EFFECT OF β-CAROTENE INCORPORATED MINERAL-VITAMIN PERMIX ON AMELIORATION OF INFERTILITY IN CROSSBRED CATTLE

काशी हिन्दू विश्वविद्यालय BANARAS HINDU UNIVERSITY

ESTABLISHED BY PARLIAMENT BY NOTIFICATION NO. 225 OF 1916

THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

Master of Science (Agriculture)

In

Animal Husbandry & Dairying

(Livestock Production & Management)

Supervisor

Dr. Vinod Kumar Paswan

Submitted by

Dheeraj Kumar

DEPARTMENT OF ANIMAL HUSBANDRY & DAIRYING
INSTITUTE OF AGRICULTURAL SCIENCES
BANARAS HINDU UNIVERSITY
VARANASI-221 005
INDIA

ID No.: 17412AHD001 **2019** **Enrolment No.: 398668**

काशी हिन्दू विश्वविद्यालय BANARAS HINDU UNIVERSITY

AN INSTITUTION OF NATIONAL IMPORTANCE ESTABLISHED BY AN ACT OF PARLIAMENT

Dr. Vinod Kumar Paswan
Assistant Professor
Department of Animal Husbandry & Dairying
Institute of Agricultural Sciences
Banaras Hindu University
Varanasi-221 005, INDIA.
Mobile No. 8565074108
Email: vkpaswan.vet@gmail.com

Ref. No…… **Dated……**

CERTIFICATE

To,
The Registrar (Academic)
Banaras Hindu University,
Varanasi-221 005,
U.P., India.

Through: The Head, Department of Animal husbandry & Dairying

Dear Sir,

I have great pleasure in forwarding the thesis entitled **"The Effect of β- Carotene Incorporated Mineral-Vitamin Premix on Amelioration of Infertility in Crossbred Cattle"** submitted by **Mr. Dheeraj Kumar, I.D. No. 17412AHD001, Enrolment No. 398668** in partial fulfilment of the requirements for the award of the degree of **Master of Science (Agriculture)** in **Animal Husbandry & Dairying (Livestock Production & Management)**, Department of AH&D, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi.

This is to certify that the work has been carried out solely by **Mr. Dheeraj Kumar** under my guidance and data forming the basis of this thesis, to the best of my knowledge are genuine and original and no part of the work has been submitted for any other degree or distinction.

Thanking you.

Yours faithfully

Forwarded

(Vinod Kumar Paswan)
Supervisor

BHU
capital of knowledge

EFFECT OF β-CAROTENE INCORPORATED MINERAL-VITAMIN PERMIX ON AMELIORATION OF INFERTILITY IN CROSSBRED CATTLE

by

Dheeraj Kumar

Thesis submitted in partial fulfilment of the requirements for the award of the degree of

Master of Science (Agriculture)

In

Animal Husbandry & Dairying

(Livestock Production & Management)

DEPARTMENT OF ANIMAL HUSBANDRY & DAIRYING
INSTITUTE OF AGRICULTURAL SCIENCES
BANARAS HINDU UNIVERSITY
VARANASI – 221 005
INDIA

ID. No.: 17412AHD001 **2019** **Enrolment No.: 398668**

THESIS APPROVED BY ADVISORY COMMITTEE

CHAIRMAN	**Dr. Vinod Kumar Paswan** **Assistant Professor** Department of Animal Husbandry & Dairying, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi.
MEMBER	**Dr. Dinesh Chandra Rai** **Professor and Head** Department of Animal Husbandry & Dairying, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi.
MEMBER	**Dr. R. K. Pandey** **Professor** Department of Animal Husbandry & Dairying, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi.
MEMBER	**Dr. S. P. Singh (External Member)** **Professor** Department of Agronomy, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi.

EXTERNAL EXAMINER

ACKNOWLEDGEMENT

I am one among the few fortunates to be a part of Banaras Hindu University, the seat of learning, which with good grace provided me the most peaceful atmosphere for study and overall improvement. I bow my head with great reverence to ***Bharat Ratna Mahamana Pandit Madan Mohan Malviya Ji.***

I deem it to be a rare opportunity and the proudest privilege of my life to express my profound sense of gratitude, sincere honour and veneration to my advisor ***Dr. Vinod Kumar Paswan****, Assistant Professor, Department of Animal husbandry & dairying, Institute of Agricultural Sciences, Banaras Hindu University, for his sagacious and benevolent guidance, indelible inspiration, persistent encouragement, valuable suggestions, ever ready assistance, invaluable criticism, keen interest and everlasting affection during the entire course of study and in preparation of the manuscript. I will ever cherish the fatherly affection that he bestowed upon me throughout my tenure as a student under him which helped me to cope with many difficult situations.*

I am highly grateful to members of my advisory committee ***Dr. Dinesh Chandra Rai,*** *Professor and head, Department of Animal husbandry & dairying and* ***Dr. R.K. Pandey****, Professor, Department of Animal husbandry & dairying and* ***Dr. S.P. Singh*** *Department of Agronomy, Institute of Agricultural Sciences, for their benevolent guidance, encouragement, valuable suggestions and help during the course of investigation.*

I am equally thankful to all my seniors specially Mr.Vishal Jain, Mr. Ritesh prashad Shah, Mr. Vinod Bhateshwar, Mr. Himanshu Rai, Mr. Dheeraj Bagdi, Mr. D.S. Goswami, Mr. Hetram Chhimpa, Mr. Gurjeet Singh, and Mr. Rajesh Dhakad (ACF,MP) for their valuable guidance and encouragement.

It will be unfair if I don't mention the names of my batchmates like Vishal kumar, Hitesh, Dwarki Lal, Govind Singh, Yamini S, Saloni Rai for their kind assistance throughout

mycourse work and my friends Balwant, Mukesh Chahar, Rahul, Anuj, Kailash, Pawan, Shubash, Sunil, Satdev my best friend Ramsingh Suthar (Income Tax Department) and my B.Sc. junior Anjali Nagori (Ad), Ashok Sharma, Hansa Jhanjhra, Sunil, Rambabu, Bhupendra, Minakshi, Mogly, Anil Swami, Vishnu Bishnoi, Manish Bishnoi, Neeraj Tandi, Ajeet, Ajay, Salinder that made me grow more stronger while I request others their forgiveness for not indicating them hereby name, who will always remember in my heart.

Special thanks Neki Joshi, Mukesh Bhutna, Babulal and Gagan Sidana for their vital support and sparing their valuable time to compile and complete my research document.

I allocate my highest respect and heartfelt regards from my inner core of heart to my ever loved parents, ***Shri Ramchander Bishnoi*** *and* ***Smt. Kamla Devi,*** *who have given me life and taught the concepts of life and their dedicated efforts to educate me to this level, with boundless affection. I will never forget my grandparents* ***late Shri Bhupram Bishnoi*** *and* ***late Smt. Roshani Devi*** *and my source of inspiration, drive and support without whom I may not be studying in this prestigeous institution, It gives me immense satisfaction to express gratitude to my elder* ***Mr. Rahul Bishnoi (Technical Assistant, ARS Mandor)*** *and my younger sister* ***Ekta Bishnoi*** *for their never ending love, affection, understanding and giving me real strength to move forward and face the challenges in life.*

At last I bow my head with devotion to ***Lord Ganpati, Guru Jambheshwar Bhagwan, Lord Balaji*** *and* ***Kashi Vishwanath*** *who in unison are the sole owner of everything I have.*

Date: …June 2019

Place: Varanasi ***(Dheeraj Kumar)***

LIST OF SYMBOLS AND ABBREVIATIONS

%	Percentage
@	At the rate of
°C	Degree centigrade
e.g.	For example
et al.	Co-authors
etc.	Et cetera
Fig.	Figure
G	Gram
hrs	Hour
i.e.	That is
Kg	Kilogram
ml	Mililitre
ppm	Parts per million
no.	Number
IU	International unit
µg	Microgram
SE	Standard error
viz.	(Videlicet) Namely
P	Phosphorus
Ca	Calcium
Mn	Manganese
Cu	Copper
Fe	Iron
Zn	Zinc

LIST OF TABLES

LIST OF FIGURES

CONTENTS

Chapter-I

INTRODUCTION

Cattle occupy a unique role in human history, having been domesticated since at least the early Neolithic age. Archeozoological and genetic data indicate that cattle were first domesticated from wild aurochs (*Bos primigenius*) approximately 10,500 years ago. They must have been very valuable to early human settlements, for they quickly became ubiquitous across world cultures and are considered the oldest form of wealth. Cattle are dual-purpose animals, raised for both milk and draught purposes.

India has livestock population of 512.05 million, out of which the cattle population is approximately 190.9 million (19th Indian livestock census, 2012). India ranks first among the world's milk producing nations since 1998 and has the largest bovine population in the world. However as per the FAO statistics, 2011; the per cow milk production in India averaged at 1169 kg/year which amounts to only 12% of the same for USA, which stands highest at 9678 kg/year. In India, animal husbandry is not merely a subsidiary to agriculture but it is a major activity which plays a vital role in livelihood, employment, economy, food security, nutrition and contributes to balanced growth, gender equality and in reducing rural poverty. Livestock sector contributes approximately 4.11% to Indian GDP.

The major cause of low average milk yield per animal is higher number of non-producing cattle. These non-productive animals share the already scarce feed, fodder, water and other resources and are burden to the farmer. Through intensive efforts and proper use of synchronization technique and available resources they can be turned into productive ones.

Infertility is the diminished or absent capacity to produce viable offspring. There are many causes of infertility in cattle. If pregnancy/calving rates are below the optimum level it becomes imperative to find out the cause.

Bovine fertility is affected by nutritional and non nutritional factors. The non nutritional factors include micro-climate of the stable, lay-out of the stable, hygiene and genetic manipulations.

Severe underfeeding or overfeeding may cause irregularity of the oestrous cycle and thus affect conception and may lead to higher incidence of dystocia. The most direct relationship between nutrition and fertility exist in the first three months of lactation when the energy demand of the high-producing dairy cow becomes increasingly difficult to sustain. Energy deficiency leads to delayed or silent heat and if conception occurs, placental development is affected and may cause abortion. Protein deficiency on the other hand may disturb hormone metabolism and hence fertility, although reduction in milk production is the first indication and fertility becomes secondary.

The most common cause of infertility in cattle is poor nutrition (Navarre *et* al., 2010). Minerals have a great impact on animal's reproductive physiology. Their imbalance causes various problems leading to lowered reproductive efficiency and resultant monetary loss to the dairy industry. Adequate micro minerals supplementation is required as most of the roughages, greens, concentrates and even most of commercial feeds available to Indian market are deficient in trace mineral elements. Often, correcting an imbalance in mineral levels can solve the nagging problem of infertility by improving reproductive performance and health with little additional cost. (Kumar *et al.,* 2011*)*

In addition to energy and proteins, minerals have a significant effect on fertility. Deficiency of either calcium or phosphorus or a wide calcium : phosphorus ratio or deficiency of sodium leads to irregularity of oestrous, silent heat or cessation of the cycle. The trace elements implicated directly with bovine fertility are copper, cobalt, zinc, manganese, iodine, and selenium. In addition vitamins A, E and have also been shown to influence fertility performance.

These vitamins are added in feeds as preformed vitamins except for vitamin which is synthesized by rumen microorganisms from dietary cobalt. Each of these vitamins is involved in a number of varied metabolic and physiological functions. Thus vitamin A is important in maintenance of the integrity of the epithelial membranes, while vitamin E is an important biological antioxidant. Vitamin E is also involved in synthesis of gonadotrophins, while vitamin B^{12} is involved in energy metabolism in the ruminant animal.

The main source of vitamin A for ruminants is B-carotene which is available in abundant quantities from green foliage. In the intestinal mucosa, B-carotene is converted to vitamin A. The importance of B-carotene had been viewed solely as a source of vitamin A until 1955. Since then, stabilized vitamin A has become available for use in animal feeds and research has extended into the comparison between vitamin A and B-carotene. It was not until the 1970s that B-carotene was suspected to have effects independent of its role as a vitamin A precursor in bovine reproduction.

Beta carotene is important for oestradiol synthesis, stimulation of progesterone synthesis and scavenging of free radicals during hormone production. Beta-carotene is a precursor for vitamin A and the importance of beta-carotene in bovine reproduction is equivocal. Recent investigations of beta-carotene and vitamin A have focused on ovarian function especially on luteal development, progesterone production and fertility. Its deficiency resulted in extended duration of oestrus, delayed ovulation, retarded development of corpus luteum and a higher incidence of ovarian cysts which led to low conception rates and abortions in early pregnancy. (Jukola *et al.*, 1996). Plasma beta-carotene concentrations during the peripartum period may affect ovulation in the first follicular wave postpartum in dairy cows (Kawashima *et al.,* 2009). Ovulation and pregnancy require high concentration levels of vitamin A in the follicle/CL. Only beta-carotene plasma levels can modulate or determine Vitamin A concentration in the follicle/follicular fluid. Beta-carotene supplementation improves reproduction (Akar *et al.*, 2006) and milk yield parameters (Arechiga *et al.*, 1998).

The various nutritive factors that also adversely affect the oestrus cycle include nutritive deficiencies of calcium, phosphorus, copper, manganese, zinc, iron, total protein, cholesterol etc.

Modie (1965) described the role of calcium in sensitising the tabular genetalia for the action of hormones. A very high percentage of calcium and phosphorus is located in the bone and most of these minerals can be mobilized when needed for use in the metabolic events of body tissue. Calcium and phosphorus are closely related to many metabolic events in the body Jacobson *et al.* 1972*).*

The usual symptoms of phosphorus deficiency are delayed onset of puberty in heifers and failure of oestrus in cows (Roberts, 1971). Bhaskaran and Abdulla Khan (1981) reported that marginal deficiency of phosphorus is sufficient to cause disturbance in pituitary ovarian axis without manifestation of deficiency.

Low serum copper level in cows and heifers, result In suppression of oestrus and oestrus cycle (Sane *et al.* 1958). There seems to be an interaction between estrogen and copper (Turpin *et al.,* 1951). Delayed or depressed oestrus has also been encountered in cattle grazing on copper deficient pasture (Allcroft *et al.* 1949).

Lack of manganese inhibits the synthesis of cholesterol and its precursors. This in turn limits the synthesis of sex hormones and possibly other sex steroids with consequent infertility (Daisey, 1972).

Zinc deficiency causes abnormal oestrus and cystic degeneration of the ovaries. Zinc enhances the actions of FSH and LH (Church, 1979)

Iron deficiency results in or are' complicated by anaemia, debility lack of appetite and consequently a reduce intake of feed. As with cobalt deficiency, a secondary inanition, a failure of oestrum and delayed onset of puberty occur. Protein helps in maintenance of osmotic pressure, synthesis of several hormones and absorption including transport of organic and inorganic constituents of body. Protein is an essential component for both dam and growing foetus (West and Todd, 1967).

Cholesterol being the precursor of steroid hormones is expected to vary in postpartum period. (Jadhav *et ai.* 1977). The cholesterol level is directly related with dietary energy intake.

Velhankar (1973) reported that high level of dietary energy revealed higher cholesterol content and better reproductive performances. Hancock (1948) obtained induced ovulatory oestrus in

73.7 per cent of the treated cases with varying dilution of Jugol's solution as intrauterine infusion.

Thus, the objective of the present study was to examine the effects of beta carotene and nutritive minerals on infertility of cross bred cows using feeding experiments and to provide useful evidence supporting the application of beta carotene in the diets of cross bred cows. Experimental procedures were approved by the department of animal husbandry & dairying, IAS, Banaras Hindu University, Varanasi (Uttar Pradesh).

The study was conducted in a parity, body weight (BW, 400 ± 50 kg) were divided into two groups with 12 replicates in each group. The experiment includes two treatments: one is 5.0 g/day of β-carotene (50 g/day of Rovimix, containing 10% β-carotene, DSM Nutritional Products) and other is control. Beta-carotene was mixed with wheat bran in a cup and top dressed to individual treatment cows on the feed offered in the morning, and control cattle received nothing. The intake of β-carotene was observed visually. Data are recorded for the 12 replicates that received the treatments for 45 days i.e. 1st April to 15th May.

The present investigation entitled **"The Effect of β- Carotene Incorporated Mineral-Vitamin Premix on Amelioration of Infertility in Crossbred Cattle"** was initiated with the following objectives in crossbred herd in dairy farm.

1) To study the effect of β-carotene incorporated mineral-vitamin premix on infertility in infertile crossbred cattle of B.H.U. dairy farm.
2) Blood mineral (Ca, P, Cu, Mn, Zn, Fe) status before and after treatment in crossbred cattle of B.H.U. dairy farm.
3) Serum biochemical (Total protein, Cholesterol) status before and after treatment in crossbred cattle of B.H.U. dairy farm.

Chapter II

REVIEW OF LITERATURE

The present investigation **"The Effect of β- Carotene Incorporated Mineral-Vitamin Premix on Amelioration of Infertility in Crossbred Cattle"** relates to study the effect of β-carotene incorporated mineral-vitamin premix on infertility, Blood mineral (Ca, P, Cu, Mn, Zn, Fe) status and Serum biochemical (Total protein, Cholesterol) status before and after treatment in cross bred cattle. Knowledge about the previous works done is necessary to get an idea about the relevance and the prospects of the work to be done. A review of work conducted in cattle, in following aspects and other related studies is presented here under:

Hemken and Bremel (1982) reported that reproductive performance in cattle was improved with dietary supplementation with vitamin A. The active form of vitamin A for

reproduction in rats and other species has not been determined; however, vitamin A does meet the reproductive requirements. The ruminant animal does have differences in carotene metabolism from the rat. Before the role off or reproduction in cattle can be clarified, the specific function of carotene or the specific form of vitamin A for reproduction needs to be determined. There is improvement in conception rate, intensity of estrus, and changes in luteinizing hormone patterns when cattle received supplementation of low carotene diets. These diets were supplemented with vitamin A.

Bindas *et al.*(1984) conducted study on seventy-eight recently calved Holstein cows to receives beta carotene supplementation or act as controls to determine effects of on beta carotene reproduction and carotene, luteinizing hormone, progesterone, insulin, glucose, and glucagon concentrations in blood plasma. Cows were fed a corn silage-based complete ration. At day 30, supplemented cows received 600 mg synthetic beta carotene daily for 60 days. Plasma carotene reached a peak of 2.45 micrograms/ml compared to 1.50 micrograms/ml in controls. Progesterone increased as lactation progressed. Somatic cells were not different between supplemented and control cows. Supplementation of beta-carotene did not improve reproductive efficiency or alter luteinizing hormone, progesterone, insulin, glucose, or glucagon in blood plasma or affect somatic cells in milk.

Rakesh *et al.* (1985) conducted study on thirty-six Holstein cows fed a corn silage-based ration and thirty-four fed an alfalfa-grass silage-based ration were assigned according to calving date to receive either 300 mg/head per day of synthetic in a gelatin capsule or an empty gelatin capsule daily for the first 100 days postpartum. Supplemental vitamin A was provided at 3919 IU/kg of ration dry matter. Cervix diameters for cows supplemented with beta carotene were smaller at 21 days and 28 days postpartum. Days from parturition to first observed estrus were less when beta carotene was added and less when corn silage was Means of other reproductive traits were more favorable for cows treated with beta carotene (fewer services per conception and shorter intervals between parturition and conception). No effects of roughage or beta-carotene on milk production or milk fat percentages were significant.

Chew *et al.* (1993) stated that many advances have been made in understanding the many diverse roles of vitamin (retinoids). Among these are the critical roles that vitamin A

plays in regulating reproduction in both the male and female. The identification of retinol-binding proteins produced by the pig uterus and concepts marks an exciting this aspect of carotenoid function event. It will be pivotal to future efforts in elucidating the mechanism by which retinoids regulate concepts development and steroidogenesis. However, relatively little is known of the possible direct role played by its provitamin, beta carotene, in controlling reproduction in the pig. However, future research likely will address.

Arechiga *et al.* (1998) conducted study was tested the efficacy of timed artificial insemination (AI) and beta-carotene supplementation for improvement of reproduction. Experiments 1 and 2 were conducted during months, and experiment 3 was conducted during cool months. Cows were fed rations supplemented with at 0 or 400 mg/d per cow for > or = 15 d before the first AI. Cows were inseminated at each observed estrus after 70 d (Experiment 1) or at 50 d postpartum (Experiments 2 and 3) or were included in a timed AI program d 0 ((i.e., approximately 40 or 60 d postpartum), 8 µg of GnRH agonist; d 7, 25 mg of $PGF_{2\alpha}$ d 9, 8 micrograms of GnRH agonist; d 10, AI) for first breeding. Pregnancy rate at first AI was similar among groups, but the percentage of cows that were pregnant by 90 d postpartum was greater for cows in the timed AI group in Experiments 1 (16.6% vs. 9.8%) and 2 (34.3% vs. 14.3%) but not in Experiment 3 (24.1% vs. 28.7%). Overall, beta- carotene had no effect on reproductive function. For cows fed supplemental for > or = 90 d, however, pregnancy rate at 120 d postpartum was increased in Experiment 1 (35.4% vs. 21.1%). In conclusion, timed A.I. can improve pregnancy rates during periods of heat stress.

Maurice and Lonergan (2003) documented that energy status is generally considered to be the major nutritional factor that influences reproductive processes, with prolonged low energy intake impairing fertility. In cattle, a strong correlation between negative energy balance in early lactation and resumption of ovulation postpartum is evident. While ovulation may not occur in animals on low dietary intakes, follicle growth and atresia will occur. The practical significance of this occurrence is a lengthening of the calving to first ovulation interval, and often an extension of the calving to conception interval. These effects, however, are not immediately evident and dietary restriction for several months may be required to prevent follicle growth and ovulation. On the other hand, including supplementary fats in inadequate diets, results in increased concentrations

of LH.

Larson (2005) reported that the role of trace minerals in animal production is an area of strong interest for producers, feed manufactures, veterinarians and scientists. As animal trace mineral status declines immunity and enzyme functions are compromised first, followed by a reduction in maximum growth and fertility, and finally normal growth and fertility decrease prior to evidence of clinical deficiency. In order to maintain animals in adequate trace mineral status, balanced intake and absorption are necessary.

Akar and Gazioglu (2006) reported that in cows with retained placenta, serum vitamin A values were significantly lower in the 2^{nd} week after parturition when compared with the other weeks. In the control group, the values were significantly higher the 6^{th} week than those in the 2^{nd} and 4^{th} weeks. The calving to the first oestrus and calving to conception intervals were considerably longer in the retained placenta group. They concluded that poor fertility parameters in cows with retained placenta, as compared to the control group, could relate to the low content of vitamin A and especially serum beta carotene.

Alosilla *et al.* (2007) conducted an experiment to evaluate the bioavailability of five sources of vitamin A. Fifty three yearling Angus Brahman cattle, consisting of 39 steers and 14 heifers, were stratified by BW and sex and randomly assigned to 6 high-concentrate diet groups receiving no vitamin A supplementation (control) or vitamin A supplemented from the following sources: Microvit A (Adisseo, Acworth, GA), Rovamix A (DSM, Parsippany, NJ), Sunvit A, Lutavit A, and Microvit A DLC (Adisseo). At all collection times, Microvit A led to numerically, but not significantly, greater concentrations of retinol in liver than did all other treatments. However, at the end of the experiment, there was no significant difference in liver retinol concentration among Microvit A, Rovamix A, Lutavit A, and Microvit A DLC diets.

Ceylan *et al.* (2008) reported on serum Ca, P, Mg, Zn, and Cu concentrations in cows affected with reproductive disorders. The mean concentration of Ca in cows with repeat breeding was found to be significantly higher ($P<0.01$) than that in the control and anoestrous group. The P concentration in cows with repeat breeding and anoestrus was significantly lower ($P<0.01$) than in the control group. As the etiology of the reproductive diseases is very broad, even a slight decrease in serum levels of Zn and Cu may induce or

predispose animals to repeat breeding and anoestrus. In the light of these findings, the rations of animals with reproductive disorders are recommended to be supplemented with Zn, Cu, and P.

Kawashima *et al.* (2009) reported that beta-carotene functions independently of vitamin A in the reproductive performance of dairy cows. The concentrations of beta-carotene in plasma decrease during the dry period, and reach a nadir in about the first week postpartum. They used 22 multiparous Holstein cows, which were fed a total mixed ration consisting of grass, corn silage and concentrate, and collected blood samples for beta-carotene and progesterone analysis from week 3 prepartum to week 3 postpartum when the period of day 0-6 after parturition was regarded as the parturient week (week 0). Plasma beta-carotene concentrations at week 3 prepartum were greater in ovulatory cows (2.97+/-0.24 mg/L) than in anovulatory cows (1.53+/-0.14 mg/L; $P<0.001$), after that its concentrations in ovulatory cows decreased and reached the lowest level at week 1 postpartum, although its concentrations in anovulatory cows remained unchanged. No differences in plasma beta-carotene concentrations between the two groups were observed postpartum.

Ondarza *et al.* (2009) conducted a study in well-managed, high- producing commercial dairy herd to test the impact of supplementing beta- carotene (425 mg/d per cow) to lactating multiparous Holstein cows with normally low serum beta-carotene (<3 µg/mL). Overall pregnancy rate was unaffected by treatment, but after 105 d of beta-carotene supplementation, pregnancy rate was 22% for beta-carotene-supplemented cows compared with 11% for control cows.

Kumar *et al.* (2011) reported that micro minerals have a great impact on animal's reproductive physiology and its imbalance causes various problems leading to lowered reproductive efficiency and resultant monetary loss to the dairy industry. Adequate micro minerals supplementation is required as most of the roughages, greens, concentrates and even most of commercial feeds available to Indian market are deficient in trace mineral elements. As terrain and agro climatic area of India is quite diverse, so one therapeutic treatment may not be suitable for other regions. Hence there is a need to map of the various nutrient statuses in soil, fodder and animal, so that accordingly an area specific mineral

may besupplemented.

Spears (2011) reported deficiencies of many minerals and vitamins required by cattle can result in metabolic disorders that impair animal health. Nutrient deficiencies during pregnancy in cows often result in metabolic disorders and increased mortality in their calves. Assuring adequate trace mineral and vitamin status is critical for optimizing the immune response during the transition period.

AY *et al.* (2012) conducted this study examined the effects of beta carotene injections before estrus synchronization with $PGF_{2\alpha}$ on fertility parameters in cows. A total of 124 postpartum (pp) cows were used. In GI (n=25), (Carofertin, 0.4 mg/kg BW, I/M) was injected on d 15 and 45 post partum. In GII (n=25), was administered on d 15 post partum. In GIII (n=25), was administered on d 45 post partum. In GIV (n=25), was injected on d 35 and 45 post partum. GV (n=24) was set aside as a control group. Positive correlation between the serum concentrations of at insemination and at day 7 of the luteal phase and the concentrations of progesterone and the conception rate, lead to a conclusion that peri-ovulatory and vitamin A supplementation could improve follicular growth and development of the corpora luteal in cows with fertility impairment.

Amin (2013) conducted a study on the relationship between nutrition and reproduction it is a topic of increasing importance and concern among dairy producers, veterinarians, feed dealers and extension workers. Nutritional deficiencies cause various reproductive problems. Progress in improving the fertility of domestic animals has been achieved by studying genetic, nutritional, endocrine, disease and managemental factors as they contribute to both fertilization failure and embryo mortality.

Yasothai *et al.* (2014) reported that cow requirements for minerals are influenced by several factors including age, stage of pregnancy and stage of lactation. Trace mineral absorption and use performs an essential role in dairy cow reproduction and hoof health. The trace minerals play critical roles in the proper functioning of enzymes, hormones and cells. Deficiencies can, and often do, result in less-than-optimal performance and lost opportunity cost.

Lotfollahzadeh and Golctin (2016) conducted a study to evaluate the effect of

intramuscular administration of vitamin A during dry period in pregnant dairy cows which have already received it in their daily ration on vitamin A status of neonatal calves. Single intramuscular injection of 2000000 IU vitamin A was carried in 10 dairy cows at 7 months of pregnancy (group 1) and in another 10 dairy cows at 8 months of pregnancy (group 2). Ten pregnant dairy cows received saline injection as placebo and were selected as the control group Daily supplementation of vitamin A in late pregnancy in dairy cows may not compensate for the calves need for vitamin A and single injection of this vitamin A during dry period either in 7 or 8 month of pregnancy can significantly increase level of vitamin A in their neonatal calves.

Metabolism and function of B-carotene

B-carotene metabolism

Glover,(1960) and Sharma *et al.*(1977) discussed the various pathways in which carotenoids might be converted to vitamin A as shown in Figure 1. These researchers indicated that two primary oxidative reactions might occur, one at the central 15,15' double bond (central cleavage) and the other at one or more of the other double bonds (excentric cleavage). The central cleavage yields two molecules of vitamin A while excentric cleavage yields one short and one long 8-apo-carotenal, such as cyclocitral and B-apo-8'- carotenal. Ganguly and Sastry, (1985), and Villard and Bates,(1986) confirmed earlier reports by Bendich,(1987) and Olson, (1969) that B-carotenoid 15,15'-dioxygenase is the enzyme responsible for central cleavage of B-carotene. This enzyme has been partly purified and characterized from intestinal mucosa of various animal species.

The enzyme catalyzing excentric cleavage has however not been characterized, though Juttner and Hoflacher, (1985) demonstrated the stoichiometric conversion of B-carotene to B-apo-carotenal. This cleavage is known to occur in microorganisms, plants, and also in humans (Ganguly and Sastry, 1985 and Villard and Bates, 1986).

Olson, (1989) postulated that experimental data as well as theoretical arguments tend to favour the central cleavage mechanism. Conversion of carotene to vitamin A occurs in the intestinal wall and also in the liver.In theory one molecule of B-carotene should form, on

hydrolysis two molecules of vitamin A. The efficiency of conversion is however rarely as high as this and in addition carotenes are not absorbed from the gut as efficiently as Vitamin A (Olson, 1989). The Agricultural Research Council, (1978,1980) states that 6pg, l-4pg, and 5-8pg of B-carotene in the diet are equivalent to 1m9 of vitamin A alcohol in pig, poultry, and ruminants, respectively while NRC, (1978) and Lotthammer, (1979) considered 1 mg of B-carotene to be equivalent to 400 IU of vitamin A in cattle.

Factors affecting conversion of B-carotene to vitamin A

Olson, (1989) managed to partly purify the enzyme B-carotenoid 15,15' dioxygenase from the intestines and other organs of several unnamed species. This enzyme was demonstrated to require molecular oxygen. This author further reported that the enzyme was inhibited by sulphydryl-binding reagents and ferrous ion chelating agents and had a pH optimum of between 7.5-8.5. Activity of 0-carotenoid 15,15' dioxygenase of the intestine is also affected by both the protein content of the diet and vitamin A status of the animal.

Various researchers have reported that high supplementation with vitamin A has a depressing effect on the conversion of 0-carotene to vitamin A (Kalyanakrishnan, *et al.* 1951 and Wise, *et al.* 1947), and as such more 0-carotene is saved for other functions. Thompson, (1975) stated that once a 0-carotene molecule has been split or terminally catabolized, it could not be resynthesized again. For this reason it might be desirable to keep the amount of 0-carotene which is converted into vitamin A to a minimum by ensuring that the daily ration supplied optimum amounts of vitamin A. Villard and Bates, (1986) and Grownowska-Sengerand Wolf, (1970) found that B-carotenoid 15, 15' dioxygenase activity in vitamin A-sufficient rats is 50% that in vitamin A depleted rats which suggests a homeostatic control mechanism for carotenoid cleavage. These researchers also suggested that the dietary carotenoids may induce activity of the intestinal enzyme. Grownowska- Senger and Wolf, (1970) also reported that the activity of the intestinal enzyme is depressed by approximately 50% in rats receiving low (5%)15 protein diets. Kamath and Arnrich, (1973) reported that the effects of high protein intake did not give consistent results. In one study however, appear-ance of retinyl esters from 8-carotene in the intestine and liver of rats fed a 40% protein diet was twice that of rats fed a 10% protein diet. Another factor, quoted by NRC (1978), affecting conversion ratio is biological source of the carotene. Meinecke, *et al.* (1986) found that B-

carotene given as an intramuscular injection is utilized immediately by the body and not stored as Vitamin A. They observed that B-carotene was rapidly absorbed and metabolized with a half-life of about four hours.

Thompson, (1975) reported a considerable variation in the efficiency of converting B-carotene to vitamin A by different species. Goodman *et al.* (1966) and Blomstrand and Werner (1967) reported that approximately 15% of B-carotene is absorbed unchanged in cattle, horses, and humans, while in most other species, dietary B-carotene is largely converted to vitamin A before absorption (Ribaya-Mercado *et al.* 1989). They further reported absence of B-carotene in blood plasma of guinea pigs and rabbits.

Physiological functions of vitamin A

Besides the well known functions of vitamin A in the visual process, vitamin A is also known to have many systematic effects on various physiological processes including growth, embryonic development, fertility, haemopoiesis, immune response, epithelial cell differentiation, synthesis of corticosterone, prophylaxis of neoplasms and bone development. On epithelial cell differentiation, deficiency affects the major barrier to infection in the bronchial, gastrointestinal, urinary and other epithelia. Weakening of these barriers by keratinizing metaplasia is probably the mechanism as a result of which the incidence, severity and duration of various infections are increased (World Health Organization, 1982).

Physiological function of B-carotene

B-carotene can be said to have all the functions of vitamin A. However this is indirect as it has to be converted by a dioxygenase to vitamin A. In addition, work done in Germany Meyer *et al.* (1975), Lotthammer *et al.* (1976), Lotthammer and Ahlswede (1977), Schams *et al.* (1977) and Ahlswede and Lotthammer (1978) indicated that B-carotene has specific functions of its own independent of vitamin A. These include wound healing, avoidance of mastitis, anti-carcinogenic, growth promoter, metabolism (activation of the thyroid), and influence on butterfat content in milk. Of the functions shared by both vitamin A and B-carotene, the major ones being fertility and immune response, the latter has been found to be more potent.

Speculated mode of action of B-carotene in influencing reproduction

Very little is known yet about the non-vitamin a related mode of action of B-carotene. However in recent years various hypotheses have been put forward which may partly explain the physiological mechanisms. It has been thought for some time that the ability of the corpus luteum to synthesise progesterone depends on its B-carotene supplies (schultz *et al.* 1973), Jackson (1981) and Meinecke *et al.* (1984) showed that the synthesis of steroid hormones by the ovaries is reduced in cows with low plasma B-carotene levels. Lotthammer *et al.* (1978) found a positive correlation between cholesterol levels and B-carotene concentration. Many of the symptoms occurring with low plasma B-carotene levels can be explained in the light of the intricate interrelationship between reproductive hormones and reduced synthesis of these hormones in 6-carotene deficiency. Moreover, Schweigert *et al.* (1986) found that the intrafollicular vitamin A concentration depends on B-carotene in plasma and is correlated with follicle quality, follicle size, and intrafollicular oestradiol-17B. These findings further confirm the independent role of B-carotene in ovulation, and more generally, to the fertility of dairy cows. Kovanen *et al.* (1979), suggested that since the low-density- lipoprotein associated cholesterol fraction in blood is the main substrate unit for ovarian production of steroid hormones, B-carotene could thus affect hormone synthesis through the metabolism of cholesterol. Jackson, (1982) postulated the theoretical sites for such action to be:

1) In the liver by facilitating production of cholesterol from cholesterol ester.

2) In the blood by acting as cholesterol "transport" agent.

3) In the ovary by assisting the uptake of cholesterol and/or by facilitating the production of progesterone.

4) At the gonads during steroidogenesis as an anti-oxidant.

The hypothesis and speculations in this area must never the less be left open in the absence of definitive proof but it is a fact that bovine fertility is better (Rensburg and Des vos, 1962), plasma cholesterol levels higher (Sinha *et al.* 1981), and return to cyclicity post-partum quicker (Peters and Riley, 1982), during seasons when plenty of green forage is available. However, these researchers suggest that it is important to differentiate between a possible B-carotene nutritional effect from those of day length, temperature and other nutritional factors, which require detailed and lengthy field studies supported by in vitro laboratory work.

B-carotene requirements

The evidence available indicated that B-carotene improves reproduction in dairy cattle. It has also been shown that 13- carotene supply from pastures varies with season. It is therefore important to establish B-carotene requirement in dairy cattle.

Jackson (1982) pointed out that critical plasma concentration for normal fertility was a matter of debate but he quoted some researchers as suggesting this to be between 100-300 mg.

In 1978, Lotthammer gave the B-carotene requirement for young cattle to be about 100 gm/day which also applies to dairy cows during the dry period. Later, Lotthammer, (1985) calculated the daily vitamin A - independent requirement of 13-carotene per animal to be 300-400 mg. Indeed, this researcher categorized plasma concentration below 400 as questionable for adequate supplies in dairy cows, while concentrations below 250 mg and 150 mg are critical and deficient for optimum fertility, respectively.

Effect on fertility

Bovine corpus luteum has been found to contain higher concentrations of B-carotene than other tissues unlike in other species (Ahlswede and Lotthammer, 1978). This high 6-carotene content in the corpus luteum is dependent on plasma B-carotene (Anwandter, 1974). However, Ahlswede and Lotthammer, (1978) found no correlation between plasma and hepatic B-carotene in dairy cattle. In addition, the plasma B-carotene content for cattle at various stages of the oestrous cycle is not uniform. Seitaridis, (1963) found that luteal phase had the highest concentration followed by follicular phase while cows with ovarian cysts had the lowest amounts. No such variation was observed with vitamin A. These researchers also reported that the content of B-carotene in the corpus luteum and in other tissues depended on the type of feed the animals were on. Indeed, the concentration of B-carotene in these tissues and blood plasma varies with season or type of forage offered (Anwandter, 1974). This was found to be higher when the pasture was green in summer and low in winter when feeding was on hay. In the tropics one would also expect low B-carotene in foliage during the dry season. Schultz *et al.* (1973) surveyed the B-carotene and progesterone concentration in the corpus luteum at slaughter and not only did they confirm that B-carotene levels were two to three times higher in summer than in winter but also found a similar pattern for progesterone levels. These

findings were also supported by Meyer *et al.* (1975) and Lotthammer and Ahlswede, (1977) who showed that plasma B-carotene is a reflection of supplementary B-carotene or the pasture quality.

Effect of B-carotene on conception was studied by Konermann, (1974) who reported that first insemination to conception interval was shortened by ten days when B-carotene supply to the herd was increased by 100 mg/day. Calving interval and the time-lag between onset of oestrus and ovulation were also shortened. The duration of oestrus was shorter with 0.5 hours while the incidence of cystic ovaries was nil compared to 50% in the control group. The interval between peak luteinizing hormone (LH) and ovulation was delayed by almost 24 hours in the (3-carotene deficient heifers. Schams *et al.* (1977) also reported a maximum delay of ovulation to be 72.5 hours compared to 49 hours after peak LH in deficient cows and animals given 8-carotene supplements, respectively. Meyer *et al.* (1975) also found that the corpora lutea of 8-carotene deficient heifers were smaller than those of the control group and reached maximum size four days later. Similar results were reported by Schams *et al.* (1977) who also noted that cystic changes in the ovaries in the form of lutea and/or follicular cysts developed in 45% of the heifers with the lowest B-carotene levels both in the blood and in various tissues. However, Schams and associates also found the level of vitamin A in the blood of the B-carotene deficient heifers to be significantly lower than that in the control group, despite a more than double supply of dietary vitamin A in the deficient group. These researchers concluded that with deficiency of B-carotene, for which low blood plasma levels are indicative, ovulation is delayed by about one day, the duration of oestrus is extended and the whole development of the *corpus luteum* is drastically retarded and impaired. This may in turn explain the abnormalities in the production of progesterone observed by Anwandter, (1974) and Schultz *et al.* (1973). Lotthammer *et al.* (1976) observed reproductive disturbances such as prolonged, poorly defined oestrous and nymphomania, observed as swelling of the vulva, moist and reddened vaginal vestibule with a low discharge of mucus during non-oestrous parts of the cycle, in heifers deprived of 6-carotene for a period 6 to 7 weeks, which was attributed directly or indirectly to the ovary. In a later study Lotthammer, (1978) observed that insemination at the wrong time risks infection of the vagina which may lead to further complication as a result of purulent inflammation.

Mingazov, (1977) did field trials on fi-carotene supplementation of dairy cows and reported that (3-carotene supplementation improved conception after the first insemination from 39 to 57 per cent. The differences persisted in the same order of magnitude during the second insemination. 13-carotene supplementation reduced the number of inseminations per conception from 2.0 to 1.4. Lotthammer, (1978) attributed the poor conception in B-carotene deficient cows to ovarian disorders and also observed that if conception occurred at all, embryonic death at around sixth and seventh week of gestation and risk of early abortion was imminent.

Wetherill, (1965) carried out a study to investigate the effect of B-carotene on puerperal problems in cattle. From this study, it was observed that blood carotene level of 300 M9% or more corrected or essentially decreased puerperal problems. Similar findings have been reported by Mihalka, (1981) who investigated the influence of B-carotene status on puerperal diseases in a Hungarian dairy cattle herd. Puerperal problems such as delayed uterine involution and retained placenta were increased when cows were B-carotene deficient (Mihalka, 1981) and a decrease in puerperal disorders improved conception rate. Lotthammer, (1978) reported no B-carotene effect on the incidences of retained placenta, although Akordor *et al.* (1986) and Inaba *et al.* (1986) found a direct correlation between retention of placenta and the plasma concentration of vitamin A and B-carotene. It has been suggested that B-carotene either plays a specific vitamin A independent role in reproduction of cattle, or it acts on uterus-placenta separation after conversion into vitamin A (Inaba *et al.* 1986).

Role of macro and micro nutrients in reproduction

In recent years the role of macro and micro nutrients in animal reproduction like disturbances in ovulation, anoestrosity as well as in the regulation of harmones have received greater attention but in relation to specific infertile conditions in formations are rather meager.

Scientists already have made some valuable contributions in this field highlighting the level of various blood minerals and their relationship with the reproduction.

In the present study the various blood minerals (viz. serum calcium, phosphorus, manganese, copper, zinc and iron) and total protein and cholesterol in crossbred cows were studied by different workers have been reviewed under the following sections.

Serum Calcium

Samad *et al.* (1980) estimated the serum calcium levels of 30 indigenous anoestrus cattle (14 heifers with genital hypoplasia and 16 cows with non-functional ovary) and 20 indigenous normally cycling cows and found no significant difference in serum calcium level of anoestrus and normally cycling cattle (9.10 ± 0.76 and 9.25 ± 0.85 mg% in heifers and cows in anoestrus vs 9.60 ± 0.47 in normally cycling animals.).

Rao and Rao (1982) studied the serum calcium level in 18 cross bred pre-pubertal heifers (9 Jersey x Ongole and 9 Holstein fresian x Ongole) at estrus under farm conditions. The levels of serum calcium either between estrus and anoestrus or between the two genetic groups were non-significant.

Prasad *et al.* (1984) reported that the mean calcium value in crossbred cows on the day of heat was significantly higher (10.18 mg per cent with a range from 8.2 mg to 12.30 mg per cent) than that in anoestrous cows (9.97 mg per cent with a range from 8.20 to 12.30 mg per cent).

Sahukar *et al.* (1984) measured the serum calcium level at oestrus in 10 crossbred cows (% Red Dane x V2 Red Sindhi) and the mean values were 9.55 ± 0.112 mg per cent.

Chetty and Rao (1986) studied the total serum protein in cyclic crossbred heifers having varying level of Jersey blood (25% to 87.5%). They reported that serum calcium was found to remain unaltered between cyclic heifers (9.99 ± 0.16 mg% and anoestrus heifers (9.66 ± 0.16 mg%).

Dutta *et al.* (1988) conduct an experiment on a total of 12 Jersey heifers, 2-3 years of age and above 120 kg body weight under same standard feeding and managerial conditions.

They reported that the difference of serum Ca level between anoestrus heifers of more than 2 years of age with normal size nonfunctional ovaries (10.73 ± 0.06 mg%) and normal cyclic heifers with normal ovaries (11.02 ± 0.05 mg%) was non-significant.

Vadnere and Singh (1989) opined that the mean plasma level of calcium was 9.67 ± 0.28 mg% in an estimation on 21 postpartum anoestrus crossbred cows.

Kumar and Sharma (1991) estimated the serum calcium levels in 26 non-de script estrus cows and were found nonsignificant difference in between fertile (9.14 ± 0.18 mg%) and nonfertile oestrus (8.60 ± 0.26 mg%) cows.

Rupde *et al.* (1993) reported that the level of serum calcium was (9.84 ± 0.659) mg% in normal breeding cows.

Shrivastava *et al.* (1995) reported that serum calcium was slightly higher (Non-significant) in normal cycling (9.46 ± 0.26 mg%) than in delayed pubertal (8.95 ± 0.298 mg%) crossbred heifers.

Tandle *et al.* (1997) reported that the level of serum calcium were (10.07 ± 0.31) and (9.33 ± 0.01 mg%) in oestrus and anoestrus non-descript cows respectively.

Joe Arosh *et al.* (1998) analysed serum samples from 6 normal cyclic and 6 anoestrus Jersey crossbred cows and observed that concentration of serum calcium was significantly lower (8.98 ± 0.38 mg%) in anoestrus than normal cyclic animals (10.71 ± 0.36 mg%).

Sood *et al.* (1999) reported that mean level of serum calcium was 6.38 ± O. 18 mg% in case of anoestrus heifers.

Kumar *et al.* (2000) observed that serum calcium concentration was found to be lower in prepartum cows (10.56 ± 0.77 mg/dl) than the postpartum cows (11.21 ± 0.93 mg/dl.)

Dutta *et al.* (2001) reported that serum calcium level was significantly lower in postpartum anoestrus cows (9.54 ± 0.22 mg%) than the normal cycling cows (10.73 ± 0.0'8 mg%).

Das *et al*, (2002) reported that in their study they do not found any significant variation in serum Ca level between the groups of cyclic and repeat breeder crossbred cows.

Sheshappa *et al.* (2002) reported that serum calcium level in postpartum crossbred cattle ranged from 8.78 to 9.75 mg% between the pre and postpartum samples.

Jain *et al.* (2003) conducted a study on fertility of crossbred Cattle by mineral supplementation. They reported that the serum calcium level during anoestrus was 8.20 ± 0.33

mg% and 6.75 ± 0.33 mg% in normal cyclic cows and in mineral supplemented group respectively, and on the day of estrus was 8.79 ± 0.31 mg% and 9.72 ± 0.31 *mg%* in normal cyclic cows and in mineral supplemented group, respectively.

Shah *et al.* (2003) reported from their study that the serum calcium level in postpartum fertile and infertile buffalo was 9.45 ± 0.10 mg% and 9.08 ± 0.15 mg% respectively.

Serum Inorganic Phosphorus

Laing (1979) opined that ovarian functions are interfered with phosphorus deficiency.

Dindorkar and Kohli (1979) reported that mean inorganic phosphorus levels were lower in anoestrus cows (5.71 mg / 100 ml) than in nonnal (8.01 mg/100 ml) cows. They concluded that phosphorus deficiency might be the cause of prolonged anoestrus in these cows.

Bhaskaran and Khan (1981) observed a significant difference in serum inorganic phosphorus level between anoestrus (4.22 ± 1.34 mg/ 100 ml) and oestrus cows (7.59 ± 2.40 mg/ 100ml).

Reddy (1982) reported that higher plasma inorganic phosphorus profiles were associated in fertility in cows.

Bhaskaran and Patil (1982) observed that the mean serum inorganic phosphorus concentrations during estrus and anoestrus in heifers were 7.59 ± 0.22 and 5.96 ± 0.16 mg/ 100 m, respectively. Their study indicated that minimum critical level of phosphorus was perhaps necessary for initiation and maintenance of ovarian activity or onset of oestrus and also indicated a possible close relationship between blood serum phosphorus level and reproductive hormones.

Prasad *et al.* (1984) studied serum phosphorus in anoestrus crossbred cows and recorded the average level as 7.80 mg per cent.

Sahukar *et al.* (1984) reported from their study that the level of inorganic phosphorus during anoestrus in crossbred cows is 6.25 mg%.

Chetty and Rao (1986) opined that the level of inorganic phosphorus in the blood of cycling heifers (5.93 ± 0.12 mg%) was significantly higher ($P < 0.01$) than in anoestrus heifers (4.57 ± 0.11 mg%).

Siviah *et al.* (1986) reported that phosphorus had a role to initiate and maintain ovarian activity leading to onset of oestrus.

Hafez (1987) reported that phosphorus deficiency in range cattle and sheep caused ovarian dysfunction which in tum lead to delayed puberty, depressed signs of oestrus and eventually cessation of oestrus.

McDonald *et al.* (1987) observed that low dietary intake of phosphorus had been associated with poor fertility and apparent dysfunction of the ovaries causing inhibition depression or irregularity of oestrus.

Dutta *et al.* (1988) found that the mean serum inorganic phosphorus levels were significantly ($P < 0.05$) lower in anoestrus heifers than in the normal cycling animals.

Vadnere and Singh (1989) reported that level of inorganic phosphorus was significantly lower in anoestrus cow (6.225 ± 0.02 mg/ dl) than normal cows.

Kumar and Sharma (1991) opined that inorganic phosphorus level was significantly lower (4.98 ± .08 mg%) in non-fertile estrus cows than in fertile estrus cows (6.44 ± 0.42 mg%).

Yadav (1993) reported from his study that phosphorus was essential for general health and reproduction.

Ashtukar *et al.* (1995) observed that there was no significant difference in serum inorganic phosphorus level between anoestrus and repeat breeder cows.

Ramkrishna *et al.* (1997) studied the level of serum inorganic phosphorus in cycling cows and anoestrus cows and recorded the level as 5.30 ± 0.117 and 4.29 ± 0.15 mg% respectively. They also emphasized the need for certain levels of inorganic phosphorus for estrogen secretion.

Tandle *et al.* (1997) concluded that serum level of inorganic phosphorus was significantly higher in conducted an experiment on 6 normal cyclical estrus cows than in anoestrus cows.

Arosh *et al.* (1998) concluded that serum P was significantly lower in anoestrus cows than normal cyclical cows. Phosphorus is often associated with reproductive abnormalities in cattle and its deficiency induces anoestrus and reduces ovarian activity.

Sood *et al.* (1999) estimated the level of serum inorganic phosphorus level in cows of Himachal Pradesh and the level was 3.68 ± 0.26 mg/dl.

Singh and Pant (1998) observed from their study on cows of Himachal Pradesh that serum P level is not significantly higher normal cows (5.91 ± 0.16 mg%) than the repeat breeder cows (4.89 ± 0.14 mg%).

Kumar *et al.* (2000) found that serum inorganic phosphorus level was lower in prepartum cows (4.95 ± 0.16 mg/dl) than the postpartum cows (5.68 ± 0.30 mg/dl).

Outta *et al.* (2001) reported that the serum inorganic phosphorus level was significantly lower in postpartum anoestrus cows (3.48 ± 0.12 mg %) than the cyclic cows (4.22 ± 0.07 mg%).

Oas *et al.* (2002) found in their study that the serum P level is significantly lower in repeat breeder crossbred cow (4.729 ± 0.150 mg/ 100 mI) than the nonnal cyclic cows (5.513 ± 0.265 mg/100mI).

Sheshappa *et al.* (2002) reported from their study on postpartum crossbred cows that the serum inorganic phosphorus level was range from 4.10 to 5.43 mg% between pre and post treatment samples.

Cetin *et al.* (2002) reported that the serum inorganic phosphorus level is higher in normal fertile cows (5.69 ± 0.12 mg/dl) than the repeat breeder cows (5.00 ± 0.23 mg/dl).

Jain *et al.* (2003) conducted a study on fertility of crossbred cattle by mineral supplementation. They reported that the serum inorganic phosphorus level in normal cyclic cows was 4.46 ± 0.14 mg% and 4.78 ± 0.14 mg% during anoestrus and on the day of estrus

respectively and in the mineral supplemented group the serum inorganic phosphorus level was 3.98 ± 0.14 mg% and 4.99 ± 0.14 mg% during anoestrus and on the day of induced estrus respectively.

Shah *et al.* (2003) opined that the serum inorganic P level in postpartum fertile and infertile buffalo was 5.86 ± 0.07 mg% and 5.62 ± 0.07 mg%,· respectively.

Serum Copper

Rowlands *et al.* (1977) recorded the mean serum copper level which was 0.73 mg/l in 351 lactating dairy cows at 40 to 100 days past-calving.

Desai *et al.* (1982) observed that serum copper level at estrus, ovulation time, luteal and follicular phases were 189.99 µg%, 189.76 µg%, 195.35 µg% and 204.79 µg% respectively in Surti buffaloes.

Dabas *et al.* (1987) estimated the serum concentration of copper to be 185 ± 11 µg/dl, 130 ± 8 µg/dl in cyclic and anoestrus cows respectively in an observation of 77 crossbred cows.

Vadnere and Singh (1989) measured the mean plasma copper level from 21 postpartum anoestrus crossbred cows and the average value was 138.47 ± 10.20 µg/dl which was significantly lower than in normal cycling cows.

Saxena and Gupta (1992) while experimented on 20 crossbred heifers that in anoestrus heifers with smooth ovarian condition the plasma level of copper (171.11 ± 15.89 µg/dl) was significantly lower in comparison to the level in heifers (255.00 ± 10.87 µg/dl) that cycling normally.

Rupde *et al.* (1993) conducted an experiment with 10 repeat breeder and 5 regular breeding cows. And found that the serum copper level was significantly lower in repeat breeder (0.847 ± 0.052 µg/ml) than regular breeder cows. (2.304 ± 0.010 µg/ml).

Rajasundaram and Rajasekaran (1994) estimated a serum copper value of 53.32 ± 0.6 µg/dl in cyclic cows and 48.25 ± 0.58 µg/dl in repeat breeding cows.

Samanta *et al.* (1995) reported that there was Significant reduction of serum eu in anoestrus with anaemic cattle (51.66 ± 5.47 µg/100 ml) as compared to control animals (110.65 ± 4.65 µg/ 100 ml).

Prasad and Rao (1997) conducted an experiment on 4 anoestrus and 4 repeat breeder cows and they reported that in normal cycling cows the Cu level was 87 µg/ 100 ml whereas both in anoestrus and repeat breeder cows the Cu level was subnormal.

Sharma *et al.* (1999) reported that serum Cu level was lower (10.10 ± 1.78 µg/dl) in anoestrus heifer having smooth ovaries. Whereas the Cu level was 130.60 ± 4.73 µg/dl in proestrus, 103.20 ± 2.94 µg/dl in estrus, 113.40 ± 60.47 µg/dl in metestrus and 116.40 ± 3.60 µg/dl in diestrus.

Kumar *et al.* (2000) reported from their study that the serum Cu level is significantly lower in prepartum cows (21.30 ± 2.53 µg/dl) than the postpartum cows (26.20 ± 0.97 µg/dl).

Mehere *et al.* (2002) reported that serum Cu level differs significantly (P < 0.0 I) during the peripartum periods in crossbred cows.

Das *et al.* (2002) reported that in crossbred cows the serum Cu level is significantly lower in repeat breeder group (0.96 ± 0.017 µg/ml) than the normal cyclic group (0.97 ± 0.023 µg/ml).

Jain *et al.* (2003) conducted a study on fertility of crossbred cows by mineral supplementation. They reported that serum copper level during anoestrus is 153.2 ± 14.61 and 140.6 ± 14.61 µg% in normal cyclic cows and in mineral supplemented group respectively and on the day of estrus the serum levels were 165.00 ± 12.89 µg% and 180.00 ± 12.89 µg% in normal cyclic cows and in mineral supplemented group, respectively.

Shah *et al.* (2003) reported that serum Cu level in postpartum fertile and infertile buffalo is 1.24 ± 0.02 µg% and 1.16 ± 0.02 µg% respectively.

Serum cholesterol

Purohit and Kohli (1977) measured serum cholesterol level in 60 Rathi cows and found lower in anoestrus (188.61 mg%) than that is estrus (264.30 mg%) period.

Murtuza *et al.* (1978) obtained the total serum cholesterol levels to be 153.84 ± 5.40 mg%, 119.41 ± 6.90 mg%, 147.19 ± 6.72 mg% and 181. 43 ± 13.69 mg% in heifers, empty dry cows, late pregnant cows and early lactating cows of Hariana breed respectively.

Hafez (1980) reported that the immediate precursor for all the steroids is pregnenolone derived from cholesterol.

Rawlands *et al.* (1980) reported that in bovine low bloo cholesterol levels to poor fertility.

Rao *et al.* (1982) measured the serum cholesterol levels in Holstein Fresian x Ongole crossbred cows (normal cyclic and different stages of reproduction) they reported that the serum cholesterol level was higher in estrus when compared to that in proestrus and di-estrus stage.

Sharma *et al.* (1984) observed the serum cholesterol levels as 91.15 ± 628 mg%, 99.62 ± 8.23 mg% and 102.62 ± 8.95 mg% in anoestrus, cyclic and repeat breeding. Crossbred cows respectively. The difference was however non-significant.

Aminuddin *et al.* (1984) estimated that serum cholesterol levels in 30 normally reproducing (15 cows and 15 heifers) and 45 anoestrus (15 physically good anoestrus cows, 12 physically poor anoestrus cows and 15 anoestrus heifers) Rathi cattle of arid tract of Rajasthan. Serum cholesterol level was found to be significantly lower in anoestrus heifers (177.50 ± 3.79 mg%) than that in normally reproducing heifers (217.46 ± 3.16 mg%).

Sahukar *et al.* (1985) opined that the serum cholesterol level was highest at estrus (290.00 ± 9.31 mg/100 ml). An increasing trend of cholesterol level was noticed up to 8 month followed by a sudden fall in cholesterol concentration.

Dutta *et al.* (1988) estimated the serum cholesterol levels in, 12 Jersey heifers of age varying from 2 to 3 years. And it was reported to be significantly lower in anoestrus heifers (99.86 ± 8.34 mg%) than that in normal cycling heifers (129.80 ± 8.20 mg%*).*

Kumar and Sharma (1991) estimated blood serum cholesterol in 26 nondescript cows and it was found to be significantly higher (105.22 ±2.06 mg%) in fertile estrus than that in non fertile (99.17 ± 1.37 mg%) estrus.

Phogat *et al.* (1992) reported that the plasma cholesterol levels decreased with parturition and increases thereafter.

Singh *et al.* (1996) conducted an experiment on 121 crossbred cow of Jersey, Holstein fresian and Gir. They concluded that serum cholesterol level is significantly higher in estrus cows (120.55 ± 2.01) mg/100 ml than that of the non-oestrus cows (78.54 ± 0.49) mg/100 ml.

Bhaga *et al.* (1997) observed that serum cholesterol level increase up to 21 days of progesterone treatment for induction of oestrus in buffalo heifers.

Singh and Pant (1998) observe in their study on cows in Himachal Pradesh that the serum cholesterol level is significantly higher (240.9 ± 11.68 mg%) in cows which conceive normally than that of the cows which fail to conceive after repeated attempts (125.4 ± 4.03 mg%).

Nayyar *et al.* (1998) found no significant difference in serum cholesterol level between the low age (27.5 ± 1.3 months) pubertal buffalo heifers (55.05 mg/dl) and high age (33.5 ± 0.8 months) pubertal buffalo heifers (55.095 mg/dl).

Kumar *et al.* (2000) reported from their study that serum cholesterol level in case of prepartum cows in lower (193.15 ± 8.08 mg/dl) than the postpartum cows (202.13 ± 5.35 mg/dl).

Kabir *et al.* (2001) reported that in case of rural buffalo the serum cholesterol breed is significantly higher in cyclic animals (146.35 ± 8.41 mg/ dl) than the acyclic animals (113.57 ± 1.91 mg/dl).

Cetin *et al.* (2002) reported that serum cholesterol level is higher in repeat breeder cows (138.93 ± 8.59 mg/dl) than the fertile cows (128.20 ± 4.83 mg/dl).

Shah *et al.* (2003) reported that the serum cholesterol level in postpartum fertile and infertile buffalo is (180.97 ± 4.37) and (188.25 ± 6.30 mg%).

Serum total protein

Little (1974) reported that the decrease serum protein immediately after parturition was due to decrease synthesis of albumin by liver and due to diversation for the synthesis of milk protein.

Hewett (1975) studied a negative relationship between protein, phosphorus and urea nitrogen.

Naidu and Rao (1982) studied on 50 crossbred anoestrus animals (25 heifers and 25 cows) and 20 normal cycling animals. (10 heifers and 10 cows) of Chittor district of Andhra Pradesh. The total serum proteins in cycling heifers (9.20 ± 1.85 gm%) and cows (9.43 ± 1.22 gm%) was recorded to be significantly higher (P < 0.0 1) than that in anoestrus heifers (7.17 ± 1.52 gm%) and cows (7.87 ± 1.50 gm%).

Chetty and Rao (1986) estimated the total serum protein of anoestrus and cyclic crossbred heifers having varying level of Jersey blood (250/0 to 87.50/0) maintained by the farmers under traditional husbandry practices. They reported that total serum protein in cycling heifers (8.24 ± 0.17 gm%) was noted to be significantly higher (P < 0.01) than that in anoestrus heifers (7.06 ± 0.16 gm%).

Pedroso *et al.* (1986) opined that blood total protein concentrations were highest in pregnant cows than those, which were non-pregnant.

Mehta *et al.* (1989) cited increased level of serum protein in cows with the advancement of pregnancy.

Khan *et al.* (1990) reported that the average serum protein levels were slightly lower in pregnant buffaloes than the nonpregnant one.

Quayam *et al.* (1990) reported that serum protein value remained unaltered during the prepartum. It declines significantly at the time of parturition in Murrah buffaloes.

Setia *et al.* (1992) total plasma protein levels declined significantly (P < 0.05) on the day of parturition as compared with prepartum phases.

Montana *et al.* (1994) observed that total protein level in serum increased during the first three months of gestation in ewes.

Singh *et al.* (1996) conducted an experiment on 121 crossbred cows of Jersey. Holstein-Friesian and Gir. They concluded that serum total protein level is higher in oestrus cows (9.50 mg/ 100 ml) than the non-oestrus cows (8.69 mg/ 100 ml).

Singh and Pant (1998) observed in their study on cows in Himachal Pradesh that serum total protein level is not significantly higher in normal cows (7.70 ± 0.11 gm%) than the repeat breeder cows (7.60 ± 0.10 gm%).

Prabhakar *et al.* (1999) reported that there was gradual, decline in total plasma proteins at the time of parturition in buffaloes.

Kumar *et al.* (2000) reported from his study that the total serum protein level is higher in prepartum cow (10.33 ± 0.71 gm/dl) than the postpartum cows (9.35 ± 0.26 gm/dl).

Sheshappa *et al.* (2002) reported from their study on postpartum crossbred cattle that the level of serum total protein ranges from 8.68 to 9.13 between pre and post treatment samples.

Kabir *et al.* (2001) reported that in case of rural buffalo the serum total protein level is not significantly higher in cyclic animals (8.46 ± 0.11 gm/dl) than the acyclic animals (7.92 ± 0.11 gm/dl).

Cetin *et al.* (2002) reported that total serum protein level is higher in case of repeat breeder cows (8.50 ± 0.14 *gm/dl)* than the fertile cows (8.26 ± 0.13 gm/dl)

Manowar and Singh (2002) reported that the serum total protein level is lower in non-cycling heifers (5.34 ± 0.16 gm/dl) than the cycling heifers (7.35 ± 0.24 gm/dl).

Shah *et al.* (2003) reported that the serum total protein level in postpartum fertile and infertile buffalo is 8.87 ± 0.07 mg% and 8.83 ± 0.07 mg% respectively.

Serum Zinc

Wegner *et al.* (1973) estimated serum zinc level 117 ± 39 µg/dl with the range of 85 to 175 µg/dl in 10 Holstein-Friesian lactating dairy cattle.

Ghergariu *et al.* (1986) stated that in 651 healthy crossbred cows mean serum Zn value was 120 ± 20 µg/dl.

Dabas *et al.* (1987) obtained the serum Zn concentration 310 ± 13 µg/dl in cyclic and 305 ± 9 µg/dl in anoestrus crossbred cows.

Sharma *et al.* (1988) recorded the serum Zn level in 24 healthy Kankrej heifers and significant difference between the normal cyclic heifers (0.44 ± 0.03 µg/ml) and anoestrus heifers (0.66 ± 0.20 µg/ml) were found.

Saxena *et al.* (1991) opined that plasma Zn values in heifers was ranged from 133.10 ± 15.16 to 336.36 ± 9.00 µg/dl and heifers having average zinc level of 230.00 µg/dl attained puberty earlier than those having lower plasma zinc concentration.

Saxena *et al.* (1992) observed that plasma level of zinc was lowest in heifers (274.44 ± 17.81 µg/dl) exhibiting smooth ovarian condition compared to normally cycling heifers (604.37 ± 85.71µg/dl).

Behera *et al.* (1993) reported a significant difference in serum Zn level of un-treated cycling heifers (103.53 ± 2.40 mg%) and delayed matured heifers (53.96 ± 1.9 mg%).

Jain, G.C. (1994) observed in crossbred cows that the average level of Zn in anoestrus and repeat breeder animals were 1.45 µg/ ml and 0.96 µg/ml respectively.

Joy and Nair (1995) found no significant difference in serum Zn concentration between fertile and anoestrus cows.

Samanta *et al.* (1995) reported that the level of zinc in anoestrus cow having anaemia was 444.94 ± 49.75 µg% and in normal cow was 384.24 ± 39.20 µg%.

Prasad and Rao (1997) observed that mean serum zinc concentration of normal cow was *185* µg/ 100 ml and anoestrus cow had lower serum zinc concentration (ranged 31 to 64 µg/100 ml) than normal.

Kalita *et al.* (1999) in an experiment showed that serum Zn concentration was significantly low in postpartum anoestrus (2.44 ± 0.21 ppm) than in normal cycling cows (3.03 ± 0.21 ppm).

Mehere *et al.* (2002) reported from their study that the serum Zn level exhibited significant ($P < 0.05$) variation among different groups of crossbred cows during the peripartum period.

Das *et al.* (2002) reported that serum Zn level in crossbred cows was significantly lower in repeat breeder group (1.80 ± 0.033 µg/ml) than normal cyclic group (2.09 ± 0.057 µg/*ml).*

Jain *et al.* (2003) conducted a study on fertility of crossbred cows by mineral supplementation. They reported that serum Zn level was 370.5 ± 38.64 µg% and 138.6 ± 38.04 µg% in anoestrus and normal cyclic cows respectively. Whereas, serum Zn level on the day of estrus in these groups were 459.4 ± 54.77 µg% and 253.4 ± 54.47 µg% respectively.

Shah *et al.* (2003) reported that the serum Zn level in postpartum fertile and infertile buffalo was 1.28 ± 0.03 µg% and 1.33 ± 0.04 µg%, respectively.

Serum Manganese

Groppel· and Anke (1971) reported that manganese deficient animals showed no signs of estrus despite normal ovulation. Several services were required per conception.

Howes and Dyer (1971) and Anke *et al.* (1973) found that low Mn rations to cows caused depressed or delayed oestrus and conception followed by increased abortion, still births and lowered *birth* weights.

Arthur (1978) opined that Mn probably acted in enzyme system influencing oestrus, ovulation, foetal development, udder development etc. A deficiency of Mn would accordingly cause delayed oestrus, reduced fertility, abortions, resorptions, deformed youngs, poor growth etc.

Hafez (1987) observed in gilts and cows with manganese deficient diet caused ovarian disturbances exhibited weak signs of oestrus to anoestrus.

Oas *et al.* (1993) observed that the concentration of Mn in blood of anoestrus cows was 16.31 ± 4.68 µg%.

Rupde *et al.* (1993) reported that serum Mn level was found to be significantly lower in repeat breeder (0.170 ± 0.009 µg/m1) than in normal cyclic cows (0.464 ± 0.007 µg/ml).

Jain (1994) studied on anoestrus cows and opined that the average level of Mn in anoestrus cow was 0.98 µg/ml.

Joy and Nair (1995) recorded serum Mn level serum from fertile, anoestrus and repeat breeder cows, which was nonsignificant among different groups.

Samanta *et al.* (1995) observed that there was decrease level of serum Mn in anoestrus cattle. They also found the level of Mn in normal cycling cattle was 6.69 ± 0.46 µg% and in anoestrus was 4.10 ± 0.07 µg%.

Oas (1997) reported that the serum Mn content of heifer and adult cattle in new alluvial zone of West Bengal was 0.54 ± 0.052 and 0.62 ± 0.047 µg/ml respectively.

Prasad and Rao (1997) in a study with 4 anoestrus and 4 repeat breeder cows observed that the concentration of Mn both in anoestrus and repeat breeder was higher than normal (18 µg/100 ml). the concentration of serum Mn in repeat breeder and in anoestrus animals were ranged from 37-50 µg/100 ml.

Kalita *et al.* (1999) observed no significant difference among anoestrus (0.38 ± 0.05 ppm), repeat breeder (0.42 ± 0.04 ppm) and normal cycling cows (0.45 ± 0.04 ppm.).

Kumar *et al.* (2000) reported that the serum manganese level was higher in prepartum cows (13.33 ± 3.33 µg/dl) than the postpartum cows (10.0 ± 0.00 µg/dl).

Mehere *et al.* (2002) observed that highly ($P < 0.01$) significant variation prevail in the serum Mn levels of different group of crossbred cows during the peripartum period.

Das *et al.* (2002) reported that in crossbred cows there was no significant variation in serum Mn level between the repeat breeder and normal cyclic groups.

Jain *et al.* (2003) conducted a study on fertility of crossbred cows by mineral supplementation. They reported that serum Mn level during anoestrus in normal cyclic cows and in mineral supplemented group were 38.10 ± 14.53 µg% and 31.50 ± 4.53 µg% respectively. Whereas, on the day of oestrus the manganese level in those groups were 40.50 ± 4.53 µg% and 42.80 ± 4.53 µg% respectively.

Shah *et al.* (2003) reported that the serum Mn level in postpartum fertile and infertile buffalo was 0.08 ± 0.004 µg% and 0.09 ± 0.006 µg% respectively.

Serum Iron

Vadnere and Singh (1989) observed on 21 postpartum anoestrus crossbred cows that the serum iron level was 194.300 ± 8.35 µg/dl in anoestrus cows which was significantly lower than normal cyclic cows.

Rupde *et al.* (1993) opined that the serum iron level did not show any variation between repeat breeder and normal cyclic cows. The level of irop were recorded in repeat breeder was 2.467 ± 0.031 µg/ml and in normal cyclic cows was 2.455 ± 0.028 µg/ml respectively.

Jain (1994) said that the serum iron level in anoestrus cattle was 1.64 µg/ml.

Yessein *et al.* (1994) observed that the serum level of iron was higher (226.47 ± 10.14 µg%*)* in fertile cows than infertile cows having ovarian inactivity (198. 57 ± 7.58 µg%).

Samanta *et al.* (1995) reported lower level of iron in anoestrus cow with anaemia (485.05 ± 49.63 µg%) than normal cycling cows (678.42 ± 18.25 µg%).

Oas (1997) opined that the serum iron concentration in heifer and adult cattle were 3.13 ± 0.361 µg/ml and 2.791 ± 0.308 µg/ml respectively in new alluvial zone.

Prasad and Rao (1997) reported that serum iron concentration of anoestrus cows were lower than that of normal cows (30 µg/100 ml).

Ramkrishna (1997) observed that serum level of iron among anaemic anoestrus cows were lower (86.33 ± 6.27 µg/100 ml) than normal cycling cows.

Singh and Pant (1998) stated that concentration of iron in serum of repeat breeder cow was higher 113.7 ± 5.56 µg/dl than normal cycling animal (107.6 ± 5.06 µg/dl).

Kalita *et al.* (1999) measured the mean concentration of serum iron in normal cycling cows was 5.22 ± 0.32 ppm whereas the value was low in anoestrus cows (3.34 ± 0.27 ppm).

Kumar *et al.* (2000) opined that the serum iron conc.entration was lower in prepartum cows (90.30 ± 7.51 µg/dl) than that of postpartum cows (120.50 ± 5.l4 µg/dl).

Mehere *et al.* (2002) reported from their study that in case of crossbred cows serum iron level did not show significant variation during the peripartum periods.

Oas *et al.* (2002) reported that serum iron level in repeat breeder cows (3.594 ± 0.043 µg/ml) than the normal cyclic cows was (3.424 ± 0.053 µg/ml).

Shah *et al.* (2003) reported that serum iron level in postpartum fertile and infertile buffalo was 1.26 ± 0.03 µg% and 1.20 ± 0.04 µg%, respectively.

Effect of mineral supplementation on serum mineral concentration

Sampath and Kumar (1984) stated that supplementation of trace element particularly Cu and Co could favour a significant increase in blood level of the same in case of lactating cattle.

Aken *et al.* (1991) reported from their study that plasma levels of Ca, P, Mg, Cu, Zn and Fe could be elevated to the normal level by supplementation in case of pregnant milking cows suffering from mineral deficiency.

Vijchulata *et al.* (1994) concluded that mineral supplementation in 2nd and 3rd lactation of Holstein cows receiving a guinea grass and concentrate based diet brought about a rise in the plasma concentration of Ca, Fe and Zn.

Prasad *et al.* (1995) reported that in Indian condition feeding system is based upon straw, stover and grain by products, which are deficient in one or more minerals and as a result the animals become deficient. If this deficient animals is provided with optimum mineral containing rations the serum mineral concentrations become elevated.

Mee *et al.* (1995) concluded that supplementation of mineral vitamin mixture containing Cu, I, Se and Co significantly increased the concentration of trace elements in the blood of cows in the supplemented groups.

Mahanta *et al.* (1997) reported that supplementation of Ca through bone meal could result in higher blood Ca level (9.25 mg/dl in treatment group Vs. 9 mg/dl in control group), provided all the animals would have received an isocaloric and isonitrogenous ration with an identical Ca: P ratio.

Olsen *et al.* (1999) opined that a combination of Cu; Co, Mn and Zn in an organic form (Protein mineral complex) significantly raised serum Cu (0.85 µg /ml compared to 0.76 µg /ml in inorganic and 0.72 µg/ml in control group) and serum Zn (1.18 µg/ml compared to 0.85 µg/ml in control) levels in postpartum 1st calved cows.

Singh and Saraswat (2000) concluded that dietary Cu @ 5 mg/head/day could increase plasma Cu concentration and it was found to be beneficial against the rate of 10 mg/head/day as the latter dose affecting the availability of other minerals due to antagonistic reaction.

Sarkar (2000) observed that supplementation of a mineral mixture containing salts of Ca, P, Mg, Co, Cu, I, Fe and Zn brought about a significant increase in the plasma concentrations of Fe, Cu and Zn in both 1^{st} and 2^{nd} lactating cows. In this experiment the concentrations (µg /ml) of Fe, Cu and Zn were 5.6, 1043, 1.03 and 5.78, 1.53 and 1.05, respectively in case of 1st and 2nd lactating cows. The corresponding values increased ($P < 0.05$) to 5.64, and 1.08 in 1st lactating and 5.83, 1.56 and 1.10 in 2^{nd} lactating cows during the post supplemental period.

Sarkar (2002) reported that supplementation of Cu, Zn and Mn brought an increase in the plasma concentrations of these trace elements in the crossbred anoestrus cows.

Kumar *et al.* (2002) observed that feeding of mineral mixture increased circulating plasma Mn++ concentration in all age groups of non-cyclic heifers. The Mn concentrations (ppm) were found to be 0.71 ± 0.015, 0.71 ± 0.022 and 0.92 ± 0.037 of 10-13 months, 18.21 months and 27-30 months non-cyclic heifers respectively after 42 days of supplementation.

Jain *et al.* (2003) reported that there was significant increase in serum mineral during oestrus phase of the cows supplemented with sakes mineral mixture. During anoestrus the serum level of Ca, P, Cu, Zn and Mn· were 6.75 ± 0.33 *mg%,* 3.98 ± 0.14 mg%, 140.6 ± 14.61 µg%, 138.6 ±38.04 µg% and 31.50 ± 4.53 µg% whereas the estimated values were 9.72 ± 0.31 *mg%,* 4.99 ± 0.14 mg%, 180 ± 12.89 µg%, 253.4 ± 54.47 µg% and 42.80 ± 4.53 µg% respectively of Ca, P, Cu, Zn and Mn during oestrus phase.

Shah *et al.* (2003) opined from their study that there was no significant difference in the mean serum Ca level in normal cycling buffaloes. Similar results were also found in anoestrus buffaloes. In case of inorganic phosphorus the mean serum level in normal cycling cows was found to be non-significant whereas in the anoestrus and treated group (with mineral mixture) it was found to be highly significant.

Effect of mineral supplementation on reproductive performance

Groppel and Anke (1971) stated that manganese deficient animals showed no signs of oestrus despite normal ovulation several services were required per conception.

Morrow (1980) reported that phosphorus deficiency was found to hindered the vitamin A synthesis from carotene.

Bhaskaran and Patil (1982) observed a close relationship between blood serum phosphorus and reproductive hormones.

Agarwal *et al.* (1983) reported a very good oestrus induction rate (83.33%) and first service conception rate (80%) with oral supplementation of Cu and Co in anoestrus cattle.

Reddy *et al.* (1984) reported that trace elements have some important role in controlling ovulatory mechanisms.

Kumar (1986) treated 31 anoestrus buffaloes and 10 heifers with Tonophosphan and mineral mixture. 81-85% of the females exhibited oestrus within an average of 22-30 days of treatment and 76.91 % females conceived.

Dhoble and Gupta (1986) found that Ca and P play a vital role in the regulation of hormones and enzymes for initiation of oestrus.

Hefez (1987) reported that deficiency in manganese cause disturbances in the ovarian function.

Quayam *et al.* (1988) reported from their study that deficiency of phosphorus in the buffalo ration, caused delay in activation of normal ovarian function until occurrence of favourable condition.

Reddy *et al.* (1994) treated 20 rural postpartum anoestrus buffaloes by cyclomin-7 bolus. They found 55% exhibited oestrus while 81.81 % conceived.

Mee *et al.* (1995) observed that the mineral vitamin supplement had no effect on the incidence of abortion (1.4%), dystocia (2.30%), foetal maldisposition (7.30%), perinatal mortality (6.9%) or retained foetal membranes (4.0%).

Sarkar *et al.* (1996) opined that Co-Fe-Cu plus tablet (containing 40 mg cobalt sulphate, 100 mg iron sulphate, 200 mg copper sulphate and 450 mg manganese sulphate) given orally and successfully used to treat anoestrus in 27 of 34 cows.

Campbell and Miller (1998) reported that both vitamin E and Zn, supplemented to Holstein and Jersey primigravid heifers and cows, reduced days to first observed oestrus, indicating improved reproductive health during the early postpartum period.

Tiwari *et al.* (1999) observed that the cows showing the symptoms of anoestrus condition responded favorably to the supplementation of Cu (1.75 ppm), Co (0.06 ppm), Mn (1.0 ppm) and Zn (16.50 ppm) by showing -the occurrence of oestrus (62.2%), improvement of pregnancy rate (66.66%) of embryo transfer as well as in super ovulatory response.

Gosai *et al.* (1999) observed in their study on 41 Melssani and Surti buffaloes that supplementation of minerals and vitamins reduces the postpartum estrus intervals. Postpartum oestrus interval was 70.94 ± 3.41 days in treated group where as 102.29 ± 9.51 days in control group. The conception rate were 73.33% and 42.86% in treated and control group respectively.

Olsen *et al.* (1999) observed in a study that supplementation of Cu, Co, Mn and Zn had· no beneficial effect on reproduction of cows. The high levels (two times NRC, 1996) of trace

element supplementation caused decrease in reproduction in terms of less oestrus and breeding activity.

Lall *et al.* (2000) concluded that supplementation with a mineral mixture @ 50 gm/ animal/ day in 70 buffaloes and 30 adult heifers. More than 3 years of age showing anoestrus for more than 5-6 month showed that more than 70% of these animals exhibited oestrus and conceived within a period of 2-4 weeks.

Kumar *et al.* (2002) reported that 2 out of 6 non-cyclic heifers of 27-30 months when supplemented with mineral mixture, exhibited oestrus for a shorter duration of about 12 hours at the end of experiment.

Jain *et al.* (2003) reported 80% oestrus with conception rate of 60% at an average interval of 12 days in anoestrus cattle after supplementation of mineral mixture for 21 days.

Chapter III

MATERIALS AND METHODS

The present investigation entitled **"The Effect of β- Carotene Incorporated Mineral-Vitamin Premix on Amelioration of Infertility in Crossbred Cattle"** was carried out at Dairy Farm, Department of Animal husbandry and dairying, Banaras Hindu University, Varanasi (U.P.).

Place of work and animals

The study was planned and conducted on 24 cross-bred non-cyclic, non-pregnant cattle of B.H.U. dairy farm. Cattle, which were not observed in estrus for at least six months considered as non-cyclic animals. On the basis of clinical observations, cattle affected with any kind of infectious causes or structural pathological abnormalities were excluded from the study.

Selection of cattle

1. Breed

Cattle were selected of same breed, all cattle are cross bred (sahiwal×H.F.)

2. Age

Age of all cattle was around 3-6 years.

3. Body weight

Body weight of all cattle around 350-500 kg.

4. Body condition

Body condition of cattle is good muscular , attractive, shiny hair coat and active eyes.

General management practices

Management practices are the main determinant of the expression of natural behavioural patterns of the animals and any alteration of this natural behavioural patterns lead

to health disorders. Managemental practices followed in the present study have been discussed in different heading as follows.

Housing

The experimental animals were maintained in loose housing system under group Management practice with proper drainage, soft bedding, feeding and watering facilities. The feeding mangers were covered with shed type roof consisting of asbestos sheets at moderate height with low slope. At one corner of paddock, there was provision of drinking water trough with running tap water. The housing system is designed in such a way that it provides ample air movement and protects animals from extreme weather. Feeding manger and watering trough was as per BIS standard. This system of housing facilitated free movement, sufficient exercise to the animals and the animals exhibit their natural behaviour.

Feeding

The nutrient requirements of all experimental animals were mostly met with *adlibitum* green fodder and measured amount of concentrate. The green fodders, grown in the Institute farm, were supplied according to the seasonal availability. During summer and rainy seasons predominantly maize and sorghum were fed whereas in winter, fodders like barseem, oat, were fed. Feedings were spread in 3 to 4 feeding during day and night. The concentrate was fed @1.5 kg/day/animal for body maintenance in general. Concentrate mixture had 20% CP and 70% TDN consisted of 33% maize, 21% ground nut cake (oiled), 12% mustered cake (oiled) 20% wheat bran, 11% de-oiled rice bran, 2% mineral mixture and 1% common salt.

Supplementation of feed

1. Rovimix Cal-P strong (DSM nutritional product) (each 100 g contain)

Calcium	26 g
Phosphorus	14.25 g
Rovimix vitamin B_{12}	400 mcg

Rovimix vitamin D_3 16000 IU

2. Rovimix ovn dairy premix (DSM nutritional products)

Each 100g contains:	
Rovimix vitamin-A	2.000 MIU
Rovimix vitamin-D3	0.400 MIU
Vitamin-E	20.000MIU
Biotin	0.400 gm
Niacin	10.000gm
Beta-carotene	10.000gm
Iron	12.000gm
Copper	4.000gm
Manganese	15.000 mg
Zinc	16.000gm

Magnesium	80.000gm
Cobalt	0.400gm
Iodine	0.300gm
Selenium	0.120gm
Chromium	0.500gm
Potassium	5.000gm
Sodium	6.000gm

Dose.

1. 50 gm Rovimix ovn dairy premix (DSM nutritional products) in evening

2. 50 gm Rovimix Cal-P strong (DSM nutritional product) in morning

Table 3.1: TREATMENT GROUP

S.N	TAG NO.	COW/HEIFER	AGE (yr)
1	739	HEIFER	4

2	729	HEIFER	3
3	758	HEIFER	3
4	920	HEIFER	4
5	817	COW	5
6	688	HEIFER	3
7	757	HEIFER	4
8	697	HEIFER	4
9	815	COW	3
10	704	HEIFER	3
11	767	HEIFER	7
12	726	HEIFER	3

Table 3.2: CONTROL GROUP

S.N.	TAG NO.	COW/HEIFER	AGE
1	672	HEIFER	4
2	816	COW	5
3	804	COW	6
4	818	COW	5
5	819	COW	6
6	730	HEIFER	3
7	910	HEIFER	6
8	732	HEIFER	4
9	701	HEIFER	4
10	698	HEIFER	3

11	755	HEIFER	4
12	753	HEIFER	4

Beta carotene assessment

Blood samples were taken on day of the start of mineral supplement treatment, on 0 day and on 45 day, from jugular vein in sterilized collecting tubes. Tuberculin syringe was used to measure 400 μL (0.4 mL) of the fresh blood.

iCheck™ Carotene (BioAnalyt Germany) a portable photometer (provided by DSM) was used to determine beta carotene concentrations. It determined total carotenoid concentration in the fresh blood samples by measuring the color reaction in the test vial and calculated the carotene content in mg/L.

Collection of blood

About 10 ml of blood samples were drawn from the Jugular vern with 18g' sterilized needles from each animal, (both experimental and control). Blood samples were transferred immediately in dry, sterilized glass test tubes and kept at 450C angle in room temperature after proper coding.

Collection of serum

Serum samples were collected carefully into different sterilized micro-centrifuse tubes with the help of sterilized Pasteur pipettes and kept at -20^{o}C temperature till analysis.

Biochemical Parameters

Serum total protein, cholesterol, macro minerals (Ca and P), micro minerals (Cu, Fe, Zn and Mn) were estimated from, each serum samples.

Estimation of serum total protein

The level of total protein was determined by the Biuret method as described by Wotton(1964).

Estimation of serum cholesterol

The level of serum cholesterol was estimated in Microlab 200 Autoanalyser using commercial kits.

Estimation of serum Macro minerals

Serum Calcium

Serum calcium was estimated (Trudeau and Freier, 1967) with 1 ml of serum sample diluted to 1 : 50 in 0.1% (w/v). Lanthanum chloride which was used in the standard solution as well as in the blank serum samples were kept in plastic vials for subsequent analysis by atomic absorption spectrophotometer (A.A.S.). The result was expressed in µg/100 ml.

Serum phosphorus

Colorimetric method (Fiske and Subba Row ,1925) was used to estimate the phosphorus in serum samples quantitatively and the result was expressed in mg/100 ml.

Estimation of Serum trace minerals (Cu, Zn, Mn and Fe)

Serum trace (Cu, Zn, Mn and Fe) elements were estimated as per the method described by Sandel (1950) and modified by Arneza *et al.* (1977) using atomic absorption spectrophotometer (A.A.S.). The results were expressed in ppm (Parts per million). 1 ml of serum sample from each animal of control and experimental groups was digested separately with 20 ml of tri-acid mixture (concentrated nitric acid, concentrated sulphuric acid, concentrated perchloric acid, ratio 9: 2: I, respectively) and kept on hot plate at 180-200^{o}C until the mixture become clear watery in colour. Samples are then cooled at room temperature and transferred to 50 ml volumetric flasks and made up to the final volume of 50 ml. Which were transferred to properly labeled separate sterilized plastic vials and kept for subsequent analysis by atomic absorption spectrophotometer using standards at lab of A.H&D. Department. The results were expressed in (µg/dl).

Observations used for heat detection

All the cows were checked and parameters like duration of onset of estrous post protocol, total duration of estrous and the signs of estrous like-restlessness and mounting behavior, discharge and its amount, bellowing and tonicity of uterus were recorded.

Breeding

All cows were artificially inseminated with frozen semen of high fertility.

Statistical analysis

The Data obtained during investigation were subjected to statistical analysis using independent t-test.

RESULTS AND DISCUSSION

Investigation here to correct the reproductive performance of infertile cows has been tried with β-carotene incorporated mineral-vitamin permix.

Animals of the experimental treatment group responded well with the treatment scheduled. The percentage of animals had oestrus symptoms in treatment group were 50% with the mean interval of 22 days.

Table 4.1: Reproduction performance of experimental animals

Group	No. of Animals	No. of Animals showed oestrus	% of Animals in oestrus	Mean time interval for induction of oestrus (in days)	Conception rate (%)
Control	12	2	16.66	28	50
Treatment	12	6	50	22	66.66

Induction of oestrus (50%) and conception rate (66.66%) of animals in treatment group was corroborated with the findings of Poral *et al.* (1976), Sawale and Dhoble (1999) and Kadu *et al.* (2001). But the value indicated higher than reported by Babar *et al.* (1999) Singh and Saxena (2000) and Ahmed *et al.* (2003). However, lower than the results were reported by Rao *et al.* (1971), Signal (1995) and Dabas (1997).

In control group only 2 animal out of 12 exhibited the oestrus symptom with the mean interval of 28th day. Conception rate was observed 50%.

Beta carotene

Table 4.2: Status of Beta Carotene (mg/l) level in blood –

Days	Mean±SE		P-value
	Control	Treatment	
0	2.62±0.12	2.71±0.14	0.326
45	2.55±0.13	3.31±0.17	0.001

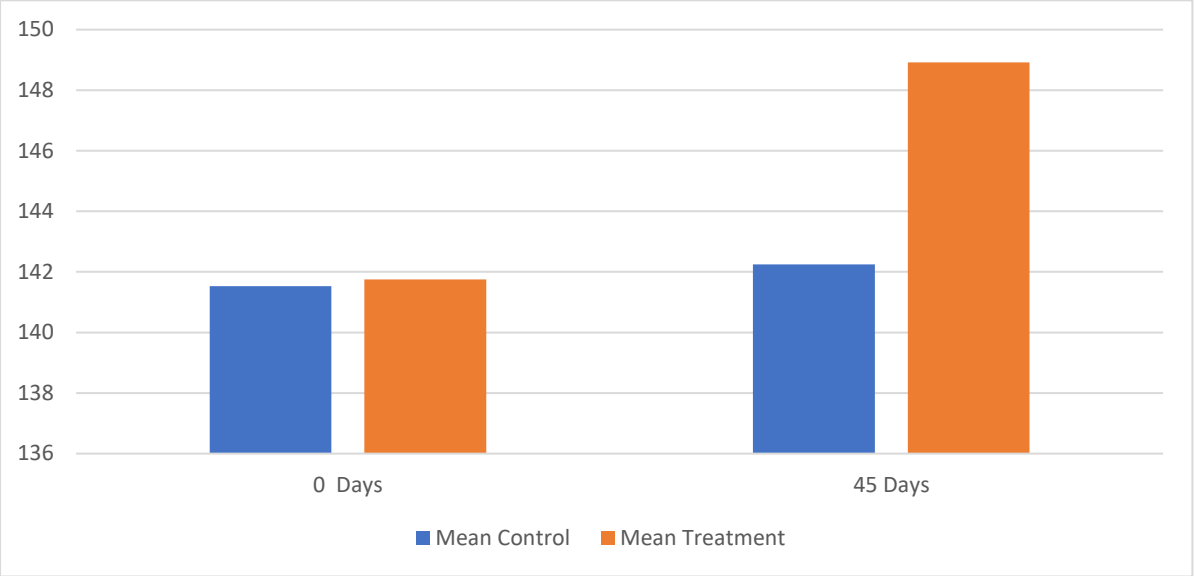

Fig 4.1: Diagram showing the status of Beta Carotene (mg/l) level in blood.

In treatment group, mean beta carotene level at day 0 i.e. before the start of the treatment, was 2.55±0.13mg/L. At day 45 of the treatment, the blood mean beta carotene levels rose to 3.31±0.17 mg/L. Six cows showed oestrus. The day of showing oestrus ranged from day 8 to day 45, with an average of 22 days.

In control group, mean beta carotene level at day 0 i.e., was 2.62±0.12 mg/L. At day 45, the blood mean beta carotene level 2.71±0.14 mg/L. 2 cows showed oestrus. The day of showing oestrus ranged from day 16 to day 45, with an average of 28 days.

Similarly Rakes *et al.*, (1985) reported feeding 300 mg beta carotene per head for 100 days resulting in improved reproductive parameters and Arechiga *et al.*, (1998) showing better results after 90 days of feeding in comparison to 15 days of feeding beta carotene in diet.

One of the possible roles of the beta carotene is its antioxidant effect as reported by Arechiga *et al.* (1998). Moreover, Arechiga *et al.* (1998) reported higher fertility rates in beta carotene supplemented cows in another study. In the present study, beta carotene might have an antioxidant effect and, high beta carotene levels might show its antioxidant effect in pregnant cows. The overall mechanism of beta carotene is not clearly understood and there are still controversies about its effect on reproduction Halilogu *et al.* (2002). While work erssuchas Graves-hoagland *et al.* (1989), Iwanska *et al.* (1997) and Akar *et al.* (2006) reported that beta carotene has a positive effect, there are also reports from Gossen *et al.* (2004, 2005) indicating

it has no effect whereas Bindas *et al.* (1984) and Yildiz *et al.* (2005) reported negative effect of beta carotene on reproductive parameters in cows.

It was thus observed that in anoestrous cows with low body score, the supplementation of beta carotene alone resulted in expressing of oestrus between day 8 to day 45 of supplementation, this is in accordance with the other studies (Arechiga *et al*. 1998, Iwanska and Strsinska, 1997) stating that there could be various factors responsible for the reproductive performance and that the beta carotene supplementation gives best results when there is a significant deficiency of beta carotene in the animal.

Total protein

Table 4.3: Status of Total protein (mg%) level in blood serum

Days	Mean±SE		P-value
	Control	Treatment	
0	7.79±0.27	7.82±0.17	0.463
45	7.24±0.17	9.31±0.14	0.000

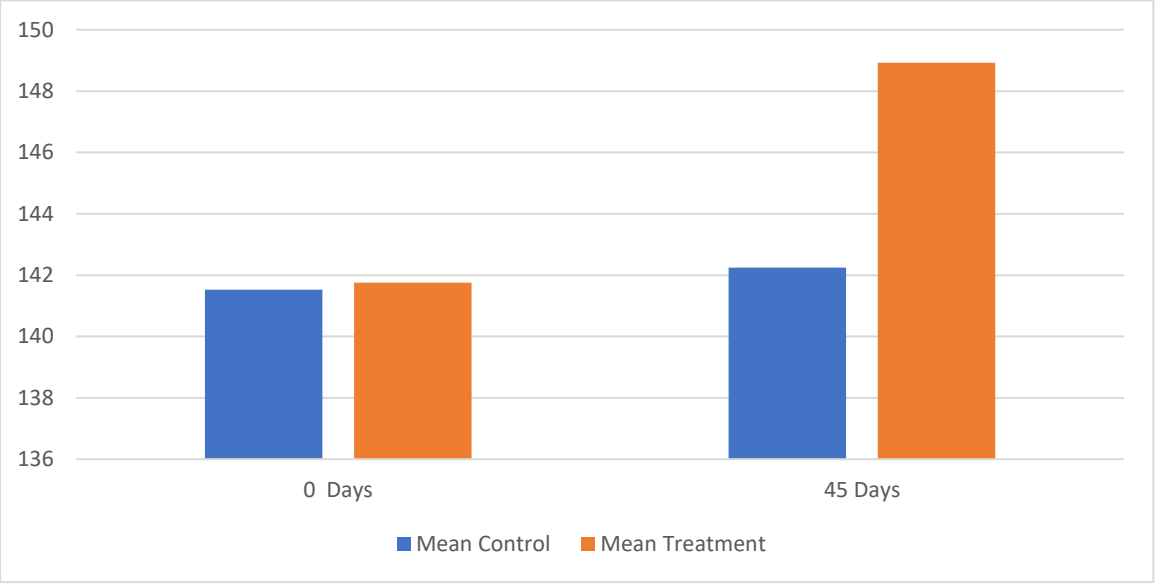

Fig 4.2: Diagram showing the status of total protein (mg%) level in blood serum.

The serum level of total protein in anoestrus condition was 7.0886 ± 0.323 gm% where as it was 9.4614 ± 0.3147 gm% during oestrus condition. The observation corroborated with the

findings of Naidu and Rao (1982), Chetty and Rao (1986), Singh et al. (1996) and Kabir et al. (2001).

Cholesterol

Tabel 4.4: Status of Cholestrol (mg%) level in blood serum

Days	Mean±SE		P-value
	Control	Treatment	
0	131.17±0.17	131.50±0.15	0.080
45	132.91±0.15	147.53±0.41	0.004

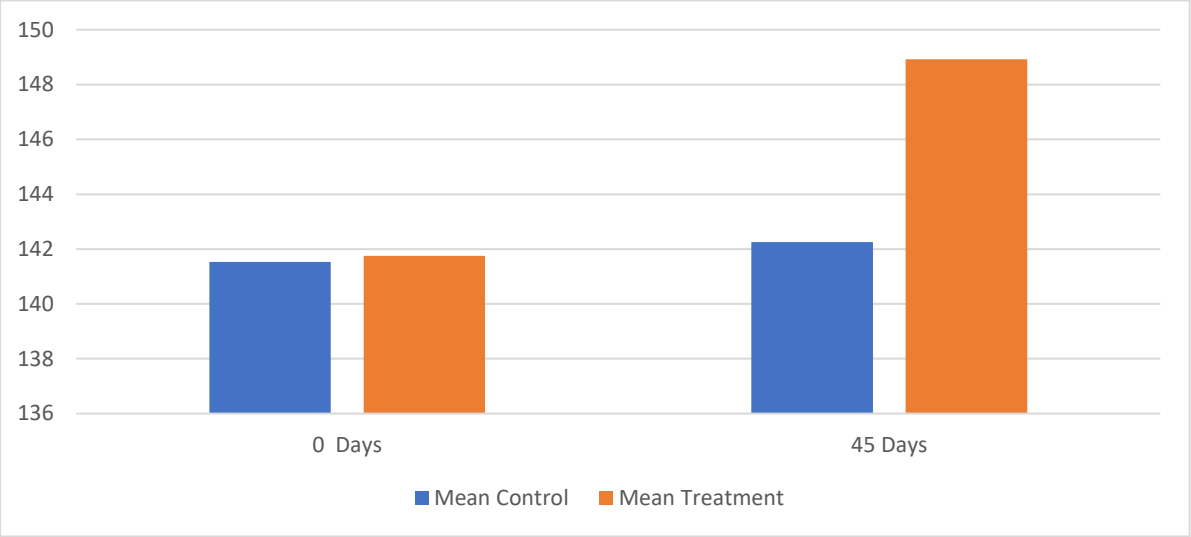

Fig 4.3: Diagram showing the status of cholestrol (mg%) level in blood serum.

The serum cholesterol concentration of cows in anoestrus and oestrus condition were 131.57 ± 7 mg% and 147.29 ± 8.31 mg% respectively. Similar findings were reported by Murtuza *et al.* (1978). Dutta et *al.(* 1988) and Cetin *et al.* (2002). The values obtained in this study was lower than that reported by Purohit and Kohli (1977), Aminuddin *et al.* (1984) and Sahukar et *al.* (1985) and higher than the finding reported by Sharma *et al.* (1984) and Kuma and Sharma (1991).

Major elements

Calcium

Table 4.5: Status of Ca (mg%) level in blood serum

Days	Mean±SE		P-value
	Control	Treatment	
0	9.31±0.24	9.23±0.06	0.389
45	9.79±0.21	10.01±0.10	0.080

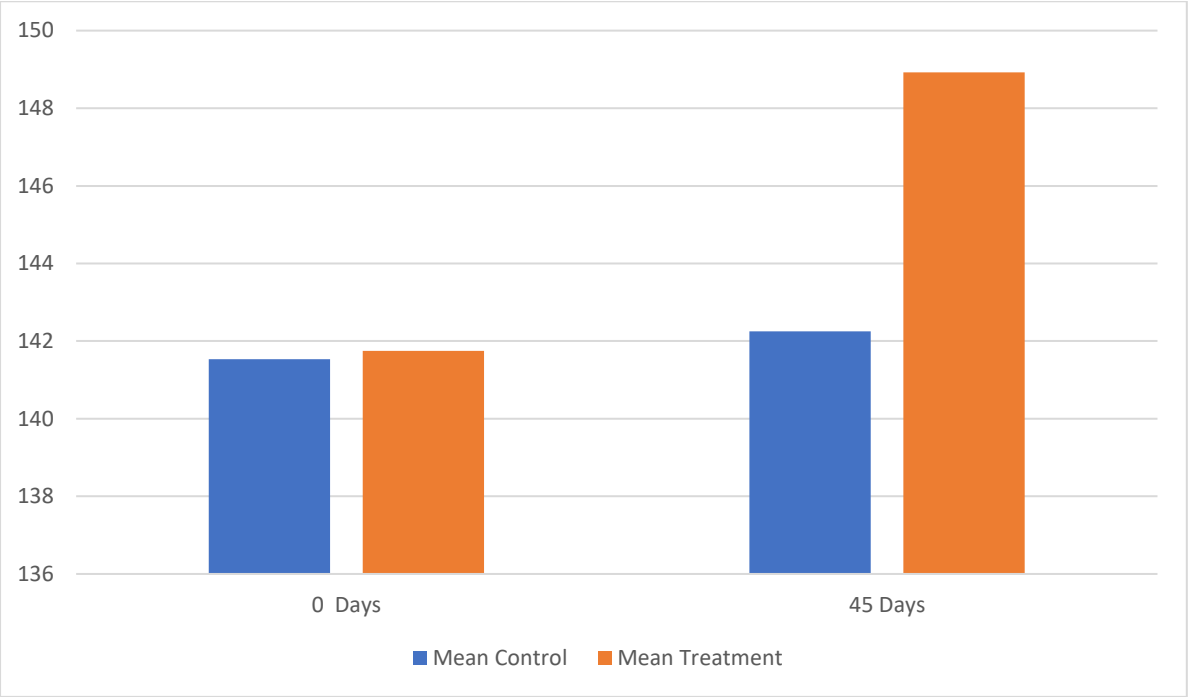

Fig 4.4: Diagram showing the status of Ca (mg%) level in blood serum.

The serum calcium level in anoestrus and oestrus conditions were 8.9786 ± 0.6532 mg% and 10.5286 ± 0.4848 mg% respectively. The results were corroborated with the findings of Prasad et *al.* (1984), Ramkrishna *et al.* (1997), Tandle et *al.* (1997), Joe Arash *et al.* (1998) and Dutta *et al.* (2001). The observation is higher than the observation of Samad et *al.* (1980), Sahukar*et al.* (1984), Sood*et al.* (1999), but present value has been found lowerthan the findings of Dutta *et al.* (1988) and Kumar *et al. (2000).*

Phosphorus

Table 4.6: Status of P (mg%) level in blood serum

Days	Mean±SE		P-value
	Control	Treatment	
0	4.71±0.26	4.68±0.08	0.466
45	4.83±0.24	5.23±0.08	0.103

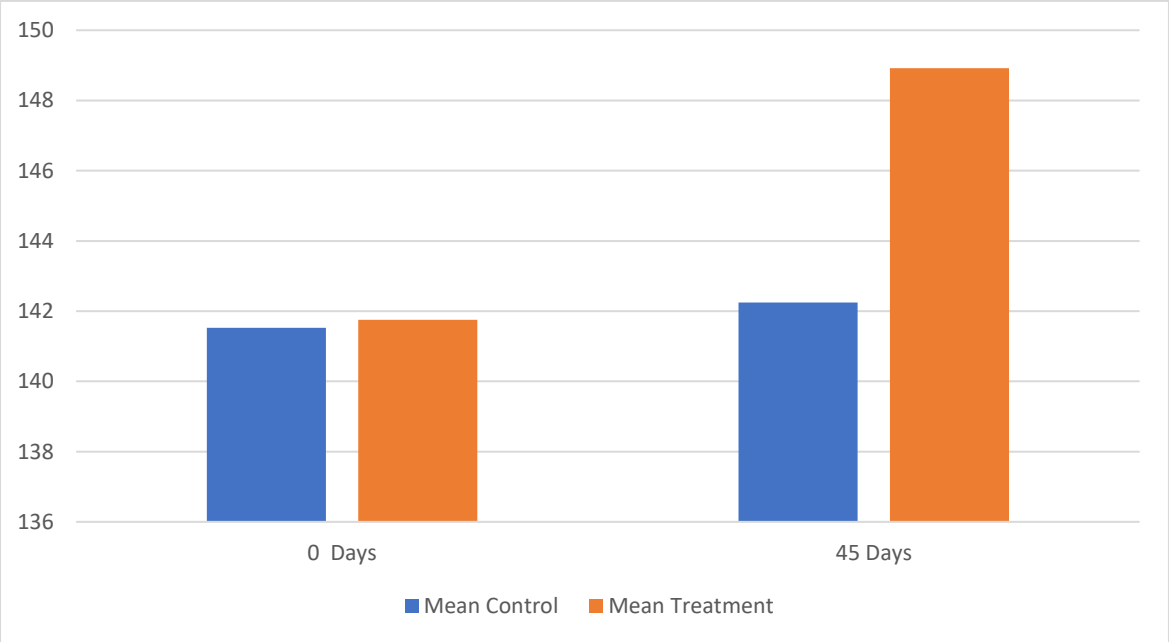

Fig 4.5: Diagram showing the status of P (mg%) level in blood serum.

The serum phosphorus level in anoestrus and oestrus conditions were 4.6114 ± 0.3218 mg% and 5.3957 ± 0.3295 % respectively. Similar results were also reported by Chetty and Rao (1985), Ramkrishna *et al.* (1997), Kumar *et al.* (2000), Das *et al.* (2002), Cetin et *al.* (2002) and Jain et *al.* (2003).

Trace Minerals

The serum Mn (µg/dl), Cu (µg/dl), Zn (µg/dl) and Fe (µg/dl) were estimated in the given tables.

Manganese

Table 4.7: Status of Mn (µg/dl) level in blood serum

Days	Mean±SE		P-value
	Control	Treatment	
0	51.50±0.25	51.72±0.24	0.478
45	48.97±0.16	55.72±0.11	0.001

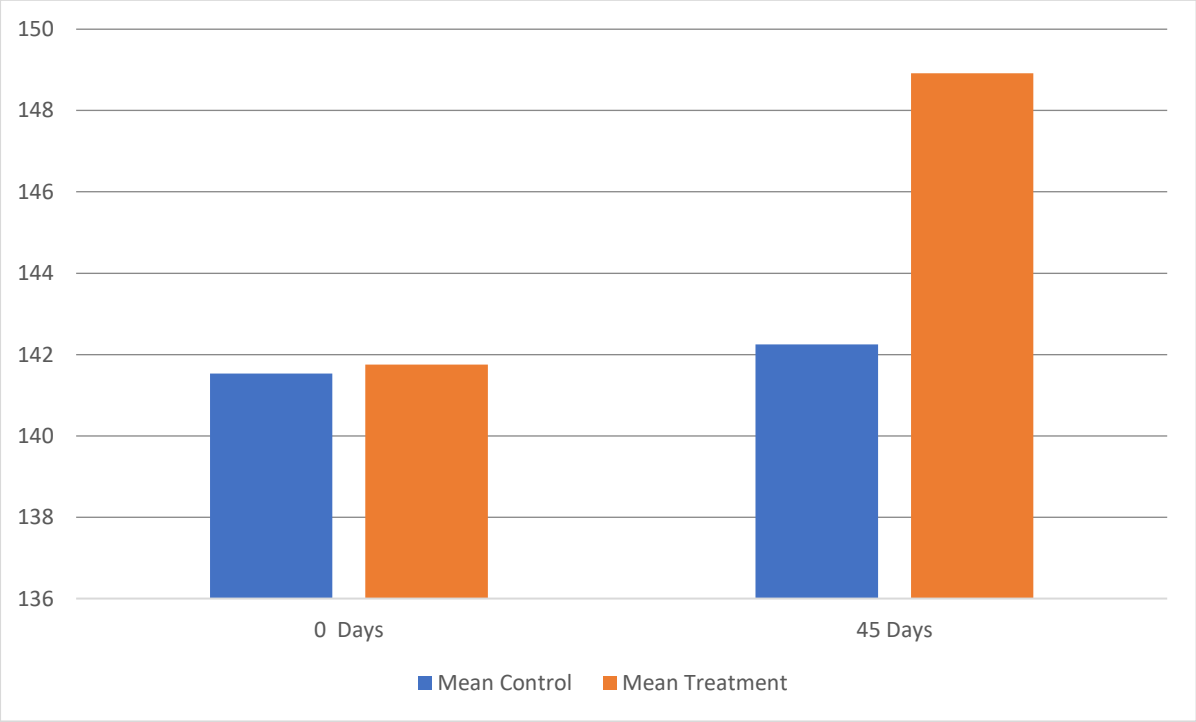

Fig 4.6: Diagram showing the status of Mn (µg/dl) level in blood serum.

The serum Mn level of in anoestrus and oestrus conditions were 44.629 ± 5.072 and 62.114 ± 4.201 respectively. The findings simulate with the findings by Samanta *et al.* (1995) Das (1997), Prasad and Rao (1997). Kalita *et al.* (1999). And the result was higher than reported by Kumar *et al.* (2000) and Jain *et al. (2003).*

Copper

Table 4.8: Status of Cu (µg/dl) level in blood serum

Days	Mean±SE		P-value
	Control	Treatment	
0	144.67±0.46	144.47±0.26	0.361
45	143.65±0.37	181.31±0.27	0.000

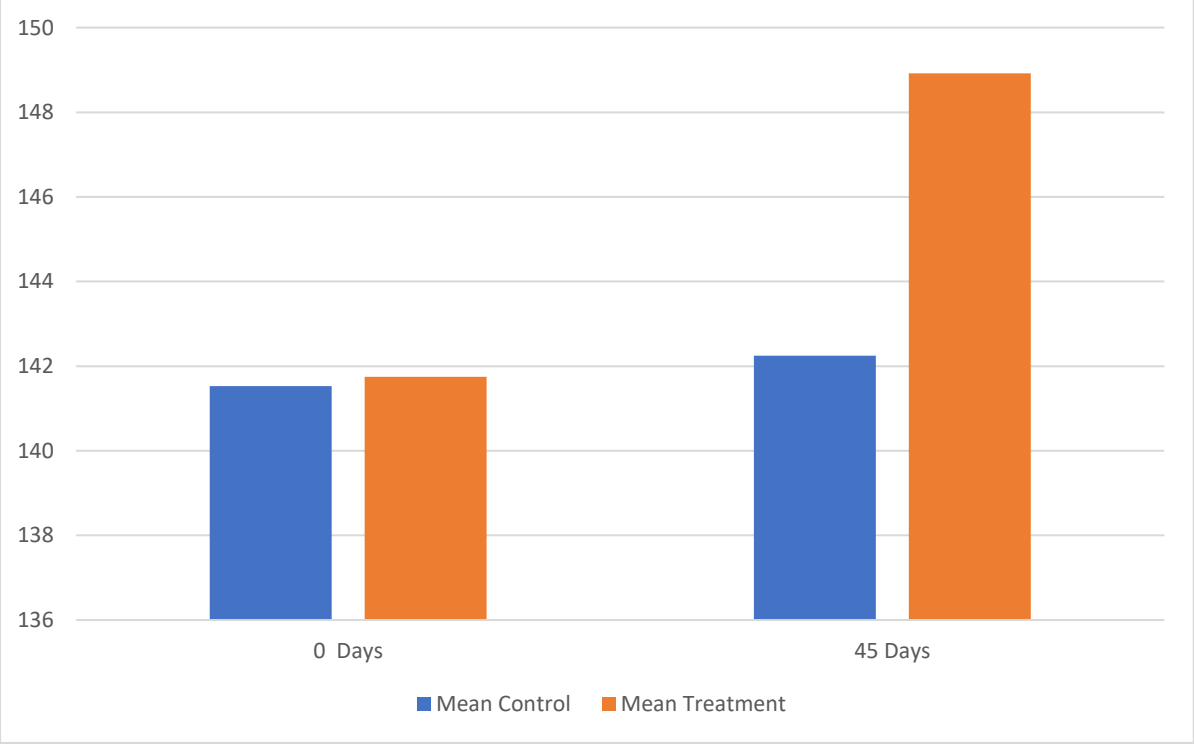

Fig 4.7: Diagram showing the status of Cu (µg/dl) level in blood serum.

The serum copper level during anoestrus and oestrus conditions were 145 ± 7.23 µg/dl and 183.29 ± 4.75 (µg/dl). Thevalues were corroborated with the findings of Dabas et *al.* (1987)and Jain et *al.* (2003) but lower than reported by Desai et *al.* (1982), Saxena and Gupta (1992) and higher as reported by Samanta et *al.*(1995) and Sharma *et al.* (1999).

Zinc

Table 4.9: Status of Zn (µg/dl) level in blood serum

Days	Mean±SE		P-value
	Control	Treatment	
0	241.23±0.44	241.08±0.28	0.390
45	231.95±0.52	303.31±0.22	0.000

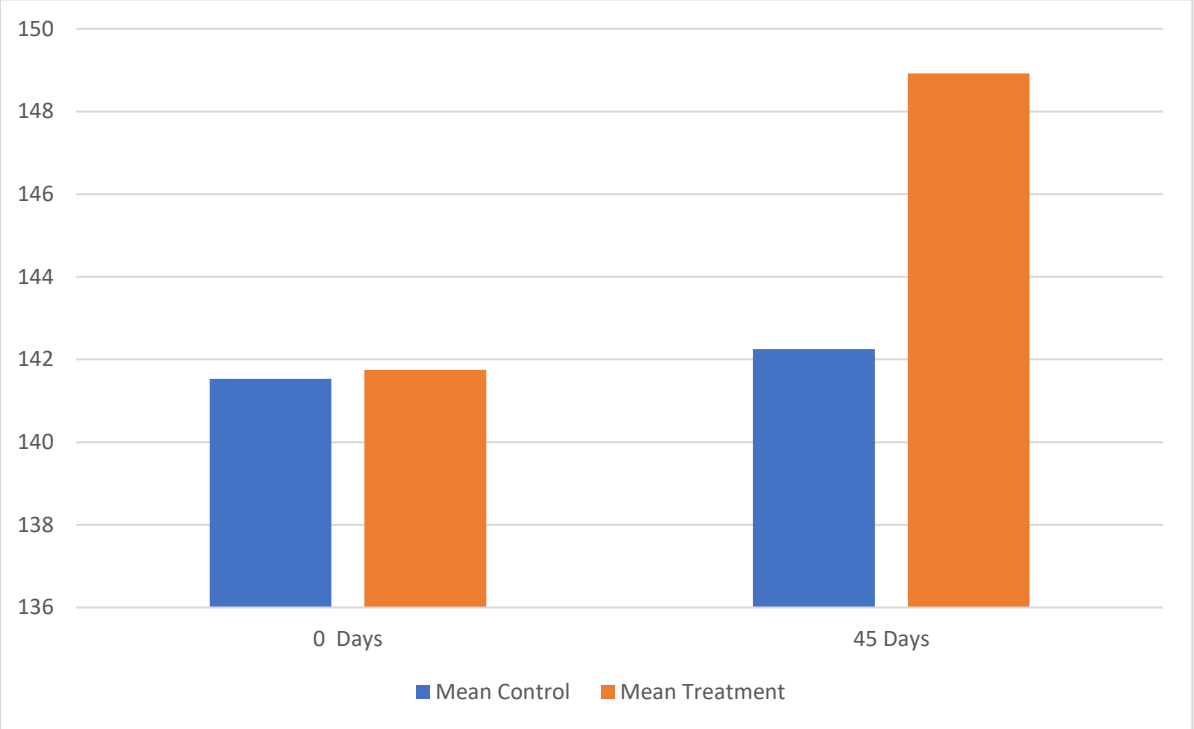

Fig 4.8: Diagram showing the status of Zn (µg/dl) level in blood serum.

The serum level of Zn in anoestrus and oestrus condition were 232 ± 6.59 (µg/dl) and 301 ± 7.73 (µg/dl) respetively. Similar results were reported by Dabas *et al.* (1987), Prasad and Rao

(1997) and Kalita *et al.* (1999) but the result is lower than reported by Samanta *et al.* (1995) and Jain *et al. (2003).*

Iron

Table 4.10: Status of Fe (µg/dl) level in blood serum

Days	Mean±SE		P-value
	Control	Treatment	
0	141.53±0.30	141.75±0.22	0.279
45	142.25±0.33	148.92±0.13	0.000

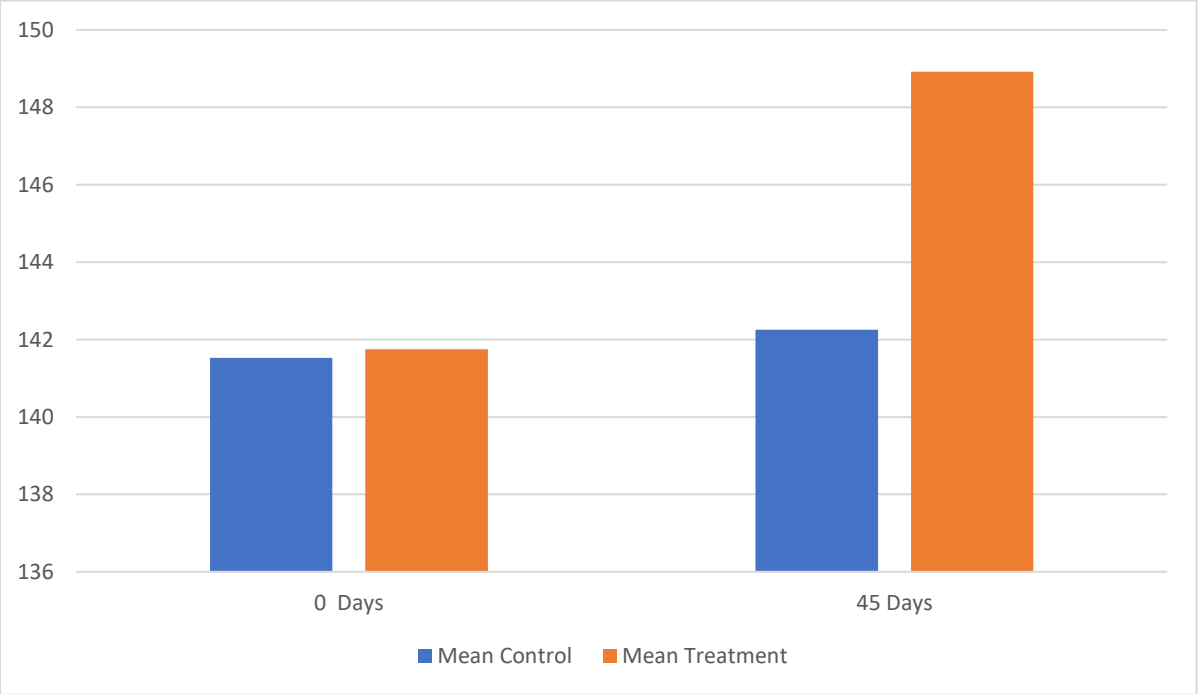

Fig 4.9: Diagram showing the status of Fe (µg/dl) level in blood serum.

The serum Fe level in anoestrus and oestrus condition were 139.57 ± 1.2 (µg/dl) and 149.29 ± 7.42 (µg/dl) respectively. The result is supported by the earlier results reported by Rupde *et al.* (1993) and Jain (1994). The values were higher than reported by Das (1997), Kumar *et al.* (2000) and Singh and Pant (1998) and lower than reported by Samanta *et al.* (1995) and Das *et al.* (2002).

SUMMARY AND CONCLUSION

The present investigation entitled **"The Effect of β-Carotene Incorporated Mineral-Vitamin Premix on Amelioration of Infertility in Crossbred Cattle"** was conducted in a commercial Dairy Herd in Dairy Farm, Department of Animal Husbandry and Dairying, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi (Uttar Pradesh) during 2018-19. An attempt has been made to summarize the findings of the present study along with conclusion in this chapter under following:

In the present study, total 24 infertile crossbred cows were selected to induce oestrus taken for correction of anoestrosity with β-carotene incorporated vitamin-permix. Total 8 out of 24 animals exhibited oestrus symptom after the treatment. Among the animals received β-carotene incorporated vitamin-permix (treatment group) 50% showed the oestrus symptoms. Among the animals under control 16.66% came into heat.

Estimation of serum total protein, cholesterol, major elements (Ca and P) and trace minerals (Mn, Cu, Zn and Fe) were performed At 0 days and 45 days.

In treatment group (β-carotene incorporated vitamin-permix) 6 animals came into heat. Serum β-carotene, total protein, cholesterol, Mn, Cu, Zn and Fe level was significantly higher (significant at 5% level) in oestrus condition than in anoestrus condition in this group. The other parameters show their elevation in oestrus condition over the anoestrus condition but this elevation is not statistically significant.

In control group only 2 animal came into the heat. Here no parameter show any significant change in between the anoestrus and oestrus condition.

The observations for 12 cows of treatment group, provided with mineral mixture with beta carotene supplementation:

a) The blood level of beta carotene rose from 3.26 ± 0.34 mg/L at day 0 to 5.60 ± 0.33 mg/L at day 40 of feed supplementation.

b) The blood serum level of total protein rose from 7.82 ± 0.17 mg% at day 0

to 9.31 ± 0.14 mg% at day 45 of feed supplementation.

c) The blood serum level of cholestrol rose from 131.50 ± 0.15 mg% at day 0 to 147.53 ± 0.41 mg% at day 40 of feed supplementation.

d) The blood serum level of Ca rose from 9.23 ± 0.06 mg% at day 0 to 10.01 ± 0.10 mg% at day 45 of feed supplementation.

e) The blood serum level of beta P rose from 4.68 ± 0.08 mg% at day 0 to 5.23 ± 0.08 mg% at day 45 of feed supplementation.

f) The blood serum level of Mn rose from 50.72 ± 0.24 µg/dl at day 0 to 52.79 ± 0.11 µg/dl at day 45 of feed supplementation.

g) The blood serum level of Cu rose from 144.47 ± 0.28 µg/dl at day 0 to 181.31 ± 0.27 µg/dl at day 45 of feed supplementation.

h) The blood serum level of Zn rose from 241.08 ± 0.28 µg/dl at day 0 to 303.31 ± 0.22 µg/dl at day 45 of feed supplementation.

i) The blood serum level of Fe rose from 141 ± 0.22 µg/dl at day 0 to 148.92 ± 0.33 µg/dl at day 45 of feed supplementation.

Therefore, following conclusions were drawn from this study

1. Beta carotene deficiency is responsible for anestrus in cows, beta carotene supplementation definitely improves the anoestrous condition with overall fertility.
2. Blood biochemical profile and serum mineral concentration improve towards optimum health after supplementation of β-carotene.

BIBLIOGRAPHY

Agarwal, S.K., Patil, B.R. and Purbey, L.N. (1983). A note of effect ofsome Indigenous drugs in restoring fertility in anoestrus cows. *Journal of Veterinary Physiology and Allied Sciences*, **2**(2): 43-46.

Agricultural Research Council (1980).The nutrient requirement of ruminant livestock. *Commonwealth Agricultural Bureaux Slough*.

Agricultural Research Council (1978).The nutrient requirement of ruminant livestock. *Commonwealth Agricultural Bureaux Slough*.

Ahlswede, L. and Lotthammer, K.H. (1978). Investigations on a specific, vitamin A independent influence of 13-carotene on the fertility of cattle. 5thcommunication: Investigation of organs (ovaries, corpora lutea, liver, adipose tissue, uterine secretion, and adrenal) weight and concentrations assays. *DtschTierarztlwochenschr*, **85**:7-12.

Ahmed, F.A., Tamuli, M.K. and Akhtar, F. (2003). Effect of placentres crossbred cattle. *Indian Veterinary Journal*, **80**: 1077-1079.

Akar, Y. and Gazioglu, A. (2006). Relationship between vitamin a and β-carotene levels during the postpartum period and fertility parameters in cows with and without retained placenta. *Bulletin of the Veterinary Institute in Pulawy*, **50***:* 93-96.

Akhter, M., Hoque, M. N., Shamsuddin, M., Alam, M. G. S. and Bari, F. Y. (2013). Ultrasonography for the management of reproductive disorders in dairy cows. *IOSR-JAVS*, **5**: 61-66.

Akordor, F.Y., Stone, J.B. Walton, J.S. Leshie, K.E. and Buchanan-Smith, J.G. (1986). Reproductive performance of lactating Holstein cows fed supplemental 13-carotene, *Journal of Dairy Science*, **69**:2173-2178.

Alosilla, C.E.J., McDowell, L.R., Wilkinson, N.S., Staples, C.R., Thatcher, W.W., Martin, F.G. and Blair, M. (2007). Bio availability of vitamin a sources for cattle. *Journal of Animal Science*, **85**:1235-1238.

Amin, R.U. (2013). Nutrition: Its role in reproductive functioning of cattle.*Veterinary Clinical Science*, **2**(1):01-09.

Aminuddin, P.K., Pareck and Ohosal, A.K. (1984). Blood Profile in normal and anestrus Rathi cows of arid tract of Rahasthan. *Indian Journal of Animal Scence.* **54**(8): 751-755.

Anwandter, Q. C. G. (1974). Progesteron and β-Carotin-Gehalt des bovinen corpus luteum wahrend der verschiedenenphasen des sexualzyklus in abhangigkeit von der Jahreszeit (Doctoral dissertation, Dissertation, Tierarztl. Hochschule, Hannover).

Arechiga, C.F., Staples, C.R., McDowell, L.R. and Hansen, P.J. (1998). Effects of timed insemination and supplemental beta-carotene on reproduction and milk yield of dairy cows under heat stress. *Journal of Dairy Science*, **81**(2):390-402.

Arechiga, C.F., Vazquez-Flores, S., Ortiz, O., Hernandez-Ceron, J., Porras, A., McDowell, L. R. and Hansen, J.P. (1998). Effect of injection of β-carotene or vitamin E and selenium on fertility of lactating dairy cows. *Theriogenology*, **50**: 65-76.

Arneza, J.S., Hathi, D.S., Singh, B. and Verma, P.N. (1977). Statusof some micro minerals in neonatal buffalo calves and their mother. *Indian Journal of Dairy Science,* **30**: 255-257.

Arthur, E.C. (1978). Feeds and Feeding. Prentice-Hall of India Private Limited, New Delhi.

Arthur, O.H. (1975). Veterinary reproduction and obstetrics.E.L.B.S., Fourth Edition, William Clowers and sons Limited. Beceles and London.

Ay, S.S., Kaya, D., Kucukaslan, I., Agaoglu, A.R., Emre, B., Handler, J. and Aslan, S. (2012). Beneficial effects of Beta-carotene injections prior to treatment with

PGF2α on the fertility of postpartum dairy cows. *Revue de Médecine Vétérinaire*, **163**(8/9): 387-392.

Bendich, A.(1987). Role of antioxidant vitamins on immune function. In: Proceedings of Technical symposium, Daytona Beach Florida, USA.

Bindas, E.M., Gwazdauskas, F.C., Aiello, R.J., Herbein, J.H., McGilliard, M.L. and Polan,C.E. (1984). Reproductive and metabolic characteristics of dairy cattle supplemented with beta-carotene. *Journal of Dairy Sciences*,**67**(6):1249-55.

Campbell, M.H. and Miller, J.K. (1998). Effect of supplemental dietary vitamin E and Zinc on reproductive performance of dairy cows and heifers fed excess Iron. *Journal of Dairy Science*,**81**(1): 2693-2699.

Ceylan, A., Serin, I., Aksit, H. and Seyrek, K. (2008). Concentrations of some elements in dairy cows with reproductive disorders. *Bulletin of the Veterinary Institute in Pulawy*,**52:** 109-112.

Chetty, A.V. and Rao, A.R. (1986). Levels of blood constituents in anoestrus condition. *Livestock Adviser*,**11**(6): 34-37.

Chew, B.P. (1993). Effects of Supplemental beta-Carotene and Vitamin A on reproduction in swine. *Journal of Animal Science*,**71**:247-252.

Church, D.C. (1979). Digestive physiology and nutrition of ruminants. Volume 2- Nutrition, 2nd Ed., 0 and B Books,Inc., United States of America.

Dabas, Y.P.S. (1997). Herbal therapy for long standing anoestrus in bovines. *Indian Veterinary Journal.* **74**: 797-798.

Dabas, Y.P.S., Singh, S.P. and Saxena, O.P. (1987). Serum concentration of certain minerals in anoestrus cows and buffaloes. *Indian Journal of Animal Reroduction*,8(2): 98-10 l.

Das, S., Bandopadhya, S.K., Basu, S., Ghosh, B.B. and Dutta gupta,R. (2002). Blood mineral profile of normal cyclic and repeat breeder crossbred cows under rural condition. *Indian Journal of Animal Reproduction*,**23**(2): 167-169.

Das, S.S., Sarkar, S., MondaI, T. and Basak, D.N. (1993).Manganese responsive infertility of cattle in relation to soil and plant in Hoogly district of West Bengal. *Indian Journal of Animal Health*,**32**(1): 57-61.

Das, U., Roy, S., Roy, B.N., Ghosh, S.P. and De Sarkar, M.K.(1997). Some blood constituents of crossbred cattle in twoagro-climatic zones of West Bengal. *Indian Journal Animal Health*,**36**(1): 11-1 3.

Desai, M.C., Thakkar, T.P., AninDharsana, R. and Janaki-raman,K. (1982). Note on serum copper and iron in Surti buffalo1n relation to reproduction and gonadotropins. *Indian Journal of Animal Science*,**52**(6): 443-444.

Dhoble, R.L. and Gupta, S.K. (1986). *Indian Journal of AnimalHealth,* **125** : 123.

Dindorkar, C.V. and Kohli, I.S. (1979). Variations in blood serum phosphorus, calcium and Magnesium values of cattle having regular estrus cycles and those in anoestrum.

Doisey, E.R. (1972). Trace Subst. Environ. Health-6 Proc. Univ.Mo. Annu. Conf., 6th 1972, p. 193 (1972), in "Trace Element Metabolism in Animals". (W.G. Hoekstra *et al.*eds.), Vol. 2., P 664. Univ. Park Press, Baltimore.

Dugwekar, Y.O., Pangawkar, O.R. and Sharma, RD. (1980).Introduction of estrus in anoestrus cows treated with Fertivet (chomiphene). *Veterinary Bulletin.,* **50**(10): 904.

Dutta, A., Aruah, B., Sarmah, B.C., Baruah, K.K. and Goswami,R.N. (2001). Macro mineral levels in cyclic, postpartum anoestrus and repeat breeding local cows in lower brahmaputra valley of Assam. *Indian Journal of Animal Reproduction.,***22**: 41-44.

Dutta, J.C., Baruah, RN., Dutta, L. and Talukdar, S.C. (1988).Blood biochemical studies in anoestrus and normal cyclingcattle. *Indian Veterinary Journal.* **65**(3): 239-241.

Esper, C.R., Pinheiro, L.E.L., Farm Ceschini, P.H. and Leite, F.G.(1979). Effect of an intra uterine iodine infusion in anoestrus cows. *Cientifica. Brazil,* **6**(7): 33-36.

FAO (1997). Production Year Book". Food and Agricultural Organisation of the United Nations, Rome.

Fiske, C.H. and Subba, R. (1925). Determination of Inorganic Phosphorus in blood.*J. Bioi. Chern.* **66**: 375.

Galhotra, A.P., Vaskar, V.V. and Goutam, O.P. (1970). *H.A.U.Journal Research* 1 (3) : 66-73 cited by Chaudhury *et al.*(1983). *Livestock Adviser*,**8**(7): 40-43.

Ganguly, J. and Sastry, P.S. (1985). Mechanism of conversion of ß-carotene in to vitamin A-central cleavage versus random cleavage.In *World Nutritional Determinants*, (Vol. 45, pp. 198-220). Karger Publishers.

Ghezgariu, S., Danielescu, N., Moldovan, N. and Pop, A. (1986).Serum copper and zinc in healthy cows in relation toseason and physiological state. *Revista de Cresterea Animal clor*, **36** (7): 39-42.

Glover, J. (1960). The conversion of B-carotene into vitamin A. *Vitamins Hormones*, **18**: 371-38.

Gonzalez-Montana, J.R., Alonsa-Diez, A.J., Alonso-Alonso, M.P.,Prieto-Montana, *F.*,Graciapartida, P. and Trenti, F. (1994).Serum protein levels during pregnancy in sheep.Proceeding 18th world Duiatrics congress: 26u1 Congress ofItalian [c.f. VETCO 1988-1998].

Gosai, N.M.; Kavani, S.F. and Panchal, M.T. (1999). Effect of supplementation of Minerals with Amino acids and vitamins (A & 03) on post-partum Fertility in buffaloes. Absract. XV Annual Convention and National Symposium on Bio techniques in Optimizing Fertility in Farm Animals(Feb, 10-12), pp.78.

Groppel, B. and Anke, M. (1971). Arch. Fur. Experiment. Vet. **25** :52. [c.f. Church, D.C. (1979). Digestive" Physiology and Nutrition of Ruminants. Volume, 2 Nutrition. Pp.133.].

Grownowska-Senger, A. and Wolf, G. (1970). Effect of dietary protein on the enzyme from rat and human intestines which converts B-carotene into vitamin A. *Journal of Nutrition*,**100**: 300-305.

Hafez, E.S.E. (1980). Reproduction in farm animals. 4th edition K. V.Varghese Publishing house. Bombay.

Hafez, E.S.E. (1987). "Reproduction in Farm Animals" Philadelphia,leaandFebiger, 5th Edition.

Hancock, J.L. (1948). The clinical analysis of reproductive failure in cattle. *Veterinary Research,* **60**: 513.

Hemken, R.W. and Bremel, D.H. (1982). Possible role of beta-carotene in improving fertility in dairy cattle. *Jounral of Dairy Sci*ence,**65**(7):1069-73.

Hewett, C. (1976). Importance of variation in the blood profile in cows in dairy hard.*Veterinary. Bulletin.* **46**(2):1060.

Howes, A.D. and Dyer, I.A. (1971). Diet and supplemental mineral effects on manganese metabolism in new born calves. *Journal of Animal Science.,* **32**: 141.

Inaba, T., Inove, A. Shimizu, R. Nakano, Y.and Mori, J. (1986). Plasma concentration of progesterone, estrogens, vitamin A, and B-carotene in cows retaining fetal membranes. Japanese . *Journal of Veterinary Science*,**48**:505-508.

Jackson P.S.(1982). Beta-carotene and some bovineblood parameters. In proceedings: Roche vitamin Symposium on Recent research on the vitamin requirements of ruminants. November London.

Jadhav, S.S., Velhankar, D.P. and qeshpande, B.R. (1977).Variation in the serum cholesterol levels of Gir cows during pregnancy, parturition and pueerperium. *Indian Vet. J.,* **54**: 536-540.

Jain, Aklank, Pathak, R.K. and Jain, P.K. (2003). Effect of mineral supplementation on fertility of crossbred cows. *Indian Veterinary Medical Jounalr,***27**: 259-260.

Jain, G.C. (1994). Mineral profiles during anoestrus and repeat breeding in bovine. *Indian journal of Animal Science,* **9**: 241-245.

Jochle, W., Schneemann, W. and Grunert, E. (1989). Clinicalresponse in anoestrus dairy cows with high or low blood progesterone breeds to treatment with the PGF analoguealfaprostol. *Zuchthygiene,* **24**(2): 57-66.

Joe Arosh, A., Kathiresan, D., Devanathan, T.G.,Rajasundaram,R.C. and Raj Asekaran, J. (1998). Blood biochemical profile in normal cycling and anoestrus cows. *Indian journal of Animal Science.*, **68**(11): 1154-1156.

Joy, G. and Nair, K.P. (1995). Phosphorus and trace elements status of anoestrus and repeat breeder crossbred cows. *Journal of Veterinary Animal Science*,**46**(2): 91-94.

Jukola, E., Hakkarainen, J., Saloniemi, H. and Sankari, S. (1996). Blood selenium, vitamin E, vitamin A, and β-carotene concentrations and udder health, fertility treatments, and fertility. *Journal of Dairy Science*,**79**(5): 838-845.

Jüttner, F. andHöflacher, B. (1985). Evidence of β-carotene 7, 8 (7′, 8′) oxygenase (β-cyclocitral, crocetindial generating) in microcystis. *Archives of microbiology*, **141**(4): 337-343.

Kabir, K.K., Varshney, J.P., Rawal, C.V.S. and Ansari, M.R. (2001). Studies on serum progesterone and certain blood biochemical indices in cyclic and acyclic non-de script rural

Kabir, K.K., Varshney, J.P., Rawal, C.V.S., Srivastava, R.S. and Ansari, M.R. (2001). Comparative efficacy of herbal preparations in the management of anoestrus in non descriptrural buffaloes. *Indian Journal of Animal Reproduction.* **22**(2):143-145

Kaikini, A.S., Pargaonkar, D.R., Patil, R.K. and Dindorkar, C.V.(1977). Break through therapy for anoestrum in cattle. *Indian Veterinary Journal*,**54**(8): 667-672.

Kalita, D.J., Sarmah, B.C. and Bhattacharya, B.N. (1999). Mineral profile and fertility of cows. *Indian Veterinary Journal*,**76**: 971-972.

Kalyainakishnan, M., Paul, T.M., Anantakrishnan, C.P. and Sen, K.C. (1951). Studies on vitamin A in milk. The effect of feeding vitamin A supplement to cows and buffaloes. *Indian Journal of Dairy Science*, **4**: 45-52.

Kamath, S.K. and I. Arnrich, (1973). Effect of dietary proteins on the intestinal biosynthesis of retinal from C-beta-carotene in rat. *Journal of Nutrition*, **103**: 202- 206.

Kamomal, H., Kaneda, Y., Domeki, I., Iwamura, S., Agrai, T., Ogasa,A. and Nakahara, T. (1988). The effect of PMSG on ovarian function in ovarian quiescent heifers. *Japanes Journal of Veterinary Science,* **50**(6): 1222-1231.

Kawashima, C., Kid, A.K., Schweigert, F.J. and miyamoto, A. (2009). Relationship between plasma beta-carotene concentrations during the peripartum period and ovulation in the first follicular wave postpartum in dairy cows. *Amin reproduction science*,**111**(1):105-111.

Khan, T.M., Khan, M.K., Basra, M.A., Chaudhury, Z.I. and Rashid,J. (1990). Effect of age and physiological status on serum cholesterol and total serum proteins in buffaloes. *Pakistan Veterinary Journal*,10(4):165-167.

Kodagali, S.B., Deshpande, B.R. and Sane, C.R. (1978). Trial with "Fertivet" (AR-EXLAB) for inducing ovulations in anoestrus cows with standing follicles paper presented at the first Asian Congress of Fertility and Sterility held at Bombay in Feb. (1977). *Indian VeterinaryJournal*,**55**(4): 339 341.

Konermann, H. (1974). Fertility problems in cattle breeding, causes and possible counter-measures. *Veterinary mededical review*. **1**:33-58.

Kovanen, P.T., Basu, S.K.,Goldstein, J.L. and Brown, M. S.(1979). Low density lipoproteins receptors in bovine adrenal cortex. II. Low density lipoproteins binding to membranes prepared from fresh tissue. *Endocrinology*,**104**:610-620.

Kubasic, N.P., Hollaner, G.D. and Brodows, R.G. (1984). Evaluation of direct solid phase RIT for progesterone useful for monitoring lutcal function. *Clinical Chemistry*,**30**(2): 284- 286.

Kudlac, E. (1981). Inducing fertile oestrus in anoestrus cows usinga synthetic analogue of PGF2a, Oestrophaninj (SPOFA);Biologizace-a-chemizaceZivocisue-Vyroby-Vetrinaria, **17**(1): 51-60.

Kumar Rakesh, Sharma, I.J. and Agrawal, KG. (2000).Periparturient levels of certain organic and inorganic components in the blood of cows and buffaloes. **21**(1): 117-120.

Kumar, A., Singh, C. and Singh, A.P. (2002). Effect of feedingmineral mixture on plasma manganese (Mn^{++}) concentration in cattle. *The Indian Journal of Animal Sciences,* **72**(6): 510-512.

Kumar, S. (1986). Use of Tonophosphan in treatment of anoestrusbuffaloes. *Indian Veterinary Medical Journal,* **10**(4): 228-229.

Kumar, S. and Sharma, M.E. (1991). Level of Haemoglobin and certain serum biochemical constituents in rural cows during fertile and non-fertile estrus. *Indian Veterinary Journal.* **68**:361-364.

Kumar, S., Pandey, A.K., Rao, M. Mutha, R. and Razzaque, W.A A. (2010). Role of ß carotene vitamin A in animal reproduction. *Veterinary World*,**3**(5):236-237.

Kumar, S., Pandey, A.K., Razzaque, W.A.A. and Dwivedi, D.K. (2011). Importance of micro minerals in reproductive performance of livestock. *Veterinary World*,**4**(5): 230-233.

Lagerlof, N. (1954). Reported to the Government of India on A.I. Sexual Health Control and Veterinary Education Publication. F.A.O. Rome, Italy.

Laing, J.A. (1970). Fertility and infertility in the domestic animals.2nd edition, Published by the Williams and Whkins, Baltimore.

Laing, J.A. (1979). Fertility and infertility in domestic animals.3rd edition, The English Language Book Society and Bailliere Tindal, London.

Lal, D., Dixit, V.B., Arora, U., Kumar, B. and Chauhan, T.R. (2000).Effect of mineral supplementation on reproductiveperformance of anestrous buffaloes under field conditions.*Indian Journal of Animal Nutrition,* **17**(1): 74-79.

Larson, C.K. (2005). Role of trace minerals in animal production. In *Nutrition Conference, Department of Animal Science, University of Tennessee.*

Little, W. (1974). *Research in Veterinary Science.* **17:** 193.

Lotfollahzadeh, S. and Golchin, P. (2016). Effect of parenteral administration of vitamin a in dairy cattle during dry period, on vitamin a status of neonatal calves. *Iranian Journal of Ruminants Health Research*,**1**(1):1-10.

Lotthammer, K.H. (1979). Importance of B-carotene for the fertility of dairy cattle. Feed stuffs **51**:16-50.

Lotthammer, K.H.(1978). On the importance of β-carotene for the fertility of dairy cattle. *DtcshTierzuchter*, **12**:520-522.

Lotthammer, K.H., Schams, D. and Scholz, H. (1978). Investigation on a specific, vitamin A independent influence of 6-carotene on the fertility of lactating cows. *Zuchthygiene*,**13**:76-77.

Lotthammer, K.H.andAhlswede, L. (1977). Investigation on a specific, vitamin A independent influence of 6-carotene on the fertility of cattle. 3rd communication: Blood serum assays (6 carotene, vitamin A SGOT, total cholesterol, Glucose, inorganic phosphate). *DtschTierarztlWochenschr*,**84**:220-226.

Luktuke, S.N. and Sharma, C. (1978). Studies on the incidence of"TrueAnestrum" in rural cattle and buffaloes. *Indian Veterinary Journal*,**55**(22): 940-942.

Mahanta, S.K., Khan, S.A.,Bedi, S.P.S. and Khan, M.Y. (1997).Effect of different calcium supplements on utilization ofnutrients and blood parameters in buffaloes. *IndianJournal of Animal Nutrition*, **14**(2): 115-118.

Maity, S.C. (1994). Comparative efficacies of Non-hormonal drugsfor inducing oestrus and conception in Rural Heifers ofWest Bengal M.V.Sc. thesis, Bidhan Chandra Krishi Viswavidyalaya, West Bengal.

Manowar, S.A.L. and Singh, C. (2002). Serum protein concentrationin crossbred (Friesian x Hariana) non-cycling heifers of different ages, cycling heifers and cycling lactating cows .*Indian Journal of Animal Science.*,**72**(10): 867-869.

Maryland(1974). [c.f. Underwood, E.J. (1977. Trace Elements in Human and Animal Nutrition. 4th Ed.,Acadamic Press.].

Maurice, P.B. and Lonergan, P. (2003). Effects of nutrition on fertility in dairy cows. *Advances in Dairy Technology*,**15:**19.

McDonald, P., Edwards, R.A. and Greenhalgh, J.F.D.(1987).Animal Nutrition. LONGMAN. ELBS, 4th edition.

Mee, J.F., Rogers, P.A.M. and Farrel, K.J. (1995). Effect offeeding a mineral-vitamin supplement before calving, onthe calving performance of a trace element deficient dairy herd. Veterinary Record, **137**(20): 508-512.

Megahed, G.A., Anwar, M.M. and Salwa, M.S. (1995). Thyroidactivity in Egyptian buffalo cows with ovarian in activityand trials for treatment by intrauterine infusion of Lugol'siodine solution. Proceedings of the third scientific congress Egyptian Society for cattle diseases, **2**:211217.

Mehere, Y.S., Talvelkar, B.A., Deshmukh, B.T., Nagvekar, A.S. andJngole, S.D. (2002). Haematological and trace element profile during peripartum period in crossbred cows. *Indian Journal Animal Science.,***72**(2): 148-150.

Mehta, J.8., Bhatia, J.8., Kholi, I.8., Pareck, P.K., Bishnei, B.L. andGupta, A. K. (1989). Studies on serum alkaline phosphatase and protein in various reproductive states in cow. *Indian Journal of Animal Reproduction.* **19**(2): 138-140.

Meinecke, B., Bittner,W. and Gips, H. (1986). The effect of 8-carotene on fertility in cows: Clinical and endocrinological findings. *Zuchthyg*, **21**: 225-232.

Meyer,J., Ahlswede, L. and Lotthammer, K.H. (1975). Investigations on a specific, vitamin A independent influence of B-carotene on the fertility of cattle. 1st communication: Body development and ovarian function. Dtsch. Tierarztl.Wochenschr, **82**:444-449.

Mihalka, S. (1981). The influence of B-carotene status on puerperal disease in Hungarian dairy cattle. *Magyar AllatorvosokLapja*,**36**:450-454. (Roche translation)

Mingazov, T.A.(1977a). Effect of vitamin E on the assimilation of 13-carotene from microbial preparations and on the reproductive function of cows. *Chem. Abstract*,**86**: 432.

Murtuza, M., Pandey, M.D. and Rawat, J.S. (1978). Serum enzymes and serum cholesterol in Hariana cattle under different physiological status. *Indian VeterinaryJournal*,**55**(12): 958-962.

Naidu, K.V. and Rao, A.R. (1982). A study on the etiology of anoestrus in crossbred cows. *Indian VeterinaryJournal*,**59**(10): 781-784.

Navarre, B.C. (2010). Animal health notes. Extension veterinarian LSU age enter research and extension.

Nayyar, S., Sharma, K.B., Malik, V.S., Singh, R., Singh, N., Pattan, P.J.S. and Sodhi, S.P.S. (1998). Blood Biochemical and Hormonal composition during the prepubertal period as related to the· age at puberty in buffalo Heifers. *Iandian Journal of Animal Reproduction.*,**19**(2): 113-116.

Olson, J.A. (1989). Provitamin A function of carotenoids: the conversion of β-carotene into vitamin A. *The Journal of Nutrition*, **119**(1): 105-108.

Olson, P.A., Brink, D.R., Hickok, D.T., Carlson, M.P., Schneider, N.E.,Dentscher, G.H.,Adams, D.C., Colburn, D.J. andJohnson, A.B. (1999). Effect of supplementation of organic and inorganic combination of copper, cobalt, manganese and zinc about nutrient requirement levels on postpartum two year old cows. *Journal of Animal Science*, **77**(3): 522-532.

Ondarza, M.B.D.E., Wilson, J.W. and Engstrom, M. (2009). Effect of supplemental β-carotene on yield of milk and milk components and on reproduction of dairy cows. *Aminalreprodution science*,**25**(4): 510-516.

Panchal, M.T., Derashari, H.J., Kavani, F.S. and Merja, R.M. (1996).Induction of estrus in buffalo using a herbal medicine.*Indian Journal of Animal Reproduction.*,**17**(2): 107-108.

Pargaonkar, D.K. and Kaikini, A.S.(1989). Studies on hormonal profiles of nondescript cows during various phases of reproduction. *Indian Journal of Animal Reproduction.*, **10**(2): 85-88.

Patel, D.M., Sarvaiya, N.P., Patel, A.V., Parmar, A.P. and Gugurkar,Y.G. (2003). Induction of estrus and hormonal profile in buffalo treated with norgestometearim plant. *Indian Journal of Animal Reproduction.*,**24**(1): 67-68.

Peters, A.R. and Riley, G.M.(1982). Is the cow a seasonal breeder. *Br. vet. J*,**138**:533-538.

Phogat, J.B., Bugalia, N.S., Verma, S.K. and Singh, I. (1992).Biochemical and haemotological studies during periparturient period of buffaloes. *Indian Veterinar yJournal*,**69**(2):142-144.

Poral, M.L., Saxena, H.K., Shrivastava, A.M. and Karandikar (1976).Efficiency of different medicameuts on anoestrus buffaloes. *Indian Veterinary Journal*,**53**: 435-437.

Prabhakar, S., Nanda, A.S. and Ghuman, S.P.S. (1999). Sequential changes in some blood indices during peripartal period in buffaloes. *Indian Journal*,**76**: 1067-1070.

Prasad, K.S.N. and Rao, S.V.N. (1997). Blood mineral profile of anestrus and repeat breeder crossbred cows. - A field study. *Indian Journal of Animal Nutrition.* **14**(2): 135-137.

Prasad, R.S., Kharche, K.G. and Shrivastava, a.p. (1984). Studies on blood calcium and phosphorus in anoestrus crossbred cows. *Indian Journal of Animal Reproduction.*,**5**(1): 74-77.

Purohit, M.K. and Kohli, I.S. (1977). Variations in the serum-cholesterol level in Rathi cows during estrus *Indian Veterinary Journal*,**54**(4): 268-270.

Purohit, O.N. and Bishnoi, B.L. (1993). Some blood biochemical studies in Rathi cows and heifers before and after induction of oestrus with Fertivet. *Indian Journal of Animal Reproduction.*,**14**(2): 74.

Quayam, S.A., Devanathan, T.O. and Pattabiraman, S.R. (1988).Studies on the influence of mineral, biochemical andhaematological concentrations at 5 days postpartum on the occurrence of postpartum oestrus in the she buffaloes. *Indian Veterinary Journal,* **65**(3): 226-238.

Quayam, S.A., Devanathan, T.O. and Pattabiraman, S.R. (1990).Serum total protein and blood glucose levels during pre,peri and postpartum Murrah buffaloes. *Indian Animal Science.,* **60**(2): 140-142.

Rajasundaram, R.C. and Rajasekaran, J. (1994). Studies on minerals and hormones in reproductive failure in bovine.Ph.D. Thesis submitted to TANUVAS.

Rakes, A.H., Owens, MP., Britt, J.H. and Whitlow, L.W. (1985). Effects of adding beta-carotene to rations of lactating cows consuming different for ages. *Journal Dairy sci*ence,**68**(7):1732-7.

Ramkrishna, K.V. (1997). Comparative studies on certainbiochemical constituents of anoestrus crossbred Jersey rural cows *Indian Journal of Animal Reproduction.* **18**(1): 33-35.

Rao, D.G., Prasad, A.B.A. Rao, K.S. and Jayaramakrishna, V.(1982). Serum total cholesterol levels in FriesianxOngole crosses in different stages of reproduction. *Indian Veterinary Journal*,**59**:107-109.

Reddy, L.V. (1982). Studies on blood glucose, cholesterol, proteins,ascorbic acid and inorganic phosphorus in cycling cows and anoestrus heifers. M.Sc. thesis. University of Agricultural Science, Bangalore. *Indian Journal of Animal Reproduction.*,**8**(2): 118-121.

Reddy, L.Y.S. and Reddy, S.M. (1988). Interrelationship of some biochemical constituents in the blood serum of fertile and in fertile cows. *Mysore Journal of Agricultural Science,* **22**:359-362.

Reddy, V.S.C., Sharma, G.P., Raju, M.S. and Reddy, C.E. (1990).Effect of "Clofert-vet" treatment in postpartum anoestrus crossbred cows and Murrah buffaloes. *Indian Indian Journal of Animal Reproduction.*,**11**(I): 75-76.

Rensburg, S.W.J. and Desvos, W.H. (1962). Ovulatory failure in bovines. *Journal veterinary Research.*,**29**:55-60.

Roberts, S.1. (1971). Veterinary obstetrics and Genital Diseases. 2nd Edition, CBS Publishers and Distributors. India.Roberts, S.J. (1971). Veterinary obstetrics and Genital diseases. 2nd edition, CBS Publishers and distributors, India.

Robertson, R.D., Richard, H.P., Pater, C.W. and Douglas, M.S.(1979). Assessment of ovulation by ultrasound and plasma determination. *Obstr. Gynaec.* **54**(6): 686-691.

Rowlands, G.J., Little, W. and Kitchenman, B.A. (1977). Relationships between blood composition and fertility in dairy cows a field study. *Journal of Dairy Research.*,**44**: 1.

Royet, K.D., Sharma, B.K. and Panwar, C.D. (1990). Effect of iodine therapy in anoestrus bovines. *Indian Journal of Animal Reproduction.*,**11**(2): 144-145.

Rupde, N.D., Rode, A.M., Sarode, D.B., Zade, N.N., Jagtap, D.G.and Kaikini, A.S. (1993). Serum Biochemical profiles in repeat breeder cows *Indian Journal of Animal Reproduction.*,**14**(2): 79-81.

Sahukar, C.S., Pandit, P.K., Chauhan, R.A.S. and Porwal, M.L. (1984). Electrolyte during various reproductive phases incrossbred cows. *Indian Journal Animal Science.*,**54**(10): 993-995.

Sahukar, C.S., Pandit, R.K., Chauhan, R.A.S. and Porwar, M.L.(1985). Cholesterol and alkaline phosphatase during various reproductive phases in crossbred cows *Indian Journal Animal Science.*,**55**(6): 421-423.

Sak, P.B., Navarre-Sitchler, A.K., Miller, C.E., Daniel, C.C., Gaillardet, J., Buss,H.L., and Brantley, S.L. (2010). Controls on rind thickness on basaltic and esiteclasts weathering in Guadeloupe. Chemical Geology, 276(3-4):129-143.

Samad, A., Ali, K.M. and Rahaman (1980). Studies on certain blood constituents of anoestrus cattle. *Indian Veterinary Journal.* **57** (2): 135-138.

Samanta, A.K., Sarkar, S., Bose, Z., Uttagupta, R., Senapati, P.K.,Bhowmik, M.K. (1995). Influence of macro and micro minerals on anaemia related to production of grazing cattle. *Indian Veterinary Journal,***72**: 1031-1034.

Sampath, S.R. and Kumar, S.N.R. (1984). Mineral supplementationfor improved performance of dairy animal. In proceeding of symposium on recent advances in mineral nutrition Dept. of Animal Nutrition, Haryana Agril.University, Hissar.

Sane, C.R.,Oeshpande, S.P., Deshpande,. B.R. and Kaikini, A.S.(1958). Herd infertility in Gir cattle due to copper deficiency. *Indian Veterinary Journal*,**35**(4): 154-161.

Sarkar, J. (2000). Bio availability of nutrients in dairy cows. M.V.Sc.Thesis submitted to W.B.U.A.F.S., Kolkata, West Bengal.

Sarkar, N.K. (2002).Studies on status of some major and trace elements and effect of supplemental trace elements on nutrient utilization in anestrous dairy cattle. M.V.Sc.Thesis. Dept. of Animal Nutrition, W.B.U.A.F.S., Kolkata.

Sawale, A.G. and Dhoble, R.L. (1999). Efficacy of new herbal MAUdrug for induction of oestrus in postpartum anoestruscows. XV Annual convention and national symposium onBiotechniques in optimizing fertility in farm animals.

Saxena, M.S. and Gupta, S.K (1992). Plasma levels of macro and micro elements in relation to occurrence of pubertal estrusin crossbred heifers. *Indian Journal of Animal Nutrition,* **8**(4): 265-268.

Schams, D., Hoffmann, B. and Lotthammer, H.K.(1977). Investigation on a specific vitamin A independent influence of B-carotene on fertility of cattle. 4th communication: Effect on hormonal parameters.*DtschTieraarztlwochenschr*,**84**:307-310.

Schweigert,F.J., Lutterbach, A., Rambeck, W.A. and Zucker, H.(1986). Vitamin A and B-carotene concentration in bovine follicullar fluid in relation to follicular size. Journal of Veterinary Medical, **33**:360-364.

Seitaridis, K.(1963). Investigation on the vitamin A and B-carotene levels in the blood plasma in cattle herds with fertility disorders, with particular reference to the carotene supply levels. Hanover, veterinary University, dissertation.

Setia, M.S., Dkggal, R.S. and Singh, R. (1992). Biochemical constituents of blood in buffaloes and cows during late pregnancy and different stages of lactation. *Buffalo Journal*,**8**(2): 123-129.

Shah, R.G., Dhami, A.J., Patel, K.P., Patil, N.V. and Kavani, F.S.(2003). Biochemical and trace minerals profile in fertile and infertile postpartum Surti buffaloes. *Indian Journal of Animal Reproduction*,**24**(1): 16-21.

Sharma, K.B., Sashi N., Malik, V.S., Singh, R. and Sodhi,S.P.S. (1999). Level of hormone and minerals in cyclic, anoestrus and subestrus buffalo heifers. *Indian Journal of Animal Science*, **69**(4): 214-216.

Sharma, M.C., Shankar, V., Gupta, A.P., Verma, R.P. and Mishra,R.R. (1984). Biochemical studies in cyclic, anestrus and repeat breeding crossbred cows *Indian Journal of Animal Reproduction*,**4**(2): 51-53.

Sharma, R.V., Mathur, S.N., Dmitrovskii, A.A., Das, R.C. and Ganguly, J. (1977). Studies on the metabolism of beta-carotene and apo-beta-carotenoids in rats and chickens. *Biochimica et biophysica acta*, **486**(1): 183-194.

Sharma, V.K., Vadodaria, V.P., Kavani, F.S. and Siddiquee, G.M.(1988). Studies on circulatory levels of trace minerals in normal cyclic and primary infertile Kankrej heifers. *Indian Veterinary journal*, **65**(4): 332-335.

Sheshappa, H., Honnappagol, S.S., Tandle, M.K. and Amanullah Mohd. (2002). Study on some blood biochemical parameters in postpartum crossbred cows treated with GnRH and PGF2a. *The Indian Journal of Animal Reproduction*,**23**(1): 79-80.

Shrivastava, A.P. and Kadu, M.S. (1995). Blood biochemical profiles in normal cycling and delayed pubertal crossbred heifers. *The Indian Journal of Animal Reproduction*,**16**(2): 91-92.

Singh, C.S.P.; Singh, S.K. and Singh, Balraj (1981). Studies on the incidence of infertility in cows. *Indian Veterinary Journal*, **58**(11): 909-912.

Singh, LD., Sinha, S.N, Singh, Bahraj and Singh, M.P.(1984).Studies on efficacy of certain drugs on anoestrus cattle. *The Indian Journal of Animal Reproduction*, **4**(2): 18-21.

Singh, M. and pant, H.C. (1998). Blood Biochemical profile of normal and repeat breeder cows in Himachal Pradesh. *The Indian Journal of Animal Reproduction,***19**(2): 156-157.

Singh, R.S. and Saxena, M.S. (2000). Clinical efficacy of different doses of emetic nuts in anoestrus cattle and buffaloes.SSAR XVI annual convention and national symposium on reproduction management for optimizing production fromlivestock.

Singh, S.K., Singh, V.P. and Singh, H.S. (1996). Studies on physiological and Biochemical attributes of three breed crossbred cows during estrus.*The Indian Journal of Animal Reproduction,***12**(1): 17-19.

Sinha, R.A., Thakuria, B.N.,Baruah,R.N. and Sarma, B.C. (1981). Effects of breed, age, sex and season on total serum cholesterol in cattle. *Indian Veterinary Journal*,**58**:529-534.

Sivaiah, K., BabuR.K. and Sreerama M.A.(1986). Serum calcium and inorganic phosphorus levels in ongoIe crossbred cows. *Indian Veterinary Journal,* **63**(10): 84-806.

Snedecor, O.W. and Cochran, W.O. (1980), Statistical methods, 8thedition. Iowa state university press, Ames. Iowa.

Sood, P., Singh, M., Vashista, N.K. and Nigam, J.M. (1999). Studies on some biochemical attributes of cervical mucus and blood serum in cows of Himachal Pradesh. *The Indian Journal of Animal Reproduction.,***20**(1): 28-30.

Spears, J.W. (2011). Role of mineral and vitamin status on health of cows and calves**.***WCDS Advances in Dairy Technology*,**23**: 287-297.

Tandle, M.K., Mohammed, Amunullah, Honnappagol, S.S.,kartikesh, S.M., Jaghiwassam, Sonwane, S.D. and Amanullah, M. (1997). *The Indian Journal of Animal Reproduction.* **18**(1): 44-45.

Thakur, M.S., Pandey, S.K. and Panday, S.K. (1984). Comparative efficiency of intrauterine infusion of Lugols iodine and secrodyl and secodyl injection in anoestrus cows. *Livestock Adviser*, **9**(12): 57-59.

Thompson, S.Y. (1975). Vitamin A in animal nutrition. Basle, Switzerland; F.Hoffmann-la Roche Co. Ltd.

Trojacanec, S., Bobos, S. and Pajic, M. (2012). Influence of Beta-caroterne and vitamin a supplementation on the ovarian activity of dairy cow with chronic fertility impairment. *VeterinarskiArhiv*,**82**(6): 567-575.

Turpin, R., Jerome, H. and Sihmittjubean, H. (1951). Action de doses progreswsives du diet hystibboestrol Sur la cupremicIe rat. C.R. Acad. Sci. CXLVI : 1703 [c.r.Hidiroglou, M.(1979). Trace element deficiencies and fertility in ruminants. A Review: *Journal Dairy Science.,* **62**(8): 1195-1206.

Uderwood, E.J. (1977). Trace Elements in Human andAnimal Nutrition. *4thEd. Academic Press*. pp. 83

Vadnere, S.Y. and Singh Surendra (1989). Blood plasma level of iodine, calcium, inorganic phosphorus, copper and iron in postpartum anoestrus in crossbred cows. *The Indian Journal of Animal Reproduction.,***10**(2): 145-146.

Van Aken, D., De-Bont, J., Van Holm, L. and Ranawana, S.S.E.(1991). A study on mineral status of cattle in a dairy farm in Srilanka. *Indian Veterinary Journal,* **68**(4): 371-374.

Velhankar, D.P. (1973). Ph.D. thesis. Konkan Krishi Vidyapeeth,Cited by Jadhav *et al.* (1977). *Indian Veterinary Journal*,**54**(7): 536-540.

Vijchulata, P.,Chanvilaikakoran, D.,Djajanegara, A. andSukmawati, A. (1994). Macro and micro elements in plasma and milk of dairy cows receiving concentrates withor without mineral supplements substainable animal production and the environment. In Process of the 7m MAP Animal Science Congress, Bali, Indonesia. 11-16 July, Vol-02, *Contributed paper*, 231-232.

Villard, L. and C.J. Bates, 1986. Carotene dioxygenase activity in rat intestine effects of vitamin A deficiency and pregnancy. *Bureau of Journal Nutrition*,**56**: 115-122**.**

Viswanatha (1991). The diagnosis of physiopathological conditionsof reproduction in bovine female and their treatment.Vijaya Viswanatha Enterprises. 204, R.M.V., Extension, Bangalore - 560096.

Wegner, T.N., Ray, D.E., Lox, C.D. and Stott, G.H. (1973). Effect of stress on serum zinc and plasma corticoids in dairy cattle. *Journal Dairy Science*,**56**(6): 748-752.

Weiss, R.R. (1975). Influence of B-carotene and vitamin A on certain sexual functions in young bulls. Dissertation veterinary medicine. University of Honnover. Abstract.

West, E.S. and Todd, H.S. (1967). Text Book of Biochemistry. 4thedition. The McMillan Co., New York, U.S.A.

Wetherill, I.G.D.(1965). Retained placenta in the bovine. A brief review. Can. The *Veterinary Journal*,**6**:290-295.

Yadav, S.L.,Sanena, V.P. and Dhiman, P.C. (1993). Role of minerals in feeding of dairy animals. Part-I (calcium and phosphorus). Livestock Adviser, **13**(5): 31.

Yasothai, R. (2014). Importance of minerals on reproduction in dairy cattle. *International Journal of Environment Science and Technology*,**3**(6): 2051-2057.

Yessein, S., Shawki, H., Bashandy, M.M., Essawy, S. and Abdullah, I. (1994). Clinico-pathological studies in female infertile buffoles. *The Indian Journal of Animal Reproduction*, **15**(1):14-18.

Yosathai, R. (2014). Importance of minerals on reproduction in dairy cattle.