of the soil, level and type of fertiliser applied to the crop, botanical composition, and maturity of the plant **(Swift et al, 2007)**.

Generally, forages contain high levels of potassium, fairly high levels of calcium and lower levels of magnesium and phosphorus. The mineral composition of these varies; cereals are usually rich in phosphorus, oil seed residues are rich in phosphorus and potassium, and sugar beet products are rich in calcium and potassium. In several dairy cow diets, inorganic mineral supplements are added to fulfill the dietary demands of the cow. Limestone, calcium or magnesium phosphate, sodium chloride and magnesium oxide are common sources of minerals included in concentrate mixtures and mineral supplements and, in addition, sodium chloride is often fed at appetite.

2.1.4.3.1. Magnesium and conception:

Magnesium usually does not have direct impact on the reproductive status of animals, since in body it remains in almost antagonistic relation with calcium and any disturbance in Ca-P-Mg homeostasis can impart some influence on reproduction. Moreover reduced reproductive efficiency encountered loss of appetite due to magnesium deficiency **(Kumar, 2003).**

Although serum Mg levels were low, differences between FSC groups were only detected in nulliparous heifers such that serum Mg was higher at estrus in those that failed to conceive. Serum Mg levels were similar among heifers and cows, suggesting that low serum Mg was not likely a cause of poor fertility **(Small et al, 1996).**

in the early pregnancy **Shabunin et al, (2017)** showed Under fetal growth restriction cows on the 19th–23rd and 28th–32nd days of gestation the magnesium serum level was 22.2% and 23.5% lower, but for embryo death Group it was lower by 19.0% and 27.2%, respectively, in comparison with the control group of normal physiological pregnancy.

2.1.4.4. Zinc:

Zinc is known to be essential for proper sexual maturity (development of secondary sexual characteristics), reproductive capacity (development of gonadal cells) in males and all reproductive events (estrus, pregnancy and lactation), more specifically with onset of estrus in female. Among these decreased fertility and abnormal reproductive events are of prime importance in females **(Kumar, 2003)** where as in male poor semen quality, reduced testicular size and libido are the usual clinical findings **(Mass, 1987).** Apart from this zinc has a critical role in repair and maintenance of uterine lining following parturition and early return to normal reproductive function and estrus **(Greene et al., 1998).**

Favier et al, (2002), Beletskaya et al, (2014), and Neve, (1998) showed that fetal growth restriction and embryo death in dairy cows are directly connected with zinc deficiency. This element plays the most important role in the protection of DNA and transcriptional proteins from free-radical oxidation (by the induction of Cu, Zn-superoxide dismutase), and it participates in inhibition of proteinase and neutralization of lipopolysaccharides and toxic metals. Zinc is an essential element in the processes of DNA synthesis and repair, growth, proliferation, differentiation, and migration of cells and embryo- and immunogenesis. Zinc deficiency provokes the decrease of sex and corticosteroid hormones, somatomedin secretion, intensification of proinflammatory cytokine expression, and inhibition of the processes of cell proliferation and embryo growth **(Neve, 1998).** Therefore, developmental delay and embryo death in dairy cows at early gestation stages are the consequences of zinc deficiency and disorders of its metabolism in the organism. Experiment in vivo **(Chen et al, 2015)** showed that additional introduction of zinc to animals effectively protected them from lipopolysaccharide-induced intrauterine growth restriction and embryo and fetus death.

in the early pregnancy **Shabunin et al, (2017)** showed that Zinc serum level in cows of fetal growth restriction Groups and fetal death group on the 19th–23rd days of gestation was lower by 30.2% and by 35.5% in comparison with control group . Zinc blood level in cows of fetal growth restriction Group on the 28th–32nd and 38th–45th days of gestation was lower by 17.2% and 10.9%, while for those with embryo death it was lower by 25.5% and 23.1%, respectively, in comparison with control group.

2.1.4.5. Selenium:

The safety margin (difference between normal requirement and toxic dose) for selenium is so narrow that its deficiency is quite rare in farm animals than its toxicity, but causes weak, silent or irregular estrus, retained fetal membranes, early embryonic death, still birth or weak offspring and abortions in females **(Randhawa and Randhawa, 1994)** and reduced sperm mortality in males. Improvement in conception rate at first service following selenium supplementation **(McClure et al., 1986).**

In the early pregnancy **Shabunin et al, (2017)** showed that Selenium blood level in cows of fetal growth restriction decreased by 26.2% on the 28th–32nd days of gestation and in animals of fetal death by 29.1% in comparison with the control group.

2.1.4.6. Copper:

Copper is one of the important mineral for reproduction point of view as such its deficiency is reported to be responsible for early embryonic death and resorption of the embryo **(Miller et al., 1988)**, increased chances of retained placenta and necrosis of placenta **(O'Dell, 1990)** and low fertility associated with delayed or depressed estrus **(Howell and Hall, 1970).** In addition to this, proper copper supplementation is must for quality semen production **(Puls, 1994).**

Copper treatment is reported to improve conception rate as the copper treated cow require 1 service and the untreated cow require 1.15 services per conception **(Hunter, 1977).**

In the early pregnancy **Shabunin et al, (2017)** showed that Copper blood level in cows of fetal growth restriction and fetal death on the 19th–23rd days of gestation was lower by 21.6% and 21.6% , respectively, in comparison with control group.

2.2. Early pregnancy diagnosis on 28th day by using ultrasonography :

Pregnancy diagnosis is a routine tool used in the reproductive management of dairy cows to identify both pregnant and nonpregnant cows **(Pierson and Ginther, 1984; Kastelic et al., 1988; Fricke, 2002).**

Scully et al., (2014) used visual scores were used during US examination, pregnancy diagnostic 97%, on d 21to 28. Uterine echotexture was scored from 1 to 3 (1 = even texture, no nonechogenic spots; 2 = intermediate amounts of nonechogenic spots along the endometrium; and 3 = maximum amounts of nonechogenic spots on the endometrium). A final pregnancy diagnosis was made on d 30 (US exam 2) post-AI by an independent operator. Cows were retrospectively assigned as either pregnant or not pregnant based on the presence of an embryonic heartbeat.

Lamb, (2014) publicated in a prolonged review document that, Transrectal ultrasonography, more commonly called ultrasound, can be used to detect pregnancy as early as day 26 of gestation for heifers and day 28 of gestation for cows with a high degree of accuracy. For a skilled technician, the procedure is as fast as rectal palpation and may provide additional information in terms of embryo/fetus viability, incidence of twins, and potentially the sex of the fetus (usually performed around day 55 of gestation). Prior to the development of ultrasound for pregnancy diagnosis in cattle, technicians were unable to accurately determine the viability or number of embryos/fetuses. Since the heartbeat of a fetus can

be detected at approximately 22 days of age, one can accurately assess whether or not the pregnancy is viable. Producers also should be aware that early embryonic loss is a natural occurrence in cattle and may be evident between the time of pregnancy diagnosis and calving, and that this is not the result of the actual pregnancy diagnosis procedure. For example, we have observed a 4.2 percent incidence of embryonic loss in beef heifers initially examined through ultrasound at day 30 of gestation and subsequently palpated rectally between day 60 and day 90 after insemination. In beef cows, embryonic loss ranges from 3 to 8 percent from 30 to 75 days of gestation, whereas in dairy cattle, pregnancy loss from 28 to 56 days after artificial insemination is 13.5 percent. Therefore, ultrasonography provides a tool to accurately differentiate between the failure of a female to conceive and the incidence of embryonic mortality, because a heartbeat is detectable at 22 days of gestation.

Ultrasound also gives producers an opportunity to identify the sex of the fetus, which becomes distinguishable between day 55 and day 80 of gestation. Many cattle operations are developing strategies to use fetal sexing as either a marketing or purchasing tool. At approximately day 55 of gestation, male and female fetuses can be differentiated by the relative location of the genital tubercle and development of the genital swellings like the scrotum in male fetuses and the vulva in female fetuses. Ultrasound was used in an experiment conducted by the authors, in which the sex of 112 fetuses in Angus heifers was determined with 98.2 percent accuracy. For beef cattle producers, fetal sexing remains limited to purebred operations, especially in conjunction with an embryo transfer program. Determination of sex, especially after the successful transfer of embryos to recipients, allows marketing of male and female embryos before the pregnancy is carried to term. This strategy can be used effectively in dairy operations trying to produce bull calves of a particular mating for sale to bull studs. From a commercial standpoint, heifer development operations use fetal sexing as a marketing tool to provide potential buyers with females pregnant with fetuses of a specific sex. As more technicians become proficient at fetal sexing, commercial operations will use this technology to enhance the marketability and efficiency of their cattle operations. As with the costs of rectal palpation, ultrasound costs vary widely for many reasons, such as the number of females to be handled, the distance that a veterinarian must travel, or the time and facilities used. Generally, ultrasound costs will range from $4.00 to $25.00 per female. As with rectal palpation, ultrasound provides an immediate diagnosis that allows a producer to make a decision while the cow is in the chute.

2.3. Postpartum period in cows and use of ultrasound:

The postpartum period has been studied extensively because of its importance in the economic outcome of cattle operations. The main characteristic of this physiological state is a period of acyclic infertility immediately after parturition **(Callahan et al., 1971; Short et al., 1990;**

Zollers et al., 1991; Roche et al., 1992; Schrick et al., 1993; Breuel et al., 1993; Inskeep, 1995).

The postpartum period is characterized by involution of the uterus and re-establishment of ovarian function, with the main purpose being to prepare the animal for a new pregnancy. Uterine involution results from three overlapping processes: contraction, loss of tissue and tissue repair **(Gier and Marion, 1968; Kindahl et al., 1992); Yavas and Walton, 2000).** Using ultrasonography, it was confirmed that uterine involution in dairy cows was completed by 40 days after calving **(Okano and Tomizuka, 1987).**

2.3.1. Uterine involution:

After parturition the uterus is grossly enlarged and consequently a reduction in size will follow. The process is known as the involution of the uterus. Its complex nature is due to the placenta in bovines being of a cotelydonary character (**Rasbech, 1950).**

Contraction, tissue repair and loss of tissue are processes that will decrease uterine weight from approximately 9 kg to 1 kg during a 30 days period post partum (p.p) (**Rasbech, 1950 and Sheldon, 2004**).

The involution can be evaluated by rectal palpation and if the involution follows a normal pattern the entire uterus will generally be palpable at day 8-10 p.p. The reduction in weight follows an exponential pattern where a substantial decrease is seen immediately after parturition; 4 days p.p the uterine volume constitutes 50%, and by 8 days 33% of its pregnant dimensions (**Rasbech, 1950**).

The speed of the involution increases by day 10-14 p.p and is probably caused by the uterine contractions that appear at this time. The number of day's p.p when the uterus is considered being fully involuted varies among different studies but both **Morrow et al. (1968) and Rasbech, (1950)** suggests that the involution is completed by day 20-25 p.p.

The criteria for a terminated involution are: uterus in a normal position in the pelvic cavity, uterine horns being symmetrical or almost symmetrical and no thickening of the uterine wall. In addition to the uterine changes the cervix will also undergo shrinkage.

A decrease of smooth muscle and collagen as well as voiding of fluids causes this. It constricts quickly after calving and by 4 days p.p it will only admit the entrance of two fingers (**Noakes, 2009**). It takes 3-5 days longer for the cervix and the uterus in an abnormal cow to reach the same size as it does for a normal cow.

The number of lactations influences the cervical and uterine involution. Multiparous cows have a prolonged involution, which may be explained by the increased uterine size in these animals (**Morrow et al., 1969**).

Lochia is a discharge that can be seen up to 18 days p.p. It contains necrotized uterine caruncles and sloughs off in a mixture of blood from the ruptured umbilicus as well as foetal fluids with help from the myometrial contractions. It can be discharged either from the uterus (uterine lochia) or from the cervical canal and the vagina (lochia).

Uterine lochia is discharged immediately after calving and contains approximately 1400-1600 ml of fluid. It is initially dark red. After 6- 8 days p.p the volume has decreased to 500-600 ml and its character is brown-red and gelatinous. By 12-14 days it is dark red and almost impossible to measure due to its small volume. Uterine lochia contains no slime.

Lochia from the cervical canal and the vagina is initially transparent and thereafter changes character to a more chocolate coloured discharge. Before ceasing at day 16 p.p it has a cherry red colour. The total volume is approximately 500-2000 ml. Regardless of origin the lochia should not carry a disagreeable odour. Both uterine lochia and lochia are normal physiologic events during the involution and must not be mistaken for indication of pyometra or metritis (**Rasbech, 1950; Morrow et al., 1969 and Sheldon, 2004**).

New epithelial cells coat when the caruncles have been sloughed off the endometrial surface. It takes approximately 6-8 weeks before the endometrium is fully regenerated (**Sheldon, 2004**).

The involution of the uterus is influenced by a number of factors: age, time of year, abnormalities associated with calving (such as dystocia, retained foetal membranes, hypocalcaemia, ketosis, twin births and metritis) and a delayed return to normal cyclic activity in the ovaries (**Noakes, 2009**).

A good indicator of energy balance can be obtained by approximating the body condition score (BCS). This is of great importance since BCS affects fertility and is correlated to the reproductive performance (**Roche et al., 2009**)

3.2.1.1. Prostaglandin F2α:

There are many aspects that influence the course of events during the p.p period and among them oxytocin, prostaglandin F2α (PG) and the removal of the foetus are important (**Noakes, 2009**).

Among hormones PG plays a key role. In normal cows a long duration of PG release precedes parturition, which will enable a quick involution. PG is believed to have a positive effect on the involution by initiating uterine contractions. On the contrary, in abnormal cows with retained fetal membranes or uterine infection, multiple large irregular amounts of PG will be released and this in addition to the inflammatory process will render a delay in uterine involution. There is a positive correlation between complete uterine involution and resumption of ovarian cyclic activity. Conclusively multiple releases of PG will delay the commencement of ovarian cyclic activity (**Kindahl et al., 1992**).

3.2.1.2. Elimination of bacterial contamination:

The vulva, vestibule, vagina and cervix function as anatomical barriers that protect the uterus from being contaminated by bacteria. These structures enable a normal uterus to remain sterile during pregnancy. During and after calving, however, the relaxed vulva and the dilated cervix allow the entrance of bacteria in to the uterus (**Sheldon, 2004)**.

Bacterial contamination of the uterus p.p is common and Swedish studies by **Fredriksson et al., (1985)** demonstrate that 33% of the animals show bacterial growth during the first week after calving. By the second week the number of cows positive for bacterial culture has increased to 44%. These numbers are in line with Swedish results from **Bekana et al. (1996)** and English results by **Sheldon (2004)** but differ from Irish results by **Griffin et al**. **(1974)** who claim that the number of cows with a uterine infection during the early p.p period, despite a normal calving, is almost 90%. Though necrotized caruncles, blood and cell debris provides a perfect medium for bacteria to grow, very few bacteria persist and cause metritis or endometritis. In approximately 10- 17% of the cows' bacteria persist and cause uterine disease (**Borsberry & Dobson, 1989**).

Whether or not the bacteria are eliminated depends on the involution, uterine contractions, endometrial regeneration and defense mechanisms such as leukocyte migration, phagocytosis and inflammatory mediators. Due to this inflammatory response an elevated rectal temperature is often seen in dairy cattle within the first ten days after calving (**Sheldon, 2004).**

Factors that delay the elimination of bacterial contamination are the size of the bacterial load, the character of the bacterial flora, retained foetal membranes (RFM), and depression in the cow's immune status (**Sheldon, 2006 and Noakes, 2009).** Common problems among dairy cows are retained foetal membranes (RFM), which may lead to uterine infection. Foetal membranes are defined as retained if they have not been excluded within 24 hours after calving. There are different definitions of uterine infections, i.e *puerperal metritis, clinical endometritis, subclinical endometritis* and *pyometra***.** Fever, systemic illness and an enlarged uterus discharging watery or purulent fluid characterizes puerperal metritis if clinical signs are detected < 21 days p.p. Clinical endometritis is defined by a purulent discharge in the vagina (>21 days p.p) or a mucupurulent discharge (>26 days p.p), but is not associated with systemic illness. A uterine infection can be defined as a subclinical endometritis when signs of endometritis are absent, but when a cytology sample from the uterus contains more than 18% neutrophiles (21-33 days pp) or more than 10% neutrophiles (34-47 days pp). Pyometra is characterized by an accumulation of purulent material in the uterus in addition to a persisting corpus luteum and a closed cervix **(Sheldon et al., 2005)**. Retention of foetal membranes and or in combination with bacterial infection has proven to delay uterine involution (**Königsson et al., 2001**).

In cows with RFM 100% are positive regarding bacterial findings during the first 3 weeks p.p. This may be explained by the fact that RFM interferes with the uterine contractility and enables bacteria to grow rapidly by providing them a substrate (**Königsson et al., 2001**). Regardless of having RFM or not, by 7 weeks 100% are bacteriological negative (**Fredriksson et al., 2010**).

The most common species of bacteria isolated from the uterine lumen p.p are *Escherichia coli, Streptococci, Arcanobacterium pyogenes, Bacillus licheniformis, Prevotella spp* and *Fusobacterium necrophorum.* Among bacteria that are associated with uterine infection (pathogenic species) the most common findings are *E.coli, A. Pyogenes, F. necrophorum, A. pyogenes, Bacillus licheniformis, Clostridius perfringens, P. melaninogenicus, Enterococcus faecalis, Klebsiella pneumonia, E. coli M. haemolyticam, Micrococcus species, F. necrophorum, Pasteurella multocida, Providencia stuartii, Peptostreptococcus species, Proteus species, Staphylococcus aureus Staphylococcus species, coagulase negative, Non-haemolytic Streptococci* - *Haemolytic Streptococci, Streptococcus acidominimus, and ,Aspergillus species.*

When studies have been made to evaluate whether the appearance and odour of vaginal mucus reflects the bacterial findings in the uterus, it has been found that *A. pyogenes, Proteus species* and *F. necrophorum* are related with purulent or mucupurulent vaginal mucus. Furthermore, *A.pyogenes, E.coli, non-haemolytic streptococci and M. haemolytica* are associated with a foul mucus odour (**Williams et al., 2005**).

Using a method described by **Williams et al., (2005)**, collecting mucus with a gloved hand and where the hand does not remain inside the vagina for more than 30 s, it has been validated that the examination does not cause a bacterial contamination of the uterus nor prolongs uterine involution (**Sheldon et al., 2002**). The bacterial status of the p.p uterus is of great importance since bacteria is known to cause inflammation and damage the endometrium and thereby prolong the uterine involution (**Sheldon et al., 2003**). Additionally it affects ovarian activity by suppressing pituitary lutenizing hormone secretion and thereby influencing the growth and the function of the follicles **(Sheldon et al., 2002).**

3.2.2. Return of ovarian function:

A large healthy estrogenic follicle at the time of ovulation is important for establishment of a successful pregnancy **(Perry et al., 2005).** However, as well as effects on luteal function, uterine infection also perturbs ovarian follicle growth and function **(Sheldon et al., 2002).** Cows with uterine disease have smaller ovarian follicles and lower peripheral plasma estradiol concentrations. Furthermore, this might be a

localised effect of uterine infection on ovarian function because when uterine bacterial growth scores were high fewer first (1/20 vs. 15/50, $P < 0.05$) or second postpartum dominant follicles (1/11 vs. 13/32, $P < 0.05$) were selected in the ovary ipsilateral to the previously gravid uterine horn than the contralateral ovary **(Sheldon et al., 2002).**

At the level of the hypothalamus and pituitary, the oestradiol-induced preovulatory LH surge is blunted when bacterial endotoxin is infused into the uterus or administered intravenously **(Peter et al., 1989, Peter et al.,** 1990 **and** Battaglia et al., 1999**).** Indeed, LPS or various intermediary cytokines such as interleukin (IL)-1 or tumour necrosis factor (TNF)-α block gonadotrophin releasing hormone (GnRH) secretion and the pituitary responsiveness to GnRH pulses **(**Rivest et al., 1993**,** Battaglia et al., 1999 **and** Williams et al., 2001**).** However, uterine infection does not appear to affect peripheral plasma FSH concentration profiles, or ovarian follicle wave emergence.

3.2.3. Ultrasound:

Usage of ultrasound when diagnosing the involution of the uterus is a good technique as it enables quantification and visualization of results. In addition to this the diagnosis of the involution is a far more objective method than rectal palpation. However, if a combination of ultrasound and rectal palpation is used, the involutionary progress admits even more precise results **(Okano & Tomizuka, 1987).** Furthermore this technique provides quick and non-invasive measure to the inner reproductive organs **(**Sheldon et al., 2002**).**

Basic principles of ultrasonography may be explained as high frequency sound waves emitted from a transducer and depending on the tissue these waves encounter, they may either be propagated or reflected to the transducer. Reflected sound waves are then converted to an image on the ultra sound screen. It is the density of the tissue that will determine to what extent the sound waves will be reflected. Dense tissues such as bone or the bovine cervix will reflect almost all of the sound waves and render an almost white image on the screen. Such tissues are referred as hyper echoic. Less dense tissues such as the uterus or the ovaries will reflect the sound waves to a varying degree, which in turn will produce an image of various shades of grey **(Sheldon et al., 2005)**.

The probe is held in a perpendicular angle in relation to the uterine horn, and therefore the horn is represented as a cross-section. The crosses indicate the uterine horn boundaries and measures 1.41 cm after uterine involution. Within the boundaries a dark irregular line can be seen, which confines the uterine lumen. Bovine uterine horns are naturally curled and to the right in the same image another section of the same uterine horn can be seen. Clear liquids do not reflect the sound waves but simply propagate them and are referred as non-echoic. This will render a black image on the screen and an example of this could be fluid in the uterine lumen or follicular fluid in the follicles of the ovaries **(Sheldon et al., 2002).** Air reflects the sound waves completely and it is therefore of great importance not only to use gel as a coupling medium but also ensure that the transducer is placed directly on the rectal mucosa as air otherwise will reduce the image quality **(Sheldon et al., 2005)**.

The frequency reflects the numbers of vibrations from the sound source per second. When using a low frequency a large area can be investigated due to the sound waves penetrating the tissue, but on the expense of detail in the image. With higher frequency greater detail is accomplished but within a smaller area. Normally a transducer with a frequency between **5.0-7.5 MHz** is used when studying the ovaries and the uterus. The transducer is preferably a linear array scanner; transrectal ultrasonography acquires sound waves that are emitted perpendicular to the transducer. The resulting image will be rectangular, where the image of tissues closest to the transducer will appear at the top of the screen **(Pierson et al., 1988).**

3. MATERIALS AND METHODS

3.1. Animals :

This study was carried out in two farms (El-Bayoumy private farm and El-Reef El-Araby Private farm).

The animals were divided into 2 main groups:

Group I: include the 42 nulliparous heifers.

Group II: include 13 multiparous cows.

3.1.1. Group (I):

This group includes forty- two (42) nulliparous Holstein Friesian - heifers aging from 393-669 days and weighing about mean 369 kg body wt in a private farm (heifers' station) in Gamasa, Al-Ismailia province. All animals had good nutrition and water supply, well sheltered and vaccinated according to the vaccination program of the station. All animals are reared in the station coming from the dairy cows' station after each parturition and weaning, and moreover also had registered records per each head. The animals are fed on TMR (Total Mixed Ration) with balanced composition**.**

The choosed animals were classified into 2 subgroups according to conception rate as follow:

3.1.1.1. Subgroup A:

n=28; heifers that had been conceived and become pregnant; 27 heifers had been conceived from the first service per conception, and one from 2^{nd} services.

All heifers have normal structure of ovary and uterus.

3.1.1.2. Subgroup B:

n=14; heifers that had been failed to be conceived after more than 2 services per conception. 5 heifers have RCL and administrated PGF2 alpha and 2 heifers have follicular cyst on the right ovary.

The animals were submitted for blood sampling on day of insemination (A.I), and 28 days after insemination for estimation of heamatochemical parameters related to estrus, conception and early pregnancy. Ultrasound examination for early pregnancy diagnosis on day 28 of pregnancy (28 days after AI) for detection of pregnancy and conception rate and percentage.

3.1.2. Group (II):

This group include 13 multiparous cows (parity no =2-3) aging from 3-5 years with average BCS ranged between 5-6, and weighing about mean 456 kg body weight in a private farm in El-Reef El-Araby – Giza . All animals were in the postpartum period after calving. All animals had good nutrition and water supply, well sheltered and vaccinated according to the vaccination program of the farm. The animals were fed on TMR (Total Mixed Ration) with balanced composition. This group of postpartum cows was subdivided into 2 subgroups

3.1.2.1. Subgroup A:

N=8; Postpartum cows that are normal PPP and normally cyclic animals

3.1.2.2. Subgroup B:

N=5; Postpartum cows that have cystic ovaries; 4 of them have luteal cyst and the another one has follicular cyst

3.2. Blood sampling:

Lindquist, (2019) advised that blood samples obtained from cattle are very useful for a variety of means, from testing for disease to checking pregnancy status. The quick and relatively simple means of taking blood is low stress and almost painless for the animal. However, knowing where and how to place the needle to quickly get a sample is important. Your veterinarian can show you where and how to take a blood sample, though this article will also guide you in following the proper procedures for two locations with two different types of blood sampling. Two key locations are used for taking blood samples: From the coccygeal vein of the tail-head, and the jugular vein of the neck. There are also two methods of blood sampling: With the classic needle and syringe, and exiting the contents of the syringe into a vaccutainer tube, and with a double-pointed needle that draws blood into a vaccutainer tube, no syringe required.

To make things simpler, the classic needle and syringe is coupled with obtaining a blood sample from the tail-head, and the double-pointed needle holder is coupled with taking blood from the jugular vein. You can use either blood-drawing method from either the tail-head or the neck.

3.2.1. Group (I):

At the time of insemination and 28 days later, blood samples were collected from all animals by jugular venipuncture into nonheparinized (20-mL) evacuated tubes. Blood samples were refrigerated overnight to allow clot formation, then centrifuged 3000 r.p.m. for 10 min and sera removed and stored frozen till the further examination.

3.2.2. Group (II):

The blood samples were collected from each cow by jugular vein puncture 4 times after calving with 10 days interval between samples per each cow coinciding with ultrasound examination of uterus and ovary.

The blood samples were collected into non heparinized (20-mL) evacuated tubes. Blood samples were refrigerated overnight to allow clot formation, then centrifuged 3000 r.p.m. for 10 min and sera removed and stored frozen till the further examination.

3.3. Serum analysis:

3.3.1 BUN:

Blood urea nitrogen level was assessed according to (**Stephens, 2018**).

3.3.2. Blood glucose:

Blood glucose concentration was estimated through a commercial kit being operated on enzymatic colorimetric mechanism **(Trinder, 1969).**

3.3.3. Serum Phosphorus:

Estimation of inorganic phosphate in serum was assessed according to (**Muñoz e. al, 1983**).

3.3.4. Sodium:

The sodium level was estimated according to **Jacobs & Hoffman (1931).**

3.3.5. Potassium:

The potassium level was estimated according to **Jacobs & Hoffman (1931)**

3.3.6. Magnesium:

The magnesium content of blood serum may be accurately determined by atomic absorption measurements. Analysis can be carried out on as little as 0.05 ml of serum and the only preliminary treatment necessary is dilution of the sample with water containing about 1 per cent of ethylenediaminetetracetic acid or of strontium chloride.

3.3.7. Zinc:

Serum zinc concentrations were measured and determined by potentiometric stripping analyzer (PSA) according to **Kojouri, et al, (2009).**

3.3.8. Selenium:

Serum selenium concentrations were measured and determined by potentiometric stripping analyzer (PSA) according to **Kojouri, et al, (2009).**

3.3.9. Copper:

Serum copper concentrations were measured and determined by potentiometric stripping analyzer (PSA) according to **Kojouri, et al, (2009).**

3.4. Experiments:

3.4.1. Estimation of blood parameters in heifers (Group I):

Estimation of blood parameters and mineral profile in the nulliparous heifers in both group A (Conceived heifers) and group B (Failed heifers) on both day of AI and 28 days Post AI

3.4.2. Relation between blood parameters and reproductive efficiency in nulliparous heifers:

3.4.2.1. Relation between blood parameters and pregnancy:

In which, comparison occurred between the values of blood parameters in the day of AI (as control) and the values of them after 28 days of the same conceived heifers (group A = 28). From which estimates the parameters which affected on and by pregnancy. Through estimation their significance (P value) statistically by using [Wilcoxon Signed Rank Test].

3.4.2.2. Relation between blood parameters and estrus in heifers:

In this experiment, a comparison was carried out between the levels of blood parameters on the day of AI or the day of estrus in the conceived heifers (Group A = 28) as a control group and their levels in the same day in the failed heifers (Group B = 14). From which, estimated the parameters which affect negatively or positively on conception in the day of AI. Through estimation their significance p value statistically by using [Mann Whitney U test ***SPSS***].

3.4.3. Estimation of blood parameters in the Postpartum dairy cows (Group II):

Estimation of levels of blood parameters from serum analysis according to the German method by mention their detailed results in both normal postpartum cows (Group A = 8) and Cystic postpartum cows (Group B = 5). Calculating the Mean and Standard deviation of each parameter within 4 successive examinations with 10 days intervals.

3.4.4. Relation between blood parameters and postpartum period in cows:

By comparison between the levels of blood parameters of normal postpartum cows (Group A = 8) and Cystic postpartum cows (Group B = 5).

From which, relationship and significance values can be detected statistically by using [Mann Whitney U test ***SPSS***].

3.5. Ultrasound Examination:

3.4.1. Group I:

An ultrasound examination of heifers was done by Means of 7 MHz linear-array, real-time, B-mode ultrasound scanner 240 Vet (SonoScape Company).

All groups of heifers are examined by the ultrasound transrectally on the 28th day post AI for early diagnosis of pregnancy coinciding with blood sampling from jugular vein puncture.

Diagnosis of early pregnancy (on the 28th day) by US depend in this study on presence of embryonic vesicle in the top of uterine horn which characterized by anaechoic cavity surrounded with hyperechoic wall and contain hyperechoic fetus, estimation of fetal heart beat/rate, asymmetry of uterine horn , and estimation of CL on the ipsilateral ovary according to **Agag, (1996)** and **Kandiel, (2002).**

3.4.2. Group II:

on the other hand the group of multiparous cows was examined by transrectal ultrasound during the postpartum period on 4 successive examination with 10 days interval between each examination per each cow coinciding with blood sampling from jugular vein .

The ultrasound examination of postpartum cows in this study is restricted on estimation of uterine diameter (for assessment of uterine involution) in addition to estimation of ovarian follicles and other structures (for assessment of ovarian activity) till the first post partum estrus.

The diameter of the post-gravid horn (mm) was estimated by taking the largest distance from the uterine serosa from side to side through cross section in the uterine horn by crossing the ultrasound probe against the horn length.

The ovarian follicles was detected on both ovaries by the US and classified into small sized follicles, medium sized follicles, and large sized follicles.

3.6. Statistical Analysis:

The obtained data are tabulated and statistically analyzed, where appropriate, by using **IBM SPSS Program Statistics (2015).**

4. RESULTS

4.1. Estimation of blood parameters in heifers (Group I):

4.1.1. Subgroup A : Conceived Heifers:

The number of heifers which diagnosed as pregnant after 28 days of AI was 28 of 42 heifers (28/42) with pregnancy rate 66.6%. Representative ultrasonography of pregnant uterus in heifers at day 28 post AI (ultra sonogram 1&2).

Estimation of the blood parameters at the day of insemination (day 0) was as following: The mean ±SD serum level of blood metabolites or (BUN and blood glucose) was 14.26 ± 1.45 and 54.11 ± 5.94 mg/dl respectively. The mean ±SD serum level of mineral including phosphorus , magnesium , sodium , potassium , zinc ,copper, and selenium was 3.79 ± 0.20 mmol/l , 0.68 ± 0.05 mmol/l , 124.94 ± 0.96 mmol/l , 4.91 ± 0.70 mmol/l , 11.56 ± 0.49 μmol/l ,10.76 ± 0.60 μmol/l ,and 5.32± 0.56 μmol/l respectively (Table1 & Figure1).

After 28 days of AI, the mean ±SD serum level of BUN, blood glucose, Ph, Mg, Na , K , Zn , Cu , and Se in conceived nulliparous heifers, was 33.75 ± 5.57 mg/dl, 61.65 ± 4.54 mg/dl , 3.85± 0.16 mmol/l, 0.66 ± 0.076 mmol/l, 122.53 ± 1.61 mmol/l , 4.68 ± 0.22 mmol/l, 12.53 ± 0.43 μmol/l, 9.42 ± 0.78 μ mol/l, and 6.29 ± 0.70 μ mol/l respectively (Table1 & Figure1).

There was a highly significant ($P < 0.0001$) increase in BUN, glucose, Zinc, and selenium and a highly significant ($P < 0.0001$) decrease at day 28 day post AI as compared to those at day of AI (estrus) in conceived nulliparous heifers. In the meantime, there was non significant difference in the Mean ±SD of P, Mg, Na, and K+ on both times in these animals.

Table 1: Serum mean ±SD levels of blood parameters on day of AI and 28 days later

Sample	BUN (mg /dl)	blood glucose (mg/dl)	P (mmol/l)	Mg (mmol/l)	Na mmol/l	K (mmol/l)	Zn (µmol/l)	Cu (µmol/l)	Se (µmol/l)
Day of AI	14.26 ±1.45	54.095 ±5.94	3.79 ±0.20	0.68 ±0.05	124.9 ±0.96	4.91 ±0.70	11.56 ±0.49	10.76 ±0.60	5.32 ±0.56
28 Days after AI	33.75 ±5.57	61.65 ±4.54	3.85 ±0.16	0.66 ±0.08	122.5 ±1.61	4.68 ±0.22	12.53 ±0.43	9.42 ±0.78	6.29 ±0.70

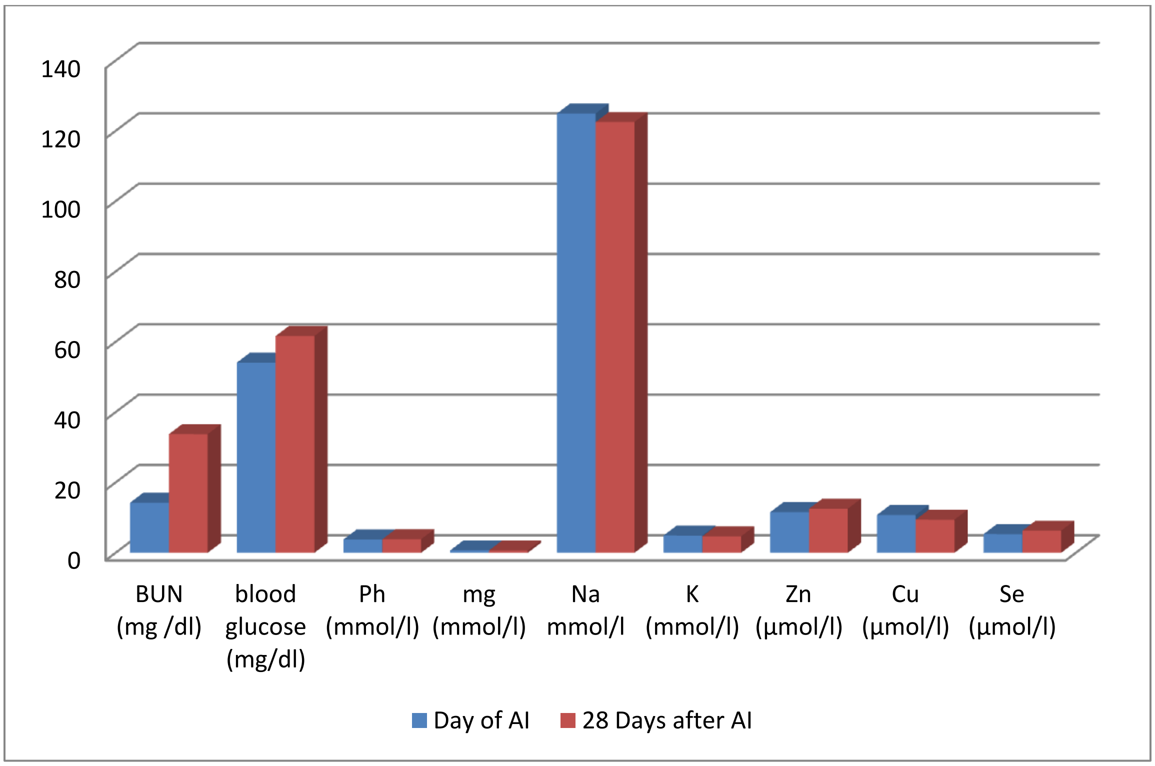
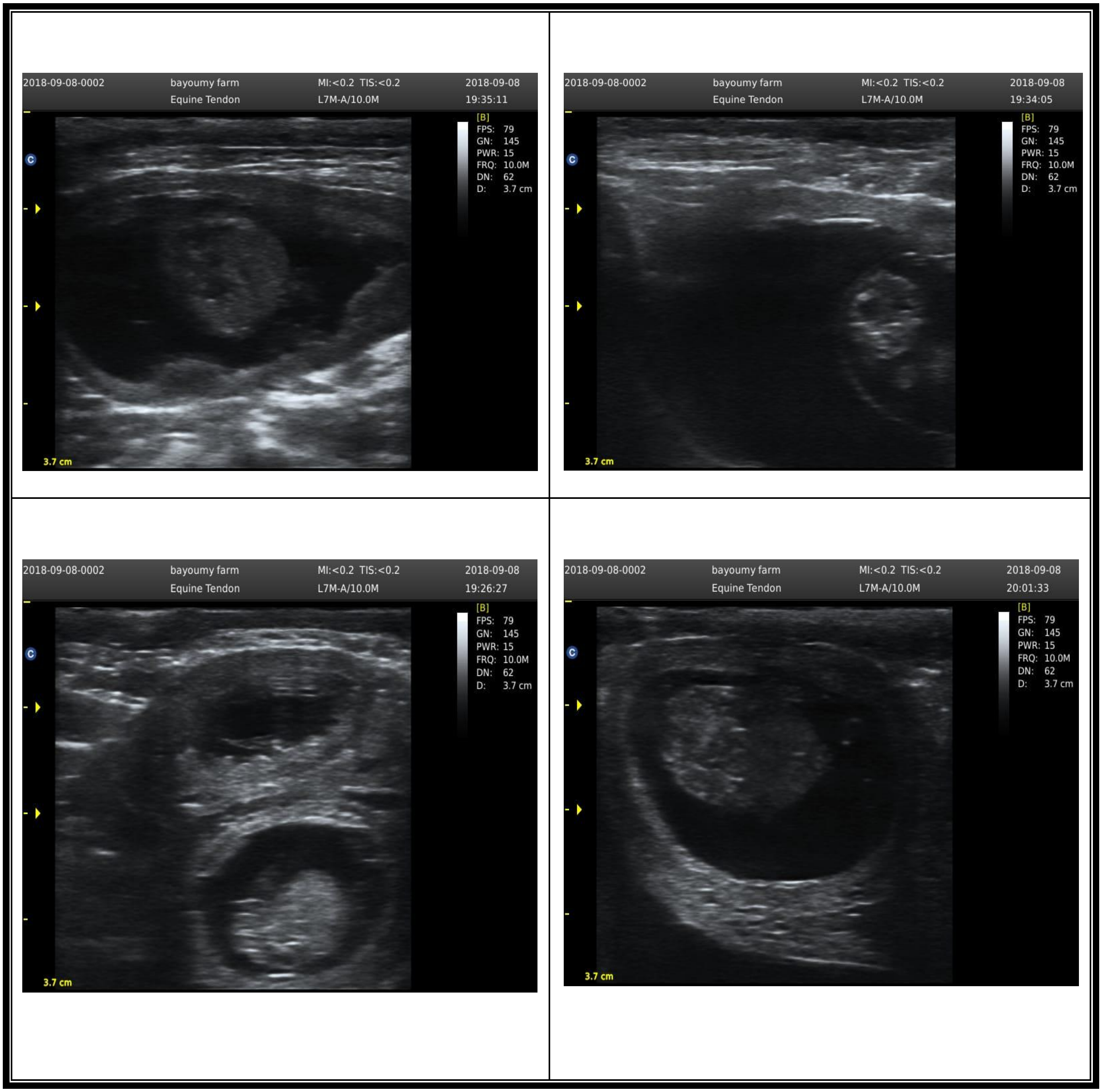
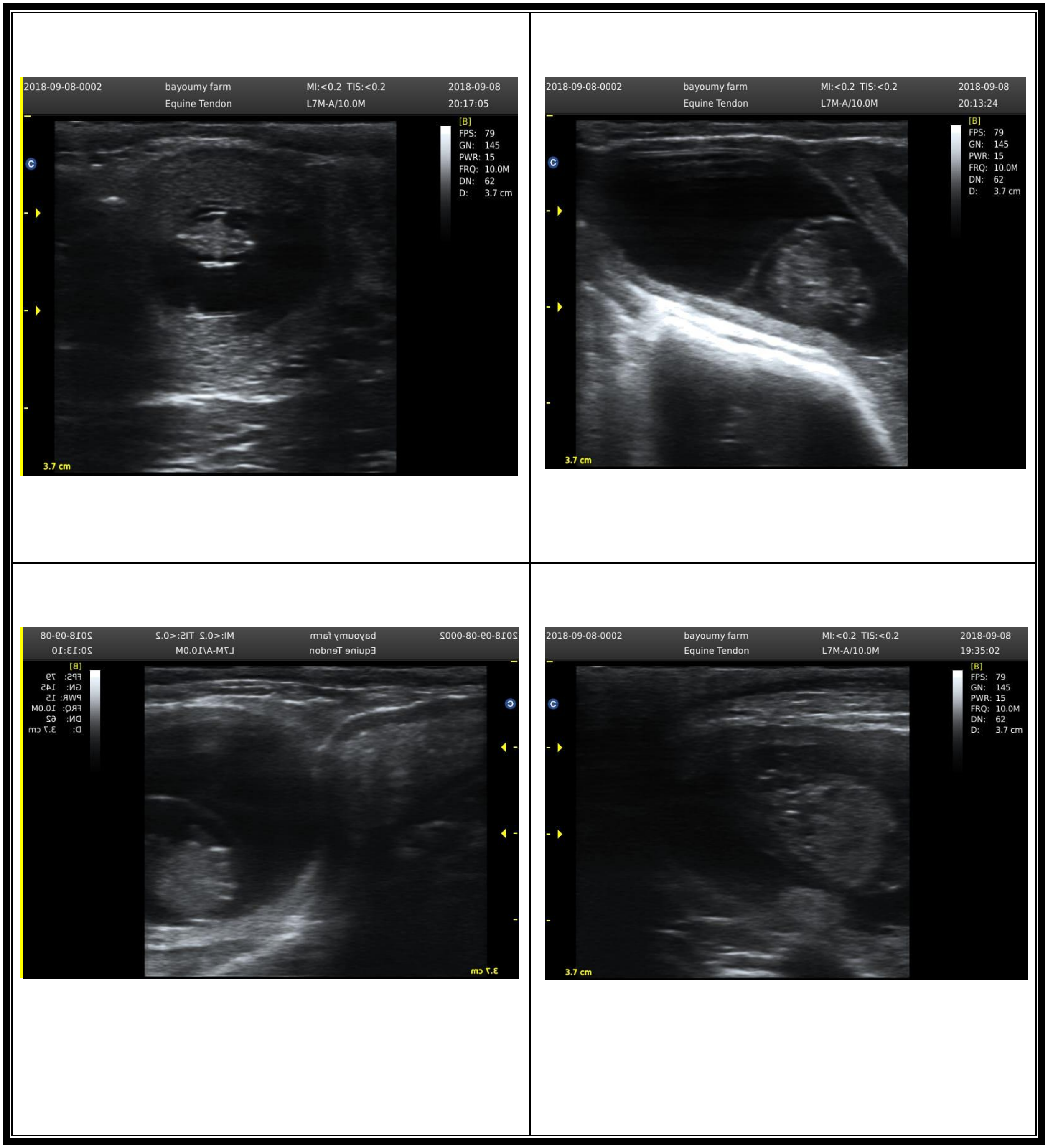


Figure 1: Blood parameters on day of AI and 28 days post AI in the conceived heifers.



Ultrasonogram (1) Representative ultrasound image of conceived heifers at day 28 post AI. Note, the fetus appeared as hyperechoic mass with CVRL measuring about 1 cm inside anechoic amniotic vesicle surrounded with hyperechoic wall



Ultrasonogram (2) Representative ultrasound image of conceived heifers at day 28 post AI. Note, the fetus appeared as hyperechoic mass with CVRL measuring about 1 cm inside anechoic amniotic vesicle surrounded with hyperechoic wall

4.1.2. Subgroup B : Non-Conceived (Failed) heifers:

The result of serum analysis for blood parameters in non- conceived heifers on the day of AI and 28 days after AI are presented in table (2) and fig (2). There was a non significant difference in the recorded serum levels of blood parameters in the non-conceived heifers between the day of AI and 28 days after AI, except the mean ± SD serum levels of BUN ($P<0.01$), sodium ($P<0.0001$) and potassium ($P<0.0001$) was noticeably decreased at 28 after AI as presented in table (2) and figure (2).

Table 2: Serum levels of blood parameters on day of AI and 28 days later in the non conceived heifer

	BUN (mg /dl)	Blood glucose (mg/dl)	Ph (mmol/l)	Mg (mmol/l)	Na (mmol/l)	K (mmol/l)	Zn (µmol/l)	Cu (µmol/l)	Se (µmol/l)
Day of AI	19.63 ±1.072	41.91 ±2.84	3.82 ±0.24	0.68 ±0.06	125.05 ±0.45	5.52 ±0.32	11.45 ±0.32	12.75 ±0.53	4.71 ±0.44
28 Days later	19.05 ±1.08	41.13 ±2.67	3.76 ±0.28	0.68 ±0.05	116.42 ±4.40	4.78 ±0.12	11.39 ±0.30	12.84 ±0.45	4.66 ±0.65

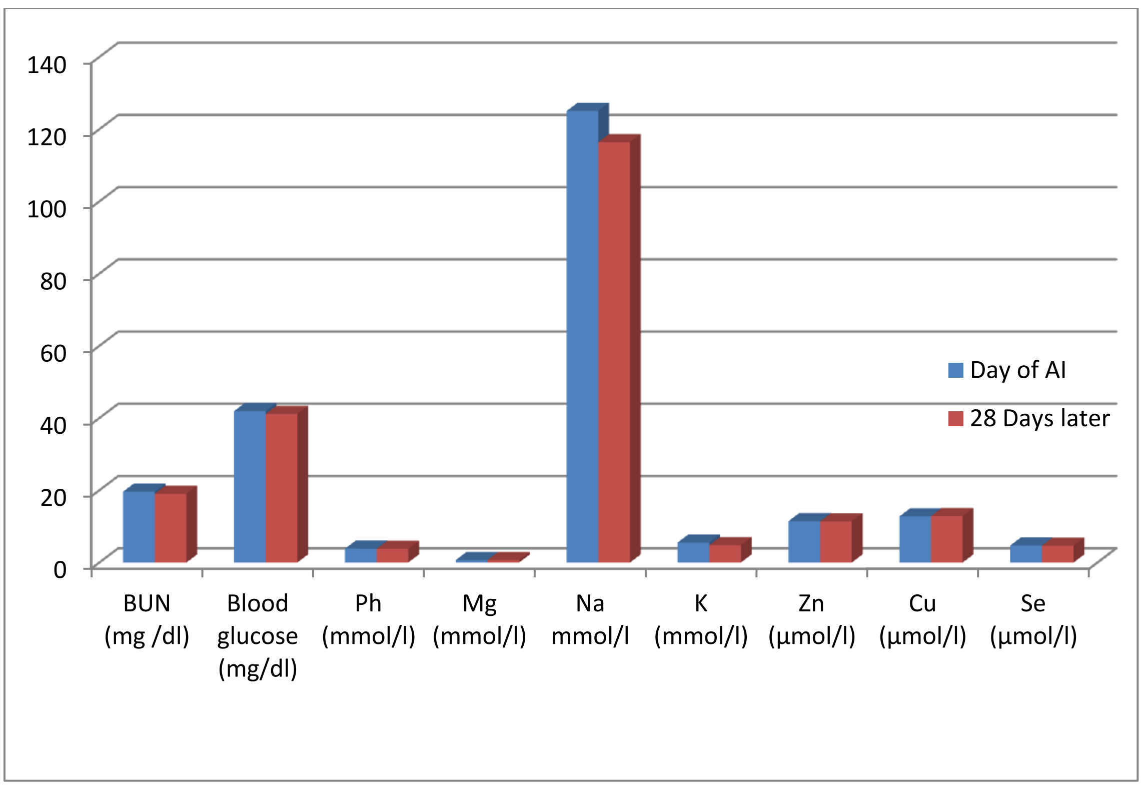


Figure 2: Changes in blood parameters in non-conceived heifers on day of AI and at 28 days later.

4.2. Relation between blood parameters and reproductive efficiency in heifers:

4.2.1. Relation between blood parameters and pregnancy

4.2.1.1. BUN and Blood glucose:

The BUN and blood glucose levels were significantly (P<0.01) higher at the 28th day of pregnancy (33.75 ± 5.57 and 61.65 ± 4.54 mg/dl) when compared to the day of estrus or AI (14.26±1.45 and 54.095 ± 5.94 mg/dl) respectively as presented in table (1) & (2) and Figure (3), (4) & (5).

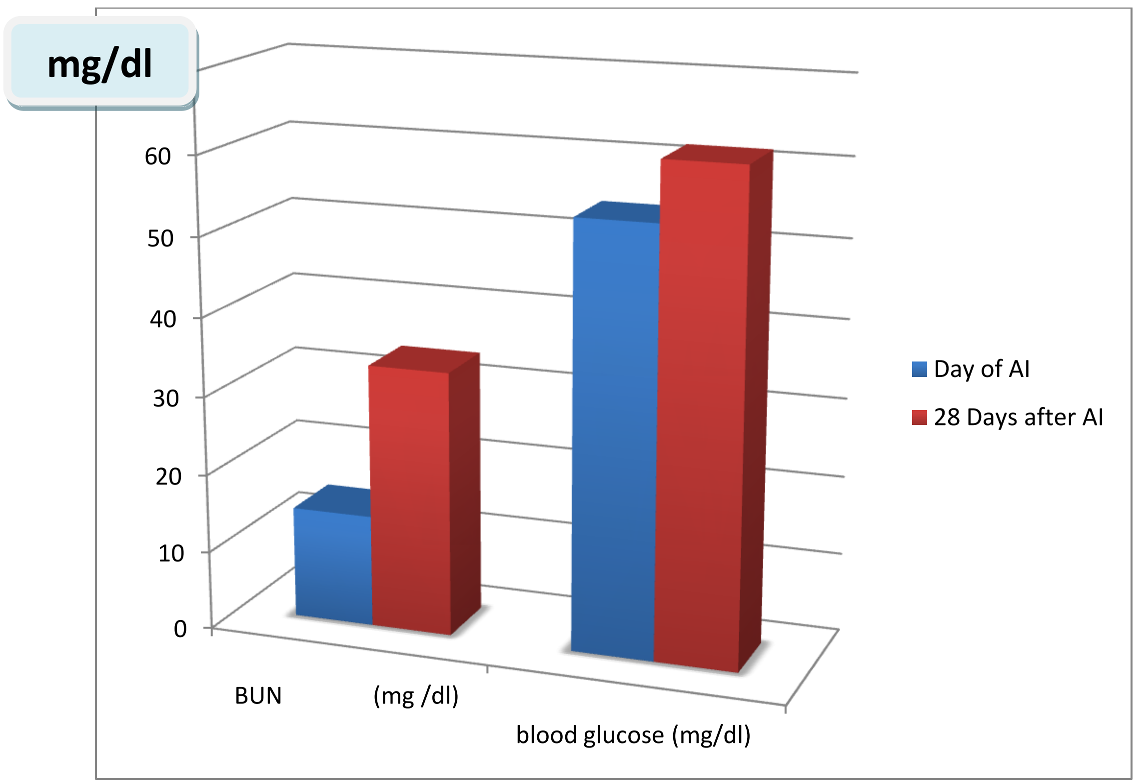


Figure 3: Relationship between BUN and blood glucose and conception and pregnancy in heifers.

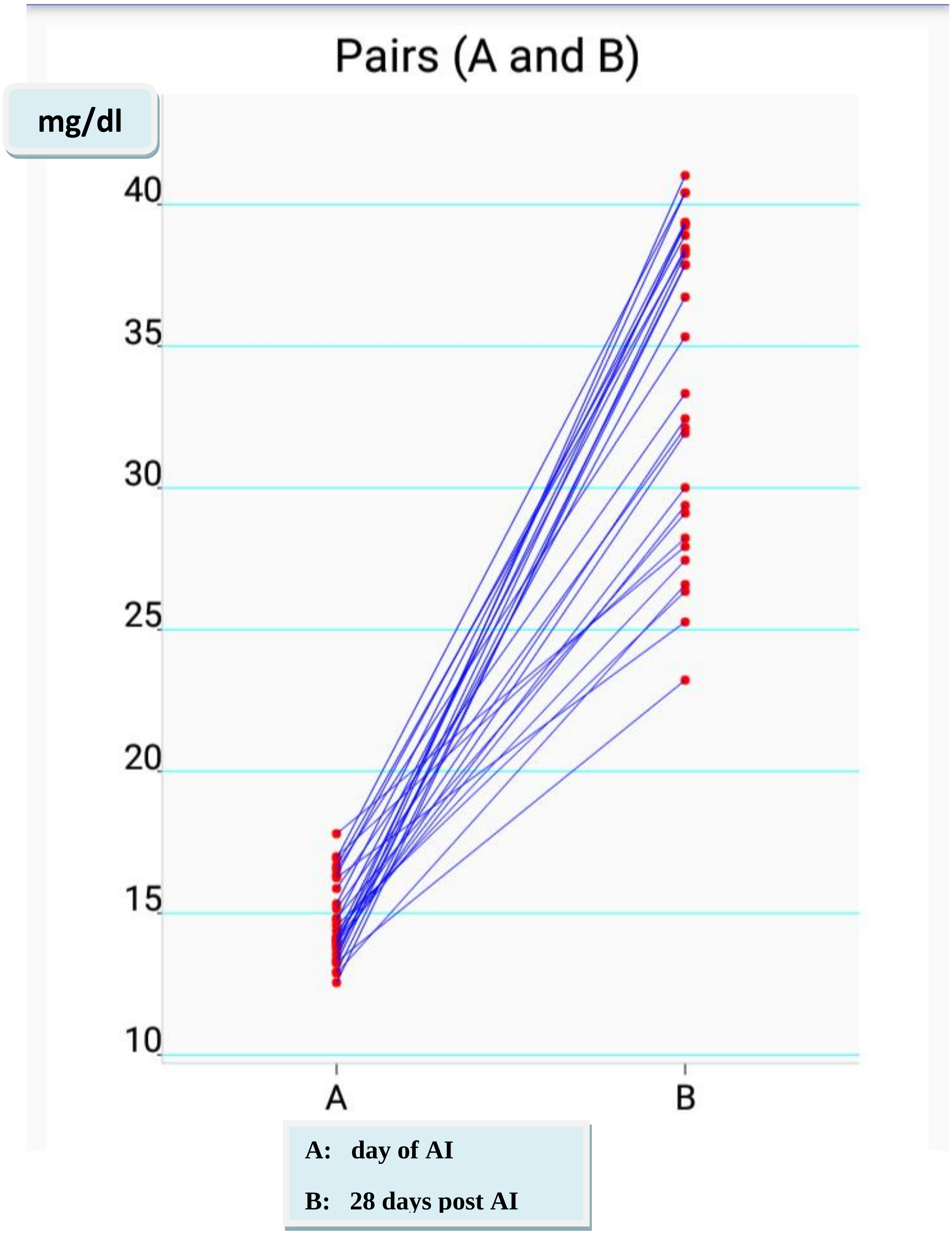


Fig 4: Serum BUN levels during conception and pregnancy in the conceived heifers.

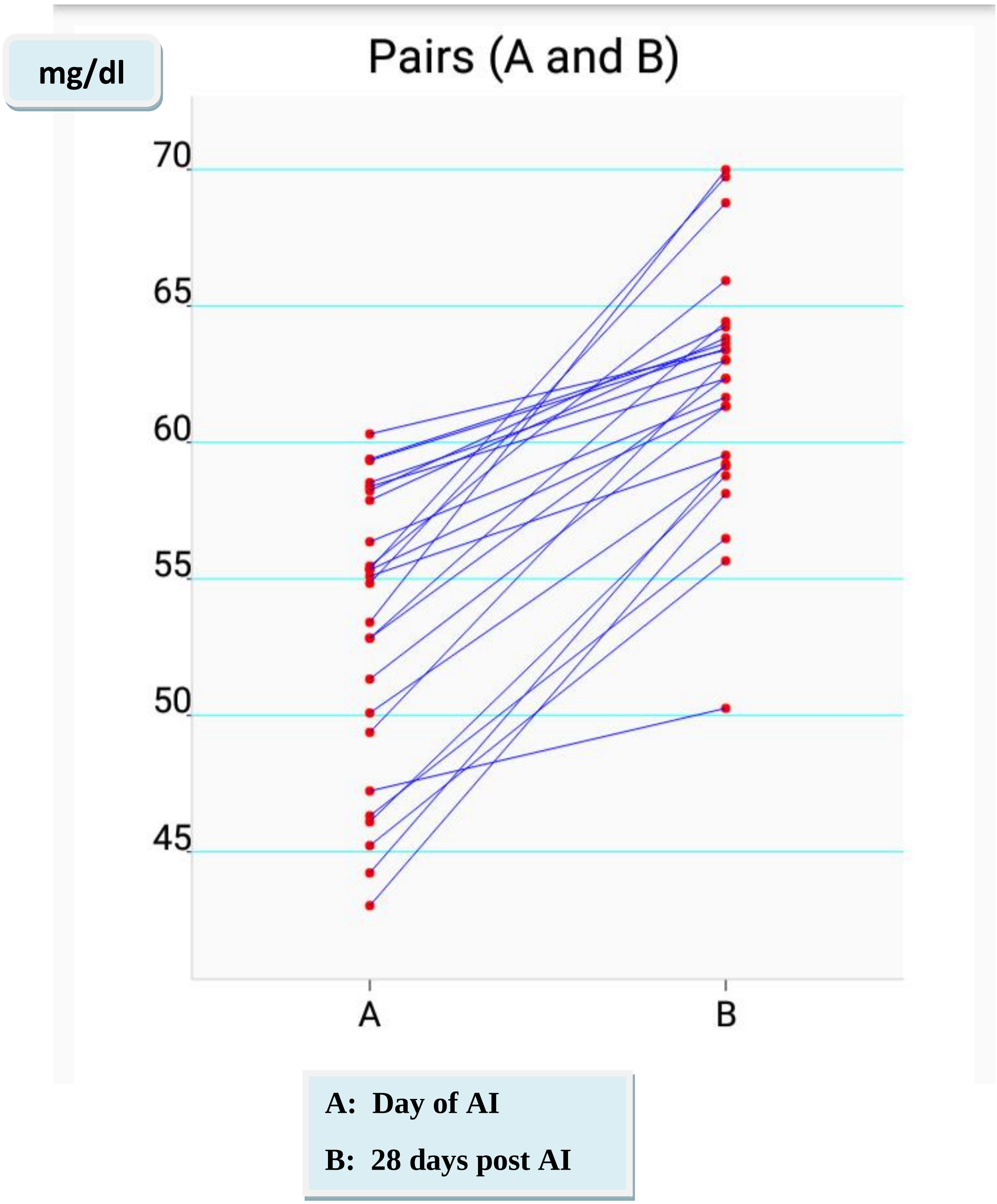


Figure 5: Blood glucose level in the conceived heifers at the day of AI and 28th day of pregnancy

4.2.1.2. Phosphorus and Magnesium:

There was a non-significant difference ($p > 0.05$) in phosphorus and magnesium levels on both day of AI and the 28th day of pregnancy in the conceived heifer group (Figure 6).

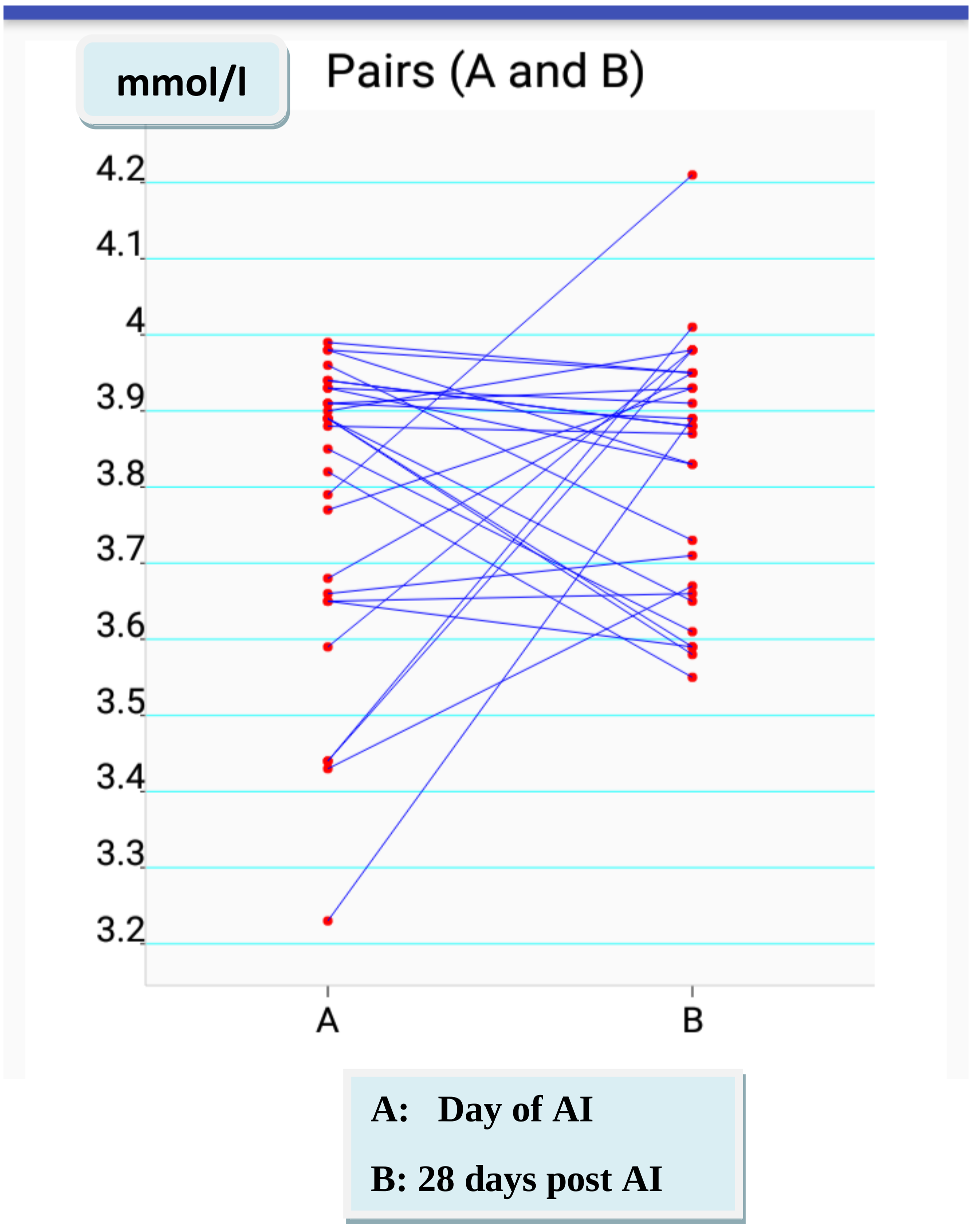


Figure 6: Serum levels of phosphorus in the conceived heifers on the day of AI and the 28th day of pregnancy

4.2.1.3. Sodium and Potassium:

There was a significant decrease ($P < 0.01$) in the sodium levels on the 28th day of pregnancy in comparison with its level on the day of AI in the conceived heifer group (Figure 7).

On the other hand there was a significant increase ($P < 0.01$) in the Potassium level on the 28th day of pregnancy higher than its level on the day of AI in the conceived heifers (Figure 8).

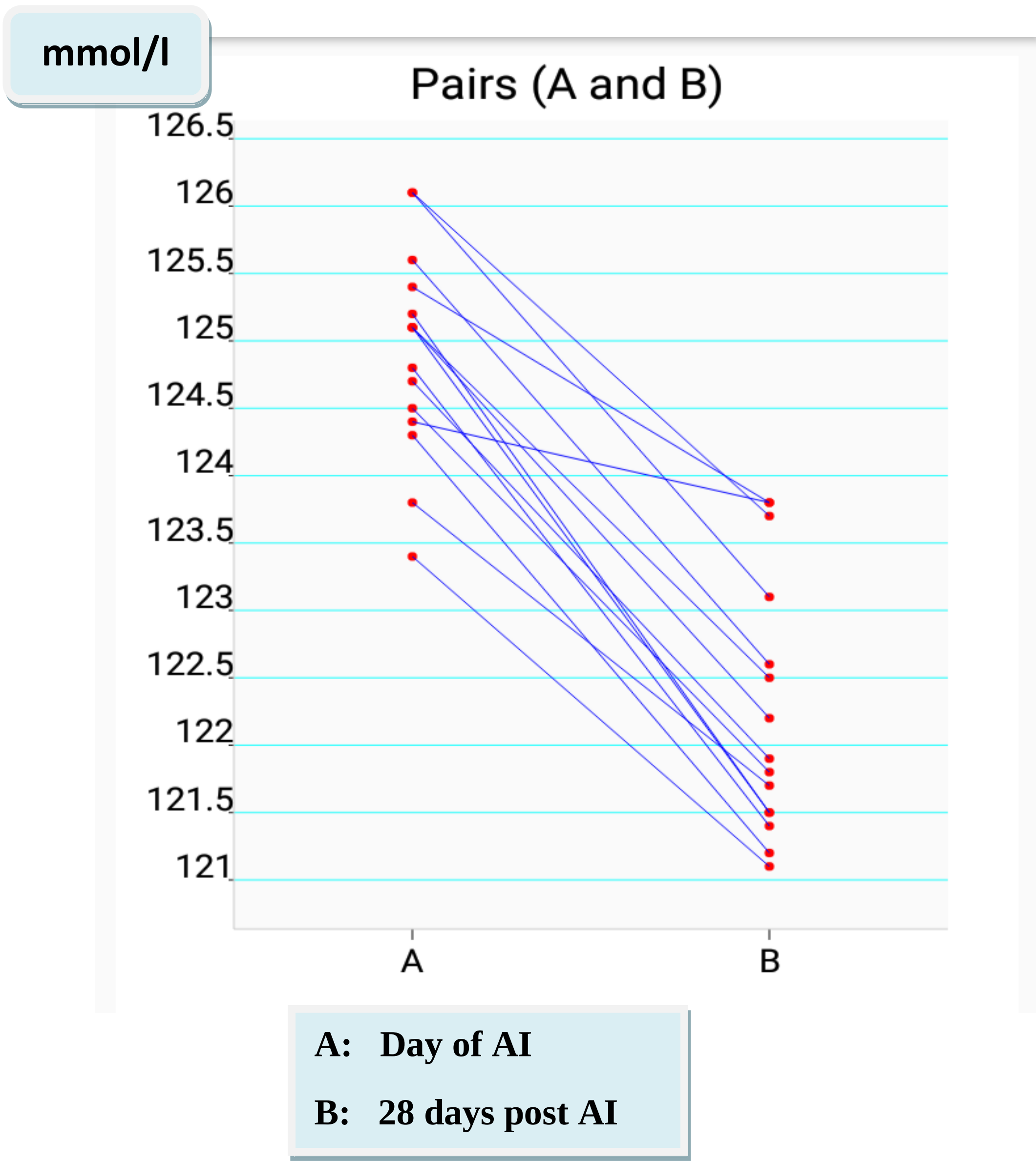


Figure 7: Serum Sodium level on the day of AI and 28 days post AI in the conceived heifers group

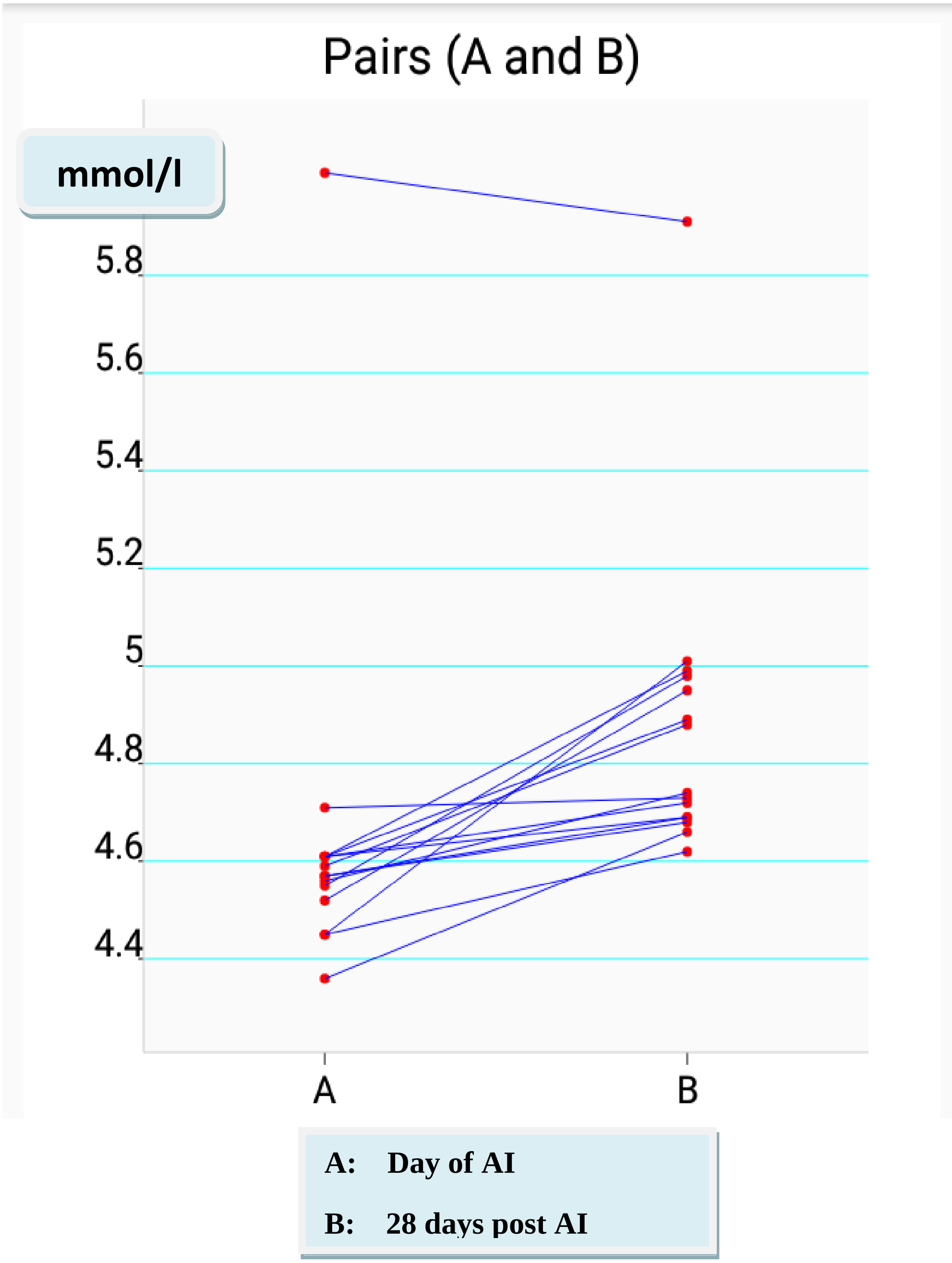


Figure 8: Serum Potassium level on the day of AI and 28 days post AI in the conceived heifers group

4.2.1.4. Zinc, Copper, and Selenium:

There was a significant increase ($P < 0.01$) in Zinc on the 28th day of pregnancy compared to their levels on the day of AI (Figure 9).

On the other hand there was a significant decrease ($P < 0.01$) in Copper on the 28th day of pregnancy lower than its levels on the day of AI (Figure 10).

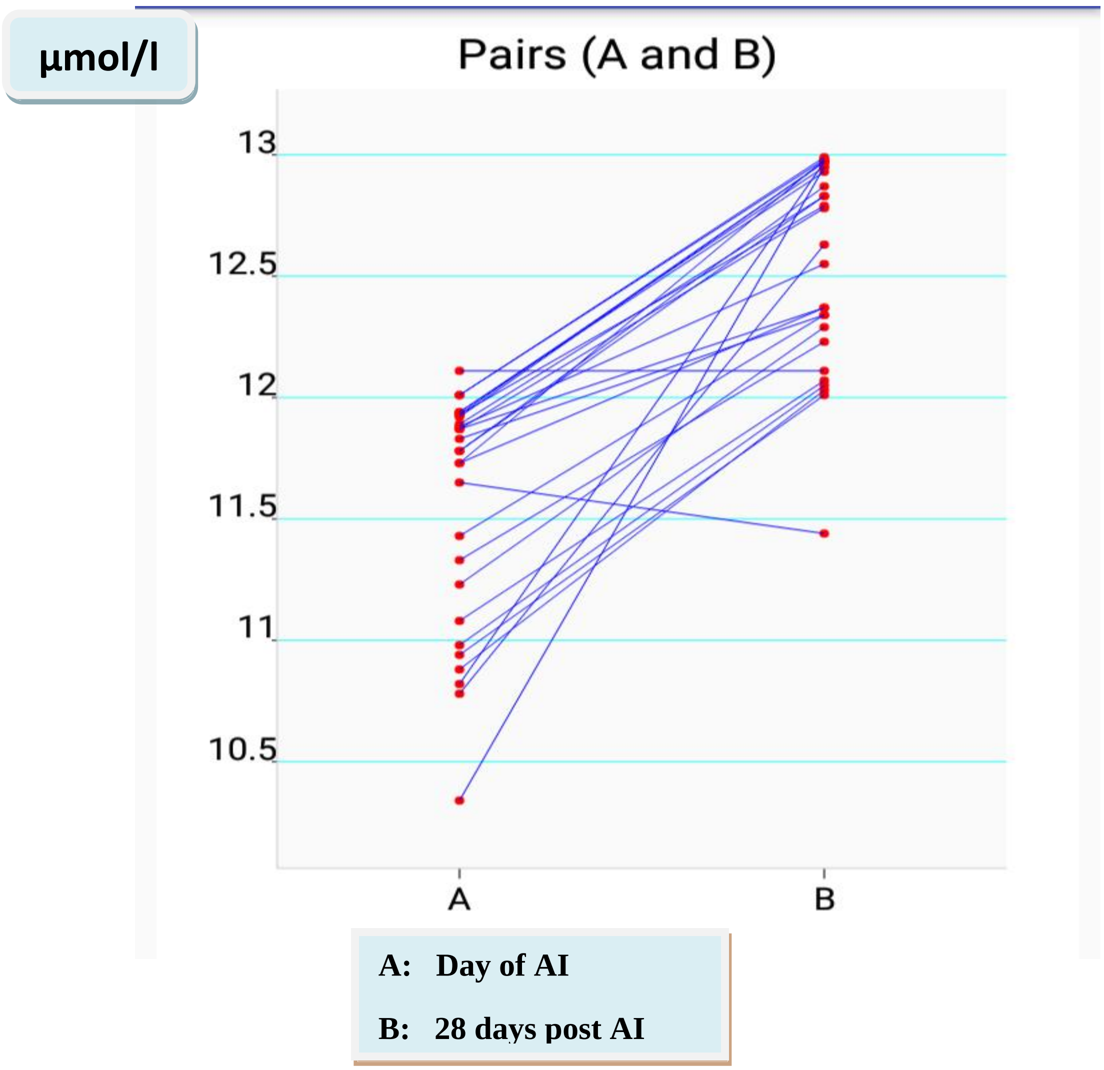


Figure 9: Serum zinc level on the day of AI and 28 days post AI in the conceived heifers group

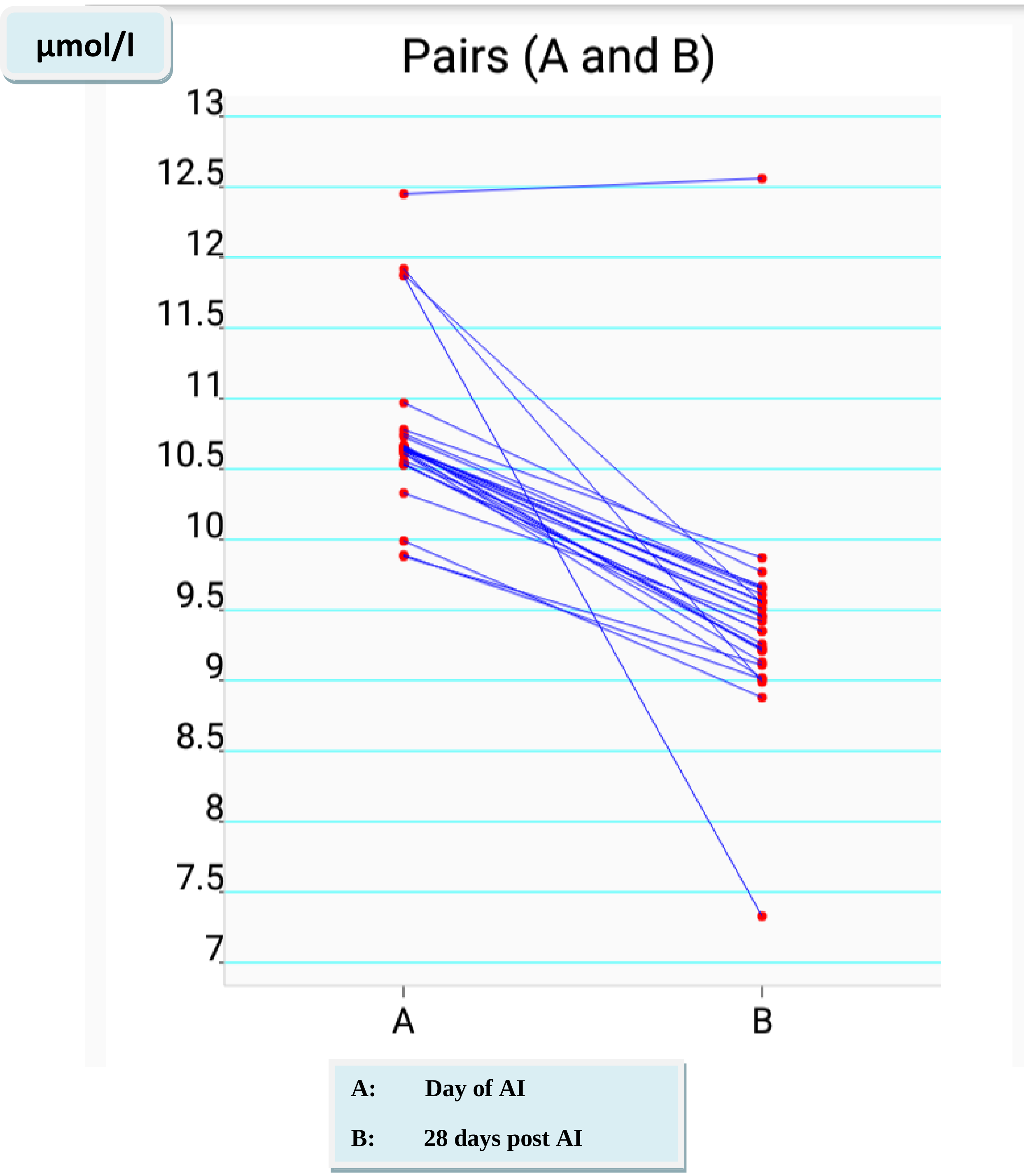


Figure 10: Serum cupper level on the day of AI and 28 days post AI in the conceived heifers group

4.2.2. Changes in blood parameters at estrus in conceived and non-conceived heifers:

4.2.2.1. BUN and Blood glucose:

There was a significant increase ($P < 0.01$) in the BUN level on the day of AI (estrus) in the failed (non-conceived) heifers group when compared to its level in the conceived heifers group (19.63 ± 1.07 and 14.26 ± 1.45 mg/dl) respectively, as presented in table (3) and figure (11& 12).

On the other hand the blood glucose level was significantly ($P < 0.01$) higher in conceived heifers than in failed group on the day of AI or estrus (54.1 ± 5.94 and 41.91 ± 2.84 mg/dl) respectively, as presented in table (3) and figure (11) & (13).

Table 3: Mean ± SD level of BUN and blood glucose in both conceived and failed heifers.

	BUN (mg/dl)				Blood glucose (mg/dl)			
	At Day of AI (estrus)		after 28 days of AI		At Day of AI (estrus)		after 28 days of AI	
	Conceived	Failed	Conceived	Failed	Conceived	Failed	Conceived	Failed
Mean ± SD	14.26 ± 1.45	19.63 ±1.07	33.75 ± 5.57	19.05 ±0.99	54.1 ± 5.9	41.91 ±2.84	61.65 ±4.55	41.13 ±2.67

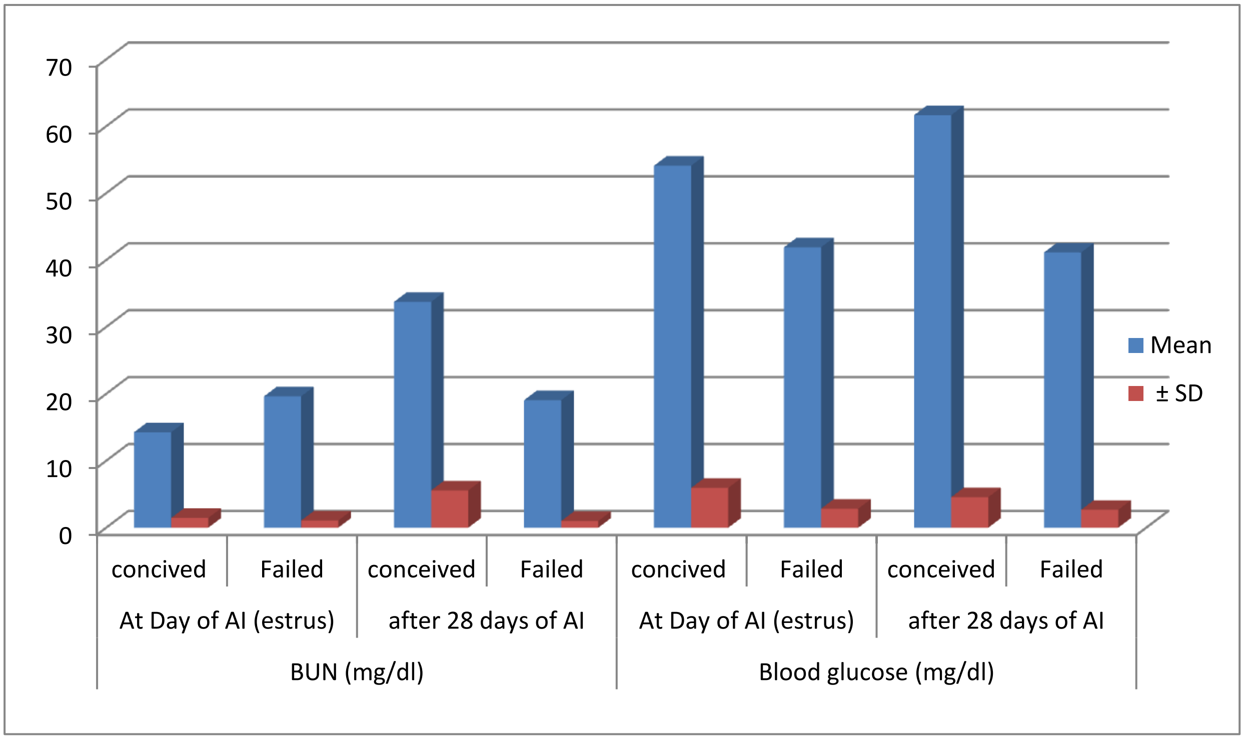


Figure 11: Relationship between BUN and blood glucose and conception and pregnancy in heifers.

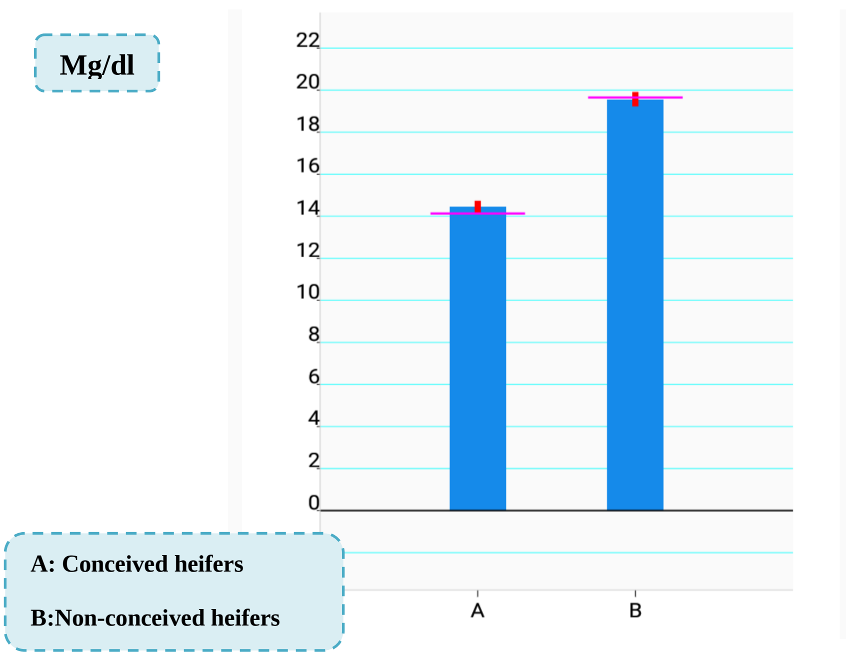


Figure 12: Relation between BUN and Conception in heifers

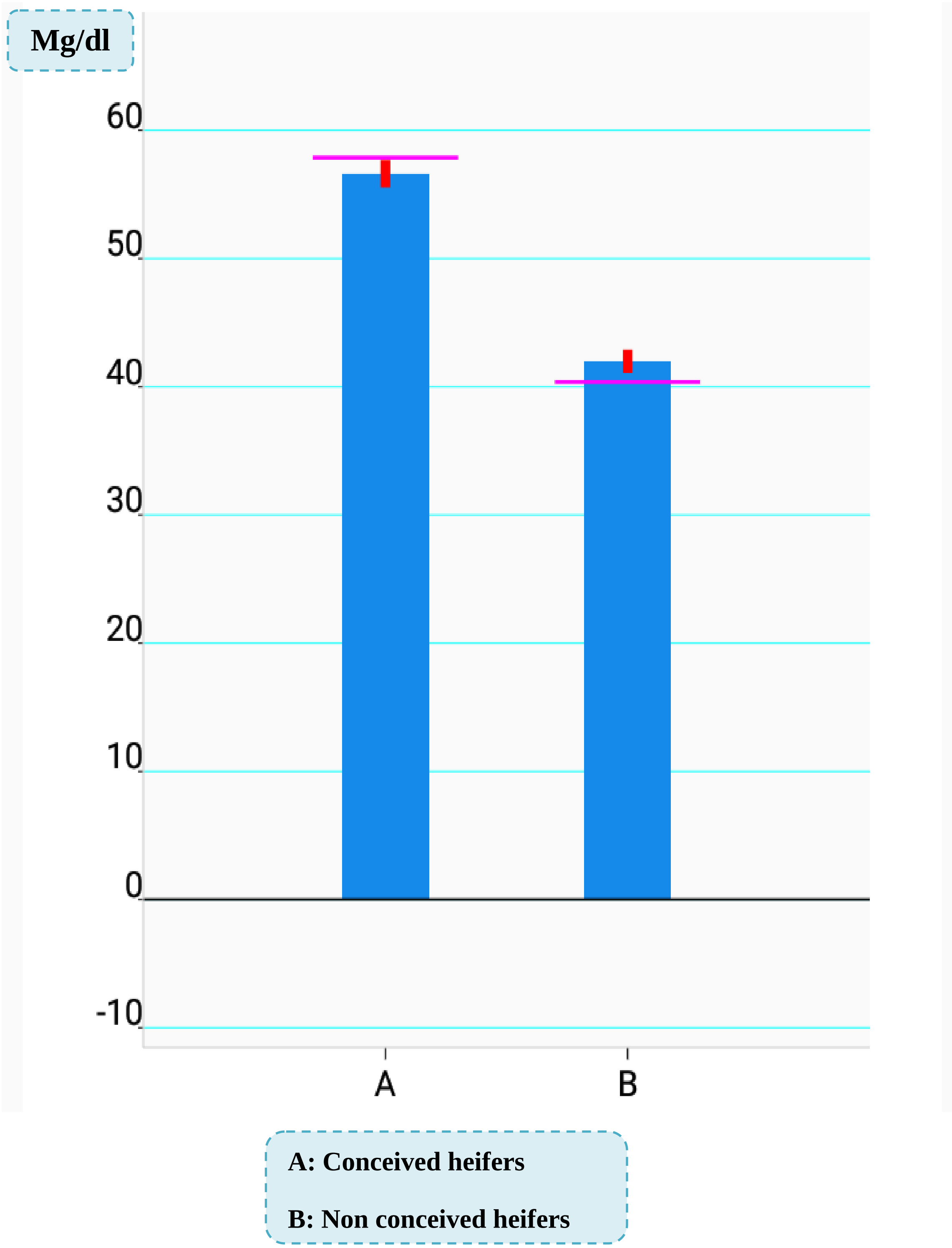


Figure 13: Relation between blood glucose and conception in heifers

4.2.2.2. Phosphorus and Magnesium:

There were non-significant differences ($P > 0.05$) in both P and Mg levels on the day of AI or estrus in both conceived heifers and failed heifers group. This indicated that Phosphorus and Magnesium not play a role in the conception process of heifers (Figure 14 & 15).

4.2.2.3. Sodium and Potassium:

The sodium levels showed non significant differences ($P > 0.05$) on the day of AI in both conceived heifers and failed heifers group (Figure 16).

On the other hand, the potassium was significantly increased ($P < 0.01$) on the day of AI or estrus in the failed heifers group when compared to the conceived group (Figure 17). With a mean level (5.52 ± 0.32 and 4.91 ± 0.70 respectively) as presented in table (2). This indicated a negative correlation between the potassium level at day of estrus and conception in the heifers.

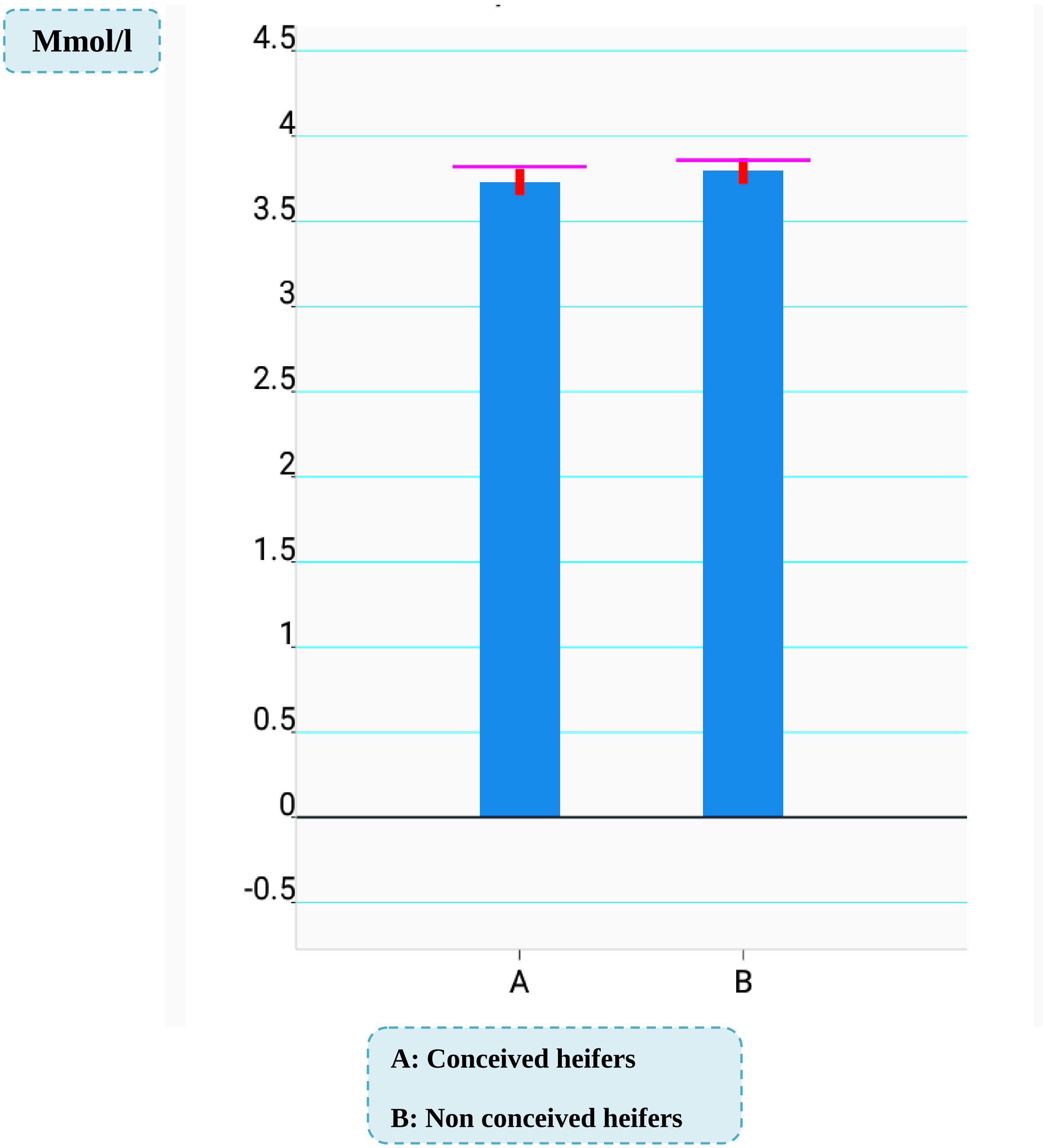
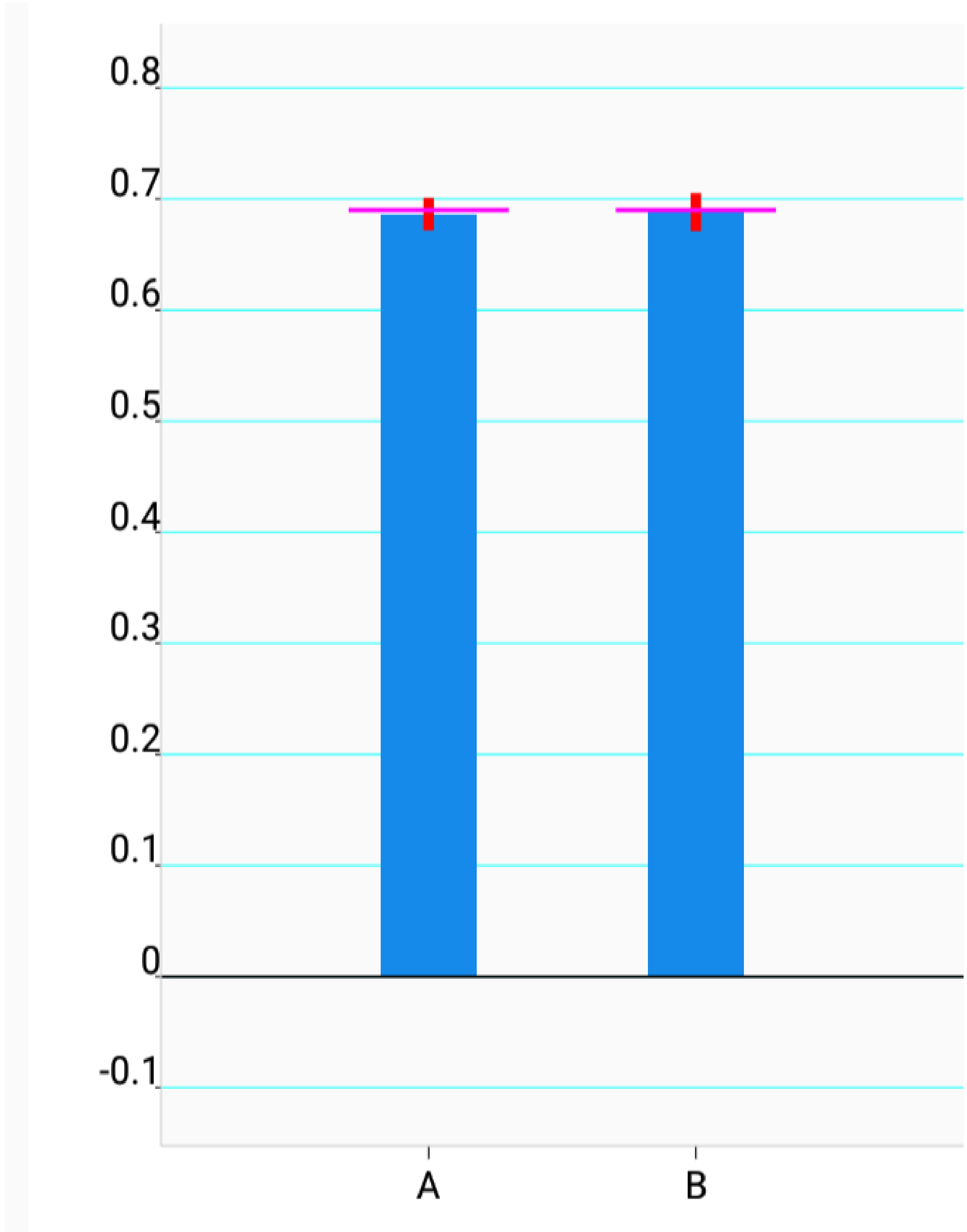


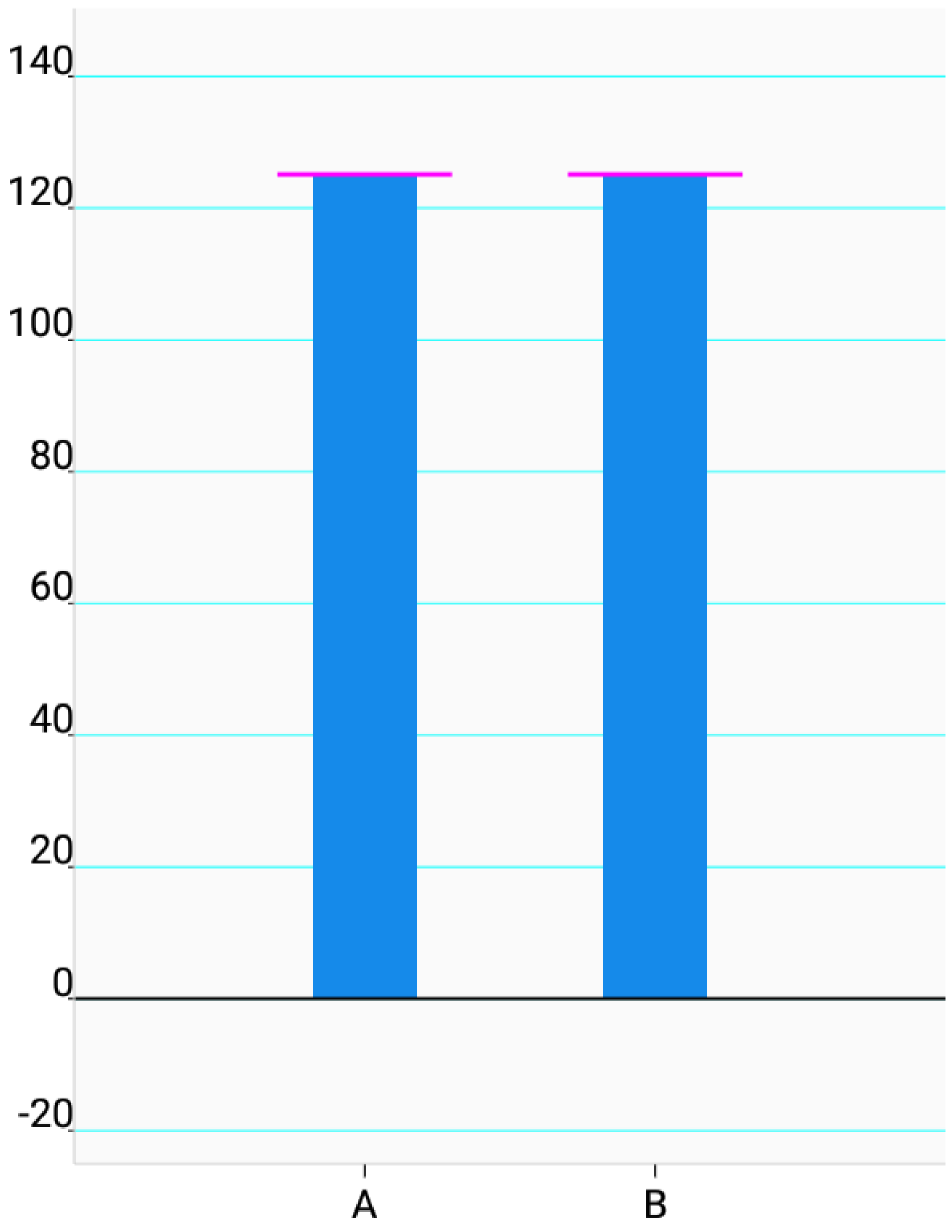
Figure 14: Relation between Phosphorus and conception in heifers



A: Conceived heifers

B: Non conceived heifers

Figure 15: Relation between Magnesium and conception in heifers



A: Conceived heifers

B: Non conceived heifers

Figure 16: Relation between Sodium and conception in heifers

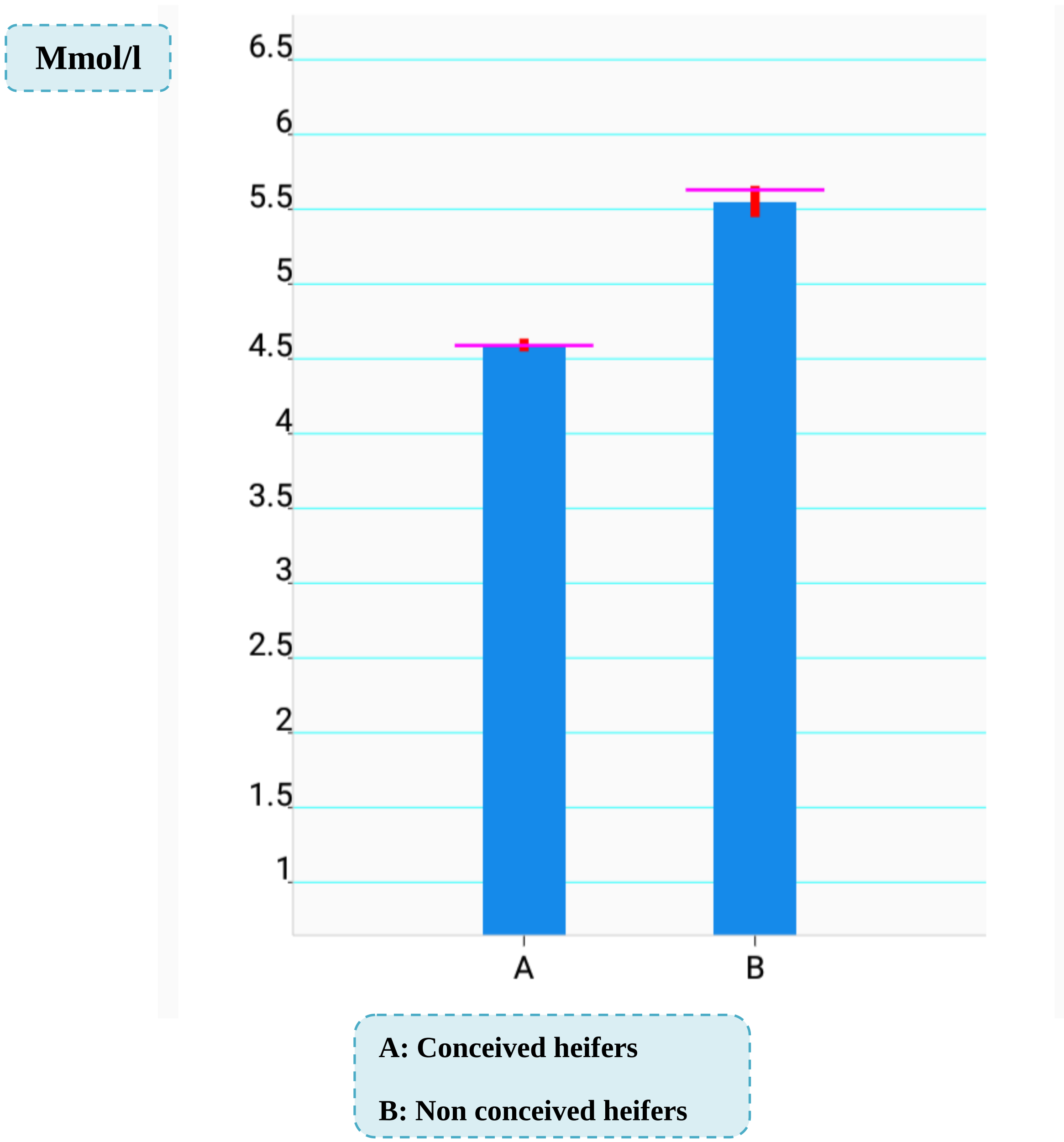


Figure 17: Relation between Potassium and conception in heifers

4.2.2.4. Zinc, Copper, and Selenium:

The Zinc levels showed non significant differences (P > 0.05) on the day of AI in both conceived heifers and failed heifers group (Figure 18). With a mean level 11.56±0.49 and 11.45±0.43 µmol/l respectively. This proved that Zinc is not important trace elements for estrus, ovulation, and conception.

On the other hand there was a significant decrease (P < 0.01) in Copper concentration on the day of AI in the group of conceived heifers when compared to its level in the failed heifers. With a mean level 10.51±0.53 and 12.75±0.53 µmol/l respectively (Figure 19).This indicated a negative relationship between the serum level of Cu and conception or reproductive efficiency in heifers. The increase of copper level on the day of AI or estrus decreases the conception rate.

On the contrary, the Selenium level was significantly increased (P < 0.01) on the day of AI or estrus in the group of conceived heifers over the group of failed heifers (5.90 ± 0.28 and 4.69 ± 0.15 µmol/l respectively (Figure 20). This indicated a positive relation between the Selenium and conception rate on the day of estrus of insemination.

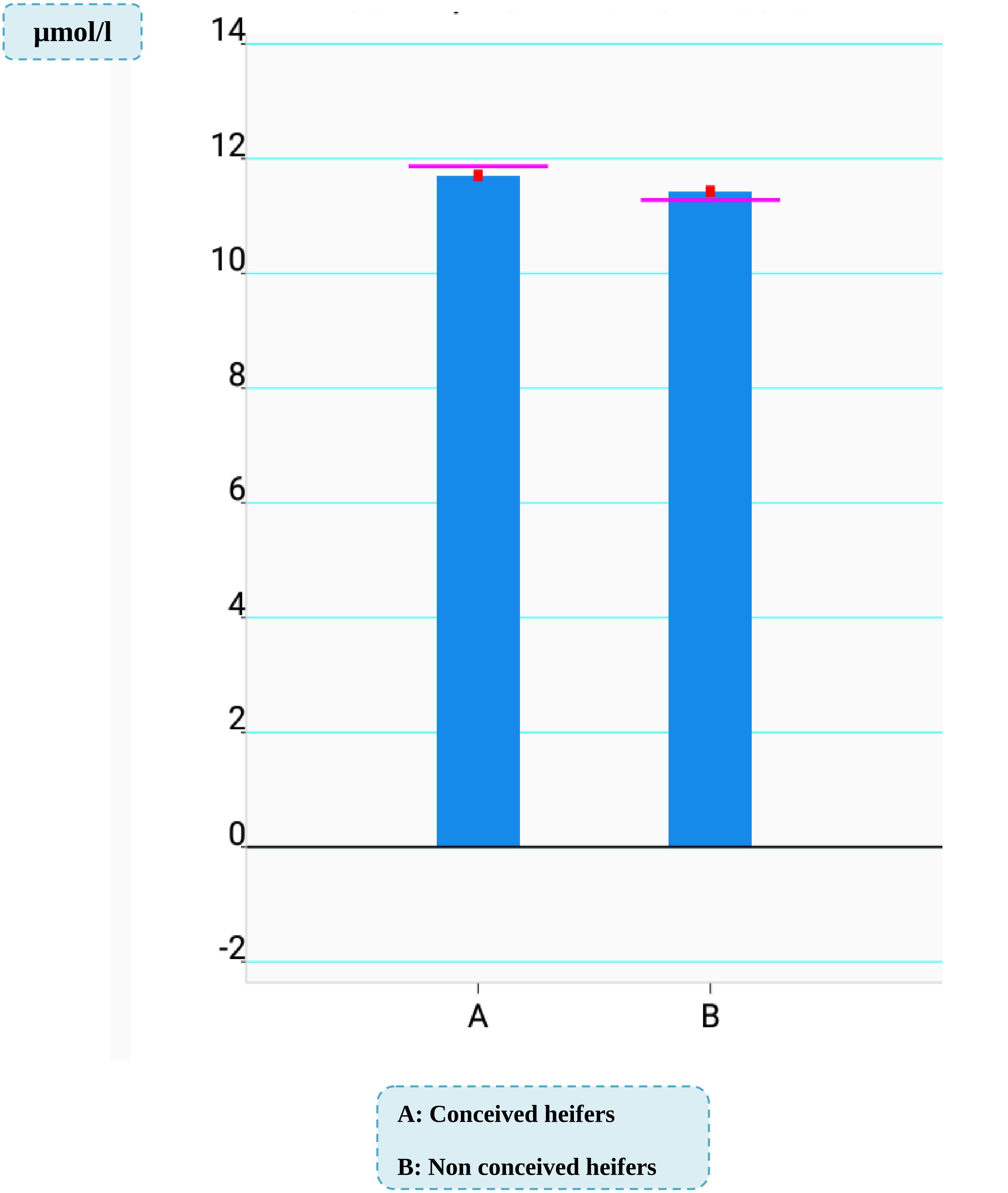


Figure 18: Relation between Zinc and conception in heifers

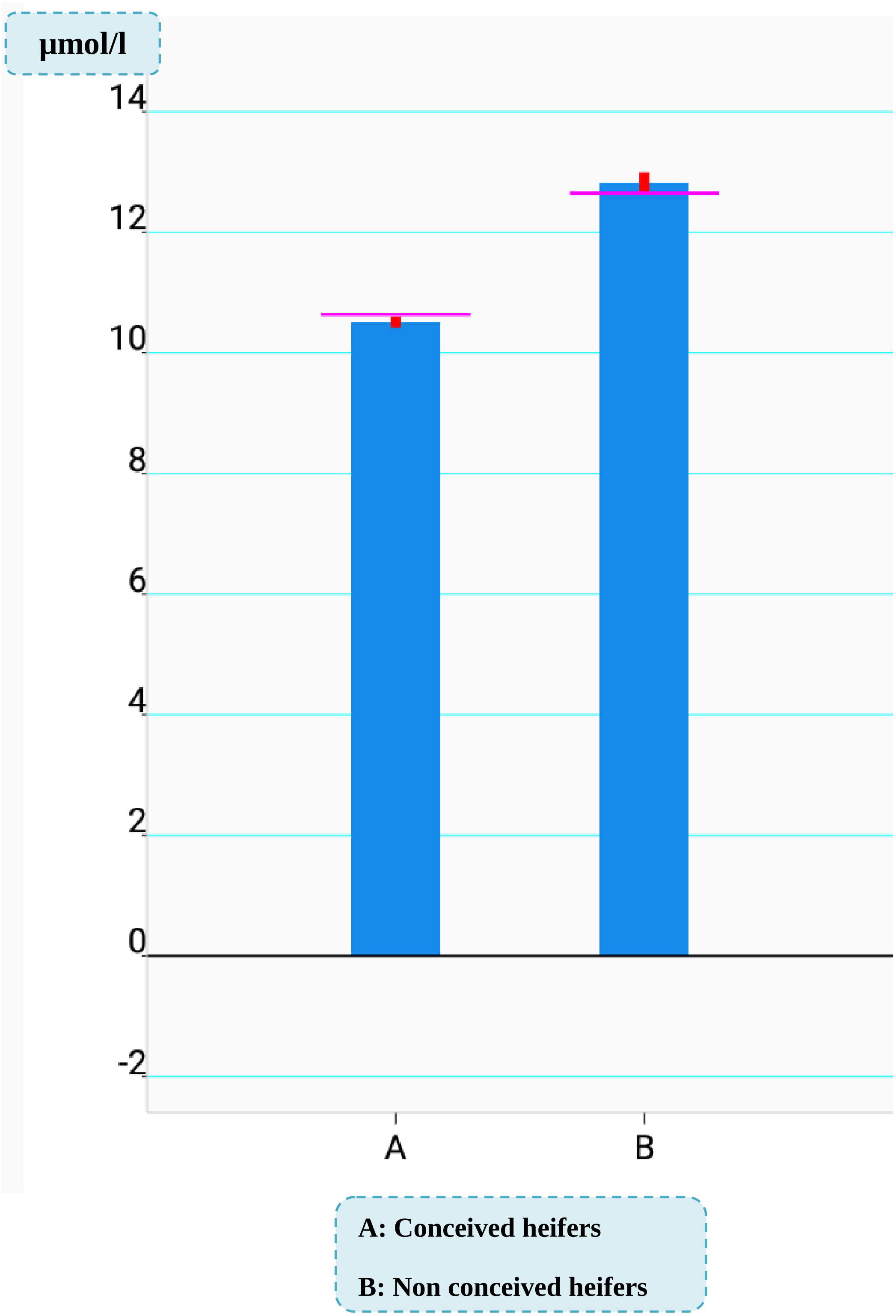
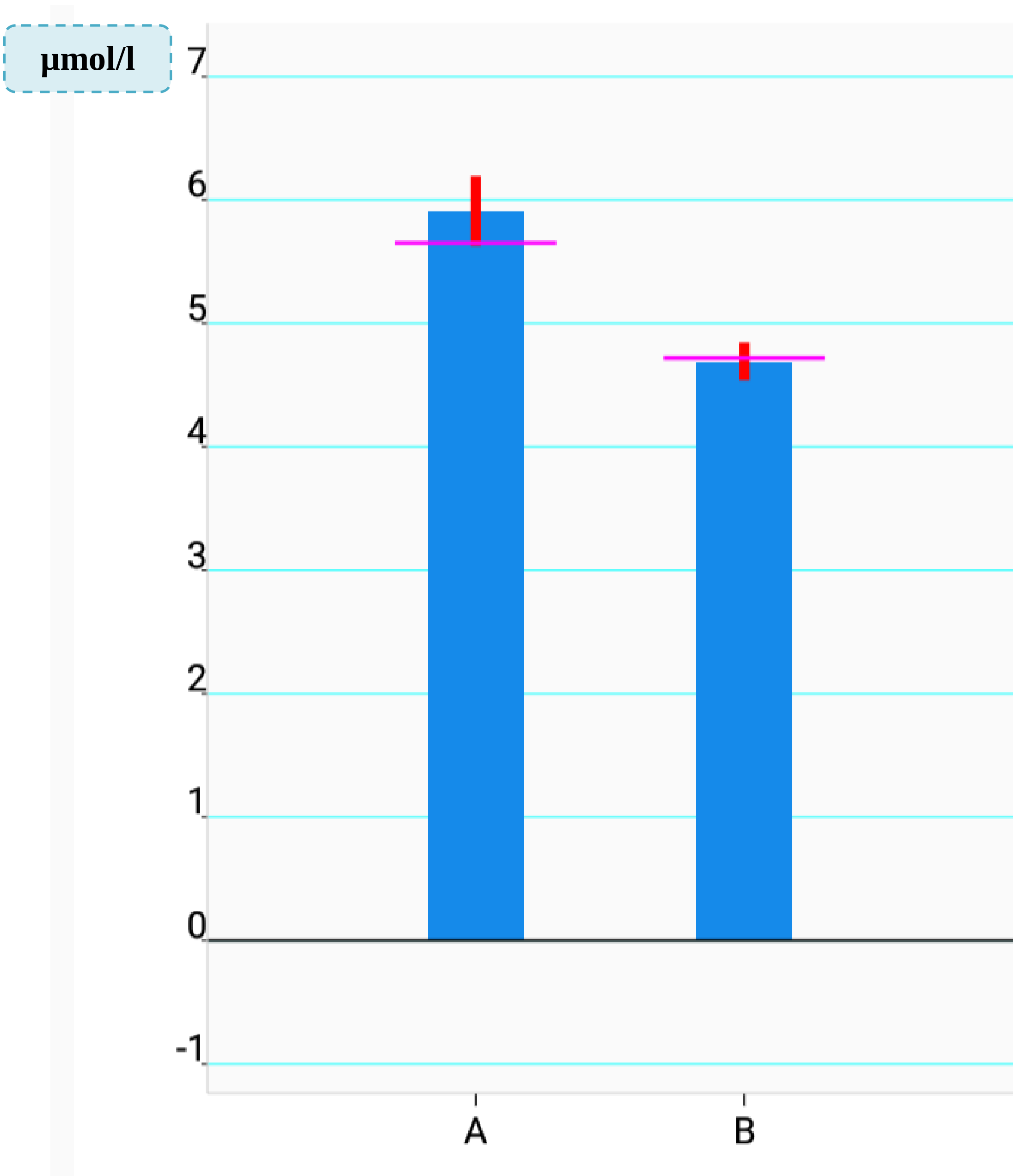


Figure 19: Relation between Copper and conception in heifers



A: Conceived heifers

B: Non conceived heifers

Figure 20: Relation between Selenium and conception in heifers

Table 4: Serum level of Zn, Cu, and Se in conceived and failed group (Mean ± SD)

Zn (µmol/l)				Cu (µmol/l)				Se (µmol/l)			
At Day of AI (estrus)		after 28 days of AI		At Day of AI (estrus)		after 28 days of AI		At Day of AI (estrus)		after 28 days of AI	
conceived	Failed	conceived	Failed	conceived	Failed	Conceived	Failed	conceived	Failed	Conceived	Failed
11.56	11.45	12.53	11.39	10.76	12.75	9.42	12.84	5.37	4.71	6.29	4.66
0.49	0.32	0.43	0.3	0.6	0.53	0.78	0.44	0.56	0.44	0.7	0.65

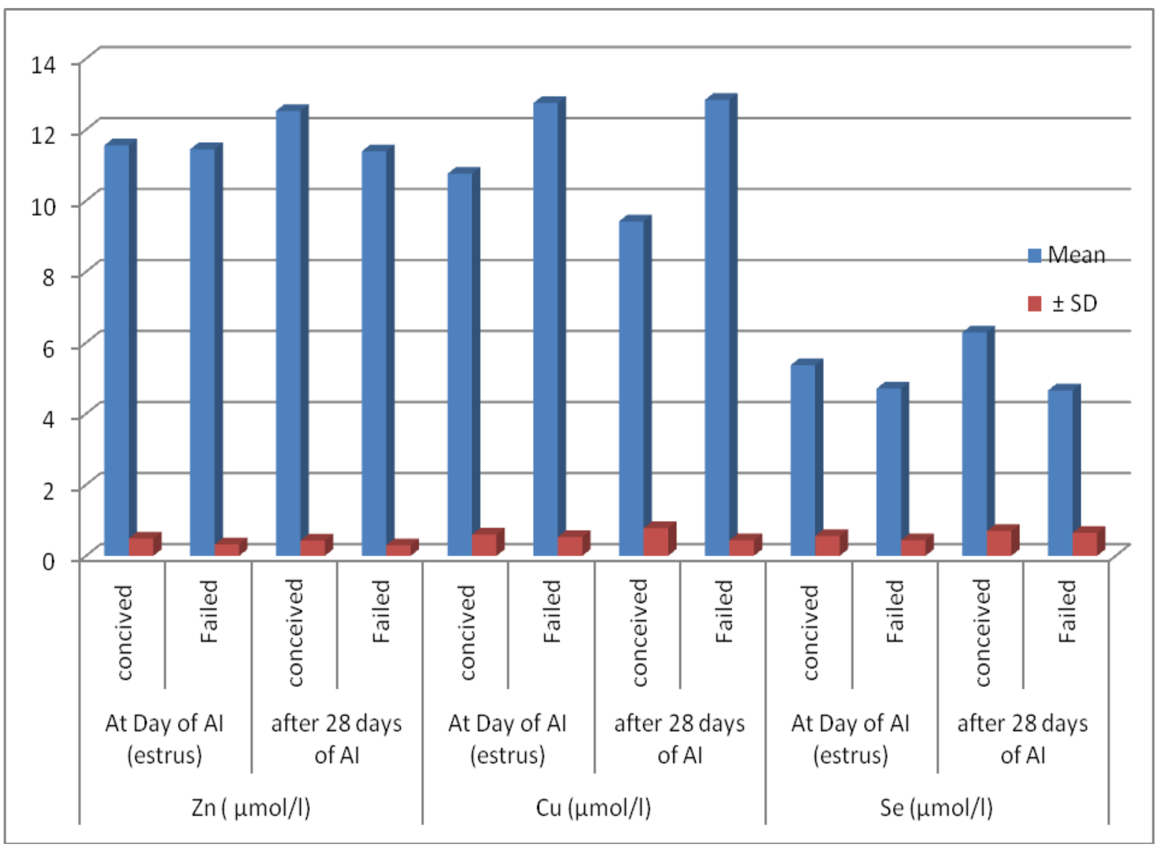


Figure 21: Relationship between Zn, Cu, and Se and conception in nulliparous heifers

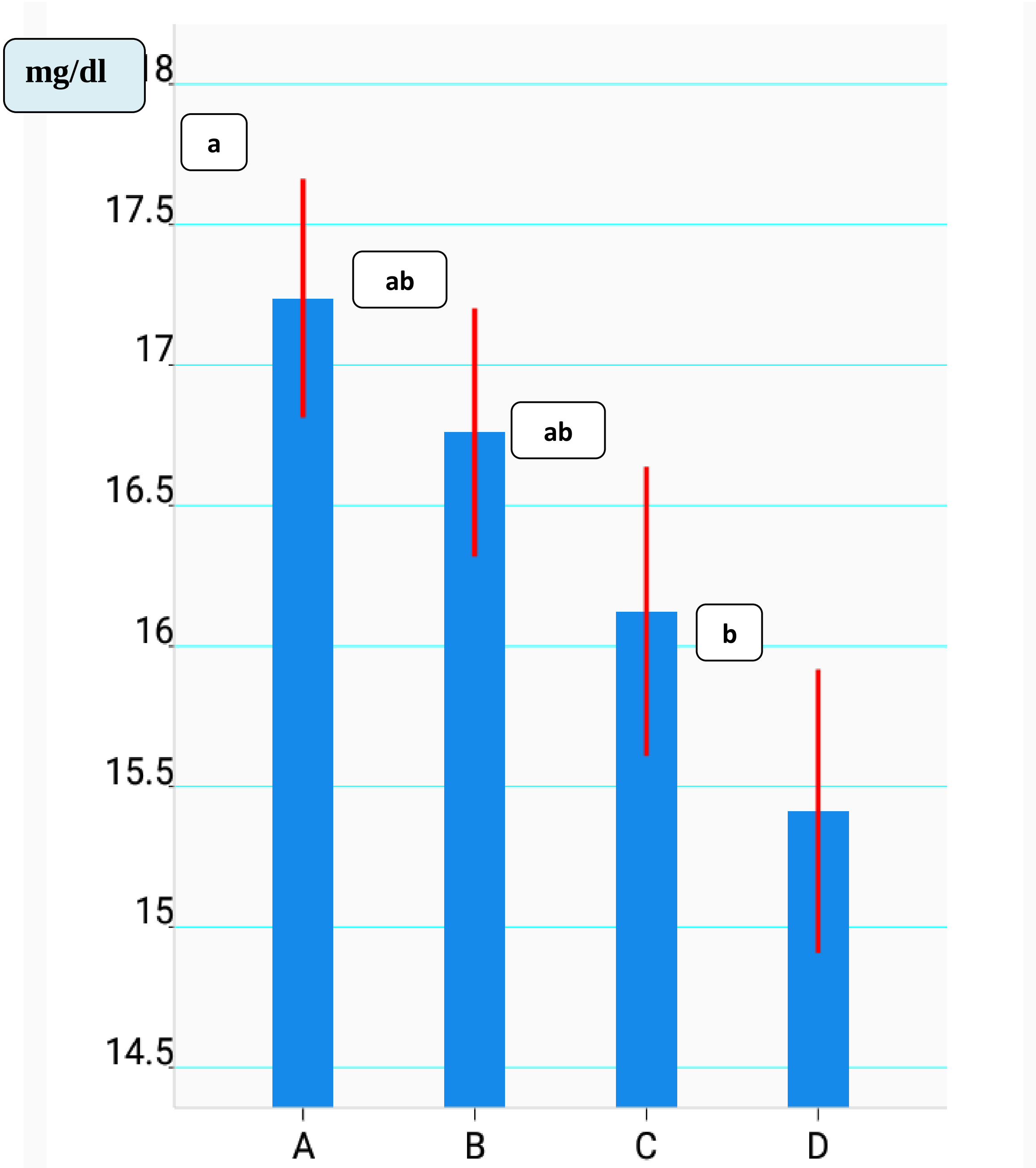
4.3. Estimation of blood parameters and mineral profile in postpartum dairy cows (Group II):

4.3.1. Subgroup A : Normal P.P Cows:

4.3.1.1. BUN and Glucose:

There was gradual non significant decrease ($P > 0.05$) in BUN level with advance of postpartum period (table 5 and figure 22). There was a significant difference ($P < 0.05$) between the levels of BUN in the 1st examination and 4th examination (figure 22). There was a negative significant correlation between BUN and time P.P. (R= - 0.479, P= 0.006) in normal cyclic cows.

The blood glucose had a gradual highly significant ($P < 0.0001$) increase with time postpartum. With high significant increase in the 3rd ($P<0.01$) and 4th ($P<0.0001$) examinations compared to 1st P.P. examination. These findings were illustrated in table (5), & fig (23). There was a positive significant correlation (R= - 0.804, P<0.0001) between glucose and time P.P. in cyclic cows.



A, B, C, & D: times of examination and blood sampling with 10 days interval

Figure 22: levels of BUN during the postpartum period in normal cyclic cows

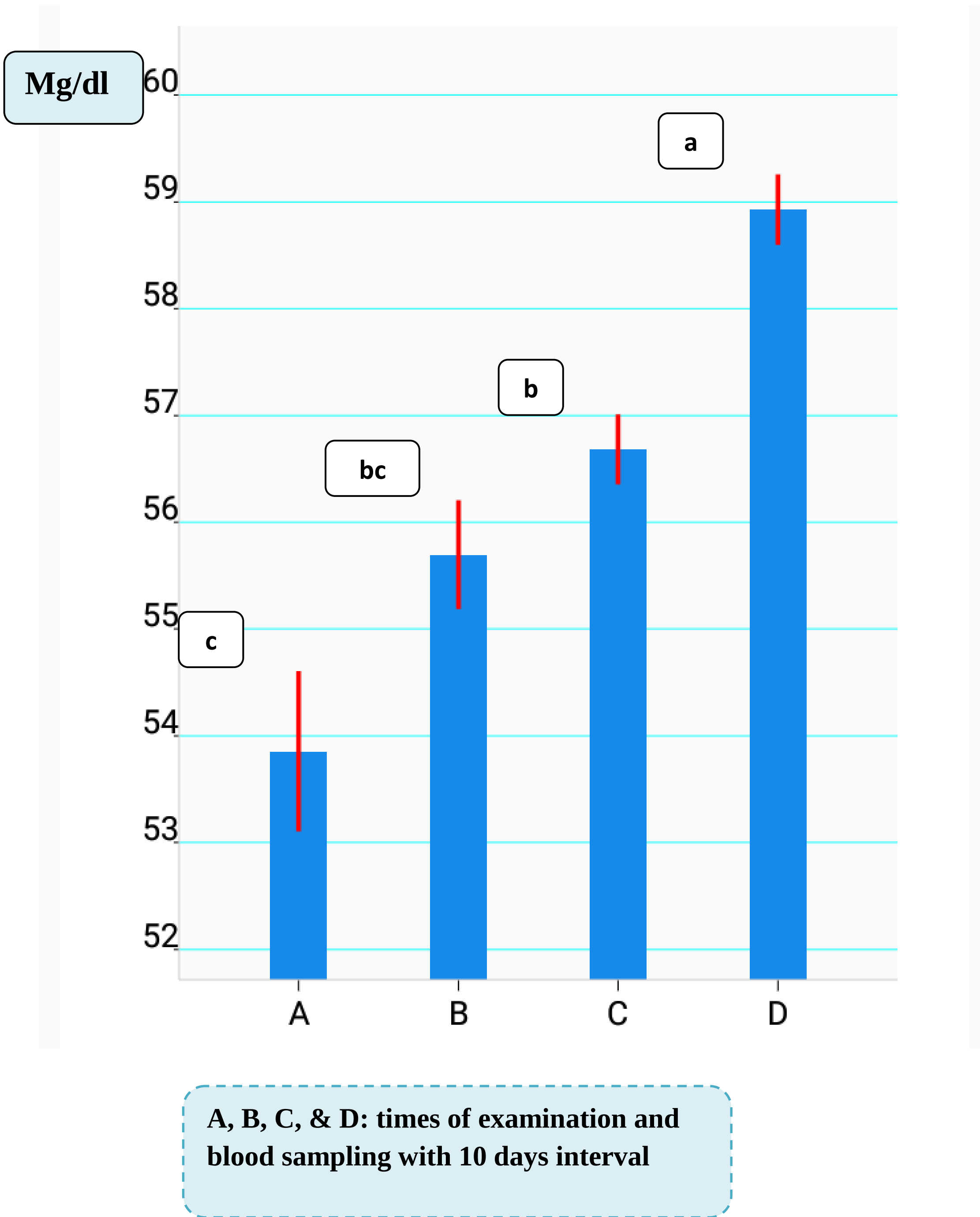


Figure 23: levels of Blood glucose during postpartum period in normal cyclic animals

Table 5: Serum level of BUN and glucose during P.P. in normal cyclic PP cows

Cow ID	BUN (mg/dl)				Blood Glucose (mg/dl)			
	8 d P.P	18 d P.P	28 d P.P	38 d P.P	8 d P.P	18 d P.P	28 d P.P	38 d P.P
Mean ± SD	17.24 ± 1.20	16.76 ± 1.25	16.12 ± 1.46	15.41 ± 1.43	53.85± 2.13	55.73± 1.25	56.68± 0.93	58.92± 0.93

4.3.1.2. Phosphorus and Magnesium

There was a non significant difference (P > 0.05) in Mg level during postpartum events (Table 6 and Figure 24). But, phosphorus level had a highly significant (P < 0.0001) difference varied with time P.P. the maximal level was recorded on day 28 P.P., the lowest levels were observed at day 8 P.P. (Figure 25). There was a non-significant correlation between Mg and time P.P. (R= - 0.2, P= 0.27) in normal cyclic cows.

Table 6: Serum level of P and Mg during PPP in normal cyclic PP cows

Cow ID	Phosphorus (mmol/l)				Magnesium (mmol/l)			
	8 d P.P	18 d P.P	28 d P.P	38 d P.P	8 d P.P	18 d P.P	28 d P.P	38 d P.P
Mean ± SD	1.6 ± 0.04	2.02 ± 0.12	2.15 ± 0.19	1.94 ± 0.1	0.67 ± 0.03	0.69 ± 0.02	0.7 ± 0.02	0.68 ± 0.02

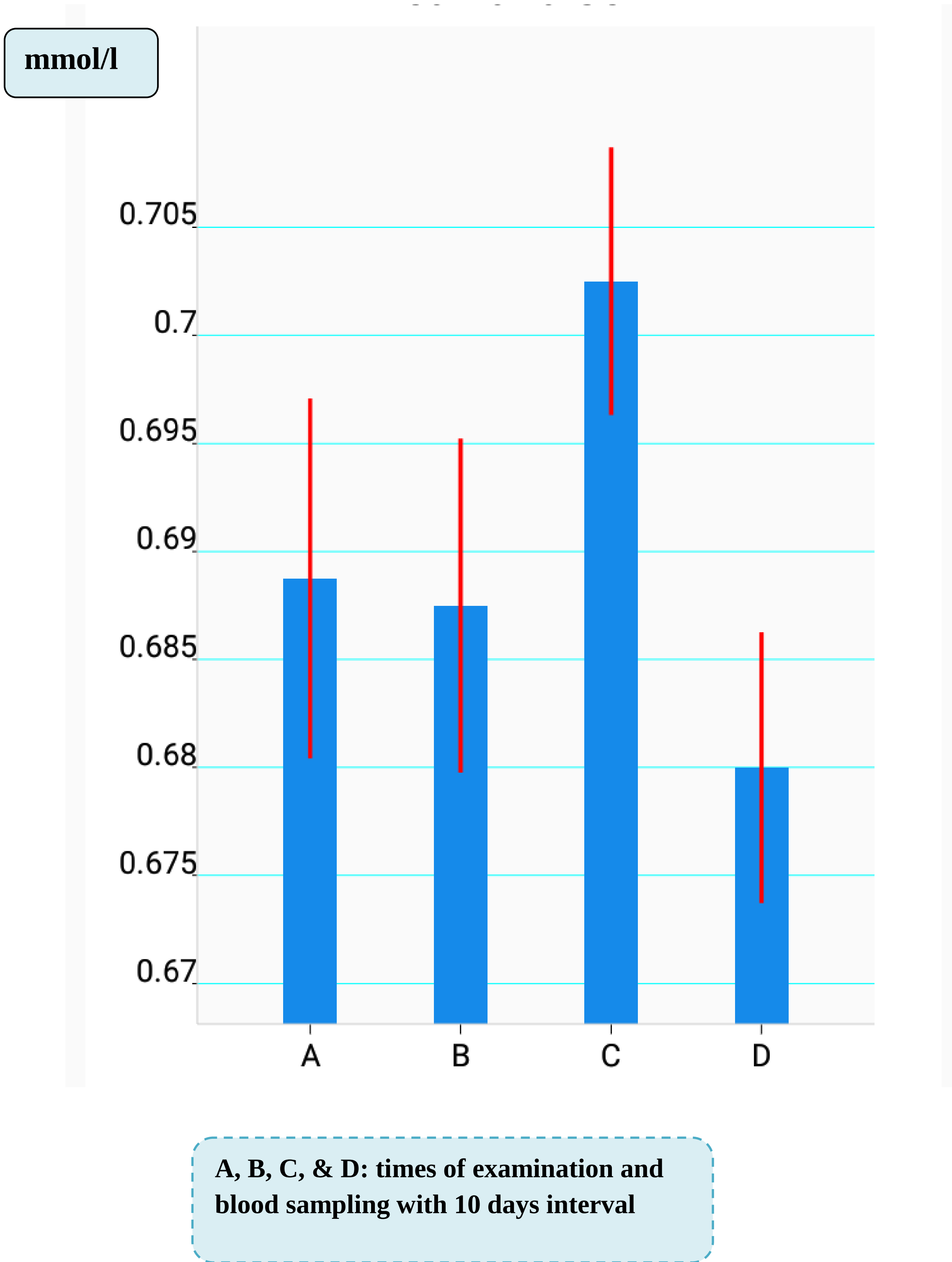
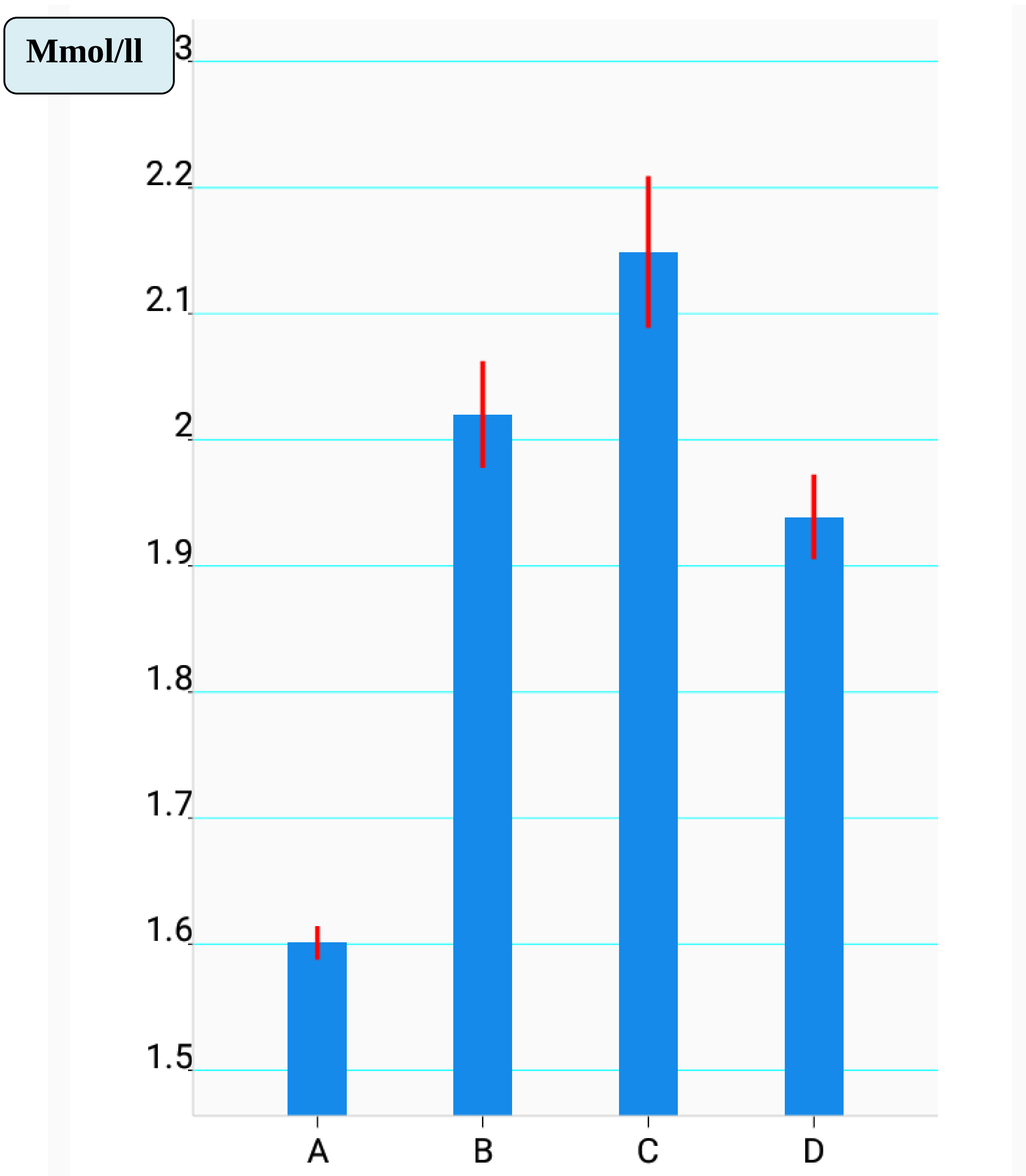


Figure 24: Mg level during the postpartum period in normal cyclic cows



A, B, C, & D: times of examination and blood sampling with 10 days interval

Figure 25: Phosphorus level during the postpartum period in normal cyclic cows

4.3.1.3. Sodium and potassium:

There was a non significant difference (P > 0.05) in the serum level of sodium and potassium during the P.P. period (Table 7 and Figure 26 & 27).

Table 7: Serum level of Na and K during PPP in normal cyclic PP cows

Cow ID	Na (mmol/l)				K+ (mmol/l)			
	8 d P.P	18 d P.P	28 d P.P	38 d P.P	8 d P.P	18 d P.P	28 d PP	38 d P.P
112	125.3	125.4	125.8	125.1	4.56	4.62	4.86	4.83
113	123.5	125.2	125.6	124.6	4.23	4.83	4.91	4.88
117	124.5	124.8	125.1	125.3	4.81	4.75	4.71	4.77
209	124.5	123.5	125.3	125.4	4.25	4.26	4.37	4.27
215	124.6	125.1	125.2	124.7	4.72	4.19	4.57	4.19
220	125.6	123.5	125.1	123.5	4.25	4.26	4.62	4.37
224	125.1	124.5	124.6	124.5	4.19	4.38	4.55	4.53
225	125.7	126.1	125.3	125.1	4.86	4.56	4.27	4.66
Mean ± SD	124.8 ± 0.72	124.8 ± 0.91	125.2 ± 0.36	124.8 ± 0.61	4.48 ± 0.28	4.48 ± 0.24	4.61 ± 0.22	4.56 ± 0.26

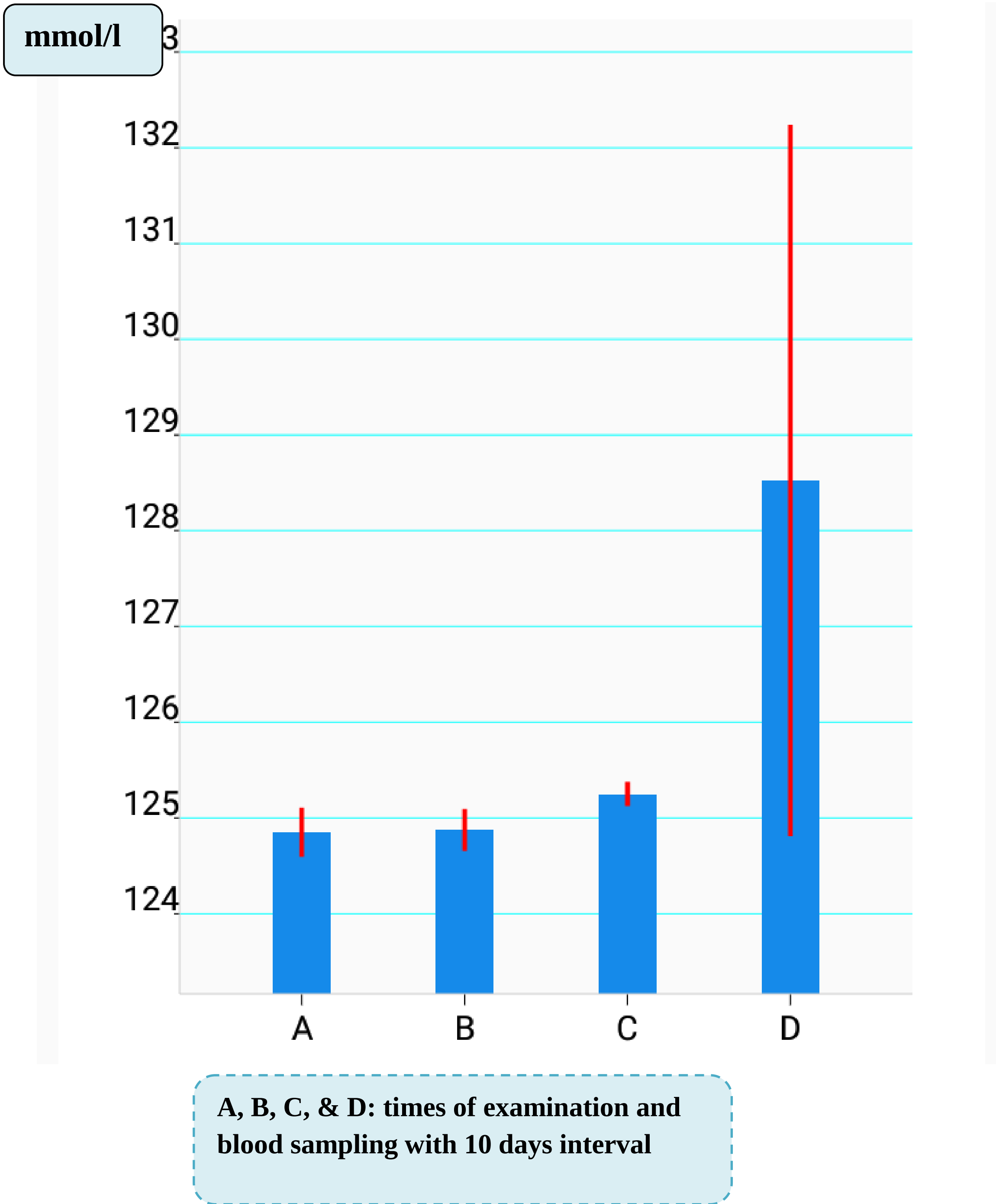
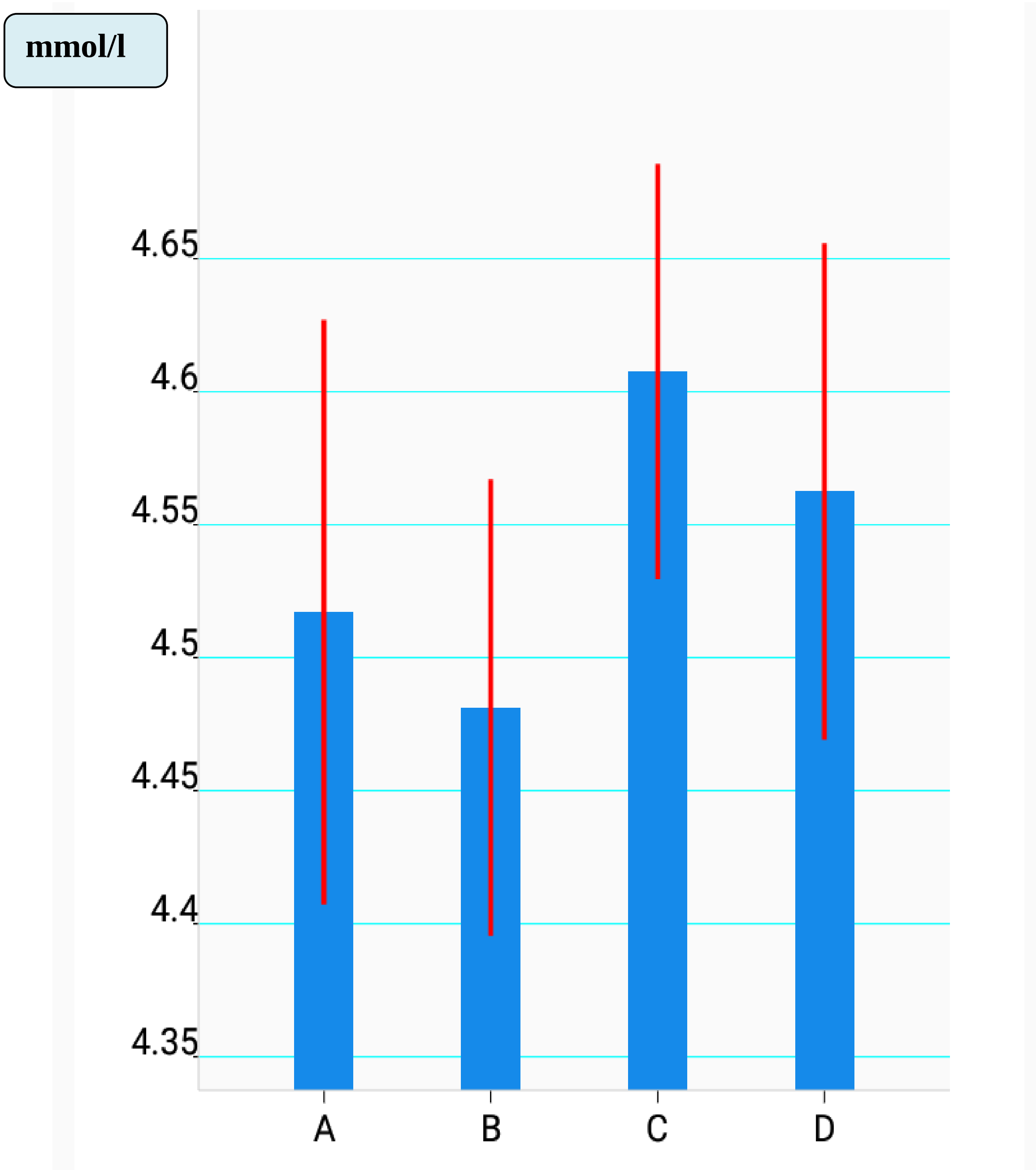


Figure 26: Sodium level during the postpartum period in normal cyclic cows



A, B, C, & D: times of examination and blood sampling with 10 days interval

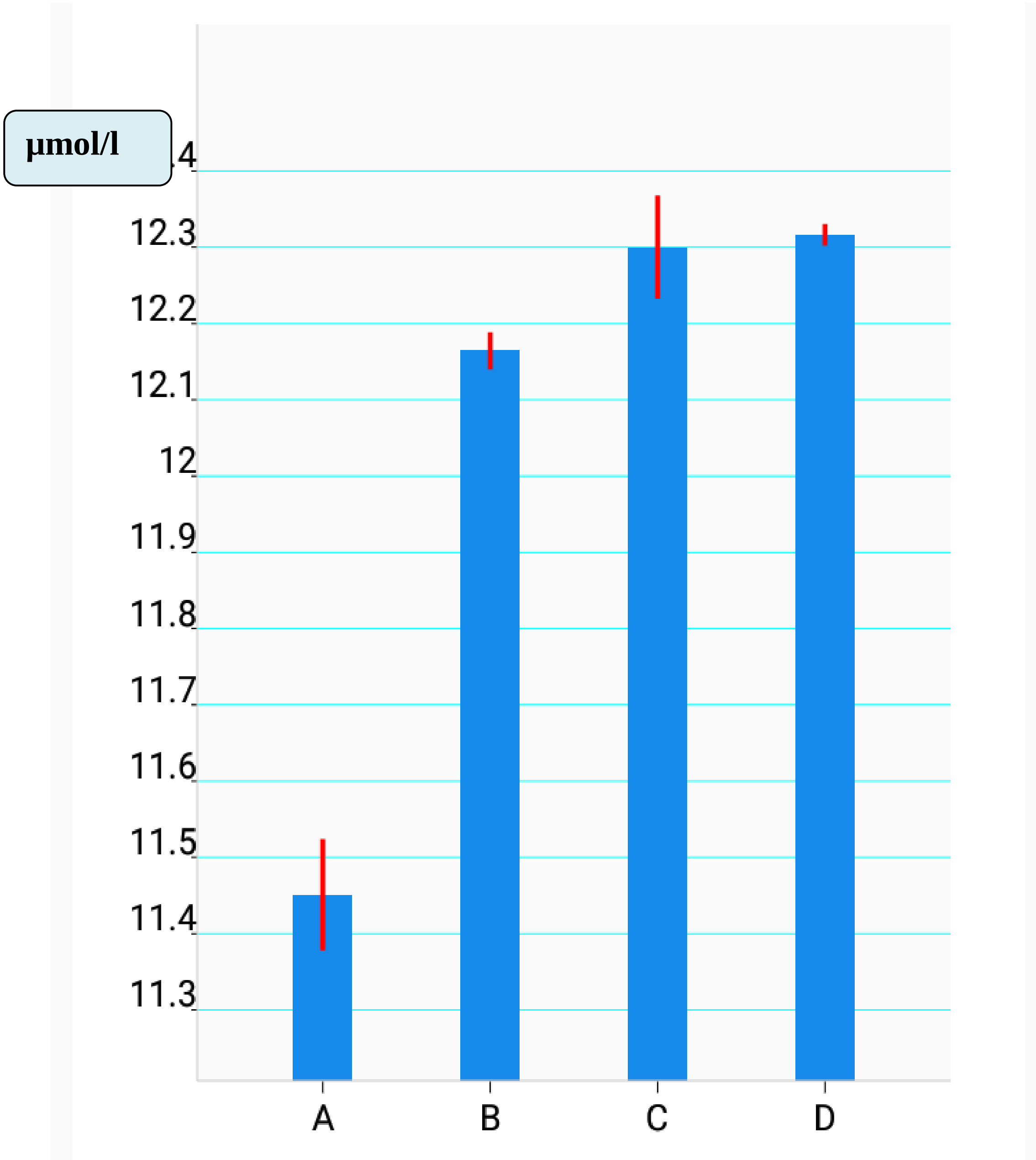
Figure 27: Potassium level during the postpartum period in normal cyclic cows

4.3.1.4. Zinc, Copper, and Selenium:

There were gradual significant increases ($P < 0.0001$) in the level of both zinc and selenium with time P.P. reaching to peak at 38 days P.P. There was a significant ($P < 0.0001$) difference when compared the recorded level of day 8 and 38 P.P. These findings were presented in table (8), and figure (28) & (29).While the serum copper level had a non significant increase ($P > 0.05$) in the 2nd and 3rd examinations PP.

Table 8: Serum level of Zn, Cu, and Se during PPP in normal cyclic PP cows

Cow ID	Zn (µmol/l)				Cu (µmol/l)				Se (µmol/l)			
	8 d P.P	18 d P.P	28 d P.P	38 d P.P	8 d P.P	18 d P.P	28 d P.P	38 d P.P	8 d P.P	18 d P.P	28 d P.P	38 d P.P
112	11.23	12.21	12.23	12.32	9.85	10.45	10.79	9.75	4.52	5.56	5.96	5.98
113	11.58	12.28	12.28	12.34	9.78	10.36	10.85	9.88	4.88	5.21	5.65	5.95
117	11.23	12.11	12.21	12.29	9.91	10.52	10.95	9.92	4.81	4.99	5.51	5.78
209	11.57	12.09	12.27	12.33	9.96	10.62	10.86	9.88	5.12	5.69	5.88	6.09
215	11.67	12.17	12.19	12.27	9.92	10.53	10.93	9.87	4.52	4.98	5.11	5.28
220	11.72	12.16	12.18	12.26	9.94	10.39	10.89	9.96	4.81	4.77	5.15	5.61
224	11.29	12.13	12.22	12.31	9.84	10.48	10.81	9.95	5.09	5.23	5.88	6.01
225	11.35	12.14	12.23	12.34	9.59	10.57	10.76	9.97	4.77	5.11	5.54	5.81
Mean ± SD	**11.45 ± 0.2**	**12.16 ± 0.1**	**12.23 ± 0.04**	**12.31 ± 0.03**	**9.85 ± 0.12**	**10.49 ± 0.18**	**10.85 ± 0.07**	**9.9 ± 0.07**	**4.81 ± 0.22**	**5.19 ± 0.31**	**5.58 ± 0.33**	**5.81 ± 0.26**



A, B, C, & D: times of examination and blood sampling with 10 days interval

Figure 28: Zn level during the postpartum period in normal cyclic animals

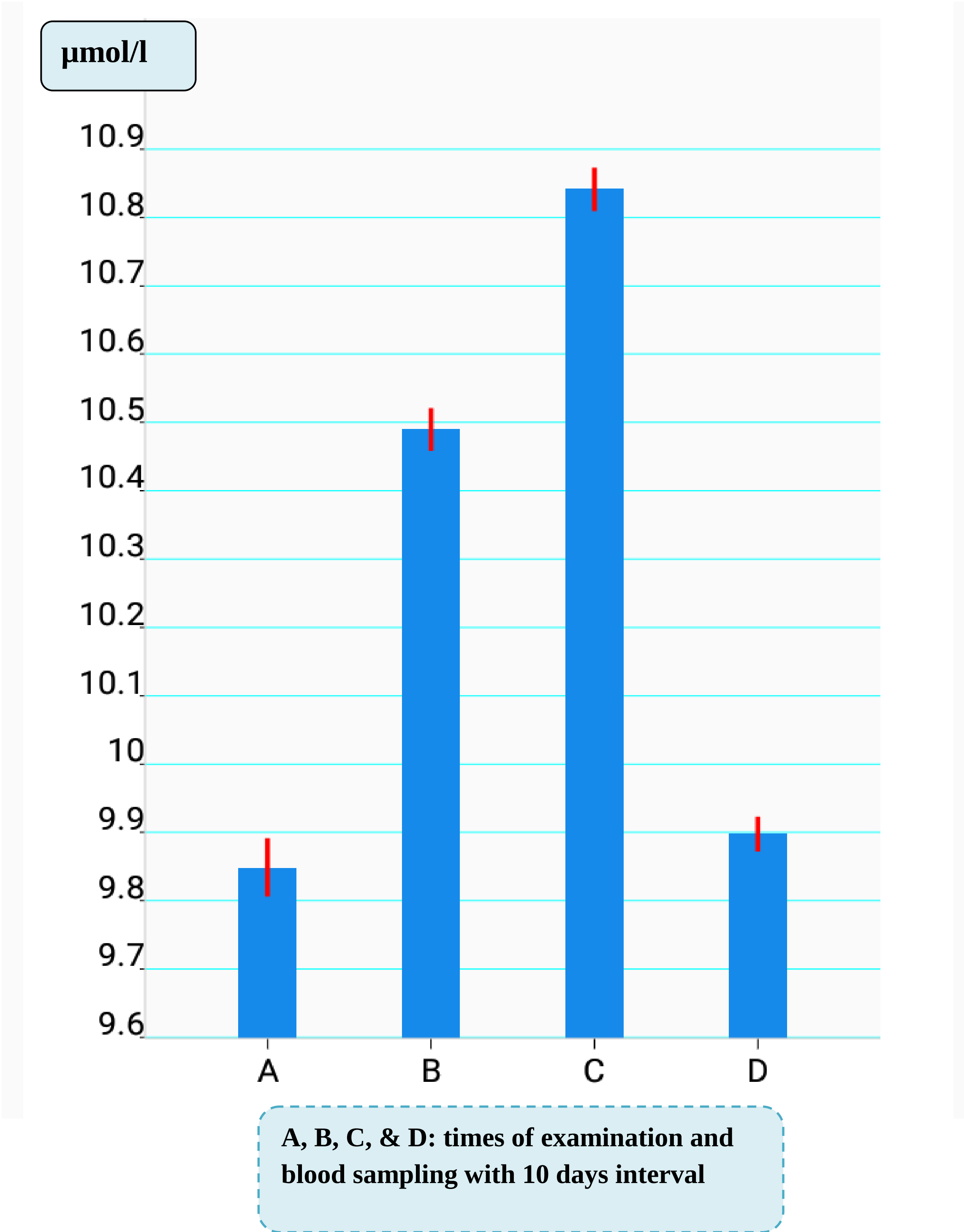


Figure 29: Copper level during the postpartum period in normal cyclic animals

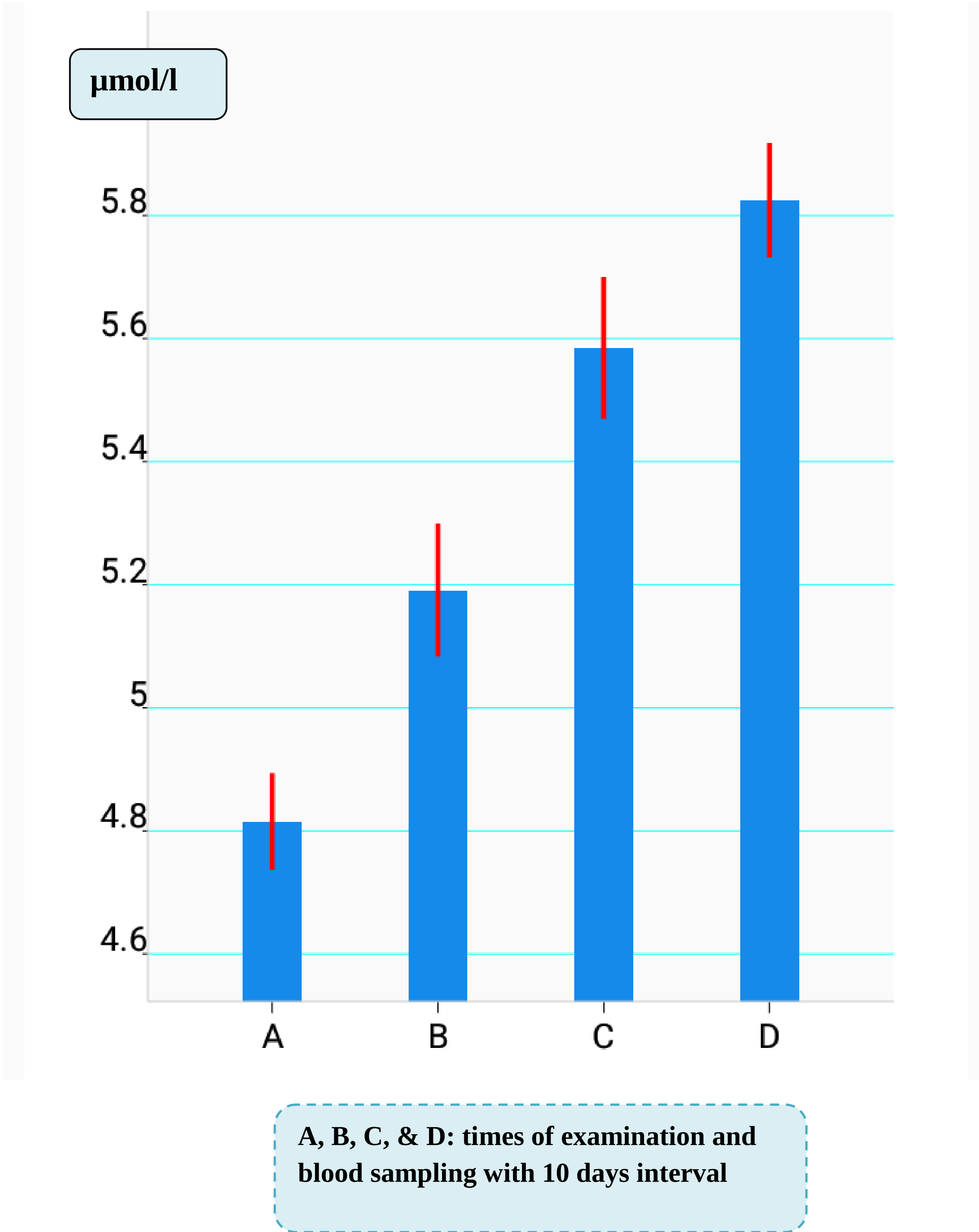


Figure 30: Selenium level during the postpartum period in normal cyclic cows

4.3.2. Subgroup B: Cows with cystic ovary:

4.3.2.1. BUN and blood glucose:

There was an increase in both BUN and blood glucose level in cystic cows over in normal PP cows. There was a negative significant correlation (R= - 0.562, P= 0.005) between BUN and time P.P. in cystic cows. While there was a non-significant correlation between blood glucose and time P.P. (R= - 0.261, P= 0.229).

Table 9: Serum levels of BUN and glucose during PPP in cystic PP cows

Cow ID	BUN (mg/dl)				Blood Glucose (mg/dl)			
	8 d P.P	18 d P.P	28 d P.P	38 d P.P	8 d P.P	18 d P.P	28 d P.P	38 d P.P
15*	20.89	20.35	19.52	19.88	61.56	63.46	62.15	61.88
44*	21.56	21.33	20.56	20.89	61.87	62.89	62.51	61.47
128*	20.86	20.33	20.12	20.06	63.43	65.47	64.12	63.88
219*	20.89	21.66	20.88	19.25	60.88	62.15	62.09	61.17
212*	20.23	19.98	19.83	19.56	59.56	61.35	61.88	61.52
Mean	20.89	20.73	20.18	19.93	61.46	63.06	62.55	61.98
SD	0.47	0.72	0.55	0.62	1.42	1.56	0.91	1.09
Mean ± SD	20.89 ± 0.47	20.73 ± 0.72	20.18 ± 0.55	19.93 ± 0.62	61.46 ± 1.41	63.06 ± 1.56	62.55 ± 0.91	61.98 ± 1.09

4.3.2.2. Phosphorus and Magnesium

There was a non significant difference ($P>0.05$) in P and Mg levels in cystic cows with time P.P. Significant difference in P and Mg levels were recorded at the 3rd examination ($P>0.01$) and the 4th examination ($P<0.0001$) between cystic and cyclic cows, respectively. Phosphorus level was positively correlated with time

P.P., (R=0.546, P<0.0001) and (R=0.459, P=0.028) in normal cyclic and cystic cows respectively.

Table 10: Serum levels of P and Mg during P.P. in cystic P.P. cows

Cow ID	phosphorus (mmol/l)				Magnesium (mmol/l)			
	8 d P.P	18 d P.P	28 d P.P	38 d P.P	8 d P.P	18 d P.P	28 d P.P	38 d P.P
15*	1.59	1.57	1.62	1.67	0.72	0.71	0.73	0.71
44*	1.61	1.58	1.63	1.62	0.75	0.75	0.68	0.75
128*	1.74	1.98	1.99	1.98	0.7	0.73	0.74	0.73
219*	1.71	2.14	2.09	2.01	0.69	0.71	0.68	0.73
212*	1.56	1.85	1.98	1.86	0.73	0.68	0.73	0.74
Mean ± SD	**1.64 ± 0.08**	**1.82 ± 0.25**	**1.86 ± 0.22**	**1.83 ± 0.18**	**0.72 ± 0.02**	**0.72 ± 0.03**	**0.71 ± 0.03**	**0.73 ± 0.01**

4.3.2.3. Sodium and Potassium

There was non-significant difference in Na and K level with reference to the time P.P. in postpartum cows. Moreover, there was non-significant difference, in Na and K between cystic and normal cyclic PP cows.

Table 11: Serum levels of Na and K during PP in cystic PP cows

Cow ID	Na (mmol/l)				K+ (mmol/l)			
	8 d P.P	18 d P.P	28 d P.P	38 d P.P	8 d P.P	18 d P.P	28 d P.P	38 d P.P
15*	126.5	125.3	126.1	124.2	4.95	4.25	4.61	4.35
44*	125.3	125.5	124.8	125.1	4.85	4.51	4.76	4.51
128*	126.1	125.4	125.7	125.1	4.23	4.68	4.39	4.65
219*	123.6	125.2	124.6	124.8	4.67	4.71	4.63	4.22
212*	123.5	124.5	125.2	126.1	4.82	4.33	4.95	4.17
Mean ± SD	**125 ± 1.39**	**125.2 ± 0.40**	**125.3 ± 0.60**	**125.1 ± 0.70**	**4.7 ± 0.28**	**4.5 ± 0.20**	**4.67 ± 0.20**	**4.38 ± 0.20**

4.3.2.4. Zinc, copper, and selenium:

The selenium level in cystic PP cows were significantly (P<0.001) lower than its level in normal PP cows along PP examination period. On the other hand there were no significant differences in levels of both zinc and copper between 2 groups during the PP examination period.

Table 12: Serum level of Zn, Cu, and Se during PPP in cystic PP cows

Cow ID	Zn (µmol/l)				Cu (µmol/l)				Se (µmol/l)			
	8 d P.P	18 d P.P	28 d P.P	38 d P.P	8 d P.P	18 d P.P	28 d P.P	38 P.P	8 d P.P	18 d P.P	28 d P.P	38 d P.P
15*	11.89	12.13	12.25	12.31	9.98	10.35	10.88	9.91	3.26	3.15	3.21	3.51
44*	11.56	12.14	12.21	12.29	9.87	10.54	10.75	9.87	2.35	2.15	2.58	2.7
128*	11.98	12.01	12.25	12.28	9.81	10.48	10.91	9.99	2.21	2.51	2.81	2.85
219*	11.63	12.15	12.21	12.25	9.86	10.46	10.95	9.93	3.21	3.28	3.26	3.18
212*	11.51	12.15	12.21	12.29	9.87	10.38	10.79	9.82	2.25	3.15	3.58	3.27
Mean ± SD	**11.71 ± 0.2**	**12.12 ± 0.1**	**12.23 ± 0.1**	**12.28 ± 0.2**	**9.88 ± 0.1**	**10.44 ± 0.1**	**10.86 ± 0.1**	**9.91 ±0.1**	**2.66 ±0.53**	**2.85 ±0.49**	**3.09 ±0.39**	**3.1 ± 0.33**

4.4. Relation between blood parameters and Postpartum period in cows:

4.4.1. BUN and blood glucose:

There were highly significant differences ($P < 0.0001$) in BUN and blood glucose level in cystic cows over in normal PP cows (Figure 35 & 36) with total mean of BUN (16.38 ± 1.45 and 20.43 ± 0.68 mg/dl respectively) and blood glucose (59.32 ± 2.28 and 62.27 ± 1.32 mg/dl respectively).

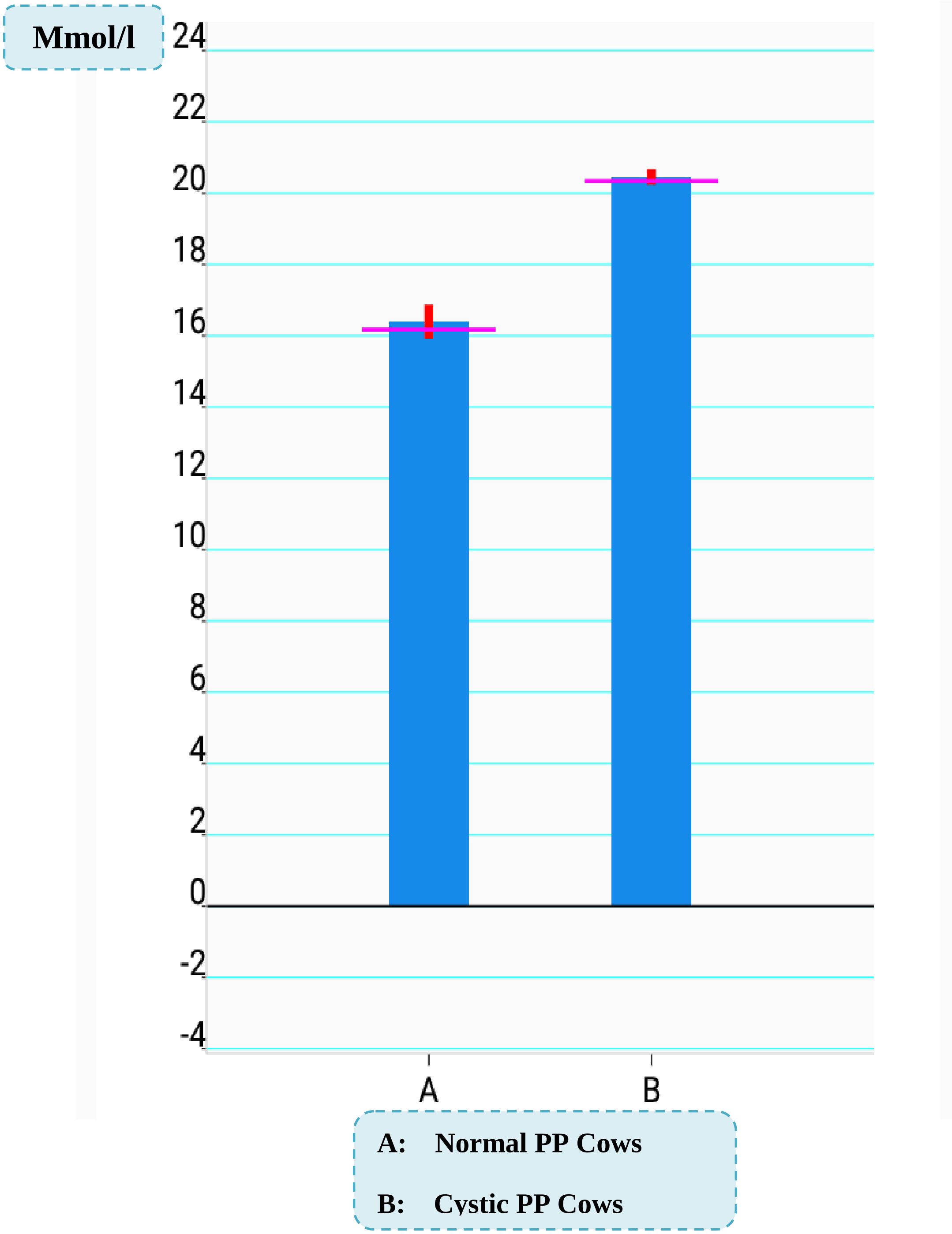


Figure 31: BUN in normal cyclic and cystic PP cows

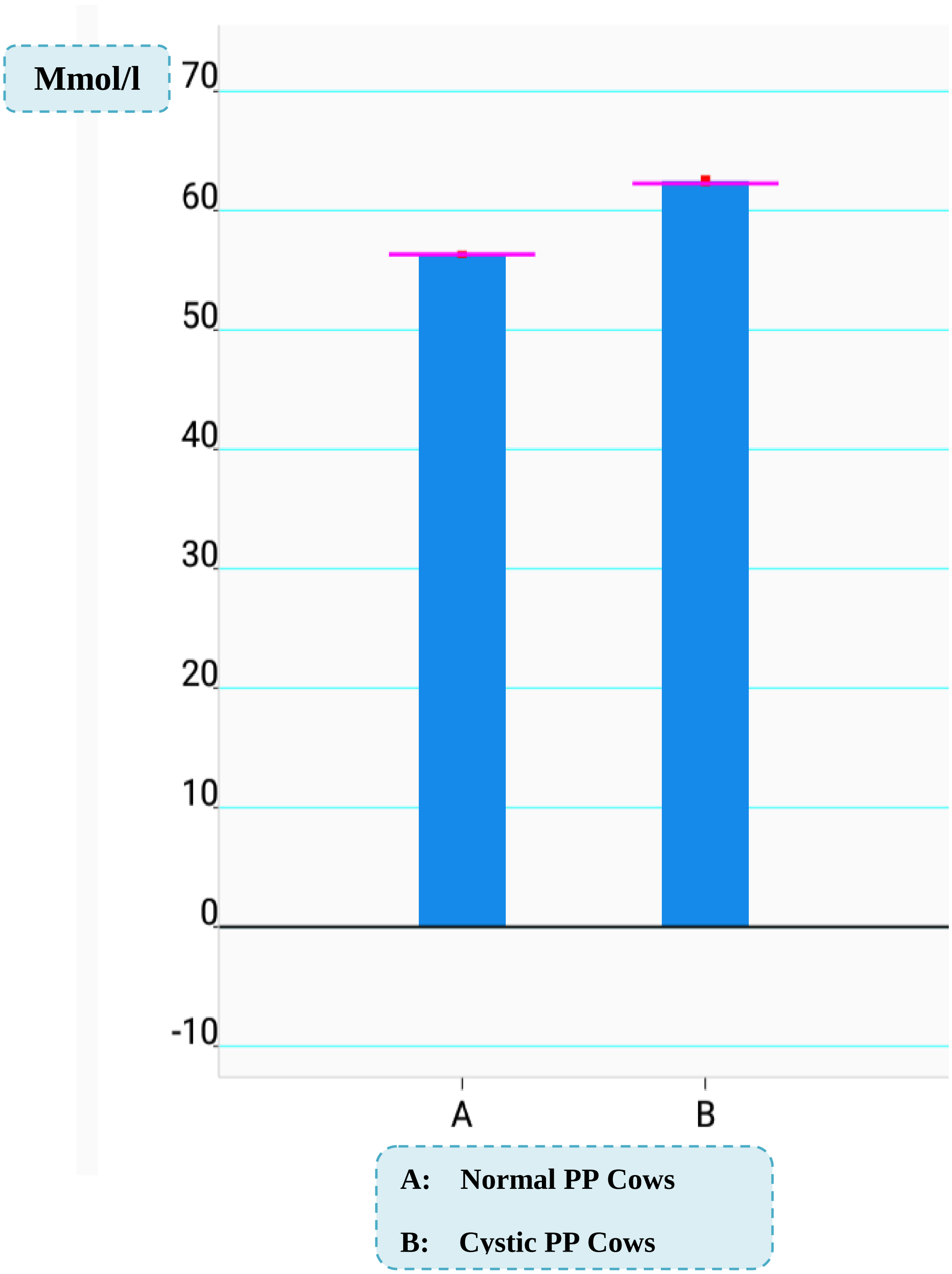


Figure 32: Blood glucose in normal cyclic and cystic PP cows

4.4.2.Phosphorus and magnesium:

There was a non significant difference ($P > 0.05$) in phosphorus level in both cystic and normal cows (Figure 34). But the Mg had a significant increase ($P < 0.0001$) in cystic PP cows over that of normal PP cows (Figure 33).

The overall mean of phosphorus in normal and cystic group was (1.93 ± 0.03 and 1.79 ± 0.06 mmol/l respectively) and that of Mg was (0.69 ± 0.01 and 0.72 ± 0.01mmol/l respectively) as presented in figure 25.

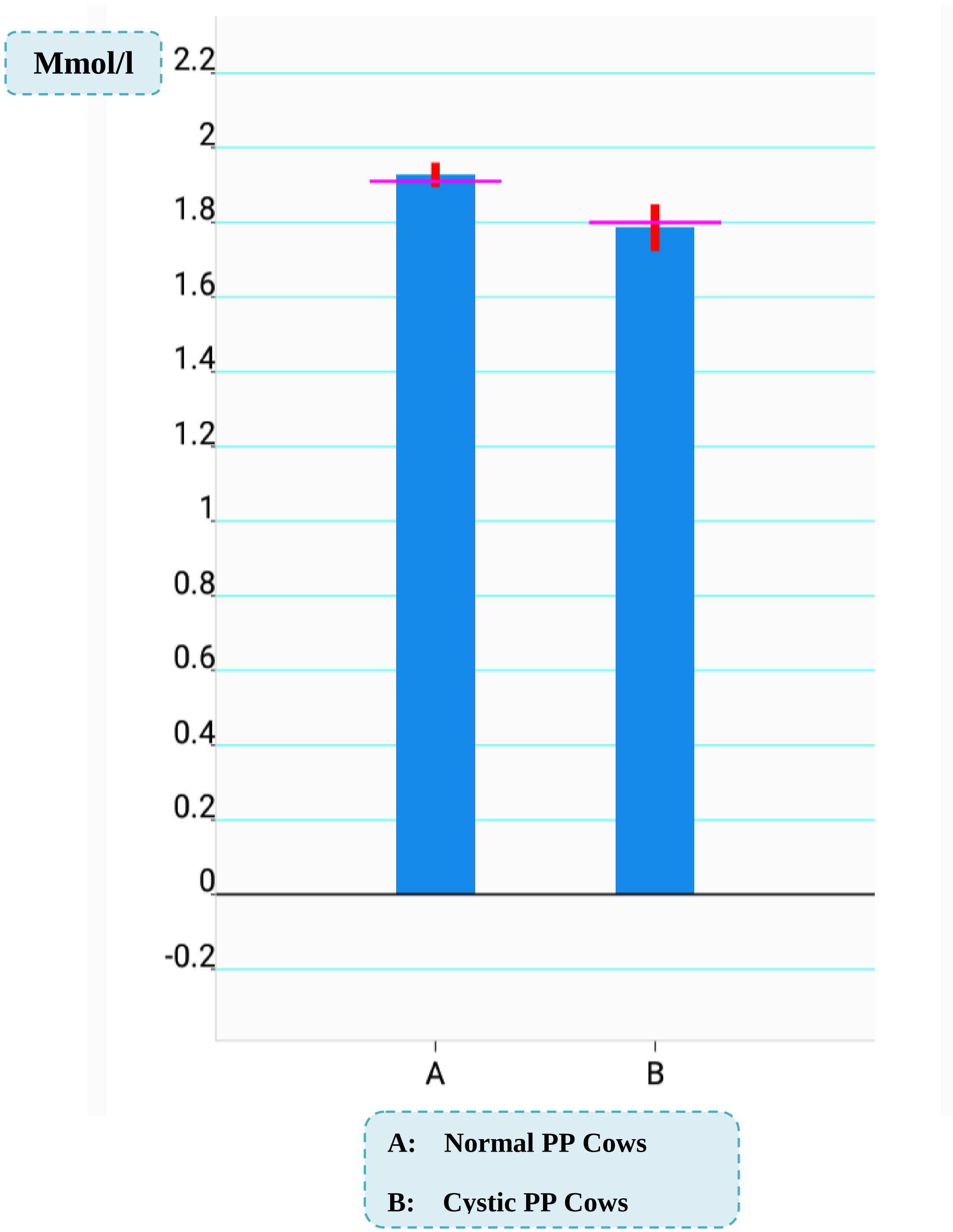


Figure 33: Phosphorus level in cyclic and cystic PP cows

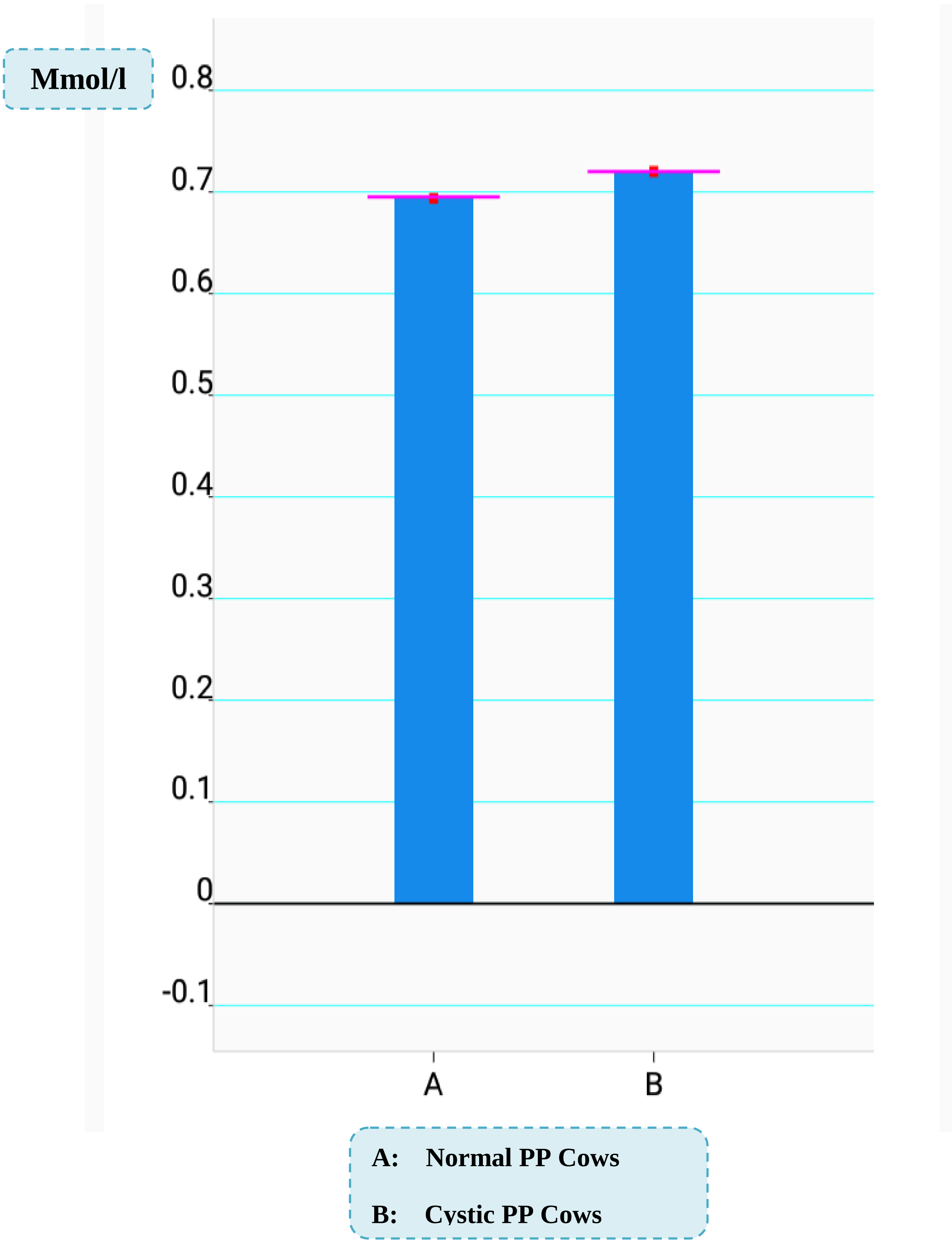


Figure 34: Magnesium level in cyclic and cystic PP cows

4.4.3.Sodium and Potassium:

There was a non-significant difference ($P > 0.05$) in Na and K level between cystic and normal PP cows as presented in figures (35) & (36).

The overall means of Na and K in normal and cystic cows were (124.9 ± 0.19, 125.1 ± 0.14, 4.54 ± 0.06, and 4.56 ± 0.03 mmol/l respectively).

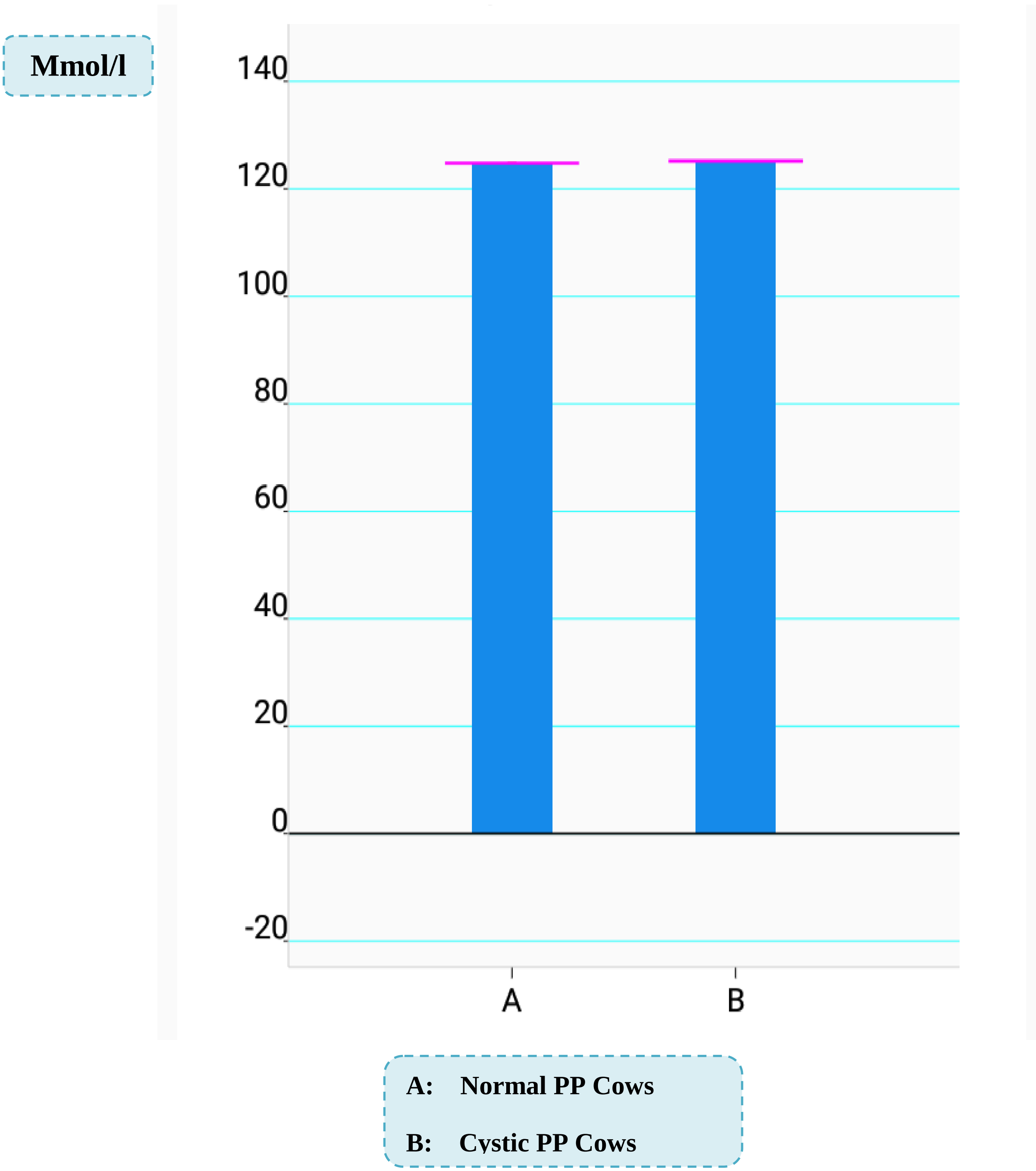


Figure 35: Sodium level in cyclic and cystic PP cows

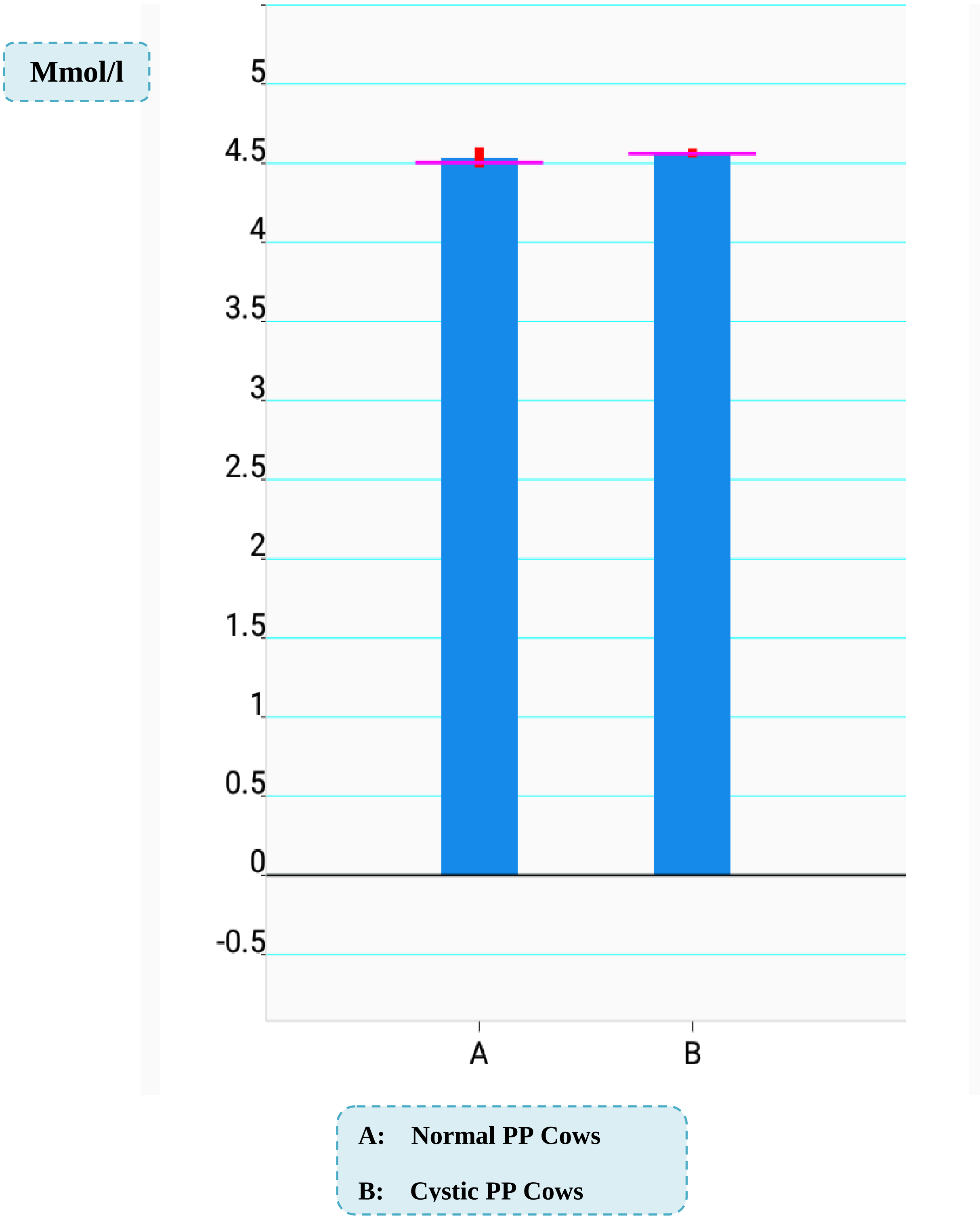


Figure 36: Potassium level in cyclic and cystic PP cows

4.4.4. Zinc, Copper, and Selenium:

There was a significant increase ($P < 0.001$) in selenium level in normal PP cows over than its level in cystic PP cows (Figure 39).

On the other hand there was a non-significant difference ($P > 0.05$) in the levels of both zinc and copper between the 2 groups (Figures 37 & 38).

Overall means of Zn, Cu, and Se in both normal and cystic PP cows were 12.06 ± 0.03, 12.08 ± 0.02, 10.27 ± 0.03, 10.27 ± 0.02, 5.36 ± 0.08, and 2.94 ± 0.17 µmol/l respectively.

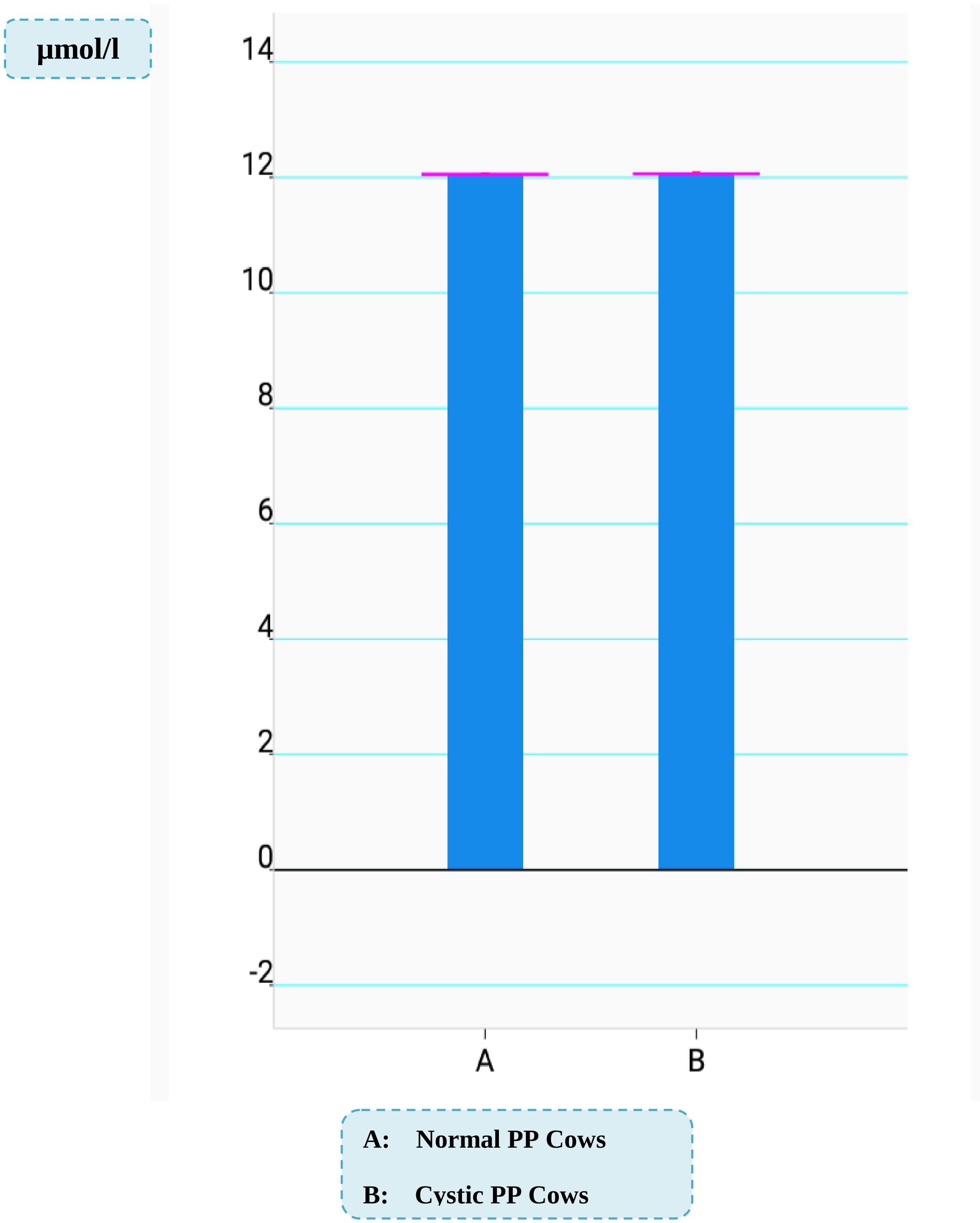
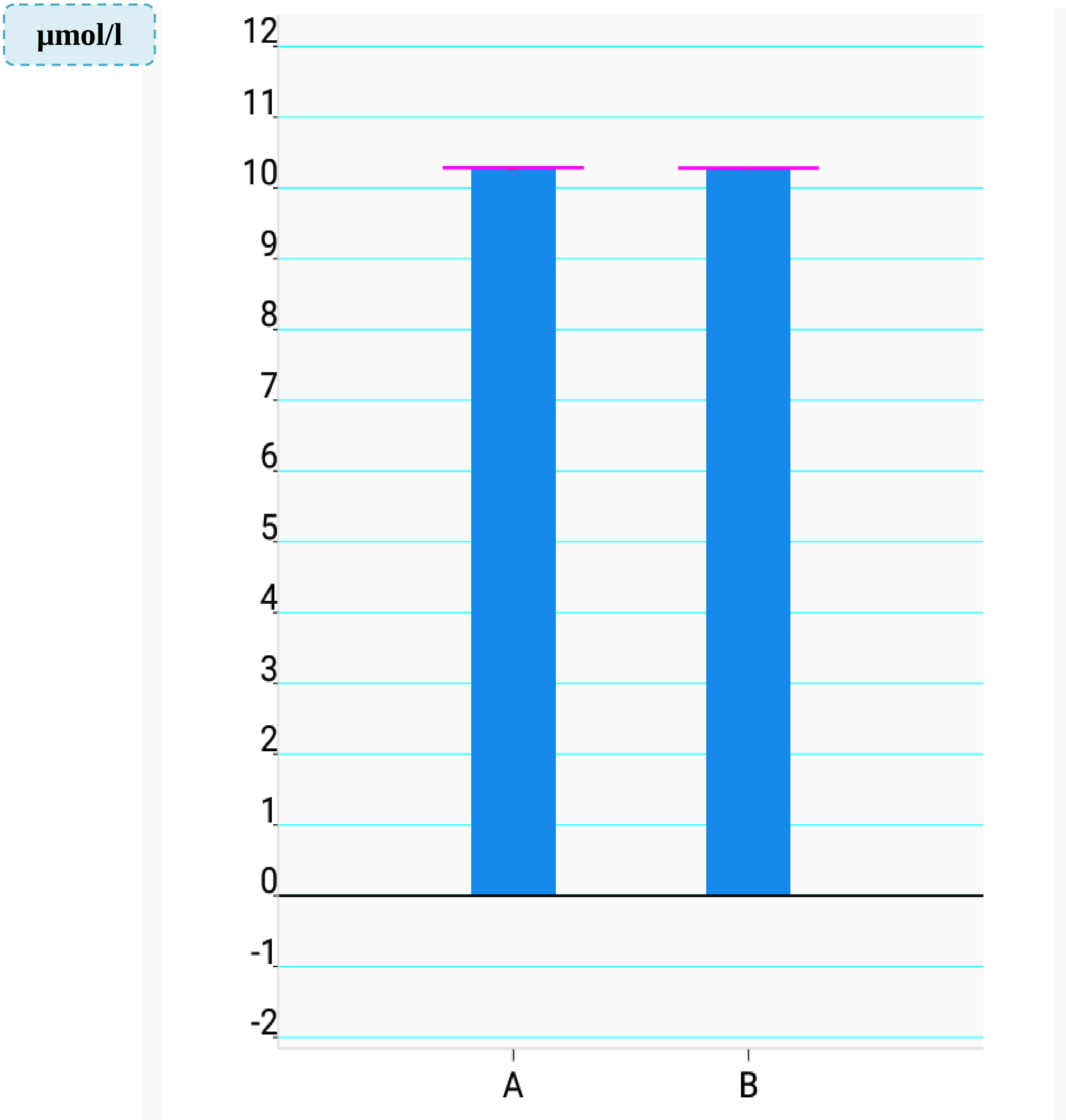


Figure 37: Zinc level in cyclic and cystic PP cows



A: Normal PP Cows

B: Cystic PP Cows

Figure 38: Copper level in cyclic and cystic PP cows

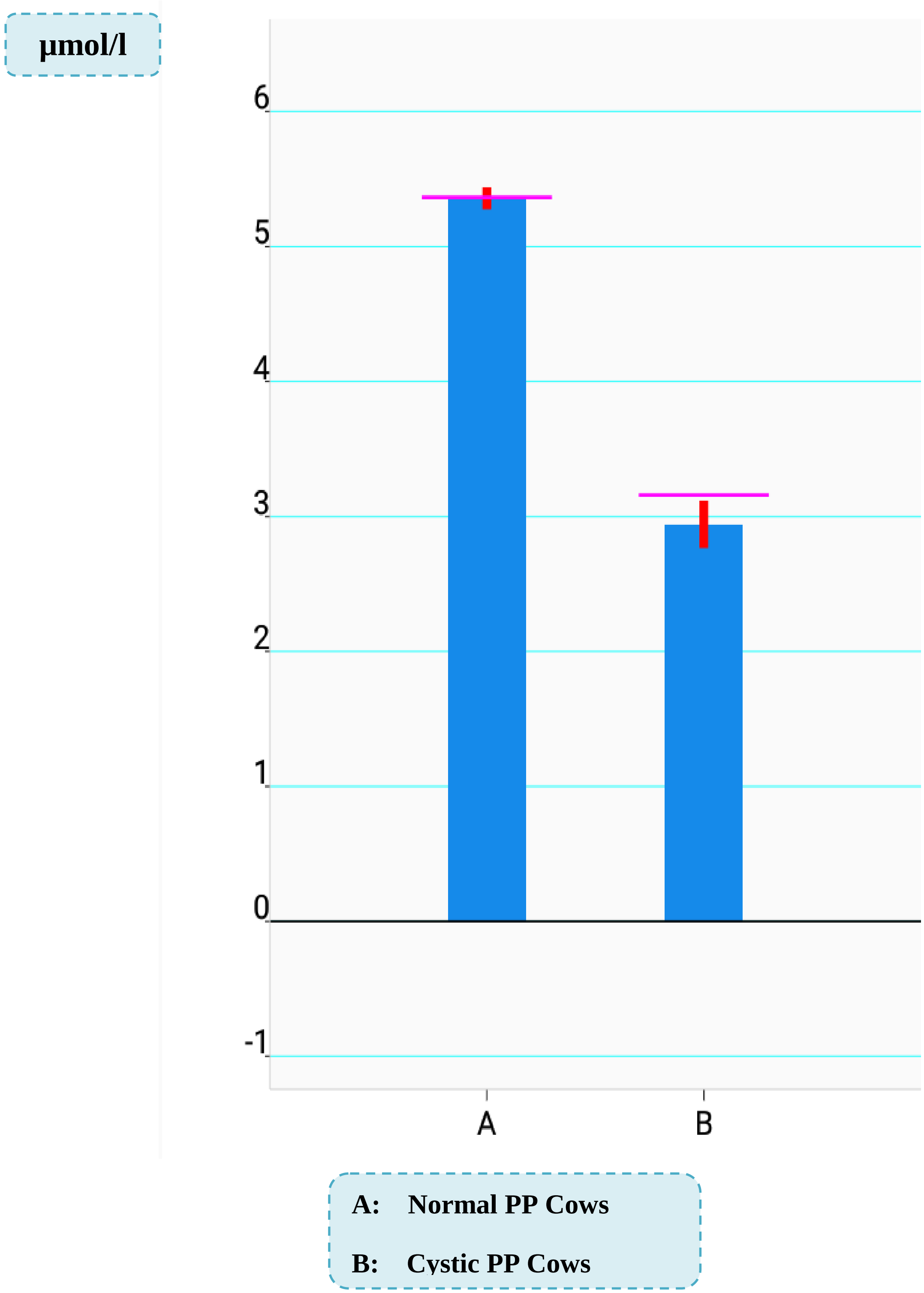


Figure 39: Selenium level in cyclic and cystic PP cows

4.5. Ultrasound examination of postpartum uterine involution and ovarian activity :

4.5.1. Uterine involution:

4.5.1.1. Uterine horn diameter:

The overall mean uterine horn diameter was 67.63 ± 8.53 mm at mean 8.16 ± 3.56 days P.P.(around the 1^{st} week PP), 38.45 ± 6.45 mm at mean 18.16 ± 3.56 days PP , 23.59 ± 5.86 mm at mean 28.16 ± 3.56 days PP, and 22.83 ± 4.36 mm at mean 38.16 ± 3.56 days PP as presented in (Ultrasound images 3 – 11).

Thus the uterus was completely involutes at mean 29.86 ± 5.42 days PP in the 2 last successive examinations.

The uterine caruncles were detected in some cows only during the 1^{st} US examination at mean day 8.16 ± 3.56 PP and measured in a mean of 9.56 ± 1.25 mm.

4.5.2. Ovarian changes:

4.5.2.1. Ovarian follicles:

During the 1^{st} examination at 8.16 ± 3.56 days PP we detect small and medium sized follicles with mean diameter 3.45 ± 2.23 mm and 7.75 ± 0.43 mm respectively.

During the 2^{nd} examination at 18.16 ± 3.56 days PP we detected medium and large sized follicles with mean diameters 8.12 ± 0.13 mm and 13.67 ± 1.56 mm respectively.

During the 3^{rd} examination at 28.16 ± 3.56 days PP we detected large sized follicles with mean diameter 18.54 ± 1.65 mm.

During the 4^{th} examination at 38.16 ± 3.56 days PP we detected large sized follicles with mean diameter 18.35 ± 0.85 mm.

4.5.2.2. Corpus Luteum:

The CL of pregnancy was detected in some cows (n= 4) whose calving date was preceding about 5 to 7 days before the rest of group i.e. about 14 days PP and measured with mean diameter 19.65 ± 2.23 mm. The ovary ipsilateral to the non gravid horn has been shown to resume ovarian activity more rapidly than the contra-lateral ovary, so most of early ovulation occurs from the ovary contra-lateral to the gravid horn and occur between the 1^{st} and 2^{nd} examinations.

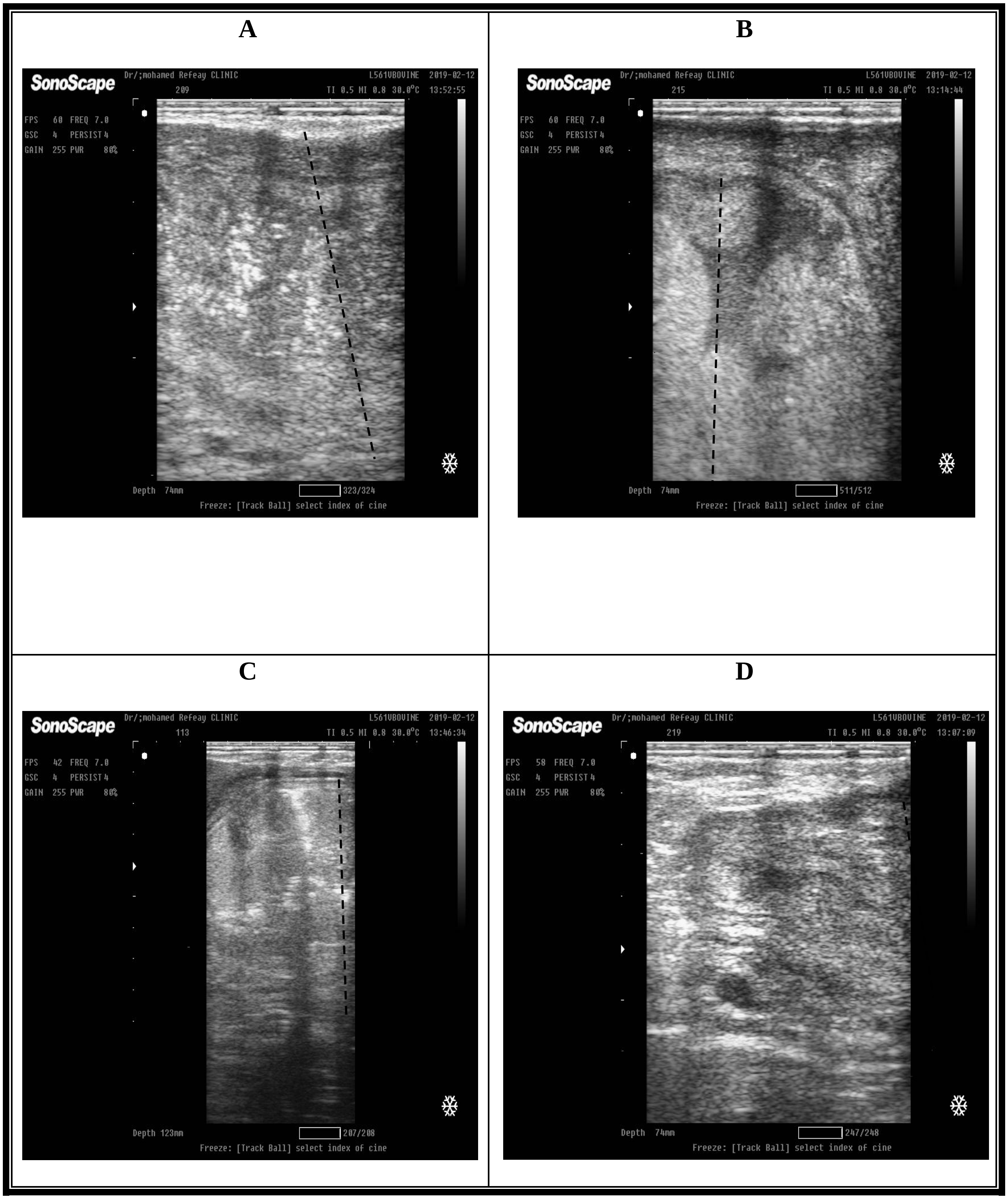
So the CL of 1^{st} estrus was detected mostly on the ovaries ipsilateral to the non-gravid horn on the 2^{nd} examination at 18.16 ± 3.56 days PP and 3^{rd} examination at 28.16± 3.56 days PP and measured with mean diameters 22.15 ± 0.82 mm and 26.25 ± 1.56 mm respectively.

4.5.2.3. Cystic ovary

Postpartum cystic ovary was detected in 5 cows (Subgroup B).

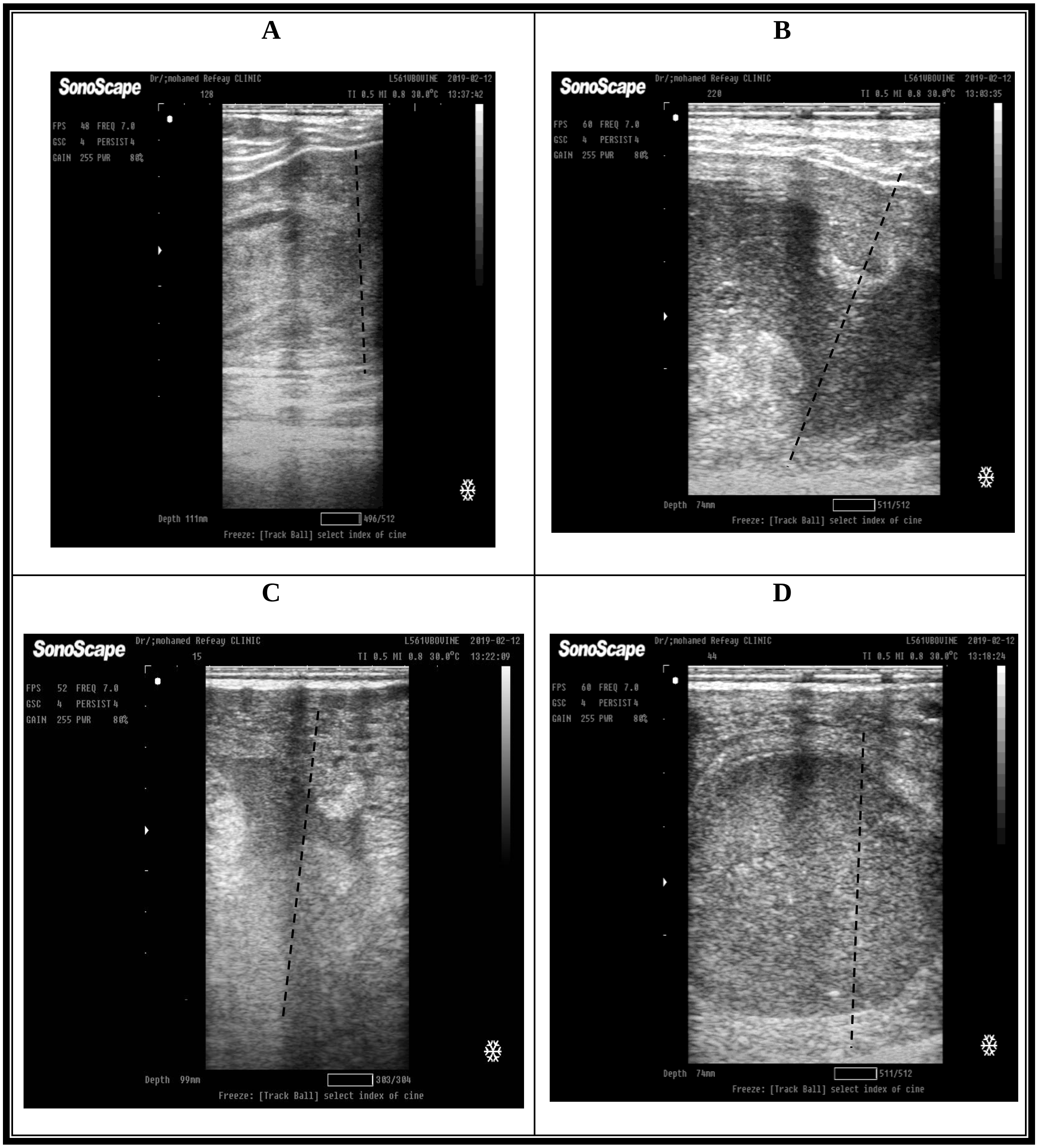
4 cows showed luteal cyst on the left ovary at mean 18.16 ± 3.56 days PP and 28.16 ± 3.56 days PP, and measured with mean diameters 29.85± 1.82 mm and 29.52± 1.22 mm respectively.

While one cow has been showed very large follicular cyst on the day 19 PP and 29 PP, and measured with diameter of 29.6 mm and 50.4 mm respectively.



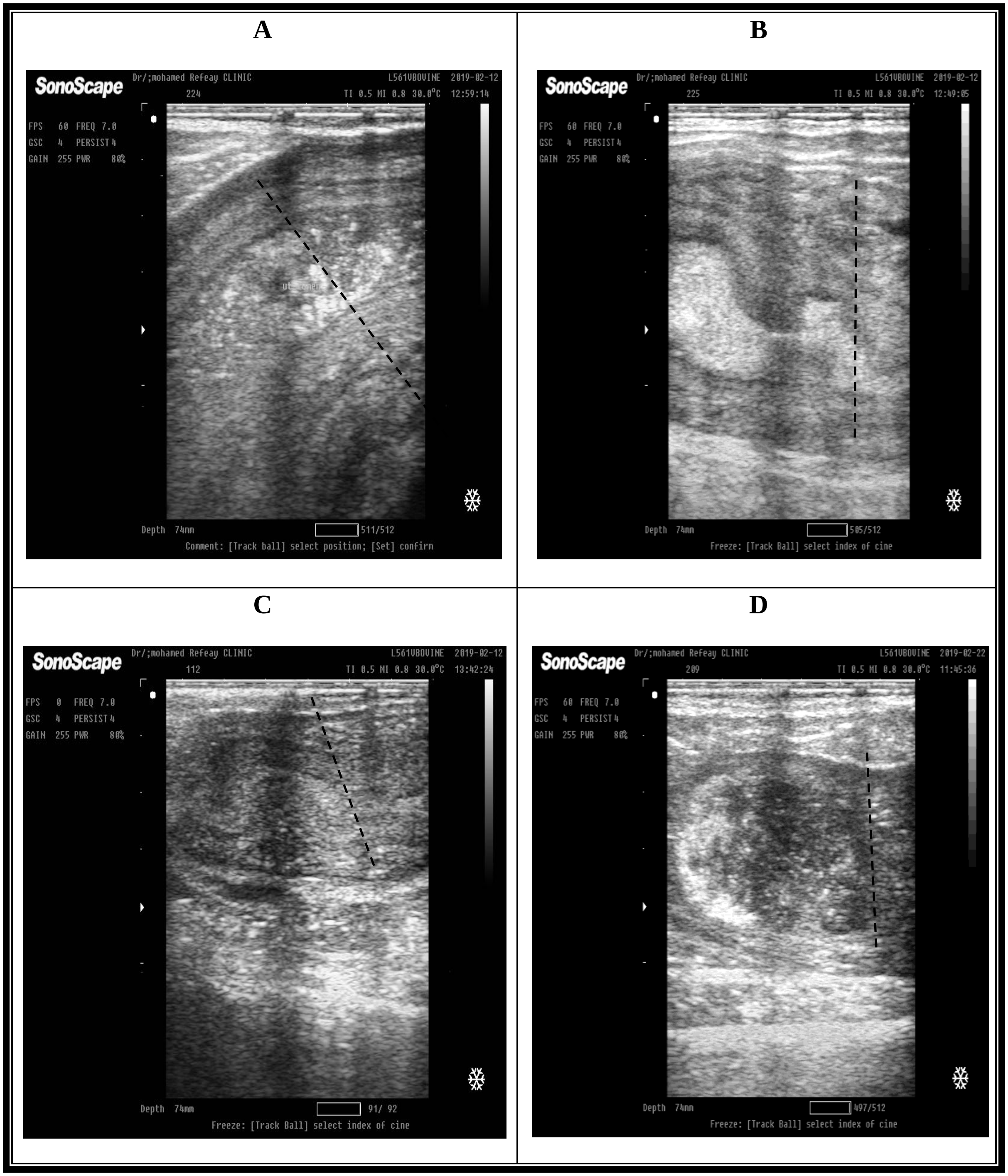
Ultrasonogram 3: Representative ultrasound image of PP cows at 1st examination:

A: Uterus on day 5 PP (U.D = 71.56 mm) containing intrauterine mixed echogenecity lochia, B: Uterus on day 6 PP (U.D = 69.25mm) showing uterine caruncle, C: Uterus on day 7 PP (U.D = 69.1mm), D: Uterus on day 8 PP (U.D = 66.25mm)



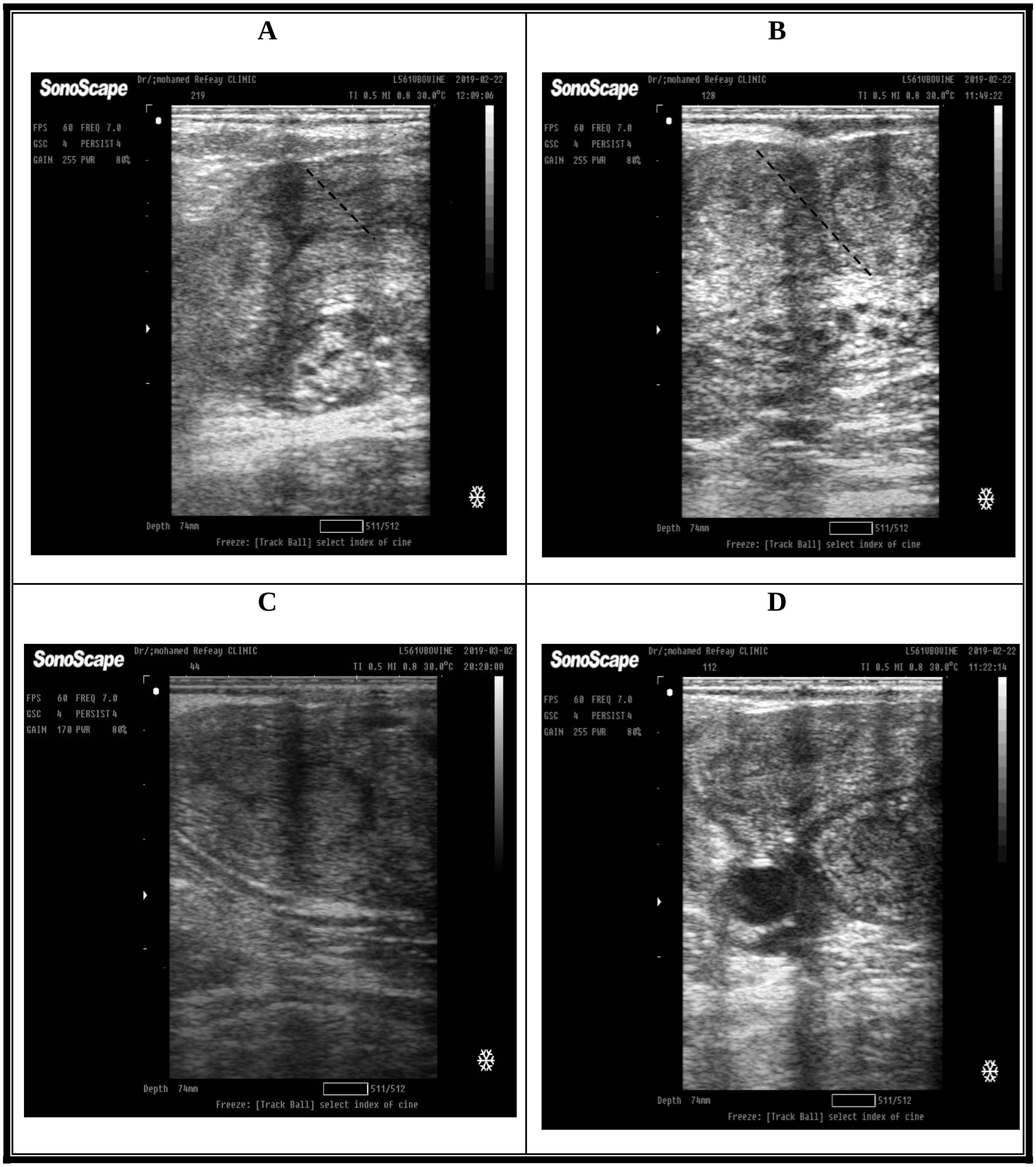
Ultrasonogram 4 Representative ultrasound image of PP cows at 1st examination:

A: Uterus on day 9 PP (U.D = 67.21mm), B: Uterus on day 10 PP (U.D = 69.1) with caruncles and mixed echogenic lochia, C&D: Uterus on day 11 PP (U.D = 63.32mm & 65.11mm respectively)



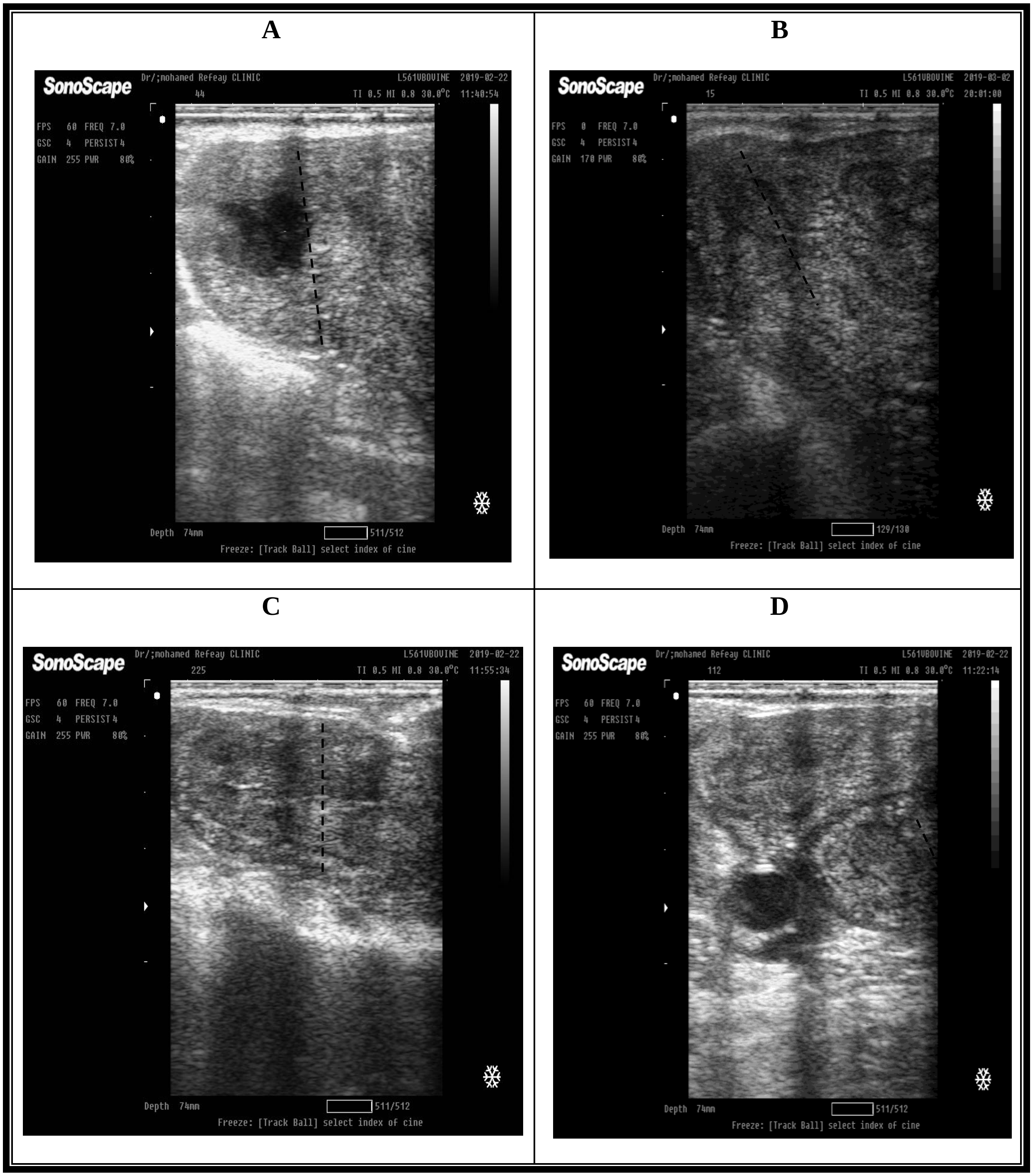
Ultrasonogram 5 Representative ultrasound image of PP cows at 1st examination:

A&B: Uterus on day 11 PP (U.D = 62.35mm & 61.54mm respectively), C: Uterus on day 14 PP (U.D = 55.32mm), D: Uterus on day 15 (U.D = 57.12m



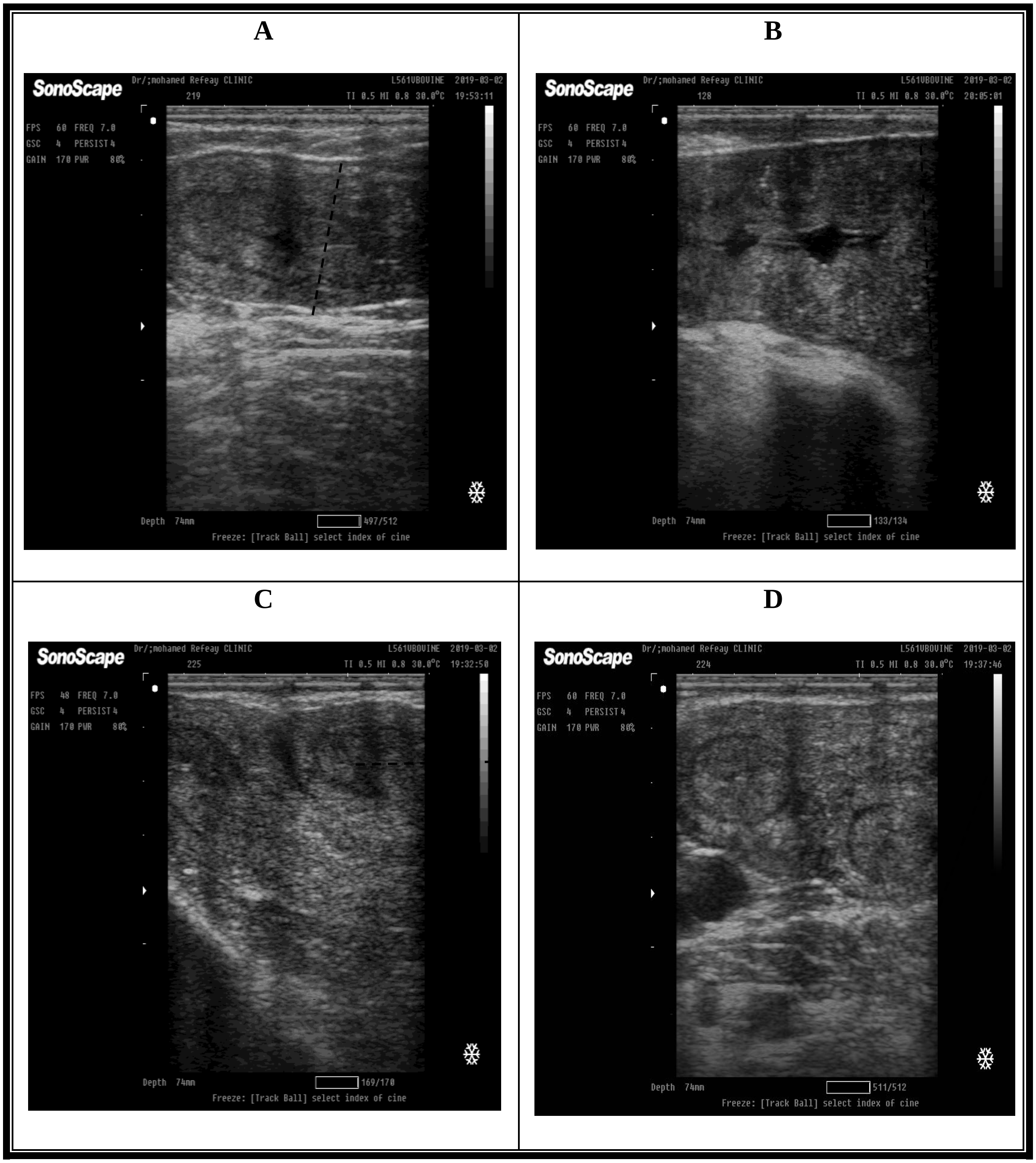
Ultrasonogram 6 Representative ultrasound image of PP cows at 2nd examination:

A: Uterus on day 18 PP (U.D = 35.25mm), B: Uterus on day 19 PP (U.D = 39.51mm), C: Uterus on day 20 PP (U.D = 41.43mm), D: Uterus on day 21 PP (U.D = 38.56mm)



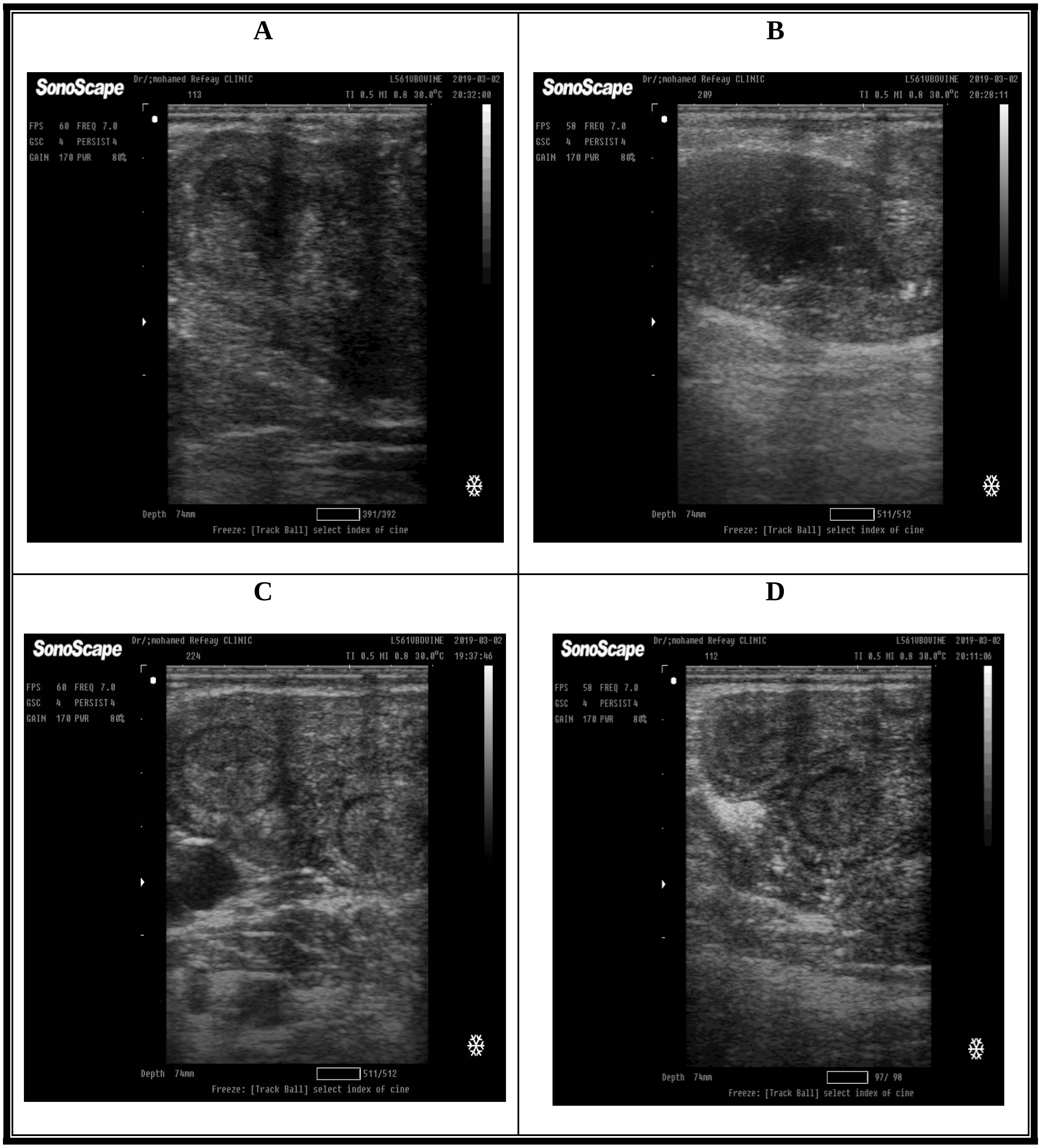
Ultrasonogram 7 Representative ultrasound image of PP cows at 3rd examination:

A, B, & C: Uterus on 21 days PP (U.D = 41.2 mm, 37.23 mm, & 40.45 mm respectively), D: Uterus on 24 days PP (U.D = 32.89 mm)



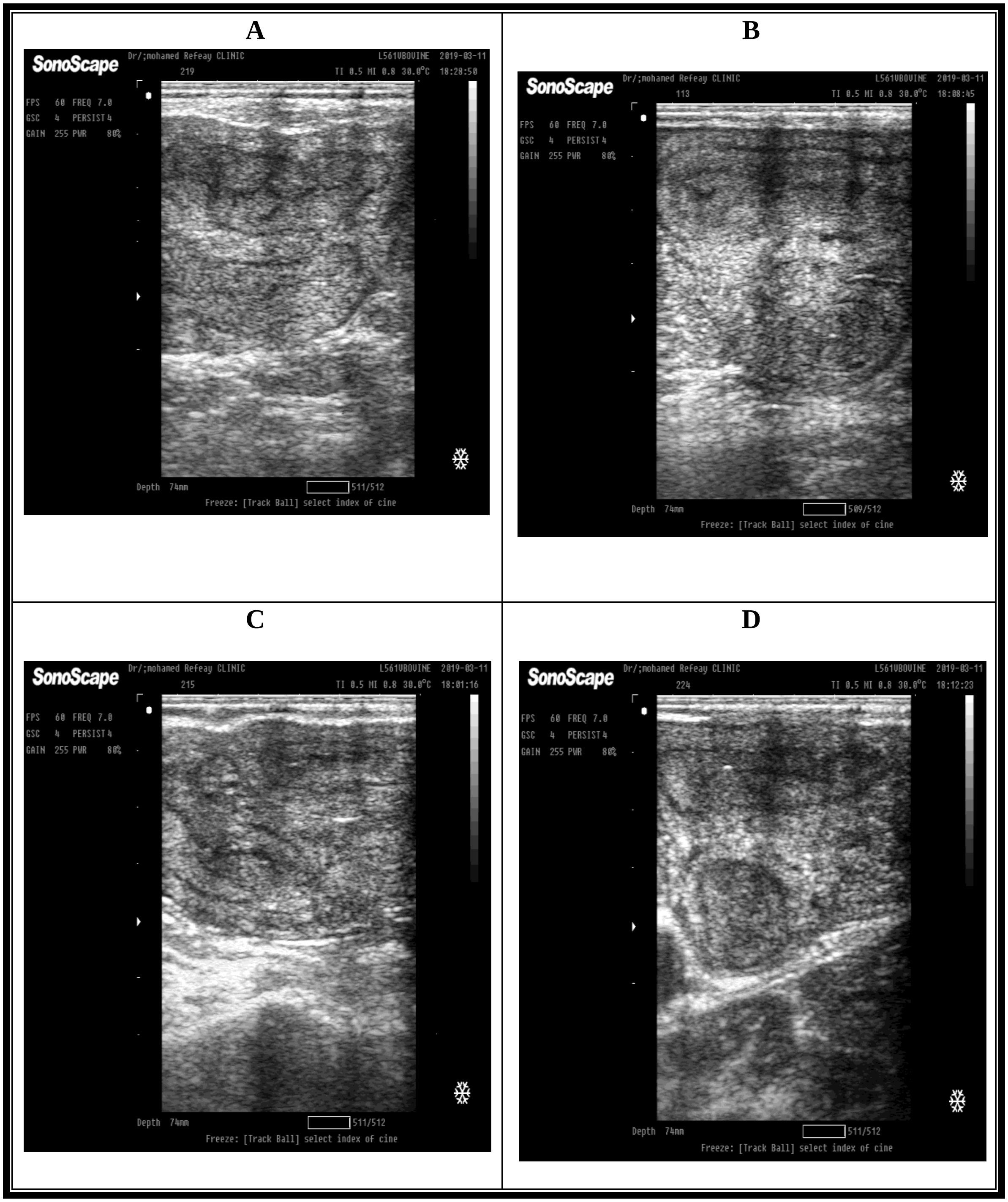
Ultrasonogram 8 Representative ultrasound image of PP cows at 3rd examination:

A: Uterus on day 28 PP (U.D = 26.23 mm), B: Uterus on day 29 PP (U.D = 25.12 mm), C: Uterus on day 30 PP (U.D = 24.25 mm), D: Uterus on day 31 (U.D = 23.33 mm)



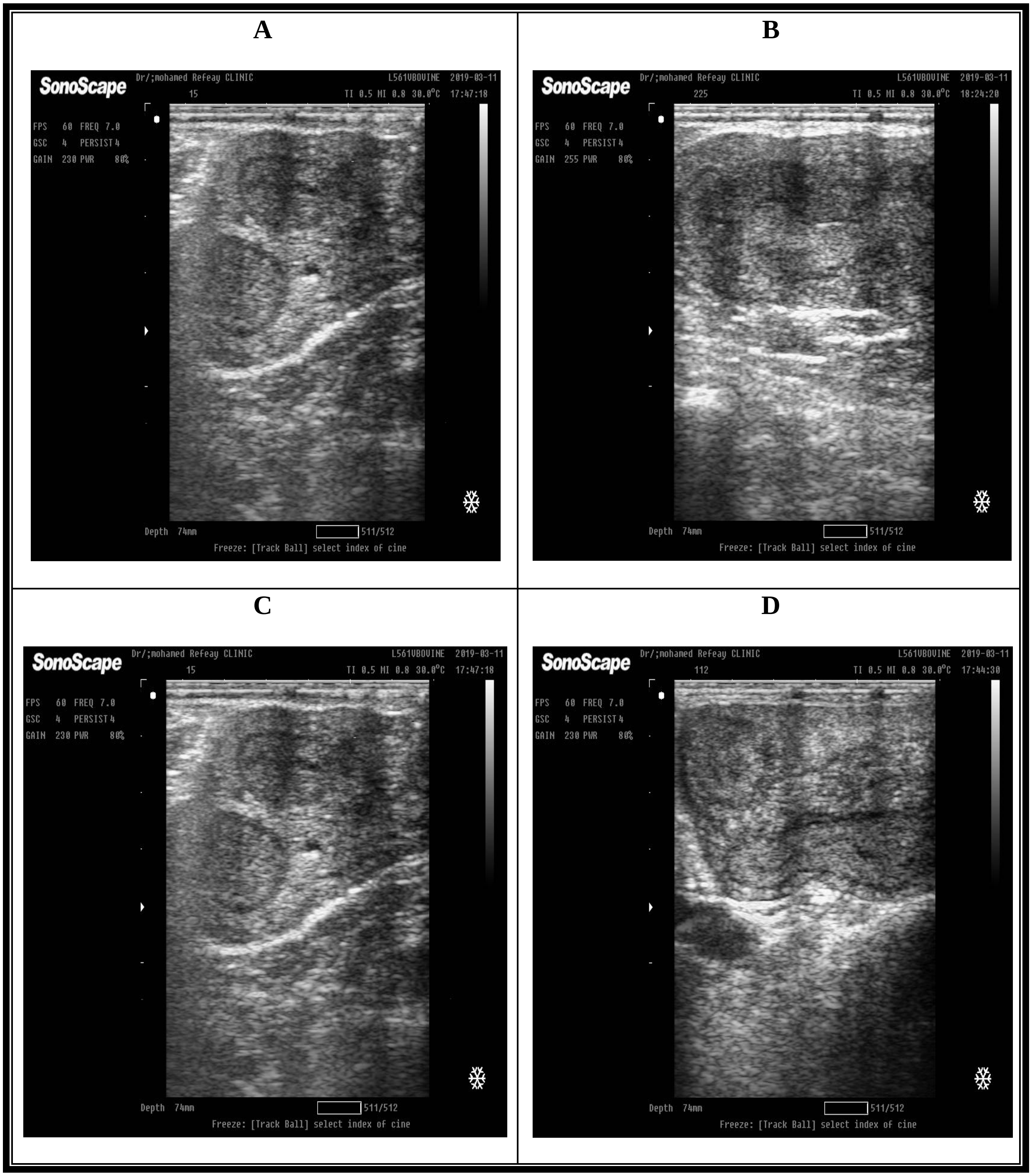
Ultrasonogram 9 Representative ultrasound image of PP cows at 4th examination:

A, B, & C: Uterus on day 31 PP (U.D = 24.21 mm, 24.98mm, & 23.02mm respectively), D: Uterus on day 34 PP (U.D = 23.01 mm)



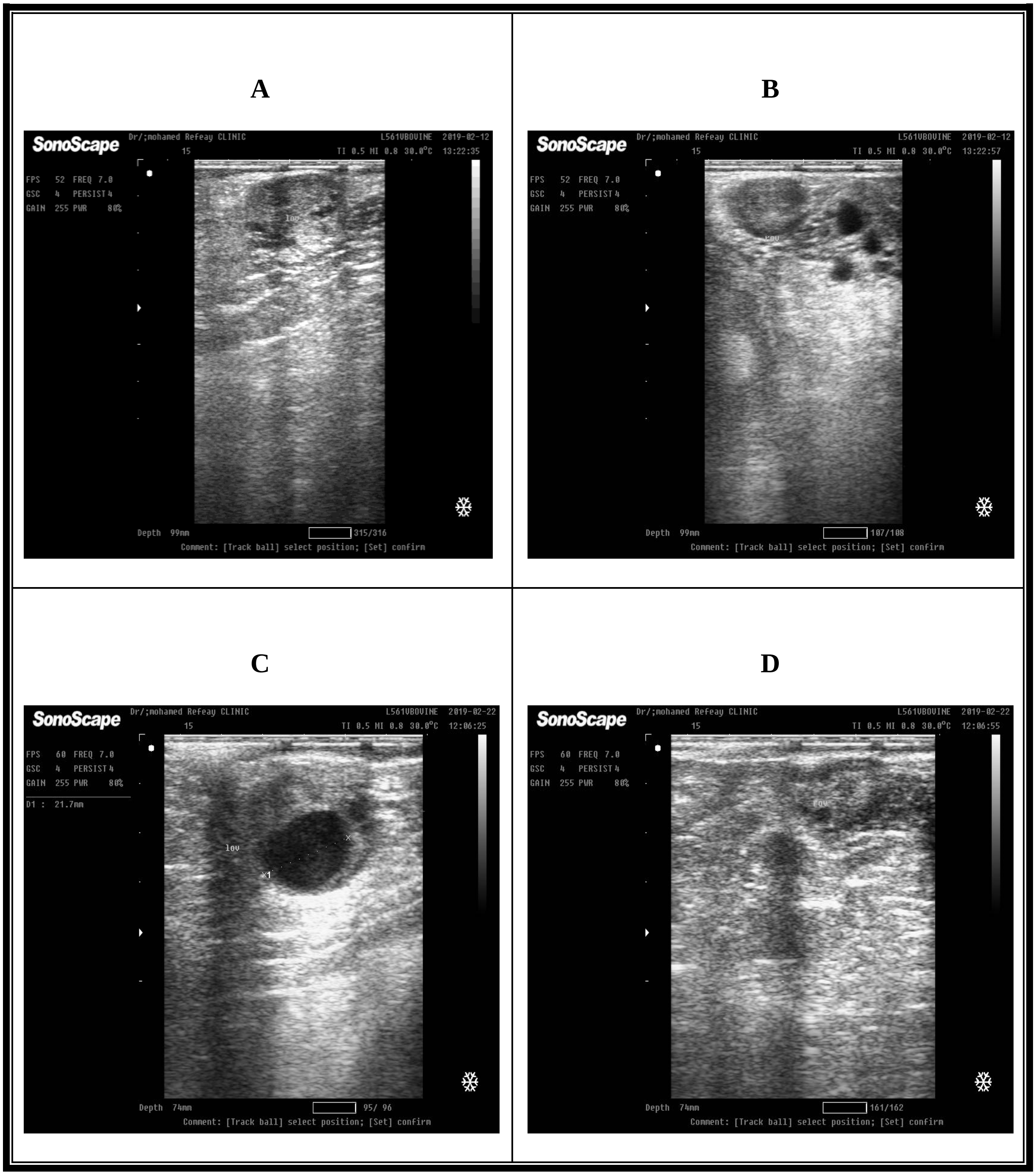
Ultrasonogram 10 Representative ultrasound image of PP cows at 4th examination:

A: Uterus on day 38 PP (U.D = 23.11mm), B: Uterus on day 39 PP (U.D = 22.98mm), C: Uterus on day 40 PP (U.D = 22.84 mm), D: Uterus on day 41 PP (U.D = 22.56 mm)



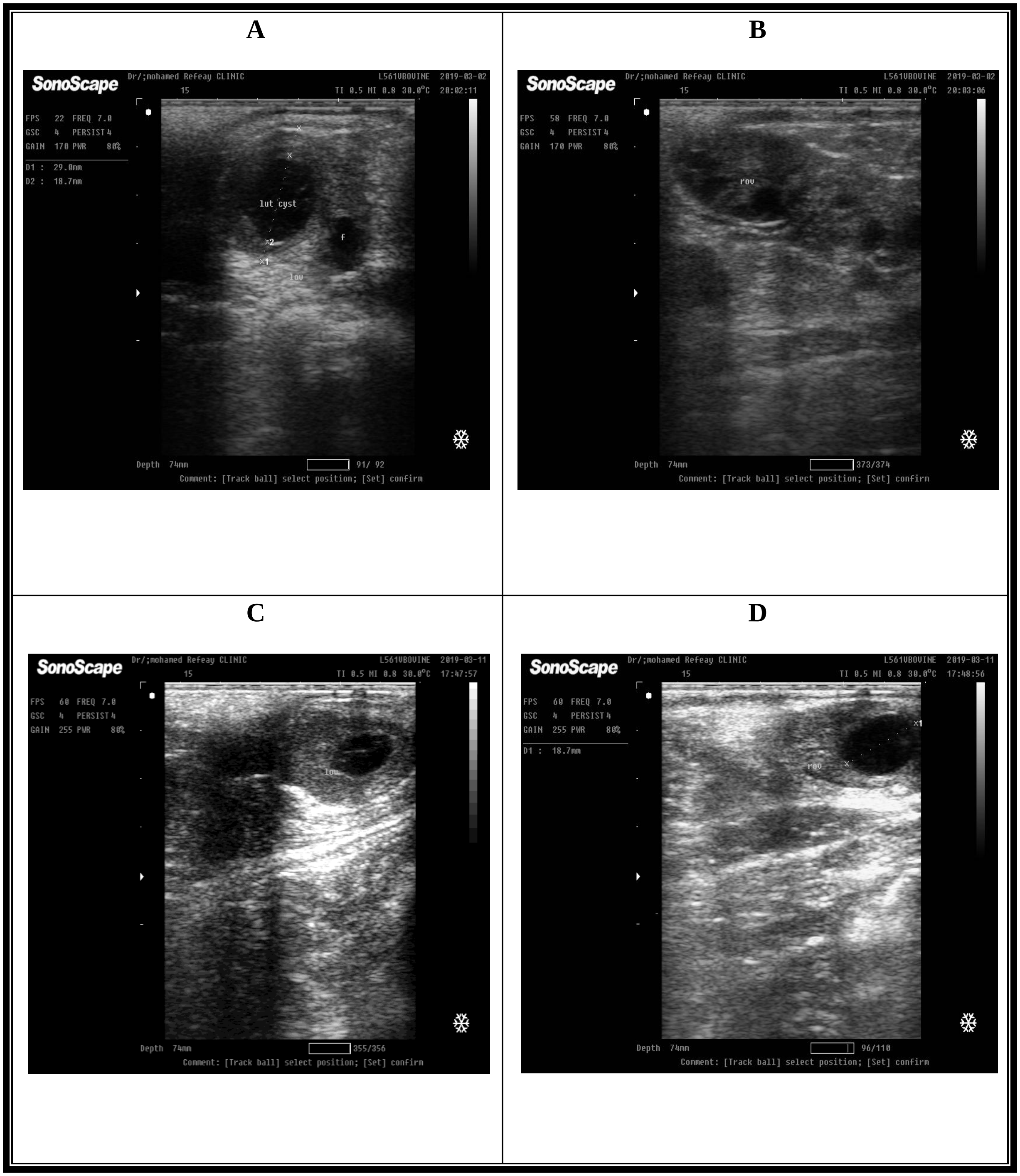
Ultrasonogram 11 Representative ultrasound image of PP cows at 4th examination:

A: Uterus on day 41 PP (U.D = 22.11mm), B: Uterus on day 41 PP (U.D = 21.78mm), C: Uterus on day 41 PP (U.D = 21.79 mm), D: Uterus on day 44 PP (U.D = 20.55 mm



Ultrasonogram 12 Representative ultrasound image of PP cows :

A: Left ovary 5 days PP showing small sized follicles, B: Right ovary 5 days PP showing no follicles, C: Left ovary 15 days PP showing luteal cyst 21mm and small sized follicle 4.53 mm, D: Right ovary 15 days PP showing small sized follicles

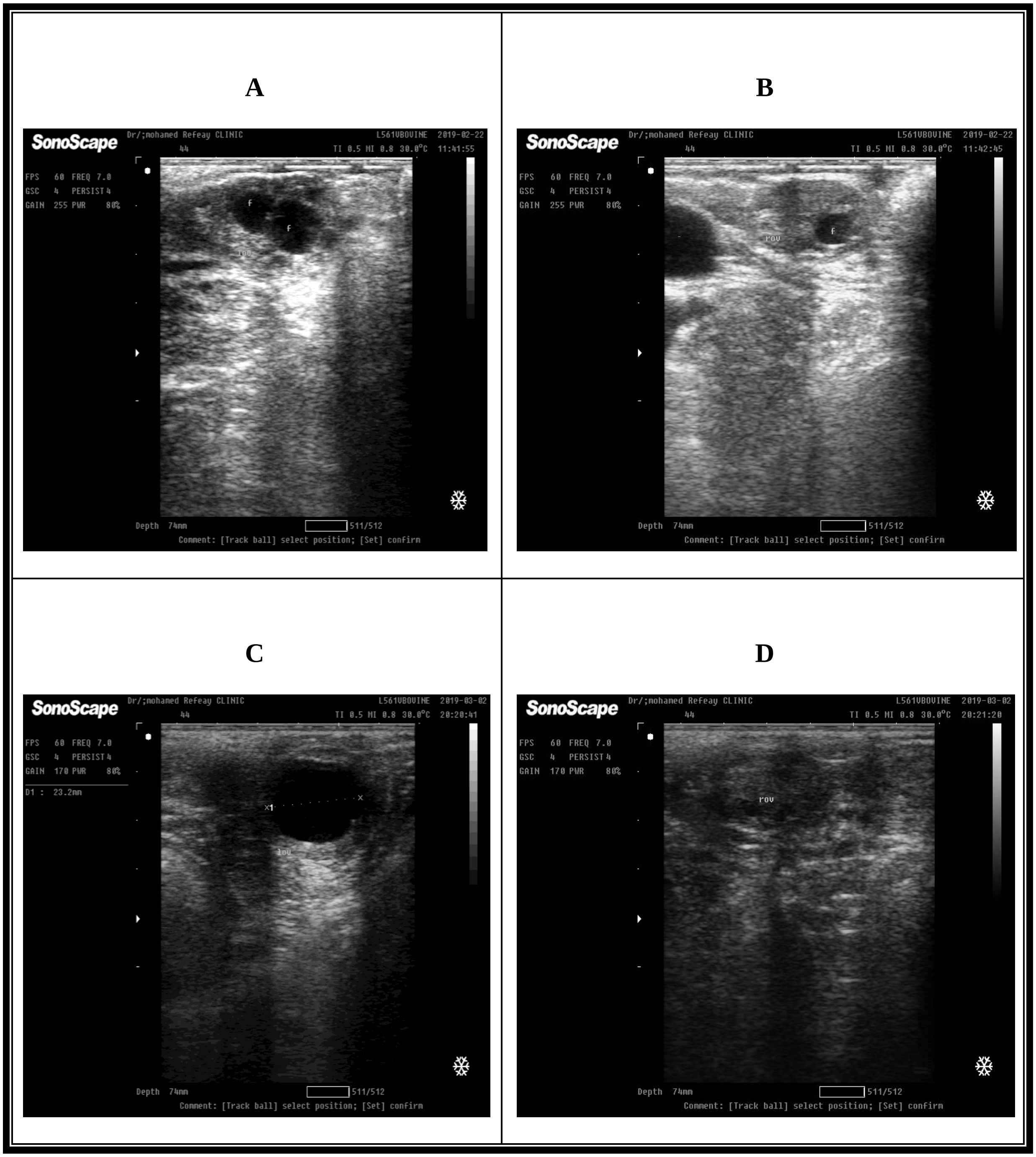


Ultrasonogram 13 Representative ultrasound image of PP cows:

A: Left ovary 25 days PP showing luteal cyst 29mm and medium sized follicle 9 mm

B: R. Ovary 25 days PP showing medium sized follicles 6.53, 5.61, and 7 mm, C: Left ovary 35 days PP showing luteal cyst 21mm

D: Right ovary 35 days PP showing large dominant follicle 18.7 mm



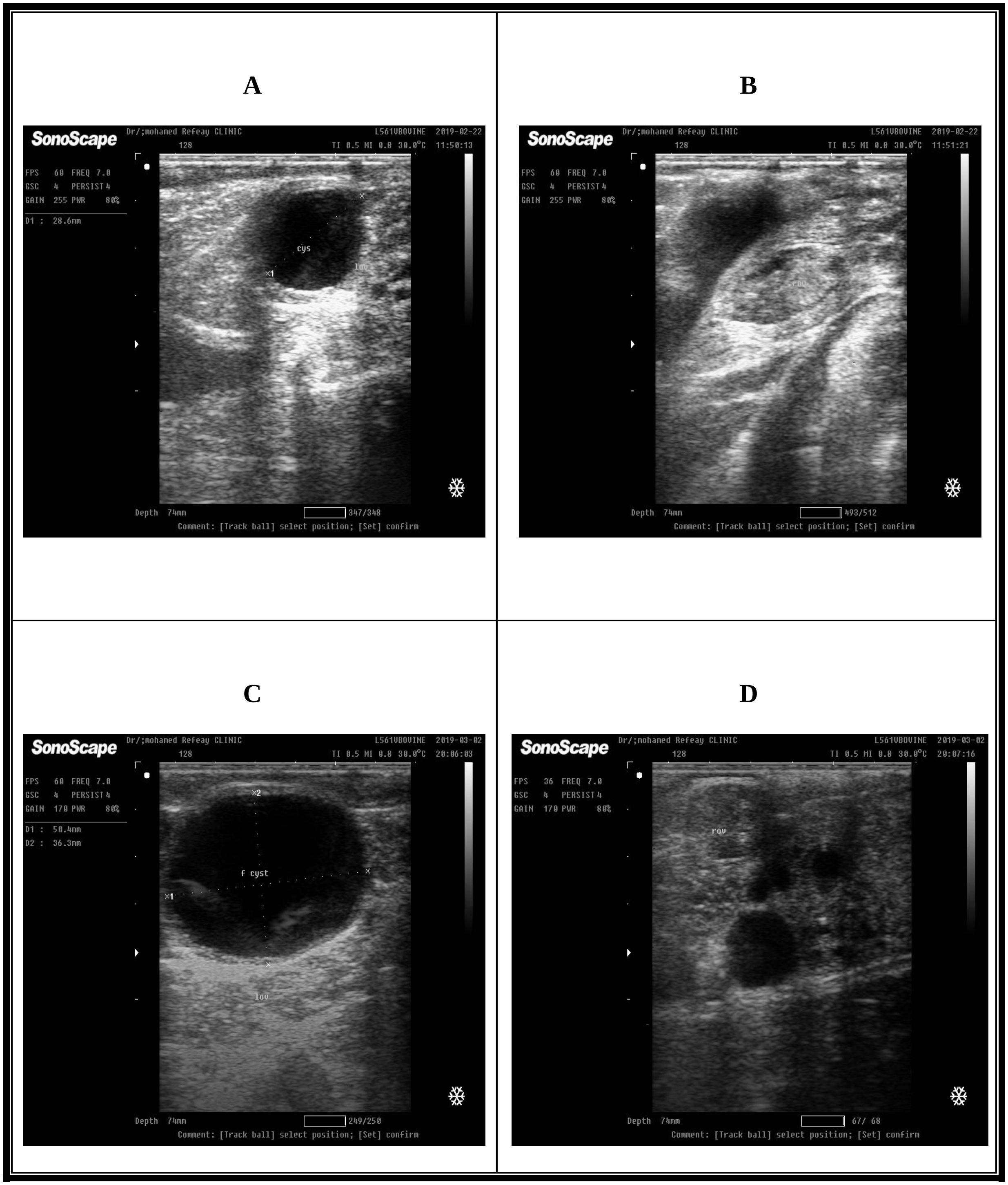
Ultrasonogram 14 Representative ultrasound image of PP cows:

A: Left ovary on 22 days PP showing 2 dominant follicles

B: Right ovary 22 days PP showing medium sized follicle 6.12mm

C: Left ovary 32 days PP showing dominant follicle measuring 27.2 mm

D: Right ovary 32 days PP showing medium sized follicle



Ultrasonogram 15 Representative ultrasound image of PP cows:

A: L. ovary 19 days PP showing luteal cyst measuring 29.6 mm, B: R. ovary 19 days PP, C: L ovary 29 days PP showing very large follicular cyst measuring 50.4 mm in diameter, D: R ovary on 29 days PP

5. DISCUSSION

The hematochemical blood parameters, Macro and trace elements, and mineral have been shown to have direct and indirect effects on the estrus, conception, pregnancy, and postpartum events. Therefore they are very important part of reproductive efficiency in cattle.

The Blood Urea Nitrogen (BUN) or serum urea nitrogen is the end product of the hepatic detoxification of ammonia **(Hammond, 1992)**. In spite of BUN is synthesized by liver, it has a relation and indirect effect on the reproductive efficiency and fertility in cattle **(Elrod and Butler, 1993; Elrod et al., 1993; Ferguson et al., 1993; Butler et al., 1996).**

In this study, the measurement of BUN in the serum obtained from the blood collected from the jugular vein of the nulliparous heifer at the estrus or day of AI, was revealed that the overall Mean ± SD of heifers in both groups (n=42) was 15.09 ± 4.56 mg/dl. This result came in appointment with the results of **Tshuma et al., (2010)** in Bonsmara heifers (n=396) (Overall BUN = 5.27 ± 1.80 mmol/l ≈ 14.98 ± 5.04 mg/dl). But on the classification of heifers into 2 groups according to the conception or pregnancy, it was showed that the level of BUN had been significantly (P < 0.0) increased in non conceived heifers group (14/42) on the day of AI with mean concentration 19.63±1.072 mg/dl above that in the conceived heifers (28/42) (Mean±SD was14.26±1.45 mg/dl). These results came in accordance with the results of **Elrod and Butler,(1993); Elrod et al., (1993); Ferguson et al., (1993); Butler et al., (1996); and Tshuma et al., (2010)** in which the BUN increase in the non-fertile females than the fertile ones.

This could be attributed to the opinion of **Elrod and Butler, (1993); Elrod et al., (1993); Ferguson et al., (1993); Butler et al., (1996)** who stated that High dietary protein (nitrogen) intake resulting in BUN of greater than 17 to 18 mg/dl has been associated with an altered uterine environment and decreased fertility (reduced conception rate, decreased pregnancy rate).

Although all heifers in this study was fed on the same TMR (total mixed ration), some individuals have high BUN concentration in their blood and this was explained by individual variations and genetic differences in the nitrogen utilization efficiency, metabolism of ammonia and kidney excretion of BUN, thus according to **(Schoeman, 1989; Stoop et al., 2007; Bouwman et al., 2010; Hossein-Zadeh and Ardalan, 2011).**

On the other side, there was a significant increase ($p < 0.01$) in BUN concentration in early pregnancy (28 days) with mean 33.75±5.57 mg/dl than its mean concentration in the day of AI (14.26±1.45 mg/dl) in the same group of conceived heifers. These results agreed with **Abdullah et al., (2017)** who stated that, the plasma BUN concentration remained at a significantly higher level during day 0 to day 20 post-AI in pregnant crossbred cows than in the non pregnant group. Maximum concentrations were observed on day 28 post-AI (38.34 ± 2.70 mg/dl). This may indicate that the source of BUN is the fetal kidney. The current results were disagreed with **Ali et al., (2014)** who reported that Blood urea level was considerably lower in non–pregnant group as compared to pregnant group on day 1 (34.19±1.41 mg/dl vs. 40.34±2.00 mg/dl). Mean values of blood urea concentration for non–pregnant and pregnant groups on day 21 were (36.18±1.42 mg/dl) and (42.22±2.19 mg/dl) respectively.

On the other hand, the normal cyclic Postpartum multiparous cows (8/13) showed no significant increase ($P > 0.05$) in BUN during the 1st 3 weeks of Postpartum period with gradual non significant NS ($P > 0.05$) decrease from the 1st week PP to the 5th week PP from mean concentration (17.24 ± 1.2 mg/dl) to (15.41± 1.43 mg/dl). These results agreed with **Piccione et al., (2012)** and disagreeing with **Veena e.al, (2015)** who showed gradual increase in BUN concentration from the 1st week to the 6th week PP. while the cystic Postpartum multiparous cows showed increase in BUN concentration with mean concentration ranged from (20.89±0.47 to 19.93±0.62 mg/dl) and this was agreed with **Jafari et al., (2015)** Who clarify the cause by lowering level of insulin, insulin resistance, and increase of blood cortisol level in case of cystic ovaries coinciding with highly significant increase in blood glucose level. This result was clarified by that increase of BUN during puerperium will rise the PH of uterus retarding the uterine involution and correspondingly ovarian cyst formation, while the higher blood glucose during P.P.P. is conjugated with insulin resistance and lowering in case of ovarian cyst.

The blood glucose was shown also to be important blood parameter in the reproductive efficiency of both nulliparous heifers and postpartum multiparous cows. In this study, the mean blood glucose level was significantly ($P < 0.01$) increased in both day of AI and 28 days later in the group of conceived heifers (54.1±5.94 and 61.65±4.55 mg/dl respectively) as compared with the group of non conceived heifer in day of AI and 28 days later also (41.91±2.84 and 41.13±2.67 mg/dl). These results came in accordance with **Pandey et al, (2015) and Forshell et al, (1991).**

But within the subdivided groups, there was a significant ($P < 0.01$) increase in blood glucose level in the 28th day of pregnancy over than its level in the day of AI in the conceived heifers (61.65±4.55 and 54.1±5.94 mg/dl respectively). This is due to the great demand for more energy for beginning the fetal development in the early pregnancy and afterwards it decease significantly with pregnancy advance due to higher consumption. This was in agreement with **Pandey et al., (2015), Padodar et al., (2014), Green et al., (2012), and Forshell et al, (1991).** But disagreed with **Ali et al., (2014)** who recorded that glucose level is decreased on the early pregnancy at 28th day of pregnancy when compared to non pregnant animals. in which they stated that Pregnant cows exhibited relatively lower blood glucose concentration in contrast to non–pregnant cows on day 1 (44.22±0.79 mg/dl and 40.88±1.07 mg/dl respectively). Similarly the blood glucose content measured on day 21 (post–insemination) was higher in non–pregnant group (44.86±0.97 mg/dl) as compared to pregnant group (40.15±1.24 mg/dl). We concluded that there was a positive relationship between the blood glucose level and conception or pregnancy in heifers. Similar conclusions were reported by **Pandey, et al., (2015), Padodar, et al., (2014), Green et al, (2012), and Forshell, et al, (1991).** Where disagreed with **De Silva, et al., (1981) and Ali et al, (2014).**

In the postpartum multiparous cows, the Blood glucose plays a strong association with early postpartum period and later fertility of cows. We found that there were considerably higher normal glucose levels in the normal cyclic post partum cows with gradual significant ($P < 0.05$) increase during early PostPartum Period coinciding with normal uterine involution, ovaraian recyclicity, and first PostPartum estrus (mean level increase from 53.85±2.13 to 58.92±0.93 mg/dl from day 8-38 PP). This result came in agreement with **Kappel et al., 1994** which showed gradual increase of glucose after calving at days 4, 11, 18, 25, and 39 postpartum.

The association between adequate blood glucose level and early postpartum period and later fertility of cows has been illustrated and reviewed by **Lucy, (2016)**; inadequate blood glucose during early lactation theoretically compromises the function of tissues that depend on glucose as a substrate for carbon skeletons and intracellular energy supply. Metabolites such as NEFA and BHBA, as well as the hormones insulin and IGF1, all of which are controlled by glucose, may also play a role in controlling tissue function. The first 30 days postpartum is a critical time for the cow with respect to the impact that metabolites and metabolic hormones can have on reproduction. Two essential processes that may be directly affected by glucose, the restoration of ovarian cyclicity and uterine involution, will be discussed.

The bulk of the research performed about metabolites and metabolic hormones in postpartum cows has focused on the re-initiation of ovarian cyclicity. There is a positive association between insulin, IGF1, and the day Postpartum that the cow begins to cycle **(Velazquez et al., 2008).**

LeRoy et al. (2008) concluded that glucose and insulin were the most likely molecules to exert an effect on hypothalamic gonadotropin releasing hormone (GnRH) secretion in the postpartum dairy cow. Increasing glucose supply so that both circulating insulin and IGF1 are increased, therefore, should theoretically cause an earlier resumption of cyclicity postpartum by causing the cow to release more GnRH and have more luteinizing hormone (LH) in the system, which is stimulatory to the ovary. There is also strong synergism for insulin, IGF1 and LH at the ovarian level that shortens the interval to first postpartum ovulation **(Kawashima et al., 2012 and Lucy 2011).** In most cases, changes in circulating concentrations of nutrients and metabolites that occur in the postpartum cow are exactly opposite to those that would benefit the function of PMN. For example, glucose is the primary metabolic fuel for PMN **(Moyes, 2015).** The glucose is stored as glycogen within the PMN. Observed that cows developing uterine disease had less circulating glucose and lower glycogen concentration in their PMN. The conclusion was that the lower glycogen reserve led to a reduced capacity for oxidative burst in PMN that predisposed the cow to uterine disease.

On the other hand, in the present study, the cystic PP cows (n=5) showed significantly higher blood glucose level during PPP (mean 63.56±1.65 mg/dl) and thus was indicated by **Jafari et al., (2015)** Who clarify the cause by lower level of insulin, insulin resistance and increase of blood cortisol level in case of cystic ovaries.

Macro minerals include calcium, phosphorus, magnesium, potassium, sulphur, sodium and chloride. Cobalt, copper, iodine, iron, manganese, molybdenum, selenium and zinc are considered micro or trace minerals. Micro or trace mineral deficiencies are associated with soil type and are usually geographically related. Abnormal levels of some minerals such as iron and cobalt do not usually cause a problem with reproduction. Other minerals, including the above mentioned minerals, can significantly affect reproduction (**Yaremcio, 2000**).

In this study, there were no signifint ($P > 0.05$) differences in P and Mg levels between all groups of both nulliparous heifers and PP multiparous cows, except non significant increase in P level during the presence of large dominant follicles on ovaries in PP normal group. This was in agreement with **Sathish Kumar, (2003) and Chaudhary and Singh, (2004)** who didn't mentioned significant differences rather than focusing on that P must be within the normal range.

The actual differences were found in trace elements including Zn, Cu, and Se which showed significant differences between the different groups as well as between the days of examination.

There was obvious significant ($P < 0.01$) increase in Zinc on 28 days of pregnancy and non significant ($P > 0.05$) increase on day of AI in the group of conceived heifers (11.56±0.49 and 12.53±0.43 µmol/l respectively) as compared with the group of failed heifers (11.45±0.32 and 11.39±0.30 µmol/l respectively). This result was matching the conclusion and the mean of **Shabunin et al., (2017) and Small.e.al, (1996)** in their zinc level, In addition to **McClure et al., (1986)** which proved Improvement in conception rate at first service following selenium supplementation**.** Both Zinc and Selenium are very important trace elements for estrus, ovulation, conception, and fetal development in cattle. This agreed with **Sathish Kumar, (2003), Favier et al, (2002), Beletskaya et al, (2014), and Neve, (1998)** in their results.

In the Group A of conceived heifers, there was another relation has been found between the early pregnancy and both Zn and Se serum concentration. In which the serum level of both Zn and Se was markedly and significantly ($P < 0.01$) increase on the 28th day of pregnancy as compared to the day of AI (11.56±0.49 and 12.53±0.43 µmol/l respectively).

This positive relationship indicated that Zn and Se are so important for the fetal development, and thus what was proved by **Chen et al., (2015)** who found that developmental delay and embryo death in dairy cows at early gestation stages are the consequences of zinc deficiency and disorders of its metabolism. Similar results were recorded by **Shabunin et al, (2017)** who showed that Zinc blood level in cows of fetal growth restriction group on the 28th–32nd and 38th–45th days of gestation was lower by 17.2% and 10.9%, while for those with embryo death it was lower by 25.5% and 23.1%, respectively, in comparison with control fetal development group. **Shabunin et al, (2017)** also showed that Selenium blood level in cows of fetal growth restriction decreased by 26.2% on the 28th–32nd days of gestation and in animals of fetal death by 29.1% in comparison with the control group (pregnant with normal fetal development).

On the other hand, the group of PP normal cyclic cows showed gradual slight significant ($P < 0.05$) increases in the level of both Zinc and Selenium reaching to peak during the 3rd, 4th, and 5th week of PPP due to their role in ovulation and finishing the regeneration of involutedly uterus.

While the serum cupper level increase within the 2nd to 4th week PP. This was in agreement with **Piccione et al., (2012).**

Concerning to the use of ultrasound examination in the current study, it was a non invasive highly helpful diagnostic tool. The technique was a must for confirmation of the research results, early diagnosis of pregnancy, evaluation of Postpartum events of uterine involution and ovarian recyclicity. Moreover it was very helpful in the classification and grouping in animals. Many authors used the same technique in their research work **(Okano & Tomizuka, 1987; Pierson et al., 1988; Agag, 1996; Kandil, 2002; Sheldon et al 2002; and Sheldon et al., 2005)**

6. **SUMMARY**

This study was carried out including two main parts.

7.1. The first part:

Was conducted on 42 nulliparous Holstein Friesian – heifers belonged to private farm in Gamasa - Ismailia governorate. All heifers was aging from 393 to 669 days and weighing about mean 369 kg. All animals had good nutrition and water supply, well sheltered. All animals are vaccinated according to the vaccination program of the station. All animals are reared in the station coming from the dairy cows' station after each parturition and weaning. Moreover, these heifers had registered records per each head. All animals were fed on TMR (Total Mixed Ration) with balanced composition.

Animals were subdivided in 2 main subgroups according to p

Subgroup A: include 28 pregnant or conceived heifers (28/42) 66.6%

Subgroup B: include 14 non conceived heifers (14/42) 33.3%

Working protocol relied on blood sampling from all heifers on the day of AI or estrus and 28 days after AI for serum analysis of BUN, blood glucose, P, Mg, Na, K, Zn, Cu, and Se as important blood parameters related with reproductive efficiency, coinciding with diagnosis of early pregnancy by Ultrasound on the day 28^{th} of pregnancy.

The obtained results had been showed the following;

1. **BUN and Glucose** was showed increase on the day 28th after AI in the conceived group as compared with the day of AI of the same group and also as compared with the non conceived heifers on the day 28 after AI. BUN was increased in the day of AI or estrus in the later failed or non-conceived heifers.
2. There were obvious increase in Zinc and Selenium on both day of AI and day 28 later in the group of conceived heifers (11.56±0.49 and 12.53±0.43 µmol/l respectively) as compared with the group of failed heifers (11.45±0.32 and 11.39±0.30 µmol/l respectively).
3. There was a decrease in copper concentration on both the day of AI and day 28 later in the group of conceived heifers (10.76±0.60 and 9.42±0.78 µmol/l respectively) as compared with the group of non conceived heifers (12.75±0.53 and 12.84±0.44 µmol/l respectively).
4. There was a marked difference in the levels of P, Mg, and K+ on both times in both groups.
5. The ultrasound was used for pregnancy diagnosis on 28 days Post AI and the pregnancy rate was 66.6% and the embryo appear as a hyper echoic mass measuring about 1 cm in diameter of CVRL , present inside anechoic amniotic vesicle

7.2. The Second Part:

In this part, 13 multiparous recently calved Holstein cows belonged to private farm in Al-Reef El-Arabic – in Giza governorate which was aging from 3-5 years were used. All animals had good nutrition and water supply, well sheltered. All animals were vaccinated according to the vaccination program of the station. All animals were reared in the station coming from the dairy cows' station after each parturition and weaning, and moreover also had registered records per each head. All animals are fed on TMR (Total Mixed Ration) with balanced composition.

They are subdivided in 2 main subgroups according to conception rate

Subgroup A: include 8 normal cyclic PP cows

Subgroup B: include 5 cystic PP cows

The work protocol was planned on examination of PPP by using diagnostic ultrasound with 10 days interval between each examination coinciding with blood sampling from jugular vein on each examination for estimation of BUN, Glucose, Ph, Mg, Na, K, Zn, Cu, and Se by atomic absorbent spectroscopy.

The obtained results was showed the following

In Subgroup A

There was gradual decrease in BUN and increase in blood glucose level with advancing of postpartum period in the normal PP cows.

The blood glucose was higher coinciding with the 1^{st} PP estrus and mature follicles presence on ovary and normal fast uterine involution.

There was no significant difference in Mg level during PP events, but phosphorus was higher during the follicular development on the ovary

Also there was no difference in the serum level of sodium and potassium during the PP progress.

There were gradual increases in the level of both Zinc and Selenium reaching to peak during the 3rd, 4th, and 5th week of PPP due to their role in ovulation and finishing the regeneration of involuted uterus.

While the serum cupper level increase within the 2nd to 4th week PP.

In Subgroup B

There was increase in both BUN and blood glucose level in cystic cows over in normal PP cows

There was no significant difference in Mg level in both cystic and normal cows. But the P was higher around 18-28 days PP in normal PP cows than that of cystic PP cows, thus indicate that P is important for fertility and ovulation.

Also there was no noticeable difference in both Na and K level between cystic and normal PP cows.

There was noticeable decrease in selenium level in cystic PP cows lower than its level in normal PP cows

On the other hand there was no noticeable difference in levels of both zinc and copper between 2 groups.

The ultrasound examination was carried out on the examination of uterine involution and ovarian recyclicity with 10 days interval between each examination and the obtained results showed,

Normal PP cyclic cows (n=8);showed normal developing follicles and luteal structures, in which the uterine involution was completed at mean days 29.35±3.65 days PP with mean diameter of UH was 22.83±1.35 mm.

In cystic PP cows (n=5); 4 of them showed luteal cyst on the left ovary and another one showed large follicular cyst measuring about 50.4 mm in diameter.

7. CONCLUSIONS

From the current work it had been concluded that blood parameters has a meaning effect on the reproductive efficiency of both nulliparous Holstein heifers and postpartum multiparous cows.

The parameters that was showed significant relation with the reproductive efficiency in cattle was including BUN, blood glucose, Ph, Zn, Cu, and Se.

Increase of BUN level over the maximum normal limit (16-17 mg/dl) will lead to negative bad effect on the fertility of cows and heifers, due to its direct effect on changing the uterine PH to the acidic side, thus prevent and interfere with the implantation of newly formed zygote leading mostly to Repeat Breeder cows or heifers. While it's significant huge increase during the 28th day of pregnancy is representing it as a preliminary indicator of early pregnancy which can be confirmatory when supported by other pregnancy diagnosis aids like as the diagnostic ultrasonography.

The Blood Glucose, Ph, Zn, and Se were proved to be important elements for successful estrus, conception, pregnancy, and PP changes progress.

Blood Glucose level must be within the higher limit of normal standard (52 to 58 mg/dl) during the estrus or ovulation day, while the hypoglycemia (45 mg/dl or less) will interfere with ovulation and follicular development, but the early pregnancy is associated with slight increase in the Blood Glucose level (62 mg/dl or more) due to great energy demand for beginning of fetal development.

The cystic ovarian syndrome in the PP cows is also associated with considerable increase in blood glucose level (64 mg/dl or more) due to insulin resistance which is a characteristic biochemical feature of cystic ovary, thus can prove that increase of blood glucose level over 63 mg/dl during the post partum period is an indicator of cystic ovarian syndrome

Zinc is an important trace mineral for conception and fetal growth and development during the early pregnancy, but with attention to its level because its toxicity margin is very narrow.

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(9) ARABIC SUMMARY

9. الملخص العربى

تم اجراء هذه الرسالة على مرحلتين أساسيتين :

1. المرحلة الأولى :

اشتملت على مجموعة من عجلات الأبقار (الهولشتين) عددهم 42 تراوحت أعمارهم بين 393– 669 يوم ومتوسط وزن 369 كجم وزن حى بوحدة العجلات بمزرعة خاصة بمنطقة جمصة بطريق الإسماعيلية الصحراوى .

وقد تم تقسيم الحيوانات إلى مجموعتين :

1–1 المجموعه الأولى :

ضمت 28 عجلة واللاتى تم تخصيبهم بالتلقيح الاصطناعى وتم تأكيد العشار أو الحمل باليوم الثامن والعشرون باستخدام الموجات فوق الصوتية التشخيصية بنسبة حمل 66.6% من إجمالى عدد العجلات .

2–1 المجموعه الثانية :

ضمت 14 عجلة واللاتى فشلن فى الإخصاب باستخدام التلقيح الاصطناعى وكانت نسبتهم 33.3% .

و تم على أساس تجميع عينات دم من الحيوانات فى يوم ا لتلقيح أو متزامنًا مع الشياع ومرة أخرى بعد مرور 28 يوم من التلقيح متزامنًا مع تشخيص وتأكيد نسبة الحمل باستخدام الموجات فوق الصوتية التشخيصية .

تم تحليل بعض عناصر الدم فى العينات وشملت :

يوريا نيتروچين الدم و جلوكوز الدم وعناصر الفسفور والماغنسيوم والصوديوم والبوتاسيوم والزنك والنحاس والسيلينيوم .

وأثبتت النتائج وجود علاقة ما بين معدل الإخصاب ومعدل الحمل ويوريا الدم والجلوكوز

إذ أثبتت النتائج أن ارتفاع نسبة يوريا الدم فى يوم التلقيح أو أثناء الشياع كانت سببًا فى انخفاض معدل الخصوبة فى الأبقار وبالتالى معدل الحمل .

وأيضًا على العكس كان هناك ارتفاع واضح فى معدل الجلوكوز فى يوم التلقيح وأيضًا اليوم الثامن والعشرون من الحمل فى العجلات التى تم تخصيبها مقابل انخفاض معدل الجلوكوز فى العجلات التى فشلت فى الإخصاب .

أما بالنسبة للمعادن فكان هناك ارتفاع فى نسب الزنك والسيلينيوم وانخفاض فى نسبة النحاس أثناء الفترة الأولى من الحمل (28 يوم) .

أما بقية العناصر لم تتاثر تاثيرًا ظاهريًا .

2. المرحلة الثانية :

وفيها تمت الدراسة وتم العمل على عدد 13 بقرة فى فترة النفاس تتراوح أعمارهن ما بين 3 إلى 5 سنوات فى مزرعة خاصة بالريف العربى .

وتم تقسيمهم أيضًا إلى مجموعتين :

2–1 المجموعه الأولى :

وضمت عدد8 بقرات واللاتى مررن بفترة نفاس طبيعية .

2–2 المجموعه الثانية :

وضمت 5 بقرات كان بهم تكيسات على المبايض خلال فترة النفاس .

وشملت الدراسة فى هذا الجزءِ على شِقين أساسيين :

الشِق الأول : متابعة الرحم والمبايض والتغيرات التى تحدث بهما باستخدام الموجات فوق الصوتية التشخيصية وذلك كل 10 أيام بعد الولادة وحتى اليوم 38 –40 .

الشِق الثانى : سحب عينات دم متزامنًا مع فحص السونار وذلك لتحليل العناصر ذاتها التى حُللت فى الجزء الأول من العمل .

وأثبتت النتائج الآتى :

بالنسبه للشِق الأول ، تم قياس الآتى :

1 – متوسط قُطر الرحم بعد الولادة على مدار ٤ فحصات بمعدل ١٠ أيام ما بين كل فحص والآخر وكانت النتائج بمتوسط ٦٣,٦٧ مم، ٤٥,٣٨ مم ، ٥٩,٢٣ مم ، و ٨٣,٢٢ مم على التوالى .

2 – معدل التبويض وحجم البويضات فى نفس الأيام المذكورة بعاليه ، وكانت النتائج بمتوسط ٤٥.٣ مم ، ٨,١٢ مم ، ١٨,٨٥ مم ، و ١٨,٣٥ مم كمتوسط لأحجام البويضات .

أما بالنسبة للشِق الثانى وهو تحليل عناصر الدم :

المجموعه الأولى :

1 – انخفاض تدريجى فى متوسط نيتروچين يوريا الدم مع تقدم فترة النفاس .
2 – ارتفاع تدريجى فى معدل الجلوكوز مع تقدم فترة النفاس .
3 – عدم وجود تغير ملحوظ فى قياسات المغنيسيوم والصوديوم والبوتاسيوم على مدار فترة النفاس .
4 – زياده طفيفة فى مستوى الفسفور متزامنًا مع نضج البويضات .
5 – زيادة تدريجية فى مستوى الزنك والسيلينيوم مع الوصول لأعلى مستوى فى الفترة ما بين الأسبوع الثالث إلى الخامس بعد الولادة .
6 – زيادة فى مستوى النحاس عند الفترة ما بين الأسبوع الثانى والرابع بعد الولادة .

المجموعه الثانية :

1 – وجود زيادة ملحوظة فى مستويات يوريا الدم والجلوكوز فى المجموعة الثانية مقارنة بالمجموعه الأولى .
2 – انخفاض ملحوظ فى مستويات السيلينيوم فى المجموعة الثانية مقارنة بالمجموعة الأولى
3 – لا يوجد تغير ملحوظ فى مستويات العناصر الأخرى بين المجموعتين .

من هذه الدراسه امكن استنتاج مايلى:

1- ان العناصر التى تم قياسها لها تاثير واضح على عملية الاخصاب فى العجلات

2- ان للحمل تاثير معنوى على مستوى تلك العناصر فى دم الابقار

3- ان هذه العناصر يمكن استخدامها كوسيله مبكره لتشخيص الحمل مع الموجات الفوق صوتيه التشخيصيه.

4- ان هناك عناصر هامه تساعد فى تحسين فترة النفاس عن طريق استعادة الرحم لحجمه ووظيفته الطبيعيه مثل الجلوكوة والزنك والسيلينيوم.

5- يمكن وقاية الابقار من الاصابه بمرض تكيس المبايض عند ضبط كمات هذه العناصر فى العليقه المقدمه للحيوان

السيره الذاتيه للباحث

الباحث من مواليد الشرقيه 1989/4/1

اتم الباحث دراسته الابتدائيه بمدرسة ناصر الابتدائيه عام 2000

اتم الباحث دراسته الاعداديه بمدرسة الالفى الاعداديه عام 2003

اتم الباحث دراسته الثانويه بمدرسة جمال عبد الناصر الثانويه عام 2006

حصل الباحث على بكالوريوس العلوم الطبيه البيطريه من كلية الطب البيطرى – جامعة بنها عام 2011

يشغل الباحث وظيفة معيد بكلية الطب البيطرى – جامعة بنها منذ عام 2011

التحق الباحث بالقوات المسلحه المصريه كضابط احتياط وانهى الخدمه العسكريه عام 2014

جامعة بنها

كلية الطب البيطرى

قسم التوليد والتناسل والتلقيح الاصطناعى

بعض الدراسات على فترة النفاس
فى حيوانات المزرعة باستخدام الموجات الفوق صوتية التشخيصية

رسالة مقدمة إلى

كلية الطب البيطرى- جامعة بنها

من

ط.ب/ عمرو محمد سعد مصطفى

(بكالوريوس فى العلوم الطبية البيطرية - كلية الطب البيطرى - جامعة بنها - 2011)

للحصول على

درجة الماجستير فى العلوم الطبية البيطري

(التوليد والتناسل والتلقيح الاصطناعى)

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2020