EFFICACY OF PROGESTERONE SUPPLEMENTATION THROUGH DIFFERENT ROUTES ON CONCEPTION RATE AND BLOOD BIOCHEMICAL PROFILE IN REPEAT BREEDING BUFFALOES

Dissertation

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By

Anil Sharma
(L-2001-V-66-D)

Department of Veterinary Physiology
College of Veterinary Science
PUNJAB AGRICULTURAL UNIVERSITY
LUDHIANA - 141 004
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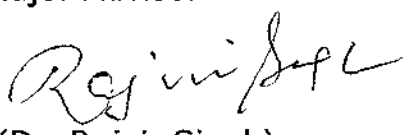
CERTIFICATE – I

This is to certify that the dissertation entitled "Efficacy of progesterone supplementation through different routes on conception rate and blood biochemical profile in repeat breeding buffaloes" submitted for the degree of Ph.D, in the subject Veterinary Physiology (Minor subject: Animal Reproduction, Gynaecology and Obstetrics) of the Punjab Agricultural university, Ludhiana, is the bonafide research work carried out by Anil Sharma (L-2001-V-66-D) under my supervision and that no part of this dissertation has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

Major Advisor



(Dr. Rajvir Singh)
Prof & Head
Dept of Vety Physiology
PAU, Ludhiana

CERTIFICATE – II

This is to certify that the dissertation entitled "Efficacy of progesterone supplementation through different routes on conception rate and blood biochemical profile in repeat breeding buffaloes" submitted by Anil Sharma (L-2001-V-66-D) to the Punjab Agricultural University, Ludhiana in partial fulfillment of the requirements for the degree of Ph.D, in the subject of Veterinary Physiology (Minor subject: Animal Reproduction, Gynaecology and Obstetrics) has been approved by the Student's Advisory Committee after an oral examination on the same, in collaboration with an External Examiner.

Major Advisor

Dr Rajvir Singh
(Prof & Head)

Dr Rajvir Singh
(Head of the Department)

Dr Darshan Singh
(Dean, Post-Graduate Studies)

External Examiner

Dr K.B. Sharma
Head
Dept of Veterinary Physiology
CSKHPKVV, Palampur (H.P.)

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Abstract

Effect of progesterone (P_4) supplementation during post-mating period through different routes on conception rate and various hormonal and blood biochemical profile was studied in repeat breeding Murrah buffaloes. The treated repeat breeding buffaloes constituted of three groups i.e. T_i (ear implant), T_m (I/M injections) and T_o (oral supplementation) groups. Eight normal cycling buffaloes were kept in control normal group (CN) and eight repeat breeding buffaloes in control repeat breeding group (CR). Increased conception rates in T_i (75%), T_m (62.5%) and T_o (50%) were observed as compared to CR group (25%). P_4 supplementation successfully improved the plasma P_4 concentrations in repeat breeding buffaloes from day-4 onwards.

The thyroidal activity (T_3 and T_4) was higher whereas the plasma insulin concentrations lower during estrus phase in all the control and treatment groups irrespective of progesterone supplementation. The blood glucose and plasma total cholesterol concentrations were also higher during estrus phase as compared to other phases of the estrous cycle in all the groups. The macro/micro-mineral status did not show much variation during the study period.

It is concluded that P_4 supplementation can effectively be used as a remedy to repeat breeding in buffaloes without any adverse effect on hormonal and blood biochemical milieu of the animal.

Keywords: Buffaloes, Repeat breeding and Progesterone

Signature of Major Advisor

Signature of the student

CHAPTER-I

INTRODUCTION

The buffalo in India is in a strongly competitive position as a producer of milk when compared with other farm animal species and no doubt still their enormous potential remains to be realized. The productivity of this species with extraordinary potential has been considerably affected by inherent problems that reduce the reproductive life as well as the productive potential of the animal. For optimum production, a dairy buffalo should display estrus within 60 days after parturition, conceive with minimum number of inseminations and calve every year (Singh *et al* 1989). However, buffalo is known to suffer from several reproductive maladies contributing to its low reproductive efficiency. The maintenance costs remaining the same, such conditions results in shorter productive life of the animal, longer inter-calving intervals and reduced calf crop leading to huge economic losses to the dairy industry.

Female infertility in bovines has always been a multidimensional phenomenon. The reproductive efficiency and thus the productive performance of buffalo are compromised by a number of factors leading to infertility. Among various reproductive

problems reducing the reproductive efficiency of dairy animals, repeat breeding is probably the most frustrating one. Repeat breeder animal is the one that fails to conceive or maintain pregnancy after repeated inseminations (at least three) with fertile semen. In bovines, incidence of repeat breeding to the tune of 30-40% has been recorded.

The normal process of estrus, ovulation and conception are controlled by interplay of various hormones released in a very precise manner. A disturbance in the hormonal profile has been recognized as critical to maintain successful pregnancy (Maurer and Echternkamp 1982, Pope *et al* 1982) and such hormonal imbalances have been reported to accompany the infertile insemination.

Progesterone is considered to be the hormone of pregnancy and an adequate supply of progesterone from corpus luteum is of prime importance in order to maintain a proper synchrony between the needs of developing embryo and its environment. Role of progesterone in conception, early embryonic development and influencing the uterine environment is well established. Luteal insufficiency has been recognized as an important factor leading to repeat breeding in dairy cows (Kimura *et al* 1987 and Shukla *et al* 2000), and the problem can be corrected by exogenous administration of progesterone (Thuemmel *et al* 1992 and Sharma *et al* 2003).

Progesterone supplementation has been found to improve conception rates in cattle (Thuemmel *et al* 1992), sheep (Ashworth *et al* 1991), pigs (Jindal *et al* 1997) and buffaloes (Andurkar *et al* 1997 and Sharma *et al* 2003). However, the information of physiological mechanism and relative efficacy of progesterone supplementation through different routes in ameliorating the problem of repeat breeding due to luteal insufficiency is scanty.

During early pregnancy, apart from progesterone other hormonal, biochemical constituents and various macro/micro-minerals of blood also play an important role in the reproductive efficiency of animal. Insulin is the important metabolic hormone stimulating follicular development and corpus luteum function (Studer *et al* 1993). The hypothalamic centers are influenced by circulating levels of thyroid hormones, glucose, cholesterol and other unidentified factors (Boyd 1977) and the deficiencies of certain macro/micro minerals can affect reproduction (Hurley and Doane 1989). Therefore supplementing repeat breeding animals with exogenous progesterone for enhancing the pregnancy rate might be expected to bring about certain changes in the hormonal or biochemical milieu of the blood. The present investigations were therefore undertaken with the following objectives

- To study the hormonal and blood biochemical profile in the control and repeat breeding buffaloes
- To monitor the effect of progesterone supplementation during post-mating period/early pregnancy through different routes viz. oral, intramuscular and subcutaneous progesterone implants on conception rate, hormonal and blood biochemical profile in repeat breeding buffaloes
- To compare the efficacy of the three treatment regimes on conception rate, hormonal and blood biochemical profile in repeat breeding buffaloes

CHAPTER – II

REVIEW OF RELEVANT LITERATURE

2.1 Repeat breeding

A goal of the reproductive management program should be, to have the buffalo spend as much of her life without encountering the productive and reproductive problems so that the maximum profits are achieved from the dairy herd. Thus, it is critical that the reproductive management program focus on getting a large percent of the buffaloes pregnant quickly after the voluntary waiting period (VWP). But there are many reproductive problems like repeat breeding, anestrus, cystic ovaries, silent heat that are responsible for heavy economic losses in the dairy herds. Among various problems, repeat breeding is considered to be a major one throughout the world because the animal fails to conceive after repeated inseminations thereby increasing the inter-calving intervals, thus adversely affecting our basic target of getting a calf per year. So the study of incidence, losses and causes of repeat breeding must be understood to reach at any conclusion for minimizing the losses due to the problem of repeat breeding. The literature is thus reviewed for understanding the various aspects of repeat breeding like incidence, losses and causes.

2.1.1 Incidence and losses

There are many studies indicating the varied incidence rates of repeat breeding affecting the reproductive efficiency. As early as in 1949, Tanabe and Casida studied the nature of reproductive failures in cows of low fertility and observed that 66% of the repeat breeder cows with no clinically detectable abnormality had fertilized ova, however at 34 days post-breeding, the percentage having normal embryos had decreased to 23%. The incidence of repeat breeding is usually less in buffaloes as compared to cows. The incidence of repeat breeding in buffaloes showed a range from 8.33% to 16.9% (Fayez *et al* 1992 and Singh *et al* 1998). Varied incidences of repeat breeding in cows ranging from 10.1% to 20.6% were reported (Singh *et al* 1998, Forbes 2000 and Gustaffson and Emanuelson 2002). The incidence of repeat breeding also varies with the different breeds. Urade (2001) reported a lower incidence (8.08%) of repeat breeding in Sahiwal cows and their crossbreds and still lower (4.26%) is reported by Narladkar *et al* (1994) in Deoni cows and their crossbreds. The incidence of repeat breeding is also affected by the management practices. The buffaloes kept under organized dairy farm conditions showed lower incidence of repeat breeding as compared to the rural buffaloes. Jain (1989) reported the incidence of repeat breeding to the tune of 10.27% and 9.8% in rural and farm buffaloes, respectively.

Repeat breeding syndrome is a major source of economic wastage in dairy herds, contributing to lower dairy profits via wastage of semen and insemination costs, increasing intervals to conception, veterinary treatments (including calving induction in seasonal calving areas), increased culling and replacement costs and lost genetic gain through increased generation intervals (Bartlett *et al* 1986). There are increased dry period and calving intervals in repeat breeders and moreover such animals require more number of services per conception with longer service periods resulting in very heavy economic losses (Pandey *et al* 1994). Early embryonic deaths (within 20 days of breeding) would seem to account for 75-80% of all embryonic and fetal deaths resulting in substantial loss in production (Sreenan and Diskin 1983). Several studies in Europe and America have indicated that 50% of the premature culling of dairy cattle i.e. before 5-6 years is due to reproductive disorders amounting to a great loss of milk production as well as off springs. Among various causes of culling, the maximum (20.6%) culling was solely due to repeat breeding (Forbes 2000).

2.1.2 Causes

There are many factors that may contribute to repeat breeding. Anatomical aberrations of the reproductive tract,

anovulation, and lower survival rate of an embryo, endocrine disorders and increased chromosomal abnormalities are possible causes of lowered fertility in repeat breeder females (Ayalon 1984). Moreover, early embryonic loss could occur through inadequate uterine environment, as the endometrial secretions are essential for stimulating and mediating changes in conceptus growth and differentiation throughout early pregnancy. It is now well documented that these uterine secretions are regulated by the ovarian steroids (Wilmut *et al* 1986). So, the adequate levels of these steroids are of great importance to maintain a successful pregnancy. According to a study conducted by Singh *et al* (1998) on 2242 cows, it was observed that the clinical abnormalities were found in 747 (33.3%), managemental causes in 229 (10.2%), anatomical defects in 215 (9.6%) and functional abnormalities in 26 (1.2%). Based on another study on 1697 buffaloes, Rao and Kotayya (1980) reported that 14.54% animals showed no anatomical or pathological abnormality but failed to conceive leading to repeat breeding indicating functional disturbances as one of the cause of repeat breeding. Rao (1982) also reported that reproductive failures were of anatomical (6.01%), functional (73.14%) and of non-infectious nature (20.85%) and among such causes, functional disturbances (main being the repeat breeding) accounts for 2.29%. Nutrition-reproduction interaction has been a

topic of increasing interest and concern among dairy producers, veterinarians, feed dealers and extension workers. Changes in nutritional status have an effect on the ovarian activity. This can occur with or without significant variation in circulating gonadotropin concentrations and can be correlated with changes in circulating concentrations of metabolic hormones, including insulin, IGF-I, GH, and leptin. Nutrition can also affect the expression of mRNA encoding components of the ovarian IGF system to regulate the sensitivity/response of follicles toward gonadotropins. Deficiencies of various trace minerals, inadequate vitamin intakes, energy protein imbalances and excessive protein intakes are mentioned as contributors to infertility and poor reproductive performance leading to repeat breeding. Among various causes of repeat breeding, even environmental pollution has been found to have high correlation with repeat breeding in buffaloes (Abdel *et al* 1994).

The repeat breeding was also found to be associated with luteal insufficiency or low plasma progesterone levels after insemination during the early luteal phase. Kimura *et al* (1987) reported that in repeat breeder cows, 62% had an abnormal pattern of milk progesterone levels indicating luteal phase defects. It was observed that among those animals 54% showed delayed rise of progesterone until days 6-11 after insemination, 15% had low levels

of progesterone during luteal phase and 31% had a combined pattern of both, suggesting that the delayed formation of corpus luteum with lowered secretion of progesterone during the luteal phase is one of the causes of repeat breeding in dairy cows. Similarly Kang *et al* (1994) also found that out of 15 repeat breeder cows, five showed a delay rise of progesterone until day 7-10, two had a lower progesterone concentration (<150 ng/ml) whereas two showed combined pattern of both delayed rise and low concentration of progesterone during luteal phase indicating that the luteal dysfunction is one of the important causes of repeat breeding. An endocrine asynchrony between the conceptus and the maternal milieu, either at the oviductal or uterine levels may account for the impaired fertility observed in repeat breeding females, confirming the assumption of the multi-causality of the syndrome. Endocrine asynchrony including elevated blood plasma progesterone concentrations at estrus has been shown to result in impaired fertility and repeat breeding (Gustafsson *et al* 1986, Bage *et al* 1997). It has been inferred that spontaneously occurring asynchrony between the embryo and the maternal environment was the prime cause of embryonic mortality. Moreover, higher progesterone concentration and a lower ratio of estrogen and progesterone on days 3-6 were very conducive for the embryo (Maurer and Echternkamp 1982).

2.2 Luteal function: An important factor for the maintenance of pregnancy

Corpus Luteum is the main organ secreting progesterone during the early embryonic life of the animal that in turn maintains the pregnancy. Abortion was reported in animals after ablation of corpus luteum (CL) at 92^{nd} and 163^{rd} day of pregnancy. The pregnancy was maintained by replacement therapy in 8 or 9 cows having CL ablation done at 60^{th} day of pregnancy indicating that progesterone is an essential hormone for maintenance of pregnancy (Mc Donald *et al* 1953). So, it is inferred that the conceptus growth is progesterone dependent; an inadequate level of progesterone may results in starvation of the conceptus. A delayed rise in post-ovulatory progesterone or lower luteal phase levels result in poor embryo development and little or no interferon secretion on day-16 (Mann *et al* 1999). The concentration of interferon-tau in uterine flushes on day-16 is related to circulating concentrations of progesterone between days 4 and 5 (Mann *et al* 1998). Therefore in this context, sufficient progesterone means a concentration high enough to trigger a normal pattern of uterine secretory function by day-4 or 5 after insemination. Luteal insufficiency or delayed rise of progesterone is considered to be an important cause of early embryonic mortality, leading to repeat breeding syndrome (Kimura *et al* 1987) and one of the reasons for low conception rate in dairy

animals (Britt and Holt 1988). Pregnant cows have higher concentration of blood progesterone within first 10 days of insemination (Mann *et al* 1999). Progesterone is transferred locally from the ovarian/oviductal venous drainage to the uterine artery in cattle (Weems *et al* 1988). The local transfer results in higher concentration of progesterone within the uterus ipsilateral to the CL (Pope *et al* 1982). Although the local progesterone may be important for uterine function and pregnancy, changes in systemic progesterone may be affecting gonadotropin secretion that may ultimately change patterns of follicular growth in dairy animals. Since progesterone is responsible for maintaining a quiescent favorable environment in the uterus for embryo development, it is logical to consider that supplementation with this hormone in one or the other forms may improve conception rate by improving the progesterone levels in repeat breeding females and thus helps in enhancing the conception rates and ameliorating the problem of repeat breeding (Sharma *et al* 2003).

2.2.1 Progesterone concentrations during estrous cycle/early pregnancy in normal and repeat breeding animals

Progesterone concentrations are at their nadir at the time of estrus in normally cycling animals. Basal levels of progesterone

during estrus are associated with the insemination success and increased conception rates. But on the other hand, if the concentrations of progesterone are high at the time of estrus it results in poor conception rates and thus leading to repeat breeding. Usually after ovulation, luteinization starts and progesterone levels starts rising from day-4 onwards. The corpus luteum on an average matures on day-5 in normally cycling animals. In the normal diestrus phase levels of progesterone rises and reaches a plateau (Kanai and Shimizu 1984). In few studies delayed rise in progesterone levels have been reported as a major reason for embryonic mortality in several species (Kimura *et al* 1987 and Kang *et al* 1994). Previous studies in buffaloes also showed a delayed rise in progesterone levels in repeat breeding animals. This rise was observed on day5 and 6 after the day of onset of estrus (Sharma *et al* 2003). A functional corpus luteum is maintained in pregnant animals but in non-pregnant animals it regresses at around day-15. In case the animal fails to conceive, there is a release of prostaglandin ($PGF_{2\alpha}$) from the progesterone impregnated non-pregnant uterine horn that reaches the ovary through counter current mechanism causing the lysis of corpus luteum which is the source of progesterone secretion (Hafez 1980). So, a precipitous fall in the progesterone concentration is observed around day-16 in non-pregnant animals and thus a rapid decrease

in the levels is found during 5 days before the onset of next estrus. Such findings were confirmed by measuring the lower concentrations of progesterone in urine and serum in repeat breeding as compared to normal cycling buffaloes (Fayez *et al* 1992). The average serum progesterone concentrations at the time of estrus in normally cycling buffaloes were 0.5 ng/ml, which rises to a peak value of 1.6 ng/ml on day-15 after estrus (Ahmad *et al* 1977). The plasma progesterone concentrations were higher in normal as compared to repeat breeding cows and buffaloes during different days of the estrous cycle (Agarwal *et al* 1982). The peak concentrations of plasma progesterone were found during diestrus (4.43 ± 0.91 ng/ml) and minimum concentrations during estrus phase (0.49 ± 0.09 ng/ml) of the estrous cycle in buffaloes (Jindal *et al* 1988). The levels of progesterone on day-8 and day-18 post estrus were higher in the animals those conceived and later confirmed as pregnant as compared to non pregnant on per rectal examination at day-45 post estrus (Gupta *et al* 1998). High progesterone concentrations (1.0 ng/ml or more) on 20th day or 23rd day post insemination was positive sign for pregnancy (Nanda *et al* 1984) but in contrast poor conception rates are observed if the progesterone concentrations were high (1.0 ng/ml or more) during the estrus phase or at the time of insemination indicating that the low plasma progesterone concentration at that time is an essential

criterion to achieve good pregnancy rates in bovines (Srivastava *et al* 1999).

2.2.2 Supplementation of progesterone and conception rates

As the luteal insufficiency is one of the major causes of repeat breeding (Kang *et al* 1994), exogenous administration of progesterone during early luteal phase could logically correct the condition thus reducing the incidence of repeat breeding. Several workers have reported enhanced conception rates and embryonic development by exogenous administration of progesterone using different routes, dose and time of administration with variable results. Most of the workers have restricted only to the effect of the exogenous progesterone on improving conception rate thus ameliorating the problem of repeat breeding. However, in some of the studies the physiology of such treatments has been addressed directly or indirectly. Such an administration can improve plasma systemic levels of progesterone during early pregnancy, thus compensating for the luteal insufficiency in these animals (Sharma 2001 and Sharma *et al* 2003). Only a few reports are available on this aspect on buffaloes where the plasma progesterone profile has been studied after exogenous administration of progesterone on conception rate (Sharma *et al* 2003). Maintenance of desired progesterone levels during early pregnancy in the animals with

luteal insufficiency improve the uterine environment for embryo survival and development (Balkrishnan *et al* 1994) and brings about a certain change in uterine luminal secretion (Devanathan *et al* 1997). Role of progesterone in affecting the uterine luminal secretions has been reported in various species as in sheep (Ashworth 1991), cattle (Thuemmel *et al* 1992), pigs (Jindal *et al* 1997) and buffaloes (Andurkar *et al* 1997).

Intra-muscular (I/M) route is considered to be the route of choice by some workers. Panchal *et al* (1991) reported that I/M administration of hydroxy progesterone caproate in repeat breeding buffaloes as a single depot on day 4^{th} post insemination, significantly increases pregnancy rates in treatment group as compared to that observed in the control group. Similarly, Awasthi *et al* (2002) also tried I/M administration of 500 mg hydroxyl progesterone caproate on days 6-8 after insemination in 20 repeat breeder cows and found that conception rate increased (70%) in treatment group as compared to 20% in control group. In other study, parentral administration of progesterone during 5-10 days of estrous cycle had improved the embryo viability and conception rate significantly in repeat breeding cows (Rosen and Struman, 1989 and Walton *et al,* 1990). Increased levels of plasma progesterone were also obtained by administering Reposital type progesterone (75 mg/day) in repeat breeder cows which in turn was associated with

the insemination success (Thuemmel *et al* 1992). Progesterone was also administered orally (Devanathan *et al* 1999) in the form of hydroxyl progesterone hexonate-Tablets (progesterone 500 mg) to crossbred repeat breeder cows that resulted in 53.84% of first service conception. Pregnancy rates were also enhanced by the supplementing norgestomet, progesterone or MGA in the suckled beef cows after timed inseminations (Stevenson *et al* 2003). Improved pregnancy rates to 60% over 30% in untreated control cows were also reported by intra vaginal implants of progesterone in 28 dairy cows inserted between days 5-12 after AI (Robinson *et al* 1989 and Stevenson and Mee 1991). Therefore, it was suggested that P_4 could be effectively used in one form or the other as a remedial measure to improve the plasma progesterone concentrations and conception rate during early pregnancy especially in cases of repeat breeding due to luteal insufficiency.

2.3 Metabolic hormones: Their relationship with reproduction

2.3.1 Insulin

The interaction of metabolic hormones with the ovary has been described for insulin and IGF-1. Insulin coordinates the utilization of various fuels in various body tissues. Insulin and/or insulin dependent changes in the glucose availability may act on the ovary and change the ability of the ovarian cells to grow or respond

to ganadotropins. By modulating GnRH pulsatility and GnRH release (Butler and Smith 1989, Tanaka *et al* 2000). An increase in insulin, IGF-1 along with the LH pulsatility during the post-partum period has been reported (Butler 2000, Lucy 2000 and Monget *et al* 2002). Mechanisms that increase LH pulsatility through their actions on the hypothalamus and pituitary also coordinate an increase in responsiveness of the ovary to LH. An increased LH pulsatility in cows during the post-partum and blood insulin and IGF-1 concentrations increase as well (Butler 2000 and Lucy 2000). Thus there is a coordinated series of events that act to promote follicular development and eventually ovulation. Further, smaller populations of antral follicles or an abnormal endocrine status during folliculogenesis have been reported to be one of the major causes of repeat breeding (Maurer and Ec ternkamp 1985). Growth factors modulate survival, proliferation and differentiation of follicular cells along with gonadotropins (Gong and Webb 1996, Totey *et al* 1996). Insulin and IGF family are likely to influence the process of selection of dominant follicles. Insulin is believed to potentiate the action of FSH on granulosa cell differentiation (Gong and Webb 1996). Hence high responsiveness of granulosa cells to FSH during final follicular growth results from intra follicular system of amplification. *In vitro* studies revealed that insulin and IGF are important mediators of follicular development, steroidogenesis, oocyte maturation and

subsequent embryo development (Totey *et al* 1996, Daliri *et al* 1999). Administration of insulin increases intra follicular development and peripheral IGF-1 levels in cattle (Simpson *et al* 1994). The increased follicular phase IGF-1 concentrations following insulin may influence the development of follicles and CL function (Leewenberg *et al* 1996). Elevated IGF-1 levels at the pre-ovulatory phase has also been positively correlated with progesterone production (Peclaris *et al* 1999) indicating that insulin may be considered as the alternate therapy to improve the conception rate in infertile animals. By administering purified bovine insulin, S/C on days 8, 9 and 10 of the estrous cycle the progesterone concentrations were successfully improved, indicating that insulin treatment may be used for improving fertility in repeat breeding cattle (Selvaraju *et al* 2002).

2.3.2 Thyroidal hormones (Triiodothyronine and Thyroxine)

Thyroidal hormones (T_3 and T_4) affect many diverse tissues and influence major processes such as metabolism, growth differentiation, lactation and reproduction. The reproduction in farm animals is closely linked with dynamic activity of the thyroid gland (Soliman 1964, Afiefy *et al* 1970). Thyroidal hormones are thought to be necessary for normal secretion and utilization of gonadotropins and gonadal hormones (Dalvi *et al* 1993).

Inadequate thyroid function reduces conception rate and ovarian activity. These hormones affect the metabolic pools of nitrogen and available energy necessary for the reproductive system and the developing embryo. Therefore abnormal decrease in thyroid hormones may interfere with the normal pregnancy (Hafez 1980).

Various workers studied the thyroidal activity in dairy animals in relation to reproduction, however not much work is reported in buffaloes. The plasma T_3 concentrations ranged from 0.84-1.88 ng/ml in buffaloes (Khurana and Madan 1985) and plasma T_4 concentrations varied from 14-16 n mol/L in different age groups of Murrah buffaloes (Esguerra *et al* 1993). The plasma T_4 concentrations were found to be 36.73 ± 1.61, 36.83 ± 1.14, 38.50 ± 2.55 and 33.14 ± 0.96 ng/ml and T_3 concentrations were 1.13 ± 0.12, 1.33 ± 0.08, 1.29 ± 0.07 ng/ml during proestrus, estrus, metestrus and diestrus phase of the estrous cycle (Jindal *et al* 1988). In general, thyroidal activity is high during estrus as compared to other phases of the estrous cycle (Khurana and Madan 1985, Kumar *et al* 1991). Activity seems to decrease during metestrus and diestrus phase of the estrous cycle (Kumar 1990, Sharma 2003). Normal T_4 concentrations in non-pregnant buffaloes were found to be 5.5 ± 1.4 ug/100 ml (Pichaicharnarong *et al* 1982). The T_4 levels increased during pregnancy and reached the peak at 8-9 months that again declined at full term (9-10

months). However, no significant difference between the values of T_3 and T_4 (4.0 ± 0.74 pg/ml and 0.55 ± 0.15 ng/ml) in repeat breeding and T_3 and T_4 (4.34 ± 0.22 pg/ml and 0.54 ± 0.14 ng/ml) in normal cycling buffaloes.

2.4 Blood biochemical constituents

Nutrition-reproduction interaction studies indicate that various metabolites and nutrients, acting at various levels, can have a significant influence on productive and reproductive efficiency of the animal. The relevant literature on the blood glucose and total plasma cholesterol has been reviewed.

Though volatile fatty acids are the main source of energy in the ruminants, basal levels of blood glucose must be maintained for normal functioning of various physiological processes, and abnormally low levels of blood glucose can adversely affects the productive and reproductive processes. Blood glucose levels are higher in fertile than infertile cows and is indicative of energy status of the animal (Mc Clure 1965). Glucose availability could influence LH secretion through a central sensor in the lower brain stem. Historic data indicates higher levels of blood glucose during estrus phase as compared to other phases of the estrous cycle in cows (Hodgson 1932). Low blood glucose level has been reported as one of the causes of anestrus, impaired fertility, repeat breeding,

ferilization failure and early embryonic mortality (Mc Donald and Pineda 1989, Das 1993). It is suggested that the secretions of gonadotropins might either reduce or altogether cease because of hypothalamic failure to utilize glucose thereby affecting the activity of adenohypophysis (Mc Clure 1968). In some studies significantly lower blood glucose levels have been reported in repeat breeding animals (Fayez *et al* 1992, Jani *et al* 1995 and Ramakrishna 1996). However, a few workers have observed even a higher blood glucose concentration in repeat breeding cows as compared to normal cycling cows (Islam *et al* 1994). Any disturbance in glucose metabolism could contribute to unexplained repeat breeding (Islam *et al* 1994). Significantly higher blood glucose concentrations were observed in repeat breeding cows that subsequently became pregnant than in non-pregnant cows (Selvaraju *et al* 2002).

Cholesterol is the precursor of all steroid hormones. Any major variation in plasma cholesterol levels during various physiological processes could affect the reproduction process. A positive correlation between higher cholesterol concentration and better reproductive performance has been established (Verma and Pandey 1975). In cycling cattle and buffaloes, plasma total cholesterol concentrations were higher during estrus as compared to other phases of the estrous cycle (Kumar *et al* 1986, Sharma *et al* 2003). Higher levels of estrogen at the time of estrus exert an

anabolic effect to increase serum cholesterol (Selvaraju *et al* 2002). Higher cholesterol levels during estrus could be due to higher estrogen titers during estrus favoring the biosynthesis of cholesterol through its influence on carbohydrate metabolism (Verma *et al* 1984). The total plasma cholesterol concentrations during estrus ranged from as low as 55.26 ± 1.86 mg/dl (Kumar *et al* 1991) to as high as 290.00 ± 9.31 mg/dl (Sahukar *et al* 1985). Total plasma cholesterol levels in Murrah buffaloes were found to be 40.97 ± 1.97, 55.26 ± 1.86, 45.97 ± 1.80 and 45.93 ± 1.37mg/dl during proestrus, estrus, metestrus and diestrus phase of the estrous cycle, respectively (Kumar *et al* 1991). Purohit and Kohli (1977) also recorded higher serum cholesterol levels in Rathi cows during estrus (264.30mg%) than cows not in estrus (188.61mg%). However, significantly higher plasma cholesterol levels were reported in normal crossbred cows at day-13 as compared to day-0 of the estrous cycle (Islam *et al* 1994). Highest serum cholesterol was noticed at estrus (290.00 ± 9.31mg/dl) that declined during metestrus and showed a further fall with the advancement of pregnancy (Sahukar *et al* 1985). A low average plasma cholesterol concentration in dry non-pregnant buffaloes (36mg/dl) as compared to pregnant buffaloes (126mg/dl) has also been reported (Verma and Pandey 1975). Some workers observed little variation in plasma cholesterol concentrations between normal and repeat breeding

animals during different phases of the estrous cycle (Lamothe *et al* 1972). However, some workers reported significantly higher concentrations of blood cholesterol in normal cycling as compared to repeat breeding cows (Burle *et al* 1995, Ramakrishna 1996) while others found significantly higher concentrations of total plasma cholesterol in repeat breeding cows as compared to normal cows (Dutta *et al* 1991, Salem *et al* 1994).

2.5 Minerals

Mineral deficiency or imbalance is often cited as a cause of poor reproduction. Minerals imbalances are considered to be the important determinant of the poor reproductive efficiency as they act as cofactors, as activators of enzymes or stabilizers of secondary molecular structures and can affect the cell metabolism (Valle and Wacker 1976), so their abnormal levels may adversely affect the physiological function in general and reproduction in particular. Reproductive failure may be induced by deficiencies of single or combined elements or by their imbalances. Concomitant infertility in cattle is believed to be associated with enzymatic dysfunctions resulting from these deficiencies (Hidiroglou 1979). Mineral deficiencies usually involve several minerals as well as other conditioning factors; however, the deficiency symptoms of one mineral may predominate and affect the performance of the

ruminant. The relevant literature pertaining to the macro and micro-minerals, included in the present study, is reviewed.

2.5.1 Plasma macro-minerals

Minerals like Ca, P and Mg may influence directly or indirectly by affecting the ability of animal to utilize other minerals or certain enzyme system affecting reproductive efficiency (Dhoble and Gupta 1986). Most experimental work relating calcium to reproduction has centered on the effect of the calcium phosphorus ratio. Milking cows should always be provided adequate amounts of calcium to maximize production and minimize health problems. 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 intake is low. Calcium (Ca) is required for maintenance of normal reproductive cycle and it has sensitizing action on reproductive organs through various hormones (Kumar *et al* 1986). Higher ratio of Ca/P in blood of normally cycling as compared to repeat breeding buffaloes has been reported (Moustafa *et al* 1994). The concentrations of blood phosphorous were significantly higher in normal cycling as compared to repeat breeding cows (Burle *et al* 1995, Ramakrishna 1996). Lower levels of Ca, inorganic phosphorous and Mg in repeat breeding as compared to normal

cycling animals have also been reported in buffaloes (Paul *et al* 2000) and cows (Dutta *et al* 2001).

Deficiencies of Mg can lead to widespread problems in metabolism of cells (Jain 1994). Further it was stated that Mg play key role in the imbalance of other mineral status of repeat breeding animals (Balakrishnan and Balagopal 1994). The average plasma level of Mg was 52.33 ug/ml in repeat breeding buffaloes and highest values of Mg were found on day-1 (63.0 ± 7.37 ug/ml) and lowest on day-19 (42.75 ± 2.26 ug/ml) of the estrous cycle (Jain 1994).

Certain studies suggested that feeding high levels of potassium might delay the onset of puberty, delay ovulation, impair corpus Luteum (yellow body) development and increase the incidence of anestrus and repeat breeding in heifers. Deficient estrogen levels triggered the influx of K into the serum thus increasing the serum levels of K. Serum K values were found to be significantly high at estrus phase and similarly, better conception rates were reported with high Na levels at the estrus (Gangwar *et al* 1984). Average Na, K, and Cl concentrations in normal cows were found to be 161.70 ± 2.71, 4.37 ± 0.07 and 89.93 ± 0.96 mEq/L whereas the concentrations in repeat breeding cows were found to be 172.13 ± 3.68, 4.48 ± 0.11 and 86.80 ± 2.09, respectively (Cetin *et al* 2002). However, no significant difference was observed

between the values of serum sodium and potassium among the normal and repeat breeding cows (Gangwar *et al* 1984).

2.5.2. Plasma micro-minerals:

Variations in the optimum levels of some of the trace elements have been found to be associated with fertile/infertile estrus (Jain and Madan 1984) and reproductive disorders (Jain 1993). Deficiencies of copper (Cu) and zinc (Zn) may lead to impaired ovarian function, anestrus, silent estrus, delayed estrus, abortions and poor conception rates. Iron (Fe) is an important element of RBC's and hemoglobin and its deficiency may cause impaired functioning of reproductive system via deficient oxygen supply to cells and tissues. The average plasma normal values of Fe and Cu in cattle were found to be 146 and 93 ug/dl, respectively (Underwood 1977). Values of Cu, Zn and Fe were higher on days 16, 1 and 3 of the estrous cycle while lower values were observed on day-19, day-16 and on day-16 of the estrous cycle, respectively in repeat breeding buffaloes (Jain 1994). However, no significant difference was found in the values of Cu, Fe and Zn in repeat breeding buffaloes and normal buffaloes (Paul *et al* 2000). Lower concentrations of Cu, Zn and Fe in repeat breeding cows were also noticed as compared to normal cows (Rupde *et al* 1993). Higher Cu levels were found at estrus (Osman 1985). Plasma Fe levels were

significantly higher in metestrus and diestrus phases of the synchronized estrous cycle in buffalo heifers (Kumar 1986) and plasma Zn levels were found to be higher during diestrus phase of the estrous cycle whereas plasma Cu and Co did not showed any variation. Significantly higher levels of serum Cu in buffaloes during the follicular phase as compared to luteal phase were also observed (Kulkarni *et al* 1994). Higher concentrations of Zn and Fe were reported in pregnant than in non-pregnant buffaloes, however the concentration of Cu was found to be lower in the pregnant buffaloes than in non-pregnant buffaloes (Mandal *et al* 1996). Significantly lower levels of Cu in blood from normally cycling as compared to repeat breeding buffaloes were also recorded (Moustafa *et al* 1994). The average serum concentrations of Fe in normal and repeat breeding crossbred cows were found to be 1.45 ± 0.05 and 0.69 ± 0.03 ppm; Zn 1.80 ± 0.16 and 0.97 ± 0.01 ppm and Cu 1.11 ± 0.09 and 1.13 ± 0.12 ppm, respectively (Dutta *et al* 2002). Lower zinc concentrations in repeat breeding as compared to normal buffaloes was reported (Fayez *et al* 1992). Lower levels of serum Fe, Zn and Cu were found in repeat breeding cows as compared to normal cycling buffaloes. However, some studies indicated higher concentration of plasma Fe in repeat breeding cows as compared to normal farm cows (Singh and Pant 1998).

CHAPTER-III

MATERIALS AND METHODS

3.1 Selection and maintenance of animals

The study was conducted on forty female Murrah buffaloes including thirty-two repeat breeding and eight normal cycling Murrah buffaloes. All the animals were 400-550 kg of body weight, between 4-7 years of age and in their 2^{nd} – 4^{th} parity. All the animals were maintained at the dairy farm of Punjab Agricultural University, Ludhiana. The animals were kept under the semi loose housing system with half walled open sheds and pucca floors.

3.1.1 Basis of selection

The animals were apparently healthy and normal cycling. They were subjected to per-rectal examination to rule out any infectious, anatomical or pathological abnormality of the genitalia. The animals were selected on the basis of reproductive history. All the animals were showing normal estrous cyclicity. An animal was considered to be repeat breeder if it failed to conceive even after at least three inseminations with the good quality frozen semen.

3.1.2 Feeding and Management

All the animals were fed according to the normal feeding schedule followed at the dairy farm of Punjab Agricultural University, Ludhiana. The animals were fed with adequate amounts of chaffed/unchaffed green fodder depending upon the availability along with wheat bhusa. Besides green fodder, animals were also given the concentrate mixture ration containing 70% TDN, 13-16% DCP. The concentrate mixture was given at the rate of 0.5-1.0 kg/day/animal. In addition, 2% mineral mixture was also added to the ration. All the animals had an access to water *ad libitum*. All the animals were vaccinated and dewormed at regular intervals following the recommended schedule.

3.2 Grouping of animals

After selection, the animals were divided into five groups; one group was left as normal cycling control group, whereas 32 repeat breeding Murrah buffaloes were randomly divided into four groups. Out of these four groups of repeat breeding animals, one was kept as control repeat breeding group and the other three groups were given exogenous progesterone through different routes. The grouping was done as below:

3.2.1 Control Normal group-CN: This group consisted of eight normal cycling female Murrah buffaloes without the history of

repeat breeding. No treatment was given to these animals and this group acted as control normal cycling group.

3.2.2 Control Repeat breeding group-CR: This group consisted of eight repeat breeding female Murrah buffaloes. No treatment was given to these animals and this group acted as the control repeat breeding group.

3.2.3 Treatment group–T_i (Implant): This group consisted of eight repeat breeding female Murrah buffaloes. The Crestar ear implants (Intervet Co. Pvt. Ltd) were inserted into left ear of each animal of this group on day-4 after insemination (day-0 being the day of insemination). The implants were kept in the place up to day-12 and then removed. The insertion and removal of implants were done with the help of specific applicator provided by the company.

3.2.4 Treatment group–T_o (Oral): This group consisted of eight repeat breeding female Murrah buffaloes. Oral progesterone supplementations with micronized progesterone (Microgest, Martin & Harris) were done to each animal of this group. Each animal was fed one tablet containing 100 mg of progesterone daily in the feed starting from day-4 through day-12 (day-0 being the day of insemination).

3.2.5 Treatment group–T_m (I/M): This group consisted of eight repeat breeding female Murrah buffaloes. 2 ml progesterone

injections (Duraprogen 500 mg; UniChem Co.) were administered by I/M route, to each animal in the neck muscle. Two injections were given to each animal; first I/M injection on day-4 and the second on day-9 after insemination (day-0 being the day of insemination).

3.3 Detection of estrus

All the animals belonging to each group were detected for the onset of estrus twice daily (morning and evening), starting at least two days prior to the expected date of onset of estrus. The following methods were employed for detection of estrus:

3.3.1 Parading a teaser bull: A vasectomized teaser bull was paraded twice daily (morning and evening) in the herd for estrus detection.

3.3.2 Behavioral signs: The animals were observed for various behavioral signs of estrus viz. bellowing, frequent micturition, and restlessness and smelling/licking of vulva by other animals and acceptance to the teaser bull.

3.4 Insemination of buffaloes

After these animals were detected in estrus, they were artificially inseminated with good quality frozen semen obtained from the semen collection center of Punjab Agricultural University,

Ludhiana; within 24 hours of the onset of estrus. Double insemination technique was followed i.e. the animals were inseminated two times; once at 12 hours and second at 24 hours after the onset of estrus. The day of insemination was designated as the day-0.

3.5 Confirmation of pregnancy

An animal was considered to be pregnant on the basis of following criteria:

3.5.1 Non-return of estrus: Non-return to estrus for at least up to two consecutive estrous cycles.

3.5.2 Mature Corpus Luteum: Presence and maintenance of the mature corpus luteum on the surface of the ovary for at least up to two consecutive estrous cycles.

3.6 Blood Sampling

Blood samples were taken from all the animals on the pre-designated intervals. All the samples were collected in the morning hours. Blood samples were obtained aseptically in a heparinzed vials by jugular venipuncture.

3.6.1 Sampling schedule

Blood samples were taken starting from day-0 through day-20 and a total of 10 blood samples were collected from each animal

belonging to control and treatments groups. The sampling schedule was as follows:

Sample No.	Day of estrous cycle
1	0
2	3
3	4
4	5
5	6
6	9
7	10
8	12
9	16
10	20

3.6.2 Collection and processing of blood

15-20 ml of blood was collected aseptically in the 30 ml heparinized glass vials from all the animals by jugular venipuncture. The blood samples after collection were quickly transferred to the laboratory in an ice bucket for further processing. 1 ml of the whole blood from each sample was used for the preparation of protein free filtrate for blood glucose estimation; rest of the samples was centrifuged in the refrigerated centrifuge at 3000 rpm for 30 minutes. The plasma was separated out and stored in the small

aliquots at -20°C for further analyses of various hormones, micro and macro-minerals and other biochemical constituents of the blood plasma.

3.7 Laboratory analyses of blood plasma

3.7.1 Analyses for hormones

The plasma samples were analysed for the progesterone (P_4), metabolic hormones [Triiodothyronine (T_3), Thyroxine (T_4) and Insulin] by Radio Immuno Assay (RIA) technique.

The principle of this technique (RIA) is that the unlabelled and labeled analyte compete for a limited number of available binding sites on the antibody that has equal affinity for the standard and the analyte in the sample. Increasing quantities of the unlabelled analyte reduced the amount of labeled analyte bound to antibody. The level of radioactivity bound is, therefore, inversely related to the concentration of analyte in the patient sample or standard. After an adequate incubation period, the bound and free fractions are separated and the radioactivity was quantitated on the Gamma Counter (Beckman Gamma Counter; Model-DP-5500-B system). Levels of hormones in the samples were then graphically determined from a standard curve.

Estimation of hormones was undertaken in the RIA Lab in Department of Veterinary Physiology, Punjab Agricultural University,

Ludhiana. For each hormone, all the samples were analyzed in the single assay to avoid any inter assay variation. Repeated thawing and freezing of plasma samples were avoided.

3.7.1.1 Progesterone (P_4) assay

The plasma samples were analyzed for determining the progesterone concentrations using the RIA kits procured from ICN Pharmaceuticals, Inc, Diagnostics Division, Costa Mosa, CA. Assay procedure includes the following steps:

(1) All the standards, samples, coated tubes and progesterone ^{125}I are brought to room temperature prior to use.

(2) The required number of anti-progesterone coated tubes in the test tube rack.

(3) 100 Í L of each standard, sample and control were pipetted into its respective coated tubes.

(4) 1.0 mL of progesterone- ^{125}I was added to all tubes and the tubes were vortex briefly.

(5) All the tubes were incubated in water bath at 37° ± 1°C for 120 minutes.

(6) The contents of the tubes were then aspirated.

(7) The radioactivity was then counted for 1 minute using the gamma counter calibrated for ^{125}I.

3.7.1.2 Metabolic hormones

3.7.1.2.1 Insulin Assay

The plasma samples were analyzed for determining the insulin concentrations using the RIA kits procured from ICN Pharmaceuticals, Inc, Diagnostics Division, Costa Mosa, CA. The principle of this technique (RIA) is that the unlabelled and labeled analyte compete for a limited number of available binding sites on the antibody that has equal affinity for the standard and the analyte in the sample. Increasing quantities of the unlabelled analyte reduced the amount of labeled analyte bound to antibody. The level of radioactivity bound is, therefore, inversely related to the concentration of analyte in the patient sample or standard. After an adequate incubation period, the bound and free fractions are separated and the radioactivity was quantitated on the Gamma Counter (Beckman Gamma Counter; Model-DP-5500-B system). Levels of hormones in the samples were then graphically determined from a standard curve

3.7.1.2.2 Triiodothyronine (T_3) Assay

The plasma samples were analyzed for determining the triiodothyronine (T_3) and thyroxine (T_4) concentrations using the RIA kits procured from ICN Pharmaceuticals, Inc, Diagnostics

Division, Costa Mosa, CA. The sensitivity of the T_3 test kit was 6.7 ng/dL. The assay procedure includes the following steps:

(1) All the standards, samples, coated tubes are brought to room temperature prior to use.

(2) The required number of anti-T_3 coated tubes in the test tube rack.

(3) 100 ÍL of each T_3 serum, sample and control were pipetted into its respective coated tubes.

(4) 1.0 mL of T_3 tracer solution was added to all tubes and the tubes were vortex briefly.

(5) All the tubes were incubated in water bath at 37° ± 1°C for 60 minutes.

(6) The contents of the tubes were then aspirated.

(7) Add 1.0 mL of distilled water to all the tubes.

(8) The tubes were reaspirated.

(9) The radioactivity was then counted for 1 minute using the gamma counter.

3.7.1.2.3 Thyroxine (T_4) assay

The plasma samples were analyzed for determining the thyroxine (T_4) concentrations using the ICN RIA kits. The sensitivity of the test was 0.7 Íg/dL. The assay procedure includes the following steps:

(1) All the standards, samples, coated tubes are brought to room temperature prior to use.

(2) The required number of anti-T_4 coated tubes in the test tube rack and they were numbered.

(3) 25 Í L of each T_4 serum, sample and control were pipetted into its respective coated tubes.

(4) 1.0 mL of T_4 tracer solution was added to all tubes and the tubes were vortex briefly.

(5) All the tubes were incubated at room temperature (18-25°C) for 60 minutes.

(6) The contents of the tubes were then aspirated.

(7) The radioactivity was then counted for 1 minute using the gamma counter.

3.8 Estimation of blood biochemical constituents

The biochemical constituents of the blood plasma were estimated by the following methods:

3.8.1 Blood glucose

The blood glucose was estimated by the method of Follin and Wu (1920) and is based on the principle that glucose present in protein free filtrate causes the reduction of the alkaline copper solution that further reduces the phosphomolybdate to molybdenum

blue, which is measured photo-metrically at a wavelength of 420 nm.

3.8.2 Plasma total cholesterol

Plasma total cholesterol was measured by the method of Henley (1957). The method is based on the principle that cholesterol in acetic acid solution gives a red color with ferric chloride and sulphuric acid, that is read at a wavelength of 560 nm.

3.9 Estimation of Minerals

3.9.1 Micro-minerals (Cu, Zn, Fe and Co)

The micro-minerals were estimated using the Atomic Absorption Spectrophotometer by the method of Ludmilla (1976). This method is based upon the principle that the monochromatic band of light is passed through a flame into which the solution of a sample has been atomized. At the correct wavelength there is a selective absorption that is dependent only on the concentration of the absorbing agent.

For the determination of various micro-minerals (copper, cobalt, iron, zinc), the plasma samples were digested in the triple acid i.e. nitric acid, per-chloric acid and sulphuric acid in the ratio of 10:3:1. For digestion, 2 mL of the plasma samples were taken in the 100 mL conical flask and 13-15 mL of the triple acid was added to it. Then the conical flasks containing the mixture were kept

overnight and the reaction mixture was then digested at hot plate till the volume reduced to 1 ml in the conical flask. After that three washings were given to each sample and the final volume was made to 10 ml by the addition of triple distilled water. The prepared final volumes were kept in the plastic vials and were stored in the refrigerator till further estimation.

3.9.2 Macro-minerals

3.9.2.1 Calcium (Ca): Ca was estimated using the same procedure as used for the estimation of micro-minerals i.e. using the Atomic Absorption Spectrophotometer.

3.9.2.2 Phosphorous (P): The estimation of phosphorous depends upon the principle that the proteins present in the serum/plasma are precipitated by the addition of trichloroacetic acid. The protein free filtrate is treated with ammonium molybdate solution, which combines with phosphate to form phosphomolybdate. The molybdate thus formed is thus reduced with ferrous sulfate and the blue colored complex is measured calorimetrically at 660 nm wavelength.

3.9.2.3 Chloride (Cl): The estimation of Cl depends on the principle that Cl is precipitated from the protein free blood filtrate (prepared by Folin & Wu method) by means of silver nitrate in the presence of nitric acid and the excess of silver nitrate is titrated

with standard thicyanate solution, using ferric ammonium sulphate solution as an indicator.

3.9.2.4 Sodium (Na) and Potassium (K): Determination of Na and K were done using flame photometer. In this process, the solution under test is passed under carefully controlled conditions as a very fine spray in the air supply to a burner. In the flame the solution evaporates and the salt dissociates to give neutral atoms. Some of these, though only a very small proportion, move into a higher energy state. It is the light emitted when these excited atoms fall back to the ground state, which is used in flame photometery. Light of characteristic wavelength is emitted and passes through a suitable filter and the amount of current produced is thus measured.

3.10 Statistical Analyses

The data were analyzed by applying the students't' test and the values of various parameters on different days were compared to day-0 value by calculating the critical difference (C.D.) values as per the method described by Snedecor and Cochran (1968).

CHAPTER-IV

RESULTS AND DISCUSSION

Effect of progesterone supplementation through different routes on hormonal, blood biochemical profile and conception rate in repeat breeding Murrah buffaloes was studied and the results of various parameters are presented in Tables 1 to 31 and Figures 1 to 22.

4.1 Conception rate

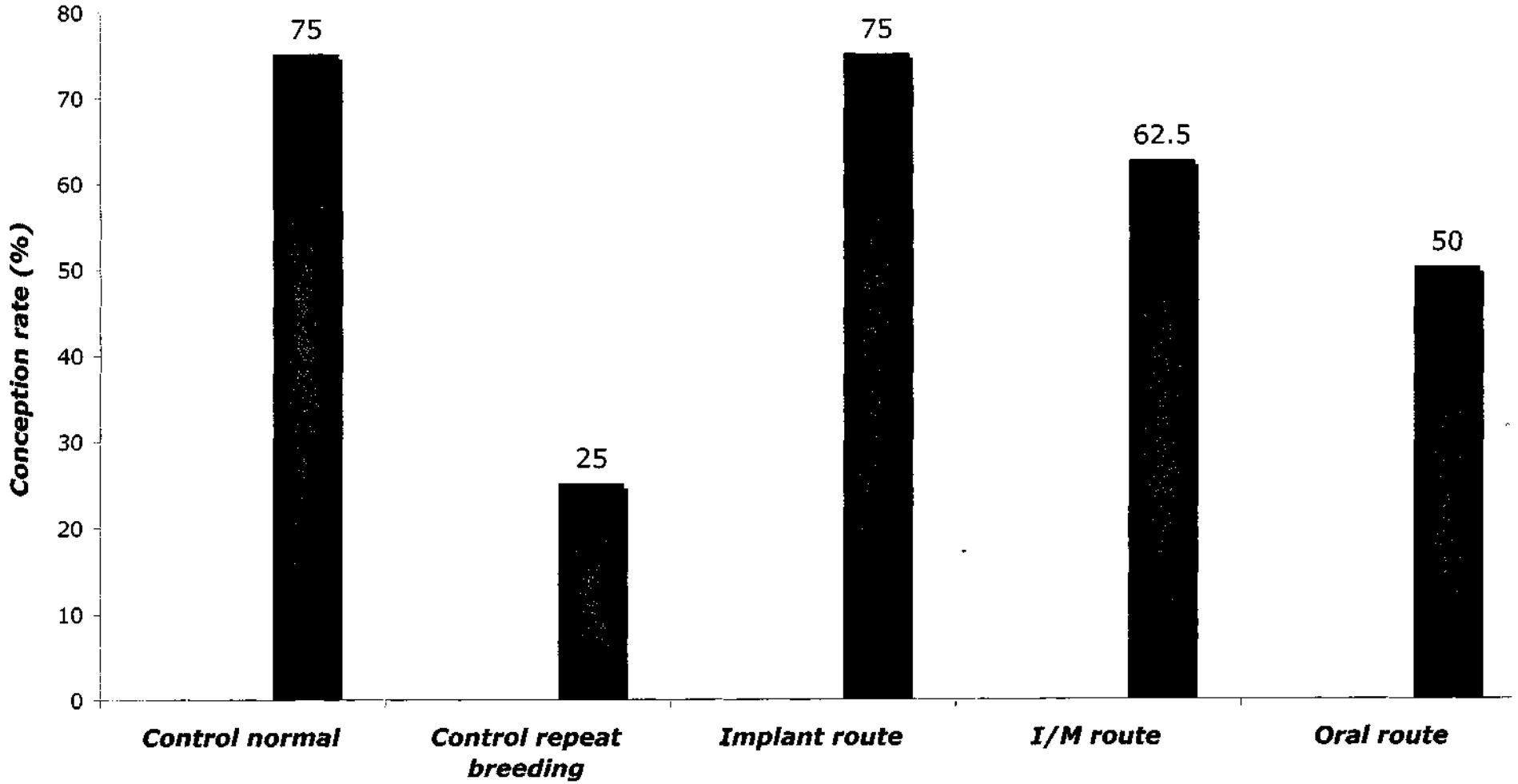
The results of conception rate in the control (CN and CR) and treatment (T_i, T_m and T_o) groups are presented in Table-1 & Figure-1.

In control repeat breeding group (CR) in which no treatment was given, two out of eight animals became pregnant resulting in the conception rate of only 25% whereas, the conception rate in the normal cycling buffaloes (CN) without the history of repeat breeding was 75% in which six out of eight animals became pregnant. The conception rate in the Crestar implant group (T_i) was found to be the highest (75%) in which six out of eight animals became pregnant, followed by the I/M group (T_m) in which five out of eight animals became pregnant resulting in the conception rate of 62.5%. Lowest conception rate among the treatment groups was found in

Table-1 Conception rate in control (CN and CR) and treatment (T_i, T_m and T_o) groups of buffaloes

GROUPS	TOTAL NO. OF BUFFALOES	BUFFALOES CONCEIVED	BUFFALOES NOT CONCEIVED	CONCEPTION RATE (%)
Control Normal (CN)	8	6	2	**75.0**
Control Repeat Breeding (CR)	8	2	6	**25.0**
T_i group (Implant)	8	6	2	**75.0**
T_m group (I/M)	8	5	3	**62.5**
T_o group (Oral)	8	4	4	**50.0**

Figure-1 ***Conception rate in control (CN and CR) and treatment groups (Implant, I/M and Oral)***



the oral progesterone supplementation group (T_o) in which only four out of eight animals conceived resulting in 50.0% conception rate.

Lower conception rates in untreated repeat breeding animals are well established (Walton *et al* 1990, Awasthi *et al* 2002, Sharma *et al* 2003). Low pregnancy rates in these animals (ranging from 16 to 42%) and present study (25%) are indicative of fertility problems in the animals. In most of the studies improved pregnancy rates in repeat breeding animals after progesterone supplementation have been reported. Earlier workers have tried to supplement progesterone in repeat breeding cows and buffaloes either through I/M or oral routes (Panchal *et al* 1991 and Singh *et al* 2002). The enhanced pregnancy rates in the present study in T_m (I/M) and T_o (oral) groups are comparable with those reported by Kavani and Kodagali (1986), Rosen and Struman (1989) and Devanathan *et al* (1999). Previous studies in this lab (Sharma *et al* 2003) also indicated enhanced conception rate from 50.0% (in untreated buffaloes) to 67% by giving exogenous progesterone by I/M route in repeat breeding buffaloes. Although, few reports indicated enhanced conception rates in repeat breeding cows using intra-vaginal implants (Robinson *et al* 1989), however no positive effect was observed by Walton *et al* (1990). In some studies increasing progesterone levels by implants initially failed but the conception rates were improved at subsequent service (Stevenson and Mee

1991). No such study seems to be conducted in buffaloes using progesterone implants in repeat breeding animals.

Certain reports indicate that the progesterone supplementation in repeat breeding animals failed to produce any positive effects (Sreenan and Diskin 1983, Drew *et al* 1982, Almeida *et al* 1995 and Bage 2003). The possible reason for this failure could be that suprabasal progesterone levels were present at the time of estrus, the progesterone treatment was not given at the proper time or the conception rates were already near normal. Further, Singh *et al* (2002) suggested that progesterone supplementation was useful only in repeat breeding females with no other reproductive abnormality; the progesterone supplementation in normal animals may even have adverse effects.

4.2 Hormonal analyses

4.2.1 Progesterone (P_4)

The mean plasma progesterone concentrations in control (CN and CR) and treatment (T_i, T_m and T_o) groups are presented in Table-2 and Fig-2, and in pregnant and non-pregnant buffaloes are presented in Table-3 and Fig-3.

The mean plasma progesterone concentrations on day-0 of the estrous cycle in both control normal (CN) and control repeat breeding (CR) groups were found to be 0.25 ± 0.04 and 0.19 ±

Table-2 Plasma progesterone concentrations (ng/ml) in control (CN and CR) and treatment (T_i, T_m and T_o) groups of buffaloes (Mean ± S.E.)

DAY OF ESTROUS CYCLE/ EARLY PREGNANCY	CONTROL GROUPS		TREATMENT GROUPS		
	CN	CR	T_i group*	T_m group*	T_o group*
0	0.25 ± 0.04[a]	0.19 ± 0.03[a]	0.15 ± 0.04[a]	0.19 ± 0.02[a]	0.16 ± 0.03[a]
3	0.36 ± 0.07[a]	0.29 ± 0.09[a]	0.28 ± 0.08[a]	0.21 ± 0.04[a]	0.24 ± 0.05[a]
4	0.69 ± 0.13[b]	0.39 ± 0.11[a*]	0.58 ± 0.11[b]	0.55 ± 0.18[b]	0.51 ± 0.12[b]
5	1.93 ± 0.31[b]	1.26 ± 0.27[b*]	1.88 ± 0.25[b]	1.71 ± 0.28[b]	1.68 ± 0.22[b]
16	1.78 ± 0.61[b]	0.58 ± 0.34[b*]	1.67 ± 0.58[b]	1.51 ± 0.48[b]	1.13 ± 0.55[b]
20	1.69 ± 0.59[b]	0.52 ± 0.28[b*]	1.58 ± 0.42[b]	1.46 ± 0.41[b]	1.04 ± 0.47[b]

[a, b]values differ significantly (P<0.05) as compared to day-0 values within the column

**values in CR group differ significantly (P<0.05) as compared to respective values in all other groups*

****T_i, T_m, T_o.....Progesterone supplementation through ear implant, I/M and oral routes***

Figure-2 ***Plasma progesterone (P_4) concentrations (ng/ml) in control (CN and CR) and treatment groups of buffaloes (Implant, I/M and Oral)***

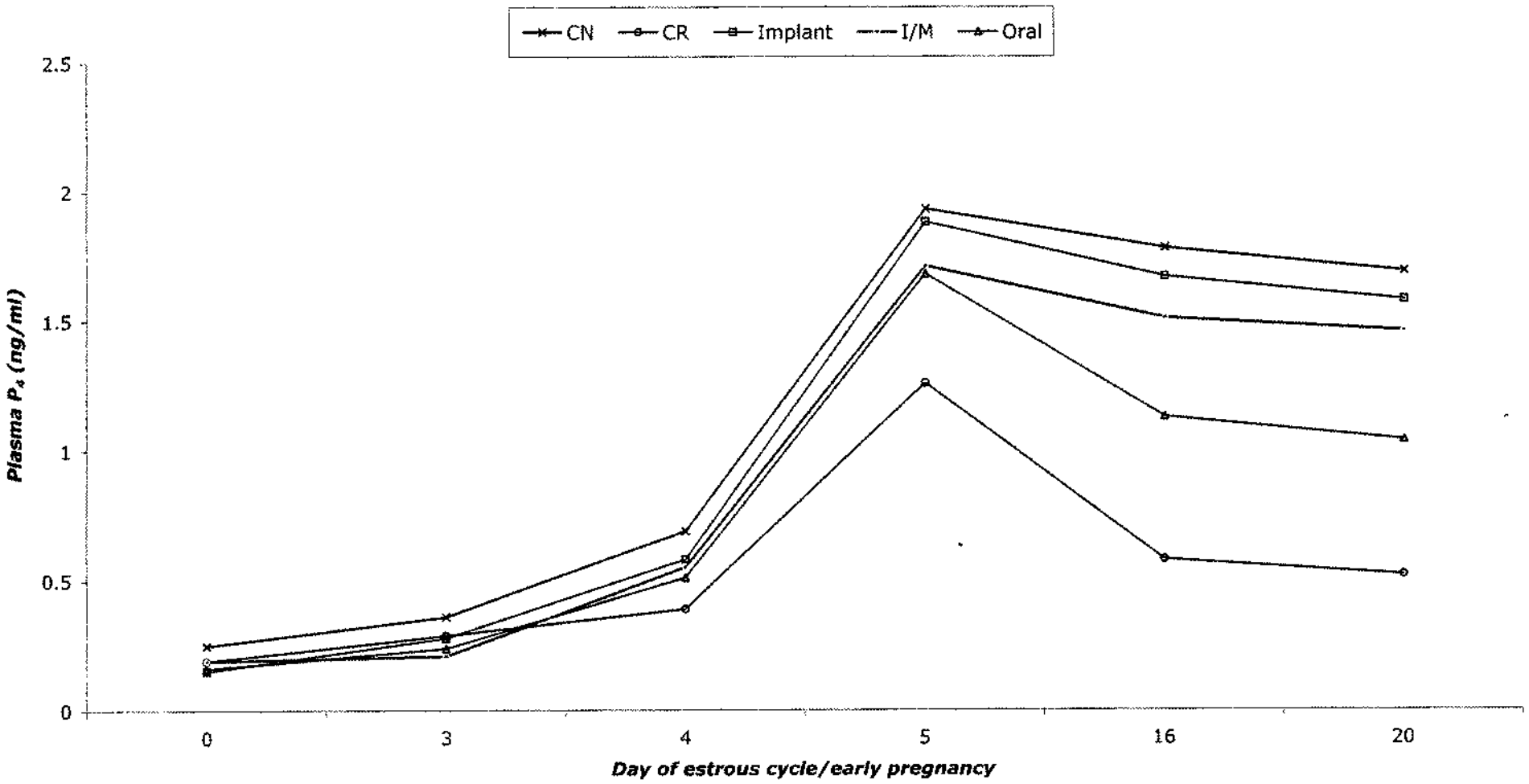
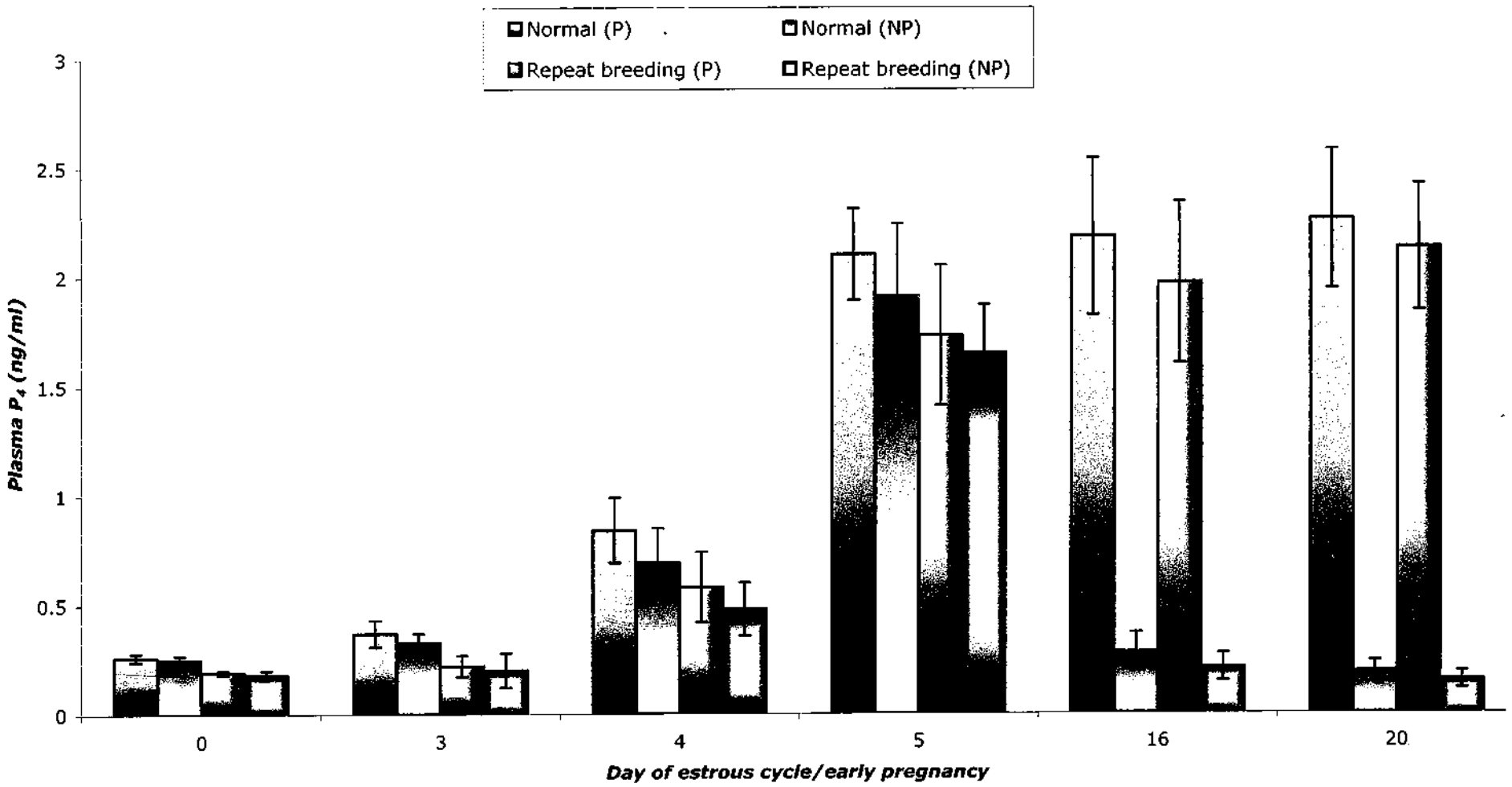


Table-3 Plasma progesterone concentrations (ng/ml) in pregnant and non-pregnant buffaloes belonging to normal cycling and repeat breeding buffaloes (Mean ± S.E.)

DAY OF ESTROUS CYCLE/ EARLY PREGNANCY	NORMAL BUFFALOES		REPEAT BREEDING BUFFALOES	
	PREGNANT	NON-PREGNANT	PREGNANT	NON-PREGNANT
0	0.26 ± 0.02[a]	0.25 ± 0.02[a]	0.19 ± 0.01[a]	0.18 ± 0.02[a]
3	0.37 ± 0.06[a]	0.33 ± 0.04[a]	0.22 ± 0.05[a]	0.20 ± 0.08[a]
4	0.84 ± 0.15[b]	0.69 ± 0.16[b]	0.58 ± 0.16[b]	0.48 ± 0.12[b]
5	2.10 ± 0.21[b]	1.91 ± 0.33[b]	1.73 ± 0.32[b]	1.65 ± 0.22[b]
16	2.18 ± 0.36[b]	0.28 ± 0.09[a*]	1.97 ± 0.37[b]	0.21 ± 0.06[a*]
20	2.26 ± 0.32[b]	0.19 ± 0.05[a*]	2.13 ± 0.29[b]	0.15 ± 0.04[a*]

-values with different superscripts differ significantly (P<0.01) as compared to day-0, within the column
**values in the non-pregnant buffaloes differ from pregnant buffaloes in normal cycling and repeat breeding buffalo groups*

Figure-3 ***Plasma progesterone (P_4) concentrations (ng/ml) in pregnant (P) and non-pregnant (NP) buffaloes belonging to normal cycling and repeat breeding groups (Mean ± S.E.)***



0.03 ng/ml with no significant difference. On day-3, the concentrations in both CN and CR groups were 0.36 ± 0.07 and 0.29 ± 0.09 ng/ml, again without any significant difference (Table-2). Moreover, the P_4 concentrations did not differ significantly on day-3 as compared to day-0 of the estrous cycle in either of the groups. On day-4, a significant rise in plasma progesterone concentration (0.69 ± 0.13 ng/ml) was observed in control normal (CN) buffaloes whereas the levels did not change much as compared to day-0 in control repeat breeding (CR) buffaloes. On day-5, a significant rise in plasma progesterone levels was observed in both CN and CR groups (Table-2 and Fig-2); however these levels were much lower in repeat breeding buffaloes (1.26 ± 0.27 ng/ml) as compared to normal cycling buffaloes (1.93 ± 0.31 ng/ml). Lower plasma progesterone levels or abnormal pattern of progesterone variation in repeat breeding animals during early pregnancy/luteal phase has been reported (Kimura *et al* 1987, Kang *et al* 1994, Shukla *et al* 2000, Sharma 2001, Jindal *et al* 2004) in cattle and buffaloes. In case of normal cycling buffaloes (CN), a higher level of plasma progesterone was maintained on day-16 (1.78 ± 0.61 ng/ml) and day-20 (1.69 ± 0.59 ng/ml) of the cycle/pregnancy; however, these levels were lower as compared to day-5 plasma progesterone concentrations. On the other hand, the repeat breeding buffalo (CR), a significant decline in plasma

progesterone concentrations was observed on day-16 (0.58 ± 0.34 ng/ml) and day-20 (0.52 ± 0.28 ng/ml) of the cycle/pregnancy, though, these levels were higher as compared to day-0 and day-3 plasma progesterone concentrations.

In bovines, the plasma progesterone concentrations are usually at their nadir on the day of estrus. After ovulation the progesterone production starts increasing due to the process of luteinization. The corpus luteum usually matures within 4-5 days and plasma progesterone levels keep rising. If the animal conceives, the functional corpus luteum and thus the higher plasma progesterone concentrations are maintained throughout pregnancy. In case the animal fails to conceive the non-pregnant, progesterone-impregnated uterus starts producing $PGF_{2\alpha}$ that reaches the ovary through counter current exchange mechanism between uterine vein and ovarian artery leading to regression of the corpus luteum (Hafez 1980). The mean progesterone levels after fertile and non-fertile first service inseminations were reported to be similar from 21 days before until 13 days after insemination. However, levels declined in non-pregnant animals while those in pregnant animals continued to rise until day-20 (Bulman and Lamming 1978).

In the present study, irrespective of the group (CN or CR), the animals that conceived maintained higher progesterone levels

throughout the study period, whereas in the non-pregnant animals there was a precipitous fall in the progesterone concentrations on day-16 that was in accordance with previous observations (Ahmad *et al* 1977, Jindal *et al* 1988 and Sharma *et al* 2003). Further, the plasma progesterone levels remained low on day-20 (Table-3). As discussed earlier (Section 4.1), six out of eight in control normal (CN) and two out of eight in control repeat breeding (CR) buffaloes conceived leading to conception rates of 75% and 25%, respectively (Table-1). In present study, higher plasma progesterone levels on days-16 and 20 in normal cycling buffaloes (CN) as compared to control repeat breeding buffaloes (CR) can be attributed to higher conception rates. As is evident from the Table-2, in group-CN, six presumed pregnant buffaloes maintained higher levels on day-16 and 20 as compared to only two buffaloes in case of CR group.

The plasma progesterone concentrations on day-0 and day-3 of the estrous cycle did not differ significantly among the three treatment groups (T_i, T_m and T_o). These plasma progesterone concentrations were similar to those found in group CR on respective days and were relatively lower as compared to normal cycling buffaloes group CN (Table-2), indicating that the progesterone treatment could not bring about any significant change in plasma progesterone concentrations up to day-3 of the cycle in repeat breeding buffaloes. In all the treatment groups, the

plasma progesterone concentrations showed a significant rise on day-4 following progesterone treatment (Table-2). This rise on day-4 was similar to group-CN buffaloes; however, the group CR buffaloes did not show any significant rise on this day. In all the control as well as treatment groups, there was a further rise in plasma progesterone concentration on day-5 of the cycle, however these concentrations were relatively lower in control repeat breeding buffaloes (group-CR).

In physiologically normal animals, after the formation of corpus luteum, ovulation occurs that reaches a mature stage by day-4. In this study, lower levels of plasma progesterone in control repeat breeding buffaloes on day-4 is a clear indication of luteal insufficiency due to some problem with the process of luteinization during early pregnancy. Luteal insufficiency has been recognized as a possible cause for lower conception rate and repeat breeding (Kang *et al* 1994 and Sharma *et al* 2003). In the present study lower conception rate (25%) in untreated repeat breeding buffaloes has been observed as compared to normal cycling buffaloes (75%).

In normal cycling animals and also in all the three treatment groups, there was a further rise in plasma progesterone concentration (Fig-2). In control repeat breeding group (CR), also the mean plasma progesterone concentrations on day-5 was significantly higher with respect to day-0 concentrations, however

this value was lower (1.26 ± 0.27 ng/ml) as compared to day-5 plasma progesterone concentration of other groups. This lower value may probably be due to delayed/faulty luteinization and hence a delay in the post-ovulatory rise in progesterone.

On day-16 and day-20 of the cycle the plasma progesterone concentrations remained high in normal cycling as well as progesterone supplemented groups however, these concentrations were slightly lower as compared to day-5 levels. This fall in the progesterone concentrations may possibly be due to regression of corpus luteum in some of the animals that did not conceive or fail to maintain pregnancy. In case of control repeat breeding buffaloes, the progesterone concentrations on day-16 and 20 were significantly low as compared to respective concentrations in other groups. Interestingly, these levels in group-CR were also lower as compared to day-5 levels in the same group.

In this study, in all the groups, the mean plasma progesterone levels were at their nadir on the day of estrus that started rising during metestrus (day-3) and kept rising during diestrus phase (days 4 & 5). In all the groups plasma progesterone concentrations followed the same pattern during the study period. In the treatment groups, the concentrations were similar to those found in normal cycling animals, however, mean plasma progesterone concentrations in control repeat breeding group (CR)

were lower as compared to normal cycling as well as treatment groups. These observations have indicated that the exogenous progesterone administration in the treatment groups was effective enough to improve the plasma progesterone concentrations comparable to normal cycling buffaloes during early pregnancy satisfying one of the major aims of the study.

In this study when the data (plasma progesterone concentrations) were analyzed irrespective of the progesterone supplementation, the pregnant and non-pregnant buffaloes belonging to all the groups (control and treatment) showed a similar pattern up to day-5 (diestrus/early pregnancy), which was expected because of the fact that whether the animal conceives or not there is a formation of mature corpus luteum at the site of ovulation (Hafez 1980). A functional corpus luteum was maintained in pregnant animals whereas, in non-pregnant buffaloes, it regressed at around day-15/16 as observed by per-rectal examination. In case the animal fails to conceive, there is release of $PGF_{2\alpha}$ from the progesterone impregnated non-pregnant uterine horn. This $PGF_{2\alpha}$ reaches ovary through counter current mechanism causing the lyses of corpus luteum that is the main organ responsible for secreting progesterone (Hafez 1980). A precipitous fall in plasma progesterone concentrations after regression of corpus luteum have been reported by Ahmad *et al* (1977) and Jindal *et al* (1988) in

buffaloes. In the present study, lower mean plasma progesterone concentrations on days-16 and 20 (Fig-3) in non-pregnant buffaloes as compared to pregnant buffaloes were quite evident (Table-3). Interestingly, the plasma progesterone concentrations on these days were found to be higher in control normal and all the treatment groups as compared to the control RB buffaloes. This difference could be attributed to less number of buffaloes maintaining a mature corpus luteum up to this period (lower conception rate-25%) in untreated repeat breeding buffaloes (group-CR) as compared to other groups. In the present study, three different routes have been employed in order to enhance the early pregnancy plasma progesterone concentrations with an objective to overcome the effects of presumed luteal insufficiency. Among the three treatment groups day-16 and 20 plasma progesterone concentrations were relatively higher in T_i (progesterone supplementation through ear implant) and lower in group T_o (oral progesterone supplementation), which was a reflection of the number of buffaloes that maintained pregnancy (conception rates-75% and 25%, respectively; Table-1).

4.2.2 Metabolic hormones

4.2.2.1 Triiodothyronine (T_3)

The mean concentrations of circulating plasma triiodothyronine (T_3) in control (CN and CR) and treatment (T_i, T_m and T_o) groups are presented in Table-4 and Fig-4, and in pregnant and non-pregnant buffaloes are presented in Table-5 and Fig-5.

Overall range of mean plasma T_3 concentrations in control normal (CN) and control repeat breeding (CR) groups varied from 0.45 ± 0.05 to 0.81 ± 0.08 ng/ml and 0.37 ± 0.04 to 0.68 ± 0.06 ng/ml, respectively. Highest mean plasma T_3 concentrations in control normal (CN) and control repeat breeding (CR) groups were found on day of estrus i.e. day-0 (0.81 ± 0.08 and 0.68 ± 0.06 ng/ml, respectively). Except on day-20, the plasma T_3 concentrations were relatively higher in group-CN than in group-CR ($p<0.05$). The mean plasma triiodothyronine varied from 0.40 ± 0.04 to 0.71 ± 0.08 ng/ml in T_i, 0.38 ± 0.03 to 0.69 ± 0.04 ng/ml in T_m and 0.40 ± 0.03 to 0.70 ± 0.05 ng/ml in T_o groups. As in case of control animals, in the treatment groups also the mean plasma T_3 concentrations were found to be higher on day of estrus in T_i (0.71 ± 0.08 ng/ml), T_m (0.69 ± 0.04 ng/ml) and T_o (0.70 ± 0.05 ng/ml) groups that were not significantly different (Table-4). No significant change in the T_3 concentrations was observed during rest of the cycle in any of the control or treatment groups.

Table-4 Plasma triiodothyronine concentrations (ng/ml) in control (CN and CR) and treatment (T_i, T_m and T_o) groups of buffaloes (Mean ± S.E.)

DAY OF ESTROUS CYCLE/ EARLY PREGNANCY	CONTROL GROUPS		TREATMENT GROUPS		
	CN	CR	T_i group*	T_m group*	T_o group*
0	0.81 ± 0.08[a]	0.68 ± 0.06[a]	0.71 ± 0.08[a]	0.69 ± 0.04[a]	0.70 ± 0.05[a]
3	0.45 ± 0.05[b]	0.37 ± 0.04[b]	0.40 ± 0.04[b]	0.38 ± 0.03[b]	0.40 ± 0.03[b]
4	0.49 ± 0.04[b]	0.39 ± 0.05[b]	0.42 ± 0.06[b]	0.39 ± 0.05[b]	0.38 ± 0.04[b]
5	0.52 ± 0.03[b]	0.41 ± 0.07[b]	0.38 ± 0.03[b]	0.42 ± 0.04[b]	0.41 ± 0.06[b]
16	0.61 ± 0.07[b]	0.42 ± 0.06[b]	0.48 ± 0.04[b]	0.44 ± 0.06[b]	0.45 ± 0.05[b]
20	0.59 ± 0.09[b]	0.58 ± 0.07[a]	0.50 ± 0.07[b]	0.54 ± 0.08[b]	0.58 ± 0.09[a]

-values with different superscripts differ significantly (P<0.01) as compared to day-0, within the column

**** T_i, T_m, T_o.....Progesterone supplementation through ear implant, I/M and oral routes***

Figure-4 ***Mean plasma triiodothyronine (T_3) concentrations (ng/ml) in control (CN & CR) and treatment groups of buffaloes (Implant, I/M and Oral)***

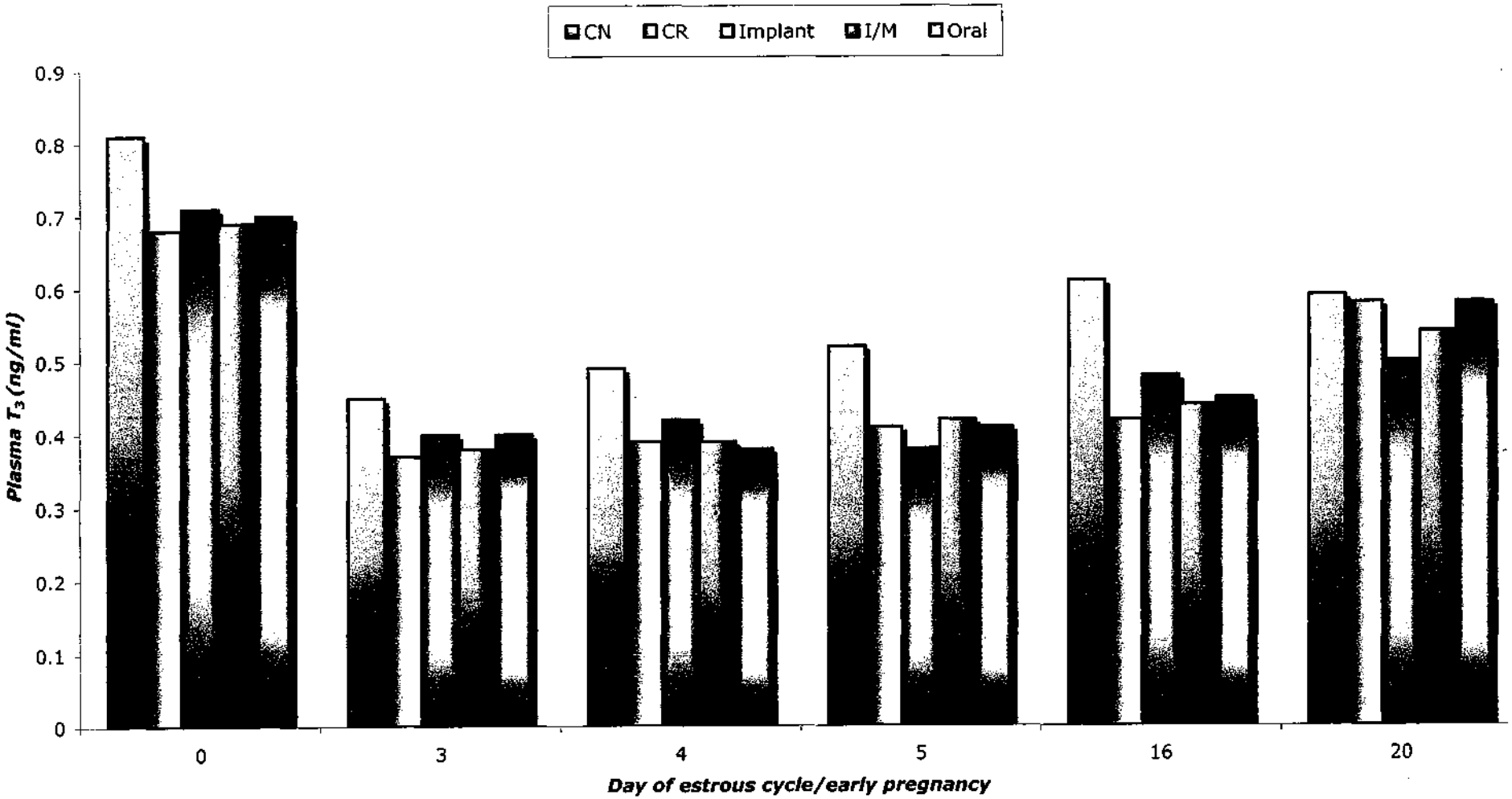
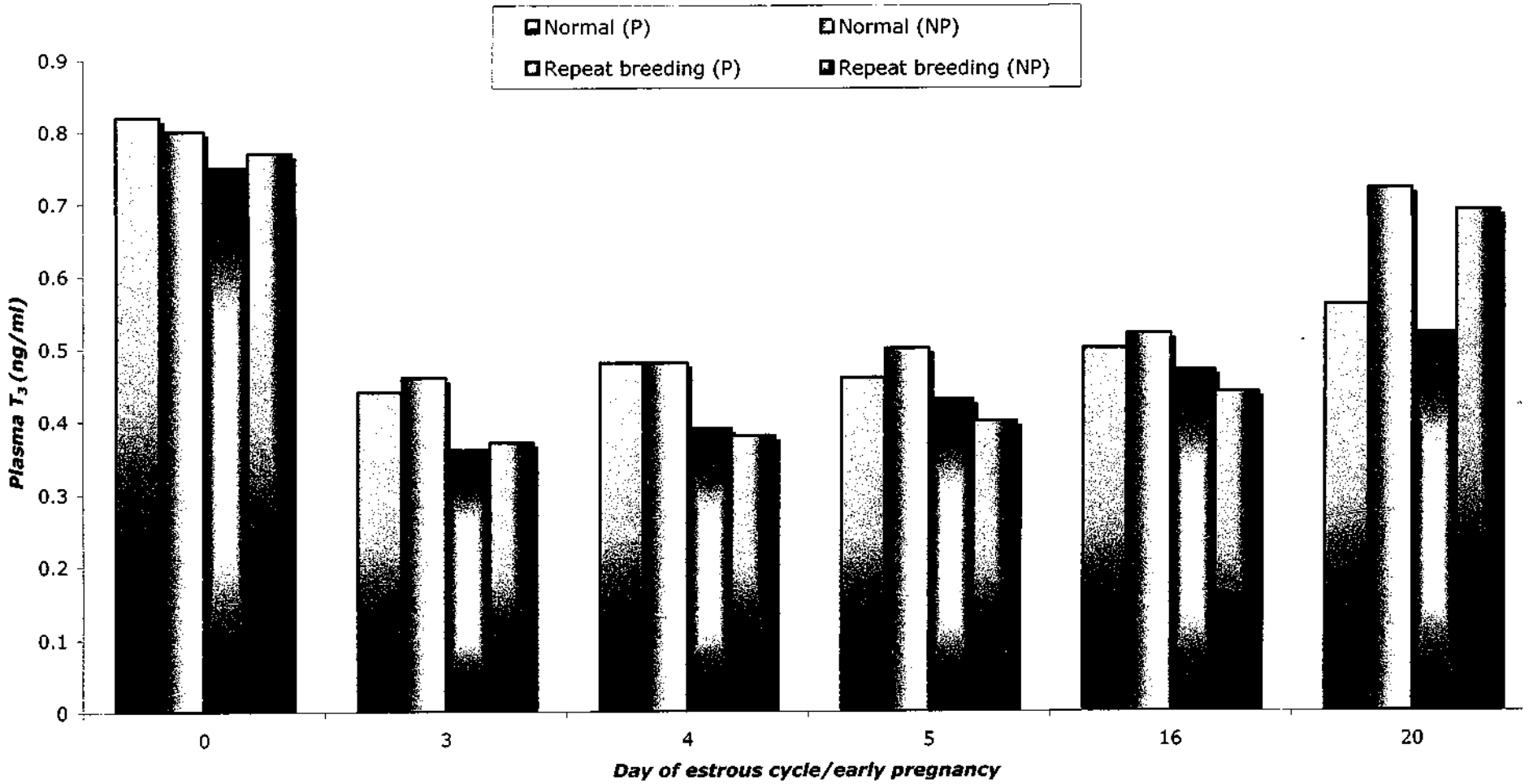


Table-5 Plasma triiodothyronine concentrations (ng/ml) in pregnant and non-pregnant buffaloes belonging to normal cycling and repeat breeding groups (Mean± S.E.)

DAY OF ESTROUS CYCLE/ EARLY PREGNANCY	NORMAL BUFFALOES		REPEAT BREEDING BUFFALOES	
	Pregnant	Non-pregnant	Pregnant	Non-pregnant
0	0.82 ± 0.07[a]	0.80 ± 0.09[a]	0.75 ± 0.07[a]	0.77 ± 0.08[a]
3	0.44 ± 0.06[b]	0.46 ± 0.07[b]	0.36 ± 0.04[b]	0.37 ± 0.06[b]
4	0.48 ± 0.08[b]	0.48 ± 0.05[b]	0.39 ± 0.06[b]	0.38 ± 0.05[b]
5	0.46 ± 0.06[b]	0.50 ± 0.07[b]	0.43 ± 0.08[b]	0.40 ± 0.07[b]
16	0.50 ± 0.09[b]	0.52 ± 0.06[b]	0.47 ± 0.07[b]	0.44 ± 0.06[b]
20	0.56 ± 0.11[b]	0.72 ± 0.09[a]	0.52 ± 0.06[b]	0.69 ± 0.11[a]

-values with different superscripts differ significantly ($P<0.01$) as compared to day-0, within the column

Figure-5 ***Mean plasma triiodothyronine (T_3) concentrations (ng/ml) in pregnant (P) and non-pregnant (NP) buffaloes belonging to normal cycling and repeat breeding groups***



groups. In all the treatment groups, the plasma T_3 concentrations were similar to control repeat breeding buffaloes. The concentrations in both the groups were found to be significantly lower ($p<0.05$) on day-3 onwards as compared to day-0 of the estrous cycle.

In repeat breeding buffaloes (control as well as treated), the plasma T_3 concentration followed the same pattern as that in normal cycling animals during the study period. The T_3 levels were high during estrus in both pregnant and non-pregnant animals and the levels did not change much during other phases of the cycle. However the values were slightly lower in repeat breeding animals as compared to normal cycling buffaloes (Table-5). An interesting finding was that in non-pregnant buffaloes the T_3 concentrations were higher on day-20 in control normal cycling group-CN (0.72 ± 0.09 ng/ml) as well as repeat breeding buffaloes (0.69 ± 0.11 ng/ml). No such rise was seen in case of pregnant buffaloes belonging to either group; i.e. normal cycling pregnant (0.56 ± 0.11 ng/ml) and repeat breeding pregnant (0.52 ± 0.06 ng/ml) buffaloes.

4.2.2.2 Thyroxine (T_4)

The mean concentrations of circulating plasma thyroxine (T_4) in control (CN and CR) and treatment (T_i, T_m and T_o) groups are

depicted in Table-6 and Fig-6 and in pregnant and non-pregnant buffaloes are presented in Table-7 and Fig-7.

Overall range of mean plasma T_4 concentrations in control normal (CN) and control repeat breeding (CR) groups varied from 37.72 ± 4.64 to 61.52 ± 6.63 ng/ml and 36.13 ± 3.87 to 52.43 ± 4.85 ng/ml, respectively. As in case of plasma T_3, the mean plasma T_4 concentrations were also found to be higher on day-0 in CN group (52.43 ± 4.85 ng/ml) and CR group (49.75 ± 4.28 ng/ml). No significant difference was found between the plasma T_4 concentrations in the two groups. Higher levels of T_4 have been reported during estrus (Khurana and Madan 1985). These plasma T_4 concentrations were relatively lower on day-3 of the cycle and thereafter these concentrations did not showed much change (Table-6). The plasma T_4 concentrations did not show much change during the rest of the cycle in both CN and CR groups of buffaloes.

The mean plasma thyroxine varied from 36.34 ± 3.12 to 54.15 ± 4.48 ng/ml in T_i, 37.88 ± 3.85 to 53.28 ± 5.12 ng/ml in T_m and 37.54 ± 3.74 to 50.67 ± 4.62 ng/ml in T_o groups. The mean plasma T_4 concentrations followed the same pattern as that by the control groups i.e. higher concentrations on day of estrus and not much change during rest of the cycle (Table-6 and Fig-6).

Irrespective of progesterone supplementation, when data were analyzed on the basis of pregnancy status, similar trend was

Table-6 Plasma thyroxine concentrations (ng/ml) in control (CN and CR) and treatment (T_i, T_m and T_o) groups of buffaloes (Mean ± S.E.)

DAY OF ESTROUS CYCLE/ EARLY PREGNANCY	CONTROL GROUPS		TREATMENT GROUPS		
	CN	CR	T_i group*	T_m group*	T_o group*
0	52.43 ± 4.85[a]	49.75 ± 4.28[a]	54.15 ± 4.48[a]	53.28 ± 5.12[a]	50.67 ± 4.62[a]
3	37.72 ± 4.64[b]	36.13 ± 3.87[b]	36.34 ± 3.12[b]	37.88 ± 3.85[b]	37.54 ± 3.74[b]
4	42.68 ± 4.15[b]	38.48 ± 3.57[b]	37.16 ± 3.82[b]	38.42 ± 3.62[b]	37.62 ± 3.92[b]
5	39.82 ± 3.95[b]	40.62 ± 4.12[b]	38.46 ± 4.22[b]	39.44 ± 4.12[b]	38.44 ± 4.14[b]
16	41.62 ± 4.26[b]	39.28 ± 4.26[b]	39.28 ± 4.26[b]	40.26 ± 4.34[b]	41.28 ± 3.88[b]
20	45.27 ± 5.28[b]	44.68 ± 5.64[a]	41.28 ± 4.47[b]	42.64 ± 4.14[b]	44.38 ± 4.62[a]

-values with different superscripts differ significantly ($P<0.01$) as compared to day-0, within the column

**** T_i, T_m, T_o.....Progesterone supplementation through ear implant, I/M and oral routes***

Figure-6 ***Mean plasma thyroxine (T_4) concentrations (ng/ml) in control (CN & CR) and treatment groups of buffaloes (Implant, I/M and Oral)***

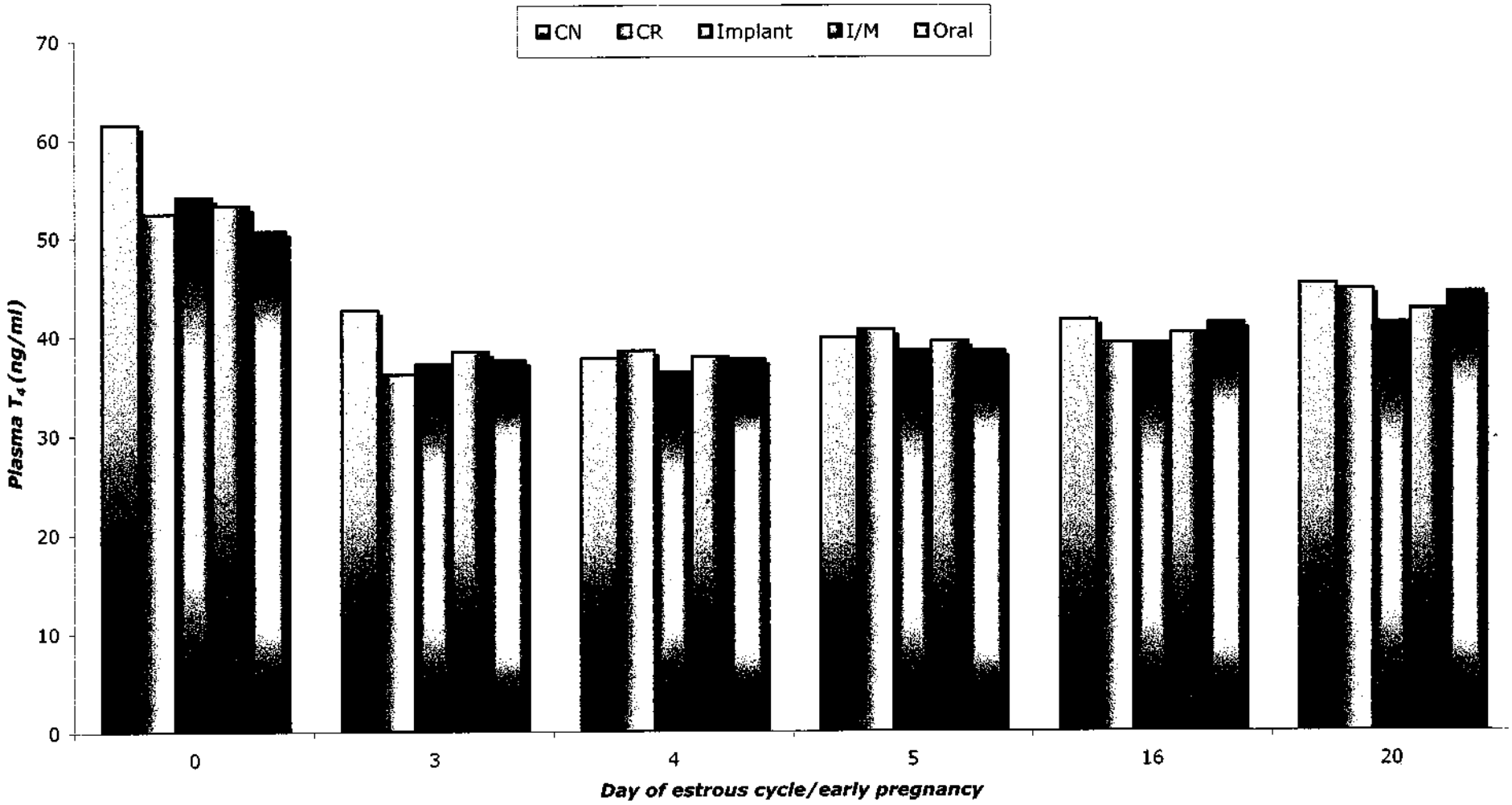
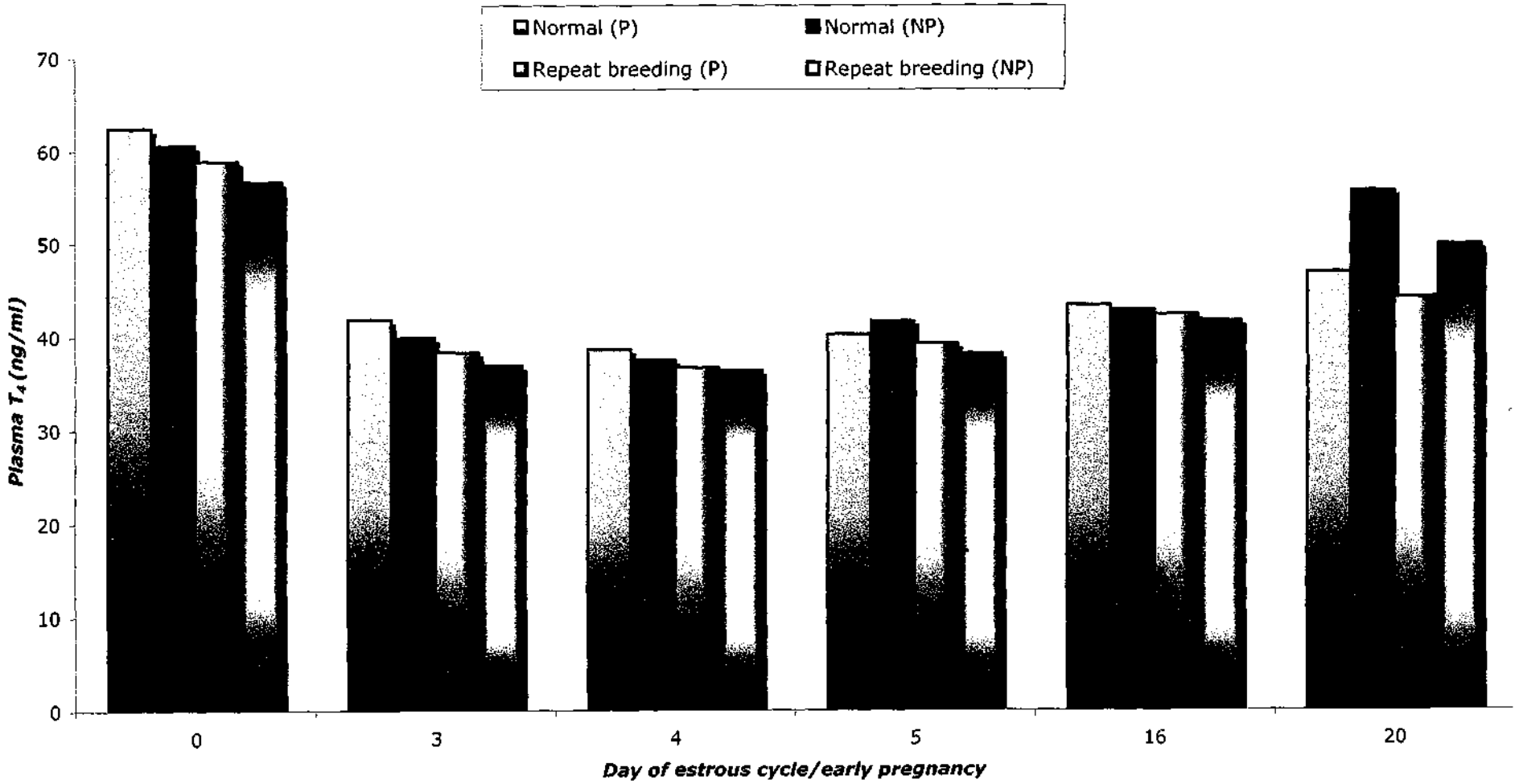


Table-7 Plasma thyroxine concentrations (ng/ml) in pregnant and non-pregnant buffaloes belonging to normal cycling and repeat breeding groups(Mean ± S.E.)

DAY OF ESTROUS CYCLE/ EARLY PREGNANCY	NORMAL BUFFALOES		REPEAT BREEDING BUFFALOES	
	Pregnant	Non-pregnant	Pregnant	Non-pregnant
0	62.42 ± 6.12[a]	60.56 ± 6.24[a]	58.84 ± 6.26[a]	56.64 ± 5.96[a]
3	38.61 ± 4.84[b]	37.46 ± 3.88[b]	36.64 ± 3.64[b]	36.34 ± 3.78[b]
4	41.84 ± 3.96[b]	39.84 ± 3.64[b]	38.26 ± 3.88[b]	36.88 ± 3.42[b]
5	40.22 ± 4.68[b]	41.62 ± 4.16[b]	39.18 ± 4.14[b]	38.14 ± 3.86[b]
16	43.34 ± 5.12[b]	42.82 ± 4.46[b]	42.34 ± 4.36[b]	41.64 ± 4.28[b]
20	46.88 ± 5.66[b]	55.62 ± 5.24[a]	44.14 ± 5.76[b]	49.78 ± 5.14[a]

-values with different superscripts differ significantly ($P<0.01$) as compared to day-0, within the column

Figure-7 ***Mean plasma thyroxine (T_4) concentrations (ng/ml) in pregnant (P) and non-pregnant (NP) buffaloes belonging to normal cycling and repeat breeding groups***



found in the control normal (CN) and repeat breeding (control as well as treatment groups) buffaloes. Overall range of mean plasma T_4 concentrations in normal cycling control group (pregnant and non-pregnant) varied from 37.46 ± 3.88 to 62.42 ± 6.12 ng/ml. Highest levels were found on day-0 in both pregnant (62.42 ± 6.12 ng/ml) and non-pregnant (60.56 ± 6.24 ng/ml) buffaloes. The levels were found to be low on day-3 in pregnant (38.61 ± 4.84 ng/ml) as well as non-pregnant (37.46 ± 3.88 ng/ml) buffaloes. Thereafter the concentrations of T_4 remained more or less constant except for a rise on day-20 in non-pregnant buffaloes (55.62 ± 5.24 ng/ml; Fig-7). In repeat breeding buffaloes (control as well as treated), the plasma T_4 concentration followed the same pattern as that in normal cycling animals during the study period. The levels of T_4 were high during estrus in both pregnant and non-pregnant animals and the levels did not change much during other phases of the cycle. Just like plasma T_3, the mean plasma T_4 concentrations also showed a little rise (though not significant statistically) on day-20 in non-pregnant buffaloes belonging to normal cycling (44.14 ± 5.76 ng/ml) and repeat breeding buffalo groups (49.78 ± 5.14 ng/ml).

In the present study, the thyroidal activity as reflected by plasma T_3 and T_4 concentrations followed an almost similar trend in

all the groups. The higher T_3 and T_4 concentrations on the day of estrus in control as well as treatment groups are in agreement with those reported by earlier workers in cattle and buffaloes (Sharma 2001 and Jindal *et al* 2004). Similar values were reported by Baysu and Dundary (1985) and Deshpande *et al* (1995). Increased thyroidal activity has also been reported by Boccabella and Alger (1964) and Jindal (1988). This rise in thyroidal activity during estrus may possibly be due to the estural stress on the animals (Khurana and Madan 1985). Higher estrogen levels during estrus may be responsible for an elevation in the thyroidal activity during estrus phase (Jindal *et al* 1988). Estradiol benzoate administration in rats has been reported to influence the thyroidal activity in a similar way (Boccabella and Alger 1964, Chen and Walfish 1978). Estrogen stimulates the thyroidal activity either by directly acting on it and increasing the iodine uptake leading to an elevation in the T_3 and T_4 levels in blood or through stimulation of TSH release from the pituitary gland (D' Angelo and Fisher 1969). In the present study a significant fall in the mean plasma T_3 and T_4 has been found on day-3 and the levels remained low up to day-16 (metestrus and diestrus phase or early pregnancy). A lower thyroidal activity immediately after ovulation has been reported by Vadodaria *et al* (1978) which substantiates the results of the present study. Lower levels of T_3 and T_4 during metestrus and diestrus have been

reported by Jindal *et al* (1988) and Kumar *et al* (199|) in buffalo heifers and lactating buffaloes. In the present investigation elevated mean plasma T_3 and T_4 concentrations were observed on day-20 in non-pregnant buffaloes belonging to any of the groups irrespective of the progesterone supplementation (Table-4, 6). This elevation may be due to the fact that some of the animals in both the groups failed to conceive/maintain pregnancy and returned to estrus around this day, and higher thyroidal activity around estrus is well established.

4.2.2.3 Insulin

The results of mean plasma insulin concentrations in control (CN & CR) and treatment (T_i, T_m and T_o) groups are presented in Table-8 and Fig-8 and those in pregnant and non-pregnant buffaloes are presented in Table-9 and Fig-9.

In the present study, the plasma insulin varied from 20.34 ± 2.26 to 25.22 ± 2.84 μU/ml in the control normal cycling buffaloes. The plasma insulin concentrations from 5-50 μU/ml in ruminants have been reported by Mc Atee and Trenkle (1971). Plasma insulin concentrations were found to be low on the day of estrus (day-0) in control normal group-CN (20.34 ± 2.26 μU/ml) and control repeat breeding group-CR (14.12 ± 2.16 μU/ml) buffaloes which were significantly low as compared to the study period. The

Table-8 Plasma insulin concentrations (μU/ml) in control (CN and CR) and treatment (T_i, T_m and T_o) groups of buffaloes (Mean ± S.E.)

DAY OF ESTROUS CYCLE/ EARLY PREGNANCY	CONTROL GROUPS		TREATMENT GROUPS		
	CN	CR	T_i group*	T_m group*	T_o group*
0	20.34 ± 2.26^{a*}	14.12 ± 2.16^{a}	15.24 ± 2.38^{a}	16.18 ± 1.85^{a}	15.28 ± 2.24^{a}
3	25.22 ± 2.84^{b*}	20.92 ± 1.84^{b}	20.88 ± 1.72^{a}	21.84 ± 2.26^{b}	21.78 ± 2.16^{b}
4	24.45 ± 1.82^{b*}	21.12 ± 1.78^{b}	19.88 ± 2.16^{b}	20.76 ± 1.88^{b}	19.82 ±1.26^{b}
5	24.26 ± 2.16^{b*}	19.94 ± 2.26^{b}	18.84 ± 2.24^{b}	19.74 ± 2.22^{b}	19.84 ± 1.20^{b}
16	23.12 ± 1.78^{b*}	20.28 ± 2.10^{a}	20.06 ± 1.86^{b}	20.82 ± 2.14^{b}	20.68 ± 1.18^{b}
20	23.20 ± 1.45^{b*}	18.42 ± 1.84^{a}	19.12 ± 2.12^{b}	19.82 ± 1.76^{b}	18.24 ± 2.32^{a}

-values with different superscripts differ significantly (P<0.05) as compared to day-0, within the column

**values in CN group differ significantly (P<0.05) as compared to values in all other groups*

**** T_i, T_m, T_o.....Progesterone supplementation through ear implant, I/M and oral routes***

Figure-8 ***Mean plasma insulin concentrations (μU/ml) in control (CN & CR) and treatment groups of buffaloes (Implant, I/M and Oral)***

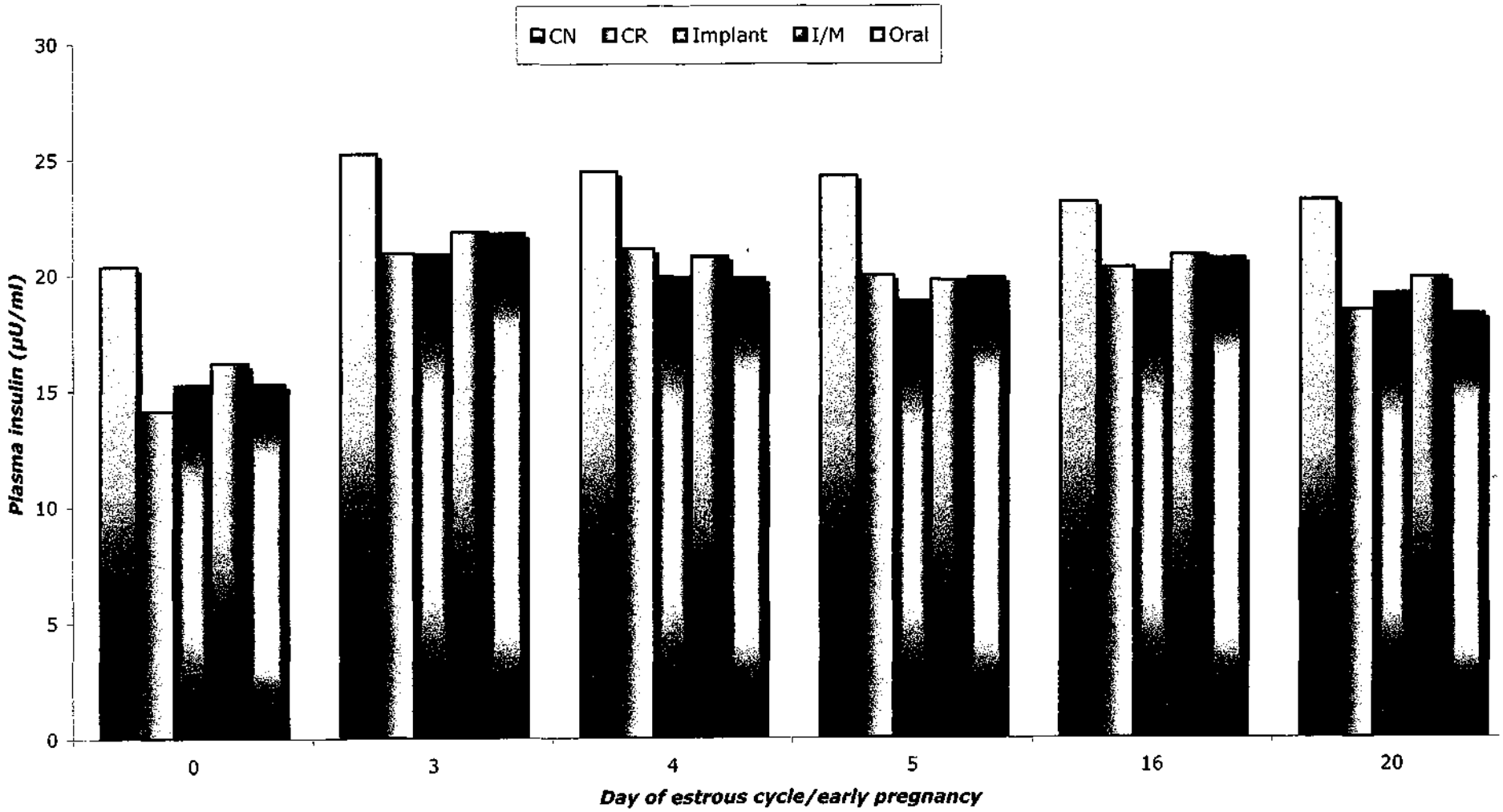
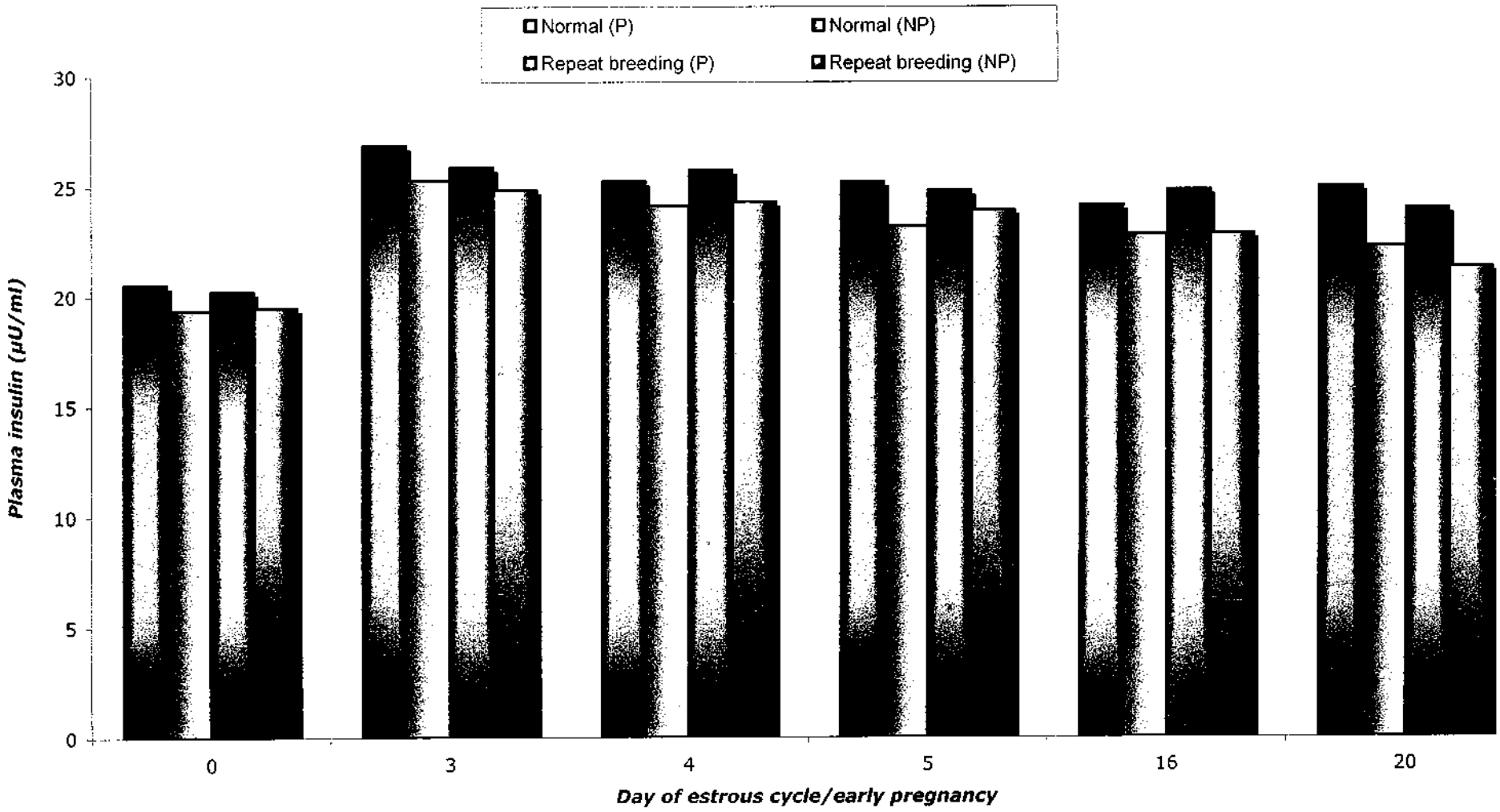


Table-9 Plasma insulin concentrations (μU/ml) in pregnant and non-pregnant buffaloes belonging to normal cycling and repeat breeding groups (Mean ± S.E.)

DAY OF ESTROUS CYCLE/ EARLY PREGNANCY	NORMAL BUFFALOES		REPEAT BREEDING BUFFALOES	
	Pregnant	Non-pregnant	Pregnant	Non-pregnant
0	20.54 ± 2.14[a]	19.36 ± 2.10[a]	20.22 ± 1.72[a]	19.46 ± 1.86[a]
3	26.86 ± 1.80[b]	25.28 ± 1.78[b]	25.86 ± 2.16[b]	24.82 ± 2.20[b]
4	25.22 ± 1.10[b]	24.10 ± 2.12[b]	25.74 ± 2.22[b]	24.28 ± 1.22[b]
5	25.18 ± 1.24[b]	23.16 ± 1.82[b]	24.78 ± 1.84[b]	23.88 ± 1.26[b]
16	24.12 ± 1.18[b]	22.78 ± 2.26[b]	24.82 ± 2.14[b]	22.82 ± 1.18[b]
20	24.96 ± 1.26[b]	22.24 ± 1.24[a]	23.94 ± 1.88[b]	21.28 ± 1.24[a]

-values with different superscripts differ significantly ($P<0.01$) as compared to day-0, within the column

Figure-9 ***Mean plasma insulin concentrations (μU/ml) in pregnant (P) and non-pregnant (NP) buffaloes belonging to normal cycling and repeat breeding groups***



concentrations plasma insulin on day-3 of the estrous cycle were 25.22 ± 2.84 μU/ml and 20.92 ± 1.84 μU/ml in groups CN and CR, respectively. Thereafter the levels did not change much up to day-20. Overall mean plasma insulin concentrations ranged from 15.24 ± 2.38 to 20.88 ± 1.72 μU/ml in T_i, 16.18 ± 1.85 to 21.84 ± 2.26 μU/ml in T_m and 15.28 ± 2.24 to 21.78 ± 2.16 μU/ml in T_o groups. The plasma insulin concentrations followed the same pattern in all the three treatment groups i.e. the concentrations were found to be low on the day of estrus (day-0), and thereafter no change during rest of the cycle in all the three treatment groups (Table-8).

When the data were analyzed on the basis of pregnancy status, irrespective of progesterone supplementation no difference was observed in the plasma insulin concentrations between pregnant and non-pregnant buffaloes of normal cycling group of buffaloes (Table-9). Overall range in normal cycling buffaloes (pregnant and non-pregnant) varied from 19.36 ± 2.10 to 26.86 ± 1.80 μU/ml and in repeat breeding buffaloes (pregnant and non-pregnant) from 19.46 ± 1.86 to 25.86 ± 2.16 μU/ml. Lowest plasma insulin concentrations in both pregnant and non-pregnant buffaloes belonging to normal cycling as well as repeat breeding groups were observed on the day of estrus. In general progesterone supplementation through any of the route did not bring about any change in the plasma insulin concentrations.

Plasma insulin levels vary depending upon glucose concentrations as it is the most important stimulus for insulin secretion (Pamela and Richard 1984). Insulin hormone is considered as regulator of blood glucose but biological effects of insulin do not confine to the transport of glucose only. Insulin stimulates anabolism of carbohydrates, fats, proteins and nucleic acids from the building blocks (Mc Donald 1980). Insulin coordinates the utilization of various fuels in various body tissues. Insulin and/or insulin dependent changes in the glucose availability may act on the ovary and change the ability of the ovarian cells to grow or respond to ganadotropins by modulating GnRH pulsatility and GnRH release (Butler and Smith 1989, Tanaka *et al* 2000). *In Vitro* studies revealed insulin and IGF as important mediators of follicular development, steroidogenesis, oocyte maturation and subsequent embryo development (Totey *et al* 1996, Daliri *et al* 1999). The increased IGF-1 concentrations during follicular phase following insulin administration may influence the development of follicles and CL function (Studer *et al* 1993 and Leewenberg *et al* 1996). Increased ovulation rates (Harrison and Randel 1986) and increased progesterone concentrations (Selvaraju *et al* 2002) were reported after exogenous administration of insulin indicating that insulin treatment may be used for improving fertility in repeat breeding cattle. So the estimation of insulin was carried out to investigate

any relation between the progesterone treatments on insulin concentrations. However, in these studies, no significant difference in plasma insulin concentrations between normal and repeat breeding buffaloes was found which indicates that in these animals the lower insulin concentrations might not be the etiological factor for repeat breeding. Even the progesterone supplementation through any of the routes did not bring about any change in the insulin levels. Further, in this study the plasma progesterone and insulin levels were found to be low as compared to luteal phase (Table-2 and 8). Lower levels of insulin on the day of estrus could be attributed to estural stress. The glucose sparing effect of stress hormones by inhibiting the insulin secretion, glycogenolysis and gluconeogenesis is well established (Mc Donald 1989). Also in this study, the blood glucose levels were found to be higher during estrus as compared to luteal phase.

4.3 Blood biochemical constituents

4.3.1 Blood glucose

The results of blood glucose concentrations in control (CN & CR) and treatment (T_i, T_m and T_o) groups are presented in Table-10 and Fig-10 and those in pregnant and non-pregnant buffaloes are presented in Table-11 and Fig-11.

Table-10 Blood glucose concentrations (mg/dl) in control (CN and CR) and treatment (T_i, T_m and T_o) groups of buffaloes (Mean ± S.E.)

DAY OF ESTROUS CYCLE/ EARLY PREGNANCY	CONTROL GROUPS		TREATMENT GROUPS		
	CN	CR	T_i group*	T_m group	T_o group*
0	58.76 ± 3.41[a]	49.26 ± 3.12[a]	48.56 ± 3.26[a]	50.46 ± 3.10[a]	49.14 ± 3.14[a]
3	47.24 ± 2.42[b]	41.34 ± 2.68[b]	40.46 ± 2.64[b]	41.24 ± 2.42[b]	40.26 ± 2.88[b]
4	47.78 ± 2.75[b]	41.48 ± 2.46[b]	40.64 ± 2.82[b]	40.32 ± 2.36[b]	40.42 ± 2.64[b]
5	48.12 ± 2.24[b]	42.14 ± 2.54[b]	40.88 ± 2.42[b]	40.84 ± 2.48[b]	41.64 ± 2.46[b]
6	47.88 ± 2.68[b]	42.34 ± 2.84[b]	41.58 ± 2.68[b]	41.26 ± 2.54[b]	41.48 ± 2.34[b]
9	48.26 ± 2.24[b]	41.68 ± 2.72[b]	40.52 ± 2.34[b]	41.82 ± 2.42[b]	42.24 ± 2.24[b]
10	48.78 ± 2.62[b]	42.32 ± 2.84[b]	41.48 ± 2.56[b]	42.16 ± 2.46[b]	42.34 ± 2.12[b]
12	49.24 ± 2.54[b]	43.46 ± 2.26[b]	41.42 ± 2.48[b]	42.64 ± 2.62[b]	42.26 ± 2.06[b]
16	50.38 ± 2.64[b]	44.56 ± 2.32[b]	42.34 ± 2.54[b]	43.10 ± 2.86[b]	43.42 ± 2.42[b]
20	51.28 ± 2.36[b]	46.58 ± 2.84[a]	42.48 ± 2.26[b]	43.48 ± 3.14[b]	44.16 ± 2.68[a]

-values with different superscripts differ significantly (P<0.01) as compared to day-0, within the column

**** T_i, T_m, T_o.....Progesterone supplementation through ear implant, I/M and oral routes***

Figure-10 ***Mean blood glucose concentrations (mg/dl) in control (CN & CR) and treatment groups of buffaloes (Implant, I/M and Oral)***

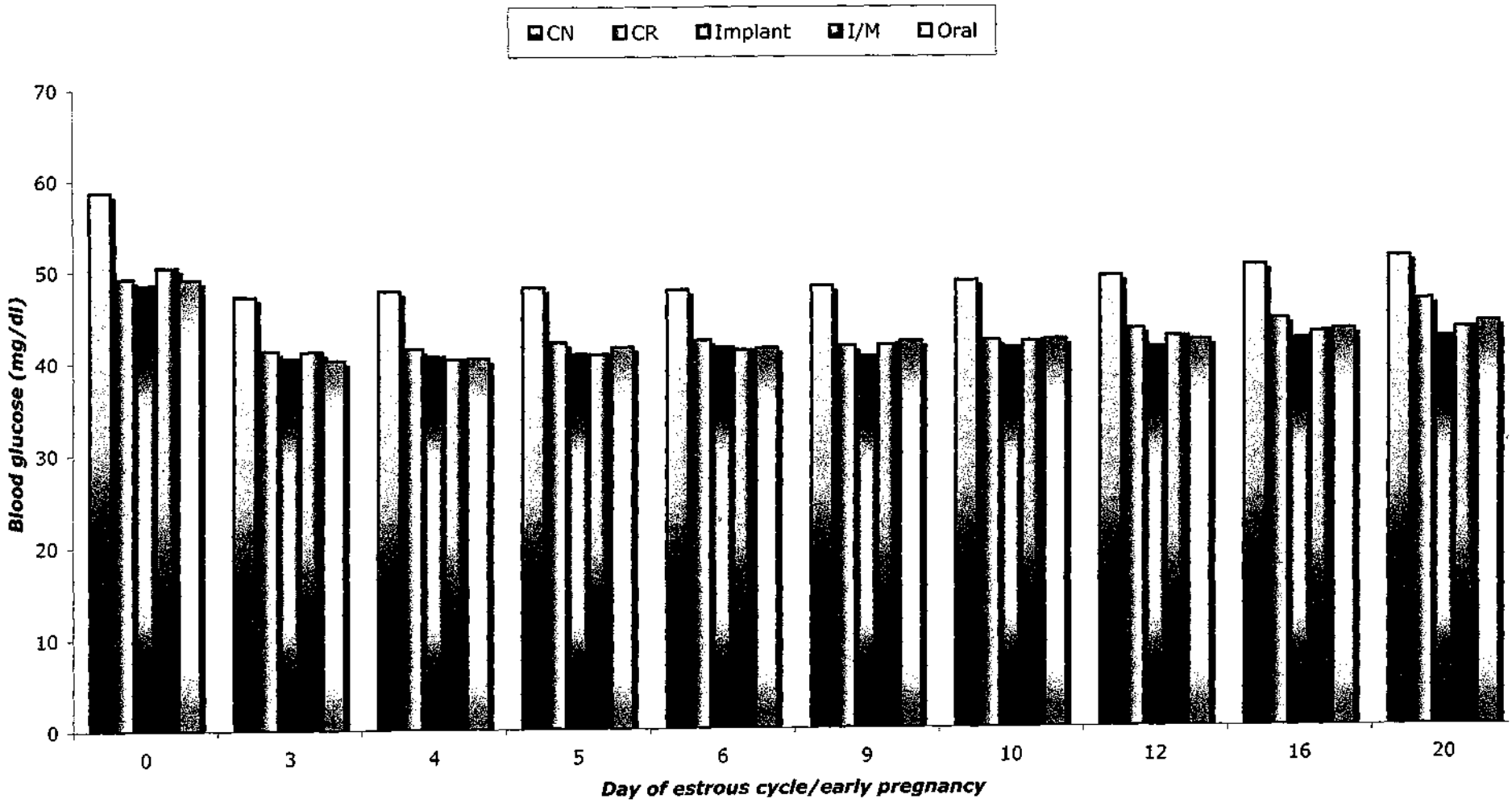
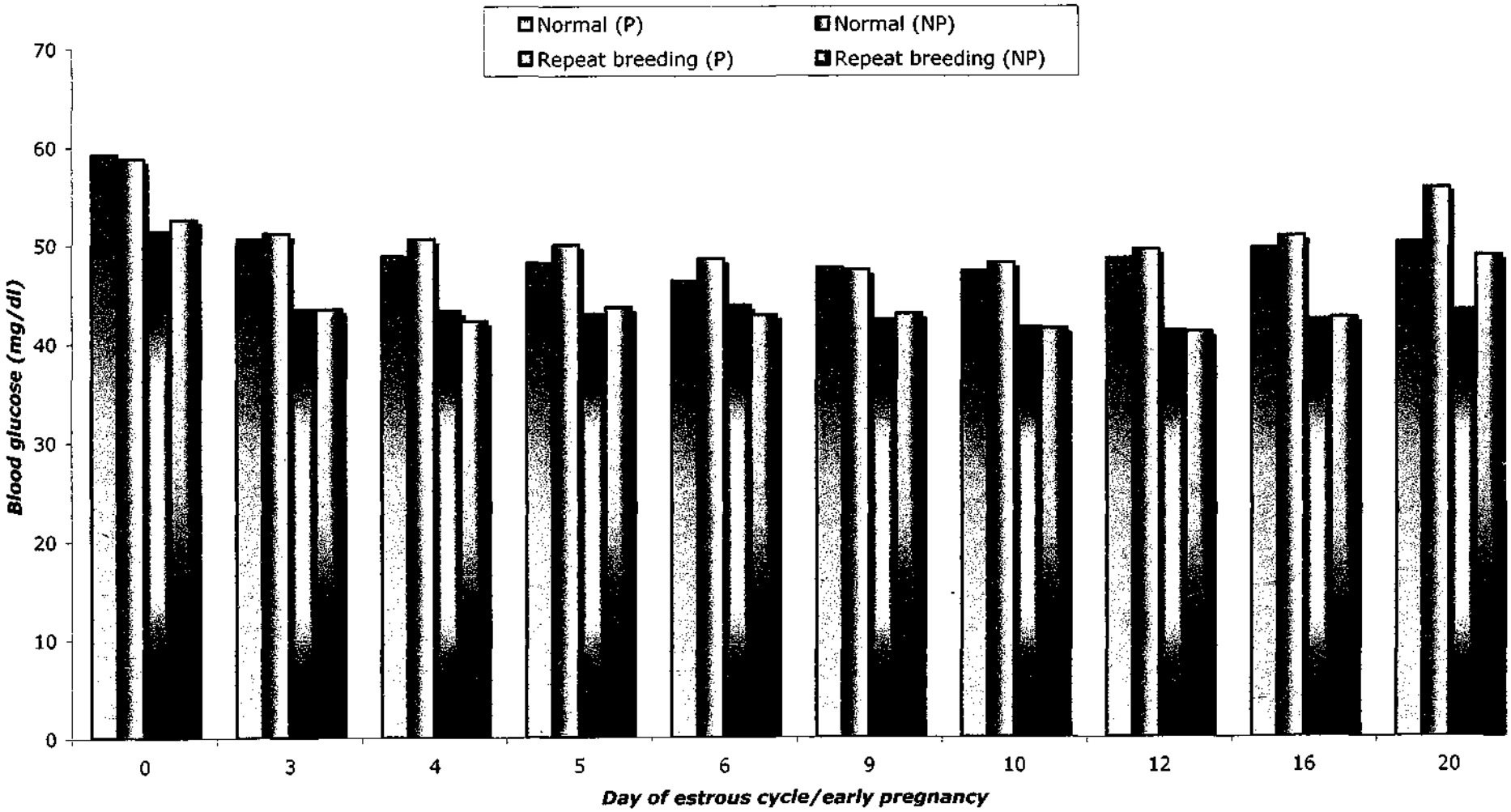


Table-11 Blood glucose concentrations (mg/dl) in pregnant and non-pregnant buffaloes belonging to normal cycling and repeat breeding groups (Mean ± S.E.)

DAY OF ESTROUS CYCLE/ EARLY PREGNANCY	NORMAL BUFFALOES		REPEAT BREEDING BUFFALOES	
	Pregnant	Non-pregnant	Pregnant	Non-pregnant
0	59.28 ± 3.36[a]	58.82 ± 3.46[a]	51.46 ± 3.48[a]	52.64 ± 3.78[a]
3	50.64 ± 2.78[b]	51.16 ± 2.64[b]	43.46 ± 2.26[b]	43.48 ± 3.14[b]
4	48.82 ± 2.64[b]	50.62 ± 2.84[b]	43.32 ± 2.54[b]	42.26 ± 2.84[b]
5	46.12 ± 2.48[b]	49.94 ± 3.12[b]	42.94 ± 2.52[b]	43.62 ± 2.26[b]
6	46.28 ± 2.26[b]	48.58 ± 2.46[b]	43.86 ± 2.46[b]	42.84 ± 2.52[b]
9	47.62 ± 2.68[b]	47.42 ± 2.24[b]	42.32 ± 2.64[b]	42.94 ± 2.68[b]
10	47.26 ± 2.68[b]	48.14 ± 2.36[b]	41.62 ± 2.86[b]	41.48 ± 2.12[b]
12	48.58 ± 2.54[b]	49.46 ± 2.26[b]	41.26 ± 2.52[b]	41.14 ± 2.58[b]
16	49.64 ± 2.44[b]	50.84 ± 2.96[b]	42.34 ± 2.34[b]	42.56 ± 2.86[b]
20	50.16 ± 2.96[b]	55.74 ± 3.66[a]	43.28 ± 3.16[b]	48.84 ± 3.46[a]

-values with different superscripts differ significantly (P<0.01) as compared to day-0, within the column

Figure-11 ***Mean blood glucose concentrations (mg/dl) in pregnant (P) and non-pregnant (NP) buffaloes belonging to normal cycling and repeat breeding groups***



The mean concentrations of blood glucose on day-0 (day of onset of estrus) were found to be significantly higher ($P<0.05$) as compared to other days of the estrous cycle in both the CN (58.76 ± 3.41 mg/dl) and CR (49.26 ± 3.12 mg/dl) groups. These concentrations were higher in case of normal cycling buffalo group-CN as compared to group-CR (Table-10). In general, the blood glucose concentrations were lower in repeat breeding buffaloes as compared to normal cycling buffaloes (Fayez *et al* 1992, Jani *et al* 1995 and Ramakrishna 1996). In control normal (CN) group, mean concentrations of blood glucose from day-3 through day-20 varied from 47.24 ± 2.42 to 51.28 ± 2.36 mg/dl and in control repeat breeding group (CR) from 41.34 ± 2.68 to 46.58 ± 2.84 mg/dl (Table-10). The mean concentrations of blood glucose in control repeat breeding (CR) buffaloes (49.26 ± 3.12 mg/dl) was significantly lower ($P<0.01$) as compared to control normal (CN) buffaloes (58.76 ± 3.41 mg/dl) on day of estrus (day-0).

The overall range of mean blood glucose concentrations in the treatment groups varied from 40.46 ± 2.64 to 48.56 ± 3.26 mg/dl in T_i, and 40.32 ± 2.36 to 50.46 ± 3.10 mg/dl in T_m and 40.26 ± 2.88 to 49.14 ± 3.14 mg/dl in T_o groups, respectively. Blood glucose concentrations were similar to those observed in control repeat breeding buffaloes, but slightly lower than the normal cycling control buffaloes (group-CN). All the three treatment groups

followed a similar trend as shown by control group of buffaloes with higher values on day-0 and not much change thereafter (Table-10).

In pregnant and non-pregnant buffaloes, belonging to normal cycling group as well as repeat breeding buffalo groups (irrespective of progesterone supplementation), the blood glucose concentrations from day-3 through day-16 remained more or less constant in all the animals without any significant difference between the two groups (normal cycling buffaloes and repeat breeding buffaloes or the pregnant or non-pregnant buffaloes) within each group. The blood glucose concentrations were higher ($P<0.05$) on day-20 in non-pregnant buffaloes belonging to normal cycling group (55.74 ± 3.66 mg/dl) and repeat breeding group (48.84 ± 3.46 mg/dl), however no such rise was observed in pregnant buffaloes.

Optimum levels of glucose in the body of animal are very important for maintaining the reproductive efficiency and variations in the glucose levels seems to be linked with estrus cyclicity, fertility and functioning of reproductive organs (Mc Clure 1965, Herrick 1977). In the present study, the mean blood glucose concentrations were found to be higher on day-0 i.e. the day of onset of estrus that might be due to estural stress. Increased cortisol during stress has been known to cause gluconeogenesis and thus an increase in the blood glucose levels. Even the historical data indicates higher blood glucose levels during estrus (Hodgson 1932). Blood glucose levels

can directly or indirectly affect the neuro-endocrine functioning of the reproductive axis at hypothalamic, hypophyseal or gonadal levels, thus altering the process of reproduction (Booth 1990). Further, hypoglycemia at estrus and shortly after service can cause lowering of the glucose of genital mucosa causing lack of energy for the spermatozoa to fertilize ova (Mc Clure 1968). Glucose availability influences both tonic and surge modes of LH secretion, presumably through its effects on GnRH. Glucose thus appears to be involved through a central sensor in the lower brain stem that could be an important glucosensor in the release of LH and thus presumably reflecting its role in modulating GnRH release (Murahashi *et al* 1996).

4.3.2 Total plasma cholesterol

The results of total plasma cholesterol concentrations in control (CN & CR) and treatment (T_i, T_m and T_o) groups are presented in Table-12 and Fig-12 and those in pregnant and non-pregnant buffaloes are presented in Table-13 and Fig-13.

In the present study, the total plasma cholesterol concentrations ranged from 131.62 ± 9.52 to 170.48 ± 12.58 mg/dl in normal buffaloes and from 88.58 ± 9.42 to 124.74 ± 10.98 mg/dl in repeat breeding buffaloes (Table-13). These values are in

Table-12 Plasma cholesterol concentrations (mg/dl) in control (CN and CR) and treatment (T_i, T_m and T_o) groups of buffaloes (Mean ± S.E.)

DAY OF ESTROUS CYCLE/ EARLY PREGNANCY	CONTROL GROUPS		TREATMENT GROUPS		
	CN	CR	T_i group*	T_m group*	T_o group*
0	168.12 ± 11.28^{a*}	118.54 ± 10.56^{a}	125.74 ± 10.36^{a}	119.62 ± 10.54^{a}	122.48 ± 10.58^{a}
3	134.38 ± 9.42^{b*}	94.42 ± 9.14^{b}	96.58 ± 9.58^{b}	92.84 ± 8.64^{b}	94.24 ± 8.56^{b}
4	138.24 ± 9.66^{b*}	92.78 ± 8.48^{b}	93.42 ± 8.26^{b}	89.68 ± 9.56^{b}	96.52 ± 8.26^{b}
5	136.48 ± 9.24^{b*}	89.56 ± 8.52^{b}	94.24 ± 8.62^{b}	91.26 ± 8.74^{b}	93.84 ± 8.62^{b}
6	138.62 ± 8.94^{b*}	91.26 ± 8.48^{b}	90.48 ± 8.64^{b}	88.48 ± 8.56^{b}	91.74 ± 9.14^{b}
9	132.54 ± 8.32^{b*}	93.54 ± 8.64^{b}	89.42 ± 8.46^{b}	90.24 ± 8.64^{b}	88.84 ± 8.84^{b}
10	134.84 ± 8.86^{b*}	95.28 ± 9.62^{b}	92.74 ± 8.26^{b}	93.48 ± 8.42^{b}	89.28 ± 8.12^{b}
12	133.36 ± 9.26^{b*}	94.84 ± 8.26^{b}	89.48 ± 8.64^{b}	92.78 ± 8.56^{b}	91.76 ± 8.46^{b}
16	128.58 ± 8.56^{b*}	99.74 ± 9.16^{b}	90.62 ± 8.56^{b}	94.26 ± 8.84^{b}	92.64 ± 8.62^{b}
20	121.64 ± 10.88^{b*}	109.72 ± 10.48^{a}	92.24 ± 9.24^{b}	96.36 ± 9.48^{b}	100.86 ± 10.74^{b}

-values with different superscripts differ significantly (P<0.01) as compared to day-0, within the column

**values in CN group differ significantly (P<0.05) as compared to respective values in all other groups*

**** T_i, T_m, T_o.....Progesterone supplementation through ear implant, I/M and oral routes***

Figure-12 ***Mean plasma cholesterol concentrations (mg/dl) in control (CN & CR) and treatment groups of buffaloes (Implant, I/M and Oral)***

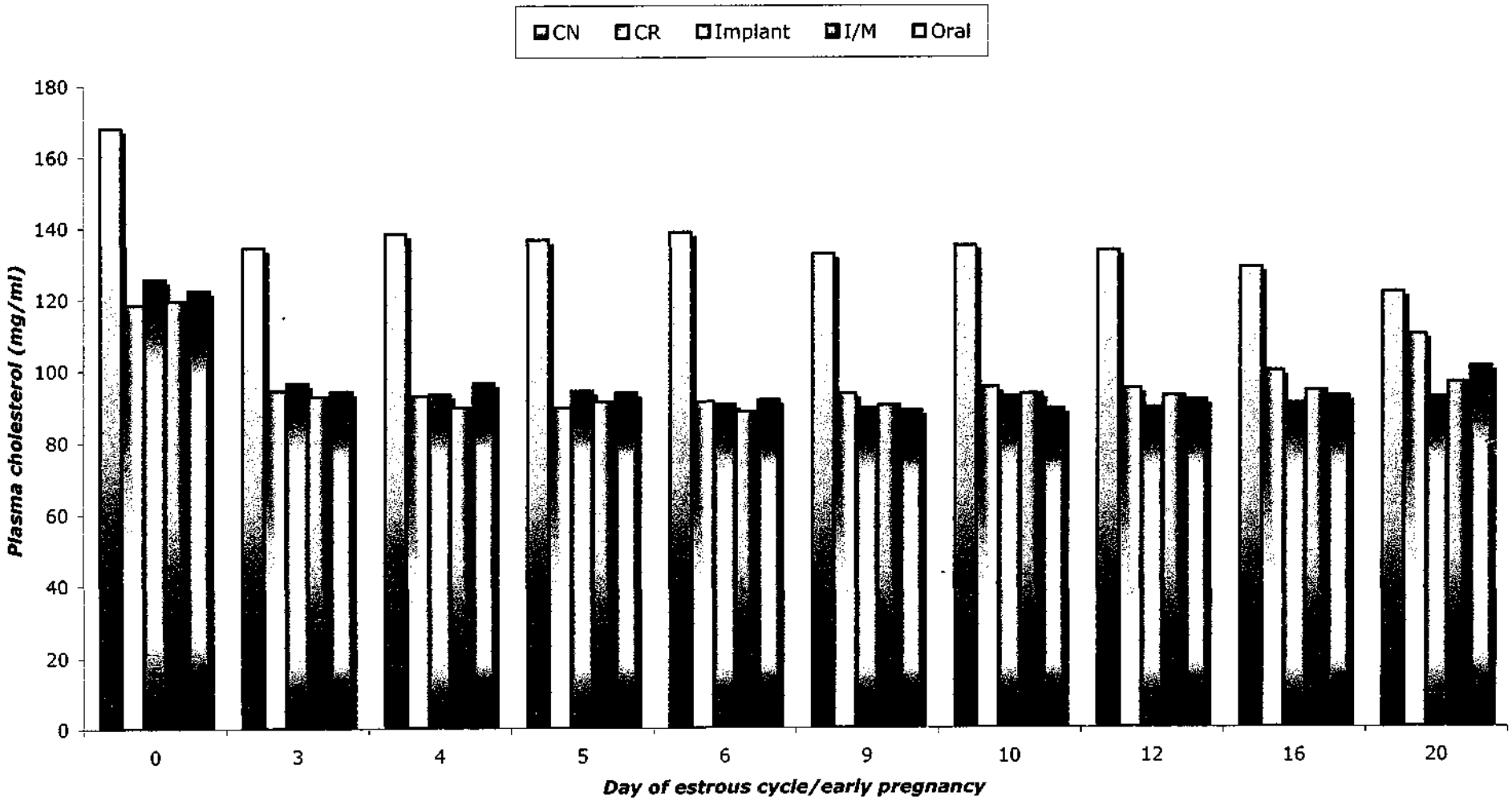


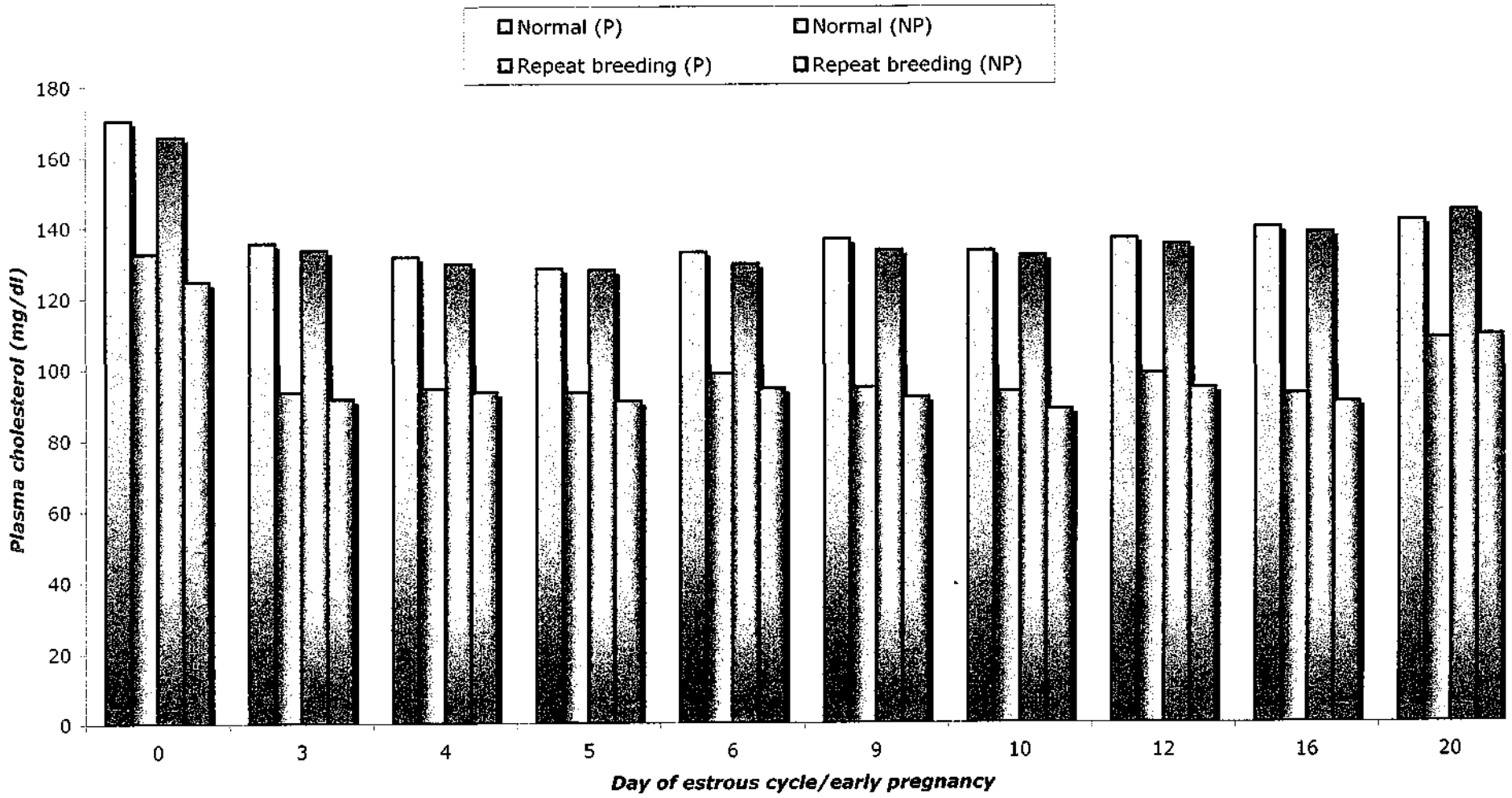
Table-13 Plasma cholesterol concentrations (mg/dl) in pregnant and non-pregnant buffaloes belonging to normal cycling and repeat breeding groups (Mean ± S.E.)

DAY OF ESTROUS CYCLE/ EARLY PREGNANCY	NORMAL BUFFALOES		REPEAT BREEDING BUFFALOES	
	Pregnant	Non-pregnant	Pregnant	Non-pregnant
0	170.48 ± 12.58^{a*}	132.58 ± 11.36^{a}	165.84 ± 13.46^{a*}	124.74 ± 10.98^{a}
3	135.54 ± 9.62^{b*}	93.42 ± 9.48^{b}	133.64 ± 11.34^{b*}	91.52 ± 9.14^{b}
4	131.62 ± 9.52^{b*}	94.48 ± 9.54^{b}	129.62 ± 9.58^{b*}	93.42 ± 8.68^{b}
5	128.32 ± 9.58^{b*}	93.36 ± 9.26^{b}	127.84 ± 9.64^{b*}	90.96 ± 8.46^{b}
6	132.84 ± 8.86^{b*}	98.62 ± 8.86^{b}	129.58 ± 8.24^{b*}	94.36 ± 8.84^{b}
9	136.64 ± 8.56^{b*}	94.74 ± 8.92^{b}	133.42 ± 8.66^{b*}	91.96 ± 9.64^{b}
10	133.24 ± 8.56^{b*}	93.52 ± 9.46^{b}	131.84 ± 9.54^{b*}	88.58 ± 9.42^{b}
12	136.68 ± 9.36^{b*}	98.56 ± 9.28^{b}	134.96 ± 8.58^{b*}	94.52 ± 8.56^{b}
16	139.82 ± 8.82^{b*}	92.82 ± 9.64^{b}	138.42 ± 9.32^{b*}	90.42 ± 8.76^{b}
20	141.64 ± 10.48^{b*}	108.48 ± 9.46^{b}	144.48 ± 12.52^{b*}	109.36 ± 11.58^{b}

-values with different superscripts differ significantly (P<0.05) as compared to day-0, within the column

values in non-pregnant buffaloes differ significantly (p<0.05) as compared to pregnant buffaloes in normal and repeat breeding buffalo groups

Figure-13 ***Mean plasma cholesterol concentrations (mg/dl) in pregnant (P) and non-pregnant (NP) buffaloes belonging to normal cycling and repeat breeding groups***



agreement with those observed by various workers in repeat breeding animals (Shanker *et al* 1983, Ramakrishna 1996). This range of values during estrous cycle/early pregnancy was higher than that reported in this lab by Kumar (1989) and Sharma (1990) and lower than that reported by Arosh *et al* (1998) and Singh and Pant (1998) for normal cycling animals. The mean concentrations of total plasma cholesterol in control repeat breeding (CR) buffaloes was 118.54 ± 10.56 mg/dl and in control normal (CN) buffaloes, it was 168.12 ± 11.28 mg/dl mg/dl on day of estrus (day-0). These concentrations from day-3 through day-20 varied from 121.64 ± 10.88 to 138.62 ± 8.94 mg/dl in control repeat breeding (CR) and 89.56 ± 8.52 to 109.72 ± 10.48 mg/dl in control normal (CN) group. The lower concentrations of total plasma cholesterol in repeat breeding control group as compared to normal cycling buffaloes is in accordance with previous workers (Shanker *et al* 1983, Ramakrishna 1996).

In both the control normal (CN) and control repeat breeding (CR) groups the total plasma cholesterol concentrations on day-0 were significantly higher ($P<0.05$) as compared to other days of the estrous cycle up to day-16. On day-20, in case of control repeat breeding (CR) group of buffaloes, the cholesterol levels showed a rise.

A similar trend was observed in the treatment groups i.e. the higher mean total plasma cholesterol concentrations on the day of estrus as compared to other phases of the cycle. In general, the mean total plasma cholesterol concentrations were similar to those found in control repeat breeding (CR) group but significantly lower ($p<0.05$) than the control normal (CN) group of buffaloes, indicating no apparent effect of progesterone supplementation on total plasma cholesterol.

Irrespective of the progesterone supplementation, the data when sorted out on the basis of pregnancy status of the animal, all the animals broadly followed the same pattern of changes in total plasma cholesterol concentrations (Table-13) i.e. higher concentrations on the day of estrus as compared to the luteal phase. It is evident from the Table-13 and Fig-13, that plasma cholesterol concentrations were higher in pregnant animals than non-pregnant animals in groups CN as well as in group CR, indicating a positive correlation between the plasma cholesterol levels (during early pregnancy) and maintenance of pregnancy. Higher cholesterol concentrations during early pregnancy might contribute to higher energy status for development and luteinization of follicles; the low cholesterol concentrations during this period can adversely affect the early embryo which might lead to repeat breeding (Sharma 2001).

The cholesterol acts as a precursor of steroid hormones. Its levels can affect the circulatory adequacy of the hormones responsible for normal reproductive efficiency. In these investigations, the plasma cholesterol concentrations were significantly higher around the time of insemination (estrous period). These findings are in agreement with those reported by other researchers (Purohit and Kohli 1977, Kumar 1986). Though not estimated in the present study, higher levels of estradiol at the time of estrus might be one of the major factors to favour an increased biosynthesis of endogenous cholesterol. The estrogen has an effect on carbohydrate metabolism that in turn causes increased production of cholesterol from acetate (Purohit and Kohli 1977). In the present investigation, the progesterone supplementation through any of the route did not bring about any significant change in the total plasma cholesterol concentrations.

4.4 Minerals

4.4.1 Macro-minerals:

The results regarding various macro-mineral concentrations in control (CN and CR) and treatment (T_i, T_m and T_o) group of buffaloes are presented in Tables 14 to 31 and Figures 14 to 22.

4.4.1.1 Calcium (Ca)

The results of plasma Calcium (Ca) concentrations in control (CN and CR) and treatment (T_i, T_m and T_o) groups are presented in Table-14 and Fig-14, and in pregnant and non-pregnant buffaloes are presented in Table-15.

The plasma Calcium (Ca) concentrations in control normal (CN) and control repeat breeding (CR) groups ranged from 9.22 ± 0.78 to 11.14 ± 0.56 mg/dl and 9.64 ± 0.65 to 10.88 ± 0.44mg/dl, respectively during the study period. These concentrations are in accordance with the concentrations reported by previous workers (Jani *et al* 1995). The plasma calcium concentrations were found to be slightly low on day-0 in control (CN and CR) and treatment (T_1, T_2 and T_3) groups and from day-3 onwards, these concentrations remained more or less constant throughout the study period. Lower plasma calcium concentrations at the time of estrus were also reported by Verma *et al* (1984), Jindal *et al* (1990) and Sharma *et al* (1999). Said *et al* (1966) suggested that the low levels of calcium due to increased estrogen as at estrus or by stillbesterol administration, are possibly responsible for the increased irritability of the animal and uterine contractibility. Estrogen has its effect in increasing the deposition of calcium in bones and hence it depresses the blood calcium levels in mammals (Dickson 1970). Irrespective of progesterone supplementation, when data were sorted out on the

Table-14 Plasma calcium concentrations (mg/dl) in control (CN and CR) and treatment (T_i, T_m and T_o) groups of buffaloes (Mean ± S.E.)

DAY OF ESTROUS CYCLE/ EARLY PREGNANCY	CONTROL GROUPS		TREATMENT GROUPS		
	CN	CR	T_i group*	T_m group*	T_o group*
0	10.41 ± 0.48	10.24 ± 0.46	10.18 ± 0.76	10.22 ± 0.64	10.20 ±0.74
3	11.14 ± 0.56	10.88 ± 0.44	10.64 ± 0.68	10.88 ±0.76	10.42 ±0.68
4	10.84± 0.64	10.64 ± 0.52	10.46 ± 0.82	10.68 ± 0.84	10.36 ±0.54
5	10.68 ± 0.52	10.26 ± 0.68	10.24 ± 0.68	10.56 ± 0.78	10.28 ± 0.62
6	10.22 ± 0.48	10.12 ± 0.58	9.88 ± 0.74	10.12 ± 0.56	10.30 ± 0.56
9	9.86 ± 0.52	9.86 ± 0.44	9.74 ± 0.88	10.08 ± 0.50	9.86 ± 0.62
10	9.68 ± 0.62	9.64 ± 0.56	9.66 ± 0.42	9.78 ± 0.68	9.76 ± 0.78
12	10.54 ± 0.58	9.76 ± 0.48	9.84 ± 0.84	9.86 ± 0.74	9.86 ± 0.84
16	9.36 ± 0.46	9.88 ± 0.86	9.46 ±0.76	9.88 ± 0.84	9.72 ± 0.78
20	9.22 ± 0.78	10.24 ± 0.64	9.56 ±0.88	9.72 ± 0.78	9.68 ± 0.56

**** T_i, T_m, T_o.....Progesterone supplementation through ear implant, I/M and oral routes***

Figure-14 ***Mean plasma calcium (Ca) concentrations (mg/dl) in control (CN & CR) and treatment groups of buffaloes (Implant, I/M and Oral)***

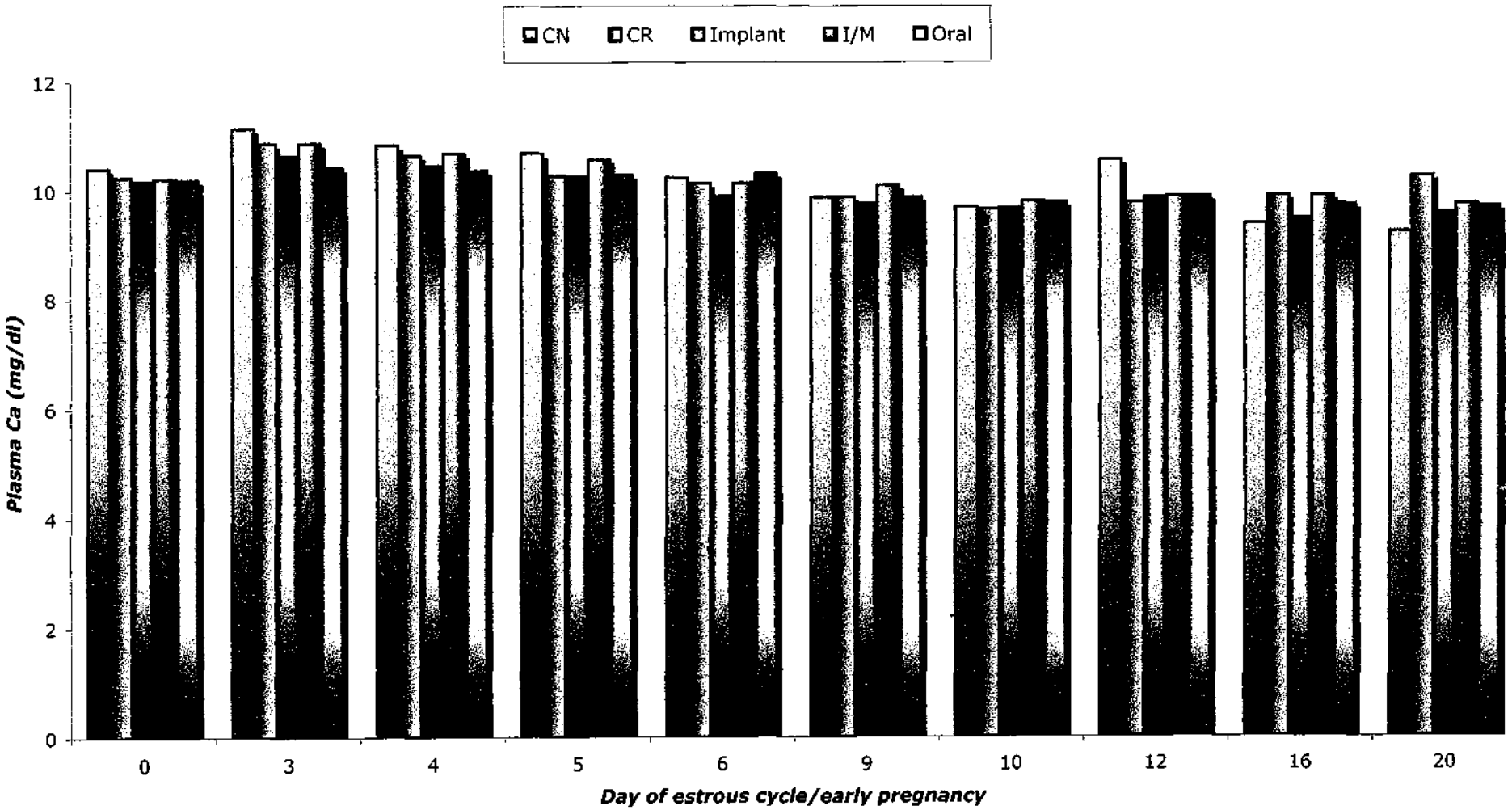


Table-15 Plasma calcium concentrations (mg/dl) in pregnant and non-pregnant buffaloes belonging to normal cycling and repeat breeding buffalo groups (Mean ± S.E.)

DAY OF ESTROUS CYCLE/ EARLY PREGNANCY	NORMAL BUFFALOES		REPEAT BREEDING BUFFALOES	
	Pregnant	Non-pregnant	Pregnant	Non-pregnant
0	10.18 ± 0.76	10.24 ± 0.46	10.41 ± 0.48	10.18 ± 0.76
3	10.64 ± 0.68	10.88 ± 0.44	11.14 ± 0.56	10.64 ± 0.68
4	10.46 ± 0.82	10.64 ± 0.52	10.84± 0.64	10.46 ± 0.82
5	10.24 ± 0.68	10.26 ± 0.68	10.68 ± 0.52	10.24 ± 0.68
6	9.88 ± 0.74	10.12 ± 0.58	10.22 ± 0.48	9.88 ± 0.74
9	9.86 ± 0.62	10.08 ± 0.50	9.86 ± 0.52	10.08 ± 0.50
10	9.76 ± 0.78	9.78 ± 0.68	9.64 ± 0.56	9.78 ± 0.68
12	9.86 ± 0.84	9.86 ± 0.74	9.76 ± 0.48	9.86 ± 0.74
16	9.72 ± 0.78	9.88 ± 0.84	9.88 ± 0.86	9.88 ± 0.84
20	9.68 ± 0.56	9.72 ± 0.78	10.24 ± 0.64	9.72 ± 0.78

basis of pregnancy status, the plasma calcium concentrations broadly followed the same pattern and no significant change was observed between the mean calcium concentrations among the control (CN and CR) and treatment (T_i, T_m and T_o) groups.

4.4.1.2 Phosphorous (P)

The results of plasma phosphorous (P) concentrations in control (CN and CR) and treatment (T_i, T_m and T_o) groups are presented in Table-16 and Fig-15, and in pregnant and non-pregnant buffaloes are presented in Table-17.

The plasma phosphorous (P) concentrations in control normal (CN) and control repeat breeding (CR) groups ranged from 4.76 ± 0.66 to 6.82 ± 0.84 mg/dl and 4.74 ± 0.66 to 6.46 ± 0.76 mg/dl, respectively during the study period. The P concentrations found in the present study are in agreement with the concentrations reported by Jani *et al* (1995) and Jindal *et al* (1990), but lower than those reported by Sharma *et al* (1999). The plasma phosphorous concentrations were found to be significantly high ($p<0.05$) on day of estrus (day-0) in control (CN and CR) and treatment (T_i, T_m and T_o) groups as compared to the respective values on other days of the estrous cycle. Higher plasma phosphorous concentrations during estrus phase were also reported by Jindal *et al* (1990) and Sharma *et al* (1999). However, these values remained more or less constant from day-3 through day-20. Low phosphorous level can

Table-16 Plasma phosphorous concentrations (mg/dl) in control (CN and CR) and treatment (T_i, T_m and T_o) groups of buffaloes (Mean ± S.E.)

DAY OF ESTROUS CYCLE/ EARLY PREGNANCY	CONTROL GROUPS		TREATMENT GROUPS		
	CN	CR	T_i group*	T_m group*	T_o group*
0	6.82 ± 0.84[a]	6.46 ± 0.76[a]	6.38 ± 1.12[a]	5.86 ± 0.48[a]	6.14 ± 0.56[a]
3	5.76 ± 0.42[b]	5.24 ± 0.56[b]	5.24 ± 0.96[b]	4.24 ± 0.52[b]	4.88 ± 0.42[b]
4	5.84 ± 0.51[b]	5.52 ± 0.48[b]	5.48 ± 0.84[b]	4.38 ± 0.48[b]	5.22 ±0.62[b]
5	5.48 ± 0.62[b]	5.46 ± 0.62[b]	5.64 ± 0.96[b]	4.86 ± 0.44[b]	5.14 ± 0.66[b]
6	5.14 ± 0.31[b]	4.84 ± 0.44[b]	5.58 ± 1.18[b]	4.46 ± 0.56[b]	4.84 ± 0.72[b]
9	5.78 ± 0.48[b]	4.82 ± 0.72[b]	4.82 ± 0.84[b]	4.74 ± 0.62[b]	4.78 ± 0.58[b]
10	4.84 ± 0.82[b]	4.74 ± 0.66[b]	4.48 ± 0.92[b]	4.68 ± 0.42[b]	4.68 ± 0.46[b]
12	4.76 ± 0.66[b]	4.82 ± 0.54[b]	4.94 ± 0.88[b]	4.84 ± 0.76[b]	4.76 ± 0.52[b]
16	4.84 ± 0.84[b]	4.84 ± 0.52[b]	4.88 ± 0.78[b]	4.92 ± 0.74[b]	4.84 ± 0.58[b]
20	5.12 ± 0.72[b]	5.62 ± 0.74[a]	5.24 ± 1.02[b]	5.12 ± 0.86[b]	5.56 ± 0.58[a]

-values with different superscripts differ significantly (P<0.01) as compared to day-0, within the column

**** T_i, T_m, T_o.....Progesterone supplementation through ear implant, I/M and oral routes***

Figure-15 ***Mean plasma phosphorous (P) concentrations (mg/dl) in control (CN & CR) and treatment groups of buffaloes (Implant, I/M and Oral)***

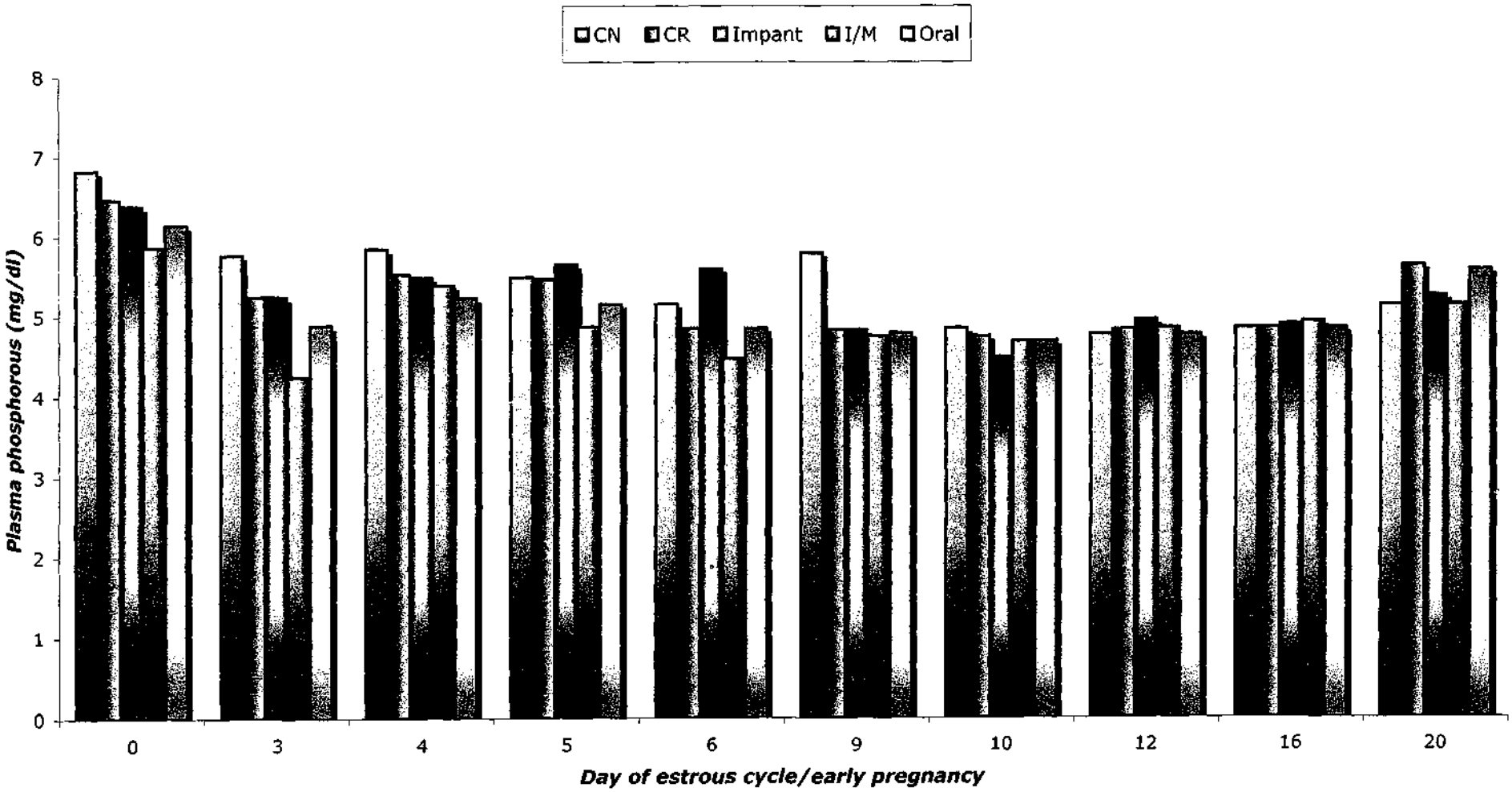


Table-17 Plasma phosphorous concentrations (mg/dl) in pregnant and non-pregnant buffaloes belonging to normal cycling and repeat breeding groups (Mean ± S.E.)

DAY OF ESTROUS CYCLE/ EARLY PREGNANCY	NORMAL BUFFALOES		REPEAT BREEDING BUFFALOES	
	Pregnant	Non-pregnant	Pregnant	Non-pregnant
0	6.38 ± 1.12[a]	6.14 ± 0.56[a]	6.22 ± 0.84[a]	6.46 ± 0.76[a]
3	5.24 ± 0.96[b]	4.88 ± 0.42[b]	5.76 ± 0.42[b]	5.24 ± 0.56[b]
4	5.48 ± 0.84[b]	5.22 ±0.62[b]	5.84 ± 0.51[b]	5.84 ± 0.51[b]
5	5.46 ± 0.62[b]	4.86 ± 0.44[b]	5.48 ± 0.62[b]	5.48 ± 0.62[b]
6	4.84 ± 0.44[b]	4.46 ± 0.56[b]	4.84 ± 0.44[b]	5.14 ± 0.31[b]
9	4.82 ± 0.72[b]	4.74 ± 0.62[b]	4.82 ± 0.72[b]	5.78 ± 0.48[b]
10	4.48 ± 0.92[b]	4.68 ± 0.42[b]	4.74 ± 0.66[b]	4.82 ± 0.54[b]
12	4.94 ± 0.88[b]	4.94 ± 0.88[b]	4.94 ± 0.88[b]	4.84 ± 0.52[b]
16	4.88 ± 0.78[b]	4.88 ± 0.78[b]	4.88 ± 0.78[b]	5.62 ± 0.74[a]
20	5.24 ± 1.02[b]	5.24 ± 1.02[b]	5.24 ± 1.02[b]	4.82 ± 0.54[b]

values with different superscripts differ significantly ($P<0.01$) as compared to day-0, within the column

lead to imbalance of calcium and phosphorous ratio resulting in lowered fertility rates (Mc Clure 1965 and Bansal *et al* 1978).

Irrespective of progesterone supplementation, when data were sorted out on the basis of pregnancy status, the plasma phosphorous concentrations broadly followed the same pattern. Significantly high ($p<0.05$) mean phosphorous concentrations were found in the control (CN and CR) and treatment (T_i, T_m and T_o) groups on the day of estrus as compared to other phases of the estrous cycle. Although not observed in the present study, higher serum phosphorous concentrations in normal cycling as compared to repeat breeding cattle were also reported by previous workers (Eltohamy *et al* 1989 and Jani *et al* 1995).

4.4.1.3 Chloride (Cl)

The results of blood chloride (Cl) concentrations in control (CN and CR) and treatment (T_i, T_m and T_o) groups are presented in Table-18 and Fig-16, and in pregnant and non-pregnant buffaloes are presented in Table-19.

The blood chloride (Cl) concentrations in control normal (CN) and control repeat breeding (CR) groups ranged from 78.84 ± 5.12 to 104.46 ± 6.24 mEq/L and 78.64 ± 6.12 to 96.26 ± 5.34 mEq/L, respectively during the study period. These concentrations are in agreement with the chloride concentrations reported by previous

Table-18 Plasma chloride concentrations (mEqlL) in control (CN and CR) and treatment (T_i, T_m and T_o) groups of buffaloes (Mean ± S.E.)

DAY OF ESTROUS CYCLE/ EARLY PREGNANCY	CONTROL GROUPS		TREATMENT GROUPS		
	CN	CR	T_i group*	T_m group*	T_o group*
0	104.46 ± 6.24[a]	96.26 ± 5.34[a]	98.62 ± 5.12[a]	96.34 ± 5.24[a]	99.36 ± 5.12[a]
3	87.64 ± 6.36[b]	81.42 ± 4.64[b]	82.46 ± 4.88[b]	81.26 ± 4.64[b]	83.34 ± 4.26[b]
4	84.26 ± 5.88[b]	80.64 ± 5.36[b]	81.92 ± 4.74[b]	80.64 ± 4.12[b]	81.64 ± 3.84[b]
5	80.84 ± 5.62[b]	78.92 ± 6.24[b]	79.48 ± 4.62[b]	80.42 ± 3.88[b]	82.46 ± 4.48[b]
6	82.46 ± 4.84[b]	78.64 ± 6.12[b]	80.34 ± 4.36[b]	81.48 ± 4.62[b]	83.38 ± 4.86[b]
9	85.24 ± 4.66[b]	77.46 ± 5.76[b]	78.62 ± 3.84[b]	78.34 ± 4.84[b]	81.92 ± 5.28[b]
10	79.36 ± 5.82[b]	80.36 ± 5.34[b]	77.36 ± 4.46[b]	77.46 ± 5.62[b]	80.46 ± 5.12[b]
12	78.84 ± 5.12[b]	81.62 ± 4.88[b]	78.96 ± 5.26[b]	78.68 ± 5.12[b]	79.62 ± 4.84[b]
16	81.42 ± 5.26[b]	79.48 ± 5.46[b]	76.64 ± 5.64[b]	76.24 ± 4.62[b]	77.46 ± 4.26[b]
20	86.82 ± 5.48[b]	88.92 ± 5.32[a]	81.24 ± 5.36[b]	84.44 ± 4.46[b]	89.84 ± 5.48[a]

-values with different superscripts differ significantly (P<0.01) as compared to day-0, within the column

**** T_i, T_m, T_o.....Progesterone supplementation through ear implant, I/M and oral routes***

Figure-16 ***Mean plasma chloride (Cl) concentrations (mEq/L) in control (CN & CR) and treatment groups of buffaloes (Implant, I/M and Oral)***

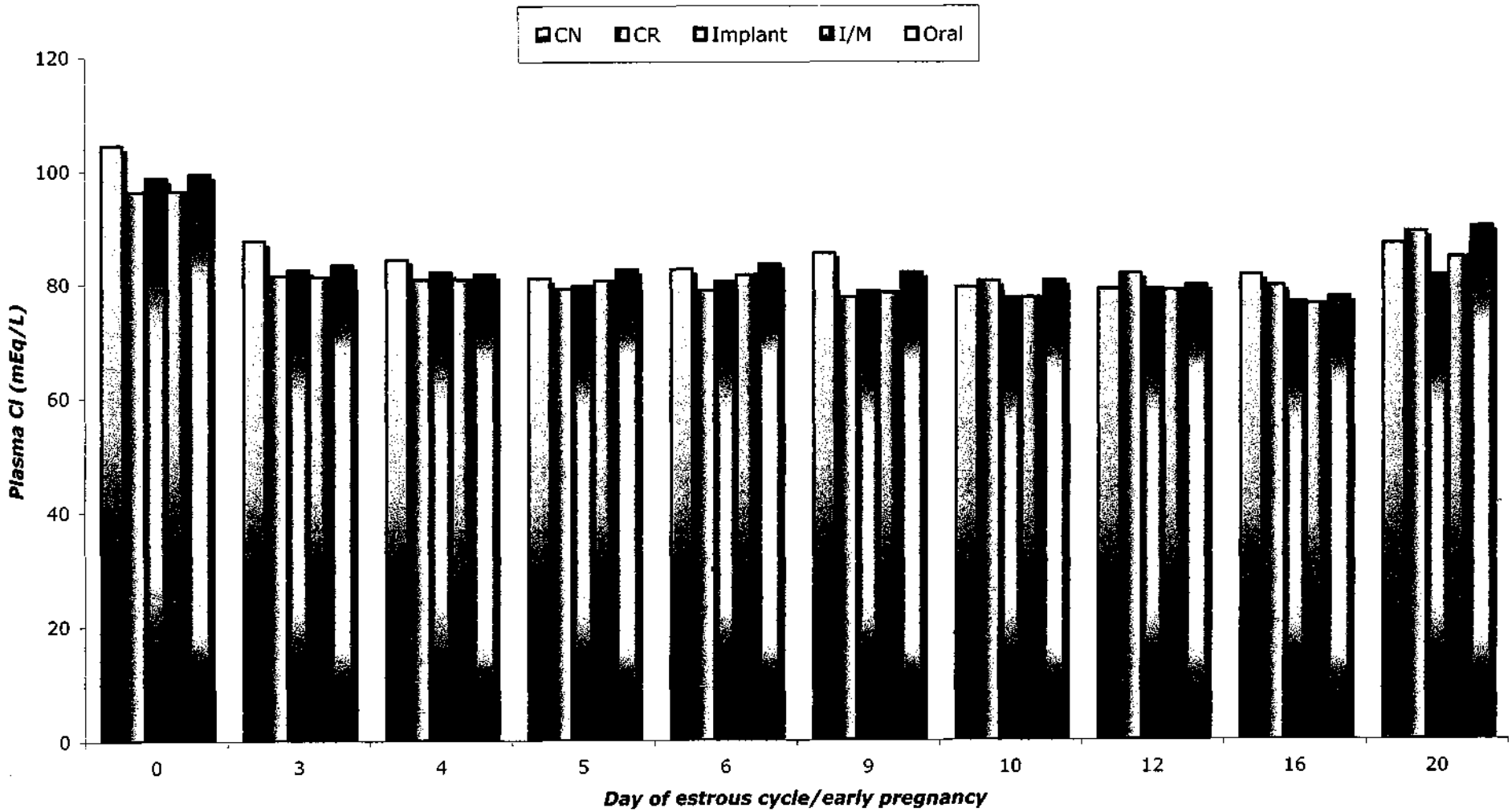


Table-19 Plasma chloride concentrations (mEq/L) in pregnant and non-pregnant buffaloes belonging to normal cycling and repeat breeding groups (Mean ± S.E.)

DAY OF ESTROUS CYCLE/ EARLY PREGNANCY	NORMAL BUFFALOES		REPEAT BREEDING BUFFALOES	
	Pregnant	Non-pregnant	Pregnant	Non-pregnant
0	98.62 ± 5.12[a]	96.26 ± 5.34[a]	104.46 ± 6.24[a]	99.36 ± 5.12[a]
3	82.46 ± 4.88[b]	81.42 ± 4.64[b]	87.64 ± 6.36[b]	83.34 ± 4.26[b]
4	81.92 ± 4.74[b]	80.64 ± 5.36[b]	84.26 ± 5.88[b]	81.64 ± 3.84[b]
5	80.42 ± 3.88[b]	78.92 ± 6.24[b]	80.84 ± 5.62[b]	82.46 ± 4.48[b]
6	81.48 ± 4.62[b]	80.34 ± 4.36[b]	78.64 ± 6.12[b]	80.34 ± 4.36[b]
9	78.34 ± 4.84[b]	78.62 ± 3.84[b]	77.46 ± 5.76[b]	78.62 ± 3.84[b]
10	77.46 ± 5.62[b]	80.46 ± 5.12[b]	80.36 ± 5.34[b]	77.36 ± 4.46[b]
12	78.68 ± 5.12[b]	79.62 ± 4.84[b]	78.96 ± 5.26[b]	81.62 ± 4.88[b]
16	76.24 ± 4.62[b]	77.46 ± 4.26[b]	76.64 ± 5.64[b]	79.48 ± 5.46[b]
20	84.44 ± 4.46[b]	89.84 ± 5.48[a]	81.24 ± 5.36[b]	88.92 ± 5.32[a]

-values with different superscripts differ significantly (P<0.01) as compared to day-0, within the column

workers (Eltohamy *et al* 1989 and Sharma *et al* 1999). The blood chloride concentrations were found to be significantly high ($p<0.05$) on day of estrus (day-0) in control (CN and CR) and treatment (T_i, T_m and T_o) groups as compared to the respective values on other days of the estrous cycle. Higher blood chloride concentrations during estrus phase were also reported by Agarwal *et al* (1985) and Jindal *et al* (1990). Estrogen administration induced a significant rise in blood chloride (Said *et al* 1966). However, Sharma *et al* (1999) reported no significant difference in the blood chloride concentrations during different phases of the estrous cycle. The blood chloride concentrations remained more or less constant from day-3 through day-16 of the cycle in all the groups. In control repeat breeding group (CR), the blood chloride concentrations did not differ significantly on day-0 and day-20 of the cycle, but these concentrations were found to be significantly high on day-0 as compared to day-20 in the control normal group (CN). Irrespective of progesterone supplementation, when data were sorted out on the basis of pregnancy status, the blood chloride concentrations broadly followed the same pattern and the blood chloride concentrations were found to be significantly high ($p<0.05$) on the day of estrus (day-0) as compared to other days of the estrous cycle in the control (CN and CR) as well as treatment (T_i, T_m and T_o) groups.

4.4.1.4 Sodium (Na)

The results of plasma sodium (Na) concentrations in control (CN and CR) and treatment (T_i, T_m and T_o) groups are presented in Table-20 and Fig-17, and in pregnant and non-pregnant buffaloes are presented in Table-21.

The plasma sodium (Na) concentrations in control normal (CN) and control repeat breeding (CR) groups ranged from 124.12 ± 4.84 to 131.54 ± 5.54 mEq/L and 121.36 ± 4.56 to 128.64 ± 6.14 mEq/L, respectively during the study period. These concentrations are in agreement with the concentrations reported by previous workers (Jindal *et al* 1990 and Sharma *et al* 1999). The plasma sodium concentrations did not differ significantly between the control and treatment groups (CN, CR, T_i, T_m and T_o). These concentrations remained more or less constant throughout the study period. Irrespective of progesterone supplementation, when data were sorted out on the basis of pregnancy status, again no significant difference was found in the plasma sodium concentrations in the pregnant and non-pregnant buffaloes belonging to normal cycling and repeat breeding buffalo groups.

4.4.1.5 Potassium (K)

The results of plasma potassium (K) concentrations in control (CN an0d CR) and treatment (T_i, T_m and T_o) groups are presented in Table-22 and Fig-18, and in pregnant and non-pregnant buffaloes

Table-20 Plasma sodium concentrations (mEq/l) in control (CN and CR) and treatment (T_i, T_m and T_o) groups of buffaloes (Mean ± S.E.)

DAY OF ESTROUS CYCLE/ EARLY PREGNANCY	CONTROL GROUPS		TREATMENT GROUPS		
	CN	CR	T_i group*	T_m group*	T_o group*
0	131.54 ± 5.54	123.26 ± 5.54	124.24 ± 4.54	126.36 ± 5.14	125.42 ± 4.46
3	128.26 ± 4.56	121.36 ± 4.56	125.26 ± 5.14	125.24 ± 4.88	124.36 ± 5.14
4	127.36 ± 5.24	122.52 ± 5.12	125.84 ± 4.26	124.38 ± 5.16	123.68 ± 4.88
5	126.24 ± 4.48	123.64 ± 4.84	124.38 ± 5.46	123.26 ± 4.66	122.38 ± 5.12
6	124.12 ± 4.84	124.56 ± 4.54	126.36 ± 4.32	122.62 ± 4.54	121.46 ± 5.24
9	126.32 ± 5.32	125.36 ± 4.26	127.48 ± 5.12	123.36 ± 4.68	123.64 ± 4.72
10	128.54 ± 4.24	126.48 ± 5.12	125.32 ± 4.84	124.64 ± 5.12	126.24 ± 5.12
12	126.62 ± 5.48	128.64 ± 6.14	126.78 ± 5.18	125.26 ± 5.46	125.46 ± 4.32
16	128.48 ± 4.84	125.54 ± 5.32	127.54 ± 4.82	126.78 ± 5.62	126.38 ± 5.14
20	130.24 ± 6.45	126.78 ± 5.26	128.36 ± 5.14	125.12 ± 5.16	127.54 ± 4.84

** T_i, T_m, T_o.....Progesterone supplementation through ear implant, I/M and oral routes*

Figure-17 ***Mean plasma sodium (Na) concentrations (mEq/L) in control (CN & CR) and treatment groups of buffaloes (Implant, I/M and Oral)***

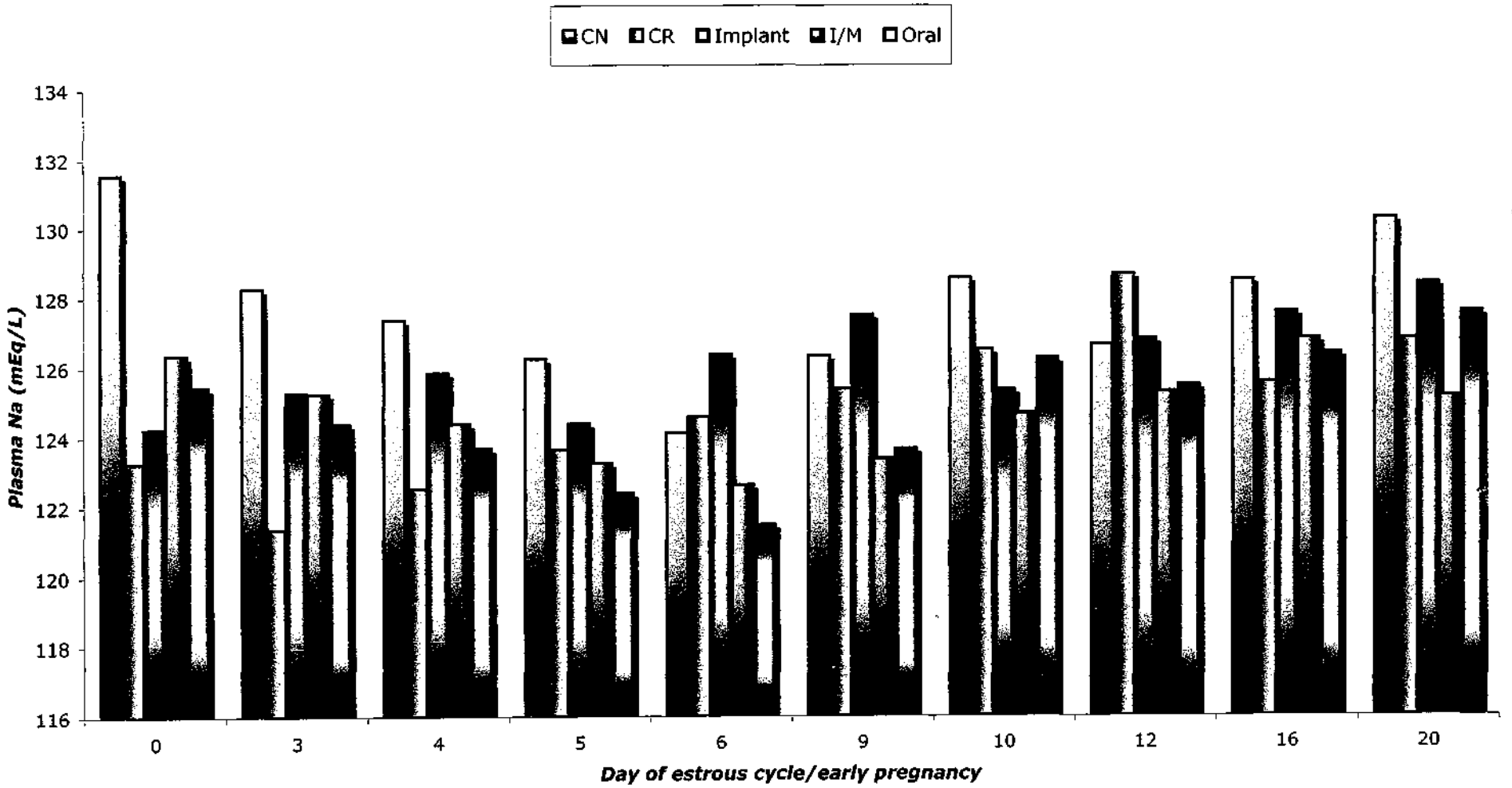


Table-21 Plasma sodium concentrations (mEq/L) in pregnant and non-pregnant buffaloes belonging to normal cycling and repeat breeding groups (Mean ± S.E.)

DAY OF ESTROUS CYCLE/ EARLY PREGNANCY	NORMAL BUFFALOES		REPEAT BREEDING BUFFALOES	
	Pregnant	Non-pregnant	Pregnant	Non-pregnant
0	124.24 ± 4.54	123.26 ± 5.64	131.54 ± 5.26	123.26 ± 5.54
3	125.26 ± 5.14	121.36 ± 4.56	128.26 ± 4.56	121.36 ± 4.56
4	125.84 ± 4.26	122.52 ± 5.12	127.36 ± 5.24	122.52 ± 5.12
5	124.38 ± 5.46	124.38 ± 5.46	126.24 ± 4.48	123.64 ± 4.84
6	126.36 ± 4.32	126.36 ± 4.32	124.12 ± 4.84	123.26 ± 4.66
9	127.48 ± 5.12	127.48 ± 5.12	126.32 ± 5.32	122.62 ± 4.54
10	125.32 ± 4.84	125.32 ± 4.84	128.54 ± 4.24	123.36 ± 4.68
12	125.26 ± 5.46	125.46 ± 4.32	126.62 ± 5.48	124.64 ± 5.12
16	126.78 ± 5.62	126.38 ± 5.14	128.48 ± 4.84	125.54 ± 5.32
20	125.12 ± 5.16	127.54 ± 4.84	130.24 ± 6.45	126.78 ± 5.26

are presented in Table-23.

The plasma potassium (K) concentrations in control normal (CN) and control repeat breeding (CR) groups ranged from 3.84 ± 0.36 to 4.12 ± 0.24 mEq/L and 3.76 ± 0.22 to 4.10 ± 0.32 mEq/L, respectively during the study period. The plasma potassium concentrations observed in the present study are in accordance with Jindal *et al* (1990) and Sharma *et al* (1999). No significant difference was found in the plasma sodium concentrations in control and treatment groups (CN, CR, T_i, T_m and T_o). These concentrations remained more or less constant throughout the study period. Irrespective of progesterone supplementation, when data were sorted out on the basis of pregnancy status, again no significant difference was observed in the plasma potassium concentrations in the pregnant and non-pregnant buffaloes belonging to normal cycling and repeats breeding buffalo groups.

4.4.2 Micro-minerals

The results regarding various micro-mineral concentrations in control (CN and CR) and treatment (T_i, T_m and T_o) groups of buffaloes are presented in Table 24-31 and Figs 19-22.

4.4.2.1 Copper (Cu)

The results of plasma copper (Cu) concentrations in control

Table-22 Plasma potassium concentrations (mEq/l) in control (CN and CR) and treatment (T_i, T_m and T_o) groups of buffaloes (Mean ± S.E.)

DAY OF ESTROUS CYCLE/ EARLY PREGNANCY	CONTROL GROUPS		TREATMENT GROUPS		
	CN	CR	T_i group*	T_m group*	T_o group*
0	3.92 ± 0.32	3.82 ± 0.24	3.84 ± 0.32	3.78 ± 0.32	3.88 ± 0.26
3	3.84± 0.36	3.76 ± 0.22	3.72 ± 0.26	3.84 ± 0.26	3.72 ± 0.24
4	3.96 ± 0.28	3.88 ± 0.26	3.86 ± 0.31	3.82 ± 0.28	3.86 ± 0.28
5	4.12 ± 0.24	3.96 ± 0.24	3.94 ± 0.28	3.88 ± 0.30	3.94 ± 0.30
6	4.04 ± 0.22	4.02 ± 0.22	3.78 ± 0.30	3.92 ± 0.32	4.04 ± 0.28
9	3.98 ± 0.32	4.10 ± 0.32	4.02 ± 0.24	4.08 ± 0.30	4.12 ± 0.30
10	3.84 ± 0.26	3.94 ± 0.24	4.12 ± 0.32	4.12 ± 0.32	4.18 ± 0.32
12	3.96 ± 0.18	4.06 ± 0.22	4.18 ± 0.28	4.18 ± 0.34	4.04 ± 0.28
16	4.04 ± 0.20	3.84 ± 0.26	3.94 ± 0.22	4.04 ± 0.28	3.94 ± 0.26
20	4.12 ± 0.24	3.92 ± 0.20	3.88 ± 0.24	4.10 ± 0.26	3.88 ± 0.22

** T_i, T_m, T_o.....Progesterone supplementation through ear implant, I/M and oral routes*

Figure-18 ***Mean plasma potassium (K) concentrations (mEq/L) in control (CN & CR) and treatment groups of buffaloes (Implant, I/M and Oral)***

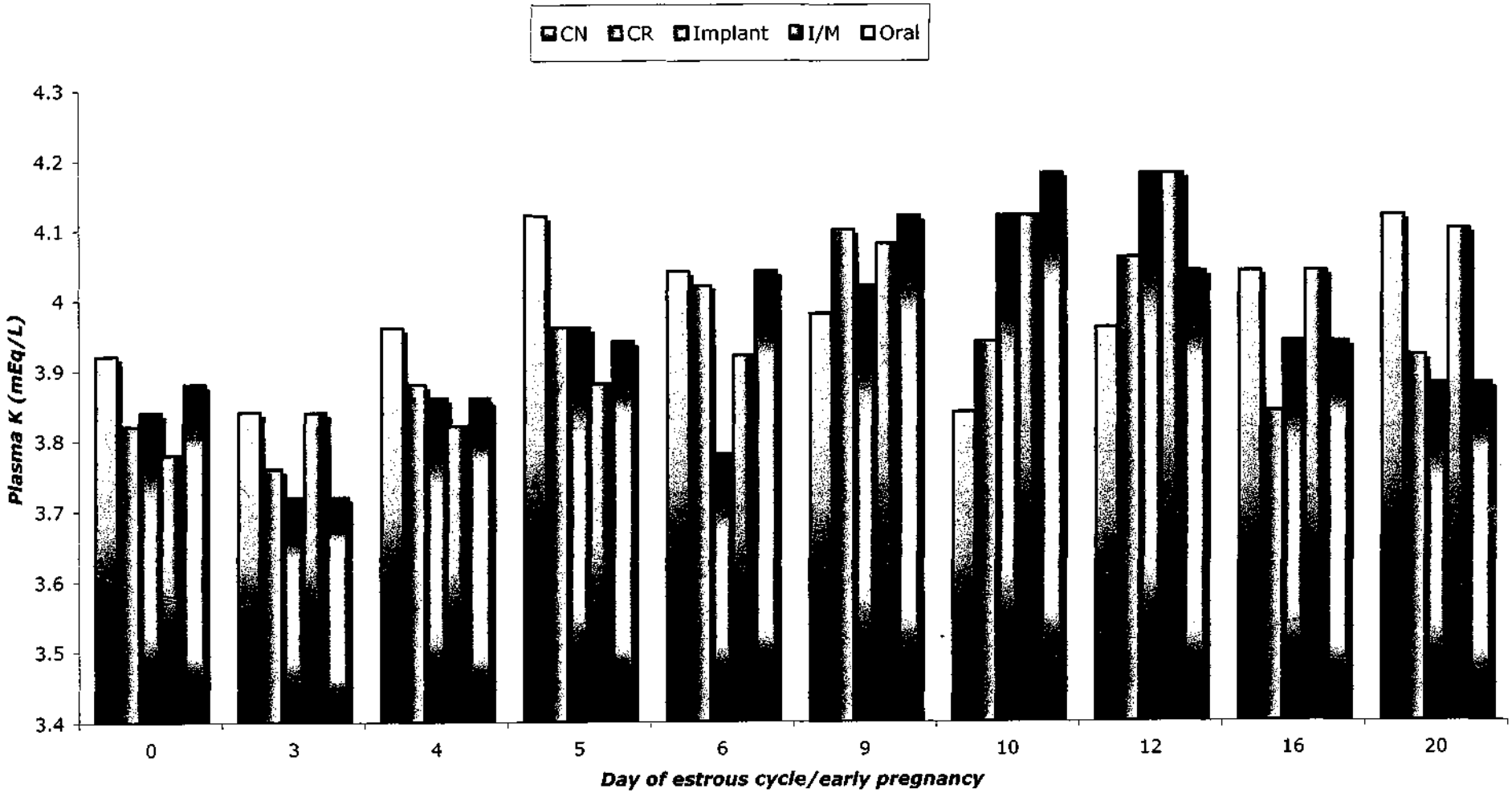


Table-23 Plasma potassium concentrations (mEq/L) in pregnant and non-pregnant buffaloes belonging to normal cycling and repeat breeding groups (Mean ± S.E.)

DAY OF ESTROUS CYCLE/ EARLY PREGNANCY	NORMAL BUFFALOES		REPEAT BREEDING BUFFALOES	
	Pregnant	Non-pregnant	Pregnant	Non-pregnant
0	3.84 ± 0.32	3.78 ± 0.32	3.92 ± 0.32	3.82 ± 0.24
3	3.72 ± 0.26	3.84 ± 0.26	3.84± 0.36	3.76 ± 0.22
4	3.86 ± 0.31	3.82 ± 0.28	3.96 ± 0.28	3.88 ± 0.26
5	3.94 ± 0.28	3.96 ± 0.24	4.12 ± 0.24	3.94 ± 0.30
6	3.78 ± 0.30	4.02 ± 0.22	3.78 ± 0.30	4.04 ± 0.28
9	4.02 ± 0.24	4.10 ± 0.32	4.02 ± 0.24	4.12 ± 0.30
10	4.12 ± 0.32	4.12 ± 0.32	4.12 ± 0.32	4.12 ± 0.32
12	4.12 ± 0.32	4.04 ± 0.28	4.06 ± 0.22	4.18 ± 0.34
16	4.18 ± 0.34	3.94 ± 0.26	3.84 ± 0.26	4.04 ± 0.28
20	4.04 ± 0.28	3.88 ± 0.22	3.92 ± 0.20	4.10 ± 0.26

(CN and CR) and treatment (T_i, T_m and T_o) groups are presented in Table-24 and Fig-19, and in pregnant and non-pregnant buffaloes are presented in Table-25.

The plasma copper (Cu) concentrations in control normal (CN) and control repeat breeding (CR) groups ranged from 115.24 ± 7.54 to 131.32 ± 7.46 µg/dl and 110.46 ± 8.18 to 119.46 ± 6.26 µg/dl, respectively during the study period. The levels of copper were found to be slightly high on day-0 in control (CN and CR) and treatment (T_i, T_m and T_o) groups as compared to respective values on other days of the estrous cycle. However, these concentrations remained more or less constant throughout the study period in all the groups. Irrespective of progesterone supplementation, when data were sorted out on the basis of pregnancy status, the plasma copper concentrations broadly followed the same pattern and no significant change was observed between the mean copper concentrations between the pregnant and non-pregnant buffaloes belonging to control (CN & CR) and treatment (T_i, T_m and T_o) groups.

4.4.2.2 Iron (Fe)

The results of plasma iron (Fe) concentrations in control (CN and CR) and treatment (T_i, T_m and T_o) groups are presented in Table-26 and Fig-20, and in pregnant and non-pregnant buffaloes are presented in Table-27.

Table-24 Plasma copper concentrations (μg/dl) in control (CN and CR) and treatment (T_i, T_m and T_o) groups (Mean ± S.E.)

DAY OF ESTROUS CYCLE/ EARLY PREGNANCY	CONTROL GROUPS		TREATMENT GROUPS		
	CN	CR	T_i group*	T_m group*	T_o group*
0	131.32 ± 7.46	119.46 ± 8.18	126.62 ± 8.26	125.98 ± 8.48	128.62 ± 7.86
3	123.47 ± 7.16	113.82 ± 8.56	115.54 ± 7.86	108.84 ± 7.46	119.84 ± 8.26
4	126.64 ± 6.82	111.62 ± 7.76	110.46 ± 7.54	112.48 ± 6.84	116.32 ± 8.42
5	121.21 ± 6.54	114.31 ± 7.24	118.34 ± 7.16	118.34 ± 7.22	121.46 ± 7.56
6	122.16 ± 6.86	116.67 ± 6.28	121.98 ± 6.82	121.42 ± 7.64	126.36 ± 7.28
9	119.84 ± 7.16	110.46 ± 6.52	124.86 ± 7.56	120.46 ± 7.48	117.38 ± 8.16
10	115.24 ± 7.54	114.81 ± 6.48	121.48 ± 8.46	110.62 ± 6.84	111.86 ± 7.64
12	117.37 ± 6.28	119.42 ± 6.84	116.34 ± 8.28	118.48 ± 7.64	119.42 ± 6.84
16	124.16 ± 7.56	114.61 ± 6.26	113.12 ± 7.48	121.46 ± 8.54	124.32 ± 6.56
20	118.44 ± 8.46	115.14 ± 7.86	123.46 ± 6.86	124.42 ± 7.94	115.48 ± 7.64

** T_i, T_m, T_o.....Progesterone supplementation through ear implant, I/M and oral routes*

Figure-19 ***Mean plasma copper (Cu) concentrations (µg/dl) in control (CN & CR) and treatment groups of buffaloes (Implant, I/M and Oral)***

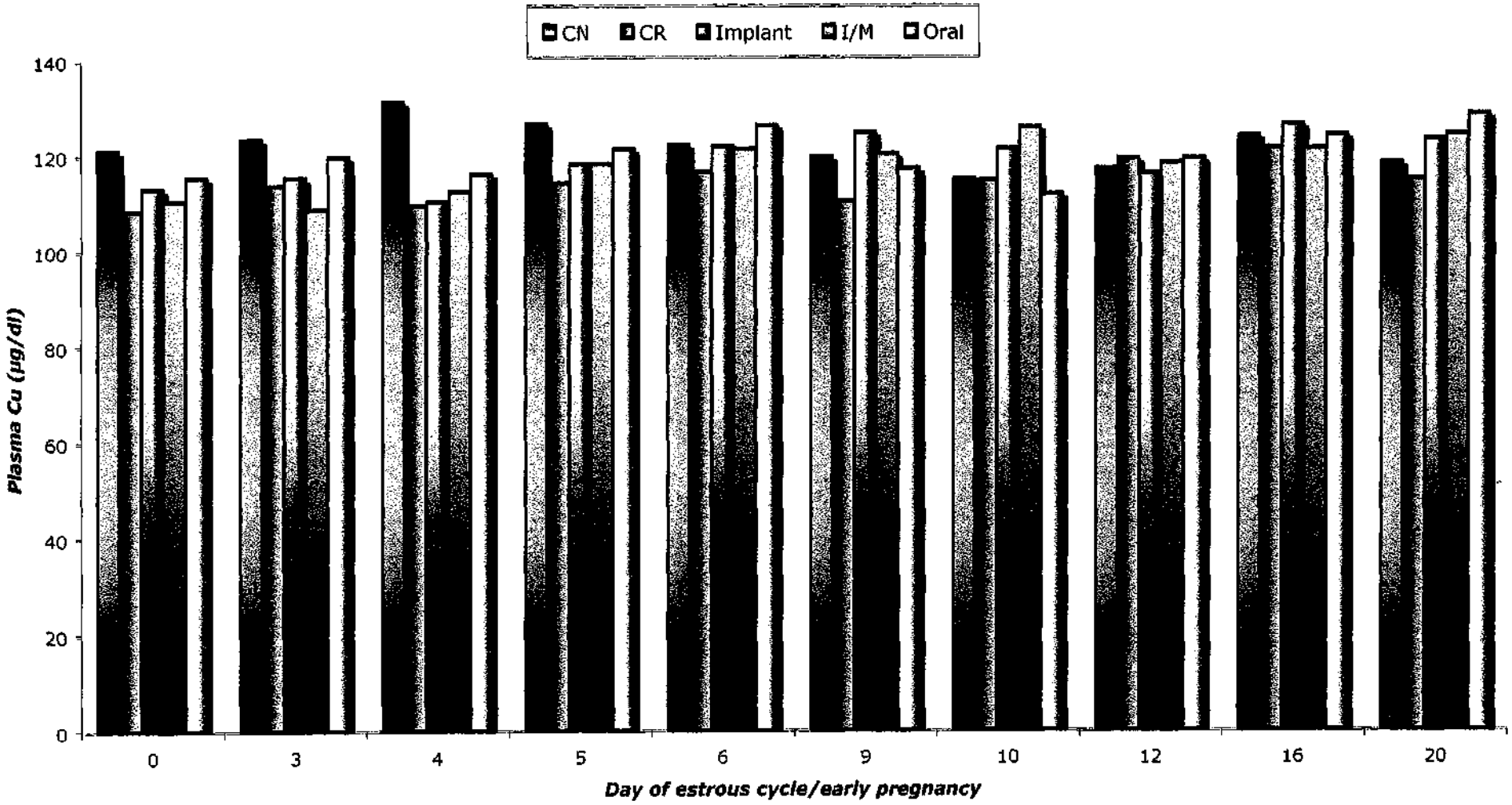


Table-25 Plasma copper concentrations (µg/dl) in pregnant and non-pregnant buffaloes belonging to normal cycling and repeat breeding groups (Mean ± S.E.)

DAY OF ESTROUS CYCLE/ EARLY PREGNANCY	NORMAL BUFFALOES		REPEAT BREEDING BUFFALOES	
	Pregnant	Non-pregnant	Pregnant	Non-pregnant
0	130.32 ± 6.56	116.46 ± 7.48	131.32 ± 7.46	115.24 ± 7.54
3	115.54 ± 7.86	108.84 ± 7.46	123.47 ± 7.16	113.82 ± 8.56
4	110.46 ± 7.54	112.48 ± 6.84	126.64 ± 6.82	111.62 ± 7.76
5	118.34 ± 7.16	118.34 ± 7.22	118.34 ± 7.16	116.67 ± 6.28
6	110.46 ± 7.54	121.48 ± 8.46	121.98 ± 6.82	114.31 ± 7.24
9	114.81 ± 6.48	124.86 ± 7.56	124.86 ± 7.56	116.67 ± 6.24
10	119.42 ± 6.84	121.48 ± 8.46	121.48 ± 8.46	110.46 ± 6.52
12	114.61 ± 6.26	116.34 ± 8.28	119.42 ± 6.84	114.81 ± 6.48
16	111.86 ± 7.64	124.32 ± 6.56	124.16 ± 7.56	114.61 ± 6.26
20	119.42 ± 6.84	115.48 ± 7.64	118.44 ± 8.46	115.14 ± 7.86

Table-26 Plasma iron concentrations (μg/dl) in control (CN and CR) and treatment (T_i, T_m and T_o) groups (Mean ± S.E.)

DAY OF ESTROUS CYCLE/ EARLY PREGNANCY	CONTROL GROUPS		TREATMENT GROUPS		
	CN	CR	T_i group*	T_m group*	T_o group*
0	126.42 ± 13.45[a]	121.48 ± 13.74[a]	123.46 ± 14.32[a]	118.32 ± 14.64[a]	120.34 ± 13.45[a]
3	152.34 ± 13.28[b]	148.64 ± 12.48[b]	142.24 ± 13.64[b]	140.48 ± 13.59[b]	138.26 ± 12.84[b]
4	151.46 ± 12.62[b]	144.36 ± 12.26[b]	148.84 ± 13.48[b]	142.64 ± 13.69[b]	144.64 ± 12.63[b]
5	148.78 ± 11.51[b]	138.46 ± 13.85[b]	144.78 ± 12.54[b]	138.42 ± 12.56[b]	141.42 ± 13.84[b]
6	154.64 ± 12.84[b]	142.28 ± 14.82[b]	138.64 ± 13.86[b]	132.38 ± 12.84[b]	134.68 ± 14.85[b]
9	158.36 ± 13.72[b]	148.48 ± 13.64[b]	134.28 ± 12.54[b]	126.82 ± 13.15[b]	129.48 ± 13.41[b]
10	146.98 ± 12.82[b]	140.82 ± 13.92[b]	140.46 ± 11.28[b]	129.68 ± 13.68[b]	124.24 ± 12.95[b]
12	148.24 ± 11.84[b]	136.46 ± 13.46[b]	138.38 ± 10.64[b]	135.26 ± 14.54[b]	128.46 ± 13.84[b]
16	145.46 ± 12.64[b]	134.84 ± 11.57[b]	132.56 ± 12.82[b]	136.62 ± 12.62[b]	132.74 ± 12.56[b]
20	150.28±13.28[b]	130.46 ± 12.46[b]	142.48 ± 13.76[b]	139.46±12.84[b]	136.86 ± 12.89[b]

-values with different superscripts differ significantly (P<0.01) as compared to day-0, within the column

**** T_i, T_m, T_o.....Progesterone supplementation through ear implant, I/M and oral routes***

Figure-20 ***Mean plasma iron (Fe) concentrations (µg/dl) in control (CN & CR) and treatment groups of buffaloes (Implant, I/M and Oral)***

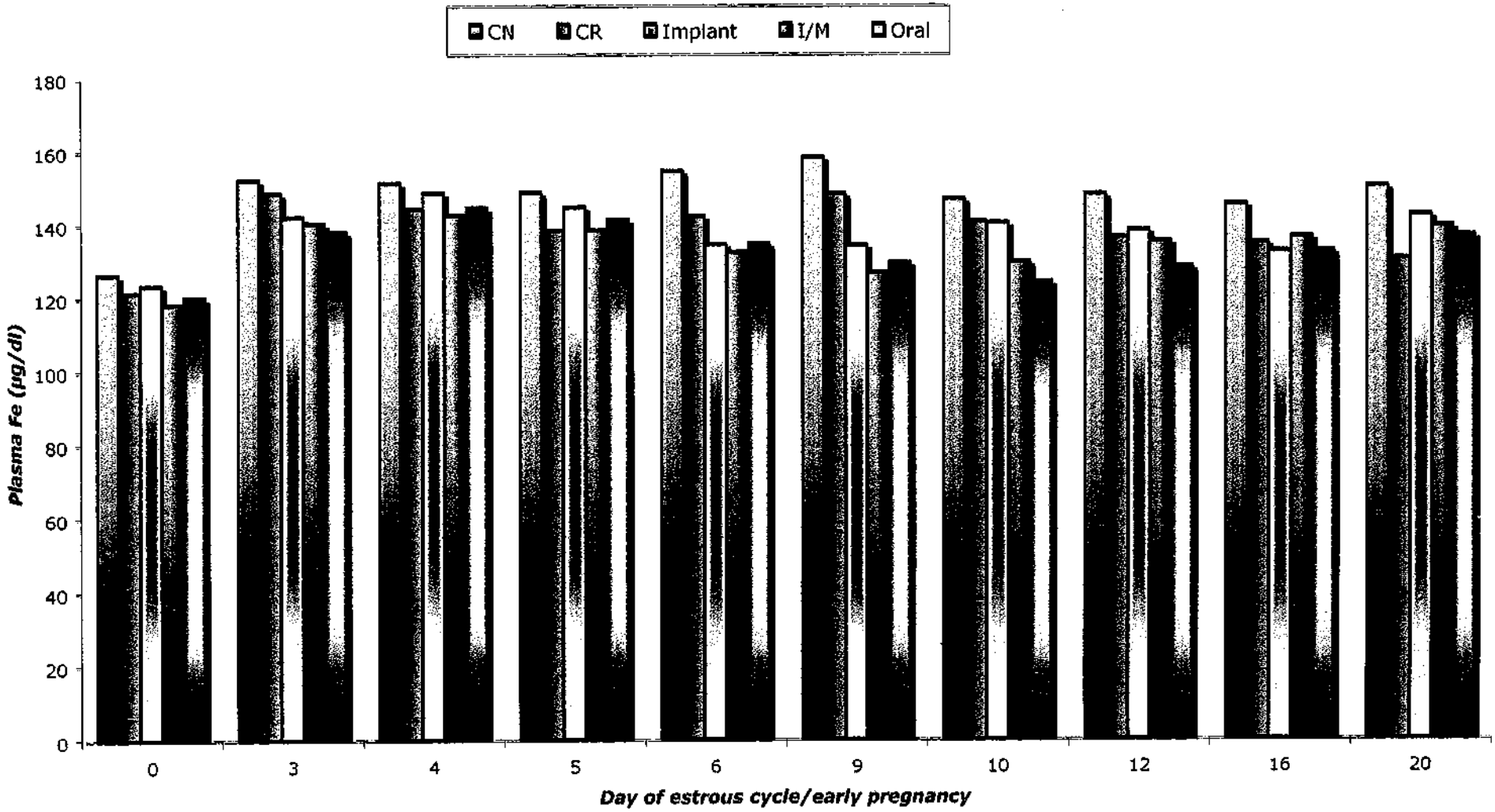


Table-27 Plasma iron concentrations (μg/dl) in pregnant and non-pregnant buffaloes belonging to normal cycling and repeat breeding groups (Mean ± S.E.)

DAY OF ESTROUS CYCLE/ EARLY PREGNANCY	NORMAL BUFFALOES		REPEAT BREEDING BUFFALOES	
	Pregnant	Non-pregnant	Pregnant	Non-pregnant
0	123.46 ± 14.32[a]	120.34 ± 13.45[a]	126.42 ± 13.45[a]	121.48 ± 13.74[a]
3	142.24 ± 13.64[b]	138.26 ± 12.84[b]	152.34 ± 13.28[b]	148.64 ± 12.48[b]
4	148.84 ± 13.48[b]	144.64 ± 12.63[b]	151.46 ± 12.62[b]	144.36 ± 12.26[b]
5	144.78 ± 12.54[b]	141.42 ± 13.84[b]	148.78 ± 11.51[b]	138.46 ± 13.85[b]
6	154.64 ± 12.84[b]	142.28 ± 14.82[b]	152.34 ± 13.28[b]	141.42 ± 13.84[b]
9	158.36 ± 13.72[b]	148.48 ± 13.64[b]	151.46 ± 12.62[b]	134.68 ± 14.85[b]
10	146.98 ± 12.82[b]	140.82 ± 13.92[b]	140.46 ± 11.28[b]	129.48 ± 13.41[b]
12	135.26 ± 14.54[b]	128.46 ± 13.84[b]	138.38 ± 10.64[b]	135.26 ± 14.54[b]
16	136.62 ± 12.62[b]	132.74 ± 12.56[b]	132.56 ± 12.82[b]	136.62 ± 12.62[b]
20	139.46±12.84[b]	136.86 ± 12.89[b]	142.48 ± 13.76[b]	139.46±12.84[b]

-values with different superscripts differ significantly (P<0.01) as compared to day-0, within the column

The plasma iron (Fe) concentrations in control normal (CN) and control repeat breeding (CR) groups ranged from 126.42 ± 13.45 to 158.36 ± 13.72 µg/dl and 121.48 ± 13.74 to 148.64 ± 12.48 µg/dl, respectively during the study period. The plasma iron concentrations were found to be significantly low ($p<0.05$) on day-0 in control (CN and CR) and treatment (T_i, T_m and T_o) groups as compared to the respective values on other days of the estrous cycle. However, these concentrations remained more or less constant from day-3 through day-20. Data when sorted ón the basis of pregnancy status, irrespective of progesterone supplementation, the plasma iron concentrations broadly followed the same pattern. Significantly low ($p<0.05$) plasma iron concentrations were found on day of estrus (day-0) as compared to other days of the cycle in pregnant and non-pregnant buffaloes belonging to control (CN & CR) and treatment (T_i, T_m and T_o) groups.

4.4.2.3 Zinc (Zn)

The results of plasma zinc (Zn) concentrations in control (CN and CR) and treatment (T_i, T_m and T_o) groups are presented in Table-28 and Fig-21, and in pregnant and non-pregnant buffaloes are presented in Table-29.

The plasma zinc (Zn) concentrations in control normal (CN) and control repeat breeding (CR) groups ranged from 88.16 ± 3.94 to 110.37 ± 5.68 µg/dl and 84.64 ± 3.88 to 108.46 ± 5.64 µg/dl,

Table-28 Plasma zinc concentrations (μg/dl) in control (CN and CR) and treatment (T_i, T_m and T_o) groups of buffaloes (Mean ± S.E.)

DAY OF ESTROUS CYCLE/ EARLY PREGNANCY	CONTROL GROUPS		TREATMENT GROUPS		
	CN	CR	T_i group*	T_m group*	T_o group*
0	105.94 ± 5.84	98.71 ± 5.45	101.93 ± 5.66	99.21 ± 5.85	102.64 ± 5.48
3	110.37 ± 5.68	108.46 ± 5.64	111.36 ± 5.84	106.34 ± 5.26	109.91 ± 5.62
4	102.81 ± 5.48	102.84 ± 5.26	105.85 ± 5.46	96.76 ± 4.88	101.64 ± 5.54
5	98.19 ± 5.12	96.79 ± 5.84	100.27 ± 5.24	91.34 ± 4.76	98.71 ± 4.72
6	92.34 ± 4.66	92.46 ± 4.56	97.24 ± 4.34	87.49 ± 4.24	94.37 ± 4.56
9	91.48 ± 4.84	88.13 ± 4.54	91.76 ± 4.56	92.56 ± 3.98	91.75 ± 3.58
10	92.34 ± 4.56	89.64 ± 3.88	93.64 ± 4.84	89.42 ± 3.84	88.54 ± 3.64
12	91.16 ± 3.94	90.97 ± 4.24	89.46 ± 5.21	88.29 ± 4.65	87.91 ± 3.88
16	90.54 ± 4.58	88.54 ± 4.68	89.85 ± 5.64	86.48 ± 4.87	88.45 ± 4.21
20	93.78 ± 4.23	87.23 ± 4.97	89.56 ± 5.48	88.41 ± 5.26	89.32 ± 4.45

** T_i, T_m, T_o.....Progesterone supplementation through ear implant, I/M and oral routes*

Figure-21 ***Mean plasma zinc (Zn) concentrations (µg/dl) in control (CN & CR) and treatment groups of buffaloes (Implant, I/M and Oral)***

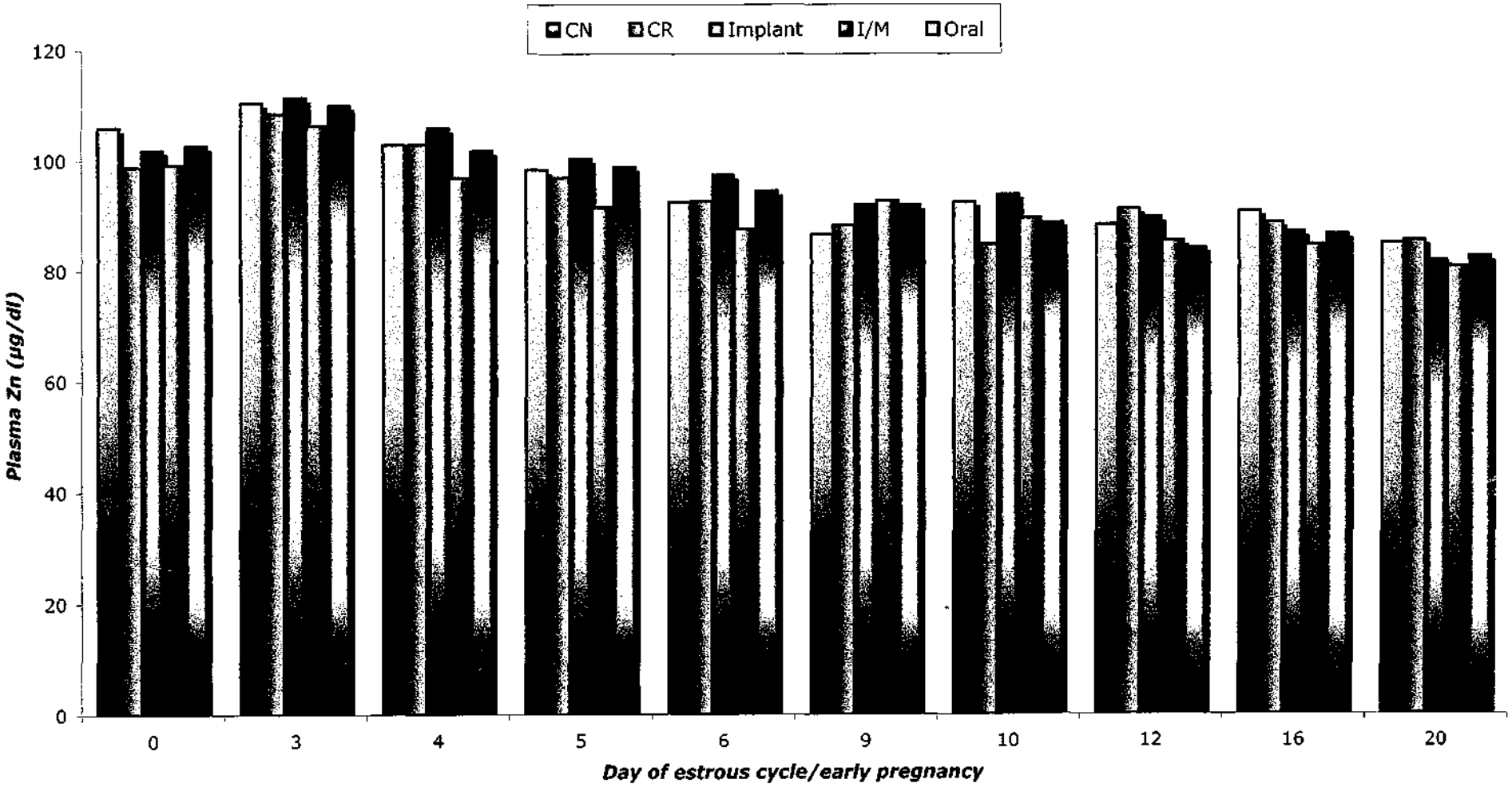


Table-29 Plasma zinc concentrations (μg/dl) in pregnant and non-pregnant buffaloes belonging to normal cycling and repeat breeding groups (Mean ± S.E.)

DAY OF ESTROUS CYCLE/ EARLY PREGNANCY	NORMAL BUFFALOES		REPEAT BREEDING BUFFALOES	
	Pregnant	Non-pregnant	Pregnant	Non-pregnant
0	101.93 ± 5.66	102.84 ± 5.26	105.94 ± 5.84	100.27 ± 5.24
3	111.36 ± 5.84	98.71 ± 5.45	110.37 ± 5.68	108.46 ± 5.64
4	105.85 ± 5.46	108.46 ± 5.64	102.81 ± 5.48	102.46 ± 5.26
5	91.34 ± 4.76	102.24 ± 5.06	98.19 ± 5.12	96.79 ± 5.84
6	89.49 ± 4.24	92.56 ± 3.98	93.64 ± 4.84	92.46 ± 4.56
9	92.56 ± 3.98	89.42 ± 3.84	92.46 ± 5.21	96.79 ± 5.84
10	91.75 ± 3.58	84.64 ± 3.88	91.85 ± 5.64	91.76 ± 4.56
12	88.54 ± 3.64	90.97 ± 4.24	95.56 ± 5.48	93.64 ± 4.84
16	86.85 ± 5.64	88.54 ± 4.68	93.97 ± 4.24	89.46 ± 5.21
20	89.56 ± 5.48	88.23 ± 4.97	92.54 ± 4.68	90.85 ± 5.64

respectively during the study period. The plasma zinc concentrations were found to be slightly lower on day-0 in control (CN and CR) and treatment (T_i, T_m and T_o) groups. The concentrations rise in all the groups on day-3 of the estrous cycle and then a gradual fall was observed from day-4 onwards after which the concentrations remained more or less constant up to day-20. Irrespective of progesterone supplementation, when data were sorted out on the basis of pregnancy status, the plasma zinc concentrations broadly followed the same pattern with no significant difference between the mean zinc concentrations between the pregnant and non-pregnant buffaloes belonging to control (CN & CR) as well as treatment (T_i, T_m and T_o) groups.

4.4.2.4 Cobalt (Co)

The results of plasma cobalt (Co) concentrations in control (CN and CR) and treatment (T_i, T_m and T_o) groups are presented in Table-30 and Fig-22, and in pregnant and non-pregnant buffaloes are presented in Table-31.

The plasma cobalt (Co) concentrations in control normal (CN) and control repeat breeding (CR) groups ranged from 0.74 ± 0.08 to 0.91 ± 0.10 µg/dl and 0.71 ± 0.04 to 0.86 ± 0.12 µg/dl, respectively during the study period. The plasma cobalt concentrations were found to be slightly high on day-0 in control (CN and CR) and treatment (T_i, T_m and T_o) groups as compared to

Table-30 Plasma cobalt concentrations (μg/dl) in control (CN and CR) and treatment (T_i, T_m and T_o) groups (Mean ± S.E.)

DAY OF ESTROUS CYCLE/ EARLY PREGNANCY	CONTROL GROUPS		TREATMENT GROUPS		
	CN	CR	T_i group*	T_m group*	T_o group*
0	0.91 ± 0.10	0.86 ± 0.12	0.88 ± 0.10	0.90 ± 0.11	0.86 ± 0.10
3	0.88 ± 0.05	0.85 ± 0.08	0.85 ± 0.08	0.87 ± 0.09	0.85 ± 0.09
4	0.85 ± 0.04	0.81 ± 0.05	0.87 ± 0.06	0.82 ± 0.06	0.84 ± 0.06
5	0.86 ± 0.09	0.78 ± 0.04	0.79 ± 0.07	0.79 ± 0.07	0.81 ± 0.04
6	0.84 ± 0.08	0.75 ± 0.08	0.76 ± 0.04	0.75 ± 0.05	0.78 ± 0.07
9	0.81 ± 0.07	0.79 ± 0.07	0.80 ± 0.06	0.77 ± 0.08	0.75 ± 0.08
10	0.79 ± 0.06	0.74 ± 0.06	0.76 ± 0.05	0.74 ± 0.04	0.71 ± 0.06
12	0.82 ± 0.05	0.76 ± 0.09	0.74 ± 0.09	0.78 ± 0.08	0.79 ± 0.09
16	0.78 ± 0.06	0.71 ± 0.04	0.72 ± 0.07	0.72 ± 0.07	0.76 ± 0.07
20	0.74 ± 0.08	0.75 ±0.07	0.73 ± 0.09	0.76 ± 0.06	0.71 ± 0.08

** T_i, T_m, T_o.....Progesterone supplementation through ear implant, I/M and oral routes*

Figure-22 ***Mean plasma cobalt (Co) concentrations (µg/dl) in control (CN & CR) and treatment groups of buffaloes (Implant, I/M and Oral)***

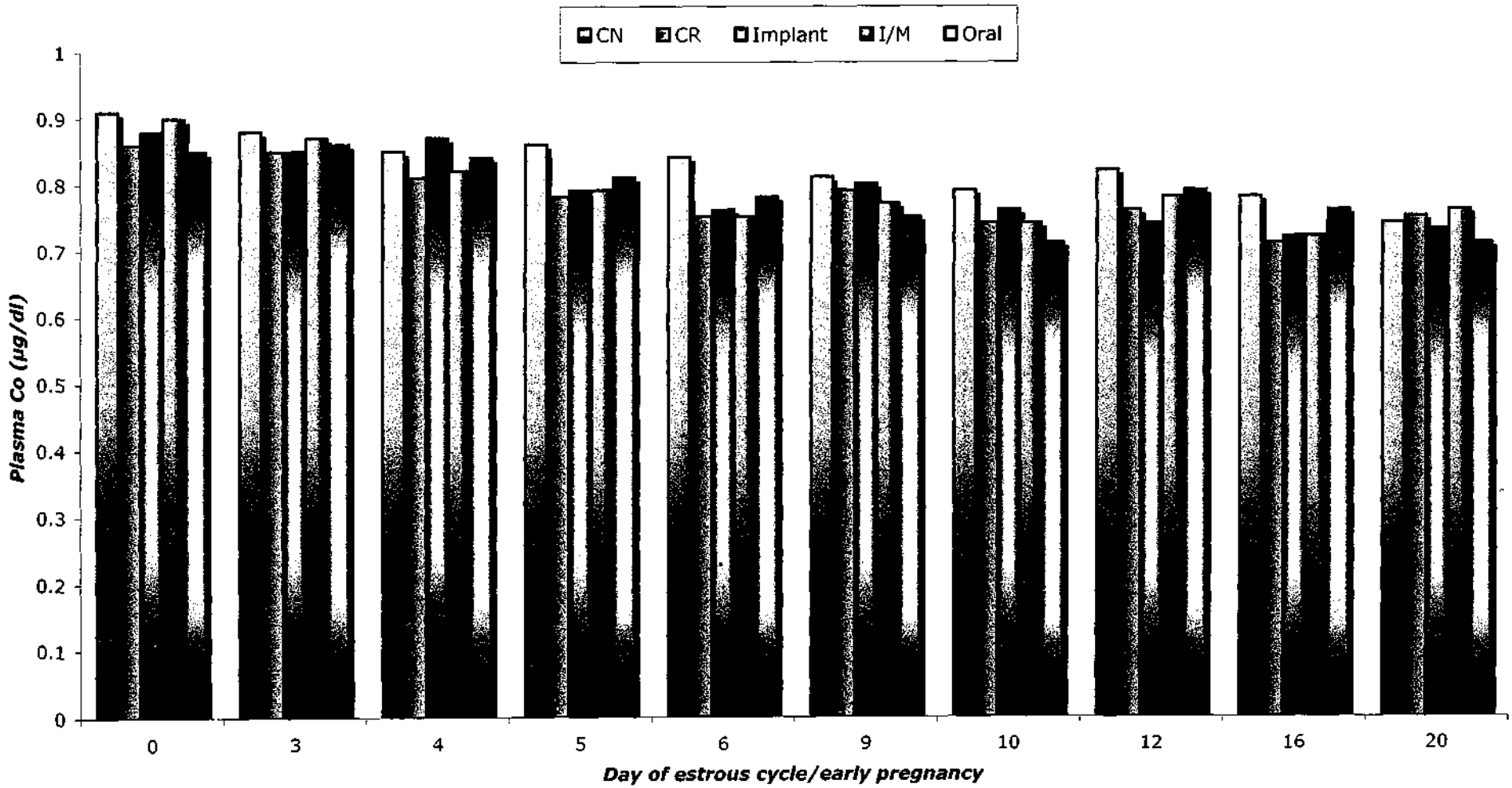


Table-31 Plasma cobalt concentrations (μg/dl) in pregnant and non-pregnant buffaloes belonging to normal cycling and repeat breeding groups (Mean ± S.E.)

DAY OF ESTROUS CYCLE/ EARLY PREGNANCY	NORMAL BUFFALOES		REPEAT BREEDING BUFFALOES	
	Pregnant	Non-pregnant	Pregnant	Non-pregnant
0	0.89 ± 0.09	0.87 ± 0.06	0.85 ± 0.08	0.86 ± 0.10
3	0.88 ± 0.10	0.86 ± 0.12	0.81 ± 0.05	0.85 ± 0.09
4	0.85 ± 0.08	0.85 ± 0.08	0.85 ± 0.04	0.84 ± 0.06
5	0.87 ± 0.06	0.81 ± 0.05	0.86 ± 0.09	0.81 ± 0.04
6	0.80 ± 0.06	0.78 ± 0.04	0.84 ± 0.08	0.75 ± 0.08
9	0.76 ± 0.05	0.79 ± 0.07	0.81 ± 0.07	0.79 ± 0.07
10	0.74 ± 0.09	0.74 ± 0.06	0.76 ± 0.04	0.74 ± 0.06
12	0.76 ± 0.04	0.76 ± 0.09	0.80 ± 0.06	0.76 ± 0.09
16	0.80 ± 0.06	0.71 ± 0.04	0.76 ± 0.05	0.79 ± 0.07
20	0.76 ± 0.05	0.74 ± 0.08	0.72 ± 0.07	0.74 ± 0.06

the respective values on other days of the estrous cycle. However, these concentrations remained more or less constant throughout the study period and no significant difference was observed among any of the control (CN & CR) or treatment (T_1, T_2 and T_3) group4s. Irrespective of progesterone supplementation, when data were sorted out on the basis of pregnancy status, no significant change was observed between the mean cobalt concentrations between the pregnant and non-pregnant buffaloes belonging to control (CN & CR) as well as treatment (T_i, T_m and T_o) groups. The concentrations of trace elements found in the present study are in accordance with the earlier reports by Jindal *et al* (1990), Sharma *et al* (1999) and Sharma (2001).

Minerals play a key role in enzymatic and hormonal systems acting at cellular levels. They act as cofactors, activators of enzymes or stabilizers of secondary molecular structures (Valee and Wacker 1976). Variations in compositions of some of the blood elements in farm animals during different reproductive periods affect or upset the proper functioning of the reproductive organs (Rowlands *et al* 1977). The deficiency of one or more of these can result in impaired reproductive functioning of the animal such as infertility, repeat breeding, embryonic loss, poor conception rate, anestrus etc. by affecting various physiological activities (Underwood 1977, Mc Dowell 1992). Lower plasma concentrations

of various macro/micro-minerals in repeat breeding animals as compared to normal cycling animals have been reported by various workers (Eltohamy *et al* 1989, Rupde *et al* 1993, Prasad and Rao 1997).

The problem of repeat can be attributed to large number of factors and the mineral deficiencies may be responsible for causing repeat breeding in about 10% of the animals (Jain 1989). The animals used at the present study were maintained at an organized dairy farm and their feed was supplemented with recommended dose of mineral mixture, so it was less likely that these animals were having any mineral deficiencies. In these studies, no difference in the concentrations of these elements was found in the control and treatment groups of buffaloes. These results indicated that progesterone supplementation through any route did not bring about any change in the macro/micro-mineral status of the animal. Also, no differences in the concentrations of macro/micro-minerals was observed between pregnant and non-pregnant buffaloes belonging to normal cycling and repeat breeding buffalo groups, irrespective of progesterone supplementation. These observations further substantiate the hypothesis that the deficiency of these minerals might not have been the cause of repeat breeding in these animals.

CHAPTER-V

SUMMARY AND CONCLUSION

Among various reproductive maladies contributing to low reproductive efficiency, repeat breeding is the major one. Taking into account the various factors causing repeat breeding in dairy animals, the hormonal imbalance during early pregnancy is regarded as an important one. Since progesterone maintains a quiescent and hospitable uterine environment, optimum levels of this hormone are essential for the establishment and maintenance of pregnancy. The present investigation was therefore undertaken to study the effect of progesterone supplementation during early pregnancy, through different routes on conception rate and other blood biochemical constituents in repeat breeding buffaloes. The parameters studied included various hormones (P_4, insulin, T_3 and T_4), biochemical constituents (blood glucose and total plasma cholesterol), macro-minerals (Ca, P, Na, K and Cl) and micro-minerals (Cu, Co, Fe and Zn).

The study was conducted on eight normal cycling (CN) and thirty-two repeat breeding Murrah buffaloes in their 2^{nd}-4^{th} parity. The repeat breeding buffaloes were randomly divided into control (CR) and

three treatment groups (T_i, T_m and T_o) with equal number of animals in each group. No treatment was given to the buffaloes belonging to CN group. Repeat breeding buffaloes belonging to group T_i were inserted with Crestar ear implant on day-4 that was removed on day-12 after insemination (day-0 being the day of insemination). In T_m group, two I/M injections of hydroxy-progesterone caproate (Duraprogen), was given, first on day-4 and second on day-9 after insemination (day-0 being the day of insemination). The animals belonging to group T_o were administered with oral preparation of progesterone (Microgest) starting from day-4 through day-12 after insemination (day-0 being the day of insemination). The blood samples were collected by jugular veni-puncture on days 0, 3, 4, 5, 6, 9, 10, 12, 16 and 20 of the cycle in both the control (CN and CR) as well as treatment (T_i, T_m and T_o) groups. All the animals were maintained on usual farm ration and were kept on standard managemental practices.

In the present investigation, progesterone supplementation successfully improved the conception rate in treatment groups T_i (75%), T_m (62.5%) and T_o (50%) over the control repeat breeding group-CR in which the conception rate was found to be only 25%. In all the control (CN and CR) and treatment (T_i, T_m and T_o) groups, on day-0 the plasma progesterone was found at its nadir and the levels

started rising with the formation of the corpus luteum. The plasma progesterone concentrations in the control normal (CN) and treatment (T_i, T_m and T_o) groups were significantly higher than the control repeat breeding group (CR) on days 4, 5, 16 and 20 of the cycle. Data when analyzed on the basis of pregnancy status, irrespective of progesterone supplementation, the pregnant and non-pregnant animals belonging to normal cycling as well as repeat breeding groups, the plasma progesterone concentrations were at their base levels on day-0, thereafter rising gradually and the levels remained high in pregnant buffaloes up to day-20 i.e. the period of study, whereas the levels declined from day-16 in case of non-pregnant buffaloes.

In the present study, no significant variation was found in the levels of plasma insulin, triiodothyronine and thyroxine among the control and treatment groups (CN and CR, T_i, T_m and T_o). In all the groups, plasma insulin concentrations were found to be significantly lower whereas the thyroidal activity (T_3 and T_4 concentrations) higher during estrus phase as compared to other phases of the estrous cycle. These concentrations remained more or less constant during the study period.

Among biochemical constituents, the blood glucose and total plasma cholesterol concentrations were found to be higher on the day

of estrus as compared to other days of the cycle/pregnancy. No significant difference in these parameters was found between control (CN and CR) and treatment (T_i, T_m and T_o) groups. In the present study, no difference was observed in the macro and micro-mineral status of the animal, indicating that the mineral deficiency might not be the cause of repeat breeding in these animals. Thus the use of exogenous progesterone is an appropriate substitutional approach to lower the repeat breeding problems due to luteal insufficiency, as has been done in the present study.

CONCLUSIONS

1. Progesterone supplementation through any of the routes (ear implant, I/M injection or oral) in repeat breeding buffaloes can be effectively used to improve the conception rate, thus ameliorating the problem of repeat breeding.
2. Highest conception rate of 75% was observed in group-T_i (progesterone ear implant), followed by I/M progesterone supplemented group-T_m (62.5%) and oral progesterone supplemented group-T_o (50%).
3. The mean plasma progesterone concentration was at its nadir on the day of estrus, which started rising with the development of

the corpus luteum. Higher levels were maintained in the pregnant animals, whereas the plasma progesterone concentrations declined after the regression of corpus luteum (day-16) in the non-pregnant buffaloes.

4. Progesterone administration in all the three treatment groups of repeat breeding buffaloes successfully improved the plasma progesterone concentrations from day-4 onwards.
5. Circulating levels of insulin, T_3 and T_4 were higher during estrus as compared to other phases of the cycle/early pregnancy in all the groups and were not affected by progesterone supplementation treatment.
6. Blood glucose and total plasma cholesterol levels were higher during estrus as compared to the other phases of the cycle/early pregnancy.
7. The plasma progesterone administration as a remedy to repeat breeding in buffaloes does not seem to have any adverse effect on insulin, thyroidal activity, other blood biochemical parameters and macro/micro mineral status of the animal.

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VITA

Name of the student	:	Anil Sharma
Father's name	:	Dr. Yash Pal Sharma
Mother's name	:	Mrs. Rajni Sharma
Nationality	:	Indian
Date of birth	:	September 24, 1975
Permanent home address	:	1379/2, Krishna Nagar, Moga-142 001, Punjab
Educational qualifications	:	
Bachelor's degree	:	B.V.Sc & A.H.
University and year of award	:	PAU, Ludhiana, 2005
OCPA on 10.00 basis	:	6.59/10.00
Master's degree	:	M.V.Sc (Veterinary Physiology)
University and year of award	:	PAU, Ludhiana, 2001
OCPA on 10.00 basis	:	7.95/10.00
Title of Master's thesis	:	Effect of progesterone on blood biochemical profile and conception rate in repeat breeding buffaloes
Ph.D	:	Veterinary Physiology
OCPA on 10.00 basis	:	7.69/10.00
Awards/Fellowships/Scholarships	:	University merit scholarship B.V.Sc & A.H. (1994-1995) Fellowship: M.V.Sc (1999-2001) Fellowship: Ph.D (2001-2004)